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Synthesis of symmetrical pseudopeptides as potential inhibitors of the human immunodeficiency virus-1 protease

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Summary — It is demonstrated that HIV-1 protease is essential for the assembly and infectivity of the acquired immunodeficiency syndrome (AIDS) virus. This protease is an aspartyl protease with a 2-fold symmetry axis. Potential inhibitors were synthesized and consisted of a spacer linking 2 peptidic chains. They had to satisfy the following constraints: a C_2 symmetry axis, a backbone similar to the peptidic substrates, and side-chains filling the subsites $S_n...S'_n$. The compounds were synthesized *via* peptidic synthetic methods and evaluated in an enzymatic test with HIV-1 protease. Several compounds displayed an inhibitory activity at 10 μM. They possessed the spacers CO, CO-CO, CO-CH_2-CHOH-CO and the terminal chain Phe-O-iC_4H_9. However, the structural-variation-based optimization of these different compounds failed and no potent inhibitors were prepared.

HIV-1 / aspartyl protease / peptidic synthesis

Introduction

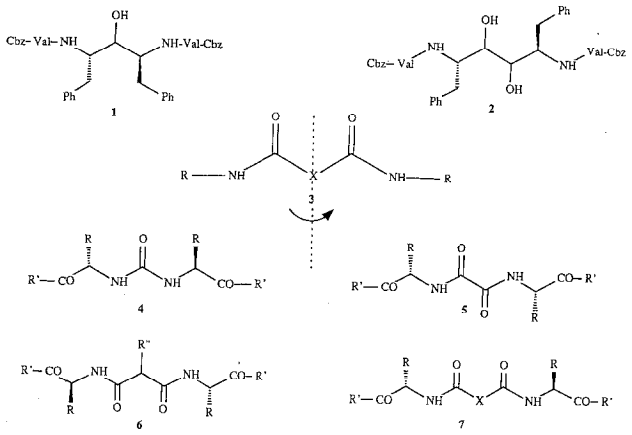
The 3 major genes of human immunodeficiency virus type 1, *gag*, *pol* and *env*, encode polyprotein products that undergo post-translational proteolytic processing during the replication cycle [1]. The product of the *env* gene is the glycoprotein gpl60, which is cleaved by a cellular protease [2], but the polyproteins produced from the *gag* and *gag-pol* genes are processed by a particular virally encoded protease [3]. This cleaves at specific sites on the polyprotein, releasing itself [4] and other viral proteins. Mutation of the protease results in morphologically immature and non-infectious viral particles [5, 6]. Thus, the inhibition of HIV protease has attracted widespread interest and represents a potential target for the development of therapies against AIDS. As with a number of retroviral proteases, it is a member of the well-characterized aspartic protease family [7]. From structural analysis of these enzymes, it is now clear that the active site is composed of 2 catalytic aspartyl residues located within a similar tripeptide sequence, Asp-Thr-Gly [8]. The catalytic mechanism implicates a water molecule polarized by hydrogen-bonding interactions with the carboxyl side chains of the aspartic acids of the enzymatic site. This is a powerful nucleophilic agent for the scissile peptide bond of the substrate [9].

HIV-1 protease is active as a dimer produced by the assembly of two 99 amino-acid polypeptides resulting in a molecular framework analogous to those of the monomeric aspartic proteases, such as pepsin, renin or penicillopepsin [10]. Using this knowledge, it was shown that the structure of HIV-1 protease has a crystallographic 2-fold rotational (C_2) symmetry identical to that of the Rous sarcoma virus (RSV) protease [11]. HIV-1 protease is highly specific for the precursor polyproteins and there are 4 cleavage sites within the *gag* polyprotein precursor, which give rise to the mature core proteins pl7, p24, p7 and p6 [12]. Comparison of the sequences of the scissile bond does not indicate a strict homology between these sites, although many of them contain the sequence Phe/Tyr-Pro [13]. Several studies of structure–activity relationships emphasized the favorable role of a hydrophobic substituent at the P_1-P'_1 cleavage point of the substrates. Nevertheless, the hydrolysis rate is also dependent upon the nature of the side chain of the other amino acids, particularly in the P_3 and the P'_2 positions [14].

Extensive studies have been carried out in recent years giving rise to a number of potent HIV-1 protease inhibitors as potential anti-AIDS drugs and almost all of these compouds were derived from classical strategies for the design of protease inhibitors widely used in the field of renin inhibitors [15]. These strategies are based on the synthesis of peptide substrate analogs in which the scissile bonds have been replaced by a non-hydrolyzable isostere, such as a reduced amide, a hydroxylethylene sequence or a fluoranited ketone [16, 17]. This last method illustrates the classical

approach by analogy with the transition state during the hydrolysis step. It has been demonstrated that such a compound mimics the geometrical tetrahedric transition state during the hydrolysis step, involving a high affinity with the enzymatic site. However, the development of peptide-based inhibitors into effective drugs has been hampered by the high lipophilicity and poor water solubility of the compounds often giving weak pharmacological properties [18]. For this reason, it is essential to develop a new strategy to inhibit HIV-1 protease. The C_2 symmetry properties of the enzyme could be used to design new potential inhibitors as the C_2 symmetry of the different subsites of the enzymatic cleft was clearly demonstrated by X-ray crystallography [19] of the cocrystallized enzyme inhibitors. Thus, this unique property could be used to prepare compounds with the same symmetry, the structure of which should complement the enzymatic site. The value of the affinity of such a compound would depend only upon the degree of complementarity with the site. Initially, the advantage of such a strategy would be to prepare compounds with minimal peptidic character, but also with marked specificity relative to other aspartic proteases since these symmetry properties are unique to HIV-protease. Furthermore, it was thought that small molecules with better pharmacological properties and bioavailability than the previous peptidic inhibitors could be obtained. The structural properties of these compounds must satisfy 3 major constraints: they need a C_2 symmetry axis similar to that of the enzyme; the axis of the backbone chain of the molecule must coincide with that of the peptidic chain of the substrates; and the different substituents must fill the enzyme subsites $S_n \ldots S'_n$.

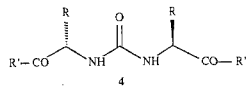
A similar strategy was successfully developed by the pharmaceutical firm Abbott [20, 21], which designed and synthesized compounds **1** and **2** as potent inhibitors of HIV-1 protease. However, these can also be related to the classical transition-state analogy-based compounds by the presence of 1 or 2 hydroxyl functions, which are essential for their activity, on the central part of the molecule. It was therefore of interest to examine whether the symmetry properties alone were essential for productive interactions with the enzymatic site and potent inhibition of the enzyme. For this purpose, we have undertaken the synthesis and assessment of the potency for inhibition of HIV-1 protease of a series of symmetrical compounds **3** designed initially from a dicarboxylic spacer and peptidic derivatives, the sequences of which were chosen from among the P'_2, P'_1, P_1 or P_2 groups of the HIV-1 protease substrates [1]. The initial goal of our work was to obtain information about the structural parameters implicated in the molecular recognition of the enzyme site to improve the design of new, non-peptidic inhibitors. However, the synthesized compounds **3** differ from compounds **1** and **2** by the opposite direction of their peptidic backbone since Abbott's compounds were prepared from a diamino spacer. In this paper we describe the initial results obtained with this strategy.



Chemistry

The compounds synthesized were amide derivatives and were prepared by the classical methods. Ureas **4** (compounds **9–12**, table I) and oxalamides **5** (compounds **13–41** and **47**, table II) were directly synthesized by the condensation of triphosgene or oxalyl chloride, respectively, with the amino derivatives **8** in the presence of 4-methylmorpholine (scheme 1, *Methods A* and *B*, respectively) [22]. A similar route was used to prepare the tripeptide derivative **47**. Malonamide derivatives **6** (compounds **42–44**, table III) were obtained using the azide method [23] (scheme 1, *Method D*). Thus, the diazidomalonyls were prepared *in situ* from the diazotation reaction of the corresponding hydrazino derivative and condensed with the amine to give fairly good yields. The amino malonyl derivatives **45–46** and the Pro-Ile-Val derivative **48** were prepared directly from *N*-protected aminomalonic acid and malonic acid respectively by the DCC/HOBt route [24, 25] (scheme 1, *Method C*) with moderate yields. Similarly (table IV), this method was used for the synthesis of the fumaric and maleic derivatives **52** and **55** and the amides **56–60** derived from hydroxysuccinic and tartaric acids. For these latter compounds, a protective group on the hydroxyl function was not useful and the derivatives were obtained by a straightforward route. On the other hand, the pimelic and maleic derivatives (compounds **61–66** and **53–54**)

Table I. Structures and enzymatic inhibitory activity of the urea derivatives **4**.



Compound	*R*[a]	*R'*	*Method*	*Yield (%)*	*mp (°C)*	*Solvent*[b]	*Prot inh*[c]	*Anal formula*[d]
9	$PhCH_2$	Val-OMe	*A*	60	216	M/DIPO	I	$C_{31}H_{42}N_4O_7$
10	$PhCH_2$	Val-OH	[e]	65	105	M/DIPO	I	$C_{29}H_{36}N_2O_5$
11	$PhCH_2$	O-iC_4H_9	*A*	55	150	M/DIPO	I	$C_{27}H_{36}N_2O_5$
12	iC_4H_9	O-iC_4H_9	*A*	60	65	[f]	41	$C_{21}H_{40}N_2O_5$

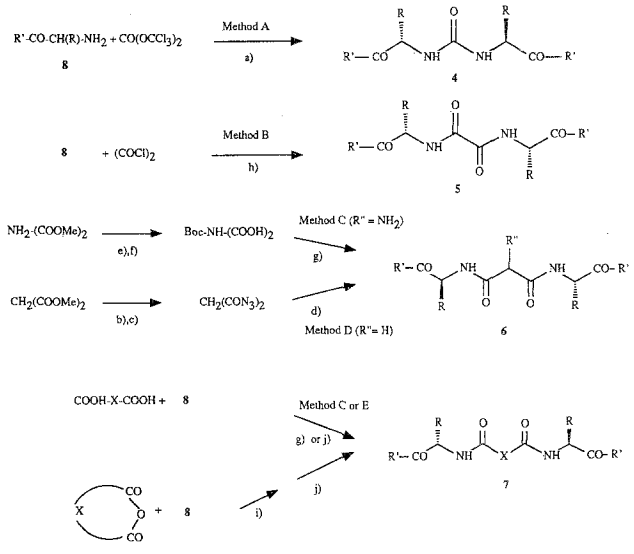
[a]The configuration is (*S*). [b]M: methanol, DIPO: isopropyl ether. [c]% inhibition of HIV-1 protease at 10^{-5} M; I: inactive (less than 10% inhibition). [d]Analyses for C, H, N. [e]Obtained by saponification of **9**. [f]Purified by chromatography.

were directly synthesized from the diacid and amino synthons by condensation *via* the mixed anhydride method (scheme 1, *Method E*). For the succinamide compounds **50–51**, the preferred route was the mono condensation of the amino derivatives with succinic anhydride, followed by a condensation reaction *via* the mixed anhydride method (scheme 1). The asymmetric compound **49** branched with a benzyl group in the P_1 position was synthesized from the corresponding succinic acid [26] and condensed in the presence of BOP [27]. The hydroxylated compounds **67–69** were synthesized directly from the reduction by $NaBH_4$ of the corresponding keto derivatives.

The di- or tripeptides linked to the diacid spacer were synthesized by the classical method using peptidic condensation in the presence of isobutyl chloroformate and 4-methylmorpholine in THF [28]. The promising enzymatic results observed among the first oxalamides **5** synthesized (compound **17**) with the Phe-O-iC_4H_9 sequence initiated the synthesis of several aromatic structural analogues of Phe esters, which were prepared from the commercially available amino acids or synthesized, as for compounds **34** and **36**, by previously reported methods [29, 30]. Preliminary enzymatic results with the oxalamide derivatives demonstrated, in particular, that the β-branched chain of esters of Phe can mimic the Val residue and, for this reason, various esters of phenylalanine were prepared. Almost all of the compounds were synthesized through the azeotropic method with TsOH. However, for compounds **21–26** where the terminal moieties are derived from (*S*)-3-methyl-2-butanol, methyl (*S*)-2-hydroxy-3-methylbutanoate [31] or 1,2-dimethylpropanol the corresponding esters were prepared efficiently by the mixed anhydride method using 2,4,6-trichlorobenzoyl chloride as the activating group [31] in the presence of 4-methylmorpholine.

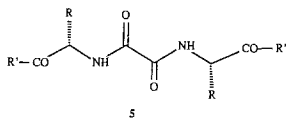
Biological results and discussion

Enzymatic activity was determined using an HIV-1 aspartic protease expressed in high levels in *Escherichia coli* as previously reported [32]. This method uses a synthetic octapeptide, Val-Ser-Gln-Asn-Phe(pNO_2)-Pro-Ile-Val, which spans the cleavage site between the p17 and p24 proteins of the virus and is hydrolyzed by HIV-1 protease. Protease activity was evaluated by the amount of the cleaved pentapeptide produced and measured by a spectrophotometric assay (307 nm). Inhibitory activity was expressed relative to an uninhibited control. Pepstatine was the reference inhibitor.

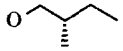


Scheme 1.

Table II. Structures and enzymatic inhibitory activity of the oxalamide derivatives **5**.

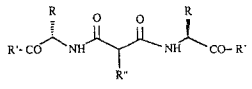


5

Compound	*R*[a]	*R'*	*Method*	*Yield (%)*	*mp (°C)*	*Solvent*[d]	*Prot inh*[e]	*Anal formula*[f]
13	$PhCH_2$	Val-OMe	B	55	95	h	I[g]	$C_{32}H_{42}N_4O_8$
14	$PhCH_2$	O-CH_2Ph	B	50	161	EtOH	I	$C_{34}H_{32}N_2O_6$
15	$PhCH_2$	O-*n*Pent	B	51	110	EtOH	I	$C_{30}H_{40}N_2O_6$
16	$PhCH_2$	O-*n*Bu	B	45	144	EtOH	I	$C_{28}H_{36}N_2O_6$
17	$PhCH_2$	O-*i*Bu	B	44	175	EtOH	40	$C_{28}H_{36}N_2O_6$
18	$PhCH_2$[b]	O-*i*Bu	B	45	179	EtOH	I	$C_{28}H_{36}N_2O_6$
19	$PhCH_2$[c]	O-*i*Bu	B	45	153	EtOH	19	$C_{28}H_{36}N_2O_6$
20	$PhCH_2$	O-*n*Pr	B	45	166	EtOH	I	$C_{26}H_{36}N_2O_6$
21	$PhCH_2$		B	57	137	EtOH	22	$C_{30}H_{40}N_2O_6$
22	$PhCH_2$[b]	" "	B	30	121	EtOH	I	$C_{30}H_{40}N_2O_6$
23	$PhCH_2$[c]	" "	B	53	114	EtOH	45	$C_{30}H_{40}N_2O_6$
24	$PhCH_2$		B	55	111	EtOH	37	$C_{30}H_{40}N_2O_6$
25	$PhCH_2$		B	48	99	EtOH	I	$C_{30}H_{40}N_2O_6$
26	$PhCH_2$		B	61	124	EtOH	I	$C_{32}H_{40}N_2O_6$
27	4-BzlO-$PhCH_2$	O-*i*Bu	B	52	153	EtOH	24	$C_{42}H_{48}N_2O_8$
28	4-MeO-$PhCH_2$	O-*i*Bu	B	62	149	EtOH	I	$C_{30}H_{40}N_2O_8$
29	4-NO_2-$PhCH_2$	O-*i*Bu	B	65	224	EtOH	49	$C_{28}H_{34}N_4O_{10}$
30	4-F-$PhCH_2$[c]	O-*i*Bu	B	40	175	*i*PrOH	30	$C_{28}H_{34}F_2N_2O_6$
31	3-F-$PhCH_2$[c]	O-*i*Bu	B	62	169	h	24	$C_{28}H_{34}F_2N_2O_6$
32	4-Cl-$PhCH_2$[c]	O-*i*Bu	B	33	189	M/C	20	$C_{28}H_{34}Cl_2N_2O_6$
33	4-OH-$PhCH_2$	O-*i*Bu	B	64	222	AcOEt	27	$C_{28}H_{36}N_2O_8$
34	4-PyridylCH$_2$[c]	O-*i*Bu	B	38	149/174[i,h]		I	$C_{26}H_{34}N_4O_6$
35	4-c$C_6H_{11}CH_2$	O-*i*Bu	B	62	75	h	I	$C_{28}H_{48}N_2O_6$
36	4-NaphthylCH$_2$[c]	O-*i*Bu	B	68	163	EtOH/CX	19	$C_{36}H_{40}N_2O_6$
37	3-IndolylCH$_2$	O-*i*Bu	B	18	170	EtOH	I	$C_{32}H_{38}N_2O_6$
38	$(CH_3)_2CH\text{-}CH_2$	O-*i*Bu	B	26	77	h	35	$C_{22}H_{40}N_2O_6$
39	$CH_3(CH_2)_3$	O-*i*Bu	B	29	98	EtOH	I	$C_{22}H_{40}N_2O_6$
40	$PhCH_2$	NH-*i*Bu	B	17	> 290	EtOH	NT[j]	$C_{28}H_{38}N_4O_4$
41	$(CH_3)_2CH\text{-}CH_2$[c]	NH-*i*Bu	B	26	206	C/H	21	$C_{22}H_{42}N_4O_4$
47	(CO-Pro-Ileu-Val-OMe)$_2$		B	30	Oil	h	I	$C_{36}H_{60}N_6O_{10}$

[a]The configuration is (*S*), except for the compounds with footnotes b) and c). [b](*R*) configuration. [c]Racemic mixture. [d]C: chloroform; M: methanol; H: hexane. [e]Percentage inhibition of HIV-1 protease at 10^{-5} M. [f]Analyses for C, H, N. [g]I: Inactive (less than 10%). [h]Purified by chromatography. [i]Mixture of diastereoisomers purified by chromatography. [j]Not tested.

Table III. Structures and enzymatic inhibitory activity of the malonyl derivatives **6**.



6

Compound	*R''*	*R*[a]	*R'*	*Method*[b]	*Yield (%)*	*mp (°C)*	*Prot inh*[c]	*Anal formula*[d]
42	H	$PhCH_2$	OMe	D	80	128	28	$C_{23}H_{26}N_2O_6$
43	H	$PhCH_2$	O-*i*Bu	D	60	81	41	$C_{29}H_{38}N_2O_6$
44	H	$PhCH_2$	Val-OMe	D	50	65	I	$C_{33}H_{44}N_4O_8$
45	Boc-NH	$PhCH_2$	O-*i*Bu	D	45	Oil	45	$C_{34}H_{47}N_3O_8$
46	NH_2	$PhCH_2$	O-*i*Bu	[e]	100	60	I	$C_{29}H_{40}N_4ClO_6$
48	H	Pro-Ileu-Val-OMe		C	15	90	I	$C_{37}H_{62}N_6O_{10}$

[a]The configuration is (*S*). [b]The compounds were purified by chromatography. [c]Percentage inhibition of HIV-1 protease at 10^{-5} M, I: inactive (less than 10% inhibition). [d]Analyses for C, H, N. [e]From **45** by the hydrolysis with 3 N HCl/AcOEt.

Analysis of the results from the enzymatic assay showed weak inhibitory activity for the synthesized compounds described above since only a few compounds possessed an IC_{50} value in the range of 10 μM and were 10 000 times less active than the most potent compounds reported up to now. In the oxalamide derivatives (table II), only the β-branched chains of the isobutyl and 2-methyl-1-butyl groups displayed an inhibitory activity (compounds **17**, **23** and **29**). It is possible that they are mimicking the side chain of valine, an amino acid located in the P_2 position in the different substrates and inhibitors [21] of HIV-1 protease as in compounds **1** and **2**.

With the derivatives **4** and **6** (tables I and III), in which an orthogonal orientation was given to the C_2 symmetrical axis of the molecules by using the spacers CO and malonyl, the degree of inhibition was not improved. We noticed the inactivity of the amino compound **46** which was unable to bind with the carboxylic group of aspartic acid in the enzymatic site to give an additional anchorage point.

The influence of lengthening the spacer with a C_4 or C_7 chain was studied with compounds **7** reported in table IV. In addition, several spacers with 1 or 2 hydroxyl groups such as hydroxy succinic, tartaric or hydroxy pimelic acid were also selected to evaluate the importance of this function in the central part of the molecule. Only the derivatives of hydroxy succinic acid **57** possessing the chiral center (*R*) display a level of activity similar to the previous active compounds. On the other hand, the introduction of an additional hydroxyl function with the derivatives of tartaric acid **58–60** led to a drop in the activity. These results do not agree with previous results obtained with the potent C_2 symmetrical reference compounds **1** and **2** in which the favorable role of an additional hydroxyl function in the central part of the molecule was demonstrated [33].

Several hypotheses can be put forward to explain these disappointing results. The unsuitable length or the relatively locked nature of the spacer prevents a good match with the enzymatic site. Non-specific interactions with the enzyme surface can explain the failure of the structural-variation-based optimization of the inhibitory activity.

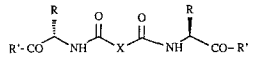
In summary, it seems to be difficult to prepare potent inhibitors of HIV-1 protease when only the concepts of symmetry and productive binding interactions between the enzymatic sites and the side chains of the amino acids of the substrates are considered. The design of efficient spacers requires the introduction of the complementary structural elements of the S'_1 and S_1 subsites separated by a suitable distance and associated with a polar function capable of binding to the aspartic acids of the site. This work is currently in progress in our laboratory.

Experimental protocols

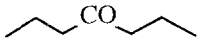
Chemistry

Melting points were determined on a Mettler FP 61 apparatus. ^1H and ^{13}C-NMR spectra were recorded using Brucker AC200E or AM300 spectrometers and tetramethylsilane as an internal standard. Chemical-shift data are reported in parts per million (δ in ppm) where s, d, dd, t, q and m designate singlet, doublet, doublet of doublets, triplet, quartet and multiplet,

Table IV. Structures and enzymatic inhibitory activity of the diamides **7**.



7

Compound	*X*	*R*[a]	*R'*	*Method*	*Yield (%)*	*mp (°C)*	*Solvent*[b]	*Prot inh*[c]	*Anal formula*[d]
49	$CH(CH_2\text{-}Ph)CH_2$	iC_3H_7	O-Me	[e]	55	130	[f]	I	$C_{23}H_{34}N_2O_6$
50	$(CH_2)_2$	$PhCH_2$	O-*i*Bu	[e]	55	130	M/DIPO	23	$C_{30}H_{40}N_2O_6$
51	$(CH_2)_2$	$PhCH_2$	O-Bzl	[e]	40	160	M/DIPO	I	$C_{36}H_{36}N_2O_6$
52	CH=CH*(E)*	$PhCH_2$	O-*i*Bu	C	55	186	AcOEt/H	I	$C_{30}H_{38}N_2O_6$
53	CH=CH*(E)*	iC_3H_7	O-Me	E	45	235	[f]	I	$C_{16}H_{26}N_2O_6$
54	CH=CH*(E)*	iC_3H_7	O-Bzl	E	40	200	[f]	I	$C_{28}H_{34}N_2O_6$
55	CH=CH*(Z)*	$PhCH_2$	O-*i*Bu	C	30	Oil	[f]	16	$C_{30}H_{38}N_2O_6$
56	$CH_2CHOH(S)$	$PhCH_2$	O-*i*Bu	C	55	76	[f]	29	$C_{30}H_{40}N_2O_7$
57	$CH_2CHOH(R)$	$PhCH_2$	O-*i*Bu	C	65	90	[f]	50	$C_{30}H_{40}N_2O_7$
58	$(CHOH)_2(R,R)$	$PhCH_2$	O-*i*Bu	C	55	118	[f]	I	$C_{30}H_{40}N_2O_8$
59	$(CHOH)_2(S,S)$	$PhCH_2$	O-*i*Bu	C	55	119	AcOEt/H	I	$C_{30}H_{40}N_2O_8$
60	$(CHOH)_2(R,S)$	$PhCH_2$	O-*i*Bu	C	35	121	AcOEt/H	I	$C_{30}H_{40}N_2O_8$
61	$(CH_2)_5$	$PhCH_2$	O-Bzl	E	70	128	M/DIPO	I	$C_{39}H_{42}N_2O_6$
62	$(CH_2)_5$	iC_3H_7	O-Me	E	50	103	[f]	I	$C_{19}H_{34}N_2O_6$
63	$(CH_2)_5$	iC_3H_7	O-Bzl	E	85	108	M/DIPO	I	$C_{31}H_{42}N_2O_6$
64		$PhCH_2$	O-Bzl	E	55	135	M/DIPO	I	$C_{39}H_{40}N_2O_7$
65	"	iC_3H_7	O-Bzl	E	60	110	M/DIPO	I	$C_{31}H_{40}N_2O_7$
66	"	iC_3H_7	O-Me	E	45	115	M/DIPO	I	$C_{19}H_{32}N_2O_7$
67		$PhCH_2$	O-Bzl	[g]	90	143	[f]	I	$C_{39}H_{42}N_2O_7$
68	"	iC_3H_7	O-Bzl	[g]	95	125	AcOEt/H	I	$C_{31}H_{42}N_2O_7$
69	"	iC_3H_7	O-Me	[g]	80	135	[f]	I	$C_{19}H_{34}N_2O_7$

[a]The configuration is (*S*).[b]M: methanol; DIPO: isopropyl ether; H: hexane. [c]Percentage inhibition of HIV-1 protease at 10^{-5} M; I: inactive (less than 10% inhibition). [d]Analyses for C, H, N. [e]See *Experimental protocols*. [f]Purified by chromatography. [g]Compounds **67**, **68** and **69** were obtained from the corresponding keto derivatives.

respectively. Mass spectra were obtained using a Ribermag R10-10 mass spectrometer. Infrared spectra were obtained with a Perkin Elmer 1420 ratio recording infrared spectrometer. Microanalyses were performed by the CNRS (Department of microanalytical services), Vernaison, France. The analyses are indicated only by the symbols of the elements when the results obtained were within ± 0.4% of the theoretical values.

Most of the amino acids were purchased from Aldrich-chimie (Strasbourg, France) or Novabiochem (Meudon, France). Several were synthesized according to the published procedures: 3-(2-naphthyl)-(D,L)-alanine [29], and 3-(4-pyridyl)-(D,L)-alanine [30]. Almost all of the amino esters were prepared as crude materials from the amino acids and appropriate alcohols in the presence of *p*-toluenesulfonic acid in toluene; the mixture was heated to reflux and the water formed was trapped in a Dean-Stark receiver [34]. The amino amides such as H-Phe-NH-iC_4H_9 and H-Nle-NH-iC_4H_9 were synthesized by reaction of the ethyl carbonate mixed anhydride of the Boc amino acids with the appropriate amines in the presence of 4-methylmorpholine; this intermediate was deprotected with anhydrous HCl in EtOAc. Methyl (*S*)-2-hydroxy-3-methylbutanoate was prepared according to a previously described method [31].

The di- or tripeptides such as H-Pro-Ile-Val-OMe and H-Phe-Val-OMe were prepared according to the mixed anhydride method [28] by reaction of the isobutylcarbonate of the appropriate Boc amino acids or Boc peptides with the appropriate amino esters in the presence of 4-methylmorpholine. The Boc protection was removed with anhydrous HCl in EtOAc [35]. Two amino esters derived from chiral alcohols were prepared as follows.

L-Phenylalanine[(1S)-1-methyloxycarbonyl-2-methylpropyl] ester
Benzyloxycarbonyl phenylalanine (2.99 g, 10 mmol) was dissolved in THF (25 ml) and treated with 4-methylmorpholine (1.16 ml, 1.062 g, 10.5 mmol). To this solution was added 2,4,6-trichlorobenzoyl chloride (1.64 ml, 2.56 g, 10.5 mmol) in THF (25 ml). The reaction mixture was stirred for 1.5 h, then filtered and concentrated. The resulting oil was dissolved in benzene (20 ml) and treated with methyl (*S*)-2-hydroxy-3-methylbutanoate (1.32 g, 10 mmol) in benzene (15 ml), followed by addition of 4-(*N,N*-dimethylamino)pyridine (DMAP) (3 g, 25.2 mmol). After 18 h, the reaction mixture was diluted with ether (130 ml) and washed with HCl 3 N (2 x 30 ml), 5% $KHCO_3$ (2 x 30ml) and saturated NaCl (30 ml) solutions. The organic layer was dried (Na_2SO_4), filtered and concentrated. The crude oil was purified by column chromatography (SiO_2, CH_2Cl_2/pentane/*i*-PrOH, 80:20:0 then 98:0:2) to yield the pure protected amino ester (3.87 g, yield: 94%).

To a solution of the previous ester (3.16 g, 7.66 mmol) in absolute EtOH (20 ml) was added 0.34 g of 10% Pd/C. The suspension was vigorously stirred under an H_2 atmosphere for 5 h. The catalyst was removed by filtration and washed with absolute EtOH. The filtrate was concentrated and the residue triturated with ether, filtered, washed with ether and dried to give 1.96 g (yield: 81%) of the aminoester. ^{1}H-NMR (CD_3OD) δ: 7.55–7.45 (m, 5H), 5,15 (d, *J* = 4.2 Hz, 1H), 4.65 (t, *J* = 7 Hz, 1H), 3,95 (s, 3H), 3.6–3.25 (m, 2H), 2.45–2.35 (m, 1H), 1.15 (d, *J* = 6.9 Hz, 3H), 1.05 (d, *J* = 6.9 Hz, 3H).

L-Phenylalanine [(1*S*)-1,2-dimethylpropyl]ester was prepared according to the above procedure.

*General methods for the synthesis of compounds **4**, **5**, **6** and **7***
Method A. To a suspension of the amine salt (2 eq) in anhydrous THF at 0°C were added 4-methylmorpholine (4 eq) and a solution of triphosgene (bis-(trichloromethyl)carbonate) (1/3 eq) in anhydrous THF. After 1 h the reaction mixture was filtered, the filtrate evaporated and the residue dissolved in CH_2Cl_2 and washed (1 N HCl, H_2O, $KHCO_3$, H_2O). The organic layer was dried ($MgSO_4$) and concentrated to provide the crude product.

Method B. To oxalyl chloride (0.44 ml, 0.635 g, 5 mmol) in anhydrous THF (5 ml) was added dropwise, under stirring at –30°C, a solution of 4-methylmorpholine (1.10 ml, 1.01 g, 10 mmol) in anhydrous THF (5 ml). A solution of the amino ester (10 mmol) in anhydrous THF (5 ml) was added dropwise over 30 min with stirring at –30°C. After stirring for 12 h at room temperature, the reaction mixture was filtered. The solution was concentrated to provide the crude compound.

Method C. To a solution of the diacid (1 eq) in anhydrous THF at 0°C were added a solution of the amine salt (2 eq) with 4-methylmorpholine (2 eq) in CH_2Cl_2, a solution of HOBt (2.2 eq) in THF and a solution of dicyclohexylcarbodiimide (DCC, 2.2 eq) in CH_2Cl_2. The mixture was stirred for 1 h at 0°C and 1 h at room temperature. The dicyclohexylurea was removed by filtration, the filtrate was evaporated and the residue dissolved in EtOAc and washed (saturated $KHCO_3$, brine, 10% citric acid, brine). The organic layer was dried ($MgSO_4$) and concentrated to provide the crude product.

Method D. To a solution of dihydrazide malonate (1 eq) in a mixture of glacial acetic acid (12 ml), 5 N chlorhydric acid (5 ml) and water (50 ml) at –5°C was added a solution of sodium nitrite (2 eq), in water (5 ml). The yellow solution was extracted with ether (60 ml), the organic layer was washed with ice-cold water (40 ml), dried ($MgSO_4$) and added dropwise to a solution of the amine (2 eq) in a mixture of CH_2Cl_2/Et_2O (50:50, 30 ml). The solution was stirred overnight at room temperature, then washed (1 N HCl, water, 1 N $KHCO_3$, water), dried ($MgSO_4$) and concentrated to provide the crude product.

Method E. To a solution of the diacid (1 eq) in anhydrous THF at –15°C were added 4-methylmorpholine (2 eq), isobutyl chloroformate (2 eq) and a solution of the amine (2 eq). After 1 h at –15°C the reaction mixture was filtered, the filtrate evaporated and the residue dissolved in EtOAc and washed (1 N HCl, H_2O, saturated $KHCO_3$, H_2O). The organic layer was dried ($MgSO_4$) and concentrated to provide the crude product.

*Synthesis of ureas **4***
Compounds **9**, **11** and **12** were prepared according to the general *Method B* (see table I). One example, **9**, is given here.

*Carbonyl-bis-(L-phenylalanyl-L-valine methyl ester) **9**.* According to *Method A*, the dipeptide phenylalanyl valine methyl ester hydrochloride (2.5 g, 7.9 mmol) yielded the product as a white powder which was recrystallized in a mixture of MeOH/DIPO (1.4 g, 2.4 mmol, yield: 60%), mp: 216°C (dec). ^{1}H-NMR ($CDCl_3$) δ: 7.4 (d, *J* = 8.5 Hz, 2H), 7.2–7.0 (m, 10H), 6.1 (d, *J* = 8.5 Hz, 2H), 4.7–4.6 (m, 2H), 4.4 (dd, *J* = 8.5 Hz, *J* = 6 Hz, 2H), 3.6 (s, 6H), 2.8–2.9 (m, 4H), 2.1–1.9 (m, 2H), 0.82 (d, *J* = 6 Hz, 6H), 0.79 (d, *J* = 6.0 Hz, 6H). ^{13}C-NMR ($CDCl_3$) δ: 172.0–171.8 (4C), 156.9 (1C), 136.5 (2C), 129.3 (4C), 128.1 (4C), 126.4 (2C), 57.1–54.6 (4C), 51.5 (2C), 39.0 (2C), 31.0 (2C), 18.7–17.8 (4C). Anal $C_{31}H_{42}N_4O_7$ (C, H, N),

*Synthesis of oxalamides **5***
Compounds **13–32**, **35–41** and **47** were prepared according to the general *Method B* (see table II). One example, **17**, is given here.

*Oxalyl-bis-(L-phenylalanine isobutyl ester) **17**.* Compound **17** was prepared from L-phenylalanine isobutyl ester and oxalyl chloride with a 44% yield after recrystallization in EtOH, mp: 175°C. ^{1}H-NMR ($CDCl_3$) δ: 7.65 (d, *J* = 8.6 Hz, 2H), 7.3–7.0 (m, 10H), 4.8–4.7 (m, 2H), 3.9–3.7 (m, 4H), 3.15–3.0 (m, 4H), 0.8 (d, *J* = 6.8 Hz, 12H). ^{13}C-NMR ($CDCl_3$) δ: 170.3 (2C), 158.7 (2C), 135.5 (2C), 129.2 (4C), 128;7 (4C), 127.2 (2C), 71.7 (2C), 53.8 (2C), 38.0 (2C), 27.6 (2C), 19.0 (4C). α_D^{25} = +50° (c = 1, $CHCl_3$). Anal $C_{28}H_{36}N_2O_6$ (C, H, N).

*Oxalyl-bis-(L-tyrosine isobutyl ester) **33**.* Compound **27** (2.4 g, 3.5 mmol) was reduced under an H_2 atmosphere for 16 h in the presence of 0.4 g of Pd/C (10%) in 100 ml of EtOAc at 20°C. After addition of 100 ml of MeOH, the catalyst was removed by filtration and washed with MeOH. After concentration and crystallization in EtOAc, compound **33** was prepared with a 64% yield, mp: 221.5°C. DCI-MS(NH_3): 546 (M + NH_4^+). ^{1}H-NMR (CD_3OD) δ: 7.0 (d, *J* = 8.5 Hz, 2H), 6.75 (d, *J* = 8.5 Hz, 2H), 4.6–4.7 (m, 2H), 3.9–3.95 (m, 4H), 3.0–3.2 (m, 4H), 1.85–2.0 (m, 2H), 0.95 (d, *J* = 6.8 Hz, 12H). ^{13}C-NMR (CD_3OD) δ: 172.0 (2C), 160.5 (2C), 157.4 (2C), 131.15 (4C), 128.1 (2C), 116.25 (4C), 72.45 (2C), 55.5 (2C), 37.2 (2C), 28.75 (2C), 19.2 (4C). α_D^{25} = –20.5° (c = 1, DMF). Anal $C_{28}H_{36}N_2O_8$ (C, H, N).

*Oxalyl-bis-[3-(4-pyridyl)-(D,L)-alanine isobutyl ester] **34**.* Compound **34** was prepared from 3-(4-pyridyl)-(D,L)-alanine isobutyl ester and oxalyl chloride. The crude product was purified by column chromatography (SiO_2, CH_2Cl_2/*i*PrOH, 95:5, then 93:7 and 90:10). Two fractions were collected and identified as the diastereoisomers.

The fraction (R_f: 0.39 CH_2Cl_2/*i*PrOH 90:10) was obtained with a yield of 21%, mp: 174°C. DCI-MS(NH_3): 499 (M + H^+).

^{1}H-NMR ($CDCl_3$) δ: 8.45 (d, J = 6.0 Hz, 4H), 7.7 (d, J = 8.4 Hz, 2H), 7.0 (d, J = 6.0 Hz, 4H), 4.85–4.75 (m, 2H), 3.95–3.8 (m, 4H), 3.2–2.95 (m, 4H), 1.9–1.75 (m, 2H), 0.8 (d, J = 6.7 Hz, 12H). Anal $C_{26}H_{34}N_4O_6$ (C, H, N).

The fraction (R_f: 0.26 CH_2Cl_2/*i*PrOH 90:10) was obtained with a yield of 17%, mp: 149°C. DCI-MS(NH_3): 499 ($M + H^+$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.6 (m, 2H), 7.8 (d, J = 8.6 Hz, 2H), 7.15 (m, 2H), 4.95–4.9 (m, 2H), 3.4 and 3.95 (AB part of a ABX, J = 10.6, J = 6.7, J = 6.7 Hz, 4H), 3.25 and 3.15 (AB part of a ABX, J = 13.9, J = 6.05, J = 6.95 Hz, 4H), 1.95–1.9 (m, 2H), 0.9 (d, J = 6.7 Hz, 12H). Anal $C_{26}H_{34}N_4O_6$ (C, H, N).

Synthesis of the malonamides ***6***

Malonyl-bis-(L-phenylalanine methyl ester) ***42***. According to *Method D*, phenylalanine methyl ester (1.8 g, 10 mmol) gave the product as a white powder after flash chromatography (1.7 g, 3.9 mmol, yield: 80%), mp: 128°C. ^{1}H-NMR ($CDCl_3$) δ: 7.5–7.2 (m, 12H), 5.0–4.9 (m, 2H), 3.6 (s, 6H), 3.3–3.1 (m, 6H). ^{13}C-NMR ($CDCl_3$) δ: 171 (2C), 166 (2C), 136.5 (2C), 129.3 (4C), 128.1 (4C), 126.4 (2C), 53.0–52.0 (4C), 42. (1C), 37.0 (2C). Anal $C_{23}H_{26}N_2O_6$ (C, H, N).

Malonamides **43**, **44** and **48** were prepared according to *Methods D* or *C* (see table III).

tert-Butyloxycarbonylaminomalonyl-bis-(L-phenylalanine isobutyl ester) ***45***. This compound was prepared from tert-butyloxycarbonylaminomalonic acid which was synthesized according to the following process. To a suspension of diethyl aminomalonate hydrochloride (4.2 g, 20 mmol) in CH_2Cl_2 (40 ml) were added an aqueous solution of $NaHCO_3$ (40 ml, 1.7 g, 20 mmol) and a solution of di-tert-butyldicarbonate (4.4 g, 20 mmol) in CH_2Cl_2 (5 ml). The mixture was refluxed for 3 h and, after cooling, the aqueous layer was extracted with CH_2Cl_2. The organic layers were washed (1 N HCl, brine, $NaHCO_3$, brine), dried ($MgSO_4$) and concentrated to give an oil (5.4 g, 19 mmol, yield: 95%).

To a solution of the above diester (2.8 g, 10 mmol) in EtOH (10 ml) was added dropwise a solution of 2.5 N NaOH (10 ml). After one night, the mixture was acidified and extracted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product, which was recrystallized in a mixture of MeOH/DIPO (1.8 g, 8.2 mmol, yield: 80%), mp: 115°C. ^{1}H-NMR (CD_3OD) δ: 6 (d, J = 7 Hz, 1H), 3.5 (d, J = 7 Hz, 1H), 1.5 (s, 9H). Anal $C_8H_{13}NO_6$ (C, H, N).

According to *Method C*, phenylalanine isobutyl ester *p*-toluenesulfonate (3.5 g, 9 mmol) and the previous diacid (1 g, 4.55 mmol) gave compound **45** as an oil after flash chromatography (1.3 g, 2,mmol, yield: 45%). ^{1}H-NMR ($CDCl_3$) δ: 7.4 (d, J = 7.8 Hz, 2H), 7.2–7.0 (m, 10H), 5.8 (d, J = 5.0 Hz, 1H), 4.8–4.6 (m, 3H), 3.8–3.7 (m, 4H), 3.1–3.0 (m, 4H), 1.9–1.7 (m, 2H), 1.4 (s, 9H), 0.85 (d, J = 7.0 Hz, 12H). ^{13}C-NMR ($CDCl_3$) δ: 170.4–165.8 (4C), 155.3 (1C), 135.3 (2C), 129.0 (4C), 128.4 (4C), 126.7 (2C), 80.7 (1C), 71.3 (2C), 57.8 (1C), 53.7 (2C), 37.8 (2C), 27.7–27.4 (5C), 18.7 (4C). Anal $C_{34}H_{47}N_3O_8$ (C, H, N).

Aminomalonyl-bis-(L-phenylalanine isobutyl ester) hydrochloride ***46***. The protective group of the previous ester (625 mg, 1 mmol) was removed according to a previously reported method [35], mp: 60°C. ^{1}H-NMR ($CDCl_3$) δ: 9.0 (d, J = 7 Hz, 2H), 8.0 (s, 2H), 7.2–7.0 (m, 10H), 5.6 (s, 1H), 4.8–4.6 (m, 3H), 3.8–3.7 (m, 4H), 3.1–3.0 (m, 4H), 1.9–1.7 (m, 2H), 0.80 (d, J = 7 Hz, 6H), 0.70 (d, J = 7 Hz, 6H). ^{13}C-NMR ($CDCl_3$) δ: 172.2, 173.5, 162.9 (4C), 135.3 (2C), 129.0 (4C), 128.4 (4C), 126.7 (2C), 71.8 (2C), 55.3–55.0–54.9 (3C), 37.8 (2C), 27.3 (2C), 18.7 (4C). Anal $C_{29}H_{40}N_4O_6Cl$ (C, H, N).

Synthesis of diamides ***7***

Succinyl-bis-(L-phenylalanine isobutyl ester) ***50***. To a solution of phenylalanine isobutyl ester *p*-toluenesulfonate (5.9 g, 20 mmol) cooled at 0°C in $CHCl_3$ (50 ml) were added succinic anhydride (2 g, 20 mmol) and 4-methylmorpholine (4.4 mol, 40 ml). The solution was stored at room temperature for 2 h and then evaporated. The oil was dissolved in a mixture of EtOAc/H_2O (50:50 200 ml) and acidified with 1 N HCl. The organic layer was dried ($MgSO_4$) and evaporated to dryness to give the acid ester intermediate which was recrystallized in a mixture of MeOH/DIPO (3.5 g, 15 mmol, yield: 75%), mp: 93°C. α_D^{25} = +68.1° (c = 1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 10.0 (s, 1H), 7.1–6.9 (m, 5H), 6.3 (d, J = 7.8 Hz, 1H), 4.8–4.7 (m, 1H), 3.8 (J_{AB} = 10.7, J_{AX} = 6.7, J_{BX} = 6.8 Hz, 2H), 3.0 (m, 2H), 2.6–2.2 (m, 4H), 1.9 (1H, septuplet, J = 6.7 Hz), 0.8 (d, J = 6.7 Hz, 6H). ^{13}C-NMR ($CDCl_3$) δ: 176.8 (1C), 171.6–171.2 (2C), 135.6–126.8 (6C), 71.5 (1C), 53.2 (1C), 37.7 (1C), 30.6–29.1 (2C), 27.3 (1C), 18.7 (2C). Anal $C_{17}H_{23}NO_5$ (C, H, N).

The above acid (1.2 g, 5.2 mmol) and phenylalanine isobutyl ester *p*-toluenesulfonate (2.1 g, 5.2 mmol) were treated according to the mixed anhydride method described previously for the synthesis of peptides. The crude product was recrystallized in MeOH/DIPO to give a white powder (1.95 g, 3.7 mmol, 70%), mp: 130°C. α_D^{25} = +94° (c = 1 in $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.5–7.2 (m, 10H), 6.6 (d, J = 7.7 Hz, 2H), 5.1–5.0 (m, 2H), 4.2–4.0 (m, 4H), 3.4–3.2 (m, 4H), 2.7 (s, 4H), 2.2–2.0 (m, 2H), 1.0 (d, J = 6.7 Hz, 12H). ^{13}C-NMR ($CDCl_3$) δ: 171.4–171.2 (4C), 135.7 (2C), 128.9 (4C), 128.2 (4C), 126.7 (2C), 71.2 (2C), 53.1 (2C), 37.6 (2C), 31.1 (2C), 27.3 (2C), 16.7 (4C). Anal $C_{30}H_{40}N_2O_6$ (C, H, N).

Compound **51** was obtained according to the above procedure.

Fumaryl-bis-(L-phenylalanine isobutyl ester) ***52***. According to *Method C*, phenylalanine isobutyl ester *p*-toluenesulfonate (1.97 g, 5 mmol) and fumaric acid (290 mg, 2.5 mmol) gave **52** as a white powder which was recrystallized in a mixture of EtOAc/hexane (730 mg, 1.4 mmol, yield: 55%), mp: 186°C. ^{1}H-NMR ($CDCl_3$) δ: 7.2–6.9 (m, 10H), 6.8 (s, 2H), 6.7 (d, J = 8.0 Hz, 2H), 4.9–4.8 (m, 2H), 3.8–3.7 (m, 4H), 3.0–2.9 (m, 4H), 1.8–1.6 (m, 2H), 0.9 (d, J = 6.7 Hz, 12H). ^{13}C-NMR($CDCl_3$) δ: 171.4–163.7 (4C), 135.7 (2C), 133.0 (2C), 129.2 (4C), 128.4 (4C), 126.9 (2C), 71.5 (2C), 53.5 (2C), 37.9 (2C), 27.4 (2C), 18.9 (4C). Anal $C_{30}H_{38}N_2O_6$ (C, H, N).

Compounds **53**, **54** and **55** were prepared according to the above procedure (*Methods C* or *E*).

(S)-Hydroxysuccinyl-bis-(L-phenylalanine isobutyl ester) ***56***. According to *Method C*, phenylalanine isobutyl ester *p*-toluenesulfonate (3.9 g, 10 mmol) and L-malic acid (670 mg, 5 mmol) gave **56** as a white powder after flash chromatography (1.50 g, 2.7 mmol, yield: 55%), mp: 76°C. ^{1}H-NMR ($CDCl_3$) δ: 7.6 (d, J = 8.3 Hz, 1H), 7.4–7.1 (m, 10H), 6.8 (d, J = 7.8 Hz, 1H), 5.1–4.9 (m, 3H), 4.5–4.4 (m, 1H), 4.1–4.0 (m, 4H), 3.3–3.2 (m, 4H), 2.9–2.5 (m, 2H), 2.0–1.9 (m, 2H), 1.1–1.0 (m, 12H). ^{13}C-NMR ($CDCl_3$) δ: 171.7–171.2–170.9–170.8 (4C), 135.5–135.3 (2C), 129.0–128.9–128.3–128.2 (8C), 126.9–126.7 (2C), 71.4–71.2 (2C), 68.6 (1C), 53.0–52.7 (2C), 38.2–37.9–37.5 (3C), 27.2 (2C), 18.6 (4C). Anal $C_{30}H_{40}N_2O_7$ (C, H, N).

Compounds **57**, **58**, **59** and **60** were obtained in a similar way according to *Method C*.

Pimelyl-bis-(L-phenylalanine benzyl ester) ***61***. According to *Method E*, phenylalanine benzyl ester (2 g, 6.85 mmol) and pimelic acid (550 mg, 3.4 mmol) gave **61** which was recrystallized (MeOH/DIPO) to provide a white powder (1.5 g, 2.4 mmol, yield: 70%), mp: 128°C; ^{1}H-NMR ($CDCl_3$) δ: 7.3–7.0 (m, 2H), 5.9 (d, J = 7.8 Hz, 2H), 5.1 (m, 4H), 4.8 (m, 2H), 3.0 (m, 4H), 2.0 (t, J = 7.4 Hz, 4H), 1.5 (q, J = 7.4 Hz, 4H), 1.2 (m, 2H). ^{13}C-NMR ($CDCl_3$) δ: 172.5–171.6 (4C), 135.8–135.1 (4C), 129.3 (8C), 128.5 (8C), 127.1 (4C), 67.3 (2C), 53.0 (2C), 37.8 (2C), 36.1 (2C), 28.6 (1C), 25.0 (2C). Anal $C_{39}H_{42}N_2O_6$ (C, H, N).

The compounds **62–66** were prepared in a similar way according to *Method C* or *E* (see table IV).

4-Hydroxypimelyl-bis-(L-phenylalanine benzyl ester) ***67***. To a solution of the ketone **64** (600 mg, 0.93 mmol) in THF (20 ml) cooled at –15°C were added $NaBH_4$ (13 mg, 0.35 mmol) and MeOH (10 ml) dropwise. The mixture was kept for 1 h at 15°C and quenched at 0°C with 1 N HCl (10 ml). The organic solvent was evaporated and the water was extracted with EtOAc. The organic layer was dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography to provide a white powder (560 mg, 0.85 mmol, yield: 90%). ^{1}H-NMR ($CDCl_3$) δ: 7.3–6.9 (m, 20H), 6.3 (d, J = 7.8 Hz, 2H), 5.0 (2d, J = 12.2 Hz, 4H), 4.8 (m, 2H), 3.4–3.3 (m, 1H), 3.0 (m, 4H), 2.9 (s, broad, 1H), 2.2 (t, J = 6.8 Hz, 4H), 1.6–1.4 (m, 4H). ^{13}C-NMR ($CDCl_3$) δ: 172.9–171.3 (4C), 135.5–134.8 (4C), 129.0–128.3 (20C), 70.1 (1C), 67.0 (2C), 37.5 (2C), 32.6–32.3 (4C). Anal $C_{39}H_{42}N_2O_7$ (C, H, N).

Following the previous procedure, the ketones **65** and **66** gave alcohols **68** and **69** respectively.

Benzylsuccinyl-bis-(L-valine methyl ester) ***49***. To a suspension of benzyl succinic acid (520 mg, 2.5 mmol) in anhydrous CH_2Cl_2 were added valine methyl ester hydrochloride (840 mg, 5 mmol), 1-benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (BOP) [27] (2.21 g, 5 mmol) and 4-methylmorpholine (1.4 ml). The mixture became limpid and the solution was stored for 3 h at room temperature, then washed (brine, 10% citric acid, brine, saturated $KHCO_3$ and brine) and concentrated to provide the crude product, which was purified by flash chromatography to give a white powder (590 mg, 1.3 mmol, yield: 55%), mp: 130°C. α_D^{25} = +6.0° (c = 1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.2 (m, 5H), 6.6–6.3 (m, 2H), 4.5–4.3 (m, 2H), 3.7–3.62–3.60 (3s, 6H), 3.1–2.3 (m, 5H), 2.1–1.8 (m, 2H), 0.9–0.6 (m, 12H). ^{13}C-NMR ($CDCl_3$) δ: 173.9–171.2 (4C), 136.9–126.5 (6C), 57.3–57.2 (2C), 52.0, 51.8 (2C), 45.2 (1C), 38.7–37.9 (2C), 31.2–30.8 (2C), 19.0–18.8–17.9–17.7 (4C). Anal $C_{23}H_{34}N_2O_6$ (C, H, N).

Biochemistry

Test for the inhibition of HIV-1 protease

The assay for the inhibition of HIV-1 protease was performed at Rhône-Poulenc Rorer, Institut de Biotechnologie (Vitry, France). The test used the following octapeptide as the substrate: Val-Ser-Gln-Asn-Phe(pNO$_2$)-Pro-Ile-Val, at a final concentration of 0.4 mM. The drugs were evaluated at 10 μM from a 10 mM DMF solution. The mixture of substrate and drug in a phosphate buffer solution at pH 8 was incubated at 37°C in the presence of HIV-1 protease. The hydrolysis of the substrate was followed with a spectrophotometric assay at 307 nm. The results are given in percentage inhibition of control and expressed in comparison with a non-inhibited assay. The test was carried out in triplicate and, under these conditions, pepstatin produces 70% inhibition of the enzyme activity.

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