博士論文

Efficient Use of the Nitrile Functionality as a Gateway to Diversified Peptide Bond Synthesis

（ニトリル官能基を利用した種々のペプチド結合生成反応の開発）

WANG XIAOLING

令和 4 年

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Acknowledgement

Finally, I graduated. Five years, very fast.

I spent my master and doctoral careers here. Five years is really long but it's really fast.

Looking back, I am grateful to my mentor, Prof. Hayashi, for giving me the opportunity to be a member of our lab. Five years ago, after graduating from undergraduate, I made many choices and finally chose and successfully came here to continue my studies. I am very grateful to my supervisor for the training and education that has allowed me to successfully complete my PhD program. I am also grateful to assistant Prof. Li at that time, who is also a teacher and friend. Thank you for your help and teaching, so that I understand how to do research and work efficiently. Experiments and research are full of unknowns, difficulties, and frustrations. Therefore, I am really grateful to every lab member who has accompanied me on the road of scientific research. Especially when I was in the northern laboratory, it was really full of joy. Thank you for your guidance and help at every journal seminar, group meeting, and for the members who discuss and solve problems with me when research encounters problems. I really learned a lot from the discussions. I think this is a good and proper atmosphere for research. We work hard and we find time to have fun with each other, which makes life meaningful. We all really worked hard and that makes me very happy.

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Chapter 2.

Oxidative peptide bond formation of glycine–amino acid using 2-(aminomethyl)malononitrile as a glycine unit.

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Chapter 3.

Highly sterically hindered peptide bond formation between α,α-disubstituted α-amino acids and *N*-alkyl cysteines using α,α-disubstituted α-amidonitrile

Xiaoling Wang, Jing Li and Yujiro Hayashi*

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Abbreviation

Cbz	Benzyloxycarbonyl
Ac	Acetyl
Bn	Benzyl
Boc	*t*-Butoxycarbonyl
Bz	Benzoyl
Bu	Butyl
DMF	*N,N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
dr	Diastereomeric ratio
Et	Ethyl
Me	Methyl
NMR	Nuclear magnetic resonance
Pg	Protecting group
Ph	Phenyl
rt	Room temperature
temp.	Temperature
MeCN	Acetonitrile
NOESY	Nuclear Overhauser effect and Exchange Spectroscopy
THF	Tetrahydrofuran
H_2(2,6-Cl_2TPP)	meso-tetrakis(2,6-dichlorophenyl)porphyrin)
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
TEA	Triethylamine
TFA	Trifluoroacetic acid
^{i}Pr	Isopropyl

Chapter 1. Introduction

1. Peptides and proteins

Peptides and proteins play a plethora of functional and structural roles in biological system. The great significance of peptides and proteins is supported by their abundant natural presence and strong relevance with living cells and organisms. A protein consists of one peptide or several peptides with special conformational structure and a peptide chain is a sequence of amino acids with amide junctures between the carboxylic acid and amine (Figure 1). They are produced in living systems through a series of smooth and well-organized production lines that originate in DNA. Starting from interpreting the genetic information in DNA to mRNA, the synthesis of peptide chains by an orderly assembly of proteinogenic amino acids at ribosomes takes place according to the genetic order from mRNA. This process is called translation, by which a polypeptide is produced. The polypeptide product is further transported to post-translational modifications. The real synthetic and modification stories of proteins are far more complex than what can be described here, which is a result of the abundance of genes, the sequential diversity, the length of a peptide chain and so on. Statistically, for example, in humans, millions of proteomes are thought to be producible arising from ~20, 000 human genes.[1]

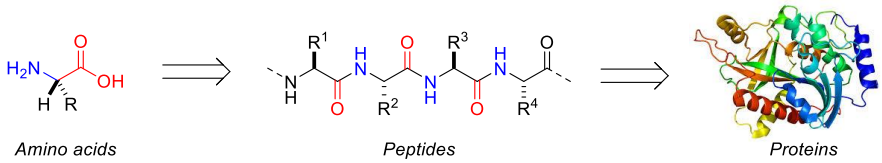

Figure 1. A brief description of peptides and proteins

Enzymes are involved in each step of the ribosomal synthesis and post-translational modifications of peptides, which indicates nicely that proteins are the irreplaceable necessities in living biological entities. In addition, peptide hormones which structurally are either peptide or protein are also good examples to have a closer look at the role of peptides and proteins. For example, human vasopressin, a peptide hormone with 9 amino acids, is also called antidiuretic hormone (ADH), arginine vasopressin (AVP) or argipressin (Figure 2).[2] It's a hormone synthesized from the AVP gene as a peptide prohormone in neurons in the hypothalamus, and is converted to AVP. Vasopressin regulates the tonicity of body fluids.

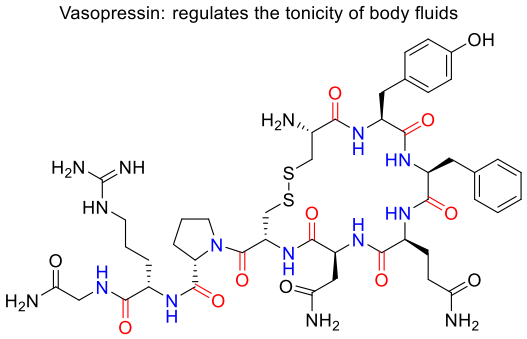

Figure 2. Peptide hormone: human vasopressin

Neuropeptides which regulate brain activities are no doubt of great significance. Nociceptin, is a neuropeptide bearing 17 amino acids (Figure 3). It attracts a lot of attentions from researcher because of its role in pain sensation and fear learning.[3]

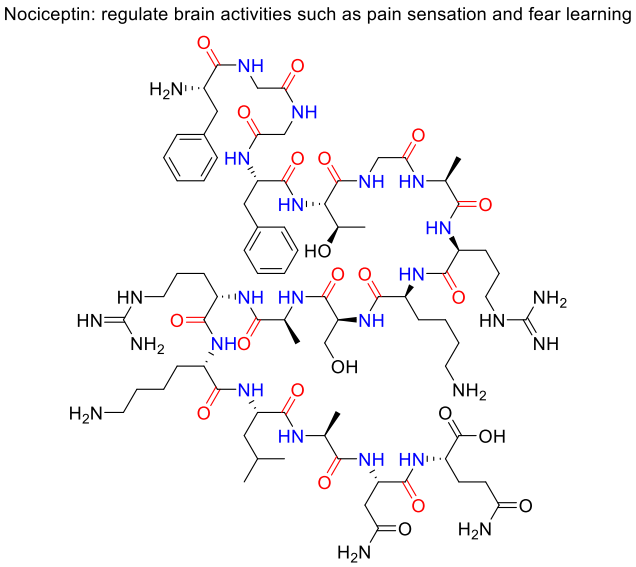

Figure 3. Neuropeptides: Nociceptin

Because of the exceptional roles of peptides and proteins discovered in biological system as well as their excellent biocompatibility, peptide therapeutics have been obtaining growing attentions in recent decades.[4] More than 50 peptide drugs are on the market with approximately 170 in clinical trials, and more than 200 candidates in preclinical development in 2019.[5] For instance, oral semaglutide, a 30-residue glucagon-like peptide-1 (GLP-1) receptor agonist, received FDA approval in 2019 for the treatment of type 2 diabetes.[6] These success in utilizing peptide therapeutics are encouraging for the whole society based on the awareness that peptides and proteins are generally more friendly to patients for reducing as much as possible the side effect and efficient in treatment.

2. Current peptide/protein synthesis

Peptide synthesis has evolved for more than a century. In 1901, E. Fischer and E. Fourneau reported the preparation of the dipeptide glycylglycine by hydrolysis of the diketopiperazine of glycine (Figure 4).[7] And this work opened the door of peptide chemistry.[8]

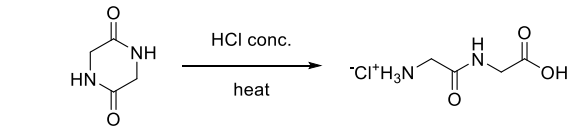

Figure 4. Fischer's synthesis of dipeptide

2.1 Normal peptide synthesis

Numerous research on developing general and mild synthetic methods towards peptides were conducted. Currently, the coupling reagent induced peptide bond formation between carboxylic acid and amine arises to be the most practical and prevalent strategy. So far, the coupling reagents developed include carbodiimides along with coupling additives, phosphonium reagents and uronium reagents (Figure 5).[9]

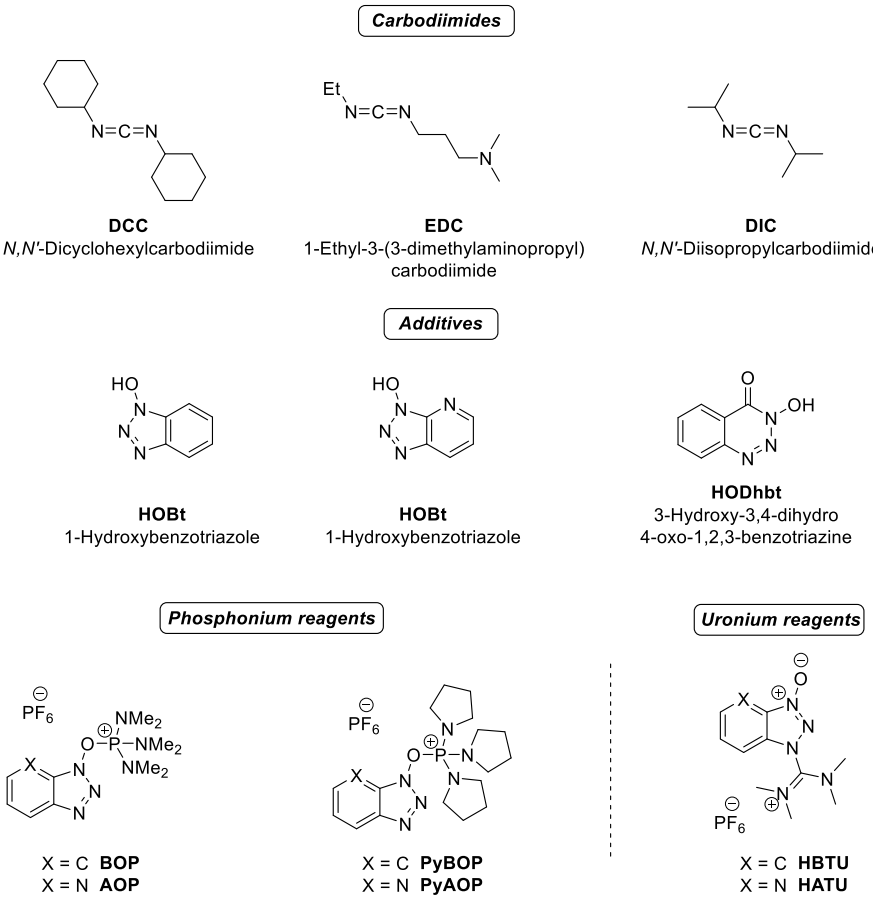

Figure 5. Examples of coupling reagents

In a normal solution phase synthesis, the peptide product was isolated by a work-up and chromatography, which usually becomes problematic due to the generation of a huge amounts of byproducts as a result of those bulky coupling reagents (Figure 6).

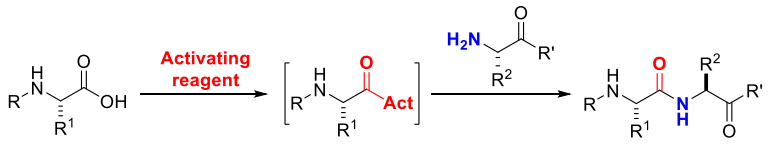

Figure 6. Solution-phase peptide synthesis by coupling reagents

Solid-phase peptide synthesis was invented by B. Merrifield in 1963,[10] which was a big breakthrough for the chemical synthesis of polypeptides or small proteins. Basically, by attaching one amino acid to an insoluble resin support at the C terminus via a covalent bond juncture, the peptide product can be easily separated from the byproduct by a simple filtration and washing procedure. And the elongation of the peptide chain can be achieved by an iterative operation including deprotection, washing, coupling, washing (Figure 7).

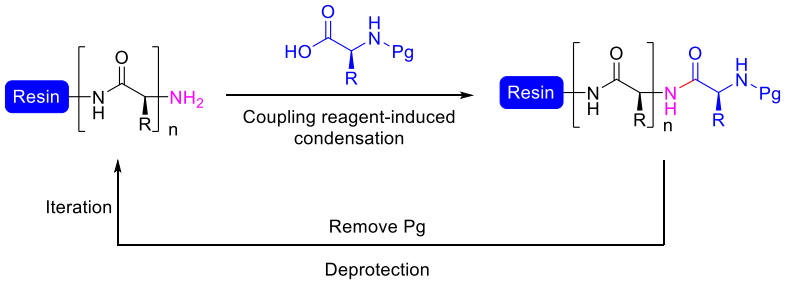

Figure 7. Solid-phase peptide synthesis by coupling reagents

2.2 Sterically hindered peptide synthesis

Normal peptides made up with the proteinogenic amino acids are of paramount significance in a broad variety of research fields. Unusual peptides whose structure are analogous to the proteinogenic peptides are also frequently found in nature, for example, fungi, bacteria, marine sponges and other lower animal forms.[11] The inclusion of unusual amino acids in the backbone of peptides creates the so-called unusual peptides. Sterically hindered amino acids are among the group of such kind of unusual amino acids. Replacement of the α-hydrogen atom of normal amino acids with an alkyl substituent affords α,α-disubstituted amino acids (Figure 8).[11,12] Naturally occurring peptides bearing one or more α,α-disubstituted amino acid residues are shown to have a rigidified conformation and improved stability against physical environment. Alkylation of the nitrogen atom of normal amino acids forms *N*-alkyl amino acids (Figure 8). Such kind of N-alkylated amino acids are widely existed in bacterial antibiotics, fungal metabolites and display restricted conformation, improved bioactivity and oral bioavailability.[11,13]

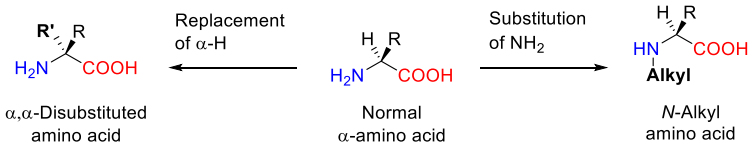

Figure 8. Unusual amino acids: sterically hindered type

The efficient synthesis of sterically hindered peptides is difficult because of the high potential of epimerization and other side reactions.[11] Racemization during the activation of the *N*-protected *N*-methyl amino acid can occur and afford an oxazolonium ion, which is easily epimerized through enolization or tautomerization (Figure 9). In addition, the dipeptidyl *N*-methyl product can undergo cyclization to afford side product diketopiperazine (Figure 10). Similar problems arise as well in the case of α,α-disubstituted peptides. The diketopiperazine cyclization can easily occur attributed to the gem dialkyl effect.[14]

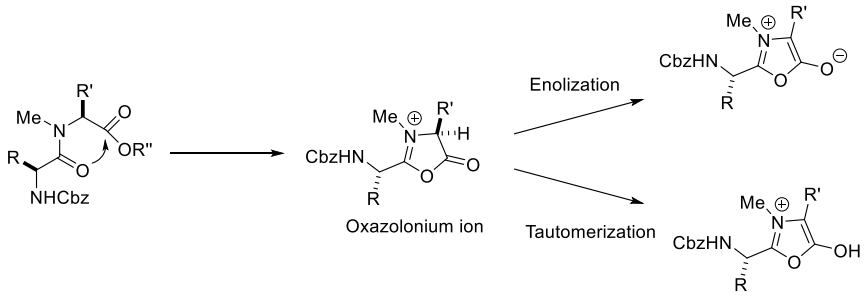

Figure 9. Racemization pathway during the synthesis of N-Methyl peptide

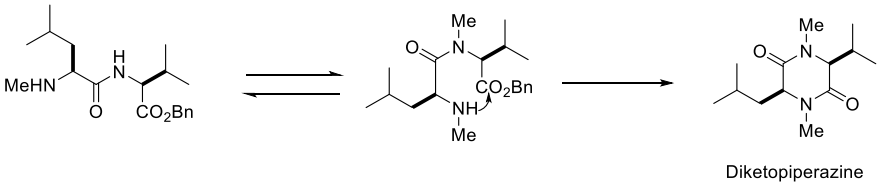

Figure 10. Diketopiperazine cyclization of N-Methyl peptide

2.3 Large peptide synthesis

The coupling between two polypeptide segments is called peptide ligation. A ligation reaction which forms a native peptide bond is generally preferred to a ligation by forming other types of covalent bonding. Peptide ligation requires a coupling reagent free, mild, often in aqueous and dilute conditions. Therefore, the coupling reagent-initiated methodology is not applicable. Several good site-selective coupling reactions have been developed, like native chemical ligation,[15] Staudinger ligation,[16] keto-hydroxyl amine ligation,[17] serine/threonine ligation[18] and so on. However, each method has their limits and current chemical society is called for innovation in this region.

3. This Work

In chapter 2, a nonconventional synthetic method for the synthesis of short peptides is developed (Figure 11). The substituted malononitrile acts as a masked acyl moiety of amide bond with formation of an acyl cyanide as the key intermediate. O_2 acts as an oxidant and provides amide's oxygen. The acyl cyanide can be then trapped by the amine substrate and provides the peptide product. The reaction condition is mild and simple with using a base under aqueous condition.

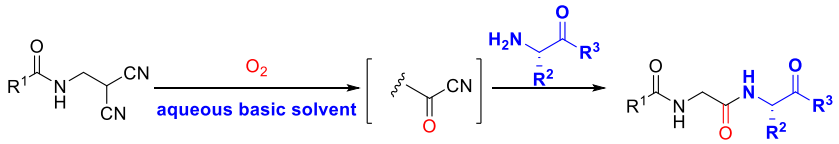

Figure 11. Oxidative peptide synthesis

In chapter 3, an efficient methodology for the synthesis of extremely sterically hindered peptides is described (Figure 12). Basically, the reaction proceeds selectively between the α,α-disubstituted α-amido nitrile and *N*-alkyl cysteine under an aqueous condition. It doesn't require any coupling reagents. The α,α-disubstituted *N*-alkyl alanyl peptide can also be easily prepared via desulfurization of its cysteinyl precursor.

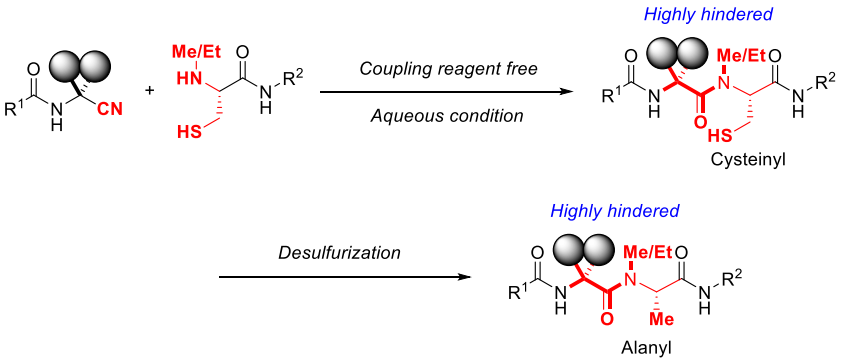

Figure 12. Highly hindered peptide synthesis

In chapter 4, preliminary investigation on large peptide ligation is shown (Figure 13). By incorporating a chiral mono α-amido nitrile into the C-terminal peptide and NH_2-cysteine into the N-terminal fragment, a thiazoline intermediate can be generated under an aqueous condition. The thiazoline intermediate is further hydrolysed to give the ligated cysteinyl peptide product.

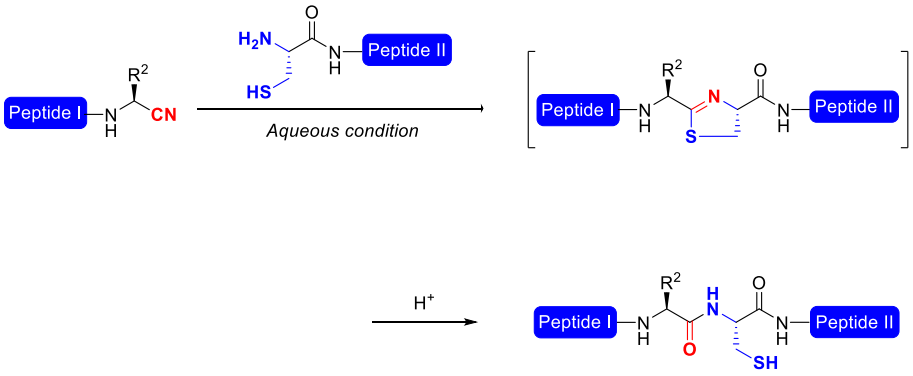

Figure 13. Peptide ligation

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Chapter 2. Oxidative peptide bond formation of glycine–amino acid using 2-(aminomethyl)malononitrile as a glycine unit

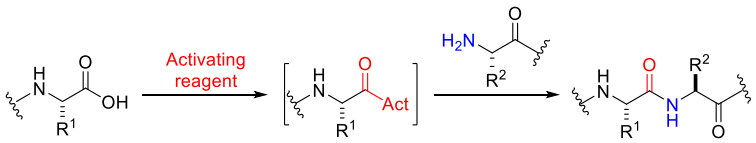
1. Introduction

Peptides and proteins are essential components in almost all living organisms and play various central functions.[1] Their abundant natural occurrence and unparalleled biological and medicinal significance have made them a vital research topic in both biological and chemical research field.[2] The chemical synthesis represents the most reliable method to provide the desired peptides and proteins in both reasonable quantity and purity compared to the other two main pathways that are natural isolation and recombinant expressions in microorganisms.[3]

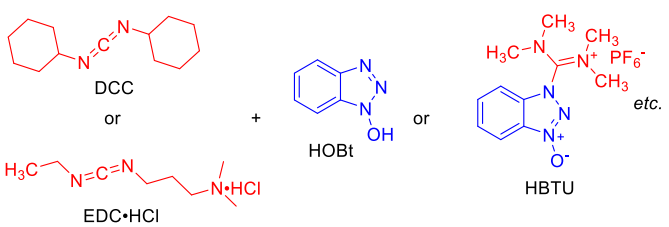
1.1 Traditional method for peptide synthesis

There are many methods for the amide bond synthesis.[4] Traditionally, the direct amide formation between C-terminal carboxylic acid and N-terminal amine amino acids and peptides is well utilized by employing coupling reagents (Figure 1).[5] Mechanistically, the carboxylic acid has to be activated by an activating reagent to give an active species which can next be trapped by the amine group, generating a CONH amide bond. This is a great method that the activation strategy by coupling reagents successfully builds the amide linkage between carboxylic acid and amine. Because there's no reactivity towards each other without the coupling reagents. Moreover, both starting materials are readily available. The often-used coupling reagents are DCC, EDC•HCl combined with a HOBt catalyst or a bifunctional coupling reagent, like HBTU. However, the activating reagents are generally expensive and they are required stoichiometrically. On the other hand, one of the most disadvantageous properties of the traditional method is that the purification is generally problematic due to the unavoidable byproduct generated from the activating reagents. The generally large molecular weight of coupling reagents leads to poor atom efficiency and makes it environmentally hazardous.

(a) Traditional peptide bond formation



(b) Often-used activating reagents



Price from TCI

HBTU	EDC•HCl	HOBt
¥23,900 100 g	¥44,700 100 g	¥5,500 25 g

Figure 1. Traditional method for peptide synthesis and often-used coupling reagents

1.2 Nonclassical amide bond formation

Although the activating strategy of carboxylic acid by coupling reagents accounts the most prevalent and efficient method in academia and industry for making amide products, a much more sustainable method in terms of green chemistry is still under exploration. To overcome the challenge from the low reactivity of carboxylic acid toward amine and to avoid the adoption of hazardous coupling reagents, novel synthetic methods are driven to emerge.

1.2.1 Catalytic amide synthesis: replacing coupling reagents by organoboron reagent

As described above, the traditional method manages to work result from the activation of the unreactive carboxylic acid, which unavoidably generates large amounts of waste. Therefore, catalytic versions of activation strategy emerge, among which boronic acids as catalysts are the most prominent (Figure 2). In 1996, Yamamoto and coworkers reported the seminal advancement using 3,4,5-trifluorobenzeneboronic acid as an extremely active amide formation catalyst with only 1 mol%. Various aliphatic and aromatic carboxylic acids and amines are compatible with the reaction condition, including some chiral carboxylic substrates.[16]

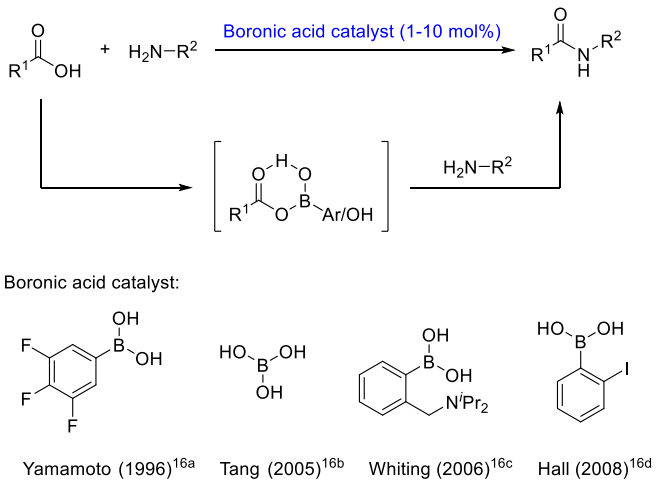


Figure 2. Boronic acid catalyzed amide synthesis

1.2.2 Amidation by using carboxylic acid surrogates: alcohol, aldehyde, alkyne

Other precursors of CONH's acyl moiety are developed in order to avoid the use of carboxylic acid through metal- or organo-catalyzed pathway.

Alcohol as starting material As a natural product with widespread occurrence and importance, alcohols fulfill the primary need as a feedstock for amide production. A remarkable breakthrough brought by

Milstein's group took the advantages of ruthenium-catalyzed dehydrogenation of alcohol and hemiaminal and achieved an efficient preparation of amide products (Figure 3). At first, the alcohol was oxidized by the ruthenium catalyst to give aldehyde, which can react with amine and forms a hemiaminal intermediate. The hemiaminal was trapped again by the ruthenium complex and another molecule of H_2 got extruded and the amide product was formed. It is noteworthy to mention that equimolar amounts of starting components are good enough to achieve good to excellent chemical transformations. The merit of this protocol includes also the generation of negligible and completely green waste which is H_2 gas. Other similar ruthenium catalysts are developed too.[17]

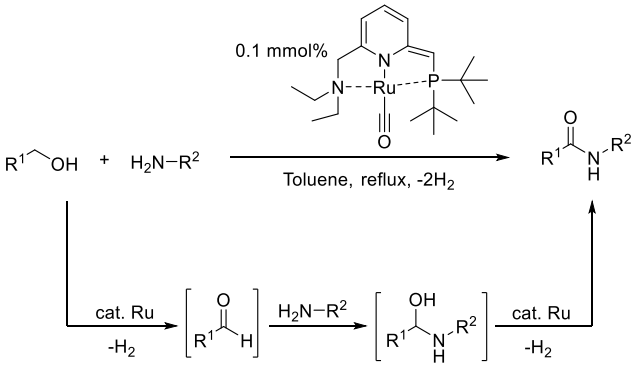


Figure 3. Ruthenium catalyzed amide synthesis from alcohol and amine

Aldehyde as starting material As indicated in the previous part, alcohol is used as precursor of acyl functionality and the corresponding aldehyde in-situ formed is a key intermediate. Aldehyde itself can also be used to make coupling with amine. An oxidative metal-catalyzed coupling between aldehyde and amine was reported and a hemiaminal intermediate was proposed to be involved in the reaction system (Figure 4a).[18,19] On the other hand, N-heterocyclic carbene catalyzed oxidative amidations of various aldehydes to the corresponding hexafluoroisopropylesters by using the readily available organic oxidant **A** were developed. The hexafluoroisopropylesters prepared in situ are shown to be highly useful active esters for amide bond formation (Figure 4b).[20] The NHC-catalyzed redox reaction works with various α-functionalized aldehydes to generate an activated carboxylate, which is then converted to an amide with a variety of amines (Figure 4c).[21]

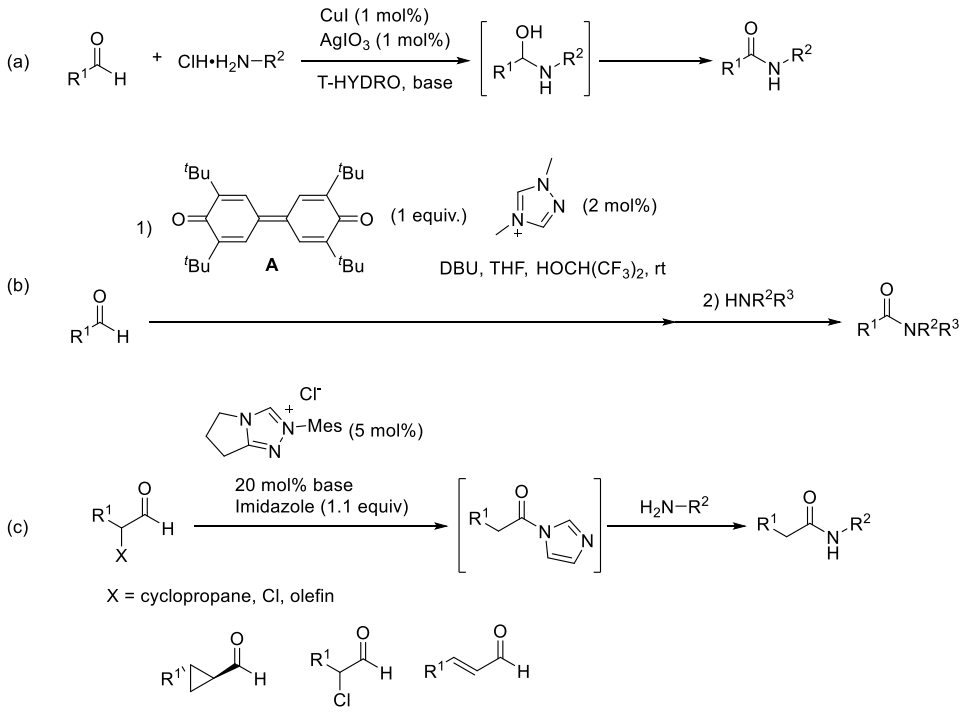


Figure 4. Amide synthesis from aldehyde and amine

Alkyne as starting material The amide synthesis is also reported to be synthesized from alkyne substrates through an oxidative pathway catalyzed by Mn catalyst (Figure 5).[22]

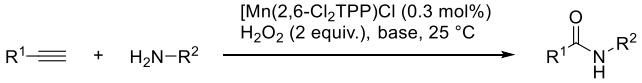


Figure 5. Amide synthesis from alkyne and amine

1.3 Oxidative amide synthesis from nitroalkane developed in my group

In 2015, my group reported an oxidative amide bond formation.[11] The amide coupling takes place between nitroalkane and amine with using I_2 or NIS under oxygen atmosphere and K_2CO_3 (Figure 6). The reaction was proposed to proceed through firstly the generation of α-iodo nitroalkane and α-diiodo nitroalkane by halogen bonded NIS–NH_2R' complex. From this intermediate onwards, the late-stage nucleophilic attack may occur through an acyl precursor (Figure 7).

Briefly, the oxidative amide synthesis was achieved with readily available nitro compounds in a straightforward operation and highly chemo-selective manner. A similar work was also published by Johnston's group but different mechanisms were proposed.[12]

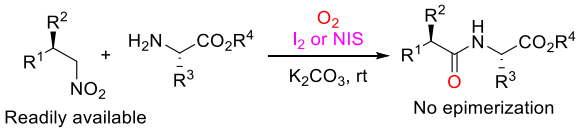


Figure 6. oxidative amide synthesis from nitroalkane

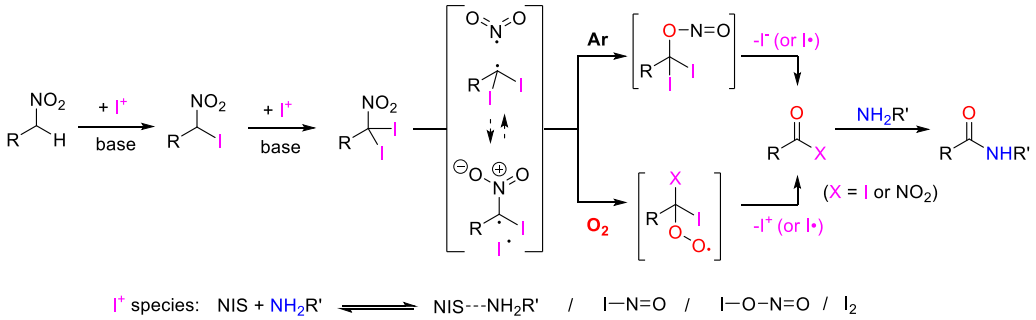


Figure 7. proposed mechanism

1.4 Oxidative amide synthesis from substituted malononitrile developed in my group

In 2016, another oxidative synthesis of amide was achieved by using α-substituted propanedinitrile and amine in the presence of O_2 and K_2CO_3 in anhydrous CH_3CN (Figure 8).[11c] The only reagents needed are K_2CO_3 and oxygen with the generation of KCN as a byproduct. Anhydrous condition was employed in order to suppress the undesired hydrolysis of an acyl cyanide intermediate in the reaction of steric hindered substrates. This method accommodates a broad range of both steric hindered propanedinitrile and amine in moderate to excellent yield. For example, benzyl, *t*-butyl, 2-benzyl *t*-butyl and adamantane substituted malononitriles are used. Primary amine nucleophiles substituted by allyl, *t*-butyl, cyclohexyl and secondary amine nucleophiles like *N*-methyl benzyl amine and pyrrolidine are suitable starting materials to synthesize sterically hindered amides.

In a word, various sterically hindered amides were synthesized in a simple reaction system with using K_2CO_3 and O_2 which are small molecules. It represents a nonconventional amide synthesis with high atom efficiency under mild conditions.

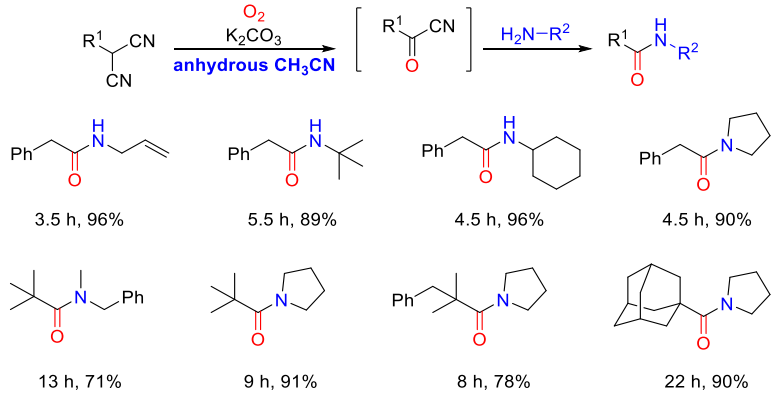


Figure 8. Selected examples of oxidative sterically hindered amide synthesis

2. This work

An ideal synthetic method for peptides requires no coupling reagents, catalysts, and toxic metals, often in aqueous solution and a simple work-up, which has been a long-standing goal.[13] Therefore, new simple coupling methodology for peptides synthesis is still in great demand.

Encouraged by the merits of the oxidative amide synthesis from substituted malononitrile, an idea was proposed. If protected amino acids and peptides can be employed as a nucleophile and a substituted 2-(aminomethyl)malononitrile can be employed as an electrophile, it would be a new method for the synthesis of an amide linkage between glycine and amino acid with a generation of the elongated peptides, without coupling reagent and with minimum waste generation (Figure 9). However, as a chiral substrate was found to give epimerization under the previous basic oxidative condition, only glycine-derived starting material is used. As amino acids and peptides are soluble in aqueous solvent, the challenge in the present reaction is whether the reaction proceeds efficiently in such an aqueous solvent. In this chapter, I will describe the realization of this scenario.

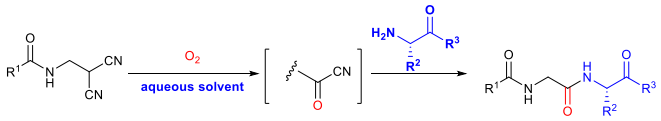


Figure 9. This work: peptide synthesis with Gly-amino acid in aqueous solution

3. Optimization of the reaction conditions

I began the initial investigation with optimizing the reaction conditions using Boc-protected L-phenylalanine substituted propanedinitrile **1a** and L-phenylalanine methyl ester **2a** en route to a Boc-L-Phe-Gly-L-Phe-OMe tripeptide **3a** (Table 1). Starting substituted propanedinitrile **1a** was easily prepared from Boc-protected L-phenylalanine and aminomethylene propanedinitrile[14] by the coupling reaction, followed by the reduction with $BH_3 \cdot NH_3$ (Figure 10).

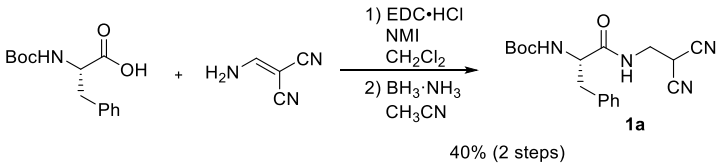
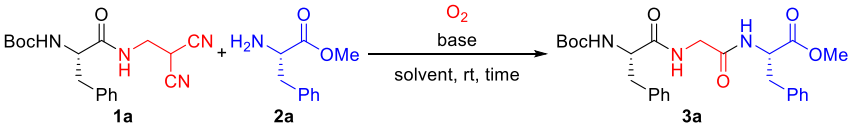


Figure 10. Synthesis of **1a**

Previous oxidative amide bond formation was conducted in anhydrous conditions, and in some cases in the presence of MS4Å to remove water completely.[11c] However, as a solubility of protected amino acids and peptides toward an organic solvent is generally poor, the aqueous solvent has to be employed. We choose a solvent system of DMF/H_2O = 9/1 in the initial investigation. Although K_2CO_3 and Cs_2CO_3

are suitable bases under previous anhydrous conditions, a complex mixture was obtained (entry 1 and 2). It was happy to find that the reaction proceeded even under aqueous conditions to afford the product **3a** in 52% yield when KOAc was employed as a base (entry 3). The yield was further improved to 62% in the presence of CsOAc (entry 4). Next, I screened the amount of water using CsOAc as a base. A mixture of DMF and H_2O (6/1) also gave an acceptable yield, but the yield decreased when amount of water increased such as DMF/H_2O (3/1) (entry 5, 6). DMSO gave a similar result with DMF (entry 9), but CH_3CN was found to be a poor solvent although it was the best solvent under anhydrous condition (cf. entry 8 and ref. 11c). Therefore, the optimal reaction conditions were found to use CsOAc as a base in a solvent of a mixture of DMF and H_2O at room temperature. We selected the ratio (6/1) of DMF and H_2O considering the better solubility of other amino acid.

Table 1. The effect of base and solvent in the reaction of **1a** and **2a**[a]



Entry	Base	Solvent	Time/h	Yield/%[b]
1	K_2CO_3	DMF/H_2O=9/1	17	<5
2	Cs_2CO_3	DMF/H_2O=9/1	18	<5
3	KOAc	DMF/H_2O=9/1	15	52
4	CsOAc	DMF/H_2O=9/1	12	62
5	CsOAc	DMF/H_2O=6/1	12	56
6	CsOAc	DMF/H_2O=3/1	24	42
7[c]	CsOAc	DMF/H_2O=6/1	4	47
8	CsOAc	CH_3CN/H_2O=6/1	20	<5
9	CsOAc	DMSO/H_2O=6/1	12	54

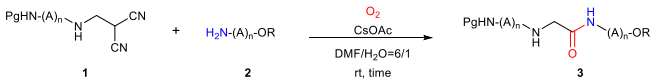
[a]Unless otherwise shown, the reaction was performed by employing **1a** (0.2 mmol), **2a** (0.4 mmol), base (0.4 mmol), solvent (total volume = 2 mL) under O_2 atmosphere at room temperature at the indicated time. [b]Isolated yield. [c]The reaction temperature is 40 °C.

4. Generality

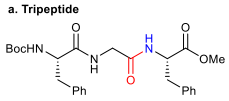
With the optimal condition in hand, the generality of the reaction was investigated (Table 2). A wide range of tripeptides was synthesized by applying various *N*-terminal free amine of amino acid esters with acceptable yields (Table 2a). Not only Boc-protected L-phenylalanine substituted propanedinitrile **1a** but also Cbz-protected L-leucine substituted and Boc-protected L-serine benzyl ether substituted propanedinitriles are suitable C-terminal glycine units. Suitable nucleophilic amino acids are Phe-OMe, Phe-Ot-Bu (Bu = butyl), Tyr-OMe, Leu-OMe, Ile-OMe, Met-OMe, Ser(Ot-Bu)-Ot-Bu, Thr(Ot-Bu)-Ot-

Bu, Glu(OMe)-OMe. Trp-OMe, Cys(STr)-OMe (Tr = Trityl), Lys(NHBoc)-OMe, His(NTr)-OMe and Arg(Pbf)-OMe.15 Thus, the tripeptides such as Boc-Phe-Gly-Phe-OMe (**3a**), Boc-Phe-Gly-Phe-Ot-Bu (**3b**), Boc-Phe-Gly-Tyr-OMe (**3c**), Boc-Phe-Gly-Leu-OMe (**3d**), Boc-Phe-Gly-Ile-OMe (**3e**), Boc-Phe-Gly-Met-OMe (**3f**), Boc-Phe-Gly-Ser(Ot-Bu)-Ot-Bu (**3g**), Boc-Phe-Gly-Thr(Ot-Bu)-Ot-Bu (**3h**), Boc-Phe-Gly-Glu(OMe)-OMe (**3i**), Boc-Phe-Gly-Trp-OMe (**3j**), Boc-Phe-Gly-Cys(STr)-OMe (**3k**), Boc-Phe-Gly-Lys(NHBoc)-OMe (**3l**), Boc-Phe-Gly-His(NTr)-OMe (**3m**), Boc-Phe-Gly-Arg(Pbf)-OMe (**3n**), Cbz-Leu-Gly-Phe-OMe (**3o**), Cbz-Leu-Gly-Met-OMe (**3p**) and Cbz-Leu-Gly-Trp-OMe (**3q**) and Boc-Ser(OBn)-Gly-Lys(NHBoc)-OMe (**3r**) were synthesized in good yield, in which Gly-amino acid bond was constructed oxidatively. Moreover, tetrapeptides such as Boc-Phe-Gly-Leu-Phe-Ot-Bu (**3s**), Cbz-Leu-Gly-Leu-Phe-Ot-Bu (**3t**), Boc-Phe-Leu-Gly-Phe-OMe (**3u**), Boc-Phe-Leu-Gly-Ile-OMe (**3v**), and Boc-Phe-Leu-Gly-Tyr-OMe (**3w**) were also synthesized in a moderate yield, in which Gly-amino acid bond was prepared (Table 2b).

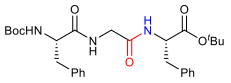
Table 2. Oxidative peptide synthesis[a]



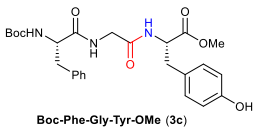
a. Tripeptide



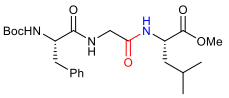
Boc-Phe-Gly-Phe-OMe (3a)
58%, 13.5 h[b]



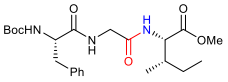
Boc-Phe-Gly-Phe-Ot-Bu (3b)
50%, 20 h



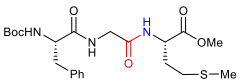
Boc-Phe-Gly-Tyr-OMe (3c)
52%, 16 h



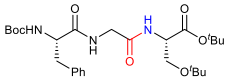
Boc-Phe-Gly-Leu-OMe (3d)
65%, 13 h



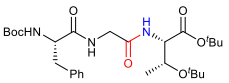
Boc-Phe-Gly-Ile-OMe (3e)
60%, 13 h



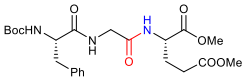
Boc-Phe-Gly-Met-OMe (3f)
47%, 12 h



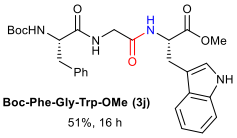
Boc-Phe-Gly-Ser(Ot-Bu)-Ot-Bu (3g)
56%, 12 h[c]



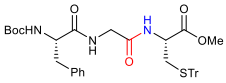
Boc-Phe-Gly-Thr(Ot-Bu)-Ot-Bu (3h)
51%, 17 h



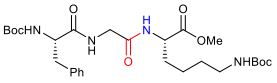
Boc-Phe-Gly-Glu(OMe)-OMe (3i)
32%, 12 h



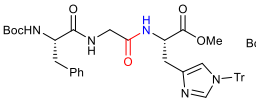
Boc-Phe-Gly-Trp-OMe (3j)
51%, 16 h



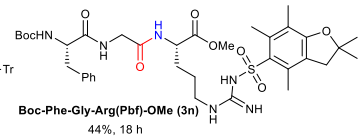
Boc-Phe-Gly-Cys(STr)-OMe (3k)
43%, 19 h



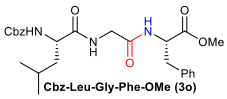
Boc-Phe-Gly-Lys(NHBoc)-OMe (3l)
53%, 16 h



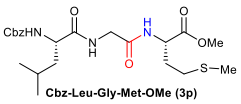
Boc-Phe-Gly-His(NTr)-OMe (3m)
49%, 20 h



Boc-Phe-Gly-Arg(Pbf)-OMe (3n)
44%, 18 h

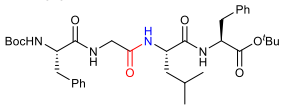


Cbz-Leu-Gly-Phe-OMe (3o)
57%, 16 h

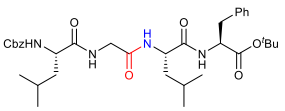


Cbz-Leu-Gly-Met-OMe (3p)
43%, 15.5 h

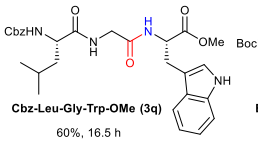
b. Tetrapeptide



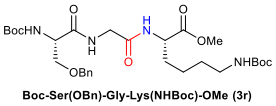
Boc-Phe-Gly-Leu-Phe-Ot-Bu (3s)
60%, 18.5 h



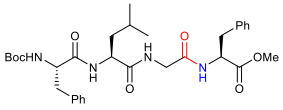
Cbz-Leu-Gly-Leu-Phe-Ot-Bu (3t)
47%, 16 h



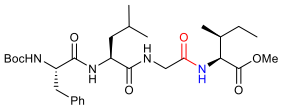
Cbz-Leu-Gly-Trp-OMe (3q)
60%, 16.5 h



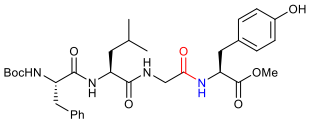
Boc-Ser(OBn)-Gly-Lys(NHBoc)-OMe (3r)
45%, 19.5 h



Boc-Phe-Leu-Gly-Phe-OMe (3u)
50%, 15 h

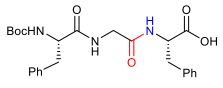


Boc-Phe-Leu-Gly-Ile-OMe (3v)
53%, 17.5 h

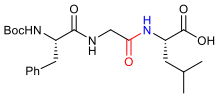


Boc-Phe-Leu-Gly-Tyr-OMe (3w)
51%, 18 h

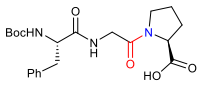
C. Tripeptide by using unprotected amino acid[d]



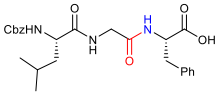
Boc-Phe-Gly-Phe-OH (3x)
67%, 22 h



Boc-Phe-Gly-Leu-OH (3y)
64%, 22 h



Boc-Phe-Gly-Pro-OH (3z)
66%, 20 h



Cbz-Leu-Gly-Phe-OH (3aa)
55%, 24 h

[a]Unless otherwise shown, the reaction was performed by employing 1 (0.2 mmol), 2 (0.4 mmol), CsOAc (0.4 mmol), DMF/H2O=6/1 (total volume: 2 mL) under O2 atmosphere at room temperature at the indicated time. The yield is an isolated yield. [b]The reaction was conducted with 0.5 mmol of 1. [c]DMF/H2O=9/1 was used. [d]The reaction was performed by using amino acid lithium salt in DMF/H2O = 7/1. See SI for more detail.

It should be noted that there is no necessity to protect the phenol moiety of tyrosine (**3c**, **3w**), and the reaction is compatible with methyl sulfide of methionine (**3f**, **3p**) even though the reaction was conducted under oxygen atmosphere. Indole of tryptophan (**3j**, **3q**) and ester moiety of glutamic methyl ester (**3i**) did not disturb the reaction. Protected functionalized amino acids such as cysteine (**3k**), lysine (**3l**, **3r**), histidine (**3m**), and arginine (**3n**) can be successfully employed. As all the substrates are soluble in an aqueous solvent and aqueous condition did not affect the reaction, the reaction proceeded efficiently to afford elongated peptides in a good yield. More important, unprotected leucine (**3y**), phenylalanine (**3x**, **3aa**) and proline (**3z**) were successfully coupled demonstrating the orthogonality of the current method with condensative peptide couplings (Table 2c). Amino acid lithium salt was used in order to have a better solubility.

5. Unsuccessful results

5.1 Synthesis of pentapeptide failed

As described in the generality table, this method can be applicable to the preparation of tripeptides and tetrapeptides with utilizing a mono amino acid ester or a dipeptide as nucleophile. Apart from this, the synthesis of pentapeptide by reacting Boc-protected L-phenylalanine substituted propanedinitrile **1a** with tripeptide NH_2-L-Phe-L-Leu-L-Met-OMe was examined. However, no desired product was obtained (Figure 11). The nucleophilic approachability of the dipeptidyl substrate reduces probably because of the increased bulkiness and the shielding of the amine moiety from the rest part of the peptide chain.

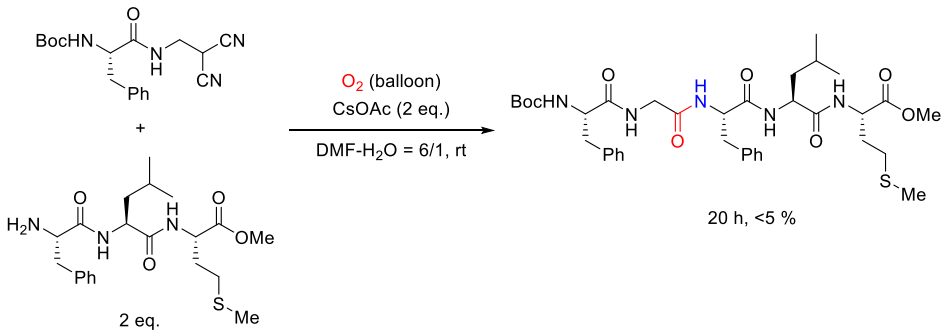


Figure 11. Unsuccessful synthesis of pentapeptide

5.2 Synthesis of *N*-Methyl peptide failed

Moreover, considering that the intermediate acyl cyanide has good steric approachability, there's a chance that the *N*-methylated nucleophile is capable of trapping it and delivering a sterically hindered peptide product. After preparing *N*-Me-L-Phe-OMe, it was applied to the standard aqueous condition and a messy reaction profile was observed (Figure 12a). Given the increased steric hindrance in *N*-Me-L-Phe-

OMe, a water-free condition with using DMF only was tried next. However, it did not give any improvement (Figure 12b).

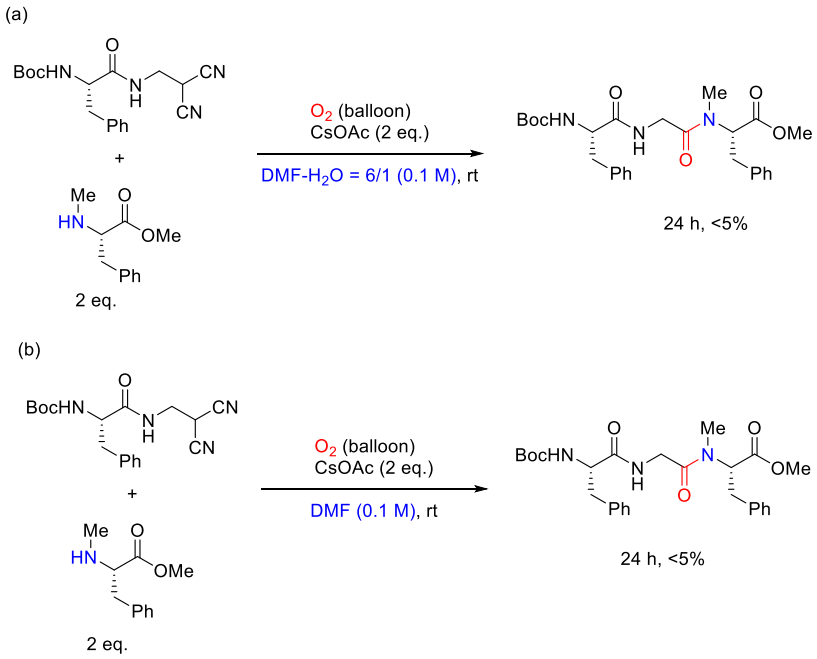


Figure 12. Unsuccessful synthesis of *N*-Me peptide

6. Unexpected discovery

As seen in the generality table, the trityl-protected cysteine methyl ester was able to give its corresponding tripeptide Boc-L-Phe-Gly-L-Cys(STr)-OMe (**3k**) in an acceptable yield. Before that, the unprotected cysteine methyl ester was used directly to react with **1a**. A very quick vanishment of **1a** was observed and a thiazolidine compound was isolated in 90% yield (Figure 13).

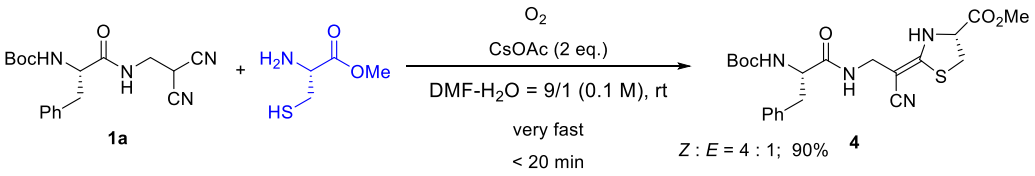


Figure 13. Unexpected discovery while using Cys-OMe

The reaction was proposed to take place following the described procedure here (Figure 14). The cysteine's thiol attacks one of the nitrile groups followed by a cyclization to give a thiazoline intermediate because of the presence of an adjacent amine moiety. One molecule of NH_3 was eliminated in this process. At this stage, the remaining unreacted CN group can induce an epimerization and generate the thiazolidine product with a conjugated system. The thiazolidine product was a geometric mixture of *Z* and *E* in 4 to 1 ratio.

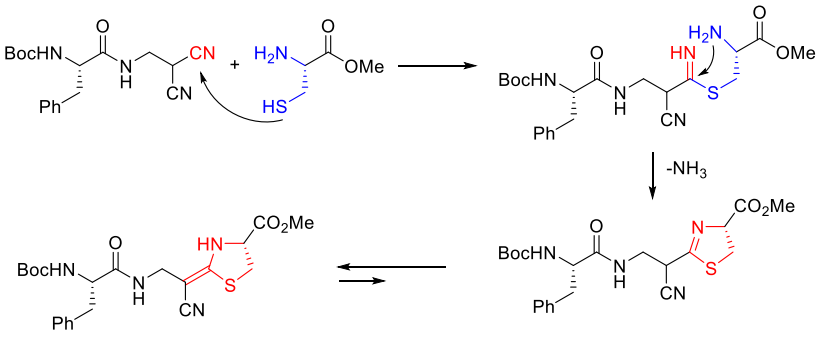


Figure 14. Proposed reaction pathway towards thiazolidine

The determination of its structure and geometry of the olefin moiety was conducted. Since **4** was shown to be existed as a mixture of rotamers, which was proved by taking its **^{1}H NMR** (measured in DMSO-d_6) under variable temperatures (Figure 15). In addition, **^{1}H NMR** (measured in DMSO-d_6) suggested that **ss1** was a mixture of two isomers (Figure 16).

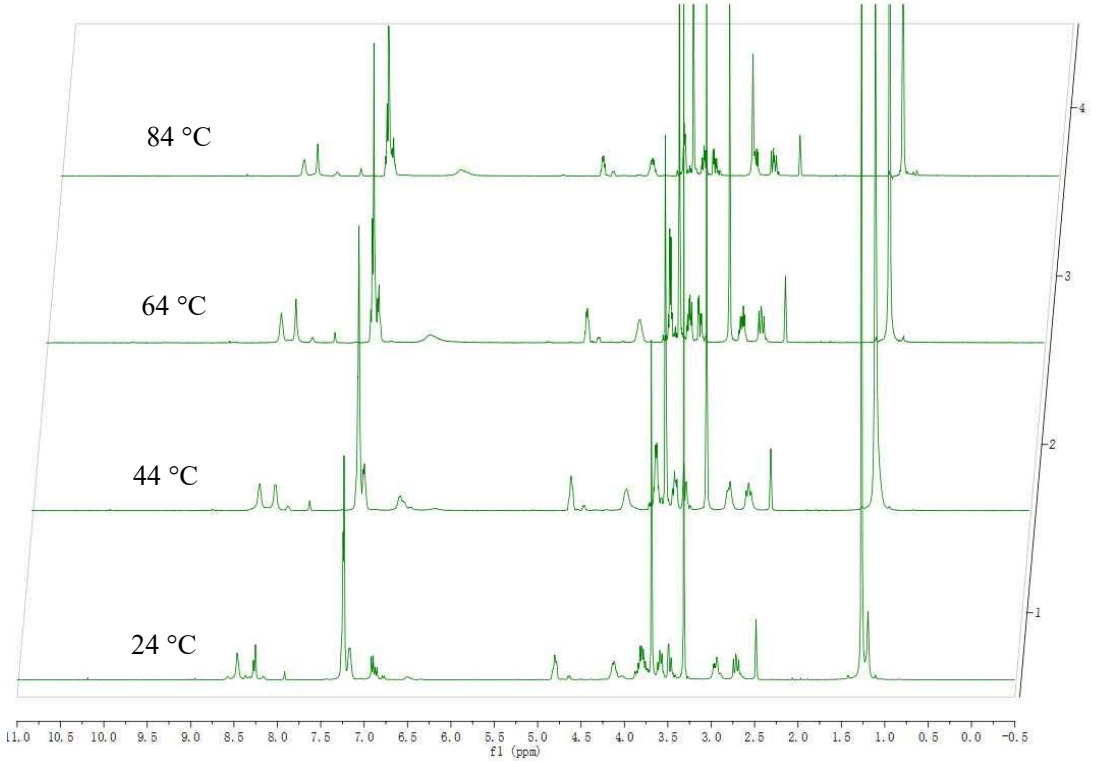


Figure 15. Variable temperature **^{1}H NMR** (DMSO-d_6) to prove the rotamers of **4**

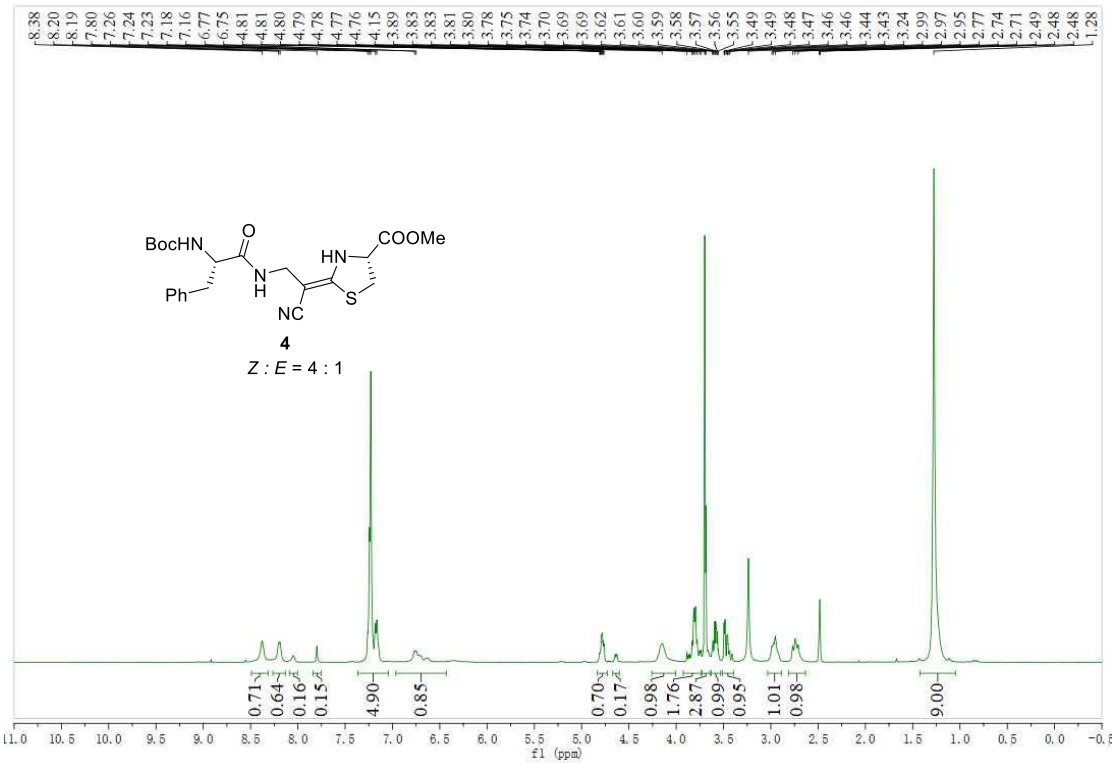


Figure 16. **^{1}H NMR** of **4** measured at 44 °C in DMSO-d_6 for determining the ratio of the two isomers

Based on the 1D NOESY study, the two isomers were ascribed to the double bond region with the Z isomer as the major compound (Figure 17).

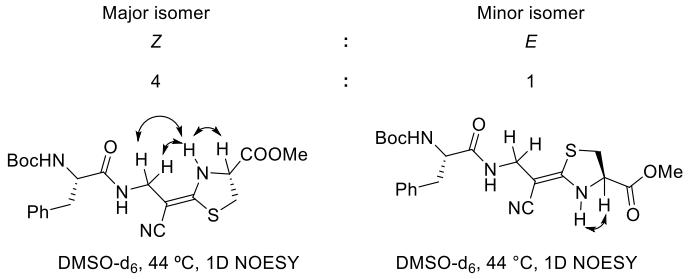


Figure 17. Result of 1D NOESY study of **4**

7. Proposed mechanism

A possible reaction mechanism was proposed when the oxidative sterically hindered amidation was developed (Figure 18) and it's most likely the same to the mechanism in this case. The substituted malononitrile undergoes deprotonation by the base to form a malononitrile anion. Single electron transfer occurs between the malononitrile anion and molecular O_2 to generate two radicals, which couples together to give a peroxide anion intermediate. With formation of a dioxirane intermediate, one of the two CN groups is eliminated. The dioxirane is then cleaved by another molecule of malononitrile anion and generates a tetrahedral intermediate, which further collapses to give two molecules of acyl cyanide species. At this stage, the acyl cyanide species are trapped by amine to give the amide products.

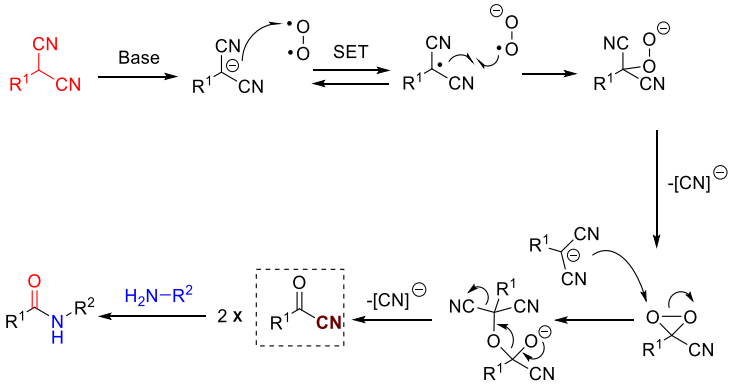
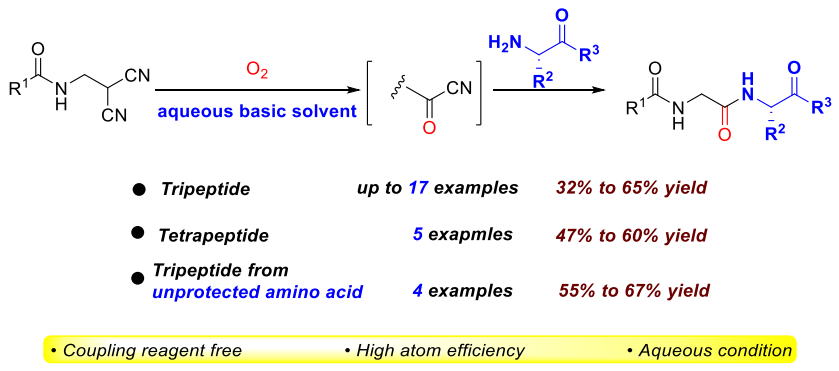


Figure 18. Propose mechanism of oxidative amide synthesis

8. Conclusion

In conclusion, an oxidative synthetic method of the peptide bond of glycine-amino acid has been developed under O_2 atmosphere in the presence of CsOAc in aqueous solution without coupling reagents and catalyst. Substituted 2-(aminomethyl)malononitrile acts as a glycine unit to react with a wide variety of amino acids to afford tripeptides, tetrapeptides under mild reaction conditions. Although the present method is limited to the generation of an amide linkage between glycine and other amino acids, it offers an alternative method for the chemical synthesis of amide linkage in the peptides.



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Chapter 3. Highly sterically hindered peptide bond formation between α,α-disubstituted α-amino acids and *N*-alkyl cysteines using α,α-disubstituted α-amidonitrile

1. Introduction

Peptides and proteins make up a family of compounds that are of utmost importance in nature because of their ubiquitous presence and essential bioactivity.[1,2] In recent decades, peptide chemistry has flourished in multidisciplinary areas, such as organic chemistry,[3-5] biochemistry,[6,7] material science,[8-10] pharmaceutical science,[11-14] and others.[15] The 20 standard natural amino acids coded by DNA are typical feedstocks of myriad coupling reactions towards peptides and proteins that are of interest. However, there are distinct disadvantages in using peptides in terms of biological and therapeutic practicality because of their instability towards proteinase and peptidase in vivo and because of their poor membrane permeability.[11-14] The unusual α,α-disubstituted α-amino acids[16-20] and *N*-alkyl α-amino acids[21-24] (Figure 1a, 1b), which are two key classes of non-coded and sterically hindered amino acids, could help address this inherent conundrum, when one or more such bulky residues are introduced to the backbone of natural peptides and proteins.

There are four types of peptide bonds that are produced by the three classes of amino acids: the natural amino acids, α,α-disubstituted α-amino acids, and *N*-alkyl amino acids (Figure 1c). Type A is a peptide bond composed of natural amino acids. Types B and C are peptide bonds composed of either an α,α-disubstituted α-amino acid or an *N*-alkyl amino acid. Type D is a bond composed of both an α,α-disubstituted α-amino acid and an *N*-alkyl amino acid.

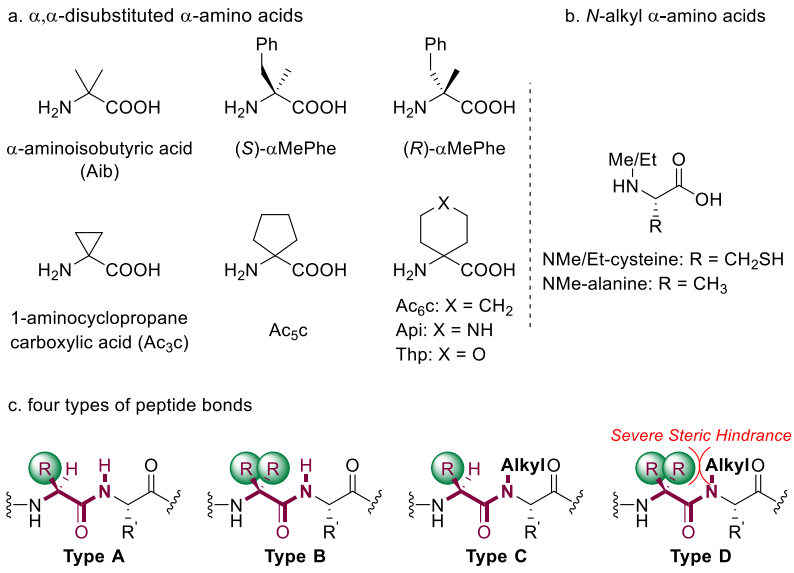


Figure 1. Unusual amino acids used in this study and four types of peptide bonds

1.1 Peptides with unusual α,α-disubstituted amino acids

It is well known that peptides incorporating the hindered α,α-disubstituted α-amino acids (type B) are conformationally restricted and display intrinsic helix-forming preference.[16-18,25-27] Such organized structure can bring enhanced biological activities in peptides. For instance, peptaibols are an established family of peptide antibiotics that contain a large proportion of 2-aminoisobutyric acid (Aib), forming transmembrane ion channels (Figure 2).[28] A wide body of experiments, like NMR, IR studies in solution and X-ray diffraction investigation in solid state of Aib-containing or Aib-modified Alamethicin fragments have shown that the Aib residues are significant for their well-defined stable helical conformation. Furthermore, deeper study on the effect of position of Aib residues in these antimicrobial peptides elucidates their potential of modulating the antibioactivity.[29]

Alamethicin I: Ac-**Aib**-Pro-**Aib**-Ala-**Aib**-Ala-Gln-**Aib**-Val-**Aib**-Gly-Leu-**Aib**-Pro-Val-**Aib**-**Aib**-Glu-Gln-Phl

Suzukacillin: Ac-**Aib**-Pro-Val-**Aib**-Val-Ala-**Aib**-Ala-**Aib**-**Aib**-Gln-**Aib**-Leu-**Aib**-Gly-Leu-**Aib**-Pro-Val-**Aib**-**Aib**-Glu-Gln-Phl

Antiamoebin: Ac-Phe-**Aib**-**Aib**-**Aib**-Iva-Gly-Leu-**Aib**-**Aib**-Hyp-Gln-Iva-Hyp-**Aib**-Pro-Phl

Emerimicin III: Ac-Phe-**Aib**-**Aib**-**Aib**-Val-Gly-Leu-**Aib**-**Aib**-Hyp-Gln-Iva-Hyp-Ala-Phl

Trichotoxon A_40: Ac-**Aib**-Gly-**Aib**-Ala-**Aib**-Glu-**Aib**-**Aib**-**Aib**-Ala-**Aib**-**Aib**-Pro-Leu-**Aib**-Iva-Gln-Valol

Hypelcin A: Ac-**Aib**-Pro-**Aib**-Ala-**Aib**-**Aib**-Gln-Leu-**Aib**-Gly-**Aib**-**Aib**-Pro-Val-**Aib**-**Aib**-Gln-Gln-Leuol

Figure 2. Examples of peptaibol family: sequence of channel-forming antibiotics. (Hyp: hydroxyproline; Phl: L-phenylalaninol)

Peptaibols are a family of naturally occurring Aib-containing peptide antibiotics. Driven by this discovery, introduction of other α,α-disubstituted amino acids into normal peptide chain has become a useful and interesting adjustment in biological study. Artificial peptides containing 1-aminocyclopentane-1-carboxylic acids (Ac_5c) showed stronger cell-penetrating ability and improved tolerance against serum.[30] The number of Ac_5c introduced were shown to be key to the secondary structures of the peptides. Peptide which had three Ac_5c, preferred 3_{10}/α-helices and showed the strongest cell-penetrating ability.

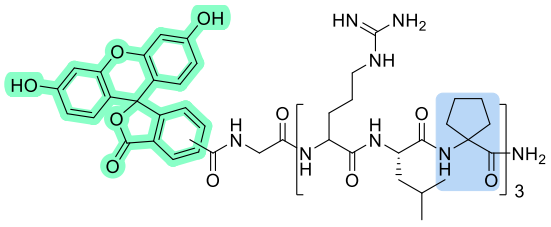


Figure 3. Artificial cell-penetrating helical peptides: 5(6)-CF-Gly-(L-Arg-L-Leu-Ac_5c)$_3$-NH_2

1.2 Peptides with unusual *N*-Alkyl amino acids

Natural products with interesting biological activity are also known to contain *N*-alkyl substituted peptides (type C) such as cyclosporine A and omphalotin.[21-24] Cyclosporine A is a cyclic peptide that contains seven N-methyl amino acid and commercially available as an orally available immunosuppressant.

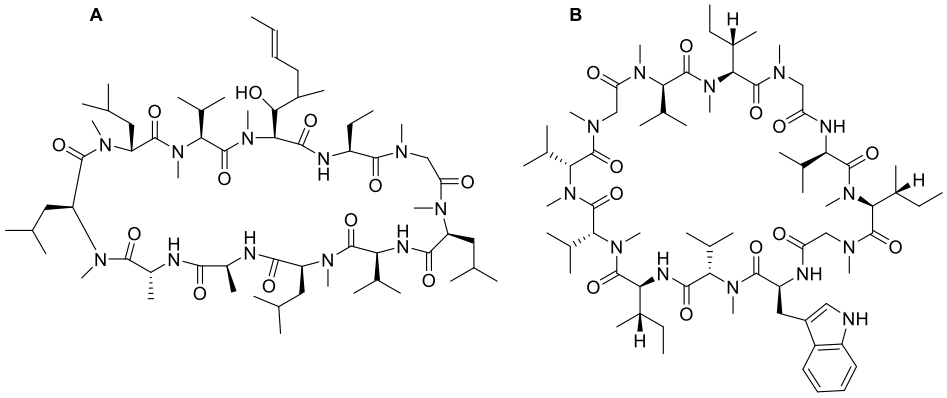


Figure 4. Naturally occurring multiply *N*-methylated cyclic peptides: (A) cyclosporine A and (B) omphalotin

The introduction of an *N*-alkyl substituent into an amide bond (type C) has become a well-established method to modulate the bioavailability of bioactive peptides.[21-23] For instance, synthetic oligo(*N*-substituted glycines), which are termed peptoids (Figure 5). Peptoids and other analogous peptoids present stronger metabolic stability and membrane permeability than the corresponding *N*–H derivatives.[31,32]

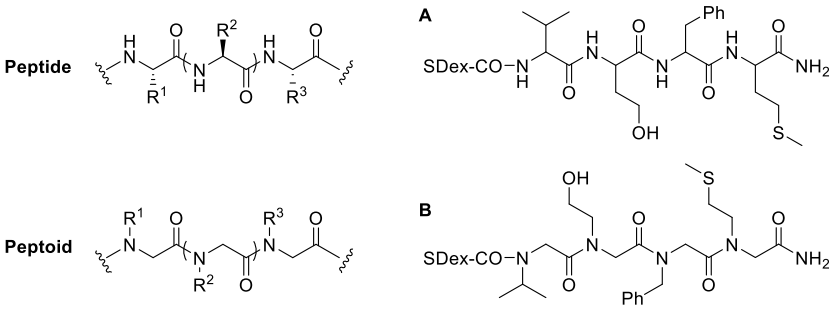


Figure 5. The comparison of peptide and peptoid and examples of peptide and peptoid

Overall, compared with their proteogenic counterparts, peptides of types B and C are generally chemically more stable, less conformationally unpredictable, can penetrate cell membrane easier, and are more resistant to enzymatic degradation.

1.3 Synthetic methods towards type B peptide bond

In 1987, Obrecht and Heimgartner developed 3-(dialkylamino)-2,2-dimethyl-2*H*-azirine as an Aib equivalent which reacts with carboxylic acid to introduce the sterically hindered Aib residue (Figure 6a). By applying a protected Aib amino acid, the type B peptide bond can be installed. On the other hand, DCC coupling reagent and HOBt strategy can be employed to introduce other amino acids. A problem that arises is the ease of epimerization in the oxazolone intermediate once a chiral mono substituted amino acid is used as coupling partner.

In 2021, Maruoka's group reported a bioinspired protocol utilizing *p*-hydroxyphenyl group as a potential activating group to generate amino ester at the C-terminal (Figure 6b). A commercially available hypervalent iodine reagent was used to oxidize the ester, followed by the treatment of Py•HF and gave the highly reactive acyl fluoride intermediate. Therefore, a smooth nucleophilic attack of the acyl fluoride species took place by the amine substrate with the formation of sterically hindered peptide.

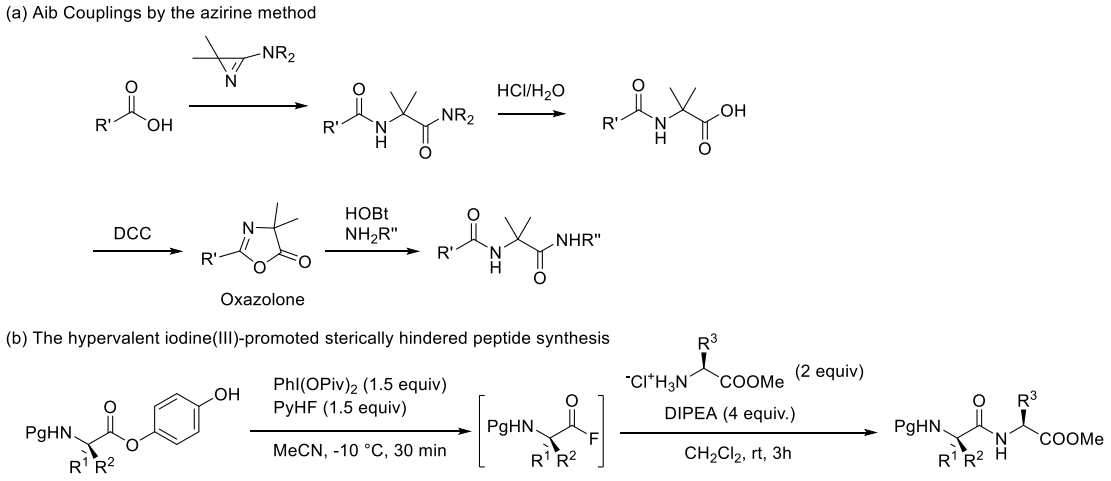


Figure 6. Synthesis of type B peptide bond

1.4 Synthetic methods towards type C peptide bond

Recently, some efficient methods have been reported to access a broad variety of *N*-methylated peptides. In 2020, Fuse's group reported an efficient assembly of *N*-methylated dipeptides involving an acyl *N*-methylimidazolium cation as an active species without severe epimerization by using equivalent amounts of amino acids (Figure 7a). First of all, the mixed carbonic anhydride was prepared from *N*-terminal protected amino acids, followed by the formation of *N*-methylimidazolium intermediate. It was then trapped by the amine substrates and gave the *N*-methyl peptide products.

Later in the same year, Masuya and coworkers reported a new activating reagent for the synthesis of *N*-methyl peptides (Figure 7b). They adopted the mixed anhydride strategy utilizing isostearic acid halide (ISTAX) to activate the *N*-protected amino acids. C-terminally unprotected *N*-methyl amino acid was used directly, which was in situ silylated by *N,O*-bis(trimethylsilyl)acetamide (BSA). The final connected

N-methyl peptides were generated by the reaction between the *N*-protected amino acid anhydride and C-silylated *N*-methyl amino acid. This method provided various peptide products with a free carboxylic acid end with perfect diastereoselectivity and good to excellent yield.

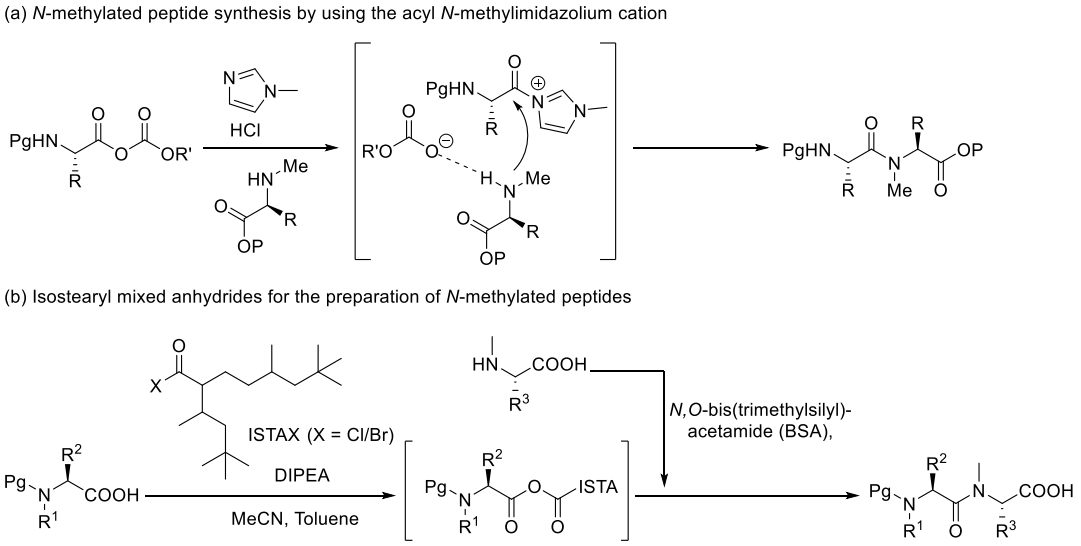


Figure 7. Synthesis of type C peptide bond

1.5 Synthetic methods towards type D peptide bond

Successful synthetic methods to access sterically hindered peptide bonds such as types B[33] and C[34,35] have been reported that are mostly based on modifications of conventional methods, although the synthesis of such a bond can be challenging because of epimerization and low yields arising from the high steric bulk of the reagents.[36,37] By contrast, the synthesis of peptide bonds of type D is especially difficult because of the severely increased steric hindrance.

Indeed, to my knowledge, there are only two reports of this type of bond formation: One involves the use of acyl fluoride (Figure 8a)[38] and the second involves thiocarboxylic acid and *t*-BuNC by Danishefsky's group (Figure 8b).[39] In both approaches, acid anhydride is formed as an intermediate, and the process proceeds through an intramolecular *O,N*-acyl transfer reaction. There are only Aib and Ac_5c amino acids with a total of six examples in the former case, and Aib amino acid with one example in the latter case.

As a free carboxylic acid is involved in the reaction, the nucleophile is restricted to an *N*-methyl mono amino acid, and only dipeptides are approachable. A peptide composed of several amino acids cannot be employed as a nucleophile. Thus, the extremely sterically hindered peptide bond formation between α,α-disubstituted α-amino acids and peptide with *N*-alkyl amino acids is essentially an unmet challenge, and the biological and physical functions of such peptides have not yet been explored.

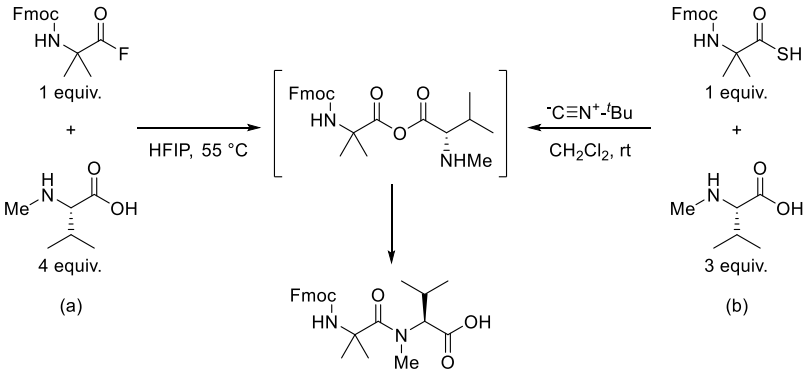
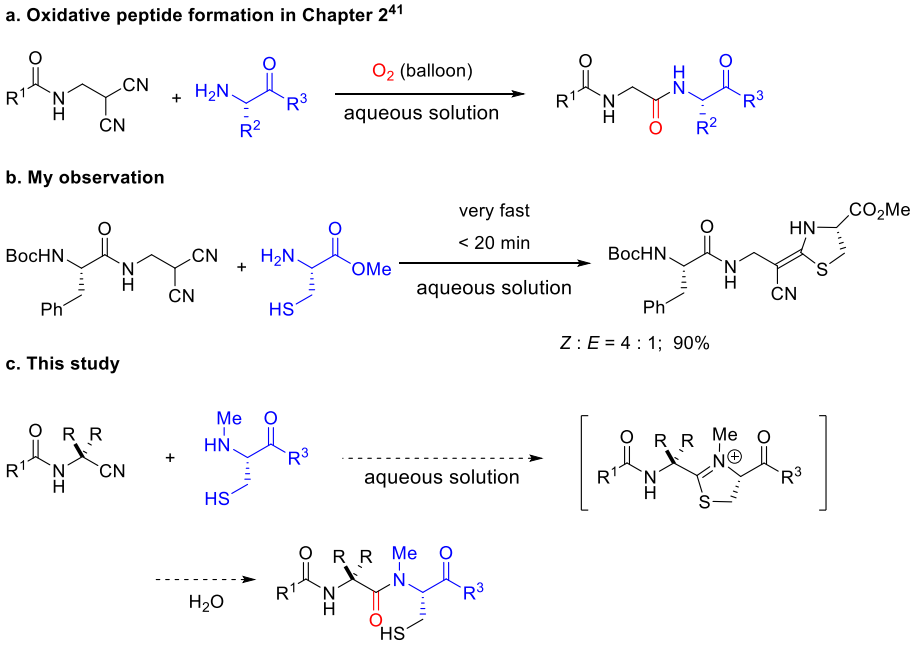


Figure 8. Synthesis of type D peptide bond

2. Previous work and discovery and this work

In 2016, my group reported an oxidative synthesis of sterically hindered amides from substituted malononitrile and amine under O_2 atmosphere.[40] In chapter 2, I expanded this approach to the synthesis of tripeptides and tetrapeptides with the formation of a glycine peptide bond under aqueous conditions (Scheme 1a).[41] During this study, I found that cysteine methyl ester can selectively and rapidly (<20 min) react with one of the cyano groups to afford a thiazolidine derivative in 90% yield (Scheme 1b). Based on the high reactivity of nitrile and cysteine,[42-45] it's expected that *N*-methylthiazolium would be formed if the *N*-methyl cysteine derivative was used. This ion would be hydrolyzed to afford a peptide bond (Scheme 1c). Moreover, given that the reaction (Scheme 1b) is very fast, α,α-disubstituted α-amidonitrile could be used as a starting material, in which case a sterically hindered peptide bond (type D) would be formed. In this chapter, I describe the successful realization of this scenario.



Scheme 1. Oxidative peptide synthesis in chapter 2 and this work

3. Optimization of reaction conditions

3.1 Synthesis of starting material: Boc-L-Phe-Aib-CN 1a

The research was commenced by optimizing the reaction conditions using Boc-L-Phe-Aib-CN (**1a**) and NMe-L-Cys-L-Phe-O^{t}Bu (**2a**) as model substrates en route to tetrapeptide **3a** (Table 1). Notably, the starting material is easily synthesized by Strecker's method followed by a general coupling (Figure 9). **1a** was obtained in 62% yield over 2 steps from Boc-L-phenylalanine.

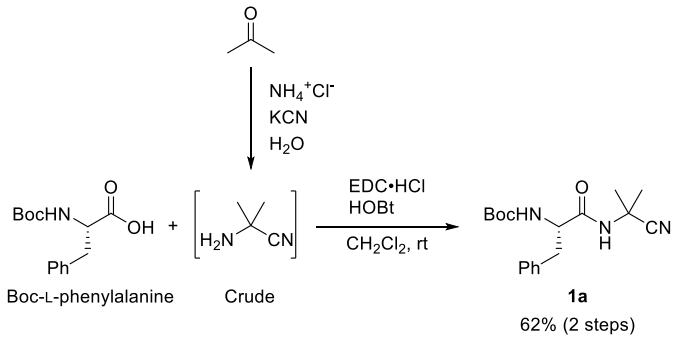
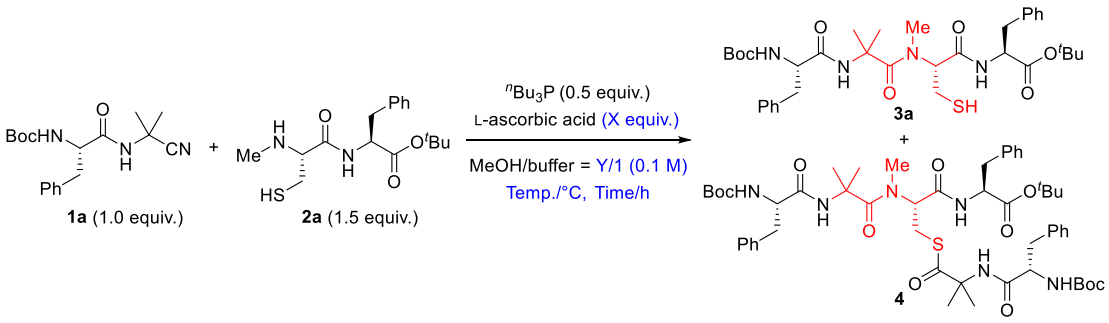


Figure 9. Synthesis of dipeptidyl α-amido nitrile

3.2 Optimization of reaction conditions using Boc-L-Phe-Aib-CN 1a

To keep Cys thiols in a reducing environment, the reaction was conducted in the presence of ^{n}Bu$_3$P *vide infra* and L-ascorbic acid.[46-48] As peptide synthesis that is compatible with the green chemistry paradigm is strongly desired,[49] a mixture of MeOH and buffer as solvent was used. Considering the extremely high steric bulk of the two components, the reaction was first conducted at 80 °C, and the desired product **3a** was obtained in 63% yield after 9 h, together with 12% of **4**, which was generated by overreaction of the desired product **3a** and starting material **1a** (entry 1). Suppression of this latter side reaction could be achieved by conducting the reaction at a lower temperature. Thus, **3a** was obtained in an improved yield of 74% with a longer reaction time without the formation of **4** (entry 2). Because the solubility also decreases with a reduction in the temperature, more MeOH was used, thereby improving the yield of **3a** slightly to 78% (entry 3). Higher concentration did not accelerate the reaction (entry 4), and slightly elevated reaction temperature increased the generation of the overreaction product (entry 5). When the amount of L-ascorbic acid was reduced to 1.3 equivalent, the yield of **3a** was improved to 87% (entry 6). Further reducing the amount of L-ascorbic acid did not improve the yield (entry 7). The increase in the amount of ^{n}Bu$_3$P did not improve the amount of product either (entry 8). Therefore, the optimal reaction conditions were found to be 1.5 equivalent of compound **2a** in the presence 1.3 equivalent of L-ascorbic acid under MeOH/buffer (2:1) in a reducing reaction system at 30 °C.

Table 1. Optimization of reaction conditions[a]



Entry	X	Y/1[b]	Temp./°C	Time/h	Yield of **3a**/%	Yield of **4**/%
1	2	1/1	80	9	63	12
2	2	1/1	30	72	74	<5
3	2	2/1	30	72	78	<5
4	2	2/1[c]	30	72	78	<5
5	2	2/1	40	48	67	10
6	1.3	2/1	30	72	87	<5
7	1.0	2/1	30	72	84	<5
8[d]	1.3	2/1	30	72	85	<5

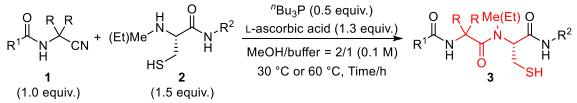
[a]Unless otherwise shown, the reaction was conducted using **1a** (0.2 mmol), **2a** (0.3 mmol), $^{n}Bu_3P$ (0.1 mmol) and L-ascorbic acid (as indicated) in MeOH/buffer (2 mL in the indicated ratio) at the specified temperature under argon atmosphere for the indicated time. See SI for more details. [b]A pH 7.4, 0.2 M phosphate buffer was used. [c]The reaction was done in 0.2 M. [d]1.0 equivalent of $^{n}Bu_3P$ was used.

4. Generality

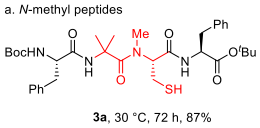
With the optimal reaction conditions in hand, the generality of the reaction was investigated (Table 2). Various tetrapeptides along with two pentapeptides (**3h**, **3o**) and a decapeptide (**3r**) with a sterically hindered amide bond were synthesized in good yield from an α,α-disubstituted α-amidonitrile and peptides possessing *N*-alkyl cysteine. The amide bond formation between α-aminoisobutyric acid (Aib) and *N*-methyl cysteine derivatives proceeded well when different amino acids were positioned next to the Aib residue (**3a**, **3b**, **3c**, **3d**). As the substituents are smaller, the reaction proceeded faster. A dipeptide derivative of an α-amidonitrile with a cyclopropane ring (Ac_3c) showed a much higher reactivity compared with its Aib congener (**3e**). The reaction also proceeded smoothly with a substrate bearing a tryptophan amino acid side chain (**3f**). In addition to Boc and Cbz protecting groups, the Fmoc group was also compatible, affording a Fmoc-His(NTr)-terminated tetrapeptide **3g** in good yield when the reaction was conducted at 40 °C to increase the solubility of the substrate. An *N*-methylated tripeptide also attached smoothly to a dipeptide to generate pentapeptide **3h**. As the size of the cyclic alkyl ring at the α-position of amidonitrile increases, the steric hindrance of the amide bond increases. However, the formation of such a severely hindered amide bond still succeeded by simply elevating the temperature to

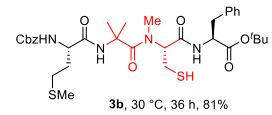
60 °C. An α-amidonitrile with a cyclopentane ring (Ac_5c) was successfully employed to afford tetrapeptides terminated with several amino acids such as Boc-Phe-, Boc-Lys(NHCbz)-, and Boc-Arg(Tos) (**3i**, **3j**, and **3k**, respectively) in good yields. A cyclohexyl substituent (Ac_6c) at the α-position of amidonitrile also provided the desired tetrapeptides **3l** and **3m** in good yields upon prolonging the reaction time. Heterocycles such as tetrahydropyran (Thp) and piperidine (Api) were tolerated as substituents at the α-position of amidonitrile. Indeed, amide bond formation was found to be faster in these substrates, and the reaction reached completion within a short time, affording tetrapeptides in high yields with Phe (**3n**, **3p**) and Glu(OBn) (**3q**) amino acids, and pentapeptide (**3o**). A coupling between two pentapeptides also took place to afford a decapeptide (**3r**) with almost no erosion of the chemical yield, with the formation of an amide bond between the terminal α,α-dimethyl α-amidonitrile and *N*-methyl cysteinyl peptide. In addition to *N*-methyl cysteinyl peptide, *N*-ethyl type nucleophile, which is more sterically hindered and synthetically challenging, also showed good to excellent performance with Aib and cyclopropane (Ac_3c) substituents at the electrophilic side (**3s**, **3t**). The chiral (*S*)- and (*R*)-α-methyl phenylalanyl *N*-methyl bonded peptides **3u** and **3v** were also accessible using this method, with the latter forming much faster than the former.

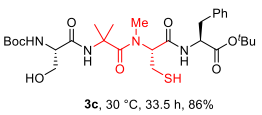
Table 2. Synthesis of various types of peptides with the formation of an extremely hindered peptide bond[a]

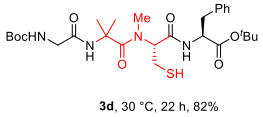


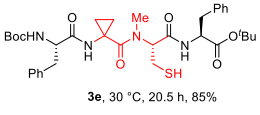
a. *N*-methyl peptides

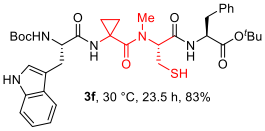

3a, 30 °C, 72 h, 87%

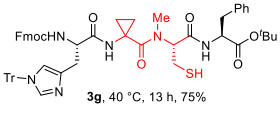

3b, 30 °C, 36 h, 81%

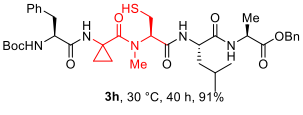

3c, 30 °C, 33.5 h, 86%

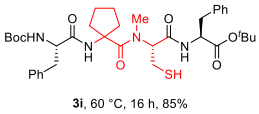

3d, 30 °C, 22 h, 82%

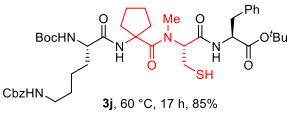

3e, 30 °C, 20.5 h, 85%

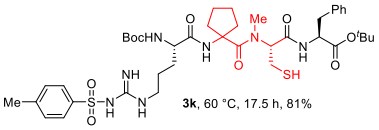

3f, 30 °C, 23.5 h, 83%

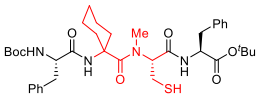

3g, 40 °C, 13 h, 75%

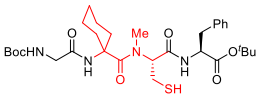

3h, 30 °C, 40 h, 91%

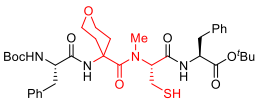

3i, 60 °C, 16 h, 85%

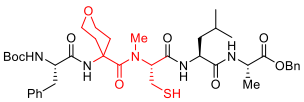

3j, 60 °C, 17 h, 85%

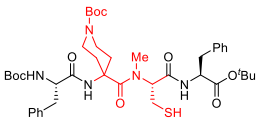

3k, 60 °C, 17.5 h, 81%

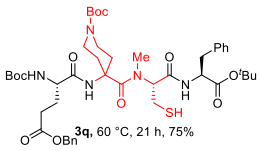

3l, 60 °C, 36 h, 79%

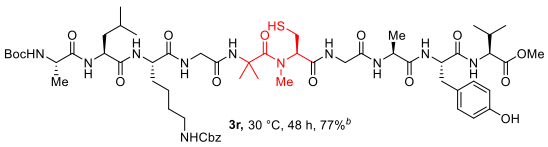

3m, 60 °C, 36 h, 82%


3n, 60 °C, 19.5 h, 88%

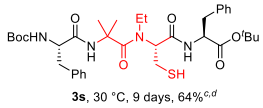

3o, 60 °C, 22 h, 78%

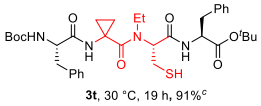

3p, 60 °C, 22 h, 84%

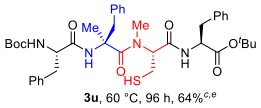

3q, 60 °C, 21 h, 75%

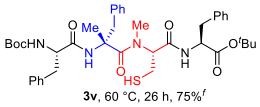

3r, 30 °C, 48 h, 77%[b]

b. others


3s, 30 °C, 9 days, 64%[c,d]


3t, 30 °C, 19 h, 91%[c]


3u, 60 °C, 96 h, 64%[c,e]


3v, 60 °C, 26 h, 75%[f]

[a]Unless otherwise shown, the reaction was conducted using **1** (0.2 mmol), **2** (0.3 mmol), $^{n}Bu_3P$ (0.1 mmol) and L-ascorbic acid (0.27 mmol) in MeOH/buffer = 2/1 (2 mL) at the temperature specified under argon atmosphere for the indicated time. See SI for more details. [b]The reaction was conducted in 0.1 mmol scale, and HCl·*N*-methyl cysteinyl pentapeptide was used and $NaHCO_3$ (0.15 mmol) was added. [c]The reaction was conducted in 0.1 mmol scale. [d]Additional $^{n}Bu_3P$ (0.025 mmol) was added after 3 days. [e]Additional $^{n}Bu_3P$ (0.05 mmol) was added after 57 h. [f]The reaction was conducted in 0.09 mmol scale.

5. Synthesis of α,α-disubstituted *N*-Me-Alanyl peptide through radical desulfurization

In addition, the α,α-disubstituted *N*-methyl alanyl peptide **5** was obtained in good yield through radical desulfurization of its corresponding cysteinyl precursor **3a** using tris(2-carboxyethyl)phosphine (TCEP) (Figure 10).[51,52] Thus, the present method can afford not only amide bonds from α,α-disubstituted amino acids and *N*-methyl cysteine but also amide bonds of α,α-disubstituted amino acids and *N*-methyl alanine.

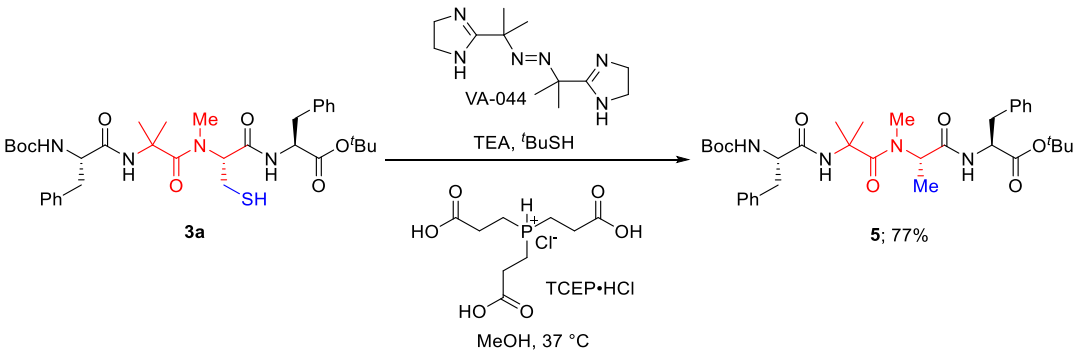


Figure 10. Synthesis of α,α-disubstituted *N*-Me-Alanyl peptide

6. Role of $^{n}Bu_3P$ and L-ascorbic acid

6.1 $^{n}Bu_3P$ and L-ascorbic acid are used as reducing reagents

$^{n}Bu_3P$ and L-ascorbic acid are often used together to keep the cysteine's thiol in a reducing state.[xx] $^{n}Bu_3P$ is known to act as a reducing reagent which can cleave the disulfide bond (Figure 11a). However, a side reaction proceeds concomitantly that the thiol moiety is cleaved by $^{n}Bu_3P$ via a radical mechanism (Figure 11b).[xx] The generation of thiyl radical is adventitious and it can be trapped by $^{n}Bu_3P$, followed by fragmentation and generation of a R radical (R•) and tributylphosphine sulfide. The R• can then abstract a hydrogen atom and generate the desulfurized product.

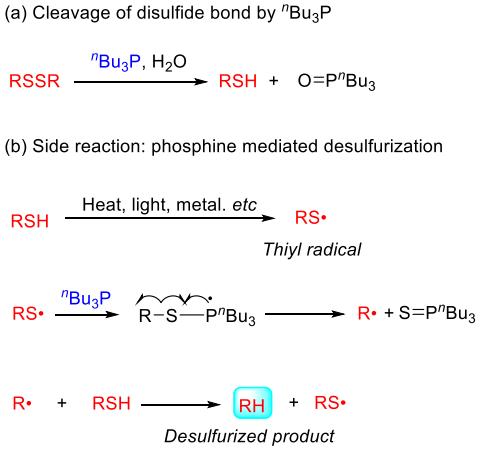


Figure 11. Role of $^{n}Bu_3P$ and Phosphine mediated desulfurization

In order to suppress this side reaction, L-ascorbic acid was used to quench the thiyl radical generated in the reaction system adventitiously. L-Ascorbic acid is an antioxidant of thiol. The reaction mechanism is summarized below (Figure 12). The ascorbate ($AscH^-$) is equilibrating with L-ascorbic acid ($AscH_2$) in the reaction system. The thiyl radical is quenched by $AscH^-$ and gives RSH and radical anion $Asc^{\bullet-}$. A disproportionation process exists in the system. The $Asc^{\bullet-}$ can provide an electron to another molecule of $Asc^{\bullet-}$, which gives one $AscH^-$ and one dehydroascorbic acid (DHA, the oxidized form of ascorbic acid). AscH- and DHA can also provide 2 molecules of $Asc^{\bullet-}$.

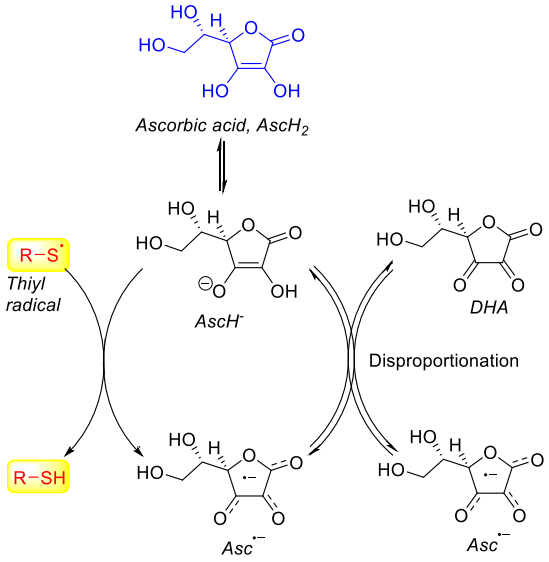
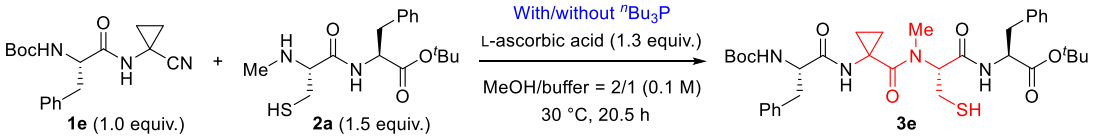


Figure 12. Principle of the scavenging of thiyl radical by ascorbate

6.2 Study of $^{n}Bu_3P$ regarding its possible activation effect in the reaction

In addition, there's a possibility that $^{n}Bu_3P$ can activate the nitrile group by a nucleophilic attack. Therefore, the results with and without using $^{n}Bu_3P$ are compared (Table 3). Control experiments with and without $^{n}Bu_3P$ were conducted with using Boc-L-Phe-Ac_3c-CN **1e** and NMe-L-Cys-L-Phe-O^{t}Bu **2a**. The corresponding product **3e** was obtained in 85% isolated yield with 6% of the starting material **1e** recovered under the standard optimized condition (Table 3, entry 1). When $^{n}Bu_3P$ was not present in the reaction, **3e** was obtained in 83% isolated yield with 7.6% of **1e** recovered under a vigorously degassed system (Schlenk-like technique was used) (Table 3, entry 2). Therefore, we concluded that the reaction proceeded with the same efficiency in the absence of $^{n}Bu_3P$ in vigorously degassed solvent under argon atmosphere. This result rules out the possibility that $^{n}Bu_3P$ acts as a catalyst. Notably, the reaction gave much lower yield if an argon balloon was attached to the system after removing oxygen completely by degassing instead of maintaining the oxygen-free condition by Schlenk-like technique.

Table 3. control experiments of **1e** with and without $^{n}Bu_3P$.[a]



Entry	With/without $^{n}Bu_3P$	Yield/%	Rsm **1e**/%
1	$^{n}Bu_3P$ (0.5 equiv.) degassed system	85	6
2	No $^{n}Bu_3P$ Vigorously degassed	83	7.6

[a]Unless otherwise shown, the reaction was conducted using **1e** (0.2 mmol) following the same procedure of generality study in table 2.

7. Study of epimerization at the α-position of cysteine residue

In this reaction, there is a possibility of epimerization at the cysteine residue. An acidic proton is present in *N*-methylthiazolium intermediate, and deprotonation and/or retro-Michael/Michael reaction of thiolate might occur (Figure 13).

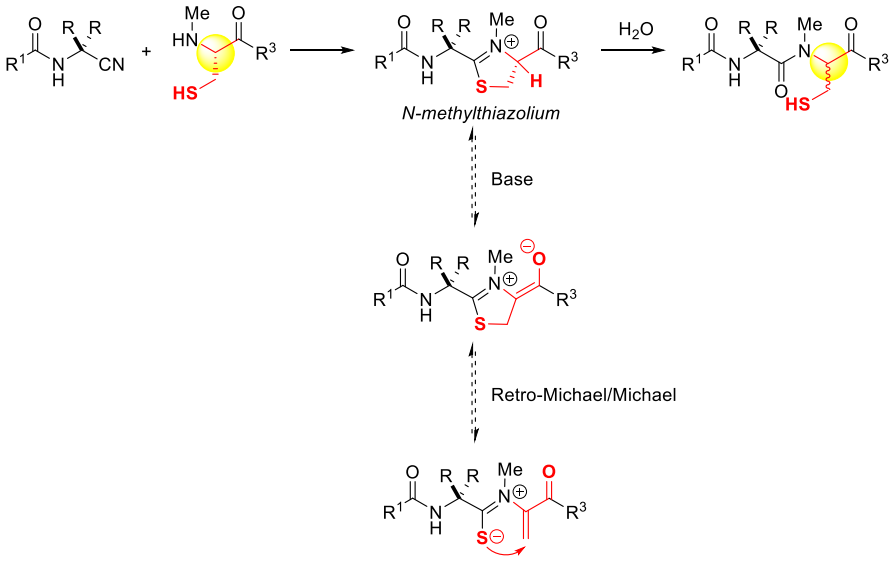


Figure 13. Possible epimerization pathway

In order to check the epimerization, I carried out the reactions of Boc-Gly-Aib-CN (**1d**) with NMe-L-Cys-L-Phe-O^{t}Bu (**2a**), and **1d** with NMe-D-Cys-L-Phe-O^{t}Bu (**epi-2a**), and examined the epimerization analyzed by HPLC. First of all, epi-**3d** was synthesized from **1d** and **epi-2a** in 76% yield after 17 h under the standard reaction condition (Figure 14).

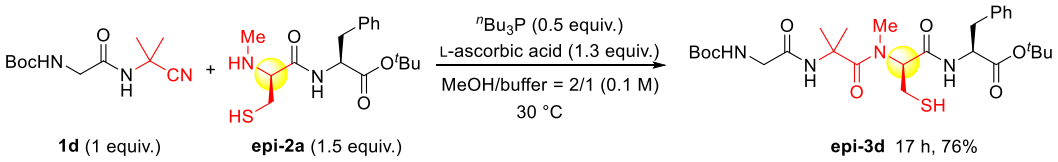


Figure 14. Synthesis of Boc-Gly-Aib-NMe-D-Cys-L-Phe-O^{t}Bu **epi-3d**

Next, HPLC analysis of the crude product of **3d** was conducted. **3d** was shown to be a single diastereomer with dr > 99:1 (Figure 15).

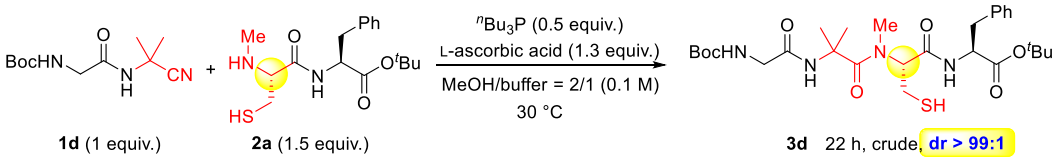


Figure 15. Synthesis of Boc-Gly-Aib-NMe- L-Cys-L-Phe-O^{t}Bu **3d**

Moreover, a control experiment was conducted using **1d** and **2a** in deuterated solvent under the same condition for making **3d** in normal solvent shown in table 2 (Figure 16). The incorporation of deuterium is not observed at the α-position of cysteinyl residue based on the comparison of ^{1}H NMR data. In addition, as expected, the thiol's H in **3d** was exchanged with deuterium, which is reasonable.

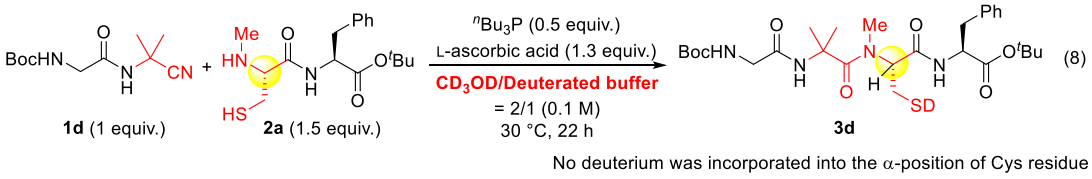


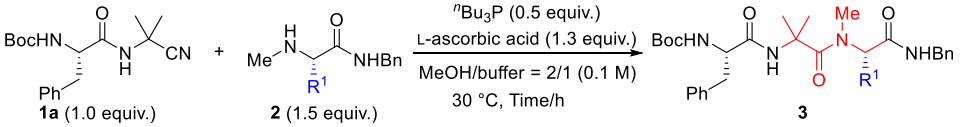
Figure 16. Control experiment for synthesizing Boc-Gly-Aib-NMe- L-Cys-L-Phe-O^{t}Bu **3d** in deuterated solvent system

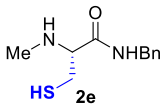
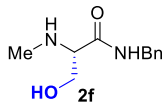
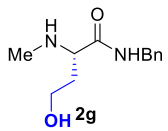
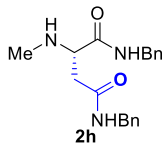
Therefore, based on the HPLC analysis and control experiment in deuterated solvent system, it was concluded that there is no epimerization at the α-position of the cysteine residue.

8. Study of reactivity of serine, homoserine and aspartic acid analogues

Because an *N*-terminal cysteine residue is required in this protocol. I examined other amino acids than cysteine. The reaction of **1a** with the following serine, homoserine, and aspartic acid analogues were investigated. Serine, homoserine, aspartic acid analogues were found to not react with the nitrile substrate. The data is summarized in **table 4**. Boc-L-Phe-Aib-CN **1a** reacts with NMe-L-Cys-NHBn **2e** to give its

Table 4. Experiments between 1a and cysteine derivative and analogues of other amino acids[a]



Entry	Substrate **2**	Time/h	Yield/%
1	 **2e**	18	**3w**: 71
2	 **2f**	24	0; rsm: **1a** 100% based on internal standard
3	 **2g**	24	0; rsm: 1a 100% based on internal standard
4	 **2h**	24	0; rsm: 1a 100% based on internal standard

[a]Unless otherwise shown, the reaction was conducted using **1a** (0.2 mmol) following the same procedure of generality study in table 2.

corresponding peptide **3w** in 71% yield after 18 h at 30 ºC under the standard optimized condition (Table 4, entry 1). However, the relevant serine, homoserine and aspartic acid derivatives **2f**, **2g**, **2h** were shown to not react with **1a** and **1a** was recovered completely based on internal standard under the same condition after 24 h (Table 4, entries 2, 3 and 4). This result indicates that a thiol moiety is essential for the reaction, which supports the assumption of the reaction mechanism.

9. Study of NH_2-cysteinyl substrate

Next, I investigated the reaction using NH_2 cysteinyl derivative as a nucleophile instead of **2a** (Figure 17). Namely, the reaction between L-Cys-L-Phe-O^{t}Bu **6** and Boc-L-Phe-Aib-CN **1a** was examined under the same reaction conditions. A stable thiazoline compound **7** was obtained in good yield and do not hydrolyze to peptide bond.

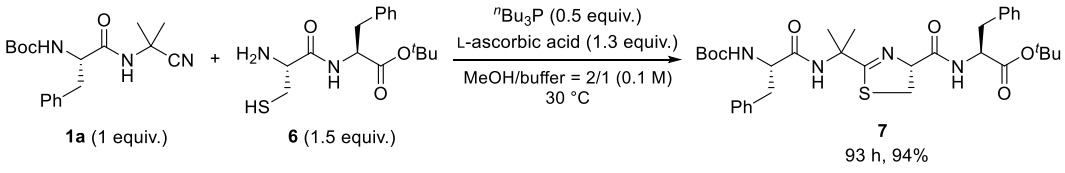
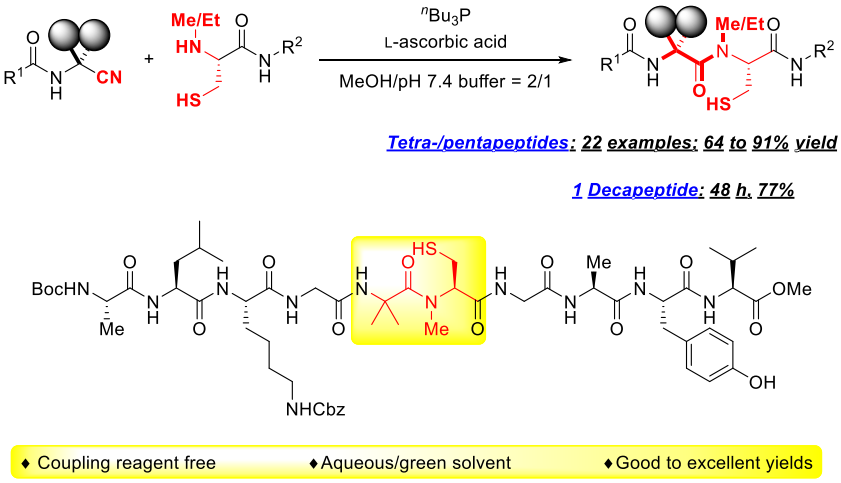


Figure 17. Experiment using **1a** and NH_2-Cysteinyl peptide

10. Conclusion

In conclusion, an efficient synthetic method has been developed to access extremely sterically hindered peptide bonds via a sequential cyclization between α,α-disubstituted α-amidonitrile and *N*-alkyl cysteine and immediate hydrolysis of the iminium intermediate. A wide variety of non-proteinogenic α,α-disubstituted amino acids, including chiral and non-chiral amino acids, show great performance in coupling with *N*-methyl or *N*-ethyl peptides. Notably, no coupling reagents are employed, and this method can also be used to connect two relatively long peptide chains. Thus, it represents a useful method for chemical ligation. The realization of synthesizing such sterically hindered peptides will open new avenues to investigate their conformation, physical, and biological properties.



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Chapter 4. Peptide ligation between α-amidonitrile and N-terminal cysteine

1. Introduction

Peptides and proteins are essential components in living organisms and play unparalleled central functions. They are well assembled from proteinogenic amino acids in living cells involving a complex sequence of ribosomal synthesis and further diversified by post-translational modifications.[1] Chemical synthesis allows reliable access to the desired peptides and proteins in terms of a reasonable quantity and quality for biological study, material science and pharmaceutical science and so on. Solid phase peptide synthesis, as the most potent method, is able to generate a peptide chain with maximum 50 amino acids approximately.[1] The assembly of those large peptide segments relies on site-selective chemical ligation tool. The ligation reaction should be orthogonal to all the functional groups in peptides, simple, mild and preferentially to be conducted in aqueous condition. Various ligation strategies have been developed to play a role.

1.1 Native chemical ligation

In 1994, native chemical ligation originated by Kent and coworkers represents the most widely used synthetic methods for large peptide synthesis.[2] It leads to a native cysteine amide bond by reacting one peptide bearing a thioester end with the other having a cysteine terminal. First of all, trans-thioesterification took place between the C-terminal thioester and N-terminal cysteine, followed by an intramolecular *N,S*-acyl transfer, which generated an elongated peptide product with formation of a cysteinyl peptide bond (Figure 1). This reaction normally works in a very dilute buffer condition. Excess thiol (RSH) is used to maintain a sufficient level of concentration of peptide thioester. Other additives like TCEP, L-ascorbate are added to keep the thiol in a reducing state.

However, the β-branched amino acids, like valine and isoleucine are rarely chosen as a reacting site due to their bulkiness which blocks the nucleophilic attack from N-terminal cysteine.[3] The difficulty in harnessing the β-branched amino acids as a ligation partner has made the synthesis of large peptides less efficient given the fact that more than 18% of amino acids that are adjoining to cysteine in proteins are β-branched.[4] In addition, apart from the bulky residues, proline-based thioester has shown its extreme inertness towards trans-thioesterification. The reason has been elucidated that the electrophilicity of proline thioester is diminished by the donation of lone pair from its vicinal amide oxygen to the π* orbital of thioester.[5]

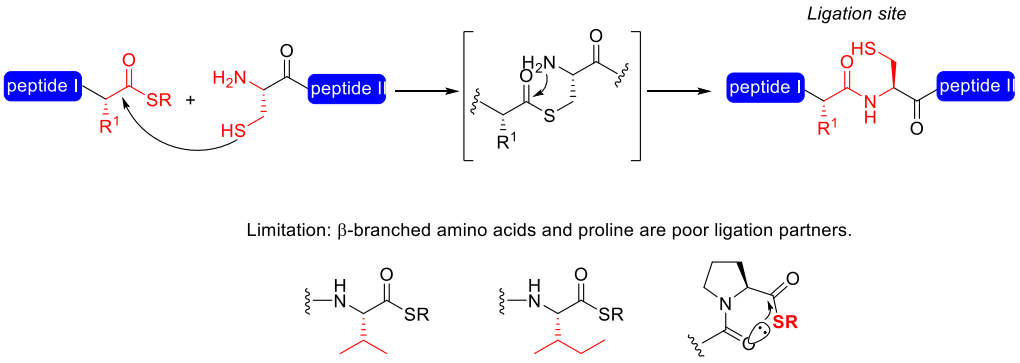


Figure 1. Native chemical ligation and limitations

1.2 Staudinger ligation

Staudinger ligation is one of the most important bioconjugation techniques utilizing Staudinger reaction reported in 1919 as a key step.[6] The reaction principal is delineated below (Figure 2). Basically, the ligation proceeds between a phosphane and an azide moiety which is attached separately to a peptide chain. At first, the Staudinger reaction occurs to give an iminophosphorane intermediate in the ylide form by extrusion of a molecule of N_2. C-N amide bond linkage forms immediately through an intramolecular trap of the negatively charged nitrogen atom by its carbonyl carbon. Finally, the phosphorous moiety is removed by hydrolysis with formation of phosphane oxide and an amide bonded peptide product. Since the phosphorous part is cleaved concomitantly with the generation of the final product, it's called traceless Staudinger ligation. Although it's a good alternative to native chemical ligation, limitations in stereochemical aspects, scope, purification are reported.[6b,6c]

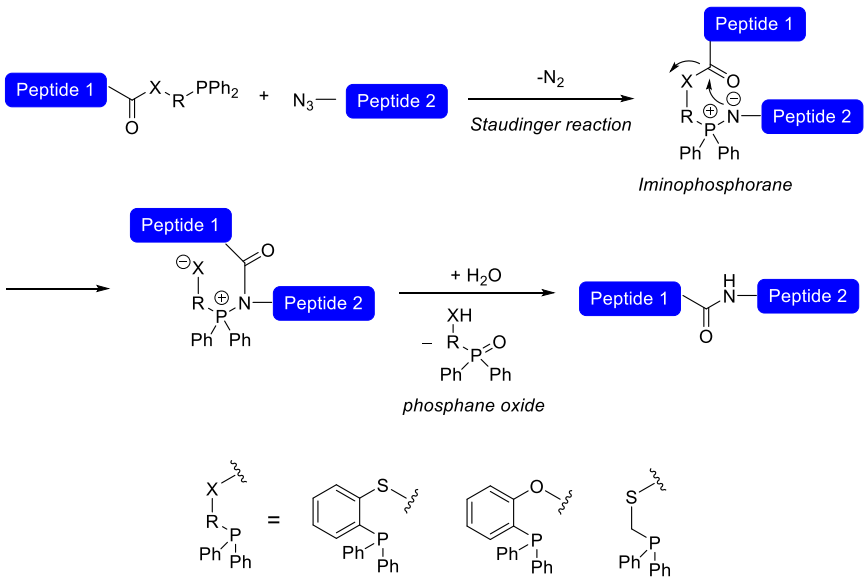


Figure 2. Staudinger ligation

1.3 Ketoacid-hydroxylamine ligation (KAHA ligation)

The KAHA ligation firstly reported by Bode's group in 2006 employs C-terminal peptide α-ketoacids (KAs) and N-terminal peptide hydroxylamines (HAs), which react chemoselectively to form amides (Figure 3).[7a] This reaction proceeds in aqueous media without reagents or catalysts. Two types of KAHA ligation are developed according to the type of N-terminal hydroxylamine.[7c] A likely mechanism has been reported for type I KAHA ligation (Figure 4a).[7b] In the first step, a nitrone intermediate is formed through a nucleophilic addition between amine moiety of N-terminal hydroxylamine and the α-keto moiety of C-terminal ketoacid. α-Lactonization occurs by the attack of carboxylate to the nitrone carbon, followed by the formation of oxaziridine. Finally, the amide linkage is generated driven by the loss of a molecule of CO_2. For type II KAHA ligation, the mechanism is unclear at present stage. The labeling experiment indicates that the oxygen in the generated amide bond originates from the keto moiety of ketoacid but not the hydroxylamine's oxygen (Figure 4b).

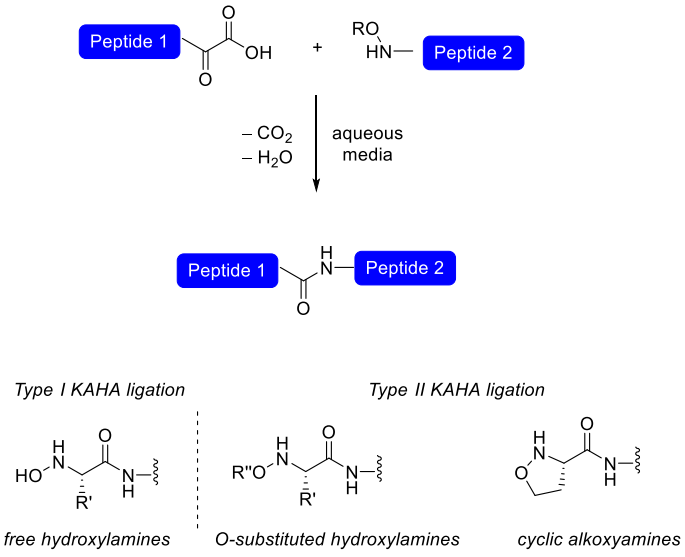


Figure 3. Ketoacid-hydroxylamine ligation

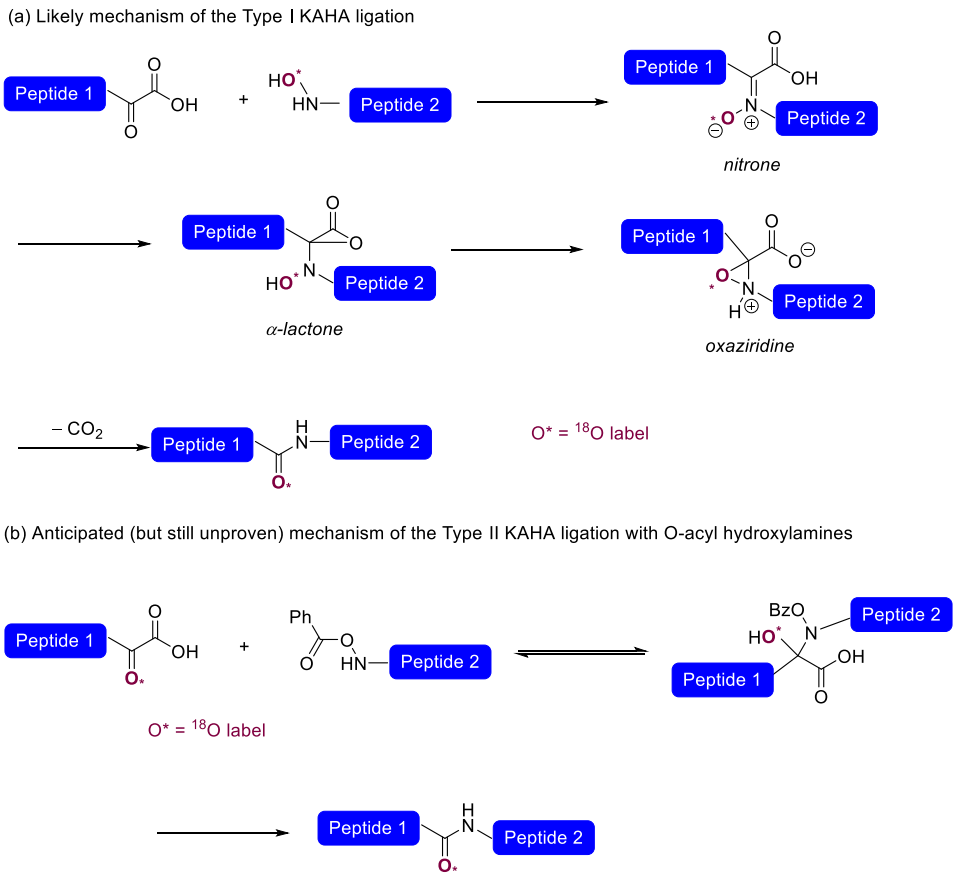


Figure 4. (a) Likely mechanism of the Type I KAHA ligation; (b) Anticipated (but still unproven) mechanism of the Type II KAHA ligation with O-acyl hydroxylamines

1.4 Serine/threonine ligation

Serine/threonine ligation (STL) is a peptide ligation method with forming a native serine or threonine peptide bond published by Li' group (Figure 5).[8] The C-terminal peptide is terminated by a salicylaldehyde ester while the N-terminal fragment bears a serine or threonine terminus. The reaction which takes place here is that oxazolidine intermediate with a *S* or *R* stereochemical hemiaminal carbon is delivered through formation of an imine followed by 5-endo-trig cyclization. From here, only the S-configured oxazolidine is observed to undergo the O-to-N acyl transfer and generate the S-configured N-acyl oxazolidine. By treatment of an acid, hydrolysis occurs to give the serine or threonine amide bond.[8]

It is suggested by the same group that 17 of the 20 natural amino acids are suitable ligation partners with Asp, Glu and Lys not compatible. Because the C-terminal Asp, Glu, and Lys derived salicylaldehyde (SAL) esters are instable when the first amino acid contains nucleophilic side-chain functionalities. Pro and Arg are not recommended because of the retarded reaction profile.[8b]

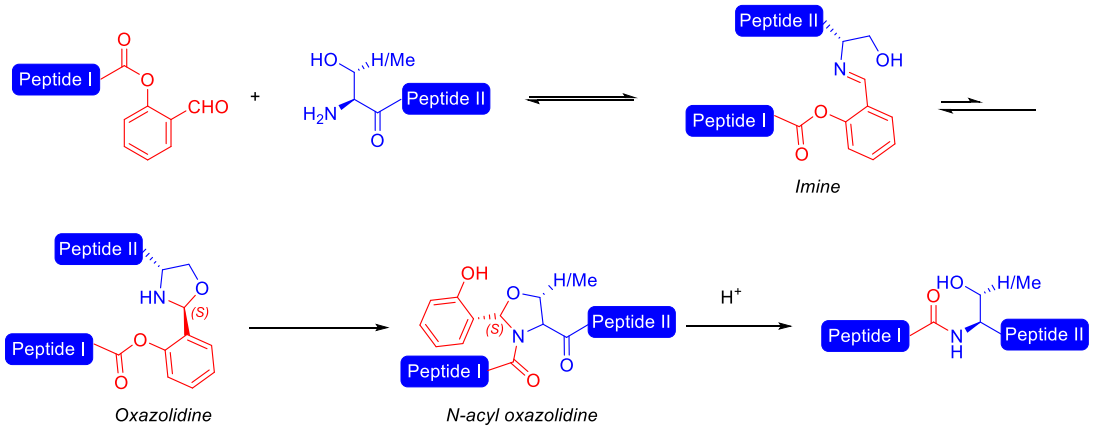


Figure 5. Serine/threonine ligation

2. This work

In chapter 3, the synthesis of the extremely hindered peptides from α,α-disubstituted amido nitrile and *N*-alkyl cysteine was described. In this work, a new peptide ligation protocol is proposed based on the high reactivity between CN and cysteine. At first, a thiazoline intermediate would be generated by using a C-terminal chiral mono substituted amido nitrile and N-terminal NH_2-cysteinyl peptide. It would be hydrolyzed to give a peptide product with formation of cysteinyl peptide bond. Obviously, native chemical ligation has achieved nicely in the synthesis of such kind of ligation product. As indicated in the previous introduction of native chemical ligation, thioesters made from bulky β-branched residues and proline react extremely slow with N-terminal cysteine.[3] β-branched amino acids are frequently found to be on the adjacent left of cysteine residue.[4] Thus, there're challenges in developing a general and easy-to-access method for peptide ligation with forming Val-Cys, Ile-Cys and Pro-Cys linkage.

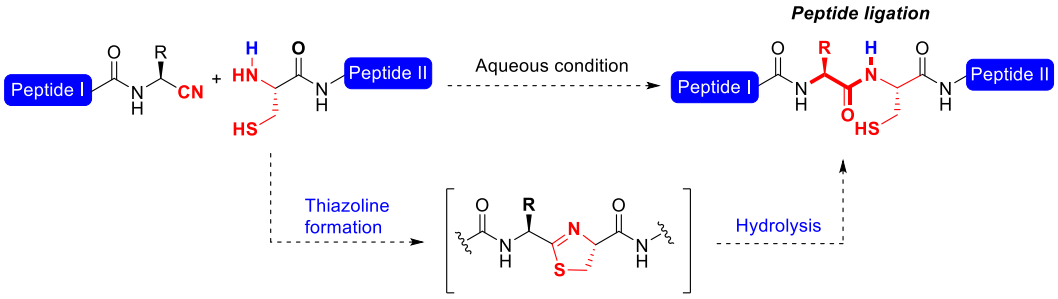


Figure 6. This work

3. Reactivity between CN and Cysteine

Literature survey indicates that the formation of thiazoline from CN and cysteine have been reported.[9] In 2020, Powner and coworkers reported a racemic synthesis of thiazoline intermediate from N-acyl amido nitrile and cysteine amino acid in a pH 9.5 buffer (Figure 7).[10] Opening of the thiazoline compound

into a racemic cysteinyl peptide was obtained in some different pH conditions.

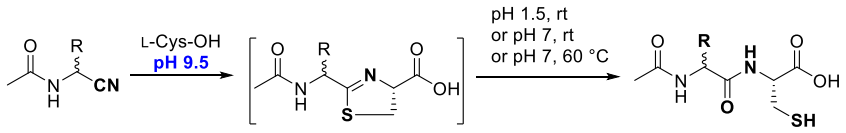


Figure 7. Racemic synthesis of thiazoline

However, later in the same year, Siegel reported an epimerization-free access towards thiazoline from Boc-L-Val-CN and L-Cys-OMe in a pH 6 buffer (Figure 8).[11] They further convert it to thiazole by oxidation.

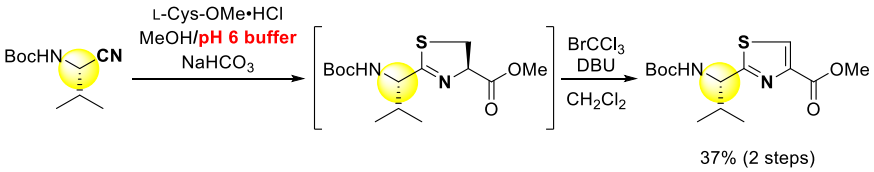


Figure 8. Epimerization-free synthesis of thiazole

These data indicates that the α-center is of high risk of epimerization and pH of the reaction system was the key to suppress epimerization.

4. Results and discussion

4.1 synthesis of thiazoline

Model substrate Boc-L-Phe-CN **1a** was prepared from Boc-L-phenylalanine via amidation followed by dehydration (Figure 9). Its corresponding thiazoline product **3a** was obtained in excellent yield as a single diastereomer following the literature condition (Figure 10a).[11] **epi-3a** was also synthesized as almost a single isomer (Figure 10b).

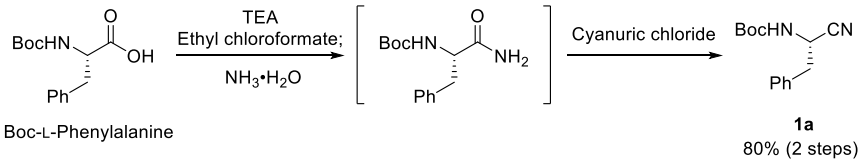


Figure 9. Synthesis of starting material **1a**

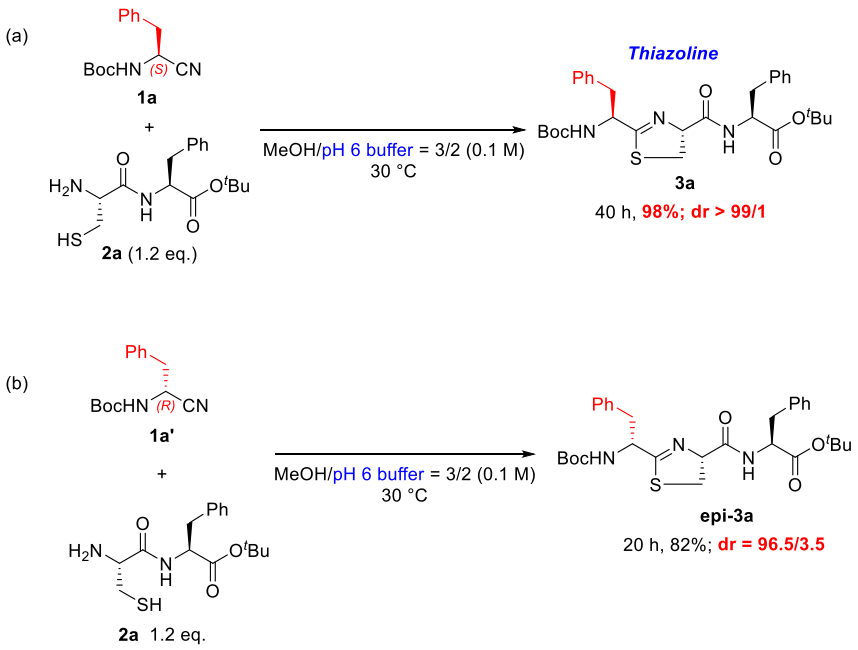
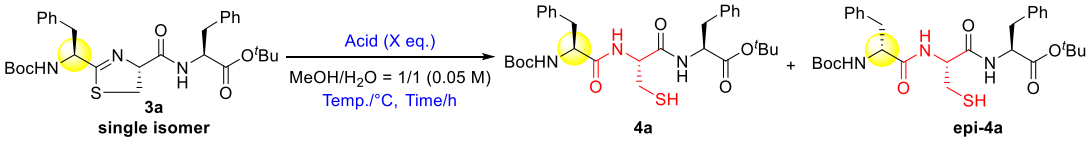


Figure 10. Synthesis of thiazoline

4.2 Optimization of hydrolysis of thiazoline

With obtaining thiazoline **3a** as a single diastereomer in hand, reaction conditions for the hydrolysis to afford the cysteine bonded tripeptide **4a** were performed. Acids were screened and the results are summarized in table 1. When **3a** was treated with formic acid at 40 ºC, the reaction was clean and 50% conversion of **3a** was observed after 42 h. However, epimerization of **4a** occurred (Table 1, Entry 1). Similar epimerization took place when HBF_4 was utilized, although higher chemical conversion was obtained (Table 1, entry 2). Surprisingly, when a stronger acid HCl was used under the same condition, **4a** was obtained as mixture in a better diastereomeric ratio that's 91.1/8.9 (Table 1, entry 3). Based on these results so far, stronger acid was favored in order to reach a higher diastereoselectivity. Therefore, much stronger triflic acid was employed (Table 1, entry 4). Higher chemical conversion with a similar dr of **4a** were obtained compared with the result of HCl in entry 3. Since triflic acid is too strong and less accessible, HCl was selected as the best acid and the reaction temperature, amount of HCl were screened subsequently. As only 69% of **4a** was observed while using HCl after 2 h (Entry 3). What I did next was prolonging the reaction time and checking the diastereomeric ratio. After 11.5 h, **4a** was found to be obtained without decreasing in diastereoselectivity with 85% of conversion (Table 1, entry 5). In order to determine the isolated yield, the reaction was conducted in 0.2 mmol scale and 91% of product **4a** was

Table 1. Optimization of hydrolysis of thiazoline[a]



Entry	pKa	Acid	Temp./°C	Time/h	*dr*[b]	Conversion/%[c]	Yield/%[d,e]	Comment
1	3.77	HCOOH (2 eq.)	40	42	85.9/14.1	50	nd	dissolve
2	1.8 (MeCN)	HBF_4 (2 eq.)	40	2.5	85.8/14.2	79	nd	10 mg scale of **3a**
3	-8.0	HCl (2 eq.)	40	2	91.1/8.9	69	nd	10 mg scale of **3a**
4	-14	TfOH (2 eq.)	40	2	88.9/11.1	86	nd	10 mg scale of **3a**
5	-8.0	HCl (2 eq.)	40	11.5	91.4/8.6	85	nd	10 mg scale of **3a**
6	-8.0	HCl (2 eq.)	40	13	91.3/8.7	97	91	dissolve
7	-8.0	HCl (2 eq.)	0	24	97.4/2.6	10	nd	solubility was not good
8	-8.0	HCl (0.2 eq.)	40	19	95.5/4.5	92	87	dissolve
9	-8.0	HCl (0.2 eq.)	23	84	97.6/2.4	61	nd	solubility was not good
10	-8.0	HCl (0.2 eq.)	23	27	97.1/2.9	81	nd	MeOH/H_2O=2/1 (dissolve better)
11	-8.0	HCl (0.2 eq.)	23	16.5	95.5/4.5	99	97	MeOH/H_2O=3/1 (dissolve well)

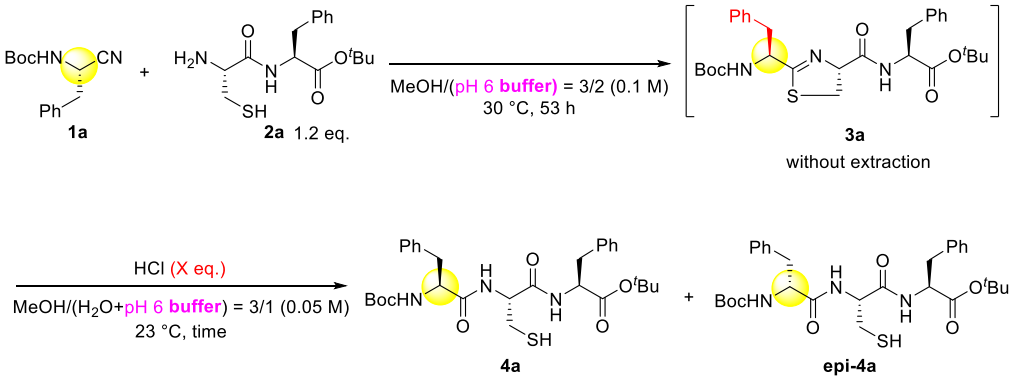
[a]Unless otherwise mentioned, the reaction was conducted using **3a** (0.2 mmo) under a mixture of MeOH and H_2O in 1:1 ratio (4 mL). HCl was titrated immediately before using. [b]dr was determined by HPLC analysis on a chiral column. [c]The conversion was determined by HPLC integration. [d,e]Isolated yield; nd = not determined.

isolated (Table 1, entry 6). Furthermore, when the reaction temperature was reduced to 0 ºC, an excellent dr was definitely obtained after 24 h but with a very limited transformation due to the poor solubility (Table 1, entry 7). Lowering the amount of HCl to a catalytic amount at 40 ºC, a 95.5/4.5 dr ratio with 87% of yield were obtained (Table 1, entry 8). Further conducting the reaction at 23 ºC produced a result with 97.6/2.4 ratio (Table 1, entry 9). However, the conversion was suppressed due to poor solubility. By increasing the amount of MeOH to a 2 to 1 mixture with buffer, excellent chemical yield and diastereoselectivity within 27 h were obtained (Table 1, entry 10) and the reaction time was further reduced to 16.5 h when a MeOH/buffer in 3 to 1 ratio was used (Table 1, entry 11). Therefore, the best hydrolysis condition of **3a** was determined as using 0.2 eq of HCl at 23 ºC in a 3/1 mixture of MeOH and buffer.

4.3 First trial with one-pot synthesis of cysteinyl peptide 4a

After finding the best condition for hydrolysis of thiazoline, one-pot synthesis of tripeptide **4a** was conducted (Figure 11). First of all, the synthesis of **3a** was conducted in MeOH and pH 6 buffer. Next, 1.8 eq. of HCl was added to the reaction mixture after diluting the reaction by adding MeOH and H_2O. After 17 h, no reaction was observed based on TLC analysis. Therefore, additional 0.2 eq. of HCl was added to the reaction system but still almost no reaction took place after 6 h. By adding another 0.4 eq.

of HCl and stirring for 44 h the generation of hydrolysis product was observed. However, epimerization of **4a** occurred by giving a diastereomeric mixture in 85 to 15 ratio in the end. The reaction was also found to be very slow and **3a** got severely epimerized with giving a mixture in 70 to 30 ratio. Since no epimerization was observed when a step-wise sequence for synthesizing **4a** was utilized as described in section 4.1 and 4.2, the epimerization here was most likely because of the existence of phosphate buffer.



X	Time/h	Result
1.8	17	no reaction
+0.2 (total 2.0)	6	almost no reaction
+0.4 (total 2.4)	44	**3a** remained. dr of **4a**: **L/D = 85/15** dr of **3a**: **L/D = 70/30**

Figure 11. One-pot synthesis of tripeptide **4a**. Notes: 1) The reaction was conducted in 0.2 mmol scale; 2) HCl was titrated immediately before using; 3) dr was determined based on HPLC analysis on a chiral column.

To figure out the effect of phosphate buffer, an extraction of **3a** was performed before the acid treatment (Figure 12). After removal of phosphate buffer by an extraction of **3a**, the residue was diluted by the addition of MeOH and H_2O, followed by the treatment of 0.4 eq. of HCl. **4a** was obtained in this case with 83% yield as almost a single diastereomer.

Based on these two experiments, it's concluded that buffer should be avoided due to epimerization in the hydrolysis step in a one-pot synthesis.

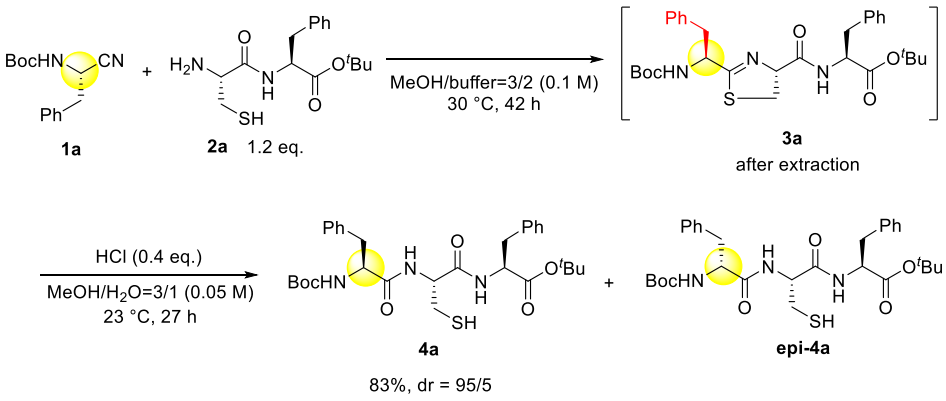


Figure 12. Synthesis of tripeptide **4a** by removing phosphate buffer

4.4 Reinvestigation of the synthesis of thiazoline 3a and one-pot synthesis to afford tripeptide 4a

As described in section 4.3, phosphate buffer was not suitable for one-pot operation because of epimerization. The synthesis of thiazoline **3a** was reinvestigated. By adding $NH_4^+Cl^-$ in 3 equivalent and using MeOH/H_2O as solvent, the reaction proceeded well to give 50% of **3a** in 96 to 4 diastereomeric ratio after 2 h at 50 ºC (Figure 13). The reaction was stopped before completion, so the yield was moderate and the remaining starting material **1a** was recovered almost completely.

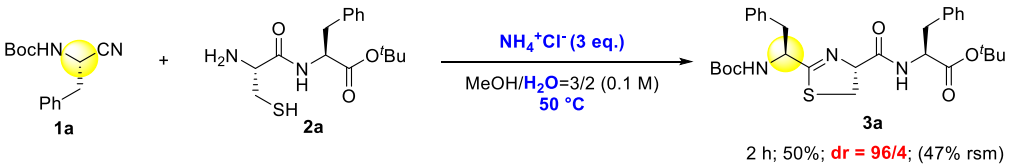


Figure 13. Synthesis of **3a** by avoiding phosphate buffer

Then, the thiazoline formation and hydrolysis were conducted in a one-pot sequence. In this case, 1.8 eq. of HCl was enough to convert the thiazoline intermediate to peptide with obtaining **4a** in 92% yield with an excellent diastereoselectivity (Figure 14). The ammonium chloride might serve as a proton source in the cyclization step and it does not disturb the acid-promoted hydrolysis.

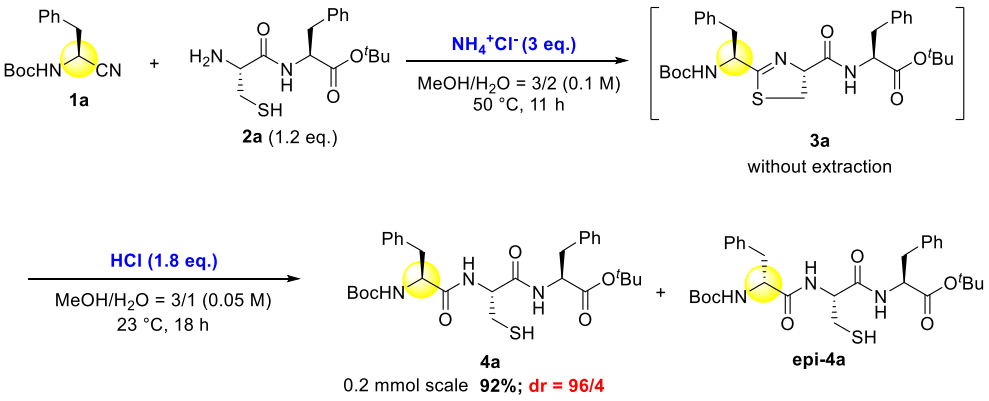


Figure 14. One-pot synthesis of **4a** starting from thiazoline formation using $NH_4^+Cl^-$

4.5 Initial trial with forming Pro-Cys ligation

After finding the best reaction condition, dipeptidyl substrate with formation of Pro-Cys bonding was examined. Boc-L-Phe-L-Pro-CN **1b** was prepared for the synthesis of tetrapeptide **4b**. Gratifyingly, proline-derived thiazoline intermediate **3b** was obtained in 99% yield after 24 h with perfect diastereomeric purity (Figure 15). The isolated thiazoline intermediate **3b** was treated with 0.2 eq. of HCl in MeOH and H_2O at 23 ºC, desired tetrapeptide **4b** was obtained in 80% yield as a single diastereomer after 46 h. The one-pot synthesis of **4b** will be conducted in due course.

The success in making Pro-Cys ligation within 58 h in a total yield of around 62% indicates that it would be a promising method for the synthesis of large peptide with formation of Pro-Cys linkage, which is not employable by native chemical ligation.

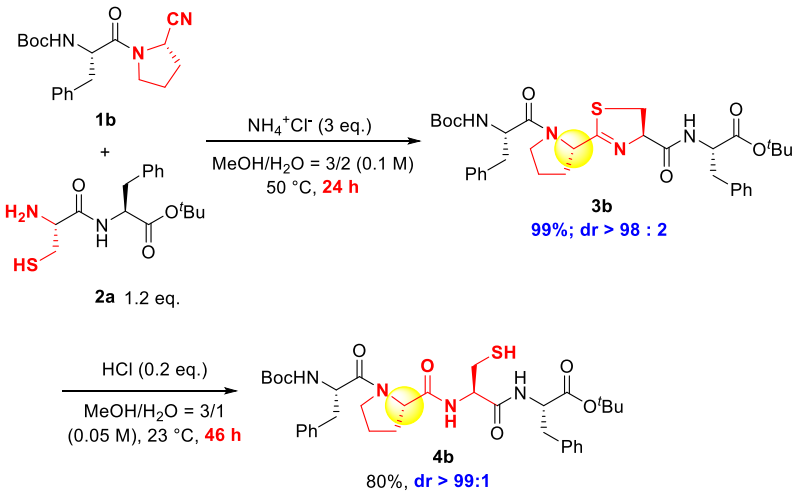


Figure 15. Synthesis of tetrapeptide with forming Pro-Cys peptide bond

5. Conclusion

As an initial investigation of harnessing chiral mono substituted α-amido nitrile as C terminus and N-terminal NH_2-Cysteine for large peptide ligation, I have succeeded in the synthesis of short peptides in a

mild and aqueous condition without epimerization. Notably, tetrapeptide bearing a Pro-Cys peptide bonding was achieved within 58 h while a much longer half life time for Pro-Cys ligation was reported in native chemical ligation. The further investigation in terms of large peptide ligation will be conducted in the coming future.

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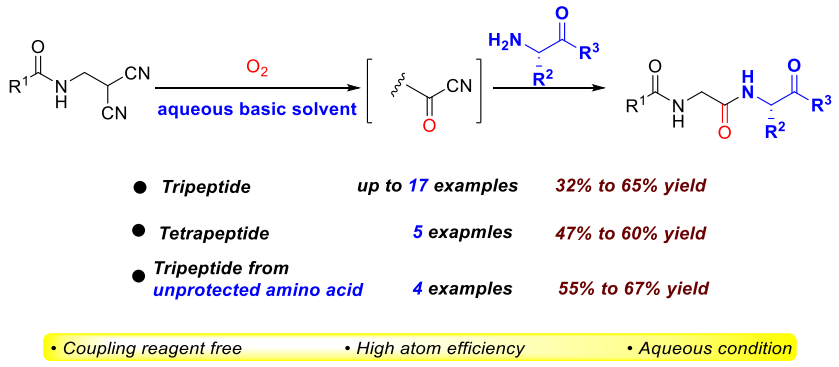
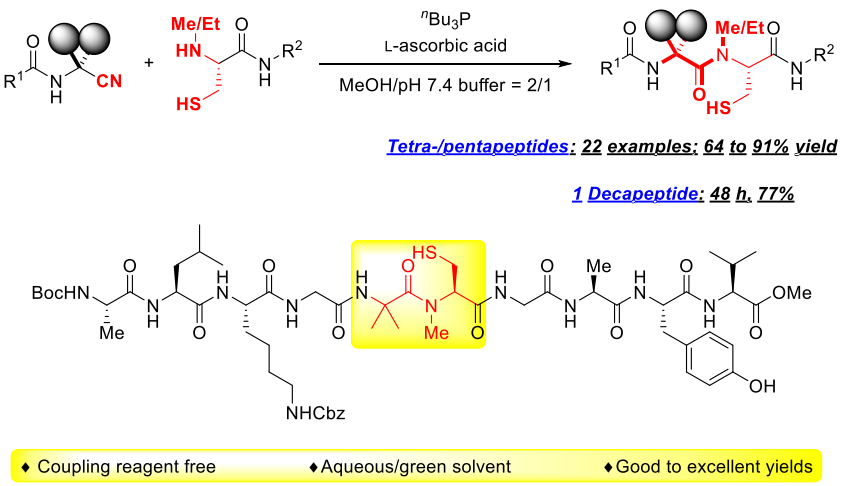
Chapter 5. Conclusion

Research on three projects were conducted during the PhD course, which mainly focused on developing new and efficient methodology for the synthesis of peptides. Two of them have been completed and the last project got very promising results at the present stage.

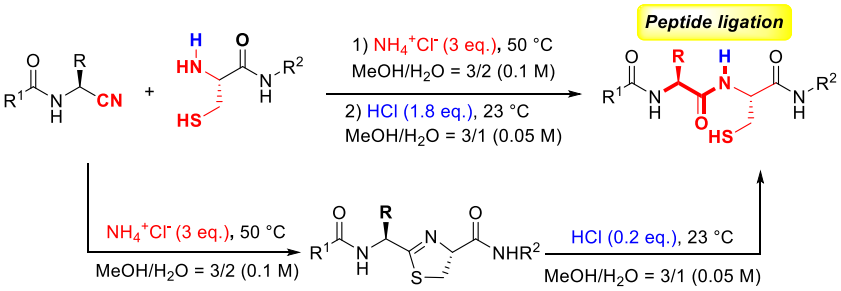
In chapter 2, an oxidative synthetic method of the peptide bond of glycine-amino acid has been developed under O_2 atmosphere in the presence of CsOAc in aqueous solution without coupling reagents and catalyst. Substituted 2-(aminomethyl)malononitrile acts as a glycine unit to react with a wide variety of amino acids to afford tripeptides, tetrapeptides under mild reaction conditions. Although the present method is limited to the generation of an amide linkage between glycine and other amino acids, it offers an alternative method for the chemical synthesis of amide linkage in the peptides.



In chapter 3, an efficient synthetic method has been developed to access extremely sterically hindered peptide bonds via a sequential cyclization between α,α-disubstituted α-amidonitrile and *N*-alkyl cysteine and immediate hydrolysis of the iminium intermediate. A wide variety of non-proteinogenic α,α-disubstituted amino acids, including chiral and non-chiral amino acids, show great performance in coupling with *N*-methyl or *N*-ethyl peptides. Notably, no coupling reagents are employed, and this method can also be used to connect two relatively long peptide chains. Thus, it represents a useful method for chemical ligation. The realization of synthesizing such sterically hindered peptides will open new avenues to investigate their conformation, physical, and biological properties.



In chapter 4, as an initial investigation of harnessing chiral mono substituted α-amido nitrile as C terminus and N-terminal NH_2-Cysteine for large peptide ligation, I have succeeded in the synthesis of short peptides in a mild and aqueous condition without epimerization. Notably, tetrapeptide bearing a Pro-Cys peptide bonding was achieved. And the further investigation in terms of large peptide ligation will be conducted in the coming future.



Experimental Section

Chapter 2. Oxidative Peptide Bond Formation of Glycine-Amino Acid using 2-(Aminomethyl)malononitrile as a Glycine Unit

Experimental procedures and Characterization data

Table of Contents

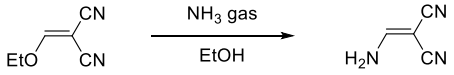
1. Materials and Methods

General Methods

General Remarks: All reactions were carried out under argon atmosphere and monitored by thin-layer chromatography using Merck 60 F254 precoated silica gel plates (0.25 mm thickness). Specific optical rotations were measured using a JASCO P-1020 polarimeter and a JASCO DIP-370 polarimeter. FT-IR spectra were recorded on a JASCO FT/IR-410 spectrometer and a Perkin Elmer spectrum BX FT-IP spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on an Agilent-400 MR (400 MHz for ^{1}H NMR, 100 M Hz for 13C NMR) instrument. Data for ^{1}H NMR are reported as chemical shift (δ ppm), integration multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quintet = quin, septet = sep, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = double of triplets, td = triplet of doublets, m = multiplet, brs = broad singlet), coupling constant (Hz), Data for ^{13}C NMR are reported as chemical shift. High resolution ESI-TOF mass spectra were measured by Themo Orbi-trap instrument.

2. Experimental procedure

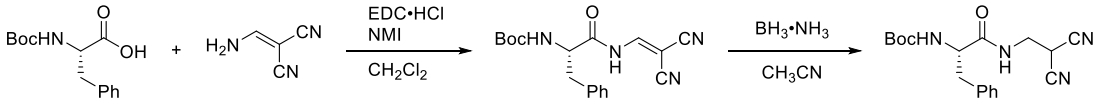
2.1 Synthesis of 2-(aminomethylene)malononitrile SS1[1].



ss1

NH_3 gas was introduced to a saturated solution of 2-(ethoxymethylene)malononitrile[1a] (5.25 g, 43 mmol) in ethanol under stirring. After 12 min, the solvent was removed under reduced pressure. The crude compound was purified by silica gel column chromatography (CH_2Cl_2/EtOAc = 25%/75%) and gave compound **SS1**, which was used directly for the next step without further determination of the structure.

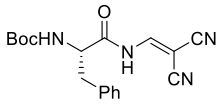
2.2 Synthesis of dicyano compound 1a and physical information



ss1 **s1** **1a**

To a mixture of **SS1** (1.34 g, 14.4 mmol) in CH_2Cl_2 (96 mL) was added *N*-(*tert*-Butoxycarbonyl)-L-phenylalanine (4.4 g, 16.6 mmol), 1-Methylimidazole (118 mg, 1.44 mmol), EDC·HCl (3.8 g, 19.8 mmol). The mixture was stirred at room temperature overnight and then evaporated to remove volatiles. After addition of EtOAc, the suspension was filtered and the filtrate was concentrated under reduced pressure. Purification by silica gel column chromatography (EtOAc/Hexane=28%/62%) gave the desired product **S1**. To a mixture of **S1** (1.76 g, 5.17 mmol) in CH_3CN (59 mL) was added $BH_3 \cdot NH_3$ (1.6 g, 51.7 mmol). The mixture was stirred at room temperature for 2 h (the reaction was monitored by TLC using MeOH/DCM=1/7). Then the reaction mixture was slowly added to a mixture of cold 1N HCl/EtOAc = 2/1 and extracted with EtOAc. The organic phase was collected and concentrated under reduced pressure. The crude compound was further purified by silica gel column chromatography (EtOAc/Hexane = 50%/50%) and gave compound **1a**.

<u>*tert*-Butyl (*S*)-(1-((2,2-dicyanovinyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate</u>



s1

S1 was obtained in 63% yield as white solid.

^{1}H NMR (399 MHz, DMSO-d_6) δ 12.05 (d, *J* = 12.2 Hz, 1H), 8.38 (d, *J* = 12.0 Hz, 1H), 7.49 – 7.07 (m, 5H), 4.69 – 4.44 (m, 1H), 2.95 (dd, *J* = 13.9, 3.6 Hz, 1H), 2.73 (dd, *J* = 13.7, 10.9 Hz, 1H), 1.28 (s, 9H).

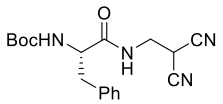
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 156.0, 153.7, 137.5, 129.6 (2C), 128.5(2C), 126.9, 114.5, 112.4, 79.0, 63.0, 56.1, 36.2, 28.5 (3C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 363.1428; Found: 363.1424

FT-IR (neat): 2361, 2233, 1684, 1614, 1497, 1367, 1252, 1165, 700 cm^{-1}

$[\alpha]_D^{24}$ +2.47 (c 1.0, DMSO)

<u>*tert*-Butyl (*S*)-(1-((2,2-dicyanoethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate</u>



1a

1a was obtained in 65% yield as white solid.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.66 (t, *J* = 6.1 Hz, 1H), 7.33 – 7.09 (m, 5H), 6.99 (d, *J* = 8.6 Hz, 1H), 4.85 (t, *J* = 6.2 Hz, 1H), 4.16 (td, *J* = 10.7, 4.1 Hz, 1H), 3.77 (dt, *J* = 12.8, 6.3 Hz, 1H), 3.69 – 3.59 (m, 1H), 2.92 (dd, *J* = 13.7, 4.0 Hz, 1H), 2.71 (dd, *J* = 13.7, 10.8 Hz, 1H), 1.25 (s, 9H).

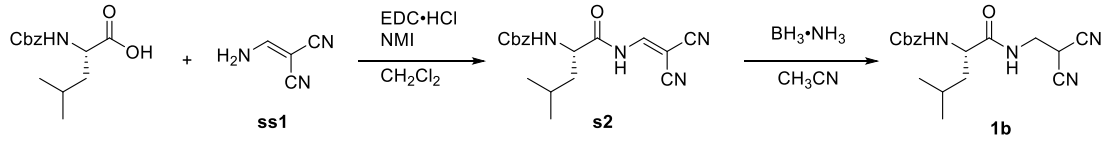
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 155.7, 138.4, 129.6 (2C), 128.5 (2C), 126.7, 113.7(2C), 78.5, 56.1, 38.6, 37.8, 28.5 (3C), 24.1.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 365.1584; Found: 365.1586

FT-IR (neat): 3291, 2979, 1668, 1526, 1367, 1249, 1168, 910, 734 cm^{-1}

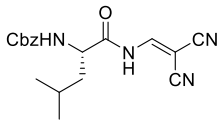
$[\alpha]_D^{27}$ -0.64 (c 1.0 $CHCl_3$)

2.3 Synthesis of dicyano compound 1b and physical information



To a mixture of **SS1** (0.46 g, 4.94 mmol) in CH_2Cl_2 (33 mL) was added N-Benzyloxycarbonyl-L-leucine (1.44 g, 5.44 mmol), 1-Methylimidazole (40.6 mg, 0.494 mmol), EDC·HCl (1.56 g, 8.15 mmol). The mixture was stirred at room temperature overnight and then quenched with 1N HCl and extracted with CH_2Cl_2. The organic face was concentrated under reduced pressure. Purification by silica gel column chromatography (EtOAc/Hexane=30%/70%) gave the desired product **S2**. To a mixture of **S2** (1.0 g, 2.94 mmol) in CH_3CN (33 mL) was added $BH_3{\cdot}NH_3$ (0.97 g, 29.4 mmol). The mixture was stirred at room temperature for 1 h. Then the reaction mixture was slowly added to a mixture of cold 1N HCl /EtOAc = 2/1 and extracted with EtOAc. The organic phase was collected and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc/Hexane = 50%/50%) and gave compound **1b**.

Benzyl (*S*)-(1-((2,2-dicyanovinyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate



s2

S2 was obtained in 61% yield as white solid.

^{1}H NMR (399 MHz, $CDCl_3$) δ 10.22 (d, *J* = 11.8 Hz, 1H), 8.16 (d, *J* = 12.0 Hz, 1H), 7.56 – 7.02 (m, 5H), 5.36 (d, *J* = 5.4 Hz, 1H), 5.10 (d, *J* = 19.7 Hz, 2H), 4.43 (s, 1H), 1.84 – 1.40 (m, 3H), 0.95 (d, *J* = 6.2 Hz, 3H), 0.91 (d, *J* = 6.1 Hz, 3H).

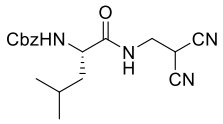
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 157.1, 151.2, 135.3, 128.7(2C), 128.6 (2C), 128.3, 112.3, 110.6, 68.1, 66.2, 53.5, 39.4, 24.6, 22.8, 21.5.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 363.1428; Found: 363.1427.

FT-IR (neat): 3020, 2963, 2400, 2235, 1703, 1615, 1508, 1216, 1044, 754, 669 cm^{-1}

$[\alpha]_D^{28}$ +8.97 (c 2.2 $CHCl_3$)

Benzyl (*S*)-(1-((2,2-dicyanoethyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate



1b

1b was obtained in 72% yield as white solid.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.55 (brs, 1H), 7.43 – 7.21 (m, 5H), 5.48 (d, *J* = 7.6 Hz, 1H), 5.09 (s, 2H), 4.32 – 4.19 (m, 1H), 4.18 – 4.12 (m, 1H), 3.77 – 3.64 (m, 1H), 3.63 – 3.54 (m, 1H), 1.72 – 1.57 (m, 2H), 1.55 – 1.44 (m, 1H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.89 (d, *J* = 6.1 Hz, 3H).

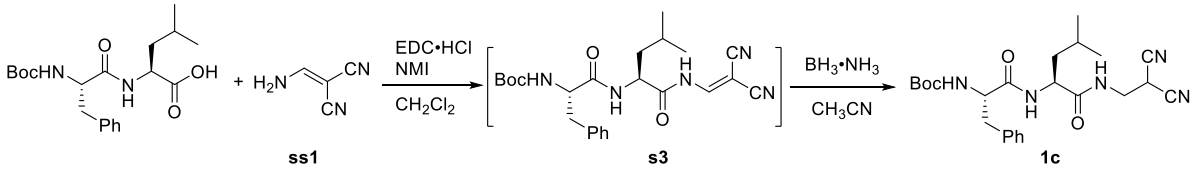
^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 156.5, 135.9, 128.6 (2C), 128.4, 127.9 (2C), 111.3 (2C), 67.4, 53.3, 40.7, 39.7, 24.7 (2C), 22.7, 21.9.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 365.1584; Found: 365.1586.

FT-IR (neat): 3300, 3067, 2959, 2259, 1672, 1534, 1455, 1368, 1239, 1173, 1122, 1043, 911, 736, 698 cm^{-1}

$[\alpha]_D^{25}$ -24.00 (c 1.9 $CHCl_3$)

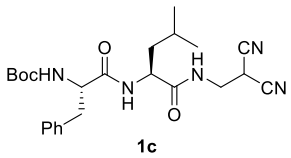
2.3 Synthesis of dicyano compound 1c and physical information



ss1 **s3** **1c**

To a mixture of **SS1** (227 mg, 2.44 mmol) in CH_2Cl_2 (22 mL) was added Boc-L-Phe-L-Leu-OH[2] (1.06 g, 5.44 mmol), 1-Methylimidazole (20 mg, 0.244 mmol), EDC·HCl (644 mg, 3.36 mmol). The mixture was stirred at room temperature overnight and then quenched with 1N HCl and extracted with CH_2Cl_2. The organic phase was concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (EtOAc/Hexane = 30%/70%) and gave compound **S3**. (It was directly used for the next step without further determination of the structure). To a mixture of **S3** (300 mg, 0.661 mmol) in CH_3CN (7.5 mL) was added $BH_3{\cdot}NH_3$ (204 mg, 6.61 mmol). The mixture was stirred at room temperature for 1 h. Then the reaction mixture was slowly added to a mixture of cold 1N HCl /EtOAc = 2/1 and extracted with EtOAc. The organic phase was collected and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (CH_2Cl_2/MeOH=92%/8%) and gave compound **1c**.

***tert*-Butyl ((*S*)-1-(((*S*)-1-((2,2-dicyanoethyl)amino)-4-methyl-1-oxopentan-2-yl)amino)- 1-oxo-3-phenylpropan-2-yl)carbamate**



1c

1c was obtained in 29% yield as white solid (**mixture of two rotamers**).

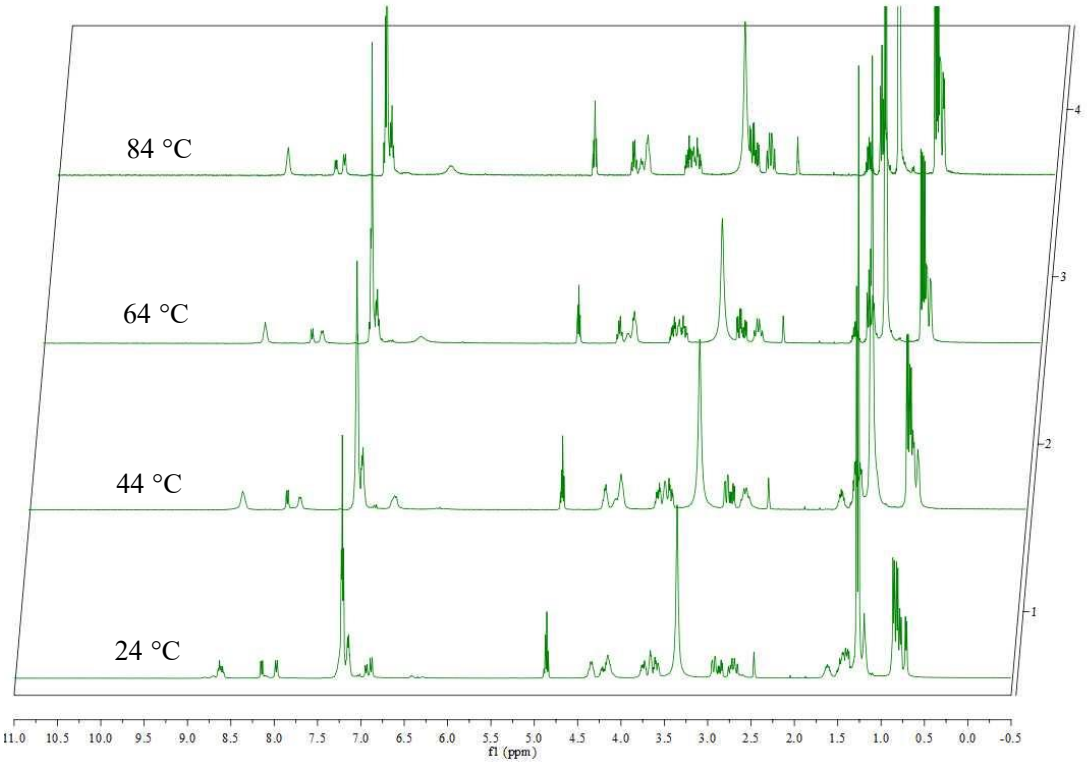
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.83 – 8.54 (m, 1H), (8.14 (d, *J* = 8.3 Hz) and 7.97 (d, *J* = 8.2 Hz), 1H), 7.34 – 7.10 (m, 5H), (6.94 (d, *J* = 8.0 Hz) and 6.88 (d, *J* = 8.4 Hz), 1H), 4.92 – 4.81 and 4.41 – 4.04 (m, 1H), 4.41 – 4.07 (m, 2H), 3.82 – 3.54 (m, 2H), (2.93 (dd, *J* = 13.8, 4.0 Hz) and 2.86 (dd, *J* = 13.5, 5.9 Hz), 1H), 2.79 – 2.61 (m, 1H), 1.73 – 1.56 (m, 1H), 1.53 – 1.36 (m, 2H), 1.28 and 1.26 (s, 9H), (0.86 (d, *J* = 6.8 Hz) and 0.78 (d, *J* = 6.0 Hz), 3H), (0.81 (d, *J* = 6.4 Hz) and 0.71 (d, *J* = 6.4 Hz), 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6 and 173.5 (1C), 172.0 and 171.9 (1C), 155.7 and 155.7 (1C), 138.6 and 138.1 (1C), 129.6 (2C), 128.4 (2C), 126.6 and 126.5 (1C), 113.6 and 113.6 (2C), 78.7 and 78.5 (1C), 56.3 and 56.0 (1C), 51.3 and 51.2 (1C), 41.6 and 41.3 (1C), 38.5, 38.0 and 37.5 (1C), 28.6 and 28.5 (3C), 24.4 and 24.3 (1C), 24.1 and 24.1 (1C), 23.5 and 23.4 (1C), 22.0 and 21.7 (1C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 478.2425; Found 478.2424.

FT-IR (neat): 3287, 2961, 2256, 1650, 1524, 1368, 1251, 1169, 1049, 910, 735, 700 cm^{-1}

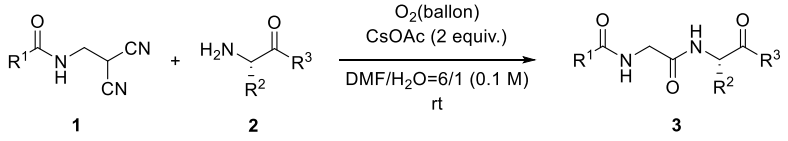
$[\alpha]_D^{28}$ -4.81 (c 1.0 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d_6) spectra of compound 1c to prove the rotamer.

2.4 Typical procedure of oxidative peptide synthesis using amino acid esters/dipeptide and physical

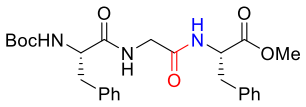
information



1 **2** **3**

To a mixture of the amino acid ester **2** (0.4 mmol) in DMF/H_2O = 6/1 (2 mL) was added CsOAc (76.8 mg, 0.4 mmol). The mixture was stirred and CsOAc dissolved and then was filled with O_2. The dicyano compound **1** (0.2 mmol) was then added and stirred at room temperature. The mixture was then diluted by EtOAc and quenched with 1N HCl and extracted with EtOAc. The organic phase was washed with water/brine = 1/1 and dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography, eluted with Hexane/EtOAc.

Methyl (*tert*-butoxycarbonyl)-L-phenylalanylglycyl-L-phenylalaninate



Boc-Phe-Gly-Phe-OMe (**3a**)

3a was obtained in 58% yield as yellow solid. Purification by EtOAc/Hexane = 65%/35%

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.26 (d, *J* = 7.4 Hz, 1H), 8.10 (s, 1H), 7.29 – 7.09 (m, 10H), 6.96 – 6.87 (m, 1H), 4.46 (dd, *J* = 13.7, 8.0 Hz, 1H), 4.13 (dd, *J* = 12.8, 5.5 Hz, 1H), 3.81 – 3.60 (m, 2H), 3.56 (s, 3H), 3.05 – 2.83 (m, 3H), 2.67 (dd, *J* = 13.3, 11.0 Hz, 1H), 1.25 (s, 9H).

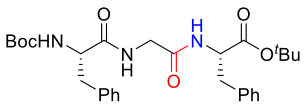
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 172.2, 169.2, 155.7, 138.7, 137.4, 129.6 (2C), 129.5 (2C), 128.7 (2C), 128.4 (2C), 127.0, 126.5, 78.5, 56.1, 54.1, 52.3, 42.1, 37.8, 37.2, 28.5 (3C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 506.2262; Found: 506.2260

FT-IR (neat): 3301, 2979, 1657, 1525, 1455, 1367, 1251, 1171, 1022, 911, 734, 701 cm^{-1}

$[\alpha]_D^{26}$ +30.74 (c 1.1 $CHCl_3$)

***tert*-Butyl (*tert*-butoxycarbonyl)-L-phenylalanylglycyl-L-phenylalaninate**



Boc-Phe-Gly-Phe-O*t*-Bu (**3b**)

3b was obtained in 50% yield as white solid (**mixture of two rotamers**). Purification by EtOAc/Hexane = 60%/40%;

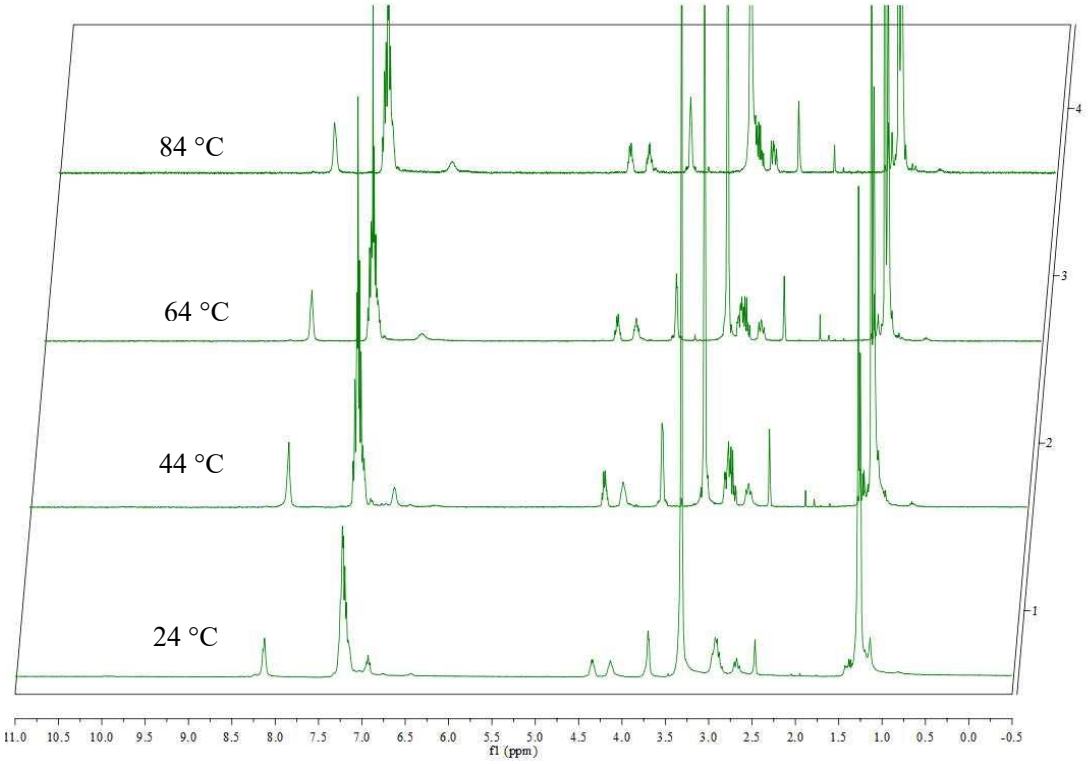
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.18 – 8.05 (m, 2H), 7.37 – 7.05 (m, 10H), 6.97 – 6.88 (m, 1H), 4.34 (dt, *J* = 7.3, 6.1 Hz, 1H), 4.13 (dd, *J* = 13.0, 6.0 Hz, 1H), 3.70 (d, *J* = 5.5 Hz, 2H), 2.99 – 2.84 (m, 3H), 2.68 (dd, *J* = 13.5, 10.9 Hz, 1H), 1.28 (s, 9H), 1.25 and 1.26 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 170.8, 169.1, 155.7, 138.7, 137.4, 129.6 (2C), 129.6 (2C), 128.6 (2C), 128.4 (2C), 127.0, 126.5, 81.2, 78.4, 56.1, 54.5, 42.1, 37.8, 37.6, 28.6 (3C), 27.9 (3C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 548.2731; Found: 548.2733

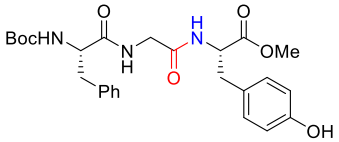
FT-IR (neat): 3299, 2979, 1652, 1523, 1455, 1368, 1252, 1160, 911, 734, 700 cm^{-1}

$[\alpha]_D^{25}$ +28.43 (c 1.0 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d_6) spectra of compound 3b to prove the rotamer.

Methyl (*tert*-butoxycarbonyl)-L-phenylalanylglycyl-L-tyrosinate



Boc-Phe-Gly-Tyr-OMe (3c)

3c was obtained in 52% yield as white solid. Purification by EtOAc/Hexane = 75%/25%

^{1}H NMR (399 MHz, DMSO-d_6) δ 9.20 (s, 1H), 8.25 – 8.15 (m, 1H), 8.11 (t, *J* = 5.2 Hz, 1H), 7.31 – 7.07 (m, 5H), 7.06 – 6.85 (m, 1H) 7.06 – 6.85 (m, 2H), 6.63 (d, *J* = 8.3 Hz, 2H), 4.37 (dd, *J* = 13.9, 8.0 Hz, 1H), 4.13 (dd, *J* = 12.9, 6.0 Hz, 1H), 3.81 – 3.62 (m, 2H), 3.55 (s, 3H), 3.01 – 2.83 (m, 2H), 2.77 (dd, *J* = 13.8, 8.5 Hz, 1H), 2.68 (dd, *J* = 13.4, 11.1 Hz, 1H), 1.25 (s, 9H).

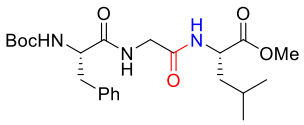
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 169.1, 156.5, 155.7, 138.7 (2C), 130.4 (2C), 129.6 (2C), 128.4 (2C), 127.3, 126.5, 115.5 (2C), 78.5, 56.1, 54.4, 52.2, 42.1, 37.8, 36.6, 28.6 (3C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 522.2211; Found: 522.2211

FT-IR (neat): 3311, 1660, 1517, 1454, 1367, 1250, 1170, 1023, 910, 733 cm^{-1}

$[\alpha]_D^{30}$ +22.72 (c 1.8 $CHCl_3$)

Methyl (*tert*-butoxycarbonyl)-L-phenylalanylglycyl-L-leucinate



Boc-Phe-Gly-Leu-OMe (3d)

3d was obtained in 65% yield as yellow solid. Purification by EtOAc/Hexane=75%/25%

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.19 – 8.06 (m, 2H), 7.29 – 7.19 (m, 4H), 7.15 (td, *J* = 8.2, 3.8 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 4.33 – 4.25 (m, 1H), 4.18 – 4.09 (m, 1H), 3.72 (d, *J* = 5.7 Hz, 2H), 3.59 (s, 3H), 2.98 (dd, *J* = 13.7, 3.9 Hz, 1H), 2.70 (dd, *J* = 13.6, 10.8 Hz, 1H), 1.64 – 1.41 (m, 3H), 1.26 (s, 9H), 0.86 (d, *J* = 6.4 Hz, 3H), 0.82 (d, *J* = 6.4 Hz, 3H).

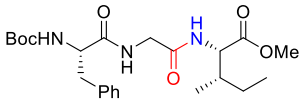
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2, 172.3, 169.2, 155.7, 138.7, 129.6 (2C), 128.4 (2C), 126.5, 78.5, 56.1, 52.3, 50.6, 42.1, 37.7, 28.5 (3C), 24.6, 23.1, 21.8 (2C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 472.2418; Found: 472.2418

FT-IR (neat): 3316, 2959, 2252, 1668, 1518, 1368, 1251, 1160, 1025, 907, 734, 649 cm^{-1}

$[\alpha]_D^{25}$ +1.38 (c 2.4 $CHCl_3$)

Methyl (*tert*-butoxycarbonyl)-L-phenylalanylglycyl-L-isoleucinate



Boc-Phe-Gly-Ile-OMe (3e)

3e was obtained in 60% yield as yellow solid (**mixture of two rotamers**). Purification by EtOAc/Hexane = 75%/25%

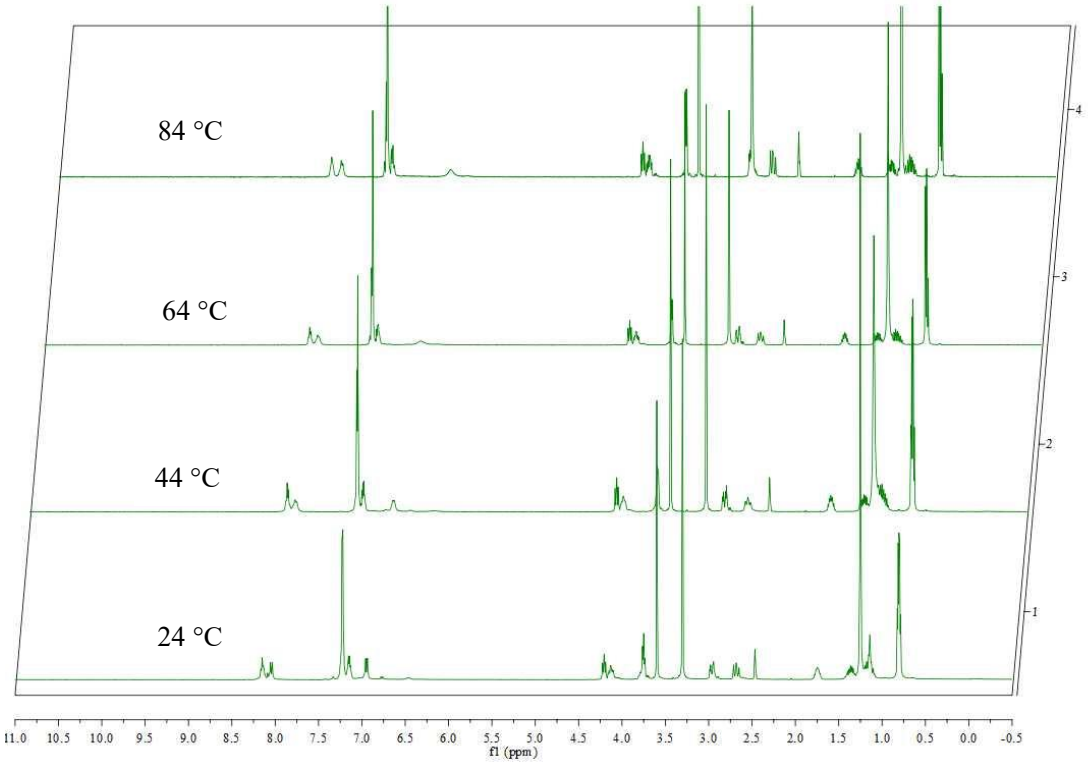
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.19 – 8.10 (m, 1H), 8.05 (dd, *J* = 12.8, 8.2 Hz, 1H), 7.29 – 7.19 (m, 4H), 7.15 (td, *J* = 8.3, 4.1 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 4.25 – 4.18 (m, 1H), 4.18 – 4.08 (m, 1H), 3.84 – 3.69 (m, 2H), 3.60 and 3.60 (s, 3H), 2.97 (dt, *J* = 13.6, 3.8 Hz, 1H), 2.69 (dd, *J* = 13.5, 11.0 Hz, 1H), 1.81 – 1.69 (m, 1H), 1.47 – 1.31 (m, 2H), 1.25 (s, 9H), 0.89 – 0.71 (m, 6H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 172.3, 169.3, 155.7, 138.7, 129.6 (2C), 128.4 (2C), 126.5, 78.4, 56.7, 56.1, 52.1, 42.2, 37.8, 36.8, 28.5 (3C), 25.1, 15.8, 11.5.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 472.2418; Found: 472.2417

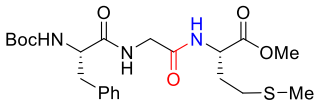
FT-IR (neat): 3304, 2969, 1655, 1526, 1455, 1366, 1252, 1170, 1022, 912, 734, 700 cm^{-1}

$[\alpha]_D^{29}$ +14.23 (c 1.2 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d$_6$) spectra of compound 3e to prove the rotamer.

Methyl (*tert*-butoxycarbonyl)-L-phenylalanylglycyl-L-methioninate



Boc-Phe-Gly-Met-OMe (3f)

3f was obtained in 47% yield as yellow solid (**mixture of two rotamers**). Purification by EtOAc/Hexane = 80%/20%

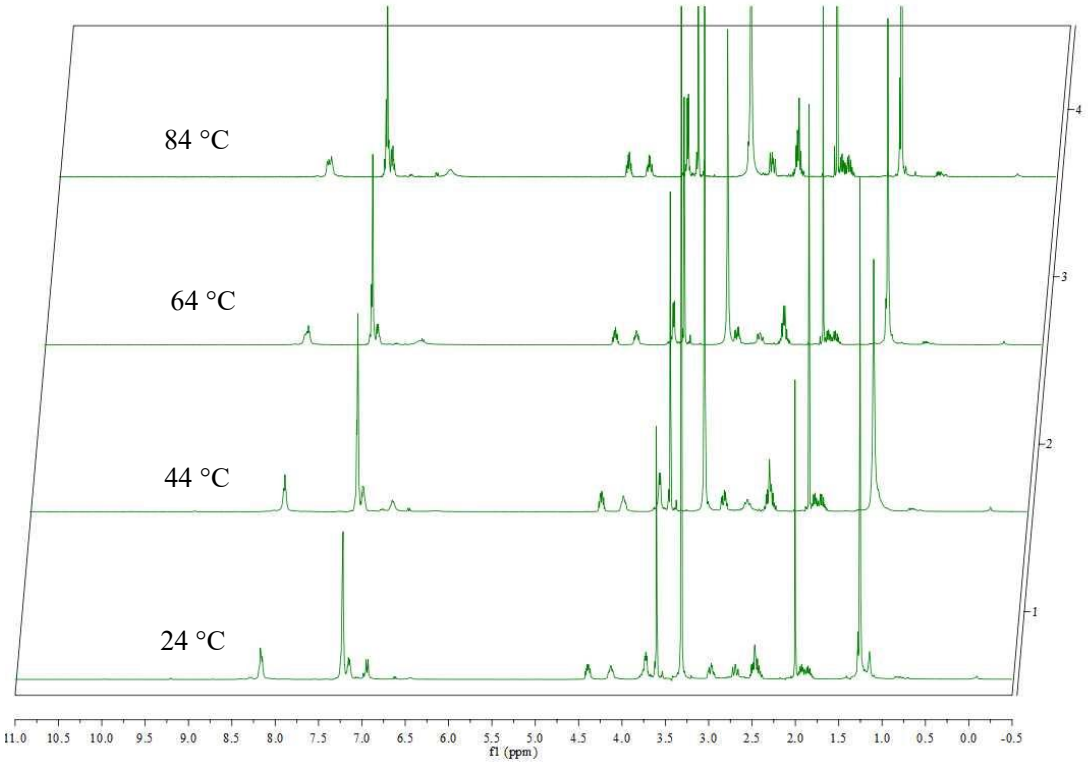
^{1}H NMR (399 MHz, DMSO-d$_6$) δ 8.24 – 8.09 (m, 2H), 7.29 – 7.20 (m, 4H), 7.16 (dd, *J* = 8.1, 4.2 Hz, 1H), 6.95 (dd, *J* = 12.9, 8.3 Hz, 1H), 4.44 – 4.35 (m, 1H), 4.12 (dd, *J* = 12.5, 6.1 Hz, 1H), 3.82 – 3.68 (m, 2H), 3.60 and 3.60 (s, 3H), 3.03 – 2.93 (m, 1H), 2.69 (dd, *J* = 13.5, 10.9 Hz, 1H), 2.54 – 2.38 (m, 2H), 2.00 (s, 3H), 1.97 – 1.79 (m, 2H), 1.26 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d$_6$) δ 172.5, 172.4, 169.4, 155.7, 138.7, 129.6, 128.4, 126.5, 78.5, 56.1, 52.4, 51.2, 42.2, 37.7, 31.1, 29.8, 28.5, 15.0 and 14.9 (1C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 490.1982; Found: 490.1981

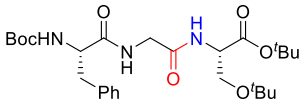
FT-IR (neat): 3308, 2978, 1658, 1527, 1440, 1367, 1251, 1170, 1022, 912, 733, 701 cm^{-1}

$[\alpha]_D^{28}$ +11.35 (c 1.8 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d$_6$) spectra of compound 3f to prove the rotamer.

***tert*-Butyl *N*-(*tert*-butoxycarbonyl)-L-phenylalanylglycyl-O-(*tert*-butyl)-L-serinate**



Boc-Phe-Gly-Ser(O*t*-Bu)-O*t*-Bu (3g)

3g was obtained in 56% yield as yellow solid (**mixture of two rotamers**). Purification by EtOAc/Hexane = 55%/45%

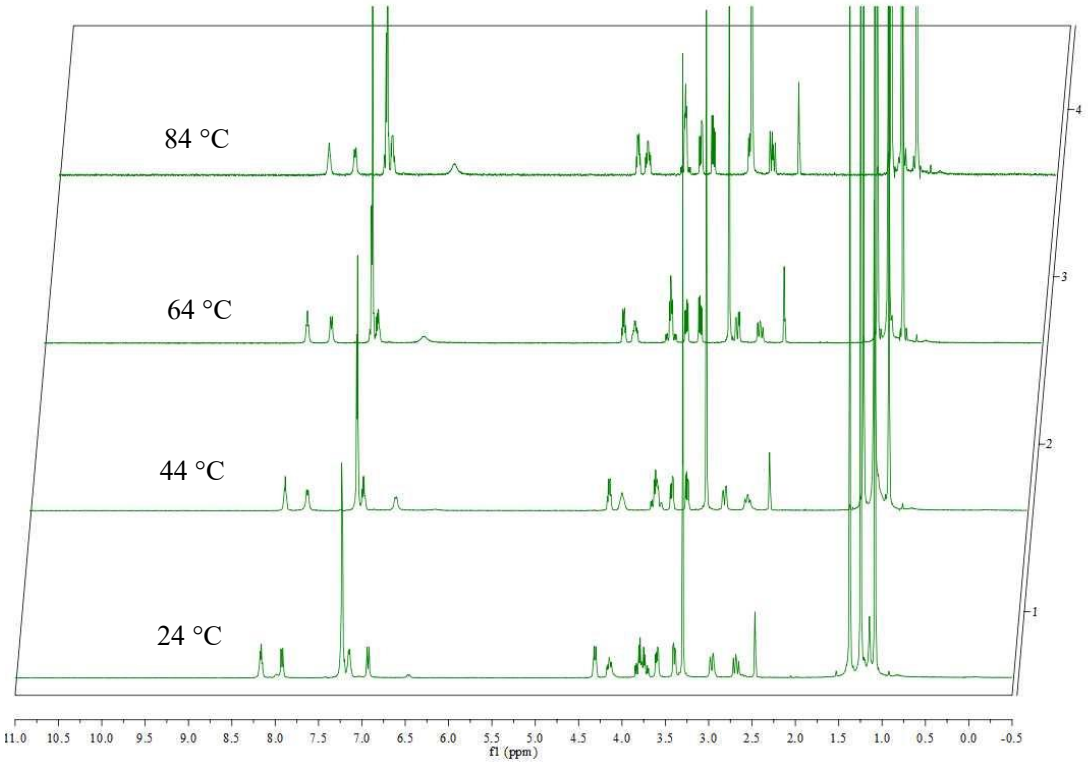
^{1}H NMR (399 MHz, DMSO-d$_6$) δ 8.14 (t, *J* = 5.3 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.34 – 7.19 (m, 4H), 7.18 – 7.09 (m, 1H), 6.89 (d, *J* = 8.6 Hz, 1H), 4.31 (dt, *J* = 8.1, 4.2 Hz, 1H), 4.21 – 4.09 (m, 1H), 3.86 – 3.68 (m, 2H), 3.59 (dd, *J* = 8.9, 4.4 Hz, 1H), 3.40 (dd, *J* = 9.0, 4.3 Hz, 1H), 2.97 (dd, *J* = 13.7, 3.5 Hz, 1H), 2.69 (dd, *J* = 13.5, 10.9 Hz, 1H), 1.37 (s, 9H), 1.25 (s, 9H), 1.08 and 1.08 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d$_6$) δ 172.4, 169.6, 169.2, 155.7, 138.7, 129.6(2C), 128.4(2C), 126.5, 81.1, 78.4, 73.1, 62.2, 56.1, 53.6, 42.2, 37.9, 28.5(3C), 28.1(3C), 27.5(3C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 544.2993; Found: 544.2993

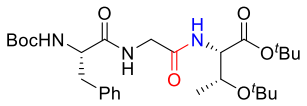
FT-IR (neat): 3299, 2977, 1645, 1521, 1392, 1366, 1249, 1169, 734 cm^{-1}

$[\alpha]_D^{27}$ +18.07 (c 1.1 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d$_6$) spectra of compound 3g to prove the rotamer.

***tert*-Butyl *N*-(*tert*-butoxycarbonyl)-L-phenylalanylglycyl-O-(*tert*-butyl)-L-threoninate**



Boc-Phe-Gly-Thr(O*t*-Bu)-O*t*-Bu (3h)

3h was obtained in 51% yield as white solid (**mixture of two rotamers**). Purification by EtOAc/Hexane=50%/50%

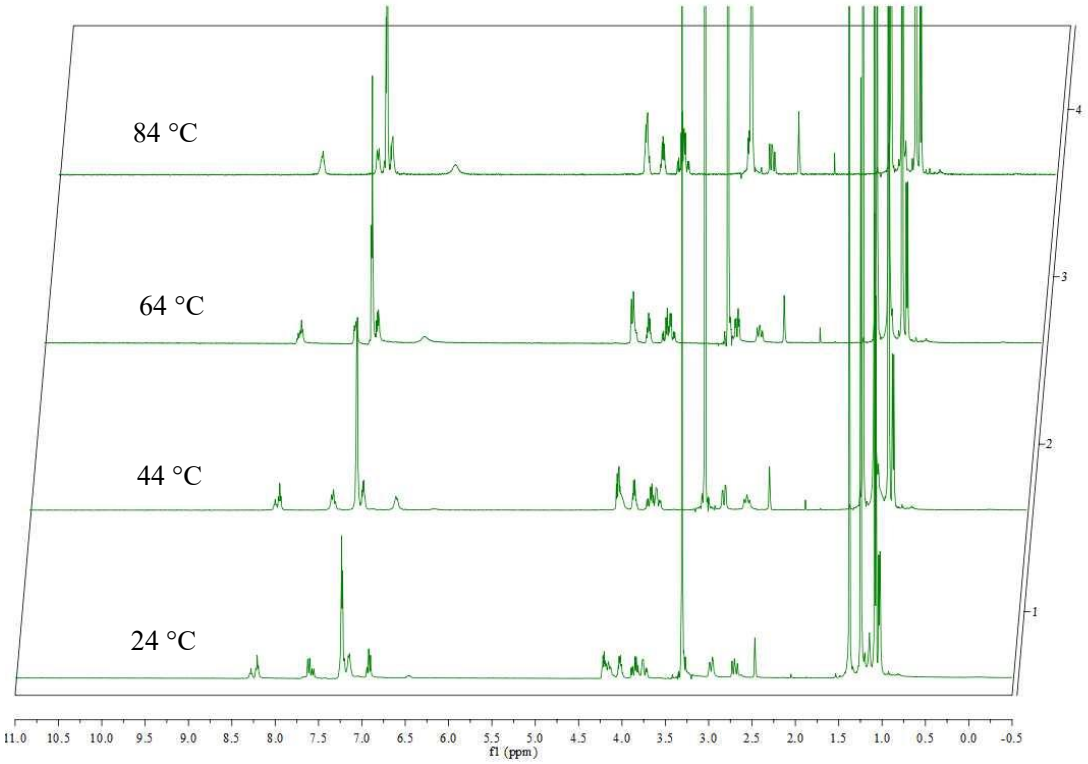
^{1}H NMR (399 MHz, DMSO-d$_6$) δ 8.24 (dt, *J* = 28.2, 5.6 Hz, 1H), (7.61 (d, *J* = 8.8 Hz) and 7.56 (d, *J* = 8.8 Hz), 1H), 7.31 – 7.10 (m, 5H), 6.91 (t, *J* = 8.2 Hz, 1H), 4.27 – 4.10 (m, 2H), 4.07 – 3.99 (m, 1H), 3.92 – 3.80 (m, 1H), 3.74 (dd, *J* = 16.9, 5.2 Hz, 1H), 2.97 (dd, *J* = 13.6, 3.0 Hz, 1H), 2.70 (dd, *J* = 13.5, 11.2 Hz, 1H), 1.38 (s, 9H), 1.22 (s, 9H), 1.09 and 1.07 (s, 9H), (1.03 (d, *J* = 6.4 Hz) and 1.02 (d, *J* = 6.0 Hz), 3H).

^{13}C NMR (100 MHz, DMSO-d$_6$) δ 172.5, 169.7, 169.5, 155.6, 138.7, 129.6 (2C), 128.4 (2C), 126.5, 81.2 and 81.2 (1C), 78.4, 73.7, 67.3, 58.3, 56.2, 42.4, 38.0, 28.8 (3C), 28.5 (3C), 28.1 (3C), 20.3 and 20.4 (1C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 558.3150; Found: 558.3151

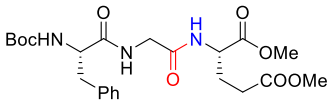
FT-IR (neat): 3299, 2978, 1652, 1520, 1367, 1251, 1169, 1084, 912, 734, 700 cm^{-1}

$[\alpha]_D^{27}$ +4.37 (c 1.2 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d_6) spectra of compound 3h to prove the rotamer.

Dimethyl (*tert*-butoxycarbonyl)-L-phenylalanylglycyl-L-glutamate



Boc-Phe-Gly-Glu(OMe)-OMe (3i)

3i was obtained in 32% yield as yellow solid (**mixture of two rotamers**). Purification by EtOAc/Hexane = 80%/20%

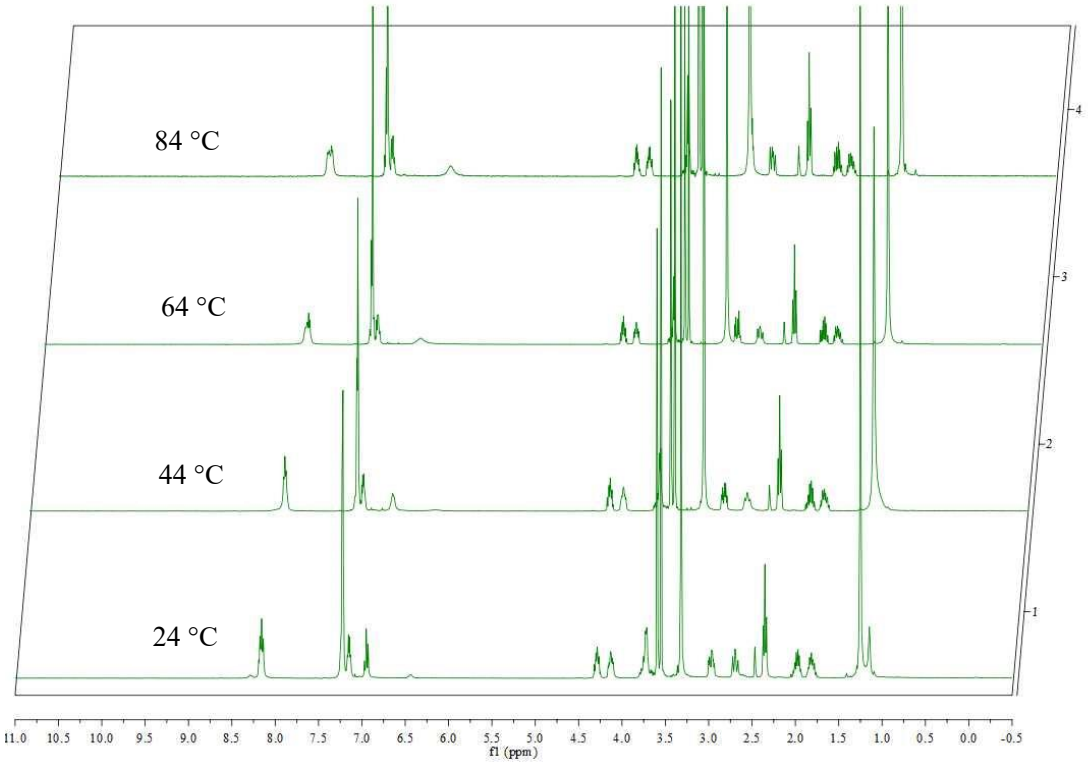
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.21 – 8.10 (m, 2H), 7.32 – 7.19 (m, 4H), 7.18 – 7.09 (m, 1H), 6.95 (t, *J* = 8.3 Hz, 1H), 4.34 – 4.23 (m, 1H), 4.17 – 4.08 (m, 1H), 3.82 – 3.68 (m, 2H), 3.60 and 3.59 (s, 3H), 3.55 (s, 3H), 3.02 – 2.91 (m, 1H), 2.69 (dd, *J* = 13.4, 10.9 Hz, 1H), 2.35 (t, *J* = 7.5 Hz, 2H), 1.98 (dt, *J* = 13.3, 7.7 Hz, 1H), 1.89 – 1.75 (m, 1H), 1.25 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 172.4, 172.4, 169.4, 155.7, 138.7, 129.6 (2C), 128.4 (2C), 126.5, 78.5, 56.1, 52.4, 51.8, 51.5, 42.2, 37.7, 29.9 and 29.9 (1C), 28.5 (3C), 26.6.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 502.2160; Found: 502.2159

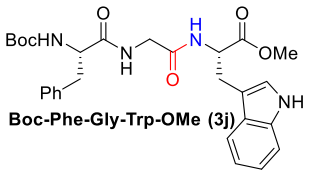
FT-IR (neat): 3310, 2979, 1740, 1659, 1526, 1439, 1367, 1251, 1170, 1022, 915, 734 cm^{-1}

$[\alpha]_D^{28}$ +5.29 (c 2.4 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d$_6$) spectra of compound 3i to prove the rotamer.

Methyl (*tert*-butoxycarbonyl)-L-phenylalanylglycyl-L-tryptophanate



Boc-Phe-Gly-Trp-OMe (3j)

3j was obtained in 51% yield as yellow solid (**mixture of two rotamers**). Purification by EtOAc/Hexane = 60%/40%

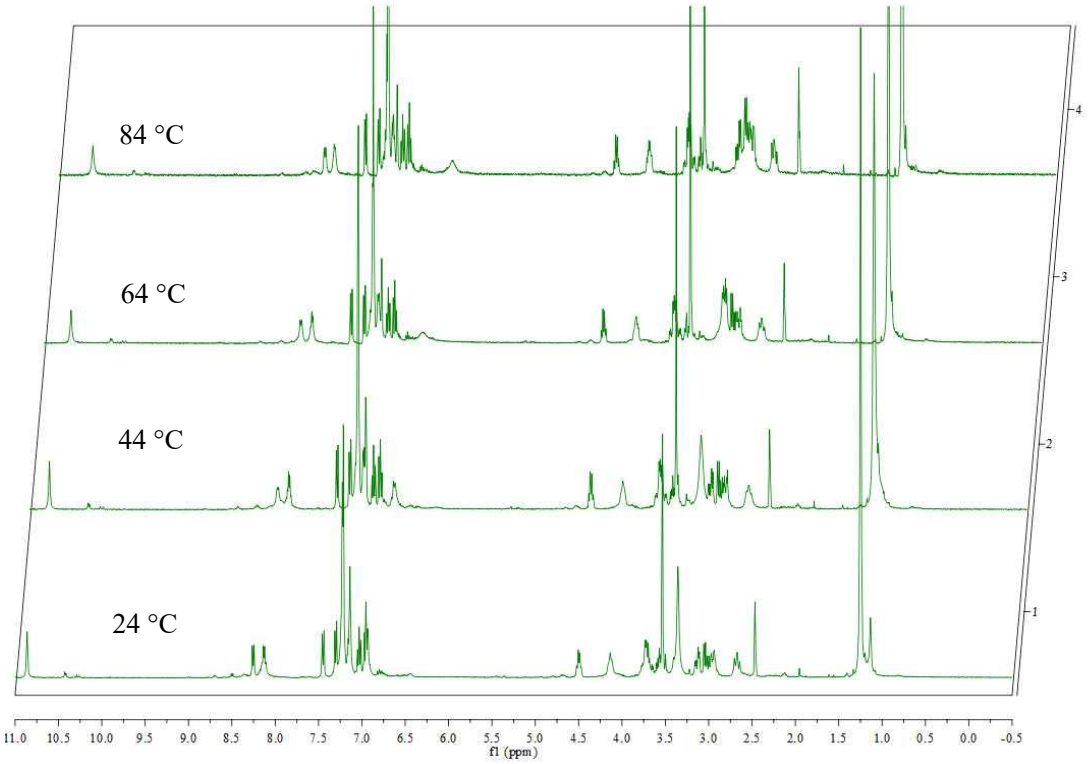
^{1}H NMR (399 MHz, DMSO-d$_6$) δ 10.82 (s, 1H), 8.21 (d, *J* = 7.4 Hz, 1H), 8.09 (d, *J* = 5.9 Hz, 1H), 7.44 (d, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 4.2 Hz, 4H), 7.16 – 7.09 (m, 2H), 7.02 (t, *J* = 7.4 Hz, 1H), 6.95 (t, *J* = 7.4 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 4.55 – 4.46 (m, 1H), 4.18 – 4.09 (m, 1H), 3.80 – 3.64 (m, 2H), 3.58 and 3.57 (s, 3H), 3.13 (dd, *J* = 14.5, 6.0 Hz, 1H), 3.07 – 2.90 (m, 2H), 2.76 – 2.63 (m, 1H), 1.24 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d$_6$) δ 172.5, 172.3, 169.1, 155.7, 138.7, 136.5, 129.6 (2C), 128.4 (2C), 127.5, 126.5, 124.2, 121.4, 118.9, 118.3, 111.9, 109.6 and 109.6 (1C), 78.5, 56.1, 53.6, 52.2, 42.2, 37.8, 28.5 (3C), 27.6.

HRMS (ESI) m/z: Calcd for $[M+H]^+$ 523.2551; Found: 523.2547

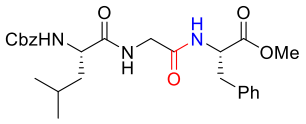
FT-IR (neat): 3308, 2917, 1662, 1523, 1456, 1367, 1251, 1168, 910, 735, 700 cm^{-1}

$[\alpha]_D^{24}$ +21.29 (c 3.6 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d_6) spectra of compound 3j to prove the rotamer.

Methyl ((benzyloxy)carbonyl)-L-leucylglycyl-L-phenylalaninate



Cbz-Leu-Gly-Phe-OMe (3k)

3k was obtained in 57% yield as yellow solid. Purification by EtOAc/Hexane = 60%/40%

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.38 – 7.17 (m, 8H), 7.14 – 7.04 (m, 2H), 6.99 – 6.90 (m, 1H), 6.85 (d, *J* = 7.2 Hz, 1H), 5.35 (d, *J* = 7.7 Hz, 1H), 5.14 – 4.97 (m, 2H), 4.83 (dd, *J* = 13.9, 6.3 Hz, 1H), 4.25 – 4.14 (m, 1H), 4.01 (dd, *J* = 17.0, 5.5 Hz, 1H), 3.81 (dd, *J* = 16.7, 4.6 Hz, 1H), 3.67 (s, 3H), 3.12 (dd, *J* = 13.8, 5.9 Hz, 1H), 3.05 (dd, *J* = 13.8, 6.5 Hz, 1H), 1.71 – 1.56 (m, 2H), 1.56 – 1.43 (m, 1H), 0.92 (d, *J* = 5.6 Hz, 6H).

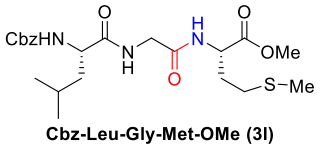
^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 171.7, 168.3, 156.3, 136.0, 135.7, 129.2 (2C), 128.6 (2C), 128.5 (2C), 128.2, 128.0 (2C), 127.1, 67.1, 53.4, 52.3, 42.9, 41.2, 37.8, 29.7, 24.6, 22.9, 21.8.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 506.2262; Found: 506.2261.

FT-IR (neat): 3303, 2956, 1658, 1531, 1455, 1218, 1043, 910, 733, 699 cm^{-1}

$[\alpha]_D^{29}$ +22.21 (c 1.8 $CHCl_3$)

Methyl ((benzyloxy)carbonyl)-L-leucylglycyl-L-methioninate



Cbz-Leu-Gly-Met-OMe (3l)

3l was obtained in 43% yield as yellow solid. Purification by EtOAc/Hexane = 50%/50%

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 5H), 7.25 – 7.00 (m, 2H), 5.58 (s, 1H), 5.15 – 4.99 (m, 2H), 4.72 – 4.63 (m, 1H), 4.25 – 4.15 (m, 1H), 4.10 (dd, *J* = 16.8, 5.4 Hz, 1H), 3.87 (dd, *J* = 16.8, 4.9 Hz, 1H), 3.69 (s, 3H), 2.57 – 2.42 (m, 2H), 2.21 – 2.09 (m, 1H), 2.05 (s, 3H), 1.98 (dt, *J* = 21.3, 7.2 Hz, 1H), 1.73 – 1.59 (m, 2H), 1.58 – 1.46 (m, 1H), 0.97 – 0.83 (m, 6H).

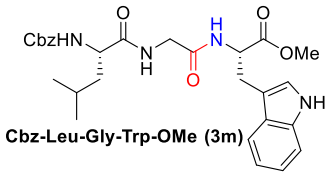
^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 172.2, 168.9, 156.4, 136.0, 128.5 (2C), 128.2, 128.0 (2C), 67.1, 53.8, 52.5, 51.5, 43.1, 41.1, 31.3, 29.9, 24.6, 22.9, 21.8, 15.4.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 490.1982; Found: 490.1982.

FT-IR (neat): 3301, 2956, 1660, 1536, 1439, 1238, 1172, 1121, 1042, 735, 698 cm^{-1}

$[\alpha]_D^{28}$ +11.58 (c 3.0 $CHCl_3$)

Methyl ((benzyloxy)carbonyl)-L-leucylglycyl-L-tryptophanate



Cbz-Leu-Gly-Trp-OMe (3m)

3m was obtained in 62% yield as yellow solid. Purification by EtOAc/Hexane = 60%/40%

^{1}H NMR (399 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.44 (d, *J* = 7.7 Hz, 1H), 7.33 – 7.21 (m, 6H), 7.18 – 7.07 (m, 3H), 7.07 – 7.00 (m, 1H), 6.93 (s, 1H), 5.58 (d, *J* = 6.9 Hz, 1H), 5.04 (t, *J* = 11.4 Hz, 1H), 4.90 (d, *J* = 12.3 Hz, 1H), 4.82 (dd, *J* = 12.9, 5.8 Hz, 1H), 4.23 – 4.11 (m, 1H), 3.86 (dd, *J* = 16.7, 5.4 Hz, 1H), 3.68 (dd, *J* = 16.3, 4.7 Hz, 1H), 3.57 (s, 3H), 3.28 – 3.18 (m, 2H), 1.67 – 1.49 (m, 2H), 1.46 – 1.36 (m, 1H), 0.94 – 0.73 (m, 6H).

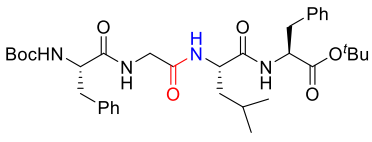
^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 172.2, 168.8, 156.5, 136.0, 136.0, 128.5 (2C), 128.1, 127.9 (2C), 127.2, 123.5, 121.9, 119.3, 118.2, 111.4, 109.2, 67.1, 53.6, 52.7, 52.4, 42.8, 41.1, 27.2, 24.6, 22.9, 21.7.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 545.2371; Found: 545.2365

FT-IR (neat): 3311, 2956, 1659, 1530, 1439, 1251, 1043, 910, 735 cm^{-1}

$[\alpha]_D^{29}$ +22.28 (c 3.6 $CHCl_3$)

***tert*-Butyl (*tert*-butoxycarbonyl)-L-phenylalanylglycyl-L-leucyl-L-phenylalaninate**



Boc-Phe-Gly-Leu-Phe-O*t*-Bu (3n)

3n was obtained in 67% yield as yellow solid. Purification by EtOAc/Hexane = 50%/50%

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.33 – 8.19 (m, 1H), 8.16 – 8.04 (m, 1H), 7.86 – 7.77 (m, 1H), 7.38 – 7.08 (m, 10H), 6.95 (dd, *J* = 15.3, 8.3 Hz, 1H), 4.39 – 4.24 (m, 2H), 4.13 (dd, *J* = 12.5, 6.2 Hz, 1H), 3.69 (s, 2H), 3.04 – 2.83 (m, 3H), 2.69 (dd, *J* = 13.6, 10.7 Hz, 1H), 1.54 (dd, *J* = 13.3, 6.7 Hz, 1H), 1.48 – 1.34 (m, 2H), 1.26 (s, 9H), 1.25 (s, 9H), 0.84 (d, *J* = 6.5 Hz, 3H), 0.81 (d, *J* = 6.5 Hz, 3H).

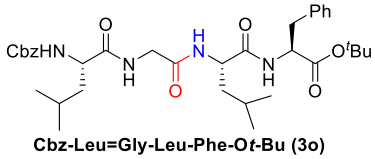
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 172.3, 170.7, 168.7, 155.7, 138.7, 137.6, 129.6(4C), 128.6 (2C), 128.4 (2C), 126.9, 126.6, 80.9, 78.5, 56.2, 54.6, 50.9, 42.4, 41.6, 37.8, 37.1, 28.6 (3C), 27.9 (3C), 24.5, 23.5, 22.1.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 661.3572; Found: 661.3570

FT-IR (neat): 3292, 2976, 1641, 1541, 1455, 1367, 1251, 1159, 1048, 910, 851, 734, 699 cm^{-1}

$[\alpha]_D^{28}$ +10.39 (c 2.6 $CHCl_3$)

***tert*-Butyl ((benzyloxy)carbonyl)-L-leucylglycyl-L-leucyl-L-phenylalaninate**



Cbz-Leu=Gly-Leu-Phe-O*t*-Bu (3o)

3o was obtained in 47% yield as yellow solid. Purification by EtOAc/Hexane = 60%/40%

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.39 – 7.26 (m, 4H), 7.24 – 7.09 (m, 5H), 7.03 (s, 1H), 6.94 (s, 1H), 5.69 (d, *J* = 6.6 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 4.98 (d, *J* = 12.2 Hz, 1H), 4.71 (dd, *J* = 14.2, 6.5 Hz, 1H), 4.59 – 4.46 (m, 1H), 4.22 (s, 1H), 4.01 (d, *J* = 12.8 Hz, 1H), 3.84 (d, *J* = 15.2 Hz, 1H), 3.10 – 2.92 (m, 2H), 1.75 – 1.43 (m, 6H), 1.34 (s, 9H), 1.10 – 0.62 (m, 12H).

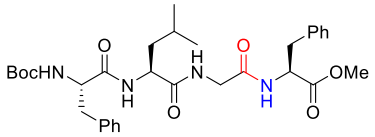
^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 171.6, 170.6, 168.8, 156.5, 136.2, 136.0, 129.5, 128.5, 128.2, 128.2, 128.0, 126.8, 82.4, 67.1, 53.8, 53.6, 51.9, 43.1, 41.2, 41.0, 38.1, 27.9 (3C), 24.7(2C), 22.9, 22.8, 22.0(2C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 661.3572; Found: 661.3573.

FT-IR (neat): 3286, 2956, 1639, 1539, 1368, 1233, 1155, 732, 697 cm^{-1}

$[\alpha]_D^{27}$ +14.55 (c 0.9 $CHCl_3$)

Methyl (*tert*-butoxycarbonyl)-L-phenylalanyl-L-leucylglycyl-L-phenylalaninate



Boc-Phe-Leu-Gly-Phe-OMe (3p)

3p was obtained in 50% yield as yellow solid (**mixture of two rotamers**). Purification by EtOAc/Hexane = 75%/25%

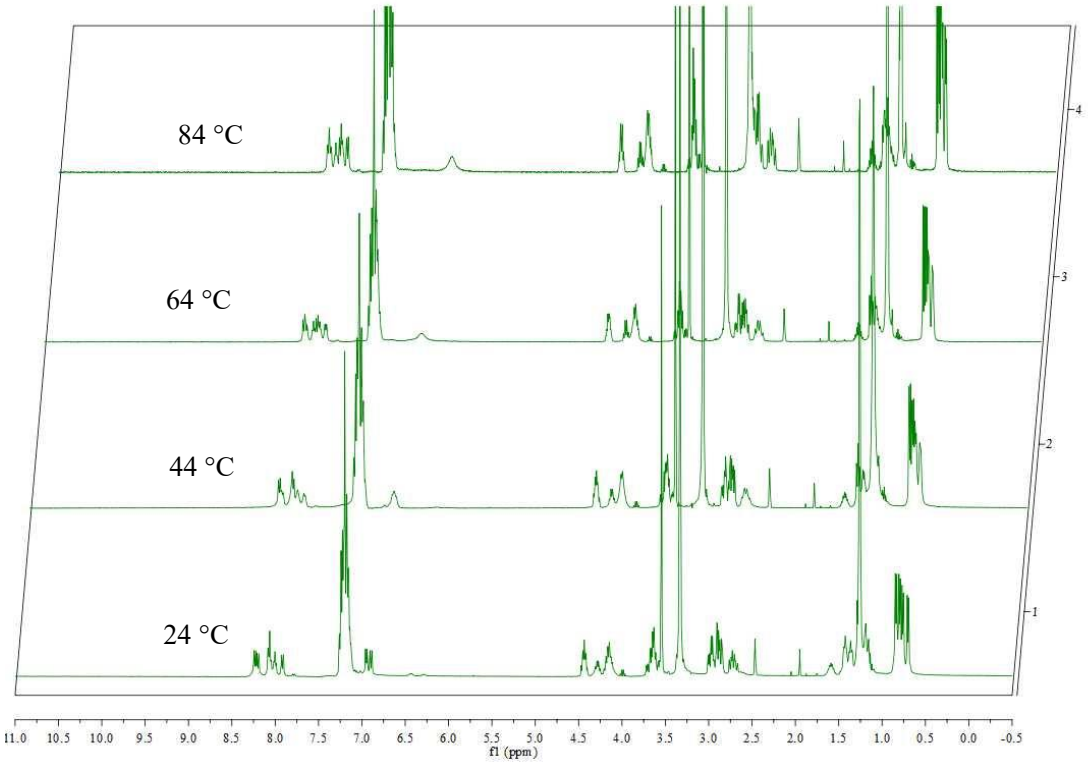
^{1}H NMR (399 MHz, DMSO-d_6) δ (8.23 (d, *J* = 7.6 Hz) and 8.20 (d, *J* = 7.6 Hz), 1H), 8.11 – 8.04 (m, 1H), 8.02 – 7.89 (m, 1H), 7.39 – 7.01 (m, 10H), (6.95 (d, *J* = 7.2 Hz) and 6.90 (d, *J* = 8.4 Hz), 1H), 4.49 – 4.39 (m, 1H), 4.34 – 4.08 (m, 2H), 3.76 – 3.60 (m, 2H), 3.54 (s, 3H), 3.02 – 2.82 (m, 3H), 2.77 – 2.65 (m, 1H), 1.72 – 1.53 (m, 1H), 1.40 (ddd, *J* = 14.7, 10.1, 3.5 Hz, 2H), 1.26 and 1.26 (s, 9H), (0.84 (d, *J* = 6.4 Hz) and 0.76 (d, *J* = 6.4 Hz), 3H), (0.80 (d, *J* = 6.4 Hz) and 0.70 (d, *J* = 6.4 Hz), 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 172.2 and 172.2 (1C), 172.0, 169.1 and 169.0 (1C), 155.8 and 155.7 (1C), 138.5 and 138.1 (1C), 137.4, 129.6 (2C), 129.5 (2C), 128.7 (2C), 128.4 (2C), 127.0, 126.6 and 126.5 (1C), 78.7 and 78.5 (1C), 56.4 and 56.1 (1C), 54.1 and 54.0 (1C), 52.3, 51.4 and 51.4 (1C), 41.9, 41.4 and 41.0 (1C), 37.9 and 37.5 (1C), 37.3, 28.5 and 28.5 (3C), 24.3 and 24.3 (1C), 23.6 and 23.5 (1C), 22.0 and 21.7 (1C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 619.3102; Found: 619.3096

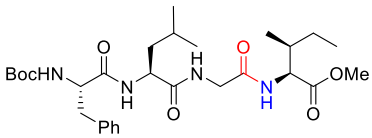
FT-IR (neat): 3287, 2956, 1642, 1528, 1455, 1367, 1250, 1171, 1030, 911, 734, 700 cm^{-1}

$[\alpha]_D^{23}$ +15.10 (c 0.7 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d$_6$) spectra of compound 3p to prove the rotamer.

Methyl (*tert*-butoxycarbonyl)-L-phenylalanyl-L-leucylglycyl-L-isoleucinate



Boc-Phe-Leu-Gly-Ile-OMe (3q)

3q was obtained in 53% yield as yellow solid (**mixture of two rotamers**). Purification by EtOAc/Hexane = 70%/30%

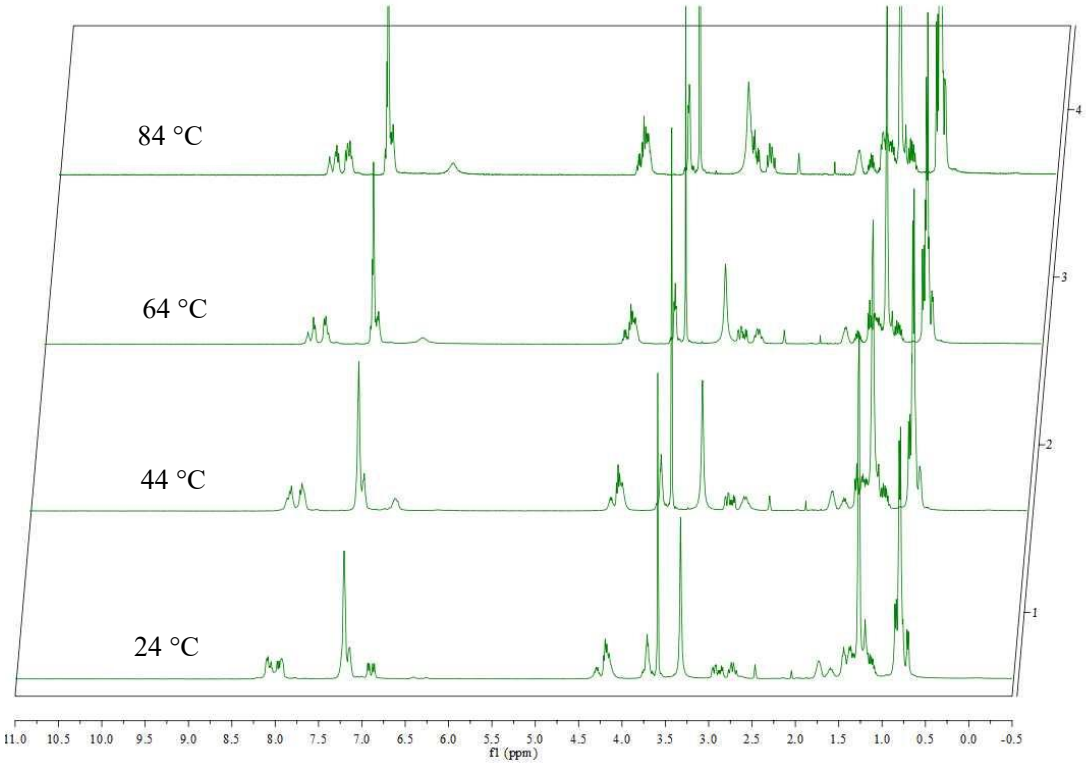
^{1}H NMR (399 MHz, DMSO-d$_6$) δ 8.13 – 7.85 (m, 1H), 8.15 – 7.86 (m, 1H), 8.13 – 7.85 (m, 1H), 7.33 – 7.06 (m, 5H), (6.92 (d, *J* = 7.2 Hz) and 6.86 (d, *J* = 8.4 Hz), 1H), 4.39 – 4.25 (m, 1H), 4.23 – 4.06 (m, 1H), 4.23 – 4.06 (m, 1H), 3.82 – 3.64 (m, 2H), 3.59 (s, 3H), 2.97 – 2.83 (m, 1H), 2.78 – 2.65 (m, 1H), 1.79 – 1.67 (m, 1H), 1.65 – 1.53 (m, 1H), 1.27 and 1.26 (s, 9H), 1.51 – 1.01 (m, 4H), 0.97 – 0.44 (m, 12H).

^{13}C NMR (100 MHz, DMSO-d$_6$) δ 172.7 and 172.6 (1C), 172.3, 172.0 and 172.0 (1C), 169.2 and 169.2 (1C), 155.7 and 155.6 (1C), 138.5 and 138.0 (1C), 129.6 (2C), 128.4 (2C), 126.6 and 126.5 (1C), 78.6 and 78.5 (1C), 56.7, 56.4 and 56.1 (1C), 52.1, 51.4, 42.1, 41.5 and 41.0 (1C), 37.9 and 37.5 (1C), 36.9 and 36.8 (1C), 28.5 and 28.5 (3C), 25.1, 24.3 and 24.3 (1C), 23.5, 21.9 and 21.7 (1C), 15.8 and 15.8 (1C), 11.5 and 11.5 (1C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 585.3259; Found: 585.3254

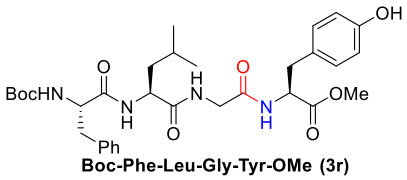
FT-IR (neat): 3289, 2961, 1646, 1530, 1367, 1251, 1170, 911, 734 cm^{-1}

$[\alpha]_D^{26}$ +2.47 (c 0.8 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d$_6$) spectra of compound 3q to prove the rotamer.

Methyl (*tert*-butoxycarbonyl)-L-phenylalanyl-L-leucylglycyl-L-tyrosinate



Boc-Phe-Leu-Gly-Tyr-OMe (3r)

3r was obtained in 51% yield as yellow solid (**mixture of two rotamers**). Purification by EtOAc/Hexane = 70%/30%

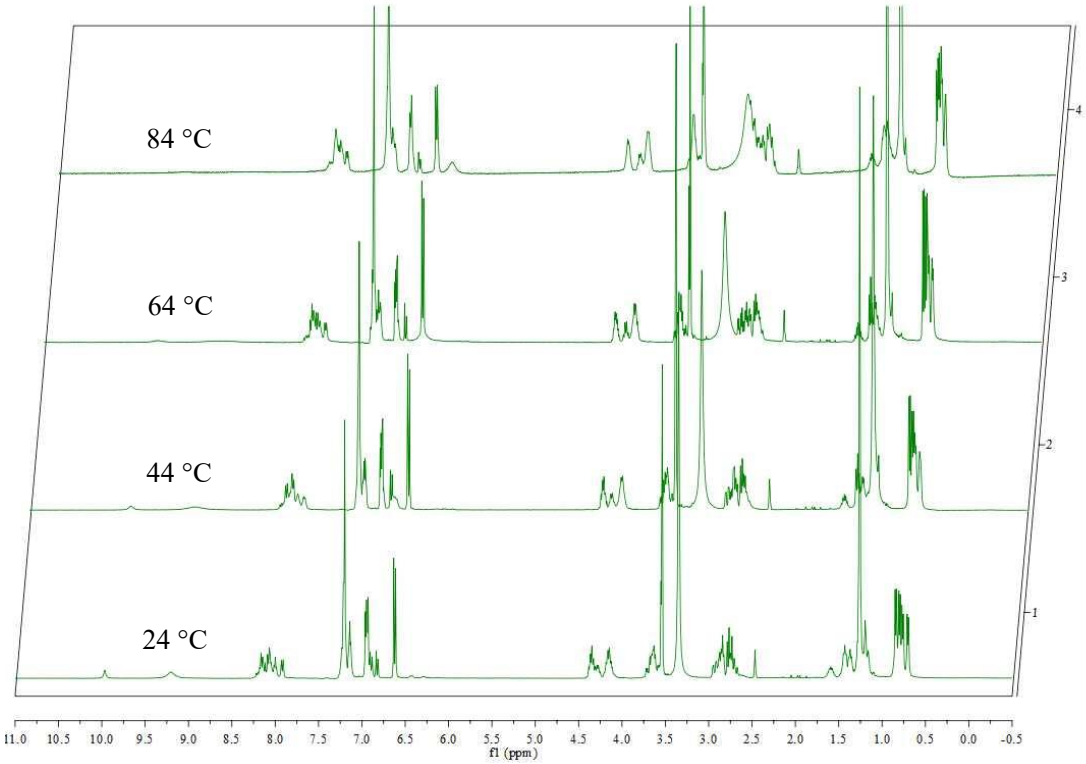
^{1}H NMR (399 MHz, DMSO-d$_6$) δ 9.97 and 9.21 (s, 1H), 8.24 – 8.04 (m, 1H), 8.24 – 8.04 (m, 1H), 8.02 – 7.86 (m, 1H), 7.33 – 7.07 (m, 5H), 7.02 – 6.78 (m, 2H), 7.00 – 6.77 (m, 1H), 6.66 – 6.26 (m, 2H), 4.41 – 4.08 (m, 1H), 4.41 – 4.08 (m, 1H), 4.41 – 4.08 (m, 1H), 3.77 – 3.60 (m, 2H), 3.54 (s, 3H), 2.98 – 2.82 (m, 2H), 2.80 – 2.63 (m, 2H), 1.67 – 1.54 (m, 1H), 1.47 – 1.34 (m, 2H), 1.26 (s, 9H), (0.84 (d, *J* = 6.4 Hz) and 0.76 (d, *J* = 6.4 Hz), 3H), (0.80 (d, *J* = 6.8 Hz) and 0.70 (d, *J* = 6.0 Hz), 3H).

^{13}C NMR (100 MHz, DMSO-d$_6$) δ 172.6 and 172.4 (1C), 172.3 and 172.0 (1C), 169.0 and 169.0 (1C), 156.4 and 152.1 (1C), 155.8 and 155.7 (1C), 138.5 and 138.1 (1C), 130.7 and 130.4 (2C), 129.6 and 129.1 (2C), 128.4 (2C), 127.4 and 127.3 (1C), 126.6 and 126.5 (1C), 119.7 and 116.9 (1C), 115.5 (2C), 78.7 and 78.5 (1C), 56.4 and 56.1 (1C), 54.4 and 54.4 (1C), 52.3 and 52.2 (1C), 51.4 and 51.4 (1C), 41.9, 41.4 and 41.0 (1C), 37.9 and 37.5 (1C), 36.6 and 36.1 (1C), 28. and, 28.5 (3C), 24.3 and 24.3 (1C), 23.6 and 23.5 (1C), 21.9 and 21.7 (1C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 635.3051; Found: 635.3047.

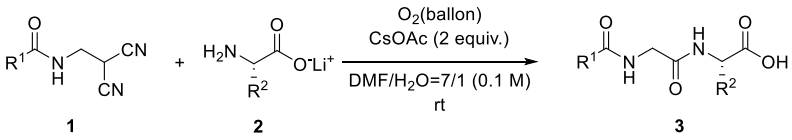
FT-IR (neat): 3296, 2959, 1652, 1517, 1367, 1259, 1170, 910, 801, 735 cm^{-1}

$[\alpha]_D^{18}$ +16.29 (c 1.6 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d_6) spectra of compound 3r to prove the rotamer.

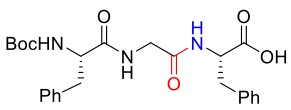
2.5 Typical procedure of oxidative peptide synthesis using unprotected amino acid and physical information



1 **2** **3**

To a mixture of the amino acid lithium salt **2** (0.4 mmol) in DMF/H_2O=7/1 (2 mL) was added CsOAc (76.8 mg, 0.4 mmol). The mixture was stirred and then was filled with O_2. The dicyano compound **1** (0.2 mmol) was then added and stirred at room temperature. The mixture was then diluted by EtOAc and quenched with 1N HCl and extracted with EtOAc/^{t}BuOH=10/1. The organic phase was dried over Na_2SO_4 and concentrated using air blower under 80 °C and then concentrated in vacuo. The crude product was purified by silica gel column chromatography, eluted with CH_2Cl_2/MeOH containing 0.5% acetic acid.

(*tert*-Butoxycarbonyl)-L-phenylalanylglycyl-L-phenylalanine



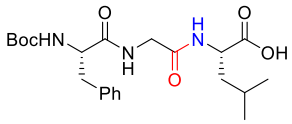
Boc-Phe-Gly-Phe-OH (3s)

3s was obtained in 67% yield as white solid. Purification by CH_2Cl_2/MeOH = 91%/9% (0.5% acetic acid was added to the eluent).

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.18 – 8.02 (m, 2H), 7.34 – 7.06 (m, 10H), 6.95 – 6.83 (m, 1H), 4.47 – 4.35 (m, 1H), 4.19 – 4.07 (m, 1H), 3.81 – 3.55 (m, 2H), 3.02 (dd, *J* = 13.8, 5.0 Hz, 1H), 2.98 – 2.91 (m, 1H), 2.85 (dd, *J* = 13.7, 8.9 Hz, 1H), 2.67 (dd, *J* = 13.3, 10.9 Hz, 1H), 1.25 (s, 9H).
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.1, 172.3, 169.0, 155.7, 138.7, 137.8, 129.6 (2C), 129.5 (2C), 128.6 (2C), 128.4 (2C), 126.9, 126.5, 78.5, 56.1, 53.9, 42.1, 37.9, 37.3, 28.6 (3C).
HRMS (ESI) m/z: Calcd for $[M+H]^+$ 470.2286; Found: 470.2284.
FT-IR (neat): 3406, 2499, 2073, 1656, 1456, 1165, 1119, 976, 735, 701 cm^{-1}

$[\alpha]_D^{25}$ +15.13 (c 1.1 DMSO)

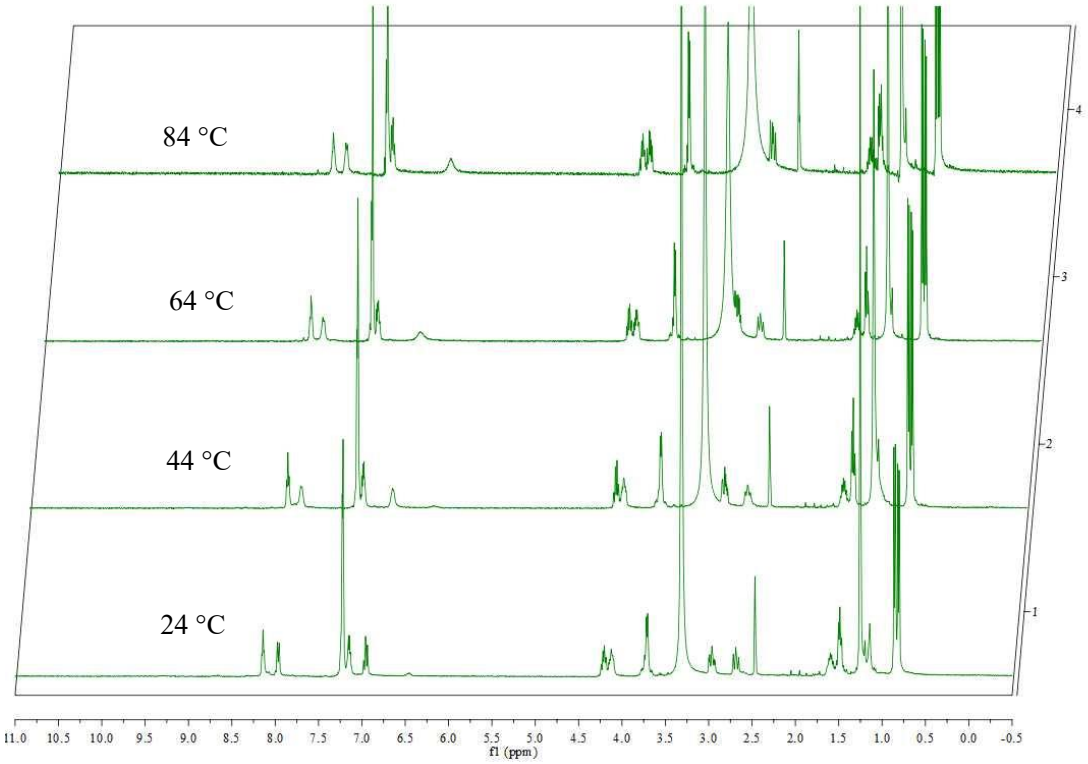
(*tert*-Butoxycarbonyl)-L-phenylalanylglycyl-L-leucine



Boc-Phe-Gly-Leu-OH (3t)

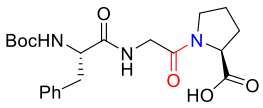
3t was obtained in 64% yield as yellow solid (**mixture of two rotamers**). Purification by CH_2Cl_2/MeOH = 92%/8% (0.5% acetic acid was added to the eluent).
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.13 (t, *J* = 5.6 Hz, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.27 – 7.19 (m, 4H), 7.18 – 7.12 (m, 1H), 6.95 (t, *J* = 8.5 Hz, 1H), 4.26 – 4.09 (m, 2H), 3.80 – 3.69 (m, 2H), 3.02 – 2.92 (m, 1H), 2.69 (dd, *J* = 13.5, 11.0 Hz, 1H), 1.66 – 1.44 (m, 3H), 1.26 (s, 9H), 0.86 (d, *J* = 6.5 Hz, 3H), 0.82 (d, *J* = 6.4 Hz, 3H).
^{13}C NMR (100 MHz, DMSO-d_6) δ 174.3, 172.3, 169.0, 155.7 and 155.8 (1C), 138.7, 129.6 (2C), 128.4 (2C), 126.5, 78.5, 56.1, 50.6, 42.2, 37.7, 28.6 (3C), 24.6, 23.2 and 23.3 (1C), 21.8 and 21.8 (2C).
HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 458.2262; Found: 458.2260.
FT-IR (neat): 3310, 2956, 2359, 1651, 1455, 1393, 1366, 1251, 1164, 699, 578 cm^{-1}

$[\alpha]_D^{25}$ +9.07 (c 0.83 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d$_6$) spectra of compound 3t to prove the rotamer.

(*tert*-Butoxycarbonyl)-L-phenylalanylglycyl-L-proline



Boc-Phe-Gly-Pro-OH (3u)

3u was obtained in 66% yield as yellow solid (**mixture of two rotamers**). Purification by CH_2Cl_2/MeOH = 91%/9%.

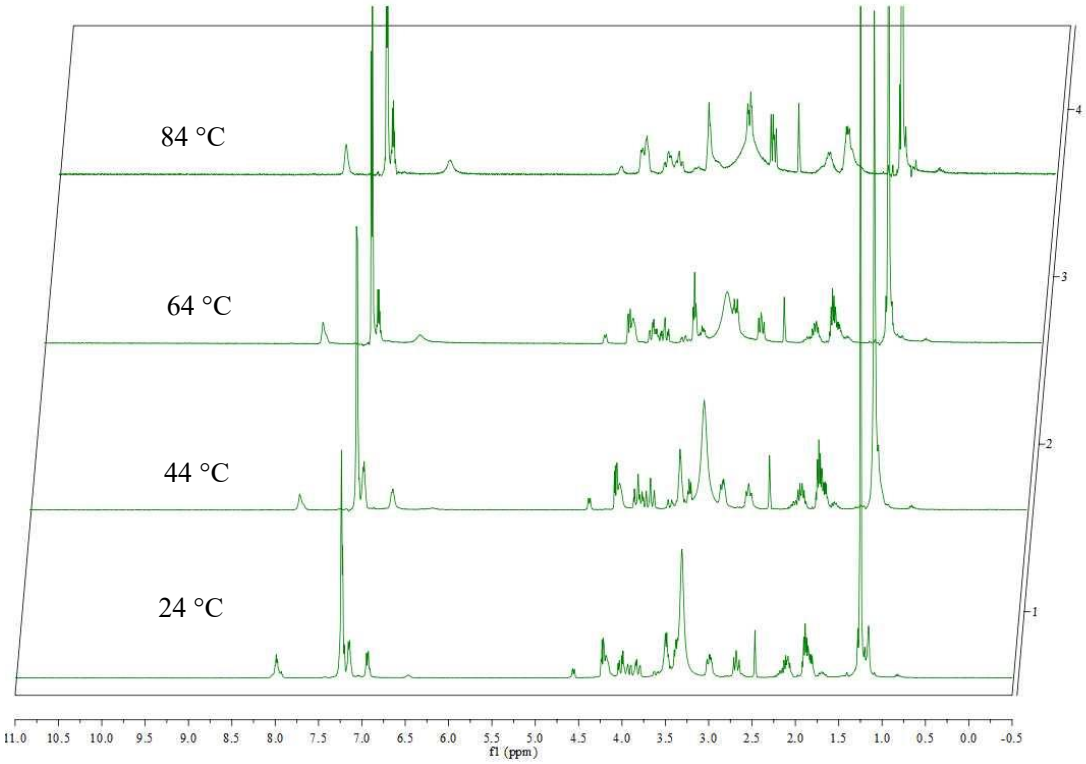
^{1}H NMR 44 °C (399 MHz, DMSO-d$_6$) δ 7.89 (s, 1H), 7.35 – 7.03 (m, 5H), 6.81, 6.35 (s, 1H), 4.59 – 4.51 and 4.28 – 4.12 (m, 1H), 4.28 – 4.12 (m, 1H), 4.05 – 3.77 and 3.65 – 3.57 (m, 2H), 3.56 – 3.37 (m, 2H), 3.02 (dd, *J* = 13.5, 3.9 Hz, 1H), 2.71 (dd, *J* = 12.8, 11.2 Hz, 1H), 2.30 – 1.98 and 1.75 – 1.64 (m, 2H), 1.95 – 1.78 (m, 2H), 1.26 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d$_6$) δ 173.9 and 173.6 (1C), 172.3 and 172.2 (1C), 167.4 and 167.1 (1C), 155.7, 138.8 and 138.7 (1C), 129.6 (2C), 128.4 (2C), 126.5, 78.4, 59.0 and 58.4 (1C), 56.1, 46.7 and 45.9 (1C), 41.6 and 41.5 (1C), 37.9, 31.2 and 29.1 (1C), 28.5 and 28.2 (3C), 24.7 and 22.3 (1C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 442.1949; Found: 442.1950

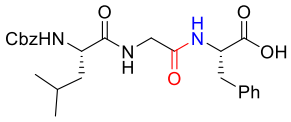
FT-IR (neat): 2918, 2370, 1637, 1456, 1366, 1168, 1022, 753, 700 cm^{-1}

$[\alpha]_D^{24}$ -33.54 (c 0.6 $CHCl_3$)



Variable temperature ^{1}H NMR (399 Hz, DMSO-d$_6$) spectra of compound 3u to prove the rotamer.

((Benzyloxy)carbonyl)-L-leucylglycyl-L-phenylalanine



Cbz-Leu-Gly-Leu-OH (3v)

3v was obtained in 55% yield as yellow solid. Purification by CH_2Cl_2/MeOH = 91%/9%.

^{1}H NMR (399 MHz, DMSO-d$_6$) δ 8.09 (t, *J* = 5.6 Hz, 1H), 8.02 (d, *J* = 7.9 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.37 – 6.98 (m, 10H), 4.97 (s, 2H), 4.45 – 4.33 (m, 1H), 4.06 – 3.93 (m, 1H), 3.65 (dd, *J* = 16.8, 4.4 Hz, 2H), 3.01 (dd, *J* = 13.8, 5.1 Hz, 1H), 2.84 (dd, *J* = 13.7, 8.8 Hz, 1H), 1.65 – 1.50 (m, 1H), 1.48 – 1.32 (m, 2H), 0.83 (d, *J* = 7.0 Hz, 3H), 0.81 (d, *J* = 6.9 Hz, 3H).

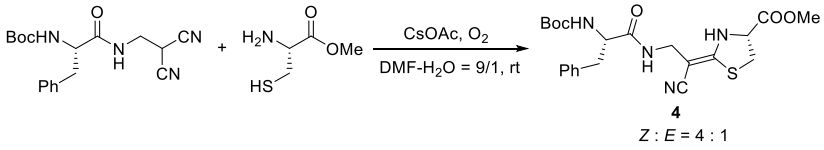
^{13}C NMR (100 MHz, DMSO-d$_6$) δ 173.1, 173.0, 169.0, 156.5, 137.8, 137.4, 129.5 (2C), 128.7 (2C), 128.6 (2C), 128.2 (2C), 128.1, 126.9, 65.8, 53.9, 53.6, 42.1, 41.0, 37.3, 24.6, 23.4, 21.8 (2C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$ 492.2105; Found: 492.2102.

FT-IR (neat): 3324, 2957, 2492, 1655, 1533, 1455, 1256, 1026, 951, 700 cm^{-1}

$[\alpha]_D^{26}$ +5.73 (c 0.47 DMSO)

3. Procedure towards the thiazolidine formation between the dicyano compound and cysteine methyl ester and physical information

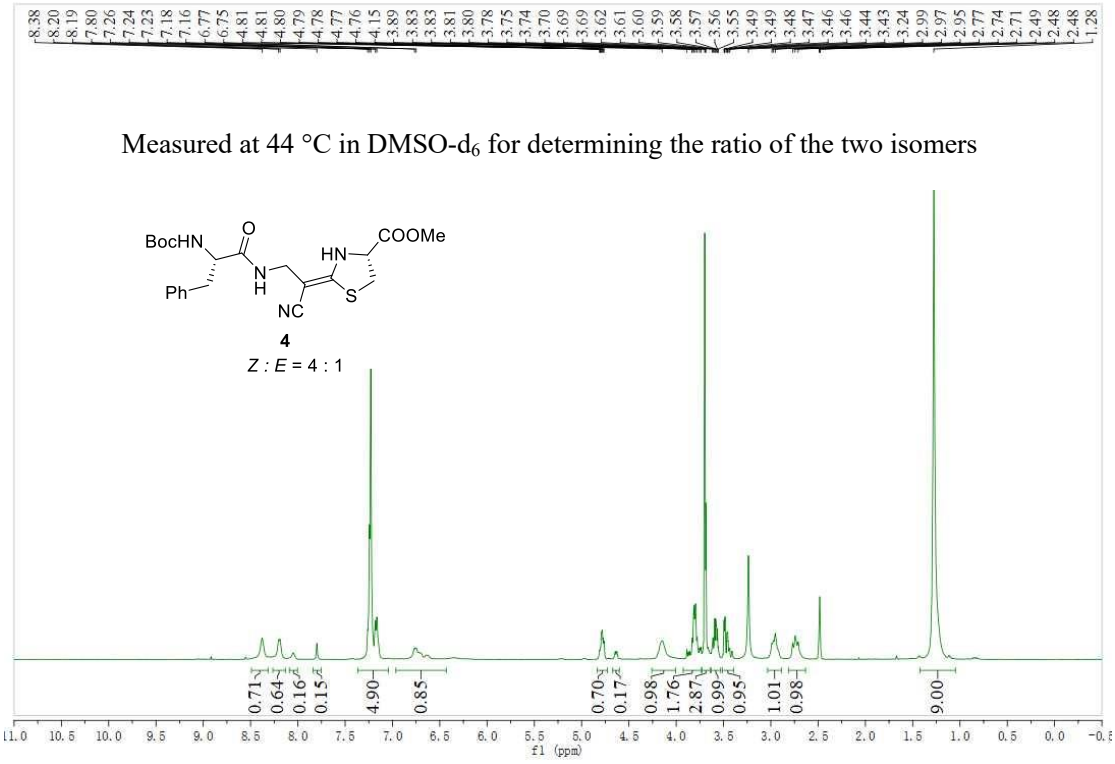


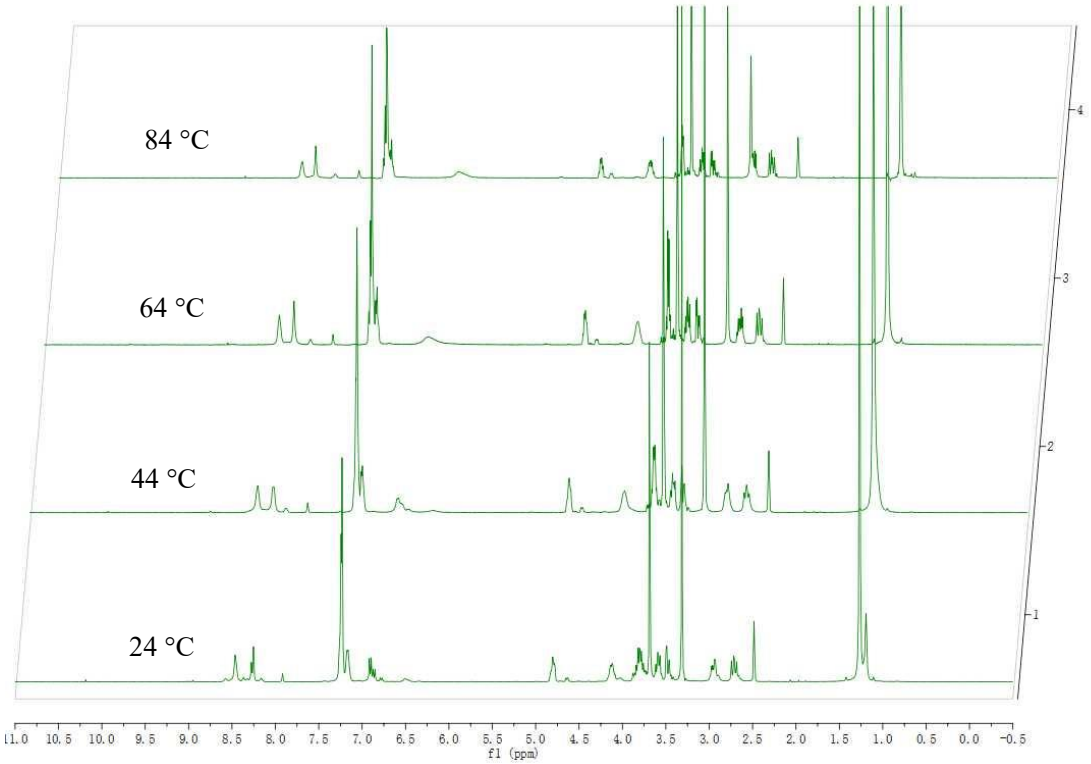
4

Z : *E* = 4 : 1

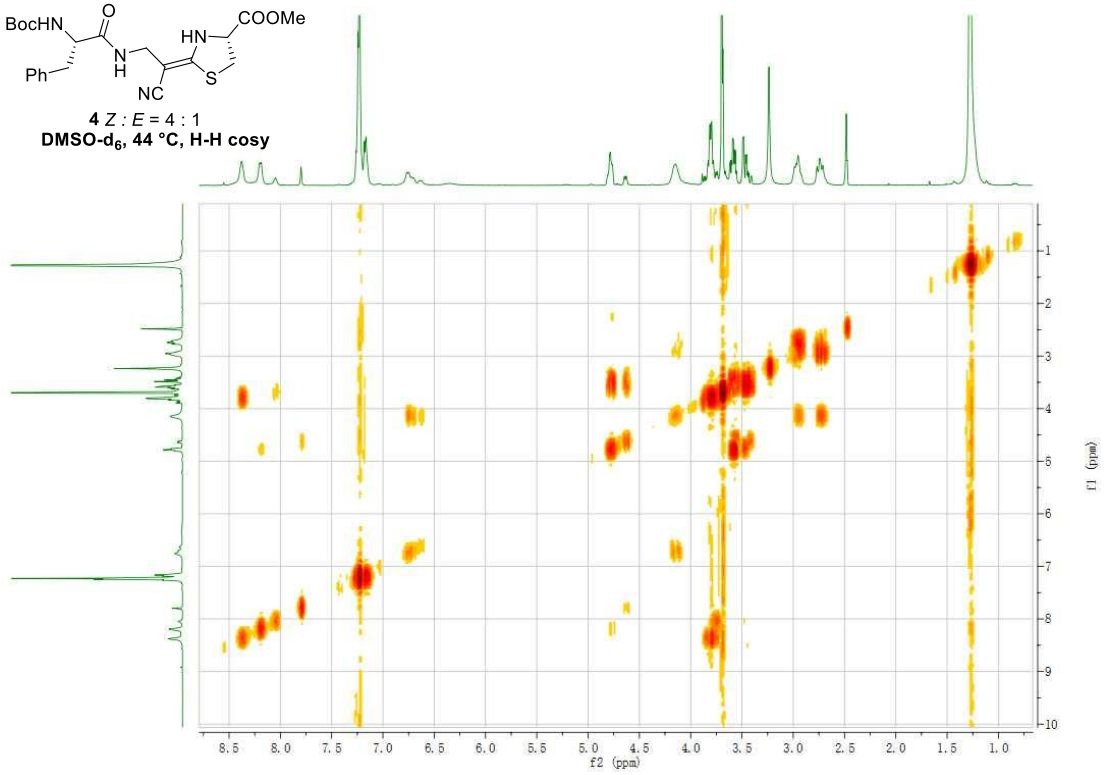
The reaction was conducted according to the general procedure in 0.2 mmol scale by using DMF/H_2O in 9/1 ratio as solvent. **4** was obtained in 90% yield. **4** existed as a mixture of isomers and rotamers at room temperature. The ratio of two isomers was determined at 44 ºC in DMSO-d_6. The NMR data of **4** was shown below.

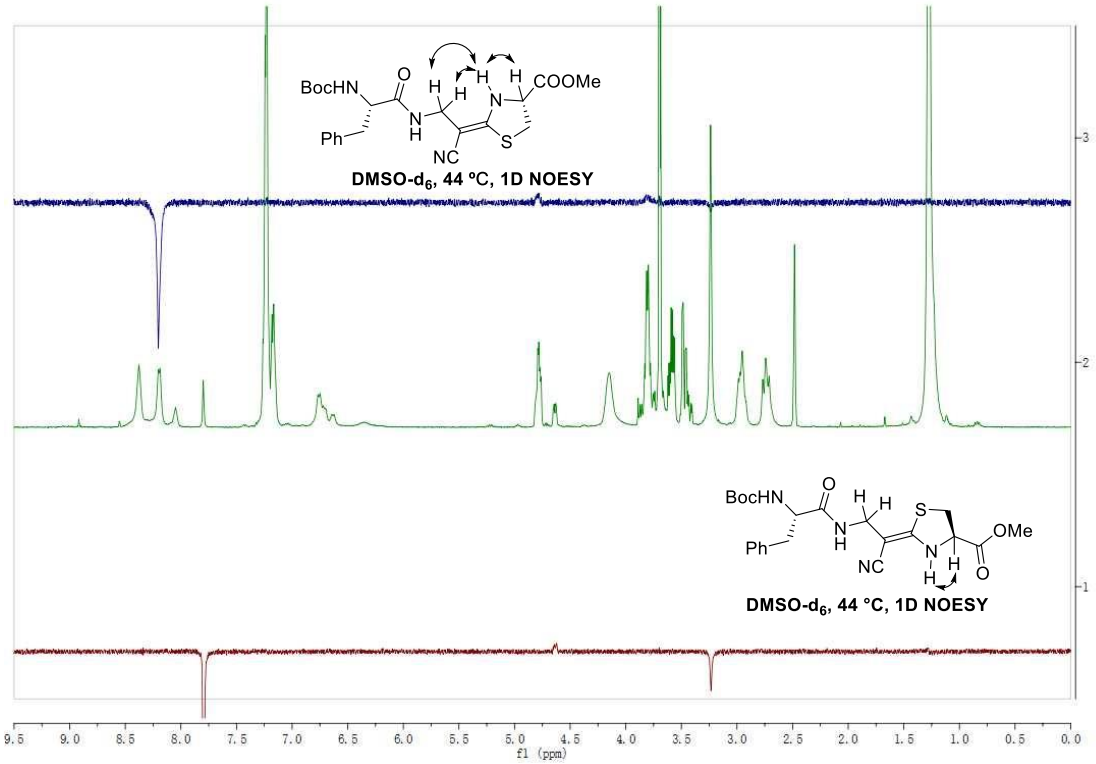
^{1}H NMR (399 MHz, DMSO-d_6) (**Measured at 44 ºC**) δ 8.38 (s, 0.8H), 8.20 (d, *J* = 6.2 Hz, 0.8H), 8.05 (s, 0.2H), 7.80 (s, 0.2H), 7.37 – 7.04 (m, 5H), 6.97 – 6.44 (m, 1H), 4.83 – 4.73 (m, 0.8H), 4.66 – 4.61 (m, 0.2H), 4.15 (s, 1H), 3.92 – 3.73 (m, 2H), 3.70 and 3.69 and 3.69 (s, 3H), 3.63 – 3.53 (m, 1H), 3.51 – 3.40 (m, 1H), 3.03 – 2.89 (m, 1H), 2.81 – 2.63 (m, 1H), 1.28 (s, 9H).
^{1}H NMR (399 MHz, $CDCl_3$) δ 8.17 and 8.09 (s, 1H), 7.36 – 7.12 (m, 5H), (7.09 (t, *J* = 6.4 Hz) and 6.94 (t, *J* = 6.5 Hz), 1H), 5.18 – 4.95 (m, 1H), 4.70 – 4.55 (m, 1H), 4.48 – 4.25 (m, 1H), 4.03 – 3.83 (m, 2H), 3.80 and 3.77 (s, 3H), 3.63 – 3.43 (m, 2H), 3.15 – 2.91 (m, 2H), 1.39 and 1.37 (s, 9H).
^{13}C NMR of major isomer (100 MHz, $CDCl_3$) δ 173.4, 170.2, 165.4, 155.2, 136.3, 129.3, 128.6, 127.0, 122.4, 80.2, 70.4, 62.7, 55.6, 53.1, 38.8, 38.1, 33.5, 28.3.
HRMS (ESI) m/z: Calcd for $C_{22}H_{28}N_4NaO_5S^+$ $[M+Na]^+$: 483.1673; found: 483.1671.
FT-IR (neat): 3308, 2918, 2186, 1744, 1706, 1653, 1591, 1522, 1366, 1248, 1169, 1020, 701 cm^{-1}

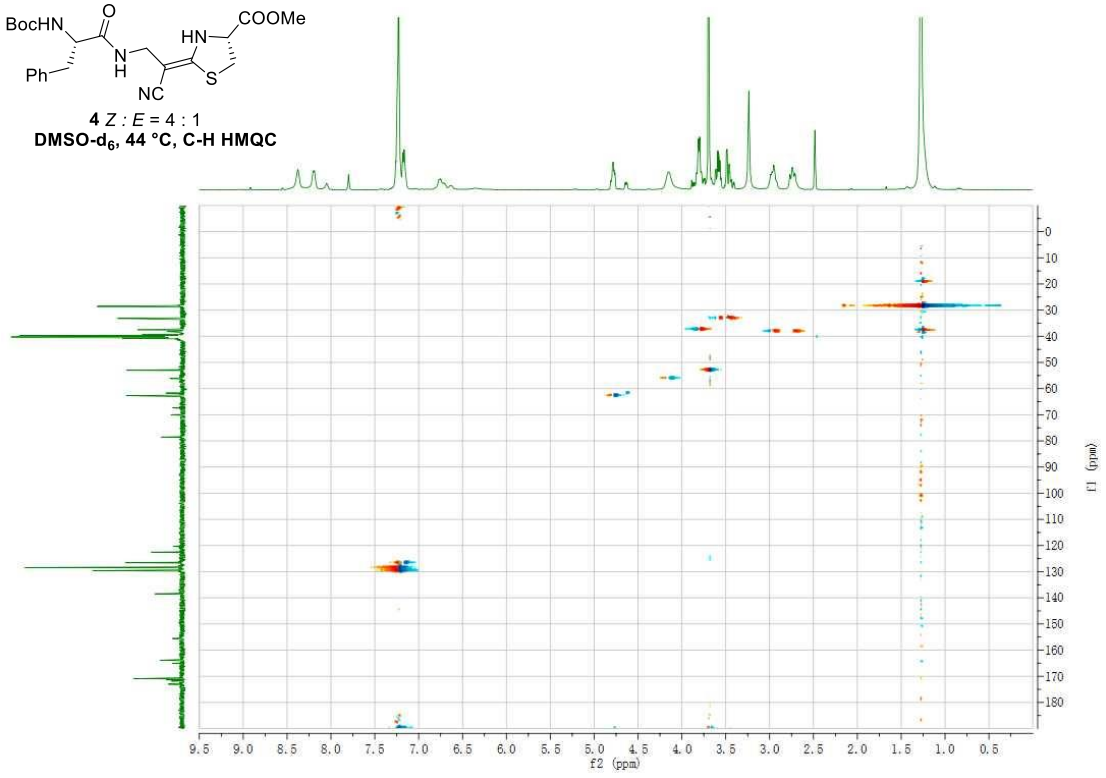




Variable temperature ^{1}H NMR to prove the rotamers of 4




BocHN
O
H
H
H
H
COOMe
N
N
H
Ph
NC
S
DMSO-d6, 44 °C, 1D NOESY
BocHN
O
H
H
S
N
H
OMe
Ph
NC
N
H
H
O
DMSO-d6, 44 °C, 1D NOESY
f1 (ppm)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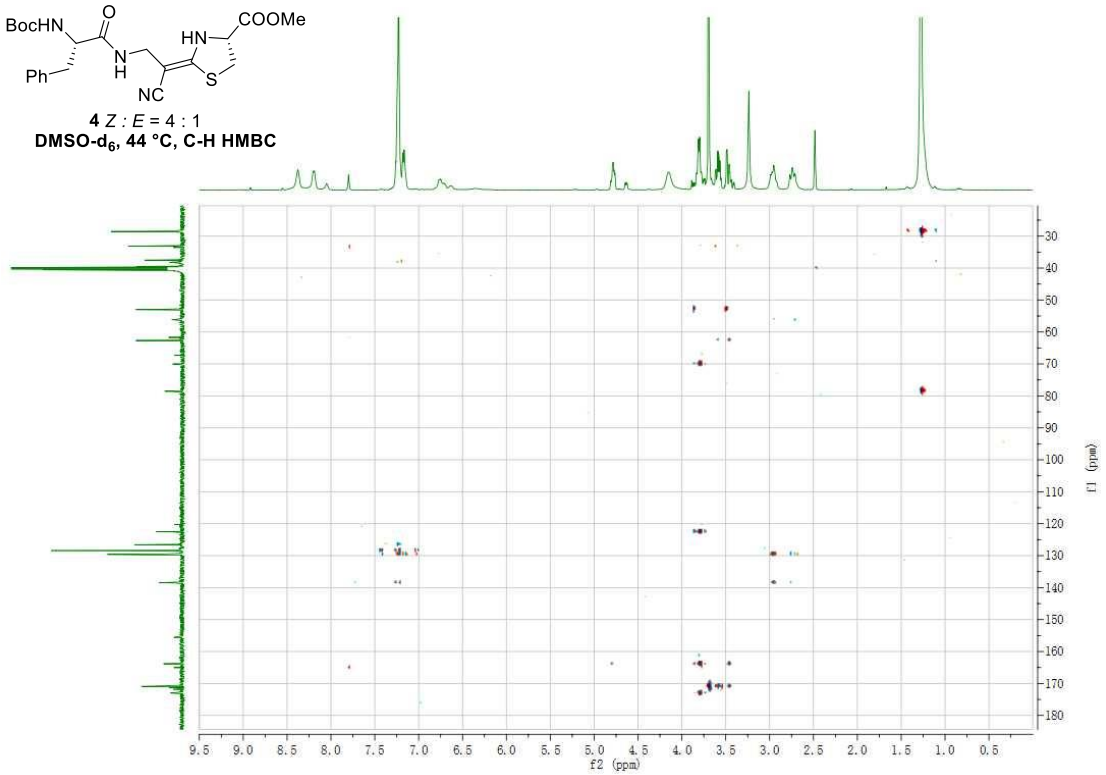
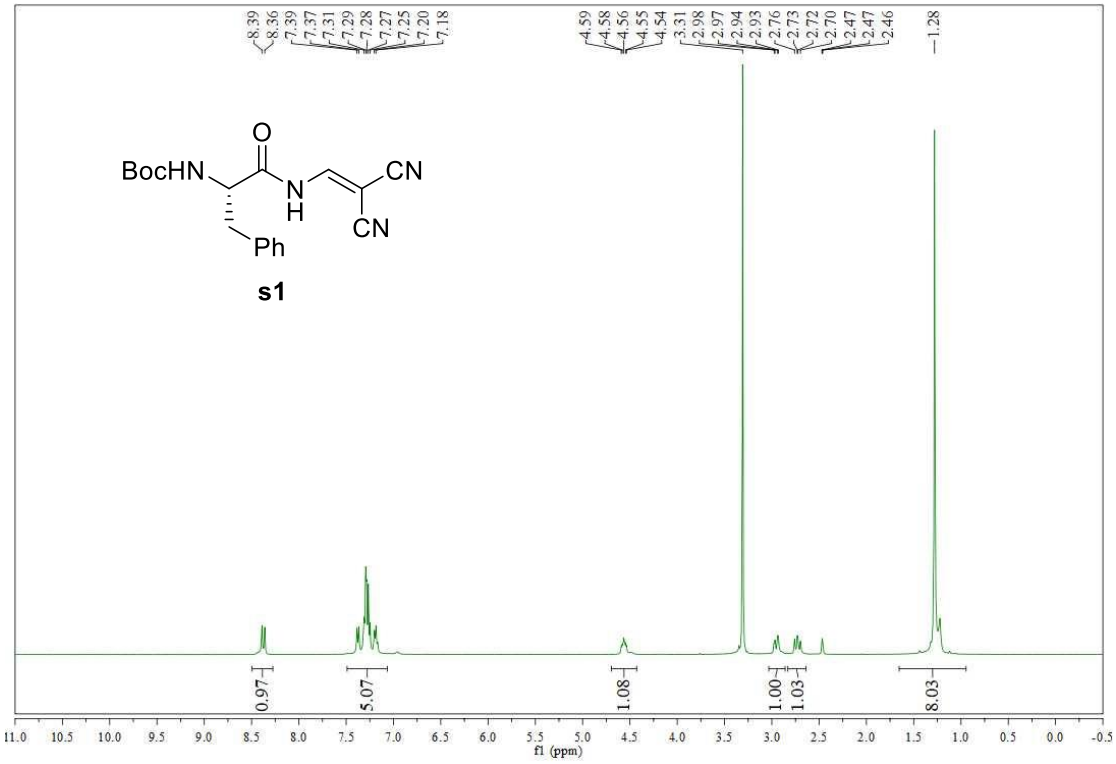
HN
N
H
Ph
NC
S
4 Z : E = 4 : 1
DMSO-d6, 44 °C, C-H HMQC
f2 (ppm)
f1 (ppm)


BocHN
O
N
H
HN
COOMe
Ph
NC
S
4 Z : E = 4 : 1
DMSO-d6, 44 °C, C-H HMBC
f2 (ppm)
f1 (ppm)

4. Reference

1. (a) C. B. Mishra, R. K. Mongre, S. Kumari, D. K. Jeong and M. Tiwari, *RSC Adv.*, 2016, **6**, 24491; (b) S. M. Schmitt, K. Stefan and M. Wiese, *J. Med. Chem.*, 2016, **59**, 3018.
2. S. Kokinaki, L. Leondiadis and N. Ferderigos, *Org. Lett.*, 2005, **7**, 1723.

5. Spectra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7.39
7.37
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7.29
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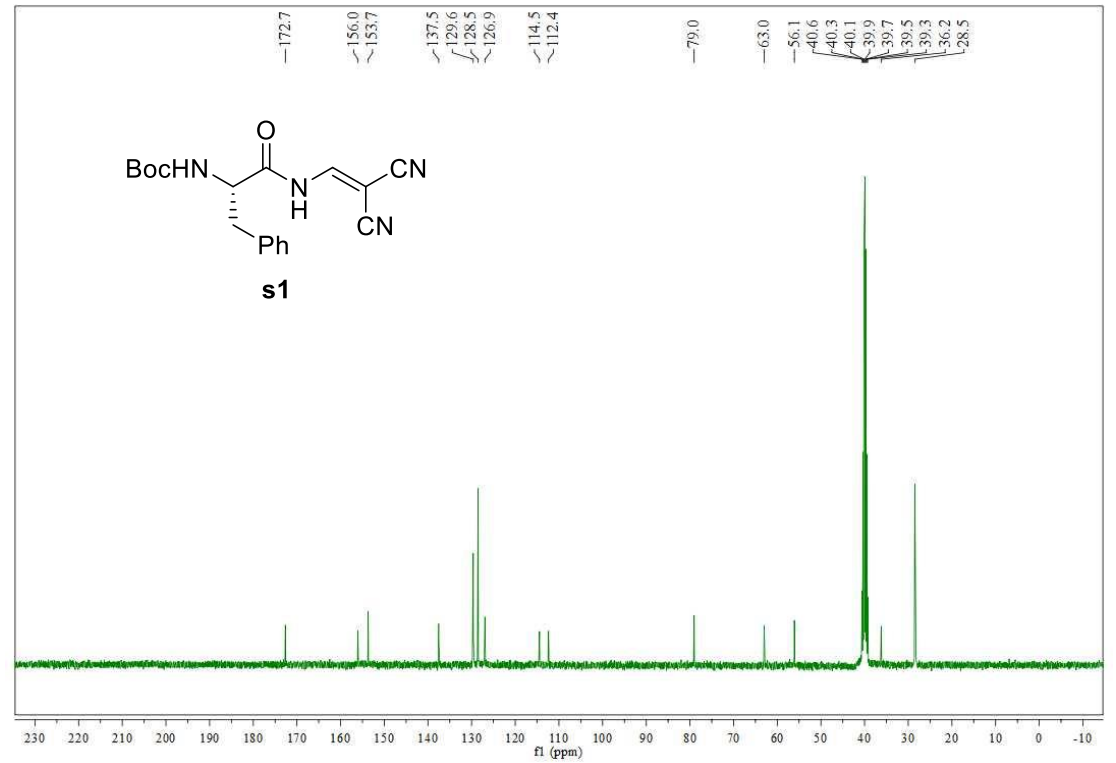
7.27
7.25
7.20
7.18
4.59
4.58
4.56
4.55
4.54
3.31
2.98
2.97
2.94
2.93
2.76
2.73
2.72
2.70
2.47
2.47
2.46
1.28
BocHN
O
N
H
CN
CN
Ph
s1
0.97
5.07
1.08
1.00
1.03
8.03
f1 (ppm)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153.7
137.5
129.6
128.5
126.9
114.5
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79.0
63.0
56.1
40.6
40.3
40.1
39.9
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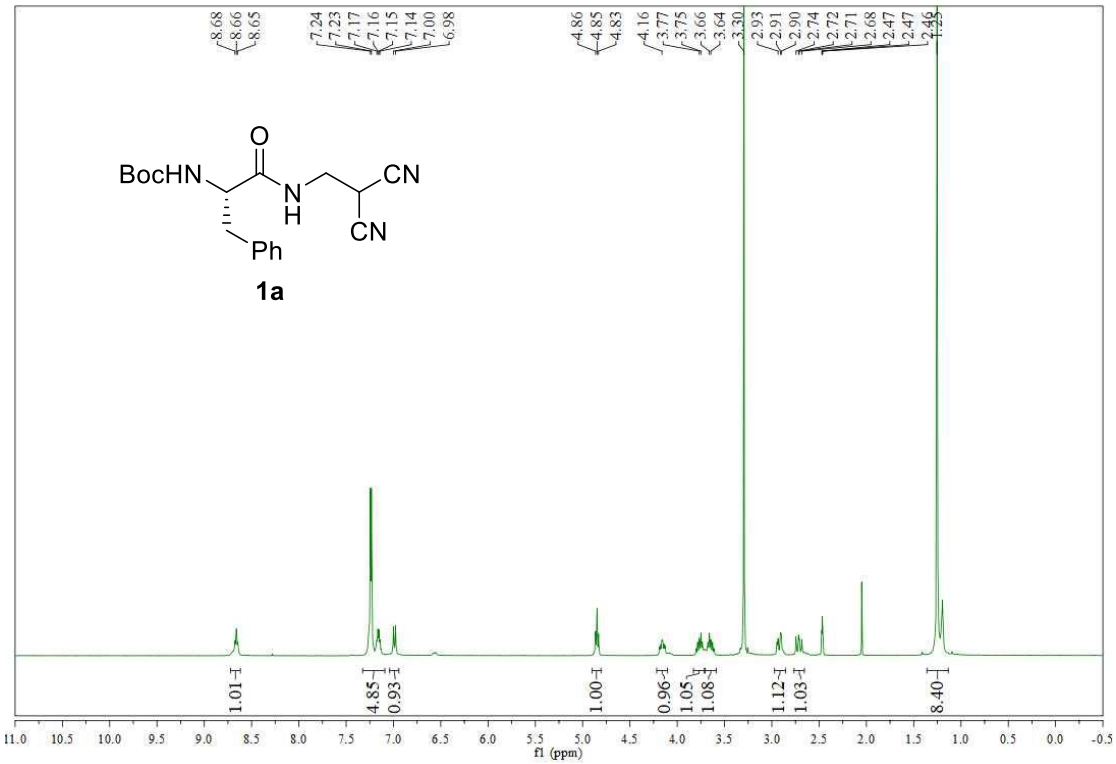
39.3
36.2
28.5
BocHN
O
N
H
CN
CN
Ph
s1
f1 (ppm)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8.65
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7.17
7.16
7.15
7.14
7.00
6.98
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4.83
4.16
3.77
3.75
3.66
3.64
3.30
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2.71
2.68
2.47
2.47
2.46
BocHN
O
N
H
CN
CN
Ph
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1.00
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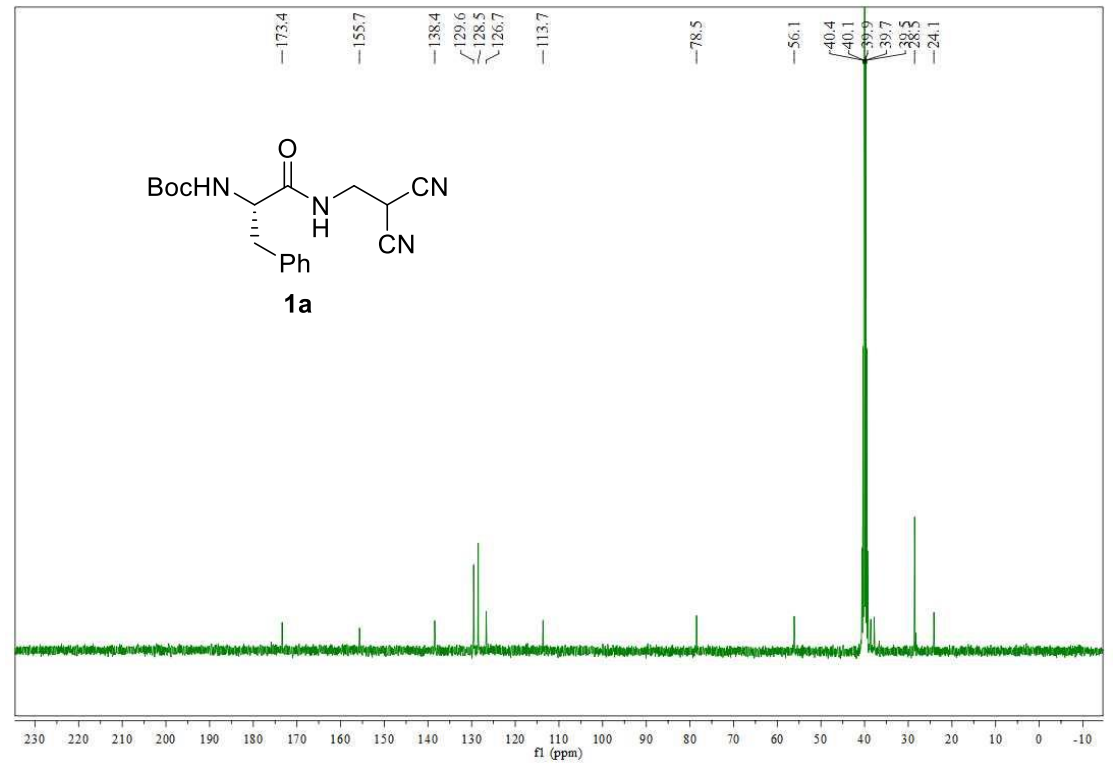
1.05
1.08
1.12
1.03
8.40
f1 (ppm)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138.4
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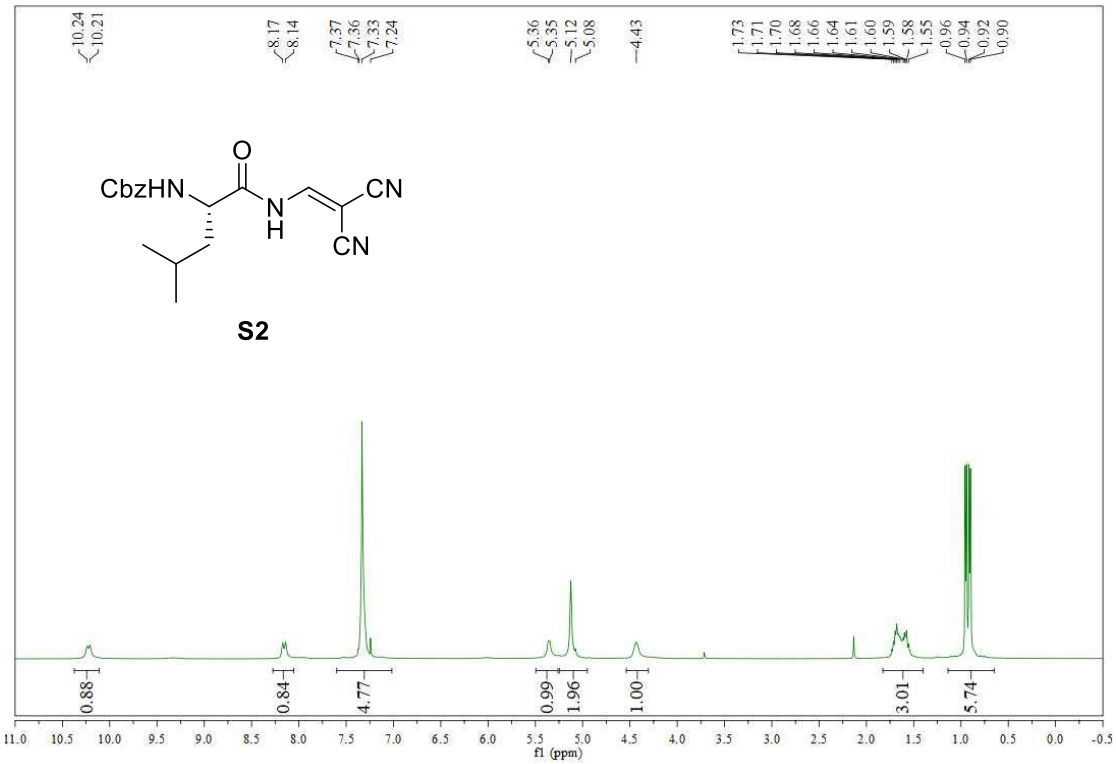
126.7
113.7
78.5
56.1
40.4
40.1
39.9
39.7
28.5
24.1
BocHN
O
N
H
CN
CN
Ph
1a
f1 (ppm)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1.71
1.70
1.68
1.66
1.64
1.61
1.60
1.59
1.58
1.55
0.96
0.94
0.92
0.90
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O
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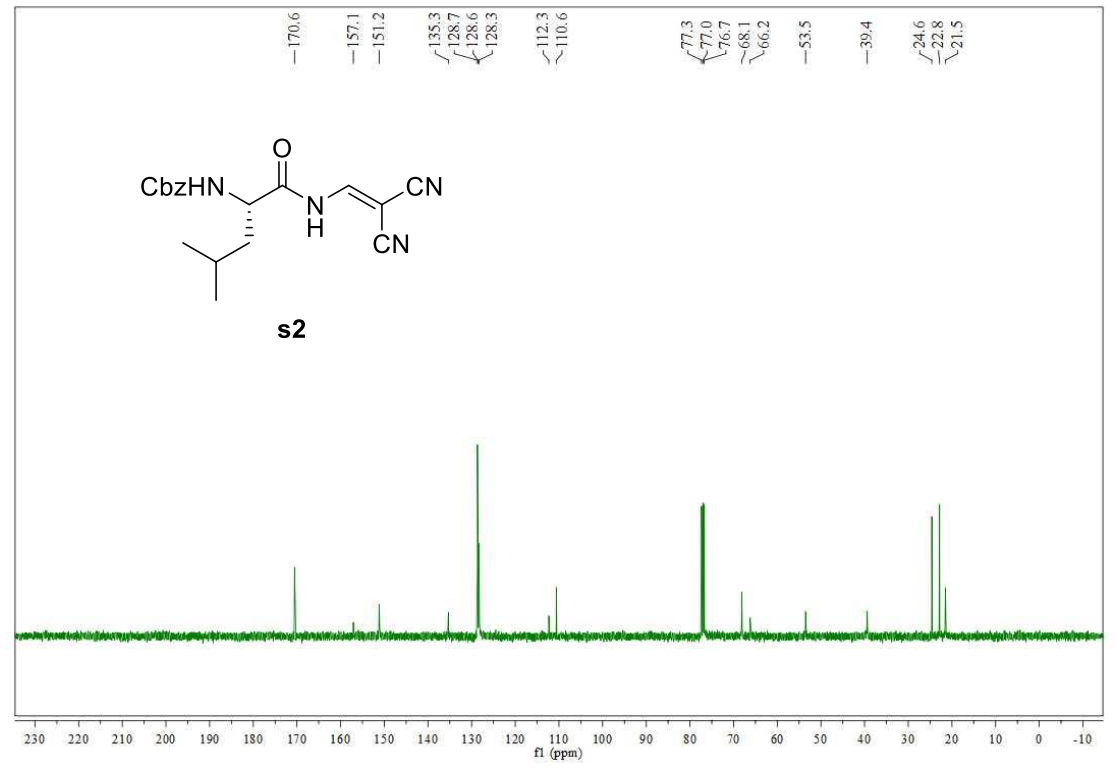
5.74
f1 (ppm)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128.6
128.3
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110.6
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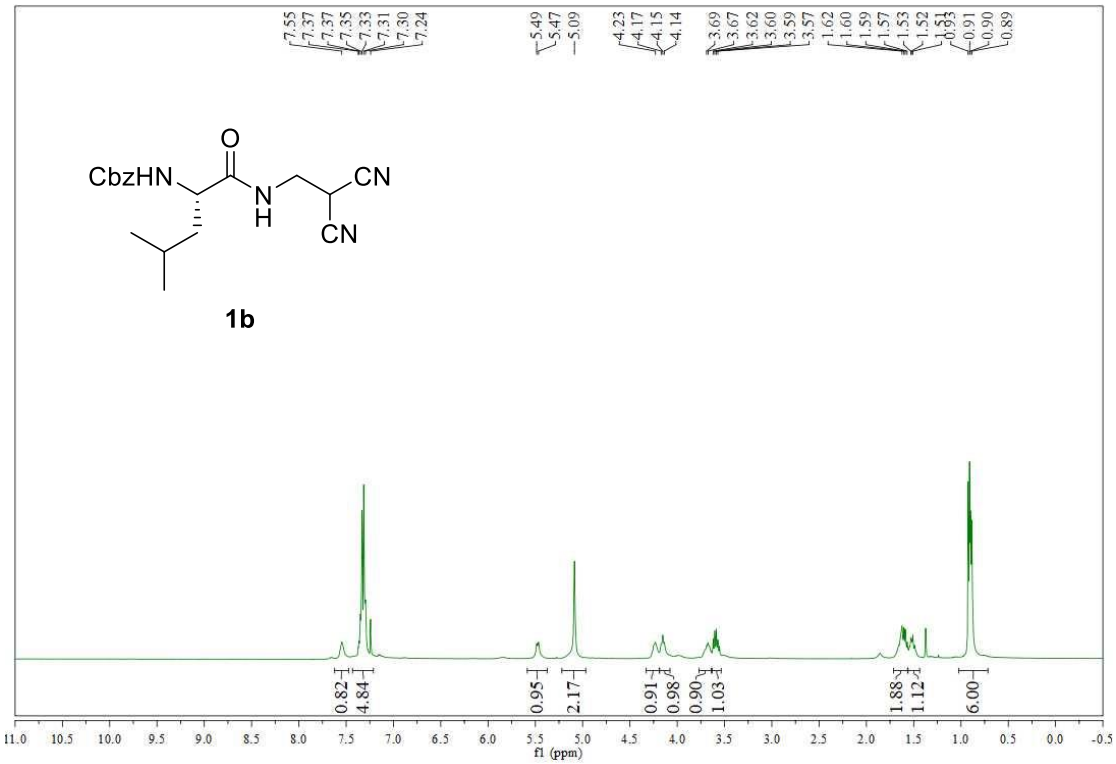
76.7
68.1
66.2
53.5
39.4
24.6
22.8
21.5
CbzHN
O
N
H
CN
CN
s2
f1 (ppm)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7.30
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5.47
5.09
4.23
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3.62
3.60
3.59
3.57
1.62
1.60
1.59
1.57
1.53
1.52
1.51
0.93
0.91
0.90
0.89
CbzHN
O
N
H
CN
CN
1b
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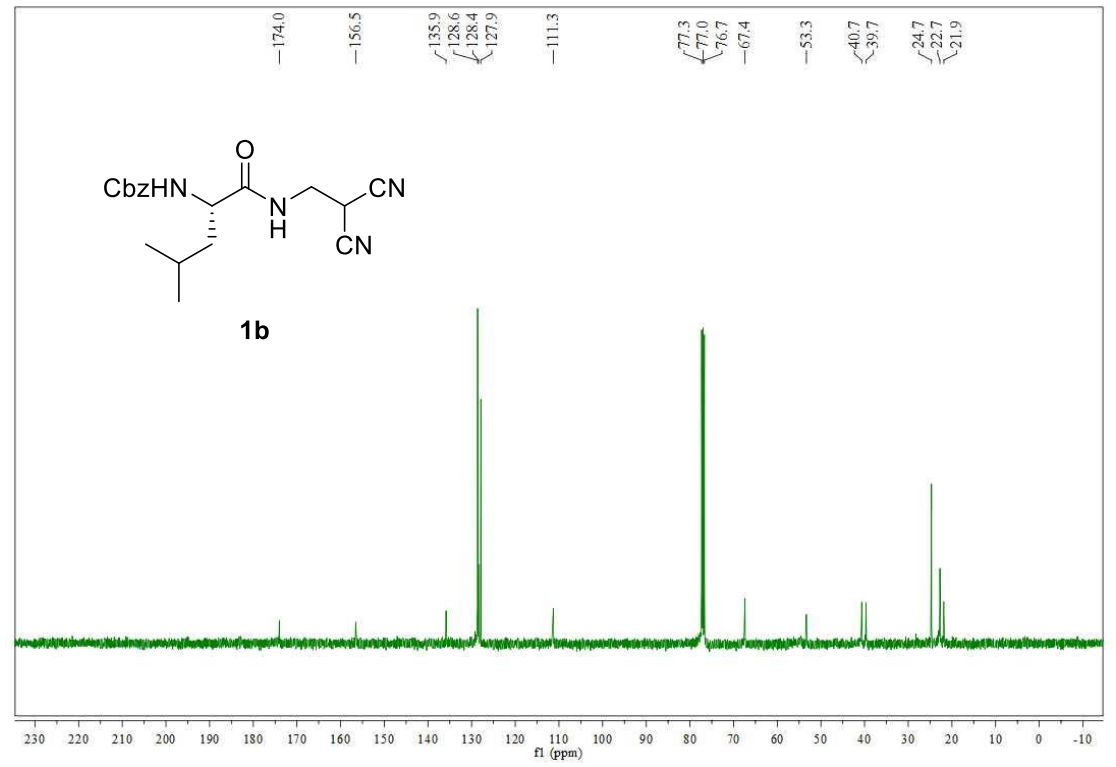
1.88
1.12
6.00
f1 (ppm)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127.9
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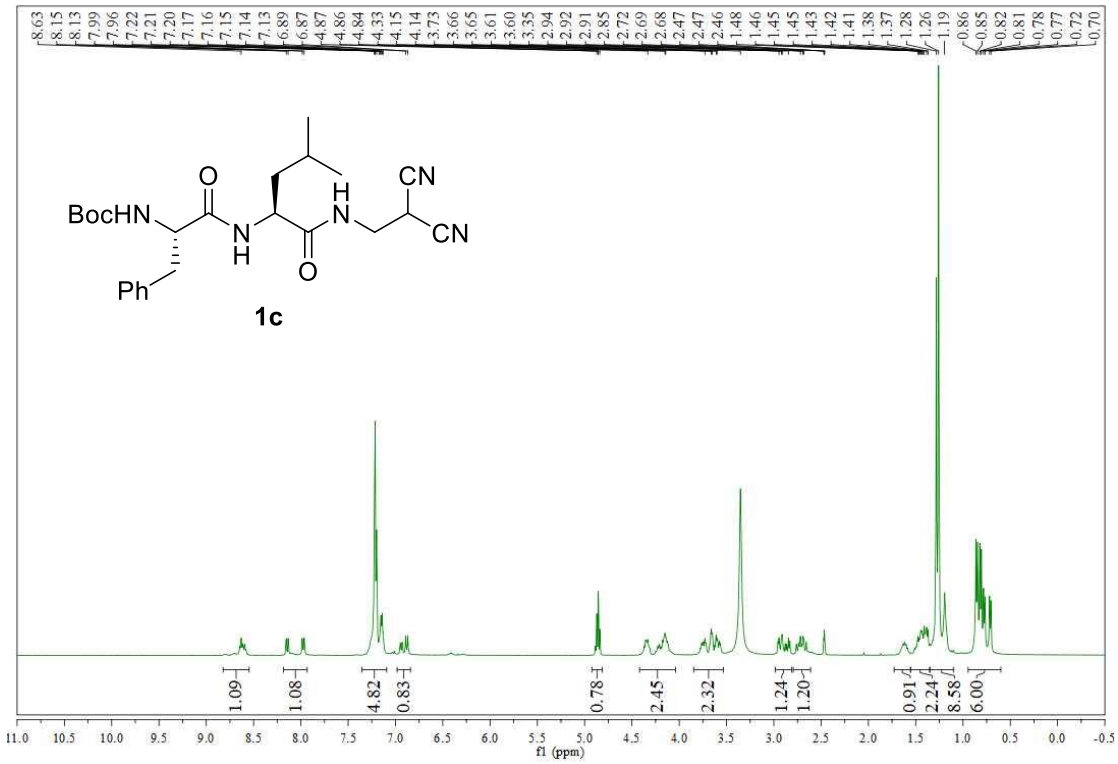
22.7
21.9
CbzHN
O
N
H
CN
CN
1b
f1 (ppm)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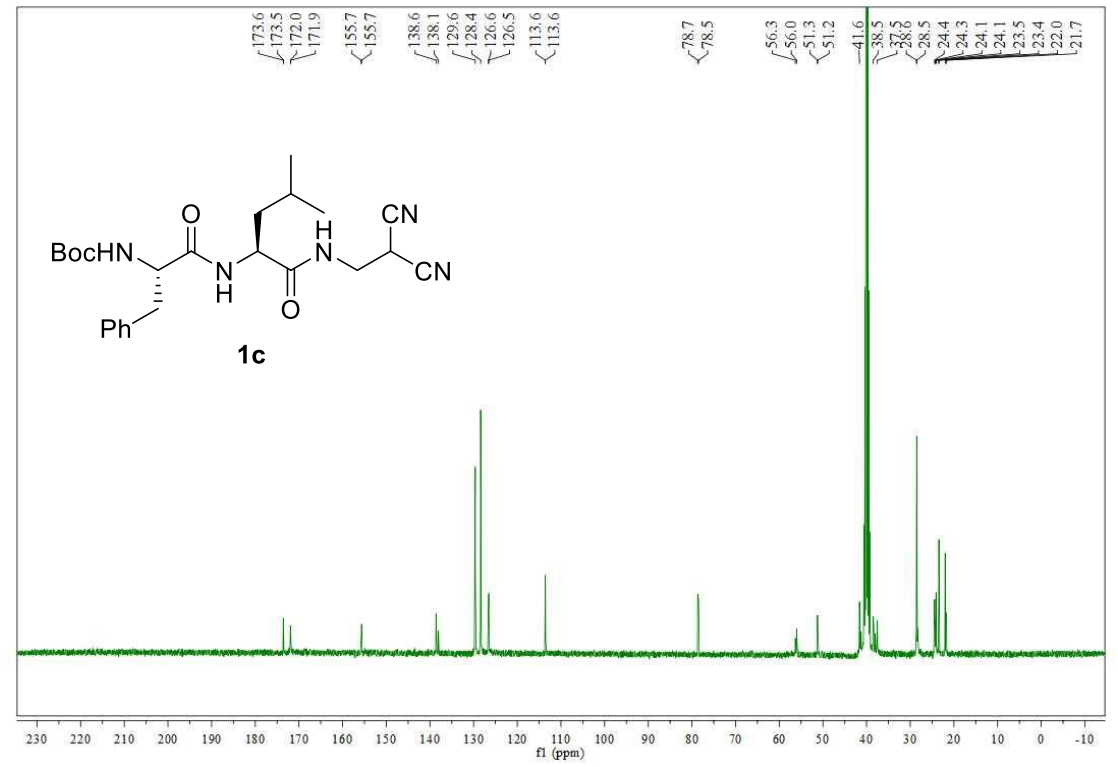
H
H
N
O
CN
CN
Ph
1c
f1 (ppm)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H
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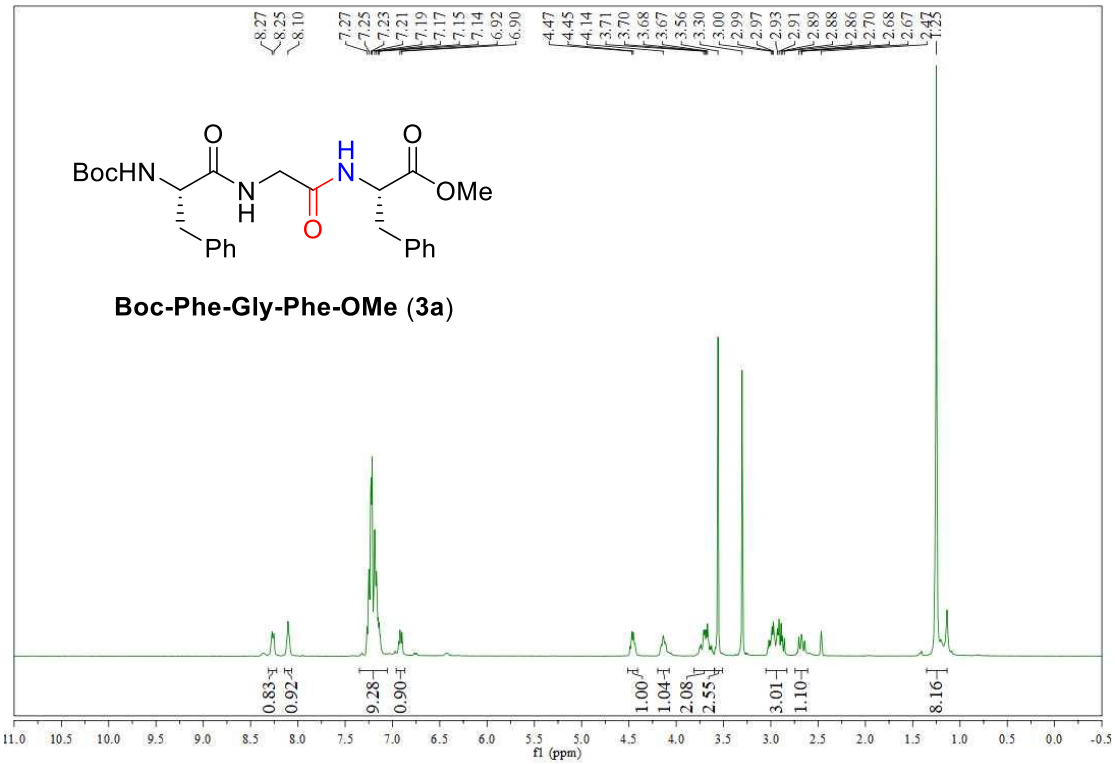
CN
CN
Ph
1c
f1 (ppm)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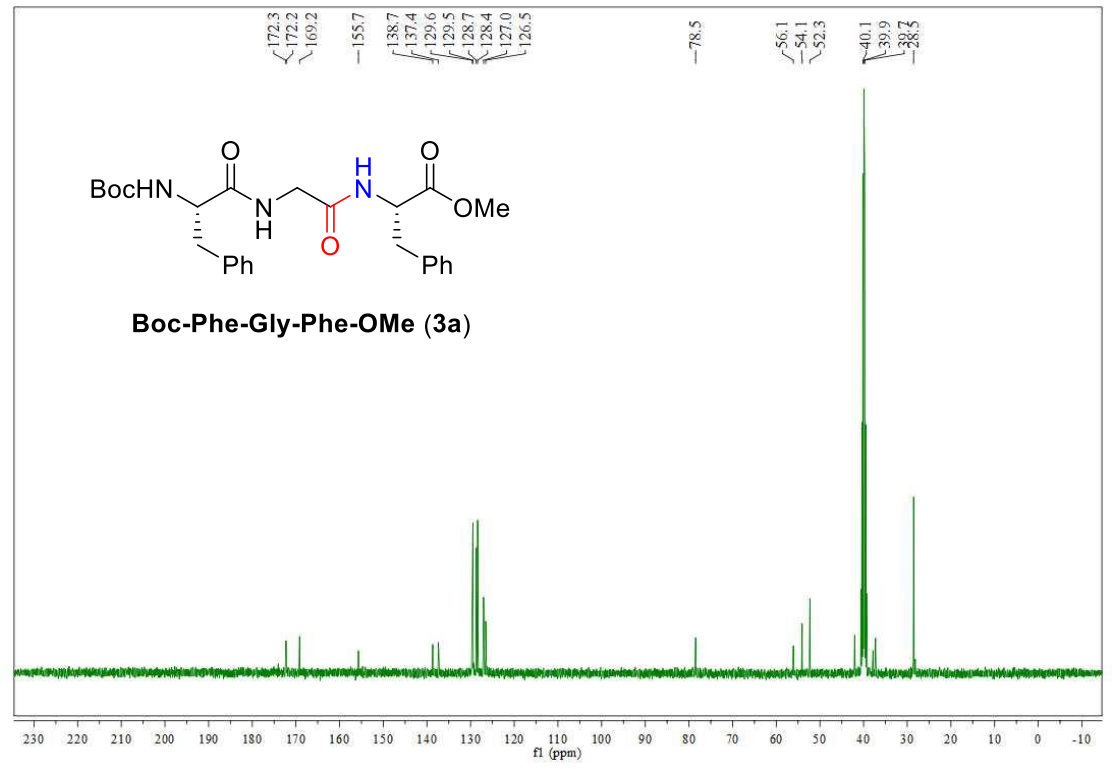
H
O
H
N
O
OMe
Ph
Ph
Boc-Phe-Gly-Phe-OMe (3a)
f1 (ppm)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H
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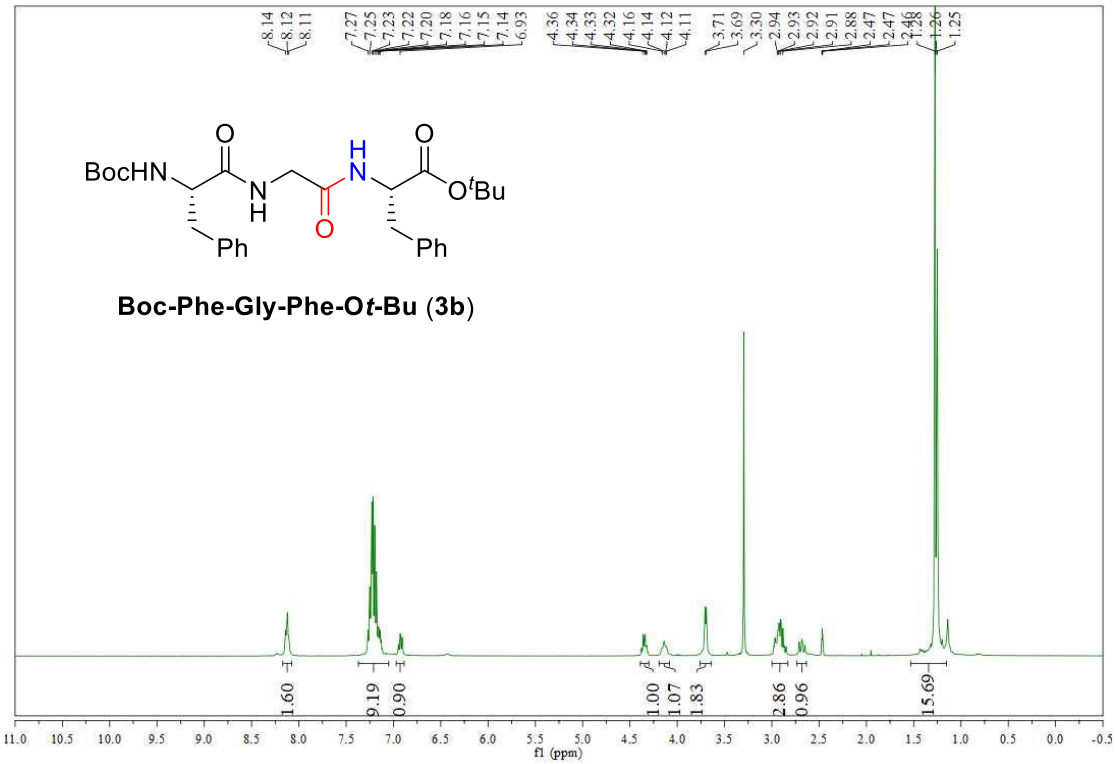
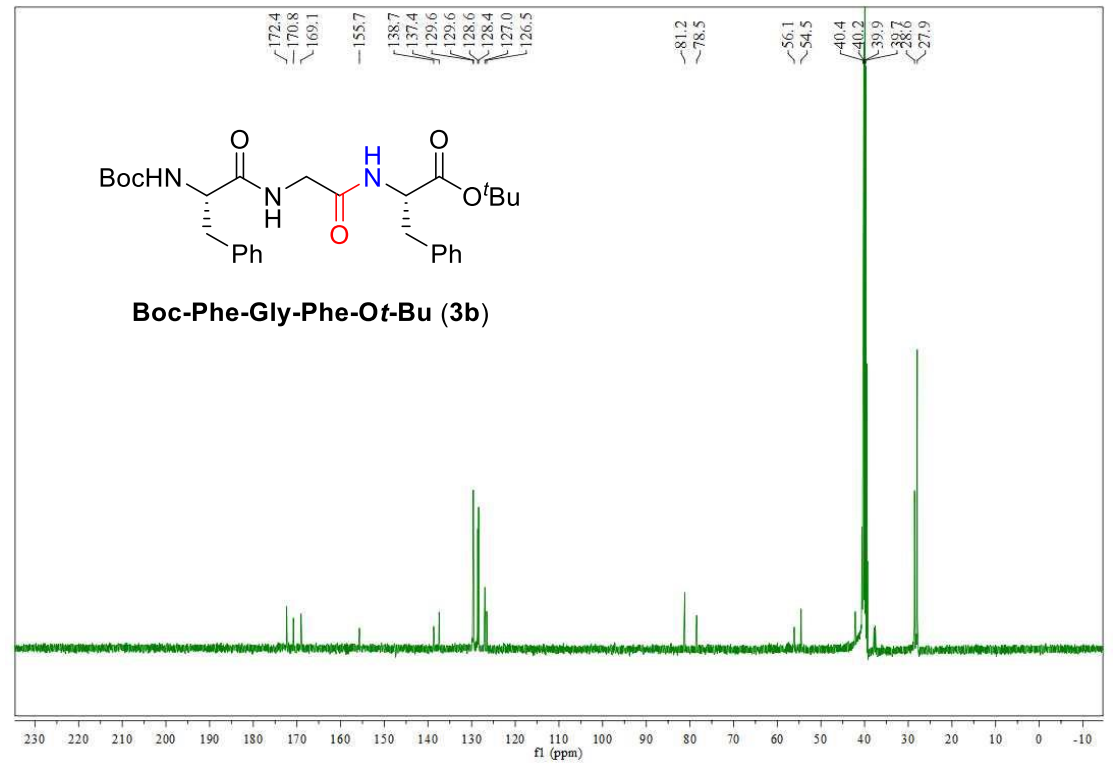
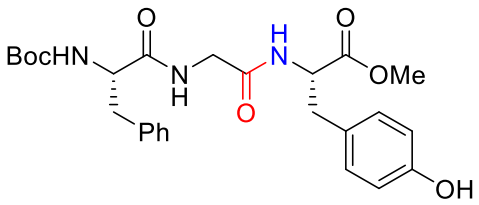
N
O
OMe
Ph
Ph
Boc-Phe-Gly-Phe-OMe (3a)
f1 (ppm)



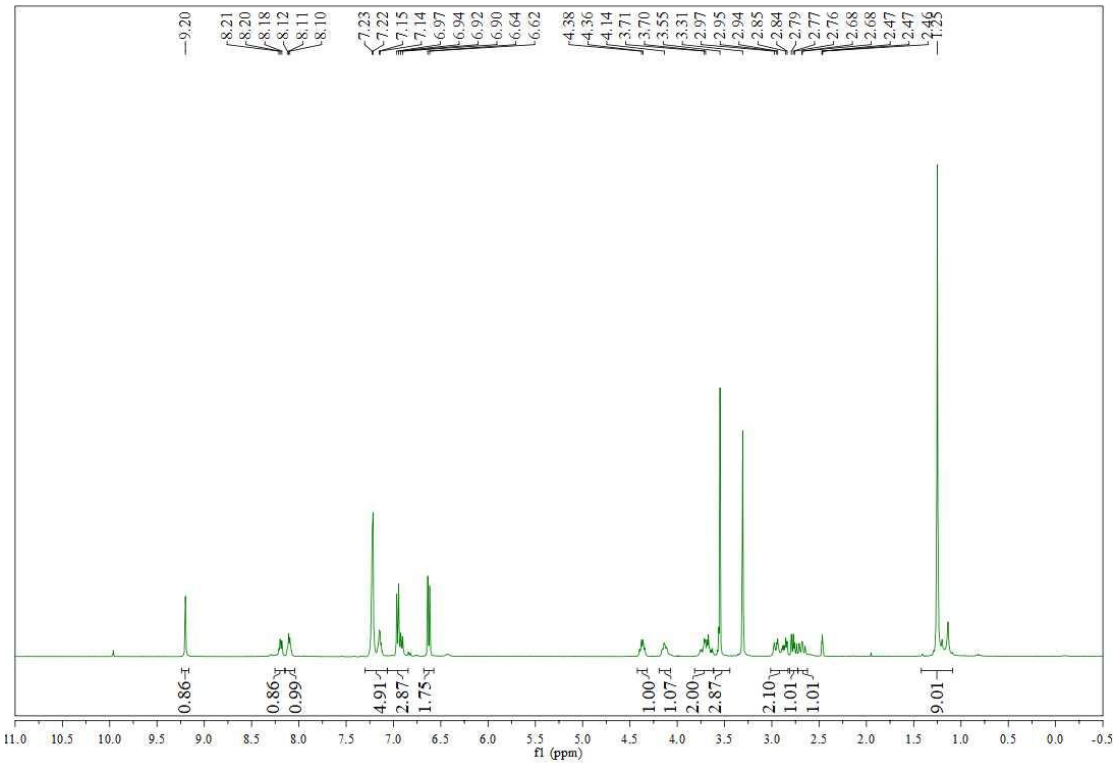
Boc-Phe-Gly-Phe-O*t*-Bu (3b)

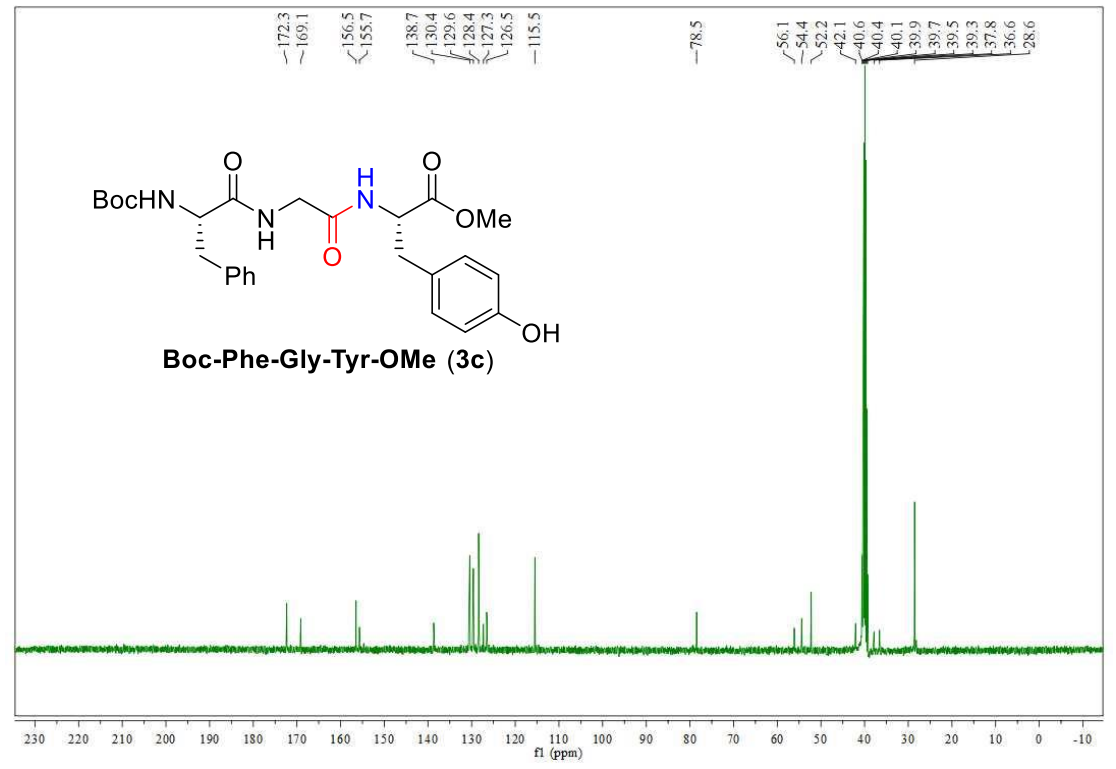


Boc-Phe-Gly-Phe-O*t*-Bu (3b)

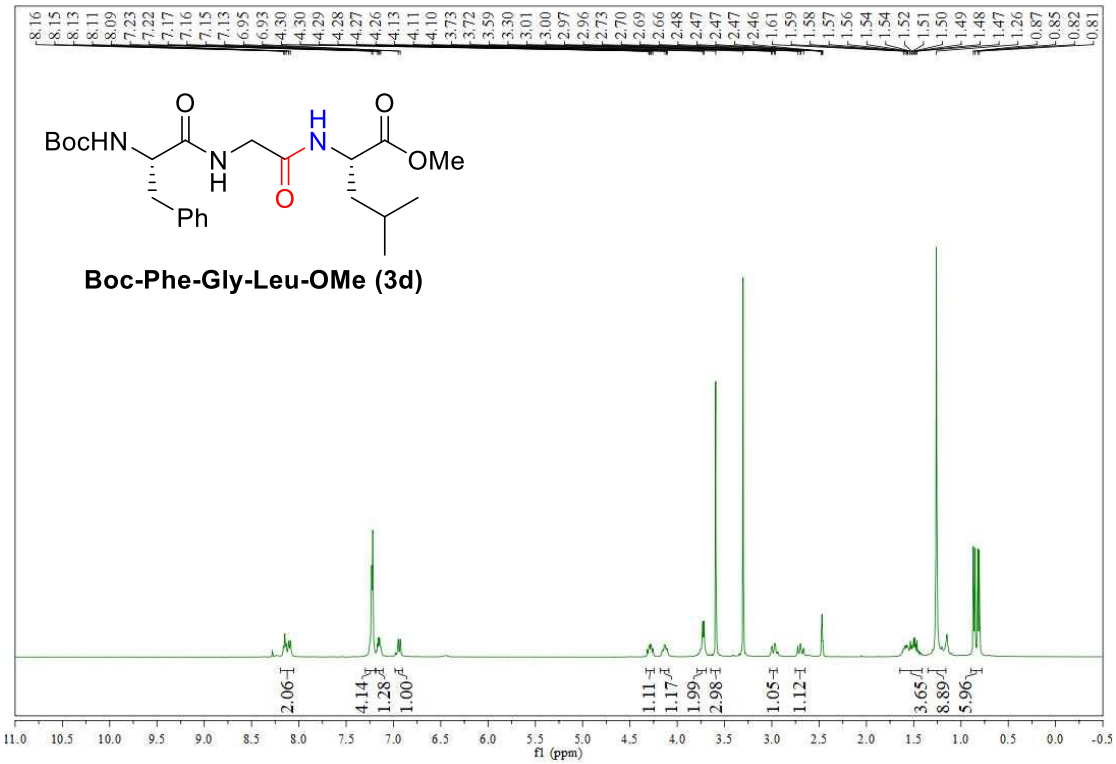


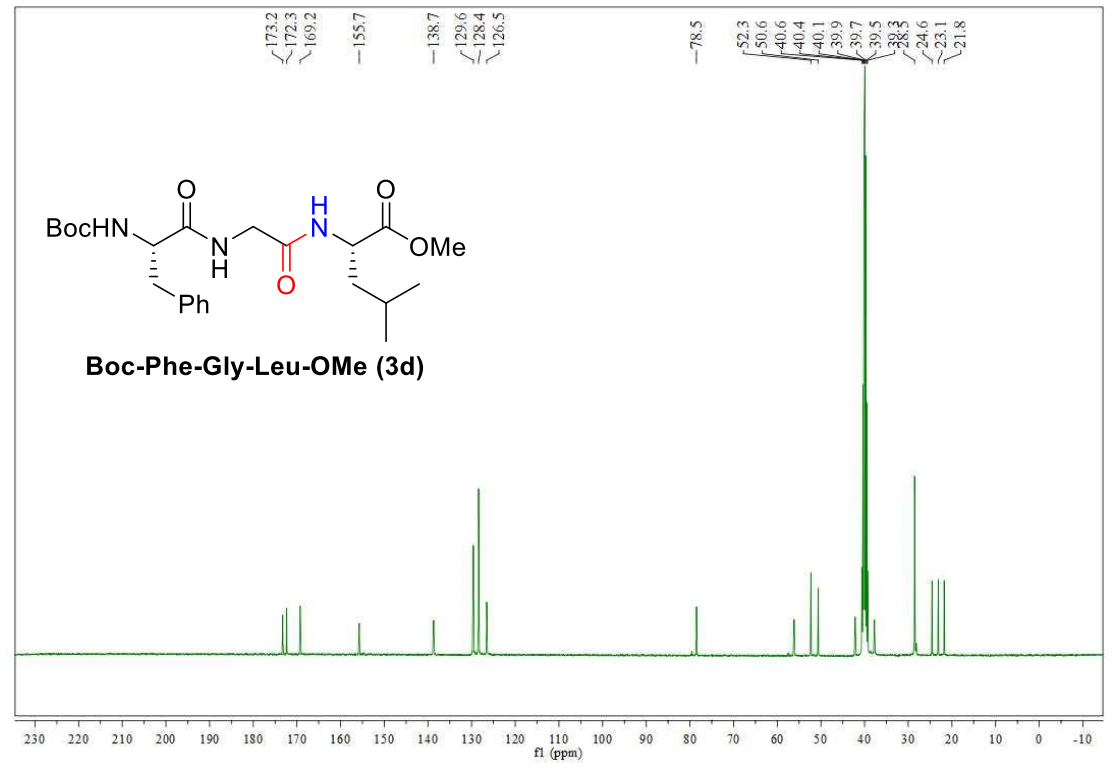
Boc-Phe-Gly-Tyr-OMe (3c)

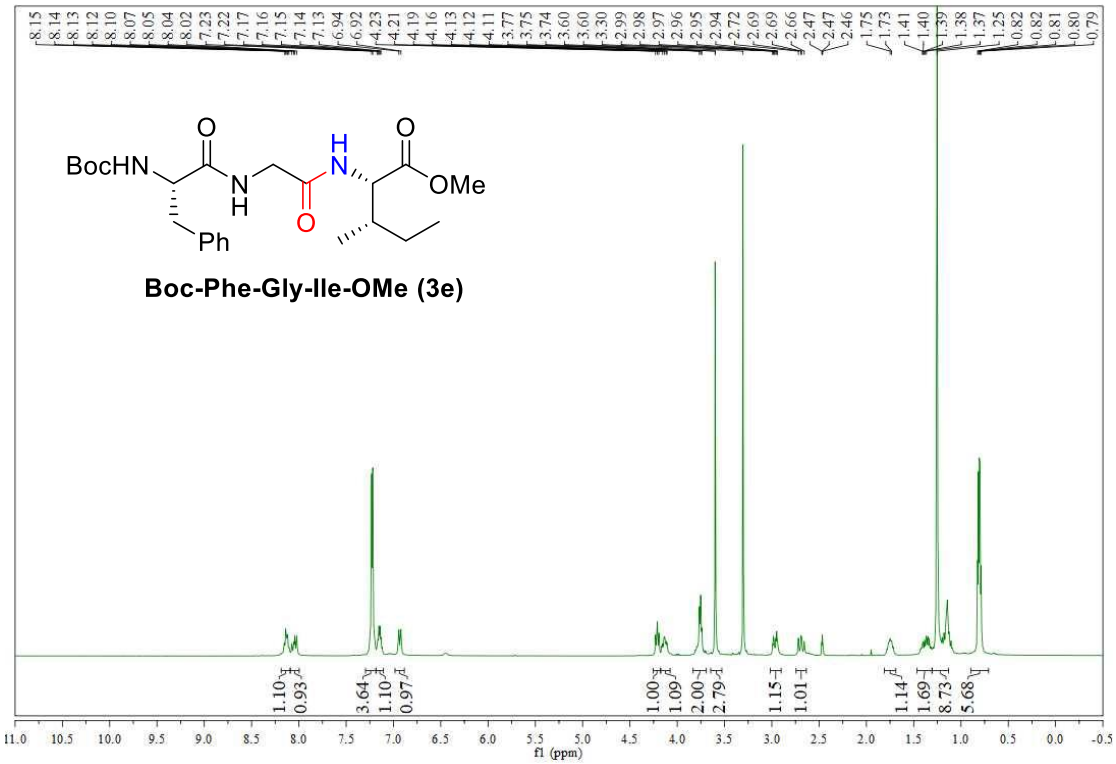


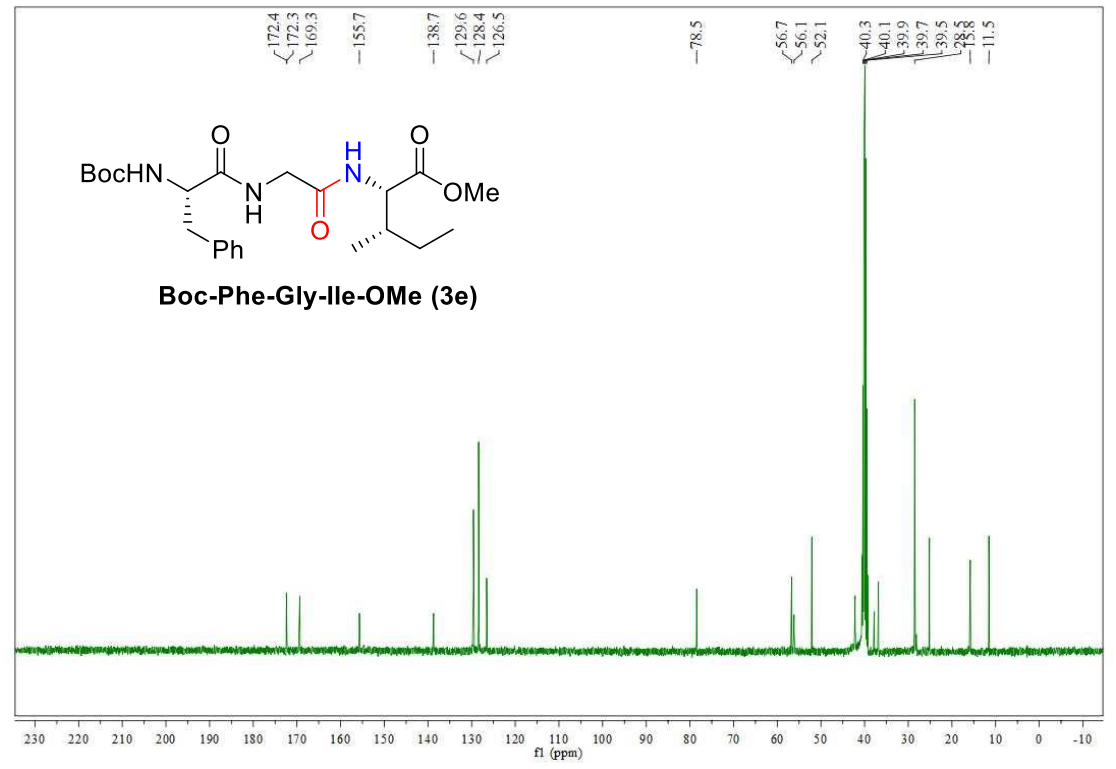


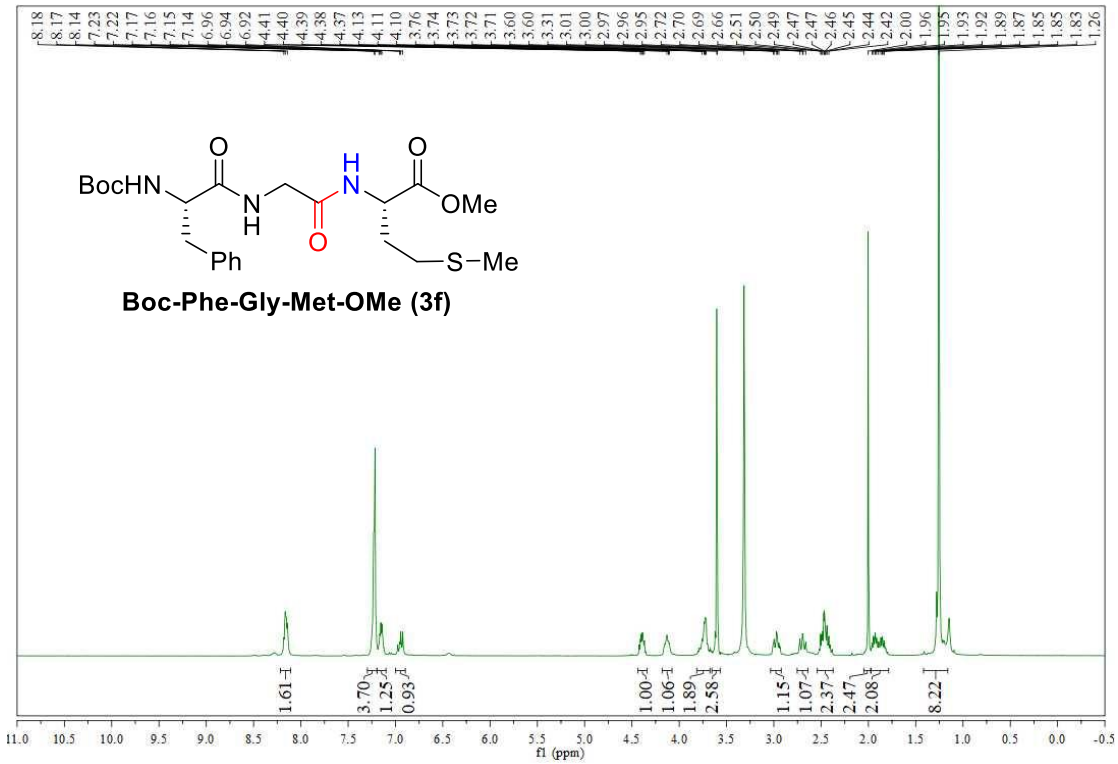
Boc-Phe-Gly-Tyr-OMe (3c)

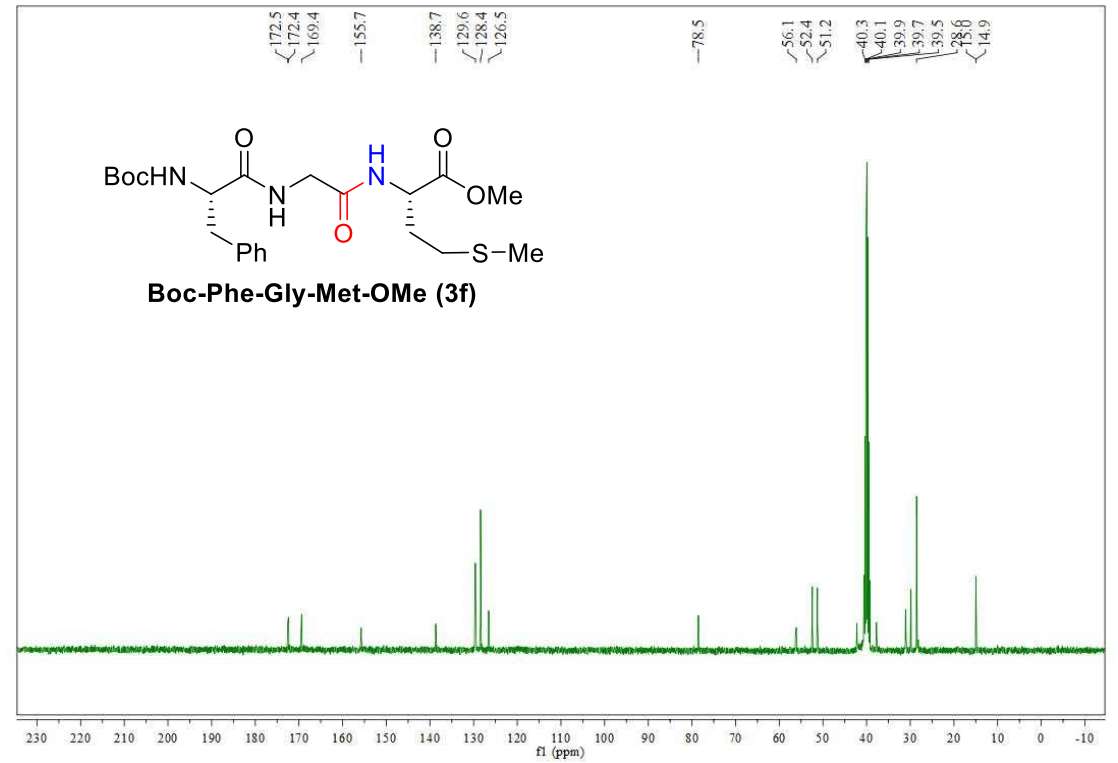

BocHN
O
N
H
O
H
N
O
OMe
Ph
Boc-Phe-Gly-Leu-OMe (3d)
f1 (ppm)

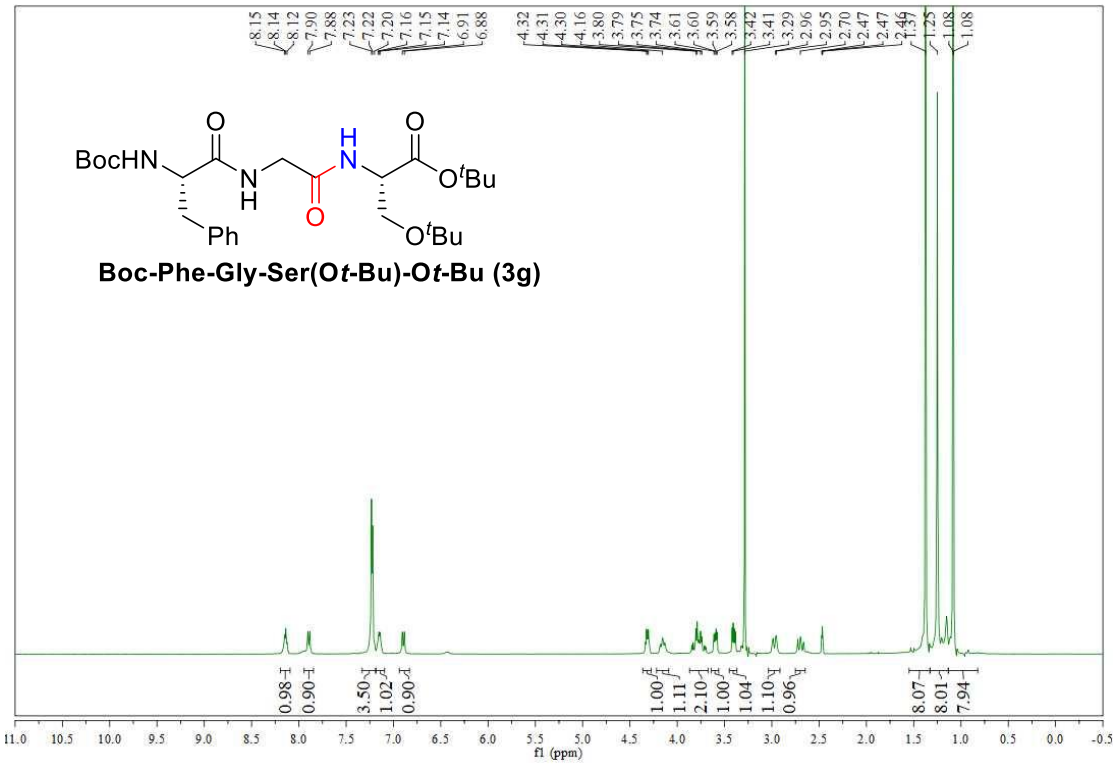

BocHN
O
N
H
O
H
N
O
OMe
Ph
Boc-Phe-Gly-Leu-OMe (3d)
f1 (ppm)

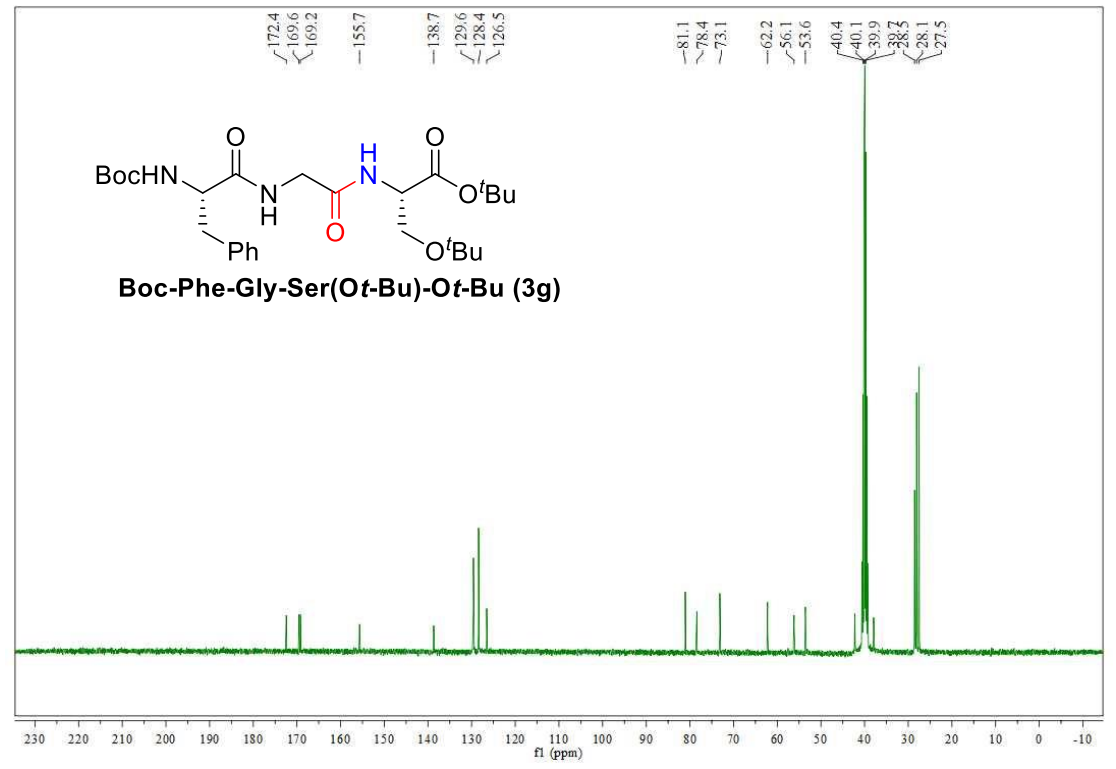

BocHN
O
N
H
O
H
N
O
OMe
Ph
Boc-Phe-Gly-Ile-OMe (3e)
f1 (ppm)

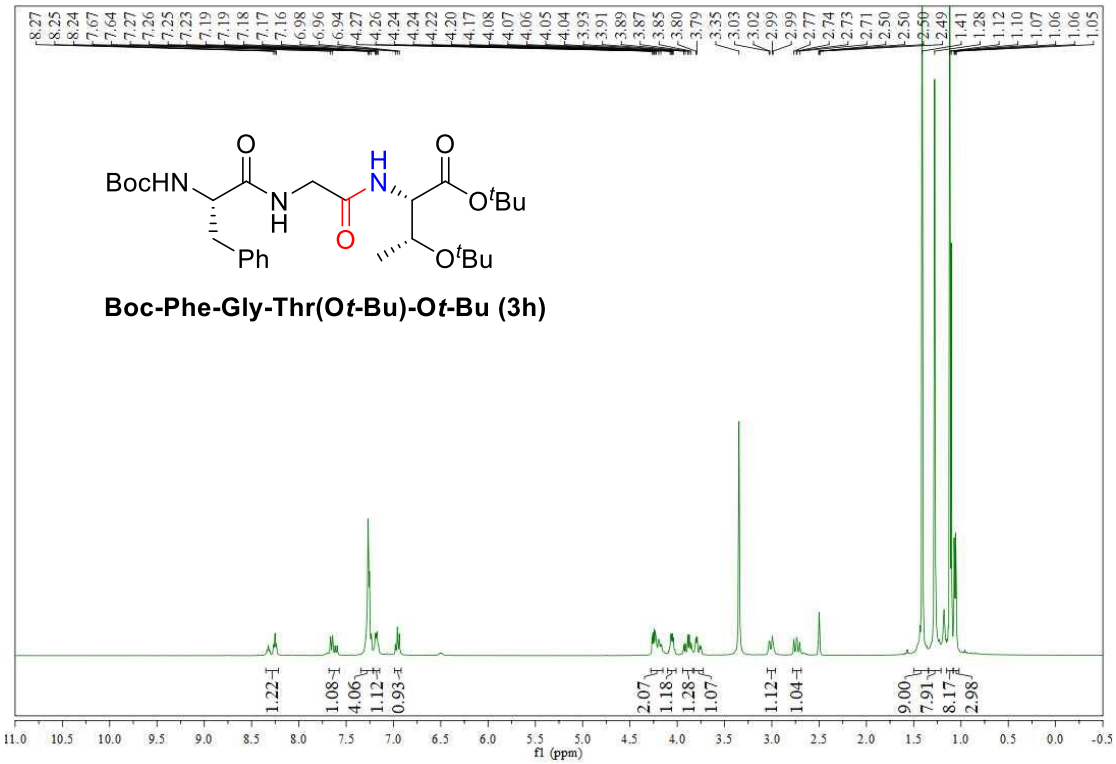

BocHN
O
N
H
O
H
N
O
OMe
Ph
Boc-Phe-Gly-Ile-OMe (3e)
f1 (ppm)

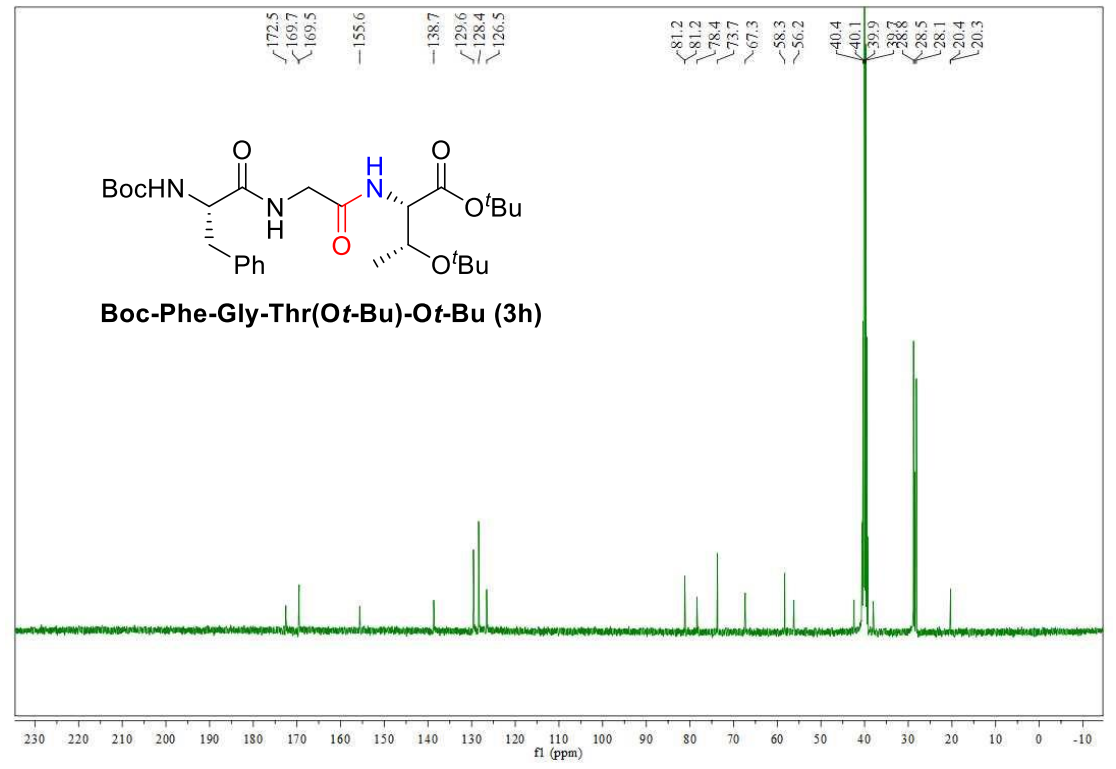

BocHN
O
N
H
H
N
O
O
OMe
Ph
S−Me
Boc-Phe-Gly-Met-OMe (3f)
f1 (ppm)

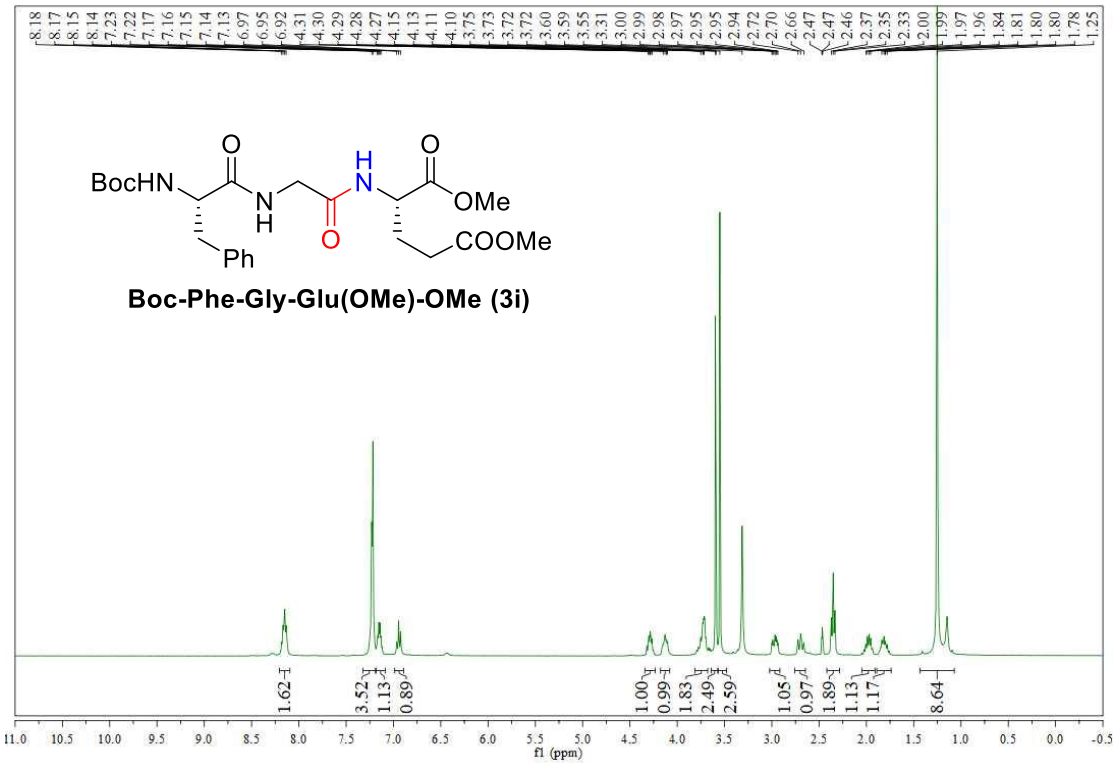

BocHN
O
N
H
H
N
O
O
OMe
Ph
S−Me
Boc-Phe-Gly-Met-OMe (3f)
f1 (ppm)

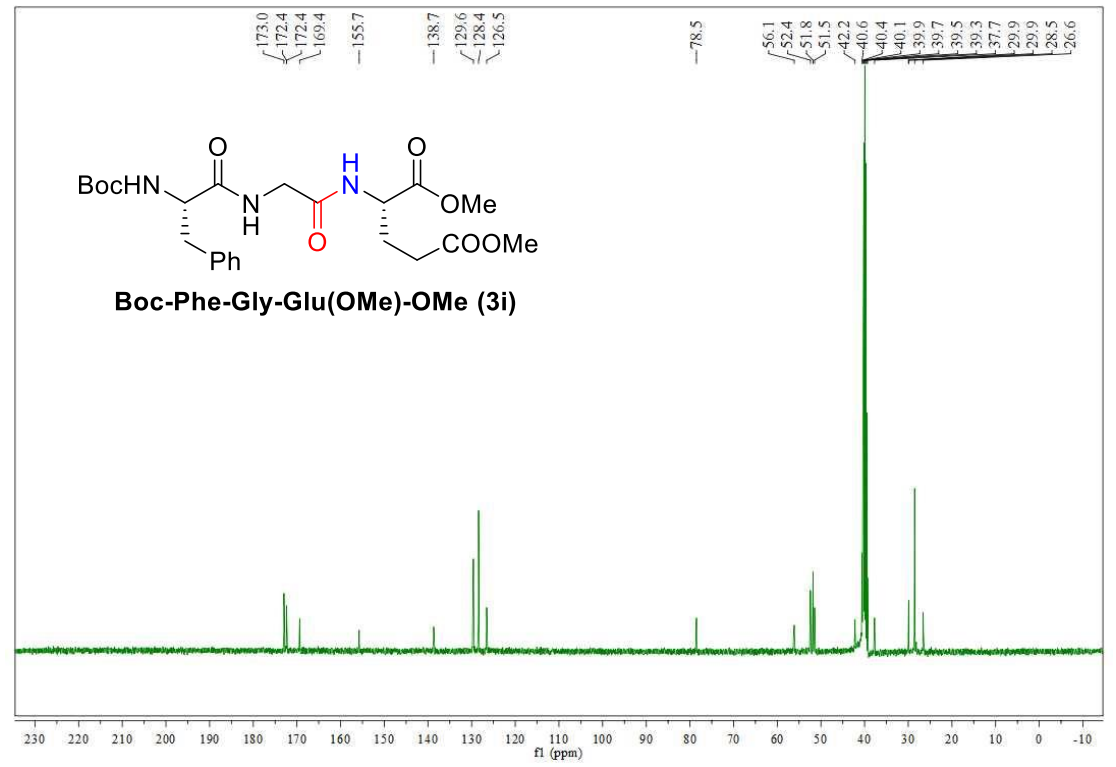

BocHN
O
N
H
H
N
O
O
O
O
tBu
Ph
O
tBu
Boc-Phe-Gly-Ser(Ot-Bu)-Ot-Bu (3g)
f1 (ppm)

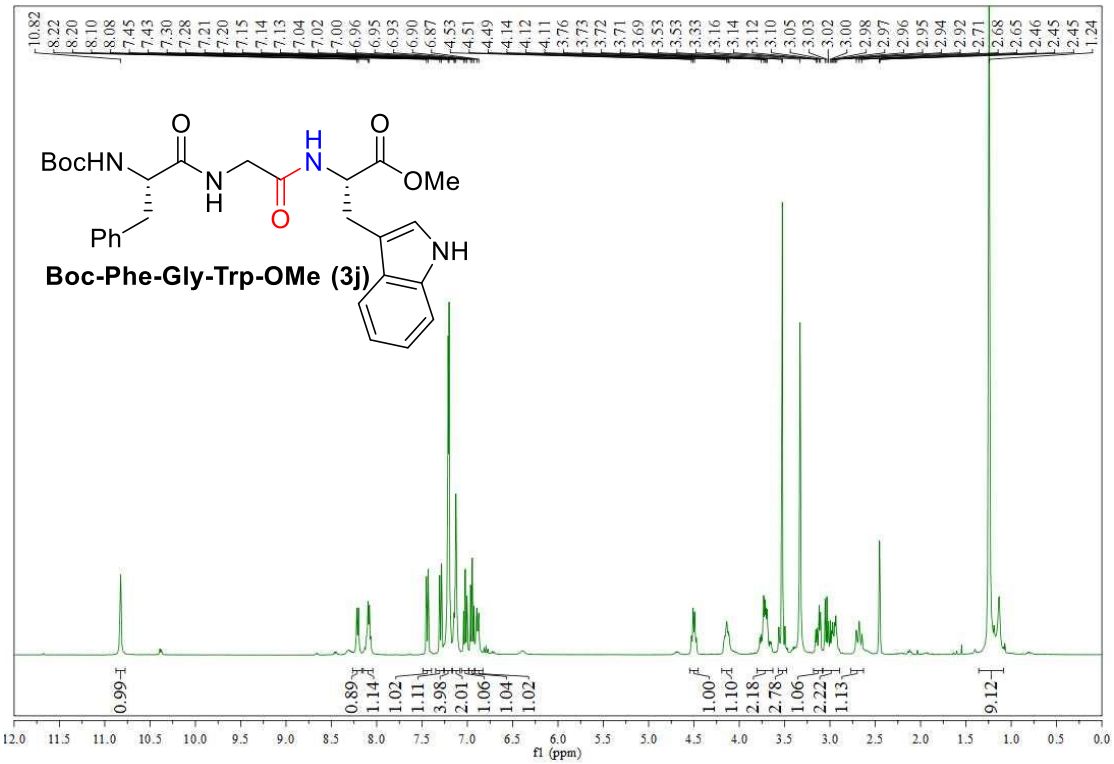

BocHN
O
N
H
H
N
O
O
O
O
tBu
Ph
O
tBu
Boc-Phe-Gly-Ser(Ot-Bu)-Ot-Bu (3g)
f1 (ppm)

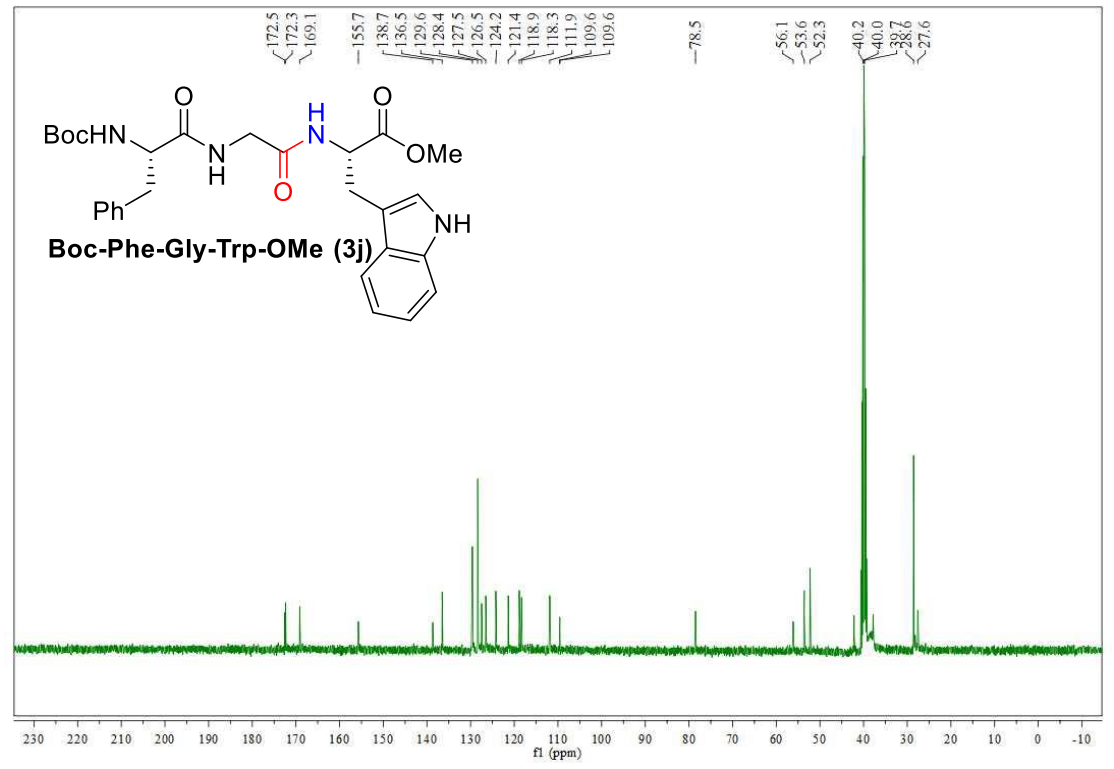

BocHN
O
N
H
Ph
H
N
O
O
O^{t}Bu
O^{t}Bu
Boc-Phe-Gly-Thr(O*t*-Bu)-O*t*-Bu (3h)
f1 (ppm)

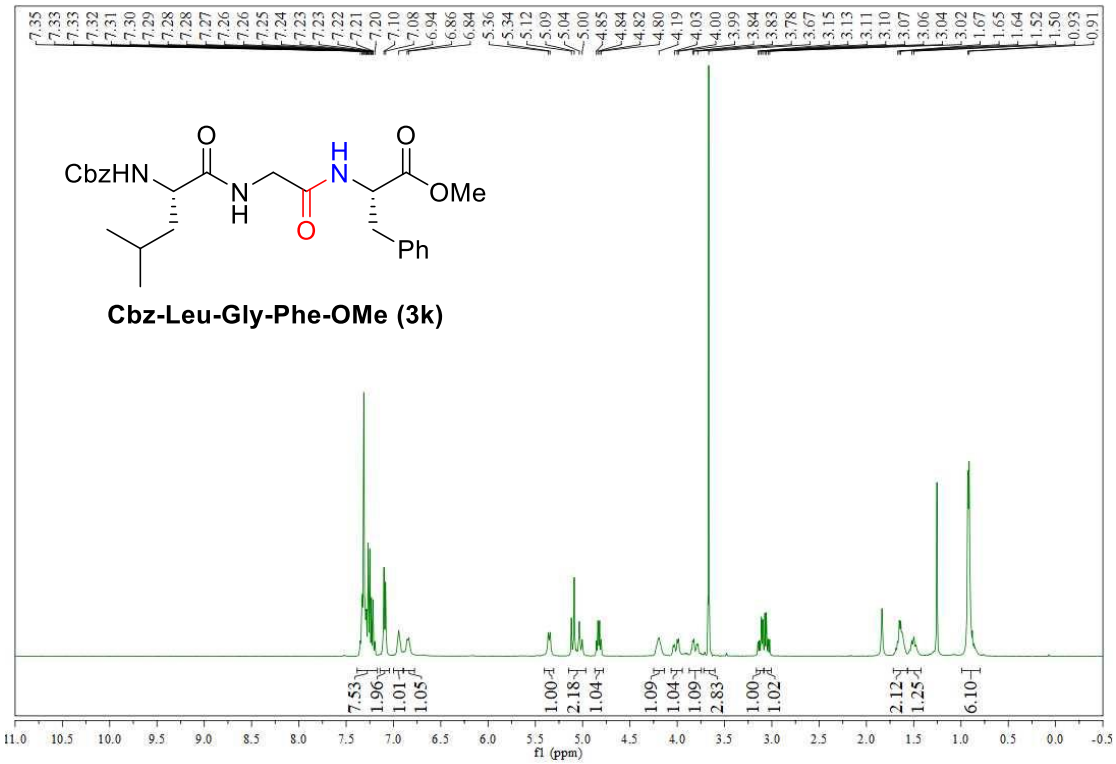

BocHN
O
N
H
Ph
H
N
O
O
O^{t}Bu
O^{t}Bu
Boc-Phe-Gly-Thr(O*t*-Bu)-O*t*-Bu (3h)
f1 (ppm)

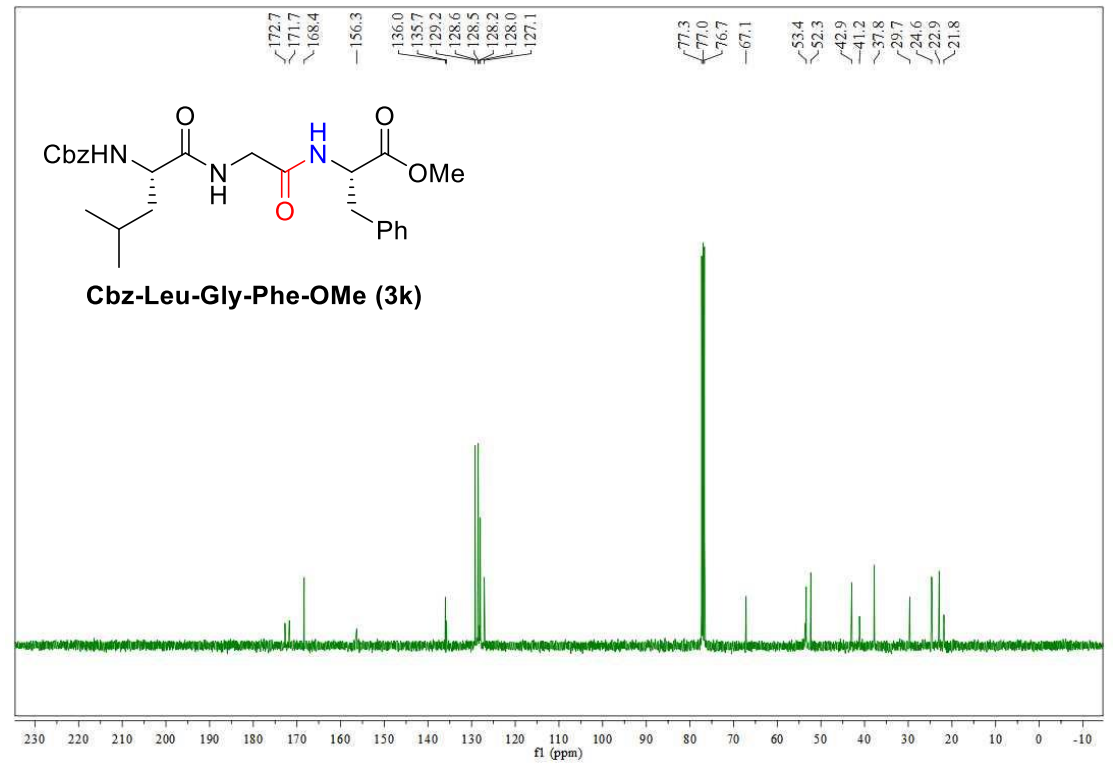

BocHN
O
N
H
O
H
N
O
OMe
COOMe
Ph
Boc-Phe-Gly-Glu(OMe)-OMe (3i)
f1 (ppm)

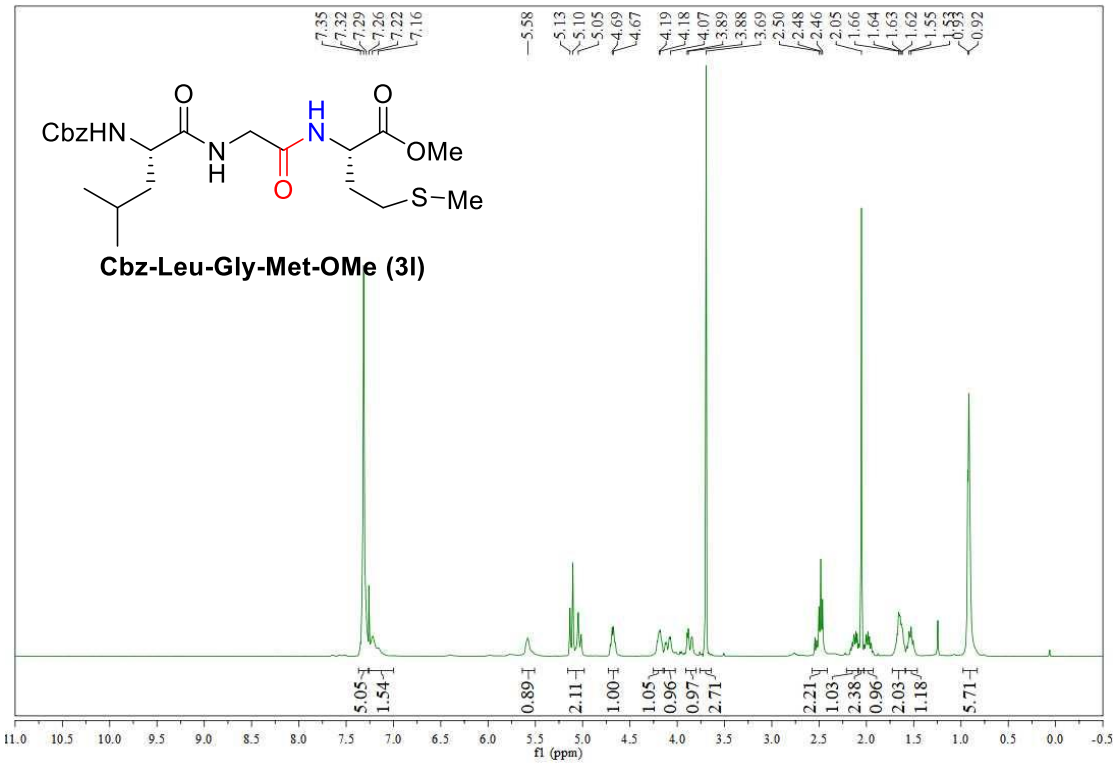

BocHN
O
N
H
O
H
N
O
OMe
COOMe
Ph
Boc-Phe-Gly-Glu(OMe)-OMe (3i)
f1 (ppm)

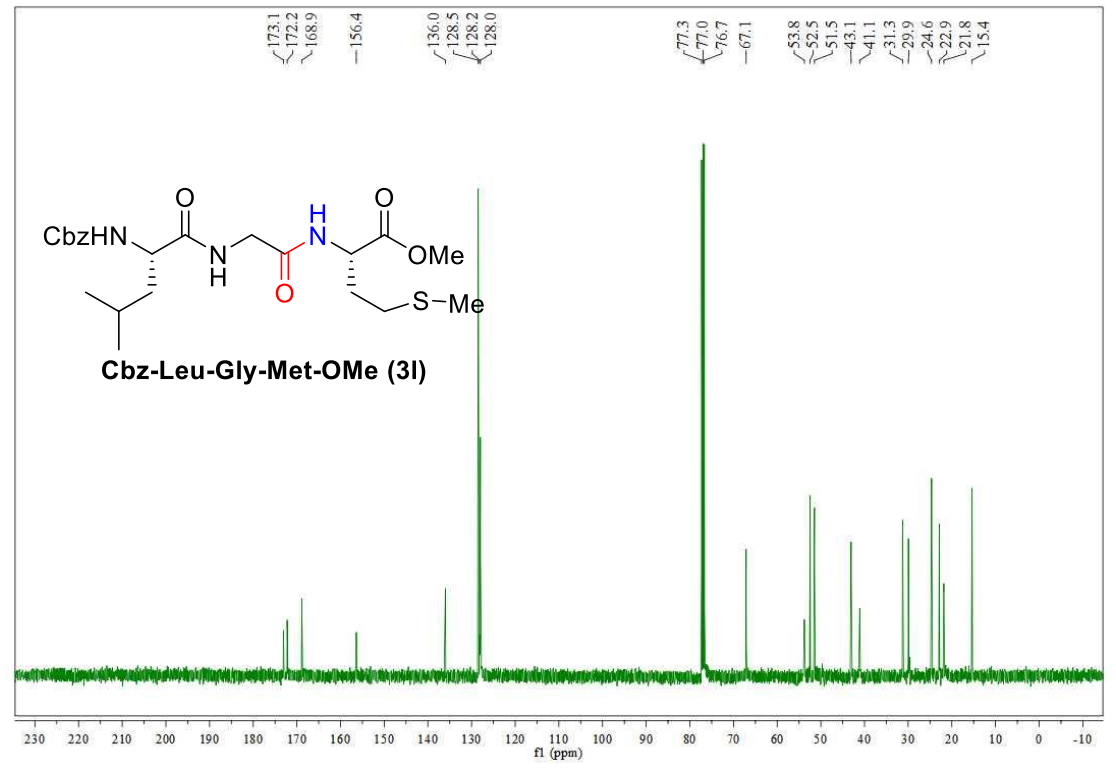

Boc-Phe-Gly-Trp-OMe (3j)
f1 (ppm)

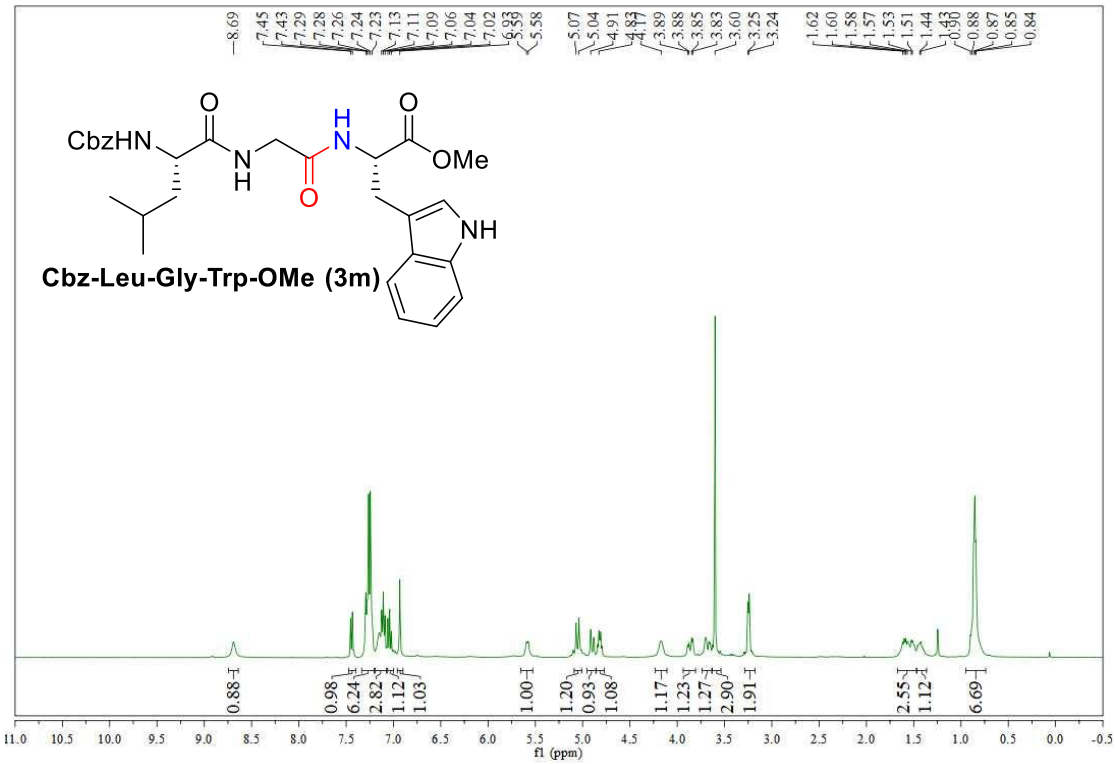

Boc-Phe-Gly-Trp-OMe (3j)
f1 (ppm)

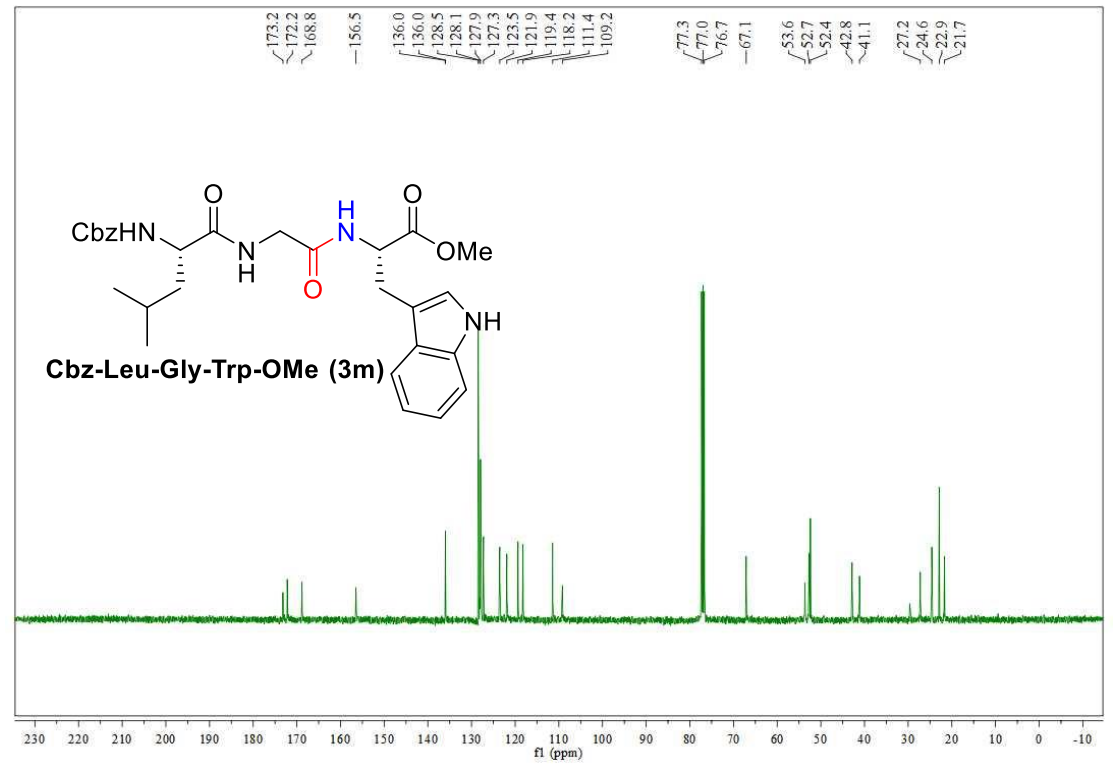

CbzHN
O
N
H
H
N
O
O
OMe
Ph
Cbz-Leu-Gly-Phe-OMe (3k)
f1 (ppm)

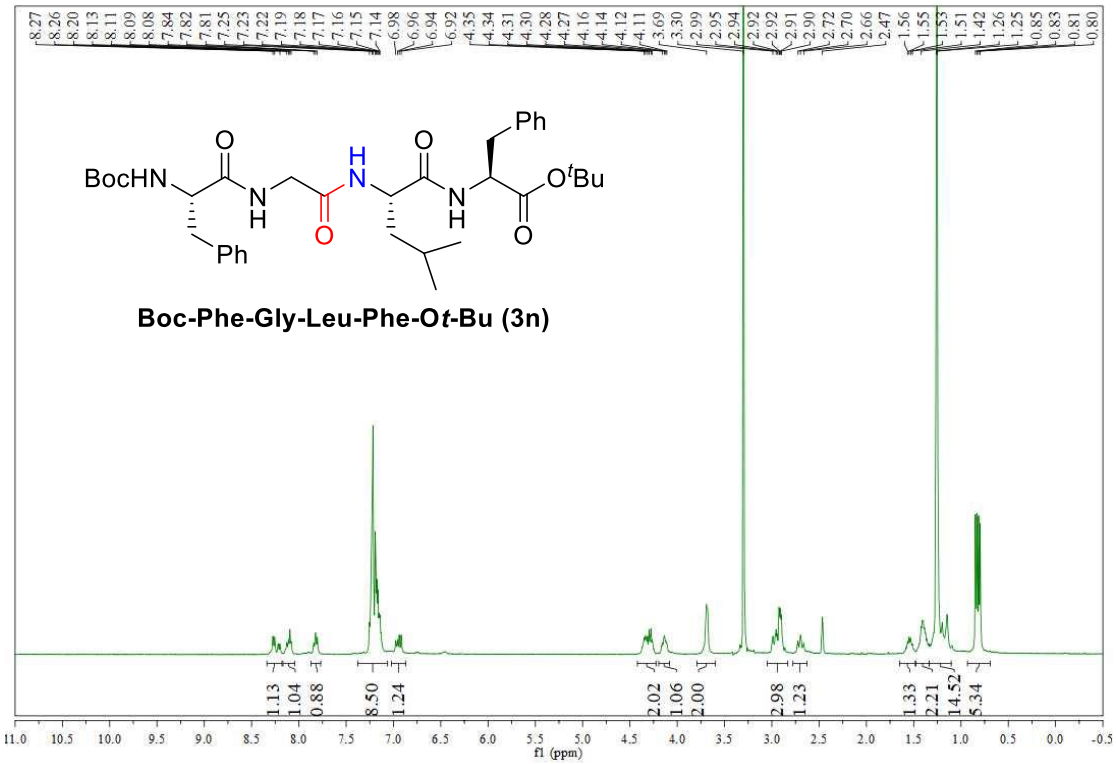

CbzHN
O
N
H
H
N
O
O
OMe
Ph
Cbz-Leu-Gly-Phe-OMe (3k)
f1 (ppm)

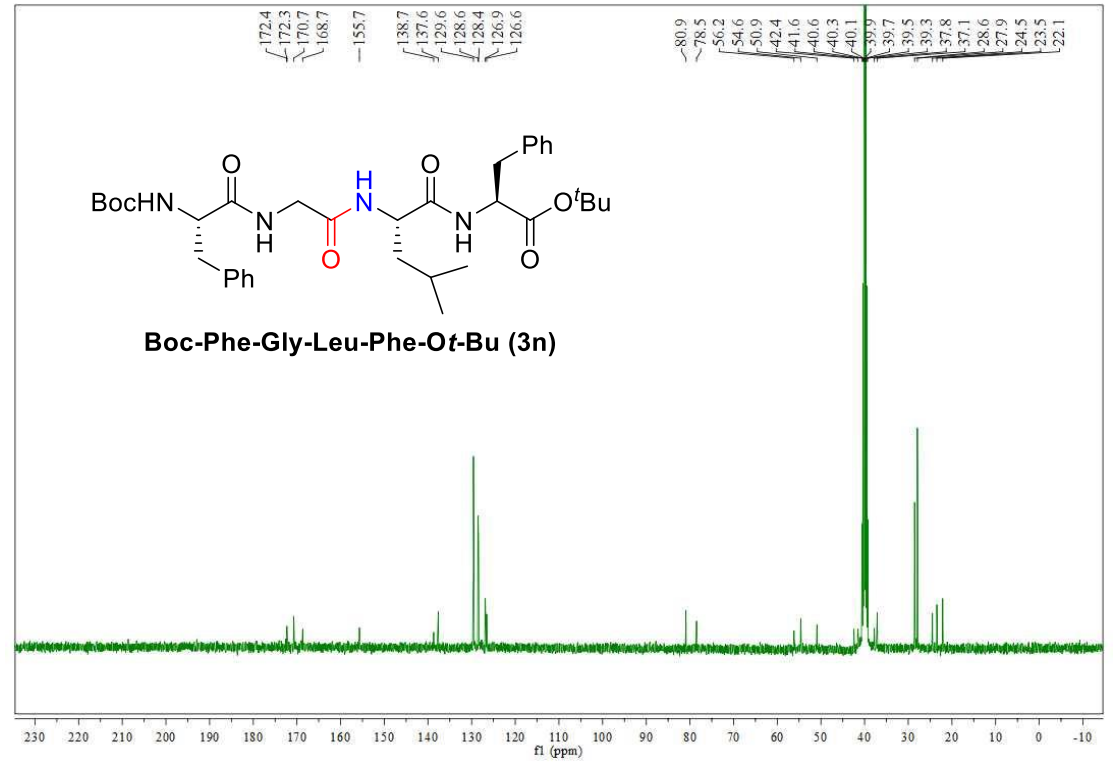

CbzHN
O
N
H
O
H
N
O
OMe
S−Me
Cbz-Leu-Gly-Met-OMe (3l)
f1 (ppm)

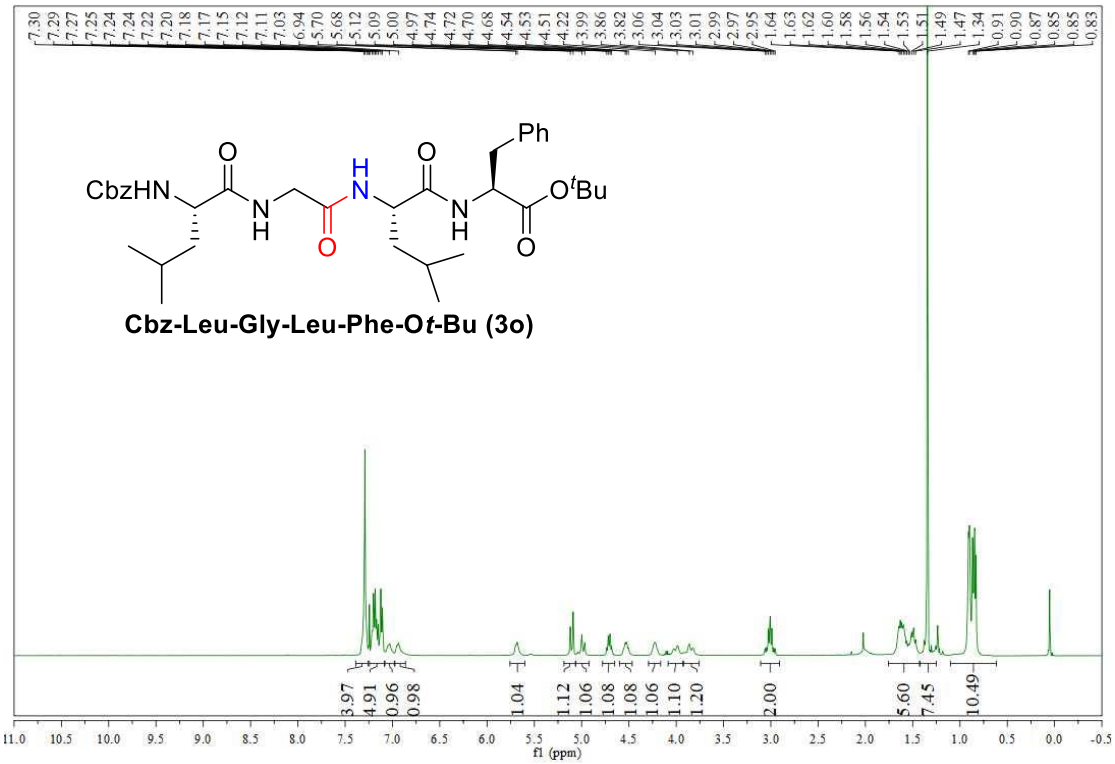

CbzHN
O
N
H
O
H
N
O
OMe
S−Me
Cbz-Leu-Gly-Met-OMe (3l)
f1 (ppm)

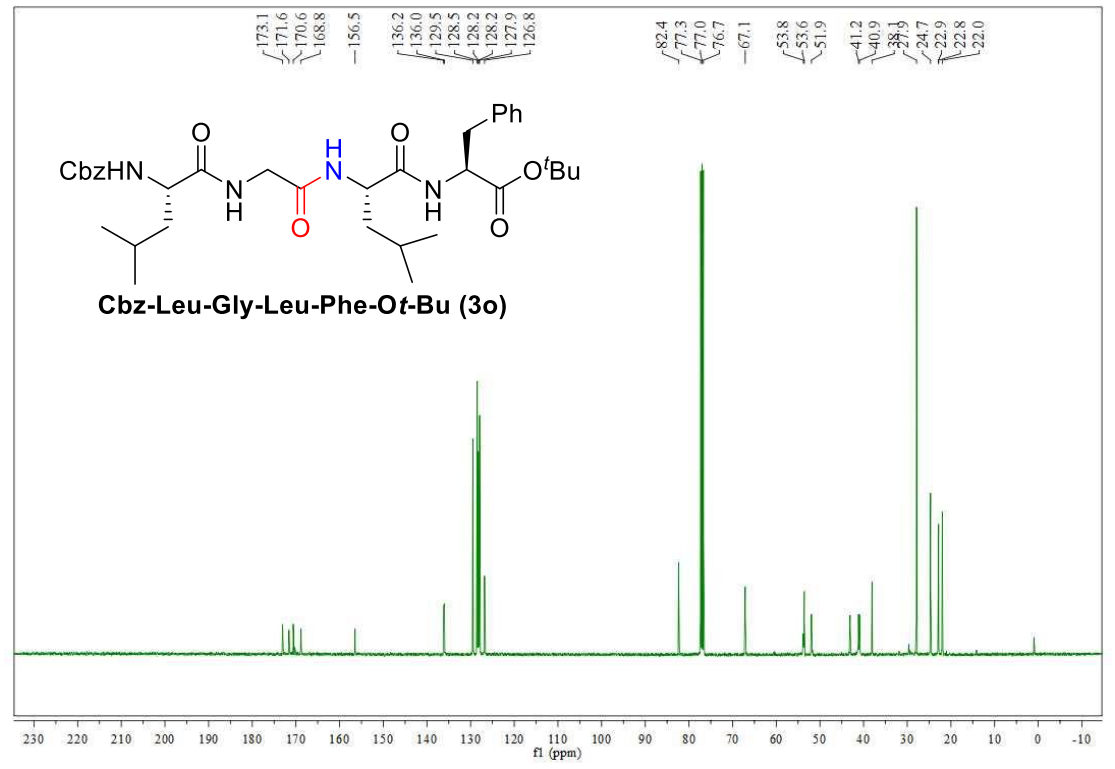

CbzHN
O
N
H
O
H
N
O
OMe
NH
Cbz-Leu-Gly-Trp-OMe (3m)
f1 (ppm)

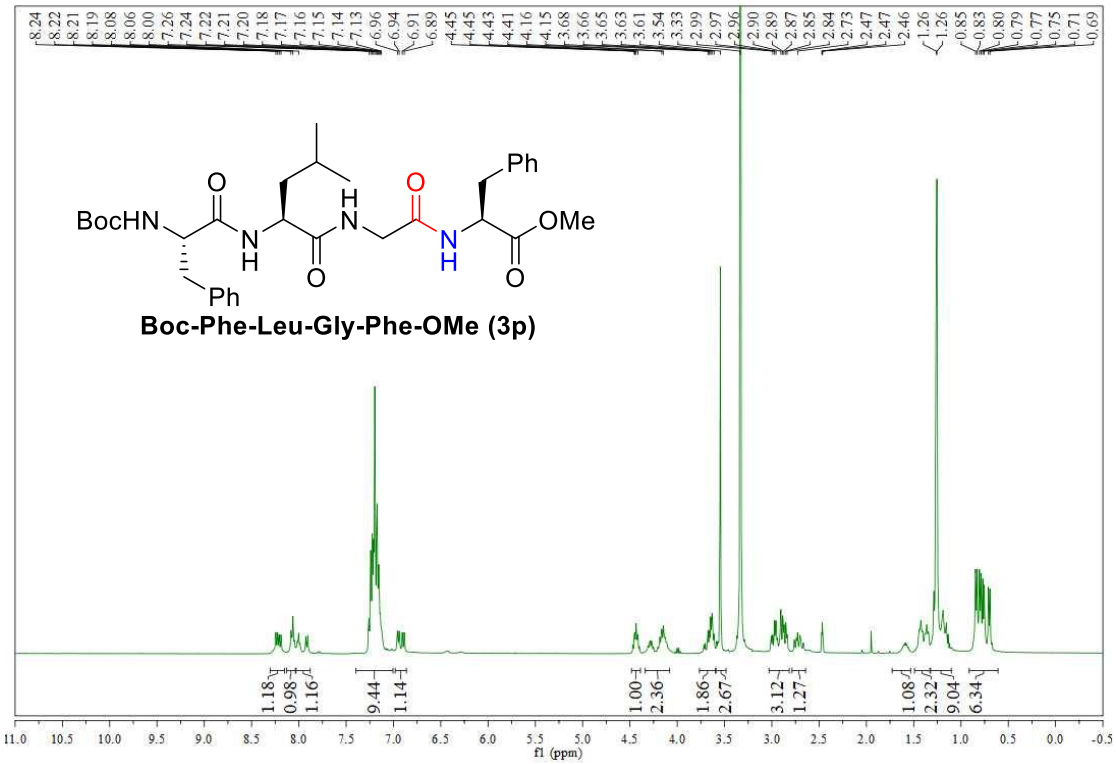

CbzHN
O
N
H
O
H
N
O
OMe
NH
Cbz-Leu-Gly-Trp-OMe (3m)
f1 (ppm)

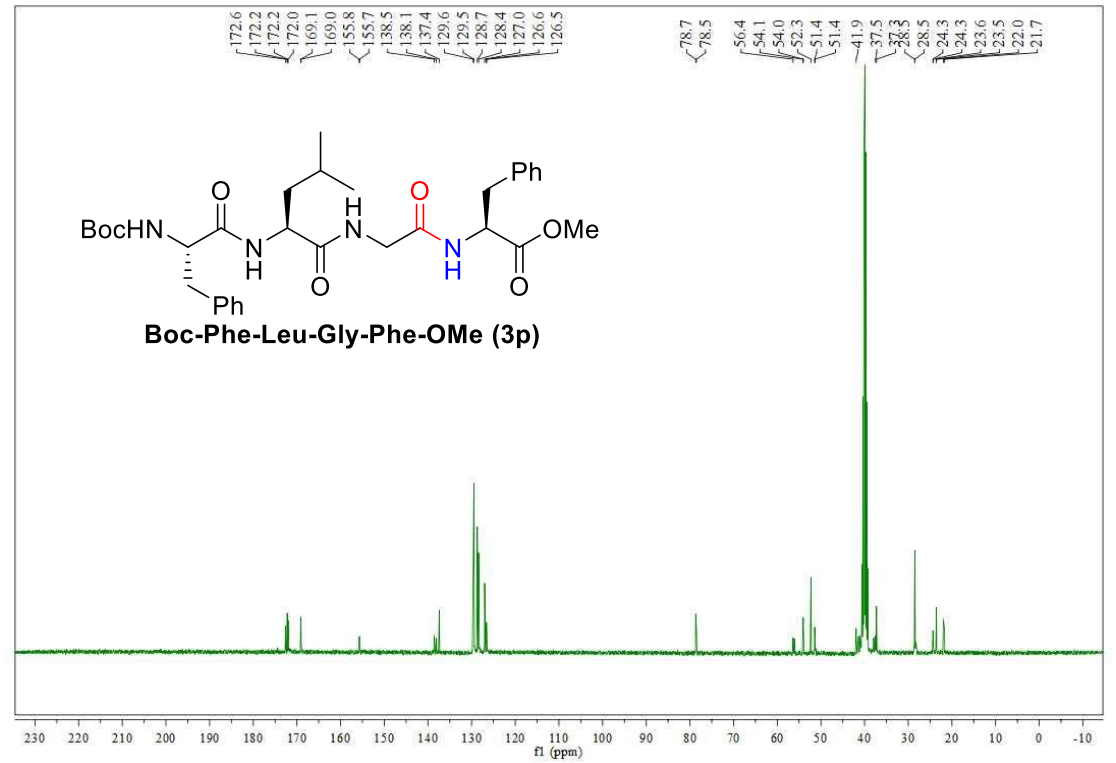

Boc-Phe-Gly-Leu-Phe-Ot-Bu (3n)
f1 (ppm)

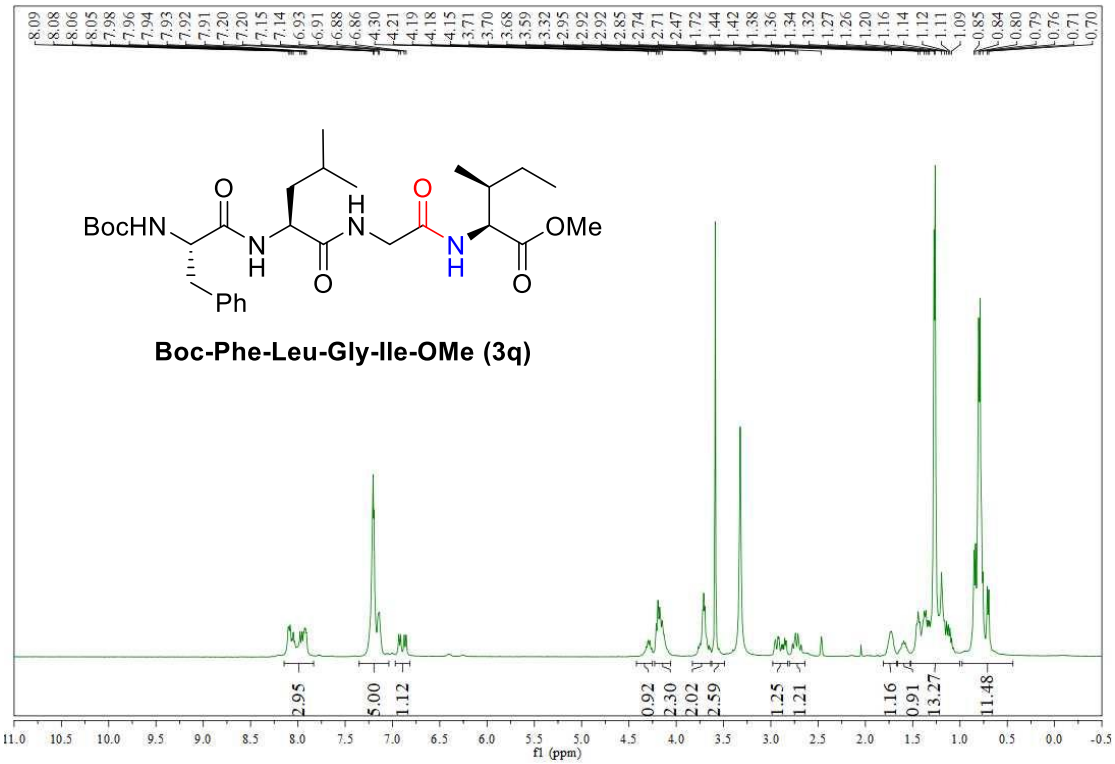

Boc-Phe-Gly-Leu-Phe-Ot-Bu (3n)
f1 (ppm)

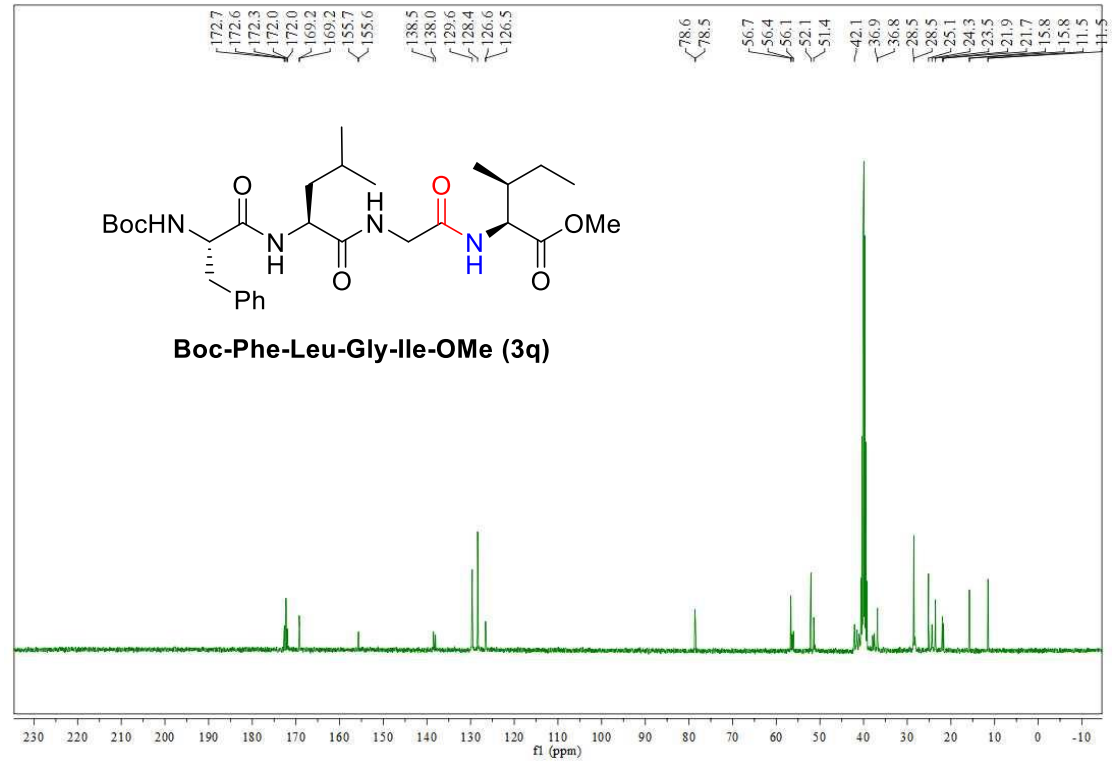

CbzHN
O
N
H
H
N
O
N
H
O
Ph
O
O
O^{t}Bu
Cbz-Leu-Gly-Leu-Phe-O*t*-Bu (3o)
f1 (ppm)

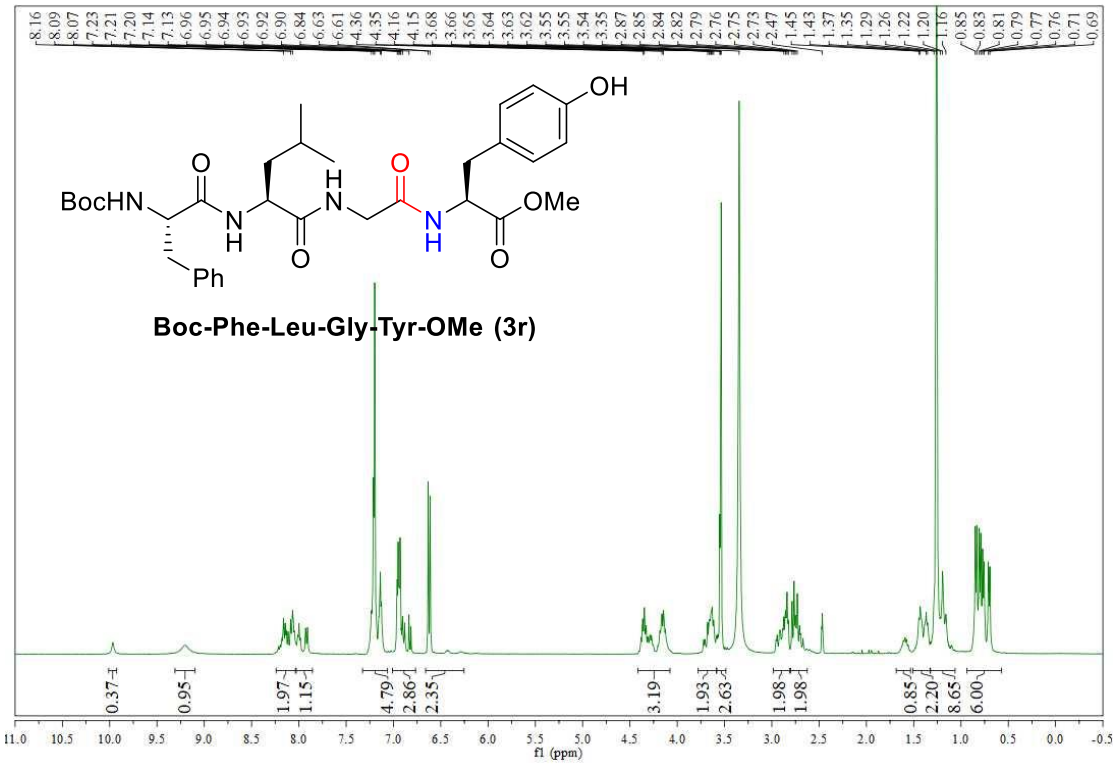

CbzHN
O
N
H
H
N
O
N
H
O
Ph
O
O
O^{t}Bu
Cbz-Leu-Gly-Leu-Phe-O*t*-Bu (3o)
f1 (ppm)

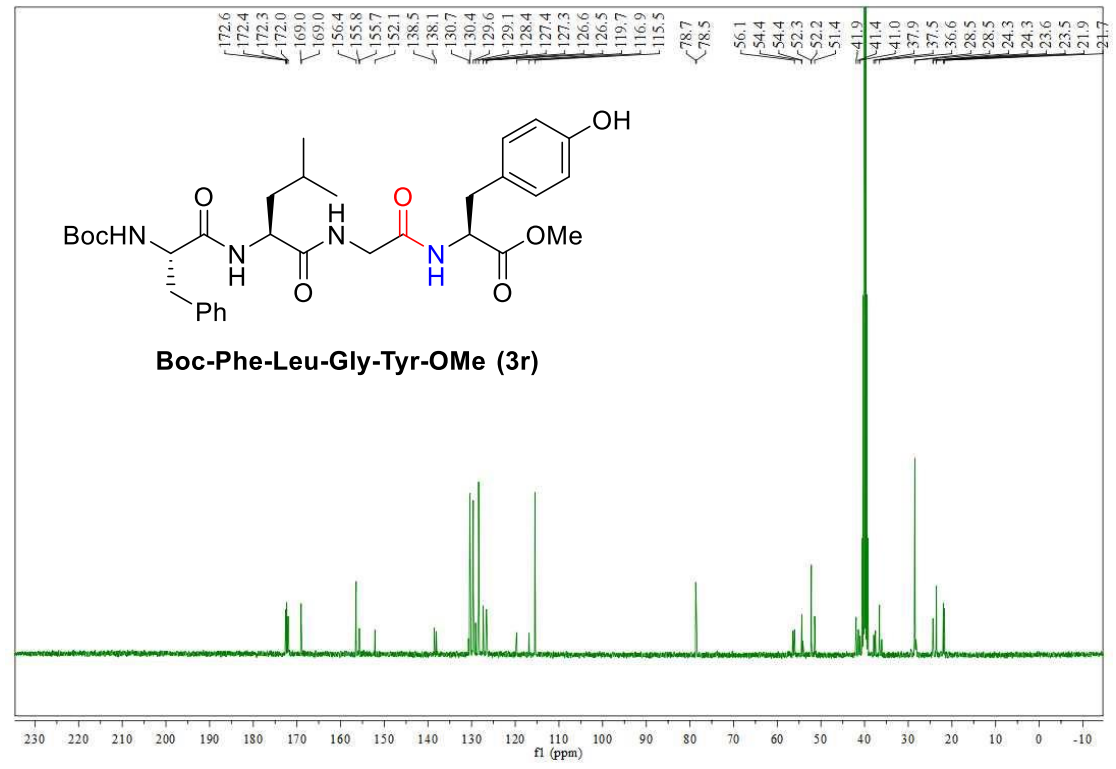

BocHN
O
N
H
O
H
N
O
N
H
Ph
OMe
O
Ph
Boc-Phe-Leu-Gly-Phe-OMe (3p)
f1 (ppm)

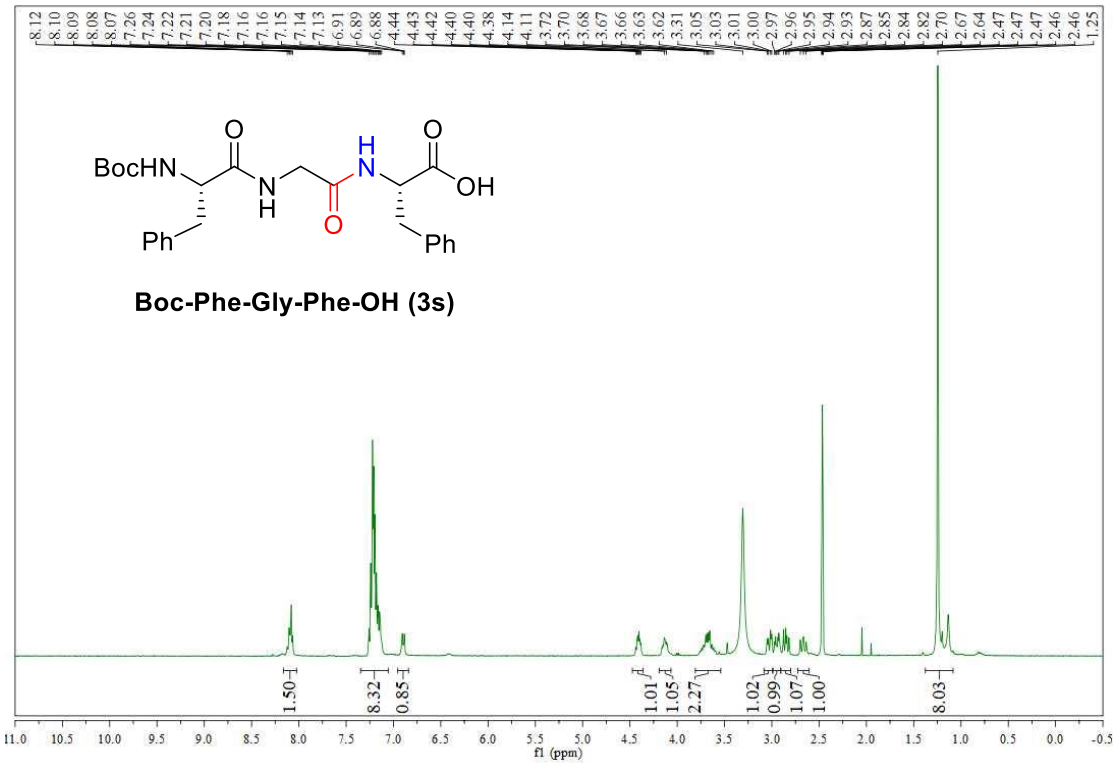

BocHN
O
N
H
O
H
N
O
N
H
Ph
OMe
O
Ph
Boc-Phe-Leu-Gly-Phe-OMe (3p)
f1 (ppm)

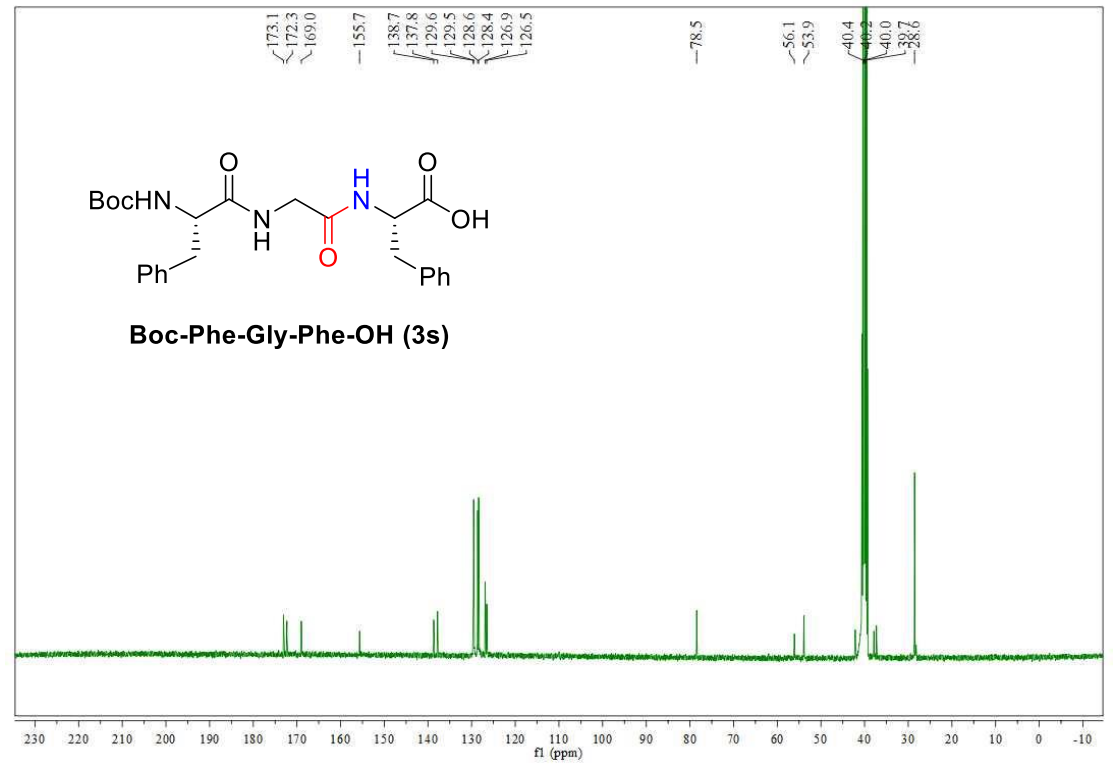

BocHN
O
N
H
O
H
N
O
N
H
OMe
O
Ph
Boc-Phe-Leu-Gly-Ile-OMe (3q)
f1 (ppm)

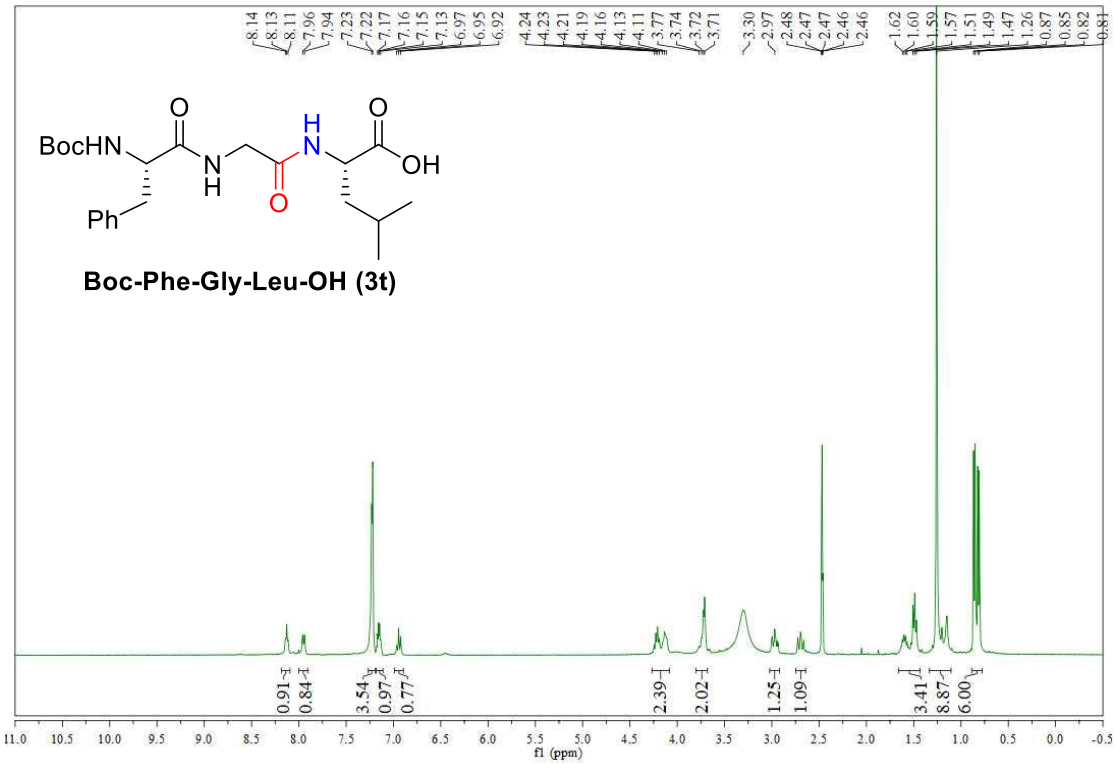

BocHN
O
N
H
O
H
N
O
N
H
OMe
O
Ph
Boc-Phe-Leu-Gly-Ile-OMe (3q)
f1 (ppm)

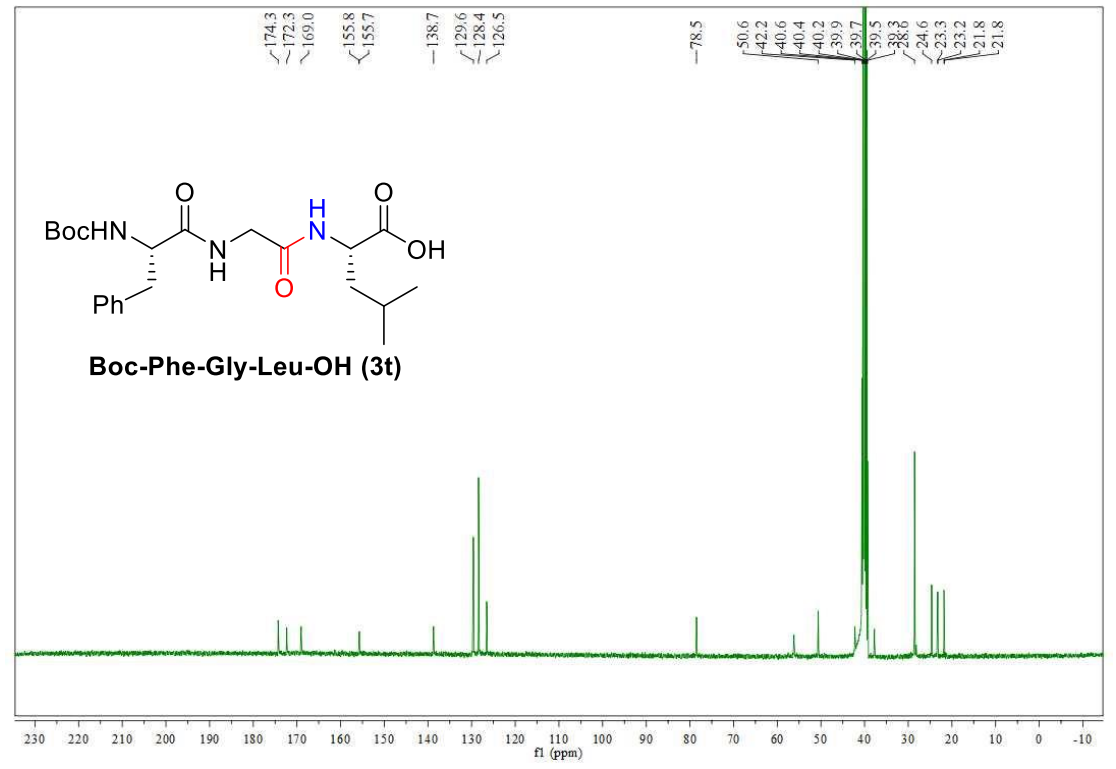

BocHN
Ph
OMe
OH
Boc-Phe-Leu-Gly-Tyr-OMe (3r)
f1 (ppm)

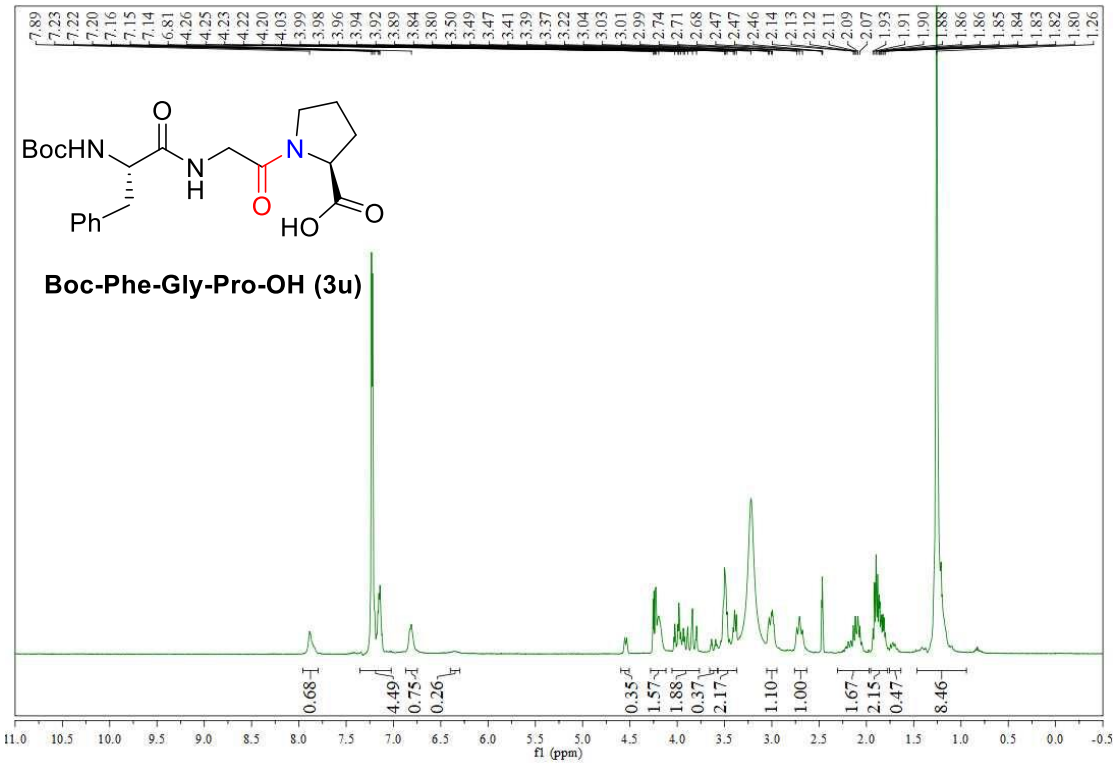

BocHN
Ph
OMe
OH
Boc-Phe-Leu-Gly-Tyr-OMe (3r)
f1 (ppm)


BocHN
O
N
H
H
N
O
O
OH
Ph
Ph
Boc-Phe-Gly-Phe-OH (3s)
f1 (ppm)

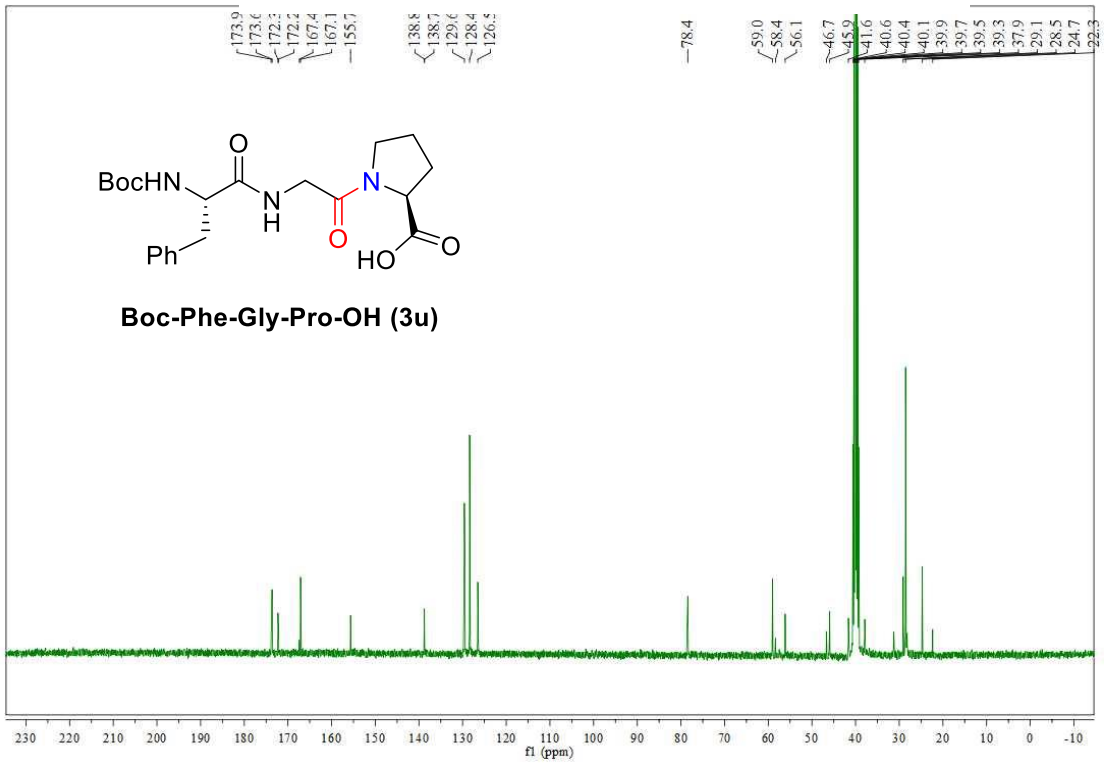

BocHN
O
N
H
H
N
O
O
OH
Ph
Ph
Boc-Phe-Gly-Phe-OH (3s)
f1 (ppm)

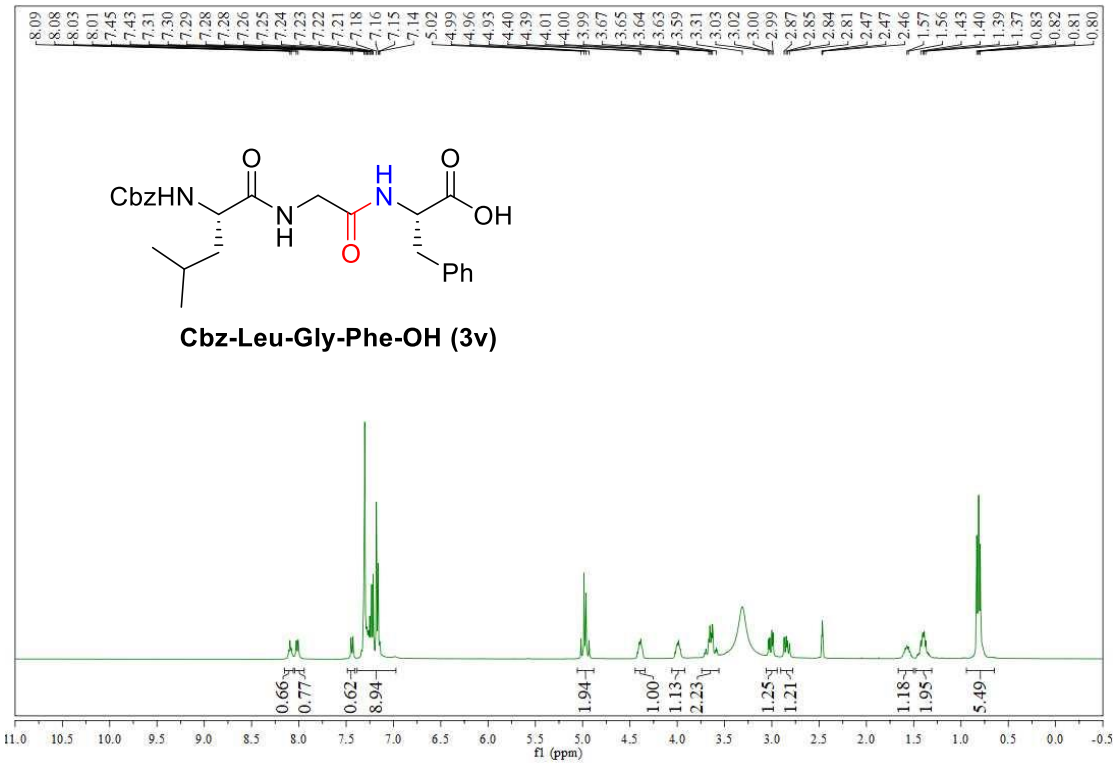

BocHN
O
N
H
H
N
O
O
OH
Ph
Boc-Phe-Gly-Leu-OH (3t)
f1 (ppm)

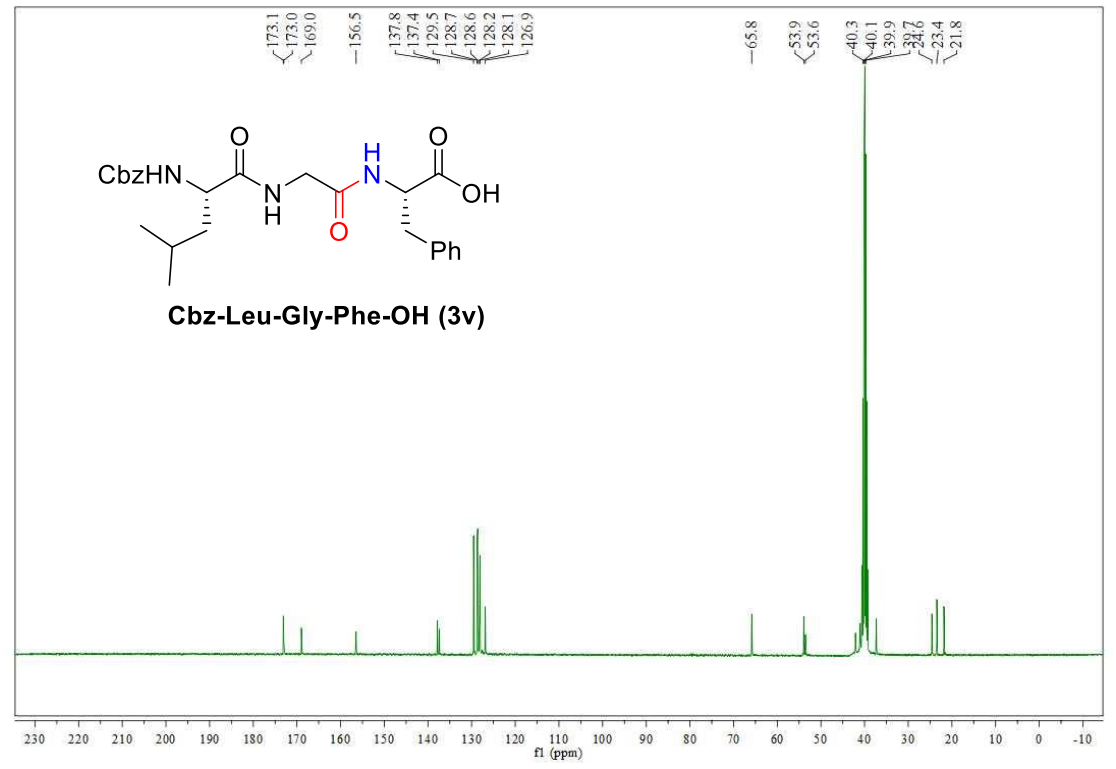

BocHN
O
N
H
H
N
O
O
OH
Ph
Boc-Phe-Gly-Leu-OH (3t)
f1 (ppm)

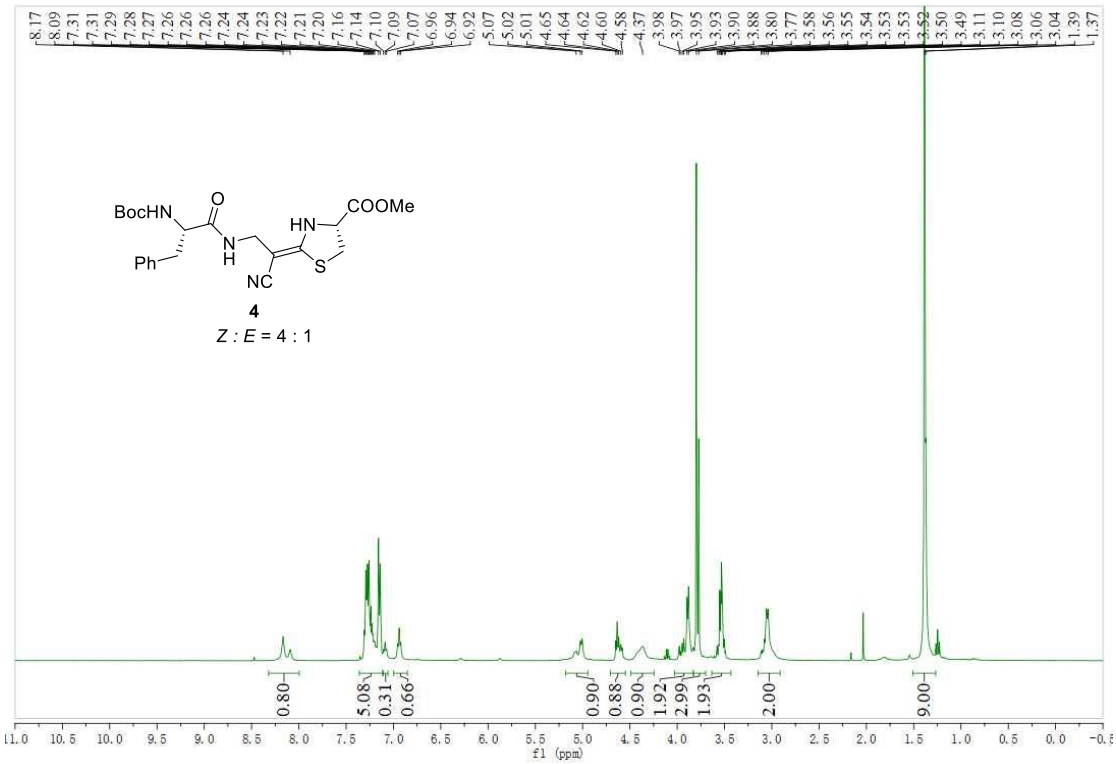


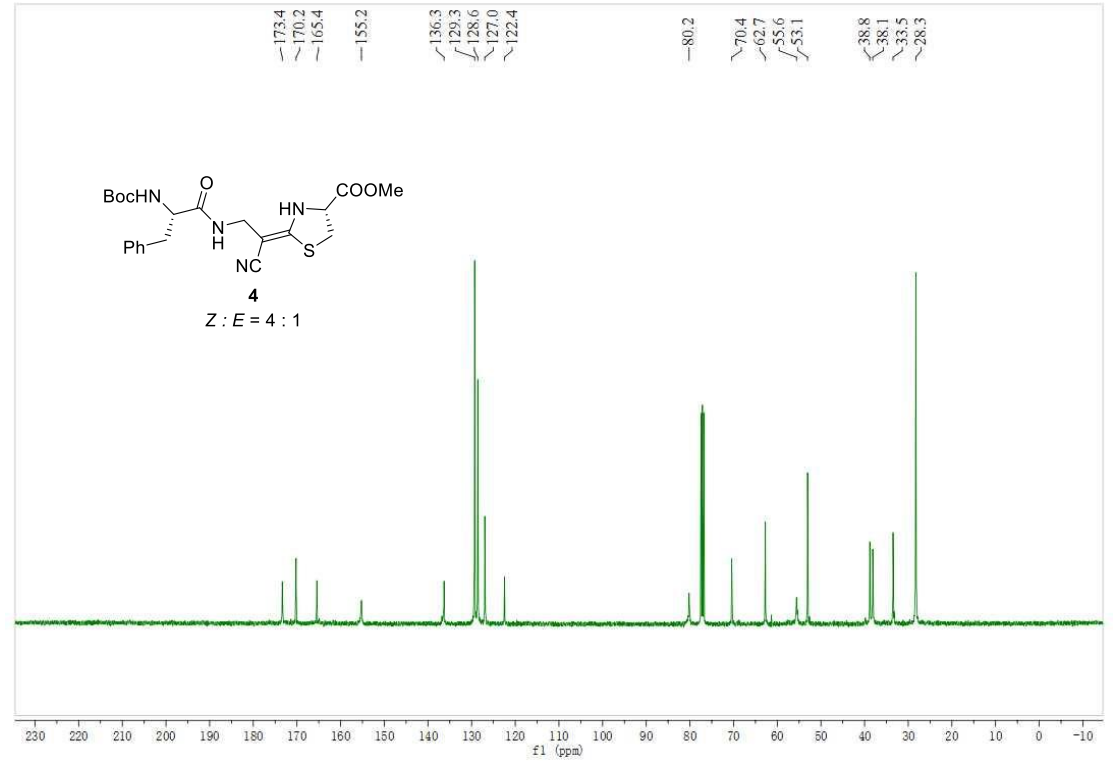
^{1}H NMR (399 Hz, DMSO-d6) of compound **3u** at 44 °C

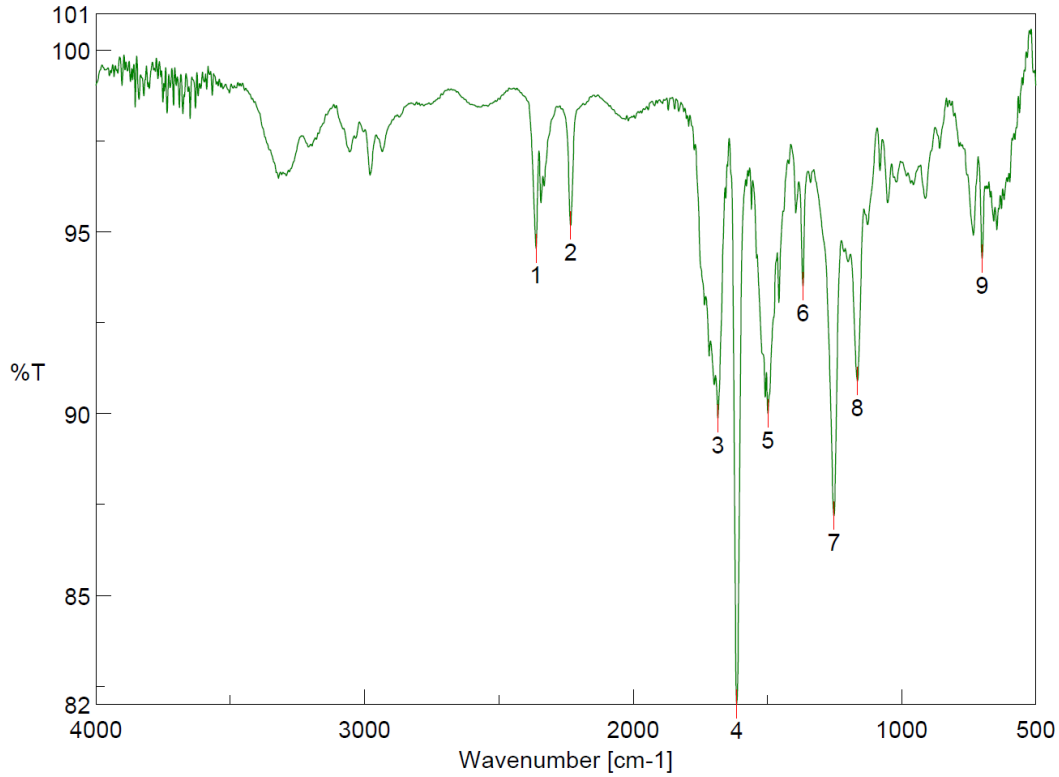



CbzHN
O
N
H
O
H
N
O
OH
Ph
Cbz-Leu-Gly-Phe-OH (3v)
f1 (ppm)


CbzHN
O
N
H
O
H
N
O
OH
Ph
Cbz-Leu-Gly-Phe-OH (3v)
f1 (ppm)

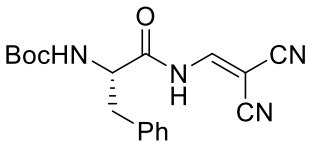

BocHN
O
N
H
Ph
HN
COOMe
S
NC
4
Z : E = 4 : 1
f1 (ppm)


BocHN
O
N
H
Ph
HN
COOMe
S
NC
4
Z : E = 4 : 1
f1 (ppm)

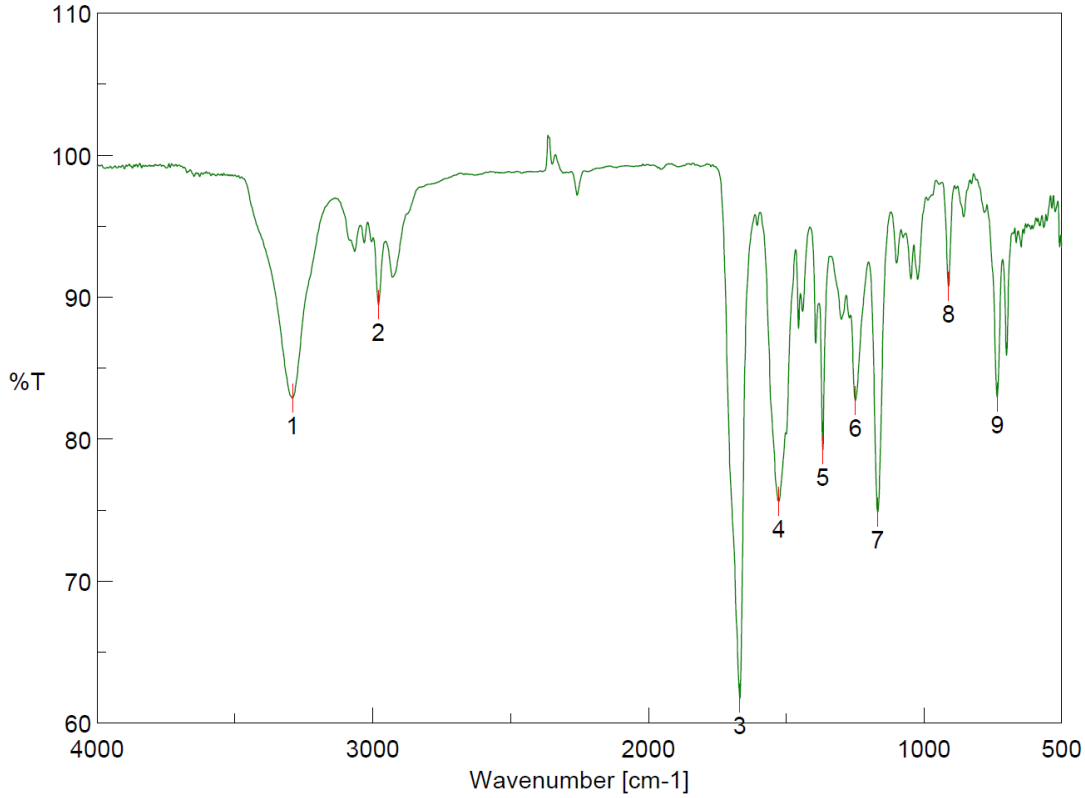


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	2361.41	94.5398	2	2233.16	95.1702
3	1683.55	89.8756	4	1614.13	82.0058
5	1497.45	89.9942	6	1367.28	93.5045
7	1251.58	87.1849	8	1164.79	90.8964
9	700.034	94.2641			

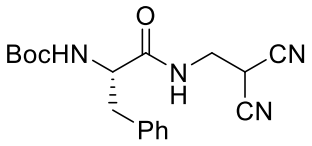


s1

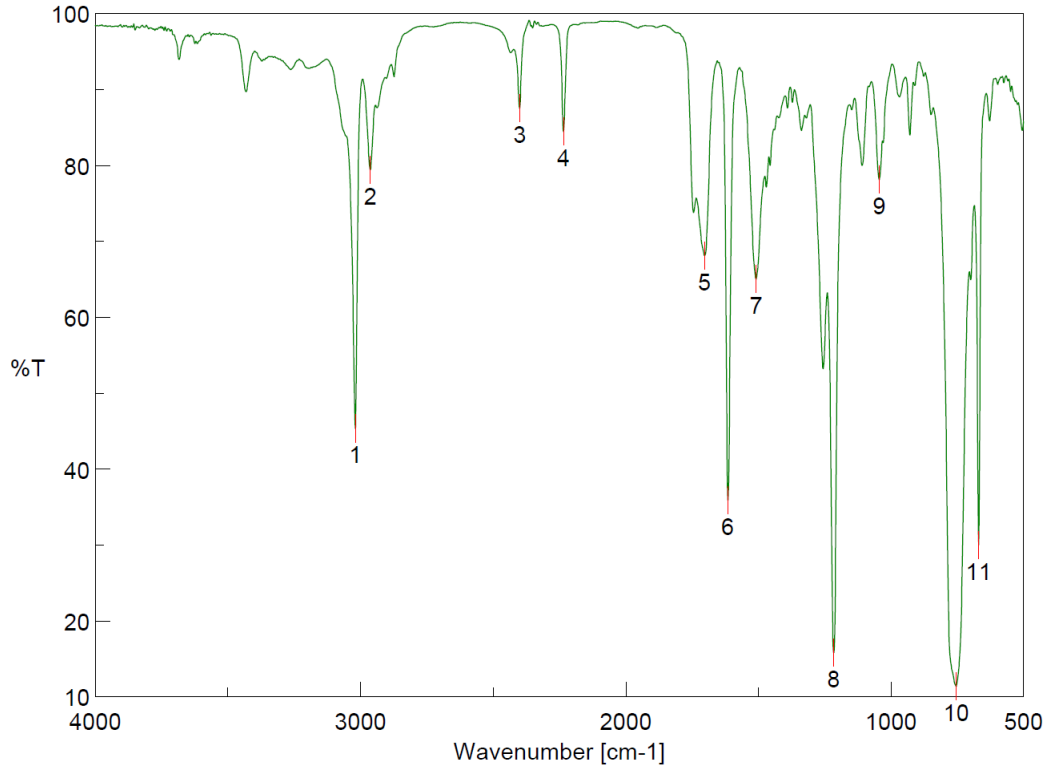


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3290.93	82.8717	2	2979.48	89.4431
3	1668.12	61.7319	4	1526.38	75.6103
5	1367.28	79.2345	6	1248.68	82.6922
7	1167.69	74.8617	8	910.236	90.744
9	733.782	82.9559			

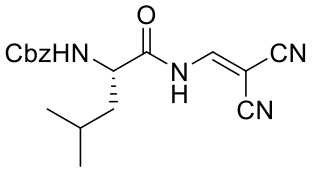


1a

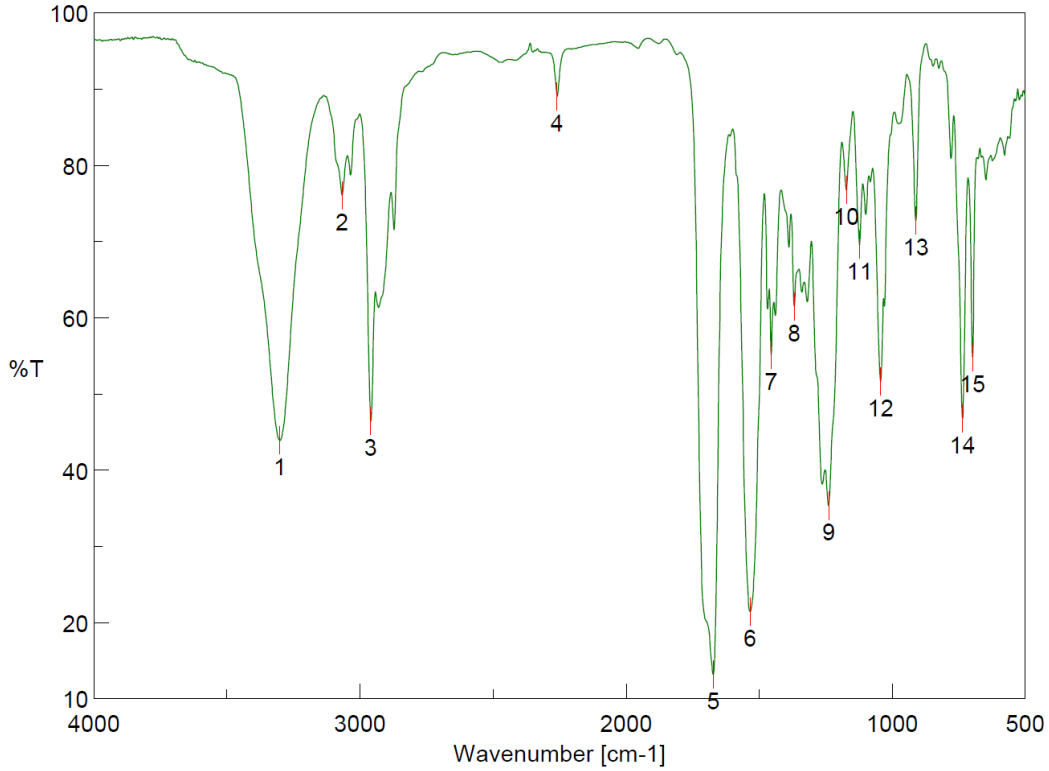


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3019.98	45.3128	2	2963.09	79.3906
3	2399.98	87.4939	4	2235.09	84.4156
5	1702.84	68.0827	6	1615.09	35.88
7	1508.06	64.9972	8	1215.9	15.752
9	1044.26	78.0936	10	754.031	11.349
11	669.178	29.9544			

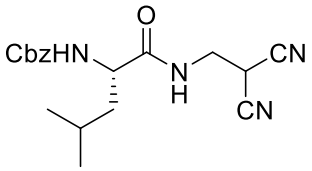


S2

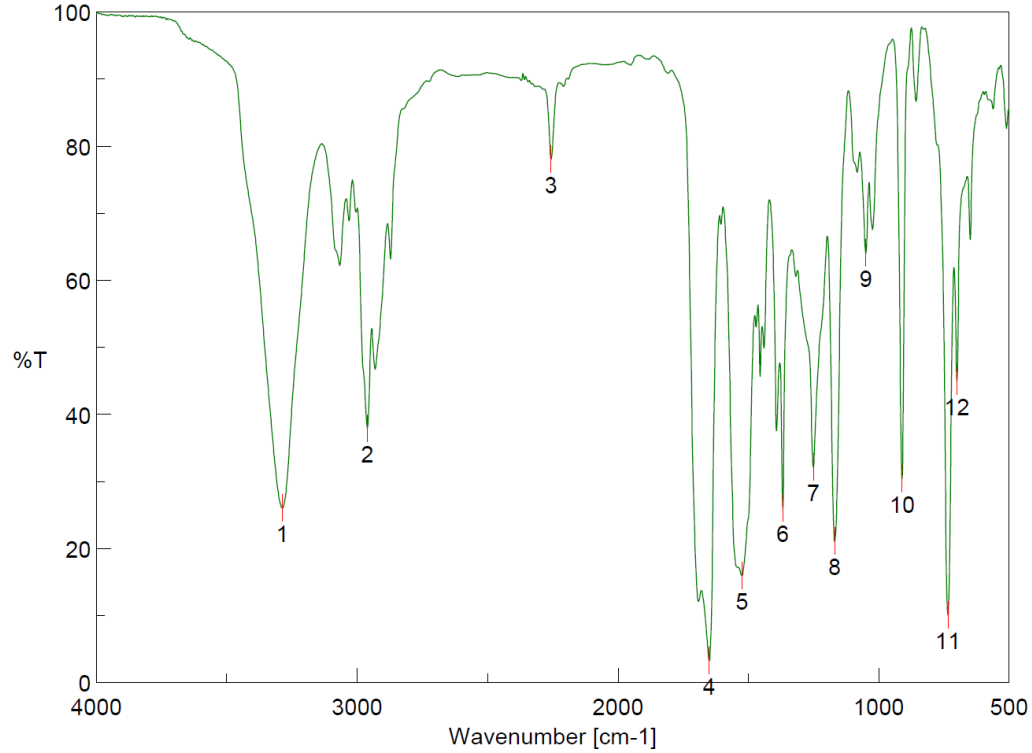


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3300.57	43.8452	2	3067.23	76.0267
3	2959.23	46.4196	4	2259.2	89.0162
5	1671.98	13.1513	6	1534.1	21.4172
7	1455.03	55.0796	8	1368.25	61.4621
9	1239.04	35.2847	10	1172.51	76.7304
11	1122.37	69.5005	12	1043.3	51.5826
13	911.201	72.7499	14	735.71	46.7239
15	698.105	54.7491			

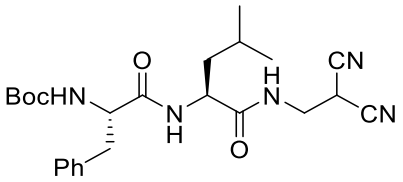


1b

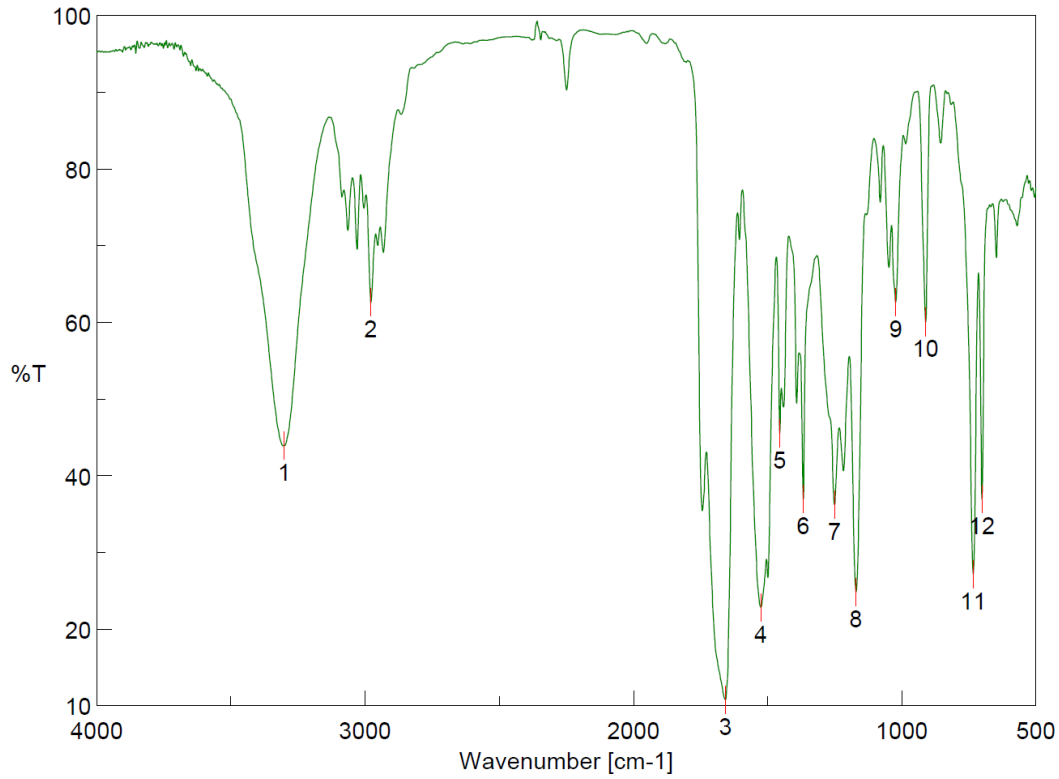


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3287.07	26.0277	2	2961.16	37.9286
3	2256.31	78.0101	4	1649.8	3.2532
5	1524.45	15.903	6	1368.25	25.9993
7	1250.61	32.091	8	1168.65	21.0487
9	1049.09	64.0349	10	910.236	30.4007
11	734.746	10.0449	12	700.034	44.9413

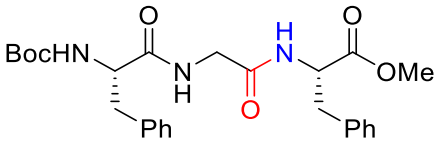


1c

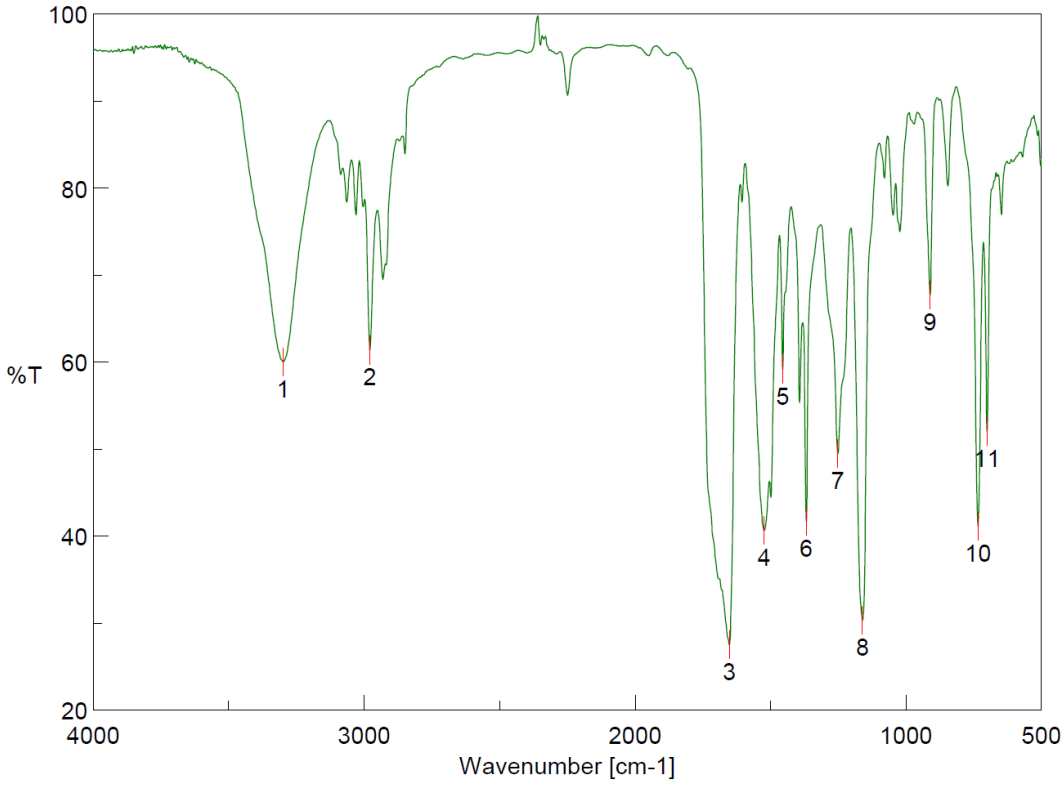


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3301.54	43.8714	2	2978.52	62.5503
3	1656.55	10.7298	4	1525.42	22.8094
5	1455.03	45.4611	6	1367.28	36.9288
7	1250.61	36.1734	8	1170.58	24.7846
9	1022.09	62.5819	10	911.201	59.9884
11	733.782	27.123	12	700.998	36.9299

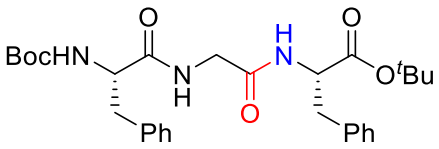


Boc-Phe-Gly-Phe-OMe (3a)

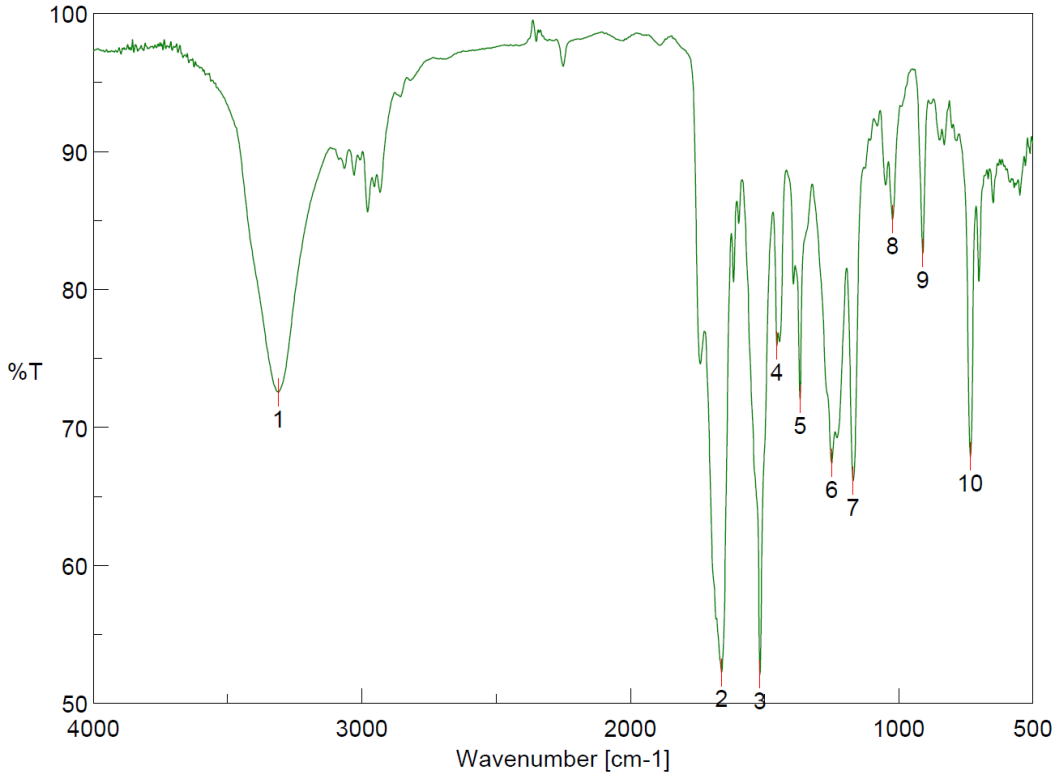


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3298.64	60.0093	2	2978.52	61.3614
3	1651.73	27.4668	4	1523.49	40.6265
5	1455.03	59.1203	6	1368.25	41.7211
7	1251.58	49.5033	8	1159.97	30.2829
9	911.201	67.674	10	733.782	41.0872
11	700.034	51.9936			

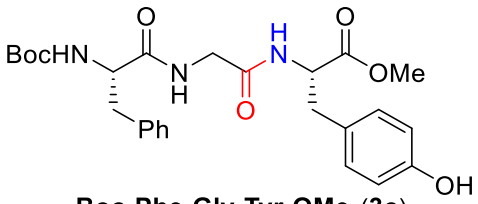


Boc-Phe-Gly-Phe-O*t*-Bu (3b)

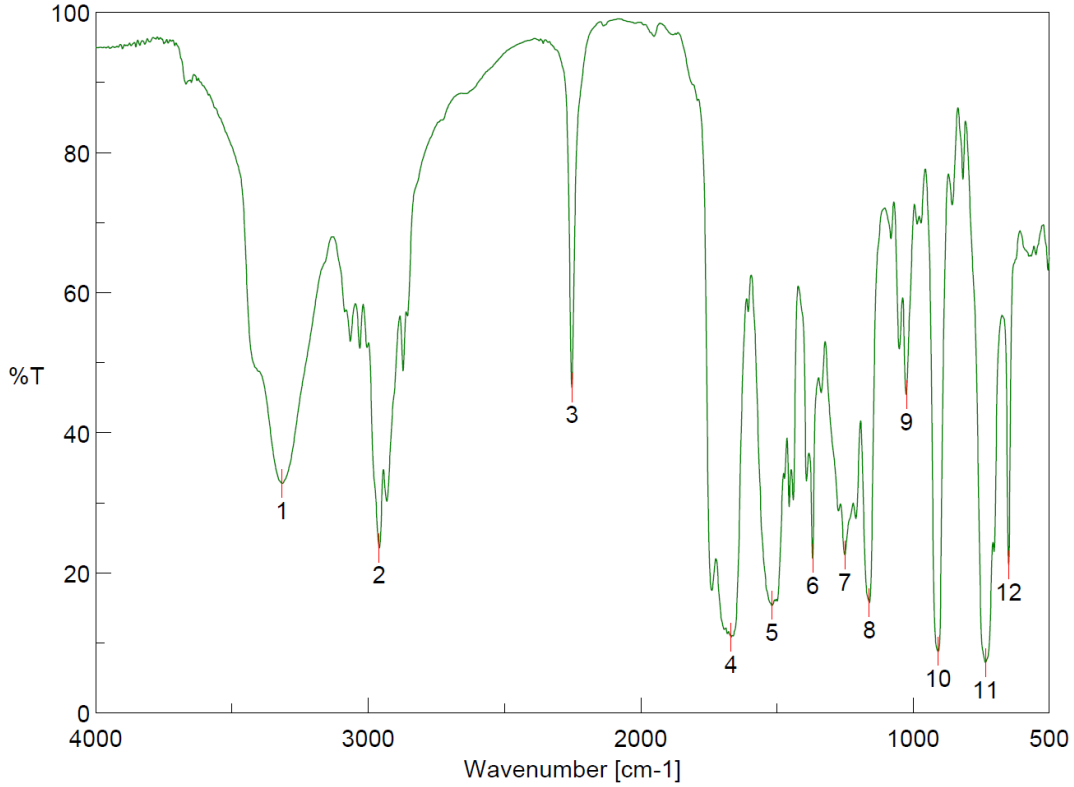


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3311.18	72.5242	2	1660.41	52.2481
3	1516.74	52.0785	4	1454.06	75.9124
5	1367.28	72.0527	6	1249.65	67.3978
7	1169.62	66.1049	8	1023.05	85.067
9	910.236	82.6095	10	732.817	67.8636

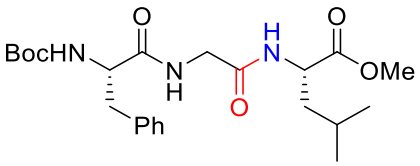


Boc-Phe-Gly-Tyr-OMe (3c)

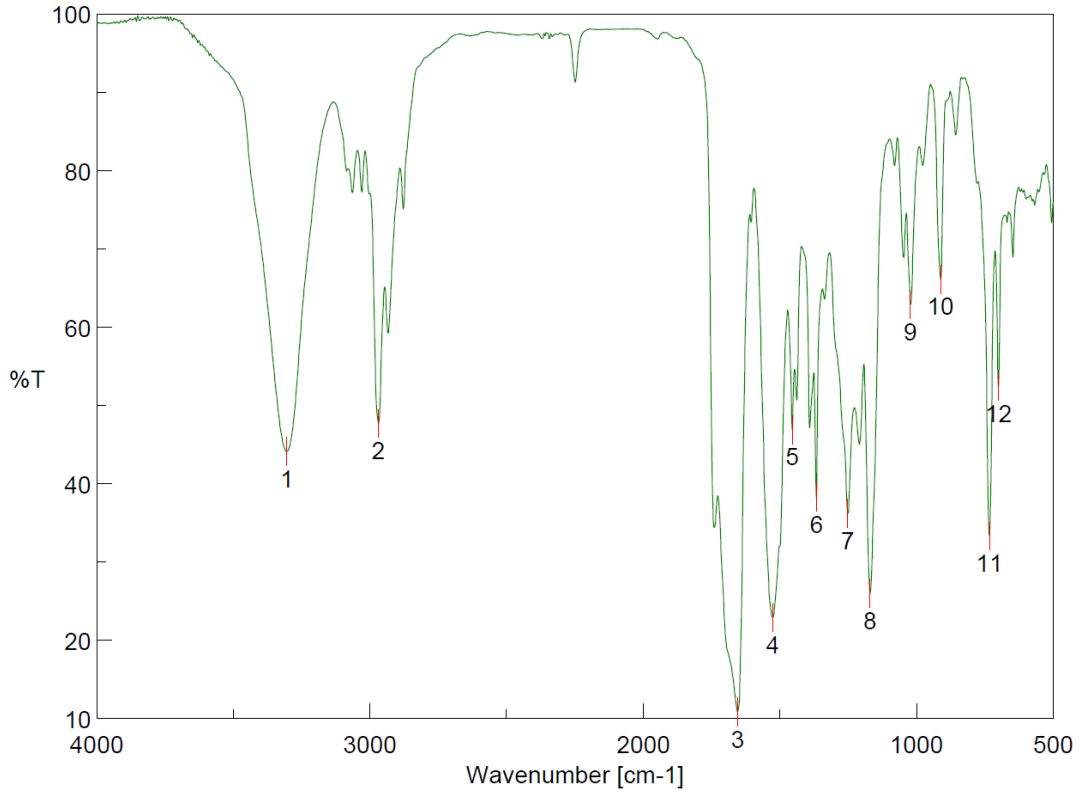


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3316	32.7416	2	2959.23	23.5087
3	2252.45	46.4209	4	1668.12	10.8064
5	1517.7	15.3303	6	1368.25	22.0287
7	1250.61	22.5466	8	1159.97	15.7076
9	1024.98	45.4259	10	907.344	8.72998
11	733.782	7.17211	12	648.929	21.1374

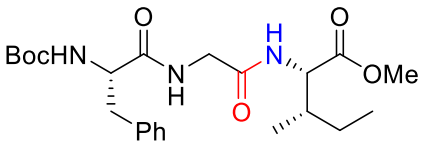


Boc-Phe-Gly-Leu-OMe (3d)

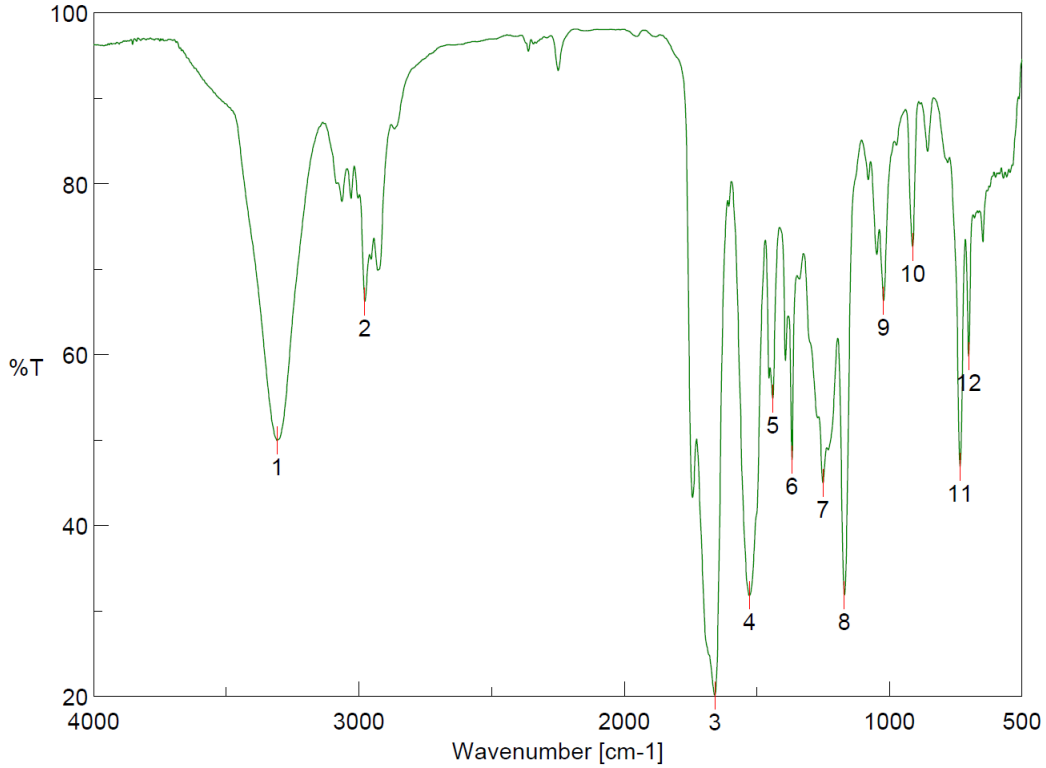


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3304.43	44.096	2	2968.87	47.6925
3	1654.62	10.8463	4	1526.38	22.9138
5	1455.03	46.8776	6	1366.32	38.3292
7	1251.58	36.1985	8	1169.62	26.0006
9	1022.09	62.8908	10	912.165	66.1071
11	733.782	33.304	12	700.034	52.4097

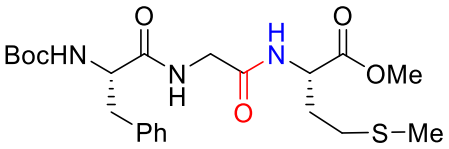


Boc-Phe-Gly-Ile-OMe (3e)

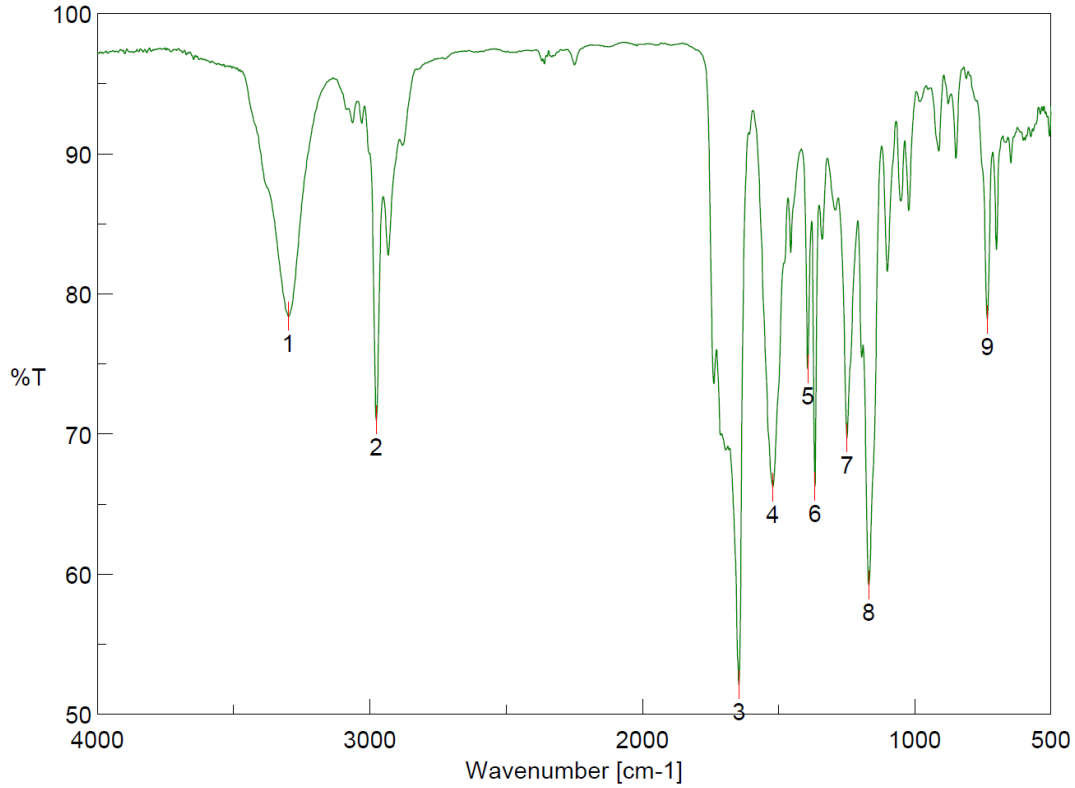


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3308.29	49.9198	2	2977.55	66.1941
3	1658.48	20.1148	4	1527.35	31.7812
5	1439.6	54.863	6	1367.28	47.6799
7	1250.61	44.9626	8	1169.62	31.8551
9	1022.09	66.2899	10	912.165	72.6073
11	732.817	46.8598	12	700.998	59.8015

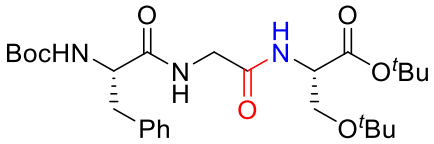


Boc-Phe-Gly-Met-OMe (3f)

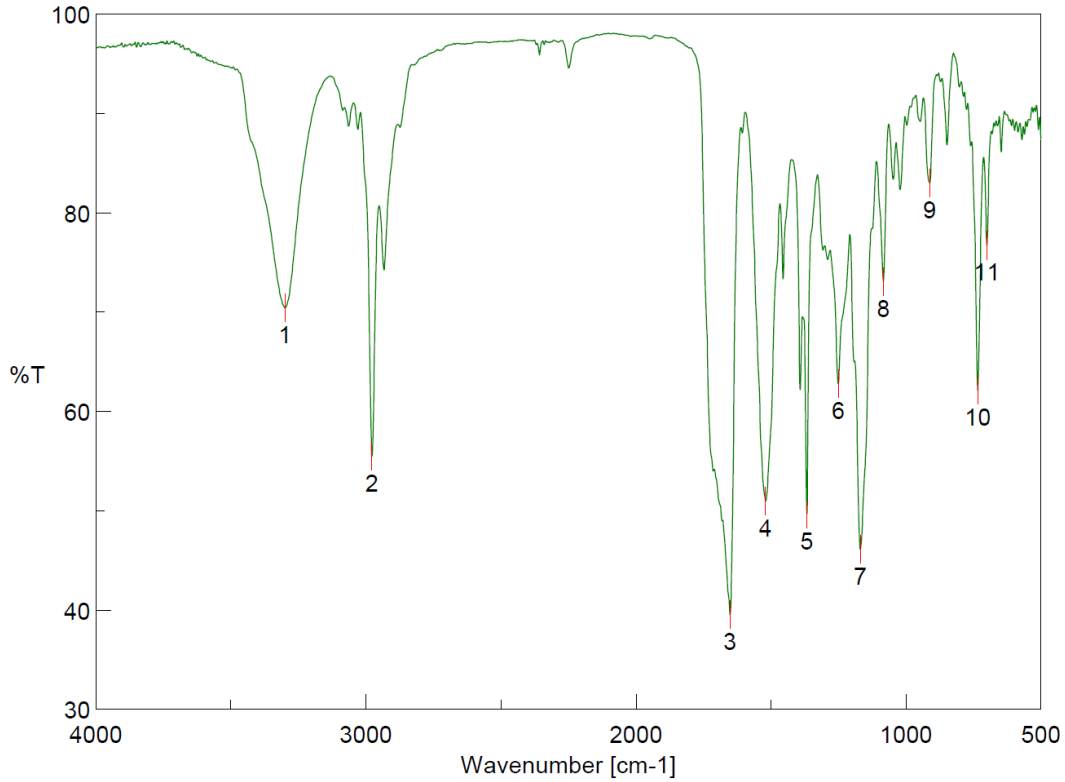


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3298.64	78.3825	2	2976.59	70.9697
3	1644.98	52.0729	4	1520.6	66.2115
5	1392.35	74.6342	6	1366.32	66.2503
7	1248.68	69.7086	8	1168.65	59.2233
9	733.782	78.1645			

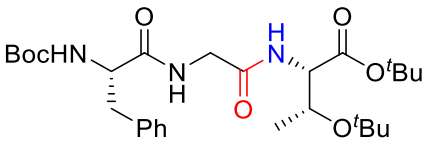


Boc-Phe-Gly-Ser(O*t*-Bu)-O*t*-Bu (3g)

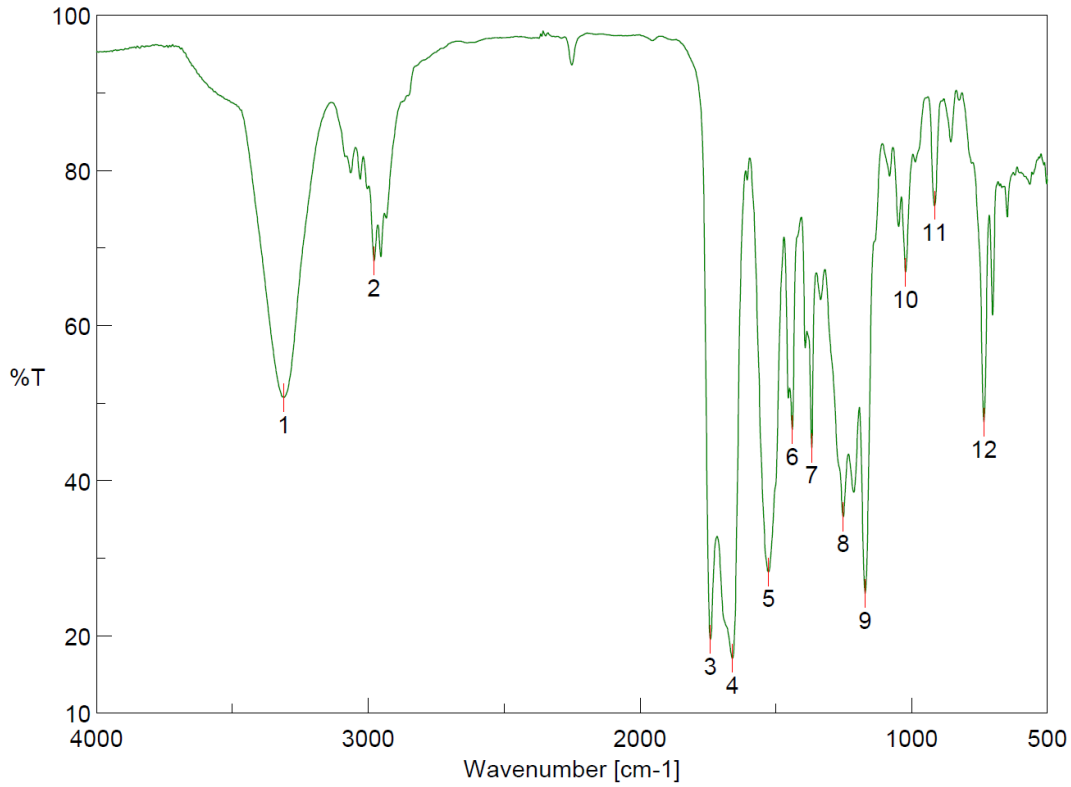


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3299.61	70.391	2	2977.55	55.4201
3	1651.73	39.4972	4	1519.63	50.9488
5	1367.28	49.6368	6	1250.61	62.7666
7	1168.65	46.111	8	1083.8	73.0417
9	912.165	82.9818	10	733.782	62.0768
11	700.034	76.708			

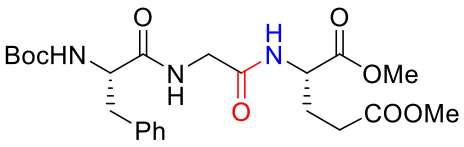


Boc-Phe-Gly-Thr(O*t*-Bu)-O*t*-Bu (3h)

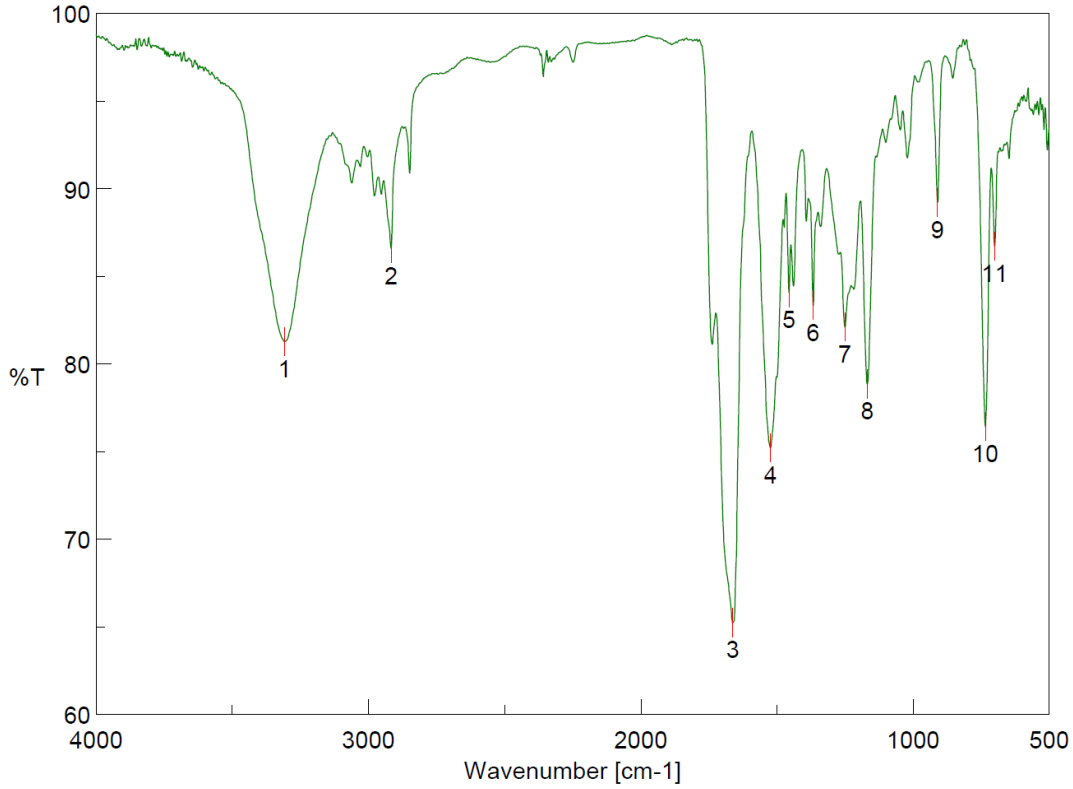


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3310.21	50.6815	2	2978.52	68.2958
3	1740.44	19.4793	4	1659.45	17.035
5	1526.38	28.1887	6	1438.64	46.5453
7	1367.28	44.1716	8	1251.58	35.2838
9	1170.58	25.4031	10	1022.09	66.8709
11	915.058	75.4201	12	733.782	47.5129

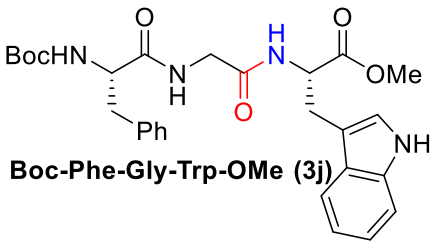


Boc-Phe-Gly-Glu(OMe)-OMe (3i)

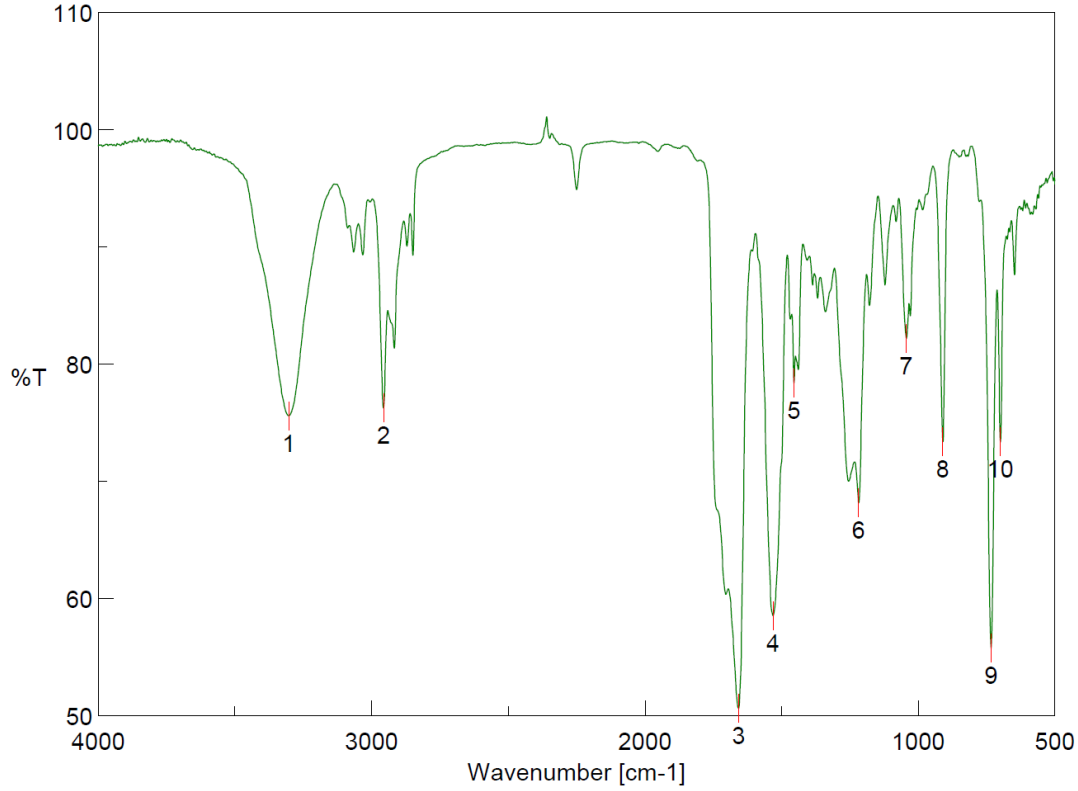


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3308.29	81.275	2	2916.81	86.59
3	1662.34	65.2255	4	1523.49	75.2082
5	1455.99	84.0545	6	1367.28	83.3322
7	1250.61	82.0954	8	1167.69	78.8467
9	910.236	89.2091	10	734.746	76.4169
11	700.034	86.6986			

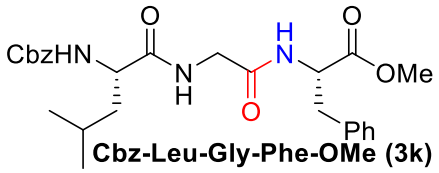


Boc-Phe-Gly-Trp-OMe (3j)

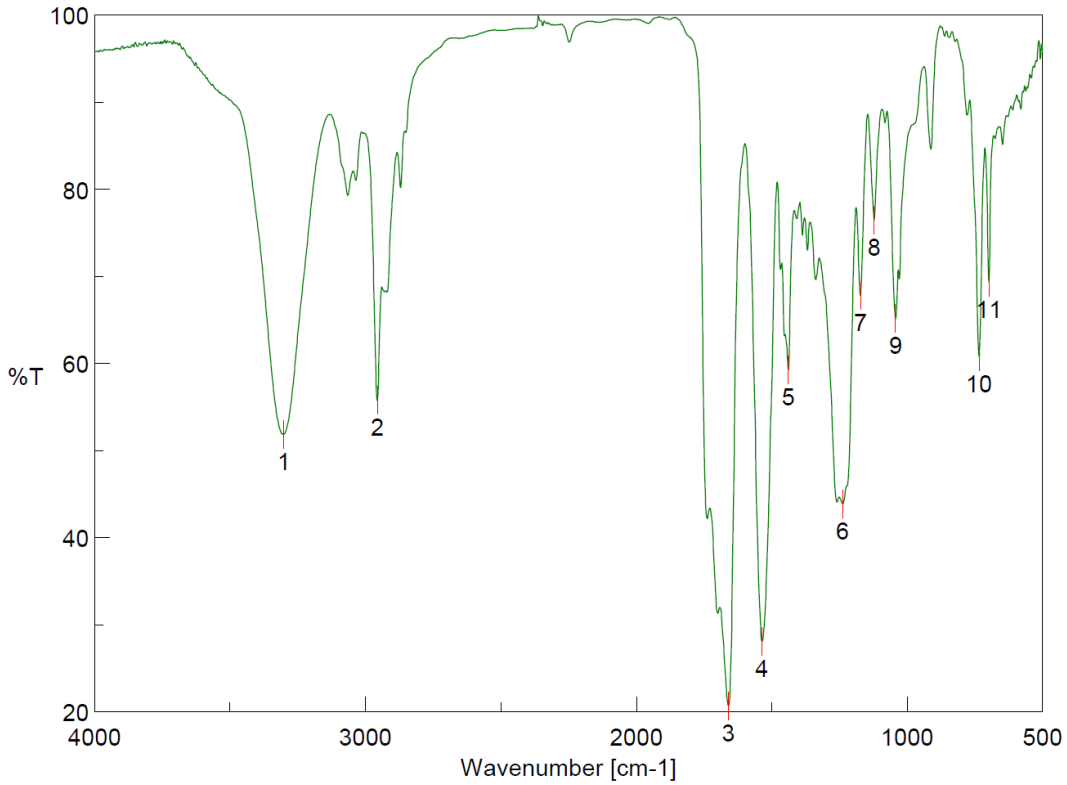


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3303.46	75.58	2	2956.34	76.2501
3	1657.52	50.6284	4	1531.2	58.5182
5	1455.03	78.3637	6	1217.83	68.1593
7	1043.3	82.1589	8	910.236	73.3698
9	732.817	55.7579	10	699.069	73.3381



Cbz-Leu-Gly-Phe-OMe (3k)



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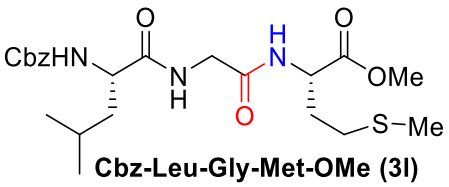
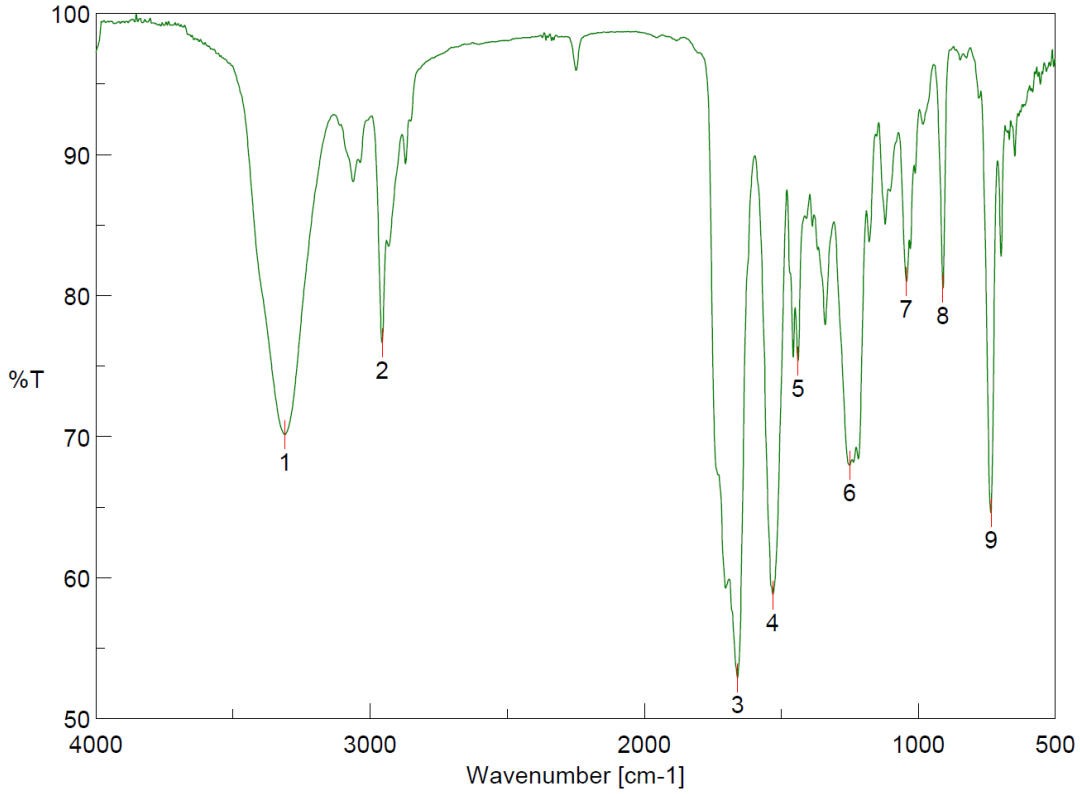
No.	位置	強度	No.	位置	強度
1	3301.54	51.839	2	2956.34	55.7298
3	1660.41	20.6665	4	1536.02	28.0911
5	1438.64	59.25	6	1238.08	43.841
7	1172.51	67.7781	8	1121.4	76.4294
9	1042.34	65.1036	10	734.746	60.7577
11	698.105	69.3008			

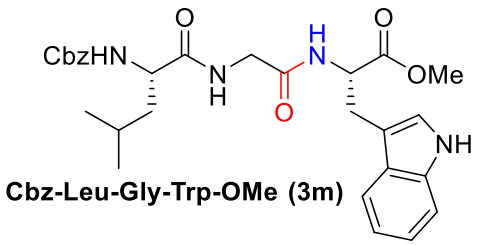


Cbz-Leu-Gly-Met-OMe (3l)

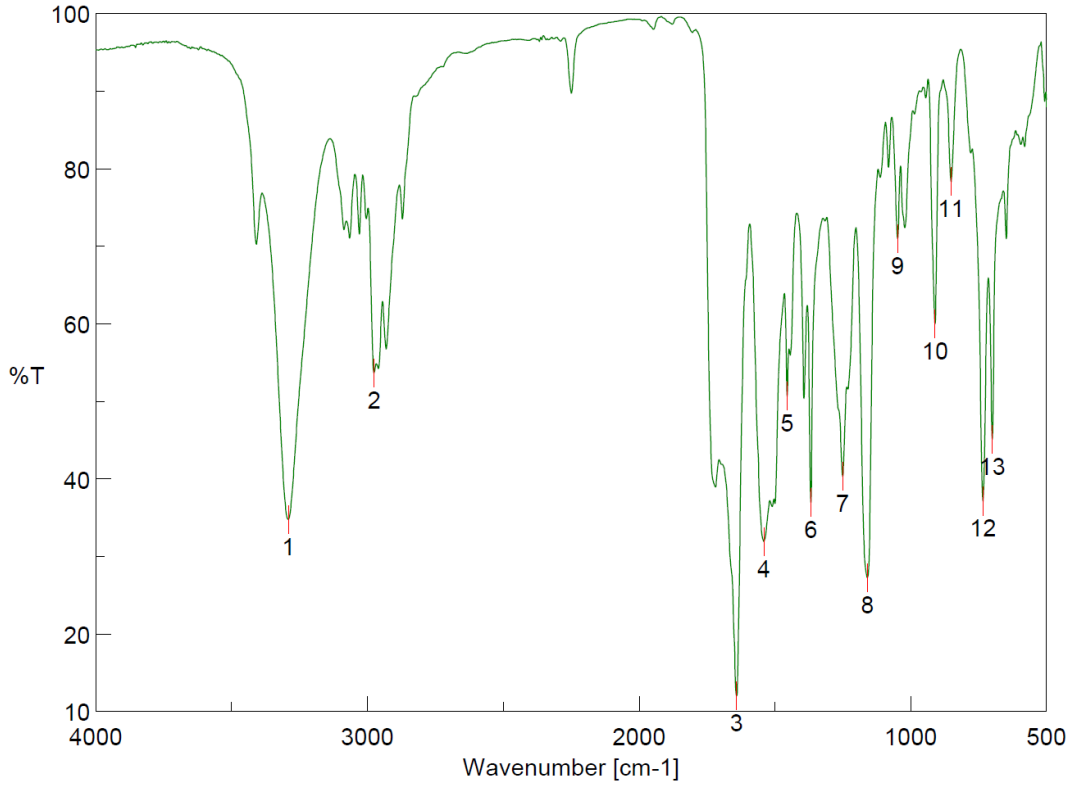


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3311.18	70.1075	2	2956.34	76.6451
3	1659.45	52.9004	4	1530.24	58.7539
5	1438.64	75.3581	6	1250.61	67.9598
7	1043.3	80.9608	8	910.236	80.5249
9	734.746	64.5908			

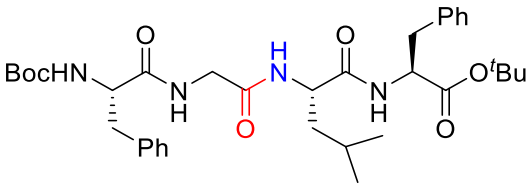


Cbz-Leu-Gly-Trp-OMe (3m)

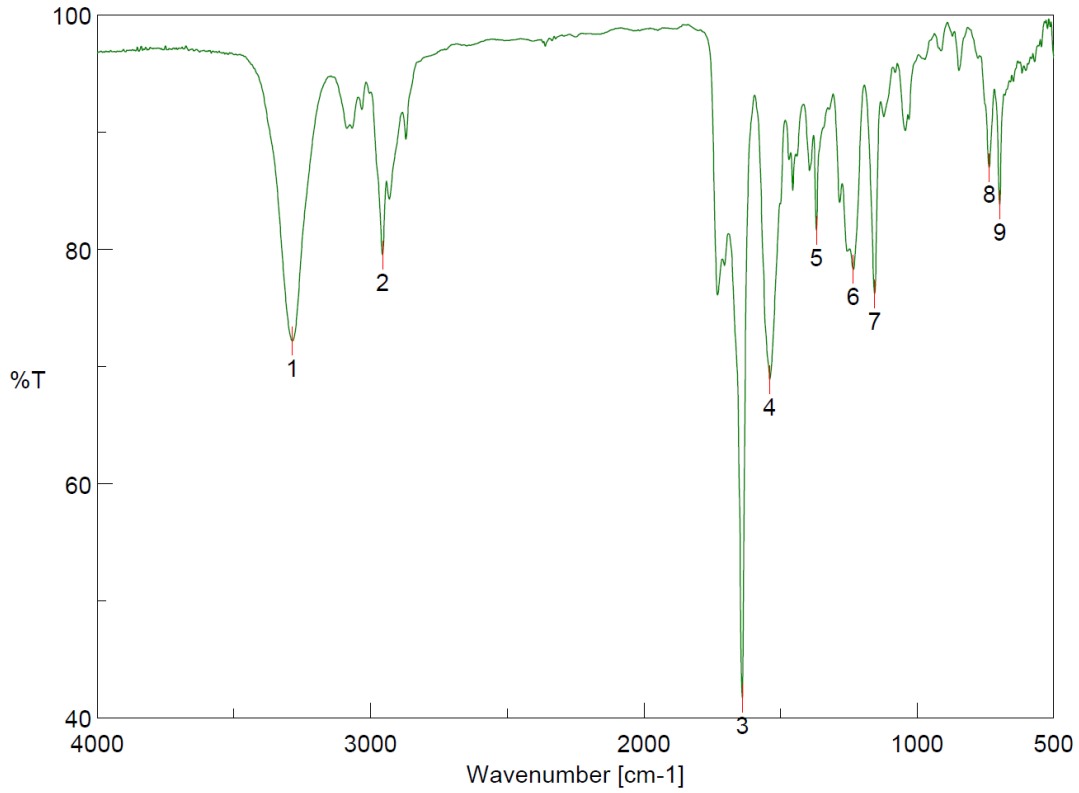


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3291.89	34.7281	2	2975.62	53.6266
3	1641.13	11.9959	4	1540.85	31.8723
5	1455.03	50.6314	6	1367.28	36.9033
7	1250.61	40.3131	8	1159.01	27.2415
9	1048.12	70.9378	10	910.236	59.9538
11	851.418	78.3084	12	733.782	37.0725
13	699.069	45.1068			

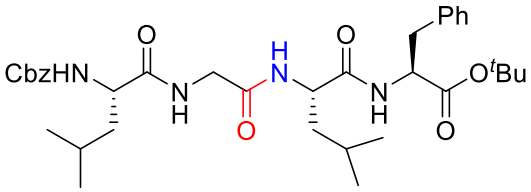


Boc-Phe-Gly-Leu-Phe-O*t*-Bu (3n)

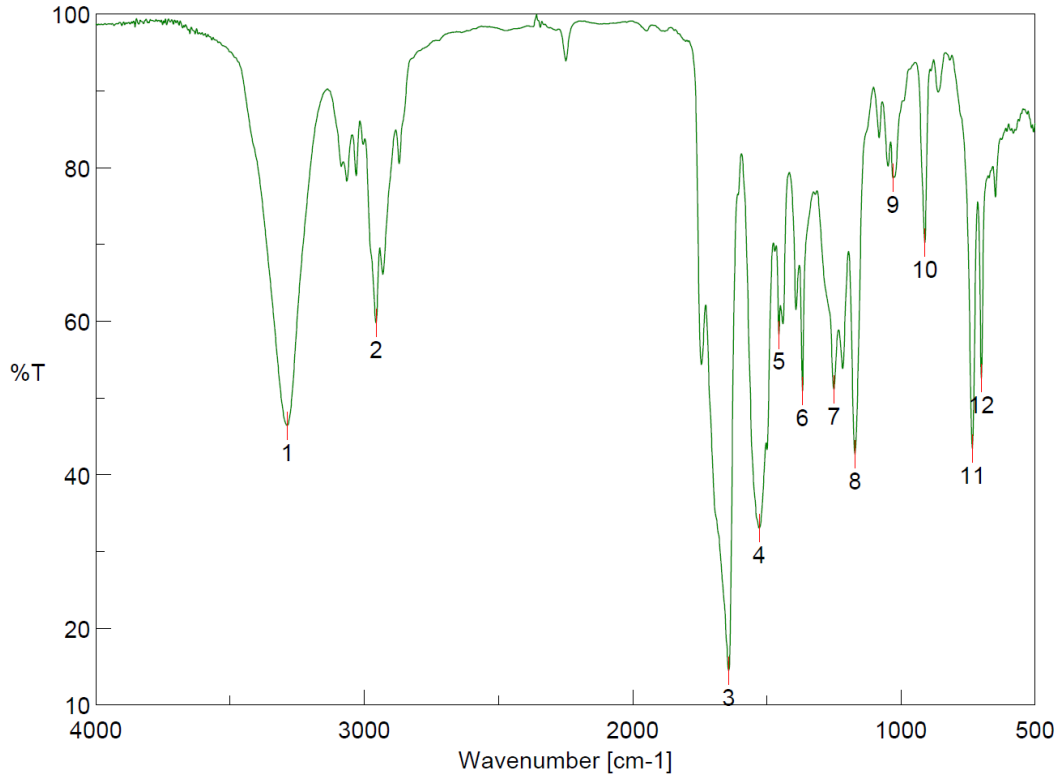


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3286.11	72.1915	2	2956.34	79.5364
3	1639.2	41.6939	4	1538.92	68.8885
5	1368.25	81.6261	6	1233.25	78.2821
7	1155.15	76.1955	8	735.71	86.9833
9	697.141	83.8246			

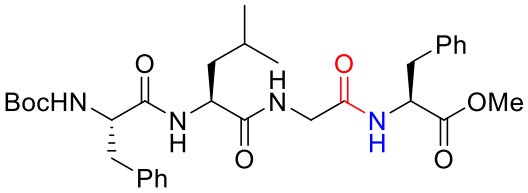


Cbz-Leu-Gly-Leu-Phe-O*t*-Bu (3o)

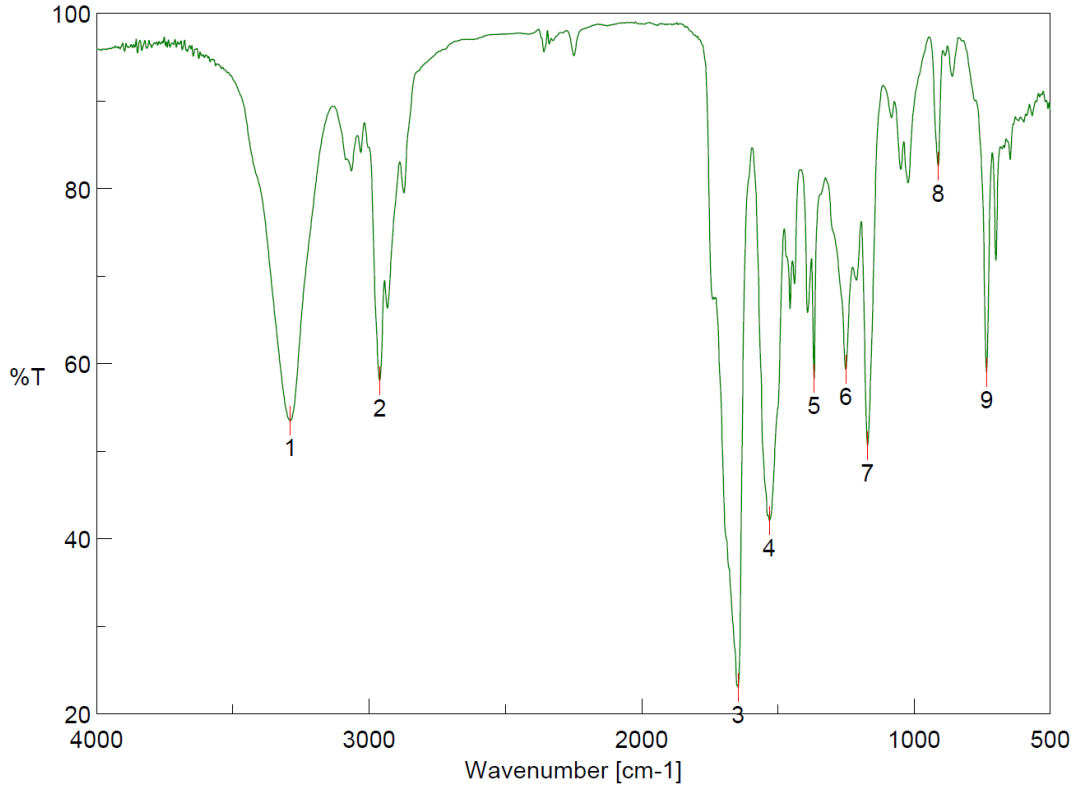


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3287.07	46.3847	2	2956.34	59.7417
3	1642.09	14.4701	4	1528.31	33.0283
5	1455.03	58.1901	6	1367.28	50.9039
7	1249.65	51.0945	8	1171.54	42.625
9	1029.8	78.6058	10	911.201	70.1885
11	733.782	43.3694	12	700.034	52.4965

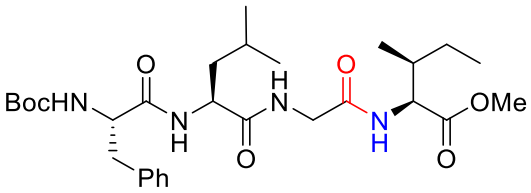


Boc-Phe-Leu-Gly-Phe-OMe (3p)

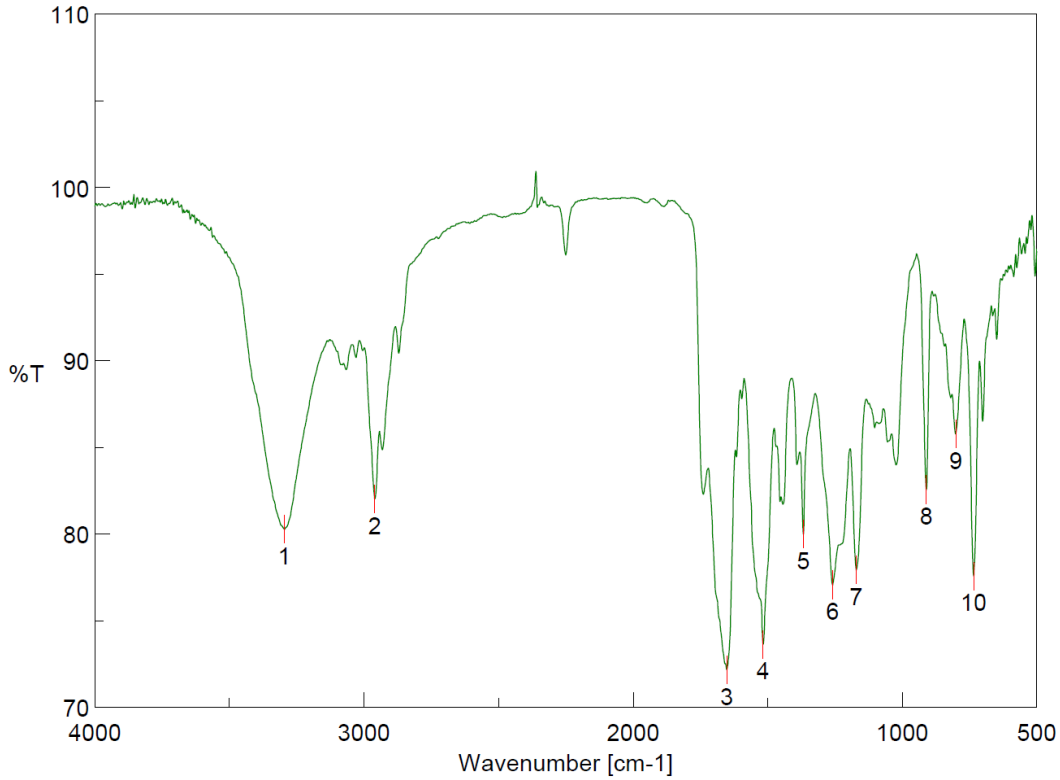


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3289	53.4656	2	2961.16	57.9913
3	1645.95	23.0177	4	1530.24	42.0124
5	1367.28	58.253	6	1250.61	59.3112
7	1170.58	50.5711	8	911.201	82.532
9	733.782	58.9794			

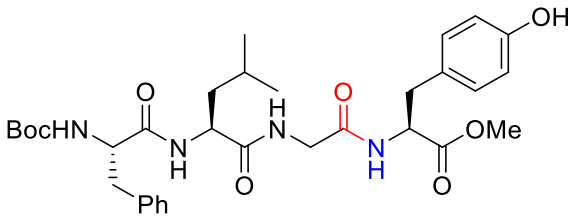


Boc-Phe-Leu-Gly-Ile-OMe (3q)

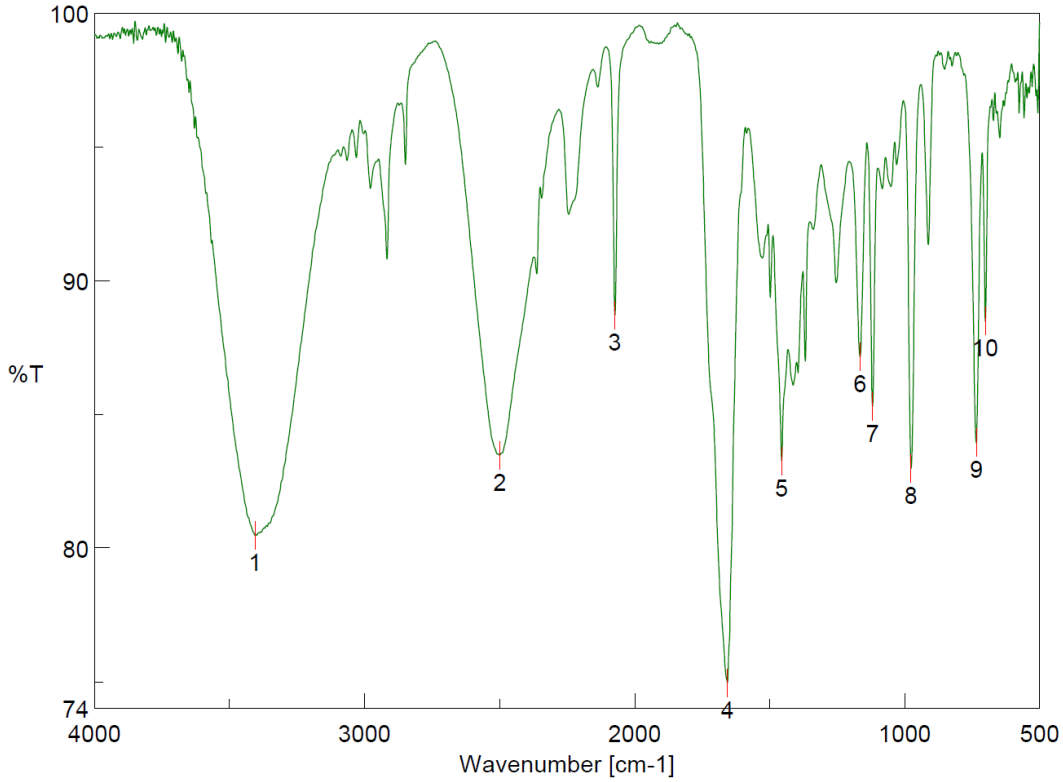


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3295.75	80.2578	2	2959.23	82.0158
3	1651.73	72.164	4	1516.74	73.6263
5	1367.28	79.9733	6	1259.29	77.0601
7	1170.58	77.913	8	910.236	82.543
9	801.278	85.7612	10	734.746	77.5714

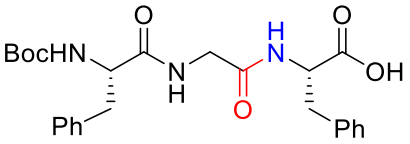


Boc-Phe-Leu-Gly-Tyr-OMe (3r)

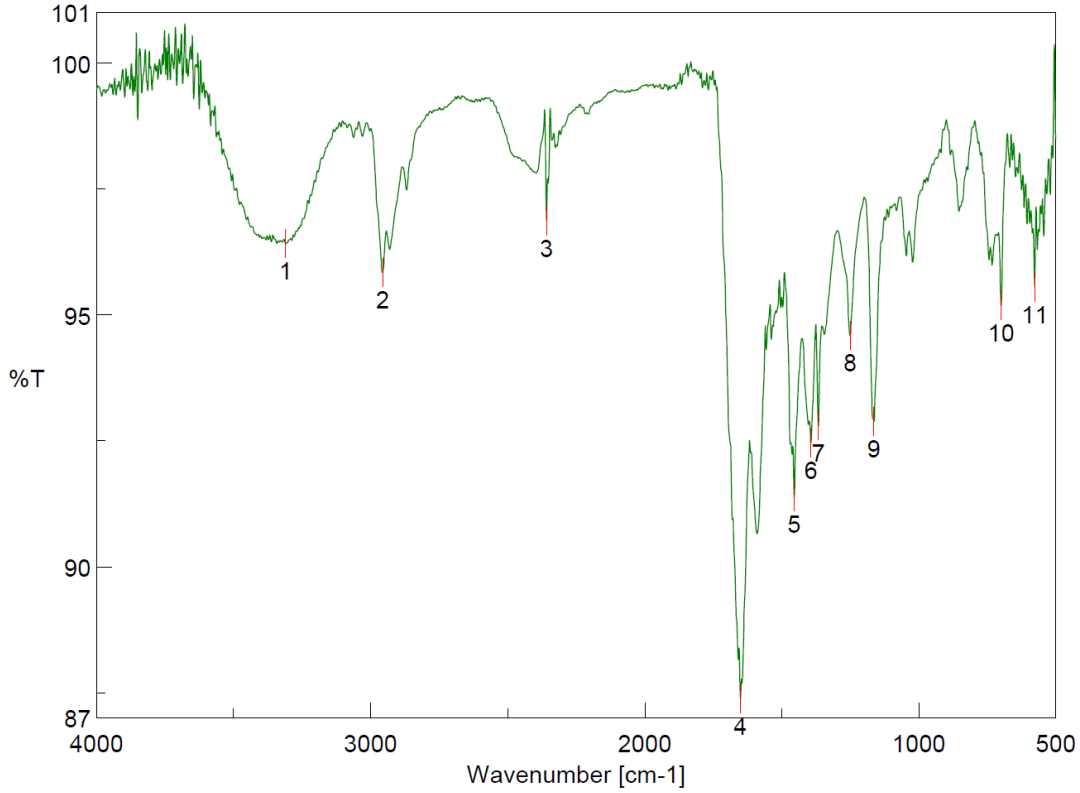


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3405.67	80.4743	2	2499.29	83.4713
3	2073.1	88.7074	4	1655.59	74.948
5	1455.99	83.2477	6	1164.79	87.1448
7	1118.51	85.2991	8	975.804	82.9677
9	734.746	83.9333	10	700.998	88.4719

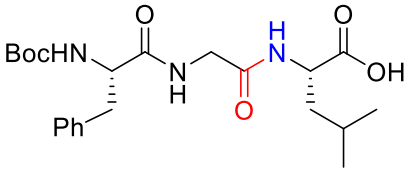


Boc-Phe-Gly-Phe-OH (3s)

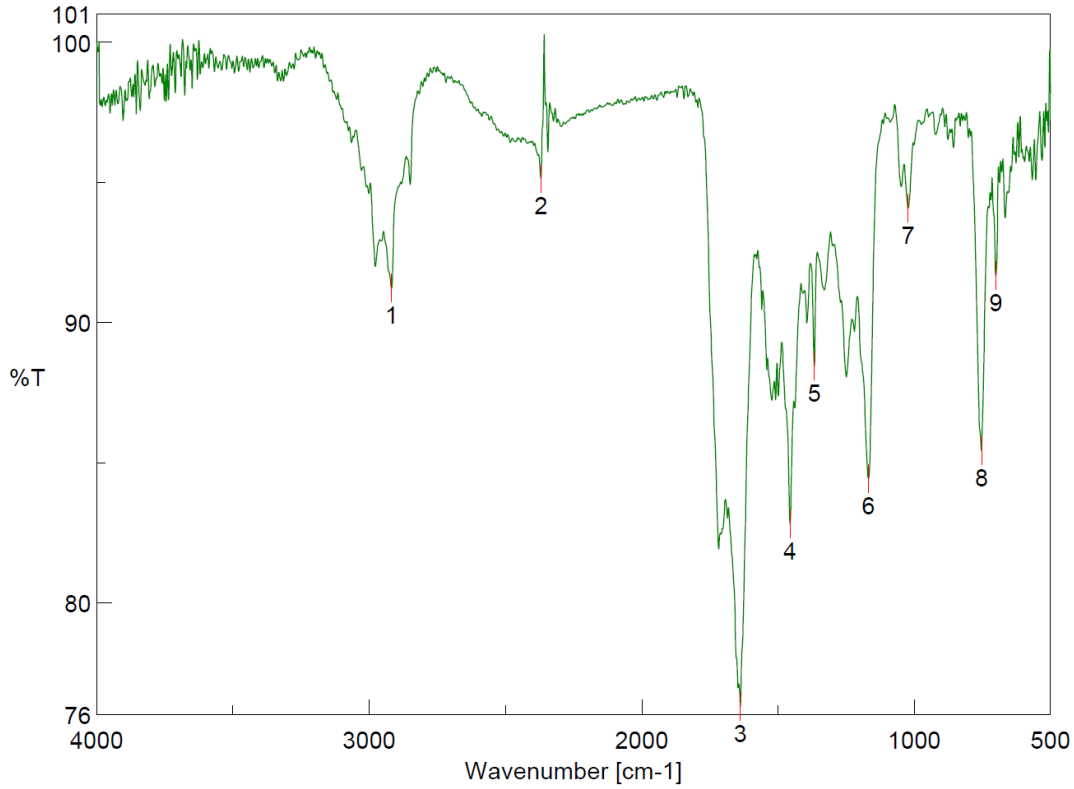


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3310.21	96.4073	2	2956.34	95.8378
3	2358.52	96.8568	4	1650.77	87.3806
5	1455.03	91.3791	6	1393.32	92.4463
7	1366.32	92.7953	8	1250.61	94.5888
9	1163.83	92.8854	10	699.069	95.1729
11	577.576	95.5465			

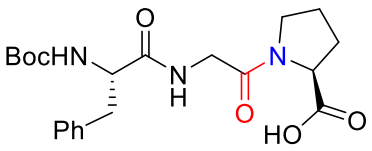


Boc-Phe-Gly-Leu-OH (3t)

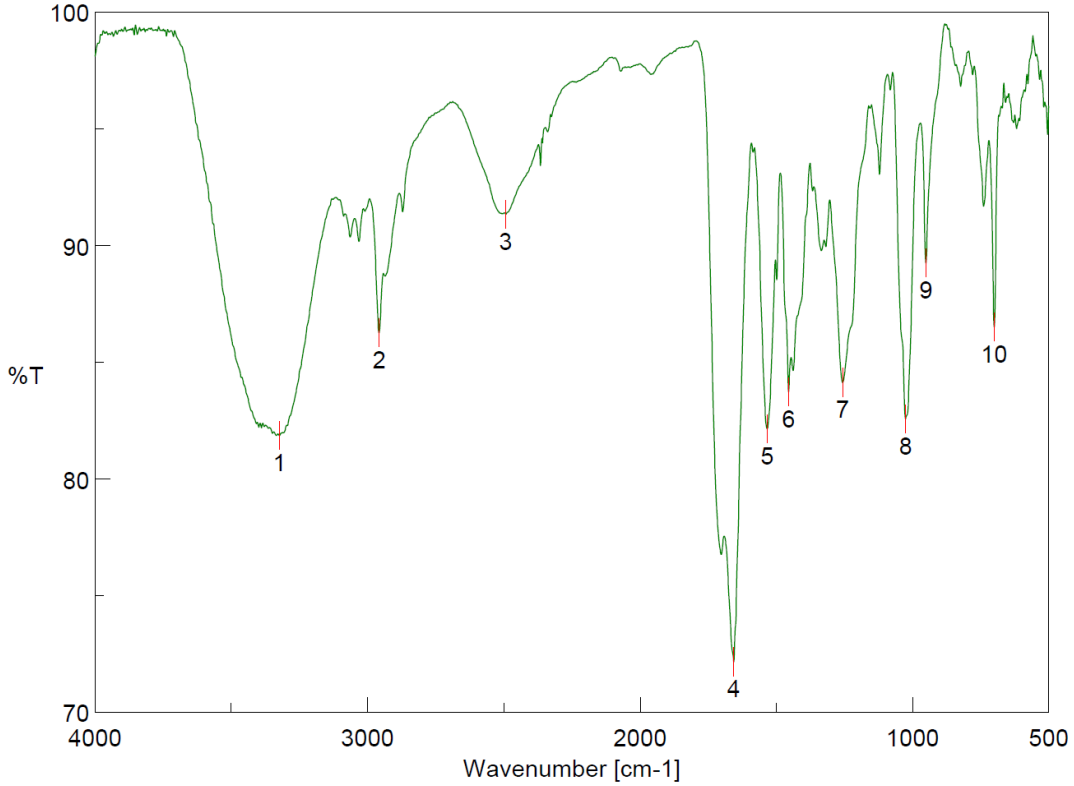


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	2917.77	91.2052	2	2370.09	95.1349
3	1637.27	76.2818	4	1455.99	82.8108
5	1366.32	88.4422	6	1167.69	84.4209
7	1022.09	94.0591	8	753.066	85.421
9	700.034	91.6616			

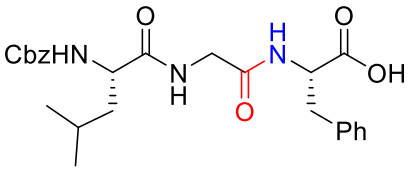


Boc-Phe-Gly-Pro-OH (3u)

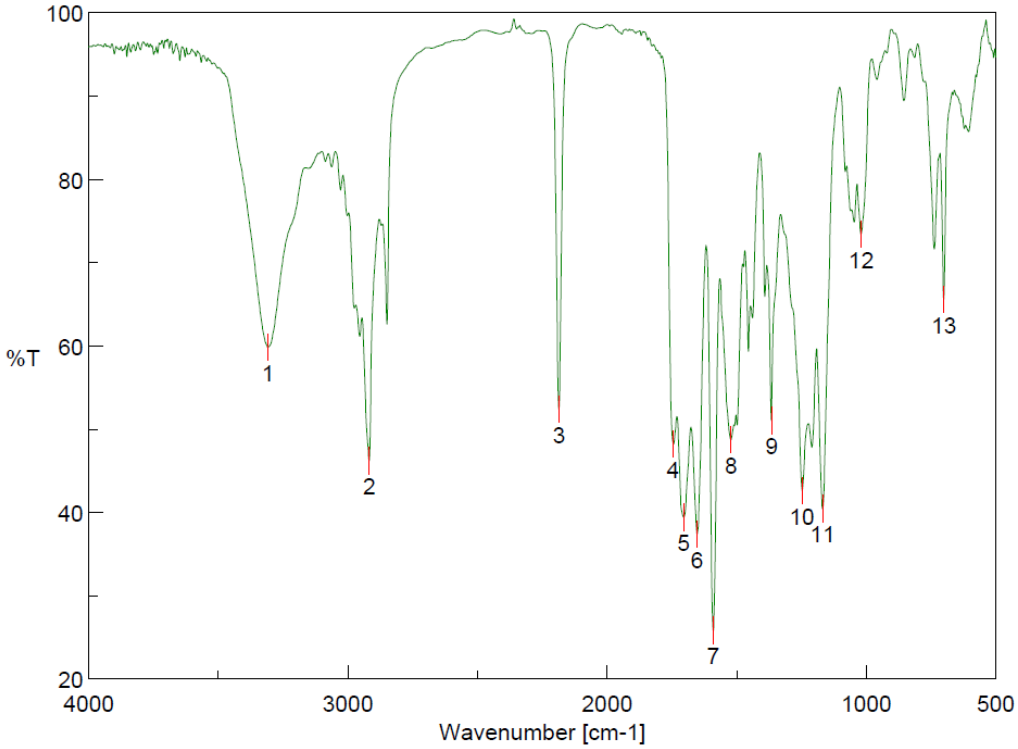


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3323.71	81.8571	2	2957.3	86.2596
3	2492.54	91.3369	4	1655.59	72.1555
5	1533.13	82.1429	6	1455.03	83.7237
7	1256.4	84.1271	8	1025.94	82.5779
9	950.734	89.2438	10	700.034	86.5

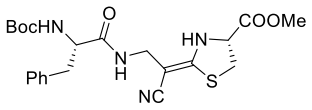


Cbz-Leu-Gly-Phe-OH (3v)



[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3308.29	59.8034	2	2917.77	46.194
3	2185.92	52.395	4	1744.3	48.2333
5	1705.73	39.4341	6	1652.7	37.4086
7	1590.99	25.8206	8	1522.52	48.723
9	1366.32	51.0309	10	1247.72	42.6406
11	1168.65	40.4419	12	1020.16	73.4107
13	700.998	65.54			



4

Z : *E* = 4 : 1

Chapter 3. Highly sterically hindered peptide bond formation between α,α-disubstituted α-amino acids and *N*-alkyl cysteines using α,α-disubstituted α-amidonitrile

Experimental procedures and Characterization data

Table of contents

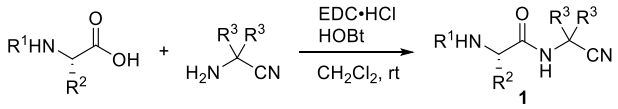
1. Materials and Methods

General Methods

General Remarks: All reactions were carried out under argon atmosphere and monitored by thin-layer chromatography using Merck 60 F254 precoated silica gel plates (0.25 mm thickness). Specific optical rotations were measured using a JASCO P-1020 polarimeter and a JASCO DIP-370 polarimeter. FT-IR spectra were recorded on a JASCO FT/IR-410 spectrometer and a Perkin Elmer spectrum BX FT-IP spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on an Agilent-400 MR (400 MHz for ^{1}H NMR, 100 M Hz for ^{13}C NMR) instrument. Data for ^{1}H NMR are reported as chemical shift (δ ppm), integration multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quintet = quin, septet = sep, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = double of triplets, td = triplet of doublets, m = multiplet, brs = broad singlet), coupling constant (Hz), Data for ^{13}C NMR are reported as chemical shift. High resolution ESI-TOF mass spectra were measured by Themo Orbi-trap LTQ XL instrument.

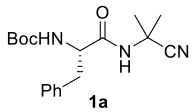
2. Experimental procedure

2.1 Synthesis of α,α-disubstituted α-amido nitrile 1 (1a to 1o)



1

***tert*-Butyl (*S*)-(1-((2-cyanopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate**



1a

To a mixture of L-Boc-Phe-OH (3.98 g, 15.0 mmol) and α,α-disubstituted α-amino nitrile[1] (1.26 g, 15.0 mmol) (It was used in crude form) in CH_2Cl_2 (60 mL, 0.25 M) was added EDC•HCl (3.45 g, 18.0 mmol) and HOBt•H_2O (230 mg, 1.50 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 67%/33%) and gave **1a** in 62% yield over 2 steps as a white solid. m.p. 168-170 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.40 – 7.17 (m, 5H), 5.91 (s, 1H), 5.12 (s, 1H), 4.31 – 4.19 (m, 1H), 3.13 (dd, *J* = 13.7, 6.5 Hz, 1H), 3.00 (dd, *J* = 13.6, 8.0 Hz, 1H), 1.57 (s, 3H), 1.51 (s, 3H), 1.43 (s, 9H).

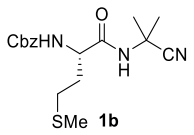
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 155.9, 136.5, 129.4, 128.6, 126.9, 120.5, 80.3, 55.8, 46.1, 38.6, 28.3, 27.0, 26.7.

HRMS (ESI) m/z: Calcd for $C_{18}H_{25}N_3NaO_3^+$ $[M+Na]^+$: 354.1788; found: 354.1790.

FT-IR (neat): 3300, 2980, 1661, 1536, 1456, 1393, 1367, 1252, 1171, 1020, 757, 699 cm^{-1}

$[\alpha]_D^{23}$-3.58 (c 1.9 $CHCl_3$)

Benzyl (*S*)-(1-((2-cyanopropan-2-yl)amino)-4-(methylthio)-1-oxobutan-2-yl)carbamate



1b

To a mixture of L-Cbz-Met-OH (1.50g, 5.29 mmol) and α,α-disubstituted α-amino nitrile[1] (445 mg, 5.29 mmol) (It was used in crude form) in CH_2Cl_2 (21 mL, 0.25 M) were added EDC•HCl (1.22 g, 6.35 mmol) and HOBt•H_2O (81.0 mg, 0.530 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 40%/60%) and gave **1b** in 72% yield over 2 steps as a colorless oil. **1b** existed as a mixture of rotamers.

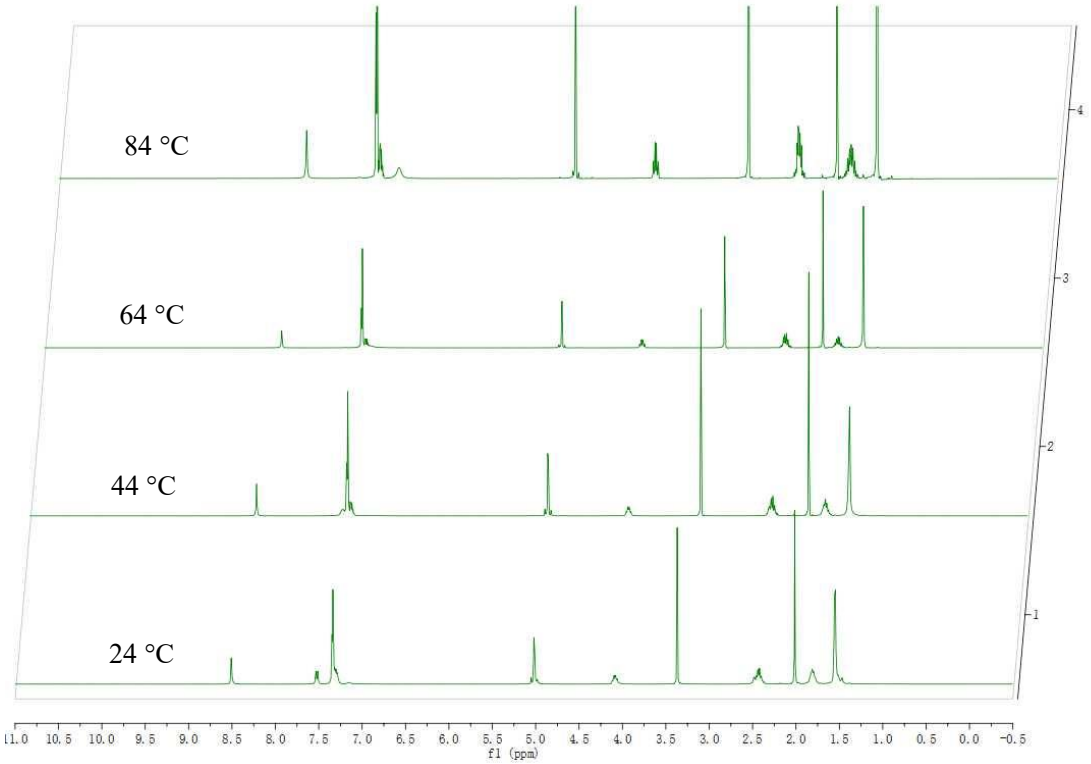
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.51 (s, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.42 – 7.20 (m, 5H), 5.02 and 5.01 (s, 2H), 4.13 – 4.02 (m, 1H), 2.50 – 2.35 (m, 2H), 2.01 (s, 3H), 1.88 – 1.73 (m, 2H), 1.56 (s, 3H), 1.55 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.0, 156.4, 137.4, 128.8, 128.2, 128.2, 122.0, 65.9, 54.1, 46.1, 32.1, 30.1, 26.9, 26.7, 15.1.

HRMS (ESI) m/z: Calcd for $C_{17}H_{23}N_3NaO_3S^+$ $[M+Na]^+$: 372.1352; found: 372.1354.

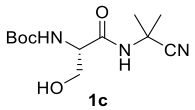
FT-IR (neat): 3300, 3061, 2919, 2243, 1671, 1538, 1455, 1391, 1368, 1241, 1049, 741, 698 cm^{-1}

$[\alpha]_D^{25}$-27.79 (c 2.2 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of 1b

***tert*-Butyl (*S*)-(1-((2-cyanopropan-2-yl)amino)-3-hydroxy-1-oxopropan-2-yl)carbamate**


1c

To a mixture of L-Boc-Ser-OH (1.00 g, 4.87 mmol) and α,α-disubstituted α-amino nitrile[1] (410 mg, 4.87 mmol) (It was used in crude form) in CH_2Cl_2 (19.5 mL, 0.25 M) was added EDC•HCl (1.12 g, 5.85 mmol) and HOBt•H_2O (75.0 mg, 0.487 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 34%/66%) and gave **1c** in 33% yield over 2 steps as a yellow solid. m.p. 136-138 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.38 (s, 1H), 5.81 (d, *J* = 6.2 Hz, 1H), 4.14 (s, 1H), 4.04 – 3.93 (m, 1H), 3.77 – 3.60 (m, 2H), 1.68 (s, 3H), 1.66 (s, 3H), 1.43 (s, 9H).

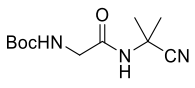
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 156.5, 120.6, 80.8, 62.2, 55.1, 46.3, 28.2, 27.0, 26.9.

HRMS (ESI) m/z: Calcd for $C_{12}H_{21}N_3NaO_4^+$ $[M+Na]^+$: 294.1424; found: 294.1428.

FT-IR (neat): 3307, 2981, 2247, 1676, 1532, 1393, 1368, 1250, 1168, 1062, 855, 759 cm^{-1}

$[\alpha]_D^{26}$-86.02 (c 1.0 $CHCl_3$)

***tert*-Butyl (2-((2-cyanopropan-2-yl)amino)-2-oxoethyl)carbamate**


1d

To a mixture of Boc-Gly-OH (1.20 g, 6.85 mmol) and α,α-disubstituted α-amino nitrile[1] (576 mg, 6.85 mmol) (It was used in crude form) in CH_2Cl_2 (27 mL, 0.25 M) were added EDC•HCl (1.58 g, 8.22 mmol) and HOBt•H_2O (105 mg, 0.685 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc =35%/65%) and gave **1d** in 70% yield over 2 steps as a colorless and viscous oil.

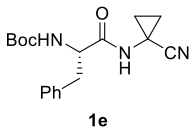
^{1}H NMR (399 MHz, $CDCl_3$) δ 7.13 (s, 1H), 5.66 (s, 1H), 3.76 (d, *J* = 5.1 Hz, 2H), 1.66 (s, 6H), 1.42 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 156.6, 120.8, 80.4, 46.3, 44.4, 28.3, 27.0.

HRMS (ESI) m/z: Calcd for $C_{11}H_{19}N_3NaO_3^+$ $[M+Na]^+$: 264.1319; found: 264.1321.

FT-IR (neat): 3311, 2981, 2243, 1682, 1530, 1368, 1170, 1054, 948, 863, 734 cm^{-1}

***tert*-Butyl (*S*)-(1-((1-cyanocyclopropyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate**



1e

To a mixture of L-Boc-Phe-OH (1.00 g, 3.77 mmol) and 1-aminocyclopropanecarbonitrile hydrochloride (492 mg, 4.15 mmol) in CH_2Cl_2 (15 mL, 0.25 M) were added triethylamine (630 μL, 4.52 mmol), EDC•HCl (867 mg, 4.52 mmol) and HOBt•H_2O (58.0 mg, 0.377 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 34%/66%) and gave **1e** in 87% yield as a white solid. m.p. 151-153 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.35 – 7.18 (m, 5H), 6.29 (s, 1H), 4.99 (s, 1H), 4.28 – 4.18 (m, 1H), 3.12 (dd, *J* = 13.7, 6.5 Hz, 1H), 3.00 (dd, *J* = 13.7, 7.8 Hz, 1H), 1.53 – 1.44 (m, 2H), 1.42 (s, 9H), 1.14 – 1.06 (m, 1H), 1.04 – 0.95 (m, 1H).

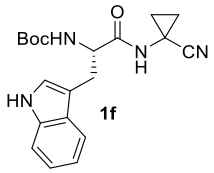
^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 155.7, 136.2, 129.3, 128.6, 127.0, 119.7, 80.4, 55.5, 38.8, 28.3, 20.0, 16.6.

HRMS (ESI) m/z: Calcd for $C_{18}H_{23}N_3NaO_3^+$ $[M+Na]^+$: 352.1632; found: 352.1624.

FT-IR (neat): 3289, 2979, 2243, 1669, 1527, 1367, 1300, 1169, 1049, 755, 700 cm^{-1}

$[\alpha]_D^{22}$-2.47 (c 1.52 $CHCl_3$)

***tert*-Butyl (*S*)-(1-((1-cyanocyclopropyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamate**



1f

To a mixture of L-Boc-Trp-OH (1.00 g, 3.29 mmol) and 1-aminocyclopropanecarbonitrile hydrochloride (390 mg, 3.29 mmol) in CH_2Cl_2 (13 mL, 0.25 M) were added triethylamine (550 μL, 3.94 mmol), EDC•HCl (756 mg, 3.94 mmol) and HOBt•H_2O (50 mg, 0.329 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 34%/66%) and gave **1f** in 50% yield as a yellow solid. m.p. 93-95 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 8.42 (s, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.22 – 7.17 (m, 1H), 7.14 – 7.09 (m, 1H), 7.04 (s, 1H), 6.55 (s, 1H), 5.27 (s, 1H), 4.38 (s, 1H), 3.27 (dd, *J* = 13.7, 4.2 Hz, 1H), 3.13 (dd, *J* = 14.2, 7.9 Hz, 1H), 1.42 (s, 9H), 1.37 – 1.30 (m, 2H), 1.00 – 0.81 (m, 2H).

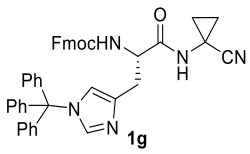
^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 155.6, 136.2, 127.2, 123.7, 122.3, 119.8, 119.7, 118.5, 111.4, 109.7, 80.5, 55.1, 28.3, 20.0, 16.6, 16.5.

HRMS (ESI) m/z: Calcd for $C_{20}H_{24}N_4NaO_3^+$ $[M+Na]^+$: 391.1741; found: 391.1741.

FT-IR (neat): 3316, 2981, 2360, 2243, 1676, 1499, 1457, 1367, 1250, 1167, 1048, 746, 669 cm^{-1}

$[\alpha]_D^{25}$+1.75 (c 0.98 $CHCl_3$)

(9H-fluoren-9-yl)methyl (*S*)-(1-((1-cyanocyclopropyl)amino)-1-oxo-3-(1-trityl-1H-imidazol-4-yl)propan-2-yl)carbamate



To a mixture of 1-aminocyclopropanecarbonitrile hydrochloride (383 mg, 3.23 mmol) in CH_2Cl_2 (13 mL, 0.25 M) were added *N*, *N*-diisopropylethylamine (562 μL, 3.23 mmol), L-Fmoc-His(NTrityl)-OH (2.00 g, 3.23 mmol), EDC•HCl (756 mg, 3.94 mmol) and HOBt•H_2O (50.0 mg, 0.329 mmol). The reaction was stirred under room temperature for 3 h. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (from Hexane/EtOAc = 20%/80% to MeOH 100%) and gave **1g** in 73% yield as a white solid. m.p. 135-137 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 9.42 (s, 1H), 7.99 (s, 1H), 7.79 – 7.71 (m, 2H), 7.57 (d, *J* = 7.3 Hz, 1H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.47 – 7.16 (m, 4H), 7.47 – 7.16 (m, 6H), 7.47 – 7.16 (m, 3H), 7.15 – 6.93 (m, 6H), 6.75 (s, 1H), 6.26 (d, *J* = 6.9 Hz, 1H), 4.89 – 4.79 (m, 1H), 4.41 – 4.30 (m, 1H), 4.17 – 4.03 (m, 2H), 3.40 (d, *J* = 14.8 Hz, 1H), 2.89 (dd, *J* = 15.0, 9.6 Hz, 1H), 1.60 – 1.45 (m, 2H), 1.43 – 1.25 (m, 2H).

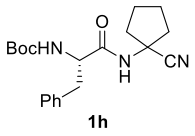
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 156.0, 143.9, 143.4, 141.2, 139.7, 134.9, 132.3, 129.5, 129.2, 128.7, 127.8, 127.8, 127.2, 127.1, 125.3, 125.0, 120.4, 120.0, 119.8, 78.4, 67.3, 53.9, 46.9, 31.5, 20.6, 16.7, 16.0.

HRMS (ESI) m/z: Calcd for $C_{44}H_{38}N_5O_3^+$ $[M+H]^+$: 684.2969; found: 684.2966.

FT-IR (neat): 3161, 3015, 2953, 2242, 1687, 1618, 1524, 1448, 1247, 1129, 1046, 756, 702, 665 cm^{-1}

$[\alpha]_D^{27}$ +7.71 (c 1.22 $CHCl_3$)

***tert*-Butyl (*S*)-(1-((1-cyanocyclopentyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate**



To a mixture of L-Boc-Phe-OH (2.00 g, 7.54 mmol) and α,α-disubstituted α-amino nitrile[1] (830 mg, 7.54 mmol) (It was used in crude form) in CH_2Cl_2 (30 mL, 0.25 M) were added EDC•HCl (1.73 g, 9.05 mmol) and HOBt•H_2O (116 mg, 0.754 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 45%/55%) and gave **1h** in 57% yield over 2 steps as a white solid. m.p. 135-136 ºC.

1h existed as a mixture of rotamers

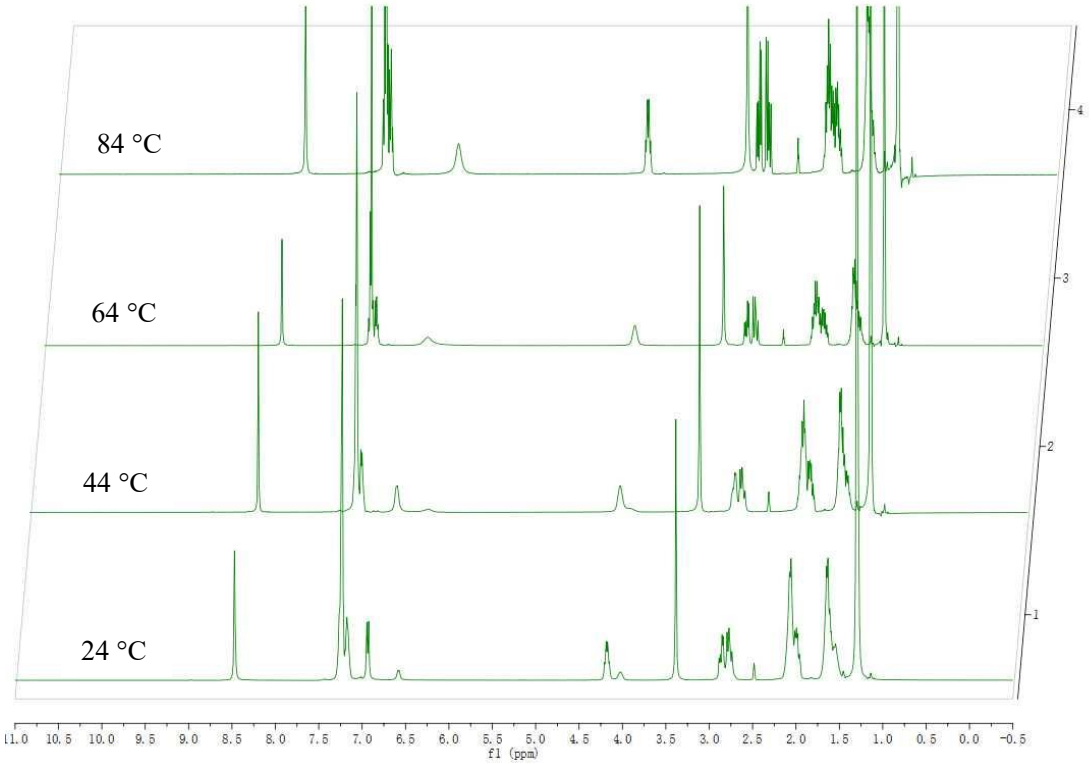
^{1}H NMR (measured at 84 °C) (399 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.35 – 7.09 (m, 5H), 6.39 (s, 1H), 4.26 – 4.15 (m, 1H), 2.93 (dd, *J* = 13.8, 5.9 Hz, 1H), 2.82 (dd, *J* = 13.8, 8.6 Hz, 1H), 2.20 – 1.93 (m, 4H), 1.77 – 1.53 (m, 4H), 1.33 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 155.6, 138.0, 129.7, 128.4, 126.7, 121.8, 78.5, 55.8, 54.4, 38.3, 38.05, 37.99, 28.5, 22.9, 22.9.

HRMS (ESI) m/z: Calcd for $C_{20}H_{27}N_3NaO_3^+$ $[M+Na]^+$: 380.1945; found: 380.1942.

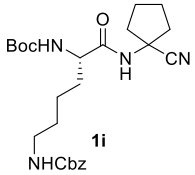
FT-IR (neat): 3303, 2978, 2239, 1661, 1536, 1455, 1367, 1253, 1172, 1022, 860, 754, 700 cm^{-1}

$[\alpha]_D^{26}$ -11.46 (c 1.06 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of 1h

Benzyl *tert*-butyl (6-((1-cyanocyclopentyl)amino)-6-oxohexane-1,5-diyl)(*S*)-dicarbamate



1i

To a mixture of L-Boc-Lys(NHCbz)-OH[2] (1.10 g, 2.89 mmol) and α,α-disubstituted α-amino nitrile[1] (319 mg, 2.89 mmol) (It was used in crude form) in CH_2Cl_2 (12 mL, 0.25 M) were added EDC•HCl (665 mg, 3.47 mmol) and HOBt•H_2O (44.0 mg, 0.289 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc =35%/65%) and gave **1i** in 54% yield over 2 steps as a colorless oil.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.39 (s, 1H), 7.38 – 7.25 (m, 5H), 7.20 (t, *J* = 5.4 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 4.98 (s, 2H), 3.91 – 3.80 (m, 1H), 2.99 – 2.88 (m, 2H), 2.17 – 1.98 (m, 4H), 1.76 – 1.59 (m, 4H), 1.53 – 1.43 (m, 2H), 1.35 (s, 9H), 1.42 – 1.26 (m, 2H), 1.26 – 1.13 (m, 2H).

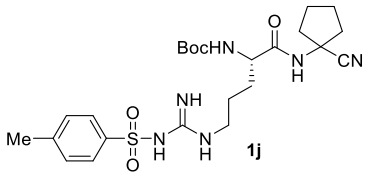
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 156.5, 155.7, 137.7, 128.8, 128.1, 121.9, 78.5, 65.5, 54.4, 40.5, 38.2, 38.1, 31.9, 29.5, 28.6, 28.4, 23.1, 22.9.

HRMS (ESI) m/z: Calcd for $C_{25}H_{36}N_4NaO_5^+$ $[M+Na]^+$: 495.2578; found: 495.2577.

FT-IR (neat): 3315, 2943, 2239, 1696, 1530, 1455, 1367, 1251, 1168, 1026, 862, 753, 698 cm^{-1}

$[\alpha]_D^{25}$-32.34 (c 1.0 $CHCl_3$)

***tert*-Butyl (*S*)-(1-((1-cyanocyclopentyl)amino)-1-oxo-5-(3-tosylguanidino)pentan-2-yl)carbamate**



1j

To a mixture of L-Boc-Arg(Tos)-OH (1.00 g, 2.33 mmol) and α,α-disubstituted α-amino nitrile[1] (257 mg, 2.33 mmol) (It was used in crude form) in CH_2Cl_2 (9.3 mL, 0.25 M) were added EDC•HCl (537 mg, 2.80 mmol) and HOBt•H_2O (36.0 mg, 0.233 mmol). The

reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 9%/91%) and gave **1j** in 62% yield over 2 steps as a white solid. m.p. 102-104 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.73 (d, *J* = 7.4 Hz, 2H), 7.22 (d, *J* = 8.1 Hz, 2H), 6.50 (s, 2H), 6.31 (s, 1H), 5.99 (s, 1H), 4.26 – 4.15 (m, 1H), 3.36 – 3.12 (m, 2H), 2.50 – 2.41 (m, 2H), 2.37 (s, 3H), 2.32 – 2.19 (m, 2H), 2.18 – 2.04 (m, 2H), 1.82 – 1.69 (m, 2H), 1.82 – 1.69 (m, 2H), 1.64 – 1.52 (m, 2H), 1.39 (s, 9H).

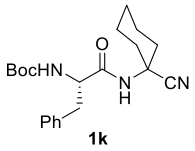
^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 157.0, 156.1, 142.3, 140.3, 129.3, 126.0, 121.4, 80.0, 54.9, 54.2, 40.1, 38.6, 38.5, 28.7, 28.3, 26.0, 23.0, 22.9, 21.4.

HRMS (ESI) m/z: Calcd for $C_{24}H_{37}N_6O_5S^+$ $[M+H]^+$: 521.2541; found: 521.2542.

FT-IR (neat): 3329, 2978, 2239, 1677, 1548, 1367, 1254, 1168, 1132, 1082, 815, 755, 677, 560 cm^{-1}

$[\alpha]_D^{27}$-15.12 (c 1.60 $CHCl_3$)

***tert*-Butyl (*S*)-(1-((1-cyanocyclohexyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate**



1k

To a mixture of L-Boc-Phe-OH (2.92 g, 11.0 mmol) and α,α-disubstituted α-amino nitrile[1] (1.24 g, 10.0 mmol) (It was used in crude form) in CH_2Cl_2 (40 mL, 0.25 M) were added EDC•HCl (2.53 g, 13.2 mmol) and HOBt•H_2O (153 mg, 1.00 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 60%/40%) and gave **1k** in 80% yield over 2 steps as a white solid. m.p. 130-132 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.37 – 7.19 (m, 5H), 5.86 (s, 1H), 5.09 (s, 1H), 4.30 – 4.21 (m, 1H), 3.14 (dd, *J* = 13.8, 6.4 Hz, 1H), 3.01 (dd, *J* = 13.7, 7.9 Hz, 1H), 2.28 – 2.17 (m, 1H), 2.16 – 2.06 (m, 1H), 1.72 – 1.53 (m, 6H), 1.52 – 1.47 (m, 1H), 1.43 (s, 9H), 1.29 – 1.17 (m, 1H).

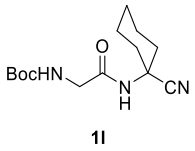
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 155.8, 136.5, 129.4, 128.7, 127.0, 119.3, 80.4, 55.9, 51.3, 38.3 (2C), 35.2, 34.9, 28.3, 24.6, 21.8.

HRMS (ESI) m/z: Calcd for $C_{21}H_{29}N_3NaO_3^+$ $[M+Na]^+$:394.2101; found: 394.2101.

FT-IR (neat): 3324, 2939, 2864, 2239, 1659, 1528, 1455, 1393, 1367, 1305, 1249, 1169, 1016, 866, 758, 698 cm^{-1}

$[\alpha]_D^{24}$-10.83 (c 1.46 $CHCl_3$)

***tert*-Butyl (2-((1-cyanocyclohexyl)amino)-2-oxoethyl)carbamate**



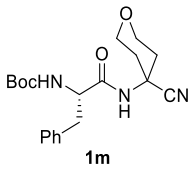
1l

To a mixture of Boc-Gly-OH (1.00 g, 5.71 mmol) and α,α-disubstituted α-amino nitrile[1] (709 mg, 5.71 mmol) (It was used in crude form) in CH_2Cl_2 (23 mL, 0.25 M) were added EDC•HCl (1.31 g, 6.85 mmol) and HOBt•H_2O (88.0 mg, 0.571 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 34%/66%) and gave **1l** in 69% yield over 2 steps as a white solid. m.p. 167-169 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 6.62 (s, 1H), 5.21 (s, 1H), 3.76 (d, *J* = 6.0 Hz, 2H), 2.40 – 2.21 (m, 2H), 1.84 – 1.64 (m, 6H), 1.64 – 1.59 (m, 1H), 1.46 (s, 9H), 1.37 – 1.27 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 156.6, 119.7, 80.5, 51.3, 44.8, 35.1, 28.2, 24.6, 21.8.
HRMS (ESI) m/z: Calcd for $C_{14}H_{23}N_3NaO_3^+$ $[M+Na]^+$: 304.1632; found: 304.1634.
FT-IR (neat): 3312, 2937, 2243, 1685, 1527, 1455, 1366, 1249, 1166, 1053, 782, 553 cm^{-1}

***tert*-Butyl (*S*)-(1-((4-cyanotetrahydro-2H-pyran-4-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate**

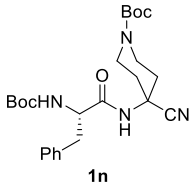


1m

To a mixture of L-Boc-Phe-OH (1.00 g, 3.77 mmol) and α,α-disubstituted α-amino nitrile[1] (476 mg, 3.77 mmol) (It was used in crude form) in CH_2Cl_2 (15 mL, 0.25 M) were added EDC•HCl (867 mg, 4.52 mmol) and HOBt•H_2O (58.0 mg, 0.377 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 40%/60%) and gave **1m** in 31% yield over 2 steps as a white solid. m.p. 82-84 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.38 – 7.18 (m, 5H), 6.10 (s, 1H), 5.07 (s, 1H), 4.32 – 4.21 (m, 1H), 3.88 – 3.76 (m, 2H), 3.76 – 3.64 (m, 2H), 3.14 (dd, *J* = 13.8, 6.6 Hz, 1H), 3.03 (dd, *J* = 13.8, 7.9 Hz, 1H), 2.32 – 2.14 (m, 2H), 1.79 – 1.64 (m, 2H), 1.43 (s, 9H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 156.0, 136.3, 129.3, 128.7, 127.1, 118.6, 80.6, 63.5, 63.5, 55.9, 49.0, 38.2, 35.1, 34.8, 28.3.
HRMS (ESI) m/z: Calcd for $C_{20}H_{27}N_3NaO_4^+$ $[M+Na]^+$: 396.1894; found: 396.1891.
FT-IR (neat): 3298, 2977, 2250, 1665, 1535, 1367, 1250, 1168, 1104, 1014, 733, 700 cm^{-1}
$[\alpha]_D^{27}$-9.31 (c 2.46 $CHCl_3$)

***tert*-Butyl (*S*)-4-(2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-4-cyanopiperidine-1-carboxylate**

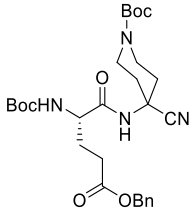


1n

The corresponding α,α-disubstituted α-amino nitrile here was made from 4-piperidone monohydrate hydrochloride via 2 steps based on the reported procedure.[1,3] To a mixture of L-Boc-Phe-OH (1.30 g, 4.90 mmol) and α,α-disubstituted α-amino nitrile (1.32 g, 5.88 mmol) (It was used in crude form) in CH_2Cl_2 (20 mL, 0.25 M) were added EDC•HCl (1.13 g, 5.88 mmol) and HOBt•H_2O (75.0 mg, 0.490 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 65%/35%) and gave **1n** in 34% yield over 3 steps as a white solid. m.p. 90-92 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.38 – 7.18 (m, 5H), 6.14 (s, 1H), 5.07 (s, 1H), 4.31 – 4.21 (m, 1H), 3.85 – 3.64 (m, 2H), 3.34 – 3.17 (m, 2H), 3.13 (dd, *J* = 13.8, 6.6 Hz, 1H), 3.03 (dd, *J* = 13.8, 7.8 Hz, 1H), 2.29 – 2.21 (m, 1H), 2.19 – 2.09 (m, 1H), 1.65 – 1.54 (m, 2H), 1.44 (s, 9H), 1.42 (s, 9H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 155.8, 154.2, 136.3, 129.3, 128.8, 127.1, 118.3, 80.7, 80.3, 55.9, 49.9, 39.6, 38.0, 34.3, 34.1, 28.3, 28.2.
HRMS (ESI) m/z: Calcd for $C_{25}H_{36}N_4NaO_5^+$ $[M+Na]^+$: 495.2578; found: 495.2585.
FT-IR (neat): 3304, 2979, 2239, 1684, 1531, 1426, 1367, 1250, 1160, 1099, 860, 757, 700 cm^{-1}
$[\alpha]_D^{26}$+2.90 (c 1.42 $CHCl_3$)

***tert*-Butyl (*S*)-4-(5-(benzyloxy)-2-((*tert*-butoxycarbonyl)amino)-5-oxopentanamido)-4-cyano-piperidine-1-carboxylate**



1o

The corresponding α,α-disubstituted α-amino nitrile here was made from 4-piperidone monohydrate hydrochloride via 2 steps based on the reported procedure.[1,3] To a mixture of L-Boc-Glu(Bn)-OH (1.00 g, 2.96 mmol) and α,α-disubstituted α-amino nitrile (668 mg, 2.96 mmol) (It was used in crude form) in CH_2Cl_2 (12 mL, 0.25 M) were added EDC•HCl (682 mg, 3.56 mmol) and HOBt•H_2O (45.0 mg, 0.296 mmol). The reaction was stirred under room temperature for 2 h. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 50%/50%) and gave **1o** in 71% yield over 3 steps as an amorphous solid.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.46 (s, 1H), 7.38 – 7.28 (m, 5H), 7.02 (d, *J* = 7.8 Hz, 1H), 5.07 (s, 2H), 4.00 – 3.88 (m, 1H), 3.62 – 3.48 (m, 2H), 3.25 – 3.14 (m, 2H), 2.37 (t, *J* = 7.6 Hz, 2H), 2.18 – 2.04 (m, 2H), 1.93 – 1.72 (m, 2H), 1.93 – 1.72 (m, 2H), 1.38 (s, 9H), 1.35 (s, 9H).

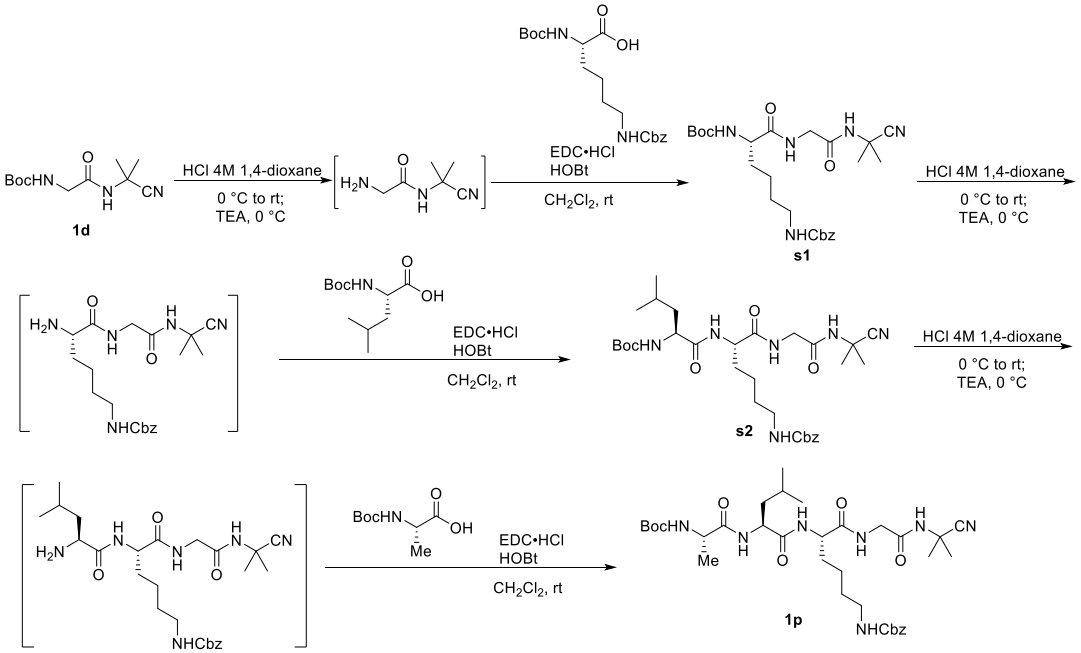
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5, 172.4, 155.8, 154.1, 136.6, 128.8, 128.4, 128.3, 119.9, 79.6, 78.7, 65.9, 53.9, 49.3, 33.6, 33.4, 30.5, 28.5, 28.4, 27.2.

HRMS (ESI) m/z: Calcd for $C_{28}H_{40}N_4NaO_7^+$ [M+Na]+: 567.2789; found: 567.2794.

FT-IR (neat): 3320, 2978, 2239, 1697, 1524, 1425, 1392, 1367, 1250, 1162, 1071, 861, 754, 698 cm^{-1}

$[\alpha]_D^{26}$-15.62 (c 1.11 $CHCl_3$)

2.2 Synthesis of α,α-disubstituted α-amido nitrile 1p



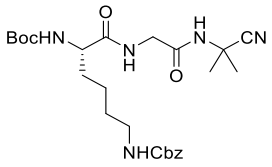
4M HCl 1,4-dioxane solution (27.3 mL) was added at 0 °C to Boc-Gly-Aib-CN **1d** (3.30 g, 13.67 mmol). The reaction mixture was stirred at room temperature for 20 min (white precipitate forms). 18 mL of triethylamine was slowly added to the reaction mixture at 0 °C. The ice bath was removed and 100 mL of CH_2Cl_2 was added to the reaction mixture. Boc-L-Lys(NHCbz)-OH[2] (1.93 g, 13.67 mmol), EDC•HCl (3.15 g, 16.41 mmol) and HOBt monohydrate (210 mg, 1.370 mmol) were added to the reaction system and stirred at room temperature overnight. The reaction was quenched by 1N HCl aqueous solution,

extracted with CH_2Cl_2. The organic phase was collected, dried over Na_2SO_4, evaporated under reduced pressure, concentrated under vacuo. The crude product was purified by silica gel column chromatography (EtOAc/Hexane = 99%/1%) and gave the desired product **s1** in 70% yield as an amorphous solid.

4M HCl 1,4-dioxane solution (8.7 mL) was added at 0 °C to Boc-L-Lys(NHCbz)-Gly-Aib-CN **s1** (2.20 g, 4.35 mmol). The reaction mixture was stirred at room temperature and cooled to 0 °C when the reaction was completed monitored by TLC. 6 mL of triethylamine was slowly added to the reaction mixture at 0 °C. The ice bath was removed and 50 mL of CH_2Cl_2 was added to the reaction mixture. Boc-L-Leu-OH (1.01 g, 4.35 mmol), EDC•HCl (1.00 g, 5.22 mmol) and HOBt monohydrate (334 mg, 2.17 mmol) were added to the reaction system and stirred at rt overnight. The reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2. The organic phase was collected, dried over Na_2SO_4, evaporated under reduced pressure, concentrated under vacuo. The crude product was purified by silica gel column chromatography (EtOAc/Hexane = 99%/1%) and gave the desired product **s2** in 67% yield as an amorphous solid.

4M HCl 1,4-dioxane solution (4.8 mL) was added at 0 °C to Boc-L-Leu-L-Lys(NHCbz)-Gly-Aib-CN **s2** (1.48 g, 2.40 mmol). The reaction mixture was stirred at room temperature and cooled to 0 °C when the reaction was completed monitored by TLC. 3 mL of triethylamine was slowly added to the reaction mixture at 0 °C. The ice bath was removed and 25 mL of CH_2Cl_2 was added to the reaction mixture. Boc-L-Ala-OH (0.450 g, 2.40 mmol), EDC•HCl (0.550 g, 2.90 mmol) and HOBt monohydrate (182 mg, 1.20 mmol) were added to the reaction system and stirred at room temperature overnight. The reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2. The organic phase was collected, dried over Na_2SO_4, evaporated under reduced pressure, concentrated under vacuo. The crude product was purified by silica gel column chromatography (EtOAc/Hexane = 99%/1%) and gave the desired product **1p** in 48% yield as a white solid.

Benzyl *tert*-butyl (6-((2-((2-cyanopropan-2-yl)amino)-2-oxoethyl)amino)-6-oxohexane-1,5-diyl)(*S*)-dicarbamate



s1

s1 was obtained in 70% yield as an amorphous solid. **^{1}H NMR** (399 MHz, DMSO-d_6) δ 8.23 (s, 1H), 8.06 (t, *J* = 5.3 Hz, 1H), 7.45 – 7.24 (m, 5H), 7.21 (t, *J* = 5.0 Hz, 1H), 6.95 (d, *J* = 7.4 Hz, 1H), 4.99 (s, 2H), 3.90 – 3.81 (m, 1H), 3.79 – 3.62 (m, 2H), 3.03 – 2.89 (m, 2H), 1.56 (s, 3H), 1.55 (s, 3H), 1.76 – 1.43 (m, 2H), 1.36 (s, 9H), 1.43 – 1.05 (m, 2H), 1.43 – 1.05 (m, 2H).

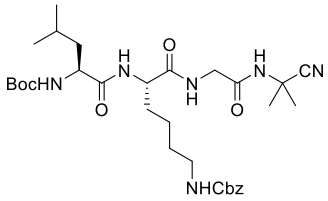
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 169.2, 156.5, 156.0, 137.7, 128.8, 128.2 (2C), 121.9 (CN, deduced by HMBC), 78.6, 65.6, 54.9, 46.0, 42.2, 40.5 (overlapping with DMSO-d_6, deduced by HMQC), 31.8, 29.5, 28.6, 26.8, 23.2.

HRMS (ESI) m/z: Calcd for $C_{25}H_{37}N_5NaO_6^+$ $[M+Na]^+$: 526.2636; found: 526.2634.

FT-IR (neat): 3314, 2938, 2243, 1688, 1525, 1455, 1392, 1367, 1251, 1169, 1027, 756, 699, 666 cm^{-1}

$[\alpha]_D^{26}$-0.96 (c 2.1 $CHCl_3$)

Benzyl ((*S*)-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-4-methylpentanamido)-6-((2-((2-cyanopropan-2-yl)amino)-2-oxoethyl)amino)-6-oxohexyl)carbamate



s2

s2 was obtained in 67% yield as an amorphous solid. **^{1}H NMR** (399 MHz, DMSO-d_6) δ 8.25 (s, 1H), 8.18 (t, *J* = 5.5 Hz, 1H), 7.87 (d, *J* = 7.3 Hz, 1H), 7.44 – 7.23 (m, 5H), 7.19 (t, *J* = 5.4 Hz, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 4.98 (s, 2H), 4.22 – 4.11 (m, 1H), 4.00 – 3.88 (m, 1H), 3.73 (dd, *J* = 17.1, 6.1 Hz, 1H), 3.67 (dd, *J* = 17.0, 5.9 Hz, 1H), 3.01 – 2.89 (m, 2H), 1.83 – 1.49 (m, 1H), 1.83 – 1.49 (m, 2H), 1.56 (s, 3H), 1.55 (s, 3H), 1.35 (s, 9H), 1.48 – 1.03 (m, 2H), 1.48 – 1.03 (m, 2H), 1.48 – 1.03 (m, 2H), 0.85 (d, *J* = 6.8 Hz, 3H), 0.83 (d, *J* = 8.0 Hz, 3H).

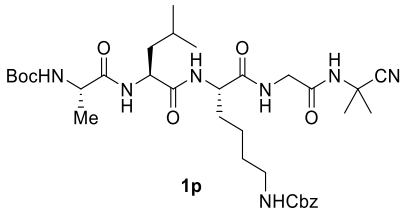
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2, 172.3, 169.1, 156.5, 155.8, 137.7, 128.8, 128.2 (2C), 121.9, 78.5, 65.5, 53.2, 53.1, 46.0, 42.1, 40.9, 40.6, 32.0, 29.5, 28.6, 26.9, 26.8, 24.7, 23.4, 22.9, 22.0.

HRMS (ESI) m/z: Calcd for $C_{31}H_{48}N_6NaO_7^+$ $[M+Na]^+$: 639.3477; found: 639.3472.

FT-IR (neat): 3300, 2956, 2243, 1693, 1663, 1532, 1455, 1391, 1367, 1252, 1167, 1026, 755, 698 cm^{-1}

$[\alpha]_D^{23}$-20.04 (c 1.05 $CHCl_3$)

<u>Benzyl ((6*S*,9*S*,12*S*)-12-((2-((2-cyanopropan-2-yl)amino)-2-oxoethyl)carbamoyl)-9-isobutyl-2,2,6-trimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazahexadecan-16-yl)carbamate</u>



1p

1p was obtained in 48% yield as a white solid. m.p. 100-102 °C.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.23 (s, 1H), 8.19 – 8.13 (m, 1H), 8.00 (d, *J* = 7.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.44 – 7.24 (m, 5H), 7.21 (t, *J* = 5.6 Hz, 1H), 6.94 (d, *J* = 7.7 Hz, 1H), 4.98 (s, 2H), 4.33 – 4.24 (m, 1H), 4.15 – 4.07 (m, 1H), 3.98 – 3.90 (m, 1H), 3.71 (dd, *J* = 16.7, 5.6 Hz, 1H), 3.66 (dd, *J* = 16.4, 5.3 Hz, 1H), 2.98 – 2.90 (m, 2H), 1.76 – 1.47 (m, 1H), 1.76 – 1.47 (m, 2H), 1.56 (s, 3H), 1.55 (s, 3H), 1.35 (s, 9H), 1.47 – 1.15 (m, 2H), 1.47 – 1.15 (m, 2H), 1.47 – 1.15 (m, 2H), 1.12 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.82 (d, *J* = 6.4 Hz, 3H).

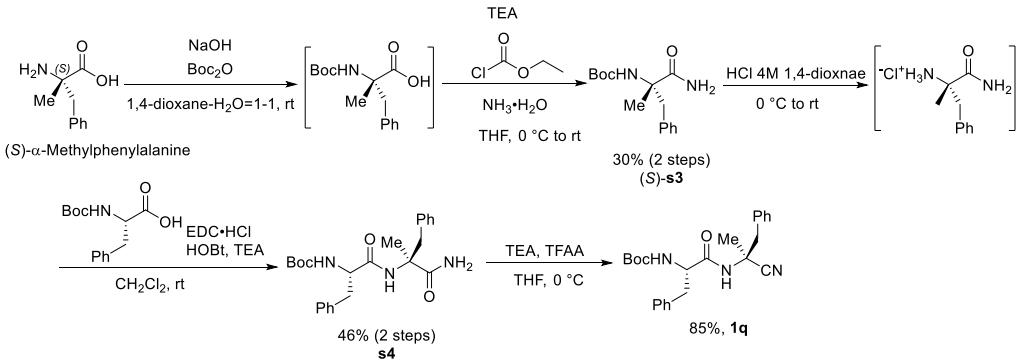
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 172.6, 172.3, 169.1, 156.5, 155.5, 137.7, 128.8, 128.2 (2C), 121.9, 78.5, 65.5, 53.3, 51.2, 50.0, 46.0, 42.2, 41.2, 40.6 (overlapping with DMSO-d_6, deduced by HMQC), 31.8, 29.6, 28.6, 26.9, 26.8, 24.4, 23.5, 23.0, 22.0, 18.3.

HRMS (ESI) m/z: Calcd for $C_{34}H_{53}N_7NaO_8^+$ $[M+Na]^+$: 710.3848; found: 710.3847.

FT-IR (neat): 3286, 3067, 2934, 2243, 1691, 1633, 1540, 1455, 1367, 1251, 1169, 1024, 757, 697 cm^{-1}

$[\alpha]_D^{25}$-15.62 (c 0.51 $CHCl_3$)

2.3 Synthesis of chiral α, α-disubstituted α-amido nitrile 1q and 1r



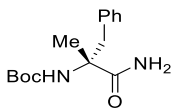
(*S*)-α-Methylphenylalanine monohydrate (500 mg, 2.53 mmol) was suspended in 1,4-dioxane-H_2O = 1/1 (10 mL, 0.25 M) at room temperature, followed by addition of aqueous 1N NaOH solution (2.6 mL) and

Boc_2O (939 mg, 4.30 mmol). After stirring for 24 h, the reaction mixture was diluted by EtOAc, quenched by 1N HCl and extracted with EtOAc. The organic phase was collected, dried over Na_2SO_4, evaporated under reduced pressure and concentrated in vacuo. The crude product Boc-(*S*)-α-methylphenylalanine (400 mg, 1.43 mmol) was dissolved in THF (2.9 mL, 0.5 M) and cool to 0 °C. Triethylamine (199 μL, 1.42 mmol) was added to the reaction mixture and stirred for 15 min, followed by ethyl chloroformate (136 μL, 1.43 mmol). After stirring for 30 min at 0 °C, 30% NH_3•H_2O (5 mL) was added to the reaction mixture and the ice bath was removed. After stirring for 1 h, the reaction was diluted by water, extracted with EtOAc, dried over Na_2SO_4, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 40%/60%) and gave the desired product (*S*)-**s3** in 30% yield over 2 steps as a white solid.

4M HCl 1,4-dioxane solution (1.9 mL) was added to (*S*)-**s3** (106 mg, 0.38 mmol) at 0 °C. After stirring for 40 min at room temperature, the volatiles were evaporated under reduced pressure. The crude product was dissolved in CH_2Cl_2 (2.5 mL, 0.15 M) at room temperature, followed by addition of triethylamine (130 μL, 0.95 mmol), Boc-L-Phe-OH (100 mg, 0.38 mmol), EDC•HCl (88.0 mg, 0.46 mmol) and HOBt monohydrate (29.0 mg, 0.19 mmol). After stirring overnight at room temperature, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with CH_2Cl_2, dried over Na_2SO_4, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 30%/70%) and gave the desired product **s4** in 46% yield over 2 steps as a white solid.

1q was synthesized based on the reported procedure[4] using **s4**. To the mixture of **s4** (64 mg, 0.15 mmol) in THF (0.8 mL, 0.2 M) at 0 °C was added triethylamine (59 μL, 0.42 mmol), followed by dropwise addition of trifluoroacetic anhydride (29 μL, 0.21 mmol). After stirring for 40 min at 0 °C, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with EtOAc, dried over Na_2SO_4, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 50%/50%) and gave the desired product **1q** in 85% yield as a white solid.

***tert*-Butyl (*R*)-(1-amino-2-methyl-1-oxo-3-phenylpropan-2-yl)carbamate**



(*R*)-**s3**

(*R*)-**s3** was obtained in 34% yield over 2 steps as a white solid. m.p. 66-68 ºC. Eluent for purification: Hexane/EtOAc = 40%/60%, then MeOH 100%.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.33 – 7.22 (m, 3H), 7.20 – 7.12 (m, 2H), 6.34 (s, 1H), 5.68 (s, 1H), 4.88 (s, 1H), 3.37 (d, *J* = 13.7 Hz, 1H), 3.12 (d, *J* = 13.7 Hz, 1H), 1.47 (s, 9H), 1.43 (s, 3H).

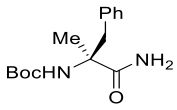
^{13}C NMR (100 MHz, $CDCl_3$) δ 176.7, 154.7, 136.1, 130.5, 128.2, 126.9, 80.3, 59.9, 41.3, 28.4, 23.9.

HRMS (ESI) m/z: Calcd for $C_{15}H_{22}N_2NaO_3^+$ $[M+Na]^+$: 301.1523; found: 301.1519.

FT-IR (neat): 3322, 1689, 1496, 1366, 1167 cm^{-1}

$[\alpha]_D^{23}$+51.81 (c 1.05 $CHCl_3$)

***tert*-Butyl (*S*)-(1-amino-2-methyl-1-oxo-3-phenylpropan-2-yl)carbamate**

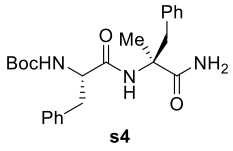


(*S*)-**s3**

(*S*)-**s3** was obtained in 30% yield over 2 steps as a white solid.

$[\alpha]_D^{23}$-57.45 (c 0.96 $CHCl_3$)

***tert*-Butyl ((*S*)-1-(((*S*)-1-amino-2-methyl-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate**



s4

s4 was obtained in 46% yield over 2 steps as a white solid. m.p. 187-188 ºC. **^{1}H NMR** (399 MHz, $CDCl_3$) δ 7.33 – 6.95 (m, 10H), 6.62 (s, 1H), 6.42 (s, 1H), 5.58 (s, 1H), 5.12 (d, *J* = 5.1 Hz, 1H), 4.16 – 4.10 (m, 1H), 3.35 (d, *J* = 13.7 Hz, 1H), 3.08 (dd, *J* = 14.0, 5.9 Hz, 1H), 3.03 – 2.89 (m, 2H), 1.52 (s, 3H), 1.33 (s, 9H).

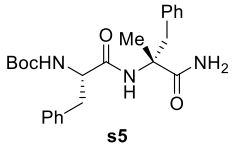
^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 170.9, 156.0, 136.4, 135.8, 130.3, 129.3, 128.8, 128.3, 127.2, 127.1, 80.7, 60.3, 56.8, 42.2, 37.3, 28.2, 23.6.

HRMS (ESI) m/z: Calcd for $C_{24}H_{31}N_3NaO_4^+$ $[M+Na]^+$: 448.2207; found: 448.2200.

FT-IR (neat): 3311, 2979, 1668, 1497, 1455, 1367, 1248, 1168, 1029, 756, 702 cm^{-1}

$[\alpha]_D^{26}$-48.27 (c 3.4 $CHCl_3$)

***tert*-Butyl ((*S*)-1-(((*R*)-1-amino-2-methyl-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-phenyl-propan-2-yl)carbamate**



s5

s5 was obtained in 55% yield over 2 steps as a white solid. m.p. 162-164 ºC. Eluent for purification: Hexane/EtOAc = 30%/70%.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.41 – 6.90 (m, 10H), 6.64 (s, 1H), 6.20 (s, 1H), 5.53 (s, 1H), 5.17 (d, *J* = 5.3 Hz, 1H), 4.15 – 4.06 (m, 1H), 3.38 (d, *J* = 13.7 Hz, 1H), 3.11 (dd, *J* = 13.7, 6.9 Hz, 1H), 2.98 – 2.80 (m, 2H), 1.44 (s, 3H), 1.36 (s, 9H).

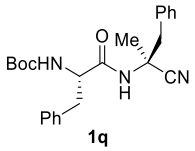
^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 171.0, 156.0, 136.5, 135.7, 130.4, 129.2, 128.8, 128.3, 127.2, 127.1, 80.7, 60.2, 57.2, 42.1, 37.7, 28.2, 23.6.

HRMS (ESI) m/z: Calcd for $C_{24}H_{31}N_3NaO_4^+$ $[M+Na]^+$: 448.2207; found: 448.2201.

FT-IR (neat): 3276, 2978, 1671, 1542, 1394, 1237, 1178, 1023, 910, 738, 699, 582 cm^{-1}

$[\alpha]_D^{27}$+18.05 (c 1.2 $CHCl_3$)

***tert*-Butyl ((*S*)-1-(((*S*)-2-cyano-1-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate**



1q

1q was obtained in 85% yield as a white solid. m.p. 137-138 ºC. **^{1}H NMR** (399 MHz, $CDCl_3$) δ 7.43 – 6.98 (m, 10H), 6.39 (s, 1H), 5.21 (s, 1H), 4.36 – 4.23 (m, 1H), 3.21 – 2.93 (m, 2H), 3.21 – 2.93 (m, 2H), 1.50 (s, 3H), 1.39 (s, 9H).

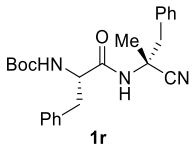
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 155.8, 136.4, 133.1, 130.5, 129.5, 128.8, 128.6, 127.8, 127.1, 119.7, 80.6, 55.8, 50.3, 43.6, 38.0, 28.2, 24.5.

HRMS (ESI) m/z: Calcd for $C_{24}H_{29}N_3NaO_3^+$ $[M+Na]^+$: 430.2101; found: 430.2093.

FT-IR (neat): 3299, 2979, 2239, 1664, 1534, 1455, 1367, 1253, 1166, 1022, 757, 701 cm^{-1}

$[\alpha]_D^{25}$-38.69 (c 1.27 $CHCl_3$)

***tert*-Butyl ((*S*)-1-(((*R*)-2-cyano-1-phenylpropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate**



1r

1r was obtained in 77% yield as a white solid. m.p. 53-55 ºC. Eluent for purification: Hexane/EtOAc = 50%/50%

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.39 – 7.08 (m, 10H), 5.98 (s, 1H), 5.16 (s, 1H), 4.28 – 4.21 (m, 1H), 3.18 (d, *J* = 13.6 Hz, 1H), 3.13 – 2.95 (m, 1H), 3.13 – 2.95 (m, 1H), 3.13 – 2.95 (m, 1H), 1.44 (s, 3H), 1.40 (s, 9H).

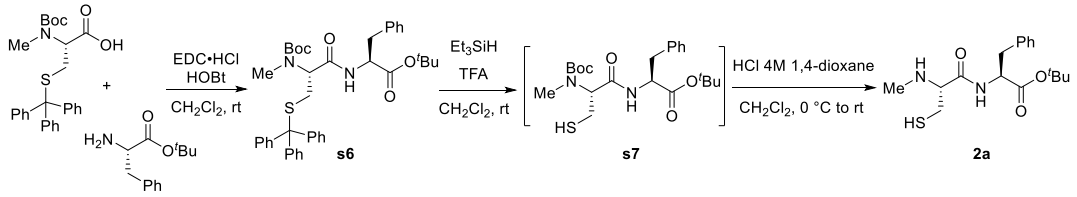
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 155.5, 136.4, 133.1, 130.4, 129.4, 128.8, 128.7, 127.9, 127.1, 119.6, 80.5, 56.0, 50.2, 44.0, 38.4, 28.2, 24.4.

HRMS (ESI) m/z: Calcd for $C_{24}H_{29}N_3NaO_3^+$ $[M+Na]^+$: 430.2101; found: 430.2098.

FT-IR (neat): 3310, 3031, 2980, 2239, 1667, 1532, 1455, 1367, 1252, 1167, 1051, 758, 702 cm^{-1}

$[\alpha]_D^{24}$+20.10 (c 0.54 $CHCl_3$)

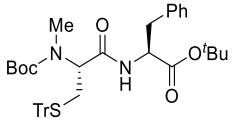
2.4 Synthesis of *N*-methyl cysteinyl peptide 2a



N-(*tert*-butoxycarbonyl)-*N*-methyl-S-trityl-L-cysteine[5] (6.55 g, 13.7 mmol) and *tert*-butyl L-phenylalaninate (3.04 g, 13.7 mmol) was dissolved in CH_2Cl_2 (55 mL, 0.25 M) at room temperature. HOBt monohydrate (210 mg, 1.37 mmol) and EDC•HCl (3.16 g, 16.5 mmol) were added to the reaction mixture. After stirring for 60 h at room temperature, the reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 60%/40%) and gave **s6** in 90% yield as an amorphous solid.

s6 (8.40 g, 12.0 mmol) was dissolved in CH_2Cl_2 (120 mL, 0.1 M) at room temperature, followed by the addition of Et_3SiH (2.2 mL, 14.0 mmol) and TFA (4.8 mL, 4 v/v%).[6] After stirring for 50 min, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 65%/35%) and gave **s7** (5.10 g) as a colorless oil. **s7** (5.10 g, 12.0 mmol) was dissolved in CH_2Cl_2 (5 mL, 2.4 M) and the reaction mixture was cooled to 0 ºC, followed by the addition of 4M HCl 1,4-dioxane solution (24 mL).[7] After stirring for 19 min at room temperature, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 30%/70%) and gave **2a** in 64% yield as an amorphous solid.

***tert*-Butyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-S-trityl-L-cysteinyl-L-phenylalaninate**



s6

s6 was obtained in 90% yield as an amorphous solid. **s6** existed as a mixture of rotamers.

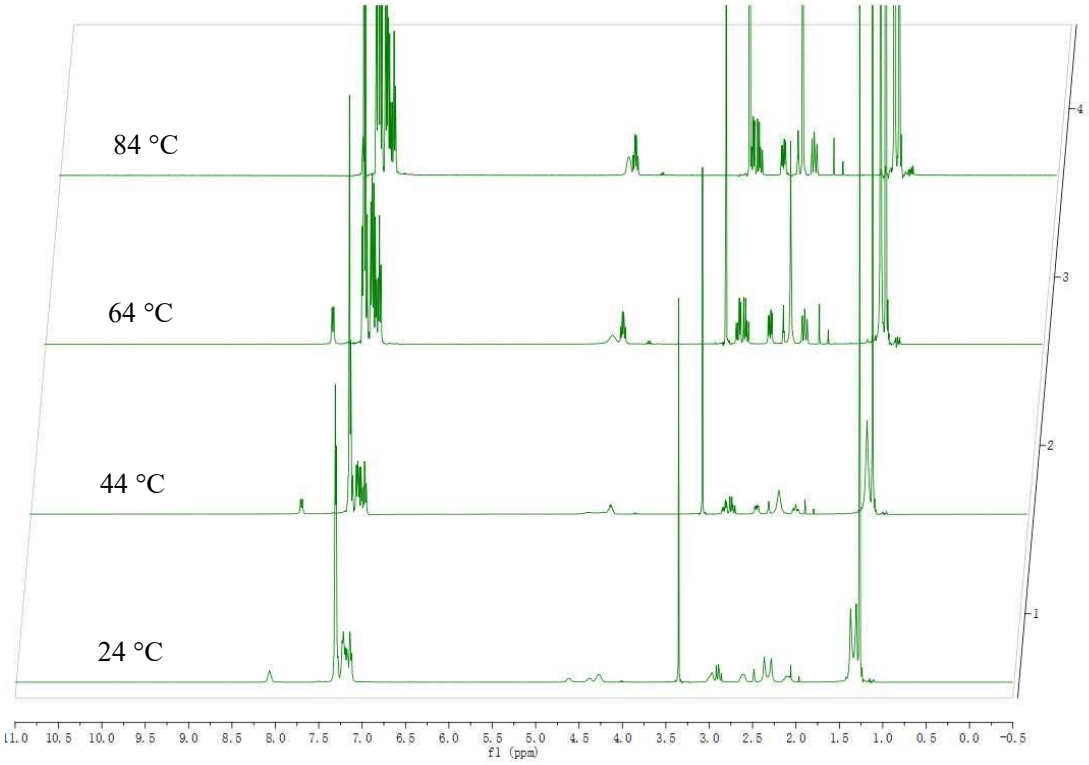
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.60 – 6.82 (m, 15H), 7.60 – 6.82 (m, 5H), 4.65 – 4.57 and 4.41 – 4.34 (m, 1H), 4.31 – 4.21 (m, 1H), 3.02 – 2.94 (m, 1H), 2.88 (dd, *J* = 13.8, 9.3 Hz, 1H), 2.65 – 2.56 (m, 1H), 2.36 and 2.28 (s, 3H), 2.13 – 2.02 (m, 1H), 1.37 and 1.30 (s, 9H), 1.26 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 170.6 and 170.5 (1C), 169.4 and 169.3 (1C), 155.5 and 154.8 (1C), 144.6, 137.8, 129.4, 128.5, 128.5, 127.2, 126.8, 81.1, 79.8 and 79.7 (1C), 66.29 and 66.25 (1C), 58.0 and 56.5 (1C), 54.5, 36.5, 31.6 and 31.2 (1C), 30.5 and 30.2 (1C), 28.4, 27.9.

HRMS (ESI) m/z: Calcd for $C_{41}H_{48}N_2NaO_5S^+$ $[M+Na]^+$: 703.3176; found: 703.3182.

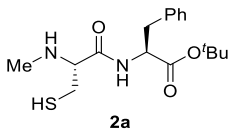
FT-IR (neat): 3406, 3063, 2978, 2932, 1685, 1495, 1445, 1367, 1320, 1155, 846, 751, 701 cm^{-1}

$[\alpha]_D^{27}$-34.66 (c 1.95 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of s6

***tert*-Butyl methyl-L-cysteinyl-L-phenylalaninate**



2a

2a was obtained in 64% yield as an amorphous solid. **^{1}H NMR** (399 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.6 Hz, 1H), 7.24 – 7.05 (m, 5H), 4.75 – 4.67 (m, 1H), 3.08 (dd, *J* = 13.9, 5.8 Hz, 1H), 3.04 – 2.99 (m, 1H), 2.94 (dd, *J* = 13.9, 7.4 Hz, 1H), 2.74 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.62 (dd, *J* = 13.9, 6.8 Hz, 1H), 2.29 (s, 3H), 1.36 (s, 9H).

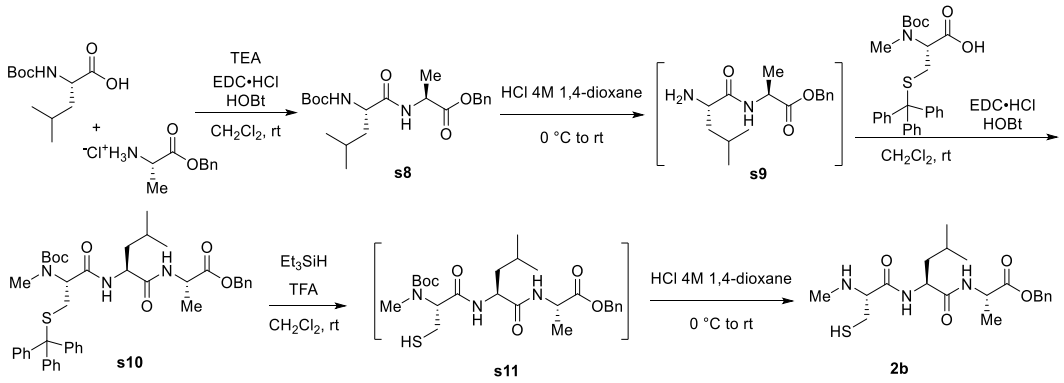
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.4, 136.3, 129.4, 128.3, 126.8, 82.1, 65.4, 53.0, 38.2, 34.7, 27.9, 26.5.

HRMS (ESI) m/z: Calcd for $C_{17}H_{27}N_2O_3S^+$ $[M+H]^+$: 339.1737; found: 339.1742.

FT-IR (neat): 3327, 2978, 2557, 1733, 1662, 1515, 1368, 1255, 1156, 846, 741, 700 cm^{-1}

$[\alpha]_D^{23}$+1.77 (c 1.54 $CHCl_3$)

2.5 Synthesis of *N*-methyl cysteinyl peptide 2b

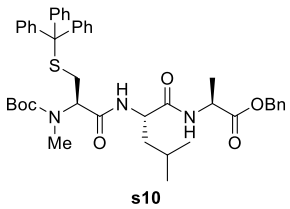


Boc-L-Leu-OH monohydrate (2.09 g, 8.65 mmol) and L-Ala-OBn hydrochloride (1.87 g, 8.65 mmol) were dissolved in CH_2Cl_2 (35 mL, 0.25 M) at room temperature, followed by the addition of HOBt monohydrate (133 mg, 0.860 mmol), EDC•HCl (1.99 g, 10.4 mmol) and triethylamine (3.01 mL, 21.6 mmol). After stirring overnight at room temperature, the reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 50%/50%) and gave **s8** in 91% yield as a colorless oil. The NMR data is consistent with the literarure.[8]

4M HCl 1,4-dioxane solution (15.2 mL) was cooled to 0 ºC and added to **s8** (3.00 g, 7.60 mmol) at 0 ºC. After stirring for 3 h at room temperature, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with EtOAc. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product (2.20 g, 7.50 mmol) was dissolved in CH_2Cl_2 (30 mL, 0.25 M) at room temperature, followed by the addition of *N*-(*tert*-butoxycarbonyl)-*N*-methyl-S-trityl-L-cysteine (3.60 g, 7.50 mmol),[5] HOBt monohydrate (114 mg, 0.750 mmol), EDC•HCl (1.70 g, 9.00 mmol). After stirring overnight at room temperature, the reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 65%/35%) and gave **s10** in 91% yield over 2 steps as a white solid.

s10 (2.00 g, 2.66 mmol) was dissolved in CH_2Cl_2 (27 mL, 0.1 M) at room temperature, followed by the addition of Et_3SiH (480 μL, 3.01 mmol) and trifluoroacetic acid (1.08 mL, 4 v/v%).[6] After stirring for 85 min, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 50%/50%) and gave **s11** (1.00 g) as a white solid. **s11** (1.00 g, 1.96 mmol) was cooled to 0 ºC, followed by the addition of 4M HCl 1,4-dioxane solution (3.9 mL). After stirring for 1 h at room temperature, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with EtOAc. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 30%/70%) and gave **2b** in 68% yield as a colorless oil.

Benzyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-S-trityl-L-cysteinyl-L-leucyl-L-alaninate



s10

s10 was obtained in 91% yield over 2 steps as a white solid. m.p. 69-71 ºC. **s10** existed as a mixture of rotamers.

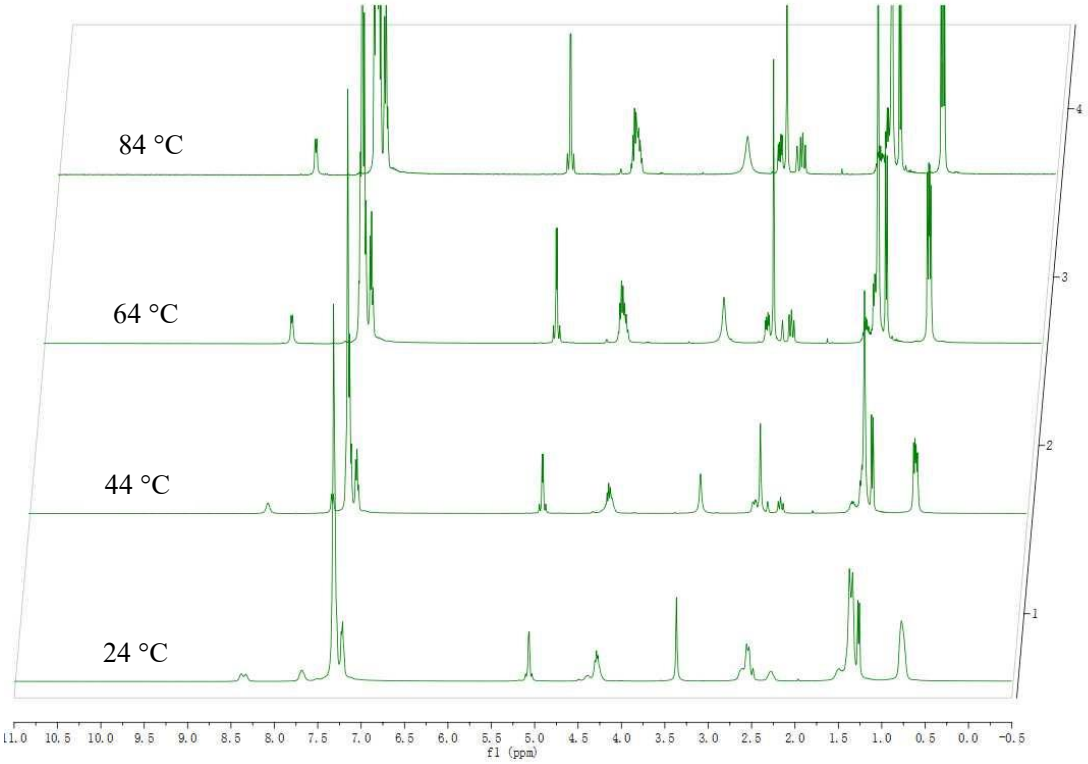
^{1}H NMR (**measured at 64 °C**) (399 MHz, DMSO-d_6) δ 8.14 (d, *J* = 6.6 Hz, 1H), 7.49 – 7.10 (m, 5H), 7.49 – 7.10 (m, 15H), 5.11 (d, *J* = 12.6 Hz, 1H), 5.06 (d, *J* = 12.6 Hz, 1H), 4.40 – 4.23 (m, 1H), 4.40 – 4.23 (m, 1H), 4.40 – 4.23 (m, 1H), 2.66 (dd, *J* = 12.4, 5.3 Hz, 1H), 2.58 (s, 3H), 2.38 (dd, *J* = 12.2, 10.2 Hz, 1H), 1.57 – 1.41 (m, 1H), 1.57 – 1.41 (m, 2H), 1.38 (s, 9H), 1.29 (d, *J* = 7.2 Hz, 3H), 0.80 (d, *J* = 6.5 Hz, 3H), 0.78 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.2, 169.1, 155.6 and 154.8 (1C), 144.7, 136.3, 129.5, 128.8, 128.5, 128.4, 128.2, 127.2, 79.9, 66.3, 58.6 and 57.7 (1C), 51.0, 48.1, 41.3 and 41.2 (1C), 31.6, 31.0, 28.4, 24.4, 23.5, 21.9, 21.7, 17.1.

HRMS (ESI) m/z: Calcd for $C_{44}H_{53}N_3NaO_6S^+$ $[M+Na]^+$: 774.3547; found: 774.3549.

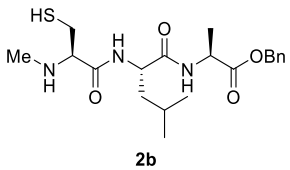
FT-IR (neat): 3306, 3060, 2957, 1747, 1656, 1541, 1446, 1389, 1367, 1321, 1161, 752, 700 cm^{-1}

$[\alpha]_D^{25}$-70.54 (c 1.35 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of s10

Benzyl methyl-L-cysteinyl-L-leucyl-L-alaninate



2b

2b was obtained in 68% yield as a colorless oil. **^{1}H NMR** (399 MHz, DMSO-d_6) δ 8.44 (d, *J* = 6.8 Hz, 1H), 8.00 (d, *J* = 8.7 Hz, 1H), 7.48 – 7.16 (m, 5H), 5.09 (d, *J* = 12.6 Hz, 1H), 5.05 (d, *J* = 12.6 Hz, 1H), 4.44 – 4.36 (m, 1H), 4.34 – 4.26 (m, 1H), 3.07 – 3.00 (m, 1H), 2.65 (dd, *J* = 13.3, 5.3 Hz, 1H), 2.57 (dd, *J* = 13.2, 6.7 Hz, 1H), 2.19 (s, 3H), 1.63 – 1.52 (m, 1H), 1.45 – 1.33 (m, 2H), 1.29 (d, *J* = 7.3 Hz, 3H), 0.82 (d, *J* = 6.3 Hz, 3H), 0.80 (d, *J* = 6.0 Hz, 3H).

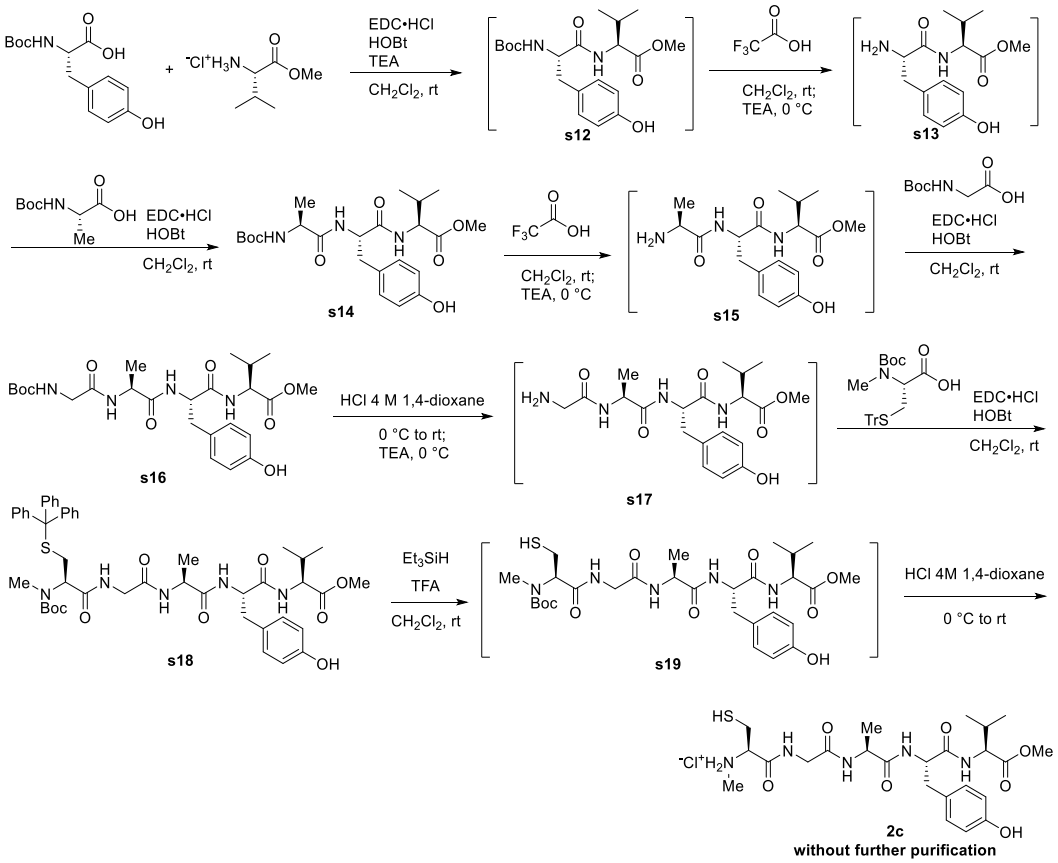
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.4, 172.1, 136.3, 128.8, 128.4, 128.2, 66.4, 65.6, 50.7, 48.1, 41.6, 34.4, 26.9, 24.5, 23.5, 21.8, 17.2.

HRMS (ESI) m/z: Calcd for $C_{20}H_{32}N_3O_4S^+$ $[M+H]^+$: 410.2108; found: 410.2108.

FT-IR (neat): 3289, 3067, 2956, 2557, 1743, 1649, 1548, 1455, 1387, 1197, 1164, 1051, 962, 751, 698 cm^{-1}

$[\alpha]_D^{25}$-78.52 (c 1.8 $CHCl_3$)

2.6 Synthesis of *N*-methyl cysteinyl peptide 2c



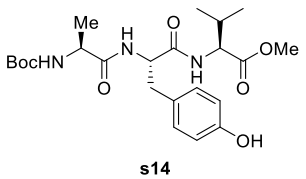
HCl•NH_2-L-Val-OMe (2.98 g, 17.8 mmol) was added to a mixture of Boc-L-Tyr-OH (5.00 g, 17.8 mmol) in CH_2Cl_2 (71 mL, 0.25 M) at room temperature. Triethylamine (3.7 mL, 26.7 mmol), EDC•HCl (4.09 g, 21.3 mmol) and HOBt monohydrate (273 mg, 1.78 mmol) were added to the reaction mixture. After stirring overnight at room temperature, the reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2, washed with sat. $NaHCO_3$ aqueous solution. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product **s12** was dissolved in CH_2Cl_2 (35 mL, 0.5 M) at room temperature, followed by the addition of trifluoroacetic acid (13.6 mL, 178 mmol). After stirring overnight at room temperature, the reaction mixture was evaporated to partially remove trifluoroacetic acid and neutralized by addition of triethylamine. 71 mL of CH_2Cl_2 was added to the resulting mixture, followed by addition of Boc-L-Ala-OH (3.36 g, 17.8 mmol), EDC•HCl (4.09 g, 21.3 mmol) and HOBt monohydrate (273 mg, 1.78 mmol). After stirring for 4 h at room temperature, the reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (EtOAc/Hexane = 85%/15%) and gave the desired product **s14** in 77% yield over 3 steps as a white solid.

Boc-L-Ala-L-Tyr-L-Val-OMe **s14** (6.42 g, 13.8 mmol) was dissolved in CH_2Cl_2 (27.6 mL, 0.5 M) at room temperature, followed by addition of trifluoroacetic acid (10.6 mL, 138 mmol). After stirring overnight at room temperature, the reaction mixture was evaporated to partially remove trifluoroacetic acid and neutralized by addition of triethylamine. 55 mL of CH_2Cl_2 was added to the resulting mixture, followed by addition of Boc-Gly-OH (2.42 g, 13.8 mmol), EDC•HCl (3.18 g, 16.6 mmol) and HOBt monohydrate (212 mg, 1.38 mmol). After stirring for 4 h at room temperature, the reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2/EtOAc = 2/1. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (EtOAc/Hexane = 95%/5%) and gave the desired product **s16** in 61% yield over 2 steps as a white solid.

4M HCl 1,4-dioxane solution (17.2 mL) was added to Boc-Gly-L-Ala-L-Tyr-L-Val-OMe **s16** (2.25 g, 4.31 mmol) at 0 °C. After stirred for 1.5 h at room temperature (white precipitate forms), the reaction mixture was cooled to 0 °C, diluted with CH_2Cl_2 (60 mL) and quenched by slow addition of triethylamine. Boc-*N*Me-L-Cys(S-Trityl)-OH (2.06 g, 4.31 mmol),[5] EDC•HCl (991 mg, 5.17 mmol) and HOBt monohydrate (66.0 mg, 0.430 mmol) were added to the resulting mixture. After stirring overnight at room temperature, the reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (MeOH/CH_2Cl_2 = 5%/95%) and gave the desired product **s18** in 74% yield over 2 steps as a white solid.

s18 (2.10 g, 2.40 mmol) was dissolved in CH_2Cl_2 (24 mL, 0.1 M) at room temperature, followed by the addition of Et_3SiH (430 μL, 2.70 mmol) and trifluoroacetic acid (0.96 mL, 4 v/v%).[6] After stirring for 1.5 h, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 95%/5%) and gave **s19** (725 mg) as a white solid. **s19** (725 mg, 1.13 mmol) was cooled to 0 ºC, followed by the addition of 4M HCl 1,4-dioxane solution (4.5 mL, 18 mmol). After stirring for 1.5 h at room temperature (white precipitate forms), the suspension was diluted with CH_2Cl_2 and filtered. The residue was washed with CH_2Cl_2, dried under vacuum, which gave **2c** in 45% yield over 2 steps as a white solid.

Methyl (*tert*-butoxycarbonyl)-L-alanyl-L-tyrosyl-L-valinate



s14

s14 was obtained in 77% yield over 3 steps as a white solid. m.p. 94-96 ºC. **^{1}H NMR** (399 MHz, DMSO-d_6) δ 9.13 (s, 1H), 8.17 (d, *J* = 7.4 Hz, 1H), 7.66 (d, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 7.3 Hz, 2H), 6.92 (d, *J* = 5.8 Hz, 1H), 6.60 (d, *J* = 7.0 Hz, 2H), 4.58 – 4.48 (m, 1H), 4.19 – 4.12 (m, 1H), 3.93 – 3.84 (m, 1H), 3.61 (s, 3H), 2.86 (dd, *J* = 13.8, 3.7 Hz, 1H), 2.69 (dd, *J* = 13.0, 8.7 Hz, 1H), 2.05 – 1.95 (m, 1H), 1.34 (s, 9H), 1.08 (d, *J* = 6.7 Hz, 3H), 0.85 (d, *J* = 6.9 Hz, 3H), 0.83 (d, *J* = 6.8 Hz, 3H).

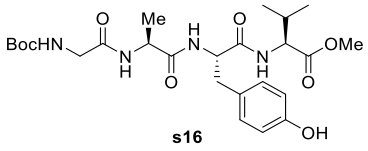
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 172.1, 171.7, 156.2, 155.3, 130.6, 127.8, 115.2, 78.5, 57.8, 53.9, 52.1, 50.3, 37.3, 30.4, 28.6, 19.3, 18.6, 18.5.

HRMS (ESI) m/z: Calcd for $C_{23}H_{35}N_3NaO_7^+$ $[M+Na]^+$: 488.2367; found: 488.2364.

FT-IR (neat): 3296, 2974, 1651, 1518, 1450, 1368, 1247, 1168, 1023, 758 cm^{-1}

$[\alpha]_D^{22}$-29.18 (c 1.13 $CHCl_3$)

Methyl (*tert*-butoxycarbonyl)glycyl-L-alanyl-L-tyrosyl-L-valinate



s16

s16 was obtained in 61% yield over 2 steps as a white solid. m.p. 204-206 ºC. **^{1}H NMR** (399 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.96 (d, *J* = 8.1 Hz, 1H), 7.85 (d, *J* = 7.3 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.92 (t, *J* = 5.8 Hz, 1H), 6.61 (d, *J* = 8.4 Hz, 2H), 4.51 – 4.42 (m, 1H), 4.28 – 4.18 (m, 1H), 4.16 – 4.09 (m, 1H), 3.60 (s, 3H), 3.51 (d, *J* = 5.8 Hz, 2H), 2.88 (dd, *J* = 13.9, 4.7 Hz, 1H), 2.66 (dd, *J* = 13.9, 9.3 Hz, 1H), 2.05 – 1.96 (m, 1H), 1.35 (s, 9H), 1.11 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H).

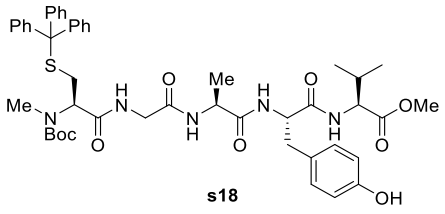
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 172., 171.8, 169.3, 156.2 (2C), 130.5, 128.1, 115.2, 78.5, 57.9, 54.3, 52.1, 48.4, 43.6, 36.9, 30.3, 28.6, 19.3, 18.8, 18.7.

HRMS (ESI) m/z: Calcd for $C_{25}H_{38}N_4NaO_8^+$ $[M+Na]^+$: 545.2582; found: 545.2585.

FT-IR (neat): 3291, 2970, 1733, 1644, 1517, 1447, 1369, 1218, 1167, 756 cm^{-1}

$[\alpha]_D^{22}$-19.05 (c 1.0 DMSO)

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-S-trityl-L-cysteinylglycyl-L-alanyl-L-tyrosyl-L-valinate



s18

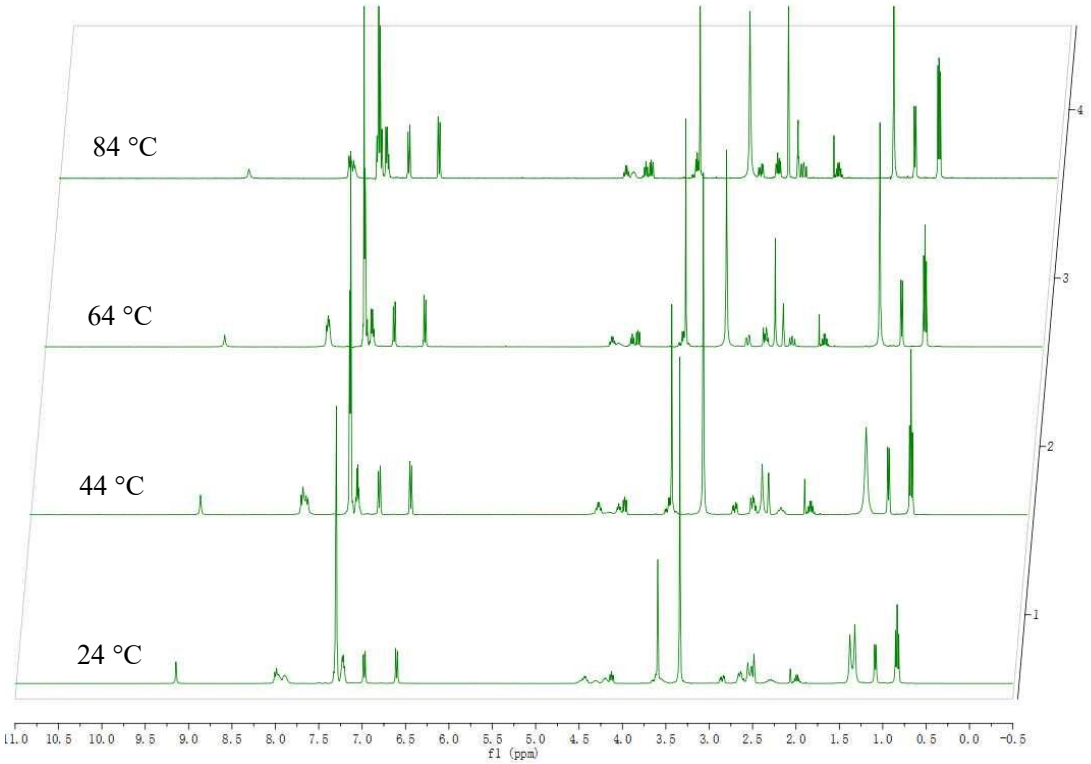
s18 was obtained in 74% yield over 2 steps as a white solid. m.p. 128-130 ºC. **s18** existed as a mixture of two rotamers. **^{1}H NMR** (399 MHz, DMSO-d_6) (**measured at 64 °C**) δ 8.92 (s, 1H), 7.81 – 7.61 (m, 1H), 7.81 – 7.61 (m, 1H), 7.81 – 7.61 (m, 1H), 7.81 – 7.61 (m, 1H), 7.47 – 7.09 (m, 15H), 6.97 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 8.4 Hz, 2H), 4.49 – 4.43 (m, 1H), 4.42 – 4.32 (m, 1H), 4.25 – 4.20 (m, 1H), 4.16 (dd, *J* = 8.2, 6.3 Hz, 1H), 3.74 – 3.52 (m, 1H), 3.74 – 3.52 (m, 1H), 3.61 (s, 3H), 2.89 (dd, *J* = 14.0, 5.2 Hz, 1H), 2.73 – 2.64 (m, 1H), 2.73 – 2.64 (m, 1H), 2.58 (s, 3H), 2.38 (dd, *J* = 12.1, 10.6 Hz, 1H), 2.05 – 1.96 (m, 1H), 1.37 (s, 9H), 1.12 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H), 0.84 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 172.1, 171.7, 169.8 and 169.7 (1C), 168.6, 156.2 (*ipso*-C of phenol), 155.6 and 154.8 (1C), 144.7, 130.5, 129.5, 128.5, 128.1, 127.2, 115.3, 80.0 and 79.9 (1C), 66.3, 59.1 and 57.7 (1C), 57.9, 54.5, 52.1, 48.5, 42.6, 36.8, 31.6, 31.4 and 31.2 (1C), 30.4, 28.5 and 28.4 (3C), 19.3, 18.7 and 18.6 (1C), 18.6.

HRMS (ESI) m/z: Calcd for $C_{48}H_{59}N_5NaO_9S^+$ $[M+Na]^+$: 904.3926; found: 904.3926.

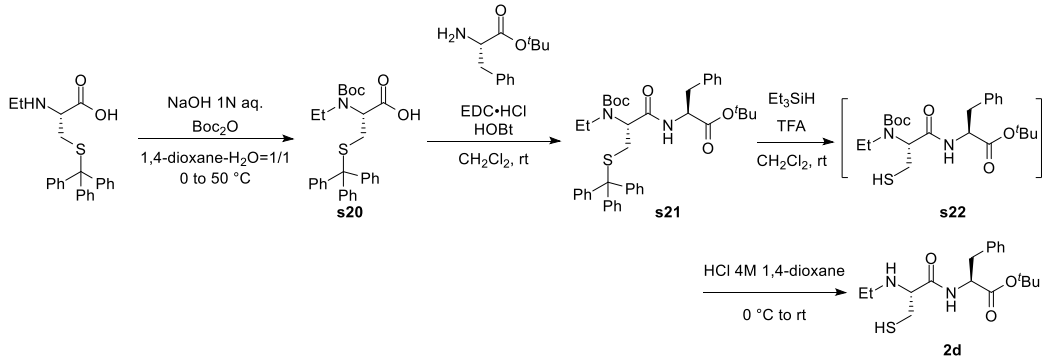
FT-IR (neat): 3297, 2972, 1736, 1646, 1517, 1445, 1369, 1317, 1218, 1158, 755, 702, 667 cm^{-1}

$[\alpha]_D^{24}$-48.64 (c 3.58 $CHCl_3$)



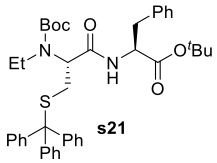
Variable temperature ^{1}H NMR to prove the rotamers of s18

2.7 Synthesis of *N*-ethyl cysteinyl peptide 2d



N-Ethyl-S-trityl L-cysteine was synthesized from S-trityl L-cysteine (1.00 g, 2.75 mmol) based on the reported procedure[9] and the crude product was roughly purified by silica gel column chromatography (CH_2Cl_2/MeOH = 88%/11%), which was dissolved in 1,4-dioxnae/H_2O = 1/1 (22 mL, 0.12 M) and cooled to 0 ºC. 1N NaOH aqueous solution (5.5 mL, 5.50 mmol) was added slowly to the reaction mixture, followed by the addition of Boc_2O (1.20 g, 5.50 mmol). After stirring overnight at 50 ºC, the reaction mixture was cooled to 0 ºC, diluted with EtOAc, quenched by 1M $KHSO_4$ aqueous solution, extracted with EtOAc. The organic phase was collected, evaporated under reduced pressure, dried over Na_2SO_4 and concentrated in vacuo. The crude product was roughly purified by silica gel column chromatography (CH_2Cl_2/MeOH = 88%/11%) and gave **s20** (700 mg). **s20** (700 mg, 1.42 mmol) was then used to synthesize **2d** according to the procedure towards the synthesis of **2a**.

***tert*-Butyl *N*-(*tert*-butoxycarbonyl)-*N*-ethyl-S-trityl-L-cysteinyl-L-phenylalaninate**



s21

s21 was obtained in 43% yield over 3 steps as an amorphous solid. **s21** existed as a mixture of two rotamers.

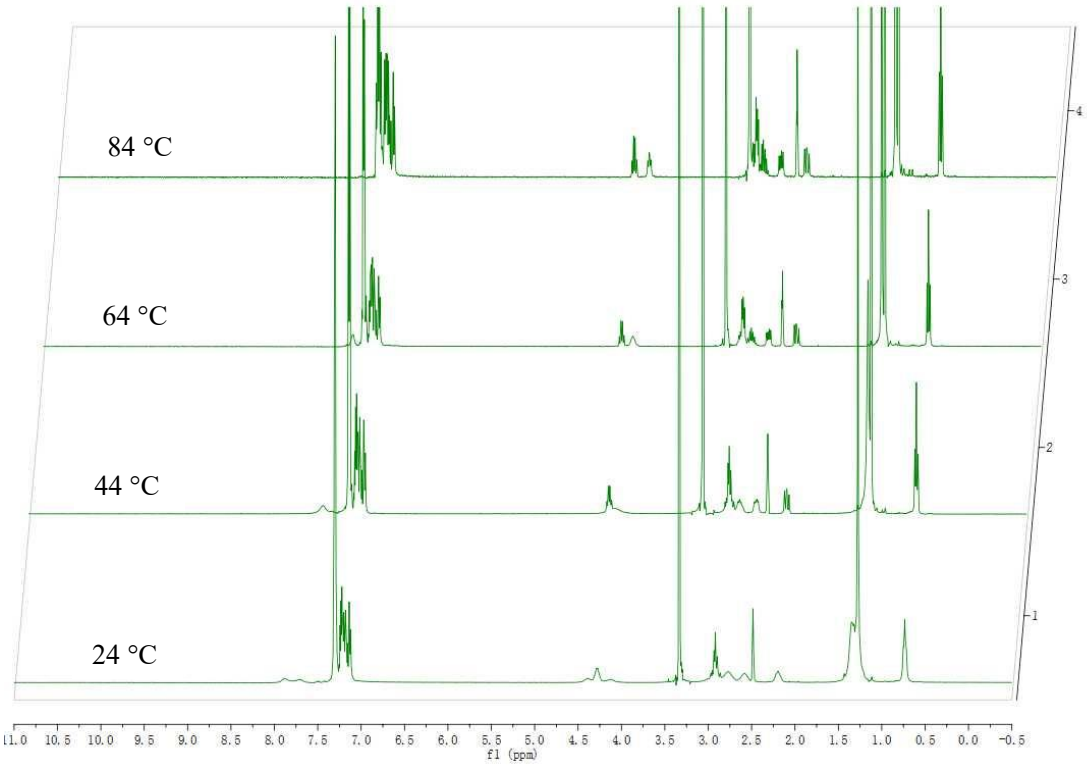
^{1}H NMR (399 MHz, DMSO-d_6) (**measured at 64 °C**) δ 7.44 (d, *J* = 7.6 Hz, 1H), 7.41 – 6.94 (m, 15H), 7.41 – 6.94 (m, 5H), 4.37 – 4.30 (m, 1H), 4.25 – 4.16 (m, 1H), 2.96 (dd, *J* = 12.9, 5.2 Hz, 1H), 3.00 – 2.88 (m, 1H), 2.91 (dd, *J* = 12.9, 6.7 Hz, 1H), 2.87 – 2.79 (m, 1H), 2.64 (dd, *J* = 12.2, 6.2 Hz, 1H), 2.32 (dd, *J* = 12.1, 8.8 Hz, 1H), 1.34 (s, 9H), 1.30 (s, 9H), 0.80 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5 (2C), 169.4, 144.7, 137.5, 129.5, 129.5, 128.6, 128.5, 127.2, 126.9, 81.3, 79.7, 66.5, 54.6 (2C), 39.9 (deduced by HMQC), 36.8, 31.9, 28.4, 27.9, 15.0 and 14.3 (1C).

HRMS (ESI) m/z: Calcd for $C_{42}H_{50}N_2NaO_5S^+$ $[M+Na]^+$: 717.3333; found: 717.3331.

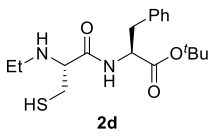
FT-IR (neat): 2977, 1732, 1686, 1495, 1407, 1367, 1290, 1254, 1156, 847, 743, 701, 622 cm^{-1}

$[\alpha]_D^{23}$-31.70 (c 1.03 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of s21

***tert*-Butyl ethyl-L-cysteinyl-L-phenylalaninate**



2d

2d was obtained in 61% yield over 2 steps as an amorphous solid. **^{1}H NMR** (399 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.5 Hz, 1H), 7.31 – 7.09 (m, 5H), 4.76 – 4.69 (m, 1H), 3.16 – 3.06 (m, 1H), 3.16 – 3.06 (m, 1H), 2.99 (dd, *J* = 13.9, 7.1 Hz, 1H), 2.76 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.67 (dd, *J* = 13.8, 6.8 Hz, 1H), 2.60 – 2.48 (m, 2H), 1.39 (s, 9H), 1.04 (t, *J* = 7.1 Hz, 3H).

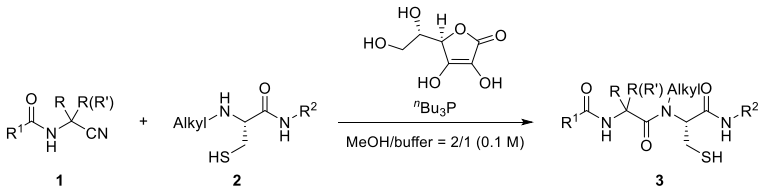
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 170.4, 136.2, 129.4, 128.3, 126.9, 82.1, 63.5, 53.0, 42.5, 38.3, 27.9, 27.1, 15.4.

HRMS (ESI) m/z: Calcd for $C_{18}H_{29}N_2O_3S^+$ $[M+H]^+$: 353.1893; found: 353.1894.

FT-IR (neat): 3315, 2975, 2554, 1733, 1661, 1513, 1368, 1253, 1156, 845, 741, 701 cm^{-1}

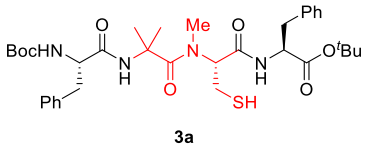
$[\alpha]_D^{25}$-6.37 (c 2.35 $CHCl_3$)

2.8 General procedure for the synthesis of 3 and physical information



N-Methyl (or ethyl) cysteinyl peptide **2** (1.5 equiv.), α-amido nitrile **1** (1 equiv.) and L-ascorbic acid (1.3 equiv.) were dissolved in MeOH (as indicated) and pH 7.4, 0.2 M phosphate buffer (as indicated), followed by the addition of $^{n}Bu_3P$ (0.5 equiv.). The reaction mixture was immediately degassed under liquid N_2 bath and filled with argon for 3 times. After stirring vigorously for the indicated time at the indicated temperature, the reaction mixture was diluted with EtOAc, dried by filtration through a short column of Na_2SO_4 (~5 g/0.1 mmol scale of substrate **1**) and washed with EtOAc. The filtrate was evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography and gave the desired product **3**.

***tert*-Butyl *N*-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-2-methylpropanoyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3a

3a was obtained in 87% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 48%/52%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.46 (s, 1H), 7.89 (d, *J* = 7.2 Hz, 1H), 7.34 – 7.07 (m, 10H), 6.99 (d, *J* = 8.4 Hz, 1H), 4.96 – 4.83 (m, 1H), 4.40 – 4.30 (m, 1H), 4.20 – 4.11 (m, 1H), 3.06 – 2.96 (m, 2H), 2.87 – 2.78 (m, 2H), 2.76 – 2.70 (m, 1H), 2.65 (s, 3H), 2.57 – 2.49 (m, 1H), 2.21 (t, *J* = 8.3 Hz, 1H), 1.31 (s, 9H), 1.30 (s, 9H), 1.26 (s, 6H).

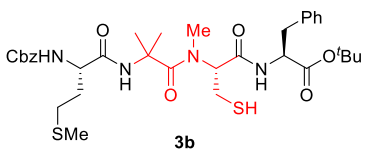
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 171.9, 170.8, 169.3, 155.8, 138.3, 138.0, 129.7, 129.6, 128.5, 128.4, 126.8, 126.7, 81.1, 78.5, 61.6, 56.3, 56.0, 54.7, 37.6, 36.7, 32.4, 28.5, 28.0, 26.5, 25.4, 22.3.

HRMS (ESI) m/z: Calcd for $C_{35}H_{50}N_4NaO_7S^+$ $[M+Na]^+$: 693.3292; found: 693.3304.

FT-IR (neat): 3299, 2979, 2544, 1662, 1498, 1393, 1367, 1250, 1161, 1095, 847, 756, 700 cm^{-1}

$[\alpha]_D^{22}$-68.95 (c 1.4 $CHCl_3$)

***tert*-Butyl *N*-(2-((*S*)-2-(((benzyloxy)carbonyl)amino)-4-(methylthio)butanamido)-2-methylpropanoyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3b

3b was obtained in 81% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 35%/65%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.47 (s, 1H), 7.82 (d, *J* = 7.5 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.40 – 7.26 (m, 5H), 7.26 – 7.12 (m, 5H), 5.00 (d, *J* = 3.8 Hz, 2H), 4.89 – 4.79 (m, 1H), 4.36 – 4.28 (m, 1H), 4.10 – 4.03 (m, 1H), 2.98 (d, *J* = 7.6 Hz, 2H), 2.89 – 2.81 (m, 1H), 2.68 (s, 3H), 2.61 – 2.54 (m, 1H), 2.46 – 2.35 (m, 2H), 2.21 – 2.16 (m, 1H), 1.99 (s, 3H), 1.84 – 1.73 (m, 2H), 1.33 (s, 3H), 1.32 (s, 3H), 1.29 (s, 9H).

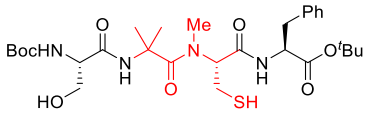
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 171.8, 170.8, 169.3, 156.5, 137.9, 137.3, 129.6, 128.7, 128.5, 128.2, 128.2, 126.8, 81.0, 66.0, 61.7, 56.4, 54.7, 54.1, 36.9, 32.6, 31.7, 30.2, 28.0, 26.4, 25.6, 22.3, 15.1.

HRMS (ESI) m/z: Calcd for $C_{34}H_{48}N_4NaO_7S_2^+$ $[M+Na]^+$: 711.2857; found: 711.2864.

FT-IR (neat): 3310, 2979, 2546, 1720, 1655, 1525, 1394, 1367, 1241, 1155, 1045, 752, 699 cm^{-1}

$[\alpha]_D^{22}$-78.74 (c 1.03 $CHCl_3$)

***tert*-Butyl *N*-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropanamido)-2-methylpropanoyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3c

3c was obtained in 86% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 20%/80%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.37 (s, 1H), 7.77 (d, *J* = 7.3 Hz, 1H), 7.30 – 7.12 (m, 5H), 6.58 (d, *J* = 8.0 Hz, 1H), 4.93 (s, 1H), 4.87 – 4.80 (m, 1H), 4.35 – 4.27 (m, 1H), 4.00 – 3.92 (m, 1H), 3.57 – 3.48 (m, 2H), 2.98 (d, *J* = 7.7 Hz, 2H), 2.93 – 2.85 (m, 1H), 2.71 (s, 3H), 2.60 – 2.51 (m, 1H), 2.20 – 2.13 (m, 1H), 1.35 (s, 9H), 1.52 – 1.30 (m, 3H), 1.32 (s, 3H), 1.27 (s, 9H).

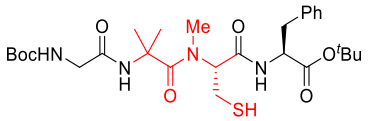
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 170.8, 170.7, 169.3, 155.6, 137.9, 129.6, 128.5, 126.8, 81.0, 78.6, 62.0, 61.3, 57.1, 56.5, 54.9, 37.0, 32.2, 28.5, 27.9, 26.2, 26.0, 22.3.

HRMS (ESI) m/z: Calcd for $C_{29}H_{46}N_4NaO_8S^+$ $[M+Na]^+$: 633.2929; found: 633.2931.

FT-IR (neat): 3313, 2979, 2550, 1715, 1660, 1525, 1394, 1367, 1251, 1160, 1091, 846, 754, 700 cm^{-1}

$[\alpha]_D^{25}$-82.25 (c 1.00 $CHCl_3$)

***tert*-Butyl *N*-(2-(2-((*tert*-butoxycarbonyl)amino)acetamido)-2-methylpropanoyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3d

3d was obtained in 82% yield as an amorphous solid and existed as a mixture of rotamers. Eluent for purification: Hexane/EtOAc = 30%/70%.

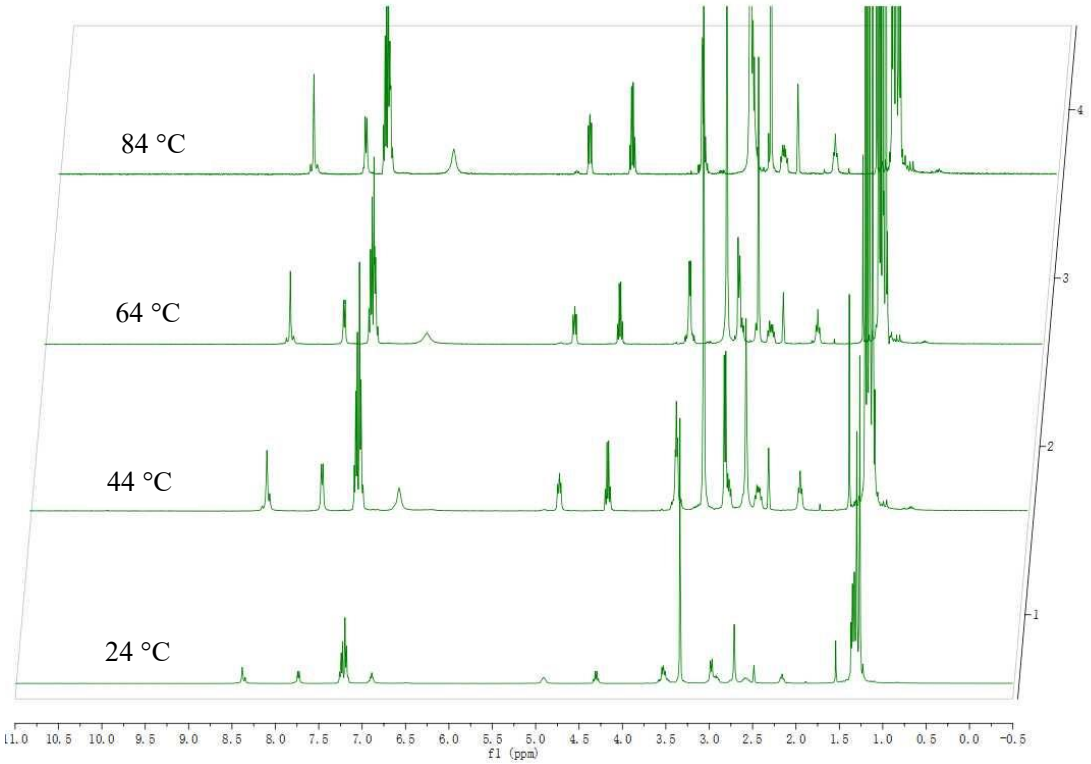
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.37 and 8.34 (s, 1H), 7.72 (d, *J* = 7.2 Hz, 1H), 7.31 – 7.11 (m, 5H), 6.88 (t, *J* = 5.8 Hz, 1H), 4.95 – 4.86 (m, 1H), 4.34 – 4.27 (m, 1H), 3.59 – 3.46 (m, 2H), 2.98 (d, *J* = 7.7 Hz, 2H), 3.04 – 2.87 (m, 1H), 2.72 (s, 3H), 2.62 – 2.53 (m, 1H), 2.20 – 2.11 (m, 1H), 1.54 and 1.35 (s, 3H), 1.36 and 1.33 (s, 3H), 1.30 (s, 9H), 1.26 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 173.0, 170.7, 169.5, 169.3, 156.2, 137.8, 129.6, 128.5, 126.8, 80.9, 78.4, 61.5, 56.4, 54.8, 46.0, 43.3, 37.0, 32.4, 28.6 and 28.5 (3C), 27.9 and 26.9 (3C), 26.3 and 26.1 (1C), 22.3.

HRMS (ESI) m/z: Calcd for $C_{28}H_{44}N_4NaO_7S^+$ $[M+Na]^+$:603.2823; found: 603.2829.

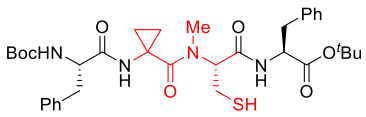
FT-IR (neat): 3303, 2979, 2550, 1717, 1664, 1523, 1393, 1367, 1249, 1162, 1090, 849, 756, 701 cm^{-1}

$[\alpha]_D^{24}$-41.00 (c 1.12 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of 3d

***tert*-Butyl *N*-(1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)cyclopropane-1-carbonyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3e

3e was obtained in 85% yield as a white solid. m.p. 180-182 ºC. Purification: the reaction suspension was centrifuged and washed with MeOH/H_2O = 1/1 mixture for 5 times. The solid was collected and dried under vacuum. The washing solvent was collected and evaporated under reduced pressure to remove MeOH, after which it was extracted with EtOAc. The organic layer was collected and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography using Hexane/EtOAc = 40%/60%. The solid product and the remaining product purified by silica gel column chromatography were combined.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.56 (s, 1H), 7.77 (d, *J* = 6.9 Hz, 1H), 7.32 – 7.12 (m, 10H), 7.07 (d, *J* = 6.9 Hz, 1H), 4.85 – 4.74 (m, 1H), 4.41 – 4.31 (m, 1H), 4.10 – 4.01 (m, 1H), 3.03 – 2.88 (m, 2H), 2.82 – 2.69 (m, 1H), 2.82 – 2.69 (m, 2H), 2.64 (s, 3H), 2.46 – 2.40 (m, 1H), 2.16 – 2.06 (m, 1H), 1.33 (s, 9H), 1.31 (s, 9H), 1.23 – 1.17 (m, 1H), 1.01 – 0.92 (m, 1H), 0.91 – 0.81 (m, 1H), 0.38 – 0.25 (m, 1H).

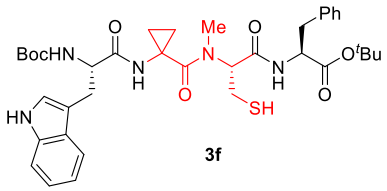
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2, 171.2, 170.7, 169.3, 155.6, 138.2, 137.7, 129.7, 129.6, 128.6, 128.4, 126.9, 126.7, 81.3, 78.5, 60.7, 55.8, 54.4, 37.5, 36.7, 35.3, 31.8, 28.6, 28.0, 22.6, 14.1, 13.6.

HRMS (ESI) m/z: Calcd for $C_{35}H_{48}N_4NaO_7S^+$ $[M+Na]^+$: 691.3136; found: 691.3132.

FT-IR (neat): 3316, 2979, 2544, 1668, 1521, 1455, 1393, 1367, 1253, 1161, 1099, 847, 754, 700 cm^{-1}

$[\alpha]_D^{23}$-118.63 (c 1.1 $CHCl_3$)

***tert*-Butyl *N*-(1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(1H-indol-3-yl)propanamido)cyclopropane-1-carbonyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3f

3f was obtained in 83% yield as a white solid. m.p. 170-172 ºC. Purification: the reaction suspension was centrifuged and washed with MeOH/H_2O = 1/1 mixture for 5 times. The solid was collected and dried under vacuum. The washing solvent was collected and evaporated under reduced pressure to remove MeOH, after which it was extracted with CH_2Cl_2. The organic layer was collected and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography using Hexane/EtOAc = 34%/66%. The solid product and the remaining product purified by silica gel column chromatography were combined.

^{1}H NMR (399 MHz, DMSO-d_6) δ 10.78 (s, 1H), 8.57 (s, 1H), 7.76 (d, *J* = 6.6 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.43 – 6.70 (m, 10H), 4.86 – 4.74 (m, 1H), 4.42 – 4.30 (m, 1H), 4.16 – 4.04 (m, 1H), 3.08 – 2.88 (m, 2H), 3.08 – 2.88 (m, 1H), 2.88 – 2.74 (m, 1H), 2.88 – 2.74 (m, 1H), 2.67 (s, 3H), 2.48 – 2.42 (m, 1H), 2.12 (s, 1H), 1.32 (s, 9H), 1.30 (s, 9H), 1.16 – 1.07 (m, 1H), 1.04 – 0.94 (m, 1H), 0.94 – 0.82 (m, 1H), 0.44 – 0.30 (m, 1H).

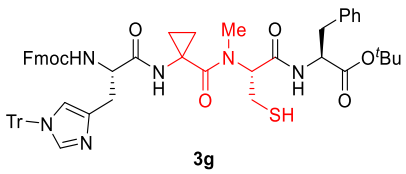
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 171.3, 170.6, 169.3, 155.6, 137.7, 136.4, 129.6, 128.6, 127.6, 126.9, 124.3, 121.3, 118.9, 118.6, 111.7, 110.3, 81.3, 78.5, 60.8, 55.3, 54.4, 36.8, 35.5, 31.8, 28.6, 28.0, 27.7, 22.6, 14.1, 13.6.

HRMS (ESI) m/z: Calcd for $C_{37}H_{49}N_5NaO_7S^+$ [M+Na]+: 730.3245; found: 730.3245.

FT-IR (neat): 3319, 2928, 2546, 1669, 1521, 1457, 1394, 1367, 1251, 1160, 1099, 741, 701 cm^{-1}

$[\alpha]_D^{25}$-94.95 (c 1.06 CH_2Cl_2)

<u>*tert*-Butyl *N*-(1-((*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-trityl-1H-imidazol-4-yl)-propanamido)cyclopropane-1-carbonyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate</u>



3g

3g was obtained in 75% yield as a white solid. m.p. 118-120 ºC. **3g** existed as a mixture of rotamers. Eluent for purification: Hexane/EtOAc = 20%/80%. Wash with 1N HCl aqueous solution if the unreacted *N*-methyl cysteinyl peptide remains after silica gel column chromatography.

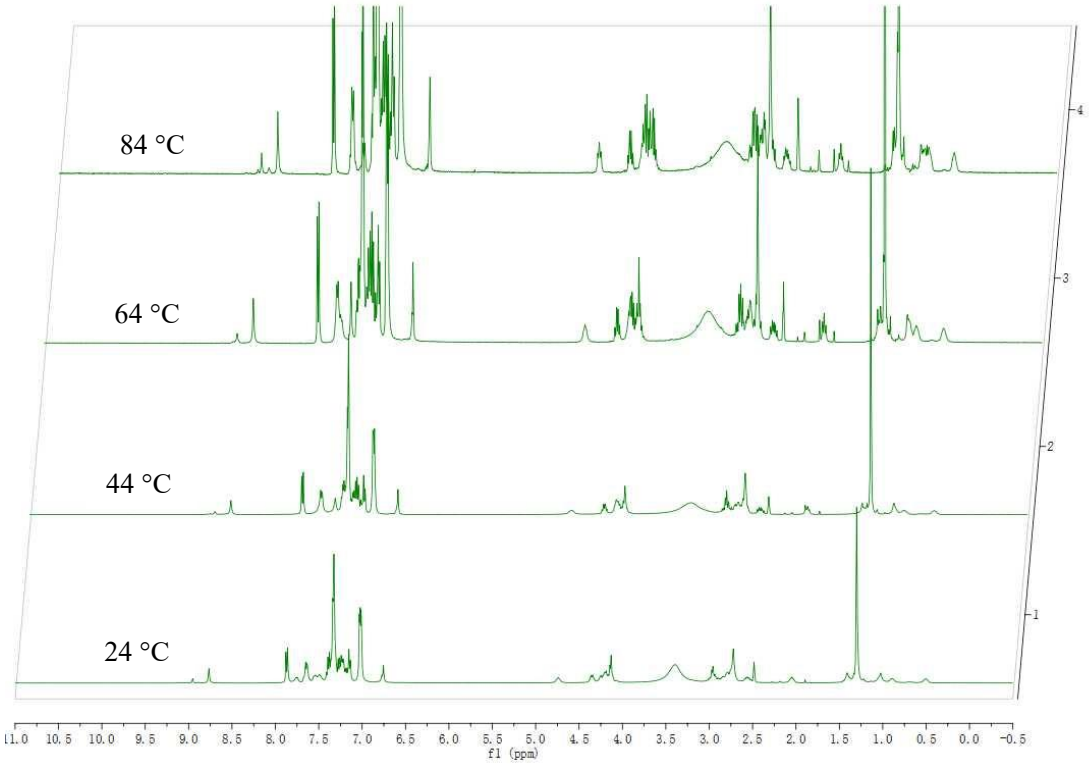
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.95 and 8.77 (s, 1H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.75 (d, *J* = 6.0 Hz, 1H), 7.69 – 7.60 (m, 2H), 7.55 (d, *J* = 6.9 Hz, 1H), 7.49 (s, 1H), 7.45 – 7.09 (m, 4H), 7.45 – 7.09 (m, 3H), 7.45 – 7.09 (m, 6H), 7.45 – 7.09 (m, 5H), 7.08 – 6.93 (m, 6H), 6.77 and 6.76 (s, 1H), 4.79 – 4.69 (m, 1H), 4.39 – 4.31 (m, 1H), 4.27 – 4.06 (m, 2H), 4.27 – 4.06 (m, 1H), 4.27 – 4.06 (m, 1H), 3.05 – 2.64 (m, 2H), 3.05 – 2.64 (m, 2H), 3.05 – 2.64 (m, 1H), 2.72 (s, 3H), 2.60 – 2.51 (m, 1H), 2.09 – 1.99 (m, 1H), 1.50 – 1.40 (m, 1H), 1.30 (s, 9H), 1.07 – 0.99 (m, 1H), 0.96 – 0.84 (m, 1H), 0.56 – 0.44 (m, 1H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 171.4, 170.6, 169.4, 156.3 and 156.2, 144.3, 144.2 and 144.1, 144.1, 142.3, 141.1, 138.0, 137.6, 129.7, 129.6, 128.6, 128.6, 128.5, 128.1, 127.5 and 127.5, 126.9, 125.7, 125.6, 120.6, 119.7, 81.3, 75.4, 66.2, 60.8, 54.7, 54.5, 47.1, 36.8 and 35.5, 32.1, 30.4, 28.0, 22.6, 20.2, 14.3, 13.7.

HRMS (ESI) m/z: Calcd for $C_{61}H_{63}N_6O_7S^+$ $[M+H]^+$:1023.4473; found: 1023.4482.

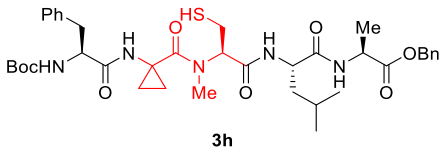
FT-IR (neat): 3296, 3017, 2565, 1673, 1521, 1448, 1396, 1368, 1249, 1155, 1036, 846, 757, 701, 663 cm^{-1}

$[\alpha]_D^{23}$-43.18 (c 0.92 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of 3g

Benzyl *N*-(1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)cyclopropane-1-carbonyl)-*N*-methyl-L-cysteinyl-L-leucyl-L-alaninate



3h

3h was obtained in 91% yield as a white solid. m.p. 170-172 ºC. Purification: the reaction suspension was centrifuged and washed with MeOH/H_2O = 1/1 mixture for 5 times. The solid was collected and dried under vacuum. The washing solvent was collected and evaporated under reduced pressure to remove MeOH, after which it was extracted with EtOAc. The organic layer was collected and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography using Hexane/EtOAc = 25%/75%. The solid product and the remaining product purified by silica gel column chromatography were combined.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.38 (d, *J* = 6.7 Hz, 1H), 7.47 (d, *J* = 7.9 Hz, 1H), 7.41 – 7.27 (m, 5H), 7.27 – 7.10 (m, 5H), 7.05 (d, *J* = 7.4 Hz, 1H), 5.06 (s, 2H), 4.76 – 4.65 (m, 1H), 4.35 – 4.21 (m, 2H), 4.11 – 4.00 (m, 1H), 2.92 (s, 3H), 2.85 – 2.66 (m, 1H), 2.84 – 2.66 (m, 2H), 2.64 – 2.52 (m, 1H), 2.17 – 2.08 (m, 1H), 1.31 (s, 9H), 1.61 – 1.05 (m, 2H), 1.61 – 1.05 (m, 2H), 1.28 (d, *J* = 7.3 Hz, 3H), 1.01 – 0.90 (m, 1H), 0.89 – 0.82 (m, 1H), 0.78 (d, *J* = 6.4 Hz, 3H), 0.75 (d, *J* = 6.4 Hz, 3H), 0.47 – 0.34 (m, 1H).

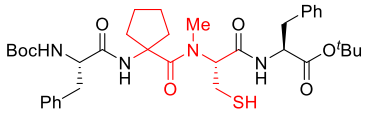
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.1, 172.6, 172.1, 171.6, 169.1, 155.6, 138.2, 136.3, 129.7, 128.8, 128.4, 128.2, 126.7, 78.5, 66.3, 61.0, 55.9, 51.0, 48.1, 41.3, 37.6, 35.3, 32.3, 28.6, 24.5, 23.5, 22.6, 21.8, 17.2, 14.2, 13.8.

HRMS (ESI) m/z: Calcd for $C_{38}H_{53}N_5NaO_8S^+$ $[M+Na]^+$: 762.3507; found: 762.3513.

FT-IR (neat): 3330, 2956, 2536, 1739, 1666, 1523, 1367, 1253, 1166, 1101, 1048, 751, 697, 508 cm^{-1}

$[\alpha]_D^{22}$-75.49 (c 1.32 DMSO)

***tert*-Butyl *N*-(1-((*S*)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)cyclopentane-1-carbonyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3i

3i was obtained in 85% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 50%/50%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.40 (s, 1H), 7.86 (d, *J* = 7.5 Hz, 1H), 7.42 – 7.06 (m, 10H), 6.98 (d, *J* = 8.4 Hz, 1H), 4.96 – 4.85 (m, 1H), 4.40 – 4.32 (m, 1H), 4.19 – 4.11 (m, 1H), 3.05 – 2.93 (m, 2H), 2.83 – 2.73 (m, 2H), 2.73 – 2.66 (m, 1H), 2.56 (s, 3H), 2.42 (dd, *J* = 13.2, 6.9 Hz, 1H), 2.39 – 2.30 (m, 1H), 2.17 (t, *J* = 8.3 Hz, 1H), 1.92 – 1.78 (m, 2H), 1.75 – 1.43 (m, 1H), 1.75 – 1.43 (m, 2H), 1.75 – 1.43 (m, 2H), 1.32 (s, 9H), 1.30 (s, 9H).

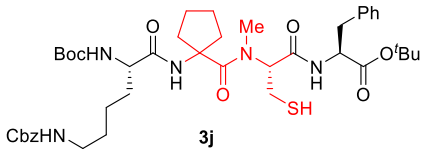
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 171.7, 170.8, 169.4, 155.7, 138.3, 137.9, 129.6, 129.6, 128.5, 128.5, 126.8, 126.7, 81.2, 78.5, 66.1, 61.3, 55.7, 54.6, 37.7, 37.5, 36.7, 35.6, 32.2, 28.6, 28.0, 24.6, 24.2, 22.4.

HRMS (ESI) m/z: Calcd for $C_{37}H_{52}N_4NaO_7S^+$ $[M+Na]^+$: 719.3449; found: 719.3447.

FT-IR (neat): 3306, 2978, 2540, 1664, 1520, 1455, 1392, 1367, 1252, 1162, 1049, 846, 756, 700 cm^{-1}

$[\alpha]_D^{22}$-94.17 (c 1.4 $CHCl_3$)

***tert*-Butyl *N*-(1-((*S*)-6-(((benzyloxy)carbonyl)amino)-2-((*tert*-butoxycarbonyl)amino)hexanamido)-cyclopentane-1-carbonyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3j

3j was obtained in 85% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 34%/66%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.31 (s, 1H), 7.82 (d, *J* = 7.4 Hz, 1H), 7.41 – 7.26 (m, 5H), 7.26 – 7.14 (m, 5H), 6.81 (d, *J* = 7.9 Hz, 1H), 4.98 (s, 2H), 4.95 – 4.85 (m, 1H), 4.38 – 4.29 (m, 1H), 3.86 – 3.78 (m, 1H), 3.04 – 2.96 (m, 2H), 2.95 – 2.87 (m, 2H), 2.85 – 2.75 (m, 1H), 2.57 (s, 3H), 2.45 – 2.37 (m, 1H), 2.16 (t, *J* = 8.3 Hz, 1H), 2.08 – 1.37 (m, 2H), 2.08 – 1.37 (m, 2H), 2.08 – 1.37 (m, 4H), 2.08 – 1.37 (m, 4H), 1.35 (s, 9H), 1.31 (s, 9H), 1.26 – 1.13 (m, 2H).

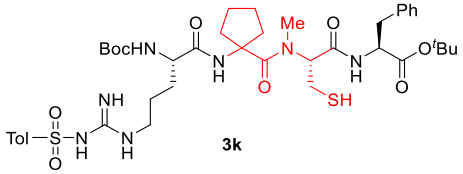
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.3, 170.8, 169.4, 156.5, 155.8, 137.9, 137.7, 129.6, 128.8, 128.5, 128.1, 126.8, 81.1, 78.4, 66.1, 65.5, 61.3, 54.7, 54.4, 40.5, 37.4, 36.8, 35.7, 31.6, 29.5, 28.6, 28.0, 24.6, 24.2, 23.4, 22.4.

HRMS (ESI) m/z: Calcd for $C_{42}H_{61}N_5NaO_9S^+$ $[M+Na]^+$: 834.4082; found: 834.4088.

FT-IR (neat): 3316, 2977, 2547, 1666, 1524, 1456, 1392, 1367, 1251, 1161, 1027, 847, 754, 700 cm^{-1}

$[\alpha]_D^{24}$-80.73(c 1.2 $CHCl_3$)

***tert*-Butyl *N*-(1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-5-(3-tosylguanidino)pentanamido)cyclopentane-1-carbonyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3k

3k was obtained in 81% yield as an amorphous solid. Purification: the crude residue was purified by silica gel column chromatography using Hexane/EtOAc = 10%/90% and the fractions that contained the desired product and NMe-Cys-Phe-OtBu reactant were collected and evaporated under reduced pressure to remove the solvent. Then the residue was dissolved again in EtOAc, washed with 1N HCl aqueous solution for 4 times, then washed with water for 3 times. The EtOAc phase was then collected, dried over Na_2SO_4, concentrated to get pure **3k**.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.83 (d, *J* = 7.4 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.35 – 7.10 (m, 7H), 7.01 (s, 1H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.75 (s, 1H), 6.54 (s, 1H), 4.95 – 4.86 (m, 1H), 4.39 – 4.31 (m, 1H), 3.90 – 3.81 (m, 1H), 3.08 – 2.91 (m, 2H), 3.07 – 2.91 (m, 2H), 2.85 – 2.76 (m, 1H), 2.57 (s, 3H), 2.44 – 2.38 (m, 1H), 2.32 (s, 3H), 2.15 (t, *J* = 8.3 Hz, 1H), 1.94 – 1.80 (m, 2H), 1.74 – 1.63 (m, 2H), 1.63 – 1.37 (m, 8H), 1.35 (s, 9H), 1.31 (s, 9H).

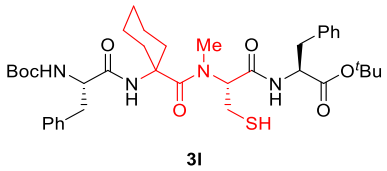
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.1, 170.8, 169.4, 157.0, 155.8, 141.5, 137.9, 129.6, 129.5, 128.5, 126.8, 126.0, 81.1, 78.5, 66.1, 61.2, 54.7, 54.0, 37.6, 36.7, 35.7, 32.2, 29.3, 28.6, 28.0, 24.6, 24.3, 22.4, 21.3.

HRMS (ESI) m/z: Calcd for $C_{41}H_{61}N_7NaO_9S_2^+$ $[M+Na]^+$: 882.3864; found: 882.3873.

FT-IR (neat): 3330, 2977, 2554, 1663, 1547, 1455, 1367, 1254, 1163, 1083, 815, 755, 680, 622, 562 cm^{-1}

$[\alpha]_D^{25}$-59.26 (c 1.08 $CHCl_3$)

***tert*-Butyl *N*-(1-((*S*)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)cyclohexane-1-carbonyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3l

3l was obtained in 79% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 67%/33%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.80 (d, *J* = 7.3 Hz, 1H), 7.39 – 7.23 (m, 5H), 7.23 – 7.08 (m, 5H), 7.00 (d, *J* = 8.6 Hz, 1H), 4.96 – 4.82 (m, 1H), 4.40 – 4.27 (m, 1H), 4.40 – 4.27 (m, 1H), 3.04 – 2.95 (m, 2H), 2.91 – 2.80 (m, 2H), 2.77 – 2.71 (m, 1H), 2.67 (s, 3H), 2.52 – 2.45 (m, 1H), 2.15 (t, *J* = 6.7 Hz, 1H), 1.96 – 1.87 (m, 1H), 1.85 – 1.71 (m, 2H), 1.69 – 1.41 (m, 6H), 1.31 (s, 9H), 1.30 (s, 9H), 1.25 – 1.06 (m, 1H).

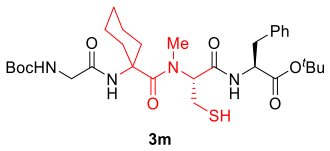
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 171.8, 170.7, 169.3, 155.8, 138.4, 137.9, 129.6, 128.5, 126.8, 126.7, 81.1, 78.6, 61.8, 58.8, 55.8, 54.7, 37.8, 36.8, 32.3 (2C), 32.0, 28.5, 28.0, 25.3, 22.2, 21.6, 21.3.

HRMS (ESI) m/z: Calcd for $C_{38}H_{54}N_4NaO_7S^+$ $[M+Na]^+$: 733.3605; found: 733.3601.

FT-IR (neat): 3307, 2933, 2540, 1665, 1498, 1455, 1367, 1252, 1162, 1046, 848, 757, 700 cm^{-1}

$[\alpha]_D^{25}$-76.09 (c 1.0 $CHCl_3$)

***tert*-Butyl *N*-(1-(2-((*tert*-butoxycarbonyl)amino)acetamido)cyclohexane-1-carbonyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3m

3m was obtained in 82% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 43%/57%. **3m** existed as a mixture of rotamers.

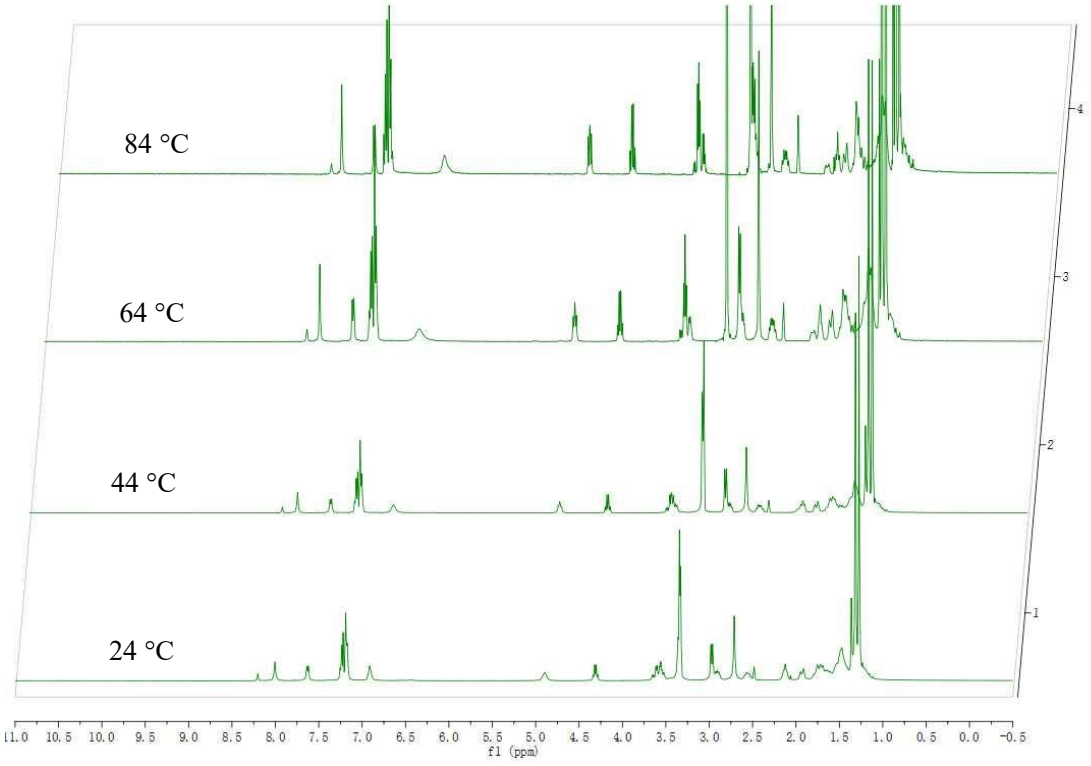
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.00 (s, 1H), 7.63 (d, *J* = 7.2 Hz, 1H), 7.37 – 7.05 (m, 5H), 6.92 (s, 1H), 4.96 – 4.84 (m, 1H), 4.36 – 4.25 (m, 1H), 3.66 – 3.50 (m, 2H), 2.97 (d, *J* = 7.7 Hz, 2H), 2.93 – 2.85 (m, 1H), 2.71 (s, 3H), 2.60 – 2.51 (m, 1H), 2.12 (t, *J* = 7.2 Hz, 1H), 1.97 – 1.88 (m, 1H), 1.82 – 1.38 (m, 9H), 1.36 and 1.31 (s, 9H), 1.27 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 173.1, 170.7, 169.4, 169.3, 156.2, 137.8, 129.6, 128.5, 126.8, 81.0, 78.5, 61.5, 58.8, 54.7, 43.4, 36.9 and 34.7 (1C), 32.7 (2C), 32.1, 28.6 and 28.5 (3C), 27.9, 25.2 and 24.6 (1C), 22.3 and 22.0 (1C), 21.4 (2C).

HRMS (ESI) m/z: Calcd for $C_{31}H_{48}N_4NaO_7S^+$ $[M+Na]^+$: 643.3136; found: 643.3134.

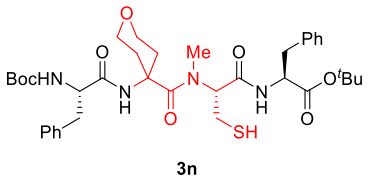
FT-IR (neat): 3317, 2933, 2546, 1668, 1523, 1455, 1367, 1250, 1160, 1052, 853, 758, 701 cm^{-1}

$[\alpha]_D^{25}$-45.26 (c 1.18 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of 3m

***tert*-Butyl *N*-(4-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)tetrahydro-2H-pyran-4-carbonyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3n

3n was obtained in 88% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 35%/65%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.47 (s, 1H), 7.91 (d, *J* = 7.3 Hz, 1H), 7.47 – 7.08 (m, 10H), 7.04 (d, *J* = 8.4 Hz, 1H), 4.98 – 4.85 (m, 1H), 4.41 – 4.33 (m, 1H), 4.33 – 4.23 (m, 1H), 3.72 – 3.60 (m, 2H), 3.61 – 3.49 (m, 1H), 3.24 – 3.13 (m, 1H), 3.07 – 2.91 (m, 2H), 2.89 – 2.66 (m, 2H), 2.89 – 2.67 (m, 1H), 2.59 (s, 3H), 2.45 – 2.36 (m, 1H), 2.21 – 2.03 (m, 2H), 1.86 – 1.75 (m, 1H), 1.75 – 1.61 (m, 2H), 1.33 (s, 9H), 1.31 (s, 9H).

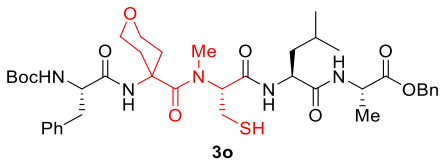
^{13}C NMR (100 MHz, DMSO-d_6) δ 171.9, 171.8, 170.8, 169.2, 155.8, 138.3, 138.0, 129.6, 129.5, 128.5, 126.8, 81.2, 78.6, 63.5, 62.6, 61.7, 56.5, 55.8, 54.6, 37.8, 36.7, 32.5 (2C), 32.1, 28.5, 28.0, 22.2.

HRMS (ESI) m/z: Calcd for $C_{37}H_{52}N_4NaO_8S^+$ $[M+Na]^+$: 735.3398; found: 735.3403.

FT-IR (neat): 3306, 2977, 2547, 1670, 1522, 1456, 1367, 1250, 1161, 1107, 1031, 845, 755, 701 cm^{-1}

$[\alpha]_D^{25}$-80.81 (c 1.03 $CHCl_3$)

Benzyl *N*-(4-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)tetrahydro-2H-pyran-4-carbonyl)-*N*-methyl-L-cysteinyl-L-leucyl-L-alaninate



3o

3o was obtained in 78% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 20%/80%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.50 (s, 1H), 8.32 (d, *J* = 6.8 Hz, 1H), 7.51 (d, *J* = 8.1 Hz, 1H), 7.42 – 7.13 (m, 10H),

7.06 (d, *J* = 8.4 Hz, 1H), 5.07 (d, *J* = 1.3 Hz, 2H), 4.83 – 4.73 (m, 1H), 4.36 – 4.25 (m, 1H), 4.36 – 4.25 (m, 1H), 4.37 – 4.24 (m, 1H), 3.67 – 3.59 (m, 2H), 3.59 – 3.53 (m, 1H), 3.24 – 3.14 (m, 1H), 2.90 (s, 3H), 3.00 – 2.83 (m, 2H), 2.82 – 2.74 (m, 1H), 2.67 – 2.57 (m, 1H), 2.24 – 2.18 (m, 1H), 2.14 – 2.05 (m, 1H), 1.89 – 1.82 (m, 1H), 1.81 – 1.71 (m, 2H), 1.59 – 1.41 (m, 2H), 1.59 – 1.41 (m, 1H), 1.31 (s, 9H), 1.29 (d, *J* = 7.3 Hz, 3H), 0.80 (d, *J* = 7.1 Hz, 3H), 0.78 (d, *J* = 6.8 Hz, 3H).

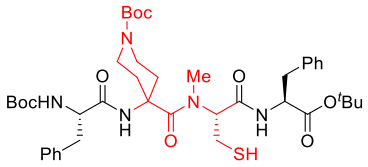
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.2 (2C), 171.9, 168.9, 155.8, 138.2, 136.3, 129.6, 128.8, 128.6, 128.4, 128.2, 126.8, 78.6, 66.3, 63.3, 62.7, 62.2, 56.8, 55.8, 51.2, 48.1, 41.1, 37.9, 33.1, 32.6, 32.3, 28.5, 24.4, 23.5, 22.5, 21.9, 17.2.

HRMS (ESI) m/z: Calcd for $C_{40}H_{57}N_5NaO_9S^+$ $[M+Na]^+$: 806.3769, found: 806.3774.

FT-IR (neat): 3302, 2959, 2554, 1653, 1525, 1455, 1389, 1367, 1251, 1165, 1107, 1052, 755, 699, 580 cm^{-1}

$[\alpha]_D^{25}$-88.84 (c 1.36 $CHCl_3$)

<u>*tert*-Butyl 4-(((*R*)-1-(((*S*)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-3-mercapto-1-oxopropan-2-yl)(methyl)carbamoyl)-4-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)piperidine-1-carboxylate</u>



3p

3p was obtained in 84% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 50%/50%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.40 (s, 1H), 7.88 (s, 1H), 7.44 – 7.12 (m, 5H), 7.44 – 7.12 (m, 5H), 7.06 (d, *J* = 8.2 Hz, 1H), 4.95 – 4.85 (m, 1H), 4.41 – 4.33 (m, 1H), 4.31 – 4.23 (m, 1H), 3.60 – 3.47 (m, 2H), 3.27 – 3.17 (m, 1H), 3.06 – 2.93 (m, 1H), 3.06 – 2.93 (m, 1H), 2.88 – 2.68 (m, 2H), 2.88 – 2.68 (m, 2H), 2.60 (s, 3H), 2.46 – 2.37 (m, 1H), 2.16 (t, *J* = 8.4 Hz, 1H), 2.00 – 1.90 (m, 1H), 1.79 – 1.59 (m, 1H), 1.79 – 1.60 (m, 2H), 1.39 (s, 9H), 1.33 (s, 9H), 1.30 (s, 9H).

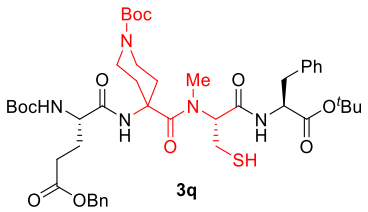
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 171.7, 170.8, 169.1, 155.8, 154.2, 138.2, 138.0, 129.6, 129.5, 128.48, 126.8, 81.2, 79.1, 78.6, 61.6, 57.3, 55.7, 54.6, 37.7, 36.6, 32.1, 31.8 (2C), 28.5, 28.5, 28.0, 22.1.

HRMS (ESI) m/z: Calcd for $C_{42}H_{61}N_5NaO_9S^+$ $[M+Na]^+$: 834.4082; found: 834.4086.

FT-IR (neat): 3308, 2978, 2544, 1671, 1522, 1427, 1393, 1367, 1250, 1158, 1048, 846, 756, 700 cm^{-1}

$[\alpha]_D^{25}$-67.45 (c 1.02 $CHCl_3$)

<u>*tert*-Butyl 4-((*S*)-5-(benzyloxy)-2-((*tert*-butoxycarbonyl)amino)-5-oxopentanamido)-4-(((*R*)-1-(((*S*)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-3-mercapto-1-oxopropan-2-yl)(methyl)carbamoyl)piperidine-1-carboxylate</u>



3q

3q was obtained in 75% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 34%/66%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.83 (s, 1H), 7.51 – 7.26 (m, 5H), 7.26 – 7.07 (m, 5H), 6.99 (d, *J* = 7.4 Hz, 1H), 5.06 (s, 2H), 4.95 – 4.85 (m, 1H), 4.40 – 4.31 (m, 1H), 4.03 – 3.94 (m, 1H), 3.66 – 3.53 (m, 2H), 3.27 – 3.18 (m, 1H), 3.07 – 2.89 (m, 1H), 3.07 – 2.89 (m, 2H), 2.85 – 2.76 (m, 1H), 2.61 (s, 3H), 2.46 – 2.30 (m, 1H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.17 – 2.10 (m, 1H), 2.03 – 1.94 (m, 1H), 1.92 – 1.65 (m, 1H), 1.92 – 1.65 (m, 4H), 1.39 (s, 9H), 1.35 (s, 9H), 1.32 (s, 9H).

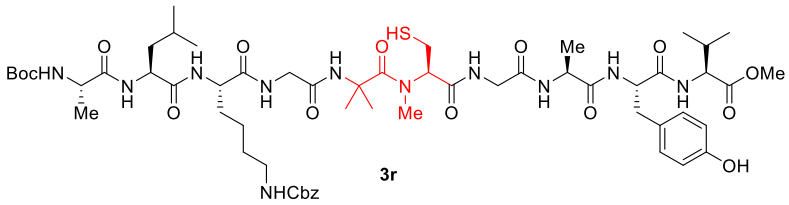
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5, 172.0, 171.7, 170.7, 169.1, 155.9, 154.2, 137.9, 136.6, 129.5, 128.8, 128.5, 128.4, 128.2, 126.8, 81.2, 79.1, 78.7, 65.9, 61.6, 57.3, 54.6, 53.8, 36.7, 31.8 (3C), 30.8, 28.53, 28.50, 28.0, 27.2, 22.1.

HRMS (ESI) m/z: Calcd for $C_{45}H_{65}N_5NaO_{11}S^+$ $[M+Na]^+$: 906.4293; found: 906.4299.

FT-IR (neat): 3311, 2978, 2547,1685, 1522, 1455, 1426, 1392, 1367, 1250, 1160, 1048, 752, 700 cm^{-1}

$[\alpha]_D^{25}$-72.51 (c 1.14 $CHCl_3$)

Methyl *N*-(2-(2-((*S*)-6-(((benzyloxy)carbonyl)amino)-2-((*S*)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-propanamido)-4-methylpentanamido)hexanamido)acetamido)-2-methylpropanoyl)-*N*-methyl-L-cysteinylglycyl-L-alanyl-L-tyrosyl-L-valinate



3r

HCl•*N*-Methyl cysteinyl peptide **2c** (86.4 mg, 0.15 mmol), the corresponding α,α-disubstituted α-amido nitrile **1p** (68.8 mg, 0.10 mmol), L-ascorbic acid (23.5 mg, 0.13 mmol) and $NaHCO_3$ (12.6 mg, 0.15 mmol) were dissolved in MeOH (670 μL) and pH 7.4, 0.2 M phosphate buffer (330 μL), followed by the addition of ^{n}Bu_3P (12 μL, 0.05 mmol). The reaction mixture was immediately degassed under liquid N_2 bath and filled with argon for 3 times. After stirring vigorously for 48 h at 30 ºC, the reaction mixture was diluted with EtOAc, filtered through Na_2SO_4 using filtration paper (Na_2SO_4 was loaded on the filtration paper). The filtrate was evaporated under reduced pressure and concentrated under vacuo. The crude product was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 95%/5%) and gave the desired product **3r** in 77% yield as a white solid. m.p. 129-131 ºC.

^{1}H NMR (399 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.32 (s, 1H), 8.25 (s, 1H), 8.11 (d, *J* = 5.5 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.87 – 7.78 (m, 1H), 7.87 – 7.77 (m, 1H), 7.72 (d, *J* = 6.9 Hz, 1H), 7.72 (d, *J* = 6.9 Hz, 1H), 7.40 – 7.24 (m, 5H), 7.20 (t, *J* = 5.5 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 7.7 Hz, 1H), 6.61 (d, *J* = 8.4 Hz, 2H), 4.98 (s, 2H), 4.66 – 4.57 (m, 1H), 4.50 – 4.42 (m, 1H), 4.34 – 4.27 (m, 1H), 4.24 – 4.18 (m, 1H), 4.15 – 4.09 (m, 1H), 4.08 – 4.01 (m, 1H), 3.99 – 3.91 (m, 1H), 3.79 (dd, *J* = 16.5, 6.7 Hz, 1H), 3.71 (d, *J* = 5.2 Hz, 2H), 3.59 (s, 3H), 3.51 (dd, *J* = 16.2, 4.4 Hz, 1H), 2.99 (s, 3H), 2.86 (dd, *J* = 13.9, 4.9 Hz, 1H), 3.06 – 2.74 (m, 2H), 3.06 – 2.74 (m, 1H), 3.06 – 2.74 (m, 1H), 2.66 (dd, *J* = 13.9, 8.8 Hz, 1H), 2.22 (t, *J* = 7.7 Hz, 1H), 2.03 – 1.94 (m, 1H), 1.77 – 1.15 (m, 1H), 1.77 – 1.15 (m, 2H), 1.77 – 1.15 (m, 2H), 1.77 – 1.15 (m, 2H), 1.77 – 1.15 (m, 2H), 1.41 (s, 3H), 1.38 (s, 3H), 1.35 (s, 9H), 1.13 (d, *J* = 7.0 Hz, 3H), 1.13 (d, *J* = 7.0 Hz, 3H), 1.00 – 0.66 (m, 6H), 1.00 – 0.66 (m, 6H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 173.0, 173.0, 172.5, 172.2, 172.1, 171.7, 169.8, 169.6, 168.9, 156.5, 156.2 (*ipso*-C of phenol), 155.6, 137.7, 130.5, 128.8, 128.2, 128.0, 115.2, 78.5, 65.6, 63.2, 57.9, 56.5, 54.3, 53.8, 52.1, 51.1, 50.0, 48.6, 42.7, 42.2, 41.2, 40.5 (overlapping with DMSO-d_6 deduced by HMQC), 36.9, 34.9, 31.5, 30.4, 29.6, 28.6, 26.1, 26.0, 24.4, 23.5, 23.1, 22.6, 22.0, 19.3, 18.7, 18.4, 18.3.

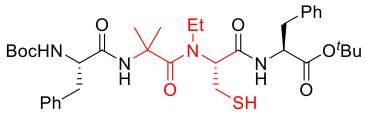
HRMS (ESI) m/z: Calcd for $C_{58}H_{89}N_{11}NaO_{16}S^+$ $[M+Na]^+$: 1250.6102; found:1250.6123.

FT-IR (neat): 3283, 3072, 2962, 2568, 1633, 1533, 1453, 1392, 1367, 1248, 1169, 1091, 1024, 756, 698 cm^{-1}

$[\alpha]_D^{25}$+7.94 (c 0.8 $CHCl_3$)

***tert*-Butyl *N*-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-2-methylpropa-noyl)-**

***N*-ethyl-L-cysteinyl-L-phenylalaninate**



3s

3s was obtained in 64% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 50%/50%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.68 (s, 1H), 7.71 (d, *J* = 6.4 Hz, 1H), 7.33 – 7.07 (m, 5H), 7.33 – 7.07 (m, 5H), 6.76 (d, *J* = 8.7 Hz, 1H), 4.39 – 4.31 (m, 1H), 4.28 – 4.21 (m, 1H), 3.90 – 3.74 (m, 1H), 3.90 – 3.74 (m, 1H), 3.32 – 3.25 (m, 1H), 3.17 – 3.08 (m, 2H), 3.07 – 3.00 (m, 1H), 2.93 (dd, *J* = 14.1, 7.4 Hz, 1H), 2.87 (dd, *J* = 13.8, 5.6 Hz, 1H), 2.75 (dd, *J* = 13.5, 9.2 Hz, 1H), 2.20 (t, *J* = 8.6 Hz, 1H), 1.37 (s, 3H), 1.31 (s, 9H), 1.25 (s, 9H), 1.17 (s, 3H), 0.97 (t, *J* = 6.7 Hz, 3H).

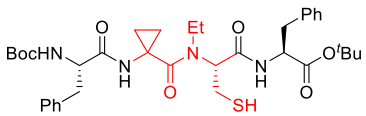
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3 (2C), 170.8, 169.7, 155.4, 138.1, 137.9, 129.8, 129.8, 128.4, 128.4, 126.7, 126.7, 80.7, 78.5, 64.1, 56.6, 55.5, 55.3, 44.7, 38.4, 37.2, 28.5, 27.9, 26.8, 25.4, 23.5, 13.4.

HRMS (ESI) m/z: Calcd for $C_{36}H_{52}N_4NaO_7S^+$ $[M+Na]^+$: 707.3449; found: 707.3452.

FT-IR (neat): 3284, 2979, 2561, 1645, 1499, 1391, 1367, 1252, 1166, 1114, 1052, 847, 755, 700 cm^{-1}

$[\alpha]_D^{26}$-53.23 (c 0.4 $CHCl_3$)

***tert*-Butyl *N*-(1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)cyclopropane-1-carbonyl)-*N*-ethyl-L-cysteinyl-L-phenylalaninate**



3t

EtOAc was added to dissolve the generated white precipitate. **3t** was obtained in 91% yield as a white solid. m.p. 78-80 ºC. Eluent for purification: Hexane/EtOAc = 40%/60%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.71 (s, 1H), 7.72 (s, 1H), 7.40 – 7.02 (m, 10H), 6.88 (d, *J* = 8.5 Hz, 1H), 4.37 – 4.27 (m, 1H), 4.26 – 4.04 (m, 1H), 4.26 – 4.04 (m, 1H), 3.54 – 3.41 (m, 1H), 3.31 – 3.20 (m, 1H), 3.03 – 2.65 (m, 2H), 3.03 – 2.65 (m, 2H), 3.03 – 2.65 (m, 2H), 2.19 – 2.11 (m, 1H), 1.29 (s, 9H), 1.28 (s, 9H), 1.06 – 0.65 (m, 3H), 1.06 – 0.65 (m, 2H), 1.06 – 0.65 (m, 1H), 0.56 – 0.40 (m, 1H).

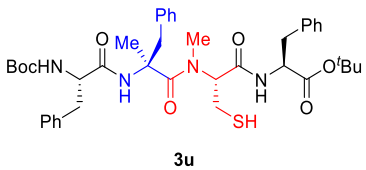
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.7, 170.8, 170.6, 169.4, 155.5, 138.1, 137.7, 129.7, 128.5, 128.4, 126.9, 126.7, 81.1, 78.5, 62.9, 55.7, 54.9, 42.5, 38.0, 37.1, 36.0, 28.5, 28.2, 27.9, 23.5, 14.3, 14.1.

HRMS (ESI) m/z: Calcd for $C_{36}H_{50}N_4NaO_7S^+$ $[M+Na]^+$: 705.3292; found: 705.3297.

FT-IR (neat): 3300, 2979, 2932, 2554, 1669, 1522, 1455, 1367, 1252, 1164, 1031, 846, 755, 700 cm^{-1}

$[\alpha]_D^{24}$-61.19 (c 1.05 $CHCl_3$)

***tert*-Butyl *N*-((*S*)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-2-methyl-3-phenylpropanoyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3u

3u was obtained in 64% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 67%/33%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.09 (s, 1H), 8.00 (d, *J* = 7.4 Hz, 1H), 7.37 – 6.89 (m, 15H), 7.37 – 6.89 (m, 1H), 5.01 – 4.88 (m, 1H), 4.43 – 4.35 (m, 1H), 4.22 – 4.13 (m, 1H), 3.43 – 3.30 (m, 1H), 3.07 – 2.96 (m, 2H), 3.07 – 2.96 (m, 1H), 2.89 – 2.77 (m, 1H), 2.89 – 2.77 (m, 1H), 2.77 – 2.71 (m, 1H), 2.65 (s, 3H), 2.49 – 2.43 (m, 1H), 2.25 (t, *J* = 8.3 Hz, 1H), 1.34 (s, 9H), 1.27 (s, 9H), 1.25 (s, 3H).

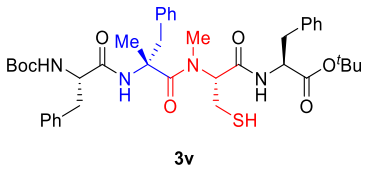
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 171.7, 170.8, 169.3, 155.8, 138.8, 138.0, 137.5, 131.7, 129.6, 129.5, 128.6, 128.4, 128.2, 126.8, 126.8, 126.6, 81.2, 78.4, 62.1, 58.8, 55.7, 54.7, 41.6, 37.6, 36.7, 32.6, 28.6, 28.0, 22.5, 21.9.

HRMS (ESI) m/z: Calcd for $C_{41}H_{54}N_4NaO_7S^+$ $[M+Na]^+$: 769.3605; found: 769.3607.

FT-IR (neat): 3305, 2979, 2932, 2547, 1664, 1497, 1455, 1367, 1250, 1161, 1081, 847, 756, 701 cm^{-1}

$[\alpha]_D^{25}$-134.18 (c 0.9 $CHCl_3$)

***tert*-Butyl *N*-((*R*)-2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-2-methyl-3-phenylpropanoyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



3v

3v was obtained in 75% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 60%/40%

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.43 (s, 1H), 7.82 (d, *J* = 6.6 Hz, 1H), 7.45 – 7.06 (m, 15H), 7.02 (d, *J* = 9.0 Hz, 1H), 5.03 – 4.90 (m, 1H), 4.43 – 4.35 (m, 1H), 4.32 – 4.24 (m, 1H), 3.21 – 2.86 (m, 2H), 3.21 – 2.86 (m, 2H), 3.21 – 2.86 (m, 2H), 2.92 (s, 3H), 2.76 – 2.61 (m, 2H), 2.24 (t, *J* = 7.9 Hz, 1H), 1.30 (s, 9H), 1.27 (s, 9H), 1.18 (s, 3H).

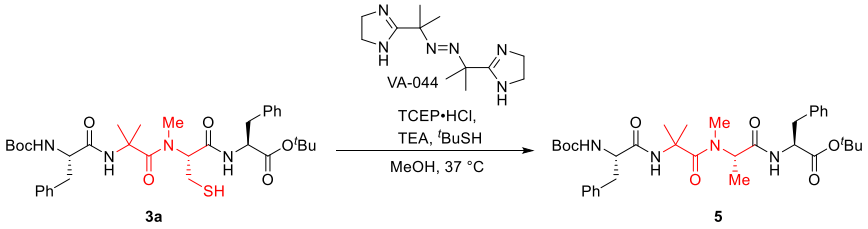
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 172.2, 170.7, 169.3, 155.8, 138.4, 138.0, 136.8, 131.7, 129.6, 128.6, 128.4, 128.3, 126.8, 126.7, 81.0, 78.5, 61.3, 59.2, 56.0, 54.9, 42.0, 37.8, 36.8, 32.7, 28.5, 27.9, 22.4, 22.1.

HRMS (ESI) m/z: Calcd for $C_{41}H_{54}N_4NaO_7S^+$ $[M+Na]^+$: 769.3605; found: 769.3604.

FT-IR (neat): 3303, 2979, 2932, 2550, 1665, 1497, 1455, 1368, 1251, 1160, 1073, 847, 755, 701 cm^{-1}

$[\alpha]_D^{25}$-6.40 (c 0.8 $CHCl_3$)

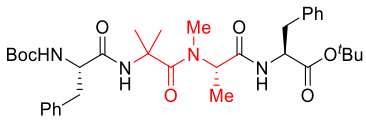
2.9 Desulfurization towards α,α-disubstituted *N*-methyl alanyl peptide 5 and physical information



3a **5**

The desulfurization was conducted with **3a** (30 mg, 0.045 mmol) following the known protocol[10,11] with using 0.2 equivalent of VA-044. And **5** was obtained in 77% yield as an amorphous solid after 42 h at 37 ºC.

***tert*-Butyl *N*-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-2-methylpropanoyl)-*N*-methyl-L-alanyl-L-phenylalaninate**



5

5 was obtained in 77% yield as an amorphous solid. **^{1}H NMR** (399 MHz, DMSO-d_6) δ 8.33 (s, 1H), 7.65 (d, *J* = 7.3 Hz, 1H), 7.36 – 7.07 (m, 10H), 6.94 (d, *J* = 8.6 Hz, 1H), 4.94 – 4.87 (m, 1H), 4.38 – 4.29 (m, 1H), 4.18 – 4.10 (m, 1H), 2.99 (d, *J* = 7.9 Hz, 2H), 2.83 (dd, *J* = 13.4, 4.1 Hz, 1H), 2.71 (dd, *J* = 13.6, 10.2 Hz, 1H), 2.62 (s, 3H), 1.30 (s, 3H), 1.30 (s, 9H), 1.28 (s, 9H), 1.25 (s, 3H), 1.04 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 171.6, 171.3, 170.9, 155.8, 138.4, 138.0, 129.7, 129.6, 128.5, 128.4, 126.8, 126.7, 81.0, 78.4, 56.1, 56.0, 54.6, 53.8, 37.6, 36.9, 31.6, 28.5, 28.0, 26.5, 25.4, 13.6.

HRMS (ESI) m/z: Calcd for $C_{35}H_{50}N_4NaO_7^+$ $[M+Na]^+$: 661.3572; found: 661.3574.

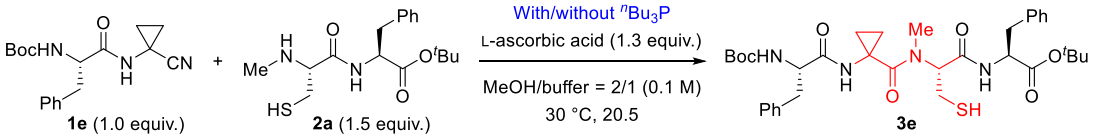
FT-IR (neat): 3305, 2980, 2933, 1660, 1522, 1393, 1367, 1249, 1162, 1084, 846, 754, 700 cm^{-1}

$[\alpha]_D^{25}$-82.66 (c 1.02 $CHCl_3$)

2.10 Control experiments to study the role of $^{n}Bu_3P$ (Table s1)

Control experiments with and without $^{n}Bu_3P$ were conducted with using Boc-L-Phe-Ac$_3$c-CN **1e** and NMe-L-Cys-L-Phe-O^{t}Bu **2a** (**Table s1**). The corresponding product **3e** was obtained in 85% isolated yield with 6% of the starting material **1e** recovered under the standard optimized condition (**Table s1**, entry 1). When $^{n}Bu_3P$ was not present in the reaction, **3e** was obtained in 83% isolated yield with 7.6% of **1e** recovered under a vigorously degassed system (Schlenk technique was used) (**Table s1**, entry 2). Therefore, we concluded that $^{n}Bu_3P$ does not act as an activating reagent in the reaction.

Table s1: control experiments of **1e** with and without $^{n}Bu_3P$.[a]

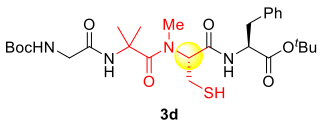


Entry	With/without $^{n}Bu_3P$	Yield/%	Rsm **1e**/%
1	$^{n}Bu_3P$ (0.5 equiv.) degassed system	85	6
2	No $^{n}Bu_3P$ Vigorously degassed	83	7.6

[a]Unless otherwise shown, the reaction was conducted using **1e** (0.2 mmol) following the same procedure of generality study in table 2.

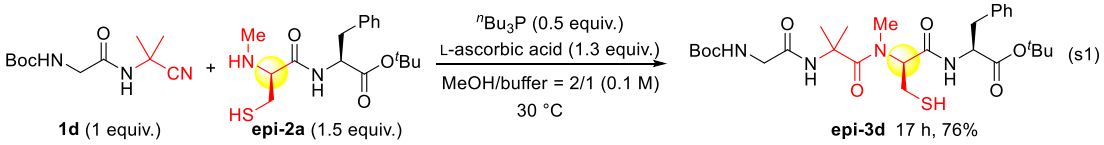
2.11 HPLC analysis of 3d, control experiment in deuterated solvent, synthesis of epi-2a and physical information of epi-2a and epi-3d

HPLC analysis and control experiment in deuterated solvent system using Boc-Gly-Aib-CN **1d** were conducted and the corresponding product **3d** was shown to be a single diastereomer. The data is summarized below.



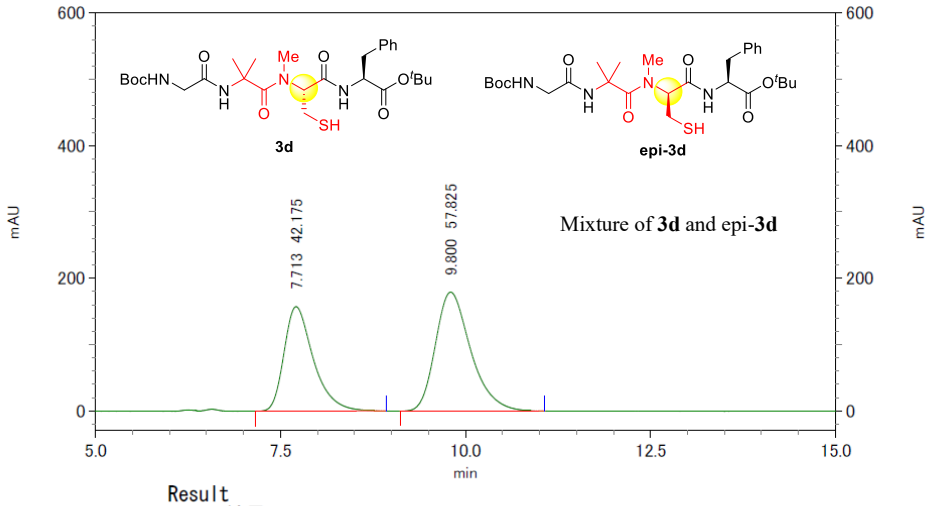
2.11.1 HPLC analysis

First of all, epi-**3d** was synthesized from **1d** and **epi-2a** in 76% yield after 17 h under the standard reaction condition (**Equation s1**).



The obtained epi-**3d** was mixed with **3d** and applied to a HPLC analysis on a chiral phase to determine the separation condition (IG column, ^{i}PrOH/Hexane = 1/4, applied as ^{i}PrOH solution, flow rate: 1 ml/min, $t_{\mathbf{3d}}$ = 7.71 min, $t_{\text{epi-}\mathbf{3d}}$ = 9.80 min). NMe-L-Cys-L-Phe-O^{t}Bu **2a** appeared in HPLC at 6.43 min

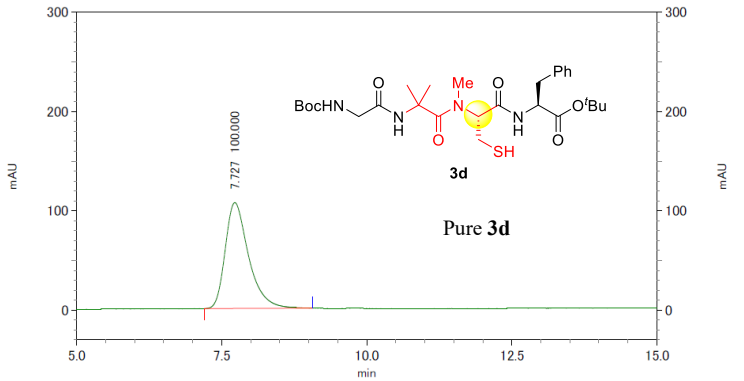
(IG column, ^{i}PrOH/Hexane = 1/4, applied as ^{i}PrOH solution, flow rate: 1 ml/min, t_{2a} = 6.43 min). The crude product of **3d** obtained based on the standard condition shown in **table 2** was then applied to HPLC analysis (IG column, ^{i}PrOH/Hexane = 1/4, applied as ^{i}PrOH solution, flow rate: 1 ml/min, t_{3d} = 7.68 min). **epi-3d** was not observed in HPLC analysis of the crude product of **3d**.



Result

1: 210.0 nm, 4.0 nm 結果

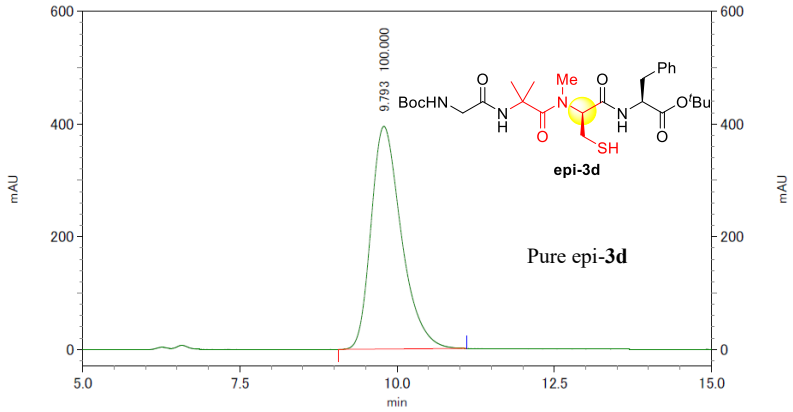
Pk #	Retention time / min	Integration/ %
1	7.713	42.175
2	9.800	57.825
合計 Total		100.000



Result

1: 210.0 nm, 4.0 nm 結果

Pk #	Retention time / min	Integration/ %
1	7.727	100.000
合計 Total		100.000

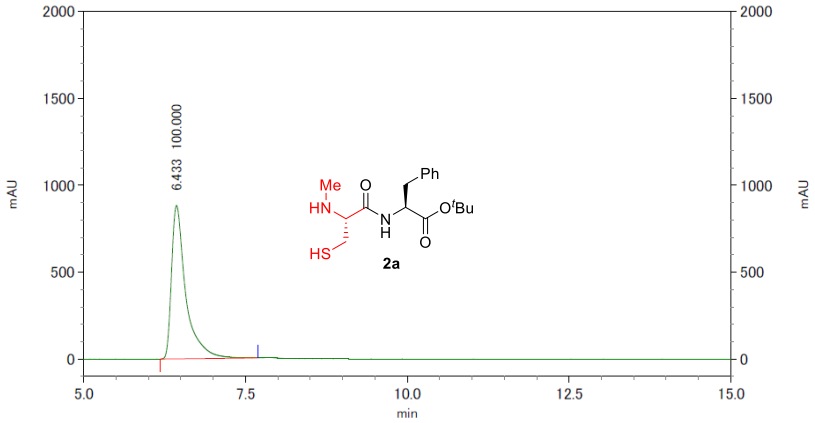


Result

1: 210.0 nm, 4.0 nm 結果

Pk #	Retention time / min	Integration/ %
1	9.793	100.000

合計	
Total	100.000

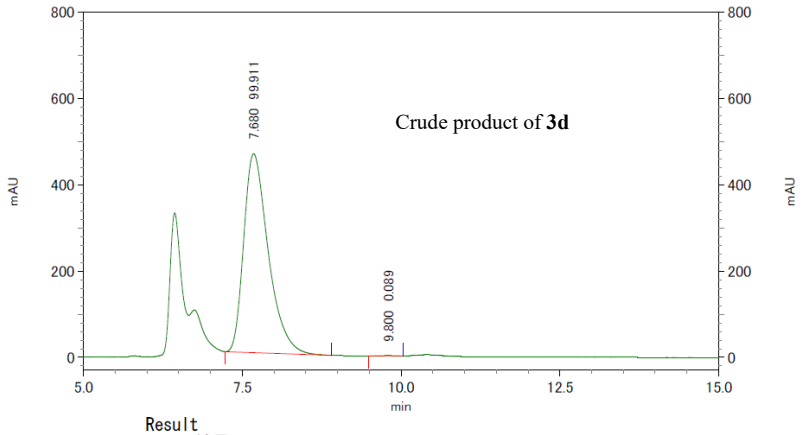


Result

1: 210.0 nm, 4.0 nm 結果

Pk #	Retention time / min	Integration/ %
1	6.433	100.000

合計	
Total	100.000



Result

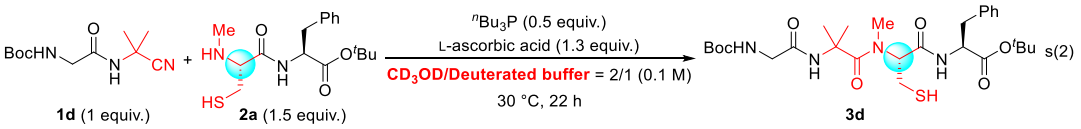
1: 210.0 nm, 4.0 nm 結果

Pk #	Retention time / min	Integration/ %
1	7.680	99.911
2	9.800	0.089
合計		100.000

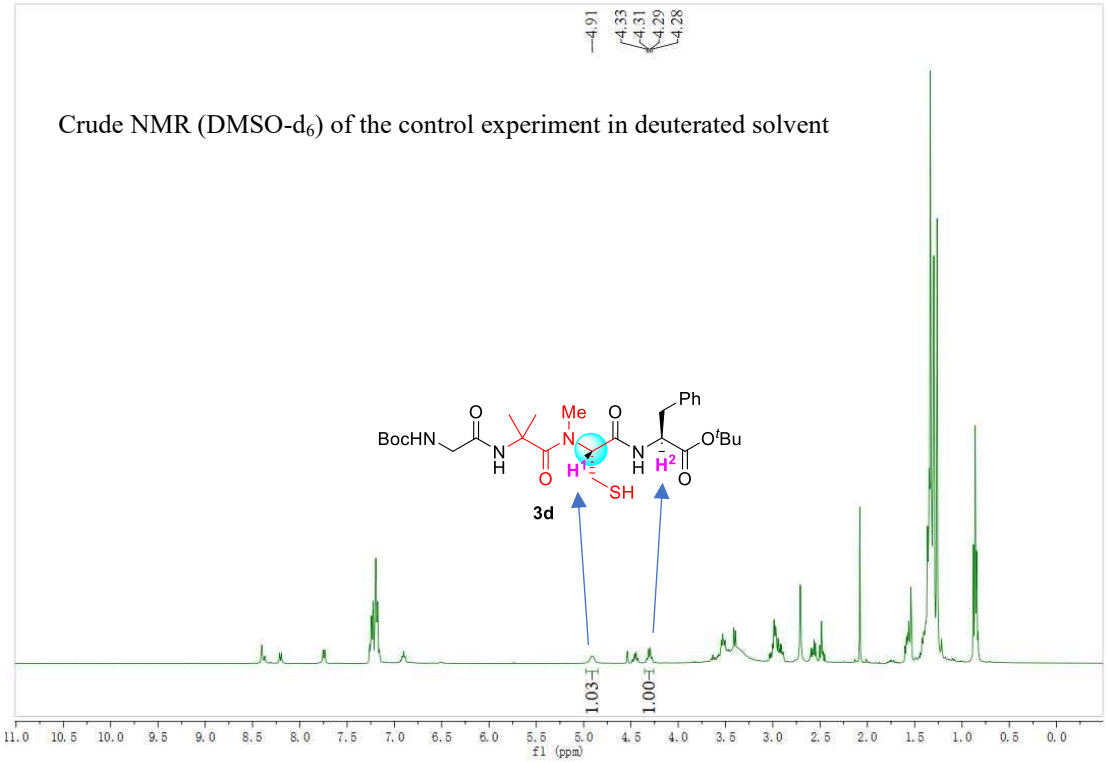
Total

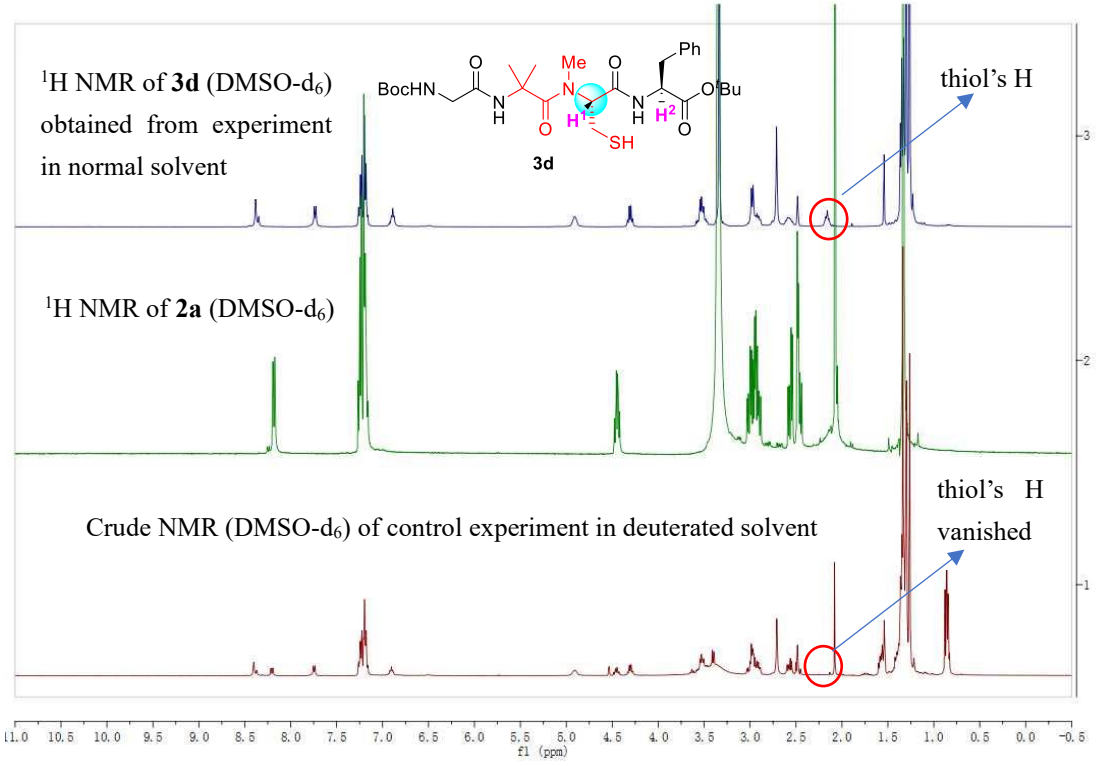
2.11.2 Control experiment in deuterated solvent

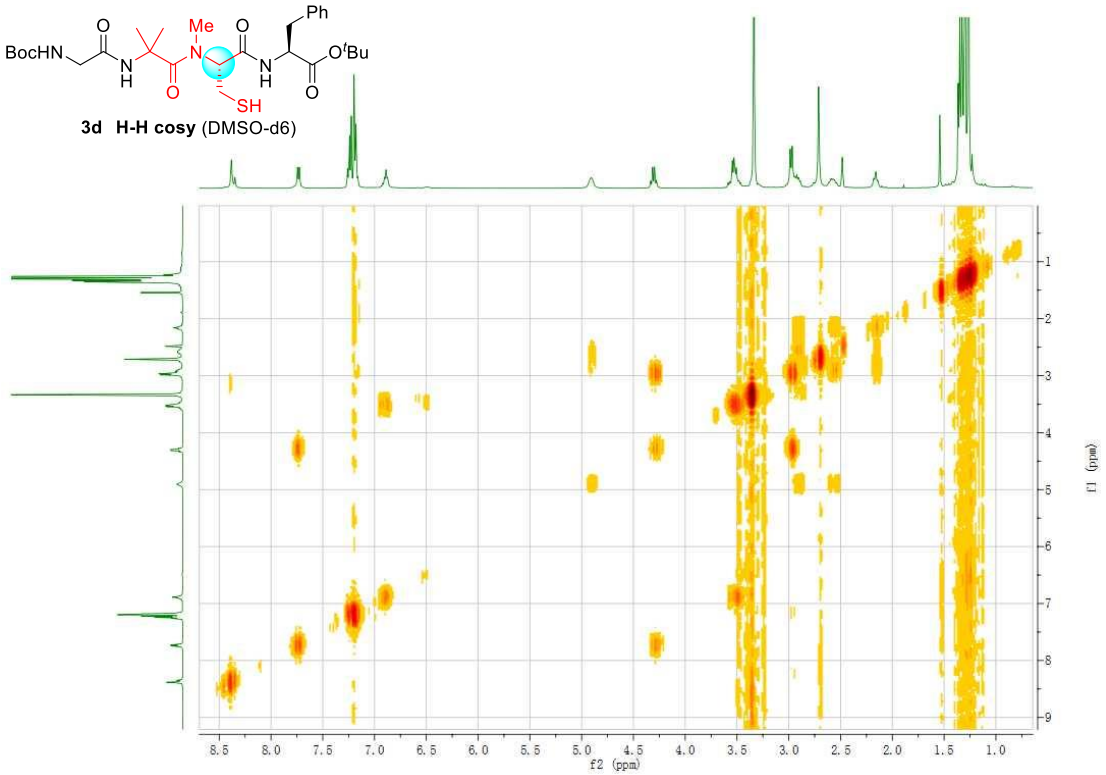
A control experiment was conducted using **1d** and **2a** in deuterated solvent under the same condition for making **3d** in normal solvent shown in table 2 (**Equation s2**). The incorporation of deuterium is not observed at the α-position of cysteinyl residue based on the comparison of ^{1}H NMR data. In addition, as expected, the thiol's H in **3d** was exchanged with deuterium, which is reasonable.



1d (1 equiv.) **2a** (1.5 equiv.) **3d**

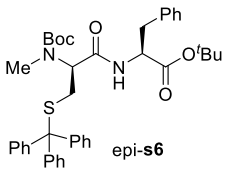

Crude NMR (DMSO-d6) of the control experiment in deuterated solvent
4.91
4.33
4.31
4.29
4.28
BocHN
Me
Ph
O'Bu
SH
3d
1.03
1.00
f1 (ppm)


1H NMR of 3d (DMSO-d6) obtained from experiment in normal solvent
BocHN
Me
Ph
O'Bu
SH
3d
thiol's H
1H NMR of 2a (DMSO-d6)
Crude NMR (DMSO-d6) of control experiment in deuterated solvent
thiol's H vanished
f1 (ppm)



2.11.3 Synthesis of NMe-D-Cys-L-Phe-O^{t}Bu epi-2a and physical information of epi-2a and epi-3d

***tert*-Butyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-*S*-trityl-D-cysteinyl-L-phenylalaninate**



epi-**s6**

epi-s6 was synthesized following the procedure for making **s6** and was obtained in 83% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 80%/20%. **epi-s6** existed as a mixture of rotamers.

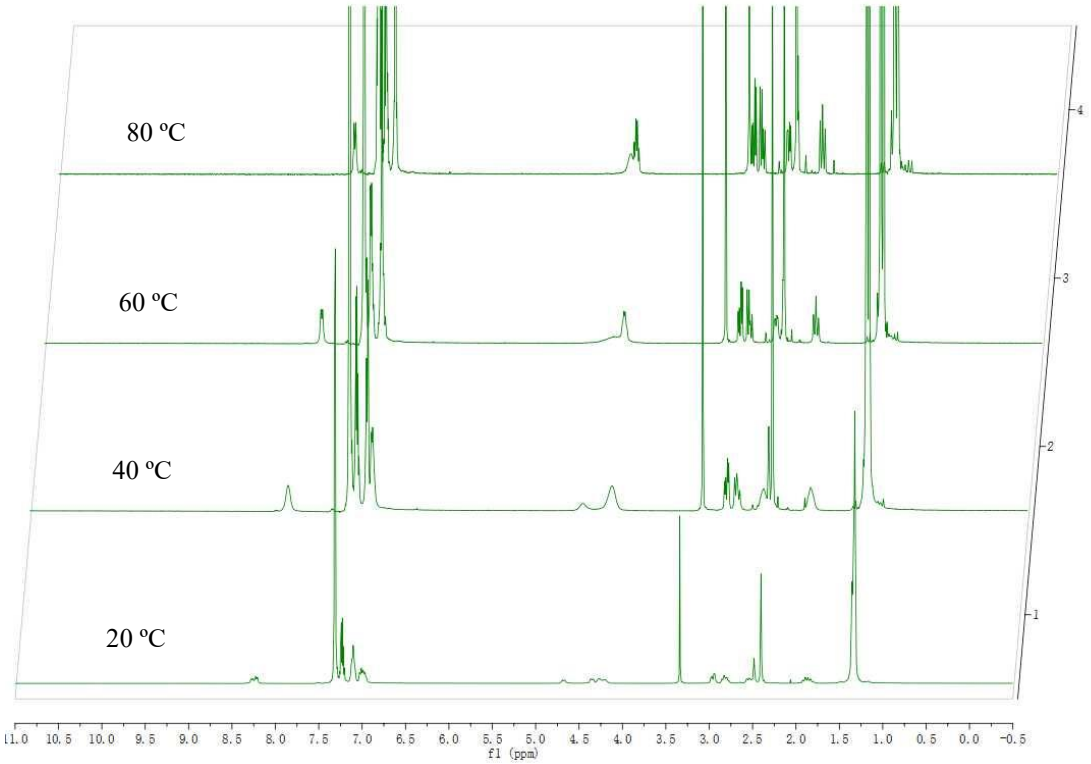
^{1}H NMR (399 MHz, DMSO-d_6) δ (8.27 (d, *J* = 7.5 Hz) and 8.22 (d, *J* = 8.1 Hz), 1H), 7.46 – 6.87 (m, 15H), 7.46 – 6.87 (m, 5H), 4.73 – 4.31 (m, 1H), 4.31 – 4.13 (m, 1H), 3.01 – 2.89 (m, 1H), 2.88 – 2.74 (m, 1H), 2.61 – 2.48 (m, 1H), 2.40 (s, 3H), 1.96 – 1.76 (m, 1H), 1.49 – 1.15 (m, 9H), 1.49 – 1.15 (m, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 170.9 and 170.7 (1C), 169.6 and 169.4 (1C) 155.6 and 155.1 (1C), 144.6, 137.7, 129.6, 129.5, 128.5, 128.4, 127.2, 126.7, 81.3, 79.8 and 79.7 (1C), 66.2 and 66.1 (1C), 57.9 and 56.1 (1C), 54.4 and 54.3 (1C), 37.0 and 36.9 (1C), 31.7 and 31.5 (1C), 30.2 and 30.0 (1C), 28.4, 28.0.

HRMS (ESI) m/z: Calcd for $C_{41}H_{48}N_2NaO_5S^+$ $[M+Na]^+$: 703.3176; found: 703.3179.

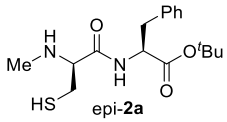
FT-IR (neat): 2978, 1730, 1687, 1495, 1445, 1367, 1321, 1252, 1155, 1034, 847, 751, 701, 621 cm^{-1}

$[\alpha]_D^{23}$+55.97 (c 1.0 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of epi-s6

***tert*-Butyl methyl-D-cysteinyl-L-phenylalaninate**



epi-**2a**

epi-2a was synthesized following the procedure for making **2a** and was obtained in 74% yield as a colorless oil. Eluent for purification: Hexane/EtOAc = 75%/25%, then EtOAc 100%.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.1 Hz, 1H), 7.34 – 7.13 (m, 5H), 4.75 – 4.65 (m, 1H), 3.16 (dd, *J* = 13.9, 6.1 Hz, 1H), 3.10 – 3.00 (m, 1H), 3.10 – 3.00 (m, 1H), 2.91 (dd, *J* = 13.9, 4.2 Hz, 1H), 2.79 (dd, *J* = 13.9, 7.1 Hz, 1H), 2.28 (s, 3H), 1.41 (s, 9H).

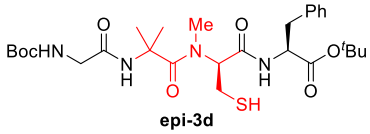
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.5, 136.3, 129.3, 128.4, 126.9, 82.2, 65.4, 53.3, 37.9, 34.7, 28.0, 26.7.

HRMS (ESI) m/z: Calcd for $C_{17}H_{27}N_2O_3S^+$ $[M+H]^+$: 339.1737; found: 339.1740.

FT-IR (neat): 3326, 2978, 2550, 1733, 1665, 1512, 1455, 1368, 1253, 1155, 741, 701 cm^{-1}

$[\alpha]_D^{20}$+62.69 (c 0.59 $CHCl_3$)

***tert*-Butyl *N*-(2-(2-((*tert*-butoxycarbonyl)amino)acetamido)-2-methylpropanoyl)-*N*-methyl-D-cysteinyl-L-phenylalaninate**



epi-3d

epi-3d was obtained in 76% yield as an amorphous solid. Eluent for purification: Hexane/EtOAc = 40%/60%, then CH_2Cl_2/EtOAc = 50%/50%. **epi-3d** existed as a mixture of rotamers.

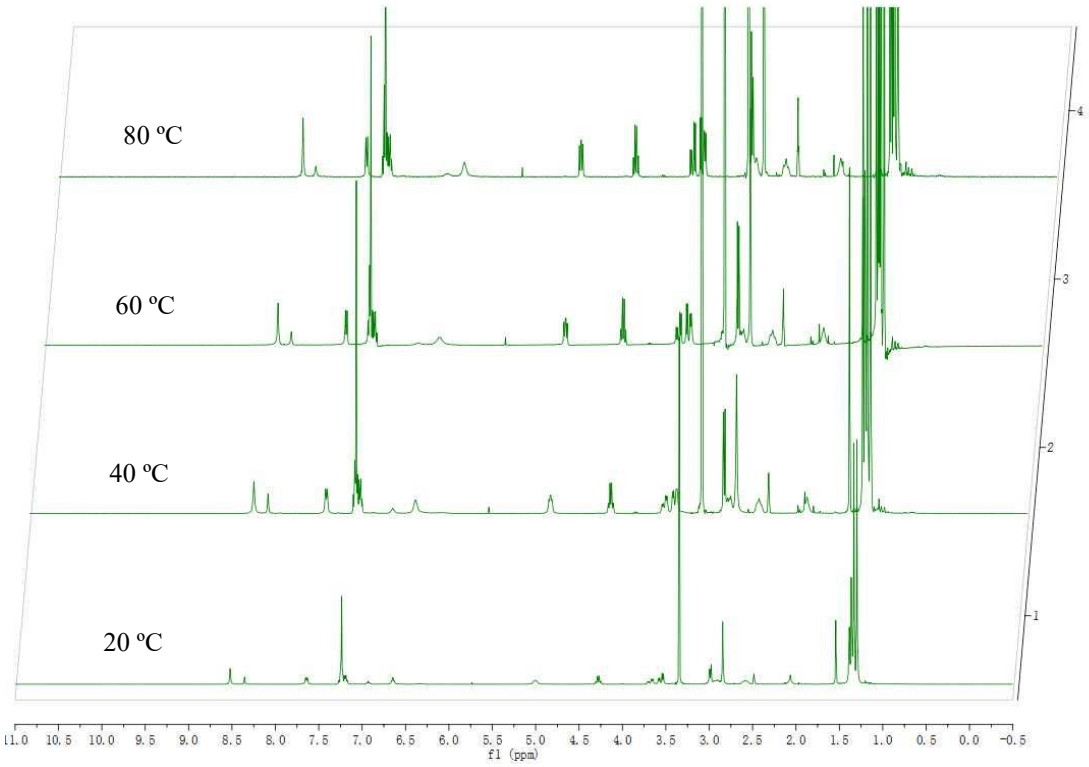
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.52 and 8.36 (s, 1H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.33 – 7.12 (m, 5H), (6.93 (t, *J* = 6.1 Hz) and 6.65 (t, *J* = 5.6 Hz), 1H), 5.06 – 4.94 (m, 1H), 4.33 – 4.23 (m, 1H), 3.73 – 3.52 (m, 2H), 3.10 – 2.74 (m, 1H), 3.10 – 2.74 (m, 1H), 3.10 – 2.74 (m, 1H), 2.84 (s, 3H), 2.63 – 2.52 (m, 1H), 2.12 – 2.01 (m, 1H), 1.58 – 1.18 (m, 9H), 1.58 – 1.19 (m, 9H), 1.58 – 1.19 (m, 3H), 1.58 – 1.19 (m, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.8, 170.8, 169.5 and 169.3 (1C), 168.9, 155.8 and 155.7 (1C), 137.3, 129.2, 128.1, 126.5, 80.9, 78.2 and 78.0 (1C), 59.7, 56.0 and 45.5 (1C), 54.4, 42.9 and 42.7 (1C), 36.6, 31.2, 28.2 and 28.1 (3C), 27.5, 26.5 and 26.4 (1C), 25.1, 21.8.

FT-IR (neat): 3302, 2980, 2934, 2557, 1716, 1669, 1523, 1393, 1367, 1249, 1159, 1089, 756, 701 cm^{-1}

HRMS (ESI) m/z: Calcd for $C_{28}H_{44}N_4NaO_7S^+$ $[M+Na]^+$: 603.2823; found: 603.2825.

$[\alpha]_D^{25}$-50.20 (c 0.6 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of epi-3d

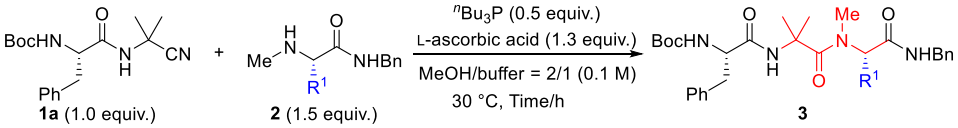
2.12 Reactivity of Serine, Homoserine and Aspartic acid analogues and corresponding synthesis and physical information

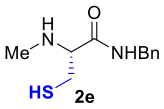
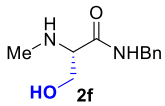
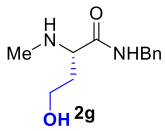
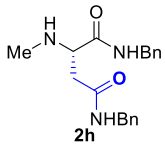
2.12.1 Reactivity of Serine, Homoserine and Aspartic acid analogues

Serine, Homoserine, Aspartic acid analogues were found to not react with the nitrile substrate. The data is summarized in **table s2**. Boc-L-Phe-Aib-CN **1a** reacts with NMe-L-Cys-NHBn **2e** to give its corresponding peptide **3w** in 71% yield after 18 h at 30 ºC under the standard optimized condition (**Table s2**, entry 1). However, the relevant Serine, Homoserine and Aspartic acid derivatives **2f**, **2g**, **2h** were shown to not react with **1a** and **1a** was recovered completely based on internal standard under the same condition after 24 h (**Table s2**, entries 2, 3 and 4). The NMR study based on internal standard (Br_2CH_2)

was shown below.

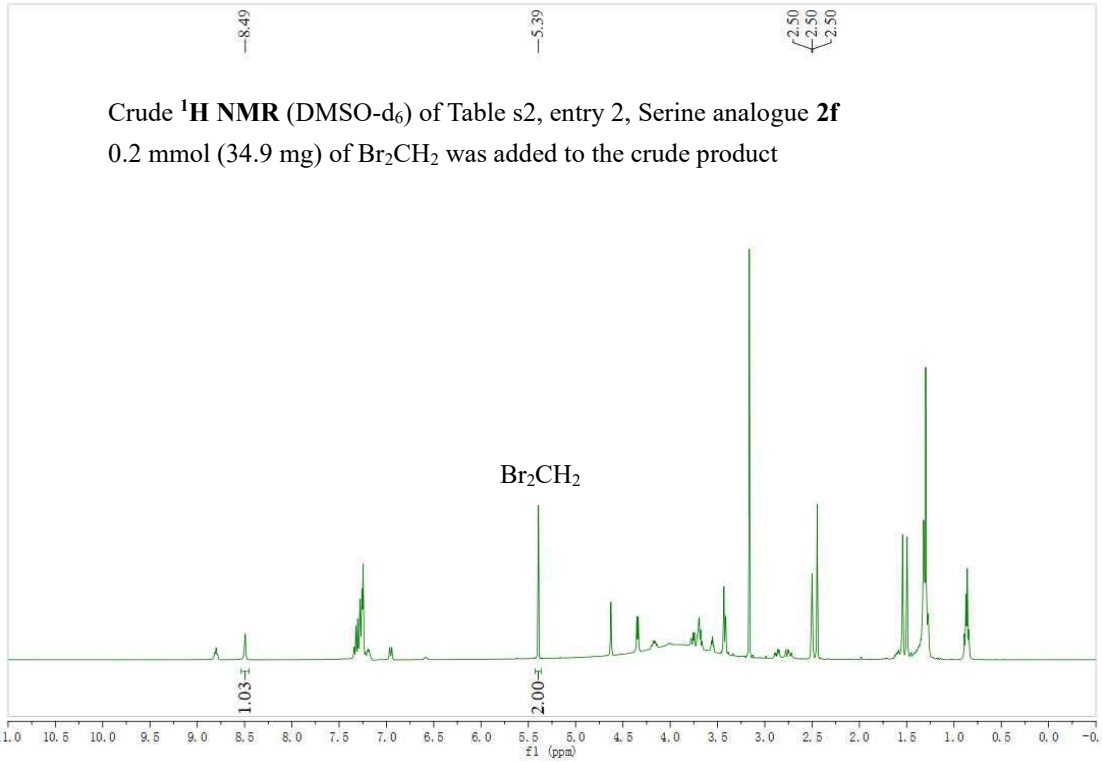
Table s2. Experiments between 1a and cysteine derivative and analogues of other amino acids[a]



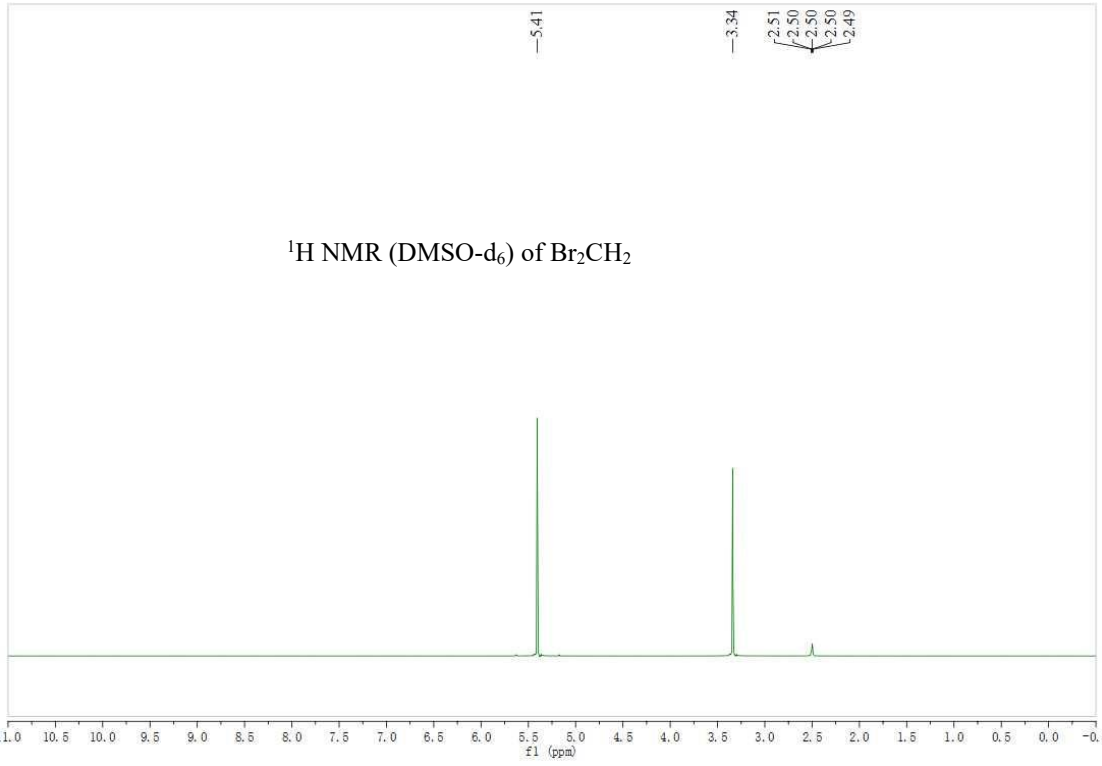
Entry	Substrate **2**	Time/h	Yield/%
1	 **2e**	18	**3w**: 71
2	 **2f**	24	0; rsm: **1a** 100% based on internal standard
3	 **2g**	24	0; rsm: 1a 100% based on internal standard
4	 **2h**	24	0; rsm: 1a 100% based on internal standard

[a]Unless otherwise shown, the reaction was conducted using **1a** (0.2 mmol) following the same procedure of generality study in table 2.

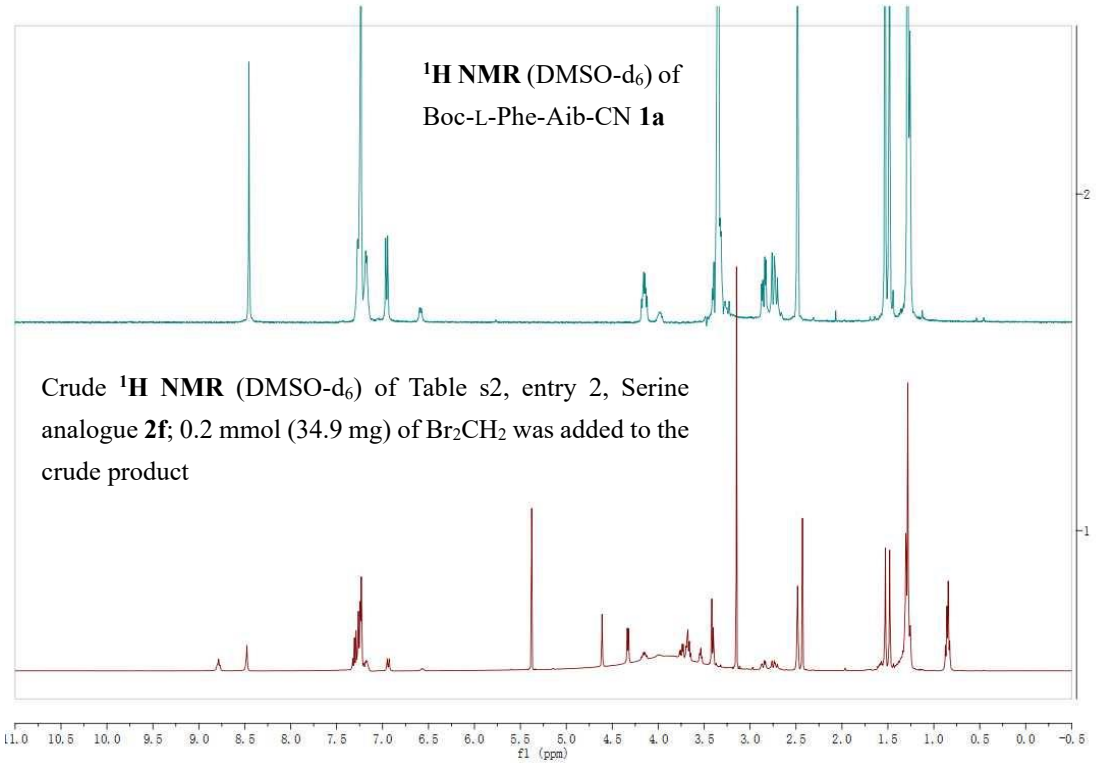
^{1}H NMR integration based on internal standard of Table s2, entry 2, Serine analogue 2f:



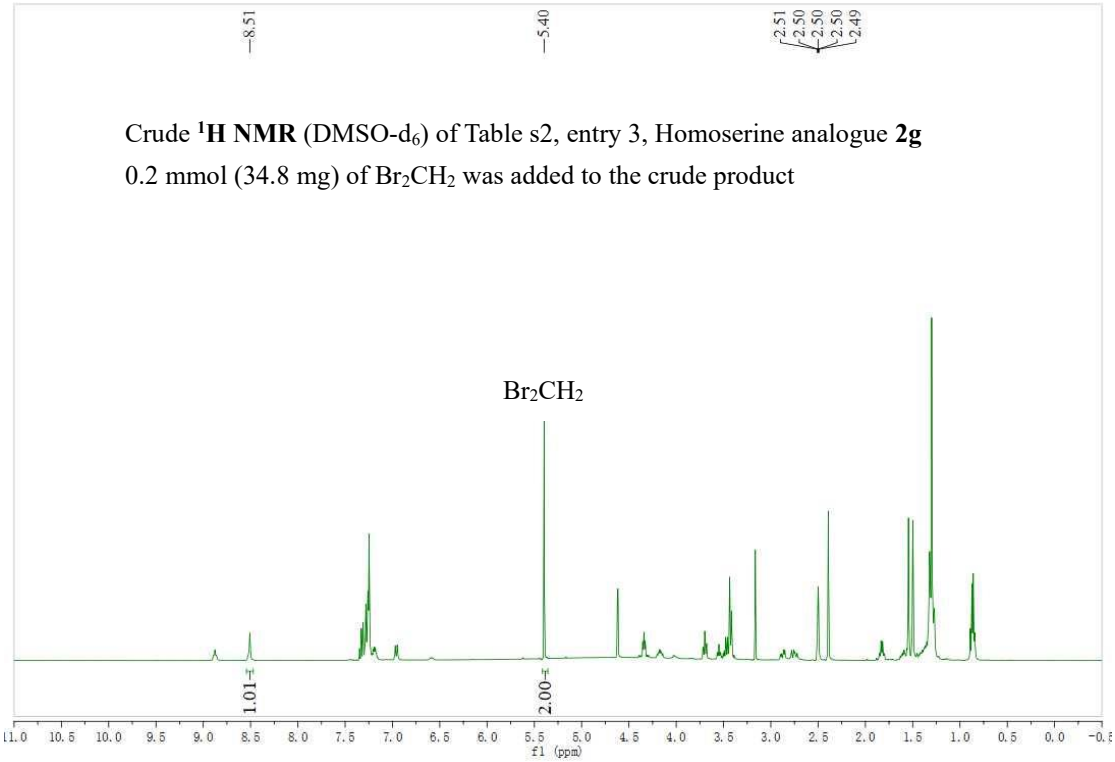
Crude **^{1}H NMR** (DMSO-d_6) of Table s2, entry 2, Serine analogue **2f**
0.2 mmol (34.9 mg) of Br_2CH_2 was added to the crude product

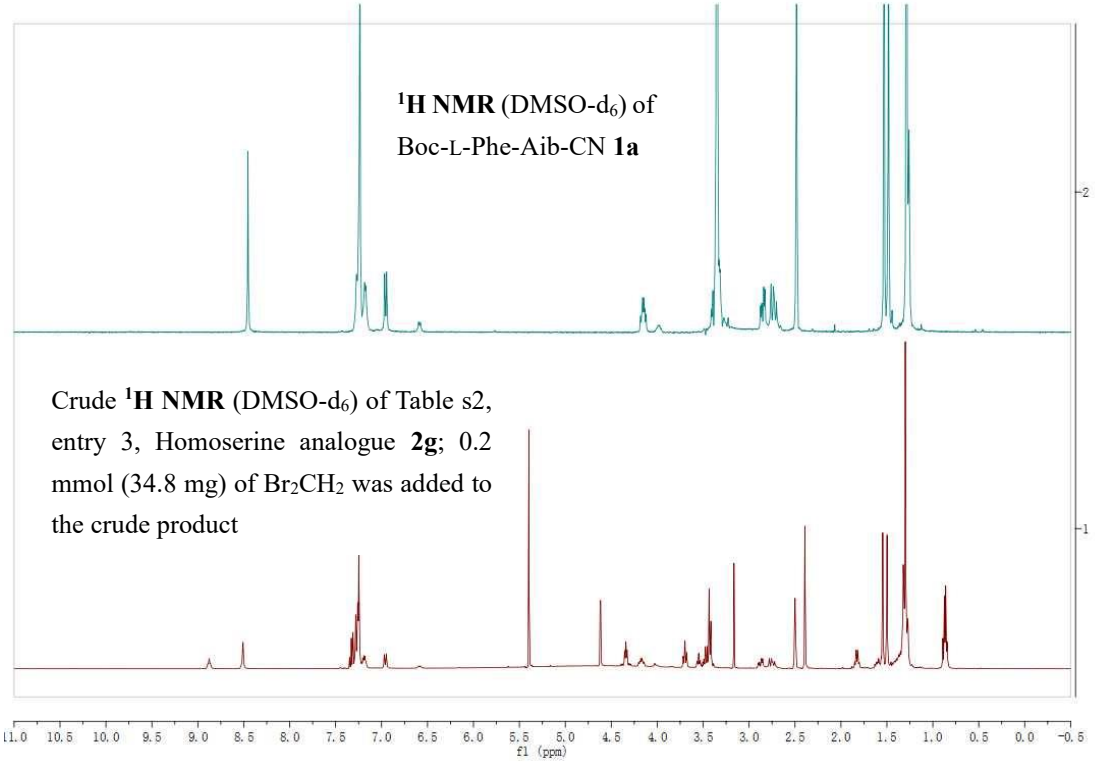


^{1}H NMR (DMSO-d_6) of Br_2CH_2

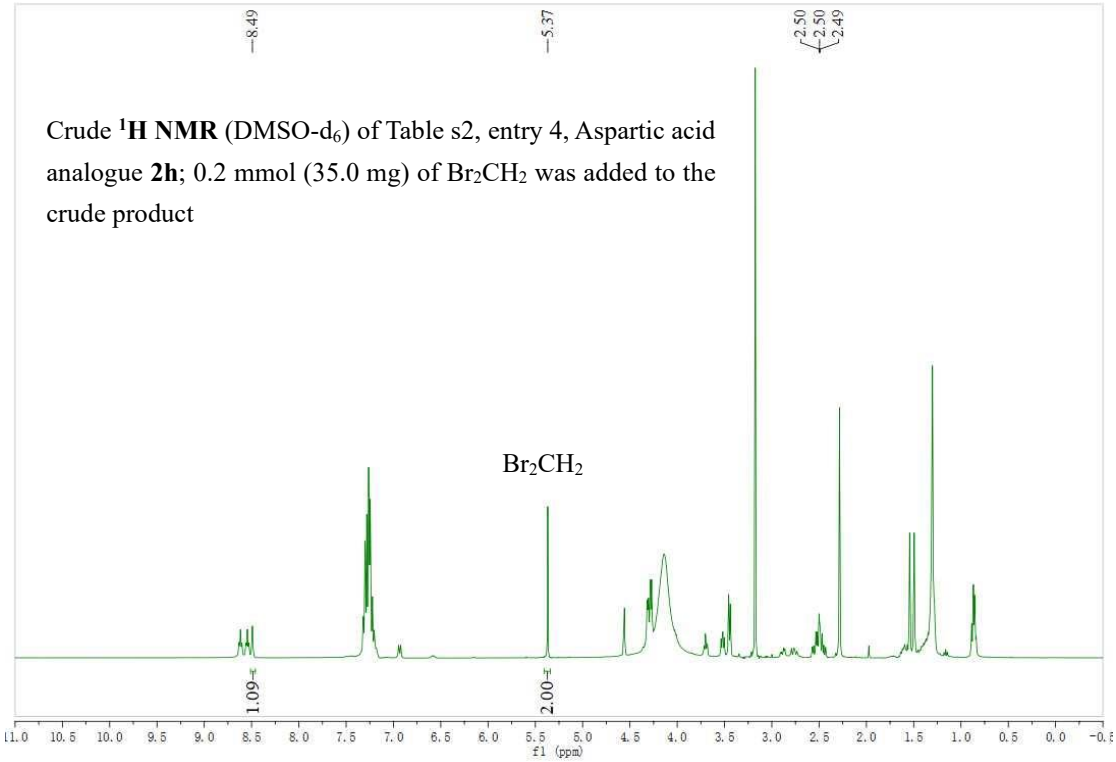


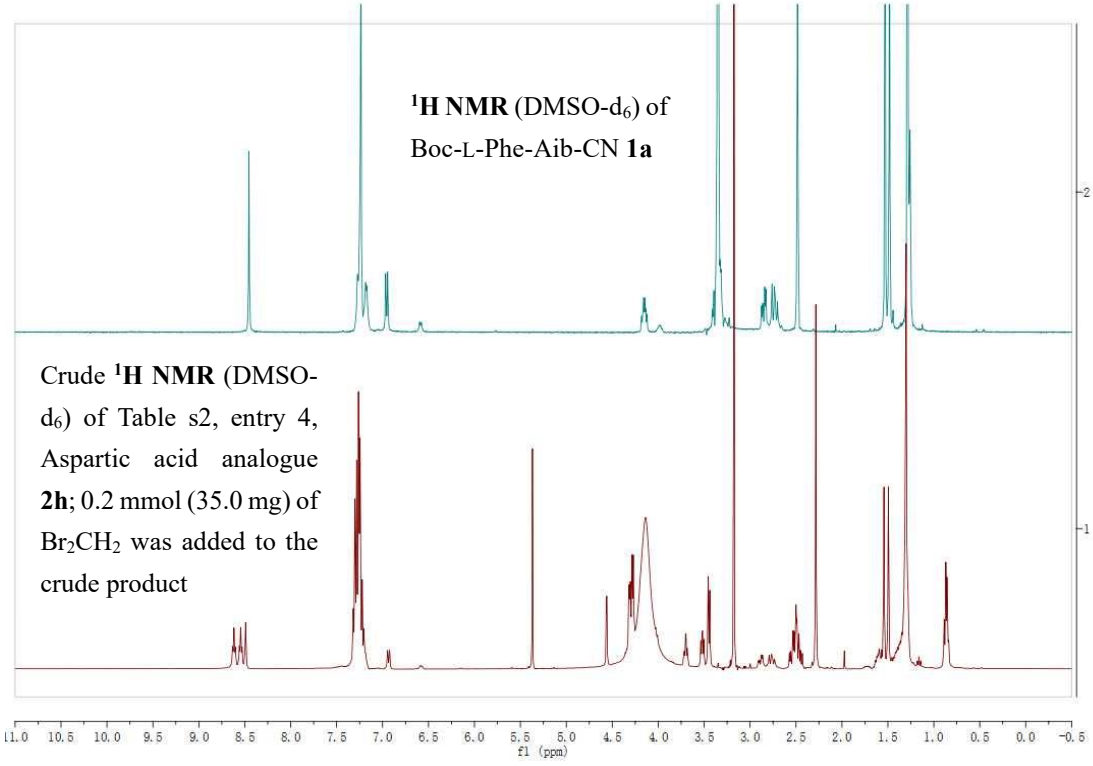
NMR integration based on internal standard of Table s2, entry 3, Homoserine analogue 2g:

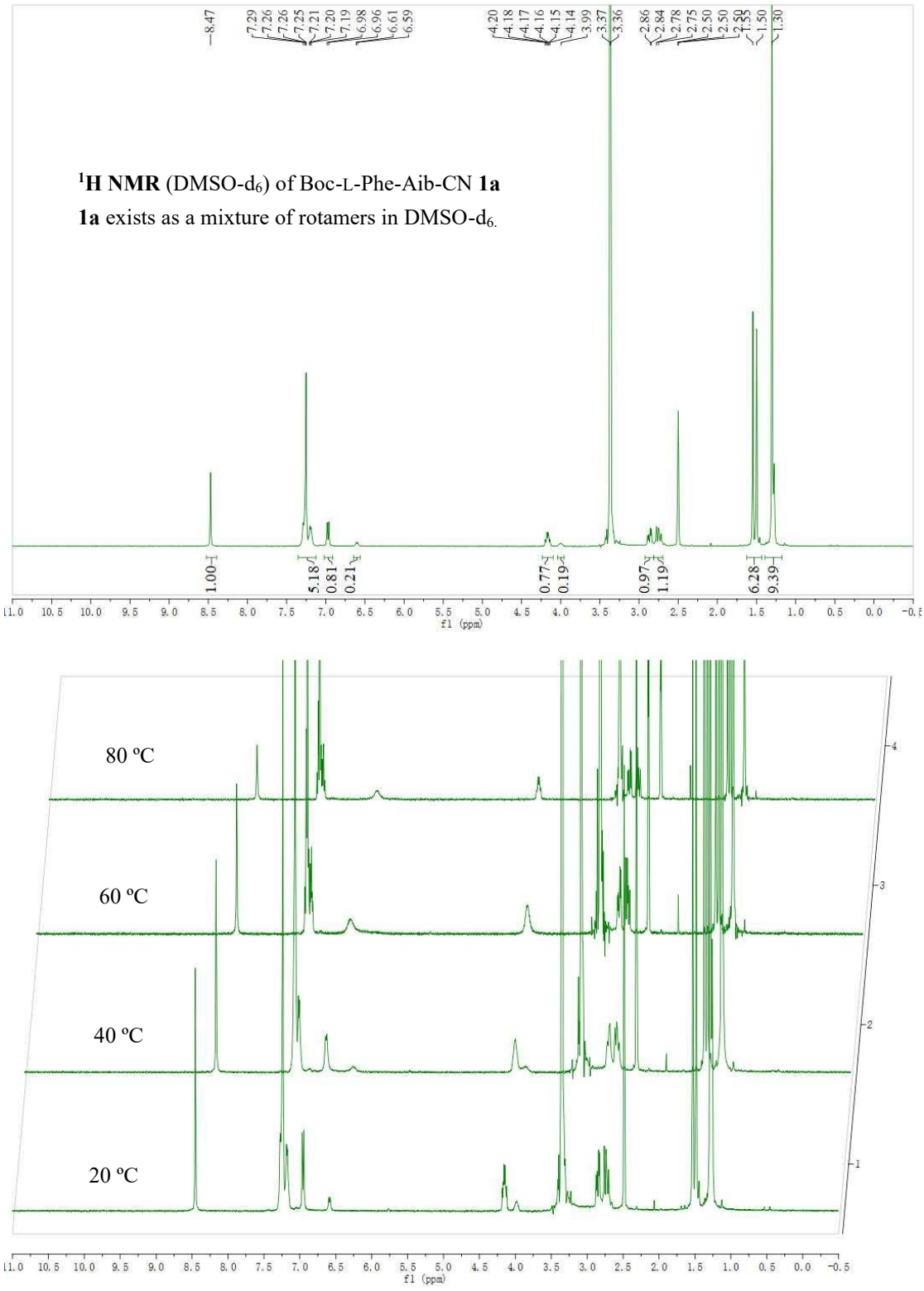




NMR integration based on internal standard of Table s2, entry 4, Aspartic acid analogue 2h:

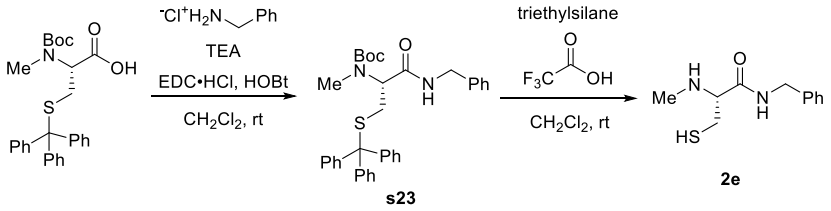

8.49
5.37
2.50
2.50
2.49
Crude ¹H NMR (DMSO-d6) of Table s2, entry 4, Aspartic acid analogue 2h; 0.2 mmol (35.0 mg) of Br2CH2 was added to the crude product
Br2CH2
1.09
2.00
f1 (ppm)


¹H NMR (DMSO-d6) of Boc-L-Phe-Aib-CN 1a
Crude ¹H NMR (DMSO-d6) of Table s2, entry 4, Aspartic acid analogue 2h; 0.2 mmol (35.0 mg) of Br2CH2 was added to the crude product
f1 (ppm)



Variable temperature [1]H NMR to prove the rotamers of Boc-L-Phe-Aib-CN 1a

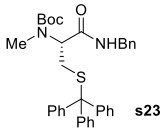
2.12.2 Synthesis of NMe-L-Cys-NHBn 2e and physical information



N-(*tert*-butoxycarbonyl)-*N*-methyl-S-trityl-L-cysteine[5] (1.00 g, 2.09 mmol) and benzylamine hydrochloride (301 mg, 2.09 mmol) was dissolved in CH_2Cl_2 (8.4 mL) at room temperature. Triethylamine (730 µl, 5.23 mmol), HOBt monohydrate (32.1 mg, 0.209 mmol) and EDC•HCl (482 mg, 2.51 mmol) were added to the reaction mixture. After stirring overnight at room temperature, the reaction was quenched by 1N HCl aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 70%/30%) and gave **s23** in 65% yield as an amorphous solid.

s23 (540 mg, 0.953 mmol) was dissolved in CH_2Cl_2 (9.5 mL), followed by the addition of triethylsilane (172 µL, 1.08 mmol) and trifluoroacetic acid (380 µL, 4% v/v).[6] After stirring for 0.5 h at room temperature, trifluoroacetic acid (1080 µL, 14.1 mmol) was added to the reaction mixture. After stirring for 50 min at room temperature, it was quenched with sat. $NaHCO_3$ aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 60%/40%; then EtOAc 100%; then MeOH/EtOAc = 80%/20%) and gave **2e** in 85% yield as a white solid.

<u>*tert*-Butyl (*R*)-(1-(benzylamino)-1-oxo-3-(tritylthio)propan-2-yl)(methyl)carbamate</u>



s23

s23 was obtained in 65% yield as an amorphous solid. **s23** existed as a mixture of rotamers.

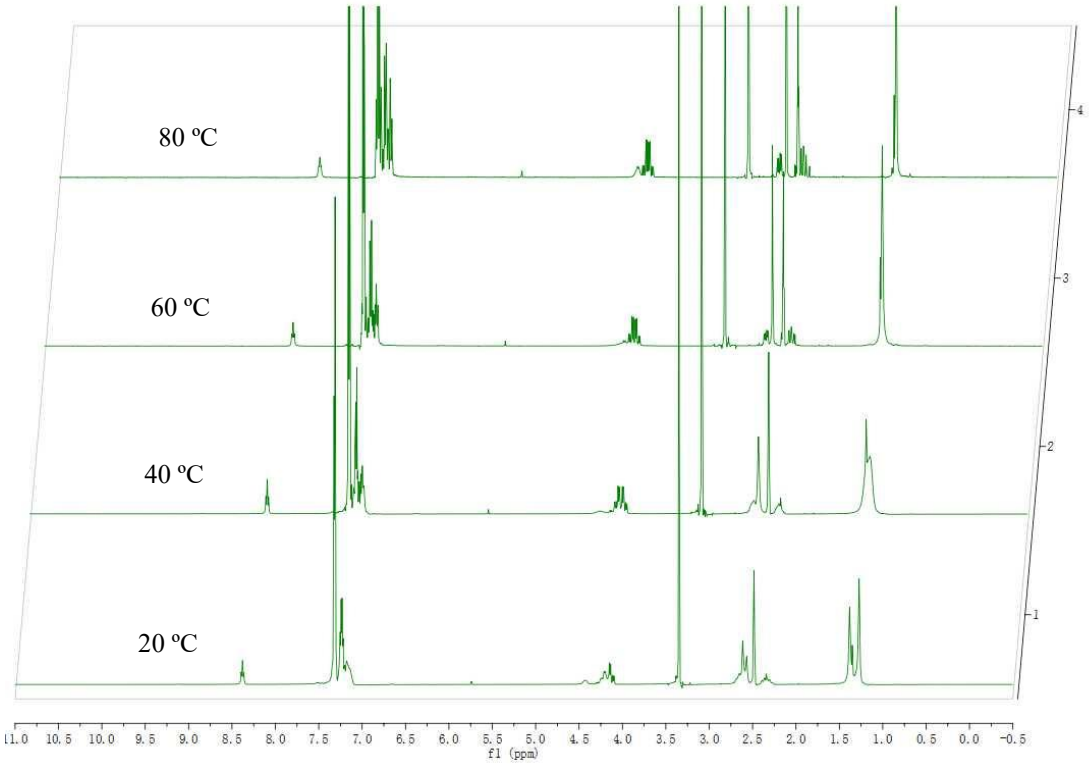
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.38 (t, *J* = 6.0 Hz, 1H), 7.62 – 6.98 (m, 15H), 7.62 – 6.98 (m, 5H), 4.49 – 4.16 (m, 1H), 4.49 – 4.16 (m, 1H), 4.12 (dd, *J* = 15.2, 5.8 Hz, 1H), 2.73 – 2.52 (m, 1H), 2.61 and 2.57 (s, 3H), 2.41 – 2.26 (m, 1H), 1.38 and 1.27 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 155.4 and 154.8 (1C), 144.7, 139.8, 129.5, 128.6, 128.5, 127.7, 127.2, 127.1, 79.8, 66.4, 59.6 and 57.8 (1C), 42.7 and 42.4 (1C), 32.8 and 32.0 (1C), 31.6 and 31.5 (1C), 28.4 and 28.3 (3C).

HRMS (ESI) m/z: Calcd for $C_{35}H_{38}N_2NaO_3S^+$ $[M+Na]^+$: 589.2495; found: 589.2490.

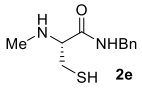
FT-IR (neat): 3330, 3060, 2977, 1685, 1522, 1490, 1445, 1390, 1367, 1321, 1252, 1158, 745, 700 cm^{-1}

$[\alpha]_D^{24}$-46.44 (c 1.1 $CHCl_3$)



Variable temperature ^{1}H NMR to prove the rotamers of s23

(*R*)-*N*-benzyl-3-mercapto-2-(methylamino)propanamide



2e was obtained in 85% yield as a white solid. m.p. 46-48 ºC

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.40 – 7.26 (m, 5H), 4.46 (d, *J* = 6.0 Hz, 2H), 3.32 – 3.23 (m, 1H), 3.04 – 2.90 (m, 2H), 2.43 (s, 3H).

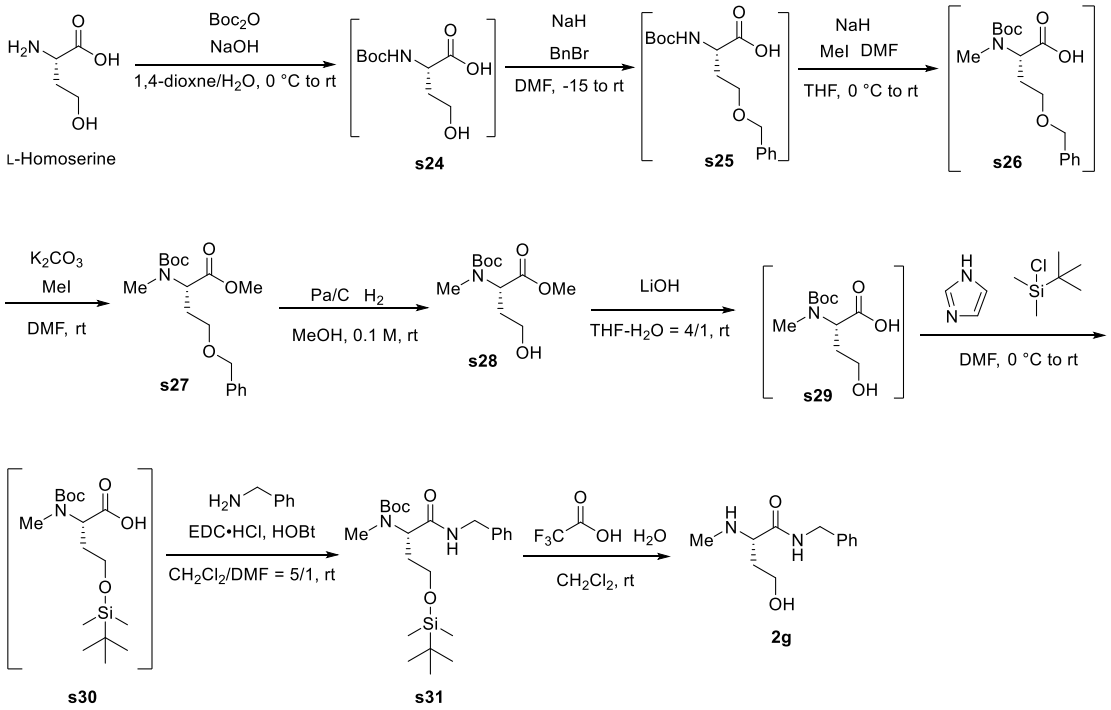
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 138.2, 128.7, 127.6, 127.4, 65.4, 43.1, 34.9, 26.5.

HRMS (ESI) m/z: Calcd for $C_{11}H_{16}N_2NaOS^+$ $[M+Na]^+$: 247.0876; found: 247.0874.

FT-IR (neat): 3307, 2936, 2550, 1654, 1523, 1454, 1244, 732, 699 cm^{-1}

$[\alpha]_D^{24}$-40.73 (c 1.08 $CHCl_3$)

2.12.3 Synthesis of NMe-L-Ser-NHBn 2f and NMe-L-Homoserine-NHBn 2g and physical information



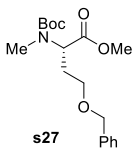
A mixture of L-Homoserine (1.00 g, 8.40 mmol) in 1.4-dixane/H_2O = 1/1 (17 mL) was cooled to 0 ºC, followed by the addition of NaOH (710 mg, 18.0 mmol) and Boc_2O (2.20 g, 10.1 mmol).[13] After stirring for 20 h at room temperature, the reaction mixture was quenched by HCl 1N aqueous solution to pH around 2, extracted with EtOAc. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product **s24** was dissolved in DMF (22 mL) and cooled to -15 ºC, followed by the addition of NaH (60% in mineral oil) (767 mg, 19.3 mmol).[13] After stirring for 3 h at -15 ºC, Benzyl bromide (1.1 mL, 9.23 mmol) was added slowly to the reaction mixture. After stirring for 2 h at room temperature (The reaction was monitored by TLC analysis, EtOAc/Hexane = 80%/20%), it was poured into prechilled water (20 mL), washed with Et_2Ox2. The aqueous phase was quenched with HCl 1N aqueous solution to pH around 4 and extracted with EtOAc. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product **s25** was dissolved in dry THF (15 mL) and cooled to 0 ºC, followed by the addition of NaH (60% in mineral oil) (467 mg, 11.6 mmol).[14] After stirring for 10 min at 0 ºC (until the gas evolution ceased), MeI (2.4 mL, 38.8 mmol) and DMF (0.68 mL, 8.80 mmol) was added to the reaction mixture. After stirring for 2 h at 0 ºC, the reaction mixture was warmed to room temperature and stirred for 14 h. Then it was quenched with H_2O, evaporated under reduced pressure to remove the volatiles. The residue was diluted with EtOAc, acidified with HCl, extracted with EtOAc. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product **s26** was dissolved in DMF (9.7 mL), followed by the addition of K_2CO_3 (2.01 g, 14.6 mmol) and MeI (2.4 mL, 38.8 mmol). After stirring for 1.5 h at room temperature, it was quenched with HCl 1N aqueous solution at 0 ºC, extracted with EtOAc, washed with water and brine. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 80%: 20%) and gave **s27** in 55% yield over 4 steps as a yellow oil.

s27 (848 mg, 2.51 mmol) was dissolved in MeOH (25 mL), followed by the addition of Pd/C (85 mg, 10 wt%). After stirring under H_2 atmosphere (H_2 balloon) for 2 days, it was filtered through a pad of celite and washed with EtOAc. The filtrate was evaporated under reduced pressure, concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 60%: 40%) and gave **s28** in 78% yield as a colorless oil. And it was used directly without further characterization.

s28 (204 mg, 0.825 mmol) was dissolved THF/H_2O = 4/1 (3.3 mL), followed by the addition of LiOH•H_2O (344 mg, 8.20 mmol). After stirring for 0.5 h at room temperature, it was evaporated under reduced pressure. The residue was diluted with EtOAc, quenched with HCl 1N aqueous solution to pH around 1 to 2, extracted with EtOAc. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure, azeotrope with benzene x2 and concentrated in vacuo. The crude product **s29** was dissolved in dry DMF (1.3 mL), followed by the addition of imidazole (179 mg, 2.62 mmol).[15] The reaction mixture was then cooled to 0 ºC, followed by the addition of TBSCl (185 mg, 1.23 mmol) in small portions. After stirring for 5 h at room temperature, EtOAc (15 mL) and HCl 1N aqueous solution (15 mL) were added to the reaction mixture, stirred vigorously for 0.5 h at room temperature to hydrolyze the carboxylic silyl ester and extracted with EtOAc. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure, and concentrated in vacuo. The crude product **s30** was dissolved in CH_2Cl_2/DMF = 5/1 (9.3 mL), followed by the addition of benzyl amine (90 μL, 0.825 mmol), EDC•HCl (188 mg, 0.983 mmol) and HOBt•H_2O (12.7 mg, 0.0825 mmol). After stirring at room temperature overnight, it was quenched by HCl 1N aqueous solution, extracted with CH_2Cl_2. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 75%: 25%) and gave **s31** in 58% yield over 3 steps as a colorless oil.

s31 (210 mg, 0.481 mmol) was dissolved in CH_2Cl_2 (2.6 mL), followed by the addition of H_2O (284 μL) and trifluoroacetic acid (735 μL, 9.62 mmol).[15] After stirring for 4 h at room temperature, it was evaporated under reduced pressure. EtOAc was added to the residue, quenched with NaOH 4N aqueous solution, extracted with EtOAc. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 87%/13%) and gave **2g** in 75% yield as a viscous amorphous.

Methyl *O*-benzyl-*N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-homoserinate



s27

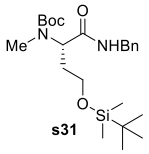
s27 was obtained in 55% yield over 4 steps as a yellow oil. **s27** existed as a mixture of rotamers.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.48 – 6.98 (m, 5H), 4.84 – 4.64 and 4.59 – 4.27 (m, 1H), 4.59 – 4.27 (m, 2H), 3.78 – 3.38 (m, 3H), 3.79 – 3.38 (m, 1H), 3.78 – 3.38 (m, 1H), 2.87 and 2.81 (s, 3H), 2.38 – 2.24 (m, 1H), 2.08 – 1.93 (m, 1H), 1.46 and 1.40 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 156.0 and 155.4 (1C), 138.2 and 138.1 (1C), 128.4, 127.7 and 127.6 (2C), 127.6, 80.3 and 80.0 (1C), 73.1 and 73.0 (1C), 66.9 and 66.3 (1C), 57.4 and 56.2 (1C), 52.0, 33.4 and 32.1 (1C), 29.9 and 29.1 (1C), 28.3 and 28.3 (3C).

HRMS (ESI) m/z: Calcd for $C_{18}H_{27}NNaO_5^+$ $[M+Na]^+$: 360.1781; found: 360.1783.
FT-IR (neat): 2976, 1744, 1699, 1454, 1392, 1366, 1327, 1152, 1009, 739, 699 cm^{-1}
$[\alpha]_D^{23}$-30.69 (c 1.1 $CHCl_3$)

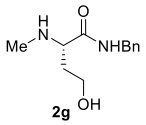
<u>*tert*-Butyl (*S*)-(1-(benzylamino)-4-((*tert*-butyldimethylsilyl)oxy)-1-oxobutan-2-yl)(methyl)carbamate</u>



s31

s31 was obtained in 58% yield over 3 steps as a colorless oil. **s31** existed as a mixture of rotamers.
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.30 (t, *J* = 5.9 Hz, 1H), 7.37 – 7.10 (m, 5H), 4.67 – 4.36 (m, 1H), 4.29 (dd, *J* = 15.1, 6.2 Hz, 1H), 4.25 – 4.15 (m, 1H), 3.66 – 3.45 (m, 2H), 2.72 (s, 3H), 2.13 – 1.94 (m, 1H), 1.85 – 1.71 (m, 1H), 1.39 and 1.32 (s, 9H), 0.84 (s, 9H), 0.00 (s, 6H).
^{13}C NMR (100 MHz, DMSO-d_6) δ 171.0, 155.7 and 155.3 (1C), 140.1 and 140.1 (1C), 128.6, 127.6 and 127.4 (2C), 127.1, 79.3, 60.1, 56.7 and 55.3 (1C), 42.6 and 42.4 (1C), 32.5 and 31.9 (1C), 31.6 and 31.1 (1C), 28.5 and 28.4 (3C), 26.3 18.4, -5.0.
HRMS (ESI) m/z: Calcd for $C_{23}H_{40}N_2NaO_4Si^+$ $[M+Na]^+$: 459.2650; found: 459.2651.
FT-IR (neat): 3330, 2957, 2929, 2857, 1686, 1524, 1455, 1390, 1366, 1329, 1255, 1152, 1102, 949, 837, 777, 699 cm-1
$[\alpha]_D^{25}$-32.36 (c 2.00 $CHCl_3$)

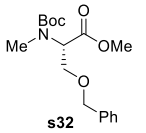
<u>(*S*)-*N*-benzyl-4-hydroxy-2-(methylamino)butanamide</u>



2g

2g was obtained in 75% yield as a viscous white amorphous.
^{1}H NMR (399 MHz, CD_3OD) δ 7.38 – 7.19 (m, 5H), 4.43 (s, 2H), 3.65 (t, *J* = 5.8 Hz, 2H), 3.51 (t, *J* = 6.2 Hz, 1H), 2.43 (s, 3H), 2.00 – 1.82 (m, 2H).
^{13}C NMR (100 MHz, CD_3OD) δ 173.4, 139.5, 129.6, 128.6, 128.3, 62.8, 59.7, 44.1, 35.6, 33.9.
HRMS (ESI) m/z: Calcd for $C_{12}H_{19}N_2O_2^+$ $[M+H]^+$: 223.1441; found: 223.1445.
FT-IR (neat): 3285, 1677, 1455, 1206, 1140, 1071, 842, 801, 724 cm^{-1}
$[\alpha]_D^{22}$+0.25 (c 0.67 MeOH)

<u>Methyl *O*-benzyl-*N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-serinate</u>



s32

s32 was synthesized from Boc-L-Ser(OBn)-OH following the procedure for synthesizing **2g** and **s32** was obtained in 61% yield as a colorless oil over 2 steps. **s32** existed as a mixture of rotamers. Eluent for purification: Hexane/EtOAc = 75%/25%.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.62 – 7.01 (m, 5H), 4.95 (t, *J* = 5.9 Hz, 0.5H), 4.63 – 4.54 (m, 1H), 4.54 – 4.39 (m, 0.5H), 4.54 – 4.39 (m, 1H), 3.95 – 3.77 (m, 2H), 3.71 (s, 3H), 2.95 and 2.90 (s, 3H), 1.47 and 1.39 (s, 9H).

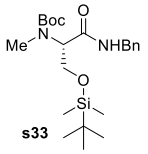
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 and 170.4 (1C), 156.2 and 153.3, 137.89 and 137.7 (1C), 128.4 and 128.3 (2C), 127.8 and 127.7 (1C), 127.6 (2C), 80.4 and 80.2 (1C), 73.2 and 72.9 (1C), 68.3 and 68.0 (1C), 60.2 and 58.2 (1C), 52.1, 33.3 and 32.3 (1C), 28.3 and 28.3 (3C).

HRMS (ESI) m/z: Calcd for $C_{17}H_{25}NNaO_5^+$ $[M+Na]^+$: 346.1625; found: 346.1631.

FT-IR (neat): 2976, 1749, 1696, 1455, 1392, 1366, 1329, 1152, 740, 698 cm^{-1}

$[\alpha]_D^{25}$-26.82 (c 1.5 $CHCl_3$)

<u>*tert*-Butyl (*S*)-(1-(benzylamino)-3-((*tert*-butyldimethylsilyl)oxy)-1-oxopropan-2-yl)(methyl)carbamate</u>



s33

s33 was obtained in 51% yield as a yellow oil over 3 steps. **s33** existed as a mixture of rotamers. Eluent for purification: Hexane/EtOAc = 75%/25%.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.45 (t, *J* = 5.6 Hz, 1H), 7.33 – 7.14 (m, 5H), 4.71 – 4.62 and 4.48 – 4.39 (m, 1H), 4.38 – 4.28 (m, 1H), 4.28 – 4.17 (m, 1H), 4.05 – 3.93 (m, 1H), 3.90 – 3.80 (m, 1H), 2.83 (s, 3H), 1.41 and 1.35 (s, 9H), 0.85 (s, 9H), 0.04 (s, 6H).

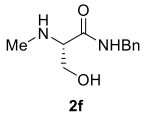
^{13}C NMR (100 MHz, DMSO-d_6) δ 169.2, 155.9 and 155.3 (1C), 140.0 and 139.9 (1C), 128.5 (2C), 127.6 and 127.5 (2C), 127.0, 79.2 and 79.1 (1C), 61.8 and 60.2 (1C), 61.3 and 60.8 (1C), 42.5 and 42.4 (1C), 32.0 and 31.7 (1C), 28.5 and 28.4 (3C), 26.0 (3C), 18.2 and 18.1 (1C), -5.1 and -5.3 (2C).

HRMS (ESI) m/z: Calcd for $C_{22}H_{38}N_2NaO_4Si^+$ $[M+Na]^+$: 445.2493; found: 445.2502.

FT-IR (neat): 3329, 2957, 2930, 2857, 1693, 1674, 1526, 1473, 1390, 1366, 1325, 1254, 1153, 897, 838, 777, 699 cm^{-1}

$[\alpha]_D^{23}$-16.81 (c 0.9 $CHCl_3$)

<u>(*S*)-N-benzyl-3-hydroxy-2-(methylamino)propanamide</u>



2f

2f was obtained in 83% yield as a colorless oil. Eluent for purification: CH_2Cl_2/MeOH = 75%/25%.

^{1}H NMR (399 MHz, CD_3OD) δ 7.40 – 7.18 (m, 5H), 4.44 (s, 2H), 3.92 (dd, *J* = 11.6, 4.3 Hz, 1H), 3.81 (dd, *J* = 11.6, 5.4 Hz, 1H), 3.66 – 3.60 (m, 1H), 2.58 (s, 3H).

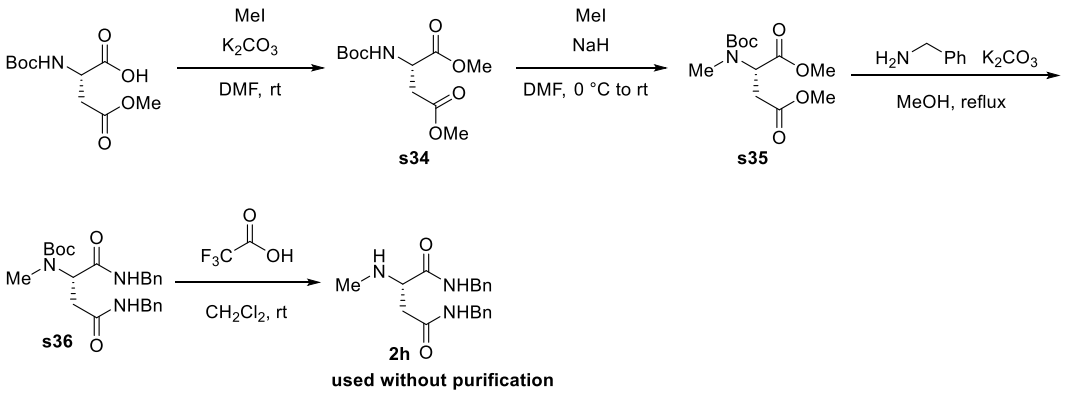
^{13}C NMR (100 MHz, CD_3OD) δ 168.7, 138.0, 128.2, 127.2, 127.0, 63.5, 60.3, 42.9, 31.7.

HRMS (ESI) m/z: Calcd for $C_{11}H_{17}N_2O_2^+$ $[M+H]^+$: 209.1285; found: 209.1285.

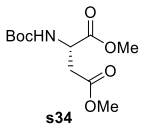
FT-IR (neat): 3289, 1674, 1572, 1433, 1203, 1138, 1070, 840, 801, 723, 700 cm^{-1}

$[\alpha]_D^{24}$-3.88 (c 1.18 MeOH)

2.12.4 Synthesis of NMe-L-Asp(NHBn)-NHBn 2h and physical information

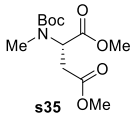


Dimethyl (*tert*-butoxycarbonyl)-L-aspartate



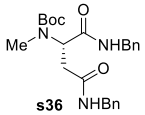
s34 was synthesized from Boc-L-Asp(OMe)-OH (500 mg, 2.02 mmol was used) following the same procedure for synthesizing **s27** and **s34** was obtained in 95% yield. The NMR data matches with the reported one.[16]

Dimethyl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-L-aspartate



s35 was synthesized following the reported procedure for synthesizing **s35**'s enantiomer.[17] **s35** was obtained in 91% yield. $[\alpha]_D^{23}$-79.32 (c 1.05 $CHCl_3$). The NMR data of **s35** matches with the reported NMR data of **s35**'s enantiomer. The data of its optical rotation was not shown in the literature.

***tert*-Butyl (*S*)-(1,4-bis(benzylamino)-1,4-dioxobutan-2-yl)(methyl)carbamate**



s35 (470 mg, 1.71 mmol) was dissolved in MeOH (3.6 mL), followed by the addition of benzylamine (410 μL, 3.76 mmol) and K_2CO_3 (7 mg, 51.3 μmol).[18] After refluxing for 24 h at 70 ºC, it was cooled to room temperature, quenched with HCl 1N aqueous solution and extracted with EtOAc. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 30%: 70%) and gave **s36** in 14% yield as a colorless oil.

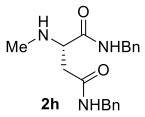
s36 existed as a mixture of rotamers.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.57 – 7.02 (m, 5H), 7.57 – 7.02 (m, 5H), 6.95 – 6.37 (m, 1H), 6.95 – 6.37 (m, 1H), 5.11 – 4.71 (m, 1H), 4.59 – 4.12 (m, 2H), 4.59 – 4.12 (m, 2H), 3.18 – 2.93 (m, 1H), 2.93 – 2.68 (m, 3H), 2.68 – 2.40 (m, 1H), 1.39 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.9, 156.3 and 155.1 (1C), 138.3 and 138.1 (2C), 128.6, 127.6, 127.3, 81.3 and 80.8 (1C), 57.8 and 56.4 (1C), 43.5, 43.3, 36.6 and 35.7 (1C), 32.3 and 31.9 (1C), 28.2.

HRMS (ESI) m/z: Calcd for $C_{24}H_{31}N_3NaO_4^+$ $[M+Na]^+$: 448.2207; found: 448.2207.
FT-IR (neat): 3312, 2977, 1655, 1542, 1454, 1391, 1366, 1253, 1149, 752, 699 cm^{-1}
$[\alpha]_D^{22}$-0.86 (c 2.30 $CHCl_3$)

(*S*)-N^1,N^4-dibenzyl-2-(methylamino)succinamide



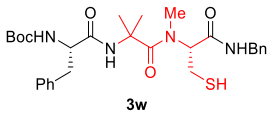
2h

2h was used directly without purification.
s36 (290 mg, 0.682 mmol) was dissolved in CH_2Cl_2 (6.8 mL), followed by the addition of trifluoroacetic acid (1.04 mL, 13.6 mmol). After stirring for 1.5 h at room temperature, it was quenched with sat. $NaHCO_3$ aqueous solution, extracted with CH_2Cl_2. The organic phase was collected, dried over Na_2SO_4 and filtered, evaporated under reduced pressure, and concentrated in vacuo. The crude product (214 mg, 97%) was used without purification.

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.53 – 8.40 (m, 1H), 8.53 – 8.40 (m, 1H), 7.33 – 7.16 (m, 5H), 7.33 – 7.16 (m, 5H), 4.29 (d, *J* = 6.1 Hz, 2H), 4.26 (d, *J* = 6.0 Hz, 2H), 3.36 – 3.29 (m, 1H), 2.44 (dd, *J* = 14.6, 4.9 Hz, 1H), 2.33 (dd, *J* = 14.6, 8.6 Hz, 1H), 2.20 (s, 3H).
^{13}C NMR (100 MHz, DMSO-d_6) δ 173.5, 170.6, 140.0, 139.9, 128.7, 127.6, 127.5, 127.1, 61.2, 42.4, 42.4, 39.0, 34.6.
HRMS (ESI) m/z: Calcd for $C_{19}H_{24}N_3O_2^+$ $[M+H]^+$: 326.1863; found: 326.1861.

2.12.5 Physical information of Boc-L-Phe-Aib-NMe-L-Cys-NHBn 3w

***tert*-Butyl ((*S*)-1-((1-(((*R*)-1-(benzylamino)-3-mercapto-1-oxopropan-2-yl)(methyl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate**



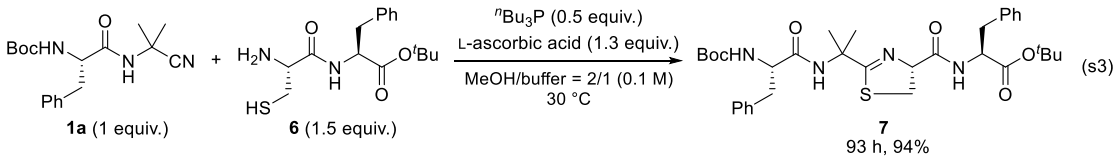
3w

3w was obtained in 71% yield as a white solid. m.p. 75-77 ºC. Eluent for purification: Hexane/EtOAc = 50%/50%.
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.09 (t, *J* = 5.9 Hz, 1H), 7.34 – 7.13 (m, 5H), 7.34 – 7.13 (m, 5H), 6.99 (d, *J* = 8.4 Hz, 1H), 4.97 – 4.59 (m, 1H), 4.34 (dd, *J* = 15.3, 6.4 Hz, 1H), 4.28 – 4.20 (m, 1H), 4.20 – 4.07 (m, 1H), 3.10 – 2.96 (m, 1H), 2.92 (s, 3H), 2.81 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.86 – 2.63 (m, 1H), 2.86 – 2.63 (m, 1H), 2.24 (s, 1H), 1.35 (s, 3H), 1.30 (s, 3H), 1.30 (s, 9H).
^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 172.4, 169.2, 155.8, 139.9, 138.3, 129.7, 128.6, 128.5, 127.5, 127.0, 126.7, 78.5, 62.6, 56.4, 56.0, 42.7, 37.4, 33.7, 28.5, 26.1, 25.9, 22.7.
FT-IR (neat): 3303. 2554, 1706, 1651, 1540, 1497, 1366, 1249, 1167, 1089, 754, 699 cm^{-1}
HRMS (ESI) m/z: Calcd for $C_{29}H_{40}N_4NaO_5S^+$ $[M+Na]^+$: 579.2612; found: 579.2613.
$[\alpha]_D^{25}$-32.79 (c 0.93 $CHCl_3$)

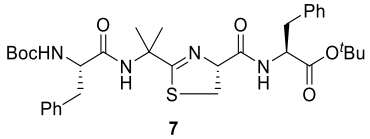
2.13 Experiment between a nitrile and NH_2-Cysteinyl dipeptide and corresponding synthesis and physical information

2.13.1 Experiment between Boc-L-Phe-Aib-CN 1a and NH_2-Cysteinyl dipeptide 6 and physical information

The corresponding NH_2 substrate L-Cys-L-Phe-O^{t}Bu **6** reacts with Boc-L-Phe-Aib-CN **1a** under the standard reaction condition and gave a stable thiazoline compound **7** and do not hydrolyze to peptide bond. **7** was obtained in 94% yield after 93 h (**Equation s3**).

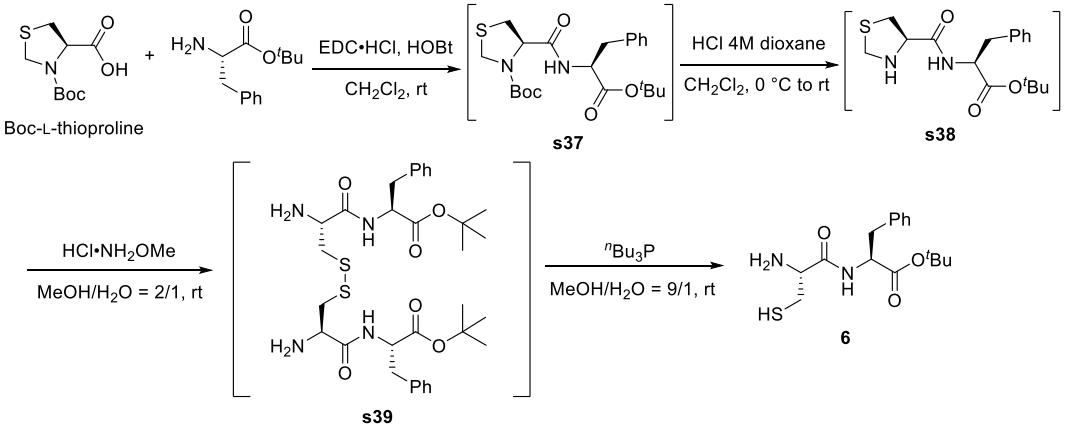


***tert*-Butyl ((*R*)-2-(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)propan-2-yl)-4,5-dihydrothiazole-4-carbonyl)-L-phenylalaninate**



7 was obtained as a white solid. m.p. 63-65 ºC. Eluent for purification: Hexane/EtOAc = 50%/50%.
^{1}H NMR (399 MHz, $CDCl_3$) δ 7.38 – 7.09 (m, 10H), 7.31 – 7.15 (m, 1H), 6.80 (s, 1H), 5.91 (s, 1H), 5.00 – 4.94 (m, 1H), 4.80 – 4.72 (m, 1H), 4.33 – 4.23 (m, 1H), 3.56 – 3.49 (m, 1H), 3.31 – 3.23 (m, 1H), 3.17 (dd, *J* = 13.6, 5.6 Hz, 1H), 3.12 – 3.03 (m, 1H), 3.12 – 3.03 (m, 1H), 2.96 (dd, *J* = 13.7, 7.3 Hz, 1H), 1.56 (s, 3H), 1.43 (s, 3H), 1.43 (s, 9H), 1.38 (s, 9H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 171.2, 171.1, 170.7, 156.3, 137.4, 136.6, 129.6, 129.4, 128.5, 128.2, 126.7, 126.6, 82.3, 79.8, 78.2, 56.5, 55.9, 53.7, 38.2, 37.1, 28.3, 28.0, 27.8, 25.8.
FT-IR (neat): 3302, 2979, 1665, 1517, 1455, 1366, 1250, 1162, 1049, 848, 755, 700 cm^{-1}
HRMS (ESI) m/z: Calcd for $C_{34}H_{46}N_4NaO_6S^+$ $[M+Na]^+$: 661.3030; found: 661.3026.
$[\alpha]_D^{25}$+5.89 (c 1.07 $CHCl_3$)

2.13.2 Synthesis of NH_2-Cysteinyl dipeptide 6 and physical information



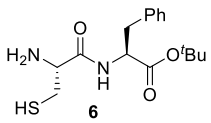
To a mixture of Boc- L-thioproline (5.50 g, 24.9 mmol) and L-Phe-O^{t}Bu (5.80 g, 24.9 mmol) in CH_2Cl_2 (100 mL, 0.25 M) were added EDC•HCl (5.72 g, 29.8 mmol) and HOBt•H_2O (382 mg, 2.49 mmol). The

reaction was stirred under room temperature overnight. Then the solvent was removed by evaporation under reduced pressure and the residue was diluted with EtOAc, followed by addition of 1N HCl aqueous solution. The organic phase was collected, washed with sat. $NaHCO_3$ aqueous solution, dried over Na_2SO_4, concentrated under reduced pressure and dried in vacuo. The crude compound **s37** (9.60 g) was used for the following step without further purification.

4M HCl 1,4-dioxane solution (110 mL) was cooled to 0 ºC and added to **s37** (9.60 g, 22.0 mmol) at 0 ºC.[7] The reaction mixture was stirred at room temperature until the reaction was deemed to complete by TLC analysis and quenched by sat. $NaHCO_3$ aqueous solution, extracted with EtOAc. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product **s38** (4.60 g, 13.7 mmol) was dissolved in MeOH/H_2O = 2/1 (68 mL). O-methylhydroxylammonium chloride (11.4 g, 137 mmol) was added to the reaction mixture. After stirring overnight under argon atmosphere at room temperature, the reaction mixture was evaporated under reduced pressure to remove the volatiles, quenched with sat. $NaHCO_3$ aqueous solution, extracted with EtOAc, dried over Na_2SO_4, concentrated under reduced pressure. The crude compound was roughly purified by silica gel column chromatography (Hexane/EtOAc = 12%/88%, then EtOAc/MeOH = 90%/10%) and gave **s39** (3.40 g).

s39 (3.40 g, 5.26 mmol) was dissolved in MeOH/H_2O = 9/1 (52.5 mL), followed by addition of ^{n}Bu$_3$P (2.60 mL, 10.5 mmol) under argon atmosphere. The reaction mixture was stirred at room temperature until the reaction was deemed to complete by TLC analysis. Then the reaction mixture was evaporated under reduced pressure to remove the volatiles. The residue was purified by silica gel column chromatography (Hexane/EtOAc = 50%/50%, then EtOAc 100%) and gave **6** (2.70 g, 8.32 mmol) in 33% yield over 4 steps as a white solid.

***tert*-Butyl L-cysteinyl-L-phenylalaninate**



6

6 was obtained in 33% yield over 4 steps as a white solid. m.p. 65-67 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.3 Hz, 1H), 7.33 – 7.09 (m, 5H), 4.80 – 4.70 (m, 1H), 3.56 – 3.48 (m, 1H), 3.13 (dd, *J* = 13.9, 6.0 Hz, 1H), 3.02 (dd, *J* = 13.9, 7.0 Hz, 1H), 2.96 (dd, *J* = 13.8, 5.8 Hz, 1H), 2.68 (dd, *J* = 13.8, 4.1 Hz, 1H), 1.42 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 170.6, 136.2, 129.5, 128.4, 126.9, 82.3, 55.9, 53.2, 38.3, 30.3, 28.0.

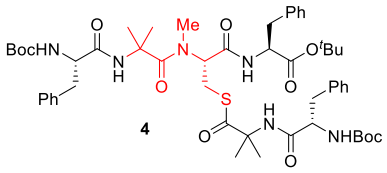
HRMS (ESI) m/z: Calcd for $C_{16}H_{25}N_2O_3S^+$ $[M+H]^+$: 325.1580; found: 325.1579.

FT-IR (neat): 3358, 2979, 2932, 2561, 1732, 1664, 1509, 1368, 1254, 1155, 845, 700 cm^{-1}

$[\alpha]_D^{24}$+22.69 (c 1.4 $CHCl_3$)

3. Physical information of the overreaction product 4

***tert*-Butyl N,S-bis(2-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanamido)-2-methylpropanoyl)-*N*-methyl-L-cysteinyl-L-phenylalaninate**



4

4 was obtained as an amorphous solid

^{1}H NMR (399 MHz, DMSO-d_6) δ 8.53 (s, 1H), 8.46 (s, 1H), 7.78 (d, *J* = 6.2 Hz, 1H), 7.43 – 7.06 (m, 5H), 7.43 – 7.06 (m, 5H), 7.43 – 7.06 (m, 5H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.82 (d, *J* = 8.9 Hz, 1H), 4.36 – 4.08 (m, 1H), 4.36 – 4.08 (m, 1H), 4.36 – 4.08 (m, 1H), 4.36 – 4.08 (m, 1H), 3.32 – 3.27 (m, 1H), 3.16 – 3.06 (m, 1H), 3.16 – 3.06 (m, 1H), 3.01 – 2.96 (m, 1H), 2.96 – 2.92 (m, 1H), 2.91 – 2.77 (m, 3H), 2.91 – 2.77 (m, 1H), 2.77 – 2.72 (m, 1H), 2.71 – 2.65 (m, 1H), 1.34 (s, 3H), 1.31 (s, 3H), 1.30 (s, 9H), 1.29 (s, 3H), 1.26 (s, 9H), 1.24 (s, 9H), 1.22 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 203.3, 172.3 (2C), 171.9, 170.7, 169.0, 155.8, 155.6, 138.4, 138.1, 138.1, 129.7, 128.5, 128.4, 128.4, 126.8, 126.7, 126.6, 80.8, 78.7, 78.4, 62.0, 57.3, 56.4, 56.1, 55.9, 55.2, 37.7, 37.6 (2C), 37.0, 28.5 (6C), 28.3, 27.9 (3C), 27.0, 26.0, 25.5, 25.2.

HRMS (ESI) m/z: Calcd for $C_{53}H_{74}N_6NaO_{11}S^+$ $[M+Na]^+$:1025.5028; found: 1025.5037.

FT-IR (neat): 3298, 2979, 1666, 1498, 1366, 1249, 1166, 1095, 1024, 848, 755, 700, 666 cm^{-1}

$[\alpha]_D^{25}$-60.17 (c 0.7 $CHCl_3$)

4. Reference

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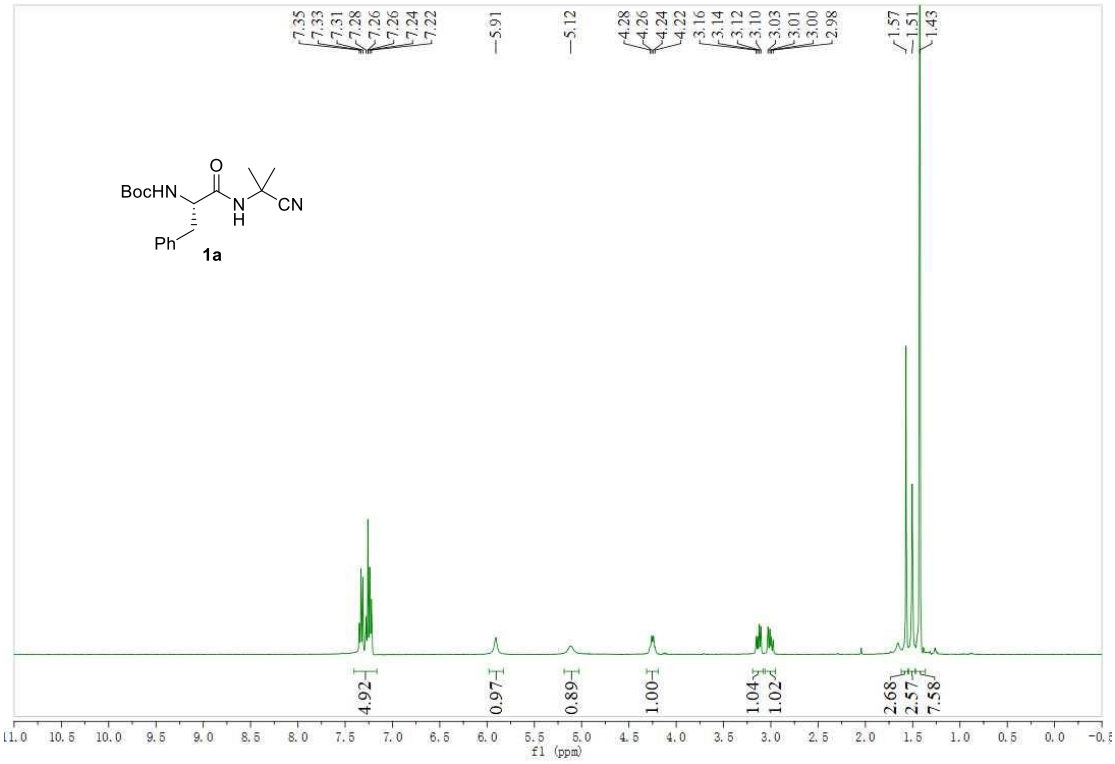
5. Spectra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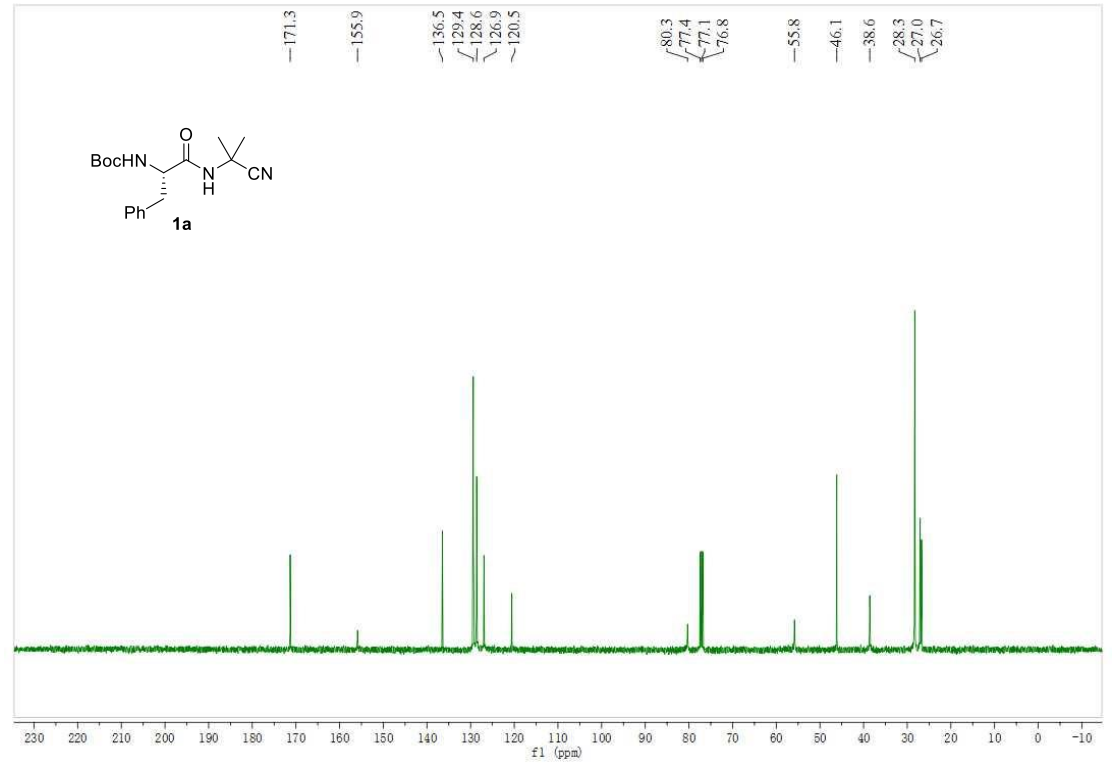
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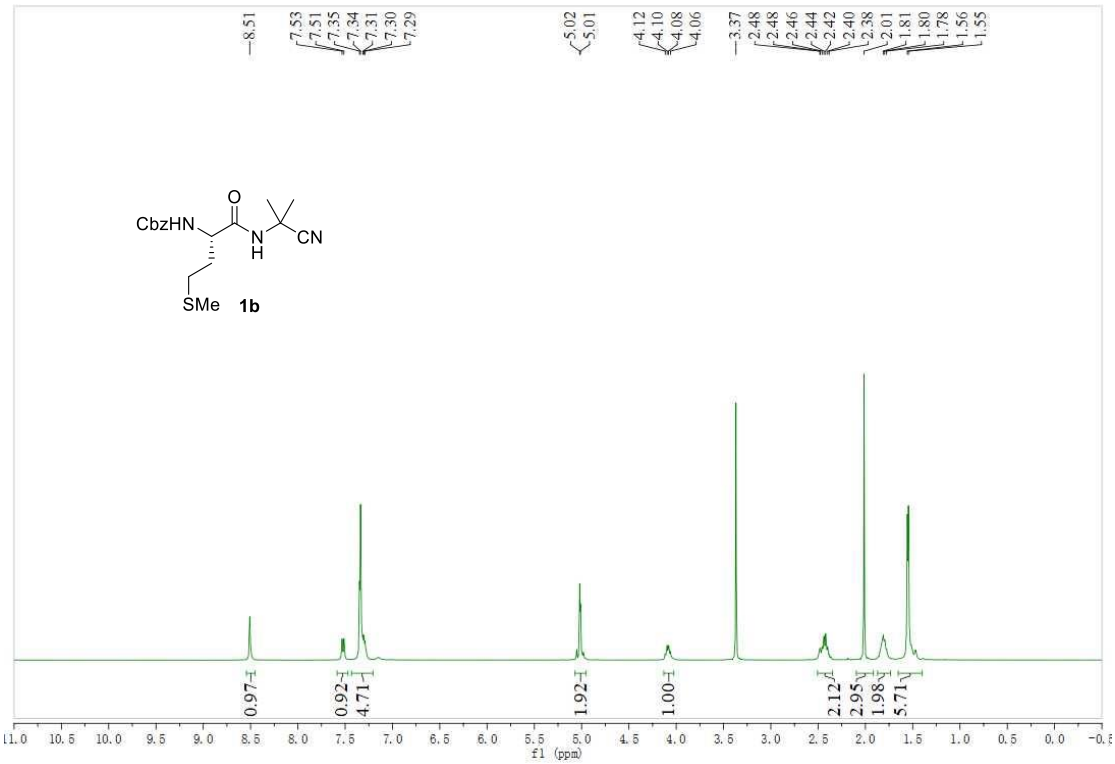
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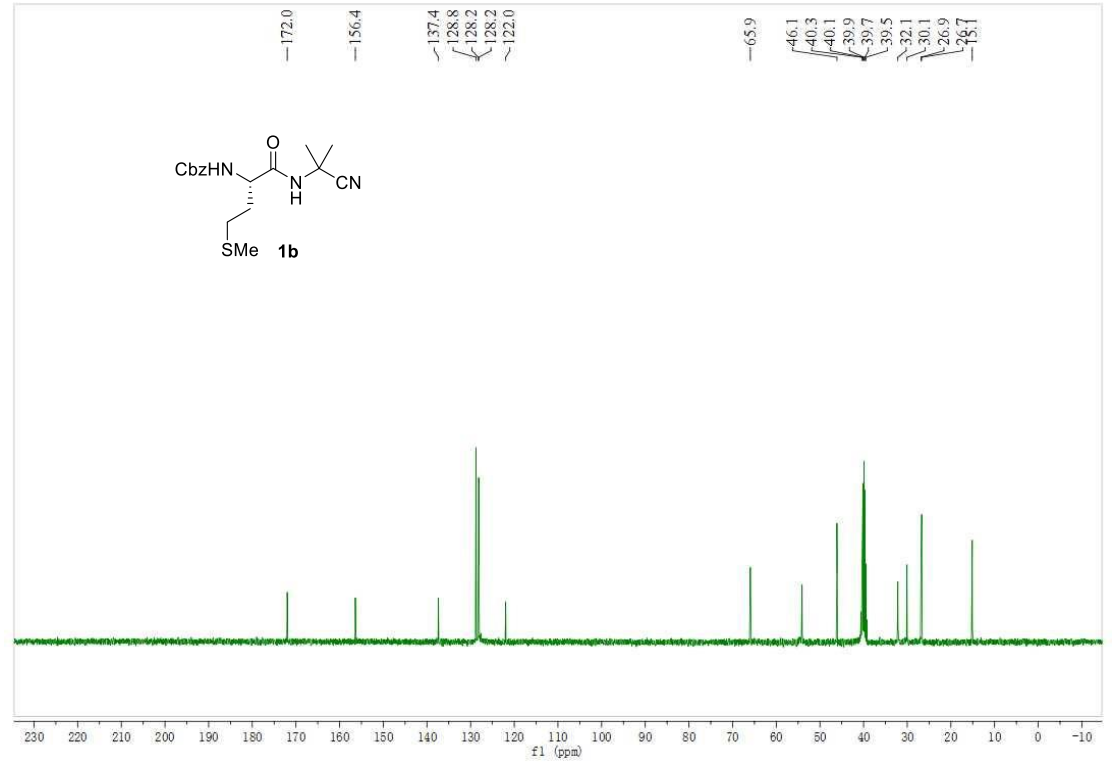
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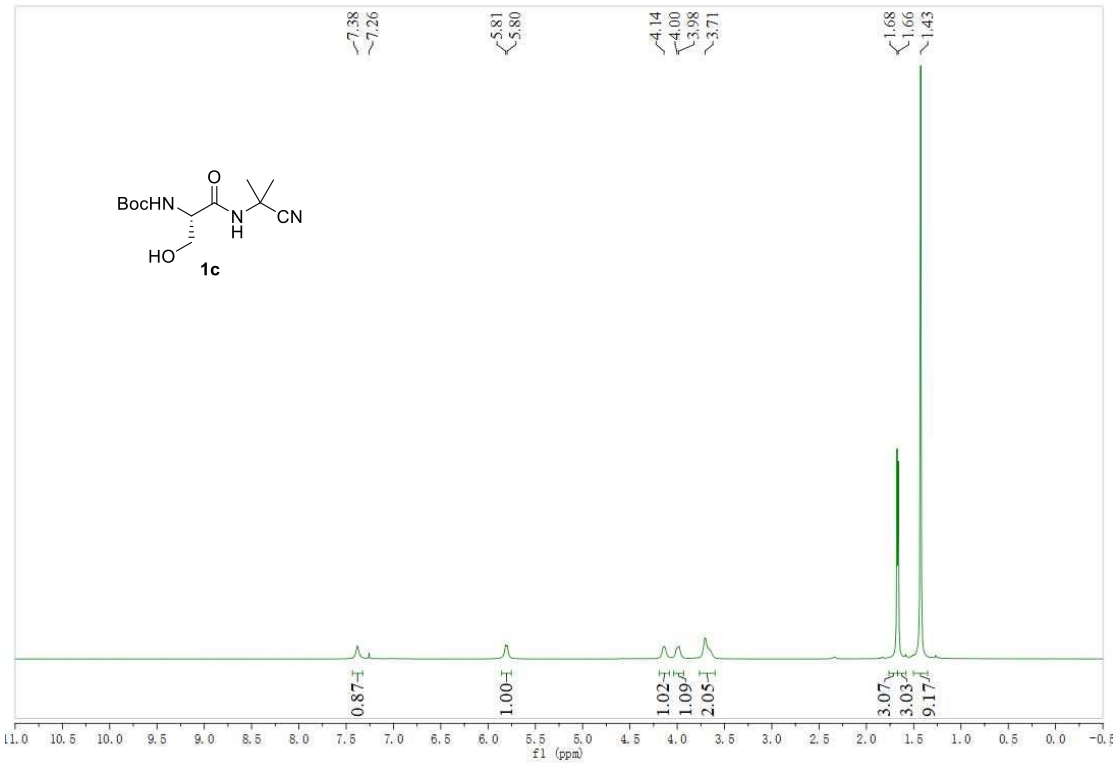
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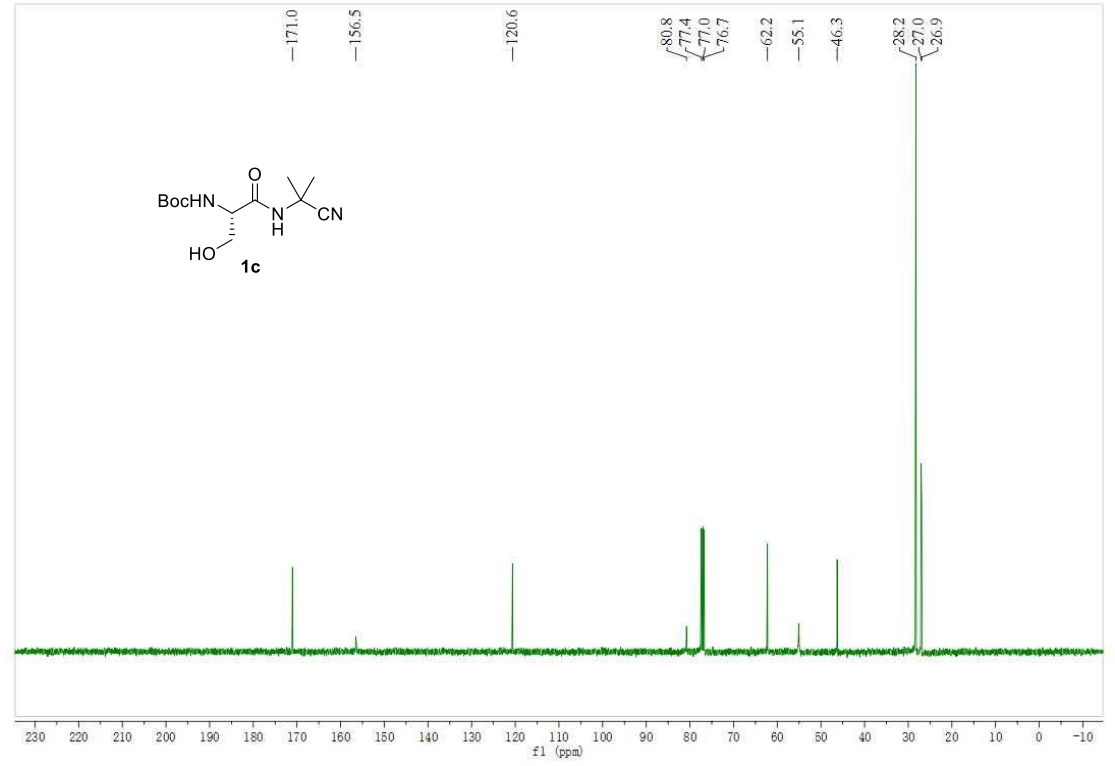
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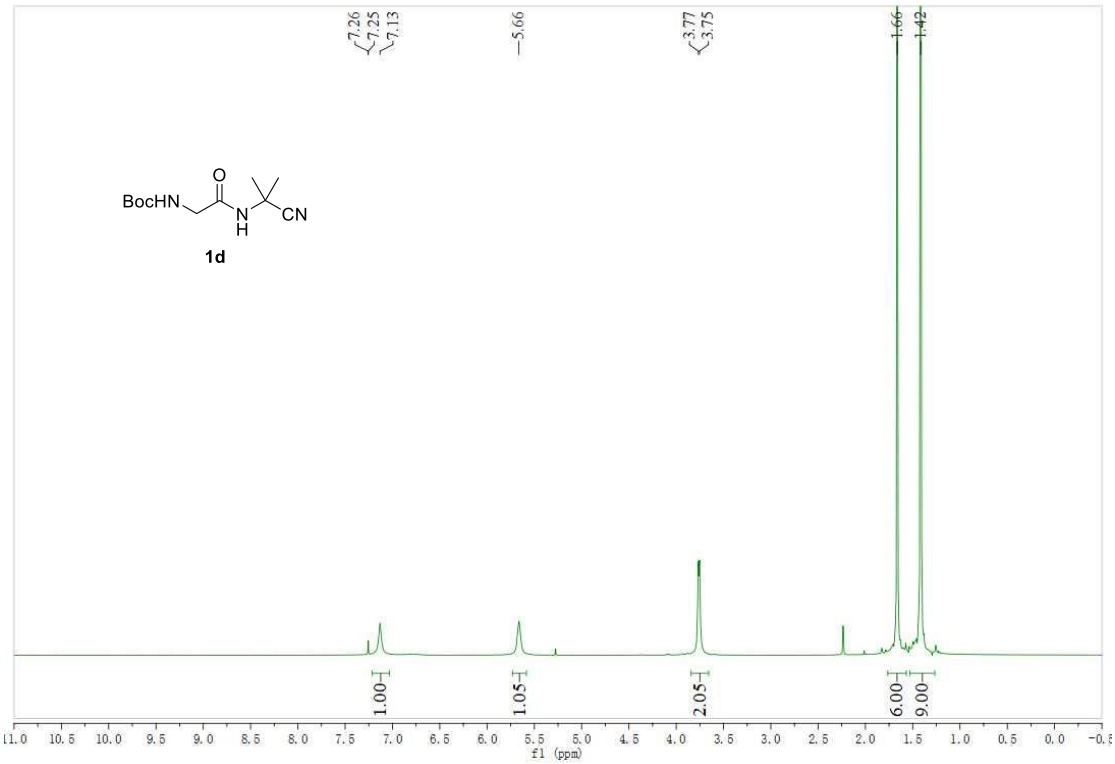
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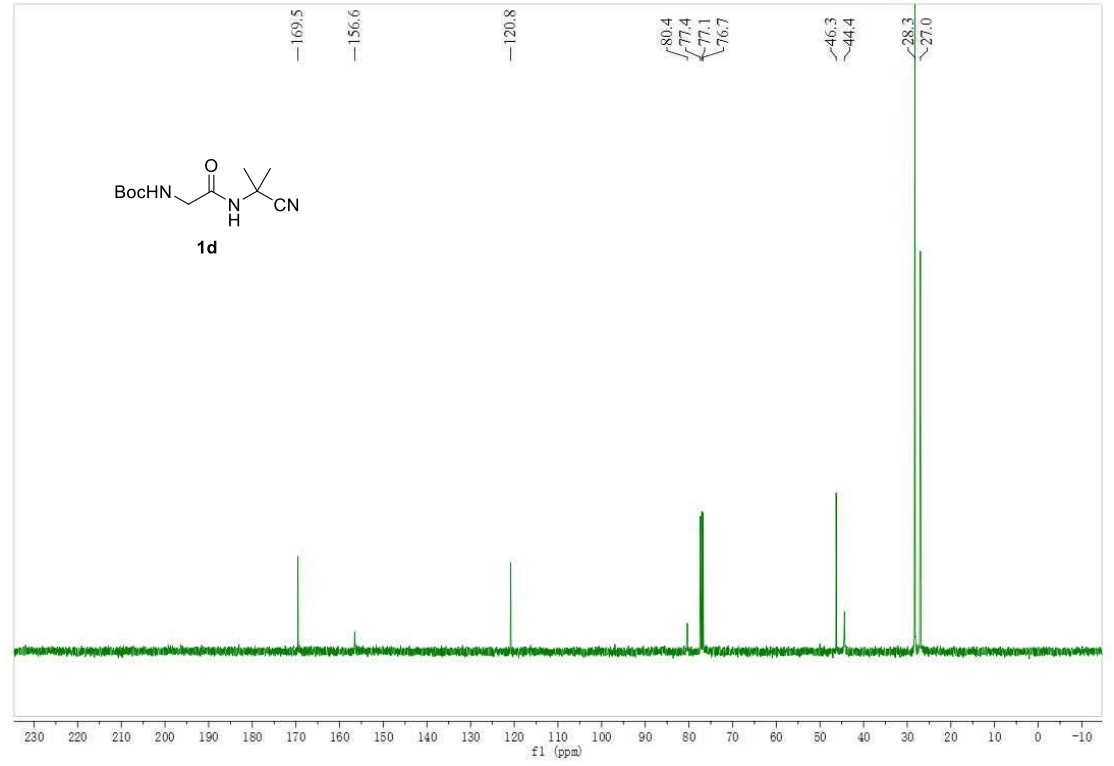
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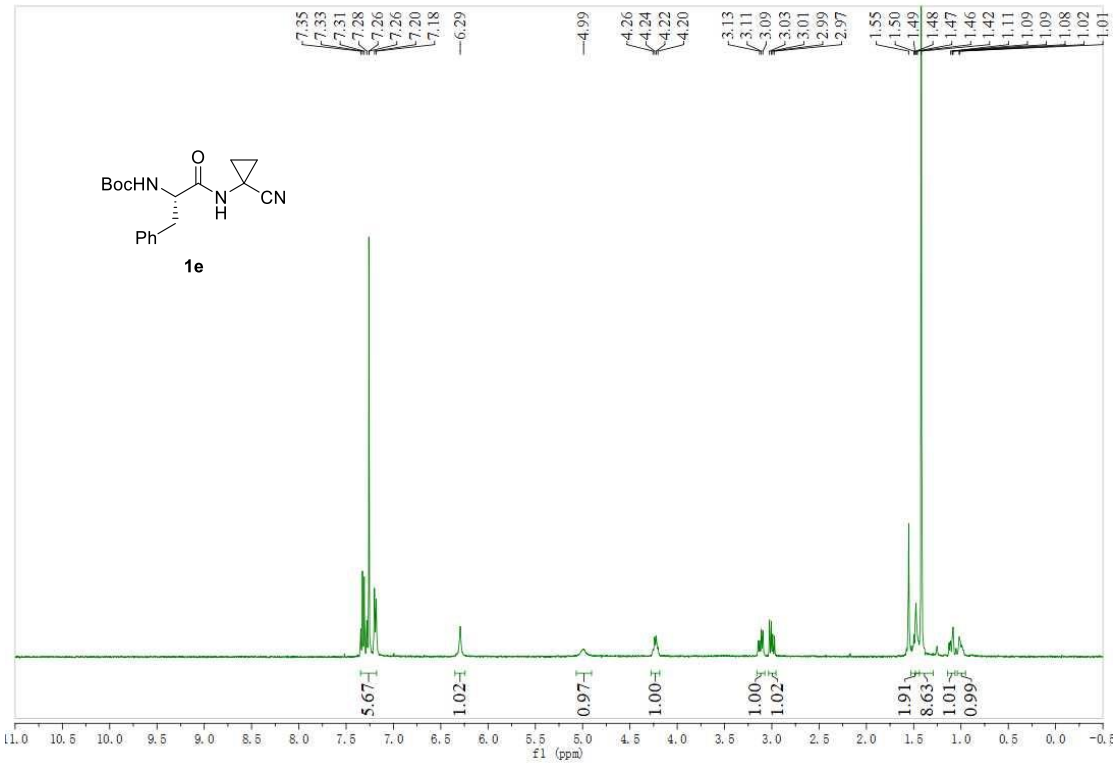
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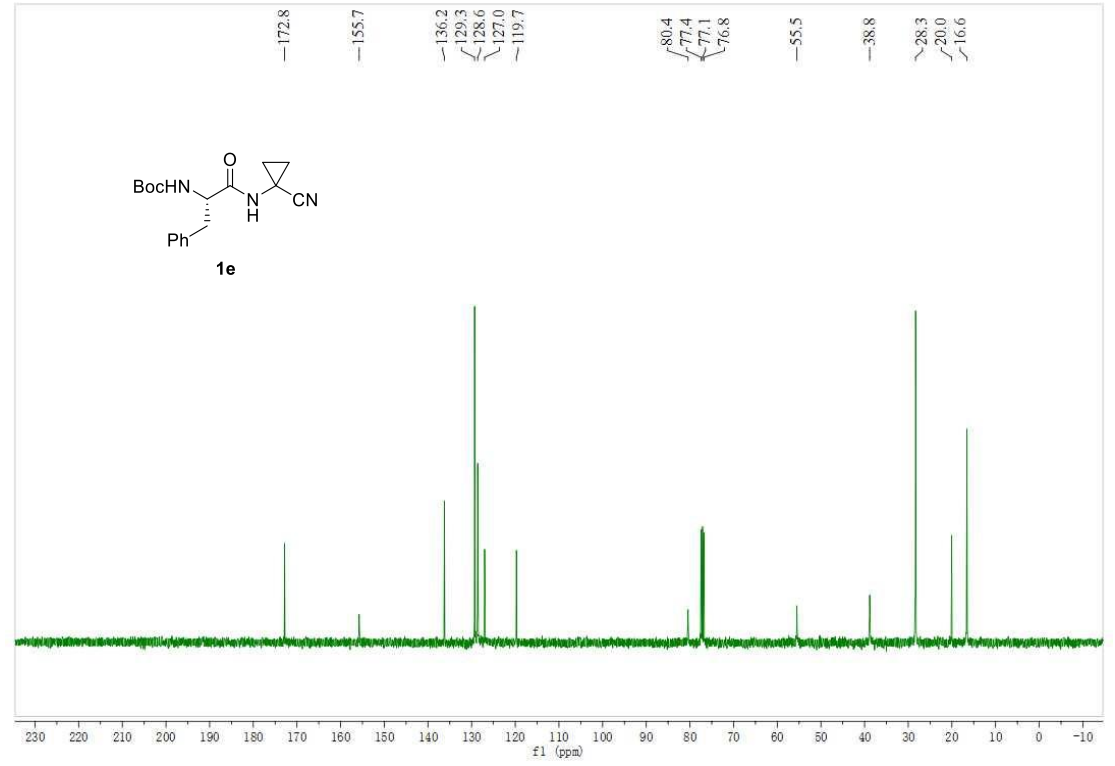
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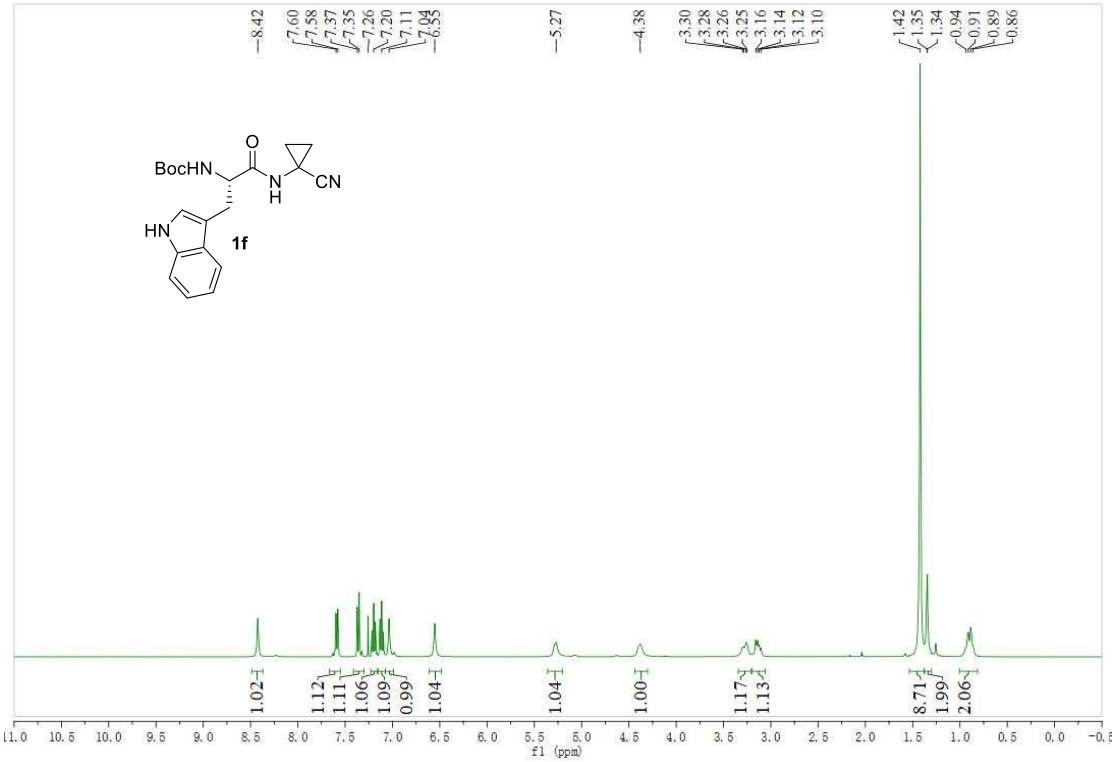
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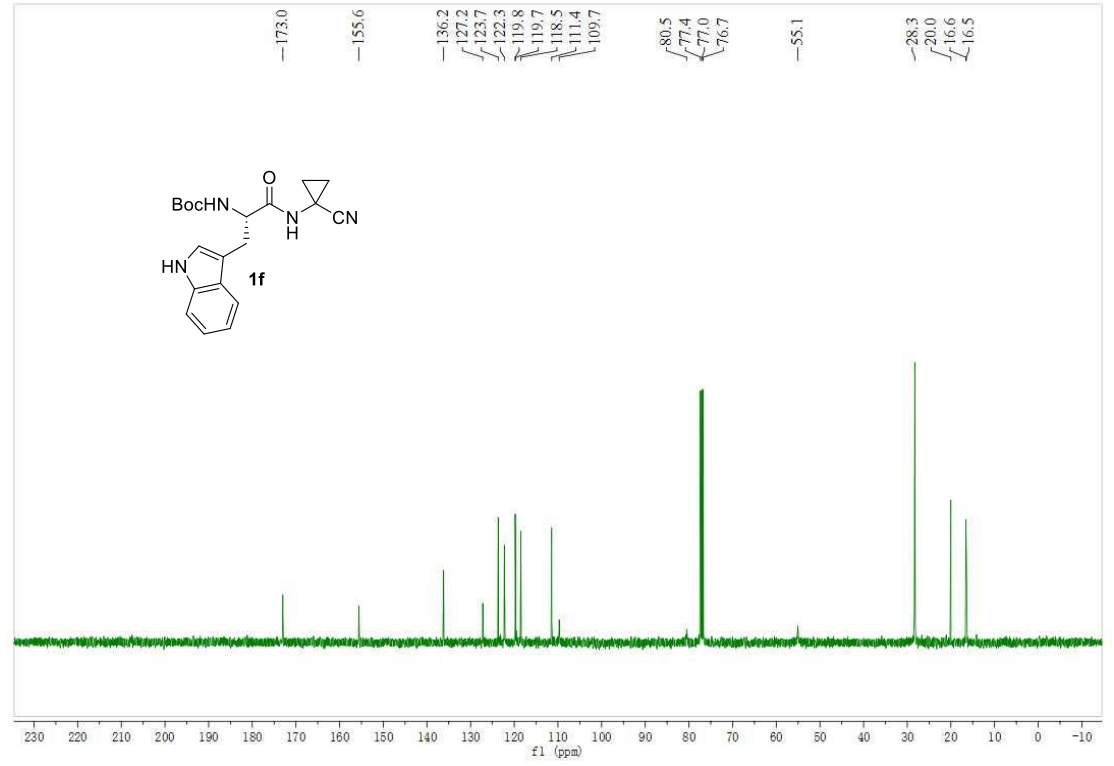
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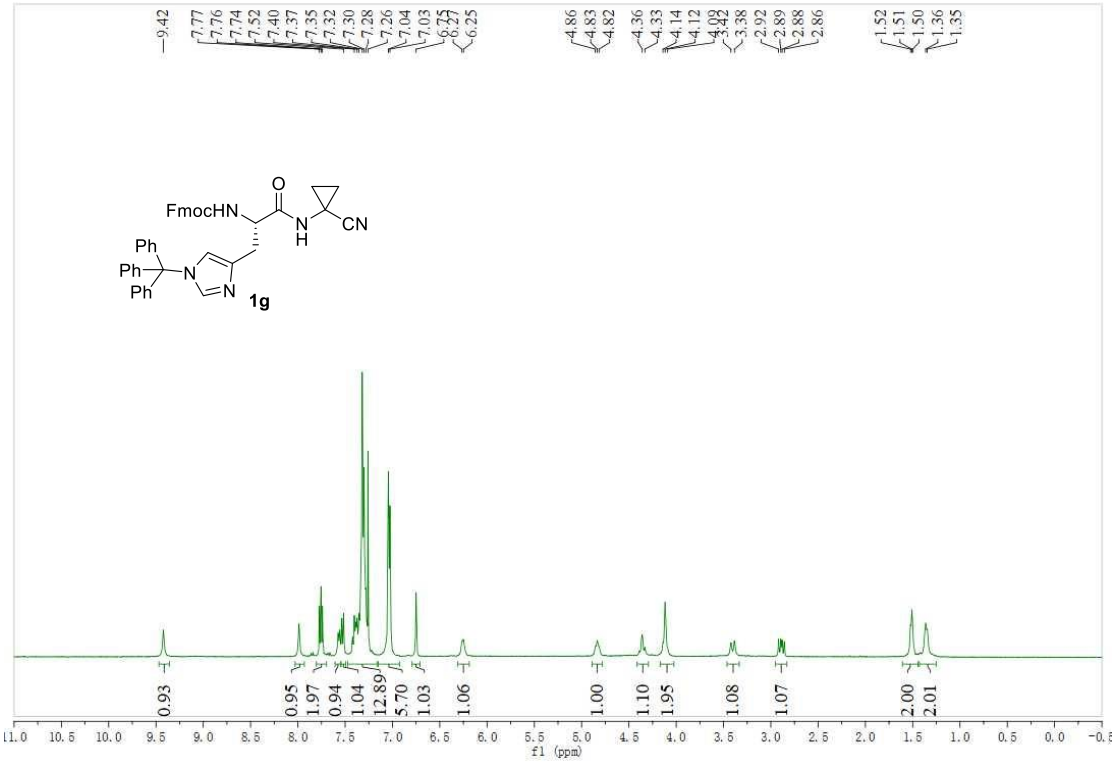
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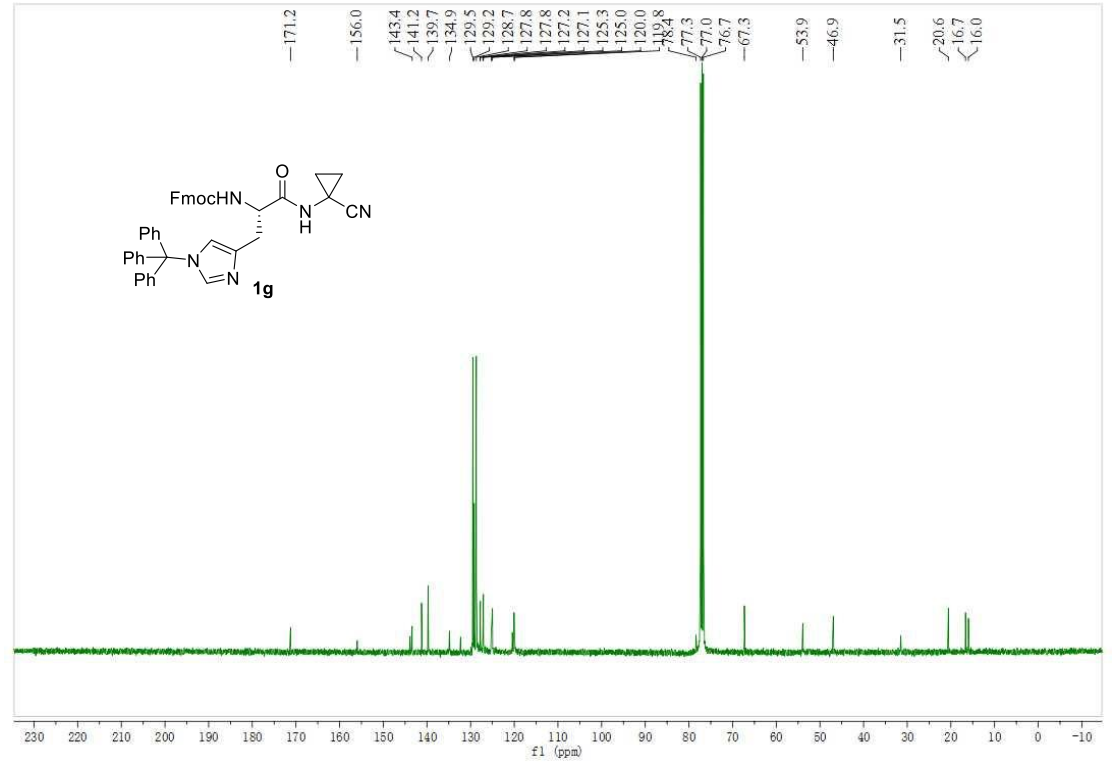
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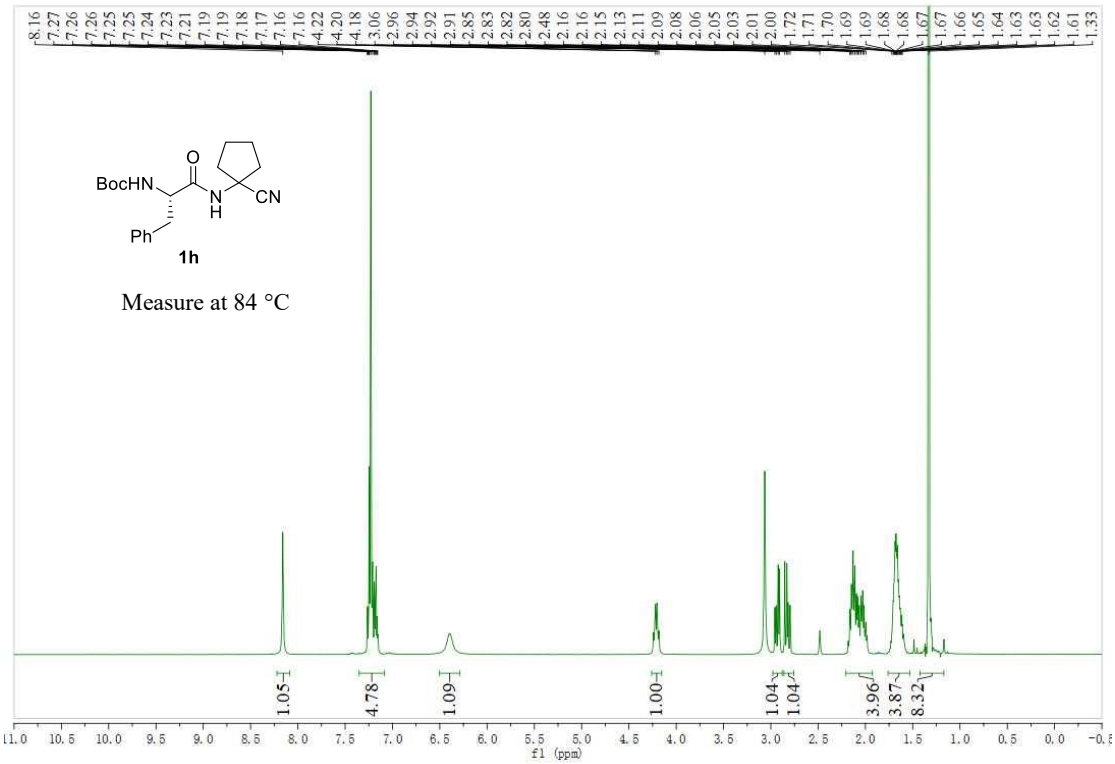
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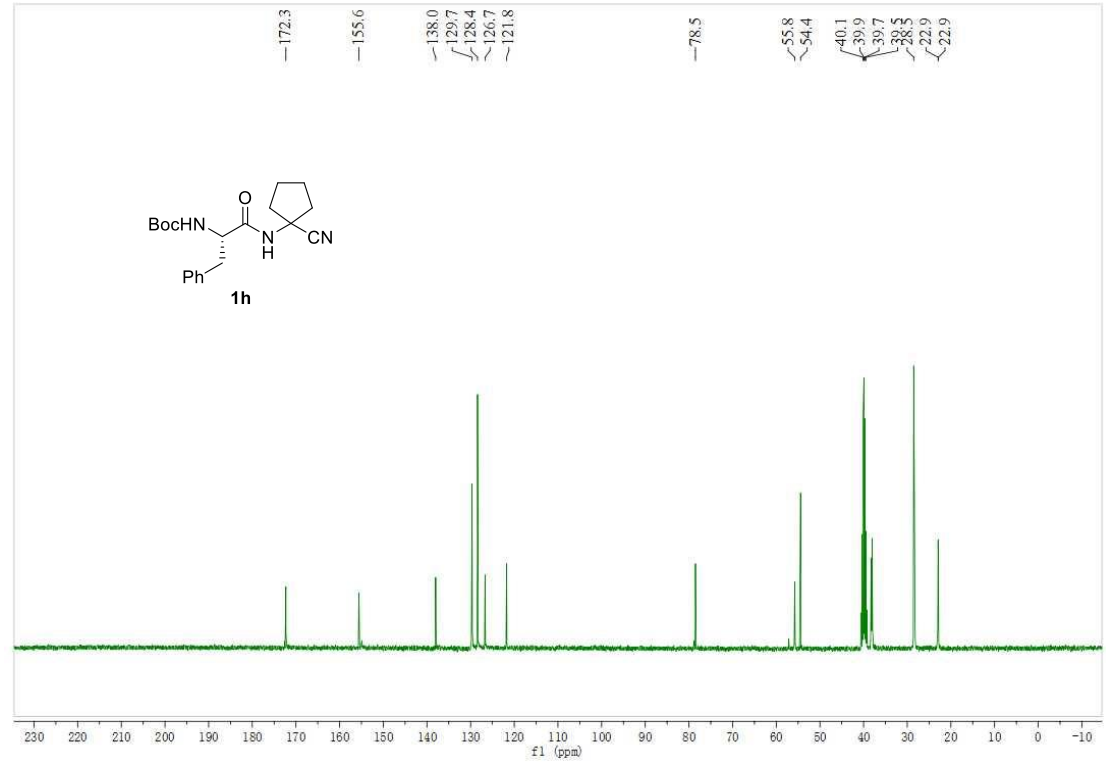
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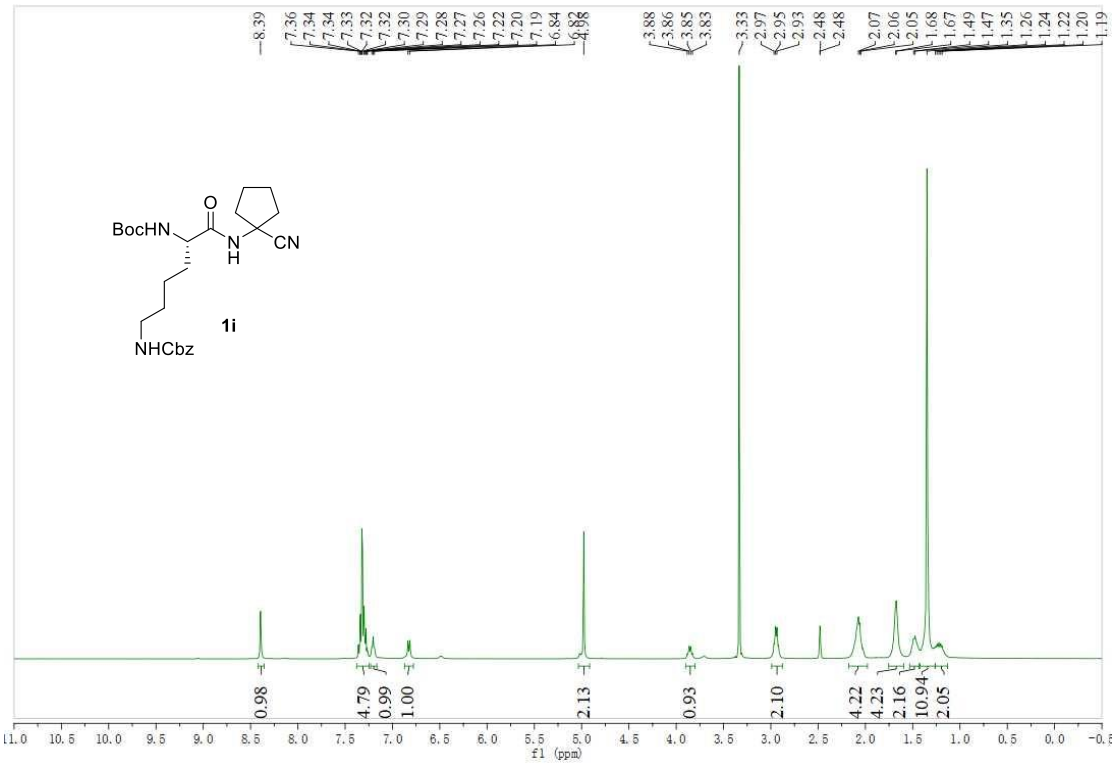
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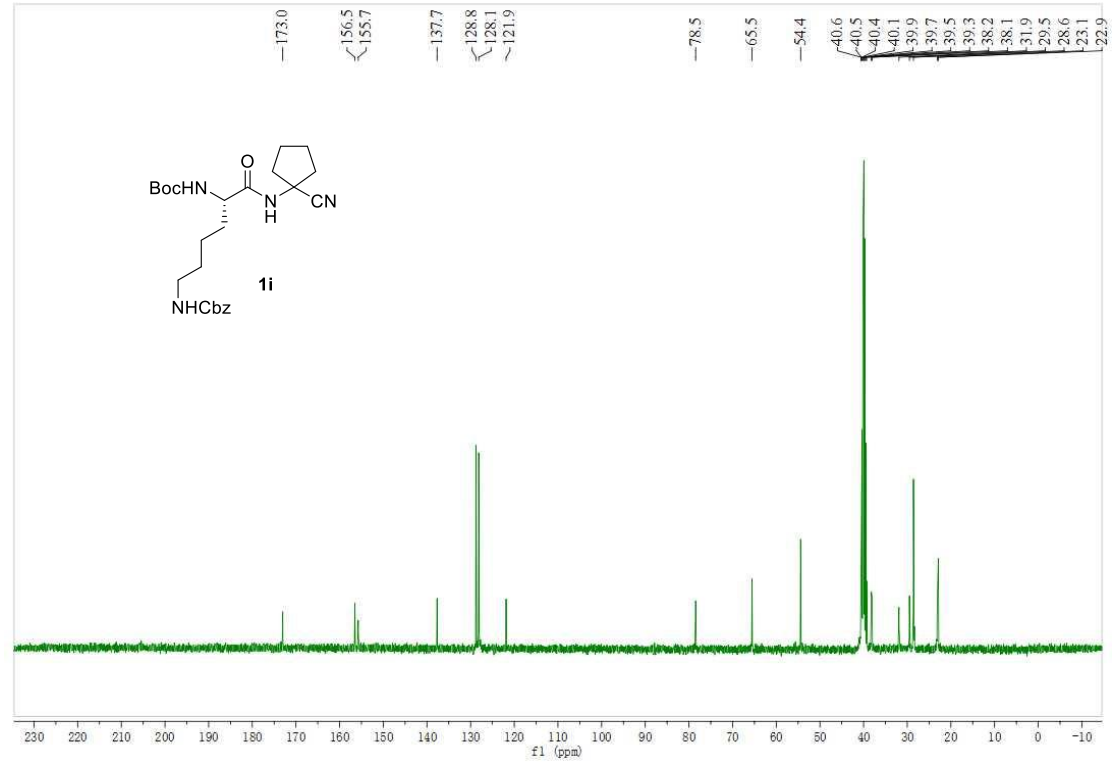
f1 (ppm)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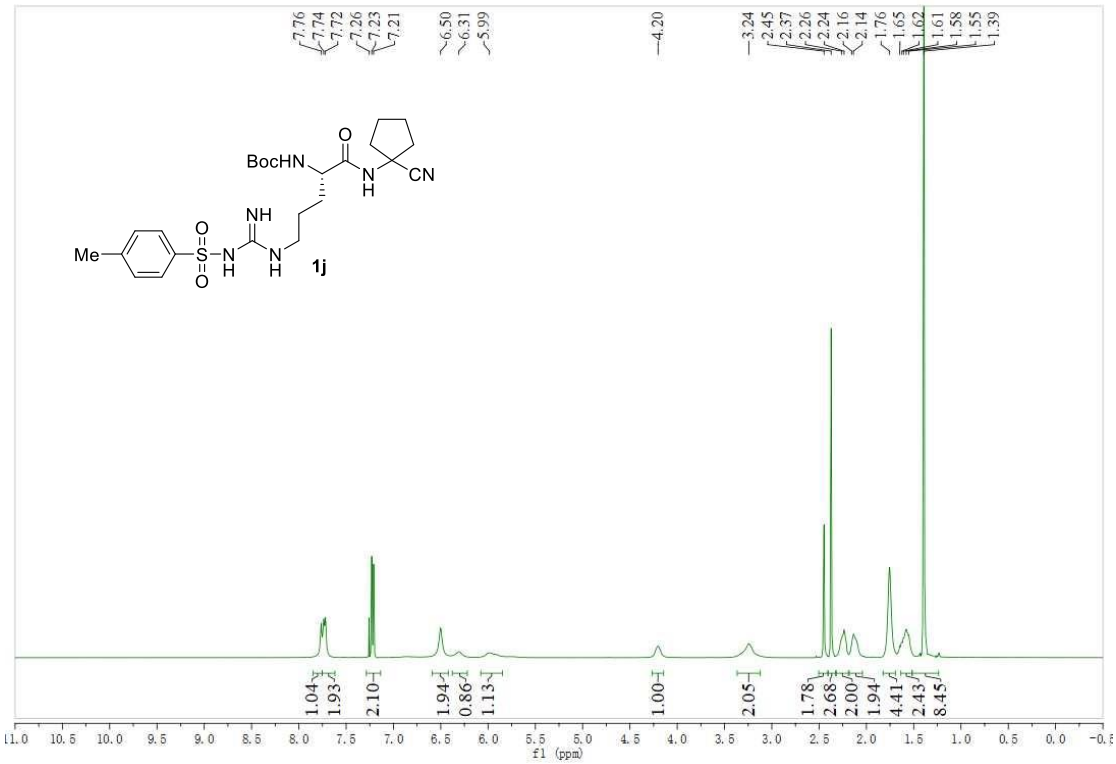
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2.16
2.14
1.76
1.65
1.62
1.61
1.58
1.55
1.39
BocHN
O
N
H
CN
NH
Me
S
O
N
H
N
H
1j
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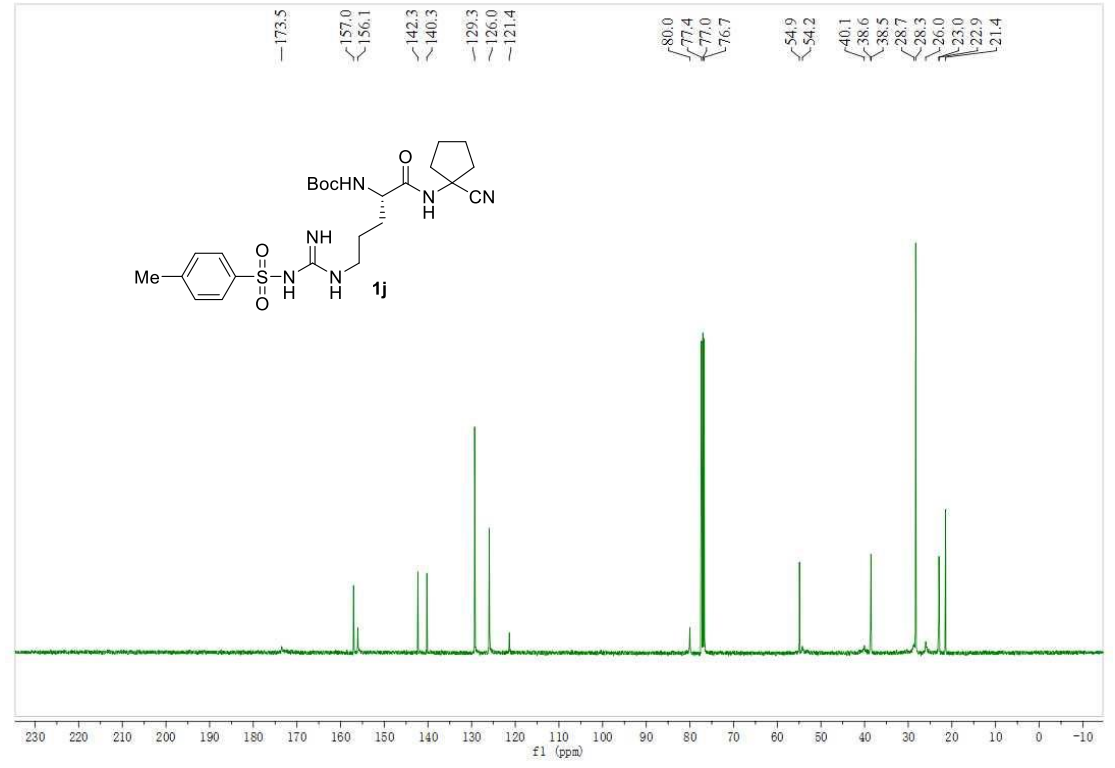
f1 (ppm)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38.6
38.5
28.7
28.3
26.0
23.0
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CN
NH
Me
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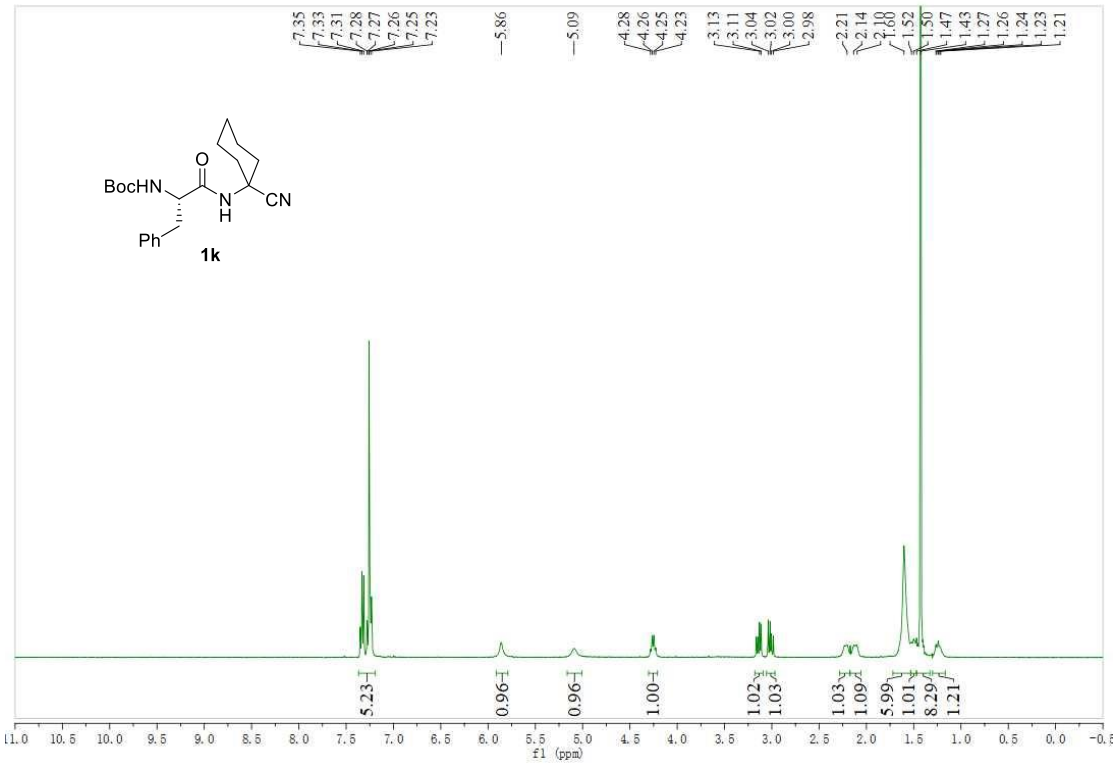
O
N
H
N
H
1j
f1 (ppm)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2.14
2.10
1.60
1.52
1.50
1.47
1.43
1.27
1.26
1.24
1.23
1.21
BocHN
O
N
H
CN
Ph
1k
5.23
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1.02
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1.01
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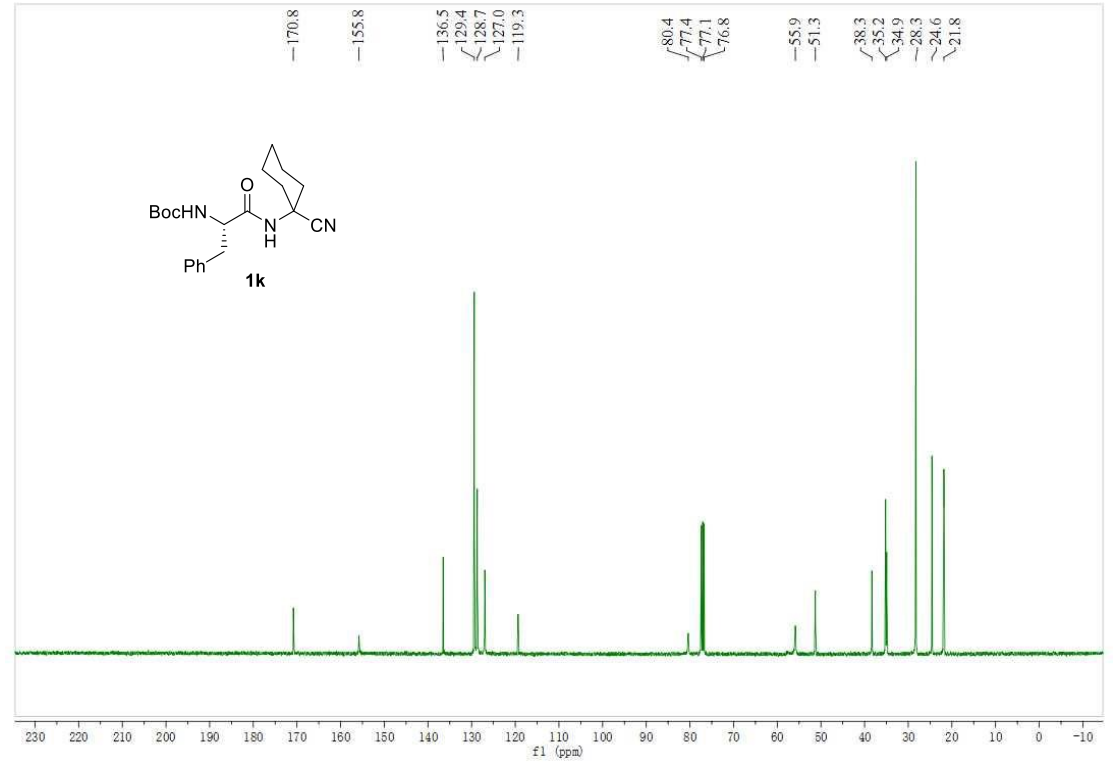
1.21
f1 (ppm)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55.9
51.3
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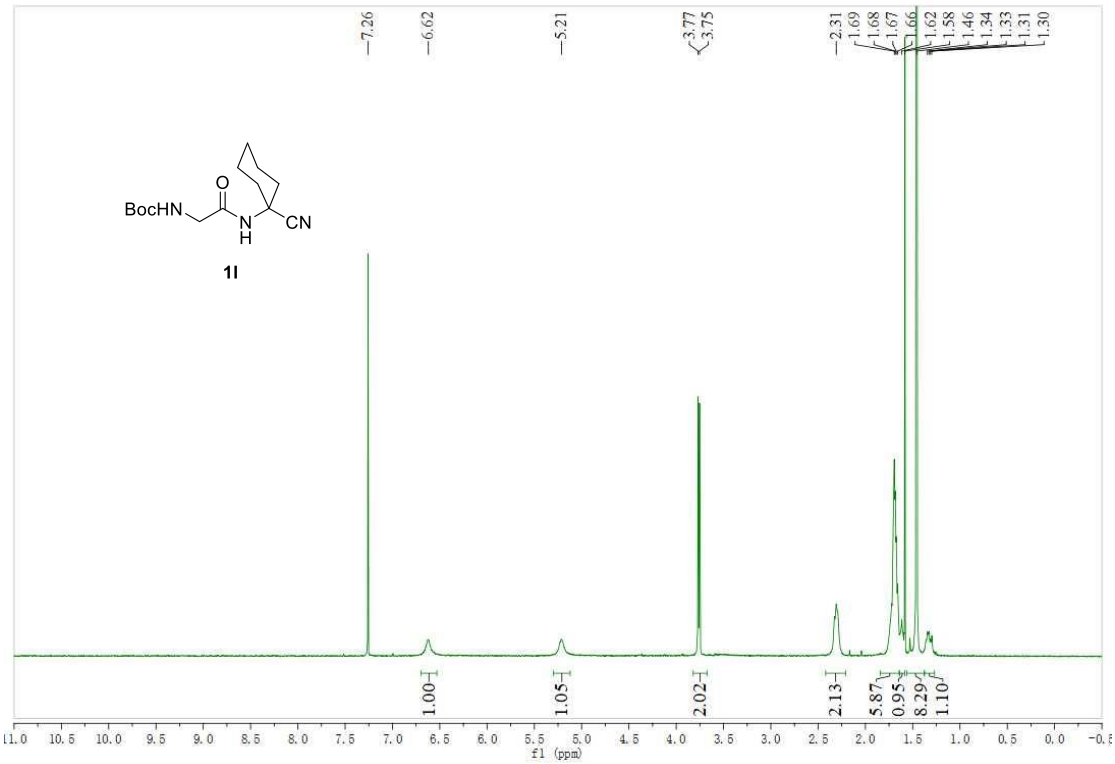
O
N
H
CN
Ph
1k
f1 (ppm)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1.33
1.31
1.30
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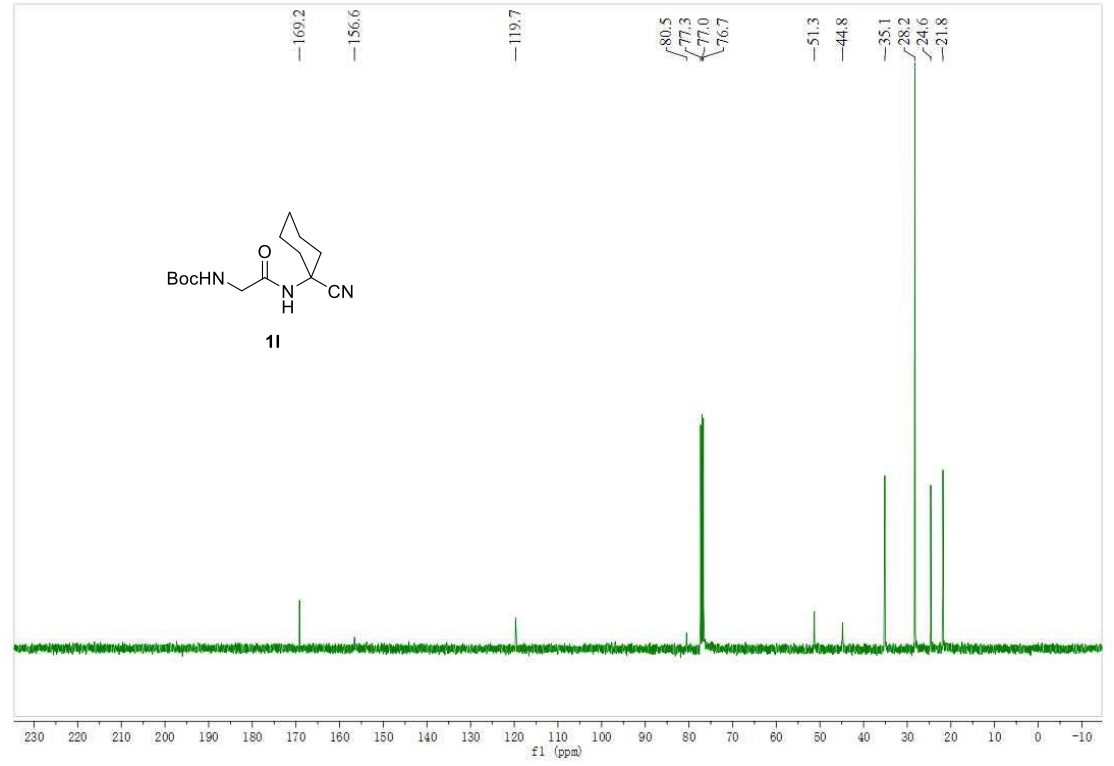
f1 (ppm)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77.0
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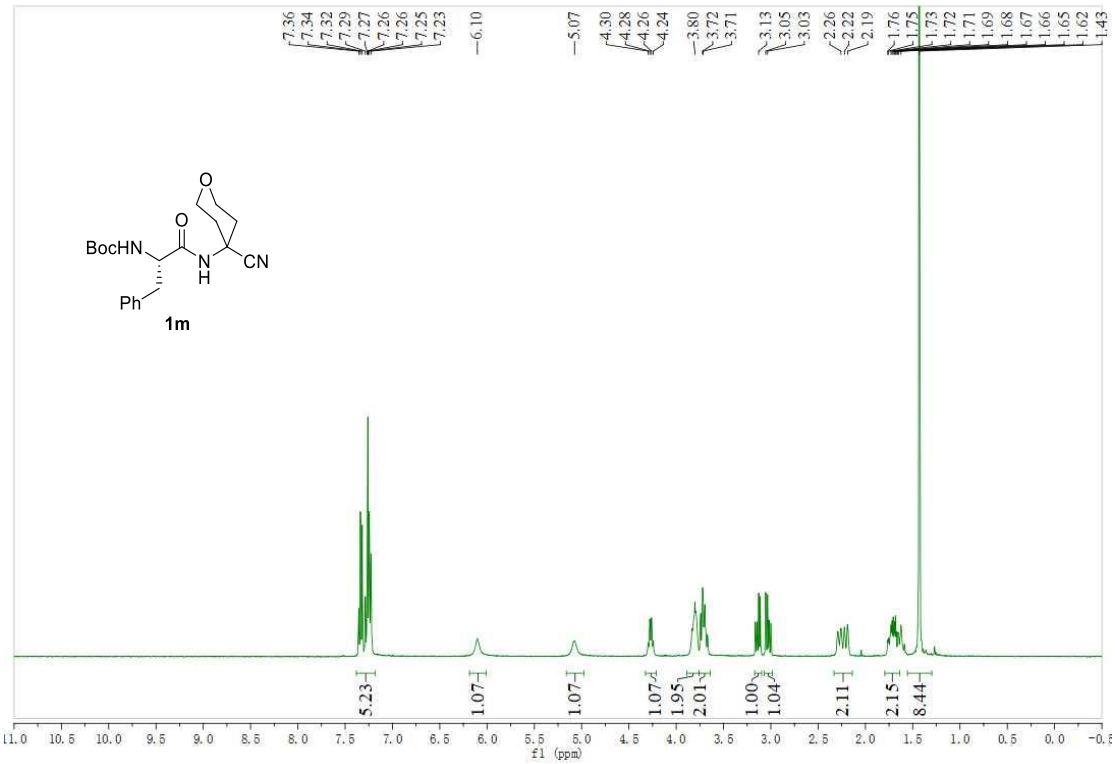
28.2
24.6
21.8
BocHN
O
N
H
CN
1l
f1 (ppm)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4.28
4.26
4.24
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3.71
3.13
3.05
3.03
2.26
2.22
2.19
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1.75
1.73
1.72
1.71
1.69
1.68
1.67
1.66
1.65
1.62
1.43
BocHN
O
N
H
CN
Ph
1m
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1.07
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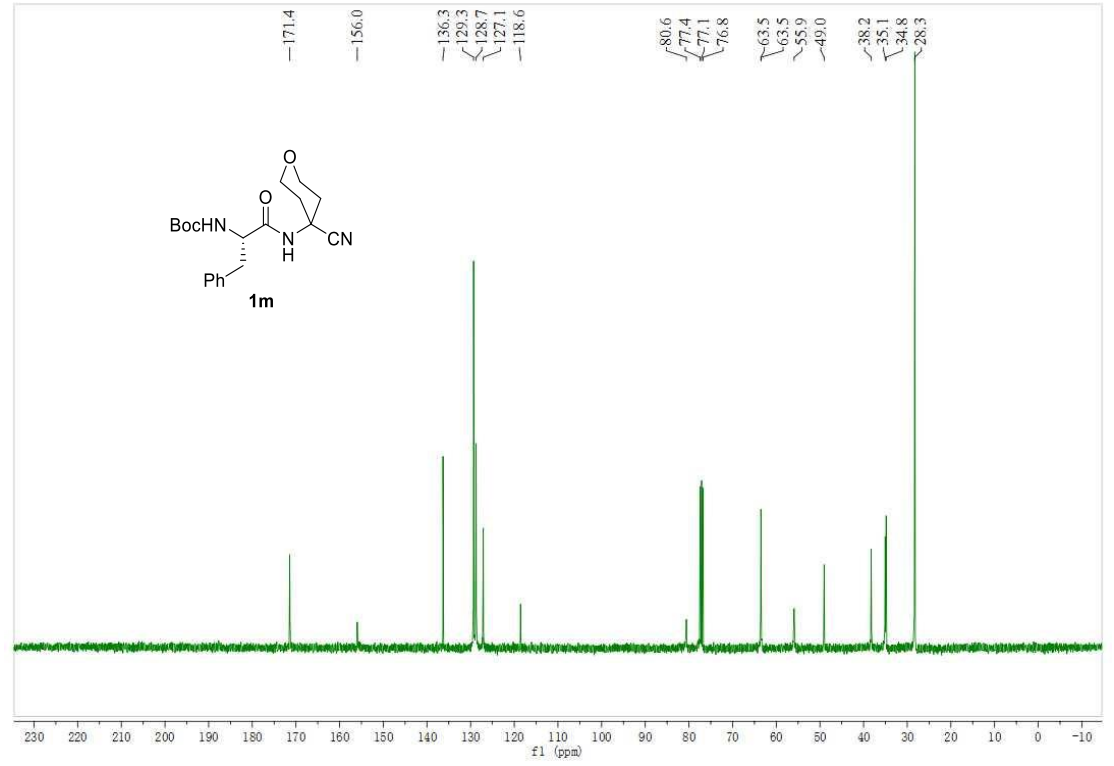
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77.4
77.1
76.8
63.5
63.5
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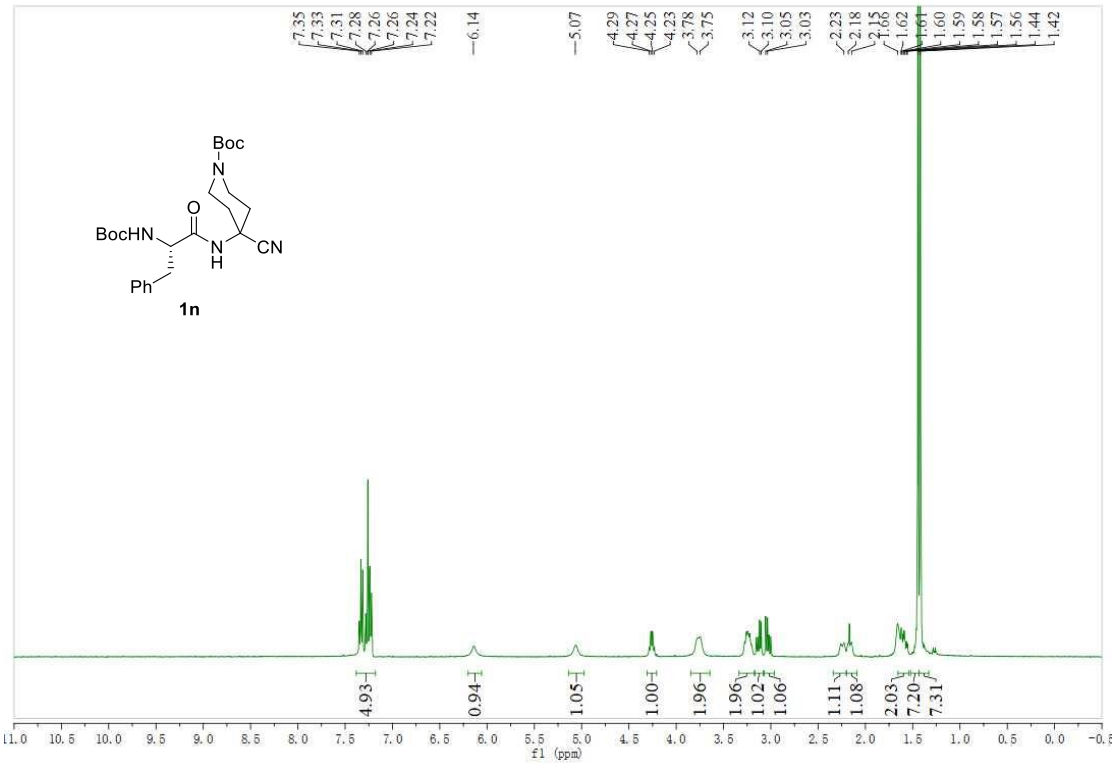
35.1
34.8
28.3
BocHN
O
N
H
CN
Ph
1m
f1 (ppm)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N
O
BocHN
N
H
CN
Ph
1n
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7.33
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7.28
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7.26
7.24
7.22
6.14
5.07
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4.27
4.25
4.23
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2.15
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1.62
1.61
1.60
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1.56
1.44
1.42
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1.11
1.08
2.03
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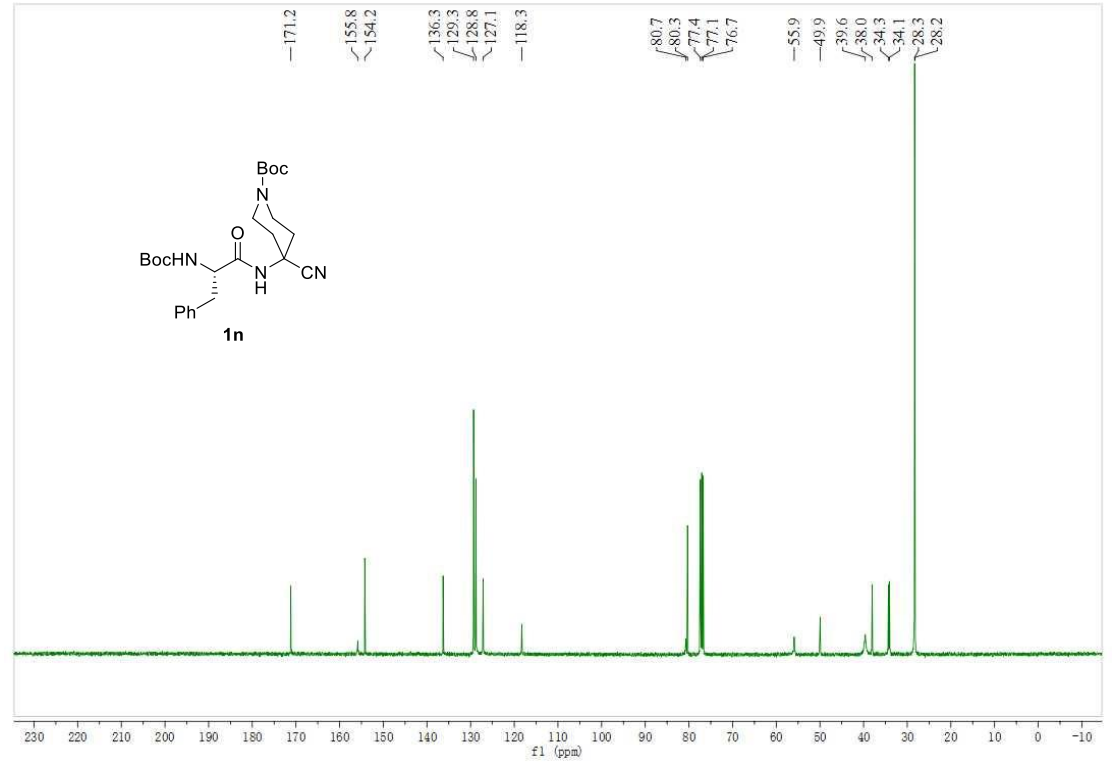
7.31
f1 (ppm)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N
H
CN
Ph
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136.3
129.3
128.8
127.1
118.3
80.7
80.3
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39.6
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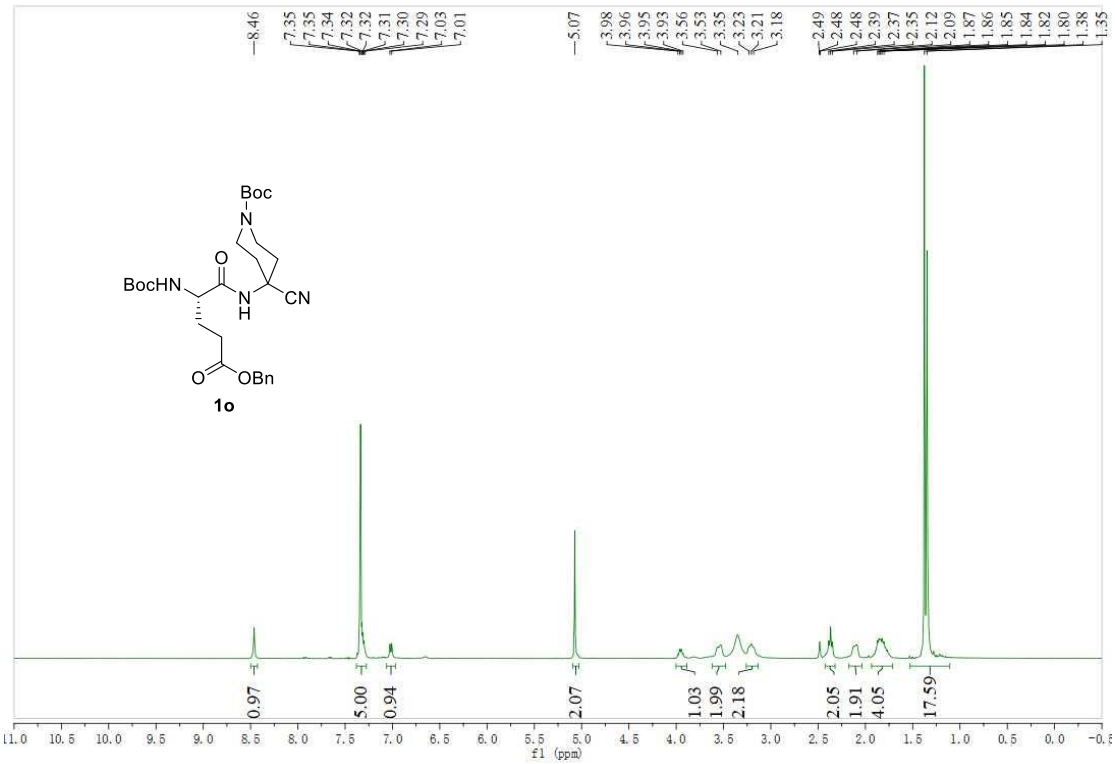
28.3
28.2
f1 (ppm)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N
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H
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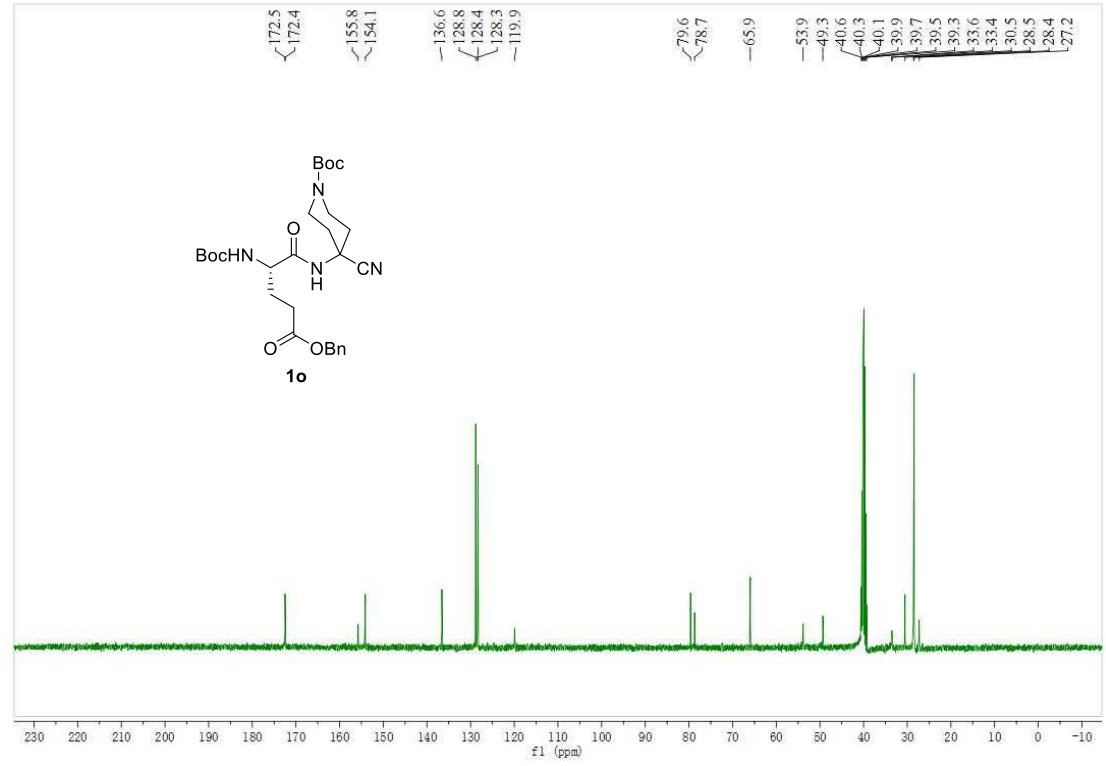
O
OBn
1o
f1 (ppm)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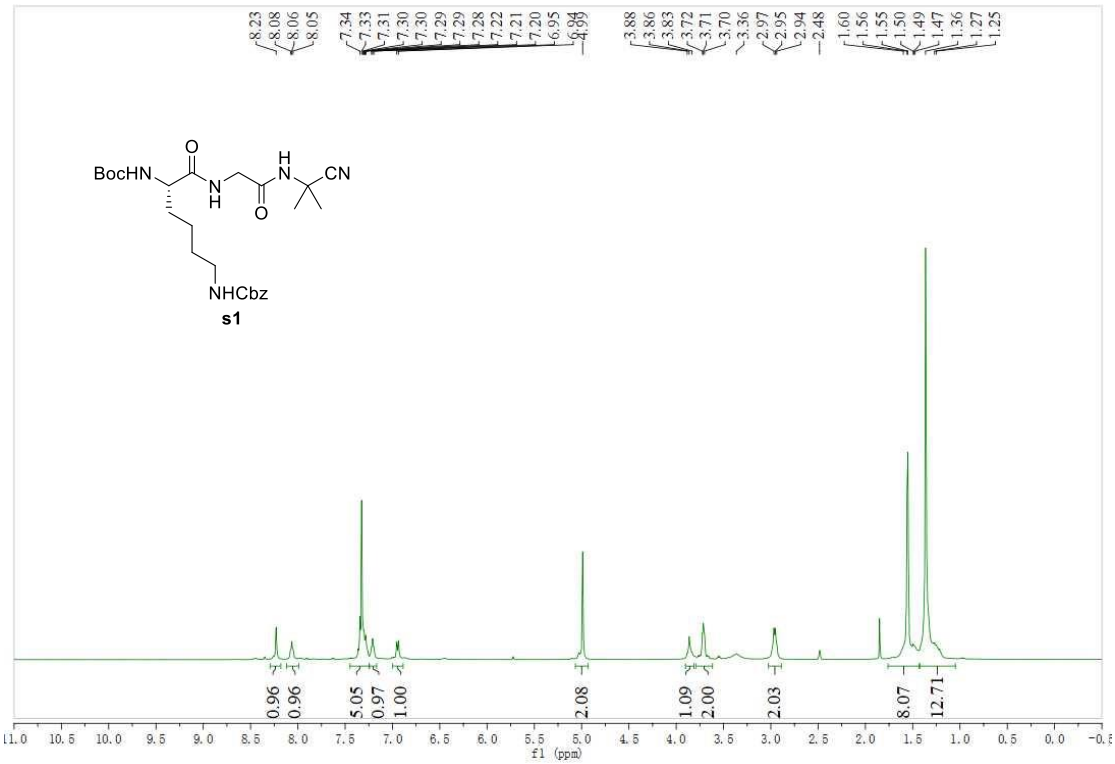
OBn
1o
f1 (ppm)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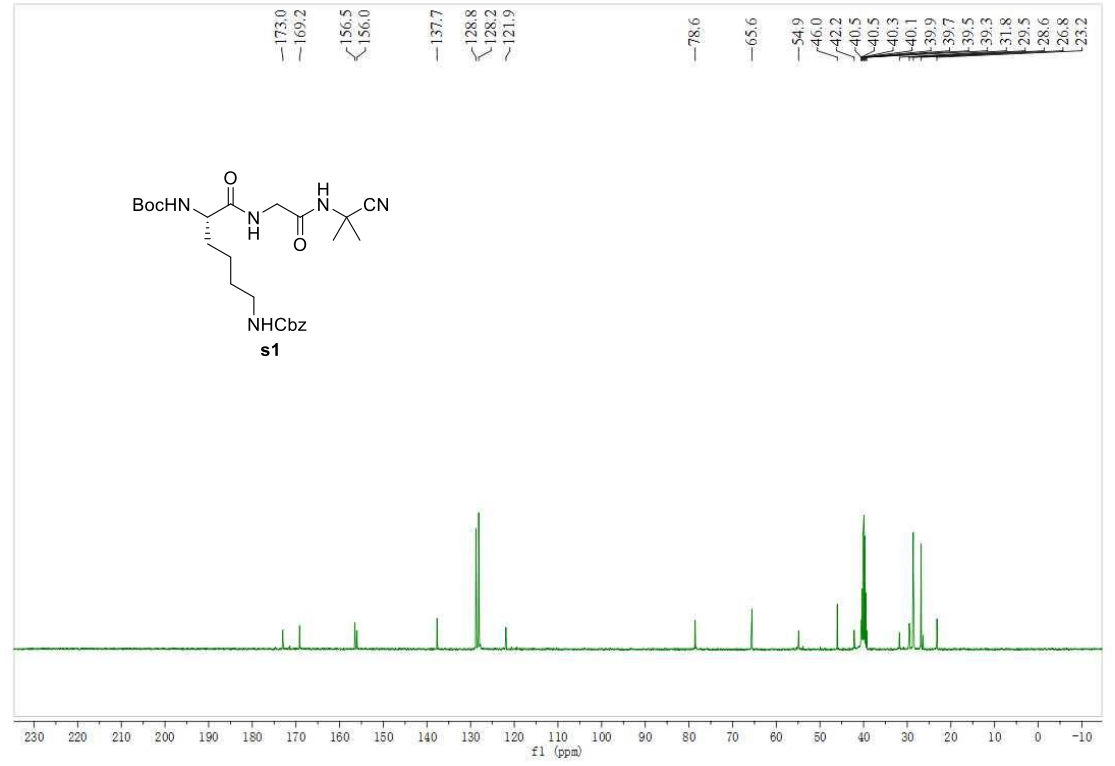
NHCbz
s1
f1 (ppm)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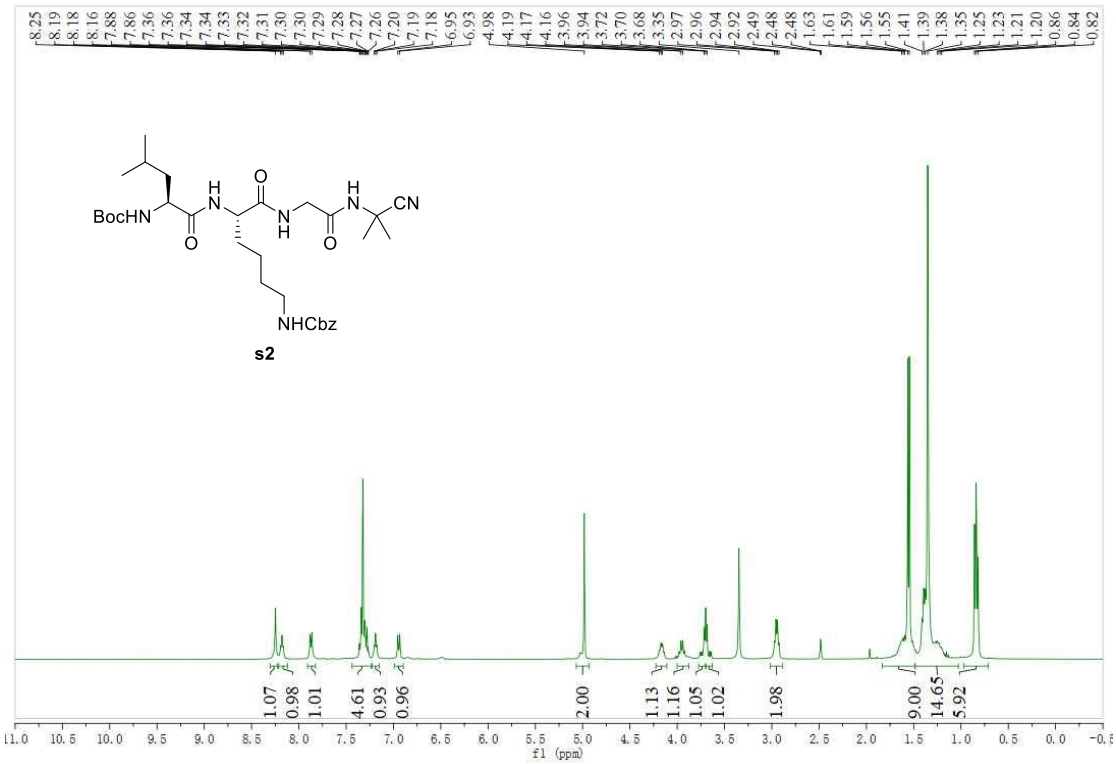
s1
f1 (ppm)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CN
s2
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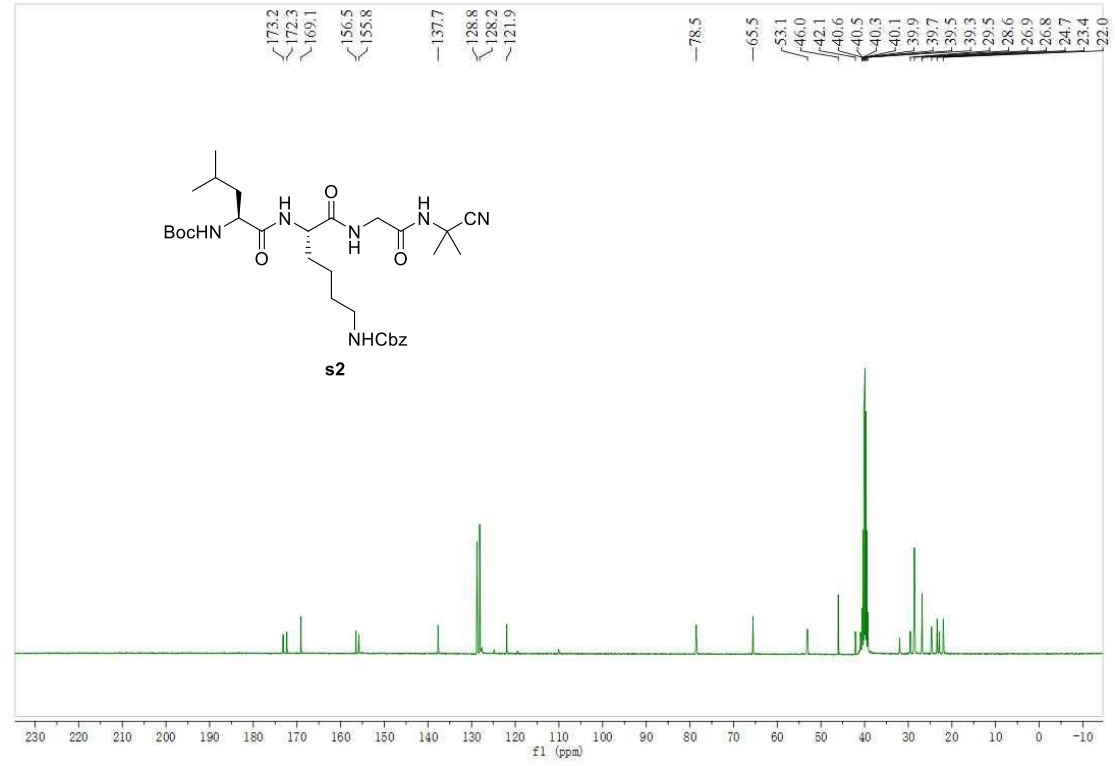
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s2
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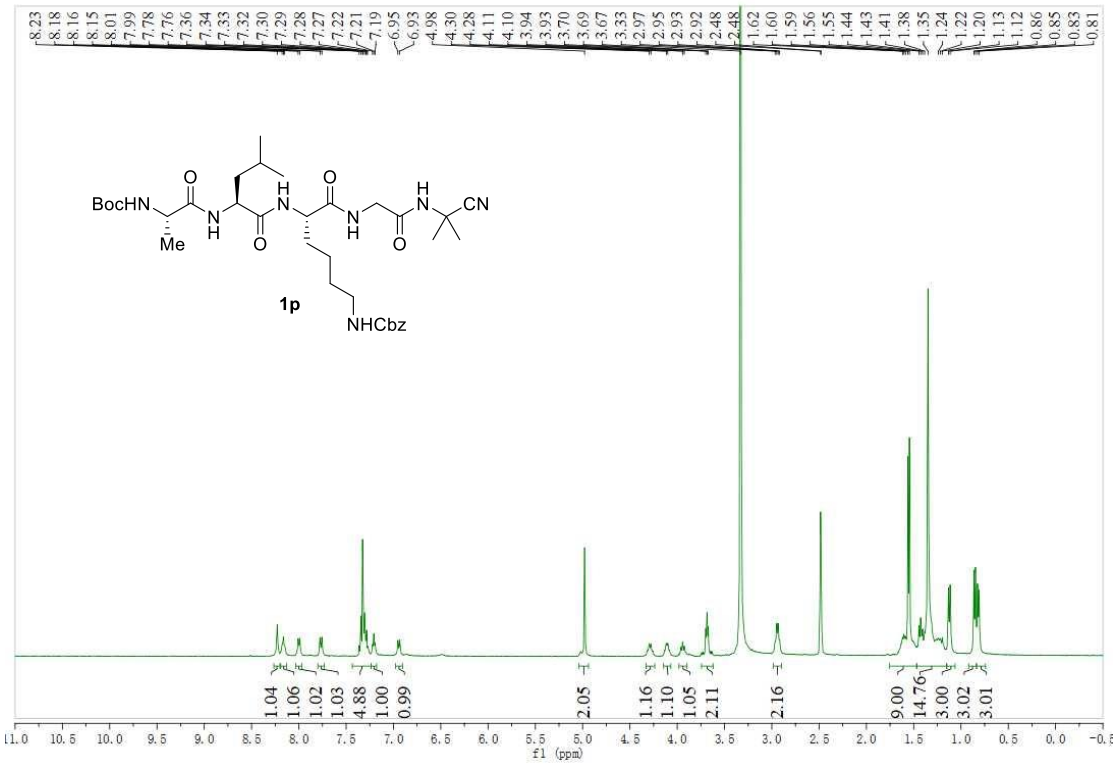
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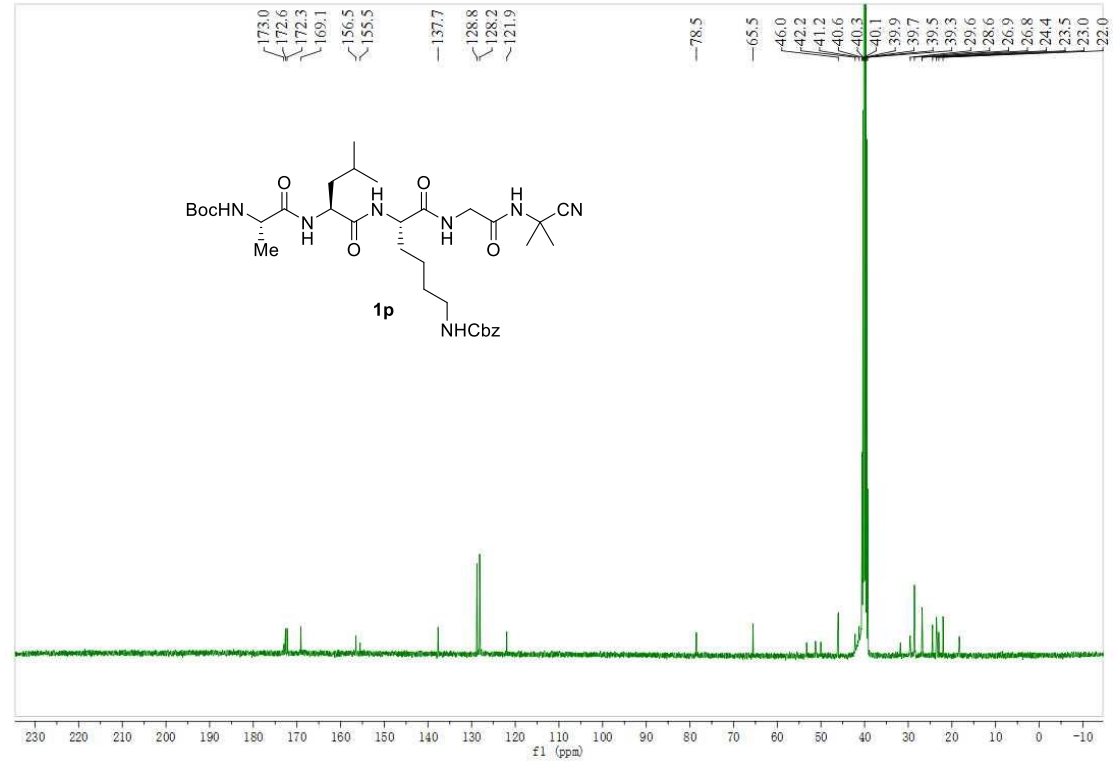
f1 (ppm)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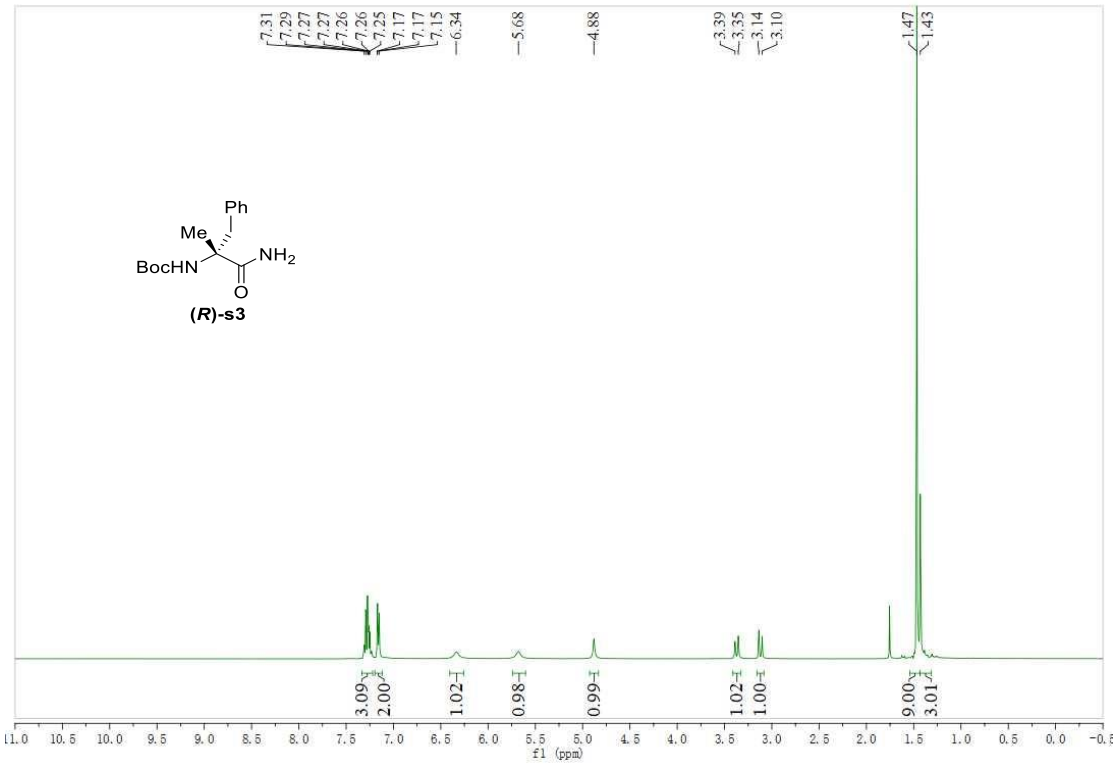
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3.35
3.14
3.10
1.47
1.43
Ph
Me
BocHN
NH2
O
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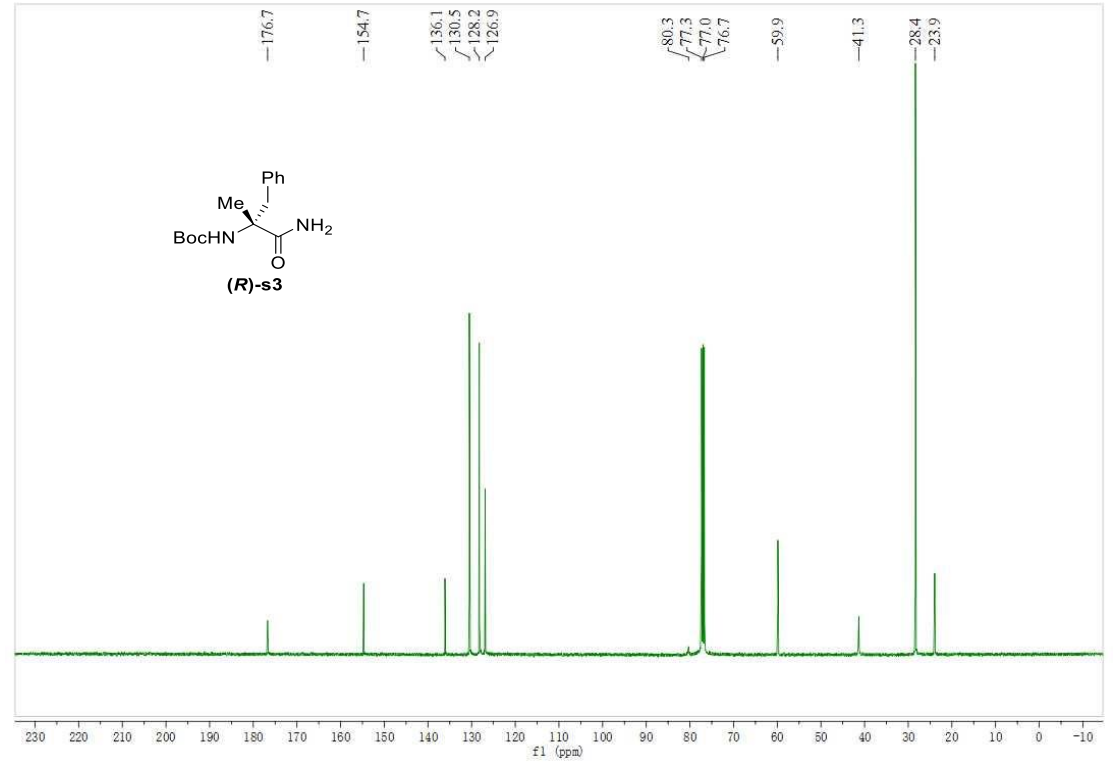
1.00
9.00
3.01
f1 (ppm)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Ph
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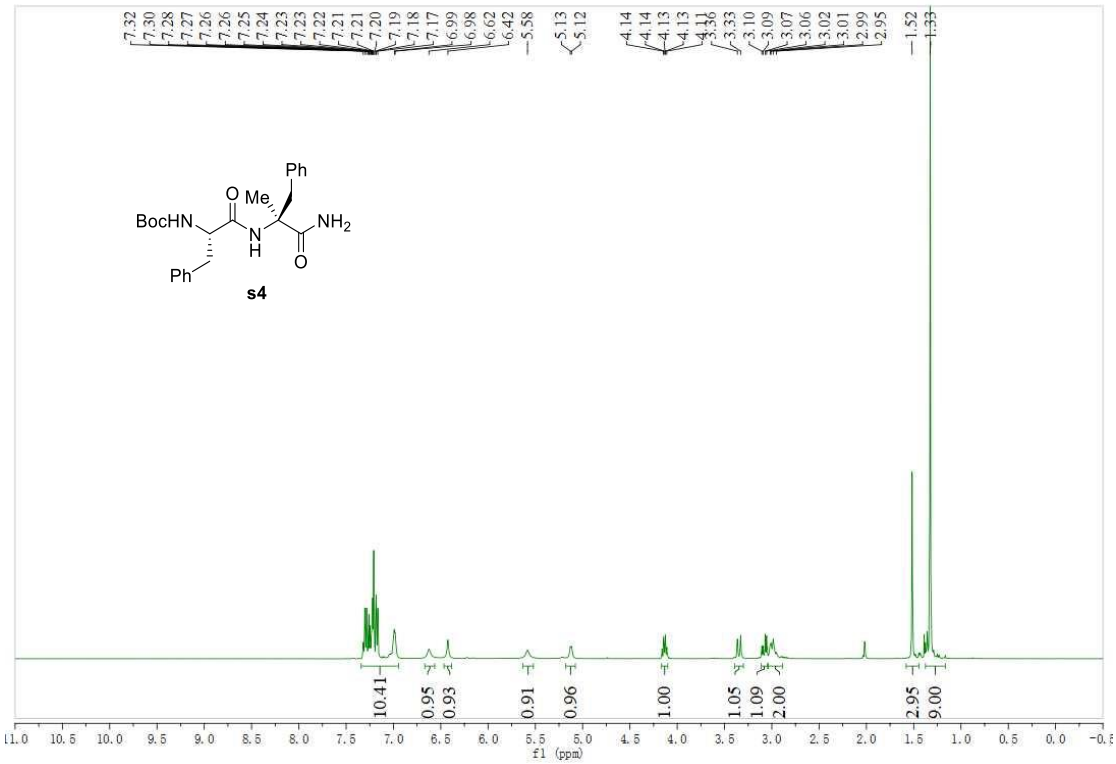
BocHN
NH2
O
(R)-s3
f1 (ppm)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5.12
4.14
4.14
4.13
4.13
4.11
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3.33
3.10
3.09
3.07
3.06
3.02
3.01
2.99
2.95
1.52
1.33
Ph
O
Me
BocHN
N
H
NH2
O
Ph
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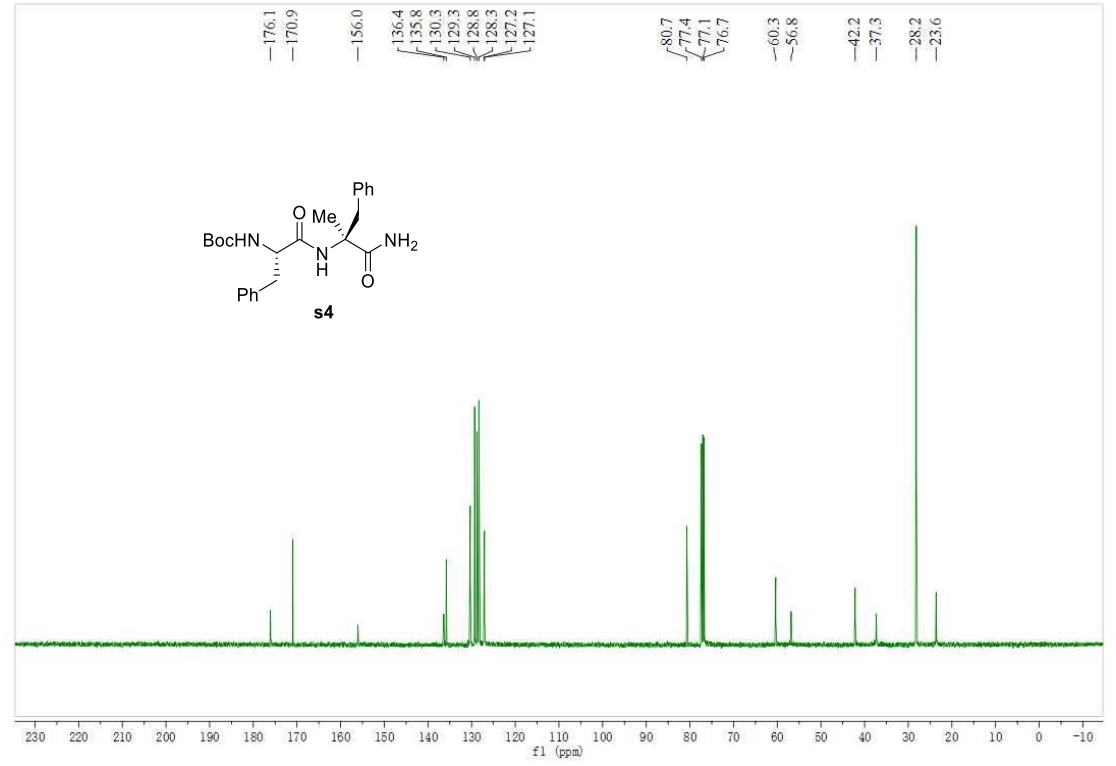
9.00
f1 (ppm)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77.4
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76.7
60.3
56.8
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H
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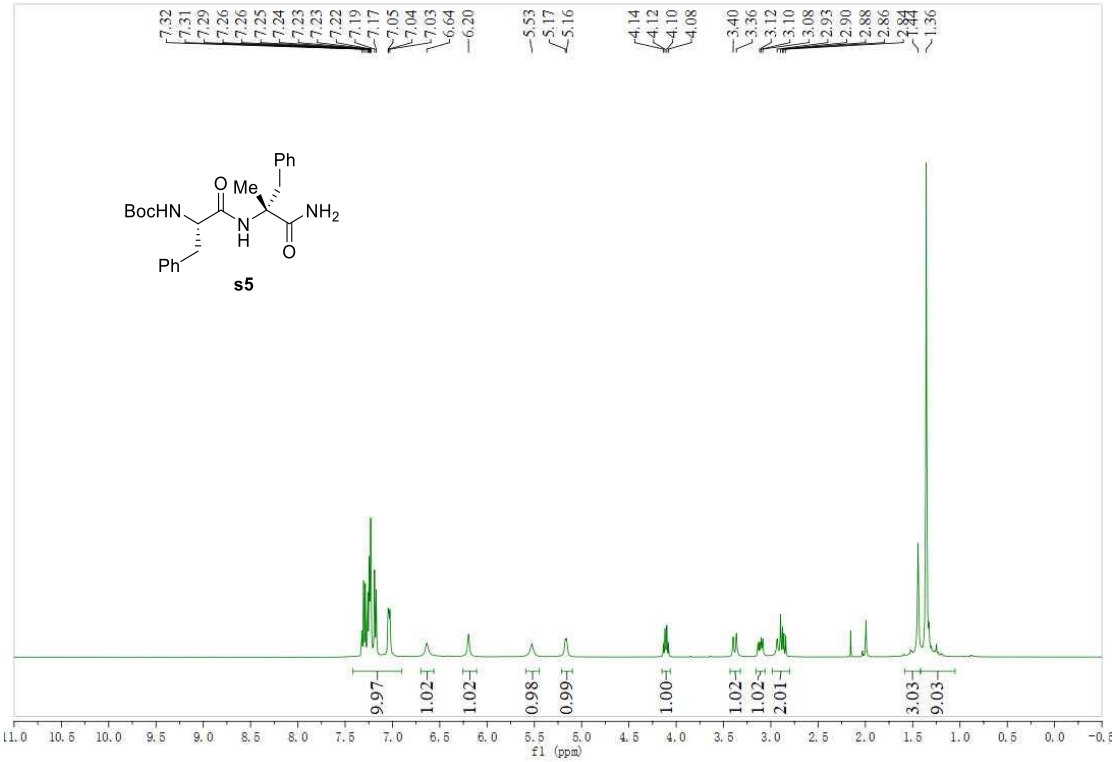
Ph
s4
f1 (ppm)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4.10
4.08
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3.36
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3.10
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Ph
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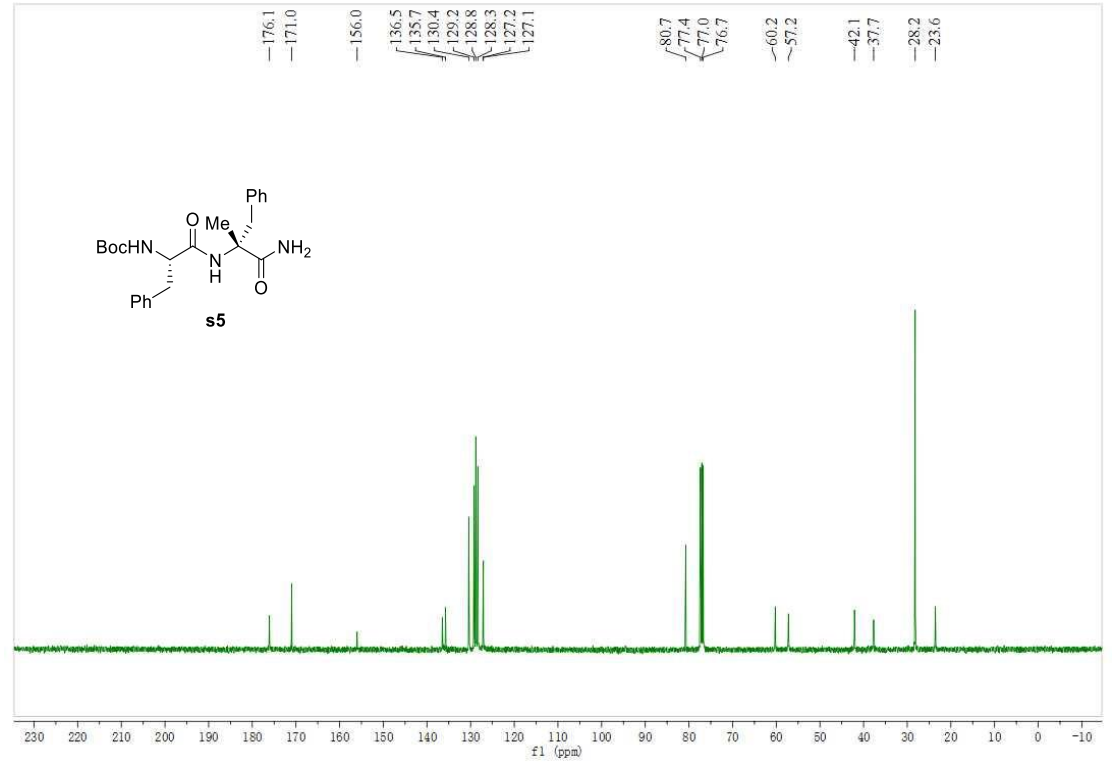
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77.4
77.0
76.7
60.2
57.2
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23.6
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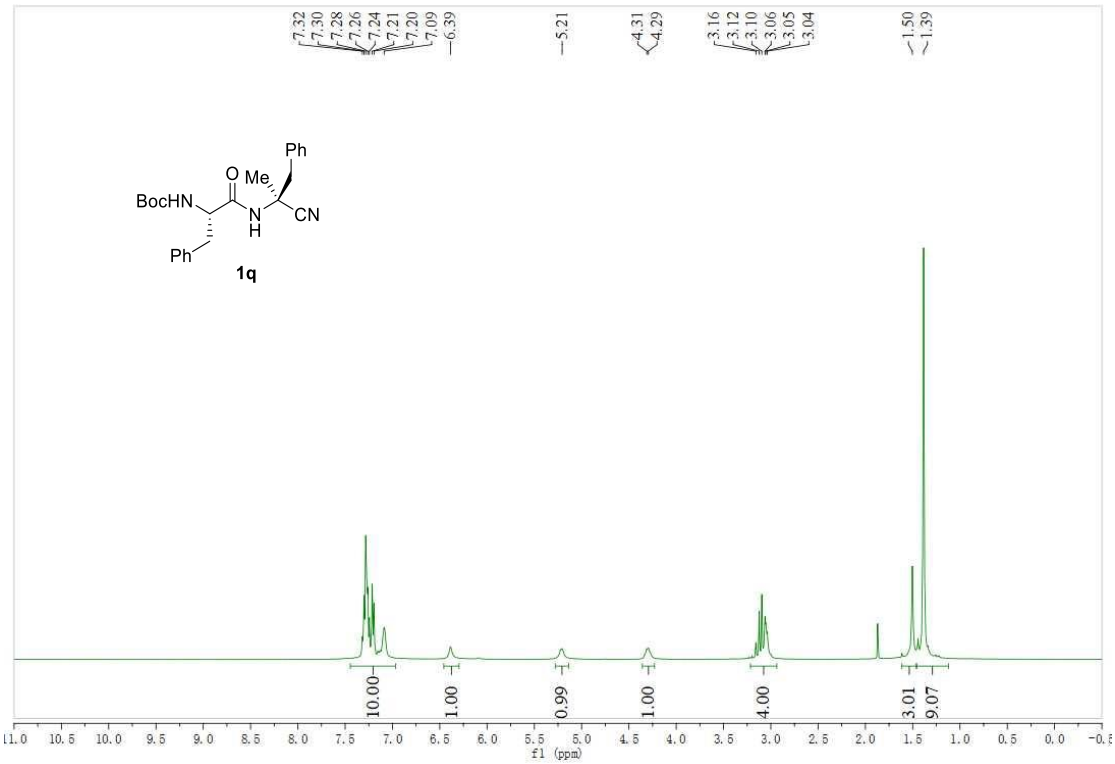
s5
f1 (ppm)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3.05
3.04
1.50
1.39
Ph
O
Me
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4.00
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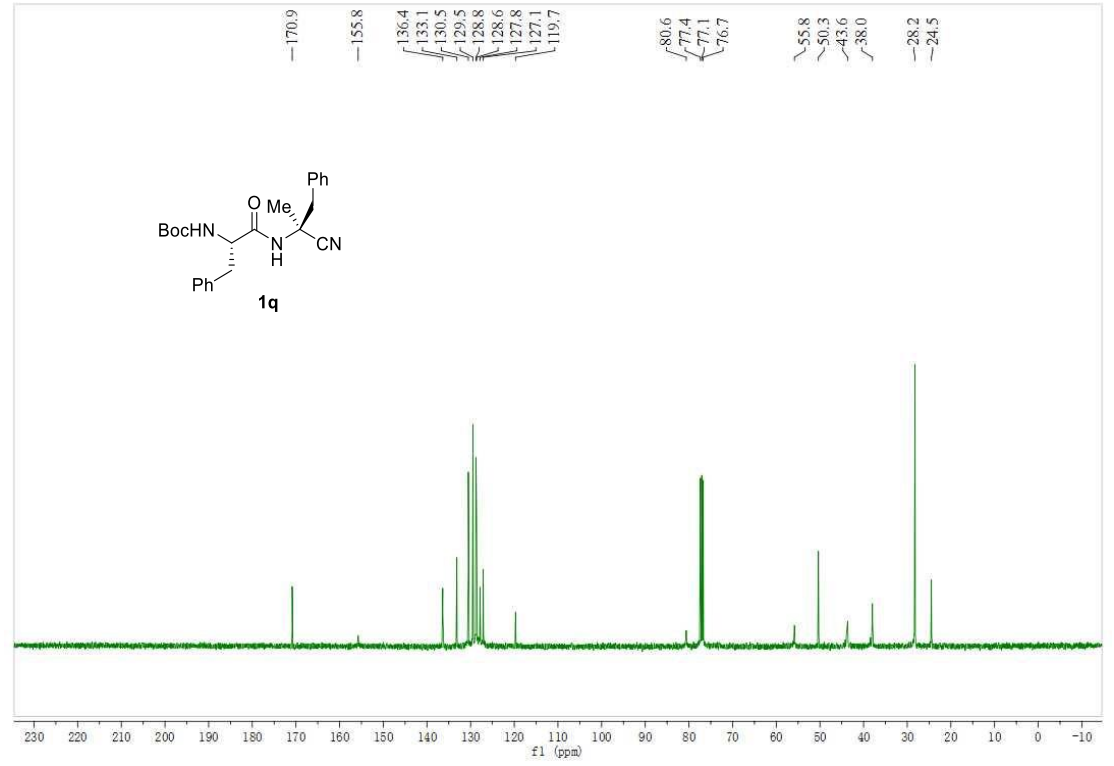
9.07
f1 (ppm)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77.4
77.1
76.7
55.8
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N
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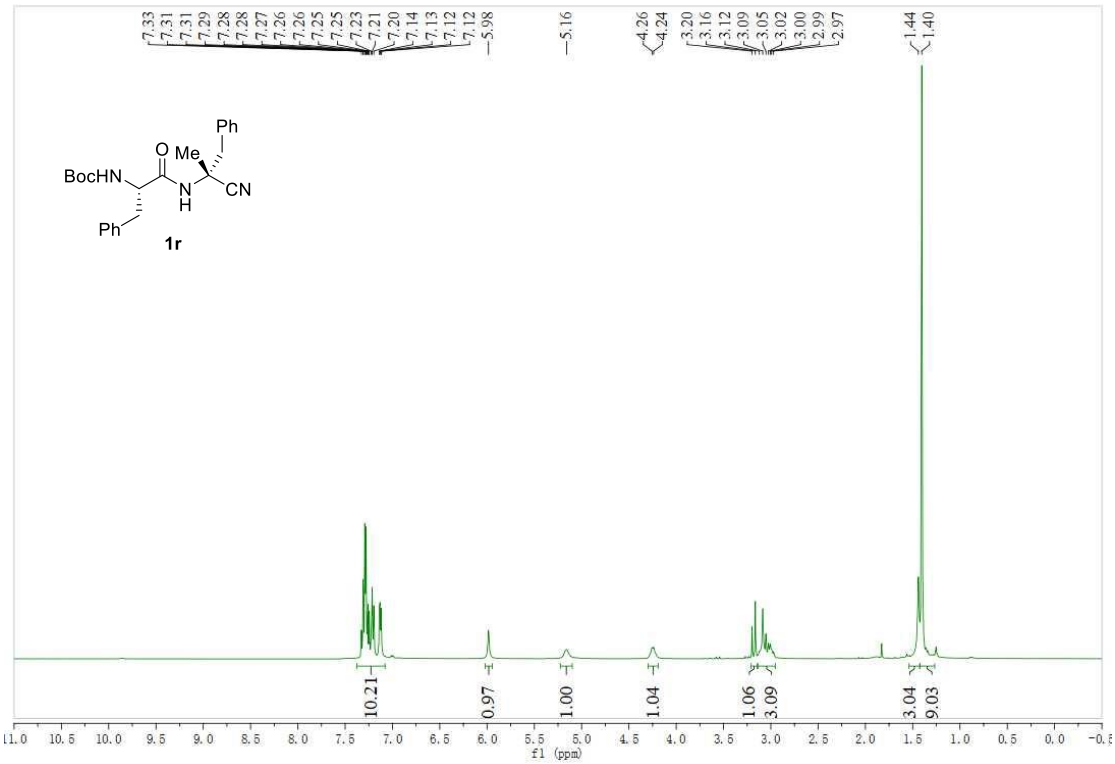
Ph
1q
f1 (ppm)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3.12
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3.02
3.00
2.99
2.97
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1.40
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BocHN
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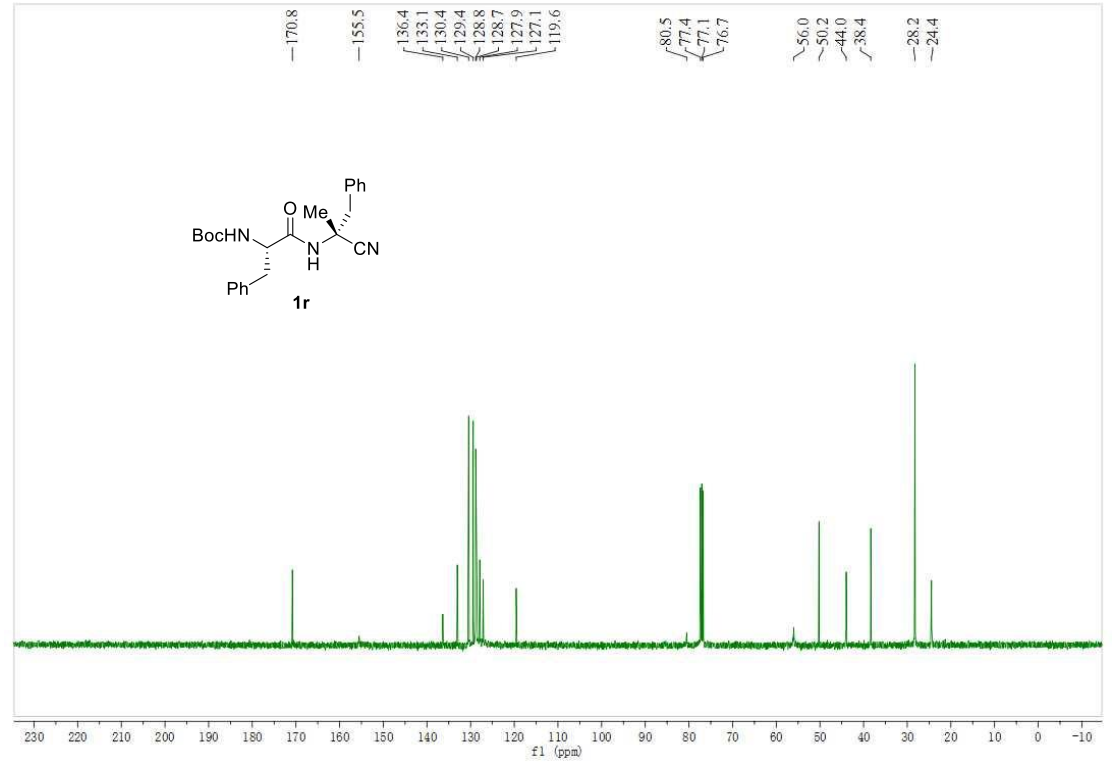
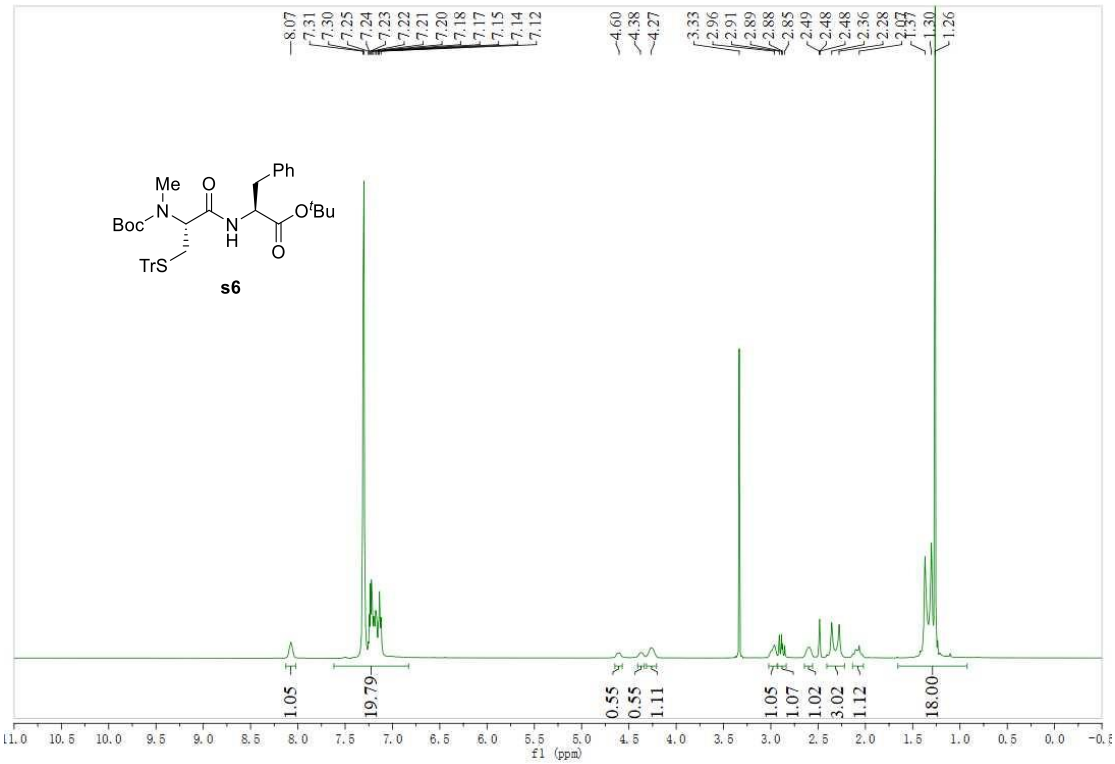
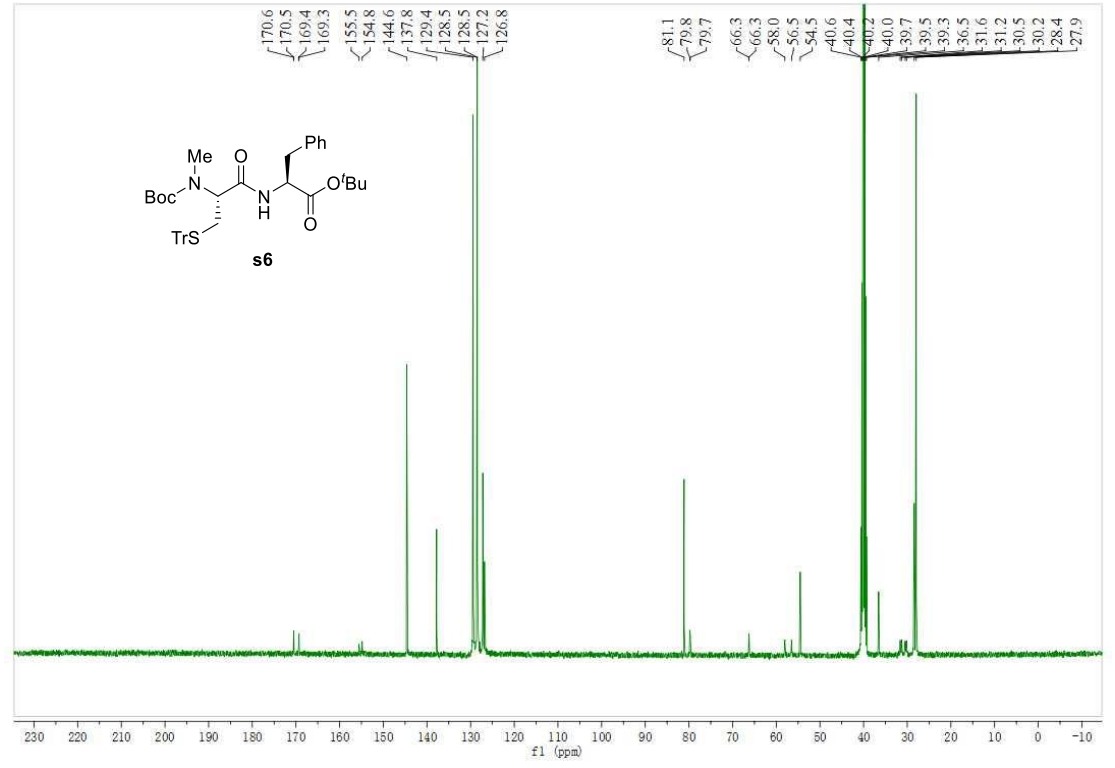
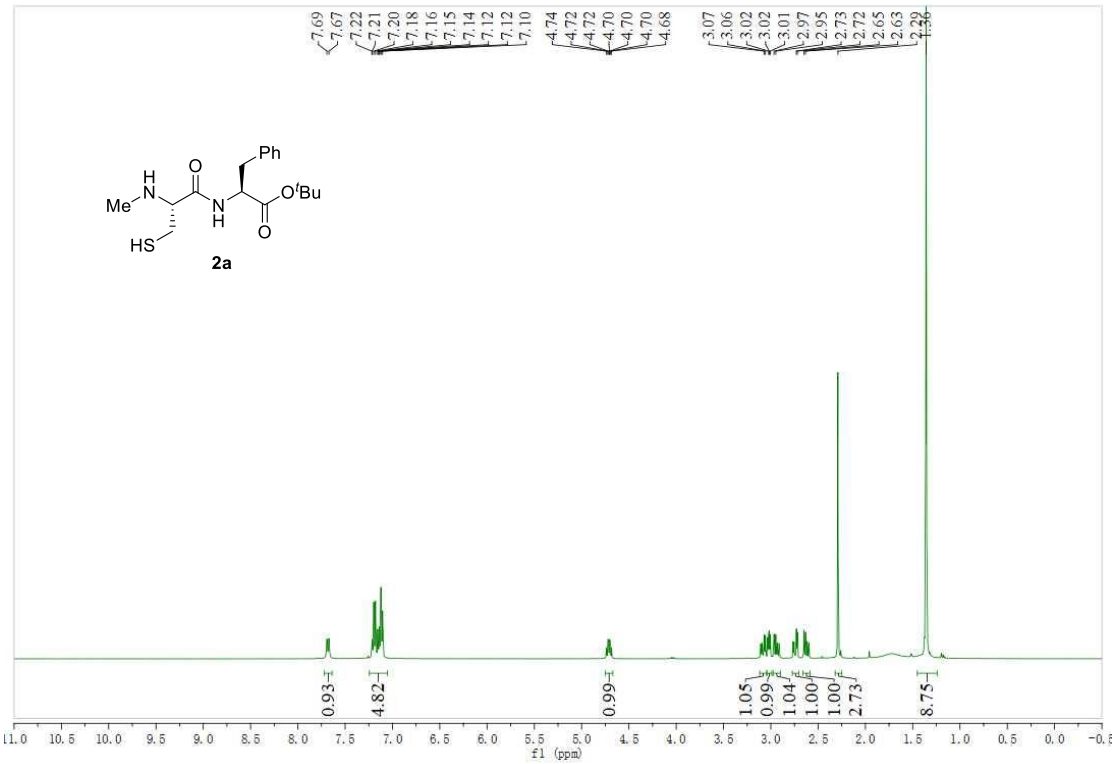
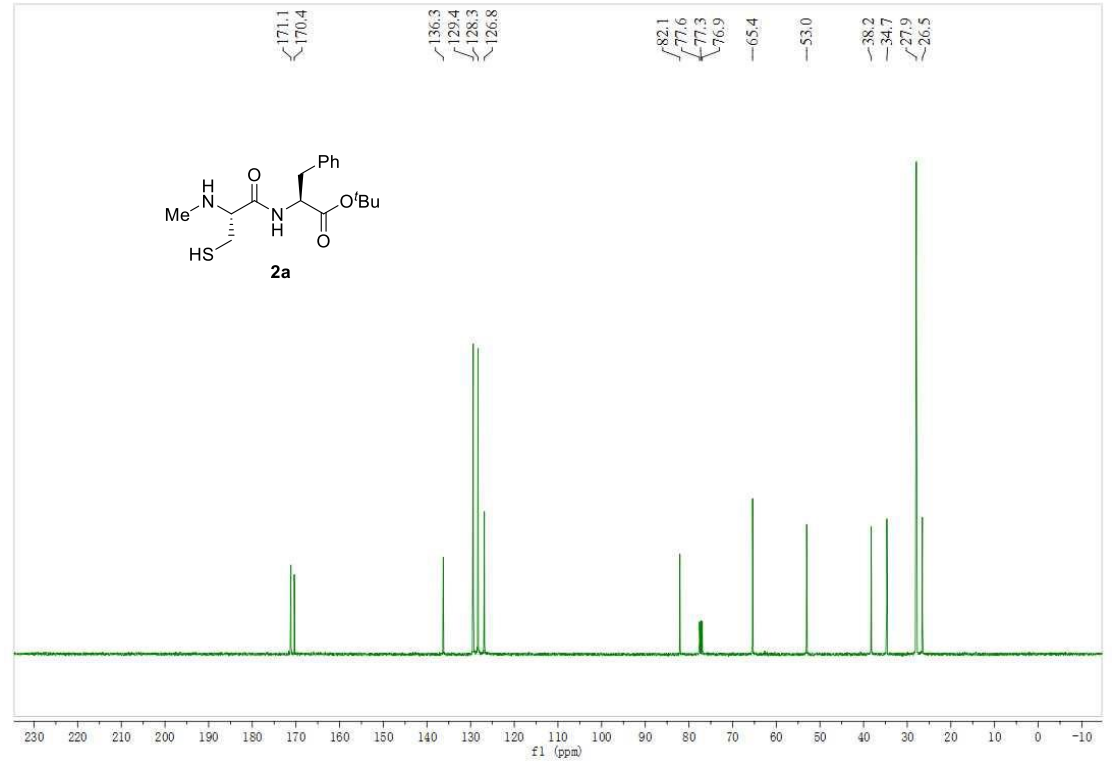
H
CN
Ph
1r
10.21
0.97
1.00
1.04
1.06
3.09
3.04
9.03
f1 (ppm)

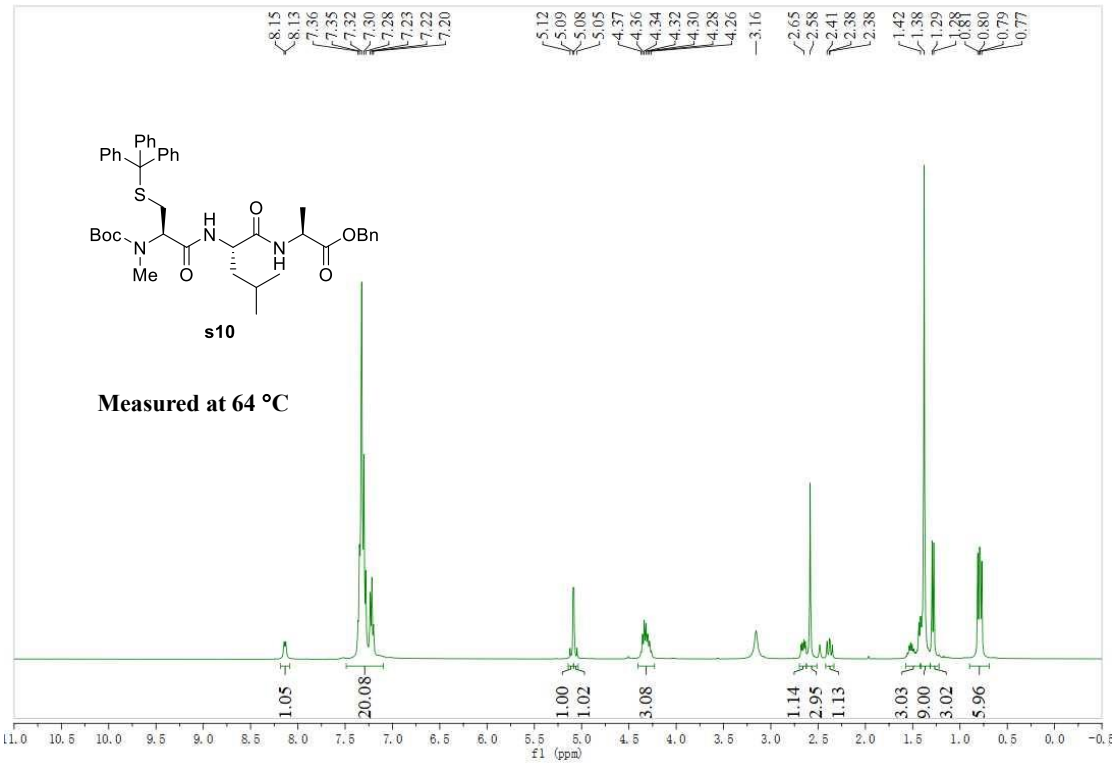

170.8
155.5
136.4
133.1
130.4
129.4
128.8
128.7
127.9
127.1
119.6
80.5
77.4
77.1
76.7
56.0
50.2
44.0
38.4
28.2
24.4
Ph
O
Me
BocHN
N
H
CN
Ph
1r
f1 (ppm)

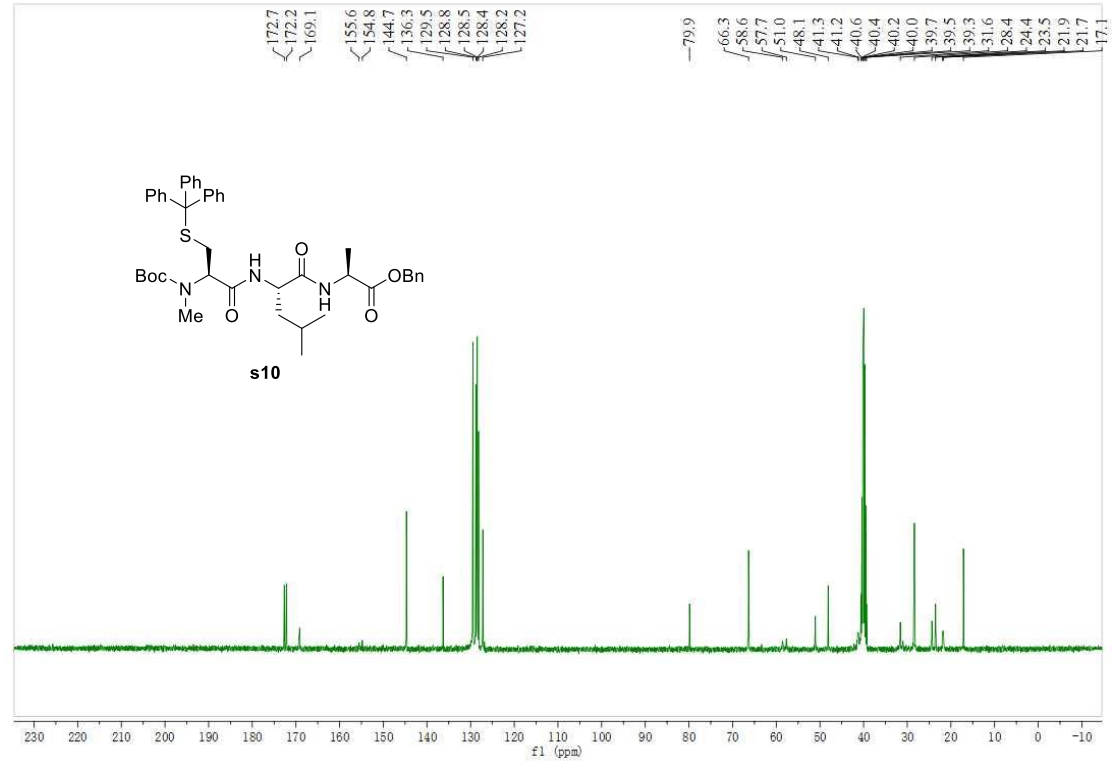

Me
O
Ph
N
Boc
N
H
O^{t}Bu
TrS
O
s6
f1 (ppm)

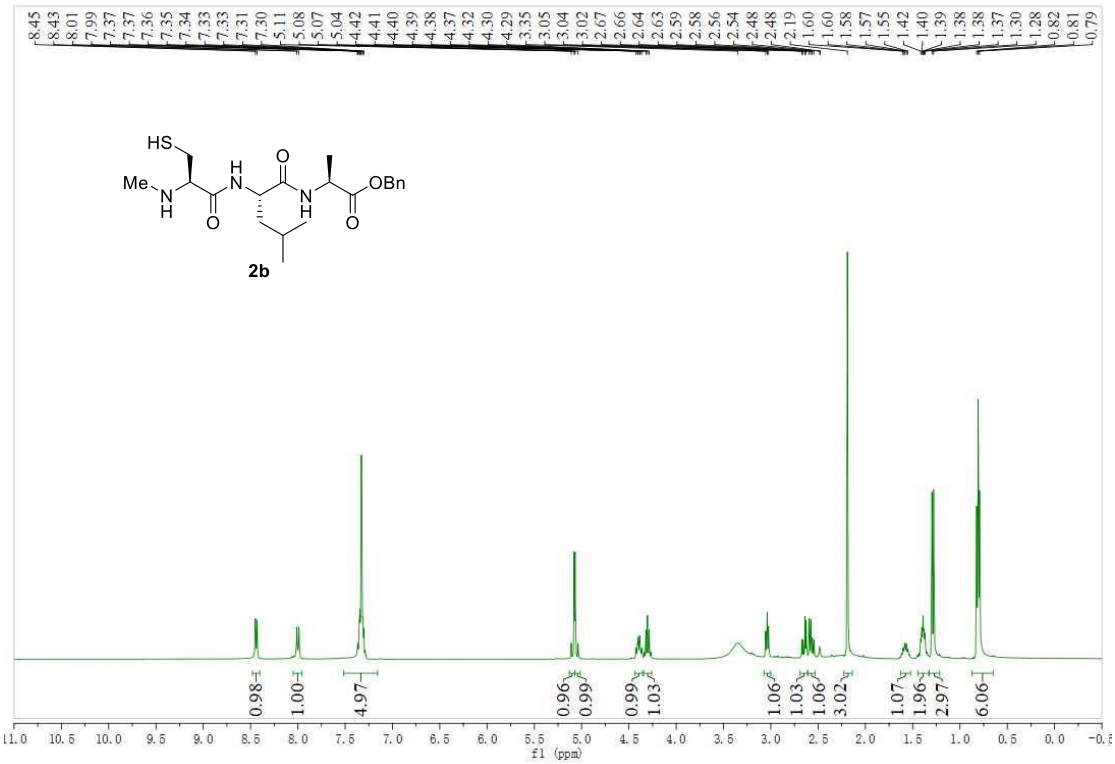

Me
O
Ph
N
Boc
N
H
O^{t}Bu
TrS
O
s6
f1 (ppm)

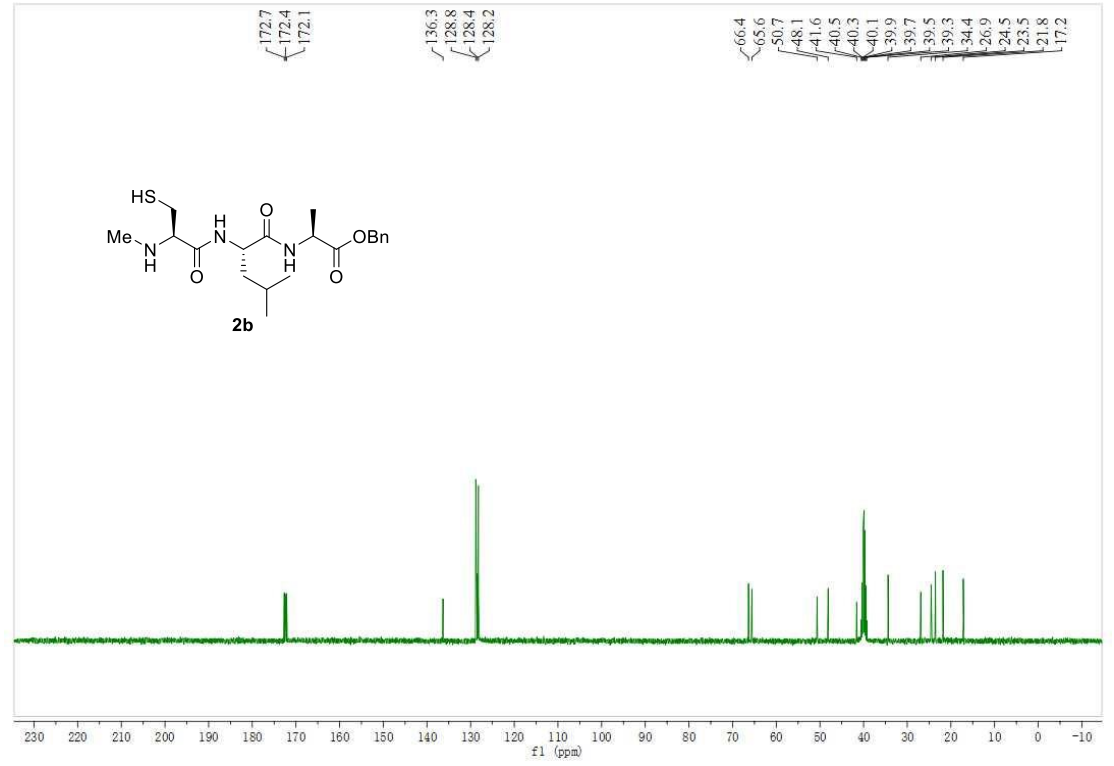

7.69
7.67
7.22
7.21
7.20
7.18
7.16
7.15
7.14
7.12
7.12
7.10
4.74
4.72
4.72
4.70
4.70
4.70
4.68
3.07
3.06
3.02
3.02
3.01
2.97
2.95
2.73
2.72
2.65
2.63
1.36
Ph
H
N
O
Me
N
H
O
O'Bu
HS
2a
0.93
4.82
0.99
1.05
0.99
1.04
1.00
1.00
2.73
8.75
f1 (ppm)

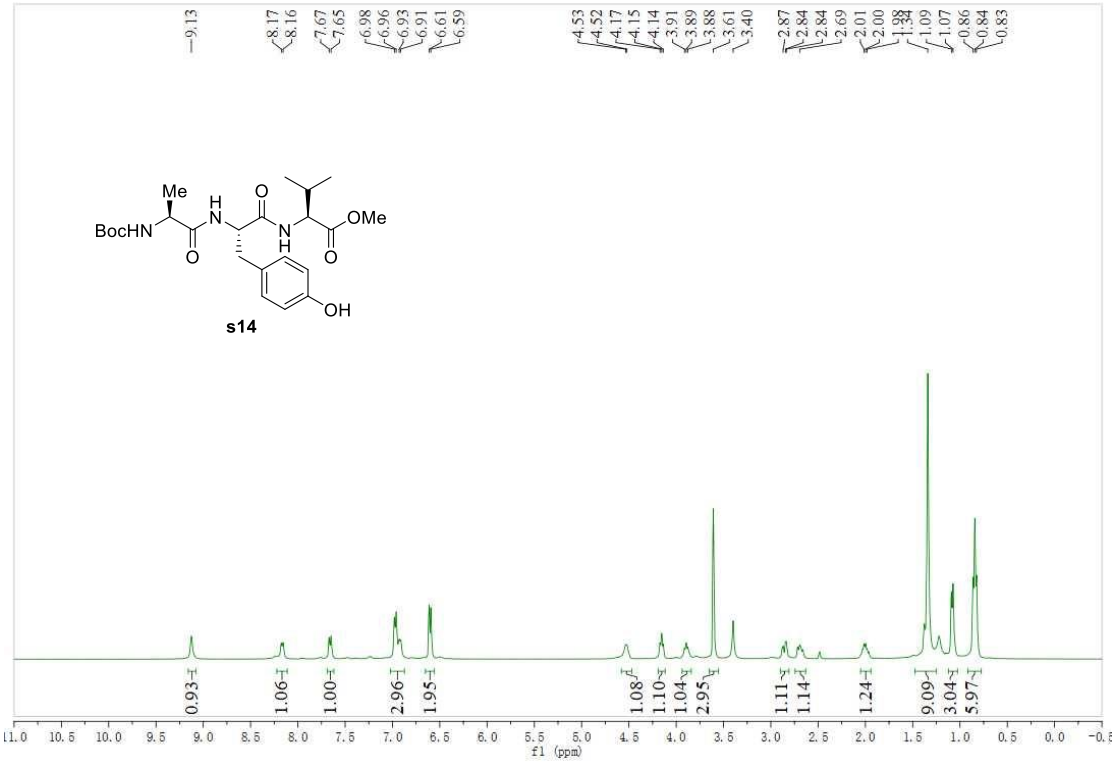

171.1
170.4
136.3
129.4
128.3
126.8
82.1
77.6
77.3
76.9
65.4
53.0
38.2
34.7
27.9
26.5
Ph
H
N
O
Me
N
H
O
O'Bu
HS
2a
f1 (ppm)

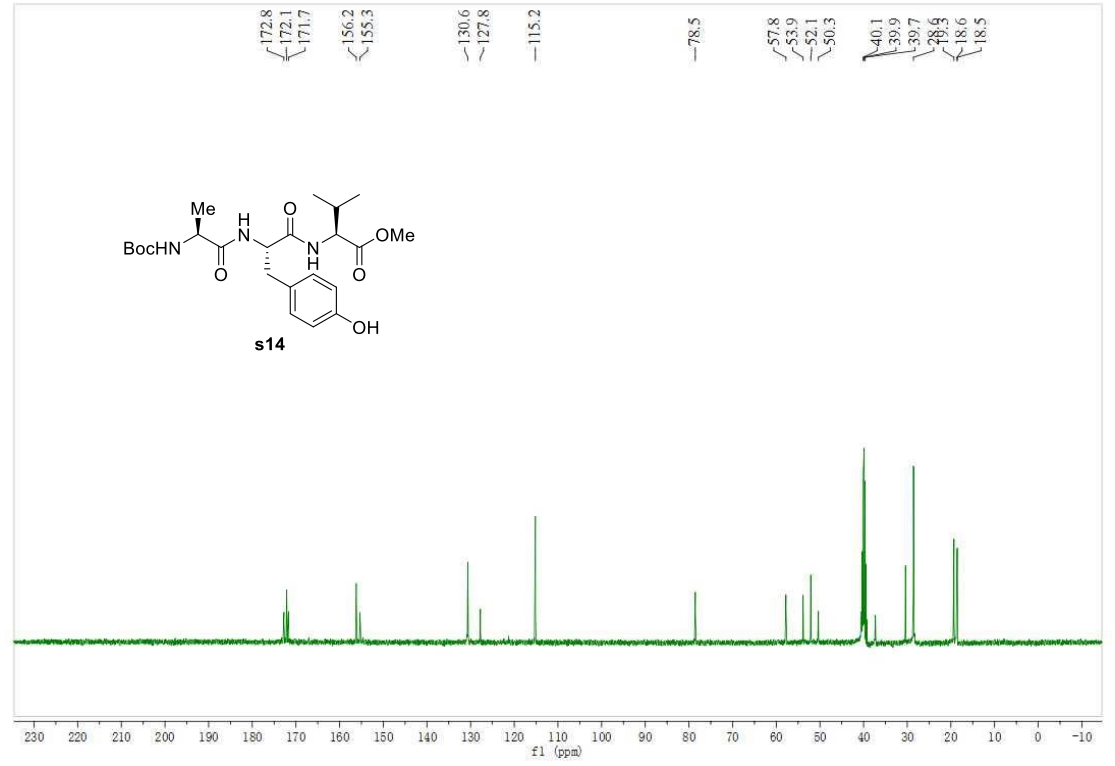

Ph
Ph
Ph
S
Boc
N
Me
O
H
N
O
N
H
O
OBn
O
s10
Measured at 64 °C
8.15
8.13
7.36
7.35
7.32
7.30
7.28
7.23
7.22
7.20
5.12
5.09
5.08
5.05
4.37
4.36
4.34
4.32
4.30
4.28
4.26
3.16
2.65
2.58
2.41
2.38
2.38
1.42
1.38
1.29
1.28
0.81
0.80
0.79
0.77
1.05
20.08
1.00
1.02
3.08
1.14
2.95
1.13
3.03
9.00
3.02
5.96
f1 (ppm)

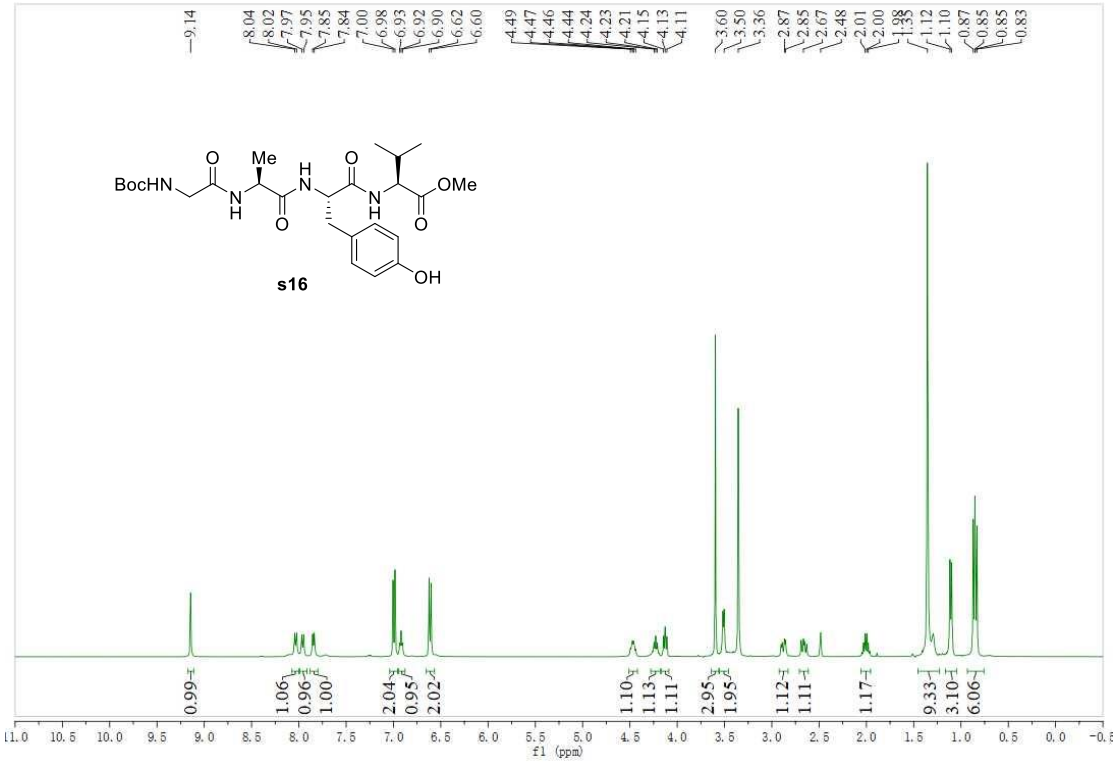

172.7
172.2
169.1
155.6
154.8
144.7
136.3
129.5
128.8
128.5
128.4
128.2
127.2
79.9
66.3
58.6
57.7
51.0
48.1
41.3
41.2
40.6
40.4
40.2
40.0
39.7
39.5
39.3
31.6
28.4
24.4
23.5
21.9
21.7
17.1
Ph
Ph
Ph
S
Boc
N
Me
O
H
N
O
N
H
O
OBn
O
s10
f1 (ppm)

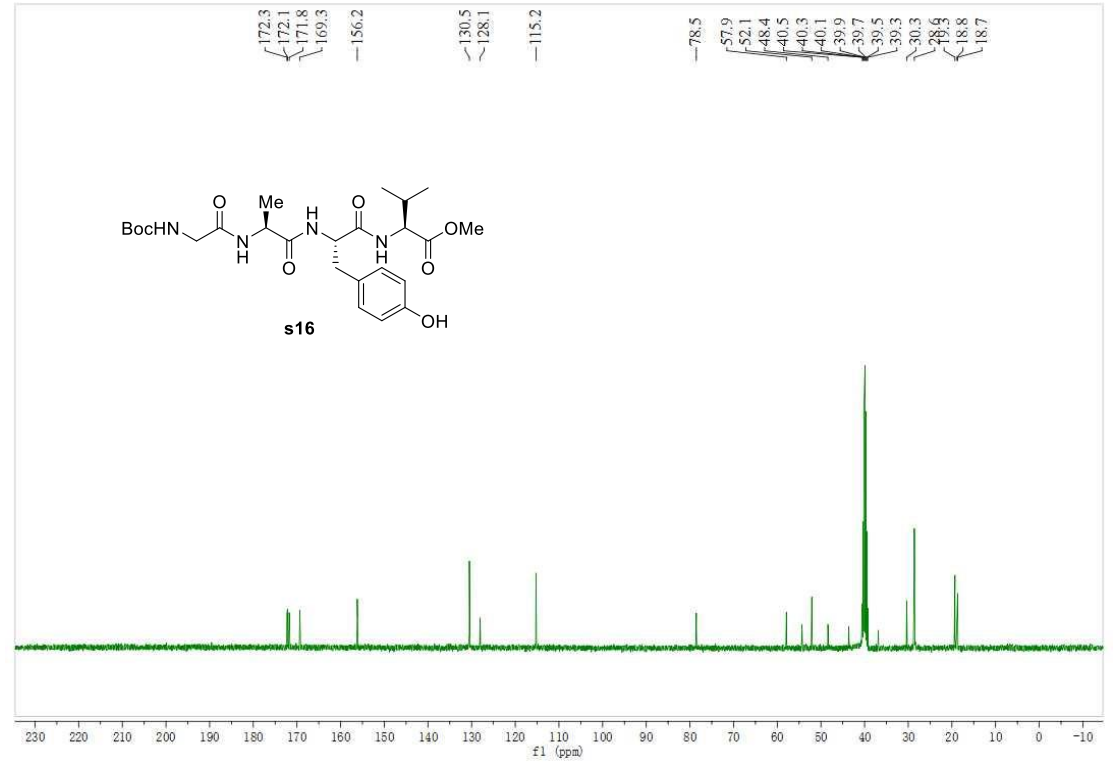

HS
Me
N
H
H
N
O
O
N
H
O
OBn
O
2b
f1 (ppm)

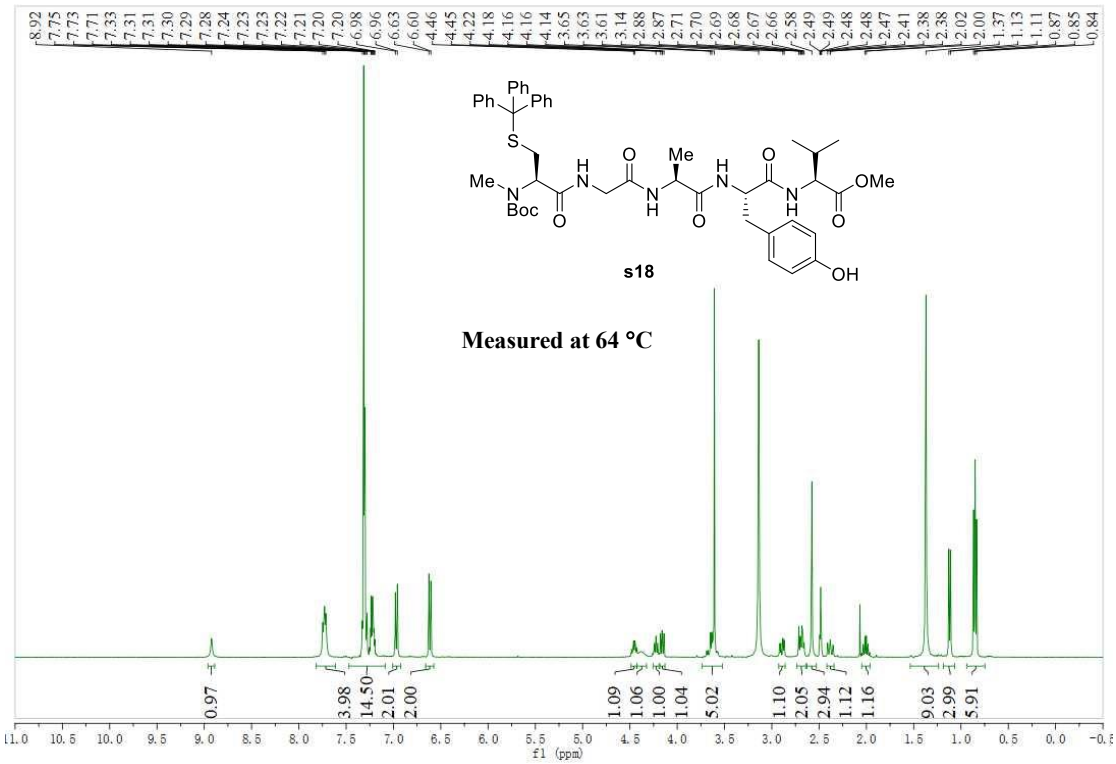

HS
Me
N
H
H
N
O
O
N
H
O
OBn
O
2b
f1 (ppm)

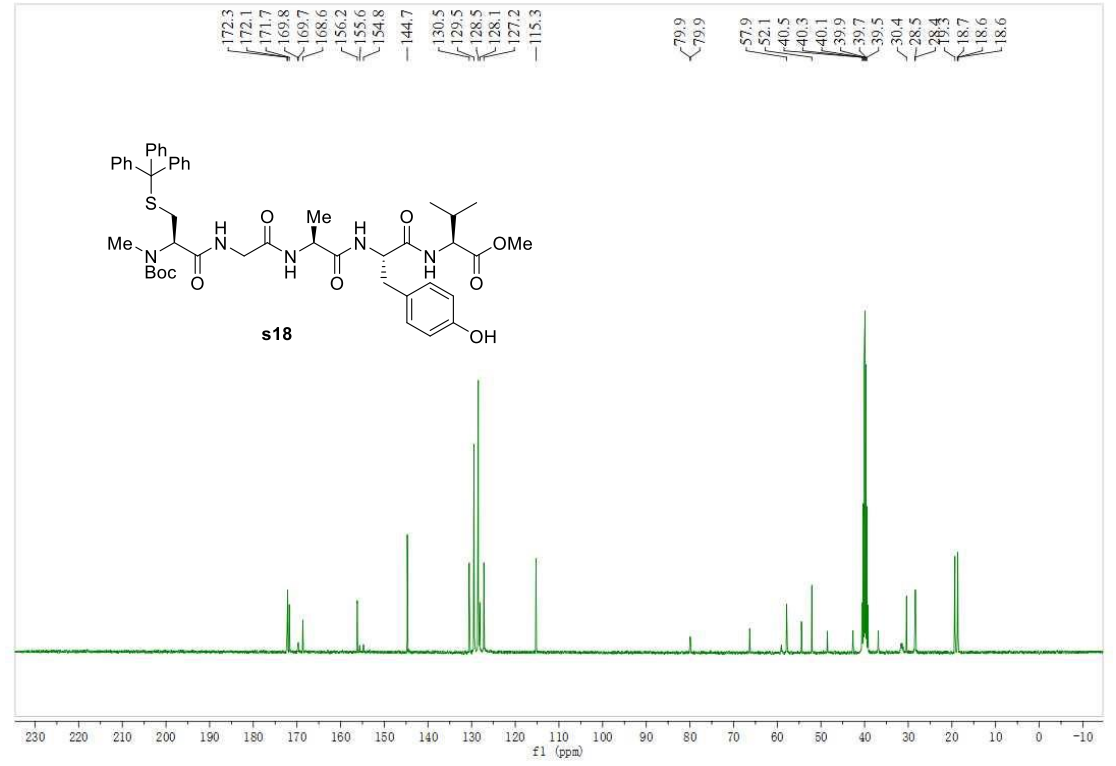

9.13
8.17
8.16
7.67
7.65
6.98
6.96
6.93
6.91
6.61
6.59
4.53
4.52
4.17
4.15
4.14
3.91
3.89
3.88
3.61
3.40
2.87
2.84
2.84
2.69
2.01
2.00
1.09
1.07
0.86
0.84
0.83
Me
BocHN
H
N
O
O
N
H
O
OMe
O
OH
s14
0.93
1.06
1.00
2.96
1.95
1.08
1.10
1.04
2.95
1.11
1.14
1.24
9.09
3.04
5.97
f1 (ppm)

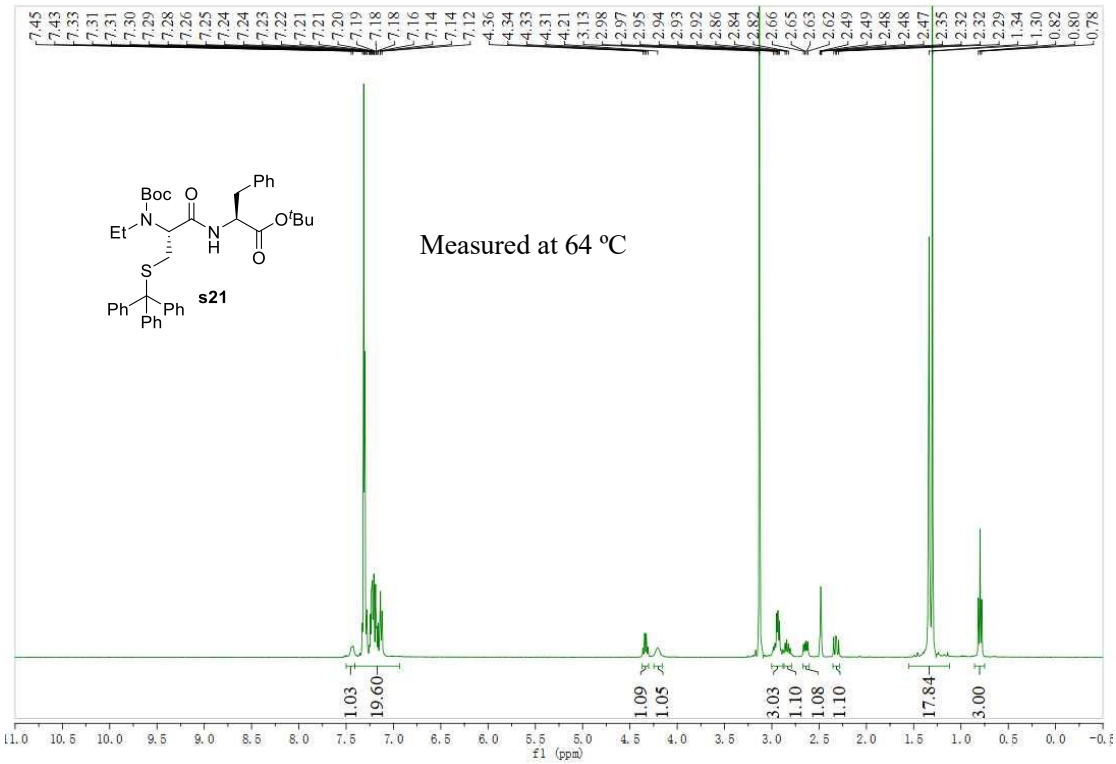

172.8
172.1
171.7
156.2
155.3
130.6
127.8
115.2
78.5
57.8
53.9
52.1
50.3
40.1
39.9
39.7
28.6
28.3
18.6
18.5
Me
BocHN
H
N
O
O
N
H
O
OMe
O
OH
s14
f1 (ppm)

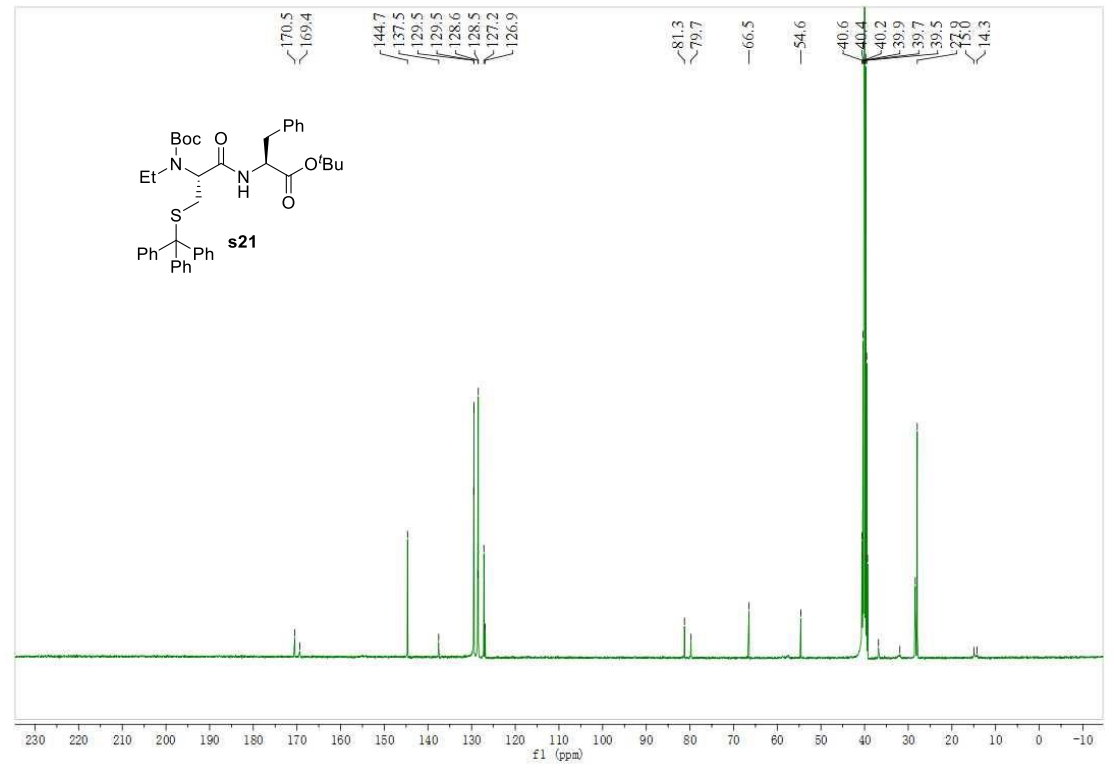

s16
f1 (ppm)

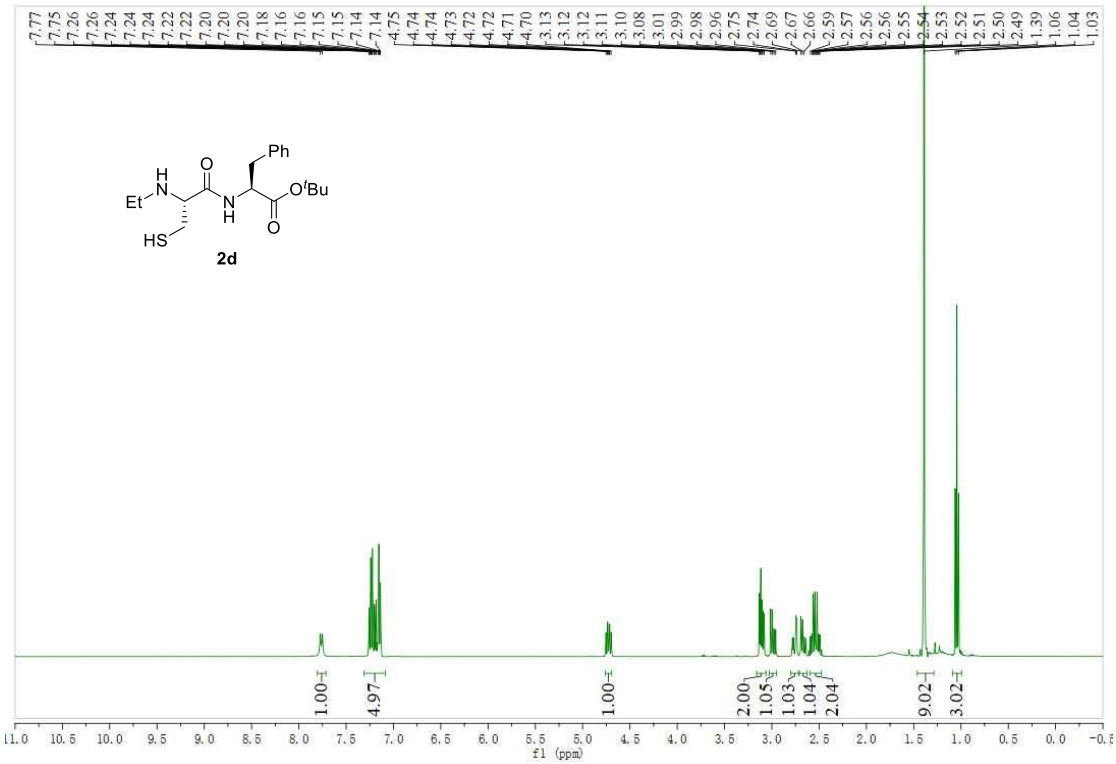

s16
f1 (ppm)

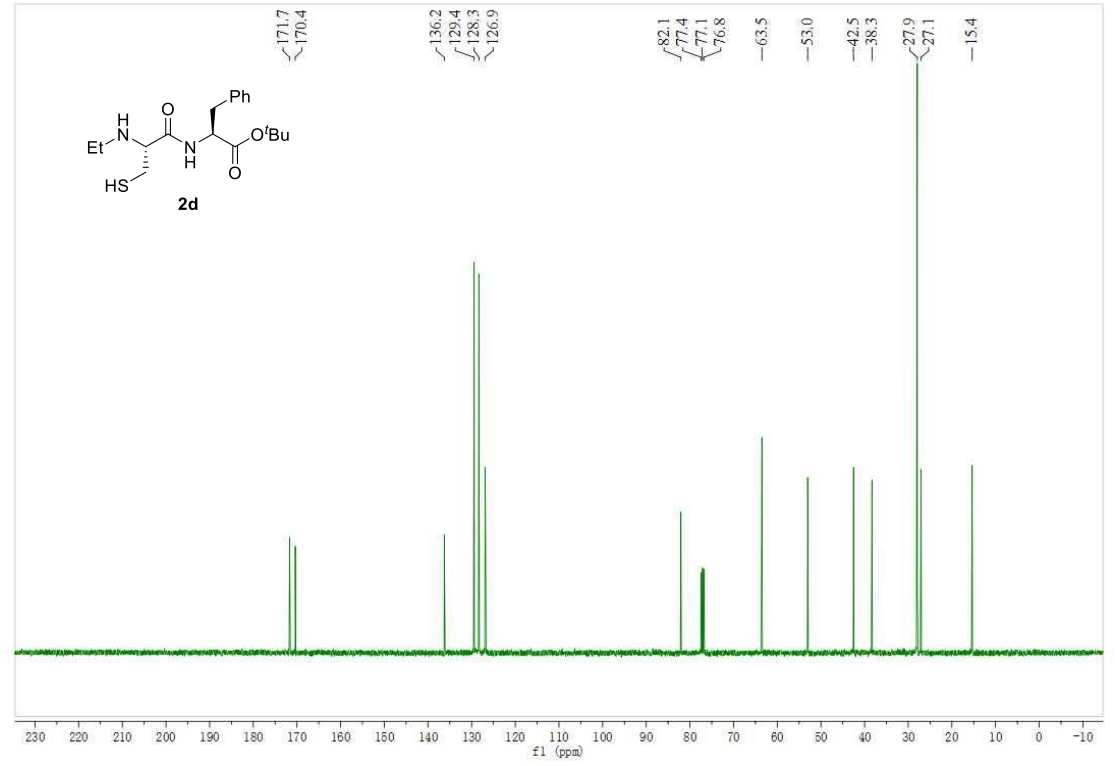

s18
Measured at 64 °C
f1 (ppm)

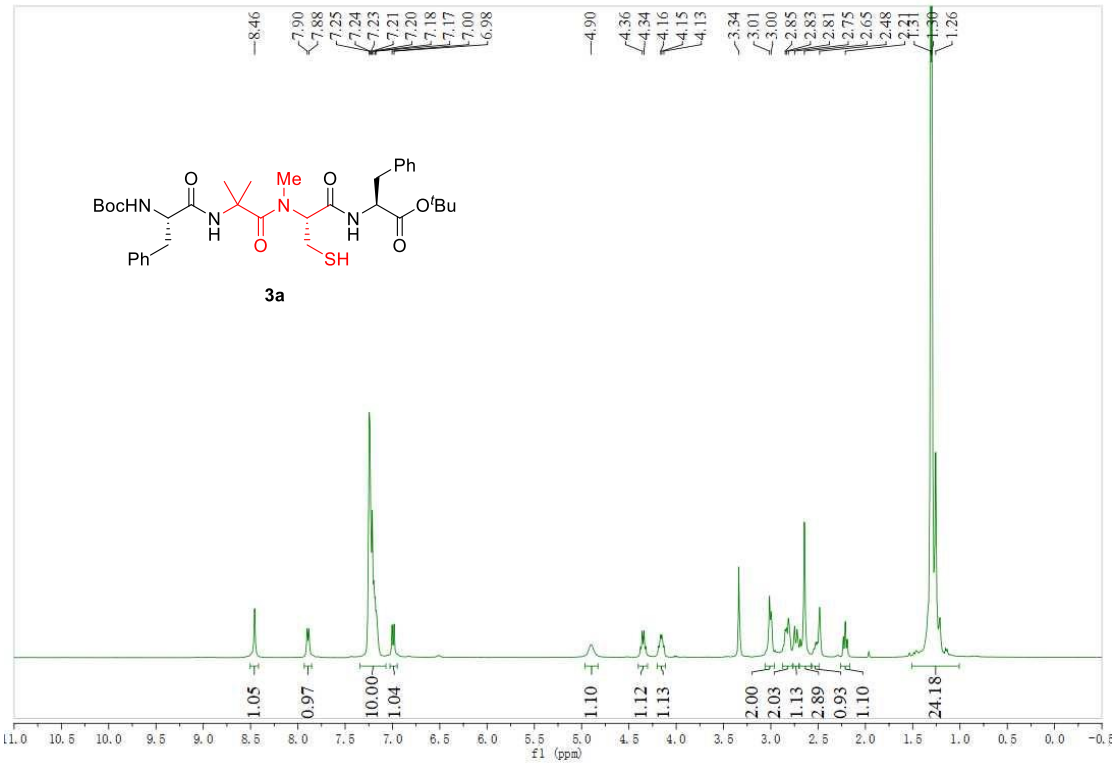

s18
f1 (ppm)


Measured at 64 ºC
s21
f1 (ppm)


s21
f1 (ppm)


Ph
Et
HS
O
O
O
O
tBu
2d
f1 (ppm)


Ph
Et
HS
O
O
O
O
tBu
2d
f1 (ppm)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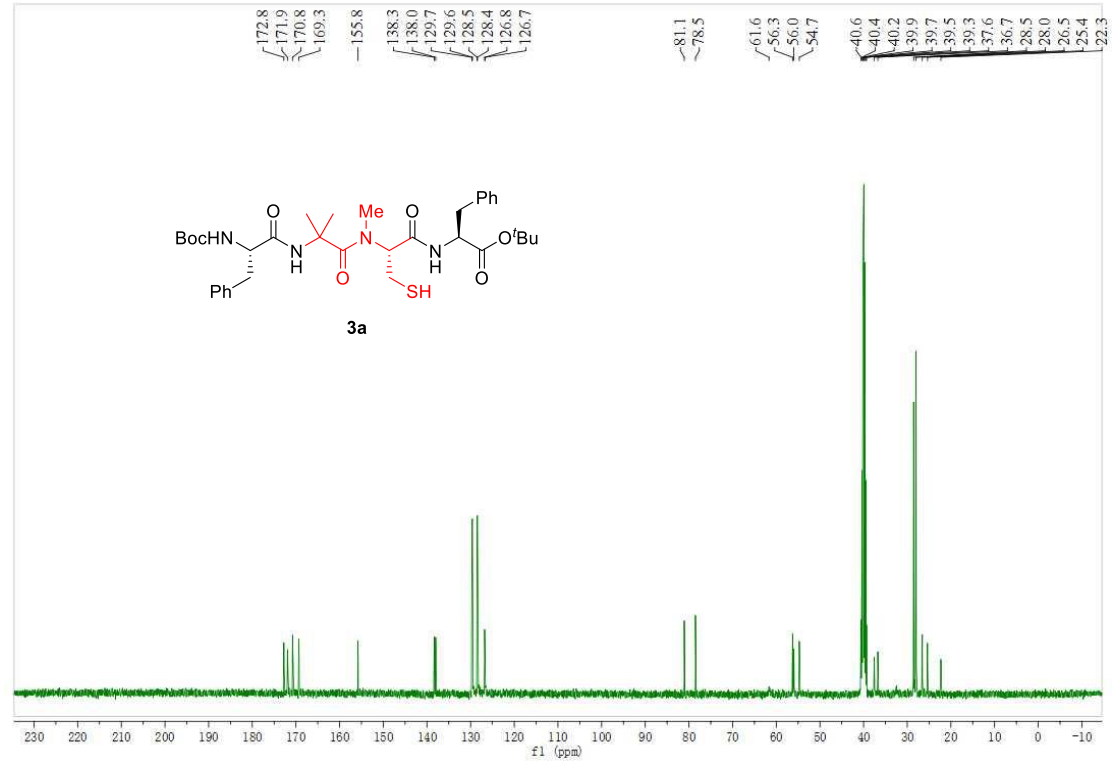
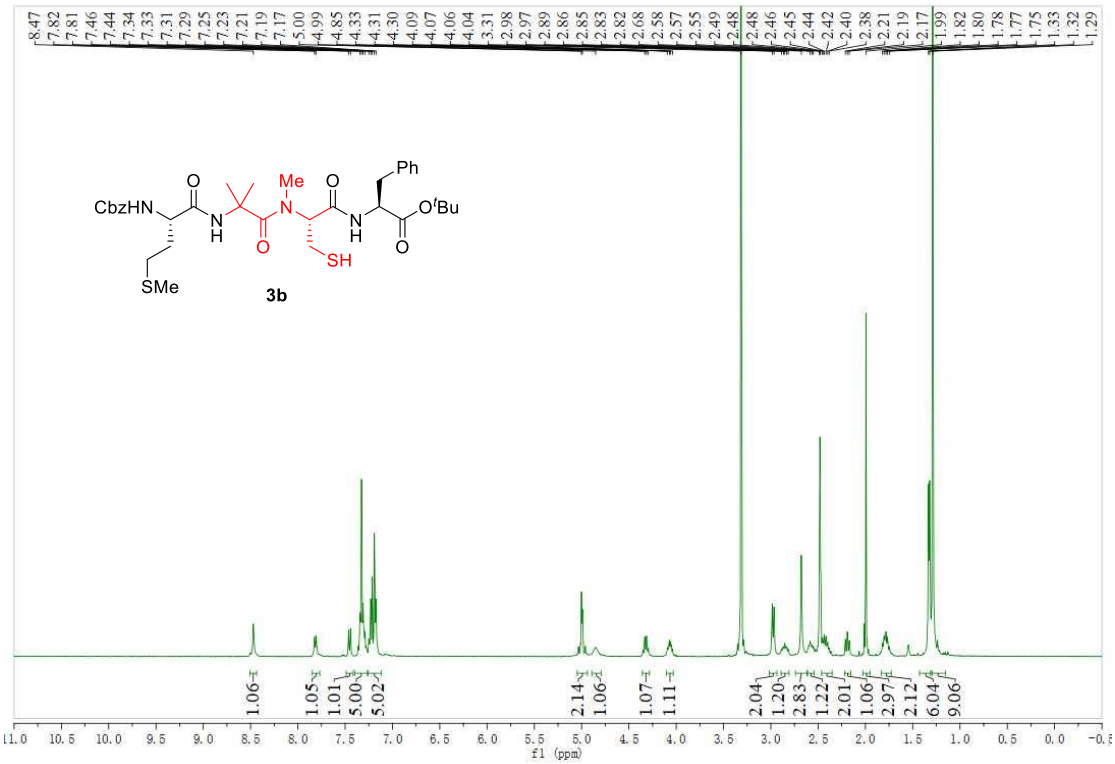
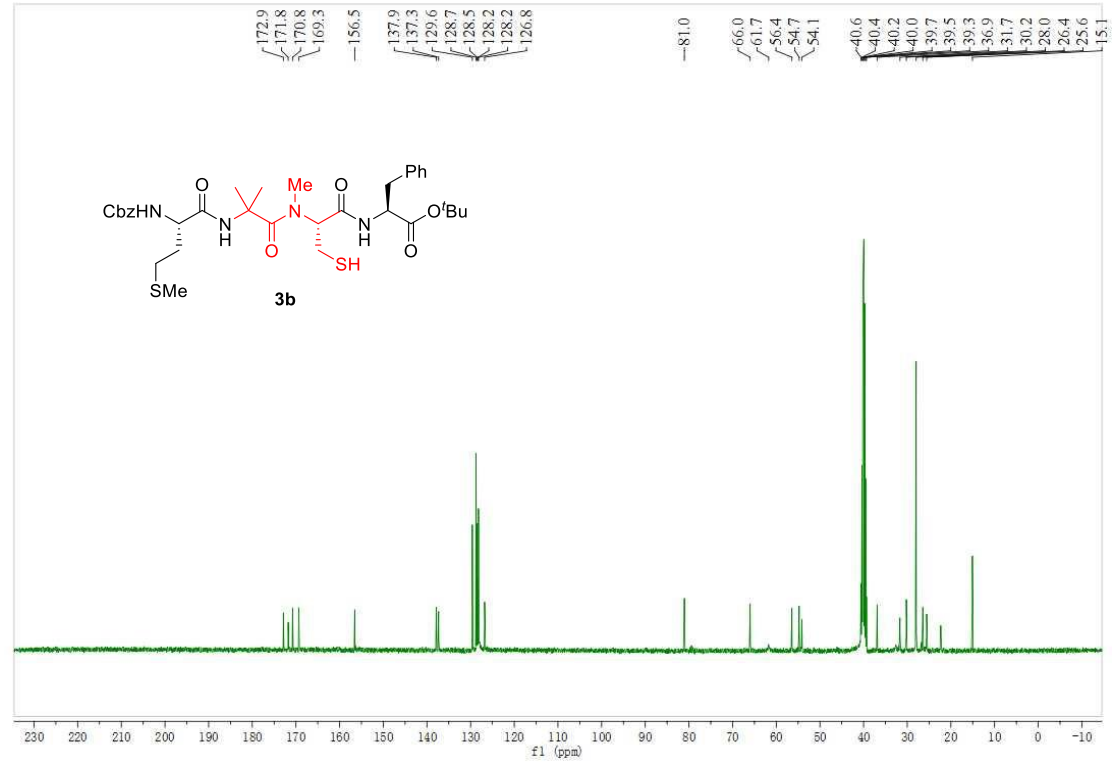
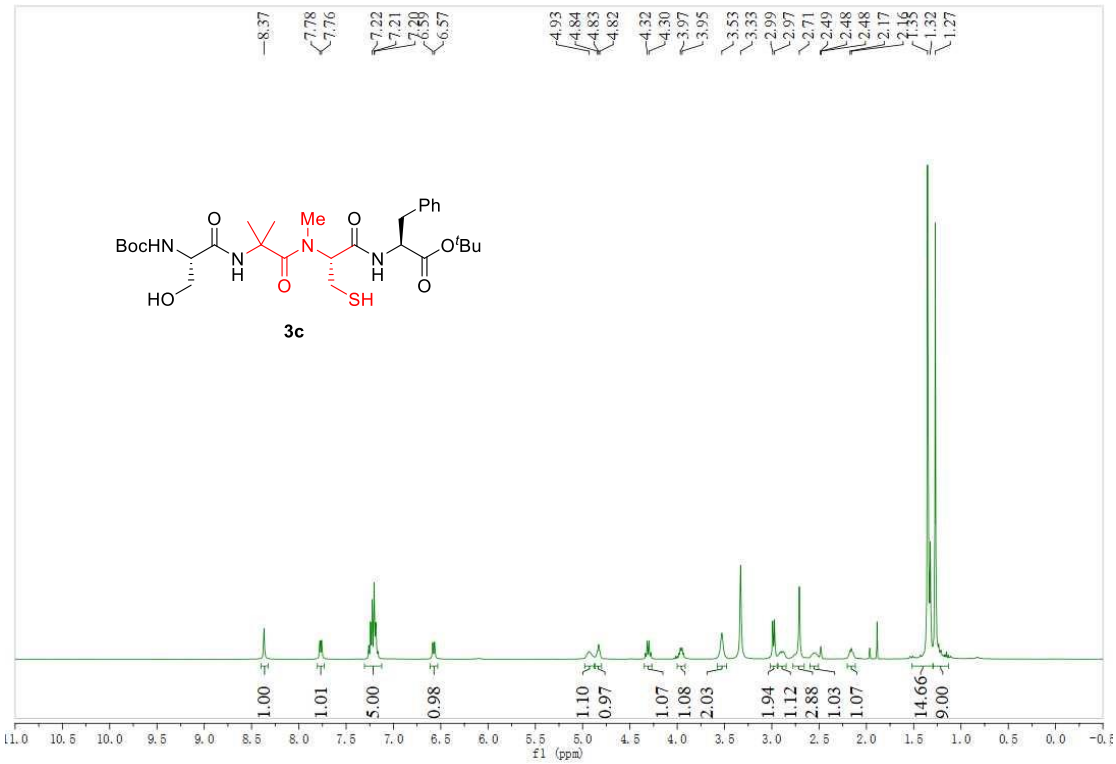
7.88
7.25
7.24
7.23
7.21
7.20
7.18
7.17
7.00
6.98
4.90
4.36
4.34
4.16
4.15
4.13
3.34
3.01
3.00
2.85
2.83
2.81
2.75
2.65
2.48
2.21
1.31
1.30
1.26
BocHN
Me
Ph
O
N
H
O
O
O
O
tBu
Ph
SH
3a
1.05
0.97
10.00
1.04
1.10
1.12
1.13
2.00
2.03
1.13
2.89
0.93
1.10
24.18
f1 (ppm)


172.8
171.9
170.8
169.3
155.8
138.3
138.0
129.7
129.6
128.5
128.4
126.8
126.7
81.1
78.5
61.6
56.3
56.0
54.7
40.6
40.4
40.2
39.9
39.7
39.5
39.3
37.6
36.7
28.5
28.0
26.5
25.4
22.3
BocHN
Me
Ph
O
N
H
O
O
O
O
tBu
Ph
SH
3a
f1 (ppm)


CbzHN
Me
Ph
O^{t}Bu
SH
SMe
3b
f1 (ppm)


CbzHN
Me
Ph
O^{t}Bu
SH
SMe
3b
f1 (ppm)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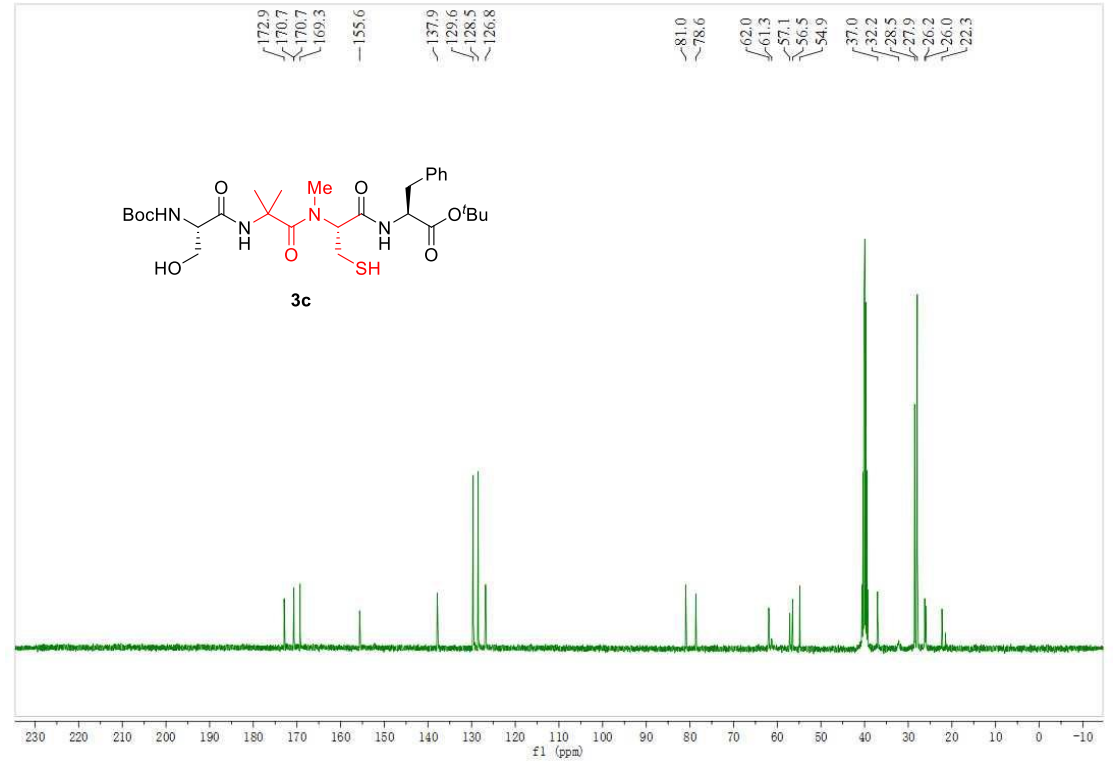
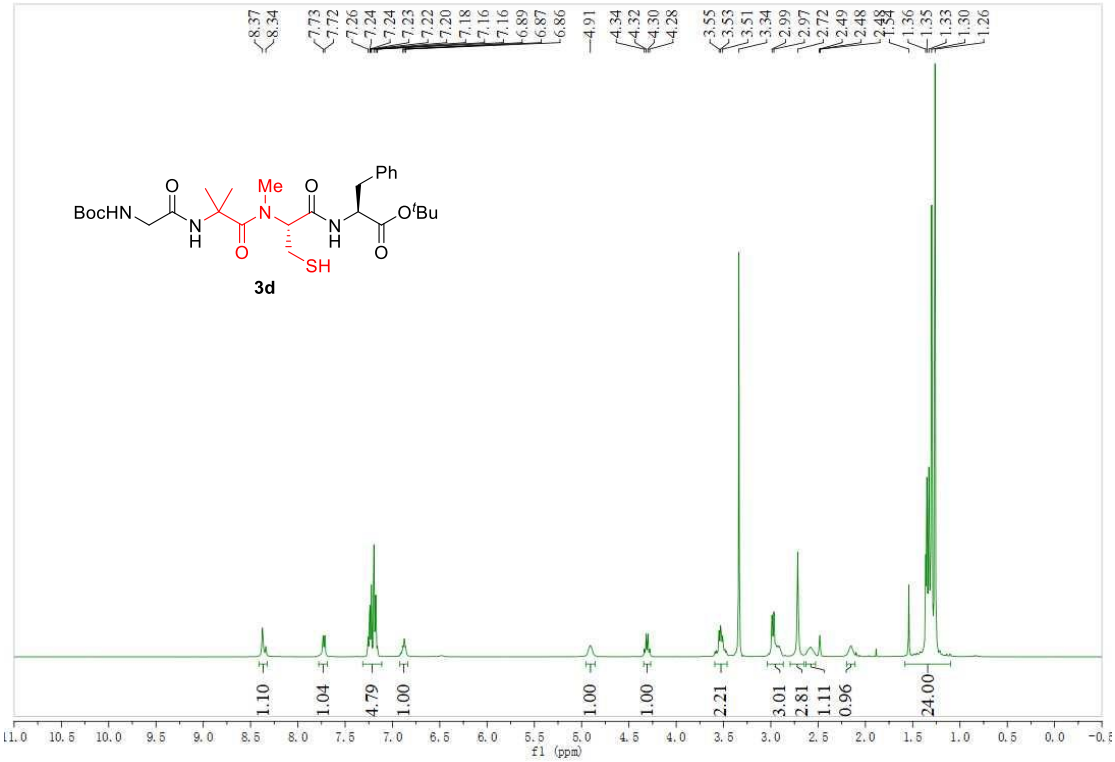
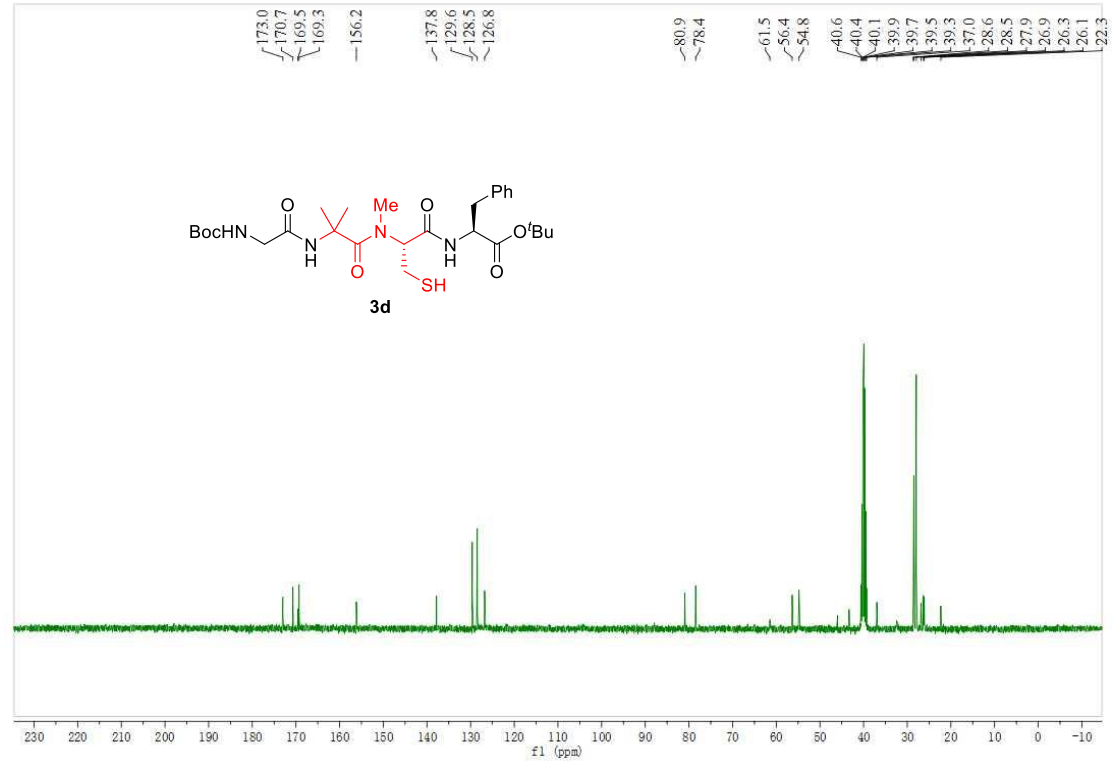
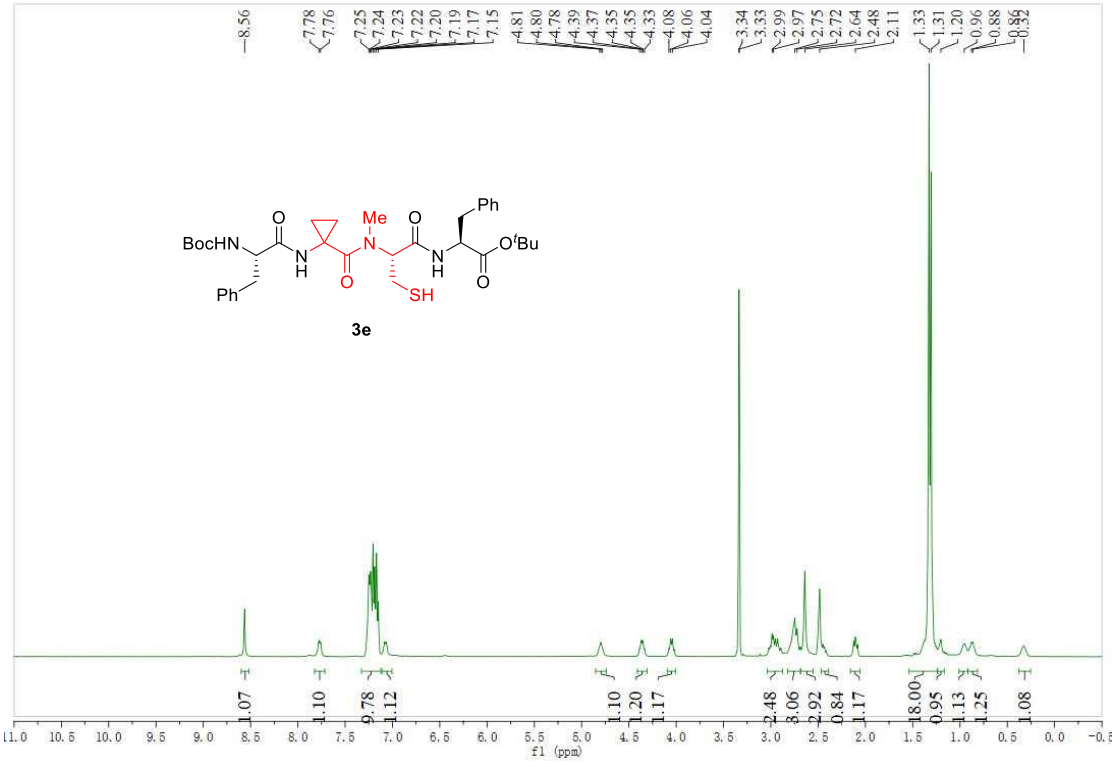
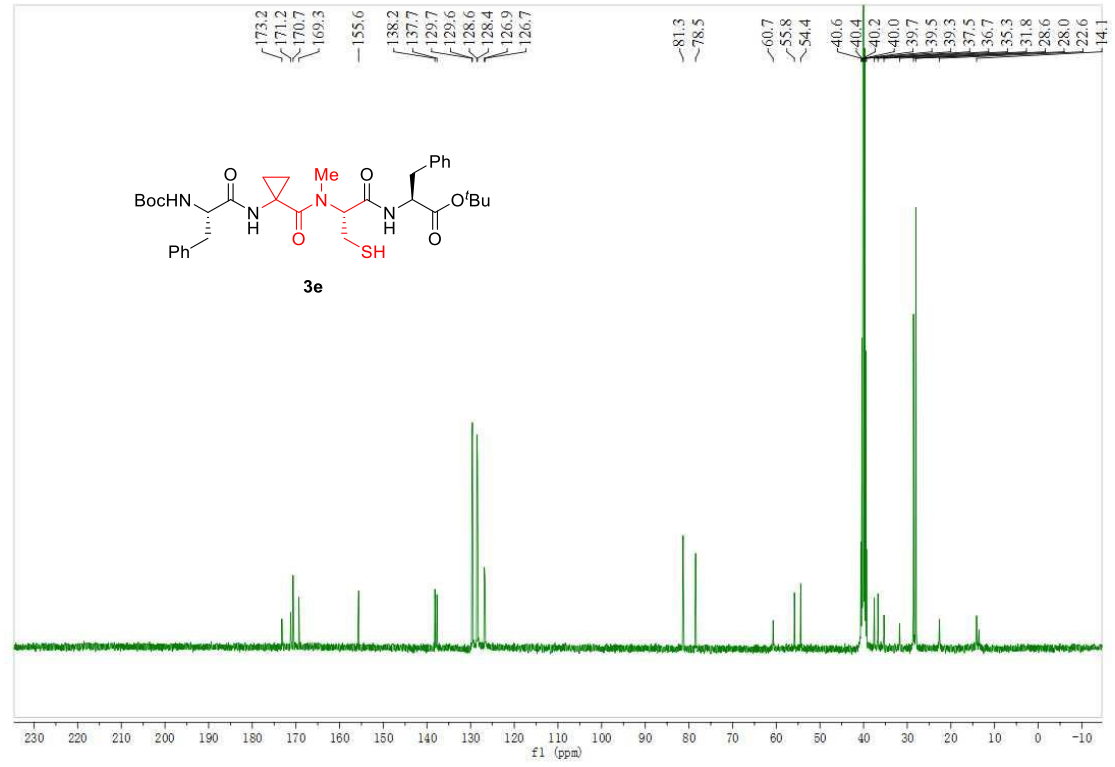
7.78
7.76
7.22
7.21
6.59
6.57
4.93
4.84
4.83
4.82
4.32
4.30
3.97
3.95
3.53
3.33
2.99
2.97
2.71
2.49
2.48
2.17
1.35
1.32
1.27
BocHN
HO
Me
Ph
O'Bu
SH
3c
1.00
1.01
5.00
0.98
1.10
0.97
1.07
1.08
2.03
1.94
1.12
2.88
1.03
1.07
14.66
9.00
f1 (ppm)

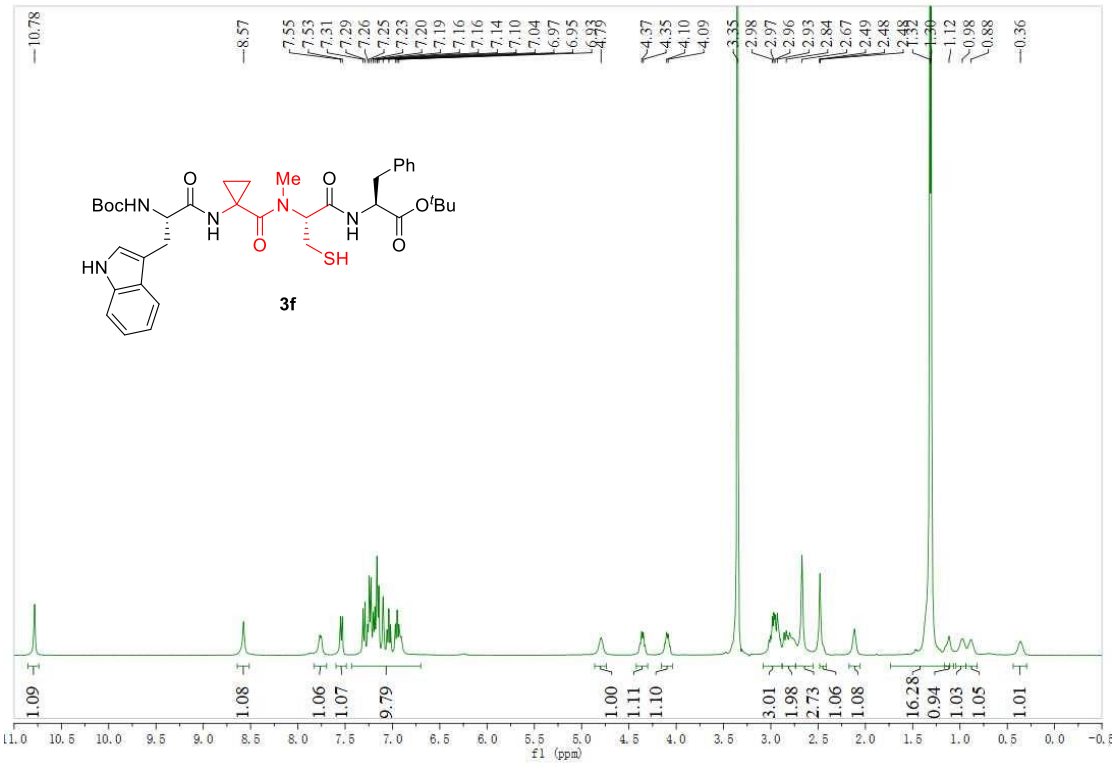

172.9
170.7
170.7
169.3
155.6
137.9
129.6
128.5
126.8
81.0
78.6
62.0
61.3
57.1
56.5
54.9
37.0
32.2
28.5
27.9
26.2
26.0
22.3
BocHN
HO
Me
Ph
O'Bu
SH
3c
f1 (ppm)

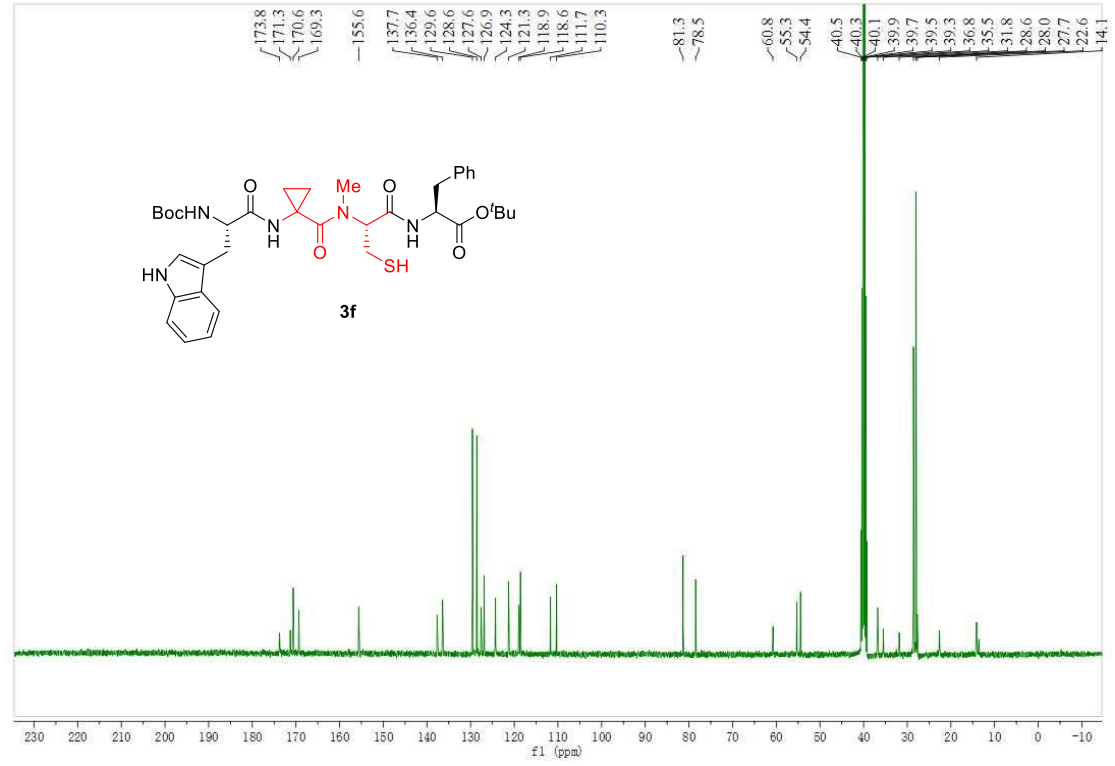

BocHN
Me
Ph
O^{t}Bu
SH
3d
f1 (ppm)

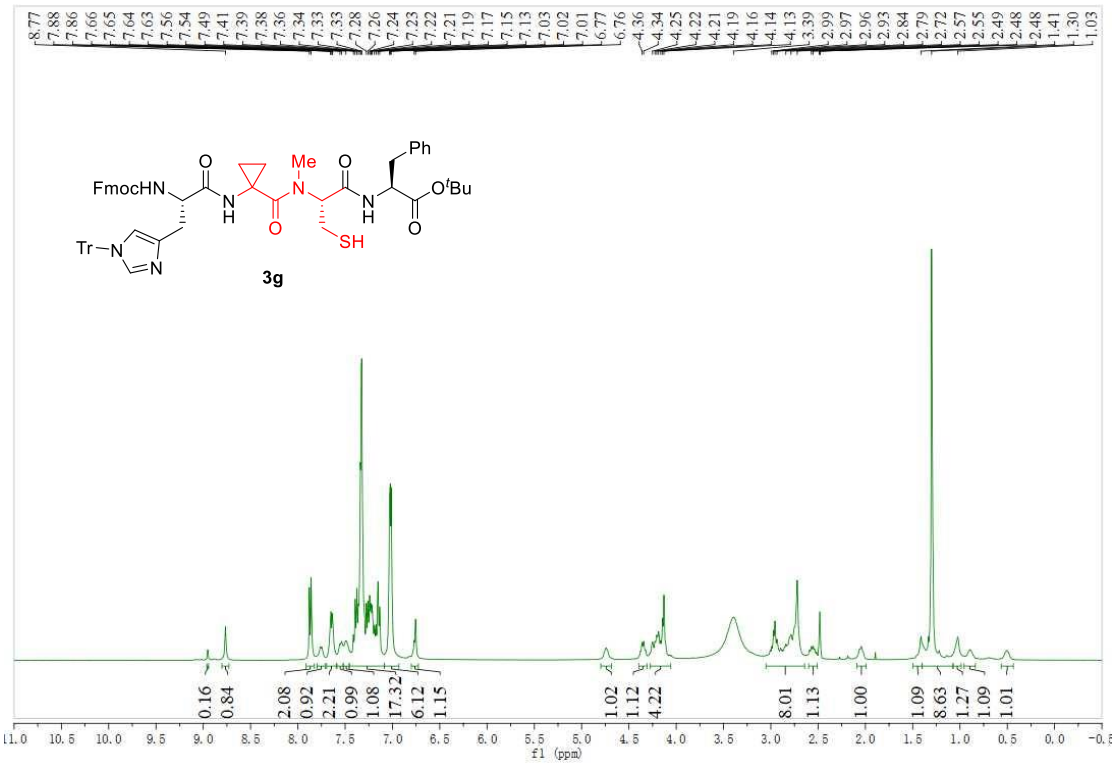

BocHN
Me
Ph
O^{t}Bu
SH
3d
f1 (ppm)

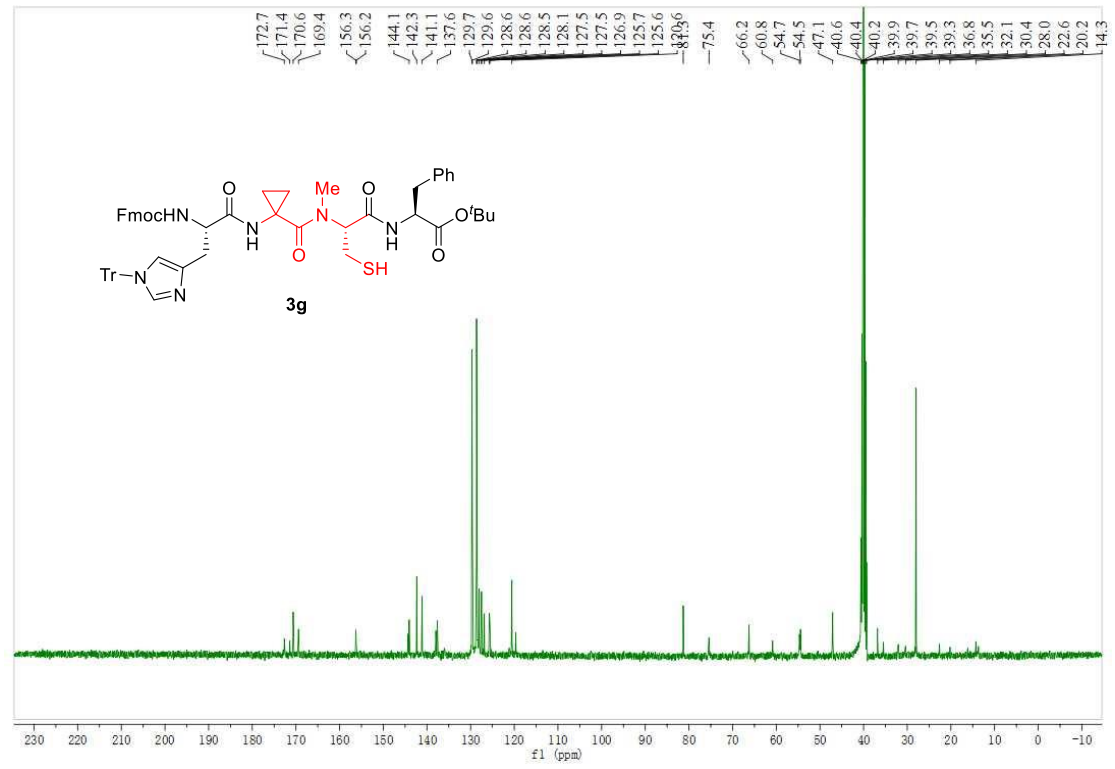

BocHN
Ph
Me
O
N
H
SH
O'Bu
3e
f1 (ppm)

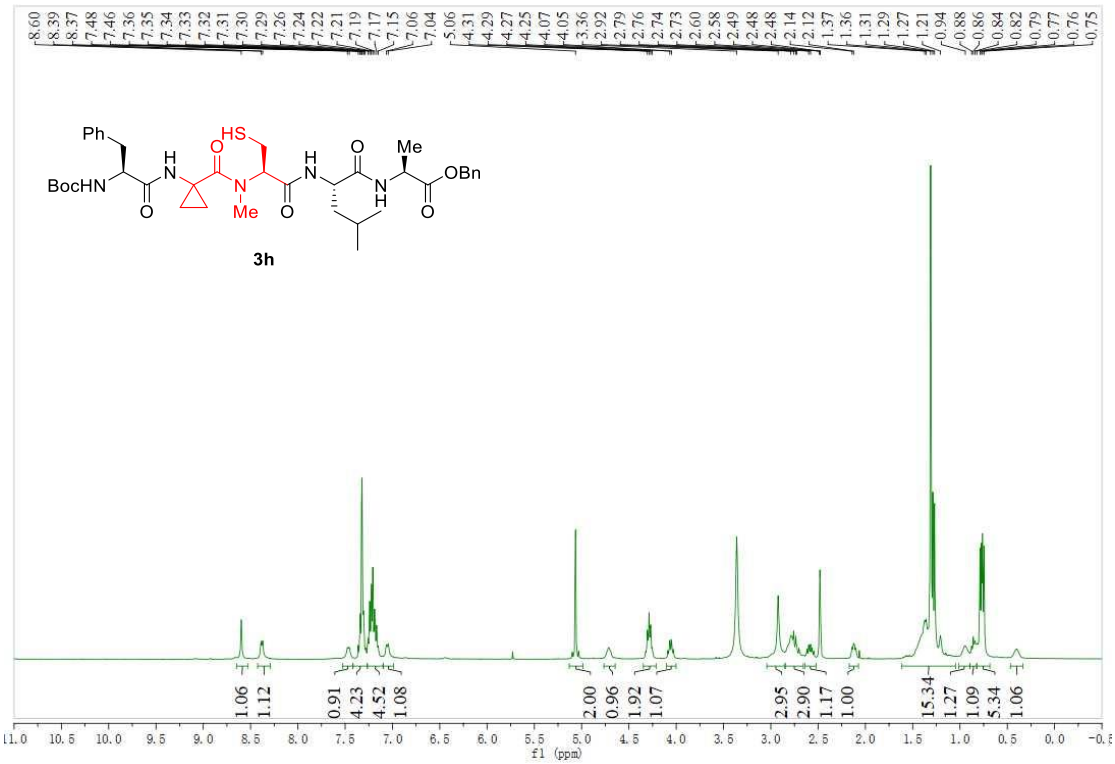

BocHN
Ph
Me
O
N
H
SH
O'Bu
3e
f1 (ppm)

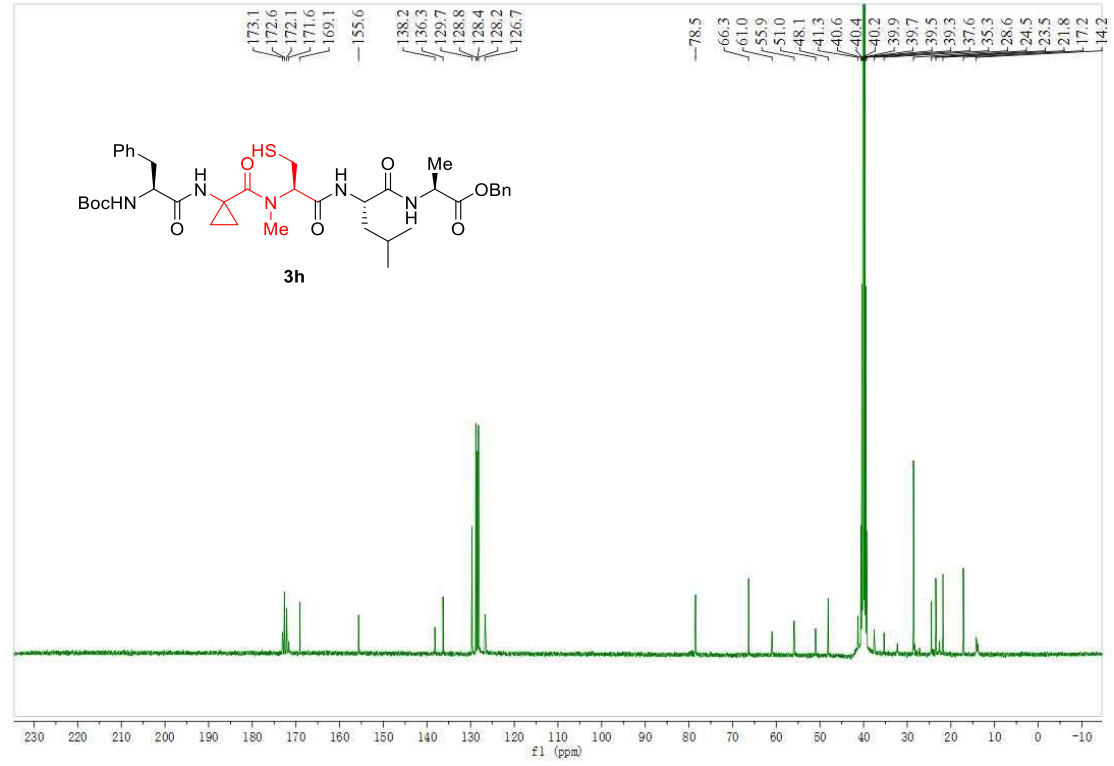

BocHN
Me
Ph
O^{t}Bu
HN
SH
3f

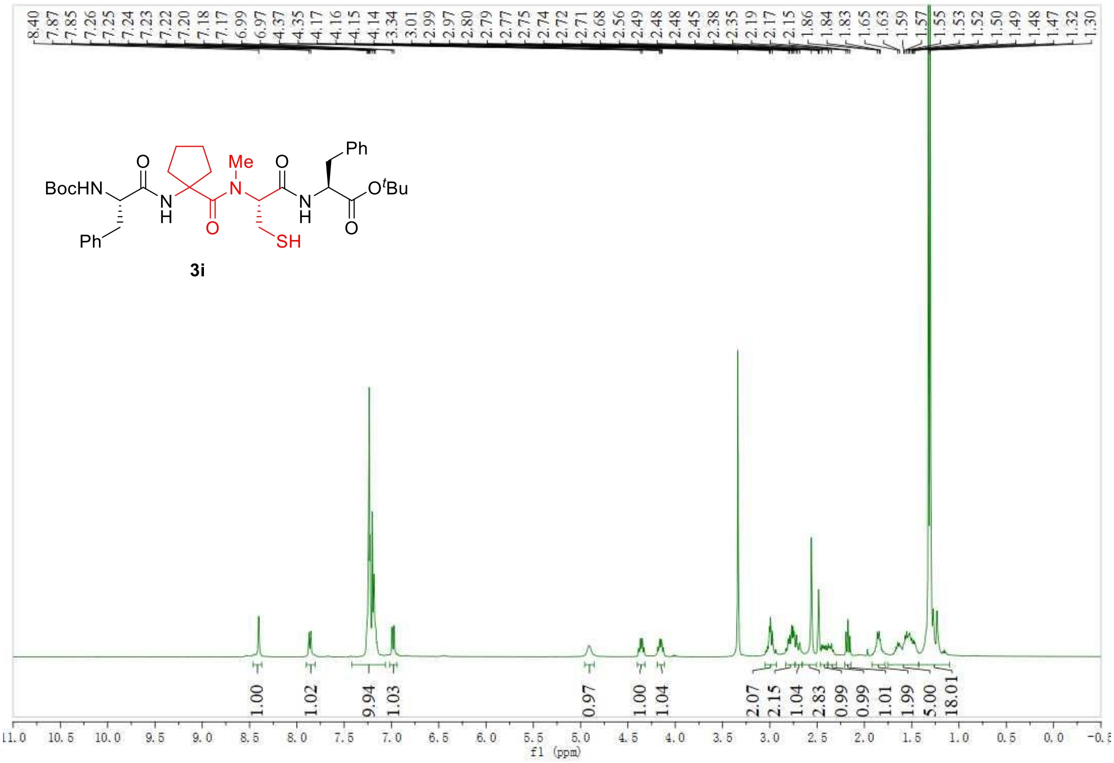

BocHN
Me
Ph
O^{t}Bu
HN
SH
3f


FmocHN
Me
Ph
O^{t}Bu
Tr
SH
3g
f1 (ppm)


FmocHN
Me
Ph
O^{t}Bu
Tr
SH
3g
f1 (ppm)


Ph
BocHN
HS
Me
OBn
3h
f1 (ppm)


Ph
BocHN
HS
Me
OBn
3h
f1 (ppm)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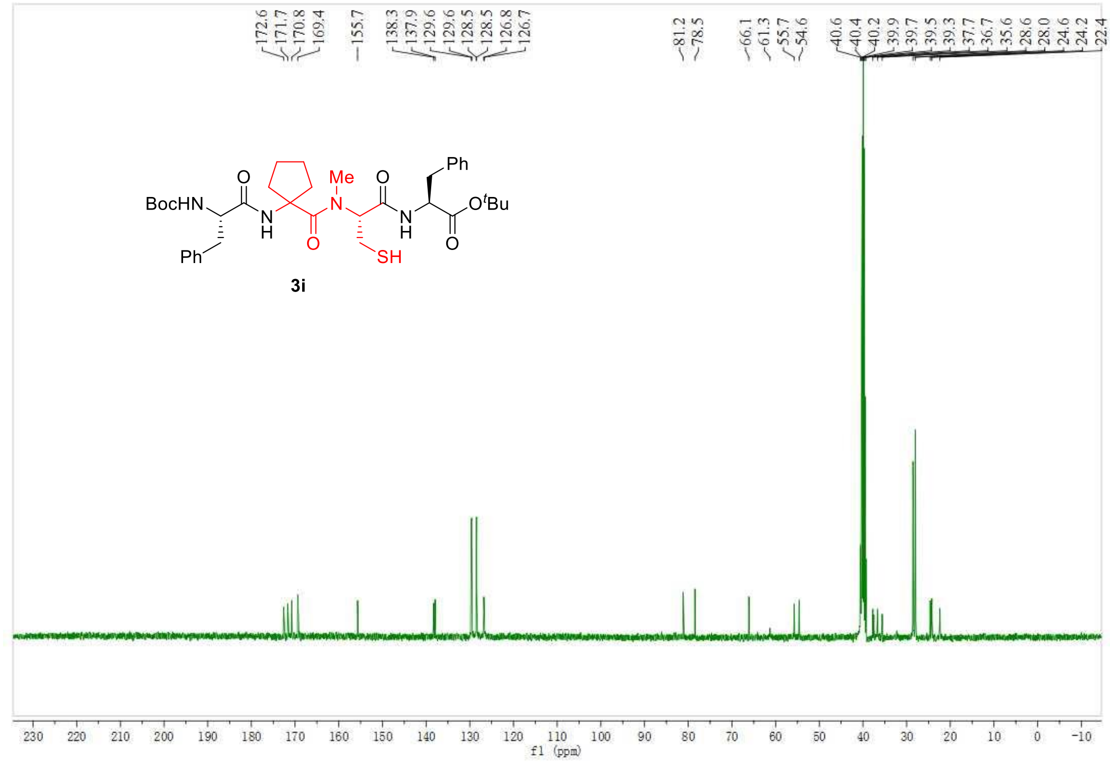
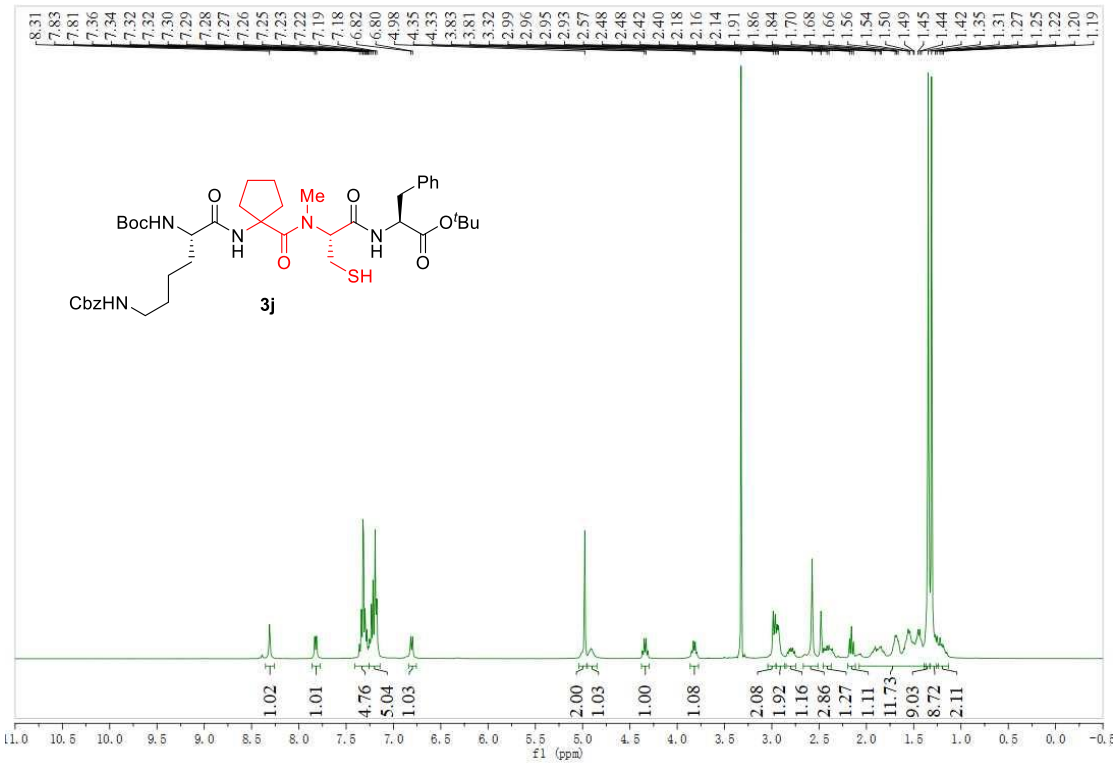
BocHN
Ph
O
N
H
O
Me
N
SH
O
N
H
Ph
O^{t}Bu
O
1.00 1.02 9.94 1.03 0.97 1.00 1.04 2.07 2.15 1.04 2.83 0.99 0.99 1.01 1.99 5.00 18.01
f1 (ppm)

3i


172.6 171.7 170.8 169.4 155.7 138.3 137.9 129.6 129.6 128.5 128.5 126.8 126.7 81.2 78.5 66.1 61.3 55.7 54.6 40.6 40.4 40.2 39.9 39.7 39.5 39.3 37.7 36.7 35.6 28.6 28.0 24.6 24.2 22.4
BocHN
Ph
O
N
H
O
Me
N
SH
O
N
H
Ph
O^{t}Bu
O
f1 (ppm)

3i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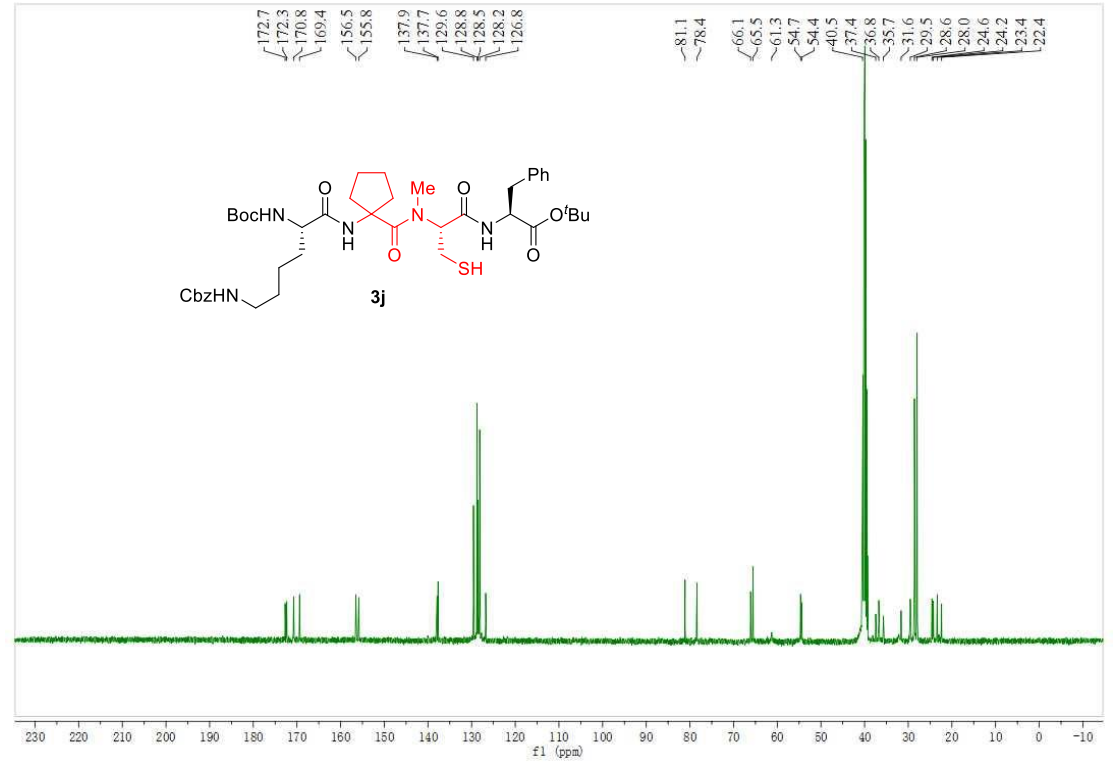
CbzHN
Me
Ph
O^{t}Bu
SH
3j
f1 (ppm)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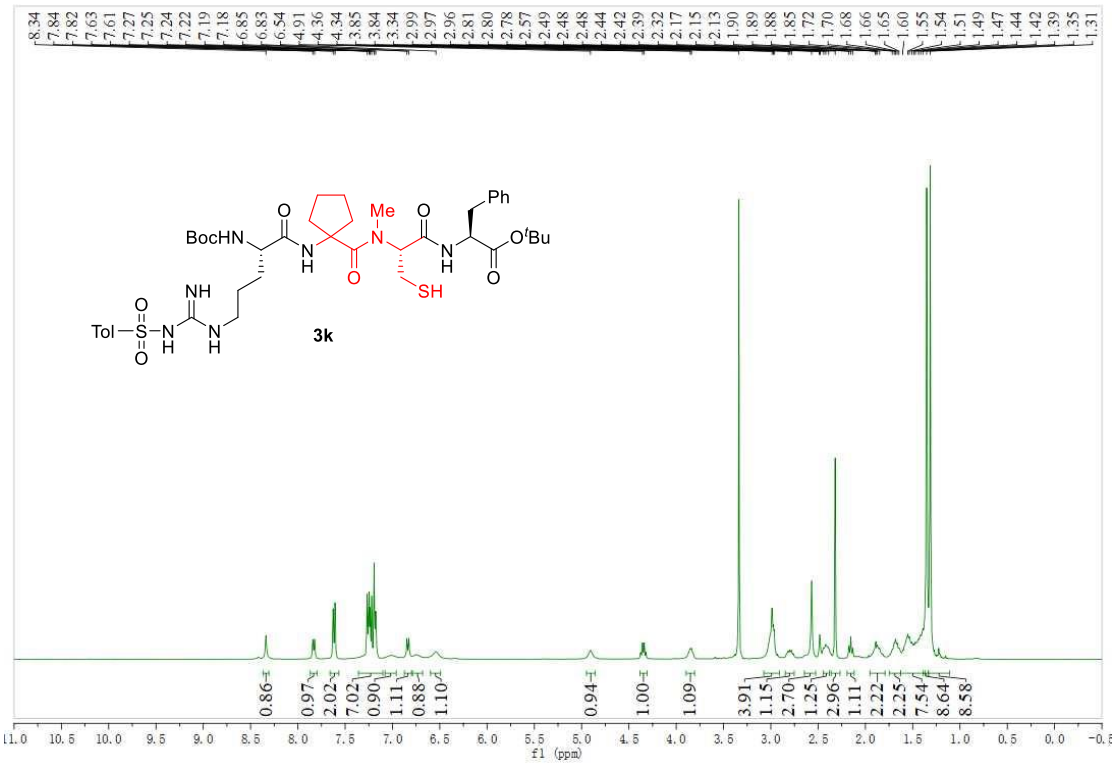
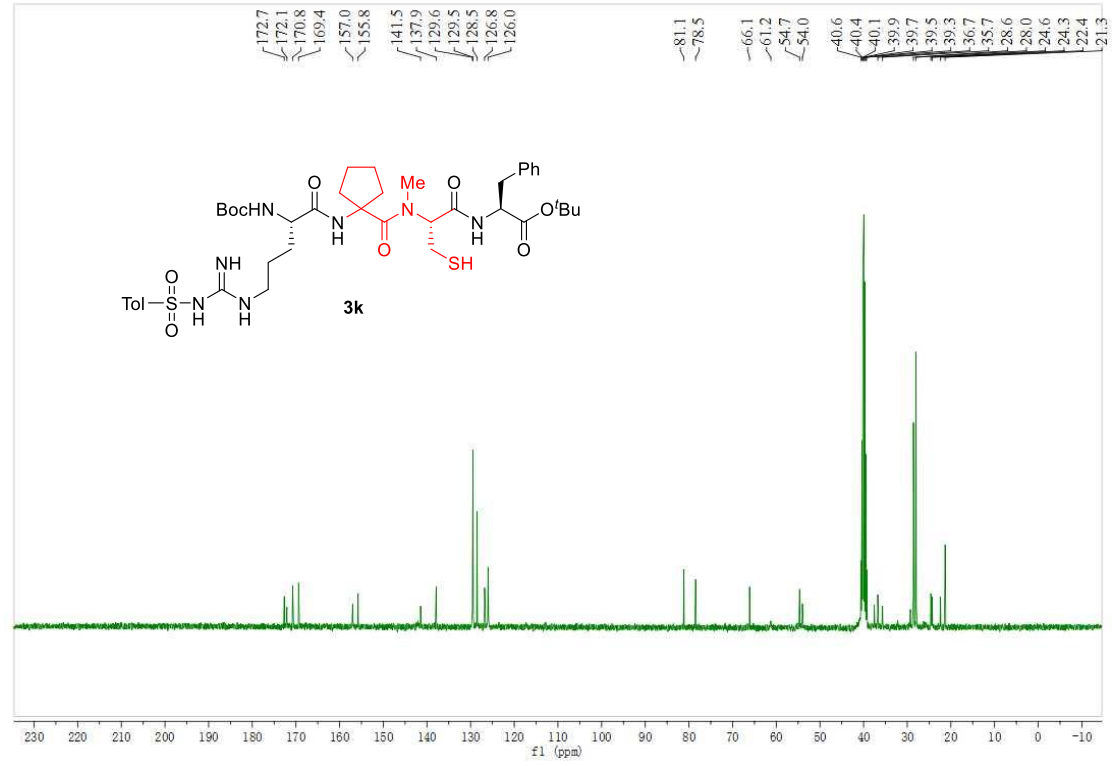
CbzHN
Me
Ph
O^{t}Bu
SH
3j
f1 (ppm)


3k
f1 (ppm)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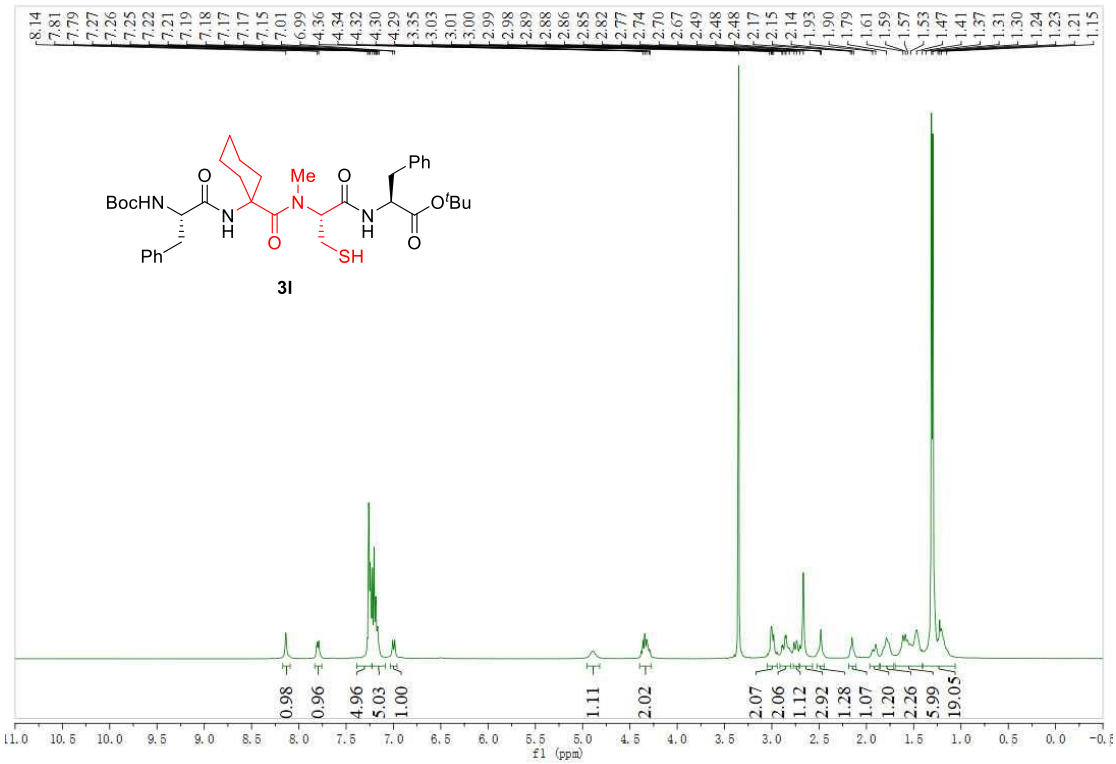
BocHN
Ph
Me
N
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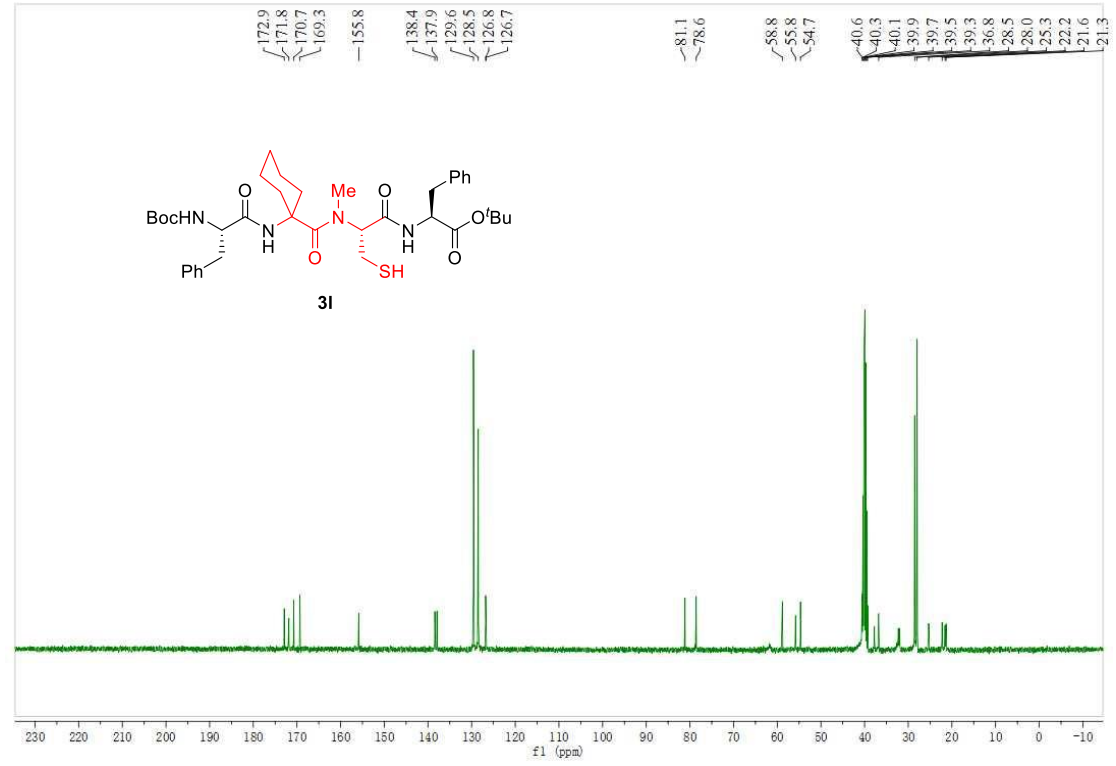
H
SH
Ph
O
tBu
3l
f1 (ppm)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SH
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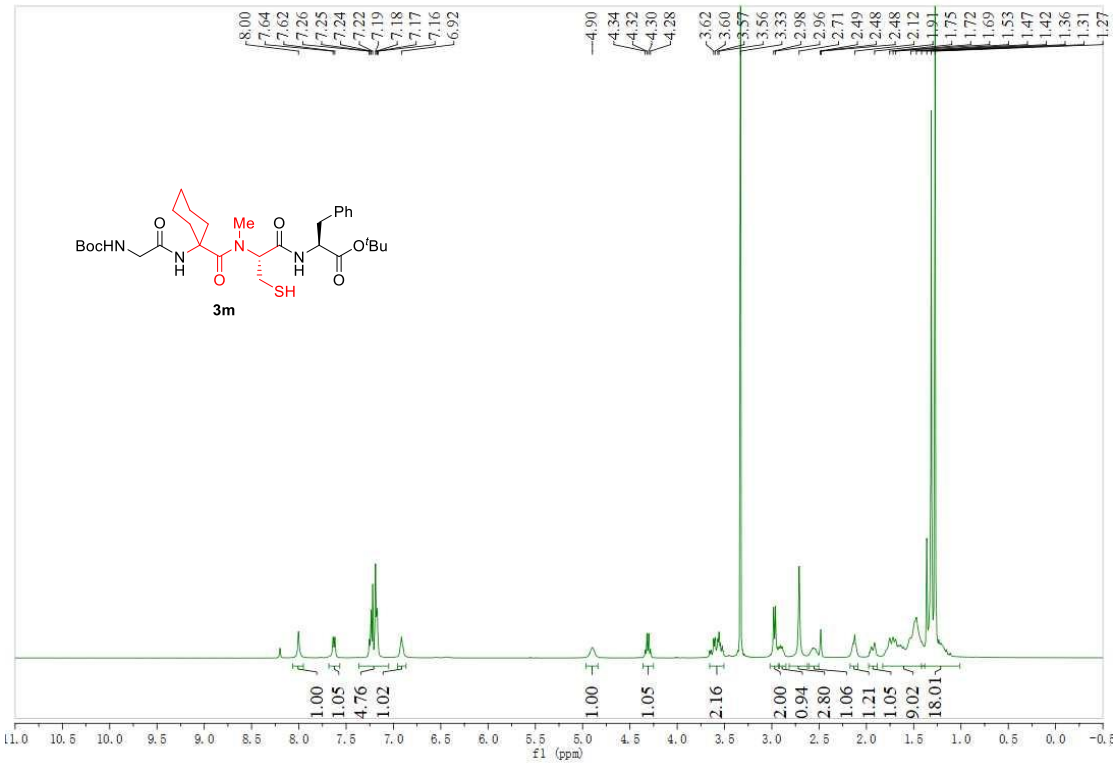
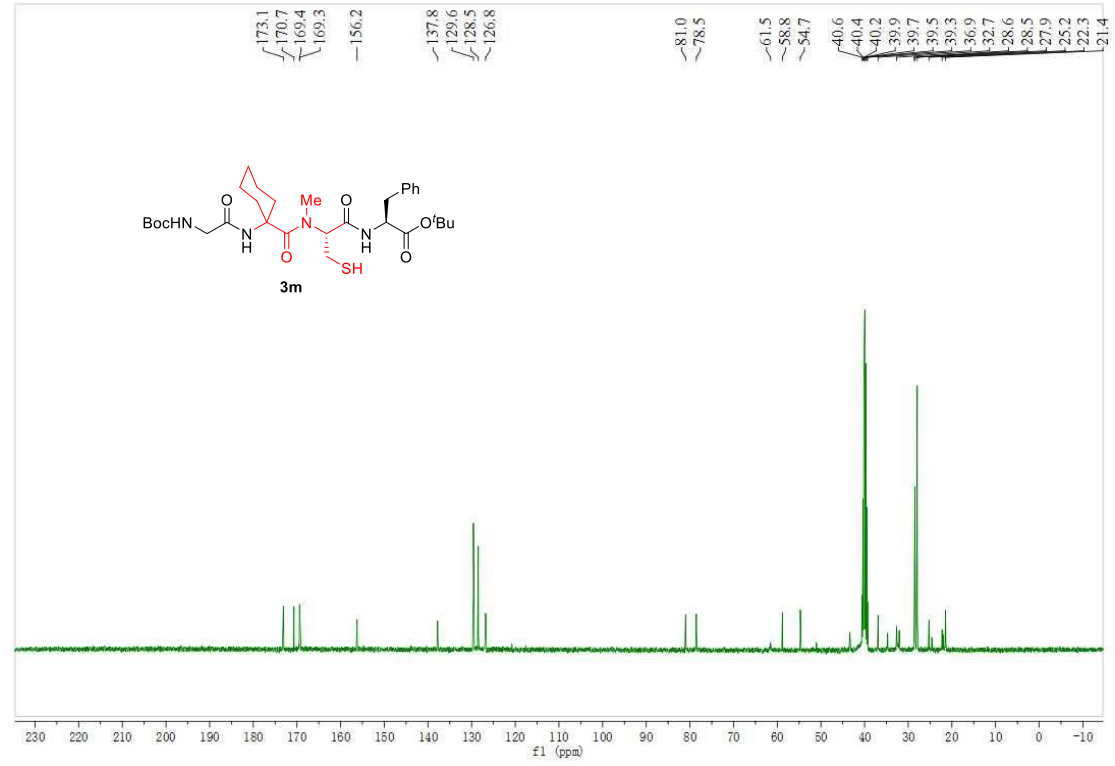
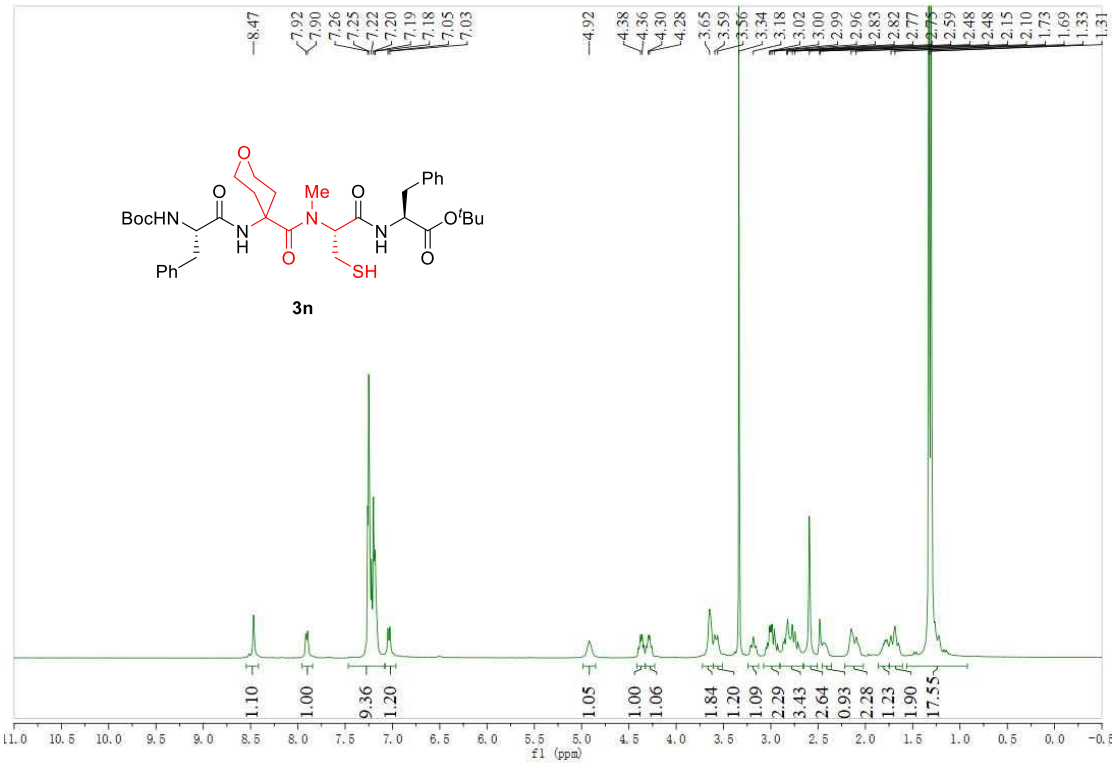
tBu
3l
f1 (ppm)


BocHN
Me
Ph
O^{t}Bu
SH
3m
f1 (ppm)


BocHN
Me
Ph
O^{t}Bu
SH
3m
f1 (ppm)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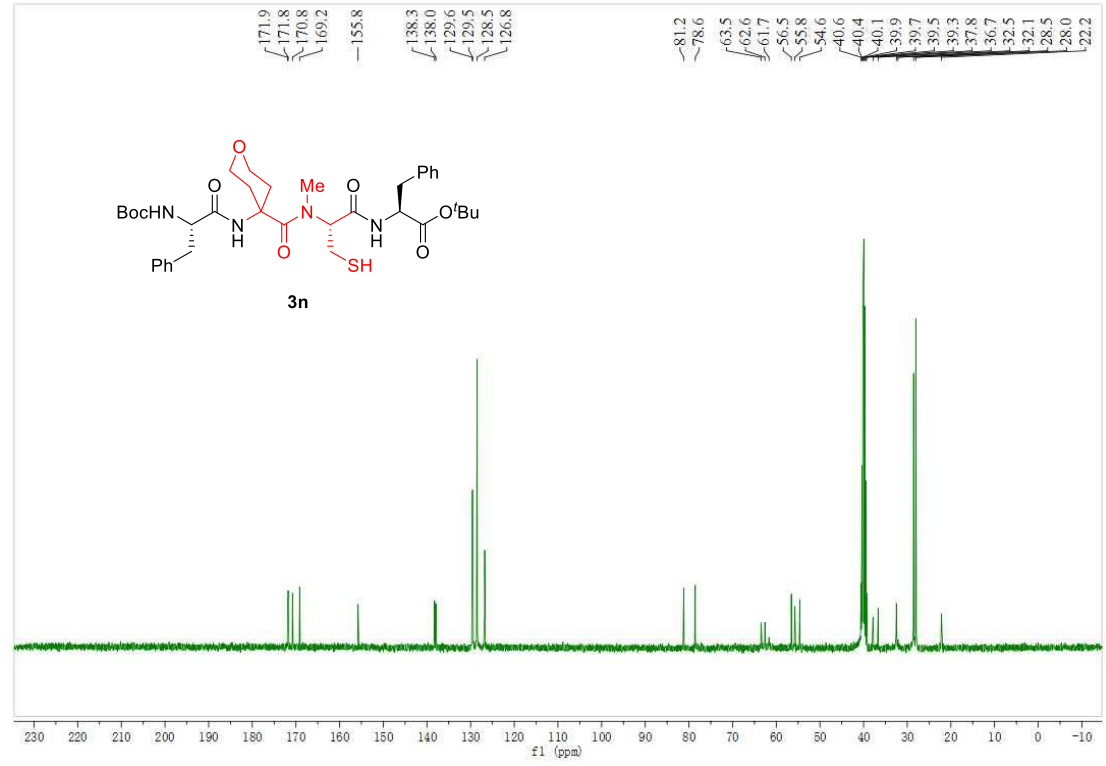
Me
Ph
O^{t}Bu
SH
3n
f1 (ppm)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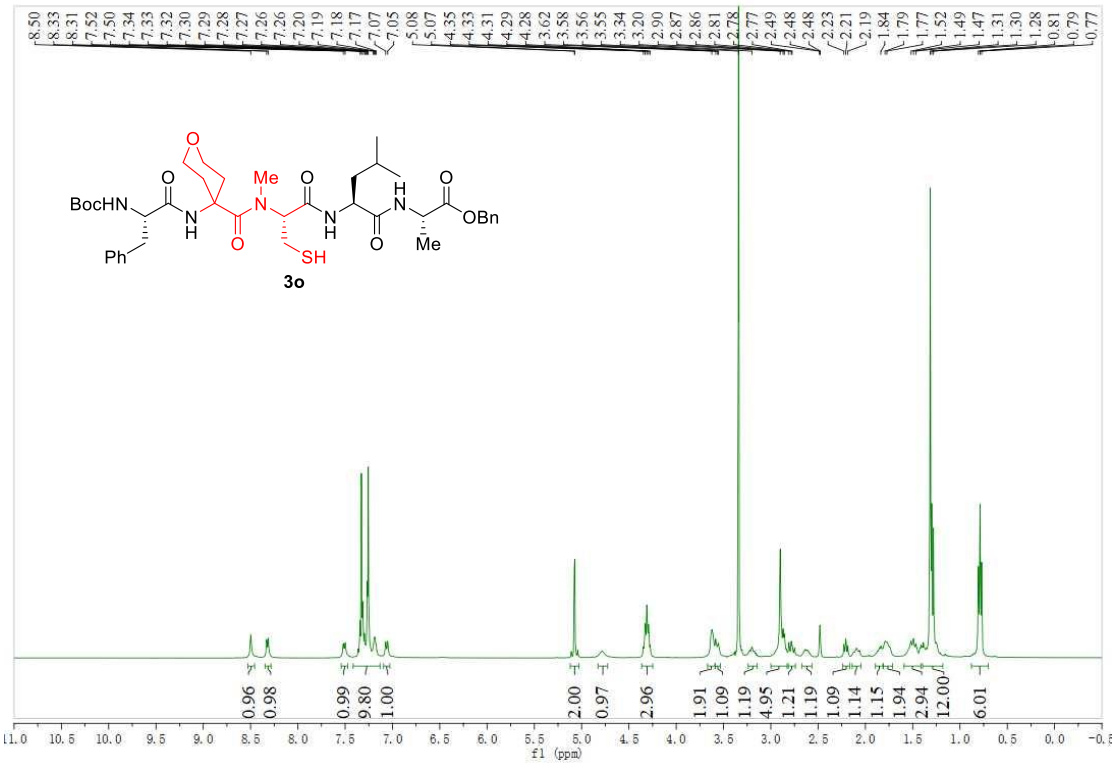
f1 (ppm)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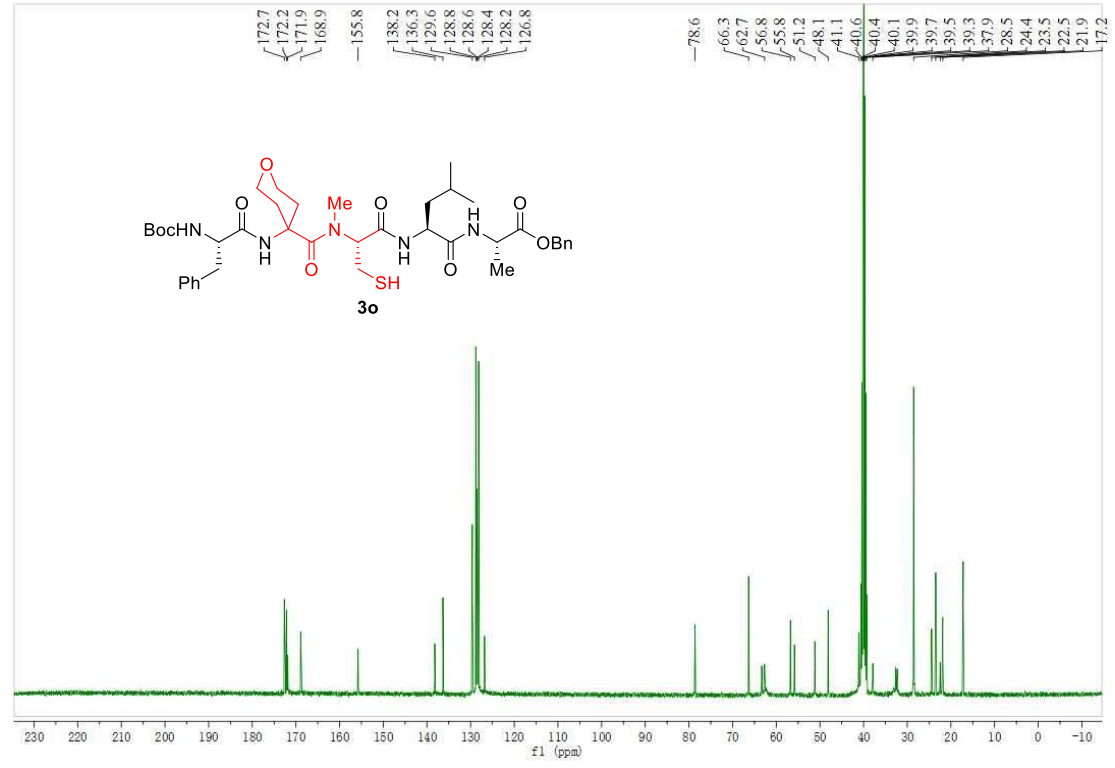
Me
SH
OBn
Me
3o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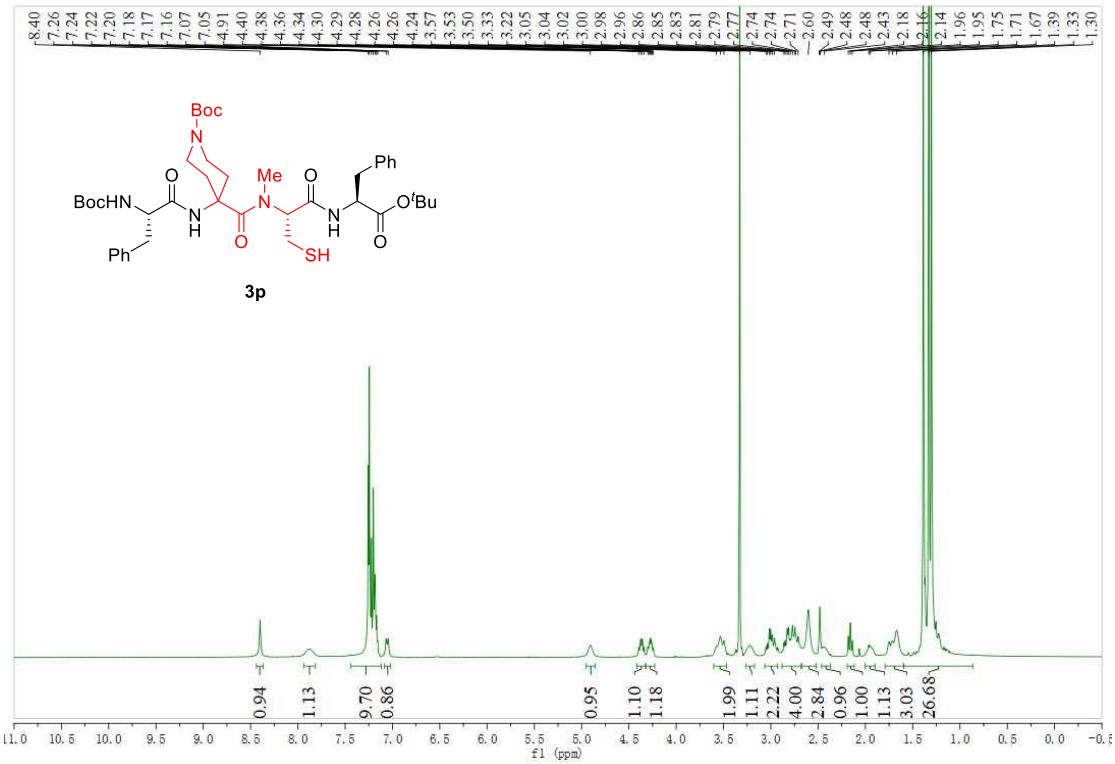
SH
OBn
Me
3o
f1 (ppm)


Boc
N
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N
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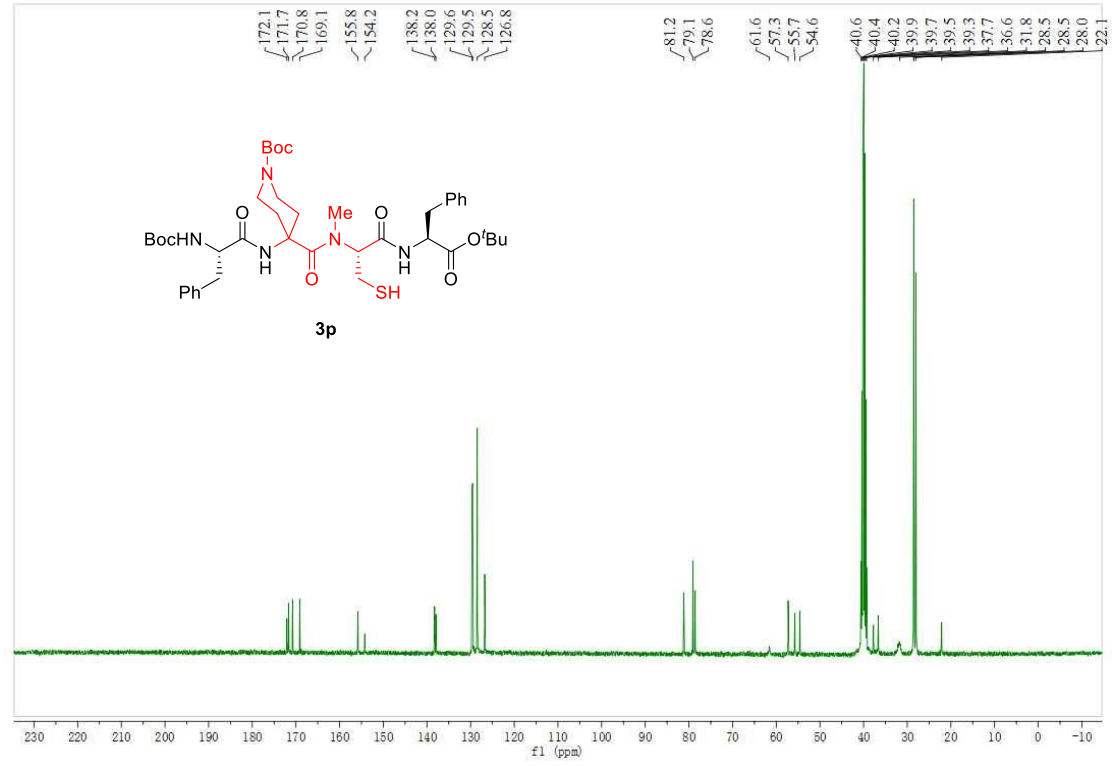
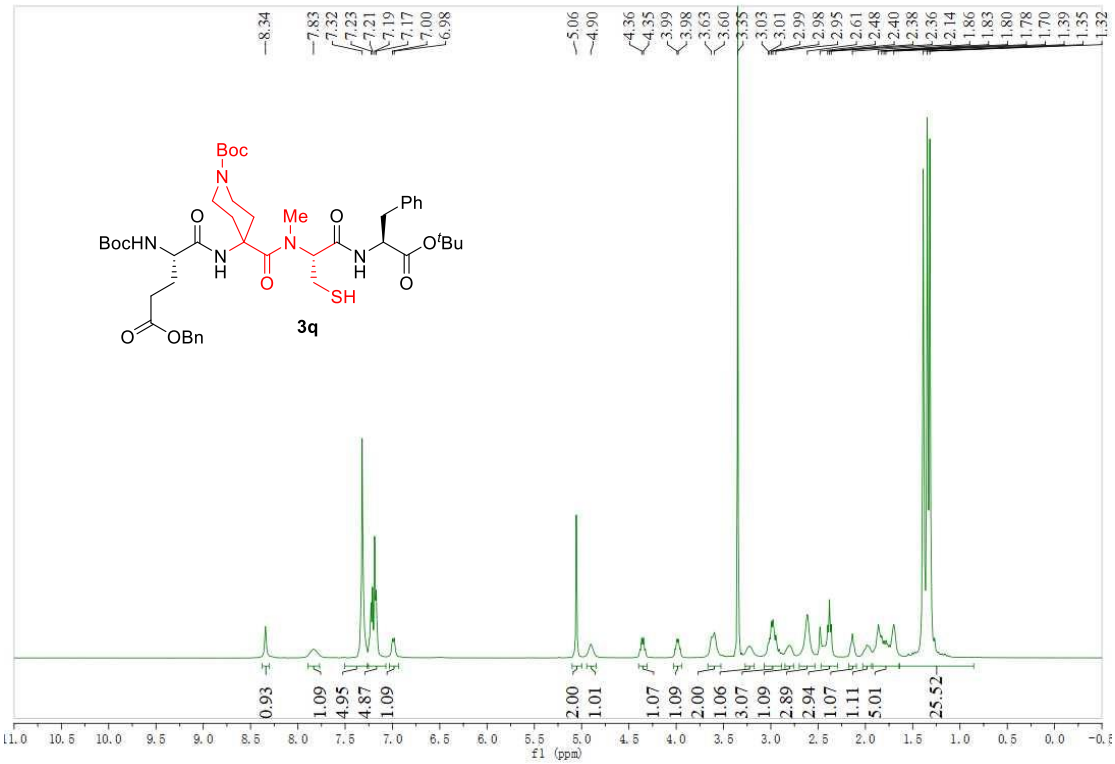
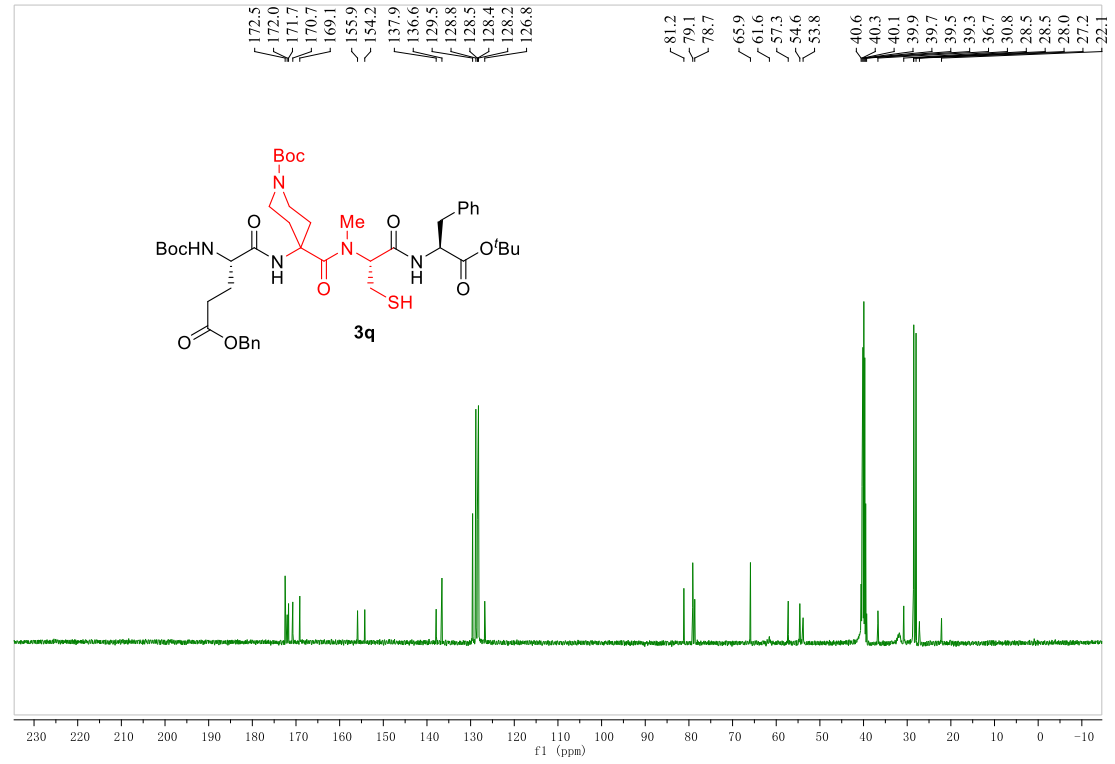
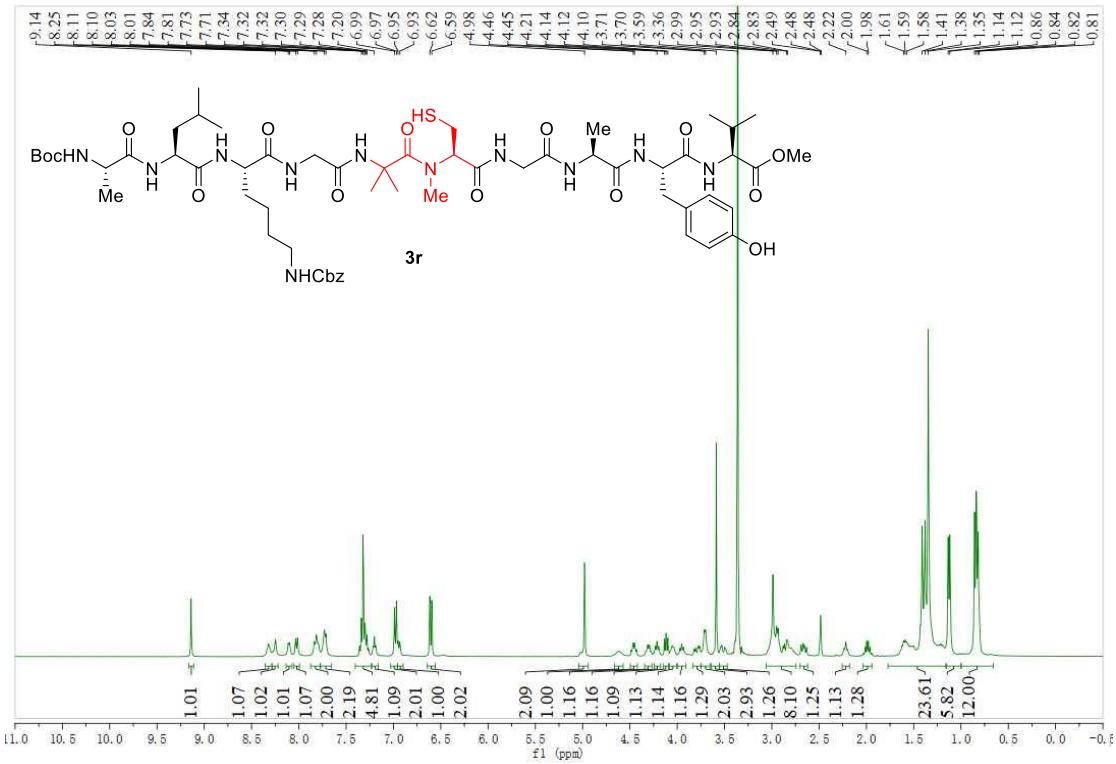
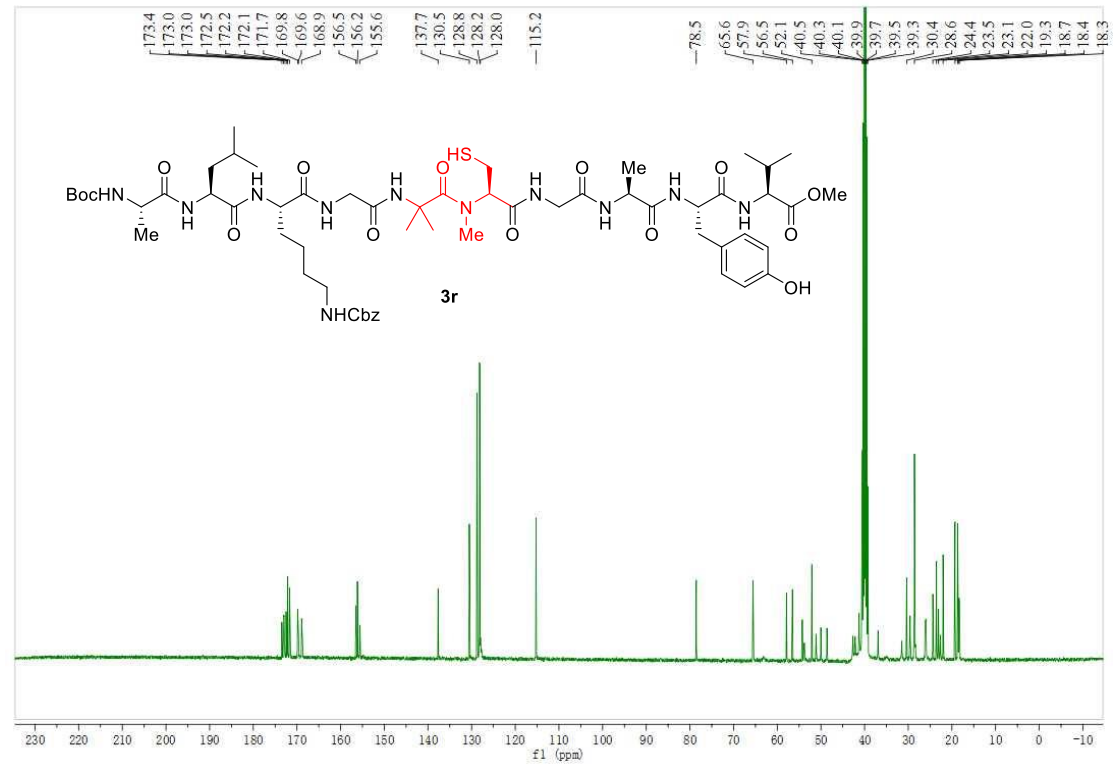
Ph
O
N
H
Ph
O
O^{t}Bu
SH
3p
f1 (ppm)

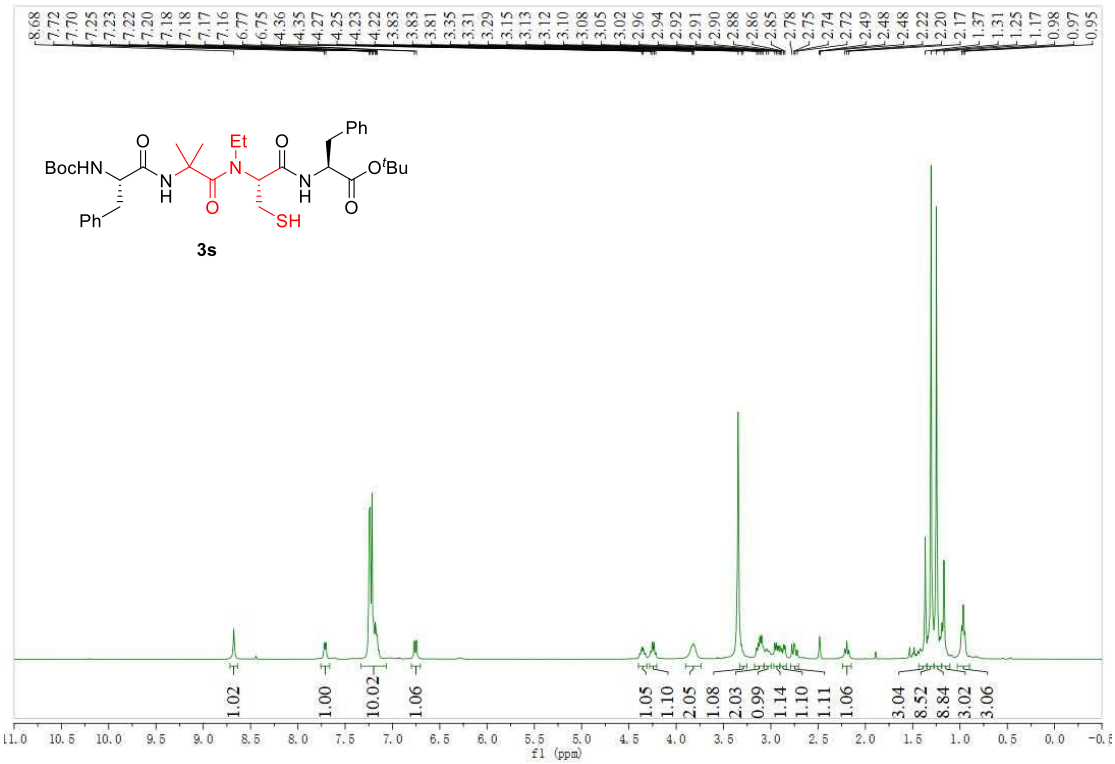

Boc
N
Me
O
BocHN
N
H
N
O
Ph
O
N
H
Ph
O
O^{t}Bu
SH
3p
f1 (ppm)

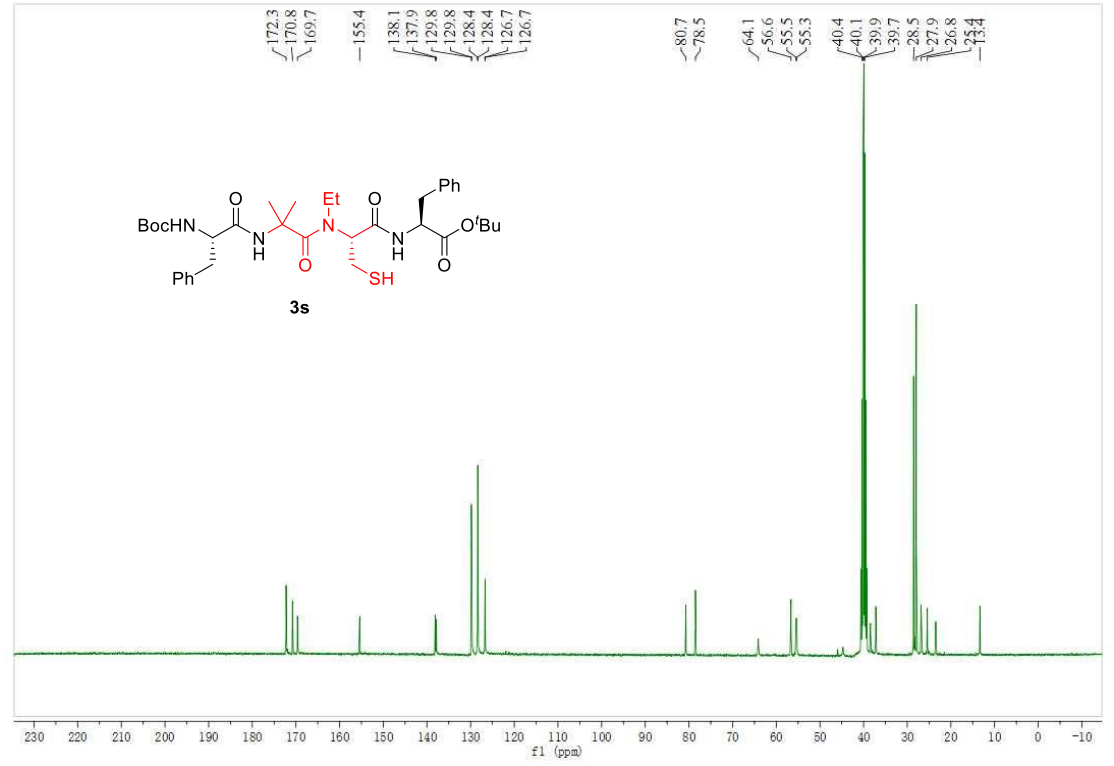

Boc
N
BocHN
O
N
H
Me
N
O
O
N
H
Ph
O
O
tBu
SH
O
OBn
3q
f1 (ppm)

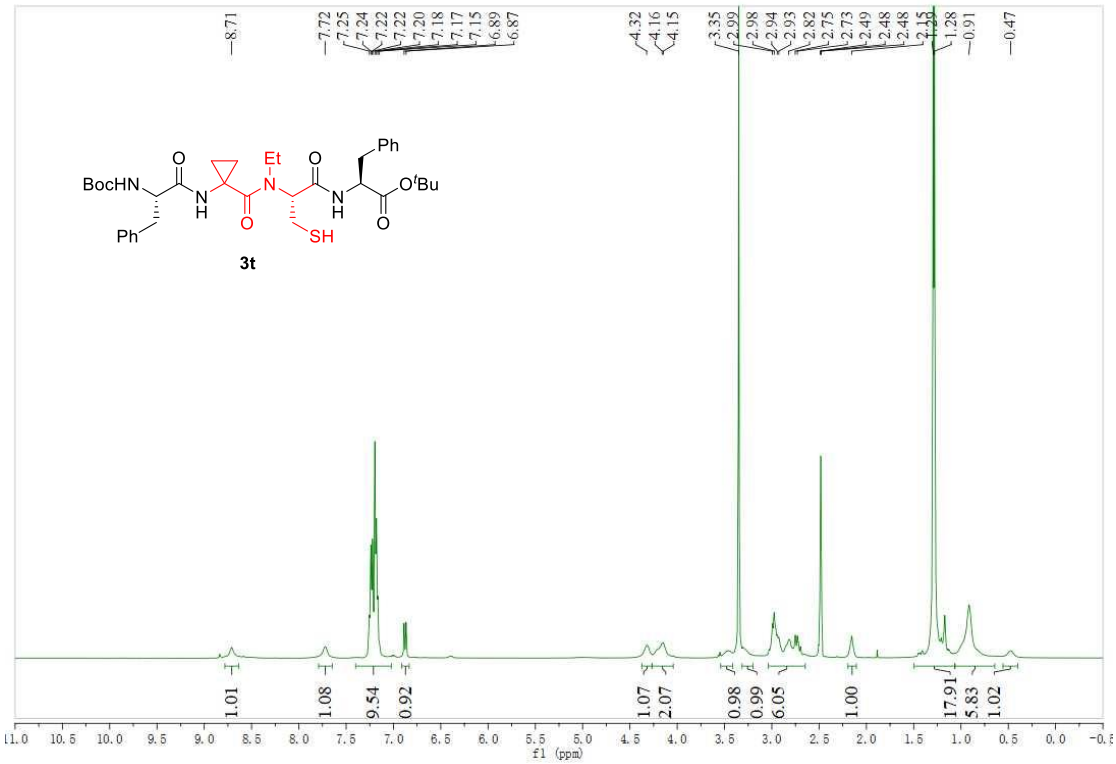

Boc
N
BocHN
O
N
H
Me
N
O
O
N
H
Ph
O
O
tBu
SH
O
OBn
3q
f1 (ppm)


BocHN
Me
HS
N
Me
OMe
NHCbz
OH
3r
f1 (ppm)


BocHN
Me
HS
N
Me
OMe
NHCbz
OH
3r
f1 (ppm)


3s
f1 (ppm)


3s
f1 (ppm)


8.71
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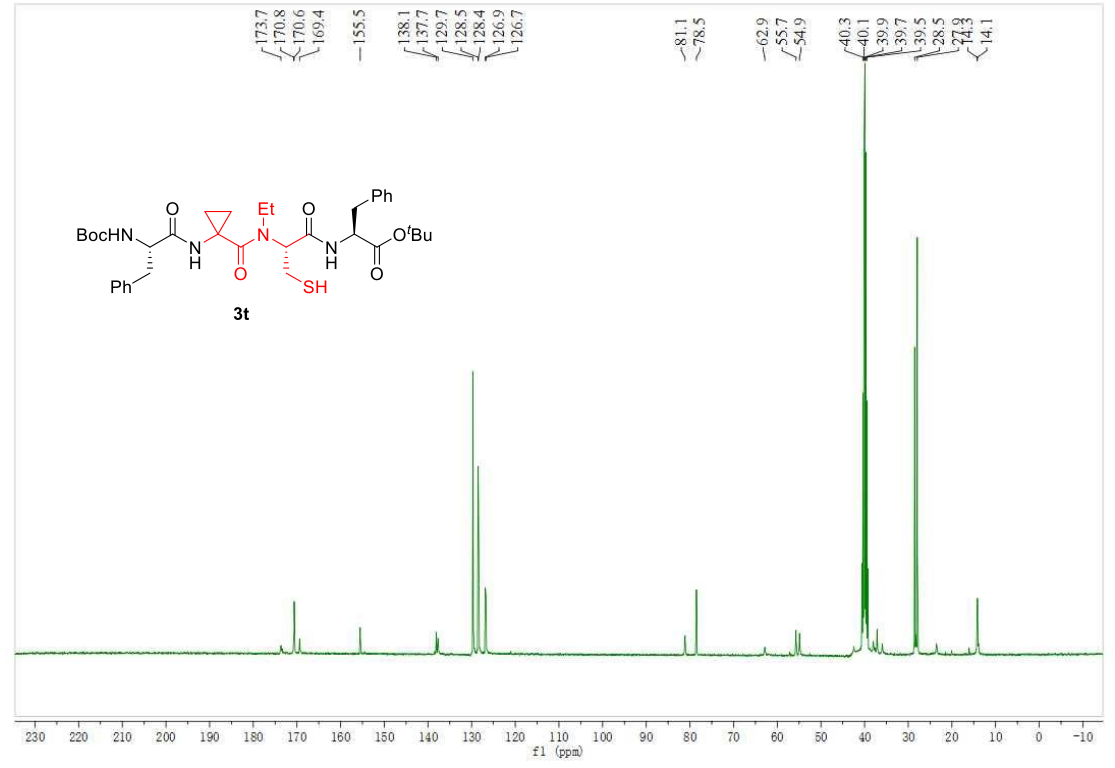
7.25
7.24
7.22
7.22
7.20
7.18
7.17
7.15
6.89
6.87
4.32
4.16
4.15
3.35
3.00
2.99
2.98
2.94
2.93
2.82
2.75
2.73
2.49
2.48
2.48
2.15
1.29
1.28
0.91
0.47
1.01
1.08
9.54
0.92
1.07
2.07
0.98
0.99
6.05
1.00
17.91
5.83
1.02
f1 (ppm)
BocHN
Et
Ph
O'Bu
SH
3t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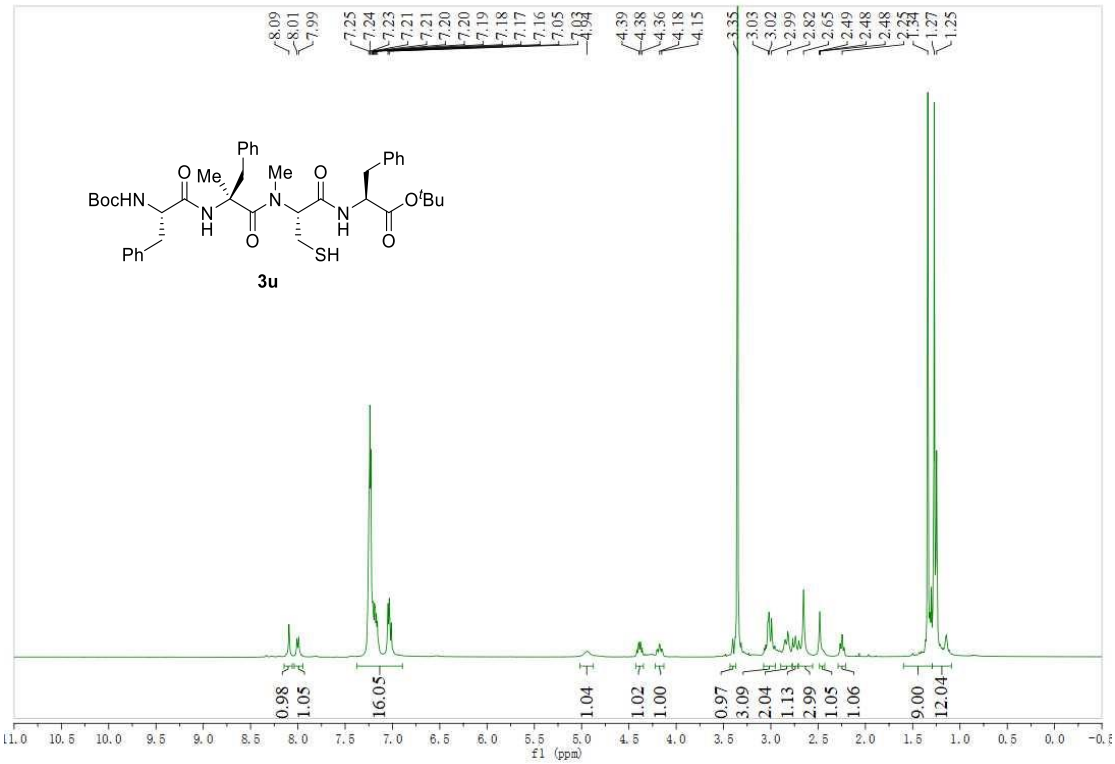
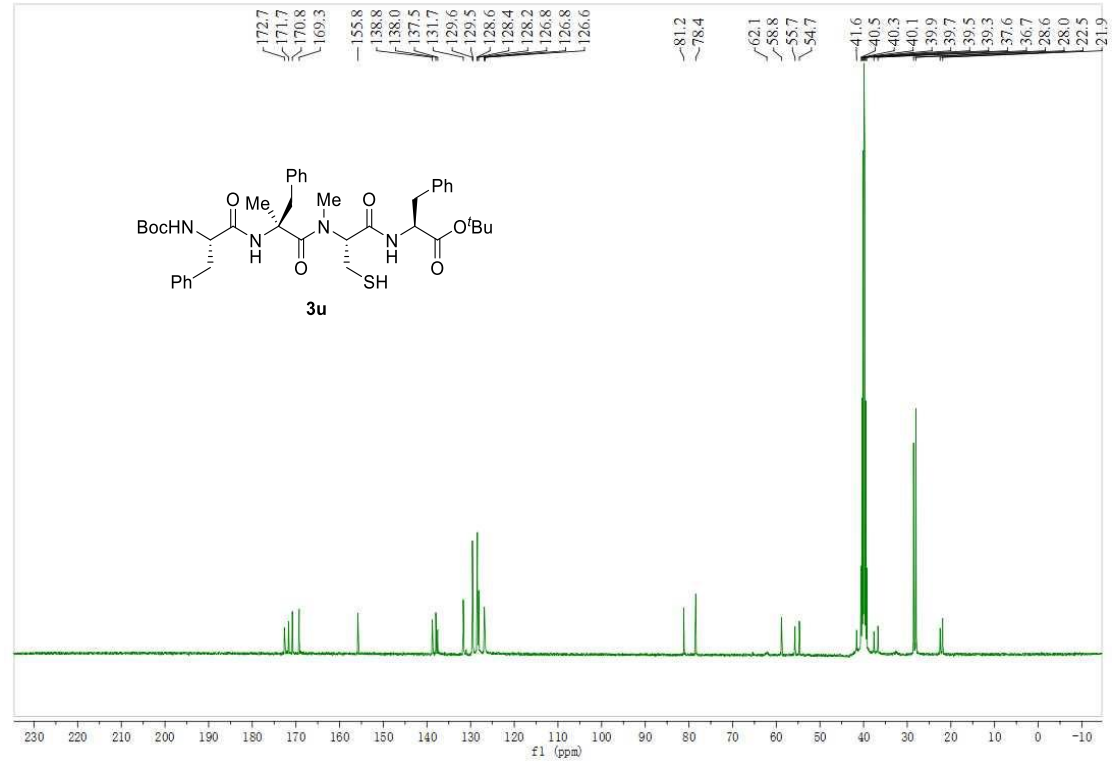
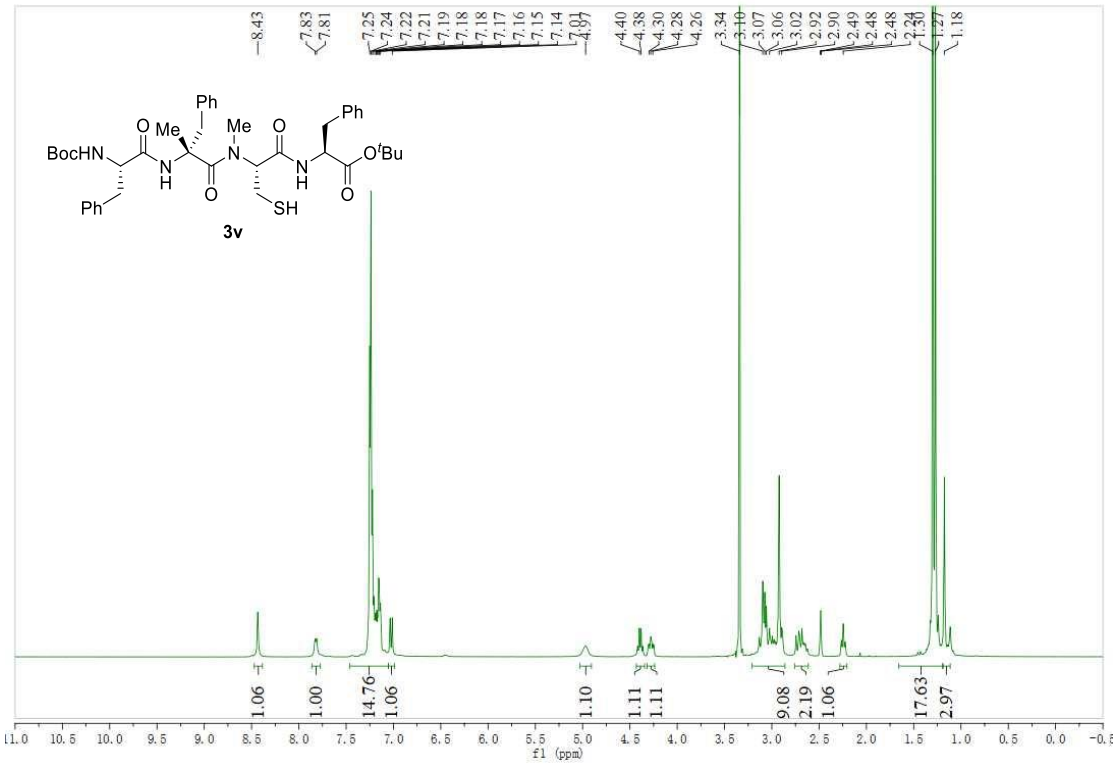
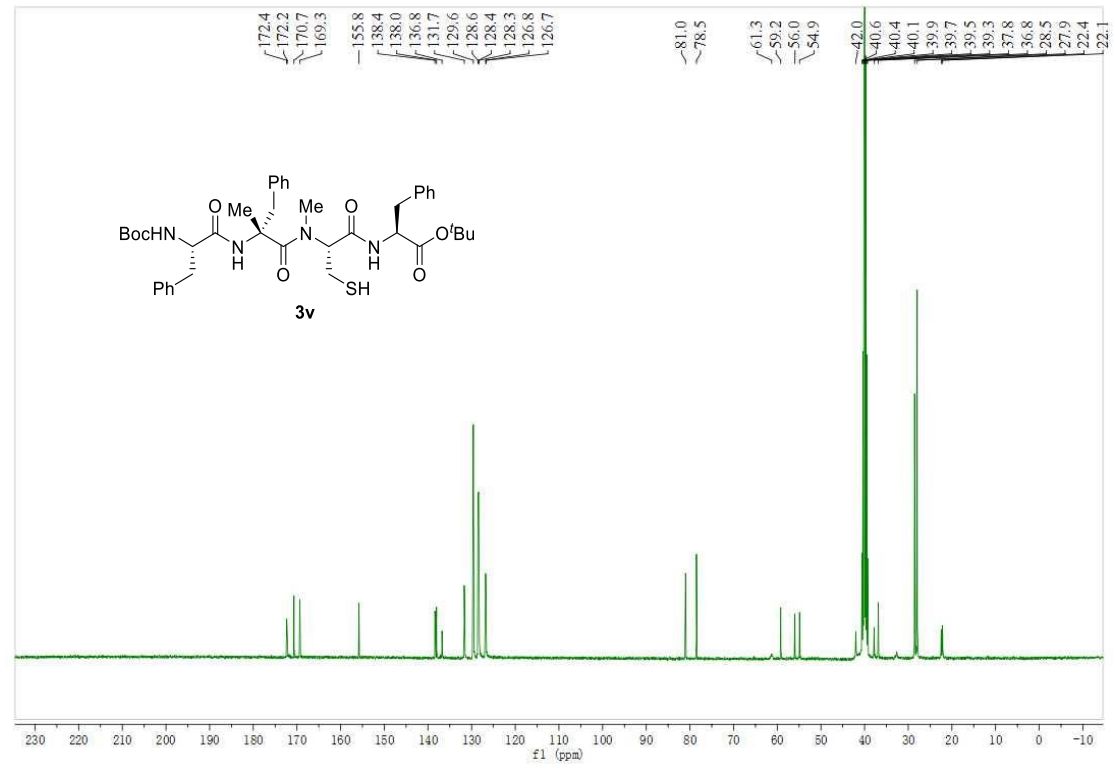
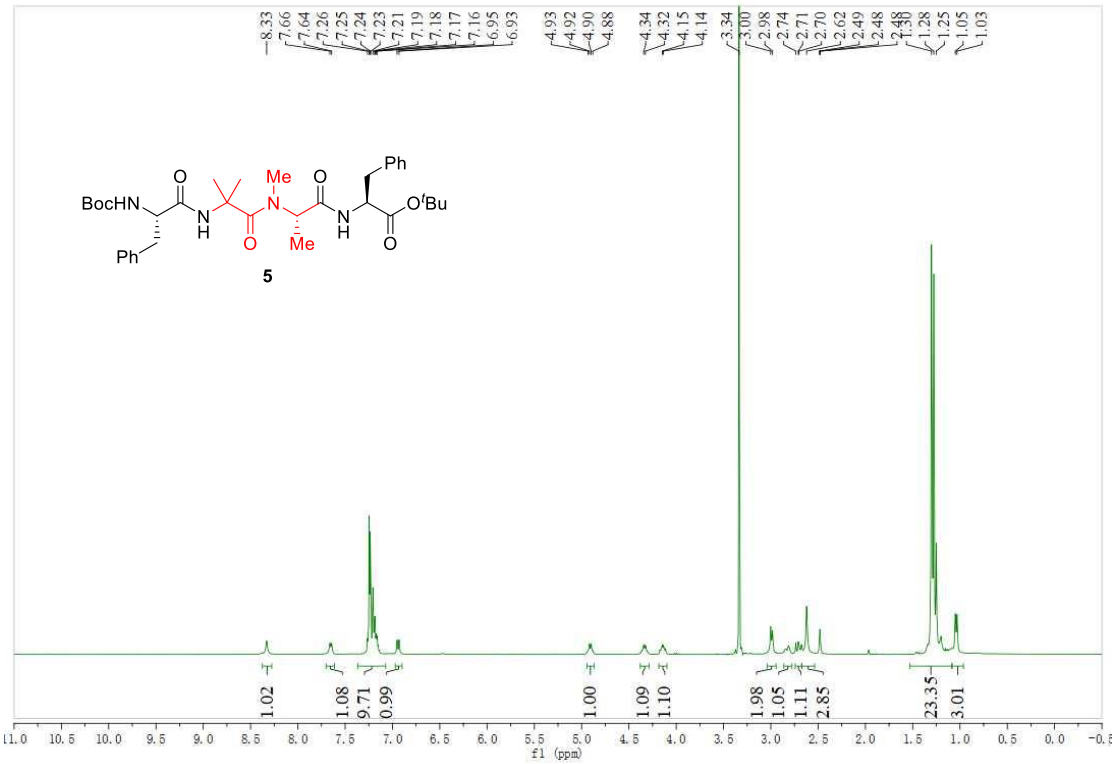
170.6
169.4
155.5
138.1
137.7
129.7
128.5
128.4
126.9
126.7
81.1
78.5
62.9
55.7
54.9
40.3
40.1
39.9
39.7
39.5
28.5
24.3
14.1
f1 (ppm)
BocHN
Et
Ph
O'Bu
SH
3t

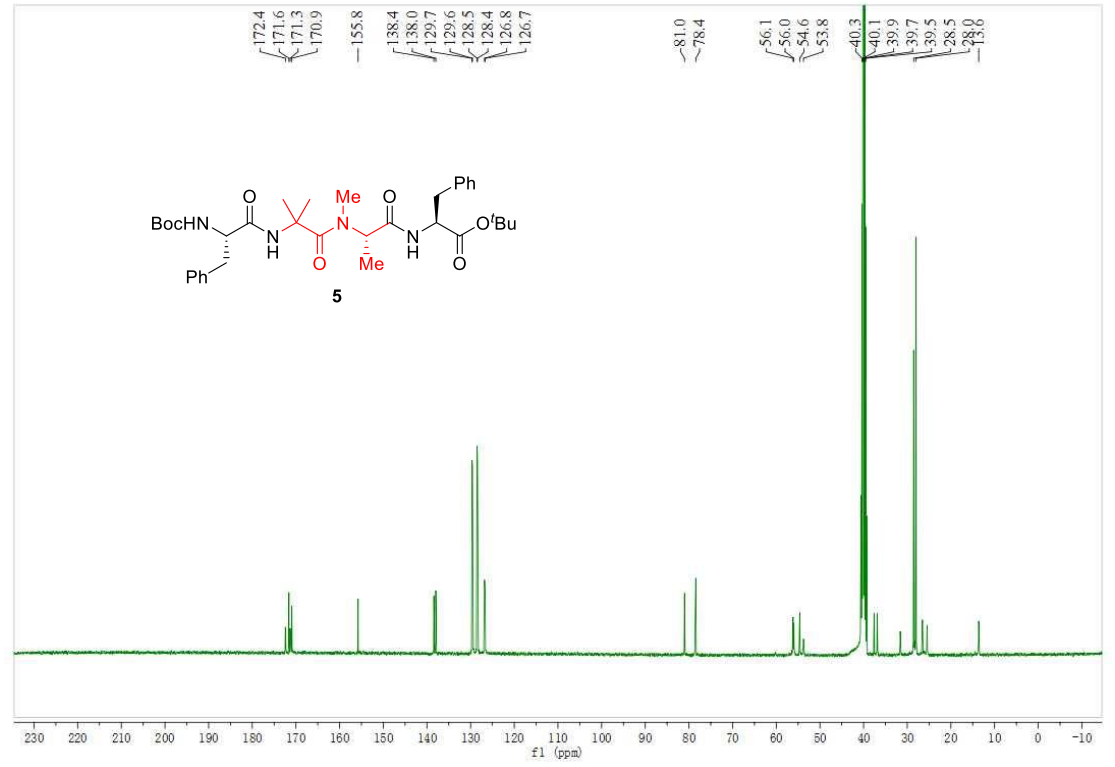

3u
f1 (ppm)

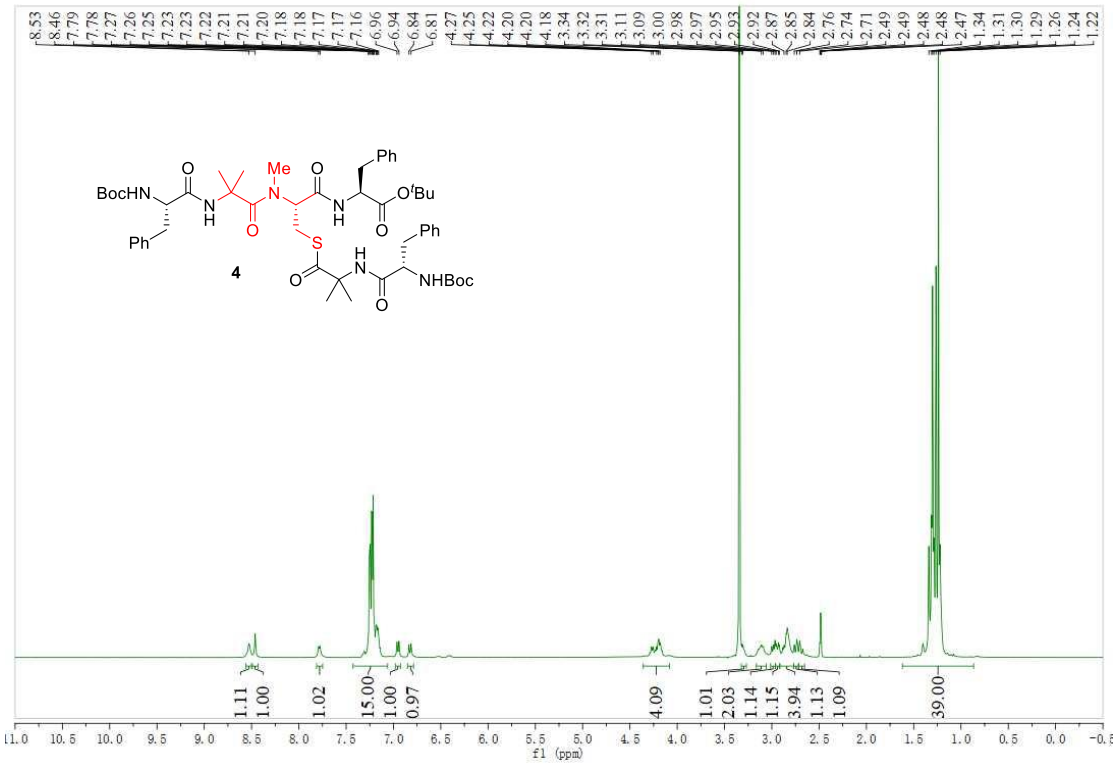

3u
f1 (ppm)


3v
f1 (ppm)


3v
f1 (ppm)


BocHN
Ph
Me
N
O
Me
N
H
O
N
H
Ph
O
O'Bu
O
5
f1 (ppm)


BocHN
Ph
Me
N
O
Me
N
H
O
N
H
Ph
O
O'Bu
O
5
f1 (ppm)


BocHN
Ph
Me
O
N
H
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Ph
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O
tBu
Ph
S
H
N
O
NHBoc
O
4
1.11
1.00
1.02
15.00
1.00
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1.01
2.03
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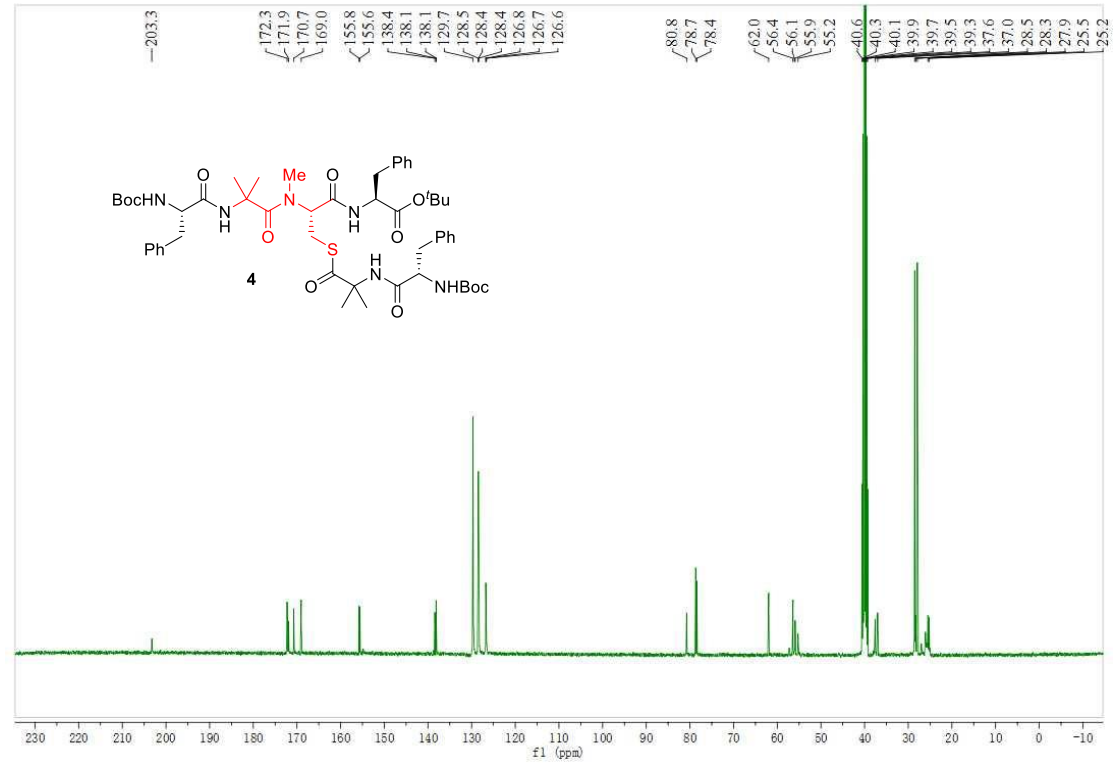
1.15
3.94
1.13
1.09
39.00
f1 (ppm)


BocHN
Ph
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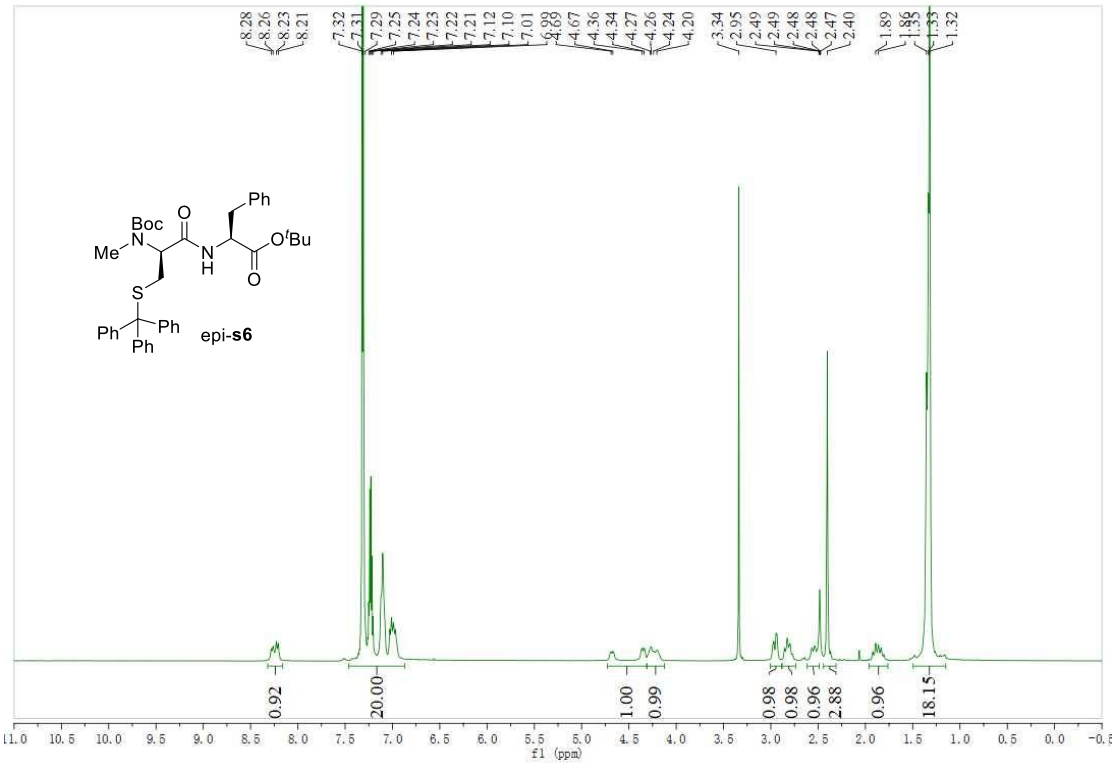
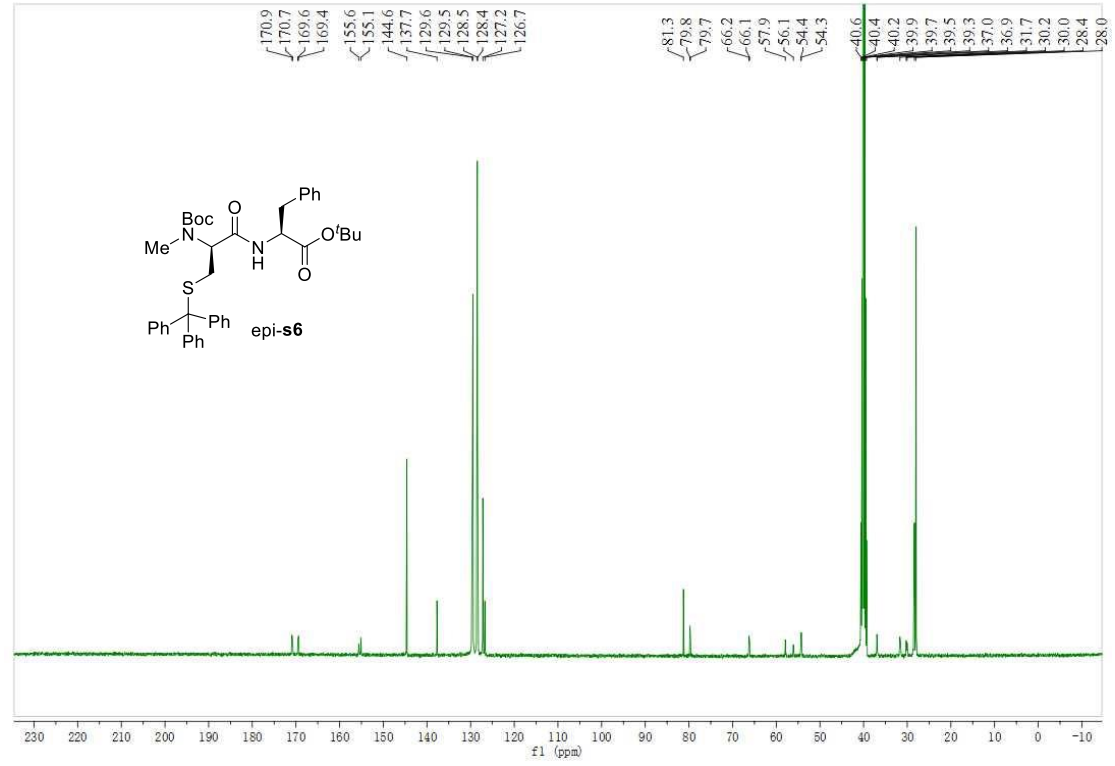
Ph
O
O
tBu
Ph
S
H
N
O
NHBoc
O
4
f1 (ppm)


epi-s6
f1 (ppm)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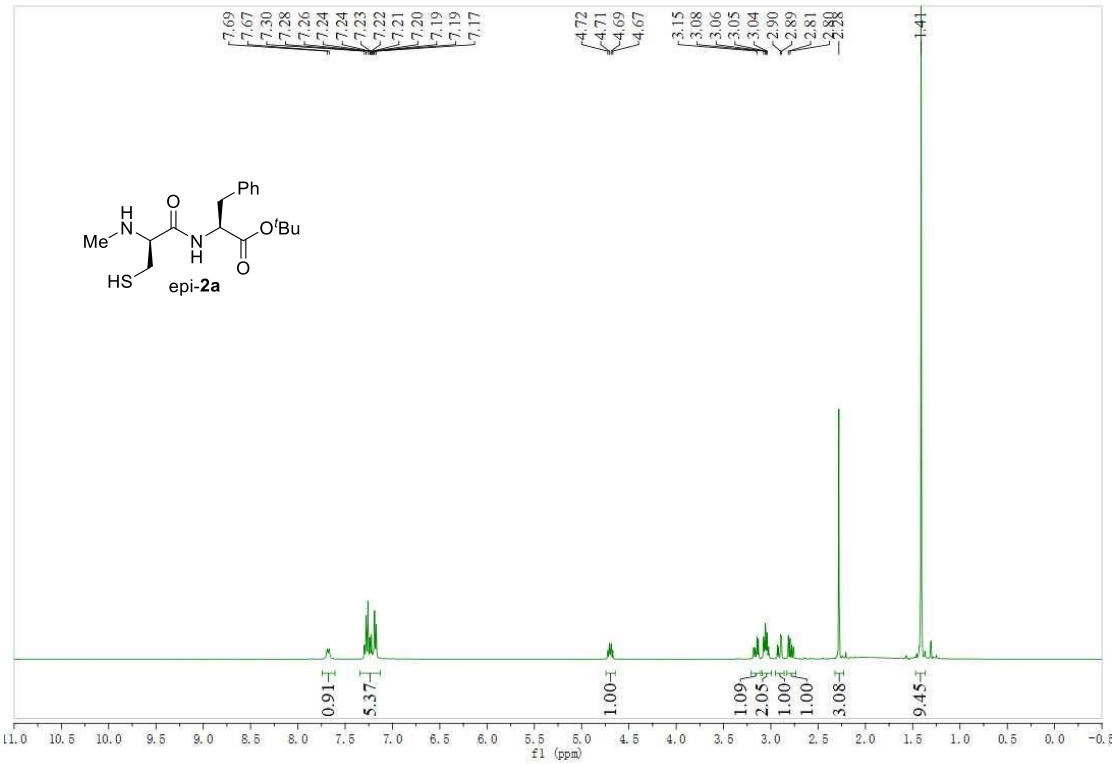
f1 (ppm)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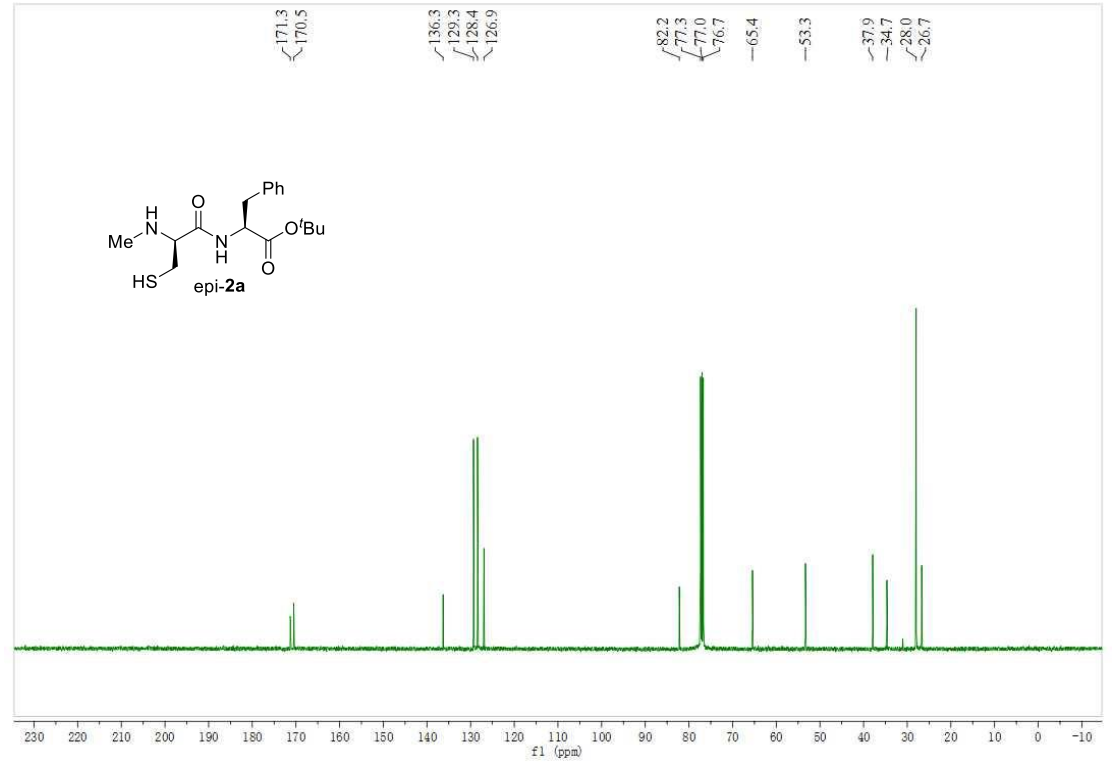
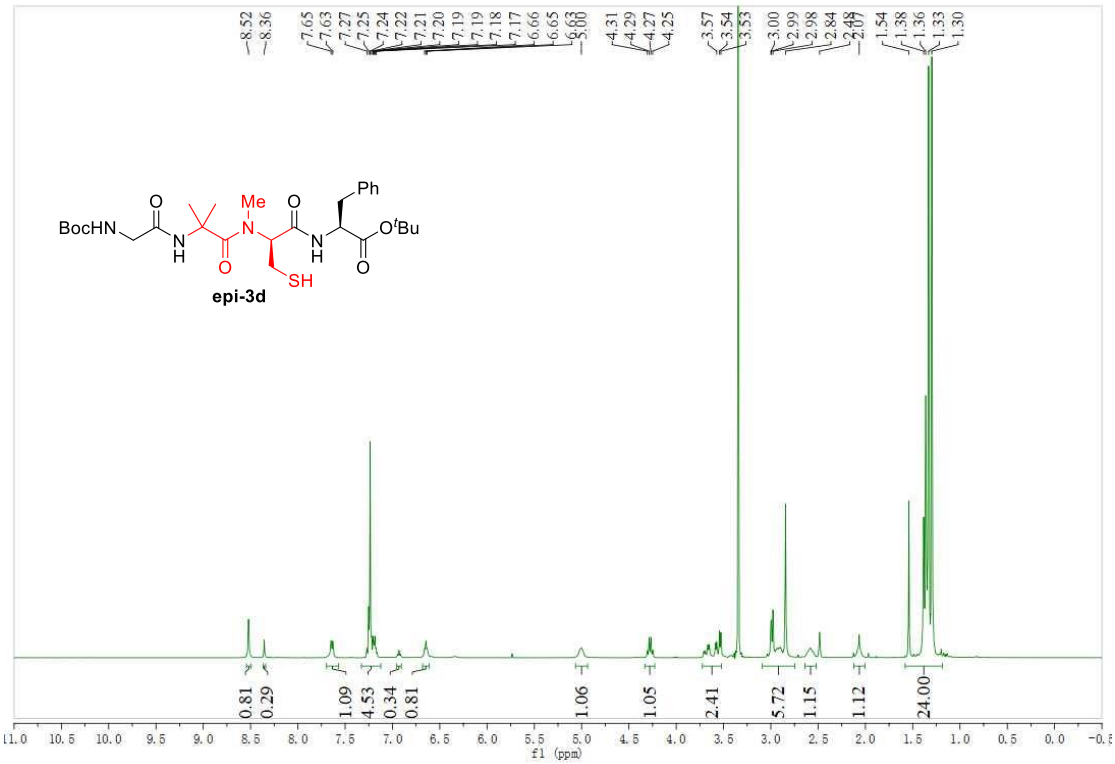
f1 (ppm)


epi-2a
f1 (ppm)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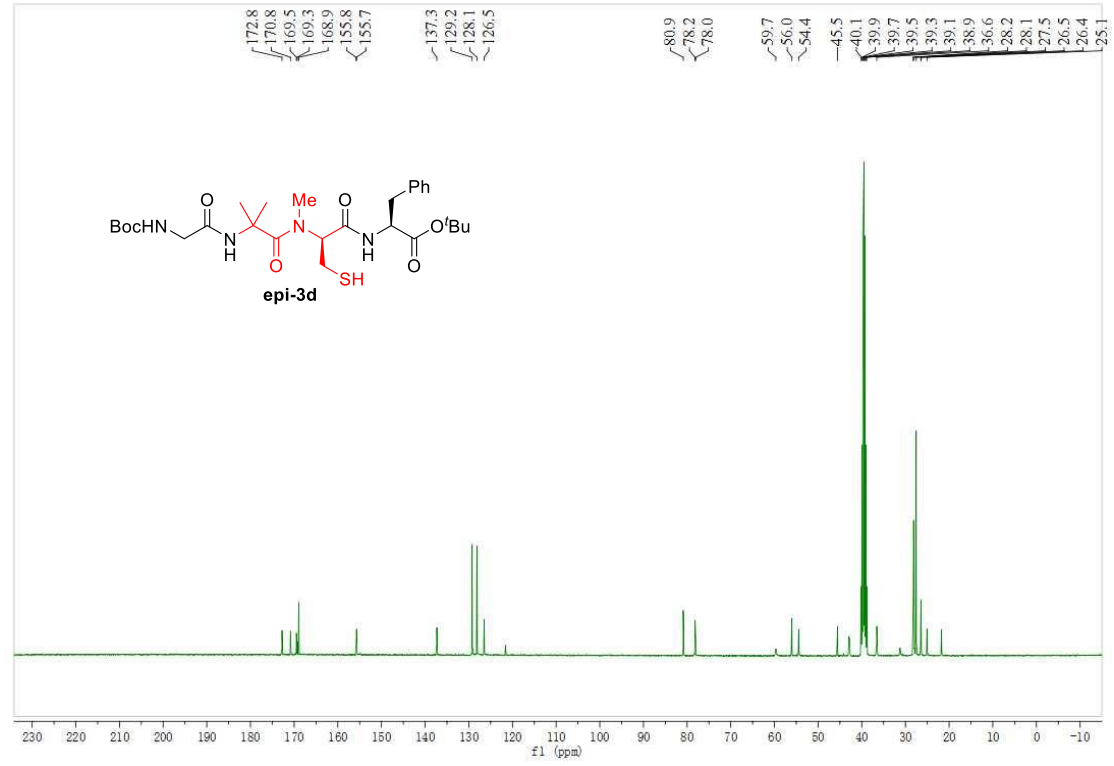
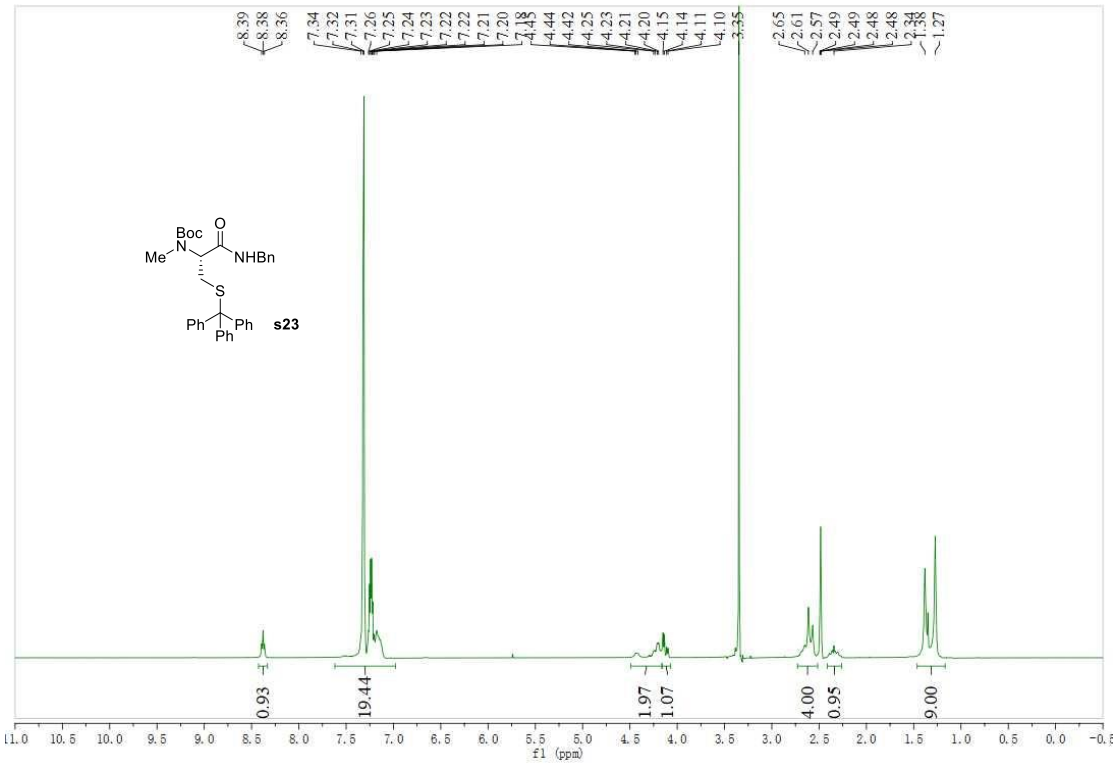
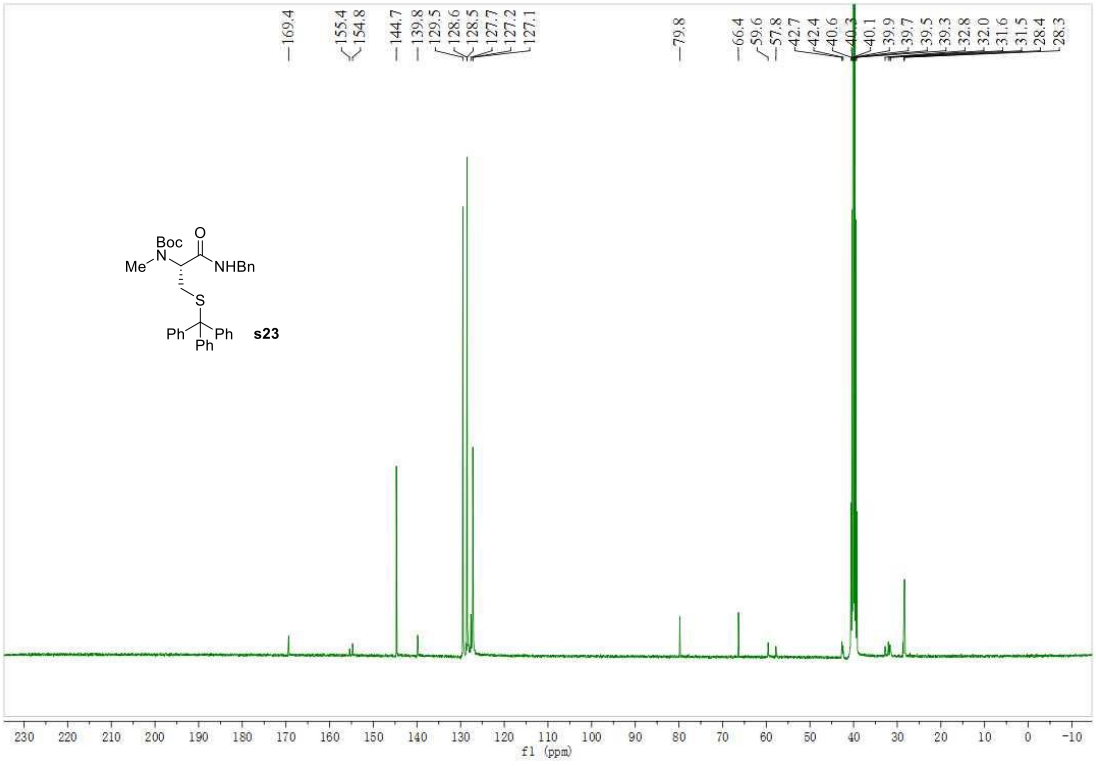
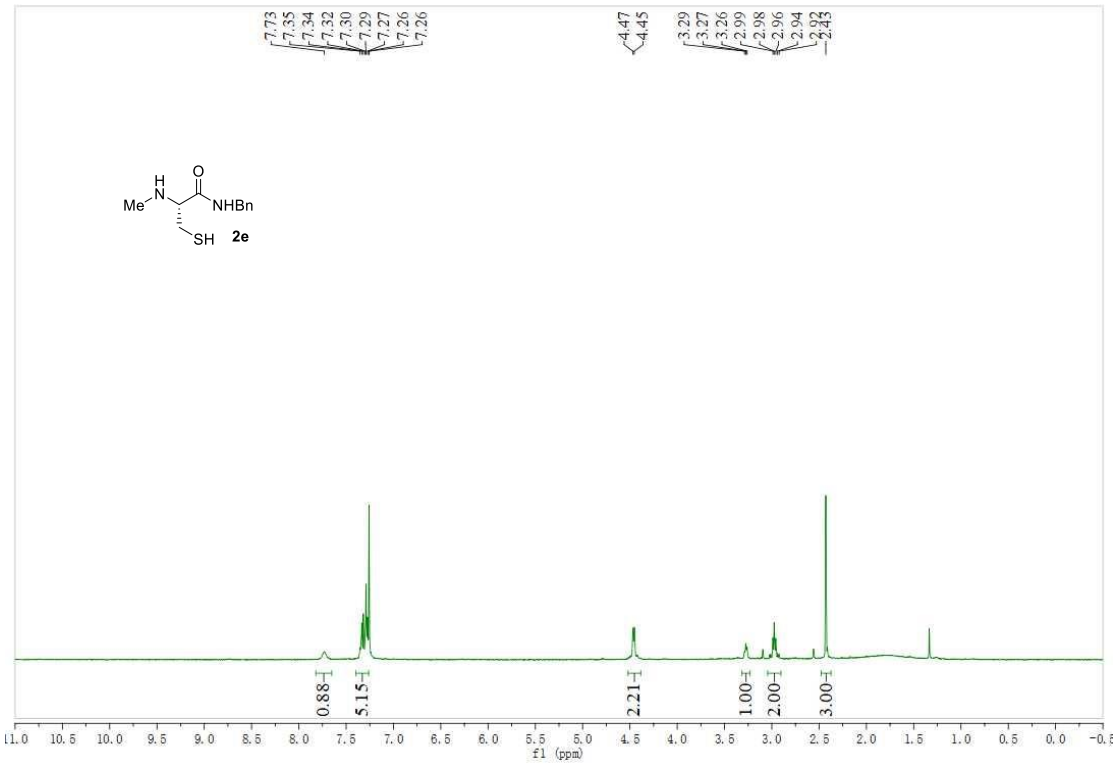

epi-3d
f1 (ppm)


Boc
O
Me
N
NHBn
S
Ph
Ph
Ph
s23
8.39
8.38
8.36
7.34
7.32
7.31
7.26
7.25
7.24
7.23
7.22
7.21
7.20
4.44
4.42
4.25
4.23
4.21
4.20
4.15
4.14
4.11
4.10
3.35
2.65
2.61
2.57
2.49
2.48
1.38
1.27
0.93
19.44
1.97
1.07
4.00
0.95
9.00
f1 (ppm)


Boc
O
Me
N
NHBn
S
Ph
Ph
Ph
s23
169.4
155.4
154.8
144.7
139.8
129.5
128.6
128.5
127.7
127.2
127.1
79.8
66.4
59.6
57.8
42.7
42.4
40.6
40.3
40.1
39.9
39.7
39.5
39.3
32.8
32.0
31.6
31.5
28.4
28.3
f1 (ppm)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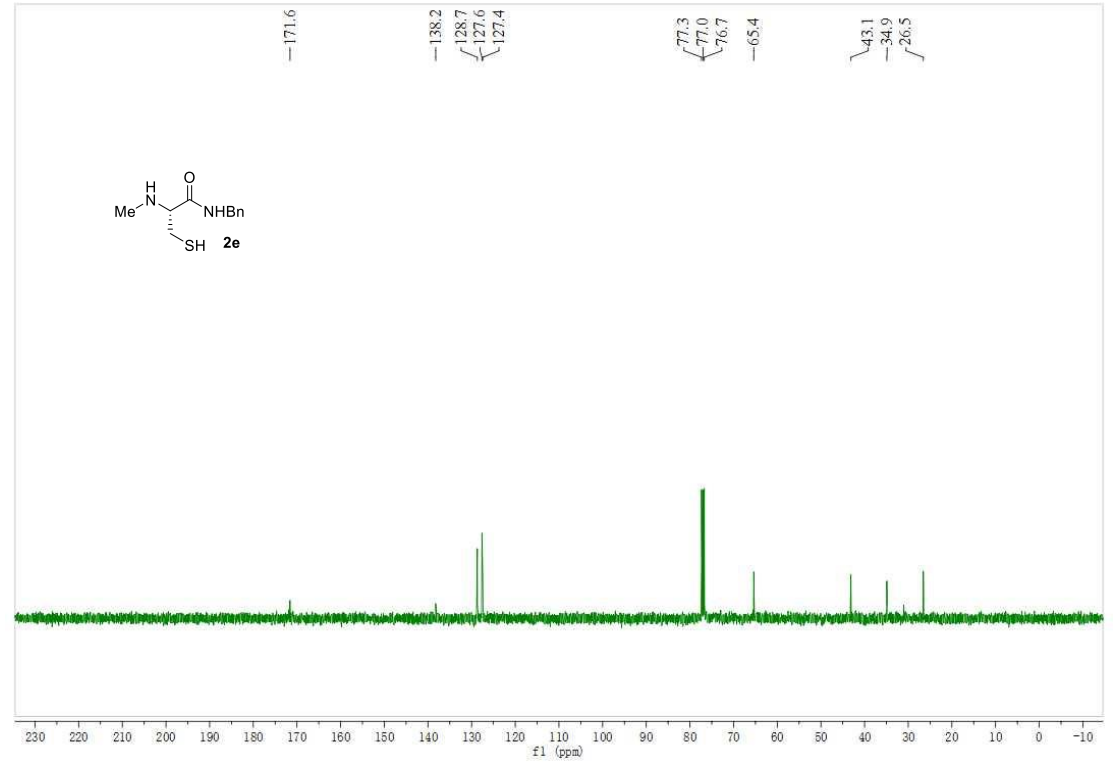
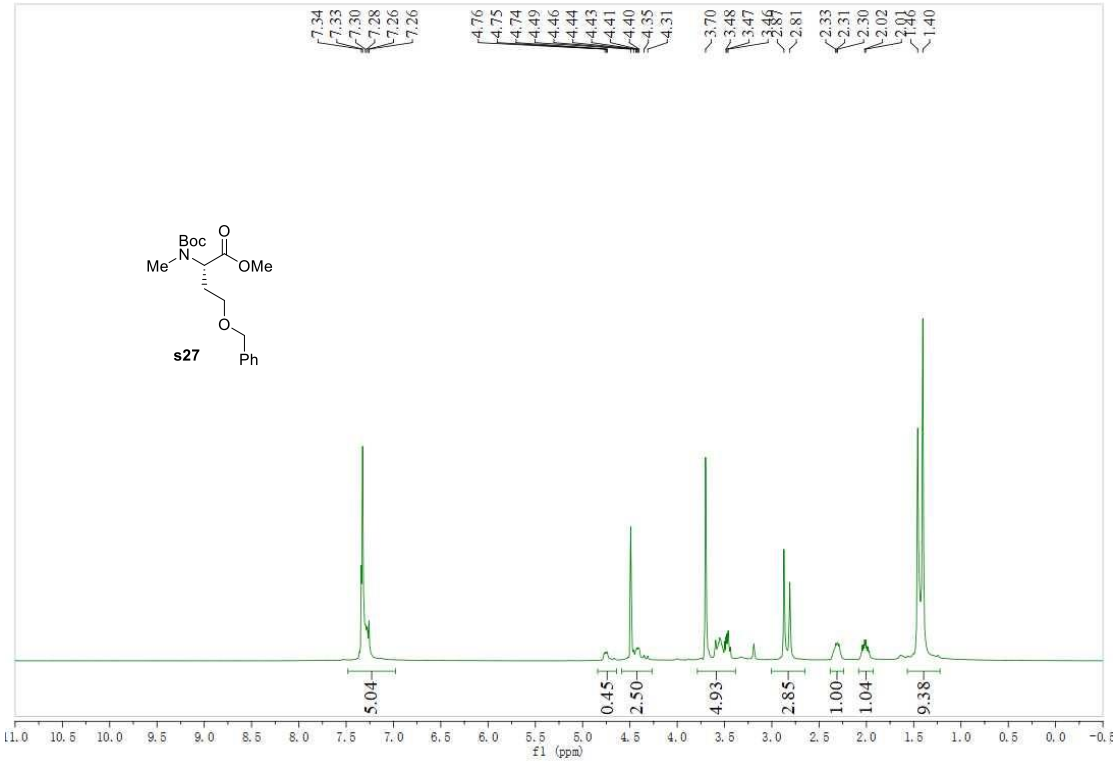
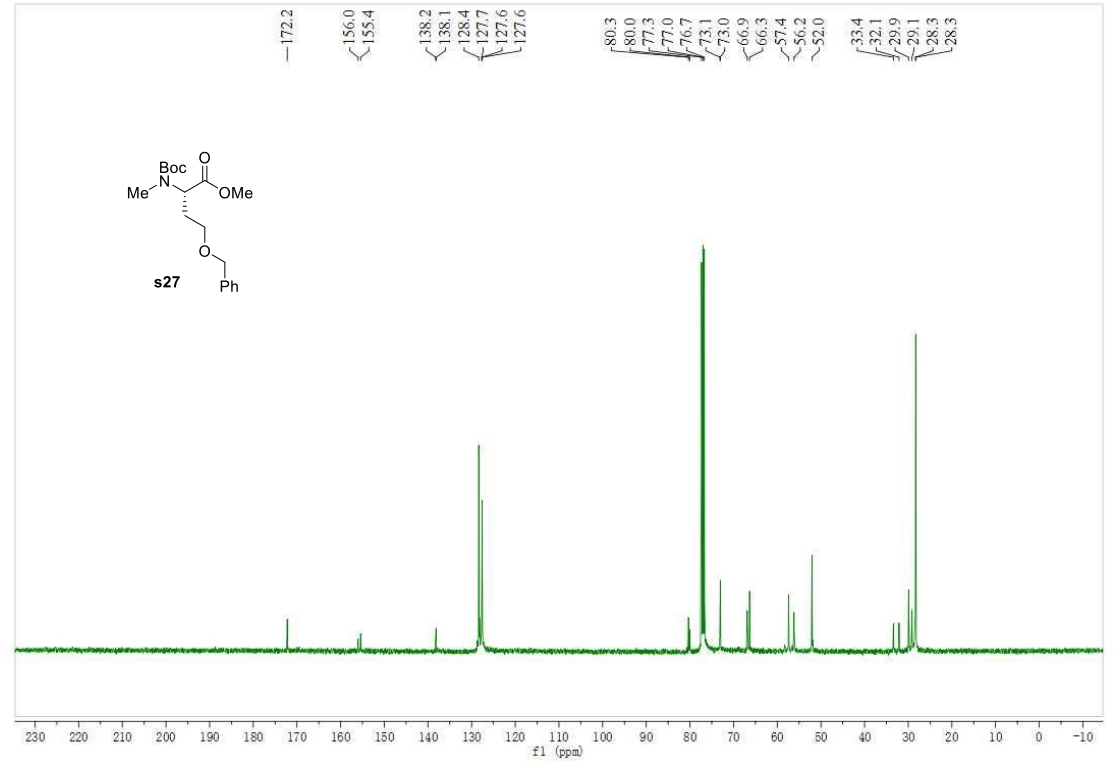
7.35
7.34
7.32
7.30
7.29
7.27
7.26
7.26
4.47
4.45
3.29
3.27
3.26
2.99
2.98
2.96
2.94
2.93
2.43
Me
N
H
O
NHBn
SH
2e
0.88
5.15
2.21
1.00
2.00
3.00
f1 (ppm)


171.6
138.2
128.7
127.6
127.4
77.3
77.0
76.7
65.4
43.1
34.9
26.5
Me
N
H
O
NHBn
SH
2e
f1 (ppm)


7.34
7.33
7.30
7.28
7.26
7.26
4.76
4.75
4.74
4.49
4.46
4.44
4.43
4.41
4.40
4.35
4.31
3.70
3.48
3.47
3.46
2.87
2.81
2.33
2.31
2.30
2.02
2.01
1.46
1.40
Boc
N
Me
O
OMe
O
Ph
s27
5.04
0.45
2.50
4.93
2.85
1.00
1.04
9.38
f1 (ppm)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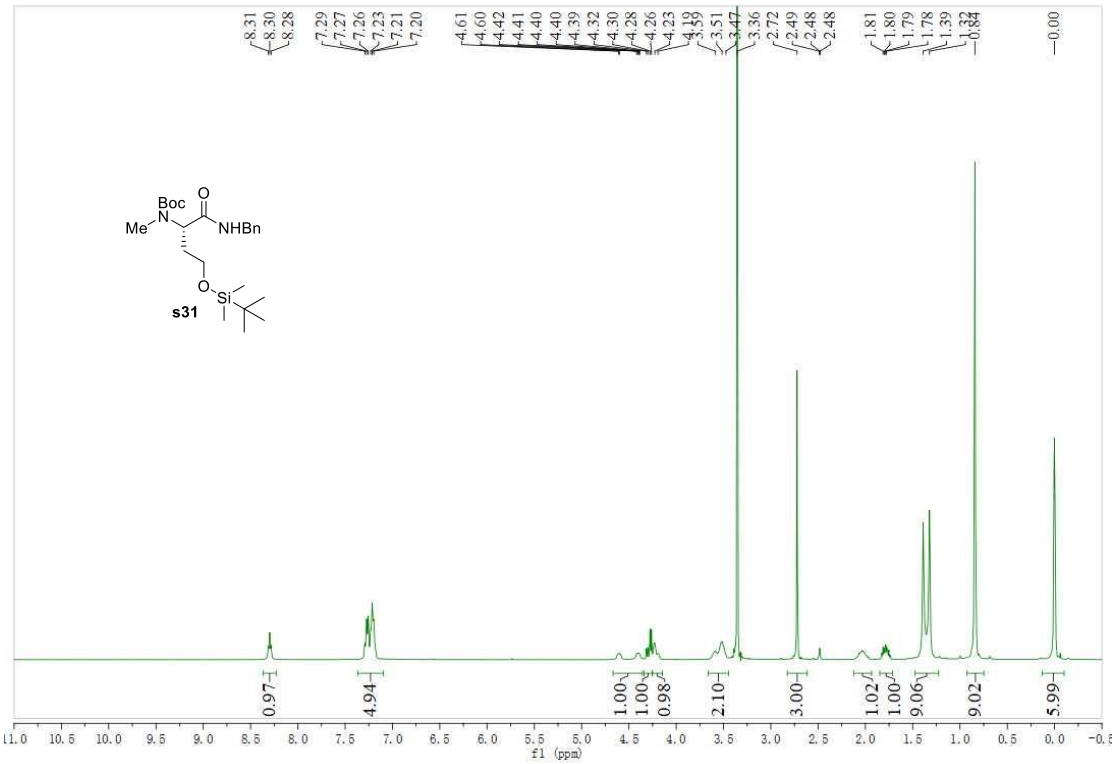
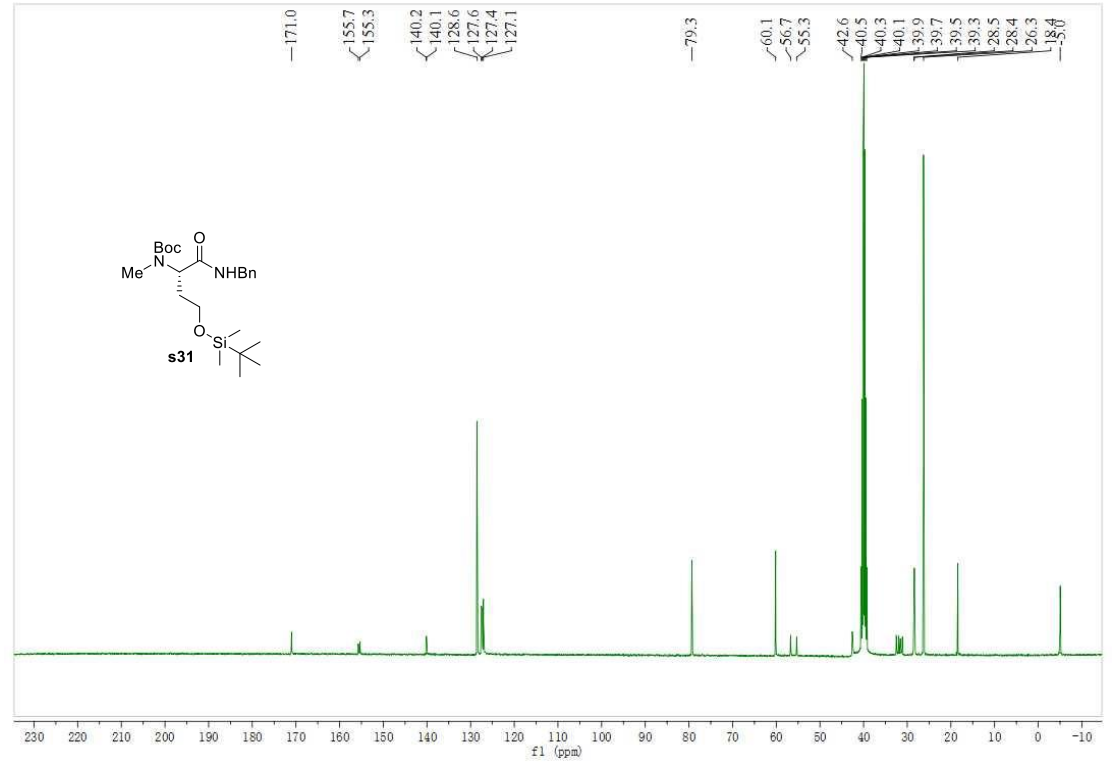
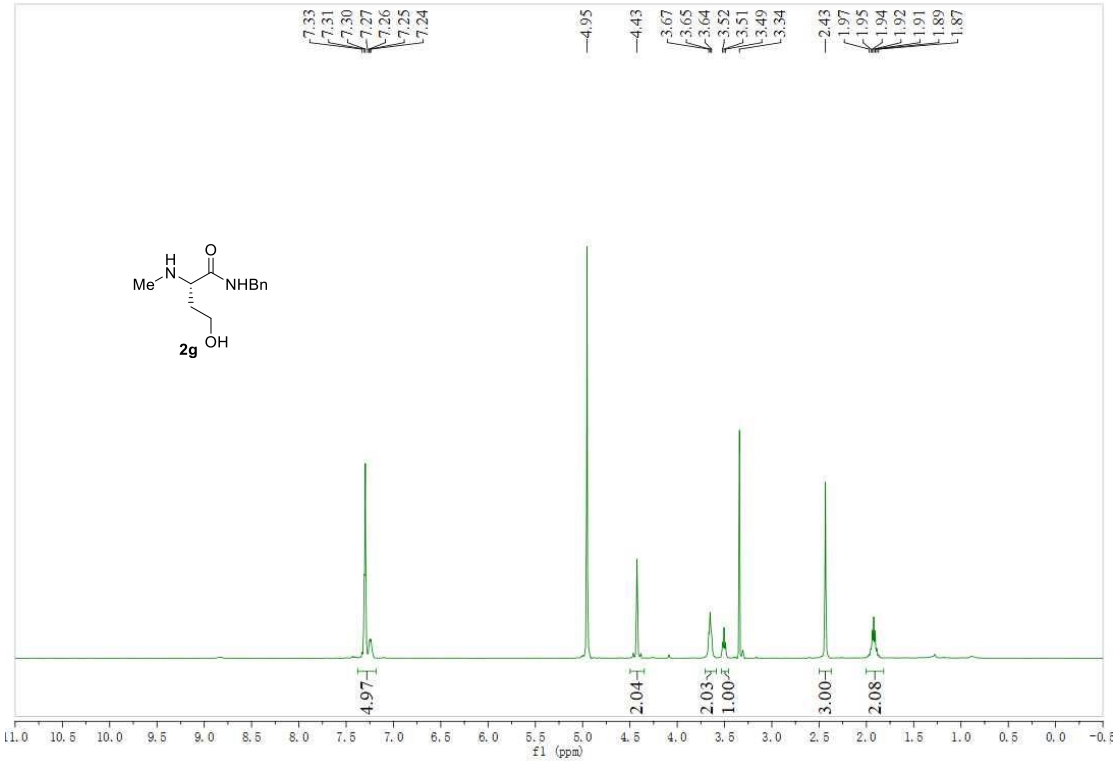
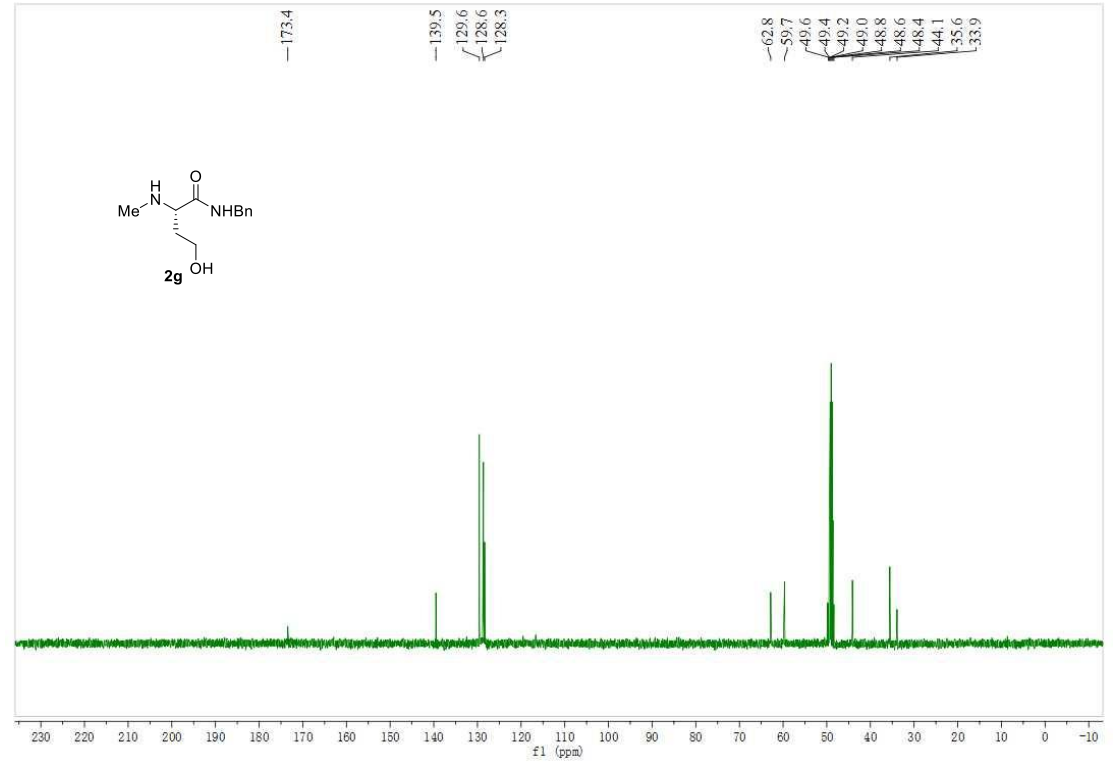
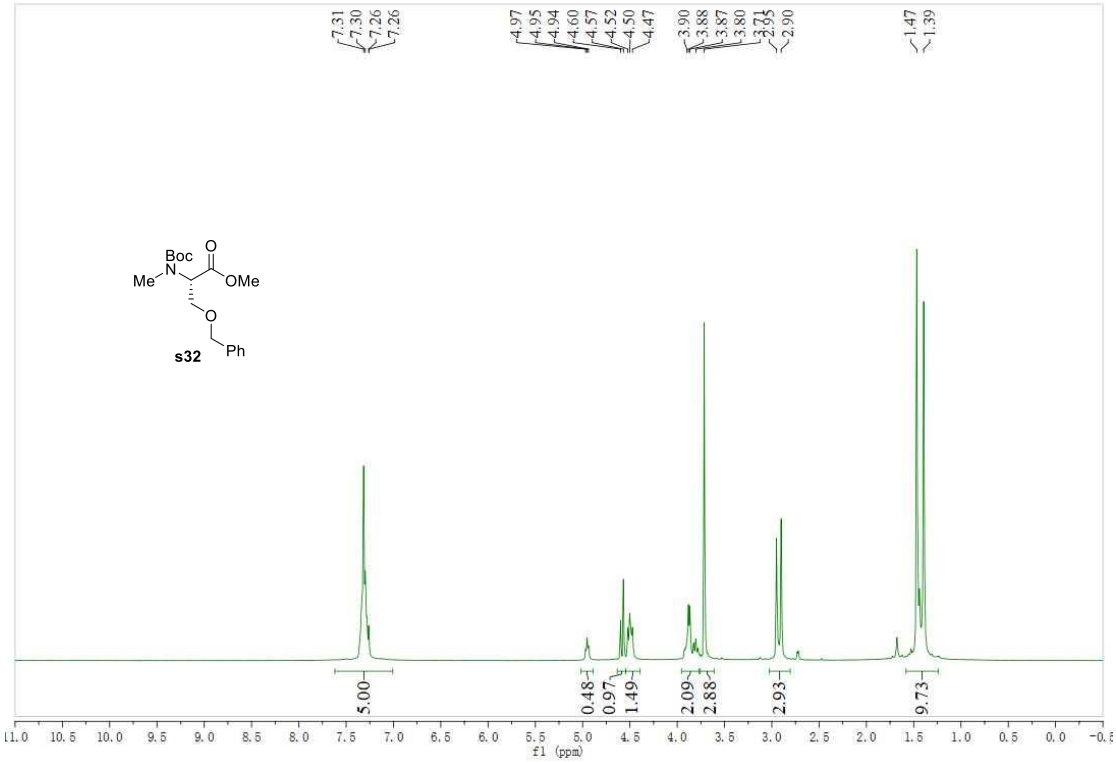
156.0
155.4
138.2
138.1
128.4
127.7
127.6
127.6
80.3
80.0
77.3
77.0
76.7
73.1
73.0
66.9
66.3
57.4
56.2
52.0
33.4
32.1
29.9
29.1
28.3
28.3
Boc
N
Me
O
OMe
O
Ph
s27
f1 (ppm)

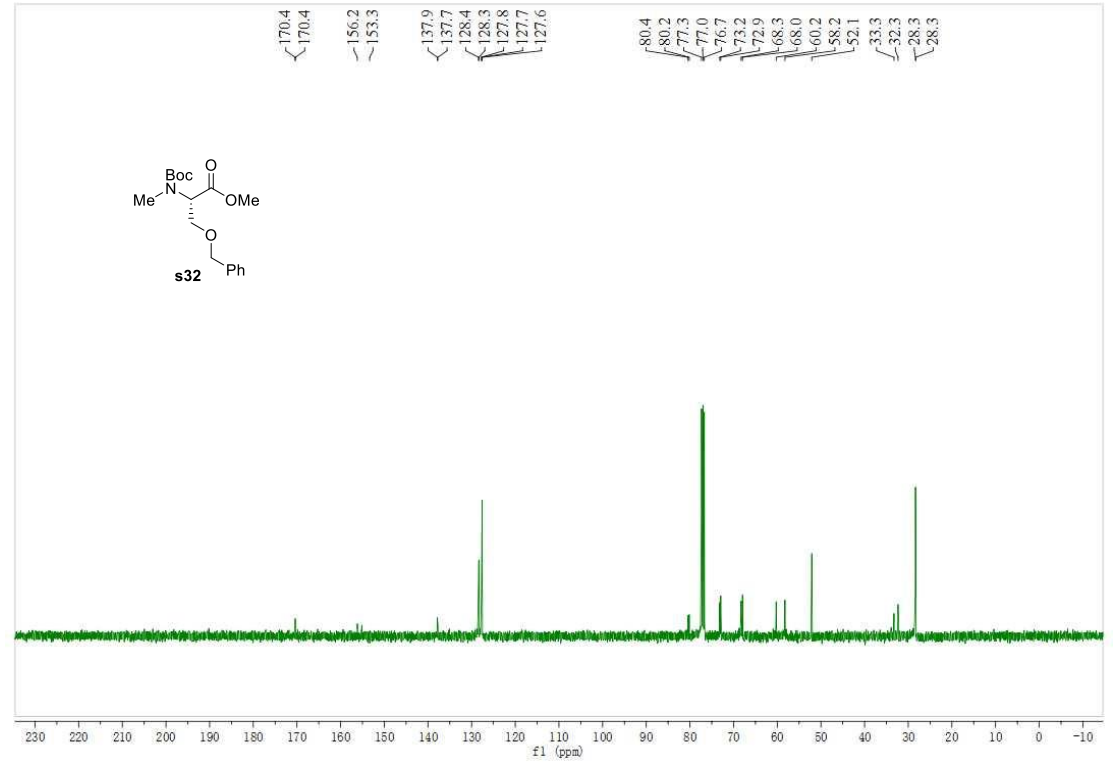

Boc
Me
N
O
NHBn
O
Si
s31
f1 (ppm)

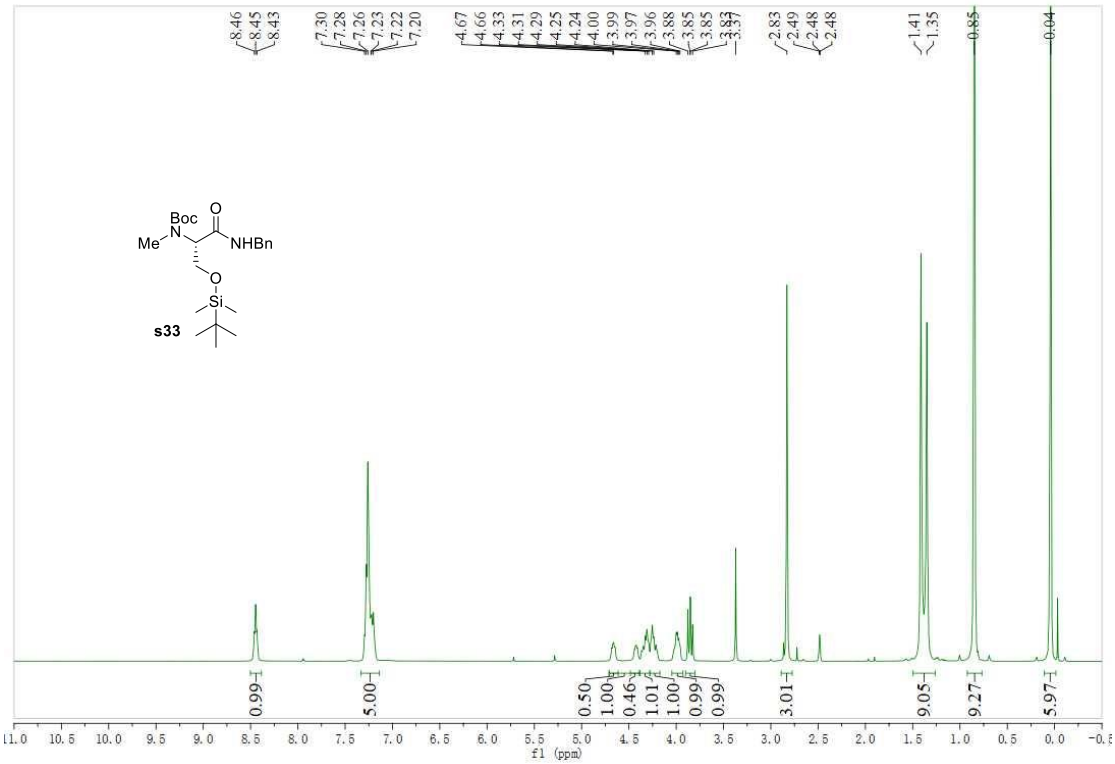

Boc
Me
N
O
NHBn
O
Si
s31
f1 (ppm)

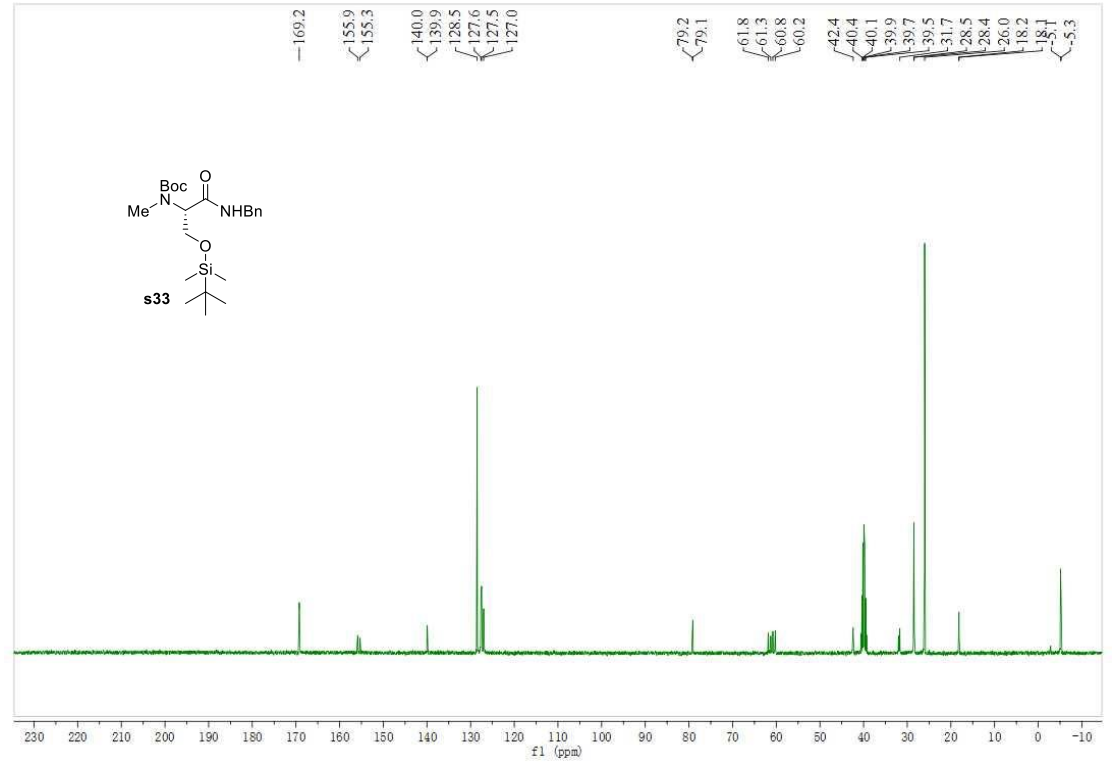

7.33
7.31
7.30
7.27
7.26
7.25
7.24
4.95
4.43
3.67
3.65
3.64
3.52
3.51
3.49
3.34
2.43
1.97
1.95
1.94
1.92
1.91
1.89
1.87
Me
N
H
O
NHBn
OH
2g
4.97
2.04
2.03
1.00
3.00
2.08
f1 (ppm)

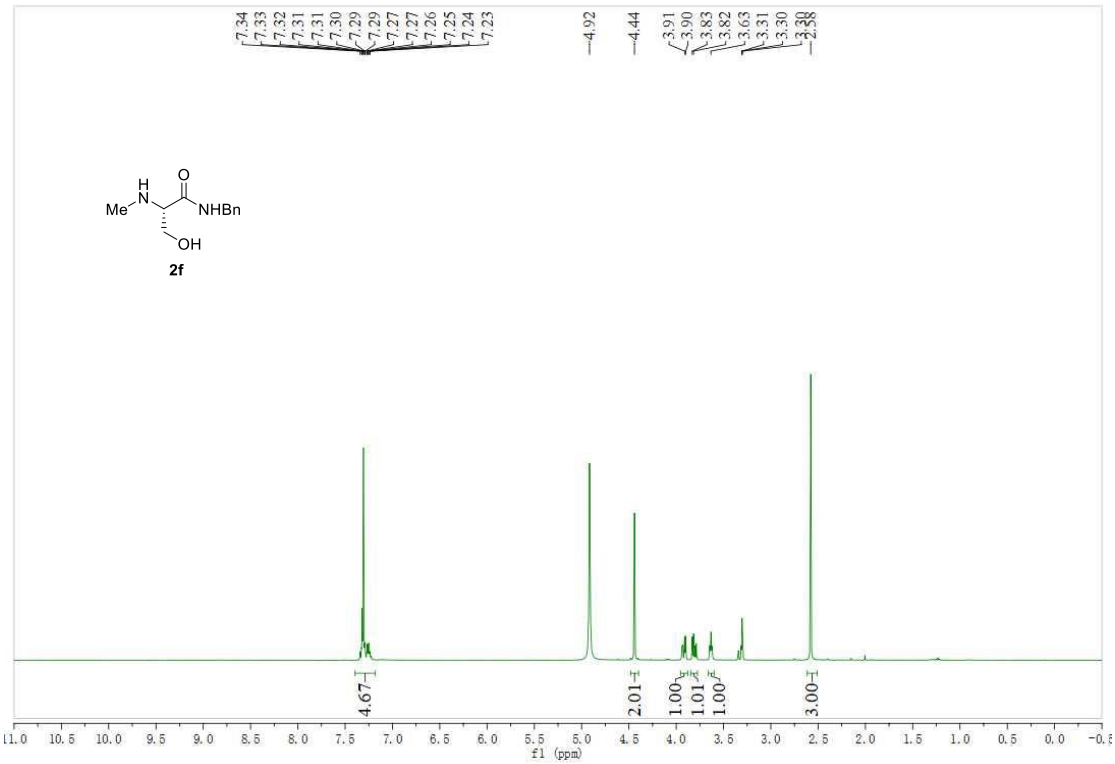

173.4
139.5
129.6
128.6
128.3
62.8
59.7
49.6
49.4
49.2
49.0
48.8
48.6
48.4
44.1
35.6
33.9
Me
N
H
O
NHBn
OH
2g
f1 (ppm)

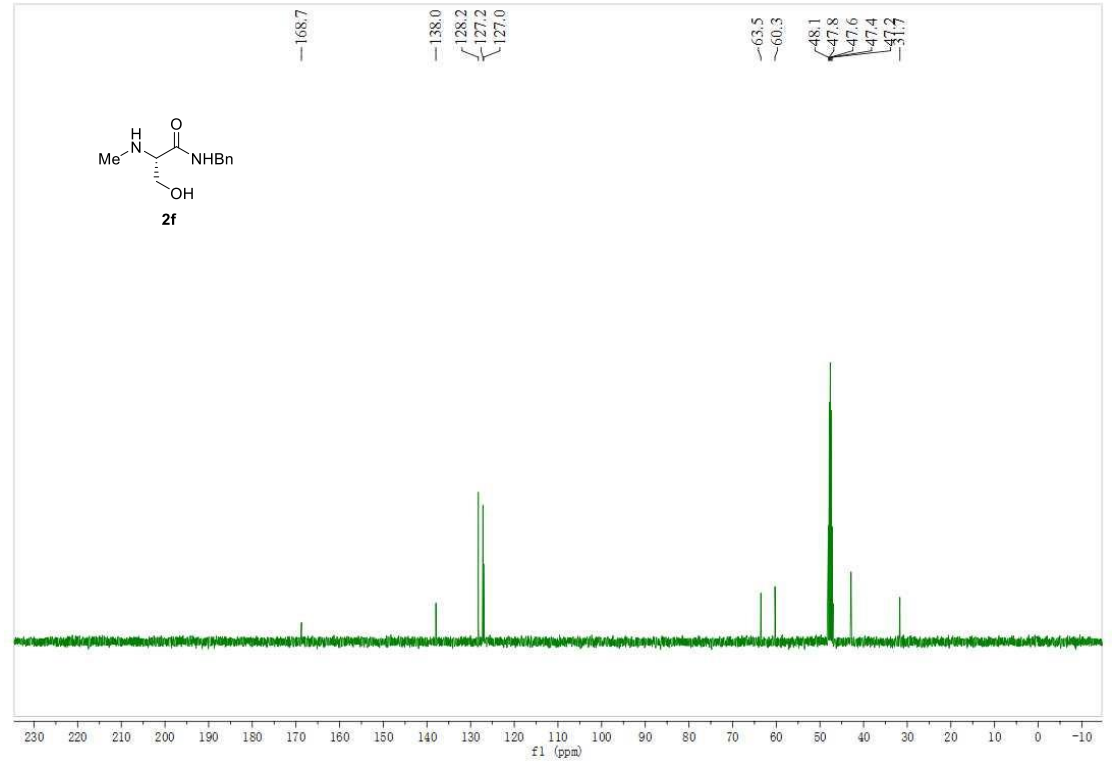

7.31
7.30
7.26
7.26
4.97
4.95
4.94
4.60
4.57
4.52
4.50
4.47
3.90
3.88
3.87
3.80
3.71
3.65
2.90
1.47
1.39
Boc
N
Me
O
OMe
O
Ph
s32
5.00
0.48
0.97
1.49
2.09
2.88
2.93
9.73
f1 (ppm)

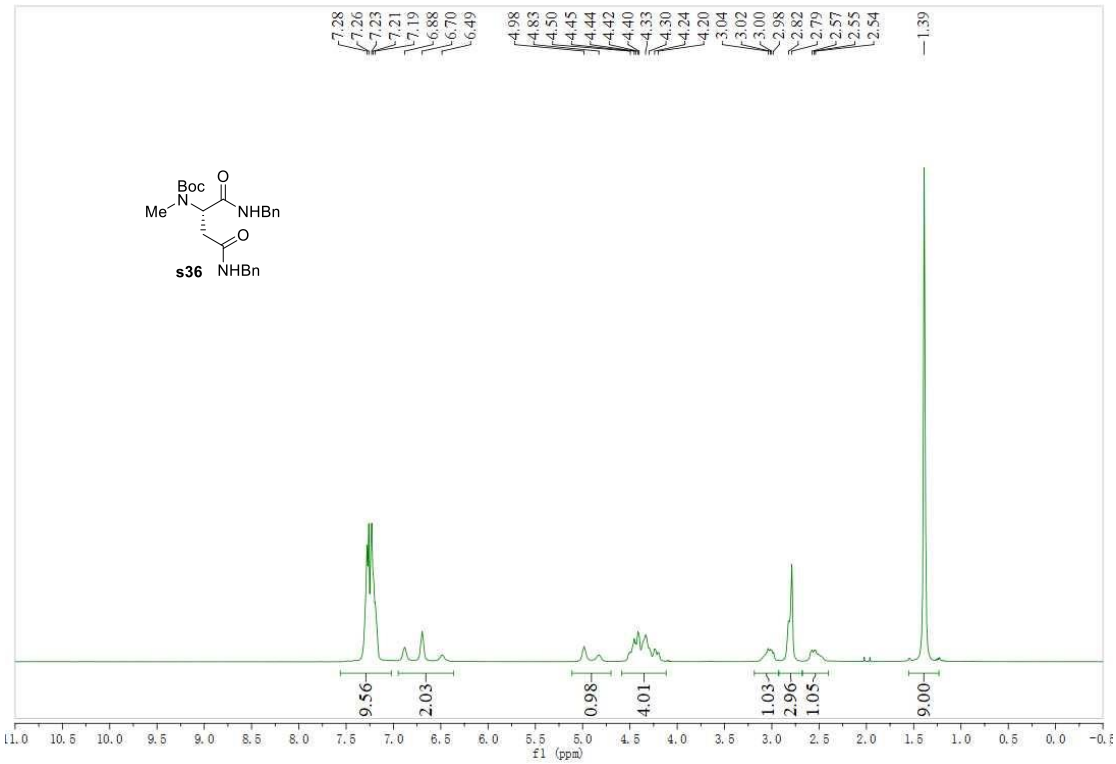

170.4
170.4
156.2
153.3
137.9
137.7
128.4
128.3
127.8
127.7
127.6
80.4
80.2
77.3
77.0
76.7
73.2
72.9
68.3
68.0
60.2
58.2
52.1
33.3
32.3
28.3
28.3
Boc
N
Me
O
OMe
O
Ph
s32
f1 (ppm)


Boc
N
Me
O
NHBn
O
Si
s33
f1 (ppm)


Boc
N
Me
O
NHBn
O
Si
s33
f1 (ppm)


7.34
7.33
7.32
7.31
7.31
7.30
7.29
7.29
7.27
7.27
7.26
7.25
7.24
7.23
4.92
4.44
3.91
3.90
3.83
3.82
3.63
3.31
3.30
2.58
Me
N
H
O
NHBn
OH
2f
4.67
2.01
1.00
1.01
1.00
3.00
f1 (ppm)


168.7
138.0
128.2
127.2
127.0
63.5
60.3
48.1
47.8
47.6
47.4
47.2
31.7
Me
N
H
O
NHBn
OH
2f
f1 (ppm)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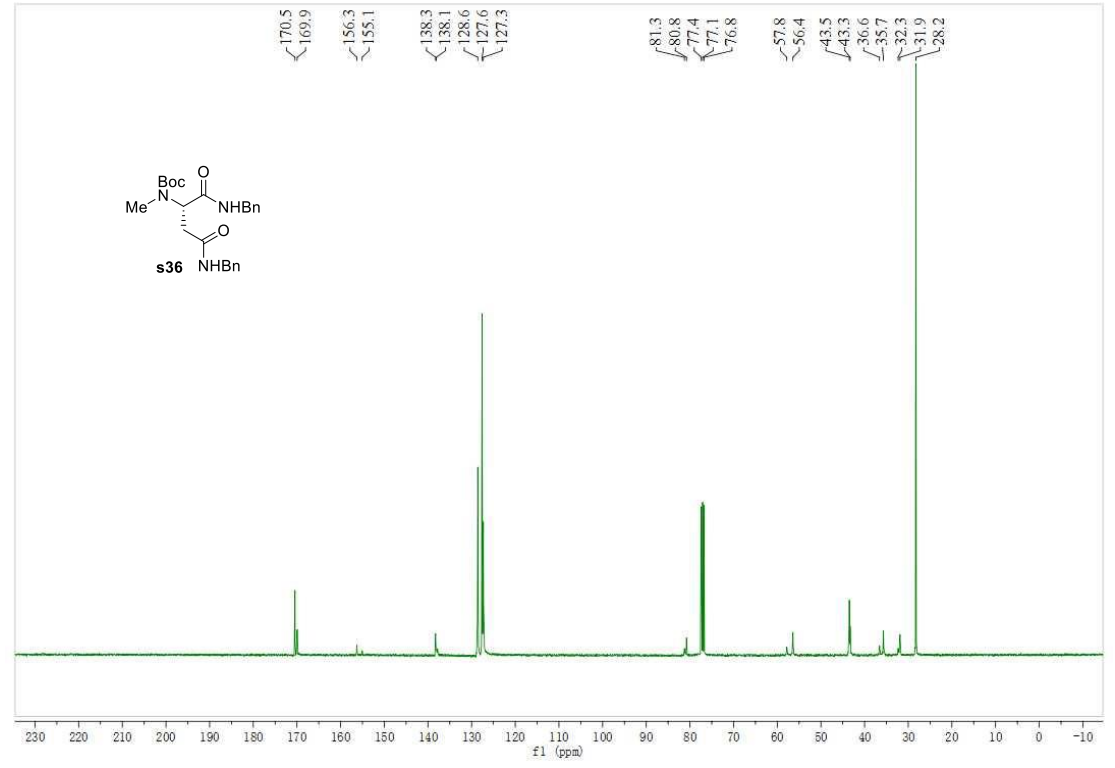
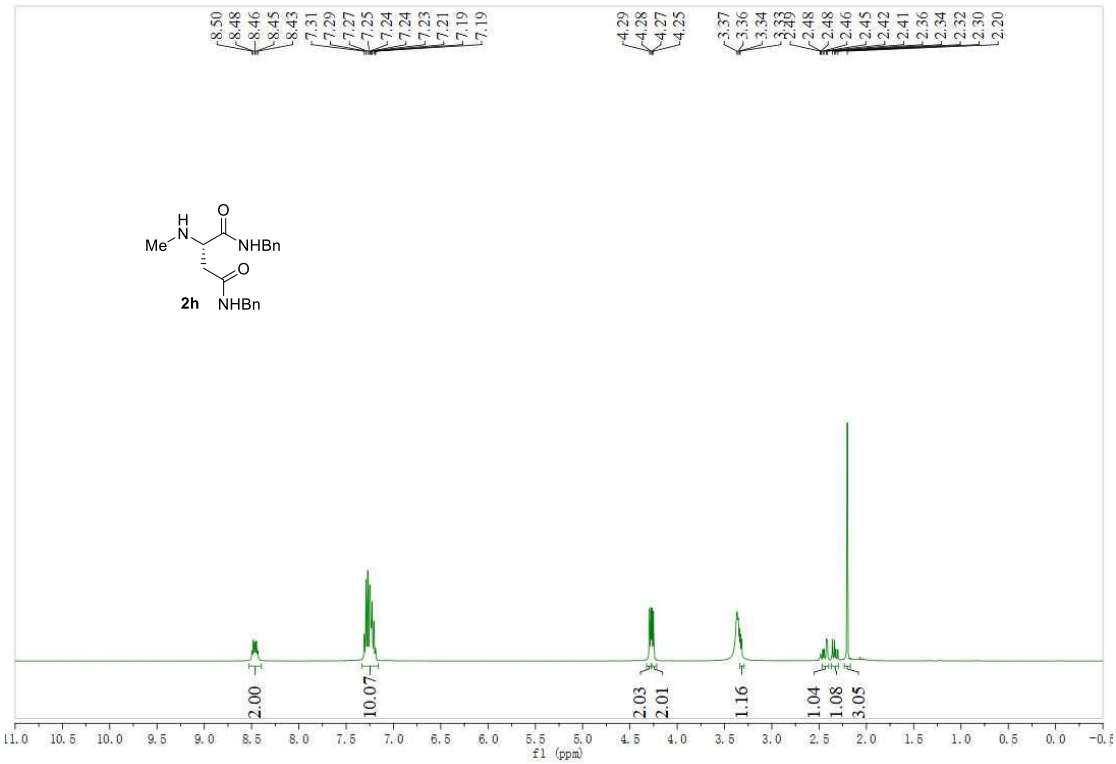
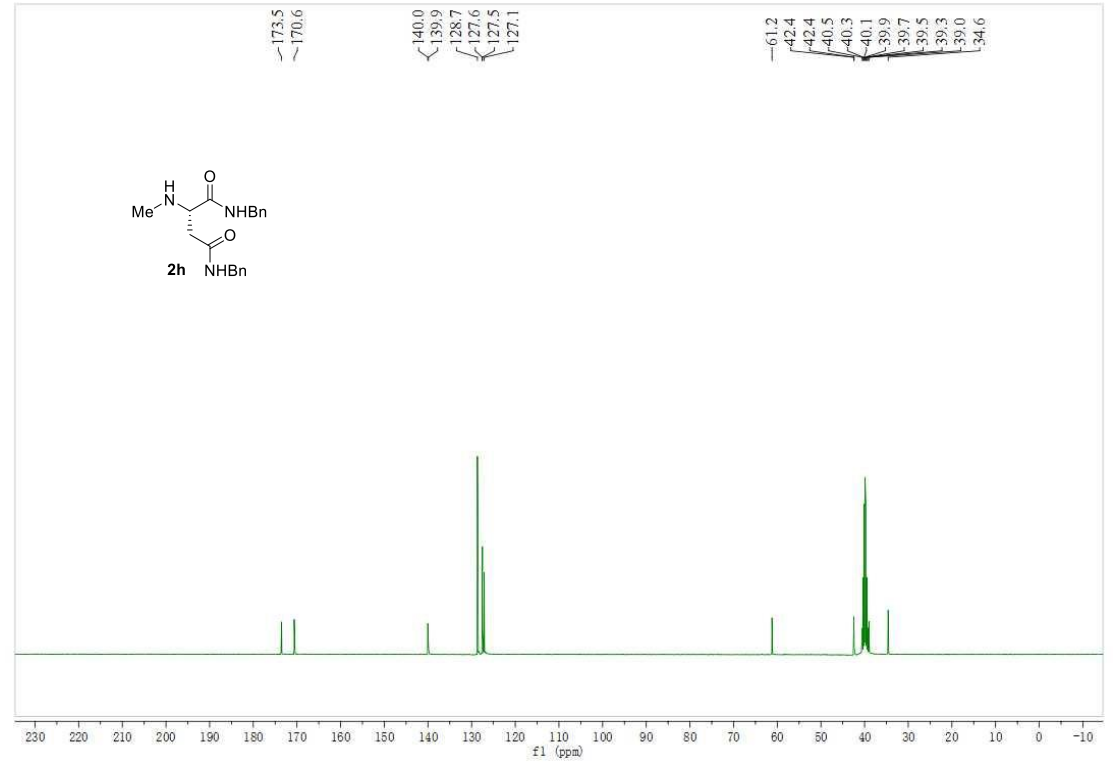
7.26
7.23
7.21
7.19
6.88
6.70
6.49
4.98
4.83
4.50
4.45
4.44
4.42
4.40
4.33
4.30
4.24
4.20
3.04
3.02
3.00
2.98
2.82
2.79
2.57
2.55
2.54
1.39
Boc
N
Me
O
NHBn
O
s36
NHBn
9.56
2.03
0.98
4.01
1.03
2.96
1.05
9.00
f1 (ppm)


170.5
169.9
156.3
155.1
138.3
138.1
128.6
127.6
127.3
81.3
80.8
77.4
77.1
76.8
57.8
56.4
43.5
43.3
36.6
35.7
32.3
31.9
28.2
Boc
N
Me
O
NHBn
O
s36
NHBn
f1 (ppm)


8.50
8.48
8.46
8.45
8.43
7.31
7.29
7.27
7.25
7.24
7.24
7.23
7.21
7.19
7.19
4.29
4.28
4.27
4.25
3.37
3.36
3.34
3.33
2.49
2.48
2.48
2.46
2.45
2.42
2.41
2.36
2.34
2.32
2.30
2.20
Me
H
N
O
NHBn
O
NHBn
2h
2.00
10.07
2.03
2.01
1.16
1.04
1.08
3.05
f1 (ppm)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170.6
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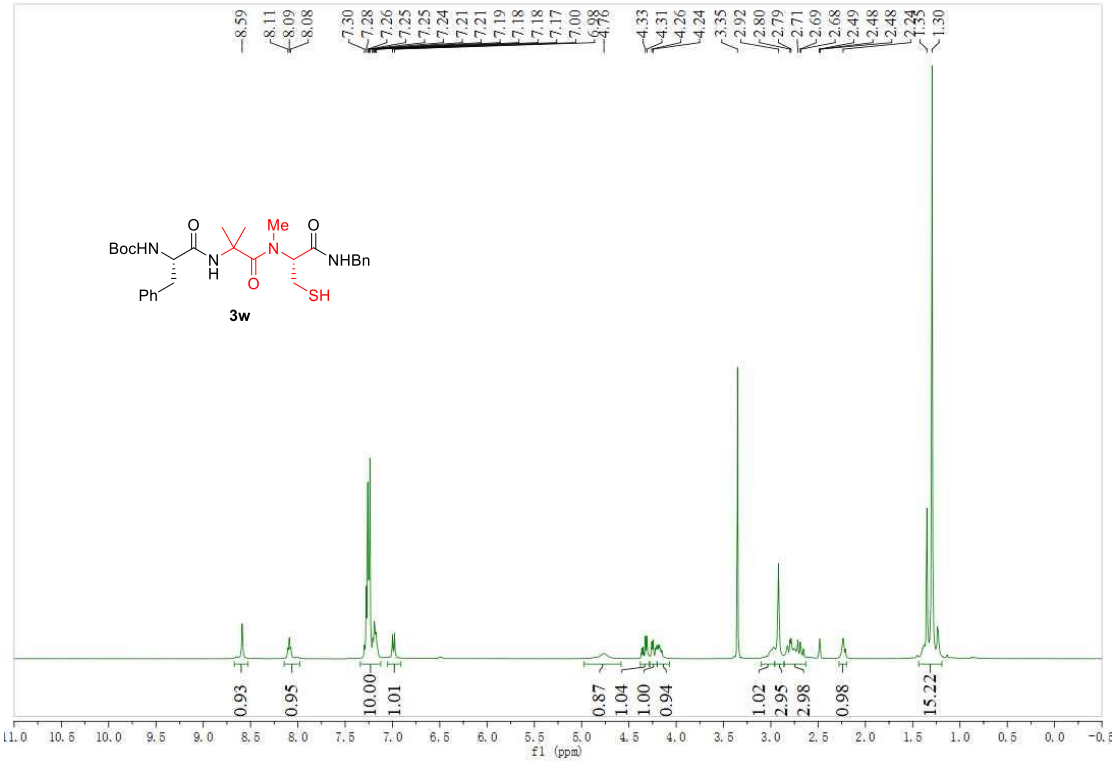
139.9
128.7
127.6
127.5
127.1
61.2
42.4
42.4
40.5
40.3
40.1
39.9
39.7
39.5
39.3
39.0
34.6
Me
H
N
O
NHBn
O
NHBn
2h
f1 (ppm)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8.11
8.09
8.08
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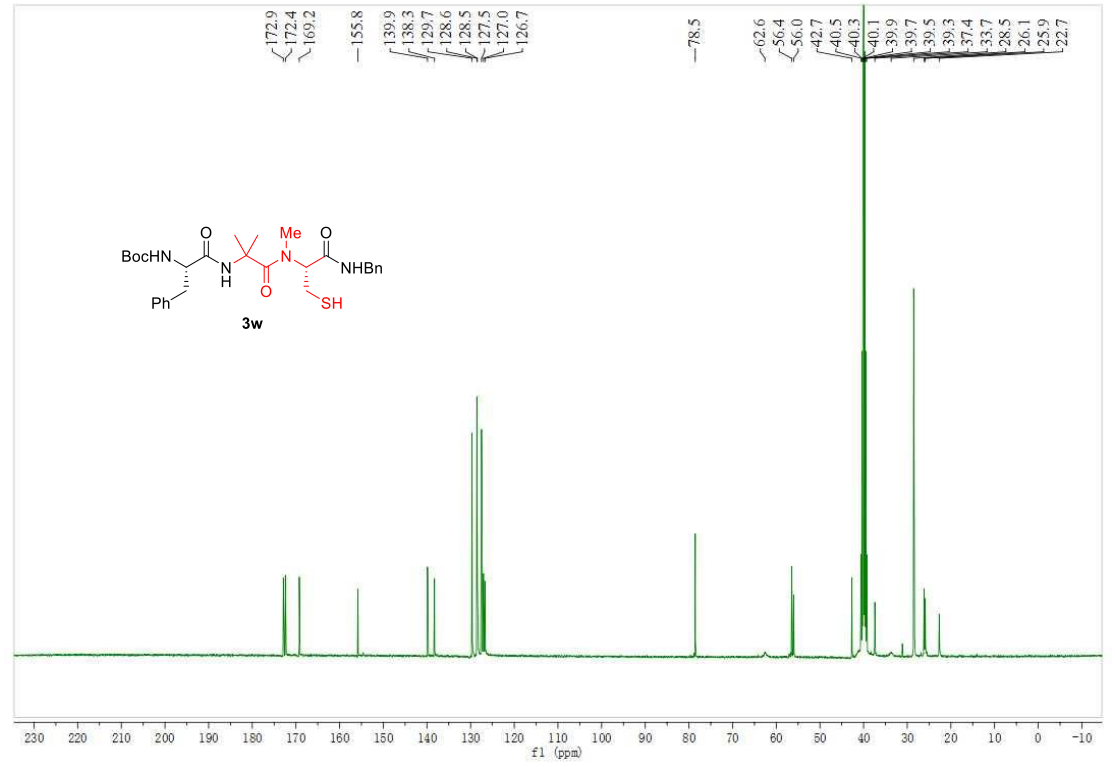
7.28
7.26
7.25
7.25
7.24
7.21
7.21
7.19
7.18
7.18
7.17
7.00
6.98
6.96
4.33
4.31
4.26
4.24
3.35
2.92
2.80
2.79
2.71
2.69
2.68
2.49
2.48
2.48
2.24
1.33
1.30
BocHN
O
N
H
O
Me
N
O
NHBn
Ph
SH
3w
0.93
0.95
10.00
1.01
0.87
1.04
1.00
0.94
1.02
2.95
2.98
0.98
15.22
f1 (ppm)


172.9
172.4
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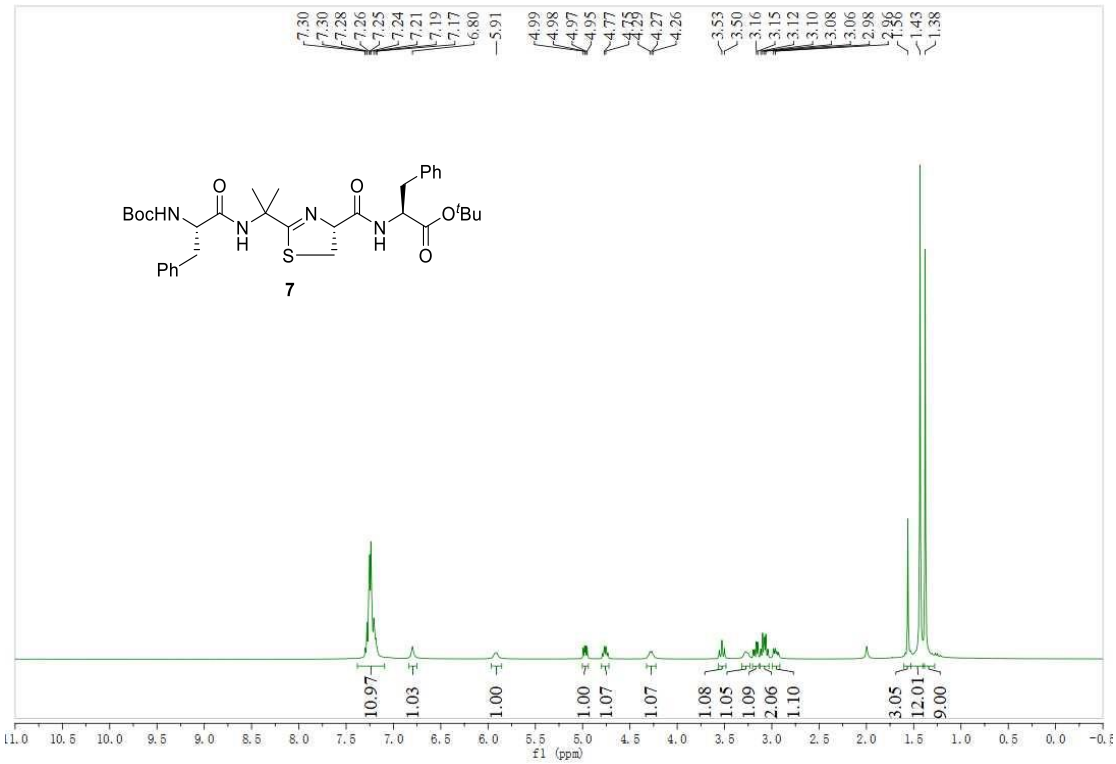
155.8
139.9
138.3
129.7
128.6
128.5
127.5
127.0
126.7
78.5
62.6
56.4
56.0
42.7
40.5
40.3
40.1
39.9
39.7
39.5
39.3
37.4
33.7
28.5
26.1
25.9
22.7
BocHN
O
N
H
O
Me
N
O
NHBn
Ph
SH
3w
f1 (ppm)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7.26
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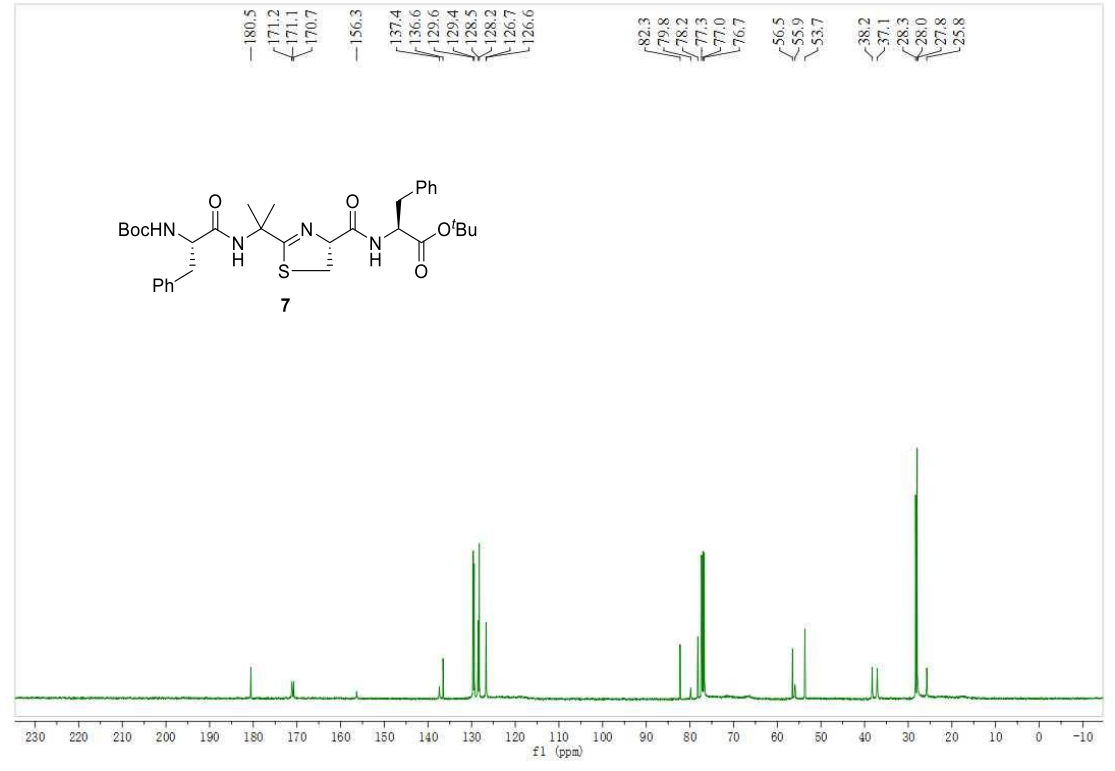
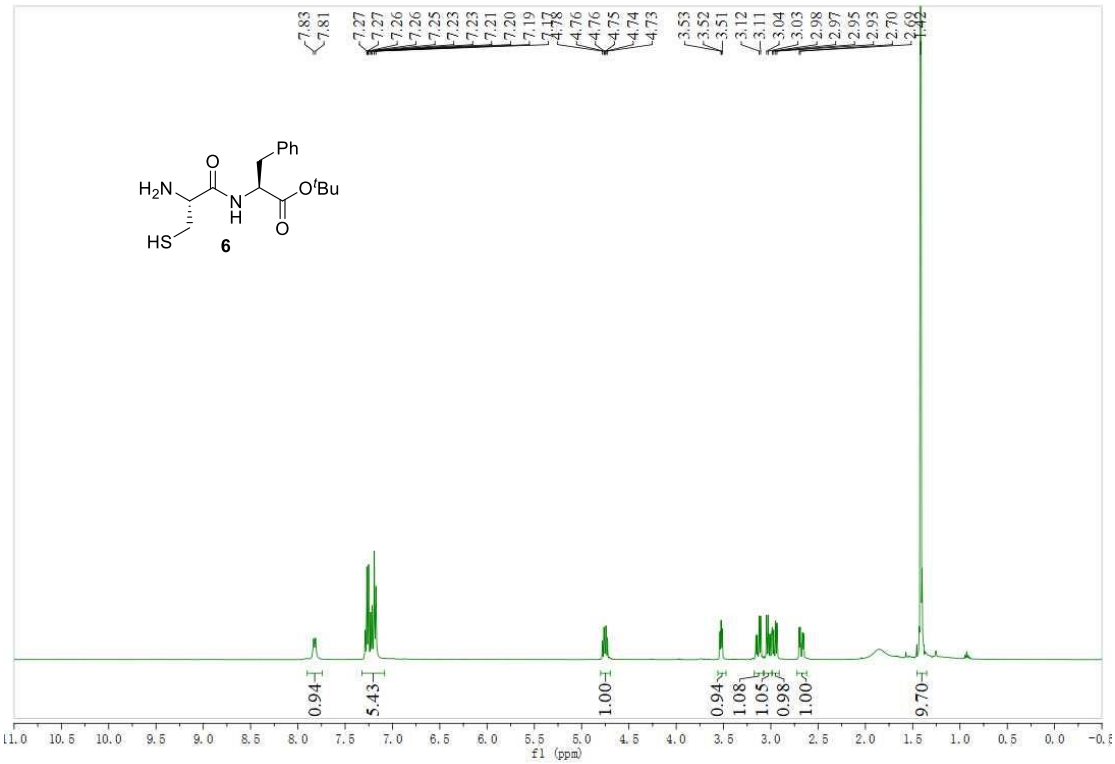
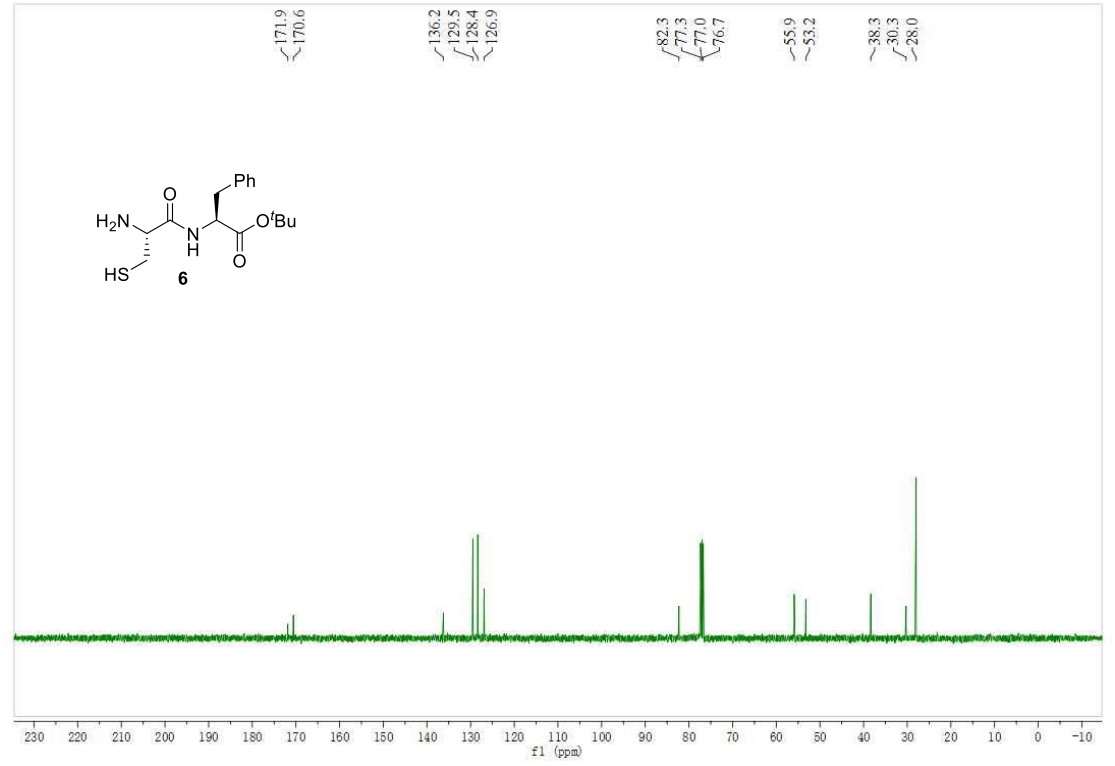
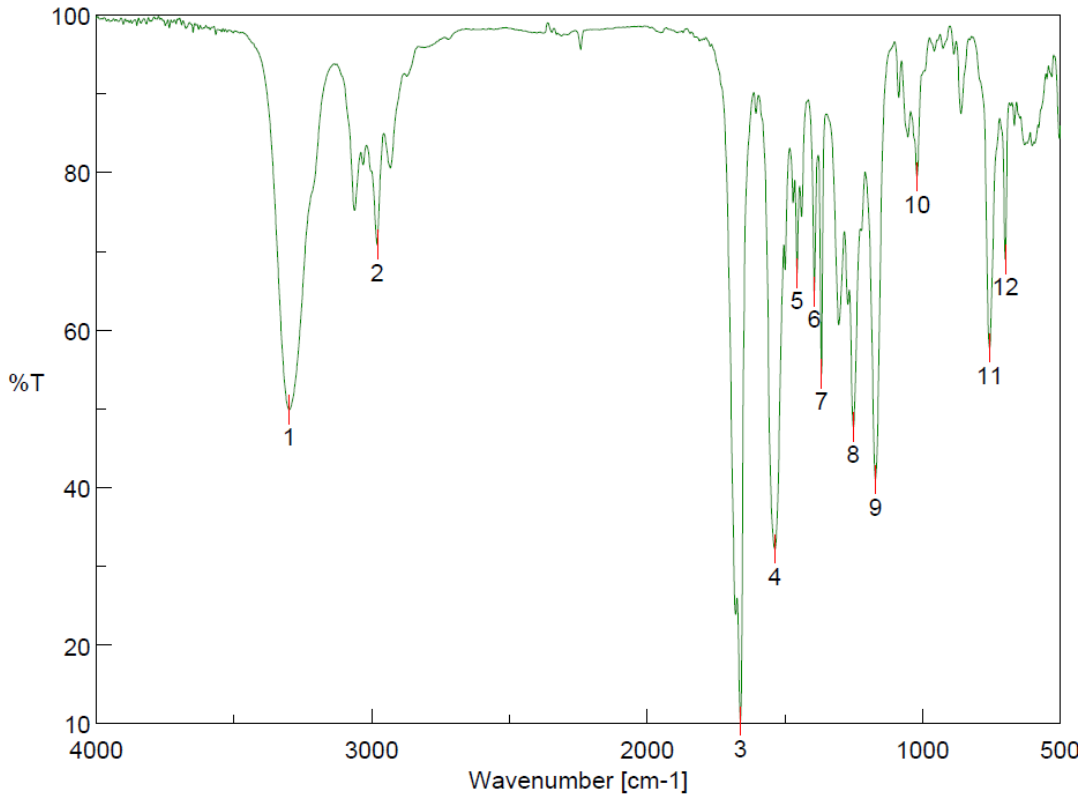
7.24
7.21
7.19
7.17
6.80
5.91
4.99
4.98
4.97
4.95
4.77
4.75
4.29
4.27
4.26
3.53
3.50
3.16
3.15
3.12
3.10
3.08
3.06
2.98
2.96
1.56
1.43
1.38
BocHN
O
N
H
O
N
S
N
H
O
Ph
Ph
O
tBu
7
10.97
1.03
1.00
1.00
1.07
1.07
1.08
1.05
1.09
2.06
1.10
3.05
12.01
9.00
f1 (ppm)


180.5
171.2
171.1
170.7
156.3
137.4
136.6
129.6
129.4
128.5
128.2
126.7
126.6
82.3
79.8
78.2
77.3
77.0
76.7
56.5
55.9
53.7
38.2
37.1
28.3
28.0
27.8
25.8
BocHN
O
N
H
O
N
S
N
H
O
Ph
Ph
O
tBu
7
f1 (ppm)

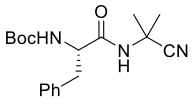

7.83
7.81
7.27
7.27
7.26
7.26
7.25
7.23
7.23
7.21
7.20
7.19
7.17
4.78
4.76
4.76
4.75
4.74
4.73
3.53
3.52
3.51
3.12
3.11
3.04
3.03
2.98
2.97
2.95
2.93
2.70
2.69
1.42
Ph
O
H2N
N
H
O
OtBu
HS
6
0.94
5.43
1.00
0.94
1.08
1.05
0.98
1.00
9.70
f1 (ppm)


171.9
170.6
136.2
129.5
128.4
126.9
82.3
77.3
77.0
76.7
55.9
53.2
38.3
30.3
28.0
Ph
O
H2N
N
H
O
OtBu
HS
6
f1 (ppm)



[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3299.61	49.8478	2	2980.45	70.8183
3	1661.37	10.333	4	1536.02	32.1659
5	1455.99	67.213	6	1393.32	64.8463
7	1367.28	54.4188	8	1251.58	47.7045
9	1170.58	40.9551	10	1020.16	79.4905
11	756.923	57.7262	12	699.069	68.9941



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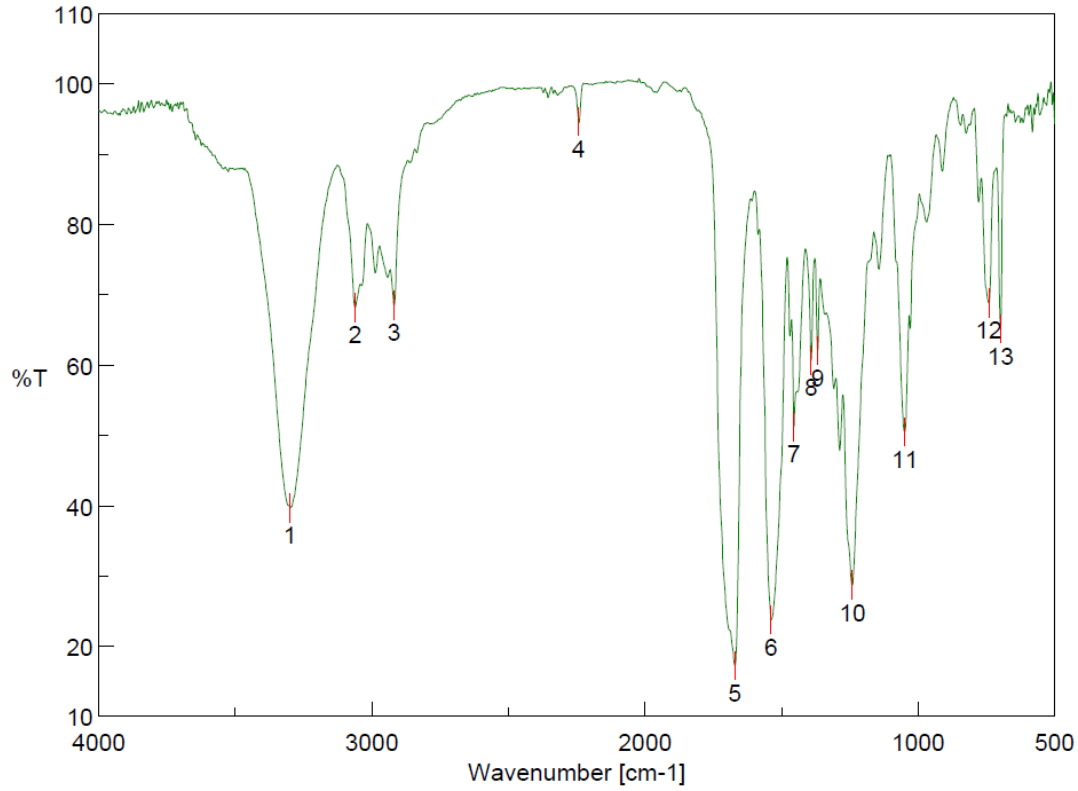
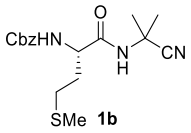


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3299.61	39.6659	2	3061.44	68.172
3	2918.73	68.4897	4	2242.81	94.6991
5	1671.02	17.2213	6	1537.95	23.7486
7	1455.03	51.2308	8	1391.39	60.6557
9	1368.25	62.0313	10	1240.97	28.6785
11	1049.09	50.4757	12	740.531	68.7954
13	698.105	65.1877			



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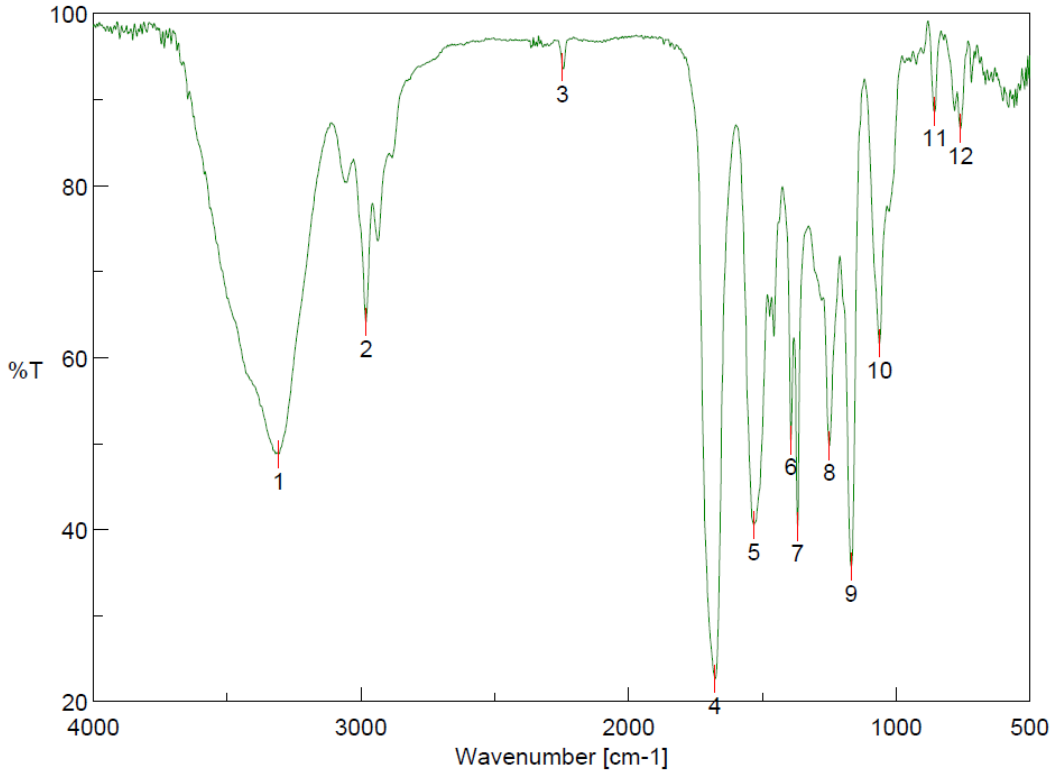
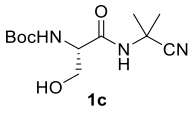
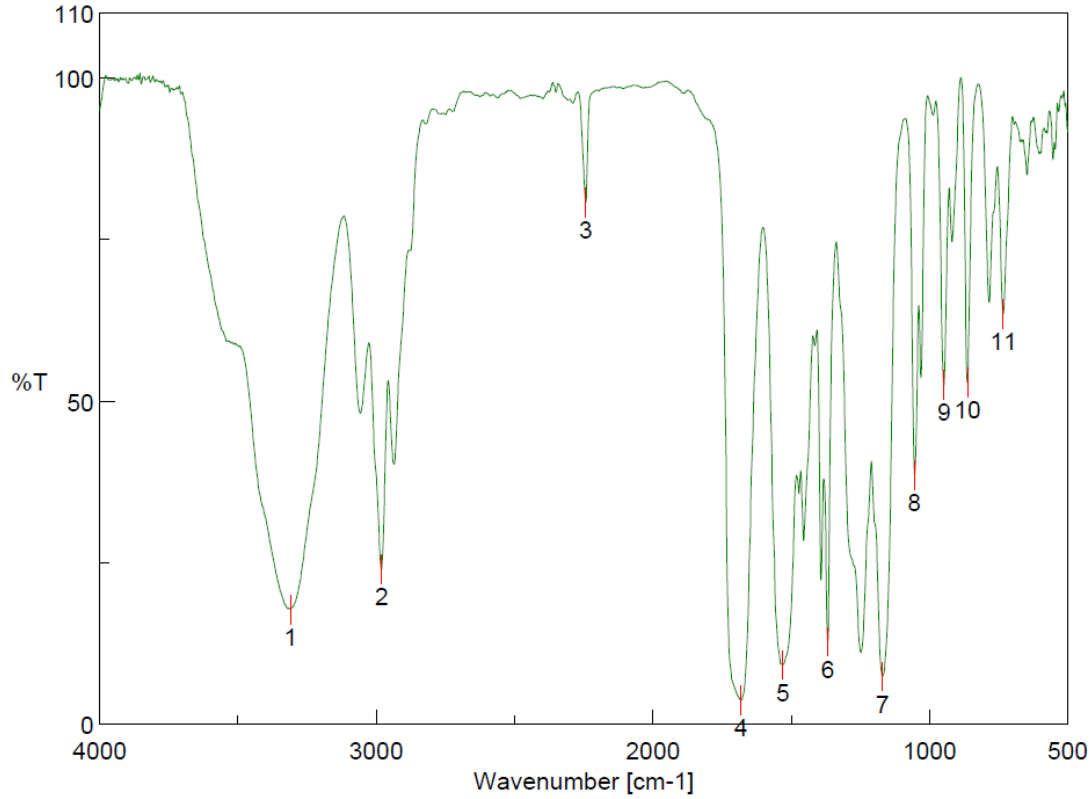


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3307.32	48.7797	2	2981.41	64.1262
3	2246.66	93.7556	4	1675.84	22.6259
5	1532.17	40.5457	6	1393.32	50.3474
7	1368.25	40.3496	8	1249.65	49.7406
9	1167.69	35.7242	10	1061.62	61.6007
11	855.275	88.5424	12	758.852	86.5809

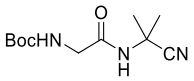


1c

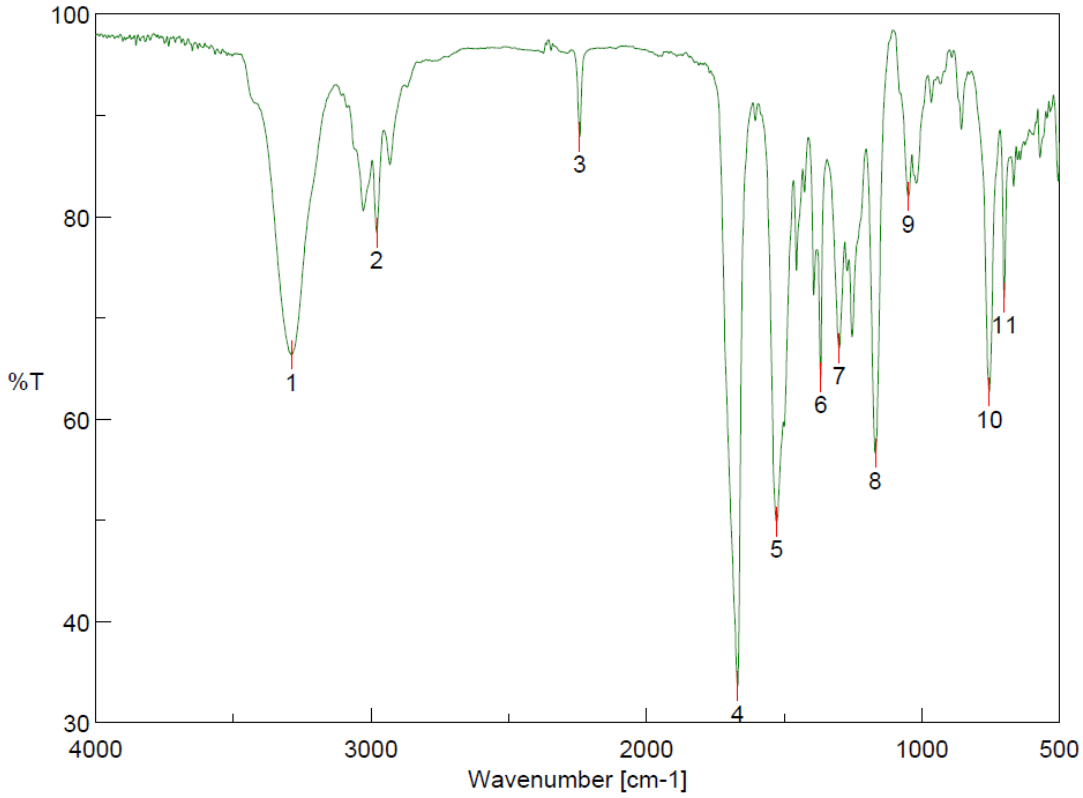


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3311.18	17.7636	2	2981.41	24.0049
3	2242.81	80.6983	4	1681.62	3.75909
5	1530.24	9.23531	6	1368.25	12.7733
7	1169.62	7.4802	8	1053.91	38.5302
9	947.842	52.5321	10	862.989	52.8896
11	733.782	63.4252			

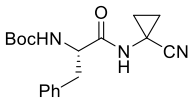


1d

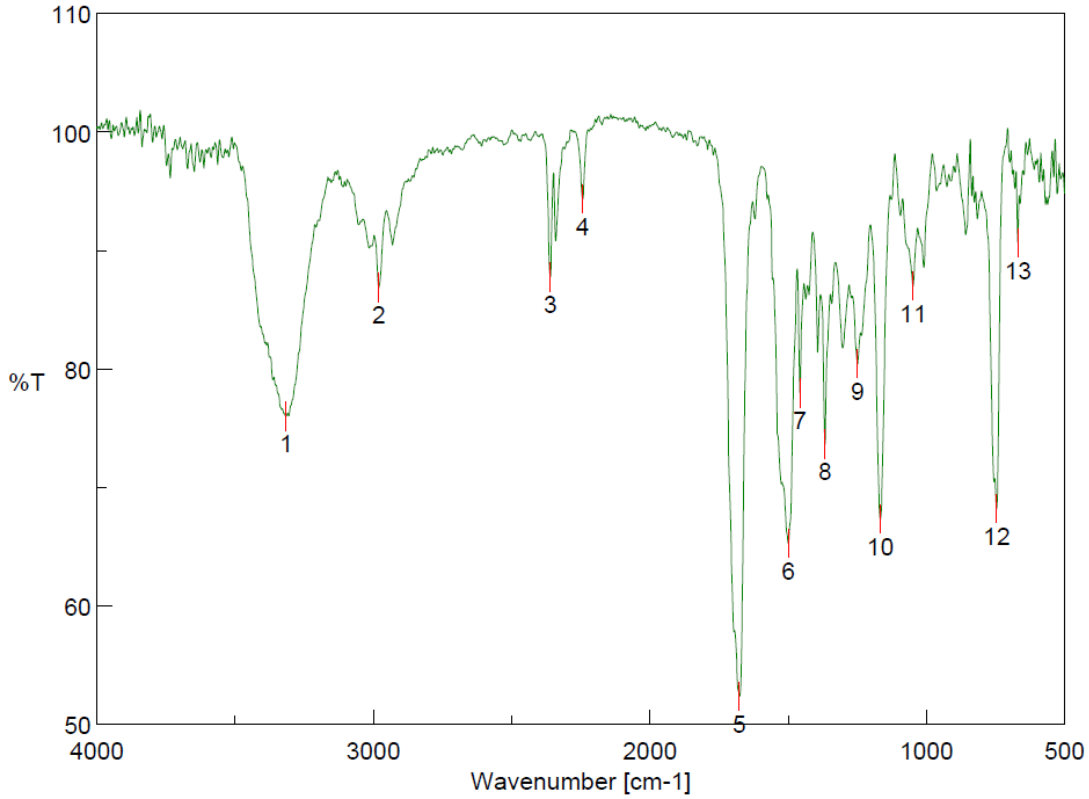


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3289	66.3206	2	2979.48	78.3871
3	2242.81	87.8631	4	1669.09	33.5986
5	1527.35	49.8781	6	1367.28	64.0987
7	1299.79	66.9743	8	1168.65	56.6224
9	1049.09	81.9527	10	754.995	62.6962
11	700.034	71.9841			

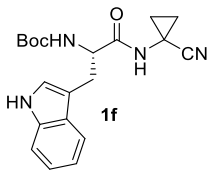


1e

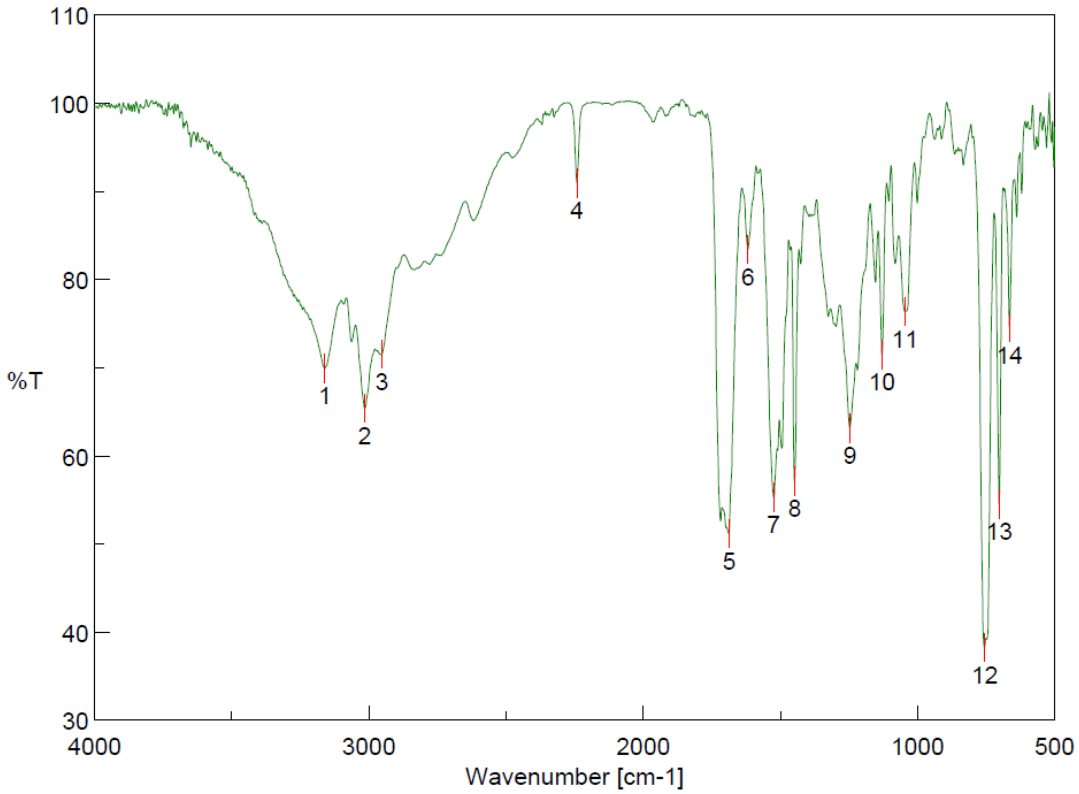


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3316	76.0266	2	2981.41	86.8551
3	2360.44	87.7515	4	2242.81	94.3484
5	1675.84	52.3739	6	1499.38	65.2823
7	1456.96	77.9742	8	1367.28	73.6485
9	1249.65	80.4413	10	1166.72	67.3504
11	1048.12	87.0084	12	746.317	68.2227
13	669.178	90.6252			

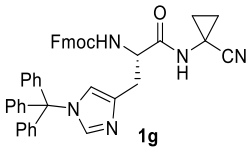


1f

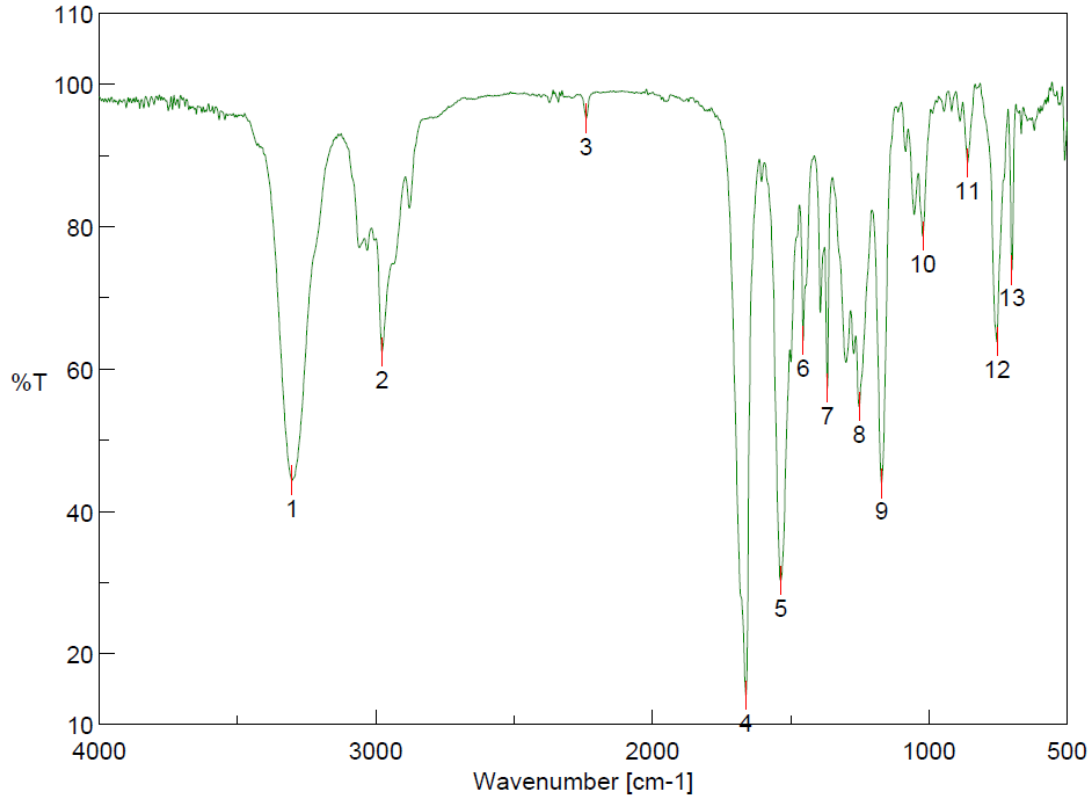


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3160.76	69.903	2	3015.16	65.3598
3	2953.45	71.5078	4	2241.84	90.9039
5	1687.41	51.1735	6	1617.98	83.3937
7	1524.45	55.2562	8	1448.28	57.1023
9	1246.75	63.1355	10	1129.12	71.4424
11	1046.19	76.3249	12	755.959	38.2736
13	701.962	54.495	14	665.321	74.5467

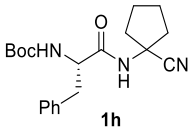


1g

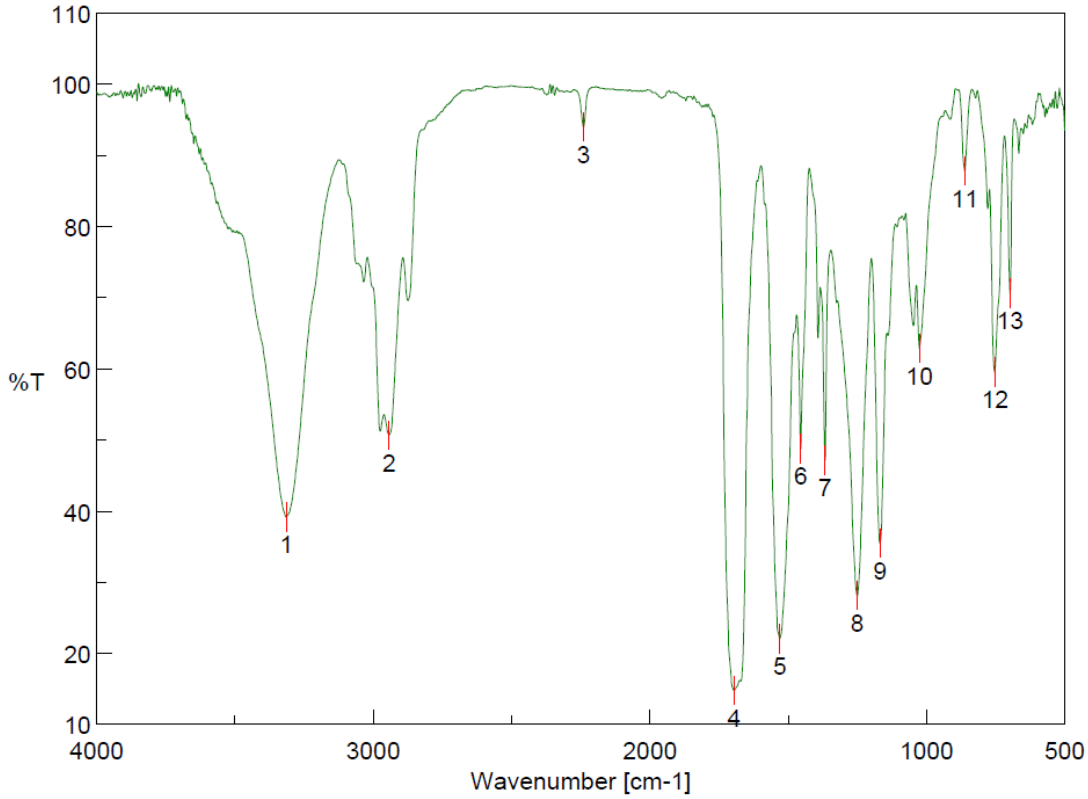


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3303.46	44.3259	2	2977.55	62.3971
3	2238.95	95.1844	4	1661.37	14.1344
5	1536.02	30.2807	6	1455.03	63.963
7	1367.28	57.3397	8	1252.54	54.7036
9	1171.54	43.8943	10	1022.09	78.6636
11	860.096	88.9477	12	754.031	63.8344
13	700.034	73.8618			

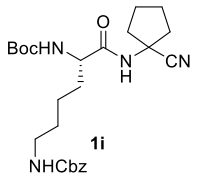


1h

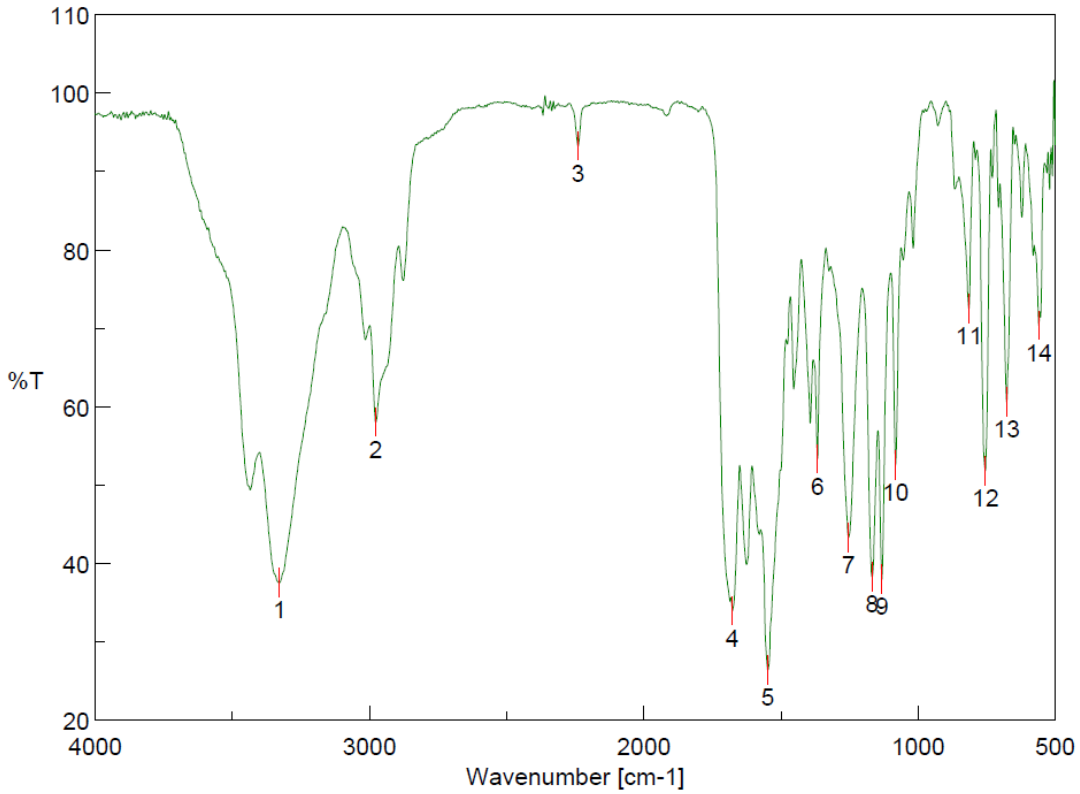


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3315.03	39.1601	2	2942.84	50.6643
3	2238.95	93.9587	4	1696.09	14.7887
5	1530.24	22.0642	6	1455.03	48.7354
7	1367.28	47.1511	8	1250.61	28.133
9	1167.69	35.4783	10	1025.94	62.8545
11	862.025	87.8354	12	753.066	59.5334
13	698.105	70.6459			

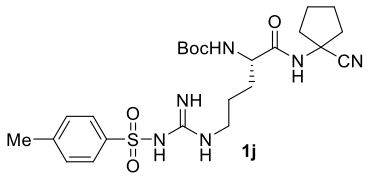


1i

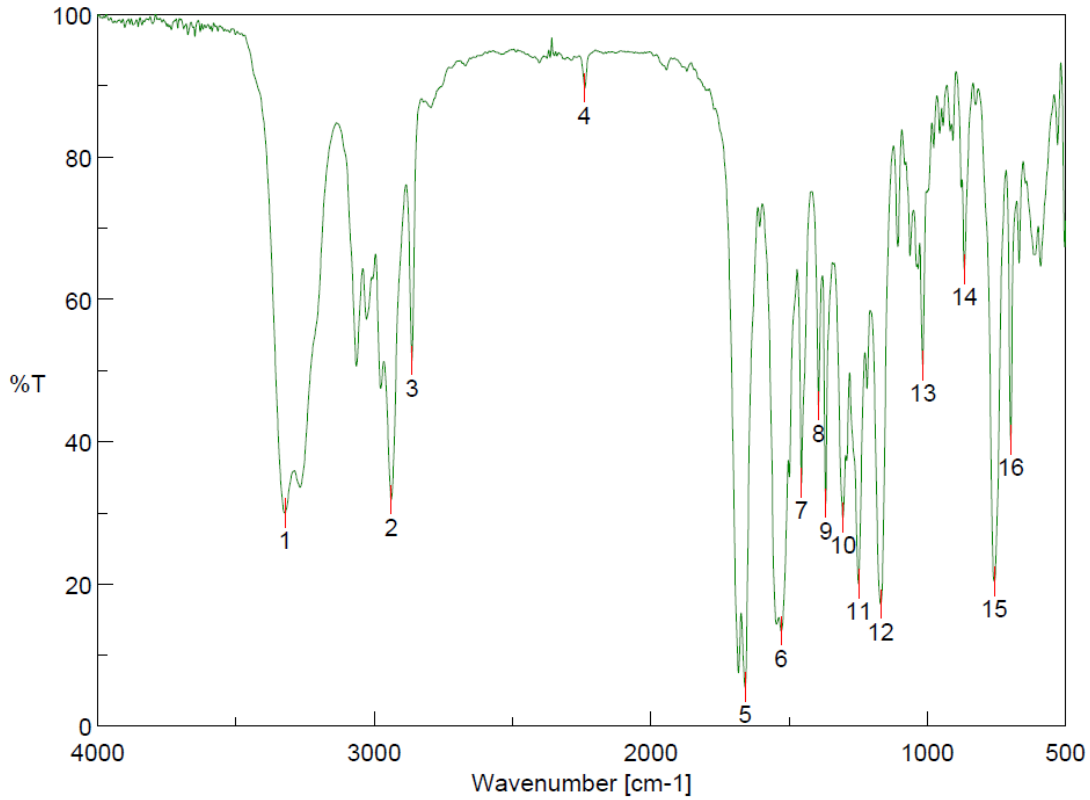


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3328.53	37.5469	2	2977.55	58.0232
3	2238.95	93.204	4	1676.8	33.993
5	1547.59	26.4248	6	1367.28	53.3778
7	1253.5	43.3484	8	1167.69	38.2982
9	1132.01	38.0016	10	1081.87	52.5307
11	814.777	72.471	12	754.995	51.8487
13	676.892	60.589	14	560.22	70.3856

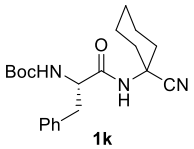


1j

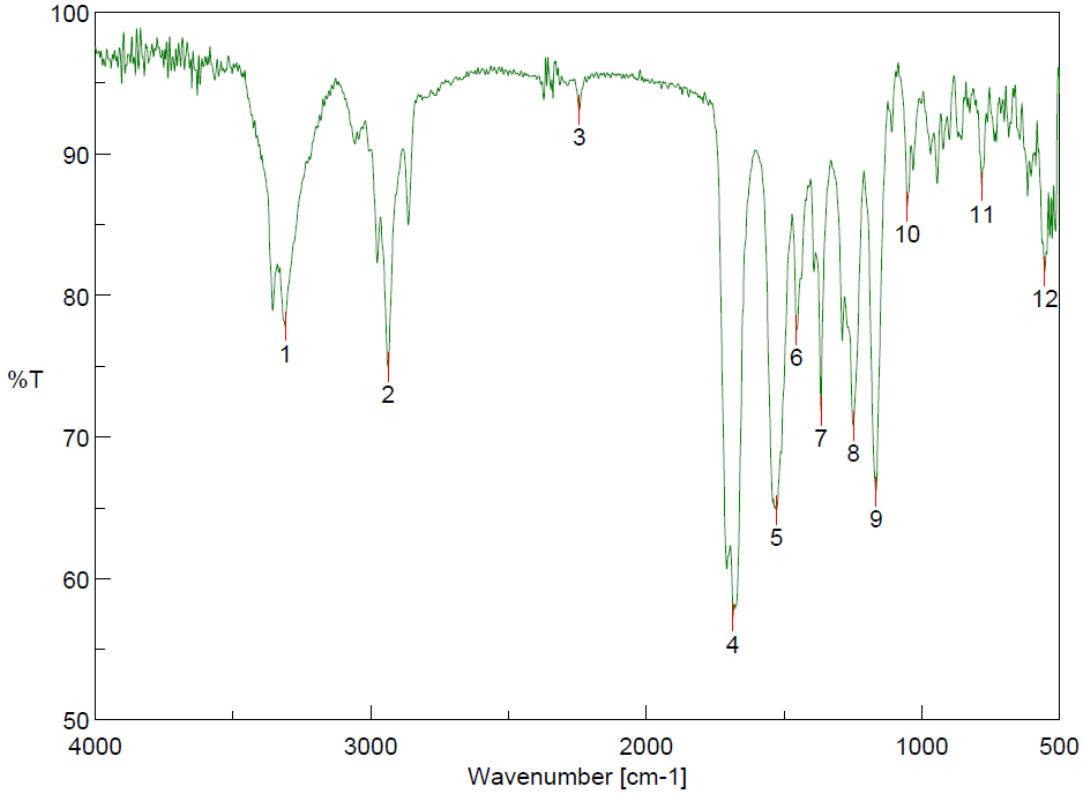


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3323.71	29.9575	2	2938.98	31.881
3	2863.77	51.4324	4	2238.95	89.695
5	1659.45	5.48373	6	1528.31	13.3855
7	1455.03	34.2102	8	1393.32	45.0703
9	1367.28	31.329	10	1304.61	29.3267
11	1248.68	20.0182	12	1168.65	17.1933
13	1016.3	50.685	14	865.882	64.2295
15	757.888	20.3756	16	698.105	40.2584

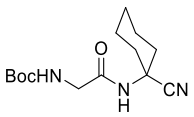


1k

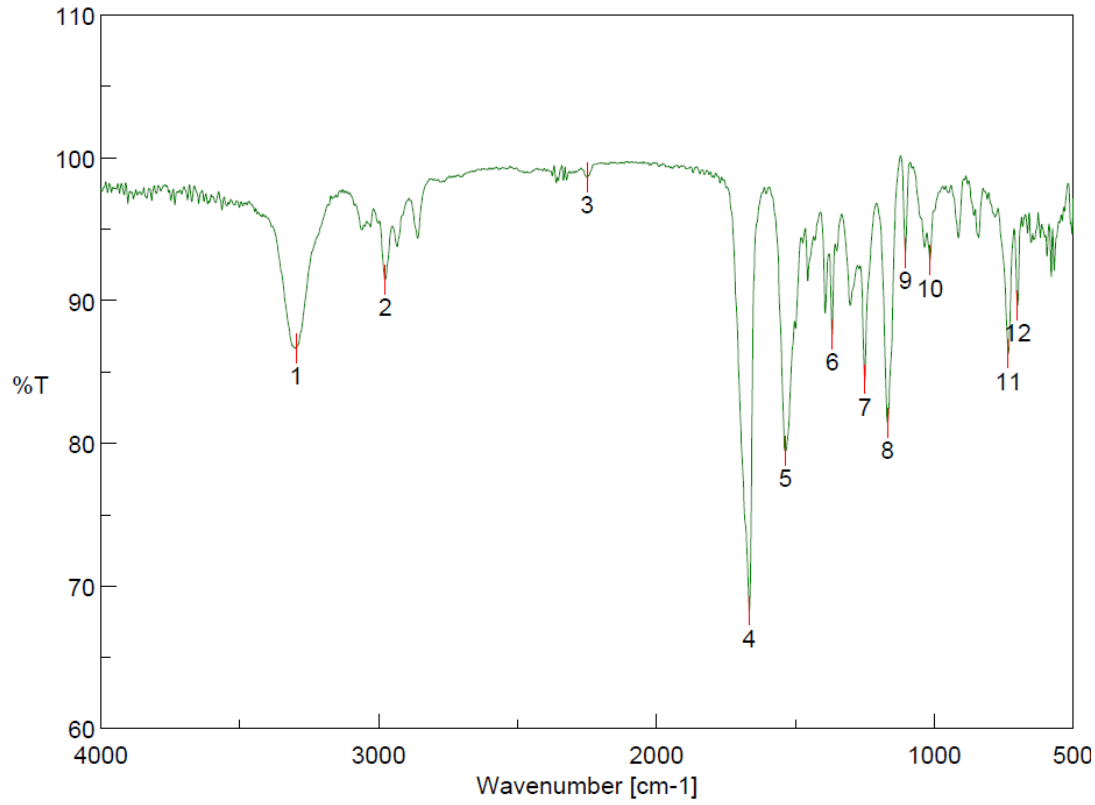


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3311.18	77.8308	2	2937.06	74.9312
3	2242.81	93.0864	4	1684.52	57.3057
5	1527.35	64.8349	6	1455.03	77.5517
7	1366.32	71.8331	8	1248.68	70.7802
9	1165.76	66.1479	10	1052.94	86.2545
11	781.993	87.6882	12	553.47	81.7088

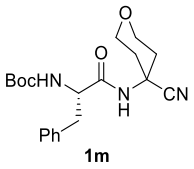


1l

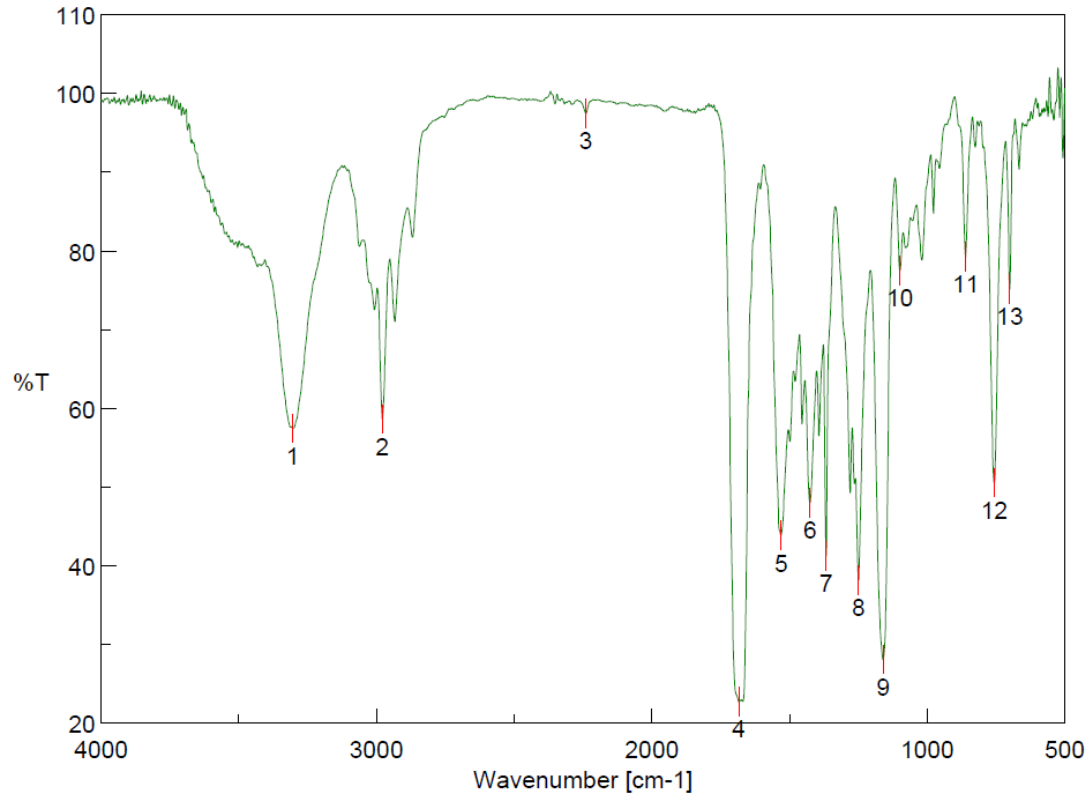


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3297.68	86.6443	2	2976.59	91.4534
3	2249.56	98.6438	4	1665.23	68.2695
5	1535.06	79.4683	6	1367.28	87.6009
7	1249.65	84.4826	8	1167.69	81.4419
9	1104.05	93.3408	10	1014.37	92.8206
11	732.817	86.2614	12	700.034	89.6593

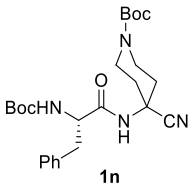


1m

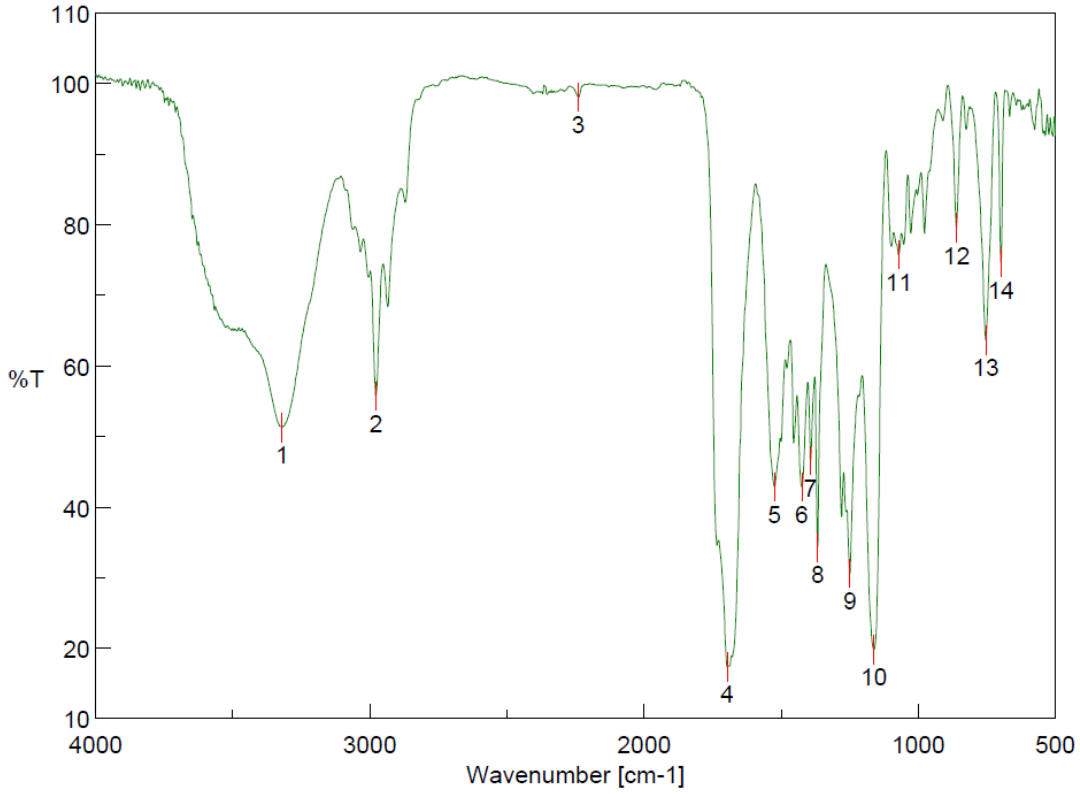


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3304.43	57.4903	2	2978.52	58.6517
3	2238.95	97.4031	4	1683.55	22.7645
5	1531.2	43.847	6	1426.1	48.0033
7	1367.28	41.2471	8	1249.65	38.1952
9	1159.97	28.1209	10	1099.23	77.5003
11	860.096	79.2302	12	756.923	50.5554
13	700.034	75.1251			

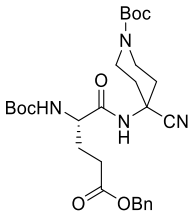


1n

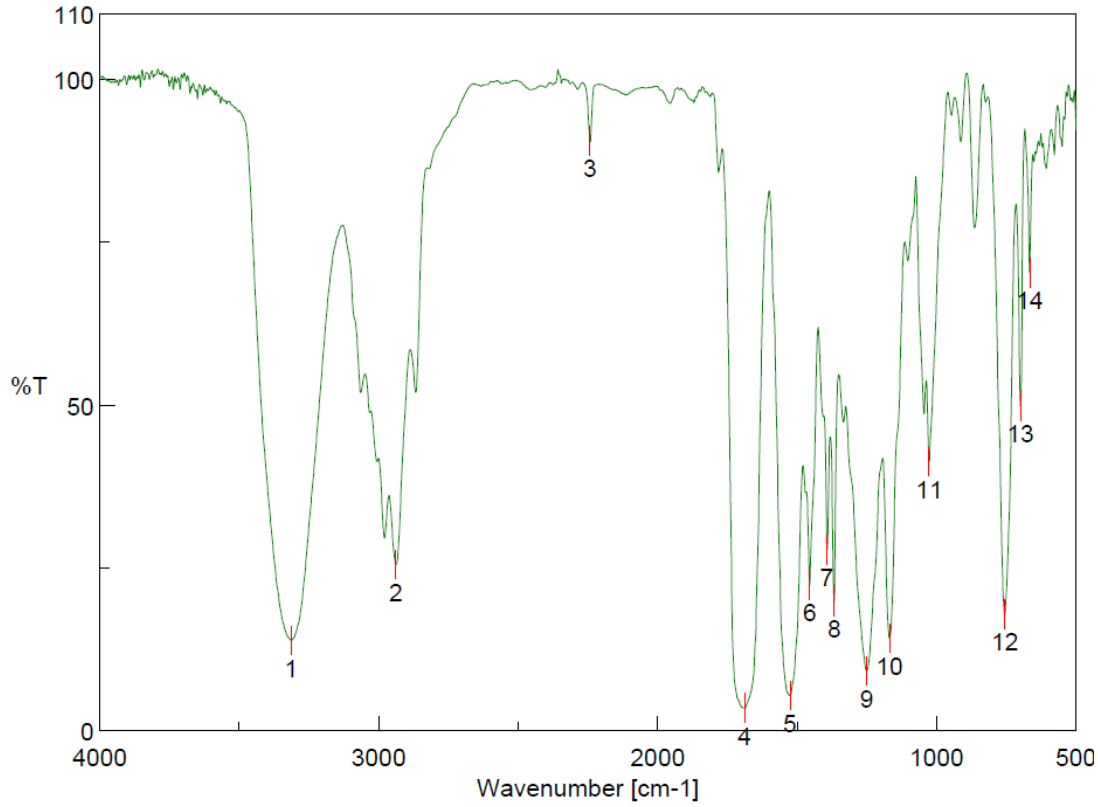


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3319.86	51.2319	2	2977.55	55.7415
3	2238.95	98.0441	4	1697.05	17.3276
5	1524.45	42.8369	6	1425.14	42.8167
7	1392.35	46.5859	8	1367.28	34.3111
9	1249.65	30.5959	10	1161.9	19.768
11	1071.26	75.7295	12	861.06	79.5469
13	754.031	63.6408	14	698.105	74.5662

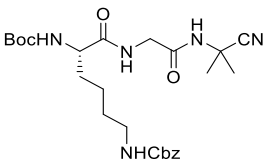


1o

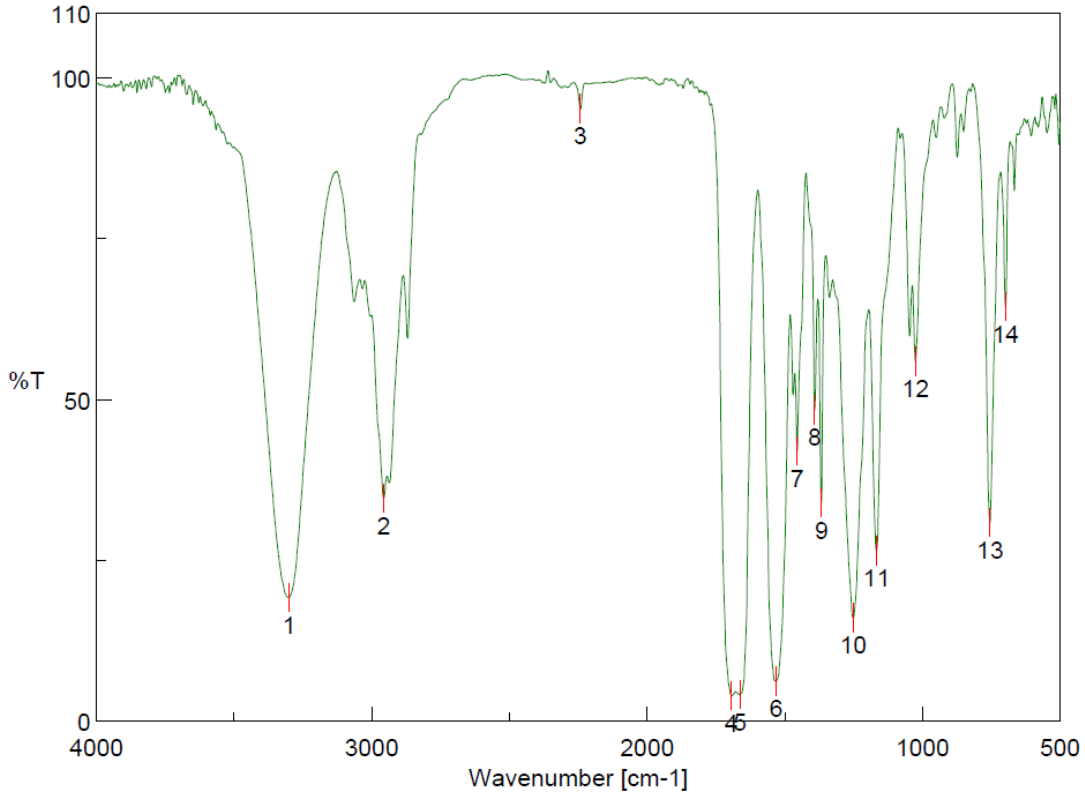


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3314.07	13.9123	2	2938.02	25.5445
3	2242.81	90.5103	4	1688.37	3.51893
5	1525.42	5.43178	6	1455.03	22.3836
7	1392.35	27.7897	8	1367.28	19.8459
9	1250.61	9.22952	10	1168.65	14.2568
11	1026.91	41.3458	12	755.959	17.8961
13	699.069	49.8123	14	666.285	70.2446

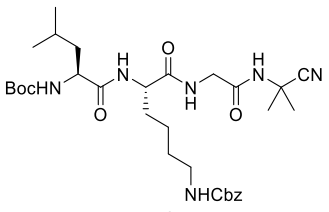


s1

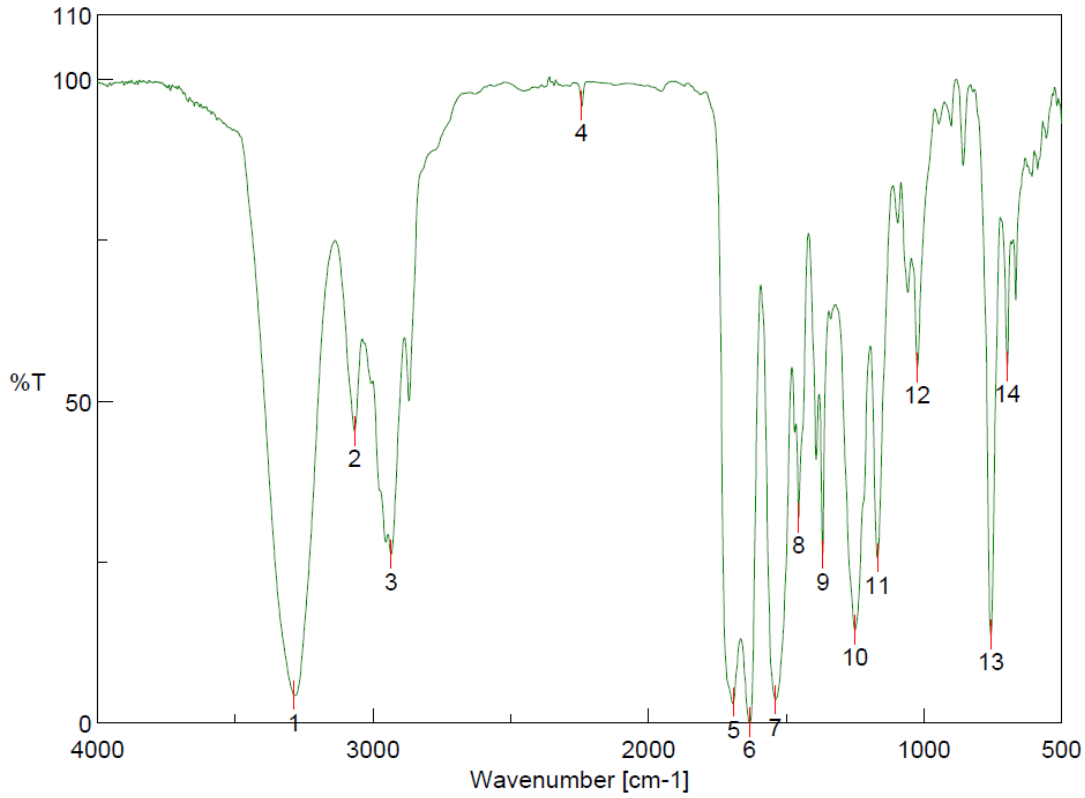


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3300.57	19.2741	2	2956.34	34.7086
3	2242.81	95.1508	4	1693.19	4.03467
5	1663.3	4.15279	6	1532.17	6.23119
7	1455.03	42.0119	8	1391.39	48.3939
9	1367.28	33.9668	10	1251.58	16.1085
11	1166.72	26.5108	12	1025.94	55.9145
13	754.995	30.8944	14	698.105	64.405

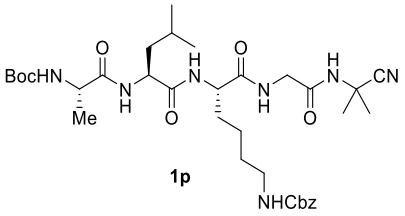


s2

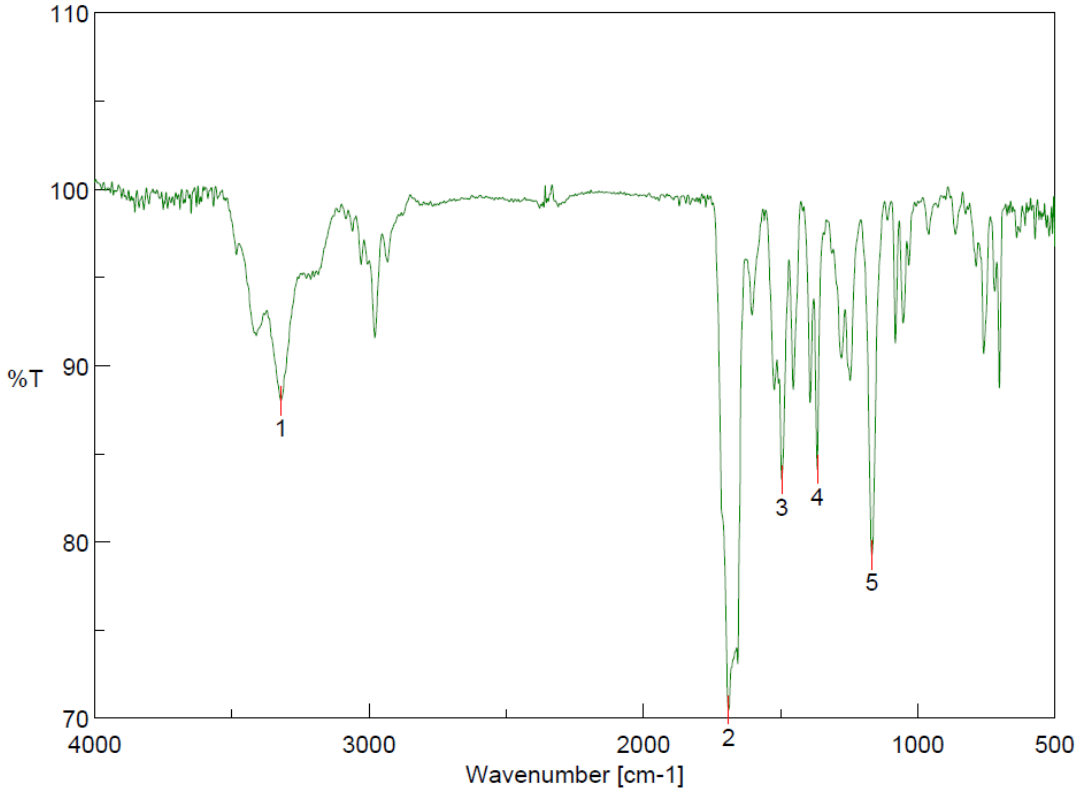


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3286.11	4.33292	2	3067.23	45.3678
3	2934.16	26.2645	4	2242.81	95.9233
5	1691.27	3.27355	6	1633.41	0.188895
7	1539.88	3.64812	8	1455.03	31.8493
9	1367.28	26.2904	10	1250.61	14.4819
11	1168.65	25.7134	12	1024.02	55.1667
13	756.923	13.783	14	697.141	55.3792

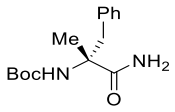


1p

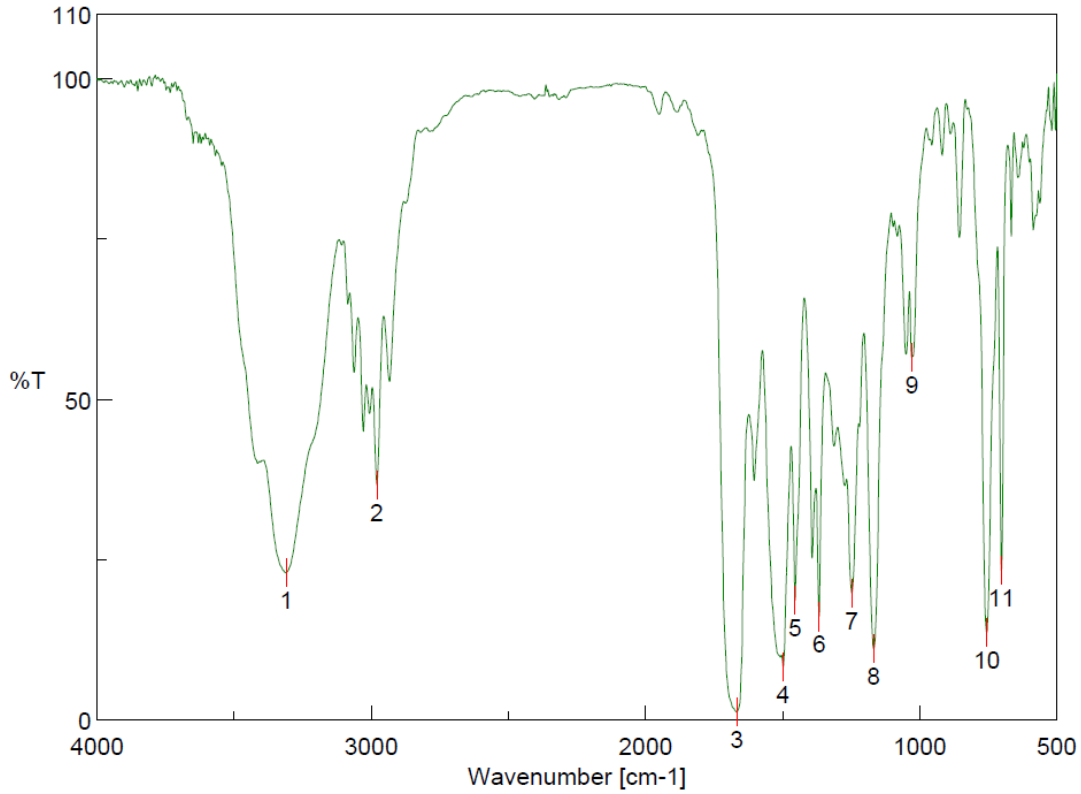


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3321.78	88.0005	2	1689.34	70.4815
3	1495.53	83.5379	4	1366.32	84.1038
5	1166.72	79.2554			

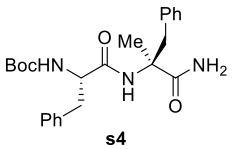


(*R*)-s3

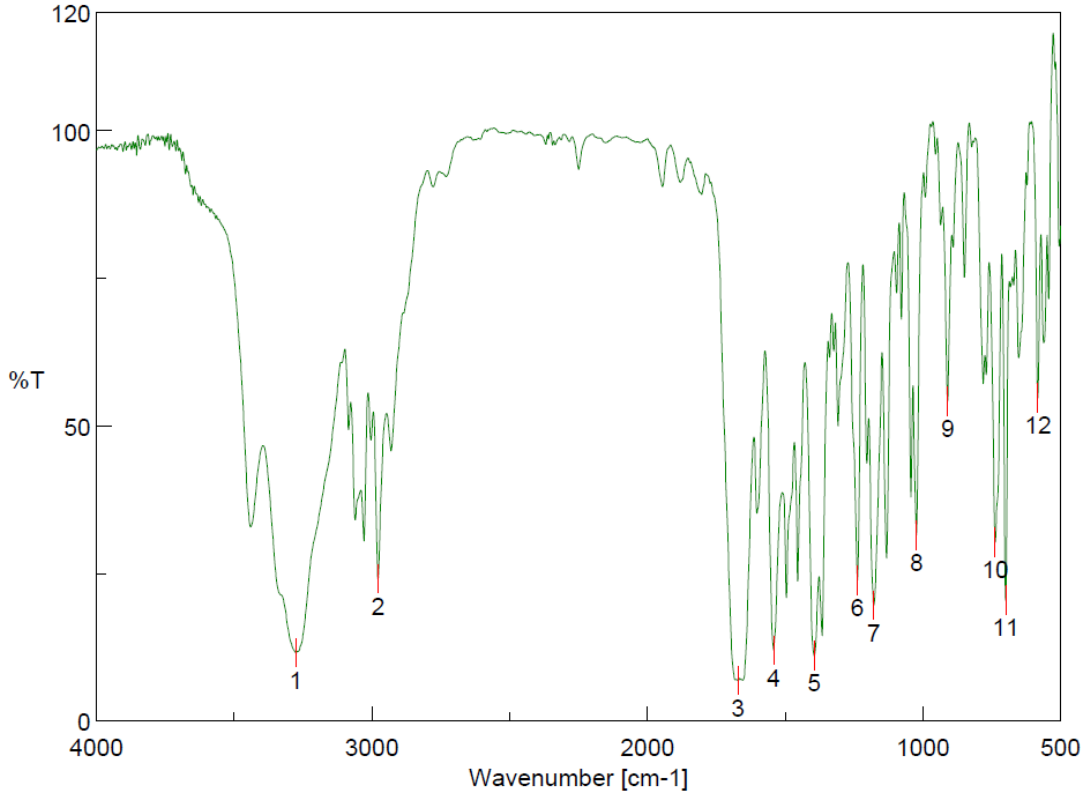


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3311.18	22.9788	2	2979.48	36.6703
3	1668.12	1.28551	4	1497.45	8.32637
5	1455.03	18.6775	6	1367.28	16.1819
7	1247.72	19.8057	8	1167.69	11.1918
9	1028.84	56.5245	10	755.959	13.7022
11	701.962	23.3613			

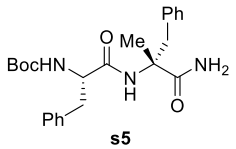


s4

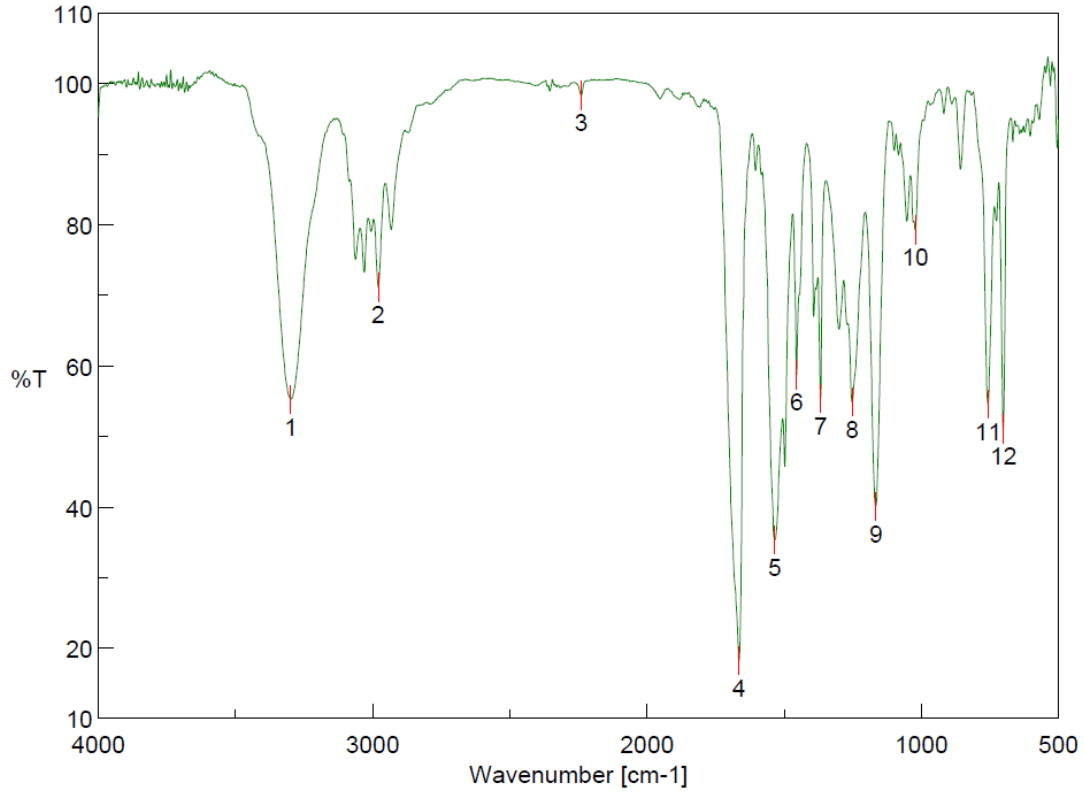


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3276.47	11.6363	2	2977.55	24.1089
3	1671.02	6.88124	4	1541.81	12.0302
5	1394.28	11.0896	6	1237.11	23.8662
7	1178.29	19.673	8	1023.05	31.4884
9	910.236	54.1643	10	737.639	30.3228
11	699.069	20.4645	12	582.397	54.7488

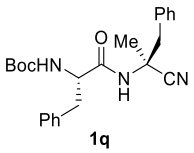


s5

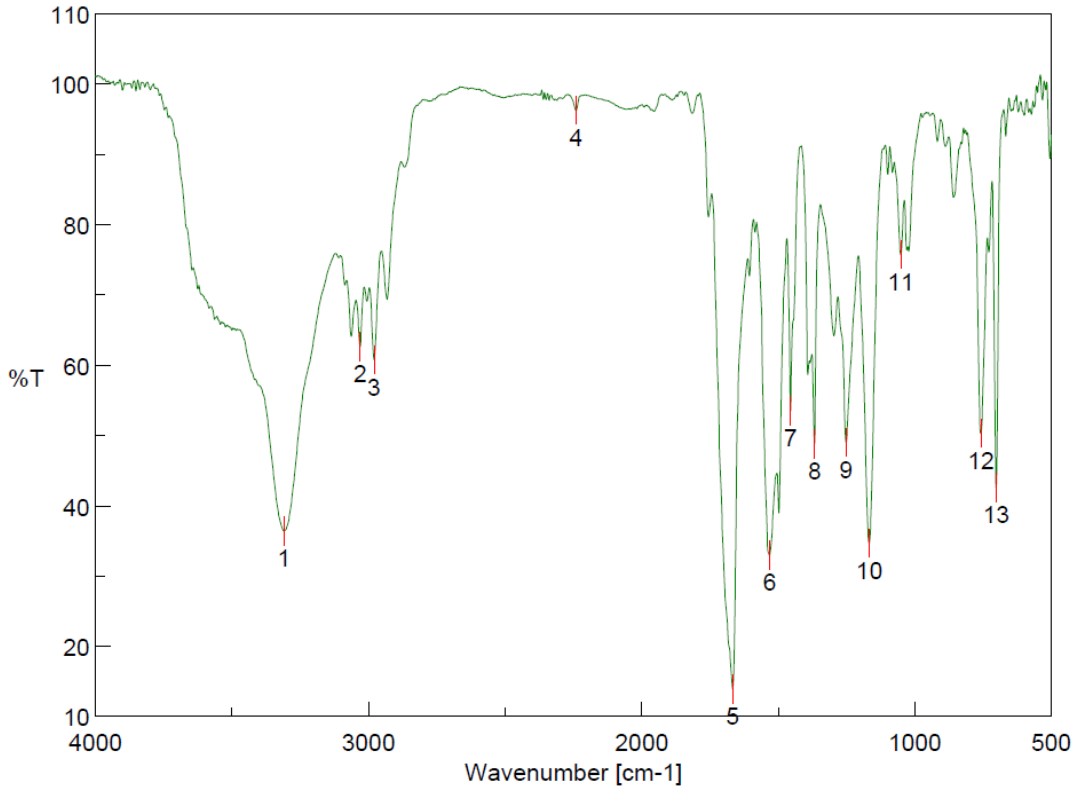


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3298.64	55.2539	2	2979.48	71.1413
3	2238.95	98.3093	4	1664.27	18.1897
5	1534.1	35.3407	6	1455.03	58.6845
7	1367.28	55.3705	8	1252.54	54.8628
9	1165.76	40.1038	10	1022.09	79.2347
11	756.923	54.5821	12	700.998	51.0669

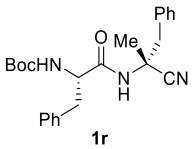


1q

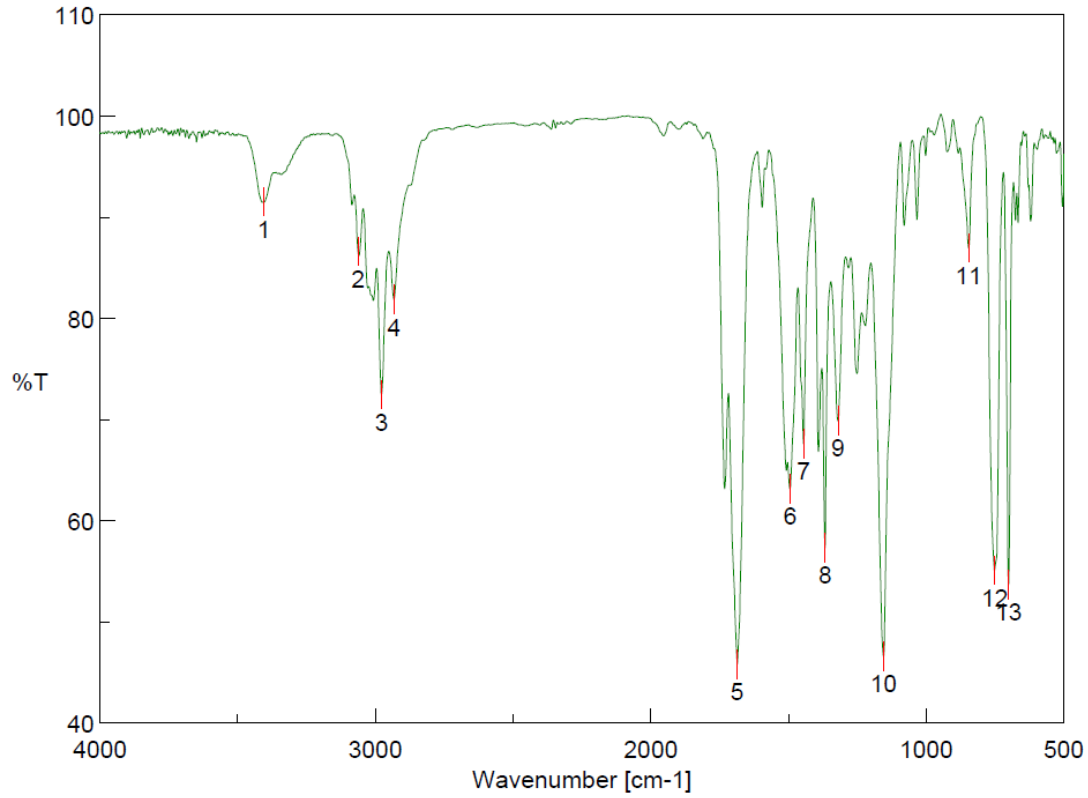


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3310.21	36.4155	2	3031.55	62.6523
3	2980.45	60.7633	4	2238.95	96.2588
5	1667.16	13.8567	6	1532.17	32.9138
7	1455.03	53.4708	8	1367.28	48.7387
9	1251.58	48.9791	10	1166.72	34.6851
11	1051.01	75.7118	12	757.888	50.2688
13	701.962	42.5775			

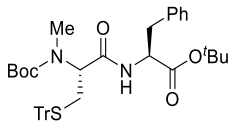


1r

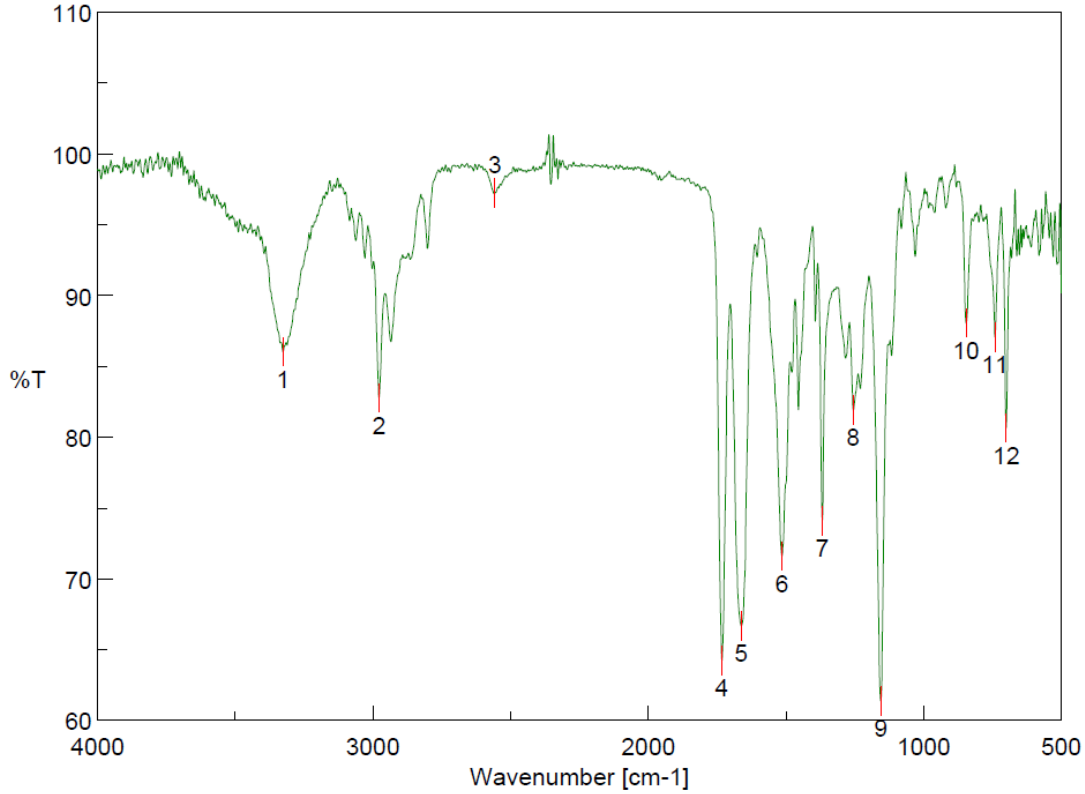


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3405.67	91.4727	2	3063.37	86.5961
3	2977.55	72.461	4	2932.23	81.8211
5	1685.48	45.8238	6	1494.56	63.1718
7	1445.39	67.5957	8	1367.28	57.3233
9	1320.04	69.8545	10	1155.15	46.6043
11	845.633	86.9272	12	751.138	55.1182
13	700.998	53.6477			

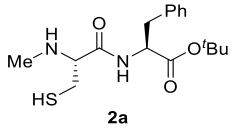


s6

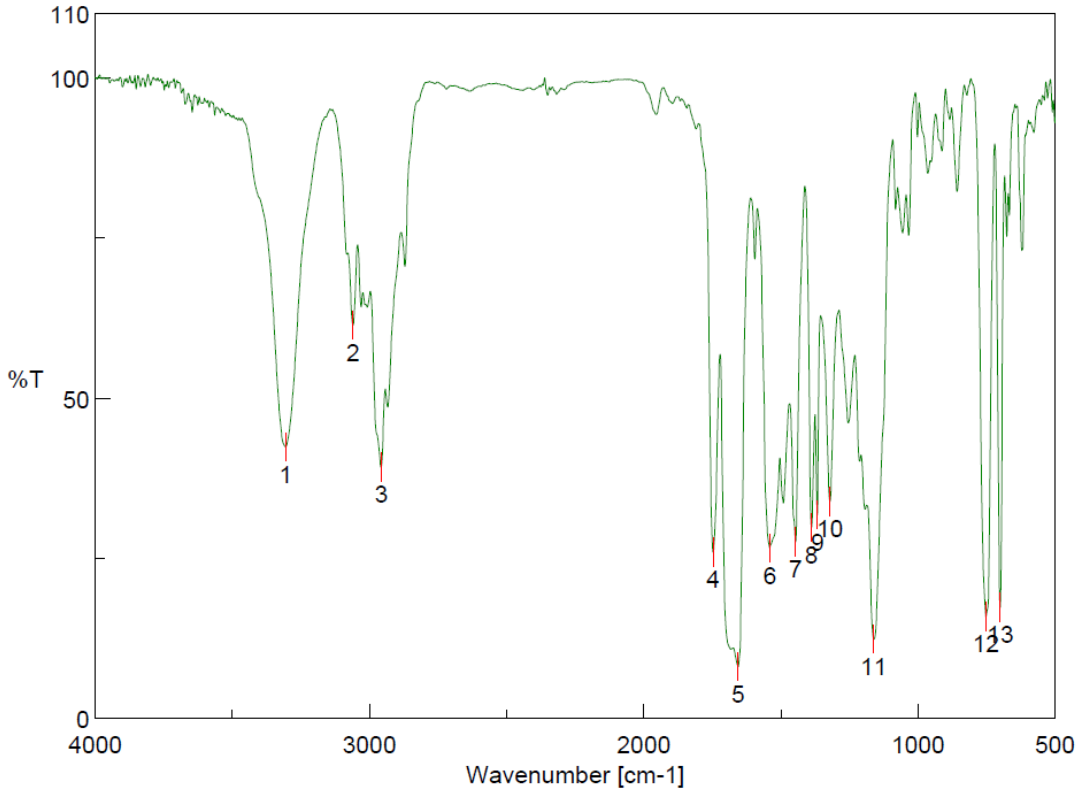


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3326.61	86.0145	2	2977.55	82.7927
3	2557.15	97.2314	4	1732.73	64.2261
5	1662.34	66.6886	6	1514.81	71.614
7	1368.25	74.1036	8	1255.43	81.9109
9	1156.12	61.3847	10	845.633	88.0407
11	740.531	87.048	12	700.034	80.6705

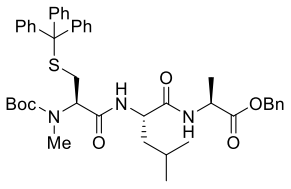


2a

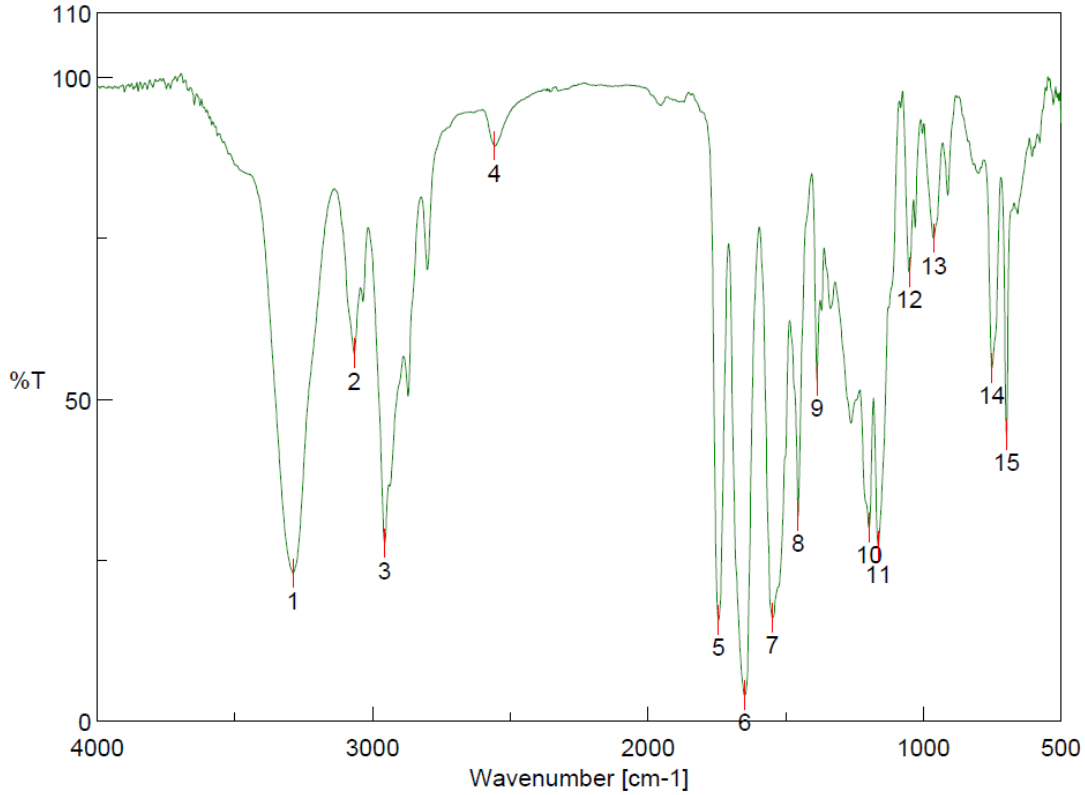


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3306.36	42.4098	2	3060.48	61.3985
3	2957.3	39.2204	4	1747.19	25.9354
5	1655.59	8.11371	6	1540.85	26.6153
7	1446.35	27.593	8	1388.5	29.8521
9	1367.28	31.7708	10	1321	33.8652
11	1160.94	12.3897	12	752.102	15.9078
13	700.034	17.3576			

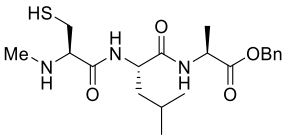


s10

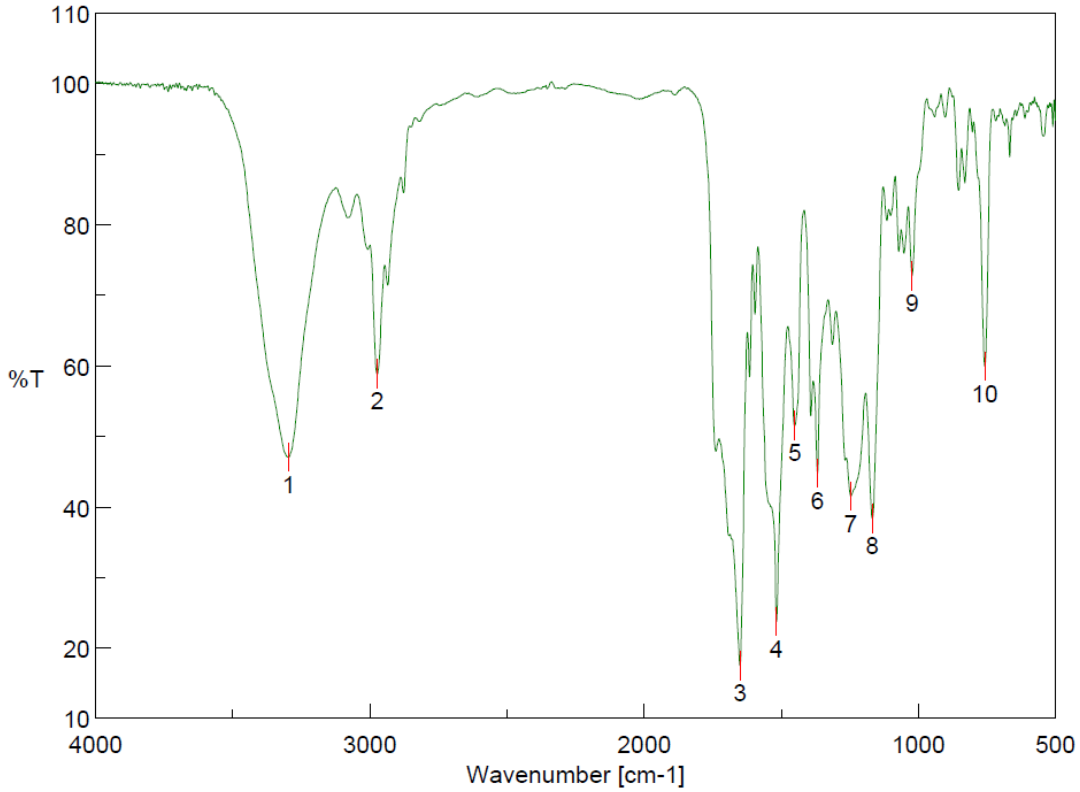


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3289	23.0209	2	3067.23	57.1222
3	2956.34	27.7102	4	2557.15	89.3559
5	1743.33	15.7565	6	1648.84	4.06289
7	1547.59	16.1104	8	1455.03	31.8829
9	1386.57	52.8706	10	1196.61	30.0732
11	1163.83	27.2135	12	1051.01	69.7518
13	962.305	75.0184	14	751.138	54.845
15	698.105	44.4421			

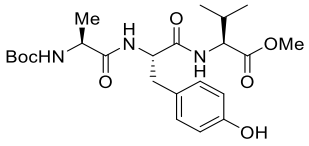


2b

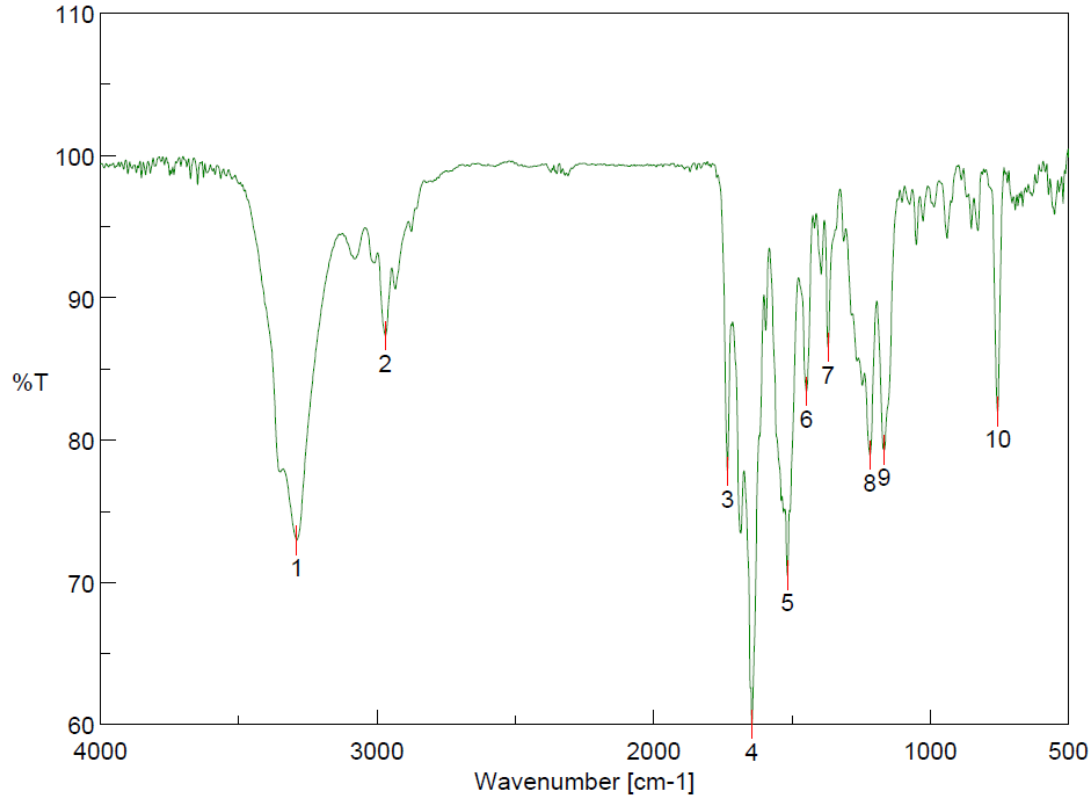


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3295.75	47.0593	2	2973.7	58.8386
3	1650.77	17.4919	4	1517.7	23.7197
5	1450.21	51.5338	6	1368.25	44.7854
7	1246.75	41.5124	8	1167.69	38.3363
9	1023.05	72.7663	10	757.888	59.924

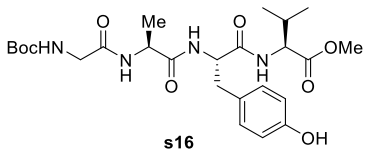


s14

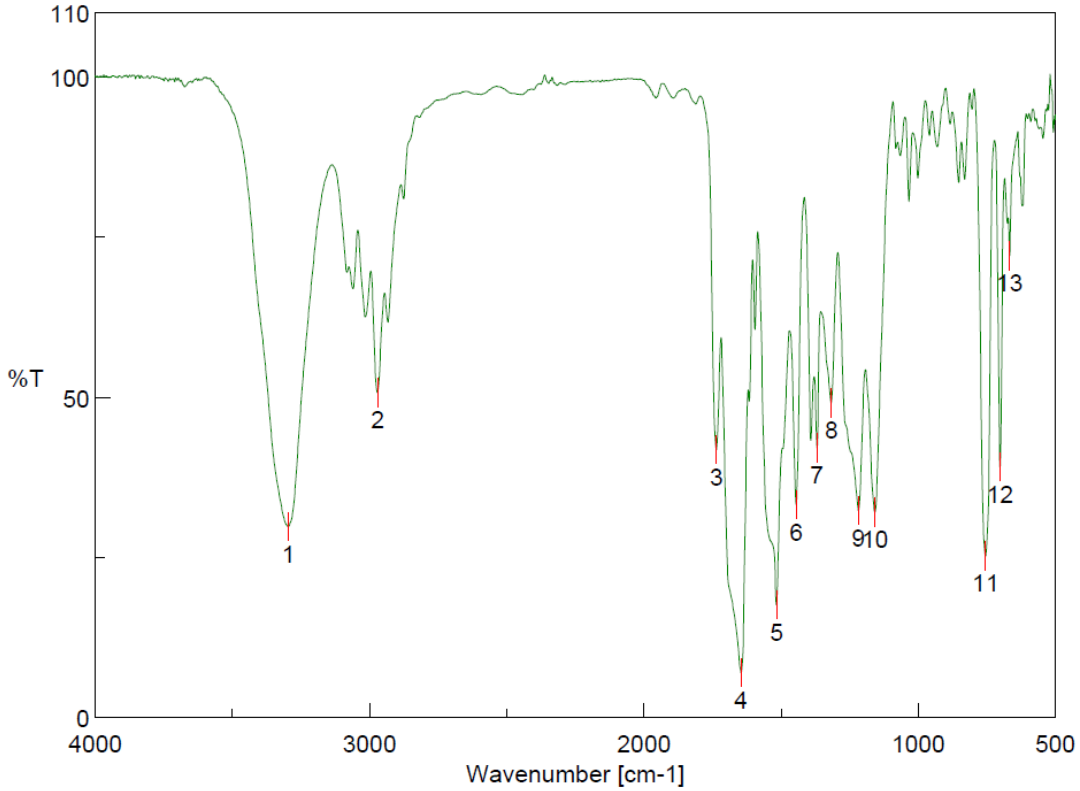


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3290.93	72.9578	2	2969.84	87.3212
3	1732.73	77.8026	4	1644.02	60.0178
5	1516.74	70.4987	6	1447.31	83.4195
7	1369.21	86.5297	8	1217.83	78.956
9	1166.72	79.2956	10	755.959	82.0212

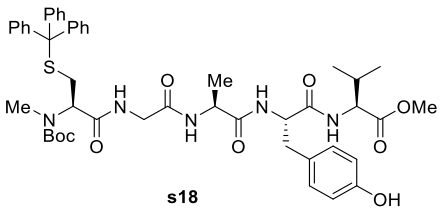


s16

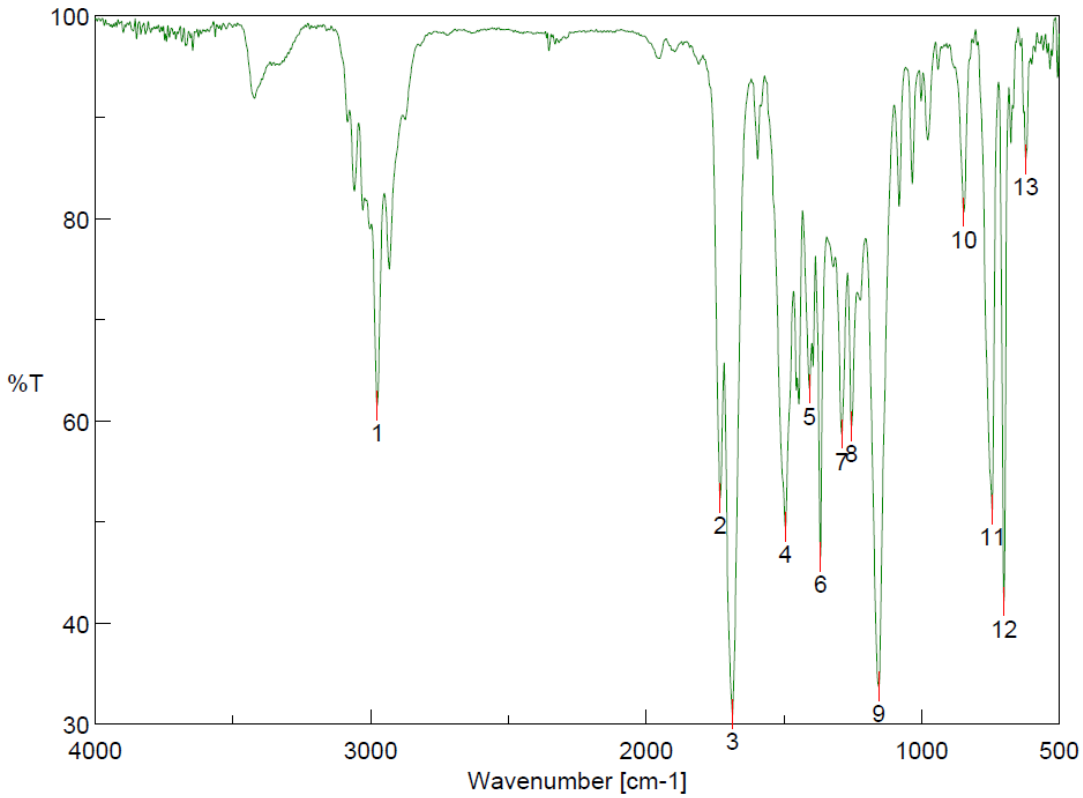


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3296.71	29.8534	2	2971.77	50.7386
3	1735.62	41.8212	4	1645.95	7.04076
5	1516.74	17.6455	6	1445.39	33.2739
7	1369.21	42.1315	8	1317.14	49.1092
9	1217.83	32.3204	10	1158.04	32.0929
11	754.995	25.2385	12	701.962	39.1494
13	667.25	72.0388			

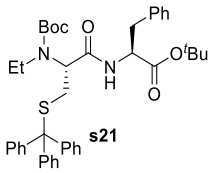


s18

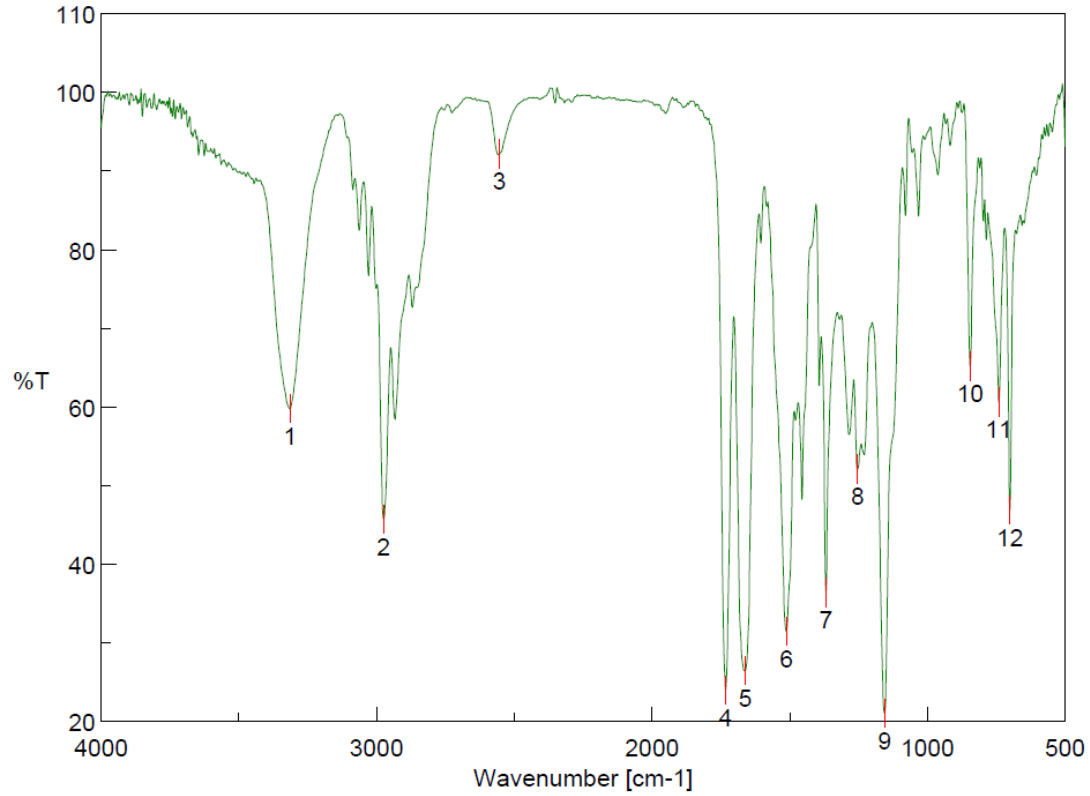


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	2976.59	61.4987	2	1731.76	52.3605
3	1686.44	30.9976	4	1494.56	49.4962
5	1406.82	63.1552	6	1367.28	46.5406
7	1290.14	58.6502	8	1254.47	59.4209
9	1156.12	33.7644	10	846.597	80.5718
11	743.424	51.2221	12	700.998	42.1416
13	621.931	85.7951			

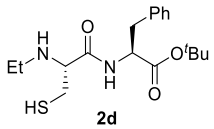


s21

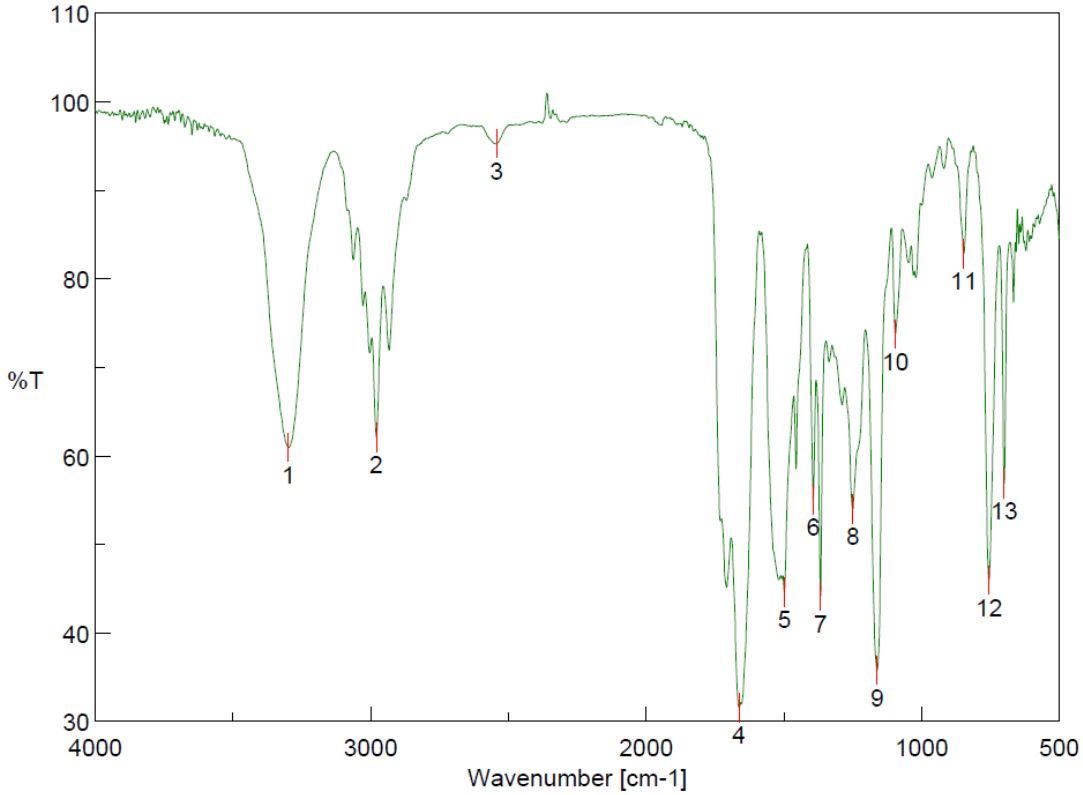


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3315.03	59.7729	2	2974.66	45.7338
3	2554.25	92.1288	4	1732.73	24.0545
5	1661.37	26.493	6	1512.88	31.4977
7	1368.25	36.4081	8	1253.5	52.0999
9	1156.12	21.104	10	844.669	65.1391
11	740.531	60.6135	12	700.998	46.9152

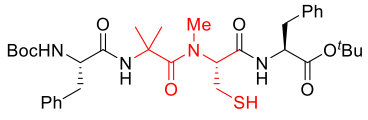


2d



[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3298.64	60.9312	2	2979.48	62.108
3	2543.65	95.2214	4	1662.34	31.5705
5	1498.42	44.5691	6	1393.32	55.0107
7	1367.28	44.1654	8	1249.65	53.9964
9	1160.94	35.7801	10	1095.37	73.7419
11	846.597	82.8104	12	755.959	45.9762
13	700.034	56.8187			



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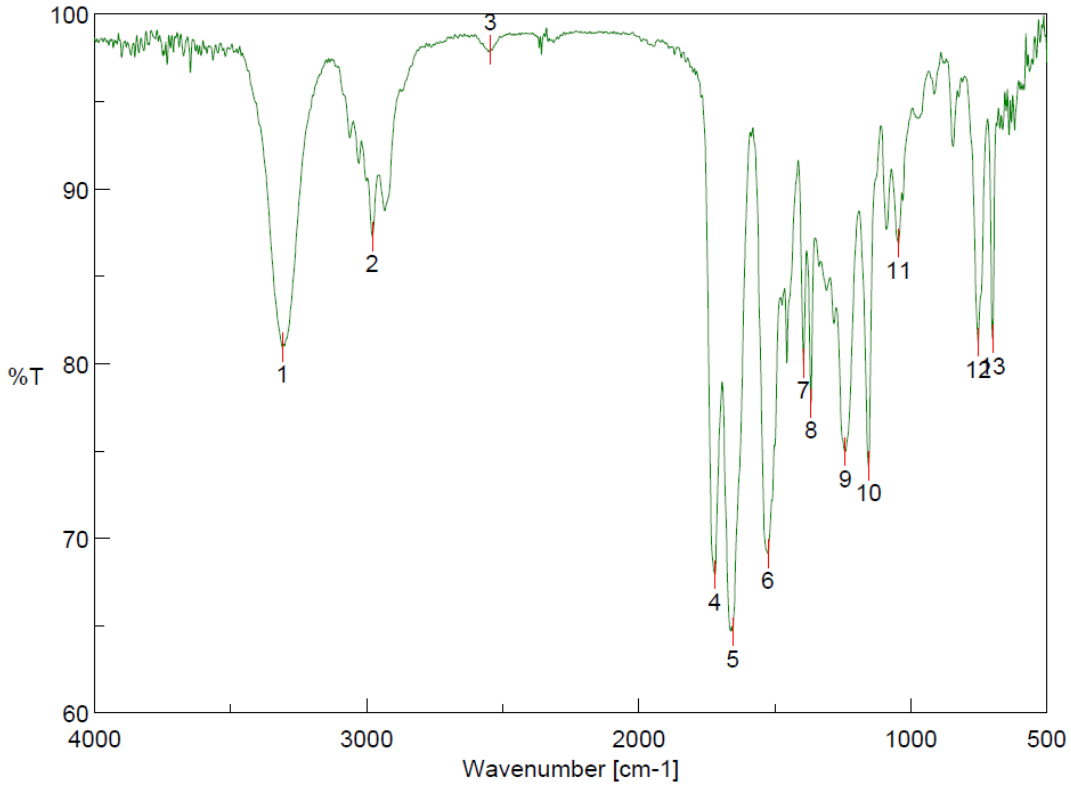
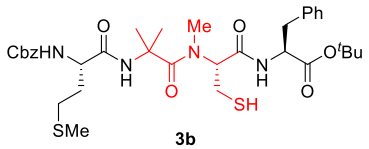
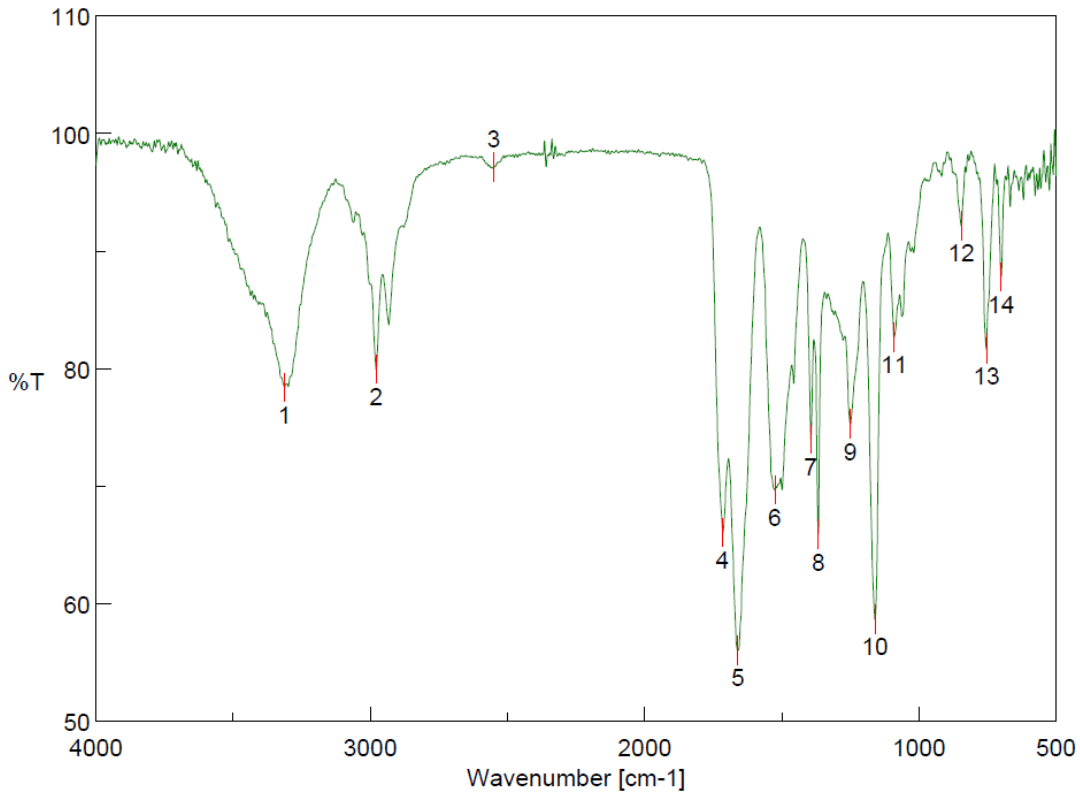


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3310.21	80.9138	2	2979.48	87.2461
3	2546.54	97.938	4	1720.19	67.919
5	1654.62	64.6371	6	1525.42	69.1288
7	1394.28	80.0071	8	1367.28	77.724
9	1240.97	74.9607	10	1155.15	74.1496
11	1045.23	86.8817	12	752.102	81.2216
13	699.069	81.4202			

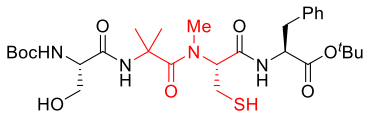


3b



[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3313.11	78.4114	2	2978.52	79.9657
3	2550.4	97.1148	4	1715.37	66.0631
5	1660.41	56.0376	6	1525.42	69.6867
7	1394.28	74.0202	8	1367.28	65.9056
9	1250.61	75.3009	10	1159.97	58.6843
11	1090.55	82.6398	12	845.633	92.1248
13	754.031	81.6888	14	700.034	87.7916



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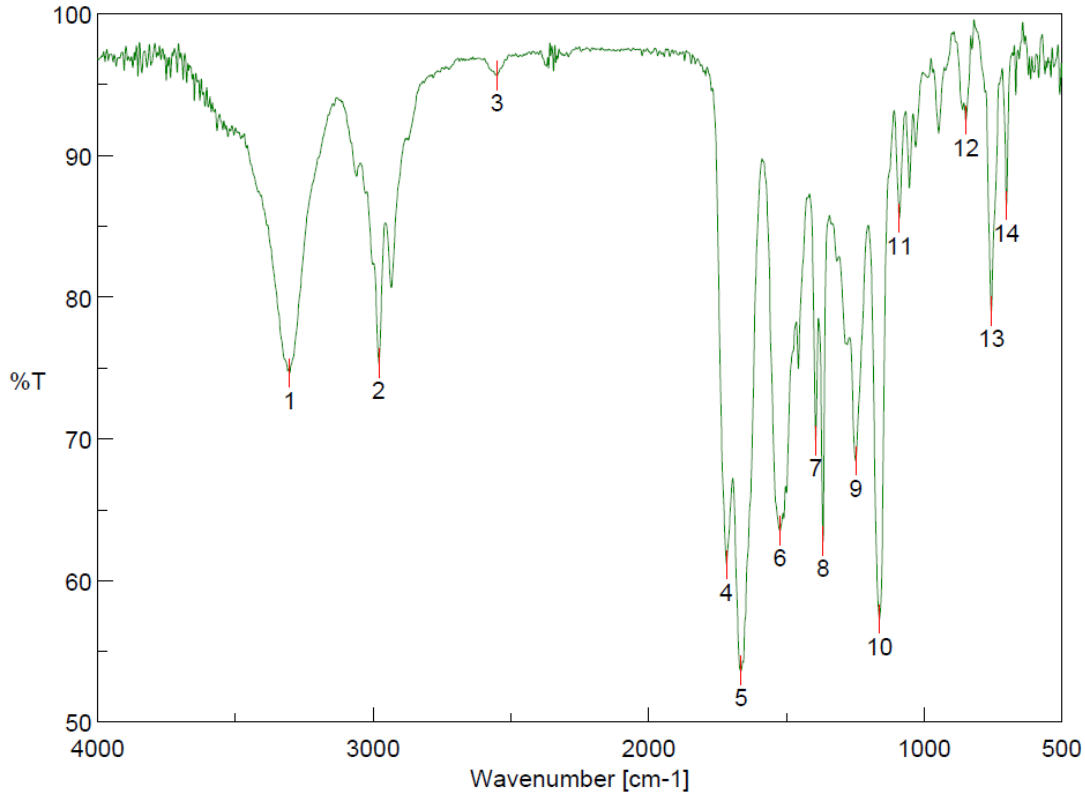
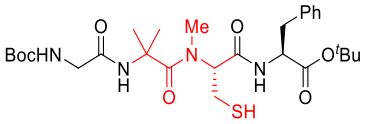


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3303.46	74.6611	2	2979.48	75.3308
3	2550.4	95.6012	4	1717.3	61.1276
5	1664.27	53.6412	6	1523.49	63.509
7	1393.32	69.8926	8	1367.28	62.7845
9	1248.68	68.4442	10	1161.9	57.2911
11	1089.58	85.5773	12	848.525	92.4981
13	755.959	78.9997	14	700.998	86.4691



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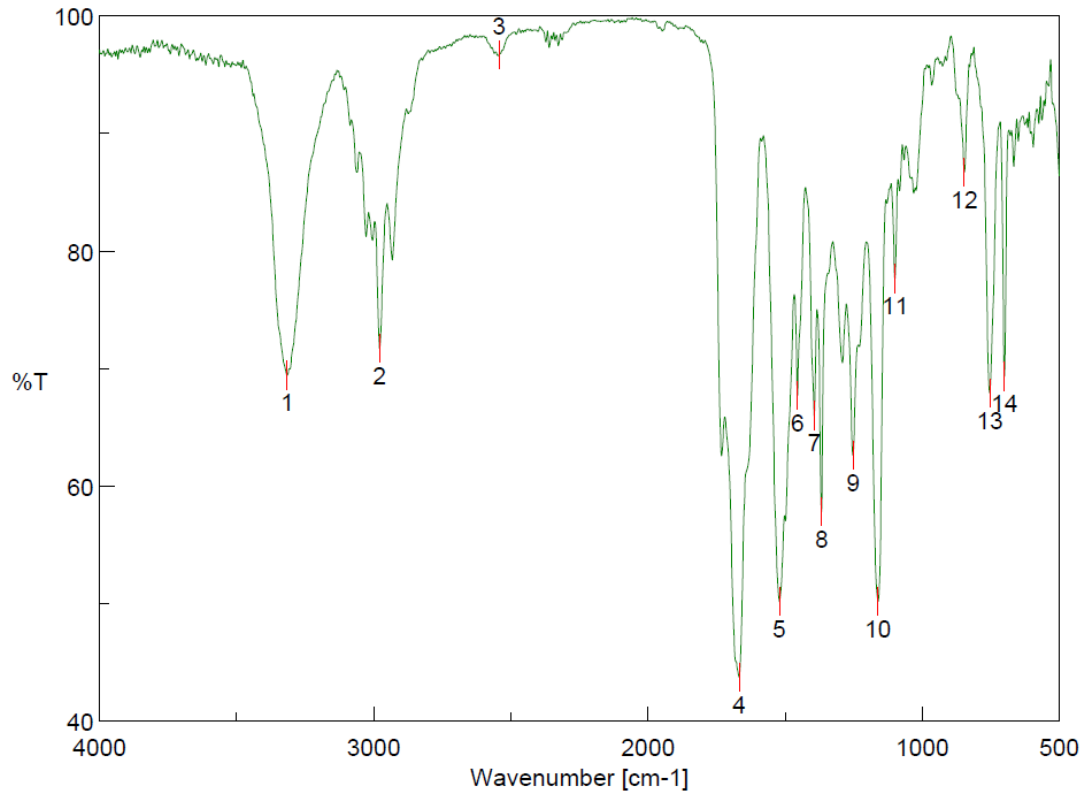
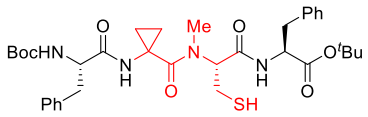


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3316	69.4184	2	2978.52	71.7214
3	2543.65	96.6838	4	1668.12	43.7618
5	1520.6	50.1925	6	1455.03	67.7031
7	1393.32	65.9987	8	1367.28	57.852
9	1252.54	62.6096	10	1160.94	50.1793
11	1099.23	77.653	12	846.597	86.7032
13	754.031	67.9151	14	700.034	69.3415



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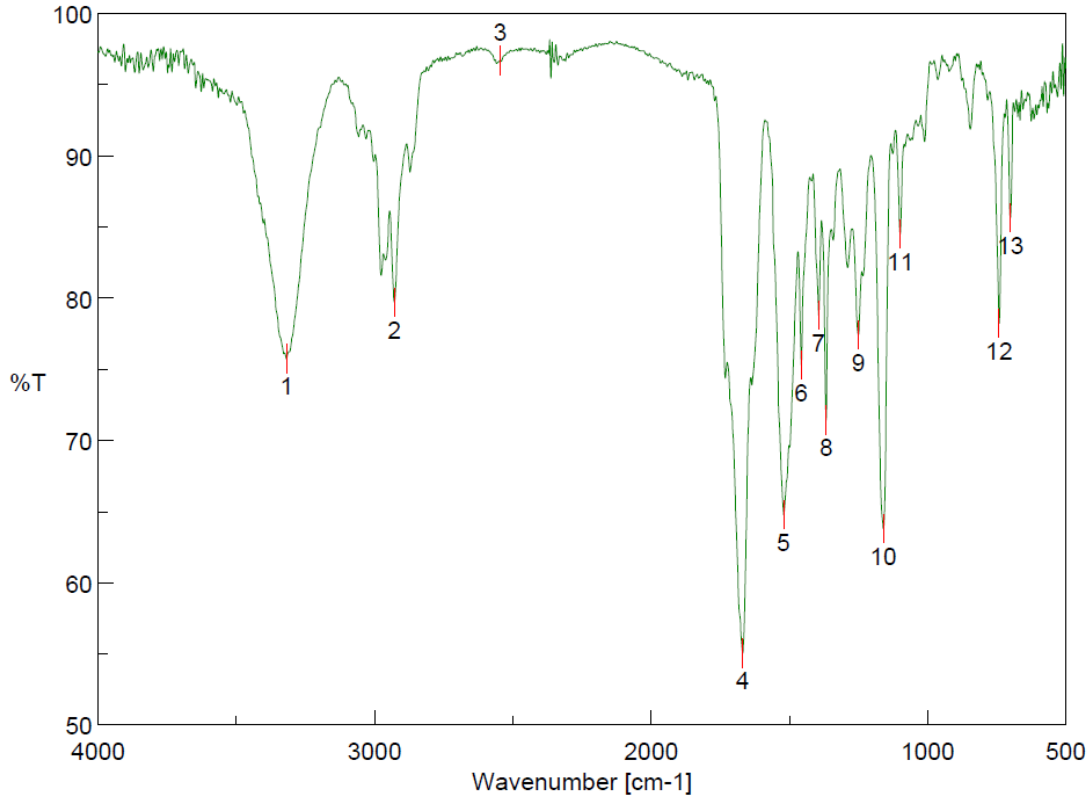
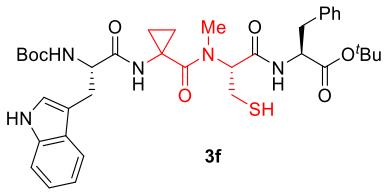


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3318.89	75.7346	2	2928.38	79.718
3	2546.54	96.6824	4	1669.09	55.061
5	1520.6	64.7707	6	1456.96	75.3016
7	1394.28	78.8106	8	1367.28	71.4396
9	1250.61	77.4247	10	1159.97	63.7932
11	1099.23	84.5069	12	741.496	78.2164
13	700.998	85.6447			



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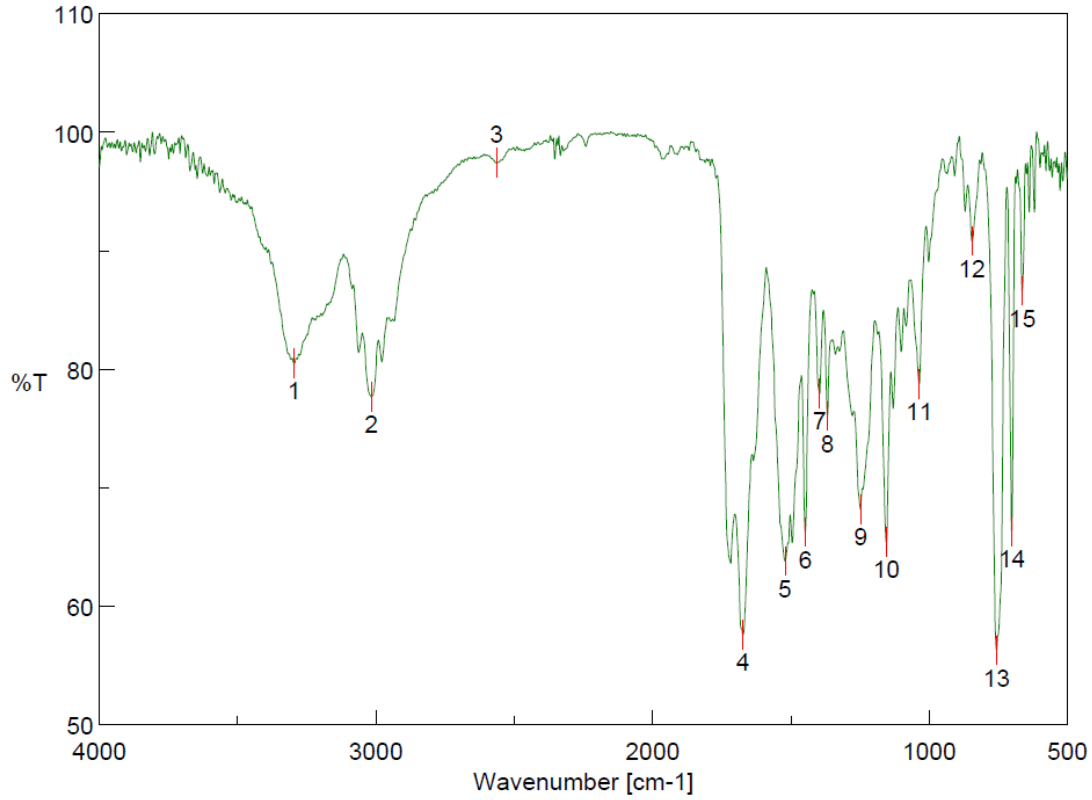
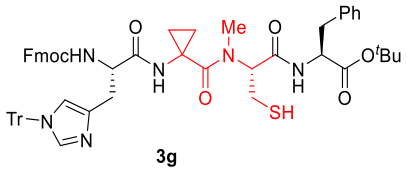
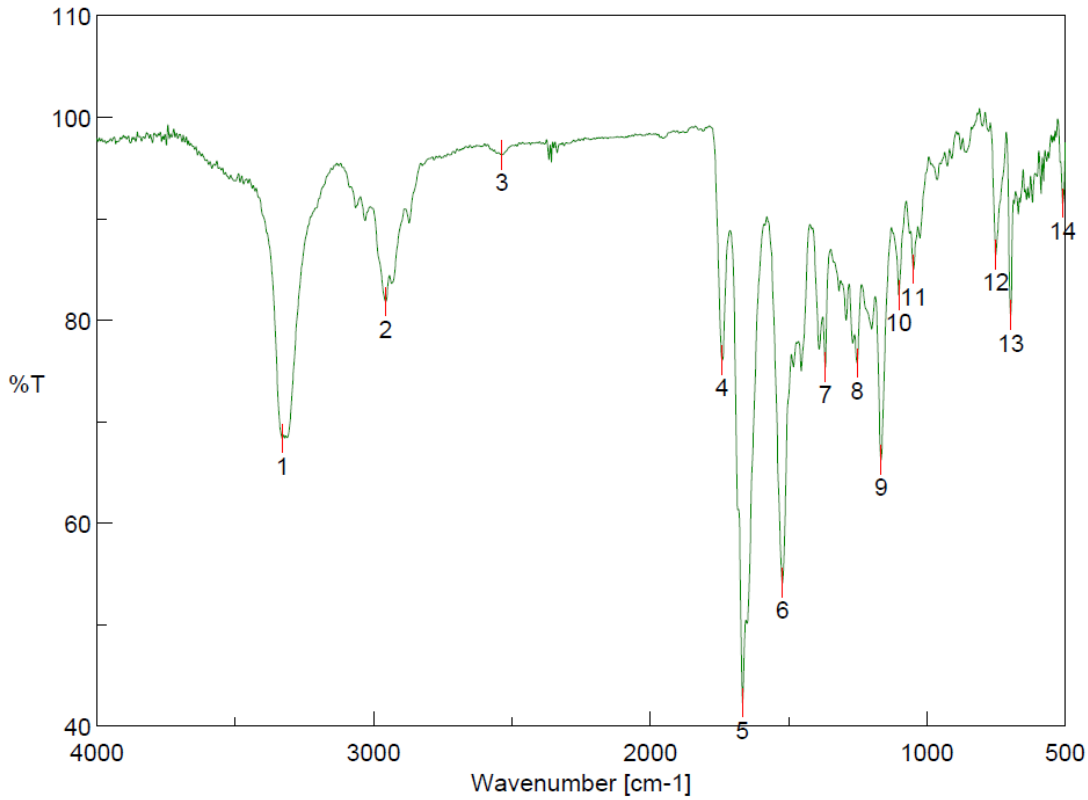


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3295.75	80.4791	2	3017.09	77.6756
3	2564.86	97.4297	4	1672.95	57.6066
5	1520.6	63.7991	6	1448.28	66.2743
7	1396.21	77.9413	8	1368.25	76.0408
9	1248.68	68.2128	10	1155.15	65.4252
11	1036.55	78.7704	12	845.633	90.7983
13	756.923	56.3104	14	700.998	66.3395
15	663.393	86.5959			

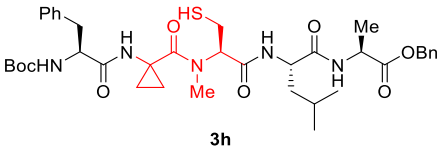


3g



[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3330.46	68.3011	2	2956.34	81.801
3	2535.93	96.2598	4	1739.48	76.0387
5	1666.2	42.3197	6	1522.52	54.1175
7	1367.28	75.3293	8	1252.54	75.7754
9	1165.76	66.2228	10	1101.15	82.4407
11	1048.12	84.9819	12	751.138	86.4134
13	697.141	80.476	14	508.151	91.4795



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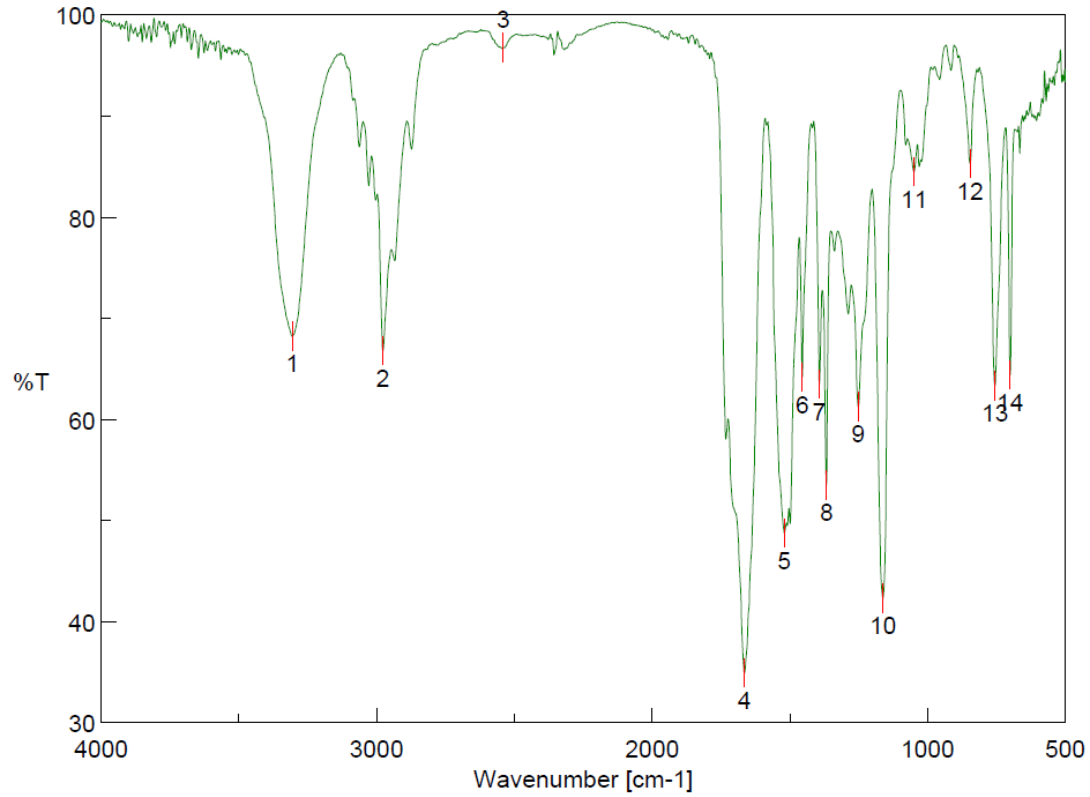
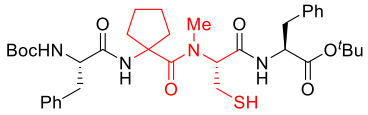


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3306.36	68.1893	2	2977.55	66.7762
3	2539.79	96.7367	4	1664.27	34.9142
5	1519.63	48.7664	6	1455.03	64.1832
7	1392.35	63.4856	8	1367.28	53.4981
9	1251.58	61.2698	10	1161.9	42.3048
11	1049.09	84.4542	12	845.633	85.2901
13	755.959	63.3232	14	700.034	64.3706



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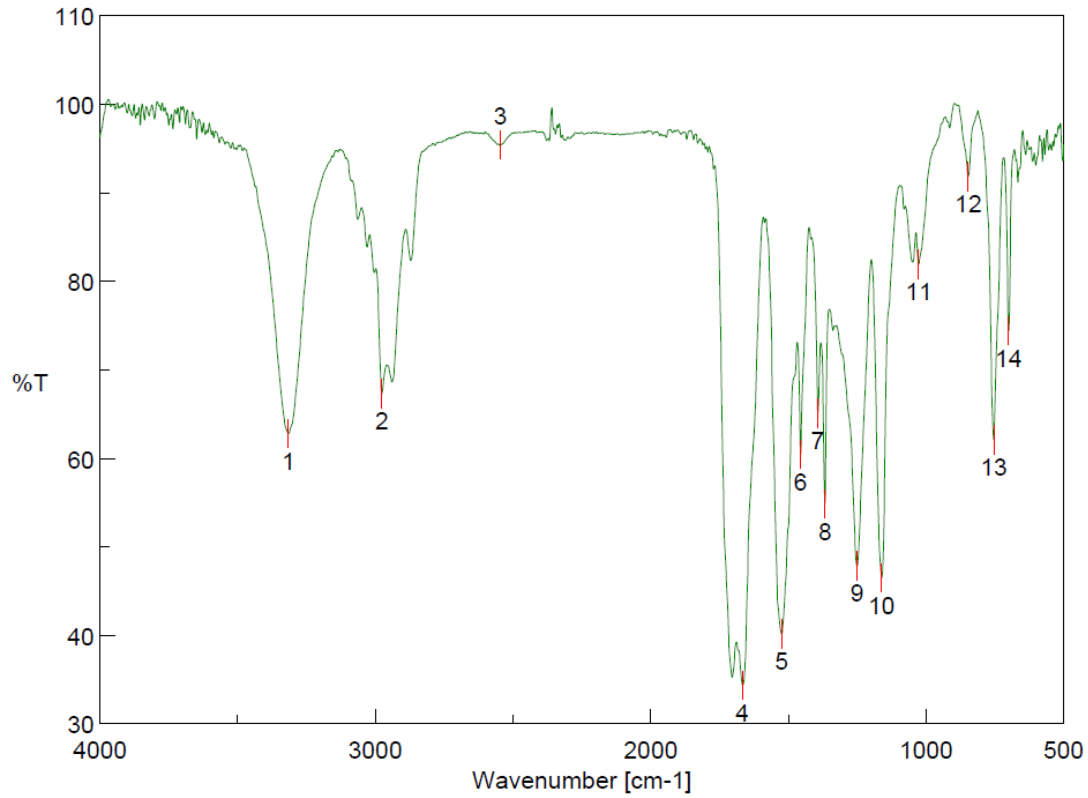
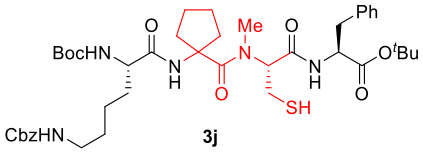


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3316	62.7941	2	2976.59	67.2797
3	2546.54	95.3756	4	1666.2	34.374
5	1524.45	40.1434	6	1455.99	60.4351
7	1392.35	65.0828	8	1367.28	54.8382
9	1250.61	47.8371	10	1160.94	46.4851
11	1026.91	81.9135	12	846.597	91.831
13	754.031	62.0941	14	700.034	74.3741



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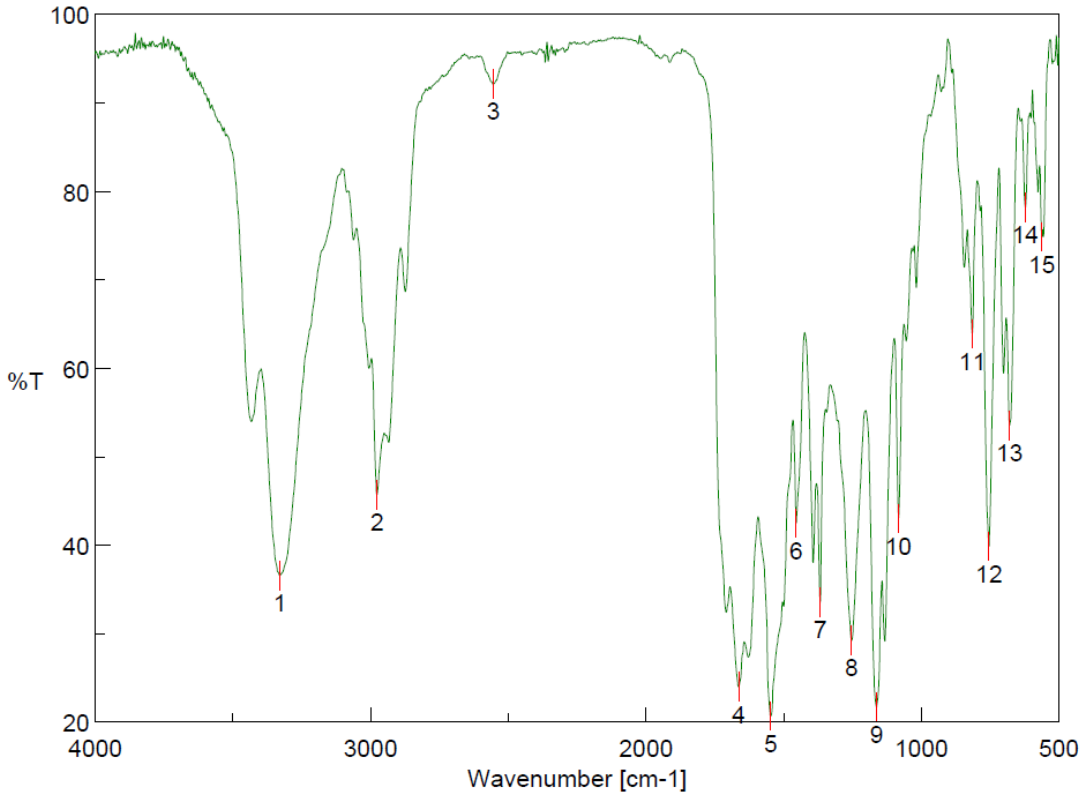
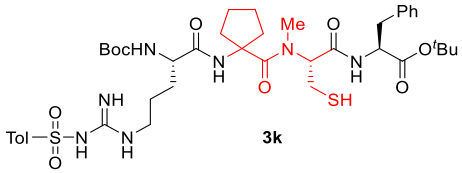
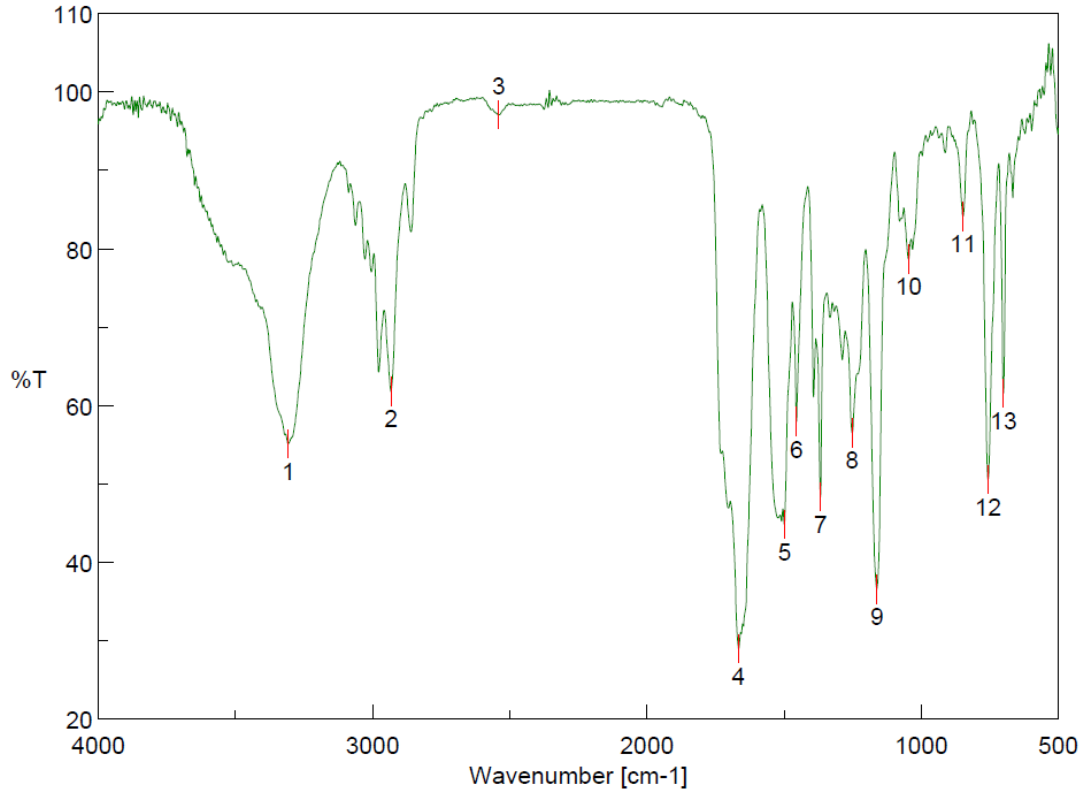


[ピーク検出結果]

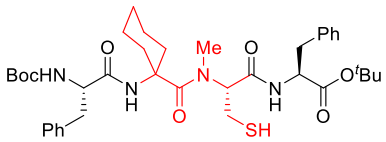
No.	位置	強度	No.	位置	強度
1	3330.46	36.5619	2	2976.59	45.6915
3	2554.25	92.0639	4	1663.3	23.9961
5	1546.63	20.6949	6	1455.03	42.5198
7	1367.28	33.5378	8	1253.5	29.2377
9	1162.87	21.744	10	1082.83	43.0067
11	814.777	63.8661	12	754.995	39.8668
13	679.785	53.5239	14	621.931	78.1536
15	562.148	74.7904			



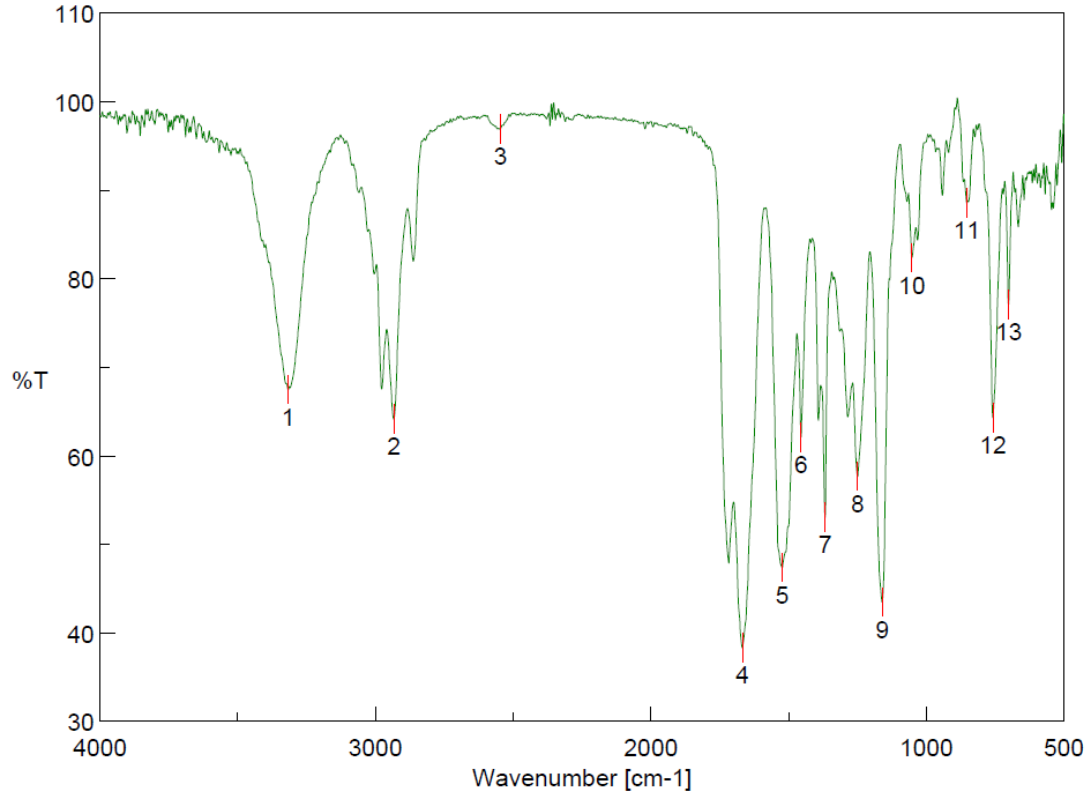


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3307.32	55.1402	2	2933.2	61.765
3	2539.79	97.0656	4	1665.23	29.0318
5	1498.42	44.8403	6	1455.03	57.9523
7	1367.28	48.3783	8	1251.58	56.5185
9	1161.9	36.6097	10	1046.19	78.6393
11	847.561	84.1239	12	756.923	50.6132
13	700.034	61.5839			

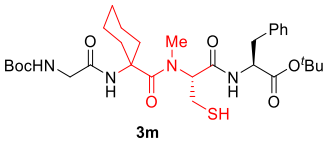


3l

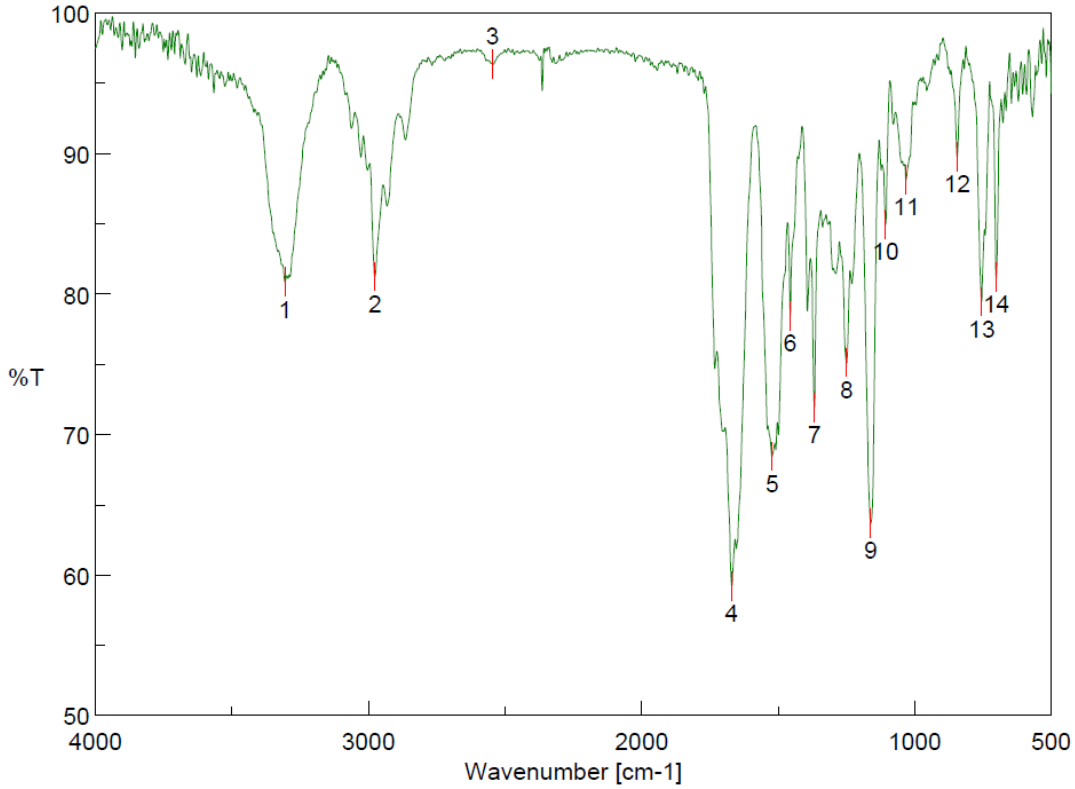


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3316.96	67.4531	2	2933.2	64.1193
3	2546.54	96.8915	4	1668.12	38.3342
5	1523.49	47.3779	6	1455.03	62.1006
7	1367.28	53.0341	8	1249.65	57.6683
9	1159.97	43.4843	10	1051.98	82.3639
11	853.347	88.5347	12	757.888	64.294
13	700.998	77.0391			

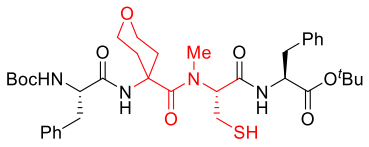


3m

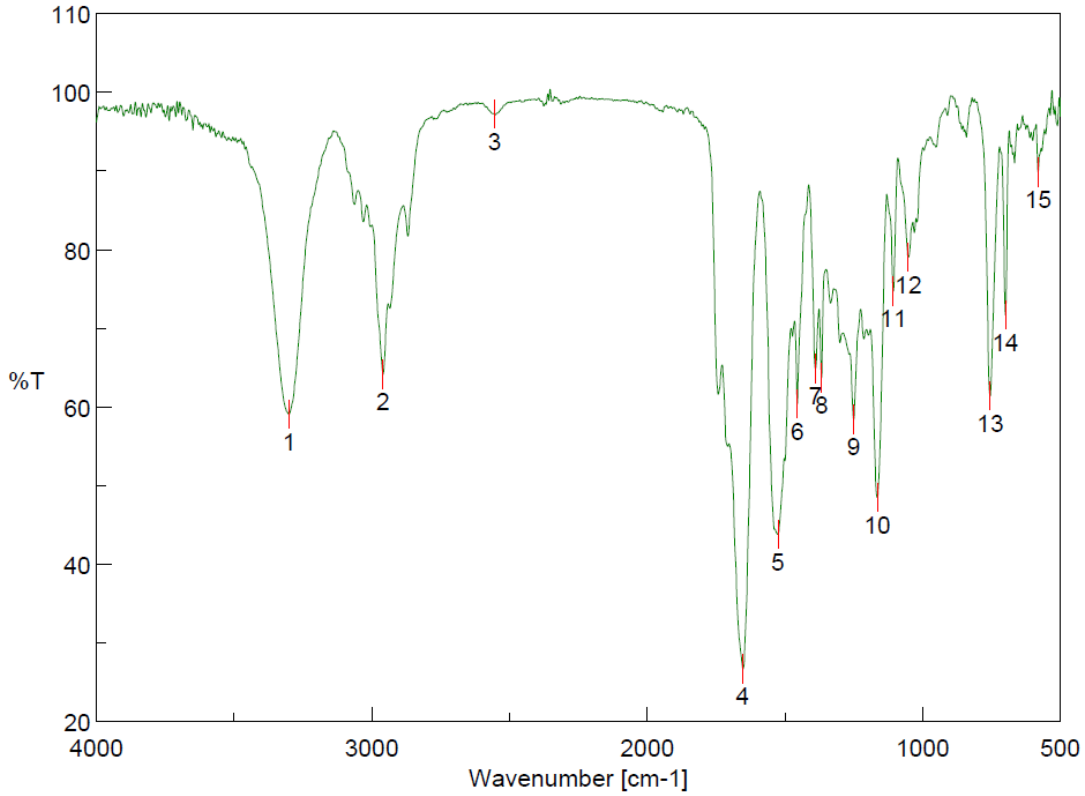


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3306.36	80.8493	2	2976.59	81.2547
3	2546.54	96.3832	4	1670.05	59.1916
5	1522.52	68.4364	6	1455.99	78.4519
7	1367.28	71.9147	8	1249.65	75.1058
9	1160.94	63.6754	10	1106.94	84.9454
11	1030.77	88.1376	12	844.669	89.7388
13	754.995	79.4534	14	700.998	81.1923

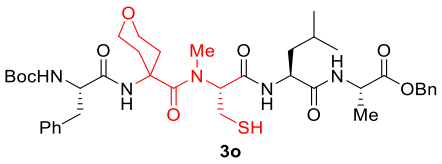


3n

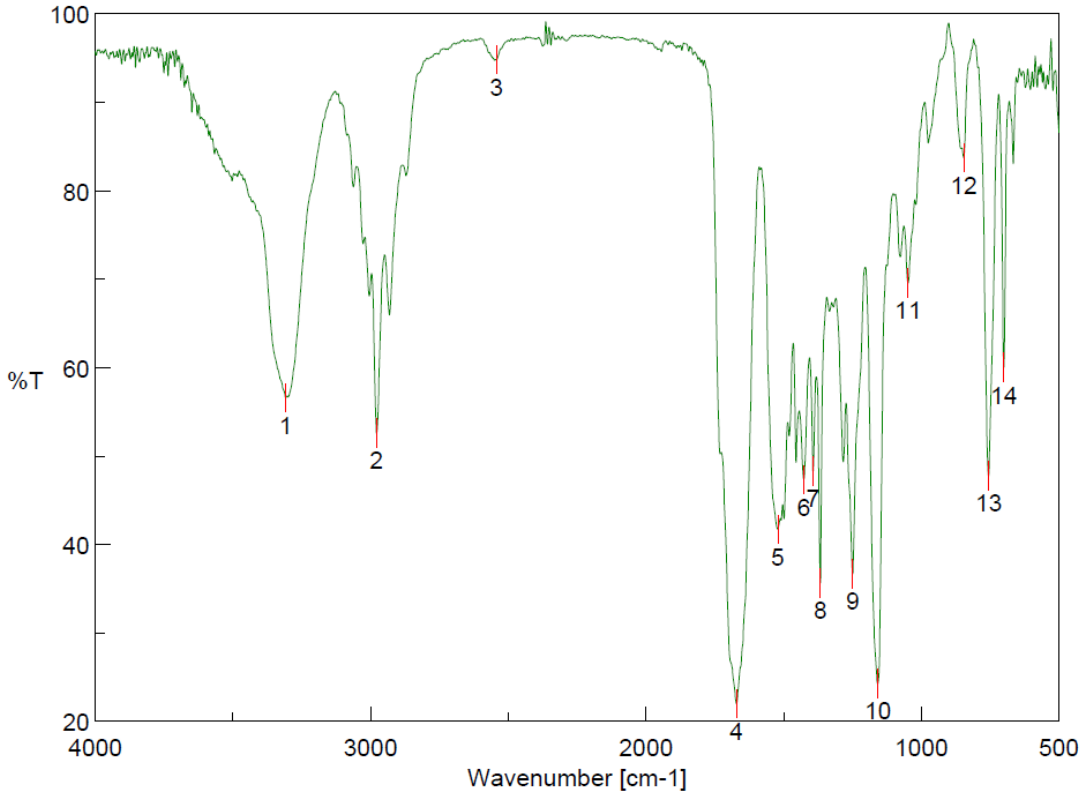


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3301.54	59.0615	2	2959.23	64.1144
3	2554.25	97.1872	4	1652.7	26.7216
5	1525.42	43.811	6	1455.03	60.4039
7	1389.46	64.8785	8	1367.28	63.7121
9	1250.61	58.4015	10	1164.79	48.5037
11	1106.94	74.7154	12	1051.98	79.0104
13	754.995	61.4106	14	699.069	71.693
15	580.469	89.8134			

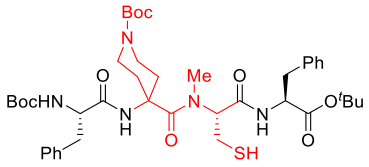


3o

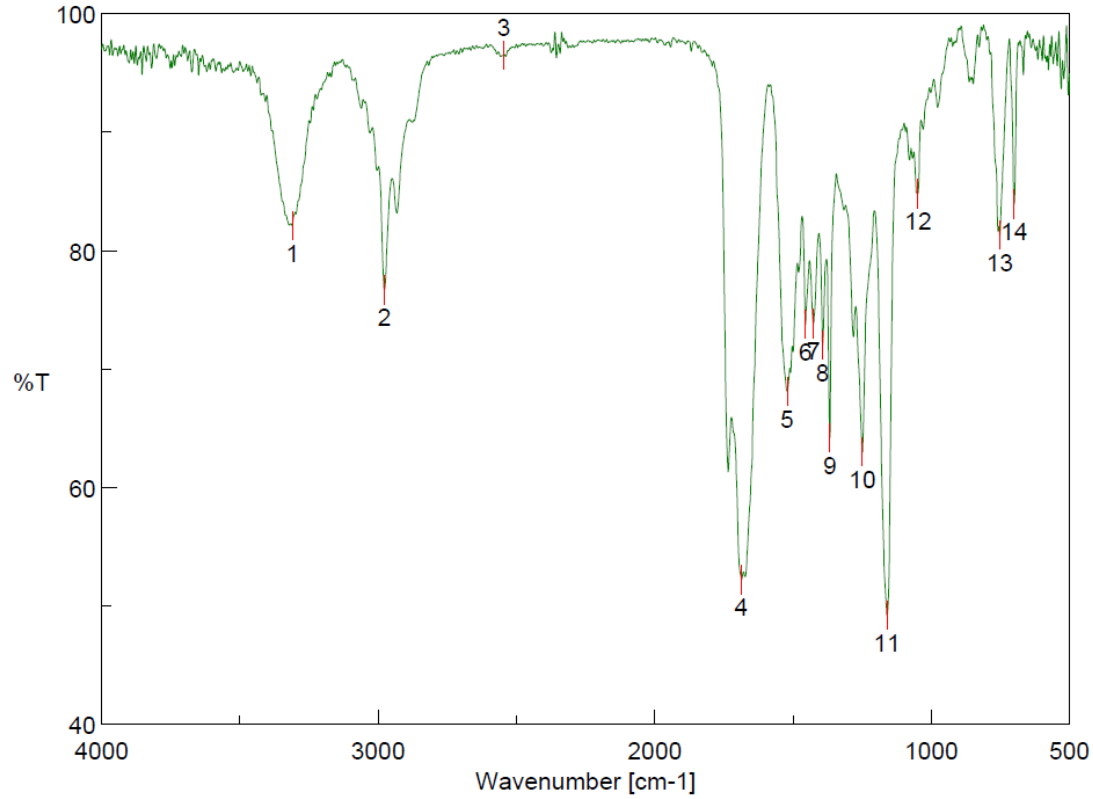


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3308.29	56.5886	2	2978.52	52.5545
3	2543.65	94.7504	4	1671.02	22.0084
5	1521.56	41.6961	6	1427.07	47.3443
7	1393.32	48.2612	8	1367.28	35.649
9	1249.65	36.675	10	1158.04	24.2669
11	1048.12	69.5402	12	845.633	83.6984
13	755.959	47.7227	14	700.034	59.9781

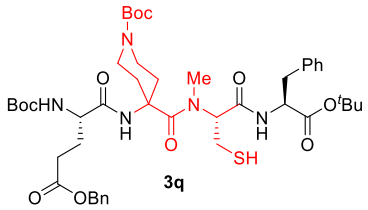


3p

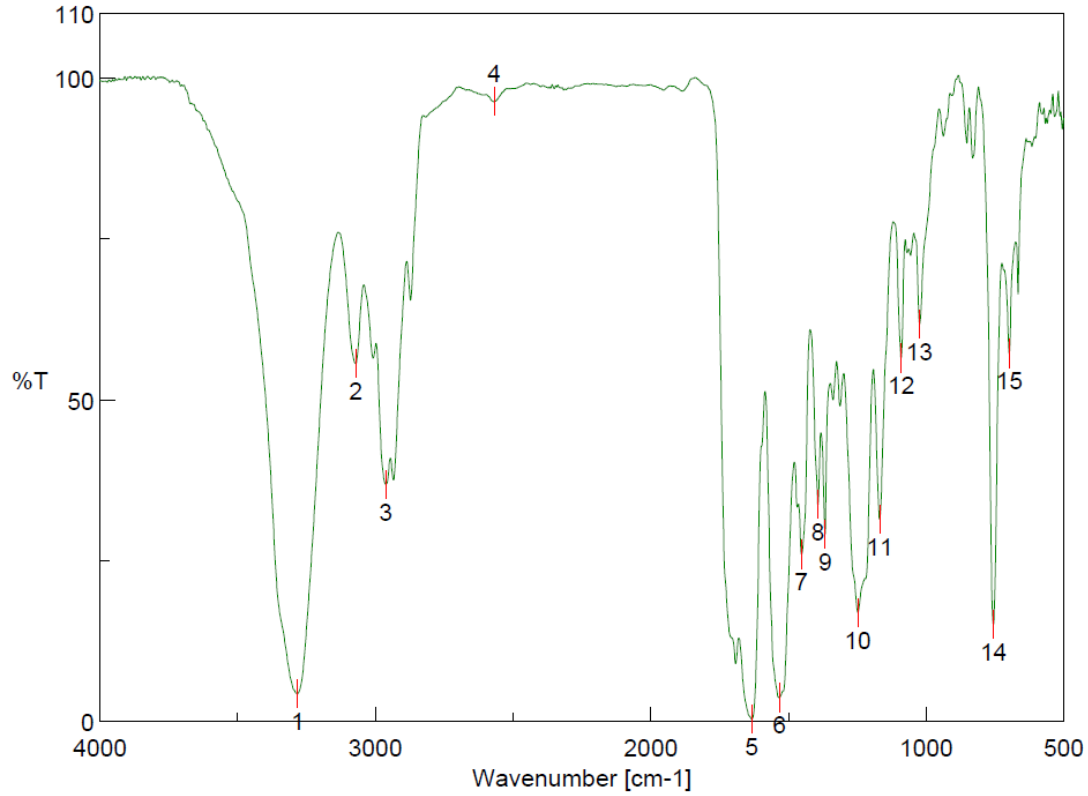


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3311.18	82.0912	2	2977.55	76.6847
3	2546.54	96.4652	4	1685.48	52.2449
5	1521.56	68.1172	6	1455.03	73.7534
7	1426.1	73.8308	8	1392.35	72.0157
9	1367.28	64.2051	10	1249.65	63.0014
11	1159.97	49.2319	12	1048.12	84.796
13	752.102	81.2995	14	700.034	83.9156

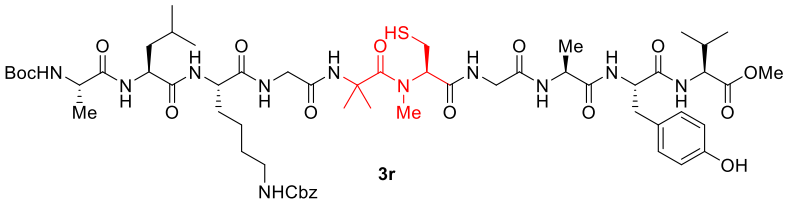


3q

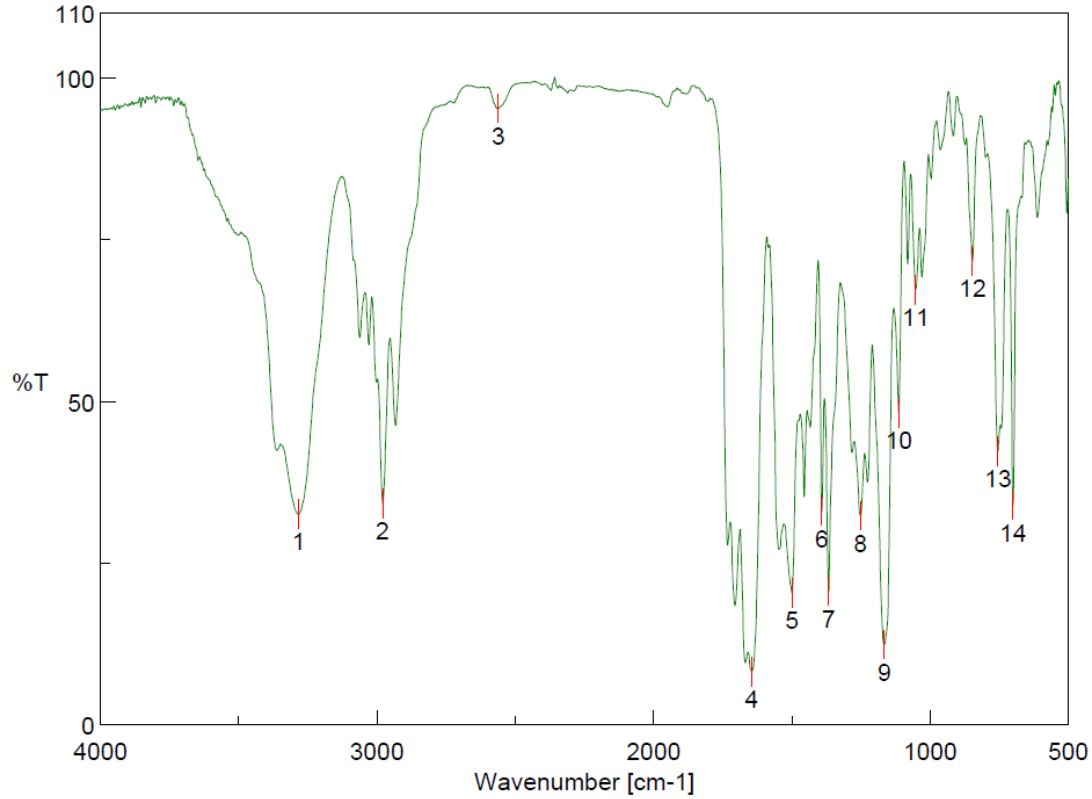


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3283.21	4.32817	2	3072.05	55.6324
3	2962.13	36.863	4	2567.75	96.3311
5	1633.41	0.427127	6	1533.13	3.74662
7	1453.1	25.9806	8	1392.35	33.7487
9	1367.28	29.0924	10	1247.72	16.953
11	1168.65	31.4632	12	1090.55	56.4856
13	1024.02	61.6857	14	755.959	15.092
15	698.105	57.1733			

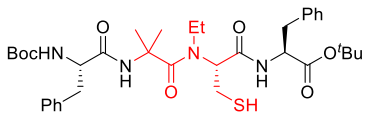


3r

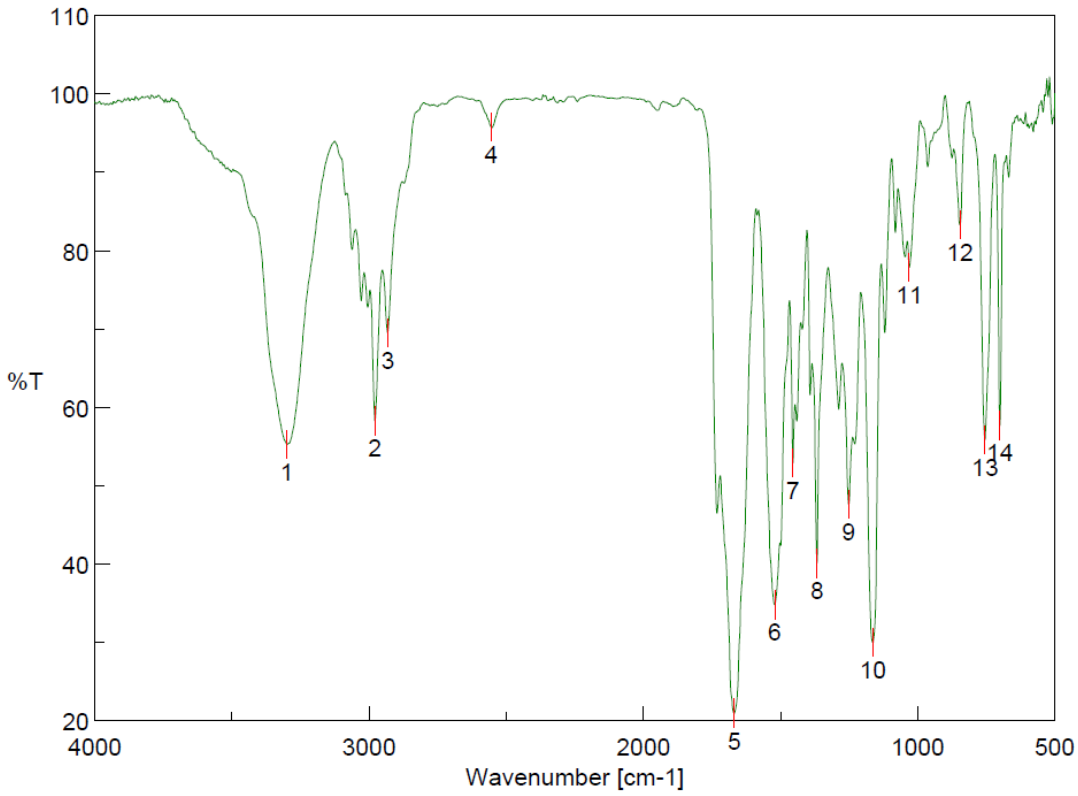


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3284.18	32.5757	2	2979.48	34.1831
3	2561	95.2547	4	1644.98	8.22675
5	1499.38	20.4274	6	1391.39	33.0433
7	1367.28	20.6523	8	1252.54	32.3584
9	1165.76	12.4276	10	1113.69	48.2179
11	1051.98	67.2534	12	846.597	71.6818
13	754.995	42.2768	14	700.034	33.9673

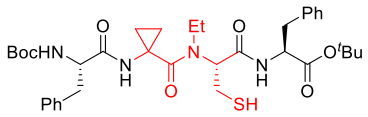


3s

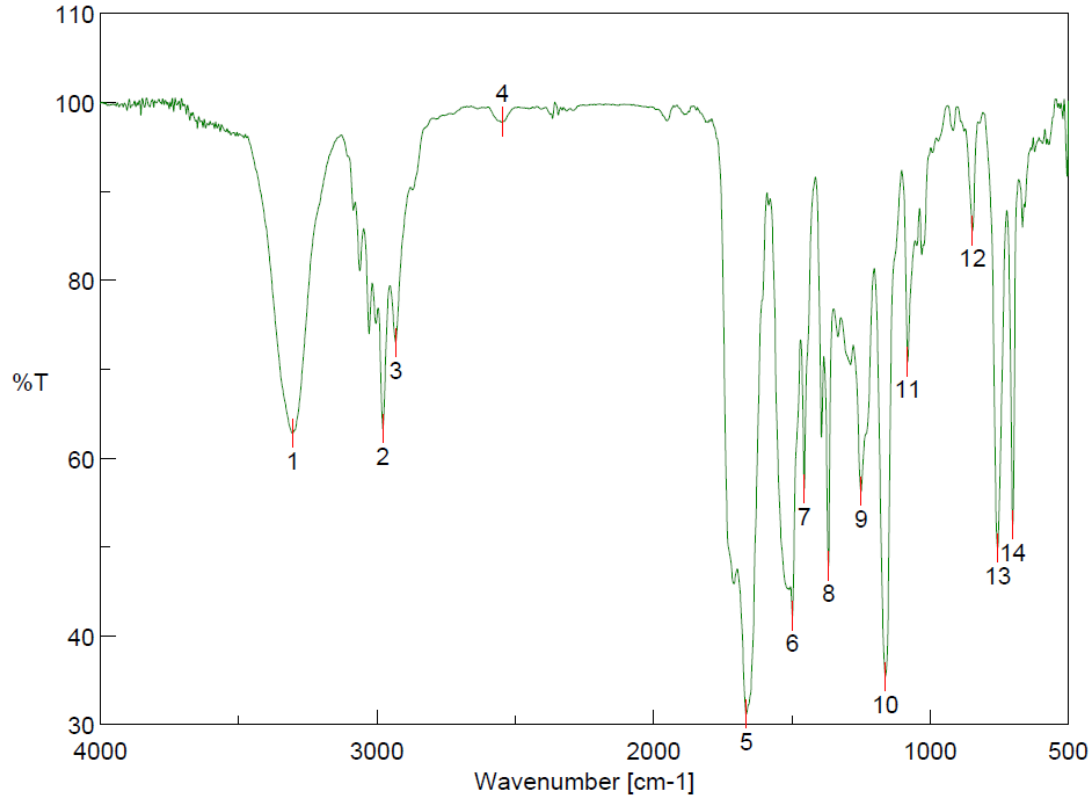


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3299.61	55.2314	2	2978.52	58.2685
3	2932.23	69.4906	4	2554.25	95.7283
5	1669.09	20.9916	6	1521.56	34.8181
7	1455.03	52.8739	8	1367.28	40.1051
9	1251.58	47.5878	10	1163.83	29.9835
11	1030.77	77.8375	12	845.633	83.2555
13	754.995	55.8867	14	700.034	57.6899

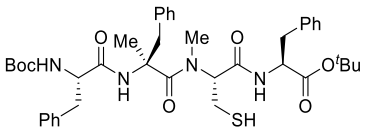


3t

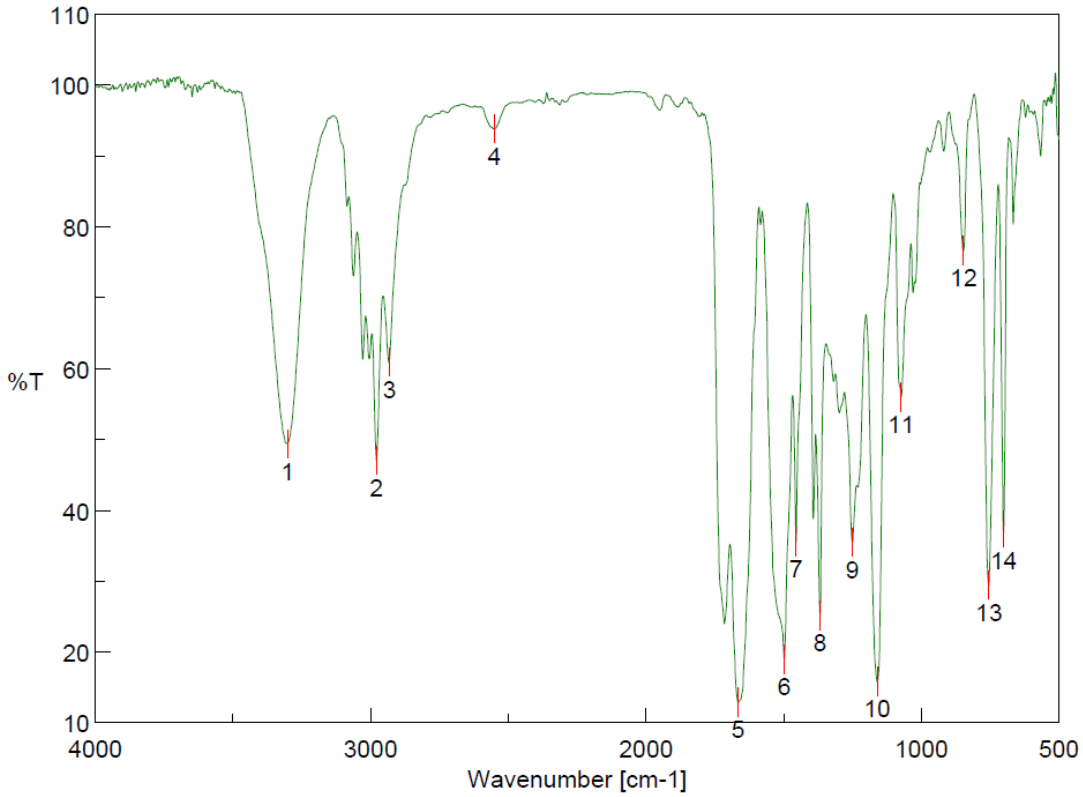


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3305.39	62.768	2	2978.52	63.2958
3	2932.23	72.9639	4	2546.54	97.7848
5	1664.27	31.1856	6	1497.45	42.2273
7	1455.03	56.5434	8	1367.28	47.8927
9	1249.65	56.2411	10	1160.94	35.4345
11	1080.91	70.7708	12	846.597	85.5227
13	755.959	49.8801	14	700.998	52.5009

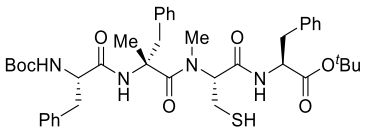


3u

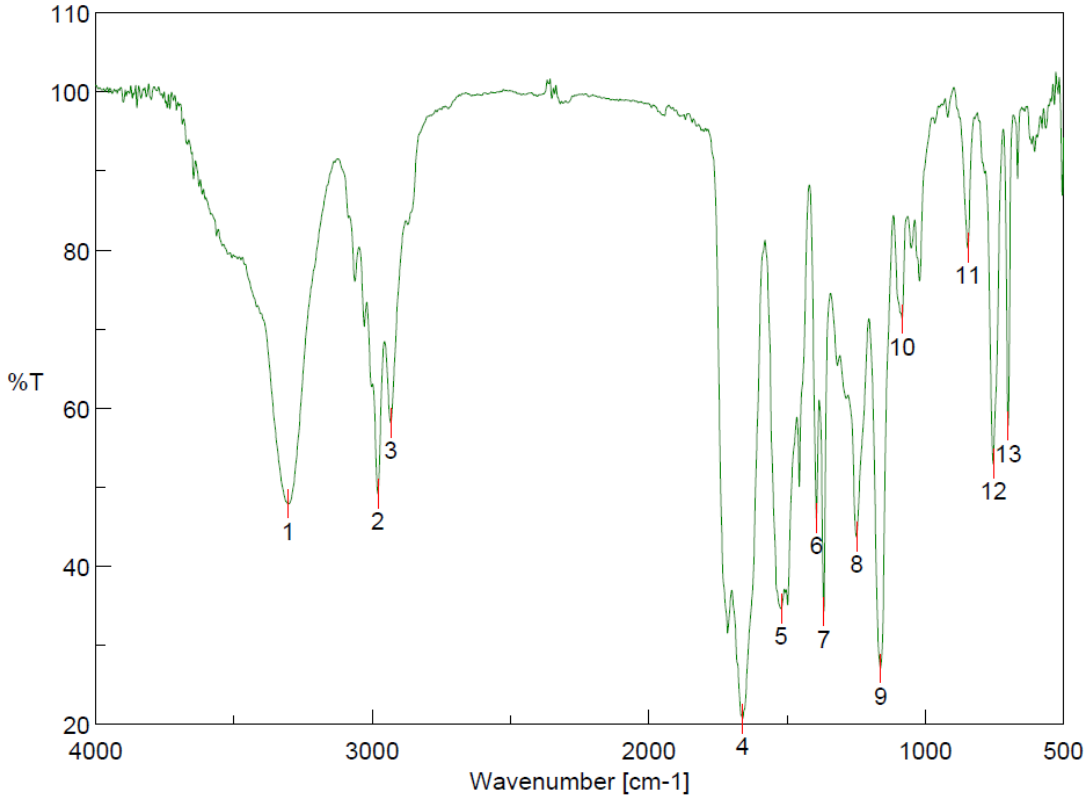


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3302.5	49.3709	2	2978.52	47.0936
3	2932.23	60.8884	4	2550.4	93.8555
5	1665.23	12.901	6	1497.45	18.965
7	1455.03	35.567	8	1368.25	25.1037
9	1250.61	35.4698	10	1159.97	15.8227
11	1073.19	55.9904	12	846.597	76.6256
13	754.995	29.4808	14	700.998	36.8089

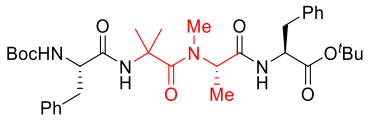


3v

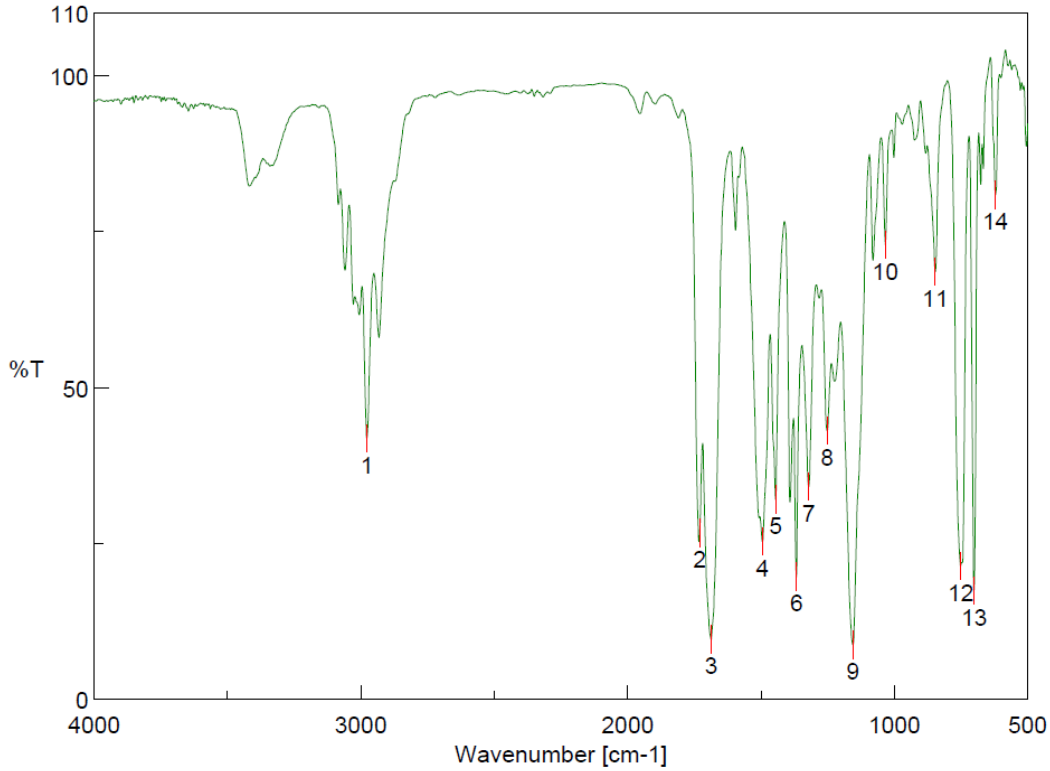


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3305.39	47.8869	2	2980.45	49.1868
3	2933.2	58.1201	4	1660.41	20.7621
5	1521.56	34.6559	6	1393.32	46.0986
7	1367.28	34.3064	8	1248.68	43.7505
9	1161.9	27.1151	10	1083.8	71.2644
11	845.633	80.2694	12	754.031	52.9743
13	700.034	57.8022			

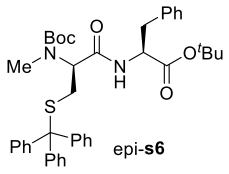


5

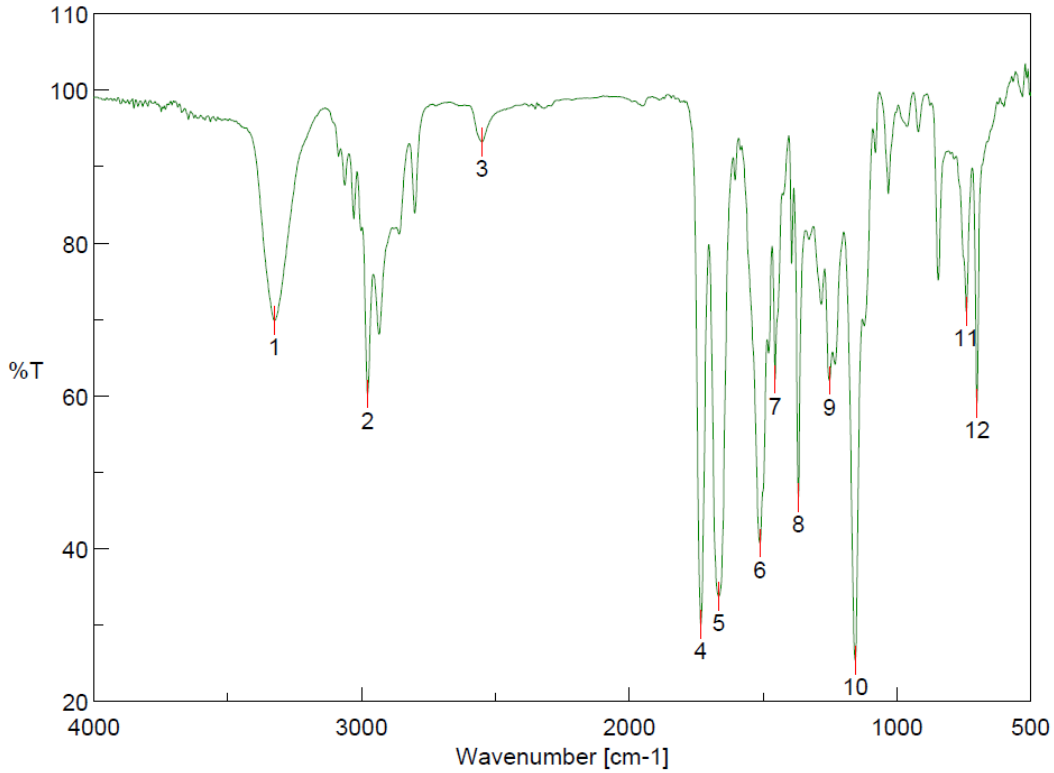


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	2977.55	41.8433	2	1729.83	26.6986
3	1687.41	9.66572	4	1494.56	25.3005
5	1445.39	32.0233	6	1367.28	19.6834
7	1321	34.083	8	1251.58	43.0609
9	1155.15	8.78128	10	1033.66	72.8681
11	846.597	68.5488	12	751.138	21.4284
13	700.998	17.5084	14	620.966	80.8352

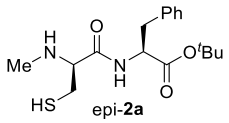


epi-**s6**

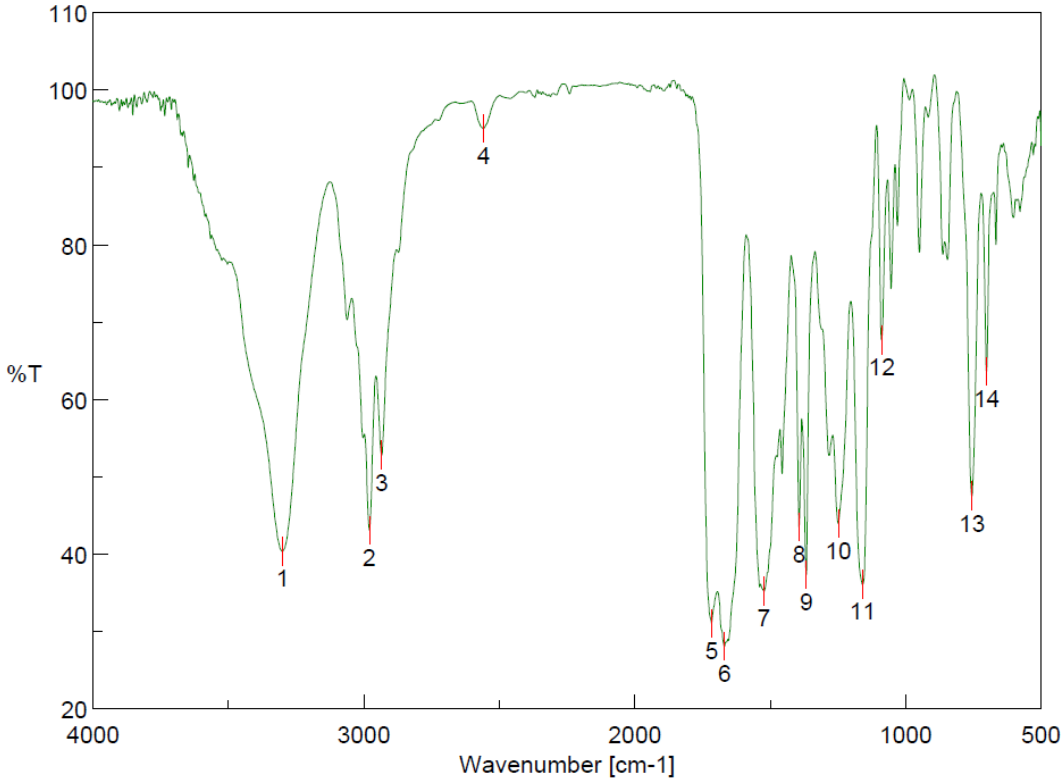


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3325.64	69.8861	2	2977.55	60.2297
3	2550.4	93.1997	4	1732.73	30.0763
5	1665.23	33.7707	6	1511.92	40.7551
7	1455.03	62.184	8	1368.25	46.6915
9	1252.54	62.0227	10	1155.15	25.4486
11	740.531	71.0545	12	700.998	59.0226

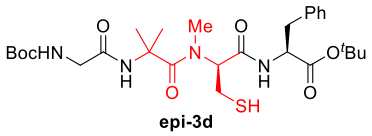


epi-**2a**

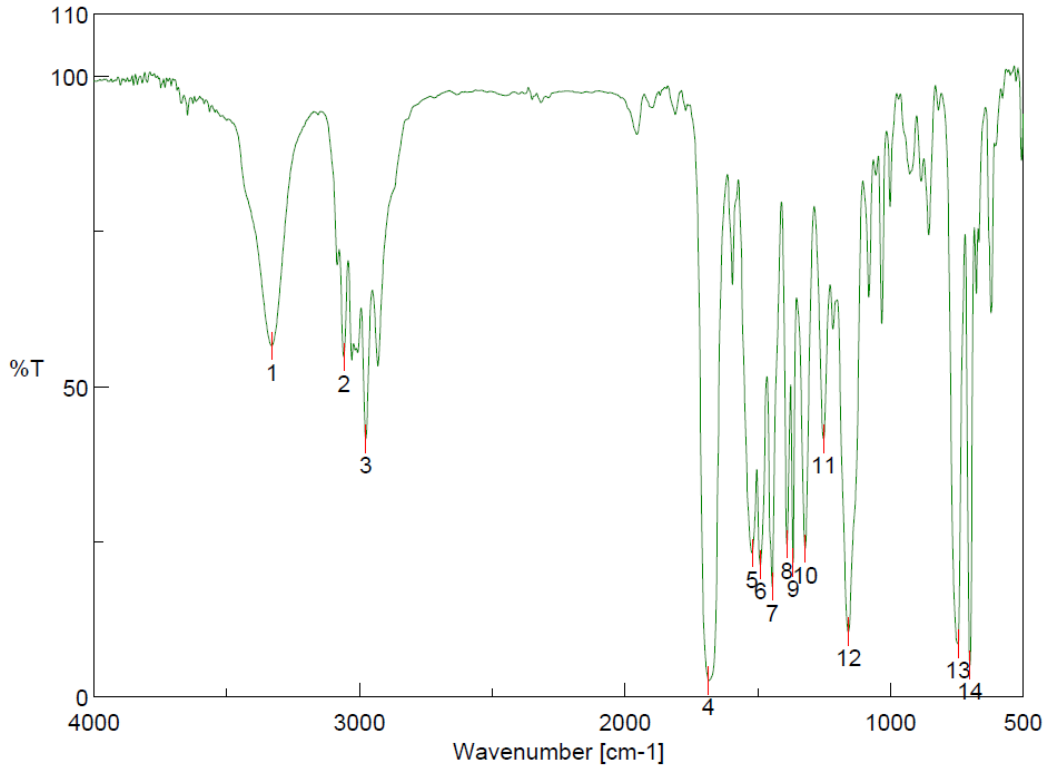


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3301.54	40.3764	2	2980.45	43.1249
3	2934.16	52.8188	4	2557.15	95.0335
5	1716.34	31.0719	6	1669.09	28.1031
7	1523.49	35.2562	8	1393.32	43.5224
9	1367.28	37.342	10	1248.68	43.9884
11	1159.01	36.1166	12	1088.62	67.6808
13	755.959	47.5095	14	700.998	63.5945

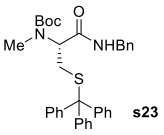


epi-3d

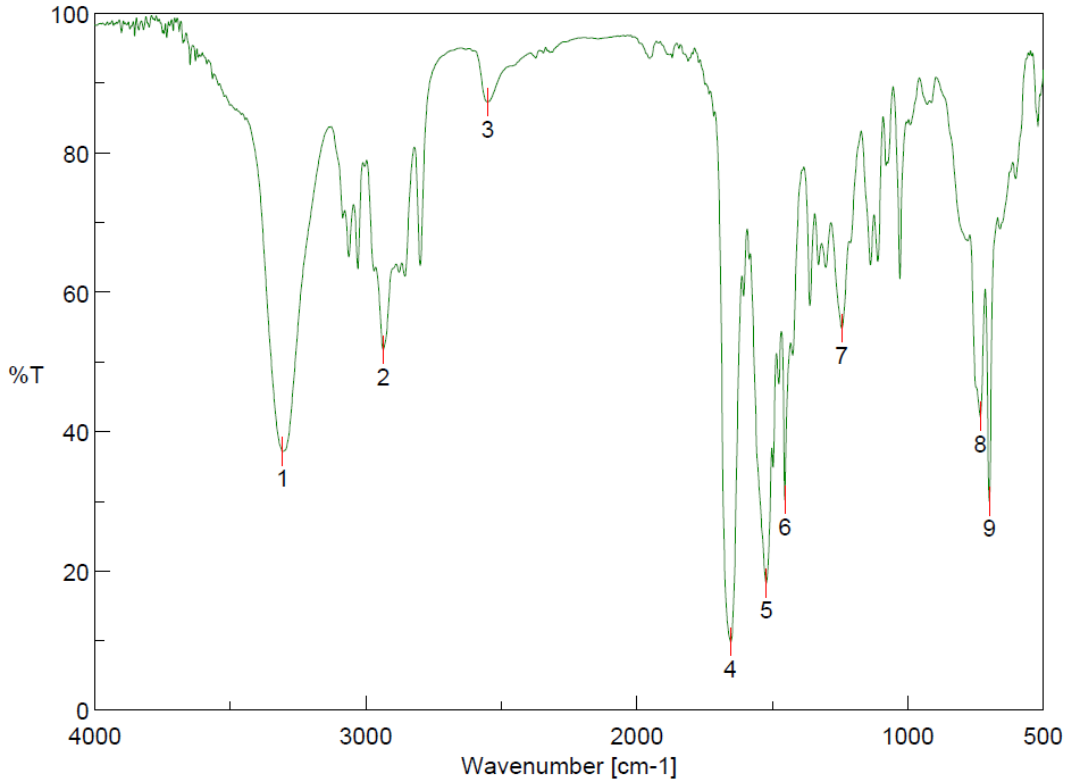


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3330.46	56.5672	2	3059.51	54.8147
3	2976.59	41.5785	4	1684.52	2.64279
5	1521.56	23.214	6	1489.74	21.2712
7	1445.39	17.7924	8	1390.42	24.6163
9	1367.28	21.7004	10	1321	23.9564
11	1251.58	41.6011	12	1158.04	10.5273
13	745.352	8.57007	14	700.034	5.17608

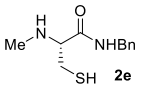


s23

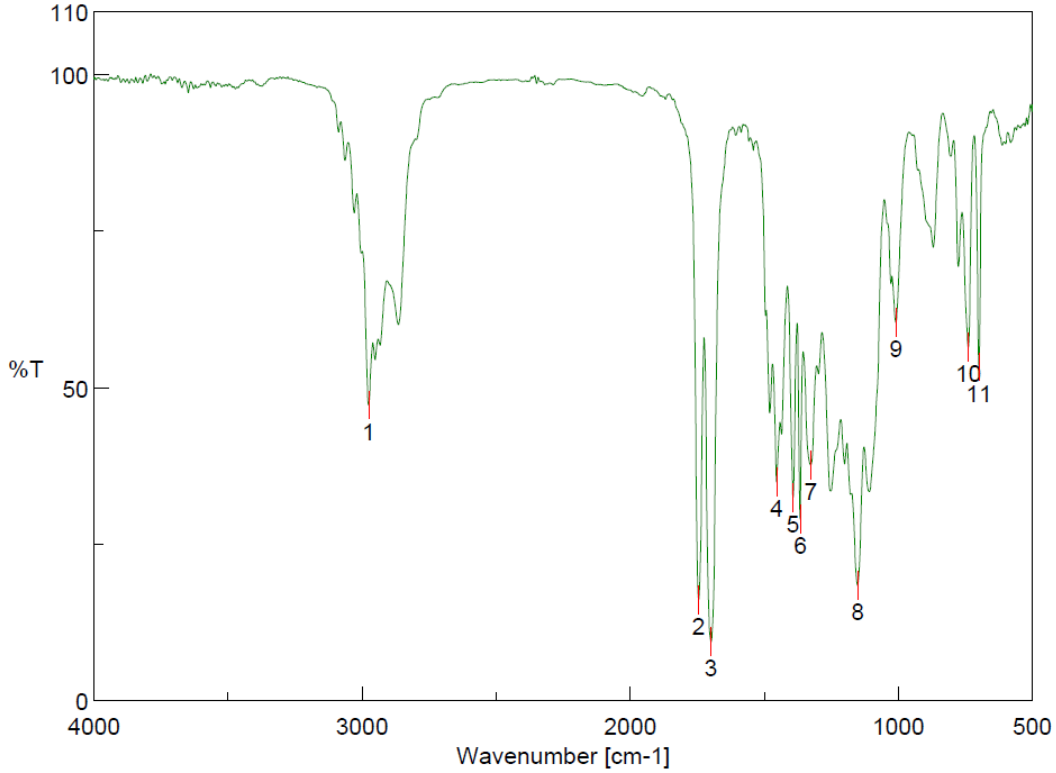


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3307.32	37.1351	2	2936.09	51.7815
3	2550.4	87.2838	4	1653.66	9.81441
5	1522.52	18.2681	6	1454.06	30.1984
7	1243.86	54.8249	8	731.853	42.1531
9	699.069	30.016			

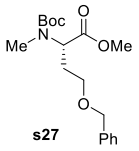


2e

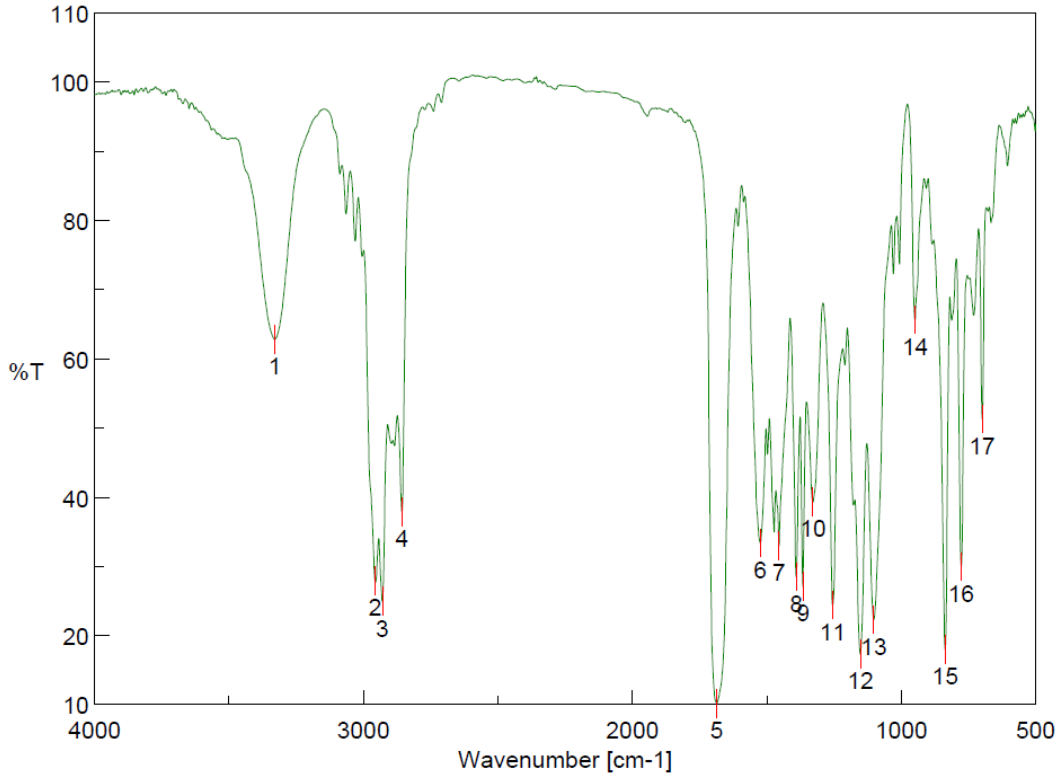


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	2975.62	47.2194	2	1744.3	16.1461
3	1698.98	9.46203	4	1454.06	34.9496
5	1392.35	32.451	6	1366.32	29.0122
7	1326.79	37.685	8	1152.26	18.4583
9	1009.55	60.4413	10	738.603	56.4318
11	699.069	53.2654			

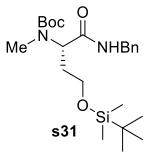


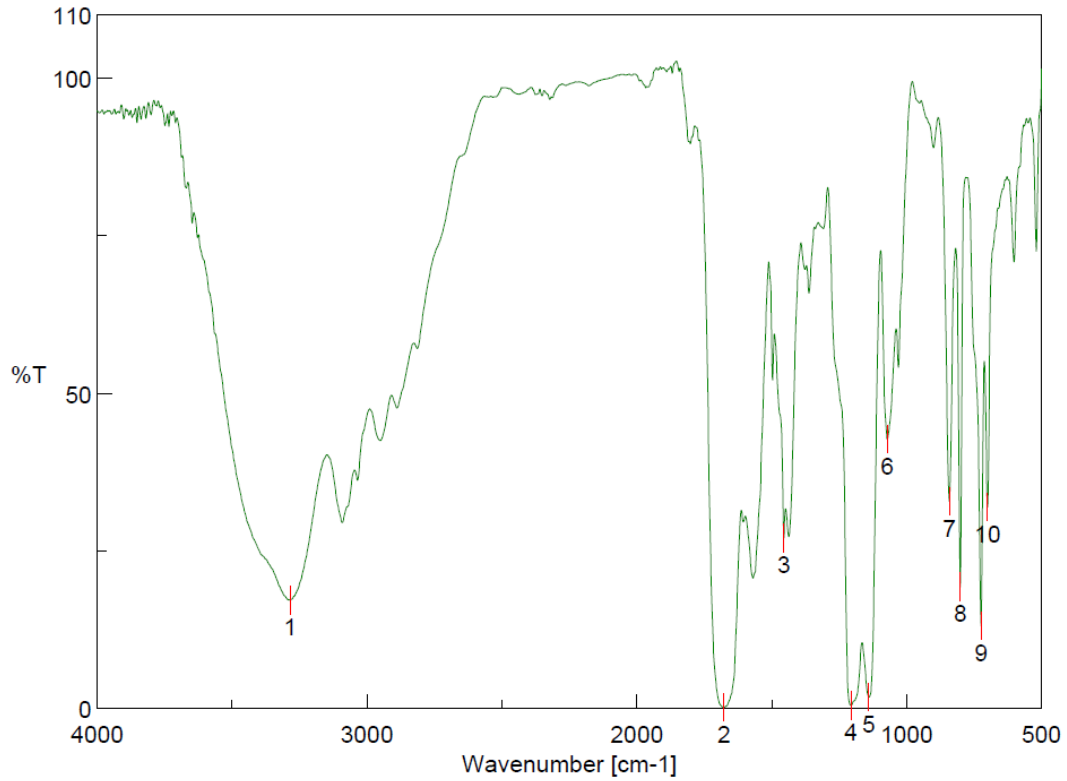
s27



[ピーク検出結果]

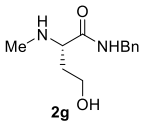
No.	位置	強度	No.	位置	強度
1	3330.46	62.7738	2	2957.3	27.943
3	2929.34	24.9304	4	2857.02	37.8271
5	1686.44	10.1742	6	1524.45	33.3656
7	1455.03	32.9229	8	1390.42	28.4596
9	1366.32	27.0792	10	1328.71	39.284
11	1255.43	24.4013	12	1152.26	17.2793
13	1102.12	22.2603	14	948.806	65.6384
15	836.955	17.9841	16	777.172	29.9861
17	699.069	51.3123			



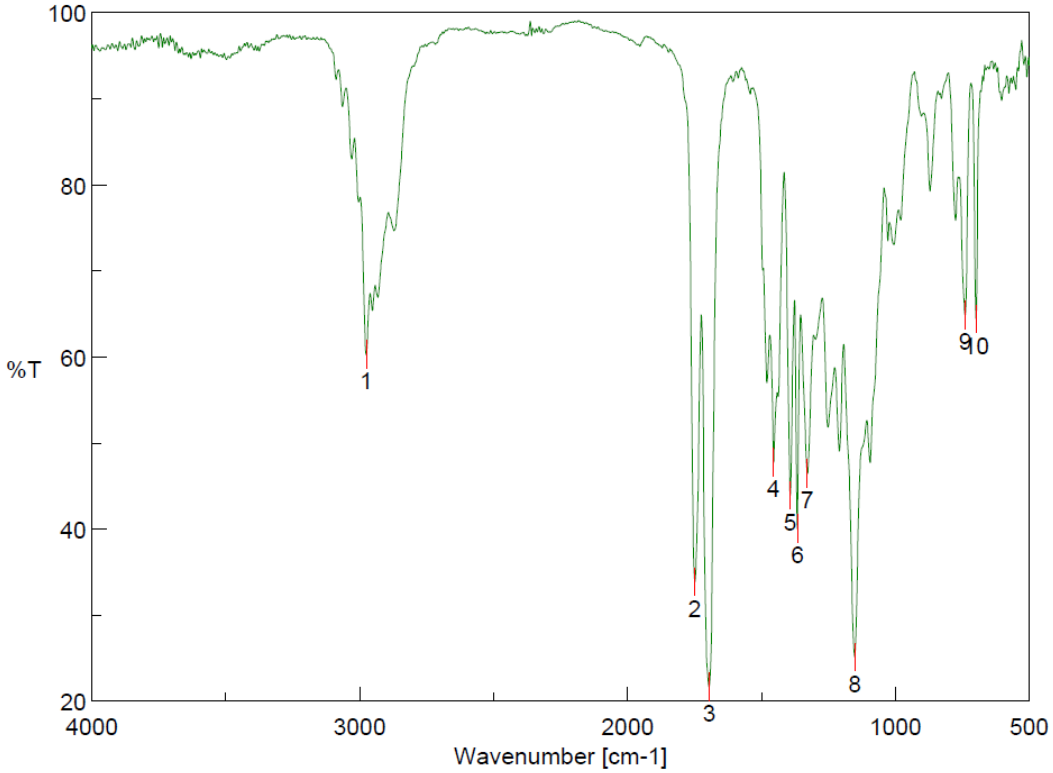


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3285.14	17.2281	2	1676.8	0.207431
3	1455.03	27.0592	4	1206.26	0.545568
5	1139.72	1.87174	6	1071.26	42.6972
7	841.776	32.8581	8	801.278	19.312
9	724.139	13.1443	10	700.034	31.9561

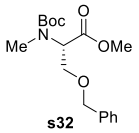


2g

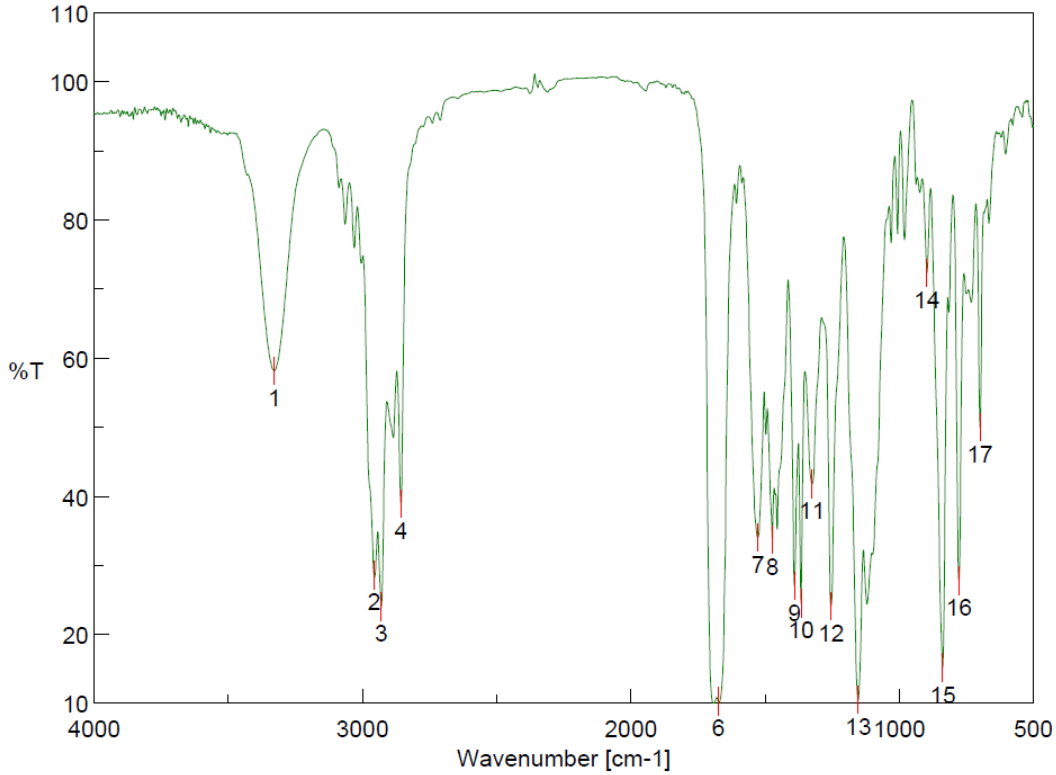


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	2975.62	60.2526	2	1749.12	33.8756
3	1696.09	21.6778	4	1455.03	47.733
5	1392.35	43.9148	6	1366.32	40.0372
7	1328.71	46.4649	8	1152.26	25.1516
9	739.567	64.8279	10	698.105	64.3874

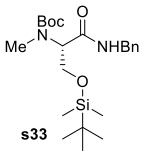


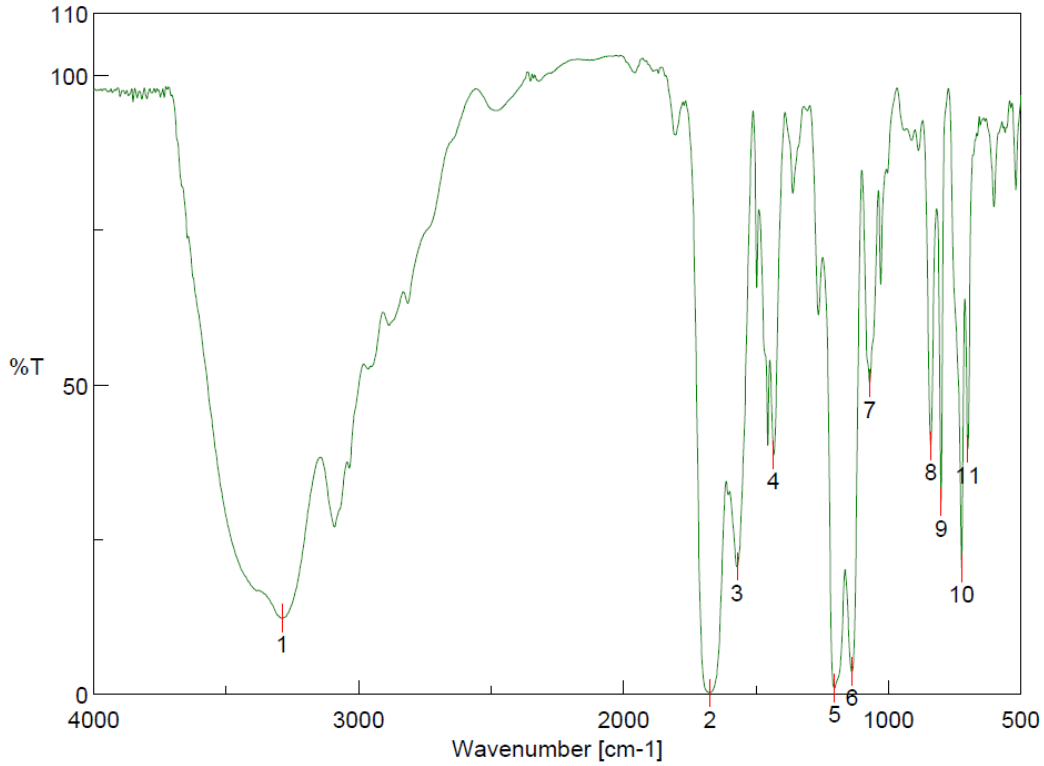
s32



[ピーク検出結果]

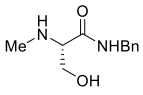
No.	位置	強度	No.	位置	強度
1	3328.53	58.1453	2	2957.3	28.5494
3	2930.31	24.0239	4	2857.02	38.9027
5	1693.19	10.0122	6	1673.91	10.2919
7	1526.38	34.0496	8	1473.35	33.6827
9	1390.42	27.0357	10	1366.32	24.4627
11	1324.86	41.8015	12	1254.47	24.1098
13	1153.22	10.5332	14	896.737	72.3173
15	837.919	15.1456	16	777.172	27.7646
17	699.069	50.054			



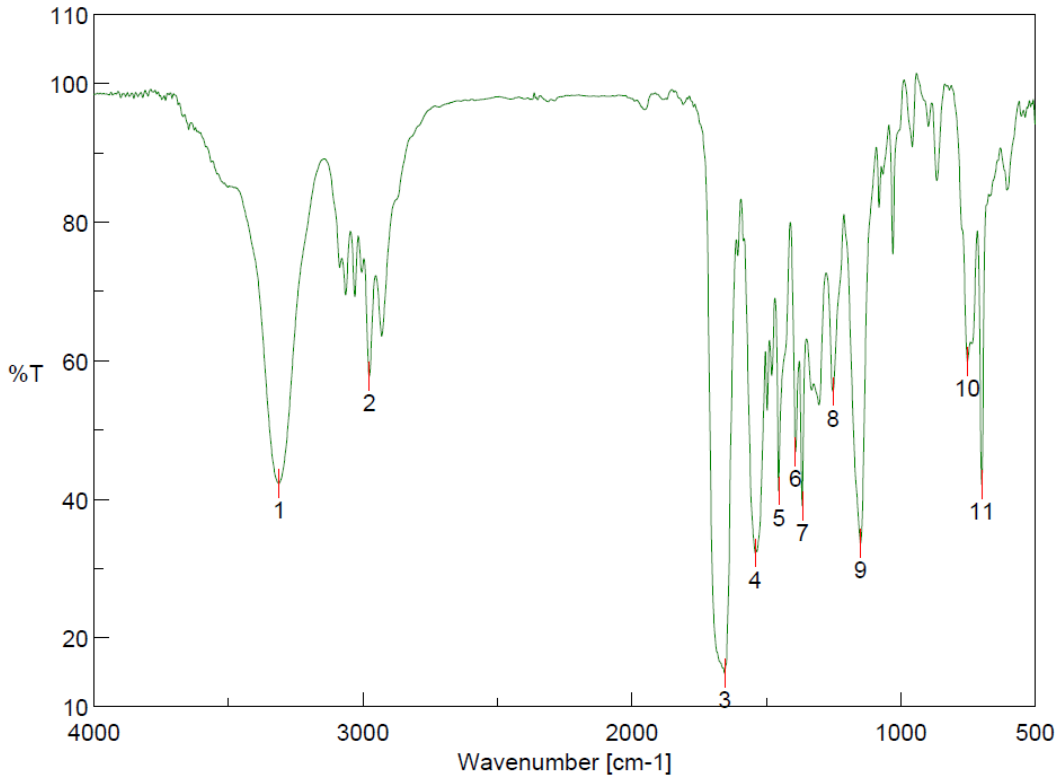


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3289	12.2913	2	1673.91	0.259418
3	1571.7	20.6462	4	1432.85	38.6869
5	1203.36	1.08766	6	1137.8	3.71495
7	1070.3	50.3289	8	839.847	40.1257
9	801.278	31.1101	10	723.175	20.4462
11	700.034	39.6859			

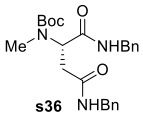


2f

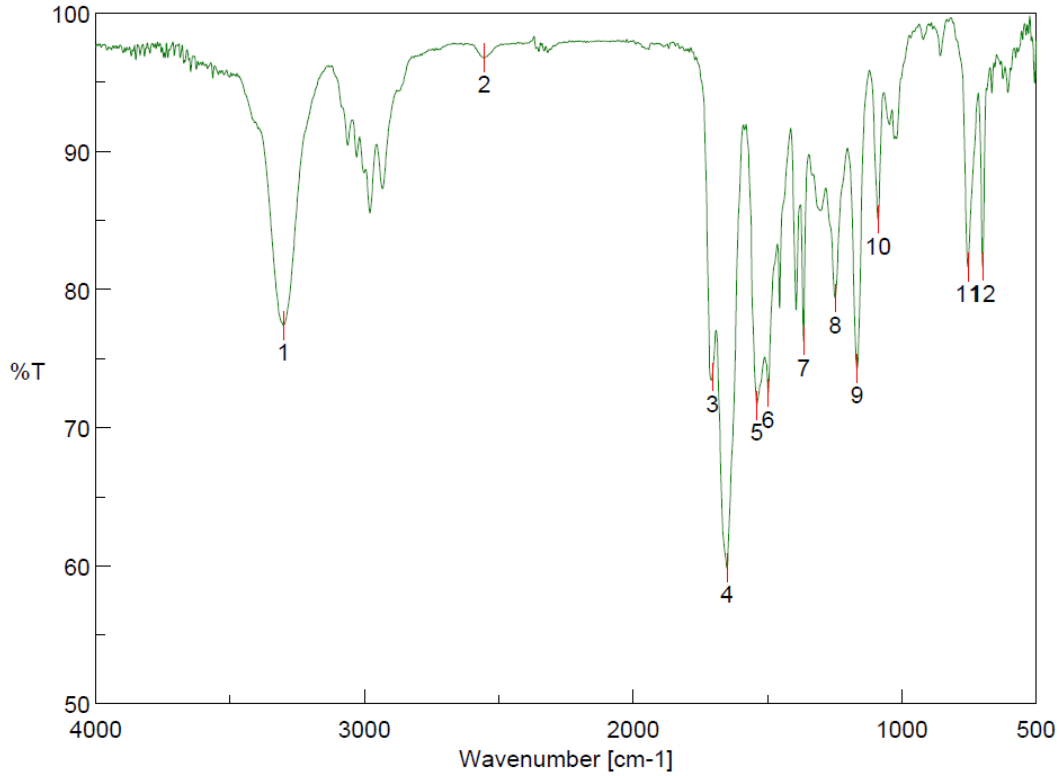


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3312.14	42.2652	2	2976.59	57.7337
3	1654.62	14.8668	4	1541.81	32.207
5	1454.06	41.1517	6	1391.39	46.8265
7	1366.32	38.9872	8	1252.54	55.5572
9	1149.37	33.605	10	752.102	59.877
11	699.069	42.1389			

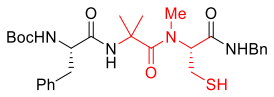


s36

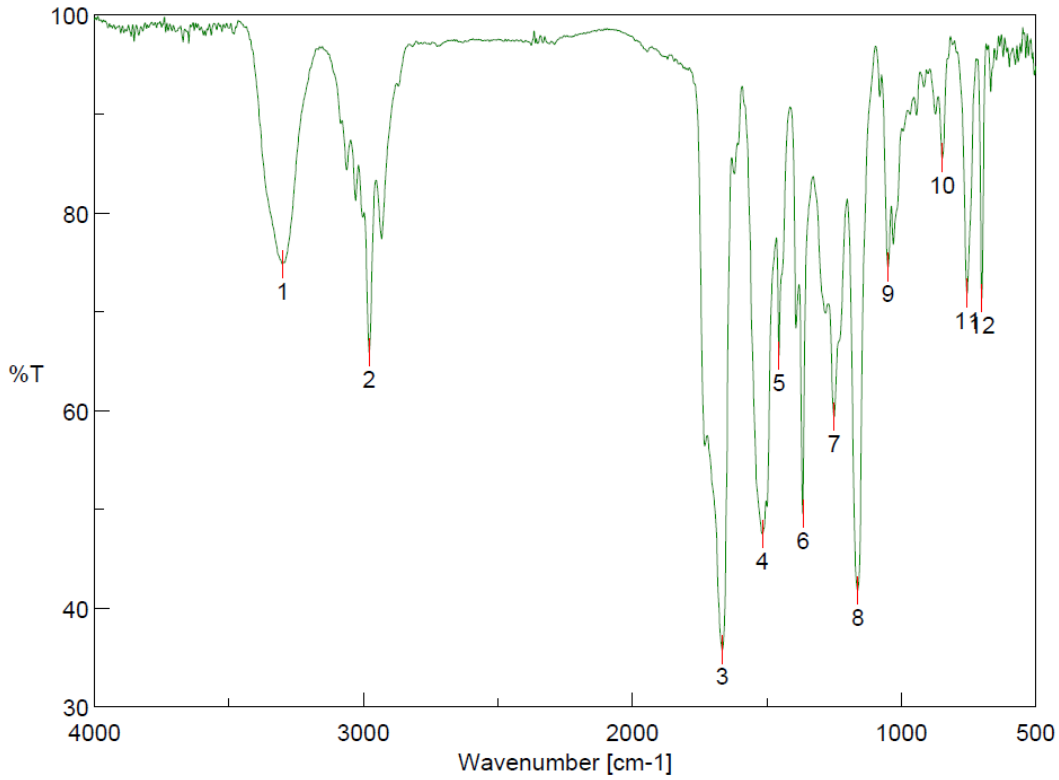


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3302.5	77.3759	2	2554.25	96.7531
3	1705.73	73.6591	4	1650.77	59.8346
5	1539.88	71.5903	6	1497.45	72.5257
7	1366.32	76.2751	8	1248.68	79.3855
9	1166.72	74.2736	10	1088.62	85.0529
11	754.031	81.6257	12	699.069	81.6687

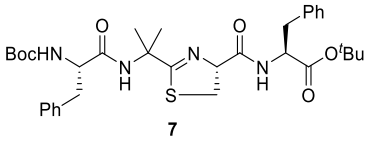


3w

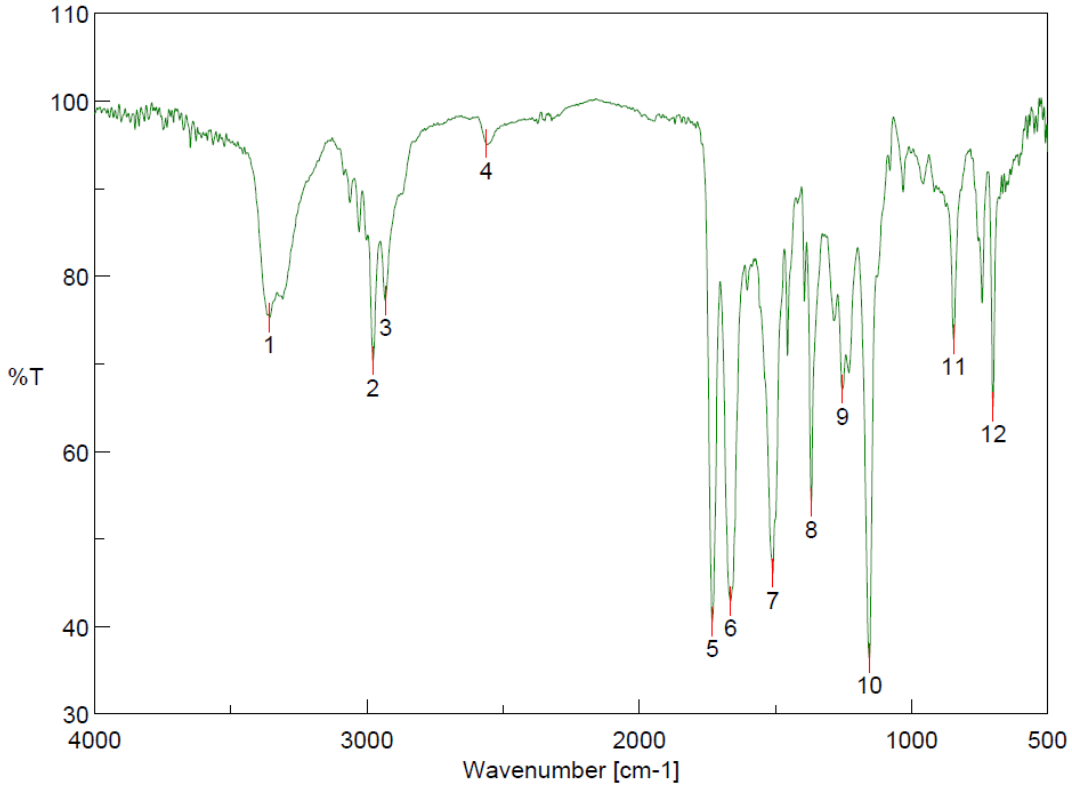


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3301.54	74.7867	2	2978.52	65.8635
3	1665.23	35.7988	4	1516.74	47.5212
5	1455.03	65.549	6	1366.32	49.5409
7	1249.65	59.3625	8	1161.9	41.7861
9	1049.09	74.4734	10	847.561	85.5086
11	754.995	71.8384	12	700.034	71.3256

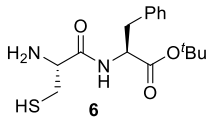


7

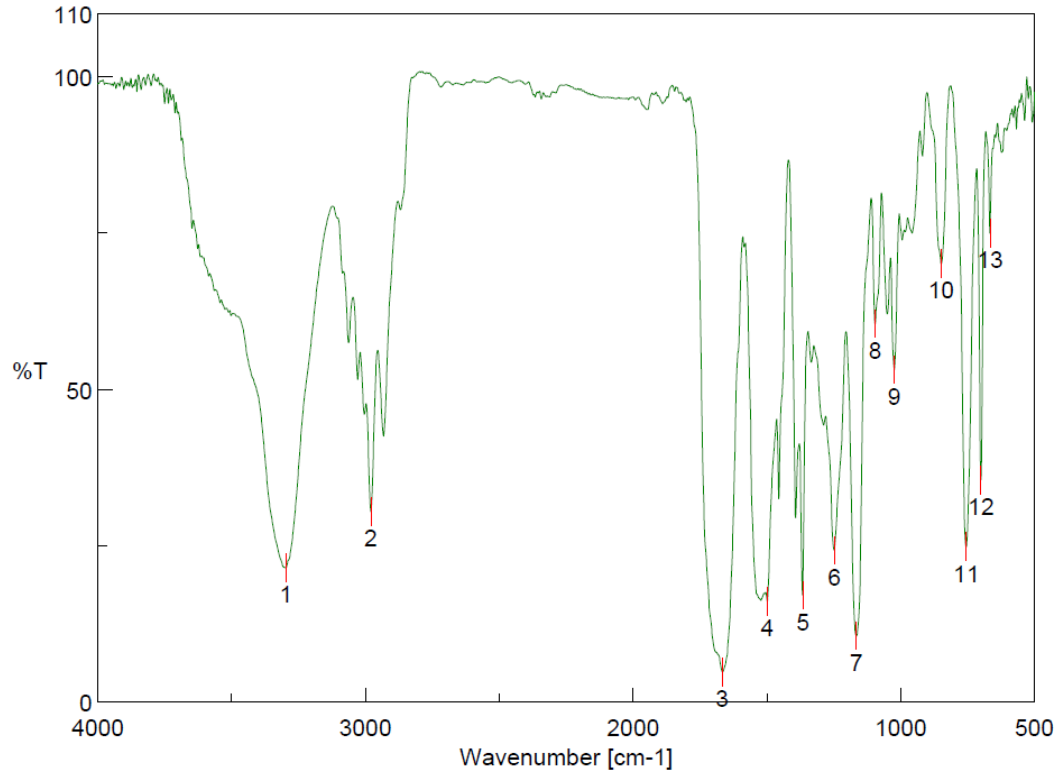


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3358.43	75.2237	2	2978.52	70.3484
3	2932.23	77.2118	4	2561	95.0727
5	1731.76	40.5543	6	1664.27	42.9194
7	1509.03	46.0761	8	1368.25	54.1634
9	1253.5	67.0921	10	1155.15	36.3639
11	844.669	72.7413	12	700.034	65.1006

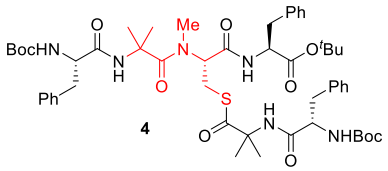


6



[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3297.68	21.4868	2	2979.48	30.4241
3	1666.2	4.87194	4	1498.42	16.1414
5	1366.32	17.1085	6	1248.68	24.29
7	1165.76	10.6172	8	1095.37	60.4665
9	1024.02	53.1056	10	848.525	70.0998
11	754.995	24.8411	12	700.034	35.5068
13	666.285	74.8976			



4

Chapter 4. Peptide ligation between α-amidonitrile and N-terminal cysteine

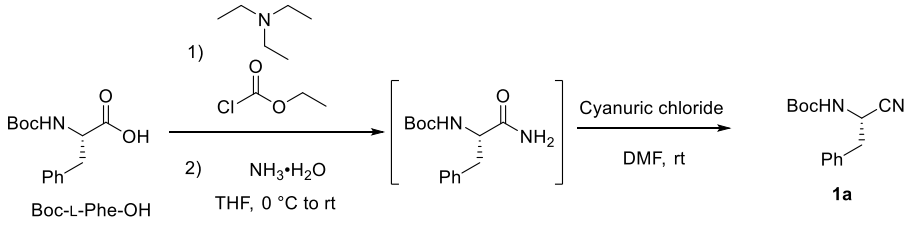
Experimental procedures and Characterization data

Table of contents

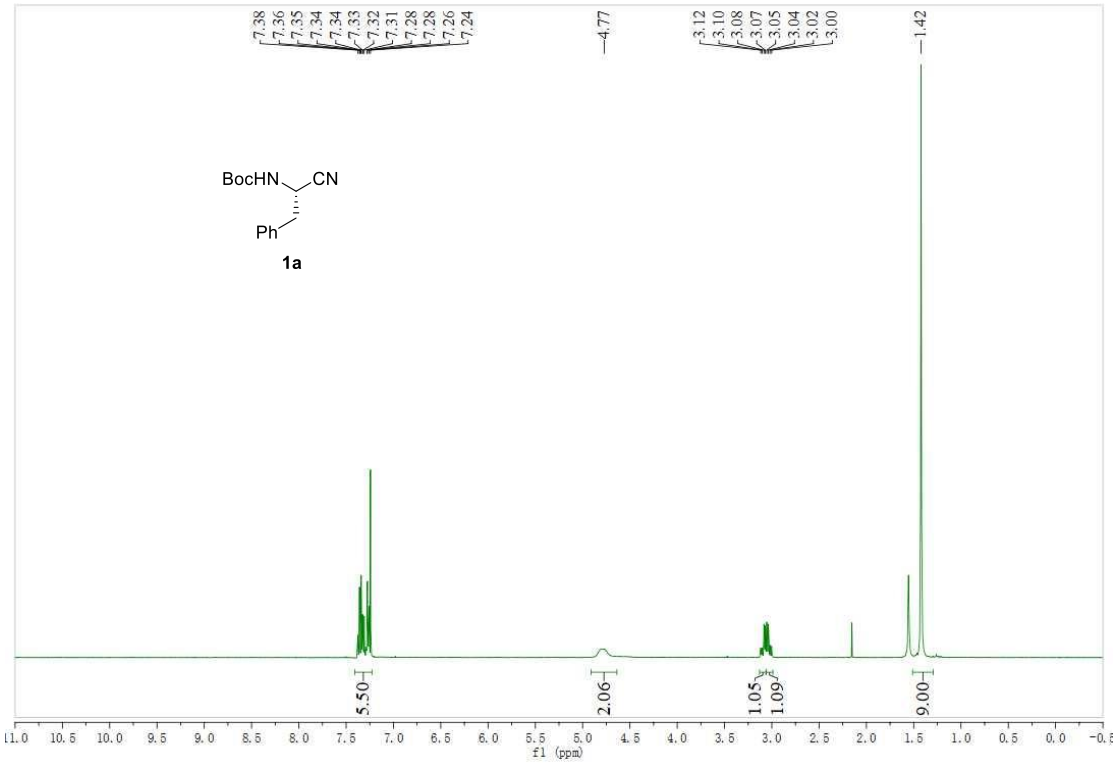
General Methods

General Remarks: All reactions were carried out under argon atmosphere and monitored by thin-layer chromatography using Merck 60 F254 precoated silica gel plates (0.25 mm thickness). Specific optical rotations were measured using a JASCO P-1020 polarimeter and a JASCO DIP-370 polarimeter. FT-IR spectra were recorded on a JASCO FT/IR-410 spectrometer and a Perkin Elmer spectrum BX FT-IP spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on an Agilent-400 MR (400 MHz for ^{1}H NMR, 100 M Hz for ^{13}C NMR) instrument. Data for ^{1}H NMR are reported as chemical shift (δ ppm), integration multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quintet = quin, septet = sep, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = double of triplets, td = triplet of doublets, m = multiplet, brs = broad singlet), coupling constant (Hz), Data for ^{13}C NMR are reported as chemical shift. High resolution ESI-TOF mass spectra were measured by Themo Orbi-trap LTQ XL instrument.

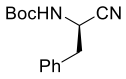
1. Synthesis of 1a and physical information



L-Boc-Phe-OH (5.30 g, 20.0 mmol) was dissolved in THF (40 mL) and cooled to 0 ºC. Triethyl amine (2.8 mL, 20 mmol) was added to the reaction mixture and stirred for 15 min at 0 ºC. Ethyl chloroformate (1.9 mL, 20 mmol) was added slowly at 0 ºC and stirred for 30 min. ammonia hydrate (~30% solution) was added to the reaction mixture and stirred for 0.5 h. The reaction mixture was evaporated to remove volatiles. The residue was extracted with EtOAc, the organic phase was collected, dried over Na_2SO_4, evaporated under reduced pressure, dried in vacuo. The crude amide product (5.3 g, 20 mmol) was dissolved in DMF (20 mL), followed by the addition of cyanuric chloride (2.8 g, 15 mmol). After stirring for 2 h, the reaction was quenched by water at 0 ºC, extracted with EtOAc, washed with water. The organic phased was collected, dried over Na_2SO_4, evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 60%/40%) and gave **1a** in 80% yield (2 steps) as a white solid. The NMR was matched with the reported data. **^{1}H NMR** (399 MHz, $CDCl_3$) δ 7.43 – 7.19 (m, 5H), 4.92 – 4.63 (m, 1H), 4.94 – 4.63 (m, 1H), 3.09 (dd, *J* = 13.7, 5.6 Hz, 1H), 3.03 (dd, *J* = 13.8, 6.9 Hz, 1H), 1.42 (s, 9H).



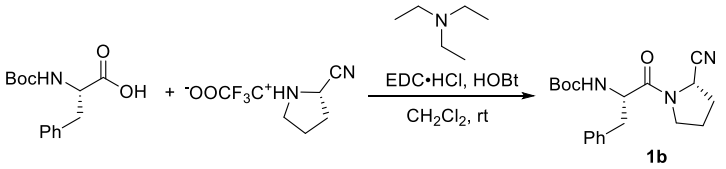
***tert*-Butyl (*R*)-(1-cyano-2-phenylethyl)carbamate**



epi-1a

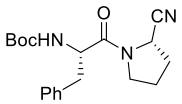
a_D^{27}+34.03 ($CHCl_3$ 1.13)

2. Synthesis of 1b and physical information



(((2*S*)-2-cyanopyrrolidin-1-ium-1-yl)difluoromethyl)-λ^2-fluoranecarboxylate was synthesized from *tert*-butyl (*S*)-2-cyanopyrrolidine-1-carboxylate.[1] *tert*-Butyl (*S*)-2-cyanopyrrolidine-1-carboxylate was synthesized from *tert*-butyl (*S*)-2-carbamoylpyrrolidine-1-carboxylate.[2] (*S*)-2-carbamoylpyrrolidine-1-carboxylate was synthesized from (*tert*-butoxycarbonyl)-L-proline.[3] The crude product (*S*)-2-amino-3-phenylpropanenitrile (14.7 mmol, around 4 mL) was quenched by triethylamine (9.02 mL) at 0 ºC slowly. Then to that mixture was added CH_2Cl_2 (58 mL, 0.25 M), L-Boc-Phe-OH (3.90 g, 14.7 mmol), EDC•HCl (3.38 g, 17.6 mmol) and HOBt•H_2O (226 mg, 1.47 mmol). The reaction was stirred under room temperature overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 45%/55%; then CH_2Cl_2/Et_2O = 80%/20%) and gave **1b** in 36% yield over 2 steps as a white solid.

***tert*-Butyl ((*S*)-1-((*S*)-2-cyanopyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate**



1b

1b was obtained in 36% yield over 2 steps as a white solid and **1b** existed as a mixture of rotamers.

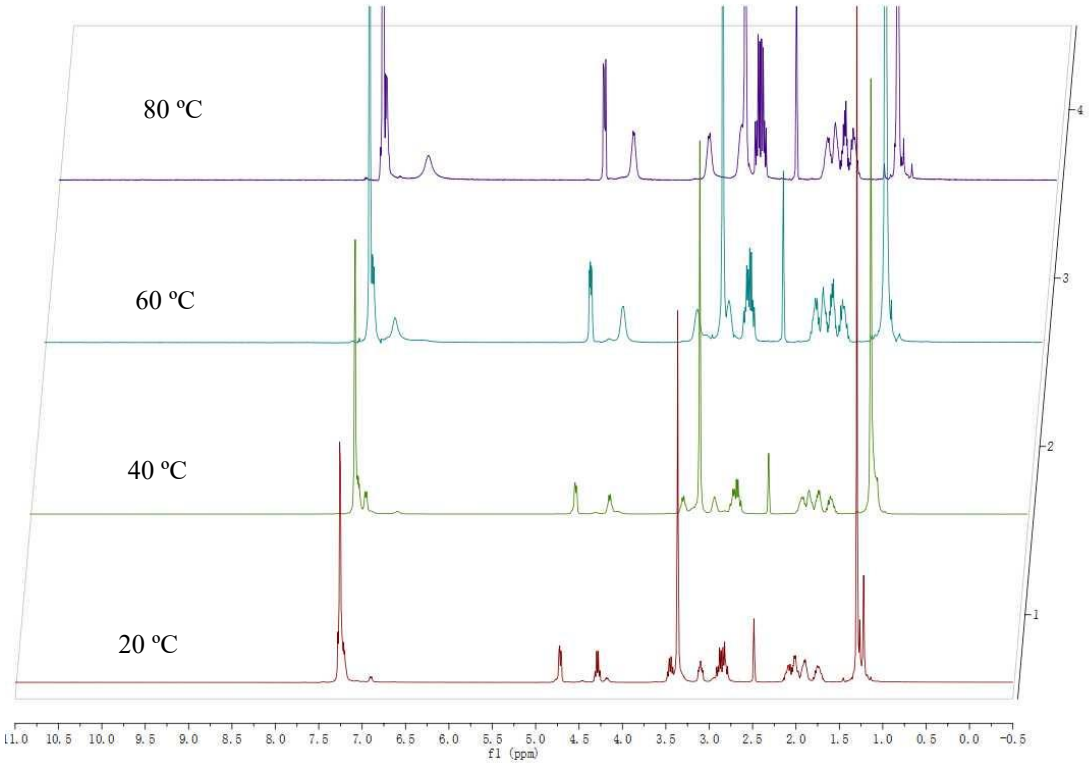
^{1}H NMR (399 MHz, DMSO-d_6) **(Measured at 80 ºC)** δ 7.34 – 7.12 (m, 5H), 6.74 (brs, 1H), 4.77 – 4.66 (m, 1H), 4.47 – 4.28 (m, 1H), 3.62 – 3.40 (m, 1H), 3.20 – 3.11 (m, 1H), 2.95 (dd, *J* = 13.5, 6.9 Hz, 1H), 2.88 (dd, *J* = 13.5, 7.6 Hz, 1H), 2.23 – 2.09 (m, 1H), 2.09 – 2.00 (m, 1H), 1.99 – 1.90 (m, 1H), 1.89 – 1.76 (m, 1H), 1.33 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) **(Major rotamer)** δ 171.2, 155.6, 137.6, 129.8, 128.6, 127.0, 119.3, 78.6, 54.2, 46.5, 46.3, 37.5, 29.8, 28.6, 25.3.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 366.1788; found: 366.1793

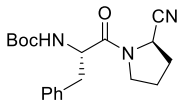
FT-IR (neat): 3330, 2979, 2243, 1707, 1649, 1498, 1430, 1392, 1367, 1249, 1169, 754, 703 cm^{-1}

a_D^{27}-9.40 ($CHCl_3$ 0.66)



Variable temperature ^{1}H NMR (DMSO-d$_6$) to prove the rotamers of 1b

***tert*-Butyl ((*S*)-1-((*R*)-2-cyanopyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate**



epi-1b

epi-1b was obtained in 36% yield over 2 steps as a colorless oil and **epi-1b** existed as a mixture of rotamers. Eluent for purification: Hexane/EtOAc = 50%/50%; then CH_2Cl_2/Et_2O = 85%/15%.

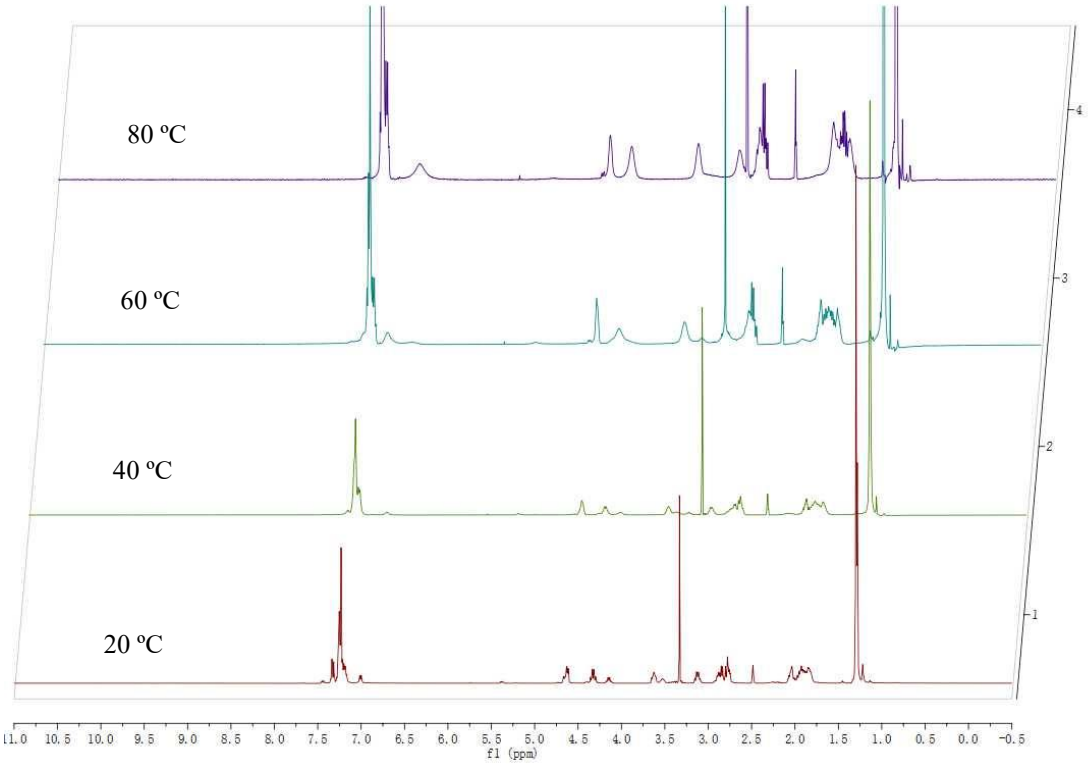
^{1}H NMR (399 MHz, DMSO-d$_6$) **(Measured at 80 ºC)** δ 7.38 – 7.12 (m, 5H), 6.83 (brs, 1H), 4.72 – 4.56 (m, 1H), 4.49 – 4.29 (m, 1H), 3.72 – 3.53 (m, 1H), 3.23 – 3.08 (m, 1H), 3.00 – 2.79 (m, 1H), 3.00 – 2.79 (m, 1H), 2.20 – 1.78 (m, 2H), 2.20 – 1.78 (m, 2H), 1.34 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d$_6$) (**Major rotamer**) δ 171.2, 155.7, 137.8, 129.7, 128.5, 126.9, 119.6, 78.6, 54.2, 46.7, 46.3, 37.1, 29.8, 28.5, 25.1.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 366.1788; found: 366.1787.

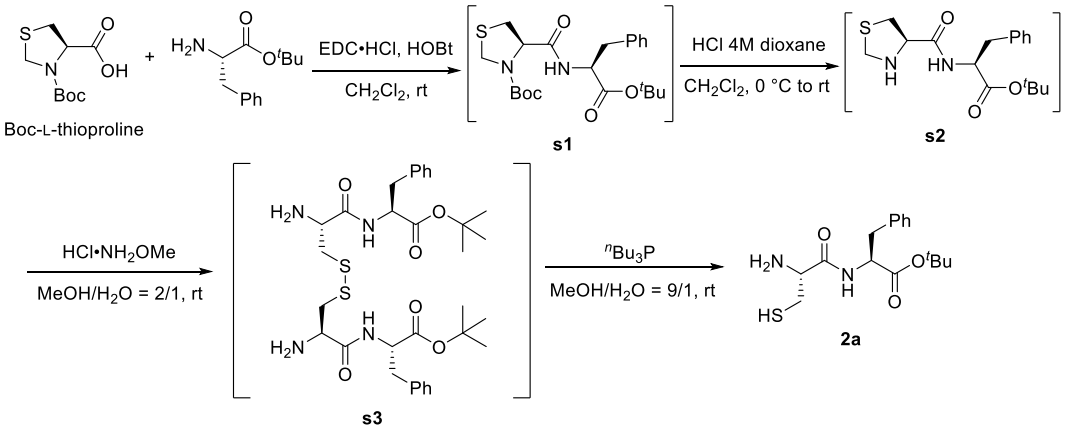
FT-IR (neat): 3314, 2978, 2243, 1705, 1652, 1497, 1429, 1366, 1250, 1168, 1054, 738, 702 cm^{-1}

a_D^{26}+95.48 ($CHCl_3$ 0.49)



Variable temperature ^{1}H NMR (DMSO-d_6) to prove the rotamers of epi-1b

3. Synthesis of NH_2-Cysteinyl dipeptide 2a and physical information

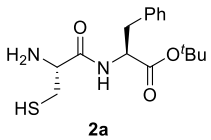


To a mixture of Boc- L-thioproline (5.50 g, 24.9 mmol) and L-Phe-O^{t}Bu (5.80 g, 24.9 mmol) in CH_2Cl_2 (100 mL, 0.25 M) were added EDC•HCl (5.72 g, 29.8 mmol) and HOBt•H_2O (382 mg, 2.49 mmol). The reaction was stirred under room temperature overnight. Then the solvent was removed by evaporation under reduced pressure and the residue was diluted with EtOAc, followed by addition of 1N HCl aqueous solution. The organic phase was collected, washed with sat. $NaHCO_3$ aqueous solution, dried over Na_2SO_4, concentrated under reduced pressure and dried in vacuo. The crude compound **s1** (9.60 g) was used for the following step without further purification.

4M HCl 1,4-dioxane solution (110 mL) was cooled to 0 ºC and added to **s1** (9.60 g, 22.0 mmol) at 0 ºC.[7] The reaction mixture was stirred at room temperature until the reaction was deemed to complete by TLC analysis and quenched by sat. $NaHCO_3$ aqueous solution, extracted with EtOAc. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product **s2** (4.60 g, 13.7 mmol) was dissolved in MeOH/H_2O = 2/1 (68 mL). O-methylhydroxylammonium chloride (11.4 g, 137 mmol) was added to the reaction mixture. After stirring overnight under argon atmosphere at room temperature, the reaction mixture was evaporated under reduced pressure to remove the volatiles, quenched with sat. $NaHCO_3$ aqueous solution, extracted with EtOAc, dried over Na_2SO_4, concentrated under reduced pressure. The crude compound was roughly purified by silica gel column chromatography (Hexane/EtOAc = 12%/88%, then EtOAc/MeOH = 90%/10%) and gave **s3** (3.40 g).

s3 (3.40 g, 5.26 mmol) was dissolved in MeOH/H_2O = 9/1 (52.5 mL), followed by addition of $^nBu_3P$ (2.60 mL, 10.5 mmol) under argon atmosphere. The reaction mixture was stirred at room temperature until the reaction was deemed to complete by TLC analysis. Then the reaction mixture was evaporated under reduced pressure to remove the volatiles. The residue was purified by silica gel column chromatography (Hexane/EtOAc = 50%/50%, then EtOAc 100%) and gave **2a** (2.70 g, 8.32 mmol) in 33% yield over 4 steps as a white solid.

***tert*-Butyl L-cysteinyl-L-phenylalaninate**



2a

2a was obtained in 33% yield over 4 steps as a white solid. m.p. 65-67 ºC.

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.3 Hz, 1H), 7.33 – 7.09 (m, 5H), 4.80 – 4.70 (m, 1H), 3.56 – 3.48 (m, 1H), 3.13 (dd, *J* = 13.9, 6.0 Hz, 1H), 3.02 (dd, *J* = 13.9, 7.0 Hz, 1H), 2.96 (dd, *J* = 13.8, 5.8 Hz, 1H), 2.68 (dd, *J* = 13.8, 4.1 Hz, 1H), 1.42 (s, 9H).

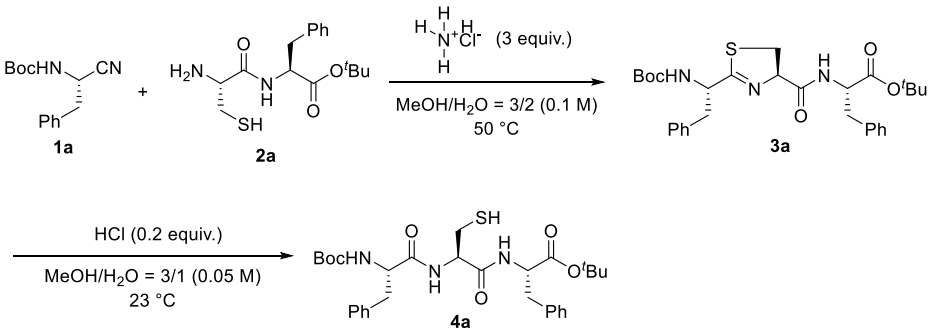
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 170.6, 136.2, 129.5, 128.4, 126.9, 82.3, 55.9, 53.2, 38.3, 30.3, 28.0.

HRMS (ESI) m/z: Calcd for $C_{16}H_{25}N_2O_3S^+$ $[M+H]^+$: 325.1580; found: 325.1579.

FT-IR (neat): 3358, 2979, 2932, 2561, 1732, 1664, 1509, 1368, 1254, 1155, 845, 700 cm^{-1}

$[\alpha]_D^{24}$+22.69 (c 1.4 $CHCl_3$)

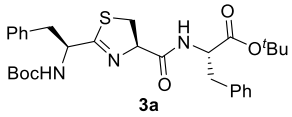
4. Synthesis of 4a and physical information



NH_2-cysteinyl peptide **2a** (0.24 mmol, 1.2 equiv.), α-amido nitrile **1a** (0.20 mmol, 1 equiv.) and ammonium chloride (0.60 mmol, 3.0 equiv.) were dissolved in a mixture of MeOH and H_2O in 3/2 ratio (total volume: 2 mL). The reaction mixture was immediately degassed under liquid N_2 bath and filled with argon for 3 times. After stirring vigorously for the time indicated at 50 ºC, it was diluted with EtOAc, filtered through a short column of Na_2SO_4 (~5 g/0.1 mmol scale of substrate **1a**), and washed with MeOH and EtOAc. The filtrate was evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography and gave the desired product **3a** (dr > 99 : 1 based on HPLC analysis on a chiral phase. The isolated product was injected as ^{i}PrOH solution, IB-N5, Hexane/^{i}PrOH = 9/1. Flow rate = 1.0 ml/min, λ = 210 nm, t_{major} = 8.49 min, t_{minor} = 7.66 min,).

3a (0.20 mmol) was dissolved in a mixture of MeOH and H_2O in 3/1 ratio (total volume: 4 mL), followed by the addition of HCl 1.02 M aqueous solution (The HCl solution was titrated before using) (0.2 equiv.). The reaction mixture was immediately degassed under liquid N_2 bath and filled with argon for 3 times. After stirring vigorously for the time indicated at 23 ºC, the reaction mixture was diluted with EtOAc, dried by filtration through a short column of Na_2SO_4 (~5 g/0.1 mmol scale of substrate **3a**) and washed with MeOH and EtOAc. The filtrate was evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography and gave the desired product **4a** (dr > 95 : 5 based on HPLC analysis on a chiral phase. The crude product was injected as ^{i}PrOH solution, IB-N5, Hexane/^{i}PrOH = 9/1. Flow rate = 1.0 ml/min, λ = 210 nm): t_{major} = 5.76 min, t_{minor} = 6.63 min).

***tert*-Butyl ((*R*)-2-((*S*)-1-((*tert*-butoxycarbonyl)amino)-2-phenylethyl)-4,5-dihydrothiazole-4-carbonyl)-L-phenylalaninate**



White foam

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.35 – 6.97 (m, 5H), 7.35 – 6.97 (m, 5H), 6.93 (d, *J* = 8.1 Hz, 1H), 5.05 (d, *J* = 7.5 Hz, 1H), 4.96 (t, *J* = 9.7 Hz, 1H), 4.84 – 4.74 (m, 1H), 4.74 – 4.66 (m, 1H), 3.60 – 3.51 (m, 1H), 3.45 – 3.35 (m, 1H), 3.18 (dd, *J* = 13.9, 5.5 Hz, 1H), 3.08 – 2.91 (m, 1H), 3.08 – 2.91 (m, 1H), 3.08 – 2.91 (m, 1H), 1.43 (s, 9H), 1.42 (s, 9H).

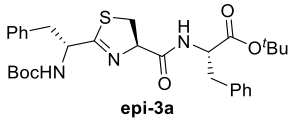
^{13}C NMR (100 MHz, $CDCl_3$) δ 175.8, 170.2, 170.1, 154.8, 136.2, 135.9, 129.5, 128.5, 128.4, 127.0, 126.9, 82.2, 80.1, 78.6, 54.2, 53.3, 39.6, 38.0, 35.5, 28.3, 28.0.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 576.2503; found: 576.2501.

FT-IR (neat): 3326, 2978, 1715, 1670, 1516, 1455, 1392, 1367, 1250, 1159, 1045, 755, 701 cm^{-1}

a_D^{27}+45.64 ($CHCl_3$ 0.80)

***tert*-Butyl ((*R*)-2-((*R*)-1-((*tert*-butoxycarbonyl)amino)-2-phenylethyl)-4,5-dihydrothiazole-4-carbonyl)-L-phenylalaninate**



epi-3a

White foam

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.34 – 7.07 (m, 5H), 7.34 – 7.07 (m, 5H), 7.00 (d, *J* = 8.0 Hz, 1H), 5.05 – 4.91 (m, 1H), 5.05 – 4.91 (m, 1H), 4.81 – 4.65 (m, 1H), 4.81 – 4.65 (m, 1H), 3.55 (dd, *J* = 11.3, 9.9 Hz, 1H), 3.44 (dd, *J* = 11.3, 8.6 Hz, 1H), 3.18 (dd, *J* = 14.1, 5.2 Hz, 1H), 3.11 (dd, *J* = 13.8, 6.1 Hz, 1H), 3.00 (dd, *J* = 13.8, 6.7 Hz, 1H), 2.89 (dd, *J* = 13.9, 7.2 Hz, 1H), 1.41 (s, 9H), 1.41 (s, 9H).

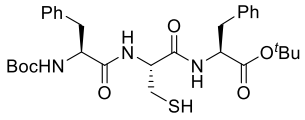
^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 170.4, 170.0, 154.9, 136.0, 129.4, 129.3, 128.4, 128.3, 126.9, 82.2, 80.0, 78.5, 54.1, 53.5, 39.5, 37.9, 35.6, 28.3, 27.9.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 576.2503; found: 576.2500.

FT-IR (neat): 3325, 2977, 1716, 1674, 1515, 1367, 1250, 1158, 700 cm^{-1}

a_D^{27}+38.54 (MeOH 0.70)

***tert*-Butyl (*tert*-butoxycarbonyl)-L-phenylalanyl-L-cysteinyl-L-phenylalaninate**



4a

White solid

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.33 – 7.10 (m, 5H), 7.33 – 7.10 (m, 5H), 6.90 (d, *J* = 7.5 Hz, 1H), 6.83 (d, *J* = 5.2 Hz, 1H), 5.05 (d, *J* = 7.4 Hz, 1H), 4.75 – 4.65 (m, 1H), 4.65 – 4.55 (m, 1H), 4.43 – 4.29 (m, 1H), 3.17 – 2.89 (m, 1H), 3.17 – 2.89 (m, 1H), 3.17 – 2.89 (m, 1H), 3.17 – 2.89 (m, 1H), 3.17 – 2.89 (m, 1H), 2.68 – 2.53 (m, 1H), 1.40 (s, 9H), 1.40 (s, 9H).

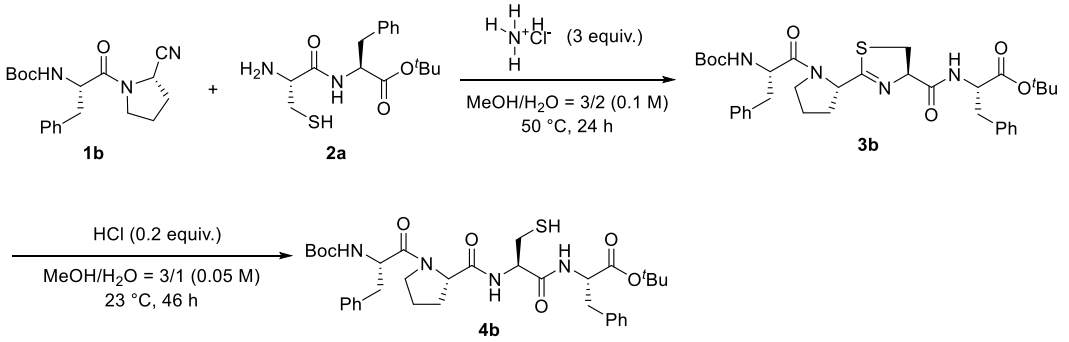
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.0, 168.7, 155.5, 136.4, 136.1, 129.4, 129.2, 128.8, 128.5, 127.1, 127.0, 82.4, 80.5, 55.8, 54.1, 54.0, 38.0, 37.9, 28.3, 27.9, 26.7.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 594.2608; found: 594.2605.

FT-IR (neat): 3285, 2978, 2554, 1695, 1645, 1521, 1391, 1367, 1250, 1161, 699 cm^{-1}

a_D^{27}-1.13 ($CHCl_3$ 0.88)

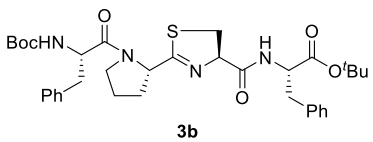
5. Synthesis of 4b and physical information



NH_2-cysteinyl peptide **2a** (0.24 mmol, 1.2 equiv.), α-amido nitrile **1b** (0.20 mmol, 1 equiv.) and ammonium chloride (0.60 mmol, 3.0 equiv.) were dissolved in a mixture of MeOH and H_2O in 3/2 ratio (total volume: 2 mL). The reaction mixture was immediately degassed under liquid N_2 bath and filled with argon for 3 times. After stirring vigorously for 24 h at 50 ºC, it was diluted with EtOAc, filtered through a short column of Na_2SO_4 (~5 g/0.1 mmol scale of substrate **1b**), and washed with MeOH and EtOAc. The filtrate was evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography and gave the desired product **3b** in 99% yield (dr > 98 : 2 based on HPLC analysis on a chiral phase. The isolated product was injected as ^{i}PrOH solution, IB-N5, Hexane/^{i}PrOH = 9/1. Flow rate = 1.0 ml/min, λ = 210 nm, t_{major} = 10.71 min, t_{minor} = 7.87 min,).

3b (65.1 mg, 0.10 mmol) was dissolved in a mixture of MeOH and H_2O in 3/1 ratio (total volume: 2 mL), followed by the addition of HCl 1.02 M aqueous solution (The HCl solution was titrated before using) (19.6 μL, 0.2 equiv.). The reaction mixture was immediately degassed under liquid N_2 bath and filled with argon for 3 times. After stirring vigorously for 46 h at 23 ºC, the reaction mixture was diluted with EtOAc, dried by filtration through a short column of Na_2SO_4 (~5 g/0.1 mmol scale of substrate **3b**) and washed with MeOH and EtOAc. The filtrate was evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography and gave the desired product **4b** in 80% yield (dr > 95 : 5 based on HPLC analysis on a chiral phase. The crude product was injected as ^{i}PrOH solution, IB-N5, Hexane/^{i}PrOH = 9/1. Flow rate = 1.0 ml/min, λ = 210 nm): t_{major} = 7.92 min, t_{minor} = 8.94 min).

***tert*-Butyl ((*R*)-2-((*S*)-1-((*tert*-butoxycarbonyl)-L-phenylalanyl)pyrrolidin-2-yl)-4,5-dihydrothiazole-4-carbonyl)-L-phenylalaninate**



3b existed as a mixture of rotamers and as a white foam.

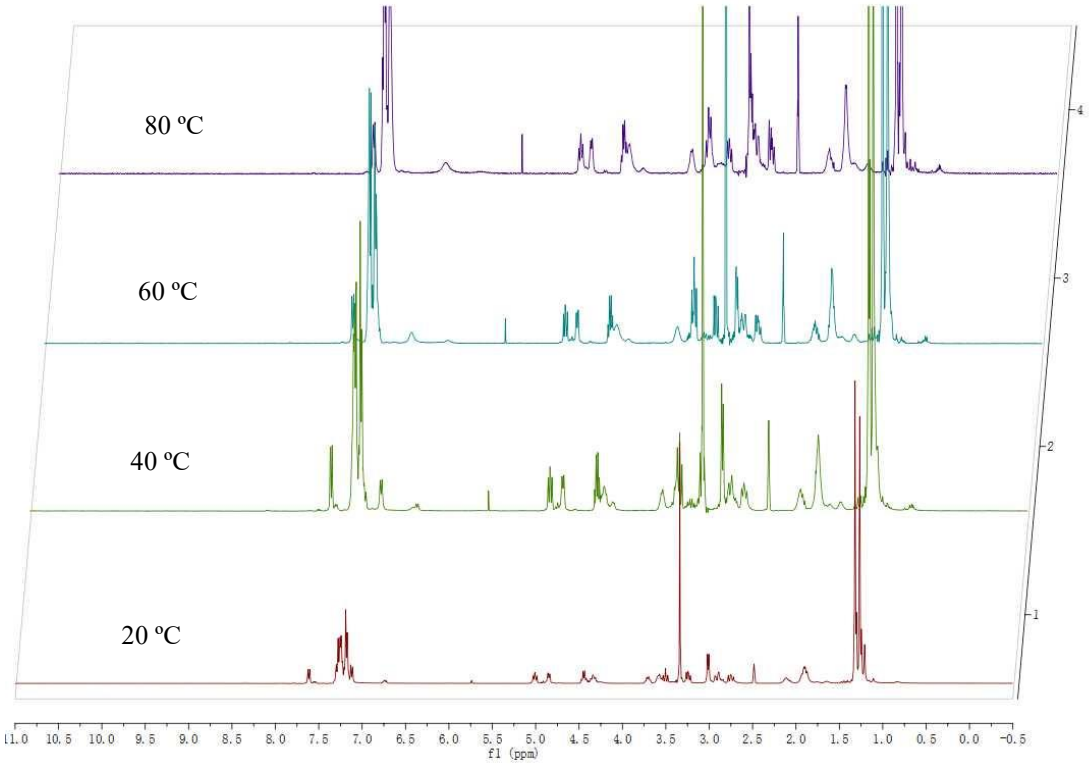
^{1}H NMR (399 MHz, DMSO-d_6) (**Measured at 60 ºC**) δ 7.46 (d, *J* = 7.9 Hz, 1H), 7.35 – 7.10 (m, 5H), 7.35 – 7.10 (m, 5H), 6.79 (brs, 1H), 5.06 – 4.96 (m, 1H), 4.93 – 4.82 (m, 1H), 4.55 – 4.46 (m, 1H), 4.45 – 4.26 (m, 1H), 3.80 – 3.34 (m, 1H), 3.80 – 3.34 (m, 1H), 3.80 – 3.34 (m, 1H), 3.28 (dd, *J* = 11.2, 7.5 Hz, 1H), 3.09 – 2.86 (m, 2H), 3.09 – 2.86 (m, 1H), 2.83 – 2.75 (m, 1H), 2.22 – 1.62 (m, 2H), 2.22 – 1.62 (m, 2H), 1.36 (s, 9H), 1.30 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) (**Major rotamer**) δ 176.3, 171.2, 170.5, 170.4, 155.8, 138.4, 137.1, 129.8, 129.7, 128.6, 128.5, 127.0, 126.7, 81.6, 78.7, 78.5, 59.1, 54.5, 54.0, 47.0, 37.2, 36.7, 35.4, 30.4, 28.6, 28.0, 24.9.

HRMS (ESI) m/z: Calcd for $C_{35}H_{46}N_4NaO_6S^+$ $[M+Na]^+$: 673.3030; found: 673.3035.

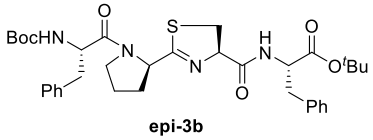
FT-IR (neat): 3373, 2978, 1715, 1654, 1509, 1429, 1366, 1249, 1160, 1044, 702 cm^{-1}

a_D^{27}-16.05 (MeOH 0.57)



Variable temperature ^{1}H NMR (DMSO-d_6) to prove the rotamers of 3b

<u>*tert*-Butyl ((*R*)-2-((*R*)-1-((*tert*-butoxycarbonyl)-L-phenylalanyl)pyrrolidin-2-yl)-4,5-dihydrothiazole-4-carbonyl)-L-phenylalaninate</u>



epi-3b

epi-3b existed as a mixture of rotamers and as a white foam.

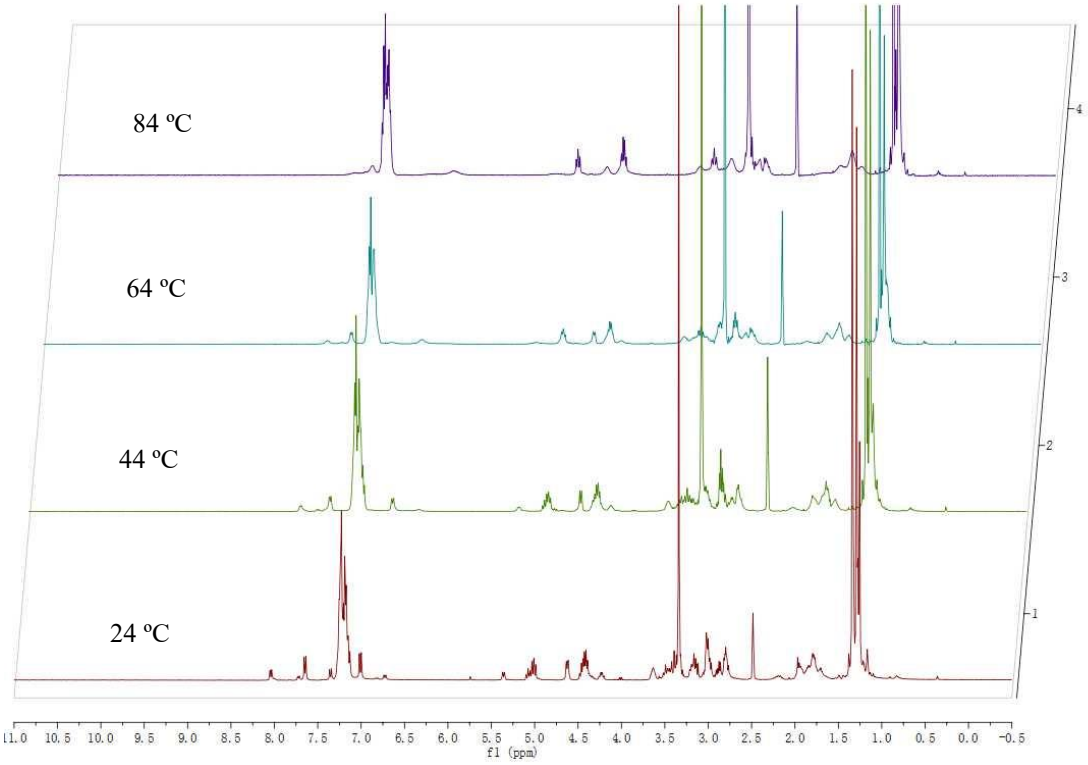
^{1}H NMR (399 MHz, DMSO-d_6) (**Measured at 84 ºC**) δ 7.40 (brs, 1H), 7.33 – 7.12 (m, 5H), 7.33 – 7.12 (m, 5H), 6.45 (brs, 1H), 5.02 (t, *J* = 8.5 Hz, 1H), 4.81 – 4.20 (m, 1H), 4.81 – 4.20 (m, 1H), 4.81 – 4.20 (m, 1H), 3.76 – 3.15 (m, 2H), 3.76 – 3.15(m, 1H), 3.76 – 3.15 (m, 1H), 3.04 – 2.76 (m, 1H), 3.04 – 2.76 (m, 1H), 3.04 – 2.76 (m, 1H), 3.04 – 2.76 (m, 1H), 2.15 – 1.62 (m, 2H), 2.15 – 1.62 (m, 2H), 1.39 (s, 9H), 1.33 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) (**Major rotamer**) δ 177.0, 171.1, 170.9, 170.7, 155.4, 138.5, 138.0, 137.3, 129.8, 129.7, 129.7, 129.6, 128.5, 127.0, 126.8, 81.7, 78.5, 59.2, 54.2, 53.9, 47.0, 37.6, 37.2, 35.3, 33.0, 30.9, 28.6, 28.0, 24.6.

HRMS (ESI) m/z: Calcd for $C_{35}H_{46}N_4NaO_6S^+$ $[M+Na]^+$: 673.3030; found: 673.3033.

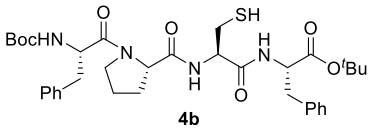
FT-IR (neat): 3360, 2978, 1730, 1709, 1674, 1642, 1514, 1445, 1367, 1246, 1161, 1044, 701 cm^{-1}

a_D^{27}+48.11 (MeOH 0.60)



Variable temperature ^{1}H NMR (DMSO-d_6) to prove the rotamers of epi-3b

<u>*tert*-Butyl (*tert*-butoxycarbonyl)-L-phenylalanyl-L-prolyl-L-cysteinyl-L-phenylalaninate</u>



4b

4b existed as a mixture of rotamers and as a white foam.

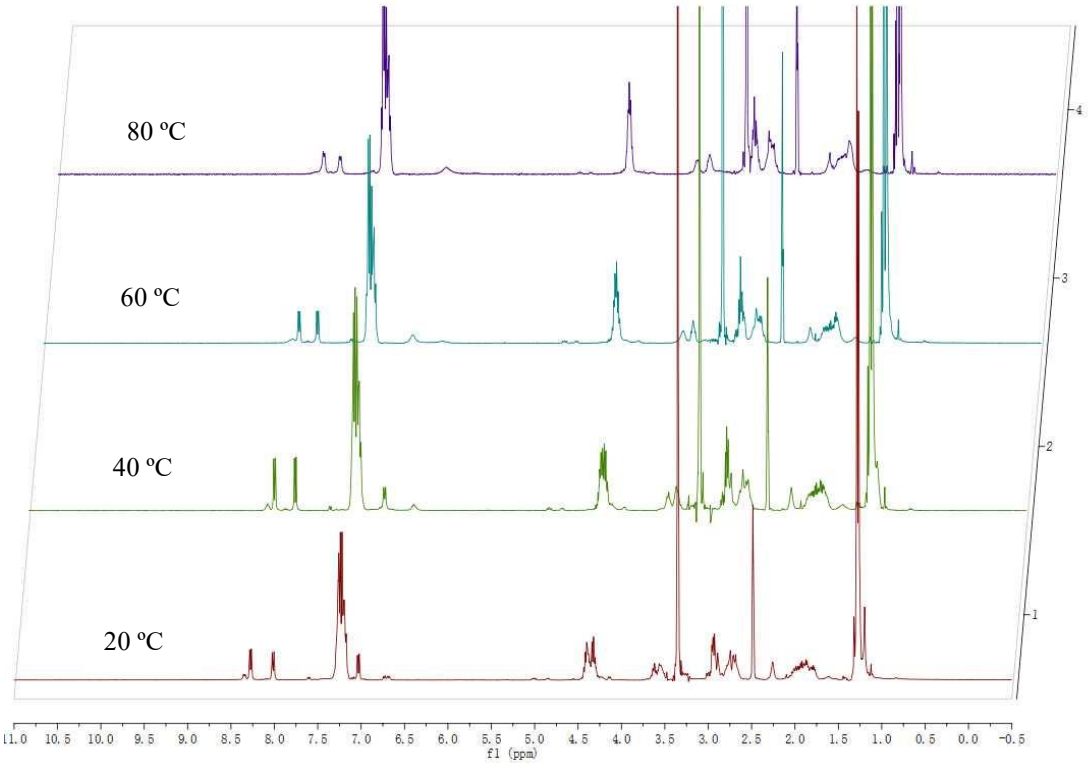
^{1}H NMR (399 MHz, DMSO-d_6) (**Major rotamer**) δ 8.29 (d, *J* = 7.3 Hz, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.39 – 7.13 (m, 5H), 7.39 – 7.13 (m, 5H), 7.05 (d, *J* = 8.3 Hz, 1H), 4.49 – 4.13 (m, 1H), 4.49 – 4.13 (m, 1H), 4.49 – 4.13 (m, 1H), 4.49 – 4.13 (m, 1H), 3.74 – 3.47 (m, 2H), 3.05 – 2.85 (m, 1H), 3.05 – 2.85 (m, 1H), 3.05 – 2.85 (m, 1H), 2.84 – 2.59 (m, 1H), 2.84 – 2.59 (m, 1H), 2.84 – 2.59 (m, 1H), 2.34 – 2.21 (m, 1H), 2.11 – 1.71 (m, 2H), 2.11 – 1.71 (m, 2H), 1.30 (s, 9H), 1.28 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 171.9, 171.0, 170.7, 170.0, 155.8, 138.5, 137.5, 129.7, 129.6, 128.6, 128.5, 126.9, 126.7, 81.2, 78.4, 59.9, 55.0, 54.7, 54.3, 47.2, 37.1, 36.7, 29.3, 28.6, 27.9, 26.9, 25.1.

HRMS (ESI) m/z: Calcd for $C_{35}H_{48}N_4NaO_7S^+$ $[M+Na]^+$: 691.3136; found: 691.3139.

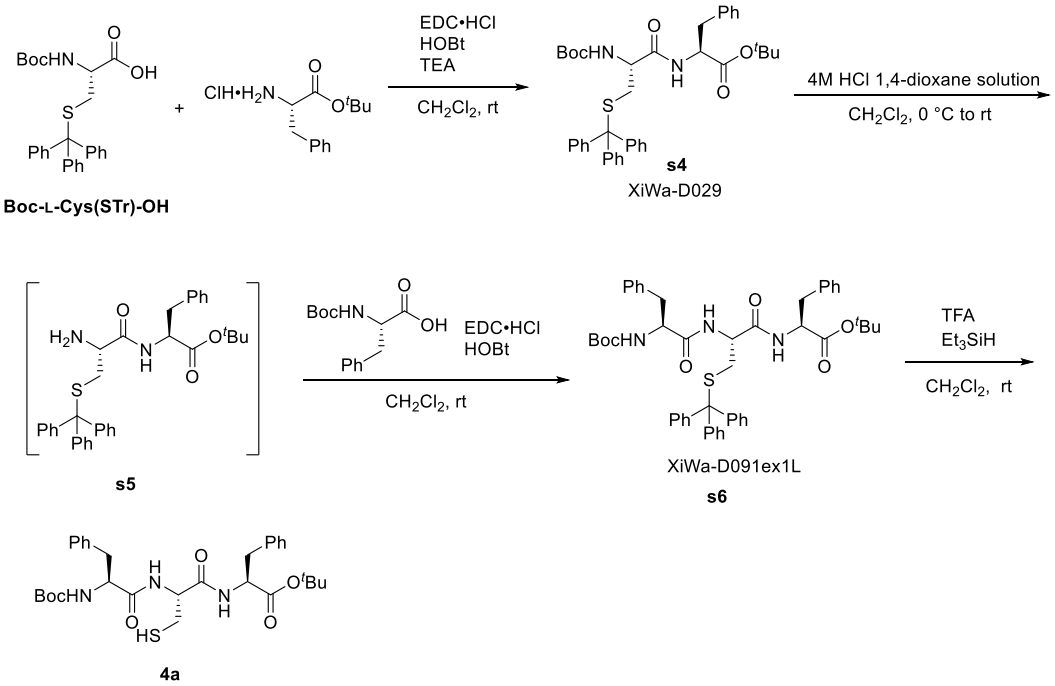
FT-IR (neat): 3299, 2977, 2546, 1709, 1635, 1520, 1454, 1367, 1249, 1162, 739, 701 cm^{-1}

a_D^{27}-25.32 ($CHCl_3$ 0.50)



Variable temperature ^{1}H NMR (DMSO-d_6) to prove the rotamers of 4b

6. Synthesis of authentic sample 4a and epi-4a



To a mixture of L-Boc-Cys(STr)-OH (1.29 g, 2.78 mmol) and L-Phe-O^{t}Bu hydrochloride (716 mg, 2.78 mmol) in CH_2Cl_2 (22 mL, 0.13 M) was added triethylamine (0.97 mL, 6.95 mmol), EDC•HCl (639 mg, 3.33 mmol) and HOBt•H_2O (42.7 mg, 0.278 mmol). The reaction was stirred under room temperature for 2 h. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was

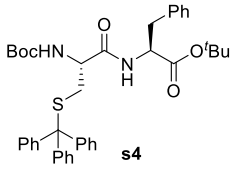
collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 70%/30%) and gave **s4** in 70% yield.

4M HCl 1,4-dioxane solution (3.5 mL) was cooled to 0 ºC and added to the mixture of **s4** (2.30 g, 3.45 mmol) in CH_2Cl_2 (3.5 mL) at 0 ºC. After stirring for 1.5 h at room temperature, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with EtOAc. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 40%/60%) and gave **s5** in 50% yield. **s5** was used directly without characterization of its structure.

To a mixture of L-Boc-Phe-OH (50.0 mg, 0.19 mmol) and **s5** (108 mg, 0.19 mmol) in CH_2Cl_2 (1.9 mL, 0.1 M) was added EDC•HCl (43.0 mg, 0.23 mmol) and HOBt•H_2O (2.8 mg, 0.019 mmol). The reaction was stirred under room temperature for overnight. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 60%/40%) and gave **s6** in 90% yield. Note: **epi-s6** was obtained in 98% yield.

s6 (87.0 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (1.1 mL, 0.1 M) at room temperature, followed by the addition of Et_3SiH (19 μL, 0.12 mmol) and trifluoroacetic acid (44 μL, 4 v/v%). After stirring for 55 min, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 50%/50%) and gave **4a** in 75% yield. Note: **epi-4a** was obtained in 77% yield as a white solid (Hexane/EtOAc = 50%/50%)

***tert*-Butyl *N*-(*tert*-butoxycarbonyl)-S-trityl-L-cysteinyl-L-phenylalaninate**



s4

white foam

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.48 – 7.10 (m, 15H), 7.48 – 7.10 (m, 5H), 6.57 (d, *J* = 6.1 Hz, 1H), 4.82 – 4.54 (m, 1H), 4.82 – 4.54 (m, 1H), 3.98 – 3.81 (m, 1H), 3.08 (dd, *J* = 14.2, 6.1 Hz, 1H), 3.04 (dd, *J* = 14.5, 5.9 Hz, 1H), 2.82 – 2.62 (m, 1H), 2.53 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.41 (s, 9H), 1.36 (s, 9H).

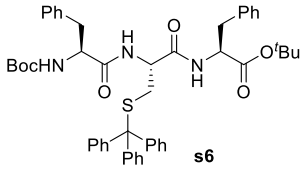
^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 155.1, 144.3, 136.0, 129.6, 129.5, 128.2, 128.0, 126.9, 126.8, 82.3, 80.2, 67.2, 53.7, 53.5, 37.9, 33.7, 28.2, 27.9.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 689.3020; found: 689.3018.

FT-IR (neat): 3319, 3060, 2978, 2932, 1726, 1670, 1495, 1445, 1367, 1252, 1158, 846, 756, 701 cm^{-1}

a_D^{25}+33.15 ($CHCl_3$ 1.90)

***tert*-Butyl N-((*tert*-butoxycarbonyl)-L-phenylalanyl)-S-trityl-L-cysteinyl-L-phenylalaninate**



s6

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.40 – 7.11 (m, 5H), 7.40 – 7.11 (m, 5H), 7.40 – 7.11 (m, 15H), 6.57 (brs, 1H), 6.28 (d, *J* = 7.4 Hz, 1H), 4.88 (d, *J* = 5.3 Hz, 1H), 4.66 – 4.57 (m, 1H), 4.35 – 4.23 (m, 1H), 4.09 – 3.94 (m, 1H), 3.14 – 2.89 (m, 1H), 3.14 – 2.89 (m, 1H), 3.14 – 2.89 (m, 1H), 3.14 – 2.89 (m, 1H), 2.72 (dd, *J* = 12.9, 6.6 Hz, 1H), 2.43 (dd, *J* = 12.9, 5.7 Hz, 1H), 1.37 (s, 9H), 1.36 (s, 9H).

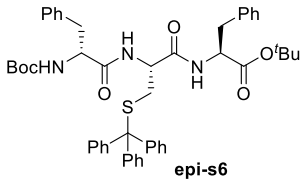
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 169.8, 168.9, 155.2, 144.3, 136.2, 136.1, 129.6, 129.5, 129.4, 129.2, 128.7, 128.3, 128.0, 127.7, 127.7, 127.0, 126.8, 82.2, 80.3, 67.1, 55.4, 53.9, 52.1, 37.9 (2C, deduced by HSQC), 33.3, 28.2, 27.8.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 836.3704; found: 836.3708

FT-IR (neat): 3299, 2978, 1650, 1522, 1496, 1444, 1367, 1250, 1159, 753, 700 cm^{-1}

a_D^{27}+5.48 ($CHCl_3$ 1.00)

***tert*-Butyl N-((*tert*-butoxycarbonyl)-D-phenylalanyl)-S-trityl-L-cysteinyl-L-phenylalaninate**



epi-s6

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.45 – 7.07 (m, 5H), 7.45 – 7.07 (m, 5H), 7.45 – 7.07 (m, 15H), 6.47 (d, *J* = 6.2 Hz, 1H), 6.13 (d, *J* = 4.7 Hz, 1H), 4.98 (d, *J* = 6.0 Hz, 1H), 4.66 – 4.55 (m, 1H), 4.32 – 4.13 (m, 1H), 3.92 – 3.75 (m, 1H), 3.15 – 2.84 (m, 1H), 3.15 – 2.84 (m, 1H), 3.15 – 2.84 (m, 1H), 3.15 – 2.84 (m, 1H), 2.54 (dd, *J* = 12.6, 7.0 Hz, 1H), 2.44 (dd, *J* = 13.0, 6.2 Hz, 1H), 1.37 (s, 9H), 1.36 (s, 9H).

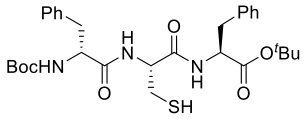
^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 169.8, 169.0, 155.1, 144.2, 136.4, 136.1, 129.6, 129.5, 129.4, 129.2, 128.6, 128.2, 128.0, 127.7, 127.6, 126.9, 126.8, 82.1, 80.1, 67.1, 55.7, 53.8, 52.1, 38.4, 37.8, 33.0, 28.2, 27.8.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 836.3704; found: 836.3701.

FT-IR (neat): 3297, 2978, 1650, 1519, 1496, 1444, 1394, 1367, 1250, 1159, 751, 700 cm^{-1}

a_D^{25}+10.92 ($CHCl_3$ 1.00)

***tert*-Butyl (*tert*-butoxycarbonyl)-D-phenylalanyl-L-cysteinyl-L-phenylalaninate**



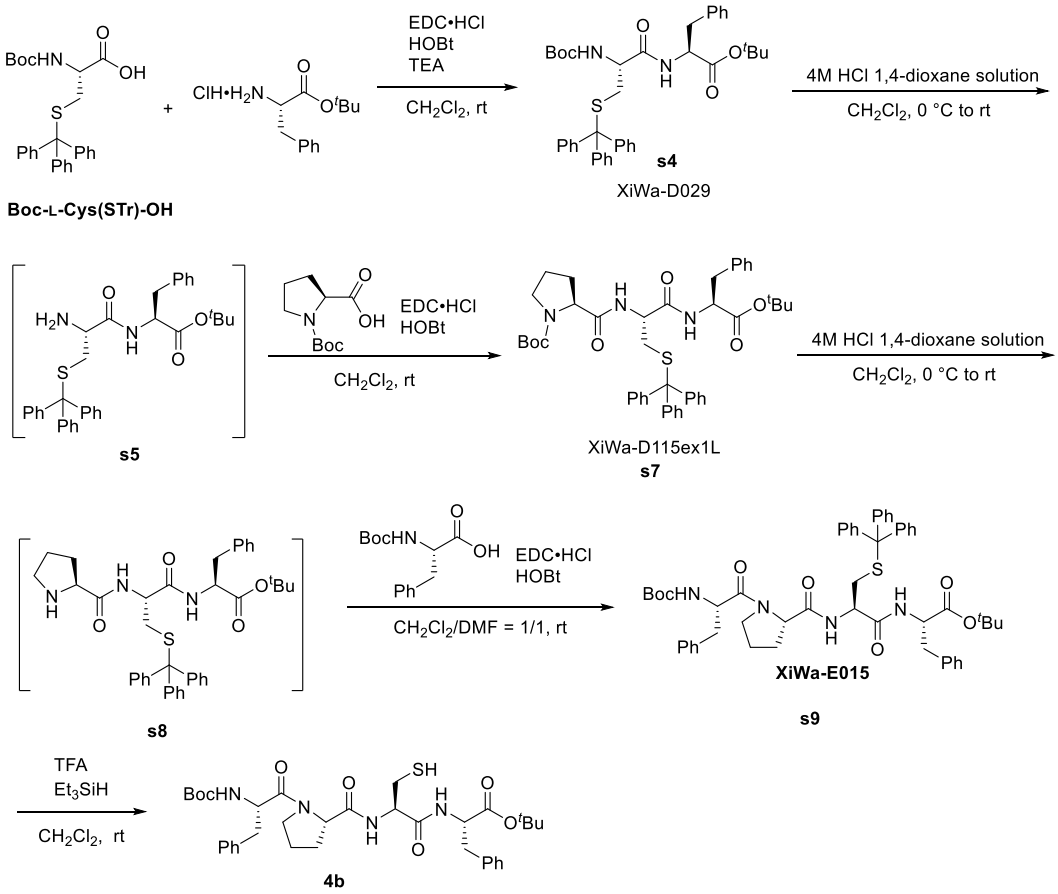
epi-4a

^{1}H NMR (399 MHz, $CDCl_3$) δ 7.36 – 7.09 (m, 5H), 7.36 – 7.09 (m, 5H), 6.86 (d, *J* = 6.4 Hz, 1H), 6.51 (d, *J* = 7.1 Hz, 1H), 5.13 (brs, 1H), 4.75 – 4.62 (m, 1H), 4.61 – 4.47 (m, 1H), 4.34 – 4.17 (m, 1H), 3.22 – 2.84 (m, 1H), 3.22 – 2.84 (m, 1H), 3.22 – 2.84 (m, 1H), 3.22 – 2.84 (m, 1H), 3.22 – 2.84 (m, 1H), 2.34 – 2.13 (m, 1H), 1.43 (s, 9H), 1.40 (s, 9H), 1.24 – 1.12 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.0, 168.7, 155.4, 136.3, 129.4, 129.3, 129.1, 128.8, 128.4, 127.2, 126.9, 82.2, 80.4, 56.7, 53.9, 53.3, 38.4, 37.7, 28.3, 27.9, 26.3.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 594.2608; found: 594.2606.
FT-IR (neat): 3300, 2977, 2568, 1691, 1644, 1522, 1455, 1367, 1293, 1251, 1164, 739, 700 cm^{-1}
a_D^{25}-2.64 ($CHCl_3$ 0.62)

7. Synthesis of authentic sample 4b and epi-4b



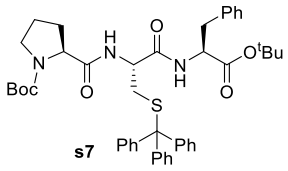
Following the procedure for synthesizing **s4** and **s5**, **s7** was obtained in 95% yield (Hexane/EtOAc = 50%/50%). White precipitate was formed after 2 or 3 min in the case of preparing **s8** and **s8** was used as a crude product after 2 h. Note: **epi-s7** was obtained in 89% yield and in the case of **epi-s8**, no precipitation.

To a mixture of L-Boc-Phe-OH (41.7 mg, 0.16 mmol) and **s8** (104 mg, 0.16 mmol) in CH_2Cl_2/DMF = 1/1 (6.4 mL, 0.025 M) was added EDC•HCl (36.1 mg, 0.19 mmol) and HOBt•H_2O (12.0 mg, 0.079 mmol). The reaction was stirred under room temperature for 5 h. Then 1N HCl aqueous solution was added to the reaction mixture and the organic phase was collected, dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by silica gel column chromatography (Hexane/EtOAc = 50%/50%) and gave **s9** in 45% yield. Note: in the case of preparing **epi-s9**, only CH_2Cl_2 (0.045 M) was used, **epi-s9** was obtained in 51% yield after 7 h (Hexane/EtOAc = 50%/50%).

s9 (50.0 mg, 0.055 mmol) was dissolved in CH_2Cl_2 (2.2 mL, 0.025 M) at room temperature, followed by the addition of Et_3SiH (88 μL, 0.55 mmol) and trifluoroacetic acid (88 μL, 4 v/v%). After stirring for 2.5 min, the reaction was quenched by sat. $NaHCO_3$ aqueous solution, extracted with CH_2Cl_2. The organic

phase was dried over Na_2SO_4 and filtered, evaporated under reduced pressure and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Hexane/EtOAc = 50%/50%) and gave **4b** in 49% yield. Note: **epi-4b** was obtained in 30% yield after 2.5 h.

***tert*-Butyl (*S*)-2-(((*R*)-1-(((*S*)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate**



s7

s7 existed as a mixture of rotamers and as a white foam.

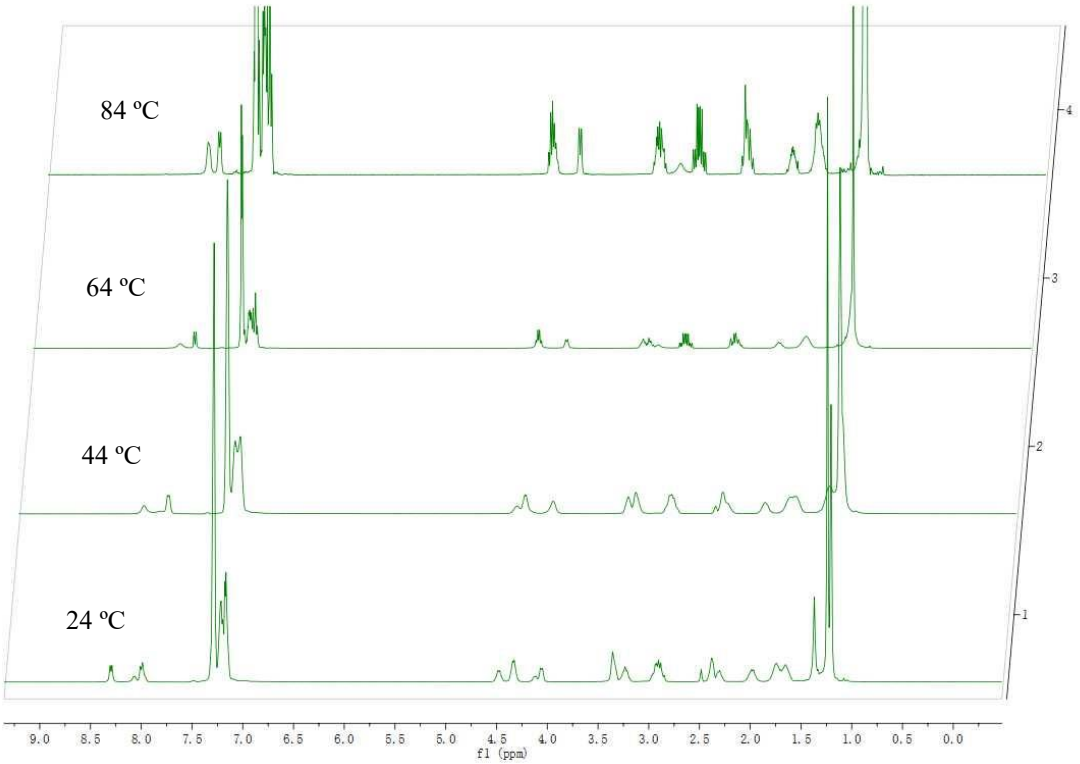
^{1}H NMR (399 MHz, DMSO-d_6) **(measured at 64 ºC)** δ 7.90 (s, 1H), 7.76 (d, *J* = 8.3 Hz, 1H), 7.43 – 7.01 (m, 15H), 7.43 – 7.01 (m, 5H), 4.47 – 4.25 (m, 1H), 4.47 – 4.25 (m, 1H), 4.16 – 4.02 (m, 1H), 3.41 – 3.32 (m, 1H), 3.31 – 3.22 (m, 1H), 2.96 (dd, *J* = 14.0, 6.5 Hz, 1H), 2.89 (dd, *J* = 14.0, 7.6 Hz, 1H), 2.48 – 2.34 (m, 2H), 2.08 – 1.92 (m, 1H), 1.86 – 1.59 (m, 1H), 1.86 – 1.59 (m, 2H), 1.27 (s, 9H), 1.27 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5 and 172.2 (1C), 170.4 and 170.3 (1C), 169.9 and 169.8 (1C), 154.4 and 153.7 (1C), 144.7, 137.4, 137.3, 129.5, 128.5, 128.4, 127.1, 126.9, 81.1, 79.2 and 78.8 (1C), 66.1, 59.8 and 59.6 (1C), 54.6 and 54.5 (1C), 52.0 and 51.7 (1C), 47.2 and 46.9 (1C), 37.4 and 37.1 (1C), 34.2 and 34.0 (1C), 31.4 and 30.1 (1C), 28.6 and 28.4 (3C), 27.9, 24.3 and 23.4 (1C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 786.3547; found: 786.3546.

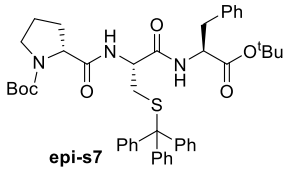
FT-IR (neat): 3310, 2978, 1734, 1684, 1674, 1670, 1540, 1508, 1395, 1366, 1158, 742, 700 cm^{-1}

a_D^{26}-19.37 ($CHCl_3$ 0.55)



Variable temperature ^{1}H NMR (DMSO-d_6) to prove the rotamers of s7

***tert*-Butyl (*R*)-2-(((*R*)-1-(((*S*)-1-(*tert*-butoxy)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate**



epi-s7

epi-s7 existed as a mixture of rotamers and as a white foam.

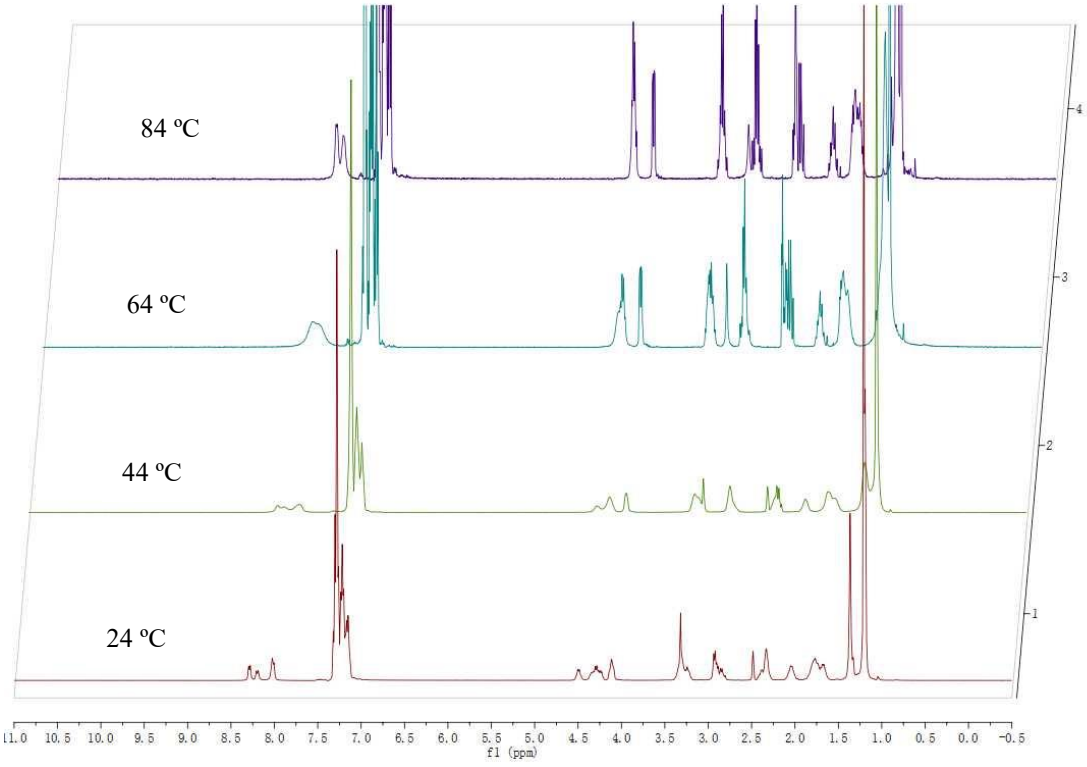
^{1}H NMR (399 MHz, DMSO-d_6) **(Measured at 84 ºC)** δ 7.79 (d, *J* = 5.7 Hz, 1H), 7.71 (brs, 1H), 7.37 – 7.10 (m, 5H), 7.37 – 7.10 (m, 15H), 4.43 – 4.29 (m, 1H), 4.43 – 4.29 (m, 1H), 4.14 (dd, *J* = 8.4, 3.6 Hz, 1H), 3.42 – 3.26 (m, 2H), 2.97 (dd, *J* = 14.0, 6.8 Hz, 1H), 2.92 (dd, *J* = 14.0, 7.4 Hz, 1H), 2.55 – 2.47 (m, 1H), 2.43 (dd, *J* = 11.9, 8.3 Hz, 1H), 2.14 – 2.00 (m, 1H), 1.90 – 1.68 (m, 1H), 1.90 – 1.68 (m, 2H), 1.33 (s, 9H), 1.28 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.5 and 172.4 (1C), 170.3 and 170.3 (1C), 170.0, 154.2 and 153.8 (1C), 144.7, 137.5, 137.2, 129.5, 128.6, 128.5, 127.2, 126.9, 126.9, 81.1 and 80.9 (1C), 79.3 and 78.8 (1C), 66.2 and 66.0 (1C), 60.0 and 59.8 (1C), 55.0 and 54.7 (1C), 51.5 and 51.4 (1C), 47.2 and 46.9 (1C), 37.3 and 37.1 (1C), 35.0 and 34.3 (1C), 31.5 and 30.3 (1C), 28.6 and 28.3 (3C), 27.9, 24.4 and 23.5 (1C).

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: 786.3547; found: 786.3555.

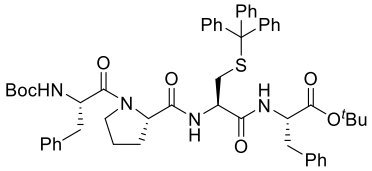
FT-IR (neat): 3300, 2976, 1732, 1669, 1496, 1393, 1367, 1158, 742, 700 cm^{-1}

a_D^{27}+55.69 ($CHCl_3$ 0.64)



Variable temperature ^{1}H NMR (DMSO-d_6) to prove the rotamers of epi-s7

***tert*-Butyl N-(*tert*-butoxycarbonyl)-L-phenylalanyl-L-prolyl-S-trityl-L-cysteinyl-L-phenylalaninate**



s9

s9 existed as a mixture of rotamers and as a white foam.

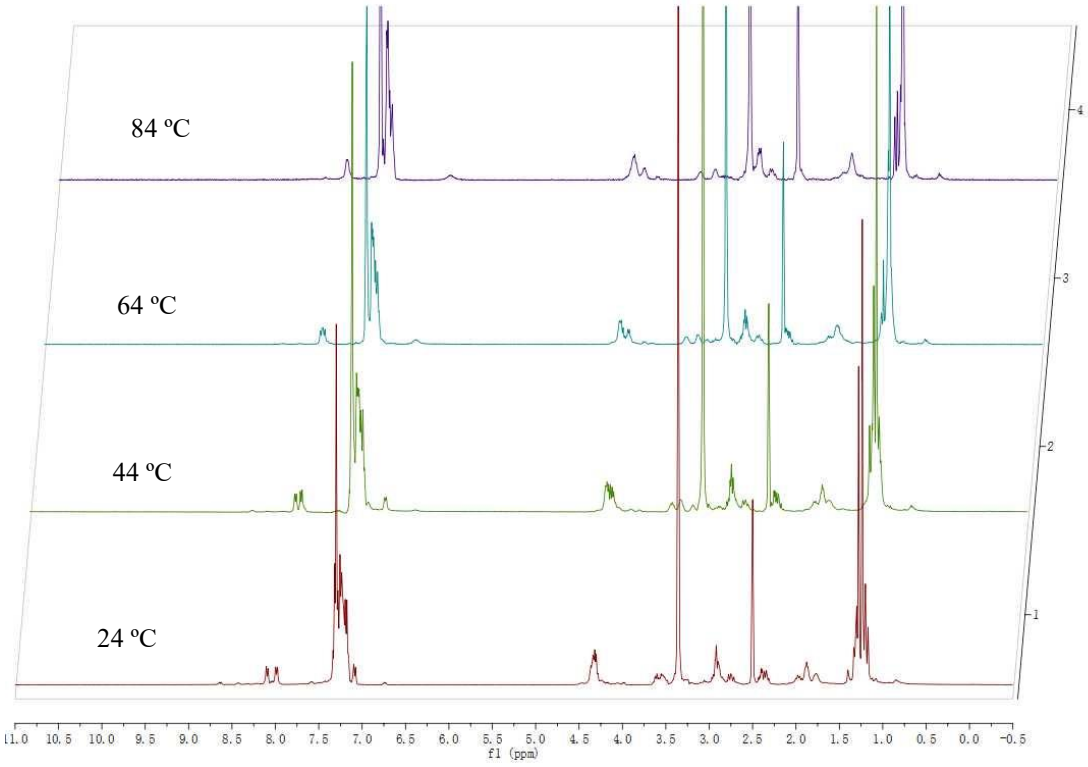
^{1}H NMR (399 MHz, DMSO-d_6) δ 8.09 (d, *J* = 7.5 Hz, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.44 – 7.13 (m, 5H), 7.44 – 7.13 (m, 5H), 7.44 – 7.13 (m, 15H), 7.09 (d, *J* = 8.2 Hz, 1H), 4.48 – 4.11 (m, 1H), 4.48 – 4.11 (m, 1H), 4.48 – 4.11 (m, 1H), 4.48 – 4.11 (m, 1H), 3.68 – 3.46 (m, 2H), 3.00 – 2.67 (m, 1H), 3.00 – 2.67 (m, 1H), 3.00 – 2.67 (m, 1H), 3.00 – 2.67 (m, 1H), 2.41 (dd, *J* = 11.7, 5.6 Hz, 1H), 2.38 – 2.28 (m, 1H), 2.05 – 1.69 (m, 2H), 2.05 – 1.69 (m, 2H), 1.28 (s, 9H), 1.23 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 171.2, 170.3, 169.8, 155.8, 144.7, 138.5, 137.4, 129.7, 129.6, 129.5, 128.5, 128.5, 127.2, 126.9, 126.6, 81.1, 78.4, 66.2, 59.9, 54.5, 54.3, 52.1, 47.6, 47.2, 37.3, 36.9, 33.9, 29.1, 28.6, 27.9, 24.9.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: $C_{54}H_{62}N_4NaO_7S^+$: 933.4231; found:933.4247.

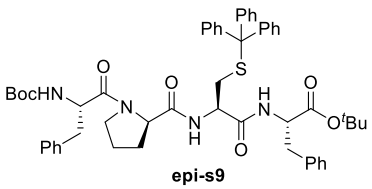
FT-IR (neat): 3300, 2977, 1683, 1515, 1496, 1445, 1367, 1250, 1161, 743, 701 cm^{-1}

a_D^{26}-20.69 ($CHCl_3$ 0.35)



Variable temperature ^{1}H NMR (DMSO-d_6) to prove the rotamers of s9

<u>*tert*-Butyl N-(*tert*-butoxycarbonyl)-L-phenylalanyl-D-prolyl-S-trityl-L-cysteinyl-L-phenylalaninate</u>



epi-s9

epi-s9 existed as a mixture of rotamers and as a white foam.

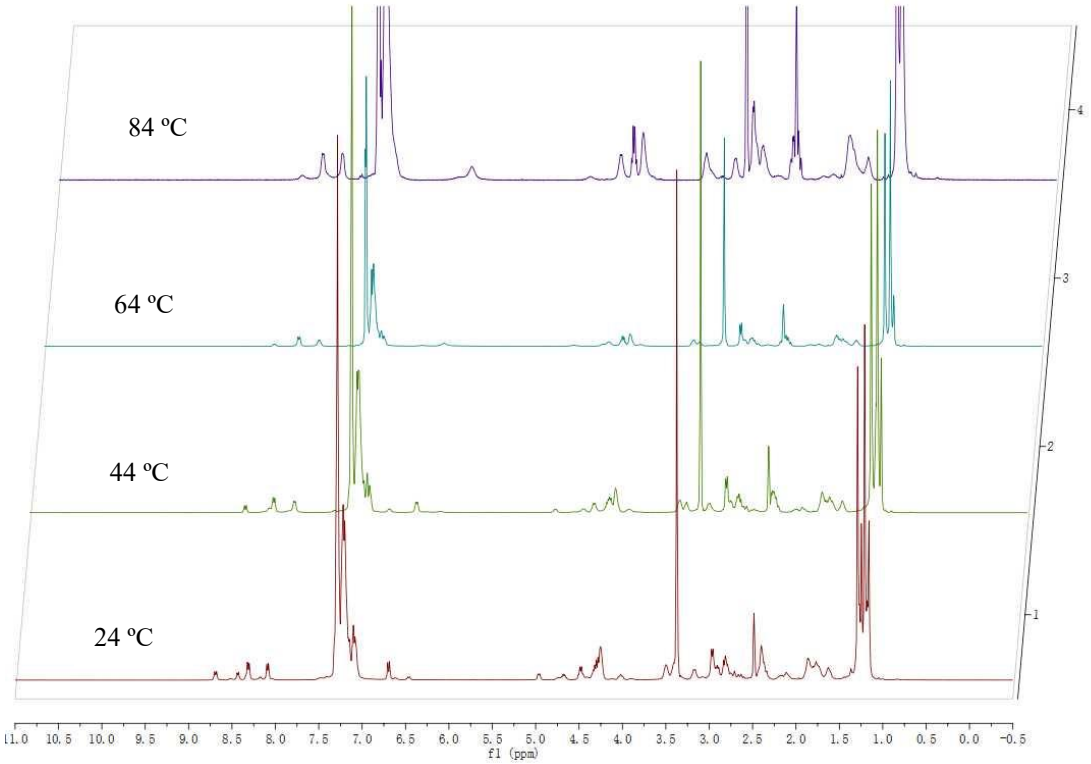
^{1}H NMR (399 MHz, DMSO-d_6) (**Measured at 84 ºC**) δ 7.97 (brs, 1H), 7.74 (brs, 1H), 7.41 – 6.99 (m, 15H), 7.41 – 6.99 (m, 5H), 7.41 – 6.99 (m, 5H), 6.24 (brs, 1H), 4.65 – 4.44 (m, 1H), 4.42 – 4.33 (m, 1H), 4.33 – 4.04 (m, 1H), 4.33 – 4.04 (m, 1H), 3.61 – 3.14 (m, 2H), 3.04 – 2.77 (m, 1H), 3.04 – 2.77 (m, 1H), 3.04 – 2.77 (m, 1H), 3.04 – 2.77 (m, 1H), 2.60 – 2.42 (m, 1H), 2.60 – 2.42 (m, 1H), 2.09 – 1.55 (m, 2H), 2.09 – 1.55 (m, 2H), 1.34 (s, 9H), 1.29 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 172.2, 171.9, 171.6, 170.6, 170.2, 170.2, 169.9, 169.8, 155.9, 155.2, 144.7, 144.6, 138.7, 137.8, 137.4, 137.0, 129.8, 129.8, 129.6, 129.5, 129.5, 128.6, 128.6, 128.5, 128.2, 127.2, 126.9, 126.8, 81.1, 81.0, 78.7, 78.4, 66.2, 65.9, 60.1, 54.8, 54.8, 54.3, 53.8, 52.0, 51.4, 47.2, 38.3, 37.5, 37.4, 34.2, 32.9, 29.4, 29.4, 28.6, 27.9, 27.8, 24.8, 22.3.

HRMS (ESI) m/z: Calcd for $[M+Na]^+$: $C_{54}H_{62}N_4NaO_7S^+$: 933.4231; found: 933.4247.

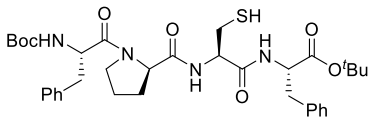
FT-IR (neat): 3315, 2977, 1683, 1674, 1635, 1508, 1497, 1446, 1367, 1246, 1160, 743, 700 cm^{-1}

a_D^{25}+12.99 ($CHCl_3$ 0.50)



Variable temperature ^{1}H NMR (DMSO-d_6) to prove the rotamers of epi-s9

***tert*-Butyl (*tert*-butoxycarbonyl)-L-phenylalanyl-D-prolyl-L-cysteinyl-L-phenylalaninate**



epi-4b

epi-4b existed as mixture of rotamers and as a white foam.

^{1}H NMR (399 MHz, DMSO-d_6) (**Measured at 84 ºC**) δ 7.92 (brs, 1H), 7.68 (brs, 1H), 7.39 – 7.04 (m, 5H), 7.39 – 7.04 (m, 5H), 6.39 (brs, 1H), 4.74 – 4.13 (m, 1H), 4.74 – 4.13 (m, 1H), 4.74 – 4.13 (m, 1H), 4.74 – 4.13 (m, 1H), 3.64 – 3.17 (m, 2H), 3.06 – 2.64 (m, 1H), 3.06 – 2.64 (m, 1H), 3.06 – 2.64 (m, 1H),

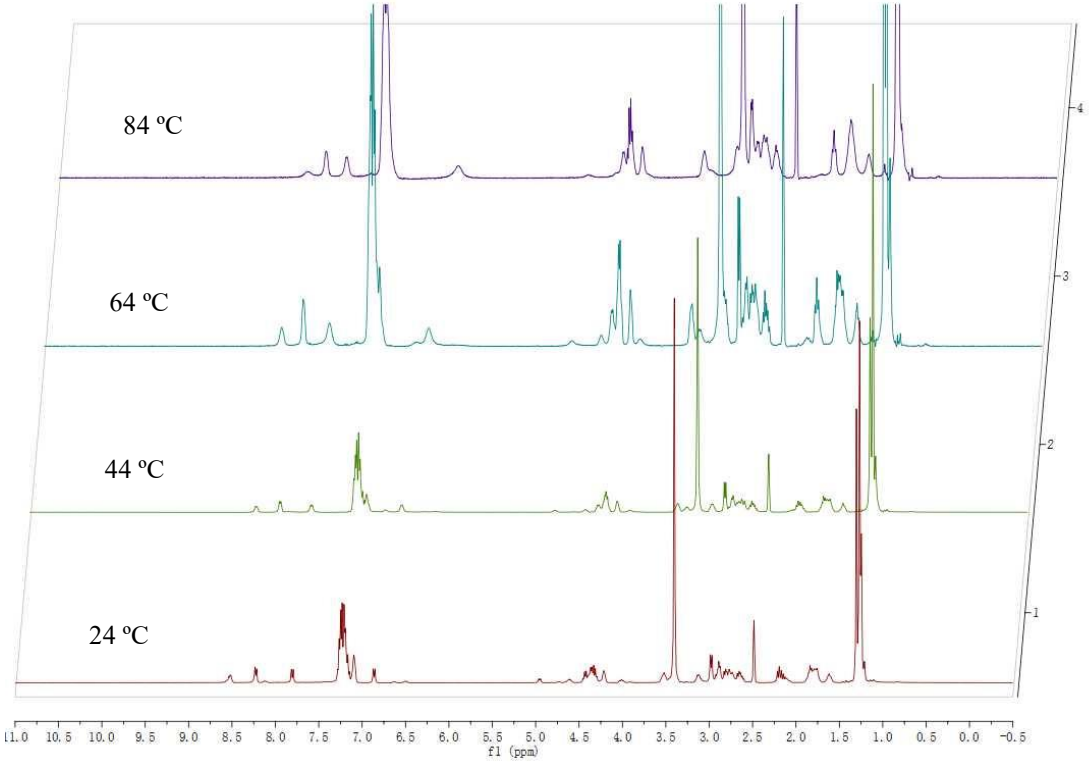
3.06 – 2.64 (m, 1H), 3.06 – 2.64 (m, 1H), 3.06 – 2.64 (m, 1H), 2.07 (t, *J* = 7.7 Hz, 1H), 1.97 – 1.58 (m, 2H), 1.97 – 1.58 (m, 2H), 1.35 (s, 9H), 1.33 (s, 9H).

^{13}C NMR (100 MHz, DMSO-d_6) (**Major rotamer**) δ 171.9, 170.9, 170.6, 170.0, 155.4, 138.7, 137.7, 137.5, 137.1, 129.8, 129.6, 129.5, 128.6, 128.5, 128.2, 126.9, 126.8, 126.4, 81.2, 78.7, 60.5, 55.0, 54.8, 54.0, 47.2, 38.0, 37.3, 29.5, 28.6, 27.9, 26.7, 24.7.

HRMS (ESI) m/z: Calcd for $C_{35}H_{48}N_4NaO_7S^+$ $[M+Na]^+$: 691.3136; found: 691.3130.

FT-IR (neat): 3309, 2978, 2550, 1637, 1520, 1511, 1500, 1454, 1367, 1246, 1160, 698 cm^{-1}

a_D^{26}-28.52 ($CHCl_3$ 0.70)



Variable temperature ^{1}H NMR (DMSO-d_6) to prove the rotamers of epi-4b

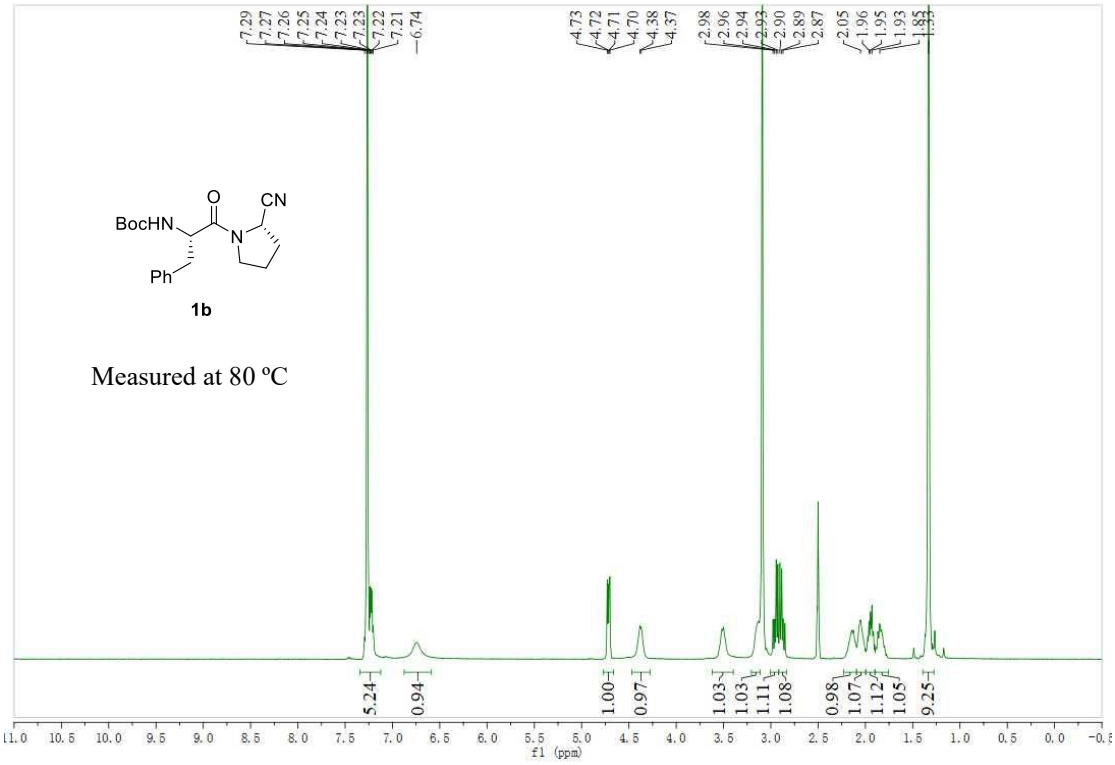
8. Reference

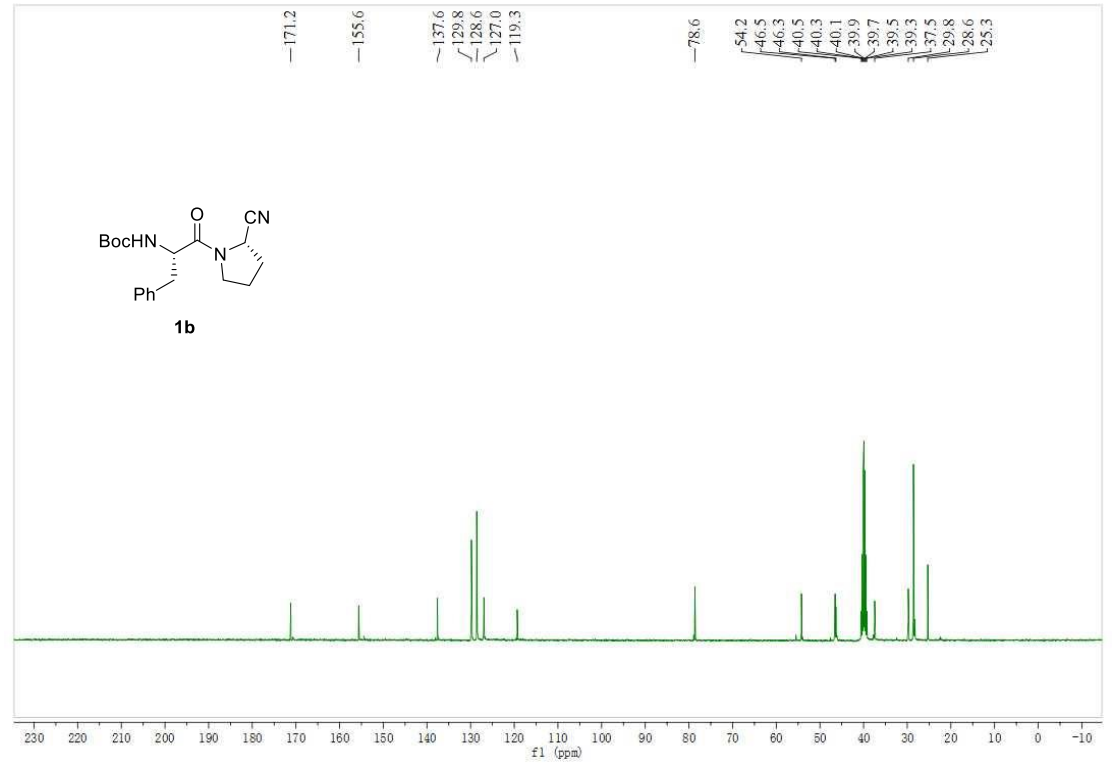
1. Vagkidis, N.; Brown, A. J.; Clarke, P.A. Evaluation of Amino Nitriles and an Amino Imidate as Organocatalysts in Aldol Reactions. *Synthesis* **2019**, *51*, 4106-4112.

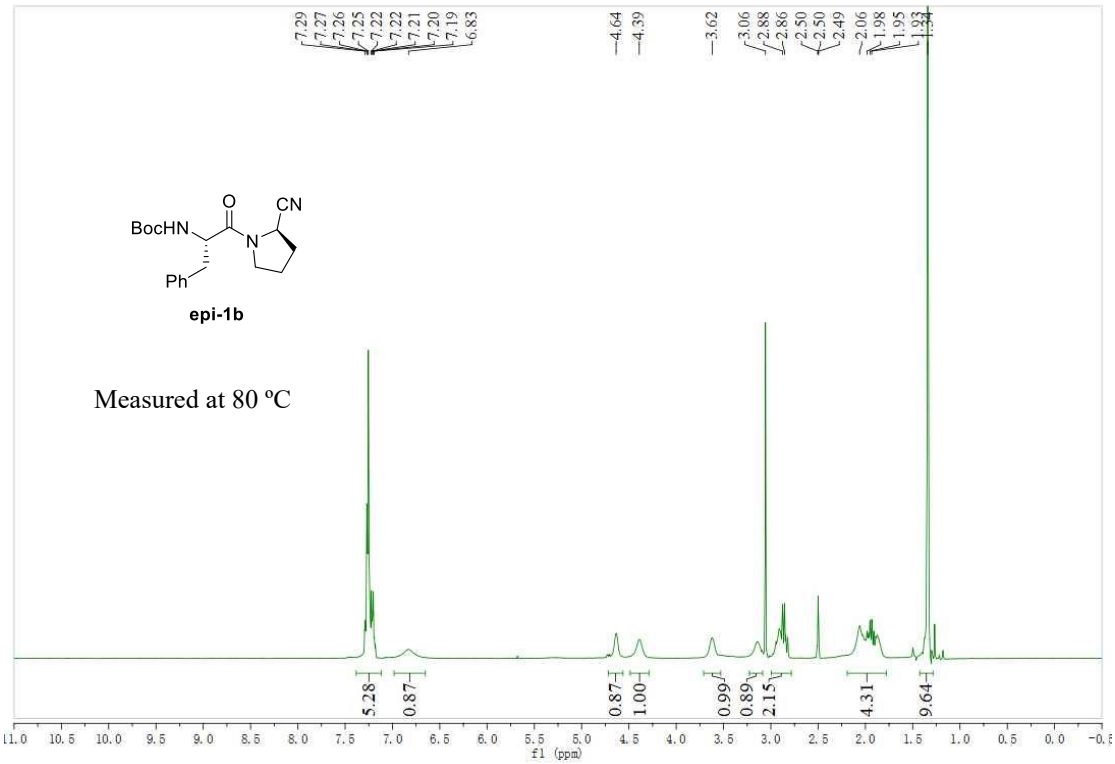
2. Maetz, P.; Rodriguez, M. A simple preparation of *N*-protected chiral α-aminonitriles from *N*-protected α-amino acid amides. *Tetrahedron Lett.* **1997**, *38*, 4221-4222.

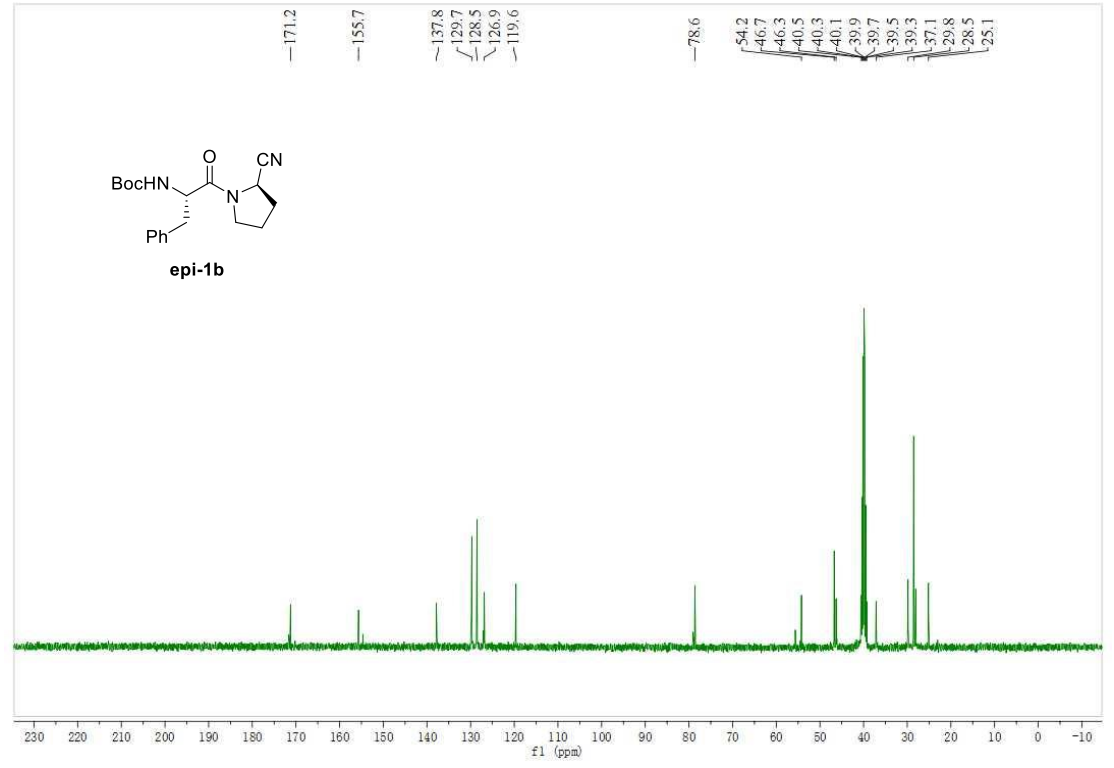
3. Hoang, C. T.; Bouille`re, F.; Johannesen, S.; Zulauf, A.; Panel, C.; Pouilhe`s, A.; Gori, D.; Alezra, V.; Kouklovsky, C. Amino acid homologation by the Blaise reaction: a new entry into nitrogen heterocycles. *J. Org. Chem.* **2009**, *74*, 4177–4187.

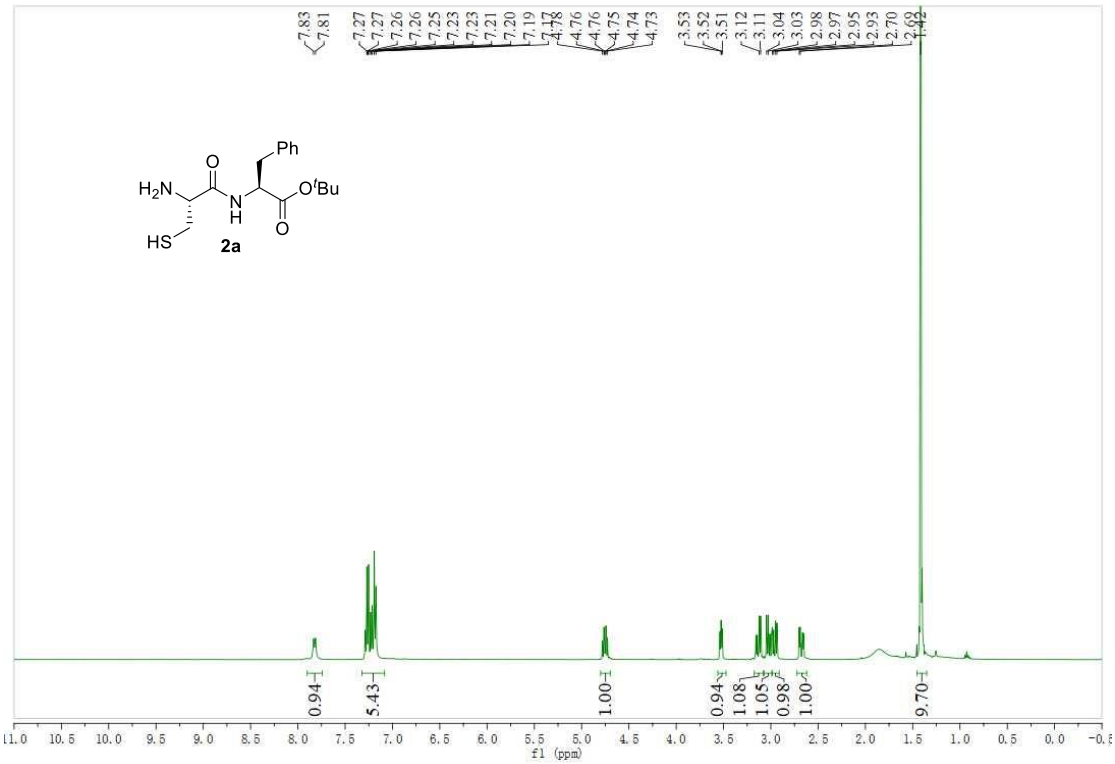
9. Spectra


BocHN
O
CN
N
Ph
1b
Measured at 80 ºC
f1 (ppm)


BocHN
O
CN
N
Ph
1b
f1 (ppm)


epi-1b
Measured at 80 ºC
f1 (ppm)


epi-1b
f1 (ppm)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7.81
7.27
7.27
7.26
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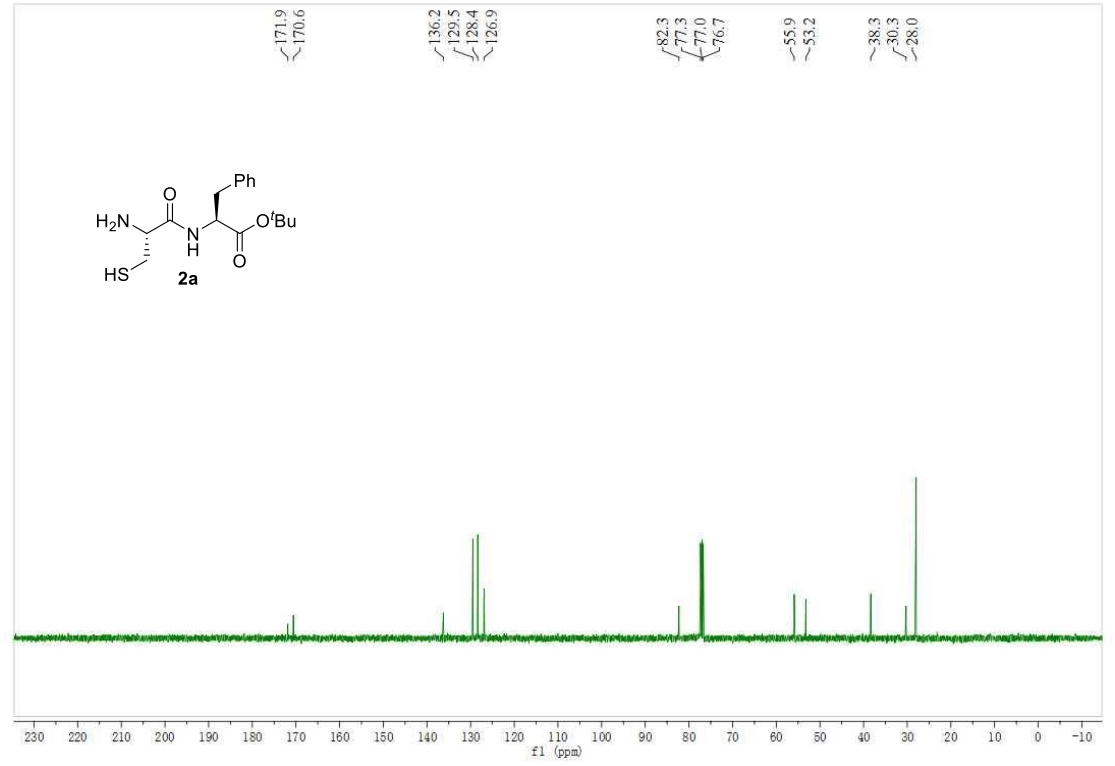
7.25
7.23
7.23
7.21
7.20
7.19
7.17
4.78
4.76
4.76
4.75
4.74
4.73
3.53
3.52
3.51
3.12
3.11
3.04
3.03
2.98
2.97
2.95
2.93
2.70
2.69
1.42
Ph
O
H2N
N
H
O
tBu
O
HS
2a
0.94
5.43
1.00
0.94
1.08
1.05
0.98
1.00
9.70
f1 (ppm)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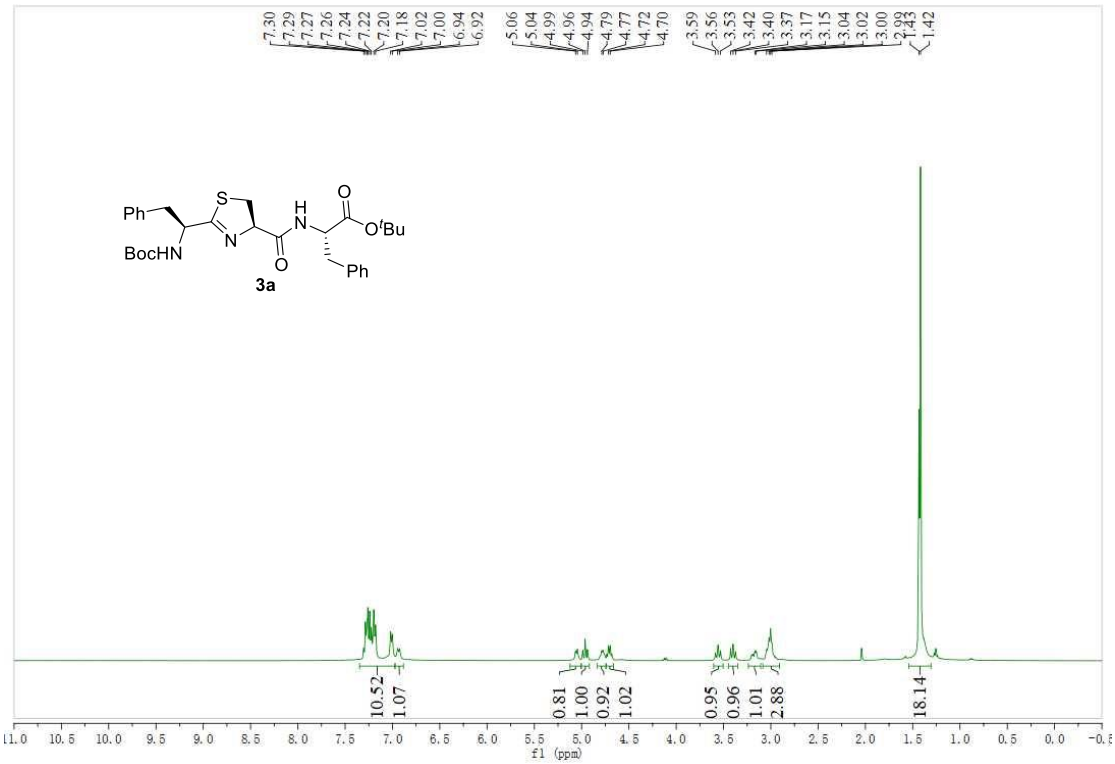
136.2
129.5
128.4
126.9
82.3
77.3
77.0
76.7
55.9
53.2
38.3
30.3
28.0
Ph
O
H2N
N
H
O
tBu
O
HS
2a
f1 (ppm)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7.29
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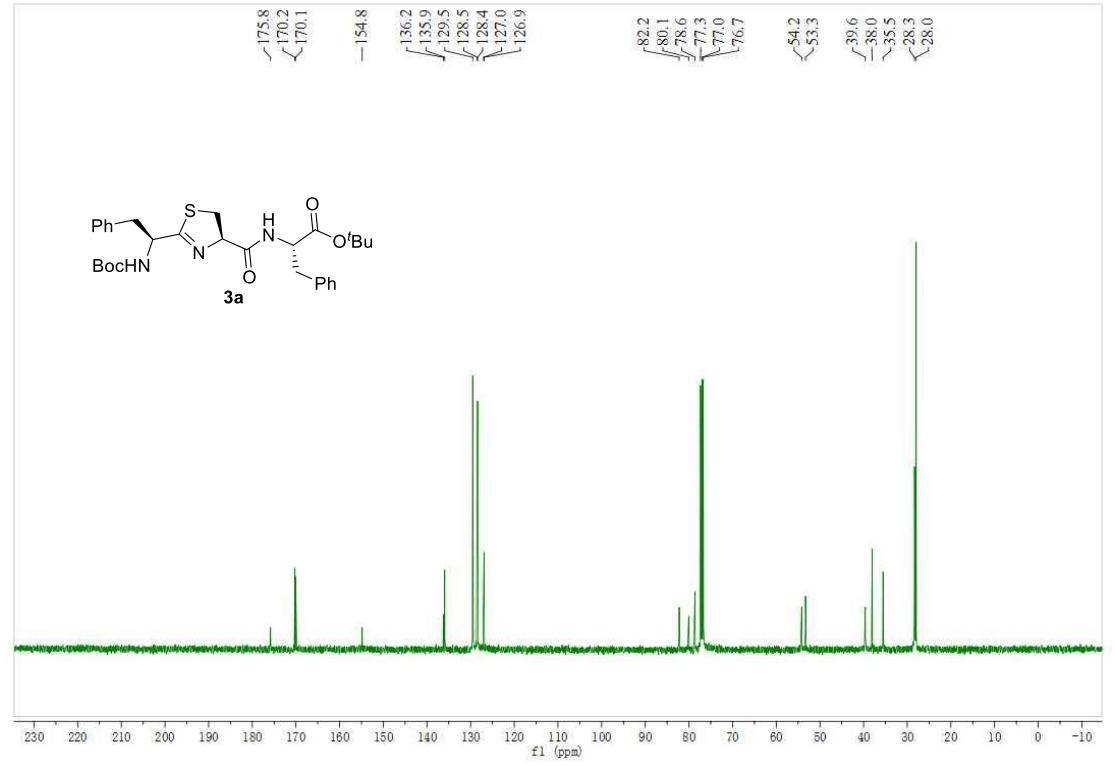
7.22
7.20
7.18
7.02
7.00
6.94
6.92
5.06
5.04
4.99
4.96
4.94
4.79
4.77
4.72
4.70
3.59
3.56
3.53
3.42
3.40
3.37
3.17
3.15
3.04
3.02
3.00
2.98
1.43
1.42
Ph
BocHN
S
N
H
N
O
O
O
O'Bu
Ph
3a
10.52
1.07
0.81
1.00
0.92
1.02
0.95
0.96
1.01
2.88
18.14
f1 (ppm)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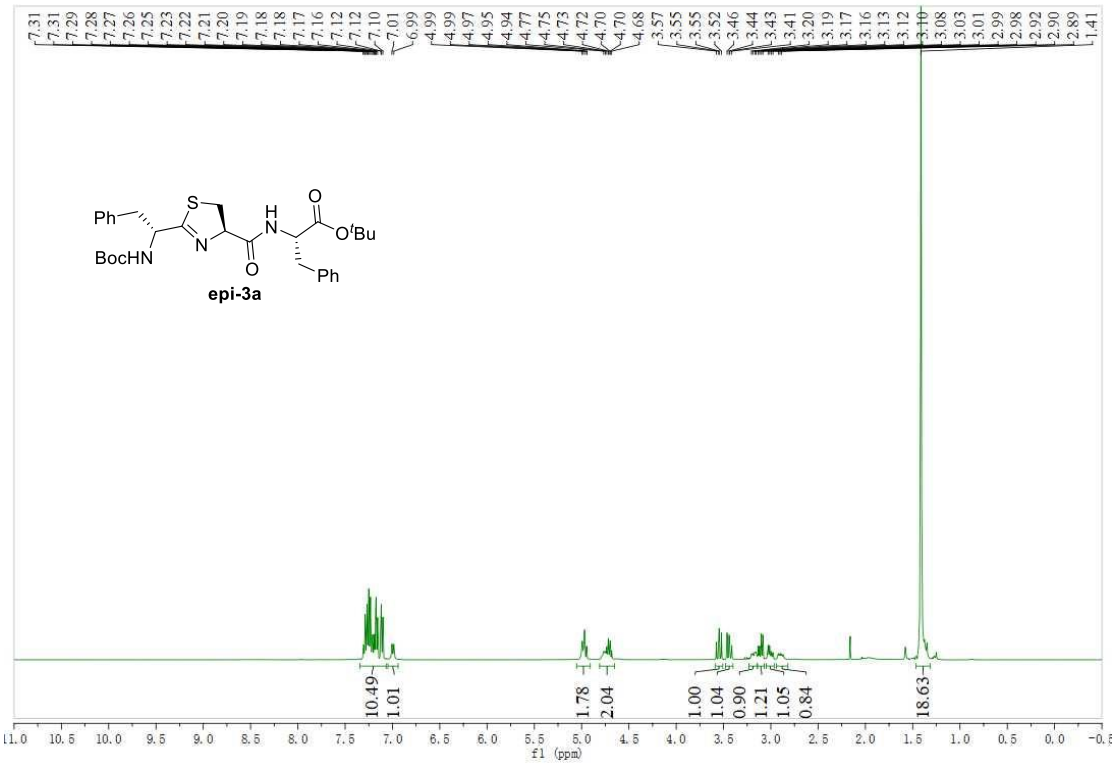
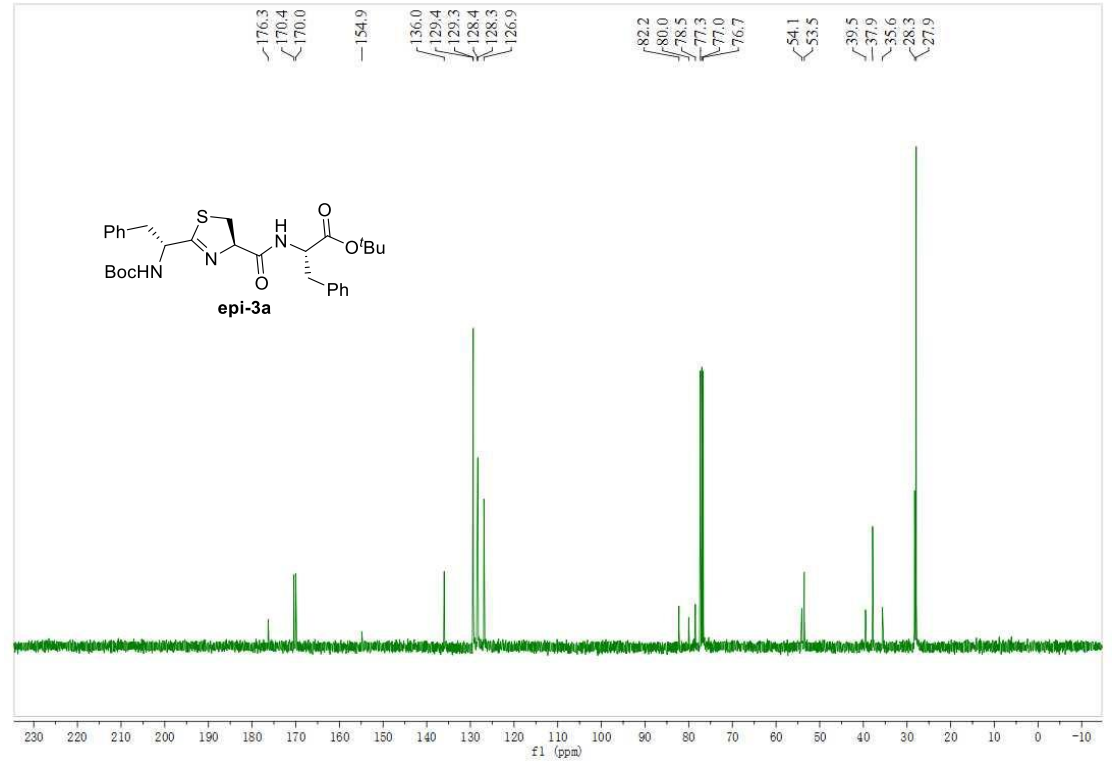
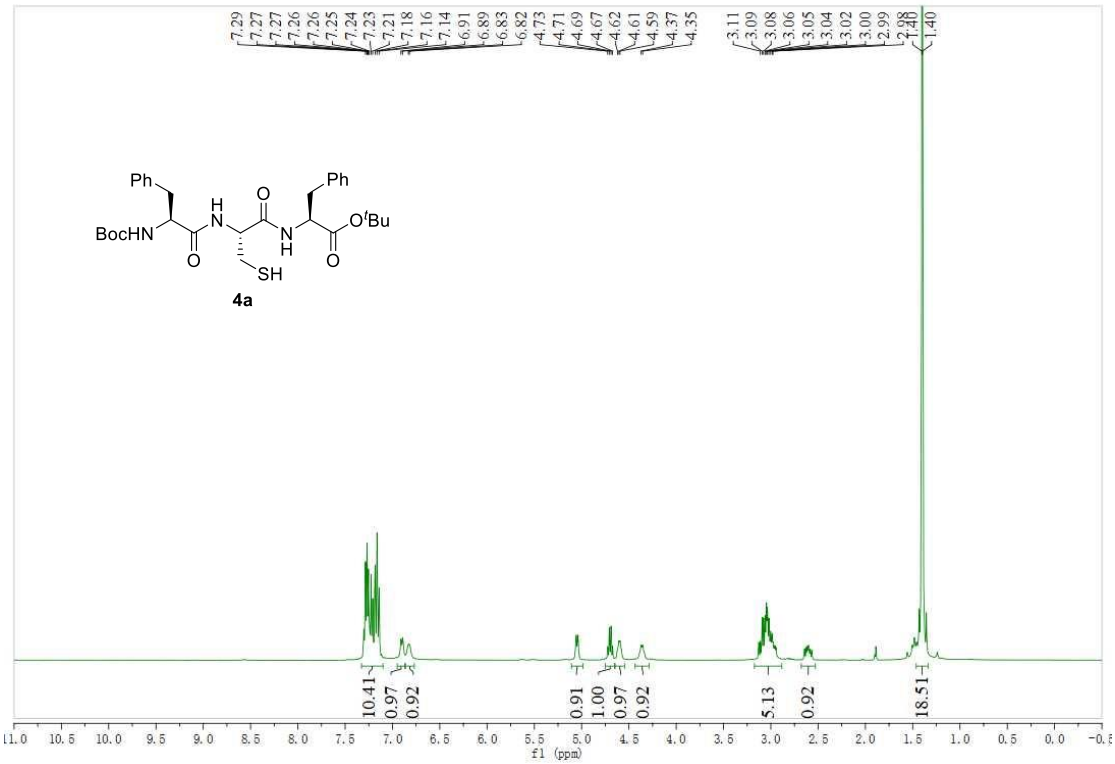
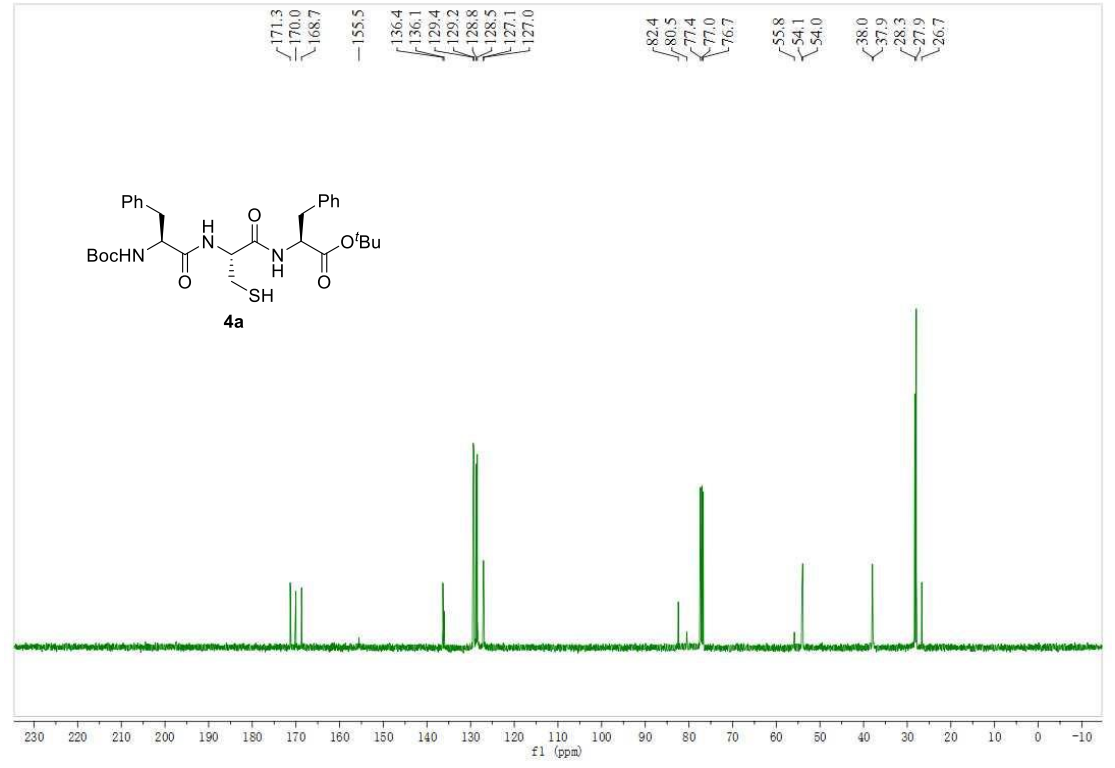
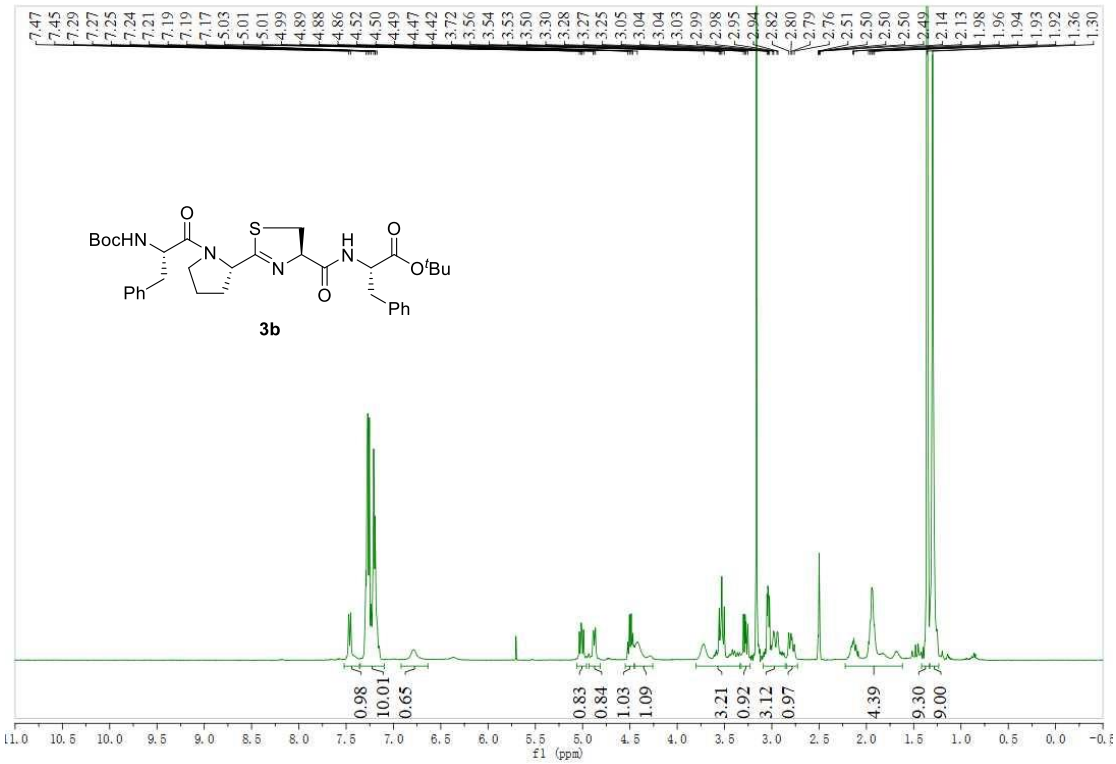
170.2
170.1
154.8
136.2
135.9
129.5
128.5
128.4
127.0
126.9
82.2
80.1
78.6
77.3
77.0
76.7
54.2
53.3
39.6
38.0
35.5
28.3
28.0
Ph
BocHN
S
N
H
N
O
O
O
O'Bu
Ph
3a
f1 (ppm)

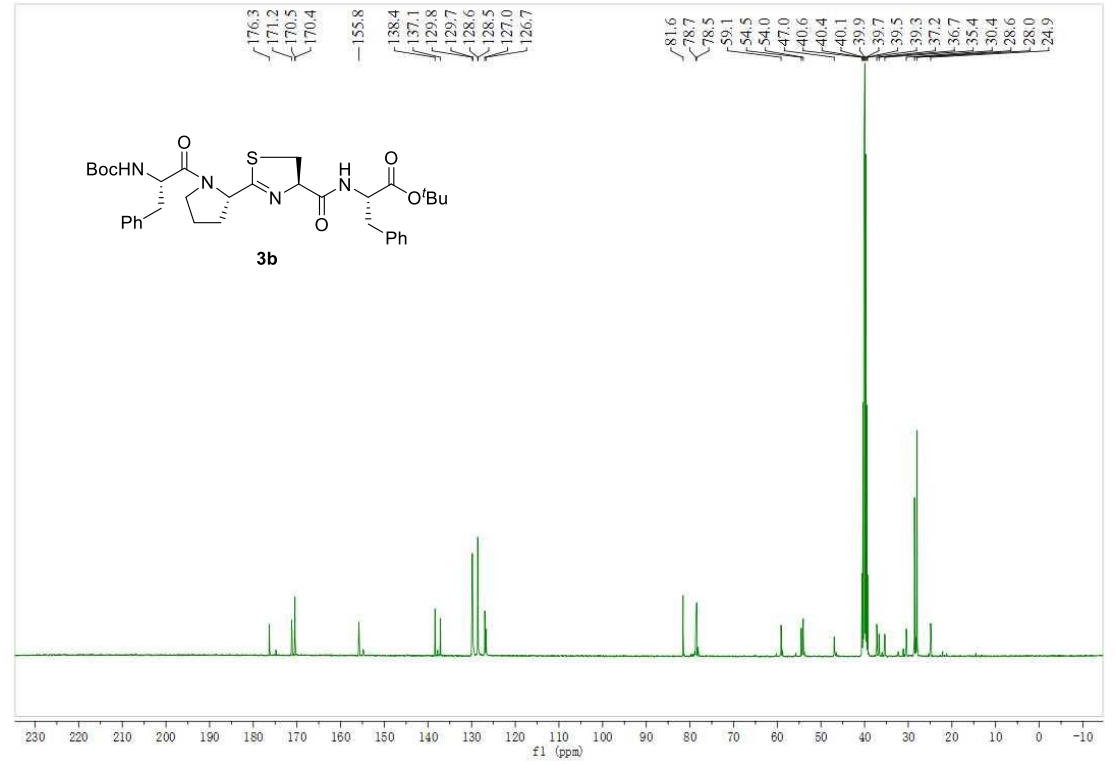

epi-3a
f1 (ppm)

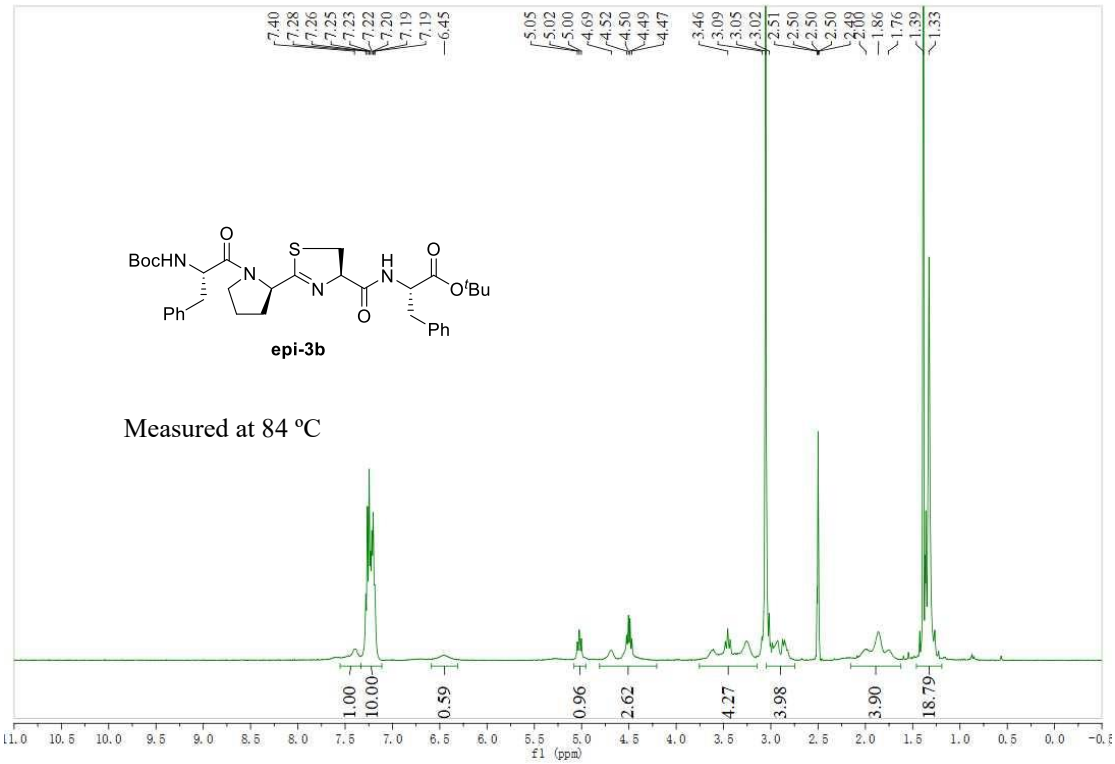

epi-3a
f1 (ppm)

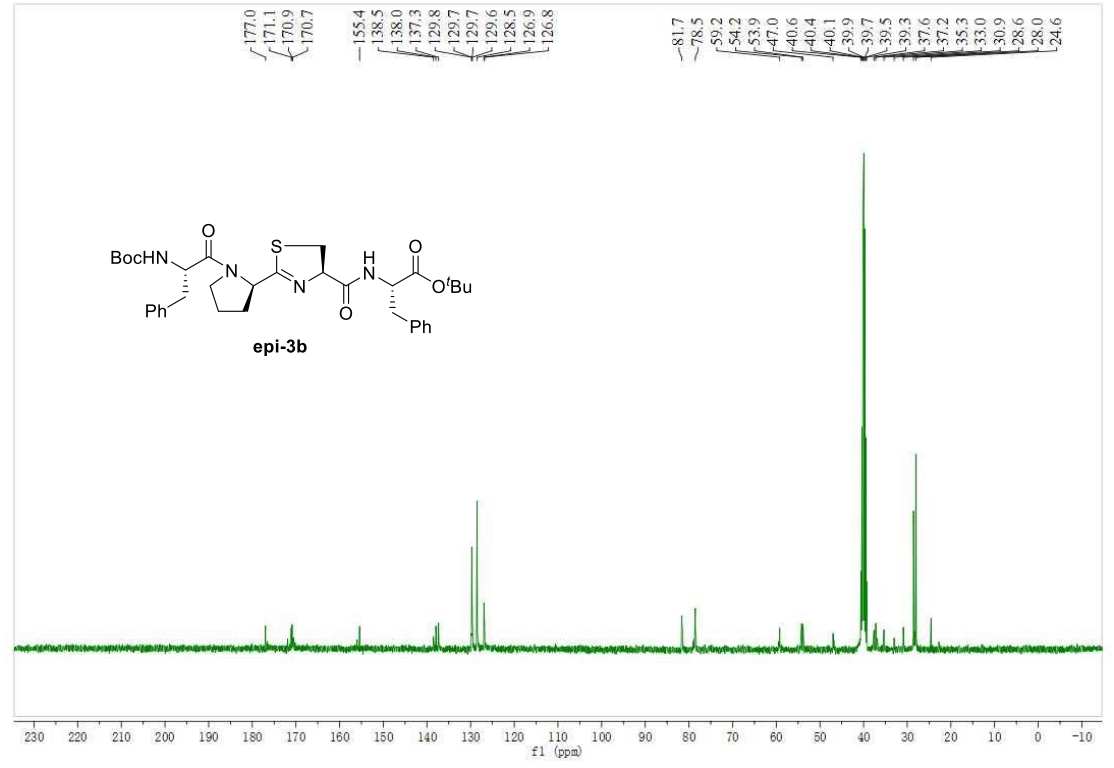

7.29
7.27
7.27
7.26
7.26
7.25
7.24
7.23
7.21
7.18
7.16
7.14
6.91
6.89
6.83
6.82
4.73
4.71
4.69
4.67
4.62
4.61
4.59
4.37
4.35
3.11
3.09
3.08
3.06
3.05
3.04
3.02
3.00
2.99
1.40
Ph
Ph
BocHN
O
H
N
N
H
O
O
O
O
tBu
SH
4a
10.41
0.97
0.92
0.91
1.00
0.97
0.92
5.13
0.92
18.51
f1 (ppm)

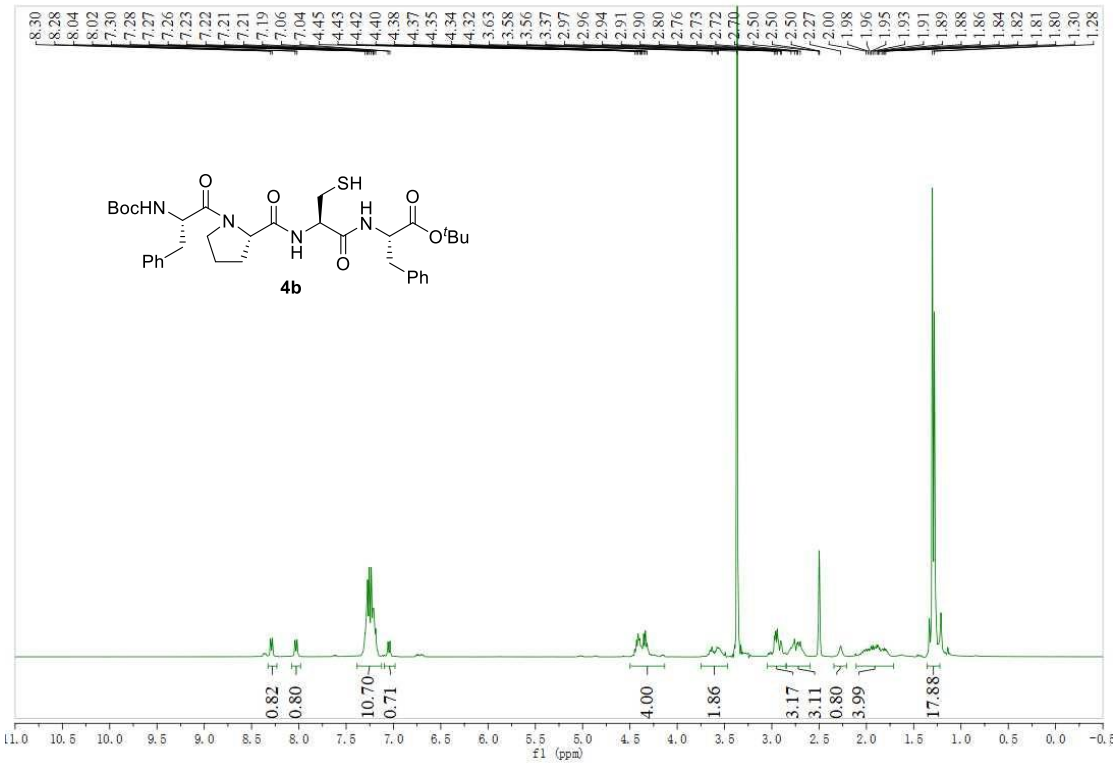

171.3
170.0
168.7
155.5
136.4
136.1
129.4
129.2
128.8
128.5
127.1
127.0
82.4
80.5
77.4
77.0
76.7
55.8
54.1
54.0
38.0
37.9
28.3
27.9
26.7
Ph
Ph
BocHN
O
H
N
N
H
O
O
O
O
tBu
SH
4a
f1 (ppm)

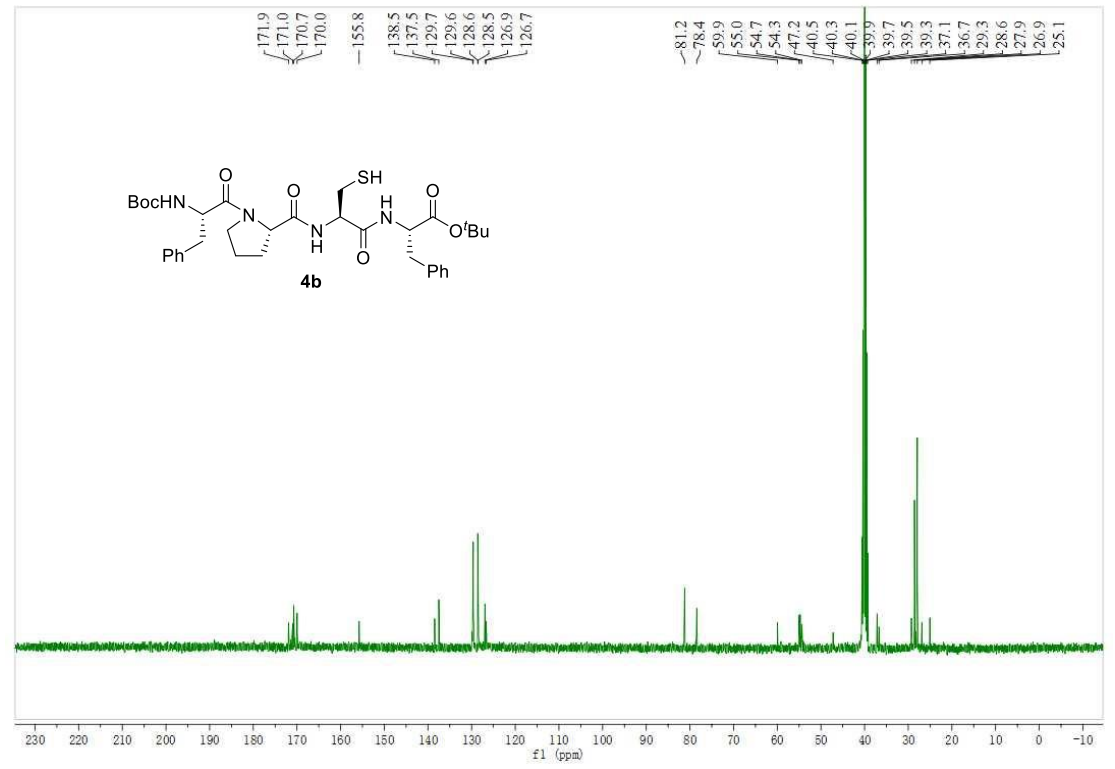

BocHN
Ph
S
N
H
N
O
O
O
O
tBu
Ph
3b
7.47 7.45 7.29 7.27 7.25 7.24 7.21 7.19 7.17 5.03 5.01 5.01 4.99 4.89 4.88 4.86 4.52 4.50 4.49 4.47 4.42 3.72 3.56 3.54 3.53 3.50 3.30 3.28 3.27 3.25 3.05 3.04 3.04 3.03 2.99 2.98 2.95 2.94 2.82 2.80 2.79 2.76 2.51 2.50 2.50 2.50 2.49 2.14 2.13 1.98 1.96 1.94 1.93 1.92 1.36 1.30
0.98 10.01 0.65 0.83 0.84 1.03 1.09 3.21 0.92 3.12 0.97 4.39 9.30 9.00
f1 (ppm)

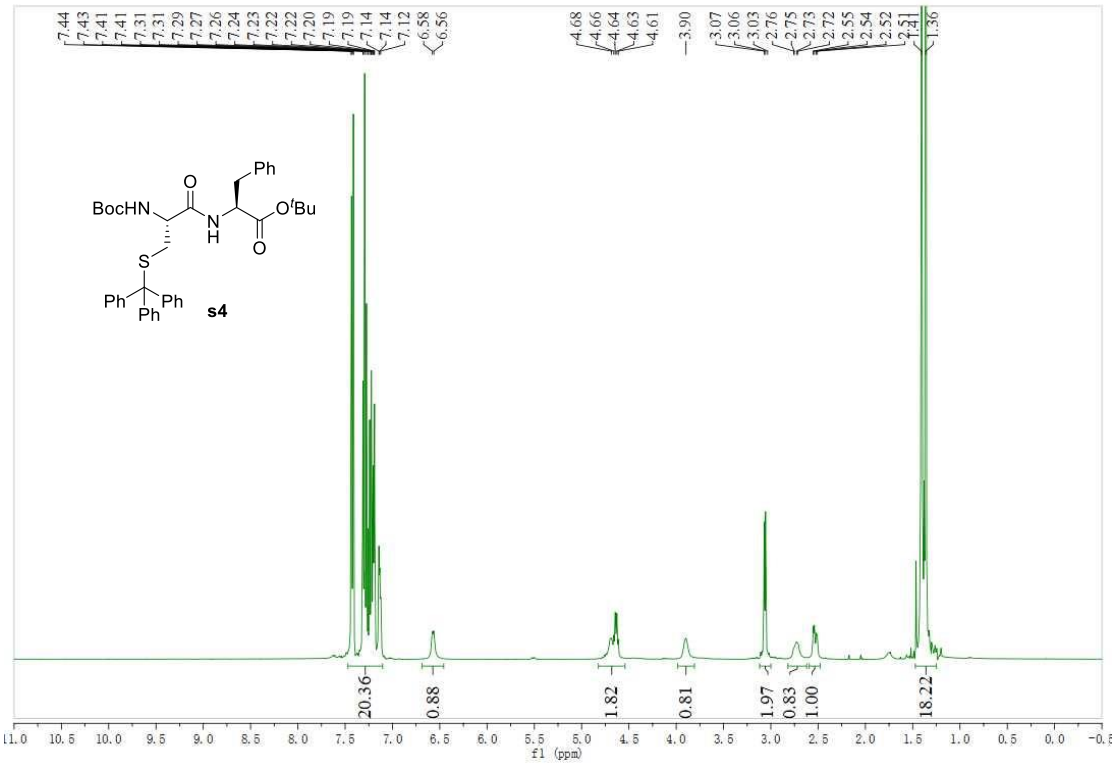

BocHN
Ph
S
N
H
N
O
O
O
O
tBu
Ph
3b
176.3 171.2 170.5 170.4 155.8 138.4 137.1 129.8 129.7 128.6 128.5 127.0 126.7 81.6 78.7 78.5 59.1 54.5 54.0 47.0 40.6 40.4 40.1 39.9 39.7 39.5 39.3 37.2 36.7 35.4 30.4 28.6 28.0 24.9
f1 (ppm)


epi-3b
Measured at 84 ºC
f1 (ppm)


epi-3b
f1 (ppm)


4b
f1 (ppm)


4b
f1 (ppm)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7.43
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7.41
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7.29
7.27
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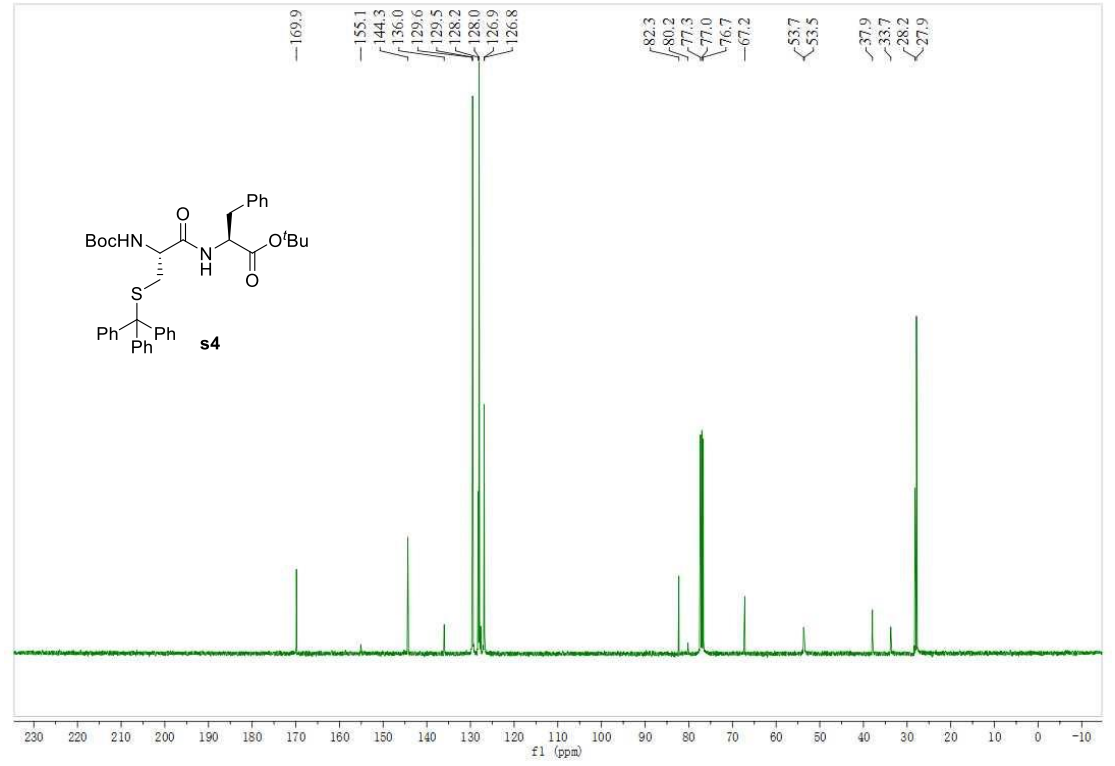
7.24
7.23
7.22
7.22
7.20
7.19
7.19
7.14
7.12
6.58
6.56
4.68
4.66
4.64
4.63
4.61
3.90
3.07
3.06
3.03
2.76
2.75
2.73
2.72
2.55
2.54
2.52
2.51
1.41
1.36
BocHN
O
N
H
Ph
O
O'Bu
S
Ph
Ph
Ph
s4
20.36
0.88
1.82
0.81
1.97
0.83
1.00
18.22
f1 (ppm)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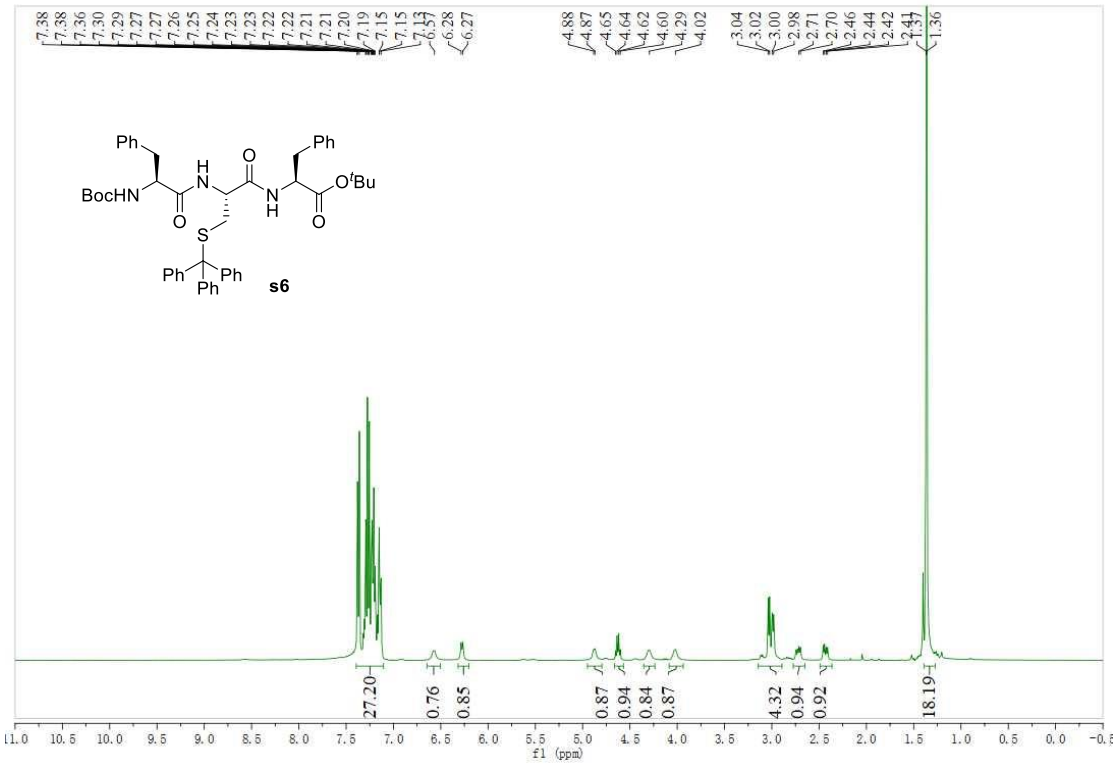
155.1
144.3
136.0
129.6
129.5
128.2
128.0
126.9
126.8
82.3
80.2
77.3
77.0
76.7
67.2
53.7
53.5
37.9
33.7
28.2
27.9
BocHN
O
N
H
Ph
O
O'Bu
S
Ph
Ph
Ph
s4
f1 (ppm)


7.38
7.38
7.36
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7.26
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7.24
7.23
7.23
7.22
7.22
7.21
7.21
7.20
7.19
7.15
7.15
6.57
6.28
6.27
4.88
4.87
4.65
4.64
4.62
4.60
4.29
4.02
3.04
3.02
3.00
2.98
2.71
2.70
2.46
2.44
2.42
2.41
1.37
1.36
Ph
BocHN
H
N
O
N
H
O
Ph
O
O
O
O
tBu
S
Ph
Ph
Ph
s6
27.20
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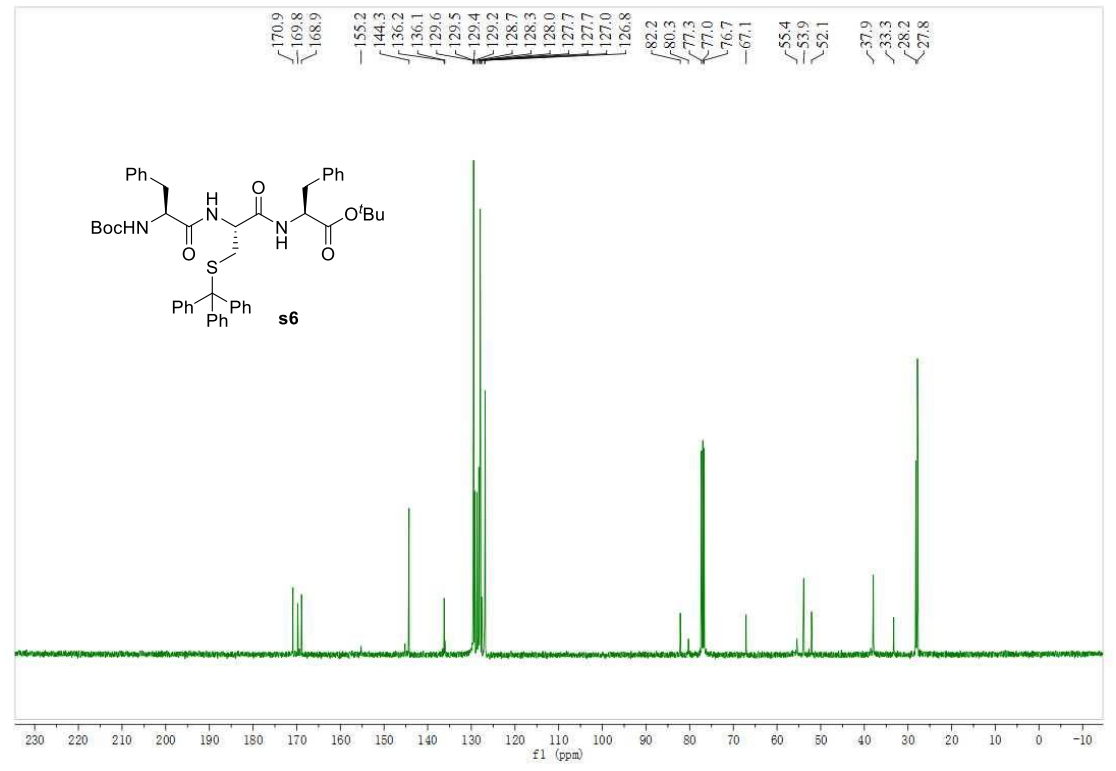
0.85
0.87
0.94
0.84
0.87
4.32
0.94
0.92
18.19
f1 (ppm)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168.9
155.2
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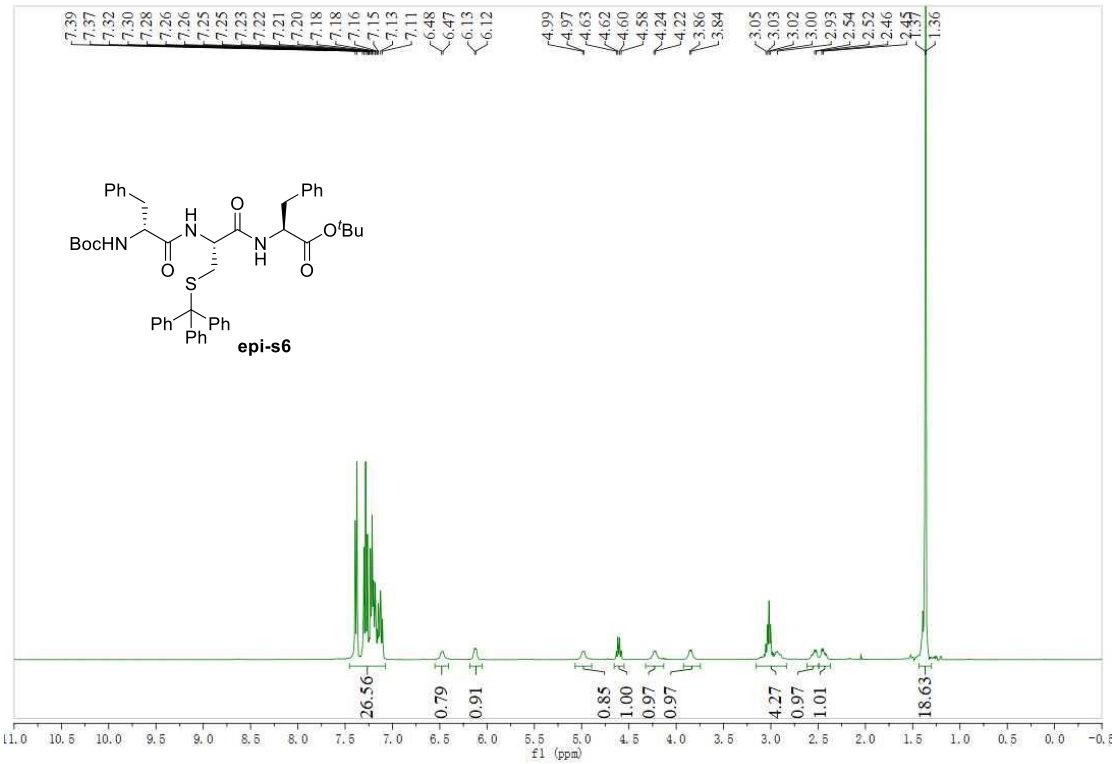
136.1
129.6
129.5
129.4
129.2
128.7
128.3
128.0
127.7
127.7
127.0
126.8
82.2
80.3
77.3
77.0
76.7
67.1
55.4
53.9
52.1
37.9
33.3
28.2
27.8
Ph
BocHN
H
N
O
N
H
O
Ph
O
O
O
O
tBu
S
Ph
Ph
Ph
s6
f1 (ppm)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7.18
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7.13
7.11
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6.47
6.13
6.12
4.99
4.97
4.63
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4.60
4.58
4.24
4.22
3.86
3.84
3.05
3.03
3.02
3.00
2.93
2.54
2.52
2.46
2.45
1.37
1.36
Ph
BocHN
H
N
O
O
N
H
Ph
O'Bu
O
O
S
Ph
Ph
Ph
epi-s6
26.56
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0.97
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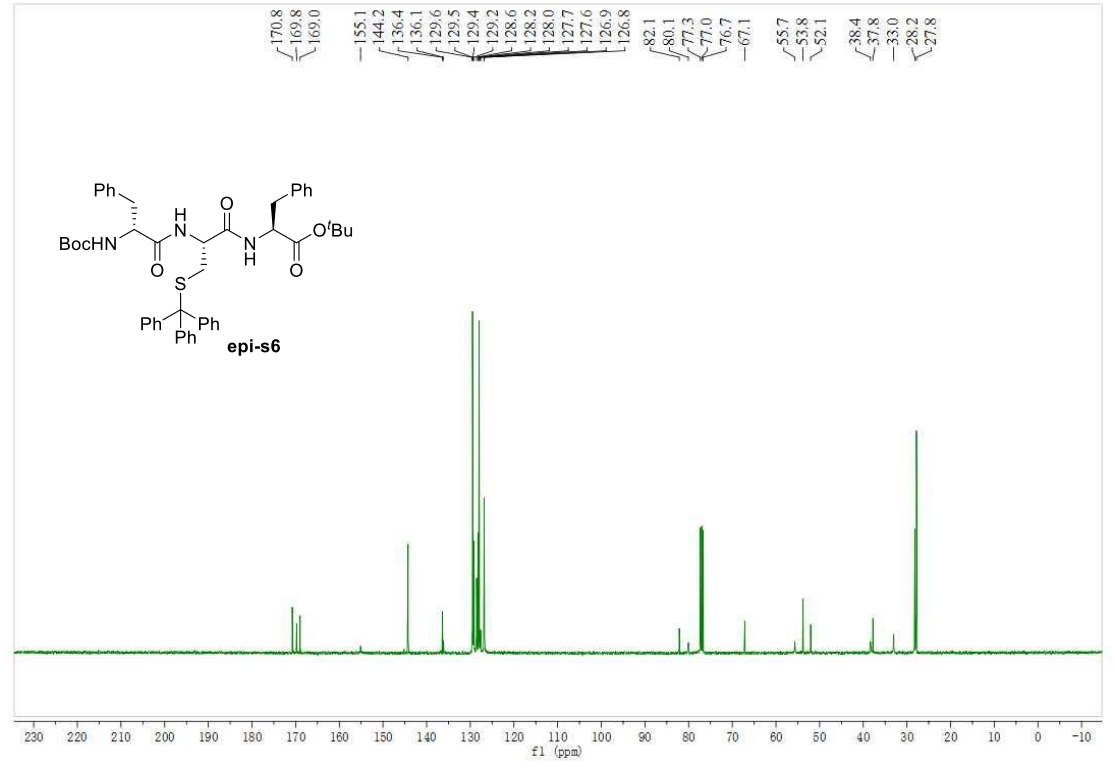
0.97
1.01
18.63
f1 (ppm)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169.8
169.0
155.1
144.2
136.4
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129.6
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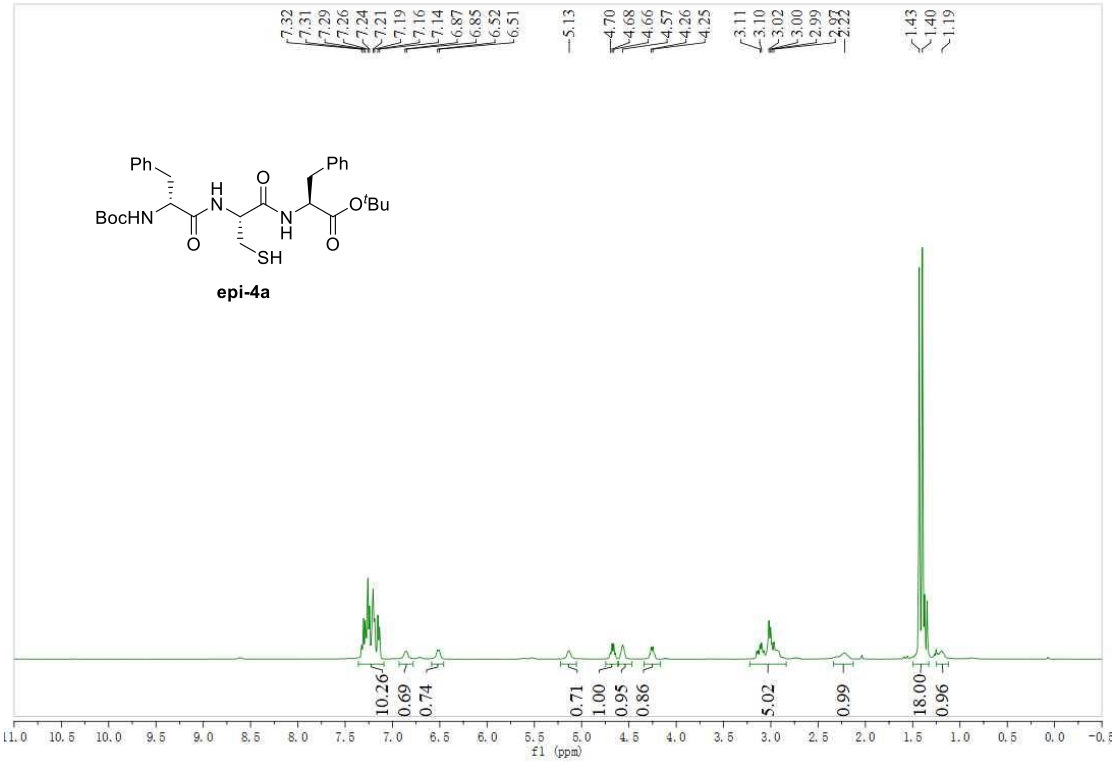
129.4
129.2
128.6
128.2
128.0
127.7
127.6
126.9
126.8
82.1
80.1
77.3
77.0
76.7
67.1
55.7
53.8
52.1
38.4
37.8
33.0
28.2
27.8
Ph
BocHN
H
N
O
O
N
H
Ph
O'Bu
O
O
S
Ph
Ph
Ph
epi-s6
f1 (ppm)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7.31
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7.21
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7.14
6.87
6.85
6.52
6.51
5.13
4.70
4.68
4.66
4.57
4.26
4.25
3.11
3.10
3.02
3.00
2.99
2.87
2.22
1.43
1.40
1.19
Ph
BocHN
O
H
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O
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H
Ph
O
O
O
tBu
SH
epi-4a
10.26
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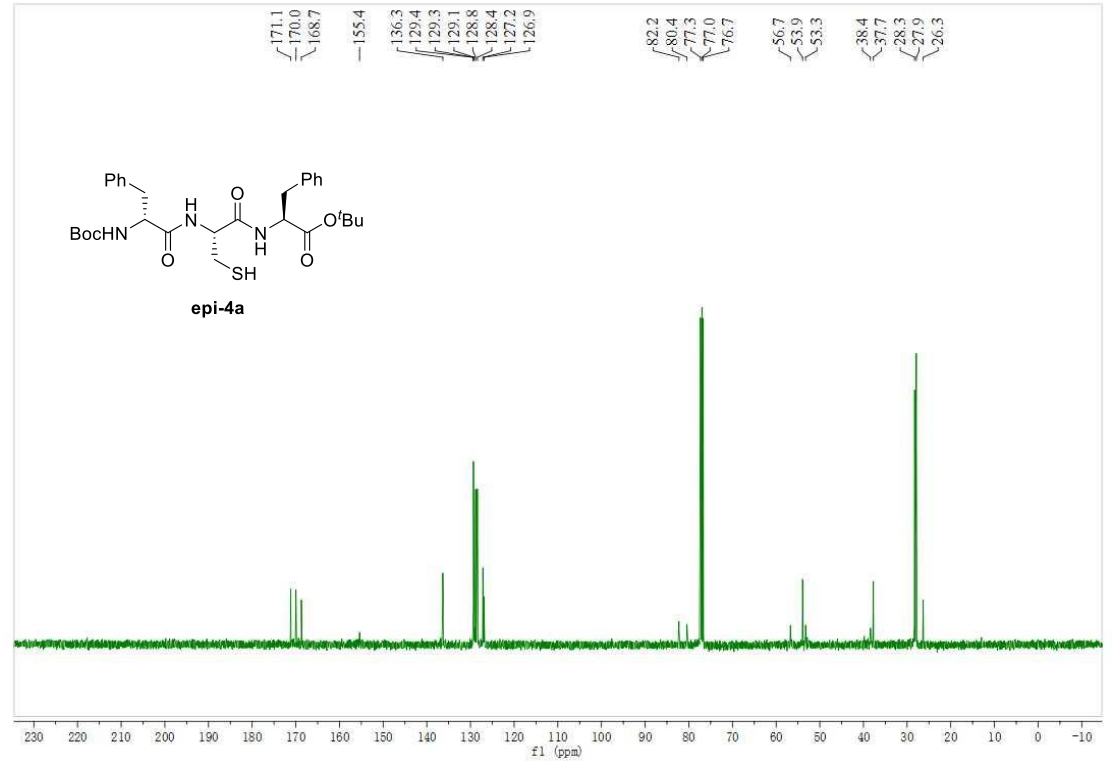
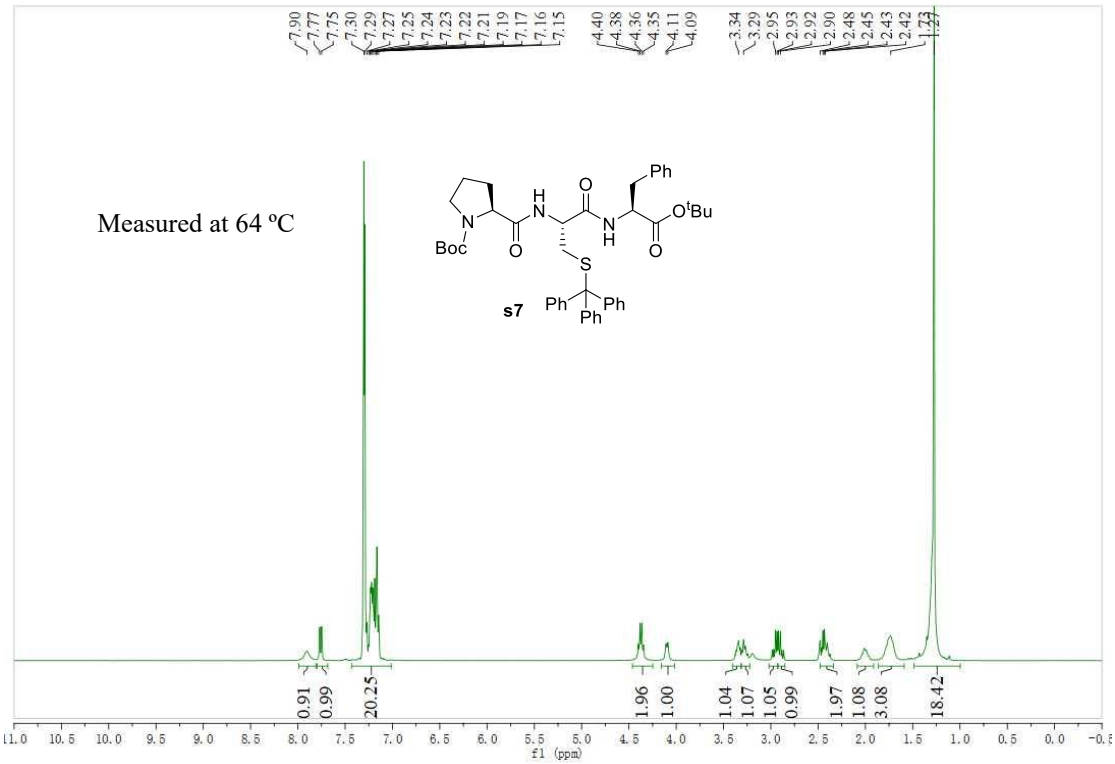
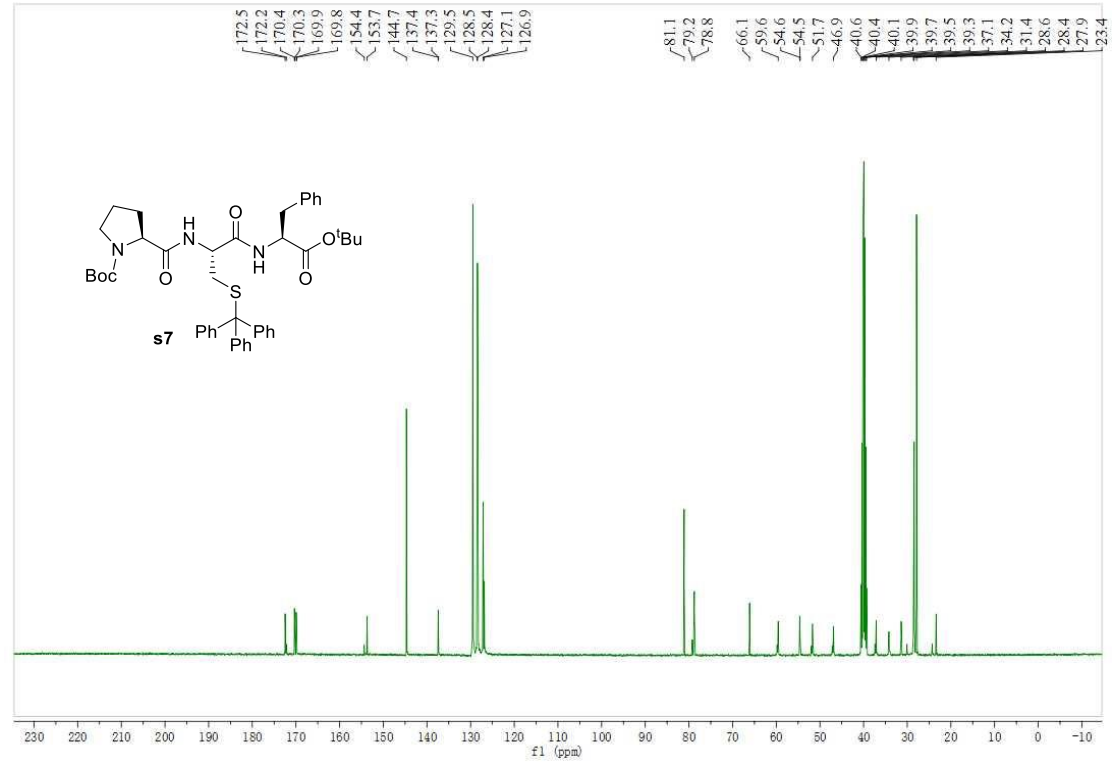
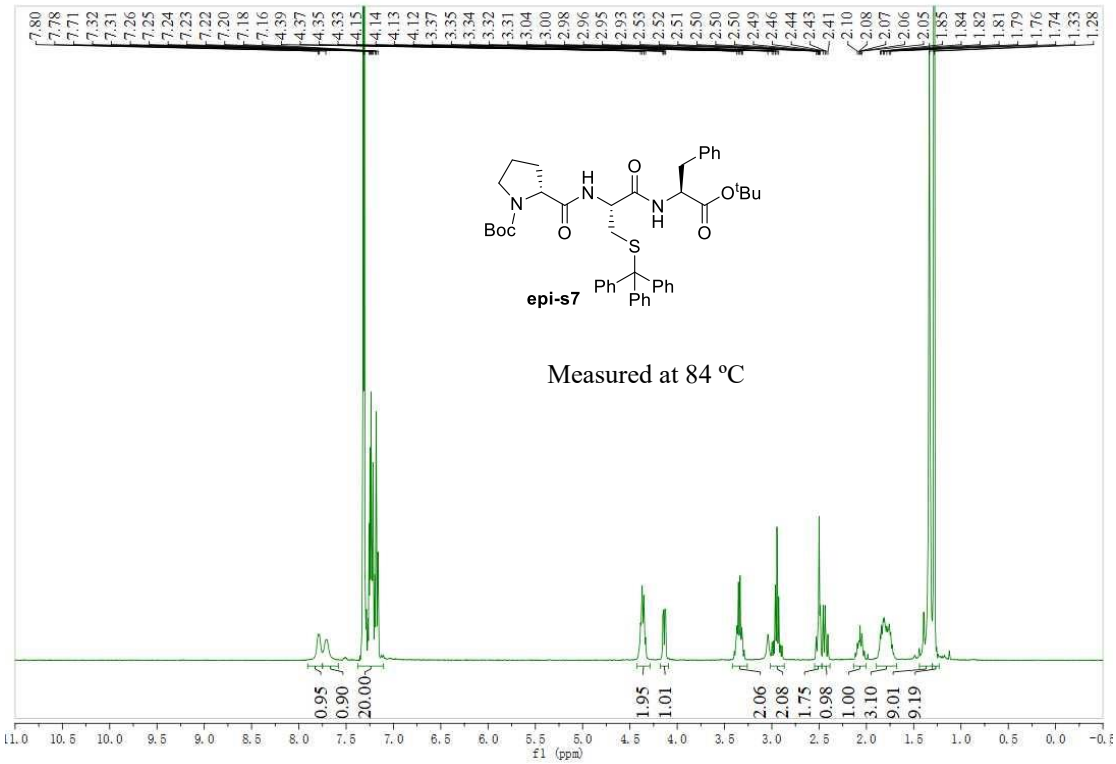
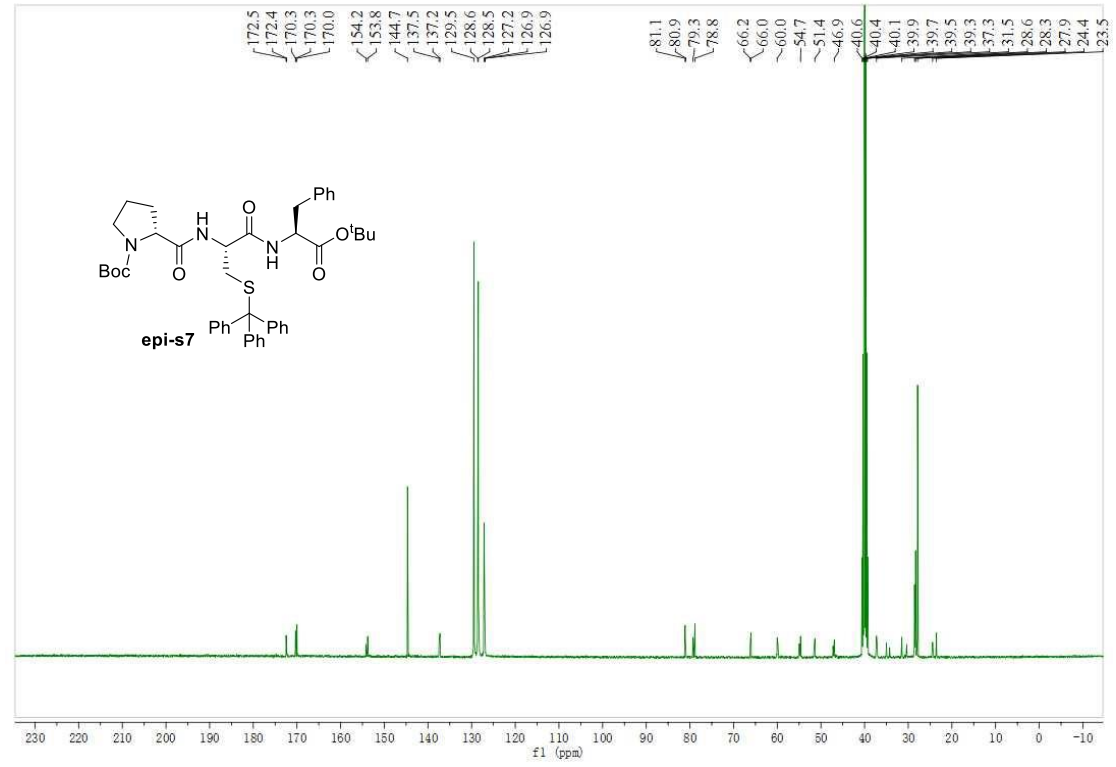
0.71
1.00
0.95
0.86
5.02
0.99
18.00
0.96
f1 (ppm)

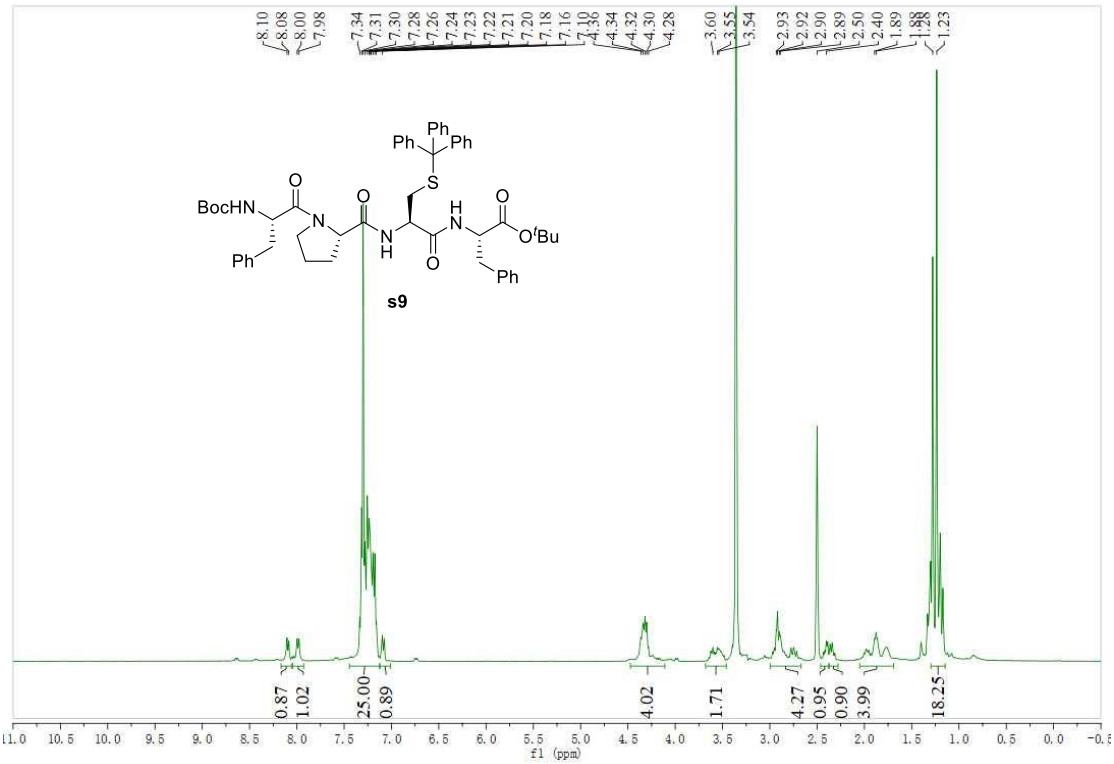

171.1
170.0
168.7
155.4
136.3
129.4
129.3
129.1
128.8
128.4
127.2
126.9
82.2
80.4
77.3
77.0
76.7
56.7
53.9
53.3
38.4
37.7
28.3
27.9
26.3
Ph
BocHN
O
H
N
O
N
H
Ph
O
O
O
tBu
SH
epi-4a
f1 (ppm)

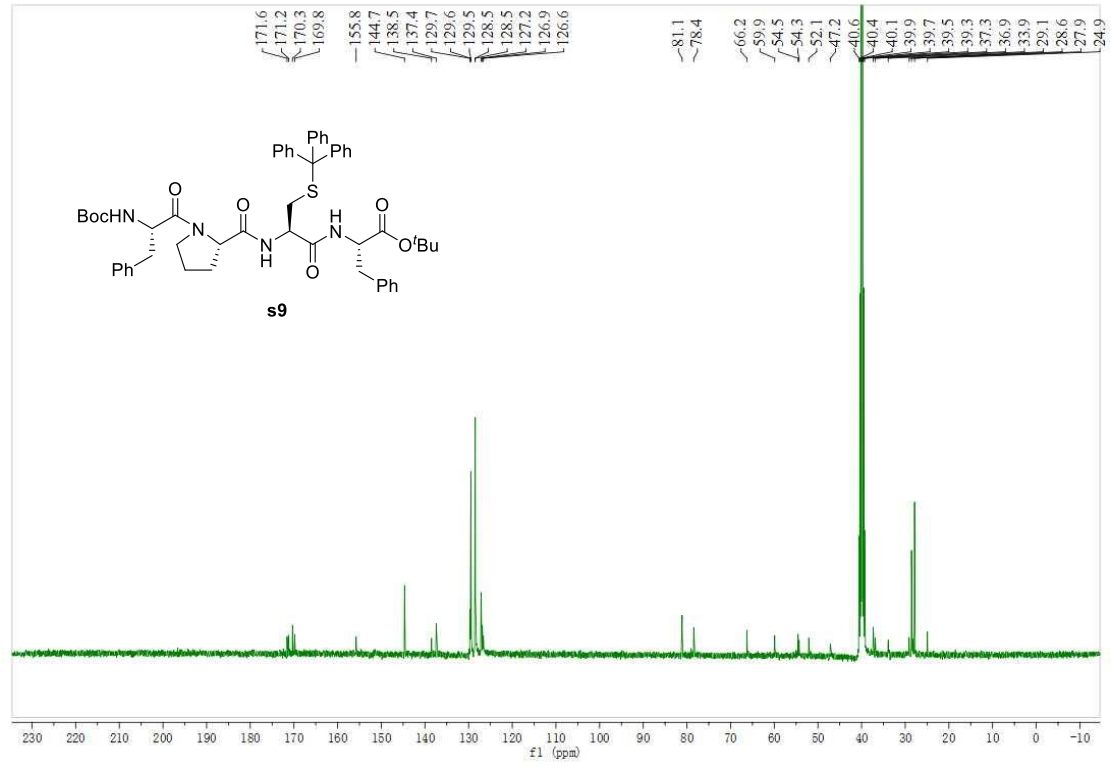

Measured at 64 ºC
Boc
Ph
O
tBu
s7
f1 (ppm)

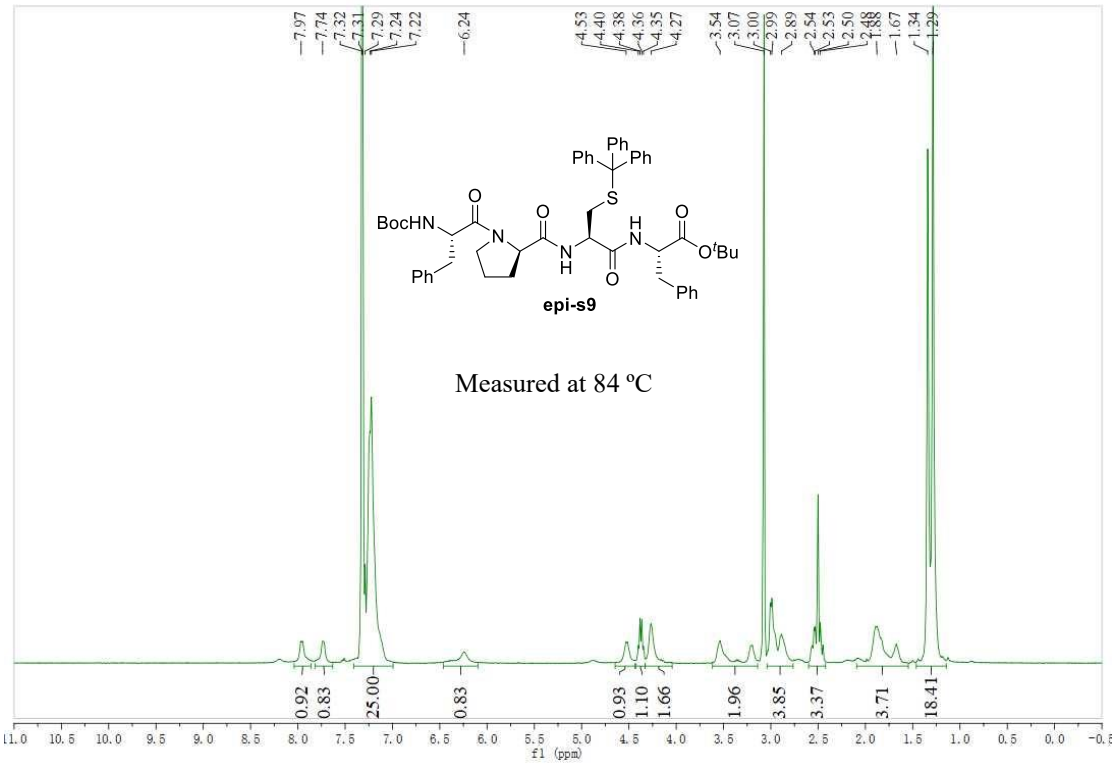

Boc
Ph
O
tBu
s7
f1 (ppm)

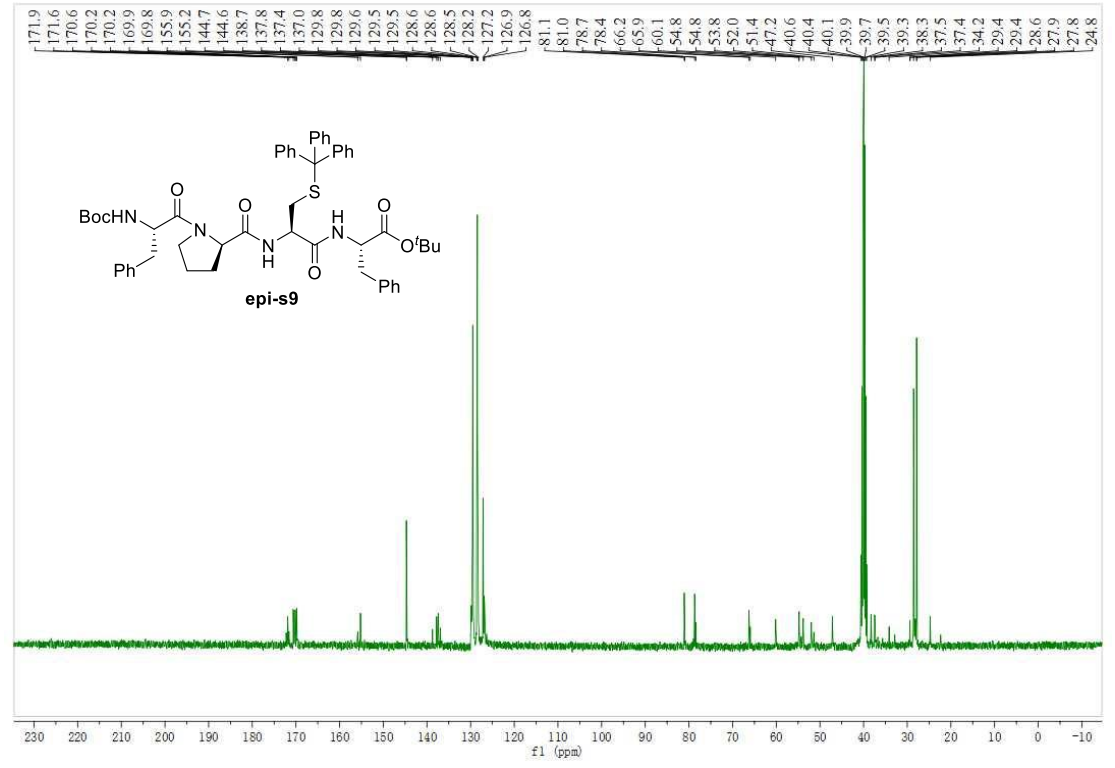

epi-s7
Measured at 84 ºC
f1 (ppm)

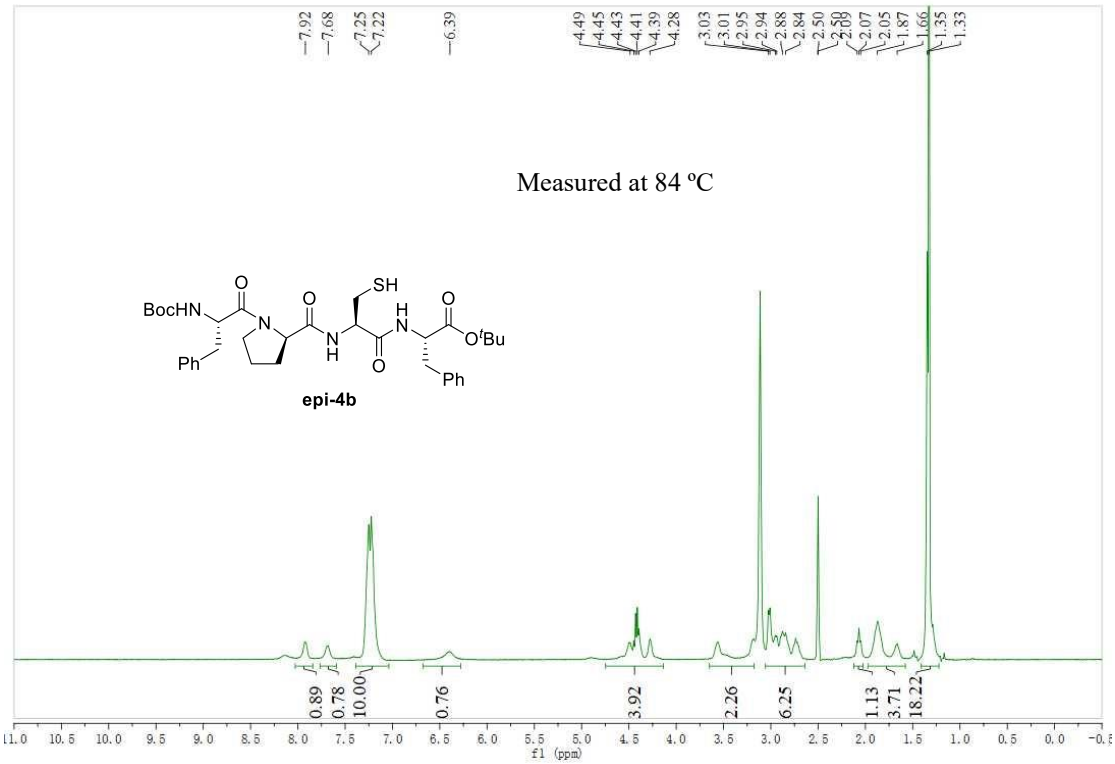

epi-s7
f1 (ppm)

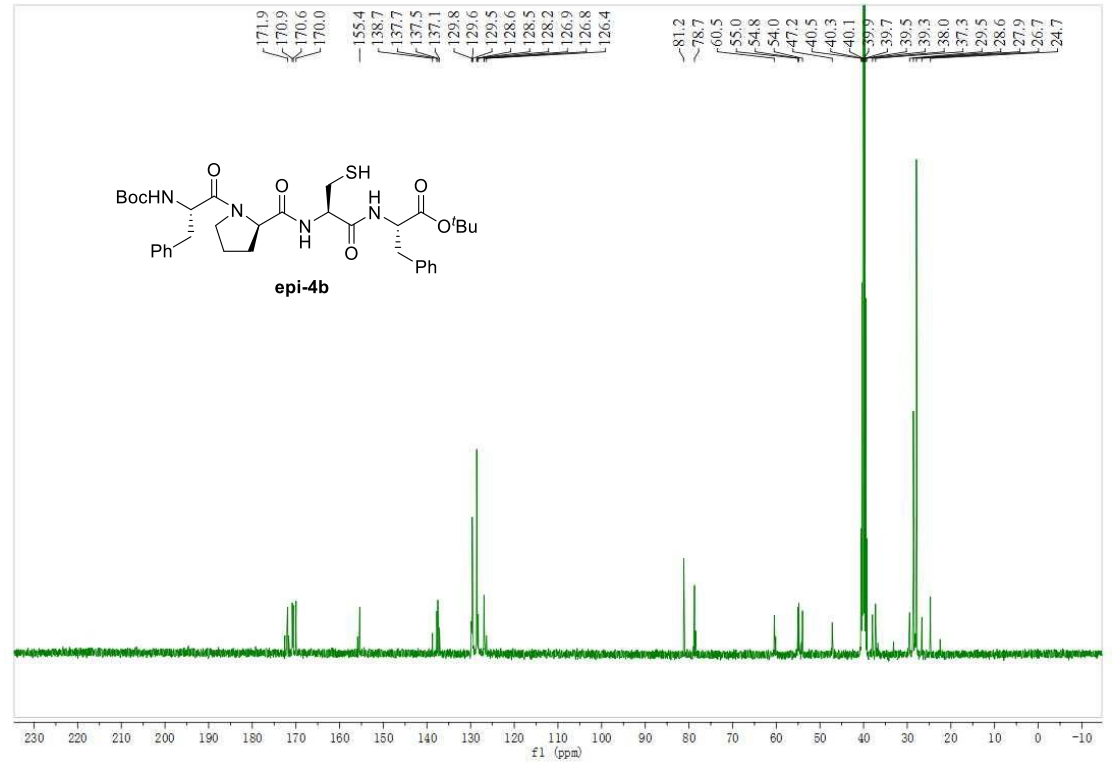

s9
f1 (ppm)


s9
f1 (ppm)


epi-s9
Measured at 84 ºC
f1 (ppm)


epi-s9
f1 (ppm)


Measured at 84 ºC
BocHN
Ph
SH
O
N
H
O
O
O
O
tBu
Ph
epi-4b
f1 (ppm)


BocHN
Ph
SH
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H
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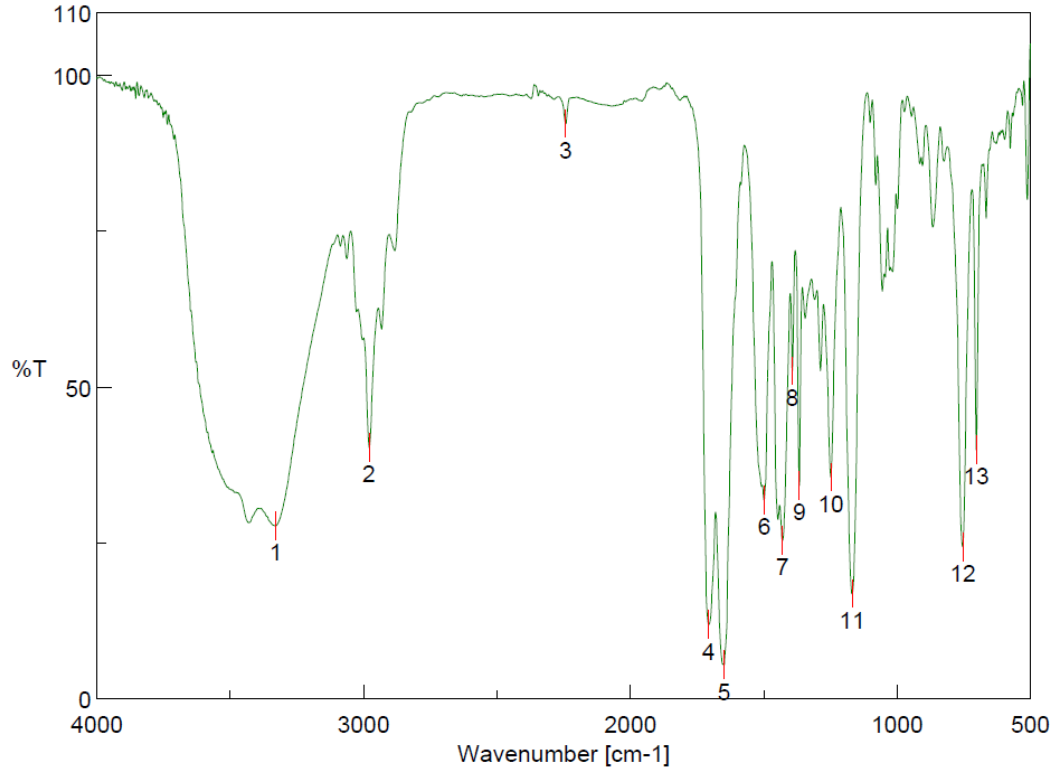
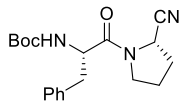
epi-4b
f1 (ppm)

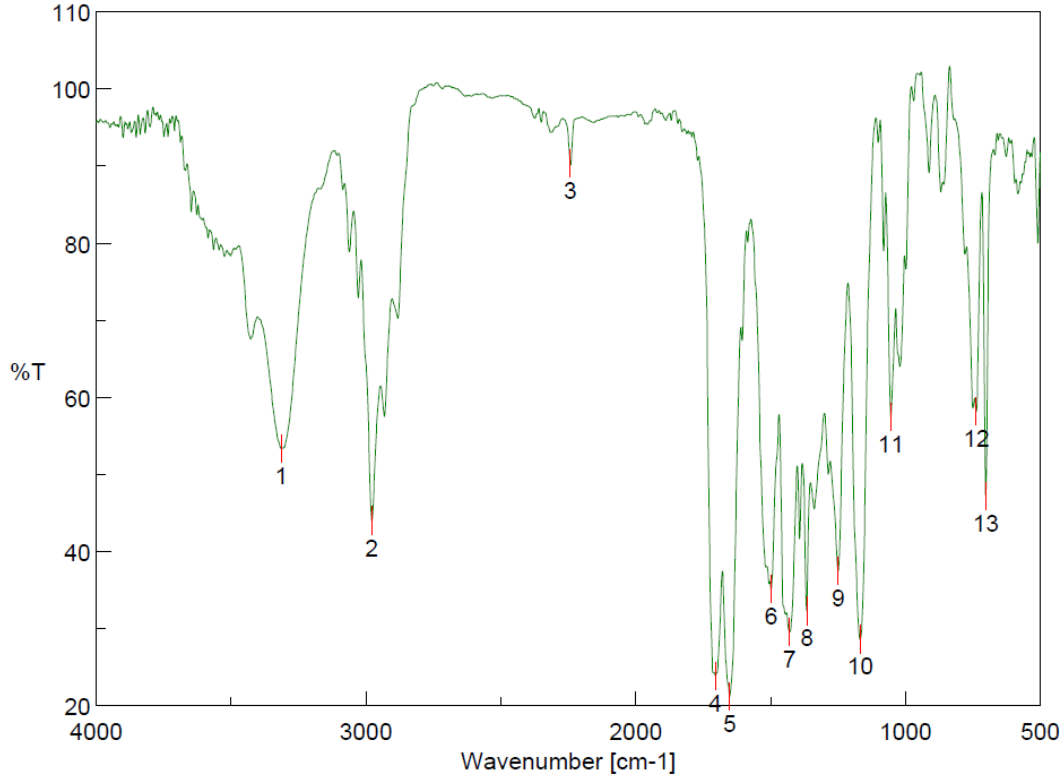


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3330.46	27.7335	2	2979.48	40.3104
3	2242.81	92.1849	4	1706.69	11.9501
5	1648.84	5.49022	6	1498.42	31.8459
7	1429.96	25.4337	8	1392.35	52.6473
9	1367.28	34.2061	10	1248.68	35.5887
11	1168.65	16.8762	12	754.031	24.3917
13	702.926	39.9595			

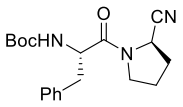


1b

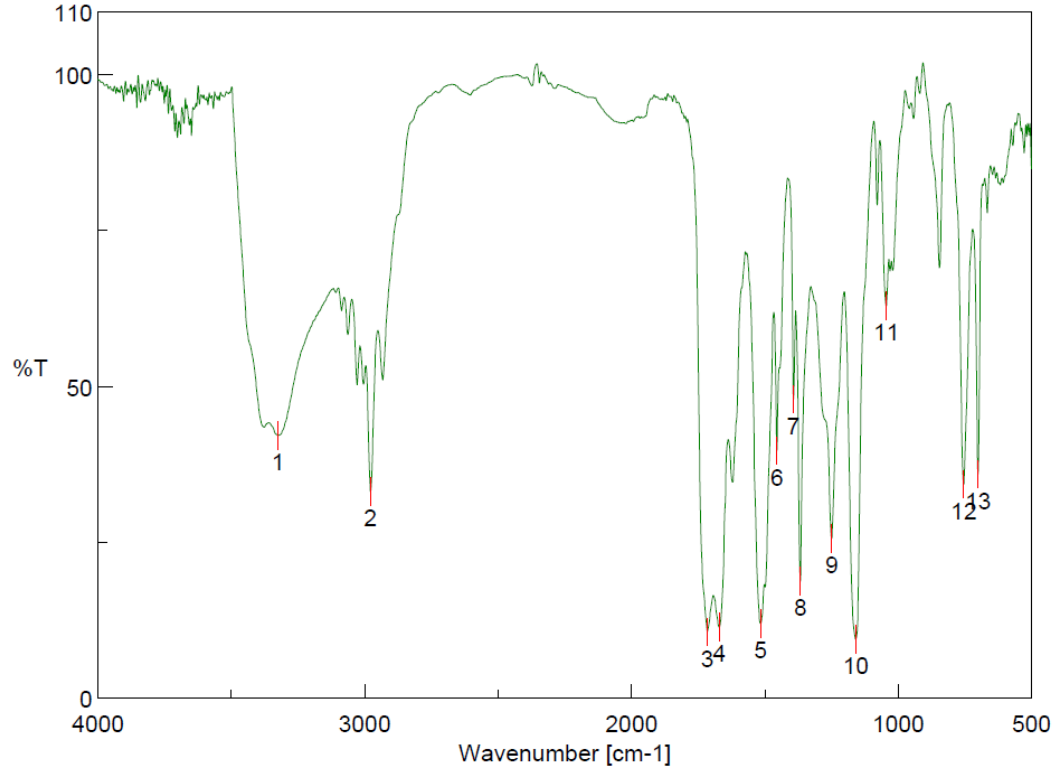


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3314.07	53.3223	2	2978.52	44.0128
3	2242.81	90.3056	4	1704.76	23.8905
5	1651.73	21.2497	6	1497.45	35.1255
7	1428.99	29.5953	8	1366.32	32.3303
9	1249.65	37.5359	10	1167.69	28.6427
11	1053.91	57.4776	12	737.639	58.178
13	701.962	47.2234			

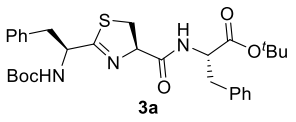


epi-1b

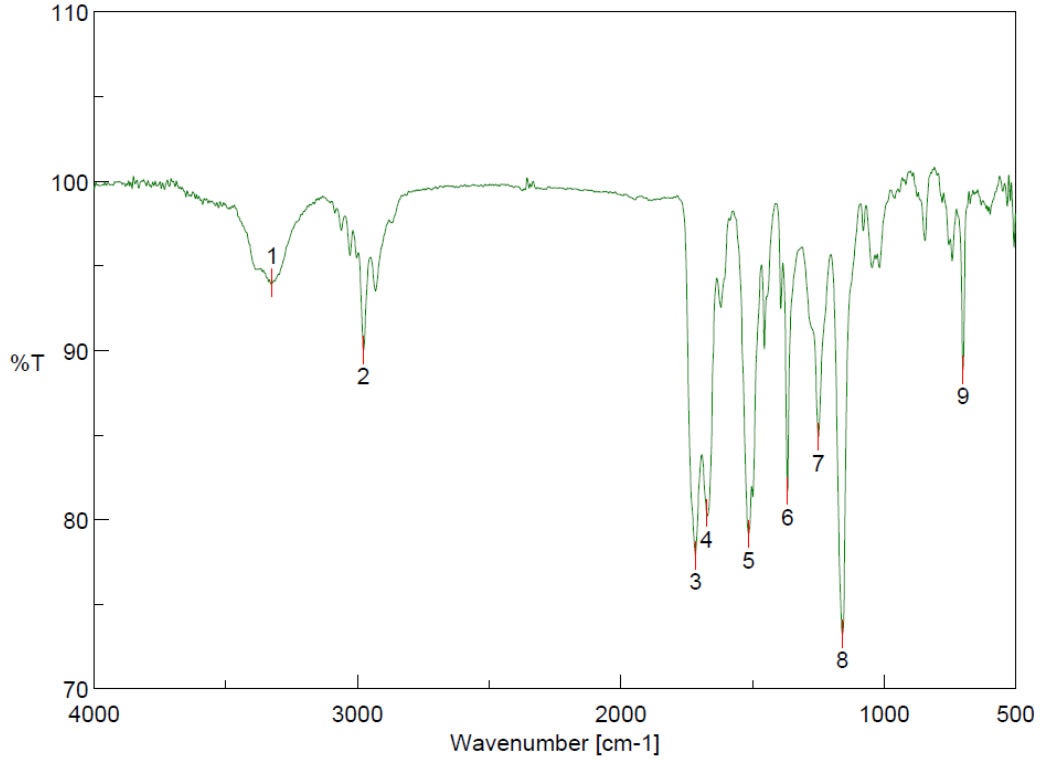


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3325.64	42.1251	2	2978.52	33.1359
3	1715.37	10.67	4	1670.05	11.475
5	1515.78	12.0041	6	1455.03	39.7517
7	1392.35	47.9512	8	1367.28	18.7787
9	1249.65	25.6219	10	1159.01	9.52166
11	1045.23	62.8942	12	754.995	34.3032
13	700.998	35.936			

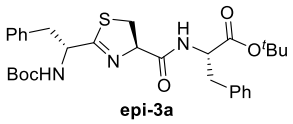


3a

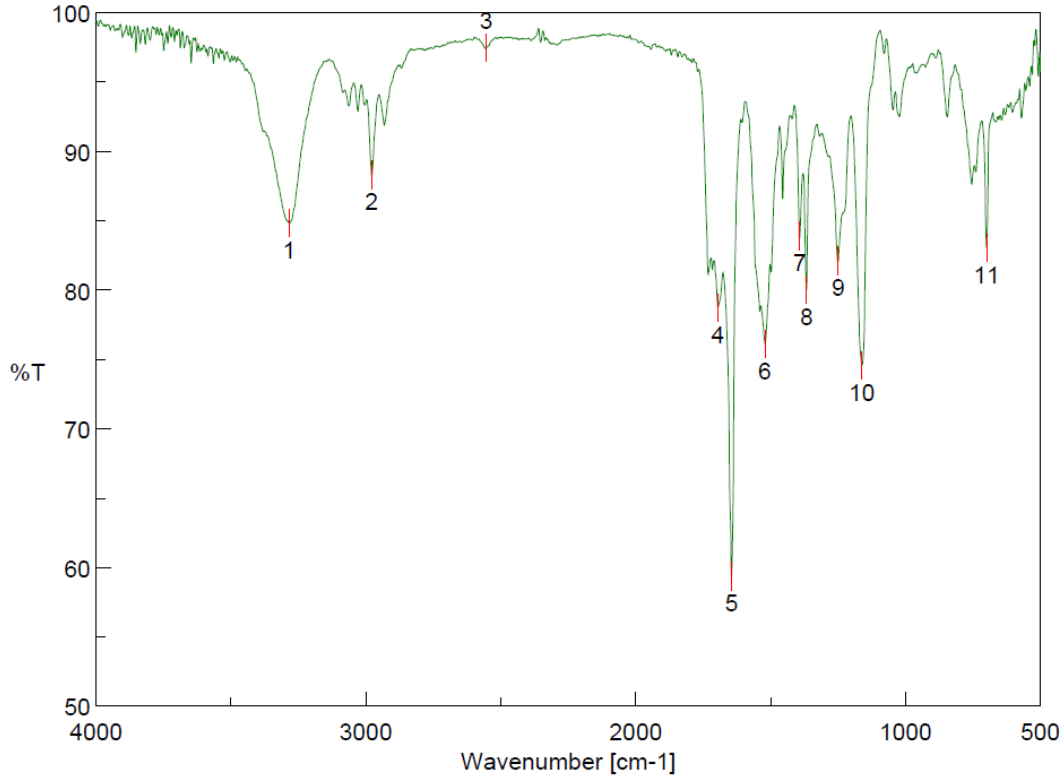


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3324.68	93.989	2	2977.55	90.0061
3	1716.34	77.8799	4	1673.91	80.4043
5	1514.81	79.1565	6	1367.28	81.6915
7	1249.65	84.9011	8	1158.04	73.253
9	700.034	88.8412			

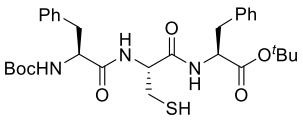


epi-3a

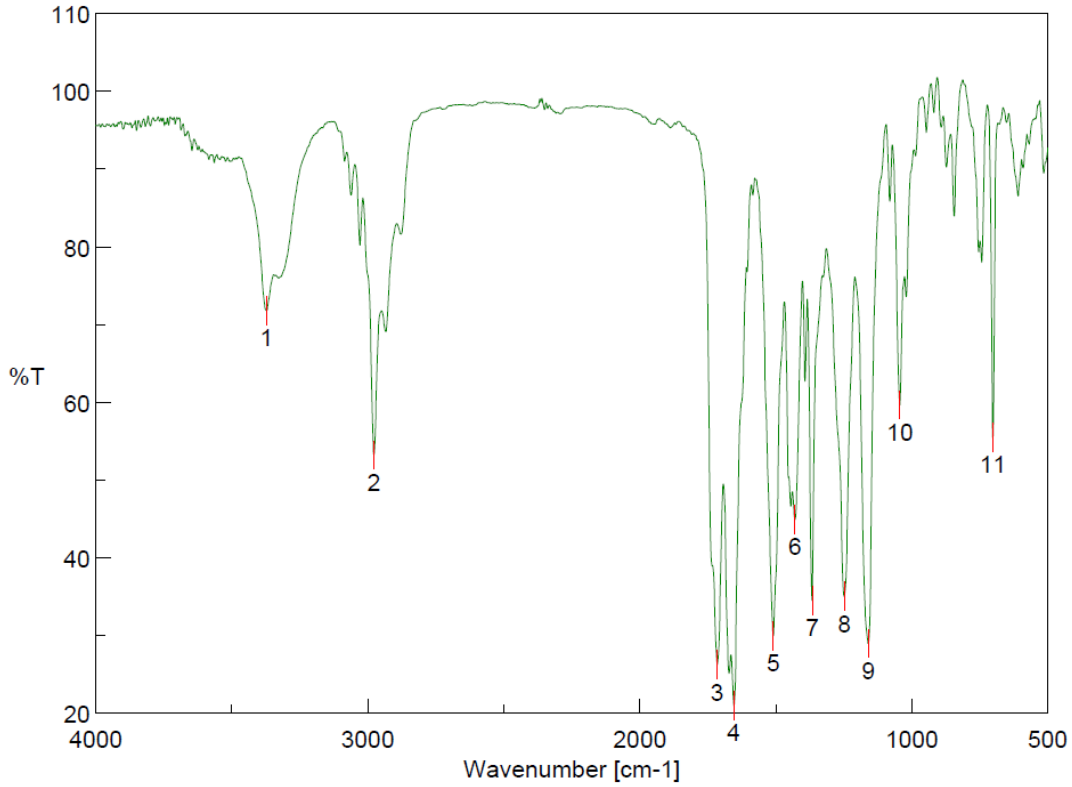


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3285.14	84.8081	2	2978.52	88.2842
3	2554.25	97.4573	4	1695.12	78.7246
5	1644.98	59.3672	6	1520.6	76.1175
7	1391.39	83.8666	8	1367.28	80.0092
9	1249.65	82.0842	10	1160.94	74.5754
11	699.069	83.0351			

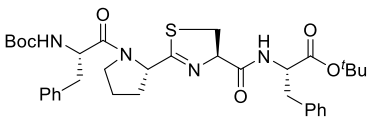


4a

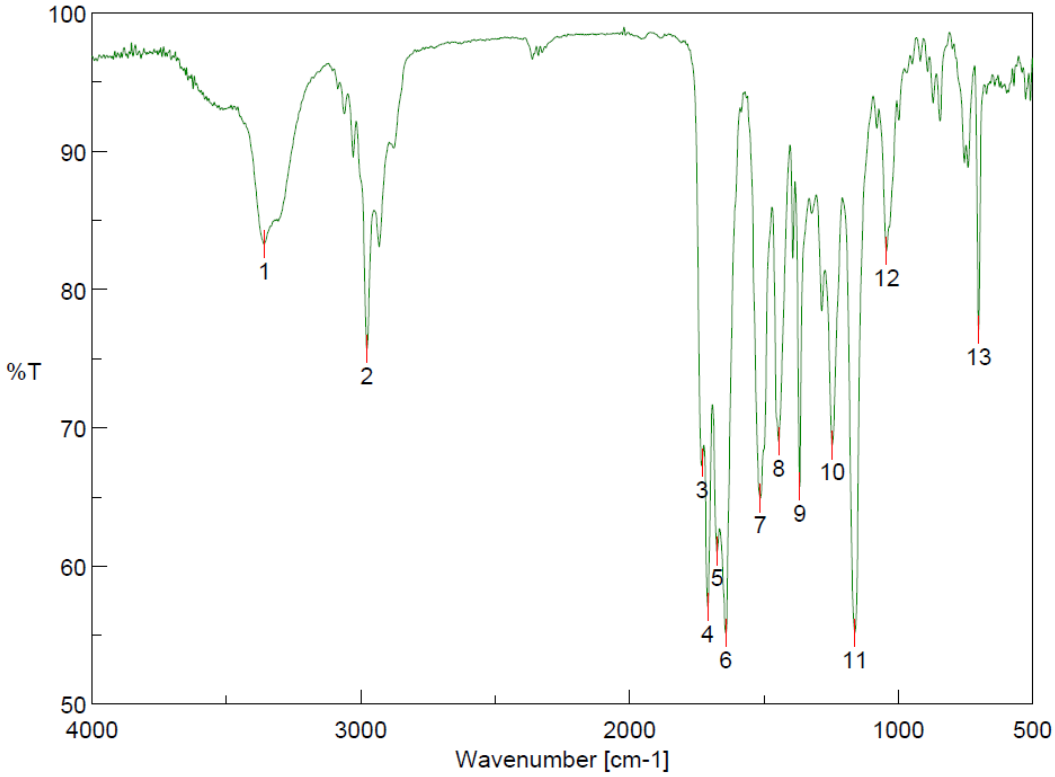


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3372.89	71.7857	2	2977.55	53.215
3	1715.37	26.2439	4	1653.66	21.0042
5	1509.03	29.9677	6	1428.99	44.9426
7	1366.32	34.5456	8	1248.68	35.0586
9	1159.97	29.0015	10	1044.26	59.6633
11	701.962	55.464			

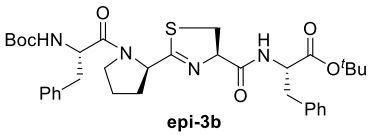


3b

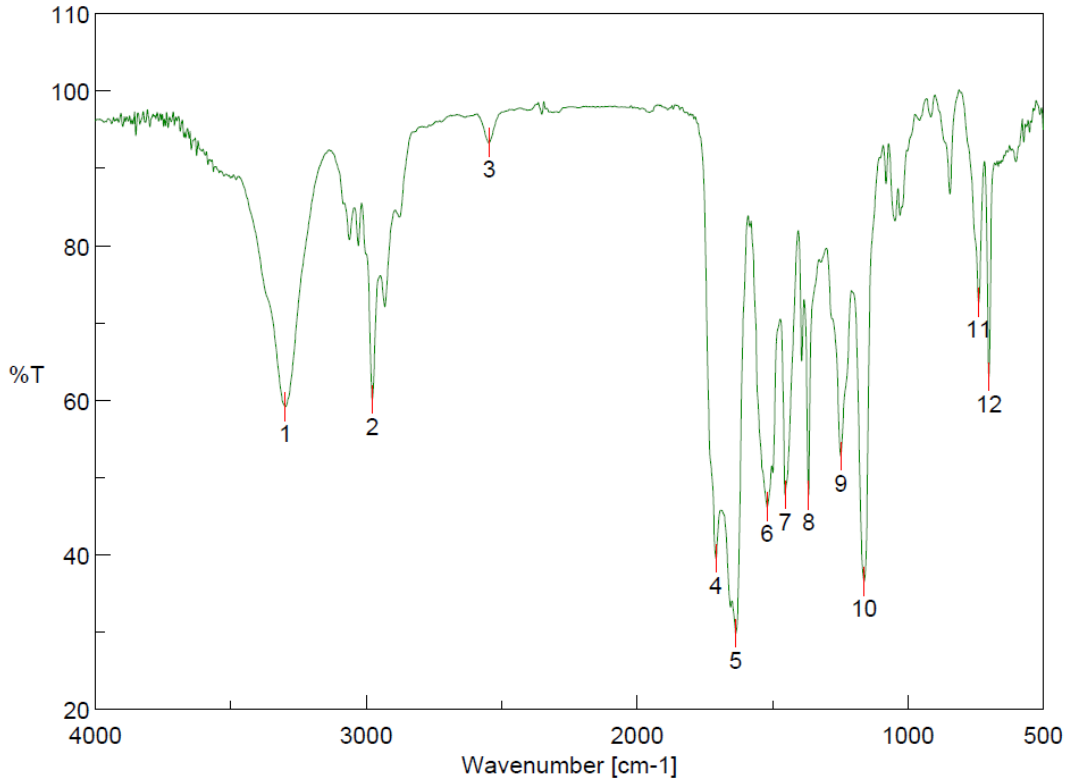


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3360.35	83.2668	2	2977.55	75.703
3	1729.83	67.4813	4	1708.62	57.061
5	1673.91	61.066	6	1642.09	55.151
7	1513.85	64.9121	8	1445.39	69.0182
9	1367.28	65.7572	10	1245.79	68.7464
11	1160.94	55.1845	12	1044.26	82.8026
13	700.998	77.08			

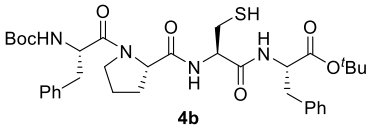


epi-3b

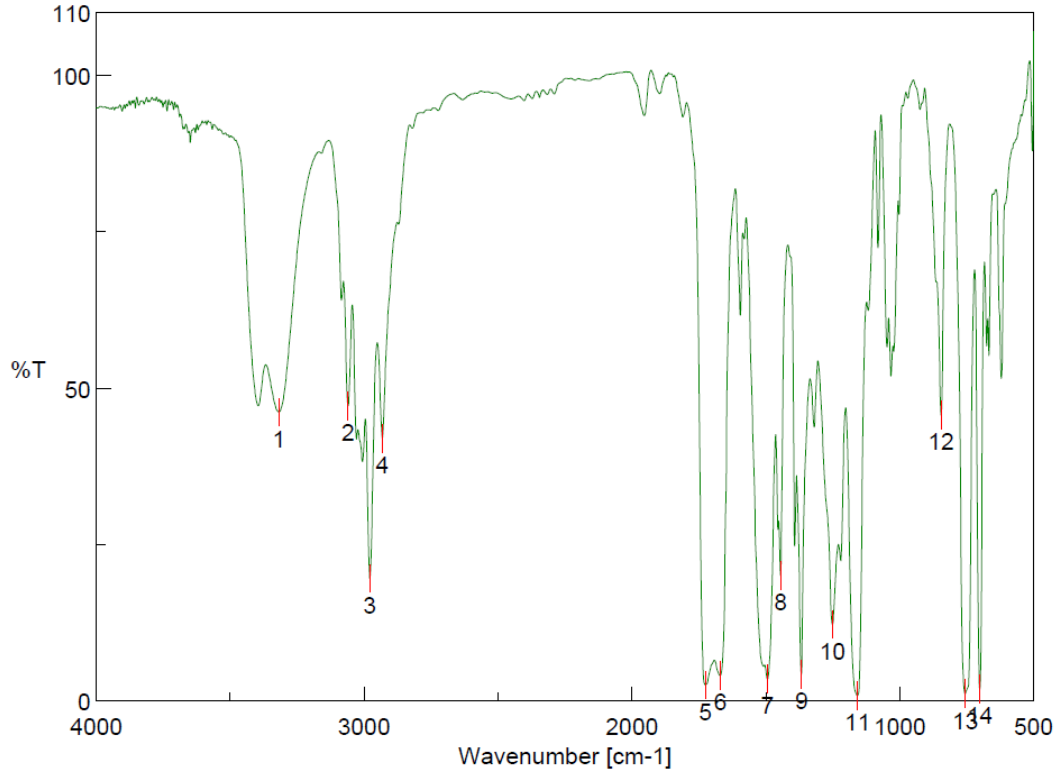


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3298.64	59.1742	2	2977.55	60.0893
3	2546.54	93.3213	4	1708.62	39.5528
5	1635.34	29.8522	6	1519.63	46.1997
7	1454.06	47.7754	8	1367.28	47.7027
9	1248.68	52.7537	10	1161.9	36.5666
11	738.603	72.6202	12	700.998	63.0197

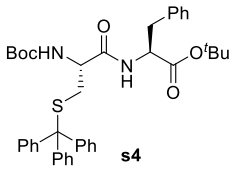


4b

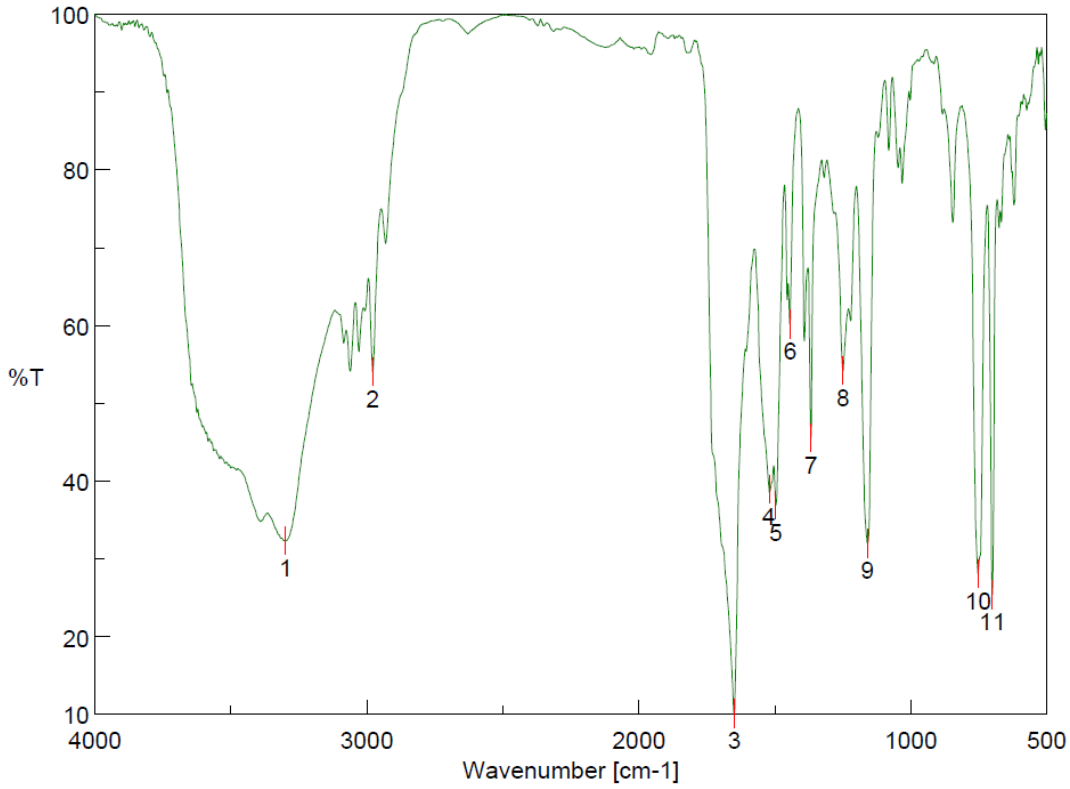


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3318.89	46.1795	2	3060.48	47.1704
3	2978.52	19.5881	4	2932.23	41.9292
5	1725.98	2.60419	6	1670.05	4.11593
7	1494.56	3.5501	8	1445.39	20.1087
9	1367.28	4.2861	10	1251.58	12.2494
11	1158.04	0.900499	12	845.633	45.6829
13	755.959	1.20968	14	700.998	1.91833

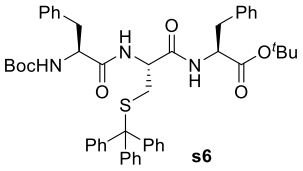


s4

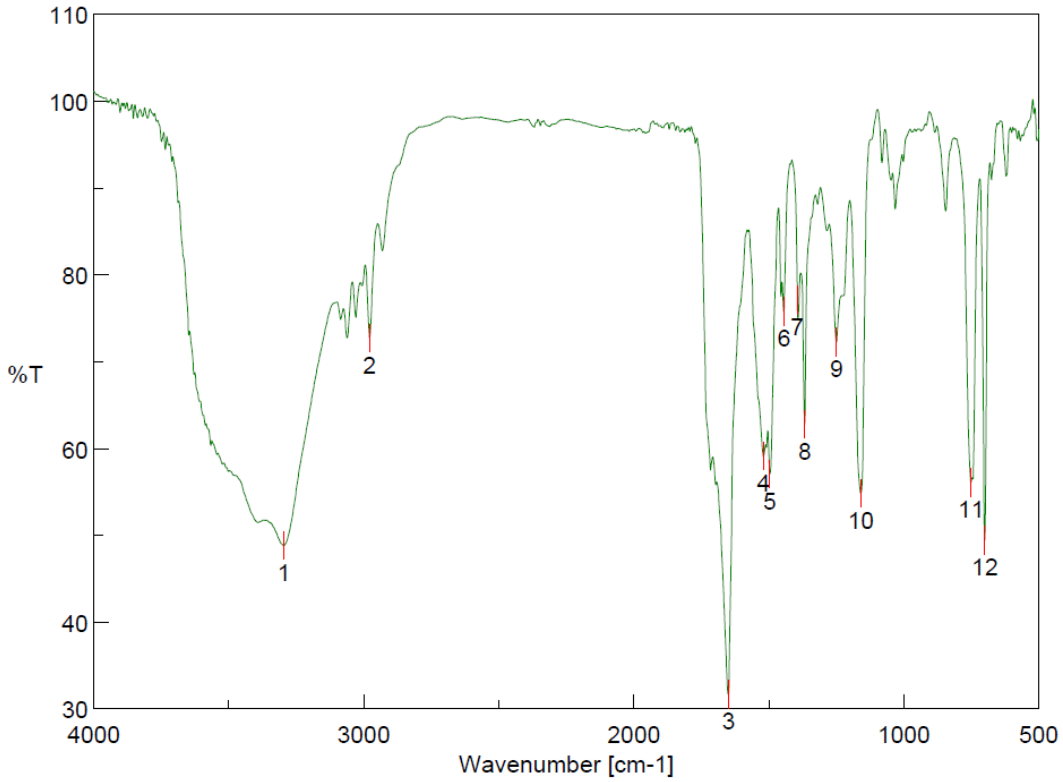


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3298.64	32.3232	2	2978.52	54.0343
3	1649.8	10.1411	4	1521.56	38.9539
5	1496.49	36.8949	6	1444.42	60.1676
7	1367.28	45.5467	8	1249.65	54.2019
9	1159.01	31.9954	10	753.066	28.0999
11	700.034	25.4002			

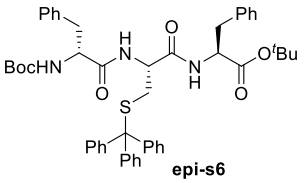


s6

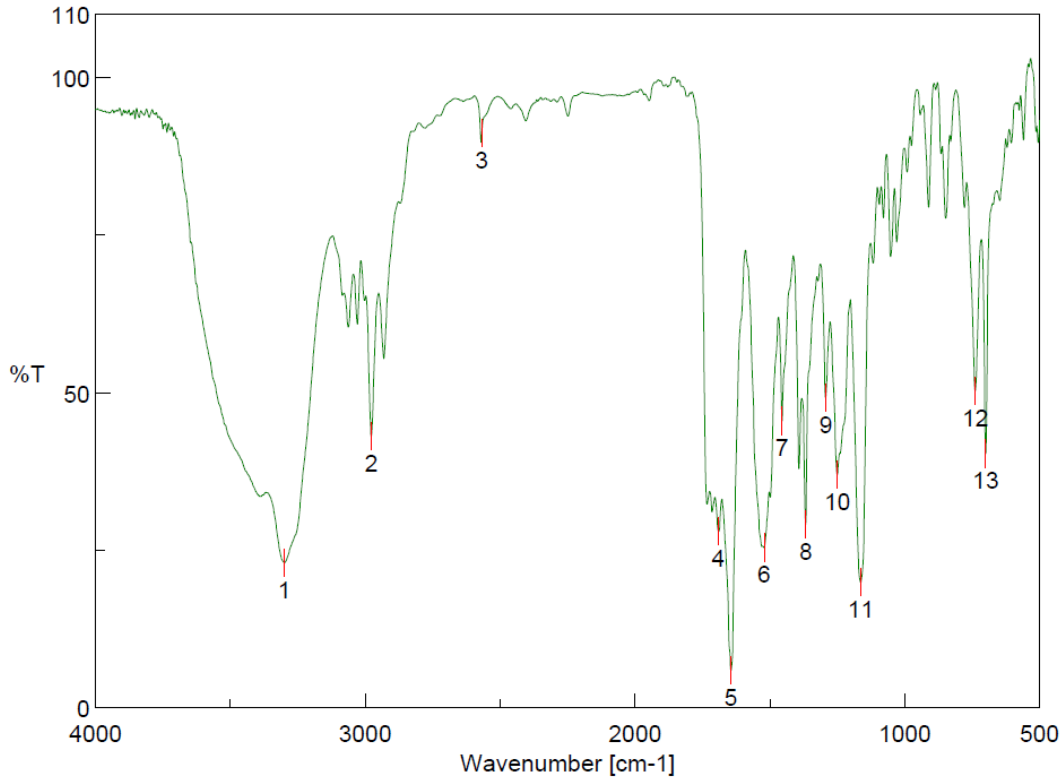


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3296.71	48.7986	2	2978.52	72.7133
3	1649.8	31.6699	4	1518.67	59.1195
5	1496.49	57.05	6	1444.42	75.7496
7	1394.28	77.0267	8	1367.28	62.8064
9	1249.65	72.2567	10	1159.01	54.8333
11	751.138	56.0648	12	700.034	49.3938

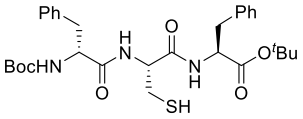


epi-s6

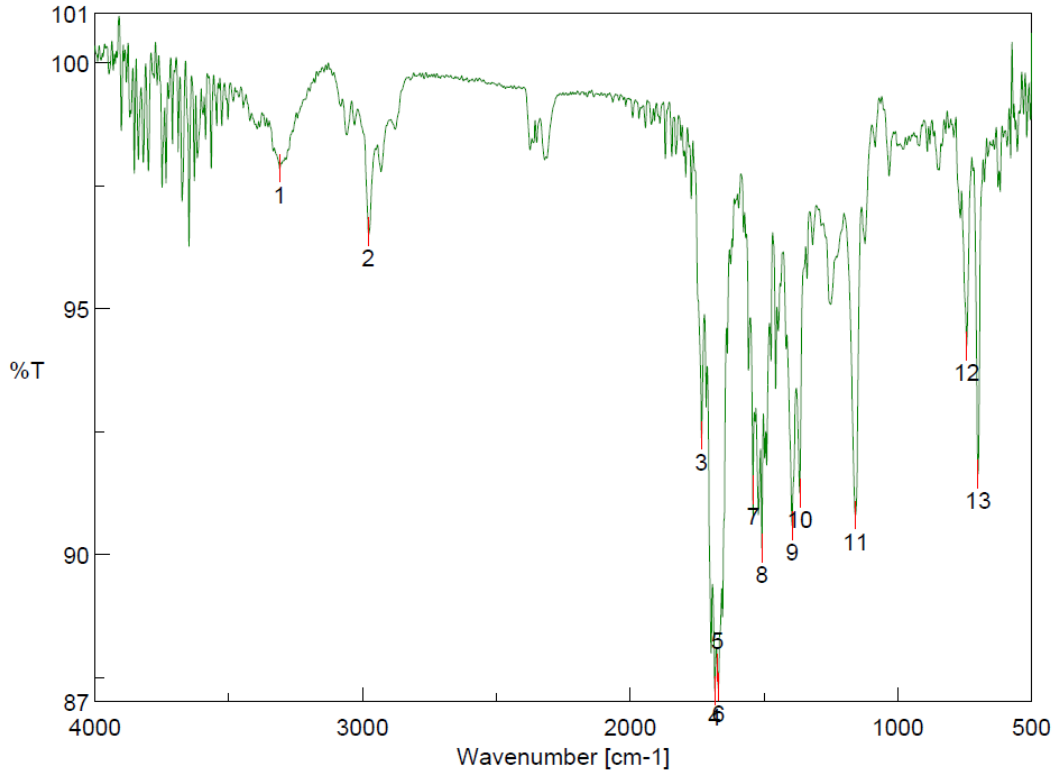


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3299.61	23.0316	2	2977.55	43.0653
3	2567.75	91.1717	4	1691.27	28.0676
5	1644.02	5.9585	6	1521.56	25.4127
7	1455.03	45.4851	8	1367.28	29.135
9	1293.04	49.1594	10	1250.61	37.0033
11	1163.83	19.9427	12	738.603	50.2731
13	700.034	40.3167			

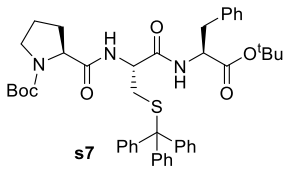


epi-4a

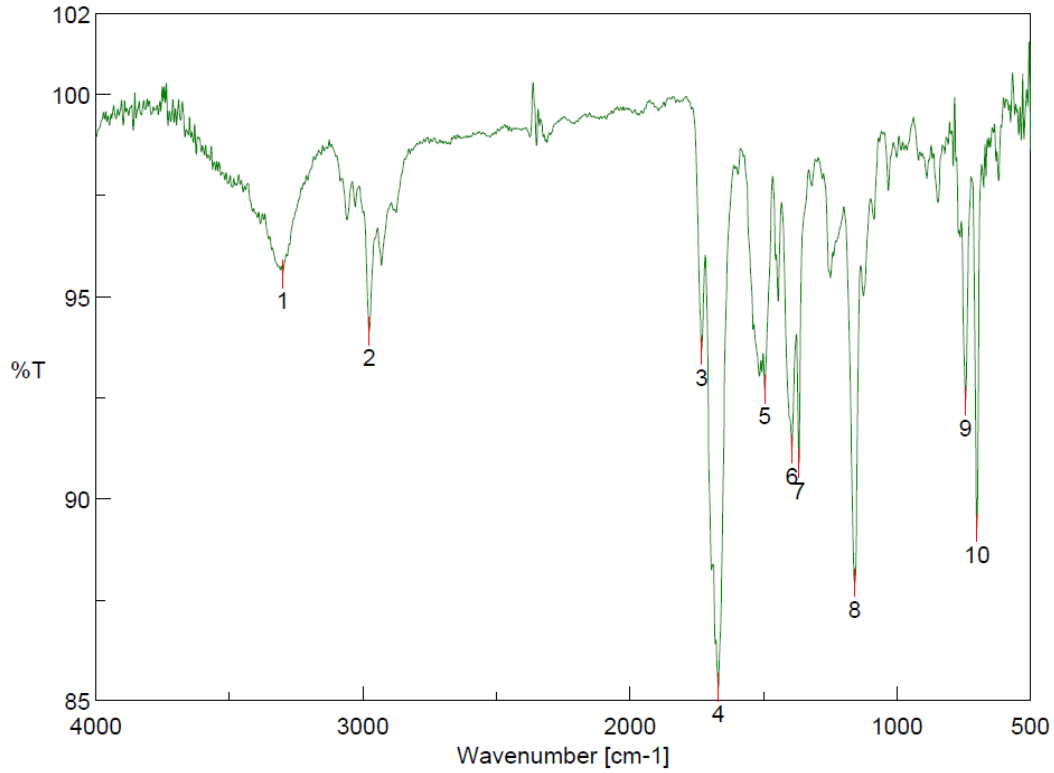


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3310.21	97.8433	2	2978.52	96.5578
3	1733.69	92.4118	4	1683.55	87.223
5	1673.91	87.693	6	1670.05	87.3025
7	1539.88	91.3068	8	1508.06	90.1274
9	1395.25	90.582	10	1366.32	91.2404
11	1158.04	90.7931	12	742.46	94.2291
13	700.034	91.6343			

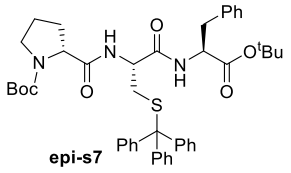


s7

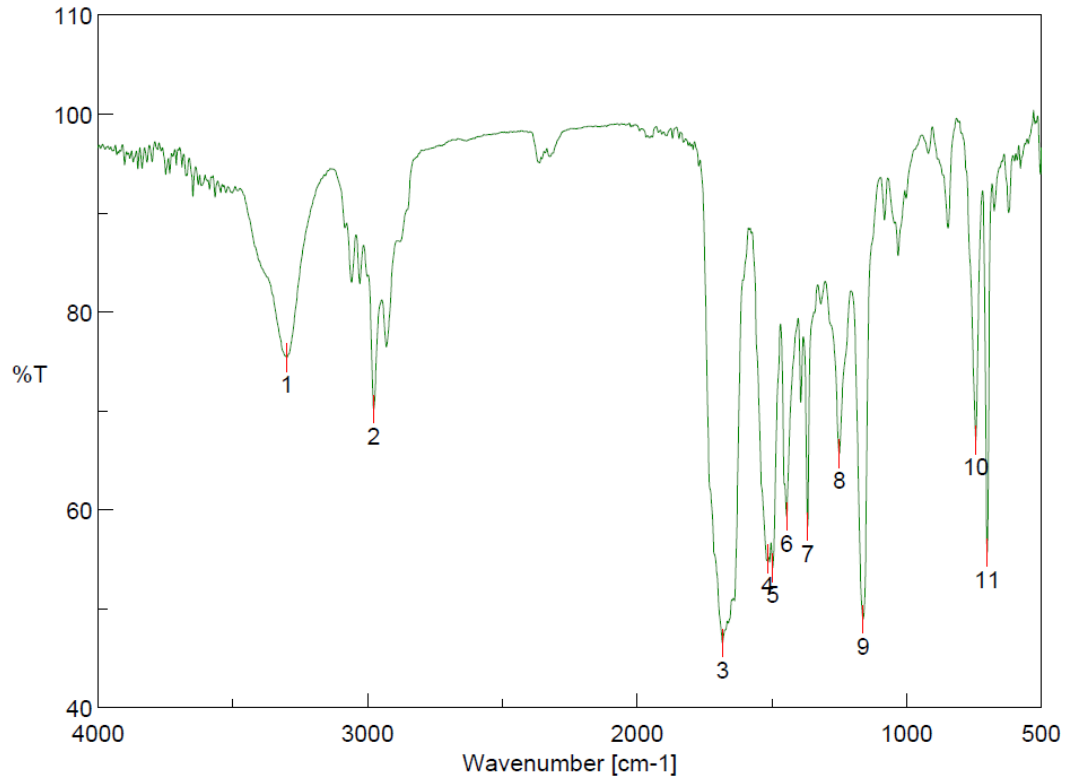


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3299.61	95.5565	2	2976.59	94.1383
3	1731.76	93.661	4	1669.09	85.3287
5	1495.53	92.7038	6	1393.32	91.2209
7	1367.28	90.8678	8	1158.04	87.9136
9	742.46	92.4068	10	700.034	89.2769

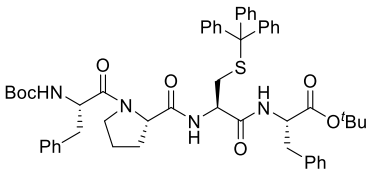


epi-s7

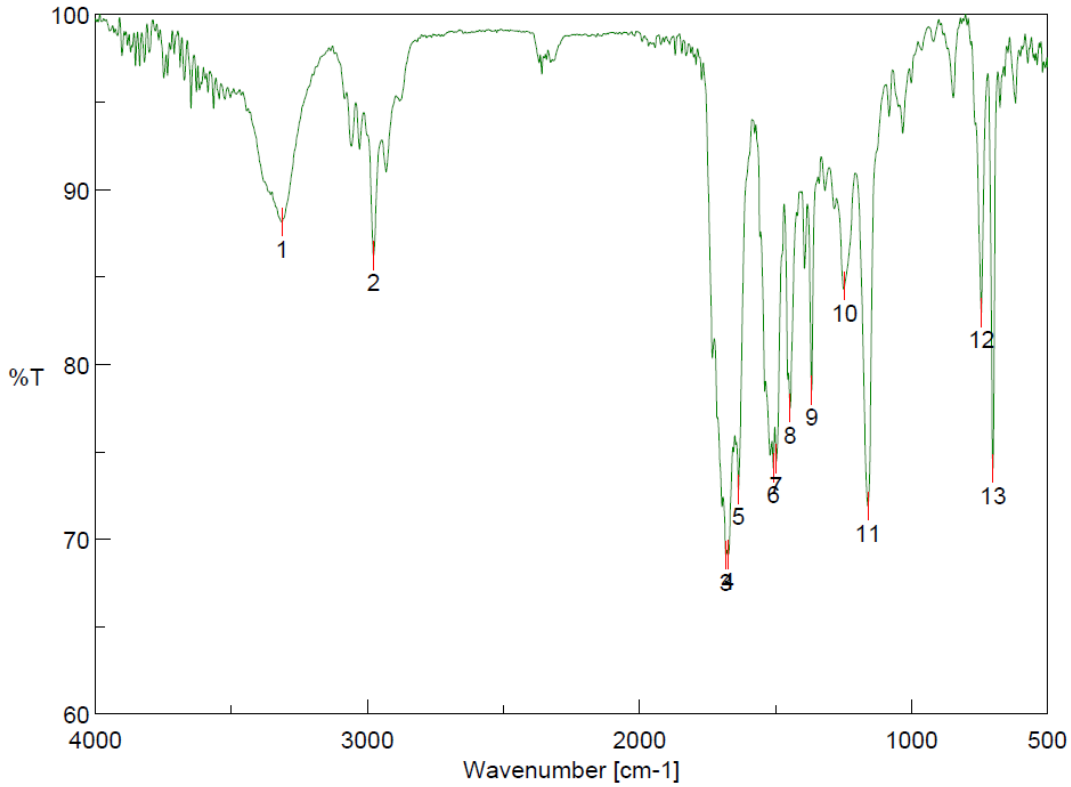


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3299.61	75.3644	2	2977.55	70.1617
3	1682.59	46.5626	4	1514.81	55.0222
5	1496.49	54.1529	6	1445.39	59.3318
7	1367.28	58.2903	8	1249.65	65.6736
9	1160.94	48.9438	10	743.424	67.0094
11	700.998	55.702			

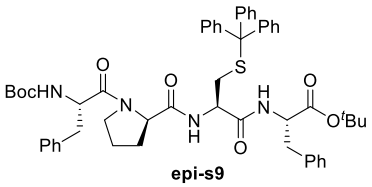


s9

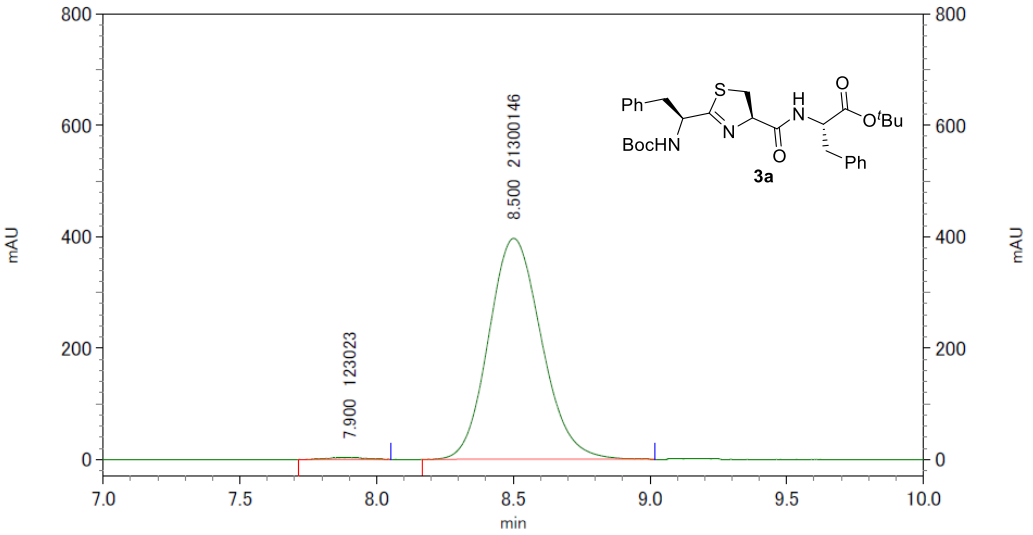


[ピーク検出結果]

No.	位置	強度	No.	位置	強度
1	3315.03	88.1335	2	2977.55	86.2048
3	1682.59	69.0736	4	1673.91	69.1237
5	1635.34	72.8334	6	1508.06	74.0595
7	1497.45	74.596	8	1446.35	77.4936
9	1367.28	78.4853	10	1245.79	84.4707
11	1159.97	71.8985	12	743.424	82.9567
13	700.034	74.0261			



epi-s9

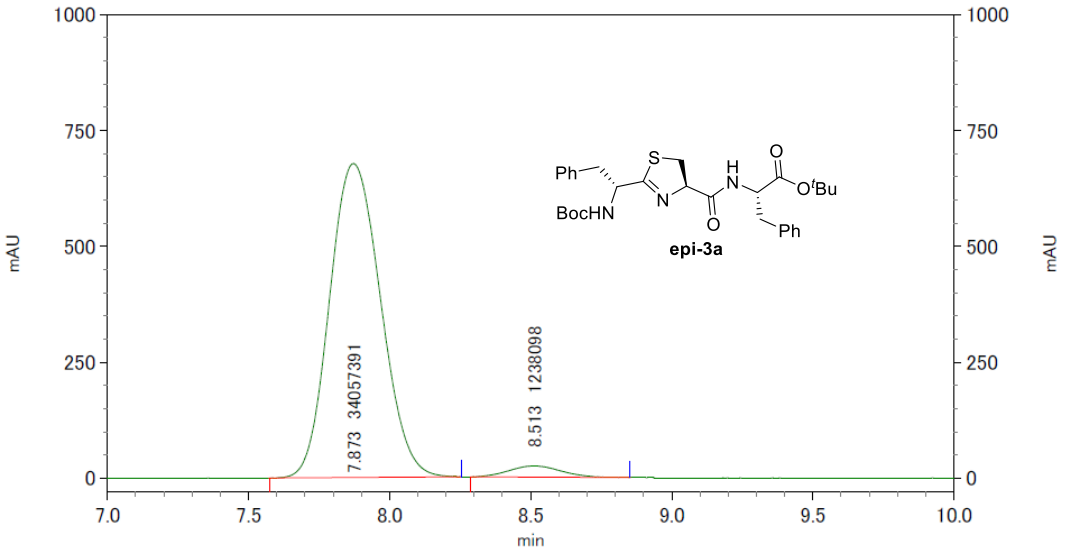


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	7.900	0.574
2	8.500	99.426

トータル	
Total	100.000

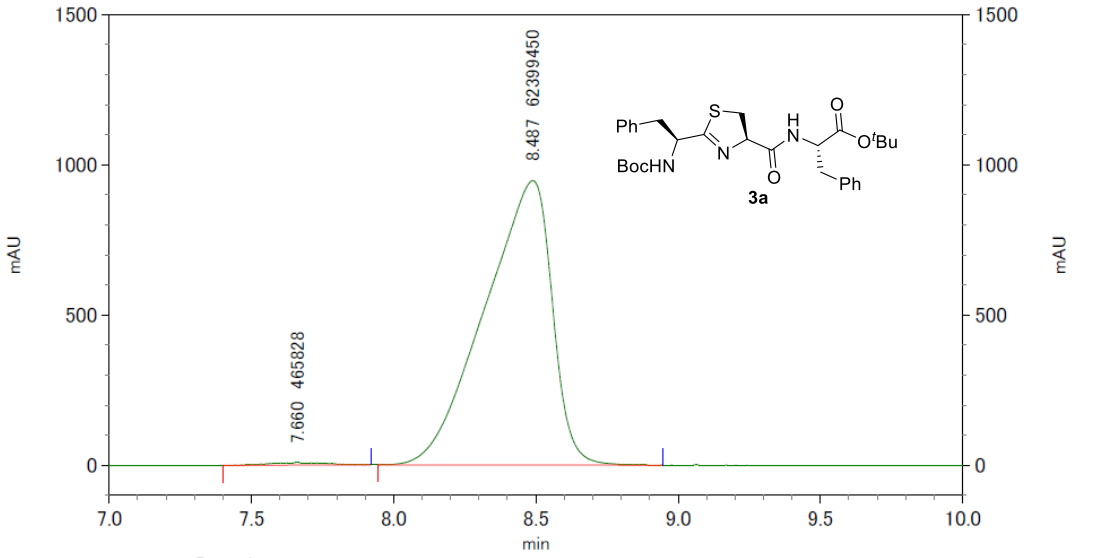


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	7.873	96.492
2	8.513	3.508

トータル	
Total	100.000

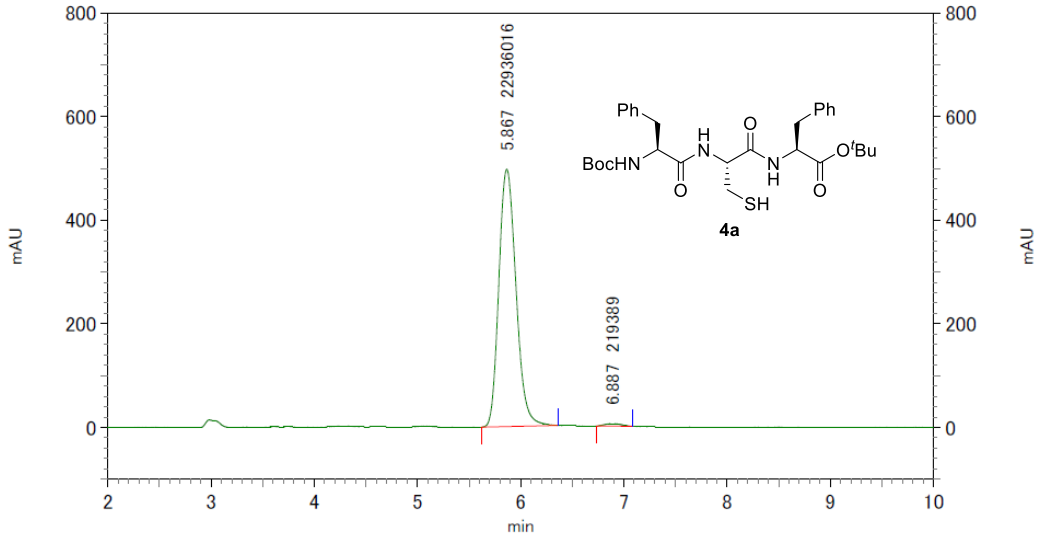


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	7.660	0.741
2	8.487	99.259
トータル		100.000
Total		

HPLC analysis of the crude product **3a** obtained from the described procedure in the supporting information

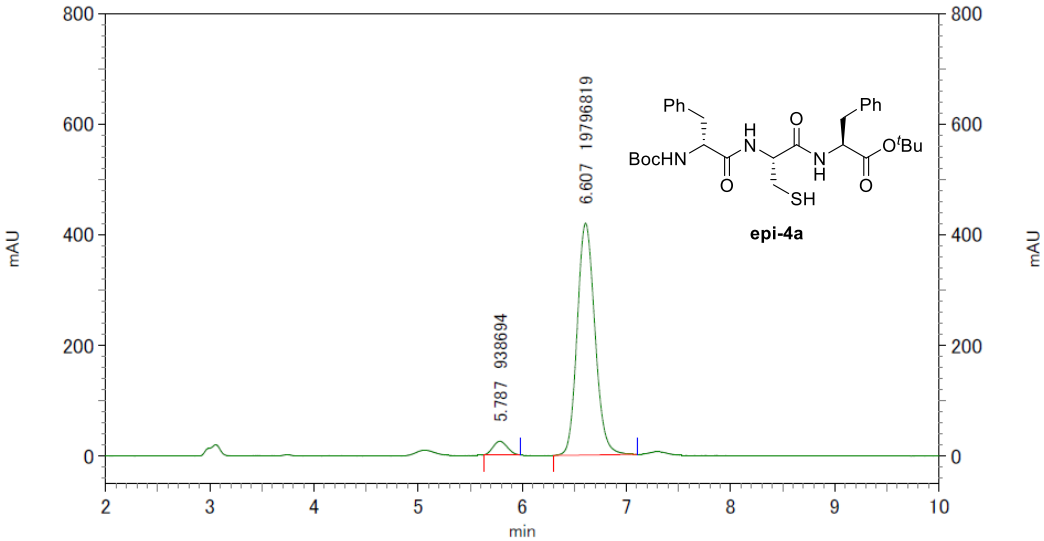


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	5.867	99.053
2	6.887	0.947

トータル	
Total	100.000

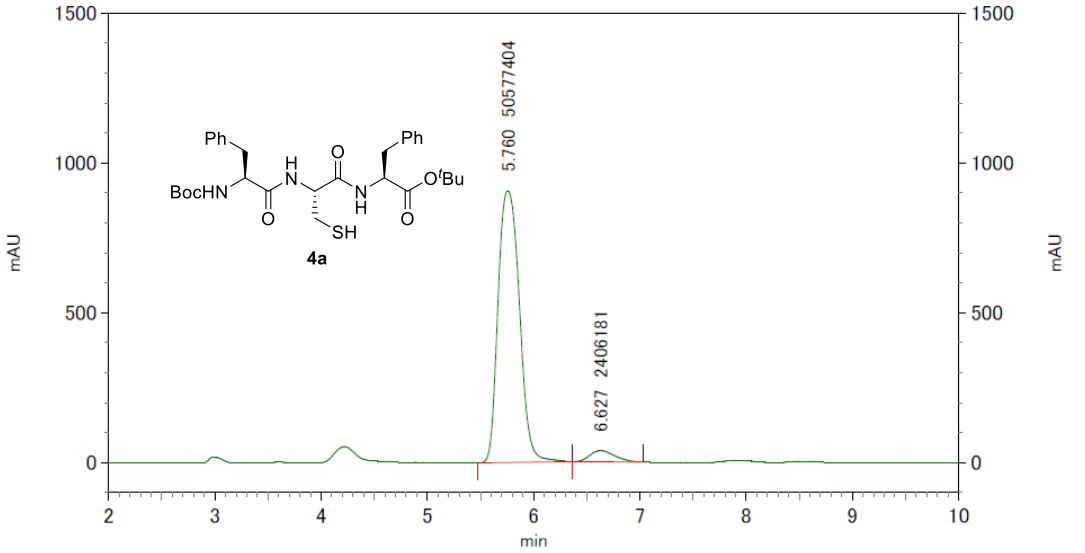


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	5.787	4.527
2	6.607	95.473

トータル	
Total	100.000

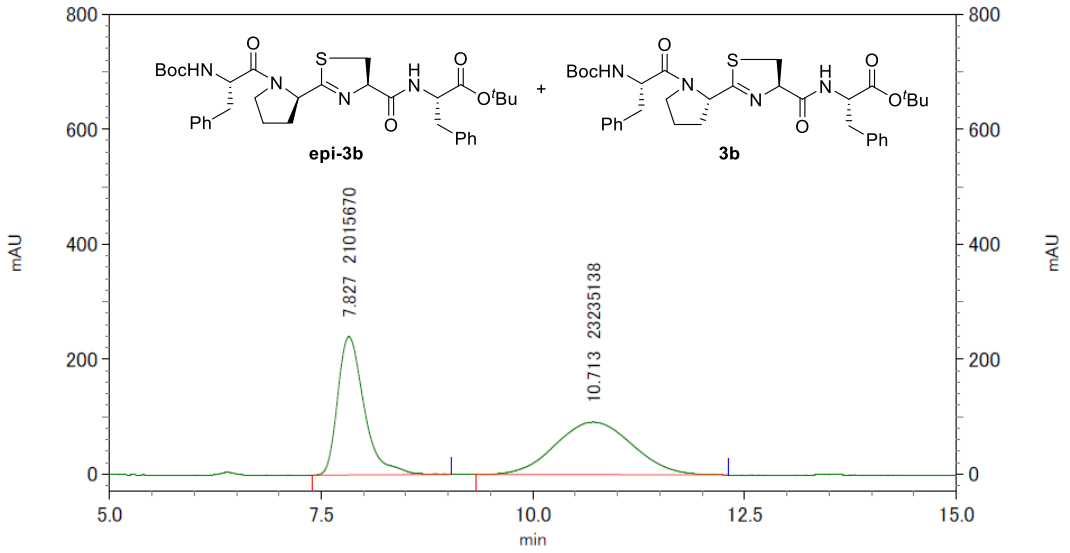


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	5.760	95.459
2	6.627	4.541
トータル Total		100.000

HPLC analysis of the crude product **4a** obtained from the described procedure in the supporting information

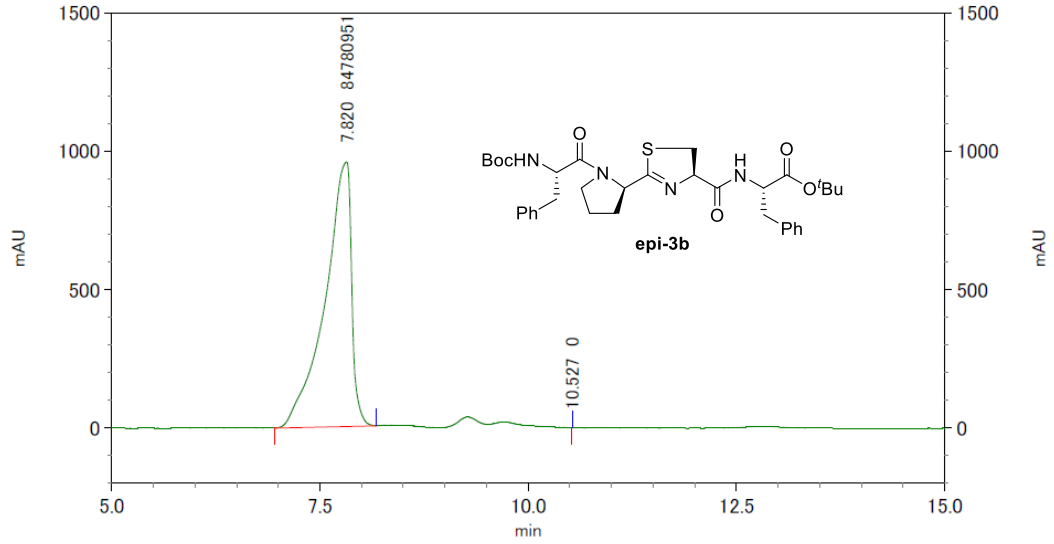


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	7.827	47.492
2	10.713	52.508

トータル	
Total	100.000

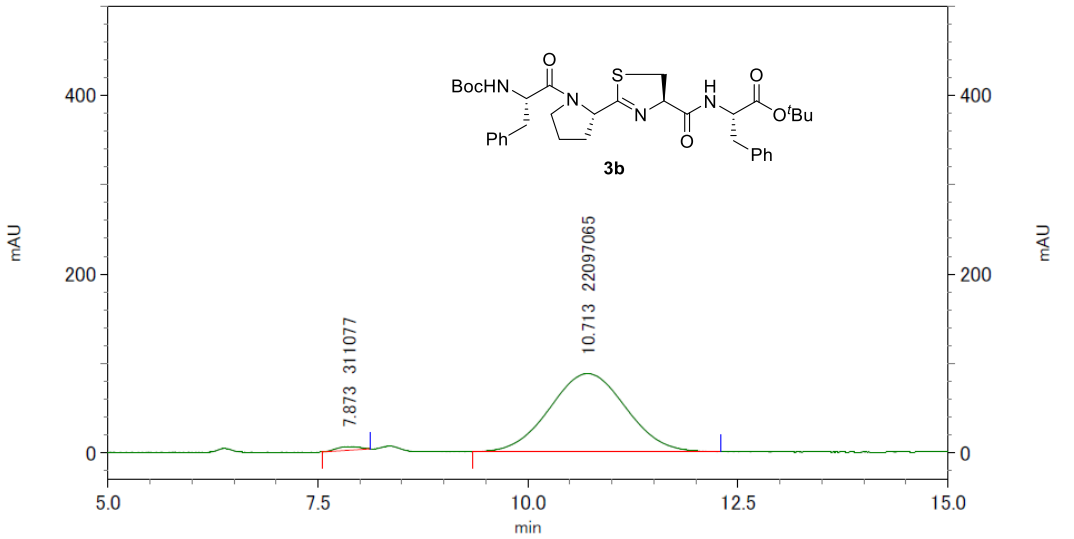


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	7.820	100.000
2	10.527	0.000

トータル	
Total	100.000

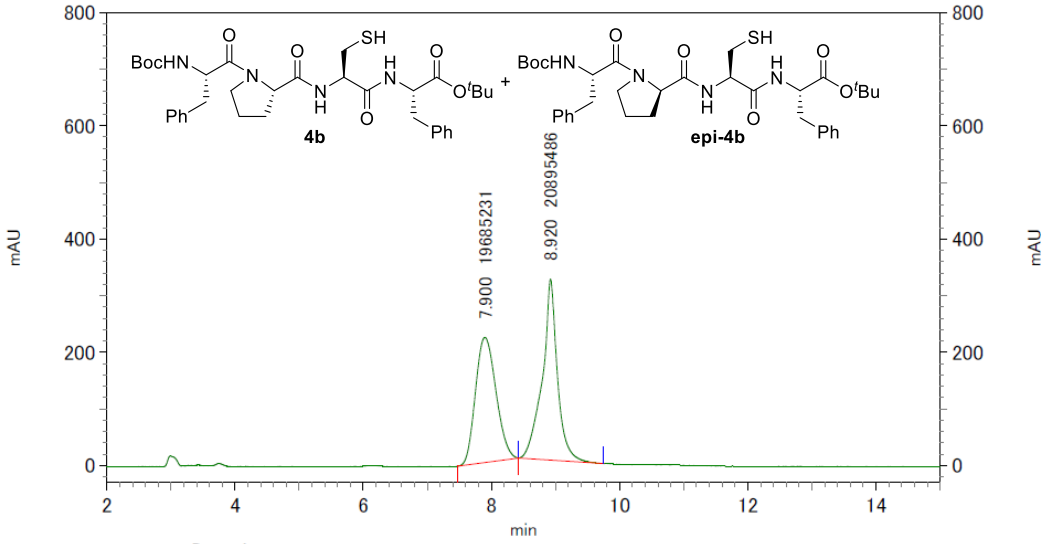


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	7.873	1.388
2	10.713	98.612
トータル		100.000
Total		

HPLC analysis of the purified product **3b** obtained from the described procedure in the supporting information

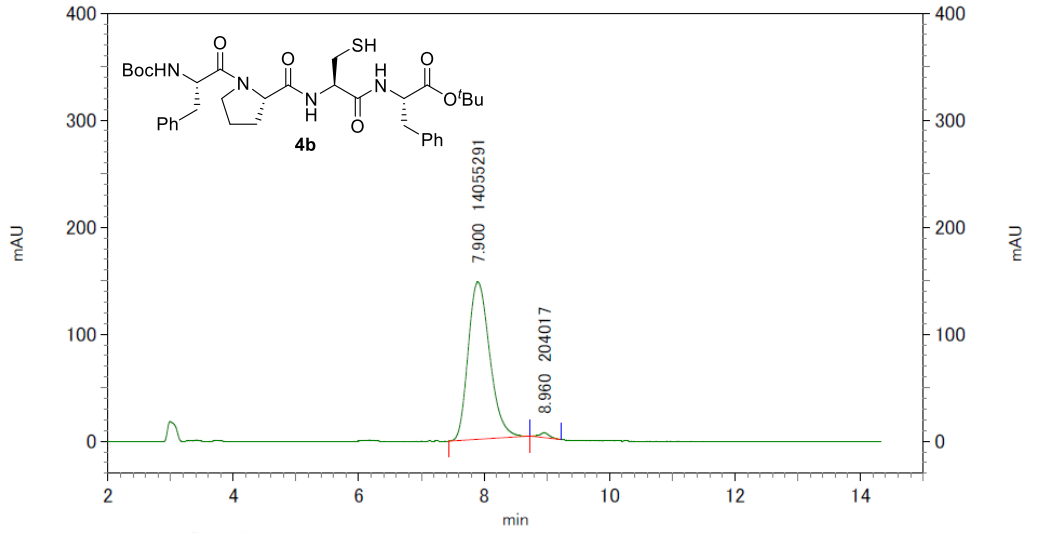


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	7.900	48.509
2	8.920	51.491

トータル	
Total	100.000

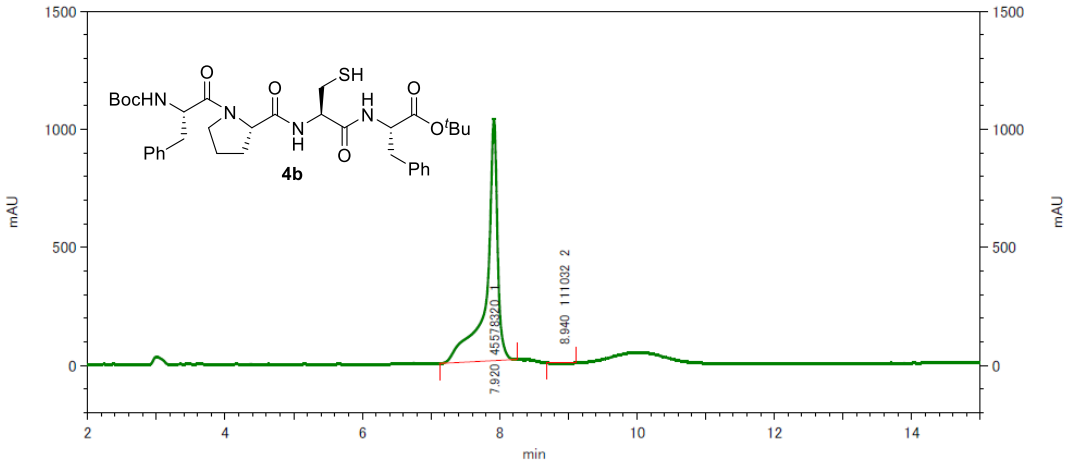


Result

1: 210 nm, 4 nm結果

Pk #	Retention time / min	Integration/ %
1	7.900	98.569
2	8.960	1.431

トータル	
Total	100.000



210 nm, 4 nm
ｽﾍﾟｸﾄﾙﾏｯｸｽﾌﾟﾛｯﾄ結果

Pk #	Retention time/min	Integration%
1	7.920	99.757
2	8.940	0.243
ﾄｰﾀﾙ		100.000

HPLC analysis of the crude product **4b** obtained from the described procedure in the supporting information