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PAPER

Non-peptidic cell-penetrating agents: synthesis of oligomeric chiral bicyclic guanidinium vectors†

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Polycationic oligo(chiral bicyclic guanidines) constitute useful non-peptidic penetrating agents for cell uptake and protein surface recognition. We report herein improved and selective procedures for the preparation of oligoguanidinium scaffolds linked through thioether bonds, with similar or different groups and functions at both ends of the chain. Two synthetic strategies were developed to obtain these compounds in relatively good yields from a common thioacetate precursor: generation of a disulfide intermediate or thiolate formation. Thus, tetraguanidinium intermediates **8** and **22** are best synthesized by the disulfide route, whereas hexamer **29**, octamer **31**, and trimer **37** arise from a combination of both the disulfide and the thioacetate routes. Finally, tetramer **28** can be readily obtained from either strategy.

Introduction

The protonated side chains of lysine and arginine play a pivotal role in many relevant biological processes, such as stabilization of the tertiary and quaternary structures of proteins, recognition of specific DNA sequences, protein–protein interactions, cell membrane signaling, and cellular uptake.[1–9] In their protonated form, these basic amino acids strongly interact with the anionic aspartate or glutamate side chains, as well as other oxoanions, such as sulfate, the phosphodiester backbone of oligonucleotides, or the phosphates present in cell-membrane lipids, *via* salt-bridges, occasionally enhanced by hydrogen bonding.

In particular, the guanidinium group of arginine constitutes one of the stronger individual binders present in biology. Its unique properties in terms of solvation, charge, pK_a and hydrogen bonding capability make it structurally and functionally fundamental in the cell machinery. Furthermore, the relevance of several arginine-rich peptides, such as TAT or Antp, in internalization or transport of non-permeable molecules across the cell membrane has been recently highlighted by numerous authors.[10,11] These naturally occurring compounds, known as cell-penetrating peptides (CPPs), are efficient carriers for oligonucleotides, proteins, fluorophores, PNAs, and even liposomes, nanoparticles or naturally transduced proteins (PTD*sa*).[12–14]

The chain length, a high content of cationic residues and the variable spacing between the charges are key structural features for CPPs' activity, whereas the backbone conformation does not seem to play a critical role.[15,16] However, their bioavailability is usually limited by *in vivo* proteolysis. Therefore, short peptidomimetics with modified backbones, carrying basic functionalities such as ammonium or guanidinium constitute valuable non-peptidic alternatives to naturally occurring CPPs because of their enhanced *in vivo* stability.

Embedding a guanidinium function into a decalin framework results in conformational rigidity, hydrogen bonding directionality and amphiphilicity.[17] Moreover, introduction of stereogenic centers α to the guanidinium NHs allows chiral recognition of the oxoanionic guests.[18] Inspired by nature's systems where concepts such as cooperativity or selective recognition are at the basis of design, oligomers of bicyclic guanidinium subunits were synthesized and studied for several purposes such as peptide recognition,[19] helical induction,[20–22] protein stabilization[23] or cellular uptake.[24,25] Indeed, a fluorescein-labelled, TBDPS protected tetraguanidinium oligomer was shown to be an efficient cell penetrating non-peptide carrier, showing better translocation through HeLa membranes than the reference Tat or Antp peptides.[24] Moreover, confocal microscopy experiments confirmed the preferential cellular localization and accumulation of this molecular vector in mitochondria (Fig. 1). Further studies

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with terbium complex conjugates confirmed mitochondrial targeting of this tetraguanidinium carrier.

More recently, it has been reported that attachment of Gamitrinibs, a molecule designed to target and inhibit Hsp90, to our tetraguanidinium vector results in an efficient inhibition of Hsp90 ATPase activity, therefore showing mitochondrial accumulation.[25]

Herein we describe improved methodologies for the synthesis of these bicyclic guanidinium oligomers in order to expand their functionalisation, to answer relevant questions about their binding behaviour, biocompatibility and cellular uptake efficiency.

Results and discussion

In earlier contributions,[24,26,27] we described the preparation of tetrameric guanidinium oligomers. However, attempts to expand iteratively this synthetic approach towards higher symmetrically or non-symmetrically functionalized oligomers were difficult to reproduce or yielded variable amounts of the desired compounds. Herein, we discuss methodological improvements and experimental conditions that we have developed to overcome the synthetic difficulties in the preparation of these polycationic oligomers. Otherwise trivial factors like the nature of the leaving groups and nucleophiles, or the choice of the appropriate counterions, turned out to be key aspects for success. Thus, two main strategies involving either formation of a disulfide intermediate or the direct deprotection of a thioacetyl group were developed to generate a thiolate nucleophile precursor (Scheme 1). The former, based on disulfide generation, involves cleavage of a *O*-silyl protecting group, and it is therefore compatible with the presence of a free primary alcohol. Although this approach looks simpler than the thioacetate route, since it skips one synthetic step, an additional cleavage step is needed for the *in situ* generation of the thiolate.

The choice of the synthetic route mainly relied on experimental considerations, such as solubility of the reaction components, nature and amount of side products, and difficulties in purification. Herein we will describe how the combination and iterative use of both modular approaches is employed for the different synthetic targets.

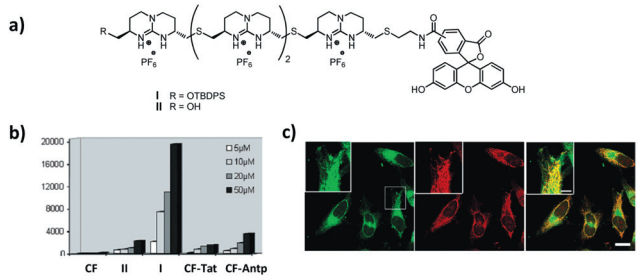
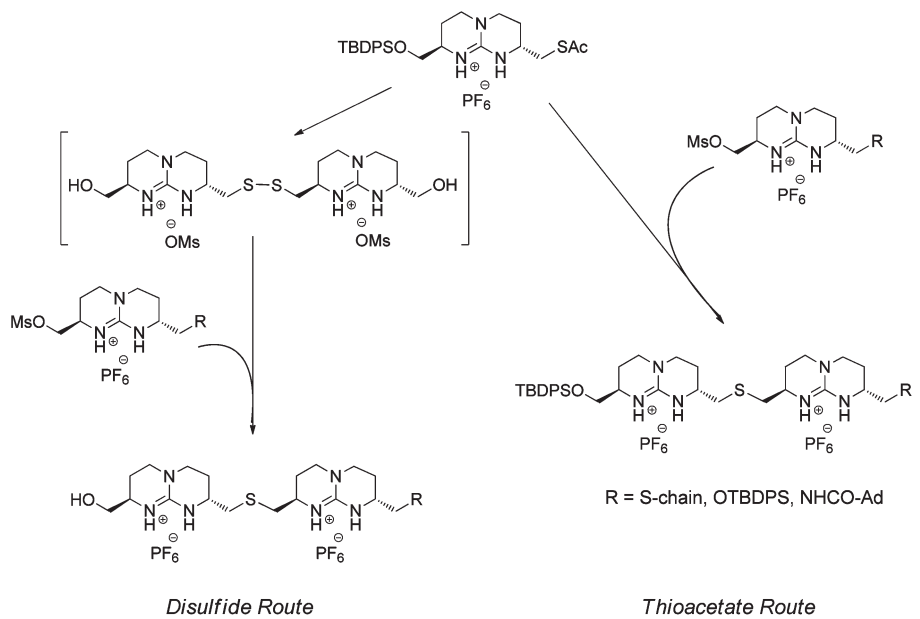


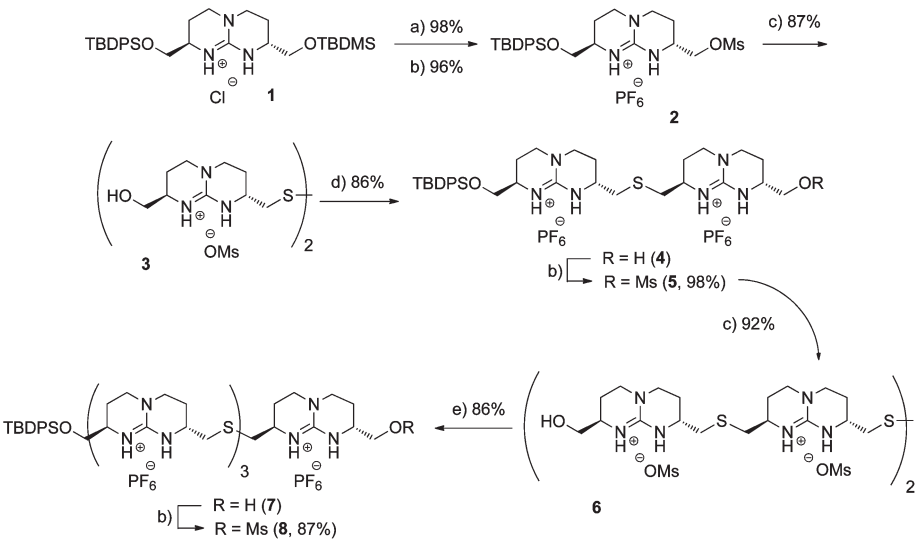
Fig. 1 (a) Structure of the fluorescein-labelled tetraguanidinium vectors. (b) Fluorescence measurements in HeLa cells incubated with these non-peptide vectors **I** and **II**, compared with Tat and Antp peptide vectors. (c) Cellular localization of vector **I** showing accumulation in mitochondria.[24]

Disulfide approach

The TBDMS group of the chiral bicyclic guanidinium salt **1**,[28] was selectively deprotected (Scheme 2), and the resulting free hydroxyl group activated as a mesylate **2**.[29] Then, the mesylate was transformed into a thioester using potassium thioacetate, _followed by treatment with methanesulfonic acid for the cleavage. Afterwards, the pH was increased to remove the excess acid and to promote formation of a disulfide bridge. Reduction of the disulfide was performed with *n*-butylphosphine and the resulting sulfide was reacted *in situ* with another equivalent of **2** to produce dimeric monoalcohol **3** as PF_6^- salt in a 45% yield.



Scheme 1 Synthetic routes for bicyclic guanidinium oligomers (Ms = mesyl, OTBDPS = *tert*-butyldiphenylsilyloxy, Ad = 1-adamantyl).



Scheme 2 The disulfide route to tetraguanidinium **8**. Reagents and conditions: (a) THF–AcOH–H_2O (1 : 3 : 1), 20 h, r.t.; (b) NMM, Ms_2O, dry THF; (c) $KSCOCH_3$ (5 equiv.), THF–H_2O (3 : 1), reflux overnight; then MsOH (15 equiv.), 16 h; (d) **2**, Cs_2CO_3, (^{n}Bu)$_2$PhP polystyrene, THF–MeOH, 3 h, r.t., under N_2; (e) **5**, Cs_2CO_3, (^{n}Bu)$_2$PhP polystyrene, THF–MeOH, 3 h, r.t., under N_2 (THF = tetrahydrofuran, NMM = *N*-methylmorpholine).

However, extensive formation of side-products was a major drawback, since it made the purification difficult and the reaction hard to reproduce. Use of more than one equivalent of mesylate did not produce significant changes in the reaction, indicating that reduction of the disulfide was not complete. This could be due to oxidation of the reducing phosphine or inactivation of the phosphino intermediate.

Use of a polymer-supported phosphine, namely di(*n*-butyl)-phenylphosphine polystyrene,[30] allowed an easier purification and improved reactivity of the intermediates resulting in an increase of the overall yield.

Within this context, note that the choice of the counterion for those polycationic molecules is not trivial. Indeed, hexafluorophosphate anion turns out to be optimal to handle these guanidinium compounds and the corresponding salts showed better solubility in apolar solvents compared to the chloride ones, due to the higher lipophilicity of this anion. This results in more facile methodologies for the purification of these oligoguanidinium hexafluorophosphate salts, involving regular column chromatographic methods. However, in some specific cases hexafluorophosphate impregnated silica (see ESI for Experimental details†), which mimics in some extent the well-known anion exchange resins, was required to ensure anionic exchange and to provide a better elution of these charged compounds through the column.

This strategy can be further employed to build-up the corresponding trimer, tetramer or pentamer without substantial decreases in yield. For instance, alcohol **4** was activated as mesylate (94%), and the whole process was repeated again affording the non-symmetric tetramer **8** in 87% yield.

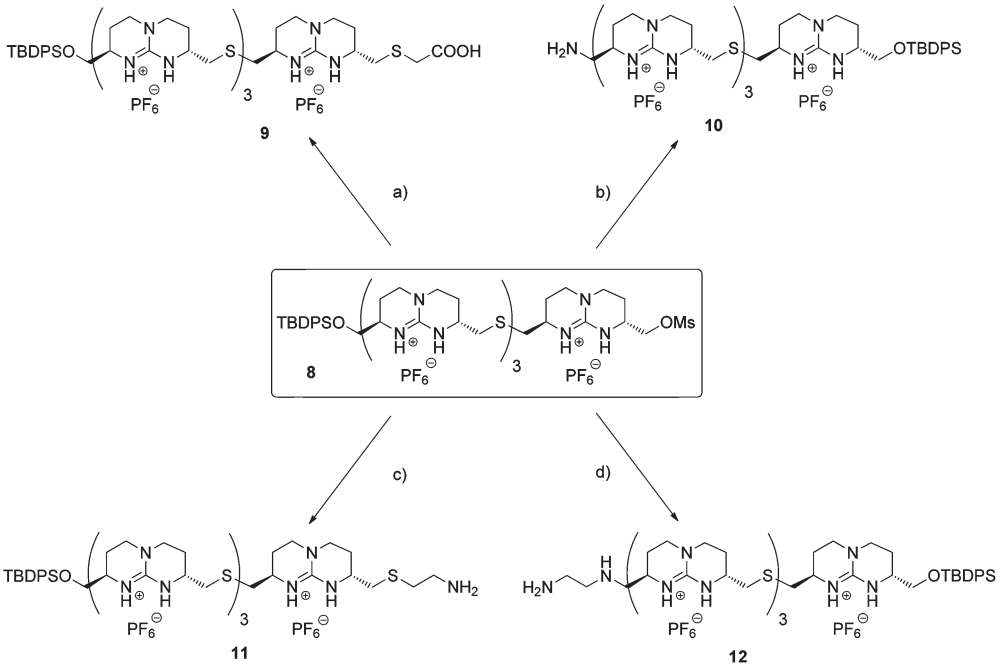
A major advantage of this synthesis is the generation of a non-symmetric oligomer, easy to functionalize *via* alcohol activation, allowing elongation of the chain and introduction of other groups such as chromophores, fluorophores or biologically active cargos. Replacement of the mesyl group in **8** with amino or carboxylic acid functions, would allow further coupling to peptidic cargos, directly attached to these groups *via* amide or the appropriate spacer (Scheme 3).

Thus, vectors **9–12** were readily prepared from compound **8**. Use of mercaptoacetate under basic conditions afforded compound **9**, whereas compound **10** was prepared using a methanol–30% aq. ammonia mixture. Longer spacers such as in **11** and **12** were introduced by *N*-alkylation of **8** with *N*-Boc-cystamine–Cs_2CO_3 or *N*-Boc-ethylenediamine–K_2CO_3, respectively, followed by deprotection. In the case of **11**, di(*n*-butyl)phenylphosphine polymeric resin was also added to avoid disulfide formation.

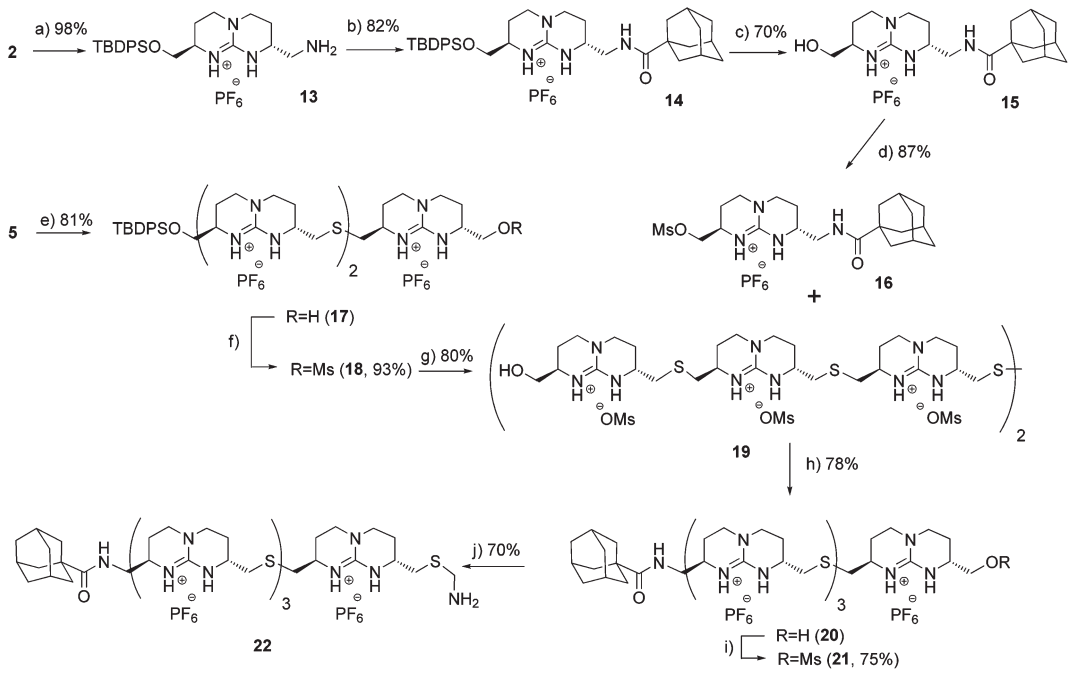
This strategy was mainly employed for the construction of oligoguanidinium vectors with cellular uptake properties, keeping the TBDPS silyl group, which provides the required lipophilicity for an efficient internalization process. Other more robust lipophilic groups, not sensitive to cleavage under acidic conditions, were also introduced. It has been established that the presence of some lipophilic residues in the molecular vectors, such as fatty acids, or simple adamantyl or pivaloyl groups, favors membrane crossing.[31] Therefore, guanidinium precursor **16** bearing a 1-adamantoyl amide instead of the TBDPS protecting group was also prepared (Scheme 4).

Reaction with mesylate **2** in a 1 : 1 methanol–30% aq. ammonia mixture, afforded the primary amine **13** which was coupled without further purification with 1-adamantanecarbonyl chloride to generate the amide derivative **14**. Deprotection of the TBDPS group with HF–Py gave **15**, able to be activated again as an electrophile.

Addition of disulfide **3** to compound **5** resulted in the triguanidinium product **17** (Scheme 4). Mesylation and disulfide generation followed to basic treatment yielded **19**, which was reacted with the above mentioned adamantyl monoguanidinium derivative **16** to give rise to the adamantyl tetraguanidinium derivative **20**. Consecutive activation of the hydroxyl group and introduction of the cistamine molecule through *S*-alkylation successfully afforded **22**, a new member of the library of oligoguanidinium vectors.



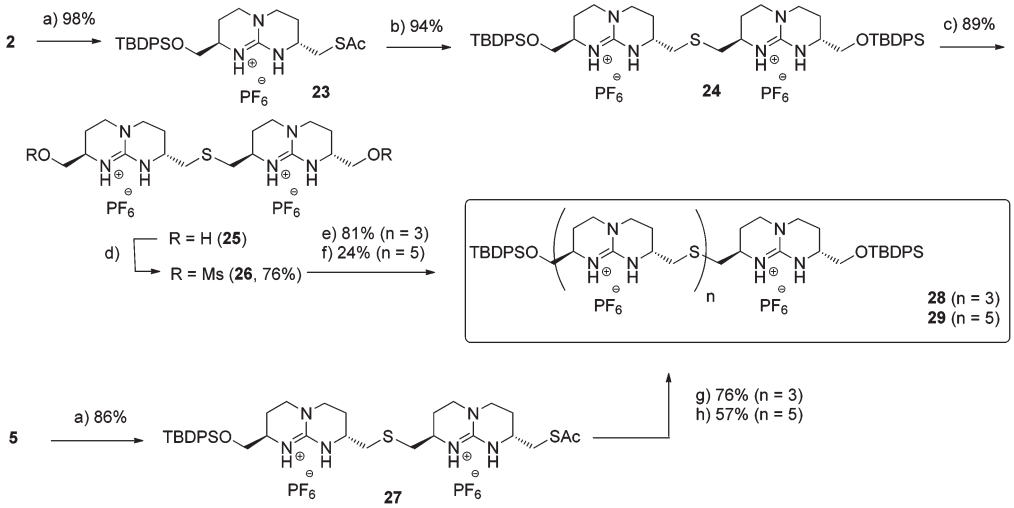
Scheme 3 Introduction of different functional groups in **8**. Reagents and conditions: (a) sodium mercaptoacetate, *t*-BuOK, MeOH, 4 h, r.t.; (b) 30% aq. ammonia–MeOH (2 : 1), 40 min, r.t.; (c) Boc-cystamine, Cs_2CO_3, MeOH, r.t. under N_2; then TFA, CH_2Cl_2; (d) Boc-ethylendiamine, K_2CO_3, reflux overnight; then TFA, CH_2Cl_2 (TFA = trifluoroacetic acid, Boc = *tert*-butoxycarbonyl).



Scheme 4 Synthetic route to **22**. Reagents and conditions: (a) 30% aq. ammonia–MeOH (1 : 1), 40 min, r.t.; (b) 1-adamantanecarbonyl chloride, Et_3N, dry CH_2Cl_2, 4 h, r.t.; (c) HF–Py, THF, overnight at r.t.; (d) Ms_2O, NMM, dry CH_2Cl_2, 3 h, r.t.; (e) **3**, Cs_2CO_3, (^{n}Bu)$_2$PhP polystyrene, THF–MeOH, 4 h, r.t., under N_2; (f) NMM, Ms_2O, dry CH_2Cl_2; (g) $KSCOCH_3$, THF–H_2O (3 : 1), reflux overnight; then MsOH, reflux 16 h; (h) **16**, Cs_2CO_3, (^{n}Bu)$_2$PhP polystyrene, THF–MeOH, 2 h, r.t., under N_2; (i) Ms_2O, NMM, dry CH_2Cl_2, 4 h, r.t; (j) Boc-cystamine, Cs_2CO_3, MeOH, 4 h, r.t.; then TFA, CH_2Cl_2.

Thioacetate approach

Use of acetyl protection to prevent disulfide formation during *in situ* generation of the thiolate and the choice of cesium carbonate as the base, substantially improved the reaction yield in thioether bond formation. Furthermore, use of polymeric phosphine to prevent disulfide formation was employed in some specific cases, especially in the synthesis of symmetric oligomers of different lengths.

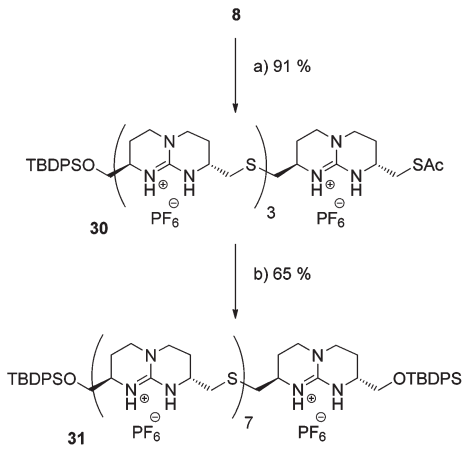


Scheme 5 Synthetic pathway for oligomers **28** and **29**. Reagents and conditions: (a) $KSCOCH_3$, ACN, reflux overnight; (b) **2**, Cs_2CO_3, ACN–H_2O (3 : 1), 3 h at r.t. under N_2; (c) MsOH, reflux overnight; (d) NMM, Ms_2O, dry CH_2Cl_2; (e) **3**, Cs_2CO_3, (^{n}Bu)$_2$PhP polystyrene, ACN–MeOH (3 : 1), 10 h at r.t., under N_2; (f) **27**, Cs_2CO_3, (^{n}Bu)$_2$PhP polystyrene, ACN–MeOH (3 : 1), 10 h at r.t., under N_2; (g) **5**, Cs_2CO_3, ACN–H_2O (3 : 1), 3 h at r.t., under N_2; (h) **8**, Cs_2CO_3, (^{n}Bu)$_2$PhP polystyrene, ACN–MeOH (3 : 1), 5 h at r.t., under N_2 (ACN = acetonitrile).

The thioacetyl derivative **3** was obtained in almost quantitative yield from precursor **2**. Subsequently, generation of the thiolate under basic conditions followed by reaction with mesylate **2** afforded diguanidinium **24**. Cleavage of the silyl groups and mesylation of the diol gave rise to compound **26** in good yields. This molecule reacted with two equivalents of a thioacetyl di- or tetraguanidinium precursors (**23** and **27**, respectively) to form the corresponding oligomers. These oligomers were also easily formed by combination of the thioacetyl diguanidinium **27** either with mesyl derivative **5** to produce tetramer **28** or with **8** to get hexamer **29**, with an improved yield (Scheme 5). Following this procedure, octaguanidinium **31** was successfully prepared in 65% yield from tetramer **8** (Scheme 6).

The thiolate route requires an extra deprotection step of the silyl group as compared with the disulfide route but it is also a method of choice for non-symmetric oligomeric derivatives because, for solubility reasons, the less polar TBDPS-protected molecules are often easier to isolate and purify in organic solvents.

In the case of triguanidinium **37**, attempts to expand the number of subunits *via* disulfide reduction were unsuccessful. Therefore, the synthesis of this compound was achieved by *S*-alkylation of compound **2**, to give compound **32** quantitatively (Scheme 7). Subsequent deprotection and activation afforded mesyl derivative **34**, which reacted with the diguanidinium thioacetyl derivative **27** giving rise to triguanidinium **35** in 87% yield. Iterative cleavage and mesylation of the resulting alcohol generated compound **37**, ready to be functionalized or coupled to different cargos, such as fluorescent probes, gene therapeutic agents, or drugs.



Scheme 6 Synthesis of octaguanidinium **31**. Reagents and conditions: (a) $KSCOCH_3$, ACN, reflux overnight; (b) **8**, Cs_2CO_3, (^{n}Bu)$_2$PhP polystyrene, ACN–MeOH (3 : 1), 7 h at r.t., under N_2.

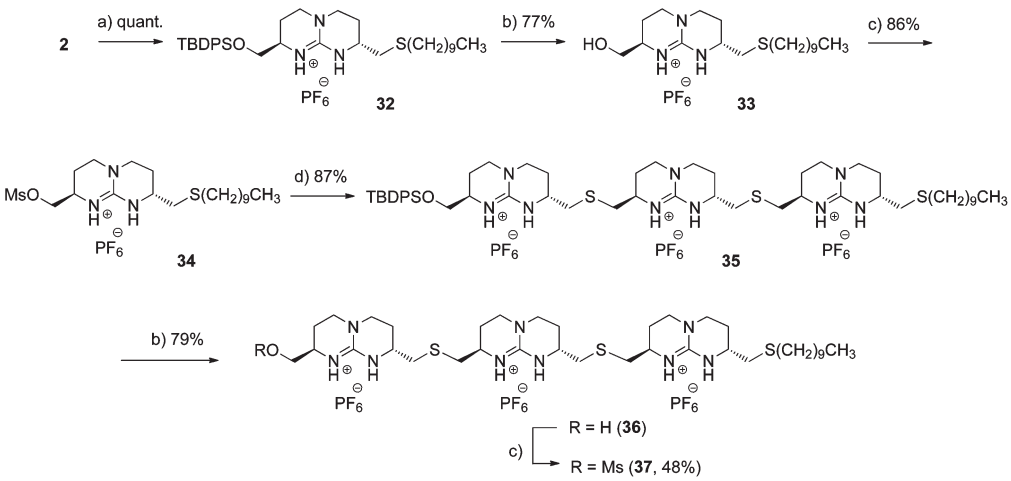
Conclusions

We have described the synthesis of diverse oligomers of chiral bicyclic guanidines with moderate to good overall yields. Disulfide and thioacetate routes were employed independently or in combination to afford oligomers up to eight guanidinium residues. Use of polymer-supported phosphines as reducing agents as well as silica gel impregnated with KPF_6 for the chromatographic separations were found to be essential for an easy purification of these polycationic scaffolds. In particular, preparation of oligomers with two different substituents at the ends of the chain, *i.e.* a reactive site and a lipophilic end, are relevant for the generation of drug-carrier conjugates for efficient cellular uptake.[24,25] On the other hand, symmetrically substituted oligomers are of interest for the recognition of peptides[19–22] and protein surfaces.[23]

Experimental section

General methods

Synthesis. All commercially available reagents (Aldrich, Fluka, Acros, NovaBiochem, Panreac) were used without further



Scheme 7 Synthesis of triguanidinium **37**. Reagents and conditions: (a) decanethiol, *t*-BuOK, acetone, r.t., under N_2; (b) TBAF, THF, 4 h, r.t.; (c) NMM, Ms_2O, dry CH_2Cl_2; (d) **27**, Cs_2CO_3, ACN–MeOH, 3 h, r.t., under N_2. (TBAF = tetrabutylammonium fluoride).

purification. Anhydrous solvents were obtained from a solvent purification system (SPS). All reactions were performed under nitrogen atmosphere unless specified. Chiral bicyclic guanidinium derivatives were synthesized in the *R*,*R* configuration. Although compounds **2–8** have been described elsewhere,[24] improved synthetic procedures are reported.

Chromatography. Thin layer chromatography (TLC) was performed on pre-coated TLC-plates SIL G-25 UV_{254} (Macherey-Nagel) glass supported with detection by UV at 254 nm and/or bromocresol green stain (in EtOH and 1 N NaOH mixture). Column chromatography was done using silica gel from SDS (Chromagel 60 ACC, 40–60 mm) and Scharlau (ASTM, 40–60 mm) following the procedure described by W. C. Still. HPLC chromatograms were recorded on an Agilent Technologies 1100 (UV-detector) analytical HPLC C18 Symmetry300 and SunFire C18 columns (4.6 × 150 mm, 5 µm). For semi-preparative HPLC a LC 18 column Symmetry (10 × 150 mm, 5 µm) was used. The mobile phase consisted of CH_3CN–H_2O mixture containing 0.05 or 0.1% TFA. The solvents were provided by Scharlau and Carlo Erba (HPLC gradient grade).

Analysis. NMR spectra were performed on a Bruker Advance 400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) equipped with a z-gradient 5 mm BBO probe with ATM Ultrashield spectrometer. Deuterated solvents used are indicated in each case. Chemicals shifts (δ) are expressed in ppm, and are referred to the residual peak of the solvent. Mass spectra were recorded in a Waters LCT Premier (ESI or APCI mode), Waters GCT (EI and CI ionization modes) or Bruker MALDI-TOF spectrometers.

Compound 2

A solution of **1** (6.00 g, 10.20 mmol) in H_2O–AcOH–THF (1 : 3 : 1, 150 mL) was stirred for 20 h at room temperature. After removing the solvent, the crude was dissolved in CH_2Cl_2 (150 mL) and washed subsequently with saturated solutions of Na_2CO_3 (100 mL) and NH_4Cl (3 × 100 mL). The organic phase was dried (Na_2SO_4) and after evaporating the solvent, the remaining oil was triturated with toluene–diethyl ether at 4 °C, resulting in the selective cleavage of the TBDMS monoalcohol product (4.74 g, 98%) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.23 (s, 1H, NH), 7.78 (s, 1H, NH), 7.67–7.63 (m, 4H, CH_{Ar}), 7.46–7.40 (m, 6H, CH_{Ar}), 3.83 (dd, J = 3.5, 12.5 Hz, 1H, CH_2OSi), 3.73 (dd, J = 5.2, 12.5 Hz, 1H, CH_2OSi), 3.63–3.47 (m, 4H, CH_2O, CH_α), 3.36–3.21 (m, 4H, $CH_{2\gamma}$), 2.13–1.86 (m, 5H, OH, $CH_{2\beta}$), 1.07 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 151.2 (C_{guan}) 135.5, 135.4, 132.6, 129.8, 128.9, 127.8 (C_{Ar}, CH_{Ar}), 65.3, 63.7 (CH_2O), 50.6, 49.3 (CH_α), 45.6, 44.6 ($CH_{2\gamma}$), 26.7 ($CH_{3t\text{-Bu}}$), 22.7 ($CH_{2\beta}$), 19.1 ($C_{t\text{-Bu}}$). FAB/LSIMS m/z 474.5 [$(M - Cl^-)^+$, 100%]. To a solution of this intermediate compound (400 mg, 0.75 mmol) in dry THF (20 mL) was added Ms_2O (336 mg, 1.88 mmol) in 5 mL of dry THF. The mixture was treated with NMM (330 µL, 3.00 mmol) and stirred for 4 h at room temperature. After the evaporation of the solvent, the resulting solid was dissolved in CH_2Cl_2 (60 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 30 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. Purification by silica gel column chromatography (CH_2Cl_2–MeOH, 98 : 2) afforded **2** (450 mg, 96%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.69–7.60 (m, 4H, CH_{Ar}), 7.50–7.39 (m, 6H, CH_{Ar}), 6.28 (s, 1H, NH), 6.12 (s, 1H, NH), 4.33 (dd, J = 4.4, 10.4 Hz, 1H, CH_2OMs), 4.19 (dd, J = 6.3, 10.4 Hz, 1H, CH_2OMs), 3.86–379 (m, 1H, CH_α), 3.73–3.64 (m, 2H, CH_2OSi), 3.63–3.57 (m, 1H, CH_α), 3.43–3.25 (m, 4H, $CH_{2\gamma}$), 3.11 (s, 3H, CH_{3MsO}), 2.15–1.86 (m, 4H, $CH_{2\beta}$), 1.09 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 150.7 (C_{guan}), 135.6, 135.5, 132.6, 132,5, 130.1, 128.0 (CH_{Ar}, C_{Ar}), 69.5 (CH_2OMs), 65.4 (CH_2OSi), 50.2, 47.8 (CH_α), 45.3, 45.0 ($CH_{2\gamma}$), 37.2 (CH_{3MsO}), 26.8 ($CH_{3t\text{-Bu}}$), 22.5, 21.9 ($CH_{2\beta}$), 1921 ($C_{t\text{-Bu}}$). HRMS calcd for $[C_{26}H_{38}N_3O_4SSi]^+$ 516.2352; found 516.2354.

Compound 4

A solution of mesylate **2** (500 mg, 0.76 mmol) and potassium thioacetate (430 mg, 3.775 mmol) in a mixture of THF–H_2O

(3 : 1, 40 mL) was refluxed for 7 h. After cooling to room temperature, MsOH (490 μL, 7.54 mmol) was added and the mixture was refluxed for 16 h. The reaction mixture was cooled to room temperature, 10 mL of Et_2O were added and then the aqueous phase was extracted and washed with $CHCl_3$ (2 × 20 mL) and finally with Et_2O (20 mL). After concentrating the aqueous layer to *ca.* 50%, $KHCO_3$ (607 mg, 9.06 mmol) was added and the solvent evaporated to dryness. Then, MeOH (50 mL) was added, the precipitate was removed by filtration and the solvent evaporated under reduced pressure. This procedure was repeated a few times increasing the amount of CH_2Cl_2 in the solvent mixture until pure CH_2Cl_2 was used, to afford the symmetric disulfide **3** (204 mg, 87%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.90–3.81 (bs, 2H, OH), 3.80–3.67 (m, 3H, CH_2O), 3.64–3.26 (m, 13H, CH_2O, CH_α, $CH_{2\gamma}$), 3.15 (dd, J = 5.2, 13.7 Hz, 2H, CH_2S), 2.87–2.78 (m, 2H, CH_2S), 2.28–2.20 (m, 2H, $CH_{2\beta}$), 1.96–1.79 (m, 6H, $CH_{2\beta}$). To a solution of **3**, Cs_2CO_3 (250 mg, 0.77 mmol) in MeOH (5 mL) and (^{n}Bu)$_2$PhP polystyrene resin (617 mg, 0.58 mmol) were added and the mixture was stirred for 40 min. Then a solution of guanidine mesylate **2** (512 mg, 0.77 mmol) in THF (12 mL) was added and the mixture was stirred for 4 h. After evaporation of the solvent, the crude residue was dissolved in CH_2Cl_2 (25 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 20 mL). The organic layer was filtered over cotton and concentrated at reduced pressure. Purification by silica gel column chromatography (CH_2Cl_2–MeOH, 96 : 4 → 94 : 6) afforded **4** (220 mg, 86%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.69–7.63 (m, 4H, CH_{Ar}), 7.49–7.39 (m, 6H, CH_{Ar}), 6.23 (s, 1H, NH), 6.21 (s, 1H, NH), 6.19 (s, 1H, NH), 6.06 (s, 1H, NH), 3.80–3.21 (m, 16H, CH_2O, CH_α $CH_{2\gamma}$), 2.95–2.81 (m, 2H, CH_2S), 2.74 (dd, J = 3.6, 13.2 Hz, 1H, CH_2S), 2.60 (dd, J = 3.8, 13.2 Hz, 1H, CH_2S), 2.18–1.82 (m, 8H, $CH_{2\beta}$), 1.08 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 150.7 (C_{guan}), 150.3 (C_{guan}), 135.2, 132.7, 132.5, 129.8, 127.7 (CH_{Ar}, C_{Ar}), 65.4, 65.2 (CH_2OSi, CH_2O), 50.1, 49.8, 47.7, 47.6 (CH_α), 45.3, 44.8 ($CH_{2\gamma}$), 36.0, 35.7 (CH_2S), 26.7 ($CH_{3t\text{-Bu}}$), 26.5, 25.8, 22.4, 22.2 ($CH_{2\beta}$), 19.1 ($C_{t\text{-Bu}}$). FAB/LSIMS m/z 781.3 $(M - PF_6^-)^+$, 318.2 $(M - 2PF_6^-)^{2+}$. HRMS calcd for $[C_{34}H_{52}N_6O_2SSi]^{2+}$ 318.1821; found 318.1816.

Compound 5

To a solution of alcohol **4** (180 mg, 0.19 mmol) and NMM (173 μL, 1.55 mmol) in dry CH_2Cl_2 (10 mL) was added a solution of Ms_2O (169 mg, 0.97 mmol) in CH_2Cl_2 (4 mL) and the mixture was stirred for 4 h. The solvent was evaporated under reduced pressure and the resulting crude dissolved in CH_2Cl_2 and washed with a 0.1 N NH_4PF_6 solution (2 × 30 mL). The organic layer was filtered over cotton and concentrated to dryness. Purification by silica gel (with KPF_6) column chromatography (CH_2Cl_2–MeOH, 96 : 4) afforded **5** (191 mg, 98%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.69–7.61 (m, 4H, CH_{Ar}), 7.49–7.39 (m, 6H, CH_{Ar}), 6.25 (s, 1H, NH), 6.18 (s, 2H, NH), 5.98 (s, 1H, NH), 4.25 (dd, J = 4.9, 10.4 Hz, 1H, CH_2O), 4.12 (dd, J = 6.1, 10.4 Hz, 1H, CH_2O), 3.86–3.22 (m, 16H, CH_2O, CH_α, $CH_{2\gamma}$), 3.05 (s, 3H, CH_{3MsO}), 2.91–2.83 (m, 2H, CH_2S), 2.69–2.53 (m, 2H, CH_2S), 2.12–1.79 (m, 8H, $CH_{2\beta}$), 1.08 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 151.4 (C_{guan}), 151.2 (C_{guan}), 135.6, 135.5, 130.0, 127.8 (CH_{Ar}, C_{Ar}), 69.4, 65.2 (CH_2OSi, CH_2O), 50.1, 49.8, 47.7, 47.4 (CH_α), 45.7, 44.8 ($CH_{2\gamma}$), 37.5 (CH_2S), 37.0 (CH_{3MsO}) 36.9 (CH_2S), 26.7 ($CH_{3t\text{-Bu}}$), 26.5, 25.7, 22.0 ($CH_{2\beta}$), 19.2 ($C_{t\text{-Bu}}$). FAB/LSIMS m/z 859.3 $(M - PF_6^-)^+$. HRMS calcd for $[C_{35}H_{53}N_6O_4S_2Si]^+$ 713.3339; found 713.3344.

Compound 7

A solution of mesylate **5** (280 mg, 0.279 mmol) and potassium thioacetate (159 mg, 1.393 mmol) in a mixture of THF–H_2O (3 : 1, 40 mL) was refluxed for 7 h. After cooling it to room temperature, MsOH (270 μL, 4.179 mmol) was added and the mixture was refluxed overnight. The reaction mixture was cooled to room temperature, evaporated to dryness and the crude was dissolved in water (30 mL). Then, 10 mL of Et_2O were added and the aqueous phase was extracted and washed with $CHCl_3$ (2 × 20 mL) and finally with Et_2O (20 mL). After concentration of about 50% of aqueous layer, $KHCO_3$ (474 mg, 4.74 mmol) was added and the solvent evaporated to dryness. Afterwards, MeOH (50 mL) was added, the precipitate was removed by filtration and the solvent evaporated at reduced pressure. This procedure was repeated a few times increasing the amount of CH_2Cl_2 in the solvent mixture until pure CH_2Cl_2, to afford symmetric disulfide **6** (157 mg, 92%). ^{1}H-NMR (400 MHz, MeOD) δ 3.86–3.72 (m, 2H, CH_2O), 3.70–3.47 (m, 10H, CH_2O, CH_α), 3.46–3.35 (m, 16H, $CH_{2\gamma}$), 3.05–2.82 (m, 6H, CH_2S), 2.79–2.62 (m, 2H, CH_2S), 2.21–1.78 (m, 16H, $CH_{2\beta}$). To a solution of **6** (218 mg, 0.180 mmol), Cs_2CO_3 (118 mg, 0.361 mmol) in MeOH (15 mL), (^{n}Bu)$_2$PhP polystyrene resin (332 mg, 0.289 mmol) was added and the mixture was stirred for 1 h. Then a solution of diguanidine mesylate **5** (327 mg, 0.325 mmol) in THF (10 mL) was added and the mixture was stirred for 6 h. After evaporation of the solvent, the crude residue was dissolved in CH_2Cl_2 (25 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 20 mL). The organic layer was filtered over cotton and concentrated at reduced pressure. Purification by silica gel column chromatography (CH_2Cl_2–MeOH, 99 : 1 → 95 : 5) afforded **7** (245 mg, 86%) as a white solid. ^{1}H-NMR (400 MHz, CD_2Cl_2) δ 7.72–7.64 (m, 4H, CH_{Ar}), 7.53–7.42 (m, 6H, CH_{Ar}), 6.34–6.09 (m, 6H, NH), 6.02 (s, 1H, NH), 3.83–3.29 (m, 28H, CH_2O, CH_α, $CH_{2\gamma}$), 3.00–2.78 (m, 6H, CH_2S), 2.76–2.56 (m, 6H, CH_2S), 2.28–1.80 (m, 8H, $CH_{2\beta}$), 1.09 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C NMR (acetone-d_6, 100 MHz) δ 151.3 (2 × C_{guan}), 151.2 (C_{guan}), 135.7, 133.2, 133.1, 130.3, 128.2 (CH_{Ar}, C_{Ar}), 66.3, 64.4 (CH_2OSi, CH_2O), 51.1, 50.6, 48.2, 48.1, 48.0 (CH_α), 45.7, 45.5, 45.4, 45.3 ($CH_{2\gamma}$), 36.3, 36.2, 36.1 (CH_2S), 26.6 ($CH_{3t\text{-Bu}}$), 26.0, 25.8, 25.7, 22.8, 22.7 ($CH_{2\beta}$), 19.2 ($C_{t\text{-Bu}}$). MS (FAB^+) m/z 1467.5 (92) $[M - PF_6]^+$, 1321.7 (100) $[M - HPF_6 - PF_6]^+$, 1175.5 (78) $[M - 2HPF_6 - PF6]^+$, 1029.5 (76) $[M - 3HPF_6 - PF_6]^+$. HRMS (FAB^+) m/z 1029.5512 (calcd 1029.5537) $[M - HPF_6 - PF_6]^+$.

Compound 8

To a solution of alcohol **7** (150 mg, 0.093 mmol) and NMM (83 μL, 0.744 mmol) in dry CH_2Cl_2 (10 mL) was added a solution of Ms_2O (81 mg, 0.495 mmol) in CH_2Cl_2 (4 mL) and the

mixture was stirred for 4 h. The solvent was evaporated under reduced pressure and the resulting crude dissolved in CH_2Cl_2 and washed with a 0.1 N NH_4PF_6 solution (2 × 30 mL). The organic layer was filtered over cotton and concentrated to dryness. Purification by silica gel (with KPF_6) column chromatography (CH_2Cl_2–MeOH, 96 : 4) afforded **8** (137 mg, 87%) as a white solid. ^{1}H-NMR (400 MHz, CD_3CN) δ 7.74–7.68 (m, 4H, CH_{Ar}), 7.56–7.44 (m, 6H, CH_{Ar}), 6.23 (s, 6H, NH), 4.33 (dd, J = 4.9, 10.4 Hz, 1H, CH_2O), 4.15 (dd, J = 6.1, 10.4 Hz, 1H, CH_2O), 3.86–3.25 (m, 28H, CH_2O, CH_α, $CH_{2\gamma}$), 3.12 (s, 3H, CH_{3MsO}), 2.90–2.79 (m, 6H, CH_2S), 2.68–2.54 (m, 6H, CH_2S), 2.15–2.07 (m, 8H, $CH_{2\beta}$), 1.88–1.76 (m, 8H, $CH_{2\beta}$), 1.09 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C NMR (acetone-d_6, 100 MHz) δ 151.9 (C_{guan}), 151.8 (2 × C_{guan}), 136.3, 136.2, 133.7, 133.6, 130.9, 128.8 (CH_{Ar}, C_{Ar}), 71.7 (CH_2OMs), 66.9 (CH_2OSi), 51.2, 48.7, 48.6 (CH_α), 46.0, 45.9, 45.5 ($CH_{2\gamma}$), 37.2, 36.6 (CH_3, CH_2S), 27.2 ($CH_{3t\text{-Bu}}$), 26.3, 26.2, 23.2, 22.6 ($CH_{2\beta}$), 19.7 ($C_{t\text{-Bu}}$). MS (FAB$^+$) m/z 1545.8 (100) $[M - PF_6]^+$, 1399.6 (70) $[M - HPF_6 - PF_6]^+$, 1253.7 (30) $[M - 2HPF_6 - PF_6]^+$, 1107.5 (6) $[M - 3HPF_6 - PF_6]^+$; HRMS calcd for $[C_{53}H_{85}F_{12}N_{12}O_4P_2S_4Si]^{2+}$ 700.2421; found 700.2412.

Compound 9

To a stirred solution of **8** (40 mg, 0.024 mmol) in THF (1 mL) was added a solution of sodium mercaptoacetic acid (84 mg, 0.07 mmol) and *t*-BuOK (80 mg, 0.071 mmol) in MeOH (0.3 mL). The resulting mixture was stirred for 4 h at room temperature. The solvent was removed, and the solid residue dissolved in CH_2Cl_2 (30 mL), washed with 1 N $NaHCO_3$ (20 mL), water (20 mL) and then dried over cotton to afford **9** (32 mg, 81%) as a white solid. ^{1}H-NMR (400 MHz, MeOD) δ 7.77–7.60 (m, 4H, CH_{Ar}), 7.53–7.44 (m, 6H, CH_{Ar}), 3.81–3.78 (m, 2H), 3.72–3.63 (m, 9H), 3.51–3.41 (m, 18H), 3.03–2.91 (m, 6H), 2.83–2.73 (m, 6H), 2.28–2.18 (m, 9H), 2.03–1.91 (m, 9H), 1.09 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (400 MHz, MeOD) δ 172.39 (CO), 151.0 (C_{guan}), 135.1, 132.6, 129.6, 127.7 (CH_{Ar}, C_{Ar}), 65.8, 53.6, 49.7, 47.8, 44.9, 36.8, 36.1, 36.0, 33.1, 26.0, 25.3, 25.0, 22.4 (CH_2, CH), 18.6 (C). FAB/LSIMS m/z 585.27 $(M - 2Cl)^{2+}$.

Compound 10

Compound **8** (50 mg, 0.03 mmol) was dissolved in 0.9 mL of 30% aq. ammonia and 0.5 mL of MeOH. The reaction was stirred at room temperature for 40 min. After evaporation, the resulting solid was dissolved in CH_2Cl_2 (50 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 25 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. HPLC chromatography was performed to isolate the corresponding compound (Column: Symmetry 300 Å C18 10 × 150 HPLC; M.P.: H_2O–CH_3CN mixture with 0.05% of TFA) affording the amine derivative **10** (18 mg, 40%) as a white solid. ^{1}H NMR (CD_3CN, 500 MHz) δ 7.73–7.70 (m, 4H), 7.53–7.43 (m, 6H), 3.76–3.68 (m, 2H), 3.66–3.46 (m, 10H), 3.43–3.25 (m, 16H), 2.94–2.78 (m, 6H), 2.68–2.52 (m, 6H), 2.20–2.02 (m, 8H), 1.93–1.72 (m, 8H), 1.08 (s, 9H); ^{13}C NMR (CD_3CN, 100 MHz) δ 151.4 (C), 150.9 (C), 135.3, 133.7, 132.5, 130.6, 127.8 (CH_{Ar}, C_{Ar}), 64.8 (CH_2O), 53.8, 52.1, 50.9, 44.6, 40.2, 38.2, 37.5, 35.3, 30.8, 27.2, 26.2, 23.6 (CH_2), 19.7 (C); HRMS calcd for $[C_{52}H_{85}C_{l2}N_{13}OS_3Si]^{2+}$ 550.7665; found 550.7654.

Compound 11

To a stirred solution of *N*-Boc-cystamine (5 mg, 0.014 mmol) and Cs_2CO_3 (9 mg 0.028 mmol) in 2.2 mL of MeOH (under N_2) was added 1.4 equiv of (^{n}Bu)$_2$PhP polystyrene resin and the resulting mixture was stirred for 30 min at room temperature. Then a solution of **8** (33 mg, 0.020 mmol) in dry THF was added and the mixture was stirred for 4 h at room temperature. The mixture was filtered and the solvent evaporated. Afterwards, the crude residue was dissolved in CH_2Cl_2 (20 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 30 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. The resulting solid was treated with 0.7 mL of TFA in 0.7 mL of DCM at 0 °C. The mixture was stirred at this temperature for 2.5 h and the reaction is quenched with a saturated solution of $NaHCO_3$. The solvent was removed to dryness and the resulting oil was purified by semi-preparative HPLC (Conditions: Column: Waters Symmetry 300 Å C18 10 × 150 mm, MP: CH_3CN (0.05% TFA)–H_2O (0.05% TFA)), affording **11** (21 mg, 88%) as a white solid. ^{1}H NMR (MeOD, 500 MHz) δ 7.77–7.68 (m, 4H), 7.57–7.45 (m, 6H), 3.74–3.47 (m, 4H), 3.42–3.25 (m, 18H), 3.07–2.98 (m, 2H), 2.90–2.81 (m, 6H), 2.74–2.44 (m, 8H), 2.42–2.34 (m, 2H), 2.16–1.72 (m, 8H), 1.07 (s, 9H); ^{13}C NMR (MeOD, 500 MHz, DEPT) δ 151.3 (C), 150.7 (C), 135.5 (CH), 133.1 (C), 133.0 (C), 130.3 (CH), 128.2 (CH), 65.9 (CH_2), 65.7 (CH_2), 50.2 (CH), 49.1 (CH), 48.7 (CH), 47.6 (CH), 47.1 (CH), 46.4 (CH), 45.0 (CH_2), 44.7 (CH_2), 41.3 (CH_2), 38.2 (CH_2), 37.1 (CH_2), 36.7 (CH_2), 36.4 (CH_2), 36.2 (CH_2), 35.4 (CH_2), 26.3 (CH_2), 26.2 (CH_3), 26.0 (CH_2), 25.4 (CH_2), 25.1 (CH_2), 22.2 (CH_2), 18.7 (C). HRMS calcd for $[C_{54}H_{89}F_{18}N_{13}OP_3S_4Si]^+$ 1526.4891; found 1526.4926.

Compound 12

A mixture of mesylate **8** (30 mg, 0.018 mmol), *N*-Boc-ethylenediamine (9 µl, 0.05 mmol) and a slight excess of K_2CO_3 in acetonitrile was stirred and refluxed overnight. After cooling, the mixture was evaporated to dryness and water was added to the residue. The aqueous phase was extracted twice with CH_2Cl_2, the organic layer was filtered over cotton and concentrated *in vacuo*. The resulting solid was treated with 0.4 mL of TFA in 0.4 mL of CH_2Cl_2 at 0 °C. The mixture was stirred at this temperature for 2.5 h and the reaction is quenched with a saturated solution of $NaHCO_3$. The solvent was removed to dryness and the resulting oil was purified by semi-preparative HPLC (Conditions: Column: Waters Symmetry 300 Å C18 10 × 150 mm, MP: CH_3CN (0.05% TFA)–H_2O (0.05% TFA)), affording **12** (19 mg, 70%) as a white solid. ^{1}H NMR (MeOD, 500 MHz) δ 7.75–7.69 (m, 4H), 7.56–7.43 (m, 6H), 3.74–3.58 (m, 4H), 3.55–3.19 (m, 18H), 3.01–2.79 (m, 8H), 2.73–2.35 (m, 12H), 2.15–1.72 (m, 8H), 1.08 (s, 9H); ^{13}C NMR (MeOD, 500 MHz, DEPT) δ 151.2 (C), 150.5 (C), 135.3 (CH), 133.2 (C), 132.8 (C), 131.0 (CH), 127.2 (CH), 66.0 (CH_2), 65.7 (CH_2), 51.2 (CH), 49.5 (CH), 48.7 (CH), 47.7 (CH), 47.3 (CH),

46.7 (CH), 45.2 (CH_2), 44.7 (CH_2), 41.8 (CH_2), 39.3 (CH_2), 38.9 (CH_2), 38.1 (CH_2), 36.7 (CH_2), 36.6 (CH_2), 35.9 (CH_2), 35.1 (CH_2), 27.3 (CH_2), 26.5 (CH_3), 26.2 (CH_2), 25.6 (CH_2), 24.8 (CH_2), 23.1 (CH_2), 18.5 (C). HRMS calcd for $[C_{54}H_{89}F_{18}N_{13}OP_3S_4Si]^+$ 1413.5913; found 1413.5867.

Compound 13

Mesylate **2** (270 mg, 0.414 mmol) was dissolved in 6.1 mL of 30% aq. ammonia and 5.2 mL of MeOH. The reaction was stirred at room temperature for 40 min. After the evaporation of the solvent, the resulting solid was dissolved in CH_2Cl_2 (500 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 250 mL). The organic layer was filtered over cotton and concentrated *in vacuo* affording the amine derivative **13** (238 mg, 98%) as a white solid. ^{1}H-NMR (400 MHz, MeOD): δ 7.67–7.63 (m, 4H, CH_{Ar}), 7.46–7.40 (m, 6H, CH_{Ar}), 4.02 (s, 2H, NH_2), 3.85–3.53 (m, 4H, CH_2O, CH_α), 3.28–3.02 (m, 6H, CH_2N, $CH_{2\gamma}$), 2.15–1.83 (m, 4H, $CH_{2\beta}$), 1.05 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, MeOD) δ 150.6 (C_{guan}) 135.0, 132.2, 129.5, 127.9, 127.4 (C_{Ar}, CH_{Ar}), 64.9 (CH_2O), 48.9 (CH_α), 44.7 (CH_2N), 44.2 ($CH_{2\gamma}$), 26.4 ($CH_{3t\text{-Bu}}$), 23.2, 22.2 ($CH_{2\beta}$), 18.7 ($C_{t\text{-Bu}}$). HRMS calcd for $[C_{25}H_{36}N_4OSi]^+$ 437.2611; found 437.2585.

Compound 14

Compound **13** (134 mg, 0.23 mmol) was dissolved in 7 mL of dry CH_2Cl_2 and Et_3N (70 µL, 0.46 mmol) was added followed by the addition of 1-adamantanecarbonyl chloride (58 mg, 0.28 mmol). The reaction was stirred for 4 h. After the evaporation of the solvent, the resulting solid was dissolved in CH_2Cl_2 (500 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 250 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. Purification by silica gel column chromatography (CH_2Cl_2–MeOH, 98 : 2) afforded the relative adamantyl derivative **14** of the initial guanidine as a white solid (82% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.69–7.60 (m, 4H, CH_{Ar}), 7.50–7.37 (m, 6H, CH_{Ar}), 6.91 (s, 1H, NH), 6.75 (t, 1H, NH), 6.55 (s, 1H, NH), 3.70 (dd, J = 5.8, 10.5 Hz, 1H, CH_2O), 3.62 (dd, J = 5.9, 10.1 Hz, 1H, CH_2O), 3.59–3.43 (m, 3H, CH_2N, CH_α), 3.39–3.26 (m, 5H, CH_α, $CH_{2\gamma}$), 2.12–1.64 (m, 19H, $CH_{2\beta}$, Adamantyl), 1.07 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 181.6 (CO), 150.5 (C_{guan}), 135.5, 132.5, 130.1, 127.9 (CH_{Ar}, C_{Ar}), 65.5 (CH_2OSi), 50.8, 50.3 (CH_α), 45.9., 45.1 ($CH_{2\gamma}$), 43.1 (CH_2N), 40.6, 40.3 ($CH_{2\beta}$), 38.7 (CH_2-Adamantyl), 36.5 (C) 36.4 (CH_2-Adamantyl), 27.9 (CH-Adamantyl), 27.7 ($CH_{3t\text{-Bu}}$), 19.3 ($C_{t\text{-Bu}}$). This compound was used directly in the next step without further purification.

Compound 16

A solution of **14** (200 mg, 0.26 mmol) in 5.2 mL THF was added to a solution of hydrogen fluoride–pyridine (70% HF–30% pyr, 0.8 mL) and was stirred at room temperature overnight at room temperature. After the evaporation of the solvent, the resulting solid was dissolved in CH_2Cl_2 and washed with 1 M $NaHCO_3$ solution and 0.1 N NH_4PF_6 solution. The organic layer was filtered over cotton and concentrated *in vacuo*. Without further purification, 80 mg of compound **15** and Ms_2O (86 mg, 0.48 mmol) was dissolved in dry CH_2Cl_2 (3 mL). The mixture was treated with NMM (70 µL, 0.63 mmol) and stirred for 4 h at room temperature. After the evaporation of the solvent, the resulting solid was dissolved in CH_2Cl_2 (100 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 50 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. Purification by silica gel column chromatography (CH_2Cl_2–MeOH, 98 : 2) afforded the mesylate **16** (87%) as a white solid. ^{1}H-NMR (500 MHz, CD_3CN) δ 6.68 (s, 2H, NH), 6.53 (s, 1H, NH), 4.30 (dd, 1H, J = 4.1, 10.4 Hz, CH_2OMs), 4.16 (dd, 1H, J = 6.8, 10.4 Hz, CH_2OMs), 3.77 (m, 1H, CH_α), 3.51 (m, 1H, CH_α), 3.36 (m, 5H, CH_2N, $CH_{2\gamma}$), 3.22 (m, 1H, CH_2N), 3.33 (m, 4H, $CH_{2\gamma}$), 3.11 (s, 3H, CH_{3MsO}), 2.07–1.68 (m, 19H, $CH_{2\beta}$, Adamantyl). ^{13}C-NMR (100 MHz, CD_3CN) δ 179.8 (CO), 150.5 (C_{guan}), 70.5 (CH_2OMs), 50.1, 47.6 (CH_α), 45.4 (CH_2N), 44.6 ($CH_{2\gamma}$), 42.4 (C), 38.9 (CH_2-Adamamtyl), 36.6 (CH_3MsO), 36.2 (CH_2-Adamamtyl), 29.9 (CH-Adamantyl), 28.2 (CH-Adamantyl), 23.1, 21.7 ($CH_{2\beta}$). This compound was used directly in the next step without further purification.

Compound 18

To a solution of disulfide **3** (50 mg, 0.08 mmol) in MeOH (3 mL), Cs_2CO_3 (52 mg, 0.16 mmol) and (^{n}Bu)$_2$PhP polystyrene resin (110 mg, 0.10 mmol) was added and the mixture was stirred for 40 min. Then a solution of **7** (145 mg, 0.15 mmol) in THF (10 mL) was added and the mixture was stirred for 3 h. After evaporation of the solvent, the crude residue was dissolved in CH_2Cl_2 (20 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 30 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. Purification by silica gel column chromatography (CH_2Cl_2–MeOH, 98 : 2 → 94 : 6) afforded **17** (148 mg, 81%) as a white solid. ^{1}H-NMR (400 MHz, CD_3CN) δ 7.74–7.68 (m, 4H, CH_{Ar}), 7.55–7.44 (m, 6H, CH_{Ar}), 6.43 (bs, 6H, NH), 3.78–3.20 (m, 22H, CH_2O, CH_α $CH_{2\gamma}$), 2.89–2.80 (m, 4H, CH_2S), 2.65–2.51 (m, 4H, CH_2S), 2.17–2.06 (m, 6H, $CH_{2\beta}$), 1.90–1.65 (m, 12H, $CH_{2\beta}$), 1.09 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, CD_3CN) δ 150.8, 150.7 (C_{guan}), 135.5, 135.4, 130.1, 128.0, 127.9 (CH_{Ar}, C_{Ar}), 65.9, 63.7 (CH_2OSi, CH_2O), 50.5, 50.2, 47.8, 47.7, 47.6 (CH_α), 45.4, 45.1, 45.0 ($CH_{2\gamma}$), 35.7, 35.6 (CH_2S), 26.2 ($CH_{3t\text{-Bu}}$), 25.4, 25.2, 22.2, 22.1 ($CH_{2\beta}$), 19.0 ($C_{t\text{-Bu}}$). HRMS calcd for $[C_{43}H_{68}N_9O_2F_6PS_2Si]^{2+}$ 489.7180; found 489.7166. To a solution of alcohol **17** (148 mg, 0.12 mmol) and NMM (64 µL, 0.58 mmol) in dry CH_2Cl_2 (10 mL) was added Ms_2O (81 mg, 0.47 mmol) and the mixture was stirred for 4 h. The solvent was evaporated under reduced pressure and the resulting crude dissolved in CH_2Cl_2 and washed successively with a 0.1 N NH_4PF_6 solution (2 × 30 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. Purification by silica gel column chromatography (CH_2Cl_2–MeOH, 100 : 0 → 96 : 4) afforded **18** (145 mg, 93%) as a white solid. ^{1}H-NMR (400 MHz, CD_3CN) δ 7.74–7.68 (m, 4H, CH_{Ar}), 7.56–7.45 (m, 6H, CH_{Ar}), 6.68–6.41 (m, 6H, NH), 4.31(dd, J = 4.1, 10.4 Hz, 1H, CH_2O), 4.14 (dd, J = 7.6, 10.3 Hz, 1H, CH_2O), 3.86–3.51 (m, 20H, CH_2O, CH_α, $CH_{2\gamma}$), 3.12 (s, 3H, CH_{3MsO}), 2.90–2.80 (m, 4H, CH_2S), 2.66–2.52 (m, 4H,

CH_2S), 2.18–2.05 (m, 6H, $CH_{2\beta}$), 1.93–1.77 (m, 6H, $CH_{2\beta}$), 1.09 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, CD_3CN) δ 150.8, 150.7 (C_{guan}), 135.5, 135.4, 133.0, 130.1, 128.0 (CH_{Ar}, C_{Ar}), 70.7, 65.9 (CH_2OSi, CH_2O), 50.2, 47.7, 47.6, 47.5 (CH_α), 45.2, 45.1, 45.0, 44.6 ($CH_{2\gamma}$), 36.6 (CH_{3MsO}), 35.7, 35.6 (CH_2S), 26.2 ($CH_{3t\text{-Bu}}$), 25.2, 25.1, 22.2, 21.6 ($CH_{2\beta}$), 18.8 ($C_{t\text{-Bu}}$). HRMS calcd for $[C_{43}H_{69}N_9O_5F_6PS_2Si_2]^+$ 1056.4044; found 1056.4033.

Compound 20

A solution of mesylate **18** (260 mg, 0.19 mmol) and potassium thioacetate (112 mg, 0.96 mmol) in a mixture of THF (5 mL) and water (1.5 mL) was refluxed for 18 h. After cooling to room temperature MsOH (200 µL, 1.93 mmol) was added and the mixture was refluxed for 24 h. The reaction was cooled to room temperature, water (150 mL) and Et_2O (150 mL) were added and after extraction, the phases were separated. The aqueous phase was washed with $CHCl_3$ (150 mL) and once again with Et_2O (150 mL). After evaporation of about 50% of the aqueous layer, $KHCO_3$ (1.1 g, 10.8 mmol) was added and the solvent was evaporated to dryness. Then MeOH (100 mL) and CH_2Cl_2 (20 mL) were added, the precipitate was removed by filtration and the solvent was evaporated. This procedure was repeated a few times with increasing amounts of CH_2Cl_2, until pure CH_2Cl_2 (150 mL), resulting in a slightly yellow solid **19** (136 mg, 80%). To a solution of this product and Cs_2CO_3 (50 mg, 0.08 mmol) in MeOH (1.5 mL), (^{n}Bu)$_2$PhP polystyrene resin (150 mg, 0.12 mmol) was added and the reaction was stirred for 30 min at room temperature. Then a solution of mesylate **16** (62 mg, 0.10 mmol) in THF (3 mL) was added and the mixture was stirred for 1 h. The mixture was filtered and the solvent evaporated. After this, the crude residue was dissolved in CH_2Cl_2 (20 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 30 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 4%), to obtain **20** (127 mg, 78%) as hexafluorophosphate salt. ^{1}H NMR (400 MHz, CD_3CN) δ 6.71–6.52 (m, 5H), 3.59–3.49 (m, 8H), 3.42–3.31 (m, 18H), 2.91–2.79 (m, 8H), 2.65–2.53 (m, 9H), 2.19–1.94 (m, 16H), 1.86–1.69 (m, 15H); ^{13}C NMR (100 MHz, CD_3CN) δ 179.6 (C), 150.8 (2 × C), 150.7 (C), 150.5 (C), 67.3 (CH_2), 63.7 (CH_2), 50.5 (CH), 49.7 (CH), 47.8 (CH), 47.7 (CH), 45.4 (CH_2), 45.2 (CH_2), 45.1 (CH_2), 38.8 (CH_2), 36.2 (CH_2), 35.7 (CH_2), 29.7 (CH), 29.2 (CH), 25.3 (CH_2), 25.2 (CH_2), 25.1 (CH_2), 23.3 (CH_2), 22.2 (CH_2). ESI-MS *m*/*z* 1294.4 $(M - CF_3COO^-)^+$, 1180.4 $(M - CF_3COOH - CF_3COO^-)^+$, 1066.4 $(M - 2CF_3COOH - CF_3COO^-)^+$.

Compound 22

To a solution of alcohol **20** (101 mg, 0.066 mmol) and NMM (58 µL, 0.52 mmol) in CH_3CN (2 mL) was added a solution of Ms_2O (36 mg, 0.20 mmol) in CH_3CN (1 mL) and the mixture was stirred for 2 h at room temperature. The solvent was evaporated, CH_2Cl_2 and washed with a 0.1 M aq. NH_4PF_6 solution. Once the organic layer was extracted, the aqueous phase was extracted once more with CH_2Cl_2. The combined organic layers were filtered over cotton and concentrated to dryness and the residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 4%), affording **21** (80 mg, 75%) as hexafluorophosphate salt. ^{1}H NMR (400 MHz, CD_3CN) δ 6.77–6.26 (m, 8H, NH), 4.32 (dd, J = 4.1, 10.4 Hz, 1H, CH_2O), 4.15 (dd, J = 7.5, 10.4 Hz, 1H, CH_2O), 3.85–3.79 (m, 4H,), 3.60–3.28 (m, 22H, CH_2N, CH_α, $CH_{2\gamma}$), 3.12 (s, 3H, CH_{3MsO}), 2.89–2.80 (m, 8H, CH_2S), 2.64–2.57 (m, 4H, CH_2S), 2.19–1.98 (m, 13H, $CH_{2\beta}$, Adamantyl), 1.86–1.69 (m, 18H, $CH_{2\beta}$, Adamantyl). ^{13}C NMR (100 MHz, CD_3CN) δ 179.8 (C), 150.8 (2 × C), 150.7 (C), 150.5 (C), 70.8 (CH_2O), 64.8 (CH_2O), 60.0, 54.3 (CH_2), 49.8, 47.8, 47.7, 47.7, 47.6 (CH), 45.2, 45.2, 45.1, 44.6 (CH_2), 44.3 (CH), 42.5 (CH_2), 38.8 (CH_2), 36.6 (CH_{3MsO}), 36.1 (CH_2), 35.8, 35.7 (CH_2), 28.2 (CH), 25.3 (CH_2), 25.2 (CH_2), 25.1, 25.1, 25.0 (CH_2), 23.2, 21.6 (CH_2), 20.2, 13.5 (CH). ESI-MS *m*/*z* 1258.4 $(M - CF_3COOH - CF_3COO^-)^+$, 1144.4 $(M - 2CF_3COOH - CF_3COO^-)^+$.

To a stirred solution of di-Boc-cystamine (0.014 mmol) and Cs_2CO_3 (0.028 mmol) dissolved in 2.2 mL of MeOH (under N_2) was added 1.4 equiv. of (^{n}Bu)$_2$PhP polystyrene resin and the resulting mixture was stirred for 30 min at room temperature. Then a solution of tetraguanidine mesylate **21** (32 mg, 0.020 mmol) in dry THF was added and the mixture was stirred for 4 h at room temperature. The mixture was filtered and the solvent evaporated. After this, the crude residue was dissolved in CH_2Cl_2 (20 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 30 mL). The organic layer was filtered over cotton and concentrated *in vacuo* and purified by silica gel column chromatography (CH_2Cl_2–MeOH, 4%). The resulting solid was treated with 0.7 mL of TFA in 0.7 mL of DCM at 0 °C. The mixture was stirred at this temperature for 2.5 h and the reaction was quenched with a saturated solution of $NaHCO_3$. The solvent was removed and for the purification, a water HPLC column was used (Symmetry 300 Å C18 10 × 150 HPLC) in a mixture of H_2O and ACN with 0.05% of TFA, affording compound **22** as trifluoroacetate salt (31 mg, 70%). ESI-MS *m*/*z* 1467.5 $(M + H)^+$, 1353.6 $(M - CF_3COO^-)^+$.

Compound 23

A solution of compound **2** (423 mg, 0.64 mmol) and potassium thioacetate (224 mg, 1.92 mmol) in CH_3CN (25 mL) was heated for 7 h at 80 °C. After evaporation of the solvent the crude was dissolved in CH_2Cl_2 and washed with distilled H_2O (2 × 40 mL) and 1 N NH_4PF_6 (2 × 40 mL). The organic phase was filtered over cotton and concentrated *in vacuo* to give a crude residue which was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 99.5 : 0.5 → 98 : 2), yielding **23** (411 mg, 98%) as a reddish solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66–7.63 (m, 4H, CH_{Ar}), 7.50–7.40 (m, 6H, CH_{Ar}), 6.57 (s, 1H, NH), 6.43 (s, 1H, NH), 3.76–3.64 (m, 2H, CH_2O), 3.63–3.56 (m, 2H, CH_α), 3.46–3.26 (m, 4H, $CH_{2\gamma}$), 3.10 (dd, J = 7.7, 14.2 Hz, 1H, CH_2S), 3.08 (dd, J = 7.8, 14.2 Hz, 1H, CH_2S), 2.41 (s, 3H, CH_3CO), 2.12–2.02 (m, 2H, $CH_{2\beta}$), 1.99–1.84 (m, 2H, $CH_{2\beta}$), 1.09 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 195.8 (CO), 150.8 (C_{guan}), 135.6, 134.4, 132.6, 130.1, 127.9 (CH_{Ar}, C_{Ar}), 65.4 (CH_2O), 49.7, 48.5 (CH_α), 45.1, 45.0 ($CH_{2\gamma}$), 32.5 (CH_2S), 30.5 (CH_3CO),

26.8 ($CH_{3t\text{-}Bu}$), 24.5, 22.7 ($CH_{2\beta}$), 19.2 ($C_{t\text{-}Bu}$). ESI-MS *m/z* 496.2 [(M − PF_6^-)$^+$, 100%]. HRMS calcd for [$C_{27}H_{38}N_3O_2SSi$]$^+$ 496.2454; found 496.2429.

Compound 24

A mixture of compound **23** (214 mg, 0.334 mmol), mesylate **2** (221 mg, 0.334 mmol) and Cs_2CO_3 (304 mg, 0.93 mmol) was dissolved in 15 mL of degassed CH_3CN–MeOH (3 : 1) at 0 °C under N_2 and the solution was stirred for 2 h. The solvent was evaporated under vacuum at room temperature. The crude was dissolved in CH_2Cl_2 (20 mL) and washed with aqueous 1 N NH_4PF_6 (2 × 10 mL). The organic phase was filtered over cotton and concentrated to dryness to give a crude residue which was purified by silica gel (with KPF_6) column chromatography (CH_2Cl_2–MeOH, 96 : 4), affording symmetric diguanidinium **24** (381 mg, 94%) as a white–yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.65–7.61 (m, 8H, CH_{Ar}), 7.49–7.37 (m, 12H, CH_{Ar}), 6.24 (s, 2H, NH), 6.10 (s, 2H, NH), 3.84–3.79 (m, 2H, CH_2O), 3.75–3.49 (m, 6H, CH_2O, CH_α), 3.48–3.18 (m, 8H, $CH_{2\gamma}$), 2.93–2.80 (m, 2H, CH_2S), 2.70–2.62 (m, 2H, CH_2S), 2.18–1.99 (m, 4H, $CH_{2\beta}$), 1.98–1.79 (m, 4H, $CH_{2\beta}$), 1.06 (s, 18H, $CH_{3t\text{-}Bu}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 150.6 (C_{guan}), 135.6, 135.5, 132.8, 129.9, 127.9 (CH_{Ar}, C_{Ar}), 65.2 (CH_2O), 49.8, 47.7 (CH_α), 45.3, 44.9 ($CH_{2\gamma}$), 36.6 (CH_2S), 26.8 ($CH_{3t\text{-}Bu}$), 25.7, 22.5 ($CH_{2\beta}$), 19.2 ($C_{t\text{-}Bu}$). FAB/LSIMS *m/z* 873.4 (M – HPF_6 − PF_6^-)$^+$, 437.2 (M − $2PF_6^-$)$^{2+}$. ESI-MS *m/z* 873.5 (M − HPF_6 − PF_6^-)$^+$, 437.3 (M − 2 PF_6^-)$^{2+}$. HRMS calcd for [$C_{50}H_{69}N_6O_2SSi_2$]$^{2+}$ 873.4741; found 873.4731.

Compound 25

A solution of **24** (385 mg, 0.330 mmol) and MsOH (24 µL, 0.4 mmol) in a mixture of THF–H_2O (3 : 1, 40 mL) was heated overnight at 76 °C. The solvent was evaporated, the acid mixture diluted in water and washed with CH_2Cl_2 (2 × 50 mL). The aqueous phase was partially evaporated under reduced pressure. Afterwards $KHCO_3$ was added until a neutral pH was reached. The water was evaporated, and the crude was dissolved in a mixture of CH_2Cl_2–MeOH (1 : 20, 50 mL). The resulting precipitate was filtered off. The polarity of the solvent mixture was gradually reduced until pure CH_2Cl_2. The solvent was then evaporated to afford compound **25** (172.5 mg, 89%) as a pale-yellow powder. ^{1}H-NMR (400 MHz, MeOD) δ 3.78–3.70 (m, 2H, CH_2O), 3.69–3.41 (m, 14H, CH_2O, CH_α, $CH_{2\gamma}$), 2.98 (dd, *J* = 5.2, 13.8 Hz, 2H, CH_2S), 2.75 (dd, *J* = 7.9, 13.8 Hz, 2H, CH_2S), 2.30–1.83 (m, 8H, $CH_{2\beta}$). ^{13}C-NMR (100 MHz, MeOD) δ 152.1 (C_{guan}), 65.0 (CH_2O), 51.7 (CH_α), 46.4 ($CH_{2\gamma}$), 36.6 (CH_2S), 26.7, 23.5 ($CH_{2\beta}$). ESI-MS *m/z* 397.3 (M − MsOH − MsO^-)$^+$, 199.1 (M − 2 MsO^-)$^{2+}$. HRMS calcd for [$C_{18}H_{34}N_6O_2S$]$^+$ 397.2386; found 397.2392.

Compound 26

Compound **25** (100 mg, 0.17 mmol) and NMM (302 µL, 0.95 mmol) were mixed in dry CH_2Cl_2 (10 mL) under N_2 at 0 °C and the mixture was stirred for 5–10 min. Then, a solution of Ms_2O (237 mg, 1.36 mmol) in dry CH_2Cl_2 (4 mL) was added and stirring was continued for 24 h. The solvent was evaporated under reduced pressure and the resulting crude dissolved in CH_2Cl_2 and washed with a 0.1 N NH_4PF_6 solution (2 × 15 mL). The organic layer was filtered over cotton and left slowly to evaporate at reduce pressure. A white precipitate was filtered affording **26** (109 mg, 76%) as a white solid. ^{1}H-NMR (400 MHz, CD_3CN) δ 6.51 (bs, 4H, NH), 4.33 (dd, *J* = 4.1, 10.7 Hz, 2H, CH_2O), 4.16 (dd, *J* = 7.4, 10.5 Hz, 2H, CH_2O), 3.85–3.76 (m, 2H, CH_α), 3.59–3.51 (m, 2H, CH_α), 3.43–3.30 (m, 8H, $CH_{2\gamma}$), 3.12 (s, 6H, CH_{3MsO}), 2.83 (dd, *J* = 5.04, 14.0 Hz, 2H, CH_2S), 2.58 (dd, *J* = 8.6, 13.8 Hz, 2H, CH_2S), 2.13–2.04 (m, 4H, $CH_{2\beta}$), 1.93–1.78 (m, 4H, $CH_{2\beta}$). ^{13}C-NMR (100 MHz, CD_3CN) δ 150.8, 150.7 (C_{guan}), 70.7, 70.6 (CH_2O), 47.6, 47.4 (CH_α), 45.0, 44.7, 44.6, 43.9, 41.5 ($CH_{2\gamma}$), 36.6 (CH_{3MsO}) 35.3 (CH_2S), 24.6, 22.0, 21.5, 21.4 ($CH_{2\beta}$). FAB/LSIMS *m/z* 699.1 (M − PF_6^-)$^+$, 553.1 (M − PF_6^- − HPF_6)$^+$, 277.1 (M − $2PF_6^-$)$^{2+}$. HRMS calcd for [$C_{20}H_{38}N_6O_6S_3PF_6$]$^+$ 699.1657; found 699.1630.

Compound 27

Compound **5** (150 mg, 0.140 mmol) and potassium thioacetate (51 mg, 0,45 mmol) were dissolved in CH_3CN (10 mL) and the mixture was refluxed and stirred overnight. After cooling it to room temperature, the solvent was removed and the crude dissolved in CH_2Cl_2. Then, the solution was washed with a 0.1 N NH_4PF_6 solution (3 × 40 mL). The organic phase was filtered over cotton and concentrated to dryness. Purification by silica gel (with KPF_6) column chromatography (CH_2Cl_2–MeOH, 95 : 5) afforded **27** (125 mg, 86%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.69–7.59 (m, 4H, CH_{Ar}), 7.49–7.37 (m, 6H, CH_{Ar}), 6.27 (s, 2H, NH), 6.15 (s, 1H, NH), 6.08 (s, 1H, NH), 3.82–3.67 (m, 2H, CH_2O), 3.65–3.54 (m, 4H, CH_α), 3.49–3.20 (m, 8H, $CH_{2\gamma}$), 3.14–2.96 (m, 2H, CH_2S), 2.93–2.80 (m, 2H, CH_2S), 2.76–2.57 (m, 2H, CH_2S), 2.34 (s, 3H, CH_3CO), 2.20–2.07 (m, 4H, $CH_{2\beta}$), 1.99–1.80 (m, 4H, $CH_{2\beta}$), 1.08 (s, 9H, $CH_{3t\text{-}Bu}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 183 (CO), 150.6 (C_{guan}), 135.6, 135.5, 129.9, 127.9 (CH_{Ar}, C_{Ar}), 65.2 (CH_2O), 49.8, 47.7 (CH_α), 45.3, 45.4 ($CH_{2\gamma}$), 36.6, 36.4 (CH_2S), 30.5 (CH_3CO), 26.8 ($CH_{3t\text{-}Bu}$), 25.7, 22.5 ($CH_{2\beta}$), 19.2 ($C_{t\text{-}Bu}$). ESI-MS *m/z* 839.3 (M − PF_6^-)$^+$. HRMS calcd for [$C_{36}H_{54}N_6O_2SSi_2PF_6$]$^+$ 839.3161; found 839.3155.

Compound 28

A mixture of **27** (174 mg, 0.177 mmol), mesylate **5** (195 mg, 0.194 mmol) and Cs_2CO_3 (144 mg, 0.442 mmol) was dissolved in 25 mL of degassed CH_3CN–MeOH at 25 °C under N_2 and stirred for 6 h. The solvent was evaporated at reduced pressure at room temperature. The crude was dissolved in CH_2Cl_2 (30 mL) and washed with 1 N NH_4PF_6 (2 × 30 mL). The organic phase was filtered over cotton and concentrated at reduced pressure to give a crude which was purified by silica gel (with KPF_6) column chromatography (CH_2Cl_2–MeOH, 92 : 8), affording **12** (PF_6) (234 mg, 76%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.72–7.60 (m, 8H, CH_{Ar}), 7.51–7.38 (m, 12H, CH_{Ar}), 6.33–5.84 (bs, 7H, NH), 3.86–3.48 (m, 12H, CH_2O, CH_α), 3.47–3.18 (m, 16H, $CH_{2\gamma}$), 2.95–2.75 (m, 6H, CH_2S), 2.74–2.50

(m, 6H, CH_2S), 2.28–1.99 (m, 8H, $CH_{2\beta}$), 1.98–1.75 (m, 8H, $CH_{2\beta}$), 1.07 (s, 18H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 151.2 (C_{guan}), 135.6, 135.5, 132.8, 129.9, 127.9 (CH_{Ar}, C_{Ar}), 65.2 (CH_2O), 49.3, 48.2 (CH_α), 45.4, 44.9 ($CH_{2\gamma}$), 37.2, 37.0 (CH_2S), 26.8 ($CH_{3t\text{-Bu}}$), 25.7, 22.5 ($CH_{2\beta}$), 19.2 ($C_{t\text{-Bu}}$). ESI-MS *m*/*z* 1267.69 $(M - PF_6^- - 3HPF_6^-)^+$.

Compound 29

Compound **27** (89 mg, 0.090 mmol) was dissolved in MeOH (10 mL). Then, $(^nBu)_2$PhP polystyrene resin (103 mg, 0.090 mmol) and Cs_2CO_3 (65 mg, 0.200 mmol) were added to the solution and stirred for 20 min under N_2. Afterwards, compound **8** (127 mg, 0.075 mmol) was added and the mixture was stirred for 2 days. The solvent was evaporated under vacuum, dissolved in CH_2Cl_2 (30 mL) and washed with 0.1 N NH_4PF_6 (2 × 20 mL). The organic layer was filtered over cotton and concentrated at reduced pressure. Purification by silica gel (with KPF_6) column chromatography (CH_2Cl_2–MeOH, 98 : 2 → 94 : 6) afforded **29** (108 mg, 57%) as a light yellow solid. ^{1}H-NMR (400 MHz, CD_3CN) δ (s, 10H, NH), 7.76–7.67 (m, 8H, CH_{Ar}), 7.57–7.43 (m, 12H, CH_{Ar}), 6.24 (bs, 8H, NH), 3.75 (dd, *J* = 4.3, 10.2 Hz, 2H, CH_2O), 3.70–3.49 (m, 14H, CH_2O, CH_α), 3.46–3.26 (m, 24H, $CH_{2\gamma}$), 2.90–2.77 (m, 10H, CH_2S), 2.67–2.52 (m, 10H, CH_2S), 2.16–2.04 (m, 12H, $CH_{2\beta}$), 1.92–1.76 (m, 12H, $CH_{2\beta}$), 1.09 (s, 18H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, CD_3CN) δ 150.4 (C_{guan}), 135.6, 135.5, 134.8, 129.9, 127.9, 127.7 (CH_{Ar}, C_{Ar}), 65.4 (CH_2O), 49.1, 47.9 (CH_α), 45.4, 44.9 ($CH_{2\gamma}$), 36.8 (CH_2S), 26.7 ($CH_{3t\text{-Bu}}$), 25.5, 22.7 ($CH_{2\beta}$), 19.1 ($C_{t\text{-Bu}}$). ESI-MS *m*/*z* 2232.0 $(M - TFA^-)^+$, 1059.5 $(M - 2\,TFA^-)^{2+}$.

Compound 30

Compound **8** (10 mg, 0.006 mmol) and potassium thioacetate (2.7 mg, 0.024 mmol) were dissolved in CH_3CN (2 mL) and the mixture was refluxed and stirred overnight. After cooling it to room temperature, the solvent was removed and the crude dissolved in CH_2Cl_2. Then, the solution was washed with a 0.1 N NH_4PF_6 solution (2 × 10 mL). The organic phase was filtered over cotton and concentrated to dryness. Compound **30** (9 mg, 91%) was characterized by NMR and used directly in the next synthetic step without further purification. ^{1}H-NMR (400 MHz, CD_3CN) δ 7.76–7.67 (m, 4H, CH_{Ar}), 7.56–7.39 (m, 6H, CH_{Ar}), 3.83 (dd, *J* = 4.3, 9.8 Hz, 1H, CH_2O), 3.68 (dd, *J* = 7.3, 9.8 Hz, 1H, CH_2O), 3.65–3.41 (m, 6H, CH_α), 3.40–3.20 (m, 18H, CH_α, $CH_{2\gamma}$), 2.97–2.54 (m, 12H, CH_2S), 2.48–2.42 (m, 2H, CH_2S), 2.76–2.57 (m, 2H, CH_2S), 2.35 (s, 3H, CH_3CO), 2.12–2.02 (m, 8H, $CH_{2\beta}$), 1.91–1.65 (m, 8H, $CH_{2\beta}$), 1.05 (s, 9H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, CD_3CN) δ 135.5, 135.4, 130.0, 128.1, 128.0 (C_{Ar}), 65.3 (CH_2O), 49.3, 48.8, 48.3, 47.5 (CH_α), 44.9, 44.5, 44.3 ($CH_{2\gamma}$), 36.4, 36.3, 36.2 (CH_2S), 30.0 (CH_3CO), 26.2 ($CH_{3t\text{-Bu}}$), 25.4, 25.1, 22.4 ($CH_{2\beta}$).

Compound 31

To a solution of **30** (74 mg, 0.044 mmol) in MeOH (5 mL), Cs_2CO_3 (30 mg, 0.092 mmol) and $(^nBu)_2$PhP polystyrene resin (53 mg, 0.044 mmol) were added and stirred for 5 min under N_2 at room temperature. Afterwards, a solution of compound **8** (100 mg, 0.059 mmol) in CH_3CN (5 mL) was added to the mixture and stirred overnight. The solvent was evaporated under vacuum and the crude was triturated with 0.1 N NH_4PF_6 (2 × 20 mL) and filtered. Purification by reverse phase-C18 column chromatography (H_2O–CH_3CN: 40 : 60 → 30 : 70) afforded **31** (93 mg, 65%) as a yellowish solid. ^{1}H-NMR (400 MHz, acetone-d_6) δ 7.74–7.69 (m, 8H, CH_{Ar}), 7.56–7.45 (m, 12H, CH_{Ar}), 7.23 (s, 1H, NH), 7.18 (bs, 6H, NH), 7.05 (s, 1H, NH), 3.87–3.70 (m, 20H, CH_2O, CH_α), 3.60–3.48 (m, 32H, $CH_{2\gamma}$), 3.05–2.96 (m, 14H, CH_2S), 2.78–2.70 (m, 14H, CH_2S), 2.29–2.15 (m, 16H, $CH_{2\beta}$), 2.06–1.90 (m, 16H, $CH_{2\beta}$), 1.08 (s, 18H, $CH_{3t\text{-Bu}}$). ^{13}C-NMR (100 MHz, acetone-d_6) δ 151.4, 151.3, 151.2 (C_{guan}), 135.9, 135.8, 133.3, 133.2, 130.5, 130.4, 128.3 (CH_{Ar}, C_{Ar}), 66.4 (CH_2O), 50.6, 48.3, 48.2 (CH_α), 45.6, 45.5 ($CH_{2\gamma}$), 36.3, 36.2 (CH_2S), 26.7 ($CH_{3t\text{-Bu}}$), 25.9, 25.8, 22.8 ($CH_{2\beta}$), 19.3 ($C_{t\text{-Bu}}$). HRMS calcd for $[C_{104}H_{166}F_{36}N_{24}O_2P_6S_7Si]^{+2}$ 1466.4525; found 1466.4358.

Compound 32

To a stirred solution of **2** (200 mg, 0.30 mmol) in dry THF (20 mL) was added a solution of 1-decanethiol (130 µL, 0.61 mmol) treated with *t*-BuOK (85 mg, 0.76 mmol) in THF–MeOH (1 : 1). The resulting mixture was stirred for 4 h at room temperature. The solvent was removed and the solid residue dissolved in CH_2Cl_2 (30 mL) and washed with a solution of 0.1 N NH_4PF_6 (2 × 30 mL). The organic phase was filtered over cotton, and concentrated *in vacuo* to give a crude residue which was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 100 : 0 → 98 : 2), affording **32** (226 mg, quant.) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.66–7.63 (m, 4H, CH_{Ar}), 7.43–7.41 (m, 6H, CH_{Ar}), 6.05 (s, 1H, NH), 5.88 (s, 1H, NH), 3.67–3.64 (m, 2H, CH_2OSi), 3.56–3.44 (m, 2H, CH_α), 3.36–3.29 (m, 4H, $CH_{2\gamma}$), 2.67–2.60 (m, 2H, CH_2S), 2.52 (t, *J* = 7.5 Hz, 2H, SCH_{2dec}), 2.21–2.09 (m, 2H, $CH_{2\beta}$), 1.96–1.80 (m, 2H, $CH_{2\beta}$), 1.61–1.48 (m, 2H, CH_{2dec}), 1.46–1.26 (m, 14H, CH_{2dec}), 1.06 (s, 9H, $CH_{3t\text{-Bu}}$), 0.88 (t, *J* = 6.8 Hz, 3H, CH_{3dec}). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 150.4 (C_{guan}), 135.6, 135.5, (CH_{Ar}), 132.6 (C_{Ar}), 130.1, 130.0, 128.0 (CH_{Ar}), 65.5 (CH_2OSi), 50.2, 48.5 (CH_α), 45.6, 45.3 ($CH_{2\gamma}$), 36.3 (CH_2S), 32.6 (SCH_{2dec}), 31.9, 29.6, 29.5, 29.5, 29.3, 29.2 29.1, 28.8 (CH_{2dec}), 26.8 ($CH_{3t\text{-Bu}}$), 25.2 ($CH_{2\beta}$), 22.6 ($CH_{2\beta}$), 19.2 ($C_{t\text{-Bu}}$), 14.1 (CH_{3dec}). HRMS calcd for $[C_{35}H_{56}N_3OSSi]^+$ 594.3916; found 594.3895.

Compound 33

Compound **32** (227 mg, 0.30 mmol) was dissolved in dry THF (15 mL). Then, 1 M THF TBAF solution (922 µL, 0.68 mmol was added to this solution and stirred under inert atmosphere overnight. The solvent was stripped off under vacuum and the compound redissolved in CH_2Cl_2 (25 mL) and was washed with a solution of 0.1 N NH_4PF_6 (15 mL). The aqueous phase was reextracted with CH_2Cl_2 (2 × 20 mL) and $CHCl_3$ (20 mL). The organic phase was filtered over cotton, and concentrated *in vacuo* to give a crude residue which was purified by silica gel column

chromatography (CH_2Cl_2–MeOH, 100 : 0 → 95 : 5), affording **33** (118 mg, 77%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.62 (s, 1H, NH), 6.38 (s, 1H, NH), 3.85 (d, J = 7.8 Hz, 1H, CH_2OH), 3.67–3.30 (m, 7H, CH_2OH, CH_α, $CH_{2\gamma}$), 2.73 (dd, J = 6.0, 13.7 Hz, 1H, CH_2S), 2.62 (dd, J = 7.9, 13.7 Hz, 1H, CH_2S), 2.55 (t, J = 7.5 Hz, 2H, SCH_{2dec}), 2.19–2.00 (m, 2H, $CH_{2\beta}$), 1.96–1.77 (m, 2H, $CH_{2\beta}$), 1.62–1.55 (m, 2H, CH_{2dec}), 1.39–1.28 (m, 14H, CH_{2dec}), 0.90 (t, J = 6.8 Hz, 3H, CH_{3dec}). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 151.0 (C_{guan}), 64.4 (CH_2OH), 50.5, 48.2 (CH_α), 45.7, 45.4 ($CH_{2\gamma}$), 36.8 (CH_2S), 32.6 (SCH_{2dec}), 31.9, 29.7, 29.6, 29.5, 29.3, 28.8, (CH_{2dec}), 25.5 ($CH_{2\beta}$), 22.7 ($CH_{2\beta}$), 14.1 (CH_{3dec}). ESI-MS m/z 356.2 [$(M - PF_6^-)^+$, 100%]. HRMS calcd for $[C_{19}H_{38}N_3OS]^+$ 356.2735; found 356.2730.

Compound 34

To a solution of **33** intermediate (118 mg, 0.24 mmol) and NMM (157 μL, 1.41 mmol) in dry THF (15 mL) was added Ms_2O (164 mg, 0.94 mmol). The reaction mixture was stirred for 4 h at room temperature. After evaporating the solvent, the resulting crude was dissolved in CH_2Cl_2 (30 mL) and washed with a 0.1 N NH_4PF_6 solution (2 × 20 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. Purification by silica gel column chromatography (CH_2Cl_2–MeOH, 100 : 0 → 96 : 4) afforded **34** (117 mg, 86%) as a yellowish oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.21 (s, 1H, NH), 6.17 (s, 1H, NH), 4.28 (dd, J = 4.3, 10.5 Hz, 1H, CH_2OMs), 4.10 (dd, J = 6.7, 10.5 Hz, 1H, CH_2OMs), 3.82–3.76 (m, 1H, CH_α), 3.49–3.45 (m, 1H, CH_α), 3.40–3.26 (m, 4H, $CH_{2\gamma}$), 3.07 (s, 3H, CH_{3MsO}), 2.60–2.58 (m, 2H, CH_2S), 2.47 (t, J = 7.4 Hz, 2H, SCH_{2dec}), 2.16–2.05 (m, 2H, $CH_{2\beta}$), 1.96–1.80 (m, 2H, $CH_{2\beta}$), 1.52–1.46 (m, 2H, CH_{2dec}), 1.30–1.19 (m, 14H, CH_{2dec}), 0.81 (t, J = 6.8 Hz, 3H, CH_{3dec}). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 150.5 (C_{guan}), 70.4 (CH_2OMs), 48.3, 47.8 (CH_α), 45.6, 44.9 ($CH_{2\gamma}$), 37.0 (CH_{3MsO}), 36.4 (CH_2S), 32.4 (SCH_{2dec}), 31.9, 29.6, 29.5, 29.5, 29.3, 29.2, 28.8 (CH_{2dec}), 25.3 ($CH_{2\beta}$), 22.7 (CH_{2dec}), 21.8 ($CH_{2\beta}$), 14.1 (CH_{3dec}). HRMS calcd for $[C_{20}H_{40}N_3O_3S_2]^+$ 434.2511; found 434.2510.

Compound 35

A mixture of diguanidinium compound thioacetate **27** (59 mg, 0.06 mmol), mesylate **34** (38 mg, 0.07 mmol) and Cs_2CO_3 (49 mg, 0.15 mmol) was dissolved in 20 mL of degassed CH_3CN–MeOH at room temperature under N_2 and stirred for 4 h. The solvent was evaporated and the crude was dissolved in CH_2Cl_2 (30 mL) and washed with 1 N NH_4PF_6 (2 × 10 mL). The organic phase was filtered over cotton and concentrated *in vacuo* to give a crude residue which was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 100 : 0 → 96 : 4), affording **35** (73 mg, 87%) as a yellowish solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.73–7.65 (m, 4H, CH_{Ar}), 7.51–7.42 (m, 6H, CH_{Ar}), 6.65–6.03 (bs, 3H, NH), 3.87–3.16 (m, 20H, CH_2OSi, CH_α,$CH_{2\gamma}$), 2.91–2.45 (m, 10H, CH_2S), 2.34–1.74 (m, 12H, $CH_{2\beta}$), 1.59–1.55 (m, 2H, SCH_{2deca}), 1.43–1.21 (m, 14H, CH_{2deca}), 1.08 (s, 9H, $CH_{3t\text{-}Bu}$), 0.89 (t, J = 7.0 Hz, 3H, CH_{3dec}). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 150.7 (C_{guan}), 135.6, 135.5 (CH_{Ar}), 132.7, 132.6 (C_{Ar}), 130.0, 127.9 (CH_{Ar}), 65.4 (CH_2OSi), 53.5, 50.1, 49.5, 47.8, 47.7, 47.3 (CH_α), 45.6, 45.5, 45.4, 45.3, 45.0 ($CH_{2\gamma}$), 36.5, 36.4, 36.2 (CH_2S), 32.6 (SCH_{2deca}), 31.9, 29.7, 29.6, 29.5, 29.3, 29.2, 28.8, 28.7 (CH_{2deca}), 26.8 ($CH_{3t\text{-}Bu}$), 26.0, 25.7, 25.6, 25.1 ($CH_{2\beta}$), 22.7, 22.5 ($CH_{2\beta}$), 18.9 ($C_{t\text{-}Bu}$), 14.1 (CH_{3deca}). HRMS calcd for $[C_{53}H_{86}N_9OS_3Si]^+$ 988.5881, found 988.5795.

Compound 37

A solution of **35** (64 mg, 0.05 mmol) and TBAF (1M THF solution, 137 μL, 0.14 mmol) in THF (20 mL) was stirred overnight. The solvent was stripped off and the crude dissolved in CH_2Cl_2 (30 mL) and washed with 1 N NH_4PF_6 (10 mL). The organic phase was filtered over cotton and concentrated *in vacuo* to give a crude residue which was purified by silica gel (with KPF_6) column chromatography (CH_2Cl_2–MeOH, 100 : 0 → 92 : 8), giving rise to **36** intermediate (42 mg, 79%) as a white solid, ready to be used in the next synthetic step. ^{1}H-NMR (400 MHz, CD_3CN) δ 7.25–7.06 (bs, 2H, NH), 6.98–6.77 (bs, 3H, NH), 3.66 (dd, J = 3.7, 10.4 Hz, 1H, CH_2OH), 3.62–3.29 (m, 19H, CH_2O, CH_α,$CH_{2\gamma}$), 2.94–2.87 (m, 3H, CH_2S), 2.78 (dd, J = 5.3, 13.7 Hz, 1H, CH_2S), 2.66–2.50 (m, 6H, CH_2S), 2.18–2.05 (m, 6H, $CH_{2\beta}$), 1.88–1.69 (m, 6H, $CH_{2\beta}$), 1.67–1.52 (m, 2H, SCH_{2dec}), 1.44–1.26 (m, 16H, CH_{2dec}), 0.91 (t, J = 6.9 Hz, 3H, CH_{3dec}). To a solution of alcohol **36** intermediate (42 mg, 0.04 mmol) and NMM (23.3 μL, 0.21 mmol) in dry THF (10 mL) was added Ms_2O (24 mg, 0.14 mmol) and the mixture was stirred for 4 h. The solvent was evaporated under reduced pressure and the resulting crude dissolved in CH_2Cl_2 and washed successively with a 0.1 N NH_4PF_6 solution (2 × 30 mL). The organic layer was filtered over cotton and concentrated *in vacuo*. Purification by silica gel (with KPF_6) column chromatography (CH_2Cl_2–MeOH, 94 : 6) afforded **37** (21 mg, 48%) as a white solid. ^{1}H-NMR (400 MHz, CD_3CN) δ 7.25–6.55 (bs, 4H, NH), 4.32 (dd, J = 3.8, 10.3 Hz, 1H, CH_2OMs), 4.19–4.09 (m, 1H, CH_2OMs), 3.86–3.76 (m, 1H, CH_α), 3.65–3.27 (m, 17H, CH_α,$CH_{2\gamma}$), 3.13 (s, 3H, CH_{3MsO}), 2.99–2.83 (m, 3H, CH_2S), 2.78 (dd, J = 5.2, 13.9 Hz, 1H, CH_2S), 2.66–2.49 (m, 6H, CH_2S), 2.16–2.05 (m, 6H, $CH_{2\beta}$), 1.90–1.73 (m, 6H, $CH_{2\beta}$), 1.66–1.51 (m, 2H, SCH_{2dec}), 1.46–1.24 (m, 16H, CH_{2dec}), 0.91 (t, J = 6.7 Hz, 3H, CH_{3dec}). ^{13}C-NMR (100 MHz, HSQC-DEPTQ, CD_3OD) δ 150.0 (C_{guan}), 70.1 (CH_2OMs), 48.0, 47.5, 47.5 (CH_α), 45.1, 44.9, 44.7, 44.4 ($CH_{2\gamma}$), 36.0 (CH_{3MsO}), 36.1, 36.0 (CH_2S), 31.7 (SCH_{2dec}), 29.3, 29.2, 29.2, 29.1, 29.0, 28.4 (CH_{2dec}), 25.3, 25.2, 25.1, 25.0 ($CH_{2\beta}$), 22.4 (CH_{2dec}), 22.2, 21.9 ($CH_{2\beta}$), 13.1 (CH_{3dec}). HRMS calcd for $[C_{38}H_{71}F_6N_9O_3PS_4]^+$ 974.4227; found 974.4218.

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