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Synthesis of sialyllactosamine clusters using carbosilane as core scaffolds by means of chemical and enzymatic approaches ☆

Koji Matsuoka [a,*], Reina Kaneko [a], Tetsuo Koyama [a], XiaoTao Ma [a], Yasuaki Esumi [b], Takemichi Nakamura [b], Ken Hatano [a], Daiyo Terunuma [a]

[a] Area for Molecular Function, Division of Material Science, Graduate School of Science and Engineering, Saitama University, Sakura, Saitama 338-8570, Japan
[b] The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-0198, Japan

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ABSTRACT

An efficient synthesis of sialyllactosamine (SiaLacNAc) clusters using carbosilanes as core scaffolds has been accomplished by means of chemical and enzymatic approaches. *N*-Acetyl-D-glucosamine (GlcNAc) clusters having *O*-glycosidic linkage or *S*-glycosidic linkage were chemically synthesized from known intermediates in high yields. The GlcNAc clusters were first used as substrates for β1,4 galactosyl transferase using UDP-galactose (UDP-Gal) as a sugar source to provide corresponding *N*-acetyllactosamine clusters. Further sugar elongation of the LacNAc clusters was demonstrated using α2,3 sialyl transferase and CMP-neuraminic acid (CMP-NANA) to yield the corresponding SiaLacNAc clusters.

Sialyl *N*-acetyllactosamine (SiaLacNAc; Neu5Acα2→3Galβ1→4GlcNAc) and *N*-acetyllactosamine (LacNAc; Galβ1→4GlcNAc) are known as extremely valuable structures of glycoconjugates such as glycoproteins and glycolipids.[1] Influenza virus recognizes the Neu5Acα2→3Gal structure of SiaLacNAc[2] and adheres to the surface of host cells, by which infection by the virus is established.[3] LacNAc is also known as a repeating sequence of the lactosaminoglycan, which is a biologically important glycan chains on aging.[4] Synthetic assembly of the trisaccharidic and disaccharidic structures was successfully accomplished by means of synthetic organic chemistry[5] and by a combination of chemistry and biochemistry such as chemo-enzymatic methodology.[6] The use of organic synthesis for construction of an oligosaccharide structure has the merit of enabling large-scale preparation and formation of unnatural saccharide sequences, but it also has disadvantages including the necessity of a synthetic scheme involving multiple laborious steps and complication of purification. The use of enzymatic constructions using glycosyl transferases and sugar nucleotides has the merit on the basis of natural pathway in biological systems. The disadvantages of enzymatic synthesis of the oligosaccharides are that only small-scale preparation is possible because of the use of costly enzymes and sugar nucleotides and that enzymatic synthesis is not suitable for preparation of unnatural saccharide structures. Thus, an appropriate combination of organic synthesis and biochemistry is a promising convenient method for efficient construction of important oligosaccharide structures.

Since monomeric oligosaccharides usually show low levels of biological activity against sugar-binding proteins, the sugar cluster effect[7] is utilized in order to enhance the low binding affinities.[8] In our recent study on glyco-silicon functional materials, successful enhancement of the interaction between carbohydrate chains and proteins was also demonstrated.[9] In this letter we report efficient assembly of SiaLacNAc and LacNAc on carbosilane core scaffolds by means of a chemo-enzymatic approach.

Figure 1 summarizes our synthetic plan for the construction of SiaLacNAc on a carbosilane scaffold. Known GlcNAc derivative and carbosilane core frames bearing functional groups at ω-positions are condensed to produce the corresponding multivalent-type GlcNAc derivative. Sugar elongations are performed on dendritic GlcNAc residues by using appropriate glycosyl transferases and sugar nucleotides.

Scheme 1 summarizes chemical synthesis of glycoclusters. The oxazoline derivative of GlcNAc **1**[10] was allowed to glycosylate with a known triol **2**[11] in the presence of CSA to afford the

☆ Glyco-silicon functional materials. Part 13. For part 12, see Ref. 9b.

* Corresponding author. Tel./fax: +81 48 858 3099.
E-mail address: koji@fms.saitama-u.ac.jp (K. Matsuoka).

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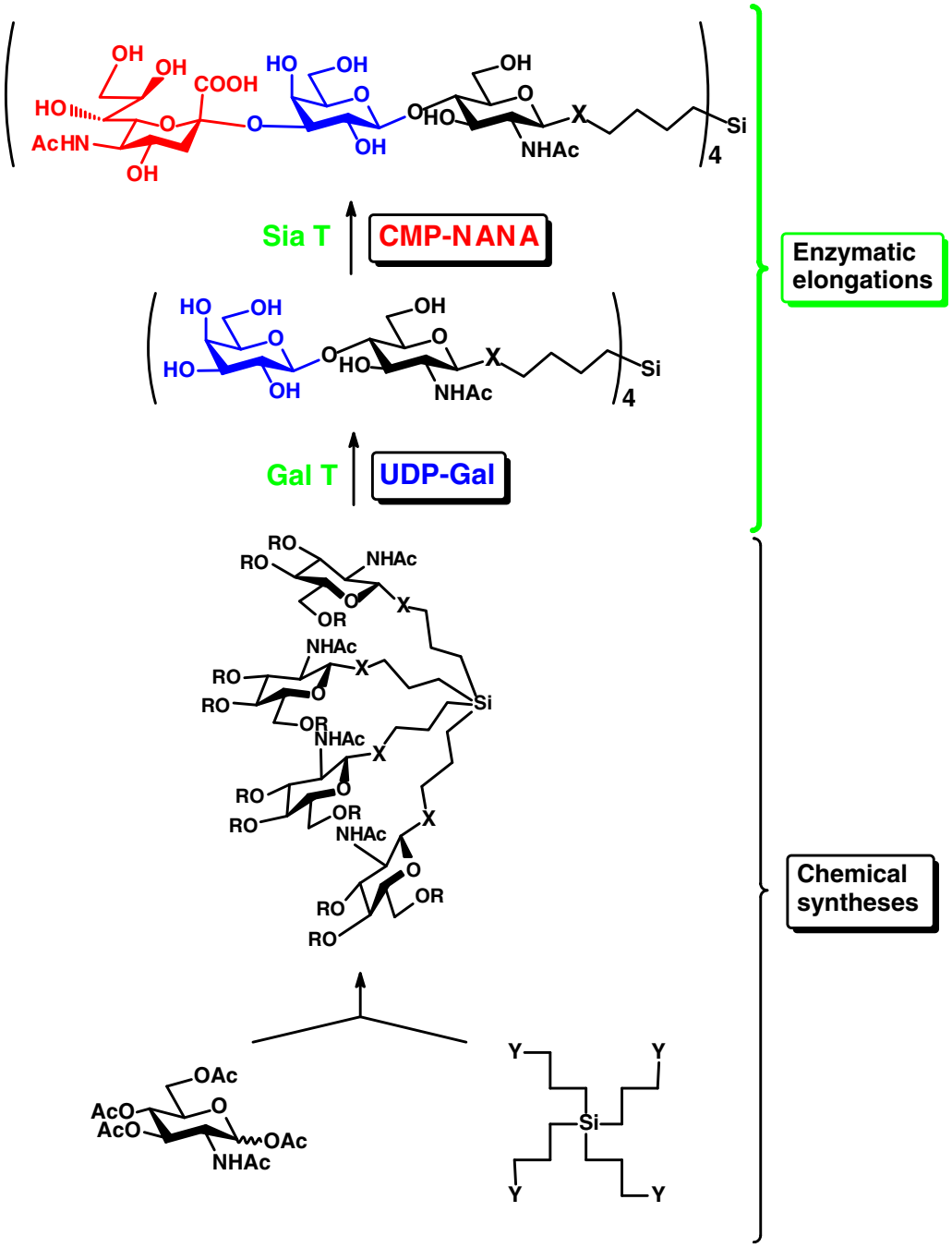
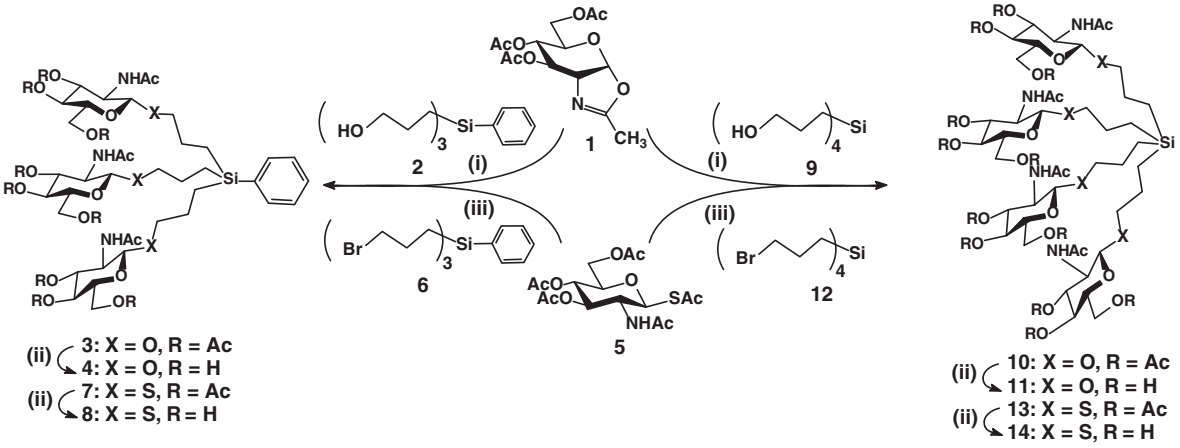


Figure 1. Synthetic plan for construction of a carbohydrate chain on a multivalent-type core scaffold.

corresponding glycocluster **3** having three GlcNAc moieties in 69% yield,[†] $[\alpha]_D^{17}$ −66° (*c* 1.1, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 6.67 (d, 3H, $J_{2,NH}$ = 8.8 Hz, N–H), 4.71 (d, 3H, $J_{1,2}$ = 8.4 Hz, H-1), integral ratio of the H atoms by ^1H NMR: SiCH_2/Ph/H-1 = 6:5:3, ESI MS calcd for $[M+Na]^+$: 1292.4881; found *m*/*z*: 1292.4914. Transesterification with MeONa/MeOH followed by saponification with 0.1 M aqueous NaOH for acetate **3** proceeded smoothly to give water-soluble glycocluster **4** in quantitative yield, ^1H NMR (D_2O) δ 4.45 (d, 3H, $J_{1,2}$ = 8.6 Hz, H-1), integral ratio of the H atoms by ^1H NMR: SiCH_2/Ph/H-1 = 6:5:3, ^{13}C NMR (D_2O) δ 100.82 (C-1). Since efficient introduction of GlcNAc into the simple core scaffold through *O*-glycosidic linkage was accomplished, preparation of similar glycoclusters having *S*-glycosidic linkage was attempted. The target compound is of great interest in comparison with a typical *O*-glycoside-type glycocluster for activity against glycosidase and glycosyl transferase. Thus, known **5**[12] was treated with tris(3-bromopropyl)phenylsilane **6**[13] in the presence of NaOMe in MeOH–DMF[14] to yield the corresponding thioglycoside **7** in 86% yield after reacetylation, $[\alpha]_D^{17}$ −27° (*c* 1.1, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 6.09 (d, 3H, $J_{2,NH}$ = 9.6 Hz, N–H), 4.60 (d, 3H, $J_{1,2}$ = 10.2 Hz, H-1), integral ratio of the H atoms by ^1H NMR: SiCH_2/Ph/H-1 = 6:5:3, ESI MS calcd for $[M+Na]^+$: 1340.4196; found *m*/*z*: 1340.4219. The chemical shift of C-1 in **7** was upfield shifted by the results of ^{13}C NMR, and a wider coupling constant between H-1 and H-2 than that of *O*-glycoside **4** was also observed. Complete removal of the protections in **7** was performed by a combination of

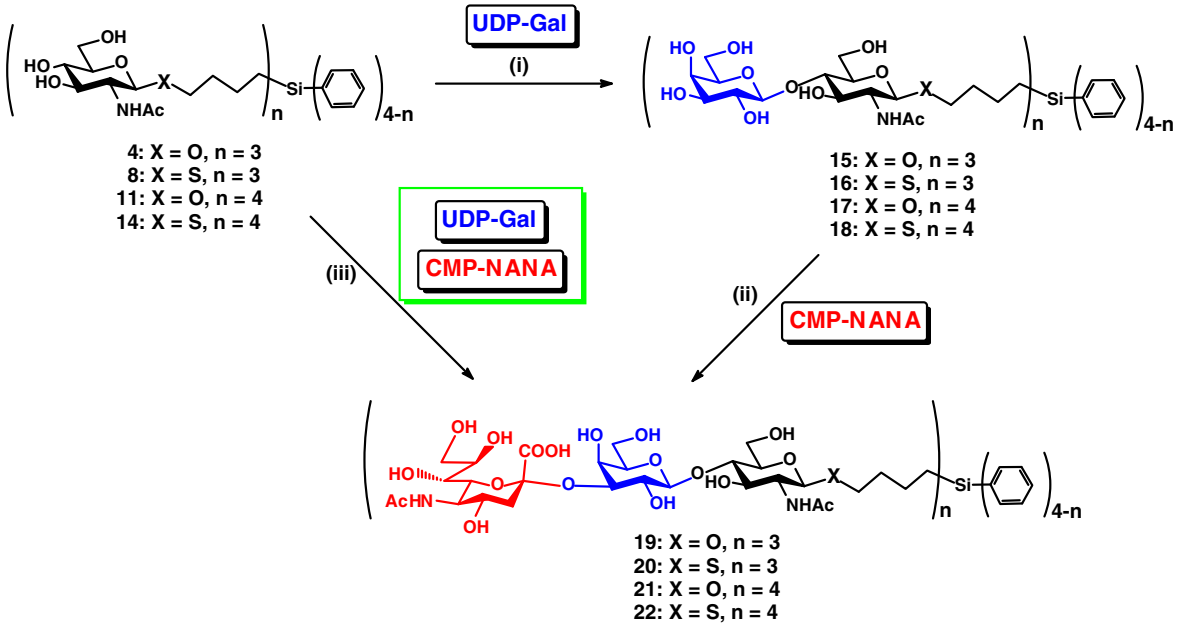
[†] All new compounds with specific rotation data gave satisfactory results of elemental analyses.



Scheme 1. Reagents and conditions: (i) CSA, CH_2ClCH_2Cl, 80 °C 2 h; (ii) NaOMe, MeOH, rt, then, 0.1 M aq NaOH; (iii) NaOMe, MeOH, 0 °C→rt, overnight, then, Ac_2O–Pyr, rt.

transesterification and hydrolysis with 0.1 M aqueous NaOH to afford trivalent-type thioglycosidic carbosilane **8** in 97% yield after chromatographic purification on Sephadex LH-20 with MeOH as the eluent, ^{1}H NMR (D_2O) δ 4.54 (d, 3H, $J_{1,2}$ = 10.2 Hz, H-1), integral ratio of the H atoms by ^{1}H NMR: SiCH_2/Ph/H-1 = 6:5:3, ^{13}C NMR (D_2O) δ 82.84 (C-1). Similar chemical modifications were then performed using known carbosilane **9**[15] and **12**.[16] Thus, oxazoline **1** was condensed with tetraol **9** to give **10** in 13% yield, $[\alpha]_D^{26}$ −14° (*c* 1.0, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 6.76 (d, 4H, $J_{2,NH}$ = 6.8 Hz, N–H), 4.73 (d, 4H, $J_{1,2}$ = 8.0 Hz, H-1), integral ratio of the H atoms by ^{1}H NMR: SiCH_2/H-1 = 8:4, in which ester functional groups were removed by the same method as that for **3** to give water-soluble **11** in 87% yield, $[\alpha]_D^{27}$ −33° (*c* 1.0, $CHCl_3$), ^{1}H NMR (D_2O) δ 4.49 (d, 4H, $J_{1,2}$ = 8.4 Hz, H-1), integral ratio of the H atoms by ^{1}H NMR: SiCH_2/H-1 = 8:4, ^{13}C NMR (D_2O) δ 100.73 (C-1), ESI MS calcd for $[M+Na]^+$: 1099.4824; found *m/z*: 1099.4858. In comparison of the yield for the preparation of **3**, the yield for the preparation of **10** was low because of poor solubility of tetraol **9** in various organic solvents. Sulfide formation between **5** and **12** gave **13** having four GlcNAc residues at each terminal in 94% yield, $[\alpha]_D^{26}$ −65° (*c* 1.1, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 6.20 (br s, 4H, N–H), 4.66 (d, 4H, $J_{1,2}$ = 9.8 Hz, H-1), integral ratio of the H atoms by ^{1}H NMR: SiCH_2/H-1 = 8:4, which was further treated under the same basic conditions as those described for **3** to afford water-soluble **14** in 92% yield, ^{1}H NMR (D_2O) δ 4.48 (d, 4H, $J_{1,2}$ = 10.2 Hz, H-1), integral ratio of the H atoms by ^{1}H NMR: SiCH_2/H-1 = 8:4, ^{13}C NMR (D_2O) δ 83.99 (C-1), ESI MS calcd for $[M+Na]^+$: 1163.3916; found *m/z*: 1163.3945.

Since chemical synthesis of GlcNAc clusters was accomplished, our attention was focused on enzymatic elongation of the sugar chains on the dendritic scaffolds. Enzymatic elongation of saccharide chains on a dendrimer was previously reported by Roy and co-workers[17a] and Narvor and co-workers[17b]; however, carbosilane dendrimers have not been used for such an objective. Carbosilanes bearing an appropriate number of GlcNAc and glycosidic linkages were treated with β1,4 galactosyl transferase (GalT) obtained from bovine milk in the presence of UDP-galactose (UDP-Gal)[18] followed by treatment of α2,3 sialyl transferase (SiaT)[19] obtained from rat liver (recombinant) in the presence of CMP-sialic acid (CMP-NANA). The schematic images are shown in Scheme 2. Trimeric *O*-glycosides of GlcNAc **4** were allowed to react with UDP-Gal in



Scheme 2. Reagents and conditions: (i) UDP-Gal, GalT, α-lactoalbumin, $MgCl_2$, 50 mM HEPES buffer (pH 7.4), 37 °C, 3 d; (ii) CMP-NANA, SiaT, BSA, mercaptoethanol, CIAP, $MgCl_2$, 50 mM HEPES buffer (pH 7.4), 37 °C, 6 d; (iii) (i), then (ii) in one-pot.

Table 1
Results of enzymatic sugar elongation on dendritic carbosilanes after isolation

Primer compounds	Two step synthesis			One-pot synthesis Yield (%)
	Galactosylation (%)	Sialylation (%)	Total yield (%)	
4	**15** 97.2	**19** 65.3	63.5	96.8
8	**16** 92.4	**20** 95.3	88.1	63.7
11	**17** ND[a]	**21** ND[a]	ND[a]	ND[a]
14	**18** 87.4	**22** 73.7	64.4	93.5

[a] ND means 'not determined' due to unsuccessful purification.

the presence of GalT, α-lactoalbmin,[20] and $MgCl_2$ in 50 mM HEPES buffer (pH 7.4) at 37 °C for 3 days to give **15** after the usual workup, ^{1}H NMR (D_2O) δ 4.46 (br s, 4H, H-1′), 4.41 (br s, 4H, H-1), integral ratio of the H atoms by ^{1}H NMR: SiCH_2/Ph/H-1/H-1′ = 6:5:3:3, ESI MS calcd for $[M+Na]^+$: 1400.55148; found *m/z*: 1400.3833. Galactosylation of the other primer compounds was performed using the same reaction conditions, and the reaction proceeded smoothly to afford the corresponding glycoclusters having an appropriate number of LacNAc residues. Further elongation of the sialic acid residue was then carried out using CMP-NANA in the presence of SiaT, bovine serum albumin (BSA), mercaptoethanol, calf intestinal alkaline phosphatase (CIAP), and $MgCl_2$ in 50 mM HEPES buffer (pH 7.4) at 37 °C for 6 days to give **19** after the usual workup, ^{1}H NMR (D_2O) δ 4.52 (br s, 3H, H-1′), 4.44 (br s, 3H, H-1), 2.74 (br s, 3H,

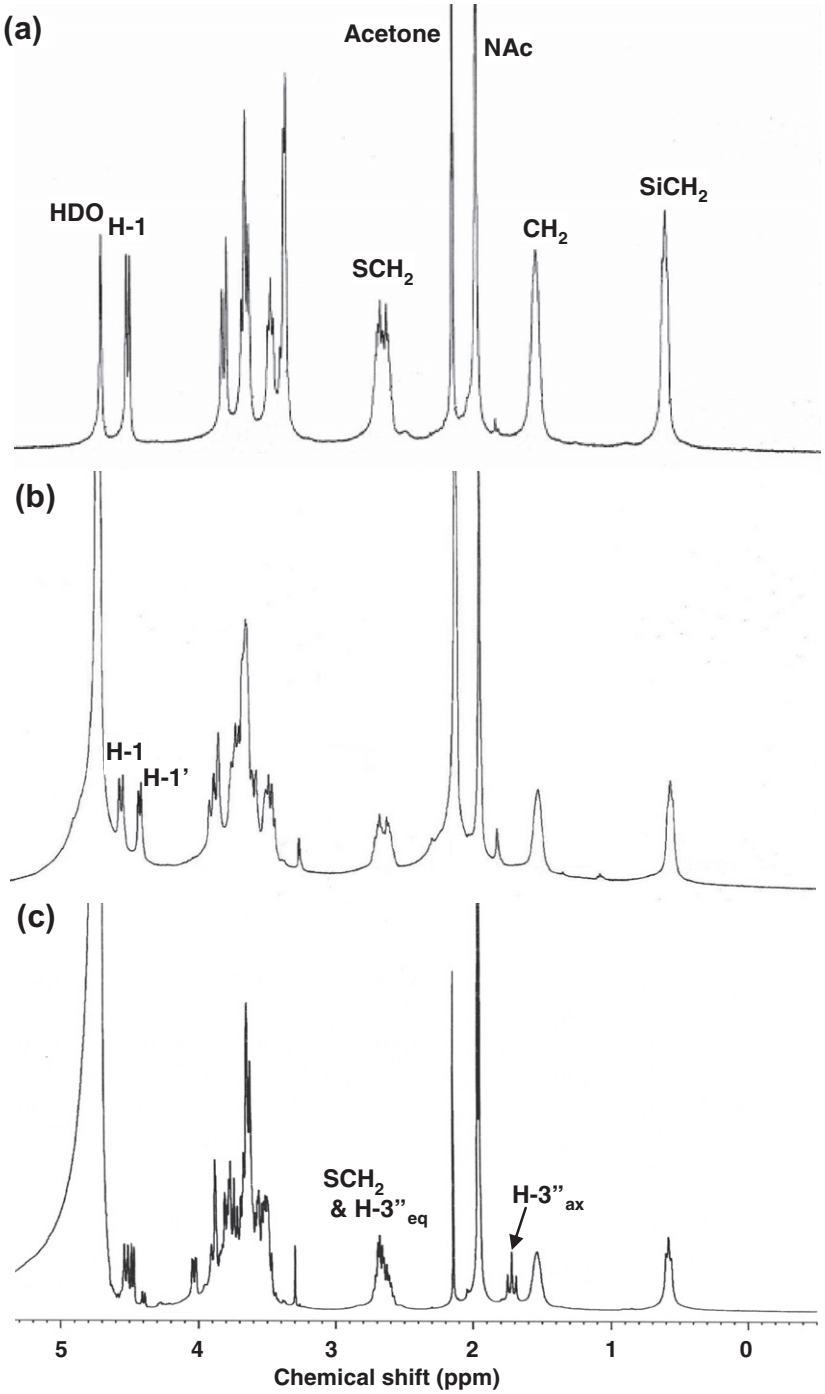


Figure 2. Comparison of ^{1}H NMR spectra on each reaction step. (a) Compound **14**, (b) compound **18**, (c) compound **22**.

H-3″), integral ratio of the H atoms by ^{1}H NMR: SiCH_2/Ph/H-1/H-1′/H-3″ = 6:5:3:3:3, MALDI-TOF MS calcd for $[M-H]^-$: 2249.8; found *m/z*: 2250.2. The results of the enzymatic elongation are shown in Table 1. These reactions were conveniently monitored by TLC, and the TLC indicated progress of the reaction step by step. After ultrafiltration, chromatographic purification was performed by means of a recycle-type SEC apparatus with water as the mobile phase.[21] **20** was isolated in 95% yield, ESI MS calcd for $[M-3H]^{3-}$: 765.25; found *m/z*: 765.26, and **22** was isolated in 74% yield, ESI MS calcd for $[M-4H+2Na]^{2-}$: 1497.47; found *m/z*: 1497.47. Unfortunately, tetrameric compounds **17** and **21** were not isolated because of the difficulty in removing impurities from the product mixtures. Comparison of the results of ^{1}H NMR of GlcNAc compound **14**, LacNAc compound **18**, and SiaLacNAc compound **22** is shown in Figure 2. These spectra clearly indicated that stepwise synthesis is sufficiently accomplished.

Given the success of the stepwise elongation of carbohydrate chains on the dendrimers, one-pot preparation was applied for this reaction sequence. Thus, galactosylation for the dendrimer was first carried out and the reaction was monitored by TLC. When the reaction was completed, sialylation using the same protocol as that for the preparation of **19** was carried out in the same reaction vial. The results of the one-pot reaction are shown in Table 1. Efficiency of the two-step synthesis was much higher than that of the stepwise synthesis.

In conclusion, we have successfully demonstrated the preparation of GlcNAc clusters using carbosilane core scaffolds and the efficient enzymatic elongation of carbohydrate moieties on multivalent-type cores. This approach is widely applicable for small-scale preparation of carbosilane dendrimers with uniformly functionalized bioactive carbohydrate moieties. Further transformations including other oligosaccharide structures on the glycoclusters are now in progress, and the results will be reported elsewhere.

Acknowledgments

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