# Octafunctionalized polyhedral oligosilsesquioxanes as scaffolds: synthesis of peptidyl silsesquioxanes‡

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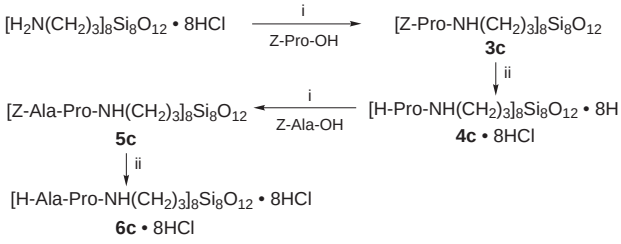
**The first use of polyhedral silsesquioxanes to organize ensembles of biologically relevant motifs is described. N-Protected amino acids and peptides can be attached to $[H_2N(CH_2)_3]_8Si_8O_{12}$ 1 and $[p\text{-}HOCH_2C_6H_4]_8Si_8O_{12}$ 2 in either a convergent fashion or a divergent fashion to produce peptidyl silsesquioxanes in excellent yield and purity.**

Symmetric core scaffolds possessing multiple reactive functional groups have recently attracted much interest as templates for the presentation of molecular domains of biological relevance. For example, symmetrical tetrafunctional xanthene and cubane have been used as scaffolds to create combinatorial libraries of trypsin inhibitors with moderate activities,[1–3] while porphyrins,[4,5] polyfunctional cyclic peptides[6] and cyclotriphosphazenes[7] have been used as scaffolds for helical bundles of peptides in models for ion channels. Similarly, calixarenes have been used as templates to assist the spatial arrangement of cyclic peptides[8] and sugars.[9] In principle, any polyfunctional molecule may serve as a scaffold to present multiple copies of a biologically relevant pendant group, but the most attractive cores are those which have particular geometrical parameters (*e.g.* size, shape, symmetry) to allow unique ligand presentation. Here, we describe the first use of polyhedral oligosilsesquioxanes as scaffolds for peptides. These robust, readily available Si/O frameworks offer interesting possibilities as cores for ensembles of biologically relevant pendant groups.

A wide range of polyhedral oligosilsesquioxanes can be prepared *via* hydrolytic condensation reactions of trifunctional organosilicon monomers.[10] Two known frameworks that possess terminal protic nucleophilic functionality are octaamine **1** and octaalcohol **2**. Octaamine **1** is readily available as a hydrochloride salt in one step (35% yield) from $H_2N(CH_2)_3Si(OEt)_3$.[11,12] Octaalcohol **2** is prepared in approximately 5% overall yield in three steps from $p\text{-}ClCH_2C_6H_4SiCl_3$.[13]

Octaamine **1** undergoes octafunctionalization with N-protected amino acids and N-protected di- and tri-peptides under standard coupling conditions.[14–16] As outlined in Scheme 1 and Table 1, high yields of coupled products can be obtained by reacting **1** with an excess of an N-protected amino acid (*e.g.* Z-Pro-OH) and *O*-(7-benzotriazol-l-yl)-1,1,3,3-tetramethyluronium (TBTU) in DMF–*N*,*N*′-diisopropylethylamine (DIPEA), and then precipitating the product with aqueous acid.§ The course of these reactions can be conveniently monitored by $^1$H NMR spectroscopy because the chemical shift for the $CH_2N$ of **1** shifts from $\delta$ 2.8 to 3.0 [$(CD_3)_2SO$] upon condensation with the amino acid. The only Si-containing species detectable by $^1$H, $^{13}$C and $^{29}$Si NMR spectroscopy in the crude materials are the desired octafunctional derivatives and they are spectroscopically pure. In most cases, the $^1$H and $^{13}$C NMR spectra of peptidyl silsesquioxane are well defined and fully assignable on the basis of COSY, HMQC and DEPT experiments.




**Scheme 1** *Reagents and conditions*: i, protected amino acid (1.2–4 equiv. per $NH_2$), TBTU (2–4 equiv. per $NH_2$), HOBt + $H_2O$ (4 equiv. per $NH_2$) and DIPEA (9 equiv. per $NH_2$) in DMF, 10–24 h, 25 °C; ii, $H_2$ (100 psi), 10% Pd/C, 1 M HCl–MeOH, 8 h, 25 °C

The syntheses of **3d** and **3e** *via* coupling reactions of **1** with N-protected peptides represent convergent syntheses of peptidyl silsesquioxanes, which are attractive when the desired peptide sequence can be prepared in advance. It is also possible to synthesize peptidyl silsesquioxanes in a divergent fashion by iteratively coupling and deprotecting Z-protected amino acids. For example, removal of the Z-group from **3c** *via* hydrogenolysis over 10% Pd/C in aqueous acidic methanol (100 psi, 25 °C, 8 h)¶ gives **4c**·8HCl in high yield, which can be subsequently coupled with Z-Ala-OH and deprotected to give dipeptidyl silsesquioxane **6c**·8HCl (Scheme 1).

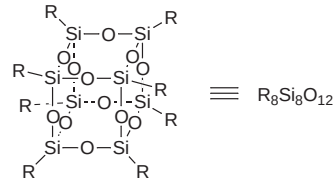
Octaalcohol **2** is also a useful scaffold for peptidyl silsesquioxanes, which may exhibit different properties because of its rigid *para*-phenylene spacer. The synthesis of peptidyl silsesquioxanes derived from **2** is analogous to the preparation of peptidyl silsesquioxanes derived from **1** (Fig. 1 and Table 2). However, the poorer nucleophilicity of the benzylic hydroxyl group requires a much longer reaction time and a generous excess of N-protected amino acid and TBTU for complete coupling to occur. For example, the reaction of **2** with 2 equiv. of Fmoc-Ala-OH and TBTU per OH for 17 h in DMF–DIPEA produces **7c** in only 26% yield. The major product (40%) is the incompletely-substituted product derived from coupling of **2** with seven Fmoc-Ala-OH; small amounts (6%) of incom-

**Table 1** Preparation of peptidyl silsesquioxanes derived from **1**

$[R^1NH(CH_2)_3]_8Si_8O_{12} \rightarrow [R^2NH(CH_2)_3]_8Si_8O_{12}$

| Starting material | $R^1$ | Product | $R^2$ | Isolated yield (%) |
|---|---|---|---|---|
| **1**·8HCl | H | **3a** | Z-Gly | 91 |
| **1**·8HCl | H | **3b** | Z-Ala | 98 |
| **1**·8HCl | H | **3c** | Z-Pro | 44 |
| **1**·8HCl | H | **3d** | Z-Phe-Leu | 94 |
| **1**·8HCl | H | **3e** | Z-Phe-Leu-Ala | 73 |
| **3c** | Z-Pro | **4c**·8HCl | H-Pro | 89 |
| **3d** | Z-Phe-Leu | **4d**·8HCl | H-Phe-Leu | 87 |
| **4c**·8HCl | H-Pro | **5c** | Z-Ala-Pro | 100 |
| **4d**·8HCl | H-Phe-Leu | **5d** | Z-Ala-Phe-Leu | 92 |
| **5c** | Z-Ala-Pro | **6c**·8HCl | H-Ala-Pro | 89 |

See Scheme 1 for reaction conditions. $R^1$ and $R^2$ refer to neutral organic substituents on N. Where indicated, starting materials and products were used or isolated as salts containing 8HCl.




**1** R = $CH_2CH_2CH_2NH_2$
**2** *p*-$C_6H_4CH_2OH$

**Fig. 1**

**Table 2** Preparation of peptidyl silsesquioxanes derived from **2**

[*p*-$R^1OCH_2C_6H_4]_8Si_8O_{12}$ → [*p*-$R^2OCH_2C_6H_4]_8Si_8O_{12}$

| Starting material | $R^1$ | Product | $R^2$ | Isolated yield (%) |
|---|---|---|---|---|
| **2** | H | **7a** | Fmoc-Ala | 26 |
| **2** | H | **7b** | Fmoc-Phe | 28 |
| **2** | H | **7c** | Boc-Ala | 72 |
| **7c** | Boc-Ala | **8c**·8TFA | H-Ala | 100 |
| **8c**·8TFA | H-Ala | **9c** | Boc-Phe-Ala | 46 |

See Scheme 1 for reaction conditions. $R^1$ and $R^2$ refer to neutral organic substituents on O. Where indicated, starting materials and products were used or isolated as salts containing 8TFA (*i.e.* $CF_3CO_2H$).

pletely-substituted products containing six Fmoc-Ala groups are also isolated. These compounds can be readily separated by flash chromatography on $SiO_2$ (20 : 1 $CH_2Cl_2$–MeOH) and fully characterized by multinuclear NMR spectroscopy and MALDI-TOF mass spectrometry. Complete functionalization of **2** by Boc-Ala-OH was accomplished by using 4 equiv. of the protected amino acid (per $CH_2OH$), 4 equiv. of TBTU and a reaction time of 7 days. Removal of Boc protecting groups can be efficiently accomplished with TFA in $CH_2Cl_2$, but the cleavage of Fmoc protecting groups with piperidine (20% in THF, 25 °C) does not occur cleanly, presumably because of base-induced decomposition of the $Si_8O_{12}$ framework.[11]

In summary, we have demonstrated the first use of polyhedral silsesquioxanes to organize ensembles of biologically relevant motifs. Peptides can be attached to octaamine **1** and octaalcohol **2** in either a convergent fashion or a divergent fashion to produce peptidyl silsesquioxanes in excellent yields and purity. In the case of octaalcohol **2**, coupling reactions are more difficult to complete due to the poor nucleophilicity of hydroxy groups, but the octasubstituted compound can be easily separated from less extensively substituted derivatives by column chromatography. These large frameworks can be synthetically manipulated by standard solution methods and characterized by NMR spectroscopy and mass spectroscopy (MALDI-TOF). We have only begun to explore the use of silsesquioxanes as scaffolds for biologically relevant pendant groups, but the ready availability of discrete polyhedral frameworks containing 6–16 silicon atoms offers many interesting possibilities in areas of molecular recognition, biomimetics and drug design. Our work in these and other areas will be reported separately.[17,18]

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## Notes and References

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§ Peptidyl silsesquioxane **3c** was prepared by adding DIPEA (0.8 ml, 46 mmol) to a solution of **1**·8HCl (0.074 g, 0.063 mmol), CBZ-Pro-OH (0.501 g, 2.02 mmol), TBTU (642 mg, 2.00 mmol), and 1-hydroxybenzotriazole hydrate (306 mg, 2.00 mmol) in DMF (3 ml). After stirring for 1 day, the crude product was precipitated by dropwise addition of the reaction mixture to ice-cold 0.2 M aqueous citric acid (200 ml). Filtration, extraction with methanol, precipitation with a 0.5 M $NaHCO_3$, washing and drying *in vacuo* (25 °C, 0.01 Torr) afforded **3c** in 44% yield (76 mg). For **3c**, which possesses proline pendants with two rotameric forms (a : b 1.3 : 1): $^1$H NMR [500.0 MHz, $(CD_3)_2SO$, 25 °C] $\delta$ 7.98 (t, NHCO, rotamer a), 7.90 (t, NHCO, rotamer b), 7.35–7.22 (m, $C_6H_5$), 5.10–4.98 (m, $CH_2C_6H_5$), 4.13 (m, COCH), 3.41 [s, $CH_2(N)CH_2$], 3.35 [s, $CH_2(N)CH_2$], 2.98 (br, $SiCH_2CH_2CH_2$), 2.05 [d, $COCH(N)CH_2$], 1.73 (br, $COCHCH_2CH_2$), 1.42 (br, $SiCH_2CH_2$), 0.53 (br, $SiCH_2$); $^{13}$C{$^1$H} [125.7 MHz, $(CD_3)_2SO$, 25 °C] $\delta$ 172.06, 153.78, 65.70, 59.63, 47.10, 31.29, 23.07 (rotamer a), 171.75, 154.05, 65.87, 60.12, 46.49, 30.23, 23.95 (rotamer b), 136.95, 128.36, 128.15, 127.75, 127.50, 127.46, 127.00, 41.01, 22.43, 8.75; $^{29}$Si NMR [99.3 MHz, $(CD_3)_2SO$, 25 °C) $\delta$ −66.2 (s); Elemental analysis for $C_{128}H_{168}O_{36}Si_8N_{16}$. Found (calc.); C, 56.22 (56.28), H, 6.14 (6.20), N, 8.08 (8.20).

¶ Hydrogenolysis of **3c** (250 mg, 0.090 mmol) was performed by using 10% Pd/C (50 mg) in a mixture of methanol (25 ml) and 1 M HCl (10 ml) for 24 h at 25 °C and 100 psig $H_2$. Filtration and evaporation of the solvent *in vacuo* (25 °C, 0.01 Torr) gave **4c**·8HCl in quantitative yield. For **4c**·8HCl: $^1$H NMR (500.2 MHz, $CD_3OD$, 25 °C) $\delta$ 9.46, 8.53 (s, NH), 4.26 [s, $CH(N)CH_2$], 3.37 [m, $CH_2N(CH)$], 3.30 [m, $CH_2N(CH)$], 3.18 (br, $SiCH_2CH_2CH_2$), 2.43 (br, $COCHCH_2CH_2$), 2.00 (br, $COCHCH_2CH_2$); $^{13}$C{$^1$H} (125.8 MHz, $CD_3OD$, 25 °C) $\delta$169.32 (CO), 61.04 (COCH), 47.48 ($CHCH_2CH_2CH_2N$), 42.90 ($SiCH_2CH_2$), 31.26, 25.14 ($CH_2$), 23.64 ($SiCH_2CH_2$), 97.6 ($SiCH_2$). $^{29}$Si NMR (99.4 MHz, $CD_3OD$, 25 °C) $\delta$ −66.5 (s); MS (MALDI). Calc. for $C_{64}H_{121}N_{16}O_{20}Si_8$ ($M + H^+$) *m/z* 1658.7, found 1658.7.

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