Cyclic Dipeptides. 3.[1] Synthesis of Methyl (*R*)-6-[(*tert*-Butoxycarbonyl)amino]-4,5,6,7-tetrahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylate and Its Hexahydro Analogues: Elaboration of a Novel Dual ACE/NEP Inhibitor[†]

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Synthetic routes to highly functionalized 1,4-thiazepinones **2** and **3** have been developed. *S*-Ac-*N*-Boc-L-Cys-D(L)-ThrOMe **7a,b** have been prepared and, after transformation into the corresponding mesylates, used as the cyclization substrates. Treatment of these compounds with $LiAlH(OMe)_3$ in THF results in mesylate elimination and thiolacetate reduction, giving rise to both a Michael acceptor (α,β-unsaturated ester) and Michael donor (thiol anion), which undergo in situ intramolecular conjugate addition leading to the stereoselective formation of only two of the four possible stereoisomers of **2**. On the other hand, $PCC/CaCO_3$ oxidation of **7a** gave in 80% yield the corresponding ketone **11**, which was in turn transformed into the enol triflate **15**. Cleavage of the thiolacetate moiety, simultaneous elimination of trifluoromethanesulfonic acid to generate an allene system, and addition of the thiol group to the central carbon of the allene to provide the enantiomerically pure cyclic compound **3** in 85% yield was effected via a one-pot reaction by means of MeONa/MeOH. Thiazepinone **3** is an interesting intermediate for the preparation of conformationally restricted dipeptide mimetics, and its elaboration into the dual ACE/NEP inhibitor **4** is reported.

Introduction

The vasoactive peptides angiotensin-II (Ang-II) and atrial natriuretic peptide (ANP) display opposing biological effects. The former stimulates vasoconstriction and sodium retention, while the latter produces vasodilation, diuresis, and natriuresis and decreases the levels of plasma renin and aldosterone.[2] Therefore, a blockade of Ang-II production concomitant with a potentiation of endogenous ANP levels could represent a beneficial approach to the treatment of various cardiovascular disorders. The activity of these two antagonistic systems is regulated essentially by metabolizing processes involving the zinc metallopeptidases, angiotensin-converting enzyme (ACE, EC3.4.15.1), and neutral endopeptidase (NEP, EC 3.4.24.11, neprilysin). ACE is responsible for the maturation of Ang-II from its inactive precursor angiotensin I (Ang-I), and NEP inactivates ANP.[3] Moreover, both peptidases are involved in the metabolism of bradykinin,[4] a vasodilatory peptide. ACE inhibitors have gained wide acceptance clinically and are commonly prescribed for the treatment of hypertension and congestive heart failure (CHF).[5] On the other hand, selective NEP inhibitors have been recently shown to have diuretic and natriuretic properties, without loss of potassium in various experimental models of hypertension,[6] but displayed hypotensive effects only in DOCA salt rats.[7] Moreover, no significant reduction in arterial blood pressure was observed in patients with CHF.[8] Several studies have shown that coadministration of selective ACE and NEP inhibitors in animal models of hypertension and CHF has a more beneficial effect over the administration of the single agents separately.[9] In recent reports, it has also been demonstrated that single molecules that possess dual ACE and NEP inhibitory activity also exhibit these synergistic properties.[10]

Conformationally restricted peptidomimetics have been used extensively to probe the topography of enzyme active sites and to generate potent inhibitors devoid of the typical therapeutic shortcomings of peptides.[11] Thi-

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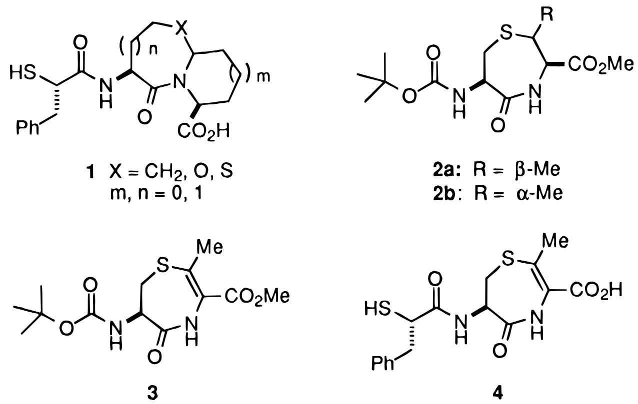


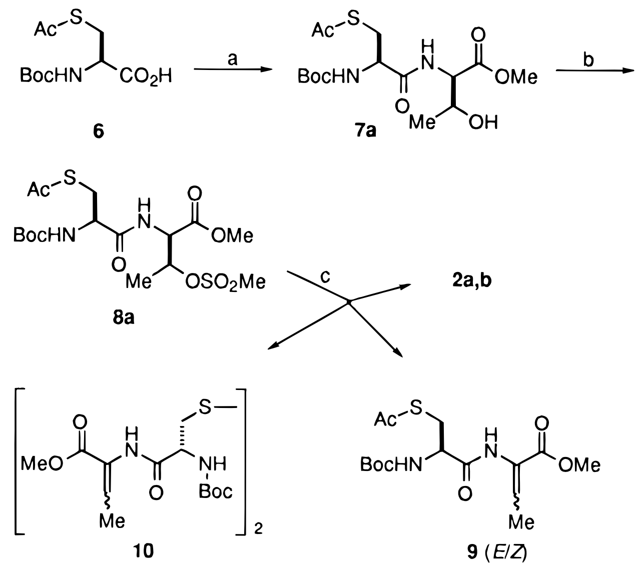
Figure 1.

azepinones and oxazepinones of general structure **1** (Figure 1) were studied by Bristol-Myers Squibb as dual inhibitors, and compound **1a** (X = S; *m*, *n* = 1; BMS-186716), in particular, was advanced into clinical development for the treatment of hypertension and CHF.[12] Extension of our previous work on antihypertensive agents[13] has led us to identify the cyclic thiazepinones **2a**,**b** and **3** as interesting conformationally restricted dipeptide mimetics en route to potential ACE/NEP dual inhibitors such as **4**. In this paper, we describe in full the synthesis of **2a**,**b**[14] and **3**[1] as well as the further elaboration of **3** into **4**.

Results and Discussion

Synthesis of Hexahydro-1,4-thiazepine Derivatives 2a and 2b. It is well-known that cyclization of linear precursors is in principle an excellent route to heterocycles. However, for seven-membered rings, this reaction is disfavored by entropic and enthalpic factors,[15] so that these heterocycles are usually obtained in good yield only when configurational and/or conformational constraints facilitate intramolecular cyclization.[16] Nevertheless, recent strategies show that the cyclization is possible in high yields, either by carbon−carbon[17] or by heteroatom−carbon[18] bond formation.

Scheme 1[a]



[a] Reaction conditions: (a) D-threonine methyl ester (**5a**), CMC, HOBt, 92%; (b) MsCl, DIPEA, quant; (c) $LiAlH(OMe)_3$, THF.

To avoid the use of orthogonally N- and C-protected amino acids, our approach to the target compounds involves formation of the amide bond first, followed by intramolecular displacement of a leaving group by a sulfur nucleophile to obtain the heterocyclic ring. Reaction between D-threonine methyl ester (**5a**)[19] and *S*-acetyl-*N*-*tert*-butoxycarbonyl-L-cysteine **6** (prepared[20] from *N*-*tert*-butoxycarbonyl-L-cystine) in the presence of *N*-cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimide methyl *p*-toluenesulfonate (CMC) and 1-hydroxybenzotriazole (HOBt) afforded after chromatographic purification the amide **7a** in 92% yield as a single stereoisomer as determined by TLC and ^{1}H NMR spectroscopy (Scheme 1). Treatment of **7a** with methanesulfonyl chloride and *N*-ethyldiisopropylamine (DIPEA) at −5 °C gave the corresponding mesylate **8a**, which proved to be unstable under the conditions of silica gel chromatography and was therefore used in the next reaction step without further purification. First attempts to convert **8a** into the cyclic derivatives **2** by a one-pot S-deacetylation−mesylate displacement sequence using methanolic ammonia or sodium borohydride led to a complex mixture of

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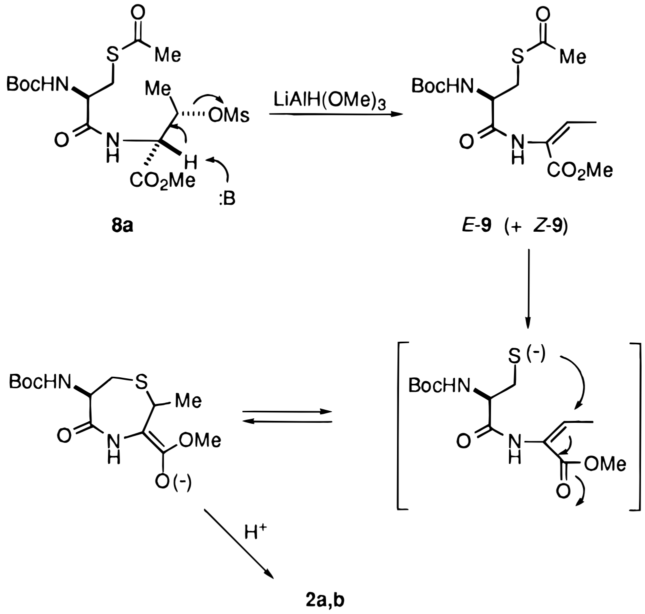
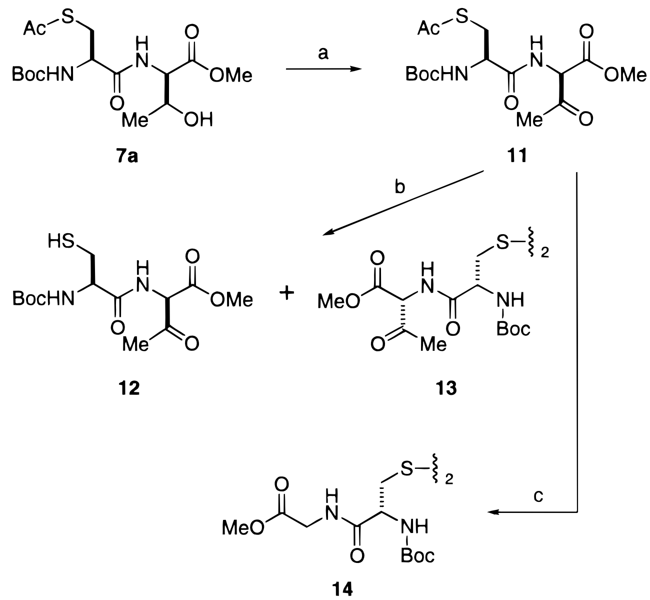
Scheme 2



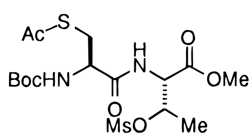
Scheme 3[a]



[a] Reaction conditions: (a) PCC, $CaCO_3$, CH_2Cl_2; (b) NH_3, CH_2Cl_2; (c) $(EtO)_3CH$, CSA, 4 Å MS.

products from which **2a** was isolated in only very small quantities after laborious chromatographic separations. Subsequently, the deacetylation−cyclization reaction was performed with lithium trimethoxyaluminum hydride (3 molar equiv) in dry THF. Under these conditions, compounds **2a** and **2b** were obtained in 15% and 35% yield, respectively, along with the olefins **9** (*E*/Z mixture, 20%) and the disulfides **10** (12%). Bubbling nitrogen into the reaction mixture suppressed the formation of **10** and led to compounds **2a** and **2b** in a yield of 24% and 56%, respectively, while the olefins **9** were always present in trace amounts.

Assignment of Relative and Absolute Stereochemistries and Elucidation of the Cyclization Reaction Pathway. The structures of **2a** and **2b** were determined by FAB-MS and ^{1}H NMR spectroscopy. The stereochemistry of the methyl at C-2 and the methoxycarbonyl group at C-3 was proven to be cis for **2a** by a nuclear Overhauser effect (NOE) between the C-2 and C-3 protons and trans in the case of **2b** (where no NOE was detected). To establish the absolute stereochemistry at C-3, **2a** was desulfurated with Ra-Ni to methyl *N*-[*N*-(*tert*-butoxycarbonyl)-L-alanyl]-L-2-aminobutanoate,[14] which proved to be identical in all respects, including specific rotation, with the material obtained from methyl (*S*)-(+)-2-aminobutyrate[21] and commercially available Boc-L-alanine. These results show not only that the L-cysteinyl moiety retains its configuration throughout the synthetic pathway but also, and more interestingly, that both **2a** and **2b** possess a C-3 stereochemistry inverted with respect to their precursor **8a**.



8b

When mesylate **8b**, prepared from L-threonine methyl ester and **6** (see Supporting Information) as described for **8a**, was cyclized by means of $LiAlH(OMe)_3$, the same diastereoisomers **2a** and **2b** were obtained (51% and 25%, respectively), although the ratio of **2a**/**2b** (2:1) in this case was inverted with respect to the previous reaction (1:2). Considering that olefin **9** might be a possible intermediate of the cyclization reaction, this compound (*E*/Z mixture) was subjected to the cyclization under the same experimental conditions as used for **8a**, leading indeed to the same products **2a** and **2b** in the ratio 2:1. This same result was also obtained starting from **9** as a single isomer[22] (double-bond geometry not determined).

In light of these results, we suggest that the cyclization might occur through a preliminary methanesulfonic acid elimination from **8a**,**b** to the olefin **9**, followed by intramolecular conjugate addition of the thiol group to give **2**. A concurrent retro-Michael reaction might give rise to an *E*/*Z* mixture of **9**, which on cyclization leads to the thermodynamically more stable isomers[23] **2a**,**b** via C-3 epimerization (Scheme 2). The obtaining of different **2a**/**2b** ratios starting from isomeric threonine mesylates **8a**,**b** could be explained assuming that these compounds might epimerize to a different extent prior to elimination, leading to different ratios of (*E*/*Z*)-**9**.[24]

Synthesis of 1,4-Thiazepinone 3. Tetrahydrothiazepinones of the type **3** were previously unknown, and we envisioned that construction of the ring could be possible via intramolecular addition of a thiol function to a ketone. Accordingly, the dipeptide **7a** (Scheme 3) was oxidized with PCC/$CaCO_3$[25] to give the corresponding ketone **11** in 80% yield. Other oxidizing reagents such as oxalyl chloride/DMSO,[26] tetrapropylammonium perruthenate/

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(22) Prepared from **8a** by reaction with potassium carbonate in refluxing chloroform (see the Supporting Information).

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(24) We thank one of the reviewers for highlighting this point.

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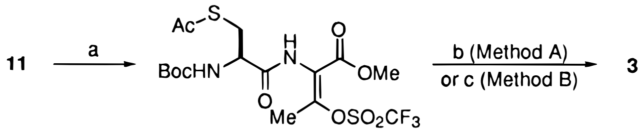
Scheme 4[a]



15

[a] Reaction conditions: (a) Tf_2O, DIPEA, CH_2Cl_2; (b) $(EtO)_3CH$, CSA, 4 Å MS; (c) MeONa, MeOH, −15 °C.

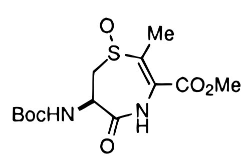
N-methylmorpholine *N*-oxide (TPAP/NMO),[27] and PCC/Al_2O_3[28] led to **11** in yields ranging between 24 and 42%. The subsequent transformation of the thiolacetate group into a free thiol proved to be troublesome, since, by treatment of **11** with NH_3, **12** was obtained in very low yield (≤10%), the main reaction product being the corresponding disulfide **13** (≈25−30%). On the other hand, the use of other nucleophiles (MeONa, LiOH) led to complex mixtures in which compounds deriving from C−C bond cleavage at the ketone group were also present. In an attempt to mask the ketone function as a ketal, **11** was heated with triethyl orthoformate in the presence of camphorsulfonic acid (CSA),[29] and quite unexpectedly, the disulfide **14** was isolated in 85% yield after preparative TLC. This result clearly demonstrates that the adopted conditions, though causing ketone cleavage, nevertheless are able to efficiently remove the sulfur protecting group, delivering a free thiol that is then oxidized to the disulfide **14**.

As a consequence of these findings, we sought a different approach to **3**. The ability of alkenyl triflates to undergo solvolytic displacement through the intermediacy of the corresponding vinyl cations is well documented in the literature.[30] Therefore, we devised a new avenue to our target molecule entailing the preparation of enol triflate **15**, followed by its intramolecular displacement by an in situ generated thiol group (Scheme 4). To this end, ketone **11** was transformed by means of triflic anhydride and DIPEA into the corresponding enol triflate **15** in 88% yield. TLC and ^1H NMR spectroscopy revealed that only one isomer of **15** was obtained (double-bond geometry not determined). By reaction with triethyl orthoformate and CSA in refluxing methanol complete conversion of substrate **15** was obtained in 6 h and **3** was isolated in 52% yield after chromatographic purification.[31] To improve the yield of the cyclization reaction, other conditions were explored,[32] and eventually it was found that treatment of **15** with MeONa in dry MeOH at −15 °C for 2 h provided **3** in 85−90% yield.

Mechanistic Considerations and Assignment of Absolute and Relative Stereochemistries. The observation of facile allene formation from vinyl triflates[33] was first described by Stang.[34] The chemistry was extended to the synthesis of allenylazetidinones by Conway et al.[35] and, more recently, by Kant and Farina,[36] who demonstrated that these compounds are stable enough to allow their isolation, chromatographic purification, and storage.[37] In view of this information, we exposed **15** to CD_3ONa in CD_3OD, checking the reaction by ^1H NMR at 10 min intervals, and we noticed (i) a prompt disappearance of the MeCOS signal; (ii) a steady decrease in intensity of the Me−C−OTf signal until complete disappearance; (iii) the appearance of a pair of doublets at δ 4.57 and 4.54 with a coupling constant of 11.8 Hz, diagnostic of the cumulene system,[38] whose intensity decreased gradually; and (iv) an early (after the first 10 min) appearance of the signal relative to the Me−C═C of **3**, whose intensity gradually increased during the following 2 h. In view of this, we are led to conclude that under the reported experimental conditions the transformation of **15** to **3** most likely involves, though not necessarily in this order, the following steps: (a) MeONa-catalyzed transesterification of the thiolacetate, delivering the nucleophile (thiol anion); (b) formation of the allene by elimination of methanesulfonic acid; and (c) nucleophilic attack of the thiol anion to the central allenic carbon, which is indeed susceptible to nucleophilic addition reaction when activated with an electron-withdrawing group,[39] leading to *7-endo-dig* cyclization. It is interesting to point out that attempts to cyclize an analogue of **15**, having a phenyl group instead of the methyl and hence no possibility to generate an allene intermediate, were unsuccessful.[40]

To definitely assess the intermediacy of an allenic compound in the cyclization reaction, we subjected the enol triflate **15** to the same reaction conditions reported by Kant and Farina[36] for the isolation of allenylazetidinones, with the purpose to isolate the allene and to transform it into **3** by means of MeONa. However, isolation of this allene was prevented by its inherent high reactivity, most likely due to the presence of a free NH group on the cumulene system.

To establish its enantiomeric purity, **3** was oxidized with *m*-CPBA to give only two diastereomeric sulfoxides **16a**,**b**, which by further oxidation led to the same sulfone.[14,41]



16a: α-sulfoxide
16b: β-sulfoxide

The structure of **16b** was confirmed by X-ray crystallographic analysis (Figure 2), which showed that the compound has the *R* configuration and also determined

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(31) No attempt has been made yet to assess the actual mechanism of this cyclization reaction.
(32) Attempts to cyclize **15** using $LiAlH(OMe)_3$ as described for the synthesis of **2** were unsuccessful, giving complex reaction mixtures in which thiazepine **3** was never detected.
(33) For a recent review on vinyl and aryl triflates, see: Ritter, K. *Synthesis* **1993**, 735−761.
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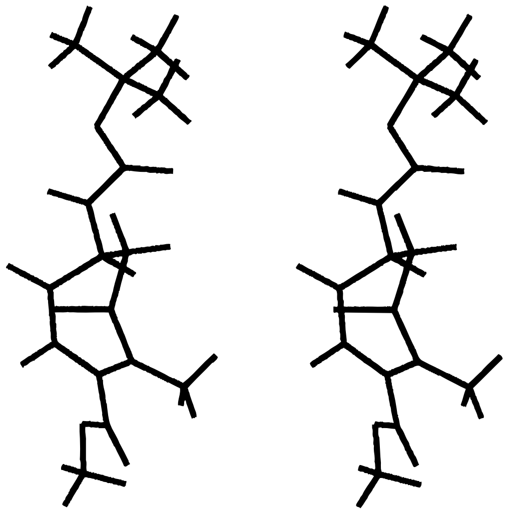
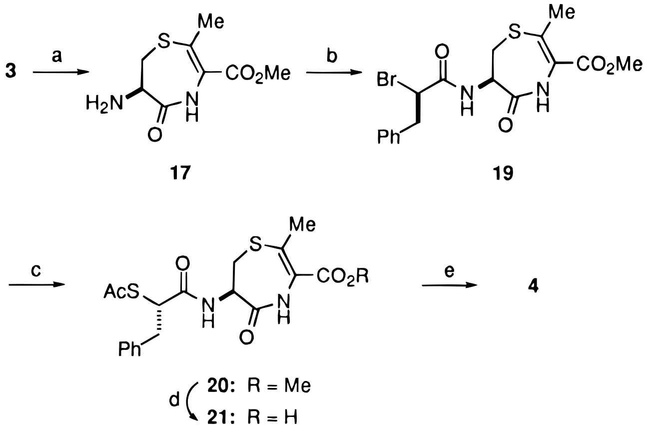


Figure 2. Stereoview of the X-ray structure of **16b**.

Scheme 5[a]



[a] Reaction conditions: (a) TFA, CH_2Cl_2; (b) **18**, CMC, HOBt, CH_2Cl_2; (c) CsSAc, DMF; (d) LiI, Py; (e) MeONa, MeOH.

the relative stereochemistry of the substituents, with the side chain at C-6 and oxygen at sulfur being cis. Hence, the diastereomeric sulfoxide **16a** was assigned the 1*S*-trans configuration.

Elaboration of 3 into the Dual Inhibitor 4. The transformation of **3** into **4** entails deprotection and subsequent acylation of the amino group at C-6, followed by hydrolysis of the ester function to deliver the free acid. To this end, **3** was treated with TFA in CH_2Cl_2 (Scheme 5) to give the amine **17**, which was subjected to acylation with (*R*)-2-bromo-3-phenylpropionic acid (**18**)[42] in the presence of CMC and HOBt to provide the amide **19** in a most satisfactory yield of 54%, considering that, due to their low stability on silica gel, both **17** and **18** were used in this reaction without previous purification. On treatment with cesium thiolacetate[43] in DMF, **19** underwent a clean displacement of bromide with complete inversion of configuration giving **20** (70%) as a sole diastereomer. Attempts to hydrolyze both the ester groups at the same time were unsatisfactory, as complex reaction mixtures were obtained; therefore, we opted for the selective cleavage of each of them. The use of LiI in pyridine allowed for the selective cleavage of the methyl ester function and the acid **21** was obtained in 66% yield along with 25% recovered **20**. Finally, reaction of **21** with MeONa/MeOH led to the target compound **4** in 50% yield after careful chromatographic purification. The biological evaluation of **4** as a dual ACE/NEP inhibitor is still ongoing, and the results will be reported in due course.

Conclusions

We have reported in detail the straightforward synthesis of two conformationally restricted dipeptides **2a** and **2b** through a three-step sequence involving (a) amide bond formation between readily available, suitably protected, L-cysteine and D- or L-threonine, (b) mesylation of a secondary hydroxy group, and (c) $LiAlH(OMe)_3$-mediated elimination of methanesulfonic acid and reduction of the thiolacetate function delivering both the nucleophile (thiol anion) and electrophile (α,β-unsaturated ester), which undergo intramolecular conjugate addition. This synthesis is also stereoselective in that it provides only two of the possible stereoisomers of methyl 6-[(*tert*-butoxycarbonyl)amino]hexahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylate in chiral nonracemic form starting from either D- or L-threonine, with the product ratios depending on the absolute configuration of the starting amino acids. Moreover, a concise synthesis of enantiomerically pure cyclic dipeptide **3** has been accomplished in four steps starting from readily available, protected amino acids. The key step is based on a one-pot cyclization procedure entailing the MeONa-mediated cleavage of a thiolacetate moiety and triflic acid elimination to an intermediate allene, which undergoes intramolecular nucleophilic addition in situ, with formation of a new S−C bond leading to the cyclization product. Compound **3** is an interesting synthon for the preparation of dual ACE/NEP inhibitors such as **4**, as well as of other peptidomimetics based on a conformationally restricted dipeptide scaffold.

Experimental Section

General Methods. All moisture-sensitive reactions were performed under an argon atmosphere using oven-dried glassware. All solvents were dried over standard drying agents[44] and freshly distilled prior to use. Reagents were from commercial suppliers and used without further purification. Extracts were dried over Na_2SO_4 and evaporated under reduced pressure with a rotary evaporator. Merck silica gel 60 was used for chromatography (70−230 mesh) and flash chromatography (230−400 mesh) columns. Analytical and preparative TLC were performed with Merck silica gel 60 F_{254} (0.2 and 2 mm thickness, respectively) precoated aluminum sheets with visualization by UV light, charring with H_2SO_4 (10% in water), or charring with anisaldehyde in ethanolic sulfuric acid.[45] Melting points are uncorrected. Optical rotations were measured at 20 ± 2 °C in $CHCl_3$ unless otherwise

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stated. IR spectra were recorded in $CHCl_3$ solutions (unless otherwise stated). ^1H NMR spectra were measured at 200 MHz. Chemical shifts are reported relative to $CDCl_3$ at δ 7.24 ppm and tetramethylsilane at δ 0.00 ppm. EI and FAB low-resolution mass spectra were recorded with an electron beam of 70 eV. Elemental analyses (C, H, N) were performed in-house.

***N*-[*S*-Acetyl-*N*-(*tert*-butoxycarbonyl)-L-cysteinyl]-D-threonine Methyl Ester (7a).** A solution of HOBt (1.37 g, 10.2 mmol) in dry dichloromethane (20 mL) was added to a cold (0−5 °C) solution of **5a** (1.70 g, 12.7 mmol) and **6** (2.22 g, 8.5 mmol) in the same solvent (100 mL). After 5 min, a solution of CMC (5.30 g, 12.7 mmol) in dichloromethane (30 mL) was added dropwise, and the mixture was kept at room temperature for 4 h. The solution was washed successively with 1 N HCl, aqueous $NaHCO_3$ (5% solution), and brine and then dried, and the solvent was evaporated in vacuo. The residue was purified by column chromatography with EtOAc to give **7a** (2.94 g, 92%) as a solid: mp 121−123 °C (from toluene/cyclohexane, 1:1); $[\alpha]_D$ −10 (*c* 1.0); ^1H NMR ($CDCl_3$) δ 7.12 (d, 1H, *J* = 8.6 Hz), 5.32 (d, 1H, *J* = 7.1 Hz), 4.60 (dd, 1H, *J* = 8.8 and 2.6 Hz), 4.41 (m, 1H + 1H), 3.77 (s, 3H), 3.27 (m, 2H), 2.40 (s, 3H), 2.30 (d, 1H), 1.45 (s, 9H), 1.21 (d, 3H, *J* = 6.6 Hz); IR 3420, 2970, 1690 cm^{-1}. Anal. Calcd for $C_{15}H_{26}N_2O_7S$: C, 47.61; H, 6.92; N, 7.40. Found: C, 47.72; H, 7.05; N, 7.36.

***N*-[*S*-Acetyl-*N*-(*tert*-butoxycarbonyl)-L-cysteinyl]-D-threonine Methyl Ester, Methanesulfonate (8a).** A solution of methanesulfonyl chloride (0.10 mL, 1.4 mmol) in dry dichloromethane (2 mL) was added dropwise to a cold (−5 °C) solution of **7a** (0.50 g, 1.3 mmol) and DIPEA (0.26 mL, 1.5 mmol) in the same solvent (20 mL). After being stirred at room temperature for 14 h, the solution was cooled at −5 °C, and further amounts of DIPEA (0.52 mL, 3.0 mmol) and methanesulfonyl chloride (0.10 mL, 1.4 mmol), each diluted in dichloromethane (2 mL), were added successively. After 30 min, the solution was diluted with cold water and neutralized with solid $NaHCO_3$. The organic layer was washed with brine, dried, and concentrated to a yellow oil (0.60 g, 100%) homogeneous by TLC: ^1H NMR ($CDCl_3$) δ 7.06 (d, 1H), 5.29 (m, 1H + 1H), 4.80 (dd, 1H), 4.36 (m, 1H), 3.79 (s, 3H), 3.30 (m, 2H), 3.01 (s, 3H), 2.39 (s, 3H), 1.48 (s, 9H), 1.41 (d, 3H).

Conversion of 8a into 2a,b, 9, and 10. A solution of **8a** (0.60 g, 1.3 mmol) in dry THF (10 mL) was added dropwise to a solution of $LiAlH(OMe)_3$ prepared by adding dry MeOH (0.63 mL, 15.6 mmol) to a suspension of $LiAlH_4$ (197 mg, 5.2 mmol) in dry and degassed THF (20 mL). After the solution was stirred for 2 h at room temperature, the excess reducing agent was decomposed with acetone (0.5 mL) and then water (0.5 mL). The reaction mixture was neutralized with citric acid, and most of the THF was removed under reduced pressure. EtOAc was added to the residue, and the organic solution was washed with brine, dried, and evaporated. Chromatography on silica gel (EtOAc/hexanes 1.5:1) afforded the following compounds:

2a: 15% yield; white solid; mp 147−149 °C; R_f 0.74 (EtOAc/hexanes 1.5:1); $[\alpha]_D$ −15 (*c* 1.4); ^1H NMR ($CDCl_3$) δ 6.50 (d, 1H, *J* = 5.2 Hz), 6.01 (d, 1H, *J* = 5.1 Hz), 4.85 (d, 1H, *J* = 5.1 Hz), 4.60 (m, 1H), 3.80 (s, 3H), 3.31 (q, 1H, *J* = 7.2 Hz), 2.90 (dq, 2H, *J* = 9.9, and 2.0 Hz), 1.50 (s, 9H), 1.20 (d, 3H, *J* = 7.2 Hz); IR 3410, 3000, 1730, 1710, 1680 cm^{-1}; FABMS (TDEG-GLY) *m/z* 319 (M + H)$^+$. Anal. Calcd for $C_{13}H_{22}N_2O_5S$: C, 49.04; H, 6.96; N, 8.80. Found: C, 49.18; H, 7.12; N, 8.84.

9 (*E/Z* mixture): 20% yield; R_f 0.58 (EtOAc/hexanes 1.5:1); ^1H NMR ($CDCl_3$) δ 7.83 (s, 1H), 6.76 (q, 1H), 5.43 (d, 0.5H), 5.39 (d, 0.5H), 4.36 (m, 1H), 3.69 (s, 3H), 3.27 (2dd, 1H), 2.86 (d, 1H), 2.31 (s, 1.5H), 2.11 (s, 1.5H), 1.70 (2d, 3H), 1.39 (s, 9H). Anal. Calcd for $C_{15}H_{24}N_2O_6S$: C, 49.99; H, 6.71; N, 7.77. Found: C, 50.21; H, 6.84; N, 7.60.

10: 12% yield; R_f 0.45 (EtOAc/hexanes 1.5:1); ^1H NMR ($CDCl_3$) δ 8.88 (s, 2H), 6.89 (q, 2H), 5.63 (d, 2H), 5.13 (m, 2H), 3.79 (s, 6H), 3.12 (2dd, 4H), 1.75 (d, 6H), 1.38 (s, 18H); IR 3440, 3320, 3000, 1740, 1510 cm^{-1}; FABMS (TDEG-GLY) *m/z* 635 (M + H)$^+$. Anal. Calcd for $C_{26}H_{42}N_4O_{10}S_2$: C, 49.21; H, 6.62; N, 8.83. Found: C, 49.17; H, 6.70; N, 8.97.

2b: 35% yield; yellowish oil; R_f 0.38 (EtOAc/hexanes 1.5:1); ^1H NMR ($CDCl_3$) δ 6.61 (d, 1H, *J* = 8.0 Hz), 5.91 (d, 1H, *J* = 5.0 Hz), 4.50 (m, 1H), 4.20 (m, 1H), 3.75 (s, 3H), 3.38 (m, 1H), 2.65 (d, 2H, *J* = 9.1 Hz), 1.41 (s, 9H), 1.40 (d, 3H, *J* = 7.2 Hz); IR 3420, 2990, 1750, 1680 cm^{-1}; FABMS (TDEG-GLY) *m/z* 341 (M + Na)$^+$, 319 (M + H)$^+$. Anal. Calcd for $C_{13}H_{22}N_2O_5S$: C, 49.04; H, 6.96; N, 8.80. Found: C, 49.28; H, 7.17; N, 8.66.

When the same reaction was performed by bubbling argon into the reaction mixture, compounds **2a** and **2b** were obtained in 24 and 56% yield, respectively, while compound **9** was present only in trace amount.

Methyl *N*-[*S*-Acetyl-*N*-(*tert*-butoxycarbonyl)-L-cysteinyl]-2-amino-3-oxobutanoate (11). $CaCO_3$ (0.86 g, 8.52 mmol) and 4 Å MS (both dried at 250 °C overnight) were added to a solution of **7a** (0.65 g, 1.7 mmol) in dry CH_2Cl_2 (200 mL). After the mixture was stirred at room temperature for 15 min, PCC (1.51 g, 7.0 mmol) was added, and the mixture was left stirring at the same temperature as other portions of PCC were added five times at 1 h intervals to achieve a reagent/substrate ratio of 24.7:1. After 18 h, a further portion of PCC was added, and 1 h later the reaction mixture was filtered through Celite and then through a short silica gel column (CH_2Cl_2/EtOAc 10:1). Evaporation of eluates afforded a residue that was purified by column chromatography (SiO_2−EtOAc/hexanes 1.5:1) to yield **7a** (0.52 g, 80%) as white crystals: mp 103−105 °C (from EtOAc); $[\alpha]_D$ +10.1 (*c* 1.0); ^1H NMR ($CDCl_3$) δ 7.43 (1H, s), 5.29−5.23 (1H, m), 5.20 (1H, dd, *J* = 7.1, 5.4 Hz), 4.39 (1H, m), 3.82 (3H, s), 3.41 (1H, dd, *J* = 14.4, 4.7 Hz), 3,21 (1H, dd, *J* = 14.4, 7.1 Hz), 2.38 (3H + 3H, s), 1.46 (9H, s); IR (Nujol) 3324, 2923, 2853, 1748, 1727, 1691, 1653, 1559, 1521 cm^{-1}. Anal. Calcd for $C_{15}H_{24}N_2O_7S$: C, 47.86; H, 6.43; N, 7.45. Found: C, 47.65; H, 6.33; N, 7.50.

Methyl *N*-[*S*-Acetyl-*N*-(*tert*-butoxycarbonyl)-L-cysteinyl]-2-amino-3-trifluoromethanesulfonyloxy-2-butenoate (15). TEA (519 μL, 3.98 mmol) and DMAP (cat.) were added to a solution of **11** (0.50 g, 1.33 mmol) in dry CH_2Cl_2 (100 mL), and the reaction mixture was stirred for 1 h at room temperature and then cooled to −78 °C. Tf_2O (447 μL, 2.65 mmol) was added over 2 min, and after 15 min at −78 °C the mixture was warmed to room temperature and washed successively with 1 N HCl, water, and brine. The organic layer was dried and evaporated, and the residue was purified by column chromatography on silica gel (CH_2Cl_2/EtOAc 10:1) to give **15** (0.62 g, 92%) as a yellowish solid: mp 129−132 °C; $[\alpha]_D$ −31.3 (*c* 1.0); ^1H NMR ($CDCl_3$) δ 8.10 (1H, s), 5.21 (1H, d, *J* = 7.4 Hz), 4.41 (1H, m, *J* = 4.6, 7.9 Hz), 3.82 (3H, s), 3.38 (1H, dd, *J* = 14.6, 4.6 Hz), 3.22 (1H, dd, *J* = 14.6, 7.9 Hz), 2.45 (3H, s), 2.38 (3H, s), 1.46 (9H, s); IR 3400, 3030, 2980, 1730, 1710, 1695 cm^{-1}. Anal. Calcd for $C_{16}H_{23}F_3N_2O_9S_2$: C, 37.79; H, 4.56; N, 5.51. Found C, 37.55; H, 4.42; N 5.38.

Methyl (*R*)-6-[(*tert*-Butoxycarbonyl)amino]-4,5,6,7-tetrahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylate (3). Method A. A solution of **15** (0.05 g, 0.10 mmol) in dry MeOH (10 mL) was reacted with triethyl orthoformate (0.03 g, 0.20 mmol) in the presence of CSA (cat.) and 4 Å molecular sieves. After the solution was heated at reflux for 6 h, solid $NaHCO_3$ (0.05 g) was added to the cooled (5 °C) mixture. Filtration and evaporation afforded a residue that was taken up into EtOAc, and this solution was washed with aqueous 5% $NaHCO_3$ solution and brine, dried, and evaporated. Purification of the crude product by silica gel chromatography ($CHCl_3/Et_2O$ 15:1) provided **3** (0.016 g, 52%) as a yellowish solid: mp 152−154 °C (from $CHCl_3/Et_2O$); $[\alpha]_D$ −20.7 (*c* 0.8); ^1H NMR ($CDCl_3$) δ 7.25 (1H, s), 5.70 (1H, d, *J* = 6.0 Hz), 4.56 (1H, m), 3.73 (3H, s), 3.39 (1H, dd, *J* = 11.4, 11.0 Hz), 3.12 (1H, dd, *J* = 11.4, 3.2 Hz), 2.35 (3H, s), 1.36 (9H, s); IR 3412, 3378, 1690 cm^{-1}; EIMS *m/z* 316 (M$^+$); HRMS calcd for $C_{13}H_{20}N_2O_5S$ 316.1093, found 316.1096. Anal. Calcd for $C_{13}H_{20}N_2O_5S$: C, 49.37; H, 6.33; N, 8.86. Found: C, 49.42; H, 6.35; N, 8.79.

Method B. To a solution of **15** (0.54 g, 1.06 mmol) in dry MeOH (160 mL) maintained at −15 °C was added MeONa (0.086 g, 1.59 mmol). After being stirred for 2 h at this temperature, the reaction mixture was gradually warmed to room temperature, neutralized with citric acid, and concentrated. The residue was dissolved in EtOAc, and this solution was washed with water, dried, and evaporated. Purification of the reaction product as reported in method A gave **3** (0.29 g, 85%), whose analytical and spectroscopic data are identical to those of the compound obtained by method A.

Methyl (*R*)-6-Amino-4,5,6,7-tetrahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylate (17). A solution of **3** (0.050 g, 0.16 mmol) in dry CH_2Cl_2 (1.0 mL) was stirred at 0 °C as a 50% solution of TFA in CH_2Cl_2 (1.0 mL) was added over 5 min. After 2 h, the reaction mixture was made alkaline by addition of solid $NaHCO_3$ and filtered. Evaporation of volatiles afforded a residue (0.034 g) that proved to be unstable on silica gel and was therefore used in the next step without purification.

Methyl [*R*-(*R,*R**)]-6-[(2-Bromo-1-oxo-3-phenylpropyl)-amino]-4,5,6,7-tetrahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylate (19).** A solution of crude **17** (0.066 g, 0.31 mmol) and **18**[42] (0.058 g, 0.25 mmol) in the same solvent (5 mL) was reacted following the same procedure reported for the preparation of **7a**. Column chromatography on silica gel (EtOAc/hexanes 1.5:1) of the reaction product provided **19** (0.058 g, 54%): $[\alpha]_D$ −7.7 (*c* 0.7); ^1H NMR ($CDCl_3$) δ 7.55 (1H, s), 7.35 (1H, d, $J = 6.2$ Hz), 7.25−7.19 (5H, m), 4.76 (1H, m), 4.42 (1H, t, $J = 7.5$ Hz), 3.76 (3H, s), 3.53−2.87 (2H + 2H, m), 2.41 (3H, s); FABMS (TDEG-GLY) *m*/*z* 427 (M^+). Anal. Calcd for $C_{17}H_{19}BrN_2O_4S$: C, 47.78; H, 4.45; N, 6.56. Found: C, 47.49; H, 4.66; N, 6.47.

Methyl [*S*-(*R,*S**)]-6-[(2-Acetylthio-1-oxo-3-phenylpropyl)amino]-4,5,6,7-tetrahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylate (20).** Compound **19** (0.065 g, 0.15 mmol) was dissolved in a 3 M solution of CH_3COSCs in DMF[43b] (0.08 mL, 024 mmol), and the reaction mixture was left at room temperature for 24 h and then diluted with Et_2O (10 mL). This cloudy solution was washed with water (5 × 5 mL), dried, and evaporated. Purification of the crude product by chromatography on silica gel (EtOAc/hexanes 1:1) gave **20** (0.045 g, 70%) as a sole diastereoisomer: ^1H NMR ($CDCl_3$) δ 7.42 (1H, s), 7.30−7.14 (5H + 1H, m), 4.68 (1H, m), 4.29 (1H, t, $J = 7.4$ Hz), 3.76 (3H, s), 3.42−2.89 (2H + 2H, m), 2.40 (3H, s), 2.29 (3H, s); IR 3200, 3100, 1692 cm^{-1}; FABMS (TDEG-GLY) *m*/*z* 422 (M^+). Anal. Calcd for $C_{19}H_{22}N_2O_5S_2$: C, 54.03; H, 5.21; N, 6.64. Found: C, 54.30; H, 5.33; N, 6.48.

[*S*-(*R,*S**)]-6-[(2-Acetylthio-1-oxo-3-phenylpropyl)amino]-4,5,6,7-tetrahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylic Acid (21).** LiI (0.060 g, 0.48 mmol) was added to a solution of **20** (0.100 g, 0.24 mmol) in dry pyridine (4.0 mL), and the resultant mixture was refluxed for 2 h. After cooling, the solution was diluted with water (15 mL) and EtOAc (15 mL) and acidified with 1 N HCl. The organic layer was separated, washed with water, dried, and evaporated. The residue was purified by passing through a silica gel column. Elution with EtOAc afforded 0.031 g (30%) of the starting compound **20**, while further elution with EtOAc/AcOH (95:5) gave **21** (0.064 g, 66%) as a foam: ^1H NMR ($CDCl_3 + D_2O$) δ 7.27−7.23 (5H, m), 4.78 (1H, m), 4.27 (1H, m), 3.36−2.90 (2H + 2H, m), 2.43 (3H, s), 2.30 (3H, s); IR 3400, 3250, 1740, 1680 cm^{-1}; FABMS (TDEG-GLY) *m*/*z* 408 (M^+). Anal. Calcd for $C_{18}H_{20}N_2O_5S_2$: C, 52.94; H, 4.90; N, 6.86. Found: C, 53.19; H, 4.77; N, 7.06.

[*S*-(*R,*S**)]-6-[(2-Mercapto-1-oxo-3-phenylpropyl)amino]-4,5,6,7-tetrahydro-2-methyl-5-oxo-1,4-thiazepine-3-carboxylic Acid (4).** MeONa (0.024 g, 0.32 mmol) was added to a cooled (0 °C) solution of **21** (0.070 g, 0.17 mmol) in dry MeOH (2 mL). After being stirred for 4 h, the reaction mixture was acidified with 1 N HCl and concentrated in vacuo at room temperature. The residue was dissolved in EtOAc, and the resultant solution was dried and evaporated. Purification of the crude product by silica gel chromatography (EtOAc/AcOH 9:1) provided **4** (0.031 g, 50%) as a foam: mp 241−244 °C dec; $[\alpha]_D$ −10.7 (*c* 1.0); ^1H NMR ($CDCl_3 + D_2O$) δ 7.49−7.21 (5H, m), 4.61−440 (1H + 1H, m), 3.34−2.71 (2H + 2H, m), 2.32 (3H, s); IR 3410, 3250, 2200, 1745, 1690, cm^{-1}; FABMS (TDEG-GLY) *m*/*z* 365 (M^+). Anal. Calcd for $C_{16}H_{18}N_2O_4S_2$: C, 52.46; H, 4.92; N, 7.65. Found: C, 52.80; H, 4.75; N, 7.46.

Crystal Data of the Sulfoxide 16b. Solution and refinement: $C_{13}H_{20}N_2O_6S$, $M = 332.4$, monoclinic, space group $P2_1$, $a = 5.962(2)$ Å, $b = 10.281(8)$ Å, $c = 13.949(7)$ Å, $\beta = 101.19(7)°$, $V = 838.8$ Å^3, $T = 23$ °C, $Z = 2$, $D_c = 1.316$ g/cm^3, monochromated Cu Kα radiation, $\lambda = 1.5418$ Å. Data were collected on a four-circle Enraf-Nonius CAD-4 diffractometer using $\omega - 2\theta$ scan. A total of 1684 reflections were collected, of which 1684 were unique. The structure was solved by direct methods (SIR 92)[46] and refined on F^2 by full-matrix least-squares procedures using the SDP package[47] to give $R = 0.049$ and $R_w = 0.045$ for 1022 observed independent reflections with $I > 3\sigma(I)$. Non-hydrogen atoms were made anisotropic, and hydrogen atoms were included in calculated positions. All calculations were performed on a VAX3100 Digital computer of the Biocrystallography Research Centre of CNR at the Department of Chemistry, University of Naples “Federico II”.[48]

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Supporting Information Available: X-ray data for compound **16b** and experimental procedures and characterization data for **8a**, **9** (single isomer), **12**−**14**, and **16a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(48) The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.