A Series of Penicillin Derived C_2-Symmetric Inhibitors of HIV-1 Proteinase: Synthesis, Mode of Interaction, and Structure-Activity Relationships

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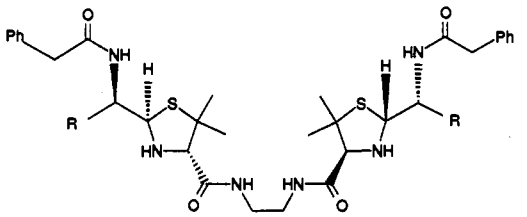
The C_2-symmetric diester 1 was identified by random screening as a novel inhibitor of HIV-1 proteinase. This led to the preparation of a series of related more potent amides from readily accessible penicillins. Many of the compounds showed potent antivirial activity in HIV-1-infected MT-4 cells and an ability to inhibit syncytia formation in infected C8166 cells, with no evidence of cytotoxicity. The compounds showed no activity against other aspartyl proteinases (renin, pepsin, and cathepsin D). Structure-activity relationships support a symmetrical interaction with the enzyme. Pharmacokinetic evaluation of the ethylamide 3 revealed it was subject to rapid plasma clearance and had low oral bioavailability.

Introduction

Human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), encodes an aspartyl proteinase which plays an essential role in viral replication. Mutation[1] or deletion[2] of the HIV-1 proteinase gene has been shown to result in the formation of immature and noninfectious virions. Inhibition of this enzyme has thus been identified as a major chemotherapeutic target for the treatment of AIDS.[3] The ability of HIV-1 proteinase inhibitors to block infection in chronically infected cells[4] offers the prospect of a more effective therapy for AIDS than that currently achieved with agents targeting reverse transcriptase.

In an earlier paper[5] we described the identification of the C_2-symmetric bis-ester 1 (Figure 1) as a selective inhibitor of HIV-1 proteinase. The lead provided by 1 led to the preparation of the more potent bis-amides 2–4, of which the latter two also inhibited the cytopathic effect of HIV-1 in cellular assays.

Initially three possible modes of binding of these inhibitors to the enzyme were considered, two symmetric modes and an asymmetric mode. Spectroscopic and X-ray crystallographic studies, presented in an accompanying paper,[6] confirmed a symmetric interaction in which the thiazolidine rings occupy the S_1 and S_1' pockets[7] and the phenyl rings the S_2 and S_2' pockets (Figure 2). In this paper we describe further structure-activity relationships of this novel series of inhibitors together with information on their kinetics of inhibition and pharmacokinetic properties.[8]



1 R = $COOCH_3$

2 R = $CONH_2$

3 R = $CONHCH_2CH_3$

4 R = $CONHCH_2Ph$

Figure 1. Penicillin-derived C_2-symmetric dimers.

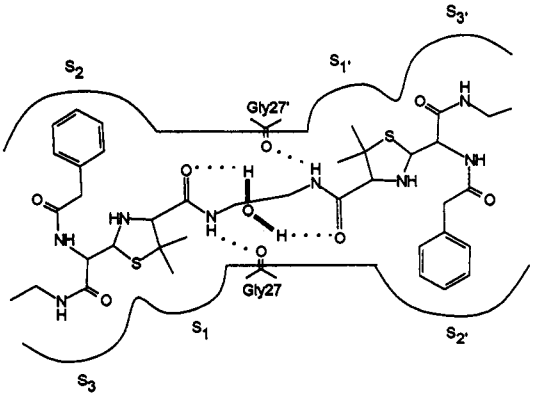


Figure 2. Schematic representation of the binding of compound 3 at the active site of HIV-1 proteinase.

Chemistry

The esters and amides 1 to 16 described in Table I were prepared from penicillin G *N*-ethylpiperidine salt 20 by the route shown in Scheme I. The salt 20 was reacted with ethyl chloroformate and the resulting mixed anhydride treated with ethylenediamine to give the penicillin dimer 21. Nucleophilic opening of the β-lactam rings of the dimer 21 with the appropriate alcohol or amine provided the analogues 1–12 and 14–16. The acid 13 was obtained by deprotection of the corresponding *tert*-butyl ester 12 with 30% hydrogen bromide in acetic acid. Reduction of the ester 1 with lithium borohydride provided the hydroxymethyl compound 17, which was acetylated to give the (benzoyloxy)methyl analogue 18. Compound 19 was prepared by reacting the thiazolidine acid 22[9] with

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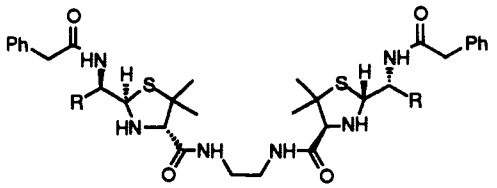
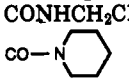
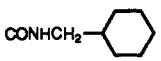
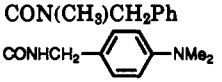
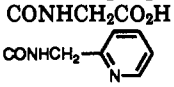
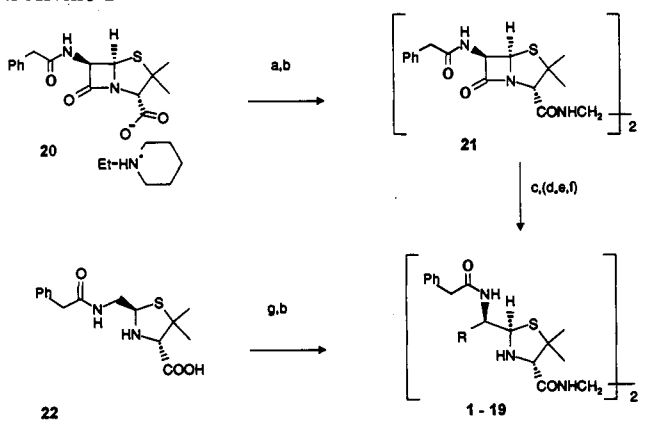
Table I. Anti-HIV Activity



no.	R	mp (°C)	formula[a]	IC_{50} (nM)[b]	$EC_{50}(\mu M)$ MT-4[c]	$EC_{50}(\mu M)$ C8166[d]
1	CO_2CH_3	113–114	$C_{36}H_{48}N_6O_6S_2 \cdot 0.5H_2O$	60	>100	NT
2	$CONH_2$	180–182	$C_{34}H_{46}N_8O_6S_2 \cdot 0.5H_2O$	3.0	>100	NT
3	$CONHCH_2CH_3$	190–193	$C_{38}H_{54}N_8O_6S_2 \cdot 1.5H_2O$	4.8	5.4	1.1
4	$CONHCH_2Ph$	193–194	$C_{48}H_{58}N_8O_6S_2$	0.9	0.29	0.06
5	CO_2CH_2Ph	amorph	$C_{48}H_{56}N_6O_8S_2 \cdot H_2O$	75	>100	
6	$CONMe_2$	125–128	$C_{38}H_{54}N_8O_6S_2 \cdot 2.5H_2O$	5.2	4.4	1.7
7	$CONHCH_2CF_3$	178–179	$C_{38}H_{48}F_6N_8O_6S_2 \cdot H_2O$	4.4	0.28	0.22
8		145–146	$C_{44}H_{62}N_8O_6S_2 \cdot H_2O$	6.9	1.8	0.23
9		185–186	$C_{48}H_{70}N_8O_6S_2$	40	8.2	0.06
10	$CON(CH_3)CH_2Ph$	128–129	$C_{50}H_{62}N_8O_6S_2 \cdot 1.5H_2O$	820	NT	NT
11		173–174	$C_{52}H_{68}N_{10}O_6S_2 \cdot H_2O$	47	<0.01	0.02
12	$CONHCH_2CO_2C(CH_3)_3$	132–133	$C_{48}H_{66}N_8O_{10}S_2 \cdot 0.5H_2O$	21	2.7	NT
13	$CONHCH_2CO_2H$	amorph	$C_{38}H_{50}N_8O_{10}S_2 \cdot H_2O \cdot CHCl_3$	39	>100	NT
14		139–140	$C_{48}H_{56}N_{10}O_6S_2 \cdot H_2O$	11	80	NT
15	$CONHCH_2CH_2OH$	151–158	$C_{38}H_{54}N_8O_6S_2 \cdot H_2O$	40	>100	NT
16	$CON(CH_2CH_2OH)_2$	amorph	$C_{42}H_{62}N_8O_{10}S_2 \cdot 0.75CHCl_3$	840	NT	NT
17	CH_2OH	111–112	$C_{34}H_{48}N_6O_6S_2 \cdot 0.5H_2O$	140	>100	NT
18	CH_2OCOPh	110–114	$C_{48}H_{56}N_6O_8S_2 \cdot H_2O$	70	NT	NT
19	H	amorph	$C_{32}H_{44}N_6O_4S_2 \cdot 1.5H_2O$	7800	NT	NT
24		121–122	$C_{35}H_{49}N_7O_5S_2 \cdot 0.5H_2O$	35	>100	NT
26		amorph	*m/z* 565.8 (MH^+)	8800	NT	NT
27		220–223	$C_{38}H_{54}N_8O_6S_2 \cdot H_2O$	1300	NT	NT
28		137–140	$C_{38}H_{54}N_8O_6S_2 \cdot 2.0H_2O$	1300	NT	NT
Ro 31-8959				11.2	0.004	0.0006
AZT					0.02	0.03

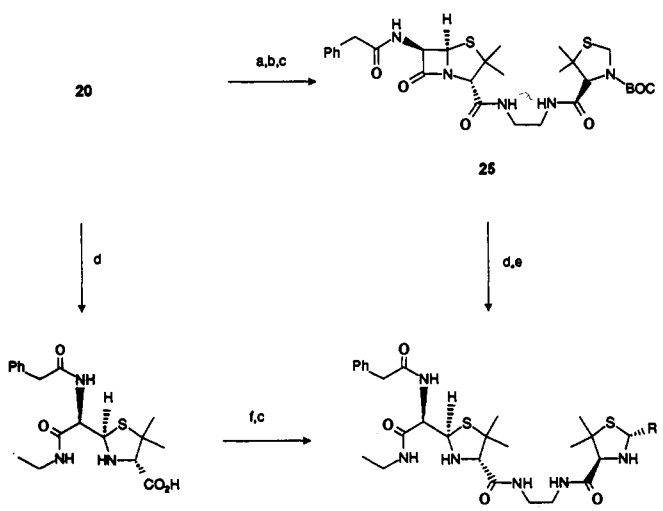
[a] Satisfactory analyses (C, H, N, and S; ± 0.4% of the theoretical values) were obtained for all compounds which were invariably obtained hydrated or solvated. Exhaustive drying of samples was generally counterproductive. [b] Inhibition of HIV-1 proteinase. [c] Inhibition of the cytopathic effect of H IV-1 in MT-4 cells. [d] Inhibition of syncytium formation in C8166 cells.

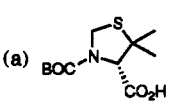
Scheme I[a]



[a] (a) $ClCO_2Et$, CH_2Cl_2, −10 °C; (b) $H_2NCH_2CH_2NH_2$; (c) alcohol or amine, CH_2Cl_2; (d) 12 to 13: 30% HBr in HOAc, CH_2Cl_2; (e) 1 to 17: $LiBH_4$, dioxane–THF; (f) 17 to 18: PhCOCl; (g) DCC, HOBT, THF, dioxane.

Scheme II[a]

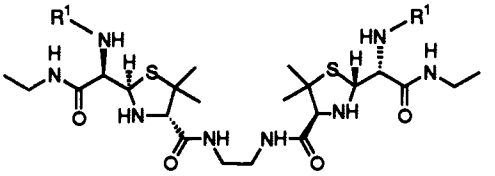


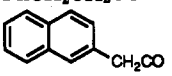
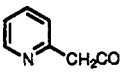
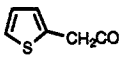
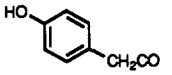
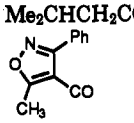
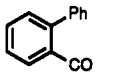
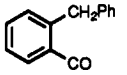
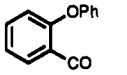
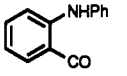
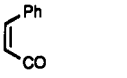
[a] (a)  , CH_2Cl_2; (b) $ClCO_2Et$, −10 °C; (c) $H_2NCH_2CH_2NH_2$; (d) $CH_3CH_2NH_2$, CH_2Cl_2; (e) CF_3CO_2H; (f) **22**, DCC, HOBT, THF.

ethylenediamine in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT). The asymmetric analogues **24** and **26** were synthesized by a DCC-mediated and a mixed-anhydride coupling procedure, respectively (Scheme II).

It was observed that acid treatment of compounds, *e.g.* **3** (Scheme III), in which the β-lactam ring(s) had been

Table II. Anti-HIV Activity[a]



no.	R^1	method	mp (°C)	formula	IC_{50} (nM)	EC_{50} (μM) MT-4	EC_{50} (μM) C8166
34	H	B	185–186	$C_{22}H_{42}N_8O_4S_2 \cdot 4.8HBr \cdot 2H_2O$	120000	NT	NT
35	PhCO	A	178–179	$C_{38}H_{56}N_8O_6S_2 \cdot H_2O \cdot 0.5CH_2Cl_2$	390	NT	NT
36	$PhCH_2CH_2CO$	B	158–159	$C_{40}H_{58}N_8O_6S_2 \cdot H_2O$	2.4	3.9	0.06
37		B	201–202	$C_{46}H_{58}N_8O_6S_2 \cdot H_2O$	820	NT	NT
38		B	amorph	*m/z* 785.6 (MH^+)	19	>100	NT
39		C	159–161	$C_{34}H_{50}N_8O_6S_4 \cdot 0.5H_2O$	15	27	5.2
40	$PhCH_2SO_2$	B	147–148	$C_{36}H_{54}N_8O_6S_4 \cdot 3.0H_2O$	58	32	13
41		B	153–154	$C_{38}H_{54}N_8O_6S_2 \cdot 2.0H_2O$	>12000	NT	NT
42	Me_2CHCH_2CO	A	224–225	$C_{32}H_{56}N_8O_6S_2 \cdot H_2O$	8.7	>100	NT
43		A	173–174	$C_{44}H_{56}N_{10}O_6S_2 \cdot H_2O$	5.4	2.5	NT
44		B	amorph	$C_{48}H_{56}N_8O_6S_2 \cdot 2.2H_2O$	9.9	0.03	0.17
45		C	176–177	$C_{50}H_{62}N_8O_6S_2 \cdot 1.5H_2O$	72	>100	NT
46		C	170–171	$C_{48}H_{56}N_8O_8S_2 \cdot 1.5H_2O$	160	>100	NT
47		C	179–181	$C_{48}H_{60}N_{10}O_6S_2 \cdot 2.0H_2O$	4800	NT	NT
48		C	186–187	$C_{40}H_{54}N_8O_6S_2 \cdot 0.75H_2O$	2.9	3.2	NT

[a] Footnotes as in Table I.

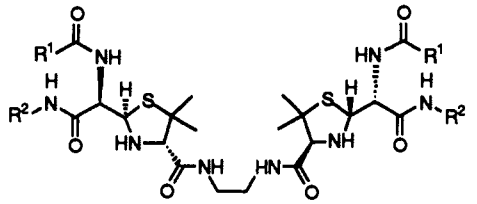
opened resulted in some epimerization at the point(s) of attachment to the thiazolidine ring (*i.e.* at the 5-position using penicillin numbering). The epimer **27** was isolated by preparative HPLC from such a mixture. The isomer **28**, epimerized at both 6-positions, was obtained by taking a *ca.* 1:1 mixture of penicillin G and 6-epipenicillin G[10] through the same synthetic procedure used for the preparation of **3** with separation of the isomeric products by HPLC.

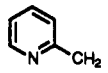
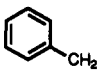
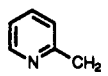
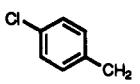
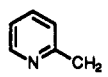
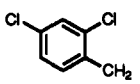
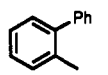
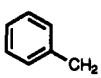
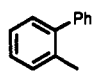
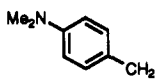
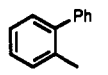
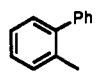
Compounds shown in Tables II and III bearing alternatives to the phenylacetamido groups of the compounds of Table I were prepared by one of the three methods shown in Scheme IV. In method A, 6-aminopenicillanic acid (6-APA, **29**) was first acylated and then converted into the dimer **32** by the mixed-anhydride procedure described in Scheme I. The β-lactam rings of **32** were opened with the requisite amine to give a number of the compounds listed in Tables II and III. In method B, the N-protected penicillin derivative **30**[11] was converted in the usual way into the dimer **32** ($R^1 = PhCH_2OCO$). This was treated with the required amine and then subsequently deprotected under carefully controlled conditions (30% HBr in acetic acid at 0 °C) to avoid epimerization. The resulting amine **34** was reacylated by standard procedures. In method C, which avoided the possibility of epimerization, the *N*-trityl derivative **31**[12] was converted to **32** ($R^1 = Ph_3C$) and this was deprotected with toluene-4-sulfonic acid in acetone to give the 6-APA dimer salt **33**. Acylation of **33** followed by ring opening completed the synthesis.

Results and Discussion

Variation of the Groups in the P_3 and P_3' Positions (Table I). The superior activity of the amides **2** and **4** compared to the esters **1** and **5** biased the synthetic strategy toward additional amides **6–16**. While none proved more active than compound **4**, except for compounds **10** and **16** (see below), they showed remarkably similar activity in inhibiting the enzyme *in vitro*. This is consistent with the S_3/S_3' pocket being near the outside of the enzyme cleft[6] and having relatively undemanding structural requirements. Consequently both lipophilic and hydrophilic groups were well tolerated. The greater activity of the amide **4** compared to its ester counterpart **5** may be explained by a stronger hydrogen-bond to the enzyme (Arg 8)[6] by the amide carbonyl in the former compound. In

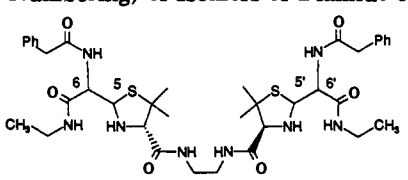
Table III. Anti-HIV Activity[a]



no.	R^1	R^2	method	mp (°C)	formula	IC_{50} (nM)	EC_{50} (μM) MT-4	EC_{50} (μM) C8166
49			B	132–134	$C_{48}H_{58}N_{10}O_6S_2 \cdot H_2O$	0.55	>10	NT
50			B	156–158	$C_{48}H_{54}Cl_2N_{10}O_6S_2 \cdot H_2O$	7.5	1.9	NT
51			B	140–142	$C_{46}H_{52}Cl_4N_{10}O_6S_2 \cdot H_2O$	14	0.53	0.24
52			B	180–181	$C_{58}H_{62}N_8O_6S_2 \cdot H_2O$	7.5	0.11	0.13
53			A	amorph	$C_{62}H_{72}N_{10}O_6S_2 \cdot 3.0H_2O$	0.23	<0.01	0.03
54		$CH_2CO_2Bu^t$	A	amorph	$C_{56}H_{70}N_8O_{10}S_2 \cdot 2.5H_2O$	4.1	1.6	NT
55		CH_2CO_2H	A	amorph	*m/z* 967.4 (MH^+)	39	>100	NT

[a] Footnotes as in Table I.

Scheme III. Configuration at C5/C5′ and C6/C6′ (Penicillin Numbering) of Isomers of Diamide **3**

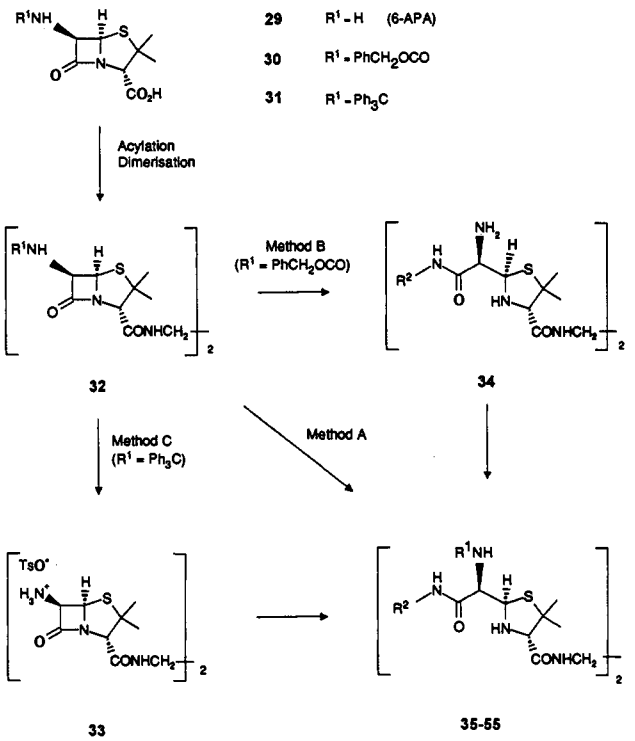


3	5,5′-(R)- ; 6,6′-(R)
27	5,5′-(S)- ; 6,6′-(R)
28	5,5′-(R)- ; 6,6′-(S)

view of the good activity shown by the N,N-disubstituted compounds **6** and **8**, the poor activity of analogues **10** and **16** is not easily explained, although there is evidence that bulky substituents at this position may disrupt the local protein structure and thus be deleterious.[6]

The bis(hydroxymethyl) compound **17** showed only modest activity, which was not significantly altered upon benzoylation to give **18**, a structural isomer of **5**. Complete removal of the ethylamide substituents to give compound **19** resulted in loss of activity. However, the removal of only one CONHEt group to give the asymmetric compound **24** (Scheme II) led to only a 10-fold drop in activity. These results indicate the importance of the amide carbonyls in hydrogen bonding to the enzyme (Arg 8)[6] and also demonstrate that the correct stereochemistry at this center is crucial in enabling the P_2/P_2' groups to fit the pockets. Consistent with this interpretation is the relative inactivity of compound **28** (Scheme III, Table I) in which the 6-/6′-positions have been epimerized. Compound **27**, epimerized at the 5-/5′-positions, also had only low activity.

Scheme IV



Variation of the Groups in the P_2 and P_2' Positions (Table II). It was evident from the X-ray structure of the compound **3**–proteinase complex[6] that the benzyl groups fit comfortably into the S_2/S_2' pockets. Not surprisingly, therefore, the diamine **34** was inactive. In addition, replacement of the phenylacetyl moieties of **3** by benzoyl groups to give compound **35** led to a 100-fold drop in inhibitory activity. The slight increase in chain

length provided by the phenylpropionamido derivative **36** gave a marginally more active analogue. Heteroaromatic replacements for phenyl such as pyridyl **38** and thienyl **39** showed good enzyme activity. The introduction of *p*-hydroxy substituents into the phenyl rings of **3** abolished activity either for steric reasons or due to the hydrophilic nature of the substituent.

Penicillins bearing isoxazolyl side chains (*e.g* oxacillin) are readily available and have been shown to possess improved pharmacokinetic properties over their phenylacetyl counterparts.[13] Thus the derivative **43** was prepared and its good activity was attributed to the phenyl groups being able to access the S_2/S_2' pockets.[6] This led to the synthesis of the 2-phenylbenzamido analogue **44** which, in contrast to its unsubstituted counterpart **35**, showed good inhibitory activity against the enzyme. An X-ray crystal structure of **44** complexed to recombinant proteinase enzyme confirmed these postulates.[6]

Kinetics and Selectivity. Due to the tight-binding nature of these inhibitors, K_i values were determined by the method of Henderson.[14] The slope of plots of $[I]/(1-v_i/v_o)$ against v_o/v_i increased with increasing substrate concentration, indicating that the mode of inhibition by these compounds was competitive. The K_i value for the inhibition of HIV-1 proteinase by compound **3** was determined to be 0.1 nM.

Compound **3** and other dimeric inhibitors listed in Tables I and II were assayed for selectivity by screening against renin, pepsin, and cathepsin D.[15] None of the compounds showed any significant inhibition of these mammalian enzymes at doses up to 100 μM.

Antiviral Activity *in Vitro*. Compounds showing activity against the isolated enzyme were tested for their ability to inhibit HIV-1 (strain RF) in cell culture (MT-4 cells) using a formazan-based microtiter assay.[16] Analogues showing good activity in this assay were further examined for their inhibitory effects on HIV-1-induced syncytium formation in C8166 cells.[17] Activities of the peptidomimetic HIV-1 proteinase inhibitor Ro 31-8959[18] currently undergoing clinical trials and the established nucleoside reverse transcriptase inhibitor AZT[19] are included for comparative purposes.

The lack of antiviral activity *in vitro* shown by some compounds can be attributed to their hydrophilicity. However, there appears to be no firm correlation between cellular activity and lipophilicity [calculated log *P* or measured log *D* (pH 7.4)[20] values], except within series of closely related compounds. For example, the 2-pyridylacetamido compound **49** (Table II) (calcd log $P = +0.08$) showed good inhibitory activity against the proteinase enzyme but was inactive in the primary cellular assay. Introduction of lipophilic chlorine atoms into the P_3/P_3' benzyl substituents of **49** to furnish **50** (calcd log $P = +1.51$) and **51** (calcd log $P = +2.93$) resulted in increased cellular potency despite some loss in activity against the enzyme. In addition, compound **4** showed activity against HIV-2 (Rod) in a secondary assay in CEM cells: **4**, $EC_{50} = 0.26$ μM; AZT, $EC_{50} = 0.03$ μM. None of the compounds showed evidence of cytotoxicity at concentrations up to 100 μM.

Pharmacokinetic Results. The pharmacokinetic profile of the ethylamide **3**[21] was examined in several species. In the three species listed in Table IV, plasma clearance was rapid; however, the drug showed a moderately high volume of distribution and an acceptable terminal elimination half-life. Studies performed with **3** administered intravenously at 5 mg/kg to rats with cannulated bile ducts revealed that 75% of the dose was excreted in the bile within 4 h (60% within the first hour). Chromatographic analysis of the bile samples showed that 80–90% of the eliminated material was unchanged **3**. The remainder of the dose administered was accounted by renal clearance (*ca.* 15%) with the balance attributed to metabolism.

Table IV. *In Vivo* Data for the Ethylamide **3** in Various Species Dosed Intravenously

species	dose[a] (mg/kg)	$t_{1/2}$ (h)	plasma clearance (mL/min)	volume of distribution[b] (L)
rat[c]	5	5	12	2.8
dog[d]	1	4	300	50
monkey[e]	5	1.2	200	17

[a] Formulated in ethanol (10–20%), polyethylene glycol (10%), and saline. [b] Steady state. [c] CD rats, $n = 2$ per time point. [d] Beagles, $n = 2$. [e] Cynomolgus, $n = 2$.

When **3** was administered orally to rats at 25 mg/kg, the bioavailability was found to be low (*ca.* 5%), consistent with high first-pass elimination. It has been shown that **3** epimerizes (at the points of the attachment of the side chains to the thiazolidine rings) in pH 2.0 buffer ($t_{1/2} \approx$ 20 min) and in the lumen of rat stomach, but epimerization at pH 5.0 was found to be negligible. However, when carbon-14 labeled **3** was administered subcutaneously to rats, its bioavailability was shown to be in excess of 75%.

Studies are in progress to examine the pharmacokinetic properties of other members of the series. Preliminary results indicate that the more active lipophilic analogues such as the benzylamide **4** are even more rapidly cleared from the plasma and present formulation difficulties.

Conclusions

We have developed a series of novel and selective dimeric inhibitors of HIV-1 proteinase which are accessible in few steps from readily available penicillins. Structure–activity relationships support a C_2-symmetric interaction with the enzyme, which was confirmed by X-ray crystallographic studies.[6] Many of the less polar compounds, *e.g.* **11** and **53**, show good activity against HIV-1 in cellular assays with no evidence for cytotoxicity. Introduction of hydrophilic groups into the molecule led to loss of cellular activity. In common with most peptide-based inhibitors of HIV-1 proteinase,[3] this series of high molecular weight compounds, typified by the inhibitor **3**, suffer from low oral bioavailability due to high hepatic clearance. Further work is in progress to design inhibitors with improved interactions with the enzyme and the catalytic Asp residues, and also to overcome pharmacokinetic deficiencies.

Experimental Section

Chemistry. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were determined on an Optical Activity AA 100 polarimeter. ^{1}H NMR spectra were recorded on a Bruker AM250 spectrometer with chemical shifts expressed in δ units (ppm) relative to tetramethylsilane. Mass spectra were recorded on a Bio Ion 20 time of flight (TOF) spectrometer. Elemental analyses were performed by the Structural Chemistry Department of Glaxo Group Research, Greenford. Thin-layer chromatography was conducted with E. Merck silica gel 60 F-254 plates. Column chromatography was performed using E. Merck silica gel 60 (230–400 mesh). Anhydrous dichloromethane and chloroform were dried over 4A molecular sieves.

[2*S*-[2α,5α,6β]]-*N*,*N*'-[1,2-Ethanediyl]bis[3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxamide] (21). Ethyl chloroformate (2.0 mL,

20.9 mmol) was added to a stirred solution of benzylpenicillin *N*-ethylpiperidine salt **20** (9.09 g, 20.1 mmol) in anhydrous $CHCl_3$ (120 mL) at −11 °C. The solution was stirred at this temperature for 2 h. Ethylenediamine (2.0 mL, 30.1 mmol) was added over 5 min, the temperature being kept below 0 °C, and a white precipitate was formed. The mixture was stirred for 2 h with the temperature being allowed to gradually reach 19 °C. The mixture was filtered and the filtrate successively washed with 0.5 N HCl and saturated aqueous $NaHCO_3$ and then dried and evaporated to a white foam (7.3 g). This was crystallized from acetonitrile to afford **21** (3.89 g, 27.9%) as white prisms: mp 201.5–202 °C; $[\alpha]_D$ +279° (*c* = 1.08, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.45 (s, 6H), 1.64 (s, 6H), 3.2–3.47 (m, 4H), 3.62 (AB, q, *J* = 18 Hz, 4H), 4.10 (s, 2H), 5.35 (d, *J* = 5 Hz, 2H), 5.72 (dd, *J* = 5, 9 Hz, 2H), 6.21 (d, *J* = 9 Hz, 2H), 7.10 (br s, 2H), 7.22–7.42 (m, 10H). Anal. ($C_{34}H_{40}N_6O_6S_2$) C, H, N, S. Concentration of the liquors provided a second crop of similar material (0.65 g, 4.7%), mp 195 °C.

[2*R*-[2α(*R),4β]]-4,4′-[1,2-Ethanediylbis[aminocarbonyl]]-bis[5,5-dimethyl-α-[(phenylacetyl)amino]-2-thiazolidineacetic acid, methyl ester] (1).** A suspension of **21** (1.04 g, 1.5 mmol) in MeOH (60 mL) was stirred at 21 °C. After *ca.* 45 min a clear solution was obtained and after a further 2.25 h the solution was evaporated to give a white foam, which was triturated with isopropyl ether (10 mL), to give **1** (1.01 g, 89.0%) as a white solid. A portion of this material (494 mg) was crystallized from EtOAc to afford white prisms (451 mg, 81.3%): mp 113–114 °C; $[\alpha]_D$ +93.5° (*c* = 1.07, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.25 (s, 6H), 1.49 (s, 6H), 3.17–3.47 (m, 8H), 3.63 (s, 4H), 3.7 (s, 6H), 4.69 (dd, *J* = 5, 9 Hz, 2H), 4.91 (d, *J* = 5 Hz, 2H), 6.35 (d, *J* = 9 Hz, 2H), 6.68 (br s, 2H), 7.22–7.44 (m, 10H). Anal. ($C_{36}H_{48}N_6O_8S_2 \cdot 0.5H_2O$) C, H, N, S.

[2*R*-[2α(*R),4β]]-4,4′-[1,2-Ethanediylbis[aminocarbonyl]]-bis[*N*-ethyl-5,5-dimethyl-α-[(phenylacetyl)amino]-2-thiazolidineacetamide] (3).** A solution of ethylamine (0.4 mL, 6.11 mmol) in CH_2Cl_2 (3.6 mL) was added to a stirred solution of **21** (2.0 g, 2.89 mmol) in CH_2Cl_2 (100 mL). The solution was stirred for 4.5 h and further ethylamine (0.4 mL, 6.1 mmol) in CH_2Cl_2 (3.6 mL) was added. The solution was stored at 0–5 °C for 2.5 days, during which time a white solid was deposited. The solid was collected by filtration, washed with cold CH_2Cl_2, dried (2.1 g), and crystallized from acetonitrile to give **3** (1.57 g, 69.4%): mp 186.5–187.5 °C; $[\alpha]_D$ +45.5° (c = 1.0, MeOH); ^1H NMR (d_6-DMSO) δ 0.98 (t, *J* = 7.5 Hz, 6H), 1.14 (s, 6H), 1.50 (s, 6H), 2.98–3.25 (m, 8H), 3.41 (d, *J* = 12 Hz, 2H), 3.51 (collapsed AB q, *J* = 16 Hz, 4H), 3.79 (dd, *J* = 8, 12 Hz, 2H), 4.32 (t, *J* = 8 Hz, 2H), 4.82 (t, *J* = 8 Hz, 2H), 7.13–7.32 (m, 10H), 7.96 (m, 4H), 8.28 (d, *J* = 8 Hz, 2H). Anal. ($C_{28}H_{54}N_8O_8S_2 \cdot 1.5H_2O$) C, H, N, S.

[2*R*-[2α(*R),4β]]-*N*,*N*′-[1,2-Ethanediyl]bis[2-[2-hydroxy-1-[(phenylacetyl)amino]ethyl]-5,5-dimethyl-4-thiazolidinecarboxamide] (17).** Sodium borohydride (415 mg, 11 mmol) was added to a stirred solution of **1** (1.01 g, 1.33 mmol) in dioxane (150 mL)–H_2O (100 mL). The mixture was stirred for 7 h and the pH adjusted to 6 with glacial HOAc. The solution was extracted with EtOAc, and the extracts were washed with brine, dried, and evaporated to give a white powder (775 mg). This was chromatographed on a column of silica gel (100 g) using $CHCl_3$–MeOH (10:1) to give **17** (230 mg, 24.7%) as a colorless glass. A portion was crystallized from acetonitrile as white prisms: mp 111–112 °C; $[\alpha]_D$ +93° (*c* = 0.97, MeOH); ^1H NMR (d_6-DMSO) 1.17 (s, 6H), 1.49 (s, 6H), 3.18 (m, 4H), 3.3–3.6 (m, 10H), 3.7–3.85 (m, 4H), 4.75 (m, 4H), 7.18–7.35 (m, 10H), 7.85 (d, *J* = 8 Hz, 2H), 8.03 (br s, 2H). Anal. ($C_{34}H_{48}N_6O_6S_2 \cdot 0.5H_2O$) C, H, N, S.

[2*R*-[2α(*R),4β]]-*N*,*N*′-[1,2-Ethanediyl]bis[2-(benzoyloxy)-5,5-dimethyl-1-[[(phenylacetyl)amino]ethyl]-4-thiazolidinecarboxamide] (18).** Benzoyl chloride (0.2 mL, 1.7 mmol) was added to a stirred solution of **17** (502 mg, 0.71 mmol) in pyridine (5 mL) at 0–5 °C under a nitrogen atmosphere. The reaction was allowed to warm to room temperature over 2 h and then diluted with water and extracted with EtOAc. The extracts were washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine and then dried and evaporated to give a white foam (480 mg). This was chromatographed on a column of silica gel (50 g) using EtOAc–Me_2CO (19:1) to give a foam which was stirred with Et_2O to give **18** (205 mg, 31.8%) as white prisms: mp 110–114 °C; $[\alpha]_D$ +66.3° (*c* = 1.01, MeOH); ^1H NMR (d_6-DMSO) δ 1.18 (s, 6H), 1.52 (s, 6H), 3.05–3.3 (m, 4H), 3.37–3.54 (m, 6H), 3.96 (dd, *J* = 8, 11 Hz, 2H), 4.1–4.42 (m, 6H), 4.85 (t, *J* = 8 Hz, 2H), 7.13–7.28 (m, 10H), 7.50 (t, *J* = 7 Hz, 4H), 7.66 (t, *J* = 7 Hz, 2H), 7.90 (d, *J* = 7 Hz, 4H), 8.10 (br s, 2H), 8.23 (d, *J* = 8 Hz, 2H). Anal. ($C_{48}H_{56}N_6O_8S_2 \cdot H_2O$) C, H, N, S.

[2*R*-*trans*]-*N*,*N*′-[1,2-Ethanediyl]bis[5,5-dimethyl-2-[[(phenylacetyl)amino]methyl]-4-thiazolidinecarboxamide] (19). 1-Hydroxybenzotriazole hydrate (500 mg, 3.26 mmol), ethylenediamine (0.10 mL, 1.5 mmol), and 1,3-dicyclohexylcarbodiimide (700 mg, 3.39 mmol) were successively added to a solution of [2*R*-[2α,4β]]-5,5-dimethyl-2-[[(phenylacetyl)amino]methyl]-4-thiazolidinecarboxylic acid (**22**)[10] (925 mg, 3.0 mmol). The mixture was stirred for 2 h and then filtered and the filtrate evaporated to dryness. The residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic portion was washed with water and then dried and evaporated. The residue was chromatographed on a column of silica gel (50 g) using EtOAc–MeOH (9:1) to give a white foam which was triturated with ether to give **19** (345 mg, 35.9%) as an amorphous white powder: $[\alpha]_D$ +57.6° (c = 0.99, Me_2SO); ^1H NMR (d_6-DMSO) δ 1.14 (s, 6H), 1.50 (s, 6H), 3.0–3.28 (m, 8H), 3.41 (s, 6H), 3.94 (dd, *J* = 9, 12 Hz, 2H), 4.64 (dt, *J* = 12, 6 Hz, 2H), 7.14 (m, 10H), 8.10 (br s, 2H), 8.25 (br t, 2H). Anal. ($C_{32}H_{44}N_6O_4S_2 \cdot 1.5H_2O$) C, H, N, S.

[2*R*-[2α(*R),4β]]-2-[2-(Ethylamino)-2-oxo-1-[(phenylacetyl)amino]ethyl]-5,5-dimethyl-4-thiazolidinecarboxylic Acid (23).** Anhydrous ethylamine (4 mL, 61.1 mmol) was added slowly to an ice-cold solution of **20** (6.5 g, 14.5 mmol) in CH_2Cl_2 (150 mL). The solution was stirred at 21 °C for 15 h. The resulting suspension was cooled and the solid was filtered off and washed with CH_2Cl_2 (30 mL). The solid was suspended in a stirred mixture of water (50 mL) and CH_2Cl_2 (50 mL) and orthophosphoric acid was added to pH 3. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (50 mL). The combined extracts were washed sequentially with water and saturated brine to give **23** (3.80 g, 69.2%) as an amorphous white solid: $[\alpha]_D$ +90° (*c* = 0.84, MeOH); ^1H NMR (d_6-DMSO) δ 0.98 (t, *J* = 7.5 Hz, 3H), 1.18 (s, 3H), 3.35 (br s, 1H), 3.53 (q, *J* = 18 Hz, 2H), 3.56 (s, 1H), 4.37 (t, *J* = 8 Hz, 1H), 4.84 (d, *J* = 8 Hz, 1H), 7.14–7.52 (m, 5H), 8.01 (t, *J* = 5 Hz, 1H), 8.24 (d, *J* = 8 Hz, 1H). This material was used in the next step, the preparation of **24**, without further purification.

[2*R*-[2α(*R),4β]2′*R*-[2′β,4′α]]-*N*-Ethyl-5,5-dimethyl-4-[[[2-[[[5,5-dimethyl-2-[[(phenylacetyl)amino]methyl]-4-thiazolidinyl]carbonyl]amino]ethyl]amino]carbonyl]-α-[(phenylacetyl)amino]-2-thiazolidineacetamide (24).** 1-Hydroxybenzotriazole hydrate (1.23 g, 8.0 mmol), *N*,*N*′-dicyclohexylcarbodiimide (1.80 g, 8.75 mmol), and ethylenediamine (0.27 mL) were successively added to a stired solution of **23** (1.52 g, 4.0 mmol) and **22**[10] (1.23 g, 4.0 mmol) in anhydrous THF (30 mL). The solution was stirred for 1.5 h, during which time a solid precipitated. HOAc (2 drops) was added and the mixture filtered with the aid of THF (10 mL). The filtrate was diluted with EtOAc (200 mL) and sequentially washed with saturated aqueous $NaHCO_3$, water, and a brine solution, then dried, and evaporated. The residue (2.4 g) was chromatographed on a column of silica gel (300 g) using 5–10% EtOH in EtOAc to give **24** (437 mg, 15.3%), which crystallized from acetonitrile as white prisms (292 mg): mp 121–122 °C; $[\alpha]_D$ +78° (c = 1.06, MeOH); ^1H NMR (d_6-DMSO) δ 0.96 (t, *J* = 7.5 Hz, 3H), 1.13 (s, 6H), 1.48 (s, 3H), 1.51 (s, 3H), 2.9–3.3 (m, 8H), 3.42 (m, 4H), 3.51 (s, 2H), 3.78 (dd, *J* = 5, 12 Hz, 1H), 3.94 (dd, *J* = 5, 12 Hz, 1H), 4.31 (t, *J* = 8 Hz, 1H), 4.64 (q, *J* = 13 Hz, 1H), 4.82 (t, *J* = 8 Hz, 1H), 7.15–7.34 (m, 10H), 7.97 (m, 2H), 8.08 (br s, 1H), 8.25 (t, *J* = 5 Hz, 1H), 8.28 (d, *J* = 8 Hz, 1H). Anal. ($C_{35}H_{49}N_7O_5S_2 \cdot 0.5H_2O$) C, H, N, S.

[2*R*-[2α(*R),4β(*S**)]]-*N*-Ethyl-5,5-dimethyl-4-[[[2-[[(5,5-dimethyl-4-thiazolidinyl)carbonyl]amino]ethyl]amino]carbonyl]-α-[(phenylacetyl)amino]-2-thiazolidineacetamide (26).** *N*-Ethylpiperidine (0.27 mL, 2.0 mmol) and [*S*]-5,5-dimethyl-3,4-thiazolidinedicarboxylic acid, 3-(1,1-dimethylethyl) ester (522 mg, 2.0 mmol), were added to a stirred solution of **20** (895 mg, 2.0 mmol) in anhydrous CH_2Cl_2 (25 mL) under a nitrogen atmosphere. The solution was cooled to −10 °C and ethyl chloroformate (0.38 mL, 4.0 mmol) was added. The solution was stirred for 2 h and then ethylenediamine (0.14 mL, 2.1 mmol) was added. The solution was allowed to warm to room temperature over 1.25 h and then washed sequentially with water, 0.5 N HCl, saturated aqueous $NaHCO_3$, and brine. The dried

solution was evaporated and the residue **25** (363 mg) was dissolved in anhydrous CH_2Cl_2 (10 mL), and ethylamine (0.4 mL, 6.0 mmol) was added. The solution was set aside for 3 days and then evaporated. The residue (435 mg) was chromatographed on a column of silica gel (40 g) using 0–5% EtOH in EtOAc to give an amorphous white solid (98 mg). The bulk of this (88 mg) was dissolved in anhydrous CH_2Cl_2 (2 mL), and trifluoroacetic acid (1 mL) was added. The solution was left for 15 min and then partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic portion was dried and evaporated and the residue (60 mg) was chromatographed on a column of silica gel (10 g) using 5–10% EtOH in EtOAc to give **26** (21 mg, 1.7%) as an amorphous white powder: $[\alpha]_D$ +80° (c = 0.38, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.13 (t, J = 7.5 Hz, 3H), 1.25 (s, 3H), 1.30 (s, 3H), 1.55 (s, 3H), 1.67 (s, 3H), 1.75 (br m, 1H), 3.15–3.5 (m, 9H), 3.60 (s, 2H), 4.22 (dd, J = 8, 18 Hz, 2H), 4.45 (t, J = 7.5 Hz, 1H), 4.83 (d, J = 7.5 Hz, 1H), 6.46 (t, J = 6 Hz, 1H), 6.70 (d, J = 8 Hz, 1H), 6.78 (t, J = 5 Hz, 1H), 7.18 (t, J = 5 Hz, 1H), 7.23–7.4 (m, 5H); MS (TOF) m/z 565.8 (MH^+) (calcd for $C_{26}H_{41}N_5O_4S_2$ 565.76). This material was 98.2% pure by analytical HPLC (S5-ODS-2 column eluted with 30% acetonitrile in H_2O).

[2*S*-[2α(*R),4β]]-4,4′-[1,2-Ethanediylbis[aminocarbonyl]]-bis[*N*-ethyl-5,5-dimethyl-α-[(phenylacetyl)amino]-2-thiazolidineacetamide] (27).** A solution of **3** (500 mg) in trifluoroacetic acid (3.5 mL) was stirred for 100 min and then evaporated *in vacuo*. The residue was dissolved in MeOH, neutralized with solid $NaHCO_3$, and filtered. The filtrate was evaporated and the residual gum treated with acetonitrile to give a white solid (100 mg), which was subjected to purification by preparative HPLC (ODS-2 column, 5 μm, 21 × 250 mm; flow rate, 12 mL/min using 40% acetonitrile in water). The component with the shortest retention time had identical HPLC mobility to starting material **3** and was not pursued. The component with the longest retention time gave upon evaporation a white solid which was crystallized from acetonitrile to give **27** (20 mg) as white prisms: mp 220–223 °C; ^{1}H NMR (d_6-DMSO) δ 1.01 (t, J = 7.5 Hz, 6H), 1.18 (s, 6H), 1.47 (s, 6H), 2.99–3.26 (m, 8H), 3.26 (d, J = 12.5 Hz, 2H), 3.52 (s, 4H), 3.64 (dd, J = 12.5, 12.5 Hz, 2H), 4.62 (dd, J = 7.5, 8 Hz, 2H), 4.74 (dd, J = 7.5, 12.5 Hz, 2H), 7.15–7.33 (m, 10H), 8.12 (br s, 2H), 8.19 (t, J = 6 Hz, 2H), 8.29 (d, J = 8 Hz, 2H). Anal. ($C_{38}H_{54}N_8O_6S_2 \cdot H_2O$) C, H, N.

[2*R*-[2α(*S),4β]]-4,4′-[1,2-Ethanediylbis[aminocarbonyl]]-bis[*N*-ethyl-5,5-dimethyl-α-[(phenylacetyl)amino]-2-thiazolidineacetamide] (28).** Using the procedure described for the preparation of **21**, a *ca.* 1:1 mixture of benzylpenicillin and 6-epibenzylpenicillin[11] (1.35 g, 4.0 mmol) was converted to the corresponding mixture of β-lactam dimer epimers as a yellow solid (1.06g). A portion of this material (0.30 g, 0.43 mmol) was treated with ethylamine as detailed in the preparation of **3** to give a white solid (307 mg). This was purified by preparative HPLC (ODS-2 column, 5 μm, 21 × 250 mm; flow rate, 12 mL/min using 40% acetonitrile in water). The component with the shortest retention time (91 mg) was shown to be a mixture of **21** and partially epimerized material. The component with the longest retention time (60 mg) was crystallized from acetonitrile to afford **28** as white prisms: mp 137–140 °C; $[\alpha]_D$ +107° (c = 1.00, Me_2SO); ^{1}H NMR (d_6-DMSO) δ 0.97 (t, J = 7.5 Hz, 6H), 1.13 (s, 6H), 1.44 (s, 6H), 2.95–3.26 (m, 6H), 3.4–3.55 (m, 6H), 3.88 (dd, J = 8, 12.5 Hz, 2H), 4.30 (t, J = 8 Hz, 2H), 4.88 (t, J = 8 Hz, 2H), 7.15–7.33 (m, 10H), 7.90 (m, 4H), 8.18 (d, J = 8 Hz). Anal. ($C_{38}H_{54}N_8O_6S_2 \cdot 2.0H_2O$) C, H, N, S.

Method A: [2*S*-[2α,5α,6β]]-*N*,*N*′-[1,2-Ethanediyl]bis[3,3-dimethyl-6-[((5-methyl-3-phenyl-4-isoxazolyl)carbonyl)-amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamide][32; R^1 = (5-methyl-3-phenyl-4-oxazolyl)carbonyl]. A solution of oxacillin sodium salt (1.0 g, 2.36 mmol) in water was acidified with 2 N HCl in the presence of EtOAc. The layers were separated, and the aqueous portion was extracted twice with EtOAc. The combined extracts were dried and evaporated to give a white foam. This was dissolved in dry CH_2Cl_2 (100 mL) and cooled to −10 °C. *N*-Ethylpiperidine (0.37 mL, 2.69 mmol) and ethyl chloroformate (0.23 mL, 2.40 mmol) were added, and the mixture was stirred at −10 °C for 0.5 h. Ethylenediamine (0.24 mL, 3.59 mmol) was added and the mixture stirred for 2.5 h at +21 °C and was then filtered. The filtrate was washed with 0.5 N HCl and saturated aqueous $NaHCO_3$ and then dried and evaporated to give a foam. This was purified by chromatography on a column of silica gel (50 g) using EtOAc as eluant to give a solid which was treated with Et_2O to give the title compound (480 mg, 49.2%) as a white solid: mp 153 °C dec; $[\alpha]_D$ +231° (c = 0.61, Me_2SO); ^{1}H NMR (d_6-DMSO) δ 1.42 (s, 6H), 1.55 (s, 6H), 2.57 (s, 6H), 3.08–3.28 (m, 4H), 4.19 (s, 2H), 5.52–5.63 (m, 4H), 7.43–7.57 (m, 6H), 7.6–7.72 (m, 4H), 8.29 (br s, 2H), 9.28 (d, J = 6 Hz, 2H). Anal. ($C_{40}H_{42}N_8O_8S_2 \cdot 0.5H_2O$) C, H, N, S.

[2*R*-[2α(*R),4β]]-4,4′-[1,2-Ethanediylbis[aminocarbonyl]]-bis[*N*-ethyl-5,5-dimethyl-α-[[(5-methyl-3-phenyl-4-isoxazolyl)carbonyl]amino]-2-thiazolidineacetamide] (43).** Ethylamine (0.13 mL, 1.99 mmol) was added to a solution of **32** [R^1 = (5-methyl-3-phenyl-4-oxazolyl)carbonyl] in CH_2Cl_2 (10 mL). The solution was stored at 21 °C for 16 h and then evaporated to dryness. The residue was crystallized from acetonitrile to furnish **43** (148 mg, 67.3%) as white prisms: mp 173–173.5 °C; $[\alpha]_D$ +32° (c = 0.73, Me_2SO); ^{1}H NMR (d_6-DMSO) δ 1.02 (t, J = 7.5 Hz, 6H), 1.14 (s, 6H), 1.52 (s, 6H), 2.50 (s, 6H + DMSO), 2.94–3.23 (m, 8H), 3.50 (d, J = 12.5 Hz, 2H), 3.97 (dd, J = 8, 12.5 Hz, 2H), 4.54 (t, J = 8 Hz, 2H), 4.88 (t, J = 8 Hz, 2H), 7.32–7.5 (m, 6H), 7.69–7.83 (m, 4H), 8.04–8.26 (m, 4H), 8.82 (d, J = 8 Hz, 2H). Anal. ($C_{44}H_{56}N_{10}O_8S_2 \cdot H_2O$) C, H, N, S.

Method B: [2*S*-[2α,5α,6β]]-*N*,*N*′-[1,2-Ethanediyl]bis[3,3-dimethyl-7-oxo-6-[[(phenylmethoxy)carbonyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamide] (32; R^1 = PhCH$_2$OCO). *N*-Ethylpiperidine (6.2 mL, 46 mmol) was added to a solution of [2*S*-[2α,5α,6β]]-3,3-dimethyl-7-oxo-6-[[(phenylmethoxy)carbonyl]amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid[12] (16.2 g, 46.0 mmol) in dry CH_2Cl_2 (250 mL). The solution was cooled to −10 °C, ethyl chloroformate (4.4 mL, 46.0 mmol) was added, and the solution was stirred at this temperature for 2h. Ethylenediamine (4.7 mL, 69 mmol) was added and the solution was allowed to warm to room temperature over 3 h and then successively washed with 0.5 N HCl, brine solution, and saturated aqueous $NaHCO_3$. The dried solution was evaporated to dryness and the residue chromatographed on silica gel (250 g) eluting with EtOAc followed by EtOAc–Me_2CO (4:1). Appropriate fractions were combined to give **32** (R^1 = $PhCH_2OCO$) (5.1 g, 30.6%) as a white solid. A portion of this material was crystallized from acetonitrile to give white crystals: mp 183 °C; ^{1}H NMR (d_6-DMSO) 1.54 (s, 6H), 1.73 (s, 6H), 3.2–3.41 (m, 4H), 4.25 (s, 2H), 5.22 (collapsed AB q, 4H), 5.16 (dd, J = 4.5, 8 Hz, 2H), 5.58 (d, J = 4.5 Hz, 2H), 7.50 (br s, 10H), 8.3–8.46 (m, 4H). Anal. ($C_{34}H_{40}N_6O_8S_2$) C, H, N, S.

[2*R*-[2α(*R),4β]]-4,4′-[1,2-Ethanediylbis[aminocarbonyl]]-bis[α-amino-5,5-dimethyl-*N*-(phenylmethyl)-2-thiazolidineacetamide] (34; R^2 = PhCH$_2$).** Benzylamine (1.2 mL, 10.8 mmol) was added to a stirred solution of **32** (R^1 = $PhCH_2OCO$) (1.3 g, 1.8 mmol) in CH_2Cl_2 (50 mL) and the solution left for 3 days. The solution was successively washed with 0.5 N HCl, saturated aqueous $NaHCO_3$, and brine solution and then dried and evaporated to give a white solid which crystallized from acetonitrile as white prisms (341 mg): mp 115 °C; $[\alpha]_D$ +25 °C (c = 1.1, MeOH). Dilution of the liquors with ether provided a second crop of similar material (414 mg). The bulk of this material (684 mg) was dissolved in CH_2Cl_2 (300 mL) and a 45% solution of HBr in HOAc (10 mL) was added with stirring. The mixture was stirred for 1.5 h then extracted with water (300 mL) and 0.5 N HCl (160 mL). The combined aqueous extracts were basified and extracted with CH_2Cl_2. The dried organic extracts were evaporated to give **34** (R^2 = $PhCH_2$) (405 mg, 37.3%) as an off-white solid which crystallized from acetonitrile to afford white prisms (114 mg, 10.5%): mp 179–180 °C, $[\alpha]_D$ +22.5° (c = 1.0, MeOH); ^{1}H NMR (d_6-DMSO) δ 1.16 (s, 6H), 1.48 (2, 6H), 1.96 (br s, 4H), 3.07–3.26 (m, 6H), 3.37 (d (obscured by H_2O), 2H), 3.94 (dd, J = 7.5, 12.5 Hz, 2H), 4.28 (m, 4H), 4.63 (t, J = 8 Hz, 2H), 7.16–7.36 (m, 10H), 8.04 (br s, 2H), 8.43 (t, J = 6 Hz, 2H); MS (TOF) m/z 671.9 (MH^+) (calcd for $C_{32}H_{47}N_8O_4S_2$ 671.89). This material was 97.2% pure by analytical HPLC (S5-ODS-2 column eluted with 40% acetonitrile in 0.05 M $(NH_4)H_2PO_4$ at pH 2.3).

[2*R*-[2α(*R),4β]]-4,4′-[1,2-Ethanediylbis[aminocarbonyl]]-bis[5,5-dimethyl-α-[[[(1,1′-biphenyl)-2-yl]carbonyl]amino]-*N*-(phenylmethyl)-2-thiazolidineacetamide] (52).** 2-Phenylbenzoic acid (233 mg, 1.17 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (257 mg, 1.33 mmol) were added to a stirred solution of **34** (R^2 = $PhCH_2$) (360 mg, 0.5 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred for

19 h when additional 2-phenylbenzoic acid (105 mg, 0.53 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (205 mg, 1.06 mmol) were added. The mixture was stirred for a further 7 h and then diluted with CH_2Cl_2 and washed with water and aqueous $NaHCO_3$. The aqueous washings were back-extracted with CH_2Cl_2 and the combined organic extracts were then dried and evaporated. The residue was chromatographed on silica gel (50 g) using EtOAc followed by EtOAc–Me_2CO (4:1) to give **52** (116 mg, 22.5%) as a white solid. A portion of this material was crystallized from acetonitrile to afford white prisms: mp 180–181 °C; $[\alpha]_D$ +75° (c = 1.0, MeOH); ^{1}H NMR (d_6-DMSO) δ 1.17 (s, 6H), 1.53 (s, 6H), 3.1–3.4 (m, 4H), 3.45 (s, 2H), 3.87 (m, 2H), 4.19–4.42 (m, 4H), 4.48 (t, J = 8 Hz, 2H), 4.97 (t, J = 8 Hz, 2H), 7.15–7.6 (m, 28H), 8.18 (broad s, 2H), 8.41 (t, J = 6 Hz, 2H), 8.70 (d, J = 8 Hz, 2H). Anal. ($C_{58}H_{62}N_8O_6S_2 \cdot H_2O$) C, H, N, S.

Method C: [2*S*-[2α,5α,6β]]-*N*,*N*'-[1,2-Ethanediyl]bis[3,3-dimethyl-7-oxo-6-[(triphenylmethyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamide] (32; R^1 = Ph_3C). 1-Hydroxybenzotriazole hydrate (1.82 g, 13.5 mmol), ethylenediamine (0.36 mL, 5.4 mmol), and *N*,*N*-dicyclohexylcarbodiimide (2.54 g, 12.3 mmol) were added in turn to a stirred solution of *N*-trityl-6-aminopenicillanic acid[13] (5.0 g, 10.9 mmol) in EtOAc (100 mL). The mixture was stirred for 3 h and then filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ and brine and then dried and evaporated to give a yellow foam (5.2 g). This was purified by chromatography on a column of silica gel (200 g) using EtOAc–light petroleum (bp 40–60 °C) (2:1) to give **32** (R^1 = Ph_3C) (2.4 g, 23.4%) as a white foam. This was stirred with ether to give an amorphous white solid (1.3 g, 12.7%): $[\alpha]_D$ +115° (c = 1.01, Me_2SO); ^{1}H NMR (d_6-DMSO) δ 1.18 (s, 6H), 1.44 (s, 6H), 2.9–3.1 (m, 4H), 3.20 (d, J = 11 Hz, 2H), 4.12 (s, 2H), 4.33 (d, J = 4 Hz, 2H), 4.38 (dd, J = 4, 11 Hz, 2H), 7.17–7.50 (m, 30H), 8.08 (broad s, 2H). Anal. ($C_{56}H_{56}N_6O_4S_2 \cdot H_2O$) C, H, N, S.

[2*S*-[2α,5α,6β]]-*N*,*N*'-[1,2-Ethanediyl]bis[6-amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamide], 4-Methylbenzenesulfonic Acid Salt (33). Toluenesulfonic acid monohydrate (2.40 g, 1.3 mmol) was added to a stirred solution of **32** (R^1 = Ph_3C) (6.065 g, 6.4 mmol) in Me_2CO (600 mL). The mixture was stirred for 3.5 h during which time a solid was precipitated. This was collected and washed with ether to give **33** (4.12 g, 79.9%) as a pale yellow amorphous solid: $[\alpha]_D$ +135° (c = 1.07, MeOH); ^{1}H NMR (d_6-DMSO) δ 1.42 (s, 6H), 1.60 (s, 6H), 2.30 (s, 6H), 3.17 (m, 4H), 4.30 (s, 2H), 5.04 (d, J = 4 Hz, 2H), 5.50 (d, J = 4 Hz, 2H), 7.13 (d, J = 8 Hz, 4H), 7.48 (d, J = 8 Hz, 4H), 8.39 (m, 2H), 8.86 (m, 6H). Anal. ($C_{32}H_{44}N_6O_{10}S_4 \cdot 2.0H_2O$) C, H, N, S.

[2*R*-[2α(*R),4β]]-4,4'-[1,2-Ethanediyl]bis[aminocarbonyl]-bis[*N*-ethyl-5,5-dimethyl-α-[(2-thienylacetyl)amino]-2-thiazolidineacetamide] (39).** A solution of **33** (2.0 g, 2.5 mmol) in water (20 mL) was basified with saturated aqueous $NaHCO_3$. The solution was washed with EtOAc and then the aqueous portion was saturated with ammonium sulfate and extracted with $CHCl_3$–EtOH (19:1). The extracts were dried and evaporated to give a white solid (865 mg). A solution of this material (500 mg) in water (15 mL) was treated with a solution of 2-thiopheneacetic acid (330 mg, 2.34 mmol) in dioxane (15 mL) followed by 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (430 mg, 3.21 mmol). The resulting solution was stirred for 20 h and then extracted with EtOAc. The extracts were washed with water, saturated aqueous $NaHCO_3$, and brine then dried and evaporated to give a white solid (690 mg): $[\alpha]_D$ +294° (c = 0.6, Me_2SO). A solution of this material (150 mg) in CH_2Cl_2 (8 mL) was treated with 10% ethylamine in CH_2Cl_2 (0.75 mL, 1.15 mmol). The solution was stirred for 8 h and then evaporated to give a solid, which was crystallized from acetonitrile–light petroleum (bp 40–60 °C) to give **39** (87 mg, 34.8%) as white prisms: mp 159–161 °C; $[\alpha]_D$ +82.0° (c = 0.6, Me_2SO); ^{1}H NMR (d_6-DMSO) δ 1.03 (t, J = 7.5 Hz, 6H), 1.16 (s, 6H), 1.51 (s, 6H), 3.08 (m, 4H), 3.19 (m, 4H), 3.44 (d, J = 10 Hz, 2H), 3.76 (m, 6H), 4.33 (t, J = 8 Hz, 2H), 4.84 (t, J = 8 Hz), 6.94 (m, 4H), 7.36 (m, 4H), 7.99 (m, 4H), 8.30 (d, J = 8 Hz, 2H). Anal. ($C_{34}H_{50}N_8O_6S_4 \cdot 0.5H_2O$) C, H, N, S.

HIV-1 Proteinase Inhibition Assay. The K_i value for **3** and IC_{50} values for the compounds of Tables I-III were determined using purified recominant HIV-1 proteinase.[22] IC_{50} values were obtained by assaying the enzyme against the synthetic substrate peptide KQGTVSFNFPQIT tritiated at the proline residue (Cambridge Research Biochemicals). The peptide was coupled *via* its N-terminal lysine to an Affi-gel 10/15 bead mixture (Bio-Rad) and the immobilized peptide (1 part) was stored in the assay buffer: 20 nM MES (pH 6.0), 2 M NaCl, 5 mM DTT, and 2 mM EDTA (2 parts) at −20 °C until required. The assay was performed in 96-well microtiter filtration plates (Pall, silent monitor) using 10 μL of a solution of the test compound dissolved at a range of concentrations in 25% Me_2SO in water, 50 μL of HIV-1 proteinase (0.2 μg/mL), and 50 μL of radiolabeled bead suspension [diluted with assay buffer (40 parts)] per well. The plates were incubated at 37 °C for 60 min on a flat-bed shaker (80 rev/min) and then filtered into the wells of a collection plate on a vacuum manifold (Pall). A 10-μL sample of each filtrate was transferred to a 96-well scintillation plate (Wallac) and mixed with 50 μL of Hi-Load Scintillant (Wallac). Radioactivity was measured using an LKB 1205 Microbeta liquid scintillation counter (Wallac). IC_{50} determinations were performed in duplicate at each concentration with mean values used for data analysis. Results were standardized relative to the initial IC_{50} value obtained for compound **3** which was thereafter used as a control.

The K_i value for compound **3** was determined by HPLC assay using the same peptide substrate and buffer conditions described above. Chromatography was performed using an isocratic reverse-phase system of 26% acetonitrile in water containing 0.1% trifluoroacetic acid at 2.5 mL/min on a 3-μm ODS-2 (Spherisorb) column (4.6 × 50 mm).

Inhibition of HIV-1 in Cell Culture. A. Inhibition of Formazan Conversion Assay.[16] MT-4 cells at 10^6 cells/mL in RPMI 1640 growth medium with HIV-1 (strain RF) at a moi of 2×10^{-3} infectious units/cell were used. Test compounds were dissolved in Me_2SO and serially diluted with RPMI 1640 growth medium in 10-fold steps from 100 to 0.01 μg/mL in 96-well microtiter plates. A 20-μL portion of infected or mock-infected cell suspension was added to each well (5×10^4 cells/well), and the plates were incubated at 37 °C in a 5% CO_2 atmosphere for 7 days in humidified containers. After incubation, 10 μL of MTT (7.5 mg/mL) was added, and the plates were incubated at 37 °C for a further 60 min. Acidified 2-propanol (150 μL) was added to each well and the absorbance was measured at 540 nm using a Multiskan MC plate reader. Conversion of yellow MTT to its blue-black formazan derivative was maximal in uninfected cells and absent in untreated infected cells. The effective concentration (EC_{50}) required to inhibit the conversion of MTT by 50% was determined from the average of duplicate assays. Compounds were assayed for toxicity at identical concentrations.

B. **Inhibition of Syncytium Formation Assay.** C8166 cells were infected with HIV-1 (strain RF) at a moi of 1 × 10 10^{-3} infectious units/cell. Aliquots of 10^5 cells were added to each well of 24-well plates containing the test compounds dissolved in Me_2SO and serially diluted with RPMI 1640 growth medium from 50 to 0.05 μg/mL. Untreated infected cells and untreated uninfected cells were included as controls. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 3–4 days in humidified containers. The cells were examined daily for evidence of HIV-I-induced syncytium formation. The syncytia were quantified by reference to the untreated infected controls and the dose of compound required to reduce the cytopathic effect by 50% (EC_{50}) was calculated.

Compound **4** was assayed against HIV-2 (Rod) in CEM cells using an identical procedure.

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