ARTICLE

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Design, synthesis and biological activity of a targeted library of potential tryptase inhibitors

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We have designed, synthesized, and tested two small collections of potential tryptase inhibitors. The first library consists of diversely *N*-substituted 3-aminopiperidin-2-ones **6**, and the second (compounds **7**) was prepared by dimerising compounds **6** through the 3-amino function using diverse carbon chains. We have established efficient routes for obtaining **6** both in solution and on solid supports. We have also compared the dimerisation on-resin and in solution. Four of the compounds showed a high degree of tryptase inhibition at 1 μM, but none surpassed the tryptase inhibition activity of BABIM.

Introduction

Tryptases are the major serine proteases of human mastocytes, and are known to be involved in numerous inflammatory processes. Tryptases are tetrameric proteins, whose monomers define a flat rectangle and orient their active sites towards the central pore.[1] Owing to their structure, the substrate specificity of tryptases is governed by the quaternary architecture rather than by the structure of the active sites. This peculiar geometry, which impedes access of proteins to the active centers, also seems to be responsible for the resistance of tryptases to protein inhibitors. So far, the only site-directed proteinaceous inhibitor known for human tryptase is LDTI (leech-derived tryptase inhibitor, a 46 residue peptide).[2]

Over the last decade, low molecular weight synthetic tryptase inhibitors have been largely studied to control inflammatory diseases such as allergies and asthma.[3] The first generation of inhibitors (Fig. 1) consisted of bis-benzamidines such as BABIM (**1**),[4] peptidilphosphonates (**2**),[5] benzisothiazolones (**3**),[6] or APC 366 (**4**).[7] All of these bind in different ways to the active sites of tryptase. The second generation of tryptase inhibitors was based on the assumption first proposed by Axys Pharm Corp., that larger compounds could interact with active sites of two adjacent monomers which are spatially close (Fig. 2).[8]

APC 2059, which belongs to this generation of tryptase inhibitors (**5b**, Fig. 2), is in phase II clinical trials.[9] The general

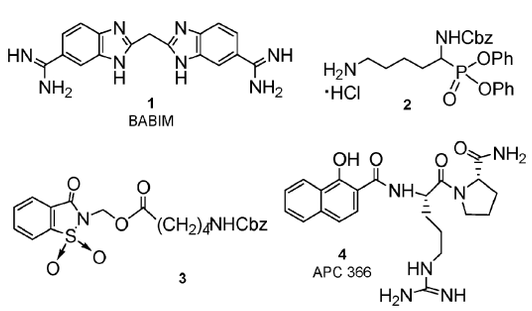

Fig. 1

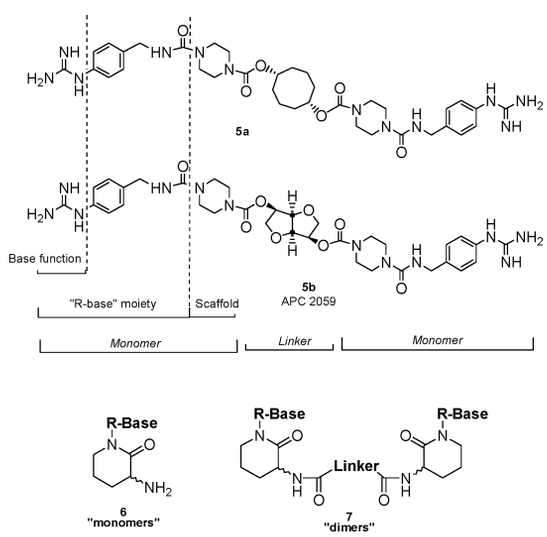

Fig. 2

structure of these compounds consists of a central linker that binds two identical or very similar moieties. These moieties contain a terminal base function, attached to an aromatic ring ("R-base"), which is also bound to the linker through a scaffold.

In connection with our previous work on the application of 3-aminolactams as pseudopeptides,[10] we planned to use this heterocycle as a surrogate of the piperazine moiety in compounds **5**. Thus, the monomeric and dimeric series **6** and **7** were designed as potential tryptase inhibitors (Fig. 3). Compounds **6** consist of a 3-aminovalerolactam scaffold bearing a terminal basic moiety, which not only has all the requisites for interaction as a monomer, but could also be used to produce the dimerised species **7**.

We selected from among already reported compounds the linkers and the "R-base" moieties which had given the best biological activity,[11] and attached them to the piperidone scaffold. Theoretically, the combination of these two factors

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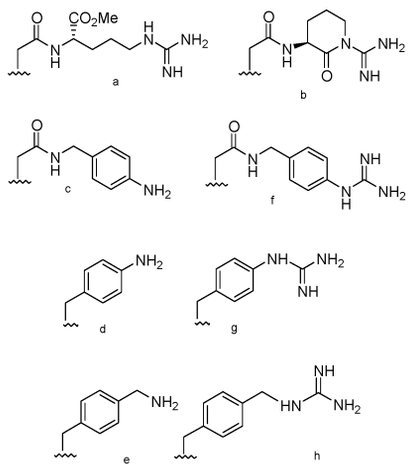


Fig. 3

should give us a small but efficient library of compounds. For the terminal base, we planned to test amines and guanidines. Concerning the R moiety, we decided to use a phenyl ring, as well as a non-aromatic valerolactam and an acyclic carbon chain. Another variable to consider was the need for the polar carbonyl group in R. Thus, we selected the eight radicals shown in Fig. 3 to prepare monomers type **6**.

To evaluate the feasibility of this scaffold replacement, we first performed a molecular overlay of compound **5a** and some of the designed compounds. For this purpose, and because **5a** is a very flexible molecule, we docked it to the tryptase active site (PDB accession code: 1A0L) in order to obtain a possible bioactive conformation (Fig. 4).

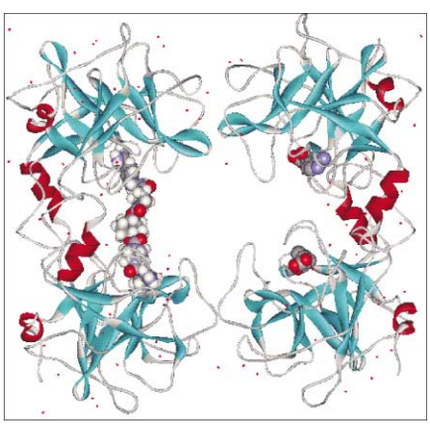


Fig. 4 Docking (InsightII package) of compound **5a** in the tryptase active site (PDB accession code: 1A0L). The two guanidino groups of **5a** are accommodated very well in two of the tryptase subunits.

The conformation of **5a** which provided better results in the docking study was used as a template for comparison to compounds **7**. As an example, Fig. 5 shows the superposition of this conformation of **5a** with compound **7f** ($n = 7$), which shows a good overlay of the different functional groups.

In view of these results, we chose to use diacids ranging from 6 to 9 carbons long,[8] to create amide bonds on the exocyclic C3-amino group of the piperidone scaffold. The combination

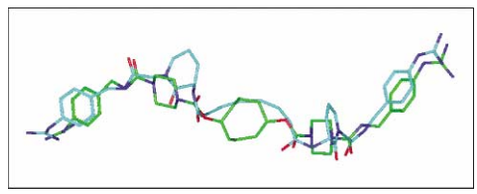


Fig. 5 Flexible superposition of **5a** with compound **7f** ($n = 7$).

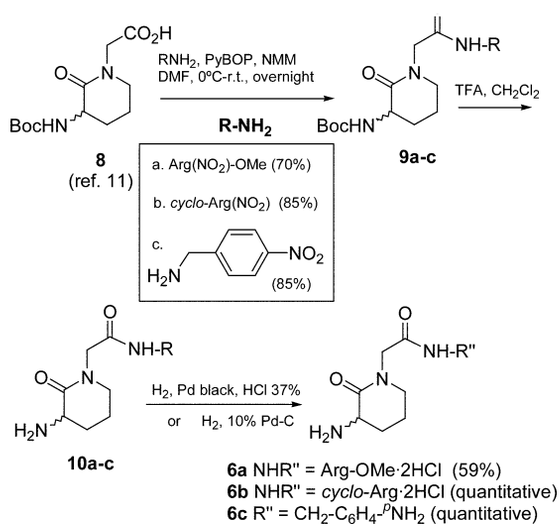
of these items would provide a small library of 32 dimers of type **7**, which should provide data on the relevance of the four parameters considered (the base, the aromatic ring, the carbonyl group, and the length of the linker).

We planned to carry out the synthesis of compounds **6** both in solution (SS) and on solid phase (SPS). Compounds **7** were prepared in solution, but we also attempted an on-resin dimerisation of compounds of type **6** to produce a resin-bound library of dimers **7**.

Results and discussion

Synthesis of compounds 6a–g in solution

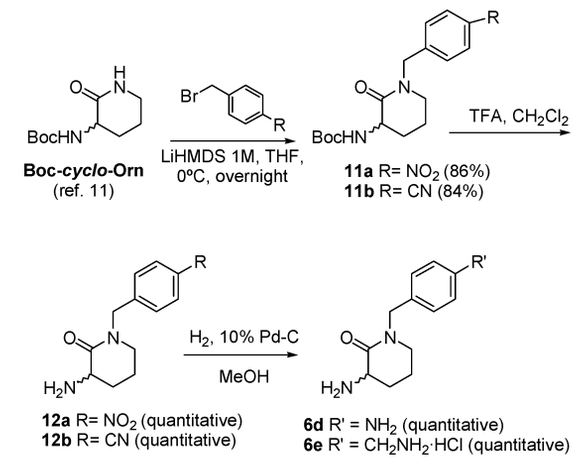
Compounds **6a–c** were prepared in three steps from compound **8** (Scheme 1). Compound **8**[11] was coupled to the chosen "R–NH_2" moieties using PyBOP and NMM as the activating agents. In this manner we obtained compounds **9a–c** in good yields, which were Boc-deprotected by treatment with TFA. Hydrogenation of compounds **10a–c** in the presence of 10% Pd–C led to the target compounds **6a–c**, which were fully characterized.



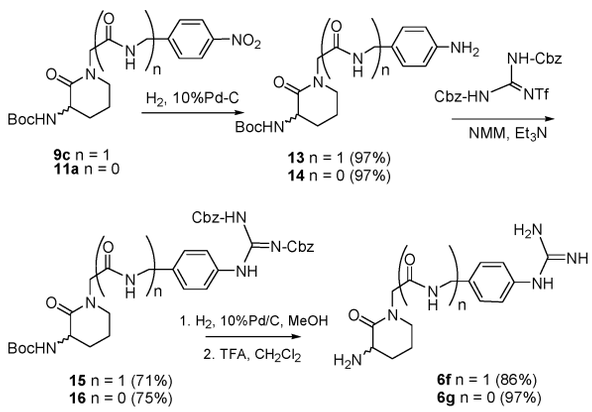
Scheme 1

Compounds **6d**,**e** were prepared from Boc-*cyclo*-ornithine[12] in three steps. Thus, selective alkylation of Boc-*cyclo*-ornithine yielded the corresponding amines **11a**,**b**. Treatment of compounds **11a**,**b** with TFA, and hydrogenation of the resulting amines **12a**,**b**, yielded the target lactams **6d**,**e** in good yields (Scheme 2).

The guanidino derivatives **6f** and **6g** were prepared from the nitro precursors **9c** and **11a** (Scheme 3). Reduction of the nitro group followed by reaction of the resulting anilines **13** and **14** with *N*,*N*′-bis(benzyloxycarbonyl)guanidinium triflate in the presence of NMM led to the fully protected guanidines **15** (71%) and **16** (75%), which presented NMR signals at δ ~163 (^{13}C), and at δ ~10–11 (^{1}H, two signals), characteristic of the guanidino group. Removal of the Cbz protecting groups and TFA cleavage of the Boc yielded the target lactams **6f** and **6g** (86 and 97%, respectively).



Scheme 2

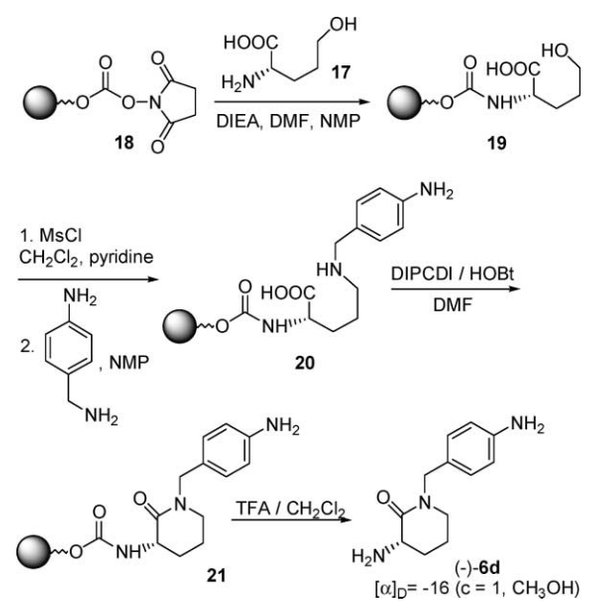


Scheme 3

SPS synthesis of compounds 6e, 6d and 6h

Regarding the SPS synthesis,[13,14] we planned to anchor the monomers of type **6** both through the exocyclic C3-amino group and through the terminal base function (amino or guanidino). The first series would allow easy diversification on the lactam nitrogen atom, whereas the second would do so on the C3-amino substituent.

To establish the first SPS synthetic route, we aimed at obtaining compound **6d** (Scheme 4). Our approach involved attachment of (*S*)-5-hydroxynorvaline to a solid support *via* its *N*-terminus and building the lactam ring by formation of the N1–C2 bond. For this purpose, enantiomerically pure (*S*)-5-hydroxynorvaline (**17**)[15] was anchored to a previously functionalised benzhydrylamine resin **18**[16] by means of a carbamate function to give compound **19**. The extent of this coupling was 70%, calculated from amino acid analysis using Ala as the internal reference. Subsequent mesylation and nucleophillic substitution with 4-aminobenzylamine yielded compound **20**. The complete formation of the product was verified by a Kaiser test, and the identity of compound **20** was confirmed by cleavage from the resin and ^{1}H-NMR characterization of the resulting benzylamino amino acid.



Scheme 4

Compound **20** was then treated with HOBt/DIPCDI to promote the intramolecular amino acid coupling, and subsequent TFA cleavage released the desired 1-substituted 3-aminolactam **6d** in 68% crude yield, based on the loading of resin **18**. The purity of the crude product was 75% as determined by HPLC analysis.

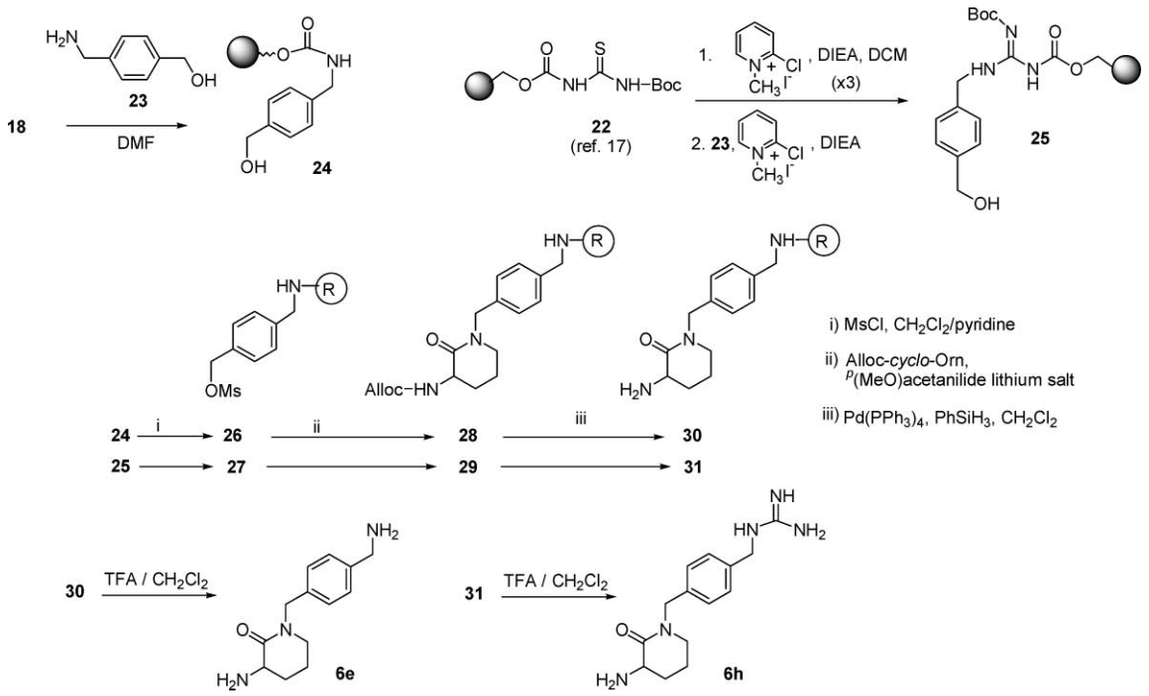
To anchor compounds **6** through the terminal function we used resins **18** and **22**,[17] which would release the suitable amino and guanidino functions (Scheme 5). The benzyl alcohol **23**[18] was anchored to the solid support **18** through a carbamate function to give compound **24** (Scheme 5). To quantify the loading of the resin-bound amino alcohol, we acetylated and cleaved (TFA) a sample of compound **24**. The resulting trifluoroacetate was analyzed by quantitative mass spectrometry and by HPLC.[19] Surprisingly, the conditions used in the conversion of **18** to **19** did not give a satisfactory yield when applied to the anchoring of compound **23**. After optimization, the best results were obtained when carbonate activation was extended to 24 h and its reaction with compound **23** was performed without additional base for 24 h.

Once the solid support was satisfactorily loaded (>80%), compound **24** was mesylated and the on-resin nucleophilic substitution with Alloc-*cyclo*-ornithine[20] was performed. Compound **28**[21] was obtained in 70–80% yield using the lithium amidate of *p*-methoxyacetanilide[22] as the base. Lower yields were obtained when the bromide was used instead of the mesylate. Compound **6e** was obtained in 57% yield from **24**, after removal of the Alloc protecting group and final cleavage with TFA/CH_2Cl_2. HPLC analysis indicated that this material was 75% pure.

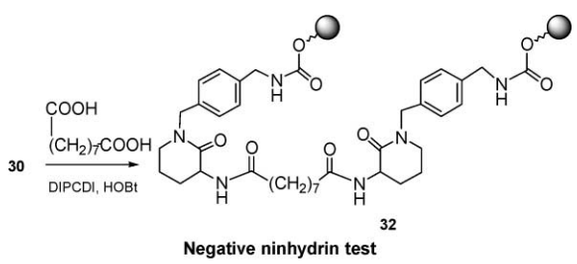
In parallel, benzyl alcohol **23**[18] was anchored on resin **22**,[17] using 2-chloro-1-methylpyridinium iodide and DIEA (Scheme 5).[23] Subsequent mesylation of the alcohol, reaction with Alloc-*cyclo*-ornithine, Alloc deprotection, and cleavage using triisopropylsilane (TIS), led to the expected compound **6h** in 85% crude yield from **25**. Compound **6h** was 95% pure according to HPLC analysis, and was identified from its spectral data.

Synthesis of a small library of dimers 7

Once we had established the SPS methods to obtain the "monomer" moieties **6**, we tried the on-resin conversion of compound **30** to the resin-bound dimer type **7** (**32**, Scheme 6). Despite the fact that working on-resin theoretically imitates infinite dilution conditions,[24] the existence of two precedents for this sort of cross-linking,[25] encouraged us to test the assay. Compound **30** was treated with 0.5 equivalents of azelaic acid in the presence of DIPCDI and HOBt as the activating agents. The resulting resin **32** showed a very encouraging negative ninhydrin test. Cleavage using 95% TFA in CH_2Cl_2 for 20 min yielded compound **7e**, which was identified from its spectral data. However, a dramatic loss of weight was observed, and **7e** was obtained only in 15% yield. Longer reaction times for the cleavage did not improve the result. In order to find a possible explanation for this low yield, we evaluated how much lactam resin **32** actually contained. For this, we performed an amino acid analysis of resin **32** using pure lactam **6e** (previously obtained in solution) as the reference.



Scheme 5

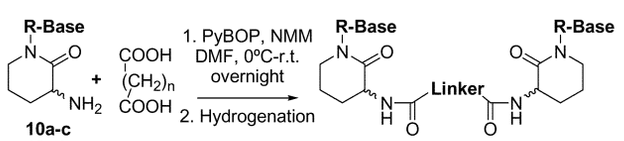


Scheme 6

Resin **32** showed only traces of the lactam, which indicated that the cleavage had occurred in the previous step. Modification of the coupling conditions, by using HBTU or HATU and DIPEA,[26] or by adding a previously prepared mixture of azelaic acid and the activating agents, did not improve the results either. We also tested the on-resin dimerisation using a Wang resin. For this, we anchored compound **23** to the CDI-activated Wang resin[17] and followed the synthetic sequence described in Scheme 5[27] to obtain the analogue of compound **30**, attached to the Wang resin (90% total yield). Unfortunately, dimerisation on this resin also produced the target dimer in poor yields.

In view of these results we decided to test the sensitivity of our compounds to the coupling reagents. Compound **30** was therefore coupled to Fmoc-Ala-OH in HATU/DIEA and HOBt/DIPCDI conditions. The yield of this reaction was calculated by the method of dibenzofulvene–piperidine adduct quantification, to be 70%, which confirmed that compound **30** was stable in the coupling conditions and that the on-resin dimerisation was not a suitable option for preparative purposes.

In contrast, the library of compounds **7** was satisfactorily prepared in solution. The experimental conditions were tested by treatment of compound **10c** with adipic acid, in the presence of PyBOP and NMM, which led to the corresponding dimer in 75% yield. Then compounds **10a**–**c**,**12a**,**b**, and Boc-deprotected **15** and **16** were treated in parallel under the same conditions, with the four chosen diacids (7 × 4 = 28 reactions). Final hydrogenation to deprotect the terminal functions, using the catalysts already established in the monomeric series, yielded the target library (Scheme 7). There was one exception: *cyclo*-arginine-derived compound **10b** yielded the acyclic arginine compounds **7a** in the four couplings. The results are summarised in Table 1. Compounds **7** showed the characteristic NMR signals observed in their corresponding monomers **6**, and additional aliphatic signals corresponding to the central linkers.[28] Structural assignment of the dimers was confirmed by ES mass spectrometry.



Substrate	Dimer products[a]	Final products
10a	7a (NO_2) x 4	7a x 4
10b	7a (NO_2) x 4	7a x 4
10c	dim-11c x 4	7c x 4
12a	dim-12a x 4	7d x 4
12b	dim-12b x 4	7e x 4
deBoc-15	7f (di-Cbz) x 4[b]	7f x 4[b]
deBoc-16	7g (di-Cbz x 4[b]	7g x 4[b]
Total number	24 out of 28	24 out of 28

a. Compounds obtained from the coupling, before hydrogenation.
b. Three compounds in each of these series were obtained in < 5% yield

Scheme 7

Tryptase inhibition activity tests

A total of 54 compounds (24 dimers of type **7** (**a**, and **c**–**g**), 6 "intermediate" dimers shown in Scheme 7, and all the lactams we have prepared) were evaluated as tryptase inhibitors using a colorimetric technique[4] (see Experimental). In the first test, only 4 compounds **7e** (n = 5, 6, 7) and **7f** (n = 7) showed a

Table 1 Synthesis of the library of dimers. The chemical yields given correspond to the 2 steps

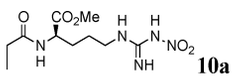
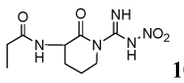
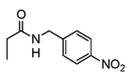
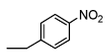
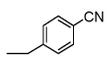
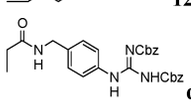
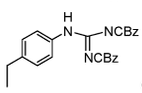
Substrate	COOH-(CH$_2$)$_4$-COOH	COOH-(CH$_2$)$_5$-COOH	COOH-(CH$_2$)$_6$-COOH	COOH-(CH$_2$)$_7$-COOH
 **10a**	**7a** (n = 4) 44%	**7a** (n = 5) 40%	**7a** (n = 6) 38%	**7a** (n = 7) 34%
 **10b**	**7a** (n= 4) 40%	**7a** (n = 5) 20%	**7a** (n = 6) 33%	**7a** (n = 7) 22%
 **10c**	**7c** (n = 4) 75%	**7c** (n = 5) 15%	**7c** (n = 6) 37%	**7c** (n = 7) 26%
 **12a**	**7d** (n = 4) 66%	**7d** (n = 5) 70%	**7d** (n = 6) 63%	**7d** (n = 7) 66%
 **12b**	**7e** (n = 4) 57%	**7e** (n = 5) 65%	**7e** (n = 6) 55%	**7e** (n = 7) 37%
 **de-Boc-15**	**7f** (n = 4) <5%	**7f** (n = 5) <5%	**7f** (n = 6) <5%	**7f** (n = 7) 34%
 **de-Boc-16**	**7g** (n = 4) <5%	**7g** (n = 5) <5%	**7g** (n = 6) <5%	**7g** (n = 7) 37%

Table 2 IC_{50} values (μM) of the four active compounds

Compound	**7e** (n = 5)	**7e** (n = 6)	**7e** (n = 7)	**7f** (n = 7)
IC_{50}/μM	0.31	0.36	0.32	1.094

percentage of inhibition at 1 μM of 50% or higher. Their IC_{50} was then calculated (Table 2).

From these biological results, we can conclude that, as expected, the dimers are more active than the monomers, that the most efficient base function is the benzyl amino group, and that the preferred length of the linker is n = 5, 6 or 7. However, since the aminolactams are less potent than the reference compound BABIM (see Experimental), new linkers and amino groups need to be explored.

Conclusion

We have designed and builts a targeted library of diversely substituted 3-aminolactam-derived monomeric (**6**) and dimeric (**7**) compounds, and have tested their activity as tryptase inhibitors. We have established the synthesis of the monomers of type **6**, both in solution and on solid phase. The two SPS methods, anchorage through the C3-amino group and anchorage through the terminal base function, resulted in equally efficient syntheses, and furnished the target monomeric compounds **6** in very pure form. However, the on-resin synthesis of dimers of type **7** gave very poor yields (15%), and parallel synthesis in solution was used to obtain the library of dimers.

Some of the compounds assayed showed moderate tryptase inhibition in the submicromolar range. Therefore, the aminolactam may be a bioisoster replacement of the piperazino group, although both the base group and linker remain to be optimised.

Experimental

General

Optical rotations were measured with a Perkin-Elmer 241 polarimeter, at 23 °C. IR spectra were recorded on a Nicolet FT-IR spectrophotometer. Unless otherwise indicated, ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, on a Mercury 400 instrument. Chemical shifts are expressed in parts per million (δ) relative to Me_4Si. Mass spectra were determined on a Hewlett Packard 5988A mass spectrometer by electronic impact (EIMS), or on a Micromass Vplatform 2 system by electrospay (ESMS). TLC was performed on SiO_2 (silica gel 60 F254, Macherey-Nagel) and developed with the eluent described for column chromatography. The spots were located with ninhydrin, potassium hexachloroplatinate, anisaldehyde, or $KMnO_4$. Organic solutions were dried over anhydrous Na_2SO_4 (Panreac, ref. 141716.0416), prior to evaporation of the solvent. Unless otherwise indicated, flash chromatography was done over SiO_2 (60 A C.C 35–70 μm, SDS). HPLC analyses were performed on a Waters system using Symmetry C_{18} 5 μm reversed phase column (4.6 × 150 mm). Purification of reagents and solvents was performed according to standard methods. Microanalyses were performed on a Carlo Erba 1106 analyzer at the Serveis Científico-Tècnics (Universitat de Barcelona). Amino acid analyses were performed using a Beckman system Gold 6300 at the Serveis Científico-Tècnics (UB).

General method for HPLC analysis

The purity of cleaved compounds was determined by C-18 reverse phase HPLC, using 90% solvent A (0.05% TFA in H_2O) and 10% solvent B (0.05% TFA in CH_3CN) for 10 min, then a gradient to 70% solvent A and 30% solvent B over 20 min with a flow rate of 1 mL min^{-1} monitored at 214 and 280 nm.

Boc-*cyclo*-Orn-Gly-Arg(NO$_2$)-OMe (9a). To a solution of compound **8**[12] (1 g, 3.68 mmol) in dry DMF (10 mL) cooled at 0 °C, NMM (1.40 mL, 12.88 mmol) and PyBOP (1.91 g, 3.68 mmol) were added. Commercial Arg(NO$_2$)-OMe (994 mg, 3.64 mmol) was then added to the solution, and the mixture was stirred overnight, at rt. The DMF was removed and the residue was partitioned in AcOEt and saturated aqueous $NaHCO_3$. The organic phase was washed with H_2O, dried and evaporated, to yield an oil which was flash chromatographed (AcOEt : MeOH, 99 : 1) to obtain compound **9a** (71%) as a foam. IR ($CHCl_3$): ν_{max} (cm^{-1}) 3320, 1634, 1682, 1745; ^{1}H NMR: δ 1.42 (s, 9H, C(CH_3)$_3$), 1.68 (br s, 2H, H-β), 1.75–1.87 (m, 1H, H-4), 1.94–1.99 (m, 4H, H-5, H-γ), 2.27 (br s, 1H, H-4′), 3.29 (dd, J = 13.2 and 6.4 Hz, 2H, H-δ), 3.38 (dd, J = 8.4 and 4.4 Hz, 1H, H-6), 3.50–3.57 (m, 1H, H-6′) 3.74 (s, 3H, CO_2CH_3), 3.83 (d, J = 16 Hz, 1H, N(CH_2)CO), 4.12 (dd, J = 14.4 and 7.2 Hz, 1H, H-3), 4.30 (d, J = 16 Hz, 1H, N(CH_2′)CO), 4.53 (d, J = 3.6 Hz,

1H, H-α), 5.62 (br s, 1H, N*H*Boc), 7.30 (d, *J* = 5.6 Hz, 1H, CON*H*), 8.45 (br s, 3H, *H*NC(= N*H*)N*H*); ^{13}C NMR: *δ* 21.2, 24.9, 28.1, 28.3, 28.6, 40.6, 49.7, 51.4, 51.8, 52.5, 79.8, 155.9, 159.2, 168.7, 171.0, 172.1. *m/z* (ES+): 488 (MH^+), 388 (100%). $C_{19}H_{33}N_7O_8$ requires: C, 46.81%; H, 6.82%; N, 20.11%; found: C, 46.35%; H, 6.76%; N, 19.97%.

Boc-*cyclo*-Orn-Gly-*cyclo*-Arg(NO$_2$) (9b). Operating as above, from compound **8**[12] (1 g, 3.68 mmol) in dry DMF (10 mL), NMM (1.40 mL, 12.88 mmol), PyBOP (1910 mg, 3.68 mmol), and *cyclo*-Arg(NO$_2$) (1160 mg, 3.68 mmol), an oil was obtained which was flash chromatographed (AcOEt : MeOH, 99 : 1) to yield piperidone **9b** (85%) as a foam. IR ($CHCl_3$): ν_{max} (cm^{-1}) 3387, 1651, 1666, 1681, 1698; ^{1}H NMR: *δ* 1.40 (s, 9H, C(C*H*$_3$)$_3$), 1.80–2.04 (m, 6H, H-4, H-5, H-β, H-γ), 2.26–2.35 (m, 2H, H-4′, H-β′), 3.31–3.37 (m, 1H, H-6), 3.50 (td, *J* = 10.8 and 3.6 Hz, 1H H-6′), 3.65–3.77 (m, 2H, H-δ, N(C*H*$_2$)CO), 3.85 (dt, *J* = 9,6 and 5,6 Hz, 1H, H-3), 4.42 (2dt, *J* = 14 and 5.6 Hz, 1H, H-δ′), 4.59 (d, *J* = 15.6 Hz, 1H, N(C*H*$_2$′)CO), 4.75 (dt, *J* = 11.6 and 8 Hz, 1H, H-α), 5.52 (d, *J* = 6.4 Hz, 1H, N*H*Boc), 7.45 (t, *J* = 7.2 Hz, 1H, NH-*cyclo*-Arg), 9.5 (br s, 1H, NC(=N*H*)NH), 10.4 (br s, 1H, NC(=NH)N*H*); ^{13}C NMR: *δ* 20.0, 21.1, 24.6, 27.9, 43.6, 49.1, 51.1, 51.4, 52.7, 79.8, 155.9, 158.5, 168.6, 170.1, 175.7, 176.1. *m/z* (ES+): 456 (MH^+), 388 (100%). $C_{18}H_{29}N_7O_7$ requires: C, 47.47%; H, 6.42%; N, 21.53%. Found: C, 47.13%; H, 6.05%; N, 21.13%.

***p*-Nitrobenzylamide 9c.** Operating as above, from compound **8**[12] (1 g, 3.68 mmol) in dry DMF (10 mL), NMM (1.40 mL, 3.68 mmol), PyBOP (1.91 g, 3.68 mmol), and *p*-nitrobenzylamine (696 mg, 3.68 mmol), an oil was obtained which was flash chromatographed (AcOEt : MeOH, 99 : 1) to obtain piperidone **9c** (85%) as a foam. IR ($CHCl_3$): ν_{max} (cm^{-1}) 1651, 1666, 1681, 1698, 1521, 1344; ^{1}H NMR: *δ* 1.30 (s, 9H, C(C*H*$_3$)$_3$), 1.80–1.91 (m, 1H, H-5), 1.96 (dt, *J* = 14 and 3.6 Hz, 1H, H-5′), 2.04–2.07 (m, 1H, H-4), 2.14–2.22 (m, 1H, H-4′), 3.30 (d, *J* = 11.6 Hz, 1H, H-6), 3.46 (d, *J* = 16.4 Hz, 1H, N(C*H*$_2$)CO), 3.53 (td, *J* = 11.2 and 4 Hz, 1H, H-6′), 3.73 (dt, *J* = 10.4 and 6.8 Hz, 1H, H-3), 4.50 (dd, *J* = 15.6 and 6 Hz, 1H, NHC*H*$_2$C$_6$H$_4$(NO$_2$)), 4.57 (dq, *J* = 15.6 and 6 Hz, 1H, NHC*H*$_2$′C$_6$H$_4$(NO$_2$)), 4.85 (d, *J* = 16 Hz, 1H, N(C*H*$_2$′)CO), 5.61 (d, *J* = 6.4 Hz, 1H, N*H*Boc), 7.46 (d, *J* = 8.4 Hz, 2H, C$_6$H$_4$(NO$_2$)-*o*), 7.84 (br s, 1H, N*H*CH$_2$-C$_6$H$_4$(NO$_2$)), 8.16 (d, *J* = 8.8 Hz, 2H, C$_6$H$_4$(NO$_2$)-*m*); ^{13}C NMR: *δ* 21.3, 28.2, 28.3, 42.5, 49.7, 51.9, 52.4, 80.3, 123.7, 127.9, 146.2, 146.9, 156.1, 168.5, 168.8. *m/z* (ES+): 407 (MH^+), 351 (100%). $C_{19}H_{26}N_4O_6$ requires: C, 56.15%; H, 6.45%; N, 13.79%. Found: C, 55.67%; H, 6.34%; N, 13.74%.

***cyclo*-Orn-Gly-Arg(NO$_2$)-OMe (10a).** Compound **9a** (200 mg, 0.41 mmol) was dissolved in CH_2Cl_2 : TFA (1 : 1, 2 mL) and the mixture was stirred at rt until disappearance of the substrate (tlc monitoring). The solvent was evaporated and the residue was washed with Et_2O to yield quantitatively compound **10a** as a foam. IR ($CHCl_3$): ν_{max} (cm^{-1}) 3395, 1740, 1679; ^{1}H NMR (CD_3OD): *δ* 1.68–1.80 (m, 3H, H-β, H-γ), 1.87–1.97 (m, 2H, H-β′, H-4), 2.02–2.06 (m, 2H, H-5), 2.30 (dd, *J* = 8.8 and 2.8 Hz, 1H, H-4′), 3.29 (t, *J* = 6.4 Hz, 2H, H-δ), 3.41 (dt, *J* = 8.4 and 3.6 Hz, 1H, H-6), 3.50 (td, *J* = 10.4 and 5.6 Hz, 1H, H-6′), 3.72 (s, 3H, CO$_2$C*H*$_3$), 3.96 (dd, *J* = 11.2 and 5.6 Hz, 1H, H-3), 3.97 (d, *J* = 17.6 Hz, 1H, N(C*H*$_2$)CO), 4.23 (d, *J* = 16 Hz, 1H, N(C*H*$_2$′)CO), 4.48 (d, *J* = 4.8 Hz, 1H, H-α); ^{13}C NMR (CD_3OD): *δ* 21.5, 26.0, 26.5, 29.5, 41.6, 50.4, 50.9, 51.3, 52.8, 53.3, 160.8, 168.2, 170.4, 173.4. *m/z* (EI-MS): 386 (MH^-), 69 (100), 51 (66). $C_{14}H_{25}N_7O_6\cdot CF_3COOH$ requires: C, 36.97%; H, 4.96%; N, 20.12%. Found: C, 37.01%; H, 5.05%; N, 19.87%.

***cyclo*-Orn-Gly-*cyclo*-Arg(NO$_2$) (10b).** Operating as above from compound **9b** (200 mg, 0.44 mmol), and TFA : CH_2Cl_2 (1 : 1, 2 mL), compound **10b** was obtained quantitatively as a foam. IR ($CHCl_3$): ν_{max} (cm^{-1}) 3366, 1697, 1652, 1609; ^{1}H NMR (CD_3OD): *δ* 1.76–1.87 (m, 1H, H-4), 1.90–2.01 (m, 3H, H-β, H-γ), 2.05 (br s, 2H, H-5), 2.22 (dt, *J* = 13.6 and 6.8 Hz, 1H, H-β′), 2.30 (ddd, *J* = 12, 6.4 and 4.8 Hz, 1H, H-4′), 3.42–3.51 (m, 2H, H-δ), 3.69–3.77 (m, 1H, H-6), 3.95 (dd, *J* = 11.6 and 6 Hz, 1H, H-3), 4.15 (d, *J* = 13.2 Hz, 1H, N(C*H*$_2$)CO), 4.17 (d, *J* = 13.2 Hz, 1H, N(C*H*$_2$′)CO), 4.22 (ddd, *J* = 10, 4.4 and 2.4 Hz, 1H, H-6′), 4.68 (dt, *J* = 11.6 and 7.6 Hz, 1H, H-α); ^{13}C NMR (CD_3OD): *δ* 21.3, 21.4, 26.2, 26.5, 45.7, 49.9, 50.6, 51.3, 52.7, 160.0, 168.1, 170.3, 176.5; *m/z* (ES+): 356.4 (MH^+), 388 (100%). $C_{13}H_{21}N_7O_5\cdot CF_3COOH$ requires: C, 38.38%; H, 4.72%; N, 20.89%. Found: C, 38.75%; H, 4.86%; N, 20.79%

***p*-Nitrobenzylamide 10c.** Operating as above from compound **9c** (200 mg, 0.49 mmol), and TFA : CH_2Cl_2 (1 : 1, 2 mL), compound **10c** was obtained quantitatively as a foam. IR ($CHCl_3$): ν_{max} (cm^{-1}) 3409, 1775, 1679, 1519, 1347; ^{1}H NMR: *δ* 1.86–2.09 (m, 3H, H-5, H-4), 2.27–2.34 (m, 1H, H-4′), 3.39–3.57 (m, 2H, H-6), 3.95 (ddd, *J* = 11.7 Hz, *J* = 6.3 and 2.7 Hz, 1H, H-3), 4.07 (d, *J* = 16.5 Hz, 1H, N(C*H*$_2$)CO), 4.18 (d, *J* = 16.2 Hz, 1H, N(C*H*$_2$′)CO), 4.52 (dd, *J* = 15.9 Hz, 2H, NHC*H*$_2$-C$_6$H$_4$(NO$_2$)), 7.53 (d, *J* = 9 Hz, 2H, C$_6$H$_4$(NO$_2$)-*o*), 8.17 (d, *J* = 9 Hz, 2H, C$_6$H$_4$(NO$_2$)-*m*); ^{13}C NMR: *δ* 21.5, 26.5, 43.5, 50.4, 51.1, 51.3, 124.5, 129.2, 147.6, 148.4, 168.1, 170.6. *m/z* (EI-MS): 307 (MH^+, 3%), 289 (10), 261 (20), 99 (30), 70 (100), 56 (51). $C_{12}H_{18}N_4O_4\cdot CF_3COOH$ requires: C, 45.72%; H, 4.56%; N, 13.33%. Found: C, 45.62%; H, 4.41%; N, 13.21%.

***cyclo*-Orn-Gly-Arg-OMe (6a).** To a solution of **10a** (120 mg, 0.25 mmol) in MeOH (10 mL), Pd black (5%) and concentrated HCl (37%, 0.1 mL) were added. The mixture was hydrogenated at rt until disappearance of the substrate (tlc monitoring). The mixture was filtered through Celite® and the filtrate was evaporated. The residue was washed with Et_2O to yield compound **6a** as a foam (59%). IR ($CHCl_3$): ν_{max} (cm^{-1}) 3389, 1681, 1650, 1633; ^{1}H NMR (CD_3OD): *δ* 1.68 (br s, 2H, H-β), 1.80 (br s, 1H, H-4), 1.95 (br s, 2H, H-γ), 2.07 (br s, 2H, H-5), 2.32 (br s, 1H, H-4′), 3.22 (br s, 2H, H-δ), 3.29 (br s, 2H, H-6), 3.72 (s, 3H, CO$_2$C*H*$_3$), 3.98 (br s, 1H, H-3), 4.07 (br s, 1H, N(C*H*$_2$)CO), 4.22 (br s, 1H, N(C*H*$_2$′)CO), 4.44 (br s, 1H, H-α); ^{13}C NMR (CD_3OD): *δ* 20.9, 25.5, 25.8, 28.5, 41.2, 49.6, 50.2, 50.7, 52.4, 52.5, 157.4, 167.1, 169.5, 172.4. *m/z* (ES+): 343 (MH^+), 165 (100%). $C_{14}H_{26}N_6O_4\cdot 2HCl$ requires: C, 44.15%; H, 7.67%; N, 22.07%. Found: C, 44.00%; H, 7.83%; N, 21.59%.

***cyclo*-Orn-Gly-*cyclo*-Arg (6b).** Operating as above, from compound **10b** (70 mg, 0.15 mmol) in MeOH (10 mL), Pd-black (5%) and concentrated HCl (37%, 0.1 mL) compound **6b** was obtained quantitatively as a foam. IR ($CHCl_3$): ν_{max} (cm^{-1}) 3584, 1731, 1693, 1650; ^{1}H NMR (CD_3OD): *δ* 2.00 (br s, 1H, H-4), 2.07 (br s, 6H, H-β, H-γ, H-5), 2.21 (br s, 1H, H-4), 2.32 (br s, 1H, H-4′), 3.44–3.49 (m, 2H, H-δ), 3.81 (br s, 2H, H-6), 3.97 (br s, 1H, H-3), 4.16 (s, 2H, N(C*H*$_2$)CO), 4.62 (br s, 1H, H-α); ^{13}C NMR (CD_3OD): *δ* 20.4, 20.6, 25.5, 25.7, 49.0, 49.7, 50.4, 51.0, 51.8, 157.9, 166.9, 169.3, 173.1; *m/z* (ES+): 311.4 (MH^+), 133 (100%). $C_{13}H_{22}N_6O_3\cdot 2HCl$ requires: C, 44.89%; H, 6.95%; N, 24.16%. Found: C, 44.45%; H, 6.63%; N, 24.01%

***p*-Aminobenzylamide 6c.** Operating as above, from compound **10c** (75 mg, 0.17 mmol), MeOH (10 mL) and 10% Pd/C (5%), compound **6c** was obtained quantitatively as a foam. IR (NaCl): ν_{max} (cm^{-1}) 3318, 3069, 1668; ^{1}H NMR (CD_3OD): *δ* 1.85–2.04 (m, 3H, H-5, H-4), 2.22–2.30 (m, 1H, H-4′), 3.35–3.51 (m, 2H, H-6), 3.92 (dd, *J* = 10 and 6.4 Hz, 1H, H-3), 3.98 (d, *J* = 16.4 Hz, 1H, N(C*H*$_2$)CO), 4.15 (d, *J* = 16.4 Hz, 1H, N(C*H*$_2$′)CO), 4.46 (dd, *J* = 14.8 and 14.4 Hz, 2H, NHC*H*$_2$′-C$_6$H$_4$(NH$_2$)), 7.34 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$(NH$_2$)-*o*), 7.45 (d, *J* = 8.7 Hz, 2H, C$_6$H$_4$(NH$_2$)-*m*); ^{13}C NMR (CD_3OD): *δ* 21.4, 26.5, 43.4, 50.3, 51.0, 51.3, 124.0, 130.0, 131.2, 140.8, 168.1, 170.4. *m/z* (EI-MS): 276 (M^+, 9%), 121 (85), 106 (100), 70 (91).

$C_{14}H_{20}N_4O_2{\cdot}CF_3COOH$ requires: C, 49.23%; H, 5.42%; N, 14.35%. Found: C, 49.13%; H, 5.15%; N, 14.11%.

***p*-Nitrobenzylpiperidin-2-one 11a.** To a solution of Boc-*cyclo*-Orn[12] (1 g, 4.67 mmol) in dry THF (30 mL) cooled at 0 °C, LiHMDS (1 M in THF, 5.6 mL, 5.6 mmol) was added under Ar atmosphere. The solution was stirred for 1 h. *p*-Nitrobenzylbromide (2.02 g, 9.34 mmol) was then added to the solution, and the mixture was stirred overnight at rt. The THF was evaporated, the residue was dissolved in CH_2Cl_2 and washed with 2 M aqueous $NaHSO_4$. The organic phase was dried and evaporated, to yield an oil which was flash chromatographed (hexane : AcOEt, 7 : 3 to 0 : 1) to obtain compound **11a** (86%), as a foam. IR ($CHCl_3$): ν_{max} (cm^{-1}) 1712, 1648 cm^{-1}; ^1H NMR: δ 1.45 (s, 9H, $C(CH_3)_3$), 1.68 (ddd, J = 12.6, 8.4, and 3.6 Hz, 1H, H-4), 1,92 (dddd, J = 13.8, 8.7, 3.9 and 2.4 Hz, 2H, H-5), 2.48 (ddd, J = 12.6, 10.2, and 4.2 Hz, 1H, H-4′), 3.29 (td, J = 6.3 and 1.2 Hz, 2H, H-6), 4.09 (dt, J = 11.7 and 5.7 Hz, 1H, H-3), 4.68 (s, 2H, $CH_2C_6H_4(NO_2)$), 5.09 (d, J = 3.9 Hz, 1H, N*H*Boc), 7.42 (d, J = 9 Hz, 2H, $C_6H_4(NO_2)$-*o*), 8.18 (d, J = 9 Hz, 2H, $C_6H_4(NO_2)$-*m*); ^{13}C NMR: δ 20.9, 27.8, 28.3, 47.4, 50.3, 52.0, 79.7, 123.8, 128.5, 144.2, 148.3, 155.8, 170.0. *m*/*z* (EI-MS): 350 (MH^+), 293 (15), 204 (37), 57 (100). $C_{17}H_{23}N_3O_5$ requires: C, 58.44%; H, 6.64%; N, 12.03%. Found: C, 58.71%; H, 6.73%; N, 11.71%.

***p*-Cyanobenzyl lactam 11b.** Operating as above, from Boc-*cyclo*-Orn[12] (1 g, 4.67 mmol), LiHDMS (1 M in THF, 5.6 mL, 5.6 mmol), and *p*-cyanobenzylbromide (1830 mg, 9.34 mmol) in THF (30 mL), an oil was obtained which was flash chromatographed (hexane : AcOEt, 7 : 3 to 0 : 1) to yield compound **11b** (84%) as a foam. IR (NaCl): ν_{max} (cm^{-1}) 1712, 1648; ^1H NMR: δ 1.46 (s, 9H, $C(CH_3)_3$), 1.66 (ddd, J = 12, 8.1, and 4.2 Hz, 1H, H-4), 1.90 (dddd, J = 14.4, 8.4, 4.5 and 2.1 Hz 2H, H-5), 2.48 (ddd, J = 12.6, 9.9, and 4.5 Hz, 1H, H-4′), 3.29 (td, J = 6.3 and 2.1 Hz, 2H, H-6), 4.08 (dt, J = 11.7 and 5.4 Hz, 1H, H-3), 4.59 (d, J = 16.5 Hz, 1H, $CH_2C_6H_4(CN)$), 4.66 (d, J = 15.3, 1H, $CH_2'C_6H_4(CN)$), 5.50 (br s, 1H, N*H*Boc), 7.36 (d, J = 8.1 Hz, 2H, $C_6H_4(CN)$-*o*), 7.62 (d, J = 8.1 Hz, 2H, $C_6H_4(CN)$-*m*); ^{13}C NMR: δ 20.9, 27.8, 28.3, 47.3, 50.5, 52.0, 79.7, 111.4, 118.5, 128.4, 132.4, 142.2, 155.8, 169.9. *m*/*z* (EI-MS): 330 (MH^+, 2%), 184 (42), 116 (57), 57 (100). $C_{18}H_{23}N_3O_3$ requires: C, 65.63%; H, 7.04%; N, 12.76%. Found: C, 65.14 %; H, 7.06%; N, 12.31%.

***p*-Nitrobenzylpiperidin-2-one 12a.** Operating as for the preparation of compound **10a**, from piperidone **11a** (200 mg, 0.57 mmol), CH_2Cl_2 : TFA (1 : 1, 2 mL), an oil was obtained which was washed with Et_2O to yield quantitatively compound **12a** as a foam. IR ($CHCl_3$): ν_{max} (cm^{-1}) 2924, 1652, 1519, 1343; ^1H NMR (CD_3OD): δ 1.96 (2dd, dd, J = 12 and 5.1 Hz, 1H, H-4), 2.04–2.18 (m, 2H, H-5), 2.41 (dddd, J = 12, 5.2, 4.2, and 2.4 Hz, 1H, H-4′), 3.48 (dd, J = 7.5 and 4.5 Hz, 2H, H-6), 4.11 (dd, J = 11.7 and 6 Hz, 1H, H-3), 4.78 (d, J = 15.6 Hz, 1H, $CH_2C_6H_4(NO_2)$), 4.87 (d, J = 15,6 Hz, 1H, $CH_2'C_6H_4(NO_2)$), 7.63 (d, J = 8.7 Hz, 2H, $C_6H_4(NO_2)$-*o*), 8.31 (d, J = 8.7 Hz, 2H, $C_6H_4(NO_2)$-*m*); ^{13}C NMR: δ 21.5, 26.5, 49.8, 50.9, 51.4, 124.7, 129.8, 145.4, 148.7, 167.5. *m*/*z* (EI-MS): 250 (MH^+, 3%), 204 (22), 69 (85), 56 (100). $C_{12}H_{15}N_3O_3{\cdot}CF_3COOH$ requires: C, 46.29%; H, 4.44%; N, 11.57%. Found: C, 46.12%; H, 4.33%; N, 11.23%.

***p*-Cyanobenzylpiperidin-2-one 12b.** Operating as for the preparation of compound **10a**, from piperidone **11b** (200 mg, 0.62 mmol), CH_2Cl_2 : TFA (1 : 1, 2 mL), compound **12b** was obtained quantitatively as a foam. IR (NaCl): ν_{max} (cm^{-1}) 3440, 2229, 1696; ^1H NMR (CD_3OD): δ 1.81–1.94 (m, 1H, H-4), 2.96–2.08 (m, 2H, H-5), 2.30 (ddd, J = 11.4, 6.3, and 4.2 Hz, 1H, H-4′), 3.36 (td, J = 4.5, and 1.5 Hz, 2H, H-6), 4.01 (dd, J = 11.4 and 5.7 Hz, 1H, H-3), 4.64 (d, J = 15.3 Hz, 1H, $CH_2C_6H_4(CN)$), 4.72 (d, J = 15.6 Hz, 1H, $CH_2'C_6H_4(CN)$), 7.46 (d, J = 8.4 Hz, 2H, $C_6H_4(CN)$-*o*), 7.70 (d, J = 8.4 Hz, 2H $C_6H_4(CN)$-*m*); ^{13}C NMR (CD_3OD): δ 21.5, 26.4, 49.2, 51.1, 51.4, 112.2, 119.5, 128.4, 129.6, 143.5, 167.7. *m*/*z* (ES+): 230 (MH^+, 100%). $C_{13}H_{15}N_3O{\cdot}CF_3COOH$ requires: C, 52.48%; H, 4.70%; N, 12.24%. Found: C, 52.56 %; H, 4.75 %; N, 11.95 %.

***p*-Aminobenzylpiperidin-2-one d6. Method A** (SS). Operating as for the preparation of compound **6c**, from nitrobenzylpiperidone **12a** (100 mg, 0.27 mmol) and 10% Pd–C (5%) in MeOH (10 mL), compound **6d** was quantitatively obtained as a foam, which was purified by precipitation from Et_2O. **Method B** (SPS). A solution of (*S*)-5-hydroxynorvaline (**17**, 10 equivalents) and DIEA (20 equivalents) in DMF/NMP (1/1) was added to resin **18**, which had previously been suspended in DMF. The reaction was shaken for 7 h under Ar atmosphere. The resin was then drained and rinsed sequentially with DMF (×4), CH_2Cl_2 (×4), MeOH (×4), Et_2O (×4), and dried *in vacuo* to obtain compound **19**. Resin **19** was then suspended in CH_2Cl_2, purged with Ar and cooled to 0 °C. MsCl (20 equivalents) and pyridine (20 equivalents) were added and the reaction was shaken at 0 °C for 1 h, and at rt for an additional 24 h. The mesylated resin was drained and washed with the above solvent sequence. For the alkylation reaction the resin was suspended in NMP, purged with Ar and heated to 75 °C. 4-Aminobenzylamine (20 equivalents) was added and the reaction was gently stirred at 75 °C for 2 days. The resin was then drained and washed as described before to obtain compound **20**. Resin **20** was suspended in DMF, purged with Ar and cooled to 0 °C. DIPCDI (10 equivalents) was added and the mixture was shaken at 0 °C for 1 h before the addition of HOBt (10 equivalents). The coupling was carried out for 24 h. After draining and washing, resin **21** was subjected to cleavage with a mixture of TFA/CH_2Cl_2 (1/1) for 5 h. Compound **6d** was isolated by precipitation from Et_2O after evaporation of the filtrates. Purity of the cleaved compound determined by HPLC (see general procedure) was 75%. **Piperidone 6d** IR (NaCl): ν_{max} (cm^{-1}) 3441, 2924, 1677; ^1H NMR (CD_3OD): δ 1.79–1.91 (m, 1H, H-4), 1.95–2.06 (m, 2H, H-5), 2.29 (ddd, J = 11.7, 6.3, and 4.2 Hz, 1H, H-4′), 3.34 (dd, J = 6.3 and 4.2 Hz, 2H, H-6), 3.98 (dd, J = 12 and 6.3 Hz, 1H, H-3), 4.59 (d, J = 15 Hz, 1H, $CH_2C_6H_4(NH_2)$), 4.68 (d, J = 15 Hz, 1H, $CH_2'C_6H_4(NH_2)$), 7.35 (d, J = 8.4 Hz, 2H, $C_6H_4(NH_2)$-*o*), 7.45 (d, J = 8.4 Hz, 2H, $C_6H_4(NH_2)$-*m*); ^{13}C-NMR (CD_3OD): δ 21.5, 26.5, 49.8, 50.9, 51.4, 124.7, 130.6, 131.5, 138.7, 167.5. *m*/*z* (EI-MS): 219 (M^+, 1%), 106 (100), 69 (67), 51 (53). $C_{12}H_{17}N_3O{\cdot}CF_3CO_2H$ requires: C, 50.45%; H, 5.44%; N, 12.61%. Found: C, 50.36%; H, 5.40%; N, 12.35%. The enantiomerically pure sample obtained by SPS showed $[a]_D$ −16 (c = 1, CH_3OH).

***p*-Aminomethylbenzylpiperidin-2-one 6e. Method A** (SS). Operating as for the preparation of compound **6a**, from cyanobenzylpiperidone **12b** (145 mg, 0.44 mmol), 10% Pd–C (5%), concentrated HCl (37%, 0.1 mL), and MeOH (5 mL), compound **6e** was quantitatively obtained as an foam. **Method B** (SPS). A solution of *p*-(hydroxymethyl)benzylamine **23** (10 equivalents) in dry DMF was added to resin **18**, which had been previously suspended in DMF and purged with Ar. The reaction mixture was shaken for 24 h, and the resin was then drained and washed sequentially with DMF (×4), CH_2Cl_2 (×4), MeOH (×4), Et_2O (×4), to obtain compound **24**. Resin **24** was suspended in CH_2Cl_2, purged with Ar and cooled to 0 °C. MsCl (20 equivalents) and pyridine (20 equivalents) were added, and the reaction was gently stirred at 0 °C for 1 h, and at rt for additional 24 h. The resulting support-bound mesylated compound **26** was drained and washed sequentially with the above solvent sequence. In a separate flask, *n*-BuLi (2.5 M in hexanes, 15 equivalents) was added dropwise to a solution of *N*-(4-methoxyphenyl)acetamide (15 equivalents) in dry THF, purged with Ar and cooled to −78 °C. The suspension was stirred for 45 min, and became slightly yellow. DMF was added

to dissolve the precipitate, and the mixture was stirred at −78 °C for another 15 min. This solution was transferred *via cannula* into a solution of Alloc-*cyclo*-Orn[20] (10 equivalents) in dry DMF, under Ar atmosphere, cooled to 0 °C. The reaction was stirred at 0 °C for 2 h, warmed to rt and then transferred *via cannula* to the syringe containing resin **26**, pre-swollen in DMF. The mixture was gently shaken for 2 days and the solid-supported compound **28** was then drained and washed with the usual solvent sequence (see above). The Alloc group was cleaved by treating compound **28** with $PhSiH_3$ (24 equivalents) and $Pd(PPh_3)_4$ (0.1 equivalent), in dry CH_2Cl_2 under Ar (2 × 20 min), and washing with CH_2Cl_2 and dioxane:H_2O (9 : 1). Quantitative deprotection was confirmed by the Kaiser test. Final cleavage from the solid support was effected in TFA : CH_2Cl_2 (1 : 1) for 5 h to yield compound **6e** as the trifluoroacetate salt. This salt was purified by precipitation with Et_2O. **Piperidone 6e**: ^{1}H NMR (CD_3OD): δ 1.92–2.01 (br s, 3H, H-5, H-4), 2.34 (br s, 1H, H-4′), 3.34 (br s, 2H, H-6), 4.01 (br s, 1H, H-3), 4.12 (s, 2H, $C_6H_4(CH_2NH_2)$), 4.59 (d, J = 14.8 Hz, 1H, $CH_2C_6H_4(CH_2NH_2)$), 4.69 (d, J = 14.8, 1H, $CH_2'C_6H_4$-(CH_2NH_2)), 7.38 (d, J = 7.6 Hz, 2H, $C_6H_4(CH_2NH_2)$-*o*), 7.47 (d, J = 7.2 Hz, 2H, $C_6H_4(CH_2NH_2)$-*p*); ^{13}C NMR (CD_3OD): δ 20.4, 25.4, 42.9, 48.5, 49.8, 50.4, 128.6, 129.4, 132.7, 137.7, 166.4; *m*/*z* (ES+): 234 (MH^+), 217 (100%). $C_{13}H_{19}N_3O{\cdot}2HCl$ requires: C, 57.66%; H, 7.85%; N, 15.52%. Found: C, 57.17%; H, 7.80 %; N, 15.35%.

***p*-Aminobenzylamide 13.** Operating as for the preparation of compound **6a**, from piperidone **9c** (250 mg, 0.61 mmol), 10% Pd/C (5%), and MeOH (15 mL), compound **13** was obtained (97%) as a foam. IR (NaCl): ν_{max} (cm^{-1}) 3423, 1697, 1681, 1650; ^{1}H NMR: δ 1.36 (s, 9H, $C(CH_3)_3$), 1.85–1.93 (m, 3H, H-5, H-4), 2.19–2.22 (m, 1H, H-4′), 3.28 (d, J = 11.2 Hz, 1H, H-6), 3.45 (dd, J = 13.6 and 7.2 Hz, 1H, H-6′), 3.62 (d, J = 15.2 Hz, 1H, $N(CH_2')CO$), 3.88 (d, J = 6.4 Hz, 1H, H-3), 4.24 (dd, J = 14.4 and 6 Hz, 1H, $NHCH_2C_6H_4(NH_2)$), 4.33 (dd, J = 14.8 and 6 Hz, 1H, $NHCH_2'C_6H_4(NH_2)$), 4.48 (d, J = 15.6 Hz, 1H, $N(CH_2')$-CO), 4.74 (br s, 2H, $C_6H_4(NH_2)$), 5.74 (d, J = 4.4 Hz, 1H, N*H*-Boc), 6.73 (d, J = 8.4 Hz, 2H, $C_6H_4(NH_2)$-*o*), 7.03 (d, J = 8.4 Hz, 2H, $C_6H_4(NH_2)$-*m*), 7.45 (br s, 1H, $NHCH_2C_6H_4(NH_2)$); ^{13}C NMR: δ 21.0, 27.9, 28.2, 29.5, 42.7, 49.1, 51.6, 79.7, 114.9, 128.0, 128.6, 145.4, 155.8, 167.8, 169.9. *m*/*z* (EI-MS): 376 (M^+, 2%), 161 (36), 121 (100), 106 (77). $C_{19}H_{28}N_4O_4$ requires: C, 60.62%; H, 7.50%; N, 14.88% found: C, 60.81%; H, 7.89%; N, 14.97%.

***p*-Aminobenzylpiperidin-2-one 14.** Operating as for the preparation of compound **6a**, from piperidone **11a** (225 mg, 0.64 mmol), 10% Pd/C (5%), and MeOH (15 mL), compound **14** was obtained (97%) as a foam. IR (NaCl) ν_{max} (cm^{-1}) 3354, 1711, 1647; ^{1}H NMR: 1.45 (s, 9H, $C(CH_3)_3$), 1.55 (ddd, J = 12, 8.7, and 4 Hz, 1H, H-4), 1.76–1.85 (m, 2H, H-5), 2.45 (dt, J = 17.4 and 5.1 Hz, 1H, H-4′), 3.15 (dd, J = 12.6 and 7.2 Hz, 1H, H-6), 3.21 (ddd, J = 12.6, 5.4, and 2.1 Hz, 1H, H-6′), 3.70 (br s, 2H, NH_2), 4.05 (dt, J = 12.3 and 5.4 Hz, 1H, H-3), 4.40 (d, J = 14.4 Hz, 1H, $CH_2C_6H_4(NH_2)$), 4.49 (d, J = 14.1 Hz, 1H, $CH_2'C_6H_4(NH_2)$), 5.58 (br s, 1H, N*H*Boc), 6.62 (d, J = 8.4 Hz, 2H, $C_6H_4(NH_2)$-*o*), 7.03 (d, J = 8.7 Hz, 2H, $C_6H_4(NH_2)$-*m*); ^{13}C NMR: δ 20.6, 27.7, 28.3, 46.0, 50.0, 51.9, 79.4, 115.0, 126.4, 129.3, 145.7, 155.8, 169.3. *m*/*z* (EI-MS): 319 (M^+, 1%), 174 (20), 106 (20), 59 (28). $C_{17}H_{25}N_3O_3$ requires: C, 63.93%; H, 7.89%; N, 13.16% found: C, 63.53%; H, 7.93%; N, 12.85%.

***p*-Guanidinobenzylamide 15.** To a solution of compound **13** (380 mg, 1.01 mmol) in dry CH_2Cl_2 (4 mL) under Ar atmosphere, *N*,*N*′-bis(benzyloxycarbonyl)guanidinium triflate (417 mg, 0.91 mmol) and NMM (0.11 mL, 1.01 mmol) were added. The solution was stirred overnight at rt. The solution was evaporated and the residue was partitioned in AcOEt and 0.1 M aqueous HCl. The organic solution was washed with 10% aqueous Na_2CO_3 and brine, dried and evaporated, to yield an oil which was flash chromatographed (CH_2Cl_2, CH_2Cl_2 : MeOH 98 : 2) to obtain compound **15** (71%). IR (NaCl) ν_{max} (cm^{-1}) 1723, 1720, 1679, 1649, 1636; ^{1}H NMR: δ 1.36 (s, 9H, $C(CH_3)_3$), 1.80–1.97 (m, 3H, H-5, H-4), 2.20 (br s, 1H, H-4′), 3.27 (br s, 1H, H-6), 3.44 (td, J = 11.4 and 4.2 Hz, 1H, H-6′), 3.51 (d, J = 12 Hz, 1H, $NHCH_2CO$), 3.77 (dt, J = 10.2 and 6.6 Hz, 1H, H-3), 4,28 (dd, J = 15 and 5.4 Hz, 1H, $NHCH_2C_6H_4$), 4.49 (dd, J = 15 and 6.6 Hz, 1H, $NHCH_2'C_6H_4$), 4.63 (d, J = 15.6 Hz, 1H, $N(CH_2')CO$), 5.13 (s, 2H, Cbz), 5.23 (s, 2H, Cbz), 5.49 (d, J = 6 Hz, 1H, N*H*Boc), 7.24 (d, J = 7.8 Hz, 2H, C_6H_4-*o*), 7.31 (m, 10H, $(Cbz)_2$), 7.49 (d, J = 8.1 Hz, 2H, C_6H_4-*m*), 10.2 (s, 1H, N*H*Guan), 11.8 (s, 1H, N*H*Guan); ^{13}C NMR: δ 21.1, 28.0, 28.2, 42.6, 49.3, 51.8, 67.3, 68.4, 79.9, 122.6, 127.8, 127.9, 128.1, 128.3, 128.4, 128.6, 128.8, 13.3, 135.1, 135.6, 136.3, 153.5, 153.7, 155.9, 163.6, 168, 169.9. *m*/*z* (ES+): 687 (MH^+, 100%). $C_{36}H_{42}N_6O_8$ requires: C, 62.96%; H, 6.16%; N, 12.24%. found: C, 62.50%; H, 6.05%; N, 11.87%.

***p*-Guanidinobenzylpiperidin-2-one 16.** Operating as above, from compound **15** (470 mg, 1.47 mmol), *N*,*N*′-bis(benzyloxycarbonyl)guanidinium triflate (609 mg, 1.32 mmol), and NMM (0.16 mL, 1.47 mmol), in dry CH_2Cl_2 (8 mL), an oil was obtained which was flash chromatographed (CH_2Cl_2, CH_2Cl_2 : MeOH 98 : 2) to obtain piperidone **16** (75%) as a foam. IR (NaCl) ν_{max} (cm^{-1}) 1717, 1647; ^{1}H NMR: δ 1.45 (s, 9H, $C(CH_3)_3$), 1.59 (td, J = 12 and 3.6 Hz, 1H, H-4), 1.82–1.85 (m, 2H, H-5), 2.47 (dt, J = 12 and 4.8 Hz, 1H, H-4′), 3.13–3.25 (m, 2H, H-6), 4.06 (dt, J = 11.7 and 5.1 Hz, 1H, H-3), 4.48 (d, J = 14.4 Hz, 1H, $CH_2C_6H_4$), 4.56 (d, J = 14.4 Hz, 1H, $CH_2'C_6H_4$), 5.14 (s, 2H, Cbz), 5.23 (s, 2H, Cbz), 5.50 (br s, 1H, N*H*Boc), 7.20 (d, J = 8.4 Hz, 2H, C_6H_4-*o*), 7.32 (m, 10H, $(Cbz)_2$), 7.52 (d, J = 8.4, Hz, 2H, C_6H_4-*m*), 10.25 (s, 1H, N*H*Guan), 11.9 (s, 1H, N*H*Guan); ^{13}C NMR: δ 20.7, 27.8, 28.3, 46.5, 50.0, 51.9, 67.4, 68.5, 79.5, 122.7, 128.0, 128.3, 128.4, 128.7, 128.8, 133.7, 134.3, 135.5, 136.3, 153.5, 153.8, 155.9, 163.7, 169.6. *m*/*z* (ES+): 630 (MH^+, 100%). $C_{34}H_{39}N_5O_7$ requires: C, 64.85%; H, 6.24%; N, 11.12% found: C, 64.37 %; H, 6.32%; N, 10.85%.

***p*-Guanidinobenzylamide 6f.** Operating as for the preparation of compound **6a**, from piperidone **15** (100 mg, 0.15 mmol) and 10% Pd–C (5%) the corresponding Cbz-deprotected **guanidine** was obtained (86%) as a foam. ^{1}H NMR: δ 1.35 (s, 9H, $C(CH_3)_3$), 1.87 (br s, 3H, H-5, H-4), 2.18 (br s, 1H, H-4′), 3.29 (br s, 1H, H-6), 3.44 (br s, 1H, H-6′), 3,67–3.74 (m, 1H, $N(CH_2)CO$), 3.92 (br s, 1H, H-3), 4,28 (br s, 3H, $N(CH_2')CO$, $NHCH_2C_6H_4$), 6.78 (br s, 2H, C_6H_4-*o*), 7.11 (br s, 2H, C_6H_4-*m*), 7.26 (s, 6H, NH_2, N*H*); ^{13}C NMR: δ 21.3, 28.6, 29.9, 42.8, 49.5, 51.6, 79.9, 124.3, 128.8, 156.3, 168,8, 170.8. The Boc group was then cleaved following the protocol described for the preparation of compound **11a**, obtaining an oil which was washed with Et_2O to yield quantitatively piperidone **6f** as a foam. ^{1}H NMR (CD_3OD): δ 1.84 (ddd, J = 16.4, 11.6, and 5.2 Hz, 1H, H-4), 1.95–1.99 (m, 2H, H-5), 2.18 (ddd, J = 12, 6.8, and 4 Hz, 1H, H-4′), 3.20 (td, J = 8.4 and 3.6 Hz, 1H, H-6), 3.42 (td, J = 10.8 and 6.4 Hz, 1H, H-6′), 3.86 (dd, J = 11.6 and 6 Hz, 1H, H-3), 3.98 (d, J = 16.8 Hz, 1H, $N(CH_2)CO$), 4.08 (d, J = 16.4 Hz, 1H, $N(CH_2')CO$), 4.35 (s, 2H, $NHCH_2C_6H_4$), 7.15 (d, J = 8.4 Hz, 2H, C_6H_4-*o*), 7.31 (d, J = 8.4 Hz, 2H, C_6H_4-*m*); ^{13}C NMR (CD_3OD): δ 20.2, 25.3, 42.3, 49.1, 49.9, 50.1, 125.5, 128.8, 133.9, 138.3, 157.0, 167.0, 169.2. *m*/*z* (ES+): 319 (MH^+), 137 (100%). $C_{15}H_{22}N_6O_2{\cdot}CF_3COOH$ requires: C, 47.22%; H, 5.36%; N, 19.44% found: C, 47.02%; H, 5.15%; N, 19.30%

***p*-Guanidinobenzylpiperidin-2-one 6g.** Operating as for the preparation of compound **6a**, from piperidone **16** (100 mg, 0.16 mmol) and 10% Pd–C (5%) the corresponding Cbz-deprotected **guanidine** was obtained (97%) as a foam. ^{1}H NMR: 1.44 (s, 9H, $C(CH_3)_3$), 1.62 (dt, J = 8.8 and 6 Hz, 1H, H-4), 1.85 (br s, 2H,

H-5), 2.38 (br s, 1H, H-4′), 3.24 (br s, 2H, H-6), 4.06 (m, 1H, H-3), 4.43 (d, J = 14.4 Hz, 1H, CH_2C$_6$H$_4$) 4.53 (d, J = 14.4 Hz, 1H, CH_2′C$_6$H$_4$), 6.89 (d, J = 7.6 Hz, 2H, C$_6$H$_4$-*o*), 7.13 (d, J = 7.6 Hz, 2H, C$_6$H$_4$-*m*), 7.26 (s, 4H, *H*NC(N*H*)*NH*$_2$); ^{13}C NMR: δ 21.1, 28.0, 28.6, 47.2, 50.5, 51.9, 79.8, 124.3, 129.3, 132.7, 154.7, 156.3, 170.1. The Boc group was then cleaved following the protocol described for the preparation of compound **11a**, and piperidone **6g** was obtained quantitatively: ^{1}H NMR (CD$_3$OD): δ 1.78 (2dd, J = 12 and 4 Hz, 1H, H-4), 1.87 (dt, J = 8.4 and 2.8 Hz, 1H, H-5), 1.95 (dt, J = 8 and 4 Hz, 1H, H-5′), 2.27 (ddd, J = 11.6, 6, and 3.6 Hz, 1H, H-4′), 3.28 (ddd, J = 7.6, 4, and 1.2 Hz, 2H, H-6), 3.91 (dd, J = 11.6 and 6 Hz, 1H, H-3), 4.48 (d, J = 15.2 Hz, 1H, CH_2C$_6$H$_4$), 4.63 (d, J = 14.8 Hz, 1H, CH_2′C$_6$H$_4$), 7.18 (d, J = 8.4 Hz, 2H, C$_6$H$_4$-*o*), 7.32 (d, J = 8.4 Hz, 2H, C$_6$H$_4$-*m*); ^{13}C NMR (CD$_3$OD): 20.3, 25.3, 47.1, 49.6, 50.3, 125.6, 129.4, 134.4, 136.0, 156.9, 166.4, 166. *m*/*z* (ES+): 262.4 (MH$^+$, 100%). C$_{13}$H$_{19}$N$_5$O·CF$_3$COOH requires: C, 48.00%; H, 5.37%; N, 18.66% found: C, 47.56%; H, 5.11%; N, 18.33%

***p*-Guanidinomethylbenzylpiperidin-2-one 6h.** Resin **22**[17] (1 equivalent) was swollen in DMF, and a solution of 2-chloro-1-methylpyridinium iodide[23] (Mukaiyama's reagent, 10 equivalents) and DIEA (5 equivalents) in dry DMF was added. After gently shaking for 2 h at rt, the resin was rinsed with DMF (×8). The *S*-alkylation process was repeated twice. A solution of *p*-(hydroxymethyl)benzylamine **23** (15 equivalents), 2-chloro-1-methylpyridinium iodide[23] (5 equivalents), and DIEA (10 equivalents) in DMF was added, the resin was shaken at rt for 18 h, and washed with DMF (×5), DCM (×5), MeOH (×5), THF (×5), Et$_2$O (×5), THF (×5), and Et$_2$O (×5). Subsequent mesylation, alkylation, deprotection and cleavage were performed as previously described for compound **6e** from resin **24**. Final cleavage from the solid support was effected in TFA : CH_2Cl_2 (1 : 1) for 5 h to yield compound **6h** as the trifluoroacetate salt, which was purified by precipitation in Et$_2$O. Compound **6h** (85% from resin **22**): ^{1}H NMR (CD$_3$OD): 1.70 (m, 1H), 1.80 (m, 2H), 2.2 (m, 1H), 3.30 (m, 2H), 3.50 (t, J = 13.4 Hz, 1H), 4.10 (m, 2H), 4.25 (m, 2H), 4.5 (d, J = 4.8 Hz, 2H), 5.15 (dd, J = 1.2 and 1 Hz, 1H), 5.28 (dd, J = 1.2 and 1 Hz, 1H), 5.9 (m, 1H), 6.80 (d, J = 7.6 Hz, 2H), 7.35 (d, J = 9.20 Hz, 2H); ^{13}C NMR (CD$_3$OD) 22.1, 28.75, 50, 52, 55, 58, 65.3, 113, 128.5, 130.1, 132.3, 158.1, 162.1, 174.9; ESMS calcd. for C$_{18}$H$_{23}$N$_5$O$_3$ *m*/*z* 357. Found: *m*/*z* 357.4.

General method for the preparation of compounds 7. Coupling of compounds **10a**, **10b**, **10c**, **12a**, **12b**, **deBoc-15**, and **deBoc-16** with the diacids was performed using the protocol reported for the preparation of compound **9a**. The target dimers **7** were obtained by cleavage of the protecting groups following the protocols reported for deprotection of the monomers. Compounds **7** were obtained as a mixture of isomers owing to the epimerisation of the starting Boc-*cyclo*-Orn.[12] Thus, each sample of **7** contained a *meso* form and a pair of enantiomers, as shown by analytical HPLC (2 peaks). The samples were washed with Et$_2$O, but the isomers were not separated. Compounds **7** were identified by ESMS, and by comparison of their spectral data with those of their monomeric precursors. Thus, the NMR spectra showed splitting of the signals corresponding to the respective monomers, and aliphatic signals characteristic of the additional carbon chain of the linkers. The samples were used as such for the bioassays.

Tryptase activity assay and determination of the inhibiting activity of compounds on tryptase. The hydrolytic activity of human lung tryptase (Promega) was measured using the chromogenic substrate Tosyl-Gly-Pro-Lys-*p*-nitroanilide (Tosyl-GPL-pNA, Sigma).[4] Assays were performed in 96 well microtiter plates, in a final volume of 0.1 ml. Tryptase enzyme was diluted to 2.5 μg ml^{-1} with TNT buffer (50 mM Tris-HCl pH 8.2, 100 mM NaCl and 0.05% Tween20) containing 100 μg ml^{-1} heparin, freshly prepared, to stabilize the tryptase. The Tosyl-GPL-pNA was diluted to 2 mM with TNT buffer. One unit of activity was defined as the amount of enzyme which gave a change of 1.0 absorbance units min^{-1} at 405 nm.

The effects of the compounds were evaluated as follows: 25 μl of compound, diluted with TNT buffer containing 10% DMSO, were pre-incubated for 1 hour at room temperature with 50 μl of TNT buffer containing 25 μl of 2.5 μg ml^{-1} human lung tryptase enzyme. The reaction was started by addition of 25 μl of 2 mM Tosyl-GPL-pNA substrate, and absorbance at 405 nm was monitored every 2 min for 10 min using a kinetic microtiter plate reader (CERES UV 900, Bio Tek Instruments, Inc.) at room temperature. Reaction rates were linear during this period (linear regression correlations >99%).

Inhibitory activities were first tested in duplicate at a single dose, 1 μM. The compounds that showed an inhibition effect of about 50% or higher were then evaluated using eight different concentrations in duplicate. IC_{50} values were obtained by non-linear regression using Prism 3.03 software. In all experiments the inhibitory effect of BABIM on tryptase activity was used as a positive control (IC_{50} values in the range of 10–25 nM).

Stock solutions (10^{-2} M) of the tested compounds were prepared in 100% DMSO. Further dilutions were performed in TNT containing 10% DMSO. The same amount of DMSO (10%; 2.5% is the final concentration in the assay) was also included in the control conditions of the assay: basal tryptase activity (without enzyme and compounds) and maximum tryptase activity (with enzyme and without compounds).

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28 We expected to obtain three stereoisomers for compounds **7** prepared from racemic lactams: the racemic *RR* and *SS* forms and the *meso* epimer *RS*. However, we could not detect the diastereomeric mixture by standard methods such as NMR and analytical HPLC. Since the compounds turned out not to be sufficiently active, we did not pursue this issue.