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Synthetic assembly of trisaccharide moieties of globotriaosyl ceramide using carbosilane dendrimers as cores. A new type of functional glyco-material

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Abstract

As a novel type of artificial receptor for Vero toxins, three pairs of carbosilane dendrimers uniformly carrying 12, 6, and 3 units of trisaccharide moieties of globotriaosyl ceramide were prepared through formation of the sulfide linkages in liquid NH_3, which revealed unexpected differences among their biological responses.

Keywords: carbohydrates; biologically active compounds; dendrimers; sulfides.

Globotriaosyl ceramide (Gb_3; Galα1-4Galβ1-4Glcβ1-Cer) is a major glycolipid located on the surface of the kidney glomerular endothelial cell and is known as the host receptor for Verotoxins (VTs; VT1 and VT2),[1] which are produced by pathogenic *Escherichia coli* O157.[2] Since the extremely selective and potent affinity of Gb_3 for VTs is mainly attributable to its trisaccharide component, clustering the trisaccharide (globotriose) moieties of Gb_3 as an artificial receptor for VTs might give potential glyco-materials of medicinal use. Thus, Nishida et al. co-polymerized an acrylamide derivative carrying the globotriosyl moiety with acrylamide, obtaining a linear co-polymer holding the trisaccharides like pendants.[3] Although this polymer showed some inhibitory effect against cytotoxicity of VT1, it did not reveal any activity against VT2.

This communication describes a novel type of assembly of the globotriosyl moieties using carbosilane dendrimers as polymers supporting them. Carbosilane dendrimers have recently been developed and found to have several unique characteristics: (1) simplicity of the synthetic process to extend the generation;[4] (2) accessibility to the polymer with definite molecular weight and a definite number of terminal functions, which depend on the polymer generation; (3) neutral nature in contrast to the usual

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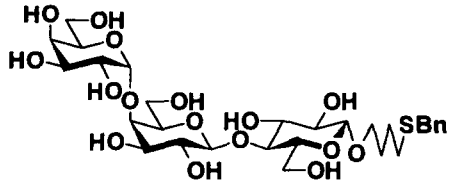

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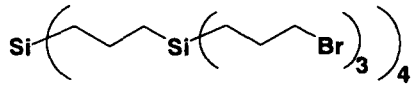


A

polyamine-type dendrimers;[5] and (4) biological inertness, and so on. Hitherto, most modifications of such dendrimers have been conducted by coupling with various functional molecules through condensation reactions; i.e., esterification or amide formation, etc. In contrast, our strategy to uniformly modify carbosilane dendrimers with globotriosyl moieties employed the coupling of both components through S_N2 reaction to form more stable sulfide linkages.[6] Thus, we designed compounds **1** as a precursor of the globotriosyl reactant and **A** as a generation 1 (G1) of the carbosilane dendrimer, since our initial target was the preparation of the G1 carrying 12 globotriosyl moieties.

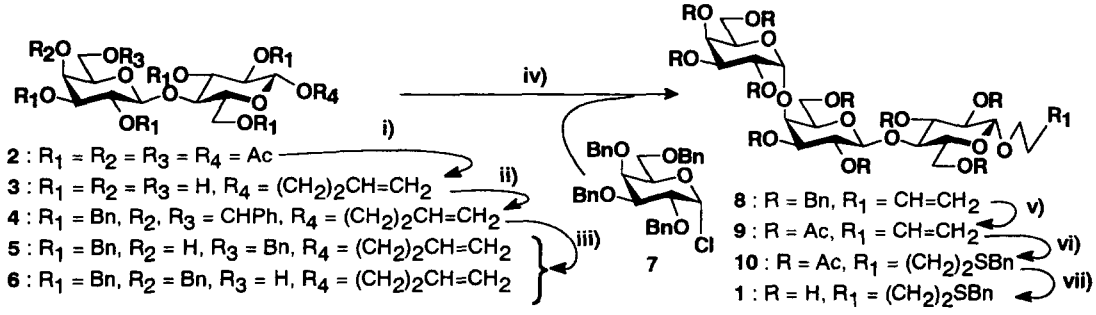
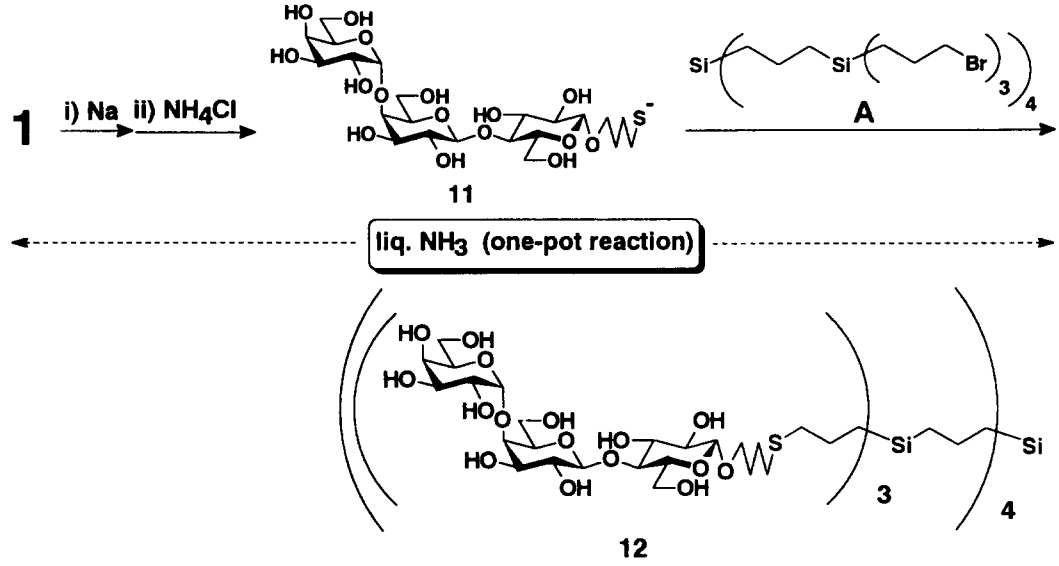


Figure 1. *Reagents and conditions*: (i) 3-Buten-1-ol, $BF_3 \cdot Et_2O$, $ClCH_2CH_2Cl$, 0°C, then NaOMe, MeOH, rt; (ii) α,α-dimethoxytoluene, CSA, DMF, 60°C, then BnBr, NaH, DMF, 0°C; (iii) $BH_3 \cdot NMe_3$, $AlCl_3$, MS4 Å, THF, rt; (iv) AgOTf, MS4 Å, Et_2O; (v) Na, liq. NH_3, −78°C, then Ac_2O, Pyr., rt; (vi) BnSH, AIBN, Dioxane, 50→80°C; (vii) NaOMe, MeOH, rt

For the synthesis of **1** (Fig. 1), the starting peracetyl-β-lactose **2** underwent glycosidation with 3-buten-1-ol in the presence of Lewis acid[7] and subsequent deacetylation, giving **3** in ca. 60% overall yield, $[\alpha]_D^{28}$ −12 (MeOH), ^{1}H NMR (D_2O) δ: 4.5 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.4 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′). After 4′,6′-*O*-benzylidenation of **3**, the remaining OH groups were all benzylated to give **4**, which was subjected to reductive cleavage by treatment with $BH_3 \cdot NMe_3$ in the presence of $AlCl_3$, giving **5** with the 4-OH in 82% yield, mp 101°C, $[\alpha]_D^{24}$ +20 ($CHCl_3$) and the 6-OH isomer **6** in 13% yield. Glycosidation of **5** with 2,3,4,6-tetra-*O*-benzyl-α-D-galactosyl chloride **7**[8] in the presence of AgOTf in ether at −20°C proceeded steroselectively to give syrupy **8** in 80% yield, ^{13}C NMR ($CDCl_3$) δ: 104 (β; C-1′), 103 (β; C-1), 101 (α; C-1″). Debenzylation of **8** without affecting the terminal double bond was conducted through Birch reduction. Thus, **8** was treated with Na in liq. NH_3 at −78°C and then acetylated to give fully acetylated *n*-butenyl glycoside **9** in 54% overall yield, $[\alpha]_D^{25}$ +38 ($CHCl_3$), ^{1}H NMR ($CDCl_3$) δ: 5.0 (d, 1H, $J_{1'',2''}$=3.6 Hz, H-1″), 4.5 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′), 4.5 (d, 1H, $J_{1,2}$=7.9 Hz, H-1). When **9** was treated with α-toluenethiol in 1,4-dioxane in the presence of AIBN, radical addition of the thiol to the double bond of **9** proceeded smoothly,[9] giving the sulfide **10** in quantitative yield, $[\alpha]_D^{26}$ +35 ($CHCl_3$), ^{1}H NMR ($CDCl_3$) δ: 5.0 (d, 1H, $J_{1'',2''}$=3.5 Hz, H-1″), 4.5 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′), 4.4 (d, 1H, $J_{1,2}$=8.0 Hz, H-1). Deacetylation of **10** gave **1** quantitatively as an amorphous solid, $[\alpha]_D^{27}$ +39 (MeOH), ^{1}H NMR (D_2O) δ: 4.9 (br s, 1H, H-1″), 4.5 (d, 1H, $J_{1',2'}$=6.7 Hz, H-1′), 4.3 (d, 1H, $J_{1,2}$=6.1 Hz, H-1), ^{13}C NMR (D_2O) δ: 103, 102 (C-1 and -1′), 100 (C-1″).

For the synthesis of **A**, the known polyhydroxyl dendrimer having the same G1 skeleton[10] was used as the precursor and was fully *O*-mesylated. The resulting compound was treated with NaBr in DMF, giving **A** in 60% overall yield, ^{1}H NMR ($CDCl_3$) δ: 3.4 (t, 24H, *J*=6.8 Hz, $12CH_2Br$), 1.8 (m, 24H, $12CH_2CH_2Br$), 1.3 (m, 8H, $4SiCH_2CH_2CH_2Si$), 0.7–0.6 (m, 40H, $20SiCH_2$).

Before coupling of **1** with **A**, the *S*-benzyl group of **1** should be removed. We developed methodology to perform the removal of the benzyl group and the coupling reaction in a one-pot manner, using liq. NH_3 as the solvent. Thus, Birch reduction of **1** was accomplished in the presence of Na in liq. NH_3 at –33°C giving a thiolate anion **11**, which was successively treated with the brominated dendrimer **A** after neutralization of the excess Na with NH_4Cl. The resulting raw product was purified with Sephadex G-25 to give **12** carrying 12 globotriosyl moieties as a white powder in 36% yield based on **A**, MALDI MS calcd for [M+Na$^+$]: 7935.0; found *m/z*: 7935.5, integral ratio of the H atoms by ^{1}H NMR: $SiCH_2$:SCH_2:H-1 and 1′=40:48:24, ^{13}C NMR (D_2O) δ: 103, 103 (C-1 and -1′), 101 (C-1″).



Examination of the relationship between the number of the globotriaosyl moieties assembled and their biological responses has also attracted much attention. Therefore, we further prepared **B**, a dumbell-type of G1 dendrimer carrying six bromine atoms, and **C**, a G0 dendrimer with three bromine atoms, for coupling with **1**. The synthetic scheme for **B** is shown in Fig. 2. The starting dichlorodimethylsilane **13** was subjected to a series of reactions such as Grignard, hydrosilation, and the second Grignard reaction to give the hexaallyl compound **14**, which further underwent successively hydroboration, *O*-mesylation, and replacement with bromo anions, giving **B** in 26% overall yield, ^{1}H NMR ($CDCl_3$) δ: 3.4 (t, 12H, *J*=6.9 Hz, $6CH_2Br$), 1.8 (m, 12H, $6CH_2CH_2Br$), 1.3 (m, 4H, $2SiCH_2CH_2CH_2Si$), 0.7–0.5 (m, 20H, $10SiCH_2$). The synthesis of **C**, ^{1}H NMR ($CDCl_3$) δ: 7.5–7.4 (m, 5H, Ph), 3.4 (t, 6H, *J*=6.8 Hz, $3CH_2Br$), 1.9 (m, 6H, $3CH_2CH_2Br$), 1.0 (m, 6H, $3SiCH_2$), was accomplished from the corresponding triol **15**[6] via the sulfonates like the synthesis of **A** and **B** (Fig. 2).

Coupling of **1** with **B** and **C** was performed in liq. NH_3 in the same way as for the preparation of **12**, giving dendrimers **16** (50% yield) and **17** (88% yield), which carry six and three globotriaosyl moieties, respectively. Compound **16**: FABMS calcd for [M+H$^+$]: 4000.5; found *m/z*: 4001.0, ^{1}H NMR (D_2O) δ: 4.9 (d, 6H, $J_{1'',2''}$=3.1 Hz, H-1″), 4.5 (d, 6H, $J_{1',2'}$=6.9 Hz, H-1′), 4.4 (d, 6H, $J_{1,2}$=6.7 Hz, H-1), –0.04 (br s, 6H, CH_3×2). Compound **17**: FABMS calcd for [M+H$^+$]: 2005.75; found *m/z*: 2005.64, ^{1}H NMR

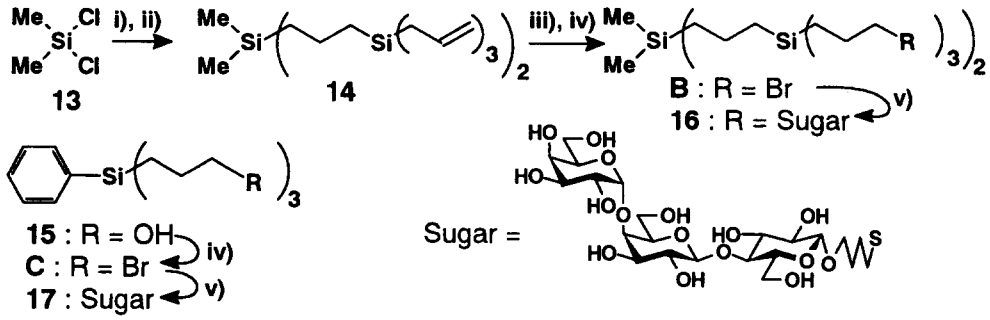


Figure 2. *Reagents and conditions*: (i) CH_2=$CHCH_2MgBr$, Ether; (ii) $HSiCl_3$, H_2PtCl_6, THF,[4] then CH_2=$CHCH_2MgBr$, Ether–THF; (iii) BH_3–THF, THF, then 3 M NaOH aq., H_2O_2; (iv) MsCl, Pyr., then NaBr, DMF; (v) **1**, Na, liq. NH_3, then NH_4Cl, liq. NH_3

(D_2O) δ: 7.3 (m, 5H, ph), 4.9 (d, 3H, $J_{1'',2''}$=3.3 Hz, H-1″), 4.5 (d, 3H, $J_{1',2'}$=7.1 Hz, H-1′), 4.4 (d, 3H, $J_{1,2}$=7.1 Hz, H-1).

Inhibitory activities of **12**, **16**, and **17** against cytotoxicity of VT1 and VT2 were examined, using cell culture assay. Unexpectedly, **12** and **16** showed a similar degree of potent activities against both VTs, while **17** did not show any activity. The detailed results of the biological assay will be reported elsewhere in due course.

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