γ-Amino-Substituted Analogues of 1-[(*S*)-2,4-Diaminobutanoyl]piperidine as Highly Potent and Selective Dipeptidyl Peptidase II Inhibitors

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Received December 4, 2003

Using 1-[(*S*)-2,4-diaminobutanoyl]piperidine as lead compound, we developed a large series of highly potent and selective dipeptidyl peptidase II (DPP II) inhibitors. γ-Amino substitution with arylalkyl groups, for example, a 2-chlorobenzyl moiety, resulted in a DPP II inhibitor with an $IC_{50} = 0.23$ nM and a high selectivity toward DPP IV ($IC_{50} = 345$ μM). Furthermore, it was shown that the basicity of the γ-amino is important and that α-amino substitution is not favorable. Piperidine-2-nitriles did not show an increase in potency but rather reduced the selectivity. Introduction of a 4-methyl or a 3-fluorine on piperidine improved selectivity and preserved the high potency.

Introduction

Due to the unique structure of proline, most common proteases are not able to hydrolyze peptide bonds before or after a proline residue. Therefore, proline-specific proteases are of great metabolic importance since many biologically active peptides include one or more proline residues. Among the serine peptidases, several members of clan SC are surprisingly specific for cleavage of the peptide bond after a proline residue.[1] These enzymes are considered to have high potential as targets for drug discovery.[2] Because clan SC contains dipeptidyl aminopeptidases such as dipeptidyl peptidase IV (DPP IV), fibroblast activation protein α (FAPα), dipeptidyl peptidase II (DPP II), DPP8, and DPP9, as well as the carboxypeptidase prolyl carboxypeptidase (PCP) and the endopeptidase prolyl oligopeptidase (POP), the position of the scissile Pro-Xaa bond varies. The DPPs are also referred to as "DPP IV activity- and/or structure-homologues" (DASH) proteins[3] to recognize the status of DPP IV as the most widely studied member of this family and to indicate the structural or functional similarity of its members with DPP IV. The development of potent and specific inhibitors for each of these enzymes can be an important tool to unravel their physiological function and to validate their potential as a therapeutic target. Whereas inhibitors of DPP IV[4,5] and POP[6] have been around for some time, investigation of inhibitors for the other related enzymes has only started recently.

DPP IV (EC 3.4.14.5) is widely expressed in mammalian tissues and can be present in both a membrane-bound and soluble form. It releases *N*-terminal dipeptides with preferentially proline or alanine at the penultimate position from substrate proteins at neutral to weakly basic pH.[7] A broad array of diverse functional properties in the immune, nerve, and endocrine systems has been suggested.[8−10] DPP IV substrates that have recently received special attention are incretins, a class of hormones involved in glucose homeostasis. Inhibition of DPP IV prolongs the in vivo half-life of these incretins (GLP-1 and GIP), and therefore, inhibitors can be valuable in the treatment of type 2 diabetes. Several DPP IV inhibitors are currently under clinical evaluation in this field.[11−13]

DPP II (EC 3.4.14.2), first identified by McDonald et al.,[14] is believed to be involved in the physiological breakdown of some proline-containing neuropeptides and in the degradation of collagen and substance P.[15,16] Recently, DPP II was suggested to be identical to human quiescent cell proline dipeptidase (QPP). This is based on a signigicant sequence homology (79.4%) found between rat DPP II and human QPP.[17] 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 positive and DPP IV negative T cells undergo apoptosis.[18,19] No sequence homology has been found between DPP II/QPP and DPP IV.

Development of highly specific and potent DPP II inhibitors will contribute to the unraveling of the physiological functions of DPP II/QPP and therapeutic potential of its inhibitors. The similarity in substrate specificity and catalytic mechanism of DPP II and DPP IV makes this a challenging task.

In previous papers, we reported a systematic search for new, potent, and selective DPP II inhibitors.[20−22] A structure−activity relationship was investigated starting from aminoacyl pyrrolidides as lead compounds. Exploration of the C-terminal (P_1) and N-terminal (P_2) building blocks led to the discovery of some potent DPP II inhibitors, characterized by a high selectivity for DPP II with regard to DPP IV.

L-2,4-Diaminobutyric acid (Dab) was selected as the most promising P_2 amino acid. The three carbon atom spacing between the basic side chain amino function and the α-amino group appeared to be optimal. Further-

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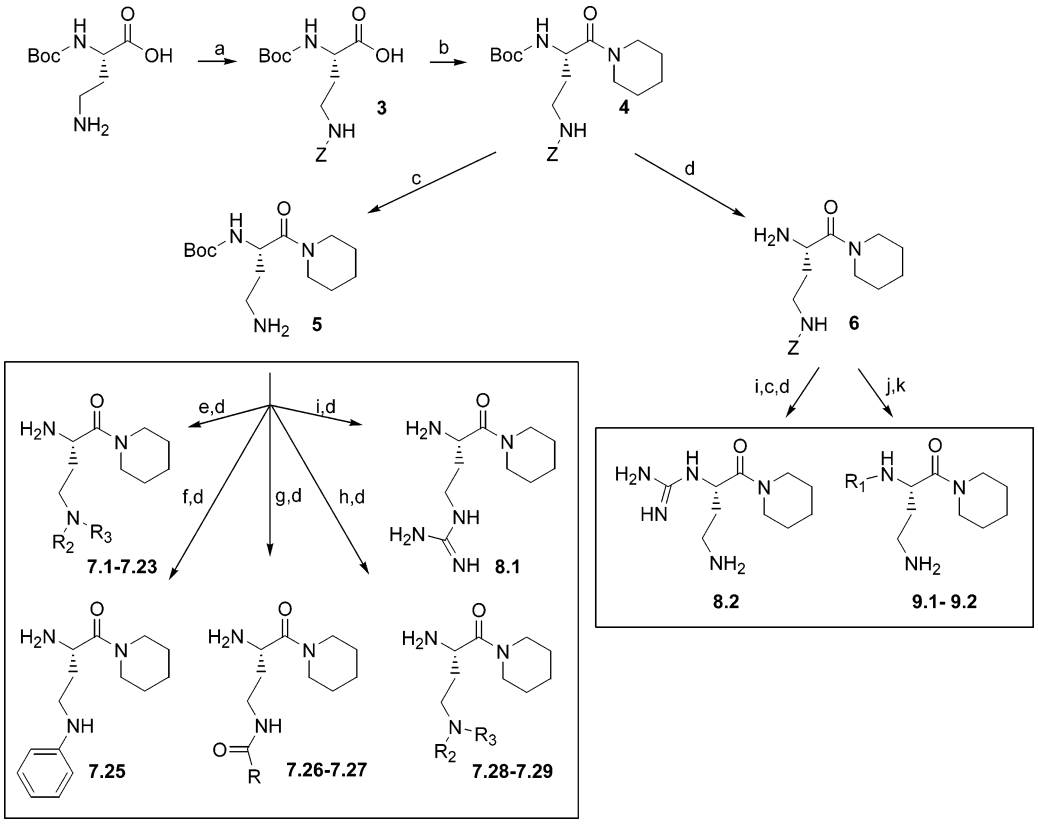
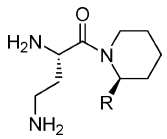
10.1021/jm031122f CCC: $27.50
Published on Web 04/07/2004

Scheme 1. Introducing α- or γ-Substitution in Dab-Pip (**1**)[a]



[a] Reagents: (a) benzylchloroformate, TEA, dioxane, H_2O; (b) TBTU, piperidine, TEA, DMF; (c) H_2, Pd/C; (d) TFA, DCM; (e) (1) aldehyde, PS-cyanoborohydride, acetic acid, (2) PS-benzaldehyde, DCM; (f) $Pd_2(Dbd)_3$, (*o*-biphenyl)P(*t*-Bu)$_2$, bromobenzene, NaOtBu, toluene; (g) acetic anhydride (**7.26**) or benzoyl chloride (**7.27**), pyridine; (h) 2-chloro-3-nitropyridine (**7.28**) or 6-chloronicotinonitrile (**7.29**), DMF, $KHCO_3$; (i) 1-*H*-pyrazole-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)]carboxamidine, DIPEA, acetonitrile/H_2O (95:5); (j) aldehyde, $NaCNBH_3$, acetic acid, MeOH; (k) HBr in acetic acid. Note: R_1, R_2, and R_3 refer to Table 2.

Table 1. Previously Reported Potent and Selective DPP II Inhibitors[a]



compound	R	DPP II inhibition IC_{50} (μM)	DPP IV inhibition IC_{50} (μM)	SI
1	H	0.13 ± 0.01	>1000	$>7.6 \times 10^3$
2	CN	0.038 ± 0.002	151 ± 5	4.0×10^3

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

more, the amino acid at the P_2 position needed to be in the L-configuration with a free α-amino function.

In search for an optimized P_1 building block, piperidine proved to be superior over pyrrolidine and thiazolidine among other cyclic or acyclic amines.[20] 1-[(*S*)-2,4-Diaminobutanoyl]piperidine (Dab-Pip) (**1**) showed an IC_{50} for DPP II of 130 nM and a selectivity index of more than 7500. Incorporation of a nitrile, leading to Dab-Pip-2-CN (**2**), increased potency by a factor of 3. Although the selectivity index of this nitrile compound (**2**) declined to approximately 4000, it still can be regarded as highly selective for DPP II (Table 1). Both compounds **1** and **2** are far more active and selective than all the earlier reported DPP II inhibitors and were retained as lead compounds.[20]

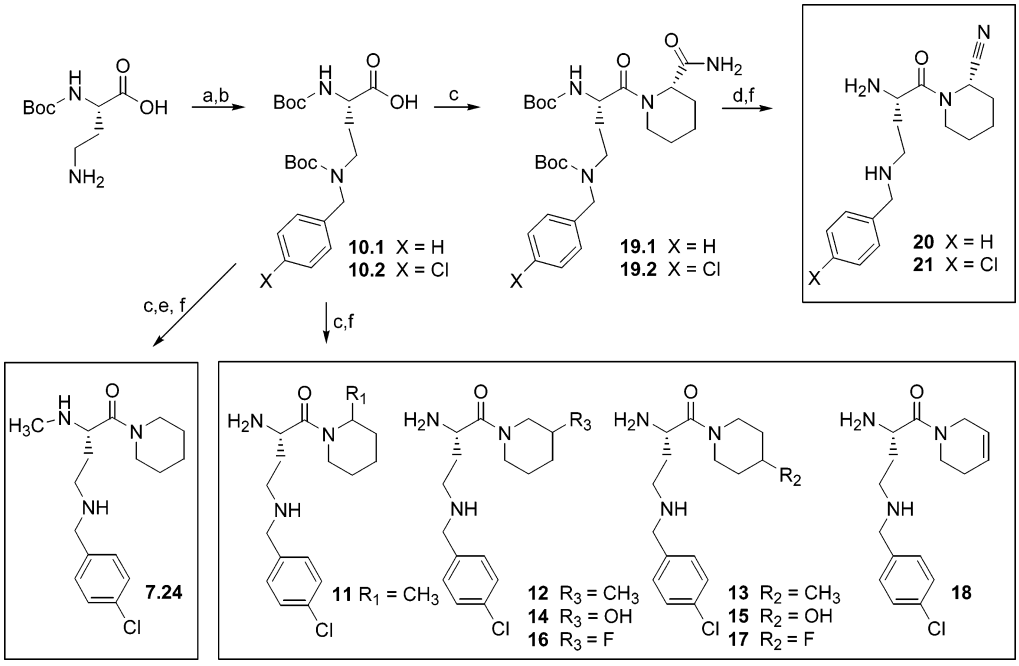
In this paper, we report a structure−activity relationship study of Dab-Pip (**1**) in which the α- and γ-amino functions were modified and the piperidine ring was substituted.

Chemistry

For selective substitution on the α- or γ-amino group of diaminobutyric acid, the orthogonally protected compound **4** was synthesized (Scheme 1). The α- or γ-amino protecting group could be selectively removed, respectively, by acidolysis using TFA leading to **5** and by hydrogenolysis affording compound **6**. Compounds **7.1**−**7.22** were prepared by parallel synthesis using a polymer-assisted solution phase (PASP) procedure. Starting from **5**, a reductive amination was carried out with the required aldehyde using polymer-bound $NaCNBH_3$. Purification was facilitated by the use of polymer-bound benzaldehyde as a scavenger for the unreacted primary amine **5**. For compounds **7.21** and **7.22**, an excess of aldehyde was used to ensure the disubstitution of the free amino function of **5**. Compound **7.23** was prepared from **5** by a successive reductive amination with 4-chlorobenzaldehyde and an excess of paraformaldehyde.

Compound **7.25** is obtained from **5** by a palladium-catalyzed amination reaction of bromobenzene.[23] Compounds **7.26** and **7.27** were prepared from **5** by reaction with, respectively, acetic anhydride or benzoyl chloride in the presence of pyridine. Compounds **7.28** and **7.29** were the result of an aromatic nucleophilic substitution

Scheme 2. Synthesis of **7.24** and Introduction of Substituted Piperidine Rings[a]



[a] Reagents: (a) benzaldehyde (**10.1**) or 4-chlorobenzaldehyde (**10.2**), $NaCNBH_3$, acetic acid, MeOH; (b) Boc_2O, TEA, dioxane, H_2O; (c) TBTU, TEA, DMF, piperidine (**7.24**)/2-, 3-, or 4-methylpiperidine (respectively, **11**, **12**, **13**)/3- or 4-piperidinol (respectively, **14**, **15**)/3- or 4-fluoropiperidine (respectively, **16**, **17**)/1,2,3,6-tetrahydropyridine (**18**)/2-piperidinecarboxamide (**19.1**, **19.2**); (d) $POCl_3$, imidazole, pyridine, DCM; (e) $(CH_3)_2SO_4$, NaH, THF, catalytic H_2O; (f) TFA, DCM. Note: R_1, R_2, and R_3 refer to Table 3.

of **5** with 2-chloro-3-nitropyridine (**7.28**) or 6-chloronicotinonitrile (**7.29**). Finally, compounds **7.1**−**7.29** were obtained after removal of the Boc-protecting group using TFA.

Compounds **8.1** and **8.2** were prepared from, respectively, **5** and **6** by refluxing in the presence of 1-*H*-pyrazole-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)]carboxamidine[24] and could be obtained by hydrogenolysis (for **8.2**), followed by Boc-removal using TFA (**8.1**, **8.2**).

α-Substitution leading to compounds **9.1** and **9.2** started from **6** by reductive amination using $NaCNBH_3$, and the compounds were finally obtained after removal of the benzyloxycarbonyl group (Z) by acidolysis with hydrogen bromide.

Synthesis of substituted piperidides (**11**−**17**, **20**, **21**) started by reductive amination of benzaldehyde or 4-chlorobenzaldehyde with Boc-Dab-OH, followed by Boc-introduction at the side chain amino function (Scheme 2). Compound **10.2** was then coupled in the presence of TBTU with, respectively, 2-, 3-, or 4-methylpiperidine (**11**, **12**, **13**), 3- or 4-piperidinol (**14**, **15**), and 3- or 4-fluoropiperidine (**16**, **17**). Final Boc-removal was done using TFA. The fluoro-substituted piperidines were prepared from the corresponding Boc-protected piperidinols with diethylaminosulfur trifluoride (DAST). The Boc-protected 1,2,3,6-tetrahydropyridine was obtained as a side product in the synthesis of 4-fluoropiperidine and was also isolated. After removal of the Boc-group, the 3- and 4-fluoropiperidine and 1,2,3,6-tetrahydropyridine were used in the coupling reaction with **10.2**.

Compound **7.24** was prepared from compound **10.2**, which after coupling with piperidine was methylated by the use of dimethyl sulfate in the presence of sodium hydride.[25] Boc-removal by TFA yielded compound **7.24** (Scheme 2).

2-Cyanopiperidides (**20**, **21**) were obtained by coupling of, respectively, **10.1** and **10.2** with 2-piperidinecarboxamide, followed by dehydration of the primary amide to the corresponding nitrile using phosphorusoxychloride[26] and subsequent acid-catalyzed Boc-removal (Scheme 2).

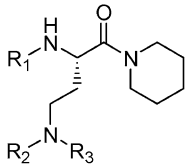
Results and Discussion

Optimization of Dab-Pip (**1**) as DPP II inhibitor was investigated by introducing substituents on the α- or γ-amino function (Table 2). All compounds were evaluated for their DPP II and DPP IV inhibitory potency. A selectivity index (SI) is proposed as a means to evaluate the selectivity of these DPP II inhibitors with respect to DPP IV and is defined as the IC_{50} value for DPP IV divided by the IC_{50} value for DPP II. Compound **7.1**, in which a benzyl group is introduced in the side chain, shows an IC_{50} value for DPP II of approximately 2 nM. Compared to Dab-Pip (**1**), this means a 65-fold increase in DPP II inhibition accompanied with a very high selectivity. Therefore, compound **7.1** can be considered as a very promising improvement of lead compound (**1**).

Several substituted benzyl, heteroaryl, naphthyl, and arylalkyl groups were introduced. Compounds **7.2**−**7.20** represent a series with substitutions leading to a basic secondary nitrogen in the side chain. Substituted benzyls (**7.2**−**7.11**) lead to equally or more potent compounds. However, no conclusion can be drawn concerning the position and nature of an optimal substituent. The 3-cyano derivative (**7.7**) is slightly less potent and less selective. In this series, the 2-chlorobenzyl-substituted compound **7.2** is the most potent and at the same time most selective DPP II inhibitor with an IC_{50} value of 0.23 nM and a selectivity index of 1.5×10^6.

Replacement of the aromatic ring of **7.1** with the heteroaromatic rings thiophene (**7.12**) or pyridine (**7.13**)

Table 2. Investigation of the Structure−Activity Relationship: Introduction of α- or γ-Substitution in Dab-Pip (**1**)[a]



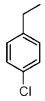
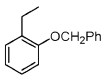
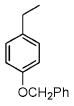
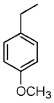
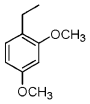
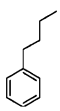
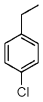
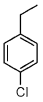
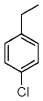
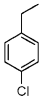
Compound	R_1	R_2	R_3	DPP II inhibition (IC_{50})	DPP IV inhibition (IC_{50})	SI
7.1	H		H	2.03 ± 0.14 nM	247 ± 46 μM	1.2 x 10^5
7.2	H		H	0.23 ± 0.01 nM	345 ± 8 μM	1.5 x 10^6
7.3	H		H	1.34 ± 0.07 nM	186 ± 8 μM	1.4 x 10^5
7.4	H		H	0.48 ± 0.04 nM	165 ± 9 μM	3.4 x 10^5
7.5	H		H	0.39 ± 0.04 nM	> 500 μM	> 1.3 x 10^6
7.6	H		H	0.41 ± 0.03 nM	192 ± 12 μM	4.7 x 10^5
7.7	H		H	2.7 ± 0.1 nM	132 ± 14 μM	5,0 x 10^4
7.8	H		H	1.85 ± 0.08 nM	> 500 μM	> 2.7 x 10^5
7.9	H		H	1.77 ± 0.06 nM	250 ± 9 μM	1.4 x 10^5
7.10	H		H	0.66 ± 0.15 nM	417 ± 34 μM	6.3 x 10^5
7.11	H		H	2.7 ± 0.3 nM	> 500 μM	> 1.8 x 10^5
7.12	H		H	0.80 ± 0.20 nM	278 ± 17 μM	3.5 x 10^5
7.13	H		H	0.75 ± 0.10 nM	125 ± 6 μM	1.7 x 10^5
7.14	H		H	1.31 ± 0.06 nM	301 ± 21 μM	2.3 x 10^5
7.15	H		H	1.1 ± 0.1 nM	179 ± 6 μM	1.6 x 10^5
7.16	H		H	0.90 ± 0.06 nM	188 ± 28 μM	2.1 x 10^5
7.17	H		H	1.91 ± 0.08 nM	> 500 μM	> 2.6 x 10^5
7.18	H		H	1.1 ± 0.1 nM	496 ± 25 μM	4.5 x 10^5

Table 2. Continued

Compound	R_1	R_2	R_3	DPP II inhibition (IC_{50})	DPP IV inhibition (IC_{50})	SI
7.19	H		H	18.6 ± 2 nM	> 1000 μM	> 5.0 x 10^4
7.20	H		H	85 ± 2 nM	64 ± 2.1 μM	7.5 x 10^2
7.21	H			0.60 ± 0.04 nM	266 ± 9 μM	4.4 x 10^5
7.22	H			4.2 ± 0.2 nM	> 125 μM	> 3.0 x 10^4
7.23	H		CH_3	0.22 ± 0.02 nM	196 ± 8 μM	8.9 x 10^5
7.24	CH_3		H	187 ± 9 nM	> 500 μM	> 2.7 x 10^3
7.25	H		H	1.5 ± 0.1 μM	> 250 μM	> 1,7 x 10^2
7.26	H		H	1.50 ± 0.03 μM	518 ± 9 μM	3.5 x 10^2
7.27	H		H	7.4 ± 0.6 μM	418 ± 19 μM	5.6 x 10
7.28	H		H	23.5 ± 1 μM	56 ± 3 μM	2.0
7.29	H		H	4.6 ± 0.1 μM	205 ± 11 μM	4.5 x 10
8.1	H		H	1.80 ± 0.04 μM	545 ± 53 μM	3.0 x 10^2
8.2		H	H	7.4 ± 0.2 μM	>> 1000 μM	>> 1.4 x 10^2
9.1		H	H	12.5 ± 0.2 μM	>> 1000 μM	>> 8.0 x 10
9.2		H	H	17.0 ± 3.5 μM	>> 1000 μM	>> 5.9 x 10

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

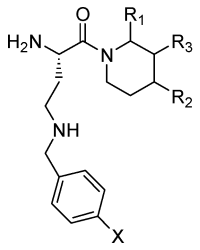
and with 1-naphthyl (**7.14**) and 2-naphthyl (**7.15**) leads to a small increase in potency and selectivity.

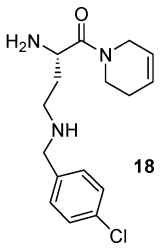
The alkyl chain length of the γ-arylalkyl substituent was also investigated by replacing the benzyl substituent (**7.1**) for phenylethyl (**7.16**) and phenylpropyl (**7.17**). Little influence in inhibitory potency is noticed. Compounds **7.1**, **7.16**, and **7.17** are therefore considered as equipotent, but regarding the selectivity toward DPP IV, compound **7.17**, bearing the phenylpropyl substituent, has a small advantage over **7.1**.

Benzyl was also replaced with the nonaromatic cyclic substituents such as cyclohexylmethyl (**7.18**), cyclohexyl (**7.19**), and 4-piperidyl (**7.20**). For the cyclohexylmethyl substituent (**7.18**), a slight increase in potency and selectivity is noticed. However, introduction of the cyclohexyl or 4-piperidyl substituents leads to a decreased inhibitory potency toward DPP II: the IC_{50} for **7.19** is approximately 19 nM, whereas for the 4-piperidyl substituent, the IC_{50} value is increased to 85 nM.

Disubstitution of the side chain aminofunction is investigated in compounds **7.21**−**7.23**. Compound **7.21**, substituted with two benzyls, is slightly more potent than the monosubstituted benzyl compound **7.1**. On the other hand **7.22**, containing two 4-chlorobenzyl substituents, is markedly less active than the monosubstituted **7.4**. For compound **7.23** in which the γ-amino function is substituted with methyl and 4-chlorobenzyl, an increase in DPP II inhibition and selectivity is noticed, compared to **7.4**. It shows an IC_{50} value for DPP II of

Table 3. Further Investigation of the Structure−Activity Relationship: Substitution of the Piperidine Ring[a]





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compound	X	R_1	R_2	R_3	DPP II inhibition IC_{50} (nM)	DPP IV inhibition IC_{50} (μM)	SI
11	Cl	CH_3	H	H	2.30 ± 0.08	>500	$>2.2 \times 10^5$
12	Cl	H	H	CH_3	1.13 ± 0.06	>500	$>4.4 \times 10^5$
13	Cl	H	CH_3	H	0.55 ± 0.01	>500	$>9.1 \times 10^5$
14	Cl	H	H	OH	54.7 ± 1.1	>1000	$>2.0 \times 10^4$
15	Cl	H	OH	H	113 ± 3	>1000	$>9.0 \times 10^3$
16	Cl	H	H	F	0.62 ± 0.03	500	8.1×10^5
17	Cl	H	F	H	0.39 ± 0.01	70 ± 4	1.8×10^5
18					1.100 ± 0.001	148 ± 7	1.3×10^5
20	H	CN	H	H	4.0 ± 0.1	68 ± 4	1.7×10^4
21	Cl	CN	H	H	6.5 ± 0.2	13 ± 0.7	2.0×10^3

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

0.22 nM and a selectivity index of almost 9.0×10^5 and is therefore, besides **7.2**, a very potent and selective DPP II inhibitor in our series.

In compounds **7.25**−**7.29**, the basicity of the γ-amino function is severely decreased by modification to amides or anilines. For all these compounds, the IC_{50} value for DPP II is raised to the micromolar level resulting in a serious potency loss of these compounds compared to Dab-Pip (**1**). The basic character of the side chain amino function has therefore a large contribution to the high potency of the DPP II inhibitors in these series. This also confirms our previous results obtained by omitting the nitrogen or modification to a carbamate.[20]

Similar substituents were also introduced on the α-amino function (**9.1**, **9.2**). The benzyl substituent in compound **9.1** results in a profound drop in potency toward DPP II. Even a small methyl substituent is not tolerated, as seen in compound **7.24** having a 390-fold higher IC_{50} value than **7.4**.

Conversion of the γ- or α-amino function to a guanidine in compounds **8.1** and **8.2**, respectively, dramatically decreases the potency, although the basic character is conserved.

The modification of the piperidine ring was started from compound **7.4** (IC_{50} = 0.48 nM; Table 3). Methyl, hydroxyl, fluorine, or carbonitrile were introduced at several positions of the piperidine ring.

Introduction of methyl at position 2, 3 or 4 in, respectively, **11**, **12**, and **13** still evokes potent DPP II inhibition with IC_{50} values in the low nanomolar level. However, the 2- and 3-methylpiperidine compounds (**11** and **12**) are less potent than **7.4** on both DPP II and DPP IV. On the other hand, substitution by methyl at position 4 (**13**) has little influence on the DPP II inhibition but strongly decreases the inhibition of DPP IV. Hence, compound **13**, exhibiting an IC_{50} value of 0.55 nM and a selectivity index of more than 9.0×10^5 can be considered as an equipotent but more selective DPP II inhibitor than **7.4**.

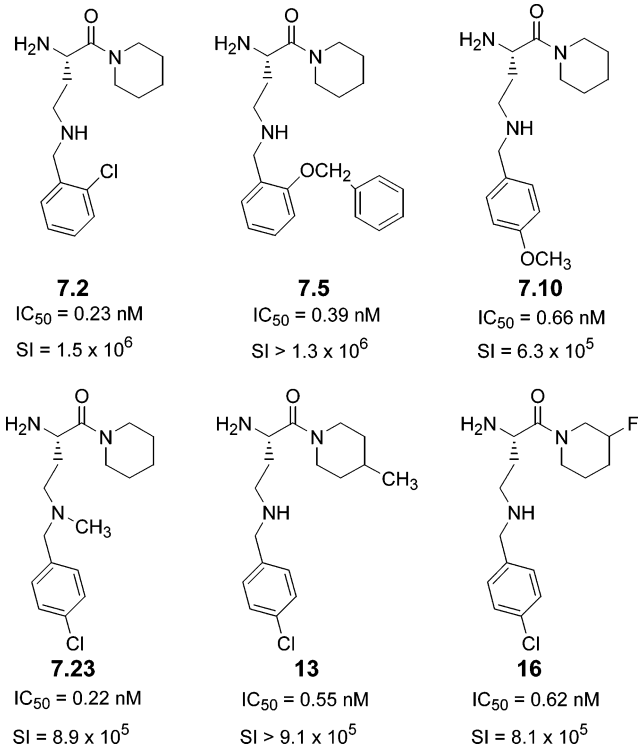

Figure 1. Structures of the most potent and selective DPP II inhibitors revealed in this structure−activity relationship investigation.

Introduction of a hydroxyl group at position 3 or 4 as seen in compounds **14** and **15** gives a fundamental decline of DPP II inhibition.

Replacement of hydrogen with fluorine in compounds **16** (at position 3) and **17** (at position 4) results in a similar level of DPP II inhibition as seen for **7.4**. However, the effect on DPP IV inhibition is different because the 3-fluoropiperidine compound **16** is less potent toward DPP IV, whereas the 4-fluoropiperidine compound **17** is more potent, compared to **7.4**. Compound **16** can therefore be regarded as a more selective DPP II inhibitor, preserving the high potency of **7.4**.

The 1,2,3,6-tetrahydropyridine substitute for piperidine in compound **18** reveals no advantage over **7.4** and even inhibits DPP II to a lesser extent.

We previously reported[20] that introduction of a 2-carbonitrile on the piperidine ring slightly improved DPP II inhibition compared to Dab-Pip (**1**), accompanied by a decreasing selectivity as the potency toward DPP IV is also increased. Indeed, looking at the outcome of compounds **20** and **21**, selectivity is severely affected as both compounds inhibit DPP IV in the low micromolar level. The increase in potency toward DPP II, however, could not be repeated as inhibition of DPP II is decreased by a factor of 2−14 compared to the parent compounds.

Conclusion

Modification of the lead compound Dab-Pip (**1**) (IC_{50} = 130 nM) led to the development of several very potent and selective inhibitors for DPP II. The most promising among them are presented in Figure 1.

Introduction of arylalkyl substituents on the γ-amino function, in a way that the basic character is respected, yielded a series of inhibitors in which the DPP II

inhibition could be increased up to 600-fold compared to Dab-Pip (**1**). The need for retaining the basic character is demonstrated by compounds **7.25**−**7.29** and confirms our previous results.[20] Disubstitution with two arylalkyls or arylalkyl and methyl is also allowed. These results could suggest that the S_2 subsite of DPP II has a large hydrophobic cavity in which a hydrogen bond or salt bridge with the γ-amino group of the inhibitor might be formed.

Substitution of the α-amino group with methyl, alkyl, or arylalkyl or modification to guanidine or carbamate[20] strongly reduces the activity. Furthermore, the P_2 amino acid should be in the L-configuration.[20]

Introduction of a 4-methyl (**13**) or 3-fluorine (**16**) on the piperidine ring shows equal potency on DPP II but improves selectivity, while 2- and 3-methyl, 3- and 4-hydroxyl, and 4-fluorine substitutions lead to less potent or less selective DPP II inhibitors or both. The result with 4-methylpiperidine confirms our hypothesis that the S_1 subsite of DPP II is larger than the one of DPP IV, the latter being optimally suited to fit five-membered rings.[27]

Introduction of a 2-nitrile on the pyrrolidine ring was a successful event to obtain DPP IV transition state inhibitors that were approximately 1000-fold more active than the parent aminoacylpyrrolidines.[28] However, this could not be extended to structurally related DPP II inhibitors. The nitriles **20** and **21** are less potent and less selective DPP II inhibitors compared to their respective parent compounds.

A number of highly potent ($IC_{50} = 10^{-10}$ M) and very selective (10^6) DPP II inhibitors were developed (Figure 1). These compounds are outstanding tools to determine the physiological function of DPP II and the therapeutic potential of DPP II inhibitors.

Experimental Section

Materials. Parallel synthesis was performed using the Quest 210 Organic Synthesizer (Argonaut Technologies). Boc-L-Dab-OH, polymer-bound $NaCNBH_3$, and polymer-bound benzaldehyde were purchased from Novabiochem. Other reagents were obtained from Sigma-Aldrich or Acros.

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). Electrospray (ES^+) mass spectra were acquired on an ion trap mass spectrometer (Esquire 3000, Bruker Daltonics). Purity was verified using two diverse HPLC systems using mass and UV detection. LC-MS were recorded on an Agilent 1100 series HPLC system using a C_{18} column (2.1 mm × 50 mm, 5 μm, Supelco, Sigma-Aldrich) coupled with a Bruker Esquire 3000 plus mass spectrometer (3−100% ACN, 30 min, 0.2 mL/min). Reversed-phase HPLC was run on a Gilson instrument (Villiers-le-bel, France) equipped with an Ultrasphere ODS column (4.6 mm × 250 mm, 5 μm, Beckman, Fullerton, CA) and a UV detector (10−100% ACN, 35 min, 214 nm, 1 mL/min). Element analyses were performed by the Laboratory of Pharmaceutical Chemistry (University Ghent) and were within 0.4% of theoretical values. Preparative TLC was performed on Silicagel $60PF_{254}$ containing gypsum.

Inhibition Measurements. DPP IV was purified from human seminal plasma as described previously.[29] DPP II was isolated from the same source using techniques described previously for purification of the enzyme from rat kidney,[30] supplemented with adenosine deaminase affinity chromatography to eliminate contaminating DPP IV.[29] Initial velocities were measured with the chromogenic substrates Gly-Pro-*p*-nitroanilide (100 μmol/L) at pH 8.3 for DPP IV and Lys-Ala-*p*-nitroanilide (1 mmol/L) at pH 5.5 for DPP II. 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 mmol/L or, in case of solubility limits, the highest concentration possible. If v_i/v_o (initial velocity in the presence of inhibitor/velocity in the presence of DMSO) was <0.5, an IC_{50} value was determined experimentally using at least six different concentrations of inhibitor. For those compounds with IC_{50} values below 5 μmol/L for one of the enzymes, the analysis was repeated using a new stock of compound. Generally, independent measures of IC_{50} differed less than 20% from each other. IC_{50} value was defined as the inhibitor concentration that caused a 50% decrease of the activity under assay conditions. The enzyme concentration is defined as E_0 and the inhibitor concentration is represented by I_0. IC_{50} values were calculated with the GraFit software[31] using the following equation:

$$\frac{v_i}{v_o} = \frac{1}{1 + \left(\frac{I_0}{IC_{50}}\right)^s} + \text{background}$$

where s is the slope factor when plotting v_i/v_o versus log I_0 and background represents the estimated minimal v_i/v_o value.

In the case when the calculated IC_{50} value was below 0.5 nmol/L, the IC_{50} value was recalculated using the following equation:[32]

$$\frac{v_i}{v_o} = \frac{(1 - \{IC_{50} + I_0 + E_0 - \sqrt{(IC_{50} + I_0 + E_0)^2 - 4E_0I_0}\}/2)}{E_0}$$

The errors given in the tables represent errors of the fit.

Synthesis. 4-{[(Benzyloxy)carbonyl]amino}-2-[(*tert*-butoxycarbonyl)amino]butanoic Acid (**3**). To Boc-L-Dab-OH (1 equiv, 23 mmol) in dioxane and H_2O (60 mL, 1:1) was added triethylamine (3 equiv, 69 mmol). The solution was cooled at 0 °C and a solution of benzylchloroformate (1.1 equiv, 25 mmol) in dioxane (5 mL) was added dropwise. The solution was allowed to stir for several hours. The dioxane was evaporated, and the aqueous layer was acidified to pH < 2 and extracted with EtOAc (2 times). The organic layer was dried over Na_2SO_4 and evaporated. The oily residue was used as such for the next step.

General Procedure for Coupling Reaction with TBTU. Compounds **4**, **7.24**, **11**−**18**, **19.1**, and **19.2**. To a mixture of the carboxyl compound (**3**, **10.1**, or **10.2**; 1.1 equiv), triethylamine (3 equiv), and TBTU (1.1 equiv) in DMF (40 mL) was added the proper amine compound (piperidine, 2-, 3-, or 4-methylpiperidine, 3- or 4-piperidinol, 3- or 4-fluoropiperidine, 1,2,3,6-tetrahydropyridine, or 2-*S*-piperidinecarboxamide[20]; 1 equiv) at 0 °C. After stirring 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 Na_2SO_4, evaporated, and purified by column chromatography.

tert-Butyl 3-Amino-1-(1-piperidinylcarbonyl)propylcarbamate (**5**). Deprotection of the benzyloxycarbonyl (Z) group was done by hydrogenolysis: to a mixture of compound obtained from the previous step in methanol (50 mL) was added Pd/C (20%) and acetic acid (1 mL). A flow of nitrogen gas was carried over the solution for 10 min, followed by a flow of H_2 gas. The reaction was monitored by TLC. After completion, again a flow of nitrogen was carried over the solution for 10 min. The mixture was filtered over Celite, and the methanol was removed in vacuo. The compound obtained was used as such in the next step.

General Procedure for Boc-Removal. Deprotection of *tert*-butyloxycarbonyl (Boc) was done by dissolving the compound in 15 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 compound could be obtained by precipitation

in diethyl ether (**6**) or was converted to the HCl salt by coevaporation several times with 4 N HCl in diethyl ether, followed by precipitation in ether and lyophilization from H_2O (compounds **7.1**−**7.29**, **8.1**, **8.2**, **11**−**18**, **20**, and **21**).

General Procedure for the Reductive Amination in Parallel using PASP Technique (Compounds 7.1−**7.22).** Compound **5** (1.8 equiv) was dissolved in DCM/acetic acid (90/10) (5 mL). The required aldehyde (1 equiv; for compounds **7.21** and **7.22**, 5 equiv of aldehyde was used) and the polymer-bound cyanoborohydride (2.5 equiv) were added. After agitation overnight, the polymer-bound benzaldehyde (2.5 equiv) was added to scavenge the excess of the starting amine compound. After agitation for 5 h, the polymer-bound reagents were filtered off, and the solvent was evaporated. Purification was done on preparative TLC. Finally, Boc-deprotection was done according to the described general procedure.

N^1-Benzyl-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.1**). ^{1}H NMR (D_2O, 400 MHz) δ 1.58−1.74 (m, 6H, CH_2), 2.29−2.48 (m, 2H, β-CH_2), 3.15−3.24 (m, 2H, γ-CH_2), 3.48−3.60 (m, 4H, CH_2), 4.36 (s, 2H, CH_2), 4.65−4.74 (m, 1H, α-CH), 7.58 (s, 5H, H_{arom}); MS (ES^+) *m*/*z* 276 (M + H)$^+$; LC-MS rt 9.7 min, *m*/*z* 276, 96%; HPLC (214 nm) rt 9.92 min, 95%.

N^1-(2-Chlorobenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.2**). ^{1}H NMR (D_2O, 400 MHz) δ 1.53−1.75 (m, 6H, CH_2), 2.29−2.42 (m, 2H, β-CH_2), 3.24−3.30 (m, 2H, γ-CH_2), 3.49−3.63 (m,4H, CH_2), 4.53 (s, 2H, CH_2Ph), 4.71−4.80 (m, 1H, α-CH), 7.49−7.67 (m, 4H, H_{arom}); MS (ES^+) *m*/*z* 310 (M + H)$^+$; LC-MS rt 8.2 min, *m*/*z* 310, 91%; HPLC (214 nm) rt 14.48 min, 95%.

N^1-(3-Chlorobenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.3**). ^{1}H NMR (D_2O, 400 MHz) δ 1.46−1.76 (m, 6H, CH_2), 2.28−2.38 (m, 2H, β-CH_2), 3.13−3.23 (m, 2H, γ-CH_2), 3.46−3.59 (m, 4H, CH_2), 4.35 (s, 2H, CH_2Ph), 4.70−4.78 (m, 1H, α-CH), 7.42−7.63 (m, 4H, H_{arom}); MS (ES^+) *m*/*z* 310 (M + H)$^+$; LC-MS rt 8.8 min, *m*/*z* 310, 95%; HPLC (214 nm) rt 15.64 min, 96%.

N^1-(4-Chlorobenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.4**). ^{1}H NMR (D_2O, 400 MHz) δ 1.46−1.75 (m, 6H, CH_2), 2.28−2.40 (m, 2H, β-CH_2), 3.12−3.38 (m, 2H, γ-CH_2), 3.45−3.62 (m, 4H, CH_2), 4.27 (s, 2H, CH_2Ph), 4.70−4.77 (m, 1H, α-CH), 7.42−7.61 (m, 4H, H_{arom}); ^{13}C NMR (D_2O, 400 MHz) 23.48, 25.13, 26.04, 26.82 (3,4,5-C(pip), β-C), 41.97, 42.22, 47.11, 47.97, 50.42 (α-C, γ-C, C−Ph, 2,6-C(pip)), 128.82, 129.49, 131.65, 135.39 (C_{arom}), 165.77 (CO); MS (ES^+) *m*/*z* 310 (M + H)$^+$; LC-MS rt 9.0 min, *m*/*z* 310, 96%; HPLC (214 nm) rt 14.89 min, 95%; Anal. ($C_{16}H_{24}ClN_3O$·2.0HCl·0.4H_2O) C, H, N.

N^1-[2-(Benzyloxy)benzyl]-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.5**). ^{1}H NMR (D_2O, 400 MHz) δ 1.38−1.73 (m, 6H, CH_2), 2.15−2.29 (m, 2H, β-CH_2), 3.05−3.17 (m, 2H, γ-CH_2), 3.28−3.58 (m, 4H, CH_2), 4.28 (s, 2H, CH_2-Ph), 4.54−4.63 (m, 1H, α-CH), 5.32 (s, 2H, OCH_2Ph), 7.14−7.17 (m, 1H, H_{arom}), 7.27−7.29 (m, 1H, H_{arom}), 7.46−7.59 (m, 7H, H_{arom}); MS (ES^+) *m*/*z* 382 (M + H)$^+$; LC-MS rt 11.7 min, *m*/*z* 382, 100%; HPLC (214 nm) rt 17.56 min, 97%.

N^1-[4-(Benzyloxy)benzyl]-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.6**). ^{1}H NMR (D_2O, 400 MHz) δ 1.38−1.75 (m, 6H, CH_2), 2.21−2.35 (m, 2H, β-CH_2), 3.11 (t, 2H, γ-CH_2), 3.44−3.63 (m, 4H, CH_2), 4.30 (s, 2H, CH_2Ph), 4.54−4.63 (m, 1H, α-CH), 5.27 (s, 2H, OCH_2Ph), 7.19−7.21 (m, 2H, H_{arom}), 7.47−7.57 (m, 7H, H_{arom}); MS (ES^+) *m*/*z* 382 (M + H)$^+$; LC-MS rt 11.2 min, *m*/*z* 382, 95%; HPLC (214 nm) rt 18.50 min, 90%.

3-({[3-(*S*)-amino-4-oxo-4-(1-piperidinyl)butyl]amino}methyl)-benzonitrile Dihydrochloride (**7.7**). ^{1}H NMR (D_2O, 400 MHz) δ 1.50−1.76 (m, 6H, CH_2), 2.32−2.37 (m, 2H, β-CH_2), 3.20−3.27 (m, 2H, γ-CH_2), 3.48−3.61 (m, 4H, CH_2), 4.41 (s, 2H, CH_2-Ph), 4.72 (t, 1H, α-CH), 7.70−7.74 (m, 1H, H_{arom}), 7.85−7.87 (m, 1H, H_{arom}), 7.94−7.95 (m, 2H, H_{arom}); MS (ES^+) *m*/*z* 301 (M + H)$^+$; LC-MS rt 3.6 min, *m*/*z* 301, 96%; HPLC (214 nm) rt 13.31 min, 97%.

N^1-(2-Methoxybenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.8**). ^{1}H NMR (D_2O, 400 MHz) δ 1.42−1.73 (m, 6H, CH_2), 2.28−2.37 (m, 2H, β-CH_2), 3.10−3.18 (m, 2H, γ-CH_2), 3.45−3.58 (m, 4H, CH_2), 3.97 (s, 3H, CH_3), 4.36 (s, 2H, CH_2Ph), 4.69 (t, 1H, α-CH), 7.12−7.22 (m, 2H, H_{arom}), 7.43−7.45 (m, 1H, H_{arom}), 7.57−7.61 (m, 1H, H_{arom}); MS (ES^+) *m*/*z* 306 (M + H)$^+$; LC-MS rt 8.1 min, *m*/*z* 306, 100%; HPLC (214 nm) rt 21.75 min, 90%.

N^1-(3-Methoxybenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.9**). ^{1}H NMR (D_2O, 400 MHz) δ 1.40−1.73 (m, 6H, CH_2), 2.28−2.36 (m, 2H, β-CH_2), 3.16 (t, 2H, γ-CH_2), 3.49−3.62 (m, 4H, CH_2), 3.92 (s, 3H, CH_3), 4.33 (s, 2H, CH_2Ph), 4.70 (t, 1H, α-CH), 7.13−7.18 (m, 3H, H_{arom}), 7.50−7.54 (m, 1H, H_{arom}); MS (ES^+) *m*/*z* 306 (M + H)$^+$; LC-MS rt 7.7 min, *m*/*z* 306, 97%; HPLC (214 nm) rt 22.24 min, 96%.

N^1-(4-Methoxybenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.10**). ^{1}H NMR (D_2O, 400 MHz) δ 1.44−1.76 (m, 6H, CH_2), 2.23−2.38 (m, 2H, β-CH_2), 3.10−3.23 (m, 2H, γ-CH_2), 3.49−3.72 (m, 4H, CH_2), 3.92(s, 3H, CH_3), 4.30 (s, 2H, CH_2Ph), 4.64−4.72 (m, 1H, α-CH), 7.13−7.16 (m, 2H, H_{arom}), 7.48−7.50 (m, 2H, H_{arom}); MS (ES^+) *m*/*z* 306 (M + H)$^+$; LC-MS rt 0.9 min, *m*/*z* 306, 100%; HPLC (214 nm) rt 14.18 min, 95%.

N^1-(2,4-Dimethoxybenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.11**). ^{1}H NMR (D_2O, 400 MHz)-δ 1.35−1.86 (m, 6H, CH_2), 2.24−2.35 (m, 2H, β-CH_2), 3.02−3.62 (m, 6H, γ-CH_2, CH_2), 3.93 (s, 3H, CH_3), 3.95 (s, 3H, CH_3), 4.30 (s, 2H, CH_2Ph), 4.65−4.74 (m, 1H, α-CH), 6.71−6.77 (m, 2H, H_{arom}), 7.27−7.29 (m, 1H, H_{arom}); MS (ES^+) *m*/*z* 336 (M + H)$^+$; HPLC (214 nm) rt 15.04 min, 91%.

4-Oxo-4-(1-piperidinyl)-N^1-(2-thienylmethyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.12**). ^{1}H NMR (D_2O, 400 MHz) δ 1.52−1.76 (m, 6H, CH_2), 2.23−2.37 (m, 2H, β-CH_2), 3.19−3.26 (m, 2H, γ-CH_2), 3.49−3.63 (m, 4H, CH_2), 4.59 (s, 2H, CH_2Ph), 4.71 (t, H, α-CH), 7.21−7.23 (m, 1H, H_{arom}), 7.32−7.38 (m, 1H, H_{arom}), 7.65−7.67 (m, 1H, H_{arom}); MS (ES^+) *m*/*z* 282 (M + H)$^+$; LC-MS rt 2.6 min, *m*/*z* 282, 91%; HPLC (214 nm) rt 14.45 min, 99%.

4-Oxo-4-(1-piperidinyl)-N^1-(4-pyridinylmethyl)-1,3-(*S*)-butanediamine Trihydrochloride (**7.13**). ^{1}H NMR (D_2O, 400 MHz) δ 1.59−1.77 (m, 6H, CH_2), 2.38−2.47 (m, 2H, β-CH_2), 3.37−3.73 (m, 6H, γ-CH_2, CH_2), 4.72 (s, 2H, CH_2Ph), 4.75−4.82 (m, 1H, α-CH), 8.25 (m, 2H, H_{arom}), 8.85 (m, 2H, H_{arom}); MS (ES^+) *m*/*z* 277 (M + H)$^+$; LC-MS rt 1.0 min, *m*/*z* 277, 95%; HPLC (214 nm) rt 8.29 min, 96%.

N^1-(1-Naphthylmethyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.14**). ^{1}H NMR (D_2O, 400 MHz)-δ 1.26−1.74 (m, 6H, CH_2), 2.27−2.37 (m, 2H, β-CH_2), 3.13−3.60 (m, 6H, γ-CH_2, CH_2), 4.60−4.80 (m, 1H, α-CH), 4.87 (s, 2H, CH_2Ph), 7.43−7.80 (m, 4H, H_{arom}), 8.12−8.19 (m, 3H, H_{arom}); MS (ES^+) *m*/*z* 326 (M + H)$^+$; LC-MS rt 10.1 min, *m*/*z* 326, 98%; HPLC (214 nm) rt 16.35 min, 90%.

N^1-(2-Naphthylmethyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.15**). ^{1}H NMR (D_2O, 400 MHz)-δ 1.11−1.60 (m, 6H, CH_2), 2.22−2.37 (m, 2H, β-CH_2), 3.07−3.19 (m, 2H, γ-CH_2) 3.30−3.61 (m, 4H, CH_2), 4.54 (s, 2H, CH_2Ph), 4.61−4.69 (m, 1H, α-CH), 7.57−7.75 (m, 3H, H_{arom}), 8.00−8.14 (m, 4H, H_{arom}); MS (ES^+) *m*/*z* 326 (M + H)$^+$; LC-MS rt 10.3 min, *m*/*z* 326, 100%; HPLC (214 nm) rt 16.83 min, 91%.

4-Oxo-N^1-(2-phenylethyl)-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.16**). ^{1}H NMR (D_2O, 400 MHz) δ 1.66−1.78 (m, 6H, CH_2), 2.06−2.34 (m, 3H, CH_2), 3.02−3.31 (m, 2H, CH_2), 3.42−3.66 (m, 7H, CH_2), 4.61−4.69 (m, 1H, α-CH), 7.30−7.55 (m, 5H, H_{arom}); LC-MS rt 12.3 min, *m*/*z* 290, 96%; HPLC (214 nm) rt 20.75 min, 95%.

4-Oxo-N^1-(3-phenylpropyl)-4-(1-piperidinyl)-1,3-(*S*)-butanediamine dihydrochloride (**7.17**). ^{1}H NMR (D_2O, 400 MHz) δ 1.64−1.73 (m, 6H, CH_2), 2.00−2.14 (m, 2H, CH_2), 2.25−2.38 (m, 2H, β-CH_2), 2.74−2.85 (m, 2H, CH_2), 3.08−3.28 (m, 4H, γ-CH_2, CH_2), 3.47−3.61 (m, 4H, CH_2), 4.71 (t, 1H, α-CH), 7.31−7.51 (m, 5H, H_{arom}); MS (ES^+) *m*/*z* 304 (M + H)$^+$; LC-MS rt 8.6 min, *m*/*z* 304, 98%; HPLC (214 nm) rt 26.30 min, 98%.

N^1-(Cyclohexylmethyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.18**). ^{1}H NMR (D_2O, 400 MHz)-

δ 0.92−1.72 (m, 16H, CH_2), 2.05−2.27 (m, 3H, CH, β-CH_2), 2.79 (d, 2H, CH_2), 3.04−3.18 (m, 2H, γ-CH_2), 3.40−3.63 (m, 4H, CH_2), 4.64 (t, 1H, α-CH); MS (ES^+) *m/z* 282 $(M + H)^+$; LC-MS rt 11.2 min, *m/z* 282, 95%; HPLC (214 nm) rt 14.35 min, 97%.

N^1-Cyclohexyl-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.19**). ^1H NMR (D_2O, 400 MHz) δ 1.17−1.45 (m, 5H, CH_2), 1.60−1.93 (m, 9H, CH_2), 2.06−2.17 (m, 2H, β-CH_2), 2.25−2.35 (m, 2H, CH_2), 3.13−3.69 (m, 7H, CH, CH_2, γ-CH_2); MS (ES^+) *m/z* 268 $(M + H)^+$; LC-MS rt 4.5 min, *m/z* 268, 97%; HPLC (214 nm) rt 12.91 min, 93%.

4-Oxo-4-(1-piperidinyl)-N^1-(4-piperidinyl)-1,3-(*S*)-butanediamine Trihydrochloride (**7.20**). ^1H NMR (D_2O, 400 MHz) δ 1.63−1.79 (m, 6H, CH_2), 1.94−2.04 (m, 2H, β-CH_2), 2.34−2.41 (m, 2H, CH_2), 2.45−2.52 (m, 2H, CH_2), 3.18−3.42 (m, 4H, CH_2), 3.52−3.75 (m, 7H, CH, CH_2), 4.62−4.70 (m, 1H, α-CH); MS (ES^+) *m/z* 269 $(M + H)^+$; HPLC (214 nm) rt 8.01 min, 97%.

N^1,N^1-Dibenzyl-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.21**). ^1H NMR (D_2O, 400 MHz) δ 1.23−1.63 (m, 6H, CH_2), 2.27−2.32 (m, 2H, β-CH_2), 3.02−3.24 (m, 4H, CH_2), 3.30−3.34 (m, 1H, CH_2), 3.42−3.47 (m, 1H, CH_2), 4.38 (s, 2H, CH_2Ph), 4.54 (t, 1H, α-CH), 7.38−7.53 (m, 10H, H_{arom}); MS (ES^+) *m/z* 366 $(M + H)^+$; LC-MS rt 19.8 min, *m/z* 366, 97%; HPLC (214 nm) rt 16.94 min, 98%; Anal. ($C_{23}H_{31}N_3O$·3.0HCl·0.9H_2O) C, H, N.

N^1,N^1-Di(4-chlorobenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.22**). ^1H NMR (D_2O, 400 MHz)-δ 1.30−1.44 (m, 2H, CH_2), 1.55−1.75 (m, 4H, CH_2), 2.32−2.43 (m, 2H, β-CH_2), 3.13−3.65 (m, 6H, γ-CH_2, CH_2), 4.48 (s, 2H, CH_2Ph), 4.50 (s, 2H, CH_2Ph), 4.65 (t, 1H, α-CH), 7.48−7.53 (m, 4H, H_{arom}), 7.59−7.63 (m, 4H, H_{arom}); MS (ES^+) *m/z* 434 $(M + H)^+$; LC-MS rt 14.5 min, *m/z* 434, 94%; HPLC (214 nm) rt 25.56 min, 100%.

N^1-(4-Chlorobenzyl)-N^1-methyl-4-oxo-4-(1-piperidinyl)-1,3-butanediamine Dihydrochloride (**7.23**). To a mixture of **5** in MeOH was added acetic acid, 4-chlorobenzaldehyde, and $NaCNBH_3$. After completion of the reaction, the solvent was evaporated and extracted with dichloromethane and 1 N NaOH. The organic layer was separated, dried over Na_2SO_4, and evaporated. The residue was dissolved in acetic acid, and an excess of paraformaldehyde and $NaCNBH_3$ was added. After completion of the reaction, the solvent was removed. The residue was extracted with 2 N NaOH and dichloromethane. The organic layer was dried and evaporated. The residue was purified by preparative TLC using EtOAc/NH_4OH (99:1) as eluent. Final deprotection of the *tert*-butyloxycarbonyl group was done as described above. ^1H NMR (D_2O, 400 MHz) δ 1.29−1.74 (m, 6H, CH_2), 2.23−2.52 (m, 2H, β-CH_2), 2.97 (s, 3H, CH_3), 3.19−3.30 (m, 2H, γ-CH_2), 3.41−3.58 (m, 4H, CH_2), 4.47 (s, 2H, CH_2Ph), 4.67−4.73 (m, 1H, α-CH), 7.43 (d, 2H, H_{arom}), 7.53 (d, 2H, H_{arom}); MS (ES^+) *m/z* 324 $(M + H)^+$; LC-MS rt 8.6 min, *m/z* 324, 99%; HPLC (214 nm) rt 16.04 min, 98%; Anal. ($C_{17}H_{26}ClN_3O$·3.7HCl·0.6diethyl ether) C, H, N.

4-Oxo-N^1-phenyl-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrochloride (**7.25**). After a mixture of (*o*-biphenyl)P(*t*-Bu)$_2$ (0.25 mmol) and $Pd_2(dba)_3$ (0.25 mmol; 20 mol % Pd) in toluene (4 mL) was stirred for 10 min under N_2 atmosphere, it was added to a solution of compound **5** (3 mmol), bromobenzene (2.5 mmol), and NaO*t*-Bu (3.5 mmol) in dry toluene (6 mL). The mixture was stirred overnight at 40 °C under N_2 atmosphere and diluted with dichloromethane and H_2O. The organic phase was separated and washed several times with H_2O, dried over Na_2SO_4, and evaporated. The residue was purified by preparative TLC using EtOAc/hexane (1:1) as eluent. Final deprotection was done as descrived above. ^1H NMR (D_2O, 400 MHz) δ 1.21−1.24 (m, 1H, CH_2), 1.43−1.68 (m, 5H, CH_2), 2.26−2.39 (m, 2H, β-CH_2), 3.37−3.76 (m, 6H, γ-CH_2, CH_2), 4.70 (t, 1H, α-CH), 7.52−7.54 (m, 2H, H_{arom}), 7.63−7.69 (m, 3H, H_{arom}); MS (ES^+) *m/z* 262 $(M + H)^+$; LC-MS rt 10.9 min, *m/z* 262, 98%; HPLC (214 nm) rt 22.66 min, 98%.

Synthesis of Compounds 7.26 and 7.27. To a solution of **5** (1 equiv) in pyridine (10 mL) was added acetic anhydride (**7.26**) or benzoyl chloride (**7.27**) (5 equiv). The mixture was stirred overnight, and the solvent was evaporated. The residue was purified by preparative TLC using EtOAc/MeOH (95:5) (**7.26**) or EtOAc/hexane (1:1) as eluent (**7.27**).

N-[3-(*S*)-Amino-4-oxo-4-(1-piperidinyl)butyl]acetamide Hydrochloride (**7.26**). ^1H NMR (D_2O, 400 MHz) δ 1.55−1.76 (m, 6H, CH_2), 2.03−2.18 (m, 5H, CH_3, β-CH_2), 3.30−3.64 (m, 6H, γ-CH_2, CH_2), 4.41−4.67 (m, 1H, α-CH); MS (ES^+) *m/z* 228 $(M + H)^+$; HPLC (214 nm) rt 6.50 min, 96%.

N-[3-Amino-4-oxo-4-(1-piperidinyl)butyl]benzamide Hydrochloride (**7.27**). ^1H NMR (D_2O, 400 MHz) δ 1.44−1.73 (m, 6H, CH_2), 2.25−2.30 (m, 2H, β-CH_2), 3.48−3.70 (m, 6H, CH_2, γ-CH_2), 4.61 (t, 1H, α-CH), 7.58−7.84 (m, 5H, H_{arom}); MS (ES^+) *m/z* 290 $(M + H)^+$; LC-MS rt 10.1 min, *m/z* 290, 98%; HPLC (214 nm) rt 15.71 min, 99%; Anal. ($C_{16}H_{23}N_3O_2$·2.0HCl·0.1diethyl ether) C, H, N.

Synthesis of Compounds 7.28 and 7.29. To a solution of the compound **5** (1 equiv) in DMF was added $KHCO_3$ (1.2 equiv) and 2-chloro-3-nitropyridine (**7.28**) or 6-chloronicotinonitrile (**7.29**) (1 equiv). The mixture was stirred at 90 °C for 8 h. The solution was extracted with EtOAC and saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4 and evaporated, and the residue was purified by preparative TLC using EtOAc/hexane (1:1) as eluent.

N^1-(3-Nitro-2-pyridinyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Hydrochloride (**7.28**). ^1H NMR (D_2O, 400 MHz) δ 1.40−1.68 (m, 6H, CH_2), 2.22−2.40 (m, 2H, β-CH_2), 3.29−3.93 (m, 6H, γ-CH_2, CH_2), 4.50−4.61 (m, 1H, α-CH), 6.87−6.96 (m, 1H, H_{arom}), 8.41−8.50 (m, 1H, H_{arom}), 8.56−8.65 (m, 1H, H_{arom}); MS (ES^+) *m/z* 308 $(M + H)^+$; LC-MS rt 9.3 min, *m/z* 308, 96%; HPLC (214 nm) rt 17.10 min, 100%.

6-{[3-(*S*)-Amino-4-oxo-4-(1-piperidinyl)butyl]amino}nicotinonitrile Hydrochloride (**7.29**). ^1H NMR (D_2O, 400 MHz)δ 1.45−1.74 (m, 6H, CH_2), 2.22−2.33 (m, 2H, β-CH_2), 3.46−3.65 (m, 6H, γ-CH_2, CH_2), 4.58−4.66 (m, 1H, α-CH), 6.97 (d, 1H, H_{arom}), 7.96 (d, 1H, H_{arom}), 8.49 (s, 1H, H_{arom}); MS (ES^+) *m/z* 288 $(M + H)^+$; LC-MS rt 4.9 min, *m/z* 288, 99%; HPLC (214 nm) rt 14.77 min, 99%.

Synthesis of Compounds 8.1 and 8.2. To a stirred solution of 1-*H*-pyrazole-1-[*N*,*N*′-bis(*tert*-butyloxycarbonyl)]-carboxamide (1.2 mmol) in ACN/H_2O (95:5, 20 mL) was added compound **5** (for **8.1**) or **6** (for **8.2**) (1.2 mmol) and DIEA (3.6 mmol). The mixture was refluxed for 2 h. After completion of the reaction, the solvent was removed under reduced pressure, and the residue was purified by preparative TLC using DCM/MeOH (95:5) as eluent. For **8.1**, final Boc-deprotection was done as described above. For **8.2,** the benzyloxycarbonyl group was first removed by hydrogenolysis, followed by Boc-removal according to the general procedure.

N-[3-(*S*)-Amino-4-oxo-4-(1-piperidinyl)butyl]guanidine Bis-(trifluoroacetate) (**8.1**). ^1H NMR (D_2O, 400 MHz) δ 1.58−1.78 (m, 6H, CH_2), 2.21−2.26 (m, 2H, β-CH_2), 3.42−3.45 (m, 2H, γ-CH_2), 3.54−3.63 (m, 4H, CH_2), 4.67 (t, 1H, α-CH); MS (ES^+) *m/z* 228 $(M + H)^+$; HPLC (214 nm) rt 5.61 min, 95%.

N-[3-Amino-1-(*S*)-(1-piperidinylcarbonyl)propyl]guanidine Bis-(trifluoroacetate) (**8.2**). ^1H NMR (D_2O, 400 MHz) δ 1.59−1.78 (m, 6H, CH_2), 2.08−2.32 (m, 2H, β-CH_2), 3.07−3.23 (m, 2H, γ-CH_2), 3.54−3.65 (m, 4H, CH_2), 4.85−4.90 (m, 1H, α-CH); MS (ES^+) *m/z* 228 $(M + H)^+$. LC-MS rt 1.1 min, *m/z* 228, 100%; HPLC (214 nm) rt 12.05 min, 99%.

General Procedure for the Reductive Amination in Solution Phase (Compounds 9.1 and 9.2). Compound **6** (1.5 equiv mmol) was dissolved in methanol (15 mL) dried over molecular sieves. The appropiate aldehyde (1equiv), acetic acid (5 equiv), and $NaCNBH_3$ (0.8 equiv) were added. The mixture was stirred overnight at room temperature. The solvent was removed in vacuo, and the residue was purified on preparative TLC. Finally, compounds **9.1** and **9.2** were obtained after deprotection of the benzyloxycarbonyl (Z) group.

General Procedure for Z-Removal by Acidolysis. Deprotection of the benzyloxycarbonyl (Z) group was done by acidolysis: the compound resulting from the previous step was dissolved in 30% HBr in acetic acid. After completion of the deprotection, which was monitored by TLC, the volatile part was removed in vacuo. The residue was coevaporated several

times with diethyl ether and was finally precipitated in diethyl ether. Finally, the precipitate was lyophilized from H_2O.

N^3-Benzyl-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine Dihydrobromide (**9.1**). ^{1}H NMR (D_2O, 400 MHz) δ 1.52−1.72 (m, 6H, CH_2), 2.27−2.37 (m, 2H, β-CH_2), 3.06−3.15 (m, 2H, γ-CH_2), 3.42−3.59 (m, 4H, CH_2), 4.26−4.37 (m, 2H, CH_2), 4.61−4.68 (m, 1H, α-CH), 7.45−7.60 (m, 5H, H_{arom}); MS (ES^+) *m/z* 276 $(M + H)^+$; HPLC (214 nm) rt 10.63 min, 98%.

4-Oxo-4-(1-piperidinyl)-N^3-(4-piperidinyl)-1,3-(*S*)-butanediamine Trihydrobromide (**9.2**). ^{1}H NMR (D_2O, 400 MHz) δ 1.64−1.78 (m, 6H, CH_2), 1.96−2.09 (m, 2H, β-CH_2), 2.36−2.50 (m, 4H, CH_2), 3.12−3.24 (m, 4H, CH_2), 3.67−3.71 (m, 7H, CH_2), 4.89−4.92 (m, 1H, α-CH); MS (ES^+) *m/z* 269 $(M + H)^+$; HPLC (214 nm) rt 5.01 min, 96%.

Synthesis of Compounds 10.1 and 10.2. Compounds **10.1** and **10.2** were obtained by a reductive amination of Boc-L-Dab-OH and benzaldehyde (**10.1**) or 4-chlorobenzaldehyde (**10.2**) according to the procedure described for compounds **9.1** and **9.2**. After evaporation of the solvent, the residue was used as such in the next step where a Boc-protection group was introduced. To a solution of the compound obtained in previous step (1 equiv) in dioxane/H_2O (1:1) mixture (30 mL) was added TEA (3 equiv) and Boc_2O (1.1 equiv). After the mixture was stirred for several hours, dioxane was evaporated, and the aqueous phase was acidified to pH < 2 and extracted 2 times with EtOAc. The combined layers were dried over Na_2SO_4 and evaporated. Purification was done by filtration over silicagel using diethyl ether as eluent.

N^1-(4-Chlorobenzyl)-N^3-methyl-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine (**7.24**). Compound **10.2** was used in a coupling reaction with piperidine as described above. The obtained and purified compound (1.25 mmol, 1 equiv) was dissolved in THF (3 mL) and a catalytic amount of H_2O. This solution was added dropwise to a suspension of sodium hydride (60% dispersion in mineral oil; 2.5 mmol, 2 equiv) in THF (4 mL) while an internal temperature of 17−20 °C was maintained. The mixture was stirred at the same temperature for 10 min, and dimethyl sulfate (2.25 mmol, 1.8 equiv) was added dropwise. The stirring was continued at the same temperature for 20 min, and the reaction was monitored by TLC. The reaction mixture was quenched with 30% ammonium hydroxide (2 mL), which was added dropwise, and the stirring was continued for 1 h. The mixture was diluted with dichloromethane and H_2O. The organic phase was separated, washed with H_2O, dried over Na_2SO_4, and evaporated. The residue was purified by preparative TLC using EtOAc/hexane (40:60) as eluent. Final deprotection was done as described above. ^{1}H NMR (D_2O, 400 MHz) δ 1.44−1.74 (m, 6H, CH_2), 2.31−2.40 (m, 2H, β-CH_2), 2.75 (s, 3H, CH_3), 3.08−3.16 (m, 2H, γ-CH_2), 3.49−3.60 (m, 4H, CH_2), 4.33 (s, 2H, CH_2Ph), 4.64 (t, 1H, α-CH), 7.49−7.51 (m, 2H, H_{arom}), 7.56−7.58 (m, 2H, H_{arom}); MS (ES^+) *m/z* 324 $(M + H)^+$; LC-MS rt 8.3 min, *m/z* 324, 99%; HPLC (214 nm) rt 22.64 min, 100%.

Synthesis of 3- or 4-Fluoropiperidine and 1,2,3,6-Tetrahydropyridine. Synthesis started from, respectively, 3-piperidinol and 4-piperidinol. A *tert*-butyloxycarbonyl (Boc) protecting group was introduced according to the described procedure. The Boc-protected 3- or 4-piperidinol (1 equiv, 2 mmol) was dissolved in dry dichloromethane (15 mL), and DAST (1.3 equiv, 2.6 mmol) was added to the solution. The mixture was stirred for 90 min at 0 °C under N_2 atmosphere. The solution was diluted with 15 mL of dichloromethane, and the reaction was quenched by means of 20 mL of saturated $NaHCO_3$ solution. The organic phase was separated, and the aequous layer was extracted with EtOAc. The combined organic phases were dried over Na_2SO_4 and evaporated. In the case where 4-piperidinol was used as starting material, the reaction resulted in a mixture of Boc-4-fluoropiperidine and Boc-1,2,3,6-tetrahydropyridine (side product), which could both be isolated by flash chromatography (gradient elution, hexane (100%) to EtOAc (100%)). With 3-piperidinol as starting material, the Boc-3-fluoropiperidine could be obtained by flash chromatography. Deprotection of the Boc-protecting group was done according to the general procedure.

Synthesis of Compounds 11−18, 19.1, and 19.2. Synthesis started from, respectively, **10.1** or **10.2** by a coupling reaction as previously described with the proper amine compound, followed by a Boc-deprotection according to the described general procedure.

N^1-(4-Chlorobenzyl)-4-(2-methyl-1-piperidinyl)-4-oxo-1,3-(*S*)-butanediamine Bis(trifluoroacetate) (**11**). ^{1}H NMR (D_2O, 400 MHz) δ 1.04−1.27 (dd, 3H, CH_3), 1.31−1.78 (m, 6H, CH_2), 2.18−2.31 (m, 2H, β-CH_2), 2.84−3.31 (m, 3H, CH, γ-CH_2), 3.49−3.59 (m, 1H), 3.99−4.23 (m, 1H), 4.25 (d, 2H, CH_2Ph), 4.54−4.68 (m, 1H, α-CH), 7.41−7.52 (m, 4H, H_{arom}); LC-MS rt 9.4 min, *m/z* 324 $(M + H)^+$, 99%; HPLC (214 nm) rt 16.86 min, 98%.

N^1-(4-Chlorobenzyl)-4-(3-methyl-1-piperidinyl)-4-oxo-1,3-(*S*)-butanediamine Bis(trifluoroacetate) (**12**). ^{1}H NMR (D_2O, 400 MHz) δ 0.96−0.98 (m, 3H, CH_3), 1.19−1.96 (m, 5H, CH, CH_2), 2.26−2.37 (m, 2H, β-CH_2), 2.55−3.24 (m, 4H, CH_2, γ-CH_2), 3.65−3.73 (m, 1H, CH_2), 4.08−4.21 (m, 1H, CH_2), 4.33 (d, 2H, CH_2Ph), 4.67−4.77 (m, 1H, α-CH), 7.50−7.60 (m, 4H, H_{arom}); LC-MS rt 8.6 min, *m/z* 324 $(M + H)^+$, 100%; HPLC (214 nm) rt 15.81 min, 100%.

N^1-(4-Chlorobenzyl)-4-(4-methyl-1-piperidinyl)-4-oxo-1,3-(*S*)-butanediamine Bis(trifluoroacetate) (**13**). ^{1}H NMR (D_2O, 400 MHz) δ 0.90−1.05 (m, 4H, CH_3, CH), 1.10−1.29 (m, 1H, CH_2), 1.66−1.90 (m, 3H, CH_2), 2.23−2.38 (m, 2H, β-CH_2), 2.69−2.90 (t, 1H, CH_2), 3.09−3.30 (m, 3H, CH_2, γ-CH_2), 3.74−3.86 (m, 1H), 4.27−4.41 (m, 3H, CH_2Ph, CH), 4.63−4.75 (m, 1H, α-CH), 7.46−7.62 (m, 4H, H_{arom}); LC-MS rt 8.4 min, *m/z* 324 $(M + H)^+$, 100%; HPLC (214 nm) rt 16.33 min, 99.1%.

1-{2-(*S*)-Amino-4-[(4-chlorobenzyl)amino]butanoyl}-3-piperidinol Bis(trifluoroacetate) (**14**). ^{1}H NMR (D_2O, 400 MHz) δ ^{1}H NMR (D_2O, 400 MHz) δ 1.36−1.94 (m, 4H, CH_2), 2.17−2.36 (m, 2H, β-CH_2), 3.04−3.21 (m, 2H, γ-CH_2), 3.35−3.94 (m, 5H, CH_2, CH), 4.20−4.26 (m, 2H, CH_2Ph), 4.55−4.65 (m, 1H, α-CH), 7.39−7.53 (m, 4H, H_{arom}); LC-MS rt 2.7 min, *m/z* 326 $(M + H)^+$, 100%; HPLC (214 nm) rt 13.52 min, 96%.

1-{2-(*S*)-Amino-4-[(4-chlorobenzyl)amino]butanoyl}-4-piperidinol Bis(trifluoroacetate) (**15**). ^{1}H NMR (D_2O, 400 MHz) δ 1.35−1.55 (m, 2H, CH_2), 1.83−1.99 (m, 2H, CH_2), 2.18−2.29 (m, 2H, β-CH_2), 3.05−3.37 (m, 4H, γ-CH_2, CH_2), 3.65−3.77 (m, 1H), 3.89−4.09 (m, 2H), 4.24 (d, 2H, CH_2Ph), 4.60−4.66 (m, 1H, α-CH), 7.38−7.52 (m, 4H, H_{arom}); LC-MS rt 1.7 min, *m/z* 326 $(M + H)^+$, 96%; HPLC (214 nm) rt 12.75 min, 99%.

N^1-(4-Chlorobenzyl)-4-(3-fluoro-1-piperidinyl)-4-oxo-1,3-(*S*)-butanediamine Bis(trifluoroacetate) (**16**). ^{1}H NMR (D_2O, 400 MHz) δ 1.51−2.13 (m, 4H, CH_2), 2.16−2.39 (m, 2H, β-CH_2), 2.81−3.59 (m, 4H, γ-CH_2, CH_2), 3.69−4.04 (m, 1H), 4.16−4.45 (m, 1H), 4.25 (d, 2H, CH_2Ph), 4.58−4.70 (m, 1H, α-CH), 4.83−5.00 (m, 1H, 3-CH), 7.38−7.51 (m, 4H, H_{arom}); LC-MS rt 6.4 min, *m/z* 328 $(M + H)^+$, 49.6% and rt 7.3 min, *m/z* 328 $(M + H)^+$, 50.4%; HPLC (214 nm) rt 15.05 min, 96%.

N^1-(4-Chlorobenzyl)-4-(4-fluoro-1-piperidinyl)-4-oxo-1,3-(*S*)-butanediamine Bis(trifluoroacetate) (**17**). ^{1}H NMR (D_2O, 400 MHz) δ 1.72−1.98 (m, 4H, CH_2), 2.19−2.29 (m, 2H, β-CH_2), 3.04−3.19 (m, 2H, γ-CH_2), 3.36−3.87 (m, 4H, CH_2), 4.24 (s, 2H, CH_2Ph), 4.61−4.68 (m, 1H, α-CH), 4.87−5.08 (m, 1H, 4-CH), 7.39−7.52 (m, 4H, H_{arom}); LC-MS rt 6.7 min, *m/z* 328 $(M + H)^+$, 100%; HPLC (214 nm) rt 15.24 min, 99%; Anal. ($C_{16}H_{23}ClFN_3O \cdot 2.0CF_3COOH \cdot 0.5H_2O$) C, H, N.

N^1-(4-Chlorobenzyl)-4-(3,6-dehydro-1-(2*H*)-pyridinyl)-4-oxo-1,3-(*S*)-butanediamine Bis(trifluoroacetate) (**18**). ^{1}H NMR (D_2O, 400 MHz) δ 2.05−2.31 (m, 4H, β-CH_2, CH_2), 3.05−3.16 (m, 2H, γ-CH_2), 3.49−3.80 (m, 2H, CH_2), 3.90−4.07 (m, 2H, CH_2), 4.23−4.27 (d, 2H, CH_2Ph), 4.59−4.70 (m, 1H, α-CH), 4.65−4.76 (m, 1H, CH), 4.84−4.96 (m, 1H, CH), 7.40−7.52 (m, 4H, H_{arom}); LC-MS rt 7.8 min, *m/z* 308 $(M + H)^+$, 99%; HPLC (214 nm) rt 15.79 min, 100%.

Synthesis of Compounds 20 and 21. Dehydration of the amide function to the nitrile was done according to the following procedure: To a solution of, respectively, **19.1** or **19.2** (1 equiv) and imidazole (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. Finally, Boc-deprotection was done according to the described procedure.

1-[2-(*S*)-Amino-4-(benzylamino)butanoyl]-2-(*S*)-piperidinecarbonitrile Dihydrochloride (**20**). ^{1}H NMR (D_2O, 400 MHz) δ 1.52−2.12 (m, 6H, CH_2), 2.28−2.41 (m, 2H, β-CH_2), 3.11−3.29 (m, 2H, γ-CH_2), 3.34−3.45 (m, 1H, 5-CH_2), 3.81−3.95 (m, 1H, 5-CH_2), 4.34 (s, 2H, CH_2Ph), 4.67−4.83 (m, 1H, α-CH), 5.65 (s, 0.5H, 2-CH), 5.81 (s, 0.5H, 2-CH), 7.56 (s, 5H, H_{arom}); MS (ES^+) *m*/*z* 301 $(M + H)^+$; LC-MS rt 4.9 min, *m*/*z* 301, 93%; HPLC (214 nm) rt 14.04 min, 97%.

1-{2-(*S*)-amino-4-[(4-chlorobenzyl)amino]butanoyl}-2-(*S*)-piperidinecarbonitrile Dihydrochloride (**21**). ^{1}H NMR (D_2O, 400 MHz) δ 1.55−2.12 (m, 6H, CH_2), 2.31−2.36 (m, 2H, β-CH_2), 3.16−3.24 (m, 2H, γ-CH_2), 3.30−3.43 (m, 1H, 5-CH_2), 3.83−3.90 (m, 1H, 5-CH_2), 4.32 (s, 2H, CH_2Ph), 4.68−4.80 (m, 1H, α-CH), 5.64 (s, 0.5H, 2-CH), 5.80 (s, 0.5H, 2-CH), 7.49 (d, 2H, H_{arom}), 7.55 (d, 2H, H_{arom}); MS (ES^+) *m*/*z* 335 $(M + H)^+$; LC-MS rt 8.7 min, *m*/*z* 335, 92%; HPLC (214 nm) rt 16.31 min, 98%.

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 W. Bollaert and N. Lamoen is greatly appreciated.

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JM031122F