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Sialylation using *N*-glycolylneuraminyl phosphite donors to synthesize Neu5Gc-containing glycans

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ABSTRACT

Efficient sialylations using *N*-glycolylneuraminic acid (Neu5Gc) phosphite donors having an acetyl or benzyl group on the glycolyl moiety are described in the synthesis of Neu5Gc-containing glycans. Both phosphite donors **1** and **2** were readily coupled with primary and secondary acceptor alcohols in propionitrile at −78 °C to provide the desired glycosides with good α-selectivities.

1. Introduction

Among the various sialic acids, *N*-glycolylneuraminic (Neu5Gc) and *N*-acetylneuraminic (Neu5Ac) acids are the most abundant and can be readily found in Gram-negative bacteria, echinoderms, and vertebrates.[1,2] In the case of humans, most of the sialic acid is Neu5Ac due to a deficiency of CMP–Neu5Ac hydroxylase, which produces CMP–Neu5Gc in the cytosol.[3] However, minor amounts of Neu5Gc-containing glycans have been detected in tumor-associated epitopes involving breast and colon cancer, and in the brain ganglioside GM_3.[4,5]

It has been reported that a number of infectious bacteria and viruses can recognize cell-surface glycans that bear sialic acids. In view of the recent pandemic threat of the avian influenza virus, the shift of the virus receptor from Neu5Gc to Neu5Ac is a significant factor in determining infectious properties among humans.[6]

Recently, the potent activities of echinoderm gangliosides on the stimulation of nerve cells have been reported.[7] Especially, the neurite outgrowth activities of such distinctive gangliosides that contain Neu5Gc residues have been shown to be more comparable to those of GM_1 ganglioside in the presence of nerve growth factor (NGF).

In chemical synthesis of such bioactive Neu5Gc-containing gangliosides, the use of N-substituted sialic acid building blocks can be an efficient strategy in terms of both yields and α-selectivities.[8] Upon completion of the sialylation reactions using these sialyl donors carrying the C-5 amino-protecting groups such as *N*-TFA,[9a] azide,[9b] carbamate,[9c,d] phthalimide,[9e] and oxazolidinone,[9f–h] the sialic acid unit can be subsequently transformed into the glycolyl form by substituting the functionalities with a glycolylamide.[10] The liberated C-5 amino group, however, may undergo acyl migration from the C7/8-hydroxyl-protected acetyl group, even under acidic conditions,[9c] and therefore, the direct use of a Neu5Gc building block would be a straightforward strategy in avoiding such undesirable migration reactions on important intermediates. To date, a few reports have appeared describing the synthesis of Neu5Gc gangliosides involving N-glycolylneuraminylation using less reactive methylthio/phenylthio-Neu5Gc donors.[11] In this report, N-glycolylneuraminylations using more reactive Neu5Gc phosphite donors **1** and **2** are described. Furthermore, acetyl protected **1** and benzyl protected **2** were also prepared to compare the reactivities as well as α-selectivities involving sialylations with acceptors **8**, **11**, **14**, and **16**.

2. Results and discussion

2.1. Synthesis of sialyl phosphite donors 1 and 2

The synthesis of sialyl phosphite donors **1** and **2** was initially carried out as shown in Scheme 1. Thiophenyl sialosides **4**[11a,12] and **5**, which possess glycolylamide moieties with either benzyl or acetyl protection, were prepared from known thioglycoside **3**[13] according to Sugata and Higuchi's procedure.[11d] First, sialoside **3** was fully deacetylated using methanesulfonic acid in MeOH under reflux conditions. Next, the exposed amino group was immediately

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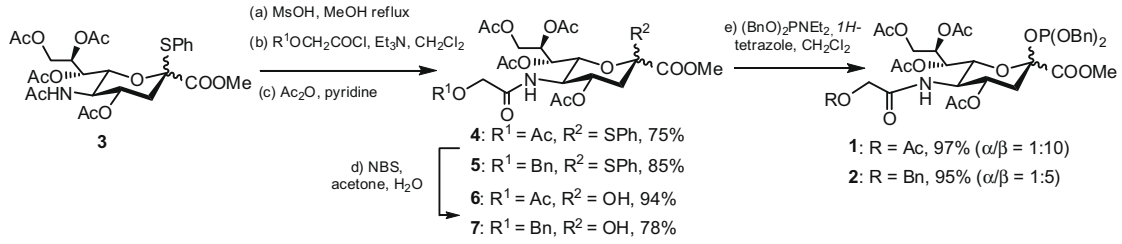


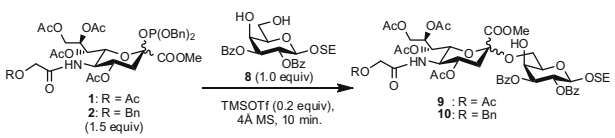
Scheme 1. Synthesis of the sialyl phosphite donors **1** and **2**.

protected with either an acetoxyacetyl or a benzyloxyacetyl group using the corresponding acid chlorides. The remaining hydroxyl groups were acetylated to afford desired sialosides **4** (75% yield) and **5** (85% yield) in three steps. Sialosides **4** and **5** were then transformed into phosphite donors **1** and **2**, respectively, via two additional steps:[14] hydrolysis of the phenylthio moiety of **4** and **5** using NBS in acetone–H_2O to give hemiketals **6** and **7** in 94% (**6**) and 78% (**7**) yields, followed by introduction of the phosphite group by treatment with *N*,*N*-dibenzylphosphoramidite and 1*H*-tetrazole to afford **1** (97% yield, α:β = 1:10) and **2** (95% yield, α:β = 1:5), respectively.

2.2. Sialylation reactions using donors 1 or 2

To investigate both reactivity and α-selectivities of the Neu5Gc phosphite donors **1** and **2**, the formation of naturally occurring Neu5Gcα(2→6)Gal linkages was carried out. As shown in Table 1, galactose 4,6-diol **8**,[15] which has benzoyl groups on the C-2 and C-3 positions for further β-selective galactosylations, was employed as a practical acceptor. In the first trials as shown in entries 1 and 2, phosphite **1** or **2** was activated with 0.2 equivalent of TMSOTf in dichloromethane at −78 °C. In the case of entry 1, the reaction went to completion within 10 min to give disaccharide **9** in 88% yield; however, the desired α-anomer was the minor component (α:β = 1:1.5, entry 1). In the choice of donor **2**, the α-selectivity was improved to afford the disaccharide **10** in 71% yield (α:β = 2.3:1, entry 2). In order to investigate a possible 'nitrile effect', the sialylation reactions were carried out in acetonitrile and propionitrile (entries 3–5).[16] In the case of acetonitrile at −40 °C with donor **2** (entry 3), although the α-selectivity was improved (α:β = 3.0:1), the yield (73%) was similar to that of entry 2 (71%). On the other hand, in the cases of propionitrile at −78 °C with donor **1**, the desired disaccharide **9** was obtained in 90% (α:β = 4.7:1, entry 4).[17] The best performance was obtained in the use of donor **2** with acceptor **8** to give disaccharide **10** in 87% yield with excellent α-selectivity (α:β = 10:1). As can be seen from the data in Table 1, donor **2** which has benzyl protection on the glycolyl

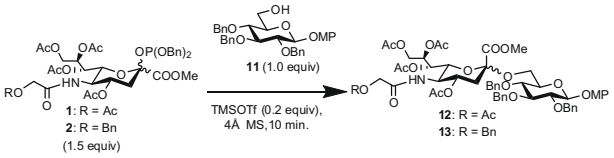
Table 1
Sialylation reactions using sialyl phosphite donors **1** or **2** with galactose acceptor **8**



Entry	Donor	Solvent	Temperature (°C)	Product (yield %)[a]	α/β ratio[b]
1	**1**	CH_2Cl_2	−78	**9** (88)	1:1.5
2	**2**	CH_2Cl_2	−78	**10** (71)	2.3:1
3	**2**	CH_3CN	−40	**10** (73)	3.0:1
4	**1**	EtCN	−78	**9** (90)	4.7:1
5	**2**	EtCN	−78	**10** (87)	10:1

[a] Isolated yields.
[b] Anomeric ratio was determined by ^{1}H NMR spectroscopy, SE: 2-trimethylsilylethyl.

Table 2
Sialylation reactions using sialyl phosphite donors **1** or **2** with glucose acceptor **11**



Entry	Donor	Solvent	Temperature (°C)	Product (yield %)[a]	α/β ratio[b]
1	**1**	CH_2Cl_2	−78	**12** (86)	1:10
2	**2**	CH_2Cl_2	−78	**13** (90)	1:10
3	**1**	EtCN	−78	**12** (85)	8:1
4	**2**	EtCN	−78	**13** (86)	8:1

[a] Isolated yields.
[b] Anomeric ratio was determined by ^{1}H NMR spectroscopy, MP: 4-methoxyphenyl.

moiety, exhibited significantly better α-selectivity than the corresponding acetyl-protected donor **1**.

To study formation of the Neu5Gcα(2→6)Glc unit, which has been identified from the echinoderm gangliosides,[7,9] sialylations of donors **1** and **2** with glucoside **11**[18] were investigated (Table 2). To determine preferential dependence of donors **1** and **2** by comparison of the results shown in Table 1, all sialylations in Table 2 were carried out in either dichloromethane or propionitrile at −78 °C. Initially as indicated in entries 1 and 2, unexpected high β selectivities were obtained for the synthesis of disaccharide **12** (86% yield,α:β = 1:10) and **13** (90% yield,α:β = 1:10) in dichloromethane, respectively. On the other hand, excellent α-selectivities (α:β = 8:1) were obtained using propionitrile at −78 °C (Table 2, entries 3 and 4), which were in good agreement with those of Table 1 (entries 4 and 5, respectively). From all the entries in Table 2, no significant difference between donors **1** and **2** was found from the points of α-selectivity and yields.

Sialylation reactions between donor **1**, which showed the best results in Tables 1 and 2, and galactose C3–OH of either galactal **14** or lactose derivative **16** were also investigated in the synthesis of the Neu5Gc-terminated α(2→3)sialylgalactose units (Scheme 2). In the former case, the nucleophilic C3-hydroxyl group of 4,6-di-*O*-benzyl-D-galactal (**14**) was coupled with donor **1** to produce α-sialoside **15** (61% yield) in a stereoselective manner. Compound **15** is an intermediate in the course of forming a Neu5Gcα(2→3)galactose building block.[17] In the latter case, donor **1** was coupled with lactose acceptor **16**[19] to give trisaccharide **17** as the major product (42% yield) in the course of a GM_3-type trisaccharide synthesis.

3. Conclusion

We have herein described a direct sialylation approach toward the synthesis of the Neu5Gc-containing glycans using *N*-glycolylneuraminyl phosphite donors **1** and **2**. In the use of acceptors **8**, **11**, **14**, and **16**, the best yields and α-selectivities were achieved