Synthesis and Structure-Activity Relationships of a Series of Penicillin-Derived HIV Proteinase Inhibitors Containing a Stereochemically Unique Peptide Isostere

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A series of HIV-1 proteinase inhibitors was synthesized based upon a single penicillin derived thiazolidine moiety. Reaction of the C-4 carboxyl group with (*R*)-phenylalaninol gave amide **10** which was a moderately potent inhibitor of HIV-1 proteinase ($IC_{50} = 0.15\ \mu M$). Further modifications based on molecular modeling studies led to compound **48** which contained a stereochemically unique statine-based isostere. This was a potent competitive inhibitor ($K_i = 0.25$ nM) with antiviral activity against HIV-1 *in vitro* (5 μM). Neither modification to the benzyl group in an attempt to improve interaction with the S_2' pocket, nor introduction of a hydrogen bond donating group to interact with residue Gly48′ resulted in improved inhibitory or antiviral activity.

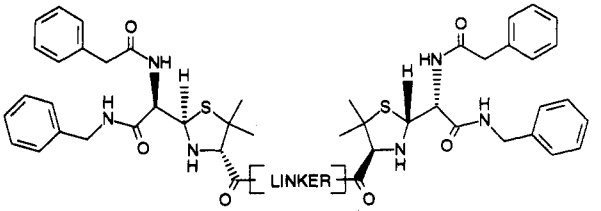
Introduction

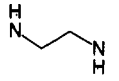
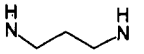
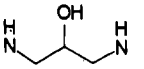
Human immunodeficiency virus (HIV)[1] is the causative agent of acquired immunodeficiency syndrome (AIDS). A late stage in the replication of HIV involves the key proteolytic processing of precursor proteins by a viral enzyme, HIV proteinase.[2] Site directed mutagenesis of this enzyme produces viral particles that are morphologically immature and noninfectious,[3] and thus inhibition of HIV proteinase represents an important therapeutic target for the treatment of AIDS.[4] The enzyme is a member of the aspartyl protease family and has been shown to be a C_2 symmetric homodimer.[5]

A number of groups have reported substrate-based inhibitors of HIV proteinase in which the scissile bond has been replaced with a noncleavable isostere, *e.g.*, reduced amide,[6] hydroxyethylene,[7] and hydroxyethylamines.[8] Although this approach has resulted in highly potent enzyme inhibitors, the combination of high molecular weight and peptidic character may result in pharmacokinetic problems. A recent report from workers at Abbott[9] demonstrated that reduction in molecular weight may be achieved whilst retaining potency and that this has resulted in compound A-80987 which has an improved *in vivo* profile.

As an alternative to a peptide based approach we embarked on a screening program which identified a penicillin derived inhibitor of HIV proteinase.[10] This led to a series of potent inhibitors based on compound **1**[11] (Table I), which showed activity against the virus *in vitro*. Determination of the structure of a cocrystal of a HIV proteinase-inhibitor complex[12] confirmed the symmetric binding mode of these novel inhibitors (Figure 1). It is interesting to note that despite the potency of **1**, and its analogues, they did not interact significantly with the catalytic aspartates, Asp25 and Asp25′, and therefore attempts were made to alter the ethylenediamine linker in order to exploit this potentially favorable interaction (Table I).[13] Extension of the linker, to give **2**, resulted in a 500-fold decrease in activity which was thought to be due to disruption of the lipophilic interaction in the S_1 and S_1' pockets. The three carbon linker allowed the introduction of a secondary hydroxyl group that interacted with the catalytic aspartates and resulted in a 100-fold increase in activity for compound **3** relative to **2**. These results suggested that the problem of accommodating the longer linker, necessary for the introduction of the hydroxyl, might be circumvented by removing the constraint of having a second penicillin unit.

Table I. Anti-HIV Activity of Penicillin Dimers



no.	linker	IC_{50}[a] (μM)	EC_{50}[b] (μM) (MT-4)
1		0.0009	0.29
2		0.54	-
3		0.005	2.6

[a] Inhibition of HIV-1 proteinase. IC_{50} determinations were performed in duplicate at each concentration with mean values used for data analysis. Results were standardized, with a control used in all assays. [b] Inhibition of the cytopathic effect of HIV-1 in MT-4 cells. Cytotoxicity ED_{50} (MT-4), ≥100 μM.

This paper describes the synthesis and structure-activity relationship of a series of "monomer" penicillin analogues, *i.e.*, compounds in which one of the penicillin units of the dimer has been replaced. Specific groups were introduced into the monomer analogues to interact with key residues at the active site resulting in a novel series of potent HIV proteinase inhibitors.

Chemistry

The starting material for all compounds described was the *N*-ethylpiperidine (NEP) salt of penicillin G, **4** (Scheme I). Nucleophilic ring opening with benzylamine was achieved at room temperature over 6 h to afford compound

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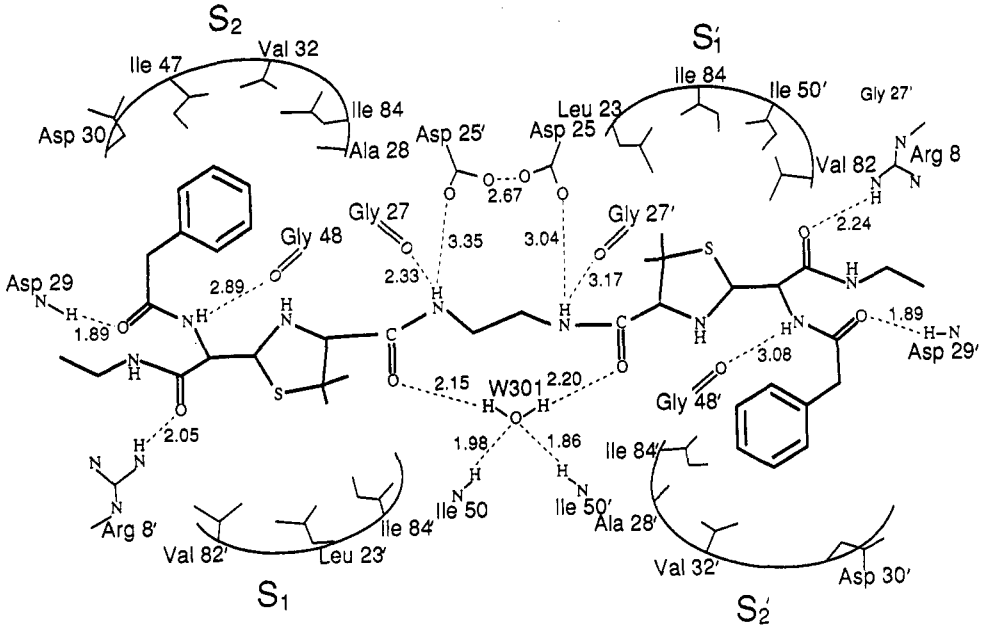
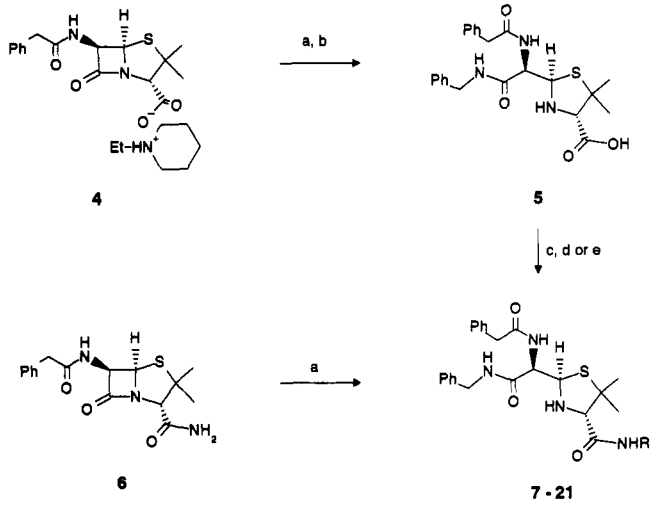


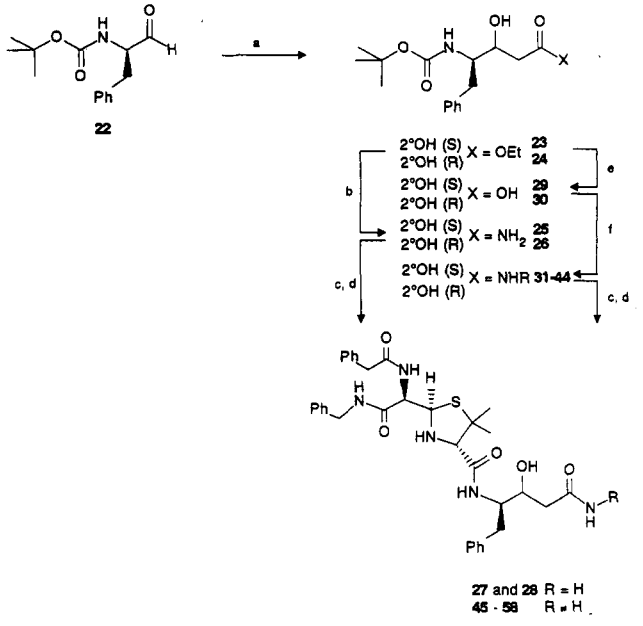
Figure 1. Schematic representation of a cocrystal structure of a penicillin derived dimer inhibitor with HIV proteinase. The figures denote interheavy atom distances and are in angstroms.

Scheme I[a]



[a] (a) $PhCH_2NH_2$, CH_2Cl_2; (b) H_3PO_4 (aqueous); (c) method A, RNH_2, TBTU, DIPEA, DMF; (d) method B, RNH_2, DCC, HOBT, DMF; (e) method C, $RNH_3^+Cl^-$, $KHCO_3$, THF, DCC, HOBT, DMF.

Scheme II[a]



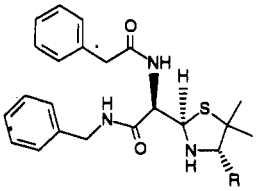
[a] (a) LDA, CH_3CO_2Et, −78 °C, THF; (b) NH_3, MeOH, (c) HCl, dioxan; (d) **5**, TBTU, DIPEA, DMF; (e) dioxan–water, pH 10; (f) RNH_2, TBTU, DIPEA, DMF.

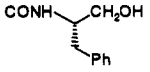
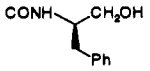
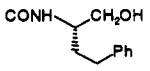
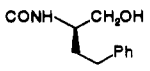
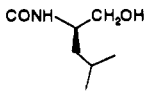
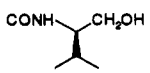
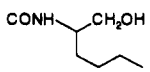
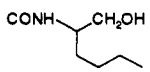
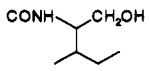
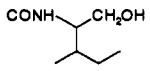
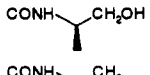
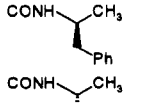
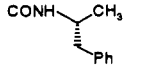
5[13] in good yield, and this was coupled to a series of amines using dicyclohexylcarbodiimide (DCC) as the coupling agent to give **8** to **21**. Subsequently 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU),[14] with *N*,*N*-diisopropylethylamine (DIPEA) in DMF was found to give cleaner reactions and higher yields (generally 60–80%). The β-lactam of the penicillin amide **6**[15] was opened with benzylamine in a similar manner to give compound **7**. Although stable at room temperature compounds **7**–**21** were prone to epimerization at the 5-position (penicillin numbering) at high temperatures and under acidic conditions.[16]

The esters **23** and **24** were synthesized from *N*-(*tert*-butoxycarbonyl)-(*R*)-phenylalanine (BOC-(*R*)-Phe) using the procedure described by Rich *et al.*[17] (Scheme II). Aminolysis of the esters with methanolic ammonia afforded the primary amides **25** and **26**. The *tert*-butoxycarbonyl protecting group was removed using $HCl_{(g)}$ in dioxan, and the resulting amine HCl salts were coupled to acid **5** using TBTU and DIPEA in DMF to give the inhibitors **27** and **28**. Esters **23** and **24** were also hydrolyzed to the respective acids, **29** and **30** as described by Rich *et al.*[17] A general method employing TBTU with DIPEA in DMF was used for coupling these acids with a series of amines to afford **31** to **44** (Table III). The BOC protecting group was removed, and the amine HCl salts were coupled to the penicillin analogue **5** as described above to give compounds **45** to **58** (Table IV).

Compound **63**, with the amide group reversed, was synthesized from *N*-(benzyloxycarbonyl)-(*R*)-phenylalanine (CBZ-(*R*)-Phe) **59** (Scheme III). Activation of the acid with ethyl chloroformate and subsequent addition of diazomethane followed by $HCl_{(g)}$ afforded the α-chloroketone **60**. The ketone was reduced using sodium borohydride at 0 °C to afford a mixture of diastereoisomers. One diastereoisomer was isolated in a pure form by

Table II. Inhibitory Activity Against HIV-1 Proteinase[a]



no.	R	method	formula[b]	IC_{50}[c] (μM)
7	$CONH_2$		$C_{23}H_{28}N_4O_3S\cdot0.5H_2O$	>230
8	$CONHCH_2CH_2OH$	A	$C_{25}H_{32}N_4O_4S$	180
9		B	$C_{32}H_{38}N_4O_4S\cdot0.25C_4H_8O_2$	>170
10		B	$C_{32}H_{38}N_4O_4S\cdot H_2O$	0.15
11		C	$C_{33}H_{40}N_4O_4S\cdot0.1C_6H_{14}O\cdot0.5H_2O$	26
12		C	$C_{33}H_{40}N_4O_4S\cdot0.1C_6H_{14}O\cdot0.5H_2O$	25
13		A	$C_{29}H_{40}N_4O_4S\cdot0.5H_2O$	>4.6
14		A	$C_{28}H_{38}N_4O_4S\cdot0.3H_2O$	>4.7
15		A	$C_{29}H_{40}N_4O_4S\cdot0.4H_2O$	>4.6
16		A	$C_{29}H_{40}N_4O_4S$	0.25
17		A	$C_{28}H_{38}N_4O_4S\cdot0.5CH_4O$	>0.54
18		A	$C_{28}H_{38}N_4O_4S\cdot0.25H_2O$	>0.54
19		A	$C_{26}H_{34}N_4O_4S$[d]	>20
20		A	$C_{32}H_{38}N_4O_3S\cdot0.5H_2O$	>180
21		A	$C_{32}H_{38}N_4O_3S$	>180

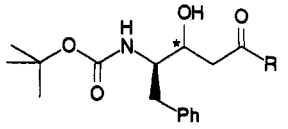
[a] Assay was performed as described in Experimental Section. [b] Satisfactory analyses (C, H, N, and S; 0.4% of theoretical values) were obtained for all compounds. [c] See Table I, footnote *a*. [d] Satisfactory accurate mass of $(M + H)^+$ and HPLC analysis.

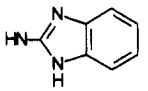
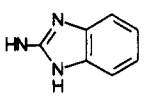
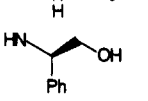
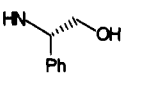
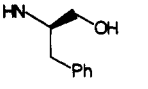
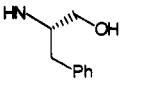
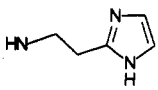
recrystallization, but as both diastereoisomers were required, the mother liquor was treated with sodium azide to afford **61** as a (2:1) mixture of diastereoisomers. The azide was reduced to an amine using triphenylphosphine in THF–water, and this was subsequently coupled to 2-phenylacetic acid using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide HCl salt (EDC) to give **62** as a mixture of diastereoisomers in 78% yield over two steps. Finally, the CBZ protecting group was removed by hydrogenation (Pd–C), and the resulting amine was coupled to acid **5** using DCC which gave the inhibitor **63** as a (2:1) mixture of diastereoisomers. The stereochemistry of the major isomer was not determined.

Results and Discussion

The penicillin based dimers are a potent series of HIV proteinase inhibitors, yet the primary amide **7** was inactive against the enzyme, thus demonstrating that a single opened penicillin unit alone was insufficient to bind to the active site. We speculated that the minimum requirement for inhibitor binding needed to replace one penicillin unit of the dimer would be the introduction of a hydroxyl group to interact with the catalytic aspartates and at least one lipophilic group to interact with the S_1' pocket. Readily available compounds that fulfill these

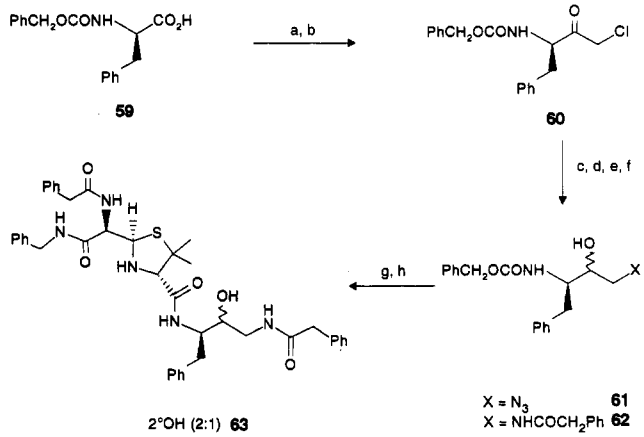
Table III. Structure and Physical Data for Intermediates 31–44



no.	*stereochemistry secondary OH	R	formula[a]
31	S	$NHCH_2CH(CH_3)_2$	$C_{20}H_{32}N_2O_4$
32	R	$NHCH_2CH(CH_3)_2$	$C_{20}H_{32}N_2O_4$
33	S	$NHCH_2Ph$	$C_{23}H_{30}N_2O_4$
34	R	$NHCH_2Ph$	$C_{23}H_{30}N_2O_4$
35	S		$C_{24}H_{30}N_4O_4\cdot0.8H_2O$
36	R		$C_{24}H_{30}N_4O_4\cdot H_2O$
37	R		$C_{24}H_{32}N_2O_5\cdot0.1H_2O$
38	R		$C_{24}H_{32}N_2O_5$
39	R		$C_{25}H_{34}N_2O_5$
40	R		$C_{25}H_{34}N_2O_5$
41	R		$C_{21}H_{30}N_4O_4\cdot0.5H_2O$
42	R	$NHCH_2CH_2OH$	$C_{18}H_{28}N_2O_5$
43	R	$NHCH_2CH(OH)CH_2OH$	$C_{19}H_{30}N_2O_6\cdot0.5H_2O$
44	R	$NHCH_2CH_2CH_2OH$	$C_{19}H_{30}N_2O_5$

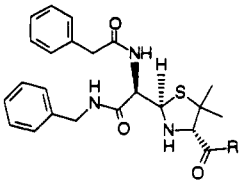
[a] Satisfactory analyses (C, H, and N; 0.4% of theoretical values) were obtained for all compounds.

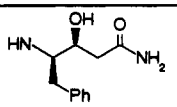
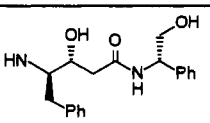
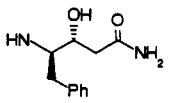
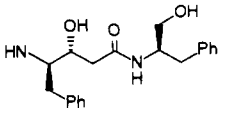
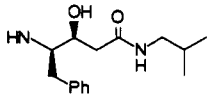
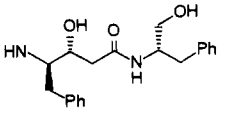
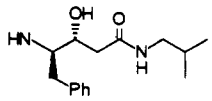
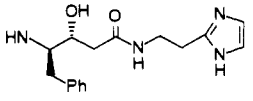
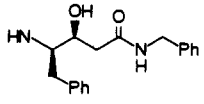
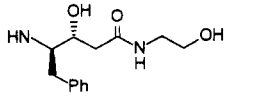
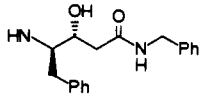
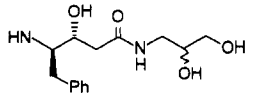
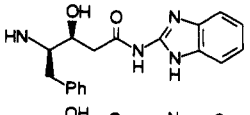
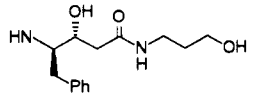
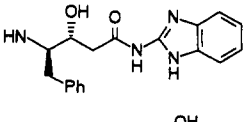
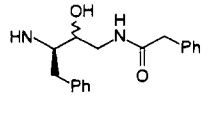
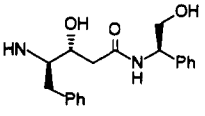
Scheme III[a]



[a] (a) $ClCO_2Et$, Et_3N, −15 °C; (b) CH_2N_2, Et_2O, $HCl_{(g)}$; (c) $NaBH_4$, 0 °C; (d) NaN_3, 90 °C, DMF; (e) $P(Ph)_3$, THF–H_2O, reflux; (f) $PhCH_2CO_2H$, EDC, HOBT; (g) H_2, Pd/C; (h) EDC, HOBT.

Table IV. Inhibitory Activity Against HIV-1 Proteinase and Anti-HIV-1 Activity[a]



no.	R	formula[b]	IC_{50}[c] (μM)	EC_{50}[d] (μM) (MT-4)	no.	R	formula[b]	IC_{50}[c] (μM)	EC_{50}[d] (μM) (MT-4)
27		$C_{34}H_{41}N_5O_5S \cdot 1.3H_2O$	1.9	–	52		$C_{28}H_{49}N_5O_5S \cdot H_2O$	0.17	10.6
28		$C_{34}H_{41}N_5O_5S \cdot H_2O$	0.082	>100	53		$C_{43}H_{51}N_5O_6S \cdot H_2O$	0.17	>100
45		$C_{38}H_{49}N_5O_5S \cdot 0.8H_2O$	2.8	–	54		$C_{43}H_{51}N_5O_6S \cdot H_2O$	0.22	>100
46		$C_{38}H_{49}N_5O_5S \cdot 0.5H_2O$	0.069	>150	55		$C_{39}H_{47}N_7O_5S \cdot 1.5H_2O$	0.3	–
47		$C_{41}H_{47}N_5O_5S \cdot 0.5H_2O$	2.9	–	56		$C_{36}H_{45}N_5O_6S \cdot 0.5H_2O$	1.1	–
48		$C_{41}H_{47}N_5O_5S \cdot 0.1CHCl_3$	0.0046	4.7	57		$C_{37}H_{47}N_5O_7S \cdot H_2O$	0.095	40
49		$C_{42}H_{47}N_7O_5S \cdot H_2O$	>3.8	–	58		$C_{37}H_{47}N_5O_6S \cdot H_2O$	0.054	5.7
50		$C_{42}H_{47}N_7O_5S \cdot 0.5H_2O$	0.0038	>130	63		$C_{41}H_{47}N_5O_5S \cdot 0.5H_2O$	0.053	>100
51		$C_{42}H_{49}N_5O_6S \cdot 1.5H_2O$	0.19	>100					

[a] Assays were performed as described in Experimental Section. [b] Satisfactory analyses (C, H, N, and S; 0.4% of theoretical values) were obtained for all compounds. [c] See Table I, footnote *a*. [d] See Table I, footnote *b*.

requirements include reduced amino acids and therefore a series of these were coupled to the opened penicillin acid to give compounds **8** to **19** (Table II). Two compounds, **10** and **16** showed moderate activity against HIV-1 proteinase, although unfortunately neither of these analogues showed antiviral against HIV-1 *in vitro*. The importance of the lipophilic group and the hydroxyl group was confirmed when compounds **19** and **20**, respectively, showed no activity. Furthermore, modification to the lipophilic group of **10** and **16** resulted in loss of activity in compounds **11–15**, **17**, and **18**.

The striking difference in activity between compounds **9** and **10** (>174 μM *vs* 0.15 μM, respectively), which differed only in stereochemistry at one center, was rationalized by inspection of molecular models. Each of the modeled ligands was assumed to bind as one-half of a "dimer", *i.e.*, with the thiazolidine ring system in the S_1 pocket and the phenylacetamido group in the S_2 pocket so as to maintain hydrogen bonding contacts to Arg8′ and Asp29 (Figure 1). The hydroxyl group was placed so as to interact with the catalytic residues, Asp25 and Asp25′, and this was achieved by manipulation of the torsion angles between the thiazolidine and the carbon bearing the hydroxyl. This procedure resulted in an average interheavy atom distance between the Asp oxygens and the hydroxyl of approximately 2.9 Å. Inspection of the "active" *R* isomer model, superimposed onto the cocrystal structure of the penicillin dimer–proteinase complex,[12] suggested that the benzyl group satisfied the requirements of the S_1' pocket (Figure 2). Inspection of the "inactive" *S* isomer with the hydroxyl group modeled between the catalytic aspartate residues suggested that the benzyl group lay between the S_1' and S_2' pockets satisfying the requirements of neither. We were later able to confirm the proposed binding mode of **10** through X-ray crystallographic studies.[18]

Having achieved moderate inhibition with compound

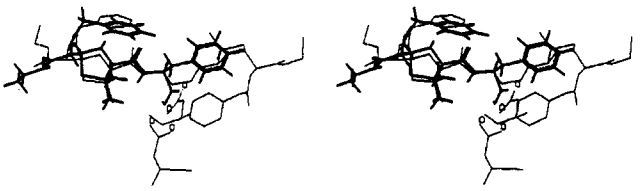
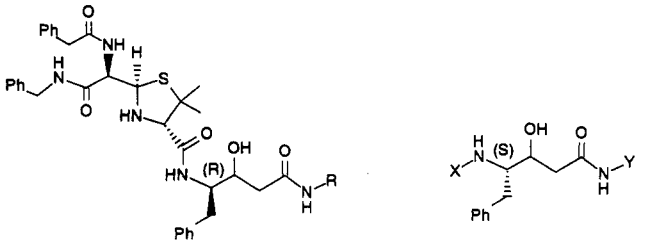


Figure 2. Stereoview of modeled **10** (thick lines) superimposed onto the cocrystal structure of a penicillin dimer with HIV proteinase—only the inhibitor and catalytic Asp residues included for clarity.



General structure of modeled inhibitors (I) General structure of statine analogues (II)

Figure 3. General structures of proteinase inhibitors.

10 we next sought to introduce further key groups to improve binding at the active site. A prominent feature of the cocrystal structure of the penicillin dimer–proteinase complex is the tetrahedral hydrogen bonding arrangement between the NH of the Ile50 and Ile50′ residues in the "flap", a conserved crystallographic water (water 301) and the linker carbonyl groups in the ligand (Figure 1). The compounds described above lack the potential for this interaction as they only possess one such carbonyl group. It was apparent from modeling based on the crystal structure that two carbonyl groups separated by a four atom spacer was preferred, and this motif was incorporated into the design process to provide the general structure **I** (Figure 3). Adjustment of torsion angles along this linking region facilitated the placement of the carbonyl groups in a position to participate in a hydrogen bonding arrangement. In addition, this second amide group was reversed in comparison to the penicillin dimers in order to facilitate a potential hydrogen bonding interaction between the amide NH and the carbonyl of Gly27′. Therefore compounds **27** and **28** were synthesized, and the latter compound was found to be a more potent inhibitor of HIV-1 proteinase than compound **10** (Table IV).

It was further postulated from the model that a lipophilic amine coupled to the linker acid could satisfy the requirements of the S_2' pocket although there is a considerable degree of flexibility in the system. Compounds **45–48** (Table IV) were synthesized, of which **48** showed very good activity as a competitive inhibitor against the enzyme, IC_{50} 4.6 nM (K_i = 0.25 nM),[19] and also showed antiviral activity against HIV-1 *in vitro*. Compound **48** showed good selectivity, with inhibition of HIV-1 proteinase at least 10 000 times greater than for the mammalian aspartic proteinases, pepsin, cathepsin D, and renin. The unusual amino acid, statine and its analogues, **II** (Figure 3), have been used as dipeptide isosteres for inhibitors of aspartic proteinases.[20] These analogues have (*S*) stereochemistry at the asymmetric center from which the lipophilic group (P_1) is attached. The group in **48** was unusual because here the center had (*R*) stereochemistry and the lipophilic group was in fact P_1'. This is the first example of a "P_1" benzyl group of a statine analogue that takes a direct and novel path to the S_1' pocket and represents a stereochemically unique dipeptide isostere that inhibits HIV aspartic proteinase.

The benzylimidazole derivative **50** in this series of analogues also had good activity in the enzyme assay, although it did not possess antiviral activity *in vitro*. Compound **50** was modeled into the enzyme active site in a similar manner to the procedure described above. Two binding modes were considered possible for the benzimidazole. The first placed the benzimidazole into the S_2' pocket, while the second positioned the ring system in the S_3' pocket although it also extended out further to the surface of the enzyme. Further inspection of the models suggested that in the first case the benzimidazole was too bulky for the S_2' pocket; however, there was a possibility that the NH of the five-membered ring may form a hydrogen bond with the carbonyl of Gly48′ in the latter case. This is currently under investigation by X-ray crystallography.[18] Attempts were therefore made to incorporate groups to interact with both S_2' (cf. **48**) and Gly48′ (cf. **50**). Compounds **51–54** were synthesized, but they showed no improvement in activity against the enzyme (Table IV).

A series of compounds **55–58** (Table IV) was synthesized in order to interact specifically with Gly48′. These compounds had the advantage of reduced lipophilicity which might improve or alter the antiviral activity *in vitro* and pharmacokinetic profile. Although none had improved activity against the enzyme compared to **48** or **50**, compound **58** showed similar levels of antiviral activity *in vitro* to **48**. Finally, reversing the amide bond in **48** gave **63** which was less active, probably due to the increased distance of the amide NH from the Gly27′ carbonyl. This result is therefore consistent with the modeling studies that led to the proposal for the general structure **I** (Figure 3).

Conclusion

We have developed a series of penicillin based monomer inhibitors of HIV-1 proteinase that has the key feature of a stereochemically unique statine-based isostere. Compound **48** attains potent activity against the enzyme (K_i = 0.25 nM) whilst having a significantly reduced molecular weight compared to the original leads to **1** to **3**. Despite the structural diversity of compounds **50–58** only **58** showed similar levels of antiviral activity *in vitro* compared to compound **48**. Further investigations into penicillin based monomer inhibitors are currently in progress.

Experimental Section

Melting points were determined with a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were determined on an Optical Activity AA100 polarimeter. Proton nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL200, Varian VXR400, or a Bruker AM250 spectrometer. Chemical shifts were reported as δ units (ppm) relative to tetramethylsilane as internal standard. Infrared (IR) spectra were recorded on a Nicolet 5SXC FT-IR spectrometer. High-resolution mass spectra were determined using a Kratos-Concept. Merck Kieselgel 60 (230–400 mesh) was used for flash column chromatography. Reagents were from commercial sources and used without further purification. The HIV-1 proteinase assay and the microtiter-based MT-4 assay were carried out according to the procedures described previously.[11]

[2*R*-[2α(*R),4β]]-5,5-Dimethyl-2-[2-oxo-1-[(phenylacetyl)amino]-2-[(phenylmethyl)amino]ethyl]-4-thiazolidinecarboxylic Acid (5).** Benzylamine (37 mL, 339 mmol) was added to a suspension of penicillin G (*N*-ethylpiperidine salt) (50.5 g, 113 mmol), and the mixture was stirred at room temperature for

6 h. The solvent was removed *in vacuo*, and the residue was recrystallized from MeOH/EtOAc. The white solid was partitioned between EtOAc (1.5 L) and orthophosphoric acid (350 mL, 2 N). After vigorous stirring the organic phase was separated, and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with water and brine before drying ($MgSO_4$). The solvent was removed *in vacuo* to afford **5** as a white foam, (43.6 g, 87.5%): ^{1}H NMR (DMSO-d_6) δ 1.18 (s, 3H), 1.52 (s, 3H), 3.55 (m, 3H), 4.1–4.4 (m, 2H), 4.50 (t, 1H, J = 7 Hz), 4.90 (d, 1H, J = 7 Hz), 7.1–7.4 (m, 10H), 8.38 (d, 1H, J = 8 Hz), 8.58 (t, 1H, J = 4 Hz); IR (Nujol) 3300, 1718, 1643 cm^{-1}; $[\alpha]^{20}_D$ +69.4° (*c* 0.89, MeOH), (lit.[16] $[\alpha]^{22}_D$ +88° (*c* 1, MeOH)). Anal. ($C_{23}H_{27}N_3O_4S\cdot0.7H_2O$) C, H, N, S.

[2*R*-[2α(*R),4β]]-4-Carbamoyl-5,5-dimethyl-α-[(phenylacetyl)amino]-*N*-(phenylmethyl)-2-thiazolidineacetamide (7).** To [2*S*-[2α,5α,6β]]-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamide[16] (**6**) (140 mg, 1.0 mmol) in dry CH_2Cl_2 (10 mL) was added benzylamine (0.33 mL, 3.0 mmol), and the solution was stirred at room temperature for 3 days. The solvent was removed *in vacuo*, and the resulting foam recrystallized from EtOH/cyclohexane to afford **7** as a white solid (350 mg, 79%): mp 114–115 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 3H), 1.38 (s, 3H), 1.84 (br, 1H), 3.04 (s, 1H), 3.45 (m, 1H), 3.54 (s, 2H), 4.15 (dd, 1H, J = 13, 4 Hz), 4.5–4.8 (m, 2H), 5.05 (d, 1H, J = 6 Hz), 5.25 (s, 1H), 5.41 (s, 1H), 6.72 (d, 1H, J = 7 Hz), 7.1–7.5 (m, 10H); IR ($CHBr_3$) 3390, 3316, 1677, cm^{-1}; $[\alpha]^{20}_D$ +74.0° (*c* 1.0, MeOH). Anal. ($C_{23}H_{26}N_4O_3S\cdot0.5H_2O$) C, H, N, S.

General Method A: TBTU Coupling of Acids with Amines. To a solution of acid (1.4 mmol) in DMF (12 mL) were added sequentially DIPEA (1.5 mmol, 1 extra equiv for $RNH_2\cdot HCl$), amine (or amine HCl salt) (1.5 mmol), and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (1.5 mmol). The resulting solution was stirred at room temperature for 3–22 h, as appropriate, before partitioning between EtOAc and water. The organic phase was removed, and the aqueous phase was extracted with EtOAc (2×). The combined organic phases were washed with 2 N HCl, saturated aqueous $NaHCO_3$, and brine before drying ($MgSO_4$). The solvent was removed *in vacuo*, and the resulting residue was generally purified by flash silica column chromatography.

General Method B: DCC Coupling of Amines with 5. To a solution of **5** (1.6 mmol) in DMF (6 mL) was added 1-hydroxybenzotriazole (HOBT) (1.7 mmol). After stirring at room temperature for 10 min amine (0.86 mmol) was added, and the solution was stirred for a further 15 min. Finally dicyclohexylcarbodiimide (DCC) (1.7 mmol) was added, and the solution was stirred for 4–5 h. The resulting precipitate was removed by filtration, and the filtrate was partitioned between EtOAc and water. The phases were separated, and the organic phase was washed with 2 N HCl, saturated aqueous $NaHCO_3$ and brine before drying ($MgSO_4$). The solvent was removed *in vacuo*, and the resulting residue was purified by flash silica column chromatography.

[2*R*-[2α(*R),4β(*R*)]]-5,5-Dimethyl-α-[(phenylacetyl)amino]-*N*-(phenylmethyl)-4-[[[1-(2-phenyl-1-(hydroxymethyl))ethyl]amino]carbonyl]-2-thiazolidineacetamide (10).** Compound **10** was synthesized according to general method B: mp 83–86 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (s, 3H), 1.48 (s, 3H), 2.6–2.9 (m, 2H), 3.45 (d, 1H, J = 9 Hz), 3.50 (s, 2H), 3.75 (dd, 1H, J = 9, 7 Hz), 4.0 (m, 1H), 4.10–4.40 (m, 2H), 4.40 (t, 1H, J = 7 Hz), 4.80 (t, 1H, J = 5 Hz), 4.90 (t, 1H, J = 7 Hz), 7.1–7.3 (m, 15H), 7.68 (d, 1H, J = 7 Hz), 8.30 (d, 1H, J = 7 Hz), 8.50 (t, 1H, J = 7 Hz); IR (Nujol) 3275, 1638, cm^{-1}; $[\alpha]^{22}_D$ +87.8° (*c* 1.0, MeOH). Anal. ($C_{32}H_{38}N_4O_4S\cdot H_2O$) C, H, N, S.

General Method C: DCC Coupling of Amine Hydrochloride Salts with 5. To a solution of the amine hydrochloride (1.0 mmol) in water (0.2 mL) was added $KHCO_3$ (1.2 mmol). After effervescence had subsided, THF (3 mL) and H_2O (0.2 mL) were added, and the solution was stirred for 0.75 h. Compound **5** (1.2 mmol), HOBT (1.2 mmol), and DCC (1.0 mmol) were then added sequentially, and the mixture was stirred for a further 2 h before filtering. The filtrate was evaporated *in vacuo*, and the resulting residue was partitioned between dilute aqueous $NaHCO_3$ and EtOAc. The organic phase was washed with water and brine before drying ($MgSO_4$). The solvent was removed *in vacuo*, and the residue was purified by flash silica column chromatography.

***N*-[4-Ethoxy-2-hydroxy-4-oxo-1-(phenylmethyl)butyl]carbamic Acid, 1,1-Dimethylethyl Ester, (1′*R*,2′*S*)-(23) and (1′*R*,2′*R*)-(24).** Compounds **23** and **24** were synthesized from *N*-(*tert*-butoxycarbonyl)-D-phenylalanine methyl ester according to the procedure described by Rich *et al.*[17]

Compound **23**: mp 135–138 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, J = 7 Hz), 1.38 (s, 9H), 2.4–2.7 (m, 2H), 2.7–3.1 (m, 2H), 3.58 (br, 1H), 3.7–4.1 (m, 2H), 4.15 (q, 2H, J = 7 Hz), 4.52 (m, 1H), 7.1–7.4 (m, 5H); IR (Nujol) 3352, 1736, 1682 cm^{-1}; $[\alpha]^{22}_D$ +22.6° (*c* 1.1, MeOH). Anal. ($C_{18}H_{27}NO_5$) C, H, N.

Compound **24**: mp 77–80 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7 Hz), 1.40 (s, 9H), 2.36 (dd, 1H, J = 16, 2 Hz), 2.58 (dd, 1H, J = 16, 9 Hz), 2.92 (d, 2H, J = 7 Hz), 3.48 (m, 1H), 3.72 (m, 1H), 3.97 (m, 1H), 4.13 (q, 2H, J = 7 Hz), 4.95 (d, 1H, J = 9 Hz), 7.1–7.4 (m, 5H); IR (Nujol) 3374, 1730, 1720, 1682 cm^{-1}, $[\alpha]^{22}_D$ +37.5° (*c* 1.0, MeOH). Anal. ($C_{18}H_{27}NO_5$) C, H, N.

(1′*R*,2′*S*)-*N*-[4-Amino-2-hydroxy-4-oxo-1-(phenylmethyl)butyl]carbamic acid, 1,1-Dimethylethyl Ester (25). $NH_{3(g)}$ was bubbled for 1 h through a solution of **23** (800 mg, 2.4 mmol) in dry methanol (5 mL) at 0 °C. The solution was stirred at room temperature for 3 days before removing the solvent *in vacuo*. The resulting residue was purified by flash silica column chromatography eluting with $CHCl_3$/MeOH (10:1) to afford **25** as a white solid, (350 mg, 48%): mp 206–208 °C; ^{1}H NMR (DMSO-d_6) δ 1.28 (s, 9H), 2.0–2.4 (m, 2H), 2.5 (m, 1H), 3.00 (d, 1H, J = 13 Hz), 3.50 (m, 1H), 3.78 (m, 1H), 4.98 (d, 1H, J = 5 Hz), 6.52 (d, 1H, J = 9 Hz), 6.80 (s, 1H), 7.0–7.3 (m, 6H); IR (Nujol) 3341, 1685, 1654 cm^{-1}; $[\alpha]^{22}_D$ +13.2° (*c* 0.2, DMSO). Anal. ($C_{16}H_{24}N_2O_4$) C, H, N.

(1′*R*,2′*R*)-*N*-[4-Amino-2-hydroxy-4-oxo-1-(phenylmethyl)butyl]carbamic acid, 1,1-Dimethylethyl Ester (26). Compound **26** was synthesized from **24** according to the procedure described for **25**: mp 173–175 °C; ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 9H), 2.14 (d, 2H, J = 5 Hz), 2.57 (dd, 1H, J = 14, 9 Hz), 2.82 (dd, 1H, J = 14, 5 Hz), 3.62 (m, 1H), 3.88 (m, 1H), 4.85 (d, 1H, J = 5 Hz), 6.40 (d, 1H, J = 9 Hz), 6.77 (s, 1H), 7.0–7.3 (m, 6H); IR (Nujol) 3411, 3363, 3189, 1685, 1650 cm^{-1} $[\alpha]^{22}_D$ +44.4° (*c* 0.54, DMSO). Anal. ($C_{16}H_{24}N_2O_4$) C, H, N.

***N*-[2,4-Dihydroxy-4-oxo-1-(phenylmethyl)butyl]carbamic acid, 1,1-Dimethylethyl Ester, (1′*R*,2′*S*)-(29) and (1′*R*,2′*R*)-(30).** Compounds **29** and **30** were synthesized from **23** and **24**, respectively, according to the procedure described by Rich *et al.*[17]

Compound **29**: mp 177–180 °C; ^{1}H NMR (DMSO-d_6) δ. 1.30 (s, 9H), 2.18 (dd, 1H, J = 16, 7 Hz), 2.45 (m, 1H), 2.59 (m, 1H), 3.00 (m, 1H), 3.50 (m, 1 H), 3.80 (m, 1H), 6.60 (d, 1H, J = 9 Hz), 7.0–7.4 (m, 5H); IR (Nujol) 3356, 1725, 1687 cm^{-1}; $[\alpha]^{22}_D$ +117° (*c* 0.45, DMSO). Anal. ($C_{16}H_{23}NO_5$) C, H, N.

Compound **30**: mp 149–152 °C (lit.[21] 146–148 °C); ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 9H), 2.20 (dd, 1H, J = 16, 7 Hz), 2.35 (dd, 1H, J = 16, 2 Hz), 2.57 (dd, 1H, J = 13, 2 Hz), 2.80 (dd, 1H, J = 13, 3 Hz), 3.65 (m, 1H), 3.90 (m, 1H), 6.50 (d, 1H, J = 9 Hz), 7.1–7.4 (m, 5H); IR (Nujol) 3379, 1688 cm^{-1}; $[\alpha]^{22}_D$ +48.0° (*c* 0.92, MeOH) (lit.[21] $[\alpha]^{22}_D$ +37.0° (*c* 1, MeOH)). Anal. ($C_{16}H_{23}NO_5$) C, H, N.

(1′*R*,2′*R*)-*N*-[1-[2-Hydroxy-4-oxo-4-[(2-methylpropyl)amino]-1-(phenylmethyl)butyl]]carbamic Acid, 1,1-Dimethylethyl Ester (32). Compound **32** was synthesized from **30** according to general procedure A: mp 139–141 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (d, 6H, J = 6 Hz), 1.30 (s, 9H), 1.65 (m, 1H), 2.15 (d, 2H, J = 5 Hz), 2.5–2.9 (m, 2H), 2.85 (t, 2H, J = 9 Hz), 3.62 (m, 1H), 3.88 (m, 1H), 4.86 (d, 1H, J = 5 Hz), 6.45 (d, 1H, J = 7 Hz), 7.1–7.3 (m, 5H), 7.77 (t, 1H, J = 5 Hz); IR (Nujol) 3358, 1688, 1666, 1616, 1518 cm^{-1}; $[\alpha]^{21}_D$ +43.9° (*c* 1.1, MeOH). Anal. ($C_{20}H_{32}N_2O_4$) C, H, N.

General Method D: Synthesis of Compounds 27, 28, and 45–58. To a solution of *N*-BOC protected amine (0.5 mmol) in dioxane (3 mL) was added a solution of $HCl_{(g)}$ in dioxan (3–8 M, 3–1.5 mL). After stirring at room temperature for 2 h the solvent was removed *in vacuo*. The resulting solid was dissolved in DMF (8 mL), and to this were added DIPEA (1.1 mmol), compound **5**, (0.5 mmol) and TBTU (0.5 mmol) sequentially. After stirring for 2–18 h (as required) the solution was partitioned between EtOAc and water. The organic phase was removed, and the aqueous phase was extracted with EtOAc (2×). The combined organic phases were washed with 2 N HCl, saturated aqueous

$NaHCO_3$, and brine before drying ($MgSO_4$). The solvent was removed *in vacuo*, and the residue was purified by flash silica column chromatography to afford the title compounds, yield 30–80%.

[2*R*-[2α(*R),4β(*R**,*R**)]]-5,5-Dimethyl-α-[(phenylacetyl)amino]-*N*-(phenylmethyl)-4-[[[1-[2-hydroxy-4-[1-(2-methylpropyl)amino]]-4-oxo-1-(phenylmethyl)butyl]amino]carbonyl]-2-thiazolidineacetamide (46).** Compound **46** was synthesized according to the general procedure D: mp 176–178 °C; ^1H NMR (DMSO-d_6) δ 0.80 (d, 6H, J = 6 Hz), 1.18 (s 3H), 1.50 (s, 3H), 1.60 (m, 1H), 2.05–2.30 (m, 2H), 2.60–2.95 (m, 4H), 3.45–3.60 (m, 3H), 3.90 (m, 2H), 4.00 (m, 1H), 4.15–4.40 (m, 2H), 4.45 (t, 1H, J = 7 Hz), 4.85 (t, 1H, J = 7 Hz), 5.20 (d, 1H, J = 5 Hz), 7.1–7.4 (m, 15H), 7.60 (d, 1H, J = 7 Hz), 7.80 (t, 1H, J = 7 Hz), 8.32 (d, 1H, J = 7 Hz), 8.50 (t, 1H, J = 6 Hz); IR (Nujol) 3290, 1641 cm^{-1}; $[\alpha]^{22}_D$ +86.0° (*c* 0.44, DMSO). Anal. ($C_{38}H_{49}N_5O_5S\cdot0.5H_2O$) C, H, N, S.

(1′*R*)-*N*-[3-Chloro-2-oxo-1-(phenylmethyl)propyl]carbamic Acid, Phenylmethyl Ester (60). *N*-(Benzyloxycarbonyl)-D-phenylalanine, **59** (1.5 g, 5.0 mmol), in THF (12.6 mL) was stirred at −15 °C, and triethylamine (0.73 mL, 5.3 mmol) and ethyl chloroformate (0.5 mL, 5.3 mmol) were added. After stirring at this temperature for a further 20 min the white precipitate was filtered, and the filtrate was added to an ethereal solution of diazomethane [30 mL; prepared from diazald (1.4 g)[22]] in a salt–ice bath. After stirring for 30 min, the yellow solution was allowed to warm to room temperature overnight. A stream of $HCl_{(g)}$ was then bubbled through the solution until the color was discharged. The mixture were diluted with EtOAc and washed with saturated aqueous $NaHCO_3$, 2 N HCl, saturated aqueous $NaHCO_3$, and brine before drying ($MgSO_4$). The solvent was removed *in vacuo* to afford a white solid which was recrystallized from 75% aqueous ethanol to afford **60** as a white solid (644 mg, 38%): mp 101–102 °C; ^1H NMR ($CDCl_3$) δ 2.9–3.2 (m, 2H), 3.92 (d, 1H, J = 13 Hz), 4.12 (d, 1H, J = 13 Hz), 4.76 (m, 1H), 5.09 (s, 2H), 5.28 (d, 1H, J = 7 Hz), 7.0–7.4 (m, 10H); IR ($CHBr_3$) 3426, 1713 cm^{-1}; $[\alpha]^{21}_D$ −25.9° (*c* 0.56, $CHCl_3$). Anal. ($C_{18}H_{18}ClNO_3$) C, H, Cl, N.

(1′*R*,2′*RS*)-*N*-[3-Azido-2-hydroxy-1-(phenylmethyl)propyl]carbamic Acid, Phenylmethyl Ester (61). A solution of **60** (17.2 g, 51.6 mmol) in THF (250 mL) and water (100 mL) was stirred in an ice–bath, and sodium borohydride (2.87 g, 75.5 mmol) added. After stirring at this temperature for 1.5 h the solvent was evaporated to a solid residue, which was suspended in $CHCl_3$ before adding water to give a clear solution. Following adjustment of the pH to *ca.* 1 with addition of 2 N HCl, the organic layer was separated, dried ($MgSO_4$), and the solvent removed *in vacuo* to afford a white solid. The crude product was recrystallized from a mixture of EtOAc/cyclohexane (4:1) to afford white crystals (1 major diastereoisomer), and the mother liquor was concentrated to give the alcohol as a mixture of diastereoisomers. The mixture was dissolved in DMF (100 mL) and to this sodium azide (3.7 g, 57.0 mmol) was added, before heating the reaction mixture at 90 °C for 18 h. After cooling, the reaction mixture was partitioned between EtOAc and water. The organic phase was separated, washed with water, dried and evaporated *in vacuo* to afford a brown gum. This was purified by column chromatography, $CHCl_3$/MeOH (95:5) as eluant to afford a pale yellow solid **61** as a (2:1) mixture of diastereoisomers (7.6 g, 43%); mp 67–69 °C; ^1H NMR ($CDCl_3$) δ 2.5–3.0 (m, 3H), 3.2–3.5 (m, 2H), 3.70 (m, 1H), 3.85 (m, 1H), 5.12 (m, 1H), 5.10 (s, 1H), 7.0–7.4 (m, 11H); IR ($CHBr_3$) 3425, 2105, 1712 cm^{-1}; $[\alpha]^{22}_D$ +41.4° (*c* 0.67, DMSO); HRMS *m/z* 341.162567 $(M + H)^+$ (calcd for $C_{18}H_{20}N_4O_3$, 341.161366).

(1′*R*,2′*RS*)-*N*-[2-Hydroxy-1-(phenylmethyl)-3-[[(phenylmethyl)carbonyl]amino]propyl]carbamic acid, Phenylmethyl Ester (62). A mixture of **61** (7.50 g, 22.0 mmol) and triphenylphosphine (5.94 g, 22.0 mmol) was dissolved in 60% aqueous THF (400 mL) and then heated under reflux for 23 h. After cooling the clear solution was diluted with EtOAc and extracted with 2 N HCl. The aqueous extracts were combined, treated with $NaHCO_3$ until basic, and back-extracted into EtOAc. The organic layer was dried and evaporated *in vacuo* to afford a foam (4.8 g) which was directly taken on to the next step without purification. The foam was dissolved in CH_2Cl_2 (340 mL) and stirred with HOBT (2.3 g, 17.0 mmol), phenylacetic acid (2.36 g, 17.0 mmol), and EDC (3.26 g, 17.0 mmol) at room temperature for 17 h. The mixture was partitioned between CH_2Cl_2 and water. The organic layer was separated and washed with 2 N HCl, saturated aqueous $NaHCO_3$, and brine before drying ($MgSO_4$). The solvent was removed *in vacuo*, and the resulting solid recrystallized from methanol to afford a white solid **62** as a 1:1 mixture of diastereoisomers (1.92 g, 78%): mp 182–186 °C dec; ^1H NMR (DMSO-d_6) δ 2.3–3.7 (m, 8H), 4.6–5.0 (m, 3H), 7.0–7.4 (m, 16H), 7.7–8.0 (m, 1H); IR (Nujol) 3342, 3304, 1688, 1680 cm^{-1}; $[\alpha]^{23}_D$ +27.5° (*c* 0.51, DMSO); HRMS *m/z* 433.214783 $(M + H)^+$ (calc for $C_{26}H_{29}N_2O_4$, 433.212733).

[2*R*-[2α(*R),4β(*R**)]]-4-[[[2-Hydroxy-3-[[(1-oxo-2-phenyl)ethyl]amino]-1-(phenylmethyl)propyl]amino]carbonyl]-5,5-dimethyl-α-[(phenylacetyl)amino]-*N*-(phenylmethyl)-2-thiazolidineacetamide (63).** Compound **62** (500 mg, 1.66 mmol) in ethanol (280 mL) was hydrogenated with 10% palladium on charcoal (682 mg) under atmospheric pressure and at room temperature for 5 h. After degassing, the mixture was filtered through Kieselguhr. The filtrate was evaporated *in vacuo* to afford a gum (300 mg) which was taken directly on to the next step without purification. The gum (280 mg) was dissolved in DMF (10.5 mL), and **5** (446 mg, 1.0 mmol), DIPEA (200 μL, 1.15 mmol) and TBTU (320 mg, 1.0 mmol) were added sequentially. After stirring at room temperature for 18 h the mixture was diluted with EtOAc and washed with dilute aqueous HCl, saturated aqueous $NaHCO_3$, and brine before drying ($MgSO_4$). The solvent was removed *in vacuo* to afford a foam which was purified by column chromatography; CH_2Cl_2/MeOH (1:1) acted as eluant to afford a brown foam **63** as a 2:1 mixture of diastereoisomers (150 mg, 14%): ^1H NMR (DMSO-d_6) δ 0.96–1.05 (2s, 3H), 1.45–1.50 (2s, 3H), 2.6–3.1 (m, 4H), 3.20–3.60 (m, 5H), 3.68 (m, 0.3H), 3.80 (m, 0.7H), 4.02 (m, 1H), 4.1–4.5 (m, 3H), 4.85 (m, 1H), 5.05 (d, 0.3H, J = 5 Hz), 5.25 (d, 0.7H, J = 5 Hz), 7.0–7.4 (m, 20H), 7.5–7.7 (m, 2H), 7.85 (t, 0.7H, J = 4 Hz), 8.02 (t, 0.3H, J = 4 Hz), 8.24 (m, 1 H), 8.45 (m, 1H); IR ($CHBr_3$) 3406, 1658 cm^{-1}; $[\alpha]^{20}_D$ +55.6° (*c* 0.50, DMSO). Anal. ($C_{41}H_{47}N_5O_5S\cdot0.5H_2O$) C, H, N.

Molecular Modeling. The molecular models described were constructed by modification of the crystallographic structure of the penicillin dimer–HIV proteinase complex.[12] Construction of the models was achieved with the aid of the INSIGHTII[23] modeling system, using where possible units from within the fragment library. Each modeled ligand was assumed to bind as one half of a "dimer", *i.e.*, with the thiazolidine ring system in the S_1 pocket and the phenylacetamido group in the S_2 pocket so as to maintain hydrogen bonding contacts to Arg8′ and Asp29 (Figure 1). The resulting models were then minimized in the static enzyme structure (including crystallographic water molecules) using DISCOVER[24] utilizing the CVFF force field.[25] The results were visualized using INSIGHTII running on a Silicon Graphics 4D35TG.

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