PREPARATION OF ALL THE FOUR DIASTEREOMERS OF β-PHENYLCYSTEINE METHYL ESTER THROUGH CHROMATOGRAPHIC OPTICAL RESOLUTION OF THE 2,2-DIMETHYLTHIAZOLIDINE DERIVATIVES

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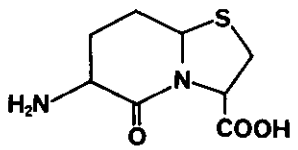
Abstract — cis And trans-2,2-dimethyl-4-carbomethoxy-5-phenylthiazolidines prepared from the corresponding erythro- and threo-β-phenylcysteines were resolved into the enantiomers by use of a chiral HPLC column, from which all of the four chiral β-phenylcysteine methyl esters were obtained.

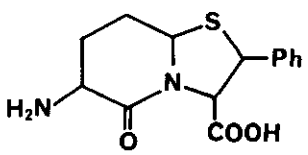
We are interested in elucidating the conformation of bioactive peptides, especially the active conformation. Generally, small peptides can take on a variety of conformations, and consequently they are mixtures of conformers with comparable energy in solution. It is, however, considered that such a peptide would take on a definite conformation when it exhibits the biological activity through its interaction with the appropriate biomacromolecules, e.g. receptors or enzymes. The knowledge of their active conformation seems important as the basis for the design of useful analogs.

As an approach to elucidate the active conformation we developed a bicyclic turn-shaped dipeptide (BTD), which can be incorporated into bioactive peptides and compel them to take on folded conformation at the point of its incorporation. If the BTD-containing analog retains potent biological activity, valuable informations can be obtained on the active conformation of the peptide[1,2]. In the series of investigation chiral β-phenylcysteines were needed as a building block for the synthesis of phenyl substituted BTD.

At the start of our project no report was found in the literature for the synthesis of chiral β-phenylcysteines. So, we developed a method to prepare all the possible



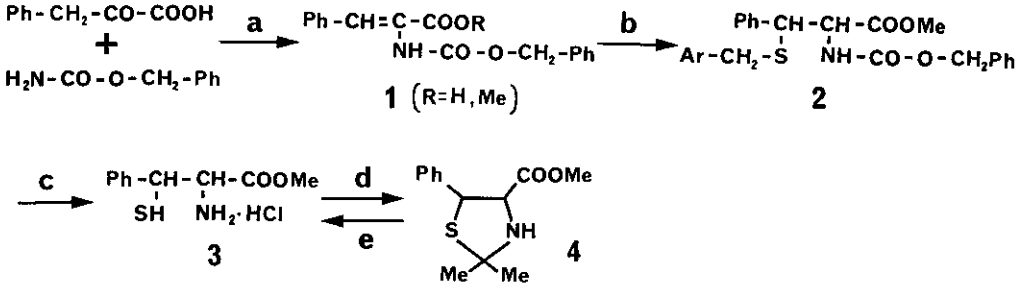
BTD



8-Phenyl BTD

diastereomers of β-phenylcysteine methyl esters. The method will be described below. Very recently, a report describing the preparation of chiral β-phenylcysteines by a quite different route has been published[3].

Two series of papers were found in the literature for the synthesis of β-phenylcysteines[4,5]. We traced both, but could not obtain satisfactory results, so we developed an original route shown below. At first, 3-phenylpyruvic acid was condensed with benzyl carbamate under acid catalysis to afford methyl 2-benzyloxycarboxamido-cinnamic acid (**1a**)[6], which was converted to the methyl ester (**1b**) by treatment with diazomethane. Compound (**1b**) was then reacted with p-methoxybenzyl mercaptan under base catalysis to give S-methoxybenzyl-N-carbobenzyloxy-β-phenylcysteine methyl ester (**2**) as a mixture of the diastereomers (72% yield). The threo (**2a**, mp 91-92°C) and erythro (**2b**, mp 101-102°C) isomers could be separated by careful fractional crystallization from ether/hexane or THF/hexane, but only with refined technique. So, the diastereomeric mixture was converted in statu quo to the S, N-deprotected β-phenylcysteine methyl ester hydrochloride (**3**) by treatment with trifluoromethanesulfonic acid followed by counterion exchange through a cloumn of anion-exchanger resin (90% yield), since the stereoisomers was separated more easily at the later stages. The threo isomer **3a** had lower solubility in 2-propanol than the erythro isomer **3b**, and readily obtained in pure form (mp 183-185°C). The isomer **3b** (mp 162-163°C) could be purified from the mother liquor residue by recrystallization from 2-propanol/ether. Both isomers were converted to the N, S-



Reagents a: reflux in C_6H_6 with TsOH, then CH_2N_2; b: $ArCH_2SH$ + NaOMe/MeOH; c: CF_3SO_3H/CH_2Cl_2, then anion exchanger resin; d: Me_2CO/MeOH, e: heat in 2N-HCl 3 min. (Ar=p-MeO-Ph)

Scheme 1

isopropylidene derivatives, **4a** and **4b**, by treatment with acetone (quantitatively). The isomer **4b** had lower solubility in hexane than the isomer **4a**, and easily obtained in pure form. By combination of the fractional recrystallization at the two stages, compounds **3** and **4**, both isomers were obtained in pure form. Pure **4a** and **4b** showed mps. of 39-41°C

Table 1. Physical Properties of the Thiazolidines

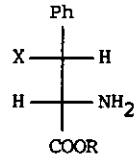
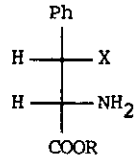
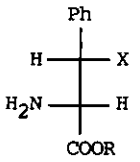
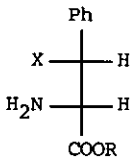
	trans (4a)		cis (4b)	
4R	r.t.	10.8 min.	r.t.	5.2 min.
	mp	oil	mp	92-93.5°
	$[\alpha]_D$	-41.4°(c 1.5)	$[\alpha]_D$	-55.9°(c 1.5)
4S	r.t.	5.8 min.	r.t.	13.2 min.
	mp	oil	mp	89-92.5°
	$[\alpha]_D$	+35.6°(c 1.7)	$[\alpha]_D$	+54.4°(c 1.5)

HPLC data: column Chiralcel-OJ (DAICEL) 4.6 mm diameter, 25 cm length; solvent EtOH 100%; flow rate 1.0 ml/min.; detection UV λ=254 nm. Specific rotations were determined in chloroform.

and 94-95°C, respectively.

Recently, a variety of chiral columns for HPLC are available commercially. Fortunately, both isomers, **4a** and **4b**, were found to be separated readily on a column of O-p-toluylcellulose (Chiralcel-OJ DAICEL) as seen from the big retention time difference (see Table 1). Thus, we had all of the four possible diastereomers of the compound **4** in hand. Their physical properties are summarized in Table 1. By heating with 2N hydrochloric acid for a few minutes, compound **4** isomers were converted to the corresponding chiral isomers of compound **3**. The chiral **3a** showed mp of 180-182°C while chiral **3b** was obtained as foams and failed to be crystallized. Their molecular rotation values are reproduced in Table 2 together with the data of β-phenylserine derivatives,

Table 2. Comparison of the Molecular Rotations of β-Phenylserine and β-Phenylcysteine Diastereomers

	threo (3a)		erythro (3b)	
L		X=OH $[M]_D$=-88° X=SH $[M]_D$=-63°		X=OH $[M]_D$=+147° X=SH $[M]_D$=+197°
D		X=OH $[M]_D$=+87° X=SH $[M]_D$=+59°		X=OH $[M]_D$=-148° X=SH $[M]_D$=-201°

For pheynylserines R=H and the rotations were determined in 6N HCl (ref. 8). For pheynylcysteines R=Me and the rotations were measured as The HCl-salts in MeOH (c 1.0).

for which assignment of the stereochemistry was established already[7]. Assignment of the stereochemistry of compound **3** isomers was carried out by comparison of the molecular

rotation data with those of β-phenylserine isomers. Since good correspondence was observed between the two series of compounds, the assignments seem to be reasonable (see Table 2).

It is interesting to note that the retention times of the thiazolidine compounds seem to reflect the absolute stereochemistry at 5-position where the phenyl group is attached, i.e. the 5S-isomer showed shorter retention times than the 5R-isomer in both cases indicating that the latters have the higher affinity to the toluylated cellulose stationary phase. This observation may be useful for the assignment of absolute stereochemistry of the compound separated using a similar system.

The method reported here is simple and seems useful for the preparation of chiral β-phenylcysteine stereoisomers, which would have potential utility as a peptide building block or a chiral synthon.

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