2′,6′-Dimethylphenoxyacetyl: A New Achiral High Affinity P_3-P_2 Ligand for Peptidomimetic-Based HIV Protease Inhibitors

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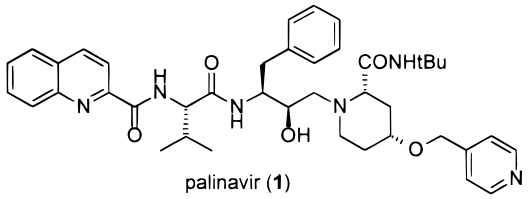
Starting from palinavir (**1**), our lead HIV protease inhibitor, we have discovered a new series of truncated analogues in which the P_3-P_2 quinaldic-valine portion of **1** was replaced by 2′,6′-dimethylphenoxyacetyl. With EC_{50}'s in the 1−2 nM range, some of these compounds are among the most potent inhibitors of HIV replication in vitro, reported to date. One of the most promising members in this series (compound **27**, BILA 2185 BS) exhibited a favorable overall pharmacokinetic profile, with 61% apparent oral bioavailability in rat. X-ray crystal structures and molecular modeling were used to rationalize the high potency resulting from incorporation of this structurally simple, achiral ligand into the P_3-P_2 position of hydroxyethylamine-based HIV protease inhibitors.

Introduction

Inhibition of the virally encoded protease of the human immunodeficiency virus (HIV) results in the production of immature, noninfectious virions.[1] The discovery has led to new strategies for the treatment of AIDS, based on the inhibition of this essential viral protein. Most successful to date in this area is the use of peptidomimetic structures that resemble the transition state for the cleavage of the enzyme's natural substrates.[2] Recently, several HIV protease inhibitors have received FDA approval and are providing clinicians with new tools for combating the deadly infection.[3]

Our own efforts in this field have culminated with the discovery of palinavir (**1**, Figure 1), a potent inhibitor of the protease and viral replication in vitro.[4] This peptidomimetic structure incorporates a hydroxyethylamine transition state mimic and a novel 4-hydroxypipecolic acid fragment which spans the $S_{1'}$-$S_{3'}$ pockets of the protease active site.[5] While palinavir exhibited a good pharmacological profile in several laboratory animal species, we felt that it could still benefit from further simplification and size reduction.[6] SAR studies leading to the discovery of palinavir had revealed that manipulation of the right-hand side of the molecule (the 4-substituent on the pipecolic ring) could be used to modulate the overall physicochemical properties of the inhibitor while maintaining antiviral properties.[4] In designing simpler and smaller versions of **1**, we wanted to retain this very useful feature of this class of inhibitors, and our efforts thus concentrated on modifications of the left-hand side of the molecule (i.e. the P_3-P_2 segment). We describe herein the discovery of 2′,6′-dimethylphenoxyacetyl, a new achiral, high affinity P_3-P_2 ligand for peptidomimetic-based HIV protease inhibitors.[4a,b]



Viral strain	IC_{50} (nM)[a]	K_i (nM)[b]	EC_{50} (nM)[b]
HIV-1 IIIB	4	0.031	5
HIV-2 ROD	10	0.13	25

[a] HPLC-based peptide substrate cleavage assay. [b] See reference 4a.

Figure 1.

Results and Discussion

Early efforts in the field of HIV protease inhibitors resulted in the incorporation of rather large peptide-like fragments in the P_3-P_2 positions, often resulting in a disappointing pharmacokinetic profile.[7] As a result, considerable efforts have been directed at the design of smaller, less peptidic P_3-P_2 replacements, some of which are shown in Figure 2. In many instances, substantial increases in potency were achieved through the design of ligands which were able to take advantage of hydrophobic (Val32, Ile50, Ile84) as well as hydrogen-bonding (Asp29, Asp30) interactions with the enzyme active site. Most notable are the mono- (**2**) and bicyclic tetrahydro-

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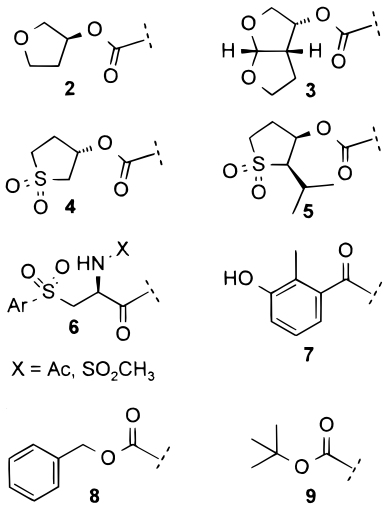
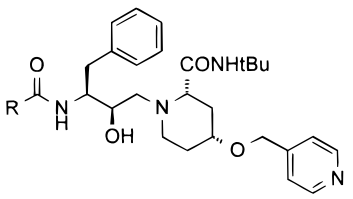


Figure 2.

furanyl (**3**) groups, which, through a combination of conformational restriction, hydrogen-bonding functionality, and molecular weight reduction, conferred an improved antiviral profile to several classes of HIV protease inhibitors.[8] Along the same lines, cyclic (**4**, **5**)[8a] as well as acyclic sulfones (**6**)[9] were designed for improved potency. While in several instances these P_3-P_2 replacements satisfied requirements of improved antiviral activity, reduced molecular weight, and superior pharmacokinetic profiles, they often resulted in increased structural complexity relative to the readily available amino acid residues they were designed to replace. One notable exception is the 3-hydroxy-2-methylbenzoyl moiety (**7**) found in inhibitors such as Viracept (nelfinavir, AG 1343)[10a,b] and the closely related LY316340,[10c] and some C_2 symmetric inhibitors.[10d] A common feature of P_3-P_2 ligands introduced to date is the incorporation of a carbonyl group for linkage to P_1 (via amide or carbamate functions) to ensure conservation of a hydrogen bond with the structural water (W301) that bridges inhibitors to the flaps of the enzyme.[2,5]

Discovery of a New P_3-P_2 Ligand. During SAR studies leading to the discovery of palinavir, we had noticed that synthetic intermediates spanning P_1 to $P_{3'}$ and capped at the N-terminal with simple carbamate protecting groups, such as carbobenzyloxycarbonyl (Cbz, **8**) and *tert*-butyloxycarbonyl (Boc, **9**), produced modest inhibitors of the enzyme. The binding affinity of these compounds could be improved by optimization of the $P_{1'}$-$P_{3'}$ enzyme interactions.[4c] For example, replacement of the quinaldic-valine fragment in **1** by Cbz gave compound **10** (Table 1), which, when tested in an HIV-1 protease HPLC-based assay (see Experimental Section for details), had an IC_{50} of 400 nM. This 100-fold drop in potency compared to **1** is not surprising considering that two critical hydrogen bonds (Val NH to G48′ and quinaldyl CO to D29′),[5] which certainly contribute to the high potency of **1**, have been sacrificed in **10**. Nevertheless, we thought that **10** could serve as a starting point for the design of new P_3-P_2 ligands. Our objectives were to recover and perhaps improve potency relative to **1**, establish a favorable pharmacokinetic profile, and maintain molecular weight and structural complexity at a minimum. Table 1 summarizes our initial attempts at improving the potency of **10**.

Table 1



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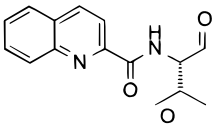
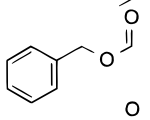
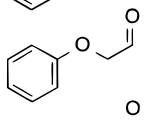
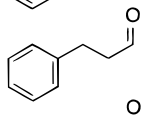
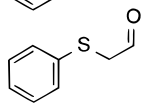
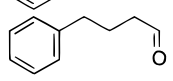
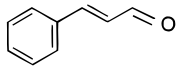
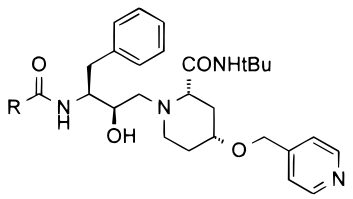
1		4.0
10		400
11		1100
12		1400
13		4200
14		>20,000
15		11000

Hoping to reestablish a hydrogen bond with residues present in the S_2 subsite of the enzyme, the alkoxy oxygen atom and methylene group in **10** were interchanged, leading to phenoxyacetyl derivative **11** suffering an additional 3-fold decrease in potency (IC_{50} = 1100 nM). Replacement of the same oxygen atom in **10** by a methylene group gave **12** (IC_{50} = 1400 nM), equipotent with **11**. The lower potency of amide derivatives **11** and **12** relative to carbamate **10** could be the consequence of an inductive decrease in basicity of the carbonyl oxygen, resulting in a weaker hydrogen bond with W301. This hydrogen bond is important for bringing the enzyme flaps down onto inhibitors through a water-mediated hydrogen bond to protein flap residues, effectively locking them into place inside the active site.

Replacement of the ether linkage of **11** with a thioether function (compound **13**) or incorporation of an additional methylene group in the chain (compound **14**) gave analogues that did not significantly inhibit enzymatic activity ($IC_{50} \geq 4\ \mu M$). The increased length of the chain linking the ring to the carbonyl group presumably does not allow the aromatic portion to settle into the S_2 pocket, and considerable interference occurs between the inhibitor and residues in the enzyme active site. Similarly, cinnamic derivative **15** forces the N-terminal group into a rigid linear arrangement, which appears to be detrimental to binding with the protease. We conclude that the modest activity seen in the case of **10** is most likely due to weak lipophilic interactions between the phenyl ring and the S_2 pocket and to a strong hydrogen bond interaction between the carbamate carbonyl and structural W301. These interactions

Table 2



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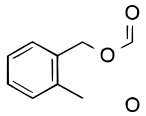
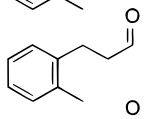
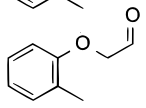
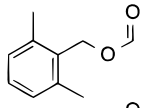
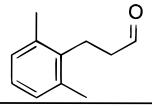
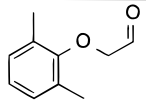
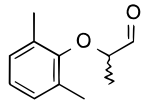
16		210
17		2500
18		120
19		420
20		2400
21		7
22		20

require flexibility in the linker portion of the ligand to allow proper positioning.

Conformational restriction has proven successful in the design of high affinity P_2 ligands. For example, rigidification through incorporation into cyclic systems[8] as well as hindered rotational mobility[10] are but two concepts which have allowed the rigidification of P_2 ligands into arrangements that more closely resemble a presumed bioactive conformation. Lowering of the activation energy required by a peptidomimetic inhibitor to adopt a bioactive conformation can, in some cases, result in dramatic increases in potency.[11]

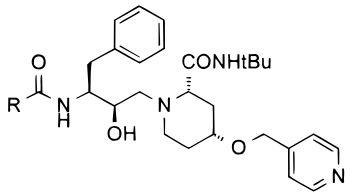
It was envisaged that incorporation of methyl groups in the *ortho*-positions of the N-terminal aromatic ring of compounds **10**−**12** might force the otherwise flexible ligands into a more rigid conformation that would lie closer in energy to the actual bioactive conformation. The added methyl groups could also provide some additional lipophilic interactions within the S_2 pocket of the enzyme, resulting in an increase in potency. In the event, attachment of a single methyl group to Cbz derivative **10** resulted in a 2-fold increase in potency (inhibitor **16**, IC_{50} = 210 nM, Table 2). In contrast, a similar modification of phenylpropionic amide **12** resulted in a 2-fold decrease in potency (inhibitor **17**, IC_{50} = 2500 nM). Much more significant, however, was the effect on phenoxyamide **11**, resulting in inhibitor **18** with a 10-fold improvement in potency (IC_{50} = 120 nM compared to 1100 nM for **11**). In this last case, the added methyl group is producing beneficial effects that override the otherwise less efficient binding of amide ligands relative to carbamates (see Table 1). Encouraged by these results, we investigated the effect of adding a second *ortho*-methyl group to inhibitors **16**−**18**. The 2′,6′-dimethyl-Cbz derivative **19** (IC_{50} = 420 nM) suffered a 2-fold loss in potency relative to monosubstituted carbamate **16**, canceling out the beneficial effect of adding one methyl group (compound **19** now has a similar activity to unsubstituted **10**). Adding a second *ortho*-methyl group to phenylpropionic amide **17** had no additional effect on potency (IC_{50} = 2500 and 2400 nM for **17** and **20**, respectively). In the case of phenoxyacetyl amide **18**, however, the presence of an additional *ortho*-methyl group resulted in a 15-fold increase in potency (from 120 nM to 7 nM for inhibitor **21**). Compared to unsubstituted derivative **11**, this represents a dramatic 150-fold increase in potency (1100 nM to 7 nM) through the simple addition of two methyl groups on the molecule (see also X-ray and modeling section). Based on enzymatic inhibitory values, 2′,6′-dimethylphenoxyacetamide **21** showed similar potency to palinavir **1** (IC_{50} = 7.0 nM and 4.0 nM, respectively).

In an attempt to further improve on the potency of **21**, phenoxypropionamide **22** was prepared as a 1:1 mixture of isomers. Orientation of the ligand through introduction of an asymmetric center (mimicking a P_2 amino acid residue) does not provide increased affinity (IC_{50} = 20 nM for the 1:1 mixture of isomers vs 7.0 nM for **21**). A large number of ring-substituted phenoxyacetyl amides, structurally related to **21**, were also evaluated for their ability to inhibit the enzymatic activity of the protease (results not shown). These included a variety of ring-polymethylated and halogenated derivatives and a combination thereof. While some compounds compared favorably, none proved superior to the original 2′,6′-dimethylphenoxyacetyl derivative **21**. Larger alkyl groups (Et, iPr, nPr) were also incorporated in the *ortho*-positions of the phenol ring, in an attempt to further optimize interactions with the S_2 pocket, but without success.

Cell Culture Activity. Inhibition of HIV-1 replication in acutely infected C8166 T cells was examined next (see Experimental Section for details). Compound **21** inhibited replication of the IIIb strain of the virus at an effective concentration (EC_{50}) of 24 nM (Table 3). No apparent cytotoxicity was noticed in this cell line at inhibitor concentrations up to 10 μM. Relative to palinavir **1** (EC_{50} = 5 nM), 2′,6′-dimethylphenoxyacetyl is a good replacement for the bulkier *N*-quinaldylvaline moiety of this class of inhibitors. To compare our novel P_2-P_3 ligand with those found in other hydroxyethylamine-based HIV protease inhibitors, we also prepared compounds **23** and **24**, featuring both the pipecolic $P_{2'}$-$P_{3'}$ portion of our molecules, and previously reported P_2 replacements. The hydroxytoluamide moiety (compound **23**) found in Viracept[8a] conferred similar antiviral properties to our compounds (compare **21** and **23**). Tetrahydrofuryl carbamate[8b] **24**, on the other hand, was found significantly less potent, indicating suboptimal compatibility with our pipecolic amide class of inhibitors.

Optimization of the $P_{3'}$ Pipecolic Substituent. As mentioned previously, the 4-substituent on the pipecolic

Table 3



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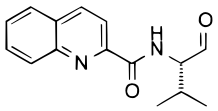
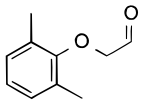
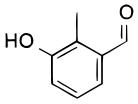
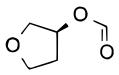
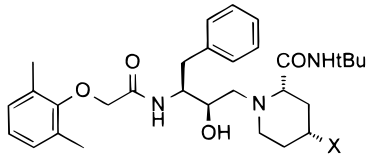
1		4	5
21		7	24
23		12	32
24		260	—

ring of our inhibitors can be used to modulate the physicochemical properties of our compounds, while maintaining inhibitory potencies against the protease. This has been rationalized by the fact that most of this substituent lies outside of the enzyme active site and is mainly exposed to solvent.[5] This feature was used to successfully improve the pharmacokinetic properties of our previous series of inhibitors, eventually leading to the discovery of palinavir.[4a,c]

During our search for structurally novel P_3-P_2 replacements, all studies were carried out on pipecolic amide derivatives bearing the 4-picolyl substituent found in our lead compound, palinavir. Further refinement of the new dimethylphenoxyacetamide series, described herein, was now directed to optimization of the 4-substituent of the pipecolic ring. A variety of heterocyclic substituents were introduced at the 4-position of the pipecolic ring of compound **21** in place of the original 4-picolyl moiety. These were linked to the six-membered ring through ether or thioether linkages and included mostly pyridyl, picolyl, and pyrimidyl derivatives. As expected, most derivatives gave IC_{50} values in the 1−10 nM range. The best compounds in terms of antiviral properties are shown in Table 4. Replacement of the ether linkage in **21** by a thioether bond (compounds **25** and **26**) resulted in a 4−6-fold improvement in EC_{50}. Furthermore, excision of the methylene group in these derivatives gave mercaptopyridine (e.g. **27**) and mercaptopyrimidine (e.g. **28**) derivatives with still superior antiviral properties. To our knowledge, compounds **27** and **28**, with EC_{50} values in the 1−2 nM range (no cytotoxicity observed in C8166 T cells at 1 μM), are among the most potent inhibitors of in vitro HIV-1 replication reported to date that target the protease enzyme. In addition, the use of the 2′,6′-dimethylphenoxyacetyl as a P_3-P_2 ligand provides for significant reduction in size and structural complexity as originally planned. We now hoped that these positive features would translate into favorable pharmacokinetic properties for this new lead series as represented by compounds **27** and **28** (see next section).

Table 4



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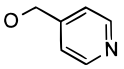
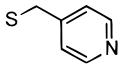
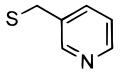
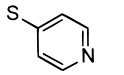
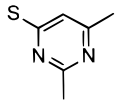
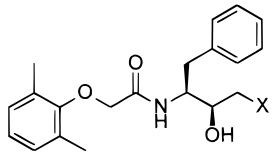
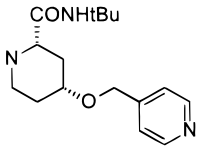
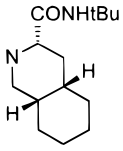
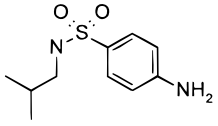
21		7.0	24
25		1.8	4
26		1.7	6
27		1.6	2
28		1.5	1

Table 5



Entry	X	IC_{50} (nM)	EC_{50} (nM)	Reference[a] (EC_{50})
21		7.0	24	palinavir (5 nM)
29		8.4	100	saquinavir (5 nM)
30		2.6	20	amprenavir (7 nM)

[a] Reference compounds (saquinavir and amprenavir) were synthesized in house and tested under identical assay conditions.

The compatibility of our novel P_3-P_2 replacement with other hydroxyethylamine-based HIV protease inhibitors was also examined, and the results are presented in Table 5. Replacement of *N*-quinaldyl-asparagine in saquinavir by 2′,6′-dimethylphenoxyacetyl gave compound **29** with an IC_{50} = 8.4 nM and EC_{50} = 100 nM (compared to EC_{50} = 5 nM for saquinavir). Our new P_3-P_2 replacement is thus less effective when combined to the hexahydroisoquinoline amide found in the Roche

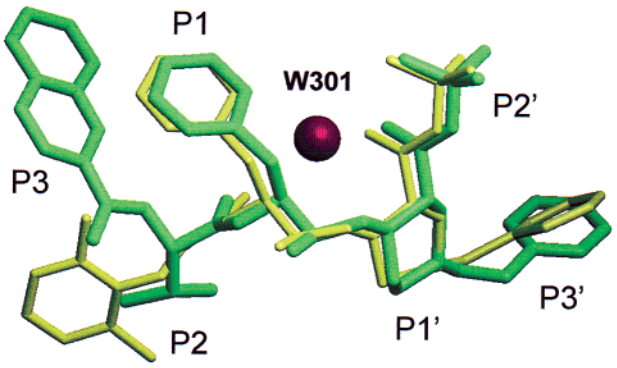


Figure 3. Palinavir (**1**) in green is superimposed with BILA 2185 BS (**27**) in yellow as bound to HIV-2 protease. The superimposition was generated by overlapping the protein residues from each protease−ligand complex crystal structure (overall rmsd fit of backbone residues was 0.29 Å). Protein is not shown. The important flap water is indicated in red (W301).

inhibitor.[12] When incorporated in place of the tetrahydrofuryl carbamate of amprenavir, however,[8b] the resulting compound (**30**) retained most of the antiviral potency of the original inhibitor (20 nM for **30** vs 7 nM for amprenavir). These observations are evidence for cooperative binding of functionalities throughout the HIV protease active site. Recent literature reports suggest that 2′,6′-dimethylphenoxyacetyl is also a useful P_3-P_2 replacement in the context of other classes of HIV protease inhibitors. For example, its incorporation into hydroxyethylene and hydroxymethylcarbonyl peptidomimetic inhibitors (such as ritonavir[13a] and KNI-272[13b]) has led to very potent analogues, allowing at the same time for significant reduction in size and structural complexity.

X-ray Crystal Structure and Molecular Modeling. Insight into the binding mode of this new series of inhibitors was gained with the help of a crystal structure of compound **27** in complex with HIV-2 protease.[5,14] Figure 3 shows a comparison between the bound conformations of **27** (which is representative of the binding mode of this class of inhibitors) and palinavir (**1**).[5] A good overlap is seen between the two structures in the regions spanning the P_1 to $P_{3'}$ positions. In particular, the critical transition state mimic hydroxyl groups and the P_2-P_1 carbonyls show similar interactions with the protein active site aspartyl residues and W301, respectively.

Figure 4 shows the dimethylphenoxyacetyl group lying in a groove between flap residues 48 and 49 and residues 27−30. In comparison to the palinavir complex, several protein residues have undergone noticeable movements to accommodate the dimethylphenoxy moiety.[5] Figure 4 also shows that this fragment does not reach into the S_3 pocket of the enzyme as did the quinaldyl group of **1**. Rather, a portion of the structure binds into the S_2 pocket, occupied by the valine residue in the case of palinavir. One of the methyl groups on the phenol ring of **27** binds similarly to one of the valine methyls in **1**. The other methyl does not appear to interact with protein residues and is exposed to solvent. The 15-fold increase in potency observed between mono- and dialkylated derivatives **18** and **21** (Table 2) is probably, in part, the result of a decrease in binding entropy for the latter, which always positions one of the

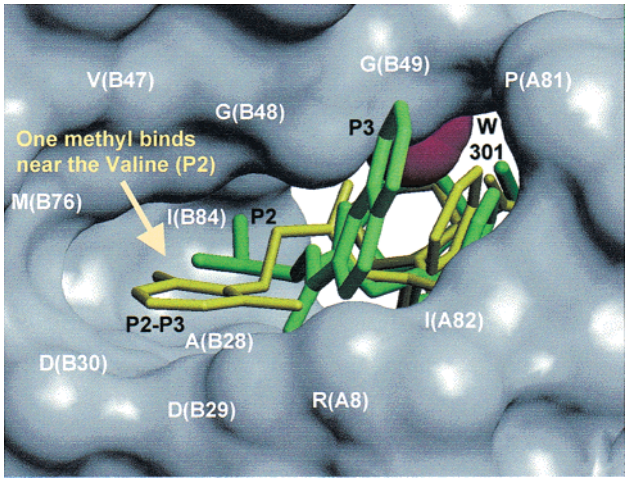
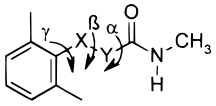


Figure 4. Palinavir (**1**, green) and BILA 2185 BS (**27**, yellow) are shown superimposed as in Figure 3. The protein (from the crystal structure in complex with **27**) is represented by a solvent accessible Connolly surface. The residues are labeled by single letter amino acid codes. A or B before the residue number indicates which monomer of HIV protease the residue belongs to.



I : X = CH_2, Y = O
II : X = Y CH_2
III : X = O, Y = CH_2

Figure 5.

methyl groups for a favorable interaction with the S_2 pocket. One can also notice that a methyl group is probably optimal in size and that larger substituents in the 2′,6′-positions of the phenyl ring might result in unfavorable contacts with protein residues (Et and iPr substituents were not tolerated in those positions).

The effect of added methyl groups on benzyloxycarbonyl versus phenylpropionamide versus phenoxyacetyl derivatives is more difficult to rationalize based on the crystal structures alone. The presence of an additional interaction between one of the methyl groups and the S_2 pocket as seen in the X-ray structures (Figure 4), alone, cannot explain the 150-fold difference in potency seen between **11** and **21**. This is especially evident when taking into consideration the effect of similar modifications on carbamate (**10**, **19**) and propionamide (**12**, **20**) derivatives (Tables 1 and 2). This would suggest that additional factors must be taken into consideration to account for the significant boost in potency achieved with dimethylphenoxyacetyl derivatives.

To probe this phenomenon, we undertook conformational studies on model fragments (*N*-methyl amides **I**, **II**, and **III**, depicted in Figure 5; see Experimental Section for details on computational methods used) representing the left-hand portion of inhibitors **19**, **20**, and **21**. Each fragment was minimized, and energies were compared to calculated crystal structure positions on a rotational potential energy plot (Figure 6). The X-ray conformation for **19** (fragment **I**) is predicted to be high in energy and away from the global minimum by a rotation of 180° about the α-angle. Both **20** and **21** are fairly close to the global minimum (1.1 and 1.3 kcal/

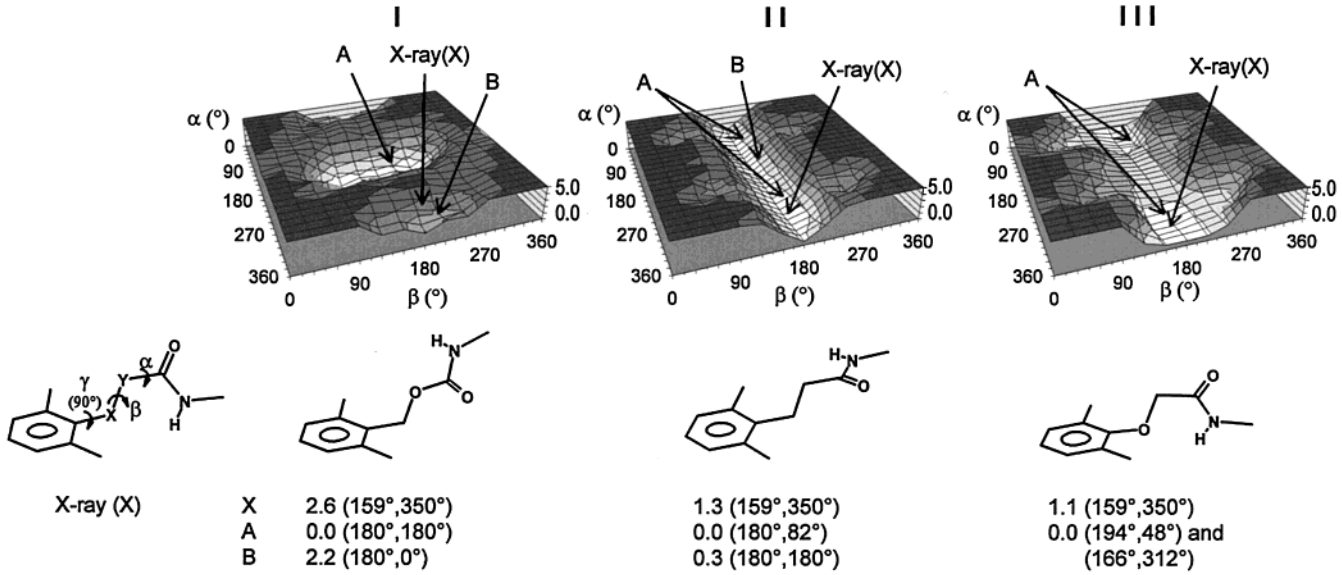


Figure 6. Conformational PM3-SM3 energy profiles of fragments **I**, **II**, and **III** are plotted against angles α and β while angle γ was held fixed at 90° (see Figure 5). Energies are shown relative to the global minimum (**A**), and the surface is truncated at 5 kcal/mol. In the case of **I** and **II**, a local minimum (**B**) was also identified. **X** indicates the position of the observed crystal structure on this energy plot. The relative energies of each conformer (**A**, **B**, and **X**) following minimization are shown in kcal/mol followed by the final values for α and β in parentheses. The structures shown for **I**, **II**, and **III** depict the lowest energy conformation (**A**) for each fragment for comparison with the crystal structure position (**X**).

Table 6

compound	I	II	III
relative conformational energy (kcal/mol)	0.2	1.5	0.0
relative protein-ligand interaction energy (kcal/mol)	3.3	0.0	2.2
relative ligand-W301 interaction energy (kcal/mol)	0.0	0.3	0.7
relative complex solvation energy (kcal/mol)	2.4	4.2	0.0
sum of relative energies (kcal/mol)	5.9	6.0	2.9
final relative energy (kcal/mol)	**3.0**	**3.1**	**0.0**

Table 7

Entry	*n*	EC_{50} (nM)	C_{max}[a] (nM)	AUC[b] (nM.h)	$T_{1/2}$[c] (h)	F% (rat)[d] (5 mg/kg po)
25	3	4	1662±408	1713±721	0.7	22±9
26	6	6	2323±1272	3263±1830	0.5	37±20
27	6	2	941±311	1772±829	0.4	61±28
28	3	1	804±386	1673±578	1.7	29±10

[a] For a po dose of 5 mg/kg. [b] After oral administration. [c] For an iv dose of 1 mg/kg. [d] Apparent bioavailability for an iv dose of 1 mg/kg.[15]

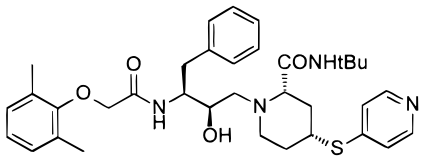
mol for fragments **II** and **III**, respectively). Compound **21**, however, is contained in a much larger well and shows much less rotation about α. It is expected that **20** will undergo a larger entropic penalty upon binding than **21**. The bound conformation of **21** is the closest of the three to its own solvation phase global minimum as predicted by PM3-SM3 calculations.[15] After consideration of protein−ligand interactions, ligand−water interactions, ligand conformational effects and electrostatic solvation energies, **21** is predicted to be bound more tightly than either **19** or **20** by at least 3 kcal/mol (Table 6). This amount of energy would lead to a difference in K_i of approximately 170-fold between **21** and **19** or **20**. Actual IC_{50} values indicate a 60-fold difference for **19** and **21** and 335-fold for **20** and **21**.

On computational grounds therefore, the higher affinity of dimethylphenoxyacetyl derivatives can be rationalized by a smaller gap in energy required by such ligands to adopt the bioactive conformation seen in X-ray structures of inhibitors such as **27** complexed to HIV-2 protease. At this stage, our calculations have not provided an explanation for the 5-fold difference seen between compounds **19** and **20**.

Bioavailability Studies.[16] Compounds **25**−**28** (Table 7) were evaluated for oral bioavailability in rat at a po dose of 5 mg/kg (formulated in methyl cellulose/Tween-80, 0.5/0.01% in water) and an iv dose of 1 mg/kg (formulated in DMA/NaH_2PO_4 0.04 M in 10% dextrose/water: 30/50/20).[16] Pharmacokinetic parameters are shown in Table 7. All compounds were able to achieve plasma concentrations (C_{max}) of 800−2300 nM, after oral administration, several orders of magnitude in excess of their respective EC_{50} values. AUC's after oral administration at 5 mg/kg were 1700−3200 nM h. Half-lives for the clearance of the compounds ($T_{1/2}$) ranged from 0.4 to 1.7 h as determined after iv administration of 1 mg/kg. Analogue **27**, however, exhibited a superior apparent bioavailability of 61%, a 2−3-fold improvement over that of other compounds investigated.

Biological Profile of Compound 27. Compound **27** (company code BILA 2185 BS) was identified as the most promising member of this novel series of P_3-P_2-truncated HIV protease inhibitors (Table 8). It inhibited

Table 8



BILA 2185 BS (**27**)

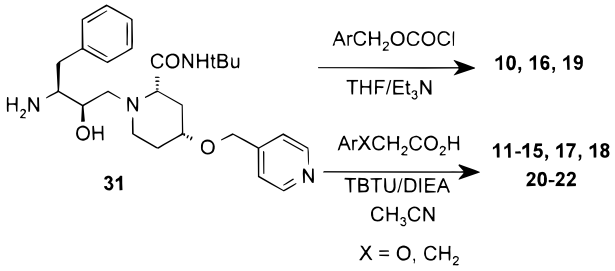
Viral strain	IC_{50} (nM)[a]	K_i (nM)[b]	EC_{50} (nM)[b]
HIV-1 IIIB	1.6	0.006	2
HIV-2 ROD	2.3	0.023	3

[a] HPLC-based peptide substrate cleavage assay. [b] See ref 4a.

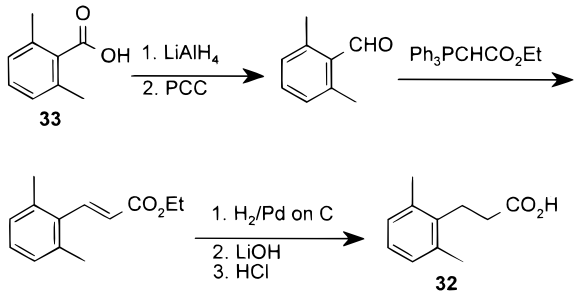
both HIV-1 and HIV-2 protease activities with IC_{50}'s = 1.6 nM and 2.3 nM, respectively. The observed inhibition constants (K_i) for compound **27** were 6 and 23 pM for the HIV-1 and HIV-2 enzymes, as determined by the method of Morrison and Walsh.[4a,17,18] Its EC_{50} for inhibition of the IIIB strain of HIV-1 in cell culture experiments was found to be 2 nM (3 nM against HIV-2 ROD). In presence of 20% human serum, a 4-fold increase in EC_{50} (from 1.3 nM to 5.3 nM) was observed. This small shift in EC_{50} was comparable to that found for palinavir under similar conditions. To our knowledge, this is one of the most potent in vitro anti-HIV protease agent reported to date. The more than 300-fold difference between the observed K_i and the EC_{50} values for compound **27** is probably the result of a number of factors including protein binding, metabolism, and its ability to enter cells. Furthermore, experimental conditions used in enzymology studies may not reflect conditions found in the cell (similar observations were made previously in the case of palinavir[4a] and hydroxyethylamide inhibitors[19]). The apparent bioavailability of **27** in the rat was 61%, and at 5 mg/kg oral doses, it achieved plasma concentrations in excess of 900 nM with a clearance half-life of 0.4 h.[16a] The apparent oral bioavailability of **27** when administered in the dog was 39% and that in chimpanzee was 5%, for an oral dose of 10 mg/kg and iv doses of 1 mg/kg and 0.5 mg/kg, respectively.[20a]

BILA 2185 BS (**27**) is a highly potent inhibitor of HIV protease activity and viral replication in vitro. Compared to palinavir (**1**), it is characterized by slightly reduced structural complexity, resulting in a lower molecular weight (619 vs 719 for palinavir), better or equal potency, and a favorable overall pharmacokinetic profile in several laboratory animal species. BILA 2185 BS was shown to induce multiple mutations in the HIV-1 protease gene, as well as in *gag* precursor cleavage sites, leading to drug resistance in vitro. The observed variants exhibit decreased viral fitness, suggesting that antiviral therapies using such inhibitors may maintain some clinical benefits.[20b,c] Unfortunately, in preliminary animal toxicology studies, BILA 2185 BS caused significant reductions in heart rates and blood pressure, which prevented further development of this compound. However, recent reports on the development of ABT-378,[13a] now undergoing phase III clinical evaluation, would suggest that the toxicity seen with BILA 2185 BS may not be caused by the 2′,6′-dimethylphenoxyacetyl moiety itself but is probably related to other features of the molecule.

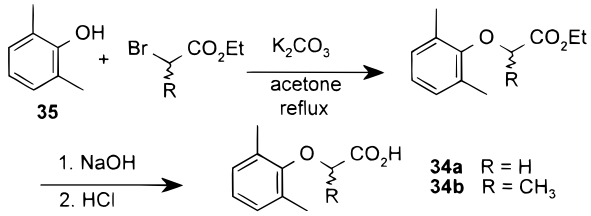
Scheme 1



Scheme 2



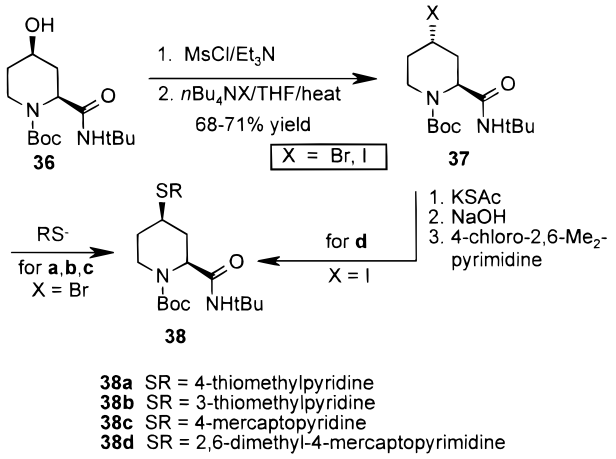
Scheme 3



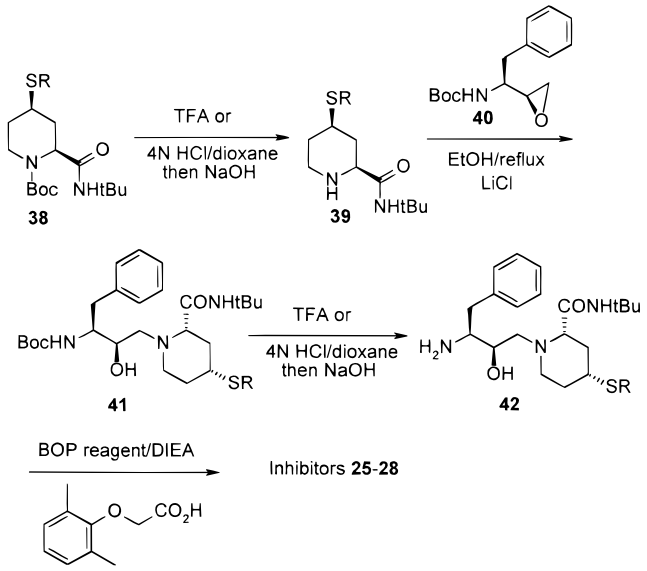
Synthesis of Inhibitors. All inhibitors were prepared by standard peptide coupling methods. The synthesis of palinavir **1** via intermediate amine **31** (Scheme 1) was reported recently.[21] Carbamate derivatives **10**, **16**, and **19** were prepared by coupling **31** with the appropriate chloroformate (Scheme 1), prepared in turn from commercially available alcohols and phosgene. Arylpropionyl, aryloxyacetyl amides, and compounds **13**−**15** were synthesized by coupling **31** with the corresponding carboxylic acids using TBTU[22] in the presence of *N*,*N*-diisopropylethylamine (DIEA) as shown in Scheme 1. 3-(2′,6′-Dimethylphenyl)propionic acid **32** was prepared by homologation of 2,6-dimethylbenzoic acid **33** (Scheme 2). Noncommercially available aryloxyacetic acids **34a,b** (R = H, CH_3, Scheme 3) were prepared by alkylation of 2,6-dimethylphenol **35** with ethylbromoacetate or ethyl 2-bromopropanoate, followed by saponification of the ester function. Compounds **23** and **24** were prepared by coupling **31** to 3-hydroxy-2-methylbenzoic acid[23] (DCC/HOBt)[24] and to the chloroformate derived from 3-(*S*)-hydroxytetrahydrofuran, respectively.

Inhibitors with modifications at the 4-position of the pipecolic ring (compounds **25**−**28**) were prepared form the corresponding pipecolic amide derivatives as shown in Schemes 4 and 5. *N*-Boc-(2*S*,4*R*)-4-hydroxypipecolic *tert*-butylamide (**36**)[21,25] was first converted to the (2*S*,4*S*)-4-iodo or 4-bromo derivatives (**37**) via mesylation and displacement with inversion using iodide or bromide. Intermediate **37** was then treated with sulfur

Scheme 4



Scheme 5



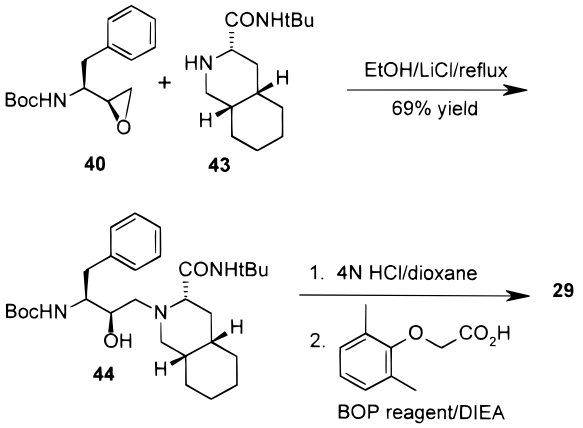
nucleophiles to deliver the desired (2*S*,4*R*)-4-substituted pipecolic amide derivatives **38a**−**c** through an overall double-inversion process. Compound **38d** was prepared from **37** (X = I) by conversion to its 4-mercapto derivative and alkylation with 4-chloro-2,6-dimethylpyrimidine. N-Deprotection of **38** gave amines **39** (Scheme 5), which were then used to open epoxide **40**[26] to give protected amino alcohol derivatives **41**. Following deprotection, amino alcohols **42** were obtained that were condensed with 2′,6′-dimethylphenoxyacetic acid in the usual fashion.

Compound **29** was prepared by condensation of epoxide **40**[26] with amine **43**[27] to give amino alcohol **44** which was elaborated to **29** in the usual way (Scheme 6). Analogue **30** was prepared by condensation of epoxide **40**[26] with isobutylamine followed by treatment with 4-nitrobenzesulfonyl chloride, to give sulfonamide **45** (Scheme 7). Deprotection followed by the usual coupling to P_3-P_2 gave nitro derivative **46**, which gave aniline **30** upon hydrogenolysis.

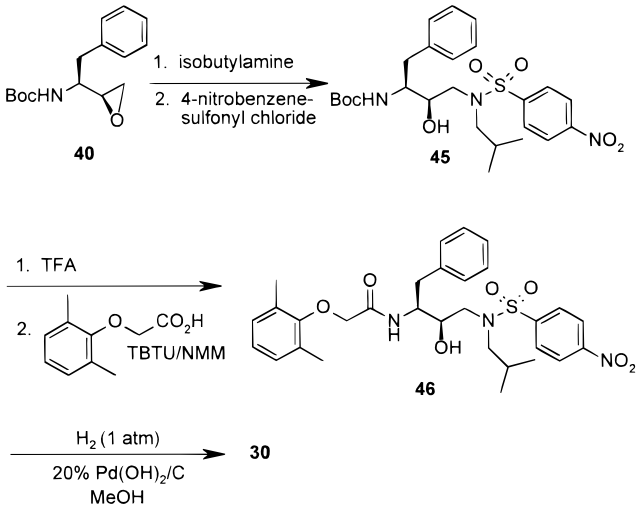
Experimental Section

General. NMR spectra were recorded on an AMX400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C unless otherwise specified) in deuterated solvents and were referenced to TMS (δ scale). *J* coupling constants are reported to the nearest 0.5 Hz. Mass spectral data was obtained in FAB mode on a MF 50 TATC instrument operating at 6 kV and 1 mA using thioglycerol or nitrobenzyl alcohol (NBA) as a matrix support.

Scheme 6



Scheme 7



HPLC homogeneities were determined under reversed-phase conditions. System A: Waters (Milford, MA) Symmetry C8 column (3.9 × 150 mm), 10 → 80% CH_3CN in 50 mM NaH_2PO_4 (pH 4.4) in 25 min then 5 min isocratic, 1.0 mL/min flow rate, UV detection at 215 nm. System B: Waters (Milford, MA) Deltapack C18 column (2 × 150 mm), 0 → 100% CH_3CN (0.1% TFA) in 0.1% TFA in 25 min then 5 min isocratic, 0.5 mL/min flow rate, UV detection at 215 nm.

Flash chromatography was performed on Merck silica gel 60 (0.040−0.063 mm). Analytical thin-layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates.

Materials. Reagents and solvents were of commercial sources and used as received without purification. Benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[22] and BOP coupling reagent were purchased from Richelieu Biotechnologies Inc. (Montréal, Canada).

The decapeptide substrate VSFNFPQITL-NH_2 was synthesized using standard solid-phase methodology. The fluorogenic substrate, 2-aminobenzoyl-Thr-Ile-Nle-Phe(p-NO_2)-Gln-Arg-NH_2, was purchased from Bachem. Protease-free BSA, MES, and EDTA were purchased from Sigma.

IC_{50} Determinations Using an HIV Protease HPLC-Based Assay.[28] Recombinant HIV-1 and HIV-2 proteases were expressed from *Escherichia coli* and purified as previously described. Enzymatic assays were performed in 100 mM MES, 300 mM KCl, 5 mM EDTA, 1 mg/mL BSA, pH 5.5. DMSO (final concentration was brought to 4.5%) was used to aid solubilization. To 20 μL of a 400 μM solution of VSFNFPQITL-

NH_2 substrate were added 10 μL of various concentrations of the inhibitor tested. The enzymatic reaction was initiated by the addition of 12 nM of enzyme in 10 μL for a final concentration of 3 nM. The assay mixture was then incubated for 45 (HIV-2 protease assay) or 60 (HIV-1 protease assay) min at 37 °C and the reaction terminated by addition of 200 μL of 1%TFA/H_2O. Cleavage products and substrate were separated by reversed-phase HPLC on a Perkin-Elmer 3 × 3CR C8 column as previously described.[28a] A nonlinear curve fit using the Hill model was applied to the percent inhibition−concentration data, and 50% effective concentrations (IC_{50}) were calculated through the use of SAS (Statistical Software System, SAS Institute Inc., Cary, NC).

Extracellular p24 Assay. Inhibition of Viral Replication.[4a] The extracellular core protein level, determined by ELISA with the HIV-1 p24 antigen (Ag) Coulter kits, was used to monitor the replication of the HIV-1 IIIB strain. C8166 T cells were infected at a MOI of 0.001 in complete medium and were incubated in the presence of serially diluted inhibitors for 3 days. The percentage of inhibition was obtained by determination of the core protein level from pools of supernatants (8 replicas). A nonlinear curve fit using the Hill model was applied to the percent inhibition−concentration data, and EC_{50}'s were obtained through the use of the SAS software. No apparent cytotoxicity was noticed in this cell line at concentrations up to 1 μM (up to 10 μM for less active compounds).

Computational Chemistry Methods. Small fragments (Figure 5, **I**, **II**, and **III**) representing the left-hand portion of our inhibitors were utilized in quantum mechanics calculations to determine the rotational minima on each potential energy surface. Semiempirical calculations using Spartan 5.0 (Wave function Inc. 18401 Von Karman Ave., suite 370, Irvine, CA, 92612) were performed using the PM3 Hamiltonian.[15a] Three angles were restrained while the remaining parameters were allowed to relax. Angle γ (Figure 5) was held at 90° throughout (close to X-ray position). Angles α and β were varied by 15° and 30°, respectively, to generate 288 relaxed points for each system. Symmetry was exploited when possible.

Each relaxed point was then treated with the SM3 solvation method[15b] as a single point calculation, and the energy was plotted as a function of angles α and β. Each predicted minimum was investigated by optimization without restraints as above. The X-ray conformation was determined by freezing angles α, β, and γ at 350°, 159°, and 87°, respectively,[5] followed by optimization as above.

Preliminary protein−inhibitor complex calculations described in the text were performed using the Discover 95.5 and the CFF95. The crystal structure position for **27**[5] was used as a starting point, followed by a series of tethered minimizations to relieve any bad contacts from the initial placement. Crystallographically determined waters were included with a dielectric constant of 1.0. Interaction energies were computed and utilized as a crude measure of protein−inhibitor interaction. Delphi was also used to gain an estimate of the electrostatic portion of the solvation energy (finite difference Poisson−Boltzmann methodology) for each of the ligands, free and in their bound complexes (Molecular Simulations Inc., 9685 Scranton Road, San Diego, CA 92121-3752).

General Procedure for the Preparation of Carbamate Inhibitors 10, 16, and 19: amine **31** (1.364 g, 3.0 mmol) and DIEA (1.05 mL, 6.0 mmol) were dissolved in CH_2Cl_2 (35 mL) and the solution was cooled in an ice bath. The chloroformate (4−5 mmol) was added and the mixture was stirred 1−2 h. The reaction mixture was then poured into water, washed with water, dried ($MgSO_4$), and concentrated under reduced pressure to give an oil that was purified by flash chromatography using 95:5 $CHCl_3$/MeOH as eluent.

Inhibitor 10: prepared using benzyl chloroformate. R_f 0.36 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −32.8° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.52 (d, J = 5.5 Hz, 2H), 7.36 (s, 1H), 7.34−7.14 (m, 13H), 7.10 (d, J = 9.5 Hz, 1H), 4.94 (d, J = 13 Hz, 1H, part of AB), 4.90 (d, J = 5.5 Hz, 1H), 4.86 (d, J = 13 Hz, 1H, part of AB), 4.60 (d, J = 14 Hz, 1H, part of AB), 4.55 (d, J = 14 Hz, 1H, part of AB), 3.78 (m, 1H), 3.72 (m, 1H), 3.39 (tt, J = 10, 4 Hz, 1H), 3.22 (broad d, J = 12 Hz, 1H), 2.97 (dd, J = 14, 3 Hz, 1H), 2.72−2.58 (m, 3H), 2.13 (dd, J = 13, 6.5 Hz, 1H), 2.11−1.98 (m, 2H), 1.93 (broad d, J = 11 Hz, 1H), 1.52 (q, J = 11.5 Hz, 1H), 1.46 (m, 1H), 1.27 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.4, 155.8, 149.4, 148.1, 139.8, 137.2, 129.2, 128.1, 127.8, 127.4, 127.1, 125.5, 121.6, 75.0, 70.3, 67.1, 66.8, 64.7, 57.3, 56.1, 50.0, 49.9, 34.8, 34.3, 30.1, 28.3. HRMS (FAB) m/z $C_{34}H_{45}N_4O_5$ (MH^+) calcd: 589.3390; found: 589.3406. HPLC homogeneity: 94.1% (system A); 94.8% (system B).

Inhibitor 16: 2-methylbenzyl alcohol (0.610 g, 5.0 mmol) was added to an ice-cold solution of phosgene in toluene (1.8 M, 5 mL, 9.0 mmol). The mixture was stirred 30 min in the cold and then another 3 h at room temperature. Volatiles were removed under reduced pressure, and the residual chloroformate was used without purification to prepare the carbamate according to the general procedure. R_f 0.47 (9:1 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −28.2° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.52 (d, J = 5.5 Hz, 2H), 7.36 (s, 1H), 7.32 (d, J = 5.5 Hz, 2H), 7.29−7.08 (m, 11H), 4.91 (d, J = 13 Hz, 1H, part of AB), 4.89 (d, J = 7 Hz, 1H), 4.83 (d, J = 13 Hz, 1H, part of AB), 4.60 (d, J = 14 Hz, 1H, part of AB), 4.55 (d, J = 14 Hz, 1H, part of AB), 3.75 (m, 1H), 3.71 (m, 1H), 3.39 (m, 1H), 3.21 (broad d, J = 12 Hz, 1H), 2.95 (dd, J = 13.5, 2 Hz, 1H), 2.70−2.57 (m, 3H), 2.19 (s, 3H), 2.16−1.97 (m, 3H), 1.92 (broad d, J = 11 Hz, 1H), 1.50 (q, J = 11.5 Hz, 1H), 1.44 (m, 1H), 1.25 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.4, 155.7, 149.4, 148.1, 139.8, 135.9, 135.0, 129.8, 129.2, 127.9, 127.8, 127.6, 125.6, 121.6, 75.0, 70.3, 67.1, 66.7, 63.3, 57.2, 56.0, 49.9, 34.8, 34.3, 30.1, 28.3, 18.2. HRMS (FAB) m/z $C_{35}H_{47}N_4O_5$ (MH^+) calcd: 603.3547; found: 603.3528. HPLC homogeneity: 93.2% (system A); 95.8% (system B).

Inhibitor 19: 2,6-dimethylbenzoic acid **33** (5.00 g, 33 mmol) was added in small portions to a suspension of $LiAlH_4$ (1.14 g, 30 mmol) in dry THF (100 mL) under an argon atmosphere. The mixture was refluxed for 3 days, cooled to room temperature, and quenched by dropwise addition of 10% HCl (100 mL). After extraction with EtOAc, drying ($MgSO_4$), and removal of volatiles under reduced pressure, crude 2,6-dimethylbenzyl alcohol was obtained as a white solid (4.30 g, 94% yield) that was used without purification: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.2−6.9 (m, 3H), 4.70 (s, 2H), 4.05 (broad s, 1H), 2.40 (s, 6H). Following the procedure described above for **16**, the alcohol was converted to its chloroformate and used to prepare carbamate **19** according to the general procedure. Inhibitor **19** was purified by reversed-phase chromatography using 0.06% TFA/0.06% TFA in CH_3CN gradients and isolated by lyophilization. R_f 0.44 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −2.5° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 9.80 (broad s, 1H), 8.66 (broad d, J = 4.5 Hz, 2H), 8.26 (broad s, 1H), 7.54 (d, J = 5.5 Hz, 2H), 7.23−7.11 (m, 5H), 7.08 (t, J = 7 Hz, 2H), 6.97 (d, J = 7.5 Hz, 2H), 4.92 (d, J = 12 Hz, 1H, part of AB), 4.78 (d, J = 12 Hz, 1H, part of AB), 4.69 (m, 2H), 3.90 (m, 2H), 3.16 (m, 2H), 2.97 (broad d, J = 13.5 Hz, 1H), 2.90 (broad t, J = 10.5 Hz, 1H), 2.42 (broad d, J = 11.5 Hz, 1H), 2.24 (m, 1H), 2.19 (s, 6H), 1.80 (q, J = 13 Hz, 1H), 1.66 (q, J = 11.5 Hz, 1H), 1.28 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 166.2, 156.2, 147.0, 138.7, 137.6, 132.4, 128.9, 128.2, 127.9, 127.8, 125.9, 122.4, 71.8, 70.0, 67.4, 65.3, 60.3, 58.1, 56.4, 51.4, 51.0, 35.6, 33.9, 28.4, 28.0, 19.0. HRMS (FAB) m/z $C_{36}H_{48}N_4O_5$ (MH^+) calcd: 617.3703; found: 617.3690. HPLC homogeneity: 98.5% (system A); 98.6% (system B).

General Procedure for Coupling of Amine 31 with Carboxylic Acids: amine **31** (1 equiv), the carboxylic acid (1.2−1.3 equiv) and DIEA (3−4 equiv) were dissolved in CH_3CN, and TBTU[22] (1.3−1.5 equiv) was added. The mixture was stirred for 1−2 h at room temperature. The solvent was removed under reduced pressure, the residue dissolved in EtOAc, and the solution washed twice with 2 N NaOH and water. After drying ($MgSO_4$), the solvent was evaporated under reduced pressure and the residue purified by flash chromatography using 95:5 $CHCl_3$/MeOH as eluent, to give the pure inhibitors as off-white foams.

Inhibitor 11: amine **31** was coupled to phenoxyacetic acid following the general procedure. R_f 0.43 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −31.4° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.51 (d, J

= 5.5 Hz, 2H), 7.83 (d, *J* = 9 Hz, 1H), 7.35 (s, 1H), 7.31 (d, *J* = 5.5 Hz, 2H), 7.28−7.19 (m, 6H), 7.15 (m, 1H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 4.98 (d, *J* = 5 Hz, 1H), 4.60 (d, *J* = 14 Hz, 1H, part of AB), 4.54 (d, *J* = 14 Hz, 1H, part of AB), 4.37 (d, *J* = 15 Hz, 1H, part of AB), 4.32 (d, *J* = 15 Hz, 1H, part of AB), 4.14 (m, 1H), 3.76 (m, 1H), 3.39 (tt, *J* = 10.5, 4.5 Hz, 1H), 3.20 (broad d, *J* = 12 Hz, 1H), 2.96 (dd, *J* = 14, 3 Hz, 1H), 2.70 (m, 2H), 2.60 (dd, *J* = 12.5, 7 Hz, 1H), 2.15 (dd, *J* = 13, 6.5 Hz, 1H), 2.10−2.00 (m, 2H), 1.94 (broad d, *J* = 11.5 Hz, 1H), 1.55 (q, *J* = 11.5 Hz, 1H), 1.46 (m, 1H), 1.26 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.3, 166.9, 157.7, 149.3, 148.0, 139.4, 129.2, 129.1, 127.8, 125.6, 121.5, 120.9, 114.5, 75.0, 70.1, 67.1, 66.6, 66.5, 57.1, 53.4, 49.9, 49.8, 34.2, 34.0, 30.1, 28.3. HRMS (FAB) *m/z* $C_{34}H_{45}N_4O_5$ (MH^+) calcd: 589.3390; found: 589.3406. HPLC homogeneity: 95.7% (system A); 94.4% (system B).

Inhibitor 12: amine **31** was coupled to 3-phenylpropionic acid following the general procedure. R_f 0.38 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −29.8° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.52 (d, *J* = 5.5 Hz, 2H), 7.78 (d, *J* = 9 Hz, 1H), 7.38 (s, 1H), 7.32 (d, *J* = 5.5 Hz, 2H), 7.29−7.20 (m, 6H), 7.18−7.09 (m, 4H), 4.91 (d, *J* = 5.5 Hz, 1H), 4.60 (d, *J* = 14 Hz, 1H, part of AB), 4.55 (d, *J* = 14 Hz, 1H, part of AB), 4.07 (m, 1H), 3.70 (m, 1H), 3.40 (m, 1H), 3.20 (broad d, *J* = 12.5 Hz, 1H), 2.91 (dd, *J* = 14, 3 Hz, 1H), 2.73−2.55 (m, 5H), 2.31 (t, *J* = 8 Hz, 2H), 2.13 (dd, *J* = 12.5, 6.5 Hz, 1H), 2.11−1.91 (m, 3H), 1.53 (q, *J* = 11.5 Hz, 1H), 1.51−1.41 (m, 1H), 1.27 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.5, 171.0, 149.4, 148.1, 141.4, 139.8, 129.2, 128.1, 127.9, 127.8, 125.7, 125.5, 121.6, 75.0, 70.1, 67.1, 66.6, 57.1, 53.7, 50.0, 49.9, 37.1, 34.5, 34.3, 31.1, 30.2, 28.3. HRMS (FAB) *m/z* $C_{35}H_{47}N_4O_4$ (MH^+) calcd: 587.3597; found: 587.3624. HPLC homogeneity: 98.2% (system A); 98.5% (system B).

Inhibitor 13: amine **31** was coupled to thiophenoxyacetic acid following the general procedure. The compound was further purified by reversed-phase chromatography using 0.06% TFA/0.06% TFA in CH_3CN gradients and isolated by lyophilization: R_f 0.40 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −1.6° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.75 (broad m, 2H), 8.28 (broad m, 1H), 8.16 (broad d, *J* = 9 Hz, 1H), 7.71 (broad, *J* = 2.5 Hz, 2H), 7.26−7.12 (m, 10H), 4.77 (broad m, 2H), 3.97 (broad t, *J* = 12.5 Hz, 1H), 3.51 (tq, *J* = 9, 3 Hz, 1H), 3.15 (t, *J* = 12 Hz, 1H), 3.09 (broad d, *J* = 14.5 Hz, 1H), 2.95 (broad d, *J* = 13.5 Hz, 2H), 2.60 (m, 1H), 2.46 (d, *J* = 12.5 Hz, 1H), 2.22 (broad d, *J* = 12 Hz, 1H), 1.81 (broad q, *J* = 12 Hz, 1H), 1.68 (broad q, *J* = 12 Hz, 1H), 1.29 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 167.8, 166.3, 154.2, 145.0, 138.5, 136.2, 129.0, 128.9, 128.1, 127.5, 126.1, 125.7, 123.2, 72.1, 69.5, 67.4, 65.0, 58.1, 54.5, 51.3, 51.1, 36.4, 35.1, 34.0, 28.3, 28.1. HRMS (FAB) *m/z* $C_{38}H_{46}N_4O_4S$ (MH^+) calcd: 605.3162; found: 605.3181. HPLC homogeneity: 99.3% (system A); 96. 9% (system B).

Inhibitor 14: amine **31** was coupled to 4-phenylbutyric acid following the general procedure. R_f 0.38 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −27.4° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.51 (d, *J* = 5 Hz, 2H), 7.67 (d, *J* = 9 Hz, 1H), 7.34 (s, 1H), 7.31 (d, *J* = 5 Hz, 2H), 7.28−7.08 (m, 8H), 7.08 (d, *J* = 8 Hz, 2H), 4.87 (d, *J* = 5 Hz, 1H), 4.60 (d, *J* = 14 Hz, 1H, part of AB), 4.54 (d, *J* = 14 Hz, 1H, part of AB), 4.08 (m, 1H), 3.69 (m, 1H), 3.39 (tt, *J* = 10.5, 4 Hz, 1H), 3.21 (broad d, *J* = 12.5 Hz, 1H), 2.91 (dd, *J* = 14, 3 Hz, 1H), 2.68 (broad t, *J* = 11.5 Hz, 2H), 2.58 (dd, *J* = 12.5, 6.5 Hz, 1H), 2.38 (t, *J* = 7.5 Hz, 2H), 1.64 (m, 2H), 1.51 (q, *J* = 11.5 Hz, 1H), 1.46 (m, 1H), 1.27 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.5, 149.4, 148.2, 141.8, 139.9, 129.2, 128.2, 128.1, 127.8, 125.6, 121.7, 75.1, 70.4, 67.2, 66.7, 57.2, 53.6, 50.0, 49.9, 35.0, 34.6, 34.4, 30.2, 28.4, 27.1. HRMS (FAB) *m/z* $C_{36}H_{49}N_4O_4$ (MH^+) calcd: 601.3754; found: 601.3739. HPLC homogeneity: 95.8% (system A); 97.0% (system B).

Inhibitor 15: amine **31** was coupled to *trans*-cinnamic acid following the general procedure. R_f 0.38 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −44.5° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.51 (d, *J* = 5.5 Hz, 2H), 8.13 (d, *J* = 9.5 Hz, 1H), 7.50 (m, 3H), 7.41−7.29 (m, 7H), 7.23 (t, *J* = 7.5 Hz, 2H), 7.13 (t, *J* = 7.5 Hz, 1H), 6.72 (d, *J* = 16 Hz, 1H), 5.00 (d, *J* = 5 Hz, 1H), 4.59 (d, *J* = 14 Hz, 1H, part of AB), 4.55 (d, *J* = 14 Hz, 1H, part of AB), 4.28 (m, 1H), 3.83 (m, 1H), 3.40 (m, 1H), 3.26 (broad d, *J* = 12 Hz, 1H), 3.00 (dd, *J* = 14, 3.5 Hz, 1H), 2.80−2.68 (m, 2H), 2.63 (dd, *J* = 12.5, 7 Hz, 1H), 2.18 (dd, *J* = 12.5, 6 Hz, 1H), 2.13−1.93 (m, 3H), 1.56 (q, *J* = 11.5 Hz, 1H), 1.49 (m, 1H), 1.29 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.7, 164.6, 149.3, 148.1, 139.7, 138.1, 135.0, 129.1, 129.0, 128.7, 127.8, 127.3, 125.6, 122.6, 121.6, 75.0, 69.8, 67.1, 66.8, 57.4, 54.1, 50.0, 49.9, 34.9, 34.8, 30.3, 28.3. HRMS (FAB) *m/z* $C_{35}H_{45}N_4O_4$ (MH^+) calcd: 585.3441; found: 585.3453. HPLC homogeneity: 99.1% (system A); 98.9% (system B).

Inhibitor 17: amine **31** was coupled to 2-methylhydrocinnamic acid following the general procedure. R_f 0.32 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −30.8° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.52 (d, *J* = 5 Hz, 2H), 7.80 (d, *J* = 9 Hz, 1H), 7.39 (s, 1H), 7.32 (d, *J* = 5.5 Hz, 2H), 7.30−7.20 (m, 4H), 7.14 (t, *J* = 7 Hz, 1H), 7.11−7.00 (m, 4H), 4.92 (d, *J* = 5 Hz, 1H), 4.60 (d, *J* = 14 Hz, 1H, part of AB), 4.55 (d, *J* = 14 Hz, 1H, part of AB), 4.07 (m, 1H), 3.71 (m, 1H), 3.40 (m, 1H), 3.21 (broad d, *J* = 12 Hz, 1H), 2.92 (dd, *J* = 14, 3.5 Hz, 1H), 2.73−2.56 (m, 5H), 2.25 (m, 2H), 2.22 (s, 3H), 2.13 (dd, *J* = 13, 6.5 Hz, 1H), 2.11−2.00 (m, 2H), 1.95 (broad d, *J* = 11 Hz, 1H), 1.54 (q, *J* = 11.5 Hz, 1H), 1.46 (m, 1H), 1.27 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.5, 171.0, 149.4, 148.1, 139.8, 139.4, 135.4, 129.8, 129.2, 128.0, 127.7, 125.8, 125.5, 121.6, 75.0, 70.1, 67.1, 66.6, 57.1, 53.7, 49.9, 49.8, 35.8, 34.5, 34.4, 30.2, 28.5, 28.3, 18.8. HRMS (FAB) *m/z* $C_{36}H_{49}N_4O_4$ (MH^+) calcd: 601.3754; found: 601.3739. HPLC homogeneity: 93.7% (system A); 94.9% (system B).

Inhibitor 18: amine **31** was coupled to 2-methylphenoxyacetic acid following the general procedure. R_f 0.42 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −30.6° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.51 (d, *J* = 6 Hz, 2H), 7.67 (d, *J* = 9.5 Hz, 1H), 7.35 (s, 1H), 7.31 (d, *J* = 5.5 Hz, 2H), 7.29−7.20 (m, 4H), 7.16 (m, 1H), 7.11 (d, *J* = 7 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 6.83 (t, *J* = 7.5 Hz, 1H), 6.58 (d, *J* = 8.5 Hz, 1H), 4.99 (d, *J* = 5.5 Hz, 1H), 4.59 (d, *J* = 14 Hz, 1H, part of AB), 4.54 (d, *J* = 14 Hz, 1H, part of AB), 4.38 (d, *J* = 14.5 Hz, 1H, part of AB), 4.31 (d, *J* = 14.5 Hz, 1H, part of AB), 4.16 (m, 1H), 3.77 (m, 1H), 3.39 (tt, *J* = 10.5, 4.5 Hz, 1H), 3.21 (broad d, *J* = 12.5 Hz, 1H), 2.98 (dd, *J* = 14, 3 Hz, 1H), 2.70 (m, 2H), 2.61 (dd, *J* = 12.5, 7 Hz, 1H), 2.19 (s, 3H), 2.15 (dd, *J* = 13, 6.5 Hz, 1H), 2.10−2.00 (m, 2H), 1.94 (broad d, *J* = 11.5 Hz, 1H), 1.55 (q, *J* = 11.5 Hz, 1H), 1.47 (m, 1H), 1.26 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.3, 167.0, 155.8, 149.3, 148.0, 139.4, 130.3, 129.2, 127.8, 126.7, 125.8, 125.6, 121.5, 120.6, 111.4, 75.0, 70.0, 67.1, 66.9, 66.6, 57.1, 53.2, 49.9, 49.8, 34.3, 33.9, 30.2, 28.2, 16.0. HRMS (FAB) *m/z* $C_{35}H_{47}N_4O_5$ (MH^+) calcd: 603.3547; found: 603.3534. HPLC homogeneity: 98.4% (system A); >99.5% (system B).

Inhibitor 20: 2,6-dimethylbenzyl alcohol (Scheme 2, prepared as described for inhibitor **19**, 2.42 g, 18 mmol) was dissolved in CH_2Cl_2 (100 mL). Celite (∼ 5 mL) and powdered 4 Å molecular sieves (∼5 mL) were added. The suspension was cooled in ice, and pyridinium chlorochromate (7.76 g, 36 mmol) was added in portions. The mixture was stirred until completion as determined by TLC analysis. The mixture was concentrated to half-volume under reduced pressure, diethyl ether (100 mL) was added, and the suspension was filtered through Celite. Volatiles were removed under reduced pressure, and the residual 2,6-dimethylbenzaldehyde was used without purification. ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.60 (s, 1H), 7.30 (t, *J* = 8 Hz, 1H), 7.10 (d, *J* = 8 Hz, 2H), 2.65 (s, 6H).

The crude aldehyde from above and methyl (triphenylphosphoranylidene)acetate (6.70 g, 20 mmol) were dissolved in toluene (40 mL), and the solution was refluxed for 4 h. After being stirred overnight at room temperature, the reaction mixture was passed through a pad of silica gel (∼100 mL) using ether as eluent. Solvents were removed under reduced pressure, and the residue was passed through another pad of silica gel (30 mL) using ether (150 mL) for elution. Removal of the solvent gave the crude cinnamate ester (∼2.4 g) as a yellow oil that was used without further purification in the next step.

The crude cinnamate from above (∼2.4 g) was hydrogenated (1 atm H_2 gas) in MeOH over 20% $Pd(OH)_2$ on carbon (100 mg) for 20 h. Filtration followed by removal of solvent gave crude methyl 3-(2′,6′-dimethylphenyl)propionate. ^{1}H NMR (200

MHz, $CDCl_3$) δ 7.00 (s, 3H), 3.70 (s, 3H), 3.00 (m, 2H), 2.49 (m, 2H), 2.35 (s, 6H). The material was dissolved in 3:1 THF−water (25 mL), and LiOH monohydrate (1.68 g, 40 mmol) was added. The mixture was stirred 20 h at room temperature. THF was removed under reduced pressure and the aqueous phase washed with 1:1 ether−hexane. After acidification to pH 1 with 6 N HCl, the product was extracted with EtOAc and washed with water and the solution dried ($MgSO_4$). Removal of the solvent under reduced pressure gave 3-(2′,6′-dimethylphenyl)propionic acid **32** as a cream colored solid (1.52 g, 48% yield overall from 2,6-dimethylbenzyl alcohol). Mp 75−77 °C. ^{1}H NMR ($CDCl_3$) δ 7.03 (s, 3H), 3.00 (m, 2H), 2.51 (m, 2H), 2.35 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.5, 137.0, 136.4, 128.6, 126.5, 33.4, 25.0, 19.9. MS (FAB) *m*/*z* 179 (MH^+). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.06; H, 7.78.

Amine **31** was coupled to acid **32** to give inhibitor **20** following the general procedure. R_f 0.40 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −44.5° (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (dd, J = 5, 1.5 Hz, 2H), 7.80 (d, J = 9 Hz, 1H), 7.39 (s, 1H), 7.33−7.28 (m, 4H), 7.24 (t, J = 7.5 Hz, 2H), 7.15 (m, 1H), 6.94 (s, 3H), 4.94 (d, J = 5.5 Hz, 1H), 4.60 (d, J = 14 Hz, 1H, part of AB), 4.56 (d, J = 14 Hz, 1H, part of AB), 4.06 (m, 1H), 3.71 (m, 1H), 3.40 (tt, J = 10.5, 4.5 Hz, 1H), 3.22 (dt, J = 12, 3.5 Hz, 1H), 2.93 (dd, J = 13.5, 3.5 Hz, 1H), 2.70 (dd, J = 11, 2 Hz, 1H), 2.68−2.46 (m, 4H), 2.22 (s, 6H), 2.16−2.00 (m, 6H), 1.95 (broad d, J = 11.5 Hz, 1H), 1.53 (q, J = 11.5 Hz, 1H), 1.46 (m, 1H), 1.26 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.4, 171.0, 149.4, 148.1, 139.8, 138.0, 135.5, 129.3, 127.8, 127.7, 125.6, 121.6, 75.0, 70.2, 67.1, 66.6, 57.1, 53.7, 50.0, 49.9, 34.8, 34.4, 30.2, 28.3, 25.5, 19.2. HRMS (FAB) *m*/*z* $C_{37}H_{51}N_4O_4$ (MH^+) calcd: 615.3910; found: 615.3931. HPLC homogeneity: 93.8% (system A); 93.7% (system B).

Inhibitor 21: 2,6-dimethylphenol **35** (500.0 g, 4.09 mol), ethyl bromoacetate (480 mL, 4.3 mol), and anhydrous potassium carbonate (690 g, 5.0 mol) were suspended in acetone (2 L), and the mixture was stirred 56 h at room temperature. NaOH (1 N, 1 L) was added and the mixture refluxed for 4 h. NaOH (150 g, 3.75 mol) was added, and reflux continued for another 48 h. After cooling and removal of acetone under reduced pressure, the aqueous phase was carefully acidified to pH 1 with concentrated HCl. The tan-colored precipitate was collected, washed with water, and dried in air. The crude material was recrystallized once from 2-propanol and then from EtOAc to give pure 2′,6′-dimethylphenoxyacetic acid **34a** (R = H) as white crystals (460 g, 62% yield): mp 137.5−139 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.4 (broad s, 1H), 7.0 (m, 3H), 4.55 (s, 2H), 2.34 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 172.2, 155.1, 130.7, 129.0, 124.5, 68.7, 16.2. MS (FAB) *m*/*z* 181 (MH^+). Anal. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.37; H, 6.74.

Amine **31** was coupled to **34a** following the general procedure: R_f 0.44 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −23.1° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.51 (d, J = 5.5 Hz, 2H), 7.78 (d, J = 9.5 Hz, 1H), 7.37 (s, 1H), 7.30 (m, 4H), 7.23 (t, J = 7.5 Hz, 2H), 7.16 (t, J = 7 Hz, 1H), 6.99 (d, J = 7 Hz, 2H), 6.91 (dd, J = 8, 6.5 Hz, 1H), 5.04 (d, J = 5 Hz, 1H), 4.60 (d, J = 14 Hz, 1H, part of AB), 4.54 (d, J = 14 Hz, 1H, part of AB), 4.25 (broad t, J = 10 Hz, 1H), 4.12 (d, J = 14.5 Hz, 1H, part of AB), 3.93 (d, J = 14.5 Hz, 1H, part of AB), 3.83 (m, J = 5 Hz, 1H), 3.40 (tt, J = 10.5, 4.5 Hz, 1H), 3.23 (broad d, J = 12.5 Hz, 1H), 3.03 (dd, J = 14, 3 Hz, 1H), 2.81−2.71 (m, 2H), 2.67 (dd, J = 12.5, 6.5 Hz, 1H), 2.19 (dd, J = 13, 6.5 Hz, 1H), 2.14 (s, 6H), 2.08 (broad d, J = 12 Hz, 2H), 1.94 (broad d, J = 11.5 Hz, 1H), 1.56 (q, J = 11.5 Hz, 1H), 1.50 (m, 1H), 1.28 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.2, 167.0, 154.3, 149.3, 148.0, 139.4, 130.2, 129.2, 128.6, 127.7, 125.6, 124.0, 121.5, 75.0, 70.1, 67.1, 66.5, 57.1, 53.1, 49.9, 49.7, 34.0, 33.8, 29.9, 28.2, 15.7. HRMS (FAB) *m*/*z* $C_{36}H_{49}N_4O_5$ (MH^+) calcd: 617.3703; found: 617.3715. HPLC homogeneity: 96.7% (system A); 97.2% (system B).

Inhibitor 22: carboxylic acid **34b** was prepared from 2,6-dimethylphenol **35** and ethyl 2-bromopropanoate as described above for **34a**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.2 (broad s, 1H), 7.0 (m, 3H), 4.60 (q, J = 7 Hz, 1H), 2.28 (s, 6H), 1.55 (d, J = 7 Hz, 3H).

Amine **31** was coupled to **34b** to give **22** as a mixture of epimers, following the general procedure: R_f 0.46 (95:5 $CHCl_3$/MeOH). ^{1}H NMR (DMSO-d_6) δ 8.51 (d, J = 5.5 Hz, 2H), 7.73 (d, J = 9 Hz, 0.5H), 7.72 (d, J = 9 Hz, 0.5H), 7.36−7.18 (m, 7H), 7.14 (m, 1H), 6.96 (t, J = 6.5 Hz, 2H), 6.90 (t, J = 7 Hz, 0.5H), 6.88 (t, J = 7.5 Hz, 0.5H), 5.02 (d, J = 5.5 Hz, 0.5H), 5.01 (d, J = 5.5 Hz, 0.5H), 4.61 (d, J = 14 Hz, 1H, part of AB), 4.54 (d, J = 14 Hz, 1H, part of AB), 4.28 (q, J = 6.5 Hz, 0.5H), 4.20 (m, 1H), 4.16 (q, J = 6.5 Hz, 0.5H), 3.77 (m, 1H), 3.41 (m, 1H), 3.21 (m, 1H), 2.99 (m, 1H), 2.80−2.60 (m, 3H), 2.23−2.04 (m, 2H), 2.15 (s, 3H), 2.08 (s, 3H), 1.92 (broad d, J = 10.5 Hz, 1H), 1.62−1.45 (m, 2H), 1.27 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.2, 170.5, 170.4, 153.8, 153.3, 149.3, 148.0, 139.3, 139.2, 130.4, 129.3, 129.1, 128.7, 127.7, 127.6, 125.5, 123.5, 121.5, 77.5, 77.1, 75.0, 70.5, 70.4, 67.1, 66.2, 56.6, 52.7, 49.9, 49.6, 33.9, 33.7, 33.5, 29.7, 28.2, 18.2, 17.9, 16.6, 16.5. HRMS (FAB) *m*/*z* $C_{37}H_{51}N_4O_5$ (MH^+) calcd: 631.3881; found: 617.3715. HPLC homogeneity: 99.1% (system A); 99.6% (system B).

Inhibitor 23: 3-hydroxy-2-methylbenzoic acid[23] (0.452 g, 3.0 mmol) and 1-hydroxybenzotriazole hydrate (0.419 g, 3.1 mmol) were dissolved in THF (35 mL), and 1,3-dicyclohexylcarbodiimide (0.619 g, 3.0 mmol) was added. After 15 min, amine **36** (1.350 g, 3.0 mmol) was added and the mixture stirred 20 h at room temperature. The precipitated dicyclohexyl urea was removed by filtration, and the filtrate was washed with water. After drying ($MgSO_4$) and removal of solvent under reduced pressure, the residue was purified by flash chromatography using 95:5 $CHCl_3$/MeOH as eluent. The product was then precipitated from EtOAc (3 mL) by addition of ether (20 mL) to give **23** as an off-white solid (0.406 g, 23% yield). R_f 0.40 (9:1 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −4.2° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 9.34 (s, 1H), 8.51 (d, J = 5.5 Hz, 2H), 7.88 (broad d, J = 8.5 Hz, 1H), 7.38−7.28 (m, 5H), 7.25 (t, J = 7.5 Hz, 2H), 7.15 (t, J = 7 Hz, 1H), 6.92 (t, J = 8 Hz, 1H), 6.75 (d, J = 8 Hz, 1H), 6.49 (d, J = 7.5 Hz, 1H), 4.94 (broad m, 1H), 4.61 (d, J = 14 Hz, 1H, part of AB), 4.55 (d, J = 14 Hz, 1H, part of AB), 4.21 (broad m, 1H), 3.77 (broad m, 1H), 3.41 (broad m, 1H), 3.25 (broad d, J = 12 Hz, 1H), 3.00 (broad d, J = 11.5 Hz, 1H), 2.78−2.62 (m, 2H), 2.2−1.89 (m, 3H), 1.81 (s, 3H), 1.51 (q, J = 11.5 Hz, 1H), 1.23 (s, 9H).^{13}C NMR (DMSO-d_6) δ 171.2, 168.8, 155.3, 149.4, 148.2, 139.9, 139.3, 129.3, 127.8, 125.6, 125.5, 121.6, 121.3, 117.3, 115.0, 75.0, 70.9, 67.1, 66.3, 56.7, 53.7, 49.9, 49.8, 34.2, 33.7, 29.8, 28.3, 12.2. HRMS (FAB) *m*/*z* $C_{34}H_{45}N_4O_5$ (MH^+) calcd: 589.3390; found: 589.3404. HPLC homogeneity: 90.5% (system A); 90.4% (system B).

Inhibitor 24: 3-(*S*)-hydroxytetrahydrofuran (1.322 g, 15.0 mmol) in CH_2Cl_2 (50 mL) was added dropwise to a ice-cooled solution of phosgene in toluene (1.93 M, 30 mL). After the mixture was stirred for 1 h at 0 °C, volatiles were removed under vacuum. The residue was dissolved in CH_2Cl_2 (20 mL), *N*-methylmorpholine (2.41 mL, 22.5 mmol) was added, and the solution cooled in an ice bath. Amine **31** (2.27 g, 5 mmol) in CH_2Cl_2 (20 mL + 20 mL rinse) was added dropwise, and the mixture stirred for 16 h at room temperature. Volatiles were then removed under reduced pressure, the residue was dissolved in EtOAc, and the solution was washed successively with saturated aqueous $NaHCO_3$ and brine. After the solution was dried over $MgSO_4$, evaporation of the solvent gave an oil that was purified by flash chromatography using 5% EtOH in $CHCl_3$ as eluent. R_f 0.43 (95:5 $CHCl_3$/MeOH). $[\alpha]^{23}_D$ −31.7° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.52 (d, J = 5.5 Hz, 2H), 7.37−7.20 (m, 7H), 7.14 (m, 1H), 6.97 (d, J = 9 Hz, 1H), 4.93 (m, 1H), 4.84 (d, J = 5 Hz, 1H), 4.60 (d, J = 13.5 Hz, 1H, part of AB), 4.55 (d, J = 14 Hz, 1H, part of AB), 3.75−3.57 (m, 5H), 3.43−3.32 (m, 1H), 3.21 (broad d, J = 12 Hz, 1H), 2.93 (dd, J = 13.5, 2.5 Hz, 1H), 2.70−2.53 (m, 2H), 2.12−1.90 (m, 5H), 1.82−1.75 (m, 1H), 1.55−1.37 (m, 2H), 1.26 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.4, 155.6, 149.4, 148.1, 139.9, 129.4, 129.1, 127.8, 125.6, 121.6, 74.9, 73.9, 72.7, 70.2, 67.1, 66.8, 66.1, 57.3, 56.0, 50.0, 34.9, 34.5, 32.1, 30.2, 28.3. HRMS (FAB) *m*/*z*

$C_{31}H_{45}N_4O_6$ (MH^+) calcd: 569.3339; found: 569.3328. HPLC homogeneity: 95.7% (system A); 98.6% (system B).

Preparation of (2*S*,4*R*)-4-bromopipecolic *tert*-butylamide 37 (X = Br): (2*S*,4*R*)-4-hydroxypipecolic *tert*-butylamide **36**[21,25] (70.10 g, 0.233 mol) was dissolved in dry THF (700 mL), and Et_3N (42 mL, 0.30 mol) was added. The solution was cooled in an ice−water bath under a nitrogen atmosphere, and methanesulfonyl chloride (22.5 mL, 0.29 mol) was added dropwise over 30 min. The resulting white suspension was stirred at room temperature for 2 h and poured into 20% aqueous NaCl, and the organic layer was separated. The organic extract was washed with 20% aqueous NaCl (650 mL), dried ($MgSO_4$), and concentrated under reduced pressure to a volume of ca. 200 mL. Hexane (350 mL) was added to the yellow solution, producing a white precipitate. After cooling overnight in a refrigerator, the solid was collected by filtration, washed with hexane, and dried in air. The mesylate of alcohol **36** (84.96 g, 96% yield) was obtained as a white solid: mp 122−123 °C. R_f 0.67 (1:4 hexane/EtOAc). $[\alpha]^{22}_D$ −41.2° (*c* 1.0, $CHCl_3$). IR (KBr) ν 3378, 1694, 1520 cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.84−5.69 (broad s, 1H), 5.07 (m, 1H), 4.68 (broad s, 1H), 4.08 (broad m, 1H), 3.25−3.13 (m, 1H), 3.07 (s, 3H), 2.92−2.83 (m, 1H), 2.06−1.95 (m, 1H), 1.81 (dd, *J* = 7, 3 Hz, 1H, part of ABX), 1.77 (dd, *J* = 7, 3 Hz, 1H, part of ABX), 1.78−1.67 (m, 1H), 1.49 (s, 9H), 1.37 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 169.1, 155.1, 81.5, 74.8, 52.7 (broad), 51.3, 39.1, 35.8 (broad), 30.5 (broad), 29.4 (broad), 28.8, 28.5. MS (ES^+) *m*/*z* 379 (MH^+), 279 (MH^+ − Boc). Anal. Calcd for $C_{16}H_{30}N_2O_6S$: C, 50.78; H, 7.99; N, 7.40. Found: C, 51.10; H, 8.34; N, 7.46.

The mesylate from above (79.67 g, 0.210 mol) and tetra-*n*-butylammonium bromide (271.41 g, 0.842 mol) were dissolved in THF (1.1 L), and the solution was refluxed for 15 h. After the mixture cooled to room temperature, ether (1.7 L) was added and the solution washed with 5% $Na_2S_2O_3$ (1 L), water (1.1 L), and brine (1.1 L). The solution was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give a white solid. MeOH (500 mL) was added to dissolve the solid, and water (220 mL) was added slowly. The mixture was left to crystallize for 2 days at 4 °C. The product was collected by filtration and dried in a vacuum over P_2O_5. Bromide **37** (X = Br), was obtained as fine white needles (52.05 g, 68% yield): mp 112−114 °C. R_f 0.78 (2:1 hexane/EtOAc). $[\alpha]^{22}_D$ −119.2° (*c* 1.0, $CHCl_3$). IR (KBr) ν 3345−3301, 1663, 1549 cm^{-1}. ^1H NMR ($CDCl_3$, mixture of rotamers) δ 6.31−6.01 (broad s, 1H), 4.81−4.41 (m, 2H), 4.23−3.82 (m, 1H), 2.92−2.67 (m, 2H), 2.28−2.15 (m, 1H), 1.97−1.81 (m, 2H), 1.49 (s, 9H), 1.32 (s, 9H).^{13}C NMR ($CDCl_3$) δ 169.2, 156.0, 81.5, 55.8, 51.4, 45.1, 42.7, 36.8, 36.6, 28.9, 28.5. MS (ES^+) *m*/*z* 263 (MH^+ − Boc). Anal. Calcd for $C_{15}H_{27}BrN_2O_3$: C, 49.59; H, 7.49; N, 7.710. Found: C, 50.00; H, 7.78; N, 7.81.

Preparation of (2*S*,4*R*)-4-iodopipecolic *tert*-butylamide 37 (X = I): following the procedure described above for the preparation of the bromide, the mesylate of **36** was converted to the iodo derivative **37** (X = I) using tetra-*n*-butylammonium iodide (71% yield): ^1H NMR ($CDCl_3$, mixture of rotamers) δ 6.27−6.02 (broad s, 1H), 4.80−4.27 (m, 2H), 4.19−3.69 (m, 1H), 2.99−2.70 (m, 2H), 2.37−2.25 (m, 1H), 2.17−1.99 (m, 2H), 1.50 (s, 9H), 1.33 (s, 9H).

General Procedure for the Preparation of Thio Derivatives 38a−c. Preparation of 38c: to an ice-cold suspension of 95% NaH (4.24 g, 0.178 mol, 1.2 equiv) in anhydrous DMF (350 mL) was added 4-mercaptopyridine (22.30 g, 0.200 mol, 1.4 equiv) in small portions. After the mixture was stirred for 1 h at 0 °C under an atmosphere of nitrogen, bromide **37** (52.05 g, 0.143 mol, 1 equiv) was added (the corresponding iodide can also be used). The mixture was stirred for 1 h at room temperature, then 18 h at 43 °C. The reaction was then cooled to room temperature, diluted with EtOAc (2 L), and washed successively with water (900 mL), 1 N NaOH (900 mL), and brine (900 mL). After drying ($MgSO_4$), volatiles were removed under reduced pressure, and the residual foam (60.16 g) was refluxed with hexane (550 mL) for 20 min. The resulting suspension was cooled to room temperature and allowed to stand overnight. The product was collected by filtration, washed with hexane (200 mL), and dried. Mercaptopyridine derivative **38c** (44.93 g, 80% yield) was obtained as a yellowish solid: mp 127−129 °C. R_f 0.56 (9:1 $CHCl_3$/MeOH). $[\alpha]^{18}_D$ −64.5° (*c* 1.0, $CHCl_3$). IR (KBr) ν 3270, 1682 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.41 (d, *J* = 6 Hz, 2H), 7.11 (d, *J* = 6 Hz, 2H), 5.84 (broad s, 1H), 4.53 (dd, *J* = 6.5, 4 Hz, 1H), 3.98 (m, 1H), 3.77 (m, *J* = 4.5 Hz, 1H), 3.33−3.20 (m, 1H), 2.64 (m, 1H), 2.16 (ddd, *J* = 14.5, 7, 5 Hz, 1H), 2.10−1.97 (m, 1H), 1.85−1.75 (m, 1H), 1.48 (s, 9H), 1.38 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 169.6, 155.3, 149.6, 147.8, 122.1, 81.3, 54.5, 51.2, 37.7, 37.6 (broad), 29.1, 28.8, 28.4. MS (FAB) *m*/*z* 394 (MH^+). Anal. Calcd for $C_{20}H_{31}N_3O_3S$: C, 61.04; H, 7.94; N, 10.68. Found: C, 60.74; H, 8.29; N, 10.67.

Following the same procedure but using iodide **37** (X = I) instead of the bromide, carbamate **38c** was obtained in 95% yield after purification by flash chromatography.

General Procedure for Deprotection of Carbamates 38. Preparation of 39c: carbamate **38c** (5.26 g, 13.4 mmol) was stirred at room temperature for 20 min with 4 N HCl-dioxane (45 mL).

Volatiles were removed under reduced pressure, and the residual solid was partitioned between EtOAc and water. The pH of the aqueous phase was adjusted to 9 with 1 N NaOH and the organic phase separated. The aqueous phase was extracted with more EtOAc (3 × 75 mL), and the combined organic extracts were washed with brine (100 mL). After drying ($MgSO_4$) and removal of solvent under vacuum, crude **39c** was obtained as a white foam (3.94 g, quantitative): ^1H NMR ($CDCl_3$) δ 8.41 (dd, *J* = 5, 1.5 Hz, 2H), 7.14 (dd, *J* = 5, 1.5 Hz, 2H), 6.50 (broad s, 1H), 3.38 (tt, *J* = 12, 4 Hz, 1H), 3.22 (m, 1H), 3.18 (dd, *J* = 12, 3 Hz, 1H), 2.77 (dt, *J* = 12, 2.5 Hz, 1H), 2.48 (dq, *J* = 13, 2.5 Hz, 1H), 2.04 (m, 1H), 1.62 (broad s, 1H), 1.51 (dq, *J* = 12, 4 Hz, 1H), 1.45−1.30 (m, 1H), 1.34 (s, 9H).

General Procedure for Coupling Amine 39 to Epoxide 40. Preparation of Carbamate 41c: amine **39c** (3.92 g, 13.5 mmol, 1 equiv) was dissolved in EtOH (45 mL), and LiCl (1.24 g, 29.5 mmol, 2.2 equiv) was added. Epoxide **40**[26] (1.76 g, 6.7 mmol, 0.5 equiv) was added, and the mixture was stirred at 60 °C for 45 min. A second portion of epoxide (1.76 g, 6.69 mmol, 0.5 equiv) was added, and stirring at 60 °C continued for 30 min. A third portion (1.82 g, 6.9 mmol, 0.52 equiv) was added, and the mixture stirred overnight at 60 °C. One last portion of epoxide (1.19 g, 4.5 mmol, 0.34 equiv) was added, and heating resumed for a total reaction time of 22 h. The reaction mixture was then evaporated under reduced pressure and the residue dissolved in EtOAc (200 mL). The solution was washed with water (135 mL) and brine (135 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel using 4% and 6% MeOH/EtOAc as eluent. Carbamate **41c** was obtained as a solid (3.97 g, 53% yield). ^1H NMR ($CDCl_3$) δ 8.41 (dd, *J* = 4.5, 1.5 Hz, 2H), 7.38−7.26 (m, 2H), 7.25−7.18 (m, 3H), 7.13 (dd, *J* = 4.5, 1.5 Hz, 2H), 6.29 (broad s, 1H), 4.70 (broad m, 1H), 3.95−3.68 (m, 3H), 3.39−3.25 (m, 2H), 2.97−2.80 (m, 3H), 2.72 (dd, *J* = 13, 4 Hz, 1H), 2.45−2.27 (m, 3H), 1.99 (m, 1H), 1.73 (dq, *J* = 12.5, 3.5 Hz, 1H), 1.65 (q, *J* = 12 Hz, 1H), 1.79−1.58 (m, 2H), 1.36 (broad s, 9H), 1.34 (s, 9H).

General Procedure for Deprotection of 41 and Coupling of Amines 42 (Scheme 5). Preparation of inhibitor 27: carbamate **42** (7.85 g, 14 mmol) was dissolved in 4 N HCl-dioxane (47 mL) and the mixture stirred for 20 min at room temperature. Volatiles were then evaporated under reduced pressure, and the resulting salt was dried under vacuum for 1 h. The crude hydrochloride was used without purification.

The salt from above (assume 14 mmol, 1 equiv), 2′,6′-dimethylphenoxyacetic acid (2.96 g, 16.5 mmol, 1.16 equiv), and BOP coupling reagent (7.79 g, 17.6 mmol, 1.25 equiv) were dissolved in dry DMF (100 mL), and diisopropylethylamine (14.8 mL, 85 mmol, 6 equiv) was added. The mixture was stirred for 2.5 h at room temperature. The reaction mixture was diluted with EtOAc (500 mL) and washed with saturated $NaHCO_3$ (2 × 500 mL), water (500 mL), and brine (500 mL). After drying ($MgSO_4$) and evaporation, crude **27** was obtained

as a foam. The inhibitor was purified by flash chromatography on silica gel using 3−10% iPrOH/EtOAc as eluents. Compound **27** was obtained as an off-white amorphous solid (3.08 g, >98% homogeneous, and 4.37 g, 88% homogeneous. Total: 7.45 g, 85% yield). R_f 0.56 (9:1 $CHCl_3$/MeOH). $[\alpha]^{22}_D$ +16.7° (*c* 1.1, $CHCl_3$). ^{1}H NMR (DMSO-d_6) δ 8.39 (dd, J = 5, 1.5 Hz, 2H), 7.79 (d, J = 9 Hz, 1H), 7.42 (s, 1H), 7.33−7.27 (m, 4H), 7.24 (t, J = 7.5 Hz, 2H), 7.16 (m, 1H), 7.00 (d, J = 7.5 Hz, 2H), 6.92 (dd, J = 8.5, 6.5 Hz, 1H), 5.05 (d, J = 5 Hz, 1H), 4.21 (m, 1H), 4.11 (d, J = 14.5 Hz, 1H, part of AB), 3.92 (d, J = 14.5 Hz, 1H, part of AB), 3.82 (m, J = 5 Hz, 1H), 3.24 (broad d, J = 12.5 Hz, 1H), 3.00 (dd, J = 14, 3 Hz, 1H), 2.88 (dd, J = 11, 2 Hz, 1H), 2.73 (dd, J = 13.5, 11 Hz, 1H), 2.65 (dd, J = 12.5, 6.5 Hz, 1H), 2.26 (m, 1H), 2.17 (dd, J = 12.5, 6.5 Hz, 1H), 2.12 (s, 6H), 2.02 (broad d, J = 12 Hz, 1H), 1.92 (broad d, J = 12 Hz, 1H), 1.60 (m, 2H), 1.25 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 170.6, 166.6, 154.0, 149.0, 146.3, 139.0, 129.8, 128.9, 128.3, 127.4, 125.3, 123.7, 121.2, 69.8, 69.5, 67.4, 56.7, 52.8, 51.1, 49.6, 34.5, 33.5, 29.9, 27.8, 15.3. HRMS (FAB) *m/z* $C_{35}H_{47}N_4O_4S$ (MH^+) calcd: 619.3318; found: 619.3308. HPLC homogeneity: 96.8% (system A); 98.9% (system B).

Inhibitor 25: alkylation of 4-iodopipecolic derivative **37** (X = I) with 4-pyridinemethanethiol,[29a,c] using NaH in DMF as described for **38c**, gave **38a** in 68% yield. Following the general procedures described above, this compound was converted into inhibitor **25**. Mp 72−76 °C. R_f 0.52 (9:1 $CHCl_3$/MeOH). $[\alpha]^{20}_D$ −10.9° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.50 (dd, J = 4.5, 1.5 Hz, 2H), 7.75 (d, J = 9 Hz, 1H), 7.36 (m, 3H), 7.31−7.21 (m, 7H), 7.16 (tt, J = 7.5, 1 Hz, 1H), 7.00 (d, J = 7.5 Hz, 2H), 6.92 (dd, J = 8.5, 6.5 Hz, 1H), 4.99 (broad d, J = 3 Hz, 1H), 4.20 (m, 1H), 4.10 (d, J = 14.5 Hz, 1H, part of AB), 3.92 (d, J = 14.5 Hz, 1H, part of AB), 3.81 (s, 2H), 3.79 (m, 1H), 3.18 (broad d, J = 11 Hz, 1H), 2.99 (dd, J = 13.5, 3 Hz, 1H), 2.71 (dd, J = 13.5, 11 Hz, 1H), 2.67−2.55 (m, 2H), 2.12 (s, 6H), 2.05−1.79 (m, 4H), 1.47 (m, 1H), 1.24 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 170.8, 166.6, 154.0, 149.2, 147.7, 139.1, 129.8, 128.9, 128.3, 127.4, 125.3, 123.7, 123.5, 69.8, 69.4, 67.8, 57.1, 52.7, 51.5, 49.6, 35.5, 33.4, 31.7, 31.0, 27.9, 15.4. HRMS (FAB) *m/z* $C_{36}H_{49}N_4O_4S$ (MH^+) calcd: 633.3475; found: 633.3465. HPLC homogeneity: 98.7% (system A); 99.4% (system B).

Inhibitor 26: alkylation of 4-iodopipecolic derivative **37** (X = I) with 3-pyridinemethanethiol,[29b,c] as described above, gave **38b** in 70% yield. Following the general procedures described previously, this compound was converted into inhibitor **26**: mp 68−74 °C. R_f 0.56 (9:1 $CHCl_3$/MeOH). $[\alpha]^{20}_D$ − 1.1° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 8.53 (d, J = 2 Hz, 1H), 8.45 (dd, J = 5, 1.5 Hz, 1H), 7.78−7.73 (m, 2H), 7.37 (s, 1H), 7.36−7.20 (m, 5H), 7.16 (m, 1H), 7.00 (d, J = 7.5 Hz, 2H), 6.92 (dd, J = 8, 6.5 Hz, 1H), 4.99 (broad d, J = 5 Hz, 1H), 4.21 (m, 1H), 4.10 (d, J = 14.5 Hz, 1H, part of AB), 3.92 (d, J = 14 Hz, 1H, part of AB), 3.83 (s, 2H), 3.80 (m, 1H), 3.18 (broad d, J = 12 Hz, 1H), 2.99 (dd, J = 13.5, 3 Hz, 1H), 2.71 (dd, J = 13.5, 11 Hz, 1H), 2.68−2.55 (m, 2H), 2.12 (s, 6H), 2.05−1.81 (m, 4H), 1.49 (m, 1H), 1.24 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 170.8, 166.6, 154.1, 149.2, 147.5, 146.1, 139.1, 135.8, 134.4, 129.8, 128.9, 128.3, 127.4, 125.3, 123.7, 123.1, 69.8, 69.4, 67.9, 57.1, 52.7, 51.5, 49.6, 35.6, 33.3, 31.0, 29.9, 27.9, 15.4. HRMS (FAB) *m/z* $C_{36}H_{49}N_4O_4S$ (MH^+) calcd: 633.3475; found: 633.3458. HPLC homogeneity: 95.8% (system A); 97.7% (system B).

Inhibitor 28: 4-iodopipecolic amide derivative **37** (X = I, 8.00 g, 19.5 mmol) and potassium thioacetate (3.35 g, 29.3 mmol) were dissolved in DMF (20 mL), and the mixture was heated to 45 °C for 2 h. The reaction mixture was then diluted with EtOAc and washed twice with water and then brine. The extract was dried ($MgSO_4$) and concentrated to give a solid residue that was purified by flash chromatography on silica gel using 4:1 to 2:1 hexane/EtOAc as eluent. (2*S*,4*R*)-4-thioacetoxypipecolic *tert*-butylamide (**38**, SR = SAc) was isolated as a solid (4.80 g, 68% yield). MS (FAB) *m/z* 359 (MH^+).

The thioacetate from above (4.80 g, 13.4 mmol) was dissolved in 1:1 MeOH/THF (64 mL) and the solution cooled in an ice−water bath. NaOH (1 N, 16.1 mL, 16.1 mmol) was added and the mixture stirred 1.5 h at 0 °C. The reaction mixture was acidified with 10% aqueous citric acid and extracted with EtOAc. The extract was washed twice with saturated $NaHCO_3$ and then with brine. After the solution was dried over $MgSO_4$, the solvent was evaporated and the residue dried under vacuum. (2*S*,4*S*)-4-Mercaptopipecolic *tert*-butylamide **38** (R = H) was used without purification (4.20 g, 99% yield, crude).

To a suspension of NaH (0.351 g, 14.6 mmol, 1.1 equiv) in DMF (44 mL) at 0 °C was added the thiol from above (4.20 g, 13.3 mmol), and the mixture was stirred for 0.5 h. 4-Chloro-2,6-dimethylpyrimidine (2.64 g, 18.6 mmol, 1.4 equiv) was added, and stirring continued at 0 °C for another hour. The reaction was then quenched by pouring into saturated $NaHCO_3$, and the product was extracted with EtOAc. The extract was washed twice with 10% aqueous Na_2CO_3 and then with brine. After drying ($MgSO_4$), the solvent was evaporated and the residue purified by flash chromatography on silica gel using 1:1 and then 3:2 EtOAc/hexane to give **38d** (4.1 g, 73% yield). MS (FAB) *m/z* 423 (MH^+).

Following the general procedures described previously, **38d** was converted into inhibitor **28**: mp 90−96 °C. R_f 0.50 (9:1 $CHCl_3$/MeOH). $[\alpha]^{20}_D$ +4.4° (*c* 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 7.78 (d, J = 9 Hz, 1H), 7.42 (s, 1H), 7.30 (d, J = 7 Hz, 2H), 7.24 (t, J = 7.5 Hz, 2H), 7.16 (t, J = 7.5 Hz, 1H), 7.09 (s, 1H), 7.00 (d, J = 7.5 Hz, 2H), 6.92 (dd, J = 8.5, 6.5 Hz, 1H), 5.04 (d, J = 5 Hz, 1H), 4.22 (m, 1H), 4.11 (d, J = 14.5 Hz, 1H, part of AB), 3.92 (d, J = 14.5 Hz, 1H, part of AB), 3.82 (m, 2H), 3.28 (s, 1H), 3.24 (m, 1H), 3.00 (dd, J = 13.5, 3 Hz, 1H), 2.85 (dd, J = 11, 2.5 Hz, 1H), 2.73 (dd, J = 13.5, 11 Hz, 1H), 2.65 (dd, J = 12.5, 7 Hz, 1H), 2.50 (s, 3H), 2.32 (s, 3H), 2.20 (m, 2H), 2.12 (s, 6H), 2.06 (broad d, J = 12 Hz, 1H), 1.96 (broad d, J = 11 Hz, 1H), 1.65 (m, 2 H), 1.25 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 171.1, 168.1, 167.0, 166.4, 165.1, 154.4, 139.5, 130.2, 129.3, 128.7, 127.8, 125.7, 124.2, 114.7, 70.2, 69.9, 68.2, 57.4, 53.2, 51.8, 50.0, 35.1, 33.8, 30.6, 28.3, 25.6, 23.2, 15.8. HRMS (FAB) *m/z* $C_{36}H_{50}N_5O_4S$ (MH^+) calcd: 648.3583; found: 648.3570. HPLC homogeneity: >99.5% (system A); 99.7% (system B).

Inhibitor 29: amine **43**[27] (0.104 g, 0.44 mmol), epoxide **40**[26] (0.060 g, 0.23 mmol), and LiCl (0.037 g, 0.87 mmol) were dissolved in EtOH (1.5 mL), and the mixture was stirred at 60 °C for 1.5 h. Another portion of epoxide **40** (0.057 g, 0.22 mmol) was added, and the mixture was stirred overnight at 60 °C. More epoxide (0.060 g, 0.23 mmol) was added, and heating continued for a total time of 24 h, at which point the reaction was judged complete by TLC. The solvent was removed under reduced pressure and the residue dissolved in EtOAc (10 mL). The solution was washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The crude material was purified by flash chromatography on silica gel, eluting sequentially with 20%, 25%, 30%, and 40% EtOAc/hexane. Carbamate **44** was obtained as a colorless oil (0.151 g, 69% yield). ^{1}H NMR ($CDCl_3$) δ 7.35−7.16 (m, 5H), 5.88 (broad s, 1H), 4.82 (broad m, 1H), 3.88 (broad m, 1H), 3.80 (broad m, 1H), 3.54 (broad m, 1H), 3.11−2.86 (m, 3H), 2.67 (dd, J = 13, 6 Hz, 1H, part of ABX), 2.61 (dd, J = 11, 3 Hz, 1H, part of ABX), 2.36−2.22 (m, 2H), 2.02−1.90 (m, 1H), 1.90−1.60 (m, 4H), 1.59−1.38 (m, 7H), 1.35 (s, 18H).

Carbamate **44** (0.143 g, 0.28 mmol) was dissolved in 4 N HCl-dioxane (2 mL). After the mixture was stirred for 20 min, volatiles were removed under a stream of nitrogen to give a white solid (0.128 g, 95% yield).

The crude salt from above (0.052 g, 0.11 mmol), 2′,6′-dimethylphenoxyacetic acid (0.023 g, 0.13 mmol), and BOP coupling reagent (0.060 g, 0.14 mmol) were dissolved in DMF (2.2 mL). Diisopropylethylamine (95 μL, 0.55 mmol) was added and the mixture stirred overnight at room temperature. The reaction mixture was then diluted with EtOAc (25 mL), washed with saturated aqueous $NaHCO_3$ (2 × 25 mL), water (25 mL), and brine (25 mL), and dried ($MgSO_4$). Evaporation of solvents under reduced pressure followed by purification by flash chromatography on silica gel using 50% EtOAc/hexane as eluent gave **29** as a white solid (0.030 g, 50% yield): mp 119−124.5 °C. R_f 0.67 (9:1 $CHCl_3$/MeOH). $[\alpha]^{20}_D$ −5.0° (*c* 1.0,

MeOH). ^{1}H NMR δ 7.33−7.25 (m, 4H), 7.25−7.18 (m, 1H), 7.01−6.90 (m, 4H), 5.99 (broad s, 1H), 4.35 (m, 1H), 4.22 (d, J = 15 Hz, 1H, part of AB), 4.05 (d, J = 15 Hz, 1H, part of AB), 3.97 (broad m, 1H), 3.83 (broad s, 1H), 3.11−2.95 (m, 3H), 2.73 (dd, J = 13.5, 5.5 Hz, 1H, part of ABX), 2.67 (dd, J = 10.5, 3.5 Hz, 1H, part of ABX), 2.37 (dd, J = 13.5, 7 Hz, 1H, part of ABX), 2.35 (dd, J = 11, 3 Hz, 1H, part of ABX), 2.08 (s, 6H), 2.02−1.61 (m, 4H), 1.58−1.21 (m, 8H), 1.33 (s, 9H). ^{13}C NMR (DMSO-d_6) δ 167.7, 167.0, 154.2, 138.6, 136.2, 129.0, 128.8, 128.0, 126.0, 124.2, 70.0, 68.1, 66.1, 58.9, 57.3, 53.7, 51.0, 34.8, 33.5, 31.2, 29.7, 28.3, 28.2, 28.1, 25.5, 24.6, 15.7. HRMS (FAB) m/z calcd. for $C_{34}H_{50}N_3O_4$ (MH^+): 564.3801; found: 564.3792. HPLC homogeneity: 94.1% (system A); 96.6% (system B).

Inhibitor 30: epoxide **40**[26] (1.30 g, 5 mmol) was dissolved in 2:1 EtOH/isobutylamine (3 mL), and the solution was stirred overnight at 50 °C under an argon atmosphere. Volatiles were then removed under vacuum, and the residue was dissolved in CH_2Cl_2 (35 mL). Saturated aqueous $NaHCO_3$ (15 mL), 4-nitrobenzenesulfonyl chloride (1.06 g, 4.8 mmol), and $NaHCO_3$ (0.42 g, 4.8 mmol) were added, and the biphasic mixture was vigorously stirred for 6 h at room temperature. The organic layer was separated, washed with water, dried ($MgSO_4$), and concentrated. The residue was crystallized from EtOAc/hexane to give **45** as a solid (1.85 g, 74% yield): mp 168−169 °C. R_f 0.75 (9:1 $CHCl_3$/MeOH). $[\alpha]^{25}_D$ +15.9° (c 1.0, $CHCl_3$). IR (KBr) ν 3475−3220, 1655, 1636, 1595 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.33 (d, J = 9 Hz, 2H), 7.98 (d, J = 9 Hz, 2H), 7.38−7.21 (m, 5H), 4.61 (broad d, J = 5 Hz, 1H), 3.80 (m, 3H), 3.21 (d, J = 5.5 Hz, 2H), 3.01 (d, J = 9.5 Hz, 2H), 2.98 (m, 1H), 2.90 (m, 1H), 1.89 (m, J = 7 Hz, 1H), 1.38 (s, 9H), 0.90 (t, J = 6 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 156.4, 150.0, 145.0, 137.5, 129.4, 128.6, 128.5, 126.7, 124.3, 80.1, 72.2, 57.5, 55.2, 52.5, 35.6, 28.2, 26.9, 20.0, 19.8. MS (FAB) m/z 522 (MH^+), 422 (MH^+ − Boc). Anal. Calcd for $C_{25}H_{35}N_3O_7S$: C, 57.76; H, 6.76; N, 8.06. Found: C, 57.59; H, 6.84; N, 8.02.

Carbamate **45** (0.480 g, 0.9 mmol) was stirred for 1 h in 1:1 TFA/CH_2Cl_2 (5 mL). Volatiles were removed under reduced pressure and the residue coevaporated twice with EtOAc (5 mL). The residue was dissolved in DMF (5 mL), and N-methylmorpholine (600 μL, 5.5 mmol, 6 equiv), 2′,6′-dimethylphenoxyacetic acid (0.180 g, 1.0 mmol), and TBTU (0.353 g, 1.1 mmol) were added. The mixture was stirred for 2 h at room temperature. The reaction was quenched by pouring into 1 N NH_4OH (100 mL), and the tan-colored precipitate was collected by filtration. The solid was washed with water and dried in air. Compound **46** was obtained as a tan-colored solid (0.57 g, quantitative): mp 60−75 °C. $[\alpha]^{25}_D$ +9.8° (c 1.0, MeOH). ^{1}H NMR ($CDCl_3$) δ 8.38 (d, J = 8.5 Hz, 2H), 7.99 (d, J = 8.5 Hz, 2H), 7.38−7.22 (m, 5H), 7.05 (broad d, J = 8 Hz, 1H), 7.05−6.93 (m, 3H), 4.25 (m, J = 4 Hz, 1H), 4.21 (d, J = 15 Hz, 1H, part of AB), 4.13 (d, J = 15 Hz, 1H, part of AB), 4.11 (m, 1H), 3.98 (broad m, J = 4 Hz, 1H), 3.32 (dd, J = 15, 4 Hz, 1H, part of ABX), 3.25 (dd, J = 15, 8 Hz, 1H, part of ABX), 3.15 (dd, J = 14, 5 Hz, 1H, part of ABX), 3.05−2.98 (dd, J = 9.5 Hz, 1H, part of ABX), 3.04 (d, J 8 Hz, 2H), 2.12 (s, 6H), 1.93 (m, J = 7 Hz, 1H), 0.92 (t, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 169.6, 153.8, 150.1, 144.8, 137.2, 130.2, 129.3, 129.2, 128.8, 128.5, 126.9, 124.9, 124.4, 72.3, 70.0, 57.9, 54.3, 52.7, 35.1, 27.0, 20.0, 19.9, 16.0. MS (FAB) m/z 584 (MH^+). Anal. Calcd for $C_{30}H_{37}N_3O_7S$: C, 61. 73; H, 6.39; N, 7.20. Found: C, 61.88; H, 6.51; N, 7.30.

Nitro derivative **46** (0.490 g, 0.8 mmol) was hydrogenated (1 atm H_2 gas) over 20% $Pd(OH)_2$/C (50 mg) in MeOH (20 mL) for 6 h. The suspension was filtered through a 0.45 μM filter, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in MeOH (3 mL), and water (25 mL) was added to precipitate the product. Crude **30** was collected by filtration, washed with water, and dried in air. The material was purified by flash chromatography on silica gel using 1:1 EtOAc/hexane as eluent. Compound **30** was obtained as a white solid (0.365 g, 78% yield). Mp 60−75 °C. R_f 0.45 (1:2 hexane/EtOAc). $[\alpha]^{25}_D$ +19.6° (c 1.0, MeOH). ^{1}H NMR (DMSO-d_6) δ 7.89 (d, J = 9.5 Hz, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.28−7.21 (m, 5H), 7.17 (m, 1H), 7.02 (m, 2H), 6.93 (dd, J = 8, 6.5 Hz, 1H), 6.61 (d, J = 9 Hz, 2H), 5.96 (broad s, 2H), 5.08 (broad d, J = 6 Hz, 1H), 4.11−4.02 (m, 1H), 4.10 (d, J = 14 Hz, 1H, part of AB), 3.92 (d, J = 14 Hz, 1H, part of AB), 3.76 (m, 1H), 3.34 (dd, J = 4 Hz, 1H, part of ABX, overlap with water signal), 3.10 (dd, J = 14, 3 Hz, 1H, part of ABX), 2.94 (dd, J = 13.5, 8.5 Hz, 1H, part of ABX), 2.80−2.65 (m, 3H), 2.14 (s, 6H), 1.98 (m, J = 6.5 Hz, 1H), 0.86 (d, J = 6.5 Hz, 3H), 0.81 (d, J = 6.5 Hz, 3H). ^{13}C NMR (DMSO-d_6) δ 166.7, 153.8, 152.3, 138.9, 129.9, 128.8, 128.5, 128.3, 127.4, 125.3, 123.8, 123.3, 112.2, 71.6, 69.6, 56.7, 52.6, 52.3, 34.2, 25.9, 19.65, 19.60, 15.2. HRMS (FAB) m/z $C_{30}H_{40}N_3O_5S$ (MH^+) calcd: 554.2689; found: 554.2700. HPLC homogeneity: 99.5% (system A); >99.5% (system B).

Conclusion

Incorporation of 2′,6′-dimethylphenoxyacetyl in the P_2-P_3 position of peptidomimetic-based HIV protease inhibitors has led to extremely potent inhibitors of the enzyme and viral replication in vitro. In particular, in the context of our palinavir class of protease inhibitors, substitution of quinaldic-valine by this new achiral, high-affinity ligand resulted in a decrease in structural complexity and molecular size, while providing some of the most potent antiviral agents reported to date in this field. Furthermore, some of the compounds displayed promising pharmacokinetic properties, including high oral bioavailability in rat and dog. On the basis of the analysis of X-ray structures and molecular modeling, the high affinity of 2′,6′-dimethylphenoxyacetyl toward the enzyme active site is attributed, in part, to binding of one of the methyl groups inside the S_2 pocket of the protease, to favorable entropic factors, and to a ligand minimal energy conformation which closely resembles that of the bioactive conformation.

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