Synthesis, Stereochemistry and Conformational Properties of Diastereomeric Cyclic Dipeptides Containing Tetrahydro-1,4-thiazine-3,5-dicarboxylic Acid

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The synthesis of linear dipeptides containing *N*-protected L-phenylalanine and (3*R*-*cis*)-tetrahydro-1,4-thiazine-3,5-dicarboxylic acid dialkyl diester residues is described. *N*-Deprotection of these dipeptides by hydrogenolysis on palladium afforded directly a mixture of *cis* and *trans* dioxopiperazines. The stereochemistry and the solution conformational properties of the cyclic dipeptides are determined.

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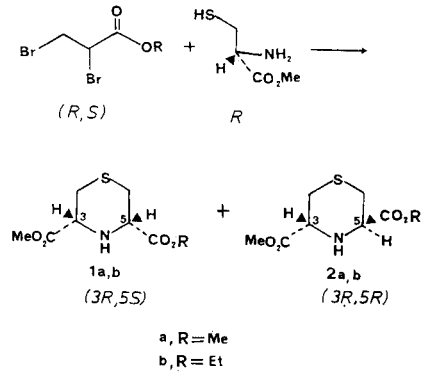
Although the physiologic role of tetrahydro-1,4-thiazine-3,5-dicarboxylic acid (Tht) is still largely unknown, the detection of this cyclic, sulfur containing imino acid in bovine brain and human urine [1] is of remarkable biological interest. Furthermore, due to its structural and chemical properties the incorporation of the Tht residue into synthetic linear and cyclic peptides should be of general interest. This residue possesses in fact, in addition to the cyclic side chain, which is bound to establish distinct conformational restrictions in the peptide backbone, two functional groups which can be involved in modifications of the properties of the primary peptide derivatives as well as in intra- and in inter-molecular interactions. As a prosecution of our studies on the synthesis and conformational properties of small cyclic peptides we report here some results concerning the synthesis of linear and cyclic dipeptides (dioxopiperazines; DOP's) containing the Tht residue as well as the stereochemistry and the solution conformation of the cyclic derivatives.

Recently Eremeev *et al.* reported the synthesis [2] and the absolute configuration [3] of the diastereoisomeric dimethyl diesters of Tht obtained by reacting methyl (*R,S*)-2,3-dibromopropionate with L-cysteine methyl ester (Scheme 1, R = Me). By using this reaction both the *cis* and the *trans* form were obtained and characterized after separation by high-performance liquid chromatography. These two diastereoisomers are described as uncrystallizable liquids which show $[\alpha]_D$ -70.2° (*c* 1.0, methanol) and $[\alpha]_D$ -8.3° (*c* 1.0, methanol). The authors [3] assign *trans* stereochemistry and *S,S* absolute configuration to the isomer with more negative optical rotation (-70.2°) by X-ray crystallographic analysis of its phenylthiohydantoine derivative. The *S,S* assignment, however, seems incorrect and should be changed into *R,R* since L-cysteine methyl ester corresponds to the Cahn-Ingold-Prelog *R*-isomer. The optical activity found by these authors for the *cis* (meso) form is not commented upon, although it can be due to contamination by the *trans* form.

Scheme 1



The reaction of methyl (*R,S*)-2,3-dibromopropionate with (*R*)-cysteine methyl ester, previously reported by Eremeev *et al.* [2], has been repeated by us (Scheme 1) by following an improved experimental procedure. We obtained the two diastereoisomeric imino diesters **1a** and **2a** in a higher overall yield (86%, lit 43% [2]) and in nearly equimolar ratio (lit **1a**/**2a** = 7/3 [2]) The two purified isomers were isolated as crystallizable solids; the meso (*cis*) form **1a** shows, as expected for a pure product, $[\alpha]_D$ 0° and melts at 43-44°. The *trans* isomer **2a** melts at 60° and shows $[\alpha]_D$ -73° (*c* 1.0, methanol). Thus, in view of the absolute configuration of the starting cysteine methyl ester, (*R,R*) configuration instead of (*S,S*) is confirmed for the (-) *trans* isomer **2a**.

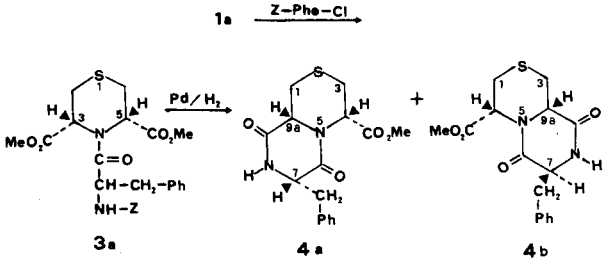
Reactivity of imino esters **1a** and **2a** in the coupling reactions with *N*-protected α-amino acids was tested by using *N*-benzyloxycarbonyl-L-phenylalanine (Z-Phe-OH) as the acidic component. Both the diastereoisomers **1a** and **2a** failed to react with Z-Phe-OH when the usual carbodiimide and mixed anhydride methods were employed to form the peptide bond. This result reflects the steric and electronic effects of the two carboxymethyl groups on the adjacent imino function. By using a more reactive acylating system, namely acid chloride/pyridine, the meso (*cis*)

form **1a** reacted with Z-Phe-Cl affording the desired dipeptide **3a** in moderate yield (40%) (Scheme 2). Under the same reaction conditions, the (*R,R*)-*trans* form **2a** was practically unreactive. It is noteworthy that the opposite trend of reactivity is found for the two diastereomers **1a** and **2a** in the reaction with phenylisocyanate and phenylisothiocyanate. In this case, as reported by Eremeev *et al.*, only the *trans* form **2a** reacts to give the corresponding hydantoine derivatives [3].

Although dipeptide **3a** was isolated as analytical pure material, its ^{1}H nmr spectrum appeared complex due to the hindered rotation around the peptide bond. Analogous isomerism has been observed in the case of the ^{1}H nmr spectra of *N*-acyl derivatives of proline and related cyclic imino acids [4].

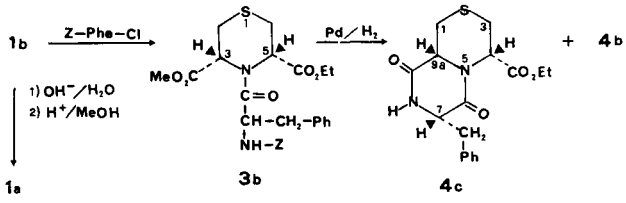
N-Deprotection of **3a** by palladium catalyzed hydrogenolysis in methanol or in dichloromethane-acetic acid (20:1) afforded directly two cyclic products which have been separated and identified as the isomeric bicyclic dioxopiperazines **4a** and **4b**. While the hydrogenolysis is a slow reaction and large amounts of catalyst are required due to the presence of the thioether group, the subsequent cyclization takes place spontaneously and the intermediate amino diester cannot be detected.

Scheme 2



In order to unequivocally assign the stereochemistry to the diastereoisomers **4a** and **4b**, the following chemical procedure has been devised: the chiral imino diesters **1b** and **2b**, containing different ester groups bound at C-3 and at C-5 (Scheme 1) have been prepared by treating (*R*)-cysteine methyl ester with ethyl (*R,S*)-2,3-dibromopropionate under the conditions already adopted to obtain **1a** and **2a**. The *cis* isomer **1b** was isolated and its stereochemistry, which is in accordance with its ^{1}H nmr spectrum, was confirmed by chemical correlation with the corresponding meso form **1a** (Scheme 3). The dipeptide **3b** was then synthesized starting from the *cis* isomer **1b** and Z-Phe-Cl. Deprotection of **3b** with palladium/hydrogen followed by separation of the reaction mixture, afforded two dioxopiperazines whose stereochemistry is revealed by the nature of the ester group which is present in each molecule. Thus 7-9a *cis* arrangement is to be assigned to the ethyl ester derivative **4c** which is formed by the intramolecular aminolysis of the COOMe bound at the 3(*R*) carbon atom of **3b**. Analogously, 7-9a *trans* arrangement can be assigned to the methyl ester derivative which is generated from the intramolecular aminolysis of the COOEt bound at the 5(*S*) carbon atom. This compound was found identical with the dioxopiperazine **4b** obtained from **3a**; the *cis* ethyl ester **4c**, on the other hand, showed spectral features almost identical to those of the corresponding methyl ester **4a**, to which 7-9a *cis* configuration is to be assigned.

Scheme 3



The two different solvent systems used to perform the palladium catalyzed deprotection of linear dipeptides **3a-b**, provided evidence of the influence that the nature of the solvent can exert on the product distribution. In particular it has been found that (Table 1) by effecting the deprotection in methanol, the formation of the 7-9a *trans* isomer **4b** is clearly preferred. An opposite trend is observed when dichloromethane-acetic acid is used as the solvent. In this case the discriminating effect of the solvent is particularly high and of preparative utility as it is shown by the almost exclusive formation of 7-9a *cis* derivative **4c** from **3b**.

Table 1
Yields of *cis*-**4a,c** and *trans*-**4b** dioxopiperazines

Starting dipeptide	Solvent	*cis*-DOP	*trans*-DOP
3a		**4a**	**4b**
	A	35	54
	B	56	9.2
3b		**4c**	**4b**
	A	17	60
	B	74	traces

A = Methanol; B = Dichloromethane/acetic acid (20:1).

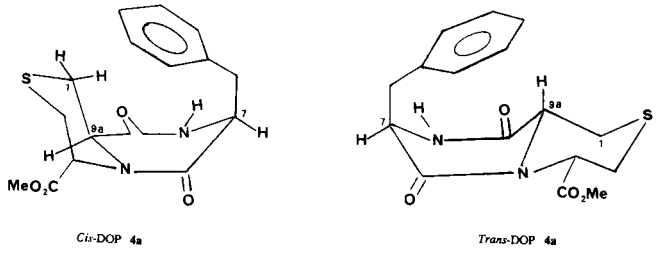
In order to gain information on the factors which control the product distribution reported in Table 1, the ^{1}H nmr spectra of the starting dipeptide **3a** in deuteriomethanol and in deuteriochloroform-deuterioacetic acid (20:1) have been examined. It has been found that in both solvents only two conformers are present in significant concentration; furthermore their relative population is different in the two cases. Whereas in deuteriomethanol the two rotamers are almost equally populated, in the other medium one form is clearly prevalent and accounts for about 75% of the total. These findings underline the rela-

tion between the rotameric distribution of the linear precursors and the final yields of dioxopiperazines. Thus, the high *cis*/*trans* ratio obtained in dichloromethane-acetic acid can be due to the stabilization by the solvent of the appropriate rotamer, as it is suggested by the ^{1}H nmr data.

The analysis of ^{1}H nmr data of the dioxopiperazines **4a** and **4b** shows that both diastereoisomers, 7-9a-*cis* and 7-9a-*trans*, adopt in deuteriochloroform folded conformations (Figure 1) which are similar to those described by Anteunis *et al.* [5] for analogous bicyclic dioxopiperazines containing the residue of phenylalanine and 1,4-thiomorpholine-3-carboxylic acid (Tmc). The most diagnostic feature in the spectrum of the *cis* diastereoisomer **4a** is the high field resonance of C-1 axial proton (δ 1.09) which falls in the shielding zone of the aromatic nucleus; a related less pronounced effect is observed on C-1 equatorial proton which resonates at δ 2.51. The corresponding CH_2S protons at C-3, which are not perturbed, resonate at δ 2.86 and 2.79. Furthermore the Phe α-CH signal occurs as an apparent triplet (after deuterium oxide exchange) with small gauche coupling constants. These data confirm the tendency of the aromatic side chain to fold on the dioxopiperazine ring.

The folding of the benzylic side chain in the case of the *trans* isomer **4b** is revealed by the H-9a resonance. This proton appears at δ 3.14 and is then sensibly shielded relative to the corresponding proton of the *cis* isomer **4a** ($\Delta\delta$ = 0.95 ppm). This shielding effect is stronger than that observed for the corresponding *trans* diastereomer of Tmc containing dioxopiperazines studied by Anteunis *et al.* [5] who found $\Delta\delta$ = 0.33 ppm. Furthermore the Phe α-CH of **4b** appears as a triplet (after deuterium oxide exchange), while the corresponding signal of the *trans* DOP containing the Tmc residue occurs as a double doublet [5].

On the basis of the above reported ^{1}H nmr data it can be concluded that both the isomeric dioxopiperazines **4a** and **4b** adopt analogous folded conformations in deuteriochloroform solution. On the other hand, in the case of cyclodipeptides containing the Phe and Tmc residues [5], the folding is more pronounced in the case of the *cis* isomer.



Cis-DOP **4a** *Trans*-DOP **4a**

Figure 1

EXPERIMENTAL

Melting points were determined with a Kofler hot stage apparatus and are uncorrected. Optical rotations were taken at 20° with a Schmidt-Haensch Polartronic D polarimeter in a 1 dm cell (*c* 1.0, chloroform, except for **1a** and **2a**). Infrared spectra were recorded with a Perkin-Elmer 983 spectrophotometer. The ^{1}H nmr spectra were measured with Varian EM-390 and XL-300 spectrometers using, unless otherwise specified, deuteriochloroform as the solvent (TMS as the internal standard). Merck silica gel 60 (230-400 mesh) (1:30) was used for column chromatography; preparative layer chromatography (plc) was carried out with Merck F_{254} silica gel. The drying agent was sodium sulfate. Light petroleum refers to 40-60° bp fraction.

Synthesis and Separation of Dimethyl (3*R*-*cis*)- and (3*R*-*trans*)-Tetrahydro-1,4-thiazine-3,5-dicarboxylates **1a**, **2a**.

All the reaction steps were performed under a nitrogen atmosphere. To a stirred solution of methyl (*R,S*)-2,3-dibromopropionate (1.31 ml, 10 mmoles) in dry ethanol (20 ml), cooled at 0°, dry triethylamine (1.4 ml, 10 mmoles) was added. The mixtures was stirred at 0° for 30 minutes. A solution of L-cysteine methyl ester hydrochloride (1.72 g, 10 mmoles) and dry triethylamine (2.8 ml, 20 mmoles) in dry ethanol (30 ml) was added dropwise. The resulting mixture was stirred at 70° for 8 hours, and then evaporated under reduced pressure. The residue was partitioned between ethyl acetate and water. The organic phase was dried and evaporated to give an oil, which was chromatographed on a column. Elution with dichloromethane-ethyl acetate (95:5 and 9:1) gave 1.891 g (86%) of the diastereomeric diesters **1a** and **2a** in nearly equimolar ratio (by ^{1}H nmr analysis). Plc [dichloromethane-ethyl acetate (8:2)] of column fractions containing mixtures of two isomers completed their separation. The less polar (Rf = 0.44) *cis* isomer **1a** had mp 43-44° (dichloromethane-light petroleum); $[\alpha]_D$ 0° (*c* 1.0, methanol), lit -8.3° [2]; ir (potassium bromide): 3443, 3325, 1742, 1219, 1205 cm^{-1}. The more polar (Rf = 0.32) *trans* isomer **2a** had mp 60° (dichloromethane-light petroleum); $[\alpha]_D$ -73° (*c* 1.0, methanol), lit -70.2° [2]; ir (potassium bromide): 3369, 1742, 1716, 1203 cm^{-1}. The ^{1}H nmr spectra of **1a** and **2a** were in agreement with literature data [2].

Synthesis of 5-Ethyl 3-Methyl (3*R*-*cis*)-Tetrahydro-1,4-thiazine-3,5-dicarboxylate (**1b**) and its (3*R*-*trans*)-Epimer **2b**.

Treatment of ethyl (*R,S*)-2,3-dibromopropionate (98%, 1.48 ml, 10 mmoles) with 10 mmoles of L-cysteine methyl ester hydrochloride, followed by usual work up and column chromatography as described for dimethyl diesters **1a** and **2a**, afforded 1.983 g (85%) of *cis* diester **1b** and its epimer **2b** in nearly equimolar ratio. Plc [dichloromethane-ethyl acetate (9:1)] of column fractions containing mixtures of two isomers completed the separation of **1b** from **2b**.

The less polar (Rf = 0.63) *cis* isomer **1b** had mp 34-34.5° (dichloromethane-light petroleum); $[\alpha]_D$ -2°; ir (potassium bromide): 3443, 3353, 3321, 1742, 1206 cm^{-1}; ^{1}H nmr: δ 1.28 (3H, t, J = 7 Hz, CH_3-CH_2-O), 2.75 (4H, m, two CH_2S), 3.72 (2H, m, two CH-CO_2), 3.78 (3H, s, O-CH_3), 4.24 (2H, q, J = 7 Hz, O-CH_2-CH_3).

The more polar (Rf = 0.46) *trans* isomer **2b**, obtained as an amorphous solid, had $[\alpha]_D$ -67°; ir (potassium bromide): 3450, 3370, 1737, 1714, 1201 cm^{-1}; ^{1}H nmr: δ 1.29 (3H, t, J = 7 Hz, CH_3-CH_2-O), 2.87 (4H, m, two CH_2S), 3.79 (3H, s, O-CH_3), 4.04 (2H, m, two CH-CO_2), 4.24 (2H, q, J = 7 Hz, O-CH_2-CH_3).

Conversion of Ethyl Methyl Diesters **1b** and **2b** into Dimethyl Diesters **1a** and **2a**.

The diester **1b** or **2b** (0.233 g, 1 mmole) was dissolved in methanolic 2% sodium hydroxide (8 ml) and stirred at room temperature for 4 hours. After evaporation of methanol under reduced pressure without heating, water and ether were added. The alkaline aqueous phase was acidified with 4*N* hydrochloric acid (until pH reached 2), cooling at 0°, and evaporated under *vacuum*, heating at 50°. The solid residue was refluxed in a solution of dry methanol (8 ml), containing 0.2 ml of concentrated sulfuric acid, for one day. After evaporation of methanol under reduced pressure without heating, saturated aqueous sodium bicarbonate and ether in excess were added. The organic phase was washed with water to neutrality, dried and evaporated to give a solid residue. Final plc [dichloromethane-ethyl acetate (8:2) as eluant] afforded pure dimethyl ester **1a** or **2a**. The optical rotations and the ir and ^{1}H nmr spectra were identical to those of the earlier characterized samples. No significant epimerization reactions were evidenced during these transformations.

Synthesis of *Z*-Phe-(3*R*-*cis*)-Tht(OMe)-OMe **3a**.

A mixture of *Z*-Phe-OH (0.632 g, 2.11 mmoles) and phosphorus pentachloride (0.44 g, 2.11 mmoles) in dry ether (18 ml) was stirred at 0° for 20 minutes and then added to a solution of *cis* diester **1a** (0.385 g, 1.76 mmoles) in dry pyridine (1.8 ml). The mixture was stirred at room temperature for 3 hours, then poured into ice-water and extracted with ether; the organic layers were washed with 2*N* hydrochloric acid, saturated aqueous sodium bicarbonate and water, dried and evaporated. The residue was chromatographed on a column, eluting with dichloromethane and dichloromethane-ethyl acetate (9:1). Plc [dichloromethane-ethyl acetate (9:1) as eluant] of nearly homogeneous column fractions afforded pure title dipeptide **3a** (0.354 g, 40%) as a foam; Rf = 0.64; $[\alpha]_D$ -32°; ir (chloroform): 3431, 1748, 1721-1713, 1650, 1501 cm^{-1}; the ^{1}H nmr spectrum [deuteriochloroform/deuterioacetic acid (20:1)] was complex and indicated that it mainly consisted of a mixture of two rotational isomers in about 3/1 ratio: δ (major conformer) 1.55 and 2.70 (2H, dd at 1.55, J = 5 and 14 Hz and m at 2.70, CH_2-S), 2.37 and 2.98 (2H, dd, at 2.37, J = 4 and 14 Hz and m at 2.98, CH_2-S), 2.86 and 3.20 (2H, two poorly resolved dd, Phe β-CH_2), 3.55 and 3.66 (6H, two s, two O-CH_3), 4.24 (1H, m, C*H*-CH_2-S coupled with the methylenic protons which resonate at 1.55 and 2.70), 5.10 and 5.17 (2H, AB q, J = 12 Hz, Ph-C*H*$_2$-O), 5.22 (1H, m, Phe α-CH), 5.35 (1H, t, J = 4 Hz, C*H*-CH_2-S coupled with the methylenic protons which resonate at 2.37 and 2.98), 7.20-7.40 (10H, m, aromatic).

Anal. Calcd. for $C_{25}H_{28}N_2O_7S$: C, 59.98; H, 5.64; N, 5.60. Found: C, 59.98; H, 5.67; N, 5.40.

Synthesis of *Z*-Phe-(3*R*-*cis*)-Tht(OEt)-OMe **3b**.

Treatment of **1b** (0.404 g, 1.73 mmoles) with *Z*-Phe-Cl as described for **1a**, followed by usual work up and chromatographic purification, gave pure oily dipeptide **3b** (0.335 g, 38%); Rf = 0.72 [dichloromethane-ethyl acetate (9:1)]; $[\alpha]_D$ -43°; ir (chloroform): 3430, 1746, 1721-1713, 1649, 1500 cm^{-1}; apart from the occurrence of the ethyl ester signals in place of those relative to one of the two methyl ester groups, the ^{1}H nmr pattern of **3b**, in deuteriochloroform-deuterioacetic acid, is very similar to that of **3a**.

Anal. Calcd. for $C_{26}H_{30}N_2O_7S\cdot\frac{1}{2}\ H_2O$: C, 59.64; H, 5.97; N, 5.35. Found: C, 59.25; H, 5.94; N, 5.11.

General Procedure for the Synthesis of Bicyclic Dioxopiperazines **4a-c**.

A solution of *N*-protected dipeptide **3a** or **3b** (0.5 mmole) in methanol (20 ml) or in dichloromethane (20 ml) containing acetic acid (1 ml) was subjected to catalytic hydrogenolysis, using 10% palladium on alumina as catalyst (0.15 g). Additional portions of catalyst (0.15 g) were carefully added for three times every 90 minutes. After 24 hours from beginning, catalyst was removed by filtration through Celite and the solution evaporated under reduced pressure. Plc (ethyl acetate as eluant) allowed the separation of homogeneous *cis* and *trans* dioxopiperazines.

The *cis* methyl ester derivative **4a** had Rf = 0.34; mp 78° (ether); $[\alpha]_D$ -31°; ir (potassium bromide): 3480, 3240, 1745, 1685-1666, 1452 cm^{-1}; ^{1}H nmr: δ 1.09 (1H, apparent t, axial 1-H), 2.51 (1H, dd, J_{vic} = 2.5 Hz, J_{gem} = 13 Hz, equatorial 1-H), 2.79 and 2.86 (2H, A and B of an ABX system, J_{vic} = 2,5 and 10 Hz, J_{gem} = 14 Hz, equatorial and axial protons at C-3), 3.05 and 3.33 (2H, A and B of an ABX system, J_{vic} = 5 and 4 Hz, J_{gem} = 14 Hz, Phe β-CH_2), 3.79 (3H, s, OCH_3), 3.93 (1H, X of an ABX system, J = 2.5 and 10 Hz, 4-H), 4.09 (1H, dd, J = 2.5 and 12 Hz, 9a-H), 4.41 (1H, m, simplified to an apparent t by exchange with deuterium oxide, Phe α-CH), 7.23-7.44 (6H, m, aromatic and NH).

Anal. Calcd. for $C_{16}H_{18}N_2O_4S\cdot\frac{1}{4}\ H_2O$: C, 56.70; H, 5.50; N, 8.27. Found: C, 56.84; H, 5.58; N, 7.91.

The *trans* methyl ester derivative **4b**, obtained as a foam, had Rf = 0.59; $[\alpha]_D$ -53°; ir (chloroform): 3391, 1753, 1688-1668, 1435 cm^{-1}; ^{1}H nmr: δ 2.79 and 3.01 (2H, A and B of an ABX system, J_{vic} = 3.5 and 5.5 Hz, J_{gem} = 14 Hz, 3-H_2), 2.85 (1H, apparent t, axial proton at C-1), 3.05-3.28 (4H, complex m, Phe β-CH_2, equatorial proton at C-1 and 9a-H at 3.14), 4.38 (1H, m, simplified to an apparent t by exchange with deuterium oxide, Phe α-CH), 4.79 (1H, dd, J = 3.5 and 5.5 Hz, 4-H), 7.13-7.38 (6H, m, aromatic and NH).

Anal. Calcd. for $C_{16}H_{18}N_2O_4S\cdot\frac{1}{4}\ H_2O$: C, 56.70; H, 5.50; N, 8.27. Found: C, 56.46; H, 5.37; N, 7.96.

The *cis* ethyl ester derivative **4c** had Rf = 0.46; mp 165-167° (ethyl acetate-ether); $[\alpha]_D$ -46°; ir (potassium bromide): 3318, 3291, 1721, 1691, 1659, 1452 cm^{-1}; ^{1}H nmr: except for the occurrence of the ethyl ester signals in place of the resonance of methyl ester group, the ^{1}H nmr pattern of **4c** is very similar to that of the *cis* dioxopiperazine **4a**.

Anal. Calcd. for $C_{17}H_{20}N_2O_4S$: C, 58.60; H, 5.79; N, 8.04. Found: C, 58.66; H, 5.84; N, 8.10.

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