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Chemical Synthesis of Sialyl Glycosides for Elucidation of Biological Phenomena

Isao OHTSUKA* Yutaka SADAKANE** Toshiyuki ATSUMI* Nobuko KAKIUCHI*

Abstract

The sialic acids are now defined as a family of carbohydrates, have very important biological functions. Thus, it considers that oligosaccharides containing them employ elucidation of biological phenomena. However, for synthesis of them glycosylations using donor of sialic acid derivative have been very hard to achieve both high yields and stereoselectivity of them. But recently, Synthesis methods of oligosaccharides containing sialic acids were developed by many researchers. Highly regio- and α-stereoselective sialyl glycoside syntheses have been achieved by using, as glycosyl donors, the 2-thioglycoside of Neu5Ac. We were interested in biological functions of oligosaccharide containing sialic acid, and tried to synthesize sialyl lewis X and synthesized trisaccharide derivative which was precursor of sialyl lewis X, α-L-Neu5Ac(2 → 3) β-D-Gal(1 → 4) β-D-GlcNAc-MP.

Key words : Sialic acid, thioglycoside, stereoselective synthesis, oligosaccharide synthesis, Sialyl Lewis X

Introduction

Sialic acid containing glycoconjugates, such as gangliosides and sialogycoproteins, have been recognized to play important roles in many biological processes[1-5]. Sialyl oligosaccharide chains of these glycoconjugates are exposed as ligands to the external environment, capable of expressing various biological functions, e.g. serving as receptors for hormones, immune responses, and so on.

The sialyl Lewis X (LeX) (Fig 1.)carbohydrate epitope, found on neutrophils, monocytes, and tumor cells, has been identified[6-8] as the ligand for selectin, a family of cell adhesion receptors that are implicated in the leukocyte traffic or extravasation to sites of inflammation, platelet adhesion, and probably tumor metastasis[9].

The most representative sialic acid, N-acetylneuraminic acid (Neu5Ac 1) is usually attached to O-3 or O-6 of galactose, or O-6 of N-acetylgalactosamine with an α(2-3)- or α(2-6)- linkage, and to O-8 of another Neu5Ac with an α(2-8)- linkage, giving diverse structures and functions. Therfore, the systematic understanding of structure – function relation of sialyl-oligosaccharides at the molecular level necessitates an efficient method for ragio- and α-stereoselective glycoside synthesis of sialic acids.

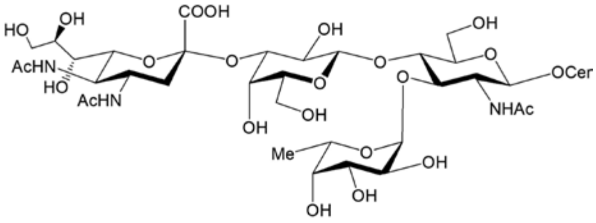
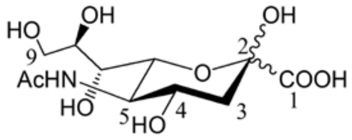


Fig 1. Sialyl Lewis X



1 (Neu5Ac)

Fig 2. Sialic acid (N-Acetylneuraminic acid)

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*School of Pharmaceutical Sciences, Kyushu University of Health and Welfare
1714-1 Yoshino-machi, Nobeoka-Shi, Miyazaki, 882-8508, Japan
**Suzuka University of Medical Science
3500-3 Minamitamakaki-cho, Suzuka city, Mie, 513-8670, Japan.

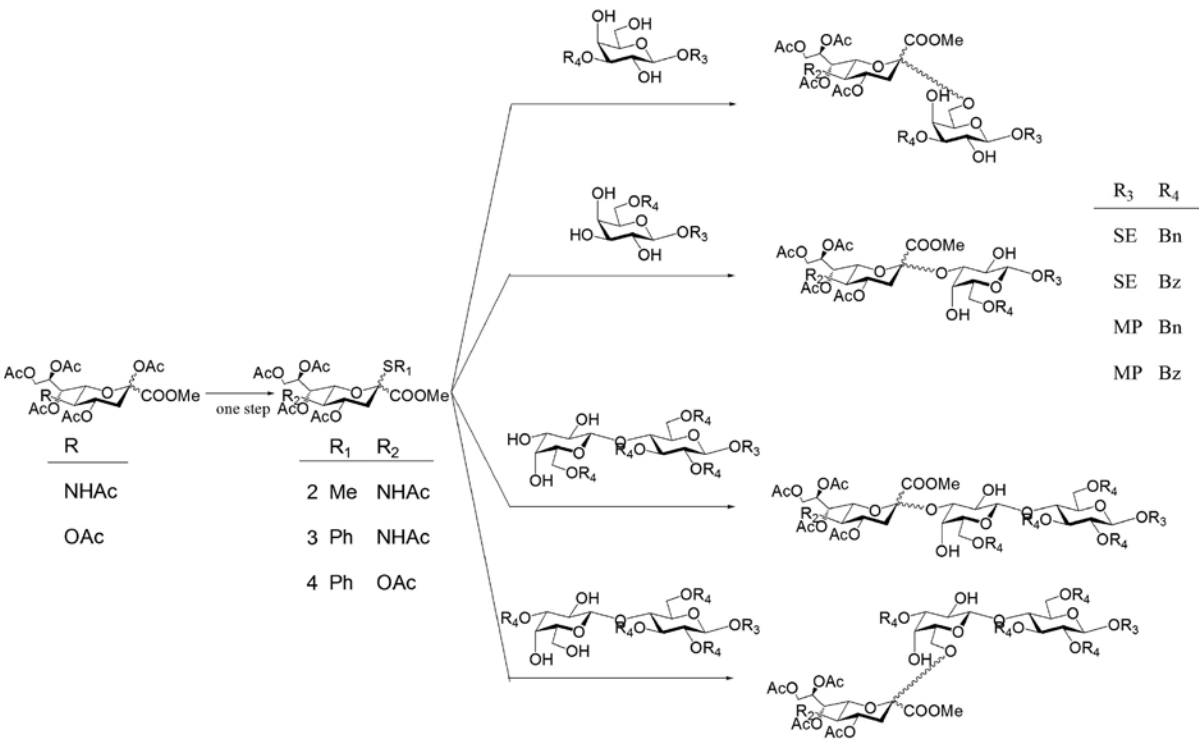

Fig 3. Syntheisi of oligosaccharide derivatives containing Neu5Ac

Sialyl glycoside synthesis

Facile and highly regio- and α-stereoselective sialyl glycoside syntheses have been achieved by using, as glycerol donors, the 2-thioglycoside (2-4) of Neu5Ac and, as glycerol acceptors, the suitably protected 2-(trimethylsilyl) ethyl (SE) or 4-methoxyphenyl (MP) glycosides of galactose or lactose, in the presence of thiophilic promoters, such as dimethyl (methylthio) sulfonium triflate (DMTST) or N-iodosuccinmide (NIS) – trifluoromethane sulfonic acid (TfOH)$^{10-11)}$ in acetonitrile (Fig 3.).

Reaction Mechanism

A possible reaction mechanism is shown in Fig 4. When the glycosyl donors (2-4) are specifically activated by thiophilic promoter (DMTST or NIS-TfOH) at low temperature, the cyclic oxocarbenium ion (c), necessary for almost all known glycosylation reactions, is formed by initially produced intermediates (a) or (b), and, subsequently, reacts with acetonitrile to generate the β-acetonitrium ion (d), which then undergoes SN2 displacement at the anomeric center by sugar-nucleophiles to give predominantly α-glcosides. However, the conformational preference of the positively charged β-acetonitrium ion (d) of Neu5Ac has been claimed to be shifted

toward the equatorial conformer (e), based on a concept of so-called reverse anomeric effect.

On the other hand, it has been demonstrated that the α-D-glucopyranosyl acetonitrium ions are stereospecifically generated from the corresponding oxocarbenium ions in dry acetonitrile$^{12-14)}$. These results indicate that the acetonitrium ions are axially oriented by anomeric effect. Recently, it has been concluded from the conformational analyses of glucopyranosyl - ammonium and glucopyranosyl - imidazolium derivatives that the reverse anomeric effect dose not exit$^{15), 16)}$.

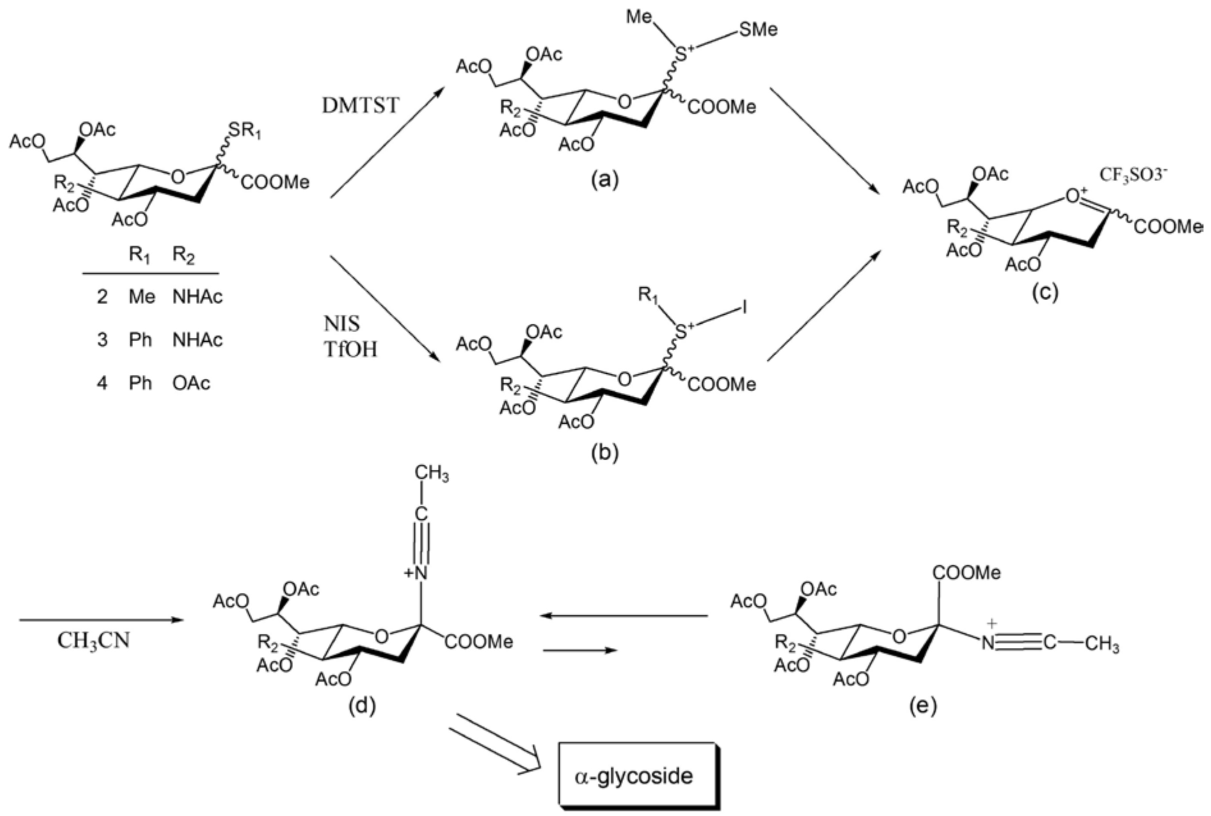

Fig 4. Reaction mechanism of sialyl glycosylation using acetonitrile

Therfore, it seems most plausible that stereospecific generation of the β-acetonitrium ion (d) of Neu5Ac from the oxocarbenium ion (c) hold the key of the α-preonderant formation of sialyl glycosides in acetonitrile. Thus, the reactive nucleophiles, such as OH-6 of acceptor have a chance toattack on other intermediate (a) ~ (c) before the complete generation of (d) to give an anomeric mixture. In the chlorinated solvent, however, nucleophiles react only with (a) ~ (c) to afford substantial amounts of the β-glycosides non stereoselectively.

This reaction mechanism could be applicable to the method employing xanthate (-SC(OEt)=S) or phosphite ($-OP(OR)_2$) as the glycosyl donor in acetonitrile medium.

Synthesis of trisaccharide derivative

Since gangliosides have various important biological functions, we tried trisaccharide derivative containing sialic acid. Sialyl lewis X (LeX) derivative (5), α-L-Neu5Ac(2→3)β-D-Gal (1→4) β-D-GlcNAc-MP, lack of L-Fucose from sialyl LeX (Fig 5).

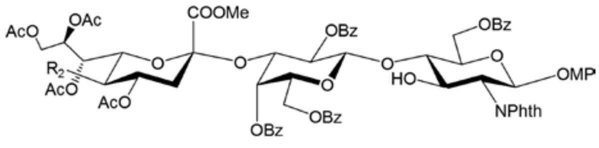

Fig 5. Target trisaccharide derivative (5)

Neu5Ac derivative (3) and D-galactose derivative 6 or 7 were coupled using NIS-TfOH.

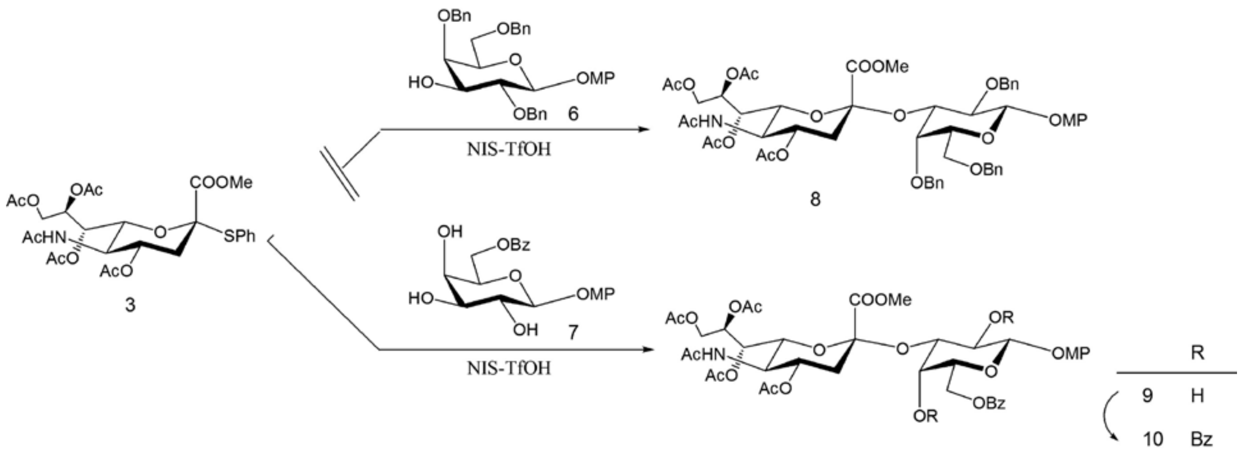

Fig 6. Synthesis of disaccharide derivative

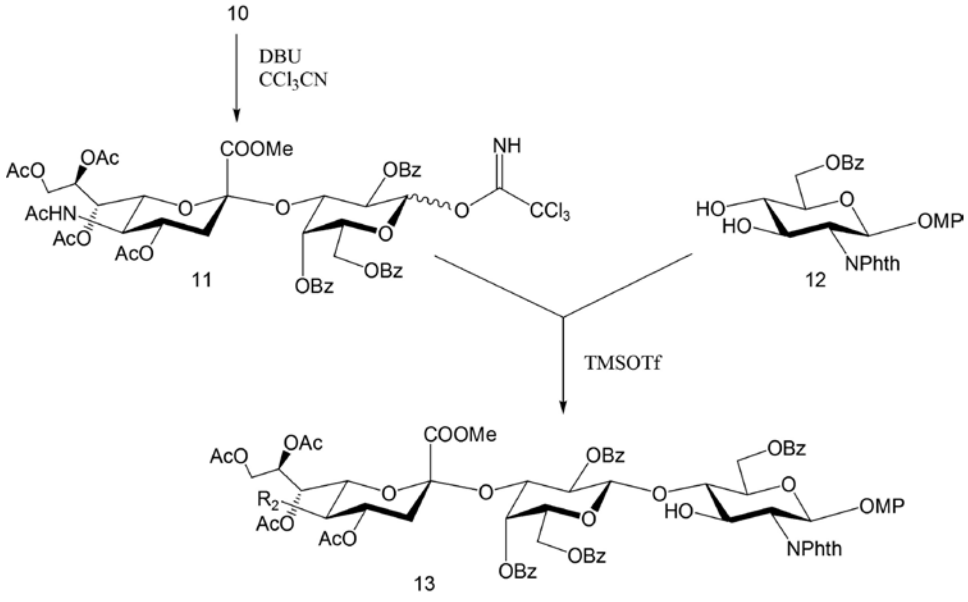

Fig 7. Synthesis of trisaccharide derivative (5)

Result, coupling of 3 and 6 was not found, but coupling of 3 and 7 led to disaccharide derivative (9, 60 % β-only)[17]. It is considered that compound 7 is less bulky than compound 6 which is protected O-2, O-4 and O-6 by benzyl ether (Fig 6).

Disaccharide derivative (9) was removed MP and then changed to trichloroacetoimidate derivative (11)[18]. Compound 11 was reacted with D-glucosamine derivative (12) using trimethylsilyl trifluoromethane sulfonate (TMSOTf), and trisaccharide derivative (5) was synthesized (Fig 7).

Conclusions

We achieved synthesis of trisaccharide derivative containing sialic acid (5). Compound 5 which have 3-OH group of D-glucosamine will be coupled with L-fucose derivative, and lead to sialyl Lewis X (LeX) derivative. Since sialyl LeX have various biological functions, this compound hopes also similar functions.

Acknowledgements

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17. note : synthesis of disaccharide derivative (9)

A solution of 3 (10.7 g, 18.3 mmol) and 6 (3.84 g, 10 mmol) containing activated type AW-300 molecular sieves (4.0 g) in dry MeCN (50 mL) and CH_2Cl_2 (5 mL) was stirred under a nitrogen atmosphere for 2 h at room temperature. After cooling to -35℃ , NIS (8.28 g, 36.8 mmol) and TfOH (540 mg, 3.6 mmol) was added, and the mixture was stirred for 2.5 h at -20℃ . The reaction was neutralized with Et3N, filtered and extracted with $CHCl_3$. The organic solvent was washed with aqueous $Na_2S_2O_3$, aqueous $NaHCO_3$ and aqueous HCl, dried, and concentrated to give clear oil. The crude product was purified by silica gel column chromatography (hexane : ethylacetate = 1 : 1) to

give 9 (6.0 g, 70%).

18. note : synthesis of trisaccharide derivative (13) A solution of 10 (975 mg, 0.9 mmol) and 11 (340 mg, 0.7 mmol) containing activated type AW-300 molecular sieves (4.0 g) in dry CH_2Cl_2 (8.0 mL) was stirred under a nitrogen atmosphere for 2 h at room temperature. After cooling to -20 ℃ , TMSOTf (31.8 μL, 0.18 mmol) was added, and the mixture was stirred for 3 h at -20℃ . The reaction was neutralized with Et_3N, filtered and extracted with $CHCl_3$. The organic solvent was washed with aqueous $NaHCO_3$, dried, and concentrated to give clear oil. The crude product was purified by silica gel column chromatography (toluene : acetone = 20 : 1 to 5 : 1) to give 5 (995 mg, 96%) as a colorless oil.

生命現象解明を目指したシアル酸を有する糖鎖の合成

大塚　功 *　　定金　豊 **　　渥美　聡孝 *　　垣内　信子 *

* 九州保健福祉大学　薬学部　生薬学講座　〒 882-8508　宮崎県延岡市吉野町 1714-1
** 鈴鹿医療科学大学　薬学部　〒 513-8670　三重県鈴鹿市南玉垣町 3500-3

要　旨

糖質に分類されるシアル酸は、様々な生体機能を有していることが知られている。従って、シアル酸を含む糖鎖を化学合成することは、生命現象の解明に寄与するものと考えられる。しかし、シアル酸を糖供与体とする糖鎖合成は困難とされており、近年多くの研究者らによって、新たな処方が開発されてきている。我々は、シアル酸が持つ生体機能の興味を持ち、これを有する糖鎖誘導体 (5) の合成を試みた。

キーワード：シアル酸、チオグリコシド、立体選択的合成、糖鎖合成、シアリルルイス X