Design, Synthesis, and SAR of Potent and Selective Dipeptide-Derived Inhibitors for Dipeptidyl Peptidases

Kristel Senten,[†] Pieter Van der Veken,[†] Ingrid De Meester,[‡] Anne-Marie Lambeir,[‡] Simon Scharpé,[‡] Achiel Haemers,[†] and Koen Augustyns*,[†]

Department of Medicinal Chemistry and Department of Medical Biochemistry, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Antwerp, Belgium

Received April 24, 2003

In this paper we report the systematic search for new, potent, and selective DPP II inhibitors. A study of the structure−activity relationship was conducted starting from aminoacyl pyrrolidides as lead compounds. Rational exploration of the P_1 and P_2 building blocks led to the discovery of some very potent DPP II inhibitors which can be characterized by their high selectivity for DPP II with regard to DPP IV. Dab-Pip and Dab-Pip-2-CN were selected as the most promising inhibitors (IC_{50} nM range) and will enable us to study the physiological role of DPP II and to differentiate between DPP II and DPP IV in biological systems.

Introduction

Dipeptidyl peptidases (DPPs, EC 3.4.14.*x*) have been identified in various mammalian tissues and catalyze the sequential release of dipeptides from peptides. Among these enzymes, DPP II (EC 3.4.14.2) and DPP IV (EC 3.4.14.5) preferentially release N-terminal dipeptide moieties (Xaa-Pro) at acidic (DPP II) or weakly basic (DPP IV) pH from some oligopeptides or polypeptides. Both enzymes are members of the recently described 'DPP IV activity- and/or structure-homologues' (DASH) proteins, comprising enzymes with a common post-proline-cleaving serine dipeptidase mechanism.[1] A large number of proteins and peptides contain proline at the penultimate position, and due to the unique structure of proline relatively few peptidases are able to cleave the peptide bond after a proline residue. Dipeptidyl peptidases from this family of DASH proteins play an important role in the regulation of the function of these molecules, as is clearly established for DPP IV-mediated degradation of incretin hormones. Although DPP IV and DPP II share proline specificity, they can be functionally and biochemically distinguished. Using a positional scanning synthetic combinatorial dipeptide substrate library, it was shown that both enzymes strongly prefer proline at P_1, but that the next most preferred residue for DPP II is norleucine (Nle), whereas for DPP IV this is alanine. For the P_2 subsite, DPP II has a preference for Lys, Nle, Met, and Ala; whereas DPP IV can tolerate a variety of residues at this position.[2]

DPP II, first identified by McDonald at al.,[3] is believed to be involved in the physiological breakdown of some proline-containing neuropeptides and in the degradation of collagen[4] together with tripeptidyl peptidase I (EC 3.4.14.9). However, its physiological functions remain elusive and the potential therapeutic value of DPP II inhibitors remains unclear. DPP II is generally localized in lysosomes and is found in a number of mammalian tissues and body fluids. Recently it is suggested to be identical to human quiescent cell proline dipeptidase (QPP), based on the significant sequence homology (79.4%) found between human QPP and rat DPP II.[5] Human QPP[6,7] is a 58-kDa glycoprotein functionally active as a homodimer formed with a leucine zipper motif.[8] It has been shown that QPP inhibitors cause apoptosis in quiescent lymphocytes, but not in activated or transformed lymphocytes. This process is believed to be independent of DPP IV, because both DPP IV^+ and DPP IV^- T cells undergo apoptosis.[6] No sequence homology has been found between DPP II/QPP and DPP IV.

DPP IV has been studied extensively over the last three decades and a broad array of diverse functional properties in the immune, nerve, and endocrine system is suggested.[9−13] DPP IV is bound to the cell membrane and expressed quite ubiquitously in mammalian tissues. In the hematopoietic system it was identified as the leukocyte antigen CD26. Inhibition of DPP IV prolongs the lifetime of incretin hormones and is therefore potentially valuable in the treatment of type 2 diabetes. Some DPP IV inhibitors are currently under clinical evaluation in this field.[10,14−16]

Development of highly specific and potent inhibitors of both enzymes will contribute to the unraveling of their physiological functions and will be helpful to differentiate between DPP II and DPP IV in biological systems. More importantly, to assess the therapeutic potential of DPP IV and DPP II inhibitors, selectivity will be an important issue. The resembling substrate specificity and catalytic mechanism make this a challenging task. In contrast to DPP IV,[9,10] only a few DPP II inhibitors are reported. They will be summarized here.

DPP IV and DPP II inhibitors often resemble the dipeptide cleavage product with a proline mimic at the P_1-site. Aminoacyl pyrrolidides (Pyrr) (**1**) and thiazolidides (Thia) (**2**) are known as potent, competitive inhibitors of DPP IV[17] and DPP II (Figure 1). Thiazolidides (**2**) are more effective inhibitors than the corresponding pyrrolidides (**1**) for both enzymes (Table 1).

* To whom correspondence should be addressed. Tel. +32-(0)3-8202703; fax: +32-(0)3-8202739; e-mail: Koen.Augustyns@ua.ac.be.
[†] Department of Medicinal Chemistry.
[‡] Department of Medical Biochemistry.

10.1021/jm0308803 CCC: $25.00
Published on Web 09/17/2003

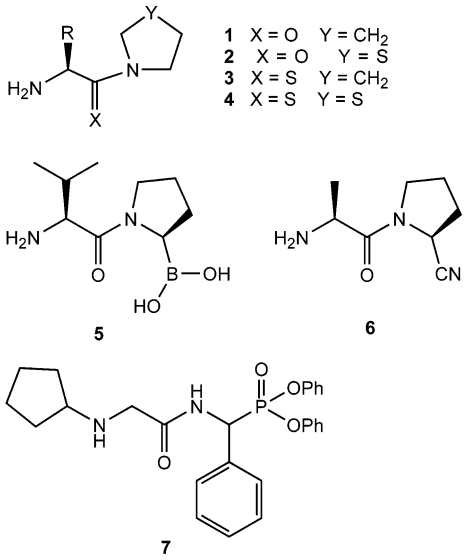


Figure 1. Examples of reported dipeptidyl peptidase inhibitors.

Table 1. Inhibitory Activity of DPP II Inhibitors Reported in Literature

compounds	DPP II/ QPP inhibition	DPP IV inhibition	SI[a]
1,[b] P_2 = Ile	K_i = 24.7 μM	K_i = 0.218 μM	0.0088
2,[b] P_2 = Ile	K_i = 8.17 μM	K_i = 0.126 μM	0.015
3,[b] P_2 = Ala	K_i = 1.43 μM	K_i = 47.6 μM	33
4,[b] P_2 = Ala	K_i = 0.277 μM	K_i = 7.88 μM	28
5[c]	K_i = 125 nM	K_i = 2 nM	0.016
6[d]	IC_{50} = 110 μM	K_i = 0.2 μM	
7[e]	IC_{50} = 3.8 μM	IC_{50} > 125 μM	>33

[a] SI = selectivity index = value for DPP IV divided by value for DPP II. [b] Values taken from ref 18. [c] Values taken from ref 6. [d] Values taken from ref 20. [e] Values taken from ref 24.

From a series with Ala, Phe, Val, Ile at P_2, Ile-Thia (**2**, P_2 = Ile) is the most potent DPP IV inhibitor, while Ile-Pyrr (**1**, P_2 = Ile) is the most selective DPP IV inhibitor with respect to DPP II.[18] Introduction of a thioamide bond results in thioxo amino acid pyrrolidides (**3**) and thiazolidides (**4**).[18] Thioxylation increased DPP II inhibition up to 10 times, whereas thioamides are 20 times less efficient at inhibition of DPP IV than the corresponding amides.[18] Thioamide analogues such as Alaψ-[CS−N]-Pyrr (**3**, P_2 = Ala) and Alaψ[CS−N]-Thia (**4**, P_2 = Ala) are the only DPP II-DPP IV inhibitors described in this series to have some selectivity toward DPP II.[18]

The boronic acid dipeptide analogue Val-boroPro (**5**), used to inhibit QPP in the apoptosis studies,[6] is in fact a more effective inhibitor for DPP IV (Table 1).

Substitution with a nitrile group in **1** or **2** at positions 2 and 4 affords competitive DPP IV inhibitors with approximately a 1000-fold increase in potency compared to the parent compounds.[19−21] Ala-Pyrr-2-CN (**6**) was reported[20] as a weak inhibitor for DPP II, but with higher potency toward DPP IV. Depending on the substituent, several nitrogen-substituted Gly-Pyrr-2-CN are even more potent and more selective DPP IV inhibitors.[14]

Incorporation of an electrophilic phosphonate group on the proline mimic at P_1 affords dipeptide proline diphenyl phosphonates, that are well-known irreversible inhibitors of DPP IV, with good selectivity over DPP II and other enzymes.[22,23] Recently, our laboratory reported a series of dipeptide α-aminoalkyl diphenyl phosphonates in which various P_1 diphenyl phosphonate building blocks were combined with commercially available or easily accessible amino acids. These compounds were used for the rapid profiling of DPP II. *N*-(Cyclopentyl)glycyl-NHCH(C_6H_5)PO(OPh)$_2$ (**7**) was the most selective and potent DPP II inhibitor in these series.[24]

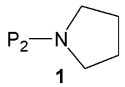
In this paper we report the systematic search for new, potent, and selective DPP II inhibitors: the structrure−activity relationship of several classes of typical serine protease inhibitors is investigated. Preliminary results were reported earlier.[25] Pyrrolidide **1** was used as lead compound. After selection of an optimal P_2 substitution, the pyrrolidine ring was optimized by substituting for different ring sizes and open amines and by introduction of substituents in position 3. The most interesting compound was further modified (thioamide; 2-carbonitrile), and an optimal compound considering potency and selectivity was selected.

Results and Discussion

Chemistry. Most compounds were prepared by parallel synthesis. Commercially available amino acids with *tert*-butyloxycarbonyl (Boc) as α-amino protection and acid-labile side-chain protecting groups were coupled with the appropriate amine using a polymer-assisted solution-phase procedure as reported earlier.[26] In this two-step synthesis we were able to produce a large number of compounds without difficult purification steps. Some compounds, however, were purified by preparative TLC before final deprotection in order to ensure the 95% purity needed for biological evaluation. Compounds **10.3**−**10.5** were prepared by thioxylation using Lawesson's reagent after the parallel synthesis of the corresponding protected amides.[18] Inhibitory activities for DPP IV of pyrrolidide analogues with different ring sizes, open ring structures, and other homologues have been reported earlier.[27] These compounds (**8**) were now also evaluated for their DPP II inhibitory capacity. The 3-substituted pyrrolidides were obtained by coupling of Boc protected amino acid (Ile or Lys) to 3-hydroxypyrrolidine. Various reactions at the hydroxyl function and cleavage of the protecting group resulted in the 3-substituted pyrrolidides.[27] An azide (**8.13**, **8.14**) was obtained from a tosylate intermediate. Treatment with benzoyl chloride afforded the benzoate (**8.15**, **8.16**). Fluorine (**8.17**) was introduced with diethylaminosulfur trifluoride (DAST). The dipeptide nitriles (**11**, **12**) were obtained by coupling of the required Boc protected amino acid with respectively 2-pyrrolidinecarboxamide or 2-piperidinecarboxamide, followed by dehydration of the primary amide function to the nitrile using phosphorus oxychloride[19] and subsequent acid-catalyzed deprotection.

Biochemical Evaluation. To establish an optimized N-terminal amino acid (P_2) for DPP II inhibition, we prepared a series of pyrrolidides (**1**). IC_{50} values for DPP II and DPP IV inhibition of compounds **1** are summarized in Table 2. A selectivity index is given as a means to evaluate the selectivity for DPP II with respect to DPP IV. Lys-Pyrr (**1.8** IC_{50} = 9.9 μM) and His-Pyrr (**1.6** IC_{50} = 1.16 μM) are the most active DPP II inhibitors in this series and are also the most selective for DPP II with respect to DPP IV. From this set of pyrrolidides (**1**) we conclude that basic (His (**1.6**), Lys,

Table 2. Inhibitory Activities and Selectivity Index of Pyrrolidides (**1**)



1

1.*x*	P_2	DPP II inhibition IC_{50} (μM)	DPP IV inhibition IC_{50} (μM)	SI[a]
1.1	Ala	179 ± 15	41 ± 6	0.2
1.2	Asn	152 ± 50	188 ± 6	1.2
1.3	Asp	>500	122 ± 2	<0.2
1.4	Cha[b]	42 ± 3	17 ± 2	0.4
1.5	Gly	>1000	>1000	1
1.6	His	1.2 ± 0.1	23.1 ± 1.0	20
1.7	Ile	110 ± 7	4 ± 1	0.04
1.8	Lys	9.9 ± 1.3	39 ± 2	3.9
1.9	Ser	65 ± 30	190 ± 120	2.9
1.10	Phe	79 ± 29	21 ± 4	0.3
1.11	Pro	>500	15 ± 2	<0.03
1.12	Thiapro	>500	>500	1
1.13	Tyr	150 ± 24	14 ± 2.4	0.09
1.14	Val	223 ± 13	4 ± 0.4	0.02

[a] SI = selectivity index = IC_{50} value for DPP IV divided by IC_{50} value for DPP II. [b] Cha = cyclohexylalanine.

(**1.8**)) amino acids are preferred above neutral amino acids at P_2. Acidic amino acids (Asp (**1.3**)), imino acids (Pro (**1.11**), Thiapro (**1.12**)) and nonsubstituted amino acids (Gly (**1.5**)) at this position are not favorable. This is in agreement with the reported substrate specificity[28] and more or less also compares with the results of a positional scanning synthetic combinatorial dipeptide substrate library (Xaa-Xaa-AMC)[2]. The results for DPP IV illustrate that more residues are tolerated at the S_2 subsite[2], confirming the preference for lipophilic, β-branched-α-amino acids (Ile (**1.7**), Val (**1.14**)).[19]

In a search for an optimized C-terminal residue (P_1), we replaced the pyrrolidine ring by several four- to seven-membered ring structures, acyclic amines, and 3-substituted pyrrolidines. Results are summarized in Table 3. Ile, previously reported and here confirmed (Table 2) to afford potent DPP IV inhibition, was used as a standard P_2 amino acid. Lys, identified as a good P_2 amino acid for DPP II inhibition in the pyrrolidide series, was used in combination with the most promising P_1 building blocks. The results of compounds **8** were compared with Ile-Pyrr (**1.7**) and Lys-Pyrr (**1.8**).

Compared to pyrrolidine, cyclopentylamine (**8.1**), pyrroline (**8.2**), azetidine (**8.9**, **8.10**), and acyclic amines (**8.18**−**8.21**) reduce potency considerably on both enzymes. As already pointed out in the Introduction, thiazolidides have been reported to give an increase in both DPP II and DPP IV inhibition compared to the corresponding pyrrolidides. Ile-Thia (**2.1**) is more active on both enzymes, keeping selectivity index to about the same value. In our hands, thiazolidide **2.2** gave unexpected results: Lys-Thia (**2.2**) is 3 times more active as DPP II inhibitor, but less active against DPP IV, increasing the selectivity for DPP II compared to Lys-Pyrr (**1.8**). Therefore the increase in potency of thiazolidides may not be generalized.

Replacing the pyrrolidine ring by piperidine in **8.3** and **8.4**, we observe a 2- to 6-fold improvement in the DPP II inhibition. As reported earlier,[25,27] this six-membered ring gives a substantial decrease in potency for DPP IV inhibitors. Introduction of piperidine at P_1 is therefore a good way of enhancing selectivity for DPP II over DPP IV. Introducing a tetrahydropyridine in the P_1-position decreases the DPP IV inhibition as reported.[27] Compared to pyrrolidine as P_1, tetrahydropyridine improves DPP II inhibition with Ile (**8.5**) in P_2; no influence is seen with Lys (**8.6**). Increasing the ring size further to a seven-membered ring decreased both DPP II and DPP IV inhibition.

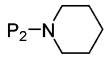
For the 3-substituted pyrrolidides, a 2-fold increase of DPP II inhibition is seen for the azide substituent (**8.13** and **8.14**). Lys-Pyrr-3-N_3 (**8.14**) has an IC_{50} of 4.9 μM and shows an enhanced selectivity for DPP II with regard to DPP IV compared to Lys-Pyrr (**1.8**). With hydroxy and benzoyloxy the DPP II inhibition decreases,

Table 3. P_1 Optimization: Inhibitory Activities and Selectivity Index

8.	P_2	P_1	DPP II inhibition IC_{50} (μM)	DPP IV inhibition IC_{50} (μM)	SI[a]
1.7	Ile		110 ± 7	4 ± 1	0.04
1.8	Lys		9.9 ± 1.3	39 ± 2	3.9
8.1	Ile		500	> 1000	> 2
8.2	Ile		288 ± 33	no data	
2.1	Ile		28 ± 9	1.7 ± 0.1	0.06
2.2	Lys		3 ± 0.3	318 ± 25	106
8.3	Ile		59 ± 15	67 ± 11	1.1
8.4	Lys		1.6 ± 0.3	247 ± 20	154
8.5	Ile		52 ± 3	52 ± 3	1
8.6	Lys		10.9 ± 1.0	> 500	> 46
8.7	Ile		228 ± 16	374 ± 62	2
8.8	Lys		51.9 ± 8.4	> 1000	19
8.9	Ile		730 ± 140	50.4 ± 2.3	0.07
8.10	Lys		159 ± 16	500 ± 25	3
8.11	Ile		299 ± 2	93 ± 6	0.3
8.12	Lys		48 ± 3	500 ± 25	10.4
8.13	Ile		43 ± 5	107 ± 6	2.2
8.14	Lys		4.9 ± 0.5	> 1000	> 205
8.15	Ile		158 ± 11	> 500	> 3.2
8.16	Lys		78.1 ± 7.8	> 1000	> 12.8
8.17	Ile		111 ± 10	3.5 ± 0.2	0.03
8.18	Ile		> 1000	360 ± 18	< 0.4
8.19	Ile		> 500	167 ± 13	< 0.3
8.20	Ile		> 500	> 500	1
8.21	Ile		213 ± 10	377 ± 16	1.4

[a] SI = selectivity index = IC_{50} value for DPP IV divided by IC_{50} value for DPP II.

Table 4. Inhibitory Activities and Selectivity Index of Piperidides (**9**)



9

9.*x*	P_2	DPP II inhibition IC_{50} (μM)	DPP IV inhibition IC_{50} (μM)	SI[a]
9.1	Arg	0.63 ± 0.11	18.1 ± 0.5	29
9.2	Cha[b]	9.9 ± 0.9	217 ± 13	22
9.3	His	0.33 ± 0.06	213 ± 42	704
8.3	Ile	62 ± 27	67 ± 11	1.1
9.4	Ser	21.7 ± 0.8	>1000	46
8.4	Lys	1.6 ± 0.3	247 ± 20	154
9.5	Lys(Z)	2.1 ± 0.2	134.9 ± 1.2	64
9.6	Orn	0.45 ± 0.08	>500	>1111
9.7	Dab[c]	0.13 ± 0.01	>1000	>7592
9.8	D-Dab	130 ± 5	≫1000	≫8
9.9	Dab(Z)	1.15 ± 0.08	500 ± 25	435
9.10	Z-Dab	191 +	>1000	
9.11	Dap[d]	1.84 ± 0.13	>1000	>544
9.12	Abu[e]	88.7 ± 7.3	250 ± 25	2.8
9.13	Nva[f]	57.1 ± 3.9	229 ± 44	4
9.14	Nle[g]	32.4 ± 2.7	250 ± 25	8

[a] SI = selectivity index = IC_{50} value for DPP IV divided by IC_{50} value for DPP II. [b] Cha = cyclohexylalanine. [c] Dab = 2,4-diaminobutyric acid. [d] Dap = 2,3-diaminopropionic acid. [e] Abu = 2-aminobutyric acid. [f] Nva = norvaline. [g] Nle = norleucine.

but the DPP IV inhibition is even more decreased. With a small substituent such as fluorine (**8.17**), the inhibition of both enzymes is not affected.

Lys-Piperidide (**8.4**) is the most potent DPP II-inhibitor in this series exhibiting an IC_{50} of 1.6 μM and a selectivity index of 154 for DPP II versus DPP IV.

Conclusively, series **8** show that S_1 specificity of DPP II is less stringent than in the case of DPP IV. This is in agreement with recent studies of the substrate specificity, in which substrates with norleucine in P_1 instead of proline are also recognized.[2] A combination of Lys at P_2 with thiazolidine, 3-azidopyrrolidine and especially piperidine considerably enhances potency and selectivity compared to pyrrolidine. These results are clearly in disagreement with the results of Leiting et al. where Lys-Thia and Lys-Pip were equipotent against both enzymes.[2] Tetrahydropyridine, hexamethyleneimine, and 3-hydroxy- and 3-benzoyloxypyrrolidine have lower affinity for the S_1 site of both DPP II and DPP IV, but they are better accepted by DPP II compared to DPP IV. Our diphenyl phosphonate inhibitor **7** seems to indicate that phenyl can also be accommodated in the S_1 site of DPP II. Considering these results, we assume that the S_1 site of DPP II is somewhat larger than the one of DPP IV. Indeed, the crystal structure of DPP IV shows a hydrophobic pocket that is optimally suited to fit five-membered rings.[29]

Recognizing the importance of the piperidine ring, a broad series of aminoacyl piperidides (Pip) (**9**) with basic and neutral amino acids at P_2 were synthesized. Results are summarized in Table 4. The significance of this piperidine ring is again confirmed: compared to the pyrrolidide series (**1**), an increase in DPP II inhibition up to 6 times is observed, whereas inhibition of DPP IV decreased simultaneously with a factor between 6 and 17. Therefore, changing pyrrolidine to piperidine results in a considerable increase in potency and selectivity for DPP II. With basic amino acids (Arg (**9.1**), His (**9.3**), and Lys (**8.4**)), high DPP II inhibitory activities are observed.

The side chain length ($(CH_2)_nNH_2$) in Lys-Pip was investigated by replacing the P_2 amino acid lysine (n = 4) (**8.4**) with respectively ornithine (n = 3) (**9.6**), 2,4-diaminobutyric acid (n = 2) (**9.7**), and 2,3-diaminopropionic acid (n = 1) (**9.11**). Decreasing the side chain length to n = 2 enhanced the DPP II inhibitory potency. Further decrease of the side chain revealed a reduction in potency (**9.11**, n = 1). Also selectivity was significantly improved since inhibition of DPP IV declined tremendously with decreasing side chain length. Dab-Pip (**9.7**) with an IC_{50} = 0.13 μM and a selectivity index of more than 7000 is the most active and most selective DPP II inhibitor in this series.

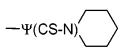
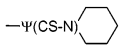
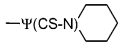
Blocking the side chain amino function in Lys-Pip and Dab-Pip with benzyloxycarbonyl (**9.5**, **9.9**, respectively) afforded for Dab(Z)-Pip (**9.9**) a decrease in potency up to 9 times, whereas selectivity was reduced by a factor 17. The significance of this unprotected side chain amino function is also revealed in compounds **9.12** to **9.14** where the amino function is omitted: the DPP II inhibition is decreased tremendously, and compounds show no selectivity at all toward DPP IV. The highest potency here is observed with norleucine (**9.14**, n = 3, IC_{50} = 32.4 μM) and decreases slightly with decreasing chain length ($(CH_2)_nCH_3$). This does not compare with Nle-Pro-AMC being a substrate with a lower K_m value than Lys-Pro-AMC.[2] From these results we learn that basic amino acids with a certain side chain length at P_2 are preferable for potent and selective DPP II inhibition. A 1000-fold decrease in potency of D-Dab-Pip (**9.8**) confirms the importance of the L-configuration of the P_2-amino acid for DPP II inhibition. A similar result is published for DPP IV inhibitors.[9] Like for DPP IV, the low potency of Z-Dab-Pip (**9.10**) shows the importance of a free α-aminofunction and proves that DPP II is an exopeptidase.

Having identified Dab-Pip (**9.7**) as the most potent and selective inhibitor so far, some analogues were prepared by replacing the piperidine ring (Table 5). The DPP II inhibitory activity is declined tremendously for Dab-piperazide (**10.2**), whereas Dab-morpholide (**10.1**) can still be considered as a potent DPP II inhibitor.

As reported by Stöckel et al,[18] thioxylation of pyrrolidides improved DPP II inhibition, whereas inhibition of DPP IV was negatively affected. Using this knowledge, we investigated the effect of thioxylating some aminoacyl piperidides. Previous findings are true for compounds **10.3** and **10.4** with respectively lysine and ornithine as P_2 amino acid. Unfortunately, this increase in DPP II inhibition and selectivity is not seen with thioxylation of the more potent Dab-Pip (**10.7**). Dab Ψ-[CS−N]-piperidide (**10.5**) exhibits an IC_{50} = 0.22 μM and is therefore a less active and less selective DPP II inhibitor compared to Dab-Pip (**10.7**).

The thiazolidides (**2.2**−**2.4**) (Table 6) show that these compounds are slightly less active as DPP II inhibitor then the corresponding piperidides (respectively **8.4**, **9.6**, and **9.7**). More importantly, selectivity is significantly decreased due to a more potent DPP IV inhibition. Thiazolidides (**2**) are therefore less selective DPP II inhibitors compared to the corresponding piperidides (**9**).

Table 5. Inhibitory Activities and Selectivity Index of Dab-Pip (**9.7**) Analogues

10.	P_2	P_1	DPP II inhibition IC_{50} (μM)	DPP IV inhibition IC_{50} (μM)	SI[a]
10.1	**Dab**		0.51 ± 0.02	> 1000	> 1961
10.2	**Dab**		29.3 ± 1.7	> 1000	> 34
10.3	**Lys**		0.49 ± 0.02	> 1000	> 2041
10.4	**Orn**		0.2 ± 0.01	> 1000	> 5050
10.5	**Dab**		0.22 ± 0.01	> 1000	> 4484
2.2	**Lys**		3 ± 0.3	318 ± 25	106
2.3	**Orn**		0.75 ± 0.02	81 ± 2	108
2.4	**Dab**		0.14 ± 0.01	289 ± 8	2064

[a] SI = selectivity index = IC_{50} value for DPP IV divided by IC_{50} value for DPP II. [b] Dab = 2,4-diaminobutyric acid.

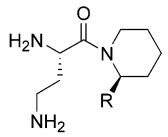
Table 6. Inhibitory Activities and Selectivity Index of Dipeptide Nitriles (**11**, **12**)

	P_2	P_1	DPP II inhibition IC_{50} (μM)	DPP IV inhibition IC_{50} (μM)	SI[a]
11	**Lys**		1.0 ± 0.3	0.32 ± 0.03	0.32
12.1[b]	**Lys**		1.5 ± 0.09	51 ± 2	35
12.2	**Dab**		0.038 ± 0.002	151 ± 5	3987

[a] SI = selectivity index = IC_{50} value for DPP IV divided by IC_{50} value for DPP II. [b] The compound tested was L-Lys-Pip-2(*R,S*)-CN.

Aminoacylpyrrolidide-2-nitriles are slow-binding, reversible inhibitors of DPP IV with approximately a 1000-fold increase in potency compared to the parent aminoacylpyrrolidides (low nM K_i) and a more than 500-fold selectivity against DPP II.[20] However, we investigated some dipeptide nitriles (**11**,**12**) for their DPP II inhibitory activity (Table 6). Introduction of a nitrile on Lys-Pyrr (**1.8**), results in a 10-fold increase in DPP II inhibition and a 100-fold increase in DPP IV inhibition. Hence, Lys-Pyrr-2-CN (**11**) is a nonselective inhibitor of both enzymes. The selectivity could be enhanced by replacing the pyrrolidine ring with piperidine. Compared to **11**, Lys-Pip-2-CN (**12.1**, mixture of diastereomers) only slightly affects DPP II inhibition while inhibitory activity of DPP IV declines with a factor 160. However compared to Lys-Pip (**8.4**), introduction of a nitrile does not improve DPP II inhibitory potency and decreases selectivity 4-fold.

Incorporation of a nitrile in Dab-Pip (**9.7**) enhances inhibition of DPP II with a factor 3. Dab-Pip-2-CN (**12.2**) exhibits an IC_{50} = 38 nM and is the most active DPP II inhibitor in our investigation. However, for **12.2** the selectivity index declines as well and is therefore less selective with respect to DPP IV compared to Dab-Pip (**9.7**).



9.7 R = H
12.2 R = CN

Figure 2. Structures of the most potent and selective DPP II inhibitors.

Conclusively, based on these compounds, introduction of a nitrile in aminoacylpiperidides moderately increases potency for DPP II, but at the same time improves DPP IV inhibition. As a result, aminoacylpiperidide-2-nitriles are very potent DPP II inhibitors. Although their selectivity generally is lower than the corresponding piperidides, they still can be considered to be highly selective for DPP II with respect to DPP IV.

Conclusions

In this structure−activity investigation we were able to identify an optimized N-terminal and C-terminal residue for potent and selective DPP II inhibition. 2,4-Diaminobutyric acid (Dab) was selected as the most promising N-terminal amino acid for the development of potent and selective DPP II inhibitors. A basic side chain amino function proved to be favorable. Omitting this function or neutralizing it by substitution with carbamate decreased DPP II inhibitory potency considerably and more importantly, negatively affects selectivity. Also histidine (His) shows excellent properties at this position. Since His is expected to be almost completely protonated under the assay conditions (pH = 5.5), we assume that a positively charged nitrogen separated by two carbons from the α-amino group represents an optimized situation. Furthermore, the amino acid at this position needs to be in the L-configuration with a free α-amino function.

In search for an optimized P_1 building block piperidine was recognized as being superior over pyrrolidine and thiazolidine. For aminoacylthiazolidides (**2**), the DPP II inhibition is slightly decreased compared to the corresponding piperidides (**9**), but they are regarded as less selective with respect to DPP IV. We showed that S_1 specificity of DPP II is less stringent than in the case of DPP IV. Considering our results, we assume that the S_1 site of DPP II is somewhat larger than the one of DPP IV. The crystal structure of DPP IV shows a hydrophobic pocket that is optimally suited to fit five-membered rings.[29]

On the basis of our compounds, introduction of a nitrile at P_1 only slightly improves DPP II inhibition but results in a decreased selectivity. Unexpectedly, thioxylation of the amide bond does not always result in an increased DPP II inhibition and a decreased DPP IV inhibition.[18]

Dab-Pip (**10.7**) has an IC_{50} of 130 nM and a selectivity index of more than 7000. Incorporation of a nitrile, leading to Dab-Pip-2-CN (**12.2**), increased potency with a factor 3 (Figure 2). Although the selectivity index of this nitrile compound (**12.2**) declined to approximately 4000, it still can be regarded as highly selective for DPP

II. Both compounds are far more active and selective DPP II inhibitors than earlier reported compounds. Especially, the high selectivity of these two compounds (**9.7** and **12.2**) must be emphasized.

Val-Pyrr (**1.14**) and Ile-Thia (**2.1**), identified as the most selective DPP IV inhibitor in our pyrrolidide and thiazolidide series respectively, are extensively used in animal studies to evaluate the effects of DPP IV inhibitors in the treatment of type II diabetes.[10] However, selectivity for DPP IV in comparison with DPP II remains rather limited. One can question the in vivo selectivity for DPP IV and results should be interpreted with caution. In this respect some of the Pro-Pro diaryl phosphonates reported[30] by our group and the *N*-substituted glycylpyrrolidide-2-nitriles,[14] with—depending on the *N*-substituent—very low DPP II inhibitory activity, seem a better choice to selectively inhibit DPP IV.

Our two compounds (**9.7** and **12.2**) should offer the opportunity to study the physiological role of DPP II and possible therapeutic benefits of DPP II inhibition. Their high selectivity will enable to differentiate between DPP II and DPP IV in biological systems. Both compounds will also serve as lead compounds for further development of DPP II inhibitors in our laboratory.

Experimental Section

Materials. Parallel synthesis was performed using the Quest 210 Organic Synthesizer (Argonaut Technologies). Boc-protected amino acids, *N*-cyclohexylcarbodiimide, *N*′-methylpolystyrene resin (PS-carbodiimide), and tris(2-aminoethyl)-amine polystyrene resin were purchased from Novabiochem. Other reagents were obtained from Sigma-Aldrich or Acros. Compounds **8.1**, **8.2**, **8.5**, **8.7**, **8.9**, **8.11**, **8.13**, **8.15**, **8.17**−**8.21** were synthesized as reported earlier.[27]

Analysis. Characterization of all compounds was done with ^{1}H NMR and mass spectrometry. ^{1}H NMR were recorded on a Bruker Avance DRX-400 spectrometer (400 MHz). Fast atom bombardment (FAB^+) mass spectra were obtained on a VG 70-SEQ hybrid mass spectrometer (Micromass, Manchester, UK), equipped with a cesium ion gun. Electrospray (ES^+) mass spectra were acquired on a Autospec-ao-TOF mass spectrometer (Micromass, Manchester, UK) or a triple quadrupole mass spectrometer (Quattro II, Micromass, Manchester, UK) or an ion trapp mass spectrometer (Esquire 3000, Bruker Daltonics). Purity was verified using two diverse HPLC systems using respectively mass and UV-detector. LC-MS were recorded on an Agilent 1100 Series HPLC system using a Discovery Cyano column (2.1 × 50 mm, 5 μm, Supelco, Sigma-Aldrich) coupled with a Bruker Esquire 3000 plus mass spectrometer (0−80% ACN, 22 min, 0.2 mL/min). Reversed phase HPLC was run on a Gilson instrument (Viliers-le-bel, France) equipped with an Ultrasphere ODS column (4.6 × 250 mm, 5 μm, Beckman, Fullerton, CA) and a UV-detection (10−100% ACN, 35 min, 214 nm, 1 mL/min). Preparative TLC was performed on silica gel 60PF_{254} containing gypsum.

Biochemical Evaluation. DPP IV was purified from human seminal plasma as described previously.[31] DPP II was isolated from the same source using techniques described previously for purification of the enzyme from porcine seminal plasma,[32] supplemented with adenosine deaminase affinity chromatography to eliminate contaminating DPP IV.[31] Enzyme activity was measured kinetically with the chromogenic substrates Gly-Pro-*p*-nitroanilide at pH 8.3 and Lys-Ala-*p*-nitroanilide at pH 5.5 for DPP IV and DPP II, respectively. Test compounds were dissolved and diluted in DMSO (final concentration DMSO during assay 5% v/v). All measurements were carried out in duplicate. The initial evaluation of compounds was carried out at 1 mM, or in case of solubility limits, the highest concentration possible. If vi/vo (velocity in the presence of inhibitor/velocity in the presence of control) was < 0.5, an IC_{50} value was determined experimentally using at least 6 different concentrations of inhibitor. For those compounds with IC_{50} values below 5 μM for one of the enzymes, the analysis was repeated using a new stock of compound. Generally, independent measures of IC_{50} differ less than 20% from each other. IC_{50} value was defined as the inhibitor concentration, which caused a 50% decrease of the activity under assay conditions. The errors given in the tables represent errors of the fit.

General Procedure for Synthesis of Compounds 1, 2, 8.3, 8.4, 8.6, 8.8, 8.10, 9, 10.1, and 10.2. These series were prepared by parallel synthesis using a PASP-protocol[26]**:** α-amino Boc-protected amino acids (0.375 mmol) (side chain protection was as followed: -Lys(Boc), -Dab(Boc), -Orn(Boc), -Dap-(Boc), -His(Trt), -Asn(Trt), -Ser(tBu), -Asp(OtBu), -Tyr(tBu), -Arg(diZ)), HOBt (0.425 mmol), and PS-Carbodiimide (0.75 mmol) were added to a dry reaction vessel. Dichloromethane (4 mL) was added, and the mixture was stirred for 10 min prior to the addition of the appropriate amine (respectively pyrrolidine (**1**), thiazolidine (**2**), 1,2,5,6-tetrahydropyridine (**8.5**, **8.6**), hexamethyleneimine (**8.7**, **8.8**), azetidine (**8.9**, **8.10**), piperidine (**8.3**, **8.4**, **9**, **10.3**−**10.5**), morpholine (**10.1**), and Boc-piperazine (**10.2**)). After the mixture was stirred at room-temperature overnight the polymer-bound polyamine (1.5 mmol) was added, and stirring was continued for 5 h. The reaction mixture was filtered, and the amide product was collected in the filtrate. The resins are washed two times with 4 mL of dichloromethane, and the combined fractions were evaporated under reduced pressure. The purity of the compounds was checked by TLC and reversed phase HPLC. Compounds were purified by preparative TLC using a mixture of EtOAc and hexane (usually 40/60) as eluent. Isolation from the silica gel of the preparative TLC was done by suspending the silica gel in EtOAc/MeOH (90:10), followed by filtration.

General Procedure for *tert*-Butyloxycarbonyl (Boc) Deprotection. Deprotection was done by dissolving in 4 mL of a TFA/dichloromethane (1:1) mixture. The solution was stirred for 3 h, and the volatile part was removed under reduced pressure. After coevaporation several times with ether, the residues were lyophilized from *tert*-butyl alcohol/water (4:1).

1-(L-Alanyl)pyrrolidine trifluoroacetate (1.1): ^{1}H NMR (D_2O, 400 MHz) δ 1.44 (d, 3H, CH_3), 1.84−1.97 (m, 4H, CH_2), 3.34−3.56 (m, 4H, CH_2), 4.26 (m, 1H, α-CH); MS (ES^+) *m*/*z* 143 $(M + H)^+$; LC-MS: rt 1.3 min, 100%, *m*/*z* 143; HPLC (214 nm): rt 3.54 min, 98%.

1-(L-Asparaginyl)pyrrolidine trifluoroacetate (1.2): ^{1}H NMR (D_2O, 400 MHz) δ 1.78−1.95 (m, 4H, CH_2), 2.71 (dd, 1H, CH_2), 2.84 (dd, 1H, CH_2), 3.28−3.56 (m, 4H, CH_2), 4.47 (t, 1H, α-CH); MS (FAB^+) *m*/*z* 186 $(M + H)^+$; LC-MS: rt 1.0 min, 100%, *m*/*z* 186; HPLC (214 nm): rt 3.60 min, 98%.

1-(L-Aspartyl)pyrrolidine trifluoroacetate (1.3): ^{1}H NMR (D_2O, 400 MHz) δ 1.84−1.97 (m, 4H, CH_2), 2.86 (dd, 1H, CH_2), 3.02 (dd, 1H, CH_2), 3.36−3.59 (m, 4H, CH_2), 4.54 (t, 1H, α-CH); MS (FAB^+) *m*/*z* 187 $(M + H)^+$; LC-MS: rt 1.1 min, 100%, *m*/*z* 187; HPLC (214 nm): rt 2.54 min, 100%.

1-(*S*-Cyclohexylalanyl)pyrrolidine trifluoroacetate (1.4): ^{1}H NMR (DMSO-d_6), 400 MHz) δ 0.70−2.00 (m, 17H, CH_2, CH), 3.20−3.70 (m, 4H, CH_2), 3.95−4.10 (m, 1H, α-CH), 8.30 (brs, 3H, NH_3^+); MS (FAB^+) *m*/*z* 225 $(M + H)^+$; LC-MS: rt 16.0 min, 100%, *m*/*z* 225; HPLC (214 nm): rt 14.34 min, 100%.

1-(L-Glycyl)pyrrolidine trifluoroacetate (1.5): ^{1}H NMR (D_2O, 400 MHz) δ 1.82−1.98 (m, 4H, CH_2), 3.36−3.42 (m, 4H, CH_2), 3.86 (s, 2H, CH_2); MS (ES^+) *m*/*z* 129 $(M + H)^+$; LC-MS: rt 1.1 min, 100%, *m*/*z* 129; HPLC (214 nm): rt 3.39 min, 95%.

1-(L-Histidyl)pyrrolidine bis trifluoroacetate (1.6): ^{1}H NMR (D_2O, 400 MHz) δ 1.90−2.01 (m, 4H, CH_2), 3.22−3.28 (m, 1H, β-CH_2), 3.44−3.57 (m, 4H, CH_2), 3.64−3.69 (m, 1H, β-CH_2), 4.65 (t, 1H, α-CH), 7.52 (s, 1H, 4H−His), 8.78 (s, 1H, 2H−His); MS (ES^+) *m*/*z* 209 $(M + H)^+$; LC-MS: rt 0.7 min, 100%, *m*/*z* 209; HPLC (214 nm): rt 2.57 min, 100%.

1-(L-Isoleucyl)pyrrolidine trifluoroacetate (1.7): ^{1}H NMR (DMSO-d_6, 250 MHz) δ 0.85 (t, 3H, δ-CH_3), 0.93 (d, 3H, γ-CH_3), 1.05−1.20 (m, 1H, γ-CH_2), 1.40−1.60 (m, 1H, γ-CH_2),

1.70−2.00 (m, 5H, β-CH, CH_2), 3.25−3.70 (m, 4H, CH_2), 3.93 (m, 1H, α-CH), 8.14 (s, 3H, NH_3^+); MS (FAB^+) *m/z* 185 $(M + H)^+$; LC-MS: rt 9.8 min, 100%, *m/z* 185; HPLC (214 nm): rt 9.68 min, 100%.

1-(L-Lysyl)pyrrolidine bis trifluoroacetate (1.8): ^1H NMR (D_2O, 400 MHz) δ 1.42−1.48 (m, 2H, CH_2), 1.63−1.71 (m, 2H, CH_2), 1.83−2.00 (m, 6H, β-CH_2, CH_2), 2.96 (t, 2H, ϵ-CH_2), 3.35−3.60 (m, 4H, CH_2), 4.26 (t, 1H, α-CH); MS (ES^+) *m/z* 200 $(M + H)^+$; LC-MS: rt 0.7 min, 95%, *m/z* 200; HPLC (214 nm): rt 4.42 min, 100%.

1-(L-Seryl)pyrrolidine trifluoroacetate (1.9): ^1H NMR (D_2O, 400 MHz) δ 1.78−1.95 (m, 4H, CH_2), 3.32−3.56 (m, 4H, CH_2), 3.81 (dd, 1H, CH_2), 3.90 (dd, 1H, CH_2), 4.29 (t, 1H, α-CH); MS (FAB^+) *m/z* 159 $(M + H)^+$; LC-MS: rt 6.3 min, 90%, *m/z* 159; HPLC (214 nm): rt 7.96 min, 90%.

1-(L-Phenylalanyl)pyrrolidine trifluoroacetate (1.10): ^1H NMR (D_2O, 400 MHz) δ 1.50−1.78 (m, 4H, CH_2), 2.56−2.62 (m, 1H, CH_2), 3.06−3.19 (m, 2H, CH_2), 3.24−3.39 (m, 3H, CH_2), 4.42 (t, 1H, α-CH), 7.21 (m, 2H, *o*-H_{arom}), 7.31−7.38 (m, 3H, *m*-, *p*-H_{arom}); MS (ES^+) *m/z* 219 $(M + H)^+$; LC-MS: rt 13.0 min, 100%, *m/z* 219; HPLC (214 nm): rt 12.43 min, 98%.

1-(L-Prolyl)pyrrolidine trifluoroacetate (1.11): ^1H NMR (D_2O, 400 MHz) δ 1.86−2.07 (m, 7H, β-CH_2-, γ-CH_2, CH_2), 2.45−2.54 (m, 1H, β-CH_2), 3.31−3.56 (m, 6H, δ-CH_2, CH_2), 4.51 (t, 1H, α-CH); MS (ES^+) *m/z* 169 $(M + H)^+$; LC-MS: rt 2.1 min, 100%, *m/z* 169; HPLC (214 nm): rt 6.44 min, 99%.

1-(*S*-Thiaprolyl)pyrrolidine trifluoroacetate (1.12): ^1H NMR (D_2O, 400 MHz) δ 1.86−2.01 (m, 4H, CH_2), 3.17 (dd, 1H, β-CH_2), 3.37−3.59 (m, 4H, CH_2), 3.63 (m, 1H, β-CH_2), 4.39 (d, 1H, δ-CH_2), 4.48 (d, 1H, δ-CH_2), 4.75−4.84 (m, 1H, α-CH); MS (ES^+) *m/z* 187 $(M + H)^+$; LC-MS: rt 2.3 min, 95%, *m/z* 187; HPLC (214 nm): rt 6.83 min, 96%.

1-(L-Tyrosyl)pyrrolidine trifluoroacetate (1.13): ^1H NMR (D_2O, 400 MHz) δ 1.51−1.80 (m, 4H, CH_2), 2.56−2.62 (m, 1H, CH_2), 2.98−3.14 (m, 2H, CH_2), 3.24−3.40 (m, 3H, CH_2), 4.37 (t, 1H, α-CH), 6.84 (d, 2H, 3-,5-H_{arom}), 7.12 (d, 2H, 2-,6-H_{arom}); MS (ES^+) *m/z* 235 $(M + H)^+$; LC-MS: rt 9.4 min, 100%, *m/z* 235; HPLC (214 nm): rt 6.09 min, 100%.

1-(L-Valyl)pyrrolidine trifluoroacetate (1.14): ^1H NMR (D_2O, 400 MHz) δ 0.97 (d, 3H, CH_3), 1.02 (d, 3H, CH_3), 1.82−1.98 (m, 4H, CH_2), 2.18−2.26 (m, 1H, β-CH_2), 3.38−3.60 (m, 4H, CH_2), 4.06 (d, 1H, α-CH); MS (ES^+) *m/z* 171 $(M + H)^+$; LC-MS: rt 0.6 min, 100%, *m/z* 171; HPLC (214 nm): rt 6.14 min, 100%.

1-(L-Isoleucyl)thiazolidine trifluoroacetate (2.1): ^1H NMR ($CDCl_3$, 400 MHz) δ 0.9−1.38 (m, 7H, CH_3, CH_2), 1.52−1.67 (m, 1H, CH_2), 1.90−2.02 (m, 1H, CH), 2.98−3.15 (m, 2H, 5-CH_2), 3.69−3.80 (m, 1H, 4-CH_2), 3.88−4.02 (m, 1H, 4-CH_2), 4.12−4.23 (m, 1H, α-CH), 4.41−4.68 (m, 2H, 2-CH_2); MS (ES^+) *m/z* 203 $(M + H)^+$; LC-MS: rt 9.8 min, 100%, *m/z* 203; HPLC (214 nm): rt 9.31 min, 100%.

1-(L-Lysyl)thiazolidine bis trifluoroacetate (2.2): ^1H NMR (D_2O, 400 MHz) δ 1.81−2.11 (m, 6H, CH_2), 3.15−3.29 (m, 4H, ϵ-CH_2, 5-CH_2), 3.82−4.04 (m, 2H, 4-CH_2), 4.60−4.82 (m, 3H, 2-CH_2, α-CH); MS (ES^+) *m/z* 218 $(M + H)^+$; LC-MS: rt 0.8 min, 98%, *m/z* 218; HPLC (214 nm): rt 5.84 min, 98%.

1-(*S*-Ornithyl)thiazolidine bis trifluoroacetate (2.3): ^1H NMR (D_2O, 400 MHz) δ 1.83−1.91 (m, 2H, γ-CH_2), 2.05−2.10 (m, 2H, β-CH_2), 3.12 (t, 2H, 5-CH_2), 3.20 (t, 1H, δ-CH_2), 3.27 (t, 1H, δ-CH_2), 3.83−4.08 (m, 2H, 4-CH_2), 4.52 (t, 0.5H, α-CH), 4.57 (t, 0.5H, α-CH), 4.59−4.84 (m, 2H, 2-CH_2); MS (ES^+) *m/z* 204 $(M + H)^+$; LC-MS: rt 0.6 min, 98%, *m/z* 204; HPLC (214 nm): rt 4.09 min, 95%.

1-[(*S*)-2,4-Diaminobutanoyl]thiazolidine bis trifluoroacetate (2.4): ^1H NMR (D_2O, 400 MHz) δ 2.34−2.40 (m, 2H, β-CH_2), 3.18−3.28 (m, 4H, γ-CH_2, 5-CH_2), 3.84−4.07 (m, 2H, 4-CH_2), 4.55−4.84 (m, 3H, 2-CH_2, α-CH); MS (ES^+) *m/z* 190 $(M + H)^+$; LC-MS: rt 0.6 min, 98%, *m/z* 190; HPLC (214 nm): rt 3.99 min, 99%.

1-(L-Lysyl)-1,2,5,6-tetrahydropyridine bis trifluoroacetate (8.6): ^1H NMR (D_2O, 400 MHz) δ 1.45−1.55 (m, 2H, CH_2), 1.69−1.81 (m, 2H, CH_2), 1.91−2.00 (m, 2H, CH_2), 2.23−2.36 (m, 2H, 5-CH_2), 3.05 (b, 2H, ϵ-CH_2), 3.62−3.81 (m, 2H, 6-CH_2), 4.03−4.17 (m, 2H, 2-CH_2), 4.55 (t, 0.5H, α-CH), 4.62 (t, 0.5H, α-CH), 5.75−5.82 (m, 1H, 4-CH), 5.96−6.06 (m, 1H, 3-CH); MS (ES^+) *m/z* 212 $(M + H)^+$; LC-MS: rt 0.9 min, 100%, *m/z* 212; HPLC (214 nm): rt 3.87 min, 100%.

1-(L-Lysyl)hexamethyleneimine bis trifluoroacetate (8.8): ^1H NMR (D_2O, 400 MHz) δ 1.51−1.66 (m, 6H, CH_2), 1.72−1.86 (m, 6H, CH_2), 1.94−2.00 (m, 2H, CH_2), 3.06 (t, 2H, ϵ-CH_2), 3.39−3.56 (m, 2H, CH_2), 3.62−3.74 (m, 2H, CH_2), 4.53 (t, 1H, α-CH); MS (ES^+) *m/z* 228 $(M + H)^+$; LC-MS: rt 1.2 min, 100%, *m/z* 228; HPLC (214 nm): rt 5.27 min, 100%.

1-(L-Lysyl)azetidine bis trifluoroacetate (8.10): ^1H NMR (D_2O, 400 MHz) δ 1.43−1.57 (m, 2H, CH_2), 1.70−1.80 (m, 2H, CH_2), 1.85−1.96 (m, 2H, CH_2), 2.35−2.48 (m, 2H, 3-CH_2), 3.05 (br s, 2H, ϵ-CH2), 4.06−4.29 (m, 3H, CH_2, α-CH), 4.39−4.44 (m, 1H, 3-CH); MS (ES^+) *m/z* 186 $(M + H)^+$; LC-MS: rt 0.6 min, 97%, *m/z* 186; HPLC (214 nm): rt 3.10 min, 98%.

1-(L-Arginyl)piperidinebistrifluoroacetate (9.1). Deprotection was done by hydrogenolysis, followed by Boc-deprotection.

^1H NMR (D_2O, 400 MHz) δ 1.50−1.80 (m, 8H, CH_2), 1.92−2.00 (m, 2H, CH_2), 3.25−3.38 (m, 2H, δ-CH_2), 3.44−3.77 (m, 4H, CH_2), 4.60−4.70 (m, 1H, α-CH); MS (ES^+) *m/z* 242 $(M + H)^+$; LC-MS: rt 0.4−0.5 min, *m/z* 242 $(M + H)^+$; HPLC (214 nm): rt 4.58 min, 91%.

1-(*S*-Cyclohexylalanyl)piperidine trifluoroacetate (9.2): ^1H NMR (D_2O, 400 MHz) δ 0.91−1.79 (m, 19H, CH_2), 3.40−3.53 (m, 4H, 2-CH_2, 6-CH_2), 4.49 (t, 1H, α-CH); MS (ES^+) *m/z* 239 $(M + H)^+$; LC-MS: rt 1.0−1.4 min, *m/z* 239 $(M + H)^+$; HPLC (214 nm): rt 23.49 min, 100%.

1-(L-Histidyl)piperidine bis trifluoroacetate (9.3): ^1H NMR (D_2O, 400 MHz) δ 1.52−1.79 (m, 6H, 3-CH_2, 4-CH_2, 5-CH_2), 3.31−3.72 (m, 6H, β-CH_2, 2-CH_2, 6-CH_2), 4.81−4.93 (m, 1H, α-CH), 7.54 (s, 1H, 4-CH−His), 8.81 (s, 1H, 2-CH−His); MS (ES^+) *m/z* 223 $(M + H)^+$; LC-MS: rt 0.3−0.5 min, *m/z* 223 $(M + H)^+$; HPLC (214 nm): rt 4.04 min, 88%.

1-(L-Isoleucyl)piperidine trifluoroacetate (8.3). ^1H NMR (DMSO-d_6, 400 MHz) δ 0.85 (t, 3H, δ-CH_3), 0.94 (d, 3H, γ-CH_3), 1.00−1.25 (m, 1H, γ-CH), 1.35−1.70 (m, 7H, γ-CH, 3-CH_2, 4-CH_2, 5-CH_2), 1.70−1.85 (m, 1H, β-CH), 3.20−3.65 (m, 4H, 2-CH_2, 6-CH_2), 4.25 (d, 1H, α-CH), 8.07 (br s, 3 H, NH_3+); MS (ES^+) *m/z* 199 $(M + H)^+$; LC-MS: rt 0.6−0.7 min, *m/z* 199 $(M + H)^+$; HPLC (214 nm): rt 11.30 min, 100%.

1-(L-Seryl)piperidine trifluoroacetate (9.4): ^1H NMR (D_2O, 400 MHz) δ 1.59−1.73 (m, 6H, CH_2), 3.50−3.64 (m, 4H, CH_2), 3.90−3.95 (m, 1H, β-CH_2), 4.02−4.06 (m, 1H, β-CH_2), 4.62−4.68 (m, 1H, α-CH); MS (ES^+) *m/z* 173 $(M + H)^+$; LC-MS: rt 0.5−0.6 min, *m/z* 173 $(M + H)^+$; HPLC (214 nm): rt 4.91 min, 93%.

1-(L-Lysyl)piperidine bis trifluoroacetate (8.4): ^1H NMR (D_2O, 400 MHz) δ 1.34−1.87 (m, 12H, CH_2), 2.95 (t, 2H, ϵ-CH_2), 3.43−3.53 (m, 4H, CH_2), 4.50 (t, 1H, α-CH), MS (ES^+) *m/z* 214 $(M + H)^+$; LC-MS: rt 0.3−0.5 min, *m/z* 214 $(M + H)^+$; HPLC (214 nm): rt 2.59 min, 86%.

1-[N-ϵ-(Benzyloxycarbonyl)-L-Lysyl]piperidine trifluoroacetate (9.5): ^1H NMR (D_2O, 400 MHz) δ 1.37−1.76 (m, 10H, CH_2), 1.84−1.98 (m, 2H, CH_2), 3.14−3.28 (m, 2H, ϵ-CH_2), 3.41−3.68 (m, 4H, CH_2), 4.47−4.57 (m, 1H, α-CH), 5.11−5.26 (m, 2H, CH_2-Z), 7.49 (s, 5H, H_{arom}); MS (ES^+) *m/z* 348 $(M + H)^+$; LC-MS: rt 1.6−1.9 min, *m/z* 348 $(M + H)^+$; HPLC (214 nm): rt 14.59 min, 100%.

1-(*S*-Ornithyl)piperidine bis trifluoroacetate (9.6): ^1H NMR (D_2O, 400 MHz) δ 1.59−1.91 (m, 8H, CH_2), 1.97−2.03 (m, 2H, CH_2), 3.11 (t, 2H, δ-CH_2), 3.53−3.66 (m, 4H, CH_2), 4.66 (t, 1H, α-CH); MS (ES^+) *m/z* 200 $(M + H)^+$; LC-MS: rt 0.4−0.6 min, *m/z* 200 $(M + H)^+$; HPLC (214 nm): rt 3.74 min, 100%.

1-[(*S*)-2,4-Diaminobutanoyl]piperidine bis trifluoroacetate (9.7): ^1H NMR (D_2O, 400 MHz) δ 1.59−1.73 (m, 6H, CH_2), 2.27−2.34 (m, 2H, β-CH_2), 3.12−3.23 (m, 2H, γ-CH_2), 3.50−3.71 (m, 4H, CH_2), 4.72 (t, 1H, α-CH); MS (ES^+) *m/z* 186 $(M + H)^+$; LC-MS: rt 0.5−0.6 min, *m/z* 186 $(M + H)^+$; UV−HPLC rt 3.62 min, 100%.

1-[(*R*)-2,4-Diaminobutanoyl]piperidine bis trifluoroacetate (9.8): ^1H NMR (D_2O, 400 MHz) δ 1.50−1.62 (m, 6H, CH_2), 2.15−2.21 (m, 2H, β-CH_2), 3.02−3.11 (m, 2H, γ-CH_2),

3.41−3.59 (m, 4H, CH_2), 4.61 (t, 1H, α-CH); MS (ES^+) *m/z* 186 ($M + H)^+$; LC-MS: rt 0.4−0.5 min, *m/z* 186 ($M + H)^+$; HPLC (214 nm): rt 4.66 min, 100%.

1-[(*S*)-2-Amino,4-benzyloxycarbonylaminobutanoyl]-piperidine trifluoroacetate (9.9): ^1H NMR (D_2O, 400 MHz) δ 1.20−1.71 (m, 6H, CH_2), 1.85−2.05 (m, 2H, β-CH_2), 3.29−3.55 (m, 6H, CH2, γ-CH_2), 4.44 (m, 1H, α-CH), 5.13 (s, 2H, CH_2), 7.43 (s, 5H, H_{arom}); MS (ES^+) *m/z* 320 ($M + H)^+$; LC-MS: rt 1.1−1.2 min, *m/z* 320 ($M + H)^+$; HPLC (214 nm): rt 14.85 min, 97%.

1-[(*S*)-2-Benzyloxycarbonylamino,4-aminobutanoyl]-piperidine trifluoroacetate (9.10). ^1H NMR (D_2O, 400 MHz) δ 1.41−1.71 (m, 6H, CH_2), 1.98−2.17 (m, 2H, β-CH_2), 3.02−3.76 (m, 6H, γ-CH_2, CH_2), 4.70−4.85 (m, 1H, α-CH), 5.17 (s, 2H, CH_2), 7.46 (s, 5H, H_{arom}); MS (ES^+) *m/z* 320 ($M + H)^+$; LC-MS: rt 0.4−0.6 min, *m/z* 320 ($M + H)^+$; HPLC (214 nm): rt 14.48 min, 96%.

1-[(*S*)-2,3-Diaminopropanoyl]piperidine bis trifluoroacetate (9.11): ^1H NMR (D_2O, 400 MHz) δ 1.57−1.81 (m, 6H, CH_2), 3.42−3.85 (m, 6H, CH_2, β-CH_2), 4.97 (m, 1H, α-CH); MS (ES^+) *m/z* 172 ($M + H)^+$; LC-MS: rt 0.4−0.5 min, *m/z* 172 ($M + H)^+$; HPLC (214 nm): rt 3.60 min, 100%.

1-[(*S*)-2-Aminobutanoyl]piperidine trifluoroacetate (9.12): ^1H NMR (D_2O, 400 MHz) δ 1.05 (t, 3H, CH_3), 1.63−1.77 (m, 6H, CH_2), 1.89−2.00 (m, 2H, β-CH_2), 3.50−3.68 (m, 4H, CH_2), 4.52 (t, 1H, α-CH); MS (ES^+) *m/z* 171 ($M + H)^+$; LC-MS: rt 0.5−0.6 min, *m/z* 171 ($M + H)^+$; HPLC (214 nm): rt 7.92 min, 96%.

1-(*S*-Norvalyl)piperidine trifluoroacetate (9.13): ^1H NMR (D_2O, 400 MHz) δ 1.01 (t, 3H, CH_3), 1.41−1.51 (m, 2H, γ-CH_2), 1.59−1.78 (m, 6H, CH_2), 1.85−1.89 (m, 2H, β-CH_2), 3.50−3.68 (m, 4H, CH_2), 4.55 (t, 1H, α-CH); MS (ES^+) *m/z* 185 ($M + H)^+$; LC-MS: rt 0.7−0.8 min, *m/z* 185 ($M + H)^+$; HPLC (214 nm): rt 9.91 min, 100%.

1-(*S*-Norleucyl)piperidine trifluoroacetate (9.14): ^1H NMR (D_2O, 400 MHz) δ 0.95 (t, 3H, CH_3), 1.30−1.42 (m, 4H, CH_2), 1.59−1.76 (m, 6H, CH_2), 1.87−1.90 (m, 2H, β-CH_2), 3.49−3.69 (m, 4H, CH_2), 4.54 (t, 1H, α-CH); MS (ES^+) *m/z* 199 ($M + H)^+$; LC-MS: rt 0.5−0.7 min, *m/z* 199 ($M + H)^+$; HPLC (214 nm): rt 11.96 min, 96%.

4-[(*S*)-2,4-Diaminobutanoyl]morfoline bis trifluoroacetate (10.1): ^1H NMR (D_2O, 400 MHz) δ 2.27−2.38 (m, 2H, β-CH_2), 3.12−3.28 (m, 2H, γ-CH_2), 3.61−3.85 (m, 8H, CH_2), 4.71 (t, 1H, α-CH); MS (ES^+) *m/z* 188 ($M + H)^+$; LC-MS: rt 0.6 min, 100%, *m/z* 188; HPLC (214 nm): rt 3.41 min, 100%.

1-[(*S*)-2,4-Diaminobutanoyl]piperazine tris trifluoroacetate (10.2): ^1H NMR (D_2O, 400 MHz) δ 2.25−2.40 (m, 2H, β-CH_2), 3.12−3.28 (m, 2H, γ-CH_2), 3.33−3.50 (m, 4H, CH_2),), 3.75−4.15 (m, 4H, CH_2) 4.76 (t, 1H, α-CH); MS (ES^+) *m/z* 187 ($M + H)^+$; LC-MS: rt 0.5 min, 95%, *m/z* 187; HPLC (214 nm): rt 3.40 min, 99%.

General Procedure for Synthesis of Thioamides (10.3−10.5). The protected amino acids amides (**9**) were prepared by paralel synthesis using the PASP-protocol. Thioxylation of these compounds was performed according to the following procedure: To a solution of the Boc-protected amino acid amides (2 equiv) in 5 mL of toluene was added 2,4-bis(*p*-methoxyphenyl)-1,3-dithiadiphosphatane 2,4-disulfide (Lawesson's reagent) (1 equiv). The reaction mixture was stirred for 2 h at 80 °C. The solvent was removed by evaporation and the crude compound was purified by preparative TLC (EtOAc/hexane, 40:60). Pure compounds were deprotected according to the general procedure.

1-(L-Lysyl)thioxopiperidine bis trifluoroacetate (10.3): ^1H NMR (D_2O, 400 MHz) δ 1.43−1.62 (m, 2H) and 1.71−1.83 (m, 8H) (γ-CH_2, δ-CH_2, 3-CH_2, 4-CH_2, 5-CH_2), 1.93−2.00 (m, 2H, β-CH_2), 3.04 (t, 2H, ϵ-CH_2), 3.80−3.96 (m, 2H) and 4.07−4.22 (m, 1H), 4.34−4.41 (m, 1H) (2-CH_2, 6-CH_2), 4.74−4.79 (m, 1H, α-CH); MS (ES^+) *m/z* 230 ($M + H)^+$; LC-MS: rt 1.4 min, 100%, *m/z* 230; HPLC (214 nm): rt 5.94 min, 100%.

1-(*S*-Ornithine)thioxopiperidine bis trifluoroacetate (10.4): ^1H NMR (D_2O, 400 MHz) δ 1.82−1.99 (m, 8H, CH_2), 2.03−2.09 (m, 2H, β-CH_2), 3.12 (t, 2H, δ-CH_2), 3.89−4.03 (m, 2H, CH_2), 4.20−4.27 (m, 1H, CH_2), 4.42−4.48 (m, 1H, CH_2), 4.90 (t, 1H, α-CH); MS (ES^+) *m/z* 216 ($M + H)^+$; LC-MS: rt 1.1 min, 100%, *m/z* 216; HPLC (214 nm): rt 7.07 min, 100%.

1-[(*S*)-2,4- Diaminobutanoyl]thioxopiperidine bis trifluoroacetate (10.5): ^1H NMR (D_2O, 400 MHz) δ 1.61−1.63 (m, 6H, 3-CH_2, 4-CH_2, 5-CH_2), 2.30−2.35 (m, 2H, β-CH_2), 3.10−3.26 (m, 2H, γ-CH_2), 3.82−3.98 (m, 2H) and 4.12−4.20 (m, 1H) and 4.36−4.44 (m, 1H) (2-CH_2, 6-CH_2), 4.91 (t, 1H, α-CH); MS (ES^+) *m/z* 202 ($M + H)^+$; LC-MS: rt 1.1 min, 95%, *m/z* 202; HPLC (214 nm): rt 5.97 min, 99%.

3-Substituted Pyrrolidide Analogues were Synthesized from 1-[*N*-(*tert*-Butyloxycarbonyl)-L-lysyl]-3(*R,S*)-hydroxypyrrolidine. 1-(L-Lysyl)-3(*R,S*)-hydroxypyrrolidine Bis Trifluoroacetate (8.12). To a mixture of *N*-α,γ-di(*tert*-butyloxycarbonyl)-L-lysine (1.1 equiv, 2.28 g), triethylamine (3 equiv, 2.53 mL), and TBTU (1.1 equiv, 2.12 g) in DMF (40 mL) was added (*R,S*)-3-hydroxypyrrolidine (1 equiv, 522 mg). After the mixture was stirred at room-temperature overnight, water was added and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with 1 N HCl (2 × 25 mL), 5% $NaHCO_3$ (2 × 25 mL), and brine (25 mL). The organic layer was dried over $NaSO_4$, evaporated, and purified by column chromatography yielding 1-[*N*-α,γ-di(*tert*-butyloxycarbonyl)-L-lysyl]-3(*R,S*)-hydroxypyrrolidine (87%). This Boc-protected compound was treated with a mixture of TFA/DCM (1/1, 10 mL) at room temperature. The title compound was obtained after evaporation and coevaporation with diethyl ether: ^1H NMR (D_2O, 400 MHz) δ 1.48−1.68 (m, 2H, CH_2), 1.71−1.80 (m, 2H, CH_2), 1.93−2.25 (m, 4H, CH_2), 3.06 (br s, 2H, ϵ-CH_2), 3.54−3.88 (m, 4H, CH_2), 4.27−4.42 (m, 1H, α-CH), 4.57−4.66 (m, 1H, 3-CH); MS (ES^+) *m/z* 216 ($M + H)^+$; LC-MS: rt 0.5 min, 95%, *m/z* 216; HPLC (214 nm): rt 2.75 min, 95%.

1-(L-Lysyl)-3(*R,S*)-azidopyrrolidine Bis Trifluoroacetate (8.14). To a solution of 1-[*N*-α,γ-di(*tert.*-butyloxycarbonyl)-L-lysyl]-3(*R,S*)-hydroxypyrrolidine (1.5 mmol, 620 mg) in dry 1.2-dichloroethane (20 mL) were added triethylamine (4.5 mmol, 574 μL) and *p*-toluenesulfonyl chloride (2.5 mmol, 457 mg) at 0 °C. The mixture was stirred at room temperature for 48 h (after 24 h an second addition of *p*-toluenesulfonyl chloride (1.5 mmol) occurred). Water (30 mL) was added, and the mixture was extracted with CH_2Cl_2 (2 × 80 mL). The organic layer was washed with 5% $NaHCO_3$ (2 × 50 mL), dried, evaporated, and purified by column chromatography ($CHCl_3$) yielding 1-[*N*-α,γ-di(*tert.*-butyloxycarbonyl)-L-lysyl]-3(*R,S*)-azidoyrrolidine (70%). A solution of this compound (0.95 mmol, 540 mg) in DMF (15 mL) was treated with NaN_3 (4.75 mmol, 390 mg) and stirred at 80 °C for 5 h. EtOAc (50 mL) was added, and the resulting mixture was washed with 5% $NaCO_3$ (2 × 30 mL). The organic layer was dried, evaporated, and purified by column chromatography yielding a pale yellow oil. Deprotection was done according to the general procedure to yield the title compound. ^1H NMR (D_2O, 400 MHz) δ 1.49−1.60 (m, 2H, CH_2), 1.72−1.82 (m, 2H, CH_2), 1.94−2.03 (m, 2H, CH_2), 2.17−2.38 (m, 2H, 4-CH_2), 3.07 (br s, 2H, ϵ-CH_2), 3.56−3.99 (m, 4H, CH_2), 4.30−4.42 (m, 1H, α-CH), 4.46−4.55 (m, 1H, 3-CH); MS (ES^+) *m/z* 241 ($M + H)^+$; LC-MS: rt 0.7 min, 100%, *m/z* 241; HPLC (214 nm): rt 3.79 min, 100%.

1-(L-Lysyl)-3(*R,S*)-benzoyloxypyrrolidine Bis Trifluoroacetate (8.16). A solution of 1-[*N*-α,γ-di(*tert*-butyloxycarbonyl)-L-lysyl]-3(*R,S*)-hydroxypyrrolidine (1.16 mmol, 480 mg) in dry pyridine (15 mL) was treated with benzoyl chloride (1.28 mol, 148 μL) at 0 °C and stirred for 3 h at room temperature. After cooling the reaction mixture to 0 °C, water was added to the residue and solvents were evaporated. Dichloromethane was added to the redidue and the mixture was washed with 5% $NaHCO_3$. The organic layer was dried, evaporated and purified by preparative TLC using EtOAc as eluent to yield the pure 1-[*N*-α,γ-di(*tert*-butyloxycarbonyl)-L-lysyl]-3(*R,S*)-benzoyloxypyrrolidine as an oil (53%). Deprotection was done according to the general procedure to yield the title compound: ^1H NMR (D_2O, 400 MHz) δ 1.44−1.60 (m, 2H, CH_2, 4H, CH_2), 2.31−2.52 (m, 2H, CH_2), 2.55−2.70 (m, 0.5H, ϵ-CH_2), 2.90 (br s, 0.5H, ϵ-CH_2), 3.08 (br s, 1H, ϵ-CH_2), 3.72−4.05 (m, 4H, CH_2), 4.34−4.46 (m, 1H, α-CH), 5.62−5.73 (m, 1H, 3-CH),

7.56−7.64 (m, 2H, *m*-H_{arom}), 7.72−7.81 (m, 1H, *p*-H_{arom}), 8.05−8.12 (m, 2H, *o*-H_{arom}); MS (ES^+) *m*/*z* 320 $(M + H)^+$; LC-MS: rt 9.9 min, 100%, *m*/*z* 320; HPLC (214 nm): rt 11.76 min, 100%.

General Procedure for the Synthesis of Dipeptide Nitriles (11, 12). To a mixture of Boc-Xaa-OH (1.1 equiv), triethylamine (3 equiv) and TBTU (1.1 equiv) in DMF was added $YaaNH_2$ (1 equiv) [2(*S*)-pyrrolidinecarboxamide was commercially available; 2(*S*)-piperidinecarboxamide was prepared from L-pipecolinic acid (1 equiv) by reaction with *N*-hydroxysuccinimide (1.05 equiv) and dicyclohexylcarbodiimide (DCC, 1.05 equiv) in DCM (yield: 90%), followed by treatment of a solution in dioxane with ammonium gas (yield: 99%).] After the mixture was stirred overnight at room temperature, water was added and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with 1 N HCl (2 × 25 mL), 5% $NaHCO_3$ (2 × 25 mL) and brine (25 mL). The organic layer was dried over $NaSO_4$, evaporated, and purified by column chromatography, yielding Boc-Xaa-$YaaNH_2$ (86%). Dehydratation of the amide function to the nitrile was done according to the following procedure:[19] To a solution of Boc-Xaa-$YaaNH_2$ (1 equiv) and imidazol (2 equiv) in pyridine at −30 °C was slowly added phosphorusoxychloride (4 equiv). The solution was allowed to attain room temperature, and the reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated and the residue was extracted with 1 N HCl and diethyl ether. The organic layer was dried and evaporated, and the residue was purified by prepartive TLC to yield the Boc protected dipeptide nitrile (60%). Boc deprotection was done according to the general procedure.

1-(L-Lysyl)-2(*S*)-cyanopyrrolidine bis trifluoroacetate (11): ^1H NMR (D_2O, 400 MHz) δ 1.50−1.53 (m, 2H, CH_2), 1.72−1.77 (m, 2H, CH_2), 1.98−2.12 (m, 2H, CH_2), 2.14−2.26 (m, 2H, CH_2), 2.32−2.43 (m, 2H, CH_2), 3.02−3.06 (m, 2H, ϵ-CH_2), 3.70−3.74 (m, 2H, 5-CH_2), 4.37 (t, 1H, α-CH), 4.83−4.88 (m, 1H, α-CH); MS (FAB^+) *m*/*z* 225 $(M + H)^+$; HPLC (214 nm): rt 3.20 min, 100%.

1-(L-Lysyl)-2(*R*,S)-cyanopiperidine bis trifluoroacetate (12.1): ^1H NMR (D_2O, 400 MHz) δ 1.42−1.63 (m, 3H, CH_2), 1.71−2.02 (m, 8H, CH_2), 2.18−2.29 (m, 1H, CH_2), 3.00−3.41 (m, 2H, 6-CH_2), 3.46−3.50 (m, 1H, ϵ-CH_2), 3.88−4.00 (m, 1H, ϵ-CH_2), 4.53−4.67 (m, 1H, α-CH), 5.69−5.88 (m, 1H, 2-CH); MS (ES^+) *m*/*z* 239 $(M + H)^+$; LC-MS: rt 1.0 min, 100%, *m*/*z* 239; HPLC (214 nm): rt 5.78 min, 99%.

1-[(*S*)-2,4-Diaminobutanoyl]-2(*S*)-cyanopiperidine bis trifluoroacetate (12.2): ^1H NMR (D_2O, 400 MHz) δ 1.50−1.68 (m, 1H, CH_2), 1.72−2.00 (m, 4H, CH_2), 2.08−2.19 (m, 1H, CH_2), 2.35−2.49 (m, 2H, CH_2), 3.10−3.29 (m, 2H, 6-CH_2), 3.48−3.53 (m, 1H, γ-CH_2), 3.85−3.98 (m, 1H, γ-CH_2), 4.70−4.82 (m, 1H, α-CH), 5.69−5.89 (m, 1H, 2-CH_2); MS (ES^+) *m*/*z* 211 $(M + H)^+$; LC-MS: rt 0.8 min, 100%, *m*/*z* 211; HPLC (214 nm): rt 6.56 min, 100%.

Acknowledgment. This work received support from The Fund for Scientific Research−Flanders (Belgium) (F.W.O.) and The Special Fund for Research−University of Antwerp (BOF UA). P. Van der Veken is a fellow of the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT). The excellent technical assistance of N. Lamoen and W. Bollaert is greatly appreciated.

References

(1) Sedo, A.; Malik, R. Dipeptidyl Peptidase IV-like Molecules: Homologous Proteins or Homologous Activities. *Biochim. Biophys. Acta* **2001**, *1550*, 2, 107−116.
(2) Leiting, B.; Pryor, K. D.; Wu, J. K.; Marsilio, F.; Patel, R. A.; Craik, C. S.; Ellman, J. A.; Cummings, R. T.; Thornberry, N. A. Catalytic Properities and Inhibition of Proline-Specific Dipeptidyl Peptidase II, IV and VII. *Biochem. J.* **2003**, *371*, 525−532.
(3) McDonald, J. K.; Leibach, F. H.; Grindeland, R. E.; Ellis, S. Purification of Dipeptidyl Aminopeptidase II (Dipeptidyl Arylamidase II) of the Anterior Pituitary Gland. *J. Biol. Chem.* **1968**, *243*, 4143−4150.
(4) Andersen, K. J.; McDonald, J. K. Lysosomal Heterogenity of Dipeptidyl Peptidase II Active on Collagen-Related Peptides. *Renal Physiol. Biochem.* **1989**, *12*, 32−40.
(5) Araki, H.; Li, Y. H.; Yamamoto, Y.; Haneda, M.; Nishi, K.; Kikkawa, R.; Ohkubo, I. Purification, Molecular Cloning, and Immunohistochemical Localization of Dipeptidyl Peptidase II from the Rat Kidney and Its Identity with Quiescent Cell Proline Dipeptidase. *J. Biochem.* **2001**, *129*, 279−288.
(6) Chiravuri, M.; Schmitz, T.; Yardley, K.; Underwood, R.; Dayal, Y.; Huber, B. T. A Novel Apoptotic Pathway in Quiescent Lymphocytes Identified by Inhibition of Post-Proline Cleaving Aminopeptidase: A Candidate Target Protease, Quiescent Cell Proline Dipeptidase. *J. Immunol.* **1999**, *163*, 3092−3099.
(7) Underwood, R.; Chiravuri, M.; Lee, H.; Schmitz, T.; Kabcenell, A. K.; Yardley, K.; Huber, B. T. Sequence, Purification, and Cloning of an Intracellular Serine Protease, Quiescent Cell Proline Dipeptidase. *J. Biol. Chem.* **1999**, *274*, 48, 34053−34058.
(8) Chiravuri, M.; Lee, H.; Mathieu, S. L.; Huber, B. T. Homodimerization via a Leucine Zipper Motif Is Required for Enzymatic Activity of Quiescent Cell Proline Dipeptidase. *J. Biol. Chem.* **2000**, *275*, 35, 26994−26999.
(9) Augustyns, K.; Bal, G.; Thonus, G.; Belyaev, A.; Zhang, X. M.; Bollaert, W.; Lambeir, A. M.; Durinx, C.; Goossens, F.; Haemers, A. The Unique Properties of Dipeptidyl-peptidase IV (DPP IV/CD26) and the Therapeutic Potential of DPP IV Inhibitors. *Curr. Med. Chem.* **1999**, *6*, 311−327.
(10) Villhauer, E. B.; Coppola, G. M.; Hughes, T. E. DPP−IV Inhibition and Therapeutic Potential. In *Annual Reports in Medicinal Chemistry;* Academic Press: New York, 2001; Vol 36, pp 191−200.
(11) De Meester, I.; Korom, S.; Van Damme, J.; Scharpé, S. CD26, Let It Cut or Cut It Down, *Immunol. Today* **1999**, *20*, 8, 367−375.
(12) Mentlein, R. Dipeptidyl-Peptidase IV (CD26)-Role in the Inactivation of Regulatory Peptides. *Regul. Peptides* **1999**, *85*, 1, 9−24.
(13) Reinhold: D.; Kahne, T.; Steinbrecher, A.; Wrenger, S.; Neubert, K.; Broche, S. The Role of Dipeptidyl Peptidase IV (DPP IV) Enzymatic Activity in T Cell Activation and Autoimmunity. *Biol. Chem.* **2002**, *383*, 1133−1138.
(14) Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Dunning, B. E.; Mangold, B. L.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Hughes, T. E. 1-[2-[(5-Cyanopyridin-2-yl)amino]ethylamino]-acetyl-2-(*S*)-pyrrolidine-carbonitrile: A Potent, Selective, and Orally Bioavailable Dipeptidyl Peptidase IV Inhibitor with Antihyperglycemic Properties. *J. Med. Chem. Lett.* **2002**, *45*, 12, 2362−2365.
(15) Augustyns, K.; Van der Veken, P.; Senten, K.; Haemers, A. Dipeptidyl Peptidase IV Inhibitors as New Therapeutic Agents in the Treatment of Type II Diabetes, *Expert Opin. Ther. Pat.* **2003**, *13*, 4, 499−510.
(16) Drucker, D. J. Therapeutic Potential of Dipeptidyl Peptidase IV Inhibitors for the Treatment of Type 2 Diabetes, *Expert Opin. Invest. Drugs* **2003**, *12*, 87−100.
(17) Schön, E.; Born, I.; Demuth, H. U.; Faust, J.; Neubert, K.; Steinmetzer, T.; Barth, A.; Ansorge, S. Dipeptidyl Peptidase IV in the Immune System. *Biol. Chem. Hoppe-Seyler* **1991**, *372*, 305−311.
(18) Stöckel-Maschek, A.; Mrestani-Klaus, C.; Stiebitz, B.; Demuth, H.-U.; Neubert, K. Thioxo Amino Acid Pyrrolidides and Thiazolidides: New Inhibitors of Proline Specific Peptidases. *Biochim. Biophys. Acta* **2000**, *1479*, 15−31.
(19) Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. 2-Cyanopyrrolidides as Potent, Stable Inhibitors of Dipeptidyl Peptidase IV. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 10, 1163−1166.
(20) Li, J.; Wilk, E.; Wilk, S. Aminoacylpyrrolidine-2-nitriles: Potent and Stable Inhibitors of Dipeptidyl-Peptidase IV (CD 26). *Arch. Biochem. Biophys.* **1995**, *323*, 1, 148−154.
(21) Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. 4-Cyanothiazolidides as Very Potent, Stable Inhibitors of Dipeptidyl Peptidase IV. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 22, 2745−2748.
(22) Boduszek, B.; Oleksyszyn, J.; Kam, C.; Selzler, J.; Smith, R. E.; Powers, J. C. Dipeptide Phosphonates as Inhibitors of Dipeptidyl Peptidase IV. *J. Med. Chem.* **1994**, *37*, 3969 3976.
(23) Belyaev, A.; Zhang, X.; Augustyns, K.; Lambeir, A.-M.; De Meester, I.; Vedernikova, I.; Scharpé, S.; Haemers, A. Structure−Activity Relationship of Diaryl Phosphonates Esters as Potent Irreversible Dipeptidyl Peptidase IV Inhibitors. *J. Med. Chem.* **1999**, *42*, 1041−1052.
(24) Senten, K.; Daniels, L.; Van der Veken, P.; De Meester, I.; Lambeir, A. M.; Scharpé, S.; Haemers, A.; Augustyns, K. Rapid

parallel Synthesis of Dipeptide Diphenyl Phosphonate Esters as Inhibitors of Dipeptidyl Peptidases. *J. Comb. Chem*. **2003**, *5*, 3, 336−344.
(25) Senten, K.; Van der Veken, P.; Bal, G.; De Meester, I.; Lambeir, A. M.; Scharpé, S.; Bauvois, B.; Haemers, A.; Augustyns, K. Development of Potent and Selective Dipeptidyl Peptidase II Inhibitors. *Biol. Med. Chem. Lett*. **2002**, *12*, 2825−2828.
(26) Senten, K.; Van der Veken, P.; Bal, G.; Haemers, A.; Augustyns, K. Polymer-Assisted Solution-Phase Parallel Synthesis of Dipeptide *p*-Nitroanilides and Dipeptide Diphenyl Phosphonates. *Tetrahedron Lett*. **2001**, *42*, 9135−9138.
(27) Augustyns, K.; Lambeir, A. M.; Borloo, M.; De Meester, I.; Vedernikova, I.; Vanhoof, G.; Hendriks, D.; Scharpé, S.; Haemers, A. Pyrrolidines: Synthesis and Structure−Activity Relationship as Inhibitors of Dipeptidyl Peptidase IV. *Eur. J. Med. Chem*. **1997**, *32*, 301−309.
(28) Mentlein, R.; Struckhoff, G. Purification of 2 Dipeptidyl Aminopeptidases-II from Rat-Brain and Their Action on Proline-Containing Neuropeptides. *J. Neurochem*. **1989**, *52*, 1284−1293.
(29) Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Wagtmann, N. Crystal Structure of Human Dipeptidyl Peptidase IV/CD26 in Complex with a Substrate Analogue. *Nat. Struct. Biol*. **2003**, *10*, 1, 1−7.
(30) Belyaev, A.; Zhang, X. M.; Augustyns, K.; Lambeir, A.-M.; De Meester, I.; Vedernikova, I.; Scharpé, S.; Haemers, A. Structure−Activity Relationship of Diaryl Phosphonate Esters as Potent Irreversible Dipeptidyl Peptidase IV Inhibitors. *J. Med. Chem*. **1999**, *42*, 1041−1052.
(31) De Meester, I.; Vanhoof, G.; Lambeir, A.-M.; Scharpé, S. Use of Immobilized Adenosine Deaminase for the Rapid Purification of Native Human CD26 Dipeptidyl Peptidase IV. *J. Immunol. Methods* **1996**, *189*, 99−105.
(32) Huang, K.; Takagaki, M.; Kani, K.; Ohkubo, I. Dipeptidyl peptidase II from porcine seminal plasma: purification, characterization and its homology to granzymes, cytotoxic cell proteinases (CCP 1−4). *Biochim. Biophys. Acta* **1996**, *1290*, 149−156.

JM0308803