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PAPER

Glycoside and peptide clustering around the octasilsesquioxane scaffold *via* photoinduced free-radical thiol–ene coupling. The observation of a striking glycoside cluster effect†

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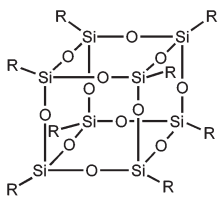
Two series of multivalent octasilsesquioxane glyco- and peptido-conjugates were synthesized using the photoinduced free-radical thiol–ene coupling (TEC). The first series was obtained by coupling *C*-glycosylpropyl thiols and cysteine containing peptides with the known octavinyl octasilsesquioxane while the second series was obtained by reacting glycosyl thiols with a new octasilsesquioxane derivative displaying eight PEGylated chains functionalized with terminal allyl groups. The evaluation of the binding properties of mannoside and glucoside clusters toward Concanavalin A by Enzyme-Linked Lectin Assay (ELLA) revealed a modest glycoside cluster effect. On the other hand, the PEGylated POSS-based glycocluster featuring eight *N*-acetyl-glucosamine residues showed high affinity toward Wheat Germ Agglutinin to give a measured IC_{50} at 3 nM. The calculated relative potency per number of sugar unit (rp/*n*) was superior to a value of 10^6, thus revealing the occurrence of a striking glycoside cluster effect.

Introduction

The cube-octameric silsesquioxanes (COSS, $R_8Si_8O_{12}$), most often referred to as polyhedral oligomeric silsesquioxanes (POSS),[1] the molecular equivalents to the cubic symmetric platonic polyhedron, are receiving considerable attention because of their rigid globular architecture displaying a precise clustering of eight ligand molecules in space. Thus, POSS can serve as nano-building blocks for constructing functional materials,[2] as supports for organometallic catalysts,[3] and as biocompatible drug carriers.[4] POSS-derived materials exhibited no significant cell toxicity demonstrating their potential as biomaterials.[5] Starting materials for the construction of complex POSS derivatives are compounds **1a–d** (Fig. 1) bearing reactive functional groups at the periphery such as amino, azido, vinyl and chloro. These compounds are commercially available or can be prepared from inexpensive organosilicon precursors.[1]



1a R = $(CH_2)_3NH_2$
1b R = $(CH_2)_3N_3$
1c R = $CH{=}CH_2$
1d R = $(CH_2)_3Cl$

Fig. 1 Functionalized POSS derivatives **1a–d**.

Thus, in the late 1900s Feher *et al.* reported the synthesis of peptidyl and glycosyl POSS by standard amide coupling of octa (aminopropyl) POSS **1a** with *N*-protected peptides and sugar lactones, respectively.[6] It now appears that this pioneering approach was plagued by two main drawbacks, one being the scarce availability of octaamine **1a** (35% from aminopropyl silane), the other being the low yields of amide coupling (20–60%). The need for efficient approaches to POSS leading to a complete and uniform conjugation at each apex to avoid the troublesome separation of partially functionalized derivatives and/or reaction intermediates quite recently led two independent research groups, one headed by Fessner[7] and the other by Chiara,[8] to use the most popular click reaction, *i.e.* the Cu-catalyzed azide-alkyne cycloaddition (CuAAC),[9] for the synthesis of triazole-linked POSS glycoconjugates. Both research groups employed the octaazide silsesquioxane **1b** as a scaffold. Unfortunately, the

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†Electronic supplementary information (ESI) available: Syntheses of sugar thiols **3a** and **3b**. ^1H and ^{13}C NMR spectra of all new compounds, ^{29}Si NMR spectrum of **4b**, stacked ^1H NMR spectra of **1c** and **4a**, **4b**, **8** and **9**, stacked ^1H NMR spectra of **12** and **13a**, **13b**, **13c**, **14** and **15**. See DOI: 10.1039/c2ob07078b

preparation of this densely nitrogenated compound presented some hazards due to the formation of azidomethane as a by-product. Moreover, while the potency and synthetic utility of CuAAC is undeniable, there is a diffuse concern about the use of this ligation tool in bioorganic synthesis due to the toxic copper catalyst as potential contaminant of the reaction product. This drawback has been recently reported in dendrimer formation[10] so that the strained-promoted azide-alkyne cycloaddition (SPAAC) approach[11] had to be employed. Fortunately enough, the click chemistry space is unlimited,[12] so that many other metal-free ligation reactions are available for the solution of specific problems.[13] One of these reactions is the century old free-radical hydrothiolation of terminal alkenes,[14] referred to as thiol–ene coupling (TEC), that is emerging as a valuable click process[15] in bioorganic[16] and polymer/dendrimer chemistry[17] as well as biomaterial synthesis.[18] Quite remarkably TEC can be initiated by using a simple initiator such as 2,2-dimethoxy-2-phenylacetophenone (DPAP) and irradiation at wavelength close to visible light, *e.g.* λ_{max} 365 nm, the latter being a condition that excludes any photodamage of biomolecules such as carbohydrates and proteins. The main features of TEC that support its click status are high efficiency, total atom economy, orthogonality to a broad range of reagents, and compatibility with water and oxygen. Notably, when an excess of thiol with respect to alkene is used, the only side product is the readily removable disulfide which in turn can be reduced back to thiol by using, for instance, dithiothreitol (DTT).[19] The only study on the use of TEC for the synthesis of POSS glycoconjugates was reported in 2004 by Lee and co-workers[20] *via* photoinduced reaction of *N*-mannosyl and *N*-lactosyl γ-thiobutyramides with octavinyl POSS **1c**. While the preparation of this manuscript was in progress, a paper has appeared describing the introduction of glucose residues on a heptavinyl POSS-polylactide conjugate (VPOSS-PLLA) *via* thiol–ene coupling.[21] Thus, we would like to report here validation/extension of TEC-based approach toward peptide and glycoside cube-shaped clusters using the commercially available **1c** and a new octaene reagent derived from it as POSS starting materials. The evaluation of the inhibition properties of selected glycoclusters thus prepared toward lectins will be also reported for the first time. This study follows our recent work on the use of TEC as a ligation tool for glycoclustering on the rigidified platform of calix[4]arene.[16c]

Results and discussion

We first set out to study the photoinduced coupling of **1c** with the simple sugar thiol 1-thio-β-D-glucopyranose[22] **2a** (Fig. 2) under previously established standard conditions for multiple TEC on calix[4]arene scaffold,[16c] *i.e.* irradiation for 1 h at λ_{max} 365 nm in the presence of DPAP as the initiator (entry 1, Table 1). The reaction was conducted at room temperature in a glass vial and no care was taken to exclude air and moisture. Despite the use of excess of **2a** from 1.5 to 4 equiv./ene of **1c**, a partial hydrothiolation of the latter was observed as evidenced by the presence of residual alkene proton signals in the 5–6 ppm region of the NMR spectrum (CD_3OD) of the crude reaction mixture.

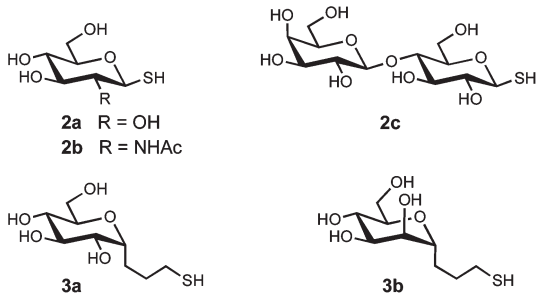


Fig. 2 Sugar thiols employed for the hydrothiolation of POSS.

Table 1 Hydrothiolation of POSS **1c** at λ_{max} 365 nm in the presence of DPAP (0.1 equiv./thiol)

Entry	Thiol	Thiol equiv./ene	Solvent	Time	Product	Yield (%)
1	**2a**	1.5–4	DMF	1 h	—	—
2	**3a**	2	DMF–THF	1 h	**4a**	94
3	**3b**	2	DMF–THF	1 h	**4b**	93
4	**5**	1.5	DMF	45 min	**8**	84
5	**6**	1.5	DMF	45 min	**9**	75
6	**7**	3	DMF–H_2O	2 h	—	—

We felt that the steric congestion around the octasilsesquioxane scaffold produced by the sequential attachment of thioglycoside fragments was responsible for these findings. Therefore, we set out to circumvent this limitation by introducing suitable tethers holding the alkenyl groups of the scaffold or the sulfhydryl group of the carbohydrate. At first we decided to test the latter possibility. To this end we prepared the *C*-glucosylpropyl thiol **3a** (Fig. 2) by thiol–ene coupling of known[23] allyl *C*-glucopyranoside with thioacetic acid and transesterification (MeONa–MeOH) of the resulting thioacetate (see ESI, Fig. S1†). Quite rewardingly the photoinduced hydrothiolation of **1c** by **3a** in the presence of DPAP was complete after 1 h as evidenced by the total disappearance of alkene proton signals in the NMR spectrum of the crude reaction mixture (Fig. 3). This indicated that all vinyl groups of the octasilsesquioxane **1c** had been saturated through eight concomitant TEC reactions.

Chromatography over Sephadex LH-20 allowed the isolation of the POSS-based octavalent glycocluster **4a** (Fig. 4) in excellent yield (entry 2, Table 1). No side reactions were observed as most of the excess of thiol was recovered unaltered while the only side product was the corresponding disulfide. A complete hydrothiolation of **1c** was also carried out using the *C*-mannosyl thiol **3b** (prepared from the known allyl *C*-mannopyranoside,[23] see ESI, Fig. S1†) to give the corresponding POSS-based glycocluster **4b** in an almost identical yield of **4a** (entry 3, Table 1). Evidence for the conservation of the structural integrity of the POSS cage in **4b** upon irradiation at λ_{max} 365 nm was unambiguously provided by ^{29}Si NMR spectroscopy showing a sharp peak at −66.2 ppm.

While a recent paper by Kolmar and co-workers reported on the preparation of POSS-peptide conjugates *via* CuAAC using

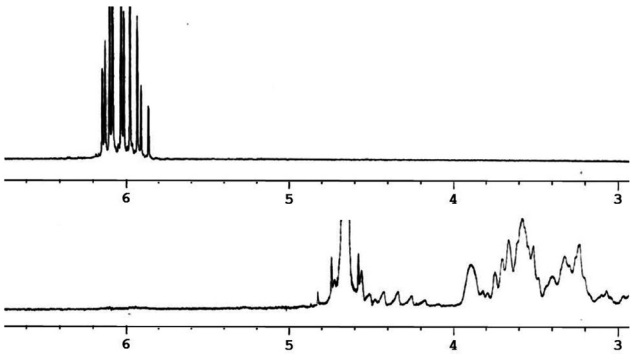


Fig. 3 ^{1}H NMR spectra of octavinyl POSS **1c** (300 MHz, $CDCl_3$) (top) and the crude reaction mixture of the coupling of **1c** with **3a** (300 MHz, D_2O) (bottom).

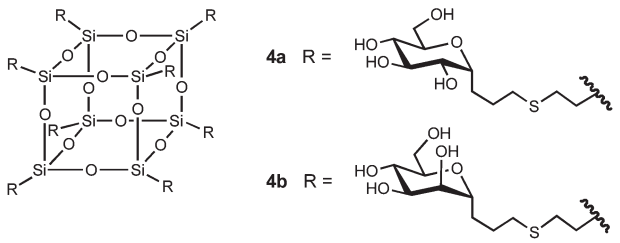


Fig. 4 Glycoconjugates prepared from octavinyl POSS **1c**.

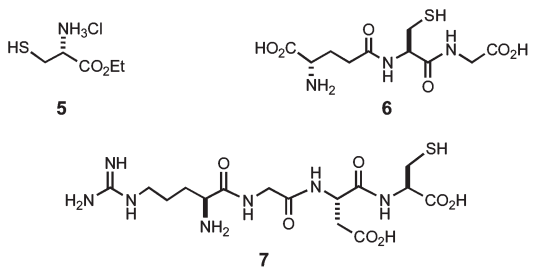


Fig. 5 Cysteine derivative and cysteine containing peptides used for the hydrothiolation of POSS.

octaazide silsesquioxane **1b** as the reagent,[24] we decided to develop a complementary metal-free approach *via* TEC using octavinyl POSS **1c**. As we intended to use cysteine-containing peptides as thiol partners, we first explored the feasibility of the photoinduced coupling of **1c** with cysteine. Specifically, we used the commercially available cysteine hydrochloride ethyl ester **5** (Fig. 5) because this compound was fairly soluble in DMF, a solvent also capable of dissolving **1c** and the photoinitiator DPAP.

Thus, the photoinduced coupling between **1c** and excess of **5** (1.5 equiv./ene of **1c**) in the presence of DPAP was successfully carried out to give the POSS-cysteine conjugate **8** (Fig. 6) in high isolated yield (entry 4, Table 1). Then, the coupling of **1c** with the natural tripeptide glutathione Glu-Cys-Gly (GSH) **6** (Fig. 5) was performed as well and also in this case complete hydrothiolation of POSS substrate was observed by ^{1}H-NMR analysis to give the POSS-GSH conjugate **9** (Fig. 6) in 75% isolated yield (entry 5, Table 1). The attempt to conjugate **1c** with a larger peptide, namely the tetrapeptide Arg-Gly-Asp-Cys

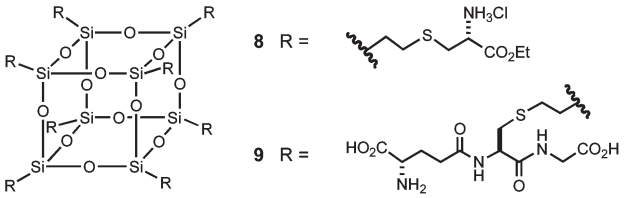
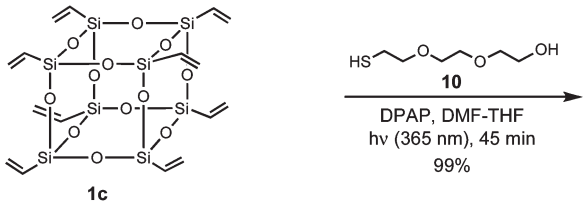
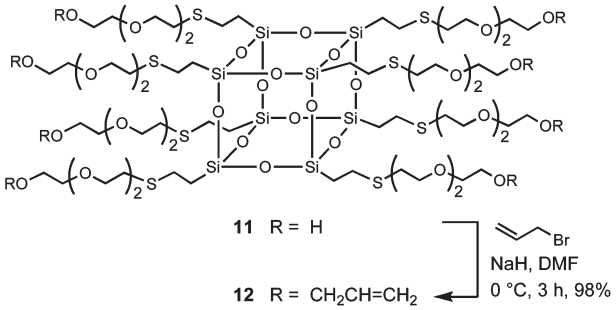


Fig. 6 POSS-peptide conjugates prepared from **1c**.





Scheme 1 Synthesis of PEGylated octaallyl POSS **12**.

(RGDC) **7** (Fig. 5) gave less satisfactory results. Although a considerable excess of **7** was employed (3 equiv./ene of **1c**), only partial hydrothiolation of **1c** was achieved as revealed by the presence of unreacted vinyl groups by NMR analysis of the crude reaction mixture (entry 6, Table 1). Therefore no efforts were made to optimize this reaction. On the other hand, the ^{1}H and ^{13}C NMR spectra of all glyco- and peptido-conjugates reported above showed the absence of olefinic signals while there was some line broadening of the other signals, very likely due to various conformations of the ligands. Moreover, MS analysis of products **4a**, **4b**, **8**, and **9** confirmed their structure.

In a second instance we set out to circumvent the incomplete conjugation due to steric hindrance by using an octaene POSS derivative in which alkene groups were attached to the scaffold through a spacer. To this end we decided to use a PEGylated tether because this hydrophilic chain is known to improve water solubility and biocompatibility. The PEG fragment was introduced by photoinduced coupling of **1c** with the known[25] thiol **10** bearing a PEG chain with a terminal hydroxyl group, to give the octahydroxy functionalized POSS **11** (Scheme 1). This in turn was treated with allyl bromide and NaH to afford the target PEG-linked octaene silsesquioxane **12** in almost quantitative yield. Notably the ^{1}H NMR spectrum of this new POSS-based reagent revealed a single set of olefinic protons in accordance with the T_8 symmetry of the system. We considered this observation as an additional evidence of the conservation of the structural integrity of the POSS cage under the conditions of photoinduced TEC.

Next, the photoinduced coupling of **12** with glycosyl thiols, *i.e.* sugars bearing the sulfhydryl group directly linked to the anomeric carbon, was explored. Thus, it was quite rewarding to find that the irradiation (λ_{max} 365 nm) of a mixture constituted of **12**, 1-thio-β-D-glucopyranose **2a** (Fig. 2) and DPAP in an aqueous solvent (MeOH–DMF–H_2O) induced the complete consumption of **12** as shown by ^{1}H NMR analysis of the crude mixture. Column chromatography of the latter allowed the isolation of pure POSS-based glycoconjugate **13a** (Fig. 7) in very good yield (entry 1, Table 2). Effective conjugation was achieved also from the reaction of **12** with the 2-acetamido-2-deoxy-1-thio-β-D-glucopyranose **2b** and the sterically more demanding disaccharide 1-thio-β-D-lactopyranose[22] **2c** (Fig. 2). In both cases the reaction afforded the corresponding glycoconjugate, being product **13b** and **13c** (Fig. 7) isolated in very good and fair yield, respectively (entries 2 and 3, Table 2). In a second instance, the photoinduced reactions of **12** with the tripeptide glutathione **6** and tetrapeptide RGDC **7** (Fig. 5) were carried out under the above conditions. These reactions did not present any problems apart the need of using the hydrochloride of **6** to achieve complete solubility of reagents and product in the selected solvent (MeOH). In both cases the silsesquioxane **12** was completely hydrothiolated after 1 h irradiation as shown by NMR analysis of the reaction mixtures. Suitable work-up and chromatography over Sephadex LH-20 afforded the corresponding peptidyl conjugates **14** and **15** (Fig. 7) in very good yields (entries 4 and 5, Table 2). Also the thioconjugates derived from **12**, *i.e.* **13a–c**, **14**, and **15**, were characterized by NMR as well as mass spectrometry. Only product **13b** failed to give a satisfactory MALDI-TOF MS spectrum (the experimental mass differed by 1.7 Da from the calculated value) but this was characterized by consistent elemental analysis of its hydrated form.

In order to ascertain whether the prepared POSS-based glycoclusters exhibited to some extent a glycoside cluster effect[26] in lectin recognition, the binding properties of some of them were studied with two lectins, one from *Canavalia ensiformis* (Concanavalin A, ConA), which is specific for the α-D-mannopyranosides and, to a lesser extent, the α-D-glucopyranosides, the other from *Triticum vulgaris* (wheat germ agglutinin, WGA), which is specific for *N*-acetyl-D-glucosamine (D-GlcNAc). First, the ability of glucosylated and mannosylated glycoclusters **4a** and **4b** to inhibit the binding of horseradish peroxidase-labelled ConA (ConA-HRP) to an α-D-mannose-polyacrylamide

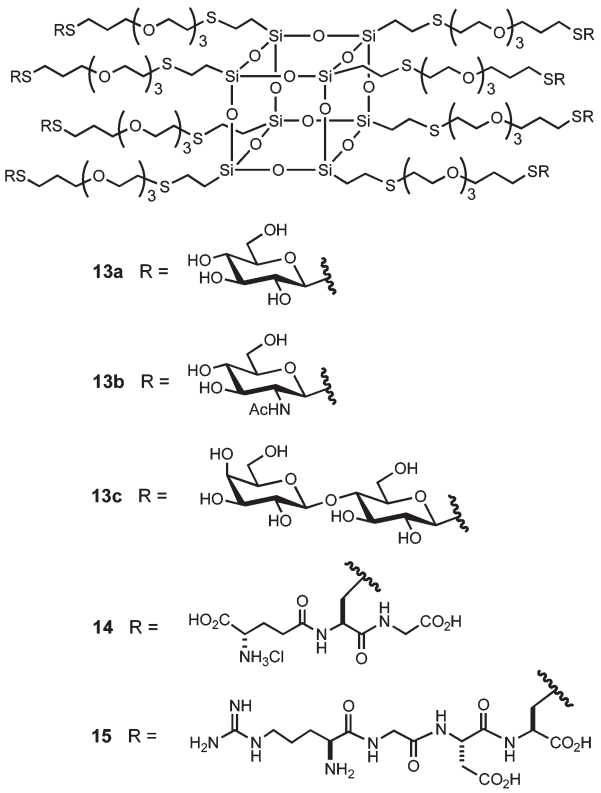


Fig. 7 Glyco- and peptido-conjugates prepared from PEGylated POSS **12**.

Table 2 Hydrothiolation of POSS **12** at λ_{max} 365 nm in the presence of DPAP (0.1 equiv./thiol)

Entry	Thiol	Thiol equiv./ene	Solvent	Time (h)	Product	Yield (%)
1	**2a**	3	MeOH–DMF–H_2O	1	**13a**	79
2	**2b**	3	DMF–H_2O	1	**13b**	82
3	**2c**	3	DMF–H_2O	1.5	**13c**	50
4	**6·HCl**	3	MeOH	1	**14**	78
5	**7**	3	MeOH	1	**15**	61

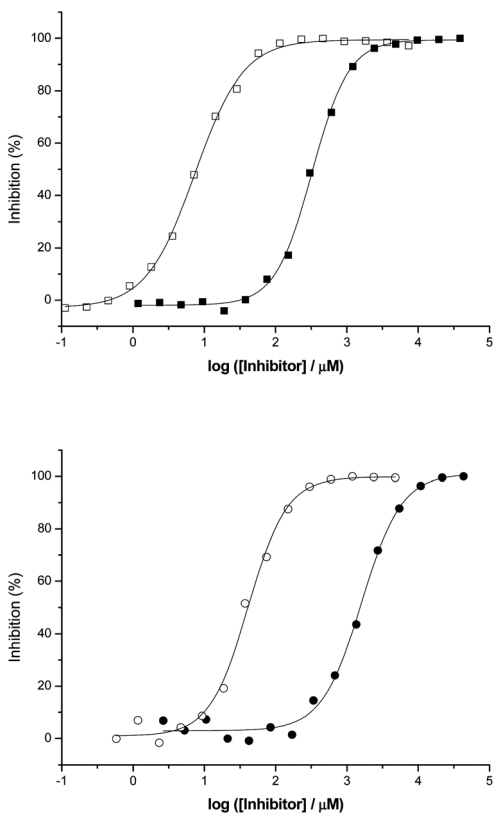


Fig. 8 Inhibition curves of methyl α-D-mannopyranoside (■) and mannosylated glycocluster **4b** (□) (top) or methyl α-D-glucopyranoside (●) and glucosylated glycocluster **4a** (○) (bottom).

Table 3 ELLA data for the inhibition of the binding of ConA-HRP to α-D-Man-PAA with glucosylated (**4a**) or mannosylated (**4b**) glycoclusters[a]

Entry	Product	n^b	IC_{50} (μM)	rp[c]	rp/n^d
1	Me α-D-Glc	1	1422 ± 129	1	1
2	**4a**	8	40.4 ± 0.7	35.2	4.4
3	Me α-D-Man	1	328 ± 27	1	1
4	**4b**	8	6.8 ± 0.9	48.2	6

[a] Each experiment was carried out in triplicate. [b] Number of sugar units in the molecule. [c] Relative potency = IC_{50}(monosaccharide)/IC_{50}(glycocluster). [d] Relative potency/number of sugar units.

Table 4 ELLA data for the inhibition of the binding of WGA-HRP to D-GlcNAc-PAA with PEGylated POSS-based glycoclusters **13a** and **13b**[a]

Entry	Product	n^b	IC_{50} (μM)	rp[c]	rp/n^d
1	D-GlcNAc	1	28 000 ± 2500	1	1
2	**13b**	8	0.003 ± 0.0006	9.3×10^6	10^6
3	**13a**	8	No inhibition[e]	—	—

[a] Each experiment was carried out in triplicate. [b] Number of sugar units in the molecule. [c] Relative potency = IC_{50}(monosaccharide)/IC_{50}(glycocluster). [d] Relative potency/number of sugar units. [e] No inhibition detected at 100 μM.

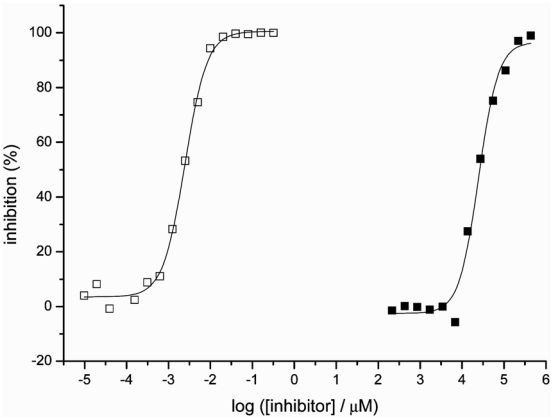


Fig. 9 Inhibition curves for the binding of WGA-HRP to D-GlcNAc-PAA by GlcNAc (■) and glycocluster **13b** (□).

conjugate (α-D-Man-PAA) was measured by an Enzyme-Linked Lectin Assay (ELLA) following a previously reported procedure[27] (Fig. 8). Methyl α-D-mannopyranoside (Me α-D-Man) and methyl α-D-glucopyranoside (Me α-D-Glc) were used as monovalent references.

As indicated in Table 3, both compounds showed modest inhibitory properties with IC_{50} values of 40 and 7 μM for **4a** and **4b**, respectively, which correspond to a relative potency (rp) of 35 (**4a**) and 48 (**4b**) in reference to the corresponding monosaccharide. When reported to the number of sugar unit (rp/*n*), the inhibition enhancement was 4.4 (**4a**) and 6-fold (**4b**) higher, indicating a weak glycoside cluster effect. It is likely that the rather short spacers between the sugars and the platform in glycoclusters **4a** and **4b** did not allow a multivalent interaction with Concanavalin A, which displays four binding sites located far away from each other (*ca.* 65 Å). These findings are in good agreement with the moderate binding affinity to ConA that is usually shown by low molecular weight glycoclusters.[28] It has to be noted, however, that this is not a general result, as in some cases higher affinity was observed.[29]

A similar assay was performed with WGA and PEGylated POSS-based GlcNAc cluster **13b** as the inhibitor while 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and glucosylated glycocluster **13a** were used as the monovalent reference and the negative control, respectively (Fig. 9).

GlcNAc cluster **13b** showed a strong inhibition effect (IC_{50} 3 nM) whereas no inhibition was observed with the glucosylated derivative **13a** at a concentration 100 μM, thus precluding unspecific binding between WGA and the silsesquioxane core (Table 4). In contrast to the results obtained from the assays with ConA, the IC_{50} found for **13b** corresponds to an extremely high relative potency when compared to the monosaccharidic GlcNAc (rp = 9.3×10^6, rp/*n* = 10^6). These unprecedented values for the inhibition of WGA by a synthetic glycocluster clearly indicated a strong multivalent effect, very likely due to a chelate binding mode.[30] Indeed WGA is a dimeric lectin containing a total of eight binding sites separated by approximately 14 Å.[31] These structural features appear fully compatible with the tridimensional orientation and the length of the spacers linking the GlcNAc moieties to the silsesquioxane platform in the glycocluster **13b**. Therefore, the multiple and simultaneous interactions of the sugar ligands with the WGA binding sites take place efficiently.

It is worth noting that ELLA experiments measure the ability of a ligand to inhibit the binding of a lectin to an immobilized glycopolymer. Therefore, the IC_{50} value is only indicative of the binding potency of the ligand to the lectin in reference to the immobilized compound. In order to fully assess the binding properties of **13b** toward WGA lectin, other assays, *e.g.* by Isothermal Titration Calorimetry (ITC) or Surface Plasmon Resonance (SPR), should be performed.

Conclusions

In conclusion, the above results demonstrate the versatility and fidelity of the free-radical thiol–ene coupling (TEC) as a tool for the introduction of sugars and peptide residues into octasilsesquioxane scaffolds to give bioorganic–inorganic hybrid materials. As exhaustive hydrothiolation of the eight vinyl groups of the octasilsesquioxanes employed did occur in all cases examined, the modest yields of some isolated products can be ascribed to the difficulty in their purification. Hence, the efficiency of TEC as a metal-free click process that can be initiated by visible light appears to be confirmed. Moreover, TEC proved to be also a useful methodology for the high yield preparation of a new functionalized octasilsesquioxane, *i.e.* the PEG-linked octaene silsesquioxane **12**. The use of this compound appears to overcome the problem of incomplete silsesquioxane conjugation due to steric hindrance. The striking glycoside cluster effect registered in inhibition experiments of a specific lectin by a glycocluster derived from **12** is notable. This particular issue needs further studies for establishing the key

structural factors of the glycocluster responsible for such effect. These studies are under way in our laboratories.

Experimental

General experimental section

Flash column chromatography was performed on silica gel 60 (40–63 mm). Optical rotations were measured at 20 ± 2 °C in the stated solvent; $[\alpha]_D$ values are given in deg mL g^{-1} dm^{-1}. ^1H NMR (300 and 400 MHz), ^{13}C NMR spectra (75 and 100 MHz), and ^{29}Si NMR (79.5 MHz) were recorded from D_2O solutions at room temperature unless otherwise specified. Peak assignments were aided by ^1H-^1H COSY and gradient-HMQC experiments. In the ^1H NMR spectra reported below, the *n* and *m* values quoted in geminal or vicinal proton–proton coupling constants $J_{n,m}$ refer to the number of the corresponding sugar protons.

The commercially available octavinyl POSS **1c**, photoinitiator 2,2-dimethoxy-2-phenylacetophenone (DPAP), cysteine hydrochloride **5**, and glutathione **6** were used without further purification. The tetrapeptide Arg-Gly-Asp-Cys (RGDC, **7**) was supplied by GL Biochem Ltd (Shangai, China). Horseradish peroxidase-labelled Concanavalin A (ConA-HRP) and *Triticum vulgaris* lectin (wheat germ agglutinin) (WGA-HRP), Bovine Serum Albumin (BSA), and SIGMA*FAST* *O*-phenylenediamine dihydrochloride (OPD) were purchased from Sigma-Aldrich. The α-D-mannose-polyacrylamide (α-D-Man-PAA) and 2-acetamido-2-deoxy-D-glucose-polyacrylamide (D-GlcNAc-PAA) were obtained from Lectinity Holding, Inc., Moscow.

The thiol–ene coupling was carried out in a glass vial (diameter: 1 cm; wall thickness: 0.65 mm), sealed with a natural rubber septum, located 2.5 cm away from the household UVA lamp apparatus equipped with four 15 W tubes (1.5 × 27 cm each).

High resolution MS analysis

For accurate mass measurements the compounds were analyzed in positive ion mode by electrospray hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (Q-TOF) fitted with a Z-spray electrospray ion source. The capillary source voltage and the cone voltage were set at 3500 V and 35 V, respectively; the source temperature was kept at 80 °C; nitrogen was used as a drying gas at a flow rate of *ca.* 50 L h^{-1}. The time-of-flight analyzer was externally calibrated with NaI from *m*/*z* 300 to 2000 to yield an accuracy near to 5 ppm. When necessary an internal lock mass was used to further increase the mass accuracy. Accurate mass data were collected by directly infusing samples (1.5 pmol μL^{-1} in 1 : 1 CH_3CN–H_2O) into the system at a flow rate of 5 μL min^{-1}. The acquisition and data processing were performed with the MassLynx 4.1 software. Compounds **4a**, **9**, **11**, **12**, **13a–c**, **14**, and **15** were analyzed by MALDI TOF mass spectrometry using a pulsed nitrogen laser (λ = 337 nm) and α-cyano-4-hydroxycinnamic acid or sinapinic acid as the matrix. The instrument was operated in positive ion reflectron mode with the source voltage set to 12 kV. The pulse voltage was optimized at 1999 V, and the detector and reflectron voltages were set to 5200 and 2350 V, respectively. Measurements were performed in the mass range *m*/*z* 800–5000 with a suppression mass gate set to *m*/*z* 500 to prevent detector saturation from matrix cluster peaks and an extraction delay of 600 ns. The instrument was externally calibrated using a polyethylene glycol mix as standard. A mass accuracy near to the nominal (50 ppm) was achieved for each standard.

Glycoconjugate 4a. A solution of octavinyl POSS **1c** (10 mg, 15.8 μmol), thiol **3a** (60 mg, 0.25 mmol), and DPAP (6.5 mg, 25.3 μmol) in DMF (300 μL) and THF (100 μL) was irradiated at r.t. for 1 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 with 3 : 1 MeOH–H_2O to give **4a** (38 mg, 94%) as a syrup; $[\alpha]_D$ = +54.4 (*c* 1.5, H_2O). ^1H NMR (300 MHz): δ 3.96–3.83 (m, 8H), 3.80–3.43 (m, 32H), 3.42–3.20 (m, 16H), 2.70–2.45 (m, 32H), 1.85–1.45 (m, 32H), 1.10–0.90 (m, 16H). ^{13}C NMR (75 MHz): δ 75.9 (CH), 73.8 (CH), 72.8 (CH), 71.6 (CH), 70.4 (CH), 61.4 (CH_2), 31.5 (CH_2), 25.6 (CH_2), 25.5 (CH_2), 23.5 (CH_2), 14.0 (CH_2). MALDI-TOF MS: *m*/*z* calcd for $C_{88}H_{168}NaO_{52}S_8Si_8$ $(M + Na)^+$ 2562.47, found 2562.47.

Glycoconjugate 4b. The octavinyl POSS **1c** (10 mg, 15.8 μmol) was treated with thiol **3b** (60 mg, 0.25 mmol) as described for the preparation of **4a** to give **4b** (37.5 mg, 93%) as a syrup; $[\alpha]_D$ = +16.4 (*c* 1.6, H_2O). ^1H NMR (300 MHz): δ 3.90–3.51 (m, 48H), 3.45–3.33 (m, 8H), 2.73–2.50 (m, 32H), 1.96–1.41 (m, 32H), 1.15–0.95 (m, 16H). ^{13}C NMR (75 MHz): δ 78.1 (CH), 73.7 (CH), 71.9 (CH), 71.2 (CH), 67.2 (CH), 61.4 (CH_2), 31.4 (CH_2), 27.1 (CH_2), 25.7 (CH_2), 25.6 (CH_2), 12.8 (CH_2). ^{29}Si NMR (79.5 MHz): δ −66.2. HRMS (ESI/Q-TOF): *m*/*z* calcd for $(C_{88}H_{170}O_{52}S_8Si_8)/2$ $(M + 2H)^{2+}$ 1269.3289, found 1269.3259.

POSS-cysteine conjugate 8. A solution of **1c** (10 mg, 15.8 μmol), cysteine hydrochloride **5** (35 mg, 0.19 mmol), and DPAP (5 mg, 19.0 μmol) in DMF (1.6 mL) was irradiated at r.t. for 45 min under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 with 1 : 1 MeOH–H_2O to give **8** (28 mg, 84%) as a syrup; $[\alpha]_D$ = +6.7 (*c* 0.8, MeOH). ^1H NMR (300 MHz): δ 4.25 (dd, 8H, *J* = 5.0, 5.5 Hz, 8 CHN), 4.22 (q, 16H, *J* = 7.2 Hz, 8 CH_2CH_3), 3.12 (dd, 8H, *J* = 5.5, 15.0 Hz, 8 H of CH_2S), 3.04 (dd, 8H, *J* = 5.0, 15.0 Hz, 8 H of CH_2S), 2.59 (t, 16H, *J* = 8.0 Hz, 8 CH_2S), 1.22 (t, 24H, *J* = 7.2 Hz, 8 CH_2CH_3), 1.05 (dd, 8H, *J* = 8.0, 15.0 Hz, 8 H of CH_2Si), 0.96 (dd, 8H, *J* = 8.0, 15.0 Hz, 8 H of CH_2Si). ^{13}C NMR (75 MHz): δ 169.2 (C), 63.6 (CH_2), 52.3 (CH), 31.3 (CH_2), 25.9 (CH_2), 13.4 (CH_3), 11.7 (CH_2). HRMS (ESI/Q-TOF): *m*/*z* calcd for $(C_{56}H_{114}N_8O_{28}S_8Si_8)/2$ $(M + 2H)^{2+}$ 913.1831, found 913.1842.

POSS-glutathione conjugate 9. The octavinyl POSS **1c** (10 mg, 15.8 μmol) was treated with glutathione **6** (58 mg, 0.19 mmol) as described for the preparation of **8** to give, after column chromatography on Sephadex LH-20 (2 : 1 H_2O–MeOH), **9** (36.5 mg, 75%) as a syrup; $[\alpha]_D$ = −17.9 (*c* 0.8, H_2O). ^1H NMR (300 MHz): δ 4.44 (bt, 8H, *J* = 5.8 Hz), 3.84 (bs, 16H), 3.70 (t, 8H, *J* = 6.2 Hz), 2.98–2.86 (m, 8H), 2.84–2.71 (m, 8H), 2.62–2.53 (m, 16H), 2.45–2.36 (m, 16H), 2.08–1.98 (m, 16H), 1.04–0.91 (m, 16H). ^{13}C NMR (100 MHz): δ 174.5 (C), 173.6 (C), 172.5 (C), 53.7 (CH), 53.0 (CH), 41.7

(CH_2), 32.7 (CH_2), 31.2 (CH_2), 26.0 (CH_2), 12.0 (CH_2). MALDI-TOF MS: *m/z* calcd for $C_{96}H_{161}N_{24}O_{60}S_8Si_8$ $(M + H)^+$ 3092.67, found 3092.66.

PEGylated POSS 11. A solution of **1c** (80 mg, 126.4 μmol), 2-[2-(2-hydroxyethoxy)ethoxy]-1-ethanethiol (**10**, 336 mg, 2.02 mmol), and DPAP (16 mg, 63.2 μmol) in DMF (1.4 mL) and THF (0.7 mL) was irradiated at r.t. for 45 min under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 with 1 : 1 MeOH–H_2O to give **11** (245 mg, 99%) as a syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76 (t, 16H, J = 4.7 Hz, 8 CH_2O), 3.72–3.65 (m, 48H, 24 CH_2O), 3.62 (t, 16H, J = 4.3 Hz, 8 CH_2O), 2.77 (t, 16H, J = 6.9 Hz, 8 CH_2S), 2.71–2.64 (m, 16H, 8 CH_2S), 2.60 (bs, 8H, 8 OH), 1.09–1.02 (m, 16H, 8 CH_2Si). ^{13}C NMR (75 MHz, $CDCl_3$): δ 72.5 (CH_2), 70.6 (CH_2), 70.3 (CH_2), 61.6 (CH_2), 31.1 (CH_2), 26.4 (CH_2), 13.0 (CH_2). MALDI-TOF MS: *m/z* calcd for $C_{64}H_{136}NaO_{36}S_8Si_8$ $(M + Na)^+$ 1985.96, found 1985.95.

PEGylated octaallyl POSS 12. NaH (16 mg, 0.40 mmol, of a 60% dispersion in oil) and then allyl bromide (35 μL, 0.40 mmol) were added to a stirred, cooled (0 °C) solution of **11** (49 mg, 25.0 μmol) in anhydrous DMF (2 mL). The mixture was stirred at 0 °C for 3 h, then diluted with 1 M phosphate buffer at pH 7 (0.5 mL), warmed to r.t., diluted with H_2O (15 mL), and extracted with AcOEt (3 × 30 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was eluted from a column of Sephadex LH-20 with MeOH to give **12** (56 mg, 98%) as a syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.94 (ddt, 8H, J = 5.6, 10.7, 16.5 Hz, 8 C*H*═CH_2), 5.30 (bd, 8H, J = 16.5 Hz, CH═C*H*$_2$), 5.21 (bd, 8H, J = 10.7 Hz, CH═C*H*$_2$), 4.05 (d, 16H, J = 5.6 Hz, 4 C*H*$_2$–CH═), 3.78–3.60 (m, 80H, 40 CH_2O), 2.82–2.62 (m, 32H, 16 CH_2S), 1.14–0.98 (m, 16H, 8 CH_2Si). ^{13}C NMR (75 MHz, $CDCl_3$): δ 134.7 (CH), 117.1 (CH_2), 72.2 (CH_2), 70.6 (CH_2), 70.3 (CH_2), 69.4 (CH_2), 31.3 (CH_2), 26.7 (CH_2), 14.1 (CH_2). MALDI-TOF MS: *m/z* calcd for $C_{88}H_{168}NaO_{36}S_8Si_8$ $(M + Na)^+$ 2303.71, found 2303.71.

Glycoconjugate 13a. A solution of **12** (14 mg, 6.1 μmol), glucosyl thiol **2a** (29 mg, 147.3 μmol), and DPAP (3.8 mg, 14.8 μmol) in 4 : 2 : 1 MeOH–DMF–H_2O (1.5 mL) was irradiated at r.t. for 1 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 with 1 : 1 MeOH–H_2O to give **13a** (18.5 mg, 79%) as a syrup; $[\alpha]_D$ = −45.5 (*c* 0.7, H_2O). ^{1}H NMR (300 MHz): δ 4.42 (d, 8H, $J_{1,2}$ = 9.8 Hz, 8 H-1), 3.78 (bd, 8H, $J_{6a,6b}$ = 12.5 Hz, 8 H-6a), 3.72–3.50 (m, 104H, 48 CH_2O, 8 H-6b), 3.42–3.26 (m, 24H), 3.20 (t, 8H, J = 8.8 Hz), 2.80–2.58 (m, 48H, 24 CH_2S), 1.90–1.77 (m, 16H, 8 $OCH_2CH_2CH_2S$), 1.15–0.93 (m, 16H, 8 CH_2Si). ^{13}C NMR (75 MHz): δ 89.8 (CH), 80.7 (CH), 77.4 (CH), 72.8 (CH_2), 71.6 (CH), 70.1 (CH_2), 70.0 (CH_2), 69.6 (CH), 61.2 (CH_2), 31.4 (CH_2), 29.8 (CH_2), 26.4 (CH_2), 14.2 (CH_2). MALDI-TOF MS: *m/z* calcd for $C_{136}H_{264}CaO_{76}S_{16}Si_8$ $(M + Ca)^+$ 3893.36, found 3893.33.

Glycoconjugate 13b. A solution of **12** (14 mg, 6.1 μmol), thiol **2b** (35 mg, 147.3 μmol), and DPAP (3.8 mg, 14.8 μmol) in DMF (200 μL) and H_2O (50 μL) was irradiated at r.t. for 1 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 with 1 : 1 MeOH–H_2O to give **13b** (21 mg, 82%) as a syrup; $[\alpha]_D$ = −12.5 (*c* 1.0, H_2O). ^{1}H NMR (300 MHz): δ 4.47 (d, 8H, $J_{1,2}$ = 10.5 Hz, 8 H-1), 3.76 (bd, 8H, $J_{6a,6b}$ = 12.3 Hz, 8 H-6a), 3.65–3.34 (m, 120H), 3.33–3.29 (m, 16H), 2.78–2.52 (m, 48H, 24 CH_2S), 1.90 (s, 24H, 8 Ac), 1.83–1.68 (m, 16H, 8 $OCH_2CH_2CH_2S$), 1.10–0.86 (m, 16H, 8 CH_2Si). ^{13}C NMR (75 MHz): δ 174.1 (C), 84.5 (CH), 80.1 (CH), 75.4 (CH), 70.0 (CH_2), 69.7 (CH_2), 69.6 (CH_2), 61.1 (CH_2), 55.0 (CH), 30.9 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 27.1 (CH_2), 26.4 (CH_2), 22.5 (CH_3), 14.2 (CH_2). MALDI-TOF MS: *m/z* calcd for $C_{152}H_{288}N_8NaO_{76}S_{16}Si_8$ $(M + Na)^+$ 4204.70, found 4203.00. Anal. Calcd for $C_{152}H_{288}N_8O_{76}S_{16}Si_8{\cdot}8H_2O$: C, 42.20; H, 7.08; N, 2.59; S, 11.86. Found: C, 42.08; H, 6.88; N, 2.38; S, 11.42.

Glycoconjugate 13c. A solution of **12** (14 mg, 6.1 μmol), lactosyl thiol **2c** (53 mg, 147.3 μmol), and DPAP (3.8 mg, 14.8 μmol) in DMF (200 μL) and H_2O (50 μL) was irradiated at r.t. for 1.5 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 with 3 : 1 H_2O–MeOH to give **13c** (16 mg, 50%) as a syrup; $[\alpha]_D$ = +6.0 (*c* 0.4, DMSO). ^{1}H NMR (300 MHz): δ 4.43 (bd, 8H, $J_{1,2}$ = 9.8 Hz, 8 H-1), 4.34 (d, 8H, $J_{1',2'}$ = 7.8 Hz, 8 H-1′), 3.83–3.79 (m, 16H), 3.72–3.40 (m, 168H), 3.26 (t, 8H, J = 8.8 Hz), 2.76–2.52 (m, 48H, 24 CH_2S), 1.88–1.76 (m, 16H, 8 $OCH_2CH_2CH_2S$), 1.10–0.91 (m, 16H, 8 CH_2Si). ^{13}C NMR (75 MHz): δ 103.4 (CH), 85.7 (CH), 79.1 (CH), 78.8 (CH), 76.3 (CH), 75.8 (CH), 75.6 (CH), 73.0 (CH), 72.5 (CH), 72.1 (CH), 71.4 (CH), 70.2 (CH_2), 69.8 (CH_2), 69.5 (CH), 69.0 (CH), 68.7 (CH), 61.5 (CH_2), 60.8 (CH_2), 38.2 (CH_2), 29.8 (CH_2), 27.1 (CH_2), 25.0 (CH_2), 22.7 (CH_2). MALDI-TOF MS: *m/z* calcd for $C_{184}H_{344}O_{116}S_{16}Si_8$ $(M)^+$ 5150.35, found 5150.53.

POSS-glutathione conjugate 14. A solution of **12** (10 mg, 4.4 μmol), glutathione chloridrate **6·HCl** (36 mg, 105.2 μmol, prepared by freeze-drying a solution of **6** in aqueous HCl), and DPAP (2.7 mg, 10.5 μmol) in MeOH (600 μL) was irradiated at r.t. for 1 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 with 3 : 1 MeOH–H_2O to give **14** (17.2 mg, 78%) as a syrup; $[\alpha]_D$ = −13.9 (*c* 0.9, H_2O). ^{1}H NMR (300 MHz): δ 4.42–4.36 (m, 8H), 3.78 (bs, 16H), 3.64–3.40 (m, 104H), 2.94–2.83 (m, 16H), 2.76–2.53 (m, 32H), 2.50 (bt, 16H, J = 7.0 Hz), 2.42–2.32 (m, 16H), 2.04–1.95 (m, 16H), 1.76–1.65 (m, 16H), 1.04–0.89 (m, 16H). ^{13}C NMR (75 MHz): δ 175.1 (C), 174.1 (C), 172.8 (C), 172.1 (C), 72.3 (CH_2), 70.1 (CH_2), 69.8 (CH_2), 63.9 (CH_2), 60.9 (CH_2), 54.4 (CH), 53.6 (CH), 53.2 (CH), 42.5 (CH_2), 41.6 (CH_2), 33.3 (CH_2), 31.8 (CH_2), 31.0 (CH), 29.1 (CH_2), 28.7 (CH_2), 26.6 (CH_2), 14.1 (CH_2). MALDI-TOF MS: *m/z* calcd for $C_{184}H_{344}O_{116}S_{16}Si_8$: *m/z* calcd for $C_{168}H_{305}N_{24}O_{84}S_{16}Si_8$ $(M + H)^+$ 4738.40, found 4738.72.

POSS-RGDC conjugate 15. A solution of **12** (5 mg, 2.2 μmol), tetrapeptide RGDC **7** (23.5 mg, 52.6 μmol), and DPAP (1.3 mg, 5.3 μmol) in MeOH (300 μL) was irradiated at r.t. for 1 h under magnetic stirring and then concentrated. The residue was eluted from a column of Sephadex LH-20 with MeOH to give **15** (7.9 mg, 61%) as a syrup; $[\alpha]_D$ = −9.9 (*c* 0.3, H_2O). ^{1}H NMR (300 MHz) selected data: δ 3.66–3.44 (m, 96H, 48 CH_2O), 3.14–3.07 (m, 16H), 1.88–1.78 (m, 16H), 1.76–1.68

(m, 16H), 1.62–1.50 (m, 16H), 1.07–0.90 (m, 16H, 8 CH_2Si). ^{13}C NMR (75 MHz) selected data: δ 172.3 (C), 170.6 (C), 170.1 (C), 69.7 (CH_2), 69.5 (CH_2), 42.5 (CH_2), 40.4 (CH_2), 33.7 (CH_2), 28.3 (CH_2), 28.0 (CH_2), 23.5 (CH_2). MALDI-TOF MS: m/z calcd for $(C_{208}H_{384}Na_2O_{92}S_{16}Si_8)/2$ $(M + 2Na)^{2+}$ 2962.65, found 2961.94.

Enzyme-linked lectin assay (ELLA). 96-well microtiter Nunc-Immuno plates (Maxi-Sorp) were coated with α-D-Man-PAA or D-GlcNAc-PAA [100 μL per well, diluted from a stock solution of 5 μg mL^{-1} in 0.01 M phosphate-buffered saline (PBS) pH 7.4 (containing 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+} for ConA assay)] for 1 h at 37 °C. The wells were then washed with T-PBS (3 × 100 μL per well, PBS containing 0.05% (v/v) Tween 20). The washing procedure was repeated after each incubation. The wells were then blocked with BSA in PBS (3% w/v, 100 μL per well) at 37 °C for 1 h. After washing, the wells were filled with 100 μL of serial dilutions of ConA-HRP or WGA-HRP (100 μL, from 10^{-1} to 10^{-7} mg mL^{-1} in PBS (pH 7.4) or PBS containing 0.1 mM Ca^{2+}, 0.1 mM Mn^{2+} (for ConA) and BSA (0.3% w/v)) and were incubated at 37 °C for 1 h. The plates were washed with T-PBS (3 × 100 μL per well), then the colour was developed using OPD (100 μL per well, 0.4 mg mL^{-1} in 0.05 M phosphate-citrate buffer) and urea hydrogen peroxide (0.4 mg mL^{-1}). The reaction was stopped after 10 min by adding H_2SO_4 (30% v/v, 50 μL per well) and the absorbance was measured at 490 nm. The concentration of ConA-HRP or WGA-HRP that gives absorbance between 0.8 and 1 was used for inhibition experiments.

Inhibition experiments. The microtiter plates were coated with α-D-Man-PAA or D-GlcNAc-PAA as described previously. Serial two-fold dilutions of each inhibitor was incubated 1 h at 37 °C in PBS on Nunclon (Delta) microtiter plates (60 μL per well) in the presence of ConA-HRP or WGA-HRP (60 μL) at the desired concentration. The above solutions (100 μL) were then transferred to the coated microtiter plates which were incubated for 1 h at 37 °C. After incubation, the plates were washed with T-PBS and the colour was revealed described above. The percentage of inhibition was plotted against the logarithm of the concentration of the sugar derivatives. The sigmoidal curves were fitted and the concentration at 50% inhibition of binding of the ConA-HRP to α-D-Man-PAA or WGA-HRP to D-GlcNAc-PAA coated plates were determined (IC_{50}). The percentages of inhibition were calculated as given in the equation below, where A = absorbance.

$$\% \text{ inhibition} = (A_{(\text{no inhibitor})} - A_{(\text{with inhibitor})})/A_{(\text{no inhibitor})} \times 100$$

The IC_{50} values were obtained from several independently performed tests in the range of ±17%. Nevertheless, the relative inhibition values calculated from independent series of data were highly reproducible.

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