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論 文 内 容 要 旨

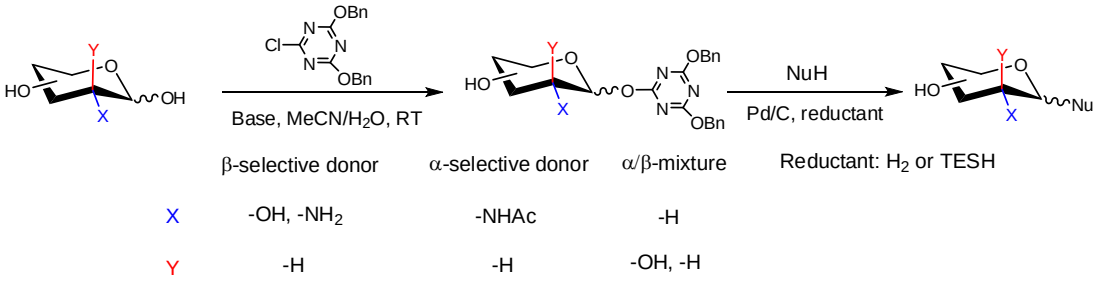
Chapter 1　Introduction

Chemical glycosylation plays crucial role for the synthesis of sugar-containing molecules which have widespread applications in biological and pharmaceutical fields, such as amphiphilic smart gelators, biosurfactants, and drugs. [1] Owing to this reason, series of glycosyl donors (e.g., thioglycoside, glycosyl halides, glycosyl trichloroacetimidates, propargyl glycoside) and advanced promoters (e.g., bismuth(V) compound, chiral phosphoric acid, Au(I)-complex) have been developed offering the efficient and chemoselective glycosylation strategies. [2] However, the multiple hydroxy groups of sugar cause serious synthetic barriers with regard to their selective activation, which usually requires laborious protection-deprotection manipulations or the strict compliance with special reaction conditions. In addition, the general side-reaction induced by acidic promoters including the hydrolysis of the inner glycosidic bond of glycosyl donors is another trouble in carbohydrate chemistry. Herein, we developed a novel chemical glycosylation with triazinyl glycosyl donors which proceeds under Pd-catalyzed reductive conditions. The triazinyl glycoside donors were directly synthesized from unprotected sugars in aqueous solutions. The subsequent glycosylation reactions were carried out with these triazinyl glycoside donors and series of alcohols under Pd-catalyzed reductive conditions, giving rise to the corresponding glycoside products stereo-selectively in good yields. Noteworthy, the oligosaccharides can be coupled with alcohols successfully without affecting their inner glycosidic bonds under this mild promoter conditions.

Chapter 2　Pd-induced glycosylation with unprotected DBT-glycosyl donors

The syntheses of 4,6-dibenzyloxy-1,3,5-triazin-2-yl glycoside (DBT-glycoside) donors were carried out through a nucleophilic substitution with unprotected sugars in the presence of base under aqueous media, giving rise to the corresponding DBT-glycosides in good yields (60%~80%) (Scheme 1). Excellent stereoselectivity was also achieved for these novel donors after the simple separation. The neighboring (C-2) groups on sugars play critical roles for the resulting stereoselectivity. Herein,

the β-selective glycosyl donors were obtained using sugars with an equatorial hydroxy group or amino group. Meanwhile, the corresponding DBT-α-glycosides were converted to the 1,2-$(DBT)_2$-α-glycoside derivatives through the further substitution. An acceptable explanation is that the hydrogen bond interactions between the triazine and neighboring -OH facilitates the further substitution for these 1,2-*cis* DBT-glycosides. In addition, with *N*-acetyl glucosamine, completely α-selective donors were synthesized with the removal of sugar oxazoline generated from the β-type product. The anomeric mixture was generated using the sugars with an axial neighboring OH (such as mannose). The DBT-glycosides of higher oligosaccharides such as melibiose, lactose, cellobiose and maltopentaose were also prepared without affecting their inner glycosidic bonds. The corresponding stereoselectivity of the achieved DBT-oligosaccharides were determined by their terminal sugars.



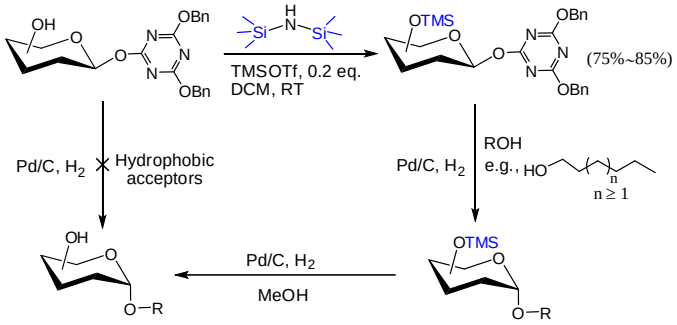
Scheme 1 Synthesis of DBT-glycoside donors for the Pd-induced reductive glycosylation

Glycosylation of the resulting DBT-glycosides was carried out in various alcohols under Pd-catalyzed hydrogenolysis conditions, giving rise to the corresponding glycoside products in good yields. When primary alcohols were used as acceptors, the high stereoselectivity was observed, suggesting an S_N2-like reaction course. The α-selective glycoside products were synthesized with the DBT-β-glycoside donors. Inversely, the coupling reaction using DBT-α-glycosides caused the β-type products. With triethylsilane (TESH) as the reductant instead of H_2, the unsaturated alcohols can be glycosylated in the absence of the hydrogen addition on the unsaturated bond. On the other hand, the longer reaction time was spent in case of using secondary or tertiary alcohols as the acceptors due to their lower nucleophilicity. The present method of hydrogenolysis allows even an acid-labile oligosaccharide to be glycosylated without cleavage of the inner glycosidic bonds.

Chapter 3 Glycosylation with silylated DBT-glycosyl donors

A convenient and mild glycosylation method has been established based on the Pd-induced reductive debenzylation. However, the DBT-glycosyl donors usually suffer from low solubility in hydrophobic solvent, causing the failed reaction in the coupling with long-chain alcohols. To improve the solubility of DBT-glycosides, the protecting groups, or to be more accurate, the solubilizing groups were introduced to the DBT-glycoside donors. In our experiments, the trimethyl silyl groups (TMS) were introduced to the DBT-glycosides to improve their solubility in hydrophobic alcohols or organic solvents (Scheme 2). These silylated glycosyl donors have proven to be especially useful starting materials in the coupling with hydrophobic alcohols. More importantly, the silyl groups could be removed under the same reductive conditions by the addition of methanol

to the reaction mixture upon glycosylation finished, affording a one-pot process including glycosylation and deprotection. In addition, the improved stereo-selectivity was also observed in the glycosylation with silylated donors, highlighting another advantage of introducing TMS solubilizing group.

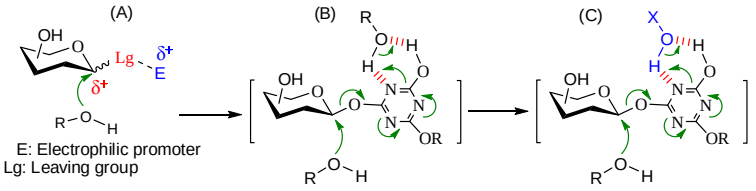


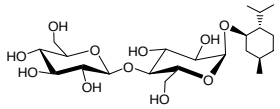
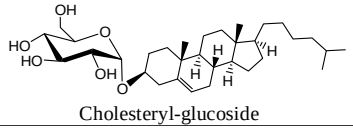
Scheme 2 Coupling of sugars and hydrophobic alcohols with the silylated DBT-glycoside donors

Chapter 4 Additive-assisted glycosylation reaction

In most chemical glycosylations, activation of the leaving group (Lg) with an electrophilic promoter (E^+) is following by nucleophilic attack of the acceptor (ROH), resulting electro-deficient anomeric carbon of glycosyl donor (Table 1, A). The interaction between leaving group and promoter is the trigger for the efficient cleavage of Lg, leading to the coupling reaction. In our reductive glycosylation, the Pd-catalyzed hydrogenolysis just promoted the debenzylation, giving rise to the 4,6-dihydroxy-1,3,5-triazn-2-yl (DHT) glycosyl intermediate. The subsequent substitution reaction was speculated to be activated by the H-bond interactions between the alcohol acceptors and DHT-glycosides (Table 1, B), causing the tremendous differences in the coupling results with good nucleophiles (primary alcohols) and poor nucleophiles (secondary and tertiary alcohols). Usually, the long reaction time and poor yield were observed using secondary and tertiary alcohols. Therefore, the H-bond donating additives (XOH), such as chloral or hexafluoroisopropanol (HFIP), were employed in the reductive glycosylation to support stronger H-bond interactions (Table 1, C). Compared with the blank case, the improvement including the reaction rate and yield was noticed under the additive-assisted coupling reaction (Table 1, entries 2 and 4). The upfield shift of the anomeric proton was found in the mixture of amine-stabilized DHT-glycosides and additives by the ^{1}HNMR analysis, suggesting the reactivity of DHT-glycosyl intermediate was improved with the utilization of H-bond donating additives. [3] In addition, the desilylation side-reaction was noticed with the hydrogenolytic glycosylation when employing the chloral or hexachloroacetone as the additive. Sometimes, a deceased stereoselectivity occurred owing to the additive-induced desilylation effect.

Table 1 Comparison of the reductive glycosylation with or without additives

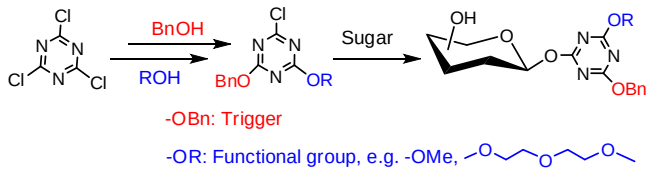


Entry	Product	Additive (1.0 *equiv*)	Time (h)	Yield (%)	α/β
1	 Menthyl-cellobioside	-	168	38	10/1
2		CCl_3CHO	24	60	α only
3	 Cholesteryl-glucoside	-	48	-	-
4		CCl_3CHO	48	40	α only

Chapter 5 Glycosylation with modified triazinyl glycosides

Dibenzyloxy triazine (DBT) behaved as an excellent leaving group in the hydrogenolytic glycosylation. We speculated that the non-symmetric triazine with only one benzyloxy group also can be used as the efficient leaving group for the reductive glycosylation. Another site on triazine could be modified with some functional groups. Hence, on the basis of this mon-symmetric concept, we reported some modified triazinyl leaving groups synthesized through the nucleophilic substitution of cyanuric chloride with different alcohols (Scheme 3). Herein, the syntheses of hydrophilic 4-(2-(2-methoxy ethoxy) ethoxy)-6-benzyloxy-1,3,5-triazin-2-yl (MEEBT) glycoside donors were carried out in aqueous solution without using any organic solvent, affording a green activation pathway. Moreover, the glycosylation of MEEBT-maltopentaoside proceeded smoothly because of the improved solubility. By contrast, the DBT-maltopentaoside was failed to be glycosylated due to its limited solubility. Despite the fact that these non-symmetric triazinyl glycoside donors behaved lower reactivity than DBT-glycoside donors, the employment of H-bond donating additives was proved to optimize this limitation. The non-symmetric triazinyl leaving groups offered new avenues for the design of direct glycosylation methods and allowed the activation of sugars proceeding under greener conditions.

Scheme 3 Synthesis of modified glycoside donors with asymmetric leaving groups



Chapter 6 Summary

A novel glycosylation reaction has been established under the Pd-catalyzed hydrogenolysis conditions. [4] The new glycosyl donors, DBT-glycosides, were prepared from unprotected sugars in aqueous media. Excellent stereoselectivity was also achieved in the syntheses of donors with the removal of 1,2-$(DBT)_2$-α-glycosides or sugar oxazoline. The neighboring group of sugar was convinced to play key roles for the resulting stereoselectivity. The acid-labile inner glycosidic bonds in oligosaccharides were not affected under the reductive conditions. Some complex glycosides were synthesized through the improved strategy by using silyl solubilizing groups and H-bond donating additives. Solubility of the glycosyl donors can also be regulated by the non-symmetric leaving groups. It is hoped that these new results will assist glycochemists in designing new glycosylation methodologies and develop the library of glycosyl donors that can be activated without adding any acid promoters.

References

[1] D. P. Galonic, D. Y. Gin, *Nature*, **2007**, 446, 1000-1007.

[2] X. M. Zhu, R. R. Schmidt, *Angew. Chem. Int. Ed.* **2009**, 48, 1900-1934.

[3] X. Liang, H. Bin, J. Yang, *Org. Lett.* **2013**, 15, 2834-2837.

[4] M. Ishihara, Y. Takagi, G. Li, M. Noguchi, S. Shoda, *Chem. Lett.* **2013**, 42, 1235-1237.

論文審査結果の要旨

糖鎖は癌、分化、免疫、感染症など，様々な領域において重要な役割を果たす分子群である。糖鎖関連の研究を進める上で必須となる技術が、糖を他の分子へ結合させる配糖化（グリコシル化）反応であるが、糖はその分子中に多数のヒドロキシ基を有するため、保護・脱保護を含む多段階の工程を要するという問題があった。一方、保護基を用いない糖の化学変換法としては、近年、論文著者が所属する研究室にて報告された、トリアジン型縮合剤を用いて無保護糖の直接活性化する方法がある。また、従来の直接配糖化反応においては、酸触媒存在下、大過剰のアルコール中で長時間加熱する必要があるため、基質が制限されることや．副反応の制御が困難であるという問題があった。本論文では、無保護糖の直接活性化を基礎とした温和な条件下、新たな配糖化反応の開発を試みた。本論文は全編６章より構成されている。

第１章は序論として、本研究の背景となる糖鎖分子（糖脂質）の応用及び配糖化反応の現状について解説し、さらに本反応発見の歴史について述べている。

第２章、2-chloro-4,6-dibenzyloxy-1,3,5-triazine (CDBT) を用いる新規な糖トリアジン誘導体を無保護糖から一段階で合成する反応を開発した結果について述べている。原料糖の２位にエカトリアルの OH 基を有する場合、得られた主生成物のアノマー位の立体は β 型であった。一方、原料糖の２位にアキシアルの OH 基を有る場合、α/β 混合型の糖トリアジン誘導体が得られた。また、糖の２位にアセチルアミノ基を有する場合、得られた糖誘導体のアノマー位の立体は α 型であった。続いて、トリアジン糖誘導体を、種々のアルコールをアクセプター兼溶媒として用い、Pd/C 触媒を用いる接触還元によるグリコシル化を行った。本反応は，トリアジン環上のベンジル基を接触還元により，対応するヒドロキシ基へと変換し，その活性中間体を利用して，配糖化反応が進行する。糖鎖構造、アクセプター求核性、溶媒、温度度等に関して詳細に条件検討を行った結果について述べている。

第３章はトリメチルシリル基を可溶化基としてトリアジン糖誘導体に導入し、疎水性アルコールと有機溶媒への溶解性を高めた糖ドナーを用いるグリコシル化プロセス検討をしている。更に、可溶化基の種類、溶媒、アクセプター当量等が配糖化反応の収率及び立体選択性に与える影響について詳しく調べている。

第４章は第二級アルコール，第三級アルコールといった、反応性の低いアルコールを求核剤として，水素結合を供与する添加剤が，配糖化反応の速度ならびに立体選択性（α/β）に与える影響について詳細に調べている。また、還元条件下、配糖化反応を促進する種々の因子について検討を行い、添加剤の種類が大きな影響を与えることを明らかにしている。

第５章は非対称のトリアジン糖誘導体合成と、それを供与体として用いる配糖化反応の検討について述べている。本修飾法の開発により、純水中、無保護糖の直接活性化が可能性であることが示されている。

第６章は総括である。

以上要するに本論文では、パラジウム触媒を用いる還元条件下、これまで合成困難であった糖脂質について、無保護糖からの合成が可能であることを実証したもので、糖質材料における新たな学問領域の創出に寄与するところが少なくない。

よって、本論文は博士(工学)の学位論文として合格と認める。