Synthesis, characterization and lectin binding study of carbohydrate functionalized silsesquioxanes

Frank J. Feher,*[a] Kevin D. Wyndham[a] and Daniel J. Knauer*[b]

[a] *Department of Chemistry, University of California, Irvine, California 92697-2025, USA. E-mail: fjfeher@uci.edu*
[b] *Department of Developmental and Cell Biology, University of California, Irvine, California 92697-1450, USA. E-mail: djknauer@uci.edu*

Received (in Bloomington, IN, USA) 21st May 1998, Accepted 25th September 1998

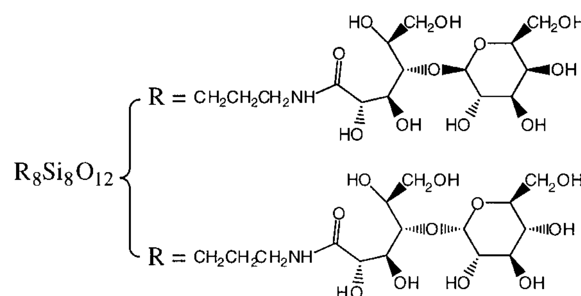
Two carbohydrate-functionalized silsesquioxanes 2 and 3 have been prepared by the reactions of $(H_2NCH_2CH_2CH_2)_8Si_8O_{12}$ 1 with *O*-β-D-galactopyranosyl-(1→4)-D-gluco-1,5-lactone and *O*-α-D-glucopyranosyl-(1→4)-D-gluco-1,5-lactone; the latter (*i.e.* 3) possesses eight maltose-derived substituents and binds with *Concanavalin A*, while the former possesses eight lactose-derived substituents that demonstrates selective binding to the hepatic asialoglycoprotein receptor.

Cell surface carbohydrates play an important role in cell recognition processes and have been implicated in a variety of pathological disorders.[1] In many instances the mechanisms by which these carbohydrates function as signals for cell recognition and subsequent biochemical transformations are not well understood, but the development of new drugs to block undesirable interactions could provide powerful tools for treating or preventing a variety of diseases.[2] One interesting approach for elucidating molecular-level details of recognition phenomena and developing new chemotherapeutics involves the use of polyfunctional molecules as 'scaffolds' to organize structurally well defined assemblies of oligosaccharide units.[3]

Here, we report the first use of polyhedral oligosilsesquioxanes (POSS) as scaffolds for the presentation of multiple carbohydrate units.

We recently introduced the use of $(H_2NCH_2CH_2CH_2)_8Si_8O_{12}$ **1** as a core for dendrimer synthesis[4a] and as a scaffold for the presentation of polypeptide chains.[4b] This water-soluble framework can be prepared *via* neutralization of its octahydrochloride salt, which is obtained in one step (>35% yield) by the hydrolytic condensation of readily available $H_2NCH_2CH_2CH_2Si(OEt)_3$. Carbohydrate substituents can be attached to the eight amine groups of **1** *via* standard coupling protocols with carbohydrate-derived lactones.[3a–e] For example, the reaction of **1** with *O*-β-D-galactopyranosyl-(1→4)-D-gluco-1,5-lactone affords octagalactose-substituted framework **2**, which is obtained in high yield as a white powder after dialysis against water and precipitation by methanol.† Similarly, the reaction of **1** with *O*-α-D-glucopyranosyl-(1→4)-D-gluco-1,5-lactone produces an excellent yield of **3**, which possesses eight equivalent maltose-derived substituents. The course of these lactone coupling reactions can be conveniently monitored by ^1H NMR spectroscopy [$(CD_3)_2SO$] because the chemical shift for the CH_2N group of **1** (δ 2.8) shifts downfield by *ca.* 0.2 ppm upon acylation. The prominent product amide NH resonance at δ 7.7 can also be integrated and used to determine the extent of reaction. Both carbohydrate-functionalized frameworks are stable in solution (water or Me_2SO, 25 °C, 7 days) and elevated temperatures (100 °C) for short durations; both frameworks are also stable in the presence of acids (*e.g.* 1 M HCl or conc. HOAc) and non-nucleophilic bases (*e.g.* DIEA in DMSO).

Both **2** and **3** were characterized by a variety of techniques, including combustion analysis, MALDI-TOF mass spectrometry and multinuclear (^1H, ^{13}C, ^{29}Si) NMR spectroscopy [D_2O or $(CD_3)_2SO$]. Assignment of all ^1H and ^{13}C resonances



for **2** and **3** could be made on the basis of COSY, HMQC and DEPT experiments, but at concentrations normally required to obtain good signal-to-noise, aggregation of these highly polar frameworks causes marked broadening of many resonances. Dynamic light scattering measurements indicate that aggregation occurs at *ca.* 0.4 mM in water and that aggregates with an effective radius of 100 nm are present at 1.0 mM. The nature of this aggregation is not known, but at 3 mM the ^{29}Si, ^1H and ^{13}C spectra [D_2O and $(CD_3)_2SO$] are consistent with two distinct environments (*ca.* 4 : 1) for the pendant groups. The integrated intensities of the featureless resonances attributable to each environment do not appear to change over a concentration range of 1–8 mM and a temperature range of 25–60 °C, but the ratio abruptly changes below 1 mM and is *ca.* 1 : 1 over the concentration range of 0.05–0.5 mM. ^1H NMR spectra (D_2O) recorded below 1 mM also exhibit dramatically better resolution with well defined first-order multiplets for the aminopropyl spacers. We suspect that the two different environments are due to restricted rotation about the amide C–N bonds, which can create distinct *cis* and *trans* conformations for the pendant groups. If this is indeed the case, our results are consistent with strong inter- and intra-molecular interactions between pendant carbohydrate groups because *cis–trans* isomerization is slow on the NMR timescale at 25 °C and the *cis*/*trans* ratio changes upon the onset of intermolecular aggregation.

We have explored biological binding affinities of **2** and **3** using the asialoglycoprotein receptor (ASGPR) and *Concanavalin A* (Con A). The ASGPR is an integral mammalian hepatocyte membrane receptor which has selective binding to terminal non-reducing β-D-galactopyranosyl residues and demonstrates increased binding with an increased number of antennary β-D-galactopyranosyl groups.[2,5,6] Early suggestions that enhanced binding to the ASGPR occurs when three galactosyl residues are situated 15, 22, and 25 Å apart and separated by flexible organic spacers (*e.g.* PEG)[7] were supported by observations that three galactose residues tethered to glycerol[6c] or TRIS[2,6a,8] with this approximate spacial relationship exhibit enhanced binding to the ASGPR over mono- or di-valent analogs. Stochastic dynamics calculations[3h] (Macromodel v. 5.5) on **2** show inter-galactose separations comparable to the distances required for enhanced-binding to the ASGPR.

The results from a competitive inhibition study of binding to ASGPR of HepG2 cells are shown in Fig. 1. As illustrated in

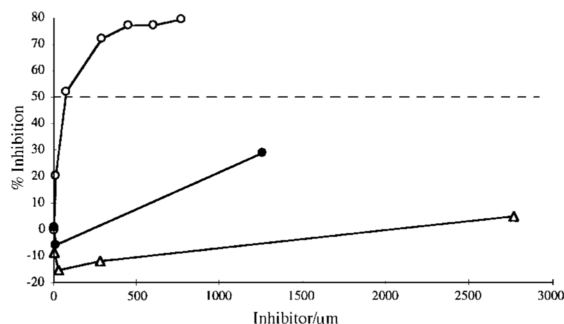


Fig. 1 Competitive inhibition of HepG2-ASGPR mediated uptake of ^{125}I-ASOM (1 μM, 0.5 ml/well) by the hydrochloride salt of **1** (△), **2** (○) and **3** (●) as measured by scintillation counting of the ^{125}I label. The reaction was performed in binding media (0.5 ml/well, pH = 7.4), incubated at 37 °C for 2 h before lysis with 10% SDS (0.5 ml/well). The inhibitory potency (IC_{50}) for unlabelled ASOM was determined to be 0.65 μM from a separate control experiment.

Fig. 1, neither the octahydrochloride of **1** nor the octa-glucose-terminated framework (*i.e.* **3**) substantially inhibit ASGPR-mediated uptake of ^{125}I-labeled Asialoorosomucoid (^{125}I–ASOM) into HepG2 cells (37 °C). In contrast, the octa-β-D-galactose substituted framework (*i.e.* **2**) strongly inhibits uptake of ^{125}I–ASOM. These results are consistent with selective recognition and binding of ASGPR to the β-galactose residues of **2** and no binding to the glucosyl residues of **3** or the ammonium groups of **1**.

It is interesting that the IC_{50} of **2** (57 μM at 37 °C) is comparable to the inhibition potency observed in a similar experiment for a glycerol molecule possessing three pendant lactose residues (IC_{50} = 9.83 μM at 4 °C).[6c] This clearly indicates that the rigid Si_8O_{12} core of **2** does not hinder binding of the galactosyl residues to the ASGPR, and it suggests that each ASGPR is interacting with three of the eight pendant groups from a single molecule of **2**.

The specific binding affinities of **2** and **3** were also assessed using *Concanavalin A* (Con A), which has four domains available for the binding of non-reducing D-glucosyl and D-mannosyl residues. As shown in Fig. 2, mixtures of Con A (35 μM) and **3** at concentrations as low as 7 μM display immediate turbidity and precipitation of a Con A-crosslinked aggregate while there is no significant turbidity when Con A (35 μM) is added to **2** at concentrations as high as 420 μM. These results are consistent with selective recognition and binding of Con A to the D-glucosyl residues of **3** and no binding to the galactosyl residues of **2**. Precipitation of the Con A/**3** aggregate can be reversed by adding D-maltose. The addition of 1.5 mol equiv. of D-maltose leads to a slight decrease in turbidity (*ca.* 25%), but the effect is minor and comparable to the decrease observed upon addition of 75 mol equiv.; complete loss of turbidity occurs upon addition of 750 mol equiv. of D-maltose. These results are similar to results for Con A binding to glycosylated PAMAM dendrimers,[3c,g] a macrocyclic sugar cluster[3a,b] and polystyrene derivatives having pendant oligosaccharides.[3h]

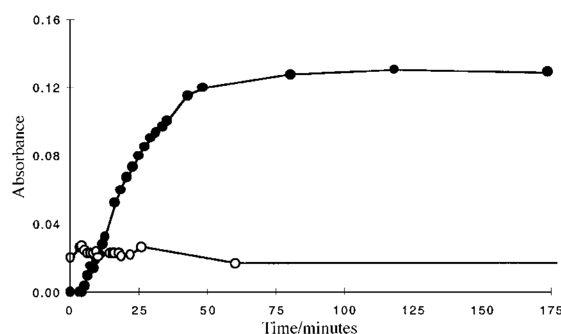


Fig. 2 Interaction of Con A (35 μM) with **2** (420 μM, ○) and **3** (7 μM, ●) as measured by absorbance at 450 nm. The reaction was performed at 25 °C and pH = 7 (0.01 M PBS).

In summary, we have synthesized carbohydrate-functionalized silsesquioxanes that exhibit highly selective and reversible complexation to carbohydrate-binding proteins. In light of the fact that $R_8Si_8O_{12}$ frameworks can be selectively monofunctionalized and subsequently modified to create new $R^1{}_7R^2Si_8O_{12}$ frameworks,[9] these observations have exciting implications for molecular recognition and the design of new site-specific drugs.

These studies were supported by the National Science Foundation (F. J. F.) and the National Institute of Health (D. J. K.). The authors gratefully acknowledge the advice and technical assistance provided by Professors David A. Brant and Charles G. Glabe (UCI), as well as the enthusiastic advice and encouragement provided by Professor A. Richard Chamberlin (UCI) and Dr Mark A. Scialdone (E. I. du Pont de Nemours).

Notes and references

† *Synthesis* of **2**: a solution of **1** (216 mg, 0.245 mmol) in MeOH (3.3 ml) was added to a solution of *O*-β-D-galactopyranosyl-(1→4)-D-glucono-1,5-lactone lactone[3e,5] (1.16 g, 3.41 mmol) in dry Me_2SO (*ca.* 3 ml); the MeOH was immediately removed by stirring under vacuum (25 °C, 0.001 Torr). The solution was stirred under nitrogen (25 °C, 24 h), filtered, and then evaporated (30 °C, 0.01 Torr) to afford a colorless resin, which was dialyzed against H_2O (25 °C, 3 × 4 l) over a period of 24 h. Evaporation (30 °C, 0.01 Torr) of the resulting solution afforded **2** as a spectroscopically pure white powder (472 mg, 53%), which was further purified by reprecipitation from H_2O–MeOH at −30 °C. Yield: 340 mg (40%). ^1H NMR [500.0 MHz, 5 mM in $(CD_2)_2SO$, 25 °C]: δ 7.66 (br, NH, 8H), 5.17–3.38 (m, carbohydrate, 168H), 3.10, 3.04 (br, CH_2N, 16H), 1.47 (br, $SiCH_2CH_2$, 16H), 0.56 (br, $SiCH_2$, 16 H). $^{13}C\{^1H\}$ NMR [125.7 MHz, 5 mM in $(CD_3)_2SO$, 25 °C]: δ 172.36 (s, CO), 104.66 (s, 1′-C), 83.16 (s, 4-C), 75.69, 73.22, 72.00, 71.70, 71.43, 71.17, 70.55, 68.23 (4′-C), 62.34 (s, 6-C), 60.72 (6′-C), 40.89 (br, CH_2N), 22.57 (br, $SiCH_2CH_2$), 8.77 (br, $SiCH_2$). $^{29}Si\{^1H\}$ NMR (99.38 MHz, 5 mM in D_2O, 25 °C): δ −65.9 (80%), −66.9 (20%). Mass spectrum (MALDI-TOF, DHB-HIQ matrix) *m/z* calc. for $C_{120}H_{224}O_{100}N_8Si_8$: [M + Na]$^+$ 3624.1, found 3623.9; [M − $C_{12}H_{19}O_{11}$ + K]$^+$ 3300.95, found 3302.0; [M − $C_{12}H_{19}O_{11}$ + Na]$^+$ 3284.99, found 3284.0; [M − $C_{12}H_{19}O_{11}$ + H]$^+$ 3261.99, found 3262.1. Elemental analysis: found (calc.) for $C_{120}H_{224}O_{100}N_8Si_8 \cdot 3H_2O$: C, 39.63 (39.40), H, 6.15 (6.34), N, 3.05 (3.06).

1 (*a*) T. K. Lindhorst and C. Kieburg, *Angew. Chem., Int. Ed. Engl.,* 1996, **35**, 1953; (*b*) I. A. Wilson and A. E. Clarke, *Carbohydrate–protein interaction*, Springer-Verlag, New York, 1988.
2 A. R. Vaino, W. T. Depew and W. A. Szarek, *Chem. Commun.,* 1997, 1871.
3 (*a*) T. Fujimoto, C. Shimizu, O. Hayashida and Y. Aoyama, *J. Am. Chem. Soc.,* 1997, **119**, 6676; (*b*) T. Fujimoto, C. Shimizu, O. Hayashida and Y. Aoyama, *J. Am. Chem. Soc.,* 1988, **120**, 601; (*c*) K. Aoi, K. Itoh and M. Okada, *Macromolecules,* 1995, **28**, 5391; (*d*) J. Murata, Y. Ohya and T. Ouchi, *Carbohydr. Polym.,* 1997, **32**, 105; (*e*) K. Kobayashi, H. Sumitomo and Y. Ina, *Polym. J.,* 1985, **17**, 567; (*f*) J. Frese, C. H. Wu and G. Y. Wu, *Adv. Drug Delivery Rev.,* 1994, **14**, 137; (*g*) D. Páge and R. Roy, *Bioconjugate Chem.,* 1997, **8**, 714; (*h*) P. R. Ashton, S. E. Boyd, C.L. Brown, N. Jayaraman, S. A. Nepogodiev and J. F. Stoddart, *Chem. Eur. J.,* 1996, **2**, 1115.
4 (*a*) F. J. Feher and K. D. Wyndham, *Chem. Commun.,* 1988, 323.; (*b*) F. J. Feher, K. D. Wyndham, M. A. Scialdone and Y. Hamuro, *Chem. Commun.,* 1998, 1469.
5 T. J. Williams, N. R. Plessas and I. J. Goldstein, *Carbohydr. Res.,* 1978, **67**, C1.
6 (*a*) R. T. Lee, P. Lin and Y. C. Lee, *Biochemistry*, 1984, **23**, 4255; (*b*) J. R. Braun, T. E. Willnow, S. Ishibashi, G. Ashwell and J. Herz, *J. Biol. Chem.,* 1996, **271**, 21 160; (*c*) A. Krebs, W. T. Depew, W. A. Szarek, G. W. Hay and L. J. J. Hronowski, *Carbohydr. Res.,* 1994, **254**, 257.
7 (*a*) Y. C. Lee, R. R. Townsend, M. R. Hardy, J. Lonngren and K. Bock, ed. T. B. Lo, T. Y. Liu and C. H. Li, New York, 1984; (*b*) A. Valentijn, G. A. vanderMarel, L. Sliedregt, T. J. C. vanBerkel, E. A. L. Biessen and J. H. vanBoom, *Tetrahedron,* 1997, **53**, 759.
8 E. A. L. Biessen, D. M. Beuting, H. Roelen, G. A. Vandemarel, J. H. Vanboom and T. J. C. Vanberkel, *J. Med. Chem.,* 1995, **38**, 1538.
9 A. Tsuchida, C. Bolln, F. G. Sernetz, H. Frey and R. Mulhaupt, *Macromolecules,* 1997, **30**, 2818.

Communication 8/06684A