Countering Cooperative Effects in Protease Inhibitors Using Constrained β-Strand-Mimicking Templates in Focused Combinatorial Libraries

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A major problem in de novo design of enzyme inhibitors is the unpredictability of the induced fit, with the shape of both ligand and enzyme changing cooperatively and unpredictably in response to subtle structural changes within a ligand. We have investigated the possibility of dampening the induced fit by using a constrained template as a replacement for adjoining segments of a ligand. The template preorganizes the ligand structure, thereby organizing the local enzyme environment. To test this approach, we used templates consisting of constrained cyclic tripeptides, formed through side chain to main chain linkages, as structural mimics of the protease-bound extended β-strand conformation of three adjoining amino acid residues at the N- or C-terminal sides of the scissile bond of substrates. The macrocyclic templates were derivatized to a range of 30 structurally diverse molecules via focused combinatorial variation of nonpeptidic appendages incorporating a hydroxyethylamine transition-state isostere. Most compounds in the library were potent inhibitors of the test protease (HIV-1 protease). Comparison of crystal structures for five protease-inhibitor complexes containing an N-terminal macrocycle and three protease-inhibitor complexes containing a C-terminal macrocycle establishes that the macrocycles fix their surrounding enzyme environment, thereby permitting independent variation of acyclic inhibitor components with only local disturbances to the protease. In this way, the location in the protease of various acyclic fragments on either side of the macrocyclic template can be accurately predicted. This type of templating strategy minimizes the problem of induced fit, reducing unpredictable cooperative effects in one inhibitor region caused by changes to adjacent enzyme−inhibitor interactions. This idea might be exploited in template-based approaches to inhibitors of other proteases, where a β-strand mimetic is also required for recognition, and also other protein-binding ligands where different templates may be more appropriate.

Introduction

Proteases are assuming major importance as enzyme targets for drug development because of their essential regulatory roles in disease.[1−6] Analysis of hundreds of crystal structures of aspartic, serine, metallo, and cysteine proteases has established that proteases universally recognize the β-strand conformation of inhibitors/substrates.[7,8] Despite this paradigm, most reported protease inhibitors are conformationally flexible[1−6] and relatively few attempts have been made to create more constrained β-strand mimetics.[9] Instead, rational design of inhibitors for proteases[1−6,10−12] and other enzymes[2,13−15] frequently begins by derivatizing peptide substrates to more pharmacologically acceptable non-peptides using analogue-, mechanism-, and structure- based approaches to maximize inhibitor−enzyme interactions.

A significant problem in optimizing enzyme inhibitors in this way is the highly cooperative nature of inhibitor−enzyme binding. Changes to one segment of an inhibitor induce reciprocal conformational changes in the local enzyme environment, which can in turn influence enzyme−inhibitor interactions at adjacent or even remote locations (the "induced fit"[16,17]). The resultant shape changes of adjacent pockets or peripheral loops of the enzyme can cause "knock-on" effects collectively termed "cooperativity", and this has been well recognized for proteases such as penicillopepsin,[18] renin,[19] cathepsin D,[20] papain,[21] and HIV-1 protease.[11,22,23] Consequently, it is normally very difficult to accurately predict how to most effectively optimize one (flexible) inhibitor region independently from another, and this is a significant obstacle to rapid development of selective enzyme inhibitors.

To address this problem, we have investigated the potential use of constrained cyclic tripeptide mimics (e.g., **1**, **2**) as templates to potentially order their immediate enzyme environment and thus dampen the induced fit resulting from changes to appendages. Macrocycles **1** and **2** were deliberately designed to structurally and functionally mimic P1−P3 or P1′−P3′ tripeptide segments of substrates for HIV-1 protease.[24,25] Crystal structures established that compounds containing these macrocycles were bound to HIV-1 protease in the same conformation as their acyclic peptide analogues and formed the same interactions.[24−26] These macrocycles have a number of advantages over acyclic

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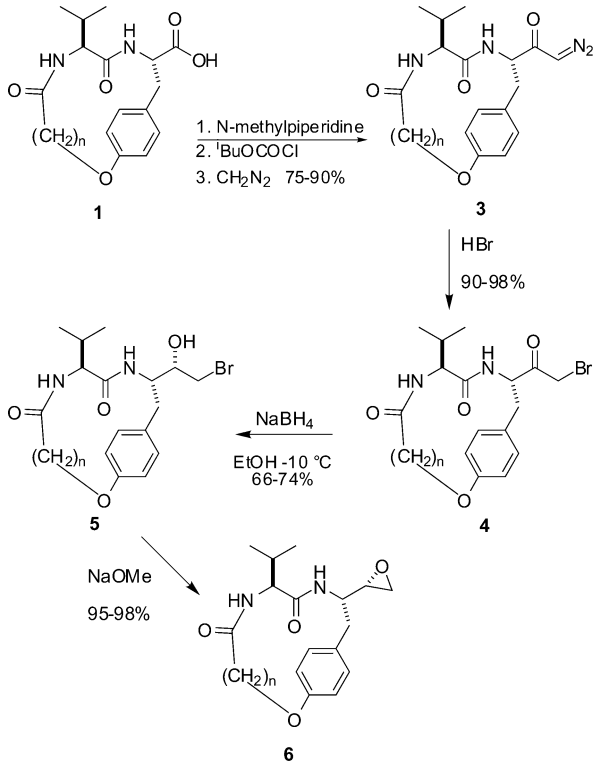
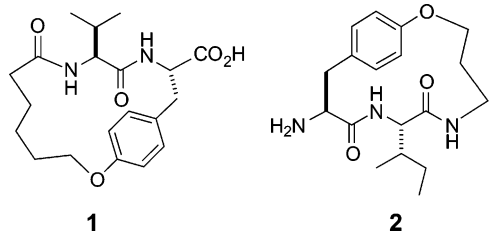
Scheme 1. Synthesis of the Macrocyclic Epoxides



peptides. They are preorganized in an extended (protease-binding) conformation in water prior to binding to a protease,[27] and this preorganization confers a significant entropy advantage for protease binding. They are more resistant to degradative proteases, and they also tend to be more bioavailable.[28,29] The use of such constrained macrocyclic templates that are equivalent to a tripeptide may allow independent regioselective optimization of protease/enzyme inhibitors via focused combinatorial libraries. The approach is illustrated here for a single target, the HIV-1 protease required for replication of the human immunodeficiency virus,[10,30−33] using inhibitors with macrocyclic components such as **1** and **2**.



Results and Discussion

Syntheses Using Macrocyclic Templates. We have reported cyclic templates **1**[24,27] and **2**[25] previously, but they are not very potent protease inhibitors in their own right ($IC_{50} = 5-60\ \mu M$, HIV-1 protease).[8] Schemes 1−3 now outline how we have derivatized **1** and **2** to a minilibrary of potent macrocyclic inhibitors of HIV-1 protease.

To facilitate combinatorial construction of inhibitors **8a**−**u** containing an N-terminal macrocycle like **1**, we first converted the cyclic acid **1** to epoxide **6** (Scheme 1). This involved formation of a mixed anhydride with isobutyl chloroformate, then reaction with diazomethane to give the diazoketone **3**. Treatment of diazoketone **3** with a dry solution of HBr in EtOAc smoothly led to displacement of N_2 and formation of the α-bromoketone **4**. Reduction of **4** with sodium borohydride gave the desired (*S*)-bromohydrin **5** in a 7:1 excess over the unwanted (*R*)-diastereomer and was easily obtained pure by preparative HPLC. Upon treatment with sodium methoxide in MeOH, the bromohydrin **5** cyclized rapidly and cleanly to the epoxide **6**, which was the precursor to inhibitors **8a**−**u**.

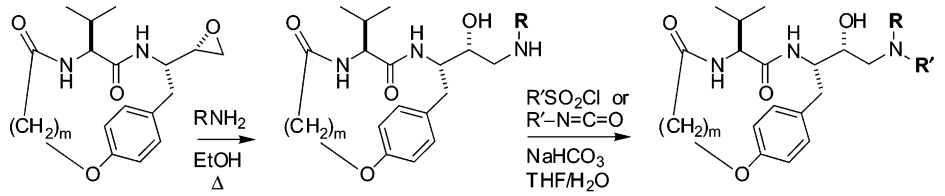
Scheme 2 illustrates a general synthesis that lends itself to combinatorial preparation of putative inhibitors for HIV-1 protease from N-terminal macrocyclic epoxides such as **6**, the macrocycle being a replacement for N-terminal tripeptides (like Nle/Leu-Val-Phe) from a protease substrate. The epoxide is regioselectively opened by primary amines to give a library of hydroxyethylamine derivatives **7**. These secondary amine products were dried in vacuo to remove excess amine and solvent before acylation to **8** by various sulfonyl chlorides, giving a sulfonamide library (Table 1, **8a**−**l**, **q**−**t**), or by various isocyanates to give a urea library (Table 1, **8m**−**p**).

Similarly, Scheme 3 shows that C-terminal tripeptides of a protease substrate can be replaced by macrocycles by coupling their amines **2** to an array of structurally varied epoxides **6** or **9**[34,35] in parallel syntheses to create a minilibrary of prospective protease inhibitors **10** (Table 2). Compound **10g** was instead prepared from **2** by reductive amination using Boc-Phe-CHO. These approaches offer scope for improvements in potency, selectivity, and pharmacological properties because of the diversity of structures that can be prepared.

N-Terminal Macrocyclic Inhibitors. The *N*-alkylsulfonamide derivatives **8** of the N-terminal macrocycle **6** are potent inhibitors of HIV-1 protease (Table 1). To evaluate substituent preferences at P1′, compounds **8a**−**g** were made with a common 4-aminobenzenesulfonamide unit at P2′. Short-branched alkanes of four to six carbon atoms (e.g., **8a**−**c**) are preferred at P1′ because a decrease in inhibitor potency was observed for longer unbranched *N*-alkyl substituents (**8d**, **8e**). Phenethyl and 4-methoxyphenethyl substituents (**8f**, **8g**) at P1′ also fit well in the protease.

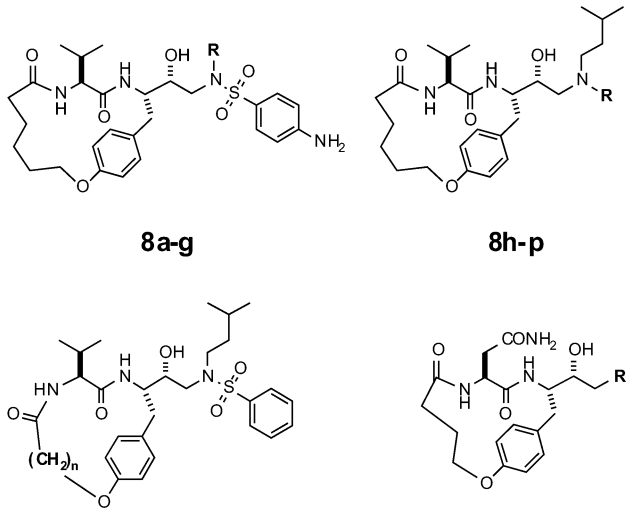
To evaluate substituent preferences at P2′, the favorable isoamyl group at P1′ of **8a** was retained and a series of different aromatic sulfonamides **8h**−**l** and ureas **8m**−**p** were synthesized. Aromatic sulfonamides were highly potent. Para substituents had little effect on potency but greatly increased solubility for **8a**−**h**, while the 3-nitro substituent **8i** was predictably deleterious because of unfavorable polar interactions. The sterically demanding triisopropylbenzenesulfonyl **8j** and quinoline-8-sulfonyl **8l** showed marked decreases in activity, whereas the 2-naphthylsulfonamide **8k** was very potent, suggesting that the S2′ subsite can accommodate larger groups of a complementary shape. The use of isocyanates to prepare ureas **8m**−**p** led generally to less potent inhibitors except for the *tert*-butyl derivative **8o**. Changing the ring size of the N-terminal macrocycle had some effect with the 16-membered ring (**8r**) being better than the 17-membered (**8s**) or 15-membered (**8q**) rings.

Scheme 2. Synthesis of N-Terminal Macrocyclic Inhibitors



6 **7** **8**

Table 1. Inhibition of HIV-1 Protease by Compounds **8a**−**v**



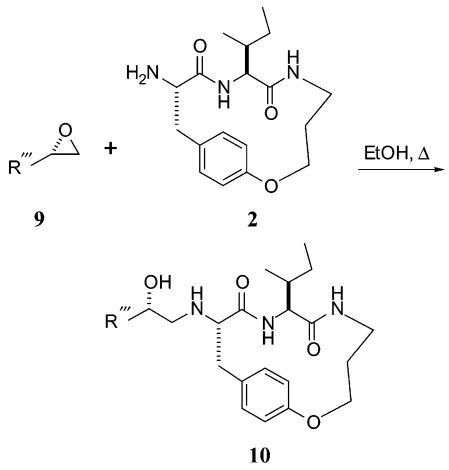
	R	K_i (nM)[a]
8a	isoamyl	1.7
8b	2-butyl	2
8c	cyclopentyl	3
8d	*n*-hexyl	14
8e	6-hexanoic acid	60
8f	2-phenyethyl	8
8g	2-(4-methoxyphenyl)ethyl	3
8h	4-acetamidobenzenesulfonyl	10
8i	3-nitrobenzenesulfonyl	145
8j	2,4,6-triisopropylbenzenesulfonyl	4700
8k	2-naphthalenesulfonyl	1
8l	quinoline-8-sulfonyl	295
8m	benzyl urea	314
8n	cyclohexyl urea	207
8o	*tert*-butyl urea	12
8p	L-valine urea	20000
8q	$(CH_2)_3$	4
8r	$(CH_2)_4$	0.4
8s	$(CH_2)_5$	1.6
8t	*N*-isoamylbenzenesulfonamido	1
8u	Pro-Ile-Val-NH_2	12
8v	L-pipecolinic acid *tert*-butylamide	12

[a] pH 6.5, I = 0.1 M, 37 °C, 50 μM substrate [Abz-NF*-6] for inhibition of synthetic HIV-1 protease using a reported fluorometric assay.[43,44]

Changing Val at P2 in **8q** to Asn and removal of the *p*-amino substituent slightly increased activity for **8t**. Varying both P1′ and P2′ substituents of **8u** and **8v** resulted in an order of magnitude decrease in potency.

C-Terminal Macrocyclic Inhibitors. Similarly, compounds **10a**−**h** containing a hydroxyethylamine isostere replacement for the scissile amide bond together with a C-terminal macrocycle, substituting for P1′, P2′, and P3′, were all potent inhibitors of HIV-1 protease

Scheme 3. Synthesis of C-Terminal Macrocyclic Inhibitors

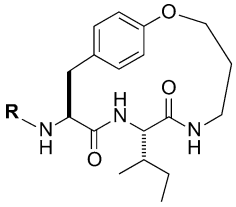


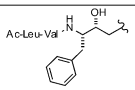
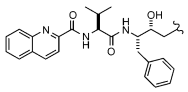
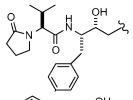
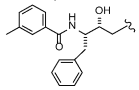
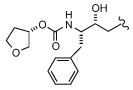
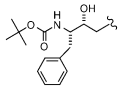
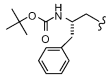
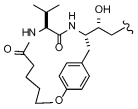
9 **2** **10**

(Table 2), spanning a >100-fold range in potency (K_i = 0.3−44 nM). When attached to the tripeptide Ac-Leu-Val-Phe-, the cycle conferred significant protease inhibition (K_i = 0.6 nM, **10a**), which was slightly enhanced when Ac-Leu was replaced by a quinolinecarboxyl substituent (K_i = 0.3 nM, **10b**) and slightly reduced when replaced by the five-membered cyclic lactam (K_i = 1 nM, **10c**). Truncation of these hexapeptide mimetics to the pentapeptide mimics (**10d**−**g**) reduced potency, with the *m*-methylbenzoyl substituent at P2 (**10d**) being 50-fold less potent than **10b**. Changing the P2 substituent to furanyl (**10e**) or *tert*-butyl (**10f**) restored 4-fold activity, which was reduced by 1 order of magnitude when the hydroxyl group was removed (**10g**). Interestingly, the N-terminal macrocyclic epoxide could be coupled to the C-terminal macrocyclic amine to produce the bicyclic **10h**,[36] which was similarly a potent protease inhibitor.

Protease-Binding Ligand Conformations. Figure 1a shows the first superimposition of a set of crystal structures that we have previously determined[24−26] for HIV-1 protease complexed individually to inhibitors (**8a**, **8q**, **8t**, **8u**, **8v**) containing an N-terminal macrocyclic component. Figure 1 demonstrates that the cycles all superimpose very well, especially at P1 and P2, despite variations in ring size (e.g., **8**, m = 3 or 5) and in P2 substituents of the N-terminal cycles (isopropyl or acetamide) and despite considerable diversity in the C-terminal (P1′, P2′) substituents. By implication, the macrocycle thus appears to organize both itself and its surrounding protease environment, while the C-terminal acyclic appendages have little effect on protease structure in the vicinity of the macrocycle.[24−26] The conformational rigidity of the cycles[27] helps to localize them within the substrate-binding protease groove, fixing the positions of P1 and P2 substituents and

Table 2. Inhibition of HIV-1 Protease by Compounds **10a**−**h**[a]



	R	K_i (nM)
10a		0.6
10b		0.3
10c		1
10d		15
10e		4
10f		4
10g		44
10h		5

[a] pH 6.5, I = 0.1 M, 37 °C, 50 μM substrate [Abz-NF*-6] for inhibition of synthetic HIV-1 protease using a reported fluorometric assay.[43,44]

minimizing cooperative enzyme−inhibitor interactions. Figure 1a demonstrates the principle that such constrained macrocycles could serve as templates to anchor an inhibitor to a specific region of the substrate-binding groove of the protease while allowing independent fine-tuning of appendages that bind adjacent regions of the protease.

Figure 1b shows superimposed crystal structures for three inhibitors (**10c**, **10f**, **10h**) containing C-terminal macrocycles bound in the active site of HIV-1 protease. The C-terminal cycles are excellent structural mimics[25,26] for C-terminal tripeptide fragments of acyclic peptide inhibitors. When the C-terminal macrocycle **2** was coupled to N-terminal macrocycle **6**, the resulting bis-macrocycle **10h** was likewise an excellent structural mimic[26,36] of a hexapeptide and a potent inhibitor of the protease.

Macrocycle-Binding Protease Conformations. Figure 2 summarizes the high crystallographic conservation of the protein residues surrounding the macrocycles, irrespective of macrocyclic inhibitor observed, and shows that the same contacts are made between

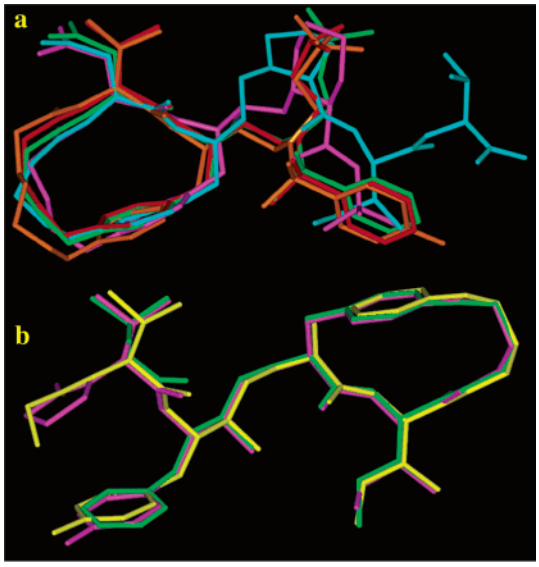


Figure 1. Superimposed inhibitor−protease crystal structures (protease omitted for clarity) for HIV-1 protease bound to (a) N-terminal macrocyclic inhibitors **8a** (orange, PDB code 1d4l),[29] **8q** (red, 1b6n),[26] **8t** (green, 1b6o),[26] **8u** (blue, 1b6j),[26] and **8v** (purple, 1b6l)[26] and (b) C-terminal macrocyclic inhibitors **10c** (purple, 1b6k),[26] **10f** (green, 1b6m),[26] and **10h** (yellow, 1b6p).[26]

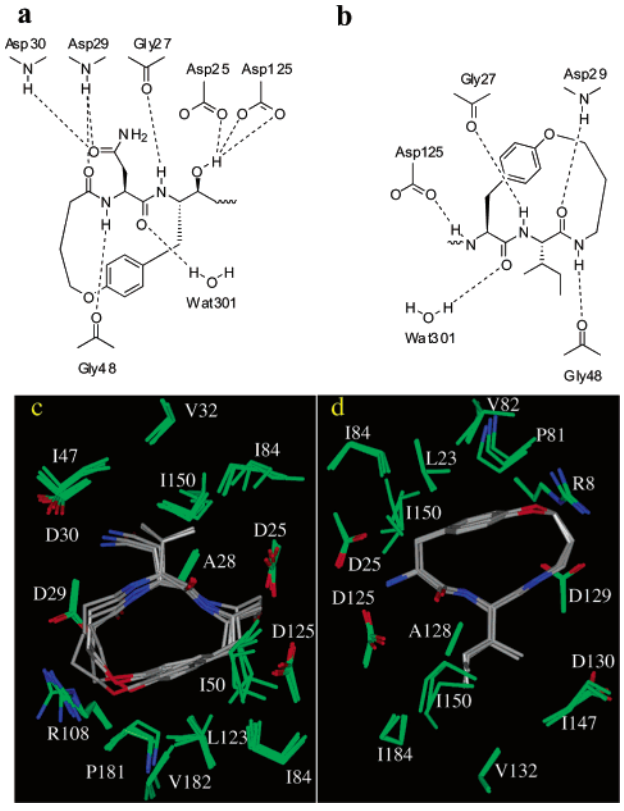


Figure 2. Protease environment surrounding macrocyclic inhibitors. Conserved hydrogen bond interactions between protease and (a) N-terminal macrocyclic inhibitors **8a**, **8q**, **8t**, **8u**, **8v** and (b) C-terminal macrocyclic inhibitors **10c**, **10f**, and **10h**. (c) Superimposed N-terminal inhibitor−protease crystal structures showing all amino acid side chains within 4.5 Å. (d) Superimposed C-terminal inhibitor−protease crystal structures showing all amino acid side chains within 4.5 Å.

each macrocycle and each protease residue. Figure 2a displays the hydrogen-bonding interactions between the N-terminal macrocycles and HIV-1 protease. All hydro-

gen bonds are conserved across the five macrocyles (**8a**, **8q**, **8t**, **8u**, **8v**) with only one minor exception. The interaction between the linkage carbonyl oxygen and the main chain amide of aspartate 29 (3.6 Å) is outside the distance cutoff of 3.3 Å but indicates the potential for a hydrogen bond. Figure 2b shows the corresponding hydrogen-bonding interactions between the C-terminal macrocycles (**10c**, **10f**, **10h**) and HIV-1 protease, all hydrogen bonds being conserved. Parts c and d of Figure 2 show all surrounding protease residues within 4.5 Å of the macrocyclic inhibitors. Clearly all such residues are highly superimposable with the only exceptions being I50 and I150, which reside in the flap region and are well documented as being highly flexible. One other possible discrepancy could be at I84 (Figure 2c), the relative disorder arising as a result of two different side chains at P2 (asparagine vs valine).

Conclusions

N-terminal macrocycles (e.g., Figure 1a) and C-terminal macrocycles (e.g., Figure 1b) have been established as excellent structural mimics[24,26] for the respective N- and C-terminal tripeptide fragments of acyclic peptide inhibitors. The crystal data superpositions (Figures 1 and 2) and protease inhibitor potencies (Tables 1, 2) reported here support the idea that both macrocyclic types anchor the inhibitors to specific regions of the substrate-binding groove of the protease, guiding the binding locations of the acyclic appendages and conferring high inhibitor potency to the compounds. These results demonstrate *predictable* ligand binding of macrocycle-containing inhibitors, the crystal structural results[24−26] being in excellent agreement with computer-modeled structures[24,25,36] for which there is a template guiding the interactions with the protease. Proteases in general still present significant challenges for drug discovery because of the extended substrate groove and the need for larger molecular weight inhibitors to obtain protease selectivity. For those proteases where crystal structures are available, this templating approach may help the exploitation of structure-based design methods.

Rational de novo ligand design for a given receptor is still a very difficult proposition largely because of the unpredictability of cooperative ligand−receptor fitting that accompanies ligand binding. We have tested a novel strategy for minimizing the induced fit that accompanies ligand−receptor interaction, a strategy that is less susceptible to cooperative influences associated with the induced fit of ligand components to an enzyme. The strategy involved the use of a macrotemplate, which spans an extended surface area within the enzyme active site (equivalent to a tripeptide herein), and modification through focused combinatorial chemistry to create a series of potent inhibitors that bind in predictable ways within the active site of the enzyme. The approach was illustrated here for the single target HIV-1 protease and may represent a valuable new method of inhibitor design and optimization for other enzymes/receptors as well. Macrocycles, by virtue of dampening the induced fit over an extended surface area, may be especially useful for building new protease inhibitors where the structure of the protease is unknown, when inhibitor elaboration is not predictable because of the unpredictable induced fit of inhibitor−protease fitting, or to counter drug resistance due to protein alterations in response to tightly fitting inhibitors. This macrocyclic approach is not designed to solve ADMET problems but, although they contain a significant number of hydrogen bond donors/acceptors, there are increasing numbers of macrocyclic molecules entering man as registered pharmaceuticals.[37]

We have demonstrated that macrocycles, derived from the amino acid sequence of a protease substrate, can function as templates for inhibitor optimization with the resultant molecules all having low nanomolar inhibitory potencies. The success of this strategy is attributed to the template occupying three contiguous subsites in the protease, facilitating the maintenance of all the hydrogen-bonding and pocket-filling interactions made by a peptide with the protease while fixing the surrounding protease environment in the vicinity of the macrocycle.

This methodology might next be tested for other proteases including those for which structural information is unavailable but where substrate sequence and type of protease are known. We have demonstrated that cyclic inhibitors for other aspartic, serine, metallo, and cysteine proteases are also β-strand mimetics.[38] With judicious incorporation of appropriate restraints[9] to regulate structures of macrocycles to optimally mimic β strands, they might similarly serve as constrained templates for regioselective optimization of inhibitors for the respective proteases. More generally the idea of rationally incorporating rigid templates[39,40] into inhibitor/antagonist structures, specifically in order to minimize the problem of cooperativity in ligand−receptor fitting, may represent a valuable rational approach to de novo ligand design.

Experimental Section

General Methods. NMR spectra were recorded on a Varian Gemini 300 or Bruker ARX-500 spectrometer at 298 K and were referenced internally to the residual solvent peak: CD_3OH δ_H 3.31, δ_C 49.0; DMSO-d_6 δ_H 2.50, δ_C 39.7; CD_3CN δ_H 1.94, δ_C 1.39; $CDCl_3$ δ_H 7.27, δ_C 77.0 ppm. Routine mass spectra were measured on a PE-SCIEX API-3 or Perceptive Biosystems Mariner API-TOF instrument equipped with an LC pump and Rheodyne injector. Reaction mixtures were sampled and diluted with 70% MeCN/30% H_2O and were introduced into the mass spectrometer at 30−50 μL/min. As a result of the electrospray ionization process, only molecular ions MH^+ were observed for each component in the sample. High-resolution mass spectra of purified products were measured on a Finnigan 2000. Preparative reverse-phase HPLC was conducted on a Waters 600 system equipped with a Rheodyne preparative injector with a 5 mL loop volume on a Phenomenex Luna C18 10 μm column, 250 mm × 22 mm, at 20 mL/min using gradient elution (solvent A is water and 0.1% TFA; solvent B is 90% MeCN, 10% water, and 0.1% TFA) and UV detection at 280 nm.

Enzyme Assay. Synthetic HIV-1 PR (SF2 isolate)[41,42] with mutations (C67B, C95B, Q7K, L33I, where B = L-α-amino-*n*-butyric acid) was solubilized in 6 M Gu·HCl (0.05 mg mL^{-1}), then refolded for 60 min in buffer A (20 mM phosphate, pH 7.0, 20% v/v glycerol, 10 mg mL^{-1} BSA). Enzyme activity was measured using a continuous fluorimetric assay described previously.[43,44] At time zero, the buffer A/protease solution was added to 50 μM substrate [2-(aminobenzoyl)-Thr-Ile-Nle-Phe-(*p*-NO_2)-Gln-Arg-NH_2], buffer B (MES, pH 6.5, 37 °C, 100 mM NaCl, 10% v/v glycerol) and varying concentrations of the inhibitor. K_m values for HIV-1 PR were calculated using a Hanes plot of [s]/*v* vs [s], and the K_i was then calculated from either Dixon plots of 1/*v* vs [s] or Henderson plots of [I]/{1 −

(V_i/V_o)} vs V_o/V_i in cases where the K_i was found to approach the concentration of the enzyme. Under these assay conditions, the cyclic compounds were confirmed to be competitive inhibitors. Under these conditions, the reference compound DM-323 gave IC_{50} = 1 nM, in agreement with the literature value.[45]

Synthesis and Characterization. (9*S*,12*S*)-12-(1′-Oxo-2′-diazoethyl)-7,10-dioxo-9-isopropyl-2-oxa-8,11-diazabicyclo[12.2.2]octadeca-14,16,17-triene (*n* = 4), 3a. A solution of (**1a**, $n = 4$)[27] (280 mg, 0.77 mmol) in THF (10 mL) and *N*-methylpiperidine (112 μL, 0.92 mmol) was cooled to −15 °C under N_2, and then isobutyl chloroformate (120 μL, 0.92 mmol) was added. After a further 5 min at −15 °C, a solution of diazomethane (∼5 mmol) in ether (20 mL) was added and the solution was slowly allowed to warm to room temperature. EtOAc was added and the solution was washed with water and brine, dried over $MgSO_4$, and evaporated. The solid residue was triturated with ether, then dried under high-vacuum, giving the diazoketone (**3a**, $n = 4$) as a pale yellow solid (224 mg, 75%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.17 (d, J = 9.7 Hz, 1H, tyr NH), 7.40 (d, J = 9.3 Hz, 1H, val NH), 7.12 (dd, J = 8.5, 2.3 Hz, 1H, ArH), 7.01 (dd, J = 8.2, 2.2 Hz, 1H, ArH), 6.77 (dd, J = 8.4, 2.6 Hz, 1H, ArH), 6.66 (dd, J = 8.2, 2.5 Hz, 1H, ArH), 6.07 (bs, 1H, CHN_2), 4.60 (m, 1H, tyr αH), 4.16−3.97 (m, 3H, val αH and CH_2OAr), 3.10 (m, 1H, try βH), 2.46 (m, 1H, tyr βH), 2.21−2.09 (m, 1H, CH_2CO), 1.90−1.79 (m, 1H, CH_2CO), 1.69 (m, 1H, val βH), 1.65−1.10 (e, 4H, $(CH_2)_2$), 0.79 (d, J = 6.7 Hz, 3H, CH_3), 0.73 (d, J = 6.8 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 193.89, 171.30, 170.58, 154.94, 131.26, 129.95, 129.17, 118.34, 117.36, 67.88, 57.49, 56.99, 53.52, 36.16, 34.74, 31.34, 25.67, 21.53, 19.21, 18.53. HRMS *m/e* 386.1933. Calcd for $C_{20}H_{26}N_2O_4$ 386.1954.

(9*S*,12*S*)-12-(1′-Oxo-2′-bromoethyl)-7,10-dioxo-9-isopropyl-2-oxa-8,11-diazabicyclo[12.2.2]octadeca-14,16,17-triene (*n* = 4), 4a. A solution of dry HBr in EtOAc (∼0.2 M, 8 mL) was added to a solution/suspension of the diazoketone **3a** (485 mg, 1.26 mmol) in THF (20 mL) at 10 °C. After 2 min, the solution was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and evaporated to give the bromoketone **4a** as a white powder (497 mg, 90%). R_f = 0.45 (100% EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (dd, J = 8.4, 2.2 Hz, 1H, ArH), 6.96−6.79 (m, 3H, ArH), 6.07 (d, J = 9.6 Hz, 1H, tyr NH), 5.72 (d, J = 8.9 Hz, 1H, val NH), 5.30 (ddd, J = 12.1, 9.7, 4.6 Hz, 1H, tyr αH), 4.30−4.15 (m, 2H, OCH_2). ^{1}H NMR, AB system, δ_A 4.15, δ_B 4.10 (J_{AB} = 13.2 Hz, CH_2Br), 4.02 (dd, J = 8.9, 7.2 Hz, 1H, val αH), 3.44 (dd, J = 13.8, 4.6 Hz, 1H, tyr βH), 2.53 (dd, J = 13.8, 12.1 Hz, 1H, tyr βH), 2.30−1.36 (m, 7H, $(CH_2)_3$ and val βH), 0.87 (d, J = 6.8 Hz, CH_3), 0.86 (d, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) δ 199.66, 172.28, 170.58, 155.92, 131.16, 129.78, 127.89, 118.84, 116.71, 77.21, 67.77, 58.19, 56.85, 37.48, 35.85, 31.69, 25.98, 21.52, 18.89, 18.36. HRMS *m/e* 438.1153, M^+; calcd for $C_{20}H_{27}N_2O_4{}^{79}Br$ 438.1154.

(9*S*,12*S*,1′*S*)-12-(1′-Hydroxy-2′-bromoethyl)-7,10-dioxo-9-isopropyl-2-oxa-8,11-diazabicyclo[12.2.2]octadeca-14,-16,17-triene (*n* = 4), 5a. A solution of **4a** (1.0 g, 2.3 mmol) in EtOH (20 mL) was cooled to −10 °C, and then $NaBH_4$ (87 mg, 2.3 mmol) was added. After the mixture was stirred for 5 min, glacial AcOH (1 mL) was added and the solution was concentrated in vacuo and purified by reverse-phase HPLC [linear gradient from 10% MeCN/90% H_2O/0.1% TFA to 90% MeCN/10% H_2O/0.1% TFA over 90 min and retention time of 19.7 min], giving the (*S*)-bromohydrin **5a** (750 mg, 74%) as a white powder after lyophilization. The minor (*R*)-diastereomer was not isolated. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.64 (d, J = 10.0 Hz, 1H, tyr NH), 7.26 (d, J = 9.4 Hz, 1H, val NH), 7.01 (dd, J = 8.4, 2.2 Hz, 1H, ArH), 6.92 (dd, J = 8.4, 1.9 Hz, 1H, ArH), 6.74 (dd, J = 8.4, 2.6 Hz, 1H, ArH), 6.61 (dd, J = 8.3, 2.5 Hz, 1H, ArH), 4.15−3.90 (m, 4H, CH_2OAr and tyr αH and val αH), 3.60−3.46 (m, 2H, CHBr and C*H*OH), 3.33 (dd, J = 10.4, 6.5 Hz, 1H, CHBr), 3.05 (dd, J = 13.4, 4.0 Hz, 1H, tyr βH), 2.24 (dd, J = 13.4, 12.2 Hz, 1H, tyr βH), 2.14−1.99 (m, 1H, CH_2-CO), 1.92−1.80 (m, 1H, CH_2CO), 1.66 (m, 1H, val βH), 1.70−1.08 (e, 4H, $(CH_2)_2$), 0.72 (d, J = 6.9 Hz, 3H, CH_3), 0.67 (d, J = 6.9 Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ 171.41,170.21, 154.71, 131.39, 131.23, 129.40, 118.28, 117.27, 73.65, 67.99, 57.04, 53.58, 38.14, 36.33, 34.97, 31.54, 25.74, 21.72, 19.20, 18.47. ISMS *m/e* 441, 443 (MH^+).

(9*S*,12*S*,1′*S*)-12-(1′,2′-Epoxyethyl)-7,10-dioxo-9-isopropyl-2-oxa-8,11-diazabicyclo[12.2.2]octadeca-14,16,17-triene (*n* = 4), 6a. A solution of the bromohydrin **5a** (700 mg, 1.59 mmol) and NaOMe (100 mg, 1.85 mmol) in MeOH (20 mL) was stirred at room temperature for 30 min, and then H_2O (4 mL) was added. The solution was concentrated in vacuo to about 5 mL, and the precipitate was filtered off, washed with a small volume of cold 30% MeOH/70% H_2O, and dried under high vacuum, giving the epoxide **6a** (543 mg, 95%) as a white powder. ^{1}H NMR (300 MHz, CD_3OD) δ 7.90 (d, J = 9.8 Hz, 1H, tyr NH), 7.47 (d, J = 8.8 Hz, 1H, val NH), 7.14 (dd, J = 8.4, 2.2 Hz, 1H, ArH), 7.01 (dd, J = 8.2, 2.3 Hz, 1H, ArH), 6.42 (dd, J = 8.4, 2.6 Hz, 1H ArH), 6.77 (dd, J = 8.2, 2.7 Hz, 1H, ArH), 4.28−4.03 (m, 3H, tyr αH and CH_2OAr), 3.90 (t, J = 8.8 Hz, 1H, val αH), 3.09 (dd, J = 13.3, 4.1 Hz, 1H, tyr βH), 3.04 (ddd, J = 6.5, 3.9, 2.7 Hz, 1H, epoxide CH), 2.75 (dd, J = 5.0, 3.9 Hz, 1H, epoxide CH_2), 2.69 (dd, J = 5.0, 2.7 Hz, 1H, epoxide CH_2), 2.46 (dd, J = 13.3, 12.5 Hz, 1H, tyr βH), 2.19−2.05 (m, 2H, $-CH_2CO$), 1.86−1.69 (m, 2H, val βH and CH_2), 1.60−1.22 (m, 3H, CH_2CH_2), 0.87 (d, J = 6.7 Hz, 3H, CH_3), 0.83 (d, J = 6.7 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 171.23, 170.50, 154.82, 131.29, 130.71, 129.07, 118.49, 117.42, 68.06, 57.59, 53.09, 50.17, 43.48, 36.28, 34.94, 31.15, 25.82, 21.75, 18.99, 18.94. HRMS *m/e* 360.2053 M^+; calcd for $C_{20}H_{28}N_2O_4$ 360.2049.

(10*S*,13*S*)-13-(1′-Oxo-2′-diazoethyl)-8,11-dioxo-10-isopropyl-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-15,17,18-triene (*n* = 5), 3b. A solution of the acid (**1b**, $n = 5$)[27] (480 mg, 1.28 mmol) and *N*-methylpiperidine (240 μL, 1.94 mmol) in dry THF (15 mL) was stirred at −15 °C under N_2, and then isobutyl chloroformate (200 μL, 1.54 mmol) was added. After a further 5 min at −15 °C, a solution of diazomethane (about 6 mmol) in ether (20 mL) was added and stirring was continued for 30 min. Then the mixture was allowed to warm to room temperature. Excess diazomethane was removed by bubbling N_2 through the solution, and then EtOAc was added. The mixture was washed with water, and the organic layer, together with any solid product, was separated and evaporated to dryness. The solid residue was triturated with ether/EtOAc 1:1 and dried under high vacuum, giving the diazoketone **3b** as a pale-yellow solid (460 mg, 90%). HRMS *m/e* 372.2042 ($M - N_2$); calcd for $C_{20}H_{20}N_2O_4$ 372.2049.

(10*S*,13*S*)-13-(1′-Oxo-2′-bromoethyl)-8,11-dioxo-10-isopropyl-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-15,17,18-triene (*n* = 5), 4b. A suspension of the diazoketone **3b** (500 mg, 1.25 mmol) in THF (10 mL) was stirred at 10 °C, while a solution of dry HBr in EtOAc (0.2 M, 8 mL) was added. After a further 2 min, the solution was diluted with EtOAc, washed with 1 M HCl, $NaHCO_3$, and brine, and dried over $MgSO_4$. Removal of solvent gave the bromoketone **4b** as a white solid (555 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (m, 2H), 6.85 (m, 2H), 4.63 (br, 1H), 5.96 (br, 1H), 5.21 (m, 1H), 4.30−4.05 (m, 5H), 3.39 (dd, J = 14.0, 4.2 Hz, 1H), 2.59 (m, 1H), 2.36−2.20 (m, 1H), 2.06−1.85 (m, 2H), 1.83−1.43 (m, 3H), 1.41−1.10 (m, 3H), 0.98−0.75 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 199.9, 172.2, 170.8, 157.6, 130.3, 127.6, 116.6, 67.7, 57.6, 57.2, 36.9, 35.5, 32.2, 31.7, 29.2, 25.1, 24.5, 19.1, 18.2. ESI-MS 453/455 MH^+.

(10*S*,13*S*,1′*S*)-13-(1′-Hydroxy-2′-bromoethyl)-8,11-dioxo-10-isopropyl-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-15,17,18-triene (*n* = 5), 5b. The bromoketone **4b** (550 mg, 1.21 mmol) was stirred with EtOH (50 mL) at −10 °C, and then $NaBH_4$ (50 mg) was added. The mixture was stirred at −10 °C for 10 min, the glacial acetic acid (400 μL) was added, and the solvent was evaporated. The solid residue was dissolved in DMF (1 mL), MeCN (10 mL), and water (20 mL) and purified by reverse-phase HPLC (82% water/18% MeCN, linear gradient increasing in MeCN at 1% per min), giving the bromohydrin **5b** (*S*)-isomer (360 mg, 66%), t_R = 20 min, and the minor (*R*)-isomeric bromohydrin (50 mg, 9%), t_R = 22 min, as white powders after lyophilization.

Major bromohydrin (*S*)-isomer: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.71 (d, J = 9.7 Hz, 1H, Tyr-NH), 7.17 (d, J = 9.2 Hz, 1H, Val-NH). ^{1}H NMR, AA′XX′ system, δ 6.99 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to CH_2), 6.70 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to O), 4.22−3.93 (m, 4H, Val αCH and Tyr αCH and CH_2O), 3.61−3.45 (m, 2H), 3.35 (dd, J = 11.1, 7.8 Hz, 1H, CHBr), 3.04 (dd, J = 13.5, 3.5 Hz, 1H, Tyr βH), 2.31 (dd, J = 13.5, 12.5 Hz, 1H, Tyr βCH), 2.21−2.05 (m, 1H, CHCO), 1.95−1.84 (m, 1H, CHCO), 1.73 (m, 1H, Val βCH), 1.65−0.94 (e, 6H, $(CH_2)_3$), 0.76 (d, J = 6.7 Hz, 3H, Val-γCH_3), 0.70 (d, J = 6.8 Hz, 3H, Val-γCH_3). ^{13}C NMR (DMSO-d_6) δ 171.0, 170.3, 156.4, 130.7, 130.3, 115.8, 73.8, 67.1, 56.6, 53.3, 38.0, 35.8, 34.2, 31.8, 29.0, 24.6, 24.6, 19.3, 18.2. ESI-MS m/z 455/457 (MH^+),

Minor bromohydrin (*R*)-isomer: ^{1}H NMR (300 MHz, acetone-d_6), δ AA′XX′ system, 7.09 (m, $J_{AX} + J_{AX'}$ = 8.5 Hz, 2H, ortho to CH_2), 6.75 (m, $J_{AX} + J_{AX'}$ = 8.5 Hz, 2H, ortho to O), 6.89 (d, J = 9.5 Hz, 1H, Tyr-NH), 6.36 (d, J = 8.5 Hz, 1H, Val-NH), 4.53−4.42 (m, 1H), 4.28−4.17 (m, 2H), 4.14−4.04 (m, 1H), 4.01−3.94 (m, 1H), 3.50 (dd, J = 10.2, 5.0 Hz), 3.37 (dd, J = 10.2, 7.5 Hz), 2.88 (dd, J = 13.4, 4.1 Hz), 2.75 (dd, J = 13.5, 12.1 Hz, 1H), 2.20−2.00 (m, 2H), 1.90 (m, 1H, Val-βCH), 1.80−1.10 (e, 6H, $(CH_2)_3$), 0.83 (d, J = 6.8 Hz, 3H, Val-γ CH_3), 0.75 (d, J = 6.9 Hz, 3H, Val-γ CH_3). ^{13}C NMR (DMSO-d_6) δ 171.0, 170.7, 156.5, 130.8, 130.3, 115.9, 73.1, 67.2, 56.5, 53.2, 36.8, 35.4, 34.2, 31.8, 29.3, 24.7, 19.3, 18.2. ESI-MS m/z 455/457 (MH^+).

(10*S*,13*S*,1′*S*)-13-(1′,2′-Epoxyethyl)-8,11-dioxo-10-isopropyl-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-15,17,18-triene (*n* = 5), 6b. The (*S*)-bromohydrin **5b** (360 mg, 0.79 mmol) was dissolved in THF (5 mL) and MeOH (15 mL), and then NaOMe (60 mg) was added. The solution was stirred at room temperature for 15 min. Then water (2 mL) was added and the mixture was concentrated to about 5 mL on a rotary evaporator. The crystalline precipitate was filtered off and washed with a small amount of 30% MeOH/70% water and then dried under high vacuum, giving the epoxide **6b** (290 mg, 98%). ^{1}H NMR (300 MHz, CD_3OD) δ NH exchanged, AA′XX′ system, 7.07 (m, $J_{AX} + J_{AX'}$ = 8.5 Hz, 2H, ortho to CH_2), 6.80 (m, $J_{AX} + J_{AX'}$ = 8.5 Hz, 2H, ortho to O), 4.30−4.10 (m, 3H, H-3, H-13), 3.95 (d, J = 8.2 Hz, 1H, val-αH), 3.10−3.00 (m, 2H, H-14, H-1′), 2.74 (dd, J = 5.0, 3.9 Hz, 1H, H-2′), 2.70 (dd, J = 5.0, 2.7 Hz, 1H, H-2′), 2.50 (dd, J = 13.6, 12.7 Hz, H-14), 2.17−2.10 (m, 2H, H-7), 1.85−1.51 (m, 3H), 1.55−1.41 (m, 1H), 1.38−1.21 (m, 1H), 1.21−1.08 (m, 2H), 0.89 (d, J = 6.8 Hz, Val-γ CH_3), 0.83 (d, J = 6.8 Hz, Val-γ CH_3). ^{13}C NMR (CD_3-OD) δ 174.6, 173.0, 158.7, 131.2, 131.1, 117.5, 68.7, 59.4, 54.4, 52.7, 45.2, 37.1, 35.9, 33.1, 31.2, 26.3, 26.1, 19.4, 18.9. HRMS m/e 374.2214; calcd for $C_{21}H_{30}N_2O_4$ 374.2206.

(10*S*,13*S*,1′*R*)-13-[1′-Hydroxy-2′-(1″-amino-3″-methylbutyl)ethyl]-8,11-dioxo-10-isopropyl-2-oxa-9,12-diazabicyclo-[13.2.2]nonadeca-15,17,18-triene (*n* = 5, R = isoamyl), 7. The epoxide (**6b**, n = 5) (123 mg, 0.33 mmol) and isoamylamine (380 μL, 10 equiv) were stirred and heated at 80 °C in dry DMSO (800 μL) for 30 h. Water (4 mL) was added, and the mixture was stirred at room temperature for 5 min. The precipitate was filtered off, washed with water (1 mL), and dried under high vacuum, giving the amine **7** (140 mg, 92%). Homogeneity was confirmed by analytical RP-HPLC (27% MeCN/73% H_2O/0.1% TFA), retention time of 7.3 min. λ_{max} (MeOH) = 225, 275 nm. ^{1}H NMR (300 MHz, CD_3OH) δ 7.98 (d, J = 9.4 Hz, 1H, Tyr-NH), 7.39 (d, J = 9.1 Hz, 1H, Val-NH). ^{1}H NMR, AA′XX′ system, 7.08 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to CH_2), 7.08 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to O), 4.24 (ddd, J = 12.3, 6.3, 4.0 Hz, 1H, H-3′), 4.14 (ddd, J = 15.9, 11.8, 3.8 Hz, 1H, H-3′), 4.06 (dd, J = 9.3, 7.0 Hz, 1H, Val-αCH), 4.10−4.00 (m, 1H), 3.80 (m, 1H), 3.20 (dd, J = 13.7, 3.6 Hz, H-14′), 3.25, 2.90 (m, 4H, CH_2NHCH_2), 2.43 (dd, J = 13.7, 12.2 Hz, H-14′), 2.20−2.19 (m, 2H, CH_2CO), 1.87 (m, 1H, Val-βCH), 1.80−1.05 (e, 9H, H-4′, H-5′, H-6′, H-5, and H-6), 0.97 (d, J = 6.4 Hz, 6H, H-4″, H-5″), 0.90 (d, J = 6.8 Hz, 3H, Val-γ CH_3), 0.83 (d, J = 6.7 Hz, 3H, Val-γ CH_3). ^{13}C NMR (CD_3-OH) δ 174.7, 173.7, 158.5, 131.1, 131.1, 117.5, 70.7, 68.6, 59.0, 55.5, 51.8, 47.7, 36.5, 35.7, 35.6, 33.1, 30.6, 27.1, 26.0, 25.8, 22.5, 22.4, 19.8, 18.3. HRMS m/e 461.3234; calcd for $C_{26}H_{43}N_3O_4$ 461.3254.

General Procedure for the Synthesis of Inhibitors (8a−t). A solution of the macrocyclic epoxide **6** (10 mg, 28 μmol) in ethanol (2 mL) was stirred and refluxed with 10−20 equiv of the desired amine overnight. The solution was evaporated to dryness under high vacuum to remove as much of the excess amine as possible. The residue was dissolved in THF (3 mL) and saturated aqueous $NaHCO_3$ (100 μL). Then the desired sulfonyl chloride or isocyanate (2−5 equiv) was added with stirring at room temperature. Acylation proceeded rapidly, generally less than 30 min, and was monitored for complete consumption of the intermediate secondary amine **7** by mass spectrometry. Inhibitors that required no further elaboration were evaporated and redissolved in MeCN/H_2O and purified by reverse-phase HPLC (column, Phenomenex Luna C18, 250 mm × 22 mm, flow rate 20 mL min^{-1}, linear gradient from 45% MeCN to 90% MeCN (+0.1% TFA) over 15 min followed by a further 15 min at 90% MeCN. Fractions containing products were lyophilized to give white powders (typically 2−8 mg).

Inhibitors **8a**−**g** were prepared using 4-acetamidobenzenesulfonyl chloride and therefore required an additional hydrolysis step to deprotect the *p*-amino group. Methanol (3 mL) and 2 M HCl (1 mL) were added, and the solution was stirred and heated at 60 °C for 1−3 h. Cleavage of the acetyl group was conveniently followed by mass spectrometry, and no undesirable degradation of the macrocycle amide bonds was observed. The solution was evaporated, and the residue was purified by reverse-phase HPLC as above.

4-Amino-*N*-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13*S*-yl)ethyl]-*N*-(3-methylbutyl)benzenesulfonamide, 8a. t_R = 10.9 min. ^{1}H NMR (300 MHz, CD_3OH) δ 7.92 (d, J = 9.7 Hz, 1H, Tyr-NH), 7.51 (m, 2H, $J_{AX} + J_{AX'}$ = 8.7 Hz, ortho to SO_2), 7.19 (d, J = 9.2 Hz, 1H, Val-NH), 7.07 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to CH_2), 6.77 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to O), 6.73 (m, 2H, $J_{AX} + J_{AX'}$ = 8.7 Hz, ortho to NH_2), 4.27−4.05 (m, 3H, Tyr-αH and H-3), 4.07 (dd, J = 9.2, 6.0 Hz, 1H, Val-αH), 3.76 (ddd, J = 8.5, 6.5, 4.2 Hz, 1H, H-1′), 3.40 (dd, J = 14.5, 4.2 Hz, H-2′), 3.35−3.25 (m, 2H, H-4′), 3.18 (dd, J = 13.6, 3.6 Hz, 1H, H-14), 3.10 (m, 1H,), 2.93 (dd, J = 14.5, 8.6 Hz, H-2′), 2.41 (dd, J = 13.6, 12.5 Hz, 1H, H-14), 2.20−2.11 (m, 2H, H-7), 1.86 (m, 1H, Val-βCH), 1.75−1.60 (m, 2H), 1.60−1.10 (m, 7H), 0.87 (d, J = 6.4 Hz, 9H, Val-γCH_3, H-7′, H-8′), 0.76 (d, J = 6.8 Hz, 3H, Val-γCH_3). ^{13}C NMR (CD_3-OD) δ 174.8, 172.5, 158.3, 153.9, 132.1, 131.4, 130.3, 126.7, 117.4, 114.7, 74.2, 68.8, 58.6, 55.0, 53.0, 49.5, 38.2, 36.1, 36.0, 33.5, 30.7, 27.1, 26.1, 22.9, 22.8, 20.0, 18.2. λ_{max}(MeOH) = 266 nm (ϵ = 19 000). HRMS m/e 616.3285; calcd for $C_{32}H_{48}N_4O_6S$ 616.3295.

4-Amino-*N*-*sec*-butyl-*N*-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),-15(19),16-trien-13*S*-yl)ethyl]benzenesulfonamide, 8b. ^{1}H NMR (500 MHz, CD_3OH) δ 7.88 (d, J = 9.8 Hz, 1H), 7.49 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 9.2 Hz, 1H), 7.06 (d, J = 8.0 Hz, 2H), 6.76 (d, J = 8.3 Hz, 2H), 6.69 (d, J = 8.7 Hz, 2H), 4.22 (m, 1H, H-3), 4.17−4.05 (m, 2H, H-3, H-13), 4.06 (dd, J = 9.0, 6.0 Hz, 1H, H-10), 3.76 (m, 1H, CHOH), 3.4−3.2 (m, solvent obscured), 2.98 (dd, J = 13.5, 8.0 Hz, 1H), 2.91 (dd, J = 14.7, 8.5 Hz, 1H), 2.84 (dd, J = 13.5, 6.8 Hz, 1H), 2.39 (t, J = 12.9 Hz, 1H), 2.21−2.10 (m, 2H), 1.97 (m, 1H), 1.84 (m, 1H), 1.75−1.63 (m, 2H), 1.52−1.40 (m, 1H), 1.38−1.10 (m, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.89−0.84 (m, 6H), 0.74 (d, J = 6.8 Hz, 3H). HRMS m/e 603.3193 MH^+; calcd for $C_{31}H_{47}N_4O_6S$ 603.3211.

4-Amino-*N*-cyclopentyl-*N*-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13*S*-yl)-ethyl]benzenesulfonamide, 8c. t_R = 9.3 min. ^{1}H NMR (500 MHz, CD_3OH) δ 7.90 (d, J = 9.9 Hz, 1H), 7.51 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 9.2 Hz, 1H), 7.07 (d, J = 8.0 Hz, 2H), 6.77 (d, J = 8.0 Hz, 2H), 6.72 (d, J = 8.7 Hz, 2H), 4.22 (m, 1H), 4.16−4.04 (m, 2H), 4.07 (dd, J = 9.1, 6.0 Hz, 1H, Val-αH), 3.75 (m, 1H, C*H*OH), 3.39

(dd, J = 14.5, 3.9 Hz, 1H), 3.25 (m, 1H), 3.18 (dd, J = 13.6, 3.4 Hz, 1H), 3.07 (m, 1H), 2.94 (dd, J = 14.5, 8.6 Hz, 1H), 2.40 (t, J = 12.9 Hz, 1H), 2.21−2.09 (m, 2H), 1.85 (m, 1H), 1.74−1.63 (m, 2H), 1.60−1.40 (m, 3H), 1.38−1.10 (m, 11H), 0.90−0.84 (m, 6H), 0.76 (d, J = 6.8 Hz, 3H). HRMS 615.3182 MH^+; calcd for $C_{32}H_{47}N_4O_6S$ 615.3211.

4-Amino-*N*-hexyl-*N*-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15-(19),16-trien-13*S*-yl)-ethyl]benzenesulfonamide, 8d. t_R = 12.8 min. ^{1}H NMR (500 MHz, CD_3OH) δ 7.94 (d, J = 9.6 Hz, 1H), 7.51 (d, J = 8.6 Hz, 2H), 7.17 (d, J = 8.8 Hz, 1H), 7.07 (d, J = 8.0 Hz, 2H), 6.77 (d, J = 8.0 Hz, 2H), 6.68 (d, J = 8.6 Hz, 2H), 4.23 (m, 1H), 4.17−4.04 (m, 4H), 3.90 (m, 1H), 3.36−3.19 (m, solvent obscured), 2.86 (dd, J = 14.6, 2.9 Hz, 1H), 2.41 (t, J = 12.3 Hz, 1H), 1.93−1.84 (m, 2H), 1.81−1.09 (m, 15H), 0.89 (d, J = 6.7 Hz, 3H), 0.75 (d, J = 6.7 Hz, 3H). HRMS 653.3335 MNa^+; calcd for $C_{33}H_{50}N_4O_6SNa$ 653.3343.

6-{(4-Aminobenzenesulfonyl)-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]-nonadeca-1(18),15(19),16-trien-13*S*-yl)ethyl]amino}-hexanoic Acid, 8e. ^{1}H NMR (500 MHz, CD_3OH) δ 7.59 (d, J = 8.8 Hz, 1H), 7.50 (d, J = 8.8 Hz, 2H), 7.08 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.8 Hz, 1H), 6.78 (d, J = 8.0 Hz, 2H), 6.70 (d, J = 8.8 Hz, 2H), 4.23 (m, 1H), 4.18−4.03 (m, 3H), 3.76 (m, 1H), 3.44−3.14 (m, solvent obscured), 3.08 (m, 1H), 2.94 (m, 1H), 2.42 (t, J = 13.2 Hz, 1H), 2.30−2.23 (m, 2H), 2.19−2.11 (m, 2H), 1.87 (m, 1H), 1.75−1.64 (m, 2H), 1.64−1.40 (m, 6H), 1.40−1.06 (m, 7H), 0.88 (d, J = 6.7 Hz, 3H), 0.77 (d, J = 6.8 Hz, 3H). HRMS 683.3079 MNa^+; calcd for $C_{33}H_{48}N_4O_8SNa$ 683.3085.

4-Amino-*N*-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13*S*-yl)-ethyl]-*N*-phenethylbenzenesulfonamide, 8f. t_R = 11.3 min. ^{1}H NMR (500 MHz, CD_3OH) δ 7.59 (d, J = 8.8 Hz, 1H), 7.50 (d, J = 8.5 Hz, 2H), 7.31−7.23 (m, 2H), 7.22−7.12 (m, 3H), 7.09 (d, J = 8.0 Hz, 2H), 6.84 (d, J = 8.5 Hz, 1H), 6.79 (d, J = 8.0 Hz, 2H), 6.71 (d, J = 8.5 Hz, 2H), 4.27 (m, 1H), 4.19−4.06 (m, 3H), 3.81 (m, 1H), 3.53−3.43 (m, 2H), 3.19 (m, 1H), 2.99 (m, 1H), 2.94−2.79 (m, 2H), 2.43 (m, 1H), 2.21−2.11 (m, 2H), 2.03 (m, 2H), 1.88 (m, 1H), 1.77−1.44 (m, 4H), 1.40−1.07 (m, 5H), 0.88 (d, J = 6.8 Hz, 3H), 0.77 (d, J = 6.7 Hz, 3H). HRMS 673.3028 MNa^+; calcd for $C_{35}H_{46}N_4O_6SNa$ 673.3030.

4-Amino-*N*-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13*S*-yl)ethyl]-*N*-[2-(4-methoxyphenyl)ethyl]benzenesulfonamide, 8g. t_R = 10.9 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.74 (d, J = 9.6 Hz, 1H), 7.12 (d, J = 8.9 Hz, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.72 (d, J = 8.4 Hz, 2H), 6.60 (d, J = 8.7 Hz, 2H), 4.17 (m, 1H), 4.08 (m, 1H), 4.03 (dd, J = 9.0, 4.0 Hz 1H), 3.99 (m, 1H), 3.71 (s, 3H), 3.63 (m, 1H), 3.36 (m, 1H), 3.31 (m, 1H), 3.11 (m, 1H), 3.07 (m, 1H), 2.82 (dd, J = 14.1, 8.0 Hz, 1H), 2.69 (m, 2H), 2.33 (m, 1H), 2.16 (m, 1H), 1.91 (m, 1H), 1.76 (m, 1H), 1.64−1.49 (m, 2H), 1.35 (m, 1H), 1.27−0.96 (m, 3H), 0.77 (d, J = 6.7 Hz, 3H), 0.69 (d, J = 6.7 Hz, 3H). ESI-MS *m/e* 681.3 MH^+.

***N*-{4-[[2*R*−Hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13*S*-yl)ethyl]-(3-methylbutyl)sulfamoyl]phenyl}-acetamide, 8h.** t_R = 10.4 min. ^{1}H NMR (300 MHz, CD_3OD) δ 7.93 (d, J = 9.7 Hz, 1H), 7.85−7.73 (m, 4H), 7.20 (d, J = 9.1 Hz, 1H), 7.08 (d, J = 8.2 Hz, 2H), 6.78 (d, J = 8.2 Hz, 2H), 4.23 (m, 1H), 4.12−4.04 (m, 3H), 3.75 (ddd, J = 8.6, 6.8, 3.8 Hz, 1H, C*H*OH), 3.45 (dd, J = 14.5, 3.9 Hz, 1H), 3.42−3.3 (m, solvent obscured), 3.25−3.10 (m, 2H), 3.00 (dd, J = 14.5, 8.8 Hz, 1H), 2.41 (dd, J = 13.5, 12.4 Hz, 1H), 2.26−2.07 (m, 2H), 2.16 (s, 3H), 1.87 (m, 1H), 1.80−1.06 (m, 9H), 0.88 (d, J = 6.4 Hz, 6H), 0.87 (d, J = 6.8 Hz, 3H), 0.76 (d, J = 6.8 Hz, 3H). HRMS *m/e* 681.3307 MH^+; calcd for $C_{36}H_{49}N_4O_7S$ 681.3316.

3-Nitro-*N*-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15,(19),16-trien-13*S*-yl)ethyl]-*N*-(3-methylbutyl)benzenesulfonamide, 8i. t_R = 15.3 min. ^{1}H NMR (500 MHz, CD_3OD) δ 8.62 (t, J = 2.0 Hz, 1H), 8.50 (ddd, J = 8.0, 2.2, 1.0 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 7.87 (t, J = 8.0 Hz, 1H), 7.07 (d, J = 8.5 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 4.24 (m, 1H), 4.14 (m, 1H), 4.10−4.04 (m, 2H), 3.72 (m, 1H), 3.53−3.45 (m, 2H), 3.31−3.24 (m, solvent obscured), 3.21−3.14 (m, 2H), 2.40 (t, J = 12.9 Hz, 1H), 2.21−2.10 (m, 2H), 1.87 (m, 1H), 1.75−1.64 (m, 2H), 1.62−1.08 (m, 8H), 0.90 (d, J = 6.5 Hz, 6H), 0.87 (d, J = 6.8 Hz, 3H), 0.77 (d, J = 6.8 Hz, 3H). HRMS *m/e* 647.3066 MH^+; calcd for $C_{32}H_{47}N_4O_8S$ 647.3115.

***N*-[2*R*-Hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13*S*-yl)ethyl]-2,4,6-triisopropyl-*N*-(3-methyl-butyl)benzene-sulfonamide, 8j.** t_R = 26.3 min. ^{1}H NMR (500 MHz, CD_3OH) δ 7.95 (d, J = 9.9 Hz, 1H), 7.25 (s, 2H), 7.19 (d, J = 9.1 Hz, 1H), 7.05 (d, J = 8.2 Hz, 2H), 6.77 (d, J = 8.2 Hz, 2H), 4.23 (m, 1H), 4.18−4.03 (m, 5H), 4.83 (m, 1H), 3.64 (dd, J = 14.7, 2.7 Hz, 1H), 3.41 (m, 1H), 3.23−3.09 (m, 3H), 2.93 (m, 1H), 2.38 (t, J = 13.3 Hz, 1H), 2.21−2.08 (m, 2H), 1.93 (m, 1H), 1.75−1.63 (m, 1H), 1.54−1.10 (m, 23H), 1.28−1.22 (m, 18H), 0.90 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 6.8 Hz, 3H), 0.73 (d, J = 6.2 Hz, 3H), 0.73 (d, J = 6.2 Hz, 3H). HRMS *m/e* 728.4552 MH^+; calcd for $C_{41}H_{66}N_3O_6S$ 728.4672.

Naphthalene-2-sulfonic Acid [2*R*-Hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]-nonadeca-1(18),15(19),16-trien-13*S*-yl)ethyl]-(3-methylbutyl)amide, 8k. t_R = 17.6 min. ^{1}H NMR (300 MHz, CD_3OH) δ 8.42 (m, 1H), 8.09−8.01 (m, 2H), 8.00−7.90 (m, 2H, 7.81 (dd, J = 8.7, 1.8 Hz, 1H), 7.72−7.61 (m, 2H), 7.19 (d, J = 9.0 Hz, 1H, H-9). ^{1}H NMR, AA′XX′ system, δ 7.06 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to CH_2), 6.77 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to O), 4.27−4.18 (m, 1H, H-3), 4.18−4.20 (m, 2H, H-3, H-13), 4.05 (dd, J = 9.1, 6.0 Hz, 1H, H-10), 3.77 (m, 1H, C*H*OH), 3.56−3.40 (m, 2H, H-2′, NCH_2CH_2), 3.34−3.23 (m, 1H, NCH_2CH_2), 3.20 (dd, J = 13.5, 3.8 Hz, 1H, H-14), 3.08 (dd, J = 14.4, 8.9 Hz, 1H, H-2′), 2.41 (dd, J = 13.5, 12.3 Hz, 1H, H-14), 2.21−2.09 (2H, m, H-7), 1.80 (m, 1H, Val-βH), 1.80−1.09 (m, 10H), 0.84 (d, J = 6.4 Hz, 6H, isoamyl-$(CH_3)_2$), 0.78 (d, J = 6.8 Hz, 3H, Val-γ CH_3), 0.66 (d, J = 6.8 Hz, 3H, Val-γ CH_3). HRMS *m/e* 674.3250, MNa^+; calcd for $C_{36}H_{49}N_3O_6SNa$ 674.3234.

Quinoline-8-sulfonic Acid [2*R*-Hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13*S*-yl)ethyl]-(3-methylbutyl)amide, 8l. t_R = 13.5 min. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.12 (m, 1H), 8.64 (d, J = 7.3 Hz, 1H), 8.38 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 7.73 (t, J = 7.8 Hz, 1H), 7.66 (dd, J = 8.1, 4.1 Hz, 1H), 7.07 (m, 2H), 6.82 (d, J = 8.4 Hz, 2H), 6.44−6.32 (m, 2H), 4.56−4.29 (br m), 4.27−4.03 (m, 4H), 3.99 (m, 1H), 3.44 (d, J = 14.6 Hz, 1H), 3.22 (m, 1H), 3.14 (m, 1H), 3.09 (m, 1H), 2.77 (t, J = 13.0 Hz, 1H), 2.33 (m, 1H), 2.02 (m, 1H), 1.89 (m, 1H), 1.80−1.61 (m, 2H), 1.52 (m, 1H), 1.40−1.29 (m, 4H), 1.29−1.13 (m, 3H), 0.87 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.9 Hz, 3H), 0.71 (d, J = 5.9 Hz, 3H), 0.65 (d, J = 5.9 Hz, 3H). ESI-MS *m/e* 653.3 MH^+.

3-Benzyl-1-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13*S*-yl)ethyl]-1-(3-methylbutyl)urea, 8m. t_R = 13.2 min. ^{1}H NMR (500 MHz, CD_3OD) δ 7.34−7.19 (m), 7.05 (d, J = 8.2 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 4.37 and 4.36 (AB system J_{AB} = 15.3 Hz, 2H), 4.23 (m, 1H), 4.14 (m, 1H), 4.08 (d, J = 6.2 Hz, 1H), 4.06 (m, 1H), 3.72 (m, 1H), 3.44−3.25 (m, solvent obscured), 3.15 (dd, J = 13.7, 3.7 Hz, 1H), 2.41 (t, J = 13.2 Hz, 1H), 2.21−2.11 (m, 2H), 1.88 (m, 1H), 1.76−1.64 (m, 2H), 1.59 (m, 1H), 1.54−1.08 (m, 8H), 0.94 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H), 0.81 (d, J = 6.8 Hz, 3H). HRMS *m/e* 595.3804 MH^+; calcd for $C_{34}H_{51}N_4O_5$ 595.3859.

3-Cyclohexyl-1-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),-16-trien-13*S*-yl)ethyl]-1-(3-methylbutyl)urea, 8n. t_R = 14.7 min. ^{1}H NMR (500 MHz, CD_3OD) δ 7.07 (d, J = 8.2 Hz, 2H), 6.79 (d, J = 8.4 Hz, 2H), 4.24 (m, 1H), 4.14 (m, 1H), 4.09 (d, J = 6.1 Hz, 1H), 4.05 (m, 1H), 3.68 (m, 1H), 3.52 (m, 1H), 3.39−3.27 (m, solvent obscured), 3.23 (m, 1H), 3.17 (dd, J = 13.7, 3.6 Hz, 1H), 2.40 (t, J = 13.0 Hz, 1H), 2.22−2.21 (m, 2H), 1.93−

1.82 (m, 3H), 1.78−1.65 (m, 4H), 1.65−1.10 (m, 15H), 0.93 (d, J = 6.6 Hz, 6H), 0.91 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.8 Hz, 3H). HRMS *m/e* 587.4143 MH^+; calcd for $C_{33}H_{55}N_4O_5$ 587.4172.

3-*tert*-Butyl-1-[2*R*-hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13*S*-yl)-ethyl]-1-(3-methylbutyl)urea, 8o. A solution of the amine **4** (12 mg, 26 μmol) and *tert*-butyl isocyanate (4 μL, 1.5 equiv) in DMF/THF 1:1 (1 mL) was left at room temperature for 10 min and then evaporated to dryness under high vacuum. The residual gum was purified by RP-HPLC, 50:50% MeCN/H_2O + TFA 0.1% isocratic, t_R = 5.6 min, giving a white powder (9 mg, 62%) after lyophilization. ^{1}H NMR (300 MHz, CD_3OH) δ 7.92 (d, J = 9.9 Hz, 1H, Tyr-NH), 7.26 (d, J = 9.2 Hz, 1H, Val-NH). ^{1}H NMR, AA′XX′ system, δ 7.06 (m, 2H, $J_{AX} + J_{AX'}$ = 8.4 Hz, ortho to CH_2), 6.77 (m, 2H, $J_{AX} + J_{AX'}$ = 8.6 Hz, ortho to O), 4.28−3.96 (m, 4H, H-3, H-13), 4.09 (dd, J = 9.0, 5.9 Hz, 1H, Val-αH), 3.65 (m, 1H, C*H*OH), 3.35−3.10 (m), 3.23 (dd, J = 10.4, 7.8 Hz, H-14), 2.38 (dd, J = 13.5, 12.3 Hz, H-14), 2.22−2.08 (m, 2H, H-7), 1.88 (m, 1H, Val-βH), 1.80−1.60 (m, 2H), 1.54 (m, 1H, isoamyl-CH), 1.60−1.10 (m), 1.32 (s, 9H, ^{t}Bu), 0.93 (d, J = 6.4 Hz, 3H, H-4″), 0.92 (d, J = 6.5 Hz, 3H, H-5″), 0.90 (d, J = 6.8 Hz, 3H, Val-γ CH_3), 0.82 (d, J = 6.9 Hz, 3H, Val-γ CH_3). ^{13}C NMR (CD_3OH) δ 174.8, 172.7, 161.0, 158.3, 131.7, 131.2, 117.3, 75.5, 68.6, 58.6, 55.3, 52.8, 51.3, 47.3, 37.9, 36.8, 35.8, 33.5, 30.5, 29.7, 27.3, 25.9, 25.9, 23.0, 22.8, 19.9, 18.1. HRMS *m/e* 560.3940; calcd for $C_{31}H_{54}N_4O_5$ 560.3938.

2-[3-[2*R*-Hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,-12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl)-ethyl]-3-(3-methylbutyl)ureido]-3-methylbutyric Acid, 8p. This urea derivative was prepared in two steps from the amine (**7**, n = 5) and valine methyl ester isocyanate[46] in THF, followed by hydrolysis of the methyl ester with NaOH. The solution was acidified with TFA and purified by RP-HPLC 50:50% MeCN/H_2O + TFA 0.1% isocratic, t_R = 6.0 min, giving a white powder after lyophilization. ^{1}H NMR (300 MHz, CD_3-CN) δ AA′XX′ system, 7.06 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to CH_2), 6.77 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to CH_2), 6.45 (d, J = 9.7 Hz, 1H, H-12), 6.19 (d, J = 8.7 Hz, 1H, H-9), 6.13 (br, 1H, urea NH), 4.24 (ddd, J = 12.0, 6.0, 4.0 Hz, 1H, H-3), 4.15−3.98 (m, 3H, H-3, H-10, H-13), 3.69 (m, 1H, C*H*OH), 3.44−3.13 (m, 3H), 3.08 (dd, J = 13.7, 3.9 Hz, 1H), 2.45−2.00 (m, also H_2O peak), 1.9−1.78 (m, 1H), 1.71−1.00 (m), 0.98 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.5 Hz, 6H), 0.84 (d, J = 6.8 Hz, 3H), 0.76 (d, J = 6.8 Hz, 3H). ^{13}C NMR (CD_3CN) δ 174.6, 172.5, 171.6, 161.1, 157.8, 131.6, 131.3, 117.0, 74.9, 68.4, 60.4, 57.7, 54.6, 52.3, 47.4, 37.6, 36.2, 35.7, 33.2, 30.7, 29.9, 26.8, 25.6, 25.6, 22.9, 22.8, 19.8, 19.7, 18.4, 18.1. ISMS *m/e* 605.4 MH^+; calcd for $C_{32}H_{53}N_4O_7$ 605.4.

***N*-[2*R*-Hydroxy-2-(8*S*-isopropyl-6,9-dioxo-2-oxa-7,10-diaza-bicyclo[11.2.2]heptadeca-1(16),13(17),14-trien-11S-yl)-ethyl]-*N*-(3-methylbutyl)benzenesulfonamide, 8q.** ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.66−7.73 (m, 10H, ArH, Val-NH), 7.62 (d, J = 9.2 Hz, 1H, Tyr-NH), 4.3 (m, 1H, OCH), 4.18 (m, 1H, OCH), 4.05 (m, 1H, Tyr-αCH), 3.69 (m, 1H, C*H*OH), 3.50 (m, 1H, Val-αCH), 3.25−3.34 (m, 2H, CHN, NCH), 3.18 (m, 1H, Tyr-βCH), 3.07 (m, 1H, NCH), 2.92 (m, 1H, CHN), 2.3−2.37 (m, 2H, Tyr-βCH, CHC(O)), 2.05 (m, 1H, CHC(O)), 1.78−1.96 (m, 2H, CH_2), 1.60 (m, 1H, Val-βCH), 1.44 (m, 1H, CH), 1.22−1.37 (m, 2H, CH_2), 0.77 (d, J = 7.3 Hz, 6H $(CH_3)_2$), 0.69 (d, J = 6.7 Hz, 3H, CH_3), 0.67 (d, J = 6.7 Hz, 3H, CH_3). ISMS *m/z* 574 $(M + H)^+$.

***N*-[2*S*-Hydroxy-2-(10*S*-isopropyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl-)ethyl]-*N*-(3-methylbutyl)benzenesulfonamide, 8s.** t_R = 14.8 min. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (m, 2H, ortho to SO_2), 7.63 (m, 1H, para to SO_2), 7.55 (m, 2H, meta to SO_2). ^{1}H NMR AA′XX′ system, δ 7.05 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to CH_2), 6.83 (m, 2H, $J_{AX} + J_{AX'}$ = 8.5 Hz, ortho to O), 5.79 (d, J = 8.4 Hz, 1H), 5.69 (d, J = 9.5 Hz, 1H), 4.32−4.22 (m, 2H, H-3, H-13), 4.16 (m, 1H, H-13), 3.96 (dd, J = 8.3, 6.2 Hz, 1H, Val-αCH), 3.90 (m, 1H, C*H*OH), 3.30−3.15 (m, 4H, CH_2NCH_2), 3.00 (dd, J = 14.0, 4.2 Hz, H-14), 2.68 (dd, J = 14.0, 12.5 Hz, 1H, H-14), 2.25 (m, 1H), 1.95−1.15 (e, 10H), 0.90 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H, Val-βCH_3), 0.86 (d, J = 6.9 Hz, 3H, Val-βCH_3). ^{13}C NMR ($CDCl_3$) δ 172.4, 170.8, 157.1, 138.7, 132.9, 130.2, 129.4, 129.3, 127.2, 116.3, 72.5, 67.6, 57.9, 53.5, 52.1, 49.0, 37.3, 35.5, 34.2, 32.1, 29.1, 25.8, 25.0, 24.5, 22.4, 22.3, 18.9, 18.3. HRMS *m/e* 601.3183; calcd for $C_{32}H_{47}N_3O_6S$ 601.3186.

2*S*-(12-{2-[Benzenesulfonyl-(3-methylbutyl)amino]-1*S*-hydroxyethyl}-6,10-dioxo-2-oxa-7,11-diazabicyclo[12.2.2]-octadeca-1(17),14(18),15-trien-8*S*-yl)acetamide, 8t. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.55−7.79 (m, 13H, ArH, Asn-NH_2, Asn-NH, Tyr-NH), 4.23 (m, 1H, OCH), 4.12−4.19 (m, 2H, OCH, Asn-αCH), 4.04 (m, 1H, Tyr-αCH), 3.67 (m, 1H, C*H*OH), 3.40 (m, 1H, CHN), 3.02−3.25 (m, 3H, NCH_2, Tyr-βCH), 2.98 (m, 1H, CHN), 2.20−2.45 (m, 4H, Tyr-βCH, Asn-βCH_2, CH(CO)), 1.82−2.08 (m, 3H, CHC(O), CH_2), 1.27−1.49 (m, 3H, CH, CH_2), 0.79 (d, J = 7.7 Hz, 6H $(CH_3)_2$). ESI-MS *m/e* 589 $(M + H)^+$.

1-[2-(8-Carbamoylmethyl-6,9-dioxo-2-oxa-7,10-diaza-bicyclo[11.2.2]heptadeca-1(16),13(17),14-trien-11-yl)-2-hydroxyethyl]pyrrolidine-2-carboxylic Acid [1-(1-Carbamoyl-2-methyl-propylcarbamoyl)-2-methylbutyl]amide, 8u. t_R = 39.9 min (0−45% MeCN + 0.1% TFA over 50 min). ^{1}H NMR (H_2O/D_2O, 8:2) δ 8.69 (br s, 1H, Ile-NH), 8.22 (d, J = 7.23 Hz, 1H, Val-NH), 7.71 (d, J = 9.8 Hz, 1H, 10′-NH), 7.65 (br s, 1H), 7.47 (br s, 1H,), 7.21 (d, J = 8.8 Hz, 1H, Asn-NH), 7.20 (dd, J = 2.1, 8.4 Hz, 1H, H17′), 7.15 (dd, J = 2.2, 8.4 Hz, 1H, H14′), 7.09 (br s, 1H), 7.00 (dd, J = 2.7, 8.4 Hz, 1H, H16′), 6.95 (dd, J = 2.7, 8.4 Hz, 1H, H15′), 6.72 (br s, 1H), 4.42−4.48 (m, 1H, H-3′), 4.29−4.38 (m, 3H, Asn-αCH, Ile-αCH, and H-3′), 4.22−4.29 (m, 1H), 4.19 (m, 1H, Val-αCH), 4.10−4.16 (m, 2H, Pro-αCH and H-2), 3.8 (m, 1H, Pro-δCH), 3.15−3.28 (m, 3H), 3.12 (dd, J = 3.5, 5.6 Hz, 1H, H-12′), 2.78 (dd, J = 13.5, 13.5 Hz, 1H, H-12′), 2.55 (m, 1H), 2.42−2.51 (m, 3H), 2.29 (ddd, J = 16.2, 7.6, 3.6 Hz, 1H), 2.19 (m, 1H), 1.98−2.14 (m, 6H), 1.89−1.98 (m, 1H), 1.51−1.61 (m, 1H), 1.20−1.30 (m, 1H), 1.02 (d, J = 6.8 Hz, 6H), 0.98 (d, J = 6.8 Hz, 3H), 0.94 (t, 3H, J = 7.4 Hz). ^{13}C NMR (H_2O/D_2O, 8:2) δ 10.59, 15.06, 18.21, 18.69, 23.08, 24.25, 24.98, 30.04, 30.23, 31.50, 35.63, 36.51, 38.86, 50.51, 53.84, 55.49, 58.19, 59.18, 59.74, 68.42, 68.78, 78.50, 114.98, 117.23, 117.83, 129.45, 131.27, 132.44, 156.90, 158.00, 171.93, 173.71, 174.50, 176.13. ESI-MS *m/e* 688 $(M + H^+)$.

11*S*-Amino-8*S*-*sec*-butyl-2-oxa-6,9-diazabicyclo[11.2.2]-heptadeca-1(16),13(17),14-triene-7,10-dione (2).[25] ^{1}H NMR (300 MHz, CD_3OH) δ 7.79 (broad m, 1H, $NHCH_2$), 7.22 (d, J = 7.8 Hz, 1H, Ile-NH), 7.23 (dd, J = 8.4, 2.1 Hz, 1H, ArH), 6.97−6.79 (m, 3H, ArH), 4.41−4.31 (m, 1H, H-3), 4.28−4.16 (m, 1H, H-3), 4.08 (dd, J = 10.7, 7.0 Hz, 1H, Tyr-αCH), 3.60−3.45 (m, 1H, H-5), 3.47 (t, J = 7.5 Hz, 1H, Ile-αCH), 3.28 (dd, J = 12.4, 7.0 Hz, 1H, Tyr-βCH), 2.86−2.75 (m, 1H, H5), 2.70 (dd, J = 12.4, 10.7 Hz, 1H, Tyr-βCH), 2.30−2.09 (m, 1H, H-4), 1.83−1.69 (m, 1H, H-4), 1.60−1.39 (m, 2H, Ile-βCH and Ile-γCH_2), 1.01−0.88 (m, 1H, Ile-γCH_2), 0.83 (t, J = 7.2 Hz, 3H, Ile-δCH_3), 0.75 (d, J = 6.8 Hz, 3H, Ile-γC_3). ^{13}C NMR (CD_3-OH) δ 171.5, 168.6, 160.0, 132.2, 130.4, 128.2, 118.8, 118.7, 68.6, 59.8, 56.0, 40.0, 38.0, 37.5, 27.6, 26.2, 14.9, 11.7. ISMS *m/z* 334 (MH^+).

2-Acetylamino-4-methylpentanoic Acid {1-[1-Benzyl-3-(8-*sec*-butyl-7,10-dioxo-2-oxa-6,9-diaza-bicyclo[11.2.2]-heptadeca-1(16),13(17),14-trien-11*S*-ylamino)-2*R*-hydroxy-propylcarbamoyl]-2-methylpropyl}amide, 10a. Ac-Leu-Val-Phe-$COCH_2Br$[24] was reduced to the bromohydrin and cyclized to the epoxide **9** under the conditions reported for **5a** and **6a**, respectively. Epoxide ring opening with the macrocyclic amine **2** in DMF, 70 °C, 24 h, gave **10a** as a white powder after HPLC (t_R = 55 min, Waters Delta-Pak cartridge (C18, 5 μm, 100 Å, 8 mm × 100 mm)), using a linear gradient from H_2O + 0.1% TFA to 55% MeCN + 0.1% TFA over 50 min at a flow rate of 2 mL/min. ^{1}H NMR (500 MHz, CD_3OH) δ 8.30 (d, J = 6.1 Hz, 1H, Leu-NH), 7.85 (m, 1H, NH), 7.73 (d, J = 5.8 Hz, 1H, Val-NH), 7.70 (m, 1H, Phe-NH), 7.34 (d, J = 7.6 Hz, 1H, Ile-NH), 6.80−7.30 (m, 9H, ArH), 4.38 (m, 1H, H3′), 4.20−4.26 (m, 2H, Leu-αCH, H3′), 4.13 (m, 2H, Tyr-αCH, Phe-αCH), 3.81 (m, 2H, Val-αCH, H2), 3.54 (m, 1H, H5′), 3.45 (m, 1H, Ile-αCH), 3.39 (dd, $J_{H12'H12'}$ = 12.1 Hz, $J_{H11'H12'}$ = 7.0 Hz, 1H,

Tyr-βCH), 3.28 (m, 1H, Phe-βCH_2), 3.08 (dd, $J_{H1H1} = 12.7$ Hz, $J_{H1H2} = 1$ Hz, 1H, H1), 3.00 (dd, $J_{H1H1} = 12.7$ Hz, $J_{H1H2} = 6.0$ Hz, 1H, H1), 2.79 (m, 2H, Tyr-βCH, H5′), 2.70 (dd, $J_{H4H4} = 14.0$ Hz, $J_{H4H3} = 11.0$ Hz, 1H, Phe-βCH), 2.26 (m, 1H, H4′), 2.04 (s, 3H, acetyl), 1.83 (m, 1H, Val-βCH), 1.72 (m, 1H, H4′), 1.62 (m, 1H, Leu-γCH), 1.53−1.59 (m, 2H, Leu-βCH, Ile-βCH), 1.43−1.49 (m, 2H, Ile-γCH, Leu-βCH), 0.97 (m, 1H, Ile-γCH), 0.93 (d, $J = 6.5$ Hz, 3H, Leu-δCH_3), 0.88 (d, $J = 6.5$ Hz, 3H, Leu-δCH_3), 0.85 (t, $J = 7.4$ Hz, 3H, Ile-δCH_3), 0.76 (d, $J = 6.7$ Hz, 3H, Ile-γCH_3), 0.74 (d, $J = 6.7$ Hz, 3H, Val-γCH_3), 0.59 (d, $J = 6.7$ Hz, 3H, Val-γCH_3). ISMS *m/z* 751 $(M + H)^+$. HRMS calcd for $C_{41}H_{62}N_6O_7$, 750.4680; found 750.4682.

Quinoline-2-carboxylic Acid {1-[1-Benzyl-3-(8-sec-butyl-7,10-dioxo-2-oxa-6,9-diaza-bicyclo[11.2.2]heptadeca-1(16),13(17),14-trien-11-ylamino)-2-hydroxypropylcarbamoyl]-2-methylpropyl-amide, 10b Compound **10f** was deprotected with TFA and then coupled to quinaldyl-val-OH with BOP reagent in DMF and purified by HPLC ($t_R = 22$ min, Waters Delta-Pak cartridge (C18, 5 μm, 100 Å, 8 mm × 100 mm)), using a linear gradient from H_2O + 0.1% TFA to 90% MeCN + 0.1% TFA over 35 min at a flow rate of 2 mL/min. ^{1}H NMR (500 MHz, CD_3OD) δ 8.77 (d, $J = 7.48$ Hz, 1H, Val-NH), 8.44 (d, $J = 8.45$ Hz, 1H), 8.25 (d, $J = 8.89$ Hz, 1H, Phe-NH), 8.21 (d, $J = 8.45$ Hz, 1H), 8.16 (d, $J = 8.45$ Hz, 1H), 7.99 (d, $J = 8.45$ Hz, 1H), 7.83−7.91 (m, 2H), 7.71 (m, 1H,), 7.31 (d, $J = 7.3$ Hz, 1H), 6.74−7.24 (m, 11H), 4.36 (m, 1H), 4.24 (m,1H), 4.11−4.17 (m, 3H), 3.78 (m, 1H, H2), 3.56 (m, 1H), 3.47 (m,), 3.29−3.33 (m, 2H), 3.10 (m, 1H), 3.03 (m, 1H), 2.79 (m, 1H), 2.53−2.62 (m, 2H), 2.25 (m, 1H), 2.01 (m, 1H, Val-CH), 1.73 (m, 1H), 1.58 (m, 1H), 1.43 (m, 1H), 0.97 (m, 1H), 0.85 (d, $J = 6.62$ Hz, 3H), 0.82 (t, $J = 7.33$ Hz, 3H), 0.77 (d, $J = 6.74$ Hz, 3H), 0.71 (d, $J = 6.62$ Hz, 3H). ISMS *m/z* 751 (M + H). HRMS calcd for $C_{43}H_{54}N_6O_6$ 750.4105; found 750.4097.

[1*S*-Benzyl-3-(8*S*-*sec*-butyl-7,10-dioxo-2-oxa-6,9-diazabicyclo[11.2.2]heptadeca-1(16),13(17),14-trien-11*S*-ylamino)-2*R*-hydroxypropyl]carbamic Acid Tetrahydrofuran-3-yl Ester, 10e.[25] t_R = 18 min, Waters Delta-Pak cartridge (C18, 5 μm, 100 Å, 8 mm × 100 mm) using a linear gradient from H_2O + 0.1% TFA to 90% MeCN + 0.1% TFA over 35 min at a flow rate of 1.5 mL/min. ^{1}H NMR (500 MHz, CD_3OH) δ 7.85 (m, 1H, NH), 7.37 (d, $J = 5.5$ Hz, 1H, Ile-NH), 6.83−7.32 (m, 9H, ArH), 6.44 (d, $J = 9.0$ Hz, 1H, Phe-NH), 6.06 (m, 1H, Tyr-NH), 5.05 (m, 1H, furan-H), 4.40 (m, 1H, H3′), 4.26 (m, 1H, H3′), 3.64−3.89 (m, 6H, H2, Tyr-αCH, Phe-αCH, 3furan-H), 3.58 (m, 1H, H5′), 3.47−3.53 (m, 2H, Ile-αCH, furan-H), 3.22 (m, 1H, Phe-βCH), 3.12 (m, 1H, H1), 3.00 (m, 1H, H1), 2.91 (m, 1H, Tyr-βCH), 2.78−2.84 (m, 2H, Tyr-βCH, H5′), 2.26 (m, 1H, H4′), 2.10 (m, 1H, furan-H), 1.93 (m, 1H, furan-H), 1.76 (m, 1H, H4′), 1.57 (m, 1H, Ile-βCH), 1.42 (m, 1H, Ile-γCH), 0.99 (m, 1H, Ile-γCH), 0.86 (t, $J = 5.5$ Hz, 3H, Ile-δCH_3), 0.78 (d, $J = 5.0$ Hz, 3H, Ile-γCH_3). ISMS *m/z* 611 $(M + H)^+$.

[1*S*-Benzyl-3-(8*S*-*sec*-butyl-7,10-dioxo-2-oxa-6,9-diazabicyclo[11.2.2]heptadeca-1(16), 13(17), 14-trien-11*S*-ylamino)-2*R*-hydroxypropyl]carbamic Acid *tert*-Butyl Ester, 10f.[25] t_R = 56 min, Waters Delta-Pak cartridge (C18, 5 μm, 100 Å, 8 mm × 100 mm), using a linear gradient from H_2O + 0.1% TFA to 45% MeCN + 0.1% TFA over 60 min at a flow rate of 2 mL/min. ^{1}H NMR (500 MHz, CD_3OH) δ 7.81 (m, 1H, NH), 6.91−7.41 (m, 10H, ArH, Ile-NH), 6.44 (d, $J = 9.0$ Hz, 1H, Phe-NH), 6.06 (m, 1H, Tyr-NH), 4.44 (m, 1H, H3′), 4.27 (m, 1H, H3′), 3.83 (m, 1H, H2), 3.69 (m, 2H, Tyr-αCH, Phe-αCH), 3.51 (m, 1H, Ile-αCH), 3.40 (m, 1H, H5′), 3.10−3.17 (m, 2H, Phe-βCH, Tyr-βCH), 3.03 (m, 1H, H1), 2.86−2.92 (m, 2H, H1, H5′), 2.53−2.61 (m, 2H, Phe-βCH, Tyr-βCH), 2.22 (m, 1H, H4′), 1.84 (m, 1H, H4′), 1.56 (m, 1H, Ile-βCH), 1.38 (m, 1H, Ile-γCH), 1.25 (s, 9H $(CH_3)_3$), 0.96 (m, 1H, Ile-γCH), 0.83 (t, $J = 5.0$ Hz, 3H, Ile-δCH_3), 0.73 (d, $J = 5.0$ Hz, 3H, Ile-γCH_3). ISMS *m/z* 597 $(M + H)^+$. HRMS calcd for $C_{33}H_{48}N_4O_6$, 596.3574; found 596.3579

[1-Benzyl-2-(8-*sec*-butyl-7,10-dioxo-2-oxa-6,9-diazabicyclo[11.2.2]heptadeca-1(16),13(17),14-trien-11-ylamino)ethyl]carbamic Acid *tert*-Butyl Ester, 10g.[25] t_R = 21 min, Waters Delta-Pak cartridge (C18, 5 μm, 100 Å, 8 mm × 100 mm), using a linear gradient from H_2O + 0.1% TFA to 90% MeCN + 0.1% TFA over 35 min at a flow rate of 1.5 mL/min. ^{1}H NMR (500 MHz, CD_3OH) δ 7.90 (m, 1H, NH), 6.82−7.37 (m, 11H, ArH, Phe-NH, Ile-NH), 6.3 (m, 1H, Tyr-NH), 4.39 (m, 1H, H3′), 4.25 (m, 1H, H3′), 4.08 (m, 2H, Tyr-αCH, Phe-αCH), 3.57 (m, 1H, H5′), 3.46 (m, 1H, Ile-αCH), 3.34 (m, 1H, Tyr-βCH), 2.68−3.07 (m, 6H, Tyr-βCH, Phe-βCH_2, H1, H1, H5′), 2.27 (m, 1H, H4′), 1.76 (m, 1H, H4′), 1.50 (m, 1H, Ile-βCH), 1.38−1.42 (m, 10H, Ile-γCH $(CH_3)_3$), 0.92 (m, 1H, Ile-γCH), 0.86 (t, $J = 6.0$ Hz, 3H, Ile-δCH_3), 0.73 (d, $J = 5.0$ Hz, 3H, Ile-γCH_3). ISMS *m/z* 567 $(M + H)^+$. HRMS calcd for $C_{32}H_{46}N_4O_5$, 566.3468; found 566.3447.

12*S*-[2-(8*S*-*sec*-Butyl-7,10-dioxo-2-oxa-6,9-diazabicyclo-[11.2.2]heptadeca-1(16),13(17),14-trien-11*S*-ylamino)-1*R*-hydroxyethyl]-9*S*-isopropyl-2-oxa-8,11-diazabicyclo[12.2.2]-octadeca-1(17),14(18),15-triene-7,10-dione, 10h. The epoxide (**6a**, $n = 4$) (20 mg, 56 μmol), the amine **2** (75 mg, 225 μmol), and diisopropylethylamine (10 μL) were heated and stirred in dry DMF (500 μL) at 80 °C for 24 h. The mixture was evaporated to dryness in vacuo, and then the residue was dissolved in a mixture of MeCN (3 mL), water (7 mL), and trifluoroacetic acid (0.25 mL). After filtration the solution was purified by reverse-phase HPLC on a Waters Delta-Pak cartridge (C18, 15 μm, 100 Å, 40 × 100 mm) using a linear gradient from 99.9% H_2O + 0.1% TFA to 49.95% H_2O + 49.95% MeCN + 0.1% TFA over 25 min at a flow rate of 30 mL/min; $t_R = 17$ min. Fractions containing the bicycle were combined and lyophilized, giving a white powder (15 mg, 35%). ^{1}H NMR (500 MHz, CD_3OH) δ 7.91 (d, $J = 9.5$ Hz, 1H, Tyr NH), 7.84 (dd, $J = 6.6$, 3.6 Hz, 1H, CH_2NH), 7.51 (d, $J = 9.4$ Hz, 1H, Val NH), 7.24 (d, $J = 7.6$ Hz, 1H, Ile NH), 7.13 (dd, $J = 8.4$, 2.0 Hz, 1H, 15′- or 18′-CH), 7.10 (dd, $J = 8.3$, 2.1 Hz, 1H, 14″- or 17″-CH), 7.02 (dd, $J = 8.2$, 2.2 Hz, 1H, 15′- or 18′-CH), 6.95 (m, 1H, 14″- or 17″-CH), 6.89 (m, 1H, 15″- or 16″-CH), 6.86 (m, 1H, 16′- or 17′-CH), 6.84 (m, 1H, 15″- or 16″-CH), 6.77 (dd, $J = 8.2$, 2.7 Hz, 1H, 16′- or 17′-CH), 4.39 (m, 1H, 3″-CH_2), 4.24 (m, 1H, 3″-CH_2), 4.20 (m, 1H, 3′-CH_2), 4.15 (dd, $J = 10.7$, 6.9 Hz, 11″-CH), 4.10 (m, 1H, 3′-CH_2), 4.05 (m, 1H, 12′-CH), 3.95 (dd, $J = 9.4$, 8.4 Hz, Val-αCH), 3.81 (m, 1H, C*H*OH), 3.57 (m, 1H, 5″-CH), 3.47 (dd, $J = 7.6$, 7.0 Hz, Ile-αH), 3.40 (dd, $J = 12.2$, 6.9 Hz, 12″-CH), 3.24 (dd, $J = 13.5$, 3.8 Hz, 13′), 3.10 (dd, $J = 12.4$, 2.8 Hz, 1H, CHOHCH_2NHR), 2.92 (dd, $J = 12.4$, 8.7 Hz, 1H, CHOHCH_2NHR), 2.80 (m, 1H, 5″), 2.77 (dd, $J = 12.2$, 10.7 Hz, 1H, 12″), 2.40 (dd, $J = 13.5$, 12.3 Hz, 1H, 13′), 2.26 (m, 1H, 4″), 2.11 (m, 1H, 6′), 2.10 (m, 1H, 5′), 1.79 (m, 1H, Val-βH), 1.76 (m, 1H, 6′), 1.74 (m, 1H, 4″), 1.64 (m, 1H, 4′), 1.57 (m, 1H, Ile-βH), 1.45 (m, 1H, 5′), 1.41 (m, 1H, Ile-CH_2), 1.31 (m, 1H, 4′), 0.95 (m, 1H, Ile-βCH_2), 0.87 (d, $J = 6.6$ Hz, Val-γCH_3), 0.85 (t, $J = 7.1$ Hz, 3H, Ile-δCH_3), 0.81 (d, $J = 6.8$ Hz, 3H, Val-γCH_3), 0.76 (d, $J = 6.9$ Hz, 3H, Ile-γCH_3). ^{13}C NMR (CD_3OD) δ 174.99, 173.68, 171.16, 167.32, 160.20, 156.66, 132.34, 131.98, 130.51, 130.15, 127.69, 119.57, 118.87, 118.75, 70.87, 69.25, 68.58, 63.69, 59.96, 59.67, 55.84, 51.06, 39.96, 37.77, 37.65, 36.83, 36.20, 32.79, 27.57, 27.29, 26.70, 22.59, 20.16, 18.91, 14.91, 11.92. ESI-MS *m/z* 694 $[M + H]^+$ (100%). HRMS (EI$^+$) *m/e* 693.4095, M^+; calcd for $C_{38}H_{55}N_5O_7$ 693.4102.

X-ray Crystallographic Files. X-ray crystallographic files are available through the Protein Data Bank at http://www.rcsb.org/pdb.

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