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An alternative route for the construction of carbosilane dendrimers uniformly functionalized with lactose or sialyllactose moieties†

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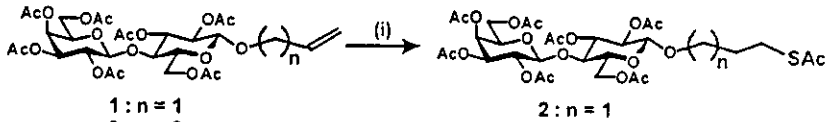
Abstract—A new approach for the formation of an acetylthio linkage on aglycon by means of a radical addition of thioacetic acid into the C=C double bond of the aglycon was examined. An introduction of a carbohydrate moiety into carbosilane dendrimers was demonstrated using a one-pot coupling reaction in MeOH–DMF in the presence of NaOMe via removal of an acetyl group of the acetylthio linkage in the saccharide moieties, producing a thiolate anion and a nucleophilic replacement of the thiolate to dendric alkyl bromide to form carbosilane dendrimers uniformly bearing lactose or sialyllactose moieties through thioether linkages in high yields.

The sialyllactose sequence (Neu5Acα2→3/6Galβ1→4Glcβ1→) is known as a receptor of hemagglutinin on the surface of the influenza virus.[1] Hitherto, several groups have reported polymeric inhibitors against such interaction between the receptors on a cell surface and the hemagglutinin of the virus.[2] In the course of our recent work on glyco-silicon functional materials, carbosilane dendrimers having globotriaose moieties showed neutralization potency against verotoxin.[3] We therefore set about synthesizing carbosilane dendrimers functionalized with sialyllactose moieties to obtain new types of inhibitor for hemagglutinin of the influenza virus.

In our ongoing synthetic study of glycoclusters, synthetic assembly of carbohydrate moieties using carbosilane dendrimers was achieved using β-cyclodextrin,[4] globotriaose[5] and functional monosaccharides.[6] In our previous investigation, the efficiency of a coupling reaction between a sialic acid derivative and a carbosilane dendrimer by means of our one-pot procedure in liquid ammonia was moderate, even when a further amount of the sialic acid derivative was used for the reaction.[6] Therefore, an alternative and highly efficient procedure for introducing sialic acid residues on the dendrimers is required. In this communication, we describe a convenient radical addition of thioacetic acid into the C=C double bond at the terminus of the sugar aglycon and a new approach for the construction of carbosilane dendrimers uniformly functionalized with carbohydrate moieties, such as lactose or sialyllactose, as potential receptors for hemagglutinin of the influenza virus.

The radical addition of mercaptan in carbohydrate chemistry was first demonstrated by Lee et al.,[7] and this reaction has been widely used.[8] A similar strategy for the introduction of a thioacetic residue into the acrylamide portion by a Michael reaction has also been



1 : n = 1
3 : n = 3

2 : n = 1
4 : n = 3

Scheme 1. *Reagents and conditions*: (i) summarized in Table 1.

Keywords: radical additions; thioacetate; carbosilane dendrimers; sulfide; glycodendrimers.

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Table 1. Results of radical addition of thioacetic acid into the C=C double bond of aglycon

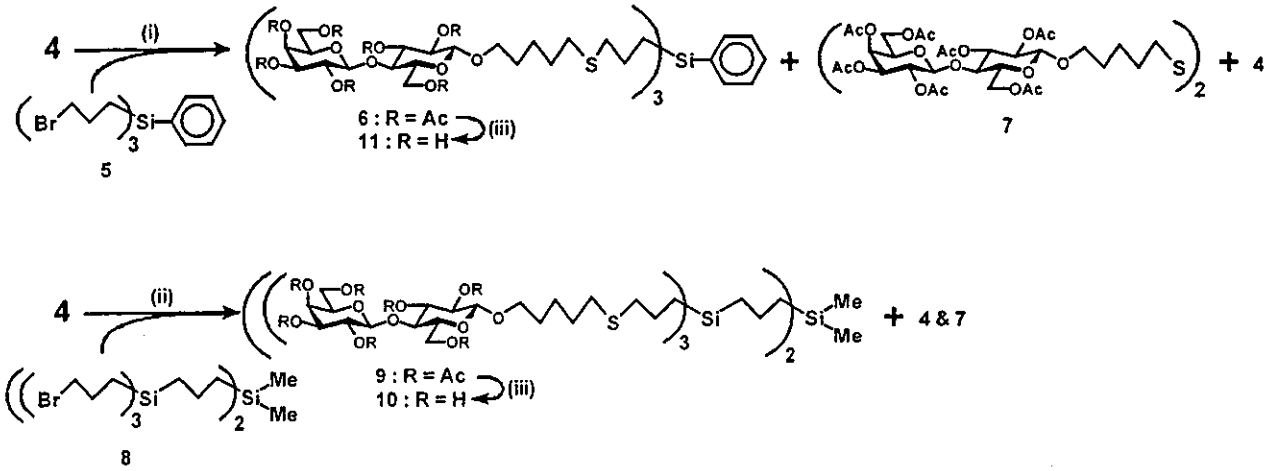
Sugar	AcSH (equiv.)	AIBN (equiv.)	Solvent	Temp. (°C)	Yield[a] (%)
1	40	0.2	–	50→80	ND[b]
1	15	0.5	Dioxane	50→80	67[c]
3	15	0.5	Dioxane	50→80	100
3	15	1	Dioxane	50→80	92
3	40	1	–	50→80	93

[a] Isolated yield.
[b] Due to an unseparable mixture of the starting material and the product.
[c] Starting materials were also recovered in 31% yields.

reported.[9] Since the radical addition of thioacetic acid,[10] however, has not been applied to an olefinic C=C double bond on a sugar aglycon, we initially optimized the radical addition of thioacetic acid using the known allyl (**1**)[11] and *n*-pentenyl (**2**)[12] lactosides as model candidates (Scheme 1). The results of the reaction are summarized in Table 1.

An allyl glycoside **1** was used as a candidate for the radical addition of thioacetic acid; however, the reaction was not completed due to the low reactivity of the allyl group in the glycoside even when a large excess of thioacetic acid was used. Consequently, another known *n*-pentenyl glycoside **3** was used under the same reaction conditions, and the radical addition proceeded smoothly to afford a nearly quantitative yield of thioacetate **4**,‡ $[\alpha]_D^{21}$ −15.1 (*c* 1.77, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 2.85 (t, 2H, *J* 7.2 Hz, CH_2SAc), 2.32 (s, 3H, SAc). Thus, the *n*-pentenyl group proved to be an effective acceptor for the radical addition of thioacetic acid.

Given the success of the introduction of a thioacetate residue into aglycon, we next turned our attention to the coupling reaction of thioacetate **4** with a dendrimer **5**[5] to produce a sulfide linkage. Scheme 2 shows the coupling reaction, and the conditions used are summa-



Scheme 2. *Reagents and conditions*: (i) summarized in Table 2; (ii) **8**, NaOMe, MeOH–DMF, rt, then, Ac_2O, Pyr., rt; (iii) NaOMe, MeOH, rt.

Table 2. Results of one-pot coupling reaction between lactosyl thioacetate and tris(3-bromopropyl)phenylsilane

Charged ratio 4:5	First step conditions	Second step conditions	Yield[a] (%)
6:1	NaOMe (6 mol equiv.), MeOH, −30 to −15°C, then concentrated	**5**, K_2CO_3 (6 mol equiv.), DMF, 50°C	19
6:1	**5**, Et_2NH (120 mol equiv.), DMF, 0°C	K_2CO_3 (6 mol equiv.), DMF, 60°C	0
6:1	NaOMe (6 mol equiv.), MeOH, rt, then concentrated	**5**, NaOMe (6 mol equiv.), THF, 50°C	0
6:1	NaOMe (7 mol equiv.), MeOH, rt, then concentrated	**5**, NaOMe (7 mol equiv.), DMF, −30°C to rt	33
6:1	**5**, NaOMe (6 mol equiv.), MeOH–DMF, −30°C, then concentrated	None	19
6:1	**5**, NaOMe (6 mol equiv.), MeOH–DMF, rt, then concentrated	None	84

[a] Isolated yield of **6** based on **5** after acetylation.

‡ All new compounds with specific rotation data gave satisfactory elemental analyses.

rized in Table 2. The reaction includes (1) *O*- and *S*-deacetylation, (2) S_N2-type displacement and (3) usual acetylation for purification by silica gel chromatography. Although a two-step procedure, i.e. deacetylation followed by addition of dendrimer **5**, gave incomplete reaction products together with the starting materials and disulfide compound **7**, FABMS calcd for [M+H$^+$]: 1475; found *m/z*: 1475, the direct coupling procedure in the presence of dendrimer **5** was found to be the most effective coupling reaction to form **6** in 84% yield after removal of byproducts by chromatography on silica gel, $[\alpha]_D^{21}$ −14.2 (*c* 1.40, $CHCl_3$), integral ratio of the H atoms by ^{1}H NMR: $SiCH_2$:SCH_2:Ph:H-1 and H-1′=6:12:5:6, FABMS calcd for [M+H$^+$]: 2444.9; found *m/z*: 2444.6.

As an extension of this new coupling reaction, we examined a combination of a dendrimer **8** and an even more complex oligosaccharide, sialyllactose. The synthetic routes for the construction of glycodendrimers are shown in Scheme 3. The coupling reaction of **4** with a dumbbell-type dendrimer **8**[5] under the same conditions as those described for the preparation of **6** proceeded efficiently to provide homogeneous **9** having six lactose moieties in 62% yield, $[\alpha]_D^{21}$ −15.3 (*c* 0.80, $CHCl_3$), integral ratio of the H atoms by ^{1}H NMR: $SiCH_3$:$SiCH_2$:SCH_2: H-1 and H-1′=6:20:24:12, FABMS calcd for [M+H$^+$]: 4877.8; found *m/z*: 4877.6. Transesterification of **9** and **6**, followed by saponification gave water-soluble glycodendrimers **10**, $[\alpha]_D^{20}$ −4.4 (*c* 0.76, H_2O), MALDI-TOF MS calcd for [M+Na$^+$]: 3134.30; found *m/z*: 3138.09, and **11**, $[\alpha]_D^{19}$ −5.0 (*c* 0.16, H_2O), FABMS calcd for [M+H$^+$]: 1561.6; found *m/z*: 1561.9 in good yields, respectively.

An *n*-pentenyl sialyllactoside **13**, $[\alpha]_D^{18}$ −6.9 (*c* 2.02, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 4.67 (d, 1H, $J_{1',2'}$ 10.0 Hz, H-1′), 4.52 (dd, 1H, $J_{2',3'}$ 10.2 Hz and $J_{3',4'}$ 3.3 Hz, H-3′), 4.45 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), was prepared from a known imidate **12**[13] coupled with 4-penten-1-ol under Tietze's conditions in 84% yield. The glycoside **13** was derivatized to thioacetate **14** by the radical addition of thioacetic acid in 99% yield, $[\alpha]_D^{29}$ −6.3 (*c* 1.29, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 2.85 (t, 2H, *J* 7.2 Hz, CH_2SAc), 2.32 (s, 3H, SAc). The acetylthio derivative of sialyllactose **14** was then coupled with dendrimer **5** under the same conditions as that described for **6** to give glycodendrimer **15** in 80% yield, integral ratio of the H atoms by ^{1}H NMR: $SiCH_2$:SCH_2:Ph:H-1 and H-1′=6:12:5:6, FABMS calcd for [M+Na$^+$]: 3761.28; found *m/z*: 3760.84, which was then deprotected in the usual manner to afford **16** in quantitative yield, FABMS calcd for [M−H$^-$]: 2433.9; found *m/z*: 2434.1. Sialyllactose derivative **14** was also allowed to react with dendrimer **8** to give **17** in 77% yield, integral ratio of the H atoms by ^{1}H NMR: $SiCH_3$:$SiCH_2$:SCH_2:H-1 and H-1′=6:20:24:12, FABMS calcd for [M+Na$^+$]: 7488.61; found *m/z*: 7488.30. Deprotection of **17** gave **18** having six sialyllactose units in quantitative yield, FABMS calcd for [M−H$^-$]: 4857.9; found *m/z*: 4859.2.

In conclusion, we have succeeded in introducing an acetylthio moiety into the aglycon of lactose and a sialylα2→3lactose sequence and incorporating those carbohydrate chains into a couple of carbosilane dendrimers through a sulfide linkage. Biological evaluation of these novel glycodendrimers is now in progress, and the results will be reported in the near future.

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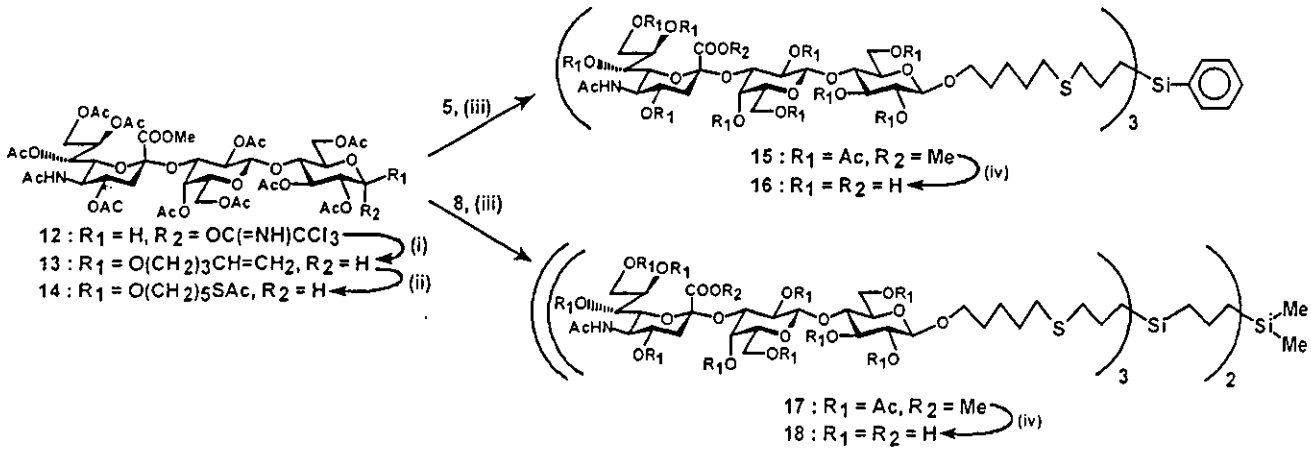

Scheme 3. *Reagents and conditions*: (i) 4-penten-1-ol, BF_3·Et_2O, MS 4 Å, CH_2Cl_2, −25→−5°C; (ii) AcSH, AIBN, 1,4-dioxane, 50→80°C; (iii) NaOMe, MeOH–DMF, rt, then, Ac_2O, Pyr., then, CH_2N_2, Et_2O; (iv) NaOMe, MeOH, rt, then, 0.05 M aq. NaOH, rt.

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Regioselective synthesis of methylated β-cyclodextrins leaving hydroxy groups

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Abstract—A series of methylated β-cyclodextrins (CDs) regioselectively leaving one or two hydroxy groups were prepared, and fluorescence spectroscopic measurements showed that they have unique binding characteristics against 2-*p*-toluidinylnaphthalene-6-sulfonate as a guest molecule in aqueous solution.

In developing an enzyme mimic, attractive molecules as starting materials are cyclodextrins (CDs), which are cyclic oligosaccharides composed of α(1→4) glucosyl residues.[1] CDs are known to function as host molecules making inclusion complexes with hydrophobic guest compounds in aqueous solution.[2] Although CDs have a number of hydrophilic functional groups, such as primary and secondary hydroxy groups, CDs, particularly β-CD, have low solubility in water.[3] Furthermore, the β-CD inclusion complexes have much lower solubility in water. In order to enhance the solubility of β-CD in an aqueous medium, totally and partially methylated β-CD derivatives have been synthesized.[4] Hitherto, regioselective modifications of the specific hydroxy groups in β-CD by methylation leaving one or two hydroxy groups for future manipulating functions have not been accomplished. Successful protection of two hydroxy groups of a primary face by an interglycosidic bridge of sulfonamide was first reported by Tabushi et al.[5] We recently reported the successful protection of two hydroxy groups of a secondary face with benzylidene acetal after protection of primary hydroxy groups by pivaloyl esters.[6] This communication describes a novel method for the synthesis of partially methylated β-CDs in a regiospecific manner and their binding properties with 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) as determined by examination of fluorescence spectra of the inclusion complexes in aqueous solution.

Heptakis 6-pivaloate **1** and the inter glycosidic benzylidene derivative **2** were prepared according to the method previously reported.[6a] Since direct benzylation of **2** has been successfully demonstrated using a combination of NaH and BnBr in DMF,[6b] direct methylation for **2** was firstly examined under similar conditions. Unfortunately, **3** was obtained in only 18% yield. Therefore, we focused our attention on a two-step procedure (i.e. de-*O*-pivaloylation in a Zemplén manner and then usual methylation). Thus, de-*O*-pivaloylation of **2** in methanol in the presence of excess sodium methoxide at 50°C gave the $3^1,2^2$-benzylidene derivative, which was further transformed by methyl iodide in the presence of NaH in DMSO to give per-methylated benzylidene derivative **3** in 89.0% yield; ^{1}H NMR ($CDCl_3$) δ: 5.93 (s, 1H, C*H*Ph).

When transformation of **1** into **2** was performed with an excess amount of α,α-dimethoxytoluene (12 equiv. mol.) followed by treatment of multi-benzylidenated products using 0.1 M of CSA in 1:1 $CHCl_3$–MeOH at 0°C, an unexpected compound **4** was formed with **2** having the same R_f (0.55, 4:1 $CHCl_3$–MeOH) on TLC as judged by the results of ^{1}H NMR spectra of the mixture. Since these compounds could not be separated, further manipulation of the mixture was performed. Thus, de-*O*-pivaloylation followed by methylation of the mixture was carried out in the same manner as that described above to afford a still-inseparable mixture of **3** and **5**; R_f (0.57, 10:1 $CHCl_3$–MeOH); ^{1}H NMR ($CDCl_3$) δ: 5.93 (s, 2/3H, C*H*Ph), 5.86 (s, 1/3H, C*H*Ph) (Scheme 1).

For the preparation of monoalcohol at the C-3 position, a reductive ring-opening reaction of the benzyli-

Keywords: cyclodextrins; methylations; regiospecificity; inclusion; host compounds.

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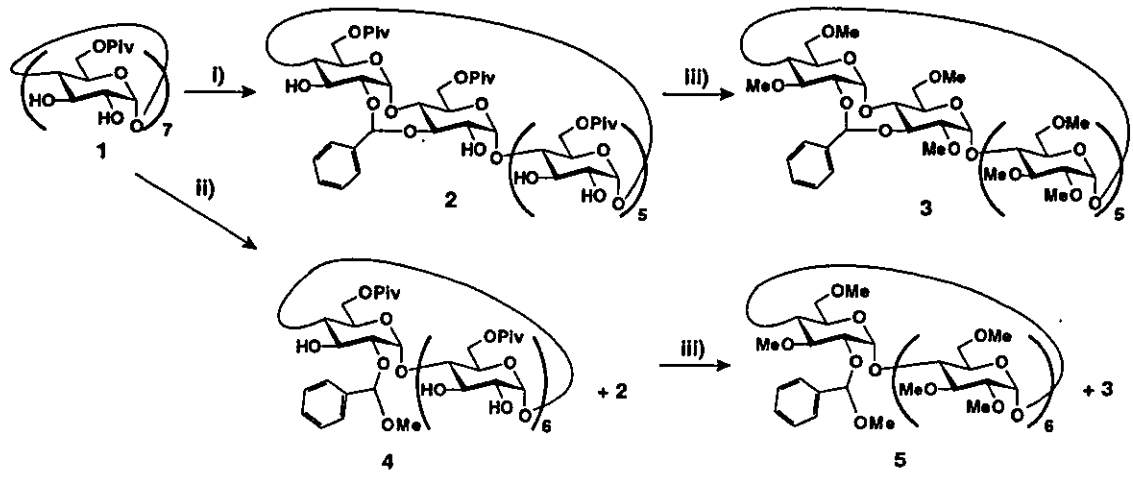
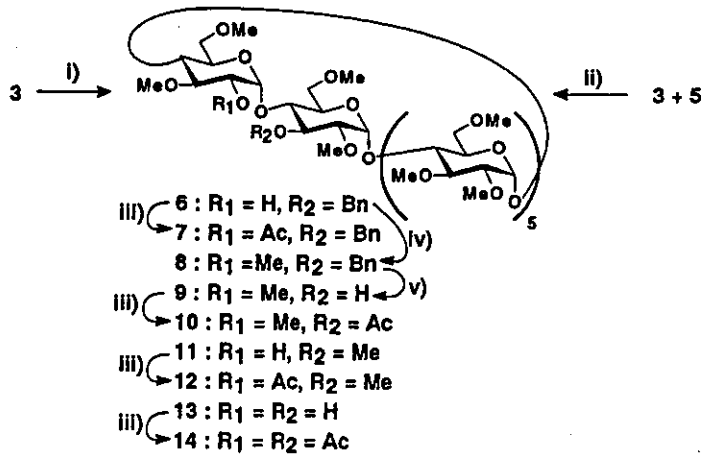
dene derivative of **3** proceeded smoothly and regioselectively in the presence of LAH and $AlCl_3$ to give **6**, having a mono hydroxy group at the C-2^2 position and a benzyl group at the C-3^1 position; $[\alpha]_D^{20}$ +141.7° (*c* 1.07, $CHCl_3$). When $BH_3{\cdot}NMe_3$ was used as the reducing reagent, the reaction did not proceed. In order to elucidate the structure of **6**, the remaining hydroxy group was acetylated to afford mono-2-*O*-acetete **7** in quantitative yield; ^{1}H NMR ($CDCl_3$) δ: 4.68 (dd, 1H, $J_{1,2}$ 3.5 Hz and $J_{2,3}$ 9.7 Hz, H-2^2), 1.71 (s, 3H, OAc). Protection of the free hydroxy group of **6** was carried out in the usual way to afford per-methylated **8** in 98% yield; $[\alpha]_D^{20}$ +151.6° (*c* 1.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 5.09 (d, 1H, J_{gem} 10.6 Hz, CH_a-Ph), 4.81 (d, 1H, CH_b-Ph). Final deprotection of the benzyl group in **8** by hydrogenolysis gave mono-3-hydroxy derivative **9** in 91% yield; $[\alpha]_D^{20}$ +156.7° (*c* 0.96, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 5.19 (s, 1H, OH-3^1), 3.97 (t, 1H, *J* 9.4 Hz, H-3^1), which was further transformed into the acetate **10**; ^{1}H NMR ($CDCl_3$) δ: 5.39 (t, 1H, $J_{3,4}$ 9.5 Hz, H-3^1), 5.28 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^1), 3.73 (t, 1H, H-4^1), 3.28 (dd, 1H, $J_{2,3}$ 10.4 Hz, H-2^1), 2.13 (s, 3H, OAc).

Since the acetal mixture of **3** and **5** could be regarded as the precursors of mono- and di-hydroxy derivatives, acid hydrolysis of the mixture was attempted, and two separate mixtures were obtained. These mixtures were purified by silica gel chromatography, giving 2^1-monoalcohol **11** in 71% yield; $[\alpha]_D^{17}$ +160.6° (*c* 1.57, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 4.36 (d, 1H, $J_{2,OH}$ 11.2 Hz, OH-2^1) and $3^1,2^2$-diol **13** in 28% yield; $[\alpha]_D^{24}$ +160.8° (*c* 1.28, $CHCl_3$). The structures of **11** and **13** were determined from ^{1}H NMR after acetylation of the remaining hydroxyl groups, 2^1-acetate **12**; ^{1}H NMR ($CDCl_3$) δ: 5.17 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^1), 4.65 (dd, 1H, $J_{2,3}$ 10.1 Hz, H-2^1), 2.16 (s, 3H, OAc) and $3^1,2^2$-diacetate **14**; ^{1}H NMR ($CDCl_3$) δ: 5.42 (t, 1H, $J_{3,4}$ 9.7 Hz, H-3^1), 5.31 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1^1), 5.06 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1^2), 4.68 (dd, 1H, $J_{2,3}$ 10.1 Hz, H-2^2), 3.25 (dd, 1H, $J_{2,3}$ 10.3 Hz, H-2^1), 2.21 (s 3H, OAc), 2.16 (s, 3H, OAc) (Scheme 2).

Since the preparation of methylated β-CD having regioselective hydroxyl groups had been accomplished, we turned our attention to evaluation of the alcoholic



Scheme 1. *Reagents and conditions*: (i) α,α-dimethoxytoluene (6 molar excess), CSA, DMF, 60°C, reduced pressure; (ii) α,α-dimethoxytoluene (12 molar excess), CSA, DMF, 60°C, reduced pressure; (iii) NaOMe, MeOH, 50°C, then NaH, MeI, DMSO, rt.



Scheme 2. *Reagents and conditions*: (i) LAH, $AlCl_3$, THF, 0°C→rt; (ii) 80% aq. AcOH, 90°C; (iii) Ac_2O, pyridine; (iv) NaH, MeI, THF, rt; (v) H_2, $Pd(OH)_2$, MeOH, rt.

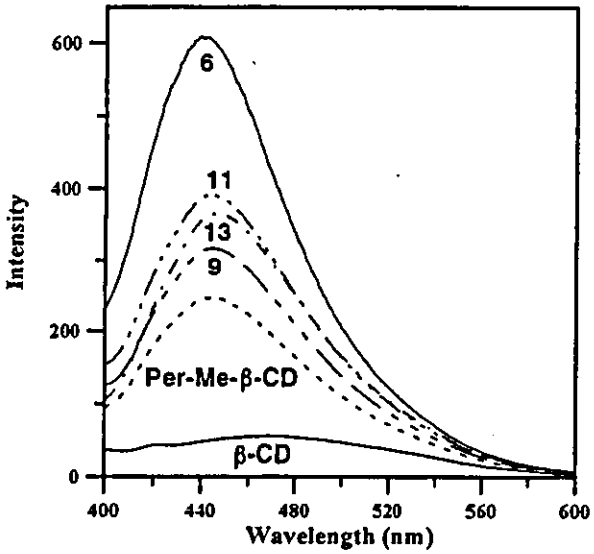


Figure 1. The fluorescence emission spectra excited at 366 nm of TNS at 20°C.

products using fluorescence measurement. After dissolving the same amount of 2-monoalcohol **11**, 3-monoalcohol **9**, benzylalcohol **6**, 2,3-diol **13**, per-methylated β-CD, and β-CD in 0.1 M phosphate-buffered water (pH 7) in the presence of 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS), respectively, fluorescence measurements were carried out at 20°C.[7] As shown in Fig. 1, fluorescence intensities of these compounds against TNS resulted in a potential binding ability for TNS. In this case, the order of the fluorescence intensities of these compounds was benzylalcohol **6**>2-monoalcohol **11**>3^1,2^2-diol **13**>3-monoalcohol **9**> per-methylated β-CD>β-CD itself. This result suggested that an alcohol in the chiral products is capable of interacting with hydrophilic functional groups in other chiral guest compounds through hydrogen bonds.

In conclusion, we have reported the successful synthesis of a series of partially methylated β-CD derivatives leaving regulated hydroxy groups and the inclusion properties against TNS in water. These regularly methylated β-CDs are potential intermediates for constructing new types of artificial enzymes.

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SYNTHESIS AND REACTIVITY OF A 5-AZIDO ANALOGUE OF NEURAMINIC ACID

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Abstract. A peracetylated phenyl 2-thioglycoside (**1**) of a 5-azido analogue neuraminic acid methyl ester was prepared from a known 5-amino precursor by a diazo transfer reaction. Glycosidation of **1** by alcohols proceeded smoothly in $CH_3CN$to give α-sialylated products in good yield.

Key Words: Azides, Glycosides, Glycosylations, Carbohydrates, Diazo transfer, Sialic acids

INTRODUCTION

N-Acetyl neuraminic acid as the usual terminal saccharide in glycoproteins and glycolipids functions as an important marker in cell-cell interaction.[1] *N*-Acetyl neuraminic acid residue of their glycoconjugates on the cell surface, for example, is known to be recognized by influenza virus hemagglutinin.[2] Although *N*-acetylated neuraminic acid is the major form in cell surface glycoconjugates, *N*-glycolylated type also exists as a minor form. This *N*-glycolylated type of sialyl lactose residue in glycolipids could be recognized by

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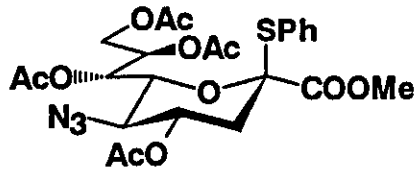
Newcastle disease virus[3] as well. Hitherto, 5-azido sialic acid as the potential inhibitor of hemagglutination by influenza virus has been prepared by the Whiteside group using enzymes such as aldolase.[4] In conjunction with our ongoing efforts directed toward chemical synthesis of sialyl oligosaccharide, 5-azido derivative of neuraminic acid having an appropriate leaving group at the C-2 position could be considered not only as a glycosyl donor but also as an attractive precursor of the free amine for future construction of a variety of sialyl oligosaccharides, including 5-glycolyl and other unnatural-type derivatives, because the azido functional group of the sialyl compound could be efficiently converted into its amino derivative under very mild conditions without decomposition of the sialyl residue. In the work described herein, we set about synthesizing peracetylated phenyl 2-thioglycoside of 5-azido sialic acid methyl ester **1** shown in Figure 1, and examine the reactivity of **1** as a donor on glycosylation without tampering the azido group.



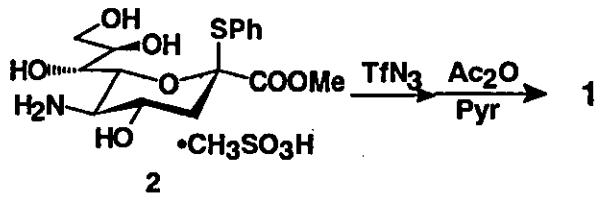
1

Figure 1.

RESULTS AND DISCUSSION

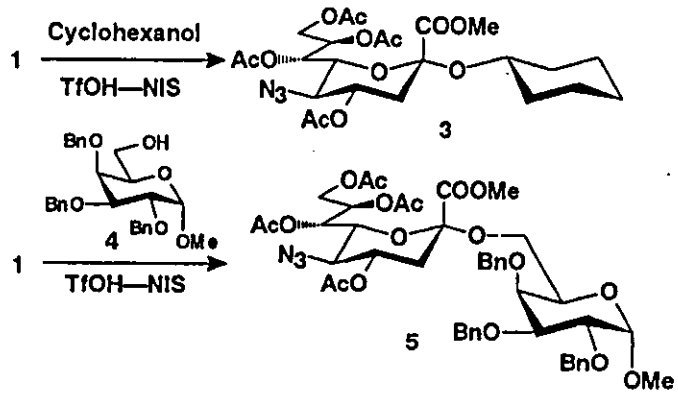
The diazo transfer reaction[5] is a simple and efficient method for obtaining an azide derivative from a precursor having a free amino group. Since the diazotransfer reaction affords a 2-azido-2-deoxy product from a hexosamine without affecting the original C-2 configuration,[6] we used this procedure to convert the 5-amino group of a sialyl derivative into a 5-azido group. Thus, phenyl 4,7,8,9-tetra-*O*-acetyl-5-amino-3,5-dideoxy-2-thio-β-D-*glycero*-D-*galacto*-2-nonulopyranosidonic acid methyl ester trifluoromethane sulfonic acid salt **2** as the candidate for the reaction was prepared by the method of Komba *et al.*.[7] Vasella *et al.* reported that the diazo transfer reaction using TfN_3 of 2-hexosamine derivatives proceeded smoothly at room temperature to give the corresponding azido compounds in high yield.[5b] Hence, the amine **2** was treated with TfN_3 in methanol at

room temperature in the presence of DMAP as an acid scavenger to yield the 5-azido derivative **1** in 80% yield after acetylation, as shown in Scheme 1. The structure of **1** was elucidated by ^{1}H NMR and IR [2116 cm^{-1} ($\nu_{N=N=N}$)] analyses. It was notable that when the reaction mixture in work-up procedure during the diazo transfer reaction was evaporated to dryness at ca. 70 °C, decomposition of the product was unexpectedly observed.



Scheme 1.

As a variety of procedures for activating thioglycosides for glycosylation have been investigated[8], we selected the TfOH—NIS activating system[9] as the most effective and convenient procedure among the procedures. Thus, the resultant azide donor **2** underwent smooth glycosylation by means of the TfOH—NIS activating system with not only a primary alcohol of methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside **4** but also a secondly alcohol of cyclohexanol, resulting in the formation of the corresponding α-glycosidic linkages in high yields after purification (Scheme 2). Since both of the resultant glycosides showed strong absorption at around 2100 cm^{-1} due to $\nu_{N=N=N}$, suggesting that no change in the azide function of the sialyl residue of the products under the conditions of glycosylation. The results of the glycosylations are summarized in the table.



Scheme 2.

Table Results of glycosidation of **1**

Acceptors	Acceptor (equiv)	Donor (equiv)	Products	Yield (%)[a]
Cyclohexanol	1	1	**3**	37.5
Cyclohexanol	1	2	**3**	60.7
4	1	1	**5**	46.3
4	1	2	**5**	81.5

[a]Isolated yield after purification.

In summary, synthesis of 5-azido sialic acid derivative was accomplished, and the azido group could endure glycosylation reaction by using the TfOH—NIS activating system without decomposition. The results of further manipulation of the 5-azide derivative will be reported elsewhere.

Experimental

Materials and Methods. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Pyridine, cylohexanol, CH_2Cl_2, and MeCN were stored over molecular sieves (MS4Å), and methanol (MeOH) was stored over MS3Å before use. MS was activated at 180 °C under diminished pressure over phosphorus pentoxide for 4-5 h before use. Melting points were measured with a Laboratory Devices MELTEMP II apparatus and were uncorrected. The optical rotations

were determined with a JASCO DIP-1000 digital polarimeter. The IR spectra were obtained using a JASCO FT/IR-300E spectrophotometer. The ^{1}H-NMR spectra were recorded at 300 MHz with a Bruker AC-300 spectrometer or at 200 MHz with a Barian Gemini-2000 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. Elemental analyses were performed with a Fisons EA1108 on samples extensively dried at 50-60 °C over phosphorus pentoxide for 4-5 h. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmmstadt, Germany). For detection of the intermediates, TLC sheets were sprayed with a solution of 85:10:5 (v/v/v) MeOH—*p*-anisaldehyde—concd sulfuric acid and heated for a few minutes. Column chromatography was performed on silica gel (Silica Gel 60; 63—200 μm, E. Merck). TfN_3 as a diazo transfer reagent was prepared as 0.4 M solution in CH_2Cl_2 by the method of Cavender and Shiner.[5-a]

Phenyl 4,7,8,9-Tetra-O-acetyl-5-azido-3,5-dideoxy-2-thio-β-D-glycero-*D*-galacto-*2-nonulopyranosidonic Acid Methyl Ester* (**1**).——To a cooled solution of known 5-amino neuraminic acid derivative[7] **2** (497 mg, 1.06 mmol) and DMAP (400 mg, 3.28 mmol) in MeOH (77 mL) at 0 °C was added dropwise TfN_3 (9.5 mL, 3.81 mmol) under argon atm, and the reaction mixture was stirred at rt overnight. When TLC indicated the complete conversion of **2**, the mixture was evaporated at 10 °C to dryness. The resulting powdery residue was dissolved in pyridine (30 mL) and acetic anhydride (50 mL) at 0 °C. After 1.5 h at rt, the mixture was concentrated at 30–40 °C, extracted with $CHCl_3$, washed successively with ice-cold 1 M aq HCl, ice-cold aq sat. $NaHCO_3$, and brine, dried over anhyd Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel (50 g) with 2:1 (v/v) *n*-hexane—ethyl acetate as the eluent to give **1** (471 mg, 79.7%), which was crystallized from 2-propanol giving white crystals: R_f 0.28 [1:1 (v/v) *n*-hexane—ethyl acetate]; mp 122-123 °C; $[\alpha]_D^{23}$ -125° (*c* 0.94, $CHCl_3$); IR (KBr) 2116 ($\nu_{N=N=N}$), 1742 ($\nu_{C=O}$), 1223 (ν_{C-O-C}) cm^{-1}; ^{1}H NMR δ ($CDCl_3$) 1.93 (dd, 1 H, $J_{3ax,3eq}$ 14.0 Hz, $J_{3ax,4}$ 11.8 Hz, H-3ax), 2.02, 2.07, 2.11, 2.17 (each s, 12 H, 4 Ac), 2.79 (dd, 1 H, $J_{3eq,4}$ 4.8 Hz, H-3eq), 3.28 (t, 1 H, H-5), 3.58 (s, 3 H, Me), 4.13 (dd, 1 H, $J_{8,9b}$ 7.0 Hz, $J_{9a,9b}$ 12.5 Hz, H-9b), 4.38 (dd, 1 H, $J_{5,6}$ 10.4 Hz, $J_{6,7}$ 2.2 Hz, H-6), 4.42 (dd, 1 H, $J_{8,9a}$ 2.3 Hz, H-9a), 5.12

(ddd, 1 H, $J_{7,8}$ 4.5 Hz, H-8), 5.40 (ddd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 5.62 (dd, 1 H, H-7), 7.45-7.27 (m, 5 H, Ph).

Anal. Calcd for $C_{24}H_{29}N_3O_{11}S_1$: C, 50.79; H, 5.15; N, 7.40%. Found: C, 50.93; H, 5.11; N, 7.30%.

*Cyclohexyl 4,7,8,9-Tetra-O-acetyl-5-azido-3,5-dideoxy-α-D-*glycero-*D-*galacto-*2-nonulopyranosidonic Acid Methyl Ester* (**3**).——A mixture of **1** (57 mg, 0.10 mmol), cyclohexanol (5 μL, 50 μmol), and molecular sieves 4A (150 mg) in MeCN (0.5 mL) was stirred for 5 h at rt under argon atm. To the suspension was added NIS (45 mg, 0.20 mmol) and TfOH (2 μL, 22 μmol) at -30 °C. To complete the reaction, the same amounts of NIS and TfOH as those started above were added, and stirring was continued overnight. The suspension was filtered through a Celite pad, and the Celite pad was washed with $CHCl_3$. The filtrate was combined, successively washed with ice-cold aq sat. $NaHCO_3$, aq 10% $Na_2S_2O_3$, and brine, dried over anhyd Na_2SO_4, and concentrated. Chromatographic purification of the residual syrup on silica gel gave **3** (17 mg, 60.7%): R_f 0.47 [1:1 (v/v) *n*-hexane—ethyl acetate]; mp 99-100 °C (from *i*-propanol); $[\alpha]_D^{18}$ -32° (*c* 1.20, $CHCl_3$); IR (KBr) 2117 ($\nu_{N=N=N}$), 1749 ($\nu_{C=O}$), 1223 (ν_{C-O-C}) cm^{-1}; ^1H NMR δ ($CDCl_3$) 1.19-1.89 (m, 11 H, H-3ax and 5 CH_2), 2.07, 2.10, 2.15, 2.19 (each s, 12 H, 4 Ac), 2.78 (dd, 1 H, $J_{3ax,3eq}$ 12.4 Hz, $J_{3eq,4}$ 4.7 Hz, H-3eq), 3.21 (t, 1 H, $J_{5,6}$ 10.2 Hz, H-5), 3.65 (m, 1 H, CH), 3.79 (s, 3 H, Me), 3.80 (m, 1 H, H-6), 4.21 (dd, 1 H, $J_{8,9b}$ 4.1 Hz, $J_{9a,9b}$ 12.7 Hz, H-9b), 4.42 (dd, 1 H, $J_{8,9a}$ 2.5 Hz, H-9a), 4.80 (ddd, 1 H, $J_{3ax,4}$ 12.3 Hz, $J_{4,5}$ 9.9 Hz, H-4), 5.35 (ddd, 1 H, $J_{7,8}$ 9.2 Hz, H-8), 5.51 (dd, 1 H, $J_{6,7}$ 1.4 Hz, H-7).

Anal. Calcd for $C_{24}H_{35}N_3O_{12}$: C, 51.70; H, 6.33; N, 7.54%. Found: C, 51.69; H, 6.35; N, 7.44%.

*Methyl O-(Methyl 4,7,8,9-tetra-O-Acetyl-5-azido-3,5-dideoxy-α-D-*glycero-*D-*galacto-*2-nonulopyranosylonate)-(2→6)-2,3,4-tri-O-benzyl-α-D-galactopyranoside* (**5**).——A suspension of **1** (100 mg, 0.176 mmol), methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside **4** (41 mg, 88 μmol), and molecular sieves 4A (300 mg) in MeCN (1.0 mL) was stirred for 4.5 h at rt under argon atm, then cooled to -30 °C. NIS (79 mg, 0.352 mmol) and TfOH (6.2 μL, 70 μmol) were added to the cold mixture with stirring, and the mixture was further stirred for 2 h at -30 °C. When TLC indicated complete

disappearance of **1**, the suspension was filtered. The same work-up procedure as described for **3** gave **5** (66 mg, 81.5%) as hard colorless syrup: R_f 0.67 [1:2 (v/v) *n*-hexane—ethyl acetate]; $[\alpha]_D^{20}$ -2.6° (*c* 1.04, $CHCl_3$); IR (KBr) 2116 ($\nu_{N=N=N}$), 1750 ($\nu_{C=O}$), 1221 (ν_{C-O-C}) cm^{-1}; ^1H NMR δ ($CDCl_3$) 1.75 (dd 1 H, $J_{3'ax,3'eq}$ 12.8 Hz, $J_{3'ax,4'}$ 12.4 Hz, H-3'ax), 2.05, 2.10, 2.13, 2.14 (each s, 12 H, 4 Ac), 2.71 (dd, 1 H, $J_{3'eq,4'}$ 4.8 Hz, H-3'eq), 3.39 (s, 3 H, OMe), 3.66 (s, 3 H, COOMe), 4.03 (dd, 1 H, $J_{1,2}$ 3.3 Hz, $J_{2,3}$ 9.1 Hz, H-2), 4.18 (dd, 1 H, $J_{8',9'b}$ 4.0 Hz, $J_{9'a,9'b}$ 12.6 Hz, H-9'b), 4.29 (dd, 1 H, $J_{8',9'a}$ 2.4 Hz, H-9'a), 5.36 (ddd, 1 H, $J_{7',8'}$ 9.1 Hz, H-8'), 5.49 (dd, 1 H, $J_{6',7'}$ 1.5 Hz, H-7').

Anal. Calcd for $C_{46}H_{55}N_3O_{17}$: C, 59.93; H, 6.01; N, 4.56%. Found: C, 59.69; H, 6.07; N, 4.71%.

ACKNOWLEDGMENT

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Notes

An Improved Preparation of *N,N'*-Diacetylchitobiose by Continuous Enzymatic Degradation of Colloidal Chitin Using Dialysis Tubing as a Convenient Separator

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Chitin is a polysaccharide composed of β-(1→4)*N*-acetyl-D-glucosamine residues and displays quite low solubility in both organic and aqueous solvents due to its highly crystallographic structure involving inter- and intra-glycosidic hydrogen bondings.[1] Therefore, acid hydrolysis of chitin to obtain a chitobiose was only possible by using strong acids such as hydrogen chloride,[2] hydrogen fluoride,[3] and sulfuric acid,[4] and the yield of the product was not high. *N,N'*-Diacetylchitobiose composed of *N*-acetyl-β-D-glucosamine-(1→4)*N*-acetyl-D-glucosamine is a biologically significant disaccharide and is a well-known general core sequence of N-linked-type glycoproteins.[5] Although successful production of *N,N'*-diacetylchitobiose by microbial degradation of chitin with bacteria has been reported, the procedure involved difficulties in handling and unreproductivity.[6] In the course of our recent study, a chitinase from *Streptomyces griseus* was found to be the most efficient enzyme among the chitinases from various species for the degradation of colloidal chitin in order to obtain a chitobiose.[7] Although in our preparation of the chitobiose enzymatic degradation of the colloidal chitin proceeded smoothly, the expensive enzyme was used just once for the reaction. Therefore, the need for an improved method for preparation of the chitobiose and the waste of the costly enzyme prompted us to try to utilize the concept of dialysis for the enzymatic method. In this paper, we describe the improved production of peracetylated chitobiose **1** by continuous enzymatic degradation of colloidal chitin in dialysis tubing, and the chitinase recycling system (Scheme 1). In our initial study for the preparation of **1**, it was found that the enzymatic degradation was stopped when the concentration of *N,N'*-diacetylchitobiose reached 3.3 mg/mL, as determined by HPLC analysis even though colloidal chitin remained. We assumed that this behavior was caused by a product inhibition due to *N,N'*-diacetylchitobiose. Therefore, we first designed system A in which the enzymatic degradation of chitin was performed

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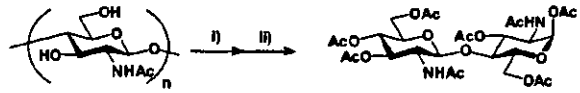
Scheme 1[a]



[a] Reagents and Conditions: (i) chitinase (50 units), buffer (pH 6.3), 40 °C; (ii) NaOAc, Ac_2O, 80 °C.

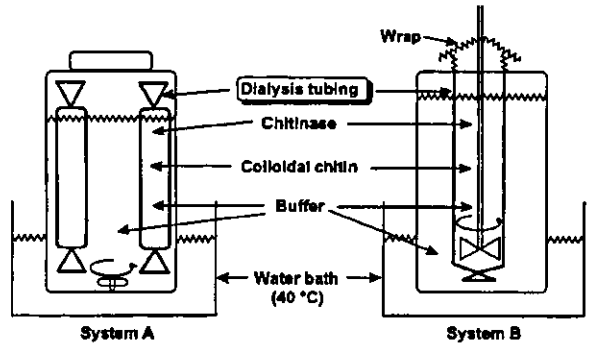


Figure 1. Schematic representation of systems A and B.

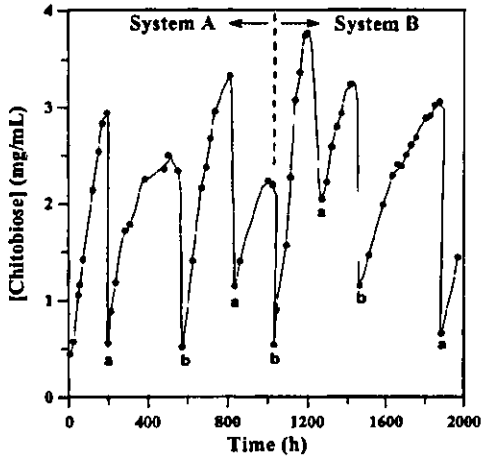


Figure 2. Time course of *N,N'*-diacetylchitobiose produced by the enzymatic hydrolysis of colloidal chitin using systems A and B. The concentration of *N,N'*-diacetylchitobiose was estimated by HPLC analysis. **a** indicates a replacement of the outer buffer solution of the dialysis tubing. **b** indicates both a replacement of the outer buffer solution and an addition of further colloidal chitin.

in dialysis tubing inside of which the product was released. A product smaller in size than that of the pore of the dialysis tubing could be obtained from the outer side of the dialysis tubing, as shown in Figure 1. The concentration of *N,N'*-diacetylchitobiose produced by the enzymatic degradation of colloidal chitin was monitored by TLC as well as HPLC, as shown in Figure 2. Since it was found in our previous study that the concentration of the product reached ca. 3.3 mg/mL, the external buffered solution of the dialysis tubing was replaced with a freshly prepared buffer when it was

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Table 1. Results of Chitinase-Assisted Hydrolysis of Colloidal Chitin To Obtain Chitobiose

method	chitin[a] (g)	chitinase (units)	1 (g)	yield[b] (%)	efficiency (g/unit)	denaturing factor
previous	315	500	200[c]	38	0.4	product inhibition
continuous[d]	99	50	60[e]	36	1.2	production of fungi

[a] Amount of chitin itself used in colloidal chitin. [b] Isolated yields based on chitin. [c] Purified by a combination of crystallization and silica gel chromatography. [d] Systems A & B. [e] Purified by single crystallization before which the raw product included ca. 100 g of **1**, as determined by HPLC analysis.

estimated that *N,N'*-diacetylchitobiose had reached that concentration or when saturation of the production of disaccharide was observed. The production of *N,N'*-diacetylchitobiose was started again immediately after the replacement of the buffer at **a** in Figure 2. Although the digestion of the colloidal chitin proceeded smoothly, further colloidal chitin was added to the dialysis tubing when the remaining chitin had been completely digested as marked by **b** in Figure 2. The combination of both systems A (sealed-type dialysis tubing) and B (open-type dialysis tubing) gave a total about 63 g of *N,N'*-diacetylchitobiose, as estimated by HPLC analysis, when 50 units of chitinase and 600 g of colloidal chitin were used. It was noteworthy that the enzyme solution was able to evaporate in vacuo at 40 °C by the use of a rotary evaporator without affecting the hydrolytic activity in order to reduce the amount of the enzyme solution when the system was changed from A to B. The *N,N'*-diacetylchitobiose produced was acetylated after removal of the solvent to give 60 g of **1** purified by only a single crystallization procedure from MeOH without silica gel chromatography. Table 1 summarizes the results of enzymatic degradation of colloidal chitin in comparison with the results of our previous study. Despite the fact that the isolated yields of **1** did not differ greatly, the efficiency of the production of chitobiose using a continuous method such as systems A and B was four times higher than that of the former method. Consequently, it seems that product inhibition owing to the *N,N'*-diacetylchitobiose produced was avoided. Indeed, both systems A and B functioned efficiently; however, an interruption of the continuous enzymatic hydrolysis was caused by denaturing of the enzyme due to growing fungi from somewhere.

Experimental Section

General Procedures. Unless otherwise stated, all commercially available solvents and reagents were used without further purification. Melting points were measured with a Laboratory Devices MELTEMP II apparatus and were uncorrected. IR spectra were obtained using a JASCO FT/IR-300E spectrophotometer. The ^{1}H NMR spectra were recorded at 400 MHz with a Bruker AM-400 in chloroform-*d* with tetramethylsilane (TMS) used as an internal standard. Ring-proton assignments in NMR were made by first-order analysis of the spectra and were supported by the results of homonuclear decoupling experiments. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmmstadt, Germany). For detection of the intermediates, TLC sheets were sprayed with (a) a solution of 85:10:5 (v/v/v) MeOH–*p*-anisaldehyde–concentrated sulfuric acid and heated for a few minutes. All extractions were concentrated below 45 °C under diminished pressure. HPLC was performed with a Kaseisorb LC NH2-300-5 column (4.6 × 250 mm, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) at ambient temperature. The column was equilibrated with acetonitrile–water (8:2, v/v) and run at a flow rate of 1.0 mL/min. For the detection of the sample on HPLC, UV 220 nm was used. Dialysis tubing (molecular weight cutoff, 12–14 kDa) of seamless cellulose was purchased from Union Carbide Corp., Danbury, CT. Colloidal chitin containing 83.5 wt % water was prepared by the previously described method.[6] Chitinase (EC 3.2.1.14, from *Streptomyces griseus* 0.26 units/mg of solid) was obtained from Sigma Chemical Co., St. Louis, MO. Buffer solution was prepared to be pH 6.3 by mixing 0.2 M aqueous acetic acid and 0.2 M aqueous disodium hydrogen phosphate in appropriate ratios. For the enzymatic degradation, the buffer was diluted with the same amount of water before use.

***O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose (1).** The first trial of enzymatic degradation of a colloidal chitin was carried out using system A. Colloidal chitin (150 g) and chitinase (50 units) in a mixture of buffer solution (800 mL) were added separately to three dialysis tubings, and the dialysis tubings were sealed. The tubings including the reaction mixture were stirred in the buffer solution (4 L) at 40 °C for 196 h, at which time the concentration of chitobiose produced in the buffer solution outside the tubings was 3 mg/mL. The buffer solution outside the tubings was replaced, and the reaction was continued for a further 284 h, at which time colloidal chitin was almost completely digested. The dialysis tubings were opened, and further colloidal chitin (150 g) was added to the buffered enzyme solution. The enzymatic degradation was performed for one more cycle as described above. At 912 h after the starting time, the enzyme solution in the dialysis tubing was evaporated to ca. 500 mL at 40 °C in vacuo. This concentrated solution was use for the next reaction using system B without purification.

The second trial of degradation was performed using system B. The enzyme solution and colloidal chitin (150 g) were added to the dialysis tubing at 40 °C with continuous stirring under conditions similar to those described above. One more cycle was performed, and the enzyme was eventually denatured by fungi.

Typical acetylation of the reactant solution outside the tubings was as follows. The solution including *N,N'*-diacetylchitobiose was concentrated to dryness. The residue was heated at 80 °C for 16 h with acetic anhydride (500 mL) and anhydrous sodium acetate (25 g) with vigorous stirring. After evaporation of the reaction mixture, water (600 mL) was added, and the precipitates were filtered, dissolved in $CHCl_3$, washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Crystallization of the resulting residue from MeOH gave pure **1**: mp 301–303

°C (lit.[7] 303−304 °C and lit.[6] 301−303 °C); IR (KBr) 2948 (ν_{C-H}), 1743 ($\nu_{C=O}$), 1658 ($\nu_{C=O}$, amide I),1536 (δ_{N-H}, amide II) cm^{-1}; ^1H NMR δ 1.93, 1.96, 2.01, 2.02, 2.06, 2.09, 2.16, 2.20 (each s, 24 H, 8 Ac), 3.63 (ddd, 1 H, $J_{4',5'}$ = 9.6 Hz, $J_{5',6'a}$ = 2.2 Hz, $J_{5',6'b}$ = 3.9 Hz, H-5′), 3.74 (t, 1 H, $J_{3,4}$ = 9.1 Hz, $J_{4,5}$ = 9.7 Hz, H-4), 3.90 (dt, 1 H, $J_{5,6a}$ = 1.9 Hz, $J_{5,6b}$ = 2 Hz, H-5), 3.98 (q, 1 H, H-2′), 4.02 (dd, 1 H, $J_{6'a,6'b}$ = 12.3 Hz, H-6′a), 4.18 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.37 (m, 1 H, H-2), 4.40 (dd, 1 H, H-6′b), 4.46 (dd, 1 H, H-6b), 4.46 (d, 1 H, $J_{1',2'}$ = 8.4 Hz, H-1′), 5.06 (t, 1 H, $J_{3',4'}$ = 9.5 Hz, H-4′), 5.13 (t, 1 H, $J_{2',3'}$ = 10.1 Hz, H-3′), 5.23 (dd, 1 H, $J_{2,3}$ = 11.1 Hz, H-3), 5.65 (d, 1 H, $J_{2,NH}$ = 9.1 Hz, NH), 6.01 (d, 1 H, $J_{2',N'H}$ = 9.3 Hz, N′H), 6.10 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1).

In summary, a convenient method for the preparation of *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose as a peracetylated chitobiose by using dialysis tubing as a convenient separating material for the *N*,*N*′-diacetylchitobiose produced from the reaction mixture of a colloidal chitin and a chitinase from *Streptomyces griseus* was described. This methodology is applicable to other enzymatic hydrolysis reactions of polymeric substances.

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Introduction of monosaccharides having functional groups onto a carbosilane dendrimer: A broadly applicable one-pot reaction in liquid ammonia involving Birch reduction and subsequent SN2 reaction☆

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Abstract

Benzylthioalkyl glycosides of D-glucuronic acid, *N*-acetyl-D-glucosamine, and *N*-acetylneuraminic acid (common monosaccharide constituents of natural oligosaccharide chains) have been prepared as sulfide precursors for the carbohydrate coating of dendric carbosilane cores and used in a generally applicable one-pot reaction (Birch reduction in liquid ammonia and subsequent SN2 reaction) to generate a thioether linkage between the monosaccharide moieties and a carbosilane dendrimer. The dendrimers were uniformly functionalized with the monosaccharides in good yields. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Carbosilane dendrimers; Glycodendrimers; Birch reduction; Sulfides; SN2 reaction

1. Introduction

The sugar clustering effect is well-known as a phenomenon, originally reported by Lee [1], that can enhance the weak individual interactions between carbohydrates and proteins. For example, Lee synthesized cluster glycosides from aminotris(hydroxymethyl)methane as the core for investigation of such interactions [2]. Recently a number of glycodendrimers intended for similar purposes have been synthesized and investigated [3]. We have also reported a novel class of glycodendrimers using carbosilane dendrimers as the core structures [4,5]. For the construction of carbosilane dendrimers uniformly functionalized with carbohydrate moieties, as shown in Fig. 1, a successful one-pot coupling procedure between the carbohydrate moieties and carbosilane dendrimers was developed. The method utilizes Birch reduction [Na in liquid ammonia (liquid NH_3)] of benzyl-thioether-functionalized carbohydrate precursors and the subsequent SN2 replacement of ω-bromo groups in dendric carbosilane scaffolds [4,5]. In our previous study, this procedure was used exclu-

☆ Part 4. Glyco-silicon functional materials. For Part 3, see Ref. [4b].

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sively to attach bioactive oligosaccharides having only hydroxyl groups (neutral sugars) such as β-cyclodextrin and a globotriaosyl moiety. We have since directed our attention toward the feasibility of a one-pot reaction for other saccharides having such functional groups as carboxyl or acetamido groups.

In this study, we demonstrate the broad applicability of a one-pot reaction for constructing carbosilane dendrimers uniformly coated with D-glucuronic acid, *N*-acetyl-D-glucosamine, or *N*-acetylneuraminic acid (Fig. 1).

2. Results and discussion

To construct a carbosilane dendrimer functionalized with monosaccharides having a carboxyl group, the glucuronic acid derivative **5** was selected for the first test case. Thus, the known trityl glucoside **1** [6] was converted in 52% yield into its carboxymethyl derivative **2** via the in situ *O*-de-tritylation followed by Jones oxidation (CrO_3) in a one-pot procedure. The convenient introduction of a sulfide function into an alkenyl group has been reported by several groups via radical addition [7]. Thus, the radical addition of α-toluenethiol to the C=C double bond of the aglycon of **2** was performed in 1,3-dioxolane in the presence of AIBN as the radical initiator to afford the 3-(benzylthio)propyl glycoside **3** in an anti-Markovnikov's manner in almost quantitative yield. The structure of the sulfide **3** was confirmed by both its ^1H NMR spectrum and elemental analysis. Zemplén *O*-deacetylation of **3** gave **4** in 69% yield after purification on a column of silica gel. Saponification of the methyl ester **4** proceeded smoothly in the presence of NaOH, giving the corresponding carboxylate anion, which was converted in 95% yield into **5** by the removal of Na cations by using ion exchange resin (H^+).

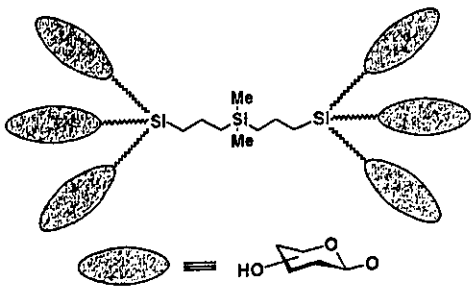

Fig. 1. An example of a carbosilane dendrimer uniformly functionalized with sugar moieties.

Since the preparation of the GlcA derivative **5** had been accomplished, we turned our attention to preparation of the 3-(benzylthio)propyl glycoside **8** of GlcNAc. For this purpose, the allyl glycoside **6** [7a] was allowed to react with α-toluenethiol in the presence of a radical initiator to provide the crystalline sulfide **7** in 98% yield. The transesterification of the acetate **7** was carried out in methanolic sodium methanoate at room temperature to give **8** in 99% yield.

Finally, we examined the preparation of a benzylthioalkyl glycoside sialic acid (which contains a carboxylic acid and an *N*-acetyl group). Thus, the known **9** was prepared as the starting material by the method previously reported [8]. Addition of a benzylsulfide moiety by a radical reaction to the allyl function of **9** also proceeded efficiently in an anti-Markovnikov's manner to afford **10** in 96% yield. Conventional *O*-de-acetylation gave the tetrol **11** in 78% yield, which was further treated with aqueous NaOH to produce the benzylthioalkyl glycoside **12** of sialic acid quantitatively (Scheme 1).

Given the success of the preparation of 3-(benzylthio)propyl glycosides **5**, **8**, and **12**, we proceeded to add these derivatives to the carbosilane dendrimer **13** [5] to produce sugar clusters. We have recently reported a simple and convenient method, namely, a one-pot reaction in liquid NH_3 for Birch reduction and subsequent SN2 reaction producing thioether linkages, for the construction of a carbosilane dendrimer functionalized with β-cyclodextrin and globotriaose moieties (sugar residues lacking additional functional groups). In order to examine the broader applicability of the one-pot reaction, the 3-benzylthiopropyl glycosides of **5**, **8**, and **12** were tested. The Birch reductive removal of the benzyl group of glycoside **5** was performed in liquid NH_3 in the presence of Na, generating the thiolate anion in situ. After neutralization of the excess Na by the addition of NH_4Cl, the thiolate anion was allowed to react with the dendrimer **13**