Solid-Phase Synthesis of Chiral Bicyclic Guanidinium Oligomers

Vera Martos,[†,‡] Pilar Castreño,[†] Miriam Royo,[§,⊥] Fernando Albericio,[||,⊥,#] and Javier de Mendoza*[,†,‡]

Institute of Chemical Research of Catalonia (ICIQ), Avgda. Països Catalans 16, 43007 Tarragona, Spain, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain, Combinatorial Chemistry Unit, Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain, and Institute for Research in Biomedicine, Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain, CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain, and Department of Organic Chemistry, University of Barcelona, Martí i Franqués 1, 08028 Barcelona, Spain

Received December 12, 2008

A simple solid-phase synthesis of thioether-linked chiral bicyclic guanidinium oligomers for cell internalization purposes has been developed. The approach is based on a Merrifield-like peptide synthesis on Rinkamide-*p*-methylbenzhydrylamine resin functionalized with Cys(methoxytrityl). A difunctionalized bicyclic guanidinium synthon, bearing both electrophile (*O*-mesyl) and protected nucleophile (*S*-methoxytrityl) group, is repeatedly grafted via a nucleophilic substitution. The sequence requires removal of methoxytrityl, reduction with 1,4-dithiothreitol to cleave any adventitious disulfides, coupling and capping with benzyl bromide. Moreover, Alloc protection of the α-amino group of the initial cysteine, provides a potential handle for cargo attachment after oligomer elongation to the desired internalizing agent and prior to cleaving it from the resin. Finally, a bicyclic guanidinium monomer containing an amino group and a carboxylic acid function has been evaluated as an alternative building block for novel amide-bridged oligomers or peptidomimetics.

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

Introduction

The therapeutic efficacy of a drug depends on its ability to reach the desired target tissues, cells, or intracellular organels. However, the ability to translocate cellular membranes still remains a major drawback in current drug development. In this context, linkage to so-called trojan horse compounds represents a promising strategy to overcome this difficulty. Molecular and drug transporters are most often functionalized with guanidinium groups because these polycations have been shown to be crucial for interaction with phosphate and other anionic moieties on the liposomal cell surface.[1]

We recently reported on a new family of tetraguanidinium compounds, in which bicyclic guanidinium subunits are linked by thioether spacers (Figure 1).[2] Cell-penetration of these conjugates was studied in HeLa cells by confocal microscopy and FACS analysis and compared with fluorescently labeled Tat_{49-57} and Antp. Interestingly, the capacity to cross the cell membrane of the reported silylated derivative **I** (R_1 = $SiPh_2{}^tBu$) outperformed its hydroxyl analogue **II** (R_1 = OH) and peptide controls at low concentrations (0.1−1 μM). Moreover, the lead compound internalized selectively into mitochondria, a property which might be of therapeutic relevance.[3] Recently, also the mitochondrial compartmentalization of an emissive terbium complex conjugated to a tetraguanidinium vector (**III**) has been observed in HeLa, 3T3, and CHO cells.[4]

However, high toxicity rates with IC_{50} around the low micromolar range were observed in both studies. Therefore, systematic structure−function studies should be developed to evaluate the influence of length, end-functionalization, linker and cargo on cellular uptake efficiency as well as on cell toxicity. For the purpose of preparing a series of bicyclic

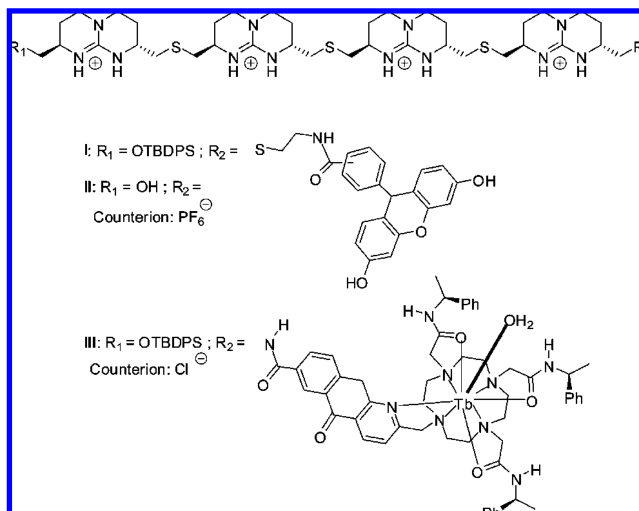

Figure 1. Chemical structure of fluoresceine derivatives **I** and **II** and terbium complex **III**, endowed with bicyclic tetraguanidinium transporter molecules.

* To whom correspondence should be addressed. E-mail: jmendoza@iciq.es.
[†] Institute of Chemical Research of Catalonia (ICIQ).
[‡] Universidad Autónoma de Madrid.
[§] Combinatorial Chemistry Unit, Barcelona Science Park.
[||] Institute for Research in Biomedicine, Barcelona Science Park.
[⊥] Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park.
[#] Department of Organic Chemistry, University of Barcelona.

10.1021/cc800207v CCC: $40.75 © 2009 American Chemical Society
Published on Web 03/06/2009

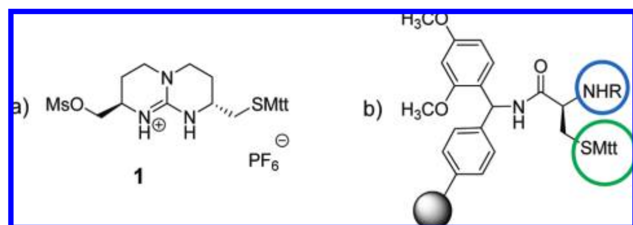

Figure 2. (a) Difunctionalized monomer **1** and (b) R-Cys(Mtt)-Rinkamide endowed with a site for guanidinium coupling via thioether formation (highlighted in green) and another for cargo attachment (highlighted in blue).

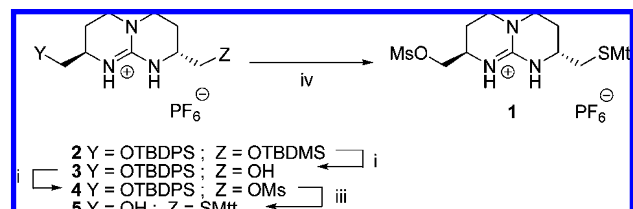

Figure 3. Synthesis of BB **1**: (i) H_2O/AcOH/THF (1:2:1), rt, 24 h, 95%; (ii) NMM, Ms_2O, dry DCM, rt, 2 h, 90%; (iii) NaOMe 0.25M, SHMtt, rt−50 °C, 18 h, 85%; (iv) NMM, Ms_2O, dry DCM, rt, 2 h, 90%.

guanidinium oligomers, a synthetic strategy on solid support to enable the iterative incorporation of guanidinium building blocks, was undertaken.

Herein we report such a solid-phase synthesis, using polythioether oligomers containing the bicyclic guanidinium units. In addition, we propose a bicyclic difunctional building block as amino acid replacement for the synthesis of peptidomimetics.

Our strategy is based in a repertoire of compatible protecting groups (Figure 2). The difunctionalized molecule **1** contains a good leaving group: mesylate (OMs) and a thiolate precursor, which is masked with the methoxytrityl (Mtt) protecting group, which can be removed using dilute trifluoroacetic (TFA) solutions in the presence of triisopropylsilane (TIS) as scavenger. As the first building block (BB) we selected Cys, protected at the thiol with Mtt, which will allow for elongation of the oligomer chain, and a semipermanent protecting group (R) for the α amino function, which should be suitable for cargo attachment. Finally, a Rinkamide resin, which ensures final detachment with TFA, was used as solid support. After incorporation of each building block, a capping step was carried out to prevent formation of deletion sequences which could jeopardize the final purification.

Results and Discussion

The synthesis of BB **1** is depicted in Figure 3 and proceeds from the orthogonally diprotected precursor **2**, which can be obtained on a large scale in eight steps from amino acids R-Asn and R-Met.[5] The silyl group (TBDMS) was removed from **2** and the free OH compound **3** was activated as mesylate **4** to incorporate the 4-methoxytrityl mercaptane moiety (SHMtt) through a nucleophilic substitution.[6,7] Simultaneously, the second hydroxyl terminus was desily-

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

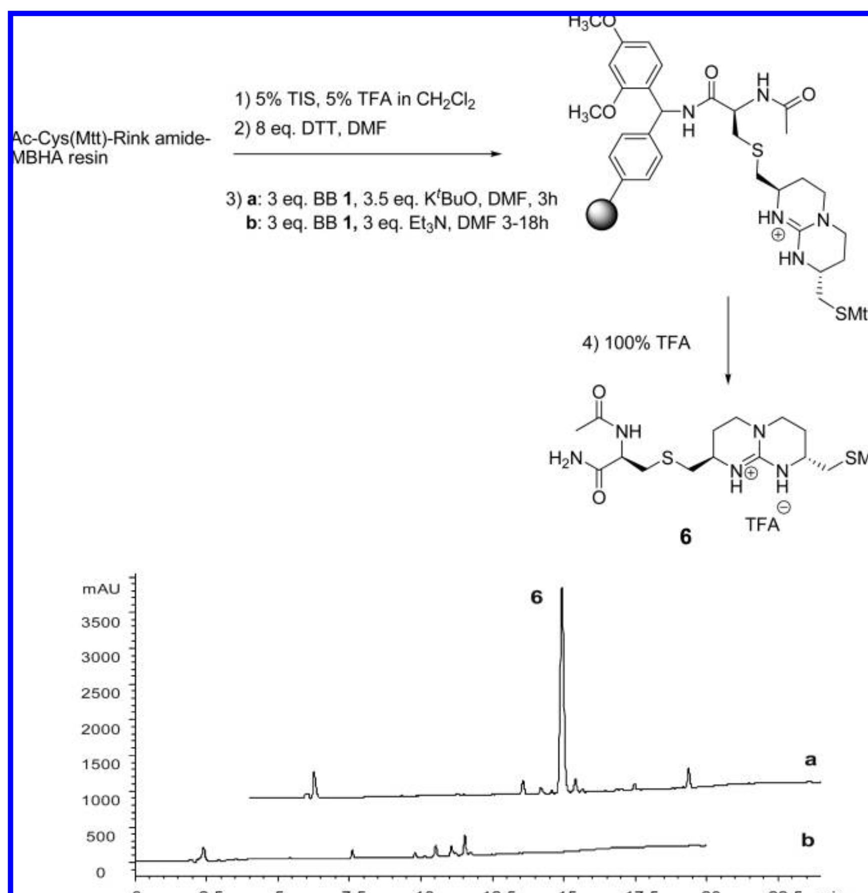

Figure 4. Reaction scheme for BB **1** coupling (top) and their HPLC crude profiles: (a) with K^{t}BuO as base and (b) with Et_3N (5−95% MeCN in H_2O with 0.1% TFA in 17 min, UV_{210}).

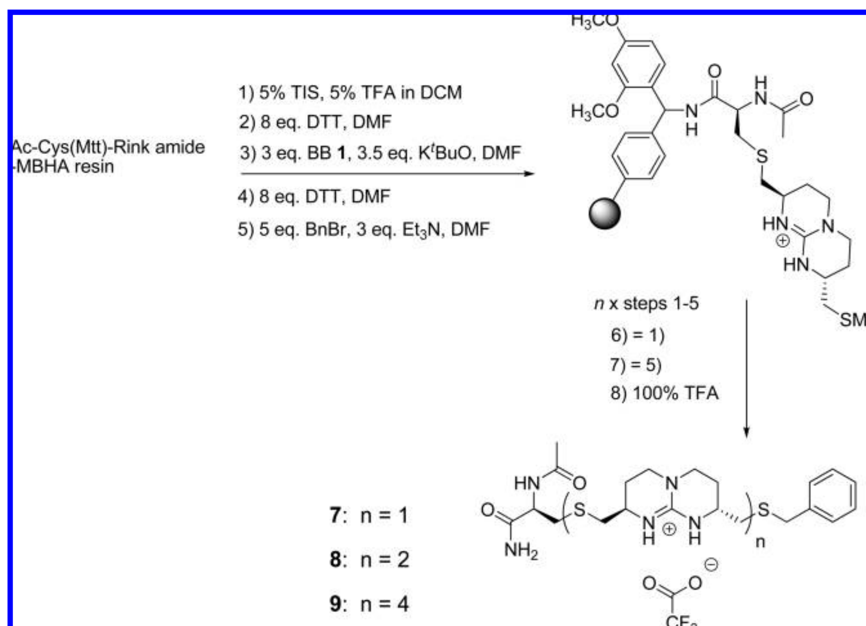

Figure 5. Synthesis of bicyclic oligomers **7** (for $n = 1$), **8** (for $n = 2$), and **9** (for $n = 4$).

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

lated to give **5**. Next, the alcohol was again converted into a mesylate to obtain the desired BB **1** in a high overall yield (65%).

Initial attempts to couple an electrophile via thioether formation on the Cys-resin were performed with R = Ac, because NHFmoc does not survive the basic coupling conditions required. Benzyl bromide was employed beforehand as a model electrophile to optimize coupling conditions.

Although Cys thiol is a strong nucleophile in basic media,[8−10] it is usually employed on solid support as anchor site for moieties via disulfide formation.[11] Alternatively, S_N reactions have been reported for solid-support anchorage of mercaptans to chlorotrityl,[12,13] Wang's bromo,[14] and CPG resins.[15] However, to the best of our knowledge, nucleophilic attack of bound mercaptans on electrophiles has never been applied on an iterative solid-phase synthesis.

Formation of disulfide subproducts is a serious drawback, especially for S_N2 reactions based on thiols. In solution, reduction agents such as triphenylphosphine or tri-*n*-butylphosphine are currently employed to avoid competing disulfides generation. In our case, phosphines were ineffective as reducing agents or even led to complex crudes after cleavage.

Instead, a solution of 1,4-dithiothreitol (DTT) in *N,N'*-dimethylformamide (DMF) was employed to treat the resins before nucleophilic coupling and to ensure the reduced state of the thiols. The Ellman test was used qualitatively before and after nucleophilic couplings, preceded in this case by an additional treatment with DTT, to assess for the presence of free thiols on the resin. With benzyl bromide as model electrophile we evaluated different coupling conditions with either Et_3N or K^{t}BuO. The results were similar for both bases and the quantitative coupling of benzyl bromide was achieved with 5 equiv of electrophile in the presence of the organic base and in the absence of phosphines (Supporting Information Figures 1 and 8).

Next, nucleophilic coupling with BB **1** was tried under similar conditions, using Et_3N or K^{t}BuO as bases. Surprisingly, no coupling was obtained with the organic base even after 18 h at room temperature, contrary to the results with K^{t}BuO (Figure 4).

Analytical HPLC pointed to a high yield coupling of monomer **1**, but an Ellman test after DTT treatment revealed that not all thiol groups had reacted. Therefore, quantitative capping with benzyl bromide was systematically performed after BB **1** anchorage. Moreover, as partial removal of Mtt during the cleavage was detected, the resin was functionalized with a non hydrolizable moiety, namely benzyl bromide, prior to cleavage.

Consequently, the following synthetic sequence was established (Figure 5): First Mtt was removed from the mercaptan, followed by treatment of the Cys resin (R = Ac) with DTT to reduce any disulfide bridge left. Next, nucleophilic coupling with BB **1** (3 equiv) under basic conditions (K^{t}BuO, 3.5 equiv) was performed and the treatment with DTT was repeated. Thereafter the resin was capped with benzyl bromide following the aforementioned protocol, and the sequence was repeated *n* times to elongate the oligomer. Before cleavage from the resin, Mtt was removed, and the final thiol was capped with benzyl bromide.

The crude HPLC profiles from monoguanidinium **7**, diguanidinium **8**, and tetraguanidinium **9** derivatives showed the major peaks corresponding to the expected products (Figure 6), accompanied by minor peaks accounting for incomplete couplings (non optimized average coupling yields were ≥75% as calculated from the HPLC).

Compounds **7**, **8**, and **9** were subsequently purified by semipreparative HPLC and 33%, 21%, and 12% yields were obtained, respectively (for final HPLC profiles and ^{1}H NMR of compounds **7**−**9**, see Supporting Information Figures 5−7).

The coupling yield might be improved by repeating the coupling step for a second time after a DTT treatment. However, in view of the easy purification of the final product and the synthetic difficulty of preparing BB **1**, a single coupling followed by a capping treatment was preferred.

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

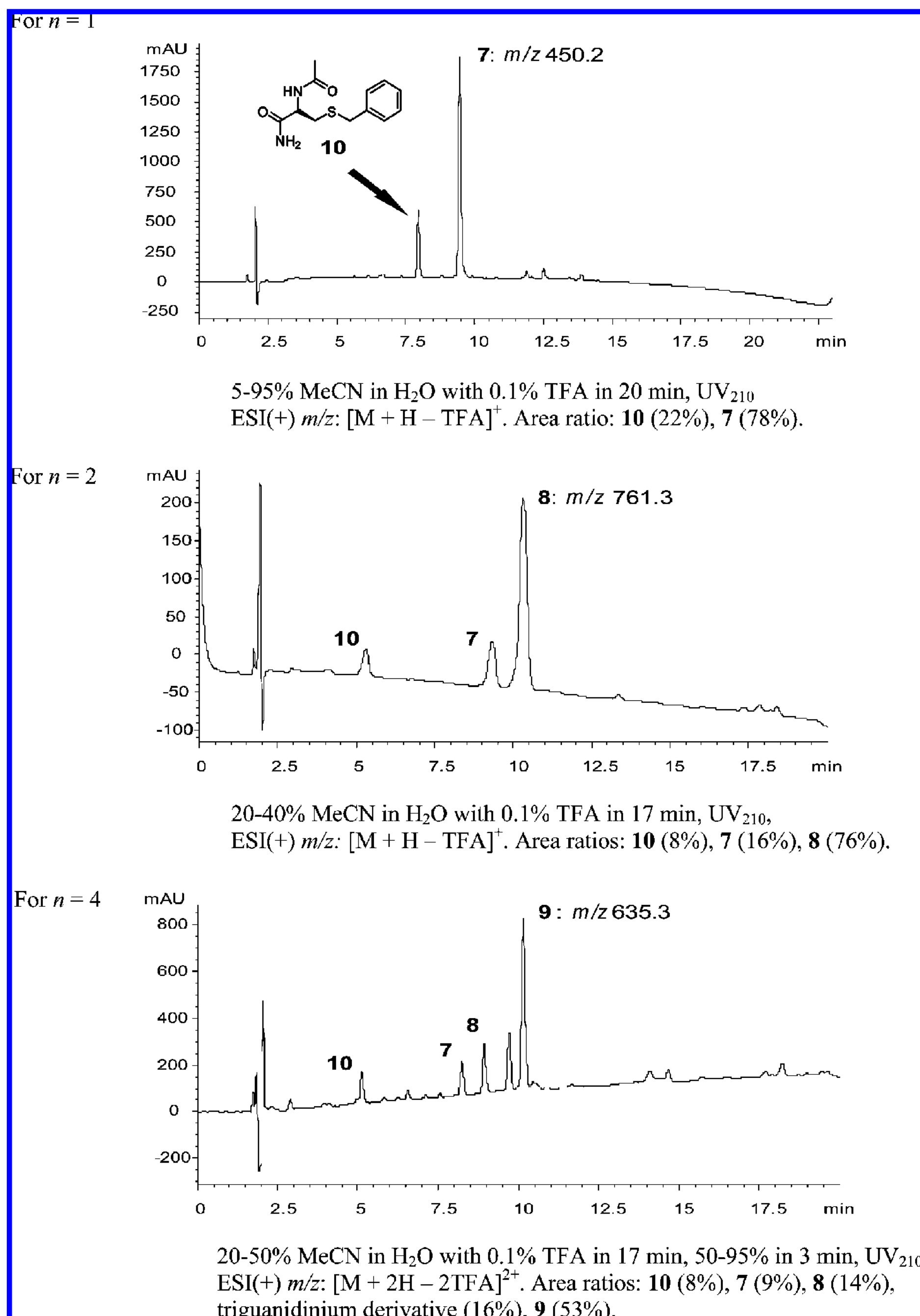

Figure 6. RP-HPLC profiles of crude compounds **7** (for $n = 1$), **8** (for $n = 2$), and **9** (for $n = 4$), ESI(+) m/z and area ratios between major peaks.

[Compound **1** was attempted to be recovered from the reaction mixture. ESI(+) and ^{1}H NMR analysis of the final reaction mixture showed that the content of non reacted BB **1** was smaller than expected. BB **1** hydrolyzes partially in the reaction mixture to its OH precursor **5** and some reacts with DMF, thus generating a mixture of closely related compounds whose separation is cumbersome.] In addition, semipreparative purification protocols enable the isolation of oligomers of different length from the same cleavage crude. Thus this strategy is suited to generate, in a short time, a series of guanidinium strands for their preliminary evaluation as internalizing vectors, although optimized conditions would be required for a large scale synthesis of the most promising candidates.

Moreover, a new building block, BB **11**, composed of two bicyclic guanidinium subunits was prepared and assayed for a faster and more convenient growth of the oligoguanidinium chain. Diguanidinium BB **11** was synthesized in a manner analogous to that of BB **1** (Figure 7).

Preliminary coupling of BB **11** was assayed on a resin functionalized with a tetraguanidinium oligomer, obtained after coupling four times BB **1.** The nucleophilic conditions were kept the same. The resulting HPLC profile of the crude after cleavage is shown in Figure 8.

The hexaguanidinium derivative was obtained from the tetraguanidinium precursor as confirmed by ESI(+) (Supporting Information Figure 9). Apparently, the coupling

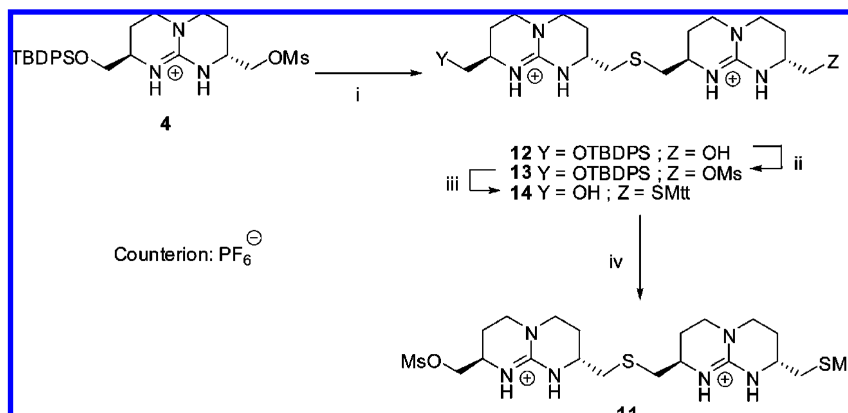

Figure 7. Synthesis of BB **11**: (i) (a) $KSCOCH_3$, MsOH, THF/water (3:1), reflux, 24 h, $KHCO_3$, 72%, (b) 4, P(Bu)3 pb, Cs_2CO_3, MeOH/THF (2:1), rt, 5h, 70%; (ii) NMM, Ms_2O, dry CH_2Cl_2, rt, 2h, 85%; (iii) NaOMe 0.25 M, SHMtt, rt−50 °C, 18 h, 79%; (iv) NMM, Ms_2O, dry CH_2Cl_2, rt, 2 h, 83%.

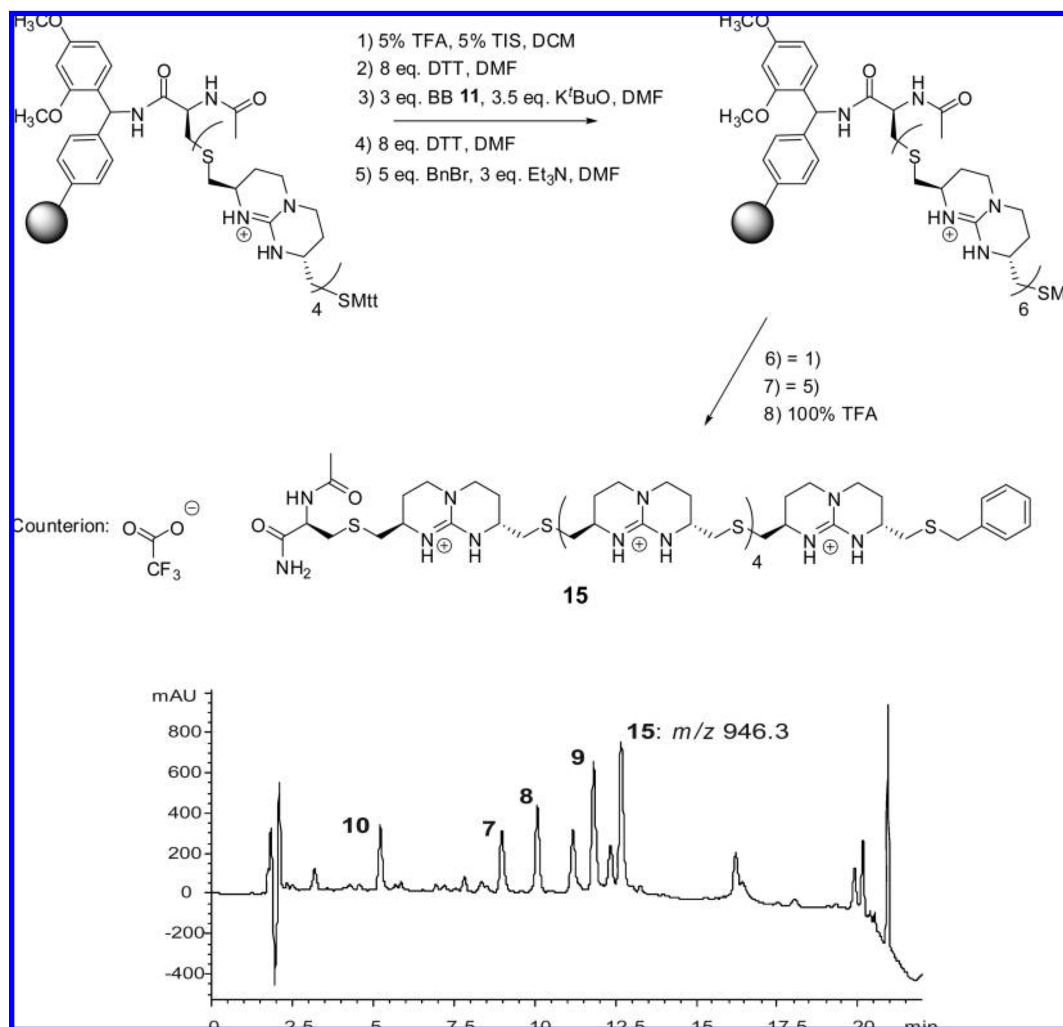

Figure 8. Reaction scheme for compound **15** (top) and its HPLC crude profile after cleavage (bottom) (20−45% MeCN in H_2O with 0.1% TFA in 17 min, 45−95% in 3 min, UV_{210}. ESI(+) *m/z* $[M + 2H - 2TFA]^{2+}$. Area ratio: **9** (47%), **15** (53%)).

of BB **11** was less efficient than that of BB **1**. However, BB **11** should be considered as an alternative to BB **1** for longer oligomers.

Introduction of the cargo should take place more conveniently at the end of the chain elongation to avoid incompatibilities with the vector synthesis. As mentioned above, Fmoc is not compatible as a semipermanent protection of the cysteine α-amino group, because it does not survive the coupling conditions. Alternatively, Alloc, which is orthogonal to Mtt and Rinkamide resin and also stable to the basic conditions used for the elongation step, was employed. Moreover, removal of Alloc from the solid support has been reported under inert conditions in the presence of phenyl-silane and tetrakis(triphenyl-phosphine)palladium, which is compatible with the guanidinium oligomer.[16] This should allow the introduction of the cargo at any step of the synthetic process.

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

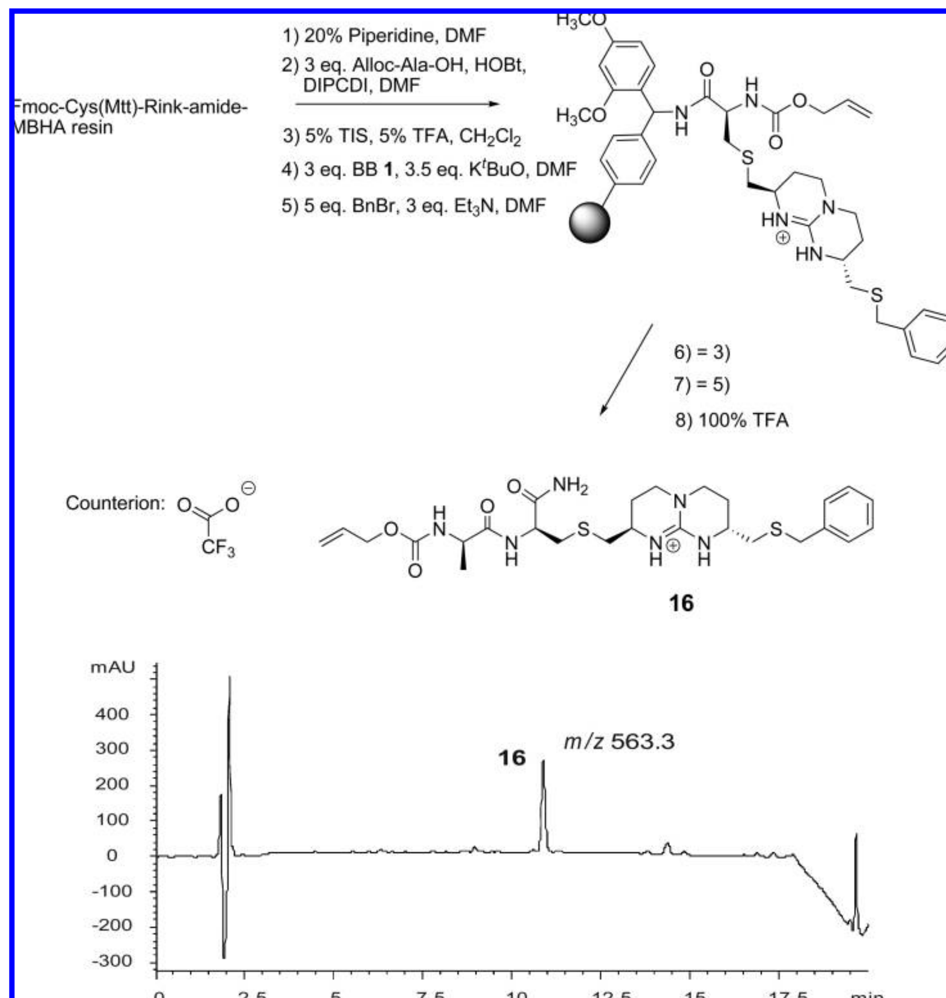

Figure 9. Incorporation of Alloc-Ala-OH into the amino anchorage point (top) and HPLC crude profile of compound **16** (bottom) (20−50% MeCN in H_2O with 0.1% TFA in 17 min, 50−95% in 3 min, UV_{210}. ESI(+) m/z $[M + H - TFA]^+$).

To assay the feasibility of the use of this protecting group with the solid-phase synthetic strategy developed for guanidinium oligomers, Alloc-L-Ala-OH was introduced at the Cys resin prior to incorporation of BB **1**.[The residue Alloc-Ala-OH was introduced to further separate the cargo from the vector, which should facilitate cargo anchorage without disrupting the biological process.]

After introduction of the first BB **1** subunit, TFA cleavage was performed and the crude was analyzed by HPLC (Figure 9).

Figure 9 shows a single peak, highlighting the stability of the Alloc protection and therefore the validation of the full synthetic strategy. Finally, compound **16** was purified by semipreparative HPLC (40% yield, see Supporting Information Figure 10).

Amino Acid-Type Bicyclic Guanidinium, BB 4. Going a step further toward the mimicking a solid-phase peptide synthesis (SPPS), we considered replacing the thioether linkage for an amide bond. Thus, a difunctionalized BB with a carboxylic function on one terminus and a protected amine on the other was targeted.

As carboxylic moiety we selected mercaptoacetic acid while for the amine protecting group we attempted Fmoc and Alloc. We did not succeed in generating a Fmoc functionalized BB because the group was not compatible with further functionalization on the other side of the bicyclic scaffold. However, we were able to obtain BB **17**, bearing an Alloc moiety, as shown in Figure 10.

Starting from the readily available mesylate **4**, a primary amine was incorporated by nucleophilic substitution in aqueous ammonia (**18**). Next, the free amine was protected with Alloc and precursor **19** was obtained.[17] Thereafter the silyl (OTBDPS) group was removed to activate the alcohol again as a mesylate. Finally the mercaptoacetic acid moiety was linked through nucleophilic substitution, leading to the final compound **17**.

The Alloc-protected BB **17** was successfully coupled to R-Cys(Mtt)-Rinkamide resin (R = Fmoc) with *N,N′*-diisopropylcarbodiimide (DIPCDI)/*N*-hydroxybenzotriazole (HOBt) as coupling agent, while amide coupling under 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (HATU)/benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) conditions failed (data not shown). Removal of the Alloc group was performed as described and monitored by the ninhydrine test. Next, acetylation of the final amine, following a standard protocol, and final cleavage of the product, led to the crude profile represented in Figure 11.

Finally, compound **21** was purified by semipreparative HPLC (see supporting Figure 11).

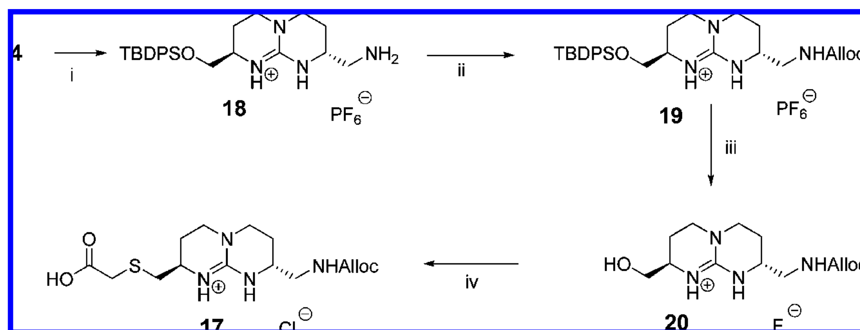

Figure 10. Synthetic scheme for BB **17**: (i) 30% NH_3 in MeOH, rt, 1 h, quant; (ii) allylchloroformate, $NaHCO_3$, 1,4-dioxane/H_2O, 0 °C−rt, 1 h, 93%; (iii) CsF, THF, rt, 24 h, quant; (iv) (a) MsCl, dist. Et_3N, dry DCM and THF (1:3), inert atm, rt, 18 h, (b) mercaptoacetic acid, KtBuO, dry THF, rt, 4 h, 54%.

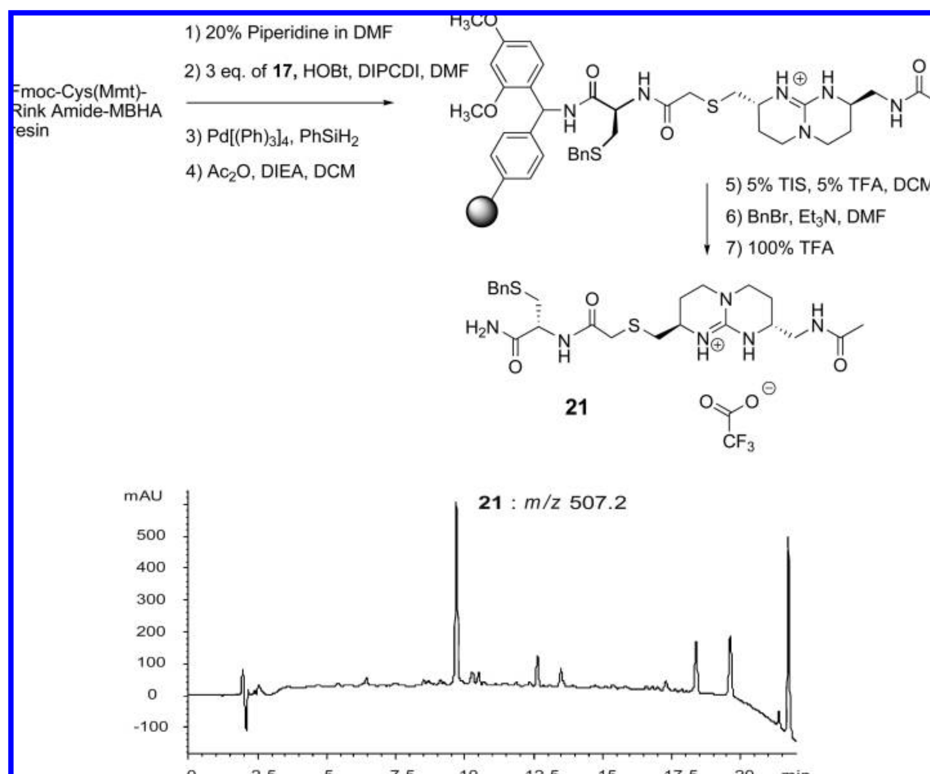

Figure 11. Synthetic scheme for product **21** (top) and HPLC profile of the crude reaction mixture (bottom) (5−60% MeCN in H_2O with 0.1% TFA in 17 min, 60−95% in 3 min, UV_{210}. ESI(+) *m/z* $[M + H - TFA]^+$).

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

This attempt shows that classical procedures from Fmoc synthesis, such as amide coupling, NHAlloc deprotection, and amine capping by acetylation, are compatible with bicyclic guanidinium scaffolds. This opens the possibility to prepare guanidinium-containing oligomers through amino acid building blocks or branched oligomers through two different chemistries.

Conclusions and Outlook

With the specific goal of synthesizing bicyclic guanidinium oligomer series, the iterative coupling of thioether-linked cationic building blocks on standard polystyrene support is described.

For a procedure enabling simple cargo incorporation, a solid support with a thiol group for oligomer elongation and an amine group for cargo anchorage has been proposed. Basic nucleophilic conditions for BB anchorage to the solid support, lead also to some disulfide formation, which is tackled with DTT treatment. Moreover, a quantitative capping reaction with benzyl bromide for complete thiol functionalization is reported.

Under the described conditions, coupling with the BB **1** takes place with ≥75% yield. Cargo anchorage to the amine moiety may proceed *before* or *after* oligomer elongation, provided the anchoring amine is protected with Alloc, fully compatible with the described strategy. Moreover, a diguanidinium BB **11** is reported, which likely paves the way to a faster growth of longer oligomers.

To the best of our knowledge, this constitutes the first example of solid-phase synthesis of thioethers conceptually following the Merrifield approach,[18] in just two steps by removal of a protecting group and incorporation of the building block. The key of our strategy is the use of a building block with a protected function, in combination with the presence of another function carrying a semipermanent

protecting group for the incorporation of the cargo, as well as a compatible resin as a permanent protecting group.

Finally an amino acid-type BB **17** based on the bicyclic guanidinium scaffold is described. This synthon could eventually be integrated in peptide solid phase synthesis affording strands with non natural amino acids. In addition, it paves the way toward new amide-bound bicyclic guanidinium oligomers.

Experimental Section

Standard Procedures for Solution-Phase Synthesis. All reactions were carried out under argon with dry solvents unless otherwise noted. Commercially available reagents were used without further purification. Solvents were distilled and dried prior to use. Reactions were monitored by thin layer chromatography (TLC) performed on DC-Fertigplatten SIL G-25 UV_{254} (Macherey-Nagel GmbH) or by analytical high performance liquid chromatography with a RP-C_{18} column (Symmetry300 C_{18} 5 μm 4.6 × 150 mm, 1 mL/min flux and 210 nm detection) on a high performance liquid chromatograph (HPLC, Agilent Technologies Series 1200). Standard column chromatography was done on silica gel by SDS (chromagel 60 ACC, 40−60 mm). Yields refer to chromatographically pure compounds. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 UltraShield spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz) and are reported in parts per million relative to the residual solvent peak. Data for ^{1}H are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet), coupling constant in Hz, and integration. Exact masses were measured on a Waters LCT Premier liquid chromatograph coupled time-of-flight mass spectrometer (HPLC/MS-TOF) with electrospray ionization (ESI). Melting points were measured with a Büchi B-540 apparatus. Standard column chromatography was done on silica gel by SDS (chromagel 60 ACC, 40−60 mm).

Compounds (2*R*,8*R*)-2-*tert*-butyldimethylsilanoxymethyl-8-*tert*-butyldiphenylsilanoxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium hexafluorophosphate (**2**), (2*R*,8*R*)-2-*tert*-butyldiphenylsilanoxymethyl-8-hydroxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium hexafluorophosphate (**3**), (2*R*,8*R*)-2-*tert*-butyldiphenylsilanoxymethyl-8-methanesulfonyloxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]-pyrimidin-1-ium hexafluorophosphate (**4**), (2*R*,8*R*)-2-*tert*-butyldiphenylsilanyloxymethyl-8-[(2*R*,8*R*)-8-hydroxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-2-ylmethylsulfanylmethyl-1-ium hexafluorophosphate]-3,4,6,7,8,9-hexahydro-2*H*-pyrimido-[1,2-*a*]pyrimidin-1-ium hexafluorophosphate (**12**) (2*R*, 8*R*)-8-[(2*R*,8*R*)-8-*tert*-butyldiphenylsilanyloxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-2-ylmethylsulfanylmethyl-1-ium hexafluorophosphate]-2-methanesulfonyloxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium hexafluorophosphate (**13**), (2*R*,8*R*)-2-*tert*-butyldiphenylsilanyloxymethyl-8-aminomethyl-3,4,6,7,8,9,-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium hexafluorophosphate (**18**) were synthesized according to procedures reported previously by our group.[5,6]

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

Alloc-Ala-OH was synthesized from L-Ala-O^{t}Bu and AllocCl reagent,[19] to avoid formation of Alloc-Ala-Ala-OH (81% yield, see Supporting Information Scheme 1).

General Procedure for the Synthesis of Compounds 1 and 11. To a solution of **5** or **14** (0.47 mmol) in DCM (20 mL) were sequentially added NMM (0.204 mL, 1.87 mmol) and Ms_2O (0.204 g, 1.17 mmol), and the reaction was stirred at rt for 3 h. The organic phase was washed with water (2 × 8 mL) and 0.1 M NH_4PF_6 (2 × 8 mL). The organic layer was dried (anhydrous Na_2CO_3), filtered, and concentrated in vacuo. Finally the crude compounds were purified by flash chromatography in silica gel.

(2*R*,8R)-2-(4-Methoxyphenyldiphenylmethylthiomethyl)-8-methanesulfonyloxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium hexafluorophosphate (1). Eluent for chromatography: DCM/MeOH 100:0 to 99.5:0.5. The final compound was obtained as a yellow solid in a 90% yield. mp: 100−102 °C. $[\alpha]^{20}_D$: −12.2 (c = 4.42, MeCN, stand. dev.: 0.67). ^{1}H NMR (CD_3Cl): δ 7.45−7.41 (m, 4H, ArH), 7.36−7.30 (m, 6H, ArH), 7.28−7.21 (m, 2H, ArH), 6.86 (d, J = 8.8 Hz, 1H, NH), 6.24 (s, 1H, NH), 6.0 (s, 1H, NH), 4.29 (dd, J = 3.7, 10.3 Hz, 1H, CH_2O), 4.13 (dd, J = 6.4, 10.3 Hz, 1H, CH_2O), 3.82−3.76 (m, 1H, CHα), 3.81 (s, 3H, CH_3O), 3.37−3.29 (m, 2H, $CH_2\gamma$), 3.27−3.18 (m, 1H, CHα), 3.17−3.10 (m, 1H, $CH_2\gamma$), 3.10 (s, 3H, CH_3), 2.94−2.85 (m, 1H, $CH_2\gamma$), 2.46 (d, J = 7.0 Hz, 2H, CH_2S), 2.12−2.00 (m, 1H, $CH_2\beta$), 1.99−1.87 (m, 2H, $CH_2\beta$), 1.80−1.67 (m, 1H, $CH_2\beta$). ^{13}C NMR ($CDCl_3$): δ 158.4 (CO), 150.5 (CGuan), 144.6, 144.6, 136.2 (CAr), 130.7, 129.3, 128.1, 126.8, 113.4 (ArH), 69.7 (CH_2O), 67.1 (C), 55.3 (CH_3O), 47.9 (CHαO), 47.4 (CHαS), 44.9 ($CH_2\gamma$), 37.2 (CH_3OMs), 35.8 (CH_2S), 24.4, 22.1 ($CH_2\beta$). Exact Mass (ESI+) m/z $[M + H - HPF_6]^+$ calcd: 566.2147 uma; found 566.2133 uma.

(2*R*,8*R*)-8-[(2*R*,8*R*)-8-(4-Methoxyphenyldiphenylmethylthiomethyl)-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-2-ylmethylsulfanylmethyl-1-ium Hexafluorophosphate]-2-methanesulfonyloxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-a]pyrimidin-1-ium Hexafluorophosphate (11). Eluent for chromatography: DCM/MeOH 98:2. The product was obtained as a colorless solid in a 83% yield. mp: 96−103 °C. $[\alpha]^{20}_D$: −80.8 (c = 1, MeCN, stand. dev.: 0.12). ^{1}H NMR (CD_2Cl_2): δ 7.49−7.43 (m, 4H, ArH), 7.39−7.31 (m, 8H, ArH), 6.88 (d, J = 9.4 Hz, 2H, ArH), 6.47 (br s, 1H, NH), 6.43 (br s, 1H, NH), 6.24 (br s, 1H, NH), 6.00 (br s, 1H, NH), 4.32 (dd, J = 4.4, 10.2 Hz, 1H, CH_2O), 4.17 (dd, J = 6.6, 10.5 Hz, CH_2O), 3.82 (s, 3H, CH_3), 3.64−3.51 (m, 2H, CHα), 3.51−3.26 (m, 7H, CHα, $CH_2\gamma$), 3.24−3.12 (m, CHα, $CH_2\gamma$), 3.11 (s, 3H, OCH_3), 2.97−2.84 (m, 3H, CHα, CH_2S), 2.67−2.58 (m, 2H, CH_2S), 2.51−2.46 (m, 2H, CH_2S), 2.22−2.10 (m, 2H, $CH_2\beta$), 2.02−1.80 (m, 4H, $CH_2\beta$), 1.74−1.65 (m, 1H, $CH_2\beta$). ^{13}C NMR (CD_2Cl_2): δ 158.4 (CAr), 150.5, 150.0 (CGuan), 144.7, 136.2 (CAr), 130.8, 129.5, 128.1, 127.0, 113.4 (CH_2Ar), 69.9 (C), 67.0 (CH_2), 55.4 (CH_3O), 48.2, 47.9, 47.8 (CHα), 45.7, 45.5, 45.0, 44.9 ($CH_2\gamma$), 37.1 (CH_3OMs), 36.4, 36.3, 36.0 (CH_2S), 25.7, 25.6, 24.5, 22.0 ($CH_2\beta$). Exact Mass (ESI+) m/z $[M + H - HPF_6]^+$ calcd: 763.3134 uma; found 763.3161 uma.

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

General Procedure for the Synthesis of Compounds 5 and 14. To a solution of 0.25 M NaOMe (38 mL, 9.5 mmol) was added MttSH (255 mg, 0.83 mmol), and the mixture was stirred at rt for 5 min. Next, a solution of mesyl precursor **4** or **13** (0.38 mmol) in MeOH (5 mL) was added with a syringe, and the reaction mixture was stirred at rt for 1 h. Thereafter the reaction was heated at 50 °C overnight. After evaporation of the solvent, the remaining residue was dissolved in DCM (12 mL), and the organic phase was washed with water (10 mL) and 0.1 M NH_4PF_6 (3 × 10 mL). After the organic layer was dried over anhydrous Na_2CO_3, it was filtered and concentrated at reduced pressure. Finally the crude was purified by flash chromatography in silica gel.

(2*R*,8*R*)-2-(4-Methoxyphenyldiphenylmethylthiomethyl)-8-hydroxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Hexafluorophosphate (5). Eluent for chromatography: DCM/MeOH 100:0 to 97:3. The product was obtained as a colorless solid in a 85% yield. mp: 90−95 °C. ^{1}H NMR ($CDCl_3$): δ 7.42−7.40 (m, 4H, ArH), 7.35−7.7.27 (m, 6H, ArH), 7.26−7.20 (m, 2H, ArH), 6.85 (d, J = 8.3 Hz, 2H, ArH), 6.30 (s, 1H, NH), 6.00 (s, 1H, NH), 3.79 (s, 3H, CH_3O), 3.64−3.59 (m, 1H, CHα), 3.56−3.41 (m, 2H, CH_2O), 3.39−3.32 (m, 1H, $CH_2\gamma$), 3.21−3.11 (m, 3H, $CH_2\gamma$), 2.86−2.78 (m, 1H, CHα), 2.51−2.40 (m, 2H, CH_2S), 1.88−1.78 (m, 2H, $CH_2\beta$), 1.69−1.59 (m, 2H, $CH_2\beta$). ^{13}C NMR ($CDCl_3$): δ 158.4 (CAr), 150.4 (CGuan), 144.7, 136.2 (CAr), 130.8, 129.4, 128.1, 127.0, 113.4 (ArH), 66.9 (C), 64.8 (CH_2), 55.3 (CH_3), 49.9, 47.8 (CHα), 45.4, 44.7 ($CH_2\gamma$), 36.5 (CH_3O), 36.3 (CH_2S), 25.2, 22.2 ($CH_2\beta$). Exact Mass (ESI+) m/z [M + H − HPF_6]$^+$ calcd: 488.2364 uma; found 488.2372 uma.

(2*R*,8*R*)-8-[(2*R*,8*R*)-8-(4-Methoxyphenyldiphenylmethylthiomethyl)-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]-pyrimidin-2-ylmethylsulfanylmethyl-1-ium Hexafluorophosphate]-2-hydroxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Hexafluorophosphate (14). Eluent for chromatography: DCM/MeOH (100:0.5 to 97:4). The pure compound was obtained as a colorless solid with 79% yield. mp: 95−100 °C. ^{1}H NMR (CD_2Cl_2): δ 7.48−7.43 (m, 4H, ArH), 7.38−7.32 (m, 6H, ArH), 7.30−3.25 (m, 2H, ArH), 6.88 (d, J = 8.7 Hz, 2H, ArH), 6.38 (br s, 1H, NH), 6.30 (br s, 1H, NH), 6.17 (br s, 1H, NH), 5.92 (br s, 1H, NH), 6.00 (br s, 1H, NH), 3.82 (s, 3H, CH_3), 3.80−3.74 (m, 1H, CHα), 3.62−3.51 (m, 4H, CHα, $CH_2\gamma$), 3.49−3.40 (m, 2H, CHα, $CH_2\gamma$), 3.40−3.28 (m, 3H, CHα, CH_2S), 3.26−3.10 (m, 2H, CHα, $CH_2\gamma$), 2.95−2.85 (m, 3H, CHα, CH_2S), 2.66−2.57 (m, 2H, CH_2S), 2.53−2.44 (m, 2H, CH_2S), 2.22−2.09 (m, 1H, $CH_2\beta$), 2.08−2.00 (m, 1H, $CH_2\beta$), 1.99−1.80 (m, 4H, $CH_2\beta$), 1.76−1.65 (m, 1H, $CH_2\beta$). ^{13}C NMR (CD_2Cl_2): δ 158.5 (CAr), 150.8, 150.1 (CGuan), 144.7, 136.2 (CAr), 130.8, 129.4, 128.1, 126.9, 113.3 (CH_2Ar), 66.9 (C), 64.3 (CH_2), 55.3 (CH_3), 50.7, 48.2, 47.8, 47.6 (CHα), 45.7, 45.5, 45.1 ($CH_2\gamma$), 36.5 (CH_3O), 36.2, 35.9 (CH_2S), 26.0, 25.7, 24.8, 22.4 ($CH_2\beta$). Exact Mass (ESI+) m/z [M + H − HPF_6]$^+$ calcd: 685.3358 uma; found 685.3345 uma.

(2*R*,8*R*)-8-Allyloxycarbonylaminomethyl-2-carboxymethylsulfanylmethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium chloride (17). To a solution of **20** (0.4 g, 0.93 mmol) in dry DCM (7 mL), freshly distilled Et_3N (0.39 μL, 2.8 mmol) was added. The suspension was cooled to 0 °C and MsCl (0.22 mL, 2.8 mmol), previously dissolved in DCM (1 mL), was added dropwise. The reaction was warmed to rt. After half an hour, THF (3 mL) was added, and the reaction was stirred overnight. Thereafter the solvent was evaporated to dryness. Next, dry THF (20 mL) was added dropwise, as well as a solution of sodium mercaptoacetic acid (363 mg, 3.08 mmol) and K^{t}BuO (341 mg, 2.88 mmol) in MeOH (10 mL). The resulting mixture was stirred at rt for 4 h. Thereafter the reaction was filtered, and the filtrate was evaporated. Then, a solution of MeOH/DCM (9:1, 10 mL) was added; the precipitate was removed by filtration, and the solvent evaporated under reduced pressure. This procedure was repeated several times increasing the amount of DCM in the solvent mixture until pure DCM was employed. Finally, the product was passed through a Cl- anion exchange column and was obtained as a colorless solid in a 54% yield. mp: 85−87 °C. $[\alpha]^{20}_D$: −31.3° (c = 1, MeOH, stand. dev.: 0.31). ^{1}H NMR (MeOD): δ 5.95−5.86 (sex, J = 5.2 Hz, 1H, CH-Alloc), 5.32 (d, J = 17.3 Hz, 1H, CH-Alloc), 5.20 (d, J = 10.0 Hz, 1H, CH-Alloc), 4.56 (br s, 2H, CH_2−Alloc), 3.67−3.60 (m, 1H, CHα), 3.58−3.49 (m, 1H, CHα), 3.46−3.37 (m, 4H, $CH_2\gamma$), 3.35 (d, J = 4.1 Hz, 2H, CH_2SCO), 3.26 (d, J = 6.2 Hz, CH_2NH_2), 2.93 (dd, J = 5.3, 14.1 Hz, 1H, CH_2S), 2.75 (dd, J = 8.0, 14.1 Hz, 1H, CH_2S), 2.21−2.14 (m, 1H, $CH_2\beta$), 2.11−2.04 (m, 1H, $CH_2\beta$), 1.96−1.79 (m, 2H, $CH_2\beta$). ^{13}C NMR (MeOD): δ 158.2 (CO), 151.2 (CGuan), 132.8 (CH), 117.6, 66.0 (CH_2), 51.5, 48.3 (CH), 44.9, 39.3 (CH_2), 36.8, 36.3 (CH_2S), 25.1 ($CH_2\beta$). Exact Mass (ESI+) m/z [M + H − HCl]$^+$ calcd: 357.1597 uma; found 357.1588 uma.

(2*R*,8*R*)-8-Allyloxycarbonylaminomethyl-2-*tert*-butyldiphenylsilanoxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium hexafluorophosphate (19). To a solution of amine **18** (186 mg, 0.319 mmol) in dioxane/water 1:1 (4 mL), cooled down to 0 °C, were added $NaHCO_3$ (67 mg, 0.799 mmol) and allyl chloroformate (58 mg, 0.479 mmol). The reaction was stirred for 2 h at rt. Next, the dioxane was evaporated, and the product was extracted from H_2O with EtOAc (5 mL) and the organic layer was further washed with 0.1 M NH_4PF_6 (2 × 5 mL). Next, the organic phase was dried with anhydrous Na_2CO_3, filtered and eliminated in vacuo. The crude compound was purified by flash chromatography in silica gel (DCM/MeOH, 98:2→96:4) and **21** was obtained as a colorless solid in a 93% yield. mp: 86−89 °C. ^{1}H NMR ($CDCl_3$): δ 7.63 (m, 4H, ArH), 7.62 (m, 6H, ArH), 6.24 (s, 1H, NH), 6.08 (s, 1H, NH), 6.06−5.96 (sex, J = 5.2 Hz, 1H, CH-Alloc), 5.37 (d, J = 17.2 Hz, 1H, CH-Alloc), 5.25 (d, J = 10.4 Hz, 1H, CH-Alloc), 4.54 (d, J = 5.2 Hz, 3.74−3.67 (m, 1H, CH_2O), 3.62−3.50 (m, 3H, CH_2O, CHα), 3.51−3.44 (m, 4H, $CH_2\gamma$), 3.32 (d, J = 6.2 Hz, CH_2NH_2), 2.17−2.05 (m, 2H, $CH_2\beta$), 1.94−1.84 (m, 2H, $CH_2\beta$), 1.04 ($C(CH_3)_3$). ESI(+) m/z [M + H − HPF_6]$^+$: 521.7 uma.

(2*R*,8*R*)-8-Allyloxycarbonylaminomethyl-2-hydroxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium fluoride (20). To a solution of **19** (0.87 g, 1.31 mmol) in THF (18 mL) was added CsF (0.59 g, 3.92 mmol). After

the reaction mixture was stirred at rt for 1 day, the solvent was evaporated, and the residue was dissolved in EtOAc (9 mL) to extract the organic phase with water (4 × 5 mL). Thereafter, the aqueous layer was evaporated and the residue was dissolved in a mixture of MeOH/DCM (9:1, 5 mL). A precipitate was discarded, and the filtrate was concentrated. This procedure was repeated with solutions of increasing amount of DCM until reaching MeOH/DCM (1:4). At this point the solvents were eliminated and product **20** was dried in vacuo and obtained in quantitative yield. mp: 85−90 °C. ^{1}H NMR (MeOD): δ 6.07−5.97 (sex, J = 5.2 Hz, 1H, CH-Alloc), 5.39 (d, J = 17.2 Hz, 1H, CH-Alloc), 5.27 (d, J = 10.4 Hz, 1H, CH-Alloc), 4.56 (d, J = 5.2 Hz, 2H, CH_2−Alloc), 3.74−3.67 (m, 1H, CH_2O), 3.62−3.52 (m, 3H, CH_2O, CHα), 3.51−3.44 (m, 4H, $CH_2\gamma$), 3.32 (d, J = 6.2 Hz, CH_2NH_2), 2.17−2.07 (m, 2H, $CH_2\beta$), 1.94−1.84 (m, 2H, $CH_2\beta$). ^{13}C NMR (MeOD): δ 156.3 (CO), 149.5 (CGuan), 131.5 (CH), 114.6, 63.7, 62.1 (CH_2), 48.8, 47.1 (CHα), 43.5, 43.2, 42.5 ($CH_2\gamma$, CH_2N), 21.5, 21.0 ($CH_2\beta$). ESI(+) m/z [M − HF + H]$^+$: 283.2 uma.

Standard Procedures for Solid-Phase Synthesis. Generally, approximately 6 mL of solvent were used per gram of resin. Initial swelling of the MBHA resin LL (200−400) HCl with loading 0.68 mmol/g, was performed with 60% TFA in DCM (1 × 1 min, 1 × 20 min), followed by washes with DCM (5 × 1 min), neutralization with DIPEA 5% (3 × 3 min), and renewed DCM washes.

Fmoc deprotection was performed twice (1 × 1 min, 1 × 15 min) using a 20% piperidine solution in DMF. Capping of amines was carried out using a standard capping reagent consisting in the mixture of acetic anhydride (Ac_2O, 5 equiv) and DIPEA (5 equiv) in DMF (20 min).

Standard washings consisted of treatments with DMF (3 × 2 min), MeOH (3 × 2 min), and DCM (3 × 2 min). Coupling of amino acid BB's, deprotection of Fmoc and Alloc, as well capping of primary amines were checked by the Kaiser test.[20]

Mtt removal was performed employing a solution of DTT (8 equiv) in DMF (3 × 20 min).

The presence of free thiols was checked by the Ellman test[21] after DTT treatment to ensure for disulfide reduction. A tiny amount of resin was introduced into a vial were it was first treated with a solution of 0.5% DTNB in DMF (150 μL) and then with some drops of DIPEA. Subsequent orange coloration of the solution embedding the beads, indicated the presence of free thiols, whereas no color change indicated full nucleophilic functionalization of the thiols.

Analytical HPLC was performed as described in the general procedures for solution phase synthesis, whereas semipreparative purification was performed on the same apparatus with an RP-C_{18} Symmetry300 5 μm 4.6 × 140 mm RP column, at 4.7 mL/min flux and 10 mg/ml concentration.

MS-spectrometry conditions were the same as for solution phase synthesis.

Synthesis of Fmoc-Cys(Mtt)-Rinkamide Resin. One gram of MBHA resin (0.68 mmol/g) was washed with DMF (5 × 1 min) before coupling was performed using Rinkamide linker (1.1 g, 2 mmol), HOBt (304 mg, 2 mmol), DIPCDI (316 mg, 2 mmol) in DMF (6 mL), in syringes fitted with a polystyrene filter placed on a shaker at rt for 18 h. After the washes, Fmoc-Cys(Mmt)-OH (1.26 g, 2 mmol) was coupled under the same conditions.

An aliquot of the Fmoc-Cys(Mmt)-Rinkamide-resin (0.1 g, 0.068 mmol) was treated with piperidine to remove Fmoc, then it was acetylated and cleaved with 100% TFA for 1 h at rt. After the mixture was evaporated and dried in vacuo, the crude compound weight (23 mg) indicated 0.054 mmol loading of the resin, ~80% yield.

(2*R*,8*R*)-2-(4-Methoxyphenyldiphenylmethylthiomethyl)-8-(1-acetamido-1-carboxamidoethylsulfanylmethyl)-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Trifluoroacetate (6). Fmoc-Cys(Mtt)-Rinkamide-MBHA resin (0.05 g, 0.034 mmol) was treated with piperidine, followed by washings and treatment with Ac_2O as described in the general procedures. Next, Mtt was removed, followed by treatment with DTT. Prior to coupling, the resin was washed with dry DMF (3 × 1 min). Next, a solution of K^{t}BuO (13 mg, 0.12 mmol) and BB **1** (72 mg, 0.102 mmol) in dry DMF (0.4 mL) was added, and the syringe was placed at rt on a shaker for 3 h. After the washes, DTT treatment was performed. Capping of the resin took place in a solution of BnBr (21 μL, 0.17 mmol) and Et_3N (14 μL, 0.10 mmol) in dry DMF (0.4 mL), shaking the syringe at rt for 3 h. After the washes, 100% TFA cleavage afforded a crude compound that was analyzed by anal. HPLC (5−95% MeCN in H_2O with 0.1% TFA in 15 min, UV_{220}, 1 mL/min, 5 μL). **6** was obtained as a colorless oil without further purification (41% yield). ^{1}H NMR (MeOD): δ 7.43−7.39 (m, 4H, ArH), 7.32−7.2 (m, 8H, ArH), 6.87−6.84 (d, J = 8.9 Hz, 2H, ArH), 4.55−4.51 (s, 1H, CHα), 3.8 (s, 3H, CH_3O), 3.54−3.47 (m, 1H, $CH_2\alpha$), 3.45−3.37 (m, 2H, $CH_2\gamma$), 3.20−3.10 (m, 1H, CH_2S), 3.06−2.96 (m, 2H, CH_2S, $CH_2\gamma$), 2.94−2.85 (m, 3H, CH_2S, $CH_2\gamma$, $CH_2\alpha$), 2.76−2.69 (m, 2H, CH_2S), 2.59−2.49 (m, 1H, CH_2S), 2.21−2.14 (m, 1H, $CH_2\beta$), 2.02 (s, 3H, C*H3*CONH), 2.02−1.94 (m, 1H, $CH_2\beta$), 1.92−1.83 (m, 1H, $CH_2\beta$), 1.79−1.69 (m, 1H, $CH_2\beta$). ESI(+) m/z [M + H − TFA]$^+$: 632.2 uma.

(2*R*,8*R*)-2-Benzylsulfanylmethyl-8-(1-acetamido-1-carboxamidoethylsulfanylmethyl)-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium trifluoroacetate (7). Fmoc-Cys(Mtt)-Rinkamide resin (0.05 g, 0.034 mmol) was treated with piperidine and then washed and treated with Ac_2O, as described in the general procedures. Next, Mtt was removed according to general procedures, followed by treatment with DTT. Prior to coupling, the resin was washed with dry DMF (3 × 1 min). Then a solution of K^{t}BuO (13 mg, 0.12 mmol) and BB **1** (72 mg, 0.10 mmol) in dry DMF (0.4 mL) was added, and the syringe was placed for 3 h at rt on a shaker. Thereafter washes and DTT treatment were performed. Capping of the resin was carried out as described for compound **6**. Next, Mtt removal was followed by renewed DTT treatment and capping with BnBr. Finally, 100% TFA cleavage afforded a crude compound which was analyzed by anal. HPLC (5−95% MeCN in H_2O with 0.1% TFA in 20 min, UV_{210}, 1 mL/min, 5 μL). Further semipreparative purification (20−90% MeCN in H_2O with 0.1% TFA in 17 min, UV_{210}, 4.7 mL/min, 75 μL) yielded pure **7** as a colorless

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

oil in a 33% yield. ^{1}H NMR (CD_3CN): δ 7.39−7.34 (m, 4H, ArH), 7.33−7.28 (m, 1H, ArH), 7.14 (br s, 1H, NH), 7.11 (br s, 1H, NH), 7.03 (br s, 1H, NH), 6.68 (br s, 1H, NH), 6.08 (br s, 1H, NH), 3.80 (s, 2H, SCH_2Ar), 3.54−3.47 (m, 1H, CHα), 3.46−3.38 (m, 1H, CHα), 3.37−3.27 (m, 4H, $CH_2\gamma$), 3.08−2.98 (m, 2H, CH_2S), 2.76−2.68 (m, 2H, CH_2S), 2.58−2.51 (m, 2H, CH_2S), 2.12−2.04 (m, 2H, $CH_2\beta$), 2.03 (s, 3H, $COCH_3$), 1.81−1.71 (m, 2H, $CH_2\beta$). ^{13}C NMR (CD_3CN): δ 150.86 (CGuan), 138.4 (CAr), 128.9, 128.6, 127.2 (CHAr), 48.5, 47.6 (CHα), 45.5, 45.0 ($CH_2\gamma$), 37.1, 35.9 (CH_2S), 25.8 (CH_2), 25.4 (CH_3). Exact Mass (ESI+) *m/z* [M + H − TFA]$^+$ calcd: 450.1997 uma; found 450.1993 uma.

(2*R*,8*R*)-8-[(2*R*,8*R*)-8-Benzylsulfanylmethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-2-ylmethylsulfanylmethyl-1-ium hexafluorophosphate]-2-(1-acetamido-1-carboxamidoethylsulfanylmethyl)-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Trifluoroacetate (8). The same procedure described for product **7** was followed starting from Fmoc-Cys(Mtt)-Rinkamide resin (0.08 mg, 0.054 mmol), repeating twice the steps between the first Mtt removal and capping with BnBr. 100% TFA-cleavage afforded a crude compound which was analyzed by anal. HPLC (20−40% MeCN in H_2O with 0.1% TFA in 17 min, UV_{210}, 1 mL/min, 5 μL) Further semipreparative purification (20−40% MeCN in H_2O with 0.1% TFA in 17 min, UV_{210}, 4.7 mL/min, 50 μL) under the same conditions yielded pure product **8** as a colorless oil in a 21% yield. ^{1}H NMR (MeOD): δ 7.44−7.36 (m, 4H, ArH), 7.36−7.29 (m, 1H, ArH), 4.64−4.60 (q, *J* = 5.3 Hz, 1H, CHα), 3.87 (s, 2H, $ArCH_2S$), 3.71−3.61 (m, 3H, CHα), 3.58−3.37 (m, 1H, CHα, $CH_2\gamma$), 3.12 (dd, *J* = 5.3, 14.0 Hz, 1H, CH_2S), 3.00−2.92 (m, 3H, CH_2S), 2.85 (dd, 1H, *J* = 8.9, 14.0 Hz, CH_2S), 2.80−2.71 (m, 4H, CH_2S), 2.64 (dd, *J* = 7.5, 13.8 Hz, 1H, CH_2S), 2.28−2.17 (m, 2H, $CH_2\beta$), 2.10 (s, 3H, $COCH_3$), 1.99−1.85 (m, 2H, $CH_2\beta$). ^{13}C NMR (CD_3CN): δ 150.8 (CGuan), 138.2 (CAr), 128.7, 128.3, 126.9 (CHAr), 52.8, 47.9, 47.7, 47.6 (CHα), 45.1, 45.0, 44.9 ($CH_2\gamma$), 36.4, 36.1, 35.6, 34.0, 30.7, 30.6 (CH_2S), 25.3, 26.2 ($CH_2\beta$), 21.2 (CH_3). Exact Mass (ESI+) *m/z* [M + H − 2TFA]$^+$ calcd: 647.2984 uma; found 647.2984 uma.

Tetra(bicyclic guanidinium) Oligomer 9. The same procedure described for product **8** was followed starting from Fmoc-Cys(Mtt)-Rinkamide resin (0.08 g, 0.054 mmol), repeating four times the steps between first Mtt deprotection and capping with BnBr. 100% TFA cleavage afforded a crude compound which was analyzed by anal. HPLC (20−50% MeCN in H_2O with 0.1% TFA in 17 min, UV_{210}, 1 mL/min, 5 μL) Further semipreparative purification (25% MeCN in H_2O with 0.1% TFA for 17 min, then in 3 min from 25−28%, 4,7 mL/min, 75 μL) yielded pure product **9** as a colorless oil in a 12% yield. ^{1}H NMR (MeOD): δ 7.46−7.36 (m, 4H, ArH), 7.35−7.30 (m, 1H, ArH), 4.65−4.59 (m, 1H, CHα), 3.85 (s, 2H, SCH_2Ar), 3.76−3.60 (m, 8H, CHα), 3.58−3.42 (m, 16H, CHγ), 3.12 (dd, *J* = 5.3, 14.0 Hz, 2H, CH_2S), 3.01−2.90 (m, 7H, CH_2S), 2.87−2.70 (m, 9H, CH_2S), 2.63 (dd, *J* = 7.7, 13.7 Hz, 2H, CH_2S), 2.29−2.18 (m, 8H, $CH_2\beta$), 2.11 (s, 3H, CH_3), 2.04−1.89 (m, 8H, $CH_2\beta$). Exact Mass (ESI+) *m/z* [M + Na]$^+$ calcd: 1519.4492 uma; found 1519.4525 uma.

2-Acetamido-2-benzylthioacetamide (10). Fmoc-Cys(Mtt)-Rinkamide resin (0.04 g, 0.027 mmol) was treated with piperidine, followed by washes and treatment with acetic anhydride, as described in the general procedures. Next, Mtt was removed, followed by treatment with DTT. Prior to nucleophilic coupling, the resin was washed with dry DMF (3 × 1 min). Thereafter a solution of BnBr (17 μL, 0.14 mmol) and Et_3N (11 μL, 0.081 mmol) in dry DMF (0.3 mL) was added, and the syringe was placed on a shaker for 3 h at rt. Thereafter the resin was cleaved according to the general procedures. The cleaved product **10** was analyzed by anal HPLC (5−95% MECN in H_2O, 0.1% TFA, in 15 min, UV_{220}, 1 mL/min, 5 μL) and obtained as a colorless oil without further purification in a 65% yield. ^{1}H NMR (MeOD): δ 7.39−7.29 (m, 4H, ArH), 7.27−7.20 (m, 1H, ArH), 4.67 (t, 1H, CHα), 3.8 (m, 2H, SCH_2Ar), 2.87 (dd, *J* = 5.8,13.8 Hz 1H, CH_2S), 2.67 (dd, *J* = 8.2, 13.8 Hz, 2H, CH_2S), 2.03 (s, 3H, CH_3O), 1.81−1.71 (m, 2H, $CH_2\beta$). ESI(+) *m/z* [M + H]$^+$: 253.1 uma.

Hexa(bicyclic guanidinium) Oligomer 15. The same procedure as for compound **9** was repeated with Fmoc-Cys(Mtt)-Rinkamide resin (0.04 g, 0.027 mmol) until incorporation of four times BB **1** and capping with BnBr. Next, a solution with K^tBuO (11 mg, 0.096 mmol) and BB **11** (86 mg, 0.082 mmol), dissolved in DMF (0.3 mL), was added, and the syringe was placed on a shaker for 3 h at rt. After the washes and DTT treatment, capping with BnBr was performed as described. After 100% TFA cleavage, crude compound **15** was analyzed by anal. HPLC (20−45% MeCN in H_2O with 0.1% TFA in 17 min, UV_{210}, 1 mL/min, 5 μL) and ESI(+). Product **15** was identified among several oligomer precursors (see Figure 7 and Supporting Information Figure 9) but was not further purified. ESI(+) *m/z* [M + 2H − 2TFA]$^{2+}$: 946.3 uma.

Compound 16. Fmoc-Cys(Mtt)-Rinkamide resin (0.04 g, 0.027 mmol) was treated with piperidine. After the washes, a solution of Alloc-Ala-OH (14 mg, 0.081 mmol), HOBt (12 mg, 0.027 mmol), and DIPCDI (13 μL, 0.081 mmol) in DMF (0.3 mL) was added to the syringe, and placed on a shaker overnight at rt. After the washes, Mtt was removed, and BB **1** was anchored using the conditions described for compound **7**. The final cleavage afforded crude **16**, which was analyzed by anal. HPLC (20−50% MeCN in H_2O with 0.1% TFA in 17 min, UV_{210}, 1 mL/min, 5 μL). The compound was further purified by semipreparative HPLC (20−50% MeCN in H_2O with 0.1% TFA in 15 min, 50−95% in 4 min, UV_{210}, 4.7 mL/min, 80 μL) and obtained in a 40% yield. ^{1}H NMR (MeOD): δ 7.46−7.23 (m, 5H, ArH-S), 6.01−5.89 (m, 1H, CHAllyl), 5.33 (d, *J* = 17.5 Hz, 1H, CHAllyl), 5.21 (d, *J* = 11.7 Hz, CHAllyl), 3.81 (s, 2H, SCH_2Ar), 3.59−3.36 (m, 6H, $CH_2\alpha$, $CH_2\gamma$), 3.25−3.06 (m, 1H, CH_2S), 3.01−2.94 (m, 2H, CH_2S), 2.87−2.80 (m, 1H, CH_2S), 2.73−2.68 (m, 1H, CH_2S), 2.63−2.53 (m, 1H, CH_2S), 2.23−2.08 (m, 2H, $CH_2\beta$), 1.90−1.77 (m, 2H, $CH_2\beta$), 1.38 (d, *J* = 7.0 Hz, 3H, CH_3−Ala). Exact Mass (ESI+) *m/z* [M + H − TFA]$^+$ calcd: 563.2474 uma; found 563.2477 uma.

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v

Compound 21. Fmoc-Cys(Mtt)-Rinkamide resin (0.03 g, 0.02 mmol) was treated with piperidine. After the washes, a solution of BB **17** (24 mg, 0.061 mmol), HOBt (9 mg, 0.061 mmol), and DIPCDI (9 μL, 0.061 mmol) was added to the syringe which was placed overnight on a shaker at rt. After washes, the Alloc group was removed by performing treatments with a solution of $Pd(P(Ph)_3)_4$ (2 mg, 0.001 mmol) and $PhSiH_3$ (18 μL, 0.1 mmol) in dry THF (0.2 mL, 3 × 15 min) at rt. Thereafter the free primary amine was acetylated as described in the general procedures. Before the final cleavage, Mtt was removed and capped with BnBr. The crude compound **21** was analyzed by HPLC (5−60% MeCN in H_2O with 0.1% TFA in 17 min, 60−95% in 3 min, 1 mL/min, 5 μL) and further purified by semipreparative HPLC (15−30% MeCN in H_2O with 0.1% TFA in 20 min, UV_{210}, 4.7 mL/min, 70 μL), although the yield could not be calculated because not enough product was recovered. Exact Mass (ESI+) m/z $[M + H - TFA]^+$ calcd: 507.2212 uma; found 507.2216 uma.

Acknowledgment. V.M. acknowledges Ministerio de Educación y Ciencia (MEC), Spain, for a predoctoral fellowship. This work was partially supported by grants from MCT (BQU2006-03794, CTQ2005-00315/BQU, CTQ2005-08948, and Consolider Ingenio 2001 CSD2006-0003), *Generalitat de Catalunya*, the ISCIII (CIBER-BBN, nanomedicine), Networking Center on Bioengineering, Biomaterials and Nanomedicine, The Institute for Research in Biomedicine, the Barcelona Science Park, and the ICIQ Foundation.

Supporting Information Available. General procedures and characterization material. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Pantos, A.; Tsogas, I.; Paleos, C. M. *Biochim. Biophys. Acta* **2008**, *1778*, 811–823.

(2) Fernández-Carneado, G.; Van Gool, M.; Martos, V.; Castel, S.; Prados, P.; de Mendoza, J.; Giralt, E. *J. Am. Chem. Soc.* **2005**, *127*, 869–874.

(3) Muratovska, A.; Lightowlers, R. N.; Taylor, R. W.; Wilce, J. A.; Murphy, M. P. *Adv. Drug Delivery Rev.* **2001**, *49*, 189–198.

(4) Kielar, F.; Congreve, A.; Law, G.-L.; New, E. J.; Parker, D.; Wong, K. L.; Castreño, P.; de Mendoza, J. *Chem. Commun.* **2008**, 2435–2437.

(5) (a) Echavarren, A.; Galán, A.; de Mendoza, J.; Salmerón, A.; Lehn, J.-M. *Helv. Chim. Acta* **1988**, *71*, 685–693. (b) Kurzmeier, H.; Schmitchen, F. P. *J. Org. Chem.* **1990**, *55*, 3749–3755.

(6) Breccia, P.; Boggetto, N.; Pérez-Fernández, R.; Van Gool, M.; Takahashi, M.; René, L.; Prados, P.; Badet, B.; Reboud-Ravaux, M.; de Mendoza, J. *J. Med. Chem.* **2003**, *46*, 5196–5207.

(7) SHMtt was initially purchased from Novabiochem (catalog number 01-63-0092) but is now synthesized in our lab as described in the Supporting Information, since it was recently discontinued.

(8) Brotzel, F.; Mayer, H. *Org. Biomol. Chem.* **2007**, *5*, 3814–3820.

(9) Takeshi-Kondo, T.; Nishiyama, A.; Ueyama, N.; Murao, H.; Manabe, H.; Ueda, Y. U.S. Patent 6,011,170, Jan. 4, 2000.

(10) Usera, A. R.; Posner, G. H. *J. Org. Chem.* **2007**, *72*, 2329–2334.

(11) McNaughton, B. R.; Miller, B. L. *Org. Lett.* **2006**, *8*, 1803–1806.

(12) Mourtas, S.; Kayakalou, C.; Nicolettou, A.; Tzavara, C.; Gatos, D.; Barlos, K. *Tetrahedron Lett.* **2002**, *44*, 179–182.

(13) Mourtas, S.; Gatos, D.; Karavoltsos, M.; Kayakalou, C.; Barlos, K. *Tetrahedron Lett.* **2002**, *43*, 3419–3421.

(14) Dahan, A.; Avi Weissberg, A.; Portnoy, M. *Chem. Comm.* **2003**, 1206–1207.

(15) Wu, X.; Pitsch, S. *Bioconjugate Chem.* **1999**, *10*, 921–924.

(16) Thieriet, N.; Alsina, J.; Giralt, E.; Guibé, F.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 7275–7278.

(17) Mandal, P. K.; McMurray, J. S. *J. Org. Chem.* **2007**, *72*, 6599–6601.

(18) Portnoy et al. (Dahan, A.; Weissberg, A.; Portnoy, M. *Chem. Commun.* **2003**, 1206−1207) have published an elegant synthesis on solid support of a polythioether dendron employing a more chemical strategy, which involves for each building block incorporation, one reduction, and two nucleophilic substitutions.

(19) Kazmaier, U.; Hebach, C.; Watzke, A.; Maier, S.; Mues, H.; Huch, V. *Org. Biomol. Chem.* **2005**, *3*, 136–145.

(20) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.

(21) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70–77.

CC800207V

Downloaded by MONASH UNIV on July 7, 2009
Published on March 6, 2009 on http://pubs.acs.org | doi: 10.1021/cc800207v