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Synthesis and activity of HIV protease inhibitors

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Abstract – We report here the synthesis and activity of HIV protease inhibitors. In the first stage hydrophobic compounds incorporating a 'carba' bond surrogate or a beta-homologated residue were synthesized. Secondly, we synthesized cyclic compounds in which we incorporated 2-quinoline carboxylic acid in the P3 position and the amino-hydroxyindane moiety in the P'3. The last part of this work was dedicated to a structure/activity study of a peptide substrate. These modifications allowed us to work up the synthesis of new pseudopeptide bonds: amino-amide and hydroxy-amide. Compounds with activity in the micromolar range were actually a starting point for the synthesis of new protease inhibitors. © Elsevier, Paris

HIV / protease / inhibitor / pseudopeptide / cyclic peptide

1. Introduction

Human immunodeficiency virus (HIV) is the etiological agent of the acquired immune deficiency syndrome (AIDS) [1]. Numerous efforts to develop therapeutic agents that inhibit or prevent the development of AIDS have been reported, and of the key steps identified to date, processing of the polyprotein encoded by the *gag pol* gene of the retrovirus has attracted much attention. This step, which yields the virally encoded enzymes HIV protease, reverse transcriptase, integrase and structural proteins (matrix, capsid and nucleocapsid) [2], is known to be realized by HIV protease itself, since mutant systems in which the catalytic sequence is modified are non-infectious [3].

The HIV protease is an aspartyl protease composed of two identical 99-amino acid, combining to form a C-2 symmetrical homodimer whose single active site contains the signature sequence Asp-Thr-Gly for aspartyl protease at amino acids 25 to 27 and 125 to 127 [4]. Amino acid substitution for the aspartic acid residue at position 25 results in inactivation of the HIV protease and loss of viral infectivity [2]. The tertiary structure of the enzyme was solved by several groups, and this information has allowed computer-based design of HIV protease inhibitors [5–8].

The general design strategy of replacing the P1–P'1 cleavage site in the substrate by transition state analogues could be used as a basis for the design of tight-binding inhibitors of the HIV protease [9].

In this work we focused attention on three cleavage sites: the reverse transcriptase/integrase site, the autocatalytic releasing site of the protease and the protease/transcriptase cleavage site. The scissile bond of such substrate sequences was removed and replaced by different peptide bond surrogates and some residues were modified. In the way to design new HIV protease inhibitors, we synthesized some cyclic structures.

2. Chemistry

2.1. Incorporation of Leu-Ψ(CH$_2$CH$_2$)-Phe and of a β-homo-phenylalanine into the substrate sequence

Structure/activity relationships of HIV protease substrates have indicated that small and hydrophobic

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residues are well-tolerated in the P3, P2, P'2 and P'3 positions. Moreover, substrate specificity studies realised by Billich [10] demonstrated a preference for aliphatic (or aromatic) and hydrophobic residues in the P1 position: numerous cleavage sites were obtained between the Leu (or Phe) and Phe (or Pro) amino acids. Recently, some compounds corresponding to the following structure with good antiprotease activities were described [11–16]:

X-Val-Leu (or Phe) Ψ Phe -Val-Y
X-Val-Val-Leu (or Phe) Ψ Phe -Val-Val-Y

(X = N-terminal protection; Y = C-terminal protection; Ψ = modified peptide bond). We therefore decided to incorporate a carba bond or a β-homo-residue into such sequences and focused our attention on two cleavage sites:

– Leu*Phe which is the reverse transcriptase/integrase cleavage site [17, 18];

– Phe*Phe (or Phe*Pro) corresponding to the autocatalytic releasing site of the protease and of the cleavage site which produced the protease and reverse transcriptase [19].

The incorporation of the carba bond introduced free rotation between the two methylene groups which allowed the molecule to adopt several conformations. The carba pseudopeptide Boc-LeuΨ(CH_2CH_2)Phe-OH was obtained unequivoqualy by the previously descrided strategy [20] and consisted of condensation of a β-homologated amino-aldehyde with an α-substituted triethyl-phosphonoacetate. We have previously demonstrated that incorporation of a β-homo-residue on the C-terminal dipeptide of gastrin resulted in compounds with antagonist activity [21]. We therefore decided to introduce a β-homo-phenylalanine into our inhibitory sequence.

All the syntheses of pseudopeptides were realised in solution by a fragment condensation strategy. For the compounds incorporating the 'carba' bond, the pseudo-dipeptide Boc-LeuΨ(CH_2CH_2)Phe-OH was synthesized and then coupled to the C-terminal dipeptide. All couplings were performed using BOP reagent. An example of each strategy is exemplified in *figures 1* and *2*.

2.2. Synthesis of cyclic peptides and pseudopeptides

Incorporation of conformational constraints (e.g. cyclic structures) in linear peptides represents a possible approach to obtain peptides with receptor selectivity [22, 23]. Moreover, some cyclic peptides, like cyclosporin, are able to cross the cell membrane [24]. We therefore decided to synthesize cyclic structures by cyclisation between the P2 and P'2 position (*figure 3*). The inhibitory potency of these compounds against the HIV protease and their activity on HIV infected cells was determined. It is well known that asparagine is often found in P2 and/or P'2 position;

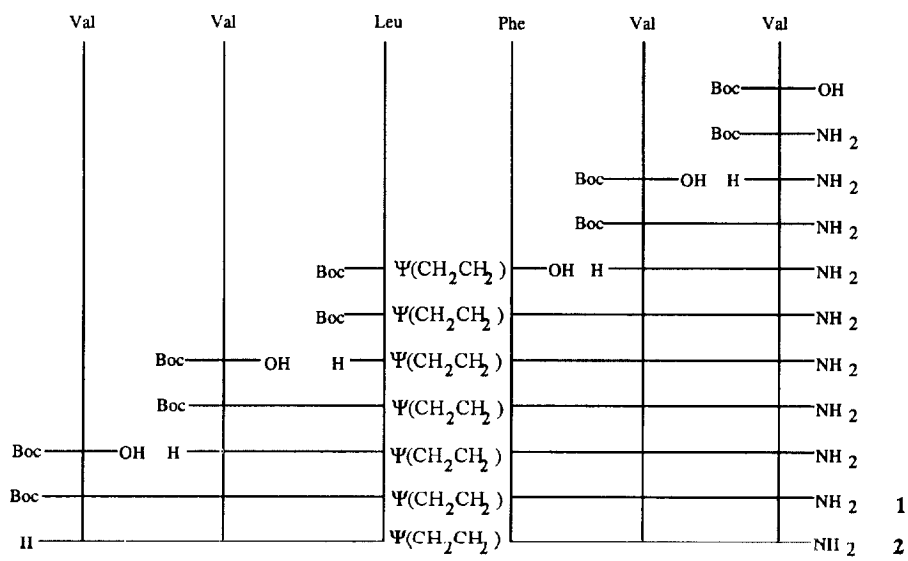

Figure 1. Synthesis of compounds **1** and **2**.

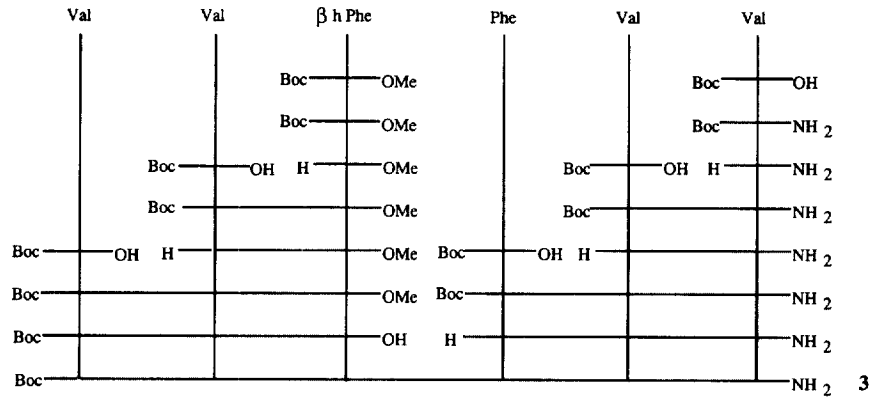

Figure 2. Synthesis of compound **3**.

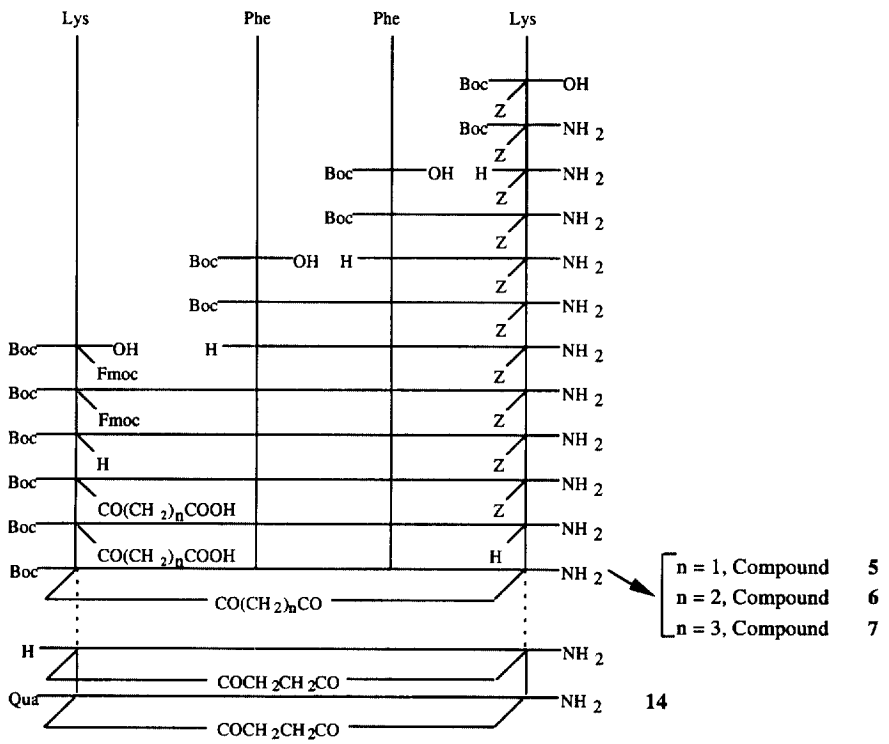

Figure 3. General strategy for the synthesis of cyclic compounds.

we decided to mimic asparagine by incorporating two aspartic acid residues bonded with an ethylenediamine bridge. Finally we have incorporated a 2-quinoline carboxylic acid (Qua) or an (–)-1(*S*)-amino-2(*R*)-hydroxyindane moiety (Ahi) synthesized according to Thompson et al. [27] which have been found to increase inhibitory activity [25, 26].

2.3. Modification in the sequence Phe-Phe-Ile-Phe

According to previously described structure–activity relationships of HIV protease substrates, the tetrapeptide corresponding to the sequence Phe-Phe-Ile-Phe could be the starting point for the design of new HIV protease inhibitors. This sequence was also chosen by Urban and al. [28, 29] as a basis for the synthesis of reduced pseudopeptide inhibitors of the retroviral protease. We have synthesized several pseudopeptides in this series (*figure 4*) including in their sequence a mimic of the transition state analogue, since it has been shown that such derivatives could produce potent HIV protease inhibitors [30].

Compound **17** [28] was obtained by reacting Boc-Phe-H [31] with H-Phe-Ile-Phe-OMe followed by subsequent reduction of the Schiff base and to reduce the resulting Schiff base [32]. The peptide bond surrogate $\Psi(CONNH_2)$ was synthesized according to Aubry et al. [33]. The hydroxyethylamine bond resulted from the condensation of a chloromethyl ketone on the amino group of the C-terminal peptide followed by reduction of the corresponding ketomethyl amine with $NaBH_4$ [34]. The two pseudotetrapeptides Boc-PheΨ(CONOH)Gly-Ile-Phe-OMe and Boc-PheΨ(CONOH)Phe-Ile-Phe-OMe were synthesized as described in *figure 5*. To further investigate

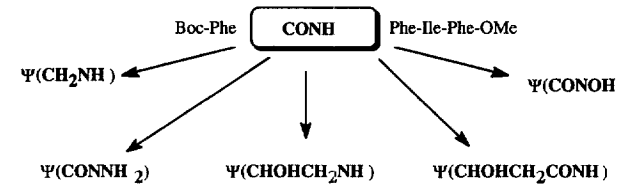

Figure 4. Modifications on the tetrapeptide Boc-Phe-Phe-Ile-Phe-OMe.

the influence of the configuration of the C-terminal residues, L amino acids in the most active compounds were substituted by their D analogues.

3. Biological results and discussion

Compounds with a carba bond or incorporating a β-homologated residue presented moderate activities against HIV protease (*table I*). Cyclic compounds **5**, **6** and **7** were marginally active (test B), with a maximum activity for compound **6** (IC_{50} in the range of 10 μM). Incorporation of a β-homo-phenylalanine, or a hydroxyethylamine surrogate resulted in a decrease of the inhibitory activity. Incorporation of both the (–)-cis amino-hydroxyindane and the 2-carboxyquinoline moiety (e.g. compound **16**, *table II*) resulted in a marginally active compound.

In the last part of this study, a structure/activity relationship was established on the peptide Phe-Phe-Ile-Phe. Compounds **25**, **26**, **28** and **29** behaved as competitive inhibitors of HIV-1 protease. This was

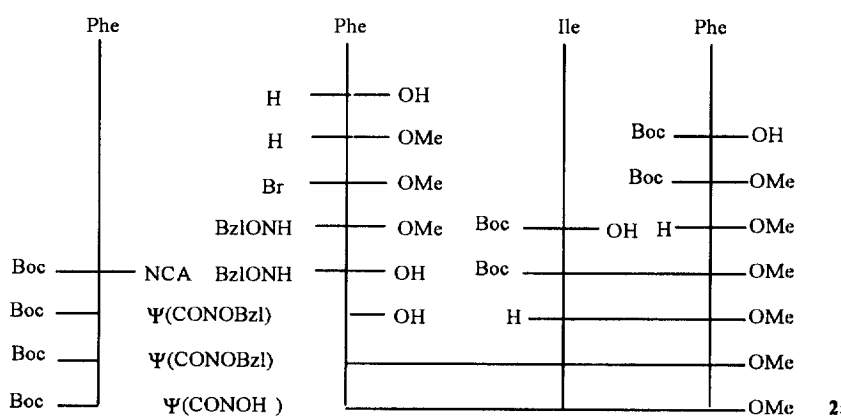

Figure 5. Synthesis of the hydroxy-amide type compound **25**.

Table I. Antiprotease activity of compounds **1–13** and **17–24** (results are expressed in % of inhibition at 10^{-5} M).

Compound	Sequence	Test A	Test B	Test C
	Reference: pepstatine A 10^{-5} M	63%	92%	78%
1	Boc-Val-Val-LeuΨ(CH_2CH_2)Phe-Val-Val-NH_2	29	77	
2	TFA.H-Val-Val-LeuΨ(CH_2CH_2)Phe-Val-Val-NH_2	40	61	
3	Boc-Val-Val-βhPhe-Phe-Val-Val-NH_2	0	11	13
4	TFA.H-Val-Val-βhPhe-Phe-Val-Val-NH_2	0	75	0
5	Boc-Lys-Phe-Phe-Lys-NH_2 $COCH_2CO$	1	22	0
6	Boc-Lys-Phe-Phe-Lys-NH_2 $CO(CH_2)_2CO$	0	62	0
7	Boc-Lys-Phe-Phe-Lys-NH_2 $CO(CH_2)_3CO$	2	32	0
8	Boc-Lys-βhPhe-Phe-Lys-NH_2 $COCH_2CO$	2	1	0
9	Boc-Lys-βhPhe-Phe-Lys-NH_2 $CO(CH_2)_2CO$	0	0	3
10	Boc-Lys-βhPhe-Phe-Lys-NH_2 $CO(CH_2)_3CO$	0	15	0
11	Boc-Lys-PheΨ($CHOHCH_2NH$) Phe-Lys-NH_2 $CO(CH_2)_2CO$	0	5	0
12	Boc-Lys-PheΨ($CHOHCH_2NH$) Phe-Lys-NH_2 $CO(CH_2)_3CO$	2	14	0
13	Boc-Asp-PheΨ($CHOHCH_2NH$) Phe-Asp-NH_2 $NH(CH_2)_2NH$	0	0	18
17	Boc-Phe Ψ(CH_2NH) Phe-Ile-Phe-OMe	42	74	22
18	Boc-Phe Ψ($CONNH_2$) Gly-Ile-Phe-OMe	0	0	3
19	Boc-Phe Ψ($CONNH_2$) Gly-Phe-Ile-Phe-OMe	0	26	7
20	Boc-AHPPA-Ile-Phe-OMe	74	90	71
21	Boc-Phe Ψ($CHOHCH_2NH$) Phe-Ile-Phe-OMe	91	100	64
22	Z-Phe Ψ($CHOHCH_2NH$) Phe-Ile-Phe-OMe	70, IC_{50} = 4.85 μM)	85	67
23	Z-Phe Ψ($CHOHCH_2NH$) Gly-Ile-Phe-OMe	3	40	14
24	Boc-Phe Ψ(CONOH) Gly-Ile-Phe-OMe	13	65	25

Table II. Antiprotease activity of compounds **14**, **15** and **16**.

Compound	Sequence	% of inhibition at the concentration x (M)	
		Test B	Test D
6	Boc-Lys-Phe-Phe-Lys-NH_2 $CO(CH2)_2CO$	62% at 10^{-5} M	
14	Qua-Lys-Phe-Phe-Lys-NH_2 $CO(CH_2)_2CO$		0% at 5×10^{-5} M
15	Boc-Lys-Phe-Phe-Lys-Ahi $CO(CH_2)_2CO$		34% at 5×10^{-5} M
16	Qua-Lys-Phe-Phe-Lys-Ahi $CO(CH_2)_2CO$		$IC_{50} = 4 \times 10^{-5}$ M

evidenced by Dixon plot (*figure 6*). The results are summarized in *tables I* and *III*. The reduced pseudopeptide **17** inhibited HIV-1 protease at pH 4.5 at 30 °C (K_i = 100 nM). This compound was previously described as a reduced-bond tight-binding inhibitor of the enzyme (K_i = 23 nM at pH 4.7 at 37 °C) [26]. Incorporation of the pseudopeptide bond Ψ($CONNH_2$) and a glycine residue yielded compounds **18** and **19** with no activity against the HIV protease pointing out the significance of the position of the hydrophobic side chain of phenylalanine. In fact, the best results were obtained with compounds **20** and **21** which respectively incorporated a statine-like residue (3S-4S Amino Hydroxy-Phenyl-Propionic-Acid: AHPPA) or a hydroxyethylamine bond. ^{1}H-NMR assignments indicated a mixture of the two diastereoisomers in the ratio 45:55 for compound **21**. In compound **21**, replacement of the Boc group by the Z group (compound **22**) did not modify the activity, whereas replacing the phenylalanyl residue in P'1 by a glycyl residue (compound **23**) decreased the inhibitory activity by at least two times.

We then investigated the capacity of pseudopeptides incorporating the motif -PheΨ(CONOH)Gly- (compound **24**). This pseudopeptide had a moderate activity. By analogy with compounds **22** and **23**, we synthesized the pseudopeptide Boc-PheΨ(CONOH)-Phe-Ile-Phe-OMe (*table III*: compound **25**); its activity was in the same range as compound **17** (K_i = 0.4 µM).

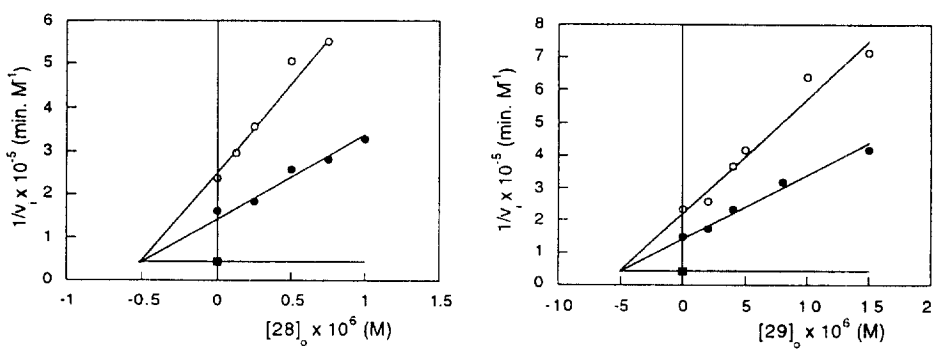

Figure 6. Dixon plots of the inhibition of HIV-1 PR by compound **17** and **29** with V-S-Q-N-F(NO_2)-P-I-V-NH_2 as substrate at pH 4.5 and 30 °C. The substrate concentrations were 175 (○), 250 (●) µM and infinite (■) and the enzyme concentration was 0.24 µM.

Table III. Antiprotease activity of compounds **25–29** at pH 4.5 and at 30 °C.

Compound	Sequence	Test D	K_i (μM)[a]
17	Boc-PheΨ(CH_2NH)Phe-Ile-Phe-OMe	IC_{50} = 1.2 x 10^{-6} M	N.D.
25	Boc-PheΨ(CONOH)Phe-Ile-Phe-OMe	IC_{50} = 3.1 x 10^{-6} M	0.4 ± 0.07
26	Boc-AHPPA-DIle-DPhe-OMe	IC_{50} = 2.3 x 10^{-6} M	0.8 ± 0.1
27	Boc-PheΨ($CHOHCH_2NH$) DPhe-D, Ile-D, Phe-OMe	16.5% at 5 x 10^{-5} M	N.I.
28	Boc-PheΨ($CHOHCH_2NH$)Phe-Glu(NHOH)-Phe-OMe	IC_{50} = 1.32 x 10^{-6} M	0.5 ± 0.01
29	Boc-PheΨ($CHOHCH_2NH$)Phe-Asp(NHOH)-PheOMe	IC_{50} = 8.7 x 10^{-6} M	5.1 ± 0.7

[a]N.I.: Inhibition at 10 μM; N.D.: not determined.

Substitution of C-terminal residues in compounds **20** and **21** by their D analogues (compounds **26** and **27**) had different effects. In the case of compound **26**, this substitution did not alter the activity. The same replacement in compound **21** resulted in a considerable decrease of the antiprotease activity (compound **27**). These differences could indicate that compounds **26** and **27** might interact in different ways with the protease. Substitution of the isoleucine 'γ-N-hydroxyglutamine' (*table III*: compound **28**) yielded compounds with significant inhibitory activity of the HIV protease whereas incorporating a 'β-N-hydroxy aspartic acid' produced a less active compound (**29**).

Compounds **1** to **7**, **10**, **12**, **13** and **17** to **24** were tested for their activity and their toxicity in HIV-1 infected and uninfected MT4 cells. None of the compounds were significantly active against HIV-1 infected MT4 cells indicating their difficulty to penetrate cells.

4. Experimentals protocols

4.1. Enzymic assays

The described peptides were tested with four tests. Three of them were performed at RHONE-POULENC-RORER laboratories (tests A, B and C).

Test A: This method uses a synthetic peptide, Succinyl-Thr-Leu-Asn-Phe-Pro-Ile-Ser-Amc (Amc = aminocoumarin), which spans the cleavage site between the protease and the reverse transcriptase. The hydrolysis by the HIV-1 protease released the peptide H-Pro-Ile-Ser-Amc. Enzymatic cleavage with aminopeptidase M released the Amc group which is fluorescent when free.

Test B: The heptapeptide, H-Ser-Gln-Asn-(pNO_2)Phe-Pro-Ile-Val-OH spanning the p17/p24 proteins cleavage was hydrolysed by HIV-1 protease. Protease activity was evaluated by the amount of the cleaved tetrapeptide produced and measured by spectrometric assay (307 nm).

Test C: In this third test, the hydrolysis of the peptide H-His-Lys-Ala-Arg-Val-Leu-(pNO_2)Phe-Glu-Ala-Nle-Ser-NH_2 was followed by the optical density decrease at 307 nm.

Test D: The octapeptide, H-Val-Ser-Gln-Asn-(pNO_2)Phe-Pro-Ile-Val-OH spanning the p17/p24 proteins cleavage site was hydrolysed by HIV-1 protease. Protease activity was evaluated by the amount of the cleaved tetrapeptide produced and measured by spectrometric assay (307 nm at 30 °C), according to Benatalah et al. [35]. The constant K_i were determined using Dixon plots. The compounds were previously dissolved in DMSO and the experimental conditions were $[E]_0$ = 0.24 μM, $[S]_0$ = 175–250 μM, $[\mathbf{25}]_0$ = 0.25–1 μM, $[\mathbf{26}]_0$ = 0.5–2.5 μM, $[\mathbf{27}]_0$ = 0.1–0.4 μM, $[a]_0$ = 0.625–5 μM, $[\mathbf{28}]_0$ = 0.125–1 μM, $[\mathbf{29}]_0$ = 2–5 μM, $[b]_0$ = 0.625–5 μM; in 0.1 acetate, 1 M NaCl, 5 mM EDTA, pH 4.5, 30 °C [36]. At infinite substrate concentration, the value of $1/V_i$ was calculated (equal to $1/k_{cat}$ x $[E]_0$). The kinetic parameter k_{cat} was previously determined in the same experimental conditions as for the inhibition studies.

4.2. In vitro inhibition of HIV-1 replication in MT4 lymphocyte cell line

MT4 cell were infected by a dose of HIV-1 producing 90% cell death after 5 days. Compounds to be tested were added in the cell culture at different concentration. Cell viability was assessed by means of the MTT [3-(4,5)-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide] assay [37]. The toxic effect of the compounds was also evaluated on non-infected MT4 cells by means of the MTT assay.

4.3. Chemistry

Amino acids were purchased from Bachem, Propeptide or Novabiochem. Urethane N-Carboxyanhydrides (UNCA) were purchased from Propeptide. Physical and analytical constants of intermediate compounds are summarized in *table IV–VII*. Melting points were determined on an open capillary apparatus and were uncorrected. Optical rotations were determinated at 20 °C using a Perkin Elmer polarimeter and were reported in ° $dm^{-1}g^{-1}$ mL. Mass spectra were recorded in the FAB positive mode using a JEOL JMS DX100 and DX300 apparatus. Products **1**, **4**, **6**, **17**, **18**, **20**, **21** and **25** were caracterised by one and two dimensional 1H-NMR spectroscopy (DMSO-d_6,

Table IV. Physical and analytical constants of intermediate compounds.

Compounds	P_f (°C)	$[\alpha]_D$ (c, solv.)	R_f[a]
(**1a**) Boc-Leu Ψ(CH_2CH_2) Phe-OH	98–100	–11 (2.0; MeOH)	0.66 (F)
(**1b**) Boc-Val-NH_2	156–157	–13 (0.99; DMF)	0.41 (D)
(**1c**) Boc-Val-Val-NH_2	169–170	–0.6 (1.02; DMF)	0.36 (D)
(**1d**) Boc-Leu Ψ(CH_2CH_2) Phe-Val-Val-NH_2	127–129	–1 (1.09; DMF)	0.47 (D)
(**1e**) Boc-Val-Leu Ψ(CH_2CH_2) Phe-Val-Val-NH_2	140–141	+4 (1.05; DMF)	0.50 (F)
(**3a**) Boc-βhPheOMe	112–113	–18 (0.9; DMF)	0.46 (A)
(**3b**) Boc-Val-βhPhe-OMe	dec. 120	–22 (0.98; DMF)	0.63 (D)
(**3c**) Boc-Val-Val-βhPhe-OMe	137–138	–4 (1.09; DMF)	0.61 (D)
(**3d**) Boc-Val-Val-βhPhe-OH	127–129	–8 (0.78; DMF)	0.27 (E)
(**3e**) Boc-Phe-Val-Val-NH_2	125–126	–5 (0.98; DMF)	0.33 (E)
(**5a**) Boc-Lys(Z)-NH_2	141–142	–14 (1.03; MeOH)	0.28 (D)
(**5b**) Boc-Phe-Lys(Z)-NH_2	190–192	–10 (0.87; DMF)	0.5 (E)
(**5c**) Boc-Phe-Phe-Lys(Z)-NH_2	218–220	–7 (1.08; DMF)	0.52 (E)
(**5d**) Boc-Lys(Fmoc)Phe-Phe-Lys(Z)-NH_2	> 230	–19 (0.99; DMF)	0.56 (F)
(**5e**) Boc-Lys($COCH_2COOH$)Phe-Phe-Lys(Z)-NH_2	> 230	–18 (1.06; DMF)	0.32 (H)
(**6a**) Boc-Lys($COCH_2CH_2COOH$)Phe-Phe-Lys(Z)-NH_2	> 230	–7 (0.88; DMF)	0.4 (F)
(**7a**) Boc-Lys($COCH_2CH_2CH_2COOH$)Phe-Phe-Lys(Z)-NH_2	> 230	+5 (1.25; DMF)	0.33 (G)
(**8a**) Boc-βhPhe-OH	100–105	–14 (0.95; DMF)	0.43 (C)
(**8b**) Boc-βhPhe-Phe-Lys(Z)-NH_2	210–212	–13 (1.08; DMF)	0.38 (E)
(**8c**) Boc-Lys(Fmoc)-βhPhe-Phe-Lys(Z)-NH_2	228–230	–32 (0.68; DMF)	0.33 (E)
(**8d**) Boc-Lys($COCH_2COOH$)-βhPhe-Phe-Lys(Z)-NH_2	> 230	–29 (1.05; DMF)	0.6 (H)
(**9a**) Boc-Lys($COCH_2CH_2COOH$)-βhPhe-Phe-Lys(Z)-NH_2	> 230	–19 (1; DMF)	0.29 (F)
(**10a**) Boc-Lys($COCH_2CH_2CH_2COOH$)-βhPhe-Phe-Lys(Z)-NH_2	> 230	–1.3 (1.03; DMF)	0.3 (G)

[a]Diastereoisomeric mixture; solvent of migration: ethyl acetate/hexane (A = 3:7; B = 5:5; C = 7:3; D = 1:0); chloroform/methanol/acetic acid (E = 180:10:5; F = 120:10:5; G = 85:10:5; H = 60:10:5; I = 40:10:5).

300 K) using DQF-COSY and Roesy experiments on an ARX-400 MHz apparatus. When unspecified all the coupling reactions were made using the method A. All the N-protected amino-amides were synthesized according to the method B. When unspecified, the terminal pseudopeptides were purified on HPLC using a mixture H_2O 0.1% TFA/CH_3CN 0.1% TFA.

4.3.1. General method for coupling amino acids: method A

TFA salts of amino acids (1 mmol) were dissolved in 10 mL of DMF, 3 mmol of NMM were added following by 1.1 mmol of N-protected amino acid and 1.1 mmol of BOP. The process of the reaction was followed by TLC. When the reaction was finished the DMF was concentrated under reduced pressure and a $NaHCO_3$ saturated solution was added. The precipitate was then collected, and washed with water and a 1 M $KHSO_4$ solution. In the absence of a precipitate, the aqueous phase was extracted with ethyl acetate and the organic layer was washed with water, a 1 M $KHSO_4$ solution, a saturated NaCl solution and dried over Na_2SO_4 and concentrated under reduced pressure.

Table V. Physical and analytical constants of intermediate compounds.

Compounds	P_f (°C)	$[\alpha]_D$ (*c*, solv.)	R_f[a]
(**11a**) Boc-Phe-CH_2Cl	86–87	–18 (0.9; DMF)	0.55 (A)
(**11b**) Boc-Phe Ψ(CHOHCH_2NH) Phe-Lys(Z)-NH_2[a]	dec. 180	–1.3 (1.3; DMF)	0.46 (F)
(**11c**) Boc-Lys(Fmoc)-PheΨ(CHOHCH_2NH)Phe-Lys(Z)-NH_2[a]	225–230	–5 (0.98; DMF)	0.34 (F)
(**11d**) BocLys($COCH_2CH_2COOH$)PheΨ(CHOHCH_2NH) Phe-Lys(Z)-NH_2[a]	> 230	–3 (1.06; DMF)	0.25 (G)
(**12a**) Boc-Lys($COCH_2CH_2CH_2COOH$)-PheΨ(CHOHCH_2NH) Phe-Lys(Z)-NH_2[a]	> 230	–2 (0.56; DMF)	0.38 (G)
(**13a**) Boc-NH-$CH_2CH_2NH_2$	oil	n.d.	0.25 (G)
(**13b**) Boc-NH-CH_2CH_2NH-Z		n.d.	0.71 (G)
(**13c**) NH_2-CH_2CH_2NH-Z	oil	n.d.	0.30 (G)
(**13d**) Boc-Asp(OBzl)-NH_2	91–92	–14 (0.98; DMF)	0.51 (C)
(**13e**) Boc-Asp(NH-CH_2CH_2NH-Z)-NH_2	112–113	–26 (1.2; DMF)	0.60 (H)
(**13f**) Boc-Phe-Asp(NH-CH_2CH_2NH-Z)-NH_2	119–122	–17 (1.06; DMF)	0.31 (E)
(**13g**) Boc-PheΨ(CHOHCH_2NH)Phe-Asp(NH-CH_2CH_2NH-Z)-NH_2[a]	136–138	+0.3 (1; DMF)	0.33 (F)
(**13h**) Boc-Asp(OBzl)-PheΨ(CHOHCH_2NH)Phe-Asp(NH-CH_2CH_2NH-Z)-NH_2[a]	190–192	–2 (1.15; DMF)	0.75 (F)
(**15a**) (–)-cis-1(*S*)-amino-2(*R*)-hydroxy indane (Ahi)	116–117	–62 (1 MeOH)	0.30 (H)
(**15b**) Boc-Lys(Z)-Ahi	128–129	–1.2 (1.2; DMF)	0.42 (B)
(**15c**) Boc-Phe-Lys(Z)-Ahi	142–143	–6 (0.67; DMF)	0.54 (D)
(**15d**) Boc-Phe-Phe-Lys(Z)-Ahi	165–167	–12 (0.78; DMF)	0.42 (D)
(**15e**) Boc-Lys(Fmoc)-Phe-Phe-Lys(Z)-Ahi	180–181	+4 (1.05; DMF)	0.60 (E)
(**15f**) Boc-Lys($COCH_2CH_2COOH$)-Phe-Phe-Lys(Z)-Ahi	220–221	–14 (1.2; DMF)	0.3 (F)

[a]Diastereoisomeric mixture; solvent of migration: ethyl acetate/hexane (A = 3:7; B = 5:5; C = 7:3; D = 1:0); chloroform/methanol/acetic acid (E = 180:10:5; F = 120:10:5; G = 85:10:5; H = 60:10:5; I = 40:10:5).

4.3.2. General method for C-terminal amide formation: method B

A solution of N-protected aminoacid (1 mmol) in DMF (1 mL) was coolled to –15 °C; NMM (1 mmol) and IBCF (1 mmol) were added. After 5 min stirring, 2 equiv. of NH_4OH were then added and the reaction mixture was stirred during 10 min. The product was precipitated by addition of water, collected by filtration, washed with water and hexane.

4.3.3. General method for peptide cyclisation: method C

The peptide (1 mmol) was dissolved in 100 mL of DMF which corresponded to a 10 mM solution. BOP (1 mmol) and $NaHCO_3$ (5 mmol) were added and the reaction mixture was stirred at room temperature. The cyclisation was followed by analytical HPLC. The cyclised product was obtained using a classical work-up.

4.3.4. General method for β homologation of amino acids: method D

To a solution of N-protected amino acids (60 mmol) in 60 mL of DME cooled to –15 °C, NMM (60 mmol) and IBCF (60 mmol) were added. The NMM salts were filtered and washed with small amount of DME. The filtrate was collected, cooled to 0 °C, and 70 mmol (in 250 mL of ether) of diazomethane previously prepared were added dropwise. After 2 h the reaction mixture was concentrated under reduced pressure and the residue was cristallized with cold hexane. The diazomethyl ketone (42 mmol) was dissolved in methanol (100 mL) at 0 °C. After adding triethylamine (5 mL), silver benzoate (0.5 g) was slowly added. Then 200 mL of a saturated $NaHCO_3$ solution followed by 200 mL of ethyl acetate were added. The organic layer was washed with water, dried with Na_2SO_4 and concentrated under reduced pressure.

Table VI. Physical and analytical constants of intermediate compounds.

Compounds	P_f (°C)	$[\alpha]_D$ (*c*, solv.)	R_f[a]
(**17a**) Boc-Ile-Phe-OMe	108–109	–29 (1.06; MeOH)	0.45 (A)
(**17h**) Boc-Phe-Ile-Phe-OMe	166–167	–73 (1.1; MeOH)	0.53 (D)
(**17c**) Boc-Phe-NMeOMe	oil	–15 (1.05; MeOH)	0.37 (B)
(**17d**) Boc-Phe-H	85–86	–44 (1.02; MeOH)	0.50 (B)
(**18a**) Z-NHNHCH$_2$COOEt	68–69		0.45 (C)
(**18b**) Boc-Phe Ψ(CONNHZ) Gly-OEt	98–99	–17 (1.07; MeOH)	0.67 (B)
(**18c**) Boc-Phe Ψ(CONNHZ) Gly-OH	102–103	–15 (0.97; MeOH)	0.31 (E)
(**18d**) Boc-Phe Ψ(CONNHZ) Gly-Ile-Phe-OMe	145–146	–16 (1.03; DMF)	0.64 (E)
(**19a**) Boc-Phe Ψ(CONNHZ) Gly-Phe-Ile-Phe-OMe	159–162	–26 (0.93; DMF)	0.60 (C)
(**22a**) Z-Phe-CH$_2$Cl	97–98	–22 (0.92; MeOH)	0.65 (A)
(**23a**) Boc-Gly-Ile-Phe-OMe	110–113	–20 (1.02; MeOH)	0.58 (E)
(**24a**) Br-CH$_2$-CONH-Ile-Phe-OMe	120–123	–18 (0.92; MeOH)	0.67 (C)
(**24h**) BzlONH-CH$_2$-CONH-Ile-Phe-OMe	164–165	–53 (1.1; MeOH)	0.25 (B)
(**24c**) Boc-Phe Ψ(CONOBzl)Gly-Ile-Phe-OMe	175–176	–45 (0.98; DMF)	0.38 (B)
(**25a**) (*R*)-Bromo-benzyl acetic acid	71–72	+17 (1.03; MeOH)	0.45 (E)
(**25h**) (*R*)-Bromo-benzyl acetic acid methyl ester	oil	+20 (0.96; MeOH)	0.54 (A)
(**25c**) BzlONH-L, Phe-OMe	oil	–19 (1.06; DMF)	0.62 (A)
(**25d**) BzlONH-Phe-OH	80–81	n.d.	0.25 (G)
(**25e**) Boc-Phe Ψ(CONOBzl)Phe-OH	110–111	n.d.	0.46 (E)
(**25f**) Boc-Phe Ψ(CONOBzl)Phe-Ile-Phe-OMe	150–151	–18 (1.2; DMF)	0.61 (B)

[a]Diastereoisomeric mixture; solvent of migration: ethyl acetate/hexane (A = 3:7; B = 5:5; C = 7:3; D = 1:0); chloroform/methanol/acetic acid (E = 180:10:5; F = 120:10:5; G = 85:10:5; H = 60:10:5; I = 40:10:5).

4.3.5. General method for deprotection of Boc: method E

The Boc protected peptide was treated with TFA during 30 min at room temperature then the TFA was concentrated under reduced pressure. The residue was precipitated by addition of ether, and the solid collected and washed several times with ether.

4.3.6. General method for the synthesis of the hydroxyethylamine bond: method F

The diazomethylketone of a N-protected amino acid (obtained with method D) was dissolved in ether at 0 °C and a solution of ether/HCl was added dropwise. Ether was concentrated and the residue was cristallized with hexane. The above chloromethyl ketone (1 equiv.) was dissolved in DMF and reacted with the TFA salt of peptide (1.1 equiv.) in the presence of 2 equiv. of $NaHCO_3$ and 1 equiv. of NaI. The resulting ketomethyl amine was reduced in situ by adding $NaBH_4$ (1.1 equiv.) dropwise at 0 °C, to yield the pseudopeptide. The reaction was followed by TLC. When the reaction was finished, the excess of hydride was quenched by addition of methanol 95% and the solvent was concentrated under reduced pressure. Then ethyl acetate was added and the organic layer was washed with a 1 M $KHSO_4$ solution, water, a saturated NaCl solution and dried over Na_2SO_4 and concentrated under reduced pressure.The product was crystallized from a diethyl ether/hexane mixture.

4.3.7. Synthesis of compounds 1 and 2

The pseudodipeptide Boc-LeuΨ(CH_2CH_2)Phe-OH was synthesized according to the published procedure [12] and then condensed to the dipeptide TFA•H-Val-Val-NH_2. After removal of the Boc protection with TFA followed by coupling with

Table VII. Physical and analytical constants of intermediate compounds.

Compounds	P_f (°C)	$[\alpha]_D$ (*c*, solv.)	R_f[a]
(**26a**) Boc-D, Ile-D, Phe-OMe	106–107	+24 (1.09; DMF)	0.45 (A)
(**27a**) Boc-D, Phe-D, Ile-D, Phe-OMe	155–156	+63 (0.99; MeOH)	0.53 (D)
(**28a**) Boc-Glu(OBzl)-Phe-OMe	86–88	–29 (1.06; MeOH)	0.48 (B)
(**28b**) Boc-Phe-Glu(OBzl)-Phe-OMe	94–95	–15 (1.1; DMF)	0.61 (B)
(**28c**) Boc-PheΨ(CHOHCH$_2$NH)Phe-Glu(OBzl)-Phe-OMe[a]	186–188	–7 (0.98; DMF)	0.50 (B)
(**28d**) Boc-Phe Ψ(CHOHCH$_2$NH)Phe-Glu(NHOBzl)-Phe-OMe[a]	200–202	–10 (0.95; DMF)	0.51 (D)
(**29a**) Boc-Asp(OBzl)-Phe-OMe	70–71	–27 (1.02; DMF)	0.60 (B)
(**29b**) Boc-Phe-Asp(OBzl)-Phe-OMe	80–82	–11 (1.13; DMF)	0.52 (B)
(**29c**) Boc-Phe Ψ(CHOHCH$_2$NH)Phe-Asp(OBzl)-Phe-OMe[a]	175–176	–6 (1.06; DMF)	0.35 (B)
(**29d**) Boc-Phe Ψ(CHOHCH$_2$NH)Phe-Asp(NHOBzl)-Phe-OMe[a]	198–200	–8 (1.02; DMF)	0.32 (E)

[a]Diastereoisomeric mixture; solvent of migration: ethyl acetate/hexane (A = 3:7; B = 5:5; C = 7:3; D = 1:0); chloroform/methanol/acetic acid (E = 180:10:5; F = 120:10:5; G = 85:10:5; H = 60:10:5; I = 40:10:5).

Boc-Val-OH, partial deprotection of the Boc group and coupling again with Boc-Val-OH, compound **1** was obtained. It was purified by column chromatography (SiO_2) using a mixture chloroform/methanol/acetic acid (120:10:5) as eluant. $[\alpha]_D$ = –4 (1.16; DMF), $(M + H)^+$ = 759. ^{1}H-NMR: 0.689–0.897 ppm (br: 30 H: H δ Leu_3 and Hγ $Val_{1,2,5\ and\ 6}$), 1.178 ppm (m: 2 H: CH_2 β Leu_3), 1.210 ppm (m: 2 H: Leu_3/CH_2), 1.355 ppm (s: CH_3 Boc), 1.399 ppm (m: Phe_4/CH_2), 1.453 ppm (m: CH γ Leu_3), 1.780 ppm (m: CH β Val_6), 1.837–2.067 ppm (br: 3 H, CH β Val; 1.892 ppm CH β Val_1, 1.917 ppm CH β Val_2, 1.963 ppm CH β Val_5), 2.351–2.703 ppm (m: 2.429 ppm CH_2 β' Phe_4, 2.452 ppm CH α Phe_4), 2.815 ppm (dd: CH_2 β, *J* = 5.88 Hz and 13.24 Hz, Phe_4), 3.607 ppm (m: 1 H, CH α Leu_3), 3.756 ppm (m: 1 H, CH α Val_1), 3.982–4.123 ppm (br: 2 H, 4.034 ppm CH α Val_6, 4.077 ppm CH α Val_2), 4.710 ppm (m: 1 H, CH α, *J* = 6.99 Hz and 14.71 Hz Val_5), 6.788 ppm (d: 1 H, NH α, *J* = 8.82 Hz, Val_1), 7.007 ppm (s: 1 H, NH_2 anti Val_6), 7.055–7.243 ppm (m: 5 H, CH aromatics Phe_3), 7.286 ppm (s: 1 H, NH_2 syn Val_6), 7.507–7.637 ppm (m: 3 H; 7.551 ppm NH α Val_2, 7.583 ppm NH α Val_6, 7.585 ppm NH α Leu_3), 7.942 ppm (d: 1 H, *J* = 7.72 Hz, NH α Val_5).

The purified compound **1** was treated with TFA to yield, after precipitation with ether, compound **2**. $[\alpha]_D$ = –7 (1.12; DMF), $(M + H)^+$ = 65.

4.3.8. Synthesis of compounds 3 and 4

Starting from Boc-Phe-OH, Boc-βhomo-Phe-OMe was obtained using method D (yield: 65%). Coupling with two valine residues gave the tripeptide Boc-Val-Val-βh-Phe-OMe. After saponification with 2 N NaOH in methanol, the tripeptide acid was precipitated by acidification of the reaction mixture to pH 1–2 with $KHSO_4$ 1 M. The precipitate was collected, washed with water then hexane. The crude product was condensed, using the method A, to the previously prepared C-terminal tripeptide TFA.H-Phe-Val-Val-NH_2. The resulting product precipitated during the reaction. After 2 h at room temperature, compound **3** was collected (yield 80%). The crude product was treated with TFA and after precipitation with ether yielded **4** (96%). $[\alpha]_D$ = –11 (0.78; DMF), $(M + H)^+$ = 721 (without TFA salt). ^{1}H-NMR: 0.754–0.911 ppm (m: 24 H: Hγ $Val_{1,2,5\ and\ 6}$), 1.785–1.858 ppm (br: 1 H: CH β Val_2), 1.877–2.078 ppm (br: 3 H: CH β $Val_{1,5\ and\ 6}$), 2.101–2.265 ppm (m: 3 H; 2.172 ppm CH_2 ββ' Phe_3, CH_2 γ Phe_3), 2.412 ppm (m: 1 H: CH_2 γ' Phe_3), 2.733 ppm (m: 1 H, CH_2 β Phe_4), 3.023 ppm (m: 1 H, CH_2 β' Phe_4), 3.585–3.678 ppm (br: 1 H, CH α Val_1), 4.029 ppm (tr: 1 H, CH α *J* = 8.19 Hz and 14.89 Hz Val_2), 4.123 ppm (tr: 1 H, CH α *J* = 8.19 Hz and 14.51 Hz Val_6), 4.157–4.262 ppm (br: 2 H, CH β Phe_3, CH α Val_5), 4.662–4.680 ppm (br: 1 H, CH α Phe_4), 6.935–7.294 ppm (m: 5 H, CH aromatics), 7.320–7.332 ppm (br: 2 H, NH_2 Val_6), 7.664 ppm (d: 1 H, NH α, *J* = 8.19 Hz, Val_6), 7.946 ppm (d: 1 H, NH β, *J* = 8.19 Hz, β$hPhe_3$), 8.032–8.142 ppm (m: 2 H; 8.066 ppm NH α Val_5, 8.112 ppm NH α Phe_4), 8.280 ppm (d: 1 H, NH α, *J* = 8.15 Hz, Val_2).

4.3.9. Synthesis of compounds 5–7 and 14–16

As an example, the synthesis of compound **6** is described. Starting from Boc-Lys(Z)-OH and with the use methods A, B and E we obtained the tetrapeptide Boc-Lys(Fmoc)-Phe-Phe-Lys(Z)-NH_2 **5d** (overall yield: 87%). Deprotection of the Fmoc group with 10 equiv. of diethylamine in DMF followed by addition of succinic anhydride (1 equiv.) and diisopropylethylamine (1 equiv.) in DMF. The DMF was concentrated under reduced pressure and 50 mL of a $KHSO_4$ solution 1 M was added followed by 50 mL of ethyl acetate. The organic layer was washed with 1 M $KHSO_4$, water, brine, dried over Na_2SO_4 and concentrated under reduced pressure to yield Boc-Lys($COCH_2CH_2COOH$)-Phe-Phe-Lys(Z)-NH_2 **6a** (yield 73% from **5d**). The Z group was removed by hydrogenation on Pd/C and the resulting product was cyclized according to the method C to yield **6**. $[\alpha]_D$ = –3 (0.88; DMF), $(M + H)^+$ = 750. ^{1}H-NMR: 1.131 ppm (br: CH_2 γ Lys_1), 1.204 ppm (br: CH_2 γ

Lys_4), 1.286 ppm (br: CH_2 δ Lys_1), 1.335 ppm (br: CH_2 δ Lys_4; s: CH_3 Boc), 1.420 ppm (br: CH_2 ββ' Lys_1), 1.495 ppm (br: 1 H, CH_2 β Lys_4), 1.640 ppm (br: 1 H, CH_2 β' Lys_4), 2.302 ppm (s: 4 H, CH_2 succinic bridge), 2.795 ppm (m: 2 H, CH_2 ββ' Phe_2), 2.833 ppm (m: 1 H, CH_2 β pro R, J = 14.8 Hz and 7.67 Hz, Phe_3), 2.980 ppm (br: 2 H, CH_2 ε Lys_1), 2.980 ppm (br: 1 H, CH_2 ε Lys_4), 3.095 ppm (br: 1 H, CH_2 ε' Lys_4), 3.313 ppm (m: 1 H, CH_2 β pro S, J = 14.8 Hz and 5.73 Hz, Phe_3), 3.822 ppm (m: 1 H, CH α Lys_1), 4.041 ppm (m: 1 H, CH α Lys_4), 4.357 ppm (br: 1 H, CH α Phe_2), 4.520 ppm (br: 1 H, CH α Phe_3), 6.696 ppm (d: 1 H, NH α, J = 7.35 Hz, Lys_1), 6.973 ppm (d: 2 H, J = 7.35 Hz, NH_2 Lys_4), 7.149–7.25 ppm (m: CH aromatics Phe_2 and Phe_3), 7.476 ppm (t: 1 H, NH ε, J = 7.35 Hz Lys_1), 7.735 ppm (m: 1 H, NH ε Lys_4), 7.746 ppm (m: 1 H, NH α Lys_4), 7.992 ppm (d: 1 H, J = 7.57 Hz, NH α Phe_3), 8.055 ppm (d: 1 H, J = 7.35 Hz, NH α Phe_2).

Compound ***5***: $[\alpha]_D$ = –11 (1.06; DMF), $(M + H)^+$ = 736.

Compound ***7***: $[\alpha]_D$ = +0.3 (1.25; DMF), $(M + H)^+$ = 764.

Compound ***14*** was obtained after removal of the Boc protecting group from **6** and coupling with 2-quinoline carboxylic acid by the method A, after treatment with TFA (yield 82%). $[\alpha]_D$ = –21 (0.68; DMF), $(M + H)^+$ = 805.

Compound ***15***: starting from the amino hydroxy indane moiety, and following the previously described strategy for the synthesis of peptide **6**, compound **15** was obtained in a 44% overall yield. $[\alpha]_D$ = –11 (1.3; DMF), $(M + H)^+$ = 882.

Compound ***16*** was obtained after removal of the Boc protecting group from **15** and coupling with 2-quinoline carboxylic acid by the method A (yield 82%). $[\alpha]_D$ = –25.6 (1; DMF), $(M + H)^+$ = 937.

*4.3.10. Synthesis of compounds **8** to **10***

The tetrapeptide Boc-Lys(Fmoc)-βhPhe-Phe-Lys(Z)-NH_2 **8c** was obtained from Boc-Phe-Lys(Z)-NH_2 **5b** and by using methods A, B and E, (and method D to synthesize Boc-βhPhe-OH) (overall yield: 89%).

Compound ***9*** was obtained using the same procedure as for compound **6**: $[\alpha]_D$ = –9 (*c* 1; DMF), $(M + H)^+$ = 764.

Compound ***8***: $[\alpha]_D$ = –19 (1.05; DMF), $(M + H)^+$ = 750.

Compound ***10***: $[\alpha]_D$ = –0.3 (1.03; DMF), $(M + H)^+$ = 778.

*4.3.11. Synthesis of compounds **11** and **12***

Boc-Lys(Fmoc)-PheΨ[CH(R,S)$OHCH_2NH$]Phe-Lys(Z)-NH_2 **11c**, was obtained from **5b** as a diastereoisomeric mixture using methods A, B, E, and F (overall yield: 70%). Cyclic pseudopeptides were synthesized as described for compound **6** using succinic or propionic anhydride to yield **11** and **12** respectively.

Compound ***11***: $[\alpha]_D$ = –1.2 (1.06; DMF), $(M + H)^+$ = 766.

Compound ***12***: $[\alpha]_D$ = –1 (0.6; DMF), $(M + H)^+$ = 780.

*4.3.12. Synthesis of compound **13***

Boc-Asp($NHCH_2CH_2NHZ$)-NH_2 **13e** was obtained by amidification of Boc-Asp(OBzl)-OH followed by removal of the benzyl group and coupling (method A) with $H_2NCH_2CH_2NHZ$. By using methods A, B E and F the tetrapeptide Boc-Asp(OBzl)-PheΨ[CH(*R,S*)$OHCH_2NH$]Phe-Asp($NHCH_2CH_2Z$)-NH_2 **13h**, (overall yield: 47%) was obtained. Catalytic hydrogenation with Pd/C, followed by cyclisation yielded compound **13**. $[\alpha]_D$ = –1.3 (1.1; DMF), $(M + H)^+$ = 682.

*4.3.13. Synthesis of compound **17***

Boc-Pheψ(CH_2NH)Phe-Ile-Phe-OMe **17** was obtained from Boc-Phe-H [18] using a classical methodology [19] (76% overall yield from H-Phe-OMe). $[\alpha]_D$ = –10 (1.1; DMF), $(M + H)^+$ = 673. ^{1}H-NMR: 0.738–0.854 ppm (m: 6 H, 0.807 ppm CH_3 γ Ile_3, 0.819 ppm CH_3 ε Ile_3), 0.894–1.046 ppm (br: 1 H, CH_2 δ Ile_3), 1.231–1.413 ppm (12 H, br: CH_2 δ' Ile_3 and CH_2; 1.285 ppm, s: CH_3 Boc), 1.602–1.760 ppm (br: 1 H, CH β Ile_3), 2.649–2.760 ppm (br: 2 H, CH_2 ββ' Phe_4), 2.858–3.056 ppm (m: 4 H, CH_2 ββ' Phe_2 and Phe_1), 3.544 ppm (s: 3 H, CH_3 methyl ester), 3.822–3.978 ppm (br: 1 H, CH α Phe_4), 3.994–4.173 ppm (br: 1 H, CH α Phe_2), 4.232 ppm (t: 1 H, CH α Ile_3, J = 8.46 Hz and 16.55 Hz), 4.313–4.445 ppm (br: 1 H, CH α Phe_1), 6.939–7.333 ppm (m: 17 H, CH aromatics, NH Phe_2 and Phe_4), 8.365–8.602 ppm (m: 2 H; NH Phe_1 and Ile_3).

*4.3.14. Synthesis of compounds **18** and **19***

*Boc-PheΨ(CONNH$_2$)Gly-OH (**18c**)*: Hydrazino ethyl acetate hydrochloride (1 equiv.) was dissolved in THF containing NMM (2 equiv.).The reaction mixture was cooled to 0 °C, Z-OSu (1.2 equiv.) was added and the reaction mixture was stirred for 4 h at 0 °C and 1 h at room temperature. The solvent was removed in vacuo. Water and dichloromethane were added and the mixture stirred for 30 min. The organic layer was collected, washed with brine, dried over Na_2SO_4 and concentrated in vacuo (67% yield). Z-NH-NH-CH_2-COOEt was recrystallized in a ethyl acetate/hexane (1:1) mixture (yield 85%). Boc-Phe-OH (1 equiv. in methylene chloride) was condensed to the above product (1 equiv.) in the presence of isobutyl chloroformate and N-methylmorpholine. The reaction was monitored by TLC. $KHSO_4$ 1 M was added and the product was extracted with methylene chloride.The organic layer was washed with water and brine before drying with Na_2SO_4 and concentrated in vacuo. The oily product Boc-Phe-N(NH-Z)-CH_2-COOEt crystallized upon trituration in a ether/hexane mixture (yield 58%). It was hydrolyzed with 1 N NaOH (yield 80%). This compound was used without any further purification and condensed using the method A to the C-terminal dipeptide H-Ile-Phe-OMe or to the tripeptide H-Phe-Ile-Phe-OMe to provide after hydogenolysis in the presence of Pd/C compounds **18** and **19** respectively.

Compound ***18***: $[\alpha]_D$ = –10 (1.03; DMF), $(M + H)^+$ = 612. ^{1}H-NMR: 0.771–0.855 ppm (m: 6 H, 0.802 ppm CH_3 γ Ile_3, 0.821 ppm CH_3 ε Ile_3), 0.928–1.115 ppm (br: 1 H, CH_2 δ Ile_3), 1.283 ppm (s: 9 H, CH_3 Boc), 1.326–1.454 ppm (br: 1 H, CH_2 δ' Ile_3), 1.605–1.751 ppm (br: 1 H, CH β Ile_3), 2.655 ppm (dd: 1 H, CH_2 β Phe_1, J = 9.93 Hz and 13.60 Hz), 2.929 ppm (dd: 1 H, CH_2 β Phe_4, J = 8.82 Hz and 13.97 Hz), 2.973–3.072 ppm (m: 2 H; CH_2 β' Phe_1 and CH_2 β' Phe_4), 3.548 ppm (s: 3 H, CH_3 methyl ester), 3.969 ppm (s: 1 H, CH_2 α Gly_2, J = 16.91 Hz), 4.225–4.290 ppm (m: 1 H, CH α Ile_3), 4.343 ppm (s: 1 H, CH_2 α' Gly_2, J = 16.91 Hz), 4.418–4.502 ppm (m: 1 H, CH α Phe_4), 5.098–5.120 ppm (m: 1 H, CH α Phe_1), 6.525 ppm (d: 1 H, NH Phe_1, J = 8.82 Hz), 7.125–7.301 ppm (m: 12 H; CH aromatics and NH_2), 7.974 ppm (d: 1 H, NH Ile_3, J = 8.82 Hz), 8.490 ppm (d: 1 H, NH Phe_4, J = 7.35 Hz).

Compound ***19***: $[\alpha]_D$ = –16 (0.93; DMF), $(M + H)^+$ = 759.

*4.3.15. Synthesis of compounds **20** and **26***

The Boc-AHPPA-OH was condensed with H-Ile-Phe-OMe or H-DIle-DPhe-OMe using method A to yield compounds **20** and **26** respectively.

Compound ***20***: $[\alpha]_D$ = –15 (1.01; DMF), $(M + H)^+$ = 584. ^{1}H-NMR: 0.735–0.821 ppm (m: 6 H; 0.772 ppm CH_3 γ Ile_2, 0.781 ppm CH_3 ε Ile_2), 1.017 ppm (m: 1 H, CH_2 δ Ile_2), 1.250–1.433 ppm (m: 10 H; 1.302 ppm CH_3 Boc, 1.357 ppm CH_2 δ' Ile_2), 2.147 ppm (dd: 1 H, CH_2 α AHPPA, J = 3.31 Hz and 13.97 Hz), 2.26 ppm (dd: 1 H, CH_2 α' AHPPA, J = 9.56 Hz and 14.34 Hz), 2.580 ppm (dd: 1 H, CH_2 δ AHPPA, J = 9.19 Hz and 13.60 Hz), 2.810 ppm (dd: 1 H, CH_2 δ' AHPPA, J = 5.15 Hz and 13.60 Hz), 2.912 ppm (dd: 1 H, CH_2 β Phe_3, J = 8.82 Hz and 1397 Hz), 3.008 ppm (dd: 1 H, CH_2 β' Phe_3, J =

5.88 Hz and 1397 Hz), 3.543 ppm (s: 3 H, CH_3 methyl ester), 3.607–3.698 (br: 1 H, CH γ AHPPA), 3.783–3.885 (br: 1 H, CH β AHPPA), 4.192 ppm (t: 1 H, CH_2 α Ile_2, J = 7.72 Hz and 16.18 Hz), 4.450 (m: 1H, CH α Phe_3), 4.820 ppm (d: 1H, OH AHPPA, J = 5.88 Hz), 6.476 ppm (d: 1H, NH AHPPA, J = 9.19 Hz), 7.121–7.288 (m: 10 H, aromatic CH), 7.687 ppm (d: 1H, NH Ile_2, J = 8.82 Hz), 8.332 ppm (d: 1H, NH Phe_3, J = 7.35 Hz).

Compound ***26***: $[\alpha]_D$ = +2 (0.99; DMF), $(M + H)^+$ = 584.

4.3.16. Synthesis of compounds ***21–23*** *and* ***27–29***

These pseudopeptides were synthesized according to methods D and F. Boc-Phe-CH_2-Cl or Z-Phe-CH_2-Cl were condensed on different C-terminal tripeptides: H-Phe-Ile-Phe-OMe (compounds **21** and **22**); H-Gly-Ile-Phe-OMe (compound **23**); H-DPhe-DIle-DPhe-OMe (compound **27**); H-Phe-Glu(OBzl)-Phe-OMe (compound **28c**); H-Phe-Asp(OBzl)-Phe-OMe (compound **29c**). Removing the benzyl ester protection on compounds **28c** and **29c**, followed by coupling with NH_2OBzl with method A yielded after hydogenation with Pd/C compounds **28** and **29**.

Compound ***21***: $[\alpha]_D$ = −12 (0.88; DMF), $(M + H)^+$ = 703. ^{1}H-NMR: 0.701–0.933 ppm (m: 6 H, 0.809 ppm CH_3 γ Ile_3, 0.832 ppm CH_3 ε Ile_3), 0.955–1.134 ppm (br: 1 H, CH_2 δ Ile_3), 1.139–1.425 ppm (10 H; br: CH_2 δ' Ile_3 and d: CH_3 Boc), 1.547–1.765 ppm (br: 1 H, CH β Ile_3), 2.460–2.852 ppm (br: CH_2 ββ' Phe_1 and CH_2 from the hydroyethyl amine bond), 2.873–3.089 ppm (m: 4 H, CH_2 ββ' Phe_2 and Phe_4), 3.520–3.680 ppm (5 H; br: CH α Phe_1 and CH from the hydroyethyl amine bond, 3.541 ppm: d CH_3 methyl ester), 4.043 ppm (br: 1 H, CH α Phe_2), 4.224–4.560 ppm (m: 2 H, CH α Ile_3 4.277 ppm and CH α Phe_4 4.385 ppm), 6.647–6.892 ppm (m: 1 H, NH Phe_1), 6.923–7.636 ppm (m: 15 H; CH aromatics), 8.334–8.676 ppm (m: 2 H, NH Ile_3 8.455 ppm and NH Phe_4 8.549 ppm), 8.700–8.952 ppm (br: 1 H, NH Phe_2), 9.067–9.428 (br: 1H, OH).

Compound ***22***: $[\alpha]_D$ = −19 (0.98; DMF), $(M + H)^+$ = 737.
Compound ***23***: $[\alpha]_D$ = −11 (1.01; DMF), $(M + H)^+$ = 647.
Compound ***27***: $[\alpha]_D$ = +8 (1.08; DMF), $(M + H)^+$ = 703.
Compound ***28***: $[\alpha]_D$ = −6 (0.95; DMF), $(M + H)^+$ = 734.
Compound ***29***: $[\alpha]_D$ = −9 (1.02; DMF), $(M + H)^+$ = 720.

4.3.17. Synthesis of compound ***24***

To a solution of bromoacetic acid (1 equiv.) in DME cooled to 0 °C, 1 equiv. of isobutyl chloroformate was added and the mixture stirred for 5 min. H-Ile-Phe-OMe (0.75 equiv.) was added and the reaction was stirrred for 30 min at 0 °C and 1 h at room temperature. The product Br-CH_2-CO-Ile-Phe-OMe was precipitated by addition of water, collected by filtration and washed with hexane (yield 79%). The above product (0.9 equiv.) was reacted with HCl.H_2N-OBzl (1 equiv.) in DMF containing $NaHCO_3$ (2 equiv.). The reaction was followed by TLC, and compound **24b** was precipitated by addition of water. It was collected by filtration, washed with 1 M $KHSO_4$, water and hexane (yield 68%). Boc-Phe-OH was finally coupled to **24b** via the isobutylchloroformate mixted anhydride method to produce, after removal of the benzyl group by hydrogenation in the presence of Pd/C of the desired pseudopeptide **24** (yield 80%). $[\alpha]_D$ = −16 (1.03; DMF), $(M + H)^+$ = 613.

4.3.18. Synthesis of compound ***25***

Bromo-(R)-benzyl acetic acid ***25a***: H-DPhe-OH (5 g; 30 mmol) was added at 0 °C to a solution of 2.5 N H_2SO_4 (63 mL; 157 mmol) and KBr (12.5 g; 105 mmol). 12.5 g (48 mmol) of $NaNO_2$ was added portionwise. The reaction mixture was stirred in the dark for 2 h at 0 °C and 2 h at room temperature. The aqueous phase was washed with ethyl acetate (3 x 100 mL), and the organic layers collected and concentrated in vacuo. The residue was purified by column chromatography (SiO_2) using a mixture of chloroform/methanol/acetic acid (180:10:5) as eluent (yield 65%; 4.4 g).

Bromo-(R)-benzyl methyl acetate ***25b***: 3.13 g (13.65 mmol) of **25a** were dissolved in methanol (100 mL), then 5.2 g (27.3 mmol) of PTS•H_2O were added and the mixture was refluxed for 2 h. The solvent was removed and 100 mL of ethyl acetate was added. The organic layer was washed several times with a $NaHCO_3$ saturated solution, water, brine, dried on Na_2SO_4 and concentrated in vacuo (yield 97%; 3.22 g).

NHOBzl-Phe-OMe ***25c***: It was obtained by the same procedure as **24b** from 3.2 g of **25b**. It was purified by column chromatography (SiO_2) using a mixture of ethyl acetate/hexane (3:7) as eluent (yield 70%, 2.6 g).

NHOBzl-Phe-OH ***25d***: Was obtained by saponificatin with 2 N NaOH of componud **25c** (1 g) (yield 85%; 850 mg).

*Boc-Phe*Ψ*(CONOBzl)Phe-OH* ***25e***: 430 mg of Boc-Phe-NCA (1.47 mmol) were reacted in DMF with 400 mg (1.47 mmol) of compound **25d**. After 24 h at room temperature, a solution of saturated $NaHCO_3$ followed by 50 mL of ethyl acetate were added. The aqueous phase was acidified to pH 1–2 with 1 M $KHSO_4$, and extracted with ethyl acetate (2 x 50 mL), the organic layer collected, dried (Na_2SO_4) and concentrated in vacuo (yield 98%; 760 mg).

*Boc-Phe*Ψ*(CONOBzl)Phe-Ile-PheOMe* ***25f***: using method A, **25e** (500 mg; 0.96 mmol) was condensed with H-Ile-Phe-OMe (yield 83%, 110 mg).

*Boc-Phe*Ψ*(CONOH)Phe-Ile-PheOMe* ***25***: Catalytic hydrogenation of **25f** in the presence of Pd/C in DMF, yielded **25** in quantitative yield. $[\alpha]_D$ = −10 (1.09; DMF), $(M + H)^+$ = 703. ^{1}H-NMR: 0.698–0.899 ppm (m: 6 H, 0.812 ppm CH_3 γ Ile_3, 0.835 ppm CH_3 ε Ile_3), 0.932–1.100 ppm (br: 1 H, CH_2 δ Ile_3), 1.264 ppm (s: 9 H, CH_3 Boc), 1.329–1.444 ppm (br: 1 H, CH_2 δ' Ile_3), 1.565–1.734 ppm (br: 1 H, CH β Ile_3), 2.568 ppm (dd: 1 H, CH_2 β Phe_1, J = 10.84 Hz and 13.69 Hz), 2.824–2.944 ppm (m: 1 H, CH_2 β' Phe_1), 2.950–3.162 ppm (m: 4 H; CH_2 ββ' Phe_2 and Phe_4), 3.535 ppm (s: 3 H, CH_3 methyl ester), 4.245 ppm (t: 1 H, CH α Ile_3, J = 7.42 Hz and 15.97 Hz), 4.398–4.502 ppm (m: 1 H, CH α Phe_4), 4.636–4.734 ppm (m: 1 H, CH α Phe_1), 5.077 ppm (dd: 1 H, CH α Phe_2, J = 5.42 Hz and 9.13 Hz), 6.748 ppm (d: 1 H, NH Phe_1, J = 9.13 Hz), 7.102–7.337 ppm (m: 15 H; CH aromatics), 7.561 ppm (d: 1 H, NH Ile_3, J = 9.13 Hz), 8.498 ppm (d: 1 H, NH Phe_4, J = 7.42 Hz), 10.057 ppm (s: 1H, OH).

References

[1] Barré-Sinoussi F., Chermann J.C., Rey F., Nugeyre M.T., Chamaret S., Gruest J., Dauguet C., Axler-Blin C., Vézinet-Brun F., Rouzioux C., Rozembaum W., Montagnier L., Science 220 (1983) 868–871.

[2] Kohl N.E., Emini E.A., Schleif W.A., Davis L.J., Heimbach J.C., Dixon A.F., Scolnick E.M., Sigal I.S., Proc. Natl. Acad. Sci. USA 85 (1988) 4686–4690.

[3] Katoh I., Yoshinaka Y., Rein A., Shibuya M., Odaka T., Oroszlan S., Virology 145 (1985) 280–292.

[4] Pearl L.H., Taylor W.R., Nature 329 (1987) 351–354.

[5] 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 Y.N., Chang C.H., Weber P.C., Jackson D.A., Sharpe T.R., Erickson-Viitanen S., Science 263 (1994) 380–384.

[6] Roberts N.A., Martin J.A., Kinchington D., Braoadhurst A.V., Craig J.C., Duncan I.B., Galpin S.A., Handa B.K., Kay J., Krohn 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., Science 248 (1990) 358–361.

[7] 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., Science 249 (1990) 527–533.
[8] Cohen J., Science 272 (1996) 1882–1883.
[9] Wlodawer A., Erickson J.W., Ann. Rev. Biochem. 62 (1993) 543–580.
[10] Billich A., Winkler G., Arch. Biochem. Biophys. 290 (1991) 186–190.
[11] Grobelny D., Wondrak E.M., Galardy R.E., Oroszlan S., Biochem. Biophys. Chem. Commun. 169 (1990) 1111–1116.
[12] 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., Science 249 (1990) 527–533.
[13] Ghosh A.K., McKee S.P., Thompson W.J., Tetrahedron Lett. 32 (1991) 5729–5732.
[14] Peyman A., Budt K.H., Spanig J., Stowasser B., Ruppert D., Tetrahedron Lett. 33 (1992) 4549–4552.
[15] Stowasser B., Budt K.H., Jian-Qi L., Peyman A., Ruppert. D., Tetrahedron Lett. 33 (1992) 6625–6628.
[16] Sham H.L., Betebenner D.A., Wideburg N.E., Saldivar A.C., Kohlbrenner W.E., Craig-Kennard A., Vasavanonda S., Kempf D.J., Clement J.J., Erickson J.W., Plattner J.J., Norbeck D.W., FEBS Lett. 329 (1993) 144–146.
[17] Darke P.L., Nutt R.F., Brady S.F., Garsky V.M., Ciccarone T.N., Leu C.T., Lumma K.P., Freidinger R.M., Veber D.F., Sigal I.S., Biochem. Biophys. Res. Commun. 158 (1988) 297–303.
[18] Tözsér J., Blada I., Copeland T.D., Wondrak E.M., Oroszlan S., FEBS Lett. 281 (1991) 77–80.
[19] Darke P.L., Nutt R.F., Brady S.F., Garsky V.M., Ciccarone T.M., Leu C.T., Lumma P.K., Freidinger R.M., Veber D.F., Sigal I.S., Biochem. Biophys. Res. Commun. 156 (1988) 297–303.
[20] Rodriguez M., Heitz A., Martinez J., Tetrahedron Lett. 31 (1990) 7319–7322.
[21] Rodriguez M., Fulcrand P., Laur J., Aumelas A., Bali J.P., Martinez J., J. Med. Chem. 32 (1989) 522–528.
[22] DiMaio J., Nguyen T.M.D., Lemieux C., Schiller P.W., J. Med. Chem. 25 (1982) 1432–1438.
[23] Rodriguez M., Amblard M., Galas M.C., Lignon M.F., Aumelas A., Martinez J., Int. J. Peptides Protein Res. 35 (1990) 441–451.
[24] Baumann G., Zenke G., Wenger R., Hiestand P., Quesniaux V., Andersen S., Schreier M.H., J. Autoimm. 5 (Suppl. A) (1992) 67–72.
[25] Tucker T.J., Lumma Jr. W.C., Payne L.S., Wai J.M., DeSolms 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., J. Med. Chem. 35 (1992) 2525–2533.
[26] Lyle T.A., Wiscount C.M., Guare J.P., Thompson W.J., Anderson P.S., Darke P.L., Zugay J., Emini E.A., Schleif W.A., Quintero J.C., Dixon R.A.F., Sigal I.S., Huff J.R., J. Med. Chem. 34 (1991) 1228–1230.
[27] Thompson W.J., Fitzgerald P.M.D., Holloway M.K., Emini E.A., Darke P.L., McKeever B.M., Schleif W.A., Huff J.R., J. Med. Chem. 35 (1992) 1685–1701.
[28] Urban J., Konvalinka J., Stehlíková J., Gregorová E., Majer P., Soucek M., Andreánsky M., Fábry M., Stop P., FEBS Lett. 298 (1992) 9–13.
[29] Majer P., Urban J., Gregorová E., Konvalinka J., Novek P., Stehlíková J., Andreánsky M., Sedlácek J., Stop P., Arch. Biochem. Biophys. 304 (1993) 1–8.
[30] Martin S.A., Redshaw S., Thomas G.J., Progress Med. Chem. 32 (1995) 239–261.
[31] Fehrentz J.A., Castro B., Synthesis (1983) 676–678.
[32] Borch R., Bernstein M., Durst H., J. Am. Chem. Soc. 93 (1971) 2897–2904.
[33] Lecoq A., Marraud M., Aubry A., Tetrahedron Lett. 32 (1991) 2765–2768.
[34] Rich D.H., Green J., Toth M.V., Marshall G.R., Kent S.B.H., J. Med. Chem. 33 (1990) 1288–1295.
[35] Benatalah Z., Trigui N., Sicsic S., Tonnaire T., de Rosny E., Boggehto N., Reboud M., Eur. J. Med. Chem. 30 (1995) 891–900.
[36] Quasmi D., de Rosny E., René L., Badet B., Bogetto N., Reboud-Ravaux M., Biorg. Med. Chem. Lett., in press.
[37] Mosmann T., J. Immunol. Meth. 65 (1983) 55–63.