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PAPER

Silica precipitation with synthetic silaffin peptides†

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Silaffins are highly charged proteins which are one of the major contributing compounds that are thought to be responsible for the formation of the hierarchically structured silica-based cell walls of diatoms. Here we describe the synthesis of an oligo-propyleneamine substituted lysine derivative and its incorporation into the KXXK peptide motif occurring repeatedly in silaffins. N^{ε}-alkylation of lysine was achieved by a Mitsunobu reaction to obtain a protected lysine derivative which is convenient for solid phase peptide synthesis. Quantitative silica precipitation experiments together with structural information about the precipitated silica structures gained by scanning electron microscopy revealed a dependence of the amount and form of the silica precipitates on the peptide structure.

Introduction

Diatoms are unicellular eukaryotic organisms whose silica shells belong to the most outstanding nanostructures found in nature.[1] The two valves of their silica shell are precisely reproduced in every cell cycle. How the formation of highly symmetrical shell shapes is controlled on the nano- and micrometre scale is a fascinating question for chemists, biochemists, and materials scientists. Nanofabrication of silica in diatoms results from specific interactions between silicic acid and long-chain polyamines[2] or polar proteins, so-called silaffins which promote the formation of SiO_2 from silicic acid.[3–5] Silaffins are characterized by a large number of phosphorylated serine residues and post-translationally modified lysines which carry oligo-propyleneamine functionalized side chains.[6] Both modifications increase the tendency of silaffins to form large aggregates which guide the nanopattering of silica matrices at ambient temperature and pH.[7,8] The protein self-assembly is driven by ionic interactions and is a major prerequisite for the silica precipitation *in vivo*. Although any amine induces silica precipitation *in vitro*, nature employs complex gene products which allow for the fine tuning of charges and countercharges in the peptide side chains. We observed a structural dependence for synthetic polyamines[9,10] and expected therefore the local amino acid composition to also have a distinguishable influence on the precipitation process. The systematic variation of peptide composition can give a deeper insight into the secrets of hierarchical silica precipitation in diatoms. Chemical synthesis circumvents the microheterogeneity of natural silaffins and the solid-phase protocol presented here allows for the straightforward assembly of systematically diversified polycationic peptides. The biosilification studies identify a notable influence of the amino acid composition on the nanostructure of the precipitated silica.

Results and discussion

In 2004, the entire genome of the diatom *Thalassiosira pseudonana* was sequenced.[11] Silaffins and the recently described cingulins from *T. pseudonana* contain more than 18% phosphorylated serine residues and above 10% lysine residues.[12] Lysines are either post-translationally dimethylated at the ε-nitrogen or even more frequently these are extended with oligo-propyleneamine repeats. Nearly all lysines (K) are assembled in KXXK tetrapeptide domains. The 105-mer peptide Silaffin 3 (Sil-3) has mainly A, G, S, or E at either position X, whereas Q and H occur only once in the altogether 15 domains (Fig. 1a).[13]

5 out of the 15 domains of Sil-3 bear the ω-dimethylated dipropyleneamino group at the N-terminal position and ε-dimethyllysine at the C-terminal position. In the title compounds of this work (**1–3**, Fig. 1b), these two lysine derivatives flank three dipeptides of increasing polarity. The systematic variation of the isolated KXXK domains shall identify the sequence specificity of post-translationally modified lysine residues on silica precipitation.

Synthesis started with the reductive amination of the hydrochloride of α-Fmoc-protected lysine **4** (Scheme 1) with formaldehyde solution and $NaBH_4$ in dioxane yielding ε,ε-dimethyllysine **5** in 88% yield after simple extraction with $CHCl_3$. The pH was adjusted between 3 and 6 with acetic acid to avoid cleavage of the Fmoc group. The synthesis of ω,ω-dimethyl-dipropyleneamino-lysine starts from completely protected lysine derivative **6**. The α-*N*-protecting group Dde [Dde = 4,4-dimethyl-2,6-dioxo-cyclohexylidene)-ethylamino-] is stable under basic conditions

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† Electronic supplementary information (ESI) available: Scanned copies of the ^{1}H and ^{13}C NMR spectra. See DOI: 10.1039/c1ob05406f

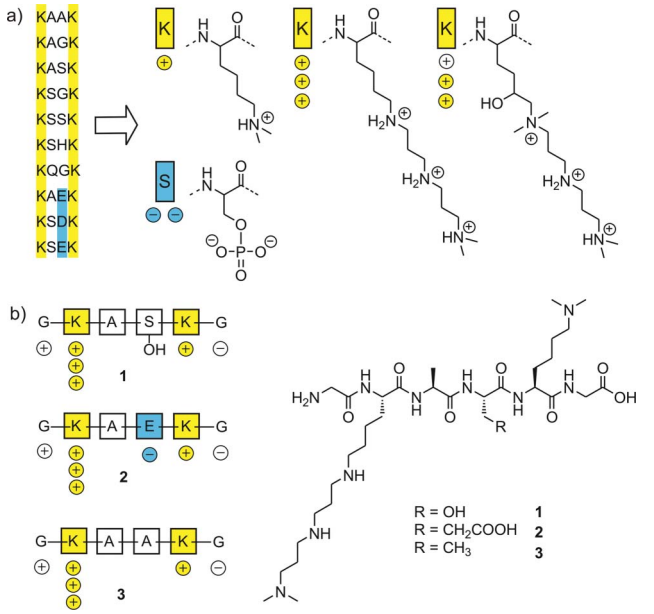
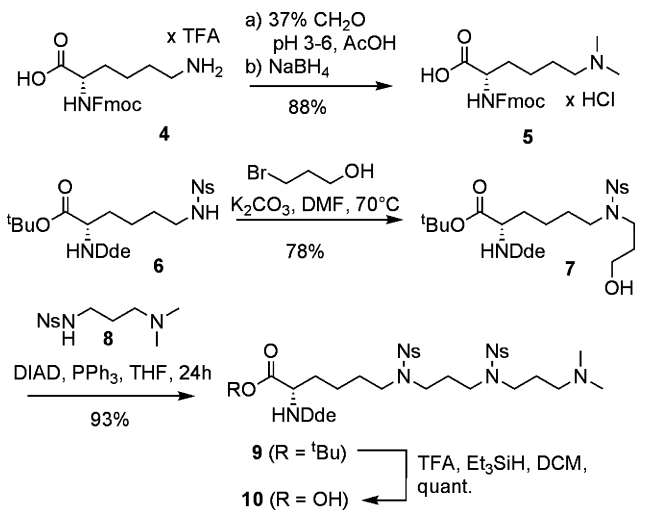


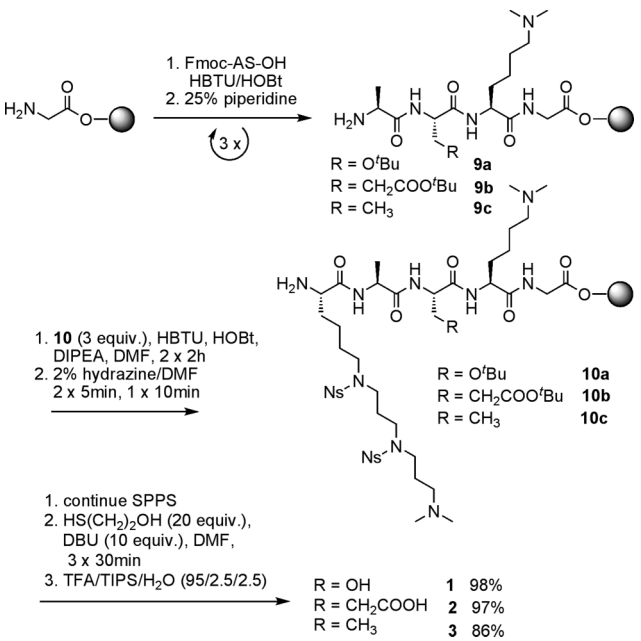
Fig. 1 a) The 10 different KXXK domains as observed in the silaffin polypeptide Sil-3 with increasing polarity (from top to bottom). The side chains of the amino acids are shown on the right with maximum charges. Serine is uncharged while phosphoserine can even bear two negative charges. KAEK occurs twice and KASK even three times in Sil-3. The three post-translationally modified lysine species as observed in the silaffins together with serine phosphate are shown on the right. b) Charge pattern of the three GKAXKG hexapeptides **1**, **2**, and **3** which contain the KXXK motif as derived from silaffin polypeptides and which were synthesized to study their silica precipitation properties.



Scheme 1 Synthesis of orthogonally protected lysine derivatives **5** and **10** for peptide synthesis.

and the carboxylate was protected as *tert*-butylester in order to avoid side reactions during the Mitsunobu reaction.[14] The first chain elongation was done according to a protocol invented by Fukuyama and co-workers.[15,16] *N*-alkylation with 3-bromo-1-propanol at the nosylated nitrogen under basic conditions delivered a primary alcohol **7** which was subjected to a second chain elongation with **8** under Mitsunobu conditions (DIAD/PPh_3) to afford **9**. Cleavage of the *tert*-butylester (**9**) with trifluoroacetic acid (TFA) and triethylsilane as a scavenger gave the orthogonally protected lysine derivative **10**. Both derivatives **5** and **10** were then used in the solid phase peptide synthesis.

Fmoc-protected **5** (Scheme 2) was attached to 2-chlorotrityl resin preloaded with glycine using Fmoc solid phase peptide synthesis (SPPS) protocols. *O*-Benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and 1-hydroxy-1*H*-benzotriazole (HOBt) were used as coupling reagents throughout the synthesis and the Fmoc-group was removed using 25% piperidine in DMF. The α-Dde-protected lysine derivative **10** was coupled under standard Fmoc-SPPS-protocols without complications. Selective removal of the Dde-group was achieved by a repeated titration of the resin with 2% hydrazine. A final coupling with Fmoc-glycine and deprotection with piperidine followed by mercaptoethanol and DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) yielded the hexapeptides still bound to the resin. Liberation from the solid support was achieved with trifluoracetic acid–triisopropylsilane and water (TFA : TIPS : H_2O, 95 : 2.5 : 2.5). Analysis of the products by MALDI-TOF showed one major peak corresponding to **1**, **2**, and **3**, respectively (Scheme 2). Other analytical methods showed the homogeneity of the peptides, too. **1**, **2**, and **3** are the first peptides with a uniform composition, in contrast to the silaffins from natural origin, which exhibit microheterogeneity.



Scheme 2 Solid-phase synthesis of peptides **1**, **2**, and **3**.

In order to investigate the influence of peptides **1**, **2**, and **3** on the biosilification process, silica precipitation assays were undertaken. In the presence of all three compounds silica precipitates were found. However, the amount was dependent on the molecular structure. Fig. 2 shows the amount of precipitated SiO_2, given as m_{Si}, as a function of different peptide concentrations at pH 6.8. While in the presence of compound **2** only small SiO_2 masses are found, m_{Si} is larger if compound **3** is added to the $Si(OH)_4$ solution. The largest amount of SiO_2 is found in case of compound **1**. Almost no SiO_2 is generated in the presence of all three compounds if the pH is shifted to 5.5.

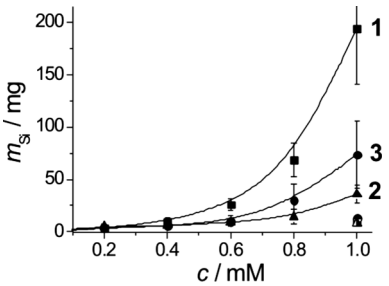


Fig. 2 Amount of precipitated silica given as m_{Si} in the presence of various concentrations of compound **1** (■), **2** (▲), and **3** (●), respectively. Precipitation was carried out at pH 6.8. At pH 5.5, even at 1 mM of the corresponding compound, only very little precipitate was found (striped symbols).

To further elucidate the structure of the silica precipitates scanning electron microscopy images were taken. Fig. 3 shows scanning electron microscopy (SEM) images of silica structures obtained in the presence of 1 mM of the three different compounds at pH 6.8. In the presence of compound **1**, silica spheres of the size of hundreds of nanometres are found together with amorphous silica. Compound **2** generates only few silica spheres but rather grainy silica structures, which demonstrates that the change in one amino acid influences the silica structure considerably. In case of compound **3**, mainly silica spheres in the range of a few 100 nm can be observed. Decreasing polarity of the side chains in the order Glu, Ser, Ala increases the size of the detected spheres of the silica precipitate. This observation is in accordance with the assumed increase of the size of the microdroplets from the postulated phase separation process during silica precipitation.[17,18] This phase separation was characterized for biogenic and synthetic polyamines without covalently linked counter ions like Glu in peptide **2**.[9] This study shows that already isolated KXXK domains can determine the morphology of silica precipitates. The chemical methods introduced here will give access to longer expansions or even the entire silaffin peptides for nanopattern formation studies.

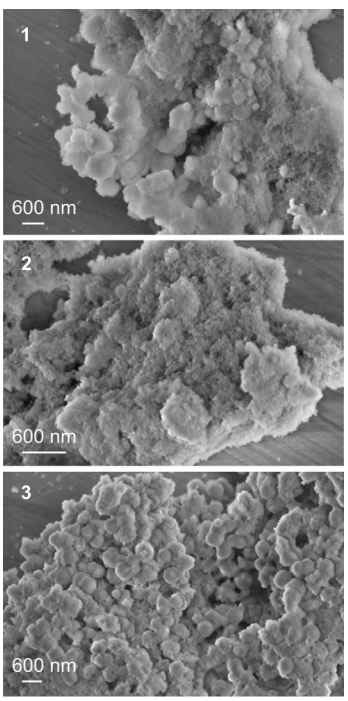


Fig. 3 Scanning electron microscopy images of the silica precipitates obtained in the presence of 1 mM of the indicated peptide **1**, **2**, or **3** at pH 6.8. The silica obtained from peptide **1** exhibits spheres together with grainy precipitate. Peptide **2** delivers grainy silica only, while spheres dominate the silica precipitated with **3**.

Conclusion

The self-assembling process of silaffins is guided by ions, which are provided by cationic side chain functionalities and by anionic modifications like serine phosphate. Polyamines are post-translationally attached to lysines in order to promote and catalyze the silica precipitation. This study identifies a correlation between the amino acid composition of synthetic KXXK domains and the character of the silica precipitates. The biosilification process is a finely balanced interaction between several organic components present in diatoms. We could show that a small change in the primary structure in the peptide side chains are directly expressed in the amount of precipitated silica as well as its morphology. The SPPS protocol for the assembly of poly-cationic peptides will allow the synthesis of even more complex peptides and thus accelerate the elucidation of structure-function relationships in the biosilification process of diatoms. Currently, longer sections of the silaffin peptidic backbone together with other post-translational modifications found in diatoms are being synthesized.

Experimental

(*S*)-6-Dimethylamino-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-hexanoic acid (5). To a solution of Fmoc-L-lysine (**4**, 5.0 g, 10.3 mmol), 37% formaldehyde solution (4.2 mL, 51.7 mmol) and acetic acid (1.8 mL) in 1,4-dioxane (30 mL) were added $NaBH_4$ (3.5 g, 93.1 mmol) in portions at 0 °C. The pH of the reaction mixture was maintained between 3 and 6 by addition of acetic acid. After half of the $NaBH_4$ was added, another portion of 37% formaldehyde-solution (4.2 mL, 51.7 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 12 h. The mixture was diluted with H_2O (30 mL) and the pH was adjusted to 6. The organic solvents were removed *in vacuo* and the remaining water phase was extracted with $CHCl_3$ (8 × 50 mL). The collected organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Titration with Et_2O and drying under high vacuum delivered **5** (3.9 g, 88%) as a white foam. (R_f 0.40 on SiO_2, 95 : 5 CH_3OH–H_2O); $[\alpha]_D^{18}$ +2.9 (*c* 1.21, EtOH); ^{1}H NMR (DMSO-d_6, 300 MHz, 300 K) δ 7.87 (d, 2H, J = 7.5 Hz, CH), 7.72–7.67 (m, 2H), 7.40 (t, 2H, J = 7.0 Hz, CH), 7.30 (t, 2H, J = 7.3 Hz, CH), 7.22 (d, 1H, J = 7.5 Hz, NH), 4.26–4.17 (m, 3H, OCH_2, CH), 3.79–3.86 (m, 1H, H_α), 2.44 (t, 2H, J = 7.5 Hz, H_ε), 2.30 (s, 6H, 2 × NCH_3), 1.73–1.54 (m, 2H, H_β), 1.50–1.37 (m, 2H, H_δ), 1.33–1.27 (m, 2H, H_γ); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 173.7 (C═O), 156.2 (C═O), 143.8 (C), 140.7 (C), 127.6 (CH), 127.1 (CH), 125.2 (CH), 120.1 (CH), 65.6 (CH_2), 56.0 (CH), 53.7 (CH_2), 46.6 (CH), 41.8 (CH_3), 30.1 (CH_2), 23.1 (CH_2), 22.7 (CH_2); HRMS (ES) Calc. for $C_{23}H_{27}N_2O_4$ 395.1976, found 395.1972.

(*S*)-2-[1-(4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-ethylamino]-6-[(3-hydroxy-propyl)-(2-nitrobenzenesulfonyl)-amino]-hexanoic acid *tert*-butyl ester (7). Dde-L-Lys(Ns)-O^{t}Bu (**6**, 10.5 g, 19.0 mmol), K_2CO_3 (11.8 g, 85.7 mmol) and TBAI (0.1 g, 0.5 mmol) were dissolved in DMF (75 mL) and heated up to 70 °C. At this temperature 3-bromopropanol (2.1 mL, 24.8 mmol) was added slowly. The mixture was stirred for 8 h at this temperature. H_2O (300 mL) was added and then the mixture was extracted with EtOAc (2 × 200 mL). The collected organic solvents were again washed with brine (3 × 150 mL). After drying over $MgSO_4$ and filtration, the organic solvents were removed *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 10 : 0 → 4 : 1, EtOAc–MeOH) to yield **7** (9.0 g, 78%) as a yellow oil. (R_f 0.25 on SiO_2, EtOAc); $[\alpha]_D^{26}$ +2.5 (*c* 0.64, $CHCl_3$); ^{1}H NMR ($CHCl_3$, 300 MHz, 300 K) δ 13.63 (d, 1H, *J* = 7.5 Hz, NH), 7.93–7.89 (m, 1H, CH), 7.66–7.60 (m, 1H, CH), 7.58–7.54 (m, 1H, CH), 4.23–4.15 (m, 1H, H_α), 3.59 (t, 2H, *J* = 5.9 Hz, CH_2OH), 3.34 (t, 2H, *J* = 7.0 Hz, NCH_2), 3.22 (t, 2H, *J* = 7.3 Hz, H_ε), 2.41 (s, 3H, CH_3), 2.41 (bs, 2H, CH_2), 2.28 (bs, 2H, CH_2), 1.86–1.78 (m, 2H, CH_2), 1.75–1.67 (m, 2H, H_β), 1.59–1.47 (m, 2H, H_δ), 1.40 (s, 9H, ^{t}Bu), 1.37–1.27 (m, 2H, H_γ), 0.96 (s, 6H, CH_3); ^{13}C NMR ($CHCl_3$, 75 MHz) δ 199.5 (C═O), 197.1 (C═O), 173.2 (C═O), 169.1 (C), 148.2 (C), 133.7 (C), 133.2 (CH), 131.8 (CH), 130.7 (CH), 124.3 (CH), 108.5 (C), 83.4 (C), 59.3 (CH_2), 56.9 (CH), 53.6 (CH_2), 52.4 (CH_2), 47.8 (CH_2), 44.9 (CH_2), 32.3 (CH_2), 31.3 (CH_2), 30.2 (C), 28.4 (CH_3), 28.2 (CH_2), 28.0 (CH_3), 22.5 (CH_2), 18.4 (CH_3); HRMS (ES) Calc. for $C_{29}H_{43}N_3O_9SNa$ 632.2612, found 632.2615.

***N*-(3-Dimethylamino-propyl)-2-nitrobenzenesulfonamide (8).** To a solution of *N*,*N*-dimethyl-1,3-diaminopropane (6.3 mL, 50.0 mmol) and Et_3N (6.7 mL, 50 mmol) in CH_2Cl_2 (100 mL) was added Ns-Cl (5.54 g, 25.0 mol) in several portions. After addition the resulting solution was stirred for 24 h. The mixture was extracted with half saturated NaCl-solution (50 mL) and H_2O (50 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was removed under high vacuum. The crude product was purified by flash chromatography (SiO_2, 5 : 1, $CHCl_3$: MeOH) to yield **8** (6.21 g, 86%) as a yellow solid. (R_f 0.23 on SiO_2, 5 : 1, $CHCl_3$: MeOH); ^{1}H NMR ($CHCl_3$, 300 MHz, 300 K) δ 8.05–8.00 (m, 1H, CH), 7.76–7.72 (m, 1H, CH), 7.59 (bs, 1H, NH), 3.11 (t, 2H, *J* = 5.9 Hz, CH_2), 2.33 (t, 2H, *J* = 5.9 Hz, CH_2), 2.16 (s, 6H, CH_3), 1.63 (app. qn, 2H, *J* = 5.9 Hz, CH_2); ^{13}C NMR ($CHCl_3$, 75 MHz) δ 148.1 (C), 133.8 (CH), 133.3 (CH), 132.4 (CH), 130.9 (CH), 125.0 (CH), 58.9 (CH_2), 45.2 (CH_3), 44.4 (CH_2), 25.3 (CH_2); HRMS (ES) Calc. for $C_{11}H_{18}N_3O_4S$ 288.1013, found 288.1006.

(*S*)-6-[{3-[(3-Dimethylamino-propyl)-(2-nitrobenzenesulfonyl)-amino]-propyl}-(2-nitrobenzenesulfonyl)-amino]-2-[1-(2,4,4,6-tetramethyl-cyclohexylidene)-ethylamino]-hexanoic acid *tert*-butyl ester (9). PPh_3 (2.51 g, 9.59 mmol) and DIAD (1.9 mL, 9.59 mmol) were dissolved in abs. THF (80 mL) at 0 °C. The mixture was stirred until a precipitate was formed (15 min). To this suspension was added **8** (2.43 g, 8.46 mmol) and stirred for another 20 min. After that time **7** (3.44 g, 5.64 mmol) in abs. THF (10 mL) was slowly added *via* syringe. The mixture was warmed to room temperature and stirred for 3 d. After evaporation of the solvent *in vacuo* the crude product was purified by flash chromatography (SiO_2, 5 : 1, $CHCl_3$: MeOH). **9** (4.60 g, 93%) was obtained as a yellow oil. (R_f 0.41 on SiO_2, 5 : 1, $CHCl_3$: MeOH); $[\alpha]_D^{18}$ –0.2 (*c* 1.23, $CHCl_3$); ^{1}H NMR ($CHCl_3$, 300 MHz, 300 K) δ 13.66 (d, 1H, *J* = 7.5 Hz, 1H, NH), 7.95–7.89 (m, 2H, CH), 7.65–7.60 (m, 4H, CH), 7.57–7.53 (m, 2H, CH), 4.17 (app. q, 1H, *J* = 6.8 Hz, H_α), 3.29–3.18 (m, 8H, CH_2, H_ε), 2.41 (s, 3H, CH_3), 2.34 (s, 2H, CH_2), 2.28 (s, 2H, CH_2), 2.23 (t, 2H, *J* = 7.2 Hz, CH_2), 2.14 (s, 6H, CH_3), 1.85–1.75 (m, 4H, CH_2, H_β), 1.70–1.61 (m, 2H, CH_2), 1.55–1.46 (m, 2H H_δ), 1.39 (s, 9H, ^{t}Bu), 1.33–1.23 (m, 2H, H_γ), 0.96 (s, 6H, CH_3); ^{13}C NMR ($CHCl_3$, 75 MHz) δ 199.3 (C═O), 196.8 (C═O), 172.9 (C═O), 169.1 (C), 147.9 (C), 133.6 (C), 132.9 (C), 131.8 (CH), 130.8 (CH), 130.6 (CH), 124.2 (CH), 124.1 (CH), 108.2 (C), 83.2 (C), 56.7 (CH_2), 56.3 (CH), 53.6 (CH_2), 52.2 (CH_2), 47.4 (CH_2), 46.0 (CH_2), 45.3 (CH_2), 45.1 (CH_3), 45.0 (CH_2), 32.2 (CH_2), 30.0 (C), 28.4 (CH_3), 28.2 (CH_3), 27.9 (CH_3), 27.6 (CH_2), 26.0 (CH_2), 22.2 (CH_2), 18.2 (CH_3); HRMS (ES) Calc. for $C_{40}H_{59}N_6O_{12}S_2$ 879.3627, found 879.3632.

(*S*)-6-[{3-[(3-Dimethylamino-propyl)-(2-nitrobenzenesulfonyl)-amino]-propyl}-(2-nitrobenzenesulfonyl)-amino]-2-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)-ethylamino]-hexanoic acid (10). **9** (615 mg, 0.69 mmol) was dissolved in CH_2Cl_2 (4.3 mL). TFA (2.0 mL, 27.3 mmol) and Et_3SiH (0.84 mL, 5.3 mmol) were added and the resulting solution was stirred for 12 h at room temperature. After evaporation of the solvent *in vacuo* and extraction of the residue with $CHCl_3$ (3×) the product was dried under high *vacuum* to yield **10** (655 mg, quant.) as a white foam. The product was directly submitted to SPPS to avoid loss of the Dde-group.

General procedure for Fmoc Solid Phase Peptide Synthesis

Fmoc-protected amino acid (4.0 eq to resin loading), HOBt (4.0 eq) and HBTU (4.0 eq) were dissolved in DMF (5 mL) and DIPEA (10.0 eq) was added. After five minutes pre-activation the solution was transferred to the pre-swelled resin. 2-Chlorotrityl chloride resin with a loading of 0.68 mmol g^{-1} was used for the synthesis. The reaction was swirled for 2 h. Double coupling was applied throughout the synthesis. After completion the solution was drained and the resin washed with DMF (3×), MeOH (3×) and CH_2Cl_2 (3×). The completion of couplings was ascertained by TNBS test. The Fmoc group was removed by treatment of the pre-swelled resin with 20% piperidine in DMF (1 × 10 min, 1 × 20 min).

Removal of Dde, Ns and final cleavage from the resin

A solution of 2% hydrazine in DMF (3 mL) was added to the resin and swirled for 5 min. The washing step was repeated two more times (1 × 5 min, 1 × 10 min). After deprotection the resin was washed with DMF (3×), MeOH (3×) and CH_2Cl_2 (3×). Coupling of the following residue and Fmoc removal was done as described above. Ns-cleavage was done by using 2-mercaptoethanol (20 eq) and DBU (10 eq) for 30 min (3×). After washing with DMF (3×), MeOH (3×) and CH_2Cl_2 (3×) the resin was dried under high *vacuum* for 12 h. Final cleavage of the peptide from the resin was done with 95 : 2.5 : 2.5 (TFA : TIPS : H_2O) for 2 h. Filtration and concentration of the filtrate *in vacuo* and precipitation with Et_2O yielded **1** (43 mg, 98%), **2** (44 mg, 97%), and **3** (37 mg, 86%), respectively, as slightly yellow solids. **1**: HRMS (ES) Calc. for $C_{32}H_{65}N_{10}O_8$ 717.4981, found 717.4984. **2**: HRMS (ES) Calc. for

$C_{34}H_{67}N_{10}O_9$ 759.5087, found 759.5088. **3**: HRMS (ES) Calc. for $C_{32}H_{65}N_{10}O_7$ 701.5032, found 701.5032.

Silica precipitation assays

a) Preparation of silicic acid. A freshly prepared solution of 1 M tetramethoxysilane in 1 mM HCl was incubated at 20 °C for exactly 15 min and immediately used as a source of monosilicic/disilicic acid.[19]

b) *In vitro* precipitation of silicic acid and SEM analysis. Silica formation was initiated by the addition of 2 μL 40 mM silicic acid, prepared as described above, to the peptide (40 μL) in 25 mM phosphate buffer. After 12 min at 25 °C, the reaction was terminated by adjusting the pH to 3.0 with HCl. Silica was spinned down by centrifugation (5 min, 14.000 × *g*). The precipitate was washed twice with water, then suspended in water, applied to an aluminium sample holder, and air dried. Silica precipitates were analysed without sputter-coating with a LEO 1530 field-emission scanning electron microscope equipped with energy dispersive X-ray analysis (EDXA, Oxford instruments).

c) Concentration assay. Precipitated silica was dissolved in 2 M NaOH for 60 min at room temperature and quantified by a modified β-silicomolybdate method.[19] 1.35 mL HCl (37%) were dissolved in 40.3 mL H_2O, 774.2 mg [$(NH_4)_6Mo_7O_{24} \times 4\ H_2O$] were dissolved in 9.7 mL water. Both solutions were mixed and the pH was adjusted to 1.12 with 2 M NaOH (molybdate solution). The sample solution containing dissolved silica (0, 20 or 40 μL) and a corresponding volume of 2 M NaOH (0, 20 or 40 μL), 160 μL H_2O, and 800 μL molybdate solution were added and the absorbance of the solution monitored at a wavelength of 370 nm. A silicon atomic absorption standard solution was used to generate calibration curves.

Acknowledgements

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References

1 F. Round, R. Crawford and D. Mann, *The Diatoms*, Cambridge University Press, Cambridge, 1990.
2 M. Sumper and G. Lehmann, *ChemBioChem*, 2006, **7**, 1419–1427.
3 N. Kröger, R. Deutzmann and M. Sumper, *Science*, 1999, **286**, 1129–1132.
4 N. Kröger, R. Deutzmann, C. Bergsdorf and M. Sumper, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 14133–14138.
5 W. D. Marner, A. S. Shaikh, S. J. Muller and J. D. Keasling, *Biomacromolecules*, 2008, **9**, 1–5.
6 N. Kröger, S. Lorenz, E. Brunner and M. Sumper, *Science*, 2002, **298**, 584–586.
7 N. Poulsen and N. Kröger, *J. Biol. Chem.*, 2004, **279**, 42993–42999.
8 M. Sumper, R. Hett, G. Lehmann and S. Wenzl, *Angew. Chem.*, 2007, **119**, 8557–8560.
9 A. Bernecker, R. Wieneke, R. Riedel, M. Seibt, A. Geyer and C. Steinem, *J. Am. Chem. Soc.*, 2010, **132**, 1023–1031.
10 A. Bernecker, J. Ziomkowska, S. Heitmüller, R. Wieneke, A. Geyer and C. Steinem, *Langmuir*, 2010, **26**, 13422–13428.
11 E. V. Armbrust, J. A. Berges, C. Bowler, B. R. Green, D. Martinez, N. H. Putnam, S. Zhou, A. E. Allen, K. E. Apt, M. Bechner, M. A. Brzezinski, B. K. Chaal, A. Chiovitti, A. K. Davis, M. S. Demarest, J. C. Detter, T. Glavina, D. Goodstein, M. Z. Hadi, U. Hellsten, M. Hildebrand, B. D. Jenkins, J. Jurka, V. V. Kapitonov, N. Kröger, W. W. Y. Lau, T. W. Lane, F. W. Larimer, J. C. Lippmeier, S. Lucas, M. Medina, A. Montsant, M. Obornik, M. S. Parker, B. Palenik, G. J. Pazour, P. M. Richardson, T. A. Rynearson, M. A. Saito, D. C. Schwartz, K. Thamatrakoln, K. Valentin, A. Vardi, F. P. Wilkerson and D. S. Rokhsar, *Science*, 2004, **306**, 79–86.
12 A. Scheffel, N. Poulsen, S. Shian and N. Kröger, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 3175–3180.
13 M. Sumper and E. Brunner, *ChemBioChem*, 2008, **9**, 1187–1194.
14 F. Degerbeck, B. Fransson, L. Grehn and U. Ragnarsson, *J. Chem. Soc., Perkin Trans. 1*, 1992, 245–253.
15 T. Fukuyama, C.-K. Jow and M. Cheung, *Tetrahedron Lett.*, 1995, **36**, 6373–6374.
16 Y. Hidai, T. Kan and T. Fukuyama, *Chem. Pharm. Bull.*, 2000, **48**, 1570–1576.
17 M. Sumper, S. Lorenz and E. Brunner, *Angew. Chem.*, 2003, **115**, 5350–5353.
18 E. Brunner, K. Lutz and M. Sumper, *Phys. Chem. Chem. Phys.*, 2004, **6**, 854–857.
19 R. K. Iler, *The Chemistry of Silica*, John Wiley & Sons, New York, 1979.

