Dimerization Inhibitors of HIV-1 Protease Based on a Bicyclic Guanidinium Subunit

Perla Breccia,[#] Nicole Boggetto,[§] Ruth Pérez-Fernández,[#] Michiel Van Gool,[#] Masayuki Takahashi,[‡] Loïc René,[||,†] Pilar Prados,[#] Bernard Badet,[||] Michèle Reboud-Ravaux,[§] and Javier de Mendoza*[,#]

Departamento de Química Orgánica, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain, Institut Jacques Monod, CNRS-Université Paris 6 & 7, 2, Place Jussieu-Tour 43, 75251 Paris Cedex 05, France, FRE2230 CNRS & Université de Nantes, F-44322 Nantes Cedex 3, France, and Institut de Chimie des Substances Naturelles-UPRS 2301 CNRS, 91198 Gif-sur-Yvette Cedex, France

Received April 11, 2003

Original inhibitors of HIV-1 protease based on a chiral bicyclic guanidinium scaffold linked to short peptidic mimics of the terminal protease sequences and to a lipophilic group were designed. These inhibitors prevent dimerization of the native protease by an interfacial structure at the highly conserved antiparallel β-strand involving both the N and C termini that substantially account for dimerization. The preorganized guanidinium spacer introduces additional electrostatic hydrogen-bonding interactions with the C-terminal Phe-99 carboxylate. Lipophilic residues linked to side chains and the guanidinium scaffold are essential for dimerization inhibition as ascertained by Zhang kinetics (**4**, K_{id} = 290 nM; **6** or **6′**, K_{id} = 150 nM; **8**, K_{id} = 400 nM) combined with a circular dichroism study on the enzyme thermal stability. Remarkably, less hydrophobic compounds result in mixed dimerization (**1a** and **3)** or active site inhibitors (**5**). Removal of the guanidinium hydrophobic groups leads to less active or inactive ligands.

Introduction

An essential step in the maturation of the human immunodeficiency virus (HIV) is the processing of the gag and gag/pol viral proteins by HIV-1 protease (PR). The crucial role of this enzyme in the production of infective virus particles makes it a prime target for therapeutic intervention in AIDS.[1] In its active form, HIV-1 PR is a 22 000 Da homodimeric aspartic protease consisting of two 99-residue polypeptide chains that self-assemble to form an approximately 45 × 23 × 25 Å dimer. The active site is shaped at the interface of the two subunits, each contributing one catalytic aspartate residue.[2] Preventing or disrupting the self-assembly of the monomers would be a unique means of inhibiting protease activity, since most active site inhibitors used in AIDS therapy have led to resistance by rapid mutation of the virus.[3] The protease homodimer is mainly stabilized by a four-stranded antiparallel β-sheet involving both the N and C termini of each monomer (H-Pro-Gln-Ile-Thr and Cys-Thr-Leu-Asn-Phe-OH, respectively) (Figure 1), which has been found to be highly conserved in HIV-1 isolates and most HIV-2 isolates.[4] More than 50% of the hydrogen bonds along the dimer interface are provided by terminal residues 1−4 and 96−99 at the antiparallel β-sheet.[5] The contacts in the area centered around N and C termini account for greater than 75% of the free energy of dimerization.[6] This suggests that using peptides as competitive inhibitors of β-sheet formation may efficiently inhibit dimer formation and hence reduce protease activity.

To target the β-sheet, agents may be generated that competitively block the assembly of the homodimer or disrupt the dimer interface.[7] Indeed, kinetic studies demonstrated that peptides corresponding to native N or C termini inhibit enzyme activity.[7a] Inhibitors based on peptides different from the native sequence were also analyzed, most exhibiting genuine dimerization inhibition.[7g,8] Interface peptides, however, may also be partly active-site-directed, since terminal segments are cleavage segments of the protease. Rather small changes in structure or ionic strength can induce a switch in binding mode. As a result, a lipophilic N-terminal blocking group, such as palmitoyl, not only improves the inhibitory potency of such peptides but also acts as an interface directing group.[9] Also, cross-linked interfacial peptides that mimic the dimerization interface have been described.[10] Finally, some synthetic compounds consisting of two tripeptide strands attached to more rigid aromatic scaffolds (pyridine and naphthalene) were shown to inhibit dimerization of HIV-1 PR at the submicromolar range.[11]

The novelty of the dimerization inhibitors presented in this paper is the use of a lipophilic bicyclic guanidinium subunit as the central tether between a chain reproducing the C-terminal native strand, to interact with both C and N termini of the monomer, and an additional hydrophobic group targeting the dimer interface. Formation of a salt bridge involving two well-oriented zwitterionic hydrogen bonds makes bicyclic guanidinium a useful subunit for the extraction of carboxylates or phosphates and, thus, an ideal complement for the C-terminal carboxylate (Phe-99).[12] By use of mutant proteases comprising deletions of either one of the terminal regions (residues 1−4 or 96−99) or both, it was demonstrated that the inner C-terminal strands are absolutely essential for dimer formation.[5b] We

* To whom correspondence should be addressed. Phone: +34 91 397 4710. Fax: +34 91 397 3966. E-mail: javier.demendoza@uam.es.
[#] Universidad Autónoma de Madrid.
[§] CNRS-Université Paris 6 & 7.
[‡] CNRS & Université de Nantes.
[||] Institut de Chimie des Substances Naturelles-UPRS 2301 CNRS.
[†] Deceased February 2000.

10.1021/jm030871u CCC: $25.00 © 2003 American Chemical Society
Published on Web 10/23/2003

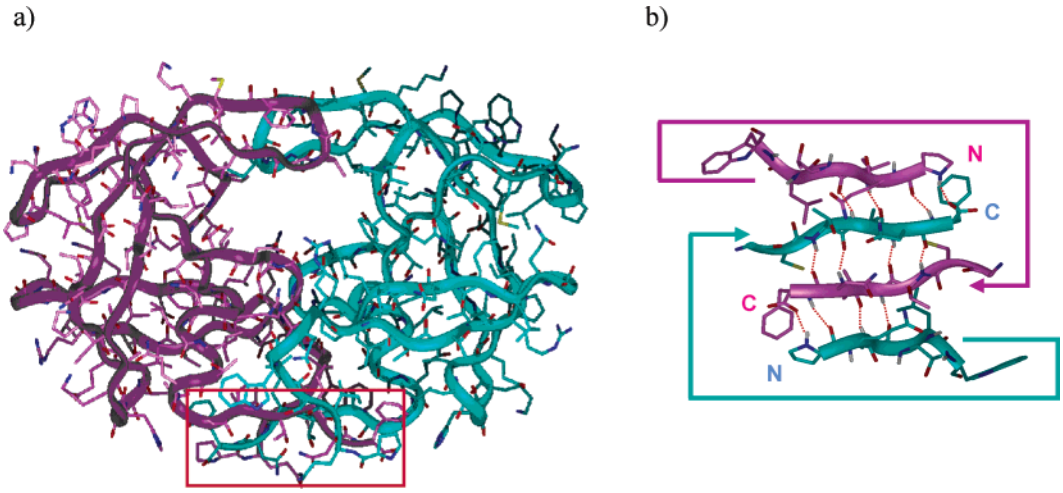


Figure 1. (a) HIV-1 PR dimer structure. (b) Antiparallel β-sheet involving N- and C-termini.

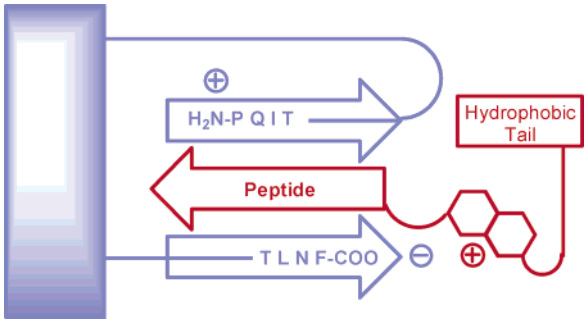


Figure 2. Dimerization inhibition scheme using a carboxylate binding subunit as a scaffold.

hypothesized that these features included in the inhibitors, preformed into a binding competent conformation, should substantially contribute to overcoming the stabilization of the protease dimer, favoring the monomer−inhibitor complex (Figure 2).

Compounds **1**−**8** were designed, differing in linkers and peptides attached to the guanidinium (Chart 1). Thus, compounds **1a**,**b**−**2a**,**b** contain an adipate chain attached to pentapeptide Ala-Thr-Leu-Asn-Phe-OMe through an ester function. Adipate contains about the right number of atoms necessary to link the peptide to the guanidinium without causing distortion to the hydrogen-bonded scaffold. The native peptide chain was slightly modified for stabilization purposes and also to facilitate its synthesis. Thus, glutamine Gln-2 and cysteine Cys-95 were both replaced by Ala. Finally, methyl or benzyl esters were employed instead of free acids in the peptide chains to prevent inhibitor self-dimerization. An *S*,*S*-configuration for the guanidinium was employed throughout.

Assuming an extended conformation for the hydrocarbon chain of the adipate linker, replacement of adipate for mercaptoacetylglycine should result in a more preorganized spacer, since (i) two carbons of the adipate skeleton are replaced by a conformationally more rigid amide and (ii) the overall chain contains one atom less, thus providing increasingly restricted conformational freedom. The two longer S−C bonds keep the length of the chain almost unchanged. Therefore, a second class of potential inhibitors (**3**−**7**) was developed, all containing the mercaptoacetylglycine spacer and a lipophilic silyl group. Compound **3**, containing an N-terminus derived sequence, was used as a control, whereas compounds **4**−**7** incorporate the mercaptoacetylglycine followed by diverse protected or deprotected C-terminus related sequences. Since the carboxylate C-terminal group of Phe-99 can freely rotate, it is unlikely that the configuration of the guanidinium moiety influences its binding properties. To confirm this prediction, an *R*,*R*-guanidinium scaffold was used in **6′** instead of the *S*,*S*-configuration employed for the rest of inhibitors. Finally, the silyl substituent of **6** was replaced by another bulky, nonaromatic, lipophilic residue, such as the thiocholesteryl substituent in compound **8**. This allows for evaluation of the influence of aromatic residues in the lipophilic tail that could participate through stacking or edge-to-face contacts with the hydrophobic interface. On the other hand, ursolic acid, a polycyclic triterpene, acts as a mixed (dimerization/active site) inhibitor.[13]

Molecular modeling was employed to evaluate the suitability of the mercaptoacetylglycine spacer between the guanidinium central core and the complementary peptide chain. To build up the initial structures, atomic coordinates for the monomer were taken from the solid-state structure of HVP PR.[2] A bicyclic guanidinium was placed in front of the Phe-99 carboxylate (an optimized guanidinium−acetate complex was used for a correct docking), and suitable fragments were selected as linking subunits. Initial minimization was performed in vacuo, with a dielectric constant $\epsilon = 1$ that is non-distance-dependent. The resulting structure was soaked in a box of water, and optimization was performed again (Figure 3).

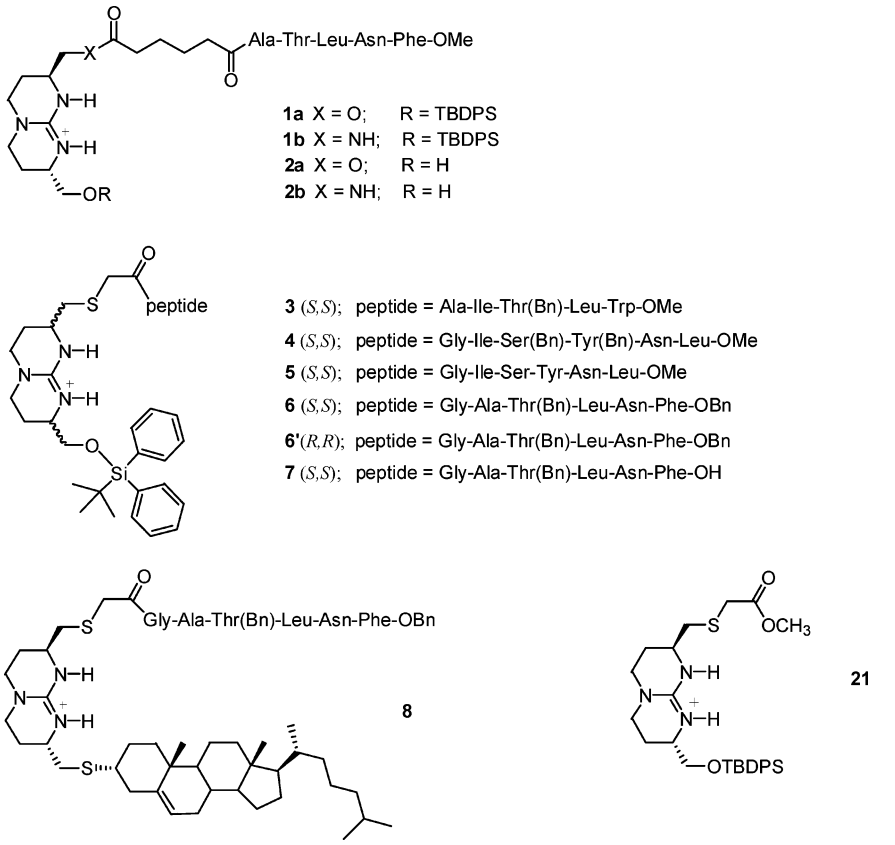
Finally, it is emphasized that lateral chains of Ser and Tyr were benzylated in most of the inhibitors to enhance the hydrophobicity of the compounds.

Results and Discussion

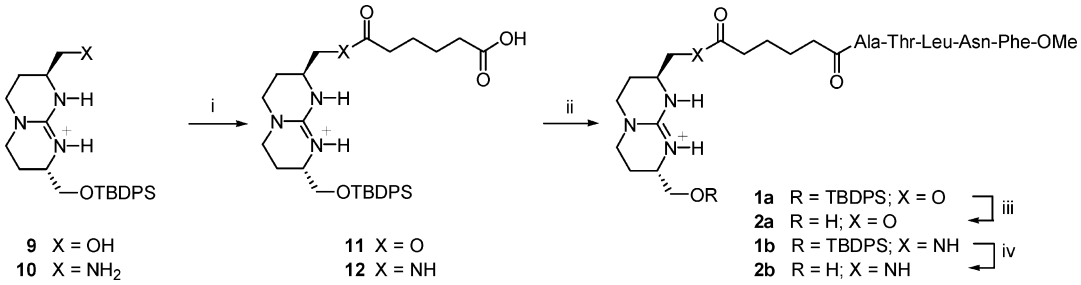
Synthesis. Silyl protected guanidinium ester or amide derivatives bearing one peptide chain and an adipate spacer (**1a** and **1b**) were easily prepared from alcohol **9**[14] or amine **10**, respectively, by successive coupling to adipic acid and Ala-Thr-Leu-Asn-Phe methyl ester (Scheme 1). Deprotection of the silyl ether afforded the corresponding alcohol derivatives (**2a**,**b**).

Compounds **3**−**7** were conveniently prepared in excellent yields from mesylate **13** via acid **14** by alkylation

Chart 1. HIV-1 PR Guanidinium-Based Inhibitors



Scheme 1. Synthesis of Compounds **1a**,**b** and **2a**,**b**[a]



[a] Reagents and conditions: (i) adipic acid, CDI, DMF; (ii) Ala-Thr-Leu-Asn-Phe-OMe, CDI, DMF; (iii) HF−Py 70%, THF; (iv) 30% HCl−CH_3CN.

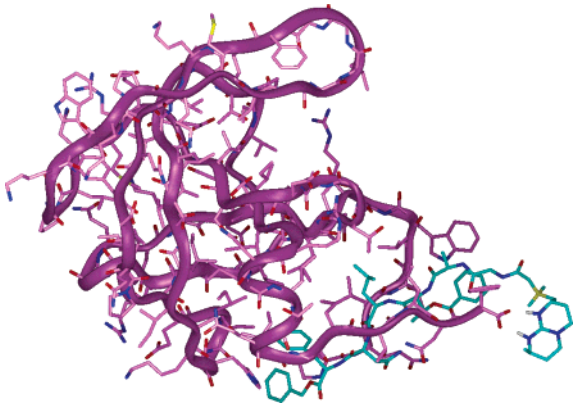


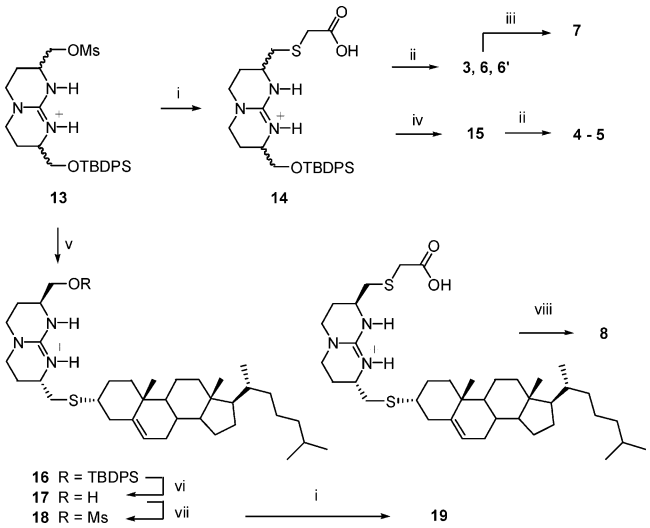
Figure 3. Optimized structure of PR−**6** complex in water. The CH_2−*O*−silyl group has been replaced by a methyl.

with sodium mercaptoacetate, followed by coupling to the corresponding peptides by Castro's reagent (BOP). The resulting products were purified by preparative HPLC. A similar strategy was followed to prepare inhibitor **8** in four steps from mesylate **13** (Scheme 2).

All peptides were synthesized in solution phase, using Boc-protected amino acids, and the compounds were purified by preparative HPLC.

Kinetic Analysis of Enzyme Inhibition. In vitro HIV-1 PR inhibition by compounds **1**−**8** was evaluated kinetically at pH 4.7 and 30 °C by means of a fluorimetric assay as reported in the literature.[11] All molecules were tested at their solubility limit. The results are summarized in Table 1. Compound **2b** (at 28 μM), endowed with an amide-linked adipate and a free OH, was inactive. However, a weak inhibitory effect was observed with the related structure **2a**, where the amide linker has been replaced by an ester. The superior inhibitory effect observed for compounds whose guanidinium subunit was attached to the spacer through an ester linkage over the same scaffolds attached through an amide linkage is also apparent by comparing amide **1b** (a weak inhibitor) with ester **1a** (an efficient one).

Scheme 2. Synthesis of Compounds **3**−**8**[a]



[a] Reagents and conditions: (i) sodium mercaptoacetate, *t*-BuOK, MeOH−THF; (ii) peptide, BOP, HOBt, NMM, DMF; (iii) H_2, Pd−C, MeOH, cat. AcOH; (iv) (a) GlyOMe, BOP, HOBt, NMM, DMF, (b) 1 N NaOH, THF; (v) thiocholesterol, *t*-BuOK, MeOH−THF; (vi) HF−Py 70%, THF; (vii) Ms_2O, NMM, THF; (viii) Gly-Ala-Thr(Bn)-Leu-Asn-Phe-OBn, BOP, HOBt, NMM, DMF.

Table 1. Inhibition of HIV-1 Protease by Guanidinium Hairpins

compound	IC_{50} (μM)[a]	K_{id} (μM)[b]	K_{ic} (μM)[c]
1a	1.9	0.55	5.25
1b	I[d]		
2a	I[e]		
2b	NI[f]		
3	5	6.50	7.00
4	3.3	0.29	
5	5		6.00
6 (*S*,*S*)	1.6	0.15	
6′ (*R*,*R*)	2	0.15	
7	11		
8	3	0.40	0.40

[a] Standard errors: ≤10%. [b] Dimerization inhibition. [c] Competitive inhibition. [d] A 28% inhibition (I) was observed at 28 μM. [e] A 28% inhibition (I) at 14 μM. [f] No inhibition (NI) detected at the solubility limit of the compound tested.

The presence of a lipophilic group attached to the guanidinium strongly enhances HIV-1 PR inhibitor activity. Thus, **1a** and **3**−**8** were found to be quite efficient inhibitors (IC_{50} = 1.6−5 μM), whereas in compounds lacking the TBDPS group the inhibitory effect is abolished (compare **2b** with **1b**) or decreased (compare **2a** with **1a**). As anticipated, the configuration of the guanidinium scaffold (*S*,*S* or *R*,*R*) does not influence its binding properties toward HIV protease (compare **6** and **6′**). However, replacing the silyl group of compound **6** with another lipophilic derivative such as the cholesteryl tail of **8**, which does not contain any aromatic ring, led to a 2-fold decrease of the IC_{50} values. It is likely that the phenyl groups of TBDPS could contribute to the binding to the hydrophobic interface, although this hypothesis requires further experiments to be fully validated. Either inhibitors with adipate (**1a**, **1b**, or **2a**) or mercaptoacetate spacers (**3**−**8**) were active. Thus, attachment of a hydrophobic moiety to the peptide strongly enhances inhibitory power, as was previously pointed out by Schramm.[9]

Replacement of the terminal methyl or benzyl ester for a free carboxylic acid caused a noticeable decrease in the inhibitory activity (i.e., 7-fold decrease in IC_{50} for **7** with respect to **6**). This is likely due to self-association of the inhibitor by intermolecular carboxylate−guanidinium ion-pairing, which decreases the effective inhibitor concentration. This is clearly demonstrated by comparing the NMR spectra of compounds **6** and **7**. While in compound **6** the guanidinium NH signals appear at 7.41−7.64 ppm (typical for non-hydrogen-bonded guanidiniums),[12] the same signals are strongly deshielded by ca. 2.5 ppm (9.95 and 10.08 ppm) in compound **7**, revealing a strong guanidinium−carboxylate interaction. Finally, it should be noted that control molecules such as peptides Gly-Ala-Thr(Bn)-Leu-Asn-Phe-OBn (see Supporting Information) and $MeO_2C(CH_2)_4$-CO-Ala-Thr-Leu-Asn-Phe-OMe (**20**) and guanidinium salts lacking peptide chains (**9**, **14**, or the synthetic intermediate **21**) did not lead to inhibition.

The mechanism of inhibition was characterized kinetically using the method developed by Zhang.[7a] Thus, initial rates v_i were determined at constant initial substrate concentrations using different enzyme concentrations $[E]_0$ for various inhibitor concentrations. Plots of $[E]_0/\sqrt{v_i}$ vs $\sqrt{v_i}$, with $\sqrt{v_i} = k_{exp}[S]_0$, were constructed. Typical parallel lines for dimerization inhibition were found for molecules **4**, **6**, **6′**, and **8** (Figure 4). Patterns found for compounds **1a** and **3** were consistent with a mixed inhibition mechanism in which the inhibitors could bind not only to the interfacial region but also to the active site. Convergent lines were obtained for compound **5**, demonstrating that this molecule acts as a competitive inhibitor without affinity for the monomer. The values of the inhibition constants K_{ic} and K_{id} were calculated using the equations

$$K_{ic} = a_0 \frac{[I]_0}{a - a_0}$$

and

$$K_{id} = b_0 \frac{[I]_0}{b - b_0}$$

respectively, where a and a_0 are the slopes and b and b_0 are the intercepts on the y axis in the presence (a, b) or absence (a_0, b_0) of inhibitor.

Stereoisomers **6** and **6′** (K_{id} = 0.15 μM) are thus inhibitors acting exclusively by inhibiting dimerization of HIV-1 PR. Replacement of the TBDPS group by a cholesteryl moiety (**8** vs **6**) decreases the inhibitory potency by 2.6-fold while maintaining the dissociative mechanism. For ursolic acid, dimerization inhibition was reported (K_{id} = 3.4 μM) with a slight competitive inhibitory component ($K_{ic} \approx 130$ μM).[13] Our results with **8** confirm that triterpenes may fit into the hydrophobic interface of PR monomers. However, guanidinium derivative **8** not only is a more potent inhibitor than ursolic acid but also has the advantage of acting as a pure dimerization inhibitor. Interestingly, compound **4** inhibits HIV-1 PR through a dissociative mechanism with a K_{id} value of 0.29 μM whereas the structurally related **5** acts as a poor active site inhibitor (K_{ic} = 16 μM). Thus, removal of the hydrophobic benzyl protecting groups at the serine and tyrosine residues of compound **4** switched the inhibition mechanism and caused the molecule to

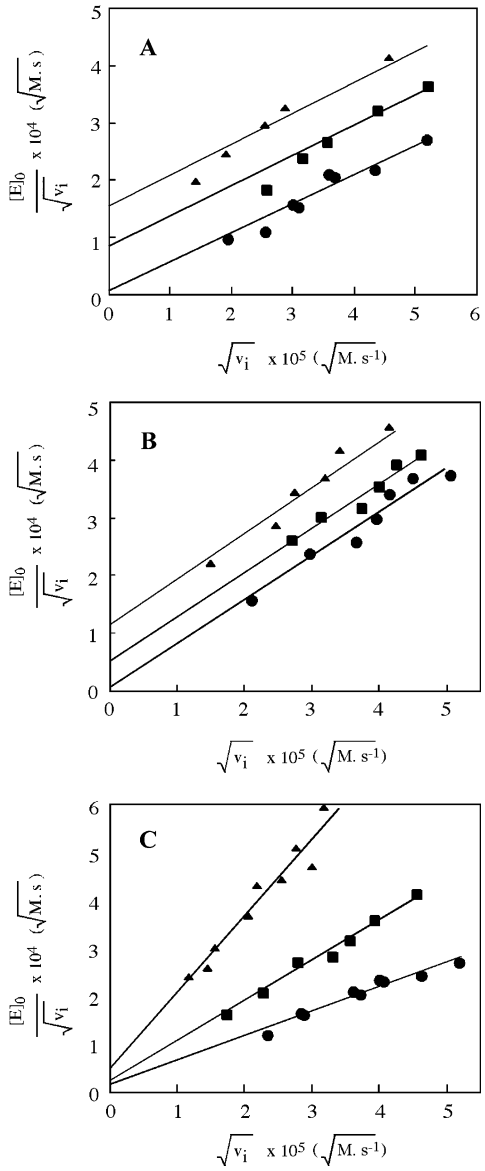


Figure 4. Plots of $[E]_0/\sqrt{v_i}$ vs $\sqrt{v_i}$ for hydrolysis of the fluorogenic substrate by HIV-1 PR at pH 4.7 and 30 °C in the absence (●) and presence of 2.5 μM (▲), 1 μM (■) of compound **6** (A) and compound **6′** (B) and presence of 12 μM (▲) and 4.5 μM (■) of compound **3** (C).

become a weak active site inhibitor. The same effect may be evoked to explain the mixed mechanism observed for **1a** (the threonine residue is not protected). Nevertheless, the presence of a protecting group is not sufficient to switch the mechanism from a mixed to a pure dimerization inhibition when the peptide strand mimics the N terminus (**3**) instead of the C terminus (compounds **4**, **6**, **6′**, **8**). By use of small peptides, more efficient dimerization inhibitors were obtained with peptides mimicking the C rather than the N terminus.[8]

Circular Dichroism. To further probe the mechanism of inhibition, we have examined the structure and thermal stability of PR alone or complexed with inhibitors by measurements of the CD signal at far-UV. An active site inhibitor should certainly stabilize the dimer form and thus the thermal stability of PR, while a dimerization inhibitor might not. The tested inhibitors (active site inhibitor acetylpepstatin and dimerization inhibitor **6**) did not affect the CD signal, indicating no

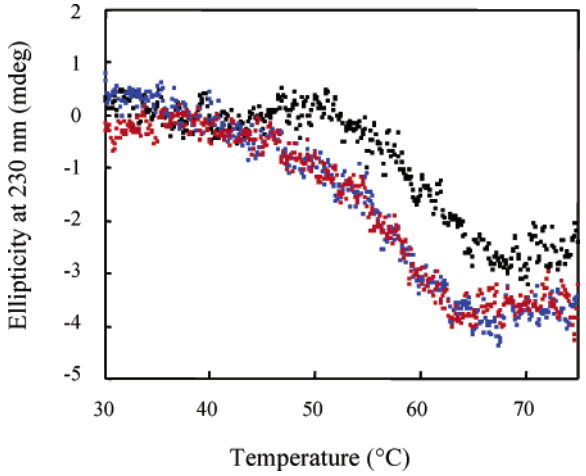


Figure 5. Thermal denaturation of HIV-1 PR (3.5 μM) in the absence (blue) and presence of compound **6** (red) and acetylpepsatin (black) at pH 4.7 in 100 mM sodium acetate, 1 mM EDTA, and 1 M NaCl containing 0.2% DMSO (v/v): temperature range, 30−75 °C (2 °C/min); [**6**] = 2 μM; [acetylpepstatin] = 0.2 μM; λ = 230 nm. Melting was followed by far-ultraviolet circular dichroism at 230 nm.

change in the secondary structure of protein and supporting our model. The enzyme was thermally denaturated in 100 mM sodium acetate buffer, pH 7.0, and monitored in the far-UV CD in the absence and in the presence of active site inhibitor acetylpepstatin and dimerization inhibitor **6** (Figure 5). The averages T_m values of 59.5, 59.5, and 61.5 °C were obtained at a ramp rate of 2 °C/min. This indicates that a significant stabilization was obtained in the acetylpepstatin complex, which had a 2 °C increase in T_m when compared to native enzyme. Conversely, no stabilization was observed by addition of compound **6**, in agreement with a different binding mode. In this case, the destabilization of PR by dimer interface disruption[6] is probably compensated by the putative stabilization due to the formation of the monomer−inhibitor complex, thus leading to identical T_m values for native and treated enzymes. The importance of PR stability of interactions implicating the C-terminal strands has been emphasized.[5b]

Conclusions and Outlook

Guanidinium derivatives are novel dimerization inhibitors of HIV-PR. Contrary to cross-linked peptides bridged by a flexible tether,[10] the compounds described in the present report display a rigid and positively charged scaffold linked simultaneously to a peptidic strand and a hydrophobic bulky group (TBDPS, cholesteryl) (**1a**,**b**, **3**−**8**). The rigid spacer likely contributes to reduction of the entropic cost of the complexation by allowing the preorganization of both peptidic and hydrophilic arms, while the positively charged guanidinium likely provides an additional and strong interaction with the carboxylate of Phe-99. It was previously reported that scaffolds such as 2,6-pyridine, 2,7-naphthalene, and dibenzofuryl linked to two peptide strands led to dimerization inhibitors. For example, K_{id} = 0.56 μM for the 2,7-naphthalenediol spacer[11a] and K_{id} = 5.40 μM for the dibenzofuryl spacer.[11b] The combination of both the steric and electronic effects within the bicyclic guanidinium scaffold explains the increase of

the inhibitory potency observed for the new inhibitors presented here ($K_{id} = 0.15\ \mu M$).[15]

Other hydrophobic subunits for more efficient contacts with the PR monomer interface are currently under investigation. Also, it must be emphasized that binding to the monomer and therefore inhibition could be substantially optimized by modification of the peptide sequences employed. Both rational and combinatorial approaches can be envisioned for this purpose. Finally, use of peptoids or nonnatural amino acids would provide a new generation of inhibitors resistant to in vivo hydrolysis by proteases. We are currently working on these modifications.

Experimental Section

Abbreviations. γ-Abu, γ-amino butyric acid; Adip, adipic; Ar, aromatic; Bn, benzyl; Boc, *tert*-butyloxycarbonyl; BOP, 1-*H*-benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; Chol, cholesteryl; CDI, *N*,*N*′-carbonyldiimidazole; DABCYL, 4-(4′-dimethylaminophenylazo)benzoyl; DCC, 1,3-dicyclohexylcarbodiimide; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; EDANS, 5-[(2-aminoethyl)amino]naphthalene-1-sulfonic acid; EDTA, ethylenedinitrilotetraacetic acid disodium salt; Guan, guanidinium; HOBt, 1-hydroxybenzotriazole; NMM, *N*-methylmorpholine; MES, 2-(*N*-morpholine)ethanesulfonic acid; Ms, mesylate; Ph, phenyl; TBDPS, *tert*-butyldiphenylsilyl; TFA, trifluoroacetic acid; τ_R, retention time in HPLC analysis.

Enzyme and Inhibition Assays. HIV-1 PR was kindly supplied by H. J. Schramm, Max-Planck Institut für Biochemie (Martinsried, Germany). It was produced by bacterial expression in *E. coli* using the plasmid pET9c-PR as decribed by Schramm[8] and Billich.[16] The stock solution protease (34 μM) was stored at −80 °C in 5 mM MES, pH 6.0, 1 mM EDTA, 1 mM DTT, 0.5 M NaCl, 5% v/v glycerol. The fluorogenic substrate DABCYL-γ-abu-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-EDANS was purchased from Bachem (Voisins-le-Bretonneux, France). Other reagents and solvents were purchased from commercial sources. The fluorescence and absorbance measurements were performed using a spectrofluorometer Perkin-Elmer LS 50B and a Uvikon 941 spectrophotometer, respectively.

Protease activity was routinely determined fluorometrically using DABCYL-γ-abu-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-EDANS in 100 mM sodium acetate, 1 mM EDTA, and 1 M NaCl at pH 4.7 and 30 °C according to the method described.[11] Excitation and emission wavelengths were 340 and 490 nm, respectively. The substrate and the compounds were previously dissolved in DMSO, with the final concentration kept constant at 3% (v/v). In a typical inhibition experiment, the enzyme and substrate concentrations were 7.5 nM and 5.2 μM, respectively. The IC_{50} values (inhibitor concentration leading to 50% inhibition) were obtained from a plot of the percent inhibition vs inhibitor concentration (0.5−28 μM) by fitting to the equation

$$\text{percent inhibition} = 100 \times \frac{[I]_0}{[I]_0 + IC_{50}}$$

The mechanism of inhibition and the corresponding kinetic constants K_{ic} (competitive inhibition) or/and K_{id} (dimerization inhibition) were determined using Zhang's method.[7a] At least seven different concentrations of enzyme ranging from 1.88 to 28 nM were tested for a given inhibitor concentration ($[S]_0 = 5.2\ \mu M$). Inhibitor concentrations were 3.15 and 6.3 μM (compound **1a**), 4.5 and 12 μM (compound **3**), 1.5 and 4.5 μM (compound **4**), 2.5, 5, and 7.5 μM (compound **5**), 1 and 2.5 μM (compounds **6** and **6′**), and 1.5 and 4.5 μM (compound **8**).

Circular Dichroism. CD was measured in a J-710 spectrometer (Jasco, Japan) using a 1 cm path length minicuvette (Hellma, Germany) on which the light was focused by a quartz lens (Jasco Europe, Italy). The temperature was controlled by a Peltier effect device. The spectra were averaged over four scans (bandwidth of 2 nm, response time of 2 s, and scan rate of 50 nm/min). The thermal denaturation of protein was studied by monitoring the change in CD signal at 230 nm (bandwidth of 10 nm, response time of 8 s) upon elevation of temperature (rate: 2 °C/min) from 30 °C.

Molecular Modeling. InsightII/Discover program modules were used, as implemented on a Silicon Graphics Indigo workstation, using the cvff (consistence valence force field) force field.[17] Initial minimizations were performed in vacuo, with a dielectric constant $\epsilon = 1$ that is non-distance-dependent. Then, the most promising complexes were soaked in a box of water and structures were optimized again.

Synthesis. All commercially available reagents were used without any further purification unless specified. The solvents were dried and distilled as described in the literature.[18] All reactions were performed under Ar atmosphere unless specified. The peptides were synthesized in solution phase using a Boc-based strategy (experimental data available in Supporting Information). Chiral bicyclic guanidinium derivatives were synthesized in *S*,*S* configuration with exception of compound **6′** (*R*,*R* configuration; same synthetic procedure as reported for **6**). The coupling reaction with the peptides were followed by analytical HPLC (column C18 Scharlau; flow = 1 mL/min; UV detector, Waters dual band with $\lambda = 230$ and 254 nm). For preparative HPLC analysis a radial column PrepLC (Waters) (flow = 20 mL/min) was used, while semipreparative HPLC was performed using an LC 18 column (Phenomenex) (flow = 6 mL/min). All solvents used in HPLC contained 0.05% TFA.

Melting points were measured with a Gallenkamp apparatus. The optical rotations were determined on a Perkin-Elmer 241 MC polarimeter using a cell (1 dm) at 20 °C (Na_D, 589 nm). ^{1}H NMR spectra were recorded on Bruker AC-300 (300 MHz), AMX-300 (300 MHz), and DRX-500 (500 MHz) spectrometers, and ^{13}C NMR spectra were performed with the same apparatus using magnetic fields at 75 and 125 MHz. In NMR assignments, the H and C of the bicyclic guanidinium derivatives are named as in the literature.[19] Mass spectra were recorded on a VG AutoSpec spectrometer using a FAB technique and on a HP1100MSD using an API-ES technique. Elemental analyses were performed on a Perkin-Elmer 2400 CHN and 2400 CHNS apparatus.

Synthesis of (2*S*,8*S*)-2-(*tert*-Butyldiphenylsilanyloxymethyl)-8-(aminomethyl)-3,4,6,7,8,9,-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Hexafluorophosphate (10). The mesylate derivative **13** (PF_6^-) (301 mg, 0.46 mmol) was dissolved in a mixture of MeOH (10 mL) and 30% aqueous NH_3 (4 mL). The resulting solution was stirred for 30 min at room temperature. Then the solvent was removed and the crude was dissolved in CH_2Cl_2 and washed with 0.1 N NH_4PF_6. After filtration over cotton and evaporation of the organic phase, the product was precipitated with CH_3CN, resulting in **10** (PF_6^-) (308 mg, 92%) as a white solid. Mp 81 °C. $[\alpha]^{25}_D$ +31 (*c* 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, acetone-d_6): δ 7.73−7.71 (m, 4H, PhSi), 7.51−7.48 (m, 6H, PhSi), 7.18 (s, 1H, NH), 7.13 (s, 1H, NH), 4.13−4.07 (m, 2H, CH_2OSi), 3.84 (m, 2H, CHα), 3.69−3.59 (m, 4H, $CH_2\gamma$), 2.61−2.52 (m, 2H, CH_2NH$_2$), 2.37−2.15 (m, 4H, $CH_2\beta$), 1.09 (s, 9H, $(CH_3)_3$C). ^{13}C NMR (75 MHz, acetone-d_6): δ 151.3 (C−guan), 135.9, 133.3, 130.5, 128.4 (ArC, ArCH), 66.2 (CH_2OSi), 51.7 (CH_2NH_2), 50.6, 47.6 (CHα), 45.5, 44.6 ($CH_2\gamma$), 26.7 [(CH_3)$_3$C], 23.3, 22.6 ($CH_2\beta$), 19.1 [(CH_3)$_3$C]. FAB/LSIMS *m*/*z*: 437.3 [$(M - PF_6^-)^+$, 100%]. HRMS for $[C_{25}H_{37}N_4OSi]^+$ 437.2736; found 437.2734.

General Procedure for Compounds 11 and 12. A mixture of adipic acid and CDI in dry DMF was stirred for 30 min. Then a solution of compound **9** or **10** in dry DMF was added and the reaction mixture was stirred at room temperature overnight. After removal of the solvent, the crude was dissolved in CH_2Cl_2 and washed with a solution of 1 N HCl, water, and brine. The organic solution was dried over Na_2SO_4, filtered, and concentrated to dryness. Purification was performed by precipitation.

Synthesis of 2-(*tert*-Butyldiphenylsilanyloxymethyl)-8-(5-carboxypentanoyloxymethyl)-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Chloride (11). Adipic acid (198 mg, 1.35 mmol), CDI (153 mg, 0.95 mmol), DMF (10 mL), **9** (Cl^-) (212 mg, 0.45 mmol), DMF (1 mL). Precipitation with a mixture of diethyl ether−CH_2Cl_2 (10:1) afforded **11** (Cl^-) (206 mg, 76%) as a white solid. Mp 80 °C. $[\alpha]^{25}_D$ +43 (*c* 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.69 (s, 1H, NH), 8.60 (s, 1H, NH), 7.67−7.63 (m, 4H, PhSi), 7.49−7.36 (m, 6H, PhSi), 4.21 (dd, J = 11.0, 5.0 Hz, 1H, CH_2COOR), 4.14 (dd, J = 11.0, 8.0 Hz, 1H, CH_2COOR), 3.82−3.56 (m, 4H, CH_2OSi, CHα), 3.40−3.28 (m, 4H, $CH_2\gamma$), 2.53−2.43 (m, 2H, CH_2COOH), 2.43−2.35 (m, 2H, OCOCH_2), 2.29−1.78 (m, 4H, $CH_2\beta$), 1.76−1.57 (m, 4H, $(CH_2)_2$), 1.09 (s, 9H, $(CH_3)_3$C). ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.5, 172.8 (CO), 151.0 (C−guan) 135.4, 132.5, 129.8, 127.7 (ArC, ArCH), 65.1 (CH_2O), 50.0, 47.0 (CHα), 44.8, 44.6 ($CH_2\gamma$), 33.9, 33.2 (CH_2CO), 26.7 [(CH_3)$_3$C], 23.9 (CH_2), 22.6, 22.4 ($CH_2\beta$), 19.0 [(CH_3)*C*]. FAB/LSIMS *m*/*z*: 566.4 [(M − Cl^-)$^+$, 100%]. Anal. Calcd for $C_{31}H_{44}N_3O_5SiCl$: C, 61.8; H, 7.4; N, 7.0. Found: C, 61.0; H, 7.1; N, 7.3.

Synthesis of 2-(*tert*-Butyldiphenylsilanyloxymethyl)-8-[(5-carboxypentanoylamino)methyl]-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Chloride (12). A mixture was prepared that contained adipic acid (69 mg, 0.472 mmol), CDI (100 mg, 0.617 mmol), DMF (1 mL), and **10** (Cl^-)[20] (112 mg, 0.237 mmol). Precipitation with diethyl ether afforded **12** (Cl^-) (101 mg, 71%) as a white solid. Mp 88 °C. $[\alpha]^{25}_D$ +42 (*c* 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.21 (s, 1H, NH−guan), 8.64 (s, 1H, NH−guan), 7.78 (m, 1H, CONH), 7.68−7.61 (m, 4H, PhSi), 7.43−7.36 (m, 6H, PhSi), 3.75−3.56 (m, 6H, CH_2OSi, CH_2NH, CHα), 3.23−3.06 (m, 4H, $CH_2\gamma$), 2.25 (m, 4H, CH_2CO−adip), 2.00−1.94 (m, 4H, $CH_2\beta$−guan), 1.64 (m, 4H, $(CH_2)_2$−adip), 1.09 (s, 9H, $(CH_3)_3$C). ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.1, 173.9 (CO), 151.4 (C−guan), 135.7, 135.5, 132.8, 129.8, 127.8, 127.7 (ArC, ArCH), 65.2 (CH_2O−guan), 49.7, 48.7 (CHα), 45.9, 44.3 ($CH_2\gamma$), 43.2 (CH_2CO−adip), 37.4, 36.9 [$(CH_2)_2$−adip], 26.7 [(CH_3)$_3$C], 25.7, 24.7 ($CH_2\beta$−guan), 19.7 [(CH_3)$_3$*C*)]. FAB/LSIMS *m*/*z*: 565.3 [(M − Cl^-)$^+$, 100%)].

General Procedure for Compounds 1a and 1b. A solution of guanidinium compound and CDI in dry DMF was stirred at room temperature for 30 min. Then, a solution of peptide Ala-Thr-Leu-Asn-Phe-OMe (TFA salt) and NMM in dry DMF was added and the reaction mixture was stirred overnight. After removal of the solvent, the crude was washed with CH_2Cl_2 and precipitated.

Synthesis of Compound 1a. A mixture was prepared that contained compound **11** (Cl^-) (130 mg, 0.216 mmol), CDI (37 mg, 0.226 mmol), DMF (5 mL). Then peptide (150 mg, 0.217 mmol), NMM (14 μL), and DMF (5 mL) were added. Reaction temperature was 50 °C. Precipitation with MeOH−H_2O (8:1) afforded **1a** (Cl^-) (196 mg, 78%) as a white solid. HPLC (gradient 10−100% CH_3CN−H_2O in 20 min; τ_R = 18.5 min; purity 95%). Mp 150 °C. $[\alpha]^{25}_D$ +33 (*c* 0.5, MeOH). ^{1}H NMR (500 MHz, COSY, MeOH-d_4): δ 7.73−7.66 (m, 4H, PhSi), 7.47−7.40 (m, 6H, PhSi), 7.27−7.21 (m, 5H, ArH−Phe), 4.71−4.66 (dd, J = 5.6, 1.8 Hz, 1H, CHα−Asn), 4.64−4.60 (m, 1H, CHα−Phe), 4.39−4.34 (m, 1H, CHα−Leu), 4.31−4.23 (m, 2H, CH_2CO), 4.05−4.01 (m, 2H, CHα−Thr, Ala), 3.72−3.68 (m, 2H, CH_2OSi), 3.65 (s, 3H, OCH_3), 3.60−3.53 (m, 4H, CH_2O, CHα), 3.39−3.35 (m, 4H, $CH_2\gamma$), 3.11−3.05 (m, 2H, CH_2−Phe), 2.69−2.63 (m, 2H, CH_2−Asn), 2.49−2.41 (m, 4H, CH_2CO−adip), 2.28−1.81 (m, 4H, $CH_2\beta$), 1.79−1.50 (m, 7H, $(CH_2)_2$−adip, $CH_2\beta$, CHγ−Leu), 1.37 (d, J = 7.2 Hz, 3H, CH_3−Ala), 1.19 (d, J = 6.2 Hz, 3H, CH_3−Thr), 1.02 (s, 9H, $(CH_3)_3$C), 0.94 (d, J = 6.2 Hz, 3H, CH_3−Leu), 0.90 (d, J = 6.2 Hz, 3H, CH_3−Leu). ^{13}C NMR (125 MHz, MeOH-d_4): δ 176.3, 175.8, 174.5 (CONH), 173.0, 172.7 (COOR), 152.3 (C−guan), 137.9, 136.7 136.6, 135.9, 134.1, 134.0, 131.2, 130.9, 130.3, 130.4, 129.5, 129.0, 128.8, 128.6, 127.9 (ArCH, ArC), 68.2 (CHO−Thr), 67.3, 66.7 (CH_2O), 60.1, 55.5, 53.6, 52.7, 51.6 (CHα−Ala, Thr, Leu, Asn, Phe), 51.5 (OCH_3), 51.4 (CHα−guan), 46.3 ($CH_2\gamma$−guan), 41.2 (CH_2−Leu), 38.4 (CH_2−Asn), 37.8 (CH_2−Phe), 36.1 (CH_2-CONH−adip), 34.5 (CH_2CO$_2$−adip) 27.4, 27.2 [(CH_3)$_3$C], 26.1 [$(CH_2)_2$−adip], 25.8 (CH−Leu), 25.4, 23.8 ($CH_2\beta$−guan), 23.6, 21.8 (CH_3−Leu), 20.4 (CH_3−Ala), 20.0 [(CH_3)$_3$*C*], 17.6 (CH_3−Thr). FAB/LSIMS *m*/*z*: 1126.7 [(M − Cl^-)$^+$, 100%]. HRMS for [$C_{58}H_{84}N_9O_{12}Si^+$] 1126.6009; found 1126.6034.

Synthesis of Compound 1b. A mixture was prepared that contained compound **12** (Cl^-) (65 mg, 0.108 mmol), CDI (18 mg, 0.111 mmol), and THF (1 mL). Then peptide (75 mg, 0.108 mmol), NMM (15 μL, 0.108 mmol), and DMF (1 mL) were added. Reaction time was 48 h at room temperature. Precipitation by dissolving in MeOH and adding some drops of water afforded **1b** (Cl^-) (94 mg, 74%) as a white solid. HPLC (gradient 10−100% CH_3CN−H_2O in 20 min; τ_R = 18.5 min; purity 97%). Mp 186 °C. $[\alpha]^{25}_D$ +18 (*c* 0.3, $CHCl_3$). ^{1}H NMR (300 MHz, COSY, MeOH-d_4): δ 7.66−7.52 (m, 4H, PhSi), 7.47−7.40 (m, 6H, PhSi), 7.27−7.07 (m, 5H, ArH−Phe), 4.71−4.45 (m, 1H, CHα−Asn), 4.35−4.08 (m, 4H, CHα−Phe, Leu, Thr, Ala), 3.98−3.85 (m, 2H, CH_2OSi), 3.68−3.41 (m, 2H, CHα−guan), 3.59 (s, 3H, OCH_3), 3.40−3.35 (m, 4H, $CH_2\gamma$−guan), 3.11−2.90 (m, 2H, CH_2−Phe), 2.65−2.48 (m, 2H, CH_2−Asn), 2.41−2.28 (m, 2H, CH_2CO−adip), 2.20−2.10 (m, 2H, CH_2CO−adip), 2.01−1.70 (m, 4H, $CH_2\beta$−guan), 1.63−1.46 (m, 7H, $(CH_2)_2$−adip, $CH_2\beta$−Leu, CHγ−Leu), 1.37 (d, 3H, J = 7.2 Hz, CH_3−Ala), 1.19 (d, J = 6.2 Hz, 3H, CH_3−Thr), 0.98 (s, 9H, $(CH_3)_3$C), 0.81 (d, J = 6.0 Hz, 3H, CH_3−Leu), 0.78 (d, J = 6.0 Hz, 3H, CH_3−Leu). ^{13}C NMR (75 MHz, MeOH-d_4): δ 181.8, 176.7, 175.7, 174.8, 173.0, 171.8, 171.5 (CO), 152.3 (C−guan), 135.1, 134.8, 133.9, 131.5, 130.8, 130.2, 129.8, 129.2, 127.7 (ArC, ArCH), 67.6 (CHO−Thr), 65.0, 64.9 (CH_2O), 58.1 (CHα−Thr), 54.9 (CHα−Phe), 52.5, 52.1, 51.8 (CHα−Asn, Leu, Ala), 51.7 (OCH_3), 49.9, 47.6 (CHα−guan), 46.9, 46.6 ($CH_2\gamma$−guan), 46.2 (CH_2NH), 43.9 (CH_2−Leu), 38.1 (CH_2−Asn), 36.6 (CH_2−Phe), 34.5 (CH_2−adip), 34.4 (CH_2−adip), 26.2 [(CH_3)$_3$C)], 25.5 (CH_2−adip), 24.8 (CH−Leu), 24.2, 23.9 ($CH_2\beta$−guan), 23.7 (CH_3−Leu), 20.0 [CH_3−Ala, (CH_3)$_3$*C*)], 17.3 (CH_3−Thr). FAB/LSIMS *m*/*z*: 1125.7 [(M − Cl^-)$^+$, 100%].

Synthesis of Compound 2a. To compound **1a** (Cl^-) (50 mg, 0.054 mmol) in dry THF (2 mL) was added dropwise 70% HF−Py (0.121 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 4 h. After neutralization with Na_2CO_3, the solvent was removed and the solid was dissolved in water and washed with diethyl ether and CH_2Cl_2. The aqueous phase was concentrated and the product was extracted from the salt with CH_3CN, affording **2a** (Cl^-) (28 mg, 70%) as a white solid. HPLC (gradient 10−100% CH_3CN−H_2O in 20 min; τ_R = 12.3 min; purity 96%). Mp 168 °C. $[\alpha]^{25}_D$ +23 (*c* 0.2, MeOH). ^{1}H NMR (300 MHz, COSY, MeOH-d_4): δ 7.45−7.31 (m, 5H, ArH−Phe), 4.86−4.72 (m, 2H, CHα−Asn, CHα−Phe), 4.54−4.34 (m, 5H, CHα−Leu, CH_2COOR, CHα−Thr, Ala), 4.18−4.08 (m, 1H, CH_2OH−guan), 3.91−3.60 (m, 2H, CH_2OH, CHα−guan), 3.78 (s, 3H, OCH_3), 3.60−3.34 (m, 5H, CH_2O, CHα−guan, $CH_2\gamma$−guan), 3.31−3.11 (m, 2H, CH_2−Phe), 2.89−2.68 (m, 2H, CH_2−Asn), 2.61−2.34 (m, 4H, CH_2-CO−adip), 2.28−2.06 (m, 2H, $CH_2\beta$−guan), 2.04−1.74 (m, 9H, $CH_2\beta$−guan, $(CH_2)_2$−adip, CHγ−Leu), 1.53−1.49 (m, 2H, $CH_2\beta$−Leu), 1.52 (d, J = 7.0 Hz, 3H, CH_3−Ala), 1.32 (d, J = 6.0 Hz, 3H, CH_3−Thr), 1.07 (d, J = 7.0 Hz, 3H, CH_3−Leu), 1.04 (d, J = 6.5 Hz, 3H, CH_3−Leu). ^{13}C NMR (75 MHz, MeOH-d_4): δ 176.5, 176.1, 175.1, 174.8, 174.7, 173.4, 173.0, 172.9 (CO), 152.7 (C−guan), 138.2 (ArC−Phe), 130.7, 129.9, 128.2 (ArCH−Phe), 68.5, 67.2, 65.4 (CHO−Thr, CH_2O−guan), 60.3, 53.9, 53.0, 52.0, 51.8 (CHα−Ala, Thr, Leu, Asn, Phe), 51.4 (CH_3O), 46.8, 46.4 (CHα−guan), 41.5 ($CH_2\gamma$−guan), 38.7, 38.0, 36.3 (CH_2−Leu, Asn, Phe), 34,7 (CH_2CO−adip), 26.3, 26.1 (CH_2−adip), 25.7 (CH−Leu), 24.0, 23.9 ($CH_2\beta$−guan), 22.1, 20.6, 17.8 (CH_3−Leu, Ala, Thr). FAB/LSIMS *m*/*z*: 888.6 [(M − Cl^-)$^+$, 100%]. HRMS for [$C_{42}H_{66}N_9O_{12}$]$^+$ 888.4830; found 888.4801.

Synthesis of Compound 2b. A solution of compound **1b** (Cl^-) (76 mg, 0.065 mmol) in a mixture of 30% HCl−CH_3CN (1:4, 5 mL) was stirred for 4 h. After removal of the solvent, the crude was dissolved in water and washed with diethyl ether and CH_2Cl_2. After evaporation of the water, the crude was dissolved in CH_3COOH and purified by preparative HPLC (CH_3CN−H_2O, 60:40), affording 13 mg of **2b** with different anions (analytical gradient 0−100% CH_3CN−H_2O in 20 min:

2b (Cl^-), τ_R = 12.8 min; **2b** ($CH_3CO_2^-$), τ_R = 15.2 min). ^{1}H NMR (Cl^-) (300 MHz, MeOH-d_4): δ 7.21−7.09 (m, 5H, ArH−Phe), 4.61−4.51, (m, 2H, CHα−Asn, Phe), 4.28−4.13 (m, 4H, CHα−Leu, Thr, Ala), 3.59−3.54 (m, 2H, CH_2O−guan), 3.58 (s, 3H, OCH_3), 3.47−3.17 (m, 6H, CHα−guan, $CH_2\gamma$−guan, CH_2N), 3.11−3.05 (m, 2H, CH_2−Phe), 2.69−2.56 (m, 2H, CH_2−Asn), 2.31 (m, 2H, CH_2CO−adip), 2.28 (m, 2H, CH_2CO−adip), 2.10−1.89 (m, 4H, $CH_2\beta$−guan), 1.85−1.61 (m, 7H, $(CH_2)_2$−adip, $CH_2\beta$−Leu, CHγ−Leu), 1.37 (d, J = 7.2 Hz, 3H, CH_3−Ala), 1.19 (d, J = 6.2 Hz, 3H, CH_3−Thr), 0.97 (d, J = 6.0 Hz, 3H, CH_3−Leu), 0.94 (d, J = 6.0 Hz, 3H, CH_3−Leu). ^{13}C NMR (Cl^-) (75 MHz, MeOH-d_4): δ 175.9, 175.5, 174.5, 174.2, 173.4, 171.5, 171.0 (CO), 154.3 (C−guan), 137.7, 130.1, 129.3, 127.7 (ArC, ArCH−Phe), 66.6 (CHO−Thr), 64.8 (CH_2O−guan), 59.7 (CHα−Thr), 55.2, 53.3, 52.4 (CHα−Leu, Asn, Phe), 51.4 (OCH_3), 51.3 (CHα−Ala), 50.9 (CHα−guan), 46.3 ($CH_2\gamma$−guan), 45.8 (CH_2-NH), 40.9 (CH_2−Leu), 38.1 (CH_2−Asn), 37.5 (CH_2−Phe), 35.8 (CH_2CO−adip), 34.2 (CH_2CO−adip), 25.7, 25.5 [$(CH_2)_2$−adip], 25.1 (CH−Leu), 23.5, 21.1 ($CH_2\beta$−guan), 23.3 (CH_3−Leu), 20.0 (CH_3−Thr), 17.3 (CH_3−Ala). FAB/LSIMS m/z: 887.5 [(M − Cl^-)$^+$, 100%].

Synthesis of (2*S*,8*S*)-2-(*tert*-Butyldiphenylsilanyloxymethyl)-8-methanesulfonyloxymethyl-3,4,6,7,8,9,-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Hexafluorophosphate (13). To a stirred solution of guanidinium salt **9**[14] (PF_6^-)[20] (1.15 g, 1.98 mmol) and Et_3N (1.5 mL, 10.8 mmol) in dry THF (40 mL) was added at 0 °C a solution of methanesulfonic anhydride (774 mg, 4.31 mmol) in dry THF (10 mL). The reaction mixture was stirred at this temperature for 1 h. Then the solvent was removed, and after addition of CH_2Cl_2, the organic phase was washed with 0.1 N NH_4PF_6, filtered over cotton, and concentrated and the crude was purified by silica gel column chromatography (2% MeOH−CH_2Cl_2), affording **13** (PF_6^-) (1.32 g, 98%) as a white solid. Mp 60−62°C. $[\alpha]^{25}_D$ +43 (*c* 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (m, 4H, PhSi), 7.62 (m, 6H, PhSi), 6.24 (s, 1H, NH), 6.08 (s, 1H, NH), 4.30 (m, 1H, CH_2OMs), 4.17 (m, 1H, CH_2OMs), 3.80 (m, 1H, CHα), 3.65 (m, 2H, CH_2OSi), 3.57 (m, 1H, CHα), 3.33 (m, 4H, $CH_2\gamma$), 3.08 (s, 3H, CH_3SO_3), 2.05−1.89 (m, 4H, $CH_2\beta$), 1.06 (s, 9H, $(CH_3)_3C$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.6 (C−guan), 135.5, 132.5, 130.0, 128.9 (ArCH, ArC), 69.5 (CH_2OMs), 66.2 (CH_2OSi), 50.1, 47.7 (CHα), 45.3, 44.9 ($CH_2\gamma$), 37.1 (CH_3SO_3), 26.7 [(CH_3)$_3$C], 22.4, 21.9 ($CH_2\beta$), 19.1 [$(CH_3)_3C$]. FAB/LSIMS m/z: 516.2 [(M − PF_6^-)$^+$, 100%]. HRMS for [$C_{26}H_{38}N_3O_4SSi$]$^+$ 516.2352; found 516.2354.

Synthesis of (2*S*,8*S*)-8-(*tert*-Butyldiphenylsilanyloxymethyl)-2-(carboxymethylsulfanylmethyl)-3,4,6,7,8,9,-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Chloride (14). To a stirred solution of **13** (PF_6^-) (680 mg, 1.03 mmol) in dry THF (50 mL) was added a solution of sodium mercaptoacetic acid (363 mg, 3.08 mmol) and *t*-BuOK (341 mg, 2.88 mmol) in MeOH (20 mL). The resulting mixture was stirred for 4 h at room temperature. The solvent was removed, and the solid residue was dissolved in CH_2Cl_2 (100 mL), washed with 1 N $NaHCO_3$, water, and HCl (1 N, 100 mL each), and then dried over Na_2SO_4. Filtration and evaporation of the solvent gave a crude that was triturated with ethyl acetate to afford **14** (Cl^-) (571 mg, 82%) as a white solid. Mp 166−168 °C. $[\alpha]^{25}_D$ +50 (*c* 0.5, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.27 (s, 1H, NH), 8.16 (s, 1H, NH), 7.65−7.60 (m, 4H, PhSi), 7.41−7.39 (m, 6H, PhSi), 3.77 (dd, J = 11.0, 4.0 Hz, 1H, CH_2OSi), 3.59 (m, 3H, CH_2OSi, CHα), 3.43 (d, J = 15.0 Hz, 1H, SCH_2CO), 3.36 (d, J = 15.0 Hz, 1H, SCH_2CO), 3.28−3.23 (m 4H $CH_2\gamma$), 2.95−2.71 (m, 2H, CH_2S), 2.12−1.85 (m, 4H, $CH_2\beta$), 1.07 [s, 9H, $(CH_3)_3C$)]. ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.9 (CO), 150.9 (C−guan), 135.5, 135.4, 132.6, 129.8, 127.8 (ArCH, ArC), 65.3 (CH_2O), 49.3, 48.0 (CHα), 45.1, 44.7 ($CH_2\gamma$), 36.7 (CH_2S), 34.5 (SCH_2-CO), 26.7 [(CH_3)$_3$C)], 24.8, 22.5 ($CH_2\beta$), 19.1 [$(CH_3)_3C$)]. FAB/LSIMS m/z: 512.4 [(M − Cl^-)$^+$, 100%]. Anal. Calcd for $C_{27}H_{38}N_3O_3SSiCl\cdot H_2O$: C, 57.1; H, 7.1; N, 7.4; S, 5.6. Found: C, 57.5; H, 6.8; N, 7.3; S, 5.5.

General Procedure for Compounds 3−6, 8, and 20. To a solution of guanidinium compound, HOBt, and BOP in DMF was added a solution of peptide and NMM in DMF. The reaction mixture was stirred at room temperature and monitored by HPLC. After removal of the solvent, the crude was purified by preparative HPLC.

Synthesis of Compound 3. A mixture was prepared that contained compound **14** (Cl^-) (51 mg, 0.093 mmol), HOBt (17 mg, 0.093 mmol), BOP (66.5 mg, 0.102 mmol), and DMF (2 mL). Then Ala-Ile-Thr(Bn)-Leu-Trp-OMe (TFA salt, 75 mg, 0.093 mmol), NMM (36 μL, 0.186 mmol), and DMF (1 mL) were added. The reaction was monitored by HPLC, and the reaction time was 2 h. Purification was by preparative HPLC (H_2O−CH_3CN 30:70, 45:65 for purification; analytical gradient 0−100% CH_3CN−H_2O in 20 min; τ_R = 19.5 min; purity >99%), affording **3** (Cl^-) (106 mg, 92%) as a white solid. Mp 180−182°C. $[\alpha]^{25}_D$ +25 (*c* 0.2, DMSO). ^{1}H NMR (500 MHz, COSY, DMSO-d_6): δ 10.88 (s, 1H, NH−guan), 8.36 (d, J = 7.0 Hz, 1H, NH−Trp), 8.19 (d, J = 6.2 Hz, 1H, NH−Ala), 8.04 (t, J = 7.6 Hz, 1H, NH−Leu), 7.90 (d, J = 8.6 Hz, 1H, NH−Ile), 7.77 (d, J = 8.5 Hz, 1H, NH−Thr), 7.69 (d, J = 4.0 Hz, 1H, ArH), 7.63−7.61 (m, 4H, ArH), 7.56−7.39 (m, 6H, PhSi), 7.34−7.24 (m, 8H, ArH), 7.12 (s, 1H, NH), 7.06 (t, J = 7.5 Hz, 1H, ArH−Trp), 6.96 (t, J = 8.0 Hz, 1H, ArH−Trp), 4.56 (m, 1H, OCH_2-Ph), 4.50−4.37 (m, 5H, CHα−Ile, Trp, Ala, Thr, OCH_2Ph), 4.30 (t, J = 7.5 Hz, 1H, CHα−Leu), 3.95−3.68 (m, 1H, CHβ−Thr), 3.65−3.48 (m, 7H, CH_2OSi, CHα−guan, OCH_3), 3.44−3.42 (m, 4H, $CH_2\gamma$−guan), 3.23 (s, 2H, SCH_2CO), 3.09−3.01 (m, 2H, CH_2−Trp), 2.84−2.55 (m, 2H, CH_2S), 1.99−1.90 (m, 2H, $CH_2\beta$−guan), 1.80−1.72 (m, 3H, $CH_2\beta$−guan, CHγ−Leu), 1.63−1.57 (m, 2H, CH_2−Leu), 1.44−1.42 (m, 3H, CH−Ile, CH_2−Ile), 1.16 (d, J = 7.0 Hz, 3H, CH_3−Ala), 1.05 (d, J = 6.0 Hz, 3H, CH_3−Thr), 1.03 (s, 9H, $(CH_3)_3C$), 0.98−0.93 (m, 3H, CH_3−Ile), 0.86−0.80 (m, 9H, CH_3−Leu, Ile). ^{13}C NMR (125 MHz, DEPT, DMSO-d_6): δ 174.5, 172.9, 169.9, 168.9, 167.9 (CONH), 153.9 (C−guan), 142.2, 139.5, 136.0, 135.9 (ArCH, ArC), 135.4, 134.9, 133.3, 131.0, 130.9 (PhSi), 128.9, 128.86, 128.4, 128.1, 127.9 (ArC, ArCH), 121.8, 109.0 (ArC, ArCH−Trp), 77.3 (CHβ−Thr), 71.4 (OCH_2Ph), 67.1 (CH_2OSi), 61.1 (CH_3O), 57.8 (CHα−Leu), 56.7 (CHα−Trp), 52.6 (CHα−Ala), 50.8 (CHα−Leu), 49.0 (CHα−Thr), 48.5, 48.4 (CHα−guan), 48.1, 47.0 ($CH_2\gamma$−guan), 46.8 (CH_2), 42.2 ($CH_2\beta$−Leu), 37.3 (CH_2S), 35.1 (SCH_2CO), 27.5 [(CH_3)$_3$C], 27.4 (CH−Ile), 25.9 ($CH_2\beta$−guan), 24.9, 23.9 (CH_3−Leu), 22.5 (CH_3−Ile), 19.7 [$(CH_3)_3C$], 19.2 (CH_3−Ala), 17.1 (CH_3−Thr), 16.1 (CH_3−Ile), 11.9 (CH_2−Ile). FAB/LSIMS m/z: 1200.0 [(M − Cl^-)$^+$, 100%]. HRMS for [$C_{65}H_{90}N_9O_9SSi^+$] 1200.6352; found 1200.6360.

Synthesis of Compound 6. A mixture was prepared that contained compound **14** (Cl^-) (97 mg, 0.176 mmol), HOBt (32 mg, 0.176 mmol), BOP (100 mg, 0.194 mmol), and DMF (4 mL). Gly-Ala-Thr(Bn)-Leu-Asn-Phe-OBn (TFA salt) (183 mg, 0.176 mmol), NMM (60 μL, 0.352 mmol), and DMF (2 mL) were then added. The reaction was monitored by HPLC (H_2O−CH_3CN, 0−100% in 20 min). Reaction time was 2 h. Purification was by preparative HPLC (H_2O−CH_3CN 30:70, 40:60 for purification; analytical gradient 0−100% CH_3CN−H_2O; τ_R = 20.5 min; purity >99%), affording **6** (Cl^-) as a white solid (210 mg, 89%). Mp 214−216 °C. $[\alpha]^{25}_D$ +31 (*c* 0.1, DMSO). ^{1}H NMR (500 MHz, COSY, DMSO-d_6): δ 8.27 (t, J = 5.7 Hz, 1H, NH−Gly), 8.17−8.13 (m, 3H, NH−Phe, Asn, Ala), 8.04 (d, J = 8.9 Hz, 1H, NH−Leu), 7.67 (d, J = 8.2 Hz, 1H, NH−Thr), 7.64−7.62 (m, 5H, PhSi, NH−guan), 7.50−7.41 (m, 7H, PhSi, NH−guan), 7.36−7.27 (m, 8H, ArH), 7.26−7.19 (m, 6H, ArH), 7.16 (d, J = 6.6 Hz, 1H, ArH), 6.90 (s, 2H, NH_2), 5.05−5.04 (d, J = 5.0 Hz, 2H, CO_2CH_2−Phe), 4.57 (dd, J = 13.2, 7.7 Hz, 1H, CHα−Asn), 4.51 (d, J = 12.0 Hz, 1H, OCH_2−Phe), 4.50−4.36 (m, 4H, CHα−Phe, Ala, Thr, Leu), 4.43 (d, J = 12.0 Hz, 1H, OCH_2−Phe), 4.00−3.96 (m, 1H, CHβ−Thr), 3.80 (dd, J = 17.0, 6.0 Hz, 1H, CH_2−Gly), 3.73 (dd, J = 17.0, 6.0 Hz, 1H, CH_2−Gly), 3.64−3.42 (m, 4H, CH_2OSi, CHα−guan), 3.34−3.27 (m, 4H, $CH_2\gamma$−guan), 3.26 (s, 2H, SCH_2CO), 2.98 (d, J = 8.0 Hz, 2H, $CH_2\beta$−Phe), 2.82 (dd, J = 14.0, 6.0 Hz, 1H, CH_2S), 2.72 (dd, J = 14.0, 6.0 Hz, 1H, CH_2S), 2.36 (dd, J = 15.0, 8.0 Hz, 2H, CH_2−Asn), 2.02−1.95 (m, 2H, $CH_2\beta$−guan), 1.81−1.75 (m, 2H, $CH_2\beta$−guan), 1.57−1.55 (m, 1H, CHγ−Leu), 1.42−1.40 (m, 2H, $CH_2\beta$−Leu), 1.24 (d, J = 7.0 Hz, 3H, CH_3−Ala), 1.10 (d, J = 6.0 Hz, 3H, CH_3−Thr), 1.03 (s, 9H, $(CH_3)_3C$), 0.82 (d, J = 6.5

Hz, 3H, CH_3−Leu), 0.78 (d, J = 6.5 Hz, 3H, CH_3−Leu). ^{13}C NMR (125 MHz, DEPT, DMSO-d_6): δ 173.4, 172.5, 172.1, 171.9, 170.1, 170.0, 169.1 (CO), 151.2 (C−guan), 139.5, 137.6, 136.5 (ArC), 136.0, 135.9 (ArCH), 133.5 (ArC), 133.3 (ArC−Phe), 131.0, 130.9 (ArCH), 129.9 (ArCH−Phe), 129.2, 129.16, 128.9, 128.8, 128.7, 128.3, 128.1, 127.5 (ArCH, ArC), 75.5 (CHβ−Thr), 71.3 (OCH_2Ph−Thr), 66.9 (CO_2CH_2Ph), 66.7 (CH_2-OSi), 57.8 (CHα−Leu), 54.7 (CHα−Phe), 51.5 (CHα−Ala), 50.3 (CHα−guan), 50.2 (CHα−Asn), 48.9 (CHα−Thr), 48.1 (CHα−guan), 45.3 ($CH_2\gamma$−guan), 42.8 (CH_2−Gly), 42.2 ($CH_2\beta$−Leu), 37.8 (CH_2−Asn), 37.5 (CH_2−Phe), 37.2 (CH_2S), 35.1 (SCH_2-CO), 27.5 [(CH_3)$_3$C], 25.6 ($CH_2\beta$−guan), 24.8 (CH_3−Leu), 23.9 (CH_3−Leu), 19.7 [(CH_3)$_3C$], 19.2 (CH_3−Ala), 17.2 (CH_3−Thr). FAB/LSIMS m/z: 1296.0 [(M − Cl^-)$^+$, 100%]. HRMS for [$C_{69}H_{91}N_{10}O_{11}SSi$]$^+$ 1295.6358; found 1295.6372.

Synthesis of Compound 7. To a solution of compound **6** (Cl^-) (20 mg, 0.014 mmol) in MeOH (5 mL) with few drops of acetic acid was added 10% Pd−C (15 mg, 0.014 mmol), and the mixture was kept under H_2 atmosphere for 5 h. The reaction mixture was filtered over Celite and evaporated, affording **7** (Cl^-) (13 mg, 80%) as a white solid. Analytical HPLC (gradient 10−100% CH_3CN-H_2O; τ_R = 19.1 min; purity 96%). Mp 220°C. [α]$^{25}_D$ +38 (c 0.1, DMSO). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.08 (s, 1H, NH−guan), 9.95 (s, 1H, NH−guan), 8.51 (m, 1H, NH−Gly), 8.33 (m, 1H, NH−Phe), 8.05 (m, 1H, NH−Asn), 7.64−7.60 (m, 5H, PhSi, NH−Ala), 7.50−7.41 (m, 7H, PhSi, NH−Leu), 7.31−7.17 (m, 5H, ArH, NH−Thr), 7.12−7.02 (m, 6H, ArH), 6.83 (s, 2H, NH_2), 4.57−4.20 (m, 7H, CHα−Asn, OCH_2, CHα−Phe, Ala, Thr, Leu), 4.00−3.96 (m, 1H, CHβ−Thr), 3.80−3.20 (m, 12H, CH_2−Gly, CH_2OSi, CHα−guan, $CH_2\gamma$−guan, SCH_2CO), 2.98−2.10 (m, 6H, CH_2−Phe, CH_2S, CH_2−Asn), 2.02−1.65 (m, 4H, $CH_2\beta$−guan), 1.57−1.15 (m, 6H, CHγ−Leu, $CH_2\beta$−Leu, CH_3−Ala), 1.06 (d, J = 6.0 Hz, 3H, CH_3−Thr), 1.00 (s, 9H, (CH_3)$_3$C), 0.77 (d, J = 6.5 Hz, 3H, CH_3−Leu), 0.68 (d, J = 6.5 Hz, 3H, CH_3−Leu). FAB/LSIMS m/z: 1250.6 [(M − Cl^-)$^+$, 100%].

Synthesis of Compound 15. To a solution of **14** (Cl^-) (50 mg, 0.090 mmol), glycine methyl ester hydrochloride (12 mg, 0.09 mmol), HOBt (12 mg, 0.090 mmol), and BOP (44 mg, 0.100 mmol) in DMF (2 mL) was added NMM (22 μL, 0.190 mmol). The resulting mixture was stirred overnight. The solvent was evaporated under reduced pressure, and the solid residue dissolved in CH_2Cl_2 (30 mL) was washed successively with a saturated solution of $NaHCO_3$ and then with a solution of HPF_6 (0.01 N). The organic phase was dried, filtered, and concentrated. Purification by silica gel column chromatography with a solvent mixture of 4% MeOH in CH_2Cl_2 afforded a white solid (58 mg, 90%). To a solution of the resulting solid (38 mg, 0.052 mmol) in THF (1 mL) was added a solution of NaOH (1 N, 0.10 mL). The mixture was stirred for 4 h at room temperature. The solvent was evaporated, and the solid residue was dissolved in CH_2Cl_2 (20 mL) and washed with 0.1 N NH_4PF_6. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The resulting solid residue was triturated with diethyl ether and dried to afford compound **15** (PF_6^-) (28 mg, 95%) as a white solid. Mp 94−95 °C. [α]$^{25}_D$ +42 (c 0.2, $CHCl_3$). ^{1}H NMR (500 MHz, COSY, $CDCl_3$): δ 10.05 (s, 1H, NH), 9.98 (s, 1H, NH), 7.82 (t, J = 4.6 Hz, 1H, NHCO), 7.65−7.62 (m, 4H, PhSi), 7.46−7.38 (m, 6H, PhSi), 3.91 (dd, J = 13.0, 4.5.0 Hz, 1 H, $NHCH_2$CO), 3.82 (dd, J = 4.5, 17.5 Hz, 1H, $NHCH_2$-CO), 3.77 (d, J = 6.0 Hz, 1 H, CH_2OSi), 3.58 (m, 3H, CH_2OSi, CHα), 3.37−3.20 (d, J = 15.1 Hz, 2H, SCH_2CO), 3.24−3.12 (m, 4H $CH_2\gamma$), 2.84 (dd, J = 14, 5 Hz, 1H, CH_2S), 2.78 (dd, J = 14, 6.5 Hz, 1H, CH_2S), 2.05−1.90 (m, 4H, $CH_2\beta$), 1.06 (s, 9H, (CH_3)$_3$C). ^{13}C NMR (125 MHz, DEPT, $CDCl_3$): δ 175.1 (CONH), 168.9 (CO), 151.4 (C−guan), 135.5, 132.8, 128.3, 127.8 (ArCH, ArC), 65.3 (CH_2O), 48.9, 48.4 (CHα), 45.3 (CH_2N), 44.5, 44.0 ($CH_2\gamma$), 36.8, 36.5 (CH_2S, SCH_2CO), 26.8 [(CH_3)$_3$C], 25.0, 22.7 ($CH_2\beta$), 19.2 [(CH_3)$_3C$]. FAB/LSIMS m/z: 569.1 [(M$^+$, 100%].

Synthesis of Compound 4. 4 was prepared according to the general procedure. A mixture was prepared that contained compound **15** (100 mg, 0.170 mmol), Ile-Ser(Bn)-Tyr(Bn)-Asn-Leu-OMe (TFA salt) (161 mg, 0.170 mmol), BOP (86 mg, 0.180 mmol), HOBt (24 mg, 0.170 mmol), DMF (12 mL), and NMM (21 μL, 0.038 mmol). Reaction time was 12 h. The residue was triturated with MeOH and filtered and the solid was purified by semipreparative HPLC (gradient CH_3CN-H_2O 75:25 to 60:40; flow 6 mL/min; τ_R = 23.6 min; purity 93%) to afford compound **4** (PF_6^-) (191 mg, 72%) as a white solid. Mp 200 °C. [α]$^{25}_D$ +10 (c 0.5, DMSO). ^{1}H NMR (500 MHz, COSY, DMSO-d_6): δ 8.27−8.23 (m, 2H, NH−Gly, Tyr), 8.14 (d, J = 8.0 Hz, 1H, NH−Ser), 8.07 (d, J = 7.7 Hz, 1H, NH−Ile), 7.95−7.92 (m, 2H, NH−Asn, Leu), 7.64−7.61 (m, 4H, PhSi), 7.60 (s, 1H, NH−guan), 7.51−7.25 (m, 16H, ArH), 7.12 (d, J = 8.7 Hz, 2H, ArHm−Tyr), 6.93 (s, 1H, NH−guan), 6.83 (d, J = 8.7 Hz, 2H, ArHo−Tyr), 5.02 (s, 2H, OCH_2Ph−Tyr), 4.62−4.48 (m, 5H, CH−Tyr, Ser, Asn, OCH_2Ph−Ser), 4.30−4.26 (m, 2H, CH−Leu, Ile), 3.84−3.77 (m, 2H, CH_2−Gly), 3.67−3.63 (m, 2H, CH_2O−Ser), 3.62 (s, 3H, OCH_3), 3.61−3.53 (m, 2H, CHα), 3.52−3.42 (m, 2H), 3.33−3.22 (m, 4H, $CH_2\gamma$), 2.99−2.95 (m, 1H, CH_2−Asn), 2.84−2.69 (m, 3H, CH_2S, CH_2−Asn), 2.55−2.37 (m, 2H, CH_2Ph−Tyr), 2.01−1.95 (m, 2H, $CH_2\beta$), 1.85−1.73 (m, 2H, $CH_2\beta$), 1.72−1.46 (m, 4H, CH−Ile, CH_2−Leu, CH−Leu), 1.43−1.35 (m, 2H, CH_2−Ile), 1.03 (s, 9H, (CH_3)$_3$C), 0.87 (d, J = 6.6 Hz, 3H, CH_3−Leu), 0.83 (d, J = 6.5 Hz, 3H, CH_3−Leu), 0.78−0.75 (m, 6H, CH_3−Ile). ^{13}C NMR (125 MHz, DEPT, DMSO-d_6): δ 173.6, 172.1, 171.9, 171.7, 171.3, 169.9, 169.8, 169.3 (CO), 151.2 (C−guan), 138.9, 138.1 (ArC), 136.0, 135.9 (ArCH), 133.5, 133.3, 131.2 (ArC), 131.0, 130.9, 130.5, 129.3, 129.1, 128.9, 128.8, 128.6, 128.4, 128.2 (ArCH), 115.1 (ArCo−Tyr), 73.2, 72.9, 70.0, 61.1 (CH_2O), 57.5, 54.7, 53.5 (CH), 52.7 (CH_3O), 51.2, 50.3, 50.2, 48.1 (CH), 45.3, 45.2, 42.9, 40.2 (CH_2), 39.9, 38.0 (CH), 37.6, 37.2, 35.1 (CH_2), 31.5 (CH), 27.5 [(CH_3)$_3$C], 25.6, 25.0 (CH_2), 23.6 (CH), 23.0 (CH_2), 22.2 (CH), 19.7 [(CH_3)$_3C$], 16.1, 11.9 (CH_3). FAB/LSIMS m/z: 1353.3 [(M − PF_6^-)$^+$, 100%]. HRMS for [$C_{72}H_{97}N_{10}O_{12}SSi$]$^+$ 1353.6777; found 1353.6739.

Synthesis of Compound 5. 5 was prepared according to the general procedure. A mixture was prepared that contained compound **15** (PF_6^-) (30 mg, 0.05 mmol), Ile-Ser-Tyr-Asn-Leu-OMe (TFA salt) (39 mg, 0.05 mmol), BOP (26 mg, 0.06 mmol), HOBt (7 mg, 0.05 mmol), DMF (12 mL), and NMM (6 μL, 0.058 mmol). Reaction time was 12 h. The product was precipitated with MeOH−H_2O and triturated with AcOEt and toluene to afford compound **5** (PF_6^-) (54 mg, 77%; HPLC gradient CH_3CN-H_2O 10−100% in 20 min; τ_R = 17.8 min; purity 94%) as a white solid. Mp 178 °C. [α]$^{25}_D$ +15 (c 0.2, DMSO). ^{1}H NMR (500 MHz,COSY, DMSO-d_6): δ 9.12 (s, 1H, OH), 8.27 (br t, 1H, NH−Gly), 8.17 (d, J = 6.5 Hz, 1H, NH), 8.04−7.80 (m, 4H, NH), 7.64−7.62 (m, 4H, PhSi), 7.48−7.30 (m, 6H, PhSi), 6.98 (d, J = 8.4 Hz, 2H, CH−Tyr), 6.92 (s, 1H, NH), 6.59 (d, J = 8.4 Hz, 2H, CH−Tyr), 4.58−4.54 (m, 2H, CH−Asn, Leu), 4.42−4.26 (m, 3H, CH−Ser, Tyr, Ile), 3.81−3.79 (m, 2H, CH_2−Gly), 3.72−3.41 (m, 7H, CH_2O, OCH_3), 3.40−3.27 (m, 9H, CHα, SCH_2CO, $CH_2\gamma$), 3.18−2.68 (m, 4H, CH_2S, CH_2−Asn), 2.41−2.36 (m, 2H, CH_2−Ph), 2.22−1.86 (m, 4H, $CH_2\beta$), 1.80−1.24 (m, 4H, CH−Ile, Leu, CH_2−Leu), 1.03 (s, 9H, (CH_3)$_3$C), 0.89 (d, J = 6.5 Hz, 3H, CH_3−Leu), 0.85 (d, J = 6.5 Hz, 3H, CH_3−Leu), 0.78−0.75 (m, 6H, CH_3−Ile). ^{13}C NMR (125 MHz, DEPT, $CDCl_3$): δ 178.7, 170.6 (CONH), 156.6, 151.8 (C−guan), 141.8, 136.0, 135.9, 133.3, 130.9, 128.9 (ArCH, ArC), 115.7 (Co−Tyr), 73.2, 67.4, 63.9, 61.1 (CH_2O), 57.0, 56.5, 56.3, 53.6, 52.7, 50.0, 49.1, 47.3, 45.5, 43.0, 38.6, 38.0, 37.9, 37.5 (CH_2S), 32.2, 31.6, 27.5 [(CH_3)$_3$C], 24.9, 24.7 ($CH_2\beta$), 23.6, 23.0, 22.1, 19.7 [(CH_3)$_3C$], 16.1, 11.8 (CH_3). HRMS for [$C_{58}H_{85}N_{10}O_{12}SSi$]$^+$ 1173.5838; found 1173.5853.

Synthesis of 8-(*tert*-Butyldiphenylsilanyloxymethyl)-2-[17-(1,5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,-11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]-phenanthren-4-ylsulfanylmethyl]-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Hexafluorophosphate (16). To a stirred solution of **13** (PF_6^-) (460 mg, 0.70 mmol) in dry THF (40 mL) was added a solution of thiocholesterol (420 mg, 1.04 mmol) and t-BuOK (115 mg, 0.97 mmol) in THF−MeOH (1:1) (20 mL). The resulting mixture was stirred for 4 h at room temperature. The solvent was removed, and the residue was dissolved in CH_2Cl_2 and washed with a solution

of 0.1 N NH_4PF_6. The organic phase was filtered over cotton and concentrated in vacuo. Purification by silica gel column chromatography (0.02% and 1% MeOH−CH_2Cl_2) afforded **16** (PF_6^-) (560 mg, 83%) as a white solid. Mp 82 °C. $[\alpha]^{25}_D$ +16 (*c* 0.5, $CHCl_3$). ^{1}H NMR (500 MHz, COSY, $CDCl_3$): δ 7.66−7.64 (m, 4 H, PhSi), 7.48−7.42 (m, 6H, PhSi), 6.17 (s, 1H, NH), 6.00 (s, 1H, NH), 5.38−5.37 (m, 1H, CH−chol), 3.70−3.68 (dd, J = 9.9, 4.7 Hz, 2H, CH_2OSi), 3.57−3.45 (m, 2H, CHα), 3.37−3.29 (m, 4H, $CH_2\gamma$), 2.80 (dd, J = 14.1, 6.3 Hz, 1H, CH_2S), 2.69 (dd, J = 14.1, 6.3 Hz, 1H, CH_2S), 2.63−2.56 (m, 1H, CHS−chol), 2.34−2.25 (m, 2H, CH_2CH−chol), 2.63−2.56 (m, 1H, CHS−chol), 2.34−2.25 (m, 2H, CH_2CH−chol), 2.22−2.16 (m, 2H, $CH_2\beta$), 1.94−1.80 (m, 5H, $CH_2\beta$, CH−chol, CH_2−chol), 1.70−1.67 (m, 1H, CH_2), 1.63−1.25 (m, 17H, CH_2−chol, CH−chol), 1.20−1.10 (m, 4H, CH_2C−chol, CH_2CH), 1.06 (s, 9H, $(CH_3)_3$C), 1.00 (s, 3H, CH_3−chol), 1.00−0.90 (m, 1H, CH−chol), 0.93 (d, J = 6.5 Hz, 3H, CH_3), 0.87 (d, J = 6.6 Hz, 3H, CH_3), 0.86 (d, J = 6.6 Hz, 3H, CH_3), 0.66 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DEPT, $CDCl_3$): δ 150.4 (C−guan), 141.0 (*C*═CH), 135.5, 132.5, 130.0, 128.0 (ArC, ArCH), 121.5 (*C*H═C), 65.4 (CH_2O), 56.7, 56.1 (CH−chol), 50.2, 50.1 (CHα), 49.0 (CH−chol), 45.6, 45.3 ($CH_2\gamma$), 45.1, 42.3 (CH−chol), 39.8, 39.7 (CH_2−chol), 39.5, 39.4 (CH_2), 36.8 (CH), 36.2 (CH_2), 35.8 (CH), 34.0, 31.8 (CH_2), 31.7 (CH), 29.8 (CH_2), 28.2 (CH), 28.0 (CH_2), 26.8 [$(CH_3)_3$C], 25.1, 24.3, 23.8 (CH_2), 22.8 (CH_3), 22.6 (CH_2), 22.5 (CH_3), 20.9 (CH_2), 19.3 (CH_3), 19.1 [$(CH_3)_3C$], 18.7, 11.8 (CH_3). ESI$^+$ *m*/*z*: 822.7 [(M − PF_6^-)$^+$, 100%]. HRMS for [$C_{52}H_{80}N_3OSSi$]$^+$ 822.5791; found 822.5779.

Synthesis of 2-[17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-4-ylsulfanylmethyl]-8-hydroxymethyl)-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Hexafluorophosphate (17). To a solution of **16** (PF_6^-) (420 mg, 0.43 mmol) in dry THF (45 mL) was added dropwise HF−Py (70%, 2.25 mL) at 0 °C. The resulting mixture was stirred overnight at room temperature. The solvent was evaporated and the residue was purified two times by silica gel column chromatography (3% and 5% MeOH−CH_2Cl_2), affording **17** (PF_6^-) (297 mg, 94%) as a white solid. Mp 243 °C. $[\alpha]^{25}_D$ +50 (*c* 0.4, $CHCl_3$). ^{1}H NMR (500 MHz, COSY, $CDCl_3$): δ 6.79 (s, 1H, NH), 6.57 (s, 1H, NH), 5.35 (d, J = 5.1 Hz, 1H, CH−chol), 3.80 (br s, 1H, OH), CH_2OH), 3.58 (dd, J = 18.7, 7.6 Hz, 2H, CH_2OH), 3.49−3.41 (m, 2H, CHα), 3.40−3.20 (m, 4H, $CH_2\gamma$), 2.76 (dd, J = 13.5, 6.6 Hz, 1H, CH_2S), 2.70 (dd, J = 13.5, 7.2 Hz, 1H, CH_2S), 2.57 (m, 1H, CHS−chol), 2.28−2.14 (m, 4H, CH_2CH−chol), 2.01−1.77 (m, 12H, $CH_2\beta$, CH_2−chol), 1.55−1.20 (m, 11H, CH_2CH−chol, CH_2−chol, CH−chol), 1.18−1.09 (m, 1H, CH−chol), 1.00−0.92 (m, 4H, CH_2−chol, CH−chol), 0.98 (s, 3H, CH_3), 0.91 (d, J = 6.5 Hz, 3H, CH_3), 0.87 (d, J = 6.6 Hz, 3H, CH_3), 0.86 (d, J = 6.6 Hz, 3H, CH_3), 0.66 (s, 3H, CH_3). ^{13}C NMR (125 MHz, DEPT, $CDCl_3$): δ 150.7 (C−guan), 141.1 (*C*═CH), 121.4 (*C*H═C), 64.6 (CH_2O), 56.7 (CH−chol), 56.2 (CH−chol), 50.2 (CHα), 48.5 (CH−chol), 45.6, 45.4 ($CH_2\gamma$), 44.8, 42.3 (CH−chol), 39.8, 39.7 (CH_2−chol), 39.5, 39.4 (CH_2), 36.8 (CH), 36.2 (CH_2), 35.8 (CH), 34.6, 31.8 (CH_2), 31.7 (CH), 29.8 (CH_2), 28.2 (CH), 28.0, 25.5, 24.3, 23.8 (CH_2), 22.7 (CH_3), 22.5 (CH_2), 22.46 (CH_3), 20.9, 19.3 (CH_2), 18.7, 11.8 (CH_3). ESI$^+$ *m*/*z*: 584.5 [(M − PF_6^-)$^+$, 100%]. HRMS for [$C_{36}H_{62}N_3OS$]$^+$ 584.4613; found 584.4633.

Synthesis of 2-[17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-4-ylsulfanylmethyl]-8-methanesulfonyloxymethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Hexafluorophosphate (18). To a solution of **17** (PF_6^-) (100 mg, 0.140 mmol) and NMM (60 μL, 0.540 mmol) in dry THF (10 mL) was added Ms_2O (61 mg, 0.340 mmol) in dry THF (15 mL). The reaction mixture was stirred for 4 h at room temperature. After evaporation of the solvent, the resulting crude was dissolved in CH_2Cl_2 and washed with a 0.1 N NH_4PF_6 solution. The organic layer was filtered over cotton and concentrated in vacuo. Purification by silica gel column chromatography (4% MeOH−CH_2Cl_2) afforded **18** (PF_6^-) (107 mg, 97%) as a white solid. Mp 183 °C. $[\alpha]^{25}_D$ +21 (*c* 0.2, $CHCl_3$). ^{1}H NMR (500 MHz, COSY, $CDCl_3$): δ 7.22 (s, 1H, NH), 7.09 (s, 1H, NH), 5.36 (s, 1H, CH−chol), 4.32−4.17 (m, 2H, CH_2OMs), 3.60−3.35 (m, 6H, CHα, $CH_2\gamma$), 3.15 (s, 3H, CH_3OSO_2), 2.88−2.63 (m, 2H, CH_2S), 2.60−2.57 (m, 1H, CHS−chol), 2.35−2.10 (m, 4H, CH_2CH−chol, $CH_2\beta$), 2.04−1.86 (m, 10H, CH_2CH−chol, CH_2−chol, $CH_2\beta$), 1.68−1.43 (m, 9H, CH_2−chol, CH−chol), 1.22−1.07 (m, 5H, CH_2, CH), 0.99 (s, 3H, CH_3), 0.91 (d, J = 6.4 Hz, 3H, CH_3), 0.87 (d, J = 6.6 Hz, 3H, CH_3), 0.86 (d, J = 6.6 Hz, 3H, CH_3), 0.67 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 150.7 (C−guan), 141.2 (*C*═CH), 121.4 (*C*H═C), 69.8 (CH_2O), 56.7 (CH−chol), 56.2 (CH−chol), 50.2, 49.0 (CHα), 47.5 (CH−chol), 45.6, 45.2 ($CH_2\gamma$), 44.8, 42.3 (CH−chol), 39.8, 39.7 (CH_2−chol), 39.5, 39.4 (CH_2), 37.2 (CH_3), 36.8, 36.2 (CH_2), 35.8 (CH), 34.1, 31.8 (CH_2), 31.78, 29.9, 29.7 (CH), 28.2, 28.0, 25.0, 24.3, 23.8 (CH_2), 22.8, 22.5 (CH_3), 21.0 (CH_2), 19.3, 18.7, 11.8 (CH_3). FAB/LSIMS *m*/*z*: 662.3 [(M − PF_6^-)$^+$, 100%]. HRMS for [$C_{37}H_{64}N_3O_3S_2$]$^+$ 662.4389; found 662.4388.

Synthesis of 8-Carboxymethylsulfanylmethyl-2-[17-(1,5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,-13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-4-ylsulfanylmethyl]-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Hexafluorophosphate (19). To a stirred solution of **18** (PF_6^-) (70 mg, 0.09 mmol) in dry THF (10 mL) was added a solution of sodium mercaptoacetic acid (31 mg, 0.26 mmol) and *t*-BuOK (29 mg, 0.24 mmol) in THF−MeOH (1:1) (20 mL). The resulting mixture was stirred for 4 h at room temperature. After removal of the solvent, the solid residue was dissolved in CH_2Cl_2 and washed successively with $NaHCO_3$, H_2O, and 0.1 N HPF_6 and then dried over Na_2SO_4. Evaporation of the solvent gave a crude that was purified by silica gel column chromatography (5% MeOH−CH_2Cl_2) to afford **19** (PF_6^-) (53 mg, 77%) as a white solid. Mp 179 °C. $[\alpha]^{25}_D$ +27 (*c* 0.204, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.57 (s, 1H, NH), 6.28 (s, 1H, NH), 5.28−5.23 (m, 1H, CH−chol), 3.62−3.20 (m, 6H, CHα, $CH_2\gamma$), 3.19 (s, 2H, SCH_2), 2.90−2.48 (m, 5H), 2.30−2.10 (m, 6H), 1.96−1.80 (m, 8H), 1.50−0.90 (m, 19H), 0.91 (s, 3H, CH_3), 0.84 (d, J = 6.5 Hz, 3H, CH_3), 0.80 (d, J = 6.6 Hz, 3H, CH_3), 0.79 (d, J = 6.6 Hz, 3H, CH_3), 0.60 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.3 (CO), 150.5 (C−guan), 141.2 (*C*═CH), 121.4 (*C*H═C), 56.7 (CH−chol), 56.2 (CH−chol), 50.2 (CH−chol), 49.7, 48.0 (CHα), 45.6, 45.4 ($CH_2\gamma$), 44.9 (CH−chol), 42.3 (CH−chol), 39.8 (CH_2−chol), 39.7, 39.5, 39.4, 37.3, 36.2 (CH_2), 35.8 (CH), 34.4 (CH_2), 34.1 (SCH_2CO), 31.4 (CH_2), 31.8 (CH), 29.8, 28.2 (CH_2), 28.0 (CH), 25.4, 25.3, 24.3, 23.9 (CH_2), 22.8, 22.6 (CH_3), 20.9 (CH_2), 19.3, 18.7, 11.8 (CH_3). FAB/LSIMS *m*/*z*: 658.2 [(M − PF_6^-)$^+$, 100%]. HRMS for [$C_{38}H_{64}N_3O_2S_2$]$^+$ 658.4439; found 658.4458.

Synthesis of Compound 8. 8 was prepared according to the general procedure. A mixture was prepared that contained compound **19** (PF_6^-) (11 mg, 0.014 mmol), Gly-Ala-Thr(Bn)-Leu-Asn-Phe-OBn (TFA salt) (10 mg, 0.014 mmol), BOP (7 mg, 0.015 mmol), HOBt (2 mg, 0.014 mmol), DMF (1 mL), and NMM (2 μL, 0.015 mmol). Reaction time was 12 h. The crude was triturated with MeOH, and the obtained solid was purified by semipreparative HPLC (CH_3CN−H_2O 75:25; flow 5 mL/min; τ_R = 21.8 min; purity 91%) to afford compound **8** (PF_6^-) (6 mg, 27%) as a white solid. Mp 207 °C. $[\alpha]^{25}_D$ +24 (*c* 0.3, DMSO). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.82−8.47 (m, 3H, NH−Gly, Phe, Asn), 8.15−7.94 (m, 2H, NH−Ala, Trp), 7.77−7.64 (m, 18H, NH−Leu, NH−guan, ArH), 7.38 (br s, 2H, NH_2), 5.81 (br s, 1H, CH−chol), 5.49 (s, 2H, CO_2CH_2Ph), 5.04−4.84 (m, 4H, CHα−Asn, CHβ−Thr, OCH_2Ph), 4.51−4.39 (m, 4H, CHα−Phe, Ala, Thr, Leu), 4.25−4.19 (m, 2H, CH_2−Gly), 4.04−3.90 (m, 3H, CHα, SCH_2), 3.79−2.52 (m, 17H), 2.45−1.29 (m, 55H), 1.11 (s, 3H, CH_3). FAB/LSIMS *m*/*z*: 1441.4 [(M − PF_6^-)$^+$, 100%]. HRMS for [$C_{80}H_{117}N_{10}O_{10}S_2$]$^+$ 1441.8395; found 1441.8413.

Synthesis of 5-[1-(1-{1-[2-Carboxy-1-(1-methoxycarbonyl-2-phenylethylcarbamoyl)ethylcarbamoyl]-3-methylbutylcarbamoyl}-2-hydroxypropylcarbamoyl)ethylcarbamoyl] Methylpentanoate (20). 20 was prepared according to a general procedure. A mixture was prepared that contained adipic acid methyl ester (8.2 μL, 0.05 mmol), Ala-Thr-Leu-Asn-Phe-OMe (TFA salt) (38.0 mg, 0.048 mmol), BOP

(34 mg, 0.06 mmol), HOBt (15.0 mg, 0.05 mmol), NMM (14 μL, 0.10 mmol), and DMF (1 mL). Reaction time was 12 h. Purification was by precipitation with EtOAc−MeOH to afford **20** (32 mg, 92%). ^{1}H NMR (300 MHz, MeOH-d_4): δ 8.12−8.00 (m, 3H, NH), 7.74 (d, J = 9.0 Hz, 1H, NH), 7.68 (d, J = 6.0 Hz, 1H, NH), 7.30−7.16 (m, 7H, ArH, NH_2−Asn), 6.92 (s, 1H, NH), 5.00−3.99 (m, 6H, CHα, CHOH), 3.59 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 2.93 (m, 2H, CH_2CO−Asn), 2.10−2.00 (m, 4H $COCH_2$−adip), 1.63−1.43 (m, 7H, $(CH_2)_2$−adip, CH_2, CH−Leu), 1.18 (d, J = 7.1 Hz, 3H, CH_3−Ala), 1.00 (d, J = 6.5 Hz, 3H, CH_3−Thr), 0.83 (d, J = 8.2 Hz, 3H, CH_3−Leu), 0.80 (d, J = 6.5 Hz, 3H, CH_3−Leu). FAB/LSIMS m/z: 721.4 [(M + H)$^+$, 42%], 743.4 [(M + Na$^+$), 5%]. HRMS for $C_{34}H_{53}N_6O_{11}$ 721.3772; found 721.3771.

Synthesis of 2-(*tert*-Butyldiphenylsilanyloxymethyl)-8-methoxycarbonylmethylsulfanylmethyl-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidin-1-ium Chloride (21). To a stirred solution of **13** (PF_6^-) (250 mg, 0.466 mmol) in MeOH (10 mL) was added at room temperature a solution of methyl mercaptoacetate (0.17 mL, 1.864 mmol) and Cs_2CO_3 (500 mg, 3.723 mmol). The reaction mixture is kept refluxing for 6 h, and after addition of water (6 mL), the solvent is removed. The crude is dissolved in CH_2Cl_2 and washed with NH_4Cl and water. The organic layer was dried over Na_2SO_4, the solvent was eliminated, and the residue was purified by silica gel column chromatography (8% MeOH−CH_2Cl_2) to afford **21** (Cl^-) (134 mg, 58%) as a white solid. Mp 62 °C. $[\alpha]^{25}_D$ +54 (*c* 0.5, $CHCl_3$). ^{1}H NMR (Cl^-) (300 MHz, $CDCl_3$): δ 9.19 (s, 1H, NH), 8.74 (s, 1H, NH), 7.64−7.59 (m, 4H, PhSi), 7.44−7.35 (m, 6H, PhSi), 3.81−3.74 (m, 1 H, CH_2OSi), 3.72 (s, 3H, OCH_3), 3.63−3.44 (m, 3H, CH_2OSi, CHα), 3.34 (m, 1H, SCH_2-CO), 3.29−3.14 (m 4H, SCH_2CO, $CH_2\gamma$), 2.99−2.93 (m, 1H, CH_2S), 2.78−2.70 (m, 1H, CH_2S), 2.18−1.82 (m, 4H, $CH_2\beta$), 1.05 (s, 9H, $(CH_3)_3C$). ^{13}C NMR (Cl^-) (75 MHz, $CDCl_3$): δ 170.7 (CO), 151.2 (C−guan), 135.6, 135.5, 132.6, 129.9, 127.9 (C−PhSi), 65.2 (CH_2O), 52.5 (OCH_3), 49.1, 48.0 (CHα), 45.2, 44.6 ($CH_2\gamma$), 37.0 (CH_2S), 34.3 (SCH_2CO), 26.8 [($CH_3)_3C$], 24.8, 22.7 ($CH_2\beta$), 19.2 [$(CH_3)_3C$]. FAB/LSIMS m/z: 526.3 [(M + H)$^+$, 100%].

Acknowledgment. This research was supported by a BIOMED 2 Networ (Grant BMH4-CT96-0823). We are grateful to J. Dumond and F. Ainoun for their help in some kinetic experiments.

Supporting Information Available: Experimental details for synthesis of peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Debouck, C. The HIV-1 Protease as a Therapeutic Target for AIDS. *AIDS Res. Hum. Retroviruses* **1992**, *8*, 153−164.

(2) (a) Wlodawer, A.; Miller, A.; Jaskolski, M.; Sathyanarayana, B.; Baldwin, E.; Weber, I.; Selk, L.; Clawson, L.; Schneider, J.; Kent, S. Conserved Folding in Retroviral Proteases Crystal Structure of a Synthetic HIV-1 Protease. *Science* **1989**, *245*, 616−621. (b) Pearl, L. H.; Taylor, W. R. A Structural Model for the Retroviral Proteases. *Nature* **1987**, *329*, 351−354.

(3) (a) Hoetelmans, R. M.; Meenhorst, P. L.; Mulder, J. W.; Burger, D. M.; Koks, C. H.; Beijnen, J. H. Clinical Pharmacology of HIV Protease Inhibitors: Focus on Saquinavir, Indinavir, and Ritonavir. *Pharm. World Sci.* **1997**, *19*, 159−175. (b) Larder, B. Mechanisms of HIV-1 Drug Resistance. *AIDS* **2001**, *15* (Suppl. 5), S27−S34.

(4) Gustchina, A.; Weber, I. T. Comparative Analysis of the Sequences and Structures of HIV-1 and HIV-2 Proteases. *Proteins* **1991**, *10*, 325−339.

(5) (a) Weber, I. T. Comparison of the Crystal Structures and Intersubunit Interactions of Human Immunodeficiency and Rous Sarcoma Virus Proteases. *J. Biol. Chem.* **1990**, *265*, 10492−10496. (b) Ishima, R.; Ghirlando, R.; Tözsér, J.; Gronenborn, A. M.; Torchia, D. A.; Louis, J. M. Folded Monomer of HIV-1 Protease. *J. Biol. Chem.* **2001**, *276*, 49110−49116.

(6) Todd, M. J.; Semo, N.; Freire, E. The Structural Stability of the HIV-1 Protease. *J. Mol. Biol.* **1998**, *283*, 475−488.

(7) (a) Zhang, Z.-Y.; Poorman, R. A.; Maggiora, L. L.; Heinrikson, R. L.; Kedzy, F. J. Dissociative Inhibition of Dimeric Enzymes. Kinetic Characterization of the Inhibition of HIV-1 Protease by Its COOH-Terminal Tetrapeptide. *J. Biol. Chem.* **1991**, *266*, 15591−15594. (b) Schramm, H. J.; Nakashima, H.; Schramm, W.; Wakayama, H.; Yamamoto, N. HIV-1 Reproduction Is Inhibited by Peptides Derived from the N- and C-Termini of HIV-1 Protease. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 847−851. (c) Schramm, H. J.; Breipohl, G.; Hansen, J.; Henke, S.; Jaeger, E.; Meichsner, C.; Riess, G.; Ruppert, D.; Rucknagel, K. P.; Schafer, W. Inhibition of HIV-1 Protease by Short Peptides Derived from the Terminal Segments of the Protease. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 980−985. (d) Franciskovich, J.; Houseman, K.; Mueller, R.; Chmielewski, J. A Systematic Evaluation of the Inhibition of HIV-1 Protease by Its C- and N-Terminal Peptides. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 765−768. (e) Schramm, H. J.; Billich, A.; Jaeger, E.; Rucknagel, K.; Arnold, G.; Schramm, W. The Inhibition of HIV-1 Protease by Interface Peptides. *Biochem. Biophys. Res. Commun.* **1993**, *194*, 595−600. (f) Valente, S.; Gobbo, M.; Licini, G.; Scarso, A.; Scrimin, P. Allosteric Regulation of an HIV-1 Protease Inhibitor by Zn^{II} Ions. *Angew. Chem., Int. Ed.* **2001**, *40*, 3899−3902. (g) For a recent review, see the following. Boggetto, N.; Reboud-Ravaux, M. Dimerization Inhibitors of HIV-1 Protease. *Biol. Chem.* **2002**, *383*, 1321−1324.

(8) Schramm, H. J.; Boetzel, J.; Buttner, J.; Fritsche, E.; Gohring, W.; Jaeger, E.; Konig, S.; Thumfart, O.; Wenger, T.; Nagel, N. E.; Schramm, W. The Inhibition of Human Immunodeficiency Virus Proteases by Interface Peptides. *Antiviral Res.* **1996**, *30*, 155−170.

(9) (a) Schramm, H. J.; de Rosny, E.; Reboud-Ravaux, M.; Buttner, J.; Dick, A.; Schramm, W. Lipopeptides as Dimerization Inhibitors of HIV-1 Protease. *Biol. Chem.* **1999**, *380*, 593−596. (b) Dumond, J.; Boggetto, N.; Schramm, H. J.; Schramm, W.; Takahashi, M.; Reboud-Ravaux, M. Thyroxine-Derivatives of Lipopeptides: Bifunctional Dimerization Inhibitors of Human Immunodeficiency Virus-1 Protease. *Biochem. Pharmacol.* **2003**, *65*, 1097−1102.

(10) (a) Zutshi, R.; Franciskovich, J.; Shultz, M.; Schweitzer, B.; Bishop, P.; Wilson, M.; Chmielewski, J. Targeting the Dimerization Interface of HIV-1 Protease: Inhibition with Cross-Linked Interfacial Peptides. *J. Am. Chem. Soc.* **1997**, *119*, 4841−4845. (b) Shultz, M. D.; Bowman, M. J.; Ham, Y. W.; Zhao, X.; Tora, G.; Chmielewski, J. Small-Molecule Inhibitors of HIV-1 Protease Dimerization Derived from Cross-Linked Interfacial Peptides. *Angew. Chem., Int. Ed.* **2000**, *39*, 2710−2713 and references therein.

(11) (a) Bouras, A.; Boggetto, N.; Benatalah, Z.; de Rosny, E.; Sicsic, S.; Reboud-Ravaux, M. Design, Synthesis and Evaluation of Conformationally Constrained Tongs, New Inhibitors of HIV-1 Protease Dimerization. *J. Med. Chem.* **1999**, *42*, 957−962. (b) Song, M.; Rajesh, S.; Hayashi, Y.; Kiso, Y. Design and Synthesis of New Inhibitors of HIV-1 Protease Dimerization with Conformationally Constrained Templates. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2465−2468.

(12) For selected examples, see the following. (a) Echavarren, A. M.; Galán, A.; de Mendoza, J.; Salmerón, A.; Lehn, J.-M. Anion-Receptor Molecules: Synthesis of a Chiral and Functionalized Binding Subunit, a Bicyclic Guanidinium Group Derived from L- or D-Asparagine. *Helv. Chim. Acta* **1988**, *71*, 685−693. (b) Kurzmeier, H.; Schmidtchen, F. P. Abiotic anion receptor functions. A facile and dependable access to chiral guanidinium anchor groups. *J. Org. Chem.* **1990**, *55*, 3749−3755. (c) Galán, A.; Andreu, D.; Echavarren, A. M.; Prados, P.; de Mendoza, J. A Receptor for the Enantioselective Recognition of Phenylalanine and Tryptophan under Neutral Conditions. *J. Am. Chem. Soc.* **1992**, *114*, 1511−1512. (d) Peczuh, M. W.; Hamilton, A. D.; Sánchez-Quesada, J.; de Mendoza, J.; Haack, T.; Giralt, E. Recognition and Stabilization of an α-Helical Peptide by a Synthetic Receptor. *J. Am. Chem. Soc.* **1997**, *119*, 9327−9328. (e) Haack, T.; Peczuh, M. W.; Salvatella, X.; Sánchez-Quesada, J.; de Mendoza, J.; Hamilton, A. D.; Giralt, E. Surface Recognition and Helix Stabilization of a Tetraaspartate Peptide by Shape and Electrostatic Complementarity of an Artificial Receptor. *J. Am. Chem. Soc.* **1999**, *121*, 11813−11820. (f) Orner, B. P.; Salvatella, X.; Sánchez-Quesada, J.; de Mendoza, J.; Giralt, E.; Hamilton, A. D. De Novo Protein Surface Design: Use of Cation-Pi Interactions To Enhance Binding between an Alpha-Helical Peptide and a Cationic Molecule in 50% Aqueous Solution. *Angew. Chem., Int. Ed.* **2002**, *41*, 117−119.

(13) Quéré, L.; Wenger, T.; Schramm, H. J. Triterpenes as Potential Dimerization Inhibitors of HIV-1 Protease. *Biochem. Biophys. Res. Commun.* **1996**, *227*, 484−488.

(14) Peschke, W.; Schiessl, P.; Schmidtchen, F. P.; Bissinger, B.; Schier, A. Building Blocks for Artificial Anion Receptors: Derivatives of Chiral Bicyclic Guanidines. *J. Org. Chem.* **1995**, *60*, 1039−1043.

(15) Recently (ref 11), the conformationally restricted linker 4-(2-aminoethyl)-6-dibenzofuranpropionic acid was introduced to link two peptide strands, leading to a poorer HIV-1 protease dimerization inhibitor (K_{id} = 5.4 μM).

(16) Billich, A.; Hammerschmid, F.; Winkler, G. Purification, Assay and Kinetic Features of HIV-1 Proteinase. *Biol. Chem. Hoppe–Seyler* **1990**, *371*, 265–272.
(17) Dauber-Osguthorpe, P.; Roberts, V. A.; Osguthorpe, D. J.; Wolff, J.; Genest, M.; Hagler, A. T. Structure and Energetics of Ligand Bindings for Proteins: Escherichia Coli Dihydrofolate Reductase-Trimethoprim, a Drug–Receptor System. *Proteins: Struct., Funct., Genet.* **1988**, *4*, 31–47.
(18) Perrin, D. D.; Perrin, D. R.; Amarego, W. L. F. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon Press Ltd.: Oxford, 1980.
(19) Segura, M.; Alcázar, V.; Prados, P.; de Mendoza, J. Synthetic Receptors for Uronic Acid Salts Based on Bicyclic Guanidinium and Deoxycholic Acid Subunits. *Tetrahedron* **1997**, *53*, 13119–13128.
(20) The counterion in the guanidinium salt can be changed by washing a solution of **9** or **10** in dichloromethane with 2 N KOH and 0.1 N NH_4PF_6 or 1 N NH_4Cl.

JM030871U