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Efficient Preparation of Glycoclusters from Silsesquioxanes

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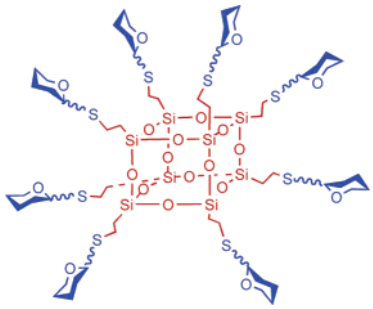
ABSTRACT



A new type of glycocluster based on polyhedral oligosilsesquioxanes (POSS) has been efficiently prepared from unprotected mannoside and lactoside employing a convergent approach of thiol-radical addition reaction. The versatility of this approach was demonstrated by functionalization of mannosides and lactosides of different-length spacers.

Cell surface oligasaccharides are involved in numerous biological processes such as cancer cell metastasis, inflammation, and infections by bacteria and viruses.[1] As the oligasaccharide−receptor interactions are often multivalent, the concept of cluster effect[2] has attracted considerable attention, and extensive efforts have been expended on building glycoclusters with a variety of scaffolds such as cyclodextrins (CDs),[3] polymers,[4] dendrimers,[5] calix[4]-allenes,[6] crown ethers,[7] and peptides.[8]

Polyhedral oligosilsesquioxanes (POSS) are an interesting class of clusters derived from the hydrolytic condensation of trifunctional organosilicon monomers. The chemistry of POSS has been extensively discussed.[9] A wide variety of other polyhedral frameworks have been developed, but cube-octameric clusters are most common. There are several reasons for the current widespread interests in POSS, the most important of which being the remarkable simplicity of

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synthesis of these compounds. In addition, functionalization of these cube molecules has expanded the range of available cluster molecules for a wide range of applications.[10] In particular, POSS provides a versatile platform of well-defined shape for construction of more sophisticated structures.[11] The use of POSS as cores for dendrimers is particularly attractive,[12] because their polyhedral structures produce spherically symmetric dendrimers with smaller generation numbers than conventional cores.[13]

To the best of our knowledge, there is only one reported example of synthesis of carbohydrate-functionalized silsesquioxane that exhibits selective and reversible complexation to carbohydrate-binding proteins.[14] The strategy involved attachment of glycodendrons to the eight amine groups of POSS core [$(H_2NCH_2CH_2CH_2)_8Si_8O_{12}$] via standard amide bond formation with carbohydrate-derived lactones, but achieved only 20−53% yields. Although this method is useful for generating glycoclusters, some drawbacks are apparent, especially its requirement to start with carbohydrate lactones. As a consequence, the spacers could not be synthesized independently, thus limiting the flexibility of this procedure. In addition, the starting material octaamine [$(H_2NCH_2CH_2CH_2)_8Si_8O_{12}$] is difficult to obtain and maintain.[15]

As part of our ongoing project involving synthesis and molecular recognition of glycoclusters, we became interested in using the rigid POSS structure as scaffold to construct new forms of carbohydrate clusters, because multivalent ligands with some rigidity can have enhanced affinity and selectivity during a binding event.[16] The unique geometry (e.g., size, shape, symmetry) and certain rigidity of POSS are likely to allow effective ligand presentation. To fulfill such potentials, attachment of carbohydrate units to the POSS core must be carried out to the maximum extent in a very high yield to avoid formation of isomers of undersubstituted regioisomers, which are very difficult to separate and purify. After some preliminary consideration of possible reactions, we chose to use the well-known photoaddition of thiols to alkenes,[17] since its usefulness has been extensively demonstrated.[18]

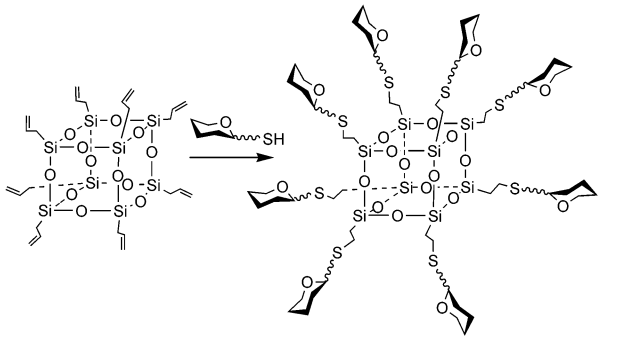

Figure 1. Graphical representation of the key synthetic step (involving the simultaneous reaction of eight carbohydrate units with the POSS core) in the synthesis of POSS-based carbohydrate cluster compounds.

Our strategy is to use radical-addition of ω-thioglycosides to commercially available octavinyl-POSS (Figure 1).

This method takes advantage of the ease of preparation of amino-terminated glycosides and the ease of introducing thiol functions to the amino groups. The use of spacer arms of varying lengths allows the distance between the carbohydrate residue and the POSS core to be varied. The conditions of highly efficient addition to thiol end-group via free-radical route in an anti-Markovnikov fasion would not incur Si−C or Si−O bond cleavage.[19] This process also eliminates the need to deprotect carbohydrate groups after their attachment to POSS, which may cause difficulties in some cases (such as cleavage of Si−C or Si−O bond).

Scheme 1 summarizes the synthetic routes of some glycosides and their derivatives to be used for the coupling reactions with γ-thiobutyrolactone.

Compound **3** was obtained by glycosylation of commercially available per-*O*-acetylated α-D-mannose **1** with benzyloxycarbonyl (Cbz)-protected 5-amino-pentanol **2**[20] using $SnCl_4$[21] as promoter in 55% yield. Deacetylation of compound **3** followed by hydrogenolysis of the Cbz-group using Pd/C catalyst gave the compound **4** in 86% yield.

For the synthesis of compound **8** (Scheme 1), benzobromolactose **5**[22] was used to glycosylate commercially available Cbz-protected 2-aminoethanol **6** in the presence of AgOTf to give compound **7** in 78% yield. Removal of the *O*-benzoyl groups under Zémplen conditions followed by catalytic (Pd/C) hydrogenolysis of the Cbz group gave compound **8** in 82% yield. Compound **11** was prepared similarly, but more

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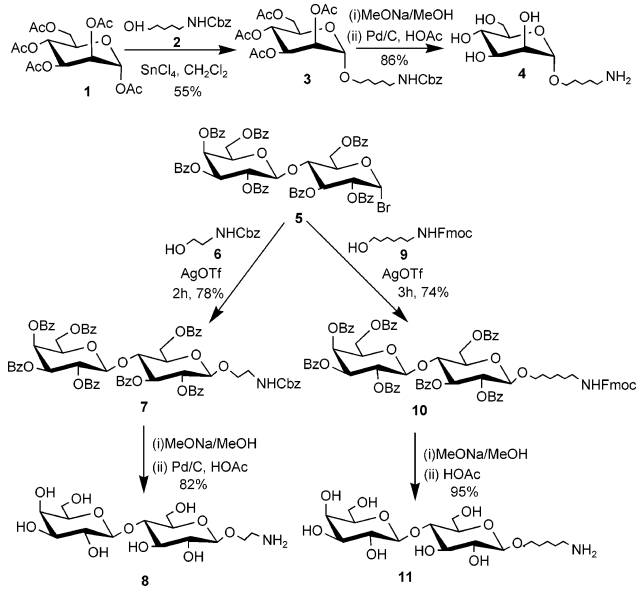
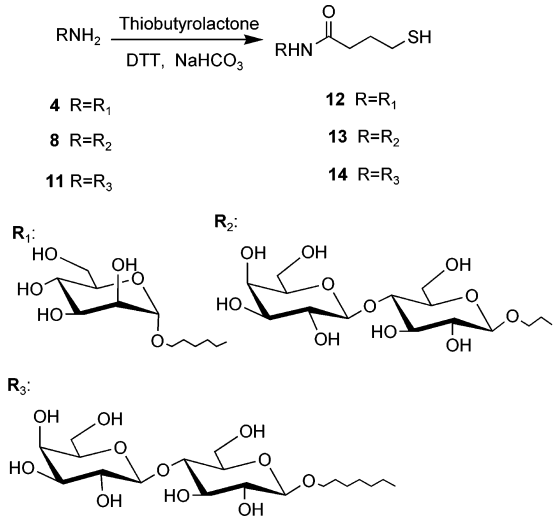
Scheme 1. Synthesis of Aminoalkyl Glycosides



simply by (i) glycosylation of Fmoc-protected 6-aminohexanol **9**[23] with benzobromolactose **5**[22] using AgOTf catalyst and then (ii) removal of the *O*-benzoyl and Fmoc groups simultaneously under Zémplen conditions. Thus, compound **11** was obtained in 70% overall yield.

The thiol compounds **12**, **13**, **14** were prepared by reacting the primary amino groups in **4**, **8**, and **11** with γ-thiobutyrolactone (6 equiv) at 50 °C in 0.25 M aqueous $NaHCO_3$ (3 equiv) in 50% ethanol solution in the presence of DTT (2.5 equiv) for 8−10 h (Scheme 2).[24] The thiol compounds were

Scheme 2. Synthesis of Thiols



purified by silica gel chromatography, and the products were isolated in excellent yields ranging from 82 to 90%.

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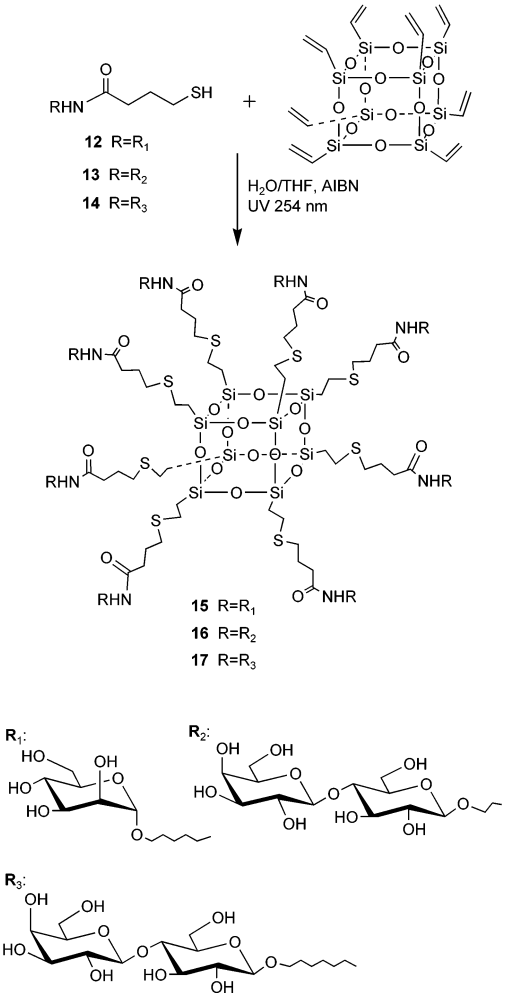
Scheme 3. Synthesis of Glycoclusters



The efficient addition of thiol-teminated glycoside residues to the POSS core was achieved (Scheme 3) when a mixture of octavinyl POSS and **12**−**14** (3.9−4.4 equiv for each vinyl group) in H_2O/THF (1:1) was irradiated with UV light (254 nm) in the presence of a catalytic amount of AIBN. After 24−48 h, the disappearance of the ^{1}H resonance at 5.95−6.18 ppm which corresponds to the vinyl group and emergence of new signals corresponding to the incorporated $SiCH_2$ residues (1.06−1.09 ppm) indicated the intended reaction was complete. Purification of the crude product from the photochemical reaction by Sephadex G-15 gel filtration (H_2O as eluent) afforded pure glycoclusters **15**, **16**, and **17** in 70%, 66%, and 73% yield, respectively. It should be noted that most of the unreacted carbohydrate thiols could be recovered in pure form after the gel filtration. Only a trace amount of disulfide (<5%) was obtained. To prevent the oxidation of the carbohydrate thiols to disulfides during storage, it is better to keep them under inert atmosphere in refrigerator. The identities of these compounds were established unequivocally from close inspection of their ^{1}H NMR and ^{13}C NMR spectra.[25] The structures were further confirmed by MALDI-TOF mass spectra.

Although the ^{1}H NMR data at 25 °C showed the broadening of both of the signals for the POSS cores and carbohydrate portions, the ratios of the integrals for the signals of saccharide residue protons and for the signals belonging to the POSS cores are in accordance with the structures of the expected products. The ^{1}H NMR spectra of **15**, **16**, and **17** show signals at 1.06−1.09 ppm revealing the presence of the $SiCH_2$ groups on the POSS cores. In addition, the signals at 2.54−2.55 ppm, corresponding to the terminal methylene (CH_2SH) of **12**, **13**, and **14** were replaced by two new signals at 2.59−2.60 ppm and 2.69−2.70 ppm as a result of the radical addition reactions.

^{13}C NMR spectra of **15** display only one anomeric carbon signal at 99.34 ppm. ^{13}C NMR spectra of **16** and **17** show two anomeric carbon signals (102.55 ppm, 101.85 ppm for **16**, 102.56 ppm, 101.82 ppm for **17**). High-field chemical shifts at 12.13 ppm for **15**, 12.51 ppm for **16**, and 12.15 ppm for **17** further confirm the presence of $SiCH_2$ groups on the POSS cores. Glycoclusters **15**, **16,** and **17** show only one signal at 173.98 ppm for **15**, 175.87 ppm for **16,** and 173.99 ppm for **17** corresponding to their amide residues**,** respectively.

The results of MALDI-TOF mass spectra also supported homogeneity of the products. The molecular masses of glycoclusters **15**, **16,** and **17** were detected as peaks of the desired molecular ions in agreement with their $[M + K]^+$ adducts, respectively.

We examined the binding of **16** or **17** by RCA120 (a β-galactose specific lectin). The study was performed by measuring the inhibitory effect of **16** or **17** on the binding of asialo-oligosaccharides from human α1-acid glycoprotein (AGP) by RCA120 using capillary affinity electrophoresis.[26] Complete inhibition was shown by 10 μM of **16** or **17**. A typical example using **16** as an inhibitor is shown in the Supporting Information. In contrast, the inhibition is not shown even at 20 mM of lactose (data not shown). This indicated that **16** and **17** showed 200 times or higher inhibitory effect than lactose, presumably due to the cluster effect.[2] As predicted by binding specificity of RCA120, **15**, which contains only mannose residues did not show any inhibition at 10 μM. The detailed binding characteristics will be reported elsewhere.

In conclusion, we have described an efficient synthetic route for a variety of POSS-based glycoclusters by photoaddition of thiols to vinyl groups. This strategy should be easily extended to attachment of other glycosides, including those of more complicated oligosaccharides to POSS cores with possible variations in spacers. This method is also amenable to generate combinatorial libraries by changing the nature of the sugar and spacing arms. Preliminary lectin binding study indicated that these novel glycoclusters showed strong inhibition of the binding of asialo-oligosaccharide mixture derived from human α1-acid glycoprotein by RCA120.

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Supporting Information Available: Detailed synthetic procedures, biological experimental procedures, NMR, and FAB or MALDI-TOF mass spectra data of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Selected data for compound **15**: ^{1}H NMR (400 MHz, D_2O, rt) δ 4.85 (s, 8H, H-1), 3.20 (bs, 16H), 2.70 (bs, 16H), 2.36 (bs, 16H), 1.90 (bs, 16H), 1.63 (bs, 16H), 1.55 (bs, 16H), 1.41 (bs, 16H), 1.09 (bs, 16H, SiCH_2); ^{13}C NMR (100 MHz, D_2O, rt) δ 173.98 (8 *C*ONH), 99.34 (C-1), 72.32, 70.35, 69.82, 67.04, 66.25, 60.48, 38.91, 34.50, 28.15, 25.07, 22.78, 12.13; MALDI-TOF MS 3608.30 $[M + K]^+$. Selected data for compound **16**: ^{1}H NMR (400 MHz, D_2O, rt) δ 4.49 (d, 8H, J = 8.0 Hz, H-1′), 4.45 (d, 8 H, J = 7.6 Hz, H-1), 3.35 (bt, 8 H), 2.70 (bs, 16H), 2.60 (bs, 16H), 2.38 (bs, 16H), 1.90 (bs, 16 H), 1.06 (bs, 16H, SiCH_2); ^{13}C NMR (100 MHz, D_2O, rt) δ 175.30 (8 CONH), 102.55, 101.85 (8C-1 and 8C-1′), 78.00, 74.93, 74.36, 73.89, 72.38, 72.12, 70.52, 68.11, 60.60, 59.71, 38.89, 34.34, 24.76, 12.51; MALDI-TOF MS 4568.50 $[M + K]^+$. Selected data for compound **17**: ^{1}H NMR (400 MHz, D_2O, rt) δ 4.47 (bs, 8H, H-1′), 4.45 (bs, 8H, H-1), 3.32 (bt, 8H), 3.19 (bs, 16H), 2.69 (bs, 16H), 2.59 (bs, 16H), 2.36 (bs, 16H), 1.89 (bs, 16H), 1.64 (bs, 16H), 1.53 (bs, 16H), 1.38 (bs, 32H), 1.08 (bs, 16H, SiCH_2); ^{13}C NMR (100 MHz, D_2O, rt) δ 173.99 (CONH), 102.56, 101.82 (C-1 and C-1′), 78.04, 74.93, 74.33, 74.10, 72.45, 72.16, 70.53, 69.95, 68.12, 60.60, 59.77, 38.96, 34.53, 28.57, 24.63, 12.15; MALDI-TOF MS: 5016.80 [M + K].

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