Establishment of Synthesis Method of Molecular Probe Containing Carbohydrate Ligand and Photoreactive Group for Elucidation of Carbohydrate – Lectin Interactions by Glycosyl Trichloroacetoimidate Strategy

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Abstract

Photoaffinity labeling technology is the most efficient method for elucidation of carbohydrate – lectin interactions. However, when carbohydrate probes are synthesized according to conventional this method, the reducing terminus of the sugar is opened to provide an acyclic structure. We were undertaken to solve this problem, and developed new molecular probe containing complete oligosaccharide structure (D-lactose) and phenyldiazirine group for elucidation of carbohydrate – lectin interactions. Next problem, establishment of synthesis method of molecular probe is important subject because carbohydrates are composed of various complex structures. We tried synthesis of oligosaccharides using orthogonal glycosylation strategy. But when glycosyl donor and glycosyl acceptor were coupled by promoter, leaveing group of glycosyl donor (-F group) was substituted to leaving group of glycosyl acceptor (-SPh group). We synthesized Galp–GlcpNAc disaccharide derivatives using glycosyl trichloroacetoimidate strategy, and then were obtained 3 types of molecular probes containing ligand and photoreactive group (1, 2, 3).

Key words : Photoaffinity Labeling, carbohydrate-lectin interaction, orthogonal glycosylation strategy, Glycosyl trichloroacetoimidate strategy, molecular probe

Introduction

Glycosphingolipids and glycoproteins in cell membranes are thought to play especially important roles in a variety of biological events such as extracellular recognition, cell-cell interaction, differentiation, oncogenesis and immunity[1-2]. Understanding of the role of carbohydrates could lead to the elucidation of many disease mechanisms, but carbohydrates often bind with low affinity to lectins[3]. If these problems are addressed, elucidation of the functions of carbohydrates will be accelerated. Formation of a covalent bond to a photoreactive group allows one to maintain the complex between the ligand and its binding protein even under denaturing conditions (Fig. 1)[4].

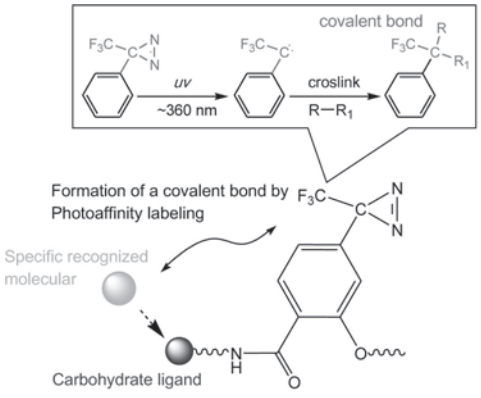

Figure 1. Identification of specific binding moleculars for ligands by photoaffinity labeling.

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Thus, photoreactive groups function as a powerful hook in fishing for specific binding proteins. The use of the phenyldiazirine group, one of these photoreactive groups, seems promising for achieving efficient crosslinking[6]. We developed new molecular tools containing oligosaccharide structure and phenyldiazirine group for elucidation of carbohydrate – protein interactions[7]. Character of new technology is that between carbohydrate ligand and photoreactive diazirine group are joined linker which formed short alkyl chain. This molecular tool have 3 components containing recognition part (carbohydrate ligand), identification part (phenyl diazirine group), and detection part (biotinyl group) (Fig. 2).

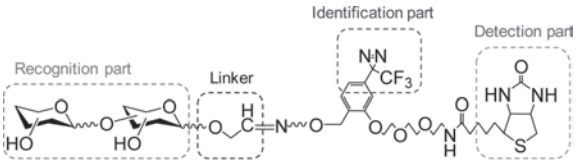


Figure 2. Synthesis of molecular probe by Photoaffinity Labeling.

However, carbohydrates, in contrast with amino acids and nucleotides, are composed of various complex structures because the configuration of each hydroxyl group, anomeric configuration to be formed in glycosylation reactions and the branching1). We were undertaken to solve this problem, and considered if Photoafinity Labeling is applied to them, it will be lead to elucidation of their functions. Photoafinity Labeling enable to search for functions of the all rounds carbohydrate – binding lectin by substitution of carbohydrate ligand. Thus, we considered that establish of synthesis method of molecular probe is important subject, and tried to development.

Disaccharides derivatives which are composed D–galactose (Gal*p*) and D–Glucosamine (Glc*p*NAc) have 3 kinds of different β–bond formations, namely 1 → 3, 1 → 4, and 1 → 6. They are recognized by differently specific lectins, Their results mean what many kinds of oligosaccharides in nature have differently informations and play independent roles on cell surfaces. We consider that dissection of potential recognition by different of glycosidic formations are very important subject for elucidation of carbohydrates – lectins interactions.

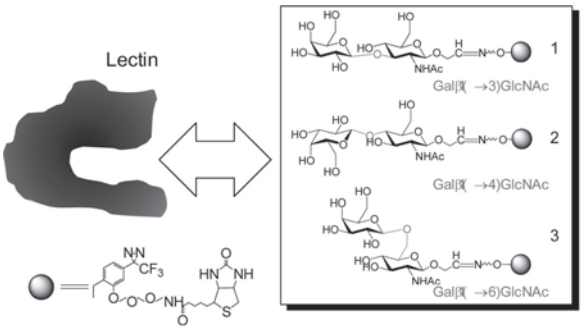


Figure 3. Dissection of potential recognition of Gal-GlcNAc derivatives (**1**, **2**, **3**).

This article tried synthesis of molecular tools containing Galp–GlcpNAc disaccharide derivatives (1, 2, 3) by different glycosidic formations (Fig. 3). Their results will be led to develop of molecular probe containing complex carbohydrate ligand.

Result and Discussion

Orthogonal glycosylation strategy[7]

The synthesis strategy is the most important component for synthesis of target compound. The orthogonal glyocylation strategy is the most straightforward method, and is the direct use of glycosylation products as donor for the next coupling reaction. The concept of this is (1) X should be unaffected under condition b) requires to activate other donor (i.e., Y), and (2) both X and Y should remain compatible with subsequent manipulations of temporary protecting groups (Fig. 4). We selected the phenylthio group for X and fluoride for Y as the leaving groups, and NIS – TfOH[8] (condition a)) and Cp_2HfCl_2 – AgOTf[9] (condition b)) as promoters.

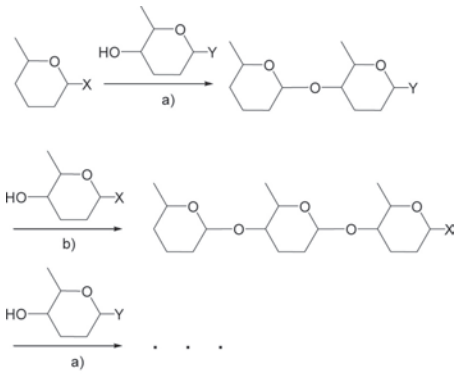
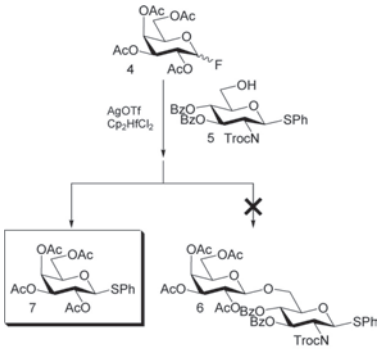


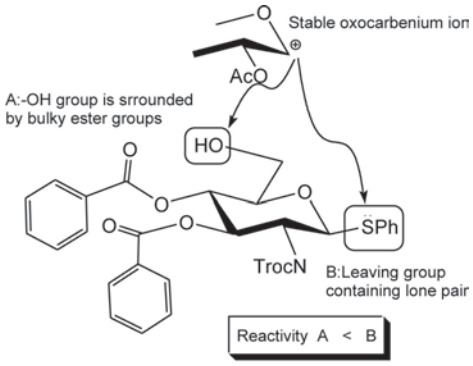
Figure 4. Orthogonal glycosylation strategy

We tried synthesis of disaccharide derivatives according to this strategy. Fluoro glycosyl derivative 4[10] as glycosyl donor was treated with thio glycosyl derivative 5[11] as glycosyl acceptor, by using Cp_2HfCl_2 – AgOTf as the glycosylation promoter. After the reaction mixture was completed as indicated by TLC monitoring, and purified by silica gel column chromatography to give pure product in 85% yield. But this product which was analyzed by ^{1}H–NMR, 13C-NMR was not confirmed disaccharide derivative 6. This reaction was only obtained monosaccharide derivative 7 (Scheme 1.).



Scheme 1.

Anomeric center of compound 7 was confirmed that –F group of 4 was exchanged to –SPh group of 5 by ^{1}H–NMR, ^{13}C-NMR. Glycosyl donor and acceptor containing per acetate or benzoate groups are low reactivity in general because ester group has effect of electron withdrawing (EWG)[12]. Fluoro glycosyl derivative 4 which was activated by Cp_2HfCl_2 – AgOTf was led to oxocarbenium ion. When –OH group of thio glycosyl derivative 5 is attacked by this reactive intermediate, disaccharide derivative 6 is obtained. But this reactive intermediate is considered more stable than other cation molecular. Furthermore, –OH gropup of glycosyl acceptor 5 also is low reactivity because it has bulky EWGs. The result, oxocarbenium ion dose not attack to –OH group, and react thio phenyl group containing lone pair. In conclusion, anomeric center of compound 4 was migrated to –SPh group from –F group (Scheme 2.).

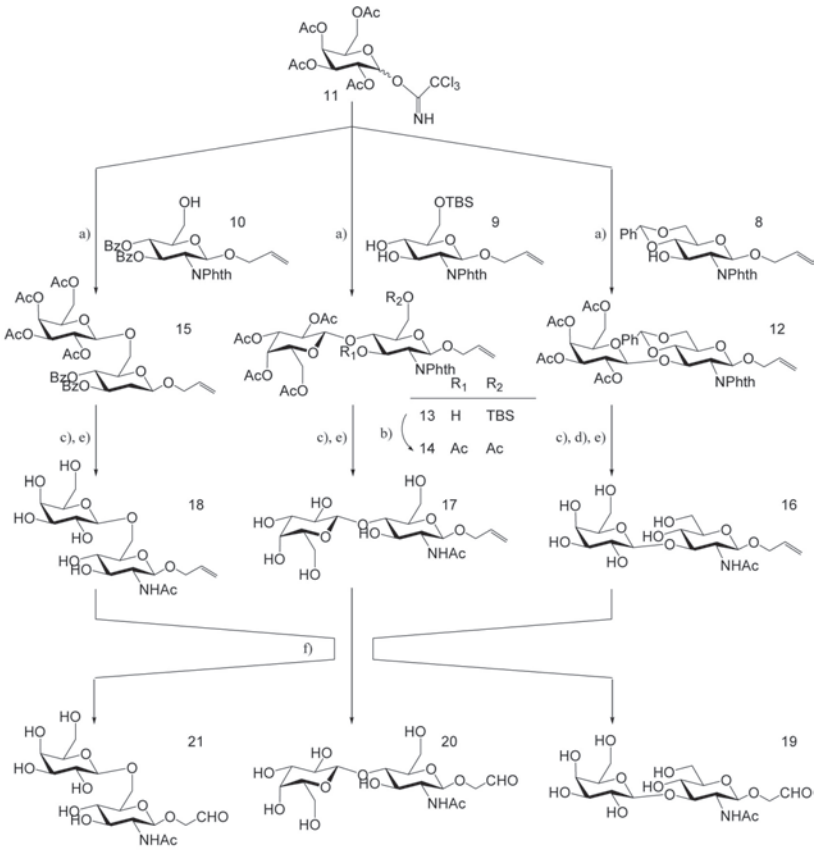


Scheme 2.

Glycosyl trichloroacetoimidate strategy[13]

We planed synthesis of carbohydrate ligands (19, 20, 21) by synthesis of disaccharide derivatives (12, 13, 14) which are obtained by coupling of D-glucosamine derivatives containing allyl group (8, 9, 10) and D-galactosyl trichloroacetoimidate derivative 11, then ozonolysis.

Disaccharide derivatives 12, 13, 15 were obtained by trichloroacetoimidate method. Synthesized glycosyl acceptors 8[14], 9, 10 were treated with gylycosyl donor 11[15] using trimethylsilyl trifluoromethanesulfonate (TMSOTf)[13] as the glycosylation promoter. After the reaction was completed as indicated by TLC monitoring, the mixture was purified by silica gel column chromatography to give pure product 12 (83%), 13 (70%), 15 (80%). The β –glycosylated linkages of them were confirmed by ^{1}H-NMR and ^{13}C-NMR spectrometry[16]. Anomeric protons of the Galp formed a glycosidic linkage of them that appeared as each a doublet with a homonuclear coupling constant of 8.3 Hz (12 : δ = 4.39 ppm, d, 1H, H–1'), 8.5 Hz (13 : δ = 5.12 ppm, d, 1H, H–1'), and 8.5 Hz (15 : δ 5.50 ppm, d, 1H, H–1'). Glycosidic formation of 13 has to be confirmed by acetylation because glycosyl acceptor 9 is 3, 4-di-OH derivative. 3 - position of 13, which was acetylated using acetic anhydrate and pyridine from 14, was confirmed by ^{1}H-NMR and ^{13}C-NMR spectrometry. A proton of 3 - position of 14 was assigned chemical shifts of δ = 5.76 ppm (t, 1H, H-3). Phthalimide groups in 12, 13, and 15 were removed using ethylenediamine in BuOH, and then acetylated. Furthermore, the removal of the acetyl and benzoyl groups were achieved by its treatment with 30 % NaOMe in MeOH to give 16



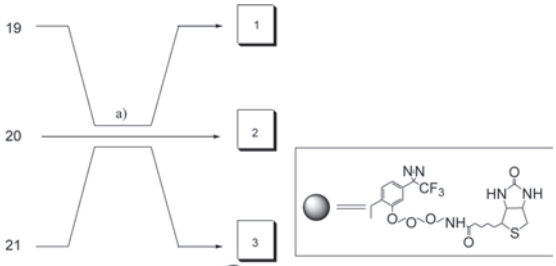
Scheme 3. Reagent: a) TMSOTf, CH_2Cl_2, **12** 83%, **13** 70%, **15** 80% ; b) 1) 90% AcOH, 2) Ac_2O, pyr., quant.; c) 1) ethylenediamine, BuOH, 2) Ac_2O,MeOH.; d) 90 % AcOH; e) NaOMe, MeOH, **16** 88% (3 steps), **17** quant. (2 steps), **18** 88% (2 steps) ; f) O_3, MeOH, then MeSMe, **19** 88%, **20** 92%, **21** 96%.

(88 % 3 steps), 17 (quant. 2 steps), and 18 (92 %, 2 steps). Then selective oxidation to the aldehyde from the alkene in 16, 17, 18 via ozonolysis[17] gave crude mixtures containing 19, 20, 21[18]. The residue was purified by LH-20 in methanol : water (1 : 1) to give compound 19 (88%), 20 (92%), 21 (96%). The structure of carbohydrate ligands 19, 20, and 21 were confirmed by ^{1}H-NMR and ^{13}C-NMR spectroscopy. Aldehyde groups of them appeared as each a singlet at δ = 8.15 ppm (19 : 1H, s), 8.15 ppm (20 : 1H, s), 8.12 ppm (21 : 1H, s) by ^{1}H–NMR spectrometry, and at 202.8 ppm (19), 202.9 ppm (20), 202.7 ppm (21) by ^{13}C-NMR spectrometry (Scheme 3.).

Synthesis of molecular tools 1, 2, 3

The oxime group was obtained by coupling the aminooxyl group and aldehyde groups under mild conditions without a promoter. They behave likely click-chemistry reaction because they are reacted under mild conditions and formed strong bond (covalent bond). 2-[2-[2-(Biotynylaminoethoxy) -ethoxy]-ethoxy]-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]-benzyloxyamine (22), which contained the activated aminooxyl group that, was obtained by the removal of the t-butoxycarbonyl (Boc) group in Affilight-CHO (Seikagaku Kogyo, Japan; biotin -N-Bocphenylaminodiazirin), was coupled with compound 19, 20, and 21 in acetonitrile : water (3 : 1), giving the molecular tool containing both a closed ring-type oligosaccharide structure and a diazirin group in quantitative yield (1, 2, 3 Scheme 4). Two major peaks were detected by HPLC analysis of the reaction residue, a finding suggested the formation of E–Z oxime isomers. This mixture was analyzed by ^{1}H-NMR, ^{13}C-NMR, and HR-FABMS spectrometry. Six triplets at δ = 7.54 (1-E), 7.52 (2-E), and 7.53 (3-E) ppm and δ =6.99 (1-Z)), 6.99 (2-Z)), 6.99 (3-Z)) ppm were assigned as the oxime protons of the E– and Z–

forms of 1, 2, and 3 by ^{1}H-NMR spectroscopy[19]. Six doublet peaks were assigned as H-1 (δ = 5.12 (1-E), 5.12 (2-E), 5.13 (3-E)ppm δ = 5.15 (1-Z),5.15(2-Z),5.15 (3-Z)ppm) and H-1' (δ = 4.27 (1-E), 4.27 (2-E), 4.43 (3-E)ppm δ = 4.25 (1-Z),4.21(2-Z),4.32 (3-Z)ppm). The E : Z ratio of them was also determined to be 1.3 : 1.0 (1), 1.4 : 1.0 (2), and 1.6 : 1.0 (3) by ^{1}H–NMR. HR-FABMS analysis of compounds 2 (E–Z) and 3 (E–Z) gave a $[M+Ha]^+$ ion peak at *m/z* 1012.3766, and 1012.3797 in agreement with the formula $C_{41}H_{61}F_3N_6O_{17}S$. Compounds 1 (E–Z) gave a [M+Na]+ ion peak at *m/z* 1036.3616, in agreement with the formula $C_{41}H_{60}F_3N_7O_{17}SNa$. The structures of 1 (E–Z), 2 (E–Z), and 3 (E–Z) were confirmed by these analytical results.



Scheme 4. Reagent: a) **22** ONH$_2$ CH_2Cl_2-CH_3CN, **1**, **2**, and **3** quant..

Conclusion

In summary, synthesis method of molecular tools containing oligosaccharide structure and phenyldiazirine group were established. Orthogonal glycosylation strategy was unable to synthesize disaccharide derivatives, but their compounds were achieved by glycosyl trichloroacetoimidate method. Their results will be applied to synthesis of molecular probe containing complex carbohydrate ligand. We hope that carbohydrate – lectin interactions by different of glucosidic formations are elucidated by molecular probes 1, 2, and 3.

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トリクロロアセトイミデート法による糖鎖
－レクチン相互作用解明のための光反応基と糖鎖リガンドを有する分子プローブの合成法の確立

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要　旨

光アフィニティーラベル法は、糖鎖 - レクチンの相互関係を明らかにする最も効果的な手法である。従来法では、プローブ合成時に還元末端糖が開環してしまっていたが、我々は、完全な糖鎖構造 (D- ラクトース) からなるリガンドとフェニルジアジリン基を有する分子プローブを開発した。次の問題点として、糖鎖は複雑な構造を有していることから、本分子プローブの合成法の確立が重要であると考えた。我々は、オルソゴナルグリコシル化法により糖鎖部分の合成を目指したが、反応時に糖受容体と糖供与体の脱離基が置換されてしまい、目的化合物が得られなかった。そこでトリクロロアセトイミデート法を用い、糖鎖合成に着手したところ、D- ガラクトース -D- グルコサミンから成る二糖誘導体が得られ分子プローブ (1、2、3) へと導かれた。

キーワード：光アフィニティーラベル法、糖鎖 - レクチン相互作用、糖鎖合成、オルソゴナルグリコシル化法、トリクロロアセトイミデート法、分子プローブ