Towards click bioconjugations on cube-octameric silsesquioxane scaffolds†

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Received 10th November 2009, Accepted 18th February 2010
First published as an Advance Article on the web 12th March 2010
DOI: 10.1039/b923393h

Cube-octameric silsesquioxane (POSS) based conjugation scaffolds for copper catalysed azide-alkyne [3+2] cycloaddition are reported. The synthetic route to octaazido and octaalkyno functionalised POSS templates without cage rearrangements is described. A set of click couplings is conducted including the first effective conjugation with a fully unprotected functional peptide towards a POSS assembled peptide octamer.

Introduction

Polyhedral oligomeric silsesquioxanes (POSS) are well defined nanosized molecules containing an inorganic silica-like core that is decorated with organic groups (Fig. 1). These hybrid nanoparticles have gained considerable research interest in recent years due to their potential application in electronics, engineering, material science and optics.[1]

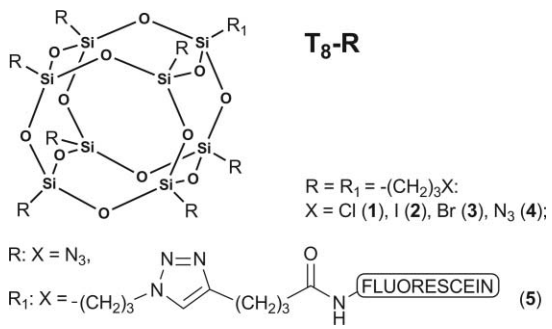

Fig. 1 Schematic representation of T_8-POSS oligomerisation scaffolds.

Though a vast number of polyhedral frameworks have been developed,[2] cube-octameric POSS bearing eight corner groups (T_8) are the most common. They form robust cage-like structures, thus allowing for a unique spatial arrangement of ligand molecules.

This siloxane core can contain either the same sort of ligands (homofunctionalised POSS)[3–7] or bear a single orthogonal function along with seven identical ligands of another type attached to the cube-octameric scaffold.[1,8–14]

Octameric POSS have been applied for the synthesis of diverse homosilsesquioxanes, were used as cores in dendrimer assembly, or employed as nanobridges.[1,15–20] Compared to gold or quantum dot nanoparticles, cube-octameric POSS have a significantly smaller inorganic core (0.5–0.7 nm),[21] thereby providing a compact scaffold enabling the display of up to eight ligand molecules in close proximity.

Despite their biocompatibility,[8,22,23] they have attracted comparably little attention for biomedical applications mainly due to the lack of robust and versatile strategies for conjugation of POSS nanoparticles with biorelevant ligands as *e.g.* peptides. Only one synthetic approach towards peptidyl silsesquioxanes has been reported to date.[24,25] This route of synthesis that is based on the acylation of octaamino or octaalcohol functionalised POSS has serious limitations concerning the choice of the ligand, thus narrowing the scope of conjugation counterparts to single amino acids or short peptides lacking reactive side chains.[24,25]

The copper-catalysed azide–alkyne cycloaddition (CuAAC) is a reliable and robust method widely used for conjugation of a variety of molecules covering almost all classes of chemical substances, including peptides.[26–31] It can be performed in various solvents and in the presence of diverse functional groups leading to the formation of a triazole ring, playing the role of a stable and rigid linker interconnecting the respective conjugation partners.[32–35] Several synthetic routes to click functionalised silsesquioxanes have been reported to date for azido substituted POSS. Monosubstituted azido-POSS were synthesised by the ring opening of epoxides using azide group as a nucleophile, or by nucleophilic substitution *via* azide/chloride exchange, respectively, from commercially available monosubstituted precursors.[36,37] Octasubstituted azidophenyl-POSS has been synthesised from the corresponding aminophenyl precursor *via* its diazonium salt,[38] and octakis(3-azidopropyl)-POSS—by azidation of a corresponding chloride.[37,39]

Though the introduction of a single azide function *via* a chlorosubstituted precursor is clean and quantitative, extensive rearrangements of T_8 cage have been reported for the synthesis of fully converted octaazide-POSS under azidation at elevated temperature.[37] Thus, only about 25% of pure octakis(3-azidopropyl)-POSS was isolated from a mixture of T_8, T_{10} and T_{12} cages.[37]

Herein, we report an easy and efficient procedure for the preparation of an octaazido functionalised octasilsesquioxane scaffold and conjugations thereupon without cage rearrangements, including octamerisation of an unprotected peptide ligand

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† Electronic supplementary information (ESI) available: **NMR** data of: **2–7**; **ESI-MS** data of: **1–9**; **IR** spectra of: **3**, **4**, **6**, **7**; **RP-HPLC** of: **5**, **6**, **8**, **9**; **TG analysis** of **1–4**, **6**. See DOI: 10.1039/b923393h

as well as effective transformation into an octaalkyne cube-octameric framework.

Results and discussion

Synthesis of octakis(3-azidopropyl)-POSS scaffold

We investigated the azidation of different octakis(3-haloalkyl)-POSS in order to find optimal conditions in terms of safety, efficacy, and formation of a fully substituted sole octaazide-POSS (Scheme 1). Octachloropropyl substituted precursor **1** was prepared using a two-step procedure by acid catalysed hydrolysis of trichloro- or trialkoxysilanes to silanols followed by condensation to siloxanes with successive substitution of terminal halogens (Scheme 1a,b).[40,41]

Although the synthesis of primary alkyl azides from their halide precursors is a well developed routine procedure,[42] initial attempts to apply it for the synthesis of **4** turned out to be problematic due to incomplete conversion, low reaction rate and sensitivity of the siloxane cage towards strong nucleophiles. Indeed, only 65% of octasubstituted T_8-POSS have been obtained in the product blend in the case of 95% conversion for an individual transformation.[43] Our observations corroborated reported rearrangements from T_8 to T_{10} and T_{12} cages;[37] in the worst case, formation of polymeric products was observed under attempted treatment of octakis(3-halopropyl)-POSS with strong nucleophiles in highly polar aprotic solvents commonly used for S_N2 reactions, particularly at elevated temperatures.[44,45] Since the reaction of **1** with NaN_3 in DMF at ambient temperature was very slow and thus could not be accomplished in one week, initially the starting POSS **1** was replaced by **2**[40] bearing a better leaving group.[46] Due to the poor solubility of $T_8[(CH_2)_3I]_8$ in polar aprotic solvents like DMF or DMSO, the azide substitution was conducted in chloroform using soluble *N*,*N*′-tetramethylguanidinium azide (TMGA)[47-49] as an azide source. In view of the concomitant formation of hazardous azidomethanes,[50] a safe alternative approach to the synthesis of **4** from corresponding halides was required.

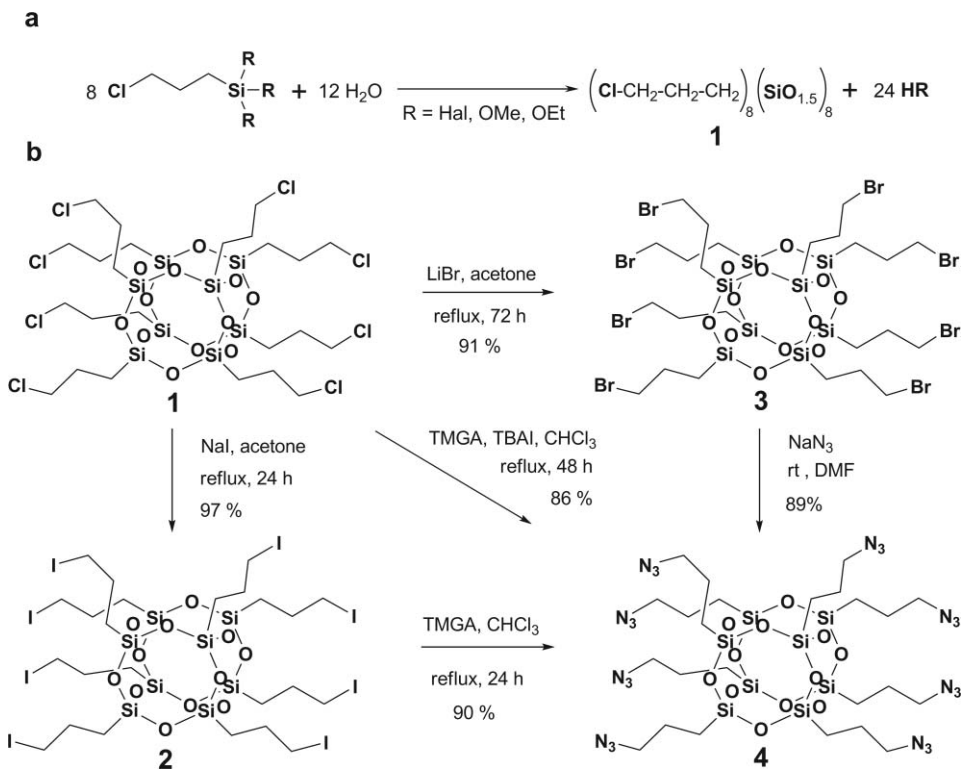
Table 1 Preparation of $T_8[(CH_2)_3N_3]_8$

T_8 alkyl halide	Azide source	Reaction conditions	Yield (%)
1	NaN_3	DMF, rt, 1 week	n. d.
1	TMGA	$CHCl_3$, TBAI, reflux, 48 h	86[a]
2	TMGA	$CHCl_3$, reflux, 24 h	90[a,b]
3	TMGA	$C_2H_5NO_2$, 40 °C, 36 h	80
3	NaN_3	DMF, rt, 20 h	89

[a] Ref. 46 [b] Conversion.

We found that the synthesis of azide **4** from the corresponding bromide **3** using sodium azide in dry, amine-free DMF is optimal as no elevated temperatures and no chromatographic purification were required. Formed POSS azide **4** was isolated by simple water precipitation from the concentrated DMF solution, followed by washing and successive lyophilisation, in very good yield (Table 1).

^{1}H NMR studies proved excellent quality of formed azide **4**. ^{29}Si NMR used to provide information about the cage symmetry and the coordination sphere of Si atoms showed that mild synthesis conditions did not affect the unique architecture of the siloxane core, as exclusively the shift attributed to T_8 cage has been detected (Fig. 2).



Scheme 1 Synthetic approaches to octakis(3-azidopropyl)-POSS **4**; **a**, hydrolytic condensation towards **1**; **b**, substitution of halide precursors yielding scaffold **4**.

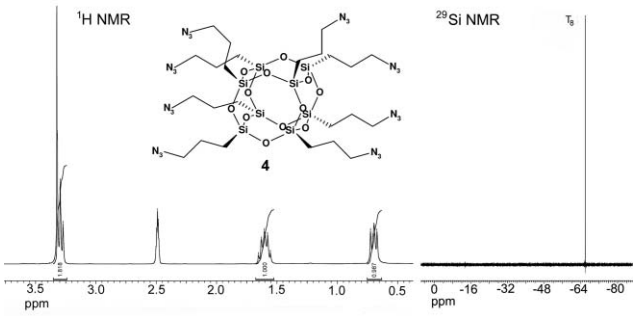


Fig. 2 ^{1}H and ^{29}Si NMR spectra of octakis(3-azidopropyl)-POSS **4**.

In our hands, ESI-FTICR MS proved to be a powerful tool for the characterisation of halide, azide, and alkyne POSS derivatives (Table 2). Intensive quasimolecular ions $[M+NH_4]^+$ were formed upon ionization of modified nanoparticles with ammonium without significant fragmentation. These studies showed that the silsesquioxane framework stabilised an ammonium ion very well, forming singly charged adducts. Therefore, ammonium acetate in solution can be used successfully for ionisation of silsesquioxanes if exclusively poorly ionisable side chain modifications are present.

Infrared (IR) spectroscopy that has been applied to study many silsesquioxane structures[51] is a useful analytical method for azide functionalised POSS. Thus, the IR spectrum of **4** shows Si–O–Si asymmetric stretching absorptions near 1112 cm^{-1}, the absorption bands of the azide group (2097 cm^{-1}, 1277 cm^{-1}), the aliphatic C–H stretching bands (2938 and 2876 cm^{-1}), and deformational vibrations of the silicon–oxygen framework in the region between 360 and 600 cm^{-1}. The broad band around 3400 cm^{-1} corresponds to physically adsorbed water (see the ESI†).[52,53]

Although POSS **4** nearly matches the so-called "rule of six",[26,54,55] and thus is expected to be fairly stable against spontaneous decomposition, the thermal behaviour of this octaazide scaffold was additionally studied by thermogravimetry coupled with mass spectrometry (TG-MS). This method provides extensive information on temperature dependent degradation pathways and has been widely used for the analysis of diverse saturated aliphatic and aromatic silsesquioxanes.[56–58] TG-MS studies demonstrated acceptable stability of **4**.[46]

Click conjugations on octakis(3-azidopropyl)-POSS scaffold

Synthesised azido-POSS template **4** was subjected to the coupling with several small molecule alkyne ligands as well as alkyne functionalised fully deprotected peptide bearing reactive groups in its side chains (Scheme 2, Fig. 3).

It is well known for siloxanes to undergo decomposition under aqueous alkaline conditions.[44,59] On the other hand, the most popular protocol for CuAAC usually requires factors potentially harmful for a POSS cage. Therefore, we focused our efforts on development of such a version of this reaction which would afford the required octaconjugated POSS derivatives under neutral media in aprotic solvent. Indeed, we found that elemental copper turned out to be a proper catalyst for the desired transformation in polar aprotic solvents like DMF, DMSO or acetonitrile. These mild conditions are appropriate for the coupling of bioligands.

Conjugates bearing hydrophobic aromatic substituents (**6**, **7**) were easily isolated by precipitation, though significant loss of reaction products occurred due to their partial solubility in reaction media as well as in the solvent used for washing.

Our subsequent study revealed that click coupling could be successfully performed in a biphasic system with both click partners dissolved in DCM, and under Sharpless conditions, namely, aqueous copper sulfate and sodium ascorbate. In this case, target conjugate **6** was isolated chromatographically in good yield.

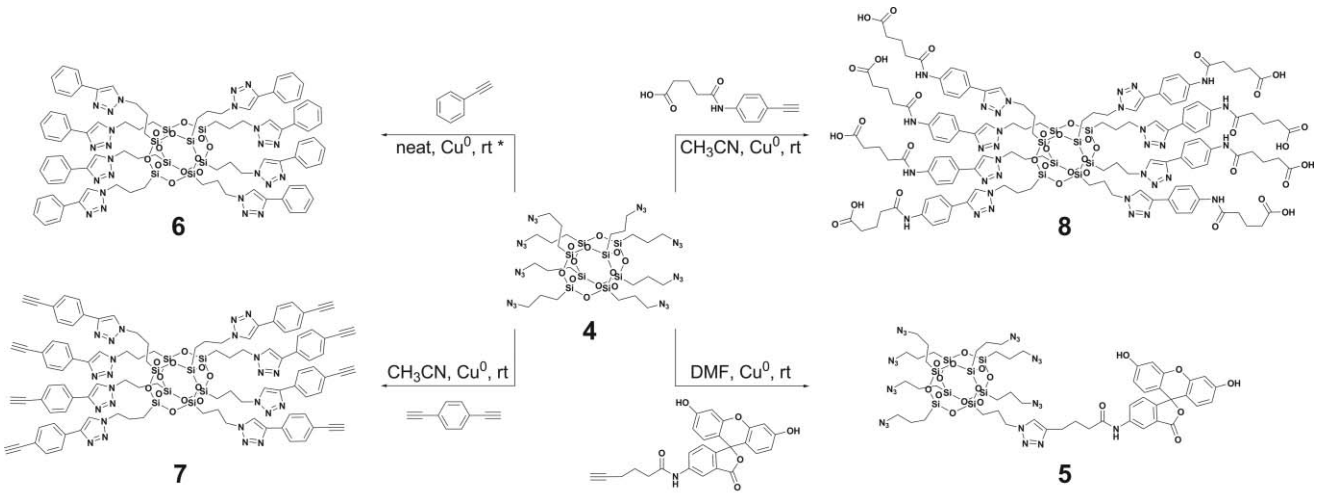
Octasubstituted conjugates bearing phenyl (**6**), *p*-ethynylphenyl (**7**), and *p*-carboxybutanamido phenyl (**8**) residues connected with a scaffold through a stable and robust triazole linker were successfully synthesised as well as an asymmetric template bearing seven functional trimethyleneazide groups and one fluorescein derived substituent (**5**). Introduction of this chromophore into optically transparent octaazide octasilsesquioxane scaffold allowed us to trace the reaction progress by monitoring UV absorption and therefore made possible its HPLC analysis and isolation (see the ESI†). Experiments on cell penetration[60] using non-cationic fluorescein labeled POSS **5** by measures of confocal microscopy are currently on the way.

Data of IR spectroscopy for click POSS derivatives showed along with a strong band associated with asymmetric stretching of a siloxane framework (1123 cm^{-1}) the disappearance of a characteristic absorption band corresponding to the azide group in the region of 2100 cm^{-1}. For the complete information refer to the ESI.†

Evidence for exhaustive ligation of all eight azide groups of octaazide **4** is provided by ^{1}H NMR spectroscopy (Table 3). Thus, the characteristic triplet of an N–CH_2 group was shifted downfield by ~1.25 ppm together with the emergence of a triazole singlet at >7.9 ppm, after 1,3-dipolar cycloaddition of terminally

Table 2 Data of high resolution ESI MS for synthesised compounds

Compound	Monoisotopic mass (calc.)	ESI-FTICR MS *m/z* (found)	ESI-FTICR MS *m/z* (calc.)
1	1031.88	1049.9145 $[M+NH_4]^+$	1049.9146 $[M+NH_4]^+$
2	1767.35	1785.4020 $[M+NH_4]^+$	1785.3996 $[M+NH4]^+$
3	1383.48	1403.5091 $[M+NH_4]^+$	1403.5085 $[M+NH_4]^+$
4	1088.20	1106.2369 $[M+NH_4]^+$	1106.2376 $[M+NH_4]^+$
5	1529.33	765,6700 $[M+2H]^{2+}$	765,6698 $[M+2H]^{2+}$
6	1904.58	953.2965 $[M+2H]^{2+}$	953.2970 $[M+2H]^{2+}$
7	2096.58	1049.2968 $[M+2H]^{2+}$	1049.2970 $[M+2H]^{2+}$
8	2936.92	979.9802 $[M+3H]^{3+}$	979.9806 $[M+3H]^{3+}$
9	9300.40	1163.6817 $[M+8H]^{8+}$, 1034.6096 $[M+9H]^{9+}$	1163.5574 $[M+8H]^{8+}$, 1034.3852 $[M+9H]^{9+}$



Scheme 2 Click conjugations of small molecules on octakis(3-azidopropyl)-POSS scaffold **4**.

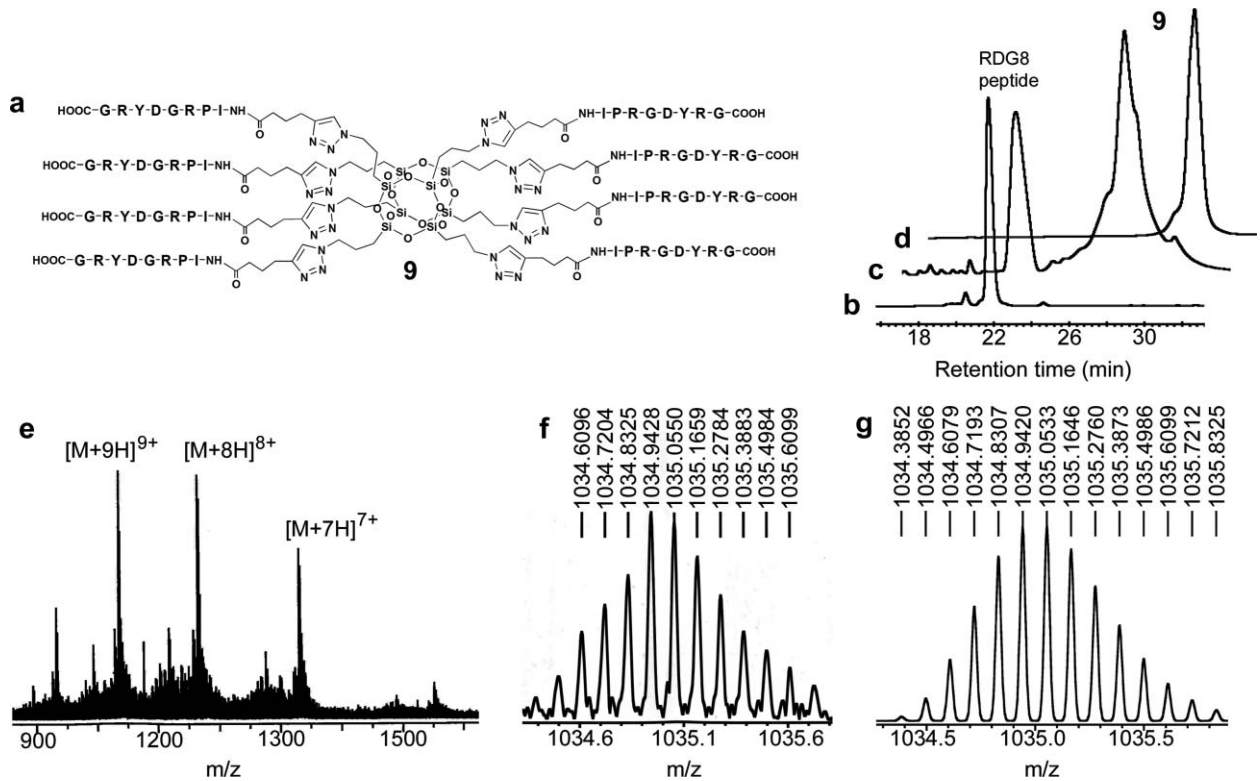


Fig. 3 Click conjugation of alkyne bearing RGD octapeptide. **a**, POSS-peptide octaconjugate; **b**, HPLC trace of the peptide ligand at 220 nm; **c**, HPLC trace of the click reaction mixture after 6 weeks (peptide ligand in excess) at 220 nm; **d**, HPLC trace of the octameric POSS-peptide conjugate **9** at 220 nm; **e**, ESI-MS analysis of the octameric POSS-peptide conjugate **9**; **f**, HR ESI-MS: isotopic pattern for ninefold charged quasimolecular ion $[M+9H]^{9+}$ of **9**; **g**, simulated isotopic pattern for $[C_{392}H_{617}N_{136}O_{116}Si_8]^{9+}$.

substituted alkyne had been accomplished. It should be noted, however, that in general proton spectroscopy cannot serve as evidence of the structural integrity of a POSS-cage, since its decomposition causes either none or only slight changes (*e.g.*, line broadening), keeping major coupling and shift patterns unaffected. Therefore, the structural integrity of the POSS cage was unambiguously confirmed by ^{29}Si NMR spectroscopy showing a sharp sole peak at -66 ± 1 ppm.

The synthesis of POSS-peptide conjugates has been reported for aminopropyl or hydroxypropyl substituted scaffolds and implies coupling of protected ligands (amino acids, di- or tripeptides) under standard activation conditions.[24,25] This method has serious limitations as stepwise coupling needs a deprotection step after each chain elongation, and convergent synthesis requires either fully protected peptides, or those without reactive groups in their side chains. Couplings are generally characterised by long

Table 3 Chemical shifts of selected POSS scaffolds and conjugates[a]

Compound	δ_H (δ_C), ppm											δ_{Si}
	1-CH_2		2-CH_2		–C≡CH		3-CH_2		Triazole		$C_{Ar\text{-}triazole}$	
3	0.75	(11.2)	1.90	(26.9)	—		3.36	(36.5)	—		—	–67.2
4	0.73	(9.0)	1.70	(22.5)	—		3.28	(53.4)	—		—	–66.5
6	0.64	(8.6)	2.02	(24.0)	—		4.35	(52.2)	7.90	(120.2)	147.7	–66.3
									—	(130.6)		
7[b]	0.61	(7.9)	1.89	(23.2)	4.22	(83.3)	4.32	(51.4)	8.54	(120.9)	145.5	–66.1
					—	(81.3)			—	(131.1)		

[a] NMR spectra were recorded in $CDCl_3$. [b] NMR spectra were recorded in DMSO-d_6.

reaction times ranging from days to weeks, and often incomplete substitutions are observed. In the present study, an integrin binding RGD peptide with a sequence IPRGDYRG[61] was taken as a model ligand for coupling onto scaffold **4**. This ligand was N-terminally modified with hexynoic acid and contained unprotected side chains of arginine, tyrosine and aspartic acid, as well as the C-terminal carboxy group. Click reaction was conducted in acetonitrile–DMF (3 : 1) at room temperature using copper wire as a catalyst. Though the reaction rate was slow, about 91% conversion into desired octamer was observed after 6 weeks. Reaction success was proved by high resolution ESI-MS (Fig. 3 and ESI†).

Conclusions

We have synthesised sterically defined frameworks for the introduction of molecules with the potential to interfere with biologic systems by copper catalysed click cycloaddition, which combine a highly symmetric siloxane inorganic core with functional azide or alkyne substituents, and demonstrated their utility in Sharpless-type couplings. Mild reaction conditions, though leading to extended reaction duration ensured the inalterability of the midmost siloxane cage for all octaconjugates of small molecular ligands.

For the first time conjugation of POSS with an RGD octapeptide bearing fully unprotected side chains was conducted leading to an eightfold presentation of a peptide ligand. It will be interesting to see to what extent the integrin binding activity of the RGD peptide is modulated upon oligomerisation on the scaffold. Detailed studies concerning conjugation of peptides of different size, polarity and biological activity using azide and alkyne bearing POSS frameworks are currently ongoing.

In conclusion, the constructs described here are promising templates for the assembly of various ligands including small molecules, sugars, peptides or proteins.

Experimental

1,3,5,7,9,11,13,15-Octakis(3-bromopropyl)pentacyclo-[9.5.1.1^{3,9}.1^{5,15}.1^{7,13}]octasiloxane (3)

$T_8[(CH_2)_3Cl]_8$[40,41] (5 g, 4.8 mmol) and anhydrous lithium bromide (25 g, 287 mmol, 60 equiv.) were dissolved in anhydrous acetone (200 mL) and the resulting mixture was heated under reflux for three days. Then, water (150 mL) and CH_2Cl_2 (300 mL) were added to the vigorously stirred reaction mixture. The organic layer was separated, washed with water (4 × 100 mL) and dried *in vacuo*. The resulting crude product was submitted to another four cycles of the procedure described above to ensure complete conversion. Finally, the pale-yellow crude material was dissolved in CH_2Cl_2 and purified by flash chromatography on Merck Silica gel 60 (0.04–0.063 mm) eluting with CH_2Cl_2, to afford 6.13 g (91.5%) of **3** as a white solid after removal of the solvent *in vacuo*.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.75 (t, $^3J_{H,H}$ = 8.1 Hz, 16H), 1.90 (mult., 16H), 3.36 (t, $^3J_{H,H}$ = 6.7 Hz, 16H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.20, 26.86, 36.46 ppm; ^{29}Si NMR (59.6 MHz, $CDCl_3$): δ–67.2 (s) ppm; IR (KBr) [cm^{-1}] ν 2958 m (C–H), 2853w, 1098vs (Si–O), 540w (O–Si–O); 475 m (Si–O). TG-MS: see the ESI.† Anal.: calc. for $C_{24}H_{48}Br_8O_{12}Si_8$ C, 20.70; H, 3.47; meas. C, 20.88; H, 3.448. HR-MS: calc. for $C_{24}H_{52}NBr_8O_{12}Si_8$ (+1): 1403.5085, meas. 1403.5091 $[M+NH_4]^+$.

1,3,5,7,9,11,13,15-Octakis(3-azidopropyl)pentacyclo-[9.5.1.1^{3,9}.1^{5,15}.1^{7,13}]octasiloxane (4)

Method A (analytic scale). POSS-bromide **3** (50 mg, 0.036 mmol) and NaN_3 (26 mg, 0.4 mmol, 11 equiv.) were dissolved in anhydrous DMF (1.5 mL). The mixture was stirred for 20 h at room temperature and concentrated *in vacuo* to a total volume of ~0.5 mL. Crude product, precipitated as a viscous oil after addition of H_2O (1 mL), was separated from supernatant solution by centrifugation and decantation, washed with water (3 × 1 mL), dissolved in CH_3CN (2 mL) and finally subjected to lyophilic drying to yield 35 mg (89%) of pure azide **4** as a colourless viscous oil.

Method B (preparative scale). POSS bromide **3** (1 g, 0.718 mmol) and NaN_3 (1 g, 15.3 mmol, 21.3 equiv., 2.7-fold excess) were dissolved in anhydrous DMF (20 mL). After stirring for 36 h at room temperature the mixture was diluted with ethylacetate (100 mL), washed with water (3 × 100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$. Solvent evaporation yielded **4** (700 mg, 89%) as a pale-yellow oil which solidified in a fridge.

^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 0.70–0.76 (m, 16H), 1.66–1.74 (m, 16H), 3.28 (t, $^3J_{H,H}$ = 6.8 Hz, 16H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.0, 22.5, 53.4 ppm; ^{29}Si NMR (59.6 MHz, $CDCl_3$): δ–66.5 (s); IR (neat) [cm^{-1}] ν 2938 m (C–H), 2876w, 2097vs (N_3), 1112vs (Si–O), 556w (O–Si–O); 486w (Si–O). TG-MS: see the ESI.† Anal.: calc. for $C_{24}H_{48}N_{24}O_{12}Si_8$ C, 26.46; H, 4.44; N, 30.86; meas. C, 26.01; H, 4.343; N, 29.05. HR-MS: calc. for $C_{24}H_{52}N_{25}O_{12}Si_8$ (+1): 1106.2376, meas. 1106.2369 $[M+NH_4]^+$

1-(3-(4-(4-(3′,6′-Dihydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9′-xanthene]-5-ylamino)-4-oxobutyl)-1*H*-1,2,3-triazol-1-yl)propyl), 3,5,7,9,11,13,15-heptakis(3-azidopropyl)penta-cyclo-[9.5.1.1^{3,9}.1^{5,15}.1^{7,13}]octasiloxane (5)

POSS azide **4** (5 mg, 0.0046 mmol, 8 equiv.) and fluorescein alkyne[62] (0.25 mg, 0.00057 mmol, 1 equiv.) were dissolved in 0.5 mL of anhydrous DMF and added to a HNO_3-preactivated Cu^0 turnings (~15 mg). The mixture was shaken at room temperature until full conversion of the starting material has been achieved (~3 weeks). Reaction progress was monitored at 220 and 485 nm by reverse phase HPLC on a Waters Symmetry 100 C8 column (150 × 3.9 mm, 5 μm) using a linear gradient of 90% aq. CH_3CN in 0.1% aq. TFA (20→100% in 30 min) at flow rate of 1 mL min^{-1}. t_R = 33.8 min.

HR-MS: calc. for $C_{50}H_{69}N_{25}O_{18}Si_8$ (+2): 765.6698, meas. 765.6700 $[M+2H]^{2+}$. t_R = 9.47 min (column: Shim-pack XR- C8, 50 × 2 mm, 2.2 μm, flow rate 0.2 mL min^{-1}, 40→100% CH_3CN).

1,3,5,7,9,11,13,15-Octakis[3-(1-phenyltriazol-4-yl)propyl]-pentacyclo-[9.5.1.1^{3,9}.1^{5,15}.1^{7,13}]octasiloxane (6)

Method A (analytic scale). Scaffold **4** (5 mg, 4.6 μmol) was dissolved in 500 μL of phenylacetylene and added to HNO_3-preactivated Cu^0 turnings (~15 mg). The mixture was then shaken at room temperature for 72 h. Formed precipitate was isolated by centrifugation, washed with CH_3CN (4 × 1 mL), and resubjected to the above procedure using fresh catalyst. Subsequent work-up followed by lyophilic drying, afforded 2.1 mg (23%) of pure **6** as a pale-yellow solid.

Method B (preparative scale). To a rapidly stirred solution of **4** (200 mg, 0.184 mmol) and phenylacetylene (450 mg, 4.41 mmol, 24 equiv.) in 10 mL CH_2Cl_2, 4 mL H_2O, 2 mL 1M aq. $CuSO_4$ and 4 mL 1M aq. sodium ascorbate were added. After 36 h the reaction mixture was diluted with 50 mL CH_2Cl_2 and 50 mL H_2O. The organic layer was washed with saturated aq. NH_4Cl (100 mL), filtered through a celite pad, dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by DCVC[63] (0→100% acetone in toluene) yielding 300 mg (86%) of **6** as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ 0.62–0.65 (m, 16H), 1.99–2.05 (m, 16H), 4.35 (t, $^3J_{H,H}$ = 6.9 Hz, 16H), 7.31 (tt, $^3J_{H,H}$ = 7.4 Hz, $^4J_{H,H}$ = 1.2 Hz, 8H, H-Ph$_{para}$), 7.35–7.39 (m, 16H, H-Ph$_{meta}$), 7.81–7.83 (m, 16H, H-Ph$_{ortho}$), 7.90 (s, 8H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 8.6 (8CH_2), 24.0 (8CH_2), 52.2 (8CH_2), 120.2 (8CH, triazole), 125.6 (16CH, Ph$_{ortho}$), 128.1 (8CH, Ph$_{para}$), 128.9 (16CH, Ph$_{meta}$), 130.6 (8C_{quat}, triazole), 147.7 (8C_{quat}, Ph) ppm; ^{29}Si NMR (59.6 MHz, $CDCl_3$): δ −67.3 (s); IR (KBr) [cm^{-1}] ν 3088 m (C–H, aromatic), 2948 m (C–H, alkyl), 1123vs (Si–O), 766 s (C–H, triazole), 490w (Si–O). TG-MS: see the ESI.† HR-MS: calc. for $C_{88}H_{98}N_{24}O_{12}Si_8$ (+2): 953.2970, meas. 953.2965 $[M+2H]^{2+}$. t_R = 9.25 min (column: Shim-pack XR- C8, 50 × 2 mm, 2.2 μm, flow rate 0.2 mL min^{-1}, 40→100% CH_3CN).

1,3,5,7,9,11,13,15-Octakis{3-[1-(4-ethynylphenyl)triazol-4-yl]propyl}pentacyclo-[9.5.1.1^{3,9}.1^{5,15}.1^{7,13}]octasiloxane (7)

To a reaction flask containing **4** (7.5 mg, 6.9 μmol) and HNO_3-preactivated Cu^0-turnings (~15 mg) 600 μL of saturated at 50 °C solution of 1,4-diethynylbenzene in acetonitrile was added. Then the reaction mixture was shaken for 96 h at room temperature. After the dilution with 0.5 mL CH_3CN a brown-yellow precipitate was isolated by centrifugation and the entire cycle was repeated. After washing of the solid (6 times with 1.5 mL CH_3CN), 14.9 mg precipitate were isolated as an intercalation product with diethynyl benzene.

^{1}H NMR (500 MHz, DMSO-d_6): δ = 0.59–0.62 (m, 16H), 1.86–1.92 (m, 16H), 4.22 (s, 8H, –C≡C*H*); 4.32 (t, $^3J_{H,H}$ = 6.8 Hz, 16H), 7.47–7.50 (m, 16H), 7.76–7.79 (m, 2H), 8.54 (s, 8H, triazole-C*H*) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 7.9 (8CH_2), 23.2 (8CH_2), 51.4 (8CH_2), 83.3 (8CH, –C≡*C*H), 81.3 (8C_{quat}, –C≡CH), 120.9 (8CH, triazole), 121.8 (8C_{quat}, Ar–C≡CH), 125.2 (16CH, Ar), 131.1 (8C_{quat}, triazole), 132.2 (16CH, Ar), 145.5 (8C_{quat}, Ar-triazole) ppm; ^{29}Si NMR (59.6 MHz, DMSO-d_6): δ = 66.1 (s) ppm; IR (KBr) [cm^{-1}] ν 3268 s (C–H, alkyne), 2925 m (C–H, alkyl), 2105w (C≡C), 1102vs (Si–O), 829 s (C–H, triazole). HR-MS: calc. for $C_{104}H_{98}N_{24}O_{12}Si_8$ (+2): 1049.2970, meas. 1049.2968 $[M+2H]^{2+}$.

1,3,5,7,9,11,13,15-Octakis {3-[4-(4-(4-carboxybutanamido)phenyl)-1*H*-1,2,3-triazol-1-yl]propyl} pentacyclo-[9.5.1.1^{3,9}.1^{5,15}.1^{7,13}]octasiloxane (8)

To a reaction flask containing **4** (5 mg, 4.6 μmol) and HNO_3-preactivated Cu^0-turnings (~15 mg) 500 μL of saturated at 50 °C solution of 5-(4-ethynylphenylamino)-5-oxopentanoic acid in acetonitrile was added. Then the reaction mixture was shaken for 96 h at room temperature. After the dilution with 0.5 mL CH_3CN a pale-green precipitate was isolated by centrifugation, redissolved in water–acetonitrile mixture and subjected to reverse phase HPLC on a Waters Symmetry 100 C8 column (150 × 3.9 mm, 5 μm) using a linear gradient of 90% aq. CH_3CN in 0.1% aq. TFA (20→100% in 35 min) at flow rate of 1 mL min^{-1}. HPLC analysis of crude reaction product has shown that 55.2% of the starting material were converted into an octamer **8**. t_R = 14.97 min

HR-MS: calc. for $C_{128}H_{155}N_{32}O_{36}Si_8$ (+3): 979.9806, meas. 979.9802 $[M+3H]^{3+}$. t_R = 6.51 min (column: Shim-pack XR- C8, 50 × 2 mm, 2.2 μm, flow rate 0.2 mL min^{-1}, 40→100% CH_3CN).

T_8-(IPRGDYRG)$_8$ (9)

To a reaction flask containing **4** (1.6 mg, 1.5 μmol, 1 equiv.) and HNO_3-preactivated Cu^0-turnings (~15 mg) 1 mL CH_3CN and 300 μL DMF, alkyne derivatised RDG8 peptide (25 mg, 23.95 μmol, 16 equiv.) was added. Then the reaction mixture was shaken at room temperature, and reaction progress was monitored by RP HPLC. After 6 weeks 91.3% conversion into the desired product was achieved, and the product was isolated by HPLC using Waters Symmetry 100 C8 column (150 × 3.9 mm, 5 μm) and linear gradient of 90% aq. CH_3CN in 0.1% aq. TFA (10→35% in 30 min) at a flow rate of 1 mL min^{-1}. t_R = 27.6 min.

HR-MS: calc. for $C_{392}H_{616}N_{136}O_{116}Si_8$ (+8): 1163.5574, meas. 1163.6817 $[M+8H]^{8+}$, calc. for $C_{392}H_{617}N_{136}O_{116}Si_8$ (+9): 1034.3852, meas. 1034.6096 $[M+9H]^{9+}$.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft through grant KO 1390/9-1 and by BMBF.

The visualization of the molecular images was conducted with QuteMol.[64]

References

1 G. Li, L. Wang, H. Ni and C. U. Pittman Jr, *J. Inorg. Organomet. Polym.*, 2001, **11**, 123–154.
2 R. H. Baney, M. Itoh, A. Sakakibara and T. Suzuki, *Chem. Rev.*, 1995, **95**, 1409–1430.
3 A. Provatas and J. G. Matisons, *Trends Polym. Sci.*, 1997, **5**, 327–332.
4 C. Zhang and R. M. Laine, *J. Am. Chem. Soc.*, 2000, **122**, 6979–6988.
5 P. P. Pescarmona and T. Maschmeyer, *Aust. J. Chem.*, 2001, **54**, 583–596.
6 F. J. Feher and T. A. Budzichowski, *Polyhedron*, 1995, **14**, 3239–3253.
7 M. G. Voronkov and V. I. Lavrent'yev, *Top. Curr. Chem.*, 1982, **102**, 199–236.
8 J. D. Lichtenhan, *Comments Inorg. Chem.*, 1995, **17**, 115–130.
9 J. D. Lichtenhan, N. Q. Vu, J. A. Carter, J. W. Gilman and F. J. Feher, *Macromolecules*, 1993, **26**, 2141–2142.
10 T. S. Haddad, H. W. Oviatt, J. J. Schwab, P. T. Mather, K. P. Chaffee and J. D. Lichtenhan, *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)*, 1998, **39**, 611–612.
11 J. D. Lichtenhan, Y. A. Otonari and M. J. Carr, *Macromolecules*, 1995, **28**, 8435–8437.
12 J. W. Gilman, D. S. Schlitzer and J. D. Lichtenhan, *J. Appl. Polym. Sci.*, 1996, **60**, 591–596.
13 *Polymeric Materials Encyclopedia*, ed. J. D. Lichtenhan, CRC Press, New York, 1996.
14 F. J. Feher and K. J. Weller, *Organometallics*, 1990, **9**, 2638–2640.
15 K. Naka, M. Sato and Y. Chujo, *Langmuir*, 2008, **24**, 2719–2726.
16 B. Hong, T. P. S. Thoms, H. J. Murfee and M. J. Lebrun, *Inorg. Chem.*, 1997, **36**, 6146–6147.
17 M. F. Roll, M. Z. Asuncion, J. Kampf and R. M. Laine, *ACS Nano*, 2008, **2**, 320–326.
18 N. R. Vautravers, P. André and D. J. Cole-Hamilton, *Dalton Trans.*, 2009, 3413–3424.
19 N. R. Vautravers, P. André, A. M. Z. Slawin and D. J. Cole-Hamilton, *Org. Biomol. Chem.*, 2009, **7**, 717–724.
20 G. Cheng, N. R. Vautravers, R. E. Morris and D. J. Cole-Hamilton, *Org. Biomol. Chem.*, 2008, **6**, 4662–4667.
21 H. Mori, Y. Miyamura and T. Endo, *Langmuir*, 2007, **23**, 9014–9023.
22 F. J. Feher and K. J. Weller, *Inorg. Chem.*, 1991, **30**, 880–882.
23 I. Lacatusu, R. Nita, N. Badea, D. Bojin and A. Meghea, *Mater. Res. Innovations*, 2009, **13**, 330–333.
24 F. J. Feher, K. D. Wyndham, M. A. Scialdone and Y. Hamuro, *Chem. Commun.*, 1998, 1469–1470.
25 T. L. Kaneshiro, X. Wang and Z.-R. Lu, *Mol. Pharmaceutics*, 2007, **4**, 759–768.
26 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
27 A. J. Dirks, S. S. van Berkel, N. S. Hatzakis, J. A. Opsteen, F. L. van Delft, J. J. L. M. Cornelissen, A. E. Rowan, J. C. M. van Hest, F. P. J. T. Rutjes and R. J. M. Nolte, *Chem. Commun.*, 2005, 4172–4174.
28 J.-F. Lutz, *Angew. Chem., Int. Ed.*, 2007, **46**, 1018–1025.
29 W. H. Binder and R. Sachsenhofer, *Macromol. Rapid Commun.*, 2007, **28**, 15–54.
30 R. A. Evans, *Aust. J. Chem.*, 2007, **60**, 384–395.
31 O. Avrutina, M. Empting, S. Fabritz, M. Daneschdar, H. Frauendorf, U. Diederichsen and H. Kolmar, *Org. Biomol. Chem.*, 2009, **7**, 4177–4185.
32 C. W. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
33 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
34 D. T. S. Rijkers, G. W. van Esse, R. Merkx, A. J. Brouwer, H. J. F. Jacobs, R. J. Pieters and R. M. J. Liskamp, *Chem. Commun.*, 2005, 4581–4583.
35 B. Jagadish, R. Sankaranarayanan, L. Xu, R. Richards, J. Vagner, V. J. Hruby, R. J. Gillies and E. A. Mash, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 3310–3313.
36 L. Petraru and W. H. Binder, *Polym. Prepr.*, 2005, **46**, 841–842.
37 V. Ervithayasupron, X. Wang and Y. Kawakami, *Chem. Commun.*, 2009, 5130–5132.
38 M. Ak, B. Gacal, B. Kiskan, Y. Yagci and L. Toppare, *Polymer*, 2008, **49**, 2202–2210.
39 Z. Ge, L. Wang, Y. Zhou, H. Liu and S. Liu, *Macromolecules*, 2009, **42**, 2903–2910.
40 U. Dittmar, B. J. Hendan, U. Flörke and H. C. Marsmann, *J. Organomet. Chem.*, 1995, **489**, 185–194.
41 S. Lücke, K. K. Stoppek-Langner, B. Krebs and M. Läge, *Z. Anorg. Allg. Chem.*, 1997, **623**, 1243–1246.
42 S. Bräse, C. Gil, K. Knepper and V. Zimmermann, *Angew. Chem.*, 2005, **117**, 5320–5374.
43 F. J. Feher, K. D. Wyndham, D. Soulivong and F. Nguyen, *J. Chem. Soc., Dalton Trans.*, 1999, 1491–1497.
44 E. Rikowski and H. C. Marsmann, *Polyhedron*, 1997, **16**, 3357–3361.
45 F. J. Feher and T. A. Budzichowski, *J. Organomet. Chem.*, 1989, **379**, 33–40.
46 D. Heyl, E. Rikowski, R. C. Hoffmann, J. J. Schneider and W.-D. Fessner, *Chem. Eur. J.*, 2010, DOI: 10.1002/chem.201000488.
47 A. J. Papa, *J. Org. Chem.*, 1966, **31**, 1426–1430.
48 C. Li, A. Arasappan and P. L. Fuchs, *Tetrahedron Lett.*, 1993, **34**, 3535–3538.
49 C. Li, T. Shih, J. U. Jeong, A. Arasappan and P. L. Fuchs, *Tetrahedron Lett.*, 1994, **35**, 2645–2646.
50 R. E. Conrow and W. D. Dean, *Org. Process Res. Dev.*, 2008, **12**, 1285–1286.
51 E. S. Park, H. W. Ro, C. V. Nguyen, R. L. Jaffe and D. Y. Yoon, *Chem. Mater.*, 2008, **20**, 1548–1554.
52 O. Cozar, L. David, V. Chis, G. Damian, M. Todica and C. Agut, *J. Mol. Struct.*, 2001, **563–564**, 371–375.
53 E. Lieber, C. N. R. Rao, T. S. Chao and C. W. W. Hoffman, *Anal. Chem.*, 1957, **29**, 916–918.
54 S. Bräse, C. Gil, K. Knepper and V. Zimmermann, *Angew. Chem., Int. Ed.*, 2005, **44**, 5188–5240.
55 M. Peer, *Spec. Chem.*, 1998, **18**, 256–263.
56 C. Bolln, A. Tsuchida, H. Frey and R. Mülhaupt, *Chem. Mater.*, 1997, **9**, 1475–1479.
57 A. Fina, D. Tabuani, F. Carniato, A. Frache, E. Boccaleri and G. Camino, *Thermochim. Acta*, 2006, **440**, 36–42.
58 Z. Zhang, G. Liang and T. Lu, *J. Appl. Polym. Sci.*, 2007, **103**, 2608–2614.
59 F. J. Feher and K. D. Wyndham, *Chem. Commun.*, 1998, 323–324.
60 C. McCusker, J. B. Carroll and V. M. Rotello, *Chem. Commun.*, 2005, 996–998.
61 S. Reiss, M. Sieber, V. Oberle, A. Wentzel, P. Spangenberg, R. Claus, H. Kolmar and W. Loesche, *Platelets*, 2006, **17**, 153–157.
62 Synthesis of *N*-(3′,6′-dihydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9′-xanthene]-5-yl)hex-5-ynamide is given in the ESI†.
63 D. S. Pedersen and C. Rosenbohm, *Synthesis*, 2001, 2431–2434.
64 M. Tarini, P. Cignoni and C. Montani, *IEEE Transactions on Visualization and Computer Graphics*, 2006, **12**, 1237–1244.

