S-p-Methylbenzyl-β-phenylcysteine: A Potential Tool for Probing Receptor Topologies

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erythro and *threo N*-Acetyl-*S-p*-methylbenzyl-β-phenyl-DL-cysteine methyl esters were obtained by addition of *p*-methyltoluene-α-thiol to 4-benzylidene-2-methyloxazol-5-one in methanol–tetrahydrofuran under basic conditions. The diastereoisomers were separated as their methyl esters by fractional crystallization and the relative configuration assigned by X-ray crystallography. Both diastereoisomers were converted into their *N*-trifluoroacetyl derivatives and resolved using Carboxypeptidase A. Hydrazinolysis of the unchanged *N*-trifluoroacetyl amino acids gave the other enantiomers of the free amino-acids. Optical purity was determined using $Eu(hfc)_3$ chemical shift reagent on *N*-trifluoroacetyl-D-amino acid methyl ester by both ^1H and ^{19}F NMR spectra. We discuss the conformation of this unique amino acid based on X-ray data and molecular mechanics calculations. Its usefulness in probing the opiate receptor site is also demonstrated.

The biological response elicited by peptide hormones and neurotransmitters is usually mediated by interactions with specific receptors.[1] The magnitude and specificity of such interactions are governed by the primary and secondary structure of the peptide, which combine inextricably to generate a molecular arrangement that is recognized by the receptor, and by complementarity, should reflect the topography of the active site of the receptor.[2]

Structural modifications of the peptide caused by C^α inverted configurations, isosteric replacement of the functional groups, altered side chain hydrophobicity, or intramolecular cyclization[3] may serve to stabilize or perturb the native conformation. Accordingly, subtle modulations in the peptide–receptor complex may manifest increased efficacy, antagonist properties or enhanced selectivity in situations of receptor heterogeneity.

Cysteine serves a specialized function in native peptides by engaging in disulfide bridge formation. This property serves to juxtapose discontiguous functional elements, to stabilize active conformations or impart enhanced proteolytic resistance.[4] Hruby and co-workers[5] have shown that penicillamine can substitute for cysteine but exerts strong influences on peptide conformations owing to the *gem*-dimethyl group.

On the other hand, phenylalanine possesses a β aromatic ring which is a critical element in the binding of several ligands to their receptor (*e.g.* enkephalin, substance P). The flat aromatic ring can interact with hydrophobic microenvironments in the receptor and may exhibit favourable interactions with disulfide bonds.[6]

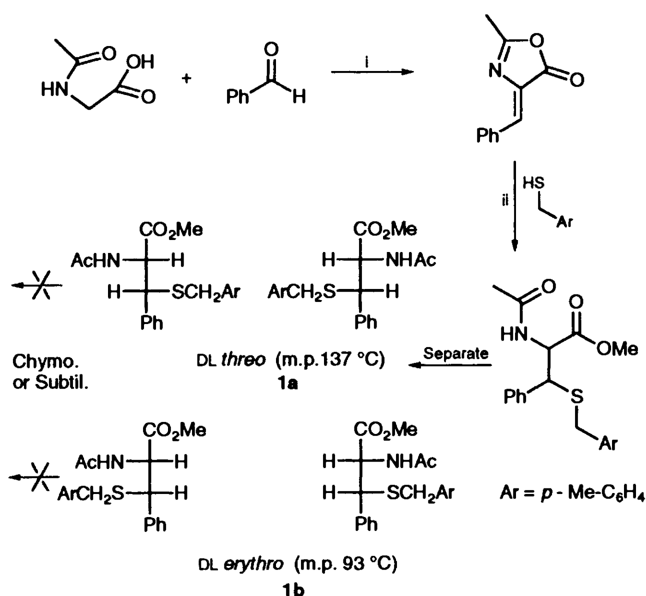
β-Phenylcysteine combines the properties of these two naturally occurring amino acids but possesses an additional asymmetric centre at the β carbon which may be useful in deciphering stereochemical preferences should the aryl group engage in receptor interactions. In this regard, Mosberg *et al.*[7] have reported the effect on opioid receptor interactions exerted by an enkephalin analogue incorporating *threo* or *erythro* β-methyl-D-cysteine.

Previous preparations of β-phenyl-DL-cysteine include the synthesis by Svoboda *et al.*[8] which led to the *erythro* diastereoisomer and the one by Holland and Mamalis[9] which gave the *threo* isomer. These two methods were reinvestigated recently in a search for new acetaldehyde sequestrating agents.[10] Chiral forms of β-phenylcysteine were independently obtained by two teams. Ploux *et al.*[11] based their approach on the regio- and stereo-controlled opening of 2-phenyl-3-menthoxycarbonylaziridine by 4-methoxytoluene-α-thiol. They however experienced difficulty in obtaining all four aziridines in enantiomerically pure form. Nagai and Pavone[12] prepared the *erythro* and *threo* DL-amino acid by the 1,4 addition of *p*-methoxytoluene-α-thiol to *N*-Cbz-dehydrophenylalanine. After N–S deprotection, they formed the acetonide which was the basis of diastereoisomeric separation by crystallization and resolution by way of a preparative chiral support. This communication reports the synthesis and enzymatic resolution of all the four possible enantiomers of *S-p*-methylbenzyl-β-phenylcysteine. The X-ray structure determination was performed for *threo N*-acetyl-*S-p*-methylbenzyl-β-phenyl-DL-cysteine methyl ester **1a** in order to assign unambiguously the correct relative stereochemistry. Since this amino acid would be eventually incorporated in biologically active peptides and linked through a disulfide bridge to another thiol containing amino acid, we have also performed molecular mechanics calculations on model structures. The conformational preferences for the side chain in relation to various backbone conformations were determined and their compatibility with previously proposed active conformations for opioid peptides was evaluated.

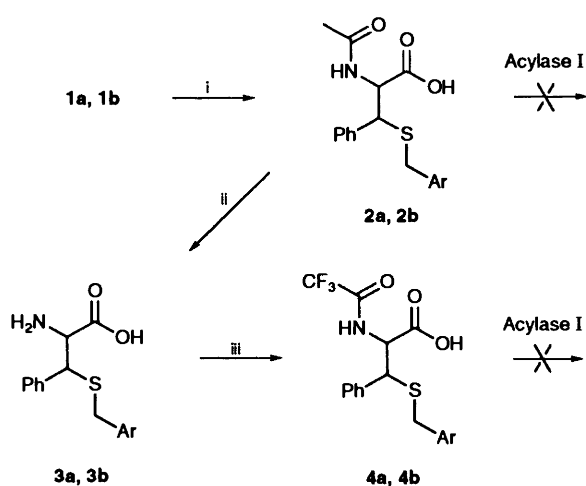
Results

Synthesis and Resolution.—The method we have adopted is a variation of the procedure developed originally by Nicolet[13] and extended later by Carter *et al.*[14] This method relies on the facile addition of thiols to unsaturated azlactones (see ref. 15 for a review of azlactone chemistry). Accordingly, 4-benzylidene-2-methyloxazol-5-one was treated with sodium *p*-methyltoluene-α-thiol in methanol–tetrahydrofuran (MeOH–THF) affording a 1:1 *erythro–threo* mixture of *N*-acetyl-*S-p*-methylbenzyl-β-phenyl-DL-cysteine methyl ester as a consequence of the opening of the saturated azlactone by the methanol in the medium (Scheme 1).

Separation of diastereoisomers proved to be very straightforward at this stage. Dissolution of the crude reaction mixture in boiling ethanol and cooling to room temperature gave one diastereoisomer in an analytically pure form and whose crystals were suitable for X-ray structure determination. The *threo* relative configuration was assigned to this diastereoisomer.



Scheme 1 *Reagents and conditions:* i, NaOAc–Ac_2O, reflux, 2 h; ii, NaOMe (cat.)–THF, MeOH, room temp., 3 h



Scheme 2 *Reagents and conditions:* i, NaOH (1.1 equiv., 0.15 mol dm^{-3}), H_2O–MeOH (5:2), reflux, 2 h; ii, N_2H_4, 60 °C, 15 h; iii, $(CF_3CO)_2O$, CF_3CO_2H, 0 °C, 10 min

Subsequent cooling of the ethanolic solution to 4 °C provided another crop of the *threo* isomer, usually contaminated with a minor amount of the *erythro* isomer. When the remaining mother liquor was evaporated to dryness and redissolved in carbon tetrachloride–pentane and cooled at −20 °C, the pure *erythro* diastereoisomer was obtained as shown by NMR spectroscopy.

Having in hand the two pure diastereoisomers, attempts to resolve the racemic mixtures using α-chymotrypsin and subtilisin were abortive for either diastereoisomer (Scheme 1). Attempts to increase the substrate binding by replacing the *N*-acetyl group with *N*-benzoyl also proved unsuccessful. Even though these trials had to be conducted in solutions rich in organic solvent (50% of methanol or dioxane in water), the inertness of these substrates may be attributed to bulky β branching.[16] It is noteworthy that only the *erythro* diastereoisomer of β-*p*-nitrophenylserine methyl ester is hydrolysed by α-chymotrypsin.[17]

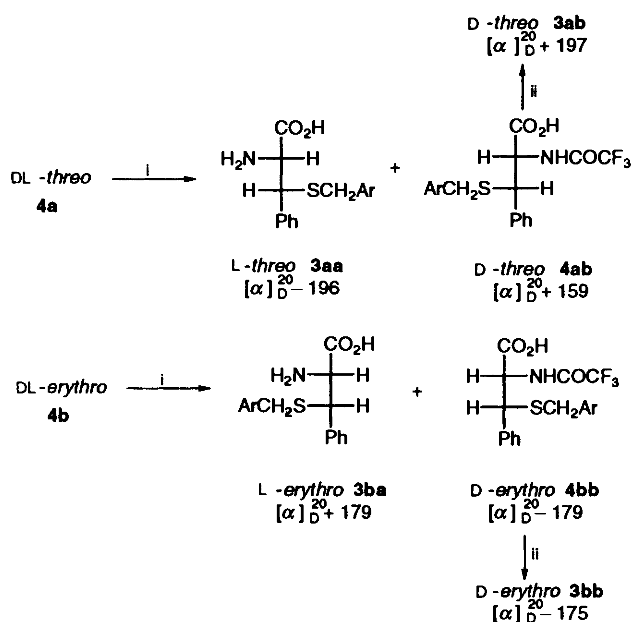
Hog renal acylase I was attempted as the next choice. Owing to the acid lability of the *p*-methylbenzyl group,[18] the methyl esters group could not be removed by acid hydrolysis. Alternatively, saponification using NaOH (0.1 mol dm^{-3}) in THF–MeOH–water mixture (1:3:1) at room temperature caused complete epimerization which could be suppressed using 2:5 methanol–water binary solvent at reflux for 2 h. The pure acids as revealed by NMR could be isolated in good yield. Both diastereoisomers, however, were resistant to the action of acylase I. Similarly, *N*-acetyl-β-phenylserine[19] and *N*-acetyl-*S*-benzylpenicillamine[20] are not attacked by acylase I.

The *N*-acetyl group was then exchanged for trifluoroacetyl with the hope that the more electrophilic carbonyl would be more susceptible to attack by enzyme. The *N*-acetyl group was removed by treatment with hydrazine at 60 °C for 15 h[21] and the crude free amino acid reacylated with trifluoroacetic anhydride in trifluoroacetic acid at 0 °C[22] in 66% overall yield for the two steps. The acids were carefully converted into their water soluble lithium salts and subjected to acylase I hydrolysis. However, the enzyme was incapable of cleaving the activated amide. This part of the work is summarized in Scheme 2.

When these trifluoroacetamides were incubated with Carboxypeptidase A (CPA) at a concentration of 0.01 mol dm^{-3},[23] slow amidolysis was observed for both diastereoisomers. The resolution was conveniently monitored by following the attenuation of the peak corresponding to the starting material by high performance liquid chromatography (HPLC) at 254 nm. The peak area decreased steadily until half of the consumption was reached whereupon it remained essentially constant for a prolonged period (1 week). Typically, 10 000 units of CPA were required to hydrolyse 10 mmol of substrate in 48 h at 20 °C. The HPLC measurements also permitted us to evaluate the relative reaction rates: (*i*) The rate was doubled when the amount of enzyme was increased by a factor of two, and this was independent of enzyme batches. (*ii*) No significant difference in the rates of hydrolysis was observed between the two diastereoisomers. In here, we are meeting the reasonable assumption that all the L-*threo* or L-*erythro* isomers were hydrolysed by the enzyme, and that the D-isomers were not acceptable substrates of CPA.

The unchanged *N*-trifluoroacetyl-D-enantiomers were isolated by acidification and diethyl ether extraction while the L-zwitterions, which were very slightly soluble in water, were precipitated almost quantitatively by concentration of the aqueous phase. The final removal of the trifluoroacetyl of the D-isomer could not be affected using the conditions 20% acetic acid reflux 4 h, described by Fones for β-phenylserine[19] and hydrazinolysis was again used for this reaction. The resistance of the trifluoroacetyl group to acidic hydrolysis can be explained by the absence of a proximate nucleophilic OH group which can promote the acid hydrolysis in the case of β-phenylserine. The resolution sequence and the related optical rotation values are given in Scheme 3. The optical rotation obtained for each enantiomorph was of the same sign as reported for β-phenylserine [*i.e.* (−)L-*threo* and (+)L-*erythro*] *in acidic medium,*[19] β-phenylcysteine methyl ester,[12] and *N*-*t*-Boc-*S*-3-nitro-2-pyridylsulfenyl-β-phenylcysteine.[11]

Since optical rotation is not a criterion for optical purity, the resolved compounds were analysed by NMR. Firstly, the racemic trifluoroacetamides **4a** and **4b** were esterified with diazomethane and then mixed with chemical shift reagent up to a molar ratio of 1:0.3 amino acid–$Eu(hfc)_3$. At this ratio the ^1H NMR spectrum showed considerable changes. The doublet of doublet (dd) resonance corresponding to $C^{\alpha}H$ reverted to a six line multiplet, while the $C^{\beta}H$ doublet and the methyl ester singlet were doubled. The *S*-benzylic CH_2AB system also experienced splitting. The ^{19}F spectrum also showed two lines for the CF_3 absorption. When this experiment was performed with the resolved compounds at the same chemical shift reagent concentration, no splitting could be detected, indicating an optical purity of at least 95%.



Scheme 3 *Reagents and conditions:* i, substrate conc. 0.01 mol dm^{-3} in water, LiOH (1.0 equiv.), LiCl (0.1 mol dm^{-3}), pH 7.5. 20 °C, CPA; ii, N_2H_4, 60 °C, 15 h

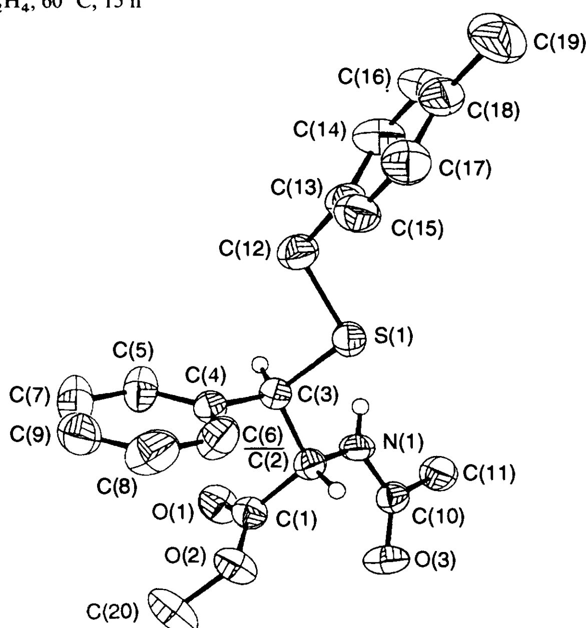


Fig. 1 ORTEP plot of **1a**

X-Ray Structure and Conformational Analysis.—A perspective view of the crystal structure of *threo* *N*-acetyl-*S*-*p*-methylbenzyl-L-phenyl-DL-cysteine methyl ester **1a** is shown in Fig. 1 for the L-enantiomer. The acetylated amino acid adopts a semi extended backbone conformation where torsional angles φ and ψ[24] adopt values of −61 and 139° respectively (Table 4). This backbone conformation gives rise to an infinite H-bond chain along the crystallographic *Z* axis where the NH (x, y, z) is hydrogen bonded to the amide carbonyl O(3) of the mirror image molecule $(x, 0.5 - y, 0.5 - z)$. Respective distances are: N ··· O(3) 2.951(2); H ··· O(3), 2.15(2) Å. The three atoms N ··· H ··· O(3) form an angle of 165(2)°. The angle between the planes of hydrogen bonded amides is 80°.

One important feature of this unique amino acid is the side chain conformation. The sulfur atom adopts the g^- orientation relative to the nitrogen atom (dihedral angle $\chi_1 = -67°$), corresponding to one of the two side chain conformations (g^-, g^+) generally found in cysteine or cystine derivatives.[25] The orientation of the β phenyl ring is *trans* (*t*) with respect to the nitrogen atom. By comparison, all staggered rotamers (g^-, *t*, g^+) have been observed by X-ray diffraction studies of phenylalanine derivatives.[26] Several *threo* β-phenylcysteine derivatives have been analysed by X-ray diffraction in our laboratory and all have shown this side chain arrangement.[27]

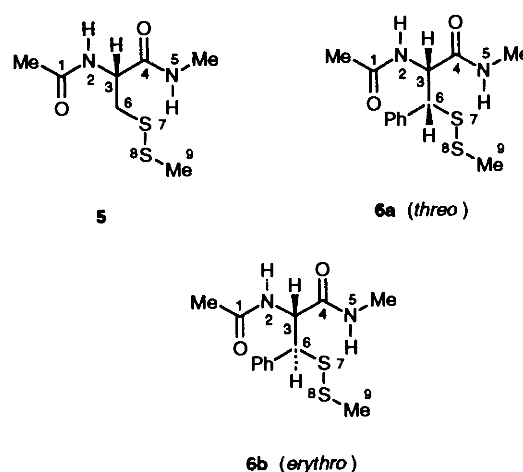


Fig. 2 Model molecules used in energy calculation. Variable torsional angles were φ: 1, 2, 3, 4; ψ: 2, 3, 4, 5; χ_1: 2, 3, 6, 7; χ_2: 3, 6, 7, 8; χ_3: 6, 7, 8, 9.

In order to evaluate the conformational repercussions induced by the β phenyl ring in an eventual disulfide bridge, we have performed a systematic conformational analysis by molecular mechanics on models structures **5**, **6a** and **6b** shown in Fig. 2. We have considered the five most stable backbone conformations A, C, D, E and F following the code of Zimmerman *et al.*[28] to identify the (φ, ψ) map local energy minima. These five backbone conformations were combined with 18 side chain orientation (corresponding to combination of g^+, *t*, g^- for χ_1, g^+, *t*, g^- for χ_2 and +90, −90° for χ_3). The resulting ninety conformers were energy minimized. From energy difference observed for each backbone conformations, the side chain rotamer populations were calculated at T = 300 K (see experimental section). Table 1 gives the calculated relative populations.

In the case of **5**, the χ_1 population is evenly distributed for backbone A and D. The g^- rotamer constitutes half of the population for backbone C and F while the g^+ rotamers is the most populated for an extended backbone (E). The *t* rotamer is nevertheless observed with all backbone conformations.

For compound **6a** (*threo*), the χ_1 population is also evenly distributed for a D backbone, but this time the g^- rotamer is strongly favoured for a C and F backbone and shows an important contribution for backbone E and D. The X-ray structure corresponds to the F backbone conformation associated with the same g^- rotamer of the side chain. The *t* rotamer is highly preferred for an A backbone and has an important contribution for the E conformation. The g^+ rotamer is only found to an appreciable extent with a D backbone conformation. Therefore, it appears for structure **6a** that the g^- rotamer is highly populated for four backbones. This is corroborated by the large (8.5 Hz) coupling constant measured between H^α and H^β in [$^2H_6$]dimethyl sulfoxide ([$^2H_6$]DMSO) for **1a** (H^α and H^β are *t* when N and S are g^- (Fig. 3). This overall tendency of the g^- rotamer for the χ_1 torsional angle may be explained in terms of reduced steric effects. In fact there are two gauche interactions with the g^- rotamer while there are three with the *t* or g^+ rotamers (Fig. 3).

In the case of compound **6b** (*erythro*), the χ_1 population analysis reveals that the *t* rotamer is favoured with a C or D

Table 1 Calculated relative side chain rotamer populations as a function of backbone conformations for model molecules **5**, **6a** and **6b** (expressed in %)

Compound (backbone)[a]	Boltzmann probability							
	χ_1			χ_2			χ_3	
	g^+	t	g^-	g^+	t	g^-	+90°	−90°
5 (A)	31	37	32	33	34	33	51	49
(C)	9	35	56	32	36	32	51	49
(D)	36	20	44	29	35	36	47	53
(E)	68	19	13	38	26	36	52	48
(F)	14	34	52	35	31	34	52	48
6a (A)	5	77	18	21	79	0	63	37
(C)	10	0	90	7	83	10	61	39
(D)	44	18	38	14	83	3	63	37
(E)	11	39	50	20	80	0	68	32
(F)	2	10	88	25	74	1	64	36
6b (A)	13	27	60	2	71	27	42	58
(C)	3	97	0	3	91	6	31	69
(D)	4	66	30	2	91	7	37	63
(E)	71	10	19	0	73	27	34	66
(F)	56	34	10	0	89	11	35	65

[a] Conformation is described according to φ, ψ Zimmerman *et al.* code letter:[28] Average torsion angles (°) were: A(−63 ± 8, −41 ± 6), C(−72 ± 3, 69 ± 4), D(−145 ± 10, 59 ± 10), E(−154 ± 10, 160 ± 8), F(−79 ± 9, 154 ± 10); χ_1 and χ_2 side chain torsion angles (°) were $g^-(-60 \pm 10)$, $g^+(+60 \pm 10)$, $t(180 \pm 10)$

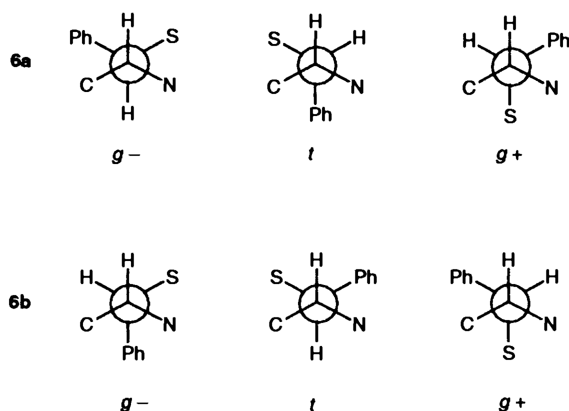


Fig. 3 Newmann projections along the C^α–C^β axis (χ_1) for the three staggered rotamers of compounds **6a** and **6b**

backbone conformation while the g^+ rotamer is favoured for an E or F backbone. The g^- rotamer is the most favoured when the backbone adopts an A conformation. It is more difficult in this case to assess an overall preference for the χ_1 dihedral angle. The Newmann projections (Fig. 3) show that there are two gauche interactions in the t rotamer while there are three in the g^+ and g^- rotamers. However, up to now, we have been unable to obtain suitable crystals of the *erythro* diastereoisomer for an X-ray diffraction study, but a large coupling constant (10.0 Hz) between H^α and H^β is also observed in [$^2H_6$]DMSO which seems to indicate a predominance of the t rotamer in this medium (H^α and H^β are t when N and S are t).

The distribution over χ_2 is much more influenced by the introduction of the β phenyl substituent. While all χ_2 rotamers were equally populated for model compound **5** independently of the backbone conformation, the t rotamer is much more favoured for both **6a** and **6b**. A small population of g^+ and g^- rotamer however persists for **6a** and **6b** respectively.

Finally, while both conformations were equally probable for χ_3 with compound **5**, slight preference (60:40) for one of the two rotamers appears with compounds **6a** and **6b** and is related to the chirality of the neighbouring C^β stereogenic centre (+90 for **6a** and −90° for **6b**).

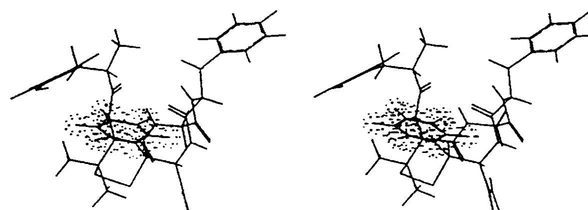


Fig. 4 Proposed active conformation for the δ selective opiate Tyr-D-Pen-Gly-Phe-Cys-NH_2[27] allowing for the insertion of a *erythro* phenyl-L-cysteine in place of L-Cys. Van der Waal surface corresponding to 0.5 times the actual radii is shown for the newly inserted aromatic ring.

Discussion

The present method for obtaining all four isomers of β-phenylcysteine has the advantage over previous methods of being suitable for large scale preparation since no chromatography is required. All purifications and separations are performed by extraction and crystallization. Enzymatic resolution is efficient and low cost especially using immobilized enzyme preparation.

N-Trifluoroacetyl-*S*-*p*-methylbenzyl-β-phenylcysteine is one of the largest amino acids to be hydrolysed by CPA. The limit of structural tolerance was investigated by preparing the corresponding *S*-α-naphthylmethyl derivative **4** (Ar = α-CH_2–$C_{10}H_7$) using similar methodology. Neither diastereoisomer was hydrolysed by CPA. These observations might be helpful in delineating the size of the dead end pocket allotted to the side chain of the amino acid hydrolysed by CPA.

Compared to cysteine or β-phenylalanine, β-phenylcysteine possesses a rigidified side chain when engaged in a disulfide bridge (*vide supra*). Therefore, if the β-aromatic ring is involved in specific binding with a putative receptor molecule, dramatic effects should be expected depending on the diastereoisomer chosen. In order to demonstrate the potential interest in using the β-phenylcysteine residue in the context of probing receptor sites, we have incorporated this particular residue with its predicted most stable conformation into a potential opiate ligand. This simulation is to be related to our preceding paper[29] on the search for the active conformation of the δ selective opiate Tyr-D-Pen-Gly-Phe-Cys-NH_2. The cysteine adopts a D backbone conformation and χ_1 and χ_2 were both *trans* which is compatible with the preferred conformation of an *erythro*-phenyl-L-cysteine (see Table 1). The peptide substituted with an *erythro*-phenyl-L-cysteine is represented in the proposed conformation in Fig. 4. Examining this structure, we observed that the newly inserted phenyl ring creates an interaction with the putative binding subsite associated with the tyramine moiety. Progress in preparing this peptide is under way, and the repercussion on binding properties will be investigated.

Experimental

Equipment.—Melting points were recorded on a Gallenkamp capillary apparatus and are uncorrected. In some cases, epimerization of diastereoisomer occurred upon slow heating; a special note is indicated in the text in this case. This behaviour was ascertained by running an NMR spectrum before and after heating the sample to complete fusion. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR and were taken as KBr pellets. ^1H, ^{19}F and ^{13}C NMR were recorded at 300, 282 and 75 MHz respectively on a Varian XL-300 and are referenced to the

residual solvent peak ($CDCl_3$ ^1H 7.24 ppm, ^{13}C 77.0 ppm, CD_3OD, ^1H 3.30 ppm, ^{13}C 49.0 ppm, [$^2H_6$]DMSO, ^1H 2.49 ppm, ^{13}C 39.5 ppm); *J*-values are given in Hz. Thin layer chromatography was carried out on glass backed Merck silica gel 60, 0.25 mm thickness. Optical rotations were measured on a JASCO DIP-140 instrument at sodium line wavelength at 20 °C in a 0.5 dm cell and the values were integrated over a period of 60 s; $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Mass spectra and exact mass were determined with a ZAB-1F mass spectrometer. Enzymatic reactions were monitored using a HP 1090M analytical HPLC equipped with an automatic injector and a 15 cm μ Bondapack reversed phase C_{18} column.

Reagents and Solvents.—*p*-Methyltoluene-α-thiol was prepared from the corresponding 4-methylbenzyl chloride using the Bunte's salt methodology (reaction with sodium thiosulfate and treatment with hot dilute sulfuric acid).[30] The material so obtained was distilled (b.p. 97 °C 12 mmHg) and was essentially odourless. *N*-Acetylglycine and 4-benzylidene-2-methyloxazol-5-one were obtained according to the procedures already published in *Organic Synthesis*.[31] The azlactone can be used without further purification but recrystallization from chloroform at 4 °C facilitated purification at the next step. Methanol was dried by distillation over magnesium and tetrahydrofuran over sodium–benzophenone. Trifluoroacetic acid (TFA) and trifluoroacetic anhydride were from Aldrich and were used without further purification. Carboxypeptidase A Type I was obtained from Sigma, 4 cm^3 of suspension accounting for 5000 units of enzymatic activity.

Preparation of threo *and* erythro N-*Acetyl*-S-p-*methylbenzyl*-β-*phenylcysteine Methyl Ester* **1a** *and* **1b**.—Into a dry 1 dm^3 three neck flask equipped with a 500 cm^3 dropping funnel under positive argon pressure was added dry methanol (150 cm^3). The solvent was cooled to 0 °C and freshly cut sodium (0.5 g) was added with vigorous stirring. When all the sodium had reacted, *p*-methyltoluene-α-thiol (24.9 g, 180 mmol) was added using a syringe. The mixture was then allowed to reach room temperature and the addition funnel was filled with a solution of 4-benzylidene-2-methyloxazol-5-one (33.4 g, 178 mmol) in dry THF (400 cm^3). The solution was added dropwise over a period of 3 h. The mixture was then treated with concentrated hydrochloric acid until pH = 2 followed by ethyl acetate (100 cm^3). The layers were separated and the organic phase was washed further with water. The organic extract was dried and evaporated under high vacuum affording a yellow oil. The crude mixture consisted of a 1:1 mixture of the *erythro*/*threo* diastereoisomer as shown by NMR.

Separation of threo N-*Acetyl*-S-p-*methylbenzyl*-β-*phenylcysteine Methyl Ester* **1a**.—The preceding crude mixture was dissolved in boiling absolute ethanol (200 cm^3) and treated with activated charcoal (1 g). The hot mixture was filtered by gravity and allowed to stand at room temperature for 3 h. Large prisms of the *threo* diastereoisomer precipitated from solution. These crystals were collected by suction filtration and washed with cold ethanol (25 cm^3) providing pure **1a** (11 g). Further cooling of the filtrate to 4 °C for 24 h afforded another crop of *threo* crystals which were slightly contaminated with the other diastereoisomer. Recrystallization in the minimum amount of boiling ethanol (*ca.* 30 cm^3) afforded 5 g of additional pure *threo* diastereoisomer. M.p. 136–137 °C (from EtOH); TLC R_f 0.30 (EtOAc–hexane 1:1); ν_{max}/cm^{-1} 3257 (NH), 3032, 2924, 1742 (CO), 1643 (CO), 1537, 1435, 1364, 1214, 1168; δ_H([$^2H_6$]DMSO) 1.856 (s, 3 H, CH_3CO), 2.254 (s, 3 H, ArMe), 3.360 (d, 1 H, CH_AH$_B$, ^2J 12.9), 3.367 (s, 3 H, CO_2CH_3), 3.531 (d, 1 H, CH$_A$$H_B$, ^2J 12.9), 4.122 (d, 1 H, CH–S, *J* 8.4), 4.774 (dd, 1 H, N–CH, *J* 8.5, 8.8), 7.00–7.10 (m, 4 H, SCH$_2$*Ar*), 7.26–7.35 (m, 5 H, CH*Ar*), 8.491 (d, 1 H, NH, *J* 8.9); δ_C($CDCl_3$) 170.57, 169.64, 137.71, 136.83, 134.04, 129.09, 128.76, 128.42, 127.93, 56.60, 52.21, 50.82, 35.22, 22.89, 21.00; *m*/*z* [electron impact (EI)] 357 (M^+), 326 (M^+ − OMe), 298 (M^+ − CO_2Me), 242, 217, 210, 183, 168 (Found: M^+, 357.1392. Calc. for $C_{20}H_{23}NO_3S$, *M*, 357.1398).

Separation of erythro N-*Acetyl*-S-p-*methylbenzyl*-β-*phenylcysteine Methyl Ester* **1b**.—The remaining mother liquors were evaporated to dryness under reduced pressure. The sticky residue was then dissolved in hot carbon tetrachloride (100 cm^3) and pentane added until the solution became cloudy. The mixture was kept at −20 °C for two days whereupon a voluminous powder precipitated. This precipitate was filtered quickly by suction and washed with the minimum amount of cold diethyl ether. The white solid was allowed to air dry affording pure **1b** (10 g), m.p. 92–93 °C (from CCl_4–pentane); TLC R_f 0.30 (EtOAc–hexane 1:1); ν_{max}/cm^{-1} 3287 (NH) 3060, 2946, 1737 (CO), 1655 (CO), 1536, 1435, 1370, 1197, 1172; δ_H([$^2H_6$]-DMSO) 1.621 (s, 3 H, CH_3CO), 2.265 (s, 3 H, ArMe), 3.343 (d, 1 H, CH_AH$_B$, ^2J 13.1), 3.585 (d, 1 H, CH$_A$$H_B$, ^2J 13.1), 3.627 (s, 3 H, CO_2CH_3), 3.942 (d, 1 H, CH–S, *J* 10.0), 4.812 (dd, 1 H, N–CH, *J* 9.1, 10.0), 7.02–7.12 (m, 4 H, SCH$_2$*Ar*), 7.22–7.34 (m, 5 H, CH*Ar*), 8.317 (d, 1 H, NH, *J* 9.0); δ_C($CDCl_3$) 170.53, 169.64, 137.02, 136.78, 134.06, 129.13, 128.88, 128.65, 128.34, 128.13, 55.36, 52.23, 50.86, 35.42, 23.03, 21.05; *m*/*z* (EI) 357 (M^+), 326, 298, 242, 217, 210, 183, 168.

Preparation of N-*Acetyl*-S-p-*methylbenzyl*-β-*phenylcysteine* **2a** *and* **2b**.—The following procedure was applied to both *erythro* and *threo* diastereoisomers. Ester **1a** or **1b** (14.3 g) was dissolved in boiling methanol (80 cm^3) (the dissolution being more difficult with **1a**) and while the solution was maintained at reflux, water (160 cm^3) was added by increments of 20 cm^3. To the resulting suspension (or solution for **1b**) was added NaOH (1 mol dm^{-3}; 45 cm^3) by 15 cm^3 increments. The reaction mixture was maintained at reflux for 2 h, after which the methanol was removed under reduced pressure. The aqueous phase was extracted with 2 portions of 100 cm^3 of ethyl acetate, placed under reduced pressure for a few minutes to remove the small amount of ethyl acetate still present in the water phase. The aqueous phase was acidified to pH 2 with concentrated hydrochloric acid and allowed to stand at 4 °C for 1 h. The white solid obtained was filtered by suction and allowed to air dry at 50 °C for 12 h to afford diastereoisomerically pure **2a** or **2b** (11.0 g, 80% yield). *threo*: M.p. 134–135 °C (fast heating); TLC R_f 0.50 (EtOAc–MeOH 1:1); ν_{max}/cm^{-1} 3500–2500vbr (CO_2H), 3357 (NH), 1707 (CO), 1659 (CO), 1535, 1430, 1280, 1237, 1138; δ_H(CD_3OD) 1.951 (s, 3 H, CH_3CO), 2.276 (s, 3 H, Ar*Me*), 3.387 (d, 1 H, CH_AH$_B$, ^2J 13.1), 3.529 (d, 1 H, CH$_A$$H_B$, ^2J 13.1), 4.223 (d, 1 H, CH–S, *J* 7.2), 4.866 (d, 1 H, N–CH, *J* 7.1), 4.91 (br s, CO_2H + NH), 7.035 (s, 4 H, S–CH$_2$*Ar*), 7.24–7.37 (m, 5 H, CH*Ar*); δ_C(CD_3OD) 173.15, 172.75, 140.10, 137.80, 135.81, 129.99, 129.89, 129.40, 128.75, 58.28, 52.43, 36.09, 22.32, 21.11; *m*/*z* (EI) 344 (MH^+), 298 (M^+ − CO_2H), 284, 274, 266, 256, 250, 238, 229, 228, 227, 220, 207, 194, 179, 178 (Found: M^+, 344.1310. Calc. for $C_{19}H_{22}NO_3S$, *M*, 344.1320). *erythro*: M.p. 176–177 °C (from EtOH–water); TLC R_f 0.50 (EtOAc–MeOH 1:1); ν_{max}/cm^{-1} 3500–2500vbr (CO_2H), 3358 (NH), 1718 (CO), 1630 (CO), 1540, 1422, 1284, 1129: δ_H(CD_3OD) 1.796 (s, 3 H, CH_3CO), 2.283 (s, 3 H, Ar*Me*), 3.446 (d, 1 H, CH_AH$_B$, ^2J 13.1), 3.620 (d, 1 H, CH$_A$$H_B$, ^2J 13.0), 4.106 (d, 1 H, S–CH, *J* 8.1), 4.92 (br s, CO_2H + NH), 4.939 (d, 1 H, N–CH, *J* 8.1), 7.07 (br s, 4 H, CH$_2$*Ar*), 7.28 (br s, 5 H, CH*Ar*); δ_C(CD_3OD) 173.00, 172.73, 139.40, 137.81, 135.78, 130.06, 130.02, 129.93, 129.37, 128.74, 57.22, 51.26, 36.27, 22.21, 21.13; *m*/*z* (EI) 344 (MH^+), 298, 284, 274, 266, 256, 250, 238, 229, 228, 227, 220, 207, 194, 179, 178.

Preparation of N-*Trifluoroacetyl*-S-p-*methylbenzyl*-β-*phenylcysteine* **4a** *and* **4b**.—**2a** or **2b** (7.5 g) was dissolved in hydrazine (100 cm^3). The reaction mixture was heated to 60 °C for 15 h, after which it was concentrated under reduced pressure. Cold water (200 cm^3) was added which resulted in the precipitation of the amino acid. The suspension was kept at 4 °C for 1 h and filtered under reduced pressure. The white precipitate was washed successively with water, ethanol and finally with diethyl ether. The solid obtained was dried in a vacuum oven at 50 °C overnight. The dry solid (4.5 g) was taken up in trifluoroacetic acid (25 cm^3), cooled to 0 °C followed by the addition of trifluoroacetic anhydride (10 g). The reaction was essentially instantaneous and therefore could be checked on TLC as soon as the addition was completed and more trifluoroacetic anhydride added if required. The reaction product was isolated by pouring the mixture into crushed ice (200 cm^3). The solid which formed immediately was broken up and allowed to stand for 30 min before being filtered under suction and washed with a copious amount of water. The white solid was dried in a vacuum oven at 50 °C overnight affording pure **4a** or **4b** (5.7 g, for the two steps). *threo*: M.p. 119–121 °C (from diethyl ether–light petroleum); TLC R_f 0.70 (EtOAc–MeOH 1:1); ν_{max}/cm^{-1} 3316 (NH), 3500–2500vbr (CO_2H), 1707s (COs), 1548, 1432, 1406, 1254, 1183br; δ_H($CDCl_3$) 2.327 (s, 3 H, CH_3CO), 3.503 (d, 1 H, CH_AH_B, ^2J 13.1), 3.638 (d, 1 H, CH_AH_B, ^2J 13.1), 4.319 (d, 1 H, S–CH, *J* 4.7), 4.957 (dd, 1 H, N–CH, *J* 4.9, 8.8), 6.978 (d, 1 H, NH, ^3J 8.6), 7.04–7.13 (m, 4 H, SCH_2Ar), 7.26–7.40 (m, 5 H, CH*Ar*) 11.0 (br s, 1 H, CO_2H); δ_C($CDCl_3$) 172.55, 156.83 (q, $^2J_{^{13}C,^{19}F}$ 37.9), 137.24, 136.76, 133.31, 129.25, 128.84, 128.80, 128.45, 128.17, 115.50 (q, $^1J_{^{13}C,^{19}F}$ 287.8), 56.77, 50.01, 35.61, 21.07; *m/z* (EI) 397 (M^+), 379 (M^+ − OH), 332, 331, 312, 292, 284, 274, 259 (Found: M^+, 397.0950. Calc. for $C_{19}H_{18}F_3NO_3S$, *M*, 397.0959). *erythro*: M.p. 139–140 °C (from diethyl ether–light petroleum); TLC R_f 0.70 (EtOAc–MeOH 1:1); ν_{max}/cm^{-1} 3300 (NH), 3500–2500vbr (CO_2H), 1710vs (COs), 1550, 1405, 1250, 1212, 1180; δ_H($CDCl_3$), 2.343, (s, 3 H, Ar*Me*), 3.558 (d, 1 H, CH_AH_B, ^2J 13.1), 3.713 (d, 1 H, CH_AH_B, ^2J 13.0), 4.273 (d, 1 H, S–CH, *J* 4.4), 5.115 (dd, 1 H, NCH, *J* 4.4, 9.3), 6.635 (d, 1 H, NH, *J* 9.3), 7.11 (br s, 4 H, CH_2Ar), 7.26–7.40 (m, 5 H, CH*Ar*) 10.8 (br s, 1 H, CO_2H); δ_C($CDCl_3$) 173.43, 157.05 (q, $^2J_{^{13}C,^{19}F}$ 38.1), 137.20, 135.26, 133.40, 129.33, 129.14, 128.85, 128.24, 115.54 (q, $^1J_{^{13}C,^{19}F}$ 287.7), 55.43, 49.91, 35.62, 21.0; *m/z* (EI) 397 (M^+), 379 (M^+ − OH), 332, 331, 312, 292, 284, 274, 259.

Resolution of erythro *or* threo N-*Trifluoroacetyl*-S-p-*methylbenzyl*-β-*phenyl*-DL-*cysteine* **4a** *and* **4b**.—**4a** or **4b** (3.98 g) was suspended in water (900 cm^3) and lithium chloride (4.24 g) was added. The suspension was stirred vigorously and treated with LiOH (0.1 mol dm^3) dropwise being careful that the pH never exceeded 7.5. This process is tedious but the use of an automatic titrator or a slow delivering system (such as a syringe pump) is advantageous. When the addition was completed (*ca.* 100 cm^3), the pH was adjusted to 7.5 and a small aliquot set apart for reference. A suspension of CPA (4 cm^3; 5000 units) was added and the pH again readjusted. After 3 h the pH had usually dropped to 7.1 and was brought back to 7.5. The reaction was allowed to proceed at room temperature for 24 h, after which the pH was readjusted and more enzyme (4 cm^3) was added. The reaction was monitored by injecting, at regular intervals, a sample (10 mm^3) of the reaction mixture and eluting it with a gradient of water–acetonitrile 0.1% TFA (100% water to 60% water 10 min/60% water 10 min/typical t_r were: **4a** 10.6 min, **3aa** 8.0 min). The extent of resolution was measured by comparing the peak area of the remaining starting material to the area found with the solution not treated with enzyme. Usually, the reaction was complete within 48 h at 20 °C. The reaction mixture wss acidified to pH 2 with conc. hydrochloric acid and the D-unhydrolysed amino acid extracted with diethyl ether (2 × 250 cm^3), dried over $MgSO_4$ and evaporated under reduced pressure to give *threo* or *erythro N*-trifluoroacetyl-*S*-*p*-methylbenzyl-β-phenyl-D-cysteine (1.95 g, 98%). *threo*: M.p. 115–116 °C (from diethyl ether–light petroleum); $[\alpha]_D^{20}$ +159 (*c* 2, MeOH); all spectroscopic data were identical to racemic compound. *erythro*: M.p. 140–141 °C (from diethyl ether–light petroleum); $[\alpha]_D^{20}$ −179 (*c* 2, MeOH); all spectroscopic data were identical to racemic compound. The L-enantiomer which often precipitated upon acidification was recovered from the separatory funnel by washing with formic acid and was added to the aqueous phase. The aqueous phase was evaporated to dryness under reduced pressure and warm water added. Evaporation was repeated until no smell of formic acid persisted. The residue was finally taken up in boiling water (100 cm^3) and allowed to cool to room temperature before filtering under reduced pressure and washing successively with ethanol and diethyl ether. The solid was dried overnight under vacuum affording the zwitterionic species (1.42 g, 94%). *threo*: M.p. 169–170 °C (decomp., from acetic acid–water) $[\alpha]_D^{20}$ −196 (*c* 2, HCO_2H); TLC R_f 0.59 (butanol–acetic acid–water 4:1:5 upper phase); ν_{max}/cm^{-1} 3500–2500vbr (CO_2H) 1650, 1603s, 1496s, 1374s, 1345; δ_H([$^2H_6$]DMSO, CF_3CO_2D) 2.251 (s, 3 H, Ar–Me), 3.467 (d, 1 H, CH_AH_B, ^2J 13.0), 3.688 (d, 1 H, CH_AH_B, ^2J 13.1), 4.209 (d, 1 H, S–CH, *J* 7.0), 4.307 (d, 1 H, N–CH, *J* 7.0), 7.075 (br s, 4 H, SCH_2Ar), 7.35 (br s, 5 H, CH*Ar*); δ_C([$^2H_6$]DMSO, CF_3CO_2D) 168.84, 136.95, 136.65, 134.21, 129.26, 129.16, 128.85, 128.44, 56.58, 49.69, 35.38, 20.79; *m/z* [chemical ionization (CI) methane] 302 (MH^+), 285 (M^+ − OH), 256 (M^+ − CO_2H), 239, 227 (M^+ − NH_2CHCO_2H) (Found: M^+, 256.1138. Calc. for $C_{16}H_{18}NS$, *M*, 256.1160. Found: M^+, 227.0892. Calc. for $C_{15}H_{15}S$, *M*, 227.0894). *erythro*: M.p. 178–179 °C (decomp., from AcOH–H_2O) $[\alpha]_D^{20}$ +179 (*c* 2, HCO_2H); TLC R_f 0.59 (butanol–acetic acid–water 4:1:5, upper phase); ν_{max}/cm^{-1} 3500–2500vbr (CO_2H), 1618vs, 1515, 1495, 1396, 1355; δ_H([$^2H_6$]DMSO, CF_3CO_2D) 2.234 (s, 3 H, ArMe), 3.531 (d, 1 H, CH_AH_B, ^2J 13.0), 3.687 (d, 1 H, CH_AH_B, ^2J 13.0), 4.248 (m, 2 H, N–CH and CH–S), 7.08 (br s, 4 H, SCH_2Ar), 7.34 (br s, 5 H, CH*Ar*); δ_C([$^2H_6$]DMSO, CF_3CO_2D) 169.03, 136.89, 136.37, 134.30, 129.42, 129.26, 129.22, 129.14, 128.68, 56.69, 48.71, 35.32, 20.83; *m/z* (CI methane) 302 (MH^+), 285 (M^+ − OH), 256 (M^+ − CO_2H), 239, 227 (M^+ − NH_2CHCO_2H).

Preparation of erythro *and* threo S-p-*Methylbenzyl*-β-*phenyl*-D-*cysteine* **3ab** *and* **3bb**.—The procedure used was essentially the same as the one described for removing the acetyl group of compounds **2a** and **2b** (see the preparation of **4a** and **4b**). *threo*: M.p. 175–176 °C (decomp., from AcOH–H_2O); $[\alpha]_D^{20}$ +197 (*c* 2, HCO_2H); all spectroscopic data identical to **3aa**. *erythro*: M.p. 179–180 °C (decomp., from AcOH–H_2O); $[\alpha]_D^{20}$ −175 (*c* 2, HCO_2H); all spectroscopic data identical to **3ba**.

Determination of Optical Purity.—Racemic **4a** or **4b** or resolved **4ab** or **4bb** (100 mg) was dissolved in diethyl ether (5 cm^3) and a solution of diazomethane in diethyl ether (prepared from 100 mg, of *N*-nitroso-*N*-methylurea in 5 cm^3 of diethyl ether and 0.3 cm^3 of 40% KOH) was added slowly with swirling at room temperature until a faint yellow colour persisted. The solvent was evaporated under reduced pressure and the residue was recrystallized from diethyl ether–light petroleum. A portion (50 mg) was dissolved in $CDCl_3$ (0.4 cm^3) and the NMR spectrum recorded. Tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) [(+)-Eu(hfc)$_3$] was added by increments of 15 mg until the effect appeared at 0.3 equivalents.

X-Ray Data Measurements and Processing.—Intensity data were collected at 293 K on an Enraf–Nonius CAD-4 automatic

Table 2 Crystal data and details of structure determination

Crystal data	
System and space group	Monoclinic, $P2_1/c$
a, *b*, *c* (Å)	8.3708(8), 24.5094(11), 9.4068(5)
β(°)	92.885(5)
V (Å^3)	1927.5(2)
Z	4
D_c (g cm^{-3})	1.232
F(000) (e$^-$)	760
μ(Mo-Kα) cm^{-1}	1.8
Crystal size (mm)	0.20 × 0.25 × 0.35
Data collection	
Temperature (K)	293
Radiation (Å)	0.709 30
θ min–max (°)	1.5, 24.9
Scan type	$\omega/2\theta$
Scan (°)	0.8 + 0.35 tan (θ)
Dataset	−9:9; 0:28; 0:11
Tot. uniq. data	3452
Observed reflection: $I > 2.5\sigma(I)$	2354
Refinement	
N_{ref}, N_{par}	2364, 227
R, R_w, S	0.041, 0.036, 1.98
Max. shift/error	0.001
Min. and max. resd. dens. (e$^-$ Å^{-3})	−0.17, 0.18

Table 3 Selected bond distances[a] (Å) and angles (°) in compound **1a**

S(1)–C(3)	1.819(2)
S(1)–C(12)	1.818(2)
O(1)–C(1)	1.198(3)
O(2)–C(1)	1.328(3)
O(2)–C(20)	1.447(3)
O(3)–C(10)	1.226(3)
N(1)–C(2)	1.448(3)
N(1)–C(10)	1.339(3)
C(1)–C(2)	1.524(3)
C(2)–C(3)	1.542(3)
C(3)–C(4)	1.508(3)
C(10)–C(11)	1.495(3)
C(12)–C(13)	1.498(3)
C(18)–C(19)	1.508(4)
C(3)–S(1)–C(12)	100.06(10)
C(1)–O(2)–C(20)	115.75(18)
C(2)–N(1)–C(10)	121.25(17)
O(1)–C(1)–O(2)	124.63(19)
O(1)–C(1)–C(2)	124.61(19)
O(2)–C(1)–C(2)	110.69(17)
N(1)–C(2)–C(1)	110.95(17)
N(1)–C(2)–C(3)	109.92(16)
C(1)–C(2)–C(3)	108.57(17)
S(1)–C(3)–C(2)	108.11(14)
S(1)–C(3)–C(4)	112.43(15)
C(2)–C(3)–C(4)	113.00(17)
C(3)–C(4)–C(5)	119.28(20)
C(3)–C(4)–C(6)	122.26(19)
O(3)–C(10)–N(1)	121.06(20)
O(3)–C(10)–C(11)	122.70(20)
N(1)–C(10)–C(11)	116.24(18)
S(1)–C(12)–C(13)	108.98(16)
C(12)–C(13)–C(14)	121.62(21)
C(12)–C(13)–C(15)	120.14(20)
C(16)–C(18)–C(19)	122.11(23)
C(17)–C(18)–C(19)	120.65(25)

[a] Benzene C–C min, 1.360(4) max, 1.387(4), average 1.376 Å.

diffractometer. Table 2 provides crystallographic and data collection details. The NRCCAD programs[32] were used for centring, indexing and data collection. The unit cell dimensions were obtained by a least-squares fit of 24 centred reflections in the range of $30 < 2\theta < 40°$. Reflections were measured with a constant scan speed of 2.7° min^{-1}. During data collection, the intensities of three standard reflections were monitored every 60 min. No decay was observed.

The structure was solved by the application of direct methods and refined by least squares using the NRCVAX program.[33] Weight based on counting statistics were used. A secondary extinction coefficient was utilized in the refinement,[34] its final value was 0.65(4). Atomic scattering factors stored in the NRCVAX program were those of Cromer and Waber.[35] Hydrogen atomic positions were calculated; they were assigned the isotropic thermal parameter of their respective attached atoms and were refined. All non-H atoms were refined anisotropically. Bond lengths and angles are listed in Table 3, selected torsional angles are given in Table 4. Tables of atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.*

Table 4 Selected torsion angles (°) in compound **1a**

C(10)–N(1)–C(2)–C(1)	−61.0(2)
O(2)–C(1)–C(2)–N(1)	139.0(2)
N(1)–C(2)–C(3)–C(4)	167.9(2)
C(2)–C(3)–C(4)–C(5)	−110.3(2)
N(1)–C(2)–C(3)–S(1)	−67.0(1)
C(12)–S(1)–C(3)–C(2)	171.9(2)
C(3)–S(1)–C(12)–C(13)	167.3(2)
S(1)–C(12)–C(13)–C(14)	110.1(2)

Energy Calculations on Model Molecules **5**, **6a** *and* **6b**.—The molecules were built from the standard SYBYL fragment library. 90 starting conformations were constructed from the combinations of five most probable backbone conformations A, C, D, E and F (φ, ψ = −60, −60; −80,80; −80, 180; 180, 180; 180, 60 corresponding respectively to the Zimmerman *et al.* letter code[28] with 18 side chain conformations (g^+, g^-, t for χ_1 and χ_2 and ±90° for the χ_3 disulfide bond). These were minimized using the MAXIMIN2 force-field of SYBYL 5.5[36] including electrostatic contributions. The partial charges were calculated using the method of Berthod and Pullmann.[37] The computations were performed on an IBM RISC 6000 machine. The amide bonds were held *trans* while the dihedral angles defined by the C$^\alpha$, C$^\beta$, C$^\gamma$Ar, C$^\delta$Ar were maintained at 90° ± 30° for **6a** and **6b**. Minimizations were performed at a convergence level of 0.001 kcal mol^{-1}. (1 cal = 4.184 J). The minimized conformations were analysed using the TABLE function of SYBYL. The Boltzmann probabilities were calculated as described by Vásquez *et al.*[38]

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* For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc., Perkin Trans. 1*, 1993, issue 1.

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