Aza-Peptide Analogs as Potent Human Immunodeficiency Virus Type-1 Protease Inhibitors with Oral Bioavailability

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A series of aza-peptide analogs with a (hydroxyethyl)hydrazine isostere has been synthesized as HIV-1 protease inhibitors using a simple synthetic scheme. Structure−activity studies based on the X-ray of a previously described inhibitor−enzyme complex led to potent inhibitors with antiviral activity in the low-nanomolar range. The *S*-configuration of the transition-state hydroxyl group was preferred in this series. Small modifications of the P_2P_3 and $P_2'P_3'$ substituents had little effect on enzyme inhibition but greatly influenced the pharmacokinetic profile. As a result of these studies, the symmetrically acylated compound **8a** and its close analog **24a** bearing a methyl carbamate in P_3 and an ethyl carbamate in P_3' position were identified as potent inhibitors with plasma concentrations exceeding antiviral ED_{50} values 150-fold following oral application in mice.

Introduction

It is widely accepted that pathogenesis of AIDS is mediated by the human immunodeficiency virus through a progressive impairment of the immune system. The different therapeutic strategies for intervention in this disease have been reviewed recently.[1] A critical role in the viral replication is played by the HIV protease, an enzyme responsible for the processing of the polyproteins to structural proteins and to enzymes essential for viral replication.[2] This pivotal role makes it a promising target for chemotherapy of AIDS. Since the initial characterization of this enzyme, rapid progress has been achieved toward the development of potent and selective inhibitors.[3] Yet it remains a challenge to combine both antiviral potency and favorable pharmacokinetic properties, thereby fulfilling one prerequisite for effective antiviral treatment.

The particular C_2-symmetry of HIV-1 protease, which functions as a dimer with each subunit contributing an amino acid triad Asp-Thr-Gly to the active site,[4] stimulated the design of symmetry-based inhibitors. On the basis of the hydroxyethylene core as a tetrahedral transition-state replacement for the peptide substrate, various potent and selective C_2-symmetric inhibitors have been described.[5] They exhibit diamino diols[6] and diamino alcohols,[7] as well as sulfones[8] or phosphinates,[9] as nonscissile dipeptide isostere units.

Our own molecular modeling studies prompted us to explore a novel pseudosymmetric type of dipeptide isostere which can be considered as an analog of an aza-peptide[10] (formula A in Figure 1). The carbon atom bearing the P_1' substituent of a hydroxyethylene (formula B in Figure 1) or a dihydroxyethyl amine isostere (formula C in Figure 1) is replaced by a nitrogen atom of a hydrazine group. This functional group eliminates one stereogenic center and allows broad variability of the substituents by acylation of the amino and hydrazino groups. More important, the synthesis of these inhibitors follows a series of simple transformations which is considered a major advantage for any future therapeutic agent in this field. This concept led to potent and selective enzyme inhibitors which have been described previously.[11] To provide further insight into the enzyme−inhibitor interactions, one of the most potent representatives of symmetrically acylated aza-peptide analogs (CGP 53820, Figure 1) has been co-crystallized with HIV-1 and HIV-2 protease.[12] The following structure−activity discussion is based on the X-ray structure obtained from HIV-1 protease. Thus, the inhibitor binds in an extended conformation with the hydroxyl group positioned symmetrically between the two active site aspartate residues. The phenyl and cyclohexyl substituents are accommodated in the P_1 and P_1' pockets of the enzyme. All HN and C=O functional groups of the backbone are arranged within hydrogen-bonding distances of H-bond donors and acceptors of the enzyme and can therefore explain the high enzyme affinity. However, in a cellular assay the antiviral activity was only moderate (ED_{90} = 1 μM). Furthermore, in a preliminary pharmacokinetic evaluation in mice, the compound did not reach plasma concentrations above the detection limit after oral application. Nev-

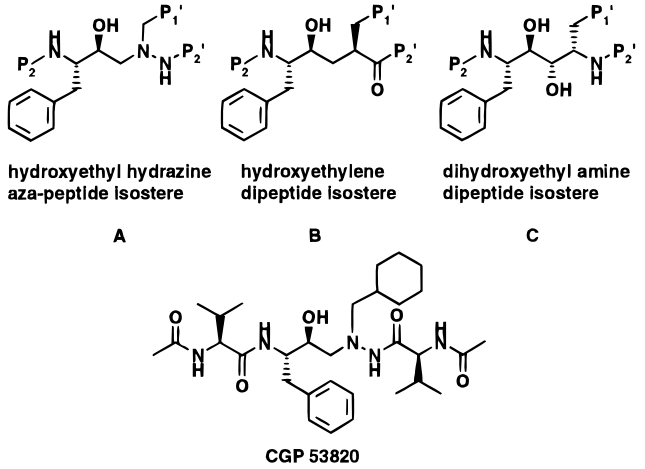

Figure 1. Comparison of transition-state dipeptide isosteres: pseudosymmetric (A) incorporated into inhibitor CGP 53820, substrate-based (B), and C_2-symmetric (C).

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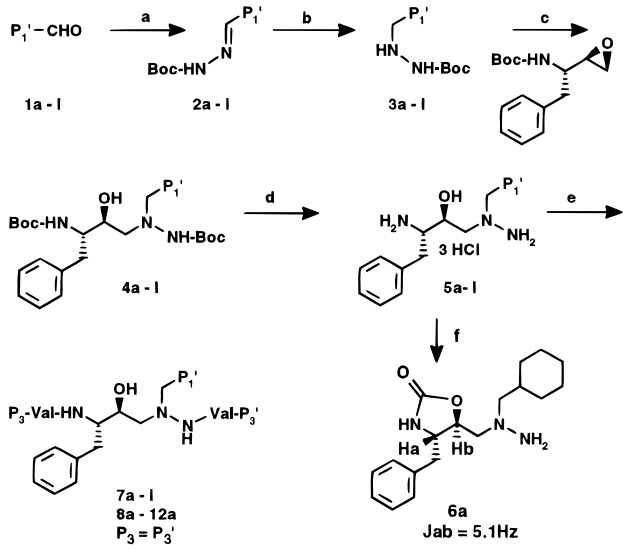
Table 1. Analytical Data for Hydrazones **2** and Hydrazines **3**

hydrazone **2**					hydrazine **3**			
no.	P_1' substituent	yield, %	mp, °C	^{1}H-NMR (ppm) P_1'-CH=N[a]	no.	yield, %	prep method[b]	^{1}H-NMR (ppm) P_1'-CH_2-NH[a]
2a	cyclohexyl	99	134−135	7.1, d, $J = 7$	**3a**	95	A	2.58, d, $J = 7$
2b	isopropyl	99	92−93	7.0, d, $J = 7$[d]	**3b**	89	A	2.55, d, $J = 7$
2c	isobutyl	81	98−99	7.27, d, $J = 7$	**3c**	96	A	2.78, t, $J = 7$
2d	*p*-MeO-phenyl	94	137−138	7.86, s	**3d**	73	D	3.83, s
2e	4-F-phenyl	96	166−167	7.81, s[d]	**3e**	94	B	3.83, s
2f	4-CN-phenyl	97	158−160	7.88, s[d]	**3f**	97	B	4.02, s[d]
2g	4-OH-phenyl	80	178 dec	7.82, s	**3g**	78	B	3.77, s
2h	4-tolyl	91	167	7.78, s[d]	**3h**	57	B	3.95, s[d]
2i	4-CF_3-phenyl	98	132	7.88, s, br[d]	**3i**	74	B	4.05, m, br[d]
2k	2,3,4-MeO-phenyl	99	nd[c]	8.05, s[d]	**3k**	95	B	3.94, s[d]
2l	2-thienyl	97	192 dec	8.1, s	**3l**	40	C	4.10, s

[a] Measured at 200 MHz and referenced to CD_3OD as the solvent unless stated otherwise. [b] Refers to experimental section. [c] Not determined. [d] $CDCl_3$ used as solvent.

Scheme 1. Synthesis of Symmetrically Acylated Aza-Dipeptide Analogs **7**−**12**[a]



[a] (a) EtOH, 80 °C, 3 h; (b) H_2, Pt/C, MeOH, room temperature (method A), H_2, Pd/C, THF, room temperature (method B), $NaBH_3CN$, TsOH, THF, room temperature (method C or D); (c) *N*-(*tert*-butyloxycarbonyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxybutane,[15a,b] MeOH, 80 °C, 16 h; (d) 4 N HCl, dioxane, room temperature, 2 h; (e) P_3-Val-OH, BOP, HOBT, NMM, DMF, room temperature, 16 h (method A), P_3-Val-OH, EDCI, HOBT, Et_3N, DMF, room temperature, 16 h (method B); (f) $COCl_2$, toluene, −60 °C.

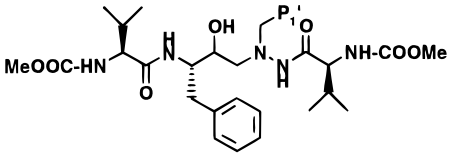
ertheless, its high enzyme affinity (IC_{50} = 8.5 nM) and its selectivity compared to other aspartic proteases[11b] made CGP 53820 an attractive lead for further optimization of the biological profile. In this paper we present our structure−activity studies leading to aza-peptide analogs as HIV-1 protease inhibitors with good antiviral acitivity and favorable pharmacokinetics following oral application in mice.

Chemistry

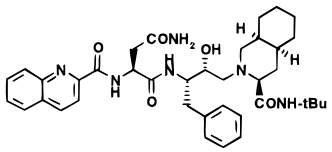
Variability of substitution and ease of synthetic transformations were emphasized in the planning of the reaction sequence. The synthesis of symmetrically substituted aza-dipeptides followed the route described in Scheme 1. Commercially available aldehydes **1a**−**l** were transformed into the corresponding hydrazones **2a**−**l** using *tert*-butylcarbazate followed by catalytic hydrogenation on Pd/carbon or Pt/carbon to give the required alkyl *tert*-butylcarbazates **3a**−**l** bearing the necessary P_1' substituents as listed in Table 1.[13] For carbazates **3d**,**l** reduction with sodium cyanoborohydride/*p*-toluenesulfonic acid[14] was preferred over catalytic hydrogenation possibly due to catalyst deactivation especially in the presence of the thienyl moiety. The required *N*-(*tert*-butyloxycarbonyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxybutane as a source of the transition-state hydroxyl group was prepared according to known procedures:[15] Peterson olefination of *N*-Boc-phenylalaninal,[16] reintroduction of the Boc protecting group, and epoxidation gave a 83:17 mixture of the *threo* and *erythro* isomers, which could be separated by crystallization. The enantiomeric excess of the desired *threo* isomer was determined by chiral HPLC to be >98%.[17] Nucleophilic ring opening with the hydrazines **3** occurred smoothly in refluxing methanol or 2-propanol to give the crystalline Boc-protected dipeptide isosteres **4a**−**l** in 45−80% yield. Acidic cleavage of the Boc protecting groups provided the key intermediates **5a**−**l**. The relative configuration was unambiguously assigned in a representative example by transforming **5a** into the corresponding oxazolidinone **6a** using phosgene in toluene at −60 °C (Scheme 1). The observed coupling constant J_{AB} of 5.1 Hz in the ^{1}H-NMR spectrum was consistent with the expected *trans*-configuration.[7c] Intermediates **5a**−**l** were coupled with the required carbamoylated valine derivatives according to standard peptide synthesis procedures ((benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate, 1-hydroxybenzotriazole, 4-methylmorpholine or 1-ethyl-3-(3-diaminopropyl)carbodiimide, 1-hydroxybenzotriazole, triethylamine) to furnish the target inhibitors **7a**−**l** (Table 2) and **8a**−**12a** (Table 3).

Optimization of the antiviral activity and modulation of physicochemical properties of the inhibitors required the possibility to introduce different acyl substituents on the amine and hydrazine functions of the dipeptide isosteres. Therefore trifluoroacetyl and Boc were chosen as orthogonal protecting groups compatible with the overall synthesis. Thus, known allylamine **13**[15a,b] was trifluoroacetylated to give protected olefin **14**, which afforded trifluoroacetyl epoxide **15** as a 87:13 mixture of *threo* and *erythro* isomers after oxidation using 3-chloroperbenzoic acid (Scheme 2).[18] This mixture was carried on to the following step without separation of the isomers. Nucleophilic opening with the previously mentioned alkyl *tert*-butylcarbazates **3a**,**d** gave the protected dipeptide isosteres *threo*-**16** and *erythro*-**16**. The predominant *threo* isomers of **16** were isolated by chromatography and crystallization and transformed into inhibitors **24a**−**29a** via subsequent deprotection and acylation steps as shown in Scheme 2. The epimeric

Table 2. Analytical Data, *in Vitro* Potency, and Antiviral Activity for HIV Protease Inhibitors **7a**−**m**: Modification of the P_1' Substituent

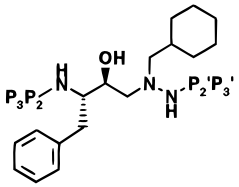


no.	P_1' substituent	config of OH	yield, %	prep method	FAB-MS $(M+H)^+$	formula[b]	IC_{50},[i] nM	ED_{50}, μM	ED_{90}, μM
7a	cyclohexyl	*S*	67	A	606	$C_{31}H_{51}N_5O_7 \cdot 0.2H_2O$[c]	84	0.018	0.1
7b	isopropyl	*S*	70	A	566	$C_{28}H_{47}N_5O_7 \cdot 0.6\ H_2O$	41	0.023	1
7c	isobutyl	*S*	28	B	580	$C_{29}H_{49}N_5O_7$	16	0.0027	0.03
7d	4-MeO-phenyl	*S*	63	A	630	$C_{32}H_{47}N_5O_8$	16	0.009	0.03
7e	4-F-phenyl	*S*	47	A	618	$C_{31}H_{44}N_5O_7F \cdot 0.7H_2O$[d]	42	0.049	1
7f	4-CN-phenyl	*S*	63	A	625	$C_{32}H_{44}N_6O_7 \cdot 0.8H_2O$[e]	62	0.068	1
7g	4-OH-phenyl	*S*	39	A	616	$C_{31}H_{45}N_5O_8$	18	0.150	1
7h	4-tolyl	*S*	47	B	636[a]	$C_{32}H_{47}N_5O_7 \cdot 0.5H_2O$	19	0.0062	0.03
7i	4-CF_3-phenyl	*S*	15	B	668	$C_{32}H_{44}N_5O_7F_3 \cdot 0.5H_2O$[f]	33	0.017	0.1
7k	2,3,4-MeO-phenyl	*S*	71	B	690	$C_{34}H_{51}N_5O_{10}$[g]	34	0.028	0.1
7l	2-thienyl	*S*	66	B	606	$C_{29}H_{43}N_5O_7S$	17	0.017	0.1
7m	cyclohexyl	*R*	31	A	606	$C_{31}H_{51}N_5O_7$[h]	1060	nd[j]	nd[j]
Ro 31-8959[19]		*R*					6.3	0.0045	0.01



[a] $(M+Na)^+$. [b] Elemental analysis within ±0.4% of the calculated values except where noted. [c] C, H; N: calcd, 11.49; found, 10.90. [d] H, N; C: calcd, 59.05; found, 54.43. [e] H, N; C: calcd, 60.15; found, 58.91. [f] N, C: calcd, 56.81; found, 58.3. H: calcd, 6.70; found, 7.2. [g] HR-FAB MS $(M+H)^+$ calcd 690.3714, obsd 690.3751. [h] HR-FAB MS $(M+H)^+$ calcd 606.3866, obsd 606.3848. [i] IC_{50} values had to be determined at suboptimal conditions at pH 6 in order to insure solubility of inhibitors with different physicochemical properties. They are expected to be 1 order of magnitude lower when determined as described in ref 19. [j] Inclusion criteria for further evaluation in cellular assays is $IC_{50} < 1\ \mu M$.

Table 3. Analytical Data, *in Vitro* Potency, and Antiviral Activity for HIV Protease Inhibitors **8a**−**29a**: Modification of the P_3P_2 and $P_2'P_3'$ Substituents



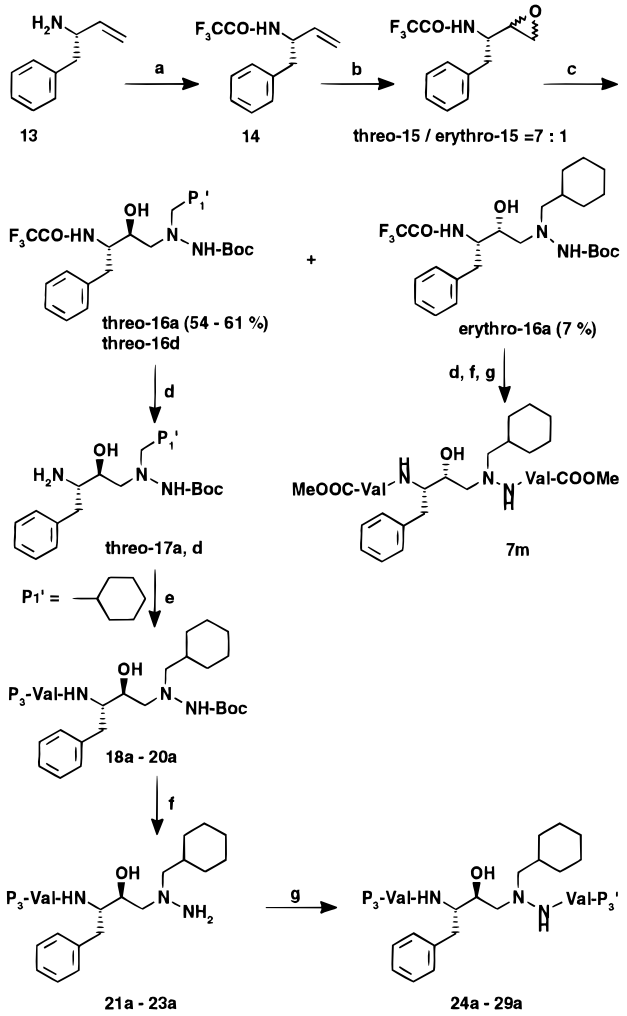
no.	P_3P_2 substituent	$P_2'P_3'$ substituent	yield, %	prep method	formula[a]	IC_{50},[c] nM	ED_{50}, μM	ED_{90}, μM
CGP 53820[12]	MeOC-Val	Val-COMe				8.5	0.3	1
8a	EtOOC-Val	Val-COOEt	92	B	$C_{33}H_{55}N_5O_7$	177	0.055	1
9a	CH_2CHCH_2OOC-Val	Val-$COOCH_2CHCH_2$	41	A	$C_{35}H_{55}N_5O_7 \cdot 0.2H_2O$	51	0.083	1
10a	$MeO(CH_2)_2OOC$-Val	Val-$COO(CH_2)_2OMe$	60	A	$C_{35}H_{59}N_5O_9 \cdot 0.4H_2O$	164	0.079	0.3
11a	$MeO(CH_2)_2O(CH_2)_2OOC$-Val	Val-$COO(CH_2)_2O(CH_2)_2OMe$	40	A	$C_{39}H_{67}N_5O_{11} \cdot 0.5H_2O$	67	0.041	10
12a	MeOOC-NMe-Val	NMe-Val-COOMe	37	B	$C_{33}H_{55}N_5O_7 \cdot H_2O$[b]	>1000	nd[d]	nd
24a	MeOOC-Val	Val-COOEt	69	B	$C_{32}H_{53}N_5O_7$	56	0.029	0.1
25a	MeOOC-Val	Val-$COO(CH_2)_2OMe$	27	B	$C_{33}H_{55}N_5O_8 \cdot 0.3H_2O$	67	0.028	0.1
26a	EtOOC-Val	Val-COOMe	40	B	$C_{32}H_{53}N_5O_7 \cdot 0.3H_2O$	76	0.072	1
27a	EtOOC-Val	Val-$COO(CH_2)_2OMe$	29	B	$C_{34}H_{57}N_5O_8$	150	0.015	0.1
28a	$MeO(CH_2)_2OOC$-Val	Val-COOMe	41	B	$C_{33}H_{55}N_5O_8 \cdot 0.3H_2O$	62	0.014	0.1
29a	$MeO(CH_2)_2OOC$-Val	Val-COOEt	32	B	$C_{34}H_{57}N_5O_8$	134	0.024	0.1
Ro-31-8959[19]						6.3	0.0045	0.01

[a] Elemental analysis within ±0.4% of the calculated values except where noted. [b] C, H; N: calcd, 10.74; found, 10.04. [c] See footnote *i* in Table 2. [d] Inclusion criteria for further evaluation in cellular assays is $IC_{50} < 1\ \mu M$.

alcohol *erythro*-**16a** as the minor component was separated by chromatography in small quantities and used to synthesize inhibitor **7m** with the *R*-configuration of the transition-state hydroxyl group. As the epimer of compound **7a**, it was distinctly different by analytical HPLC.

The same sequence of acylations (first acylation of the amino function followed by acylation of the hydrazine moiety) was initially applied to the synthesis of inhibitors **32**−**36** containing a quinolinoyl-asparaginyl (Quin-Asn)[19] side chain in P_2P_3 position (Scheme 3). However, commonly used procedures to cleave the Boc protecting group (anhydrous HCl or trifluoroacetic acid in dichloromethane) readily applicable for the synthesis of compounds **5** and **21**−**23** were not compatible with the functional groups of intermediate **30**. Instead, formic acid, when used as the reaction solvent, achieved cleavage of the Boc protecting group in good yields at

Scheme 2. Synthesis of Acylated Aza-Dipeptide Analogs **7m** and **24a**−**29a**[a]



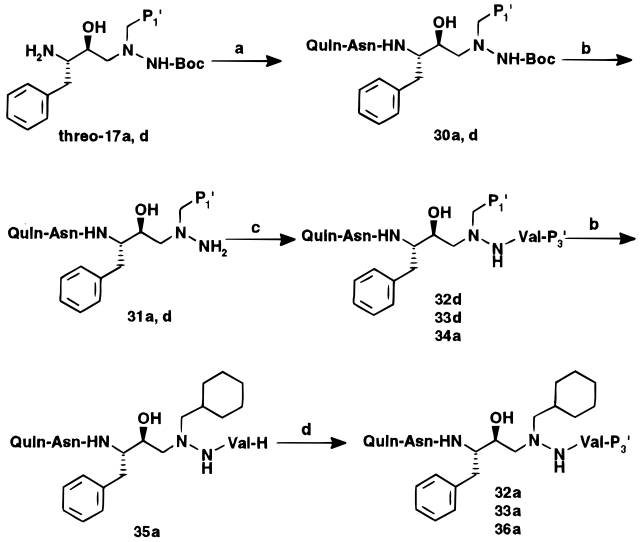
[a] (a) $(CF_3CO)_2O$, pyridine, CH_2Cl_2, 0 °C, 2 h; (b) *m*-CPBA, CH_2Cl_2, room temperature, 20 h; (c) *N*-(*tert*-butyloxycarbonyl)-*N*-2-alkylhydrazine **3**, MeOH, 80 °C, 16 h; (d) K_2CO_3, MeOH/H_2O, 80 °C, 16 h; (e) P_3-Val-OH, EDCI, HOBT, Et_3N, DMF, room temperature, 16 h; (f) 4 N HCl, dioxane, room temperature, 2 h; (g) P_3'-Val-OH, EDCI, HOBT, Et_3N, DMF, 16 h, room temperature (method B).

room temperature. Acylation of the less reactive hydrazine moiety of Quin-Asn-substituted intermediates **31** with valine derivatives turned out to be a particularly sluggish and low-yielding reaction compared to valine-substituted intermediates **21**−**23**. This observation prompted us to change the synthetic scheme and to prepare the (Cbz-valyl)alkylhydrazine **40b** first, with the $P_1'P_2'$ substituents already in place (Scheme 4). The Quin-Asn substituent was subsequently introduced by acylation of the more reactive amine terminus as the last reaction step to provide inhibitors **32b** and **33b**.

Results and Discussion

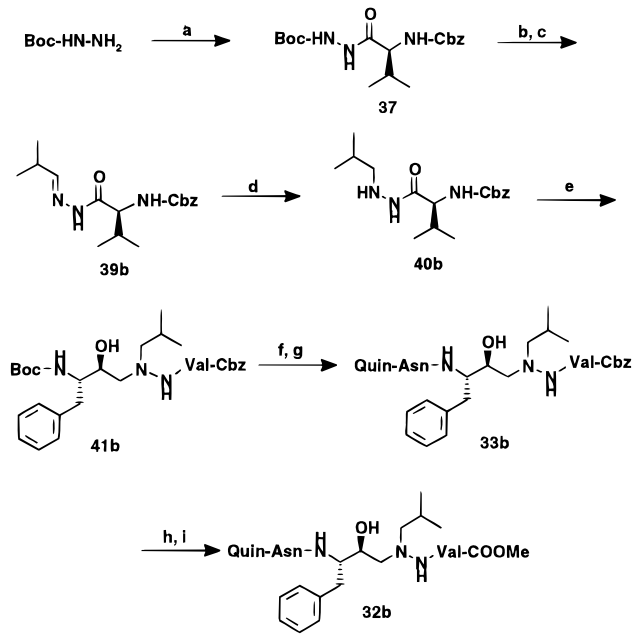
As a close analog of the previous lead compound CGP 53820, the bis[(methoxycarbonyl)valyl] derivative **7a** had already improved antiviral activity while maintaining high selectivity.[11c] Inhibitor **7m** with *R*-configuration of the alcohol served to investigate the influence of the configuration of the transition-state hydroxyl group. It inhibited the enzyme more than 10-fold weaker compared to its epimer **7a** as shown in Table 2. This observation of preferred *S*-configuration of the alcohol

Scheme 3. Synthesis of Unsymmetrically Acylated Aza-Dipeptide Analogs **32a**−**36a**[a]



[a] (a) Quin-Asn-OH, DCC, 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one or HOBT, NMM, THF; (b) HCOOH, room temperature, 7−22 h; (c) Boc-Val-OH, HBTU, NMM, CH_3CN or MeOOC-Val-OH, EDCl, HOBT, NMM, DMF or Cbz-Val-OH, HBTU, NMM, DMF; (d) ClCOOR, K_2CO_3, dioxane/H_2O.

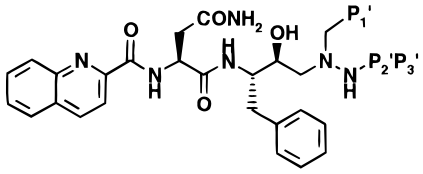
Scheme 4. Synthesis of Unsymmetrically Acylated Aza-Dipeptide Analogs **32b** and **33b**[a]



[a] (a) Cbz-Val-OH, EDCl, HOBT, NMM, EtOAc; (b) 2 N HCl, dioxane, room temperature; (c) isobutyric aldehyde, EtOH, 60 °C, 4 h; (d) $NaBH_3CN$, *p*-TsOH, THF, room temperature, 1 h; (e) *N*-(*tert*-butyloxycarbonyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxybutane,[15a,b] EtOH, 80 °C, 16 h; (f) HCOOH, room temperature, 16 h; (g) Quin-Asn-OH, EDCl, HOBT, NMM, THF; (h) H_2, Pd/C (10%), MeOH; (i) ClCOOMe, K_2CO_3, dioxane/H_2O.

in aza-peptide-derived inhibitors is in sharp contrast to hydroxyethylamine-, (hydroxyethyl)urea, and sulfonamide-derived inhibitors which also have the P_1' substituents attached to a nitrogen atom. In those compounds a distinct preference for the *R*-stereochemistry at the hydroxyl group has been reported.[20,21] It is important to note that according to the X-ray structure the alkylated hydrazine nitrogen bearing the P_1' substituent is tetrahedral with inverted conformation com-

Table 4. Analytical Data, *in Vitro* Potency, and Antiviral Activity for HIV Protease Inhibitors **32**−**36**: Modification of the P_1' and $P_2'P_3'$ Substituents



no.	P_1' substituent	$P_2'P_3'$ substituent	formula[a]	yield, %	mp, °C	IC_{50},[h] nM	ED_{50}, μM	ED_{90}, μM
32a	cyclohexyl	Val-COOMe	$C_{38}H_{51}N_7O_7$[b]	37	nd	27	0.018	0.1
32b	isopropyl	Val-COOMe	$C_{35}H_{47}N_7O_7$[c]	84	178−180	23	0.063	0.3
32d	4-MeO-phenyl	Val-COOMe	$C_{39}H_{47}N_7O_8$[d]	37	160−162	31	0.118	1
33a	cyclohexyl	Val-Cbz	$C_{44}H_{55}N_7O_7$[e]	59	nd	41	0.0098	0.03
33b	isopropyl	Val-Cbz	$C_{41}H_{51}N_7O_7$	56	208−210	37	0.012	0.03
33d	4-MeO-phenyl	Val-Cbz	$C_{45}H_{51}N_7O_8$[f]	77	nd	49	0.015	0.03
36a	cyclohexyl	Val-COOEt	$C_{39}H_{53}N_7O_7$[g]	48	nd	126	0.0094	0.03
Ro 31-8959[19]						6.3	0.0045	0.01

[a] Elemental analysis within ±0.4% of the calculated values except where noted. [b] HR-FAB MS $(M + H)^+$ calcd 718.3928, obsd 718.3918. [c] HR-FAB MS $(M + H)^+$ calcd 678.3615, obsd 678.3644. [d] HR-FAB MS $(M + H)^+$ calcd 742.3564, obsd 742.3547. [e] C, H; N: calcd, 12.35; found, 11.93. [f] HR-FAB MS $(M + H)^+$ calcd 818.3877, obsd 818.3871. [g] HR-FAB MS $(M + H)^+$ calcd 732.4085, obsd 732.4114. [h] See footnote *i* in Table 2.

pared to the α-carbon of a peptide substrate or the hydroxyethylene-based inhibitor CGP 53437.[12,22] Further examination of the X-ray structure of the lead compound revealed that the P_1' subsite could accommodate lipophilic substituents of diverse size other than cyclohexyl. Decreasing the steric bulk of the P_1' substituent by replacing the cyclohexyl moiety with isopropyl slightly increased the affinity to the enzyme but did not show a significant effect on the antiviral ED_{50} values (compounds **7a**,**b** in Table 2). Elongation of the side chain to an isobutyl substituent, on the other hand, resulted in a significant increase in affinity and provided compound **7c** with excellent antiviral potency (ED_{50} and ED_{90} values). The following compounds listed in Table 2 demonstrate that 4-substituted phenyl groups as P_1' side chains were well tolerated in the enzymatic assay. While the related compounds **7d**,**g**−**i** exhibited a similar degree of enzyme inhibition, only the 4-methoxy derivative **7d** and the 4-tolyl derivative **7h** maintained high activity in the antiviral assay and displayed ED_{50} values in the low-nanomolar range. Compound **7g** with the free phenol group had greatly diminished antiviral potency, presumably due to reduced lipophilicity.[23] Subsequently the antivirally most active compounds in this series (**7c**,**d**,**h**) were evaluated in a preliminary pharmacokinetic model in mice following oral application of a formulation containing DMSO/(hydroxypropyl)-β-cyclodextrin. As shown in Table 5, plasma concentrations in the low-micromolar range were measured for **7c** at three out of four time points. Unfortunately neither **7d** nor **7h** reached plasma concentrations exceeding the detection limit after oral application in mice.

As the next step in the optimization program, the role of the P_2P_3 and $P_2'P_3'$ substituents was investigated while the cyclohexyl substituent was kept in P_1' position. Previous findings[24] allowed the conclusion that the hydrogen bonds of the two carbonyl functions of the acylated valine residues were more important for enzyme affinity than the lipophilic interactions with the P_3 and P_3' subsites. Therefore the variations presented here are restricted to acyl residues or carbamates, which might serve as a handle to modify the physical properties in order to improve the pharmacokinetic profile of the inhibitors made so far. Parallel elongation of the acyl side chains did not show a pronounced effect on enzyme affinity as anticipated (Table 3). The bis(allyl carbamate) **9a** and the bis[(methoxyethoxy)ethyl carbamate] **11a** exhibited IC_{50} values in the same range as **7a**, whereas **8a** and **10a** were about 2-fold less potent *in vitro*. The antiviral potency was generally diminished in this series of compounds, the effect being most significant for **11a**. Its dose−response curve showed a reduced slope, such that 90% inhibition was achieved only at 10 μM concentration despite of an ED_{50} of 41 nM. N-Methylation of the valine residues as in **12a** completely abolished inhibitory potency, possibly due to the loss of two hydrogen bonds to the enzyme. Therefore we did not examine whether reducing the number of potential intramolecular hydrogen bonds to the enzyme might be beneficial for oral absorption.[25] Most interestingly, the minor change of **7a** to **8a**, although slightly reducing the antiviral potency, resulted in a significantly improved pharmacokinetic profile with maximal plasma concentrations in mice exceeding ED_{50} more than 150-fold. Replacement of two methyl carbamates with two ethyl carbamates resulted in an increase of

Table 5. Preliminary Pharmacokinetic Evaluation of Selected Compounds after Oral Application in Mice: Plasma Concentrations (μM) after 30, 60, 90, and 120 min[a]

		conctn (μM) at time given (min)				coefficient
no.	ED_{50}, μM	30	60	90	120	C_{120}/ED_{50}
7c	0.0027	<0.2	0.6	0.4	0.3	111
7d	0.009	<0.2	nd	<0.2	<0.2	nd
7h	0.0062	<0.2	<0.2	<0.2	<0.2	nd
8a	0.055	6.7	6.7	7.8	8.7	158
9a	0.083	0.8	1.2	0.9	1.0	12
10a	0.079	2.7	1.1	<0.5	<0.5	nd
24a	0.029	3.8[b]	2.2	5.8	6.0	207
25a	0.028	1.0	2.8	3.0	3.1	111
26a	0.072	3.2[b]	0.4	1.2	1.4	19
27a	0.015	1.0	2.2	1.0	1.5	100
28a	0.014	<0.5	<0.5	<0.5	<0.5	nd
29a	0.024	<0.2	<0.2	<0.2	<0.2	nd
33a	0.015	0.3	0.2	0.8	1.1	73

[a] The compounds were administered by gavage to female BALB/c mice as a suspension in DMSO/(hydroxypropyl)-β-cyclodextrin at an average dose of 120 mg/kg. The limit of detection was 0.2 μM except for compounds **10a** and **28a** (0.5 μM). Values indicated in the table represent the mean between four animals. [b] High variability, standard error ±1.9.

lipophilicity by 1 log *P* unit (log *P*: 3.61 for **8a** vs 2.55 for **7a**), which we consider one factor contributing to oral absorption.[23]

This result indicated that even more conservative changes might influence the overall profile, and therefore the acyl substituents at the amine and hydrazine terminus needed to be varied independently. Therefore the constitutional isomers **24a** and **26a** with only one ethyl carbamate were evaluated. Interestingly, only **24a** showed a clear superiority in all respects (IC_{50}, ED_{50}, C_{90}). This compound exhibited the best overall profile so far by yielding plasma concentrations more than 200-fold the ED_{50}. Compounds containing one (methoxyethoxy)ethyl carbamate maintained their high degree of antiviral activity in the nanomolar range, but their blood levels in mice were clearly inferior to those of **24a**.

Structure−activity studies that led to Ro 31-8959[19] revealed that the hydrophilic amino acid asparagine was well tolerated in P_2 position. As a consequence, the quinolinoyl-asparagine side chain has become a constituent of various potent HIV protease inhibitors with different central units.[26] Inhibitors containing this Quin-Asn side chain generally maintained a high degree of enzyme affinity (Table 4). The ED_{50} values of compounds **32a**−**33d** show a trend that antiviral potency is improved by increasing lipophilicity of either the P_1' or $P_2'P_3'$ substituents. Thus, combination of a cyclohexyl group in P_1' with the lipophilic Cbz-Val residue in $P_2'P_3'$ culminated in the best antiviral potency in this series (**33a**). However, none of these inhibitors achieved plasma concentrations exceeding 1 μM in mice.

Summary

Aza-peptide analogs containing a (hydroxyethyl)-hydrazine isostere led to potent HIV-1 protease inhibitors with high antiviral activity. The central dipeptide isostere was acylated either in a symmetric fashion with valine substituents or subsequently with different valine or asparagine substituents. Inhibitors of this type showed a distinct preference for the *S*-configuration of the transition-state hydroxyl group. Subtle changes of the carbamoylated valine residues in P_2P_3 and $P_2'P_3'$ positions, which had little effect on enzyme affinity, greatly influenced the pharmacokinetic profile. Thus, replacement of one or both methyl carbamates on the valine substituents with an ethyl carbamate produced a substantial increase in plasma concentrations upon oral application in mice. As a result, **8a** and **24a** were identified as inhibitors with the best overall profile. They combined both good antiviral potency with favorable pharmacokinetics in mice upon oral application of a DMSO/(hydroxypropyl)-β-cyclodextrin formulation with plasma concentrations exceeding ED_{50} by 150-fold.

Experimental Section

Chemistry. All reactions with air- or moisture-sensitive reactants and solvents were carried out under nitrogen atmosphere. Reagents and solvents were used as purchased without further purification. Analytical thin layer chromatography was performed on silica F_{254} glass plates (E. Merck). Components were visualized by UV light of λ 254 nm or spraying with phosphomolybdic acid. Column flash chromatography was performed on silica gel 60 (230−400 mesh ASTM, E. Merck) under a positive nitrogen pressure of approximately 20 psi. Melting points were determined in an open capillary and are not corrected. ^{1}H-NMR spectra: Bruker DRX-500 (500 MHz), Bruker AM-360 (360 MHz), Varian Gemini-300 (300 MHz) or Varian Gemini-200 (200 MHz spectrometer); chemical shifts of signals are expressed in parts per million (ppm) and referenced to the deuterated solvents used. Coupling constants are recorded in hertz (Hz). IR spectra: Bruker FT-IR-spectrometer IFS 48. MS spectra: FAB-ZAB, HF (VG analytical spectrometer). HPLC chromatography: stationary phase, Nucleosil C18, 5 μm analytical column (125 × 4.6 mm); mobile phase, 20% acetonitrile/0.1% trifluoroacetic acid (TFA) in water/0.1% TFA → 100% acetonitrile/0.1% TFA during 20 min + 10 min 100% acetonitrile/0.1% TFA; t_R refers to the retention time in minutes. Elemental analyses were performed by the Ciba Analytical Department and are within ±0.4% of the calculated values unless noted otherwise. Abbreviations: BOP, (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate; DCC, *N*,*N*′-dicyclohexylcarbodiimide; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluorophosphate; HOBT, 1-hydroxybenzotriazole hydrate; EDCI, 1-ethyl-3-(3-diaminopropyl)carbodiimide hydrochloride; NMM, 4-methylmorpholine; *p*-TsOH, 4-toluenesulfonic acid.

Cyclohexylmethanone (*tert*-Butyloxycarbonyl)hydrazone (2a) (General synthesis of hydrazones 2a−l). To a solution of 133 g (1.359 mol) of N-(*tert*-butyloxycarbonyl)-hydrazine in 3 L of absolute ethanol was added 200 mL (1.495 mol) of cyclohexanecarboxaldehyde at ambient temperature. Upon completion of the addition, the temperature rose to 34 °C, and the mixture was heated to reflux for 3 h. About 1.2 L of solvent was removed by distillation, and the residue was diluted with 500 mL of water. After cooling to 46 °C, 1 L of water was added. Crystallization of the product was completed by slow addition of 3 L of water during 1 h at ambient temperature. The resulting suspension was filtered; the filter cake was washed with 3 L of water and dried at 60 °C to give 300 g (100%) of **2a** homogeneous by TLC (80:20 hexane:ethyl acetate): mp 134−135 °C, ^{1}H-NMR ($CDCl_3$) δ 7.65 (s, br, 1H), 7.0 (d, J = 6, 1H), 2.26 (m, 1H), 1.83−1.55 (m, 5H), 1.45 (s, 9H), 1.3−1.08 (m, 5H); HPLC t_R 14.2 min; FAB MS $(M + H)^+$ = 227.

***N*-1-(*tert*-Butyloxycarbonyl)-*N*-2-(cyclohexylmethyl)-hydrazine (3a) (Reduction according to method A).** To a solution of 300 g (1.32 mol) of hydrazone **2a** in 3 L of MeOH was added 60 g of 5% Pt on carbon, and the mixture was hydrogenated during 8 h at room temperature and atmospheric pressure of hydrogen. Upon theoretical uptake of hydrogen, the catalyst was filtered, and the filtrate was concentrated under reduced pressure. The crude product was diluted with 2 L of methylene chloride and washed with 2 L of water. The organic layer was filtered through a pad of cotton wool and evaporated under reduced pressure to give 288 g (95 %) of **3a** as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 6.20 (s, br, 1H), 3.85 (s, br, 1H), 2.63 (d, J = 7, 2H), 1.78−1.65 (m, 6H), 1.42 (s, 9H), 1.3−1.05 (m, 3H), 1.0−0.82 (m, 2H); IR (dichloromethane) cm^{-1} 3440, 3040, 2990, 2920, 2850, 1710, 1450.

***N*-1-(*tert*-Butyloxycarbonyl)-*N*-2-[(4-fluorophenyl)-methyl]hydrazine (3e) (Reduction according to method B).** To a solution of 55 g (231 mmol) of hydrazone **2e** in 500 mL of THF was added 5.5 g of 5% Pd on carbon, and the mixture was hydrogenated during 30 min at room temperature and atmospheric pressure of hydrogen. Upon theoretical uptake of hydrogen, the catalyst was filtered, and the filtrate was concentrated under reduced pressure to give 52 g (94%) of **3e** as an amorphous, white solid: ^{1}H-NMR (MeOD) δ 7.35 (dd, J = 8, 5, 2H), 7.05 (t, J = 8, 2H), 3.86 (s, 2H), 1.42 (s, 9H).

***N*-1-(*tert*-Butyloxycarbonyl)-*N*-2-(2-thienylmethyl)hydrazine (3l) (Reduction according to method C).** To a suspension of 50 g (221 mmol) of hydrazone **2l** in 220 mL of THF was added 16.3 g of sodium cyanoborohydride at ambient temperature. The pH of the reaction mixture was adjusted to 3.5 by dropwise addition of a solution of 42 g (221 mmol) of *p*-TsOH in 220 mL of THF and kept constant at this value for 72 h using a pH-controlled addition valve. The reaction mixture was then diluted with 1 L of ethyl acetate and subsequently washed with 1 L of brine, 1 L of saturated

$NaHCO_3$, and 1 L of brine. The aqueous layers were re-extracted with 1 L of ethyl acetate, and the combined organic layers were dried with Na_2SO_4 and evaporated under reduced pressure. The resulting crude product was purified by column chromatography using 1 kg of silica gel (eluent: 20:1 dichloromethane:ether) and suspended in 265 mL of 1 N aqueous NaOH. After stirring during 2.5 h at ambient temperature, the mixture was extracted twice with 200 mL of dichloromethane. The organic extracts were filtered through a pad of cotton wool and evaporated under reduced pressure to give 19.95 g (40%) of **3l** as a colorless oil homogeneous by TLC (20:1 dichloromethane:ether): ^{1}H-NMR ($CDCl_3$) δ 7.3 (dd, J = 5, 1, 1H), 6.95 (m, 2H), 4.10 (s, 2H), 0.95 (s, 9H); HPLC t_R 9.6 min; FAB MS $(M + H)^+ = 229$.

***N*-1-(*tert*-Butyloxycarbonyl)-*N*-2-[(4-methoxyphenyl)-methyl]hydrazine (3d) (Reduction according to method D).** A solution of 86.5 g (502 mmol) of *p*-TsOH in 400 mL of THF was added dropwise in 30 min to a stirred solution of 125.7 g (502 mmol) of **2d** and 31.6 g (502 mmol) of $NaBH_3CN$ in 1.6 L of THF. After stirring for 4 h at room temperature, the resulting suspension was concentrated under reduced pressure. This material was redissolved in ethyl acetate and washed subsequently with saturated $NaHCO_3$ and brine. The aqueous layers were re-extracted twice with ethyl acetate; the organic layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The resulting yellow mass was taken up in 1.5 L of 1 N NaOH and stirred at ambient temperature for 90 min. Then the reaction mixture was neutralized with 2 N HCl and extracted three times with dichloromethane. The organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Vigorous stirring in diisopropyl ether yielded 66.4 g of crystalline **3d**. The concentrated filtrate afforded upon medium pressure chromatography (Lichroprep; 0% → 40% ethyl acetate in hexane) another 26.6 g (a total of 73%) of **3d**: mp 145−147 °C; TLC (2:1 hexane:ethyl acetate) R_f = 0.36; ^{1}H-NMR (MeOD) δ 7.27 and 6.87 (2d, J = 8, 2, 2H), 3.83 (s, 2H), 3.77 (s, 3H), 1.44 (s, 9H). Anal. ($C_{13}H_{20}N_2O_3$) C, H, N.

1-Cyclohexyl-5(*S*)-2,5-bis[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-6-phenyl-2-azahexane (4a) (General procedure for the synthesis of compounds 4a−l). A solution of 54.6 g (239.3 mmol) of **3a** and 60 g (227.9 mmol) of *N*-(*tert*-butyloxycarbonyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxybutane[15a,b] in 500 mL of absolute MeOH was heated to reflux temperature during 16 h. Thereafter the solvent was evaporated and the crude product precipitated by addition of 200 mL of diisopropyl ether. The solid was filtered and recrystallized by dissolving in 50 mL of dichloromethane and adding hexane to the solution to give 54.2 g of **4a** as a white solid. The mother liquor was purified by chromatography on silica gel (eluent: 3:1 hexane:ethyl acetate) to give another 7.92 g of **4a** homogeneous by TLC (1:1 hexane:ethyl acetate), combined yield 62.12 g (55.4%): mp 156−157 °C; ^{1}H-NMR (MeOD) δ 7.3−7.15 (m, 5H), 3.7 (m, 1H), 3.62 (m, 1H), 2.97−2.7 (m, 2H), 2.65−2.35 (m, 4H), 1.95−0.8 (m, br, 11H), 1.40 (s, 9H), 1.36 (s, 9H); HPLC t_R 19.2 min; EI MS m/z 491, 391, 362, 335, 241, 185, 141.

1-Cyclohexyl-5(*S*)-2,5-diamino-4(*S*)-hydroxy-6-phenyl-2-azahexane Hydrochloride (5a) (General procedure for the synthesis of compounds 5a−l). A solution of 16.4 g (33.4 mmol) of **4a** in 90 mL of 4 N HCl(g) in dioxane was stirred at room temperature. After 1 h a white precipitate formed, and the reaction mixture was diluted with 30 mL of 4 N HCl-(g) in dioxane. TLC (5:1 chloroform:MeOH) indicated completion of the reaction after 6 h at ambient temperature. The mixture was diluted with 100 mL of dioxane to give a solution which was then lyophilized overnight and yielded 12.02 g (90%) of **5a** as an amorphous white solid: ^{1}H-NMR (MeOD) δ 7.45−7.27 (m, 5H), 3.95 (m, 1H), 3.65 (m, 1H), 3.15−2.86 (m, 4H), 2.75−2.5 (m, 2H), 1.95−0.75 (m, 11H); HPLC t_R 7.8 min; FAB MS $(M + H)^+ = 291$.

4-Benzyl-5-(3-cyclohexyl-2-amino-2-azapropyl)oxazolidin-2-one (6a). To a solution of 200 mg (0.499 mmol) of **5a** in 5 mL of dichloromethane, which was cooled to −60 °C, were added 350 mL (2.49 mmol) of NEt_3 and 247 mL of a 20% solution of phosgene in toluene. After 1 h at −60 °C the reaction mixture was allowed to warm to room temperature and stirred for an additional 30 min. It was diluted with dichloromethane and washed with saturated $NaHCO_3$. The organic layer was filtered through a pad of cotton wool and evaporated to dryness. The resulting oil (200 mg) was purified by column chromatography on silica gel (eluent: 15:1 dichloromethane:MeOH) and evaporated to dryness to give 30 mg (19%) of **6a** as a colorless oil: ^{1}H-NMR (MeOD) δ 7.4−7.15 (m, 5H), 4.59 (dd, J = 6.1, 5.2, 1H), 3.91 (dd, J = 6.4, 5.1, 1H), 2.90 (d, J = 6.4, 1H), 2.63 (dd, J = 13.2, 6.1, 1H), 2.52 (dd, J = 13.2, 6.1, 1H), 2.30 (d, J = 7.1, 1H), 1.86−1.55 (m, 4H), 1.55−1.35 (m, 1H), 1.35−1.03 (m, 4H), 1.0−0.72 (m, 2H); HPLC t_R 9.98 min.

1-Cyclohexyl-5(*S*)-2,5-bis[[2-*N*-(methoxycarbonyl)-L-valinyl]amino]-4(*S*)-hydroxy-6-phenyl-2-azahexane (7a) (General synthesis of compounds 7a,b,d−g and 9a−11a using coupling method A). To form an active ester, 1.47 g (8.4 mmol) of (methoxycarbonyl)-L-valine, 1.13 g (8.4 mmol) of HOBT, and 3.71 g (8.4 mmol) of BOP were dissolved in 54 mL of a 0.3 M solution of NMM in DMF. After 15 min 1.12 g (2.8 mmol) of **5a** was added at ambient temperature, and the mixture was stirred overnight. The solvents were evaporated at 50 °C under reduced pressure, and the residue was dissolved in 300 mL of dichloromethane. This solution was subsequently washed twice with 300 mL of saturated $NaHCO_3$ and 300 mL of brine. The aqueous layers were re-extracted with 200 mL of dichloromethane. The combined organic layers were filtered through a pad of cotton wool and evaporated under reduced pressure. The resulting crude product was purified by column chromatography on silica gel (eluent: 15:1 dichloromethane:MeOH) and precipitated twice by dissolving in 5 mL of dichloromethane and adding diisopropyl ether. After lyophilization from dioxane, there was obtained 1.14 g (67%) of **7a** which was homogenous by TLC (15:1 dichloromethane:MeOH): ^{1}H-NMR (MeOD) δ 7.2 (m, 5H), 4.11 (apparent q, 1H), 3.9−3.68 (m, 3H), 3.65 (2s, 6H), 2.95−2.75 (m, 2H), 2.65−2.4 (m, 4H), 1.95 (m, 3H), 1.66 (br, 4H), 1.18−0.75 (m, 6H), 0.92 (d, J = 7, 3H), 0.90 (d, J = 7, 3H), 0.80 (d, J = 7, 6H); HPLC t_R 14.9 min; FAB MS $(M + H)^+ = 606$. Anal. ($C_{31}H_{51}N_5O_7$) C, H; N: calcd, 11.49; found, 10.90.

1-(4-Tolyl)-5(*S*)-2,5-bis[[2-*N*-(methoxycarbonyl)-L-valinyl]amino]-4(*S*)-hydroxy-6-phenyl-2-azahexane (7h) (General synthesis of compounds 7c,h−l, 8a, and 12a using coupling method B). To a stirred solution of 582 mg (3.32 mmol) of (methoxycarbonyl)-L-valine, 1.18 g (6.15 mmol) of EDCI, and 500 mg (3.70 mmol) of HOBT in 20 mL of DMF was added 1.05 mL (7.53 mmol) of triethylamine. After 30 min 502 mg (1.23 mmol) of **5h** was added, and the mixture was allowed to stir at room temperature for 16 h. The solvent was evaporated under reduced pressure and the residue dissolved in 100 mL of dichloromethane. This solution was subsequently washed with 100 mL of 10% aqueous citric acid, 100 mL of saturated $NaHCO_3$, and 100 mL of brine. The aqueous layers were re-extracted twice with 100 mL of dichloromethane. The combined organic layers were filtered through a pad of cotton wool and evaporated under reduced pressure. The residue was crystallized by dissolving in 5 mL of dichloromethane and adding diisopropyl ether. Lyophilization from dioxane gave 355 mg of **7h** (47%) as a white, amorphous solid which was homogeneous by TLC (95:5 dichloromethane:MeOH): ^{1}H-NMR (MeOD) δ 7.25−7.05 (m, 9H), 4.10 (apparent q, br, 1H), 3.96−3.55 (m, 5H), 3.63 (2s, 6H), 2.98−2.75 (m, 3H), 2.60 (d, br, J = 10, 1H), 2.27 (s, 3H), 1.85 (m, 1H), 1.67 (m, 1H), 0.77 (2d, J = 7, 2 × CH_3) 0.65 (d, J = 7, 3H), 0.60 (d, J = 7, 3H); HPLC t_R 14.1 min; FAB MS $(M + Na)^+ = 636$. Anal. ($C_{32}H_{47}N_5O_7 \cdot 0.5H_2O$) C, H, N.

1-Cyclohexyl-5(*S*)-2,5-bis[[2-*N*-(methoxycarbonyl)-L-valinyl]amino]-4(*R*)-hydroxy-6-phenyl-2-azahexane (7m). Deprotection of *erythro*-**17a** using 4 N HCl in dioxane at room temperature for 3 h followed by lyophilization of the reaction solution gave a quantitative yield of crude 1-cyclohexyl-5(*S*)-2,5-diamino-4(*R*)-hydroxy-6-phenyl-2-azahexane hydrochloride (*erythro*-**5a**). Coupling of the crude product with (methoxycarbonyl)-L-valine using method A as described for **7a** yielded 12 mg (31%) of **7m**: ^{1}H-NMR (MeOD) δ 7.2 (m, 5H), 4.08 (m, 1H), 3.76 (d, J = 7, 1H), 3.64 (s, 6H), 3.6 (m, 2H), 3.20−2.94

(m, 2H), 2.8−2.5 (m, 4H), 2.05−0.7 (m, 25H); HPLC t_R 14.7 min; HR-FAB MS ($C_{31}H_{51}N_5O_7$) $(M + H)^+$ calcd 606.3866, obsd 606.3848.

***N*-(Trifluoroacetyl)-3(*S*)-amino-4-phenyl-1-butene (14).** To a solution of 117.8 g (0.794 mol) of 3(*S*)-amino-4-phenyl-1-butene (**13**)[15a,b] in 2 L of dichloromethane was added 691 mL of pyridine at room temperature. The resulting yellow solution was cooled to −5 °C, and 167 mL (1.198 mol) of trifluoroacetic anhydride was added within 15 min. The resulting mixture was allowed to stir at 0 °C during 1 h and then diluted with 1.2 L of dichloromethane. The organic layer was subsequently washed with 3 L of 1 N aqueous HCl (3×), 3 L of water, and 3 L of brine, dried with Na_2SO_4, and evaporated under reduced pressure to give 170.4 g (88%) of **14** as an oil: ^{1}H-NMR ($CDCl_3$) δ 7.4−7.1 (m, 5H), 6.2 (s, br, 1H), 5.85 (m, 1H), 5.22 (m, 2H), 4.82 (q, J = 7, 1H), 2.95 (dd, J = 6.6, 1.9, 2H); HPLC t_R 14.0 min; FAB MS $(M + H)^+ = 244$. Anal. ($C_{12}H_{12}NF_3O$) C, H, N, F.

***N*-(Trifluoroacetyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxybutane (15).** To a solution of 255 mg (1.05 mmol) of **14** in 5 mL of dichloromethane was added 1.06 g (5.2 mmol) of 3-chloroperbenzoic acid at 0 °C. The resulting mixture was allowed to stir at ambient temperature during 20 h. The resulting suspension was diluted with 15 mL of ether and poured into 50 mL of 10% aqueous sodium sulfite. The organic layer was washed subsequently with 50 mL of 10% aqueous sodium sulfate, 50 mL of saturated $NaHCO_3$, and 50 mL of brine, dried with Na_2SO_4, and evaporated under reduced pressure. The resulting crude product was purified by column chromatography on silica gel (eluent: 19:1 dichloromethane:MeOH) to give 270 mg (90%) of **15** as a 7:1 mixture of the *threo* and *erythro* isomers: ^{1}H-NMR ($CDCl_3$, 7:1 mixture of *threo*-**15**:*erythro*-**15**) δ 7.3−7.15 (m, 5H), 6.2 (s, br, 1H), 4.43 (qd, J = 7.3, 1.7, 0.85H, *threo* isomer), 4.08 (q, br, J = 7, 0.15H, *erythro* isomer), 3.15 (m, 1H), 3.02 (dd, J = 7, 1.7, 2H), 2.76 (t, J = 4.1, 1H), 2.53 (dd, J = 4.2, 2.7, 1H); HPLC t_R 12.3 min (no separation of isomers); FAB MS $(M + H)^+ = 260$. Anal. ($C_{12}H_{12}NF_3O_2$) H, N; C: calcd, 55.60; found, 56.25. F: calcd, 21.99; found, 21.35.

1-Cyclohexyl-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-[(trifluoracetyl)amino]-6-phenyl-2-azahexane (*threo*-16a). A solution of 60 g (0.232 mol) of a (7:1 *threo*:*erythro*) mixture of *N*-(trifluoroacetyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxybutane (**15**) and 52.8 g (0.232 mol) of *N*-1-(*tert*-butyloxycarbonyl)-*N*-2-(methylcyclohexyl)hydrazine (**3a**) in 700 mL of methanol was heated to reflux for 16 h. The solvent was evaporated under reduced pressure to give a yellow oil. Crystallization was induced by adding 300 mL of dichloromethane to give 60.6 g (54%) of *threo*-**16a** as a white solid homogenous by TLC and HPLC: mp 144−145 °C; TLC (2:1 toluene:ethyl acetate) R_f = 0.66; ^{1}H-NMR (MeOD) δ 7.25 (m, 5H), 4.18 (t, br, J = 7, 1H), 3.72 (m, 1H), 3.02 (dd, J = 7, 13, 1H), 2.95-2.8 (m, 1H), 2.58 (m, 2H), 2.47 (t, J = 7, 2H), 2.01−0.9 (m, 11H), 1.43 (s, 9H); HPLC t_R 17.8 min. Anal. ($C_{24}H_{36}N_3F_3O_4$) C, H, N.

1-(4-Methoxyphenyl)-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-[(trifluoracetyl)amino]-6-phenyl-2-azahexane (*threo*-16d). A solution of 79.7 g (0.307 mol) of a (7:1 *threo*:*erythro*) mixture of *N*-(trifluoroacetyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxybutane (**15**) and 77.6 g (0.307 mol) of *N*-1-(*tert*-butyloxycarbonyl)-*N*-2-[(4-methoxyphenyl)methyl]hydrazine (**3d**) in 1 L of ethanol was heated to 80 °C for 16 h. The solvent was evaporated under reduced pressure, and the resulting crystalline residue was triturated with diisopropyl ether to give 83.6 g of pure *threo*-**16d**. The concentrated filtrate afforded upon flash chromatography (1:1 hexane:ethyl acetate) another 10 g (a total of 59%) of *threo*-**16d**: mp 170−171 °C; TLC (2:1 hexane:ethyl acetate) R_f = 0.51; ^{1}H-NMR (MeOD) δ 7.23 (m, 7H), 6.84 (d, J = 8, 2H), 4.20 (m, 1H), 3.77 (s, 3H), 3.75 (m, 3H), 2.99 (dd, J = 13, 6, 1H), 2.85 (m, 1H), 2.65 (m, 2H), 1.33 (s, 9H). Anal. ($C_{25}H_{32}N_3F_3O_5$) C, H, N.

1-Cyclohexyl-2-[(*tert*-butyloxycarbonyl)amino]-4(*R*)-hydroxy-5(*S*)-[(trifluoracetyl)amino]-6-phenyl-2-azahexane (*erythro*-16a). Preparation was as described for *threo*-**16a**. Flash chromatography (toluene:ethyl acetate, 10:1) yielded 61% of *threo*-**16a** and 7% of *erythro*-**16a**. Data for *erythro*-**16a**: TLC (2:1 toluene:ethyl acetate) R_f = 0.57; ^{1}H-NMR (MeOD) δ 7.2 (m, 5H), 4.10 and 3.68 (2m, 2H), 3.18 (dd, J = 14, 4, 1H), 2.82−2.4 (m, 5H), 2.03−0.8 (m, 11H), 1.45 (s, 9H); FAB MS $(M + H)^+ = 488$.

1-Cyclohexyl-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-amino-6-phenyl-2-azahexane (*threo*-17a). To a solution of 60 g (0.123 mol) of *threo*-**16a** in 2 L of methanol was added 615 mL of a 1 N aqueous solution of K_2CO_3. The resulting suspension was heated to reflux for 16 h. The solvent was evaporated under reduced pressure and the residue dissolved in 2 L of dichloromethane. This solution was washed with 1 L of water and 1.5 L of brine, and the aqueous layers were re-extracted with 500 mL of dichloromethane. The organic layers were combined, filtered through a pad of cotton wool, and concentrated. Crystallization of the crude product by dissolving in 100 mL of dichloromethane and adding 600 mL of diisopropyl ether gave 45.6 g (95%) of *threo*-**17a** as a white solid: ^{1}H-NMR (MeOD) δ 7.32−7.12 (m, 5H), 3.5 (m, 1H), 2.95−2.4 (m, 7H), 2.0 (d, br, 1H), 1.85−0.8 (m, 10H), 1.43 (s, 9H); HPLC t_R 13.5 min.

1-Cyclohexyl-2-[(*tert*-butyloxycarbonyl)amino]-4(*R*)-hydroxy-5(*S*)-amino-6-phenyl-2-azahexane (*erythro*-17a). Preparation was as described above for *threo*-**17a**. Data for *erythro*-**17a**: yield 202 mg (77%); ^{1}H-NMR (MeOD) δ 7.27 (m, 5H), 3.54 and 3.12 (2m, 2H), 2.96−2.4 (m, 6H), 2.05−0.8 (m, 11H), 1.42 (s, 9H).

1-(4-Methoxyphenyl)-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-amino-6-phenyl-2-azahexane (*threo*-17d). A 1 N aqueous solution (975 mL) of K_2CO_3 was added dropwise to a solution of 50 g (97.6 mmol) of *threo*-**16d** in 1 L of methanol at 70 °C. The solution was heated to reflux for 16 h. The solvent was evaporated under reduced pressure and the residue dissolved in dichloromethane. This solution was washed with water and brine, and the aqueous layers were re-extracted with two portions of dichloromethane. The organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Crystallization of the crude oil by vigorous stirring in hexane gave 35.6 g (87%) of *threo*-**17d**: mp 113−115 °C; TLC (dichloromethane:methanol) R_f = 0.37; ^{1}H-NMR (MeOD) δ 7.25 (m, 7H), 6.84 (d, J = 8, 2H), 3.77 (s, 3H), 3.86−3.64 (m, 3H), 3.56 (m, 1H), 3.00−2.55 (m, 4H), 1.31 (s, 9H). Anal. ($C_{23}H_{33}N_3O_4$) C, H, N.

1-Cyclohexyl-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(methoxycarbonyl)-L-valinyl]amino]-6-phenyl-2-azahexane (18a). To a stirred solution of 32.2 g (0.183 mol) of (methoxycarbonyl)-L-valine, 66.1 g (0.435 mol) of EDCI, and 31 g (0.228 mol) of HOBT in 500 mL of DMF was added 32 mL (0.230 mol) of triethylamine. After 30 min a solution of 32.2 g (0.183 mol) of *threo*-**17a** in 1 L of DMF was added and the mixture allowed to stir at room temperature for 16 h. The solvent was evaporated under reduced pressure and the residue dissolved in 1 L of dichloromethane. This solution was subsequently washed with 1 L of 10% aqueous citric acid, 1 L of saturated $NaHCO_3$, and 1 L of brine, and the aqueous layers were re-extracted with 500 mL of dichloromethane. The combined organic layers were filtered through a pad of cotton wool and evaporated under reduced pressure. The crude product was crystallized by dissolving in 100 mL of methanol and adding 700 mL of diisopropyl ether to give 50.1 g (79%) of **18a** as a white solid homogeneous by TLC (97:3 dichloromethane:MeOH): ^{1}H-NMR (MeOD) δ 7.3−7.12 (m, 5H), 4.03 (t, br, J = 8, 1H), 3.86 (d, J = 8, 1H), 3.7−3.55 (m, 1H), 3.66 (s, 3H), 3.0−2.75 (m, 2H), 2.65−2.33 (m, 4H), 1.95 (m, 2H), 1.85−0.76 (m, 10H), 1.40 (s, 9H), 0.86 (2d, J = 7, 6H); HPLC t_R 17.5 min.

1-Cyclohexyl-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(ethoxycarbonyl)-L-valinyl]amino]-6-phenyl-2-azahexane (19a). Preparation was as described for **18a**. Yield was 1.207 g of **19a** (84%) homogeneous by TLC (95:5 dichloromethane:MeOH): ^{1}H-NMR (MeOD) δ 7.20 (m, 5H), 4.10 (q, J = 7, 2H), 4.08 (m, 1H), 3.86 (d, J = 8, 1H), 3.62 (d, br, 1H), 3.0−2.75 (m, 2H), 2.6−2.33 (m, 4H), 1.98 (m, 2H), 1.8−0.88 (m, 10H), 1.40 (s, 9H), 1.25 (t, J = 7, 3H), 0.86 (2d, J = 7, 6H); HPLC t_R 17.7 min.

1-Cyclohexyl-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-[(methoxyethoxy)carbonyl]-L-valinyl]-

amino]-6-phenyl-2-azahexane (20a). Preparation was as described for **18a**. Yield was 1.236 g of **20a** (81%) homogeneous by TLC (95:5 dichloromethane:MeOH): ^{1}H-NMR (MeOD) δ 7.22 (m, 5H), 4.18 (t, br, J = 7, 2H), 4.05 (t, br, J = 8, 1H), 3.87 (d, J = 7, 1H), 3.61 (t, J = 7, 2H), 3.38 (s, 3H), 3.0−2.78 (m, 2H), 2.65−2.36 (m, 4H), 2.0 (m, 2H), 1.8−0.82 (m, 10H), 1.39 (s, 9H), 0.87 (apparent t, J = 7, 6H); HPLC t_R 17.1 min.

1-Cyclohexyl-2-amino-4(*S*)-hydroxy-5(*S*)-[[*N*-(methoxycarbonyl)-L-valinyl]amino]-6-phenyl-2-azahexane Hydrochloride (21a). A solution of 50 g (91.1 mmol) of **18a** in 400 mL of 4 N HCl(g) in dioxane was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure (gas adsorber filled with sodium hydroxide) and the resulting crude product suspended in 600 mL of dioxane followed by evaporation to dryness. This latter procedure was repeated with 600 mL of diisopropyl ether to give 61 g (99%) of **21a** as a white amorphous solid: ^{1}H-NMR (MeOD) δ 7.3−7.15 (m, 5H), 4.25 (m, 1H), 4.05 (m, 1H), 3.75 (s, 3H), 3.7−3.55 (m, 1H), 3.12−2.6 (m, 6H), 2.05−0.76 (m, 12H), 0.8 (2d, J = 7, 6H); HPLC t_R 11.0 min; FAB MS (M + H)$^+$ = 449.

1-Cyclohexyl-2-amino-4(*S*)-hydroxy-5(*S*)-[[*N*-(ethoxycarbonyl)-L-valinyl]amino]-6-phenyl-2-azahexane Hydrochloride (22a). Preparation was as described for **21a**. Data for **22a**: yield 1.14 g (98%); ^{1}H-NMR (MeOD) δ 7.23 (m, 5H), 4.3−4.05 (m, 2H), 4.13 (q, J = 7, 2H), 3.80 (d, J = 7, 1H), 3.05−2.7 (m, 6H), 1.98−0.85 (m, 12H), 1.25 (t, J = 7, 3H), 0.80 (d, J = 7, 6H); HPLC t_R 11.7 min.

1-Cyclohexyl-2-amino-4(*S*)-hydroxy-5(*S*)-[[*N*-(methoxyethoxycarbonyl)-L-valinyl]amino]-6-phenyl-2-azahexane Hydrochloride (23a). Preparation was as described for **21a**. Data for **23a**: yield 1.132 g (98%); ^{1}H-NMR (MeOD) δ 7.25 (m, 5H), 4.20 (m, 4H), 3.77 (d, J = 8, 1H), 3.65 (s, 3H), 3.57 (m, 2H), 3.63-3.55 (m, 6H), 2.0−0.8 (m, 12H), 0.78 (d, J = 7, 6H); HPLC t_R 10.9 min.

1-Cyclohexyl-2-[[*N*-(ethoxycarbonyl)-L-valinyl]amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(methoxycarbonyl)-L-valinyl]amino]-6-phenyl-2-azahexane (24a) (General synthesis of compounds 24a−29a using coupling method B). To a stirred solution of 27.5 g (0.146 mol) of (ethoxycarbonyl)-L-valine, 52.3 g (0.273 mol) of EDCI, and 24.5 g (0.182 mol) of HOBT in 700 mL of DMF was added 127 mL (0.91 mol) of triethylamine. After 30 min a solution of 60 g (91 mmol) of **21a** in 300 mL of DMF was added and the mixture allowed to stir at room temperature for 16 h. The solvent was evaporated under reduced pressure and the residue dissolved in 1 L of dichloromethane. This solution was subsequently washed with 1 L of 10% aqueous citric acid, 1 L of saturated $NaHCO_3$, and 1 L of brine, and the aqueous layers were re-extracted with 500 mL of dichloromethane. The combined organic layers were filtered through a pad of cotton wool and evaporated under reduced pressure. The crude product was crystallized by dissolving in 100 mL of dichloromethane and adding 700 mL of diisopropyl ether. Recrystallization by dissolving in 200 mL of hot methanol and adding 600 mL of diisopropyl ether upon cooling to room temperature gave 39 g of **24a** (79%) which was homogeneous by TLC (95:5 dichloromethane:MeOH): ^{1}H-NMR (MeOD) δ 7.20 (m, 4H), 7.13 (m, 1H), 4.08 (m, 3H), 3.83 (d, J = 7, 1H), 3.76 (d, J = 7, 1H), 3.65 (s, 3H), 3.62 (m, br, 1H), 2.95−2.78 (m, 2H), 2.68−2.43 (m, 4H), 1.93 (m, 3H), 1.70 (m, 4H), 1.41 (m, 1H), 1.23 (t, J = 7, 3H), 1.18 (s, br, 3H), 0.90 (apparent t, J = 7, 6H), 0.85 (d, br, J = 7, 2 × CH_3 + 2H); HPLC t_R 15.7 min; FAB MS (M + H)$^+$ = 620. Anal. ($C_{32}H_{53}N_5O_7$) C, H, N.

1-Cyclohexyl-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (30a). To a cooled (−10 °C) stirred suspension of 8.00 g (20.4 mmol) of 1-cyclohexyl-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-amino-6-phenyl-2-azahexane (*threo*-**17a**) and 7.28 g (22.4 mmol) of (quinolin-2-ylcarbonyl)-L-asparagine hydrochloride[27] in 200 mL of THF were added 3.6 mL (32.7 mmol) of NMM, 4.64 g (22.4 mmol) of DCC, and finally 3.67 g (22.4 mmol) of 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one. After 2 h at −10 °C, the mixture was stirred for 20 h at room temperature. The mixture was diluted with ethyl acetate and subsequently washed with saturated $NaHCO_3$ and brine. The aqueous layers were re-extracted twice with ethyl acetate, and the organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Flash chromatography (ethyl acetate) afforded 10.21 g (76%) of **30a** as a white solid: TLC (90:10:1 dichloromethane:ethanol:NH_3, concentrated) R_f = 0.61; ^{1}H-NMR (MeOD) δ 8.48 (d, J = 9, 1H), 8.20 (2d, J = 8, 2H), 8.02, 7.86, and 7.70 (3m, 3H), 7.3−6.95 (m, 5H), 4.93 (t, J = 6, 1H), 4.08 (m, 1H), 3.60 (d, br, J = 8, 1H), 3.0−2.8 (m, 4H), 2.7−2.3 (m, 4H), 1.37 (s, 9H), 2.0−0.6 (m, 11H); HPLC t_R 16.4 min. Anal. ($C_{36}H_{48}N_6O_6$) H, N; C: calcd, 65.43; found, 65.01.

1-(4-Methoxyphenyl)-2-[(*tert*-butyloxycarbonyl)amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (30d). To an ice-cold stirred solution of 10.00 g (24.0 mmol) of *threo*-**17d** and 8.55 g (26.4 mmol) of (quinolin-2-ylcarbonyl)-L-asparagine hydrochloride in 600 mL of THF were added 2.9 mL (26.4 mmol) of NMM, 5.45 g (26.4 mmol) of DCC, and finally 3.56 g (26.4 mmol) of HOBT. After 15 min at 0 °C, the mixture was stirred for 18 h at room temperature and then filtered. The filtrate was concentrated *in vacuo*; the residue was diluted with ethyl acetate and subsequently washed with two portions of saturated $NaHCO_3$ and brine. The aqueous layers were re-extracted twice with ethyl acetate, and the organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Medium pressure chromatography (Lichroprep; 9:1 dichloromethane: methanol) yielded, after trituration with diisopropyl ether, 11.0 g (67%) of **30d**: mp 135−137 °C; TLC (9:1 dichloromethane: methanol) R_f = 0.7; ^{1}H-NMR (MeOD + $CDCl_3$) δ 8.42 (d, J = 9, 1H), 8.20 and 8.16 (2d, J = 9, 2H), 7.97 (d, J = 9, 1H), 7.83 and 7.68 (2m, 2H), 7.25−6.95 (m, 7H), 6.79 (d, J = 8, 2H), 4.92 (t, J = 6, 1H), 4.06 (m, 1H), 3.75 (s, 3H), 3.64 (m, 1H), 3.2−2.7 (m, 5H), 2.52 (m, 1H), 1.9−1.5 (m, 2H), 1.27 (s, 9H); HPLC t_R 14.4 min. Anal. ($C_{37}H_{44}N_6O_7$) C, H, N.

1-Cyclohexyl-2-amino-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (31a). A solution of 7.00 g (10.6 mmol) of **30a** in 48 mL of formic acid was stirred for 17 h at room temperature. The solvent was evaporated under reduced pressure and the resulting solid partitioned between ethyl acetate and saturated $NaHCO_3$. The layers were separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*, yielding **31a** as a white solid (6.2 g, quantitative): TLC (90:10:1 dichloromethane:ethanol:NH_3, concentrated) R_f = 0.40; ^{1}H-NMR (MeOD) δ 8.48 (d, J = 9, 1H), 8.18 (m, 2H), 8.02, 7.84, and 7.72 (3m, 3H), 7.3−6.9 (m, 5H), 4.97 (t, J = 7, 1H), 4.13 and 3.88 (2m, 2H), 3.0−2.2 (m, 8H), 1.9−0.7 (m, 11H); HPLC t_R 11.7 min.

1-(4-Methoxyphenyl)-2-amino-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (31d). Preparation was as described for **31a**. The crude product was precipitated from dichloromethane by addition of diisopropyl ether to give 2.9 g (64%) of **31d** as a white solid which was used in the next step (synthesis of **32d**) without further purification and characterization.

1-Cyclohexyl-2-*N*-[[(methoxycarbonyl)-L-valinyl]amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (32a). Preparation was as described below for **33a** yielded 541 mg (37%) of **32a** as a white solid: TLC (47:3 ethyl acetate:ethanol) R_f = 0.46; ^{1}H-NMR (MeOD) δ 8.49 (d, J = 9, 1H), 8.22 (d, J = 9, 1H), 8.18 (d, J = 8, 1H), 8.02 (d, J = 8, 1H), 7.85 and 7.72 (2m, 2H), 7.25−6.9 (m, 5H), 4.9 (m, 1H), 4.07 (m, 1H), 3.75 (d, J = 7, 1H), 3.64 (s, 3H), 3.6 (m, 1H), 2.9−2.4 (m, 8H), 2.0−0.7 (m, 18H); HPLC t_R 14.6 min; HR-FAB MS ($C_{38}H_{51}N_7O_7$) (M + H)$^+$ calcd 718.3928, obsd 718.3918.

2-Methyl-4-[[*N*-(methoxycarbonyl)-L-valinyl]amino]-6(*S*)-hydroxy-7(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-8-phenyl-4-azaoctane (32b). To a mixture of 0.10 g (0.161 mmol) of 2-methyl-4-(L-valinylamino)-6(*S*)-hydroxy-7(*S*)-[[*N*-(quinolin-2-ylcarbonyl-L-asparaginyl]amino]-8-phenyl-4-azaoctane (**43b**) and 223 mg (1.6 mmol) of K_2CO_3 in 1.4 mL of dioxane and 0.7 mL of water was added 12 μL (0.16 mmol) of methyl chloroformate at 0 °C. After 16 h of stirring at room temperature, an additional 6 μL of methyl

chloroformate was added to complete the conversion (20 h). The solution was partitioned between dichloromethane and saturated $NaHCO_3$. The aqueous layer was separated and extracted twice with dichloromethane. The organic extracts were washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. Vigorous stirring in diisopropyl ether yielded 92 mg (84%) of crystalline **32b**: mp 178−180 °C; TLC (9:1 dichloromethane:methanol) $R_f = 0.46$; ^{1}H-NMR (MeOD) δ 8.49 (d, $J = 8$, 1H), 8.21 and 8.17 (2d, $J = 8$, 2H), 8.02 (d, $J = 8$, 1H), 7.86 (dd, $J = 8$, 1H), 7.70 (dd, $J = 8$, 1H), 7.3−6.9 (m, 5H), 4.9 (1H), 4.08 (t, $J = 7$, 1H), 3.75 (m, 1H), 3.65 (m, 1H), 3.62 (s, 3H), 2.95−2.4 (m, 8H), 1.9 and 1.65 (2m, 2H), 0.88 (m, 12H); HPLC t_R 13.2 min; HR-FAB MS ($C_{35}H_{47}N_7O_7$) (M + H)$^+$ calcd 678.3615, obsd 678.3644.

1-(4-Methoxyphenyl)-2-[[*N*-(methoxycarbonyl)-L-valinyl]amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (32d). To a stirred solution of 385 mg (2.20 mmol) of *N*-(methoxycarbonyl)-L-valine in 13.3 mL of a 0.3 M solution of NMM in DMF were added 421 mg (2.20 mmol) of EDCI and 385 mg (2.85 mmol) of HOBT. After 15 min 1.17 g (2.0 mmol) of **31d** was added, and the reaction mixture was stirred for 18 h. The solution was concentrated *in vacuo* and the residue partitioned between ethyl acetate and saturated $NaHCO_3$. The aqueous layer was separated and extracted twice with ethyl acetate. The organic extracts were washed with water and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. Precipitation from dichloromethane with diisopropyl ether afforded 0.56 g (37%) of crystalline **32d**: mp 160−162 °C; TLC (9:1 dichloromethane:methanol) $R_f = 0.56$; ^{1}H-NMR (MeOD) δ 8.43 (d, $J = 9$, 1H), 8.19 and 8.16 (2d, $J = 9$, 2H), 7.98 (d, $J = 9$, 1H), 7.83 and 7.68 (2m, 2H), 7.3−6.7 (m, 9H), 4.92 (m, 1H), 4.08 (m, 1H), 3.73 and 3.62 (2s, 2 × CH_3), 4.0−3.5 and 3.0−2.6 (2m, 10H), 1.67 (m, 1H), 0.63 and 0.59 (2d, $J = 7$, 6H); HPLC t_R 13.2 min; HR-FAB MS ($C_{39}H_{47}N_7O_8$) (M + H)$^+$ calcd 742.3564, obsd 742.3547.

1-Cyclohexyl-2-[[*N*-(benzyloxycarbonyl)-L-valinyl]amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (33a). A solution of 302 mg (0.397 mmol) of **34a** in 2 mL of formic acid was stirred for 22 h at room temperature. The solvent was evaporated under reduced pressure and the resulting solid partitioned between dichloromethane and saturated $NaHCO_3$. The layers were separated, and the aqueous layer was extracted twice with dichloromethane. The organic extracts were washed with brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*, yielding 0.28 g (quantitative) of 1-cyclohexyl-2-(L-valinylamino)-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (**35a**) as a white solid which was used without further purification and characterization.

Crude **35a** was dissolved in a mixture of 2.9 mL of dioxane and 2.7 mL of water. Then 528 mg (3.8 mmol) of K_2CO_3 was added followed by a solution of 126 μL (0.79 mmol) of benzyl chloroformate in 1.5 mL of dioxane. After stirring for 2 h at room temperature, the reaction mixture was partitioned between dichloromethane and saturated $NaHCO_3$. The aqueous layer was separated and extracted twice with dichloromethane. The organic extracts were washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. Flash chromatography (3% → 10% methanol in dichloromethane) yielded 186 mg (59%) of **33a** as a white solid: TLC (17:3 dichloromethane:ethanol) $R_f = 0.42$; ^{1}H-NMR (MeOD) δ 8.48 (d, $J = 9$, 1H), 8.21 and 8.18 (2d, $J = 8$, 2H), 8.02 (d, $J = 8$, 1H), 7.86 (dd, $J = 8$, 1H), 7.70 (dd, $J = 8$, 1H), 7.4−6.9 (m, 10H), 5.09 (s, 2H), 4.9 (m, 1H), 4.08 (m, 1H), 3.77 (d, $J = 7$, 1H), 3.63 (m, 1H), 2.9−2.4 (m, 8H), 2.0−0.6 (m, 18H); HPLC t_R 16.5 min; FAB MS (M + H)$^+ = 794$. Anal. ($C_{44}H_{55}N_7O_7$) C, H; N: calcd, 12.35; found, 11.93.

2-Methyl-4-[[*N*-(benzyloxycarbonyl)-L-valinyl]amino]-6(*S*)-hydroxy-7(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-8-phenyl-4-azaoctane (33b). To a stirred suspension of 1.99 g (6.15 mmol) of (quinolin-2-ylcarbonyl)-L-asparagine hydrochloride and 1.0 mL (9 mmol) of NMM in 100 mL of THF at 5 °C were added 2.71 g (5.59 mmol) of 2-methyl-4-[[*N*-(benzyloxycarbonyl)-L-valinyl]amino]-6(*S*)-hydroxy-7(*S*)-amino-8-phenyl-4-azaoctane (**42b**), 1.18 g (6.15 mmol) of EDCI, and 0.83 g (6.15 mmol) of HOBT. After 60 min at 5 °C, the mixture was allowed to stir at room temperature for 16 h. The suspension was filtered, the filtrate concentrated under reduced pressure, and the residue dissolved in ethyl acetate. This solution was subsequently washed with saturated $NaHCO_3$ and brine, and the aqueous layers were re-extracted twice with ethyl acetate. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was triturated with diisopropyl ether, filtered, and redissolved in a small amount of DMF. It was precipitated again by addition of diisopropyl ether to give 2.37 g (56%) of **33b**: mp 208−210 °C; TLC (9:1 dichloromethane:methanol) $R_f = 0.35$; ^{1}H-NMR (MeOD) δ 8.48 (d, $J = 8$, 1H), 8.21 and 8.17 (2d, $J = 8$, 2H), 8.00 (m, 1H), 7.84 (dd, $J = 8$, 1H), 7.70 (dd, $J = 8$, 1H), 7.30 (m, 5H), 7.17 (m, 2H), 7.05 (m, 2H), 6.92 (m, 1H), 5.08 (AB, $J = 14$, 2H), 4.9 (1H), 4.08 (t, $J = 7$, 1H), 3.76 (d, $J = 7$, 1H), 3.63 (d, br, $J = 9$, 1H), 2.9−2.3 (m, 8H), 1.94 and 1.66 (2m, 2H), 0.86 (d, $J = 7$, 12H); HPLC t_R 15.3 min; FAB MS (M + H)$^+ = 754$. Anal. ($C_{41}H_{51}N_7O_7$) C, H, N.

1-(4-Methoxyphenyl)-2-[[*N*-(benzyloxycarbonyl)-L-valinyl]amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (33d). To a stirred solution of 1.10 g (1.88 mmol) of **31d** in 18.2 mL of 0.3 M NMM in DMF were added 0.52 g (2.07 mmol) of Cbz-L-valine and 0.784 g (2.07 mmol) of HBTU. After stirring for 17 h at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$, water, and brine. The aqueous layers were re-extracted twice with ethyl acetate; the organic layers were dried (Na_2SO_4), filtered, and evaporated *in vacuo*. Crystallization from dichloromethane/diisopropyl ether afforded 1.18 g (77%) of **33d**: TLC (9:1 dichloromethane:methanol) $R_f = 0.58$; ^{1}H-NMR (MeOD) δ 8.46 (d, $J = 9$, 1H), 8.18 and 8.15 (2d, $J = 9$, 2H), 8.00 (d, $J = 9$, 1H), 7.83 and 7.70 (2m, 2H), 7.4−6.7 (m, 14H), 5.06 (s, 2H), 4.92 (m, 1H), 4.08 (m, 1H), 3.72 (s, 3H), 4.0−3.6 and 3.0−2.6 (2m, 10H), 1.67 (m, 1H), 0.64 and 0.60 (2d, $J = 7$, 6H); HPLC t_R 15.3 min; HR-FAB MS ($C_{45}H_{51}N_7O_8$) (M + H)$^+$ calcd 818.3877, obsd 818.3871.

1-Cyclohexyl-2-[[*N*-(*tert*-butyloxycarbonyl)-L-valinyl]-amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (34a). To a stirred solution of 5.93 g (10.6 mmol) of **31a** in 244 mL of 0.125 M solution of NMM in acetonitrile were added 4.40 g (11.6 mmol) of HBTU and 2.52 g (11.6 mmol) of Boc-L-valine. After stirring for 17 h, the precipitated crude product (5 g) was filtered and washed with acetonitrile. Further washing with hot acetonitrile, ether, and hexane yielded 4.15 g (52%) of **34a**. A 3 g portion of the product was purified by flash chromatography (49:1 dichloromethane:methanol) to give 2.32 g of pure **34a**: TLC (9:1 dichloromethane:methanol) $R_f = 0.6$; ^{1}H-NMR (MeOD + $CDCl_3$) δ 8.37 (d, $J = 8$, 1H), 8.20 and 8.14 (2d, $J = 8$, 2H), 7.92 (d, $J = 8$, 1H), 7.80 (dd, $J = 8$, 1H), 7.64 (dd, $J = 8$, 1H), 7.2−6.9 (m, 5H), 4.87 (t, $J = 7$, 1H), 4.02 (m, 1H), 3.69 (m, 1H), 3.53 (m, 1H), 2.9−2.4 (m, 8H), 1.40 (s, 9H), 2.0−0.7 (m, 18H); HPLC t_R 16.4 min; FAB MS (M + H)$^+ = 760$. Anal. ($C_{41}H_{57}N_7O_7$) C, H, N.

1-Cyclohexyl-2-[[*N*-(ethoxycarbonyl)-L-valinyl]amino]-4(*S*)-hydroxy-5(*S*)-[[*N*-(quinolin-2-ylcarbonyl)-L-asparaginyl]amino]-6-phenyl-2-azahexane (36a). Preparation as described for **33a**, using ethyl chloroformate instead of benzyl chloroformate, yielded 67 mg (48%) of **36a** as a white solid: TLC (19:1 dichloromethane:methanol) $R_f = 0.2$; ^{1}H-NMR (MeOD + $CDCl_3$) δ 8.43 (d, $J = 9$, 1H), 8.22 and 8.17 (2d, $J = 8$, 2H), 7.97 (d, $J = 8$, 1H), 7.84 and 7.71 (2m, 2H), 7.3−6.8 (m, 5H), 4.9 (m, 1H), 4.07 (m, 3H), 3.75 and 3.60 (2m, 2H), 3.0−2.4 (m, 8H), 2.0−0.7 (m, 21H); HPLC t_R 15.3 min; HR-FAB MS ($C_{39}H_{53}N_7O_7$) (M + H)$^+$ calcd 732.4085, obsd 732.4114.

[(Benzyloxycarbonyl)-L-valinyl]hydrazinecarboxylic Acid *tert*-Butyl Ester (37). To a stirred solution of 15.0 g (59.7 mmol) of (benzyloxycarbonyl)-L-valine and 7.9 mL (71.6 mmol) of NMM in 200 mL of ethyl acetate were added 12.6 g (65.7 mmol) of EDCI and 8.87 g (65.7 mmol) of HOBT. After 10 min 8.7 g (65.8 mmol) of hydrazinecarboxylic acid *tert*-

butyl ester was added. The reaction mixture was stirred for 18 h and then was diluted with ethyl acetate and saturated $NaHCO_3$. The aqueous layer was separated and extracted twice with ethyl acetate. The organic extracts were washed with water and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. Vigorous stirring in diisopropyl ether afforded 20.7 g (95%) of crystalline **37**: mp 142−143 °C; TLC (9:1 dichloromethane:methanol) $R_f = 0.57$; ^{1}H-NMR (MeOD) δ 7.35 (m, 5H), 5.08 (s, 2H), 3.95 (m, 1H), 2.05 (m, 1H), 1.44 (s, 9H), 1.00 and 0.96 (2d, $J = 7$, 6H). Anal. ($C_{18}H_{27}N_3O_5$) C, H, N.

[(Benzyloxycarbonyl)-L-valinyl]hydrazine (38). A solution of 20.7 g (56.6 mmol) of **37** in 260 mL of dioxane and 142 mL of 4 N HCl(g) in dioxane was stirred for 28 h. The reaction mixture was lyophilized. The white powder was dissolved in dichloromethane and saturated $NaHCO_3$. The aqueous layer was separated and extracted twice with dichloromethane. The organic extracts were washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated *in vacuo*. Vigorous stirring in diisopropyl ether yielded 13.9 g (92%) of crystalline **38**: mp 178−180 °C; TLC (9:1 dichloromethane:methanol) $R_f = 0.50$; ^{1}H-NMR (DMSO-d_6) δ 9.13 (s, 1H), 7.35 (m, 6H), 5.03 (s, 2H), 4.25 (s, 2H), 3.75 (t, $J = 8$, 1H), 1.90 (m, 1H), 1.44 (s, 9H), 0.86 and 0.83 (2d, $J = 7$, 6H). Anal. ($C_{13}H_{19}N_3O_3$) C, H, N.

2-Methyl-1-propanone[(Benzyloxycarbonyl)-L-valinyl]-hydrazone (39b). A solution of 13.9 g (52.4 mmol) of **38** and 4.2 mL (46 mmol) of isobutyric aldehyde in 250 mL of ethanol was heated for 4 h at 60 °C. After cooling to room temperature, the white precipitate was filtered and washed with diisopropyl ether, yielding 12.8 g (87%) of crystalline **39b**: mp 151−153 °C; TLC (9:1 dichloromethane:methanol) $R_f = 0.83$; ^{1}H-NMR (MeOD) δ 7.32 (m, 6H), 5.07 (s, 2H), 3.86 (d, $J = 8$, 1H), 2.53 (sept, $J = 7$, 1H), 2.04 (m, 1H), 1.10 (d, $J = 7$, 6H), 0.97 and 0.95 (2d, $J = 7$, 6H). Anal. ($C_{17}H_{25}N_3O_3$) C, H, N.

***N*-1-[(Benzyloxycarbonyl)-L-valinyl]-*N*-2-isobutylhydrazine (40b).** A solution of 7.17 g (41.6 mmol) of *p*-TsOH in 100 mL of THF was added dropwise in 15 min to a stirred solution of 12.8 g (41.6 mmol) of **39b** and 2.61 g (41.6 mmol) of $NaBH_3CN$ in 150 mL of THF. Stirring was continued for 1 h at room temperature. The resulting suspension was diluted with ethyl acetate and washed subsequently with brine, saturated $NaHCO_3$, and brine. The aqueous layers were re-extracted twice with ethyl acetate; the organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The resulting yellow wax was taken up in 125 mL of 1 N NaOH and stirred at ambient temperature for 90 min. Then the white suspension was neutralized with 2 N HCl and extracted three times with dichloromethane. The organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Vigorous stirring in diisopropyl ether afforded 11.75 g (87%) of crystalline **40b**: mp 152 °C; TLC (2:1 hexane:ethyl acetate) $R_f = 0.44$; ^{1}H-NMR (MeOD) δ 7.35 (m, 5H), 5.09 (s, 2H), 3.83 (d, $J = 8$, 1H), 2.71 (d, $J = 7$, 2H), 2.02 (oct, $J = 7$, 1H), 1.84 (non, $J = 7$, 1H), 0.95 (d, $J = 7$, 12H); HPLC t_R 14.5 min.

2-Methyl-4-[[*N*-(benzyloxycarbonyl)-L-valinyl]amino]-6(*S*)-hydroxy-7(*S*)-[(*tert*-butyloxycarbonyl)amino]-8-phenyl-4-azaoctane (41b). A suspension of 5.5 g (17.1 mmol) of **40b** and 4.5 g (17.1 mmol) of *N*-(*tert*-butyloxycarbonyl)-2(*S*)-amino-1-phenyl-3(*R*)-3,4-epoxybutane[15a,b] in 150 mL of ethanol was stirred at 80 °C for 16 h. The resulting solution was concentrated under reduced pressure. Crystallization from 1:1 diisopropyl ether:hexane yielded 8.0 g (80%) of **41b**: mp 195−198 °C; TLC (2:1 hexane:ethyl acetate) $R_f = 0.3$; ^{1}H-NMR (MeOD) δ 7.4−7.1 (m, 10H), 5.09 (AB, $J = 14$, 2H), 3.76 (d, $J = 7$, 1H), 3.70 and 3.62 (2m, 2H), 2.85−2.38 (m, 6H), 1.97 (oct, $J = 7$, 1H), 1.69 (non, $J = 7$, 1H), 1.33 (s, 9H), 0.90 (m, 12H); HPLC t_R 17.3 min. Anal. ($C_{32}H_{48}N_4O_6$) C, H, N.

2-Methyl-4-[[*N*-(benzyloxycarbonyl)-L-valinyl]amino]-6(*S*)-hydroxy-7(*S*)-amino-8-phenyl-4-azaoctane (42b). A mixture of 7.0 g (11.9 mmol) of **41b** in 80 mL of formic acid was stirred for 16 h at ambient temperature. The solvent was evaporated under reduced pressure and the resulting solid partitioned between ethyl acetate and saturated $NaHCO_3$. The layers were separated, and the aqueous layer was extracted twice with ethyl acetate. The organic extracts were washed with water and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. Medium pressure chromatography (Lichroprep; 0% → 8% methanol in dichloromethane) yielded 4.00 g (68%) of **42b** as an oil: TLC (9:1 dichloromethane:methanol) $R_f = 0.3$; ^{1}H-NMR (MeOD) δ 7.4−7.15 (m, 10H), 5.09 (AB, $J = 13$, 2H), 3.78 (d, $J = 7$, 1H), 3.53 and 3.00 (2m, 2H), 2.93−2.73 (m, 3H), 2.69−2.56 (m, 2H), 2.43 (dd, $J = 13$, 8, 1H), 1.98 (oct, $J = 7$, 1H), 1.70 (non, $J = 7$, 1H), 0.93 and 0.90 (2d, $J = 7$, 12H); HPLC t_R 14.5 min. Anal. ($C_{27}H_{40}N_4O_4 \cdot 0.41H_2O$) C, H, N.

2-Methyl-4-(L-valinylamino)-6(*S*)-hydroxy-7(*S*)-[[*N*-(quinolin-2-ylcarbonyl-L-asparaginyl]amino]-8-phenyl-4-azaoctane (43b). A solution of 1.00 g (1.33 mmol) of **33b** in 50 mL of methanol and 0.25 g of 10% Pd/C was stirred under 1 atm of H_2 for 6 h. The mixture was filtered through Celite, and the filtrate was concentrated *in vacuo*. Recrystallization by dissolving in dichloromethane and adding diisopropyl ether afforded 0.77 g (93%) of **43b**: mp 157−160 °C; TLC (9:1 dichloromethane:methanol) $R_f = 0.3$; ^{1}H-NMR (MeOD) δ 8.50 (d, $J = 8$, 1H), 8.22 and 8.18 (2d, $J = 8$, 2H), 8.03 (d, $J = 8$, 1H), 7.86 (dd, $J = 8$, 1H), 7.72 (dd, $J = 8$, 1H), 7.30−6.95 (m, 5H), 4.9 (m, 1H), 4.10 (t, $J = 7$, 1H), 3.62 (m, 1H), 3.0−2.4 (m, 9H), 1.84 and 1.68 (2m, 2H), 0.88 (m, 12H); HPLC t_R 10.8 min; FAB MS $(M + H)^+ = 620$. Anal. ($C_{33}H_{45}N_7O_5 \cdot H_2O$) C, H, N.

***N*-(Methoxycarbonyl)-L-valine (General method for the synthesis of carbamate derivatives of L-valine).** To a solution of 7.0 g (60 mmol) of L-valine in a mixture of 100 mL of 2 N aqueous NaOH and 30 mL of dioxane was slowly added at 0 °C 5.67 g (60 mmol) of methyl chloroformate. Upon completion of the addition, the mixture was warmed to room temperature and allowed to stir during 16 h. The reaction mixture was then extracted with 100 mL of dichloromethane, and the aqueous layer was acidified to pH 2 with 4 N aqueous HCl. It was then extracted twice with 100 mL of dichloromethane. Filtration of the organic layer through a pad of cotton wool followed by evaporation to dryness gave 5.01 g (48%) of *N*-(methoxycarbonyl)-L-valine as a white amorphous solid: mp 108−109 °C; ^{1}H-NMR (MeOD) δ 4.05 (d, $J = 5$, 1H), 3.65 (s, 3H), 2.18 (m, 1H), 0.97 (t, $J = 7$, 6H). Anal. ($C_7H_{13}NO_4$) C, H, N.

(Quinolin-2-ylcarbonyl)-L-asparagine *tert*-Butyl Ester. To an ice-cooled stirred solution of 123.3 g (0.66 mol) of L-asparagine *tert*-butyl ester in 3.7 L of THF was added 162.26 g (0.786 mol) of DCC. After stirring for 10 min, 106.1 g (0.786 mol) of HOBT and 125.03 g (0.72 mol) of quinoline-2-carboxylic acid were added. Stirring was continued for a further 18 h; then the reaction mixture was filtered. The filtrate was concentrated *in vacuo* and the resulting solid partially redissolved in 2.5 L of ethyl acetate; 2 L of saturated $NaHCO_3$ was added, and the heterogeneous mixture was filtered again. The aqueous layer of the filtrate was separated and extracted twice with 1 L of ethyl acetate. The organic layers were washed with 2 L of saturated $NaHCO_3$, water, and brine (2×), dried (Na_2SO_4), filtered, and concentrated under reduced pressure to afford a brown oil. Crystallization from 0.3 L of warm dichloromethane and 0.39 L of hexane yielded 204 g (90%) of (quinolin-2-ylcarbonyl)-L-asparagine *tert*-butyl ester: TLC (3:1 ethyl acetate:hexane) $R_f = 0.15$; ^{1}H-NMR (MeOD) δ 8.47 (d, $J = 9$, 1H), 8.19 and 8.17 (2d, $J = 9$, 2H), 8.00 (d, $J = 8$, 1H), 7.84 (q, $J = 8$, 1H), 7.69 (dd, $J = 8$, 1H), 4.9 (t, $J = 6$, 1H), 3.03 (dd, $J = 16$, 6, 1H), 2.88 (dd, $J = 16$, 6, 1H), 1.49 (s, 9H); FAB MS $(M + H)^+ = 344$.

(Quinolin-2-ylcarbonyl)-L-asparagine Hydrochloride.[27] To a stirred solution of 203 g (0.59 mol) of (quinolin-2-ylcarbonyl)-L-asparagine *tert*-butyl ester in 2.03 L of dioxane was added 2.03 L of 4 N HCl(g) in dioxane at 10 °C. The solution was stirred for 3 h at 45 °C and then cooled to room temperature. The resulting precipitate was separated, resuspended in 1.5 L of ether, and stirred vigorously. Filtration afforded 168 g (88%) of crystalline (quinolin-2-ylcarbonyl)-L-asparagine hydrochloride: mp 122−125 °C; ^{1}H-NMR (MeOD) δ 8.50 (d, $J = 8$, 1H), 8.21 and 8.17 (2d, $J = 8$, 2H), 8.00 (d, $J = 8$, 1H), 7.83 (q, $J = 8$, 1H), 7.68 (q, $J = 8$, 1H), 5.12 (t, $J = 6$, 1H), 3.02 (d, $J = 6$, 2H).

Biology. HIV protease inhibition assay: HIV-1 protease was expressed and purified according to the method described by Billich et al.[28] A peptide cleavage assay was performed using the icosapeptide H-Arg-Arg-Ser-Asn-Gln-Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Asn-Ile-Gln-Gly-Arg-Arg-OH as described by Richards et al.[29] and monitored by HPLC. The inhibitors were dissolved in DMSO, diluted with 20 mM β-morpholinoethanesulfonic acid (MES) buffer pH 6.0, and added to the icosapeptide (122 μM) in 20 mM MES buffer, pH 6.0. DMSO concentration did not exceed 5% in the assay system. The reaction was initiated by adding 10 μL of protease solution and was terminated after an incubation time of 1 h at 37 °C by the addition of 10 μL of 0.3 M $HClO_4$. The samples were centrifuged at 10000*g* during 5 min, and 20 mL of the supernatant was injected onto a reversed phase HPLC column (Nucleosil C18, 5 μm; Macherey & Nagel). The cleavage products were analyzed after elution with a water/acetonitrile gradient containing 0.1% TFA, a flow rate of 1 mL/min, and detection at 215 nm. Ro 31-8959[19] served as a reference compound in this assay with an IC_{50} of 6.3 nM. The standard error was determined to be ±13%.

Antiviral assay: The dose−response curves for HIV protease inhibitors were determined in MT-2 cells, seeded in 96-well round bottom plates (Nunc, Kamstrup, Denmark) at 2×10^4/well in 50 μL of complete medium. Serial dilutions from DMSO stock solutions of compound were freshly prepared in complete medium prior to tests. The compound was added in a volume of 50 μL and HIV-1/MN (2800 $TCID_{50}$/well) in a volume of 100 μL. The assays were performed in triplicates. After 4 days of incubation at 37 °C in a fully humidified atmosphere containing 5% CO_2, 10 μL samples of the culture supernatants were collected, and virus production was measured as virus-associated reverse transcriptase (RT) activity in these samples. The antiviral effect of the compound was determined as percent RT reduction as compared to the virus controls. The ED_{50} was determined as the concentration of compound required to inhibit 50% of RT production in this assay, similarly ED_{90} as 90% inhibition of RT formation. The assays were performed in triplicates using Ro 31-8959[19] as a reference compound.

Pharmacokinetic studies: All compounds were administered as a suspension of 12 mg of compound/mL in 19% (w/v) (hydroxypropyl)-β-cyclodextrin (Wacker Chemie, Munich, FRG)/5% DMSO in water. Twenty-eight day old female Balb/c mice (Balb/c AnCbif Tif[SPF]) were used for pharmacokinetic studies. The formulated compounds were administered by gavage. Each mouse received 200 μL resulting in an average dose of 120 mg of compound/kg of body weight. Four mice were used per time point (30, 60, 90, and 120 min). Mice were sacrificed, and heart blood was collected into heparinized tubes at given time points after administration. The blood samples were centrifuged (10000*g*, 5 min); the plasma was removed and mixed with an equal volume of acetonitrile. The protein precipitate was removed by centrifugation (10000*g*, 5 min) and the supernatant dried under vacuum. The residue was resuspended in 0.1 mL of 0.05 M phthalate buffer, pH 3.0, and 20 μL of 3 M NaCl. The mixture was extracted twice with 1 and 0.2 mL of diisopropyl ether, respectively. The diisopropyl ether fractions were pooled and dried under vacuum. The residue was dissolved in 50% acetonitrile/water prior to analysis by reversed phase HPLC. Chromatographic analysis of these samples was carried out on a 125 × 4.6 mm Nucleosil C18, 5 μm analytical column equilibrated with a mobile phase of acetonitrile containing 0.1% trifluoroacetic acid in water. The concentration of acetonitrile used was dependent on the compound under investigation. The flow rate was 1 mL/min. The compounds were detected by UV absorbance at 215 nm. Concentrations were determined by the external standard method; peak heights were used to determine concentrations by comparison with standard curves. The standard curves were constructed from HPLC analysis of blood containing known concentrations of compound which had been processed by the methods described above.

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Supporting Information Available: ^{1}H-NMR (200 MHz) data for compounds **4b**−**l**, **5b**−**l**, **7e**,**f**,**i**, and **12a** and elemental analysis data, mass spectra data, and HPLC data for compounds **7**−**43** (5 pages). Ordering information is given on any current masthead page.

References

(1) De Clercq, E. Toward Improved Anti-HIV Chemotherapy: Therapeutic Strategies for Intervention with HIV Infections. *J. Med. Chem.* **1995**, *38*, 2491−2517.

(2) (a) Darke, P. L.; Huff, J. R. HIV Protease as an Inhibitor Target for the Treatment of AIDS. *Adv. Pharmacol.* **1994**, *25*, 399−454. (b) Lang, M.; Rösel, J. HIV-1 Protease Inhibitors: Development, Status, and Potential Role in the Treatment of AIDS. *Arch. Pharm.* (*Weinheim, Ger.*) **1993**, *326*, 921−924. (c) Martin, J. A. Recent advances in the design of HIV proteinase inhibitors. *Antiviral Res.* **1992**, *17*, 265−278. (d) Debouck, C. The HIV-1 Protease as a Therapeutic Target for AIDS. *AIDS Res. Human Retroviruses* **1992**, *8*, 153−164. (e) Huff J. R. HIV Protease: A Novel Chemotherapeutic Target for AIDS. *J. Med. Chem.* **1991**, *34*, 2305−2314.

(3) (a) Boehme R. E.; Borthwick, A. D.; Wyatt, P. G. Antiviral Agents. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: New York, 1995; Vol. 30, pp 139−144. (b) Abdel-Meguid, S. S. Inhibitors of Aspartylproteinase. *Med. Res. Rev.* **1993**, *13*, 731−778. (c) Meek, T. D. Inhibitors of HIV-1 Protease. *J. Enzyme Inhib.* **1992**, *6*, 65−98.

(4) (a) Kay, J.; Dunn, B. M. Viral proteinases: weakness in strength. *Biochim. Biophys. Acta* **1990**, *1048*, 1−18. (b) Navia, M. A.; Fitzgerald, P. M. D.; McKeever, B. M.; Leu, C.-T.; Heimbach, J. C.; Herber, W. K.; Sigal, I. S.; Darke, P. L.; Springer, J. P. Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. *Nature* **1989**, *337*, 615−620. (c) Wlodawer, A.; Miller, M.; Laskolski, M.; Sathyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schneider, J.; Kent, S. B. H. Conserved Folding in Retroviral Proteases: Crystal Structure of a Synthetic HIV-1 Protease. *Science* **1989**, *245*, 616−621.

(5) (a) Ahmad, S.; Ashfaq, A.; Alam, M.; Bisacchi, G. S.; Chen, P.; Cheng, P. T. W.; Greytok, J. A.; Hermsmeier, M. A.; Lin, P.-F.; Lis, K. A.; Merchant, Z.; Mitt, T.; Skoog, M.; Spergel, S. H.; Tino, J. A.; Vite, G. D.; Colonno, R. J.; Zahler, R.; Barrish, J. C. α-Hydroxyamide Derived Aminodiols as Potent Inhibitors of HIV Protease. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1729−1734. (b) Lam, P. Y. S.; Jadhav, P. K.; Eyermann, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, J. N.; Chang, C.-H.; Weber, P. C.; Jackson, D. A.; Sharpe, T. R.; Erickson-Viitanen, S. Rational desing of potent, bioavailable, nonpeptide cyclic ureas as HIV protease inhibitors. *Science* **1994**, *263*, 380−384. (c) Barrish, J. C.; Gordon, E.; Alam, M.; Lin, P.-F.; Bisacchi, G. S.; Chen, P.; Cheng, P. W. T.; Fritz, A. W.; Greytok, J. A.; Hermsmeier, M. A.; Humphreys, W. G.; Lis, K. A.; Marella, M. A.; Merchant, Z.; Mitt, T.; Morrison, R. A.; Obermeier, M. T.; Plusec, J.; Skoog, M.; Slusarchyk, W. A.; Spergel, S. H.; Stevenson, J. M.; Sun, C.-Q.; Sundeen, J. E.; Taunk, P.; Tino, J. A.; Warrack, B. M.; Colonne, R. J.; Zahler, R. Aminodiol HIV Protease Inhibitors. 1. Design, Synthesis, and Preliminary SAR. *J. Med. Chem.* **1994**, *37*, 1758−1768 and references cited therein. (d) Babine, R. E.; Zhang, N.; Jurgens, A. R.; Schow, S. R.; Desai, P. R.; James, J. C.; Semmelhack, M. F. The Use of HIV-1 Protease Structure in Inhibitor Design. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 541−546. (e) Askin, D.; Wallace, M. A.;Vacca, J. P.; Reamer, R. A.; Volante, R. P.; Shinkai, I. Highly Diastereoselective Alkylations of Chiral Amide Enolates: New Routes to Hydroxyethylene Dipeptide Isostere Inhibitors of HIV-1 Protease. *J. Org. Chem.* **1992**, *57*, 2771−2773. (f) Bone, R.; Vacca, J. P.; Anderson, P. S.; Holloway, M. K. X-ray Crystal Structure of HIV Protease Complex with L-700,417, an Inhibitor with Pseudo C_2 Symmetry. *J. Am. Chem. Soc.* **1991**, *113*, 9382−9384.

(6) (a) Budt, K.-H.; Peyman, A.; Hansen, J.; Knolle, J.; Meichsner, C.; Paessens, A.; Ruppert, D.; Stowasser, B. HIV Protease Inhibitor HOE/BAY 793, Structure-Activity Relationship in a Series of C_2-Symmetric Diols. *Bioorg. Med. Chem.* **1995**, *3*, 559−571. (b) Randad, R. S.; Lubkowska, L.; Bhat, T. N.; Munshi, S.; Gulnik, S. V.; Yu, B.; Erickson, J. W. Symmetry-Based HIV Protease Inhibitors: Rational Design of 2-Methylbenzamides as Novel P_2/P_2' Ligands. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1707−1712. (c) Ettmayer, P.; Hübner, M.; Billich, A.; Rosenwirth, B.; Gstach, H. Novel, Extended Transition State Mimic in HIV-1

Protease Inhibitors with Peripheral C_2-Symmetry. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2851−2856. (d) Kempf, D. J.; Codacovi, L.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Saldivar, A.; Vasavanonda, S.; Marsh, K. C.; Bryant, P.; Sham, H. L.; Green, B. E.; Betebenner, D. A.; Erickson, J.; Norbeck, D. W. Symmetry-Based Inhibitors of HIV Protease. Structure-Activity Studies of Acylated 2,4-Diamino-1,5,diphenyl-3-hydroxypentane and 2,5-Diamino-1,6-diphenylhexane-2,4-diol. *J. Med. Chem.* **1993**, *36*, 320−330. (e) Kempf, D. J.; Marsh, K. C.; Paul, D. A.; Knigge, M. F.; Norbeck, D. W.; Kohlbrenner, W. E.; Codacovi, L.; Vasavanonda, S.; Bryant, P.; Wang, X. C.; Wideburg, N. E.; Clement, J. J.; Plattner, J. J.; Erickson, J. Antiviral and Pharmacokinetic Properties of C_2 Symmetric Inhibitors of the Human Immunodeficiency Virus Type 1 Protease. *Antimicrob. Agents Chemother.* **1991**, 2209−2214.

(7) (a) Wonacott, A.; Cooke, R.; Hayes, F. R.; Hann, M. M.; Jhoti, H.; McMeekin, P.; Mistry, A.; Murray-Rust, P.; Singh, O. M. P.; Weir, M. P. A Series of Penicillin-Derived C_2-Symmetric Inhibitors of HIV-1 Proteinase: Structural and Modeling Studies. *J. Med. Chem.* **1993**, *36*, 3113−3119. (b) Chenera, B.; Boehm, J. C.; Dreyer, G. B. Synthesis of C_2-Symmetric and Pseudosymmetric HIV-1 Protease Inhibitors from D-Mannitol and D-Arabitol. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 219−222. (c) Kempf, D. J.; Norbeck, D. W.; Codacovi, L.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Paul, D. A.; Knigge, M. F.; Vasavanonda, S.; Craig-Kennard, A.; Saldivar, A.; Rosenbrook, W., Jr.; Clement, J. J.; Plattner, J. J.; Erickson, J. Structure Based, C_2 Symmetric Inhibitors of HIV Protease. *J. Med. Chem.* **1990**, *33*, 2687−2689. (d) Erickson, J.; Neidhart, D. J.; VanDrie, J.; Kempf, D. J.; Wang, X. C.; Norbeck, D. W.; Plattner, J. J.; Rittenhouse, J. W.; Turon, M.; Wideburg, N.; Kohlbrenner, W. E.; Simmer, R.; Helfrich, R.; Paul, D. A.; Knigge, M. Design, Activity, and 2.8 Å Crystal Structure of a C_2 Symmetric Inhibitor Complexed to HIV-1 Protease. *Science* **1990**, *249*, 527−533.

(8) Spaltenstein, A.; Leban, J. J.; Furfine, E. S. Synthesis of C_2-Symmetric HIV-Protease Inhibitors with Sulfur-Containing Central Units. *Tetrahedron Lett.* **1993**, *34*, 1457−1460.

(9) (a) Abdel-Meguid, S. S.; Zhao, B.; Murthy, K. H. M.; Winborne, E.; Choi, J.-K.; DesJarlais, R. L.; Minnich, M. D.; Culp, J. S.; Debouck, C.; Tomaszek, T. A., Jr.; Meek, T. D.; Dreyer, G. B. Inhibition of Human Immunodeficiency Virus-1 Protease by a C_2-Symmetric Phosphinate. Synthesis and Crystallographic Analysis. *Biochemistry* **1993**, *32*, 7972−7980. (b) Peyman, A.; Wagner, K.; Budt, K.-H.; Spanig, J.; Ruppert, D.; Meichsner, C.; Paessens, A. Inhibition of Human Immunodeficiency Virus-1 Protease by a C_2-symmetrical Phosphinic Acid Amide. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1191−1194. (c) Peyman, A.; Budt, K.-H.; Spanig, J.; Stowasser, B.; Ruppert, D. C_2-symmetric Phosphinic Acid Inhibitors of HIV Protease. *Tetrahedron Lett.* **1992**, *33*, 4549−4552.

(10) (a) Lecoq, A.; Marraud, M. Hydrazino and α-Amino peptides. Chemical and Structural Aspects. *Tetrahedron Lett.* **1991**, *32*, 2765−2768. (b) Gante, J. Azapeptides. *Synthesis* **1989**, 405−413.

(11) (a) Fässler, A.; Bold, G.; Lang, M.; Schneider, P. EP-521827-A1, Pharmakologisch wirksame Hydrazinpräparate und Verfahren zu deren Herstellung; priority date, July 3, 1991; publication date, Jan. 7, 1993. (b) Fässler, A.; Rösel, J.; Grütter, M.; Tintelnot-Blomley, M.; Alteri, E.; Bold, G.; Lang, M. Novel Pseudosymmetric Inhibitors of HIV-1 Protease. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2837−2842. (c) Fässler, A.; Cozens, R.; Bold, G.; Capraro, H. G.; Mestan, J.; Rösel, J. Prodrug Esters of Pseudosymmetric HIV-1 Protease Inhibitors. Presented at the 210th ACS Meeting, Chicago, IL, 1995; Abstract Medi 28. (d) Sham, H. L.; Betebenner, D. A.; Zhao, Ch.; Wideburg, N. E.; Saldivar, A.; Kempf, D. J.; Plattner, J. J.; Norbeck, D. W. Facile Synthesis of Potent HIV-1 Protease Inhibitors containing a Novel Pseudosymmetric Dipepide Isostere. *J. Chem. Soc., Chem. Commun.* **1993**, 1052−1053. (e) Sham, H. L; Zhao, Ch.; Marsh, K. C.; Betebenner, D. A; Lin, S.; McDonald, E.; Vasavanonda, S.; Wideburg, N.; Saldivar, A.; Robins, T.; Kempf, D. J.; Plattner, J. J.; Norbeck, D. W. Potent Inhibitors of the HIV-1 Protease with Good Oral Bioavailabilities. *Biochem. Biophys. Res. Commun.* **1995**, *211*, 159−165. (f) The linear aza backbone-modified peptidomimetic has also been converted into azacyclic ureas in analogy to the C_2-symmetric ureas; see: Bold, G.; Bhagwat, S. S.; Fässler, A.; Lang, M. WO 95 02582, Cyclic hydrazine compounds; priority date, July 14, 1993; publication date, Jan. 26, 1995. (g) Sham, H. L.; Zhao, C.; Stewart, K. D.; Betebenner, D. A.; Lin, S.; Park, C. H.; Kong, X.-P.; Rosenbrook, W., Jr.; Herrin, T.; Madigan, D.; Vasavanonda, S.; Lyons, N.; Molla, A.; Saldivar, A.; Marsh, K. C.; McDonald, E.; Wideburg, N. E.; Denissen, J. F.; Robins, T.; Kempf, D. J.; Plattner, J. J.; Norbeck, D. W. A Novel Picomolar Inhibitor of Human Immunodeficiency Virus Type 1 Protease. *J. Med. Chem.* **1996**, *39*, 392−397.

(12) Priestle, J. P.; Fässler, A.; Rösel, J.; Tintelnot-Blomley, M.; Strop, P.; Grütter, M. Comparative Analysis of the X-ray Structures of HIV-1 and HIV-2 Proteases in Complex with CGP 53820, a Novel Pseudosymmetric Inhibitor. *Structure* **1995**, *3*, 381−389.

(13) Dutta, A. S.; Morley, J. S. Preparation of α-Aza-amino-acid Derivatives and Intermediates for the Preparation of α-Aza-peptides. *J. Chem. Soc., Perkin Trans. I* **1975**, 1712−1720.

(14) Rutjes, F. P. J. T.; Teerhuis, N. M.; Hiemstra, H.; Speckamp, W. N. Synthesis of Cyclic α-Hydrazino Acid Derivatives via N-Acylhydrazonium Ions. *Tetrahedron* **1993**, *49*, 8605−8628.

(15) (a) Thompson, W. J.; Fitzgerald, P. M. D.; Holloway, M. K.; Emini, E. A.; Darke, P. L.; McKeever, B. M.; Schleif, W. A.; Quintero, J. C.; Zugay, J. A.; Tucker, T. J.; Schwering, J. E.; Homnick, C. F.; Nunberg, J.; Springer, J. P.; Huff, J. R. Synthesis and Antiviral Activity of a Series of HIV-1 Protease Inhibitors with Functionality Thethered to the P_1 or P_1' Phenyl Substituents: X-ray Crystal Structure Assisted Design. *J. Med. Chem.* **1992**, *35*, 1685−1701. (b) Thompson, W. J.; Vacca, J. P.; Huff, J. R.; Lyle, T. A.; Young, S. D.; Hungate, R. W.; Britcher, S. F.; Ghosh, A. K. HIV protease inhibitors useful for the treatment of AIDS. EP 434365 A2, Dec. 18, 1990. (c) Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. A Synthesis of Protected Aminoalkyl Epoxids from α-Amino Acids. *J. Org. Chem.* **1987**, *52*, 1487−1492.

(16) The crystalline Boc-phenylalaninal proved to have an ee >98% according to the ^{1}H-NMR spectroscopic method described by DeCamp, A. E.; Kawaguchi, A. T.; Volante, R. P.; Shinkai, I. Stereocontrolled Addition of Propionate Homoenolate Equivalents to Chiral α-Amino Aldehydes. *Tetrahedron Lett.* **1991**, *32*, 1867−1870.

(17) All four possible stereoisomers of the Boc-protected epoxide could be separated on a chiral analytical HPLC column: stationary phase, Chiralcel OD; mobile phase, 98:2 hexane:2-propanol, detection at 210 nm; retention times in min, *2S3R*-isomer 12.2, *2R3S*-isomer 13.7, *2R3R*-isomer 15.5, *2S3S*-isomer 16.6. The reference compounds with *2R3S*- and *2R3R*-configurations were synthesized using commercially available D-phenylalaninol.

(18) According to analytical HPLC analysis, both the *threo* and *erythro* isomers of trifluoroacetyl epoxide **15** were enantiomerically >98% pure: stationary phase, Chiralpak AD; mobile phase, 99:1 hexane:2-propanol, detection at 215 nm; retention times in min, *2R3S*-isomer 22.1, *2S3R*-isomer 24.4, *2S3S*-isomer 28.8, *2R3R*-isomer 33.5.

(19) The Quin-Asn P_3P_2 residue is a constituent of Ro 31-8959 as reported by Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Kröhn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. Rational Design of Peptide-Based HIV Proteinase Inhibitors. *Science* **1990**, *248*, 358−361.

(20) Hydroxyethylamine-derived inhibitors like Ro 31-8959 show a marked preference for the *R*-stereochemistry at the hydroxyl group, whereas longer inhibitors with proline in P_1' position and extended C-terminus prefer the *S*-configuration for binding to the enzyme. Krohn, A.; Redshaw, S.; Ritchie, J. C.; Graves, B. J.; Hatada, M. H. Novel Binding Mode of Highly Potent HIV-Proteinase Inhibitors Incorporating the (*R*)-Hydroxyethylamine Isostere. *J. Med. Chem.* **1991**, *34*, 3340−3342.

(21) (a) Getman, D. P.; DeCrescenzo, G. A.; Heintz, R. M.; Reed, K. L.; Talley, J. J.; Bryant, M. L.; Clare, M.; Houseman, K. A.; Marr, J. J.; Mueller, R. A.; Vazquez, M. L.; Shieh, H.-S.; Stallings, W. C.; Stegeman, R. A. Discovery of a Novel Class of Potent HIV-1 Protease Inhibitors Containing the (*R*)-(Hydroxyethyl)urea Isostere. *J. Med. Chem.* **1993**, *36*, 288−291. (b) Vazquez, M. L.; Bryant, M. L.; Clare, M.; DeCrescenzo, G. A.; Doherty, E. M.; Freskos, J. N.; Getman, D. P.; Houseman, K. A.; Julien, J. A.; Kocan, G. P.; Mueller, R. A.; Shieh, H.-S.; Stallings, W. C.; Stegeman, R. A.; Talley, J. J. Inhibitors of HIV-1 Protease containing the Novel and Potent (*R*)-(Hydroxyethyl)sulfonamide Isostere. *J. Med. Chem.* **1995**, *38*, 581−584.

(22) Alteri, E.; Bold, G.; Cozens, R.; Fässer, A.; Klimkait, T.; Lang, M.; Lazdins, J.; Poncioni, B.; Rösel, J. L.; Schneider, P.; Walker, M.; Woods-Cook, K. CGP 53437, an Orally Bioavailable Inhibitor of Human Immunodeficiency Virus Typ 1 Protease with Potential Antiviral Activity. *Antimicrob. Agents Chemother.* **1993**, *37*, 2087−2092.

(23) As a measure of lipophilicity the partition coefficent (log *P*) between *n*-octanol and water for a group of aza-peptide analogs has been determined using the shaking flask method: **7a**, log *P* 2.55; **7c**, 3.02; **8a**, 3.61; **24a**, 3.74; **26a**, 2.89. Inhibitors in this related structural series generally display an decreasing coefficient of ED_{50}/IC_{50} with increasing log *P*.

(24) Simultaneous replacement of the two *N*-acetyl-L-valinyl substituents with two H-L-valinyl substituents removes two hydrogen bonds of the carbonyl groups to the amide nitrogens of Asp29 and Asp29′ of the enzyme. In a related series of aza-peptide analogs, this replacement resulted in a 3000-fold decrease in inhibitory potency as described in ref 11a.

(25) The favorable oral bioavailability of cyclosporin, which contains several N-methylated and intramolecularly hydrogen-bonded amide moieties, supports the postulate that secondary amides principally hamper the oral bioavailability of peptidic compounds. Furthermore, transport into the cell is suggested to

correlate inversely with the ability of a compound to form hydrogen bonds with water; see: Smith, A. B., III; Hirschmann, R.; Pasternak, A.; Akaishi, R.; Guzman, M. C.; Jones, D.; Keenan, T. P.; Sprengeler, P.; Darke, P. L.; Emini, E. A.; Holloway, M. K.; Schleif, W. A. Design and Synthesis of Peptidomimetic Inhibitors of HIV-1 Protease and Renin. Evidence for Improved Transport. *J. Med. Chem*. **1994**, *37*, 215−218 and references cited therein.

(26) Several protease inhibitors containing the Quin-Asn moiety besides Ro 31-8959 have been described; see: (a) SC 52151, ref 21a. (b) LY 289612, Kaldor, S. W.; Hammond, M.; Dressman, B. A.; Fritz, J. E.; Crowell, T. A.; Hermann, R. A. New dipeptide isosteres useful for the inhibition of HIV-1 protease. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1385−1390. (c) Girijavallabhan, V. M.; Bennett, F.; Patel, N. M.; Ganguly, A. K.; Basmahapatra, B.; Butkiewicz, N.; Hart, A. The synthesis of novel HIV-protease inhibitors. *Bioorg. Med. Chem*. **1994**, *2*, 1075−1083. (d) Haebisch, D. D.; Roebe, W. D.; Hansen, J. D.; Paessens, A. D. Trifluoromethyl containing pseudopeptides. EP 0528242 A, Feb. 24, 1993. (e) Tucker, T. J.; Lumma, W. C.; Payne, L. S.; Wai, J. M.; de Solms, S. J.; Giuliani, E. A.; Darke, P. L.; Heimbach, J. C.; Zugay, J. A.; Schleif, W. A.; Quintero, J. C.; Emini, E. A.; Huff, J. R.; Anderson, P. S. A Series of Potent HIV-1 Protease Inhibitors Containing a Hydroxyethyl Secondary Amine Transition State Isostere: Synthesis, Enzyme Inhibition, and Antiviral Activity. *J. Med. Chem*. **1992**, *35*, 2525−2533.

(27) Martin, J. A.; Redshaw, S. Preparation of N-(asparaginyl-aminohydroxyphenylbutyl)-decahydroisoquinoline-3-carboxamides as HIV protease inhibitors. EP 432695 A2, June 19, 1991.

(28) Billich, S.; Knoop, M.-T.; Hansen, J.; Strop, P.; Sedlacek, J.; Mertz, R.; Moelling, K. Synthetic Peptides as Substrates and Inhibitors of Human Immune Deficiency Virus-1 Protease. *J. Biol. Chem*. **1988**, *263*, 17905−17908.

(29) Richards, A.; Phylip, L. H.; Farmerie, W. G.; Scarborough, P. E.; Alvarez, A.; Dunn, B. M.; Hirel, P. H.; Konvalinka, J.; Strop, P.; Pavlickova, L.; Kostka, V.; Kay, J. Sensitive, Soluble Chromogenic Substrates for HIV-1 Proteinase. *J. Biol. Chem*. **1990**, *265*, 7733−7736.

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