New Dipeptides Containing Thiazolidine-4-carboxylic Acid Derivatives: Synthesis and Characterization Using NMR Techniques and X-Ray Data

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New dipeptides, structural analogues of known immunomodulating agents, were prepared by stereospecific condensation between 2-substituted thiazolidine-4-carboxylate esters with *N*-substituted L-proline or L-thiaproline. The structure of these compounds has been elucidated by combination of NMR methods and X-ray analysis. In addition, NMR measurements on dipeptides indicated the presence of *S-cis* and *S-trans* conformers around the amide bonds.

Key words L-thiazolidine-4-carboxylic acid; L-proline; dipeptide; NMR; diastereospecificity; X-ray analysis

Immunomodulating drugs are considered to offer therapeutic possibilities as a useful complement in the chemotherapy of cancer and diseases related to immunodeficiency. In previous work, we have described the synthesis, characterization and immunomodulating properties of synthetic compounds including some amidic derivatives of *R*-(−)-thiazolidine-4-carboxylic acid. In particular, 2-substituted-3-aminoacetylthiazolidine-4-carboxylate esters and their spiro derivatives,[1] the regioselective condensation of thiazolidine-2,4-dicarboxylic acid and its ester with isocyanate,[2] and immunodulating properties of thiadiazabicyclic compounds have been studied in details.[3] The results show that thiadiazabicyclooctanes present a high activity as immunomodulatory agents.[3] Previously, Poli Industria Chimica proposed the synthesis and immunomodulating effects of 3-(L-pyrroglutamyl)-L-thiazolidine-4-carboxylic acid derivatives.[4—7] Few dipeptides containing thiazolidine-4-carboxylic acid have been synthesized.[8—10]

Considering that introduction of N–(CO)–CH_2–N–(CO) sequence seemed to be necessary in the structure of thiazolidine-4-carboxylic acid derivatives for immunomodulating activity, we extended our investigations on synthesis and characterization of new dipeptides containing 2-substituted thiazolidine-4-carboxylate esters, structural analogues of known immunological activity.[11,12] Elucidation of the structure of these dipeptides is a challenging task which demands a combination of the data from nuclear magnetic resonance (NMR) techniques and X-ray analysis.

Chemistry

Thiazolidine-4-carboxylic acid **1** is available commercially, its synthesis is usually performed by condensation of formaldehyde with L-(−)-(*R*)-cysteine.[13] Thiazolidine-2,4-dicarboxylic acid **2** was prepared by condensation of glyoxylic acid with L-(−)-(*R*)-cysteine.[2] When allowed to react in ethanol with an aldehyde or a ketone, L-(−)-(*R*)-cysteine undergoes a condensation–cyclization reaction leading to the formation of a thiazolidinic ring; the mechanism of this reaction has been discussed.[14] Ethyl thiazolidine-4-carboxylate **3** and dimethyl thiazolidine-2,4-dicarboxylate **4** were obtained in good yields by esterification of the corresponding acid with thionyl chloride in dry ethanol and methanol, respectively, as shown in Chart 1. Dipeptides **5**—**12** were prepared by coupling *N*-protected L-proline or L-thiazolidine-4-carboxylic acid with ethyl thiazolidine-4-carboxylate **3** and dimethyl thiazolidine-2,4-dicarboxylate **4** with the well-known procedure of mixed anhydride using isobutylchloroformate and *N*-methyl morpholine. All compounds were purified by column chromatography. *N-tert*-butyloxycarbonyl and *N*-benzoyl protecting groups were not removed because of cyclization in diketopiperazine.

Results and Discussion

NMR techniques are particularly useful for the structure configuration and conformation of molecules.[15,16] In this paper, one- and two-dimensional NMR spectroscopy were used to characterize our products. The results have also been confirmed by X-ray data.

In the course of the synthesis of 2-substituted thiazolidine-4-carboxylic acid, a new chiral center in C-2 position of thiazolidine ring was generated, affording a mixture of two diastereoisomers, the C-4 position resulting from L-(−)-(*R*)-cysteine remaining unaffected.[17—19] This fact was confirmed by the ^{1}H-NMR spectrum, which showed two signals for the protons H2, H4 and H5, H5′ for compounds **2**. A mechanism involving the opening of the ring, the closure and the epimerization of C-2 position was previously described (Chart 2).[20—22] Thus, the two diastereoisomers could not be separated.

The stereochemistry of C-2 of compounds **2** with respect to C-4 can be resolved using NMR spectroscopy which permits distinction between the two diastereoisomers: two sets of resonance with different intensities were observed for H2, H4, H5a and H5b′ protons. Previously, Szilagyi and Gyorgydeak[22] observed that the sum of coupling constants between geminal protons H5a and H5b with H4 of the 2-substituted thiazolidine-4-carboxylic acid for (2*R*, 4*R*) isomer is greater than that of the (2*S*, 4*R*) isomer. In our case, for thiazolidine-2,4-dicarboxylic acid **2**, the value of the sum $^3J_{H4H5a}+{}^3J_{H4H5b}$ is 15.9 Hz and 12.5 Hz for (2*R*, 4*R*) **2a** and (2*S*, 4*R*) **2b** diastereoisomer respectively, although these conclusions differed from previous characterization of the two diastereoiso-

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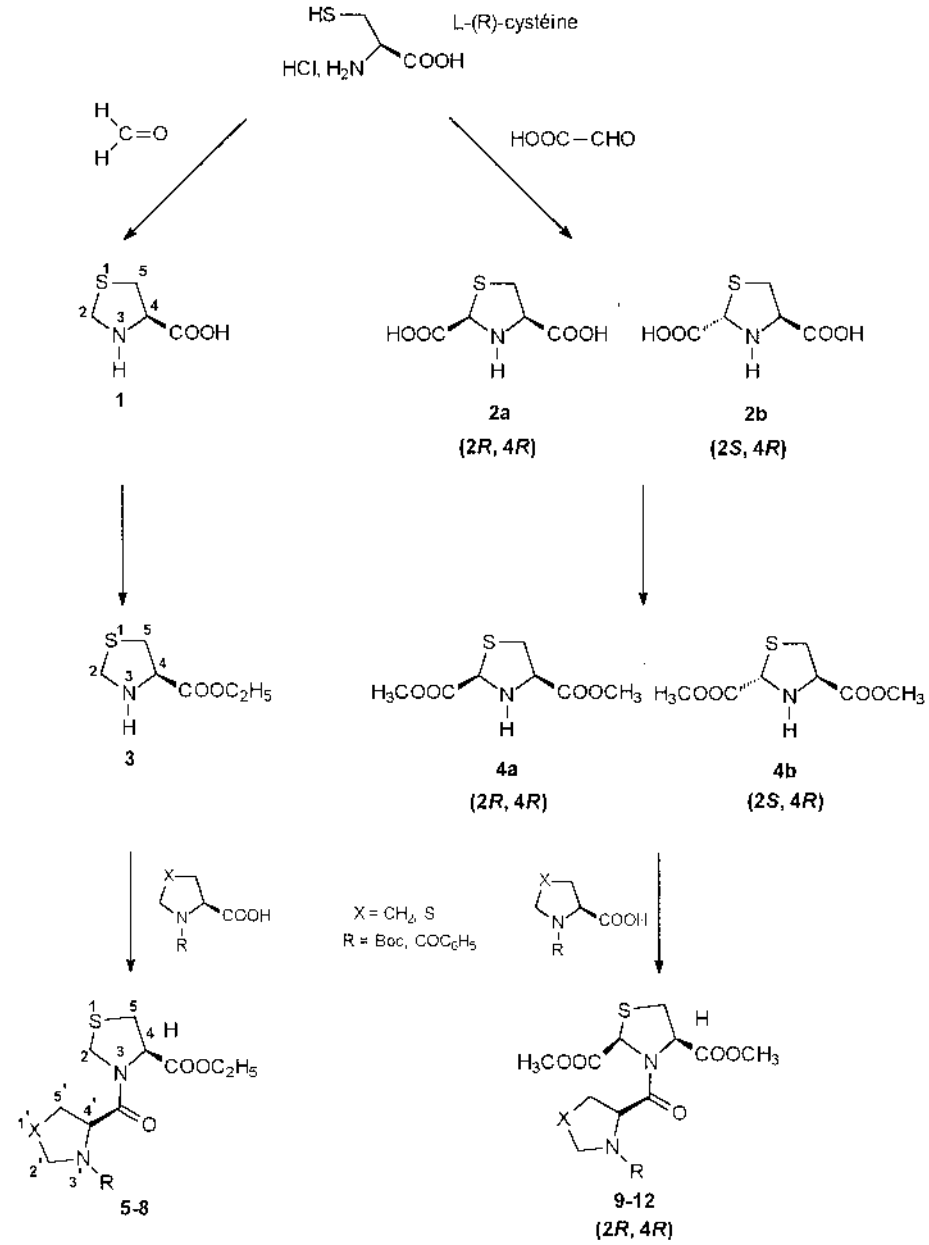

Chart 1. Synthetic Route Followed to Dipeptides **5**—**12**

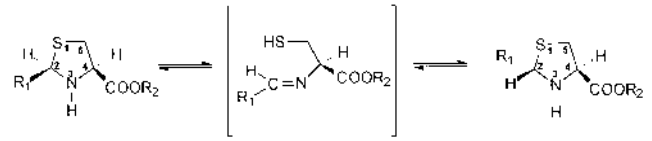

Chart 2. Epimerization at C-2 of Thiazolidine Ring

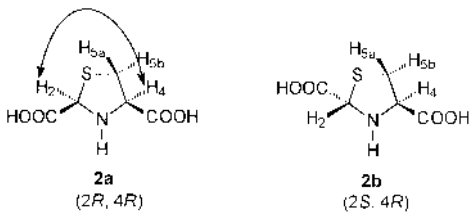

Chart 3. Configuration of the Two Isomers **2a** and **2b** and NOEs

mers in compound **2** reported in references 2 and 3.

The nuclear Overhauser effect (NOE) difference spectrum of **2** confirmed these assignments without ambiguity. For **2a**, NOEs were observed between the H2 proton signal at 4.85 ppm and H4 at 3.80 ppm showing that these protons are in a *cis* relationship to each other. For **2b**, no NOEs were observed between H2 and H4, thus confirming a (2*S*, 4*R*) configuration (Chart 3).

Methyl esterification of the diastereoisomeric mixture of compounds **2a** and **2b** by thionyl chloride in methanol also gave rise to dimethyl thiazolidine-2,4-dicarboxylate **4** in a diastereoisomeric mixture. The diastereoisomer ratio was different from that of the corresponding acid as revealed by the ^{1}H-NMR spectrum, depending on the relative stability of these two isomers in solution.[21] The proton spectrum of compound **4** also shows two different values for the sum of coupling constants for geminal protons H4 and H5a, H5b

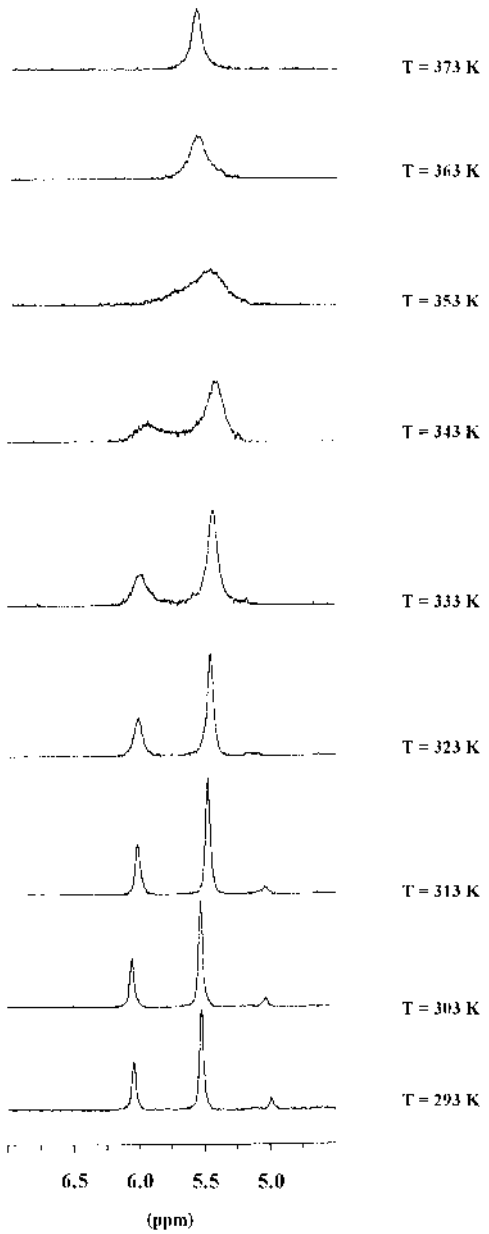

Fig. 1. The Partial ^{1}H-NMR Spectra of Compound **9** Recorded at Temperatures Ranging from 293 to 373 K, Showing Resonance Peaks for the H_2 Protons of Thiazolidinic Ring (DMSO-d_6 Was Used as Solvent)

($^3J_{H4H5a}+{}^3J_{H4H5b}$): 15.9 Hz and 11.7 Hz for (2*R*, 4*R*) **4a** and (2*S*, 4*R*) **4b** isomers respectively. This result has also been demonstrated by NOE experiment.

Using ethyl thiazolidine-4-carboxylate **3** and dimethyl thiazolidine-2,4-dicarboxylate **4**, dipeptides **5**—**8** and **9**—**12** were prepared by coupling respectively *N*-*tert*-butyloxycarbonylproline, *N*-benzoylproline, *N*-*tert*-butyloxycarbonylthiaproline and *N*-benzoylthiaproline (Chart 1). The structure of compounds **5**—**12** has been assigned from their IR and ^{1}H-NMR spectral data.

All proton NMR spectra obtained at 293 K for the dipeptides **9**—**12** exhibited two sets of resonance signals for H_2 and H_4 of the thiazolidine ring. The next step in the investigation is to identify the nature of these isomers. The easiest way to identify the origin of isomers is to vary the temperature of the sample. NMR solution samples of compounds

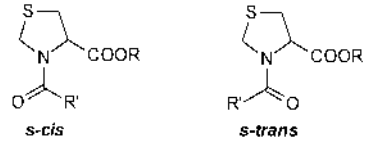

Chart 4. Conformers around the Peptide Bond of *N*-Acylthiazolidine-4-carboxylic Acid

9—**12** were prepared in dimethylsulfoxide-d_6. In Fig. 1, the variable temperature study for the sample of compound **9** is presented. The sample temperature was raised in increments from room temperature (293 K) to a temperature of 373 K. We showed the change in the 5—6 ppm region of the ^{1}H-NMR spectra in relation to temperature. Two sharp resonances were observed at 293 K and merged into one broad signal at 373 K. After reaching this temperature, the samples were cooled and the proton spectra were recorded. The resulting spectra were identical to those recorded before the heating cycle. These variable ^{1}H-NMR spectra suggest that the origin of the two isomers at room temperature is due to the presence of *S-cis* and *S-trans* conformers around the peptide bond, according to the nomenclature established by Goodman[23] for *N*-acylthiaproline (Chart 4). The rotation rate of the amide group at room temperature is often slow enough to give the splitting of the ^{1}H-NMR signals.[1,20,22] From these results, it appears that dipeptides **9**—**12** were obtained in only one diastereoisomer from a diastereoisomeric mixture of **4a** and **4b**. We can assume that only major diastereoisomer reacts, but, more probably, this result can be explained by interconversion of the two diastereoisomers **4a** and **4b**, through a ring opening mechanism involving Shiff base intermediates (Chart 2). In fact, a similar mechanism has been suggested to account for diastereoselective acylation of 2-substituted thiazolidine-4-carboxylate esters.[20,22,24,25]

Since the configuration is known at C-4 of synthesized dipeptides, attention was focused on the definition of the absolute configuration at stereocenter C-2 for compounds **9**—**12**. Nuclear Overhauser enhancement and exchange spectroscopy (NOESY and ROESY) experiments were performed with compounds **9**—**12**. However, no significant correlations for H_2—H_4 protons were observed to confirm a (2*R*, 4*R*) configuration. We finally determined the structure of only one diastereoisomer of compound **10**, dimethyl *N*-[(*N*-benzoyl)-L-prol-2-yl]-L-thiazolidine-2,4-dicarboxylate, using crystal X-ray diffraction; by analogy, dipeptides **9**—**12** should show the same configuration.

The crystal structure of **10**, recrystallized from ether/methanol (2 : 1), shows an absolute (2*R*, 4*R*) configuration, since the configuration at C-4 remained unaffected. This study also proves that the conformation of the molecule in the solid state is *S-trans*. This is in agreement with the conformation of 3-(5-oxo-L-prol-2-yl)-L-thiazolidine-4-carboxylic acid.[26] The molecular structure of **10** is shown in Fig. 2. The thiazolidine ring (S/C2/N1/C4/C5) is present in an envelope conformation. The conformational flexibility of the ring is confirmed by the different conformations found in various thiazolidine derivatives, for example, the twist conformation in *N*-acetyl-2-(*p*-tolyl)thiazolidine-4-carboxylic acid[27] and 2-oxothiazolidine-4-carboxylic acid[28] and the envelope conformation in ethyl-3-(4-nitrobenzoyl)thiazolidine-4-carboxylate.[29] This conformation is characterized by the

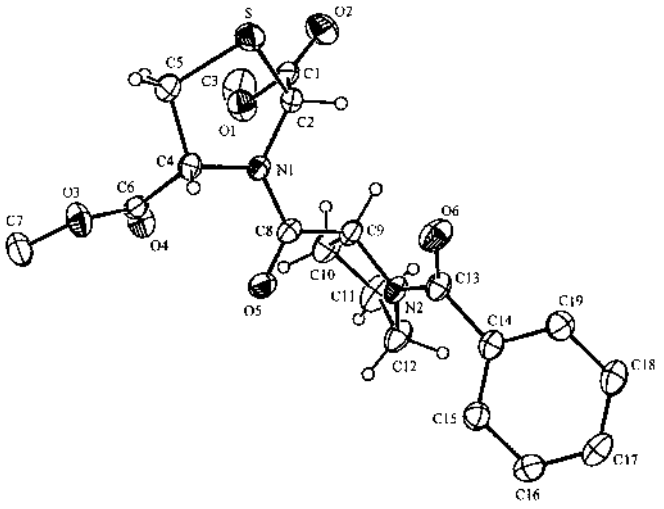

Fig. 2. ORTEP Drawing of Dimethyl *N*-[(*N*-Benzoyl)-L-prol-2-yl]-L-thiazolidine-2,4-dicarboxylate **10**

planarity of the C(2)–N(1)–C(4)–C(5) fragment (the torsion angle is only 8.1°) and by the high deviation of the S atom (0.769 Å) from the best plane defined by the atoms mentioned above. The N(1)–C(8) bond length of 1.355(4) Å is slightly longer than the N–C distance of 1.347(3) Å found in the related 3-(5-oxo-L-prol-2-yl)-L-thiazolidine-4-carboxylic acid.[26] However, this indicates a double order character of the bond, confirmed by the planarity of the C(2)–N(1)–C(8)–O(5) fragment (the torsion angle is 4.5°).

For compounds **5**—**8**, the C-2 is not a chiral center and thus, ^{1}H-NMR spectra of these compounds show no splitting of the signals. In particular, no splitting of H_2 and H_4 protons was observed, suggesting that the dipeptides have present only one *S-cis* or *S-trans* conformation in solution. Previously, published results suggest that peptide of proline and thiazolidine-4-carboxylic acid is exclusively *S-trans* conformation.[23,30,31] The conformer distribution depends on substituents at C-5 for proline and C-2 for thiazolidine-4-carboxylic acid.[31] The conformation *S-cis* can exist for 2-substituted thiazolidine-4-carboxylic acid and this form became exclusive for 2-substituted spiro derivatives.[1]

Experimental

General Remarks All compounds were characterized using the methods of elemental analyses, IR, NMR spectroscopy and X-ray analysis. Microanalyses were carried out by the Service Central d'Analyses, Centre National de la Recherche Scientifique, Vernaison (France). Infrared spectra were taken on a Shimadzu FTIR-8201PC spectrometer in potassium bromide pellets for solids and as liquid films for oils. Proton and carbon NMR spectra were recorded on a Bruker AC 200 spectrometer. The samples were dissolved in DMSO-d_6 or in $CDCl_3$. All measurements were performed at 293 K. The chemical shift values were referenced to internal tetramethylsilane (TMS from CEA Saclay, France). The signals were assigned after examination of distortionless enhancement by polarization transfer (DEPT) and 2D homonuclear ^{1}H–^{1}H correlation (COSY) spectra. Several 2D NOESY experiments were performed at different mixing times. The acquired matrices were made up of 256 FIDs of 1024 points and were zero-filled to 512 points in F1 before Fourier transformation, as described in previous work.[15,16] Chemical shift values and IR data for all compounds are summarized in this experimental part and these data are in agreement with the proposed structures. Melting points were determined on a Buchi N°510 apparatus and were uncorrected. X-Ray analysis were recorded on a CAD4 Enraf-Nonius diffractometer. Optical rotation was measured on a polarimeter ATAGO Polax L. When required, the separation of crude reaction products was performed by chromatography on silica gel column (70—230 mesh). L-(−)-*R*-Cysteine hydrochloride hydrate and proline were purchased from Aldrich Company, $[\alpha]_D^{20}=+5.2°$ (c=2.5, 1 N HCl), M_r=157.61 and $[\alpha]_D^{20}=-84°$ (c=4, H_2O), M_r=115.13, respectively.

Thiazolidine-4-carboxylic Acid (1) To a stirred solution of L-(−)-*R*-cysteine hydrochloride hydrate (15.8 g, 0.1 mol) and potassium acetate (9.8 g, 0.1 mol) in a mixture of water and ethanol (150 ml, 2 : 1, v/v) a solution of 37% aqueous formaldehyde (17 ml, 0.1 mol) was added. The reaction mixture was stirred for 1 h at room temperature and for 1 h at 90 °C. After cooling, the precipitate obtained was collected by filtration; the crude product was recrystallized from hot water. White powder, yield 91%, mp 203 °C. IR (KBr) cm^{-1}: 1627 (CO). ^{1}H-NMR (DMSO-d_6) δ: 2.80 (dd, 1H, ^3J=7.1 Hz, ^2J=10.0 Hz, H_{5a}), 3.10 (dd, 1H, ^3J=7.1 Hz, ^2J=10.0 Hz, H_{5b}), 3.75 (t, 1H, ^3J=7.0 Hz, H_4), 4.05 (d, 1H, ^2J=8.9 Hz, H_2), 4.30 (d, 1H, ^2J=8.9 Hz, H_2). *Anal.* Calcd for $C_4H_7NO_2S$: C, 36.09; H, 5.26; N, 10.43; S, 24.06. Found: C, 35.85; H, 5.05; N, 10.88; S, 24.23. $[\alpha]_D^{20}=-141°$ (c=1.3, H_2O).

Thiazolidine-2,4-dicarboxylic Acid (2a, 2b) This compound was prepared in the same manner as described for **1** using glyoxylic acid monohydrate. White powder, yield 85%, mp 180 °C. IR (KBr) cm^{-1}: 1620 (CO). ^{1}H-NMR (DMSO-d_6) isomer *cis* (2*R*, 4*R*) **2a** (70%) δ: 2.75 (t, 1H, ^3J=9.6 Hz, ^2J=9.6 Hz, H_{5a}), 3.35 (dd, 1H, ^3J=6.3 Hz, ^2J=10.1 Hz, H_{5b}), 3.80 (dd, 1H, ^3J=9.6, 6.3 Hz, H_4), 4.85 (s, 1H, H_2), isomer *trans* (2*S*, 4*R*) **2b** (30%) δ: 3.00 (dd, 1H, ^3J=5.9 Hz, ^2J=9.9 Hz, H_{5a}), 3.20 (dd, 1H, ^3J=6.6 Hz, ^2J=9.9 Hz, H_{5b}), 4.25 (t, 1H, ^3J=6.2 Hz, H_4), 5.00 (s, 1H, H_2). *Anal*. Calcd for $C_5H_7NO_4S$: C, 33.90; H, 3.95; N, 7.91; S, 18.07. Found: C, 34.03; H, 4.03; N, 7.98; S 18.20. $[\alpha]_D^{20}=-75°$ (c=1, 0.1 N NaOH).

Ethyl Thiazolidine-4-carboxylate (3) To a stirred and ice-cooled suspension of thiazolidine-4-carboxylic acid **1** (13.3 g, 0.1 mol) in anhydrous ethanol (250 ml) was added dropwise thionyl chloride (23.8 g, 0.2 mol). The mixture was stirred for another 10 h at room temperature, heated at reflux for 1 h, then evaporated under vacuum. The resulting product was hydrochloride. This product was dissolved in water (100 ml) and ethyl ether was added (100 ml). The aqueous solution was made basic by addition of sodium carbonate and the mixture was stirred. The organic phase was separated, dried over sodium sulfate and then evaporated. Yellow oil, yield 82%. IR (KBr) cm^{-1}: 3329 (NH), 1743 (CO). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (t, 3H, ^2J=7.2 Hz, $OCH_2\underline{CH_3}$), 2.90 (dd, 1H, ^3J=7.8 Hz, ^2J=10.4 Hz, H_{5a}), 3.25 (dd, 1H, ^3J=7.13 Hz, ^2J =10.0 Hz, H_{5b}), 3.90 (t, 1H, ^3J=7.4 Hz, H_4), 4.10 (d, 2H, ^2J=9.54 Hz, H_2), 4.20 (q, 2H, ^2J=7.2 Hz, $O\underline{CH_2}CH_3$). *Anal*. Calcd for $C_6H_{11}NO_2S$: C, 44.72; H, 6.83; N, 8.70; S, 19.88. Found: C, 45.03; H, 6.61; N, 8.42; S, 20.01. $[\alpha]_D^{20}=-161°$ (c=8, $CHCl_3$).

Dimethyl Thiazolidine-2,4-dicarboxylate (4a, 4b) This compound was prepared in the same manner as described for **3** using methanol. Colorless powder, yield 95%, mp 70 °C. IR (KBr) cm^{-1}: 3290 (NH), 1745 (CO). ^{1}H-NMR ($CDCl_3$) isomer *cis* (2*R*, 4*R*) **4a** (90%) δ: 2.80 (t, 1H, ^3J=10.0 Hz, ^2J=10.0 Hz, H_{5a}), 3.10 (s, 1H, NH), 3.30 (dd, 1H, ^3J=5.9 Hz, ^2J=10.1 Hz, H_{5b}), 3.85 (d, 6H, 2 $COO\underline{CH_3}$), 3.95 (m, 1H, H_4), 5.00 (s, 1H, H_2), isomer *trans* (2*S*, 4*R*) **4b** (10%) δ: 3.00 (dd, 1H, ^3J=5.5 Hz, ^2J=10.0 Hz, H_{5a}), 3.10 (1H, s, NH), 3.20 (dd, 1H, ^3J=6.2 Hz, ^2J=10.0 Hz, H_{5b}), 3.85 (d, 6H, 2 $COO\underline{CH_3}$), 4.00 (m, 1H, H_4), 5.10 (s, 1H, H_2). *Anal*. Calcd for $C_7H_{11}NO_4S$: C, 40.98; H, 5.37; N, 6.83; S, 15.61. Found: C, 40.98; H, 5.36; N, 6.82; S, 15.66. $[\alpha]_D^{20}=-83°$ (c=9, $CHCl_3$).

General Procedure for the Preparation of Dipeptides (5—12) *N-tert*-butyloxycarbonyl-L-proline, *N*-benzoyl-L-proline, *N-tert*-butyloxycarbonyl-L-thiazolidine-4-carboxylic acid and *N*-benzoyl-L-thiazolidine-4-carboxylic acid, were purchased from various suppliers or prepared by the well-known Shotten–Baumann procedure.

To a chilled solution of *N*-protected amino acid (0.01 mol) in dry tetrahydrofuran (THF), *N*-methyl morpholine (0.011 mol) and isobutylchloroformate (0.011 mol) were added at −10 °C. The mixture was kept at −10 °C for 5 min and **3** or **4** (0.01 mol) in dry THF was added. The mixture was stirred at 0 °C for 1 h, at 25 °C for 8 h, then filtered and evaporated to dryness. The remaining residue was taken up with ethyl acetate (10 ml) and washed twice with 5% $NaHCO_3$, HCl 2 N and water. The organic layer was dried over sodium sulfate. The oily residue was purified by chromatography on silica gel with ethyl acetate/hexane.

Ethyl *N*-[(*N-tert*-Butyloxycarbonyl)-L-prol-2-yl]-L-thiazolidine-4-carboxylate (5) Colorless powder, yield 42%, mp 91 °C. IR (KBr) cm^{-1}: 1656 (CO), 1705 (CO), 1751 (CO). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (t, 3H, ^2J=7.2 Hz, $OCH_2\underline{CH_3}$), 1.40 (d, 9H, $C(\underline{CH_3})_3$), 1.80—2.40 (m, 4H, $H_{1'}$, $H_{5'}$), 3.30 (m, 2H, H_5), 3.40 (m, 2H, $H_{2'}$), 4.20 (q, 2H, ^2J=7.2 Hz, $O\underline{CH_2}CH_3$), 4.50 (2d, 2H, ^2J=9.5 Hz, H_2), 4.80 (m, 1H, $H_{4'}$), 5.10 (m, 1H, H_4). ^{13}C-NMR ($CDCl_3$) δ: 14.1 ($OCH_2\underline{CH_3}$), 23.4, 23.98 (C1′), 28.3, 28.9 ($C(\underline{CH_3})_3$), 30.0 (C5′), 32.1 (C5), 46.7, 47.77 (C2′), 47.7 (C2), 52.6, 52.54 (C4′), 57.8 (C4),

65.9 (O<u>C</u>H$_2$CH$_3$), 79.7 (<u>C</u>(CH$_3$)$_3$), 154.5 (CO), 170.23 (CO), 172.75 (CO). *Anal*. Calcd for $C_{16}H_{26}N_2O_5S$: C, 53.63; H, 7.26; N, 7.82; S, 8.94. Found: C, 53.47; H, 7.39; N, 7.57; S, 9.09. $[\alpha]_D^{20}=-97°$ (c=4.5, $CHCl_3$).

Ethyl *N*-[(*N*-Benzoyl)-L-prol-2-yl]-L-thiazolidine-4-carboxylate (6) Colorless powder, yield 47%, mp 112 °C. IR (KBr) cm^{-1}: 1745 (CO), 1660 (CO), 1622 (CO). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (t, 3H, ^2J=7.2 Hz, OCH$_2$<u>CH$_3$</u>), 1.70—2.50 (m, 4H, H$_{5'}$, H$_{1'}$), 3.10—3.40 (m, 2H, H$_5$), 3.40—3.60 (m, 2H, H$_{2'}$), 4.20 (q, 2H, ^2J=7.2 Hz, O<u>CH$_2$</u>CH$_3$), 4.60 (d, 1H, ^2J=7.4 Hz, H$_2$), 4.90 (dd, 1H, ^3J=5.4, 6.9 Hz, H$_{4'}$), 5.10 (d, 1H, ^2J=7.4 Hz, H$_2$), 5.20 (dd, 1H, ^3J=4.9, 7.4 Hz, H$_4$), 7.30—7.50 (m, 5H). *Anal*. Calcd for $C_{18}H_{22}N_2O_4S$: C, 59.67; H, 6.08; N, 7.73; S, 8.88. Found: C, 59.41; H, 5.81; N, 7.98; S, 9.01. $[\alpha]_D^{20}=-116°$ (c=4.5, $CHCl_3$).

Ethyl *N*-[[(*N*-*tert*-Butyloxycarbonyl)-L-thiazolidin-4-yl]carbonyl]]-L-thiazolidine-4-carboxylate (7) Colorless powder, yield 45%, mp 80 °C. IR (KBr) cm^{-1}: 1748 (CO), 1661 (CO), 1702 (CO). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (t, 3H, ^2J=7.2 Hz, OCH$_2$<u>CH$_3$</u>), 1.40 (s, 9H, C(<u>CH$_3$</u>)$_3$), 3.10—3.50 (m, 4H, H$_5$, H$_{5'}$), 4.20 (q, 2H, ^2J=7.2 Hz, O<u>CH$_2$</u>CH$_3$), 4.50 (m, 4H, H$_2$, H$_{2'}$), 4.90 (m, 1H, H$_{4'}$), 5.20 (dd, 1H, ^3J=3.9, 6.8 Hz, H$_4$). *Anal*. Calcd for $C_{15}H_{24}N_2O_5S_2$: C, 47.87, H, 6.38, N, 7.45, S, 17.02. Found: C, 47.64, H, 6.59, N, 7.73, S, 16.88. $[\alpha]_D^{20}=-142°$ (c=2.5, $CHCl_3$).

Ethyl *N*-[[(*N*-Benzoyl)-L-thiazolidin-4-yl]carbonyl]]-L-thiazolidine-4-carboxylate (8) Colorless oil, yield 41%. IR (KBr) cm^{-1}: 1743 (CO), 1664 (CO), 1618 (CO). ^{1}H-NMR ($CDCl_3$): δ: 1.30 (t, 3H, ^2J=7.2 Hz, OCH$_2$<u>CH$_3$</u>), 3.10—3.50 (m, 4H, H$_5$, H$_{5'}$), 4.20 (q, 2H, J=7.2 Hz, O<u>CH$_2$</u>CH$_3$), 4.70 (m, 4H, H$_2$, H$_{2'}$), 5.10 (t, 1H, ^3J=8.3 Hz, H$_{4'}$), 5.20 (t, 1H, ^3J=7.7 Hz, H$_4$), 7.40—7.60 (m, 5H). *Anal*. Calcd for $C_{17}H_{20}N_2O_4S_2$: C, 53.68; H, 5.26; N, 7.37; S, 16.84. Found: C, 53.93; H, 5.09; N, 7.59; S, 16.48. $[\alpha]_D^{20}=-234°$ (c=9, $CHCl_3$).

Dimethyl *N*-[(*N*-*tert*-Butyloxycarbonyl)-L-prol-2-yl]-L-thiazolidine-2,4-dicarboxylate (9) Colorless oil, yield 35%. IR (KBr) cm^{-1}: 1743 (CO), 1745 (CO), 1683 (CO), 1705 (CO). ^{1}H-NMR ($CDCl_3$) minor conformer *S-trans* (40%) δ: 1.40 (d, 9H, C(<u>CH$_3$</u>)$_3$), 1.50—2.20 (m, 4H, H$_{5'}$, H$_{1'}$), 3.30—3.50 (m, 4H, H$_5$, H$_{2'}$), 3.80 (2d, 6H, 2 COO<u>CH$_3$</u>), 4.40 (m, 1H, H$_{4'}$), 5.05 (dd, 1H, ^3J=7.1, 8.4 Hz, H$_4$), 6.00 (s, 1H, H$_2$), major conformer *S-cis* (60%) δ: 1.40 (d, 9H, C(CH$_3$)$_3$), 1.50—2.20 (m, 4H, H$_{5'}$, H$_{1'}$), 3.30—3.50 (m, 4H, H$_5$, H$_{2'}$), 3.80 (2d, 6H, 2 COO<u>CH$_3$</u>), 4.40 (m, 1H, H$_{4'}$), 5.10 (dd, 1H, ^3J=7.0, 8.9 Hz, H$_4$), 5.50 (s, 1H, H$_2$). ^{13}C-NMR ($CDCl_3$): 23.4, 24.3 (C1′), 28.4 (C(<u>C</u>H$_3$)$_3$), 29.8, 30.77 (C5′), 31.9 (C5), 46.8 (C2′), 52.48, 52.9, 53.1 (2 COO<u>C</u>H$_3$), 57.6 (C4′), 60.9 (C4), 63.63, 63.5 (C2), 79.8 (<u>C</u>(CH$_3$)$_3$), 172.6 (CO ester), 168.7 (CO amide). *Anal*. Calcd for $C_{17}H_{26}N_2O_7S$: C, 50.75; H, 6.47; N, 6.97; S, 7.96. Found: C, 50.53; H, 6.73; N, 7.14; S 7.66. $[\alpha]_D^{20}=-89°$ (c=2.8, $CHCl_3$).

Dimethyl *N*-[(*N*-Benzoyl)-L-prol-2-yl]-L-thiazolidine-2,4-dicarboxylate (10) Colorless powder, yield 39%, mp 132 °C. IR (KBr) cm^{-1}: 1738 (CO), 1740 (CO), 1652 (CO), 1624 (CO). ^{1}H-NMR ($CDCl_3$) major conformer *S-trans* (75%) δ: 1.60—2.50 (m, 4H, H$_{5'}$, H$_{1'}$), 3.20—3.40 (m, 2H, H$_5$), 3.50—3.70 (m, 2H, H$_{2'}$), 3.80 (2d, 6H, 2 COO<u>CH$_3$</u>), 4.70 (m, 1H, H$_{4'}$), 5.10 (t, 1H, ^3J=8.0 Hz, H$_4$), 6.30 (s, 1H, H$_2$), 7.30—7.60 (m, 5H), minor conformer *S-cis* (25%) δ: 1.60—2.50 (m, 4H, H$_{5'}$, H$_{1'}$), 3.20—3.40 (m, 2H, H$_5$), 3.50—3.70 (m, 2H, H$_{2'}$), 3.80 (2d, 6H, 2 COO<u>CH$_3$</u>), 4.70 (m, 1H, H$_{4'}$), 5.70 (s, 1H, H$_2$), 6.00 (dd, 1H, ^3J=3.3, 6.5 Hz, H$_4$), 7.30—7.60 (m, 5H). ^{13}C-NMR ($CDCl_3$) δ: 25.1, 25.2 (C1′), 29.0, 29.4 (C5′), 32.2 (C5), 50.1, 49.9 (C2′), 52.1, 52.4 (COO<u>C</u>H$_3$), 58.0, 58.24 (C4′), 60.8 (C4), 63.0 (C2), 127.7, 127.7, 129.8, 129.8, 135.1 (C$_6$H$_5$), 168.7, 168.6, (<u>C</u>OC$_6$H$_5$), 169.5, 169.2 (CO), 171.5, 172.1 (CO). *Anal*. Calcd for $C_{19}H_{22}N_2O_6S$: C, 56.16; H, 5.42; N; 6.90; S, 7.88. Found: C, 55.82; H, 5.36; N, 7.05; S 8.03. $[\alpha]_D^{20}=-75°$ (c=4.3, $CHCl_3$).

Dimethyl *N*-[[(*N*-*tert*-butyloxycarbonyl)-L-thiazolidin-4-yl]carbonyl]]-L-thiazolidine-2,4-dicarboxylate (11) Colorless powder, yield 37%, mp 88 °C. IR (KBr) cm^{-1}: 1743, 1745 (CO), 1678 (CO), 1702 (CO). ^{1}H-NMR ($CDCl_3$) major conformer *S-trans* (75%) δ: 1.40 (d, 9H, C(<u>CH$_3$</u>)$_3$), 3.10—3.50 (m, 4H, H$_5$, H$_{5'}$), 3.80 (2d, 6H, 2 COO<u>CH$_3$</u>), 4.50 (m, 2H, H$_{2'}$), 4.8 (m, 1H, H$_{4'}$), 5.15 (t, 1H, ^3J=7.1 Hz, H$_4$), 6.1 (s, 1H, H$_2$), minor conformer *S-cis* (25%) δ: 1.40 (d, 9H, C(<u>CH$_3$</u>)$_3$), 3.10—3.50 (m, 4H, H5, H5′), 3.80 (2d, 6H, 2 COO<u>CH$_3$</u>), 4.50 (m, 2H, H$_{2'}$), 4.8 (m, 1H, H$_{4'}$), 5.50 (dd, 1H, ^3J=3.5, 7.0 Hz, H$_4$), 5.60 (s, 1H, H$_2$). ^{13}C-NMR ($CDCl_3$) δ: 28.3, 27.8 (C(<u>C</u>H$_3$)$_3$), 31.9 (C5), 35.8, 34.7 (C5′), 50.1 (C2′), 53.2, 52.8 (2 COO<u>C</u>H$_3$), 60.1 (C4′), 61.6 (C4), 63.6 (C2), 79.8 (<u>C</u>(CH$_3$)$_3$), 153.2 (CO), 169.7 (CO), 172.6 (CO ester). *Anal.* Calcd for $C_{16}H_{24}N_2O_7S_2$: C, 45.71; H, 5.57; N, 6.67; S, 15.24. Found: C, 45.98; H, 5.64; N, 6.32; S, 15.06. $[\alpha]_D^{20}=-131°$ (c=8.1, $CHCl_3$).

Dimethyl *N*-[[(*N*-Benzoyl)-L-thiazolidin-4-yl]carbonyl]]-L-thiazolidine-2,4-dicarboxylate (12) Colorless oil, yield 37%. IR (KBr) cm^{-1}: 1738, 1740 (CO), 1658 (CO), 1622 (CO). ^{1}H-NMR ($CDCl_3$) major conformer *S-trans* (70%) δ: 3.30—3.60 (m, 4H, H$_5$, H$_{5'}$), 3.80 (2d, COO<u>CH$_3$</u>), 4.60 (d, 1H, ^2J=9.7 Hz, H$_{2'}$), 4.80 (d, 1H, ^2J=9.5 Hz, H$_{2'}$), 5.00 (t, 1H, ^3J=7.8 Hz, H$_4$), 5.10 (t, 1H, ^3J=7.8 Hz, H$_{4'}$), 6.40 (s, 1H, H$_2$), 7.30—7.60 (m, 5H), minor conformer *S-cis* (30%) δ: 3.30—3.60 (m, 4H, H$_5$, H$_{5'}$), 3.80 (2d, COO<u>CH$_3$</u>), 4.60 (d, 1H, ^2J=9.5 Hz, H$_{2'}$), 4.80 (d, 1H, ^2J=9.5 Hz, H$_{2'}$), 5.05 (t, 1H, ^3J=7.8 Hz, H$_{4'}$), 5.80 (s, 1H, H$_2$), 6.00 (dd, 1H, ^3J=3.6, 6.9 Hz, H$_4$), 7.30—7.60 (m, 5H). *Anal*. Calcd for $C_{18}H_{20}N_2O_6S_2$: C, 50.94; H, 4.72; N, 6.60; S, 15.09. Found: C, 51.19; H, 4.56; N, 6.82; S, 14.86. $[\alpha]_D^{20}=-89°$ (c=3.5, $CHCl_3$).

X-Ray Structure Analyses of Dimethyl *N*-[(*N*-Benzoyl)-L-prol-2-yl]-L-thiazolidine-2,4-dicarboxylate (10) A colorless crystal having the approximate dimensions 0.40/0.17/0.17 mm was mounted on a CAD4 Enraf–Nonius diffractometer. The data were collected at room temperature with MoK_α radiation (λ=0.71073 Å). The unit cell was determined from 25 reflections selected by the CAD4 routines.[32]

Crystallographic data: $C_{19}H_{22}N_2O_6S$, M_w=406.45, monoclinic $P2_1$ (N° 4), a=7.889 (1), b=10.376 (2), c=12.121 (2) Å, β=101, 97 (1)°, V=970.6 (3) Å^3, Z=2, $\rho_{calc.}$=1.391 g.cm^{-3}, F (000)=428, μ=0.206 mm^{-1}, 4032 reflections measured, 1567 unique data with $I>2\sigma(I)$.

A total of 4032 intensities were reduced with the XCAD4PC data reduction program.[33] The structure was solved in the noncentrosymmetric monoclinic space group $P2_1$ with direct methods and refined by full-matrix least-squares methods (based on F^2).[34] All non-hydrogen atoms were refined with anisotropic thermal parameters and the hydrogen atoms bonded to carbons were included in calculated positions and refined with a riding model. The choice of enantiomer was checked between the *R–R* and *S–S* configurations by inversion of all positional parameters. The absolute structure Flack parameter calculated for both cases (−0.10 (10) for *R–R* and +0.29 (10) for *S–S*) and the values of final R factors, smaller for *R–R* ($R_1(F^2)$=0.0306/0.0574 and $wR_2(F^2)$=0.0739/0.0826 ($I>2\sigma$ (*I*) *vs*. all data)) than for *S–S* ($R_1(F^2)$=0.0309/0.0578 and $wR_2(F^2)$=0.0747/0.0834), argue for the expected choice of *cis* (2*R*, 4*R*) configuration. It is evident that the differences between the structural data of the two enantiomers are not significant. Nevertheless, since the configuration of the C-4 chiral atom has been clearly demonstrated previously together with the *cis* configuration (2*R*, 4*R* or 2*S*, 4*S*) of the molecule, it can be assumed that the absolute configuration at C-2 in the compound **10** is *R*.

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