Selectivity in the Inhibition of HIV and FIV Protease: Inhibitory and Mechanistic Studies of Pyrrolidine-Containing α-Keto Amide and Hydroxyethylamine Core Structures

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Abstract: This paper describes the development of new pyrrolidine-containing α-keto amide and hydroxyethylamine core structures as mechanism based inhibitors of the HIV and FIV proteases. It was found that the α-keto amide core structure **2** is approximately 300-fold better than the corresponding hydroxyethylamine isosteric structure and 1300-fold better than the corresponding phosphinic acid derivative as an inhibitor of the HIV protease. The α-keto amide is however not hydrated until it is bound to the HIV protease as indicated by the NMR study and the X-ray structural analysis. Further analysis of the inhibition activities of hydroxyethylamine isosteres containing modified pyrrolidine derivatives revealed that a *cis*-methoxy group at C-4 of the pyrrolidine would improve the binding 5- and 25-fold for the *trans*-isomer. When this strategy was applied to the α-keto amide isostere, a *cis*-benzyl ether at C-4 was found to enhance binding 3-fold. Of the core structures prepared as inhibitors of the HIV protease, none show significant inhibitory activity against the mechanistically identical FIV protease, and additional complementary groups are needed to improve inhibition.

Introduction

Human immunodeficiency virus protease (HIV PR) is an important target for the inhibition of viral replication. Though many potent *in vitro* inhibitors have been developed, most of them are either inactive or toxic *in vivo* or mutant forms of the virus emerge which are resistant.[1−8] The lack of animal systems to test the efficacy of the inhibitors further slows down the drug development process. Recently a similar protease has been identified in the life cycle of feline immunodeficiency virus (FIV)[9,10] a virus which leads to clinical symptoms comparable to those observed in human acquired immune deficiency syndrome (AIDS). Studies have shown that up to 14% of the cats surveyed in the USA and Canada[11] and 28.9% in Japan[12] are infected with FIV. In drug resistant mutants of HIV, there are at least six cases where HIV PR residues mutate to the structurally aligned residue found in FIV PR. The amino acid changes in HIV PR are V32I (I37-FIV), L90M (M107-FIV), N88D (D105-FIV), I50V (V59′-FIV), K20I (I25-FIV), and Q29K (K109-FIV).[3] Superimposition of the two proteases based on their X-ray structures indicates similarities between the two proteases and the drug resistant HIV proteases. This suggests that FIV PR may provide a good model of drug resistance in retroviral proteases and contribute to the understanding of HIV resistance to protease inhibitors. We are developing inhibitors to test against both HIV and FIV proteases, with the aim of developing inhibitors efficacious against these two mechanistically identical proteases, which will be less prone to resistance development. Another objective is to use cats as model systems on which to test HIV PR inhibitors *in vivo*.

HIV PR[13] is a 99 amino acid aspartyl protease[14] which functions as a homodimer. FIV PR is also a homodimeric

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	P_4	P_3	P_2	P_1		P_1'	P_2'	P_3'	P_4'
HIV PR	Ser	Gln	Asn	Tyr	-	Pro	Ile	Val	Gln
FIV PR	Pro	Gln	Ala	Tyr	-	Pro	Ile	Gln	Thr

Figure 1. Amino acid sequence of the natural substrates for HIV and FIV proteases about the Y~P cleavage site.

aspartyl protease which consists of 116 amino acid residues.[15] Both HIV and FIV proteases are responsible for the processing of viral *gag* and *gag-pol* polyproteins into structural proteins and enzymes essential for the proper assembly and maturation of full infectious virions.[16] In particular HIV[17] and FIV proteases[18] show high specificity for the selective cleavage of the tyrosine/phenylalanine-proline amide bonds in the Matrix-Capsid domain of the *gag-pol* polyproteins, a specificity not exhibited by mammalian cellular proteases which are not known to efficiently hydrolyze peptide bonds involving the proline nitrogen. It is this specificity that makes HIV PR an attractive target for inhibition. Figure 1 compares the amino acid sequence about the matrix capsid cleavage site (tyrosine−proline bond) in both HIV and FIV. As can be seen the residues about the cleavage site are the same at four positions, P_3, P_1, P_1', and P_2'. These similarities suggest that HIV PR inhibitors may also inhibit FIV PR to some extent.

Our group has recently developed a new α-keto amide core structure[19] which appears to be more potent than any other mechanism-based isosteric core structure (such as those of the hydroxyethylamine[20] and phosphinic acid[21] derivatives) as an HIV PR inhibitor. Activated ketones in general have been shown to inhibit aspartyl proteases such as renin[22] and serine and cysteine proteases such as α-chymotrypsin and calpain, respectively. Their modes of action, however, are not well understood.[23−26]

The activity of dipeptide isosteres is often enhanced by addition of amino acids residues to both the N- and C-terminus of the isostere to improve binding in the active site. Although this approach often provides very high binding affinity to HIV PR and some inhibitors of this type have been in clinical trials,[1] the resulting inhibitors generally exhibit metabolic instability and/or poor oral bioavailability.[27−29] It is possible to develop potent small molecule HIV PR inhibitors that span P_2-P_2' subsites in HIV PR.[6,30] In order to enhance the activity of the core isosteres containing either an α-keto amide moiety or a hydroxyethylamine, without the addition of amino acid side chains, we have investigated the effects of protecting groups and the substituents about the proline ring. This study lends itself well to a combinatorial approach as illustrated in Figure 2.

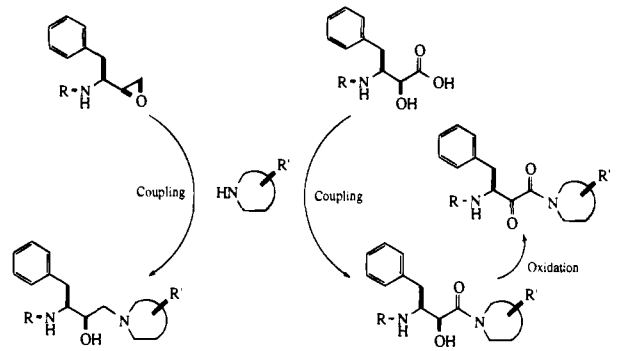

Figure 2. Illustration of an approach to rapidly access a number of potential inhibitors of HIV and/or FIV proteases to determine the protecting group and ideal substitution pattern of the "proline" moiety to provide maximum inhibition of the enzymes.

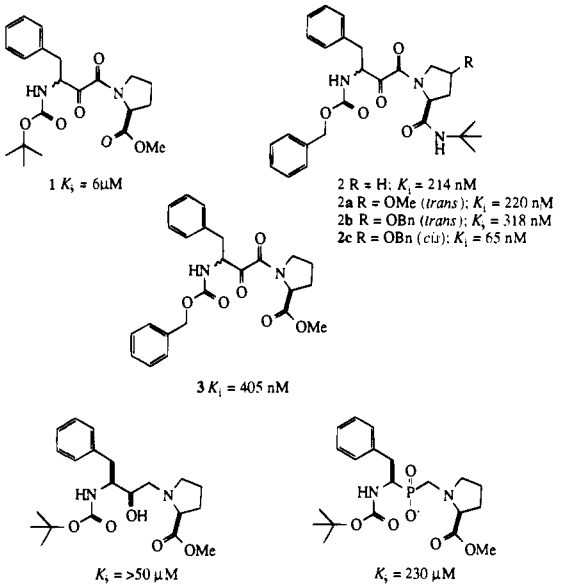

Figure 3. Comparison of keto amide core structure to other isosteric structures.

Results and Discussion

When assayed against HIV PR, the novel α-keto amide **1** (Figure 3) was found to have a K_i of 6 μM. Subsequent studies have shown that a simple modification of the N- and C-terminal protecting groups to give **2** (Figure 3) enhances the potency of this core isostere against HIV PR, to give a K_i of 214 nM.

The increase in activity in compound **2** may be due to favorable hydrophobic interactions between the protecting groups (i.e., the Cbz-protecting group and the *t*-Bu group) and the active site of HIV PR. The Boc-protecting group is also hydrophobic but is shorter and sterically more bulky, making it unable to extend effectively into the appropriate hydrophobic binding pocket. This result demonstrates how simple modifications of the core isostere can significantly improve its potency. It was felt that the potency of the α-keto amide **2** might be

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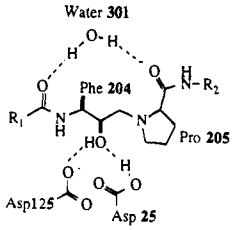

Figure 4. Schematic diagram illustrating hydrogen bond interactions between a hydroxyethylamine isostere and the active site of HIV PR, observed from the X-ray structure.

improved by introduction of additional complementary groups to the proline ring moiety. Computer modeling (Insight/Discover) indeed indicates that attachment of hydrophobic groups to the proline ring moiety will enhance binding. It was found that addition of a *cis*-benzyl ether to C-4 of the proline moiety increased binding 3-fold (K_i = 65 nM); however, no increase in binding was observed with a *trans*-benzoxy or *trans*-methoxy group at the same position.

The activity of the isostere **2** was then compared to that of the identically substituted α-hydroxy amide precursors **23** and **24**. Consistent with observations by Sakurai[31] and with studies on hydroxyethylamine dipeptide isosteres,[7,20] the *S*-diastereomer **24** (IC_{50} = 2 μM) was found to be more potent than the *R*-diastereomer **23** (IC_{50} = 300 μM) but less active than **2**. The high potency of the α-hydroxy amide **24** implies that the hydroxyl group is hydrogen bonding more effectively with the catalytic carboxylic acid groups of HIV PR than in compound **23**, similar to that observed in the X-ray structure of a hydroxyethylamine inhibitor enzyme complex[32] (Figure 4). The stereochemistry of the isosteres **23** and **24** was determined by ^{1}H NMR studies on the *R*- and *S*-Mosher esters derived from the *S*-α-hydroxy ester **19**.[33,34]

It is possible that the ketone moiety of the α-keto amide **2** is hydrated, as observed with other α-keto amides of this nature,[22,26] and is hydrogen bonding in a similar manner to that of compound **23**. However in this case, ^{13}C NMR studies on compound **2** in deuterated DMSO/D_2O (5:1) following the procedure described by Ocain and Rich[26] determined that, in the presence of water, the ketone moiety of the α-keto amide *remains unhydrated* even after incubation for 24 h (Figure 5). This would imply that the ketone moiety of **2** is quite stable in the presence of water and is therefore difficult to hydrate in the absence of a catalyst. It is likely that hydration of the ketone moiety takes place within the active site of HIV PR as illustrated in Figure 6, and the resulting hydrate is then stabilized through hydrogen bonding interactions with the aspartate residues of the enzyme. The hydrated form of **2** is considered to be a good transition state mimic based on the model presented in Figure 6.

Time dependent assays do not exhibit time dependent inhibition, indicating that if the active form of the α-keto amide **2** is indeed the hydrate, the hydration step must be rapid or **2** itself is the active form. To further investigate the mode of action, the X-ray structure of the complex between the α-keto amide **2** and HIV PR was determined, and the result indicated that the α-keto amide is hydrated, supporting the enzyme-assisted hydration mechanism (Figure 7).[36]

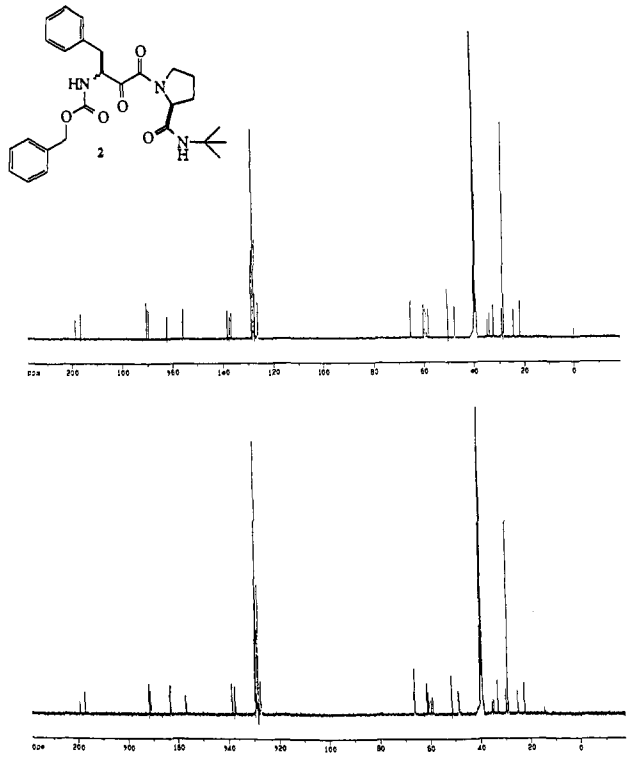

Figure 5. ^{13}C-NMR spectra of **2** in deuterated DMSO (top) and in a mixture of deuterated DMSO (0.5 mL) and D_2O (0.2 mL) after incubation for 24 h (bottom).

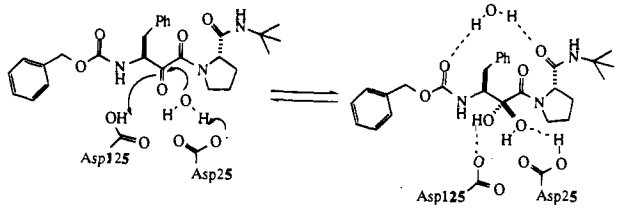

Figure 6. Schematic representation of general acid–general base mechanism for inhibitor **2** interaction with HIV PR aspartate groups.

As seen in the electron density map, the inhibitor is bound in the active site of HIV-PR in its hydrated state (Figure 7). One of the two hydroxyls is located between two aspartates, making hydrogen bonds with both of them, while the other hydroxyl interacts with only one aspartate. The keto group is also hydrogen bonded to a catalytic aspartate. The phenylalanine and proline side chains occupy proper S_1 and S_1' pockets making interactions similar to those observed in other structures.[32] The carboxybenzyl group on the N-terminus and the *tert*-butyl group of the *tert*-butyl amide on the C-terminus of the inhibitor are roughly in the S_2 and S_2' pockets, respectively. The conserved water (Wat-301), which is forming hydrogen bonds between the inhibitor and the two flaps of the enzyme, is clearly observed. Its location is rather asymmetric, unlike the case of the majority of other inhibitor complexes. The distance between the carbonyl oxygen which mimics the P_2 CO group and Wat-301 is 2.5 Å, while the distance to the P_1' CO is 3.2 Å.

We observed remarkable similarity in the binding mode of the central and C-terminal parts of compound **2** and the similar parts of an inhibitor KNI-272.[35] The differences in their binding constants are very significant (K_i for KNI-272 is almost five orders of magnitude lower than for **2**), despite the presence of

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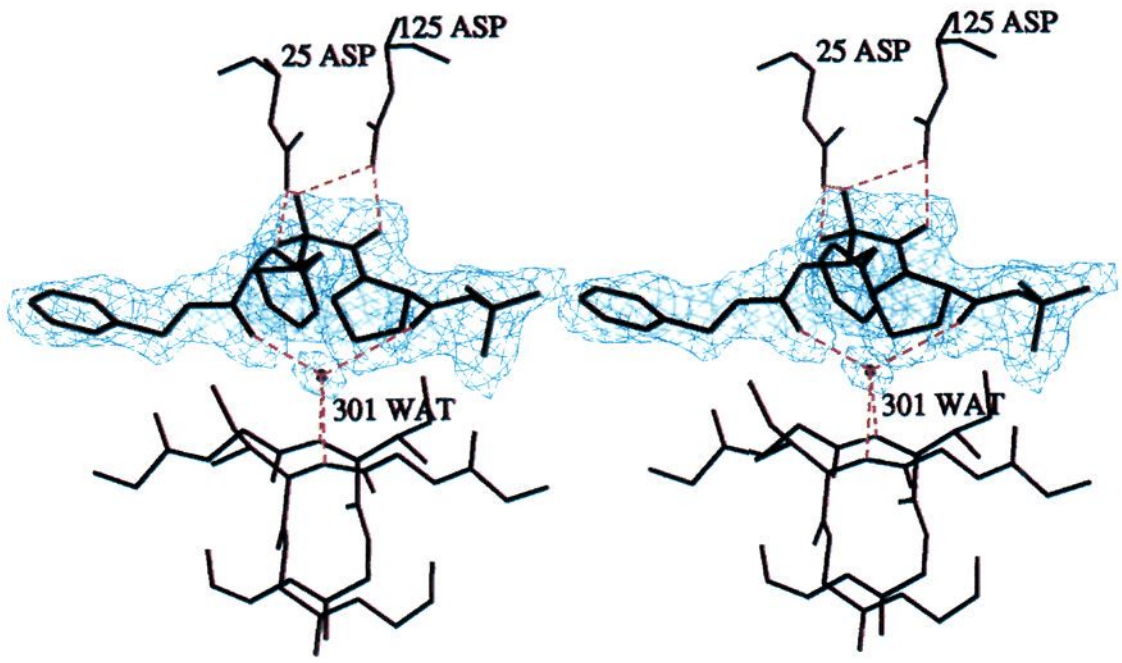

Figure 7. Crystal structure of compound **2** located in the active site of HIV PR. The inhibitor is drawn in thick lines, while the active site aspartates and flap regions of HIV PR are shown in thin lines. The $2F_o - F_c$ electron density for the inhibitor and the conserved Wat-301, contoured at 1 σ, is shown in dotted lines, while relevant hydrogen bonds are shown as dashed lines.

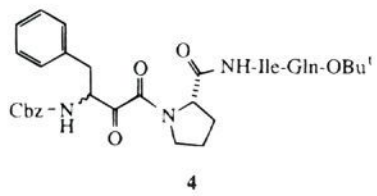

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Figure 8.

an extra hydrogen bond in the central part of the complex of HIV PR and compound **2**. Thus the increase in the potency of KNI-272 must be due to the elongation of its N-terminus, which has a bulky 5-isoquinolyloxyacetyl (IQoa) moiety. The regions of the protein interacting with this group, including the Phe-53 in one monomer and Pro-81 in another, shift significantly toward the inhibitor, making extensive hydrophobic contacts with the IQoa group.

When the α-keto amide **2** was tested against FIV PR, it was found to have no inhibitory effect when added in concentrations up to 70 mM. This result was surprising due to the similarity between the natural substrates for HIV and FIV proteases bordering the matrix/capsid cleavage site as illustrated earlier. It appears that FIV PR may require additional specific residues between the P_4-P_4' sites, than HIV PR, before it is able to recognize the core isostere **2** as a substrate. This is also supported by the observation that HIV PR will cleave an acetyl-(*6* residue) peptide substrate of sequence Gln-Ala-Tyr~Pro-Ile-Gln, whereas the smallest peptide FIV PR is known to cleave is an acetyl-(*8* residue) peptide of sequence Pro-Gln-Ala-Tyr~Pro-Ile-Gln-Thr.[37] The corresponding protected dipeptide (Cbz-Phe-Pro-NBu^t) was not cleaved by HIV or FIV proteases under normal assay conditions.[38]

In an effort to develop inhibitors of the FIV PR, it was found that the addition of suitable residues to interact with just the P_2' and P_3' sites of FIV PR was sufficient for moderate inhibition. Coupling of a side chain specific for FIV PR to the C-terminus of **2** gave **4** (Figure 8). This extended isostere **4** was found to have an IC_{50} of 25 μM and a K_i of 29 μM against FIV PR, and the activity against HIV PR was slightly enhanced (K_i of 154 nM). It appears that the isosteric core structure of HIV PR inhibitors do not bind tightly to the FIV PR, and additional complementary groups are needed to enhance the binding. This difference is also observed in the analysis of other

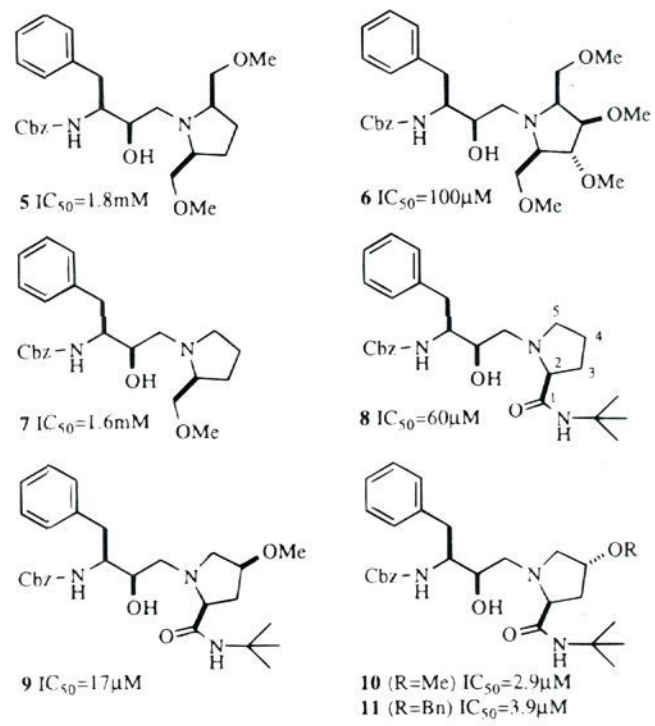

Figure 9. Inhibitory activity of variously substituted pyrrolidine analogues against HIV PR.

known HIV PR inhibitors. The potent cyclic urea based HIV PR inhibitor DMP 323 (IC_{50} = 36 nM, K_i = 0.27 nM),[6] for example was also found to be a very poor inhibitor of FIV PR (IC_{50} = 7.3 mM). Perhaps further studies on the inhibition of these two enzymes and the resistant variants of HIV PR will provide some insight into the structural basis of drug resistance.

To investigate whether the activity of the core hydroxyethylamine isostere **8** against HIV PR can be significantly enhanced by simple derivatization of the proline ring, the hydroxyethylamine derivatives shown in Figure 9 were synthesized and tested.

When the activity of the monomethylated derivative **7** is compared to that of the dimethylated derivative **5**, it can be seen that a hydrophobic moiety at the C-5 position decreases the potency of the isostere, whereas the activity increases when similar hydrophobic substitutions are made at the C-3 and C-4 positions as in compound **6**. The permethylated pyrrolidine derivative **6** was the most potent derivative of the series **5**−**7**, but significant activity was lost due to the absence of the amide bond at C-1, as can be seen by comparison of the activity of the methylated derivatives **5**−**7** to that of compound **8**. The C-4 substituted pyrrolidine derivatives **9**−**11** were shown to be more potent than **8**, with the *trans*-C-4 methoxy derivative **10** being the best (IC_{50} = 2.9 μM).

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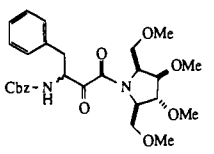

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Figure 10.

The permethylated α-keto amide derivative **12** (Figure 10) was synthesized for comparison and was found to be more potent than the corresponding hydroxyethylamine isostere **6** but significantly less potent ($K_i = 20\ \mu M$) than the original isostere **2** against HIV PR. This result again illustrates the importance of an amide bond at C-1 of the pyrrolidine derivative.

This study also suggests that the FIV PR could be used as a model for the development of HIV PR inhibitors and for the study of drug resistance. Several observations support this proposition. First, both enzymes are mechanistically identical. Second, structure-based alignment of the two enzymes displays their structure similarity (Figures 11 and 12). Third, most potent noncovalent inhibitors of the HIV PR are not good inhibitors of the FIV PR, whereas FIV PR inhibitors are often better HIV PR inhibitors. Therefore, good low molecular weight inhibitors of the FIV PR should be better for the HIV PR. It is possible to design inhibitors more selective for the FIV PR as the enzyme can accommodate slightly larger side chains in the P_1 and P_1' regions; however, this has not been proven yet. Fourth, the HIV PR isolated from the inhibitor-resistant mutants contains mutations that are found in the corresponding positions of the FIV PR. As shown in Figure 12, the six highlights represent the changed amino acids found in the HIV PR isolated from mutants resistant to HIV PR inhibitors which are identical to those found in the FIV PR in the same position. The last two observations suggest that the FIV PR is a good model for the development of HIV PR inhibitors with less resistance problem. One example to support this hypothesis is that the HIV PR isolated from a mutant resistant to a synthetic protease inhibitor contains the I50V mutation, and the mutant protease is less inhibited by the same inhibitor by 80-fold.[39] A similar proposal was made regarding the use of FIV as a model for development of reverse transcriptase inhibitors for HIV.[40] In any case, further investigation is needed to test this hypothesis.

Table 1 summarizes the inhibitory activities of the isosteres prepared in this study against the HIV and/or FIV PR.

In summary, this study provides a new class of the HIV/FIV PR inhibitors which contain new complementary groups away from the p and p′ regions in the proline moiety as useful leads for the development of more potent inhibitors (Figure 13). The mode of action of the α-keto amide inhibitor is of particular interest as it represents a new type of mechanism-based enzyme inhibition which could lead to the development of tight-binding inhibitors to overcome the problem of resistance.

Chemistry

The synthesis of the core isostere **2** has been modified from the method previously employed by our group. The α-keto ester **15** was synthesized according to the method described by Angelastro (Scheme 1).[41] It is possible to synthesize α-keto esters *via* other methodologies,[42,43] but the route employed was found to be most concise and high yielding.

It is interesting to note that the α-keto ester **15** is a potent inhibitor of cysteine and serine proteases.[24] In an attempt to synthesize the α-keto acid directly we coupled lithiated furan to the Weinreb amide of phenylalanine with the aim of using ozonolysis to give the acid directly. Unfortunately, thus far all attempts at ozonolysis of phenylalanine derivative **17** have resulted in low yields of the desired acid (Scheme 2).

It was not possible to cleanly couple the proline derivative **22** to the α-keto acid **16**, as multiple side products were produced as a result of nucleophilic attack at the electrophilic ketone moiety (Scheme 1). All attempts at forming the dithiane or dithiolane derivative of the ketone moiety of **15**, resulted in low yields after tediously long reaction times, making this approach unfavorable.

Reduction of the ketone moiety with sodium borohydride gave a separable mixture of diastereomeric alcohols **18** and **19** which could then be coupled to the desired proline derivatives after hydrolysis of the ester group. Hydrolysis of the ester moiety of **18** or **19** to the corresponding acids **20** or **21**, respectively, followed by coupling to the proline derivative **22** gave the α-hydroxy amides **23** or **24**. Dess–Martin oxidation of either α-hydroxy amide **23** or **24** gave the desired α-keto amide **2** as a 3:1 mixture of diastereomers (Scheme 3).[44] Compounds **2a–c** were synthesized in a similar manner.

When the α-keto amide **2** is dissolved in either methanol or DMSO, the ratio of isomers changes immediately from 3:1 (in chloroform) to 1:1. Racemization is due to the increased acidity of the α-hydrogen atom next to the ketone moiety in **2** and has been observed with other similar α-keto amides.[25]

The extended α-keto amide isostere **4** used to inhibit FIV PR was synthesized in a similar manner to the original dipeptide isostere **2,** with the only difference being that the *S*-α-hydroxy acid **21** was coupled to the tripeptide **25** to give the α-hydroxy amide **26**, before oxidation to the desired α-keto amide **4** as illustrated in Scheme 4.

The hydroxyethylamine inhibitors (compounds **5–11**) were prepared by coupling the desired pyrrolidine derivative to the epoxide **27**[45] *via* reflux in methanol, using triethylamine as shown in Scheme 5. The permethylated α-keto amide derivative **12** was prepared using the normal procedure used for the synthesis of α-keto amides as shown in Scheme 6.

Experimental Section

Biological Assays. For determination of IC_{50} values for HIV PR, a backbone engineered HIV-1 PR, prepared by total chemical synthesis[46] 450 nM final concentration was added to a solution (152 μL final volume) containing inhibitor, 28 μM fluorogenic peptide substrate (sequence Abz-Thr-Ile-Nle-Phe-(*p*-NO_2)-Gln-Arg-NH_2),[38] and 1.8% dimethyl sulfoxide in assay buffer: 100 mM MES buffer containing 0.5 mg/mL BSA (Bovine Serum Album, fatty acid, nuclease and protease free—to stabilize enzyme) at pH 5.5. The solution was mixed and incubated over 5 min during which time the rate of substrate cleavage was monitored by continuously recording the change in fluorescence of the assay solution. An excitation filter of 325 nm and an emission filter of 420 nm were used. This data was converted into μM substrate cleaved per minute, using a predetermined standard calibration curve of change in fluorescence against concentration of substrate cleaved.

Determination of K_i for HIV PR was performed similarly with the following modifications. The substrate concentrations used were 57, 43, 28, and 14 μM. All other concentrations were as above. The curve

(39) The inhibitor VX-478 (K_i = 0.06 nM) developed by the Vertex group was found to be 80-fold less potent (K_i = 50 nM) for the resistant mutant HIV PR with the I50V mutation: Kim, E. Presented at the 9th Meeting of Groups Studying the Structure of AIDS-Related Systems and Their Application to Targeted Drug Design, June 5–7, 1995, NIH.

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(41) Angelastro, M. R.; Peet, N. P.; Bey, P. *J. Org. Chem.* **1989**, *54*, 3913–3916.

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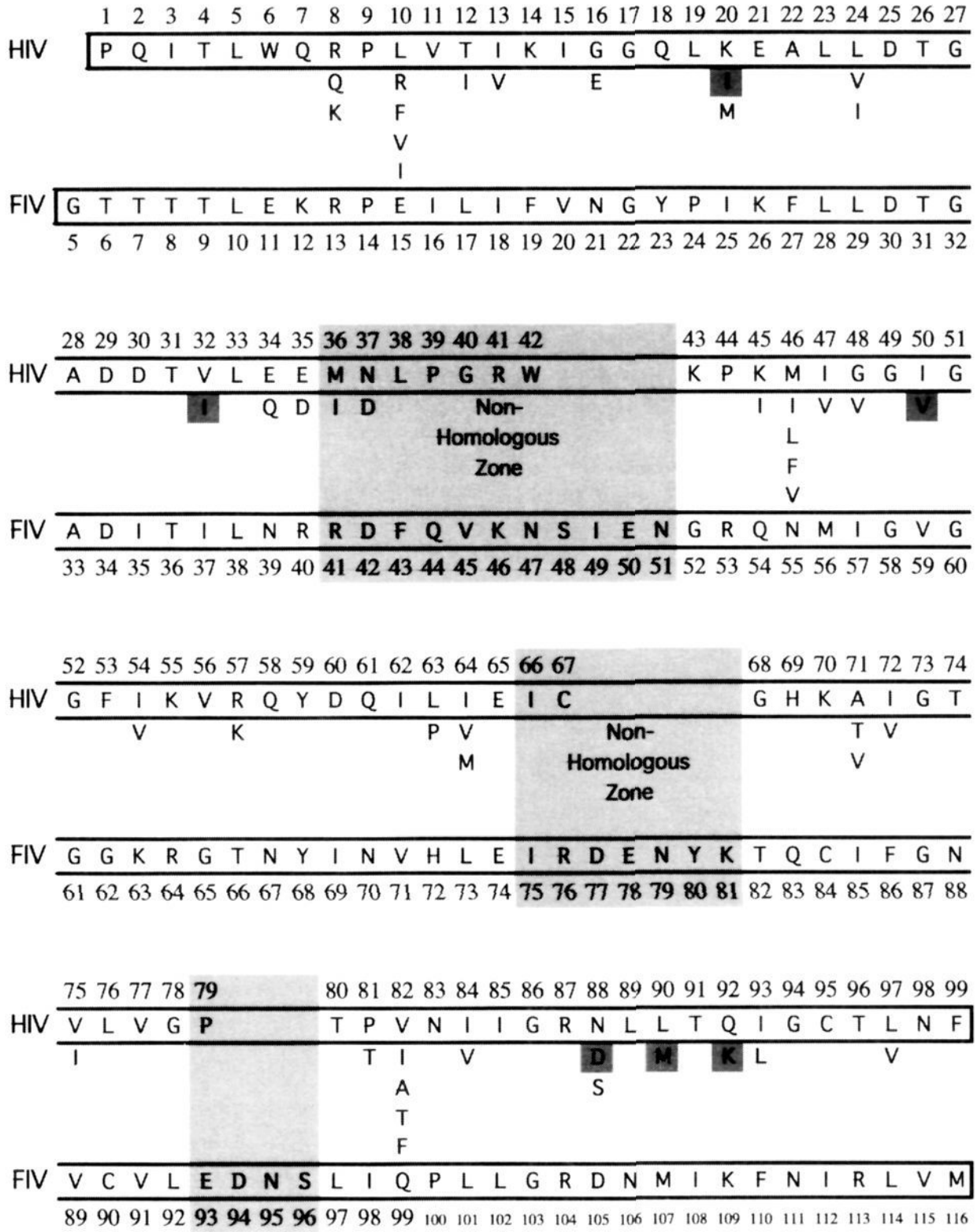

Figure 11. Structure-based sequence alignment of the HIV and FIV proteases. Amino acids appearing in the area between the HIV and FIV sequences (light gray background) are HIV PR mutations that have been shown to be linked to drug resistance.[3] Drug resistant mutations highlighted in gray are those which converge to the FIV PR sequence identity, indicating areas where FIV PR resembles drug resistant mutants of HIV PR. Dark gray areas indicate regions in which the two enzymes are structurally dissimilar.

fit for the data was determined, and the subsequent K_i was derived using a computer program based on the equation of Morrison[47] for tight binding inhibitors.

For determination of K_i and IC_{50} for FIV PR, 0.125 μg of the enzyme was added to a solution (100 μL final volume) containing inhibitor, 560 μM peptide substrate (sequence Gly-Lys-Glu-Glu-Gly-Pro-Pro-Gln-Ala-Tyr~Pro-Ile-Gln-Thr-Val-Asn-Gly), and 2% dimethyl sulfoxide in a 1:3 mixture of assay buffer (as above) and 4 M $NaCl_{aqueous}$ solution. The solution was mixed and incubated for 10 min at 37 °C, and the reaction was quenched by addition of 8 M guanidine HCl solution containing 0.2 M sodium acetate at pH 4.2 (100 μL). The cleavage products and substrate were separated by reverse phase HPLC. Absorbance was measured at 215 nm, peak areas were determined, and percent conversion to product was calculated using relative peak areas. The data were plotted as $1/V$ (V = rate substrate is cleaved in nmol/min) against inhibitor concentration, and the $-K_i$ was determined as the point at which the resulting line intersects with $1/V_{max}$ (V_{max} = 6.85 nmol/min). IC_{50} was determined as the inhibitor concentration at 50% inhibition. V_{max} (6.85 $\pm$ 0.7 nmol min^{-1}) and K_m (707 $\pm$ 70 μM) for FIV PR were determined from a plot of $1/V$ (V = rate in nmol/min) against 1/[S] ([S] = substrate concentration in nmol). The data used were generated similarly to that for K_i with the following modifications. The substrate concentrations used were 560, 448, 336, 224, 111, and 56 μM, in the absence of inhibitor.

Purification of FIV PR. A 503 base pair Eco R1-Bam H1 fragment containing the coding sequence of FIV PR was cloned from FIV-34TF10[48] into the pT7-7 vector.[49] The 5' end of the insert was modified by the addition of an Nde1 adaptor, which provided the proper reading frame with initiation of translation from the methionine encoded in the latter site. Translation resulted in production of an 18.6 kDa precursor, which autoprocessed to a 13.2 kDa FIV PR plus N- and C-terminal fragments of 3.6 and 1.8 kDa, respectively. The construct was transformed into *E. coli* strain BL21.DE3, lys S,[50] and overnight cultures were used to inoculate 15 L fermentations, performed using Circlegrow medium (Bio 101) plus 100 μL ampicillin, 20 μM chloramphenicol, at 37 °C. The cells were allowed to reach mid-log phase, then the temperature was reduced to 24 °C and IPTG was added to a final concentration of 1 mM. The fermentation was allowed to proceed for 16 h, at which time the cells were harvested by centrifugation and frozen at −70 °C in 100 g aliquots for future use.

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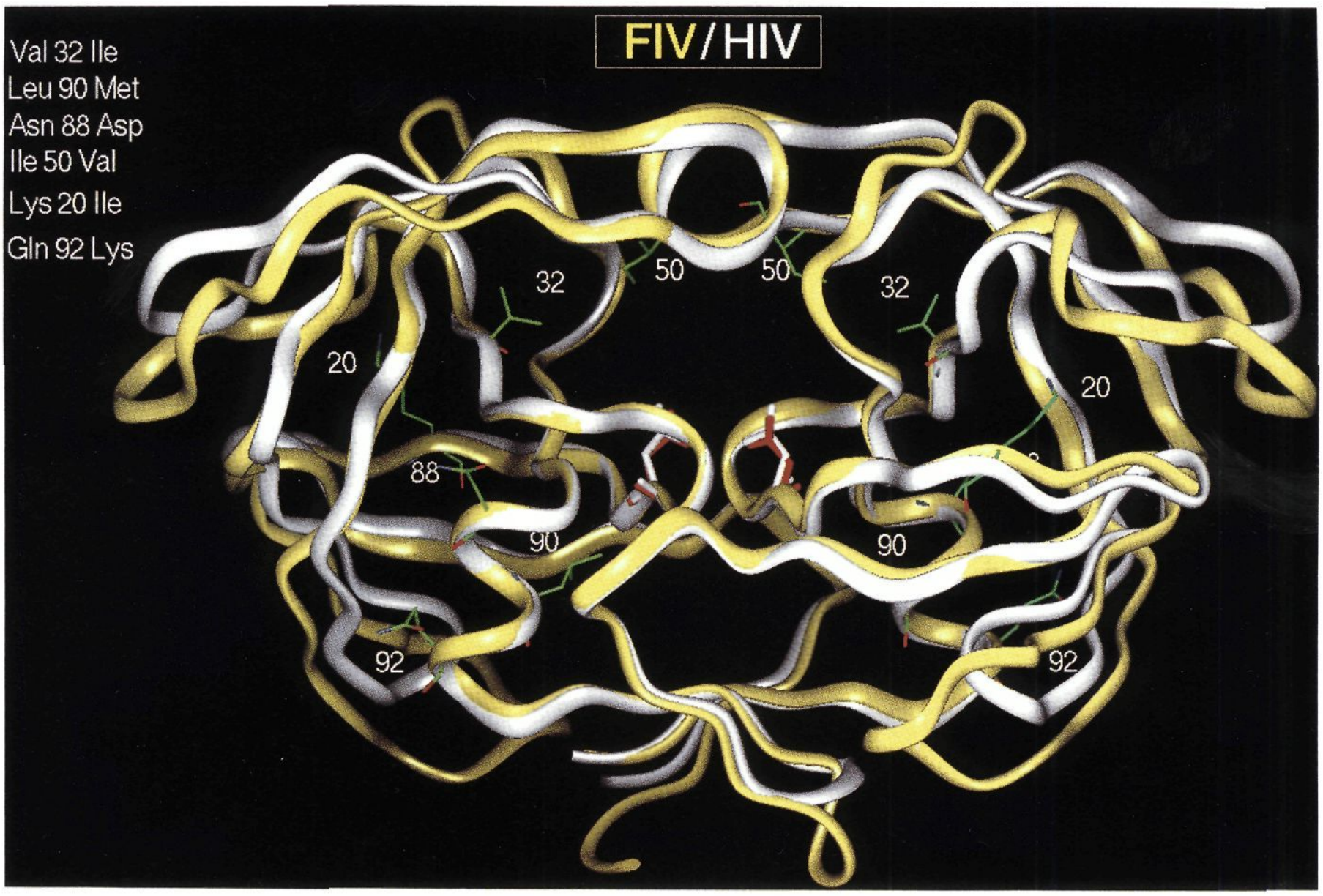


Figure 12. An overlay of the structurally similar FIV (yellow) and HIV (white) proteases details similarities between the two proteases and drug-resistant mutants of HIV PR. Mutation of the HIV PR residues (green) to the FIV sequence identity at these positions occurs through resistance to a variety of HIV PR inhibitors in clinical use or trials.[3] For reference, the catalytic aspartyl residues at the bottom of the active site are shown in red/white. The flaps are at the top center of the structure. The active site is the black area between the highlighted residues, 50, and the catalytic aspartyl residues.

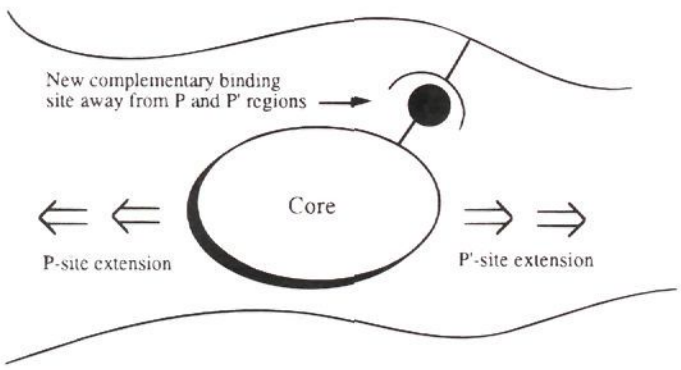


Figure 13.

Cells (100 g) were lysed by addition of 600 mL, 50 mM Tris-HCl, pH 8, 5 mM EDTA and 2 mM 2-mercaptoethanol to the frozen pellet. The cells lysed upon thawing, and the viscous mixture was homogenized at 4 °C for 2 min in a Waring blender. The sample was centrifuged at 8000 × *g* for 20 min, and the pellet was discarded. The sample was diluted to 1 L and then subjected to tangential flow against a 300 K cut-off membrane (Filtron), and the PR was washed through the membrane using five L of the same buffer. The retentate was discarded, and the flow-through supernatant was concentrated by tangential flow against a 10 K cut-off membrane. The retentate was passed over a DE52 anion exchange column (5 × 20 cm) equilibrated in the same buffer. The flow-through from this column was passed over an S-Sepharose Fast Flow matrix (2.5 × 20 cm column, Pharmacia), again equilibrated at pH 8 in the same buffer. The flow-through from S-Sepharose was made 1 M with respect to ammonium sulfate and applied to a phenyl sepharose column (Pharmacia, 1.5 × 10 cm), washed with lysis buffer containing 1 M ammonium sulfate, and then eluted with a 100−0% linear ammonium sulfate gradient. Peak fractions containing PR were pooled, concentrated using Centripreps (Amicon), and dialyzed against 10 mM Tris-HCl, pH 8, 5 mM EDTA, and 2 mM 2-mercaptoethanol. The sample was made 10 mM with respect to MOPS, adjusted to pH 5.5 with HCl, and then applied to a Resource S column (Pharmacia) equilibrated in 10 mM Tris-MOPS, pH 5.5, 5 mM EDTA, and 2 mM 2-mercaptoethanol. PR was eluted using a linear 0−300 mM NaCl gradient in the same buffer. Peak fractions were pooled, concentrated, and stored as aliquots at −20 °C for further studies. The integrity of the isolated FIV PR was confirmed by ion spray mass spectrometry.

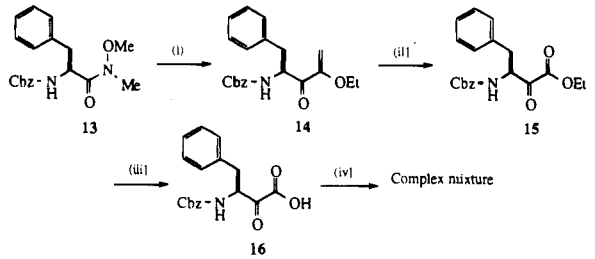
Table 1. Inhibition Activities Against HIV and/or FIV PR

	HIV PR		FIV PR	
compd	IC_{50}	K_i	IC_{50}	K_i
1	6 μM	6 μM		
2	700 nM	214 nM	>70 mM	
2a		220 nM		
2b		318 nM		
2c		65 nM		
3	3.18 μM	405 nM		
4		154 nM	25 μM	29 μM
5	1.8 mM			
6	100 μM			
7	1.6 mM			
8	60 μM			
9	17 μM			
10	2.9 μM			
11	3.9 μM			
12	20 μM			

Crystallization and X-ray Analysis of HIV PR Complexed with Inhibitor 2. Crystallization was carried out at room temperature by the hanging-drop vapor diffusion technique. HIV PR at 6.1 mg/mL in 50 mM sodium acetate, pH 5.6, 10 mM dithiothreitol, was incubated with 5-fold molar excess of inhibitor **2** (stock: 20 mg/mL in DMSO)

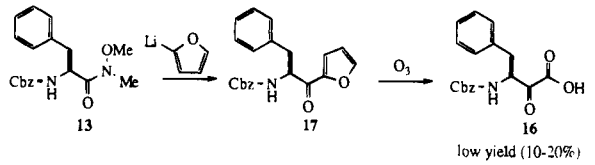
(51) Hendrickson, W. A. *Methods Enzymol.* **1985**, *115*, 252−270.

Scheme 1[a]

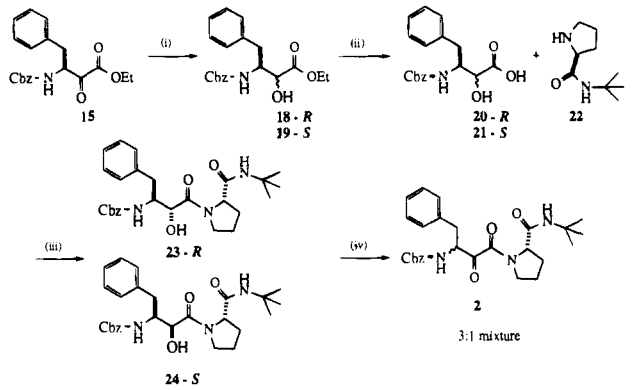


[a] (i) *t*-BuLi, ethyl vinyl ether, $MgBr_2$, THF; (ii) O_3, CH_2Cl_2; (iii) 0.17N LiOH, MeOH/H_2O, 2:1, 98%; (iv) **22**, EDC, HOBT, DIEA, DMF/CH_3CN, (1:1).

Scheme 2

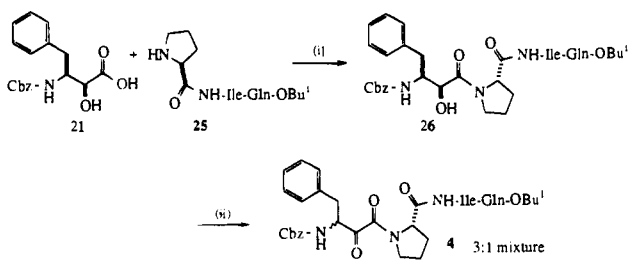


Scheme 3[a]



[a] (i) $NaBH_4$, MeOH, 0 °C, 95%; (ii) 0.17 N LiOH, MeOH/H_2O, 2:1, 98%; (iii) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1). 84%; (iv) Dess–Martin periodinane, CH_2Cl_2, 95%.

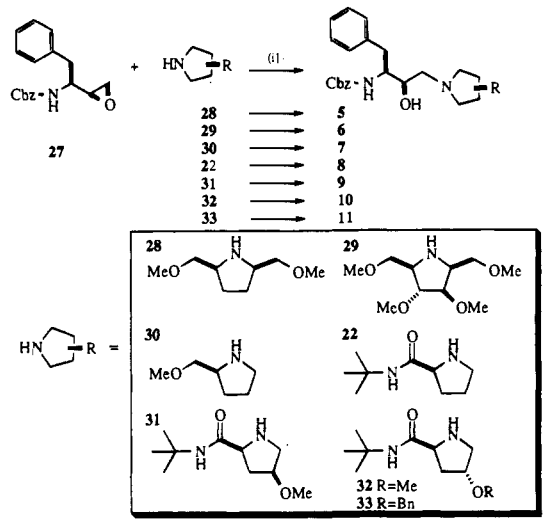
Scheme 4[a]



[a] (i) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1). 80%; (ii) Dess–Martin periodinane, CH_2Cl_2, quantitative.

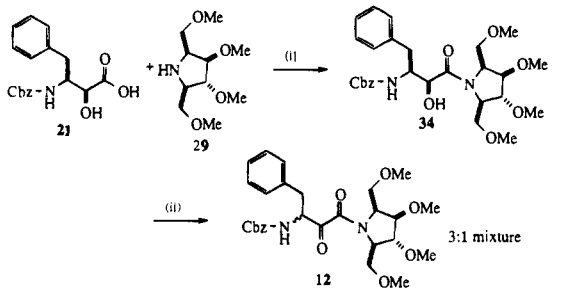
for 2 h. and 4 μL of the solution was mixed with an equal volume of reservoir buffer consisting of 75 mM sodium citrate/150 mM sodium phosphate, pH 6.2. 25% saturated ammonium sulfate, and 0.02% sodium azide. The drops were then seeded with microcrystals prepared fresh by breaking a small HIV PR/inhibitor **2** crystal in a drop of reservoir buffer (initial seeds were prepared from crystals consisting of HIV PR complexed with another inhibitor). Crystals appeared in 2 days and grew to their final size in about 10 days. They belong to an orthorhombic system, space group $P2_12_12_1$ and unit cell parameters a = 52.13 Å, b = 59.15 Å, and c = 62.06 Å. Diffraction data were collected on MAR Image Plate System, with X-rays produced using a Rigaku RU-200 generator with a Cu anode, operated at 50 kV and 100 mA, equipped with a graphite monochromator. A total of 87 096 reflections were measured, resulting after merging in 10 632 independent structure amplitudes with $I \geq \sigma(I)$, corresponding to 90.8% of theoretically possible data in a 20.0–2.1 Å resolution shell, with R_{merge} = 7.4%. The refinement with the program PROLSQ[51] resulted in a structure with R = 0.164 with rms deviations of bond lengths from ideality of 0.012 Å.

Scheme 5[a]



[a] (i) Methanol, Et_3N, reflux, 24 h, 40–60%.

Scheme 6[a]



[a] (i) EDC, HOBT, DIEA, DMF/CH_3CN, (1:1), 80%; (ii) Dess–Martin periodinane, CH_2Cl_2, quantitative.

Chemical Synthesis. General Procedures. All manipulations were conducted under an inert atmosphere (argon or nitrogen). All solvents were reagent grade. Anhydrous ether, tetrahydrofuran (THF), and toluene were distilled from sodium and/or benzophenone ketyl. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride (CaH_2). *N,N*-Dimethylformamide (DMF) and acetonitrile were distilled from phosphorous pentoxide and calcium hydride. Methanol was distilled from magnesium and iodine. Organic acids and bases were reagent grade. All other reagents were commercial compounds of the highest purity available. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel (60 F-254) plates (0.25 mm). Visualization was effected using standard procedures unless otherwise stated. Flash column chromatography was carried out on Merck silica gel 60 particle size (0.040–0.063 mm, 230–400 Mesh). Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton and carbon magnetic resonance spectra (^{1}H-NMR, ^{13}C-NMR) were recorded on either a Bruker AM-500, AMX-400, or AC250 MHz Fourier transform spectrometer. Coupling constants (J) are reported in hertz, and chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS, 0 ppm), MeOH (3.30 ppm for ^{1}H and 49.0 ppm for ^{13}C), or $CHCl_3$ (7.24 ppm for ^{1}H and 77.0 ppm for ^{13}C) as internal reference. Infrared spectra (IR) were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer. Absorptions are reported in wavenumbers (cm^{-1}).

Peptide fragments described herein were synthesized using traditional peptide coupling methodologies [EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl), HOBt (1-hydroxybenzotriazole) and DIEA

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(diisopropylethylamine)]. Esters were hydrolyzed either by base (LiOH for methyl esters) or acid (TFA for *tert*-butyl esters).

(2*R*,3*S*)- and (2*S*,3*S*)-*N*-(Benzyloxycarbonyl)-AHAP-(3-amino-2-hydroxy-4-phenylbutanoic acid) Ethyl Ester 18 and 19, Respectively. The substrate **15** (600 mg, 1.7 mmol) was dissolved in methanol (10 mL) and cooled to 0 °C. Sodium borohydride (70.3 mg, 1.9 mmol) was then added. After 20 min the reaction was quenched by addition of saturated ammonium chloride (aqueous) (10 mL). The reaction mixture was concentrated *in vacuo* to remove most of the methanol. The aqueous residue was then extracted with ethyl acetate (3 × 20 mL), washed with brine (10 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give the crude product as a mixture of diastereomers. The alcohols were separated by flash chromatography eluting with 15% ethyl acetate in hexane to give the alcohols in a ratio of 3:4, **18** and **19** (590 mg, 97%): $R_f = 0.54$ and 0.40, respectively (EtOAc/hexane, 1:2).

18 (2*R*,3*S*—*colorless oil*): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.20 (10H, m), 5.08 (1H, d, J = 9.5), 5.03 (2H, s), 4.40–4.32 (1H, m), 4.20–4.10 (2H, m), 4.07 (1H, d, J = 2.0), 3.18 (1H, d, J = 3.5), 3.00–2.80 (2H, m), 1.20 (3H, t, J = 10.0); IR (NaCl) ν_{max} 3368, 3030, 2981, 1731, 1520, 1455, 1246, 1104, 1055, 748, 699 cm^{-1}; FABHRMS (NBA) *m/e* 358.1659 ([M + H]$^+$, $C_{20}H_{23}NO_5$ requires 358.1654); (Found: C, 67.30; H, 6.50; N, 3.99. $C_{20}H_{23}NO_5$ requires C, 67.21; H, 6.49; N, 3.92).

19 (2*S*,3*S*—*crystalline*): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.18 (10H, m), 5.17 (1H, d, J = 9.5), 5.04 (2H, s), 4.43–4.38 (1H, m), 4.33 (1H, dd, J = 4.5, 2.0), 4.15–4.08 (1H, m), 3.98–3.92 (1H, m), 3.28 (1H, d, J = 5.0), 2.84–2.74 (2H, m), 1.22 (3H, t, J = 7.5); IR (NaCl) ν_{max} 3368, 3030, 2980, 1731, 1520, 1455, 1246, 1104, 1055, 748, 699 cm^{-1}; FABHRMS (NBA) *m/e* 358.1661 ((M$^+$ + H), $C_{20}H_{23}NO_5$ requires 358.1654); (Found: C, 67.22; H, 6.57; N, 3.90. $C_{20}H_{23}NO_5$ requires C, 67.21; H, 6.49; N, 3.92); mp 88–89 °C.

(2*R*,3*S*)- and (2*S*,3*S*)-*N*-(Benzyloxycarbonyl)-3-amino-2-hydroxy-4-phenylbutanoic acid 20 and 21, Respectively. The substrate (**18** or **19**) (250 mg, 0.70 mmol) was dissolved in 0.25 N LiOH in methanol/water, 2:1 (5 mL), and stirred at ambient temperature for 30 min. The pH of the reaction was adjusted to pH 7.0 with 1 N HCl (aqueous), and the methanol was removed *in vacuo*. The aqueous residue was then acidified to pH 2.0 with 1 N HCl (aqueous) and extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with water (10 mL) brine (10 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the desired acid (**20** or **21**) as a white solid (212 mg, 92%). The acids were purified by recrystallization from hot ethanol.

20 (2*R*,3*S*): ^{1}H NMR (500 MHz, CD_3OD) δ 7.31–7.18 (10H, m), 6.87 (1H, d, J = 9.5), 5.00 (1H, d, J = 10.0), 4.96 (1H, d, J = 10.0), 4.31–4.23 (1H, m), 4.07 (1H, d, J = 2.5), 2.93 (1H, dd, J = 13.5, 7.5), 2.83 (1H, dd, J = 13.5, 8.0); ^{13}C NMR (125 MHz, CD_3OD) δ 176.6 (C=O), 158.5 (C=O), 139.8 (C), 138.5 (C), 130.7 (2 × CH), 129.8 (2 × CH), 129.7 (2 × CH), 129.7 (CH), 129.1 (CH), 128.8 (CH), 127.8 (CH), 67.6 (CH), 57.1 (CH_2), 57.0 (CH_2), 39.3 (CH); FABHRMS (NBA) *m/e* 330.1353 ([M + H]$^+$, $C_{18}H_{19}NO_5$ requires 330.1341); mp 209–210 dec.

21 (2*S*,3*S*): ^{1}H NMR (500 MHz, CD_3OD) δ 7.28–7.18 (10H, m), 7.09 (1H, d, J = 12.5), 4.97 (1H, d, J = 12.5), 4.92 (1H, d, J = 12.5), 4.26 (1H, d, J = 4.0), 4.25–4.20 (1H, m), 2.81 (1H, dd, J = 14.0, 4.0), 2.76 (1H, dd, J = 14.0, 4.0); ^{13}C NMR (125 MHz, CD_3OD) δ 175.9 (C=O), 158.5 (C=O), 140.0 (C), 138.6 (C), 130.6 (3 × CH), 129.6 (CH), 129.5 (3 × CH), 129.0 (CH), 128.8 (CH), 127.6 (CH), 74.3 (CH), 67.4 (CH_2), 57.1 (CH), 36.5 (CH_2); FABHRMS (NBA/NaI) *m/e* 352.1174 ([M + Na]$^+$, $C_{18}H_{19}NO_5$ requires 352.1161); (Found: C, 65.34; H, 5.75; N, 4.33. $C_{18}H_{19}NO_5$ requires C, 65.64; H, 5.82; N, 4.25); mp 173–174 °C dec.

***N*-(*tert*-Butoxycarbonyl)-L-prolyl-*tert*-butyl Amide.** The substrate *N*-*tert*-butoxycarbonyl-L-proline (3.0 g, 13.9 mmol) was dissolved in dry CH_2Cl_2 (20 mL). HOBT (2.07 g, 15.3 mmol), EDC (2.93 g, 15.3 mmol), and *tert*-butylamine (1.6 mL, 15.3 mmol) were added, and the mixture was stirred for 18 h at ambient temperature. The reaction was diluted with ethyl acetate (100 mL), washed with water (2 × 20 mL), 1 N HCl (aqueous) (10 mL), saturated sodium bicarbonate solution (aqueous) (10 mL), water (10 mL), and brine (10 mL), and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Purification by flash chromatography, eluting with 33% EtOAc in hexane, gave *N*-*tert*-butoxycarbonyl-L-prolyl-*tert*-butyl amide as a colorless oil (1.53 mg, 40%). $R_f = 0.46$ (EtOAc/hexane, 1:1).

^{1}H NMR signals broadened due to rotamers: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.85 (0.5H, br s), (0.5H, br s), 4.27–4.03 (1H, m), 3.50–3.18 (2H, m), 2.47–2.74 (4H, m), 1.48 (9H, br s), 1.35 (9H, br s); IR (NaCl) ν_{max} 3298, 3086, 2976, 1698, 1660, 1531, 1398, 1162 cm^{-1}; FABHRMS (NBA/NaI) *m/e* 293.1832 ([M + Na]$^+$, $C_{14}H_{26}N_2O_3$ requires 293.1841).

L-Prolyl-*tert*-butyl Amide 22. *N*-*tert*-Butoxycarbonyl-L-prolyl-*tert*-butyl amide (600 mg, 2.22 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. TFA (10 mL) was then added to the solution. After 1 h at 0 °C the reaction was concentrated *in vacuo* (any remaining TFA was removed under high vacuum) to give the trifluoroacetic acid salt of the desired amine **22** as a colorless oil (800 mg, 95%). The amine was used without further purification in subsequent coupling steps.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (1H, br s), 6.90 (1H, br s), 4.52 (1H, br s), 3.35 (2H, br s), 2.49–2.34 (1H, m), 2.08–1.97 (3H, m), 1.34 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.4 (C=O), 59.6 (CH), 52.3 (C), 46.6 (CH_2), 30.4 (CH_2), 28.2 (3 × CH_3), 24.6 (CH_2); FABHRMS (NBA) *m/e* 171.1500 ([M + H]$^+$, $C_9H_{18}N_2O$ requires 171.1497).

General Peptide Coupling Procedure: (2*S*,3*R*)- and (2*S*,3*S*)-3-(*N*-Benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-L-prolyl-*tert*-butyl Amide 23 and 24. The substrate 20 or 21 (70 mg, 0.213 mmol) was dissolved in dry DMF (3 mL). HOBT (31 mg, 0.22 mmol), EDC (43 mg, 0.224 mmol), and DIEA (122 μL, 0.703 mmol) were added, and the mixture was stirred for 30 min at room temperature. The secondary amine 22 as its TFA salt (73 mg, 0.255 mmol) was added, and the reaction was stirred for 18 h. The reaction mixture was diluted with ethyl acetate (20 mL) and added to saturated ammonium chloride (30 mL). The aqueous phase was extracted with ethyl acetate (3 × 10 mL). The combined organic phases were then washed with water (2 × 5 mL), 1 N HCl (aqueous) (5 mL), saturated sodium bicarbonate solution (aqueous) (50 mL), water (5 mL), and brine (5 mL) and dried ($MgSO_4$) before concentration *in vacuo* to give the crude product. Flash chromatography eluting with ethyl acetate/hexane, 1:1 to give the desired coupled product 23 or 24 as a colorless oil (74 mg, 72%): $R_f = 0.33$ and 0.28, respectively (EtOAc/hexane, 1:1).

23 (2*R*,3*S*): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.23 (10H, m), 6.44 (1H, s), 5.16 (1H, d, J = 9.5), 5.03 (2H, s), 4.24–4.18 (1H, m), 4.11 (1H, d, J = 5.5), 3.97–3.90 (2H, m), 3.28–3.23 (1H, m), 3.12–3.06 (1H, m), 2.99–2.90 (2H, m), 2.18–2.12 (1H, m), 2.01–1.96 (1H, m), 1.89–1.83 (1H, m), 1.83–1.76 (1H, m), 1.27 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4 (C=O), 169.9 (C=O), 156.0 (C=O), 137.4 (C), 136.7 (C), 129.2 (2 × CH), 128.7 (2 × CH), 128.4 (2 × CH), 128.0 (CH), 127.9 (2 × CH), 126.9 (CH), 68.6 (CH), 66.7 (CH_2), 61.7 (CH), 52.8 (CH), 51.1 (C), 46.1 (CH_2), 38.4 (CH_2), 28.6 (3 × CH_3), 27.4 (CH_2), 24.7 (CH_2); IR (NaCl) ν_{max} 3318, 2966, 1714, 1667, 1537, 1454, 1366, 1041 cm^{-1}; FABHRMS (NBA) *m/e* 482.2677 ([M + H]$^+$, $C_{27}H_{35}N_3O_5$ requires 482.2655); (Found: C, 67.22; H, 7.32; N, 8.80. $C_{27}H_{35}N_3O_5$ requires C, 67.34; H, 7.33; N, 8.73).

24 (2*S*,3*S*) (major rotamer only): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.17 (10H, m), 6.45 (1H, s), 5.21 (1H, d, J = 8.8), 4.99 (2H, s), 4.60 (1H, dd, J = 6.8, 2.1), 4.52–4.47 (1H, m), 4.21–4.13 (1H, m), 3.81–3.66 (3H, m), 2.75–2.60 (2H, m), 2.41–2.30 (1H, m), 2.25–2.09 (1H, m), 2.05–1.90 (2H, m), 1.30 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3 (C=O), 169.6 (C=O), 156.0 (C=O), 137.3 (2 × C), 129.1 (2 × CH), 128.5 (2 × CH), 128.4 (2 × CH), 128.0 (CH), 127.8 (2 × CH), 126.5 (CH), 71.3 (CH), 66.6 (CH_2), 61.1 (CH), 54.3 (CH), 51.3 (C), 47.5 (CH_2), 33.8 (CH_2), 28.6 (3 × CH_3), 26.9 (CH_2), 25.4 (CH_2); IR (NaCl) ν_{max} 3318, 2966, 1714, 1667, 1537, 1454, 1366, 1041 cm^{-1}; FABHRMS (NBA/CsI) *m/e* 614.1645 ([M + Cs]$^+$, $C_{27}H_{35}N_3O_5$ requires 614.1631); (Found: C, 67.20; H, 7.66; N, 8.54. $C_{27}H_{35}N_3O_5$ requires C, 67.34; H, 7.33; N, 8.73).

General Dess–Martin Oxidation Procedure. (3*S*)- and (3*R*)-3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-L-prolyl-*tert*-butyl Amide 2. The substrate **23** (21 mg, 0.044 mmol) was dissolved in dry CH_2Cl_2 (2 mL), and Dess–Martin periodinane (26 mg, 0.088 mmol) was added. The reaction mixture was stirred at ambient temperature for 24 h, then diluted with ethyl acetate (10 mL), and quenched by addition of saturated sodium bicarbonate (aqueous) (5

mL) and sodium thiosulfate. The aqueous phase was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give the crude product. Flash chromatography eluting with 30% ethyl acetate in hexane gave the desired product **2** as a 3:1 mixture of diastereomers (colorless oil) (20 mg, 95%): R_f = 0.47 (EtOAc/hexane, 1:2).

Spectral data on mixture: ^{1}H NMR (400 MHz, DMSO) δ 7.86 (1H, d, *J* = 7.8), 7.71 (1H, d, *J* = 8.3), 7.64 (1H, s), 7.53 (1H, s), 7.37−7.10 (20 H, m), 5.10 (1H, ddd, *J* = 11.0, 8.3, 2.4), 5.01 (1H, d, *J* = 12.6), 4.95 (1H, d, *J* = 12.6), 4.95 (1H, d, *J* = 16.3), 4.88 (1H, d, *J* = 16.3), 4.79−4.73 (1H, m), 4.66 (1H, dd, *J* = 7.8, 4.2), 4.26 (1H, dd, *J* = 7.8, 4.5), 3.60−3.37 (3H, m), 3.33−3.24 (1H, m), 3.18 (1H, dd, *J* = 14.7, 2.4), 3.13 (1H, dd, *J* = 10.2, 3.9), 2.79 (1H, dd, *J* = 13.7, 10.2), 2.46 (1H, dd, *J* = 14.7, 11.0), 2.23−2.17 (1H, m), 2.04−1.97 (1H, m), 1.90−1.60 (6H, m), 1.24 (9H, s), 1.22 (9H, s); ^{13}C NMR (100 MHz, DMSO) δ 198.91 (C=O), 196.7 (C=O), 170.7 (C=O), 169.9 (C=O), 162.6 (C=O), 162.2 (C=O), 156.1 (C=O), 155.9 (C=O), 138.5 (C), 137.6 (C), 136.9 (C), 136.9 (C), 129.0 (CH × 2), 128.8 (CH × 2), 128.4 (CH × 8), 127.8 (CH × 2), 127.6 (CH × 2), 127.6 (CH × 2), 126.5 (CH), 126.4 (CH), 65.6 (CH_2), 65.3 (CH_2), 60.3 (CH), 59.7 (CH), 59.2 (CH), 58.2 (CH), 50.3 (C), 50.1 (C), 47.6 (CH_2), 47.4 (CH_2), 34.8 (CH_2), 34.1 (CH_2), 32.5 (CH_2), 29.1 (CH_2), 28.5 (CH_3 × 3), 28.4 (CH_3 × 3), 24.5 (CH_2), 21.7 (CH_2); IR (NaCl) υ_{max} 3325, 2966, 1715, 1670, 1634, 1531, 1454, 1258, 1051, 738, 699; FABHRMS (NBA/NaI) *m/e* 502.2295 ([M + Na]$^+$, $C_{27}H_{33}N_3O_5$ requires 502.2318); (Found: C, 67.62; H, 7.05; N, 8.96. $C_{27}H_{33}N_3O_5$ requires C, 67.62; H, 6.94; N, 8.76).

(3*S*)- and (3*R*)-3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-[2′(*S*)-(*tert*-butylamido-4′(*R*)-methoxy]pyrrolidine 2a. The α-hydroxy acid **21** was coupled to *trans*-4-methoxy-L-prolyl-*tert*-butyl amide using the general coupling procedure outlined for compound **23**, followed by Dess−Martin oxidation to give **2a** in good yield (40 mg, 70%) as a colorless foam (3:1 mixture of diastereomers): R_f = 0.33 (20% acetone in toluene).

(**3*S***) (major): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.10 (10H, m), 6.48 (1H, s), 5.33 (1H, d, *J* = 7.3), 5.16−5.01 (3H, m), 4.50 (1H, t, *J* = 6.9), 4.09−4.00 (1H, br s), 3.75 (1H, br d, *J* = 12.3), 3.66 (1H, dd, *J* = 12.6, 4.4), 3.31 (3H, s), 3.27 (1H, dd, *J* = 13.0, 5.8), 3.13 (1H, dd, *J* = 14.0, 7.6), 2.51−2.40 (1H, m), 2.12−2.00 (1H, m), 1.32 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4 (C=O), 168.6 (C=O), 162.8 (C=O), 155.8 (C=O), 136.1 (C), 135.7 (C), 129.3 (CH × 3), 128.7 (CH × 3), 128.5 (CH × 2), 128.2 (CH), 128.0 (CH), 79.1 (CH), 67.1 (CH_2), 59.9 (CH_3O), 58.6 (CH), 56.7 (CH), 52.6 (C), 51.4 (CH_2), 36.7 (CH_2), 32.4 (CH_2), 28.6 (CH_3 × 3).

(**3*R***) (minor): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.10 (10H, m), 5.82 (1H, s), 5.24 (1H, d, *J* = 7.6), 5.16−5.01 (3H, m), 4.64 (1H, t, *J* = 7.1), 4.09−4.00 (1H, br s), 3.93 (1H, br d, *J* = 13.8), 3.61 (1H, dd, *J* = 13.2, 4.7), 3.40 (1H, dd, *J* = 14.2, 4.2), 3.30 (3H, s), 2.87 (1H, dd, *J* = 14.1, 9.6), 2.27−2.20 (1H, m), 2.12−2.00 (1H, m), 1.32 (9H, s).

(**3*S***) and (**3*R***): IR (NaCl) υ_{max} 3328, 2968, 1713, 1683, 1634, 1538, 1455, 1264, 1096, 1042, 744, 699 cm^{-1}; FABHRMS (NBA/NaI) *m/e* 510.2624 ([M + H]$^+$, $C_{28}H_{35}N_3O_6$ requires 510.2604); (Found: C, 65.88; H, 7.25; N, 8.08. $C_{28}H_{35}N_3O_6$ requires C, 65.99; H, 6.92; N, 8.25).

(3*S*)- and (3*R*)-3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-[2′(*S*)-(*tert*-butylamido-4′(*R*)-benzyloxy]pyrrolidine 2b. The α-hydroxy acid **21** was coupled to *trans*-4-benzyloxy-L-prolyl-*tert*-butyl amide using the general coupling procedure outlined for compound **23**. The resulting hydroxy amide was subsequently oxidized *via* a Dess−Martin oxidation to give **2b** in good yield (35 mg, 72%) as a colorless foam (3:1 mixture of diastereomers): R_f = 0.53 (EtOAc/hexane, 1:1).

(**3*S***) (major): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.07 (15H, m), 6.48 (1H, s), 5.32 (1H, d, *J* = 7.2), 5.14 (1H, dd, *J* = 12.8, 7.4), 5.04 (2H, s), 4.56−4.45 (3H, m), 4.29−4.26 (1H, m), 3.79 (1H, br d, *J* = 12.0), 3.71 (1H, dd, *J* = 12.4, 4.6), 3.26 (1H, dd, *J* = 14.1, 5.2), 3.11 (1H, dd, *J* = 14.1, 7.7), 2.53−2.46 (1H, m), 2.17−2.10 (1H, m), 1.32 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4 (C=O), 168.6 (C=O), 162.7 (C=O), 155.8 (C=O), 137.6 (C), 136.4 (C), 135.7 (C), 129.3 (CH × 3), 128.7 (CH × 3), 128.5 (CH × 3), 128.2 (CH), 128.0 (CH), 127.7 (CH), 127.5 (CH × 2), 127.0 (CH), 77.2 (CH), 75.1 (CH_2), 67.0 (CH_2), 60.0 (CH), 58.5 (CH), 53.0 (CH_2), 51.4 (C), 36.6 (CH_2), 32.8 (CH_2), 28.6 (CH_3 × 3).

(**3*R***) (minor): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.07 (15H, m), 5.80 (1H, s), 5.22 (1H, d, *J* = 7.7), 5.13−5.08 (1H, m), 4.99 (2H, s), 4.68 (1H, dd, *J* = 7.2, 7.0), 4.56−4.45 (2H, m), 4.29−4.26 (1H, m), 3.98 (1H, br d, *J* = 13.0), 3.66 (1H, dd, *J* = 13.4, 5.1), 3.41 (1H, dd, *J* = 14.2, 4.2), 2.87 (1H, dd, *J* = 14.3, 9.6), 2.53−2.46 (1H, m), 2.17−2.10 (1H, m), 1.26 (9H, s).

(**3*S***) and (**3*R***): IR (NaCl) υ_{max} 3323, 2964, 2923, 1708, 1682, 1636, 1539, 1497, 1451, 1262, 1231, 1082, 1041, 739, 697 cm^{-1}; FABHRMS (NBA/CsI) *m/e* 718.1873 ([M + Cs]$^+$, $C_{34}H_{39}N_3O_6$ requires 718.1893); (Found: C, 69.65; H, 6.89; N, 6.78. $C_{34}H_{39}N_3O_6$ requires C, 69.72; H, 6.71; N, 7.17).

(3*S*)- and (3*R*)-3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-[2′(*S*)-(*tert*-butylamido-4′(*S*)-benzyloxy]pyrrolidine 2c. The α-hydroxy acid **21** was coupled to *cis*-4-benzyloxy-L-prolyl-*tert*-butyl amide using the general coupling procedure outlined for compound **23**. The resulting hydroxy amide was subsequently oxidized *via* a Dess−Martin oxidation to give **2c** in good yield (45 mg, 75%) as a colorless foam (3:1 mixture of diastereomers): R_f = 0.37 (EtOAc/hexane, 1:1).

(**3*S***) (major): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.12 (15H, m), 6.21 (1H, s), 5.38 (1H, d, *J* = 6.2), 5.09 (1H, d, *J* = 12.2), 5.08−4.93 (1H, m), 4.92 (1H, d, *J* = 12.4), 4.53 (1H, d, *J* = 11.7), 4.42 (1H, d, *J* = 11.7), 4.37 (1H, dd, *J* = 9.7, 2.4), 4.04 (1H, br s), 3.89 (1H, d, *J* = 12.0), 3.55 (1H, dd, *J* = 12.3, 4.3), 3.27 (1H, dd, *J* = 14.0, 4.6), 3.12 (1H, dd, *J* = 14.1, 9.0), 2.60 (1H, br d, *J* = 14.0), 2.05−1.90 (1H, m), 1.21 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.1 (C=O), 168.8 (C=O), 163.4 (C=O), 156.1 (C=O), 137.4 (C), 136.4 (C), 135.9 (C), 129.1 (CH × 2), 128.8 (CH × 2), 128.5 (CH × 2), 128.3 (CH), 128.3 (CH × 2), 128.2 (CH × 4), 127.7 (CH), 127.2 (CH), 76.7 (CH), 70.5 (CH_2), 67.1 (CH_2), 66.9 (CH_2), 60.9 (CH), 58.5 (CH), 53.5 (C), 50.9 (CH_2), 36.9 (CH_2), 28.4 (CH_3 × 3).

(**3*R***) (minor): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34−7.12 (15H, m), 5.75 (1H, s), 5.31 (1H, d, *J* = 7.2), 5.07−4.90 (3H, m), 4.65 (1H, br d, *J* = 8.7), 4.53 (1H, d, *J* = 11.7), 4.42 (1H, d, *J* = 11.7), 4.04 (1H, br s), 3.77−3.70 (2H, m), 3.38 (1H, dd, *J* = 9.8, 4.6), 2.88 (1H, dd, *J* = 14.0, 9.6), 2.57 (1H, br d, *J* = 14.4), 2.06−1.90 (1H, m), 1.22 (9H, s).

(**3*S***) and (**3*R***): IR (NaCl) υ_{max} 3307, 2965, 1698, 1651, 1538, 1455, 1257, 1097, 1041, 739, 696 cm^{-1}; FABHRMS (NBA/CsI) *m/e* 718.1872 ([M + Cs]$^+$, $C_{34}H_{39}N_3O_6$ requires 718.1893); (Found: C, 69.46; H, 6.75; N, 6.85. $C_{34}H_{39}N_3O_6$ requires C, 69.72; H, 6.71; N, 7.17).

(3*S*)- and (3*R*)-3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-L-prolyl-methyl Ester 3. The α-hydroxy acid **21** was coupled to L-proline methyl ester using the general coupling procedure outlined for compound **23**. (2*S*,3*S*)-3-(*N*-benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-L-prolyl-methyl ester was obtained in moderate yield (55 mg, 63%): R_f = 0.34 (20% acetone in toluene); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.20 (10H, m), 5.28 (1H, d, *J* = 8.5), 5.11−4.98 (2H, m), 4.70−4.58 (2H, m), 4.23−4.10 (1H, m), 3.90−3.70 (3H, m), 3.73 (3H, s), 2.81 (1H, dd, *J* = 14.0, 3.3), 2.63 (1H, dd, *J* = 14.0, 11.4), 2.38−2.20 (1H, m), 2.14−1.95 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8 (C=O), 170.4 (C=O), 156.1 (C=O), 138.0 (C), 136.3 (C), 129.0 (3 × CH), 128.3 (3 × CH), 127.8 (CH), 127.3 (2 × CH), 126.2 (CH), 71.4 (CH), 66.4 (CH_2), 59.0 (CH), 54.4 (CH), 52.3 (CH_3O), 47.0 (CH_2), 33.4 (CH_2), 28.6 (CH_2), 25.2 (CH_2); IR (NaCl) υ_{max} 3324, 2953, 1747, 1713, 1644, 1538, 1455, 1386, 1261, 1247, 1177, 1042, 746, 699; FABHRMS (NBA) *m/e* 441.2011 ([M + H]$^+$, $C_{24}H_{28}N_2O_6$ requires 441.2026).

Oxidation of (2*S*,3*S*)-3-(*N*-benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-L-prolyl-methyl ester was carried out using the general Dess−Martin oxidation procedure outlined above. Purification by flash chromatography eluting with 1% methanol in dichloromethane gave the desired α-keto amide **3** (1:1 mixture of isomers) as a colorless oil (25 mg, quantitative): R_f = 0.48 (20% acetone in toluene); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.12 (20H, m), 5.39 (1H, d, *J* = 7.2), 5.22 (1H, d, *J* = 7.2), 5.22−4.90 (6H, m), 4.83 (1H, dd, *J* = 8.1, 3.0), 4.42 (1H, dd, *J* = 9.0, 5.0), 3.80−3.70 (2H, m), 3.74 (3H, s), 3.73 (3H, s), 3.68−3.45 (2H, m), 3.37 (1H, dd, *J* = 14.3, 4.2), 3.31 (1H, dd, *J* = 14.0, 5.0), 3.19 (1H, dd, *J* = 14.4, 8.1), 2.91 (1H, dd, *J* = 14.6, 9.0), 2.20−1.60 (8H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.9 (C=O), 196.5 (C=O), 171.5 (2 × C=O), 162.2 (2 × C=O), 155.8 (2 × C=O),

136.3 (2 × C), 136.2 (2 × C), 129.4 (3 × CH), 129.2 (3 × CH), 128.6 (3 × CH), 128.5 (2 × CH), 128.4 (3 × CH), 128.1 (CH), 128.0 (2 × CH), 127.9 (CH), 127.0 (CH), 126.9 (CH), 67.0 (CH_2), 66.90 (CH_2), 59.8 (CH), 59.2 (CH), 59.0 (CH), 58.3 (CH), 52.5 (CH_3O), 52.4 (CH_3O), 47.7 (CH_2), 47.4 (CH_2), 37.1 (CH_2), 36.9 (CH_2), 31.4 (CH_2), 28.7 (CH_2), 25.0 (CH_2), 21.7 (CH_2); IR (NaCl) ν_{max} 3325, 2952, 1732, 1641, 1523, 1439, 1341, 1261, 1212, 1175, 1029, 743, 699; FABHRMS (NBA) *m/e* 439.1853 ($[M + H]^+$, $C_{24}H_{26}N_2O_6$ requires 439.1869).

L-Prolyl-L-isoleucyl-L-glutamine-*tert*-butyl Amide 25. Spectral data: ^{1}H NMR (400 MHz, CD_3OD) δ 4.35−4.30 (1H, m), 4.31 (1H, dd, J = 9.3, 5.0), 4.21 (1H, d, J = 7.8), 3.41−3.26 (2H, m), 2.48−2.36 (1H, m), 2.36−2.22 (2H, m), 2.20−1.79 (6H, m), 1.67−1.54 (1H, m), 1.46 (9H, s), 1.32−1.17 (1H, m), 0.99 (3H, d, J = 6.8), 0.93 (3H, t, J = 7.4); ^{13}C NMR (100 MHz, CD_3OD) δ 173.3 (C=O), 172.0 (C=O), 170.1 (C=O), 167.7 (C=O), 83.0 (C), 60.7 (CH), 59.9 (CH), 54.0 (CH), 47.4 (CH_2), 37.9 (CH), 32.5 (CH_2), 31.2 (CH_2), 28.4 (CH_2), 28.2 (3 × CH_3), 26.0 (CH_2), 25.0 (CH_2), 15.9 (CH_3), 11.4 (CH_3); FABHRMS (NBA) *m/e* 435.2575 ($[M + Na]^+$, $C_{20}H_{36}N_4O_5$ requires 435.2583).

(2*S*,3*S*)-3-(*N*-Benzyloxycarbonyl)amino-2-hydroxy-4-phenylbutyryl-L-prolyl-L-isoleucyl-L-glutamine-*tert*-butyl Amide 26. The coupling of the acid **21** to the amide **25** was carried out using the general peptide coupling procedure. Flash chromatography eluting with 5% methanol in dichloromethane gave the desired product **26** as a colorless oil (45 mg, 80%): R_f = 0.24 (5% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) (major rotamer only) δ 7.42−7.12 (11H, m), 6.95 (1H, d, J = 8.6), 6.79 (1H, s), 6.07 (1H, s), 5.95 (1H, d, J = 8.3), 5.03 (1H, d, J = 12.4), 4.96 (1H, d, J = 12.4), 4.65 (1H, d, J = 4.5), 4.51 (1H, dd, J = 8.0, 5.2), 4.49−4.35 (2H, m), 4.32 (1H, d, J = 7.8), 3.99−3.94 (1H, m), 3.70−3.50 (2H, m), 2.91−2.83 (2H, m), 2.28−2.21 (1H, m), 2.21−2.10 (3H, m), 2.02−1.75 (5H, m), 1.43 (9H, s), 1.14−1.06 (1H, m), 0.86 (3H, d, J = 6.4), 0.78 (3H, t, J = 7.6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5 (C=O), 172.2 (C=O), 171.5 (2 × C=O), 170.4 (C=O), 156.3 (C=O), 137.9 (C), 136.3 (C), 129.1 (2 × CH), 128.4 (4 × CH), 128.0 (CH), 127.6 (2 × CH), 126.4 (CH), 82.4 (C), 71.4 (CH), 66.5 (CH_2), 61.2 (CH), 57.8 (CH), 55.5 (CH), 52.3 (CH), 47.7 (2 × CH_2), 37.0 (CH), 33.8 (CH_2), 31.48 (CH_2), 28.76 (CH_2), 27.9 (3 × CH_3), 25.3 (CH_2), 24.8 (CH_2), 15.3 (CH_3), 11.0 (CH_3); FABHRMS (NBA/CsI) *m/e* 856.288 ($[M + Cs]^+$, $C_{38}H_{53}N_5O_9$ requires 856.2898).

(3*S*)- and (3*R*)-3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-L-prolyl-L-isoleucyl-L-glutamine-*tert*-butyl Amide 4. Oxidation of **26** was carried out using the general Dess−Martin oxidation procedure outlined above. Purification by flash chromatography eluting with 5% methanol in dichloromethane gave the desired α-keto amide **4** (2:1 mixture of isomers) as a colorless oil (37 mg, quantitative): R_f = 0.24 (EtOAc).

Compound 4 (3*S*). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.16 (11H, m), 6.98 (1H, d, J = 8.5), 6.80 (1H, s), 6.64 (1H, s), 5.64 (1H, d, J = 8.3), 5.09 (1H, d, J = 12.2), 5.02 (1H, d, J = 12.2), 4.46 (1H, dd, J = 8.2, 3.4), 4.21 (1H, t, J = 8.1), 3.67−3.60 (1H, m), 3.59−3.45 (1H, m), 3.70−3.50 (2H, m), 3.25 (1H, dd, J = 14.1, 5.5), 3.09 (1H, dd, J = 14.1, 8.5), 2.29−2.12 (4H, m), 2.12−1.78 (6H, m), 1.46 (9H, s), 1.20−1.05 (1H, m), 0.93−0.83 (6H, m, 2 × CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.1 (C=O), 175.1 (C=O), 171.1 (C=O), 170.5 (2 × C=O), 162.9 (C=O), 156.3 (C=O), 135.7 (2 × C), 129.1 (2 × CH), 128.7 (2 × CH), 128.5 (2 × CH), 128.5 (CH), 127.5 (2 × CH), 126.9 (CH), 82.4 (C), 77.2 (CH), 67.3 (CH_2), 61.0 (CH), 58.0 (CH), 52.3 (CH), 48.0 (2 × CH_2), 37.0 (CH), 31.5 (CH_2), 29.7 (CH_2), 28.2 (CH_2), 27.9 (3 × CH_3), 25.0 (CH_2), 24.8 (CH_2), 15.5 (CH_3), 10.8 (CH_3).

Compound 4 (3*R*). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.16 (11H, m), 6.84 (1H, d, J = 8.0), 6.21 (1H, s), 5.54 (1H, s), 5.51 (1H, d, J = 8.3), 5.11−4.98 (1H, m), 5.09 (1H, d, J = 12.5), 4.80 (1H, d, J = 12.5), 4.75−4.61 (1H, m), 4.59 (1H, d, J = 5.8), 4.50−4.29 (2H, m), 3.59−3.45 (1H, m), 3.29 (1H, dd, J = 14., 3.9), 2.85 (1H, dd, J = 14.0, 10.0), 2.29−2.12 (4H, m), 2.12−1.78 (6H, m), 1.46 (9H, s), 1.20−1.05 (1H, m), 0.93−0.83 (6H, m, 2 × CH_3); IR (NaCl) ν_{max} 3290, 2925, 1728, 1648, 1537, 1452, 1367, 1247, 1157; FABHRMS (NBA/CsI) *m/e* 854.2775 ($[M + Cs]^+$, $C_{38}H_{51}N_5O_9$ requires 854.2741).

(3*S*)- and (3*R*)-3-(*N*-Benzyloxycarbonyl)amino-2-keto-4-phenylbutyryl-[2′(*R*),5′(*R*)-bis(methoxymethyl)-3′(*R*),4′(*S*)-dimethoxypyrrolidine] 12. The coupling was carried out using the general peptide coupling method outlined above to give **34**, followed by oxidation using the general Dess−Martin oxidation procedure to give the desired product **12**. Flash chromatography eluting with 20% ethyl acetate in hexane gave the α-keto amide **12** (20 mg, quantitative): R_f = 0.63 (50% EtOAc in hexane). All analysis performed on mixture of isomers: NMR major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.17 (10H, m), 5.75 (1H, d, J = 8.7), 5.16−5.12 (1H, m), 5.09 (1H, d, J = 12.4), 5.04 (1H, d, J = 12.4), 4.39 (1H, dt, J = 3.9, 8.3), 3.98−3.96 (1H, m), 3.92 (1H, t, J = 6.0), 3.82−3.77 (1H, m), 3.73 (1H, dd, J = 10.0, 5.0), 3.55 (1H, dd, J = 10.0, 2.8), 3.47−3.17 (3H, m), 3.45 (3H, s), 3.40 (3H, s), 3.26 (3H, s), 3.19 (3H, s), 3.11 (1H, dd, J = 14.1, 6.92); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.62 (C=O), 164.7 (C=O), 155.7 (C=O), 136.4 (2 × C), 129.7 (2 × CH), 128.4 (2 × CH), 128.3 (4 × CH), 127.9 (CH), 126.7 (CH), 84.6 (CH), 83.6 (CH), 70.7 (CH_2), 69.7 (CH_2), 66.8 (CH_2), 60.7 (CH), 58.9 (CH), 58.7 (OCH_3), 58.7 (OCH_3), 58.4 (OCH_3), 58.4 (OCH_3), 56.7 (CH), 37.3 (CH_2). Minor isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.17 (10H, m), 5.46 (1H, d, J = 9.0), 5.11−4.87 (3H, m), 4.56−3.17 (10H, m), 3.45 (3H, s), 3.38 (3H, s), 3.31 (3H, s), 3.21 (3H, s); IR (NaCl) ν_{max} 2930, 1717, 1635, 1506, 1456, 1110; FABHRMS (NBA/CsI) *m/e* 661.1550 ($[M + Cs]^+$, $C_{28}H_{36}N_2O_8$ requires 661.1526).

2(*R*)-(Methoxymethyl)pyrrolidine 30. Spectral data: ^{1}H NMR (400 MHz, CD_3OD) δ 3.80−3.70 (1H, m), 3.64 (1H, dd, J = 10.6, 3.64), 3.50 (1H, dd, J = 10.56, 7.76), 3.40 (3H, s), 3.30 (2H, t, J = 7.6), 2.16−1.98 (3 H, m), 1.81−1.74 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 72.0 (CH_2), 60.7 (CH_3), 59.3 (CH), 46.6 (CH_2), 27.3 (CH_2), 24.8 (CH_2); FABHRMS (NBA/NaI) *m/e* 138.0902 ($[M + Na]^+$, $C_6H_{13}NO$ requires 138.0895).

2(*R*),5(*R*)-Bis(hydroxymethyl)-3(*R*),4(*S*)-dihydroxy-*N*-(benzyloxycarbonyl)pyrrolidine. A solution of 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*S*)-dihydroxypyrrolidine[52] (806 mg, 4.95 mmol) in H_2O (25 mL) was cooled to 0 °C in an ice bath, and the pH was adjusted to 9−10 with Na_2CO_3 solution (0.3 M). Benzyloxycarbonyl chloride (1.4 mL, 9.9 mmol, 2 equiv) was added dropwise, and the solution was stirred 1 h at 0 °C and then 1 h at ambient temperature. Solvent was removed *in vacuo*, and the residue was taken up in EtOAc, filtered, and concentrated *in vacuo*. Flash chromatography eluting initially with 50% ethyl acetate in hexane and then 100% ethyl acetate gave 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*S*)-dihydroxy-*N*-(benzyloxycarbonyl)pyrrolidine product as a pale yellow oil: (1.1 g, 81%); R_f = 0.27 (EtOAc); ^{1}H NMR (400 MHz, CD_3OD) δ 7.37−7.33 (5H, m), 5.13 (2H, s), 4.20−4.19 (1H, m), 4.13−4.10 (1H, m), 4.00 (1H, br s), 3.95−3.55 (5H, m); ^{13}C NMR (100 MHz, CD_3OD) (two rotamers) δ 137.8, 129.6, 129.2, 129.0, 77.8, 77.1, 68.4, 67.4, 66.7, 63.2, 62.2, 61.7, 61.6, 61.1; FABHRMS (NBA) *m/e* 320.1121 ($[M + Na]^+$ $C_{14}H_{19}NO_6$ requires 320.1110).

2(*R*),5(*R*)-Bis(methoxymethyl)-3(*R*),4(*S*)-dimethoxy-*N*-(benzyloxycarbonyl)pyrrolidine. To 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*S*)-dihydroxy-*N*-(benzyloxycarbonyl)pyrrolidine (35 mg, 0.117 mmol) in dry THF (1 mL) was added CH_3I (116 μL, 1.86 mmol, 16 equiv) followed by NaH (60% dispersion in mineral oil) (28.1 mg, 6 equiv). The reaction mixture was stirred at ambient temperature for 20 h and concentrated *in vacuo*. Flash chromatography eluting with 12% to 20% ethyl acetate in hexane gave 2(*R*),5(*R*)-bis(methoxymethyl)-3(*R*),4(*S*)-dimethoxy-*N*-(benzyloxycarbonyl)-pyrrolidine as a colorless oil: (40 mg, 97%): R_f = 0.6 (50% EtOAc in hexane). ^{1}H NMR (400 MHz, CD_3OD) δ 7.29−7.23 (5H, m), 5.04 (2H, br s), 4.10 (1H, br s), 3.74 (3H, br, s), 3.44−3.42 (4H, m), 3.34 (3H, br s), 3.31 (3H, br s), 3.23 (5H, br s); FABHRMS (NBA) *m/e* 376.1722 ($[M + Na]^+$ $C_{18}H_{27}NO_6$ requires 376.1736).

2(*R*),5(*R*)-Bis(methoxymethyl)-3(*R*),4(*S*)-dimethoxypyrrolidine 29. To 2(*R*), 5(*R*)-bis(methoxymethyl)-3(*R*),4(*S*)-dimethoxy-*N*-(benzyloxycarbonyl)pyrrolidine (40 mg, 0.181 mmol) in methanol (2 mL) was added Pd/C (10 mg). The mixture was stirred under a balloon of H_2 for 3 h. Filtration through Celite followed by concentration *in vacuo* yielded the desired product **29** as a pale yellow oil (25 mg, quant.): ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.19 (1H, br s) 3.8−3.55 (8H, m), 3.54−3.35 (12H, m); ^{13}C NMR (62 MHz, $CDCl_3$) 84.7, 83.7, 71.9, 69.1, 62.4, 59.9, 59.1, 59.0, 57.6, 57.5; FABHRMS (NBA) *m/e* 220.1543 ($[M + H]^+$ $C_{10}H_{21}NO_4$ requires 220.1549).

General Procedure for Coupling of Epoxide to Proline Derivatives. To the pyrrolidine derivative **28**−**33** or **22** (20 mg, 0.091 mmol) was added dry methanol (2 mL), Cbz-phenylalanyl epoxide **27** (27 mg, 0.091 mmol, 1.0 eq.) and triethylamine (14 μL, 0.100 mmol, 1.1 equiv). The solution was refluxed for 32 h, and then concentrated *in vacuo*.

***N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2′(*R*)methoxymethyl]pyrrolidine 7.** The pyrrolidine derivative **30** was coupled to the epoxide **27** as described in the general procedure to provide **7**. Flash chromatography eluting with ethyl acetate provided the desired product as a clear oil (40 mg, 56%): $R_f = 0.15$ (EtOAc); ^{1}H NMR (400 MHz, CD_3OD) δ 7.29−7.16 (10H, m), 4.97 (1H, d, J = 12.7), 4.92 (1H, d, J = 12.7), 3.85−3.81 (1H, m), 3.77−3.73 (1H, m), 3.42−3.25 (3H, m), 3.31 (3H, s), 3.25−3.02 (2H, m), 2.89 (1H, m), 2.64 (1H, dd, J = 13.8, 10.5), 2.61−2.52 (2H, m), 1.95−1.87 (1H, m), 1.84−1.77 (2H, m), 1.61−1.55 (1H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 158.5 (C=O), 140.3 (2 × C), 130.5 (3 × CH), 129.4 (CH), 129.2 (3 × CH), 128.8 (CH), 128.5 (CH), 127.2 (CH), 73.0 (CH), 67.1 (2 × CH_2), 59.9 (CH_2), 59.3 (2 × CH_2), 57.5 (OCH_3), 57.2 (CH_2), 36.8 (CH_2), 28.5 (CH_2), 24.2 (CH_2); IR (NaCl) ν_{max} 3330, 2939, 1699, 1538, 1454, 1252, 1203, 1134, 699; FABHRMS (NBA/NaI) *m/e* 413.2458 ([M + H]$^+$, $C_{24}H_{32}N_2O_4$ requires 413.2440).

***N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2′(*R*),5′(*R*)-bis(methoxymethyl)]pyrrolidine 5.** The pyrrolidine derivative **28**[53] was coupled, according to the above conditions, to the epoxide **27** to provide **5**. Flash chromatography eluting with 30−50% ethyl acetate in hexane provided the desired product as a clear oil (35 mg, 40%): $R_f = 0.47$ (EtOAc/hexane, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.17 (10H, m), 5.02 (2H, dd, J = 20.4, 12.1), 3.89 (1H, m), 3.50 (1H, m), 3.37 (3H, s), 3.31 (2H, d, J = 1.1), 3.24 (3H, s), 3.20−3.18 (2H, m), 2.95−2.89 (4H, m), 2.85−2.75 (2H, m), 1.87−1.83 (2H, m), 1.54−1.52 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 129.6, 128.4, 128.2, 127.9, 127.8, 126.2, 77.5, 76.8, 71.2, 66.6, 66.3, 60.4, 59.0, 58.8, 54.9, 36.2, 29.6; IR (NaCl) ν_{max} 3325, 2929, 1713, 1525, 1447, 1251, 1103, 1027; FABHRMS (NBA) *m/e* 457.2689 ([M + H]$^+$$C_{26}H_{36}N_2O_5$ requires 457.2702); (Found: C, 68.08; H 8.18; N 6.19. $C_{26}H_{36}N_2O_5$ requires C, 68.38; H, 7.95; N, 6.14).

***N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2′(*R*),5′(*R*)-bis(methoxymethyl)-3′(*R*),4′(*S*)-dimethoxy]pyrrolidine 6.** The pyrrolidine derivative **29** was coupled to the epoxide **27** as described above. Flash chromatography eluting with 50% ethyl acetate in hexane gave the desired product **6** as a pale yellow oil (20 mg, 42%): $R_f = 0.37$ (EtOAc/hexane, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.19 (10H, m), 5.02 (2H, dd, J = 12.3, 20.6), 4.09 (1H, br s) 3.90 (1H, m), 3.70 (1H, d, J = 3.7), 3.58 (1H, dd, J = 6.4, 9.3), 3.54 (2H, m), 3.37 (3H, s), 3.36 (3H, s), 3.35 (3H, s), 3.40−3.28 (5H, m), 3.28 (3H, s), 3.25−3.17 (1H, m), 2.95−2.85 (2H, m), 2.82−2.77 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 137.9, 129.7, 128.4, 128.3, 127.9, 127.8, 126.3, 84.8, 83.9, 75.1, 72.2, 70.6, 69.9, 66.9, 66.4, 61.0, 59.0, 58.9, 58.0, 57.1, 54.9, 29.7; FABHRMS (NBA) *m/e* 517.2932 ([M + H]$^+$ $C_{28}H_{40}N_2O_7$ requires 517.2914).

***N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-L-prolyl-*tert*-butyl Amide 8.** L-Proline *tert*-butyl amide **22** was coupled according to the above conditions to the epoxide **27** to give **8**. Flash chromatography eluting with 100% ethyl acetate gave the desired product **8** as a colorless oil (70 mg, 48%): $R_f = 0.17$ (EtOAc/hexane, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.14 (10H, m), 7.02 (1H, br s), 5.26 (1H, br s), 5.06 (2H, s), 3.93−3.86 (1H, m), 3.67−3.65 (1H, m), 3.29−3.16 (1H, m), 2.90−2.75 (3H, m), 2.67 (1H, d, J = 1.7), 2.48 (1H, dd, J = 4.8, 2.5), 2.20−2.05 (1H, m), 1.95−1.65 (3H, m), 1.30 (9H, s); ^{13}C NMR (63 MHz, $CDCl_3$) δ 175.7, 174.4, 137.5, 129.2, 128.5, 128.4, 128.0, 127.9, 126.5, 72.1, 68.9, 66.7, 59.8, 56.1, 55.5, 50.4, 35.4, 30.9, 29.6, 28.6, 24.3; IR (NaCl) ν_{max} 3290, 2967, 1699, 1645, 1532, 1260, 1027; FABHRMS (NBA) *m/e* 468.2810 ([M + H]$^+$ $C_{27}H_{37}N_3O_4$ requires 467.2862); (Found: C, 69.27; H, 8.31; N 8.76. $C_{27}H_{37}N_3O_4$ requires C, 69.34; H, 7.98; N, 8.99).

***N*-[1-Phenyl-2(*S*)-[(benzyloxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2′(*R*)-(*tert*-butylamido)-4′(*S*)-methoxy]pyrrolidine 9.** The pyrrolidine derivative **31** (derived from *cis*-4-hydroxy-L-proline) was coupled to the epoxide **27** as described above. Flash chromatography eluting with 50% ethyl acetate in hexane gave the desired product **9** as a pale yellow oil (40 mg, 60%): $R_f = 0.23$ (EtOAc); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.34−7.14 (10H, m), 7.04 (1H, br s), 5.01 (2H, br s,), 4.81 (1H, d, J = 8.8), 3.98−3.85 (1H, m), 3.84 (1H, t, J = 3.7), 3.58 (1H, dd, J = 6.0, 6.1), 3.35−3.25 (1H, m), 3.28 (3H, s), 3.20−3.12 (1H, m), 3.00−2.90 (2H, m), 2.85 (1H, dd, J = 13.3, 8.1), 2.71 (1H, d, J = 12.4), 2.66 (1H, d, J = 12.4), 2.56 (1H, dd, J = 10.3, 3.1), 2.30−2.10 (1H, m), 2.05−1.95 (1H, m), 1.33 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7 (C=O), 172.0 (C=O), 137.5 (C), 129.5 (2 × CH), 129.3 (C), 128.5 (3 × CH), 128.4 (2 × CH), 128.0 (CH), 127.9 (CH), 126.5 (CH), 79.9 (CH), 71.4 (CH), 68.3 (CH), 66.6 (C), 60.6 (CH_2), 59.7 (CH_2), 56.0 (CH_3O), 55.0 (CH), 50.3 (CH_2), 35.8 (CH_2), 29.7 (CH_2), 28.6 (3 × CH_3); FABHRMS (NBA/CsI) *m/e* 630.1970 ([M + Cs]$^+$ $C_{28}H_{39}N_3O_5$ requires 630.1944).

***N*-[1-Phenyl-2(*S*)-[(benzyoxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2′(*S*)-(*tert*-butylamido)-4′(*R*)-methoxy]pyrrolidine 10.** The pyrrolidine derivative **32** (derived from *trans* 4-hydroxy-L-proline) was coupled to the epoxide **27** as described above. Flash chromatography eluting with 75% ethyl acetate in hexanes gave the desired product **10** as a pale yellow oil (45 mg, 40%): $R_f = 0.20$ (EtOAc/hexane, 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33−7.13 (10H, m), 6.79 (1H, br s), 5.02 (2H, s), 4.89 (1H, d, J = 7.5), 3.88−3.85 (2H, m), 3.72−3.61 (1H, m), 3.49−3.33 (1H, m), 3.29 (3H, s), 3.20 (1H, t, J = 8.0), 2.97−2.57 (5H, m), 2.29−2.22 (1H, m), 1.95−1.88 (1H, m), 1.67 (1H, br s), 1.31 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 173.6, 156.5, 137.7, 136.3, 128.9, 128.3, 127.7, 127.6, 126.4, 79.7, 72.0, 68.0, 65.8, 60.2, 59.9, 56.6, 55.5, 50.5, 36.8, 35.3, 28.6; IR (NaCl) ν_{max} 3307, 2968, 2932, 2357, 1749, 1713, 1652, 1531, 1455, 1365, 1258, 1230, 1095, 1027, 734, 698; FABHRMS (NBA) *m/e* 498.2955 ([M + H]$^+$ $C_{28}H_{39}N_3O_5$ requires 498.2968); Found: C, 67.36; H, 8.30; N 8.49. $C_{28}H_{39}N_3O_5$ requires C, 67.57; H, 7.90; N, 8.45).

***N*-[1-Phenyl-2(*S*)-[(benzyoxycarbonyl)amino]-3(*R*)-hydroxybutan-4-[2′(*S*)-(*tert*-butylamido-4′(*R*)-benzyloxy]pyrrolidine 11.** The pyrrolidine derivative **33** (derived from *trans* 4-hydroxy-L-proline) was coupled to the epoxide **27** as described above. Flash chromatography eluting with 50% ethyl acetate in hexanes gave the desired product **11** as colorless oil (28 mg, 53%): $R_f = 0.20$ (EtOAc/hexane, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) 7.34−7.16 (15H, m), 6.76 (1H, br s), 5.01 (2H, s), 4.85 (1H, d, J = 8.2), 4.51 (1H, d, J = 11.7), 4.43 (1H, d, J = 11.7), 4.13−4.07 (1H, m), 3.90−3.80 (1H, m), 3.70−3.62 (1H, m), 3.50−3.35 (2H, m), 3.24 (1H, t, J = 8.0), 2.92−2.68 (4H, m), 2.35−2.29 (1H, m), 1.99−1.93 (1H, m), 1.63 (1H, br s), 1.30 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 137.5, 129.3, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 127.7, 126.6, 80.1, 77.9, 71.1, 68.2, 60.8, 60.1, 55.7, 37.4, 35.9, 29.7, 28.6; IR (NaCl) ν_{max} 3307, 2923, 1713, 1652, 1532, 1455, 1365, 1263, 1229, 1097, 1028, 733, 699; FABHRMS (NBA) *m/e* 706.2288 ([M + Cs]$^+$ $C_{34}H_{43}N_3O_5$ requires 706.2257).

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Supporting Information Available: ^{1}H NMR spectra of key compounds **2**−**12**, **28**, **29** and three unnumbered precursors (16 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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