Tetrahedron: Asymmetry Vol. 3, No. 11, pp. 1401-1410, 1992
Printed in Great Britain

0957-4166/92 $5.00+.00
Pergamon Press Ltd

STEREOCHEMISTRY OF THIAZOLIDINE RING FORMATION FROM AMINALS AND CYSTEINE

Aleksandra Wysłouch*, Marek Lisowski, Artur Pędyczak, Ignacy Z. Siemion

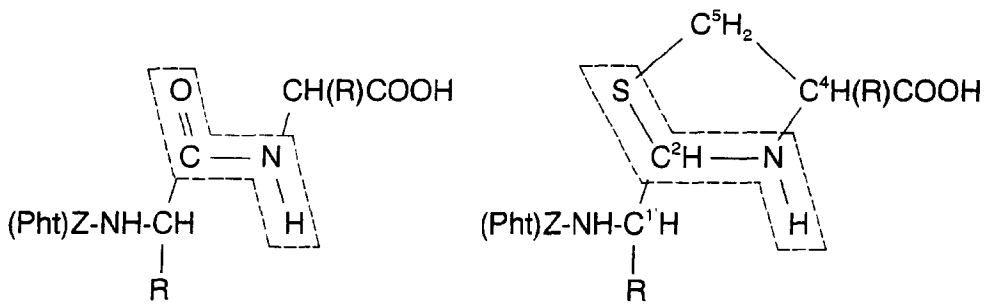
Institute of Chemistry, University of Wrocław, 14 Joliot-Curie Str, 50-383 Wrocław, Poland

(*Received* 3 *September* 1992)

Abstract: Aminals derived from Z-S-Ala, Z-R-Ala and Pht-S-Ala were coupled with R- and S-cysteine to give thiazolidine analogues of dipeptides with the configuration of the newly formed stereogenic carbon depending on the alanine configuration. ^{1}H-NMR and CD spectra were measured to check the configurational homogeneity of the products, and NOESY spectra were used to assign a configuration of the new stereogenic centre.

INTRODUCTION

Using cysteine and α-aminals (i.e. aldehydes obtained from corresponding α-aminoacids), thiazolidine analogues of dipeptides with a restricted backbone conformation could be synthesized. Our interest in the synthesis of 2-(1'-aminoalkyl)-thiazolidine carboxylic acids was manifold. Firstly, the -SCHNH- moiety mimics a *trans*-amide bond (Fig.1).



Pht - phthaloyl group
Z - benzyloxycarbonyl group

Fig. 1

Secondly, the tetrahedral character of the C-2 atom of thiazolidine ring makes this system similar to the transition state of an amide bond, which is formed by an attack of hydrolytic enzymes on peptide substrates.

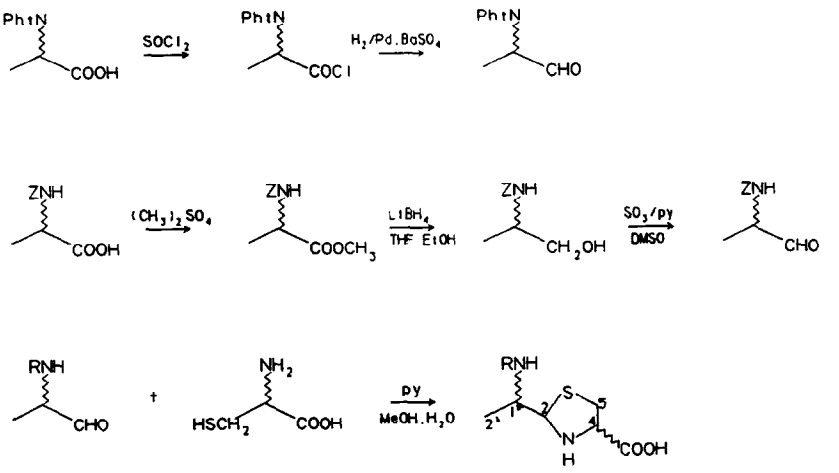
Another interesting stereochemical feature of such analogues is a restriction of rotation around the N - C^{α} bond of the C-terminal cysteine residue. The conformations resulting from rotation across this bond are characterized by the conformational angle ϕ. In thiazolidine analogues of dipeptides, this angle is equal to +120° or -120°, when R or S-cysteine is used for the synthesis, respectively. What is particularly interesting is that when the angle ϕ is equal to -120° it is close to the values typical for ß-structure of the polypeptide chain[1]. Thus starting from S-cysteine it is possible to synthesize dipeptide analogues possessing a definite (close to ß-structure) conformation in the C-terminal fragment of the molecule.

It should be also noted that the nitrogen atom of the thiazolidine ring can serve for branching of the peptide chain, and this is of interest from the synthetic point of view.

A typical method for the synthesis of 2-substituted thiazolidine-4-carboxylic acids is condensation of an appropriate aldehyde with cysteine hydrochloride in the presence of pyridine or sodium acetate[2]. Methyl esters of such derivatives were synthesized by Hamada et al.[3] by condensation of N-protected α-aminoaldehydes with cysteine methyl ester in benzene. A mixture of C-2 epimers, which was not characterized stereochemically, was obtained in the reaction. Our aim was to obtain more precise stereochemical characteristics of these interesting compounds. For this purpose we investigated in detail the stereochemical course of the reaction between N-benzyloxycarbonyl-S-alaninal and R-cysteine. The product of this reaction, (**Ia**), turned out to be only one epimer with the R configuration on the newly formed stereogenic centre (C-2 atom of thiazolidine ring). The reactions of N-benzyloxycarbonyl-S-alaninal with S-cysteine and N-benzyloxycarbonyl-R-alaninal with (R,S)-cysteine gave also the stereoisomeric products (**Ib-d**) with high diastereomeric purity. Acquired stereochemical data show that the configuration on thiazolidine C-2 atom is R or S when N-benzyloxycarbonyl-S-alaninal or N-benzyloxycarbonyl-R-alaninal are used in the reaction, respectively, regardless of the cysteine configuration. The obtained thiazolidine derivatives (**Ia-d**) are stable in the solid state but isomerize in solution giving rise to mixtures of C-2 epimers. Epimerisation of **Ia-d** in d_6-DMSO solution on standing for a few days or heating made it possible to obtain NMR data for all diastereoisomers of **I**, including the stereoisomers **Ie-h**. The reaction of N-phthaloyl-S-alaninal with R- and S-cysteine gave crystalline products that are mixtures of C-2 epimers (**IIa+IIb** and **IIc+IId**). In both cases the predominant stereoisomer (~80%) has the R configuration on the C-2 thiazolidine atom.

RESULTS AND DISCUSSION

The reaction routes from N-protected-alanine to the thiazolidine derivatives **I** and **II** are shown in Scheme 1. We chose either dimethylsulphoxide oxidation using sulphur trioxide-pyridine complex in the presence of TEA to obtain N-benzyloxycarbonyl-alaninal or the Rosenmund method, (reduction of N-phthaloyl-alanine chloride) to obtain N-phthaloyl-alaninal, as these methods are known to give no appreciable racemization[5,6]. Condensation of the aldehydes with cysteine hydrochloride in aqueous methanol containing pyridine afforded the thiazolidine derivatives **I** and **II** as white crystals.



Scheme 1.

I R=Z
II R=Pht

1. NMR spectroscopy

The ^{1}H NMR spectra of the thiazolidine derivatives **Ia-d** derived from N-benzyloxycarbonyl-alanine in d_6-DMSO show single sets of sharp signals that point to a high diastereomeric purity of these compounds. The assignment of resonances was based on the multiplicity and intensity of signals and selective ^{1}H decoupling. Observed chemical shifts and coupling constants are shown in Table 1.

Compound	$\delta_{2'}$	$\delta_{1'}$	δ_2	δ_4	δ_{5a}	δ_{5b}	$^3J_{1',2}$	$^3J_{1',2}$	$^3J_{4,5a}$	$^3J_{4,5b}$	$^2J_{5a,5b}$
Ia, Id (cis)	1.16	3.85	4.49	3.76	3.13	2.72	6.67	6.74	6.69	9.20	9.60
Ib, Ic (trans)	1.05	3.53	4.57	3.94	3.05	2.80	6.58	8.20	6.37	6.33	10.10
Ie, Ih (trans)	1.07	3.59	4.53	4.00	3.12	2.82	6.59	7.80	6.49	6.48	9.82
If, Ig (cis)	1.15	3.80	4.43	3.69	3.03	2.73	6.90	6.70	7.32	8.66	9.77
IIa	1.49	4.45	5.18	3.99	3.10	2.91	6.83	10.20	7.15	7.33	10.23
IIb	1.46	4.03	5.31	3.92	3.11	2.74	7.01	10.50	7.32	7.49	10.06
IIc	1.46	4.03	5.31	3.93	3.13	2.75	6.83	10.46	6.58	7.67	10.23
IId	1.51	4.36	4.97	3.74	3.21	2.85	7.04	9.15	6.86	8.66	10.09

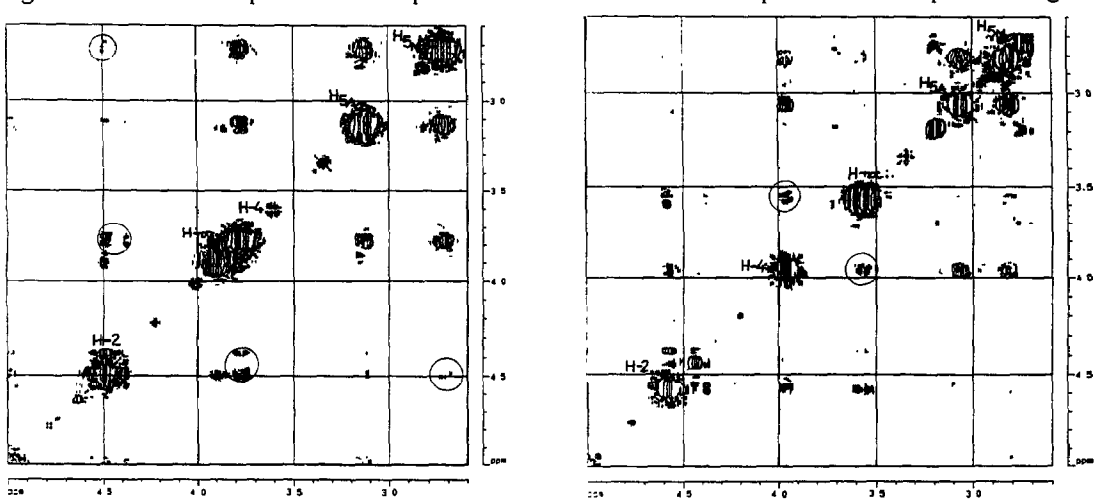
Table 1. The chemical shifts (in ppm) and coupling constants (in Hz) for thiazolidine derivatives **I** and **II** in d_6-DMSO solution (**Ia-g** and **IIa-d** are stereoisomers of 2-(1'-(N-benzyloxycarbonylamino)ethyl)-thiazolidine-4-carboxylic acid (**I**) and 2-(1'(N-phthaloylamino)ethyl)-thiazolidine-4-carboxylic acid (**II**); **Ia-Id,Ib-Ic,Ie-Ih** and **If-Ig** are the enantiomeric pairs).

The spectra consist of resonances of methyl, α-hydrogen and Z-protecting group protons derived from an aldehyde and a group of signals characteristic for the thiazolidine ring protons. The H-4 and two H-5 ring

protons give the AMX resonance pattern. The signal of the proton on the newly formed stereogenic carbon-2 atom is a well resolved doublet showing no coupling with the thiazolidine imine proton. The lack of such coupling signifies a rapid exchange of the amine hydrogen, that is probably due to its interaction with the adjacent carboxyl group situated at C-4. In the NMR spectrum of the N-phthaloyl-aminoethyl-thiazolidine derivatives **II** in d_6-DMSO, double groups of corresponding resonances were observed that proved the existence of two epimers in the sample. The amount of the predominant stereoisomer is ~80% as determined from the peak area measurement. Our attempts to separate these isomers either by crystallization or chromatography were unsuccessful. The NMR data for stereoisomers of N-phthaloyl derivatives **II** are similar to these for N-Z-derivatives **I** (Table 1). The low-field shift of signals for protons derived from the side chain and the C-2 proton of thiazolidine ring may be due to the change of electronegativity of the N-protecting group. A significant change is also visible in the values of $^3J_{H1',H2}$ coupling constants. For the Pht-derivative it is 9.15 and 10.46 Hz for the two stereoisomers, as compared to 6.74 Hz for the Z-derivative. This picture suggests that whereas in the case of **I** rotamers connected to the rotation around C1'-C2 bond are equally populated, in the case of **II** (in both stereoisomers) a definite preponderance for the conformer having antiperiplanar orientation of H-1' and H-2 atoms appears. Such a conformation places the bulky groups far from each other.

A. Configuration of the C-2 atom of thiazolidine ring

To determine the absolute configuration of the newly formed center of chirality (C-2 atom) in thiazolidine rings in **Ia-d** NOESY experiments were performed. The results of these experiments are depicted in Fig.2.



a. b.

Fig. 2 Contour map of a 300 MHz ^{1}H NOESY spectrum of: a.- **Ia**, b. - **Ib**.

Among the cross peaks in the NOESY spectrum of compound **Ia** derived from Z-N-S-alaninal and R-cysteine (Fig.2a) there are two (marked by circles) which can origin from the interaction of the H-2 atom with, respectively. H-4 (3.76 ppm) and the "high-field" (2.72 ppm) H-5_M proton. This suggests that H-2, H-4,

and "high-field" H-5_M atoms occupy the same side of the thiazolidine ring, i.e. they are situated *cis*- to each other. An almost identical pattern of NOESY cross-peaks was observed for stereoisomer **Id**, derived from Z-N-R-alaninal and S-cysteine indicating that **Ia** and **Id** must be enantiomers. The NOESY spectra of **Ib** and **Ic** (Z-N-S-alaninal + S-cysteine and Z-N-R-alaninal + R-cysteine, respectively) being exactly alike (Fig.2b) do not show any strong cross-peaks mentioned for **Ia** and **Id**. Instead, there is a characteristic cross-peak (in circle) between H-1' and H-4 proton signals. This indicates that the alkyl substituent at the C-2 thiazolidine atom must be *cis*- to the H-4 (trans to the carboxyl group).

Taking into account the NOE data, we can conclude that the configuration of the obtained 2-(1'-aminoalkyl)-4-thiazolidine carboxylic acids obtained is as follows:

Ia 1'-S, 2-R, 4-R
Ib 1'-S, 2-R, 4-S
Ic 1'-R, 2-S, 4-R
Id 1'-R, 2-S, 4-S

Epimerization of the C-2 atom of Ia-d on standing in DMSO gives corresponding products Ie-h with following configuration:

Ie 1'-S, 2-S, 4-R
If 1'-S, 2-S, 4-S
Ig 1'-R, 2-R, 4-R
Ih 1'-R, 2-R, 4-S

B. Conformation of the thiazolidine ring.

Some information about the thiazolidine ring conformation of our analogues of dipeptides can be obtained from the analysis of $^3J_{4,5}$ coupling constants. It can be observed that for all diastereoisomers having the alkyl group *trans*- to the carboxyl one ("I trans"), the coupling constants $^3J_{4,5M}$ and $^3J_{4,5A}$ are very similar and are from 6.3 to 6.5 Hz, while for diastereoisomers having the alkyl group cis to the carboxyl group ("I cis") the coupling of H-4 is bigger with the high-field H-5_M (8.52-9.20 Hz) than with the low-field H-5_A proton (6.69-7.08 Hz). These data indicate that there is a considerable difference between the ring conformational equilibria for diastereoisomers of **I** depending on the mutual orientation of the alkyl substituent at C-2 and the carboxyl group at the C-4 atom of the thiazolidine ring. The largest influence on the relative spatial orientation of the H-4 and H-5 atoms exerts the conformational transition between "N-endo" and "N-exo" forms, shown as A and B, respectively, in Fig.3 (N-endo and N-exo terms denote the "endo" or "exo" orientation between ring nitrogen and the carboxyl group on C-4).

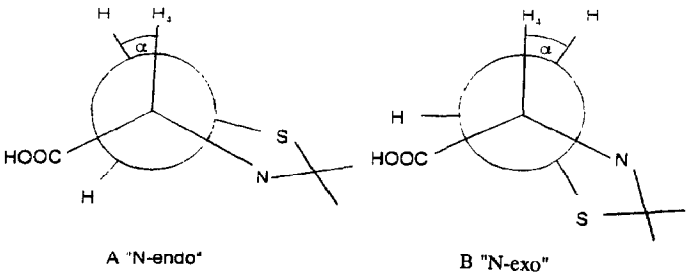


Fig. 3

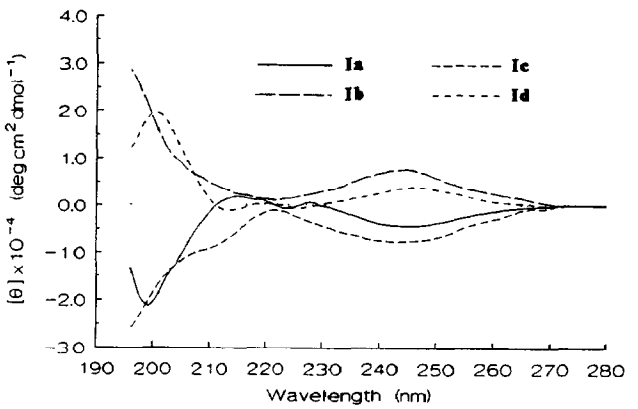
Using the Karplus relation

$$^3J_{4,5} = A\cos^2\alpha + B\cos\alpha + C$$

with coefficient values A = 10.83; B = 0.0; C = 0.9 proposed for the thiazolidine system by Kulkarni et al.[6] and the coupling constants of **Ia-d** observed in d_6-DMSO solution, we could calculate the mean conformational angles of moieties H_4-C-C-H_{5A} and H_4-C-C-H_{5M}. The values of 29.2° and 143.3° for "I cis" and 43.6° and 133.6° for "I trans" diastereoisomer obtained from the Karplus equation were in keeping with the data obtained from molecular modelling program MOPAC 5.0[7] for the lowest energy conformers of "I cis" (23.4° 143.2°) and "I trans" (42.3°, 110.5°). From these observations a conclusion can be drawn that the conformational equilibrium is dominated by the "N-endo" conformer for "I cis" and by the "N-exo" conformer for "I trans" diastereoisomers. The other observation is that for all "I cis" stereoisomers the resonance signal of the H-4 proton is in the higher field ($\Delta\delta$ = 0.2 ppm) and the H-1'proton in the lower field ($\Delta\delta$ = 0.3 ppm) than in "I trans" diastereoisomers. We do not know a simple explanation of this fact.

2. Chiroptical properties of 2-(1'-(N-benzyloxycarbonylamino)-ethyl)-thiazolidine-4-carboxylic acid (I)

The CD spectra of **Ia-d** are presented in Fig.4.



The CD spectrum of **Ia** shows a broad, negative maximum at about 243 nm, a small, positive maximum at 215 nm, and a strong, negative band at 200 nm. The CD curve of **Id**, enantiomer of **Ia**, is of the same shape but with the opposite signs of the corresponding bands. The CD spectrum of **Ic** differs strongly from the curve of **Ia**. There is a negative band at about 241 nm which is almost twice as strong as the analogous band in the spectrum of **Ia**. There are still bigger differences between the two spectra below 220 nm. In this region the CD spectrum of **Ic** shows a very pronounced, negative shoulder at about 211 nm and a negative band below 195 nm, distinctly more intense than the band at 200 nm in the CD spectrum of **Ia**. The same CD curve but of the opposite sign was observed in the case of **Ib**, enantiomer of **Ic**.

It was shown by Györgydeak et al.[8] that CD spectra of 2-polyhydroxyalkyl-thiazolidine-4-carboxylic acid derivatives, as well as of more simple compounds of this group, are dominated by the Cotton effects connected to the excitations of heterocyclic thiazolidine chromophore. The spectra show two or three Cotton effects, at 237 - 247, 217 - 221 and 198 - 203 nm. The signs and magnitudes of the effects were found to be determined by the chirality of the heterocyclic chromophore and not by a configuration of the chirality centers present in the substituent on the C-2 atom. The spectra of our series are very similar to those reported by Györgydeak et al.

It can be seen from our data that the chirality of the C-2 atom of the ring does not influence the sign of the longwavelength Cotton effect at about 240 nm. Diastereoisomers **Ia** and **Ic** which differ in the configuration on this atom, both possess the same sign of the Cotton effect discussed (the same should be noted also for diastereoisomers **Ib** and **Id**). A comparison of the CD spectra of thiazolidine-4-carboxylic acid and **Ia** shows that an R configuration of the C-2 atom should give a negative contribution to the 240 nm band. So, when the C-2 configuration is changed from R to S we should observe, if not the sign reversal, at least a distinct decrease in the negative band at 240 nm. Meanwhile, the opposite is true and this band becomes almost twice more intensive. Thus, a sign and amplitude of this effect is not used as diagnostic for determination of the configuration of the C-2 atom. More promising in this respect seems to be the next Cotton effect, present at 211 - 215 nm. A remarkable dependence of the sign of this band on the configuration of the C-2 atom suggests that it can be used as an additional criterion of the configuration of this chirality center. The data suggest that the positive Cotton effect in this region is connected to the S-configuration on C-2, and negative - to the R-configuration.

EXPERIMENTAL

Chemicals:

L-Ala and D-Ala were obtained from Reanal. N-phthaloyl and N-benzyloxycarbonyl-Ala were prepared as described by Bodanszky et al.[9] Dimethylformamide, dimethylsulphoxide, triethylamine and pyridine were distilled and dried over molecular sieves. Sulphur trioxide:pyridine complex was obtained from Aldrich.

Spectroscopic measurements:

^{1}H NMR spectra were recorded for dimethyl sulphoxide solutions on a Bruker 300 AMX and 500 AM spectrometers with internal deuterium lock in $(CD_3)_2SO$. The spin systems were analyzed using Laocoon program and the obtained results were checked by computer simulation using Racoon program. NOESY experiments were performed on a Bruker 500 AMX spectrometer.

^{1}H NOESY experiments were recorded at 300 MHz on a Bruker 300 AMX spectrometer in room temperature. The spectra were run with 4096 × 512 points (40 scans per experiment), with mixing time of 300 ms and processed with a sine-squared window function. Sine bell shifts were equal to 0 and 2 for F1 and F2, respectively.

CD spectra were measured on a Jasco J-600 spectropolarimeter at room temperature. Pathlengths of 1 and 10 mm were used (in the far and near UV spectral region respectively.). Concentration of solutions in methanol ranged from 0.005 to 0.05 mg/ml. Data are presented as molar ellipticity [Θ].

2-(1'-(N-benzyloxycarbonylamino)ethyl)-thiazolidine-4-carboxylic acids (I)

To a solution of N-Z-Ala (5g, 0.022 M) in DMF(36 ml) pulverized $KHCO_3$ was added (9g) followed by $(CH_3)_2SO_4$ (2.8 ml). The mixture was stirred at room temperature for 4 hours. 90 ml of water was added and the mixture was extracted with ethyl acetate : benzene (1:1, 3 × 120 ml). After washing with water (2 × 18 ml), 5% sodium sulphite (18 ml) and saturated aqueous sodium chloride (18 ml) the organic layer was dried over sodium sulphate and concentrated in vacuo. Addition of hexane gave N-Z-Ala methyl ester as white crystals (95% yield). The methyl ester (5 g, 0.021M) was dissolved in THF (30 ml) and $LiBH_4$ (0.93 g) in THF was added under nitrogen. After addition of ethanol the mixture was stirred until the substrate disappeared (TLC), then poured into ice water, acidified with 10% citric acid to pH = 4, concentrated in *vacuo* and extracted with CH_2Cl_2 (3 × 50 ml). The organic layers were dried over sodium sulphate. Removal of the solvent gave N-Z-alaninol (95% yield). To a cooled (-10°) solution of N-Z-alaninol (1 g) and TEA (1.99 ml) in CH_2Cl_2 (14.5 ml) sulphur trioxide - pyridine complex (2.3 g) in 14.5 ml DMSO was added. The mixture was stirred for 10 minutes at room temperature, poured into ice-saturated aqueous sodium chloride and extracted with ethyl ether. The extracts were washed with 10% citric acid and brine, dried over magnesium sulphate and concentrated in vacuo. The product, N-Z-alaninal (88% yield) was checked for the presence of a characteristic aldehyde proton signal in the NMR spectrum and used without further purification. N-Z-alaninal (0.6 g) was dissolved in methanol and a solution of cysteine hydrochloride (0.46 g) in water and pyridine (0.47 ml) was added under nitrogen.After a few minutes crystals of the thiazolidine derivative I precipitated.

1'S, 2R, 4R- 2-(1-(N-benzyloxycarbonylamino)ethyl)-thiazolidine-4-carboxylic acid-(**Ia**)
Yield: 65%. Melting point 181-183° (uncorrected), Analysis: Calculated for $C_{14}H_{18}O_4N_2S$ 54.2% C, 5.8% H, 9.0% N, 10.3% S found 53.9% C, 6.2% H, 8.7% N 10.4% S.

1'S, 2R, 4S- 2-(1-(N-benzyloxycarbonylamino)ethyl)-thiazolidine-4-carboxylic acid-(**Ib**)
Yield: 65%. Melting point 175-179° (uncorrected), Analysis: Calculated for $C_{14}H_{18}O_4N_2S$ 54.2% C, 5.8% H, 9.0% N, 10.3% S found 54.2% C, 6.2% H, 9.2% N 10.3% S.

1'R, 2S, 4R- 2-(1-(N-benzyloxycarbonylamino)ethyl)-thiazolidine-4-carboxylic acid-(**Ic**)
Yield: 65%. Melting point 175-178° (uncorrected). Analysis: Calculated for $C_{14}H_{18}O_4N_2S$ 54.2% C, 5.8% H, 9.0% N, 10.3% S found 54.4% C, 5.9% H, 9.0% N 10.4% S.

1'R, 2S, 4R- 2-(1-(N-benzyloxycarbonylamino)ethyl)-thiazolidine-4-carboxylic acid-(**Id**)
Yield: 65%. Melting point 181-183° (uncorrected), Analysis: Calculated for $C_{14}H_{18}O_4N_2S$ 54.2% C, 5.8% H, 9.0% N, 10.3% S found 54.1% C, 5.8% H, 9.0% N 10.2% S.

2-(1'-(N-phthaloylamino)ethyl)-thiazolidine-4-carboxylic acids (II)

N-phthaloyl-Ala (10 g) was heated for 50 minutes at 60° with thionyl chloride (20 ml). After evaporation of the excess of $SOCl_2$ the compound was distilled under reduced pressure to get N-Pht-Ala chloride (80% yield). The freshly distilled chloride was hydrogenated for 10 hours in xylene at 110° with 10% $Pd/BaSO_4$ as a catalyst. After filtration of the catalyst n-hexane was added to get a white precipitate of N-Pht-alaninal (85% yield) which was condensed with L- or D-cysteine hydrochloride in the same way as N-Z-alaninal. White crystals of **II** were obtained. The products was a mixture of C-2 epimers (**IIa+IIb** and **IIc+IId**, respectively) as identified by NMR.

1'S, 2 R;S, 4R- 2-(1'-(N-phthaloylamino)ethyl)-thiazolidine-4-carboxylic acids mixture (**IIa,b**).
Yield: 70%. Analysis: Calculated for $C_{14}H_{14}O_4N_2S$ 54.9% C 4.6% H 9.1% N 10.5% S, found: 55.4% C 4.6% H 8.8% N 10.7% S.

1'S, 2R;S, 4S- 2-(1'-(N-phthaloylamino)ethyl)-thiazolidine-4-carboxylic acids mixture (**IIc,d**).
Yield: 70%. Analysis: Calculated for $C_{14}H_{14}O_4N_2S$ 54.9% C 4.6% H 9.1% N 10.5% S, found: 55.1% C 4.6% H 8.9% N 10.7% S.

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