Differential Inhibition of Aminopeptidase A and Aminopeptidase N by New β-Amino Thiols

Eric N. Chauvel,[†] Catherine Llorens-Cortès,[‡] Pascale Coric,[†] Sherwin Wilk,[§] Bernard P. Roques,[*,†] and Marie-Claude Fournié-Zaluski[†]

Unité de Pharmacochimie Moléculaire et Structurale, U266 INSERM – URA D 1500 CNRS, Université René Descartes, UFR des sciences pharmaceutiques et biologiques, 4, avenue de l'Observatoire, 75270 Paris Cedex 06, France, Collège de France, Chaire de médecine expérimentale, U36 INSERM, 3 rue d'Ulm, 75005 Paris, France, and Department of Pharmacology, Mount Sinai School of Medecine, University of New York, New York, New York 10029

Received March 16, 1994[⊗]

Aminopeptidase A (APA) is a highly selective peptidase, which cleaves the N-terminal Glu or Asp residues of biologically active peptides, and has therefore been proposed to be involved in angiotensin II and CCK_8 metabolism. Highly potent and selective APA inhibitors are consequently required to study the physiological regulation of these two peptides. Using, as a model, Glu-thiol (4-amino-5-mercaptopentanoic acid), which was the first efficient APA inhibitor described but is however equipotent on APA (0.14 μM) and aminopeptidase N (APN) (0.12 μM), several β-amino thiol inhibitors have been synthesized. In these molecules, the length of the side chain was varied and the carboxylate group of Glu-thiol was replaced by other negatively charged groups, such as phosphonate, sulfonate, hydroxamate, and thiol. The inhibitory potency of one of these compounds, **22h** (*S*)-3-amino-4-mercaptobutanesulfonate, was found to be nearly 100-fold better for APA than for APN, with an affinity (0.29 μM) almost equivalent to that of Glu-thiol. Hence, this compound is the first selective APA inhibitor reported, and as such, it should be an interesting probe to explore the physiological involvement of APA in the metabolism of neuropeptides like angiotensin II and CCK_8.

Introduction

Aminopeptidase A (APA, glutamyl aminopeptidase, EC 3.4.11.7) is thought to be responsible for the transformation of angiotensin II to angiotensin III, by hydrolyzing its N-terminal aspartyl residue.[1,2] The enzyme is highly selective for acidic amino acids[3] and is also probably involved in the metabolism of other peptides containing an N-terminal Asp or Glu residue, such as CCK_8.[4,5] Aminopeptidase N (APN, EC 3.4.11.2) seems to be the enzyme that inactivates angiotensin III, by cleaving its N-terminal arginyl residue,[6] and has been proposed to sequentially degrade CCK_7.[4] The relative physiological importance of peptides issued from angiotensin I processing in the central nervous system is controversial, but there is increasing evidence that angiotensin III is a crucial component of the brain renin angiotensin system.[2,7-9] However, in peptidergic cascades, where different peptidases sequentially release different putative active ligands from a single inactive precursor, the key to analyzing the various steps is to possess highly specific and potent inhibitors of each enzyme. The pharmacological responses measured in the presence of selective inhibitors can then be unequivocally related to the physiological action of tonically released peptides, as demonstrated for the opioid peptides, enkephalins, and the atrial natriuretic peptide (for a review, see ref 10). For the renin angiotensin system, this strategy was attempted but the inhibitors used did not have the necessary selectivity. Thus, the two inhibitors that have been most commonly used for APA and APN inhibition are amastatin and bestatin, respectively.[6,8,11] However, although amastatin was initially described as a specific APA inhibitor,[12] it is in fact more potent on APN and is even a better inhibitor of the latter enzyme than bestatin[13] which is a nonspecific inhibitor of various aminopeptidases.[14]

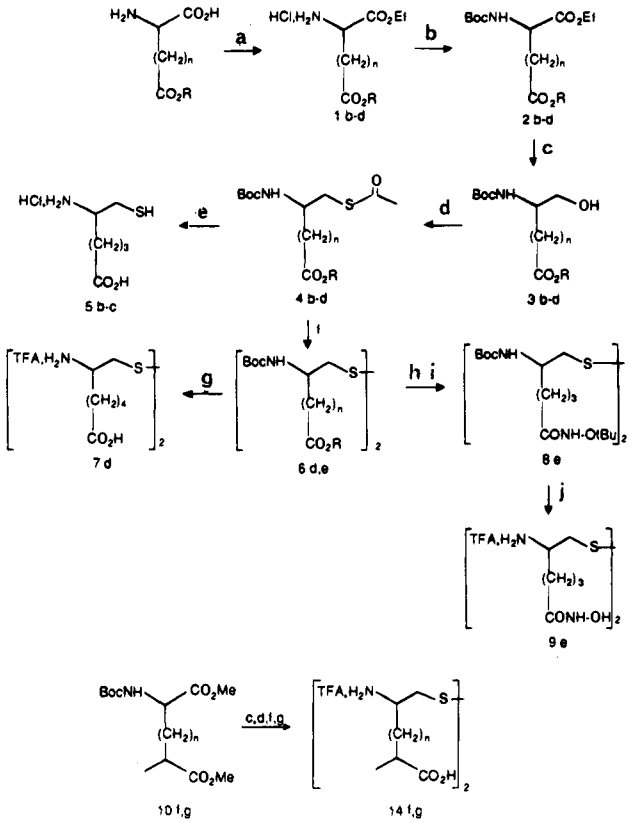
APA and APN are two enzymes belonging to the family of zinc metallopeptidases[15,16] with significant homologies in their amino acid sequences[17-20] and similarities in their physical properties.[21,22] Like neutral endopeptidase 24.11 (NEP)[15] and angiotensin converting enzyme (ACE),[23] APA and APN are characterized by a large glycosylated extracellular domain which contains the active site. On the basis of the structure and mechanism of action of the zinc metallopeptidase thermolysin,[24] efficient inhibitors can be obtained by introducing a zinc-coordinating group on molecules able to recognize the enzyme's $S_1-S'_2$ subsites (for reviews, see refs 10, 25, and 26). Potent and selective inhibitors of APN belonging to the series of mercaptans have been designed, some of them exhibiting K_i values in the 10 nM range.[27,28] In the case of APA, the best reported inhibitor, Glu-thiol,[29] which has a K_i value of 0.14 μM, is however equipotent on APN. There is therefore a need for potent specific inhibitors of APA. We have previously introduced various modifications in Glu-thiol, such as, for instance, replacement of its aliphatic side chain by phenyl or cyclohexyl moieties. This led to increased selectivity but reduced affinity for APA.[30]

In this study, we have introduced new modifications in the side chain of Glu-thiol, which was extended, to increase the selectivity versus APN and the affinity for APA, keeping in mind the respective substrate specificities of these enzymes. Moreover, the carboxylate group was replaced by other negatively charged groups. The results show that a selectivity factor for APA around 100

* To whom correspondence should be addressed. Tel: (33) 43-25-50-45. FAX: (33) 43-26-69-18.
[†] Université René Descartes.
[‡] Collège de France.
[§] University of New York.
[⊗] Abstract published in *Advance ACS Abstracts*, August 1, 1994.

0022-2623/94/1837-2950$04.50/0

Scheme 1. Synthesis of the Carboxylates[a]



[a] (a) $SOCl_2$, EtOH, Δ; (b) $(Boc)_2O$; (c) $NaBH_4$; (d) PPh_3, $(iPrOOCN{=})_2$, CH_3COSH; (e) 6 N HCl, Δ; (f) NaOH, I_2; (g) TFA; (h) NaOH; (i) H_2NOtBu, DCC, HOBt; (j) BTFA/TFA.

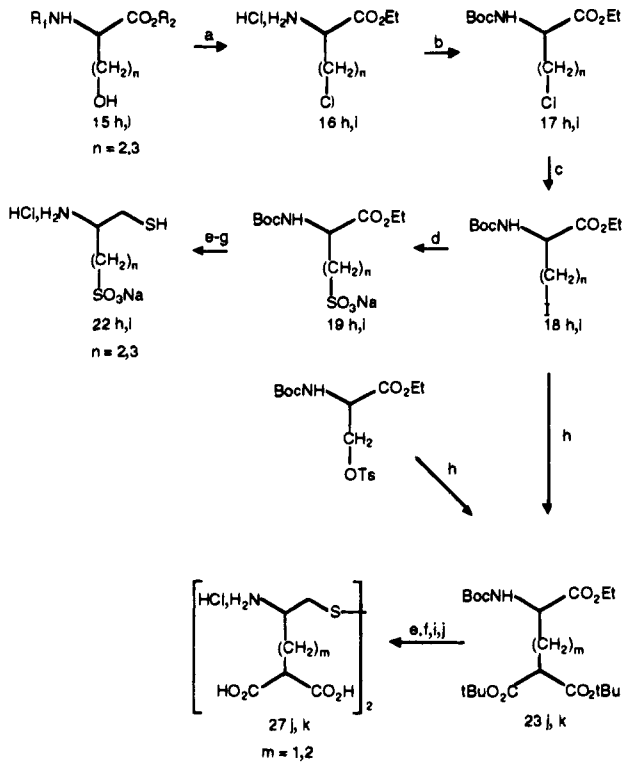
can be obtained in a compound, **22h**, (*S*)-3-amino-4-mercaptobutanesulfonate, with an inhibitory potency of 0.29 μM.

Results

Chemistry. The synthesis of the carboxylate-bearing compounds is summarized in Scheme 1. The starting materials were the commercially available 2-amino dicarboxylic acids, with or without protected side chains. After esterification of the carboxylate(s) (**1b–d**) and protection of the α-amino group (**2b–d**), the next two steps were the selective reduction of the α-ester into an alcohol (**3b–d**) as previously described,[30] using $NaBH_4$ in EtOH or EtOH/THF solutions, and the replacement of the alcohol by a thioester using the Mitsunobu reaction[31] (**4b–d**). The resulting compounds were deprotected, either directly by refluxing with 6 N HCl (**5b–c**) or in two steps by saponification and isolation of the disulfide (**6d,e**), followed by TFA treatment (**7d**). Compound **37n** was synthesized in the same way using dimethyl *N*-Boc-glutamate as starting material, except that the reduction was extended to both ester groups. The substitution of the two hydroxyls of **35n** by thioacetyl groups led to **36n**, and the final deprotection by 6 N HCl gave the desired compound. The hydroxamate **9e** was obtained from the disulfide **6e** by a coupling reaction with *O-tert*-butylhydroxylamine, and compound **8e** was then deprotected using a solution of boron tris(trifluoroacetate) (BTFA). The methyl-substituted compounds **14f,g** were obtained through reactions similar to those described for **7d**, starting from the Boc-amino diesters **10f,g**.

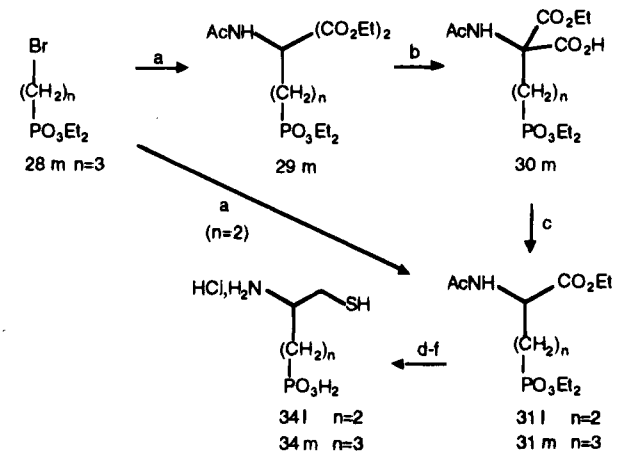
The synthesis of the sulfonates **22h,i** and the malonates **27j,k** is described in Scheme 2. From the

Scheme 2. Synthesis of the Sulfonates and the Dicarboxylates[a]



[a] (a) $SOCl_2$, EtOH, Δ; (b) $(Boc)_2O$; (c) NaI, acetone; (d) Na_2SO_3, dioxane/H_2O; (e) $NaBH_4$; (f) PPh_3, $(iPrN{=})_2$, CH_3COSH; (g) 6 N HCl, Δ; (h) $CH_2(CO_2tBu)_2$, NaH; (i) NaOH, I_2; (j) HCl, EtOAc.

Scheme 3. Synthesis of the Phosphonates[a]

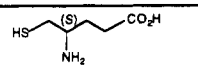
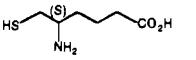
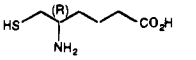
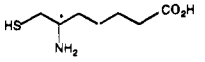
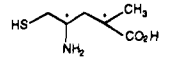
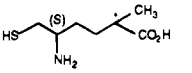
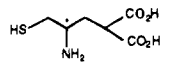
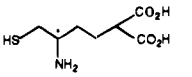
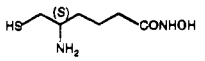
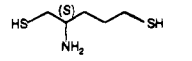
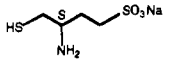
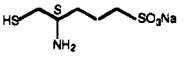
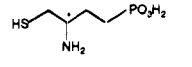
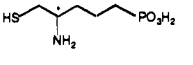


[a] (a) $AcNHCH(CO_2Et)_2$, EtONa; (b) KOH; (c) xylene, Δ; (d) $NaBH_4$; (e) PPh_3, $(iPrOOCN{=})_2$, CH_3COSH; (f) 6 N HCl, Δ.

hydroxyl α-amino acids **15h,i** the esterification in acidic conditions allowed the simultaneous substitution of the hydroxyl by a chloride to be performed (**16h,i**). After protection of the amino group (**17h,i**) and halide exchange by sodium iodide in acetone, a nucleophilic substitution of the halide by Na_2SO_3 or di-*tert*-butyl malonate led to the Boc-amino sulfonates **19h,i** or to the malonate derivatives **23j,k**. The subsequent steps of the synthesis, similar to those described in Scheme 1, gave the corresponding β-amino thiols **22h,i** and **27j,k**.

Scheme 3 depicts the synthesis of the phosphonates. Malonate alkylation of diethyl bromoalkylphosphonates followed by monosaponification and decarboxylation led to the amino phosphonoesters **31l,m** that, after the

Table 1. Inhibitory Potencies of Various β-Amino Thiols for APA and APN[a]

Nb	Formula	Ki (μM)[a] APA	APN
a		0.14 ± 0.06	0.12 ± 0.02
5b		0.13 ± 0.08	0.33 ± 0.05
5c		0.98 ± 0.2	15.5 ± 5.7
7d		1.6 ± 0.06	0.28 ± 0.01
14f		4.1 ± 0.6	2.8 ± 0.3
14g		0.87 ± 0.4	4.0 ± 0.5
27j		3.5 ± 0.4	4.5 ± 0.4
27k		0.28 ± 0.04	0.60 ± 0.04
9e		2.0 ± 0.5	0.037 ± 0.009
37n		2.4 ± 0.6	0.032 ± 0.004
22h		0.29 ± 0.06	25 ± 11
22i		0.37 ± 0.02	1.9 ± 0.2
34l		0.39 ± 0.1	12 ± 2
34m		0.51 ± 0.03	3.7 ± 0.4

[a] The K_i values are the mean ± SEM of three independent experiments performed in triplicate.

steps described in Scheme 1, gave the phosphono β-amino thiols **34l,m**.

The synthetic pathway, leading to β-amino thiols from optically pure α-amino acids, retains the chirality of the starting material. Nevertheless, this was verified in the synthesis of the sulfonates **22h,i** which were obtained from the optically pure α-amino acids **15h,i**, as the introduction of the sulfonate moiety required a reflux in an aqueous solution of Na_2SO_3 which might be responsible for a racemization of the asymmetric carbon. The thioester of the fully protected intermediate **21h** (see the Experimental Section) was saponified under an inert atmosphere, and the free mercapto group obtained was derivatized using the chiral acyl chloride (S)-phenylbutyryl chloride. The resulting compound showed a single peak on HPLC, and a 2D NMR experiment confirmed the presence of only one stereoisomer.

Inhibitory Potencies. The results of the inhibition of APA by the Glu-thiol derivatives are summarized in Table 1. The extension of the lateral chain of this compound by one methylene group (**a**, **5b**) led to the same inhibition profile as that of the parent molecule. However, the addition of another methylene (**7d**) gave a significant loss in affinity (K_i value = 1.6 μM). Introduction of a methyl at the end of the side chain increased the K_i by a factor of 12 for the analog of Glu-thiol **14f** and only by a factor of 6 for the analog of homoGlu-thiol **14g**. Introduction of two carboxyl groups at the end of the side chain led to a 20-fold loss in affinity for **27j** and to the same potency (0.28 μM) for **27k** when compared to their monosubstituted counterparts. The hydroxamate **9e** had a 15-fold lower potency than homoGlu-thiol **5b**, whereas the dithiol **37n** was 18 times less active than **5b**. The sulfonates **22h,i** and the phosphonates **34l,m** had about the same activity as the corresponding carboxylates. For APN, there was a general loss in inhibitory potency when compared to Glu-thiol. Two exceptions were the hydroxamate **9e** and the dithiol **37n**, which were good inhibitors of APN with K_i values around 35 nM. Compounds **5b** and **7d** retained the same potency on APN as their Glu-thiol counterparts.

Discussion

APA is a selective enzyme, cleaving efficiently only N-terminal acidic residues.[3] When tested for arylamidase activity, it had a 4–5-fold preference for Glu-β-naphthylamide over Asp-β-naphthylamide.[32,33] This was the basis for the development of the APA inhibitor Glu-thiol, which was also shown to be 10-fold more active than Asp-thiol in inhibiting APA (K_i values = 0.14 and 1.2 μM, respectively).[29] We have previously investigated the active site of APA by modifying the α-functions of Glu-thiol and introducing a benzene ring or a cyclohexyl in the side chain of the inhibitor.[30] This showed that APA is characterized by relatively large catalytic and S_1 subsites and that the strength of enzyme inhibition by a compound bearing an aromatic side chain is dependent on the position of the carboxylate group on the cycle.

This time, in order to enhance the fit with the S_1 subsite of APA and the selectivity toward APN, we concentrated on two points in the inhibitor: the determination of the optimal length of an aliphatic P_1 moiety and the best negatively charged group to substitute for the carboxylate. As shown in Table 1, the homo-Glu-thiol **5b** has the same K_i value for APA as Glu-thiol **a** but shows a 3-fold loss of activity for APN, therefore leading to an increase in selectivity, without any change in affinity. A further increase in side chain length, by the addition of a methylene group in **7d**, results in a 6-fold loss of inhibitory potency for APA (for the best enantiomer), while the K_i value for APN is similar to that found with Glu-thiol. The optimal length of the side chain for APA recognition therefore seems to be three methylenes, as in homoGlu-thiol **5b**. The introduction of substituents in the side chain of Glu-thiol and homoGlu-thiol gave some unexpected results. The presence of a methyl group in **14f** or of a hydrophilic carboxylate in **27j** induced a large loss of potency on both enzymes. However, the presence of the same substituents in the side chain of homoGlu-thiol (compounds **14g** and **27k**) led to different results since the K_i values for APA were not significantly modified and an improvement in APN inhibition was restricted to **14g**.

These results seem to indicate that not only the length of the side chain but a precise disposition of the carboxylate are important for optimal binding to APA, confirming our previous data with substituted aromatic side chains.[30]

Furthermore, compounds **27j,k** were synthesized to test the hypothesis of Danielsen et al.,[34] that the calcium

ion which activates APA could be positioned at the bottom of the S_1 subsite. Indeed, side chains bearing two carboxyl groups, such as in **27j,k**, have already been shown to bind Ca^{2+} with a good affinity in blood-clotting processes[35] and in calcium-binding proteins involved in mineralization.[36] However no increased inhibition of APA was observed with **27j,k**, which does not confirm the proposal of a Ca^{2+} ion located within the S_1 subsite.

The second part of this study was aimed at replacing the negatively charged carboxylate by other putatively deprotonated groups in the enzyme active site. When the carboxylate group of **5b** was changed, in **37n**, to a thiol or to an hydroxamate, in **9e**, the inhibitory potency decreased 18-fold for APA and increased 10-fold for APN, giving new selective and potent APN inhibitors. Interestingly, **9e** and **37n** differ from **5b** only by the strong decrease in the acidity of the thiol and hydroxamate groups which have replaced the carboxyl group. This suggests that a decrease in the pK_a of the side chain component modifies the binding to both enzymes. The side chain carboxylate was therefore replaced by a sulfonate or a phosphonate, with the aim of enhancing APA specificity. As expected, a selectivity factor around 100 was obtained for **22h**, together with a K_i value for APA (0.29 μM) very similar to that of Glu-thiol. The same result was observed for **34l**, but its selectivity was less pronounced (factor 31). It is interesting to note that the increased selectivity of **22h** and **34l** is not due to an enhanced recognition of APA but to a loss of potency on APN.

The addition of a methylene group in the side chains of **22h** and **34l** led to compounds **22i** and **34m** which exhibited similar inhibitory potencies toward APA but an improvement in APN active site recognition. These data are in apparent conflict with the propyl being the best side chain length, as determined with the carboxylate-containing inhibitors. This suggests that, due to the increase in size and the decrease in pK_a of the phosphonate and sulfonate moieties as compared to the side chain carboxylate, these groups exert a more important repulsive interaction in the S_1 subsite of APN at the end of an ethyl rather than a propyl side chain.

Taking into account the selectivity and affinity displayed by **22h**, this compound is, as far as we know, the best existing selective APA inhibitor. It will therefore be an interesting tool to study the diverse biological roles of APA (for a review, see ref 5).

In conclusion, the exploration of the S_1 subsite of APA presented here confirms our previous results, showing that the positioning of the negative group at the end of the side chain of a β-amino thiol inhibitor is critical for optimal enzyme recognition.[30] Having reached a reasonable selectivity, the next step is now to increase affinity for APA. One possibility could be to extend the occupation of the active site. Preliminary work with di- and tripeptides, with the aim of developing pseudopeptide inhibitors that would interact with the S_1, S_1', and S_2' subsites of APA, is encouraging.

Experimental Section

Inhibitory Potency. Aminopeptidase A, purified from rabbit kidney,[29] hydrolyzed approximately 100 μmol mL^{-1} h^{-1} of the substrate α-L-glutamyl-β-naphthylamide (GluNA). GluNA (K_m = 130 μM) was from Bachem (Bubendorf, Switzerland). Aminopeptidase N from hog kidney was purchased from Boehringer Mannheim (Meylan, France) and suspended in 3.2 M ammonium sulfate, 50 mM Tris buffer, pH 7.4. [^{3}H]-Tyr^1-Leu^5-enkephalin (30 Ci/mmol) was from Amersham. The solutions of thiol inhibitors were prepared in 50 mM Tris buffer, pH 7.4, containing dithiothreitol (DTT, 100 equiv/equiv of inhibitor). K_i values were determined from IC_{50}s, assuming a competitive inhibition, using the Chen–Prusoff relationship.

Aminopeptidase A. The procedure of Goldbarg[37] was used with a downscale modification on a microplate. APA was incubated for 1 h at 37 °C, with or without increasing concentrations of inhibitors and with 200 μM GluNA, in a total volume of 100 μL in 50 mM Tris-HCl buffer, pH 7.4, with 4 mM $CaCl_2$. The reaction was stopped by adding 10 μL of 3 N HCl; 25 μL of 87 mM (0.6%) $NaNO_2$ was then added to each well followed 3 min later by 50 μL of 0.13 M (1.5%) ammonium sulfamate. After a further 5 min, 25 μL of a 23 mM (0.6%) solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride in 95% EtOH was added and the plate was incubated for 30 min at 37 °C. The absorbance was measured at 560 nm.

Aminopeptidase N. APN was preincubated for 15 min at 25 °C with or without increasing concentrations of inhibitors, in a total volume of 100 μL in 50 mM Tris-HCl buffer, pH 7.4. [^{3}H]Tyr^1-Leu^5-enkephalin (K_m = 50 μM) was added to a final concentration of 10 nM, and the reaction was stopped after 15 min by adding 10 μL of 0.5 M HCl. The tritiated metabolite [^{3}H]Tyr was separated on polystyrene beads as described by Vogel and Altstein,[38] and the radioactivity was measured by liquid scintillation counting.

Chemistry. Amino acids were obtained from Bachem (Bubendorf, Switzerland). Homoserine, homoglutamic acid, and all the other reagents were obtained from Aldrich (Saint Quentin Fallavier, France) unless otherwise stated. The solvents were from Merck (Nogent sur Marne, France).

Melting points of the crystallized compounds were measured on an electrothermal apparatus and are reported uncorrected. Chromatography was carried out with Merck silica gel (230–400 mesh). TLC was performed on precoated silica gel plates (60F-254, 0.2 mm thick, Merck) with the following solvent systems (v/v): A1, CH_2Cl_2:MeOH, 9:1; A2, CH_2Cl_2:MeOH, 8:2; B1, *n*-hexane:EtOAc, 6:4; B2, *n*-hexane:EtOAc, 4:1; C, CH_2Cl_2:MeOH:AcOH, 9:1:0.5; D, *n*-hexane:EtOAc:AcOH, 5:5:0.5; E, $CHCl_3$:MeOH:H_2O:AcOH, 5:5:1:0.5; F, *n*-BuOH:H_2O:AcOH, 4:2:2. Plates were developed with UV light, iodine vapor, or ninhydrin. The purity of the final compounds was also checked by HPLC using a silica column (Touzart & Matignon, Vitry sur Seine, France) with CH_2Cl_2–MeOH–AcOH as solvent. The eluted peaks were monitored at 236 nm. The structure of the compounds was confirmed by ^{1}H NMR spectroscopy on a Bruker AC spectrometer (270 MHz) in DMSO-d_6 using HMDS as internal reference, and satisfactory analyses (<±0.4%) were obtained (C, H, N) for all compounds.

The following abbreviations are used, MeOH, methanol; EtOH, ethanol; EtOAc, ethyl acetate; THF, tetrahydrofuran; Boc, *tert*-butyloxycarbonyl; Boc_2O, di-*tert*-butyl dicarbonate; Et_2O, diethyl ether; DME, 1,2-dimethoxyethane; DMF, *N*,*N*-dimethylformamide; TFA, trifluoroacetic acid; HOBt, 1-hydroxybenzotriazole; DCC, dicyclohexylcarbodiimide; LDA, lithium diisopropylamide.

General Procedure for Protection of the Amino Group. Procedure A. The amino group was protected with a *tert*-butyloxycarbonyl group using the previously described method carried out in DMF.[39]

General Procedures for Esterification of the Carboxylate Group. Procedure B. The methyl or ethyl esters were prepared in acidic conditions by the Fischer method (alcohol + $SOCl_2$).

General Procedures for the Reduction of the Ester Function. Procedure C.1. The Boc-amino diester was dissolved in EtOH:water (1:1) (3 mL/mmol), and $NaBH_4$ (1 equiv) in the same solvent was added dropwise at 0 °C. After 15 min, the mixture was heated at 50 °C and stirred for 2–4 h. The EtOH was then evaporated and the resulting solution extracted with EtOAc, washed with brine, dried over Na_2SO_4, and evaporated to dryness.

Procedure C.2. The Boc-amino diester (1 equiv) was dissolved in dry EtOH:THF (8 mL/mmol) and cooled to 0 °C. $NaBH_4$ (4 equiv) and LiCl (4 equiv) in solution in the same

solvent were added dropwise at 0 °C. The mixture was then allowed to warm to room temperature and stirred overnight. The reaction was stopped with 1 N HCl, and the mixture was extracted with EtOAc, washed with water, 1 N HCl, $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated in vacuo.

General Procedure for Substitution of the Hydroxyl Group. Procedure D: Thioacetylation via the Mitsunobu Reaction.[31] Triphenylphosphine (2 equiv) was dissolved in dry THF (3.5 mL/mmol). At 0 °C, diisopropyl azodicarboxylate (2 equiv) was added and the mixture stirred for 30 min until a light yellow precipitate was formed. The alcohol (1 equiv) dissolved in THF (3 mL/mmol), and CH_3COSH (2 equiv) were added. The temperature was allowed to rise slowly to room temperature while the mixture was stirred overnight. After evaporation in vacuo, the residue was dissolved in EtOAc and washed successively with a 10% $NaHCO_3$ solution, H_2O, and brine before being dried over Na_2SO_4. After evaporation, *n*-hexane/EtOAc was added to the residue and the precipitate eliminated. The filtrate was evaporated and the residue purified by flash chromatography on a silica gel column, using *n*-hexane:EtOAc, 4:1, as eluent.

General Procedures for Deprotection Reactions. Procedure E.1: Saponification of Esters and Thioesters. The product (1 equiv) was dissolved in its corresponding alcohol (EtOH or MeOH) (5 mL/mmol), and 1 N NaOH (2.5 equiv) was added at 0 °C. The mixture was stirred for 30 min at 0 °C and for 3 h at room temperature. A solution of I_2 in EtOH was added until a persistant yellow color was obtained. The excess iodine was reduced by $Na_2S_2O_3$, and the solution was evaporated in vacuo. The residue was taken up in water, acidified with 3 N HCl, and extracted with EtOAc. The organic layer was then washed with $Na_2S_2O_3$, H_2O, and brine, dried over Na_2SO_4, and evaporated to dryness.

Procedure E.2: Deprotection of *N*-Boc and *tert*-Butyl Ester Groups by TFA. The product (1 equiv) was dissolved in CH_2Cl_2 (3 mL/mmol). Anisole (1 equiv) and TFA (10 equiv) were then added at 0 °C. After the mixture had stirred for 30 min at 0 °C and for 2 h at room temperature, c-hexane was added to facilitate the evaporation of the TFA in vacuo. The residue was taken up with c-hexane and evaporated to dryness three or four times.

Procedure E.3: General Deprotection by Refluxing in 6 N HCl. The product was taken up in a large excess of 6 N HCl, and the mixture was heated at 130 °C and stirred overnight. The mixture was then allowed to cool down, before being evaporated to dryness. The residue was dissolved in H_2O and reevaporated. This process was repeated three or four times to eliminate excess acid before lyophilization.

Procedure E.4: Deprotection by HCl in EtOAc. A 2.7 N solution of HCl in EtOAc (1 mL/mmol) was added at 0 °C to the compound solubilized in EtOAc. The mixture was then stirred for 2 h at room temperature before evaporation to dryness.[40]

General Procedure for Halide Exchange. Procedure F. The chloro derivative was added to a solution of dry NaI (2 equiv), in dry acetone (1 mL/mmol). The mixture was heated to reflux under a N_2 atmosphere for 8 h. After cooling, the mixture was filtered off and the filtrate evaporated to dryness. It was then taken up in EtOAc, washed with Na_2SO_3, H_2O, and brine, dried over Na_2SO_4, and evaporated to dryness to yield the iodo derivative that was used without purification.

Most of the physical data of the compounds synthesized are summarized in Table 2. Additional data about the end products and some intermediates follow.

(*S*)-5-Amino-6-mercaptohexanoic Acid, Hydrochloride (5b). (*S*)-2-Aminoadipic acid was esterified following procedure B and gave a white solid (100%), mp 118–119 °C, R_f(A1) 0.32. The amino group of the diethyl ester obtained was protected by a Boc group (procedure A) (100%), oily compound, R_f(B1) 0.48. The reduction of the α-ester was performed by procedure C.1. A white solid was obtained (67%), mp 45 °C, R_f(A1) 0.42. The thioacetylation was performed by procedure B. An oily product was isolated (80%), R_f(B1) 0.56. Treatment by TFA (procedure E.2) gave the titled compound (81%): mp 115–116 °C; R_f(E) 0.59; HPLC CH_2Cl_2:MeOH:AcOH, 7.5:2.5:0.1, t_R 8.3 min; $[\alpha]_D^{22} = +17.1°$ in H_2O, $c = 1.035$; ^1H NMR (DMSO) δ 1.52 (m, 4 H, CH_2CH_2CH), 2.2 (t, 2 H, CH_2CO), 2.70 (m, 2 H, CH_2S), 2.82 (d, 1H, SH), 3.15 (m, 1 H, CH), 8.15 (s, 3 H, NH_3^+), 12 (s, 1 H, CO_2H). Anal. ($C_6H_{13}NO_2S$·HCl) C, H, N.

The enantiomers (*S*)-**5b** and (*R*)-**5c** were synthesized by the same pathway. They had the same physical constants excepted for **5c**: $[\alpha]_D^{22} = -17.3°$ in H_2O, $c = 1.039$. Anal. ($C_6H_{13}NO_2S$·HCl) C, H, N.

7-*tert*-Butyl 1-Ethyl 2(*R,S*)-Aminopimelate (1d). 5-Bromovaleric acid (9.49 g, 52 mmol) was esterified with isobutylene using the procedure described by Mokotoff[41] to obtain *tert*-butyl 5-bromopentanoate as a colorless oil, 11.64 g (94%), R_f(B1) 0.64.

Alkylation of *tert*-butyl 5-bromopentanoate (5 g, 21 mmol) was performed using the procedure described by Duhamel,[42,43] with ethyl(diphenylmethylene)glycinate and LDA. Purification by flash chromatography in *n*-hexane:EtOAc (6:1) gave 7-*tert*-butyl 1-ethyl 2-[*N*-(diphenylmethylene)amino]pimelate as a yellow oil, 1.964 g (26%), R_f(B2) 0.38. The diphenylimine group of 7-*tert*-butyl 1-ethyl 2-[*N*-(diphenylmethylene)amino]-pimelate (1.93 g, 4.6 mmol) was cleaved using 1 N HCl in Et_2O[44] to give **1d** as a yellow oil, 500 mg (42%), R_f(A1) 0.34.

7,7′-Dithiobis(6(*R,S*)-aminoheptanoic acid), Bis(trifluoroacetate) (7d): HPLC CH_2Cl_2:MeOH:AcOH, 7.5:2.5:0.1, t_R 6.5 min; ^1H NMR (DMSO + TFA) δ 1.4, 1.54, and 1.67 (3 m, 3 × 2 H, $CH_2CH_2CH_2CH$), 2.25 (t, 2 H, CH_2CO), 3.0 (m, 2 H, CH_2S), 3.4 (m, 1 H, CH), 7.96 (s, 3 H, NH_3^+). Anal. ($C_{14}H_{28}N_2O_4S_2$·2TFA) C, H, N.

6,6′-Dithiobis[5-*N*-Boc-(*S*)-amino)hexane[*N*-*tert*-butyloxy)carboxamide]] (8e). At 0 °C, to a solution of **6e** (368 mg, 0.7 mmol) in dry THF were added successively H_2NOtBu, HCl (2.1 equiv), Et_3N (2.2 equiv), HOBt, H_2O (2 equiv) in THF (6 mL), and DCC (2.4 equiv) in $CHCl_3$ (5 mL). The mixture was stirred for 15 min at 0 °C, and then it was allowed to warm to room temperature overnight. It was then filtrated, evaporated to dryness, taken up in EtOAc, and washed with H_2O, citrate 10%, H_2O, $NaHCO_3$ 10%, H_2O, and brine. It was then dried over Na_2SO_4, filtered, and evaporated to dryness. Purification by gel chromatography in CH_2Cl_2:MeOH, 9:1, gave **8e** as a colorless oil, 328 mg (70%), R_f(A1) 0.47.

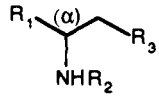
6,6′-Dithiobis[5-(*N*-Boc-(*S*)-amino)hexane(*N*-hydroxycarboxamide)] (9e). As described by Pless,[45] at 0 °C under Ar, **8e** (140 mg, 0.21 mmol) was stirred in TFA (0.5 mL); 1.7 mL of a solution of 1 M BTFA (Merck) in TFA was added. The mixture was stirred at 0 °C for 2 h. It was then evaporated to dryness at room temperature; c-hexane was added and the mixture evaporated to dryness several times. It led to **9e** as a light pink solid: 118 mg (97%); mp >260 °C; R_f(F) 0.53; HPLC CH_2Cl_2:MeOH:AcOH, 8:2:0.1, t_R 6.0 min; ^1H NMR (DMSO + TFA) δ 1.45–1.98 (m, 4 H, CH_2CH_2CH), 2.25 (t, 2 H, CH_2CO), 2.96 (m, 2 H, CH_2S), 3.64 (m, 1 H, CH), 7.96 (bs, 3 H, NH_3^+), 10.09 (s, 1 H, *NH*OH). Anal. ($C_{12}H_{26}N_4O_4S_2$·2TFA) C, H, N.

Methyl 4-(*N*-Boc-(*R,S*)-amino)-5-hydroxy-2(*R,S*)-methylpentanoate (11f). (2*R*,*S*,4*R*,*S*)-4-Methyl-*N*-Boc-glutamic acid dimethyl ester was synthesized using the procedure described by Done[46] followed by esterification (B) and amino protection (A). This compound, **10f** (3 g, 8 mmol), was reduced using procedure C.1 followed by purification by flash chromatography in CH_2Cl_2:MeOH (9:1) to afford **11f** as a yellow oil, 1.18 g (56%), R_f(A1) 0.40.

5,5′-Dithiobis(4(*R,S*)-amino-2(*R,S*)-methylpentanoic acid), Bis(trifluoroacetate) (14f): HPLC CH_2Cl_2:MeOH:AcOH, 8:2:0.1, t_R 5.9 min; ^1H NMR (DMSO + TFA) δ 1.07 (d, 3 H, CH_3CH), 1.56 and 1.97 (m, 2 H, CH_2), 2.58 (m, 1 H, $CH\gamma$), 2.92 (m, 2 H, CH_2S), 3.38 (m, 1 H, CH_α), 7.9 (s, 3 H, NH_3^+), 12.2 (s, 1 H, CO_2H). Anal. ($C_{12}H_{24}N_2O_4S_2$·2TFA) C, H, N.

1-Methyl 6-Ethyl 2-*N*-Boc-(*S*)-amino)-5(*R,S*)-methyladipate (10g). Ethyl 2-(diethylphosphono)propanoate (1.6 equiv) and NaH (1.6 equiv) in dry DME were stirred at 0 °C for 15 min until *tert*-butyl 2-(*N*-Boc-amino)-4-oxobutanoate (2.94 g, 10.8 mmol), prepared according to the procedure of Ramsamy et al.,[47] was added. The mixture was then heated to reflux for 5 h and evaporated to dryness. It was taken up in EtOAc, washed with water and brine, dried over Na_2SO_4,

Table 2. Physical Constants of the Compounds Synthesized[a]



compd	R_1	R_2	R_3	Cα	method	yield (%)	mp (°C)	TLC
1b	CO_2Et	H, HCl	$(CH_2)_2CO_2Et$	S	B	quant	118	0.71 (E)
2b	CO_2Et	Boc	$(CH_2)_2CO_2Et$	S	A	quant	oil	0.48 (B1)
3b	CH_2OH	Boc	$(CH_2)_2CO_2Et$	S	C.1	67	45	0.42 (A1)
4b	CH_2SAc	Boc	$(CH_2)_2CO_2Et$	S	D	80	oil	0.56 (B1)
5b	CH_2SH	H, HCl	$(CH_2)_2CO_2H$	S	E.3	81	115	0.59 (E)
2d	CO_2Et	Boc	$(CH_2)_3CO_2tBu$	*	A	52	oil	0.64 (B1)
3d	CH_2OH	Boc	$(CH_2)_3CO_2tBu$	*	C.2	91	oil	0.59 (A1)
4d	CH_2SAc	Boc	$(CH_2)_3CO_2tBu$	*	D	76	oil	0.68 (B1)
6d	$CH_2S-]_2$	Boc	$(CH_2)_3CO_2tBu$	*	E.1	61	oil	0.63 (B1)
7d	$CH_2S-]_2$	H	$(CH_2)_3CO_2^-$, NH_4^+	*	E.1	61	oil	0.63 (B1)
6e	$CH_2S-]_2$	Boc	$(CH_2)_2CO_2H$	*	E.1	61	oil	0.63 (B1)
12f	CH_2SAc	Boc	$CH(CH_3)CO_2Me$	*	D	42	oil	0.50 (B1)
13f	$CH_2S-]_2$	Boc	$CH(CH_3)CO_2H$	*	E.1	82	oil	0.44 (D)
14f	$CH_2S-]_2$	H, TFA	$CH(CH_3)CO_2H$	*	E.2	51	99	0.20 (E)
11g	CH_2OH	Boc	$CH_2CH(CH_3)CO_2Et$	S	C.1	50	oil	0.49 (A1)
12g	CH_2SAc	Boc	$CH_2CH(CH_3)CO_2Et$	S	D	53	oil	0.51 (B1)
13g	$CH_2S-]_2$	Boc	$CH_2CH(CH_3)CO_2H$	S	E.1	71	oil	0.42 (D)
14g	$CH_2S-]_2$	H, TFA	$CH_2CH(CH_3)CO_2H$	S	E.2	57	132	0.38 (E)
16h	CO_2Et	H, HCl	CH_2Cl	S	B	98	105	0.36 (A1)
17h	CO_2Et	Boc	CH_2Cl	S	A	82	55	0.61 (B1)
18h	CO_2Et	Boc	CH_2I	S	F	80	49	0.61 (B1)
19h	CO_2Et	Boc	CH_2SO_3Na	S	*b*	78	100	0.28 (A2)
20h	CH_2OH	Boc	CH_2SO_3Na	S	C.1	75	oil	0.12 (A2)
21h	CH_2SAc	Boc	CH_2SO_3Na	S	D	80	oil	0.20 (A2)
22h	CH_2SH	H, HCl	CH_2SO_3Na	S	E.3	quant	>260	0.23 (F)
16i	CO_2Et	H, HCl	$(CH_2)_2Cl$	S	B	92	oil	0.38 (A1)
17i	CO_2Et	Boc	$(CH_2)_2Cl$	S	A	52	oil	0.40 (B2)
18i	CO_2Et	Boc	$(CH_2)_2I$	S	F	55	oil	0.37 (B2)
19i	CO_2Et	Boc	$(CH_2)_2SO_3Na$	S	*b*	quant	150	0.24 (A2)
20i	CH_2OH	Boc	$(CH_2)_2SO_3Na$	S	C.2	51	92	0.10 (A2)
21i	CH_2SAc	Boc	$(CH_2)_2SO_3Na$	S	D	85	101	0.22 (A2)
22i	CH_2SH	H, HCl	$(CH_2)_2SO_3Na$	S	E.3	quant	157	0.39 (F)
23j	CO_2Me	Boc	$CH(CO_2tBu)_2$	*	*c*	60	oil	0.63 (B1)
24j	CH_2OH	Boc	$CH(CO_2tBu)_2$	*	C.2	70	oil	0.35 (B1)
25j	CH_2SAc	Boc	$CH(CO_2tBu)_2$	*	D	55	oil	0.60 (B1)
26j	$CH_2S-]_2$	Boc	$CH(CO_2tBu)_2$	*	E.1	82	oil	0.66 (B1)
27j	$CH_2S-]_2$	H, HCl	$CH(CO_2H)_2$	*	E.4	66	211	0.41 (F)
23k	CO_2Et	Boc	$CH_2CH(CO_2tBu)_2$	*	*c*	63	oil	0.71 (B1)
24k	CH_2OH	Boc	$CH_2CH(CO_2tBu)_2$	*	C.2	86	oil	0.25 (B1)
25k	CH_2SAc	Boc	$CH_2CH(CO_2tBu)_2$	*	D	56	oil	0.66 (B1)
26k	$CH_2S-]_2$	Boc	$CH_2CH(CO_2tBu)_2$	*	E.1	86	oil	0.73 (B1)
27k	$CH_2S-]_2$	H, HCl	$CH_2CH(CO_2H)_2$	*	E.4	97	124	0.52 (F)
31l	CO_2Et	Ac	$CH_2PO_3Et_2$	*	*d*	86	oil	0.58 (C)
32l	CH_2OH	Ac	$CH_2PO_3Et_2$	*	C.2	93	oil	0.24 (A1)
33l	CH_2SAc	Ac	$CH_2PO_3Et_2$	*	D	38	oil	0.48 (A1)
34l	CH_2SH	H, HCl	$CH_2PO_3H_2$	*	E.3	quant	oil	0.26 (F)
29m	$(CO_2Et)_2$	Ac	$(CH_2)_2PO_3Et_2$	–	*d*	84	oil	0.58 (A1)
32m	CH_2OH	Ac	$(CH_2)_2PO_3Et_2$	*	C.2	quant	oil	0.31 (C)
33m	CH_2SAc	Ac	$(CH_2)_2PO_3Et_2$	*	D	46	oil	0.46 (A1)
34m	CH_2SH	H, HCl	$(CH_2)_2PO_3H_2$	*	E.3	quant	oil	0.29 (G)
35n	CH_2OH	Boc	$(CH_2)_2OH$	S	C.2	86	oil	0.25 (A1)
36n	CH_2SAc	Boc	$(CH_2)_2SAc$	S	D	27	76	0.57 (B1)
37n	CH_2SH	H, HCl	$(CH_2)_2SH$	S	E.3	98	oil	0.77 (E)

[a] Methods and TLC systems are described in the experimental section. * = *R,S*. [b] See ref 51. [c] See refs 52 and 53. [d] See ref 50.

and evaporated to dryness. Purification by flash chromatography in c-hexane:EtOAc (9:1) gave (*S*)-1-*tert*-butyl 6-ethyl 2-(*N*-Boc-amino)-5-methylhex-4-enedioate as a yellow oil, 1.768 g (46%), R_f(B2) 0.64.

(*S*)-1-*tert*-butyl 6-ethyl 2-(*N*-Boc-amino)-5-methylhex-4-enedioate (1.92 g, 5.4 mmol) was then dissolved in MeOH. Pt/C was added as a catalyst, and the mixture was stirred under an atmosphere of hydrogen overnight. After filtration of the catalyst, the remaining solution was evaporated to dryness and gave (2*S*,5*R*,*S*)-1-*tert*-butyl 6-ethyl 2-(*N*-Boc-amino)-5-methyladipate as a yellow oil, 1.6 g (83%), R_f(B2) 0.64.

Deprotection of (2*S*,5*R*,*S*)-1-*tert*-butyl 6-ethyl 2-(*N*-Boc-amino)-5-methyladipate (1.6 g, 4.5 mmol) was carried out with procedure E.4. (2*S*,5*R*,*S*)-6-Ethyl 2-amino-5-methyladipate was obtained as a colorless oil, 1.1 g (quantitative), R_f(E) 0.54.

(2*S*,5*R*,*S*)-6-Ethyl 2-amino-5-methyladipate (1.06 g, 4.4 mmol) was then amino protected (A) and purified by flash chromatography in CH_2Cl_2:MeOH:AcOH (9:0.3:0.5), giving (2*S*,5*R*,*S*)-6-ethyl 2-(*N*-Boc-amino)-5-methyladipate as a yellow oil, 900 mg (67%), R_f(A1) 0.39.

Finally, (2*S*,5*R*,*S*)-6-ethyl 2-(*N*-Boc-amino)-5-methyladipate (900 mg, 3 mmol) was esterified under basic conditions via the cesium salt of the acid and CH_3I[48] into **10g** as a yellow oil, 790 mg (84%), R_f(A1) 0.42.

6,6′-Dithiobis(5(*S*)-amino-2(*R*,*S*)-methylhexanoic acid), Bis(trifluoroacetate) (14g): HPLC CH_2Cl_2:MeOH:AcOH, 8:2:0.1, t_R 5.8 min; ^{1}H NMR (DMSO) δ 1.05 (d, 3 H, CH_3CH), 1.4–1.6 (m, 4 H, CH_2CH_2CH), 2.3 (m, 1 H, CHCOOH), 2.95 (m, 2 H, CH_2S), 3.3 (m, 1 H, CH), 7.95 (s, 3 H, NH_3^+), 12.3 (s, 1 H, COOH). Anal. ($C_{14}H_{28}N_2O_4S_2$·2TFA) C, H, N.

Sodium 3(*S*)-Amino-4-mercaptobutanesulfonate (22h). By treatment with EtOH and $SOCl_2$, L-homoserine gave the intermediate **16h**, which was esterified on the α-carboxylate and halogenated on the side chain, white solid (98%), mp 105–106 °C, R_f(A1) 0.36.

After protection of the α-amine (procedure A, compound **17h**), the halide exchange was performed by procedure F, leading to **18h**, white solid (80%), mp 49 °C, R_f(B1) 0.61. The sulfonation was carried out as previously reported,[51] giving a yellow solid, **19h** (78%), R_f(A2) 0.28. The last steps of the synthesis, reduction of the α-ester (procedure C, compound **20h**), thioacetylation (procedure D, compound **21h**), and deprotection (procedure E.3), gave the compound desired: white solid; mp >260 °C; R_f(F) 0.23; $[\alpha]^{19}_D = +11.1°$ in H_2O, c = 2.145; HPLC CH_2Cl_2:MeOH:AcOH, 8:2:0.1, t_R 5.1 min; ^{1}H NMR (DMSO + TFA) δ 1.84–2.14 (m, 2 H, CH_2CH), 2.7 (m, 4 H, CH_2SH and CH_2SO), 3.32 (m, 1 H, CH), 7.97 (bs, 3 H, NH_3^+). Anal. ($C_4H_{10}NNaO_3S_2$·HCl) C, H, N.

(S)-*tert*-Butyl 2-(*N*-Boc-(S)-amino)-5-hydroxypentanoate (15i). To a solution of Boc-L-Glu(OH)OtBu (5.35 g, 17 mmol) in dry DME were successively added under N_2 at −15 °C 4-ethylmorpholine (1 equiv) and isobutyl chloroformate (1 equiv).[49] After 5 min, the precipitate of 4-ethylmorpholine, HCl was filtered off and washed by DME. Filtrate and washings were combined in a large flask in an ice–salt bath. A solution of $NaBH_4$ (1.5 equiv) in H_2O (8.5 mL) was added. After the solution was stirred for 15 min at the same temperature, H_2O (425 mL) was added next. Evaporation to dryness and purification in *n*-hexane:EtOAc, 6:4, gave **15i** as a colorless oil, 4.78 g (96%), R_f(A1) 0.45, R_f(B1) 0.35.

Sodium 4-Amino-5-mercaptopentanesulfonate (22i): $[\alpha]^{20}_D = -0.2°$ in H_2O, c = 0.619; HPLC CH_2Cl_2:MeOH:AcOH, 8:2:0.1, t_R 4.2 min; ^{1}H NMR (DMSO + TFA) δ 1.74 (m, 4 H, CH_2CH_2CH), 2.64 (m, 2 H, CH_2SO), 2.75 (m, 2 H, CH_2SH), 3.25 (m, 1 H, CH), 8.0 (bs, 3 H, NH_3^+). Anal. ($C_5H_{12}NNaO_3S_2$·HCl) C, H, N.

5,5′-Dithiobis(4(*R,S*)-amino-2-carboxypentanoic acid), Bis(hydrochloride) (27j): HPLC CH_2Cl_2:MeOH:AcOH, 8:2:0.1, t_R 5.3 min; ^{1}H NMR (DMSO + TFA) δ 2.25 and 2.48 (2 m, 2 × 1 H, CH_2CH), 2.78 and 2.85 (dd, 2 H, CH_2S), 3.18 (m, 1 H, CHγ), 3.82 (m, 1 H, CHα), 8.12 (bs, 3 H, NH_3^+). Anal. ($C_{12}H_{20}N_2O_8S_2$·2HCl) C, H, N.

6,6′-Dithiobis(5(*R,S*)-amino-2-carboxyhexanoic acid), Bis(hydrochloride) (27k). The intermediate **18h** (described in the preparation of **22h**) was treated with di-*tert*-butyl malonate and NaH, leading to **23k**. The reduction of the α-ester (procedure C.2) gave **24k** as an oily product (86%), R_f(B1) 0.25. The thioacetylation (procedure D) led to **25k**, oily product (56%), R_f(B1) 0.66. The saponification of the thioester (procedure E.1) and the deprotection of the *tert*-butyl groups (procedure E.4) gave the titled compound as a white solid: mp 124 °C; R_f(F) 0.52; HPLC CH_2Cl_2:MeOH:AcOH, 7.5:2.5:0.1, t_R 8.3 min; ^{1}H NMR (DMSO + TFA) δ 1.63 and 2.02 (m, 4 H, CH_2CH_2CH), 3.04 (m, 2 H, CH_2S), 3.29 (t, 1 H, CHδ), 3.42 (m, 1 H, CHα), 8.09 (bs, 3 H, NH_3^+). Anal. ($C_{14}H_{24}N_2O_8S_2$·2HCl) C, H, N.

Ethyl 2-(*N*-Acetyl-(*R,S*)-amino)-4-(diethylphosphono)-butanoate (31l). Diethyl (2-bromoethyl)phosphonate (25.5 g, 100 mmol) was alkylated using the procedure described by Chambers[50] with diethyl acetamidomalonate. **31l** was recovered decarboxylated in the aqueous phase as a yellow oil, 27 g (86%) (the yield is calculated for the decarboxylated product), R_f(C) 0.58. NMR data showed the product had undergone decarboxylation.

Sodium 3(*R,S*)-Amino-(4-mercaptobutyl)phosphonate (34l): HPLC CH_2Cl_2:MeOH:AcOH, 8:2:0.1, t_R 4.2 min; ^{1}H NMR (DMSO + TFA) δ 1.53–1.94 (m, 4 H, CH_2CH_2), 2.7 (m, 2 H, CH_2S), 3.25 (m, 1 H, CH), 7.97 (bs, 3 H, NH_3^+). Anal. ($C_4H_{12}NO_3PS$·HCl) C, H, N.

Sodium 4(*R,S*)-Amino-(5-mercaptopentyl)phosphonate (34m). HPLC CH_2Cl_2:MeOH:AcOH, 8:2:0.1, t_R 4.2 min; ^{1}H NMR (DMSO + TFA) δ 1.65 (m, 6 H, $CH_2CH_2CH_2$), 2.72 (m, 2 H, CH_2S), 3.22 (m, 1 H, CH), 7.95 (s, 3 H, NH_3^+). Anal. ($C_5H_{14}NO_3PS$·HCl) C, H, N.

2(*S*)-Aminopentane-1,5-dithiol, Hydrochloride (37n). The reduction of both ester functions of Boc-Glu(OCH_3)OCH_3 by procedure C.2 led to the diol **35n**, oily product (86%), R_f(A1) 0.25. The thioacetylation (procedure D) gave the intermediate **36n** as a white solid (27%), mp 76 °C, R_f(B1) 0.57. The deprotection of the three functional groups by procedure E.3 gave the titled compound as an oily product (98%): R_f(E) 0.77; HPLC CH_2Cl_2:MeOH:AcOH, 8:2:0.1, t_R 11.6 min; ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 1 H, SH), 1.64 and 2.05 (m, 4 H, CH_2CH_2CH), 1.97 (t, 1 H, SH), 2.52 (m, 2 H, CH_2CH_2SH), 2.88 (m, 2 H, $CHCH_2$SH), 3.4 (m, 1 H, CH), 8.43 (bs, 3 H, NH_3^+). Anal. ($C_5H_{13}NS_2$·HCl) C, H, N.

Verification of the Optical Purity of Compound 22h. Saponification under N_2 of compound **21h** by degassed aqueous NaOH (1.1 equiv) yielded its sodium salt, Boc-NHCH($CH_2CH_2SO_3Na$)CH_2SNa. This compound was condensed with (*S*)-phenylbutyric acid chloride, obtained from 2(*S*)-phenylbutyric acid treated by $SOCl_2$ in refluxing CH_2Cl_2. The resulting phenylbutyryl thioester was checked on HPLC, showing a single peak (kromasil C8, 5 μm, 100 Å, A = H_2O–TFA 0.05%, B = CH_3CN, t_R 15.8 min, gradient 30%–60% B in 15 min), and a NMR TOXY experiment was carried out, confirming the presence of only one stereoisomer, which was therefore assumed to be the *S* isomer.

Acknowledgment. We thank Dr. Ann Beaumont for stylistic revision of the paper and Hervé Meudal and Nathalie Parédes for excellent technical assistance.

References

(1) Nagatsu, I. T.; Gillespie, I. L.; George, J. M.; Folk, J. E.; Glenner, G. G. Serum aminopeptidases, "angiotensinase," and hypertension-II. Amino acid β-napthylamide hydrolysis by normal and hypertensive serum. *Biochem. Pharmacol.* **1965**, *14*, 853–861.
(2) Wright, J. W.; Quirk, W. S.; Hanesworth, J. M.; Harding, J. W. Influence of aminopeptidase inhibitors on brain angiotensin metabolism and drinking in rats. *Brain Res.* **1988**, *441*, 215–220.
(3) Yamada, R.; Mizutani, S.; Kurauchi, O.; Okano, K.; Imaizumi, H.; Narita, O.; Tomoda, Y. Purification and characterization of human placental aminopeptidase A. *Enzyme* **1988**, *40*, 223–230.
(4) Deschodt-Lanckman, M.; Bui, N. D.; Koulischer, D.; Paroutaud, P.; Strosberg, A. D. Cholecystokinin octa- and tetrapeptide degradation by synaptic membranes. II. Solubilization and separation of membrane-bound CCK-8 cleaving enzymes. *Peptides* **1983**, *4*, 71–78.
(5) Wilk, S.; Healy, D. P. Glutamyl aminopeptidase (aminopeptidase A), the BP-1/6C3 antigen. *Adv. Neuroimmunol.* **1993**, *3*, 195–207.
(6) Ahmad, S.; Ward, P. E. Role of aminopeptidase activity in the regulation of the pressor activity of circulating angiotensins. *J. Pharmacol. Exp. Ther.* **1990**, *252*, 643–650.
(7) Printz, M. P.; Jennings, C.; Healy, D. P.; Kalter, V. A glial high-affinity binding site with specificity for angiotensin II not angiotensin III: a possible N-terminal-specific converting enzyme. *J. Cardiovasc. Pharmacol.* **1986**, *8*, S62–S68.
(8) Harding, J. W.; Felix, D. The effects of aminopeptidase inhibitors amastatin and bestatin on angiotensin-evoked neuronal activity in rat brain. *Brain Res.* **1987**, *424*, 299–304.
(9) Wright, J. W.; Mizutani, 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.
(10) 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*, 87–146.
(11) Abhold, R. H.; Sullivan, M. J.; Wright, J. W.; Harding, J. W. Binding, degradation and pressor activity of angiotensin II and III after aminopeptidase inhibition with amastatin and bestatin. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 957–962.
(12) 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.
(13) Rich, D. H.; Moon, B. J.; Harbeson, S. Inhibition of aminopeptiases by amastatin and bestatin derivatives. Effect of inhibitor structure on slow-binding processes. *J. Med. Chem.* **1984**, *27*, 417–422.
(14) Suda, H.; Takita, T.; Aoyagi, T.; Umezawa, H. The structure of bestatin. *J. Antibiot.* **1976**, *29*, 100–101.
(15) Devault, A.; Lazure, C.; Nault, C.; Le Moual, H.; Seidah, N. G.; Chretien, M.; Kahn, P.; Powell, J.; Mallet, J.; Beaumont, A.; Roques, B. P.; Crine, P.; Boileau, G. Amino acid sequence of rabbit kidney neutral endopeptidase 24.11 (enkephalinase) deduced from a complementary DNA. *EMBO J.* **1987**, *6*, 1317–1322.

(16) Jongeneel, C. V.; Bouvier, J.; Bairoch, A. A unique signature identifies a family of zinc-dependent metallopeptidases. *FEBS Lett.* **1989**, *242*, 211–214.
(17) Wu, Q.; Lahti, J. M.; Air, 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.
(18) Wu, Q.; Li, L.; Cooper, M. D.; Pierres, M.; Gorvel, J. P. Aminopeptidase A activity of the murine B lymphocyte differentiation antigen BP-1/6C3. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 676–680.
(19) 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.
(20) Li, L.; Wang, J.; Cooper, M. D. cDNA cloning and expression of human glutamyl aminopeptidase (aminopeptidase A). *Genomics* **1993**, *17*, 657–664.
(21) 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.
(22) Semenza, G. Anchoring and biosynthesis of stalked brush border membrane proteins: glycosidases and peptidases of enterocytes and renal tubuli. *Annu. Rev. Cell. Biol.* **1986**, *2*, 255–313.
(23) Soubrier, F.; Alhenc-Gelas, F.; Hubert, C.; Allegrini, J.; John, M.; Tregear, G.; Corvol, P. Two putative active centers in human angiotensin I-converting enzyme revealed by molecular cloning. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9386–9390.
(24) Matthews, B. W. Structural basis of the action of thermolysin and related zinc peptidases. *Acc. Chem. Res.* **1988**, *21*, 333–340.
(25) Ondetti, M. A.; Cushman, D. W. Angiotensin converting enzyme inhibitors: biochemical properties and biological action. *Crit. Rev. Biochem.* **1984**, *16*, 381–411.
(26) Thorsett, E. D.; Wyratt, M. J. Inhibition of zinc peptidases that hydrolyze neuropeptides. *Neuropeptides and their peptidases. Ellis Horwood Series*; **1987**, 229–292.
(27) Chan, W. W. C. L.-Leucine thiol. A potent inhibitor of leucine aminopeptidase. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 297–302.
(28) Fournié-Zaluski, M. C.; Coric, P.; Turcaud, S.; Bruetschy, L.; Lucas, E.; Noble, F.; Roques, B. P. Potent and systemically active aminopeptidase N inhibitors designed from active-site investigation. *J. Med. Chem.* **1992**, *35*, 1259–1266.
(29) Wilk, S.; Thurston, L. S. Inhibition of angiotensin II formation by thiol derivatives of acidic aminoacids. *Neuropeptides (Edinburgh)* **1990**, *16*, 163–168.
(30) Chauvel, E. N.; Coric, P.; Llorens-Cortès, C.; Wilk, S.; Roques, B. P.; Fournié-Zaluski, M. C. Investigation of the active site of aminopeptidase A using a series of new thiol-containing inhibitors. *J. Med. Chem.* **1994**, *37*, 1339–1346.
(31) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, 1–28.
(32) Tobe, H.; Kojima, F.; Aoyagi, T.; Umezawa, H. Purification by affinity chromatography using amastatin and properties of aminopeptidase A from pig kidney. *Biochim. Biophys. Acta* **1980**, *613*, 459–468.
(33) Agrawal, Y.; Vanha-Perttula, T. Studies on the multiple forms of aminopeptidase A in bovine seminal vesicle secretion. *Int. J. Biochem.* **1986**, *18*, 465–471.
(34) Danielsen, E. M.; Norén, O.; Sjöström, H.; Ingram, J.; Kenny, A. J. Proteins of the kidney microvillar membrane. Aspartate aminopeptidase: purification by immunoadsorbent chromatography and properties of the detergent- and proteinase-solubilized forms. *Biochem. J.* **1980**, *189*, 591–603.
(35) Stenflo, J.; Fernlund, P.; Egan, W.; Roepstorff, P. Vitamin K dependent modifications of glutamic acid residues in prothrombin. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 2730–2733.
(36) Hauschka, P. V.; Lian, J. B.; Gallop, P. M. Direct identification of the calcium-binding amino acid, γ-carboxyglutamate, in mineralized tissue. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 3925–3929.
(37) 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.
(38) Vogel, Z.; Altstein, M. The adsorption of enkephalin to porous polystyrene beads: a simple assay for enkephalin hydrolysis. *FEBS Lett.* **1977**, *80*, 332–336.
(39) Moroder, L.; Hallett, A.; Wünsch, E.; Keller, O.; Wersin, G. Di-tert-butyldicarbonat – ein vorteilhaftes reagenz zur einführung der tert. butyloxycarbonyl-schutzgruppe. (Di-tert-butyl-dicarbonate, a useful reagent for the introduction of the tert-butyloxycarbonyl protector group.) *Hoppe-Seyler's Z. Physiol. Chem.* **1976**, *357*, 1651–1653.
(40) Stahl, G. L.; Walter, R.; Smith, C. W. General procedure for the synthesis of mono-N-acylated 1,6-diaminohexanes. *J. Org. Chem.* **1978**, *43*, 2285–2286.
(41) Mokotoff, M.; Logue, L. W. Potential inhibitors of L-asparagine biosynthesis. 5. Electrophilic amide analogues of (S)-2,3-diaminopropionic acid. *J. Med. Chem.* **1981**, *24*, 554–559.
(42) Duhamel, P.; Jamal Eddine, J.; Valnot, J. Y. Enantioselective alkylation of enolate derived from glycine influence of the imine moiety. *Tetrahedron Lett.* **1984**, *25*, 2355–2358.
(43) Duhamel, P.; Valnot, J. Y.; Jamal Eddine, J. Synthèse asymétrique à l'aide d'agents d'alkylation à nucléofuges chiraux. Alkylation énantiosélective de la glycine. (Asymmetric synthesis with the aid of chiral nucleophilic alkylating agents. Enantioselective alkylation of glycine.) *Tetrahedron Lett.* **1982**, *23*, 2863–2866.
(44) McCort-Tranchepain, I.; Ficheux, D.; Durieux, C.; Roques, B. P. Replacement of Tyr-SO_3H by a p-carboxymethyl-phenylalanine in a CCK_8 derivative preserves its high affinity for CCK-B receptor. *Int. J. Pept. Protein Res.* **1992**, *39*, 48–57.
(45) Pless, J.; Bauer, W. Boron tris(trifluoroacetate) for removal of protecting groups in peptide chemistry. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 147–148.
(46) Done, J.; Fowden, L. A. new amino acid amide in the groundnut plant (Arachis *hypogaea*): evidence of the occurrence of γ-methylene glutamine and γ-methylene glutamic acid. *Biochem. J.* **1952**, *51*, 451–458.
(47) Ramsamy, K.; Olsen, R. K.; Emergy, T. Synthesis of *N*-t-Boc-L-α-aminoadipic acid 1-*t*-butyl 6-ethyl ester from L-aspartic acid: a new route to L-α-aminoadipic acid. *Synthesis* **1982**, 42–43.
(48) Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. Facile synthesis of amino acid and peptide esters under mild conditions via cesium salts. *J. Org. Chem.* **1977**, *42*, 1286–1290.
(49) Rodriguez, M.; Llinares, M.; Doulut, S.; Heitz, A.; Martinez, J. A. facile synthesis of chiral N-protected β-amino alcohols. *Tetrahedron Lett.* **1991**, *32*, 923–926.
(50) Chambers, J. R.; Isbell, A. F. A new synthesis of amino phosphonic acids. *J. Org. Chem.* **1964**, *29*, 832–836.
(51) Garbay-Jaureguiberry, C.; Mc Cort-Tranchepain, I.; Barbe, B.; Ficheux, D.; Roques, B. P. Improved synthesis of p-phosphonomethyl and p-sulfomethyl phenylalanine. Resolution of p-phosphono, p-sulfo, p-carboxy, p-hydroxycarboxamido methyl phenylalanine. *Tetrahedron: Asymmetry* **1992**, *3*, 637–650.
(52) Hiskey, R. G.; Boggs, N. T., III Studies on prothrombin. Synthesis of peptides containing γ-carboxyglutamic acid. *Proc. Am. Pept. Symp., 5th, 1977*, 465–467.
(53) Boggs, N. T., III; Gawley, R. E.; Koehler, K. A.; Hiskey, R. G. Synthesis of DL-γ-carboxyglutamic acid derivatives. *J. Org. Chem.* **1975**, *40*, 2850–2851.