# *β*-Strand Mimicking Macrocyclic Amino Acids: Templates for Protease Inhibitors with Antiviral Activity

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New amino acids are reported in which component macrocycles are constrained to mimic tripeptides locked in a *β*-strand conformation. The novel amino acids involve macrocycles functionalized with both an N- and a C-terminus enabling addition of appendages at either end to modify receptor affinity, selectivity, or membrane permeability. We show that the cycles herein are effective templates within inhibitors of HIV-1 protease. Eleven compounds originating from such bifunctionalized cyclic templates are potent inhibitors of HIV-1 protease (Ki 0.3−50 nM; pH 6.5, $I = 0.1$ M). Unlike normal peptides comprising amino acids, five of these macrocycle-containing compounds are potent antiviral agents with sub-micromolar potencies ($IC_{50}$ 170−900 nM) against HIV-1 replication in human MT2 cells. The most active antiviral agents are the most lipophilic, with calculated values of $LogD_{6.5} \geq 4$. All molecules have a conformationally constrained 17-membered macrocyclic ring that has been shown to structurally mimic a tripeptide segment (Xaa)-(Val/Ile)-(Phe/Tyr) of a peptide substrate in the extended conformation. The presence of two trans amide bonds and a para-substituted aromatic ring prevents intramolecular hydrogen bonds and fixes the macrocycle in the extended conformation. Similarly constrained macrocycles may be useful templates for the creation of inhibitors for the many other proteins and proteases that recognize peptide *β*-strands.

## Introduction

Accelerated access during the past decade to structures of macromolecular receptors is enabling more effective and faster structure-based drug design,[1−3] which is finally realizing its long held promise as a powerful method for the rational design and development of bioactive compounds and drug leads. This has been particularly exemplified in the generation of inhibitors of proteolytic enzymes in general,[4,5] with HIV-1 protease inhibitors[6−13] being important examples of structure-based designed compounds that have shown clinical utility.

On the other hand, there is little or no structural information available for most proteases, necessitating the design of inhibitors based upon the sequence of peptide substrates that are recognized and processed by the enzyme.[5] This has led to a more circuitous route to inhibitor development, since substrates are by definition not optimized for high affinity binding to proteases. Instead, substrates need to have low (micro- to millimolar) affinities for proteases in order for there to be rapid turnover. Methods are consequently still needed to improve the process of development of protease inhibitors from the limited information provided by protease substrates. One little studied approach stems from the now established paradigm that proteases commonly, if not universally, recognize their peptide substrates/inhibitors in the form of extended "saw-tooth" *β*-strand structures.[14,15] Surprisingly, almost all known protease inhibitors are conformationally flexible molecules. Yet templates that fix peptides or other small molecules in a *β*-strand conformation could conceivably be used to build potent inhibitors that are preorganized for protease binding, leading to higher affinity for a protease due to significant entropic advantages.

We have been investigating the potential of macrocyclic peptidomimetic protease inhibitors that use a cyclic replacement for a three amino acid segment of a peptide substrate that is in a *β*-strand conformation. We have demonstrated the use of certain conformationally constrained macrocycles (e.g., **1** and **2**) as excellent structural mimics of the *β*-strand of a protease-binding peptide, reproducing both H-bond donor/acceptor properties of a peptide as well as pocket-binding properties of peptide side chains.[16−20] Such macrocycles are highly resistant to degradation by gastric, serum, and cellular proteases and are lipophilic structural components of agents with potent activity against HIV in vitro.[21] This approach to inhibitors has the potential for numerous other proteases of the aspartic, serine, and metallo classes.[22]

To date, antiviral macrocyclic peptidomimetics have been constructed by adding appendages to either the C-terminus (e.g., of **1**) or the N-terminus (e.g., of **2**) of a macrocycle.[21] In this paper, we significantly extend their utility by modifying the macrocyclic templates to accommodate both N- and C-terminal functional groups, leading to macrocyclic amino acids (e.g., **3**) that can be readily inserted into longer peptide sequences or elaborated by adding various N- and/or C-terminal nonpeptidic appendages to the cycle. These new macrocyclic

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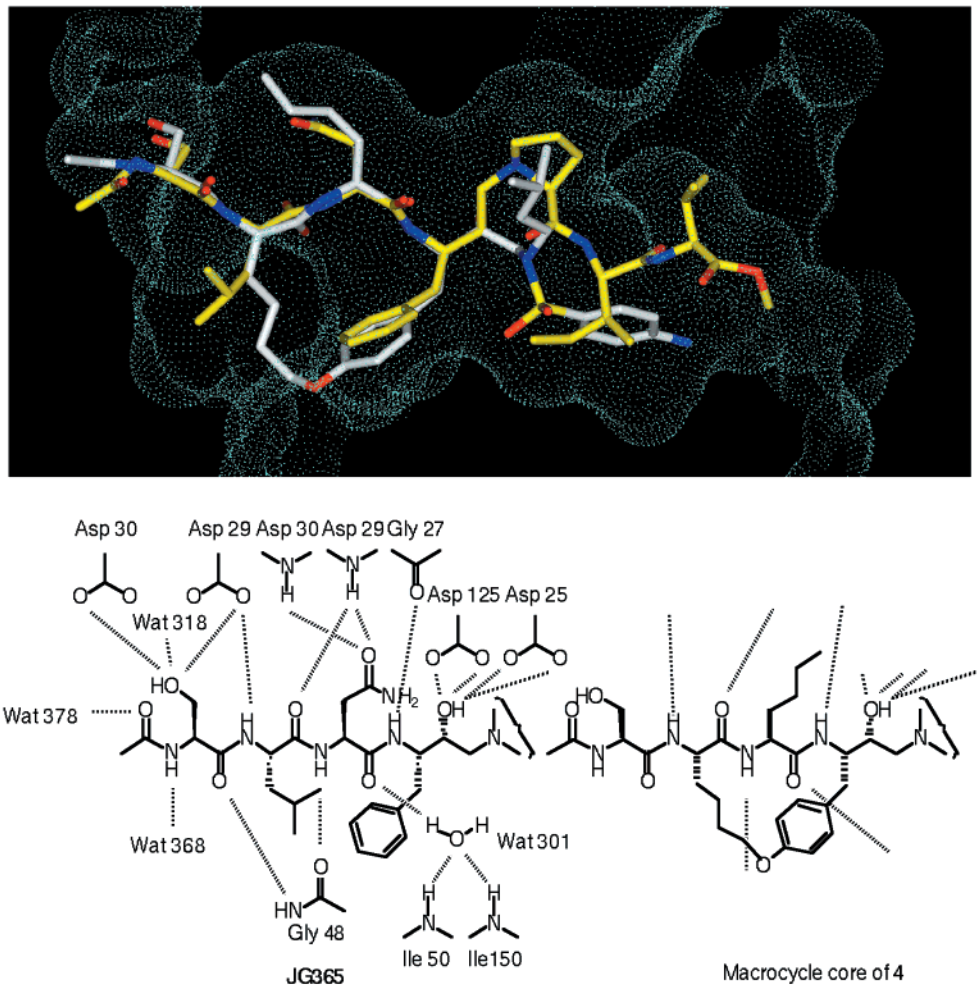
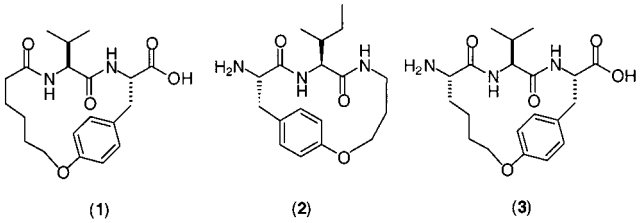


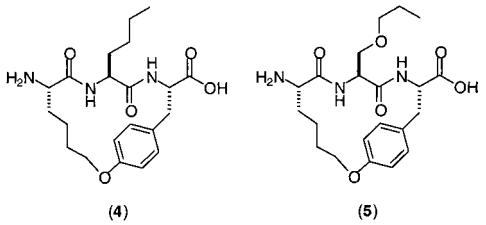

**Figure 1.** Top: Superimposition of macrocyclic inhibitor (**9g**, white) on acyclic peptide JG365 (yellow) bound within the active site of HIV-1 protease (oxygen, red; nitrogen, blue; carbon, white and yellow, respectively). The surface of the HIV-1 protease active site (pale blue) is from the protease−JG365 structure.[23] Bottom: Hydrogen bonding (dashed lines) of acyclic peptidic inhibitor JG365 (left)[23] and macrocyclic inhibitors (right)[20] to HIV-1 protease.

amino acids (e.g., **3**) are representative of an important new class of molecular building blocks or templates that rigidly maintain their structural integrity, chemical stability, and receptor-binding properties typical of the peptides that they were designed to mimic.




## Results

**Design of Macrocyclic Amino Acids.** In previous work, we had incorporated 14−17-membered rings as in **1** and **2** into inhibitors of HIV-1 protease and found their three-dimensional structures to be identical to the extended $\beta$-strand of acyclic peptides complexed within the active site of HIV-1 protease.[16,17,20,21] We also found that these cycles were unlike peptides in being proteolytically stable and conferring antiviral activity, particularly when a pentamethylene linker was used as in **1** .[21] We consequently chose the 17-membered macrocyclic templates **3**−**5** as target macrocyclic amino acids for synthesis, allowing ready derivatization to HIV-1 protease inhibitors.




An illustration of how strand-mimicking cycles such as **3**−**5** are able to reproduce the same interactions with HIV-1 protease as made by corresponding peptides[23] from acyclic inhibitors of HIV-1 protease is shown in Figure 1. The structure for a potential HIV-1 protease inhibitor (Figure 1, white, **9g**) incorporating macrocycle **4** was derived from the coordinates of a previously published HIV-1 protease bound macrocyclic inhibitor.[21b] This was energy minimized with template forcing[24] onto the structure[23] of a heptapeptide inhibitor, the substrate analogue JG365, bound to HIV-1 protease (Figure 1, yellow). A total of twelve carbon and nitrogen atoms of

the peptide backbone of JG365 between the amide nitrogen of serine and the hydroxyl-bearing carbon of the hydroxyethylamine transition state isostere (P4 to P1) were used as the template. The template forcing led to an energy minimized structure for the proposed macrocyclic inhibitor that was 4.0 kcal $mol^{-1}$ lower in energy than the non-template forced energy minimized structure (144.1 vs 140.0 kcal $mol^{-1}$), clearly indicating this to be an accessible conformation.

Superimposition of the template-forced structure (white) onto JG365 (yellow) using the same atoms reveals excellent structural mimicry (RMSD of 0.22 Å). The macrocycle fits neatly into the active site of HIV-1 protease, with carbonyl oxygens and amide nitrogens located in the appropriate positions to make the same hydrogen-bonding interactions as JG365 does with the enzyme. The serine and the amine nitrogen at the N-terminus of the cycle are also positioned and orientated appropiately to make the same H-bonds as the corresponding region of JG365. There is also ample space in the enzyme to accommodate the flexible norleucine side chain at P2, the aliphatic linker at P3, and the tyrosinyl aromatic ring at P1.

On the C-terminal side of the macrocycle, the hydroxyethylamine isotere is identical to that in JG365. The *N*-alkylbenzene sulfonamide, while occupying different space to the Pro-Ile-Val portion of JG365, occupies the same space as this same moiety in other inhibitors of HIV-1 protease.[21] In summary, the macrocyclic amino acid component of the inhibitors **9a**−**k** not only mimics the H-bonding and space-filling properties of the P3 to P1 section of JG365 but also, importantly, is an effective template or scaffold for N- and C-terminal appendages at P4, P1′, and P2′ and does not interfere with their binding in the enzyme.

Each of the three macrocycles **3**−**5** is formed by linking together P1 and P3 side chains derived from amino acids. Each cycle is significantly constrained by the presence of two trans amide bonds and the aromatic side chain of tyrosine. As a result, the amide backbone exists in the extended $\beta$-strand rather than folding into a turn conformation. Elsewhere, we show that the 17-membered constrained macrocycle is flexible enough to permit rotation of the component aromatic ring, whereas the 16-membered ring with one less methylene causes restricted rotation of the aromatic ring (Reid et al., submitted for publication). Cycle **3** contains the same valine side chain at P2 as do cycles **1** and **2**, thereby allowing a close comparison. On the other hand, compounds **4** and **5** do not contain branched side chains at P2 like **3** but rather have more flexible unbranched aliphatic substituents.

The rationale for these choices stems from the knowledge that viral resistance can originate from mutations in the protease that directly or indirectly influence the sizes of the "pockets" or indentations in the lining of the substrate-binding groove of HIV-1 protease.[25−27] Regardless of where mutations occur within the protease structure, they can potentially reduce the complementary fit between enzyme and inhibitor/substrate. The norleucine and propyl serine side chains in **4** and **5**, respectively, were considered to offer more flexibility in how they could bind at S2 in response to enzyme mutations that alter this binding site. The linear side chains are theoretically able to stretch out into a bigger enzyme pocket or curl up to accommodate a smaller binding site. We envisage that this, and other limited flexibility in the cycle, may be key features in enabling inhibitors to "breathe" and thereby still maintain potency and binding to resistant mutants by varying their shape in response to changes in the substrate-binding pocket. In this paper, we restrict our assessment of these templates to whether they confer protease inhibition and to what extent they are lipophilic enough to confer antiviral properties in cell culture. Detailed studies on the viral resistance of similar complexes will be reported subsequently.

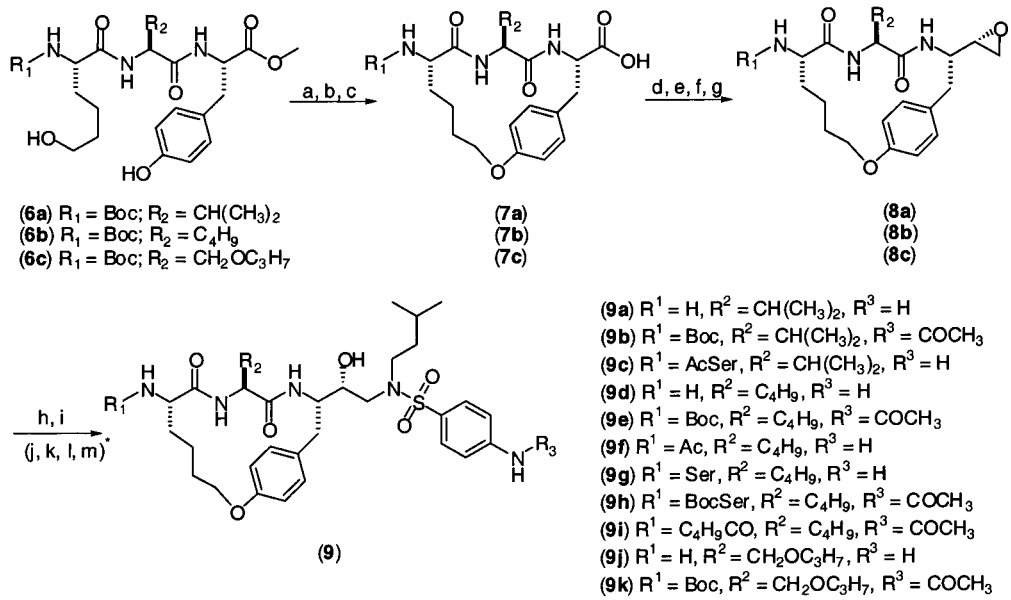
**Synthesis.** The macrocycles **3**−**5**, designed to mimic P1 to P3 residues of peptide substrates for proteases, were created from tripeptide **6** assembled using standard solution phase peptide coupling procedures. The P3 residue, (2*S*)-amino-6-hydroxyhexanoic acid, was derived from diazotization of *N*-Boc-L-lysine with sodium nitroprusside under basic aqueous conditions (pH 9).[28] Macrocyclization was effected by stepwise halogenation of the primary hydroxyl group of **6** (OH→Br→I), followed by nucleophilic displacement of halide by the phenol of the tyrosine at P1. Hydrolysis of the C-terminal ester provided the macrocyclic acids **7a**−**c** (Scheme 1).

To investigate the utility of the macrocyclic amino acids **3**−**5**, we decided to test them as inhibitors of HIV-1 protease. Like the tripeptides they mimic, these compounds are not very potent inhibitors of this enzyme in their own rite ($IC_{50}$ 5−50 $\mu$M). We therefore created a limited set of derivatives as putative inhibitors featuring one of the cycles **3**−**5**, a hydroxyethylamine transition state isostere, a series of *N*-alkyl benzenesulfonamides at the C-terminus, and either an amine, an amide, or an amino acid at the N-terminus.

Conversion of the acids **7a**−**c** to their mixed anhydrides with isobutylchloroformate and subsequent addition of diazomethane allowed one carbon homologation at the C-terminus. The resulting diazomethyl ketones were treated with 1 equiv of dry HBr, and the resulting bromomethyl ketones were reduced with sodium borohydride (d.e. > 50%). The 13(*R*)-diastereomers (major product) were separated by reverse phase high performance liquid chromatography (rpHPLC),[29] before conversion to the key epoxides **8a**−**c**. Ring opening of the epoxide with isoamylamine affected formation of the hydroxyethylamine transition state isostere, which was condensed with 4-acetamidobenzenesulfonyl chloride. Various combinations of deprotection (N-terminal deprotection with TFA or removal of both the N-terminal Boc group and the phenyl acetamido group with 2 M HCl) and N-terminal coupling with amino acids afforded the inhibitors **9a**−**k**. Inhibitors **9a**−**c** employ macrocycle **3** containing the small branched substituent of valine at P2 within the 17-membered cycle formed through connection of amino acid side chain substituents at P1 and P3. Inhibitors **9d**−**i** contain macrocycle **4** with norleucine at P2, while **9j**,**k** have macrocycle **5** and propyl serine at P2.

**Protease Inhibition.** Table 1 reports the in vitro inhibitor potencies against HIV-1 protease for a range of compounds (**9a**−**k**) containing macrocyclic amino acids **3**−**5**. All of the compounds have Ki < 50 nM under the assay conditions employed (pH 6.5, $I = 0.1$ M[30,31]).

**Scheme 1**[a]




[a] Reagents: (a) TPP, $CBr_4$. (b) (1) NaI, acetone, heat; (2) $K_2CO_3$, DMF. (c) NaOH, MeOH. (d) IBCF, THF, $CH_2N_2$. (e) HBr, THF. (f) $NaBH_4$, EtOH. (g) NaOMe, THF. (h) Isoamylamine, EtOH. (i) Benzenesulfonyl chloride, THF:$H_2O$. (j) 2 M HCl, MeOH, reflux. (k) TFA. (l) N-Boc-Serine, HBTU, DIPEA, DMF. (m) Pentanoic acid, HBTU, DIPEA, DMF. * Where necessary.

In the series **9a**, **9d**, and **9j**, the respective Ki values 0.9, 0.3, and 45 nM indicate that there is little difference between the isopropyl and the *n*-butyl side chains at P2, although the longer propyl methyl ether side chain of **9j** is less favored. The purpose of this latter side chain is related to its capacity to fit the S2 pocket in mutant proteases, and thus, **9j** may still be important in resistance studies not reported here.

At the N-terminus, the unsubstituted free amino group is surprisingly better (Table 1) than when capped by acetyl, Boc, or Ser (e.g., **9a** > **9b**, **9f**, and **9g**) although not as good as AcSer (**9c**). We had expected that the acetyl, serine, and acetylserine N-termini would have contributed additional hydrogen-bonding donors/acceptors, as would the serine side chain, that are known in peptides to interact with the enzyme (see P4−P3 region in Figure 1). However, a possible explanation for the observed higher potency for the free amino N-terminus is that the latter is protonated ($RNH_3^+$) under the assay conditions and in the enzyme and therefore may be able to H-bond more extensively with the enzyme. We have previously reported that amines are indeed protonated within the active site groove of HIV-1 protease.[17] The Boc group does not appear to be suited at P4 (**9e**, **9h**, and **9k**) since it does not increase potency of the inhibitors, and this is likely because it is exposed to solvent and because the *tert*-butyl substitutent does not fit P4 well. The N-terminal substituent at P4 in **9i** was added to increase lipophilicity for enhanced cell uptake and demonstrates a 2-fold improvement in activity against HIV-infected cells as compared to **9f**.

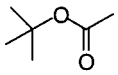
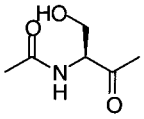
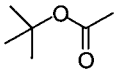
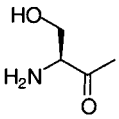
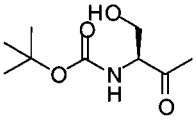
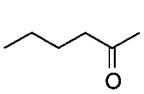
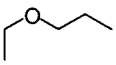
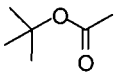
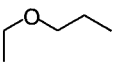
At the C-terminus, the *N*-alkyl benezenesulfonamide substituent is able to effectively occupy P1′ and P2′ and confer protease activity as well as antiviral activity, as shown elsewhere by VX478[32] and our own compounds.[21] Acetylation of the p-amino substituent on the benzenesulfonamide (**9b**, **9e**, **9h**, and **9k**) leads to 2−10-fold reduced inhibitory potency (Table 1).

**Antiviral Activity.** None of the compounds possessing free amino termini (**9a**, **9d**, and **9j**), which are likely protonated under the assay conditions and thus charged and highly water soluble, show antiviral activity at submicromolar concentrations against HIV-1-infected MT2 cells (Table 1). These compounds have calculated[33] LogD values at pH 6.5 of 1.0, 1.7, and 0.5, respectively. Similarly, compounds with Ser or AcSer added at P4 (**9c** and **9g**) are too polar ($LogD_{6.5} = 0.4, 0.6$) to penetrate membranes and did not inhibit HIV replication in cell culture at 10 $\mu$M, although some activity was seen when an additional Boc group was added to the serine to increase lipophilicity (**9h**, $LogD_{6.5} = 3.0$).

When the $LogD_{6.5}$ increases to 1.5−3.6, some activity against HIV-1 is observed (**9d**, **9f**, **9h**, and **9k**) at inhibitor concentrations of 500 nM−5 $\mu$M. When the lipophilicity is further increased to $LogD_{6.5} = 4-5$ (e.g., **9b**, **9e**, and **9i**) more substantial antiviral activity was observed at inhibitor concentrations of 100−600 nM, with values for the selective index (SI) lying between 17 and 40 (Table 1). The selectivity index (defined in the Experimental Section) is a comparitive measure of selective toxicity for the virus over MT2 cells. While overall these compounds are not as active against HIV-1 as clinically proven inhibitors (e.g., ritonavir and amprenavir), Table 1 shows that the SI value is roughly proportional to $LogD_{6.5}$ ($r^2 = 0.56$, not shown) recognizing that some of the compounds are inactive. This indicates that LogD has some value in guiding further inhibitor optimization.

Overall, the results indicate that the macrocyclic amino acids **3**−**5** can indeed function as effective templates for the creation of potent protease inhibitors with excellent antiviral properties. Although they consist of several H-bond donors and acceptors that contribute to the water solubility of the inhibitors, compounds containing the cycles are still membrane

**Table 1.**

| Compound | $R^1$ | $R^2$ | $R^3$ | Log $D_{6.5}$ | $K_i$ HIVPR (nM) | $IC_{50}$ HIV (nM) | SI |
|---|---|---|---|---|---|---|---|
| **(9a)** | H |  | H | 1.0 | 0.9 | >10000 | 4.0 |
| **(9b)** |  |  |  | 4.1 | 2.0 | 177 | 40 |
| **(9c)** |  |  | H | 0.4 | 0.34 | >10000 | 4.0 |
| **(9d)** | H |  | H | 1.7 | 0.31 | 6850 | 1.3 |
| **(9e)** |  |  |  | 4.8 | 7.5 | 390 | 23 |
| **(9f)** |  |  | H | 3.0 | 2.8 | 910 | 8.1 |
| **(9g)** |  |  | H | 0.6 | 3.0 | >10000 | 4.0 |
| **(9h)** |  |  |  | 3.0 | 12 | 2550 | 3.7 |
| **(9i)** |  |  |  | 4.6 | ND | 580 | 17 |
| **(9j)** | H |  | H | 0.5 | 45 | >10000 | 4.0 |
| **(9k)** |  |  |  | 3.6 | 45 | 825 | 6.8 |
| | **Amprenavir** | | | | | 30 | 125 |

permeable and bioavailable depending upon what is appended to the cyclic template.

## Discussion

A significant problem with current antiviral treatments based on inhibition of HIV-1 protease is the rapid onset of viral resistance and the limited number of options available after initial failure with mono or combination drug therapy. Reported inhibitors of HIV-1 protease are in general flexible molecules that are severely restricted in their mode of high affinity binding. They must bind to the protease in only a single conformation and rely on a combination of interactions with the enzyme for high affinity. When enzyme mutations occur that have an impact on the shape of the active site, significant attenuation of inhibitor activity may result, without comparable retardation of substrate processing.

Our approach to peptidomimetic inhibitors, which very closely mimic the structures of protease substrates, is based on the hypothesis that drug resistance will be more difficult for substrate mimics since the protease must still recognize its substrates to form a functional and infectious virion. This is in contrast to current

therapeutics that have pronounced structural differences from substrates. Mutations in response to substrate analogues will obviously lead to an inability to process the corresponding substrates and thus stop viral replication. Our first step toward the goal of low resistance inhibitors that closely resemble substrates has been to develop macrocyclic amino acids for incorporation to protease inhibitors that consequently maintain a substrate-like composition. Unlike peptide substrates, the macrocyclic tripeptide analogues have been shown to be resistant to hydrolytic/proteolytic degradation[17,21] and have much higher affinities for HIV-1 protease than substrates.[15−21] Some analogues (e.g., **9b**, **9d**−**f**, **9i**, and **9k**) are cell permeable (unlike substrates) and, except for **9d**, also exhibit potent antiviral activity being active at sub-micromolar concentrations in cell culture.[21]

The results in Table 1 demonstrate that macrocyclic amino acids such as **3**−**5** can successfully be used as templates to create potent inhibitors of both HIV-1 protease and replication of HIV-1 in cell culture. The results for inhibition of HIV-1 protease are consistent with the macrocycles being pre-organized in protease-binding $\beta$-strand mimetics as demonstrated by modeling in Figure 1 and by crystallographic studies of analogues incorporating similar cycles **1** and **2** bound to HIV-1 protease.[20] Both linear and branched substituents are tolerated at P2, and this suggests that the strategy of making the P2 substituent more flexible to accommodate variable sizes of S2 in mutant proteases may be feasible. Additional flexibility within a reasonably constrained macrocycle comes from the pentamethylene linker between P1 and P3. The free amino terminus is best masked by lipophilic substituents to prevent its protonation and polarization and to enhance its membrane permeability over water solubility. The C-terminal *N*-alkyl benzensulfonamide substituent is effective at P1′ and P2′ in conferring high protease-binding affinity to the macrocycles.

Inhibitory potency against HIV-1 protease correlates roughly with the number of H-bonding and pocket-filling substituents in the inhibitor. Antiviral potency requires potent protease inhibition but also correlates roughly with the degree of hydrophobicity for compounds of similar protease inhibitory potency. We are currently investigating both the capacity of HIV-1 to develop resistance to these and similar macrocyclic compounds in cell culture as well as the activities of such substrate mimetics against drug-resistant mutant proteases. Preliminary data from cellular studies supports the concept that certain substrate analogues, which are constrained to mimic a $\beta$-strand but have some limited elasticity in the projection of its side chains, are less sensitive to resistance than known inhibitors of this enzyme.

## Experimental Methods

**Abbreviations.** Ac = acetyl; Boc = *tert*-butoxycarbonyl; DIPEA = diisopropylamine; DMF = dimethylformamide; BOP = (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate; FCC = flash column chromatography; HBTU = [(benzotriazolyl)oxy]-*N*′,*N*′,*N*′,*N*′-tatramethyluronium hexafluorophosphate; IBCF = isobutylchloroformate; Nle = norleucine; Ser = serine; TFA = trifluoroacetic acid; THF = tetrahydrofuran; Tyr = tyrosine; Val = valine.

**General Methods.** $^1$H nuclear magnetic resonance (NMR) spectra were recorded on either a Bruker ARX 500 MHz or a Varian 300 MHz NMR spectrometer. Proton assignments were made using 2D COSY and TOCSY experimental data. Preparative scale rpHPLC separations were performed on a Waters Delta-Pak C18 40 × 100 mm cartridges (100 Å) using gradient mixtures of water/0.1% TFA and water (10%)/acetonitrile (90%)/0.1% TFA. Pure fractions were lyophilized under high vacuum (0.1 mm Hg) and were determined free from solvent/TFA by $^1$H and $^{13}$C NMR. Analytical scale rpHPLC was performed on a Phenomenex Luna 5$\mu$ C18(2) 250 × 4.6 mm column. Accurate mass determinations were performed on an API QSTAR mass spectrometer using electron impact ionization. Water−octanol partition coefficients (Log D) were calculated using PALLAS prolog D 2.1. Molecular modeling was performed on an SGI Octane R12000, with minimization calculations performed with the cff91 force field using the Discover Module within InsightII.[24b] Volumes given as milliliters per millimole in general procedures refer to the limiting reagent.

**Enzyme Assay.** Synthetic HIV-1 PR (SF2 isolate)[34] 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$^{-1}$) and then refolded for 60 min in buffer A (20 mM phosphate, pH 7.0, 20% v/v glycerol, 10 mg mL$^{-1}$ bovine serum albumin). At time zero, the buffer A/protease solution was added to an excess of the fluorogenic substrate Abz-NF-6*,[30] 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 $K_i$ value was then calculated from either Dixon plots of 1/v vs [s] or Henderson plots of [I]/{1-(Vi/Vo)} vs Vo/Vi in cases where the $K_i$ value was found to approach the concentration of the enzyme. Under these assay conditions, the cyclic compounds were confirmed to be competitive inhibitors.

**Virology.** Anti-HIV activity was assessed in acutely infected MT2 cells by measuring the extent to which the compounds reduced HIV specific cytopathic effects at noncytotoxic concentrations.[35] The medium used for passage and maintenance of MT2 cells was RF10, an RPMI-1640-based medium.[36] Inhibition of HIV-1 replication was confirmed by measurement of virion-associated reverse transcriptase (RT) activity in cell supernatants.[34] All tests were performed in 48 well tissue culture plates (Costar). Each drug concentration and all controls were tested in duplicate. Virus inoculum was added immediately after the addition of the drugs to the cells.

MT2 cells were counted and resuspended at a concentration of 5 × 10$^4$ per 0.25 mL of RF10 medium. Compounds at twice the final concentration required in the test were prepared in RF10 medium from a 20 mM stock of the drugs prepared in 60% v/v ethanol. A total of 0.5 mL of each dilution was pipetted into duplicate test and cytotoxicity control wells, followed by the addition of 0.25 mL of cell suspension to all wells. A total of 0.5 mL of RF10 medium was added to the virus-only controls, 0.75 mL to the cell-only controls, and 0.25 mL of HIV-1 strain to the cytotoxicity controls. A volume of 0.25 mL of strain #237288[36] diluted in the appropriate medium to contain 200 TCID50 was then immediately added to virus-only control wells and the test wells. The plates were then incubated at 37 °C in 5% $CO_2$. All wells in the test were examined by light microscopy and harvested after 6−7 days incubation, at which time viable cell numbers were determined in cell-only and cytotoxicity control wells using trypan blue dye exclusion counting.

The cell-only, virus-only, and test cultures were harvested by removing 0.8 mL of supernatant from each well to separate microtubes. The virus present in each supernate was then precipitated using poly(ethylene glycol) and assayed for virion-associated RT activity expressed as counts per minute (cpm) on a liquid scintillation counter as previously described.[35] The percentage (%) inhibition was calculated by expressing the RT activity at each dilution as a percent of the cpm in virus-only controls subtracted from 100. The SI, which enables direct comparison of the antiviral activity of the test compounds, was calculated using the formula SI = $CC_{10}/IC_{50}$, where $CC_{10}$ was the concentration of drug permitting at least 90% viability of

cells in the absence of infecting HIV-1 and $IC_{50}$ was the concentration of drug producing a 50% reduction in virion-associated RT activity in HIV-1-infected MT2.

**General Synthetic Procedures. *N*-Boc Deprotection (Procedure A).** The Boc-protected amine (1 equiv) was stirred for 1 h in TFA (1 mL/mmol) at room temperature. The TFA was removed under reduced pressure, and the residue was triturated with diethyl ether. The resulting semisolid was then used directly in the next coupling without further purification.

**Peptide Coupling (Procedure B).** To a stirred solution of the acid (1 equiv) in THF (2 mL/mmol) at room temperature was added HBTU (1 equiv) and DIPEA (2 equiv). After 5 min, a solution of the amine (1 equiv) and DIPEA (2 equiv) in THF (1 mL/mmol) was added, and stirring was continued for a further 30 min. After this time, the THF was removed under reduced pressure, and the residue was taken up into ethyl acetate (10 mL/mmol) and washed with equal portions of 1 M HCl, $NaHCO_3$, and brine. The organic layer was dried over magnesium sulfate, and the solvent was removed under reduced pressure to yield the peptide.

**Bromination of Alcohols (Procedure C).** Triphenylphosphine (1.5 equiv) was added to a cold solution (0 °C) of the alcohol (1 equiv) in THF (10 mL/mmol). Once all of the phosphine had dissolved, tetrabromomethane (1.2 equiv) was added in small portions over 10 min. After the bromomethane was completely added, the solution was stirred for a further 30 min at room temperature, and then, the solvent was removed under reduced pressure. The resulting semisolid was purified by column chromatography on silica gel.

**Macrocyclization (Procedure D).** The bromide (1 equiv) was converted to the iodide by refluxing with sodium iodide (1.5 equiv) in acetone (10 mL/mmol) for 4 h. The solution was cooled to room temperature, and the precipitate of sodium bromide was removed by filtration. The filtrate was reduced to dryness, and the iodide was dissolved in DMF (20 mL/mmol). Finely ground anhydrous potassium carbonate (5 equiv) was then added, and the resulting suspension was stirred overnight. The solvent volume was reduced under vacuum (~5 mL), and the residue was partitioned between ethyl acetate and water. The aqueous layer was discarded, and the organic layer was washed successively with dilute sodium thiosulfate solution, 2 M HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over magnesium sulfate, and the solvent was removed under reduced pressure to yield the crude macrocycle, which can be purified by flash column chromatography on silica gel.

**Ester Hydrolysis (Procedure E).** A solution of 2 M NaOH (2.5 equiv) was added to a solution of the ester (1 equiv) in methanol (5 mL/mmol) at 0 °C, and the solution was stirred for 1 h. The solution was neutralized with citric acid, and the methanol was removed under reduced pressure. Water (5 mL/mmol) was added, and the acid was extracted into ethyl acetate. The organic phase was dried over magnesium sulfate, and the solvent was removed under reduced pressure to yield the crude acid, which could be purified by rpHPLC.

**Conversion of Acids to Diazomethylketonse (Procedure F).** *N*-Methylmorpholine (1.5 equiv) was added to a solution of the acid (1 equiv) in THF (20 mL/mmol) at −15 °C. *Iso*-butyl chloroformate (1.25 equiv) was added, and the solution was stirred for 15 min at −15 °C before an ethereal solution of diazomethane (~20 equiv) was added. The reaction was stirred for a further 30 min before a stream of argon was introduced to remove the excess diazomethane. Ethyl actetate was added, and the solution was washed with brine and dried over magnesium sulfate. Removal of the solvent under reduced pressure yielded the diazomethyl ketone as a pale yellow solid.

**Conversion of Diazomethyl Ketones to Bromomethyl Ketones (Procedure G).** A solution of dry HBr in ethyl acetate (~0.2 M) was added in small portions to the diazomethyl ketone (1 equiv) in THF (25 mL/mmol) at −10 °C. The reaction was monitored by TLC and EIMS, and when complete, ethyl acetate (20 mL) was added, and the solution was washed with brine. The organic layer was dried over magnesium sulfate, and the solvent was removed under reduced pressure to yield the bromomethyl ketone.

**Conversion of Bromomethyl Ketones to Bromohydrins (Procedure H).** A solution of the bromomethyl ketone (1 equiv) in ethanol (50 mL/mmol) was cooled to −15 °C, and then, sodium borohydride (1.2 equiv) was added. After the solution was stirred for 5 min, glacial acetic acid was added (0.2 mL/mmol), and the solvent was removed under reduced pressure. The residue was dissolved in DMF, and the diasterisomers were separated and purified by rpHPLC.

**Conversion of Bromohydrins to Epoxides (Procedure I).** A solution of sodium methoxide (2 equiv) and bromohydrin (1 equiv) in tetrahydrofuran (25 mL/mmol) and methanol (75 mL/mmol) was stirred at room temperature for 15 min, and then, water (10 mL/mmol) was added. The solution was concentrated under reduced pressure, and the precipitate of the epoxide was collected on a frit. The precipitate was washed with 30% aqueous methanol and then dried under vacuum.

**Condensation of Amines with Epoxides (Procedure J).** A solution of the epoxide (1 equiv), the required amine (2−10 equiv), and DIPEA (2 equiv) in ethanol (50 mL/mmol) was refluxed overnight. The solvent and, where possible, the excess amine were removed under reduced pressure to provide the condensed product in all cases as a solid, which was purified where necessary by rpHPLC.

**Condensation of Amines with Sulfonyl Chlorides (Procedure K).** The sulfonyl chloride (3 equiv) was added to a solution of the amine (1 equiv) in 4:1 THF:water (50 mL/mmol). DIPEA (4 equiv) was added, and the solution was stirred at room temperature for 30 min. The organics were extracted into ethyl acetate, and the solvent was removed under reduced pressure. The resulting sulfonamides were purified by rpHPLC where necessary.

**(2*S*)-*tert*-Butoxycarbonylamino-6-hydroxyhexanoic Acid.** To a suspension of *N*-Boc-L-lysine (31.8 g, 129.3 mmol) in water (500 mL) at 60 °C was added 4 M NaOH (50 mL). Sodium nitroprusside (58.8 g, 197.3 mmol) was then added in portions over 1 h, while maintaining the pH at ~9.5 by the addition of a further 50 mL of 4 M NaOH as required. After the nitroprusside was completely added, the resulting red suspension was stirred for a further 6 h at 60 °C. The resulting suspension was cooled to ~10 °C, and 1 M HCl (~500 mL) was added until a pH of ~1 was achieved. The resulting solution was extracted with ethyl acetate (3 × 500 mL), the combined organic fractions were dried over magnesium sulfate, and solvent was removed under reduced pressure. The crude product was contaminated with ~30% elimination products, which were removed by flash column chromatography (silica gel, 70% EtOAc, 29% petroleum spirit, 1% HOAc) to provide (2*S*)-*tert*-butoxycarbonylamino-6-hydroxyhexanoic acid (14.6 g, 45.7%) as a white solid. $^1$H NMR ($CDCl_3$, 300 MHz): 5.21 (br d, 1H); 4.28 (m, 1H); 3.66 (t (6.0 Hz), 2H); 1.41 (s, 9H); 1.90 to 1.37 (m, 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz): 175.3, 156.5, 80.3, 62.1, 53.7, 32.4, 32.3, 28.5, 22.0.

**(2*S*)-[(2*S*)-((2*S*)-*tert*-Butoxycarbonylamino-6-hydroxyhexanoylamino)-3-methyl-butyrylamino]-3-(4-hydroxyphenyl)propionic Acid Methyl Ester (6a).** This tripeptide was synthesized sequentially from tyrosine, valine, and (2*S*)-*tert*-butoxycarbonylamino-6-hydroxyhexanoic acid using the standard Boc deprotection (procedure A) and coupling (procedure B) procedures above. $^1$H NMR ($CDCl_3$, 300 MHz): 7.09, (d (8.0 Hz), 1H); 6.89 (d (8.1 Hz), 2H); 6.69 (d (7.3 Hz), 2H); 5.40 (br d, 1H); 4.80 (m, 1H); 4.25 (m, 1H); 4.07 (m, 1H); 3.61 (s, 3H); 3.57 (t (5.5 Hz), 2H); 2.98 (d (6.3 Hz), 2H); 2.1 to 1.3 (m, 7H); 1.40 (s, 9H); 0.85 (apparent t (7.1 Hz), 6H). $^{13}$C NMR ($CDCl_3$, 75 MHz): 172.4, 171.7, 171.0, 155.7, 155.6, 130.2, 126.6, 115.5, 80.1, 69.3, 58.2, 54.2, 53.4, 52.2, 36.9, 33.3, 32.1, 31.1, 28.2, 26.4, 19.0, 18.0.

**(7*S*)-*tert*-Butoxycarbonylamino-(10*S*)-isopropyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15-(19),16-triene-(13*S*)-carboxylic Acid (7a).** Tripeptide **6a** (2.7 g, 5.2 mmol) was brominated (procedure C, FCC, 60%), followed by cyclization (procedure D, FCC, 61%), and the resulting macrocyclic ester was hydrolyzed (procedure E, 81%)

to afford the title acid. $^1$H NMR ($CD_3OD$, 300 MHz): 6.86 (d (7.6 Hz), 2H); 6.56 (d (7.8 Hz), 2H); 4.54 (m, 1H); 3.92 (m, 1H); 3.82 (m, 1H); 3.64 (m, 1H); 3.08 (m, 2H); 2.40 (t (12.2 Hz), 1H); 1.8 to 1.3 (m, 5H); 1.35 (s, 9H); 1.01 (m, 2H); 0.72 (d (6.8 Hz), 3H); 0.63 (d (6.8 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 75 MHz): 174.2, 174.1, 172.1, 159.2, 158.2, 134.3, 131.5 (br), 118.1 (br), 69.5, 68.5, 59.7, 55.1, 54.8, 38.8, 34.1, 33.1, 31.4, 30.6, 29.3, 20.2, 19.1.

**((10*S*)-Isopropyl-(13*S*)-[(*R*)-oxiranyl]-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-(7*S*)-yl)-carbamic Acid *tert*-Butyl Ester (8a).** Macrocyclic acid **7a** (460 mg, 0.93 mmol) was converted to the diazomethyl ketone (procedure F, 85%), which was subsequently converted to the bromomethyl ketone (procedure G, 69%), reduced (procedure H, rpHPLC (diastereomeric separation), 36%), and finally converted to the title epoxide (procedure I, 85%). $^1$H NMR ($CDCl_3$, 300 MHz): 7.00 (d (8.2 Hz), 2H); 6.79 (d (8.7 Hz), 2H); 6.26 (d (8.4 Hz), 1H); 5.42 (d (9.5 Hz), 1H); 5.02 (d (8.3 Hz), 1H); 4.28 (m, 2H); 4.11 (m, 1H); 3.90 (m, 2H); 3.08 (m, 2H); 2.81 (m, 2H); 2.42 (t (12.6 Hz), 1H); 1.9 to 1.1 (m, 7H); 1.38 (s, 9H); 0.85 (d (6.9 Hz), 3H); 0.82 (d (7.02 Hz), 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz): 171.8, 170.1, 157.3, 155.9, 130.1, 128.6, 116.6, 79.8, 67.8, 58.2, 54.2, 53.6, 50.9, 45.7, 35.4, 32.0, 31.9, 29.3, 28.3, 22.1, 18.9, 18.0.

**((13*S*)-{2-[(4-Acetylamino-benzenesulfonyl)-(3-methyl-butyl)-amino]-(1*R*)-hydroxy-ethyl}-(10*S*)-isopropyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15-(19),16-trien-(7*S*)-yl)-carbamic Acid *tert*-Butyl Ester (9b).** Epoxide **8a** (46 mg, 94 $\mu$mol) was condensed with isoamylamine (procedure J, 94%), followed by addition of 4-acetamidobenzenesulfonyl chloride (procedure K, rpHPLC, 44%) to afford the title compound. $^1$H NMR ($CD_3OD$, 500 MHz): 7.76, (s, 4H); 7.01 (br d, 2H); 6.70 (br d, 2H); 4.25 (m, 1H); 4.08 (m, 3H); 3.88 (m, 1H); 3.75 (m, 1H); 3.46 (m, 1H); 3.37 (m, 1H); 3.18 (m, 2H); 2.98 (m, 1H); 2.38 (t (12.5 Hz), 1H); 2.14 (s, 3H); 1.89 (m, 1H); 1.72 (m, 1H); 1.63 (m, 1H); 1.50 (m, 5H); 1.40 (s, 9H); 1.18 (m, 2H); 0.88 (m, 9H); 0.76 (d (6.5 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 173.3, 172.3, 171.6, 158.6, 158.3, 144.2, 132.2, 132.1, 130.0 (br), 129.4, 120.5, 117.7 (br), 74.3, 69.0, 58.6, 55.5, 55.3, 55.2, 53.1, 38.3, 36.4, 33.9, 33.0, 30.7, 28.7, 27.2, 24.0, 23.5, 23.0, 22.8, 20.1, 17.9. HRMS (EI) *m*/*z* calcd for $C_{39}H_{60}N_5O_9S$ ($MH^+$), 774.4107; found, 774.4106. Analytical rpHPLC: isocratic 30A:70B rt = 6.5 min; gradient 100A to 100B 20 min rt = 23.4 min.

**4-Amino-*N*-[2-((7*S*)-amino-(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)-(2*R*)-hydroxy-ethyl]-*N*-(3-methyl-butyl)-benzenesulfonamide (9a).** Amide **9b** (20.0 mg, 26 $\mu$mol) was refluxed for 2 h in methanol (1 mL) with 3 drops of 2 M HCl. After the solution was cooled to room temperature, water (1 mL) was added, and the solution was purified by rpHPLC to provide the amine as a white powder (9.7 mg, 60%) after lyophilization. $^1$H NMR ($CD_3OD$, 500 MHz): 7.50, (d (9.8 Hz, 2H); 7.10 (br s, 2H); 6.72 (br s, 2H); 6.67 (d (9.8 Hz), 2H); 4.34 (m, 1H); 1.19 (d (5.7 Hz), 1H); 4.07 (m, 2H); 3.89 (m, 1H); 3.73 (m, 1H); 3.42 (dd (14.4 Hz, 4.7 Hz), 1H); 3.32 (obscured, 1H); 3.21 (dd (13.8 Hz, 3.38 Hz), 1H); 3.07 (m, 1H); 2.87 (dd (14.7 Hz, 8.4 Hz), 1H); 2.38 (t (12.6 Hz), 1H); 1.98 (m, 1H); 1.77 (m, 2H); 1.64 (m, 2H); 1.49 (m, 2H); 1.38 (m, 1H); 1.19 (m, 2H); 0.91 (d (6.8 Hz), 3H); 0.86 (d (6.5 Hz), 6H); 0.77 (d (6.8 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 171.7, 171.4, 157.3, 154.3, 132.5, 130.4 (br), 130.3, 126.4, 116.5 (br), 114.5, 74.2, 67.8, 58.6, 55.4, 54.2, 53.1, 38.3, 36.1, 33.7, 32.4, 29.8, 27.1, 23.0, 22.8, 20.9, 20.0, 17.6. HRMS (EI) *m*/*z* calcd for $C_{32}H_{50}N_5O_9S$ ($MH^+$), 632.3477; found, 632.3473. Analytical rpHPLC: isocratic 50A:50B rt = 3.5 min; gradient 100A to 100B 20 min rt = 17.4 min.

**(2*S*)-Acetylamino-*N*-((13*S*)-{2-[(4-amino-benzenesulfonyl)-(3-methyl-butyl)amino]-(1*R*)-hydroxy-ethyl}-(10*S*)-isopropyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]-nonadeca-1(18),15(19),16-trien-(7*S*)-yl)-3-hydroxy-propionamide (9c).** Amine **9a** (3.94 mg, 6.2 $\mu$mol) was coupled with *N*-Boc-L-serine (procedure B), subsequently deprotected (procedure A, rpHPLC, 50% over two steps), and then coupled with acetic acid (procedure B, rpHPLC, 62%) to afford the title amide. $^1$H NMR ($CD_3OD$, 500 MHz): 7.48 (d (6.8 Hz), 2H); 7.06 (d (7.4 Hz), 2H); 6.75 (d (7.8 Hz), 2H); 6.69 (d (6.83 Hz), 2H); 4.40 (t (5.5), 1H); 4.26 (m, 2H); 4.09 (m, 3H); 3.70 (m, 3H); 3.20 (m, 3H); 3.10 (m, 1H); 2.92 (dd (14.5 Hz, 8.6 Hz), 1H); 2.39 (t (12.8 Hz), 1H); 2.00 (s, 3H); 1.90 (m, 1H); 1.72 (m, 1H); 1.55 to 1.35 (m, 7H); 1.19 (m, 2H); 0.87 (d (6.5 Hz), 6H); 0.86 (d (6.8 Hz), 3H); 0.75 (d (6.8 Hz), 3H). HRMS (EI) *m*/*z* calcd for $C_{37}H_{57}N_6O_9S$ ($MH^+$), 761.3903; found, 761.3892. Analytical rpHPLC: isocratic 60A:40B rt = 5.9 min; gradient 100A to 100B 20 min rt = 18.6 min.

**(2*S*)-[(2*S*)-((2*S*)-*tert*-Butoxycarbonylamino-6-hydroxy-hexanoylamino)hexanoylamino]-3-(4-hydroxy-phenyl)-propionic Acid Methyl Ester (6b).** This tripeptide was synthesized sequentially from (2*S*)-*tert*-butoxycarbonylamino-6-hydroxyhexanoic acid, norleucine, and tyrosine using the standard Boc deprotection (procedure A) and coupling (procedure B) procedures above. $^1$H NMR ($CDCl_3$, 300 MHz): 7.05 (m, 2H); 6.88 (d (8.5 Hz), 2H); 6.68 (d (8.5 Hz), 2H); 5.39 (br d, 1H); 4.72 (m, 1H); 4.39 (m, 1H); 4.08 (m, 1H); 3.67 (s, 3H); 3.51 (t (4.8 Hz), 1H); 3.1 to 2.6 (m, 3H); 1.8 to 1.1 (m, 12H); 1.38 (s, 9H); 0.80 (t (7.6 Hz), 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz): 172.6, 171.8, 171.6, 155.9, 155.5, 130.3, 126.6, 115.6, 80.2, 62.0, 54.3, 53.4, 53.2, 52.4, 38.6, 36.8, 31.9, 31.7, 28.3, 27.5, 22.3, 21.8, 13.8.

**(7*S*)-*tert*-Butoxycarbonylamino-(10*S*)-butyl-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-(13*S*)-carboxylic Acid (7b).** Tripeptide **6b** (1.17 g, 2.2 mmol) was brominated (procedure C, FCC, 64%), followed by cyclization (procedure D, FCC, 72%), and the resulting macrocyclic ester was hydrolyzed (procedure E, rpHPLC, 55%) to afford the title acid. $^1$H NMR ($CDCl_3$, 500 MHz): 7.14 (d (8.5 Hz), 1H); 7.03 (br s, 2H); 6.90 (d (8.5 Hz), 1H); 6.79 (d (8.0 Hz), 2H); 5.35 (d (8.0 Hz), 1H); 5.02 (m, 1H); 4.38 (m, 1H); 4.19 (m, 2H); 4.02 (m, 1H); 3.8 (br s, 1H); 3.40 (dd (4.5 Hz, 14 Hz), 1H); 2.65 (t (12.5 Hz), 1H); 1.8 to 1.4 (m, 6H); 1.41 (s, 9H); 1.3 to 1.1 (m, 6H); 0.81 (t (6.5 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 174.2, 171.4, 171.1, 157.7, 155.6, 131.0 (br), 128.1, 116.0 (br), 80.3, 67.8, 54.2, 52.8, 52.5, 38.1, 33.3, 32.4, 29.6, 28.3, 27.0, 22.4, 13.7.

**((10*S*)-Butyl-(13*S*)-[(*R*)-oxiranyl]-8,11-dioxo-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-(7*S*)-yl)-carbamic Acid *tert*-Butyl Ester (8b).** Macrocyclic acid **7b** (120 mg, 0.24 mmol) was converted to the diazomethyl ketone (procedure F, 91%), which was subsequently converted to the bromomethyl ketone (procedure G, 90%), reduced (procedure H, rpHPLC (diastereomeric separation), 51%), and finally converted to the title epoxide (procedure I, 93%). $^1$H NMR ($CDCl_3$, 300 MHz): 7.01 (br s, 2H); 6.85 (d (8.0 Hz), 2H); 6.48 (d (8.5 Hz), 1H); 6.18 (d (8.0 Hz), 1H); 5.40 (d (8.0 Hz), 1H); 4.28 (m, 1H); 4.17 (m, 3H); 3.95 (m, 1H); 3.1 (m, 1H); 2.95 (dd (4.4 Hz, 14 Hz), 1H); 2.8 (m, 2H); 2.40 (t (14 Hz), 1H); 1.8 to 1.4 (m, 6H); 1.41 (s, 9H); 1.3 to 1.1 (m, 6H); 0.81 (t (6.5 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 170.7, 170.7, 157.5, 154.8, 130.5, 128.7, 118.1, 80.1, 68.1, 54.3, 53.7, 52.8, 50.5, 44.7, 34.6, 33.4, 32.2, 29.6, 28.3, 27.1, 22.5, 22.1, 13.8.

**((13*S*)-{2-[(4-Acetylamino-benzenesulfonyl)-(3-methyl-butyl)-amino]-(1*R*)-hydroxy-ethyl}-(10*S*)-butyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-(7*S*)-yl)-carbamic Acid *tert*-Butyl Ester (9e).** Isoamylamine was condensed with epoxide **8b** (20 mg, 40 $\mu$mol) (procedure J, 85%), followed by addition of 4-acetamidobenzenesulfonyl chloride (procedure K, rpHPLC, 55%) to afford the title compound. $^1$H NMR ($CD_3OD$, 500 MHz): 7.84 (d (13.5 Hz), 1H); 7.65 (m, 4H); 7.28 (d (8.5 Hz), 1H); 6.95 (br s, 2H); 6.65 (br d, 2H); 4.18 (m, 1H); 4.04 (m, 2H); 3.91 (m, 1H); 3.77 (m, 1H); 3.60 (m, 1H); 3.32 (m, 1H); 3.20 (m, 2H); 3.04 (m, 2H), 2.86 (dd (6.5 Hz, 14.5 Hz), 1H); 2.27 (t (13 Hz), 1H); 2.04 (s, 3H); 1.8 to 1.4 (m, 7H); 1.30 (s, 9H); 1.2 to 1.1 (m, 8H); 0.71 (d (6.5 Hz), 6H); 0.69 (t (6.5 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 173.0, 171.9, 161.5, 158.1, 157.3, 144.1, 135.2, 131.84, 131.8 (br), 129.1, 120.4, 116.0 (br), 81.0, 74.5, 68.6, 55.5, 55.0, 53.5, 52.9, 37.9, 36.4, 34.8, 33.0, 30.7, 28.6, 28.3, 26.9, 24.0, 23.3, 23.1, 22.8, 22.6, 14.2. HRMS (EI) *m*/*z* calcd for $C_{40}H_{62}N_5O_9S$

($MH^+$), 788.4263; found, 788.4245. Analytical rpHPLC: isocratic 20A:80B rt = 7.6 min; gradient 100A to 100B 20 min rt = 24.1 min.

**4-Amino-*N*-[2-((7*S*)-amino-(10*S*)-butyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16- trien-(13*S*)-yl)-(2*R*)-hydroxy-ethyl]-*N*-(3-methyl-butyl)benzenesulfonamide (9d).** Acetamide (**9e**) (11.5 mg, 16.7 $\mu$mol) was refluxed for 2 h in methanol (1 mL) with 6 drops of 2 M HCl. After the solution was cooled to room temperature, water (1 mL) was added, and the solution was purified by rpHPLC. Lyophilization provided the title compound as a white powder (8.7 mg, 81%). $^1$H NMR ($CD_3OD$, 500 MHz): 7.91 (d (13.3 Hz), 1H); 7.49 (d (9.10 Hz), 2H); 7.35 (d (8.33 Hz), 1H); 7.07 (br s, 2H); 6.78 (br s, 2H); 6.70 (d (9.10 Hz), 2H); 4.25 (m, 1H); 4.15 (m, 1H); 4.10 (m, 1H); 4.05 (m, 1H); 3.73 (m, 1H); 3.40 (dd (3.14, 14.30 Hz), 1H); 3.27 (m, 1H); 3.22 (m, 2H), 3.11 (m, 1H); 2.94 (dd (8.53 Hz, 12.8 Hz), 1H); 2.39 (t (12.5 Hz), 1H); 1.8 to 1.1 (m, 15H); 0.88 (d (6.52 Hz), 6H); 0.84 (t (6.96 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 175.1, 173.2, 158.1, 154.1, 131.9, 130.1, 114.4, 74.4, 68.5, 55.8, 55.0, 53.5, 53.0, 38.0, 36.4, 36.2, 34.8, 31.1, 28.4, 27.0, 23.4, 22.8(2C), 22.7. The four aromatic $^{13}$C of the tyrosine residue were extremely broadened and are unassigned. HRMS (EI) *m*/*z* calcd for $C_{33}H_{52}N_5O_6S$ ($MH^+$), 646.3633; found, 646.3634. Analytical rpHPLC: isocratic 50A:50B rt = 3.9 min; gradient 100A to 100B 20 min rt = 17.9 min.

***N*-((13*S*)-{2-[(4-Amino-benzenesulfonyl)-(3-methyl-butyl)amino]-(1*R*)-hydroxy-ethyl}-(10*S*)-butyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-(7*S*)-yl)acetamide (9f).** Amine (**9d**) (11.0 mg, 16.7 $\mu$mol) was coupled with acetic acid (procedure B, rpHPLC, 64%). $^1$H NMR ($CD_3OD$, 500 MHz): 7.46 (d (7.1 Hz), 2H); 7.05 (br s, 2H); 6.75 (br s, 2H); 6.67 (d (8.1 Hz), 2H); 4.24 (m, 1H); 4.12 (m, 3H); 4.03 (m, 1H); 3.72 (m, 1H); 3.37 (m, 1H); 3.25 (m, 1H); 3.20 (m, 1H); 3.08 (m, 1H); 2.90 (dd (14.2 Hz, 7.8 Hz), 1H); 2.37 (t (12.3 Hz), 1H); 1.85 (s, 3H); 1.72 (m, 1H); 1.63 (m, 2H); 1.5 to 1.3 (m, 8H); 1.20 (m, 4H); 0.85 (d (6.3 Hz), 6H); 0.81 (t (7.5 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 173.0, 172.8, 172.5, 158.2, 154.3, 132.0, 130.1, 128.7 (br), 126.3, 117.3 (br), 114.4, 74.6, 68.6, 55.1, 54.8, 53.7, 53.1, 38.1, 36.4, 34.8, 33.0. 28.4, 27.0, 23.4, 23.2, 22.8, 22.7, 22.2, 14.2. HRMS (EI) *m*/*z* calcd for $C_{35}H_{54}N_5O_7S$ ($MH^+$), 688.3738; found, 688.3743. Analytical rpHPLC: isocratic 40A:60B rt = 5.3 min; gradient 100A to 100B 20 min rt = 20.5 min.

**[1-((13*S*)-{2-[(4-Acetylamino-benzenesulfonyl)-(3-methyl-butyl)amino]-(1*R*)-hydroxy-ethyl}-(10*S*)-butyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-(7*S*)-ylcarbamoyl)-(2*S*)-hydroxy-ethyl]carbamic Acid *tert*-Butyl Ester (9h).** Acetamide (**9e**) (17.2 mg, 25.0 $\mu$mol) was deprotected (procedure A) and coupled to *N*-Boc-serine (procedure B, rpHPLC, 52%). $^1$H NMR ($CD_3OD$, 500 MHz): 10.18 (s, 1H); 7.88 (d (12.0 Hz), 1H); 7.79 (d (10 Hz), 1H); 7.72 (m, 4H); 7.54 (d (8.8 Hz), 1H); 7.03 (br s, 2H); 6.72 (br d, 2H); 4.28 (m, 2H); 4.17 (m, 2H); 4.13 (m, 2H); 4.05 (m, 1H); 3.72 (m, 2H); 3.43 (dd (14.0 Hz, 3 Hz), 1H); 3.34 (m, 1H); 3.23 (dd (13.2 Hz, 3.2 Hz), 1H); 3.17 (m, 1H), 3.00 (dd (8.1 Hz, 14.1 Hz), 1H); 2.39 (t (12.5 Hz), 1H); 2.15 (s, 3H); 1.8 to 1.4 (m, 7H); 1.43 (s, 9H); 1.2 to 1.1 (m, 8H); 0.88 (d (6.3 Hz), 6H); 0.81 (t (6.9 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 173.1, 172.6, 172.1(2C), 158.0, 157.8, 144.3, 135.3, 132.1, 130.1(br), 129.4, 120.7, 117.3(br), 74.4, 68.3, 63.2, 58.1, 55.2, 54.3, 53.6, 52.9, 37.9, 36.4, 34.7, 33.1, 30.3, 28.6, 28.4, 26.9, 24.0, 23.3, 22.8, 22.7, 22.2, 14.2. HRMS (EI) *m*/*z* calcd for $C_{43}H_{67}N_6O_{11}S$ ($MH^+$), 875.4583; found, 875.4582. Analytical rpHPLC: isocratic 40A:60B rt = 6.9 min; gradient 100A to 100B 20 min rt = 21.0 min.

**(2*S*)-Amino-*N*-((13*S*)-{2-[(4-amino-benzenesulfonyl)-(3-methyl-butyl)amino]-(1*R*)-hydroxy-ethyl}-(10*S*)-butyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-(7*S*)-yl)-3-hydroxy-propionamide (9g).** Compound **9h** (9.48 mg, 10.8 $\mu$mol) was refluxed for 2 h in methanol (2 mL) with 6 drops of 2 M HCl. After the solution was cooled to room temperature, water (1 mL) was added, and the solution was purified by rpHPLC to provide the amine as a white powder (3.76 mg, 48%) after lyophilization. $^1$H NMR ($CD_3OD$, 500 MHz): 7.49 (d (10.7 Hz), 2H); 7.06 (br s, 2H); 6.75 (br s, 2H); 6.68 (d (9.0 Hz), 2H); 4.27 (m, 2H); 4.19 (m, 1H); 4.13 (m, 1H); 4.06 (m, 1H); 3.91 (m, 2H); 3.87 (m, 1H); 3.76 (dd (11.3 Hz, 6.9 Hz), 1H); 3.71 (m, 1H); 3.43 (dd (14.0 Hz, 3.84 Hz), 1H); 3.23 (dd (13.4 Hz, 3.4 Hz), 1H); 3.08 (m, 1H); 2.90 (dd (14.6 Hz, 7.9 Hz), 1H); 2.39 (t (12.8 Hz), 1H); 1.75 (m, 1H); 1.66 (m, 2H); 1.48 (m, 5H); 1.37 (m, 1H); 1.21 (m, 6H); 0.87 (d (7.3 Hz), 6H); 0.83 (t (7.0 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 172.9, 172.1, 167.9, 158.1, 154.5, 132.3, 131.2 (br), 130.2, 126.5, 117.5 (br), 114.5, 74.6, 68.6, 61.9, 56.2, 55.2, 54.9, 53.2, 48.2, 38.3, 36.3, 35.0, 33.3, 30.7, 28.6, 27.2, 23.6, 23.0, 22.8, 14.4. HRMS (EI) *m*/*z* calcd for $C_{36}H_{57}N_6O_8S$ ($MH^+$), 733.3953; found, 733.3933. Analytical rpHPLC: isocratic 60A:40B rt = 7.3 min; gradient 100A to 100B 20 min rt = 17.6 min.

**Pentanoic Acid (13(*S*)-{2-[(4-Amino-benzenesulfonyl)-(3-methyl-butyl)amino]-(1*R*)-hydroxy-ethyl}-(10*S*)-butyl-8,11-dioxo-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-(7*S*)-yl)amide (9i).** Amine (**9d**) (5.0 mg, 7.8 $\mu$mol) was coupled with pentanoic acid (procedure B, rpHPLC, 57%). $^1$H NMR ($CD_3OD$, 500 MHz): 7.87 (br, 2H); 7.75 (br, 1H); 7.44 (d (8.2 Hz), 2H); 7.04 (br d, 2H); 6.75 (br d, 2H); 6.66 (d (7.5 Hz), 2H); 4.23 (m, 1H); 4.15 (m, 3H); 4.05 (m, 1H); 3.69 (m, 1H); 3.36 (m, 1H); 3.22 (m, 1H); 3.09 (m, 2H); 2.91 (dd (14.5 Hz, 8.5 Hz), 1H); 2.38 (t (13.9 Hz), 1H); 2.16 (t (7.47 Hz), 2H); 1.8 to 1.1 (m, 21H); 1.0 to 0.8 (m, 12H). HRMS (EI) *m*/*z* calcd for $C_{39}H_{61}N_5O_7S$ ($MH^+$), 730.4205; found, 730.4213. Analytical rpHPLC: isocratic 20A:80B rt = 5.8 min; gradient 100A to 100B 20 min rt = 23.8 min.

**3-Allyloxy-(2*S*)-*tert*-butoxycarbonylamino-propionic Acid.** A solution of *N*-Boc-L-serine (5.0 g, 24 mmol) in DMF (80 mL) was added slowly to a suspension of 60% NaH dispersion (2.4 g, 60 mmol) in DMF (20 mL) at 0 °C. After the amino acid was completely added, allylbromide was introduced (2.6 mL, 30 mmol), and stirring was continued for 3 h at room temperature. Excess sodium hydride was quenched by the careful addition of water (5 mL), and the solvent was removed under reduced pressure. The residue was then dissolved in water, acidified to pH ~ 2 with 4 M HCl, and subsequently extracted into ethyl acetate. The organic layer was dried over magnesium sulfate, and the solvent was removed to provide the title allyl ether (5.4 g, 92%). $^1$H NMR ($CDCl_3$, 300 MHz): 5.81 (m, 1H); 5.38 (br d, 1H); 5.22 (m, 3H); 3.91 (m, 2H); 3.82 (m, 2H); 1.39 (s, 9H). $^{13}$C NMR ($CDCl_3$, 75 MHz): 172.9, 155.5, 134.0, 117.1, 79.7, 72.1, 69.8, 53.7, 28.1.

**(2*S*)-*tert*-Butoxycarbonylamino-3-propoxy-propionic Acid.** 3-Allyloxy-(2*S*)-*tert*-butoxycarbonylamino-propionic acid (5.48 g, 22.4 mmol) was dissolved in methanol (100 mL) and hydrogenated over Pd/C in a Parr hydrogenation vessel under 30 psi of hydrogen for 3 h. The catalyst was removed from the methanolic solution by filtration through super cell, and the solvent was removed to yield the title ether (4.42 g, 80%). $^1$H NMR ($CDCl_3$, 300 MHz): 5.38 (d (8.6 Hz), 1H); 4.41 (m, 1H); 3.84 (m, 1H); 3.62 (dd (9.4 Hz, 3.8 Hz), 1H); 3.36 (t (6.6 Hz), 2H); 1.52 (m, 2H); 1.40 (s, 9H); 0.83 (t (7.7 Hz), 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz): 175.2, 155.7, 80.3, 73.2, 70.1, 53.7, 28.3, 22.5, 10.4.

**(2*S*)-[(2*S*)-((2*S*)-*tert*-Butoxycarbonylamino-6-hydroxy-hexanoylamino)-3-propoxy-propionylamino]-3-(4-hydroxy-phenyl)propionic Acid Methyl Ester (6c).** This tripeptide was synthesized sequentially from (2*S*)-*tert*-butoxy-carbonylamino-6-hydroxyhexanoic acid, (2*S*)-*tert*-butoxycarbonylamino-3-propoxy-propionic acid, and tyrosine methyl ester using the standard Boc deprotection (procedure A) and coupling (procedure B) procedures above. $^1$H NMR ($CDCl_3$, 300 MHz): 7.10 (d (8.5 Hz), 2H); 6.92 (d (8.4 Hz), 2H); 6.90 (d (7.7 Hz), 1H); 6.72 (d (7.8 Hz), 1H); 5.25 (m, 1H); 4.78 (m, 1H); 4.47 (m, 1H); 4.08 (m, 1H); 3.79 (m, 1H); 3.68 (s, 3H); 3.62 (m, 1H); 3.53 (m, 1H), 3.42 (m, 1H); 3.37 (t (7.1 Hz), 2H); 3.07 (m, 2H); 1.8 to 1.4 (m, 8H); 1.40 (s, 9H); 0.83 (t (7.1 Hz), 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz): 171.6, 171.5, 169.9, 155.8, 149.5, 133.7, 130.4, 121.4, 80.2, 73.1, 62.1, 53.6, 53.3, 52.6, 52.4, 38.6, 32.2, 31.9, 31.8, 28.3, 22.6, 21.7, 10.4.

**(7*S*)-*tert*-Butoxycarbonylamino-8,11-dioxo-(10*S*)-propoxymethyl-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-triene-(13*S*)carboxylic Acid (7c).** Tripeptide **6c** (4.5 g, 8.1 mmol) was brominated (procedure C, FCC, 72%), followed by cyclization (procedure D, FCC, 36%), and the resulting macrocyclic ester was hydrolyzed (procedure E, 94%) to afford the title acid. $^1$H NMR ($CD^3OD$, 300 MHz): 6.86 (br s, 2H); 6.57 (br s, 2H); 4.56 (m, 1H); 4.18 (m, 1H), 4.02 (m, 1H); 3.95 (m, 1H); 3.73 (m, 1H); 3.29 (m, 2H); 3.16 (m, 2H); 3.06 (m, 1H); 2.44 (t (12.7 Hz), 1H); 1.6 to 1.4 (m, 6H); 1.32 (s, 9H); 0.99 (m, 2H); 0.75 (t (7.4 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 75 MHz): 174.9, 173.9, 171.2, 160.2, 159.3, 133.7, 131.9, 117.9, 74.7, 72.7, 72.4, 69.4, 55.9, 55.2, 54.9, 39.8, 34.1, 31.2, 29.3, 27.0, 23.6, 11.4.

**((13*S*)-[(*R*)-Oxiranyl]-8,11-dioxo-(10*S*)-propoxymethyl-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-(7*S*)-yl)carbamic Acid *tert*-Butyl Ester (8c).** Macrocyclic acid **7c** (220 mg, 0.42 mmol) was converted to the diazomethyl ketone (procedure F, 85%), which was subsequently converted to the bromomethyl ketone (procedure G, 72%), reduced (procedure H, rpHPLC (diastereomeric separation), 35%), and finally converted to the title epoxide (procedure I, 92%). $^1$H NMR ($CDCl_3$, 300 MHz): 7.01 (br s, 2H); 6.78 (d (8.0 Hz), 2H); 6.32 (d, 7.2 Hz), 1H); 6.05 (d (9.4 Hz), 1H); 5.18 (d (8.1 Hz), 1H); 4.3 to 3.9 (m, 5H); 3.62 (dd (8.9 Hz, 4.3 Hz), 1H); 3.40 (m, 2H); 3.23 (t (9.1 Hz), 1H); 3.03 (m, 1H); 2.89 (dd (13.2 Hz, 4.3 Hz), 1H); 2.78 (m, 2H); 2.40 (m, 1H); 1.8 to 1.6 (m, 6H); 1.40 (s, 9H); 1.31 (m, 2H); 0.82 (t (8.0 Hz), 3H). $^{13}$C NMR ($CDCl_3$, 75 MHz): 171.2, 169.0, 157.9, 155.4, 130.4, 128.8, 115.8, 73.2, 69.7, 68.2, 66.1, 54.1, 53.2, 51.9, 51.2, 46.2, 36.0, 32.3, 29.1, 28.3, 22.7, 21.2, 10.6.

**((13*S*)-{2-[(4-Acetylamino-benzenesulfonyl)-(3-methyl-butyl)amino]-(1*R*)-hydroxy-ethyl}-8,11-dioxo-(10*S*)-propoxymethyl-2-oxa-9,12-diaza-bicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-(7*S*)-yl)carbamic Acid *tert*-Butyl Ester (9k).** Isoamylamine was condensed with epoxide **8c** (9.5 mg, 18 $\mu$mol) (procedure J, 90%), followed by addition of 4-acetamidobenzenesulfonyl chloride (procedure K, rpHPLC, 36%) to afford the title compound. $^1$H NMR ($CD_3OD$, 500 MHz): 7.78 (d (9.6 Hz), 1H); 7.77 (s, 4H); 7.75 (d (8.6 Hz), 1H); 7.06 (br s, 2H); 6.76 (br s, 2H); 4.28 (m, 2H); 4.13 (m, 1H); 4.05 (m, 1H); 3.90 (m, 1H); 3.72 (m, 1H), 3.45 (m, 3H); 3.25 (m, 4H); 3.18 (m, 1H); 3.03 (dd (13.7 Hz, 8.4 Hz), 1H); 2.42 (t (12.1 Hz), 1H); 2.14 (s, 3H); 1.72 (m, 1H); 1.6 to 1.4 (m, 8H); 1.40 (s, 9H); 1.18 (m, 2H); 0.87 (d (6.7 Hz), 6H); 0.76 (t (7.0 Hz), 3H). $^{13}$C NMR ($CD_3OD$, 125 MHz): 173.5, 172.1, 171.1, 158.7, 157.6, 144.4, 135.6, 132.2, 131.8, 129.6, 120.7, 117.4, 74.3, 74.2, 72.3, 72.2, 68.9, 55.7, 54.1, 53.3, 47.8, 38.6, 37.0, 33.7, 30.9, 28.8, 27.3, 24.2, 23.9, 23.4, 23.1, 22.9, 11.0. HRMS (EI) *m*/*z* calcd for $C_{40}H_{62}N_5O_{10}S$ ($MH^+$), 804.4212; found, 804.4189. Analytical rpHPLC: isocratic 30A:70B rt = 7.3 min; gradient 100A to 100B 20 min rt = 23.9 min.

**4-Amino-*N*-[2-((7*S*)-amino-8,11-dioxo-(10*S*)-propoxymethyl-2-oxa-9,12-diazabicyclo[13.2.2]nonadeca-1(18),15(19),16-trien-13-yl)-(2*R*)-hydroxy-ethyl]-*N*-(3-methyl-butyl)benzenesulfonamide (9j).** Amide (**9k**) (3.0 mg, 3.7 mmol) was refluxed for 2 h in methanol (2 mL) with 3 drops of 2 M HCl. After the solution was cooled to room temperature, water (1 mL) was added, and the solution was purified by rpHPLC to provide the amine as a white powder (1.6 mg, 65%) after lyophilization. $^1$H NMR ($CD_3OD$, 500 MHz): 7.50 (d (8.3 Hz), 2H); 7.15 (br s, 2H); 7.05 (br s, 2H); 6.70 (d (8.3 Hz), 2H); 4.39 (m, 1H); 4.30 (m, 1H); 4.11 (m, 2H); 3.85 (m, 1H); 3.72 (m, 3H); 3.52 (d (5.6 Hz), 2H); 3.45 (dd (14.3 Hz, 3.4 Hz), 1H); 3.3 (m, 1H); 3.22 (m, 1H); 3.09 (m, 1H); 2.90 (dd (14.7 Hz, 8.7 Hz), 1H); 2.36 (t (14.4 Hz), 1H); 1.86 (m, 3H); 1.75 (m, 2H); 1.63 (m, 1H); 1.5 to 1.3 (m, 4H); 1.20 (m, 1H);, 0.87 (d (6.8 Hz), 6H); 0.81 (t (7.9 Hz), 3H). HRMS (EI) *m*/*z* calcd for $C_{33}H_{52}N_5O_7S$ ($MH^+$), 662.3582; found, 662.3563. Analytical rpHPLC: isocratic 60A:40B rt = 7.8 min; gradient 100A to 100B 20 min rt = 17.8 min.

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## References

(1) Goodwill, K. E.; Tennant, M. G.; Stevens, R. C. High-throughput X-ray crystallography for structure-based drug design. *Drug Discovery Today* **2001**, *6*, S113−S118.
(2) Wade, R. C. "Flu" and structure-based drug design. *Structure* **1997**, *5*, 1139−1145.
(3) Walkinshaw, M. D. Protein targets for structure-based drug design. *Med. Res. Rev.* **1992**, *12*, 317−372.
(4) Babine, R. E.; Bender, S. L. Molecular recognition of protein−ligand complexes: Applications to drug design. *Chem. Rev.* **1997**, *97*, 1359−1472.
(5) Leung, D.; Abbenante, G.; Fairlie, D. P. Protease Inhibitors: Current Status and Future Prospects. *J. Med. Chem.*. **2000**, *43*, 305−341.
(6) Darke, P. L.; Huff, J. R. HIV protease as an inhibitor target for the treatment of AIDS. *Adv. Pharmacol.* **1994**, *25*, 399−454.
(7) West, M. L.; Fairlie, D. P. Targeting HIV-1 protease: a test of drug-design methodologies. *Trends Pharmacol. Sci.* **1995**, *16*, 67−75.
(8) Kempf, D. J.; Sham, H. L. HIV protease inhibitors *Curr. Pharm. Des.* **1996**, *2*, 225−246.
(9) March, D. R.; Fairlie, D. P. *Designing New Antiviral Drugs for AIDS*: *HIV-1 Protease and its Inhibitors*; R. G. Landes Co.: Austin, Texas, 1996.
(10) Misson, J.; Clark, W.; Kendall, M. J. Therapeutic advances: protease inhibitors for the treatment of HIV-1 infection. *J. Clin. Pharm. Ther.* **1997**, *22*, 109−117.
(11) Wlodawer, A.; Vondrasek, J. Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249−284.
(12) Lebon, F.; Ledecq, M. Approaches to the design of effective HIV-1 protease inhibitors. *Curr. Med. Chem.* **2000**, *7*, 455−477.
(13) Furfine, E. S. The next generation of human immunodeficiency virus protease uinhibitors: targeting viral resistance. *Handb. Exp. Pharmacol.* **2000**, *140*, 49−72.
(14) Tyndall, J. D. A.; Fairlie, D. P. Conformational Homogeneity in Molecular Recognition by Proteolytic Enzymes. *J. Mol. Recog.* **1999**, *12*, 1−8.
(15) Fairlie, D. P.; Tyndall, J. D. A.; Reid, R. C.; Wong, A. K.; Abbenante, G.; Scanlon, M. J.; March, D. R.; Bergman, D. A.; Chai, C. L. L.; Burkett, B. A. Conformational Selection Of Inhibitors and Substrates By Proteolytic Enzymes: Implications for Drug Design and Polypeptide Processing. *J. Med. Chem.* **2000**, *43*, 1271−1281.
(16) Abbenante, G.; March, D.; Bergman, D.; Hunt, P. A.; Garnham, B.; Dancer, R. J.; Martin, J. L.; Fairlie, D. P. Regioselective Structural and Functional Mimicry Of Peptides: Design Of Hydrolytically Stable Cyclic Peptidomimetic Inhibitors Of HIV 1 Protease. *J. Am. Chem. Soc.* **1995**, *117*, 10, 220−10, 226.
(17) March, D.; Abbenante, G.; Bergman, D.; Brinkworth, R. I.; Wickramasinghe, W.; Begun, J.; Martin, J. L.; Fairlie, D. P. Substrate Based Cyclic Peptidomimetics Of PheIleVal That Inhibit HIIV-1 Protease Using a Novel Enzyme Binding Mode. *J. Am. Chem. Soc.* **1996**, *118*, 3375−3379.
(18) Abbenante, G.; March, D. R.; Bergman, D. A.; Hunt, P.; Fairlie, D. P. Structure−Activity Relationships for Macrocyclic Inhibitors of HIV-1 Protease. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2531−2536.
(19) Reid, R. C.; March, D. R.; Dooley, M. J.; Bergman, D. A.; Abbenante, G.; Fairlie, D. P. A novel bicyclic enzyme inhibitor as a consensus peptidomimetic for the receptor-bound conformations of 12 peptidic inhibitors of HIV-1 protease. *J. Am. Chem. Soc.* **1996**, *118*, 8511−8517.
(20) Martin, J. L.; Begun, J.; Schindeler, A.; Wickramasinghe, W. A.; Alewood, D.; Alewood, P. F.; Bergman, D. A.; Brinkworth, R. I.; Abbenante, G.; March, D.; Reid, R. C.; Hunt, P. A.; Fairlie, D. P. Molecular Recognition of Macrocyclic Peptidomimetic inhibitors by HIV-1 Protease. *Biochemistry* **1999**, *38*, 7978−7988.
(21) Tyndall, J. D. A.; Reid, R. C.; Tyssen, D. P.; Jardine, D. K.; Todd, B.; Passmore, M.; March, D. R.; Pattenden, L. K.; Bergman, D. A.; Alewood, D.; Hu, S.-H.; Alewood, P. F.; Birch, C. J.; Martin, J. L.; Fairlie, D. P. Synthesis, Stability, Antiviral Activity, and Protease-Bound Structures of Substrate-Mimicking Constrained Macrocyclic Inhibitors of HIV-1 Protease. *J. Med. Chem.* **2000**, *43*, 3495−3504. (b) pdb file: 1D4L.
(22) Tyndall, J. D. A.; Fairlie, D. P. Macrocycles Mimic The Extended Peptide Conformation Recognized By Aspartic, Serine, Cysteine and Metallo Proteases. *Curr. Med. Chem.* **2001**, *8*, 893−907.
(23) Swain, A. L.; Miller, M. M.; Green, J.; Rich, D. H.; Schneider, J.; Kent, S. B.; Wlodawer, A. X-ray crystallographic structure of a complex between a synthetic protease of human immunodeficiency virus 1 and a substrate-based hydroxyethylamine inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8805−8809.
(24) (a) Leach, A. R. Template Forcing minimises the RMSD using a specific minimisation algorithm that allows rotation around single bonds as well as translation and rotation in space. *Molecular Modelling: Principles and Applications*; Longman:

Harlow, England, 1996. (b) InsightII Modeling Envorionment, Release 2000; Accelrys Inc.: San Diego, 2001.
(25) Condra, J.; Schlief, W.; Blahy, O.; Gabryelski, L.; Graham, D.; Quintero, J.; Rhodes, A.; Robbins, H.; Roth, E.; Shivaprakash, M.; Titus, E.; Yang, T.; Teppler, H.; Squires, K.; Deutsch, P.; Emini, E. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. *Nature* **1995**, *375*, 569−571.
(26) Erickson, J. W. The Not So Great Escape. *Nat. Struct. Biol*. **1995**, *2*, 523−529.
(27) Palmer, S.; Schafer, R. W.; Merigan, T. C. Highly drug-resistant HIV-1 clinical isolates are cross-resistant to many antiretroviral compounds in current clinical development. *AIDS* **1999**, *13*, 661−667.
(28) Adamczyk, M.; Johnson, D. D.; Reddy, R. E. Collagen Cross-Links: Synthesis of Pyridinonline, Deoxypyridinoline and their Analogues. *Tetrahedron* **1999**, *55*, 63−88.
(29) For a discussion of stereoisomer preference and binding conformation, see ref 20.
(30) Bergman, D. A.; Alewood, D.; Alewood, P. F.; Andrews, J. L.; Brinkworth, R. I.; Englebretsen, D. R.; Kent, S. B. H. Kinetic-properties of HIV-1 Protease Produced by Total Chemical Synthesis with Cysteine Residues Replaced by Isosteric L-alpha-amino-*n*-butyric Acid *Lett. Pept. Sci*. **1995**, *2*, 99−107.
(31) Toth, M. V.; Marshall, G. R. A simple, continuous fluorometric assay for HIV protease. *Int. J. Pept. Protein Res*. **1990**, *36*, 544−550.
(32) Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcho, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. Crystal structure of HIV-1 protease in complex with VX478, a potent and orally bioavailable inhibitor of the enzyme. *J. Am. Chem. Soc.* **1995**, *117*, 1181−1182.
(33) Calculated using PALLAS prologD 2.1 from Compudrug Chemistry Ltd., Hungary.
(34) Schneider, J.; Kent, S. B. H. Enzymatic activity of a synthetic 99 residue protein corresponding to the putative HIV-1 protease. *Cell* **1988**, *54*, 363−368.
(35) Neate, E. V.; Pringle, R. C.; Jowett, J. B. M.; Healey, D. S.; Gust, I. D. Isolation of HIV from Australian patients with AIDS, AIDS related conditions and healthy antibody positive individuals. *Aust. N. Z. J. Med*. **1987**, *17*, 461−466.
(36) Tachedjian, G.; Tyssen, D.; Locarnini, S.; Gust, I.; Birch, C. Investigation of topoisomerase inhibitors for activity against human immunodeficiency virus: inhibition by coumermycin A1. *Antiviral Chem. Chemother.* **1990**, *1*, 131−138.

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