Investigation of Subsite Preferences in Aminopeptidase A (EC 3.4.11.7) Led to the Design of the First Highly Potent and Selective Inhibitors of This Enzyme

Christelle David,[†] Laurent Bischoff,[†] Hervé Meudal,[†] Aurélie Mothé,[†] Nadia De Mota,[‡] Sophie DaNascimento,[†] Catherine Llorens-Cortes,[‡] Marie-Claude Fournié-Zaluski,[†] and Bernard P. Roques*,[†]

Département de Pharmacochimie Moléculaire et Structurale, INSERM U266 − CNRS UMR 8600, UFR des Sciences Pharmaceutiques et Biologiques, 4, avenue de l'Observatoire, 75270 Paris Cedex 06, France, and Pathologie Vasculaire et Endocrinologie Rénale, INSERM U36, Collège de France, 3, rue d'Ulm, 75005 Paris Cedex, France

Received June 14, 1999

The study of the physiological roles of the membrane-bound zinc-aminopeptidase A (glutamyl aminopeptidase, EC 3.4.11.7) needs the design of efficient and selective inhibitors of this enzyme. An acute exploration of aminopeptidase A active site was performed by a combinatorial approach using (3-amino-2-mercapto-acyl)dipeptides able to fit its S_1, S_1', and S_2' subsites. This analysis confirmed that the S_1 subsite is optimally blocked by a glutamate or isosteric residues and demonstrated that the S_1' subsite is hydrophobic whereas the S_2' subsite recognizes preferentially negatively charged residues derived from aspartic acid. The optimization of these structural parameters led to the synthesis of nanomolar and subnanomolar inhibitors of aminopeptidase A such as $H_3N^+CH(CH_2CH_2SO_3^-)CH(SH)CO$-Ile-(3-COOH)Pro that exhibits a K_i of 0.87 nM. The best compounds were synthesized by a stereochemically controlled route. These first described highly potent inhibitors could allow studies about the role of physiological substrates of APA such as angiotensin II and cholecystokinin CCK_8 in the central nervous system.

Introduction

Aminopeptidase A (EC 3.4.11.7, APA) is a membrane-bound zinc metallopeptidase which is activated by calcium and specifically cleaves N-terminal acidic amino acids (Glu or Asp) from peptide substrates.[1,2] The determination of the amino acid sequence of APA in mice[3] or in humans[4,5] revealed a type II membrane protein of 946 residues, containing a small N-terminal cytosolic sequence and a large extracellular domain which includes the active site characterized by the consensus sequence HExxH···E found in numerous zinc metalloproteases.[6,7] Important physiological zinc enzymes belong to this family that contains the groups of neprilysin (NEP), angiotensin converting enzyme (ACE), and various aminopeptidases.[8] The main differences between these enzymes is due to their endopeptidase (or dipeptidylcarboxypeptidase) activities for the former and exopeptidase activities releasing the N-terminal amino acid for the latter. In the absence of the three-dimensional structure for these enzymes, their molecular mechanism of action has been postulated to be similar to that of the bacterial enzyme thermolysin, TLN, that is the prototype of these zinc metallopeptidases and for which a peptide hydrolysis mechanism has been deduced from crystallographic studies.[9] Thus, like in other enzymes of this large family,[10,11] the zinc atom in APA is coordinated by three residues: His 385 and His 389,[12,13] belonging to the consensus sequence, Glu 408 located at 19 residues from this sequence, and a water molecule. Furthermore, the third ligand of the ^{385}HExxH^{389} sequence, Glu 386, polarizes the zinc-coordinated water molecule and promotes its nucleophilic attack onto the carbonyl of the scissile peptide bond. As for TLN, the resulting tetrahedral intermediate might be stabilized by hydrogen bonds with Tyr 471.[14] The anionic site responsible for the exopeptidase specificity of APA, which releases N-terminal acidic amino acids, has been identified as Glu 352,[15] by analogy with Glu 350 of aminopeptidase N[16] (Scheme 1).

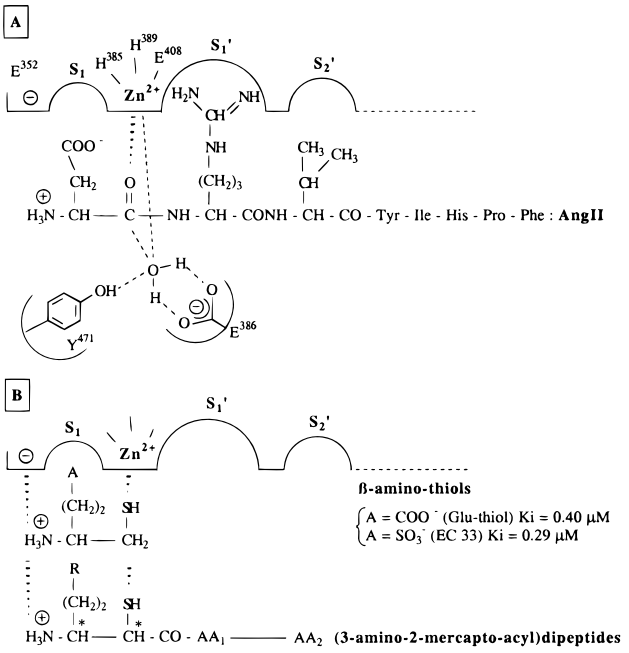
Despite significant differences in their substrate specificity, APA exhibits important analogies with aminopeptidase N (APN), another Zn-membrane-bound exopeptidase that cleaves N-terminal hydrophobic and basic residues. Thus, these peptidases have 33% of sequence homology (78% in the active site region) and are often found co-localized in various tissues where they could be involved in successive steps of enzymatic processes as this occurs in the angiotensin cascade. In this case, APA was shown to cleave in vivo angiotensin II (AngII) leading to angiotensin III (AngIII) whereas the latter was hydrolyzed by APN, giving angiotensin IV.[17] The hypertensive properties of AngII have been clearly established at the periphery, but the respective role of AngII and AngIII in brain is still questionable.[18] For a better understanding of the respective role of both aminopeptidases in brain and peripheral tissues, highly efficient and specific inhibitors of APA and APN were needed.

Amastatin[19] and bestatin[20] are currently used as APA and APN inhibitors, respectively, but neither of these compounds is selective, and amastatin was shown to be even more potent on APN (K_i = 19 nM) than on APA (K_i = 250 nM). Synthetic inhibitors for aminopeptidases have been previously obtained with β-amino-thiols of H_3N^+-CH(R)CH_2SH type that correspond to the mini-

* To whom correspondence should be addressed. Tel: (33)-1-43.25.50.45. Fax: (33)-1-43.26.69.18. E-mail: Roques@pharmacie.univ-paris5.fr.
[†] UFR des Sciences Pharmaceutiques et Biologiques.
[‡] Collège de France.

10.1021/jm9903040 CCC: $18.00
Published on Web 11/23/1999

Scheme 1. Schematic Representation of the Interaction of Angiotensin II A and Inhibitors B with APA Active Site



mal structure able to block these enzymes (Scheme 1). They are characterized by a free amino group required for the anionic site recognition, a thiol for zinc coordination and a side chain R for S_1 subsite interaction. In APN the S_1 subsite being essentially hydrophobic, potent inhibitors such as Leu-thiol [21] ($K_i = 22$ nM) and Met-thiol ($K_i = 11$ nM) [22] have been described. For APA, in which the S_1 subsite recognizes negatively charged residues, the same strategy afforded Glu-thiol (0.4 μM) and Asp-thiol (1.2 μM),[23] which have modest affinities for this enzyme. Furthermore, these inhibitors are nonspecific with K_i values of 0.25 μM and 7.5 μM on APN, respectively.

A significant improvement in the specific inhibition of APA was reported for a sulfonate-containing β-amino-thiol (EC33: $H_3N^+CH[(CH_2)_2SO_3^-]CH_2SH$)[24] with an inhibitory potency of 0.29 μM for APA and 25 μM for APN.

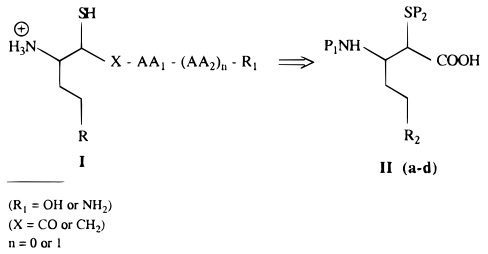
However, for accurate studies on the pharmacological roles of APA, both selectivity and affinity have to be increased.

In this paper, new inhibitors of this enzyme containing a thiol group as zinc ligand and a peptide backbone interacting with the S_1, S_1', and S_2' subsites were designed (Scheme 1). Different negatively charged side chains were selected to interact with the APA S_1 subsite, and using a combinatorial approach, residues interacting with the S_1' and S_2' subsites were investigated. A strong interaction with the S_2' subsite was obtained with negatively charged side chains. Selective inhibitors with nanomolar and subnanomolar inhibitory potencies were obtained by this way. Finally, a diastereoselective pathway was developped to synthesize these new inhibitors with a high yield, a critical requirement for possible therapeutical development.

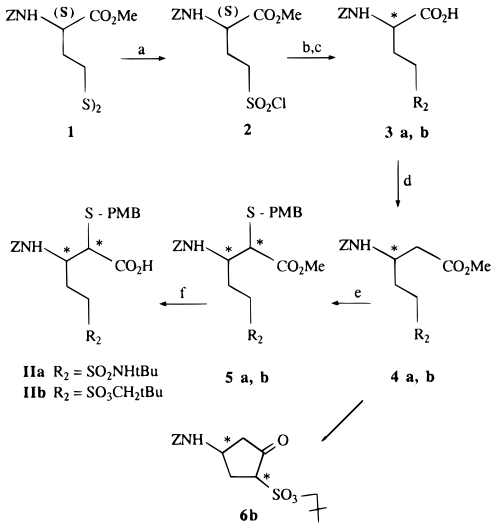
Results

Chemistry. APA is an exopeptidase, indicating that the N-terminal amino group is essential to ensure the selective hydrolysis of substrates. Moreover, APA cleaves N-terminal negatively charged amino acids (Asp or Glu). These two parameters have been taken into account in the design of the highly potent and selective inhibitors reported here. Thus, APA inhibitors corresponding to the general formula **I** were obtained by coupling various dipeptides with the synthon **II**, followed by a complete deprotection of the functional groups by classical methods of peptide synthesis (Scheme 2). However, two difficulties have to be solved: (i) this synthon must have its four functional groups orthogonally protected; (ii) it contains two asymmetric carbons whose stereochemistry has to be controlled all along the synthesis.

Scheme 2. Retrosynthetic Pathway of Aminopeptidase A Inhibitors



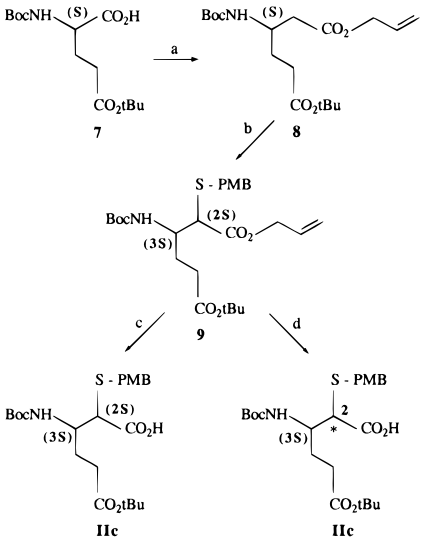
Scheme 3. Synthesis of the Synthons Sulfonamide **IIa** and Sulfonate **IIb**[a]



[a] Reagents and conditions: (a) Cl_2(g); (b) $tBuNH_2$ or $tBuCH_2OH$ in pyridine; (c) 1 M NaOH; (d) (1) iBuOCOCl, CH_2N_2; (2) Ph-COOAg, MeOH; (e) (1) LDA, (2) PMB-S-S-DNP; (f) 1 M NaOH. $* = R + S$ configuration.

In the first strategy, α-amino acids bearing the suitably protected side chain R_2 were used (Scheme 3) as starting materials. For the sulfonamide **IIa** and sulfonate **IIb** synthons, the corresponding α-amino acids were not commercially available and were prepared from a common precursor, (L)-homocystine (Scheme 3). After protection of the α-functions, the following steps consisted of the synthesis of *tert*-butyl sulfonamide (**3a**) or neopentyl sulfonate (**3b**)[25] side chains. Then, an homologation via Arndt−Eistert rearrangement resulted in the β-amino esters **4a,b** which underwent an electrophilic sulfenylation (**5a,b**) by means of the new reagent 2,4-dinitrophenyl-4-methoxybenzyl disulfide (PMB-S-S-DNP).[26] An alkaline hydrolysis of the methyl ester allowed the isolation of **IIa,b**.

Scheme 4. Synthesis of the Synthon Carboxylate **IIc**[a]



[a] Reagents and conditions: (a) (1) iBuOCOCl, CH_2N_2, (2) $PhCO_2Ag$, $CH_2{=}CH{-}CH_2OH$; (b) (1) LDA, (2) PMB-S-S-DNP; (c) $Pd(PPh_3)_4$; (d) 1 M NaOH. $*$ = *R* + *S* configuration.

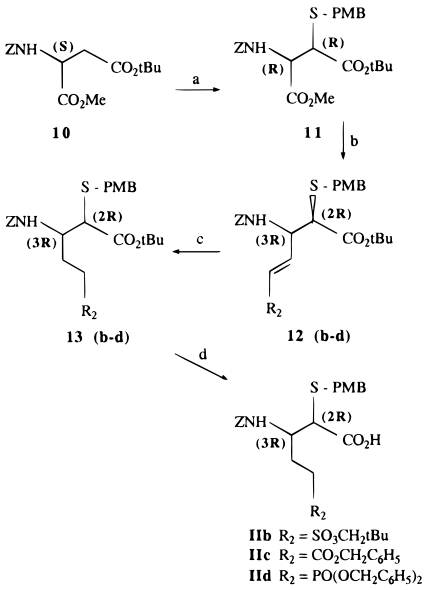
This classical method has been previously carried out for the synthesis of APN inhibitors[27] but afforded, in our case, a complete racemization at both the C_2 and C_3 carbons of these synthons during the saponification steps. The method was therefore improved for the synthesis of the carboxylate **IIc** (Scheme 4). Starting from commercially available Boc-(L)-Glu(OtBu)-OH **7**, the first step consisted of an Arndt−Eistert homologation in the presence of allylic alcohol, leading to **8**. Then, sulfenylation and deprotection of the ester moiety with $Pd(PPh_3)_4$ led to the 2*S*,3*S* diastereoisomer of **IIc**, the second 2*R*,3*S* isomer being obtained by classical saponification and separation of the epimerized form at the C_2 carbon.

However, the major problem encountered in the syntheses of sulfonate **IIb** and carboxylate **IIc** was the formation of byproducts during the sulfenylation step, resulting in very poor yields. In the sulfonate series, the main byproduct was the cyclic compound **6b** whose structure has been established by ^{1}H NMR spectroscopy and mass spectrometry. Easy formation of the carbanion adjacent to the sulfonate group and stability of the five-member ring explained the high yield of **6b** (Scheme 3).

To overcome these problems, a rapid and diastereoselective approach was developed by modification and improvement of a previously reported method using aspartic acid or glutamic acid diesters as chiral precursors[28] (Scheme 5).

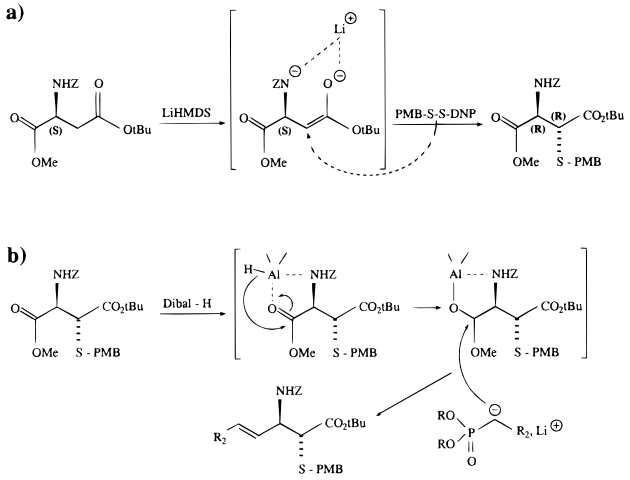
Thus, commercially available Z-(L)-Asp(OtBu)-OH was used as a starting material. The remaining α-carboxylate was esterified with diazomethane leading to **10** without the racemization observed when either Cs_2CO_3/CH_3I[29] or EDCI/DMAP/CH_3OH[30] were used as reagents. The next step consisted of the introduction of the protected thiol group in the *β* position of aspartate by electrophilic sulfenylation. As previously demonstrated,[26] the sulfenylation proceeded via an anti addition, leading essentially to the formation of **11** with the 2R and 3R configuration, the diastereomeric ratio being of 95:5 as measured by HPLC. This result is consistent

Scheme 5. Diastereoselective Synthesis of the Synthons **IIb**, **IIc**, or **IId**[a]



[a] Reagents and conditions: (a) (1) nBuLi, (2) PMB-S-S-DNP; (b) (1) nBuLi, $(RO)_2P(O)CH_2R_2$, (2) Dibal-H; (c) $[(PPh_3)CuH]_6$, H_2O; (d) TFA.

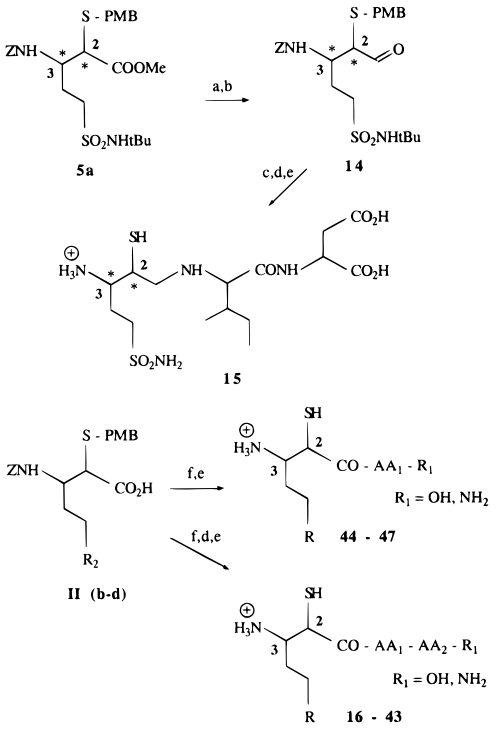
Scheme 6. (a) Proposed Mechanism of the Sulfenylation Slip and (b) Postulated Mechanism for the One-Pot Dibal-H Reduction−Wittig−Horner Olefination



with a preferential attack of the bulky sulfenylated agent, PMB-S-S-DNP, on the least hindered face of the dianion (Scheme 6a) without change in the spatial orientation of the C_3 substituents.

The α-methyl ester moiety was then used for the introduction of the side chain aimed at interacting with the S_1 subsite. Compound **11** underwent a smooth reduction of the methyl ester with Dibal-H leading to a postulated aluminoxyacetal intermediate[28] which was not isolated and was in situ submitted to a Wittig−Horner reaction (Scheme 6b). Unsaturated sulfonate **12b**, carboxylate **12c**, and phosphonate **12d** were synthesized by this way with a high stereoselectivity. The α,*β*-unsaturated esters needed a subsequent reduction in conditions allowing the conservation of the optical purity. However, sodium borohydride in alcohol led to a complete racemization at the C_2 carbon, and the presence of the thioether and benzyloxycarbonyl-protecting group was incompatible with catalytic hy-

Scheme 7. Coupling of the Synthons **II** and Deprotections of the Inhibitors[a]



[a] Reagents and conditions: (a) $NaBH_4$, LiCl; (b) DMSO, $(COCl)_2$; (c) $NaBH_4$, MeOH; (d) TFA, anisole; (e) HF, *m*-cresol; (f) BOP, DIEA, AA_1R' or AA_1-AA_2R'. $* = R + S$ configuration.

drogenation methods. Thus, another reagent $[(Ph_3P)CuH]_6$ specific for the reduction of α,β-unsaturated esters in neutral medium[31,32] was tested. The reduction was complete at 25 °C within about 3 h with only a limited change in the diastereomeric ratio at the C_2 carbon (80:20 for **13b**). Finally, in the last step, a deprotection of the tertiobutyl ester with TFA and anisole as a scavenger was carried out, giving the synthons **IIb**−**d** with high overall yields and under the 2*R*,3*R* (80%) and 2*S*,3*R* (20%) configurations. The same strategy with Z-(D)-Asp(OtBu)-OH as chiral precursor gave the synthons **IIb**−**d** with the 2*S*,3*S* (80%) and 2*R*,3*S* (20%) absolute configurations.

The coupling step of the synthons **IIa**−**d** with the suitable dipeptides was performed either by solid-phase method for the libraries or by liquid-phase method with BOP reagent for the other inhibitors (Scheme 7) followed by two subsequent deprotections by TFA and liquid HF in the presence of the suitable scavengers. The purification on a silica gel column or by semipreparative HPLC on a reverse-phase silica column allowed the separation of the isomers. The protected unnatural amino acids Sal (sulfoalanine), hSal (homo-sulfoalanine), and (3-COOH)-Pro (3-carboxyproline) introduced in **36**−**43** were synthesized as described.[33,34]

Comparative Inhibition of APA and APN Activities. In a first step, a combinatorial library of pseudotripeptides[35] derived from the sulfonamide synthon **IIa** was studied to discriminate APA and APN active sites. The inhibitory potencies of the 19 mixtures of pseudotripeptides containing well-defined amino acids in the P_1' position (Figure 1A) showed that a relatively efficient recognition of APA was obtained with Ile (45% inhibition at 10^{-6} M) and Tyr (30% at 10^{-6} M) in P_1' position, when

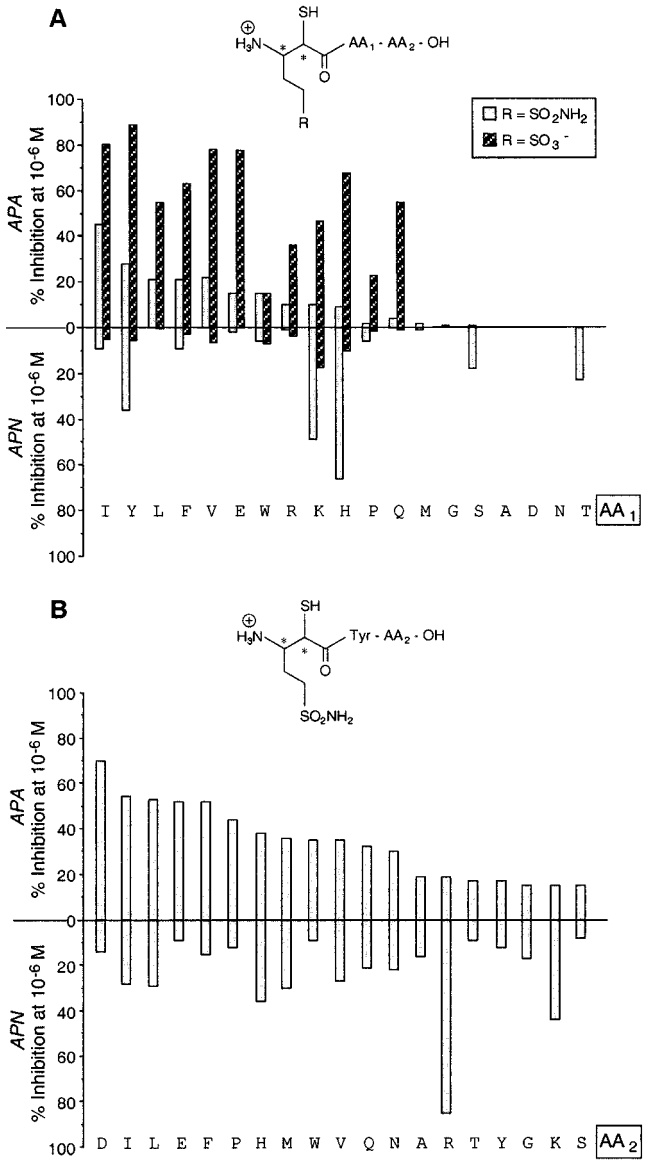
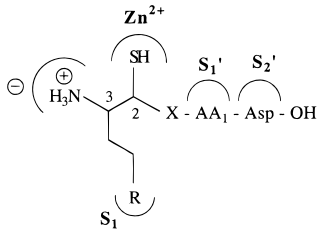


Figure 1. Histograms representing the percentage of inhibition obtained at 10^{-6} M with pseudotripeptides derived from the synthons **IIa** or **IIb** for aminopeptidase A and aminopeptidase N. The general formulas of these mixtures are given above the histogram. (A) Nineteen mixtures of pseudotripeptides were tested. The formula of AA_1 is indicated below the histogram, and AA_2 consisted of an equimolar mixture of the natural amino acids (except Cys). (B) Nineteen pseudotripeptides were tested. The formula of AA_2 is indicated below the histogram.

for APN the preferred P_1' residue was His (66% inhibition at 10^{-6} M). It was interesting to note that better results were obtained on APA with a combinatorial library containing the sulfonate synthon **IIb** in P_1 position and that an inversion of the relative activities of Tyr (88% inhibition at 10^{-6} M) and Ile (80% inhibition at 10^{-6} M) in P_1' position was observed (Figure 1A).

Accordingly a second library with the sulfonamide synthon in P_1, a Tyr residue in P_1', and 19 different natural amino acids in the P_2' position was tested to discriminate the S_2' subsites of APA and APN. The results (Figure 1B) showed a significant preference in S_2' subsites for Asp (70% inhibition at 10^{-6} M) in the case of APA and for Arg (85% inhibition at 10^{-6} M) in the case of APN.

Table 1. Inhibitory Potencies of Pseudotripeptide Inhibitors Bearing Various Side Chains in the P_1 Position for APA and APN[a]



				absolute configuration		K_i (nM)[a]	
no.	R	X	AA_1	C_2	C_3	APA	APN
16	SO_2NH_2	CO	Ile	*RS*	*RS*	80 ± 10	28000 ± 5000
15	SO_2NH_2	CH_2	Ile	*RS*	*RS*	51 ± 6	3400 ± 200
17	SO_2NH_2	CO	Tyr	*RS*	*RS*	150 ± 20	7000 ± 200
18	CO_2^-	CO	Ile	*R*	*S*	16 ± 2	3900 ± 500
19	CO_2^-	CO	Ile	*S*	*S*	13 ± 2	120 ± 2
20	CO_2^-	CO	Ile	*S*	*R*	3.56 ± 0.01	>10000
21	CO_2^-	CO	Ile	*R*	*R*	267 ± 50	>10000
22	SO_3^-	CO	Ile	*R*	*S*	50 ± 5	>100000
23	SO_3^-	CO	Ile	*S*	*S*	20 ± 1	23000 ± 2000
24	SO_3^-	CO	Ile	*S*	*R*	3.2 ± 0.1	>100000
25	SO_3^-	CO	Ile	*R*	*R*	53 ± 2	>100000
26	PO_3H^-	CO	Ile	*S*	*R*	12 ± 1	>10000
27	PO_3H^-	CO	Ile	*R*	*R*	910 ± 9	>10000

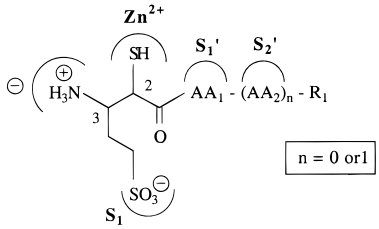
[a] The values are the mean ± SEM of three independent experiments.

Taking these results into account, Ile-Asp and Tyr-Asp were incorporated as $P_1'-P_2'$ components in the various inhibitors described in Tables 1 and 2.

In the sulfonamide series, the compounds were tested as mixtures of stereoisomers. Compound **16** inhibited APA in the 10^{-8} M range with a selectivity factor versus APN of about 350. Replacement of the amide bond in **16** by a methylenamino group in **15** did not significantly modify the K_i value for APA, but the significant increase in APN recognition resulted in a loss of selectivity (factor 68). In the same way, the replacement of Ile by Tyr in the AA_1 position (compound **17**) was responsible for both a decreased recognition of APA and an increased potency for APN in agreement with the results of the sulfonamide library (selectivity factor: 47).

The dipeptide Ile-Asp was then coupled to the carboxylate and sulfonate synthons **IIc** and **IIb**. The synthesis and separation of the four stereoisomers allowed the determination of the stereodependence of APA and APN for these inhibitors. As shown in Table 1, the 2*S*,3*R* configuration (compounds **20** and **24**) led to the best inhibitors with K_i values in the nanomolar range. In the carboxylate series, compounds **18** and **19**, which have the 3*S* configuration, were slightly less efficient (factor 4 or 5) whatever the configuration at the C_2 carbon. A significant decrease in activity was obtained for the 2*R*,3*R* isomer (compound **21**). In the sulfonate series, compounds **22**, **23**, and **25** exhibited about the same potencies in the 20−50 nanomolar range. Concerning APN inhibition, it appeared that the stereodependence was different, since only the 2*S*,3*S* isomer was relatively potent in the carboxylate series (compound **19**), leading to a selectivity factor of 10 for APA. The three other isomers could be considered as

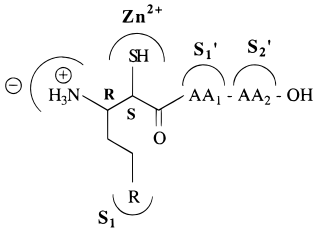
Table 2. Importance of Hydrophilic Residues in the P_2' Position of Pseudotripeptides for APA and APN Inhibition[a]



					absolute configuration		K_i (nM)[a]	
no.	AA_1	*n*	AA_1	R_1	C_2	C_3	APA	APN
44	Ile	0	/	OH	*S*	*R*	40 ± 0.3	>10000
45	Ile	0	/	OH	*R*	*R*	1200 ± 60	>10000
46	Ile	0	/	NH_2	*S*	*R*	145 ± 19	>10000
47	Ile	0	/	NH_2	*R*	*R*	4200 ± 40	>10000
28	Tyr	1	Asp	OH	*S*	*R*	4.3 ± 0.1	31000 ± 4000
29	Tyr	1	Asp	OH	*R*	*R*	27 ± 4	48000 ± 1000
30	Ile	1	Asp	NH_2	*S*	*R*	15 ± 2	44000 ± 7000
31	Ile	1	Asp	NH_2	*R*	*R*	470 ± 3	51000 ± 5000
32	D-Ile	1	Asp	OH	*S*	*R*	14.7 ± 0.6	>100000
33	D-Ile	1	Asp	OH	*R*	*R*	292 ± 14	>100000
34	Ile	1	D-Asp	OH	*S*	*R*	5.36 ± 0.02	>100000
35	Ile	1	D-Asp	OH	*R*	*R*	283 ± 6	>100000
36	Ile	1	Sal	OH	*S*	*R*	3.6 ± 0.2	>5000
37	Ile	1	Sal	OH	*R*	*R*	83.3 ± 0.6	>5000
38	Tyr	1	hSal	OH	*S*	*R*	9.3 ± 0.1	4600 ± 400
39	Tyr	1	hSal	OH	*R*	*R*	73.3 ± 0.6	7500 ± 800
40	Ile	1	(3*R*)(3-COOH)Pro	OH	*S*	*R*	0.873 ± 0.006	16400 ± 500
41	Ile	1	(3*R*)(3-COOH)Pro	OH	*R*	*R*	110 ± 10	>10000
42	Ile	1	(3*S*)(3-COOH)Pro	OH	*S*	*R*	3.83 ± 0.03	4950 ± 420
43	Ile	1	(3*S*)(3-COOH)Pro	OH	*R*	*R*	240 ± 11	>10000

[a] The values are the mean ± SEM of three independent experiments.

Table 3. Inhibitory Potencies of the Most Potent Inhibitors of APA When Tested on Neutral Endopeptidase and Angiotensin-Converting Enzyme[a]



				K_i (nM)[a]		
no.	R	AA_1	AA_2	APA	NEP	ACE
20	CO_2^-	Ile	Asp	3.56 ± 0.01	4.0 ± 0.8	1800 ± 400
24	SO_3^-	Ile	Asp	3.2 ± 0.1	22 ± 8	1200 ± 100
26	PO_3H^-	Ile	Asp	12 ± 1	5.4 ± 0.8	2200 ± 400
28	SO_3^-	Tyr	Asp	4.3 ± 0.1	34 ± 4	1800 ± 200
36	SO_3^-	Ile	Sal	3.6 ± 0.2	17 ± 2	1210 ± 10
38	SO_3^-	Tyr	hSal	9.3 ± 0.1	520 ± 30	660 ± 20
40	SO_3^-	Ile	(3*R*)(3-COOH)Pro	0.873 ± 0.006	219 ± 1	259 ± 8
42	SO_3^-	Ile	(3*S*)(3-COOH)Pro	3.83 ± 0.03	520 ± 40	1090 ± 50

[a] The values are the mean ± SEM of three independent experiments.

almost inactive. In the sulfonate series (compounds **22**−**25**) all the stereoisomers were inactive on APN ($K_i > 10^{-5}$ M), showing a very high selectivity for APA. With the phosphonate synthon **IId**, only the compounds with the 3*R* configuration (**26** and **27**) were synthesized. They were less active than the corresponding carboxylates (**20**, **21**) and sulfonates (**24**, **25**), but the selectivity versus APN was conserved.

The sulfonate synthon **IIb**, leading to the most efficient and selective inhibitors of APA, was therefore used to synthesize new inhibitors with the aim to investigate hypotheses concerning APA active site. The data are reported in Table 2.

The first question was to determine the importance of the residue which interacts with the putative S_2' subsite of APA. The absence of the C-terminal aspartate (compounds **44** and **45**) induced a significant loss of activity. The amidification of these pseudopeptides (compounds **46** and **47**) was also detrimental for APA inhibition.

The second question was the nature of the AA_1 residue, fitting the S_1' subsite of the enzymes. In the sulfonate library, the dipeptides Tyr-AA_2 seem to be preferred to Ile-AA_2 although Ile appeared also as a good residue for interaction with the S_1' subsite (Figure 1A). The inhibitory potencies of **28** and **29** for APA show that they are not significantly different from **24** and **25**, indicating that both Tyr and Ile could be introduced as P_1' components in the inhibitors. Moreover, **30** and **31**, the C-terminal amide analogues of **24** and **25**, were less potent with K_i values in the 10^{-8}−10^{-7} M range.

(D)-Amino acids were introduced in position P_1' (compounds **32** and **33**) and P_2' (compounds **34** and **35**), and the activities of the inhibitors were compared with those of the parent compounds, **24** and **25**. A slight decrease in the inhibitory potencies was observed, indicating a relatively low influence of this parameter.

Finally, the aspartate residue in P_2 position was replaced by sulfonate (Sal and hSal) analogues. The compounds **36**−**39** obtained showed a slightly decreased affinity for APA as compared to the parent compounds **24** and **25**. The C-terminal Asp was then replaced by its cyclic analogue 3-carboxy-Pro which introduced a supplementary asymmetric center (at the C_3 of the proline ring) in these newly synthesized inhibitors. As expected from computer analysis (data not shown) of the spatial orientation of the C-terminal carboxyl group in Asp and 3-carboxy-Pro, the 2*S*,3*R* configuration of the sulfonate synthon was preferred (compounds **40** and **42** compared to **41** and **43**) and the 3*R* configuration of the 3-carboxy proline moiety (**40**) led to the most efficient APA inhibitor described until now with a subnanomolar affinity (K_i = 0.8 nM).

The inhibitory potencies of the most active APA inhibitors in this series were measured versus two other zinc metalloenzymes, angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) (Table 3). All the compounds tested have K_i values in the 10^{-6} M range on ACE but act as efficient NEP inhibitors. Compounds **20** and **26** have nanomolar activities on this enzyme. However, the introduction of a sulfonate group in R position with a hSal (**38**) or (3-COOH)Pro (**40** and **42**) as P_2' compounds allowed a selectivity factor for APA between 50 and 250 to be obtained.

Discussion

The aim of this study was to develop highly efficient and selective inhibitors of APA. Previous studies have shown that β-amino-thiols derived from natural[23] or nonnatural amino acids[24] containing negatively charged side chains could inhibit APA, but also APN, with K_i values in the 10^{-6}−10^{-7} M range. However, an increase in the acidity of the charged group with the successive introduction of a carboxylate ($pK_a \sim 4.5$), a phosphonate ($pK_a \sim 2.5$), or a sulfonate ($pK_a \sim -0.5$) afforded a selectivity factor versus APN of about 100, which was not due to an improvement in the inhibitory potency for APA but to a decrease in APN recognition.[24] Consequently, the design of selective and highly potent APA inhibitors needed a complete exploration of the APA active site in order to selectively optimize this enzyme recognition essentially at the level of the S_1, S_1', and S_2' subsites (Scheme 1).

The (3-amino-2-mercapto-acyl)dipeptides obtained by this way show that a large improvement in the inhibitory potencies toward APA is obtained with compounds interacting with the three subsites S_1, S_1', and S_2'. Indeed, β-amino-thiols, only interacting with the S_1 subsite, have K_i values of about 0.3 μM. Pseudodipeptides, such as compounds **44** and **46**, are slightly more active with K_i values in $10^{-7}-10^{-8}$ M range, whereas pseudotripeptides such as **20**, **24**, **28**, **36**, **40**, and **42** have K_i values in the nanomolar range with an increased potency on APA, as compared to β-amino-thiols, by at least 2 orders of magnitude. In parallel a loss of APN recognition was observed, leading to very selective inhibitors of APA. This could be partly due to the increased hydrophilicity of these compounds, due to the presence of a negatively charged amino acid in N- and C-terminal positions since the APN active site seems to be essentially hydrophobic,[36] but also to structural parameters. Indeed in APN, the passage from β-amino-thiols ($K_i \sim 10$ nM) to thiobestatin analogues[37] ($K_i \sim 4$ μM) or to mercapto-pseudotripeptides ($K_i \sim 20$ nM) (Taté et al., unpublished results) did not increase the inhibitory potency, indicating that these compounds could not optimally fit the enzyme active site. For APN, nanomolar or subnanomolar activities have been obtained, until now, only for phosphinic inhibitors.[38] The presence of a negatively charged P_2' residue is crucial for an optimal recognition of APA active site. The putative ionic interaction involving this anionic group and its positively charged counterpart in the S_2' pocket seems to require a precise orientation since Asp and its sulfonate analogue are significantly more efficient than Glu or its sulfonate analogue. Moreover, the 3-carboxy proline derivative, which represents a constrained analogue of Asp, is preferred under its cis rather than its trans configuration, the cis configuration fitting optimally the spatial orientation of the side chain carboxylate of Asp. The enhanced affinity is very likely due to the intrinsic reduction in the degree of freedom of the 3-carboxyl part of the proline ring as compared to the aspartic side chain, thus decreasing the thermodynamically unfavorable entropy factor during the binding to the peptidase. The S_1' subsite seems to be relatively hydrophobic and interacts as well with aliphatic (Ile, Val, Leu) as aromatic (Tyr, Phe, His) residues, but surprisingly also accepts Glu or Gln residues (Figure 1). The absolute configuration of the 3-amino-2-mercapto-acyl moiety is important, indicating, once more, a precise relative orientation for the amino and mercapto functions. This stereochemical requirement is identical to that described for bestatin or amastatin for optimal inhibition of various aminopeptidases such as APN, aminopeptidase B (APB), or leucine aminopeptidase (LAP).[37,39] This suggests that the mode of interaction of the 3-amino-2-mercapto-acyl moiety could be similar for all these enzymes and that the crystallized structure of amastatin and bestatin[40,41] with LAP could represent a general model accounting for zinc-aminopeptidase inhibition.[16]

The compounds reported in Table 3 were also tested on APB and LAP activities (data not shown). As expected, according to the specificity of both enzymes, they are very poor inhibitors with K_i values greater than 10^{-4} M.

Due to the important roles of ACE and NEP in the enzymatic cleavage of vasoactive peptides, such as AngII, bradykinin, atrial natriuretic peptide, and endothelins, it was important to determine the selectivity of the inhibitors versus these two peptidases. As expected, all these compounds are poor ACE inhibitors in agreement with the high preference of this peptidase for aromatic residues in P_1 position and polycyclic residues, more or less derived from proline, in P_2' or P_1'/P_2' position.[42] Consequently the more hydrophilic content of these APA inhibitors and the presence of an additional carboxy group in P_2' position are unfavorable factors for ACE recognition.

NEP active site has also been shown to be highly hydrophobic, at least at the S_1' subsite level which is optimally fitted by benzyl[8,43] or biphenylmethyl groups.[44] The S_2' subsite seemed to have a slight preference for small and hydrophobic residues, and the not well characterized S_1 subsite has been suggested to accept preferentially, as ACE, aromatic templates.[8] Taking these data into account, it was somewhat surprising to obtain nanomolar inhibitory potencies on NEP with the inhibitors prepared in this study. However, NEP substrates or inhibitors containing an hydrophilic group in P_2' position have been described,[45] showing that the NEP S_2' subsite does not present a clear specificity. Nevertheless, negatively charged moieties in P_1 position have not yet been proposed with the aim to obtain a high NEP inhibition. Although a further reinvestigation of this NEP subsite is needed, a good selectivity for APA was obtained with compounds **40** and **42** which contain a C-terminal proline analogue, taking into account that this cyclic amino acid is well known to be unable to enter the NEP active site.[46]

On the other hand, it could be interesting to compare APA with its cytosolic counterpart, i.e., aspartyl aminopeptidase which has been recently cloned and characterized.[47] Little is known about the mechanism of action of this enzyme but the preliminary study on its specificity indicates some analogies with APA, and the specificity constants k_{cat}/K_m of various aspartyl-peptides for the cytosolic and membrane-bound aminopeptidases are quite similar. The aspartyl aminopeptidase active site seems to be characterized by a S_1 subsite specific for Asp and Glu, a S_1' subsite which is hydrophobic, and a S_2' subsite which accepts negatively charged or hydrophobic residues. Thus, the tetrapeptide Asp-Ala-Asp-Leu, which has a K_m of 50 μM and is slowly hydrolyzed, was proposed by Wilk et al.[47] as a "lead" compound for the development of potent aspartyl aminopeptidase inhibitors.

In conclusion, the complete characterization of the subsite selectivity of APA active site, with (3-amino-2-mercapto-acyl)dipeptides has led to the design of very efficient and selective inhibitors of this enzyme. In this series, the introduction of a sulfonate group in P_1 position, a hydrophobic side chain in P_1', and a (3*R*)-carboxyproline fitting the P_2' pocket allowed a very efficient and selective inhibition of this enzyme to be obtained. Such type of molecules could be useful to discriminate the respective role of AngII and AngIII in the brain renin-angiotensin system and to study the importance of APA in the catabolism of cholecystokinin CCK_8 in the central nervous system.

Experimental Section

1. Biological Tests. Enzymes. Recombinant APA was obtained as described.[48] APN from pig kidney was purchased from Boehringer Mannheim. NEP was purified to homogeneity from rabbit kidney.[49] ACE was purified from rat testis.[50]

Substrates. Inhibitory potencies were determined by using α-glutamyl-(β-naphthyl)amide (Glu-βNA, K_m = 130 μM for APA, Bachem), alanine-β-naphthylamide (Ala-βNA, K_m = 50 μM) for APN, DGNPA[51] (DNS-Gly-(pNO$_2$)Phe-β-Ala) (K_m = 37 μM) for NEP, and Z-Phe-His-Leu[52] (K_m = 50 mM) for ACE as substrates.

Assay for APA Activity. Using the procedure of Goldbarg,[53] APA (10 μL of 1/60 dilution) was incubated 30 min at 37 °C with or without increasing concentrations of inhibitor and with 200 μM Glu-βNA in a total volume of 100 μL in 50 mM Tris-HCl buffer, pH 7.4, containing 4 mM $CaCl_2$. The reaction was stopped by adding 10 μL of 3 N HCl. The diazotation of the metabolic 2-naphthylamine was obtained by adding 25 μL of 87 mM (0.6%) $NaNO_2$ followed 2 min later by 50 μL of a 0.13 M (1.5%) ammonium sulfate and then 2 min after by 25 μL of a 23 M (0.6%) solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 95% EtOH. The sample was incubated for 30 min at 37 °C. The absorbance was measured at 550 nm. A standard curve was prepared in parallel by diazotizing increasing concentrations (up to 0.2 mM) of 2-naphthylamine in 0.1 N HCl.

Assay for APN Activity. APN (final concentration 21 ng/mL) was preincubated for 10 min at 37 °C with or without increasing concentrations of a given inhibitor in a total volume of 200 μL in 50 mM Tris/HCl buffer, pH 7.4. Ala-βNA was added at a final concentration of 50 μM, and the reaction was stopped after 30 min at 37 °C by adding 10 μM $NaCH_3COO$ (1 M, pH 4.2). The fluorescence of the metabolite was measured at 400 nm (λ_{ex} = 340 nm) with a MPF44A Perkin-Elmer spectrofluorometer. A calibration curve for β-naphthylamide was obtained by addition of increasing concentrations of βNA into 210 μL of 50 mM Tris/HCl buffer pH 7.4.

Assay for Neutral Endopeptidase Activity. NEP (final concentration 250 ng/mL) was preincubated for 15 min at 37 °C with or without increasing concentrations of a given inhibitor in a total volume of 225 μL of 50 mM Tris/HCl buffer, pH 7.4. DGNPA (25 μL) was added to a final concentration of 50 μM, and the reaction was stopped after 15 min at 37 °C by adding 250 μL of dioxane.[51] The 500 μL mixture was then transferred into a quartz cell and the fluorescence measured (λ_{ex} = 342 nm, λ_{em} = 525 nm). Samples, corresponding to 0% hydrolysis, were obtained by adding the buffer and the substrate only, while samples corresponding to 100% of relative activity were prepared by adding all the reagents except the inhibitors. Both solutions were treated under the same conditions as above. The percentage of degradation was evaluated by reference to 100% of relative activity, and the IC_{50} values of tested inhibitors were determined accordingly.

Assay for Angiotensin-Converting Enzyme Activity. ACE (final concentration 0.02 pmol/100 μL, specific activity on Z-Phe-His-Leu (13 nmol/mg per min) was preincubated for 15 min at 37 °C with various concentrations of the inhibitors in 50 mM Tris/HCl buffer, pH 8.0. Z-Phe-His-Leu was added to a final concentration of 0.05 mM. The reaction was stopped after 15 min by adding 400 μL of 2 M NaOH. After dilution with 3 mL of water, the concentration of His-Leu was determined following the fluorimetric assay described by Cheung et al.[54] with a MPF44A Perkin-Elmer spectrofluorometer (λ_{ex} = 365 nm, λ_{em} = 495 nm). The calibration curve for His-Leu was obtained by addition of increasing concentrations of His-Leu into 0.1 mL of 5.0 M Tris/HCl buffer, pH 8.0, containing the denaturated enzyme.

K_i values were calculated from IC_{50} statistical values using the Cheng−Prussoff[55] relationship.

2. Chemistry. General. Reagents were used without further purification unless otherwise stated. Amino acids were obtained from Bachem (Budendorf, Switzerland) or Neosystem (Strasbourg, France); all other chemicals were purchased from Aldrich (Saint Quentin Fallavier, France) and Acros Organics (Noisy-le-Grand, France). Trifluoroacetic acid and solvents were purchased from SDS (Peypin, France). All reactions involving strong bases and air-sensitive reagents were carried out under an argon atmosphere, and starting materials were thoroughly dried under vacuum prior to use. THF was distilled from sodium/benzophenone. HPLC analyses were run on a Shimadzu LC-10AT with a reverse-phase column (250 × 4.6 mm, flow rate 1 mL/min) (Touzart & Matignon, Vitry sur Seine, France) with CH_3CN/H_2O containing 0.05% TFA as the mobile phase. Flash chromatography was carried out with Merck silica gel Geduran 60 (40−63 μm). TLC was performed on precoated silica gel plates (60F-254, 0.2 mm thick, Merck) with the eluents indicated. Plates were developped with UV light or iodine vapor. The structures of all compounds were confirmed by ^{1}H NMR spectroscopy on a Brüker AC-270 MHz or Brüker AM-400 MHz spectrometer in DMSO-d_6 or $CDCl_3$ solutions (5×10^{-3} M) using HMDS as an internal reference. The signals are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Melting points were measured on a Büchi B-540 and are given uncorrected. Mass spectra (MS) were performed by Quad Service (Poissy, France) using the electrospray ionization technique (ESI). Satisfactory elemental analyses, performed at the University of Paris VI, were obtained (C, H, N) for all compounds. When several diastereoisomers of the same molecule were synthesized, elemental analysis was carried out with only one diastereoisomer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter for $CHCl_3$ solutions at 25 °C.

The following abbreviations are used: cHex, cyclohexane; AcOEt, ethyl acetate; THF, tetrahydrofuran; EtOH, ethanol; TFA, trifluoroacetic acid; Dibal-H, diisobutylaluminum hydride; BOP, benzotiazol-1-yloxy-tris(dimethylamino)phosphonium hexa-fluorophosphate; PMB-S-S-DNP, 2,4-dinitrophenyl-4-methoxybenzyl disulfide; LDA, lithium diisopropyl amide; ee, enantiomeric enrichment; NMM, *N*-methyl morpholine.

General Procedure for Protection of the Amino Group: Procedure A. The amino group of the various synthons **IIa−d** was protected by a benzyloxycarbonyl group as described.[56]

General Procedure for the Esterification of the Carboxylate Group. The methyl or ethyl esters were prepared in acidic conditions by the Fischer method[57] (**procedure B**) or by using diazomethane solutions[58] (**procedure C**).

General Procedure for the Alkaline Hydrolysis of Esters: Procedure D. The solution of ester (1 equiv) in the corresponding alcohol (5 mL/mmol) was treated by a 1 N aqueous NaOH solution (3−5 equiv) at room temperature. The free acid was isolated by acidic workup followed by AcOEt extraction.

General Procedure for the Arndt−Eistert Homologation of Carboxylic Acids: Procedure E. The N-protected amino acids (1 equiv) were transformed into diazoketones using the mixed anhydride activation (^{i}BuOCOCl, NMM) and addition of CH_2N_2 (2 equiv). The diazoketone was then dissolved in absolute alcohol (2 mL/mmol of methanol, ethanol, or allyl alcohol), and a solution of silver benzoate (10% mol) in triethylamine (16 mL/mmol) was added as described.[59] The crude product was purified by flash chromatography on silica gel.

General Procedure for the Electrophilic Sulfenylation: Procedure F. The sulfenylation was performed following the strategy described in the literature.[26] The crude product was then diluted with cold ether in order to precipitate the excess of sulfenylating reagent which was filtered off. The crude product was purified by flash chromatography on silica gel.

General Procedure for the One-Pot Reduction−Wittig−Horner Reaction: Procedure G. To a solution of neopentyl diethoxyphosphorylmethanesulfonate,[60] benzyl diethoxyphosphoryl acetate,[61] or tetrabenzyl methylenebis(phosphonate)[62] (2.0 equiv) in dry THF (4.5 mL/mmol) was added dropwise, at −78 °C, a solution of *n*-butyllithium (2.1 equiv of a 2.5 M hexane solution). After the mixture was stirred for 30 min at this temperature, a solution of **11** (1 equiv) in dry THF (1 mL/mmol) was added, immediately followed by a dropwise

addition of diisobutylaluminum hydride (1.95 equiv of a 1.5 M toluene solution). The resulting mixture was stirred 3 h at −78 °C and warmed to room temperature for 1 h. Next, 2 N HCl and water were added, the organic layer was separated, and the aqueous phase was further extracted with ethyl acetate. The combined organic fractions were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The product was purified by flash chromatography on silica gel.

General Procedure for the Acidolysis of Tertiobutyl Ester, Tertiobutyl Ether, Tertiobutylsulfamoyl, and *N*-Tertiobutyloxycarbonyl: Procedure H. The deprotection was obtained by treatment with TFA in CH_2Cl_2 = 50/50, v/v) as described.[63]

General Procedure for the Coupling Step Using BOP Reagent: Procedure I. The coupling step with BOP reagent was performed as described.[64]

General Procedure for the Cleavage of *N*-Benzyloxycarbonyl, 4-Methoxybenzylsulfanyl, Neopentyloxysulfonyl, and Benzyl Ester Groups Using Liquid HF: Procedure J. A mixture of the protected inhibitor (1 equiv) and *m*-cresol (0.25 mL/100 mg of inhibitor) was treated with anhydrous hydrogen fluoride (5 mL) and stirred at 0 °C for 1 h. After removal of HF in vacuo, the residue was taken up with TFA and precipitated with a cold mixture of ether/*n*-hexane 50/50. After centrifugation, the precipitate was taken up in water and freeze-dried.

General Procedure for the Reduction of α,β-Unsaturated Esters: Procedure K. The reaction was performed with $[(PPh_3)CuH]_6$ as described.[31,32]

1: (2*S*), 2-(Benzyloxycarbonylamino), 4-[(3*S*), 3-Benzyloxycarbonylamino, 3- methoxycarbonyl propyldisulfanyl]-butyric Acid Methyl Ester. Starting from 5.0 g of $(H\text{-}hCys\text{-}OH)_2$ (18.6 mmol) and using successively the general procedures A and C, the compound **1** was obtained as a yellow oil (99%). ^{1}H NMR (DMSO-d_6) δ 1.8 to 2.1 (m, 2H, CH-*CH*$_2$-CH$_2$), 2.6 to 2.8 (m, 2H, CH-CH$_2$-*CH*$_2$), 3.6 (s, 3H, COOMe), 4.15 (m, 1H, *CH*-CH$_2$-CH$_2$), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 7.2 to 7.4 (m, 5H, C_6H_5), 7.8 (d, 1H, Z-*NH*). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O(TFA) 60/40, t_R = 12.3 min. Anal. ($C_{26}H_{32}N_2O_8S_2$) C, H, N.

2: (2*S*)-Benzyloxycarbonylamino-4-chlorosulfonyl-butyric Acid Methyl Ester. Gaseous chlorine was bubbled during 1 h into a solution of **1** in MeOH (0.7 mL/mmol) and CCl_4 (3.5 mL/mmol) until the mixture became yellow. After removal of the solvent under reduced pressure, 13.9 g of **2** was obtained as a yellow oil which was used without a further purification (100%). ^{1}H NMR (DMSO-d_6) δ 1.8 to 2.1 (m, 2H, CH-*CH*$_2$-CH$_2$), 2.6 (t, 2H, CH-CH$_2$-*CH*$_2$), 3.6 (s, 3H, COOMe), 4.1 to 4.25 (m, 1H, *CH*-CH$_2$-CH$_2$), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 7.2 to 7.4 (m, 5H, C_6H_5), 7.8 (d, 1H, Z-*NH*).

3a: (2*RS*), 2-Benzyloxycarbonylamino, 4-Tertiobutylsulfamoyl-butyric Acid. The *tert*-butylamine (0.6 mL/mmol) was added dropwise to a solution of **2** (13.9 g, 13.6 mmol) in $CHCl_3$ (1.8 mL/mmol), and the mixture was stirred during 2 h at room temperature. A flash chromatography on silica gel using cHex/CH_2Cl_2/AcOEt 55/30/15 as an eluent afforded a white solid, 12.1 g (84%). R_f (1/1/1) 0.57. ^{1}H NMR (DMSO-d_6) δ 1.2 (s, 9H, tBu), 1.9 to 2.2 (m, 2H, CH-*CH*$_2$-CH$_2$), 2.9 to 3.15 (m, 2H, CH-CH$_2$-*CH*$_2$), 3.6 (s, 3H, COOMe), 4.2 to 4.3 (m, 1H, *CH*-CH$_2$-CH$_2$), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 6.9 (s, 1H, SO_2-NH), 7.3 (m, 5H, C_6H_5), 7.85 (d, 1H, Z-*NH*). mp 61 °C. HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA), t_R = 5.2 min. $[\alpha]^{25}_D$ = +17.6° (c = 0.47; $CHCl_3$).

Starting from 12 g (11,4 mmol) and following the general procedure D, **3a** was obtained as a yellow oil, 10.8 g, (96%). R_f (cHex/CH_2Cl_2/AcOEt 1/1/1) 0.25 to 0.45. ^{1}H NMR (DMSO-d_6) δ 1.2 (s, 9H, tBu), 1.9 to 2.15 (m, 2H, CH-*CH*$_2$-CH$_2$), 2.85 to 3.1 (m, 2H, CH-CH$_2$-*CH*$_2$), 4.1 (m, 1H, *CH*-CH$_2$-CH$_2$), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 6.8 (s, 1H, SO_2-NH), 7.3 (m, 5H, C_6H_5), 7.7 (d, 1H, Z-*NH*), 12.2 to 12.7 (broad s,1H, COOH). Anal. ($C_{16}H_{24}N_2O_6S$) C, H, N.

3b: (2RS), 2-Benzyloxycarbonylamino, 4-Neopentyloxysulfonyl-butyric Acid. A solution of **2** (9.2 g, 26 mmol) and 2,2-dimethylpropanol (2.4 g, 27 mmol) in pyridine (40 mL) was stirred overnight at room temperature. After removal of pyridine, the residue was dissolved in ethyl acetate, and the organic layer was washed by 2N HCl, brine and then dried over Na_2SO_4. The solvent was evaporated to dryness leading to **3b** as a yellow oil, 8.8 g (87%). R_f (cHex, AcOEt 7/3) 0.26. ^{1}H NMR (DMSO-d_6) δ 0.9 (s, 9H, tBu), 2.0 to 2.3 (m, 2H, CH-*CH*$_2$-CH$_2$), 3.2 to 3.5 (m, 2H, CH-CH$_2$-*CH*$_2$), 3.6 (s, 3H, COOMe), 3.85 (s, 2H, SO_3-CH$_2$), 4.25 (m, 1H, *CH*-CH$_2$-CH$_2$), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 7.3 (m, 5H, C_6H_5), 7.8 (d, 1H, Z-*NH*). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 70/30, t_R = 6.9 min.

From 8.5 g (21 mmol) and following procedure D, **3b** was obtained as a yellow oil, 8.4 g (100%). R_f (cHex, AcOEt 1/1) 0.0 to 0.25. ^{1}H NMR (DMSO-d_6) δ 0.9 (s, 9H, tBu), 2.0 to 2.3 (m, 2H, CH-*CH*$_2$-CH$_2$), 3.2 to 3.5 (m, 2H, CH-CH$_2$-*CH*$_2$), 3.85 (s, 2H, SO_3-CH$_2$), 4.25 (m, 1H, *CH*-CH$_2$-CH$_2$), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 7.3 (m, 5H, C_6H_5), 7.8 (d, 1H, Z-*NH*). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 60/40, t_R = 7.3 min. Anal. ($C_{17}H_{25}NO_7S$) C, H, N.

4a: (3*RS*), 3-Benzyloxycarbonylamino, 5-Tertiobutylsulfamoyl-pentanoic Acid Methyl Ester. The β-amino ester was prepared by using the previously described general procedure E starting from acid **3a** (10.8 g, 29 mmol). The crude product was purified by flash chromatography on silica gel eluting with cHex/CH_2Cl_2/AcOEt 1/1/1 (R_f 0.46). A white solid was obtained 6.90 g (59%). ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 9H, tBu), 2.2 (s, 2H, CH-*CH*$_2$-CH$_2$), 2.55 (d, 2H, *CH*$_2$-COOMe), 3.0 (t, 2H, CH-CH$_2$-*CH*$_2$), 3.6 (s, 3H, COOMe), 4.1 (m, 1H, *CH*-CH$_2$-CH$_2$), 4.2 (s, 1H, SO_2-NH), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 5.4 (d, 1H, Z-*NH*), 7.3 (s, 5H, C_6H_5). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O(TFA) 70/30, t_R = 4.6 min. Anal. ($C_{18}H_{28}N_2O_6S$) C, H, N.

4b: (3*RS*), 3-Benzyloxycarbonylamino, 5-Neopentyloxysulfonyl-pentanoic Acid Methyl Ester. According to the general procedure E of the Arndt−Eistert reaction, the acid **3b** (8.4 g, 22 mmol) was homologated giving a brown oil, 7.9 g (91%). R_f (cHex/AcOEt 1/1) 0.61. ^{1}H NMR ($CDCl_3$) δ 0.9 (s, 9H, tBu), 2.0 to 2.2 (m, 2H, CH-*CH*$_2$-CH$_2$), 2.5 to 2.7 (m, 2H, CH$_2$-COO), 3.1 (t, 2H, CH-CH$_2$-*CH*$_2$), 3.6 (s, 3H, $COOCH_3$), 3.8 (s, 2H, SO_3-CH$_2$), 4.05 (m, 1H, CH-*CH*$_2$-CH$_2$), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 5.85 (d, 1H, Z-*NH*), 7.75 (m, 5H, CH arom). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 70/30, t_R = 6.6 min. Anal. ($C_{19}H_{24}NO_7S$) C, H, N.

5a: (2*RS*, 3*RS*), 3-Benzyloxycarbonylamino, 2-(4-Methoxybenzylsulfanyl), 5-Tertiobutylsulfamoyl-pentanoic Acid Methyl Ester. Using the general procedure F, the sulfenylation was carried out with 3.4 g (8.5 mmol) of **4a**. The crude product was then purified by flash chromatography on silica gel (cHex/AcOEt 4/1) giving a brown oil, 3.6 g (76%). R_f (cHex/CH_2Cl_2/AcOEt 1/1/1): 0.55. ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 9H, tBu), 1.8 to 2.1 (m, 2H, CH-*CH*$_2$-CH$_2$), 2.95 (t, 2H, CH-CH$_2$-*CH*$_2$), 3.2 (2d, 1H, CH-S), 3.65 (s, 3H, COOMe), 3.7 (2s, 5H, S-CH$_2$, OCH_3), 4.0 (m, 2H, *CH*-CH$_2$-CH$_2$, SO_2NH), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 5.5 (d, 1H, Z-*NH*), 6.75 (m, 2H, CH arom. ortho to OCH_3), 7.1 (m, 2H, CH arom. meta to OCH_3), 7.3 (m, 5H, C_6H_5). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O(TFA) 70/30, t_R = 7.0 min. Anal. ($C_{26}H_{36}N_2O_7S_2$) C, H, N.

5b: (2*RS*,3*RS*), 3-Benzyloxycarbonylamino, 5-Neopentyloxysulfonyl], 2-[4-Methoxybenzylsulfanyl]-pentanoic Acid Methyl Ester. Compound **4**, 3.8 g (9.2 mmol) was sulfenylated using the general procedure F. The crude product was chromatographed on silica gel with cHex/AcOEt 7/3 as an eluent and then purified by semipreparative HPLC, giving a yellow oil, 469 mg (9%). R_f (cHex/AcOEt 6.5/3.5) 0.48. ^{1}H NMR ($CDCl_3$) δ 0.9 (s, 9H, tBu), 1.8 to 2.2 (m, 2H, CH-*CH*$_2$-CH$_2$), 3.0 (t, 2H, CH-CH$_2$-*CH*$_2$), 3.2 (d, 1H, CH-S), 3.65 (s, 3H, COOMe), 3.75 (s, 7H, SO_3-CH$_2$, S-CH$_2$, OCH_3), 4.05 (m, 1H, *CH*-CH$_2$-CH$_2$), 5.0 (m, 2H, C_6H_5-*CH*$_2$), 5.5 (d, 1H, Z-*NH*), 6.75 (d, 2H, CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta OCH_3), 7.3 (m, 5H, *C*$_6$*H*$_5$). HPLC C_8 Kromasil (5μm, 100 Å) CH_3CN/H_2O (TFA) 70/30, t_R = 10.7 min. Anal. ($C_{26}H_{37}NO_8S_2$) C, H, N.

6b: (2*RS*, 4*RS*), 4-Benzyloxycarbonylamino, 2-Neopentyloxysulfonyl Cyclopentanone. R_f (cHex, AcOEt 6.5/

3.5) 0.24. Yellow oil (63%). ^{1}H NMR ($CDCl_3$) δ 0.95 (s, 9H, tBu), 2.4 to 2.55 (m, 2H, CH-*CH*$_2$-CH), 2.75 to 2.95 (m, 2H, CH-*CH*$_2$-CO), 3.95 (m, 3H, SO_3-CH_2, CH-CH_2-*CH*), 4.3 to 4.5 (m, 1H, *CH*-CH_2-CH), 5.1 (d, 2H, C_6H_5-*CH*$_2$), 5.0 to 5.4 (2d, 1H, Z-*NH*), 7.25 (m, 5H, *C*$_6$*H*$_5$). MS 384.2 = MH^+; 406.2 = MNa^+. Anal. ($C_{18}H_{25}NO_6S$) C, H, N.

IIa: (2*RS*, 3*RS*), 3-Benzyloxycarbonylamino, 2-(4-Methoxybenzylsulfanyl), 5-Tertiobutylsulfamoyl-pentanoic Acid. Compound **5a** (1.5 g, 2.7 mmol) was saponified according to procedure D giving **IIa**, 1.5 g (100%). ^{1}H NMR ($CDCl_3$) δ 1.2 to 1.4 (s, 9H, tBu), 1.8 to 2.2 (m, 2H, CH-*CH*$_2$-CH_2), 3.0 (t, 2H, CH-CH_2-*CH*$_2$), 3.25 (2d, 1H, CH-S), 3.7 (2s, 5H, S-CH_2, OCH_3), 4.0 (m, 1H, *CH*-CH_2-CH_2), 4.5 (s, 1H, SO_2-NH), 5.0 (m, 2H, C_6H_5-*CH*$_2$), 5.5 (d, 1H, Z-*NH*), 6.8 (m, 2H, CH arom. ortho to OCH_3), 7.1 to 7.4 (m, 7H, CH arom. meta to OCH_3, C_6H_5). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O(TFA) 60/40, t_R = 7.2 min. Anal. ($C_{25}H_{34}N_2O_7S_2$) C, H, N.

IIb: (2*RS*, 3*RS*), 3-Benzyloxycarbonylamino, 5-Neopentyloxysulfonyl-2-[4-methoxybenzylsulfanyl]-pentanoic Acid. The saponification by procedure D of 470 mg (0.86 mmol) of **5b** led to a brown oil, 480 mg (100%), which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 0.9 (s, 9H, tBu), 1.8 to 2.2 (m, 2H, CH-*CH*$_2$-CH_2), 3.0 (t, 2H, CH-CH_2-*CH*$_2$), 3.2 (d, 1H, CH-S), 3.75 (s, 7H, SO_3-CH_2, S-CH_2, OCH_3), 4.05 (m, 1H, *CH*-CH_2-CH_2), 5.0 (m, 2H, C_6H_5-*CH*$_2$), 5.5 (d, 1H, Z-*NH*), 6.75 (d, 2H, CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta OCH_3), 7.3 (m, 5H, *C*$_6$*H*$_5$). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 70/30, t_R = 6.5 min. Anal. ($C_{26}H_{35}NO_8S_2$) C, H, N.

8: (3S), 3-Tertiobutyloxycarbonylamino-hexane-dioic Acid 1-Allyl Ester, 6-Tertiobutyl Ester. Procedure E of the Arndt−Eistert reaction was performed on (2*S*), 2-tertiobutyloxycarbonylamino-pentanedioic acid 5-tertiobutylester (**7**) (10 g, 33.2 mmol). The crude product was chromatographed on silica gel with cHex/AcOEt 1/1 as an eluent leading to a yellow oil, 8.91 g (89%). R_f 0.74. ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 18H, Boc, tBu), 1.75 (m, 2H, CH-*CH*$_2$-CH_2), 2.25 (t, 2H, *CH*$_2$-COOtBu), 2.5 (d, 2H, *CH*$_2$-COOAllyl), 3.85 (m, 1H, *CH*-CH_2-CH_2), 4.5 (d, 2H, *CH*$_2$-CH=CH_2), 4.95 (d, 1H, Boc-*NH*), 5.1 to 5.3 (dt, 2H, CH_2-CH=*CH*$_2$), 5.75 to 5.95 (m, 1H, CH_2-*CH*=CH_2). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 70/30, t_R = 9.1 min. Anal. ($C_{18}H_{31}NO_6$) C, H, N.

9: (2*S*, 3*S*), 2-[4-Methoxybenzylsulfanyl], 3-Tertiobutyloxycarbonylamino-hexane-dioic Acid 1-Allyl Ester, 6-Tertiobutyl Ester. The ester **8** (1.5 g, 4.2 mmol) was sulfenylated according to procedure F. The crude product was first purified by flash chromatography with cHex/AcOEt 4/1 as an eluent and then by semipreparative HPLC CH_3CN/H_2O (TFA) 70/30 giving a yellow oil, 213 mg (10%). R_f (cHex/AcOEt 4/1) 0.27. ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 18H, Boc, tBu), 1.5 to 1.9 (m, 2H, CH-*CH*$_2$-CH_2), 2.2 (t, 2H, *CH*$_2$-COOtBu), 3.25 (d, 1H, CH-S), 3.7 (s, 5H, S-CH_2, OCH_3), 3.8 to 4.0 (m, 1H, *CH*-CH_2-CH_2), 4.5 (d, 2H, *CH*$_2$-CH=CH_2), 5.15 to 5.35 (m, 3H, Boc-*NH*, CH_2-CH=*CH*$_2$), 5.75 to 5.95 (m, 1H, CH_2-*CH*=CH_2), 6.75 (d, 2H, CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta). HPLC C_8 Kromasil (5μm, 100 Å) CH_3CN/H_2O (TFA) 70/30, t_R = 17.5 min. Anal. ($C_{26}H_{39}NO_7S$) C, H, N.

IIc: (2*S*, 3*S*), 2-[4-Methoxybenzylsulfanyl], 3-Tertiobutyloxycarbonylamino-hexane-dioic Acid 6-Tertiobutyl Ester. To a stirred solution of **9** (250 mg, 0.49 mmol) in CH_2Cl_2 (2.4 mL) under argon were added, at −15 °C, pyrrolidine (49 μL, 0.59 mmol), PPh_3 (26 mg, 0.09 mmol), and then [$(PPh_3)_4Pd$] (28 mg, 0.02 mmol). The mixture was stirred 1 h at −15 °C before quenching with H_2O (1 mL) and CH_3CN (16 mL), then extracting with 40−60 petroleum ether. The organic layer was washed with 1N HCl and brine, dried on Na_2SO_4, and concentrated under reduced pressure, giving the pure product **IIc** as a yellow oil, 250 mg (100%). ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 18H, Boc, tBu), 1.6 to 1.8 (m, 2H, CH-*CH*$_2$-CH_2), 2.2 (t, 2H, *CH*$_2$-COOtBu), 3.25 (d, 1H, CH-S), 3.7 (s, 3H, OCH_3), 3.8 (s, 2H, S-*CH*$_2$), 3.9 to 4.0 (m, 1H, *CH*-CH_2-CH_2), 5.1 (d, 1H, Boc-*NH*), 6.7 (d, 2H, CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta). HPLC C_8 Kromasil (5μm, 100 Å) CH_3CN/H_2O (TFA) 70/30, t_R = 7.1 min. Anal. ($C_{23}H_{35}NO_7S$) C, H, N.

10: (2S), 2-Benzyloxycarbonylamino-succinic Acid 1-Methyl Ester 4-Tertiobutyl Ester. Compound **10** was obtained following the general procedure C from 20 g of Z-L-Asp(OtBu)-OH (61.5 mmol), as a yellow oil (20.7 g, 99%). R_f (cHex/AcOEt/CH_2Cl_2 7/1.5/1.5) 0.31. ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 9H, COOtBu), 2.7 to 2.9 (m, 2H, CH-*CH*$_2$), 3.7 (s, 3H, COOMe), 4.5 (m, 1H, *CH*-CH_2), 5.0 (s, 2H, C_6H_5-*CH*$_2$), 5.7 (d, 1H, Z-*NH*), 7.3 (m, 5H, *C*$_6$*H*$_5$-CH_2). HPLC C_{18} Kromasil (5μm, 100 Å) CH_3-CN/H_2O (TFA) 70/30, t_R = 6.1 min. Anal. ($C_{17}H_{25}NO_6$) C, H, N.

11: (2*R*, 3*R*), 2-Benzyloxycarbonylamino, 3- (4-Methoxybenzylsulfanyl)-succinic Acid, 1-Methyl Ester 4-Tertiobutyl Ester. Compound **11** was obtained from 20.7 g (61 mmol) of **10**, using the general procedure F. Purification by flash chromatography on silica gel with cHex/CH_2Cl_2/AcOEt 8/1/1 as an eluent, gave yellow crystals (20 g, 68%). mp 71.6−73.3 °C. ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9H, tBu), 3.6 (s, 5H, S-CH_2, OCH_3), 3.7 (s, 3H, COOMe), 3.8 (d, 1H, CH-S), 4.6 (dd, 1H, *CH*-COOMe), 5.1 (s, 2H, C_6H_5-*CH*$_2$), 5.9 (d, 1H, Z-*NH*), 6.8 (d, 2H, CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta), 7.3 (m, 5H, *C*$_6$*H*$_5$). HPLC C_{18} Kromasil (5μm, 100 Å) CH_3CN/H_2O (TFA) 70/30, t_R = 14.1 min. Anal. ($C_{25}H_{31}NO_7S$) C, H, N.

12b: (2*R*, 3*R*), 3-Benzyloxycarbonylamino-2-[4-methoxybenzylsulfanyl]-5-neopentyloxysulfonyl-pent-4-enoic Acid Tertiobutyl Ester. The product **12b** was obtained from 12 g of **11** (24.5 mmol) and 15 g of neopentyl diethoxyphosphorylmethanesulfonate (49 mmol) by the general procedure G. Purification by flash chromatography on silica gel eluting with cHex/CH_2Cl_2/AcOEt 7/1.5/1.5 (R_f 0.4) gave a yellow oil, 11.6 g (68%, ee: 95/5). ^{1}H NMR ($CDCl_3$) δ 0.8 (s, 9H, SO_3-CH_2-*tBu*), 1.4 (s, 9H, tBu), 3.1 (d, 1H, CH-S), 3.65 (s, 2H, SO_3-CH_2), 3.75 (2d, 5H, S-CH_2, OCH_3), 4.7 (m, 1H, *CH*-CH=CH), 5.1 (s, 2H, C_6H_5-*CH*$_2$), 5.7 (d, 1H, Z-*NH*), 6.2 to 6.3 (dd, 1H, CH-CH=*CH*), 6.7 to 6.9 (m, 3H, CH-*CH*=CH, CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta), 7.3 (s, 5H, *C*$_6$*H*$_5$-CH_2). HPLC C_{18} Kromasil (5μm, 100 Å) CH_3CN/H_2O (TFA) 75/25, t_R = 17.9 min. Anal. ($C_{30}H_{41}NO_8S_2$) C, H, N.

12c: (2*R*, 3*R*), 3-Benzyloxycarbonylamino-2-(4-methoxybenzylsulfanyl)-hex-4-ene-1,6-dioic Acid, 6-Benzyl Ester 1-Tertiobutyl Ester. Compound **11** (2.9 g, 5.92 mmol) was treated by 3.4 g (11.8 mmol) of benzyl diethylphosphoryl acetate following procedure F. The crude product **12c** was purified by flash chromatography on silica gel eluting with cHex/CH_2Cl_2/AcOEt 7/1.5/1.5 (R_f 0.36) giving white crystals, 2.03 g (58%, ee: 95/5). ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9H, tBu), 3.1 (d, 1H, CH-S), 3.7 (2s, 5H, OCH_3, S-CH_2), 4.6 (m, 1H, *CH*-CH=CH), 5.0 (s, 2H, COO*CH*$_2$-C_6H_5), 5.1 (s, 2H, C_6H_5-*CH*$_2$-OCONH), 5.75 (d, 1H, Z-*NH*), 5.85 to 6.0 (dd, 1H, CH-CH=*CH*), 6.8 (m, 3H, 2CH arom. ortho OCH_3, CH-*CH*=CH), 7.2 (d, 2H, CH arom. meta), 7.3 (s, 10H, 2 × *C*$_6$*H*$_5$-CH_2). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 75/25, t_R = 18.2 min. Anal. ($C_{33}H_{37}NO_7S$) C, H, N.

12d: (2*R*, 3*R*), 3-Benzyloxycarbonylamino-2-[4-methoxybenzylsulfanyl]-5-[dibenzyloxyphosphoryl]-pent-4-enoic Acid Tertiobutyl Ester. From 3 g (6.13 mmol) of **11** and 6.6 g of tetrabenzyl methylene-bisphosphonate (12.2 mmol), product **12d** was obtained by procedure F. Purification by flash chromatography on silica gel eluting with cHex/CH_2Cl_2/AcOEt 5/2.5/2.5 (R_f 0.32) gave a yellow oil, 1.9 g (44%, ee: 95/5). ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9H, tBu), 3.2 (d, 1H, CH-S), 3.8 (m, 5H, OCH_3, S-CH_2), 4.6 (m, 1H, *CH*-CH=CH), 4.9 to 5.1 (m, 6H, 3 × *CH*$_2$-C_6H_5), 5.7 to 5.9 (m, 2H, Z-*NH*, CH-CH=*CH*), 6.6 to 6.8 (m, 3H, 2CH arom. ortho OCH_3, CH-*CH*=CH), 7.2 (d, 2H, CH arom. meta), 7.3 (s, 15H, 3 × *C*$_6$*H*$_5$-CH_2). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 75/25, t_R = 17.9 min. Anal. ($C_{39}H_{43}NO_8SP$) C, H, N.

13b: (2*R*, 3*R*), 3-Benzyloxycarbonylamino-2-[4-methoxybenzylsulfanyl]-5-neopentyloxysulfonyl-pentanoic Acid Tertiobutyl Ester. Compound **13b** was obtained from 12 g of **12b** (19.7 mmol) following the general procedure K. After purification by flash chromatography on silica gel (eluent: cHex/CH_2Cl_2/AcOEt 7/1.5/1.5, R_f 0.10) a yellow solid was obtained, 8.95 g (75%). ^{1}H NMR ($CDCl_3$) δ 0.9 (s, 9H, SO_3-CH_2-*tBu*), 1.4 (s, 9H, tBu), 1.8 to 2.2 (m, 2H, CH-*CH*$_2$-CH_2), 3.1 (m, 3H, CH-S, CH-CH_2-*CH*$_2$), 3.7 (d, 7H, S-CH_2, OCH_3, SO_3-

CH_2), 3.9 (m, 1H, *CH*-CH_2-CH_2), 5.1 (m, 2H, C_6H_5-*CH_2*), 5.6 (d, 1H, Z-*NH*), 6.8 (CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta), 7.3 (s, 5H, *C_6H_5*-CH_2). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 75/25, t_R = 17.5 and 18.4 min (2*S*,3*R* and 2*R*,3*R*, respectively; 20:80). Anal. ($C_{30}H_{43}NO_8S_2$) C, H, N.

13c: (2*R*, 3*R*), 3-Benzyloxycarbonylamino-2-(4-methoxybenzylsulfanyl)-hexan-1,6-dioic Acid, 6-Benzyl Ester 1-Tertiobutyl Ester. Compound **13c** was obtained from 1.4 g of **12c** (2.3 mmol) following the general procedure K. After purification by flash chromatography on silica gel (eluent: cHex/CH_2Cl_2/AcOEt 7/1.5/1.5, R_f 0.30) a yellow oil was obtained, 1.2 g (87%). ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9H, tBu), 1.6 to 2.0 (m, 2H, CH-*CH_2*-CH_2), 2.3 (t, 2H, CH-CH_2-*CH_2*), 3.1 (d, 1H, CH-S), 3.7 (s, 5H, S-CH_2, OCH_3), 3.9 (m, 1H, *CH*-CH_2-CH_2), 5.1 (2s, 4H, 2 × C_6H_5-*CH_2*), 5.5 (d, 1H, Z-*NH*), 6.8 (CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta), 7.3 (s, 10H, 2 × *C_6H_5*-CH_2). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 75/25, t_R = 17.2 and 17.3 min (2*S*,3*R* and 2*R*,3*R*, respectively; 25:75). Anal. ($C_{33}H_{39}NO_7S$) C, H, N.

13d: (2*R*, 3*R*), 3-Benzyloxycarbonylamino-2-[4-methoxybenzylsulfanyl]-5-[dibenzyloxyphosphoryl]-pentanoic Acid Tertiobutyl Ester. Following the general procedure K and starting from 2.1 g (2.9 mmol) of **12d**, compound **13d** was obtained after purification by flash chromatography on silica gel (eluent: cHex/CH_2Cl_2/AcOEt 5/2.5/2.5, R_f 0.13) giving a brown oil, 1.7 g (81%). ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9H, tBu), 1.5 to 2.0 (m, 4H, CH-*CH_2*-CH_2, CH-CH_2-*CH_2*), 3.1 (d, 1H, CH-S), 3.7 (s, 5H, S-CH_2, OCH_3), 3.9 (m, 1H, *CH*-CH_2-CH_2), 4.8 to 5.1 (m, 6H, 3 × C_6H_5-*CH_2*), 5.5 (d, 1H, Z-*NH*), 6.7 (CH arom. ortho OCH_3), 7.1 (d, 2H, CH arom. meta), 7.3 (s, 15H, 3 × *C_6H_5*-CH_2). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 75/25, t_R = 15.3 and 16.1 min (2*S*,3*R* and 2*R*,3*R*, respectively; 25:75). Anal. ($C_{39}H_{45}NO_8SP$) C, H, N.

IIb: (2*R*, 3*R*), 3-Benzyloxycarbonylamino-2-[4-methoxybenzylsulfanyl]-5-neopentyloxysulfonyl-pentanoic Acid. The acid **IIb** was obtained from 8.8 g of **13b** (14.4 mmol), according to the general procedure H. After purification by flash chromatography on silica gel eluting with cHex/Et_2O/HCOOH 4/6/0.1 (R_f 0.34), 7.9 g of **IIb** was obtained (99%). ^{1}H NMR ($CDCl_3$) δ 0.9 (s, 9H, tBu), 1.8 to 2.2 (m, 2H, CH-*CH_2*-CH_2), 3.0 (t, 2H, CH-CH_2-*CH_2*), 3.2 (d, 1H, CH-S), 3.75 (m, 7H, SO_3-CH_2, S-CH_2, OCH_3), 4.05 (m, 1H, *CH*-CH_2-CH_2), 5.0 (m, 2H, C_6H_5-*CH_2*), 5.5 (d, 1H, Z-*NH*), 6.75 (d, 2H, CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta OCH_3), 7.3 (m, 5H, *C_6H_5*-CH_2). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 75/25, t_R = 5.7 min. Anal. ($C_{26}H_{35}NO_8S_2$) C, H, N.

IIc : (2*R*, 3*R*), 3-Benzyloxycarbonylamino-2-(4-methoxybenzylsulfanyl)-hexanoic Acid 6-Benzyl Ester. The acid **IIc** was obtained from 1.0 g of **13c** (1.68 mmol), according to the general procedure H. After purification by flash chromatography on silica gel eluting with cHex/Et_2O/HCOOH 4/6/0.1 (R_f 0.31), 540 mg (60%) of **IIc** was obtained. ^{1}H NMR ($CDCl_3$) δ 1.6 to 2.0 (m, 2H, CH-*CH_2*-CH_2), 2.4 (t, 2H, CH-CH_2-*CH_2*), 3.25 (d, 1H, CH-S), 3.75 (m, 5H, S-CH_2, OCH_3), 4.05 (m, 1H, *CH*-CH_2-CH_2), 5.0 (m, 4H, 2 × C_6H_5-*CH_2*), 5.4 (d, 1H, Z-*NH*), 6.75 (d, 2H, CH arom. ortho OCH_3), 7.2 (d, 2H, CH arom. meta OCH_3), 7.3 (m, 10H, 2 × *C_6H_5*-CH_2). HPLC C_{18} Kromasil (5 μm, 100 Å) isoc. CH_3CN/H_2O (TFA) 75/25, t_R = 4.2 min. Anal. ($C_{29}H_{31}NO_7S$) C, H, N.

IId: (2*R*, 3*R*), 3-Benzyloxycarbonylamino-2-[4-methoxybenzylsulfanyl]-5-[dibenzyloxyphosphoryl]-pentanoic Acid. The acid **IId** was obtained from 1.6 g of **13d** (2.2 mmol) and according to the general procedure H after purification by flash chromatography on silica gel with Et_2O/HCOOH 10/0.1 (R_f 0.2), yield 974 mg (70%). ^{1}H NMR ($CDCl_3$) δ 1.6 to 2.0 (m, 4H, CH-*CH_2*-CH_2, CH-CH_2-*CH_2*), 3.75 (m, 7H, S-CH_2, OCH_3, CH-S, *CH*-CH_2-CH_2), 4.8 to 5.1 (m, 6H, 3 × C_6H_5-*CH_2*), 5.8 (d, 1H, Z-*NH*), 6.7 (d, 2H, CH arom. ortho OCH_3), 7.2 to 7.4 (m, 17H, CH arom. meta OCH_3, 3 × *C_6H_5*-CH_2). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 80/20, t_R = 4.4 min. Anal. ($C_{35}H_{37}NO_8SP$) C, H, N.

14: (2*RS*, 3*RS*), 3-Benzyloxycarbonylamino-2-(4-methoxybenzylsulfanyl)-5-tertiobutylsulfamoyl-pentanal. To a solution of **5a** (1.2 g, 2.18 mmol) in THF (4 mL/mmol) and absolute ethanol (4 mL/mmol) were added successively anhydrous lithium chloride (397 mg, 9.4 mmol) and sodium borohydride (354 mg, 9.4 mmol). The mixture was stirred overnight at room temperature. After addition of water and acidification to pH 1, the mixture was extracted with ethyl acetate and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The pure alcohol was obtained by flash chromatography on silica gel (cHex/AcOEt 4/6, R_f 0.53 and 0.62) as a yellow oil, 897 mg (70%). Starting from this material (322 mg, 0.6 mmol) and carrying out the Swern oxidation[65] procedure ($(COCl)_2$ 1.32 mmol and DMSO 2.8 mmol), the aldehyde **14** was obtained quantitatively as a yellow oil which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 9H, tBu), 1.7 to 2.1 (m, 2H, CH-*CH_2*-CH_2), 2.7 to 3.1 (m, 3H, CH-S, CH-CH_2-*CH_2*), 3.7 (m, 5H, S-CH_2, OCH_3), 3.95 to 4.15 (m, 2H, SO_2NH, *CH*-CH_2-CH_2), 5.0 (m, 2H, C_6H_5-*CH_2*), 5.2 (d, 1H, Z-NH), 6.7 (m, 2H, CH arom. ortho OCH_3), 7.1 (m, 2H, CH arom. meta), 7.3 (s, 5H, *C_6H_5*-CH_2), 9.15 and 9.25 (2d, 1H, CHO).

15: [[(2*RS*, 3*RS*), 3-Amino-2-sulfhydryl-5-sulfamoyl]-pentane]-(L)Ile-(L)Asp-OH. This compound was obtained from a reductive amination of the aldehyde **14** according to the procedure described by Gordon.[27] Pale yellow powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.7 to 0.9 (m, 6H, 2 × CH_3 Ile), 1.0 to 1.6 (m, 2H, $CH_2\gamma$ Ile), 1.8 (m, 1H, CHβ Ile), 2.0 (m, 2H, CH-*CH_2*-CH_2), 2.7 (m, 3H, CH-CH_2-*CH_2*, SH), 3.0 to 3.5 (m, 2H, CH-S, CHαAsp), 3.6 (m, 1H, *CH*-CH_2-CH_2), 4.1 to 4.5 (m, 1H, CHα Ile), 4.6 (m, 2H, CH_2-N), 8.0 (broad s, 3H, NH_3^+), 8.2 to 8.85 (4d, 1H, CONH-Asp). HPLC C_8 Kromasil (10 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 3.8 min. Anal. ($C_{17}H_{30}N_4O_9S_2F_3$) C, H, N.

16: [[(2*RS*, 3*RS*), 3-Amino-2-sulfhydryl-5-sulfamoyl]-pentanoyl]-(L)Ile-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA; 400 MHz) δ 0.8 (t, 3H, CH_3 Ile), 1.0 to 1.2 and 1.5 (m, 2H, $CH_2\gamma$ Ile), 1.7 to 1.9 (m, 1H, CHβ Ile), 1.9 to 2.15 (m, 2H, CH-*CH_2*-CH_2), 2.5 to 2.7 (m, 2H, $CH_2\beta$ Asp), 3.0 to 3.2 (m, 2H, CH-CH_2-*CH_2*) 3.45 (d, 1H, SH), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.8 (m, 1H, *CH*-S), 4.3 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 6.8 to 7.95 (broad s, 2H, SO_2NH_2), 7.95 (d, 3H, $^+NH_3$), 8.4 (d, 2H, CONH Asp, CONH Ile). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 8.6 min. MS 457.1 = MH^+. $[\alpha]^{22}_D = -29.8$ (c = 0.5, $HCCl_3$). Anal. ($C_{17}H_{28}N_4O_{10}S_2F_3$) C, H, N.

17: [[(2*RS*, 3*RS*), 3-Amino-2-sulfhydryl-5-sulfamoyl]-pentanoyl]-(L)Tyr-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA; 400 MHz) δ 1.8 to 2.15 (m, 2H, CH-*CH_2*-CH_2), 2.5 to 3.0 (m, 4H, $CH_2\beta$ Tyr, $CH_2\beta$ Asp), 2.9 to 3.2 (m, 3H, SH, CH-CH_2-*CH_2*), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.8 (m, 1H, CH-S), 4.5 (m, 2H, CHα Tyr, CHα Asp), 6.6 (d, 2H, CH ortho OH), 7.0 (d, 2H, CH meta OH), 7.9 and 7.95 (broad s, 3H, H_3N^+), 8.5 (m, 2H, CONH Tyr, CONH Asp). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 20/80, t_R = 2.7 min. MS 507.3 = MH^+. Anal. ($C_{20}H_{26}N_4O_{11}S_2F_3$) C, H, N.

18: [[(2*R*,3*S*), 3-Amino-5-carboxylate-2-sulfhydryl]-pentanoyl]- (L)Ile-(L)Asp-OH. Compounds **18** and **19** were obtained from the (2*RS*, 3*S*) isomer of **IIc** by procedure I and HPLC separation. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.7 (m, 6H, 2 × CH_3 Ile), 1.1 to 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.7 to 1.9 (m, 3H, CHβ Ile, CH-*CH_2*-CH_2), 2.3 (t, 2H, CH-CH_2-*CH_2*), 2.5 to 2.75 (m, 2H, $CH_2\beta$ Asp), 3.3 (m, 1H, *CH*-CH_2-CH_2), 3.85 (m, 1H, CH-S), 4.25 (m, 1H, CHα Ile), 5.5 (m, 1H, CHα Asp), 7.9 (broad s, 3H, NH_3^+), 8.3 (d, 1H, CONH Asp), 8.4 (d, 1H, CONH Ile). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O 15/85, t_R = 11.0 min. MS 421.8 = MH^+. Anal. ($C_{18}H_{28}N_3O_{10}SF_3$) C, H, N.

19: [[(2*S*, 3*S*), 3-Amino-5-carboxylate-2-sulfhydryl]-pentanoyl]- (L)Ile-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (t, 3H, $CH_3\delta$ Ile), 0.85 (d, 3H, $CH_3\gamma$ Ile), 1.1 and 1.4 (2m, 2H, CH_2Ile), 1.75 (m, 3H, CHβ Ile, CH-*CH_2*-CH_2), 2.35 (t, 2H, CH-CH_2-*CH_2*), 2.6 (m, 2H, $CH_2\beta$ Asp), 3.3 (m, 1H, *CH*-CH_2-CH_2), 3.5 (m, 1H, SH), 3.9 (m, 1H, CH-S), 4.2 (m, 1H, CHα Ile), 4.5 (m, 1H, CHα Asp), 7.9 (broad s, 3H, H_3N^+), 8.3 (d, 1H, CONH Asp), 8.45 (d, 1H, CONH Ile). HPLC

C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, $t_R = 10.2$ min. MS 421.8 = MH^+, 443.8 = MNa^+.

20: [[(2*S*, 3*R*), 3-Amino-5-carboxylate-2-sulfhydryl]-pentanoyl]-(L)Ile-(L)Asp-OH. Compounds **20** and **21** were obtained from the (2*RS*, 3*R*) isomer of **IIc** by procedure I and HPLC separation. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (t, 3H, $CH_3\delta$ Ile), 0.85 (d, 3H, $CH_3\gamma$ Ile), 1.05 and 1.4 (m, 2H, CH_2 Ile), 1.7 (m, 3H, CHβ Ile, CH-*CH_2*-CH_2), 2.25 (m, 2H, CH-CH_2-*CH_2*), 2.6 (m, 2H, $CH_2\beta$ Asp), 3.1 (m, 1H, SH), 3.4 (m, 1H, *CH*-CH_2-CH_2), 3.8 (m, 1H, CH-S), 4.2 (m, 1H, CHα Ile), 4.5 (m, 1H, CHα Asp), 7.9 (broad s, 3H, H_3N^+), 8.4 (d, 1H, CONH Asp), 8.4 (d, 1H, CONH Ile). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, $t_R = 8.9$ min. MS 422.0 = MH^+.

21: [[(2*R*, 3*R*), 3-Amino-5-carboxylate-2-sulfhydryl]-pentanoyl]-(L)Ile-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (t, 3H, $CH_3\delta$ Ile), 0.8 (d, 3H, $CH_3\gamma$ Ile), 1.0 and 1.4 (m, 2H, CH_2Ile), 1.65 (m, 2H, CH-*CH_2*-CH_2), 1.8 (m, 1H, CHβ Ile), 2.35 (m, 2H, CH-CH_2-*CH_2*), 2.5 to 2.7 (m, 2H, $CH_2\beta$ Asp), 3.3 (m, 1H, SH), 3.4 (m, 1H, *CH*-CH_2-CH_2), 4.0 (m, 1H, CH-S), 4.25 (m, 1H, CHα Ile), 4.5 (m, 1H, CHα Asp), 8.0 (broad s, 3H, H_3N^+), 8.45 (d, 1H, CONH Asp), 8.6 (d, 1H, CONH Ile). HPLC C_8 Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, $t_R = 5.6$ min. MS 422.0 = MH^+.

22: [[(2R, 3S), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)Asp-OH. Compounds **22** and **23** were obtained from the (2*RS*, 3*S*) isomer of **IIb** by procedure I and HPLC separation. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (t, 3H, $CH_3\delta$ Ile), 0.8 (d, 3H, $CH_3\gamma$ Ile), 1.05 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.75 (m, 1H, CHβ Ile), 1.95 (m, 2H, CH-*CH_2*-CH_2), 2.5 to 2.7 (m, 4H, CH-CH_2-*CH_2*, $CH_2\beta$ Asp), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.9 (m, 1H, CH-S), 4.25 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 8.0 (broad s, 3H, H_3N^+), 8.3 (d, 1H, CONH Asp), 8.6 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, $t_R = 6.0$ min. MS 458.1 = MH^+, 480.1 = MNa^+. Anal. ($C_{17}H_{28}N_3O_{11}S_2F_3$) C, H, N.

23: [[(2*S*, 3*S*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (t, 3H, $CH_3\delta$ Ile), 0.8 (d, 3H, $CH_3\gamma$ Ile), 1.05 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.75 (m, 1H, CHβ Ile), 1.95 (m, 2H, CH-*CH_2*-CH_2), 2.5 to 2.7 (m, 4H, CH-CH_2-*CH_2*, $CH_2\beta$ Asp), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.9 (m, 1H, CH-S), 4.25 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 8.0 (broad s, 3H, H_3N^+), 8.3 (d, 1H, CONH Asp), 8.6 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, $t_R = 3.7$ min. MS 458.0 = MH^+, 480.0 = MNa^+.

24: [[(2*S*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)Asp-OH. Compounds **24** and **25** were obtained from the (2*RS*, 3*R*) isomer of **IIb** by procedure I and HPLC separation. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.7 to 0.9 (m, 6H, 2 × CH_3 Ile), 1.0 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.7 to 2.0 (m, 3H, CHβ Ile, CH-CH_2-*CH_2*), 2.5 to 2.8 (m, 4H, $CH_2\beta$ Asp, CH-*CH_2*-CH_2), 3.0 (d, 1H, 5H), 3.4 (m, 1H, *CH*-CH_2-CH_2), 3.7 (m, 1H, CH-S), 4.2 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 8.0 (broad s, 3H, NH_3^+), 8.3 (d, 1H, CONH Asp), 8.5 (d, 1H, CONH Ile). MS 458.1 = MH^+.

25: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.7 to 0.9 (m, 6H, 2 × CH_3 Ile), 1.0 and 1.45 (m, 2H, $CH_2\gamma$ Ile), 1.7 to 2.2 (m, 3H, CHβ Ile, CH-CH_2-*CH_2*), 2.5 to 2.9 (m, 4H, $CH_2\beta$ Asp, CH-*CH_2*-CH_2), 3.2 (d, 1H, SH), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.95 (m, 1H, CH-S), 4.25 (m, 1H, CHα Ile), 4.5 (m, 1H, CHα Asp), 8.0 (broad s, 3H, NH_3^+), 8.3 (d, 1H, CONH Asp), 8.5 (d, 1H, CONH Ile). MS 458.2 = MH^+.

26: [[(2*S*, 3*R*), 3-Amino-5-phosphonate-2-sulfhydryl]-pentanoyl]-(L)Ile-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (t, 3H, $CH_3\delta$ Ile), 0.8 (d, 3H, $CH_3\gamma$ Ile), 1.05 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.65 to 2.0 (m, 5H, CHβ Ile, CH-*CH_2*-CH_2, CH-CH_2-*CH_2*), 2.6 (m, 2H, $CH_2\beta$ Asp), 3.1 (d, 1H, SH), 3.4 (m, 1H, *CH*-CH_2-CH_2), 3.8 (m, 1H, CH-S), 4.2 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 8.0 (broad s, 3H, H_3N^+), 8.4 (2d, 2H, CONH Asp, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, $t_R = 5.97$ min. MS 458.4 = MH^+. Anal. ($C_{17}H_{29}N_3O_{11}SPF_3$) C, H, N.

27: [[(2*R*, 3*R*), 3-Amino-5-phosphonate-2-sulfhydryl]-pentanoyl]-(L)Ile-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (t, 3H, $CH_3\delta$ Ile), 0.8 (d, 3H, $CH_3\gamma$ Ile), 1.0 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.6 to 2.0 (m, 5H, CHβ Ile, CH-*CH_2*-CH_2, CH-CH_2-*CH_2*), 2.65 (m, 2H, $CH_2\beta$ Asp), 3.25 (d, 1H, SH), 3.4 (m, 1H, *CH*-CH_2-CH_2), 3.95 (m, 1H, CH-S), 4.25 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 8.0 (broad s, 3H, H_3N^+), 8.4 (d, 1H, CONH Asp), 8.55 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, $t_R = 3.6$ min. MS 458.3 = MH^+.

28: [[(2*S*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Tyr-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 1.9 (m, 2H, CH-*CH_2*-CH_2), 2.5 to 2.7 (m, 4H, $CH_2\beta$ Asp, $CH_2\beta$ Tyr), 2.8 (d, 1H, SH), 2.9 (m, 2H, CH-CH_2-*CH_2*), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.7 (m, 1H, CH-S), 4.5 (m, 2H, CHα Tyr, CHα Asp), 6.6 (d, 2H, CH arom. ortho OH), 7.0 (d, 2H, CH arom. meta OH), 8.0 (broad s, 3H, NH_3^+), 8.4 (d, 1H, CONH Asp), 8.5 (d, 1H, CONH Tyr). HPLC C_8 Kromasil (5 μm, 100 Å) gradient CH_3CN/H_2O (TFA) 10/60, $t_R = 8.4$ min. MS 507.7 = MH^+, 529.6 = MNa^+. Anal. ($C_{17}H_{29}N_4O_{10}S_2F_3$) C, H, N.

29: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Tyr-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 1.4 to 1.6 (m, 2H, CH-*CH_2*-CH_2), 2.5 to 2.7 (m, 4H, $CH_2\beta$ Asp, $CH_2\beta$ Tyr), 2.9 (m, 2H, CH-CH_2-*CH_2*), 3.2 (d, 1H, SH), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.8 (d, 1H, CH-S), 4.5 (m, 2H, CHα Tyr, CHα Asp), 6.6 (d, 2H, CH arom. ortho OH), 7.0 (d, 2H, CH arom. meta OH), 7.9 (broad s, 3H, NH_3^+), 8.4 (d, 1H, CONH Asp), 8.6 (d, 1H, CONH Tyr). HPLC C_8 Kromasil (5 μm, 100 Å) gradient CH_3CN/H_2O (TFA) 10/60, $t_R = 8.0$ min. MS 507.8 = MH^+, 529.7 = MNa^+.

30: [[(2*S*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)Asp-NH_2. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.7 (m, 6H, $CH_3\delta$ Ile, $CH_3\gamma$ Ile), 1.0 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.7 (m, 1H, CHβ Ile), 1.85 (m, 2H, CH-*CH_2*-CH_2), 2.6 (m, 4H, CH-CH_2-*CH_2*, $CH_2\beta$ Asp), 3.45 (m, 1H, *CH*-CH_2-CH_2), 3.75 (m, 1H, CH-S), 4.1 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 8.1 (broad s, 3H, H_3N^+), 8.15 (d, 1H, CONH Asp), 8.55 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, $t_R = 4.9$ min. MS 456.9 = MH^+, 478.9 = MNa^+. Anal. ($C_{17}H_{29}N_4O_{10}S_2F_3$) C, H, N.

31: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)Asp-NH_2. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.8 (m, 6H, $CH_3\delta$ Ile, $CH_3\gamma$ Ile), 1.05 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.7 (m, 1H, CHβ Ile), 1.8 to 2.0 (m, 2H, CH-*CH_2*-CH_2), 2.6 (m, 4H, CH-CH_2-*CH_2*, $CH_2\beta$ Asp), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.9 (m, 1H, CH-S), 4.1 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 8.0 (broad s, 3H, H_3N^+), 8.15 (d, 1H, CONH Asp), 8.5 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm,100 Å) CH_3CN/H_2O (TFA) 15/85, $t_R = 3.5$ min. MS 456.9 = MH^+, 478.9 = MNa^+.

32: [[(2*S*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(D)Ile-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.8 (m, 6H, 2 × CH_3 Ile), 1.1 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.75 (m, 1H, CHβ Ile), 1.95 (m, 2H, CH-*CH_2*-CH_2), 2.6 to 2.8 (m, 4H, CH-CH_2-*CH_2*, $CH_2\beta$ Asp), 3.55 (m, 1H, *CH*-CH_2-CH_2), 3.8 (m, 1H, CH-S), 4.25 (dd, 1H, CHα Ile), 4.6 (dd, 1H, CHα Asp), 8.0 (broad s, 3H, H_3N^+), 8.35 (d, 1H, CONH Asp), 8.45 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 10/90, $t_R = 6.5$ min.

33: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(D)Ile-(L)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.8 (m, 6H, 2 × CH_3 Ile), 1.1 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.8 (m, 1H, CHβ Ile), 2.05 (m, 2H, CH-*CH_2*-CH_2), 2.6 to 2.9 (m, 4H, CH-CH_2-*CH_2*), 3.55 (m, 1H, *CH*-CH_2-CH_2), 3.9 (m, 1H, CH-S), 4.2 (dd, 1H, CHα Ile), 4.55 (dd, 1H, CHα Asp), 8.0 (broad s, 3H, H_3N^+), 8.2 (d, 1H, CONH Asp), 8.4 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 10/90, $t_R = 17.6$ min.

34: [[(2*S*, 3*R*), 3-amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(D)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.8 (m, 6H, 2 × CH_3 Ile), 1.1 and 1.45 (m, 2H, $CH_2\gamma$ Ile), 1.7 (m, 1H, CHβ Ile), 1.9 (m, 2H, CH-*CH_2*-CH_2), 2.5 to 2.7 (m, 4H, CH-CH_2-*CH_2*, $CH_2\beta$ Asp), 3.45 (m, 1H, *CH*-CH_2-

CH_2), 3.8 (m, 1H, CH-S), 4.2 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 8.05 (broad s, 3H, H_3N^+), 8.3 (d, 1H, CONH Asp), 8.5 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 10/90, t_R = 15.6 min.

35: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(D)Asp-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.8 (m, 6H, 2 × CH_3 Ile), 1.05 and 1.4 (m, 2H, $CH_2\gamma$ Ile), 1.7 (m, 1H, CHβ Ile), 1.95 (m, 2H, CH-*CH*$_2$-CH_2), 2.5 to 2.8 (m, 4H, CH-CH_2-*CH*$_2$, $CH_2\beta$ Asp), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.9 (m, 1H, CH-S), 4.3 (dd, 1H, CHα Ile), 4.5 (dd, 1H, CHα Asp), 7.95 (broad s, 3H, H_3N^+), 8.25 (d, 1H, CONH Asp), 8.5 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 10/90, t_R = 5.9 min.

36: [[(2*S*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)Sal-OH. White powder. ^{1}H RMN (DMSO-d_6 + TFA) δ 0.8 (m, 6H, 2 × CH_3 Ile), 1.1 and 1.4 (2m, 2H, $CH_2\gamma$ Ile), 1.75 (m, 1H, CHβ Ile), 1.8 to 2.0 (m, 2H, CH-*CH*$_2$-CH_2), 2.65 (m, 2H, CH-CH_2-*CH*$_2$), 2.9 (d, 2H, $CH_2\beta$ Sal), 3.0 (d, 1H, SH), 3.55 (m, 1H, CH-S), 3.85 (m, 1H, *CH*-CH_2-CH_2), 4.15 (dd, 1H, CHα Ile), 4.35 (m, 1H, CHα Sal), 7.95 (broad s, 3H, NH_3^+), 8.35 (d, 1H, CONH Ile), 8.5 (d, 1H, CONH Sal). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 4.1 min. MS 493.9 = MH^+. Anal. ($C_{16}H_{28}N_3O_{12}S_3F_3$) C, H, N.

37: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)Sal-OH. White powder. ^{1}H RMN (DMSO-d_6 + TFA) δ 0.7 to 0.9 (m, 6H, $CH_3\delta$ Ile, $CH_3\gamma$ Ile), 1.1 and 1.45 (m, 2H, $CH_2\gamma$ Ile), 1.75 (m, 1H, CHβ Ile), 1.8 to 2.0 (m, 2H, CH-*CH*$_2$-CH_2), 2.75 to 3.0 (m, 4H, CH-CH_2-*CH*$_2$, $CH_2\beta$ Sal), 3.5 (m, 1H, CH-S), 3.95 (m, 1H, *CH*-CH_2-CH_2), 4.2 (dd, 1H, CHα Ile), 4.4 (dd, 1H, CHα Sal), 7.95 (broad s, 3H, NH_3^+), 8.25 to 8.40 (4d, 2H, CONH Ile, CONH Sal). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 2.4 min.

38: [[(2*S*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Tyr-(L)hSal-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 1.4 to 1.6 (m, 2H, CH-*CH*$_2$-CH_2), 1.8 to 2.1 (m, 2H, $CH_2\beta$ hSal), 2.4 to 2.7 (m, 4H, $CH_2\beta$ Tyr, $CH_2\gamma$ hSal), 2.9 (m, 2H, CH-CH_2-*CH*$_2$), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.7 (m, 1H, CH-S), 4.2 (m, 1H, CHα hSal), 4.45 (m, 1H, CHα Asp), 6.6 (d, 2H, CH arom. ortho OH), 7.0 (d, 2H, CH arom. meta OH), 8.0 (broad s, 3H, NH_3^+), 8.5 (2d, 2H, CONH hSal, CONH Tyr). HPLC C_8 Kromasil (5 μm, 100 Å) gradient CH_3CN/H_2O (TFA) 10/60 to 60/40, t_R = 3.5 min. MS 557.9 = MH^+, 579.9 = MNa^+. Anal. ($C_{20}H_{28}N_3O_{13}S_3F_3$) C, H, N.

39: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Tyr-(L)hSal-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 1.5 to 1.7 (m, 2H, CH-*CH*$_2$-CH_2), 1.8 to 2.2 (m, 2H, $CH_2\beta$ hSal), 2.4 to 2.7 (m, 4H, $CH_2\beta$ Tyr, $CH_2\gamma$ hSal), 2.9 (m, 2H, CH-CH_2-*CH*$_2$), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.75 (m, 1H, CH-S), 4.2 (m, 1H, CHα hSal), 4.5 (m, 1H, CHα Asp), 6.6 (d, 2H, CH arom. ortho OH), 7.0 (d, 2H, CH arom. meta OH), 7.9 (broad s, 3H, NH_3^+), 8.4 (d, H, CONH hSal), 8.65 (d,1H, CONH Tyr). HPLC C_8 Kromasil (5 μm, 100 Å) gradient CH_3CN/H_2O (TFA) 10/60 to 60/40, t_R = 2.8 min. MS 557.8 = MH^+.

40: [[(2S, 3R), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)((3R)-COOH)Pro-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (t, 3H, $CH_3\delta$ Ile), 0.85 (d, 3H, $CH_3\gamma$ Ile), 1.05 and 1.5 (m, 2H, $CH_2\gamma$ Ile), 1.7 to 1.9 (m, 3H, CHβ Ile, CH-*CH*$_2$-CH_2), 2.0 to 2.2 (m, $CH_2\gamma$ Pro), 2.6 (t, 2H, CH-CH_2-*CH*$_2$), 3.1 (m, 1H, SH), 3.2 (m, 1H, CHβ Pro), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.6 to 3.8 (m, 3H, CH-S, $CH_2\delta$ Pro), 4.3 (dd, 1H, CHα Ile), 4.5 (d, 1H, CHα Pro), 8.0 (broad s, 3H, H_3N^+), 8.7 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm,100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 9.9 min. MS 484.1 = MH^+, 506.1 = MNa^+. Anal. ($C_{19}H_{30}N_3O_{11}S_2F_3$) C, H, N.

41: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)((3R)-COOH)Pro-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (t, 3H, $CH_3\delta$ Ile), 0.85 (d, 3H, $CH_3\gamma$ Ile), 1.05 and 1.5 (m, 2H, $CH_2\gamma$ Ile), 1.7 (m, 1H, CHβ Ile), 1.8 to 2.0 (2m, 2H, CH-*CH*$_2$-CH_2), 2.0 to 2.2 (m, 2H, $CH_2\gamma$ Pro), 2.65 (t, 2H, CH-CH_2-*CH*$_2$), 3.15 (m, 1H, CHβ Pro), 3.25 (m, 1H, SH), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.6 to 3.75 (2m, 2H, $CH_2\delta$ Pro), 3.8 (m, 1H, CH-S), 4.3 (dd, 1H, CHα Ile), 4.5 (d, 1H, CHα Pro), 8.0 (s broad, H_3N^+), 8.75 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 3.6 min. MS 484.1 = MH^+.

42: [[(2*S*, 3*R*), 3-amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-(L)((3S)-COOH)Pro-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.8 (t, 3H, $CH_3\delta$ Ile), 0.9 (d, 3H, $CH_3\gamma$ Ile), 1.0 and 1.5 (2m, 2H, $CH_2\gamma$ Ile), 1.8 to 2.0 (m, 3H, CHβ Ile, CH-*CH*$_2$-CH_2), 2.1 to 2.3 (m, 2H, $CH_2\gamma$ Pro), 2.7 (m, 2H, CH-CH_2-*CH*$_2$), 3.05 (m, 1H, CHβ Pro), 3.1 (d, 1H, SH), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.5 to 3.75 (m, 2H, $CH_2\delta$ Pro), 3.85 (m, 1H, CH-S), 4.35 (dd, 1H, CHα Ile), 4.5 (d, 1H, CHα Pro), 8.0 (broad s, 3H, H_3N^+), 8.65 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 14.9 min. MS 484.5 = MH^+, 522.5 = MK^+.

43: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate-pentanoyl]-(L)Ile-(L)((3S)-COOH)Pro-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.8 (t, 3H, $CH_3\delta$ Ile), 0.9 (d, 3H, $CH_3\gamma$ Ile), 1.1 and 1.6 (2m, 2H, $CH_2\gamma$ Ile), 1.8 (m, 1H, CHβ Ile), 1.9 to 2.0 (m, 2H, CH-*CH*$_2$-CH_2), 2.05 to 2.2 (m, 2H, $CH_2\gamma$ Pro), 2.7 (t, 2H, CH-CH_2-*CH*$_2$), 3.0 (m, 1H, CHβ Pro), 3.3 (d, 1H, SH), 3.55 (m, 1H, *CH*-CH_2-CH_2), 3.6 to 3.8 (m, 2H, $CH_2\delta$ Pro), 3.85 (m, 1H, CH-S), 4.35 (dd, 1H, CHα Ile), 4.5 (d, 1H, CHα Pro), 7.9 (broad s, 3H, H_3N^+), 8.7 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 4.7 min. MS 484.4 = MH^+, 522.3 = MK^+.

44: [[(2*S*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.8 (m, 6H, $CH_3\delta$ Ile, $CH_3\gamma$ Ile), 1.2 to 1.5 (2m, 2H, CH_2 Ile), 1.8 (m, 3H, CHβ Ile), 1.9 (m, 2H, CH-CH_2-CH_2), 2.5 to 2.7 (m, 2H, CH-CH_2-CH_2), 3.5 (m, 1H, CH-CH_2-CH_2), 3.75 (m, 1H, CH-S), 4.2 (m, 1H, CHα Ile), 8.1 (d, 3H, H_3N^+), 8.6 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 11.2 min. MS 342.8 = MH^+, 364.8 = MNa^+. Anal. ($C_{13}H_{23}N_2O_8S_2F_3$) C, H, N.

45: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-OH. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.8 (m, 6H, $CH_3\delta$ Ile, $CH_3\gamma$ Ile), 1.1 to 1.4 (2m, 2H, CH_2 Ile), 1.7 (m, 1H, CHβ Ile), 1.8 (m, 2H, CH-*CH*$_2$-CH_2), 2.65 (t, 2H, CH-CH_2-*CH*$_2$), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.8 (m, 1H, CH-S), 4.2 (m, 1H, CHα Ile), 7.9 (d, 3H, H_3N^+), 8.6 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 4.7 min. MS 342.9 = MH^+, 364.9 = MNa^+.

46: [[(2S, 3R), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-NH_2. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (m, 6H, $CH_3\delta$ Ile, $CH_3\gamma$ Ile), 1.1 and 1.4 (2m, 2H, CH_2 Ile), 1.7 (m, 3H, CHβ Ile), 1.8 to 2.0 (m, 2H, CH-*CH*$_2$-CH_2), 2.6 (t, 2H, CH-CH_2-*CH*$_2$), 3.1 (d, 1H, SH), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.9 (m, 1H, CH-S), 4.1 (m, 1H, CHα Ile), 7.05 and 7.45 (2 broad s, 2H, $CONH_2$), 7.9 (d, 3H, H_3N^+), 8.4 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 5.4 min. MS 342.0 = MH^+, 379.9 = MK^+. Anal. ($C_{13}H_{24}N_3O_7S_2F_3$) C, H, N.

47: [[(2*R*, 3*R*), 3-Amino-2-sulfhydryl-5-sulfonate]-pentanoyl]-(L)Ile-NH_2. White powder. ^{1}H NMR (DMSO-d_6 + TFA) δ 0.75 (m, 6H, $CH_3\delta$ Ile, $CH_3\gamma$ Ile), 1.1 and 1.4 (2m, 2H, CH_2 Ile), 1.7 (m, 3H, CHβ Ile), 1.8 to 2.0 (m, 2H, CH-*CH*$_2$-CH_2), 2.75 (t, 2H, CH-CH_2-*CH*$_2$), 3.3 (d, 1H, SH), 3.5 (m, 1H, *CH*-CH_2-CH_2), 3.9 (m, 1H, CH-S), 4.1 (m, 1H, CHα Ile), 7.05 and 7.45 (2 broad s, 2H, $CONH_2$), 7.9 (d, 3H, H_3N^+), 8.5 (d, 1H, CONH Ile). HPLC C_{18} Kromasil (5 μm, 100 Å) CH_3CN/H_2O (TFA) 15/85, t_R = 3.4 min. MS 342.2 = MH^+, 364.3 = MNa^+.

References

(1) Wilk, S.; Healy, D. P. Glutamyl aminopeptidase (aminopeptidase A), the BP-1/6C3 antigen. *Adv. Neuroimmunol.* **1993**, *3*, 195−207.

(2) Nagatsu, I.; Nagatsu, T.; Yamamoto, T.; Glenner, G. G.; Mehl, J. W. Purification of aminopeptidase A in human serum and degradation of angiotensin II by the purified enzyme. *Biochim. Biophys. Acta* **1970**, *198*, 255−270.

(3) Wu, Q.; Lahti, J. M.; Avi, G. M.; Burrows, P. D.; Cooper, M. D. Molecular cloning of the murine BP-1/6C3 antigen: a member of the zinc-dependent metallopeptidase family. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 993−997.

(4) Nanus, D. M.; Engelstein, D.; Gastl, G. A.; Gluck, L.; Vidal, M. J.; Morrison, M.; Finstad, C. L.; Bander, N. H.; Albino, A. P. Molecular cloning of the human kidney differentiation antigen gp160: human aminopeptidase A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7069−7073.
(5) Li, L.; Wang, J.; Cooper, M. D. cDNA cloning and expression of human glutamyl aminopeptidase (aminopeptidase A). *Genomics* **1993**, *17*, 657−664.
(6) Benchetrit, T.; Bissery, V.; Mornon, J. P.; Devault, A.; Crine, P.; Roques, B. P. Primary structure homologies between two Zn-metallopeptidases, the neutral endopeptidase 24.11 enkephalinase and the thermolysin through clustering. *Biochemistry* **1988**, *27*, 592−596.
(7) Roques, B. P. Zinc metallopeptidases: active site structure and design of selective and mixed inhibitors. New approach in the search for analgesics and antihypertensives. *Biochem. Soc. Trans.* **1993**, *21*, 678−685.
(8) Roques, B. P.; Noble, F.; Daugé, V.; Fournié-Zaluski, M. C.; Beaumont, A. Neutral endopeptidase 24.11. Structure, inhibition, and experimental and clinical pharmacology. *Pharmacol. Rev.* **1993**, *45* (1), 87−146.
(9) Matthews, B. W. Structural basis of the action of thermolysin and related zinc peptidases. *Acc. Chem. Res.* **1988**, *21*, 333−340.
(10) Jongeneel, C. V.; Bouvier, J.; Bairoch, A. A unique signature identifies a family of zinc-dependent metallopeptidases. *FEBS Lett.* **1989**, *242*, 211−214.
(11) Rawling, N.; Barrett, A. Evolutionary families of metallopeptidases. *Methods Enzymol.* **1995**, *290*, 205−218.
(12) Wang, J. Y.; Cooper, M. D. Histidine residue in the zinc-binding motif of aminopeptidase A is critical for enzymatic activity. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1222−1226.
(13) Vazeux, G.; Wang, J.; Corvol, P.; Llorens-Cortès, C. Identification of glutamate residues essential for catalytic activity and zinc coordination in aminopeptidase A. *J. Biol. Chem.* **1996**, *271*, 9069−9074.
(14) Vazeux, G.; Iturrioz, X.; Corvol, P.; Llorens-Cortès, C. A tyrosine residue essential for catalytic activity in aminopeptidase A. *Biochem. J.* **1997**, *327*, 883−889.
(15) Vazeux, G.; Iturrioz, X.; Corvol, P.; Llorens-Cortès, C. A glutamate residue contributes to the exopeptidase specificity in aminopeptidase A. *Biochem. J.* **1998**, *334*, 407−413.
(16) Luciani, N.; Marie-Claire, C.; Ruffet, E.; Beaumont, A.; Roques, B. P.; Fournié-Zaluski, M.-C. Characterization of Glu 350 as a critical residue involved in the N-terminal amino binding site of aminopeptidase N (EC 3.4.11.2): Insights into its mechanism of action. *Biochemistry* **1998**, *37*, 686−692.
(17) Zini, S.; Fournié-Zaluski, M.-C.; Chauvel, E.; Roques, B. P.; Corvol, P.; Llorens-Cortès, C. Identification of metabolic pathways of brain angiotensin II and III using specific aminopeptidase inhibitors: predominant role of angiotensin III in the control of vasopressin release. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11968−11973.
(18) Wright, J. W.; Mitzutani, S.; Murray, C. E.; Amir, H. Z.; Harding, J. W. Aminopeptidase-induced elevations and reductions in blood pressure in the spontaneously hypertensive rat. *J. Hypertens.* **1990**, *8*, 969−974.
(19) Aoyagi, T.; Tobe, H.; Kojima, F.; Hamada, M.; Takeuchi, T.; Umezawa, H. Amastatin, an inhibitor of aminopeptidase A, produced by Actinomycetes. *J. Antibiot.* **1978**, *31*, 636−638.
(20) Umezawa, H.; Aoyagi, T.; Suda, H.; Hamada, M.; Takeuchi, T. Bestatin, an inhibitor of aminopeptidase B, produced by Actinomycetes. *J. Antibiot.* **1976**, *29*, 97−99.
(21) Chan, W. W. C. L-Leucine thiol. A potent inhibitor of leucine aminopeptidase. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 297−302.
(22) Fournié-Zaluski, M.-C.; Coric, P.; Turcaud, S.; Bruetschy, L.; Lucas, E.; Noble, F.; Roques, B. P. Potent and systemically active aminopeptidase N inhibitors from active-site investigation. *J. Med. Chem.* **1992**, *35*, 1259−1266.
(23) Wilk, S.; Thurston, L. S. Inhibition of angiotensin III formation by thiol derivatives of acidic amino acids. *Neuropeptides* **1990**, *16*, 163−168.
(24) Chauvel, E. N.; Coric, P.; Llorens-Cortès, C.; Wilk, S.; Roques, B. P.; Fournié-Zaluski, M.-C. Differential inhibition of aminopeptidase A and aminopeptidase N by new β-amino thiols. *J. Med. Chem.* **1994**, *37*, 2950−2957.
(25) Roberts, J. C.; Gao, H.; Gopalsamy, A.; Kongsjahju, A.; Patch, R. J. Neopentyl ester protecting groups for arylsulfonic acids. *Tetrahedron Lett.* **1997**, *38*, 355−358.
(26) Bischoff, L.; David, C.; Martin, L.; Meudal, H.; Roques, B. P.; Fournié-Zaluski, M.-C. 2,4-Dinitrophenyl 4-methoxybenzyl disulfide: A new reagent for the electrophilic sulfenylation of β-amino ester enolates. *J. Org. Chem.* **1997**, *62*, 4848−4850.
(27) Gordon, E. M.; Godfrey, J. D.; Delaney, N. G.; Asaad, M. M.; Von Langen, D.; Cushman, D. W. Design of novel inhibitors of aminopeptidases. Synthesis of peptide-derived diamino-thiols and sulfur containing replacement analogues of bestatin. *J. Med. Chem.* **1988**, *31*, 2199−2211.
(28) Wei, Z. Y.; Knaus, E. E. A regioselective tandem reduction-Wittig Horner reaction involving the α-ester moiety of diethyl aspartate or glutamate. *Tetrahedron Lett.* **1994**, *35*, 2305−2308.
(29) Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; kulesha, I. D.; Tsougraki, C.; Meienhofer, J. Facile synthesis of amino acids and dipeptide esters under mild conditions via cesium salts. *J. Org. Chem.* **1977**, *42*, 1286−1290.
(30) Dhaon, M. K.; Olsen, R. K.; Ramasamy, K. Esterification of N−Protected α-amino acids with alcohol/Carbodiimide/4-(Dimethylamino)-pyridine. Racemization of aspartic and glutamic derivatives. *J. Org. Chem.* **1982**, *47*, 1962−1965.
(31) Mahoney, W. S.; Brestensky, D. M.; Stryker, J. M. Selective hydride-mediated conjugate reduction of α, β-unsaturated carbonyl compound using $[(Ph_3P)CuH]_6$. *J. Am. Chem. Soc.* **1988**, *110*, 291−293.
(32) Koenig, T. M.; Daeuble, J. F.; Brestensky, D. M.; Stryker, J. M. Conjugate reduction of polyfunctional α,β-unsaturated compounds using $[(Ph_3P)CuH]_6$. Compatibility with halogen, sulfonate, and γ-oxygen and sulfur substituents. *Tetrahedron Lett.* **1990**, *31*, 3237−324.
(33) Bischoff, L.; David, C.; Roques, B. P.; Fournié-Zaluski, M.-C. Side-chain-modified sufonic analogues of aspartic and glutamic acids: Synthesis, protection and incorporation into peptides. *J. Org. Chem.* **1999**, *64*, 1420−1423.
(34) Cotton, R.; Johnstone, A. N. C.; North, M. Asymmetric synthesis of 3-carboxyproline and derivatives suitable for peptide synthesis. *Tetrahedron* **1995**, *51*, 8525−8544.
(35) Martin, L.; Cornille, F.; Turcaud, S.; Meudal, H.; Roques, B. P.; Fournié-Zaluski, M.-C. Metallopeptidase inhibitors of tetanus toxin: a combinatorial approach. *J. Med. Chem.* **1999**, *42*, 515−525.
(36) Xie, J.; Soleilhac, J. M.; Schmidt, C.; Peyroux, J.; Roques, B. P.; Fournié-Zaluski, M. C. New kelatorphan-related inhibitors of enkephalin metabolism: improved antinociceptive properties. *J. Med. Chem.* **1989**, *32*, 1497−1503.
(37) Ocain, T. D.; Rich, D. H. Synthesis of sulfur-containing analogues of bestatin. Inhibition of aminopeptidases by α-thiol bestatin analogues. *J. Med. Chem.* **1988**, *31*, 2193−2199.
(38) Chen, H.; Noble, F.; Coric, P.; Fournié-Zaluski, M. C.; Roques, B. P. Aminophosphinic inhibitors as transition states analogues of enkephalin-degrading enzymes: A class of central analgesics. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12028−12033.
(39) Rich, D. H.; Moon, B. J.; Harbeson, S. Inhibition of aminopeptidases by amastatin and bestatin derivatives. Effect of inhibitor structure and slow-binding processes. *J. Med. Chem.* **1984**, *27*, 417−422.
(40) Kim, H.; Lipscomb, W. N. X-ray crystallographic determination of the structure of bovine Lens leucine aminopeptidase complexed with amastatin: Formulation of a catalytic mechanism featuring a gem-diolate transition state. *Biochemistry* **1993**, *32*, 8465−8478.
(41) Kim, H.; Burley, S. K.; Lipscomb, W. N. Re-refinement of the X-ray crystal structure of bovine Lens leucine aminopeptidase complexed with bestatin. *J. Mol. Biol.* **1993**, *230*, 722−724.
(42) Wyvratt, M. J.; Patchett, A. A. Recent developments in the design of angiotensin converting enzyme inhibitors. *Med. Res. Rev.* **1985**, *5*, 483−531.
(43) Roques, B. P.; Fournié-Zaluski, M. C.; Soroca, E.; Lecomte, J. M.; Malfroy, B.; Llorens, C.; Schwartz, J. C. The enkephalinase inhibitor thiorphan shows antinociceptive activity in mice. *Nature* **1980**, *288*, 286−288.
(44) Berger, J. A novel approach to analgesia: synthesis and structure activity relationship of some potent and selective inhibitors of enkephalinase A. 19th National Medicinal Chemistry Symposium, Tucson, 1984.
(45) Kanno, H.; Osanai, K.; Nishi, T.; Iino, M.; Katakura, S.; Furugohri, T.; Watanabe, Y. Synthesis and evaluation of 2-(biphenylmethyl)glutaric acid amide derivatives as neutral endopeptidase inhibitors. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1487−1490.
(46) Fournié-Zaluski, M. C.; Llorens, C.; Gacel, G.; Malfroy, B.; Swerts, J. P.; Lecomte, J. M.; Schwartz, J. C.; Roques, B. P. Synthesis and biological properties of highly potent enkephalinase inhibitors. In *Peptides*; ed. Brunfeld, K., Ed.; Scriptor: Copenhagen, Denmark, 1981; pp 476−481.
(47) Wilk, S.; Wilk, E.; Magnusson, R. P. Purification, characterization and cloning of a cytosolic aspartyl aminopeptidase. *J. Biol. Chem.* **1998**, *273*, 15961−15970.
(48) Vazeux, G.; Wilk, S.; Wilk, E.; Corvol, P.; Llorens-Cortes, C. Production and properties of a recombinant soluble form of aminopeptidase A. *Eur. J. Biochem.* **1998**, *254*, 671−678.
(49) Aubry, M.; Bertheloot, A.; Beaumont, A.; Roques, B. P.; Crine, P. The use of a monoclonal antibody for the high yield purification of kidney enkephalinase solubilized in β-octylglucoside. *Biochem. Cell Biol.* **1987**, *65*, 398−404.
(50) Pantaliano, M. W.; Holmquist, B.; Riordan, J. F. Affinity chromatographic purification of angiotensin converting enzyme. *Biochemistry* **1984**, *23*, 1037−1042.

(51) Goudreau, N.; Guis, C.; Soleilhac, J. M.; Roques, B. P. DNS-Gly-(pNO_2)Phe-βAla, a specific fluorogenic substrate for neutral endopeptidase 24.11. *Anal. Biochem.* **1994**, *219*, 87−95.
(52) Piquilloud, Y.; Reinharz, A.; Roth, M. Studies of the angiotensin converting enzyme with different substrates. *Biochim. Biophys. Acta* **1970**, *206*, 136−142.
(53) Goldbarg, J. A.; Rutenberg, A. M. The colorimetric determination of leucine aminopeptidase in urine and serum of normal subjects and patients with cancer and other diseases. *Cancer* **1958**, *11*, 283−291.
(54) Cheung, H. S.; Cushman, D. W. Inhibition of homogeneous angiotensin converting enzyme of rabbit lung by synthetic venom peptides of both rops jararaca. *Biochim. Biophys. Acta* **1973**, *293*, 451−457.
(55) Cheng, Y. C.; Prussof, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzyme reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(56) Bergmann, M.; Zervas, L. A general method of peptide synthesis. *Chem. Ber.* **1932**, *65*, 1192−1201.
(57) Brenner, M.; Huber, W. Preparation of α-amino ester by alcoholysis of methyl ester. *Helv. Chim. Acta* **1953**, *36*, 1109−1115.
(58) Von Pechmann, H. About diazomethane. *Chem. Ber.* **1894**, *27*, 1888.
(59) Ondetti, M. A.; Engel, S. L. Bradykinin analogues containing β-homoamino acids. *J. Med. Chem.* **1975**, **18**, 761−763.
(60) Cheng, P. T. W.; Poss, M. A. Produgs esters of phosphonosulfonate squalene synthetase inhibitors and method. US Patent **1997**, *5*, 618,964.
(61) Jennings, L. J.; Macchia, M.; Parkin, A. Synthesis of analogues of 5-iodo-2′-deoxyuridine-5′-diphosphate. *J. Chem. Soc., Perkin Trans.* **1992**, *1*, 2197−2202.
(62) Saady, M., Lebeau, L.; Mioskowski, C. First use of benzyl phosphites in the Michaelis-Arbuzov reaction. *Helv. Chim. Acta* **1995**, *78*, 670−678.
(63) Schwyzer, R.; Castopanagiotis, A.; Sieber, P. Two synthesis of α-melanotropins (α-MSH) with facile cleavage for the protecting groups. *Helv* **1963**, *46*, 870−889.
(64) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. Reactifs de couplage peptidique IV (1) − L'hexafluorophosphate de benzotriazolyl N-oxy-tridimethylamino phosphonium (B.O.P.). *Tetrahedron Lett.* **1975**, *14*, 1219−1222.
(65) Mancuso, A. J.; Swern, D. Activated dimethyl sulfoxide: useful reagent for synthesis. *Synthesis* **1981**, 165−185.

JM9903040