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PAPER

Applications of 3-aminolactams: design, synthesis, and biological evaluation of a library of potential dimerisation inhibitors of HIV1-protease†

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In the context of our studies on the applications of 3-aminolactams as conformationally restricted pseudodipeptides, we report here the synthesis of a library of potential dimerisation inhibitors of HIV1-protease. Two of the pseudopeptides were active on the wild type virus (HIV1) at micromolar levels (EC_{50}). Although the peptides showed lower anti-viral activity than previously reported dimerisation inhibitors, our results demonstrate that the piperidone moiety does not prevent cell penetration, and hence that such derivatization is compatible with potential anti-HIV treatment.

Introduction

Drugs currently used as inhibitors of HIV1 protease (HIV1-PR), such as ritonavir and indinavir, interact directly with the active site of the enzyme.[1] However, since the demonstration that the active form of HIV1-PR is dimeric,[2,3] dimer assembly has been seen as an alternative inhibitor target.[3,4] Molecules of the 99-residue HIV1-PR monomer dimerize by interdigitation of the five amino acids at the N- and C-termini (Fig. 1).

Meek *et al.* first proposed that small molecules able to complement these sequences could cap the monomers and would thus be good drug candidates (Fig. 2).[3] The validity of this approach was supported by Zhang *et al.*, who reported peptide-induced dissociation of HIV1-PR dimers (Fig. 2),[5] and confirmed by the observation that terminal peptides inhibit the protease by binding to its dimerisation interface.[6]

With the aim of strengthening interaction with the protease monomer, Schramm, Reboud-Ravaux *et al.* investigated dimerisation inhibition of HIV1-PR by lipopeptides,[7] and by "tweezer" compounds in which the peptide chains are separated by the charged heterocycles guanidine[8] and quinoline.[9] In parallel, Chmielewski *et al.* made a systematic study of the structure–activity relationships of compounds formed by peptide chains

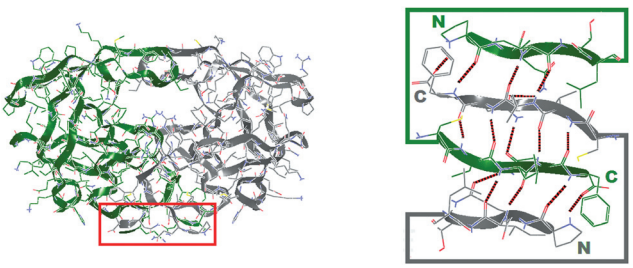

Fig. 1 HIV1 protease and its dimerisation interface.

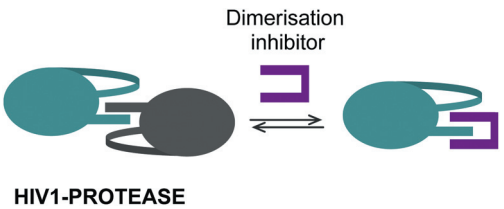

Fig. 2 Inhibition of the dimerisation.[5]

separated by more flexible linkers.[10,11] The structure–activity relationships in the field of HIV1-PR dimerisation inhibitors were extensively reviewed by Camarasa *et al.*[12] In general, the inhibitory activity of the reported compounds has been tested *in vitro* and best activities are in the range of K_{id} = 150–400 nM. More recently, Bannwarth *et al.* have reported the activity of a series of alkyltripeptides including the potent dimerisation inhibitor palmitoyl-Leu-Glu-Tyr (K_{id} = 0.3 nM).[13]

In the context of our studies on applications of conformationally restricted lactam dipeptides,[14] we report here the design and synthesis of a collection of small molecules that potentially act

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† Electronic supplementary information (ESI) available: NMR spectra of compounds **3a** and **3b**, HPLC and MS of compounds **4–18cc**, and results on cultured cells are provided. See DOI: 10.1039/c2ob25291k

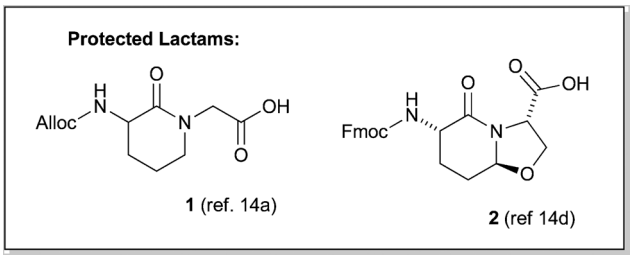


Fig. 3 Lactams used for the synthesis of the library.

by capping the monomers of HIV1-PR, thus preventing dimerisation and interrupting the virus life cycle. We have paid particular attention to balancing hydrophilicity and hydrophobicity, and to keeping molecular weight low, in order to facilitate permeation of the inhibitors through the cell membrane. Our design is based on the use of 3-aminolactams **1** and **2** (Fig. 3) as turn inducers. Compound **1** was first reported by Freidinger *et al.* as a turn inducer when inserted in a peptide chain,[15] and it has been used in our laboratory to build a library of potential tryptase inhibitors.[14a] Compound **2** is a beta-turn mimetic by itself.[14d] Compounds **1** and **2** share the ability to act as both acceptors and donors in β-strand like structures. We find that two of our compounds enter cells efficiently, and so open a new avenue for development of anti-HIV1-PR drugs.

Results and discussion

Potential dimerisation inhibitors design and synthesis

On the basis of the published data, we designed a library of compounds by combining short peptide chains, a flexible carbon chain spacer, and lactam turn-inducers **1**[14a] and **2**[14d] (Fig. 3).

The first series of compounds consisted of short peptides and of short peptides bearing a free flexible carbon chain (Fig. 4). In the second series (Fig. 5), another peptide chain, parallel to the first peptide, was bound to the spacer, so the compound would interdigitate with the antiparallel terminal chain of the HIV1-PR.

The synthesis of the first series of the library was performed on solid phase, using a standard Fmoc/^{t}Bu protocol[16] on Wang resin for the acid-terminated compounds and on MBHA Rink amide for the amide-terminated peptides (Scheme 1). Piperidine was used to cleave the Fmoc group; a DIPCDI–HOBt mixture was used as the coupling agent for the commercial amino acids; PyBOP–HOBt in the presence of DIEA was used to couple the lactam amino acids **1** and **2**, and ninhydrin (Kaiser test)[17] was used to monitor chain elongation.

For the second series (Scheme 1), we prepared the additional dipeptides separately, using the same protocol but in solution. We then coupled the dipeptide to compounds of type I still attached to the resin, using DIPCDI–HOBt, and monitored the coupling using malachite green.[18] Side-chain deprotection (when needed) and release of the compounds were performed with TFA–H_2O (95 : 5). Compounds purified by HPLC were 85–95% pure.

In this manner, 25 pure compounds were obtained in sufficient quantity to allow biological evaluation. Fig. 4 and 5 summarize the structures and the chemical yields, as well as inhibition activity (EC_{50}). Compound **7** had already been reported (IC_{50} = 1.1 μM),[19] and we prepared it as a reference for our studies. Compounds **4** and **5** correspond to the terminal portions of the HIV1-PR, and had shown some activity in early studies.[6b] Compounds **6cc**, **12cc**, **14cc**, **16cc**, **17cc** and **18cc** were obtained in <5% yield, as the cross-coupling products (indicated as "**cc**" in Scheme 1).

Anti-HIV activity in cell culture[20] (EC_{50})

All compounds were tested for activity in cultured cells infected with wild type HIV1 virus. The four most potent compounds were then tested on cells infected with the multidrug resistant HIV1 strain IRLL98DPRO.[21] The test was based on the MTT colorimetric method that measures cellular proliferation,[22] specifically applied to HIV1-PR inhibitors.[23] AZT, AMD3100, ritonavir and indinavir were used as the references. The concentrations at which these compounds were 50% effective (EC_{50}) were non-toxic to the cells (CC_{50} was also determined).[24]

The results showed that compounds **14** and **15** are active against wild type HIV1 in the μM range. HIV1-PR inhibitors indinavir and ritonavir are active at the nM scale. Although compounds **14** and **15** do not show strong anti-viral activity, our results show that the presence of lactam moieties **1** and **2** in their structures does not prevent their entry into cells. Compounds **14** and **15** showed no activity against the highly resistant mutant. In comparison with other dimerisation inhibitors reported, such as those based on a bicyclic guanidinium subunit linked to short peptide mimics of the terminal protease sequences[8] or alkyltripeptides,[13] again, compounds **14** and **15** are less potent.

Conclusion

We have built a library of potential dimerisation inhibitors of HIV1-PR and evaluated the activity of the compounds. We have found that two compounds (**14** and **15**) are active in the low micromolar range on cells infected with the wild type virus, and are not cytotoxic at the active concentration. Compounds **14** and **15** contain the piperidone moiety, showing that its inclusion in the structure does not prevent cell penetration.

Experimental section

Semipreparative HPLC was performed on a Waters Delta Prep HPLC4000, with a dual wavelength detector (model 2487) and a fraction collector. The semi-preparative column used was Symmetry C18, 19 × 100 mm, 5 μm (Waters). Analytical HPLC was performed on a Waters analytical HPLC instrument including a model binary pump (model 1525), an autoinjector (model 717 plus), and a dual wavelength detector model 2487. The analytical column used was Symmetry C18, 4.6 × 150 mm, 5 μm (Waters). Mass spectra were determined by MALDI-TOF (Proteomics Analyzer 4700, Applied Biosystems). Exact mass spectra were recorded on a LTQ-FT Ultra (Thermo Scientific) spectrometer. Samples were previously dissolved in 1 mL of H_2O–CH_3CN–FA (1 : 1 : 1), and a 1 to 100 dilution in H_2O–CH_3CN–FA (1 : 1 : 1) was used for the analysis. The UV-Vis

Compounds Type I

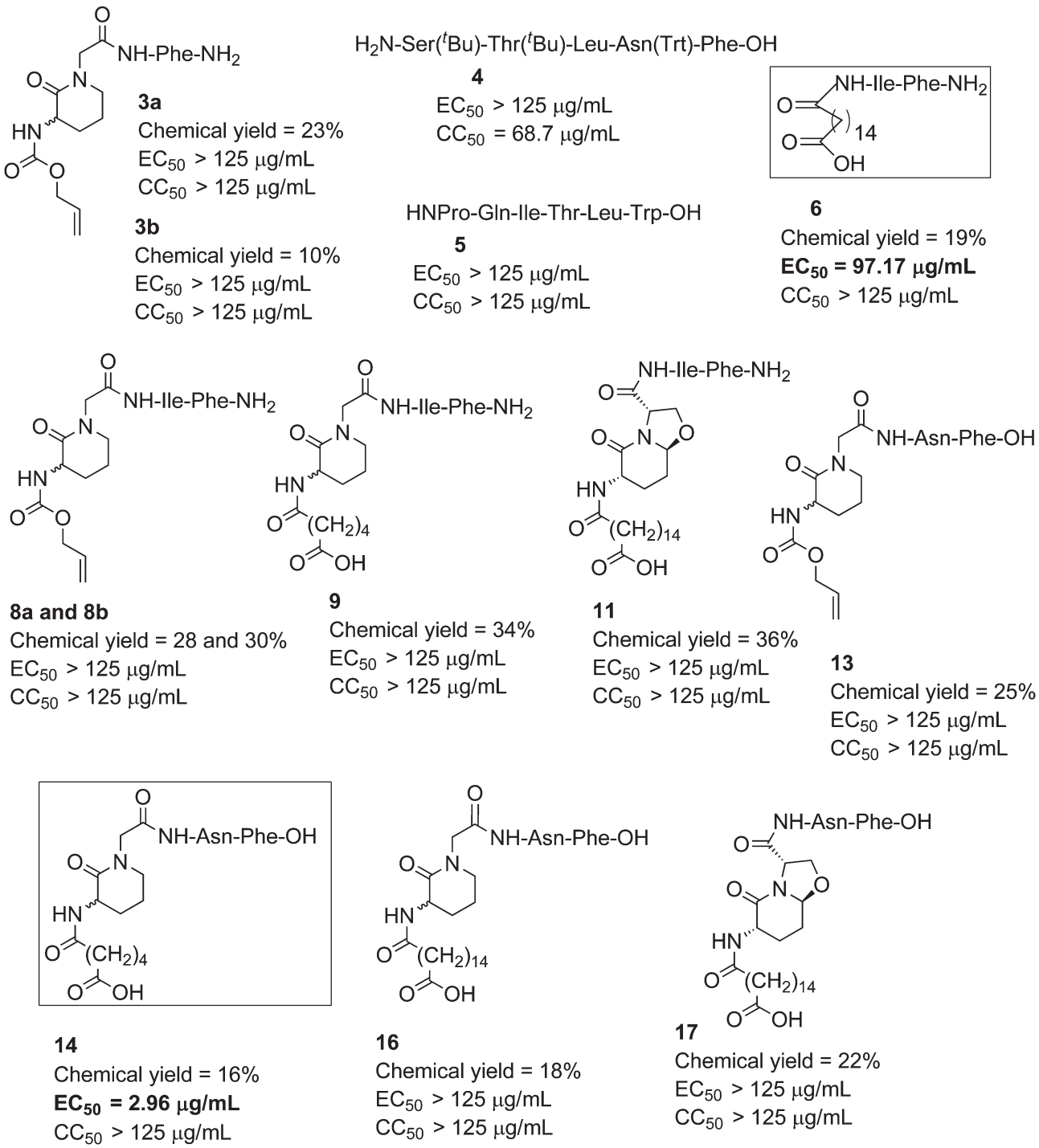


Fig. 4 Library of potential HIV1-PR inhibitors: first series.

spectrometer was a Shimadzu (model UV-2501 PC). Compounds were dried in a Freezamobile 12 EL (Virtis) lyophilizer. The centrifuge was a Beckman Coulter, model Allegra 21. The amino acid analyses were performed using a Beckman System 6300. Resins, aminoacids and coupling reagents were purchased from Novabiochem. Dry solvents were purchased from SDS or Aldrich. All final compounds showed 95–98% HPLC purities.

3-Allyloxycarbonylamino-2-oxopiperidinyl-1-(phenylalanylamide) acetamide (3a and 3b)

To a solution of compound **1**[12] (330 mg, 0.390 mmol) in CH_2Cl_2 (13 mL), a mixture of DIEA (204 μL, 1.171 mmol), PyBOP (304 mg, 0.585 mmol) and HOBt (896 mg, 0.585 mmol) was added. Then, a solution of H-Phe-NH_2·HCl

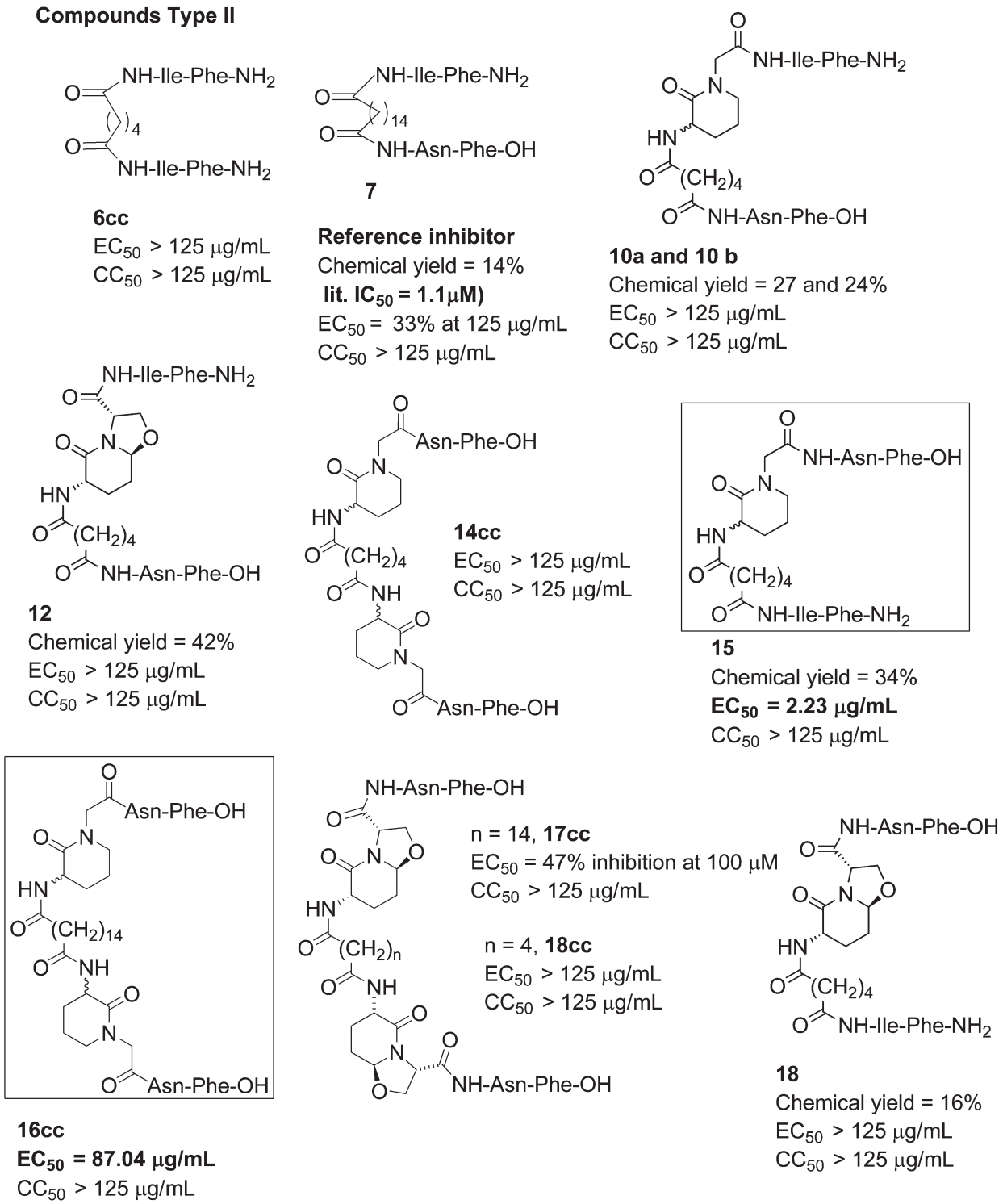
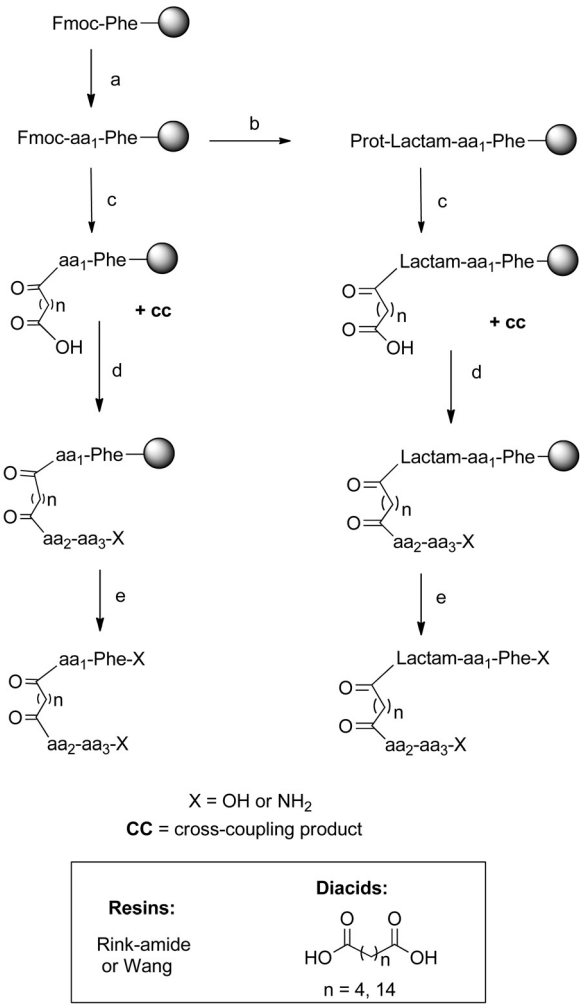


Fig. 5 Library of potential HIV1-PR inhibitors: second series.

(86 mg, 0.429 mmol) in CH_2Cl_2 (1 mL) with 2 drops of DIEA was added. The mixture was stirred for 12 h, the solvent was evaporated and the residue was purified by column chromatography (CH_2Cl_2). Semipreparative HPLC (H_2O–MeCN, 75 : 25 to 68 : 32 gradient, 15 min) allowed separation of the diastereomers. Compound **3a** (172 mg, 33%): IR (NaCl): ν 3306, 3061, 3023, 2940, 2870, 1666 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.88 (brs, 2H, H-5′), 2.26 (brs, 2H, H-4′), 3.04 (brs, 1H, H-βa), 3.18 (brs, 2H, H-βb, H-2a), 3.43 (brs, 1H, H-6′a), 3.55 (brs, 1H, H-6′b), 4.00 (brs, 1H, H-3′), 4.35 (brs, 1H, H-2b), 4.52 (brs, 2H, OCH_2), 4.68 (brs, 1H, H-α), 5.19 (d, J = 8.8 Hz, 1H, CH=CH_2), 5.27 (d, J = 16.8 Hz, 1H, CH=CH_2), 5.79–6.10 (m, 2H, CH=CH_2, NH), 6.74 (brs, 1H, NH), 7.18–7.37 (m, 5H, Ph). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 21.3 (C-5′), 28.0 (C-4′), 37.6 (C-β), 50.1 (C-6′), 52.2 (C-3′), 52.3 (C-2), 54.9 (C-α), 68.4 (OCH_2), 118.2 (CH=CH_2), 127.2



Reagents and conditions: **a**.1. 20% piperidine in DMF, 2. DIPCDI, HOBt, Fmoc-Ile or Fmoc-Asn (1:1:1); **b**. 1. 20% piperidine in DMF, 2. PyBOP, HOBt, DIEA, "Prot-Lactam" (1:1:1:2:1); **c.** 1. 20% piperidine in DMF, 2. DIPCDI, HOBt, diacid (1:1:1); **d**. H_2N-Asn-Phe-OH or NH_2-Ile-Phe-NH_2 DIPCDI, HOBt (1:1:1); **e**. 1. TFA:H_2O (95:5) 2. HPLC purification

Scheme 1 Synthesis of the compounds.

(Ph-*p*), 128.9 and 129.0 (Ph-*o*,*m*), 132.6 (CH═CH_2), 136.8 (Ph-*ipso*), 156.8 (C═O carbamate), 169.2 (C═O), 171.2 (C═O), 175.0 (C═O). MS-ESI (*m*/*z*) 425.2 $(M + Na)^+$, 403.2 $(M + H)^+$, 239.2 $(M - PheNH_2)^+$, 211.2 $(M - CONH - PheNH_2)^+$. Calcd mass for $C_{20}H_{27}N_4O_5$ 403.19760. Found: 403.19753. Calcd for $C_{20}H_{26}N_4O_5$: C, 59.69%; H, 6.51%; N, 13.92%. Found: C, 59.13%; H, 6.30%; N, 14.29%. Compound **3b** (121 mg, 23%): IR (NaCl) ν 3275, 3064, 3027, 2943, 2873, 1716, 1663 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.74–1.94 (m, 2H, H-5′), 2.08–2.24 (m, 2H, H-4′), 2.72 (brs, 2H, NH_2), 3.02–3.12 (m, 3H, H-6′, H-βa), 3.24 (d, J = 16.0 Hz, 1H, H-2a), 3.43 (d, J = 13.6 Hz, 1H, H-βb), 3.66–3.78 (m, 1H, H-3′), 4.53 (dd, J = 13.4 and 5.4 Hz, 1H, OCH_2), 4.62 (dd, J = 13.4 and 5.0 Hz, 1H, OCH_2), 4.53–4.62 (m, 1H, H-2b), 4.74 (brs, 1H, H-α), 5.24 (d, J = 10.4 Hz, 1H, CH═CH_2), 5.33 (d, J = 16.8 Hz, 1H, CH═CH_2), 5.69 (brs, 1H, NH), 5.91 (ddd, J = 22.2, 10.8 and 5.4 Hz, 1H, CH═CH_2), 6.58 (brs, 1H, NH), 7.18–7,44 (m, 5H, Ph). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 21.5 (C-5′), 28.2 (C-4′), 36.6 (C-β), 50.1 (C-6′), 52.3 (C-3′), 53.0 (C-2), 54.6 (C-α), 66.5 (OCH_2), 118.4 (CH═CH_2), 126.9 (Ph-*p*), 128.7 and 129.4 (Ph-*o*,*m*), 132.4 (CH═CH_2), 137.9 (Ph-*ipso*), 156.9 (C═O carbamate), 168.2 (C═O), 170.6 (C═O), 174.5 (C═O). MS-ESI (*m*/*z*) 425.2 $(M + Na)^+$, 403.2 $(M + H)^+$, 239.2 $(M - Phe - NH_2)^+$, 211.2 $(M - CONH - Phe - NH_2)^+$. Calcd mass for $C_{20}H_{27}N_4O_5$ 403.19760. Found: 403.19760. Calcd for $C_{20}H_{26}N_4O_5$: C, 59.69%; H, 6.51%; N, 13.92%. Found: C, 59.65%; H, 6.21%; N, 13.86%.

General protocol for the solid phase peptide synthesis

The compounds in the library were obtained by solid phase synthesis, using standard protocols[13] on Wang (Novabiochem, 0.96 mmol g^{-1}) or Rink amide (Novabiochem MBHA, 0.66 mmol g^{-1}) resins. Coupling of commercial amino acids (Novabiochem), of dipeptides H-Asn-Phe-O^{t}Bu and Ile-Phe-NH_2, as well as of adipic and hexadecandioic acids (Aldrich) were performed using DIPCDI (3 equivalents) and HOBt (3 equivalents). Oxazolopiperidone **1** and lactam **2** were coupled using PyBOP (3 equivalents), HOBt (3 equivalents), and DIEA (6 equivalents). Incorporation of amino acids was monitorized by the ninhydrin test,[14] and that of diacids by malachite green test.[15] All compounds were purified (95 to 98% purity) by semi-preparative HPLC (milli-Q H_2O with 0.1% of TFA, MeCN with 0.1% of TFA; flux, 15 mL min^{-1}; UV detection, λ = 220 and 254 nm). The chemical yields given correspond to the total syntheses described, after final HPLC purification.

H_2N-Ser(^{t}Bu)-Thr(^{t}Bu)-Leu-Asn(Trt)-Phe-OH (compound 4, 327 mg, 21%)

The synthesis was done on a 62 µmol scale using Wang resin. Purification conditions: 30 to 80% MeCN in 50 min. Analytical HPLC: t_R = 13.20 min (0 to 100% MeCN in 15 min); t_R = 11.428 min (30 to 80% MeCN in 15 min). AAA: Ser, 0.92 (1); Thr, 1.02 (1); Leu, 1.08 (1); Asn, 0.97 (1); Phe, 1.14 (1). MALDI-TOF: 957.43 $(M + Na)^+$, 979.42 $(M + K)^+$. Calcd mass for $C_{53}H_{71}N_6O_9$ 935.52770. Found: 935.52814.

HNPro-Gln-Ile-Thr-Leu-Trp-OH (compound 5, 505 mg, 53%)

The synthesis was done on a 66 µmol scale using Wang resin. Purification conditions: 30 to 90% MeCN in 30 min. Analytical HPLC: t_R = 9.53 min (0 to 100% MeCN in 15 min); t_R = 9.359 min (20 to 40% MeCN in 15 min). AAA: Pro, 0.90 (1); Glu, 1.04 (1); Ile, 0.89 (1); Thr, 1.00 (1); Leu, 1.06 (1); Trp, 0.92 (1). MALDI-TOF: 797.39 $(M + Na)^+$, 795.37 $(M + K)^+$. Calcd mass for $C_{37}H_{57}N_8O_9$ 757.42430. Found: 757.42363.

[*N*-(15-Carboxypentadecanoyl)isoleucyl]phenylalanylamide (compound 6, 7.2 mg, 19%)

The synthesis was done on a 66 µmol scale using Rink amide resin. Purification conditions: 35 to 90% MeCN in 50 min. Analytical HPLC: t_R = 14.92 min (0 to 100% MeCN in 15 min); t_R = 14.87 min (30 to 80% MeCN in 15 min). MALDI-TOF: 568.4 $(M + Na)^+$, 584.3 $(M + K)^+$. Calcd mass for $C_{31}H_{52}N_3O_5$ 546.39015. Found: 546.39019. Cross coupling product **6cc**

(2.1 mg, 4%). Analytical HPLC: t_R = 15.21 min (0 to 100% MeCN in 15 min); t_R = 15.03 min (30 to 80% MeCN in 15 min). MALDI-TOF: 829.4 $(M + Na)^+$, 845.4 $(M + K)^+$. Calcd mass for $C_{46}H_{73}N_6O_6$ 805.38792. Found: 805.38815.

N-Asparagylphenylalanyl *N*′-(isoleucylphenylalanylamide) hexadecanoyldiamide (compound 7, 7.5 mg, 14%)

The synthesis was done on a 66 μmol scale using Rink amide. Purification conditions: 40 to 90% MeCN in 50 min. Analytical HPLC: t_R = 13.66 min (0 to 100% MeCN in 15 min); t_R = 12.56 min (30 to 80% MeCN in 15 min). MALDI-TOF: 829.5 $(M + Na)^+$, 845.5 $(M + K)^+$. Calcd mass for $C_{44}H_{67}N_6O_8$ 807.50149. Found: 807.50173. The presence of the cross coupling product **6cc** was detected.

N-(Isoleucylphenylalanylamide) 3-allyloxycarbonylamino-2-oxopiperidin-1-acetamide (compounds 8a and 8b)

The synthesis was done on a 160 μmol scale using Rink amide resin. HPLC purification and separation conditions: 27% MeCN in 15 min. Compound **8a** (23 mg, 28%). Analytical HPLC: t_R = 10.94 min (0 to 100% MeCN in 15 min); t_R = 14.98 min (20 to 40% MeCN in 15 min). MALDI-TOF: 538.2 $(M + Na)^+$, 554.2 $(M + K)^+$. Calcd mass for $C_{26}H_{38}N_5O_6$ 516.28166. Found: 516.28161. Compound **8b** (25 mg, 30%). Analytical HPLC: t_R = 10.98 min (0 to 100% MeCN in 15 min); t_R = 15.36 min (20 to 40% MeCN in 15 min). MALDI-TOF: 538.3 $(M + Na)^+$, 554.2 $(M + K)^+$. Calcd mass for $C_{26}H_{38}N_5O_6$ 516.28166. Found: 516.28162.

N-(Isoleucylphenylalanine) 3-carboxypentanoylamino-2-oxopiperidin-1-acetamide (compounds 9 and 9cc)

The synthesis was done on a 198 μmol scale using Rink amide resin. HPLC purification conditions: 23 to 24% MeCN in 15 min followed by 24 to 45% MeCN in 15 min. Inhibitor **9** (38 mg, 48%). Analytical HPLC: t_R = 9.73 min (0 to 100% MeCN in 15 min); t_R = 10.08 min (20 to 40% MeCN in 15 min). MALDI-TOF: 582.2 $(M + Na)^+$, 598.2 $(M + K)^+$. Calcd mass for $C_{28}H_{42}N_5O_7$ 560.30788. Found: 560.30747. The cross-coupling product **9cc** was detected: t_R = 10.90 min (0 to 100% MeCN in 15 min); t_R = 16.41 min (20 to 40% MeCN in 15 min). MALDI-TOF: 995.5 $(M + Na)^+$, 1011.5 $(M + K)^+$.

N-(Asparagylphenylalanine) *N*′-(oxazolopiperidonylisoleucyl-phenylalanylamide) hexandiamide (compounds 10a and 10b)

The synthesis was done on a 205 μmol scale using Rink amide resin. HPLC purification and separation conditions: 26% MeCN in 15 min. Compound **(3*S*)-10a** (45 mg, 27%): analytical HPLC: t_R = 10.37 min (0 to 100% MeCN in 15 min); t_R = 14.07 min (26% MeCN in 15 min). MALDI-TOF: 871.4 $(M + Na)^+$, 887.4 $(M + K)^+$. Calcd mass for $C_{41}H_{57}N_8O_{10}$ 821.41922. Found: 821.41923. Compound **(3*R*)-10b** (40 mg, 24%). Analytical HPLC: t_R = 10.48 min (0 to 100% MeCN in 15 min); t_R = 15.00 min (26% MeCN in 15 min). MALDI-TOF: 843.3 $(M + Na)^+$, 859.3 $(M + K)^+$. Calcd mass for $C_{41}H_{57}N_8O_{10}$ 821.41922. Found: 821.41904.

N-(Oxazolipiperidonylisoleucinylphenylalanine) hexadecanamide (compound 11, 12.5 mg, 36%)

The synthesis was done on a 47 μmol scale using Rink amide MBHA resin. Purification conditions: 40 to 90% MeCN in 50 min. Analytical HPLC: t_R = 7.79 min (0 to 100% MeCN in 15 min); t_R = 4.61 min (10 to 30% MeCN in 15 min). MALDI-TOF: 750.4 $(M + Na)^+$, 766.4 $(M + K)^+$. Calcd mass for $C_{39}H_{62}N_5O_8$ 728. 45 929. Found: 728.45874.

N-(Asparaginylphenylalanine) *N*′-(oxazolopiperidonyl-isoleucyl-phenylalanylamide) hexanediamide (compounds 12 and 12cc)

The synthesis was done on a 99 μmol scale using Rink amide resin. HPLC purification conditions: 20 to 70% MeCN in 45 min. Inhibitor **12** (35.3 mg, 42%). Analytical HPLC: t_R = 10.15 min (0 to 100% MeCN in 15 min); t_R = 12.89 min (20 to 40% MeCN in 15 min). MALDI-TOF: 871.4 $(M + Na)^+$, 887.4 $(M + K)^+$. Cross-coupling product **12cc** (3.5 mg, 3%). MALDI-TOF: 1051.5 $(M + Na)^+$, 1067.5 $(M + K)^+$.

N-(Asparagylphenylalanine) 3-(allyloxycarbonylamino)-2-oxopiperidin-1-acetamide (compound 13, 37 mg, 25%)

The synthesis was done on a 288 μmol scale using Wang resin. HPLC purification conditions: 22% MeCN in 15 min. Analytical HPLC: t_R = 9.52 min (0 to 100% MeCN in 15 min); t_R = 9.12 min (20 to 40% MeCN in 15 min). MALDI-TOF: 540.2 $(M + Na)^+$, 556.1 $(M + K)^+$. Calcd mass for $C_{24}H_{32}N_5O_8$ 518.22454. Found: 518.22425.

N-(Asparagylphenylalanine) 3-carboxypentanoylamino-2-oxopiperidin-1-acetamide (compounds 14 and 14cc)

The synthesis was done on a 288 μmol scale using Wang resin. HPLC purification conditions: 10 to 17% MeCN in 15 min. Compound **14** (26 mg, 16%). Analytical HPLC: t_R = 13.86 min (0 to 100% MeCN in 15 min); t_R = 14.76 min (30 to 80% MeCN in 15 min). MALDI-TOF: 584.2 $(M + Na)^+$, 600.2 $(M + K)^+$. Cross-coupling product **14cc** (7.4 mg, 3%): t_R = 9.28 min (0 to 100% MeCN in 15 min); t_R = 8.47 min (20 to 40% MeCN in 15 min). MALDI-TOF: 999.4 $(M + Na)^+$, 1015.4 $(M + K)^+$.

N-(Isoleucylphenylalanylamide) *N*′-(aminopiperidonylglycyl-asparagylphenylalanine)hexandiamide (compound 15, 80 mg, 34%)

The synthesis was done on a 288 μmol scale using Wang resin. HPLC purification conditions: 15 to 45% MeCN in 15 min, followed by 45 to 100% in 5 min. Analytical HPLC: t_R = 13.88 min (0 to 100% MeCN in 15 min); t_R = 14.80 min (30 to 80% MeCN in 15 min). MALDI-TOF: 843.3 $(M + Na)^+$, 883.3 $(M + K)^+$. Calcd mass for $C_{41}H_{57}N_8O_{10}$ 821.41922. Found: 821.41931.

N-(Asparagylphenylalanine) 3-carboxypentadecylamino-2-oxopiperidin-1-acetamide (compounds 16 and 16cc)

The synthesis was done on a 288 μmol scale using Wang resin. HPLC purification and separation conditions: 43% MeCN in 15 min. Compound **16** (49 mg, 18%): Analytical HPLC: t_R = 12.89 min (0 to 100% MeCN in 15 min); t_R = 11.17 min (30 to 80% MeCN in 15 min). MALDI-TOF: 724.4 $(M + Na)^+$, 740.4 $(M + K)^+$. Calcd mass for $C_{36}H_{56}N_5O_9$ 702.40726. Found: 702.40752. Cross coupling product **16cc** (9.1 mg, 3%): Analytical HPLC: t_R = 11.88 min (0 to 100% MeCN in 15 min); t_R = 9.61 min (30 to 80% MeCN in 15 min). MALDI-TOF: 1139.6 $(M + Na)^+$, 1155.6 $(M + K)^+$. Calcd mass for $C_{56}H_{81}N_{10}O_{14}$ 1117.59337. Found: 1117.59344.

N-(Asparagylphenylalanine) 3-carboxypentandecanoylamino-2-oxo-7,1-oxazolidin-9-carboxamide (compound 17, 47 mg, 22%)

The synthesis was done on a 288 μmol scale using Wang resin. Purification conditions: 40 to 65% MeCN in 15 min. Analytical HPLC: t_R = 12.86 min (0 to 100% MeCN in 15 min); t_R = 11.21 min (30 to 80% MeCN in 15 min). MALDI-TOF: 752.2 $(M + Na)^+$, 768.2 $(M + K)^+$. Calcd mass for $C_{37}H_{56}N_5O_{10}$ 730.40217. Found: 730.40232. The cross-coupling product **17cc** was also isolated (9%). Analytical HPLC: t_R = 11.83 min (0 to 100% MeCN in 15 min); t_R = 9.709 min (30 to 80% MeCN in 15 min). MALDI-TOF: 1195.63 $(M + Na)^+$, 1211.64 $(M + K)^+$. Calcd mass for $C_{58}H_{81}N_{10}O_{16}$ 1173.58320. Found: 1173.58323.

N-(Isoleucylphenylalanylamide) *N*′-(oxazolopiperidonyl-asparagylphenylalanine) hexandiamide (compounds 18 and 18cc)

The synthesis was done on a 288 μmol scale using Wang resin. HPLC purification and separation conditions: 15 to 45% MeCN in 15 min. Inhibitor 180 (40 mg, 16%): analytical HPLC: t_R = 10.23 min (0 to 100% MeCN in 15 min); t_R = 13.40 min (20 to 40% MeCN in 15 min). MALDI-TOF: 871.3 $(M + Na)^+$, 887.3 $(M + K)^+$. Compound **18cc** (11 mg, 4%): analytical HPLC: t_R = 9.45 min (0 to 100% MeCN in 15 min); t_R = 9.63 min (20 to 40% MeCN in 15 min). MALDI-TOF: 1055.4 $(M + Na)^+$, 1071.3 $(M + K)^+$. Calcd mass for $C_{48}H_{61}N_{10}O_{16}$ 1033.42615. Found: 1033.42580.

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