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Discovery of Ritonavir, a Potent Inhibitor of HIV Protease with High Oral Bioavailability and Clinical Efficacy

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The structure−activity studies leading to the potent and clinically efficacious HIV protease inhibitor ritonavir are described. Beginning with the moderately potent and orally bioavailable inhibitor A-80987, systematic investigation of peripheral (P3 and P2′) heterocyclic groups designed to decrease the rate of hepatic metabolism provided analogues with improved pharmacokinetic properties after oral dosing in rats. Replacement of pyridyl groups with thiazoles provided increased chemical stability toward oxidation while maintaining sufficient aqueous solubility for oral absorption. Optimization of hydrophobic interactions with the HIV protease active site produced ritonavir, with excellent in vitro potency (EC_{50} = 0.02 μM) and high and sustained plasma concentrations after oral administration in four species. Details of the discovery and preclinical development of ritonavir are described.

Introduction

The human immunodeficiency virus encodes an aspartic proteinase (HIV protease) which is responsible for the posttranslational proteolytic processing of the *gag* and *gag-pol* polyprotein gene products into mature, functional proteins.[1−3] Accompanying these processing events, the nascent viral particles undergo a morphological transformation from an immature, noninfectious form into mature, infectious virions.[4] The critical role of functional HIV protease in the HIV replication cycle was initially demonstrated by site-directed mutagenesis wherein mutation of the catalytic aspartate residues to either asparagine[5] or alanine[6] generated viral particles which remained in the immature form. Importantly, these particles were incapable of establishing a new round of infection in susceptible T-lymphocytes.[5] Similar behavior was subsequently observed through the blockade of HIV protease by small-molecule inhibitors, thus validating HIV protease as a target for drug design for acquired immune deficiency syndrome.[7−10]

The classification of HIV protease and other retroviral proteases as members of the aspartic proteinase class was proposed on the basis of sequence alignment[11] and confirmed by X-ray crystallographic studies of both engineered[12] and synthetic[13] protein. The retroviral proteases (including HIV protease) are unique within this class in that functional protease exists as a C_2-symmetric homodimer with a single active site. Each monomeric unit contributes one of the conserved catalytic triads (Asp-Thr-Gly) common to the aspartic proteinase class. The wide availability of three-dimensional structures of complexes of HIV protease with bound inhibitors has prompted the use of this information for structure-based design of novel inhibitors.[14−17] Nonetheless, the majority of HIV protease inhibitor classes are peptidomimetics, reflecting the structure of the polyprotein substrates of HIV protease. As a result, the identification of agents with high potency and specificity for HIV protease with the proper physicochemical and pharmacodynamic properties for use as oral agents has proven challenging due to the low intestinal absorption and rapid hepatic clearance observed with most peptidomimetics.[18] Recently, substantial progress in the identification of agents with high oral bioavailability has been made.[19−22]

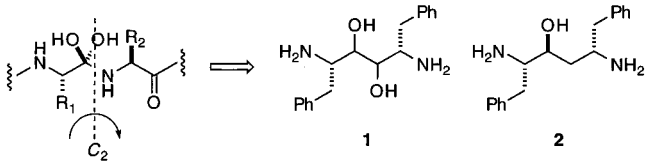

Figure 1. Design of symmetry-based inhibitors of HIV protease.

We recently reported the design of novel inhibitors of HIV protease based on the C_2-symmetric structure of HIV protease. Beginning with the tetrahedral intermediate for cleavage of an asymmetric dipeptide substrate, duplication of the N-terminus by a rotation about a C_2 axis bisecting the carbon−nitrogen single bond led to the conceptualization of the symmetric or pseudosymmetric core diamines **1** and **2** (Figure 1).[23,24] Structure−activity studies on derivatives of **1** and **2** led initially to the identification of A-77003,[24,25] which, though displaying low oral bioavailability, possessed adequate anti-HIV activity and aqueous solubility for intravenous examination in HIV patients.[26] Further studies of the influence of structural features on the oral bioavailability of this series produced the truncated inhibitor A-80987,[27] which demonstrated good oral bioavailability in both animal models and in humans.[19] Although A-80987 represented a substantial improvement in oral absorption over A-77003, the short circulating half-life of both inhibitors mirrored that of most peptidomimetics. Examination of the metabolic fate of A-77003 and A-80987 in vitro and in vivo revealed that

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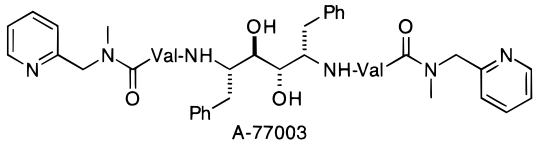
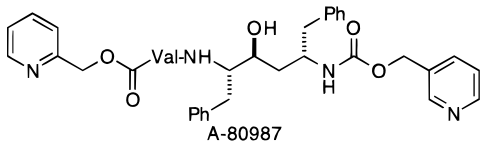
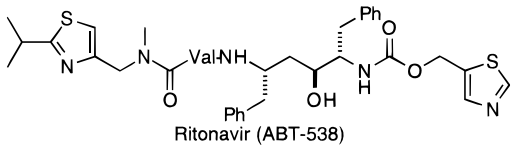
each inhibitor was primarily metabolized and cleared through N-oxidation of one or both of the pyridyl end groups.[19] Recently we communicated the identification of ritonavir (ABT-538), a symmetry-based HIV protease inhibitor with a substantially reduced rate of metabolism and high oral bioavailability in animals and humans.[19] Phase I/II clinical trials with ABT-538 produced a rapid[28,29] and profound reduction of circulating viral RNA concomitant with a large increase in CD4 cell levels.[30,31] Phase III trials also provided a significant increase in time to AIDS-defining event or death[32] and provided the basis for rapid licensing of ritonavir for treatment of HIV infection. Here we describe the details of the preclinical structure−activity studies beginning with A-80987 and leading to the identification of ritonavir.

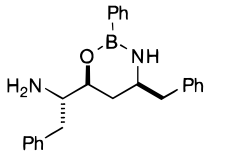






Chemistry

We[33] and others[34] have previously described the synthesis of the (*S*,*S*,*S*)-diamino alcohol **2**. For analogues of A-80987, differentiation of the two amino groups of **2** was required. This was accomplished either via statistical monoacylation as previously reported[33] or via regioselective acylation of the azoxaborylidine **3** (T. Sowin et al., unpublished results). Subsequently, an improved synthesis of **2** yielded large quantities of the monoprotected diamine **4**, which was conveniently differentiated.[35] To attach heterocyclic carbamates to bind in the S2′ region of the active site, intermediates **2**−**4** were acylated with the *p*-nitrophenyl carbonates of the corresponding heterocyclic carbinols (RCH_2OH) to produce monoamines **5** and/or **6** following either chromatographic separation or deprotection. For the P2 and P3 groups, a variety of heterocyclic N-substituted valine esters were required. Various heterocyclic alcohols or amines were prepared and acylated with L-valine methyl ester activated as either the corresponding isocyanate or *p*-nitrophenyl carbamate. Hydrolysis of the esters followed by carbodiimide-mediated coupling to **5** or **6** produced the desired HIV protease inhibitors with the different peripheral groups (P3/P2 and P2″, respectively) either proximal or distal to the central hydroxyl group of core unit **2**. General procedures are provided in the Experimental Section. The synthetic methods leading to the inhibitors are tabulated in the Supporting Information, along with yields and physical characterization of the final products.



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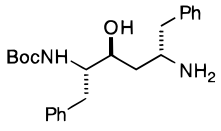

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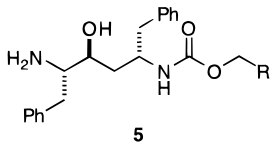

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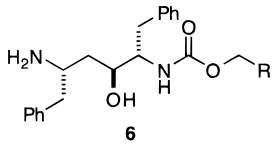


6

a, R = 3-pyridyl
b, R = 5-pyrimidinyl
c, R = 3-furanyl
d, R = 5-thiazolyl
e, R = 5-oxazolyl
f, R = 2-methyl-5-thiazolyl
g, R = 2-isopropyl-5-thiazolyl
h, R = 5-isoxazolyl

Results

The HIV protease inhibitors were evaluated in two standard biological assays as previously described.[25] The first assay measured the inhibition of purified, recombinant HIV-1 protease using a fluorogenic substrate.[36] IC_{50} values for inhibitors with potency >1 nM are provided in the tables. The majority of the compounds inhibited HIV protease at subnanomolar concentrations and thus acted as active site titrants. Accurate K_i values were not determined. Rather, the percent inhibition at a given inhibitor concentration (0.5 or 1.0 nM) is reported. This value provided a qualitative comparison between inhibitors, but varied in different experiments due to slightly different concentrations of HIV protease. The second assay measured the ability of the inhibitors to block the spread of $HIV\text{-}1_{3B}$ in the immortalized human T-cell line MT4 by measuring the cytopathic effect of HIV in those cells by uptake of a tetrazolium dye.[37] EC_{50} values represent the mean of two triplicate determinations using 10 and 32 $TCID_{50}$ per well, respectively. Potency differences of <2-fold are not considered outside of experimental error. In this assay, the activity of saquinavir[38] and indinavir[20] ranged from 0.01 to 0.05 μM. In general, the dose−response curves for each analogue were similar, and EC_{90} values were 3−4-fold higher than EC_{50} values. Compound cytotoxicity ($CCIC_{50}$) was determined in uninfected MT4 cells.

The aqueous solubility and pharmacokinetic profiles of many of the inhibitors were also evaluated. Estimated solubility in pH 7.4 phosphate and pH 4 acetate buffers were measured as previously described.[25] Plasma concentrations of inhibitor following either a 5 mg/kg intravenous (iv, $n = 2$) or 10 mg/kg oral ($n = 3$) dose in the rat were quantitated by HPLC analysis of parent compound as described.[25] Plasma half-life ($T_{1/2}$) was calculated following the iv dose. The time (T_{max}) and value (C_{max}) of highest plasma concentration and the area under the time and plasma concentration curve (AUC) were measured following the oral dose. Percent oral bioavailability (F) was calculated by comparison of the dose-normalized AUC values for iv and oral doses. Solubility and pharmacokinetic parameters are presented in the tables, along with ratio of maximum

concentration to the in vitro anti-HIV activity (C_{max}/EC_{50}).

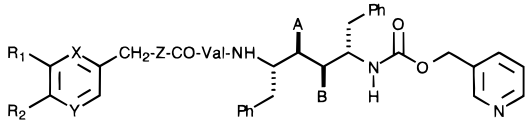
Structure−Activity Strategies. In examining the structure−activity relationships of analogues of A-80987, we identified the dual goal of improving anti-HIV activity and slowing the rate of metabolism. Underlying this premise was the supposition that significant lowering of metabolism would not only increase oral bioavailability but also affect plasma half-life. Longer half-life values would lead to higher trough levels upon repeated dosing, thereby retaining constant levels well in excess of the EC_{50}. We speculated that maintenance of high plasma levels would not only produce a more profound antiviral effect in vivo but should also delay the time before the emergence of resistant mutants due to more complete suppression of viral replication.[39] Modification of the pyridyl groups of A-80987 to reduce the rate of oxidative metabolism was examined within the framework of previous studies in this series that identified structural features promoting higher oral bioavailability, including sufficient aqueous solubility, limited hydrogen-bonding functionality, and limited size.[33] We reasoned that at least one basic nitrogen was required for sufficient solubility in a low pH dosing regimen. Modifications of the pyridyl groups of A-80987 included the addition of substituents designed to slow oxidative metabolism either sterically or electronically. Additionally, we pursued replacing both pyridyl groups with other heterocycles of sufficient basicity to contribute to aqueous solubility. Initial studies suggested that methyl substitution on the P_3 2-pyridyl group of A-80987 improved activity against HIV protease,[33] and we anticipated further improvements as we examined other substituents (vide infra).

Substituted P_3 Pyridyl Analogues. The activity of 6-alkyl-2-pyridyl analogues of A-80987 (compound **5**) is shown in Table 1. In each case, both regioisomers, resulting from attachment of the substituted valine either proximal or distal to the hydroxyl group of **2**, were prepared. Carbamate, urea, and *N*-methylurea linkers between the P_3 heterocycle and the P_2 valine were examined. As observed previously,[24,27] carbamate analogues showed slightly but consistently higher activity, both in the HIV protease assay and the antiviral assay, than the corresponding ureas or *N*-methylureas. All of the 6-alkylpyridines were more potent than the corresponding unsubstituted pyridines, e.g., A-80987.[33] Ethyl substitution appeared optimal for this series (cf. **13** and **14**) with the larger isopropyl and *tert*-butyl substituents offering no advantage. Indeed, substitution with either isopropyl or *tert*-butyl led in many cases to inhibitors with increased cellular toxicity and lower aqueous solubility. Of the subset of inhibitors of this type that were examined, compounds with larger alkyl groups showed lower C_{max} and generally lower oral bioavailability than A-80987, although C_{max}/EC_{50} ratios exceeded that of 80987 in some cases (e.g., **13**) because of increased potency. None of the analogues displayed a pharmacokinetic profile equal to that of A-80987. Notably, substitution with 6-alkyl groups did not significantly affect the circulating half-life, suggesting that pyridine oxidation was not being sufficiently retarded. P_3 methyl-substituted 3-pyridyl analogues are represented by compounds **25**−**33**. The activity of both 5-methyl- and 6-methylpyridines was similar to the 6-methyl-2-pyridines **9**−**12**. However, the pharmacokinetic profiles of the 3-pyridines were consistently poorer than the corresponding 2-pyridines. This trend was observed previously with unsubstituted P_3 pyridyl groups[25,33] and may reflect more rapid oxidation of the less hindered 3-pyridyl nitrogen.

We also examined substituents that modified the electronic nature of the P_3 pyridyl group of A-80987, including the 5-methoxy-2-pyridyl analogues **34**−**37** and the 5- and 6-methoxy-3-pyridyl analogues **38**−**45**. Compounds **34**−**45** were uniformly more active against HIV than A-80987. In most cases, the additional methoxy group provided sufficient aqueous solubility. None of the analogues matched the pharmacokinetic profile of A-80987, however. Compound **36** showed the best properties, with a C_{max} of 2.17 μM, a C_{max}/EC_{50} ratio of 22, and oral bioavailability of 17%. As with the above alkyl analogues, the plasma concentrations of **34**−**45** declined rapidly, again presumably due to rapid metabolism. In contrast to **34**−**45**, the aminopyridyl analogues **46**−**53** were less active than A-80987. 5-Amino-3-pyridyl inhibitors **50**−**51** were the most potent and showed excellent aqueous solubility at pH 4.0. However, oral administration to rats produced only low plasma levels.

Other P_3 Heterocycles. Concurrent with the above studies, we explored the replacement of the P_3 pyridyl group of A-80987 with other heterocycles. The results of those studies are provided in Tables 1 and 2. Pyrimidine analogues **54**−**65** (Table 1) showed equivalent activity to the corresponding pyridines (cf. **56**−**59** vs **7**−**10**). Furthermore, several inhibitors in this series showed significant oral bioavailability. Most promising was compound **63**, which was 5-fold more potent than A-80987 and gave plasma levels in excess of 2 μM ($C_{max}/EC_{50} > 50$). Further improvements over A-80987 were observed upon incorporation of five-membered heterocycles, specifically thiazoles and oxazoles, at the P3 position (Table 2). To a first approximation, the structure−activity relationships of the 2-alkyl-4-thiazolyl analogues **66**−**79** paralleled that of the pyridyl inhibitors **5**−**24**. In detail, however, the SAR diverged in two important ways. First, whereas in the pyridyl series alkyl groups larger than ethyl offered no advantage, the following trend was observed in the 4-thiazolyl series: *i*-Pr ≈ *t*-Bu > Et > Me > H. Second, with larger substituents on the thiazolyl groups, the activity of the *N*-methylurea analogues (Q = NCH_3) became strongly dependent upon the regioisomeric position of the core hydroxyl group. Thus, compounds **77** and **79**, in which the hydroxyl group was situated distal to the P2 valine residue (B = OH), were 10-fold more potent against HIV than the corresponding regioisomers **76** and **78**, which contained the hydroxyl group proximal to the P2 valine (A = OH). This regioisomeric divergence was not observed in carbamate analogues (cf. **74**−**75**). The culmination of these two trends resulted in the identification of compound **77** (A-83962),[19] which was ca. 8-fold more potent than A-80987 and 20-fold more potent than the corresponding pyridyl *N*-methylurea A-80613 (compound **6**).[33] Although the oral bioavailability of **77** in rats was moderate (12%), the C_{max}/EC_{50} ratio exceeded that of A-80987 by almost 2-fold. More

Table 1. Activity of P3 Pyridine Analogues

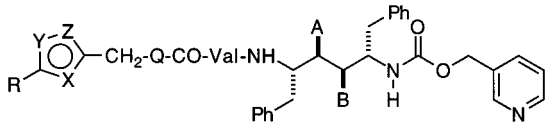


											solubility (μg/mL)							
no.	R_1	R_2	X	Y	Z	A	B	% inhibn (nM)	EC_{50} (μM)	$CCIC_{50}$ (μM)	pH 4.0	pH 7.4	$T_{1/2}$ (h)	C_{max} (μM)	T_{max} (h)	AUC (μg·h/mL)	F (%)	C_{max}/EC_{50}
5	H	H	N	CH	O	OH	H	61 (0.5)	0.22	>100	122	8.6	1.90	4.11	0.25	1.98	26	19
6	H	H	N	CH	NCH_3	H	OH	55 (0.5)	0.58	>100		178	1.22	2.18	0.15	1.22	22	3.8
7	CH_3	H	N	CH	O	OH	H	80 (0.5)	0.10	>100		3.5	0.74	1.15	0.33	0.61	7.5	12
8	CH_3	H	N	CH	O	H	OH	65 (0.5)	0.051	>100		11	1.95	1.74	0.10	0.90	16	34
9	CH_3	H	N	CH	NCH_3	OH	H	43 (0.5)	0.33	81		101	0.22	2.42	0.25	0.95	16	7
10	CH_3	H	N	CH	NCH_3	H	OH	56 (0.5)	0.26	59		137	0.68	3.39	0.29	1.66	32	13
11	CH_3	H	N	CH	NH	OH	H	69 (0.5)	0.30	75	85	1.7						
12	CH_3	H	N	CH	NH	H	OH	76 (0.5)	0.34	>100	91	1.2						
13	Et	H	N	CH	O	OH	H	76 (0.5)	0.019	82	137	4.5	0.74	1.26	0.25	0.81	16	66
14	Et	H	N	CH	O	H	OH	78 (0.5)	0.023	>100	14	0.5	0.39	0.59	0.20	0.47	8.0	26
15	Et	H	N	CH	NCH_3	OH	H	63 (0.5)	0.067	57	781	18	0.52	1.53	0.15	0.73	23	23
16	Et	H	N	CH	NCH_3	H	OH	69 (0.5)	0.25	59								
17	*i*-Pr	H	N	CH	O	OH	H	81 (0.5)	0.049	>100	46	2.2	1.31	0.31	0.25	0.31	8.3	6
18	*i*-Pr	H	N	CH	O	H	OH	83 (0.5)	0.027	20	71	2.3	0.57	0.20	0.25	0.14	5.6	7
19	*i*-Pr	H	N	CH	NCH_3	OH	H	62 (0.5)	0.18	19								
20	*i*-Pr	H	N	CH	NCH_3	H	OH	71 (0.5)	0.18	19								
21	*t*-Bu	H	N	CH	O	OH	H	76 (0.5)	0.064	19								
22	*t*-Bu	H	N	CH	O	H	OH	73 (0.5)	0.055	21								
23	*t*-Bu	H	N	CH	NCH_3	OH	H	65 (0.5)	0.60	19								
24	*t*-Bu	H	N	CH	NCH_3	H	OH	52 (0.5)	0.31	19								
25	CH_3	H	CH	N	O	OH	H	69 (0.5)	0.092	>100	98	6.2	0.28	0.48	0.30	0.21	5.9	5
26	CH_3	H	CH	N	O	H	OH	80 (0.5)	0.051	>100	29	1.0	0.38	0.33	0.15	0.07	1.6	7
27	CH_3	H	CH	N	NCH_3	OH	H	47 (0.5)	0.48	>100	85	3.4						
28	CH_3	H	CH	N	NCH_3	H	OH	63 (0.5)	0.12	>100	532	48	0.53	0.69	0.10	0.31	9.7	6
29	H	CH_3	CH	N	O	OH	H	59 (1.0)	0.087	>100		3.3	0.57	0.45	0.10	0.24	6.3	5
30	H	CH_3	CH	N	O	H	OH	56 (1.0)	0.057	>100		10	0.95	0.78	0.15	0.27	6.4	14
31	H	CH_3	CH	N	NCH_3	OH	H	60 (1.0)	0.41	>100	736	43	0.44	1.22	0.10	0.29	6.7	3
32	H	CH_3	CH	N	NCH_3	H	OH	67 (1.0)	0.13	>100	870	50	0.35	0.31	0.20	0.06	2.1	2
33	H	CH_3	CH	N	NH	OH	H	65 (1.0)	0.63	>100								
34	H	OCH_3	N	CH	O	OH	H	72 (0.5)	0.039	65	48	4.9	0.46	1.20	0.20	0.38	7.7	31
35	H	OCH_3	N	CH	O	H	OH	74 (0.5)	0.049	75	39	8.5	0.25	0.29	0.10	0.05	1.3	6
36	H	OCH_3	N	CH	NCH_3	OH	H	60 (0.5)	0.10	61	77	3.3	0.60	2.17	0.15	0.82	17	22
37	H	OCH_3	N	CH	NCH_3	H	OH	60 (0.5)	0.076	87		30	0.57	1.05	0.10	0.29	5.7	14
38	OCH_3	H	CH	N	O	OH	H	79 (0.5)	0.061	>100	329	66	1.19	0.30	0.10	0.07	1.5	5
39	OCH_3	H	CH	N	O	H	OH	79 (0.5)	0.046	>100	27	15	0.80	0.20	0.10	0.03	0.9	4
40	OCH_3	H	CH	N	NCH_3	OH	H	53 (1.0)	0.27	>100	19	1.8	0.17	0.44	0.10	0.05	0.8	2
41	OCH_3	H	CH	N	NCH_3	H	OH	64 (0.5)	0.16	>100	612	58	0.28	0.18	0.15	0.05	0.4	1
42	H	OCH_3	CH	N	O	OH	H	96 (0.5)	0.054	>100	3.2	0.6	0.43	0.56	0.15	0.16	3.3	10
43	H	OCH_3	CH	N	O	H	OH	83 (0.5)	0.019	55	46	5.3	0.33	0.19	0.10	0.03	0.7	10
44	H	OCH_3	CH	N	NCH_3	OH	H	84 (0.5)	0.063	58	127	18	0.32	1.83	0.10	0.31	7.9	29
45	H	OCH_3	CH	N	NCH_3	H	OH	84 (0.5)	0.068	61	200	69	0.22	1.75	0.10	0.32	10	26
46	H	NH_2	N	CH	O	OH	H	57 (0.5)	0.85	>100								
47	H	NH_2	N	CH	O	H	OH	59 (0.5)	0.69	>100								
48	H	NH_2	N	CH	NCH_3	OH	H	56 (1.0)	0.77	>100								
49	H	NH_2	N	CH	NCH_3	H	OH	48 (0.5)	0.34	>100								
50	NH_2	H	CH	N	O	OH	H	50 (0.5)	0.35	>100	862	22	0.72	0.20	0.10	0.02	0.5	1.0
51	NH_2	H	CH	N	O	H	OH	63 (0.5)	0.37	>100	739	33	0.50	0.14	0.10	0.03	0.3	0
52	H	NH_2	CH	N	O	OH	H	52 (0.5)	7.60	89								
53	H	NH_2	CH	N	O	H	OH	53 (0.5)	9.65	>100								
54	H	H	N	N	O	OH	H	60 (0.5)	0.20	>100	169	38	0.23	1.03	0.20	0.21	4.5	5
55	H	H	N	N	O	H	OH	58 (0.5)	0.11	>100	99	24	0.20	0.50	0.20	0.10	2.4	4
56	CH_3	H	N	N	O	OH	H	69 (0.5)	0.097	>100	45	5.9						
57	CH_3	H	N	N	O	H	OH	73 (0.5)	0.071	59	55	13	1.15	1.33	0.10	0.64	10	19
58	CH_3	H	N	N	NCH_3	OH	H	41 (0.5)	1.44	>100								
59	CH_3	H	N	N	NCH_3	H	OH	64 (0.5)	0.26	>100								
60	H	CH_3	N	N	O	OH	H	64 (0.5)	0.22	>100	77	14	0.18	0.35	0.20	0.07	2.8	2
61	H	CH_3	N	N	O	H	OH	67 (0.5)	0.10	96	38	11	0.21	0.86	0.30	0.19	6.0	9
62	CH_3	CH_3	N	N	O	OH	H	66 (0.5)	0.071	>100			0.30	0.35	0.20	0.12	5.7	5
63	CH_3	CH_3	N	N	O	H	OH	70 (0.5)	0.039	>100	562	112	0.43	2.06	0.10	0.48	10	53
64	CH_3	CH_3	N	N	NCH_3	OH	H	50 (0.5)	0.66	>100	719	93	0.30	1.52	0.15	0.55	17	2
65	CH_3	CH_3	N	N	NCH_3	H	OH	67 (0.5)	0.12	>100	769	227	0.30	1.13	0.25	0.33	6.4	10

importantly, the half-life of **77** (1.03 h) was longer than that observed for any previous *N*-methylurea-based inhibitor. Of the analogous 4-oxazolyl inhibitors **80**–**83**, compound **83** showed potency and oral bioavailability similar to those of **77** with the exception that **83** was absorbed and eliminated more rapidly than **77**.

Encouraged by the above results, we examined 2-substituents of varying polarity. Methoxymethyl substitu-

Table 2. Activity of P3 Five-membered Heterocyclic Analogues



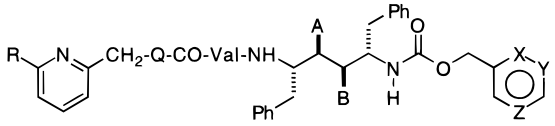
											solubility (μg/mL)							
no.	R	X	Y	Z	Q	A	B	% inhibn (nM)	EC_{50} (μM)	$CCIC_{50}$ (μM)	pH 4.0	pH 7.4	$T_{1/2}$ (h)	C_{max} (μM)	T_{max} (h)	AUC (μg·h/mL)	F (%)	C_{max}/ EC_{50}
66	H	N	S	CH	NCH_3	OH	H	41 (0.5)	0.74	83	240	53	1.40	3.13	0.37	1.66	35	4
67	H	N	S	CH	NCH_3	H	OH	82 (0.5)	0.49	>100	204	62	0.67	0.64	0.10	0.29	5.2	1
68	CH_3	N	S	CH	NCH_3	OH	H	55 (0.5)	0.21	>100	115	25	1.49	2.01	0.25	1.08	15	10
69	CH_3	N	S	CH	NCH_3	H	OH	72 (0.5)	0.18	>100	205	36	0.72	2.10	0.15	1.06	26	12
70	Et	N	S	CH	O	OH	H	75 (0.5)	0.065	59	1.6	0.09	0.49	0.99	0.10	0.42	9.5	15
71	Et	N	S	CH	O	H	OH	76 (0.5)	0.042	65	21	1.6	0.75	1.30	0.15	0.33	7.4	31
72	Et	N	S	CH	NCH_3	OH	H	58 (0.5)	0.29	61	188	12	0.74	1.46	0.20	0.87	20	5
73	Et	N	S	CH	NCH_3	H	OH	73 (0.5)	0.26	60	243	28	1.20	1.49	0.25	1.05	17	6
74	*i*-Pr	N	S	CH	O	OH	H	70 (0.5)	0.011	>100	13	0.8	0.68	0.47	0.25	0.22	3.0	43
75	*i*-Pr	N	S	CH	O	H	OH	72 (0.5)	0.010	19	6.4	2.4	0.24	0.21	0.10	0.05	1.6	21
76	*i*-Pr	N	S	CH	NCH_3	OH	H	63 (0.5)	0.29	32	80	9.4	0.75	1.65	0.25	0.95	24	6
77	*i*-Pr	N	S	CH	NCH_3	H	OH	78 (0.5)	0.029	47	66	7.9	1.03	0.98	0.33	0.53	12	34
78	*t*-Bu	N	S	CH	NCH_3	OH	H	60 (0.5)	0.37	19	49	4.6	0.36	0.83	0.33	0.51	9.3	2
79	*t*-Bu	N	S	CH	NCH_3	H	OH	71 (0.5)	0.029	17	52	4.0	0.29	0.38	0.33	0.23	5.3	13
80	*i*-Pr	N	O	CH	O	OH	H	71 (0.5)	0.018	>100	11	1.8	ns	0.17	0.10	0.02	n/a	10
81	*i*-Pr	N	O	CH	O	H	OH	86 (0.5)	0.020	52	3.8	0.7	0.33	0.46	0.20	0.20	5.0	24
82	*i*-Pr	N	O	CH	NCH_3	OH	H	60 (0.5)	0.033	62	727	264	0.29	0.29	0.10	0.09	3.3	9
83	*i*-Pr	N	O	CH	NCH_3	H	OH	79 (0.5)	0.061	63	252	39	0.22	2.13	0.25	1.10	18	35
84	*i*-Pr	N	S	CH	CH_2	OH	H	61 (0.5)	0.16	44			0.91	0.32	0.10	0.41	9.0	2
85	*i*-Pr	N	S	CH	CH_2	H	OH	87 (0.5)	0.068	100	34	3.9	0.28	0.38	0.10	0.17	3.4	6
86	$MeOCH_2$	N	S	CH	O	OH	H	66 (0.5)	0.096	>100	175	10	0.27	1.79	0.23	0.63	10	19
87	$MeOCH_2$	N	S	CH	O	H	OH	69 (0.5)	0.076	>100								
88	$MeOCH_2$	N	S	CH	NCH_3	OH	H	54 (0.5)	0.33	61	77	8.8	0.56	2.29	0.50	1.24	16	7
89	$MeOCH_2$	N	S	CH	NCH_3	H	OH	69 (0.5)	0.076	65			0.39	1.35	0.20	0.49	8.0	18
90	$(Me)_2N$	N	S	CH	O	OH	H	66 (0.5)	0.089	53								
91	4-morph	N	S	CH	O	OH	H	73 (0.5)	0.033	29	14	1.5	0.35	0.09	0.15	0.03	1.1	3
92	4-morph	N	S	CH	O	H	OH	70 (0.5)	0.033	61	63	12	0.32	0.00	n/a	0.00	0	0
93	H	N	CH	S	O	OH	H	56 (0.5)	0.37	>100	71	7.5	0.54	3.33	0.18	2.05	35	9
94	H	N	CH	S	O	H	OH	56 (0.5)	0.13	>100	6.8	2.0	0.66	2.53	0.15	0.57	8.5	19
95	Et	S	N	CH	O	OH	H	68 (0.5)	0.29	66	1.2	<0.01	1.23	0.34	0.28	0.49	9.2	1
96	Et	S	N	CH	O	H	OH	71 (0.5)	0.091	>100	1.1	<0.01	0.19	0.16	0.10	0.01	0.5	2
97	*i*-Pr	S	N	CH	O	OH	H	63 (0.5)	0.10	>100	7.9	1.0	0.62	0.10	0.20	0.03	0.8	1.0
98	*i*-Pr	S	N	CH	O	H	OH	71 (0.5)	0.076	>100			0.46	0.15	0.10	0.02	0.7	2
99	H	O	N	CH	O	OH	H	59 (0.5)	0.54	>100								
100	H	O	N	CH	O	H	OH	62 (0.5)	0.22	>100								
101	$MeOCH_2$	S	N	CH	O	OH	H	64 (0.5)	0.18	>100			0.37	0.38	0.15	0.42	11	2
102	$MeOCH_2$	S	N	CH	O	H	OH	72 (0.5)	0.085	>100	2.4	<0.01	0.27	0.12	0.10	0.02	0.6	1
103	H	S	N	$C(CH_3)$	CH_2O	OH	H	67 (0.5)	0.11	>100			0.45	0.41	0.45	0.36	9.2	4
104	H	S	N	$C(CH_3)$	CH_2O	H	OH	59 (0.5)	0.081	61			ns[a]	0.96	n/a	1.53	n/a	12
105	*t*-Bu	CH	O	N	O	OH	H	67 (0.5)	0.068	>100								
106	*t*-Bu	CH	O	N	O	H	OH	74 (0.5)	0.073	19								
107	*t*-Bu	CH	O	N	NCH_3	OH	H	49 (0.5)	1.01	56								
108	*t*-Bu	CH	O	N	NCH_3	H	OH	80 (0.5)	0.048	55								
109	CH_3O	CH	N	O	O	OH	H	70 (0.5)	0.22	61								
110	CH_3O	CH	N	O	O	H	OH	76 (0.5)	0.084	>100								

[a] ns: no iv sample; compound was too insoluble for iv dosing and was only dosed orally.

tion provided inhibitors (compounds **86**−**89**) of similar potency to the corresponding ethyl-substituted analogues **70**−**73** and moderate oral bioavailability. 2-Aminothiazoles showed only moderate anti-HIV activity (data not shown), analogous to the aminopyridyl analogues (vide supra). 2-Dialkylamino-substituted thiazoles, in contrast, demonstrated excellent potency (cf. **90**−**92**); however, the oral bioavailability of **91** and **92** was very low. Isomeric thiazoles, oxazoles, and isoxazoles were also examined as P3 pyridyl replacements. 2-Thiazolyl inhibitors **93**−**94** showed similar activity to the corresponding unsubstituted 4-thiazolyl inhibitors (**66**−**67**) and promising oral bioavailability. In contrast, both the potency and bioavailability of the 5-thiazolyl and 5-oxazolyl inhibitors **95**−**104** were inferior to that of the 4-thiazolyl analogues. Isoxazolyl-based inhibitors **105**−**110** showed good anti-HIV activity in vitro but were not evaluated for pharmacokinetic profile due to anticipated lack of aqueous solubility.

Heterocyclic P2′ Pyridyl Replacements. Given the propensity of A-80987 to undergo metabolic oxidation at both the P3 and P2′ pyridyl groups,[19] we concurrently investigated the replacement of the P2′ pyridyl group by other heterocyclic groups (Table 3). Earlier studies had demonstrated that a hydrogen-bond-accepting group two atoms from the linking methylene was critical for tight binding to the HIV protease active site. Furthermore, substituents on the P2′ heterocycle were generally not tolerated (vide infra). 5-Pyrimidinyl substitution provided inhibitors (compounds **111**−**113**) of equal potency to the pyridyl counterparts, but with lower bioavailability. Furanyl analogues **114**−**117**

Table 3. Activity of P2′ Heterocyclic HIV Protease Inhibitors



										solubility (μg/mL)							
no.	R	Q	X−Y	Z	A	B	% inhibn (nM)	EC_{50} (μM)	$CCIC_{50}$ (μM)	pH 4.0	pH 7.4	$T_{1/2}$ (h)	C_{max} (μM)	T_{max} (h)	AUC (μg·h/mL)	*F* (%)	C_{max}/EC_{50}
111	H	O	CH−N	N	OH	H	52 (0.5)	0.25	>100	39	15	0.50	1.18	0.20	0.28	6.7	4.7
112	H	NCH_3	CH−N	N	OH	H	48 (0.5)	0.68	>100	437	251	0.35	2.91	0.20	0.87	13	4.3
113	H	NCH_3	CH−N	N	H	OH	60 (0.5)	0.45	>100	444	271	0.87	0.91	0.30	0.22	5.1	2.0
114	H	O	CH	O	OH	H	78 (0.5)	0.23	>100	5.9	3.3	ns[a]	0.25	0.28	0.23	n/a	1.1
115	H	NCH_3	CH	O	OH	H	64 (0.5)	1.05	58	166	44	1.07	1.02	0.40	0.50	9.3	1.0
116	CH_3	O	CH	O	OH	H	72 (0.5)	0.090	>100		0.9	0.22	0.00	n/a	0.00	0.0	0.0
117	CH_3	O	CH	O	H	OH	75 (0.5)	0.047	>100		0.6	ns	0.16	0.14	0.02	n/a	3.3
118	H	O	S	N	OH	H	54 (0.5)	0.52	>100	3.7	2.2	ns	2.98	0.14	1.49	n/a	5.7
119	H	O	S	N	H	OH	59 (0.5)	0.55	>100	7.3	4.2	1.23	2.04	0.37	4.24	47	3.7

[a] ns: no iv sample; compound was too insoluble for iv dosing and was only dosed orally.

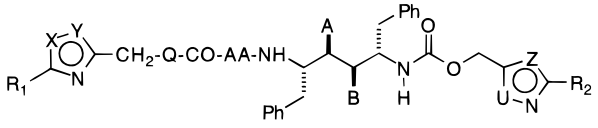
showed excellent potency but, with the exception of compound **115**, very poor bioavailability, possibly due to low aqueous solubility. In contrast, 5-thiazolyl analogues **118** and **119** showed excellent pharmacokinetic properties following oral dosing, despite limited solubility (compound **118** was too insoluble for iv dosing). Key to the large AUC following oral dosing of **119** (A-81525)[19] was a prolonged absorption profile and significantly longer half-life. Consequently, although the C_{max}/EC_{50} ratio for **119** was modest, plasma levels were detectable >6 h following a 10 mg/kg dose in rats.

Identification of Ritonavir. From the above studies, we ascertained that the pharmacokinetic profile of analogues of A-80987 could be improved by replacement of either pyridyl moiety with more highly electron-deficient heterocycles, in particular thiazolyl or oxazolyl groups. Furthermore, alkyl substitution on the P3 heterocycle led to an improvement in anti-HIV activity in vitro. We therefore anticipated that combining the elements of A-83962 (**77**) and A-81525 (**119**) would provide inhibitors of unique potency and pharmacokinetic properties. However, due to the higher pK_a of thiazole, the aqueous solubility of both **77** and **119** was significantly lower than that of A-80987 at pH 4.0, potentially posing a barrier to efficient absorption.[33,40] Compounds **120** and **121** displayed excellent anti-HIV potency. However, neither inhibitor was soluble enough in an acceptable formulation for intravenous dosing, and oral administration provided very low plasma levels (Table 4). Previous studies had shown that an *N*-methylurea linkage between the P3 and P2 groups provided higher solubility than a carbamate linkage.[25,33] Accordingly, the *N*-methylurea inhibitors **122** and **123** could be dosed in solution. Compound **123** (ABT-538, ritonavir) retained the potency of A-83962 and gave plasma levels after oral administration >100-fold in excess of the EC_{50}. Furthermore, the levels of ritonavir in plasma declined much more slowly than previous inhibitors, and levels in rats exceeded the in vitro EC_{50} for >8 h following a single 10 mg/kg dose (Figure 2). In contrast, levels of A-80987 exceeded the EC_{50} for only 2 h. The pharmacokinetic behavior of ritonavir was further investigated in mice, beagle dogs, and cynomolgus monkeys. The plasma concentration curves are shown in Figure 2. Pharmacokinetic parameters have been published elsewhere.[19] In each species, levels were sustained in excess of the EC_{50} for >8 to >12 h. The extended pharmacokinetic profile of ritonavir correlated with a suppression of the rate of metabolism in vitro using dog liver microsomes.[19] Compounds **77** and **119** were metabolized 5 times more slowly than A-80987. The rate of metabolism of ritonavir, in which both pyridyl groups have been replaced with the more electron-deficient thiazole, was ca. 20-fold lower than that of A-80987.[19]

Analogues of Ritonavir. Following the identification of ritonavir (**123**), we initiated a detailed investigation of the structure−activity relationships of analogous inhibitors. Results of those studies are provided in Table 4. Not unexpectantly, substitution of thiazole with oxazole at either the P3 or P2′ position provided inhibitors (**124**−**127**) of similar profile to ritonavir, both in potency and pharmacokinetic behavior. Each of the oxazole analogues **125**−**127** showed more rapid absorption than ritonavir, potentially due to higher aqueous solubility. However, plasma concentrations of the oxazolyl inhibitors were maintained for a shorter period of time. A variety of 2-substituents on the P3 heterocycle were examined (compounds **128**−**139**). As anticipated, activity was systematically enhanced with increasing substitution up to isopropyl (compounds **128**−**132**). Increasing the size even further (compound **133**) was detrimental, but both cyclopropyl- and cyclobutyl-substituted inhibitors (**134**−**137**) were comparable in activity to the isopropyl analogues **122** and **123**. Like ritonavir, most of the alkyl substituted analogues (**130**−**132**, **134**−**135**, **137**) showed excellent pharmacokinetics in the rat model, with oral bioavailabilities of 57−100%, C_{max} values in excess of 3 μM, AUC values of ≥9 μg·h/mL, and, in several cases, C_{max}/EC_{50} values of >100. In contrast, the morpholinyl-substituted inhibitors **138**−**139**, while retaining high potency, showed very low oral bioavailability, a difference also observed in the P2′ pyridyl analogues **91**−**92** (vide supra). We also examined the P3 5-thiazolyl compounds **140**−**141**. Although the potency of **140**−**141** declined with respect to the 4-thiazolyl counterparts **122**−**123**, the pharmacokinetic profile of **141** was notable in that high C_{max} and AUC values were obtained even though the compound had limited solubility and was thus dosed as a suspension.

We next examined changes at the *N*-methylurea linker concurrent with modifications of the P2 amino

Table 4. Analogues of Ritonavir



														solubility (μg/mL)					AUC		
no.	R_1	X	Y	Q	AA	Z	U	R_2	A	B	% inhibn (nM)	EC_{50} (μM)	$CCIC_{50}$ (μM)	pH 4.0	pH 7.4	$T_{1/2}$ (h)	C_{max} (μM)	T_{max} (h)	(μg·h/mL)	F (%)	C_{max}/EC_{50}
120	*i*-Pr	S	CH	O	Val	S	CH	H	OH	H	75 (0.5)	0.010	100	0.28	0.01	ns[a]	0.00	n/a	0.00	0	0
121	*i*-Pr	S	CH	O	Val	S	CH	H	H	OH	79 (0.5)	0.005	51	4.6	1.9	ns	0.04	0.10	0.01	n/a	10
122	*i*-Pr	S	CH	NCH_3	Val	S	CH	H	OH	H	67 (0.5)	0.19	56	7.1	3.7	2.0	2.29	1.75	1.70	16	12
123	*i*-Pr	S	CH	NCH_3	Val	S	CH	H	H	OH	79 (0.5)	0.025	57	6.9	5.3	1.2	2.62	2.0	7.75	78	105
124	*i*-Pr	O	CH	NCH_3	Val	S	CH	H	OH	H	55 (0.5)	0.98	57								
125	*i*-Pr	O	CH	NCH_3	Val	S	CH	H	H	OH	80 (0.5)	0.043	61	34	21						
126	*i*-Pr	S	CH	NCH_3	Val	O	CH	H	H	OH	86 (0.5)	0.013	61	22	9.3	0.56	1.59	0.58	2.65	23	127
127	*i*-Pr	O	CH	NCH_3	Val	O	CH	H	H	OH	70 (0.5)	<0.032	>100	57	32	0.26	2.05	0.15	1.85	35	>64
128	CH_3	S	CH	NCH_3	Val	S	CH	H	H	OH	75 (0.5)	0.79	62								
129	CH_3	O	CH	NCH_3	Val	S	CH	H	H	OH	82 (0.5)	0.077	>100								
130	Et	S	CH	NCH_3	Val	S	CH	H	OH	H	57 (0.5)	0.28	63			nd	5.12	1.00	9.37	167	18
131	Et	S	CH	NCH_3	Val	S	CH	H	H	OH	75 (0.5)	0.053	59			0.8	6.17	1.20	14.17	70	117
132	Et	O	CH	NCH_3	Val	S	CH	H	H	OH	75 (0.5)	0.018	100			2.0	8.01	0.33	8.91	57	458
133	3-pent	S	CH	NCH_3	Val	S	CH	H	H	OH	68 (0.5)	0.14	19								
134	c-Pr	S	CH	NCH_3	Val	S	CH	H	OH	H	53 (0.5)	0.15	45			0.53	3.98	2.30	10.70	175	27
135	c-Pr	S	CH	NCH_3	Val	S	CH	H	H	OH	69 (0.5)	0.047	48			1.1	6.46	2.17	21.86	58	138
136	c-Bu	S	CH	NCH_3	Val	S	CH	H	OH	H	50 (0.5)	0.23	20								
137	c-Bu	S	CH	NCH_3	Val	S	CH	H	H	OH	71 (0.5)	0.039	26			0.40	2.48	1.20	6.29	60	64
138	4-morph	S	CH	O	Val	S	CH	H	OH	H	72 (0.5)	0.080	>100	0.9	1.1	ns	0.00	n/a	0.00	n/a	0
139	4-morph	S	CH	O	Val	S	CH	H	H	OH	71 (0.5)	0.025	60	5.3	2.9	ns	0.17	0.10	0.04	n/a	7
140	*i*-Pr	CH	S	O	Val	S	CH	H	OH	H	1.9	1.1	36								
141	*i*-Pr	CH	S	O	Val	S	CH	H	H	OH	64 (0.5)	0.14	22			0.50	5.80	0.92	14.27	151	42
142	*i*-Pr	S	CH	NEt	Val	S	CH	H	OH	H	44 (0.5)	1.40	14	4.6	4.3						
143	*i*-Pr	S	CH	NEt	Val	S	CH	H	H	OH	72 (0.5)	0.25	24	4.3	2.8	ns	3.33	3.80	9.85	n/a	14
144	*i*-Pr	S	CH	NcPr	Val	S	CH	H	H	OH	44 (0.5)	0.28	19	2.8	2.8						
145	*i*-Pr	S	CH	NcPr	Val	S	CH	H	OH	H	1.9	2.72	20								
146	*i*-Pr	S	CH	O	Ala	S	CH	H	OH	H	48 (0.5)	1.69	63								
147	*i*-Pr	S	CH	O	Ala	S	CH	H	H	OH	74 (0.5)	0.10	56			0.53	1.09	0.40	1.23	19	11
148	*i*-Pr	S	CH	NCH_3	Ala	S	CH	H	OH	H	56 (0.5)	1.35	61	26	14						
149	*i*-Pr	S	CH	NCH_3	Ala	S	CH	H	H	OH	70 (0.5)	0.039	52			0.90	1.68	0.50	1.60	31	44
150	*i*-Pr	O	CH	NCH_3	Ala	S	CH	H	H	OH	66 (0.5)	0.059	100			4.0	3.60	0.25	2.86	23	61
151	*i*-Pr	S	CH	NEt	Ala	S	CH	H	OH	H	65 (0.5)	0.089	>100								
152	*i*-Pr	S	CH	NEt	Ala	S	CH	H	H	OH	73 (0.5)	0.014	52			ns	0.00	n/a	0.00	0	0
153	*i*-Pr	S	CH	NPr	Ala	S	CH	H	H	OH	50 (0.5)	0.14	19								
154	*i*-Pr	S	CH	NiBu	Ala	S	CH	H	H	OH	54 (0.5)	0.18	17								
155	*i*-Pr	S	CH	NnBu	Ala	S	CH	H	H	OH	50 (0.5)	0.29	17								
156	*i*-Pr	S	CH	NcPr	Ala	S	CH	H	OH	H	9.4	14.0	45	13	8.0						
157	*i*-Pr	S	CH	NcPr	Ala	S	CH	H	H	OH	57 (0.5)	0.55	41	16	5.3	0.61	5.19	1.80	11.99	76	9
158	*i*-Pr	S	CH	NCH_3	β-Ala	S	CH	H	OH	H	1.3	2.2	57	56	21						
159	*i*-Pr	S	CH	NCH_3	β-Ala	S	CH	H	H	OH	1.1	2.0	55	58	33	0.96	0.63	0.10	0.19	7.1	0
160	*i*-Pr	S	CH	NcPr	β-Ala	S	CH	H	OH	H	73	13.5	59			0.34	0.65	0.28	0.40	9.4	0
161	*i*-Pr	S	CH	NcPr	β-Ala	S	CH	H	H	OH	14	17.9	48	7.9	8.4						
162	*i*-Pr	S	CH	NEt	Gly	S	CH	H	H	OH	66 (1.0)	2.1	41								
163	*i*-Pr	S	CH	NPr	Gly	S	CH	H	H	OH	1.2	1.0	35								
164	*i*-Pr	S	CH	NiBu	Gly	S	CH	H	H	OH	1.6	1.2	15								
165	*i*-Pr	S	CH	CH_2O	Val	S	CH	H	H	OH	69 (0.5)	0.10	>100								
166	*i*-Pr	S	CH	CH_2O	Val	S	CH	H	OH	H	73 (0.5)	0.017	25			ns	0.00	n/a	0.00	0	0
167	*i*-Pr	S	CH		Val	S	CH	H	OH	H	50 (0.5)	0.82	>100								
168	*i*-Pr	S	CH		Val	S	CH	H	H	OH	61 (0.5)	0.21	>100								
169	*i*-Pr	S	CH	CH_2NMe	Val	S	CH	H	H	OH	69 (0.5)	0.18	25								
170	*i*-Pr	S	CH	CH_2NEt	Val	S	CH	H	H	OH	50 (0.5)	0.55	18								
171	*i*-Pr	S	CH	CH_2NPr	Val	S	CH	H	H	OH	37 (0.5)	1.1	18								
172	*i*-Pr	S	CH	CH_2NMe	Ala	S	CH	H	H	OH	36 (0.5)	0.81	59								
173	*i*-Pr	S	CH	CH_2NEt	Ala	S	CH	H	H	OH	2.3	2.0	24								
174	*i*-Pr	S	CH	CH_2NPr	Ala	S	CH	H	H	OH	4.1	2.9	18								
175	*i*-Pr	S	CH	NCH_3	Val	S	CH	CH3	OH	H	63 (0.5)	0.075	22	5.7	2.9	0.46	2.08	0.70	2.37	28	28
176	*i*-Pr	S	CH	NCH_3	Val	S	CH	CH3	H	OH	68 (0.5)	0.11	18	3.7	2.8	0.44	0.65	1.20	0.91	23	6
177	*i*-Pr	S	CH	NCH_3	Val	S	CH	*i*-Pr	OH	H	1.5	1.5	19								
178	*i*-Pr	S	CH	NCH_3	Val	S	CH	*i*-Pr	H	OH	68 (0.5)	0.20	19								
179	*i*-Pr	S	CH	NCH_3	Val	CH	O	H	H	OH	71 (0.5)	0.009	28	6.0	4.6	0.59	0.37	0.20	0.27	7.7	44
180	*i*-Pr	O	CH	NCH_3	Val	CH	O	H	H	OH	82 (0.5)	0.012	57	36	20	0.40	0.49	0.10	0.18	3.8	42
181	*i*-Pr	O	CH	NCH_3	Ala	CH	O	H	H	OH	64 (0.5)	0.042	>100								

[a] ns: no iv sample; compound was too insoluble for iv dosing and was only dosed orally.

acid of ritonavir. The rationale for this study was 2-fold. First, previous studies had identified N-demethylation of the urea linkers of A-77003 as a major metabolic pathway in addition to pyridine N-oxidation.[41] Second, valine to isoleucine mutants at position 32 in HIV protease were selected by serial passage of HIV in the

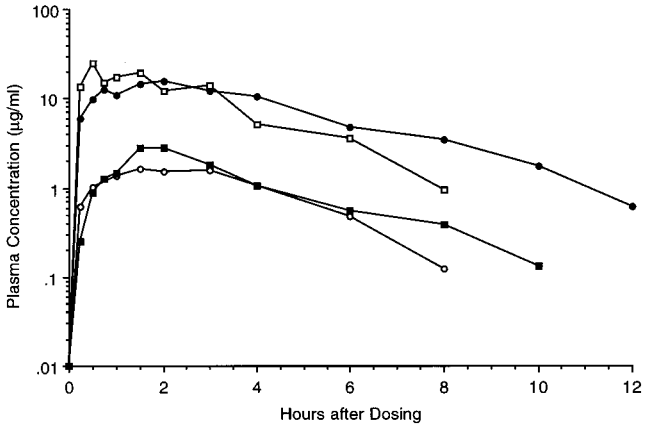


Figure 2. Mean plasma concentrations of ritonavir (ABT-538) after a single oral dose. Open circles, rat (10 mg/kg); closed circles, dog (10 mg/kg); open squares, mouse (25 mg/kg); closed squares, monkey (10 mg/kg).

presence of A-77003.[42] It was our hope that reducing the size of the P2 valine, which is common to both A-77003 and ritonavir and interacts directly with valine-32 via a hydrophobic contact,[43] might produce analogues that would not select this mutation. Inhibitors utilizing various P2 groups and urea linkers are shown in Table 4 (compounds **142**−**164**). Although substantial improvements over ritonavir were not realized, several observations are of note. The activity of *N*-ethyl- and, in particular, *N*-cyclopropylurea−valine analogues **142**−**145** declined significantly compared to that of the corresponding *N*-methylureas (**122**−**123**). In contrast, with alanine as a P2 residue, the antiviral potency of *N*-ethylurea **152** improved upon the potency of the *N*-methylurea **149** and was indistinguishable from that of ritonavir. Larger ureas were not tolerated, even with a P2 alanine (compounds **153**−**157**). Interestingly, the P2 alanine carbamates **146**−**147** were much less potent than either the *N*-methyl- or *N*-ethylureas and demonstrated a preference for one regioisomer (**147**) over the other, in contrast to the P2 valine carbamates **120**−**121**. Several of the alanine-based inhibitors, particularly **157**, showed excellent pharmacokinetic properties; however, the potent *N*-ethylurea−alanine inhibitor **152** was too insoluble to be dosed as a solution and gave no plasma levels following oral administration.

More drastic changes led uniformly to a significant fall in potency, including incorporation of β-alanine and glycine at the P2 position and extension or contraction of the P3−P2 linker functionality (compounds **158**−**174**). An exception to this trend was compound **166**, the extended analogue of **121**. Although **166** showed excellent antiviral potency, like **121** it was highly insoluble and gave no plasma levels after oral dosing. Finally, we briefly examined analogues of ritonavir with changes at the P2′ 5-thiazolyl group. Alkyl substitution on the thiazole ring resulted in decreased activity (compounds **175**−**178**). In contrast, incorporation of an unsubstituted P2′ 5-isoxazolyl group gave inhibitors (**179**−**181**) of very high potency but of relatively low oral bioavailability.

Discussion

These results describe the systematic structure−activity studies which led from A-80987, a symmetry-based HIV protease inhibitor with good potency and moderate oral bioavailability[33] to the identification of ABT-538 (ritonavir), which displays excellent potency and pharmacokinetic properties. Key to the discovery of ritonavir was the modification of chemical functionality within A-80987 to groups more stable to oxidative metabolism by the cytochrome P450 enzymes present in the intestine and liver. In a multifaceted approach, both steric and electronic modifications to the oxidatively susceptible pyridyl groups of A-80987 were investigated. Ultimately, the replacement of the pyridyl groups with less electron rich thiazolyl and oxazolyl groups led to substantial reductions in the metabolic degradation and excretion of the inhibitors.[19] As a result, high plasma levels were achieved in several animal species and humans[19] and sustained for hours following a single dose. The improved pharmacokinetic properties of ritonavir appear to be related not only to increased chemical stability but also to metabolic inhibition. Ritonavir has recently been shown to be not only metabolized by but also a potent inhibitor of the 3A4 isozyme of cytochrome P450 (CYP 3A4).[44] Inhibition of CYP by ritonavir is associated with the unhindered nitrogen atom on the unsubstituted P2′ 5-thiazolyl group, which binds directly to the heme in the CYP active site.[45] However, the sole presence of the 5-thiazolyl group is insufficient, since A-81525 (compound **119**) was 10-fold less effective at inhibiting CYP than ritonavir.[45] The difference in CYP inhibition between A-81525 and ritonavir correlates to the more rapid rate of metabolism of A-81525 in liver microsomes, presumably at the pyridyl nitrogen of A-81525. Although not measured, it is likely that rapid CYP-mediated metabolism significantly compromises the oral bioavailability of virtually all of the analogues of A-80987 described in Tables 1 and 2. Furthermore, the attachment of substituents that may be more susceptible to oxidation to the P3-thiazolyl group of ritonavir provided analogues (e.g., compounds **138** and **139**, containing morpholinyl groups) that displayed very low plasma levels. It is likely therefore that a combination of direct heme binding and chemical stability contributes to the favorable pharmacokinetic profile of ritonavir via potent inhibition of CYP in the liver and/or intestine. A consequence of this inhibition is that significant drug−drug interactions may be observed between ritonavir and other CYP3A4 substrates. Recently we have shown that this interaction can be useful for the elevation of the plasma concentrations of other investigational HIV protease inhibitors (e.g., saquinavir,[38] indinavir,[20] nelfinavir,[21] and VX-478[22]), which are also primarily metabolized by CYP3A.[45] This profound pharmacokinetic enhancement by co-dosing with ritonavir may have implications for the control of HIV infection with dual protease inhibitor therapy and has served as the basis for ongoing clinical trials utilizing dual protease inhibitor therapy (ritonavir plus saquinavir, ritonavir plus nelfinavir, and ritonavir plus indinavir).

Although replacement of the pyridyl groups of A-80987 with thiazolyl groups markedly slowed metabolism, the aqueous solubility of the resulting inhibitors, in particular at pH 4, suffered from the lower pK_a of thiazole vs pyridine. Consequently, the intestinal absorption of analogues such as compounds **120** and **121** were ap-

parently solubility-restricted. Modification of the carbamate linking group to the more soluble *N*-methylurea linker[25] was sufficient to increase oral bioavailability from 0% (**120**) to 78% (**123**, ritonavir). If, however, ritonavir was dosed as a suspension rather than a solution (using organic cosolvents), no plasma levels were detected.[19] These results suggest that a solubility of ca. 1−2 μg/mL must be exceeded in compounds of this type to permit intestinal absorption. Only two compounds (**118** and **141**) that were dosed as suspensions in rats gave significant plasma levels. In general, increased aqueous solubility of HIV protease inhibitors has been associated with higher oral bioavailability.[33,46,47] However, the superior oral bioavailability of ritonavir suggests that if a solubility threshold that allows formulation as a solution is exceeded, metabolism may play a more important role than aqueous solubility in determining the overall bioavailability and the duration of plasma levels in vivo.

The antiviral activity of ritonavir in MT4 cells was ca. 10-fold greater than that of A-80987. This difference was due primarily to increased potency against HIV protease (K_i values of 15 and 164 pM, respectively) and appears to be a result of the additional hydrophobic interaction of the P3 isopropylthiazolyl group of ritonavir with the side chain of valine-82 (V82) of HIV protease. The preliminary X-ray crystal structure of ritonavir bound to HIV protease[19] confirms this interaction and rationalizes the structure−activity relationships observed upon changing the size of the alkyl group at the 2-position of the P3 thiazolyl group in compounds **66**−**79**. Thus, both methyl groups of the isopropylthiazolyl group of ritonavir (and presumably **77**) interact with V82. In the *tert*-butyl analogue (compound **79**), the additional methyl group would project away from V82 and would not be expected to provide an increase in activity over compound **77**. The close interaction of the P3 isopropyl group with V82 also appears to affect the response of HIV to selective pressure during treatment with ritonavir. HIV strains mutated at valine-82 (to alanine, phenylalanine, and threonine) have been shown to be the primary species present during the initial rebound of plasma HIV RNA during suboptimal monotherapy with ritonavir.[39]

Within this series of C_2 symmetry-based inhibitors, the structure of ritonavir appears to be optimized for both anti-HIV activity and pharmacokinetics. Most perturbations in structure led to compounds of inferior properties. Analogues with better or comparable potency (e.g., compounds **121**, **139**, **152**, **166**, **179**, and **180**) generally suffered from diminished oral bioavailability. Only the very close bisoxazolyl analogue **127** was nearly equivalent to ritonavir. Interestingly, the P2 D-valinyl diastereomer of ritonavir was recently discovered to be virtually indistinguishable from ritonavir in activity, pharmacokinetic properties and binding/inhibition of cytochrome P450.[48] Both diastereomers were shown to adopt a similar binding orientation within the active site of HIV protease.

In this study, we used a ratio of the C_{max} obtained after a 10 mg/kg oral dose in rats to the in vitro EC_{50} for anti-HIV activity as one measure by which inhibitors were evaluated. An additional feature not reflected in that ratio is the duration of plasma levels, which was markedly pronounced in ritonavir compared to previous analogues. The importance assigned to these measures was based on the assumption that high levels of any inhibitor must be maintained in the plasma of patients to exert a strong and durable antiviral effect in vivo. Recently, this assumption has been supported by the quantification of high viral turnover in patients[28,29] and in observing that the rate of appearance of mutations in HIV protease in patients who received ritonavir monotherapy inversely correlated with the trough plasma concentrations in those patients.[39] The maintenance of high plasma levels is even more important with inhibitors that are highly bound to human serum proteins.[49,50] In separate studies (A. Molla et al., unpublished results), we discovered that high (≥99%) binding of ritonavir to human serum proteins attenuates the antiviral activity of ritonavir by ca. 20-fold. Addition of human serum to in vitro HIV cultures may give a more accurate projection of the in vivo potency of HIV protease inhibitors. Comparison of this value to the sustained plasma concentrations of a given inhibitor in humans may provide a useful predictor for antiviral efficacy in vivo.

This study details the preclinical studies that led to the identification of the HIV protease inhibitor ritonavir. Clinical trials with ritonavir have produced dramatic declines in plasma HIV RNA and substantial recovery in CD4 cell levels in HIV-infected individuals.[30,31] Accompanying these changes in surrogate markers, administration of ritonavir is associated with increased life-expectancy and time to AIDS-defining clinical events[32] and has recently been licensed by the Food and Drug Administration for the therapy of HIV infection. Ongoing clinical studies using ritonavir in combination with reverse transcriptase inhibitors display evidence of completely blocking the replication of HIV in vivo.[51] Furthermore, dual therapy with ritonavir and other protease inhibitors is under active investigation as an effective, convenient regimen.[52] The important role of ritonavir and other HIV protease inhibitors in the management of HIV infection will thus continue to be demonstrated.

Experimental Section

Synthesis of Monoamines 5 and 6. Synthesis of **5a** and **6a** have been reported.[33] Monoamines **5b**−**g** and **6b**−**e,g** were prepared according to the following general procedure described for **5d** and **6d**. Monoamines **6f** and **6h** were prepared according to the general procedure described for **6h**.

(2*S*,3*S*,5*S*)-2-Amino-5-[*N*-[[(5-thiazolyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (5d) and (2*S*,3*S*,5*S*)-5-Amino-2-[*N*-[[(5-thiazolyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (6d). 5-(Hydroxymethyl)thiazole (**182**) was prepared by a modification of the procedure of Mashraqui and Keehn[53] as follows. A solution of 7.5 g (0.123 mol) of thioformamide and 18.54 g (0.123 mol) of ethyl 2-chloro-2-formylacetate in 250 mL of dry acetone was heated at reflux for 2 h. The solvent was removed in vacuo, and the residue was purified by silica gel chromatography using $CHCl_3$ as an eluent to provide 11.6 g (60%) of ethyl thiazole-5-carboxylate (R_f = 0.25) as a light yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.39 (t, J = 7 Hz, 3 H), 4.38 (q, J = 7 Hz, 2 H), 8.50 (s, 1 H), 8.95 (s, 1 H). A mixture of 76 mmol of $LiAlH_4$ in 250 mL of THF was cooled in an ice bath and treated with a solution of 11.82 g (75.7 mmol) of the above ester in 100 mL of THF dropwise over 1.5 h. After addition, the reaction mixture was stirred for 1 h and treated cautiously with 2.9

mL of water, 2.9 mL of 15% NaOH, and 8.7 mL of water. The solid salts were filtered, and the filtrate was set aside. The crude salts were heated at reflux in 100 mL of EtOAc for 30 min. The resulting mixture was filtered, and the two filtrates were combined, dried over Na_2SO_4, and concentrated in vacuo. The product was purified by silica gel chromatography eluting sequentially with 0%, 2%, 4% CH_3OH in $CHCl_3$, to provide a 75% yield of **182** (R_f = 0.3, 4% CH_3OH in $CHCl_3$) which solidified upon standing: NMR ($CDCl_3$) δ 4.92 (s, 2 H), 7.78 (s, 1 H), 8.77 (s, 1 H); CIMS *m/z* 116 $(M + H)^+$.

A solution of 3.11 g (27 mmol) of **182** and excess 4-methylmorpholine in 100 mL of CH_2Cl_2 was cooled to 0 °C and treated with 8.2 g (41 mmol) of *p*-nitrophenyl chloroformate. After being stirred for 1 h, the reaction mixture was diluted with $CHCl_3$, washed successively with 1 N HCl, saturated aqueous $NaHCO_3$, and saturated brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (1−2% $CH_3OH/CHCl_3$) to provide 5.9 g (78%) of (5-thiazolyl)methyl (4-nitrophenyl)carbonate (R_f 0.5, 4% $CH_3OH/CHCl_3$) as a yellow solid: NMR ($CDCl_3$) δ 5.53 (s, 2 H), 7.39 (dt, J = 9, 3 Hz, 2 H), 8.01 (s, 1 H), 8.29 (dt, J = 9, 3 Hz, 2 H), 8.90 (s, 1 H); CIMS *m/z* 281 $(M + H)^+$. A solution of 500 mg (1.76 mmol) of diamine **2**[33] and 480 mg (1.71 mmol) of the above (4-nitrophenyl)carbonate in 20 mL of THF was stirred at ambient temperature for 4 h. After removal of the solvent in vacuo, the residue was purified by silica gel chromatography using first 2% and then 5% CH_3OH in $CHCl_3$ to provide a mixture of **6d** and **5d**. Silica gel chromatography of the mixture using a gradient of 0, 1, 2% CH_3OH in 98:2 *i*-$PrNH_2$−$CHCl_3$ provided 110 mg (16%) of **6d** (R_f 0.48, 96:2:2 $CHCl_3$−CH_3OH−*i*-$PrNH_2$) and 185 mg (28%) of **5d** (R_f 0.44, 96:2:2 $CHCl_3$−CH_3OH−*i*-$PrNH_2$). **5d**: NMR ($CDCl_3$) δ 1.55 (dt, J = 14, 8 Hz, 1 H), 1.74 (m, 1 H), 2.44 (dd, J = 15, 1 Hz, 1 H), 2.75−3.0 (m, 4 H), 3.44 (m, 1 H), 4.00 (br t, 1 H), 5.28 (m, 3 H), 7.1−7.4 (m, 10 H), 7.86 (s, 1 H), 8.80 (s, 1 H); CIMS *m/z* 426 $(M + H)^+$. **6d**: NMR ($CDCl_3$) δ 1.3−1.6 (m, 2 H), 2.40 (dd, J = 14, 8 Hz, 1 H), 2.78 (dd, J = 5 Hz, 1 H), 2.88 (d, J = 7 Hz, 2 H), 3.01 (m, 1 H), 3.72 (br q, 1 H), 3.81 (br d, J = 10 Hz, 1 H), 5.28 (s, 2 H), 5.34 (br d, J = 9 Hz, 1 H), 7.07 (br d, J = 7 Hz, 2 H), 7.15−7.35 (m, 8 H), 7.87 (s, 1 H), 8.80 (s, 1 H); CIMS *m/z* 426 $(M + H)^+$.

(2*S*,3*S*,5*S*)-2-Amino-5-[*N*-[[(5-pyrimidinyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (5b) and (2*S*,3*S*,5*S*)-5-Amino-2-[*N*-[[(5-pyrimidinyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (6b). 5b: 28%; ^{1}H NMR ($CDCl_3$) δ 1.57 (m, 1 H), 1.75 (m, 1 H), 2.44 (dd, 1 H), 2.78−2.89 (m, 3 H), 2.96 (m, 1 H), 3.44 (m, 1 H), 4.02 (m, 1 H), 5.10 (q, AB, 2 H), 5.40 (br, 1 H), 7.07−7.33 (m, 10 H), 8.73, (s, 2 H), 9.18 (s, 1 H). **6b**: 13%; ^{1}H NMR ($CDCl_3$) δ 1.43 (m, 1 H), 1.53 (m, 1 H), 2.43 (dd, 1 H), 2.78 (dd, 1 H), 2.88 (d, 2 H), 3.04 (m, 1 H), 3.71 (dd, 1 H), 3.83 (d, 1 H), 5.09 (s, 2 H), 5.37 (br d, 1 H), 7.07−7.32 (m, 10 H), 8.74, (s, 2 H), 9.19 (s, 1 H).

(2*S*,3*S*,5*S*)-2-Amino-5-[*N*-[[(3-furanyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (5c) and (2*S*,3*S*,5*S*)-5-Amino-2-[*N*-[[(3-furanyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (6c). 5c: 36%; ^{1}H NMR ($CDCl_3$) δ 1.57 (m, 1 H), 1.75 (d, 1 H), 2.45 (dd, 1 H), 2.80−2.87 (m, 3 H), 2.96 (m, 1 H), 3.46 (m, 1 H), 4.02 (m, 1 H), 4.95 (q, AB, 2 H), 5.14 (br s, 1 H), 7.13−7.43 (m, 13 H). **6c**: 17%; ^{1}H NMR ($CDCl_3$) δ 1.42 (m, 1 H), 1.53 (d, 1 H), 2.40 (dd, 1 H), 2.78 (dd, 1 H), 2.88 (d, 2 H), 3.02 (m, 1 H), 3.71 (dd, 1 H), 4.96 (s, 2 H), 5.28 (br d, 1 H), 7.07−7.46 (m, 13 H).

(2*S*,3*S*,5*S*)-2-Amino-5-[*N*-[[(5-oxazolyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (5e) and (2*S*,3*S*,5*S*)-5-Amino-2-[*N*-[[(5-oxazolyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (6e). A flask was charged with 1.02 g (5.96 mmol) of 5-(diethoxymethyl)oxazole[54] and cooled to 0 °C. A solution of trifluoroacetic acid/CH_2Cl_2 (1:1 (v/v), 6.7 mL) and H_2O (0.39 mL) was added, and the solution was stirred at 0 °C for 10 min. The solvent was removed in vacuo, and the residue was azeotroped with toluene. Silica gel chromatography using a gradient of EtOAc−hexane (20%, 30%, 40%) afforded 0.344 g (59%) of 5-oxazolecarboxaldehyde (**183**) as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ 7.89 (s, 1H), 8.12 (s, 1H), 9.87 (s, 1H); CIMS *m/z* 98 $(M + H)^+$. A solution of 627 mg (6.46 mmol) of **183** in CH_3OH (10 mL) at 0 °C was treated under argon with 247 mg (6.46 mol) of $NaBH_4$. After 5 min, the reaction was quenched with acetone and the solvent was removed in vacuo. Silica gel chromatography using a gradient of CH_3OH−CH_2Cl_2 (5%, 10%) afforded 408 mg (64%) of 5-(hydroxymethyl)oxazole as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.03 (t, J = 6.0 Hz, 1H), 4.70 (d, J = 6.0 Hz, 2H), 7.04 (s, 1H), 7.87 (s, 1H); CIMS *m/z* 117 $(M + NH_4)^+$, 100 $(M + H)^+$. Further reaction according to the procedure outlined above produced **5e** and **6e**. **5e**: ^{1}H NMR (DMSO-d_6) δ 1.16−1.30 (m, 1H), 1.36−1.47 (m, 1H), 2.56−2.66 (m, 2H), 2.75−2.85 (m, 1H), 2.89−3.01 (m, 1H), 3.53−3.71 (m, 3H), 4.97 (d, J = 2.4 Hz, 2H), 7.01 (d, J = 9 Hz, 1H), 7.11−7.32 (m, 14H), 8.36 (s, 1H); CIMS *m/z* 410 $(M + H)^+$. **6e**: ^{1}H NMR (DMSO-d_6) δ 1.20 (m, 1 H), 1.42 (m, 1 H), 2.60 (m, 2 H), 2.80 (m, 1 H), 2.90 (m, 1 H), 4.90 (AB, J = 12, 10 Hz, 2 H), 4.70 (s, 1 H), 7.20 (m, 14 H), 8.36 (s, 1 H); CIMS *m/z* 410 $(M + H)^+$.

(2*S*,3*S*,5*S*)-2-Amino-5-[*N*-[[(2-isopropyl-5-thiazolyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (5g) and (2*S*,3*S*,5*S*)-5-Amino-2-[*N*-[[(2-isopropyl-5-thiazolyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (6g). Prepared from isobutyramide as described for **5d/6d**. **5g**: 7%; R_f 0.25 (1% *i*-$PrNH_2/CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 1.27 (d, J = 7 Hz, 6 H), 1.5−1.7 (m, 2 H), 2.41 (dd, J = 14, 9 Hz, 1 H), 2.56 (dd, J = 14, 9 Hz, 1 H), 2.6−2.8 (m, 4 H), 3.8 (br m, 2 H), 4.45 (br, 1 H), 5.06 (s, 2 H), 7.1−7.3 (m, 11 H), 7.59 (s, 1 H); CIMS *m/z* 468 $(M + H)^+$. **6g**: 11%; R_f 0.37 (1% *i*-$PrNH_2/CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 1.28 (d, J = 7 Hz, 6 H), 1.44 (m, 1 H), 1.63 (m, 1 H), 2.46 (dd, J = 12, 5 Hz, 1 H), 2.55−2.65 (m, 3 H), 2.80 (dd, J = 12, 5 Hz, 1 H), 2.95 (br m, 1 H), 3.55−3.7 (m, 3 H), 5.06 (d, J = 13 Hz, 1 H), 5.11 (d, J = 13 Hz, 1 H), 6.98 (d, J = 9 Hz, 1 H), 7.1−7.3 (m, 10 H), 7.60 (s, 1 H); CIMS *m/z* 468 $(M + H)^+$.

(2*S*,3*S*,5*S*)-5-Amino-2-[*N*-[[(5-isoxazolyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (6h). A mixture of 1.54 g (5.41 mmol) of diamine **2**[33] and 0.673 g (5.41 mmol) of phenylboric acid in anhydrous toluene (130 mL) was heated at reflux under argon for 2 h with removal of H_2O by a Dean−Stark trap. The resulting yellow solution was allowed to cool, and the solvent was removed in vacuo to give an oil which solidified upon standing. The residue was taken up in 90 mL of THF, cooled to −40 °C, and treated dropwise under Ar atmosphere over a period of 1 h with 1.11 g (3.78 mmol) of (5-isoxazolyl)methyl (4-nitrophenyl)carbonate (prepared from 5-(hydroxymethyl)isoxazole in 40 mL of THF). The solution was allowed to warm to −20 °C over the next 0.5 h and then was stirred at 0 °C for 2.5 h and at room temperature for 1 h. After removal of the solvent in vacuo, the residue was taken up in EtOAc (200 mL), washed sequentially with 5% aqueous K_2CO_3 (4 × 25 mL) and saturated brine (25 mL), dried over Na_2SO_4, and concentrated in vacuo. Silica gel chromatography of the residue using a gradient of CH_3OH in $CHCl_3$ (2%, 4%, 6%) afforded a mixture of the desired product and its regioisomer. Purification of the mixture on two consecutive 250 g SiO_2 columns (deactivated with 1% *i*-$PrNH_2$−CH_2Cl_2) with a gradient of *i*-$PrNH_2$−CH_2Cl_2 (0.5%, 1%) afforded **6h** as a sticky solid (0.730 g, 1.78 mmol, 33%): ^{1}H NMR (DMSO-d_6) δ 1.17−1.57 (m, 5H), 2.56−2.69 (m, 2H), 2.75−2.86 (m, 1H), 2.89−3.00 (m, 2H), 3.53−3.71 (m, 3H), 5.06 (s, 2H), 6.32 (d, J = 2.4 Hz, 1H), 7.11−7.30 (m, 10H), 8.54 (d, J = 2.4 Hz, 1H); CIMS *m/z* 410 $(M + H)^+$.

Representative Procedures for Preparation of P3 Heterocyclic End Pieces. Heterocyclic end pieces were prepared according to literature methods or according to the following procedures.

Procedure A. *N*-Methyl-*N*-[(6-ethyl-2-pyridinyl)methyl]amine (184). A solution of 6 g of 4-hydroxy-1-hexene (prepared from propionaldehyde and allylmagnesium bromide) and 18 g of ethyl chloro(hydroxyimino)oxalate in 150 mL of ether was treated over 2.5 h with 21 mL of triethylamine. After addition, stirring was continued for 3 h, and the solvent was

removed in vacuo. Silica gel chromatography using ethyl acetate in CH_2Cl_2 provided 5.5 g of ethyl 5-(2-hydroxybutyl)-2-isoxazoline-3-carboxylate (**184a**): ^{1}H NMR ($CDCl_3$) δ 0.96 (t, J = 7 Hz, 3 H), 1.37 (t, J = 7 Hz, 3 H), 1.55 (m, 2 H), 1.77 (ddd, J = 14, 6, 3 Hz, 1 H), 1.90 (m, 1 H), 2.93 (td, J = 17, 8 Hz, 1 H), 3.33 (dd, J = 17, 11 Hz, 1 H), 3.78 (m, 1 H), 4.35 (q, J = 7 Hz, 2 H), 5.02 (m, 1 H). Swern oxidation (as described below for the preparation of **184e**) of **184a** followed by silica gel chromatography using 5% ethyl acetate in CH_2Cl_2 provided 5.05 g (92%) of ethyl 5-(2-oxobutyl)-2-isoxazoline-3-carboxylate (**184b**): ^{1}H NMR ($CDCl_3$) δ 1.07 (t, J = 7 Hz, 3 H), 1.36 (t, J = 7 Hz, 3 H), 2.49 (q, J = 7 Hz, 2 H), 2.71 (dd, J = 17, 7 Hz, 1 H), 2.87 (dd, J = 18, 8 Hz, 1 H), 3.03 (dd, J = 17, 6 Hz, 1 H), 3.43 (dd, J = 18, 11 Hz, 1 H), 4.35 (q, J = 7 Hz, 2 H), 5.16 (m, 1 H). A solution of 1.05 g (4.9 mmol) of **184b** in 150 mL of ethanol was treated with 4 mL of Raney nickel and 1 mL of 48% HBF_4 and stirred under 1 atm of H_2. After 6 h, the mixture was filtered, concentrated in vacuo, and purified by silica gel chromatography using 10% ethyl acetate in CH_2Cl_2 to provide 430 mg (48%) of ethyl 6-ethylpyridine-2-carboxylate (**184c**): ^{1}H NMR ($CDCl_3$) δ 1.34 (t, J = 8 Hz, 3 H), 1.43 (t, J = 7 Hz, 3 H), 2.95 (q, J = 8 Hz, 2 H), 4.48 (q, J = 7 Hz, 2 H), 7.36 (d, J = 7 Hz, 1 H), 7.74 (t, J = 7 Hz, 1 H), 7.94 (d, J = 7 Hz, 1 H). A solution of 1.2 g (6.7 mmol) of **184c** in 30 mL of THF was cooled to 0 °C and treated with 6.7 mL (6.7 mmol) of 1 M $LiAlH_4$ in THF. After 1 h, the mixture was sequentially quenched with 0.5 mL of water, 15% NaOH, and 1 mL of water, filtered, and concentrated in vacuo. Silica gel chromatography using methanol in CH_2Cl_2 provided 870 mg of 6-ethyl-2-(hydroxymethyl)pyridine (**184d**): ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J = 8 Hz, 3 H), 2.87 (q, J = 8 Hz, 2 H), 4.25 (br s, 1 H), 4.74 (s, 2 H), 7.07 (d, J = 7 Hz, 1 H), 7.11 (d, J = 7 Hz, 1 H), 7.64 (t, J = 7 Hz, 1 H). A solution of 0.67 mL (9.4 mmol) of dry DMSO in 10 mL of CH_2Cl_2 was cooled to −78 °C, treated dropwise with 0.39 mL (4.5 mmol) of oxalyl chloride, stirred under N_2 for 15 min, treated with a solution of 530 mg (3.9 mmol) of **184d** in 10 mL of CH_2Cl_2, and stirred for 20 min. After dropwise addition of 2.6 mL (18.9 mmol) of triethylamine, the solution was stirred for 20 min, quenched with water, and extracted with three portions of CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated, and the residue was purified on silica gel using ethyl acetate in CH_2Cl_2 to provide 465 mg (89%) of 6-ethyl-2-pyridinecarboxaldehyde (**184e**): ^{1}H NMR ($CDCl_3$) δ 1.36 (t, J = 7 Hz, 3 H), 2.94 (q, J = 7 Hz, 2 H), 7.40 (m, 1 H), 7.79 (m, 2 H), 10.07 (s, 1 H); CIMS m/z 136 $(M + H)^+$. A solution of 460 mg (3.4 mmol) of **184e** in 32 mL of methanol in a high-pressure flask was treated with 6 mL of methylamine. After 16 h of stirring, the solution was treated with 115 mg of 10% Pd/C and stirred under 4 atm of H_2 for 4 h. After filtration, the filtrate was concentrated in vacuo and purified by silica gel chromatography using 94:4:2 CH_2Cl_2−CH_3OH−isopropylamine mixtures to provide 379 mg (74%) of **184**: ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 7 Hz, 3 H), 2.54 (s, 3 H), 2.80 (q, J = 7 Hz, 2 H), 3.00 (br, 1 H), 3.90 (s, 2 H), 7.06 (d, J = 8 Hz, 1 H), 7.13 (d, J = 8 Hz, 1 H), 7.57 (t, J = 8 Hz, 1 H); CIMS m/z 151 $(M + H)^+$.

Procedure B. 6-Ethyl-2-(hydroxymethyl)pyridine (185). Ethyl 6-ethylpyridine-2-carboxylate was prepared according to the procedure of Kanemasa et al.[55] A solution of the above ester (1.2 g, 6.7 mmol) in 30 mL of THF was cooled to 0 °C and treated with 6.7 mL of 1 M $LiAlH_4$ in THF. The resulting solution was stirred for 1 h, quenched by sequential addition of 0.5 mL of H_2O, 0.5 mL of 15% NaOH, and 1 mL of H_2O, filtered, and concentrated in vacuo. Flash chromatography using 5−10% MeOH−$CHCl_3$ provided 870 mg (95%) of **185**: ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J = 7 Hz, 3 H), 2.87 (q, J = 7 Hz, 2 H), 4.22 (br, 1 H), 4.74 (s, 2 H), 7.06 (d, J = 8 Hz, 1 H), 7.11 (d, J = 8 Hz, 1 H), 7.63 (t, J = 8 Hz, 1 H).

Procedure C. 6-[(*tert*-Butyloxycarbonyl)amino]-3-(hydroxymethyl)pyridine (186). A solution of 2.013 g (9.32 mmol) of methyl 6-aminonicotinate, 2.235 g (10.25 mmol) of di-*tert*-butyl dicarbonate, and 122 mg (1.0 mmol) of 4-(dimethylamino)pyridine (DMAP) in acetonitrile (80 mL) was stirred at ambient temperature for 4 h, and then additional di-*tert*-butyl dicarbonate (450 mg) was added. The reaction mixture was stirred for an additional hour at ambient temperature and stored at 0 °C for 16 h. After concentration of the solvent, silica gel chromatography using a gradient (5%, 10%) of EtOAc in CH_2Cl_2 provided 1.85 g (79%) of methyl 6-[(*tert*-butyloxycarbonyl)amino]nicotinate, which was reduced in a manner analogous to the preparation of **185** to provide 1.064 g of **186**.

Procedure D. *N*-Methyl-*N*-[(2-isopropyl-4-thiazolyl)-methyl]amine (187). A suspension of 100 g (1.15 mol) of isobutyramide in 4 L of diethyl ether was stirred vigorously and treated in portions with 51 g (0.115 mol) of P_4S_{10}. The resulting mixture was stirred at ambient temperature for 2 h, filtered, and concentrated in vacuo to provide 94.2 g (80%) of crude 2-methylpropane thioamide (**187a**), which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 1.08 (d, J = 7 Hz, 6 H), 2.78 (heptet, J = 7 Hz, 1 H), 9.06 (br, 1 H), 9.30 (br, 1 H); CIMS m/z 104 $(M + H)^+$. A mixture of 94 g (0.91 mol) of **187a**, 116 g (0.91 mol) of 1,3-dichloroacetone, and 110 g (0.91 mol) of $MgSO_4$ in 1.6 L of acetone was heated at reflux for 3.5 h. The resulting mixture was allowed to cool and filtered, and the solvent was removed in vacuo to provide crude 4-(chloromethyl)-2-isopropylthiazole hydrochloride (**187b**) as a yellow oil: ^{1}H NMR (DMSO-d_6) δ 1.32 (d, J = 7 Hz, 6 H), 3.27 (heptet, J = 7 Hz, 1 H), 4.78 (s, 2 H), 7.61 (s, 1 H); CIMS m/z 176 $(M + H)^+$. A solution of 40 g of **187b** in 100 mL of water was added dropwise with stirring to 400 mL of 40% aqueous methylamine. The resulting solution was stirred for 1 h and then concentrated in vacuo. The residue was taken up in $CHCl_3$, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by silica gel chromatography using 10% CH_3OH in $CHCl_3$ provided 21.35 g (55%) of **187**: ^{1}H NMR (DMSO-d_6) δ 1.34 (d, J = 7 Hz, 6 H), 2.56 (s, 3 H), 3.30 (heptet, J = 7 Hz, 1 H), 4.16 (s, 2 H), 7.63 (s, 1 H); CIMS m/z 171 $(M + H)^+$.

Procedure E. 4-(Hydroxymethyl)-2-isopropylthiazole (188). A solution of 2.35 g (23 mmol) of **187a** and 2.89 mL (23 mmol) of ethyl bromopyruvate in 75 mL of acetone was treated with excess $MgSO_4$ and heated at reflux for 2.5 h. The resulting mixture was allowed to cool, filtered, and concentrated in vacuo to an oil, which was taken up in $CHCl_3$, washed sequentially with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified by chromatography on silica gel using $CHCl_3$ as an eluent to provide 3.96 g (86%) of ethyl 2-isopropyl-4-thiazolecarboxylate (**188a**, R_f 0.21, $CHCl_3$) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.41 (t, J = 8 Hz, 3 H), 1.42 (d, J = 7 Hz, 6 H), 3.43 (heptet, J = 7 Hz, 1 H), 4.41 (q, J = 8 Hz, 2 H), 8.05 (s, 1 H); CIMS m/z 200 $(M + H)^+$. A solution of 10 mL (10 mmol) of $LiAlH_4$ in $PhCH_3$ was diluted in a dry flask under N_2 atmosphere with 75 mL of THF. The resulting mixture was cooled to 0 °C and treated dropwise with a solution of 3.96 g (20 mmol) of **188a** in 10 mL of THF. After addition, the solution was stirred at 0 °C for 3 h, diluted with ether, and treated with a small amount of aqueous Rochelle's salt. After stirring, the slurry was filtered and washed with EtOAc, and the combined filtrates were concentrated in vacuo. The residue was purified by silica gel chromatography using 2% CH_3OH in $CHCl_3$ to provide 2.18 g (69%) of **188** (R_f 0.58, 4% CH_3OH in $CHCl_3$): ^{1}H NMR ($CDCl_3$) δ 1.39 (d, J = 7 Hz, 6 H), 2.94 (br, 1 H), 3.31 (heptet, J = 7 Hz, 1 H), 4.74 (s, 2 H), 7.04 (s, 1 H); CIMS m/z 158 $(M + H)^+$.

Procedure F. 4-(Hydroxymethyl)-2-isopropyloxazole (189). A mixture of 10 g (63 mmol) of L-serine methyl ester hydrochloride in 280 mL of dichloromethane was cooled to 0 °C and treated with 22 mL (153 mmol) of triethylamine. The resulting mixture was treated dropwise with 8.1 mL (76 mmol) of isobutyryl chloride, stirred for 20 min at 0 °C and at ambient temperature for 40 min, partitioned between dichloromethane and aqueous HCl, washed with aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, and concentrated in vacuo. Silica gel chromatography using 2% methanol in CH_2Cl_2 provided 6.33 g (53%) of *N*-isobutyryl-L-serine methyl ester (**189a**): ^{1}H NMR ($CDCl_3$) δ 1.20 (d, J = 7 Hz, 6 H), 2.47 (heptet, J = 7 Hz, 1 H), 2.57 (t, J = 6 Hz, 1 H), 3.80 (s, 3 H), 3.96 (m,

2 H), 4.68 (m, 1 H), 6.42 (br d, 1 H); CIMS *m/z* 190 $(M + H)^+$. In a dry, three-neck flask, 90 mL of anhydrous CH_2Cl_2 was cooled under Ar atmosphere to −78 °C and treated with 6.6 mL (39 mmol) of trifluorosulfonic anhydride. The resulting solution was treated dropwise with a solution of 10.5 g (52 mmol) of diphenyl sulfoxide[56] in 90 mL of CH_2Cl_2, stirred for 30 min, and then treated sequentially with 55 g (260 mmol) of K_3PO_4 and a solution of 4.9 g (26 mmol) of **189a** in 50 mL of CH_2Cl_2. After being stirred for 30 min, the mixture was warmed to 0 °C for 20 min, quenched with water, extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated in vacuo. Silica gel chromatography using ethyl acetate in CH_2Cl_2 provided 3.5 g (79%) of methyl 2-isopropyl-2-oxazoline-4-carboxylate (**189b**): ^{1}H NMR ($CDCl_3$) δ 1.22 (d, J = 7 Hz, 6 H), 2.64 (heptet, J = 7 Hz, 1 H), 3.79 (s, 3 H), 4.39 (dd, J = 10, 8 Hz, 1 H), 4.47 (t, J = 8 Hz, 1 H), 4.72 (dd, J = 10, 8 Hz, 1 H); CIMS *m/z* 172 $(M + H)^+$. A solution of 2.53 g (14.8 mmol) of **189b** in 100 mL of anhydrous toluene was treated with 7.4 g of NiO_2 and heated under Ar atmosphere to 60 °C for 30 min. After being allowed to cool, the mixture was filtered, concentrated in vacuo, and purified by silica gel chromatography using ethyl acetate in CH_2Cl_2 to provide 940 mg (38%) of methyl 2-isopropyl-1,3-oxazole-4-carboxylate (**189c**): ^{1}H NMR ($CDCl_3$) δ 1.38 (d, J = 7 Hz, 6 H), 3.15 (heptet, J = 7 Hz, 1 H), 3.91 (s, 3 H), 8.16 (s, 1 H); CIMS *m/z* 170 $(M + H)^+$. A solution of 1.20 g (7.1 mmol) of **189c** in 100 mL of THF was cooled to −25 °C and treated with 7.1 mL (7.1 mmol) of lithium aluminum hydride in THF. After 10 min, the solution was sequentially quenched with water (0.5 mL), 15% NaOH (0.5 mL), and water (1.07 mL) and filtered. The residue was washed with ethyl acetate, and the combined filtrates were concentrated in vacuo and purified by silica gel chromatography using methanol in CH_2Cl_2 to give 0.74 g (74%) of **189**: ^{1}H NMR ($CDCl_3$) δ 1.85 (d, J = 7 Hz, 6 H), 2.47 (t, J = 6 Hz, 1 H), 3.08 (heptet, J = 7 Hz, 1 H), 4.58 (d, J = 6 Hz, 2 H), 7.49 (s, 1 H); CIMS *m/z* 142 $(M + H)^+$.

Procedure G. 2-Isopropyl-4-[(*N*-methylamino)methyl]-oxazole (190). A mixture of isobutyramide (9.88 g, 112 mmol), 1,3-dichloroacetone (10.0 g, 75 mmol), $NaHCO_3$ (9.43 g, 112 mmol), and $MgSO_4$ (18.0 g, 150 mmol) in 130 mL of acetone was heated at reflux under argon for 63 h. After being allowed to cool to ambient temperature, the resulting mixture was filtered and concentrated in vacuo to a dark brown semisolid. The residue was purified by silica gel chromatography using a gradient of EtOAc−CH_2Cl_2 (5%, 10%, 20%, 40%) to obtain 6.06 g (46%) of 4-(chloromethyl)-4-hydroxy-2-isopropyloxazoline (**190a**) as an orange liquid: ^{1}H NMR ($CDCl_3$) δ 1.20−1.28 (m, 6H), 2.56−2.72 (m, 1H), 3.70 (s, 2H), 4.18 (d, J = 9.6 Hz, 1H), 4.38 (d, J = 9.6 Hz, 1H); CIMS *m/z* 178, 180 $(M + H)^+$. A solution of 4.88 g (27.5 mmol) of **190a** in 1,2-dichloroethane (20 mL) was added to a solution of 2.40 mL (32.9 mol) of $SOCl_2$ in 1,2-dichloroethane (80 mL) at 0 °C under argon. The resulting solution was heated to 70 °C for 15 min, allowed to cool to ambient temperature, and concentrated in vacuo to give crude 4-(chloromethyl)-2-isopropyloxazole (**190b**, 4.20 g, 0.0263 mol, 96%) as a brown semisolid: ^{1}H NMR ($CDCl_3$) δ 1.36 (d, J = 7.5 Hz, 6H), 3.03−3.18 (m, 1H), 4.50 (s, 2H), 7.56 (s, 1H); CIMS *m/z* 160, 162 $(M + H)^+$. Compound **190b** was treated according to procedure A above to provide **190** in 71% yield: ^{1}H NMR ($CDCl_3$) δ 1.33 (d, J = 6.9 Hz, 6H), 2.46 (s, 3H), 2.99−3.14 (m, 1H), 3.64 (s, 2H), 7.42 (s, 1H); CIMS *m/z* 155 $(M + H)^+$, 172 $(M + NH_4)^+$.

Procedure H. Ethyl 3-(2-Isopropyl-4-thiazolyl)propenoate (191). A solution of 3.10 g (15.6 mmol) of **188a** in 50 mL of dichloromethane was cooled to −78 °C and treated dropwise over 1.5 h with 15.6 mL of diisobutylaluminum hydride (1.5 M in toluene). After addition, the solution was stirred for 0.5 h and quenched with 5 mL of methanol followed by 15 mL of Rochelle's salt. After being allowed to warm, the mixture was filtered, and the filter cake was washed with chloroform. The combined filtrate was washed with aqueous Rochelle's salt, dried over Na_2SO_4, and concentrated in vacuo to give 1.37 g (56%) of crude 2-isopropyl-4-thiazolecarboxaldehyde. A slurry of 10 mmol of prewashed NaH in 25 mL of THF was cooled to 0 °C under N_2 and treated dropwise with 3.24 mL (8.84 mmol) of triethyl phosphonoacetate. After 10 min, a solution of the above aldehyde in 25 mL of THF was added, and the solution was allowed to stir for 25 min while warming to ambient temperature. After quenching with 100 mL of saturated NH_4Cl, the mixture was extracted with three portions of EtOAc, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography using 5−10% EtOAc in hexane provided 1.61 g (81%) of **191**: R_f 0.58 (20% EtOAc−hexane); ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J = 7 Hz, 3 H), 1.42 (d, J = 7 Hz, 6 H), 3.32 (heptet, J = 7 Hz, 1 H), 4.26 (q, J = 7 Hz, 2 H), 6.75 (d, J = 15 Hz, 1 H), 7.29 (s, 1 H), 7.58 (d, J = 15 Hz, 1 H).

Procedure I. 2-(4-Morpholinyl)-4-(hydroxymethyl)-thiazole (192). A solution of 3.35 g (18.8 mmol) of thiocarbonyldiimidazole in 100 mL of THF was treated with 0.82 mL (9.4 mmol) of morpholine. After being stirred at ambient temperature for 3.5 h, an additional 0.82 mL portion of morpholine was added, and stirring was continued. After 6 h, the solution was treated with excess concentrated aqueous ammonia and stirred overnight. The resulting solution was concentrated in vacuo, taken up in $CHCl_3$, separated from the aqueous phase, dried over Na_2SO_4, and concentrated. Purification of the residue by silica gel chromatography using EtOAc provided 1.85 g (76%) of 4-[(amino)thiocarbonyl]morpholine (R_f 0.17,10% CH_3OH in $CHCl_3$) as a white solid: ^{1}H NMR ($CDCl_3$) δ 3.76 (m, 4 H), 3.83 (m, 4 H), 5.75 (br, 2 H); CIMS *m/z* 147 $(M + H)^+$. The above thiourea was converted as described above for **188** to give **192** (R_f 0.16, 4% CH_3OH in $CHCl_3$) in 34% overall yield: ^{1}H NMR ($CDCl_3$) δ 2.44 (br, 1 H), 3.46 (t, J = 5 Hz, 4 H), 3.81 (t, J = 5 Hz, 1 H), 4.55 (br s, 2 H), 6.45 (s, 1 H); CIMS *m/z* 200 $(M + H)^+$.

Procedure J. 2-(Hydroxymethyl)-4-isopropylthiazole (193). A mixture of 2.11 g (12.8 mmol) of 1-bromo-3-methylbutan-2-one, 1.0 g (12.8 mmol) of ethyl thiooxamate, and 1.70 g (14 mmol) of $MgSO_4$ in 50 mL of acetone was heated at reflux for 3 h. After being allowed to cool, the mixture was filtered, concentrated in vacuo, and purified by silica gel chromatography using $CHCl_3$ to provide 0.29 g (11%) of ethyl 4-isopropyl-2-thiazolecarboxylate (R_f 0.9, 4% CH_3OH in $CHCl_3$): ^{1}H NMR (DMSO-d_6) δ 1.27 (d, J = 7 Hz, 6 H), 1.32 (t, J = 7 Hz, 3 H), 3.12 (heptet, J = 7 Hz, 1 H), 4.37 (q, J = 7 Hz, 2 H), 7.73 (s, 1 H); CIMS *m/z* 200 $(M + H)^+$. The above ester was reduced as described for compound **188** to give a 96% yield of **193** (R_f 0.3, 5% CH_3OH in $CHCl_3$).

Procedure K. 5-(Hydroxymethyl)-2-isopropylthiazole (194). A mixture of 5.0 g (48 mmol) of thioisobutyramide, 7.3 g (48 mmol) of ethyl α-chloroformylacetate, and 5.8 g (48 mmol) of $MgSO_4$ in 200 mL of acetone was heated to reflux for 3 h. After being allowed to cool, the mixture was filtered, concentrated in vacuo, and purified by flash chromatography using 9:1 hexane−EtOAc to give 8.0 g (83%) of ethyl 2-isopropyl-5-thiazolecarboxylate. A solution of 4.0 g (20 mmol) of this ester in 20 mL of anhydrous THF was added dropwise to 20 mL (20 mmol) of a cooled (0 °C) 1 M solution of $LiAlH_4$ in THF. After addition, the solution was stirred for 1 h, allowed to warm, and quenched with solid Rochelle's salt followed by ethyl acetate until bubbling ceased. The mixture was filtered and concentrated in vacuo. Flash chromatography using 3% MeOH in $CHCl_3$ provided 0.8 g (25%) of **194**: ^{1}H NMR (DMSO-d_6) δ 1.30 (d, J = 7 Hz, 6 H), 3.22 (heptet, J = 7 Hz, 1 H), 4.61 (dd, J = 6, 1 Hz, 2 H), 5.45 (t, J = 6 Hz, 1 H), 7.48 (t, J = 1 Hz, 1 H); CIMS *m/z* 158 $(M + H)^+$.

Procedure L. 5-*tert*-Butyl-3-(hydroxymethyl)isoxazole (195). A solution of 5.0 g (25 mmol) of ethyl 5-*tert*-butylisoxazole-3-carboxylate (Maybridge) in 150 mL of THF was cooled to −30 °C and treated with 25 mL (25 mmol) of lithium aluminum hydride in THF. After 30 min, the solution was sequentially quenched with 10 mL of acetone, partitioned between ethyl acetate and saturated Rochelle's salt, dried over Na_2SO_4, and concentrated in vacuo. Silica gel chromatography using ethyl acetate in CH_2Cl_2 provided 3.65 g (93%) of **195**: ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 9 H), 2.05 (t, J = 6 Hz, 1 H), 4.73 (d, J = 6 Hz, 2 H), 6.00 (s, 1 H); CIMS *m/z* 156 $(M + H)^+$.

Procedure M. *N*-Methyl-*N*-[(5-*tert*-butyl-3-isoxazolyl)-methyl]amine (196). A solution of 1.0 g (6.4 mmol) of **195** and 1.25 mL (9.0 mmol) of triethylamine in 60 mL of CH_2Cl_2 was cooled to 0 °C and treated with 0.61 mL (7.7 mmol) of methanesulfonyl chloride. After 10 min, the solvent was removed in vacuo. The residue was purified by silica gel chromatography to provide 1.49 g (99%) of 5-*tert*-butyl-3-[(methanesulfonyloxy)methyl]isoxazole (**196a**): ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 9 H), 3.08 (s, 3 H), 5.26 (s, 2 H), 6.10 (s, 1 H); CIMS *m*/*z* 251 $(M + NH_4)^+$. A solution of 754 mg (3.2 mmol) of **196a** in 7.5 mL of anhydrous methylamine was stirred in a high-pressure flask at ambient temperature for 4.5 days. After removal of the solvent in vacuo, the residue was purified by silica gel chromatography using $CH_2Cl_2-CH_3OH-$isopropylamine to provide 490 mg (90%) of **196**: ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 9 H), 2.48 (s, 3 H), 3.78 (s, 2 H), 5.94 (s, 1 H); CIMS *m*/*z* 169 $(M + H)^+$.

Procedure N. 3-Methoxy-5-(hydroxymethyl)isoxazole (197). To a solution of 2 g (13.9 mmol) of methyl 3-hydroxy-5-isoxazolecarboxylate in 40 mL of benzene was added 4 g of $AgCO_3$ and 2.5 mL of CH_3I. The reaction mixture was stirred at ambient temperature in the dark for 16 h. The resulting mixture was filtered through a pad of Celite, and the filtrate was concentrated in vacuo. Silica gel chromatography using 1:1 ethyl acetate−hexane provided 1.61 g (74%) of methyl 3-methoxy-5-isoxazolecarboxylate (**197a**): ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3 H), 4.03 (s, 3 H), 6.53 (s, 1 H). To a solution of 1.6 g (10.2 mmol) of **197a** in 20 mL of THF at −60 °C was added 1.6 mL (1.6 mmol) of $LiAlH_4$ in THF. After 15 min, the reaction was carefully quenched with water, and extractive workup followed by silica gel chromatography using 5% MeOH in $CHCl_3$ provided 0.85 g (65%) of **197**: ^{1}H NMR ($CDCl_3$) δ 1.95 (t, *J* = 6 Hz, 1 H), 3.96 (s, 2 H), 4.66 (d, *J* = 6 Hz, 2 H), 5.88 (s, 1 H); CIMS *m*/*z* 130 $(M + H)^+$.

Procedure O. *N*-Cyclopropyl-*N*-[(2-isopropyl-4-thiazolyl)methyl]amine (198). A solution of 1.8 g (10.2 mmol) of **187b** in 10 mL of $CHCl_3$ was added dropwise with stirring to 10 mL of cyclopropylamine. The resulting solution was stirred at ambient temperature for 16 h, concentrated in vacuo, and purified by silica gel chromatography using 5% CH_3OH in $CHCl_3$ to provide 0.39 g (19%) of **198**: ^{1}H NMR (DMSO-d_6) δ 0.24 (m, 2 H), 0.35 (m, 2H),1.30 (d, *J* = 7 Hz, 6 H), 2.10 (tt, *J* = 12, 3 Hz, 1 H), 3.23 (heptet, *J* = 7 Hz, 1 H), 3.77 (s, 2 H), 7.21 (s, 1 H); CIMS *m*/*z* 197 $(M + H)^+$.

Procedure P. 5-(Hydroxyethyl)-2-isopropylthiazole (199). Ester **200** was reduced with $LiAlH_4$ in the manner described for **194** to give **199** in 47% yield: ^{1}H NMR ($CDCl_3$) δ 1.40 (d, *J* = 7 Hz, 6 H), 2.96 (t, *J* = 6 Hz, 2 H), 3.30 (heptet, *J* = 7 Hz, 1 H), 3.93 (t, *J* = 6 Hz, 2 H), 6.83 (s, 1 H); CIMS *m*/*z* 172 $(M + H)^+$.

Procedure Q. Ethyl (2-Isopropyl-4-thiazolyl)acetate (200). A mixture of 1.5 g (14.5 mmol) of thioisobutyramide, 2.4 g (14.5 mmol) of ethyl α-chloroformyl acetate and 1.75 g (14.5 mmol) of $MgSO_4$ in 50 mL of acetone was heated to reflux for 16 h. After being allowed to cool, the mixture was filtered, concentrated in vacuo, and purified by flash chromatography using 1% MeOH in $CHCl_3$ to give 1.57 g (51%) of **200**: ^{1}H NMR (DMSO-d_6) δ 1.19 (t, *J* = 7 Hz, 3 H), 1.30 (d, *J* = 7 Hz, 6 H), 3.24 (heptet, *J* = 7 Hz, 1 H), 3.75 (s, 2 H), 4.08 (q, *J* = 7 Hz, 2 H), 7.30 (s, 1 H); CIMS *m*/*z* 214 $(M + H)^+$.

Procedure R. *N*-Ethyl-*N*-[(2-isopropyl-4-thiazolyl)-ethyl]amine (201). A solution of 0.20 g (1.2 mmol) of **199** in 5 mL of THF was cooled to 0 °C and treated with 0.09 mL (1.2 mmol) of methanesulfonyl chloride and 0.16 mL (1.2 mmol) of triethylamine. After being stirred for 30 min, the solution was treated with 10 mL of 40% aqueous ethylamine, heated to reflux for 4 h, and then stirred at ambient temperature for 16 h. The resulting mixture was diluted with EtOAc, washed sequentially with aqueous $NaHCO_3$ and H_2O, dried over Na_2SO_4, and concentrated in vacuo to provide crude **201** which was of sufficient purity for further reaction: ^{1}H NMR ($CDCl_3$) δ 1.11 (t, *J* = 7 Hz, 3 H), 1.38 (d, *J* = 7 Hz, 6 H), 2.70 (q, *J* = 7 Hz, 2 H), 2.96 (m, 4 H), 3.30 (heptet, *J* = 7 Hz, 1 H), 6.80 (s, 1 H); CIMS *m*/*z* 199 $(M + H)^+$.

Representative procedures for the synthesis of N-substituted amino esters. Preparation of the esters which are precursors leading to compounds **5**−**12** has been reported.[33] Other esters were prepared by the following representative procedures. Spectral characterization for the esters is provided as Supporting Information.

Procedure S. *N*-[[*N*-Methyl-*N*-[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl]-L-valine Methyl Ester (202). A solution of 66 g (0.328 mol) of 4-nitrophenyl chloroformate in 1.2 L of CH_2Cl_2 was cooled to 0 °C and treated with L-valine methyl ester hydrochloride. The resulting mixture was treated slowly, with stirring, with 69 mL (0.63 mol) of 4-methylmorpholine. The resulting solution was allowed to slowly warm to ambient temperature and was stirred overnight. After washing with three portions of 10% aqueous $NaHCO_3$, the solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography by eluting with $CHCl_3$ to provide *N*-[[(4-nitrophenyl)-oxy]carbonyl]-L-valine methyl ester: ^{1}H NMR (DMSO-d_6) δ 0.94 (d, *J* = 7 Hz, 3 H), 0.95 (d, *J* = 7 Hz, 3 H), 2.12 (octet, *J* = 7 Hz, 1 H), 3.69 (s, 3 H), 4.01 (dd, *J* = 8, 6 Hz, 1 H), 7.41 (dt, *J* = 9, 3 Hz, 2 H), 8.27 (dt, *J* = 9, 3 Hz, 2 H), 8.53 (d, *J* = 8 Hz, 1 H); CIMS *m*/*z* 314 $(M + NH_4)^+$. A solution of 15.7 g (92 mmol) of **187** in 200 mL of THF was combined with a solution of 20.5 g (69 mmol) of the above *p*-nitrophenyl carbamate. The resulting solution was treated with 1.6 g of DMAP and 12.9 mL (92 mmol) of triethylamine, heated at reflux for 2 h, allowed to cool, and concentrated in vacuo. The residue was taken up in CH_2Cl_2, washed extensively with 5% aqueous K_2CO_3, dried over Na_2SO_4, and concentrated in vacuo. Purification by silica gel chromatography using $CHCl_3$ as an eluent provided 16.3 g (54%) of **202**: R_f 0.26 (5% CH_3OH/$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 0.88 (d, *J* = 7 Hz, 3 H), 0.92 (d, *J* = 7 Hz, 3 H), 1.32 (d, *J* = 7 Hz, 6 H), 2.05 (octet, *J* = 7 Hz, 1 H), 2.86 (s, 3 H), 3.25 (heptet, *J* = 7 Hz, 1 H), 3.61 (s, 3 H), 3.96 (dd, *J* = 8, 7 Hz, 1 H), 4.44 (AA′, 2 H), 6.58 (d, *J* = 8 Hz, 1 H), 7.24 (s, 1 H); CIMS *m*/*z* 328 $(M + H)^+$.

Procedure T. *N*-[[(2-Isopropyl-4-thiazolyl)methoxy]-carbonyl]-L-alanine Methyl Ester (203). A solution of 1.12 g (5.56 mmol) of 4-nitrophenyl chloroformate in 20 mL of CH_2Cl_2 was cooled to 0 °C and treated sequentially with 0.8 g (5.1 mmol) of **188** and 0.6 mL (5.6 mmol) of 4-methylmorpholine. The resulting solution was stirred at 0 °C for 1 h, diluted with CH_2Cl_2, washed with three portions of aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo to give crude (4-(hydroxymethyl)-2-isopropylthiazolyl)methyl 4-nitrophenyl carbonate. A portion (0.53 g, 1.65 mmol) of the above carbonate was taken up in 20 mL of $CHCl_3$, treated with 0.23 g (1.67 mmol) of L-alanine methyl ester hydrochloride and 0.36 mL (3.3 mmol) of 4-methylmorpholine, and heated at reflux for 16 h. After being allowed to cool, the solvent was removed in vacuo, and the residue was purified by silica gel chromatography using 2% CH_3OH in $CHCl_3$ to provide 0.45 g (94%) of **203**: R_f 0.43 (5% CH_3OH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 1.26 (d, *J* = 8 Hz, 3 H), 1.32 (d, *J* = 7 Hz, 6 H), 3.27 (heptet, *J* = 7 Hz, 1 H), 3.63 (s, 3 H), 4.10 (pentet, *J* = 8 Hz, 1 H), 5.02 (s, 2 H), 7.47 (s, 1 H), 7.81 (d, *J* = 8 Hz, 1 H); CIMS *m*/*z* 287 $(M + H)^+$.

Procedure U. *N*-[[(2-Isopropyl-5-thiazolyl)methoxy-carbonyl]-L-valine Methyl Ester (204). A solution of 0.40 g (2.75 mmol) of **194**, 2.9 mmol of α-*N*-carbonylvaline methyl ester[24] and 0.28 mmol of DMAP in 15 mL of CH_2Cl_2 was heated at reflux for 5 h. The resulting solution was washed successively with 10% citric acid, aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuo. Silica gel chromatography of the residue using 5% EtOAc in $CHCl_3$ provided 0.25 g (29%) of **204** (R_f 0.8, 5% CH_3OH in $CHCl_3$): ^{1}H NMR δ 0.89 (d, *J* = 7 Hz, 6 H), 0.95 (d, *J* = 7 Hz, 3 H), 0.97 (d, *J* = 7 Hz, 3 H), 2.14 (m, 1 H), 3.33 (heptet, *J* = 7 Hz, 1 H), 3.74 (s, 3 H), 4.30 (dd, *J* = 9, 5 Hz, 1 H), 5.23 (s, 2 H), 5.25 (br d, 1 H), 7.63 (s, 1 H); CIMS *m*/*z* 315 $(M + H)^+$.

Procedure V. *N*-[[*N*-Ethyl-*N*-[(2-isopropyl-4-thiazolyl)-methyl]amino]carbonyl]glycine Ethyl Ester (205). A solution of 1.47 g (8 mmol) of *N*-ethyl-*N*-[(2-isopropyl-4-

thiazolyl)methyl]amine (prepared according to procedure D) and 0.9 mL (8 mmol) of ethyl isocyanatoacetate in 30 mL of THF was heated at reflux for 16 h. After being allowed to cool, the solution was concentrated in vacuo, taken up in CH_2Cl_2, washed with two portions of 10% aqueous $NaHCO_3$ and one portion of saturated brine, dried over Na_2SO_4, and concentrated. Silica gel chromatography using 0.5% methanol in $CHCl_3$ provided 0.95 g (38%) of **205**: R_f 0.70 (10% CH_3OH/$CHCl_3$).

Representative Procedures for Hydrolysis and Coupling of Substituted Amino Acids. Procedure W. (2*S*,3*S*,5*S*)-5-[*N*-[*N*-[[*N*-Methyl-*N*-[(2-isopropyl-4-thiazolyl)-methyl]amino]carbonyl]valinyl]amino]-2-[*N*-[[(5-thiazolyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (ABT-538, Compound 123). A solution of 1.42 g (4.3 mmol) of **202** in 17 mL of dioxane was treated with 17.3 mL of 0.50 M aqueous LiOH. The resulting solution was stirred at ambient temperature for 30 min, treated with 8.7 mL of 1 M HCl, and concentrated in vacuo. The residue was taken up in CH_2Cl_2, washed with water, dried over Na_2SO_4, and concentrated in vacuo to provide 1.1 g (81%) of crude *N*-[[*N*-methyl-*N*-[(2-isopropyl-4-thiazolyl)methyl]amino]carbonyl]-L-valine: CIMS *m*/*z* 314 $(M + H)^+$. A solution of 70 mg (0.223 mmol) of the above acid, 79 mg (0.186 mmol) of **6d**, 30 mg (0.223 mmol) of 1-hydroxybenzotriazole hydrate (HOBT), and 51 mg (0.266 mmol) of *N*-ethyl-N′-[(dimethylamino)propyl]-carbodiimide (EDC) in 2 mL of THF was stirred at ambient temperature for 16 h. The resulting solution was concentrated in vacuo, and the residue was purified by silica gel chromatography using 97:3 CH_2Cl_2−CH_3OH to provide 100 mg (74%) of **123** (R_f 0.4, 95:5 CH_2Cl_2−CH_3OH) as a solid: mp 61−63 °C; CIMS *m*/*z* 721 $(M + H)^+$.

Procedure X. (2*S*,3*S*,5*S*)-5-[*N*-[*N*-[[(6-Amino-3-pyridinyl)methoxy]carbonyl]valinyl]amino]-2-[*N*-[[(3-pyridinyl)-methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (53). *N*-[[[5-[(*tert*-Butyloxycarbonyl)amino]-3-pyridinyl]-methoxy]carbonyl]-L-valine (115 mg, 0.31 mmol) was coupled to **6d** (105 mg, 0.25 mmol) according to procedure W above to give, following flash chromatography using 5−7% MeOH/$CHCl_3$, 175 mg (91%) of the product amide. A portion of this amide (148 mg, 0.19 mmol) was taken up in 4 mL of 1:1 trifluoroacetic acid/CH_2Cl_2 and stirred at ambient temperature for 1 h. The solvent was removed in vacuo, and the residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated. Flash chromatography using MeOH/$CHCl_3$ provided 85 mg (66%) of **53**. CIMS *m*/*z* 669 $(M + H)^+$.

Procedure Y. (2*S*,3*S*,5*S*)-5-[*N*-[*N*-[[*N*-Methyl-*N*-[(2-methyl-4-thiazolyl)methyl]amino]carbonyl]valinyl]-amino]-2-[*N*-[[(3-pyridinyl)methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (69). A solution of *N*-[[*N*-methyl-*N*-[(2-methyl-4-thiazolyl)methyl]amino]carbonyl]-valine (66 mg, 0.23 mmol) and 39 mg (0.28 mmol) of *p*-nitrophenol in 2 mL of THF was treated with 58 mg (0.65 mmol) of dicyclohexylcarbodiimide and stirred at ambient temperature. After 16 h, the mixture was filtered, treated with 65 mg (0.16 mmol) of **6a**, and stirred under N_2 at ambient temperature. After 16 h, the solution was concentrated in vacuo and purified by silica gel chromatography using 3% CH_3OH in $CHCl_3$ to provide 104 mg (95%) of **69**.

Procedure Z. (2*S*,3*S*,5*S*)-2-[*N*-[*N*-[3-(2-Isopropyl-4-thiazolyl)propanoyl]valinyl]amino]-5-[*N*-[[(3-pyridyl)-methoxy]carbonyl]amino]-1,6-diphenyl-3-hydroxyhexane (84). Boc-L-valine was coupled to **5a** as described above, and the Boc group was removed with HCl/dioxane followed by neutralization to the free amine. A solution of 225 mg (1 mmol) of **191** in 10 mL of freshly distilled CH_3OH and 1 mL of dry THF was treated with 50 mg of Mg metal. After being stirred for 20 min, the mixture was poured over cold 2 N HCl, neutralized to pH 8, extracted with EtOAc, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography using 10% EtOAc in hexane provided 105 mg (50%) of ethyl 3-(2-isopropyl-4-thiazolyl)propanoate. This ester was hydrolyzed with LiOH and coupled to the above amine using EDC/HOBT as described in procedure W to provide **84** in 46% yield.

Biological Evaluation. Procedures for measuring the inhibition of recombinant HIV protease, the inhibition of HIV-1_{IIIB} in MT4 cells, the aqueous solubility in pH 4.0 and 7.4 buffers, and the pharmacokinetic profile of the inhibitors in rats after oral and iv dosing have been described previously.[19,24,25,33] For pharmacokinetic analysis, compounds were formulated in 5% dextrose containing 20% ethanol, 30% propylene glycol, and 2 equiv of methanesulfonic acid.

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Supporting Information Available: Tabular compilation of the physical and analytical data for protease inhibitors and spectral characterization of intermediate substituted aminoacyl esters (20 pages). Ordering information is given on any current masthead page.

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