Azasulfonamidopeptides as Peptide Bond Hydrolysis Transition State Analogues. Part 1. Synthetic Approaches

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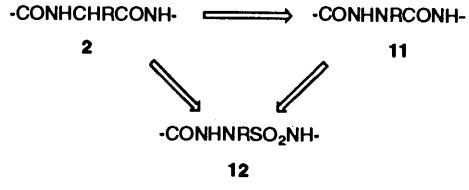
The title compounds, a novel class of peptide analogues in which an α-amino acid residue is replaced by a hydrazine-1,2-diyl sulfonyl group -NHNRSO$_2$-, are of potential interest as proteinase inhibitors. Synthetic approaches to such compounds and the X-ray molecular structures of two examples (**16** and **28**) are reported.

Sulfonamide analogues **1** of peptides **2** would, if accessible, be of considerable interest. Because the sulfur atom is tetrahedral,[1] structures **1** might be expected to model well the corresponding tetrahedral intermediates for peptide bond hydrolysis, species **3**, which are close to the transition states for such reactions. Analogues designed to relate to structures **3** are of potential value as tight-binding proteinase inhibitors, and also for the production of acyl-transfer abzymes.

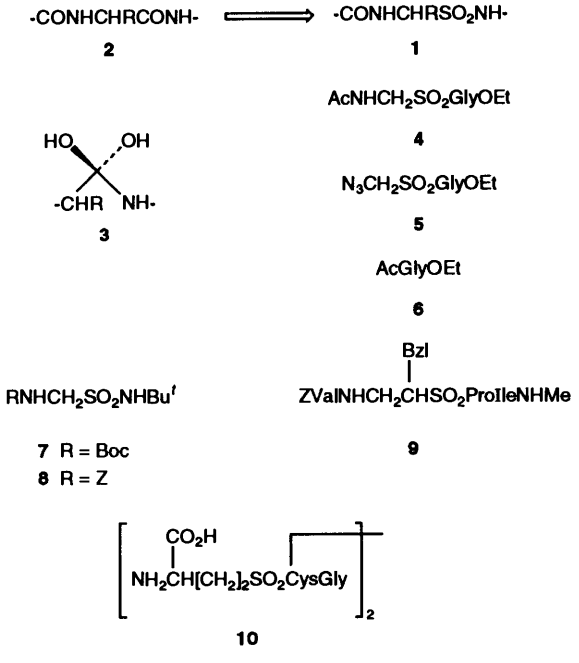
All reported efforts[2–5] to prepare peptide analogues **1** have failed, because of the fundamental fragility of α-amino sulfonic acid derivatives, which are carbonyl-addition compounds. To these disappointments we can add our own investigation of an avenue which had not been explored previously; attempts to prepare sulfonyl compound **4** by reduction of azide **5** in the presence of acetylating agents gave mixtures in which compound **6** was predominant. In fact, the only stable examples of assembly **1** which we can find in the literature are the *tert*-butyl sulfonamides **7** and **8**, both obtained by roundabout means; acidolysis of the protecting groups led to fragmentation to afford *t*-butylammonium salts as the only isolatable products.[6]

β-Amino sulfonic and γ-amino sulfonic acid derivatives are, on the other hand, generally stable; Liskamp and co-workers[5] have synthesized a number of peptide analogues containing a β-amino sulfonic acid residue, including compound **9**, a potential HIV-1 proteinase inhibitor, and the glutathione disulfide analogue **10** has also been synthesized.[7]

Peptides with modified backbones **11** in which an α-carbon is replaced by a nitrogen atom—'azapeptides'—have been extensively studied,[8] and we reasoned that analogues with this feature combined with a sulfonamide substitution for a peptide bond might be both accessible and interesting. This paper is concerned with a preliminary investigation of this novel class of transition state analogues **12**, which we call 'azasulfonamidopeptides'.



2 11 12

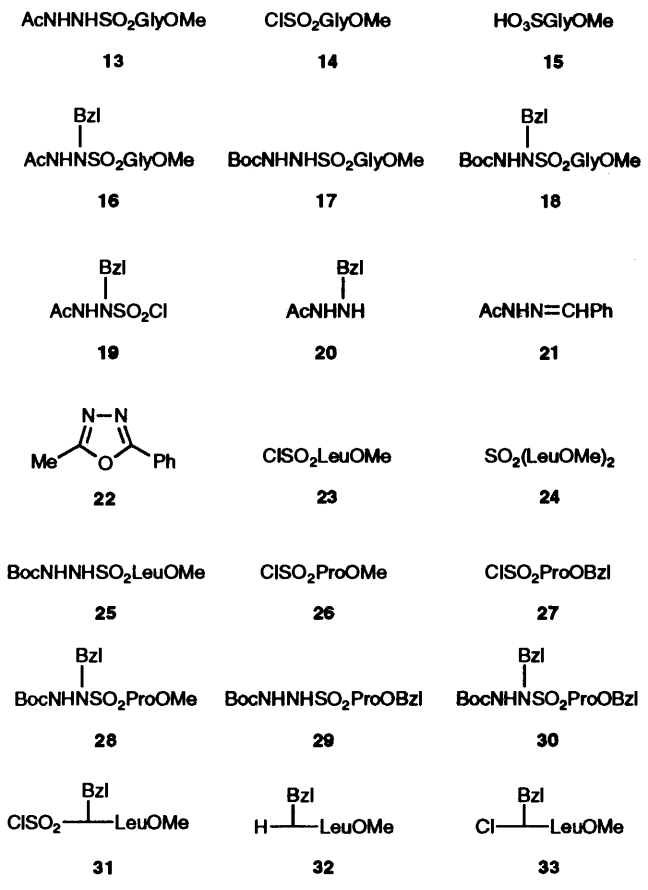


2 1 4 3 5 6 7 R = Boc 8 R = Z 9 10

Results and Discussion

There is little on record[9] concerning the preparation and properties of compounds containing the functional arrangement -NRNR'SO$_2$NR''- (hydrazinesulfonamides, alias sulfamoylhydrazines, and their derivatives). Some exploratory work was therefore indicated. As an opening exercise, we set out to make the simplest possible blocked azasulfonamidopeptide, compound **13**, *via* the primary sulfamoyl chloride **14**, which had been reported by Unterhalt and Hanewacker.[10] They employed the general preparation of primary sulfamoyl chlorides devised by Weiß and Schulze,[11] and obtained compound **14** as a crystalline solid of m.p. 45–46 °C in 60% yield from glycine methyl ester hydrochloride, by heating it with sulfuryl dichloride in acetonitrile containing a catalytic amount of antimony pentachloride. In our hands, this procedure was somewhat erratic, and gave compound **14** in yields of 20–40% and with m.p. 45–46 °C, but it was superior to the general primary sulfamoyl chloride preparation of Kloek and Leschinsky[12] as applied to the known[13] but obscure sulfamic acid **15**, which usually gave intractable mixtures on treatment with phosphorus pentachloride in benzene, although on one occasion a 90% crude yield of compound **14** with m.p. 43–45 °C was obtained. The purification of compound **14** by recrystallisation led to poor recoveries, and sometimes to decomposition.

The reaction of compound **14** with acetylhydrazine in the

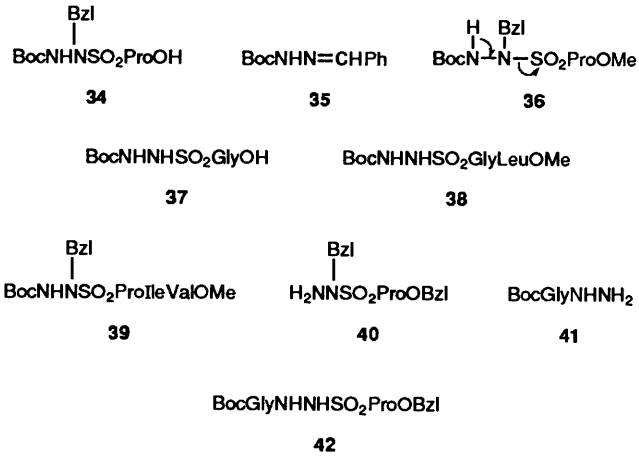


presence of triethylamine or pyridine, however, proceeded smoothly at room temperature, no doubt *via* a sulfonylimine intermediate,[14] to give the azasulfonamidopeptide **13** as a stable crystalline compound; analogues **16**–**18** were similarly obtained, all as stable crystalline materials. The complementary approach to compound **16** *via* the sulfamoyl chloride **19** was also investigated, but found to be unfeasible, because attempted generation of the latter by treatment of the hydrazide **20** with sulfuryl dichloride gave mixtures, in which the hydrazone **21** was the major product, together with some of the oxadiazole **22**.

In order to explore the generality of proceeding *via* chlorosulfonyl-α-amino esters to azasulfonamidopeptides, we attempted to prepare the leucine derivative **23** from leucine methyl ester hydrochloride by analogy with compound **14**; we failed to obtain it, except as a minor component of a complex mixture, which yielded diester **24** as well as compound **25** after treatment with *tert*-butoxycarbonylhydrazine (*tert*-butyl carbazate).

Conversion of proline methyl ester hydrochloride into chlorosulfonylproline methyl ester **26** by the method of Unterhalt and Hanewacker[10] gave modest yields, but led to the discovery that compound **26** was a stable compound amenable to purification by flash chromatography. It was found subsequently that the rather vigorous conditions of Unterhalt and Hanewacker were, in fact, unnecessary for this case; reaction of proline methyl ester in the free-base form, either previously liberated from the salt or generated *in situ* by the addition of triethylamine, with sulfuryl dichloride at ~ −10 °C gave better results. Chlorosulfonylproline benzyl ester **27**, a nicely crystalline substance, was also prepared in this way. The secondary sulfamoyl chlorides **26** and **27** were much less reactive towards acylhydrazines than was the primary sulfamoyl chloride **14**, reflecting the necessity of direct attack at sulfur—the sulfonylimine pathway is open only to primary sulfamoyl chlorides. Reaction times of 2–3 days at reflux in dichloromethane were required, but compounds **28**–**30** were obtained in satisfactory yield, all as stable crystalline materials. These results with proline derivatives suggested a route to the leucine analogue **25** *via* the secondary sulfamoyl chloride **31** with debenzylation as the final step, but reaction of compound **32** with sulfuryl dichloride under the conditions used for the preparation of the chlorosulfonamides **26** and **27** gave not the expected product **31** but a good yield of the chloramine **33**.

It remained to be seen whether routes to complex azasulfonamidopeptides could be opened up by the extension of azasulfonamidodipeptide units in either or both directions. The saponification of compounds **17** and **25** gave rise to no difficulty, but a greater excess of alkali than is usual for such reactions was required, presumably because of the presence of acidic NH groups. Indeed, an ionisable aza-NH may be necessary to inhibit alternative pathways, as attempted saponification of compound **28** gave not the free acid **34** but instead, mainly the hydrazone **35**, possibly by elimination as in structure **36** and subsequent tautomerisation. The acid **34** could be obtained, however, by hydrogenolysis of the benzyl ester group in compound **30**, which was achieved without *N*-benzyl hydrogenolysis. Standard dicyclohexylcarbodiimide–*N*-hydroxybenzotriazole (DCCI–HOBt) coupling of the acid **37** with leucine methyl ester and of the acid **34** with isoleucylvaline methyl ester gave compounds **38** and **39** respectively without any problem, demonstrating that C-terminal elongation was possible. The attachment of additional residues on the N-terminal side after deprotection, on the other hand, proved to be impossible despite many attempts. Removal of the *tert*-butoxycarbonyl protecting group from compound **30** in the conventional manner with trifluoroacetic acid (TFA), followed by evaporation of the TFA and distribution of the residue between diethyl ether and water gave, remarkably, the deprotected azasulfonamidopeptide ester in the free-base form **40**, and this free base was unreactive to acylating agents. It appeared therefore that the construction of an azasulfonamidopeptide derivative with a single azasulfonamido residue in the central position would, of necessity, have to be approached by reaction between a hydrazide of one protected amino acid and the chlorosulfonyl derivative of another; this was exemplified by treatment of hydrazide **41** with the sulfonyl chloride **27** to give compound **42**, in fair yield after chromatography.



Single-crystal X-ray structure determinations were carried out on the hydrazinesulfonamides **16** and **28**. Figs. 1 and 2 show perspective views of the two structures with the atomic numbering schemes employed; fractional atomic coordinates

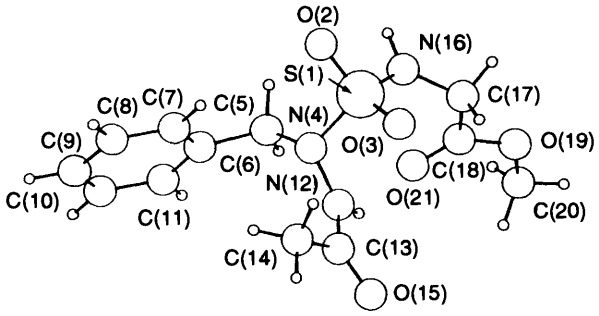


Fig. 1 The structure of compound **16** showing the atomic numbering system employed

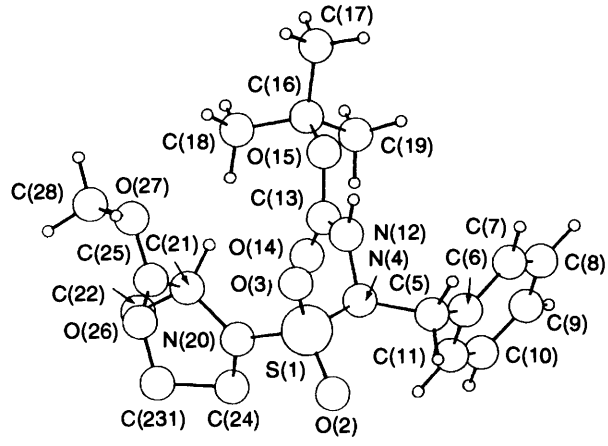


Fig. 2 The structure of compound **28** showing the atomic numbering system employed. Only one of the two disordered sites for 'C(23)', the predominant C(231) site, is shown.

and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre* and selected bonded dimensions are given in Tables 1 and 2, respectively. In both cases, the SO_2 group has a distorted tetrahedral configuration, and the azasulfonamido assembly appears to be a reasonable model for a peptide-bond-hydrolysis transition state.

In conclusion, azasulfonamidopeptides are a plausible, accessible and stable class of novel transition-state analogues, and although there are limitations on synthetic approaches to them, these have been mapped out.

Experimental

M.p.s were determined on a Kofler block, and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) or Bruker AM500 (500 MHz) spectrometer, and are quoted in ppm relative to tetramethylsilane as internal reference, for solutions in deuteriochloroform. *J*-Values are given in Hz. ^{13}C NMR spectra were recorded on the Bruker AM500 instrument operating at 125.75 MHz. NMR data are given only for two compounds which were isolated as oils on which elemental analyses were not obtained, and for one crystalline product which gave a complex spectrum because of the coexistence of two distinct conformations; but NMR spectra in full accord with their formulation as pure compounds of the structures stated were recorded on all compounds for which elemental analyses are reported. All the compounds described were chromatographically pure in appropriate TLC systems. Specific rotations ($[\alpha]_D$ units of 10^{-1} deg cm^2 g^{-1}) were determined using 1 dm cells and a Perkin-Elmer 241 automatic polarimeter. Mass spectra were recorded on a V.G. Micromass ZAB-IF, a V.G. Micromass 20-250, or a BIO-Q instrument. Flash chromatography was performed on columns of silica gel (May and Baker Sorbsil C_{60}; 40–60 μm), eluted with chloroform–methanol or diethyl ether–light petroleum (boiling range 40–60 °C, of analytical grade or redistilled before use) as appropriate. Solutions in organic solvents were dried over magnesium sulfate.

Table 1 Selected bonded dimensions for compound **16**

Atoms	Distance (Å)
S(1)–O(2)	1.421(2)
S(1)–O(3)	1.420(2)
S(1)–N(4)	1.677(2)
S(1)–N(16)	1.599(2)
N(4)–N(12)	1.396(2)
N(4)–C(5)	1.478(3)
N(12)–C(13)	1.358(3)
N(16)–C(17)	1.454(3)
	Angle (°)
O(3)–S(1)–O(2)	121.5(1)
N(4)–S(1)–O(2)	103.7(1)
N(4)–S(1)–O(3)	105.25(9)
N(16)–S(1)–O(2)	106.3(1)
N(16)–S(1)–O(3)	108.4(1)
N(16)–S(1)–N(4)	111.6(1)
N(12)–N(4)–S(1)	113.8(1)
C(5)–N(4)–S(1)	116.6(1)
C(5)–N(4)–N(12)	113.9(2)
C(13)–N(12)–N(4)	121.3(2)
C(17)–N(16)–S(1)	124.2(2)

Table 2 Selected bonded dimensions for compound **28**

Atoms	Distance (Å)
S(1)–O(2)	1.422(3)
S(1)–O(3)	1.419(3)
S(1)–N(4)	1.664(2)
S(1)–N(20)	1.600(3)
N(4)–N(12)	1.396(3)
N(4)–C(5)	1.482(4)
N(12)–C(13)	1.361(3)
N(20)–C(21)	1.456(4)
N(20–C(24)	1.490(5)
	Angle (°)
O(3)–S(1)–O(2)	120.9(2)
N(4)–S(1)–O(2)	103.9(2)
N(4)–S(1)–O(3)	110.4(2)
N(20)–S(1)–O(2)	108.5(2)
N(20)–S(1)–O(3)	106.9(1)
N(20)–S(1)–N(4)	105.1(1)
N(12)–N(4)–S(1)	111.8(2)
C(5)–N(4)–S(1)	119.2(2)
C(5)–N(4)–N(12)	113.0(3)
C(13)–N(12)–N(4)	119.4(3)
C(21)–N(20)–S(1)	122.4(2)
C(24)–N(20)–S(1)	120.3(2)
C(24)–N(20)–C(21)	113.0(3)

(N'-*Acetylhydrazino*)*sulfonylglycine Methyl Ester* **13**.—Triethylamine (0.37 cm^3, 2.66 mmol), immediately followed by acetylhydrazine[15] (0.198 g, 2.69 mmol), was added to a stirred solution of chlorosulfonylglycine methyl ester **14**[10] (0.504 g, 2.69 mmol) in dichloromethane (10 cm^3) at 0 °C. The cooling bath was removed, and after 4 h the solvent was evaporated off. Crystallisation from methanol–chloroform and flash chromatography of the liquors gave the *title azasulfonamidodipeptide* (0.41 g, 68%), m.p. 129–130 °C (Found: C, 26.8; H, 5.0; N, 18.85; S, 14.5. $C_5H_{11}N_3O_5S$ requires C, 26.7; H, 4.9; N, 18.7; S, 14.2%).

* For full details of the deposition scheme, see 'Instructions for Authors (1994)', *J. Chem. Soc., Perkin Trans. 1*, Issue 1.

(N'-*Acetyl*-N-*benzylhydrazino*)*sulfonylglycine Methyl Ester* **16**.—This was prepared from *N*-acetyl-*N'*-benzylhydrazine **20**[16] by analogy with the preceding case, except that a reaction time of 12 h was allowed, and the residue obtained after evaporation was taken up in ethyl acetate and washed successively with 1 mol dm^{-3} hydrochloric acid and brine and dried before flash chromatography, which gave the *title azasulfonamidodipeptide* in 52% yield, with m.p. 101–102 °C; δ_H(200 MHz) (two nearly equally populated conformations) 1.61 and 1.87 (3 H, both s, Ac), 3.74 and 3.79 (3 H, both s, OMe), 3.90–4.90 (4 H, m, NHCH_2 and NCH_2Ph), 5.95 and 6.28 (1 H, both br t, NHCH_2) and 7.20–7.70 (6 H, m, ArH and NHN) (Found: C, 45.8; H, 5.2; N, 13.4; S, 10.0%; *m/z* CI, 316, < 1%, MH^+. $C_{12}H_{17}N_3O_5S$ requires C, 45.7; H, 5.4; N, 13.3; S, 10.2%; M, 315).

[N'-(tert-*Butoxycarbonyl*)*hydrazino*]*sulfonylglycine Methyl Ester* **17**.—This was prepared from *tert*-butoxycarbonylhydrazine as in the preceding example, using pyridine as base, with isolation by flash chromatography of the total reaction mixture obtained after evaporation, without the washing procedures. The *title azasulfonamidodipeptide* was obtained in 72% yield, m.p. 114–115 °C (Found: C, 34.1; H, 6.15; N, 14.9; S, 11.5. $C_8H_{17}N_3O_6S$ requires C, 33.9; H, 6.0; N, 14.8; S, 11.3%).

[N-*Benzyl*-N'-(tert-*butoxycarbonyl*)*hydrazino*]*sulfonylglycine Methyl Ester* **18**.—This was prepared from *N*-benzyl-*N'*-(*tert*-butoxycarbonyl)hydrazine[17] (but prepared from *tert*-butoxycarbonylhydrazine by analogy with *N*-acetyl-*N'*-benzylhydrazine)[16] by analogy with compound **16**, except that 0.5 mol dm^{-3} citric acid was used in the washing procedure. The *title azasulfonamidodipeptide* was obtained in 62% yield, m.p. 103–104 °C (Found: C, 47.8; H, 6.1; N, 11.3; S, 8.7. $C_{15}H_{23}N_3O_6S$ requires C, 48.2; H, 6.2; N, 11.3; S, 8.6%).

[N'-(tert-*Butoxycarbonyl*)*hydrazino*]*sulfonyl*-L-*leucine Methyl Ester* **25**.—Sulfuryl dichloride (5 cm^3) and antimony pentachloride (3 drops) were added to a solution of L-leucine methyl ester hydrochloride (2.94 g, 16.2 mmol) in dry acetonitrile (15 cm^3). The mixture was heated under reflux for 48 h and then the solvent and excess of sulfuryl dichloride were removed under reduced pressure. Half of the resulting brown oil was dissolved in chloroform (10 cm^3) and, while being stirred at 0 °C, was treated with triethylamine (1.2 cm^3), followed immediately by *tert*-butoxycarbonylhydrazine (1.03 g). The mixture was left overnight at room temperature, and the solvent was then removed. Ethyl acetate was added, and the solution was washed successively with 0.5 mol dm^{-3} citric acid and brine, and dried before flash chromatography, which gave the *title azasulfonamidodipeptide* (0.69 g, 26%) as fine needles, m.p. 110–111 °C; $[\alpha]_D^{20}$ +49.1 (*c* 1.01, MeOH) (Found: C, 42.3; H, 7.5; N, 12.3; S, 9.4. $C_{12}H_{25}N_3O_6S$ requires C, 42.5; H, 7.4; N, 12.4; S, 9.4%).

Sulfonyldi-(L-*leucine Methyl Ester*) **24** was also isolated, in 19% yield, m.p. 87–88 °C; $[\alpha]_D^{20}$ −43.7 (*c* 1.01, MeOH) (Found: C, 47.5; H, 8.2; N, 8.1; S, 8.9%; *m/z* CI, 353, 60%, MH^+. $C_{14}H_{28}N_2O_6S$ requires C, 47.7; H, 8.0; N, 7.95; S, 9.1%; M, 352).

Chlorosulfonyl-L-*proline Methyl Ester* **26**.—A mixture of L-proline methyl ester hydrochloride (3.34 g, 20 mmol) and triethylamine (6.0 cm^3, 43 mmol) in dry toluene (45 cm^3) was added dropwise during 1.5 h to a stirred solution of sulfuryl dichloride (3.2 cm^3, 40 mmol) in dry toluene (30 cm^3) which was cooled in an ice–salt-bath. Excess of sulfuryl dichloride and solvent were removed under reduced pressure, and the *title sulfamoyl chloride* (1.87 g, 41%) was isolated as a light brown oil by flash chromatography. The oil crystallised on storage at −20 °C, melting on thawing at 17 °C; $[\alpha]_D^{20}$ −131.8 (*c* 1.21, $CHCl_3$) (Found: C, 31.9; H, 4.3; N, 6.0; S, 13.8%; *m/z*, CI, 245/247, 45%/15%, MNH_4^+. $C_6H_{10}ClNO_4$ requires C, 31.7; H, 4.4; N, 6.2; S, 14.1%; M, 226/228).

Chlorosulfonyl-L-*proline Benzyl Ester* **27**.—This was prepared as in the preceding case, except that after completion of the reaction the mixture was washed with water and dried; removal of the solvent and flash chromatography, or recrystallisation from chloroform–light petroleum, gave the *title sulfamoyl chloride* in 30–45% yield, m.p. 65–66 °C; $[\alpha]_D^{20}$ −127.2 (*c* 1.11, $CHCl_3$) (Found: C, 47.2; H, 4.5; N, 4.5; S, 10.5%; *m/z*, EI, 303, < 1%, M^+. $C_{12}H_{14}ClNO_4S$ requires C, 47.45; H, 4.65; N, 4.6; S, 10.6%; M, 303).

[N-*Benzyl*-N'-(tert-*butoxycarbonyl*)*hydrazino*]*sulfonyl*-L-*proline Methyl Ester* **28**.—A solution of chlorosulfonyl-L-proline methyl ester **26** (0.464 g, 2.04 mmol), *N*-benzyl-*N'*-(*tert*-butoxycarbonyl)hydrazine (0.459 g, 2.07 mmol) and triethylamine (0.3 cm^3, 2.2 mmol) in dry dichloromethane (5 cm^3) was heated under reflux for 48 h. The mixture was diluted with dichloromethane, washed successively with 0.5 mol dm^{-3} citric acid and brine, and dried. Flash chromatography, and recrystallisation from diethyl ether–light petroleum gave the *title azasulfonamidodipeptide* (0.493 g, 58%) as needles, m.p. 71–72 °C; $[\alpha]_D^{22}$ −55.2 (*c* 1.02, $CHCl_3$) (Found: C, 52.3; H, 6.6; N, 10.0; S, 7.9. $C_{18}H_{27}N_3O_6S$ requires C, 52.3; H, 6.6; N, 10.2; S, 7.8%).

[N'-(tert-*Butoxycarbonyl*)*hydrazino*]*sulfonyl*-L-*proline Benzyl Ester* **29**.—This was prepared from *tert*-butoxycarbonylhydrazine and chlorosulfonyl-L-proline benzyl ester **27** by analogy with the preceding case, but with a reflux time of 72 h. The *title azasulfonamidodipeptide* was obtained in 72% yield after recrystallisation, as needles, m.p. 83–84 °C; $[\alpha]_D^{22}$ −48.3 (*c* 1.0, $CHCl_3$) (Found: C, 51.1; H, 6.3; N, 10.6; S, 8.0. $C_{17}H_{25}N_3O_6S$ requires C, 51.1; H, 6.3; N, 10.5; S, 8.0%).

[N-*Benzyl*-N'-(tert-*butoxycarbonyl*)*hydrazino*]*sulfonyl*-L-*proline Benzyl Ester* **30**.—This was prepared from *N*-benzyl-*N'*-(*tert*-butoxycarbonyl)hydrazine by analogy with preceding cases, and was obtained after recrystallisation from ethanol–water in 64% yield, as *needles*, m.p. 105–106 °C; $[\alpha]_D^{20}$ −59.2 (*c* 1.00, $CHCl_3$) (Found: C, 58.8; H, 6.5; N, 8.5; S, 6.4. $C_{24}H_{31}N_3O_6S$ requires C, 58.9; H, 6.4; N, 8.6; S, 6.5%).

N-*Benzyl*-L-*leucine Methyl Ester* **32**.—A solution of benzaldehyde (3.30 cm^3, 32.5 mmol) in methanol (5 cm^3) was added over a period of 10 min to a stirred solution of L-leucine methyl ester hydrochloride (5.45 g, 30 mmol) and triethylamine (5.0 cm^3, 36 mmol) in methanol (40 cm^3) at 0 °C. After 5 h at room temperature, the methanol was evaporated off and the residue was dissolved in chloroform. The solution was washed with water, dried, and the solvent was evaporated off to give an oil (6.68 g).

A portion (2.34 g) of this oil was dissolved in methanol (20 cm^3), and the solution was cooled to 0 °C. Sodium borohydride (0.39 g, 10.3 mmol) was added. After a further 30 min at 0 °C, the methanol was evaporated off. The residue was dissolved in diethyl ether (50 cm^3) and washed with water (2 × 25 cm^3). The ethereal phase was extracted with 2 mol dm^{-3} hydrochloric acid (3 × 15 cm^3), and the combined extracts were washed with diethyl ether (50 cm^3). Basification of the aqueous phase with sodium hydrogen carbonate and extraction with diethyl ether (3 × 50 cm^3) gave, after drying and evaporation, the *title* N-*benzyl derivative* as an oil (1.96 g, 79% overall). For characterisation, the *hydrochloride* was prepared by mixing an ethereal solution of the free base with excess of ethereal

hydrogen chloride, evaporation and recrystallisation from methanol–diethyl ether: m.p. 167–168 °C; $[\alpha]_D^{20}$ +22.6 (*c* 1.01, MeOH) (Found: C, 61.9; H, 8.4; N, 5.1. $C_{14}H_{22}ClNO_2$ requires C, 61.9; H, 8.2; N, 5.2%).

N-*Benzyl*-N-*chloro*-L-*leucine Methyl Ester* **33**.—A mixture of *N*-benzyl-L-leucine methyl ester free base **32** (0.68 g, 2.89 mmol) and triethylamine (0.45 cm^3) in dry toluene (10 cm^3) was added during 2 h to a stirred solution of sulfuryl dichloride (0.8 cm^3) in toluene (20 cm^3) at −10 °C. After a further 2 h at room temperature, the toluene and excess of sulfuryl dichloride were evaporated off. The residue was distributed between diethyl ether (50 cm^3) and brine (10 cm^3). The ethereal phase was washed successively with 2 mol dm^{-3} hydrochloric acid (10 cm^3) and brine (10 cm^3), and dried. Evaporation and flash chromatography gave the *title chloroamine* as an almost colourless oil (0.57 g, 76%), $[\alpha]_D^{20}$ −45.1 (*c* 0.78, $CHCl_3$) (Found: C, 62.2; H, 7.6; N, 5.1%; *m/z*, CI, 270/272, 90%/30%, MH^+. $C_{14}H_{20}ClNO_2$ requires C, 62.3; H, 7.5; N, 5.2%; M, 269/271). This product was chromatographically identical with the main product of treatment of the free base **32** with an excess of sodium hypochlorite in methanol.

[N′-(tert-*Butoxycarbonyl*)*hydrazino*]*sulfonylglycine* **37**.—Sodium hydroxide (1 mol dm^{-3}; 5.0 cm^3) was added to a solution of ester **17** (0.376 g, 1.3 mmol) in methanol (5 cm^3), and the mixture was stirred at room temperature for 4 h. The residue obtained after removal of the methanol was dissolved in water and passed through a column of Amberlite CG-50 resin (acidic form). Evaporation of the water gave an oil, which crystallised on storage; recrystallisation from water gave the *title acid* (0.211 g, 59%) as plates, m.p. 125–126 °C (decomp.) (Found: C, 31.3; H, 5.5; N, 15.8. $C_7H_{15}N_3O_6S$ requires C, 31.2; H, 5.6; N, 15.6%).

[N′-(tert-*Butoxycarbonyl*)*hydrazino*]*sulfonyl*-L-*leucine*.—Sodium hydroxide (1 mol dm^{-3}; 2.8 cm^3) was added to a solution of ester **25** (0.313 g, 0.92 mmol) in methanol (1.5 cm^3), and the mixture was stirred at room temperature for 2 h. Diethyl ether (10 cm^3) and 0.5 mol dm^{-3} citric acid (10 cm^3) were added. The aqueous layer was separated, and extracted with diethyl ether (10 cm^3). The ethereal extract was combined with the ether layer from the previous separation, and the whole was extracted with 5% aq. sodium hydrogen carbonate (2 × 10 cm^3). The ethereal extracts were combined, dried, and evaporated to give, after recrystallisation from chloroform–light petroleum, the *title acid* as prisms, m.p. 145–147 °C; $[\alpha]_D^{22}$ +71.6 (*c* 1.21, MeOH) (Found: C, 40.6; H, 6.9; N, 12.7; S, 9.9. $C_{11}H_{23}N_3O_6S$ requires C, 40.6; H, 7.1; N, 12.9; S, 9.9%).

[N′-(tert-*Butoxycarbonyl*)*hydrazino*]*sulfonylglycyl*-L-*leucine Methyl Ester* **38**.—DCCI (0.621 g, 3.01 mmol) was added to a mixture of the acid **37** (0.811 g, 3.01 mmol), HOBt hydrate (0.469 g, 3.02 mmol), L-leucine methyl ester hydrochloride (0.545 g, 3.00 mmol) and triethylamine (0.45 cm^3, 3.2 mmol) in dichloromethane (8 cm^3). The solution was stirred overnight, and after evaporation of the solvent the title azasulfonamidotripeptide was isolated by flash chromatography as an oil (0.706 g, 59%); δ_H(500 MHz) 0.92 (3 H, d, *J* 5, *Me*CHCH$_3$), 0.93 (3 H, d, *J* 5, CH$_3$CH*Me*), 1.46 (9 H, s, Bu^t), 1.61–1.69 (3 H, m, C$^\alpha$HCH_2CH), 3.73 (3 H, s, OMe), 3.85–3.95 (2 H, m, Gly C$^\alpha$H$_2$), 4.62–4.64 (1 H, m, C$^\alpha$H), 5.85–6.00 (1 H, br, NH) and 7.20–7.50 (3 H, br, NH × 3); δ_C(125.75 MHz), 21.7 (*Me*CHCH$_3$), 22.7 (CH$_3$CH*Me*), 24.8 (*C*HMe$_2$), 28.1 (C*Me*$_3$), 40.9 (C$^\alpha$HCH$_2$), 46.1 (C$^\alpha$H$_2$), 51.0 (C$^\alpha$H), 52.5 (OMe), 82.5 (*C*Me$_3$) and 155.7, 169.0 and 173.8 (CO × 3).

[N-*Benzyl*-N′-(tert-*butoxycarbonyl*)*hydrazino*]*sulfonyl*-L-*prolyl*-L-*isoleucyl*-L-*valine Methyl Ester* **39**.—A solution of benzyloxycarbonyl-L-isoleucyl-L-valine methyl ester[18] (0.093 g, 0.25 mmol) and acetic acid (15 mm^3, 0.26 mmol) in ethanol (3 cm^3) was stirred with 10% palladium on charcoal (0.022 g) under hydrogen, at ambient temperature and pressure, for 6 h. The mixture was filtered through Celite and the solvent was evaporated off, to give an oil, which was partitioned between saturated aq. sodium hydrogen carbonate and dichloromethane. The organic layer was dried and evaporated to give crude L-isoleucyl-L-valine methyl ester free base as an oil.

A solution of the azasulfonamidodipeptide ester **30** (0.114 g, 0.23 mmol) in ethanol (3 cm^3) was stirred with 10% palladium on charcoal (0.19 g) under hydrogen, at ambient temperature and pressure, for 25 min. Filtration through Celite and evaporation gave an oil, which was dissolved with the preceding crude dipeptide ester, HOBt (0.034 g, 0.22 mmol), and DCCI (0.055 g, 0.27 mmol) in ethyl acetate (5 cm^3). The solution was stirred overnight, filtered, washed successively with 0.5 mol dm^{-3} citric acid (3 × 5 cm^3) and brine, and dried. Evaporation and flash chromatography gave the title azasulfonamidotetrapeptide (0.083 g, 58%) as an oil: δ_H(200 MHz) 0.89–0.96 (12 H, m, 4 × side-chain Me), 1.06–1.22 (2 H, m, MeCH_2), 1.34 (9 H, s, Bu^t), 1.44–1.73 (2 H, m, C*H*Me$_2$ and C*H*MeCH$_2$Me), 1.83–2.23 (4 H, m, C$^\alpha$HCH_2CH_2), 3.53–3.72 (5 H, m and s at 3.69, C$^\alpha$HNCH_2 and OMe), 4.33–4.40 (1 H, m, C$^\alpha$H), 4.46–4.67 (4 H, m, C$^\alpha$H × 2 and NCH_2Ph), 6.30–6.55 (1 H, br, NHN), 6.88 (1 H, br d, N*H*CH) and 7.30–7.37 (6 H, m, Ph and N*H*CH) (Found: *m/z*, FAB$^+$, 626, < 1%, MH^+. $C_{29}H_{47}N_5O_8S$ requires M, 625).

(N-*Benzylhydrazino*)*sulfonyl*-L-*proline Benzyl Ester* **40**.—A solution of the *tert*-butoxycarbonyl derivative **30** (0.131 g, 0.27 mmol) was dissolved in a mixture of TFA (0.9 cm^3) and water (0.1 cm^3). After 1 h, the solution was evaporated and the residue was partitioned between diethyl ether (5 cm^3) and water (5 cm^3). The layers were separated and the aqueous layer was extracted with diethyl ether (2 × 10 cm^3). The *title free base* was isolated from the combined ethereal extracts by evaporation and flash chromatography as a homogeneous oil (0.061 g, 57%) (Found: C, 58.5; H, 6.0; N, 10.5. $C_{19}H_{23}N_3O_4S$ requires C, 58.6; H, 6.0; N, 10.8%).

(tert-*Butoxycarbonyl*)*glycine Hydrazide* **41**.—This *compound* was prepared by successive treatment of glycine ethyl ester hydrochloride with di-*tert*-butyl dicarbonate and hydrazine under appropriate conditions, in 54% overall yield after recrystallisation from ethyl acetate: m.p. 101–103 °C (Found: C, 44.2; H, 8.0; N, 22.5. $C_7H_{15}N_3O_3$ requires C, 44.4; H, 8.0; N, 22.2%).

{N′-[(tert-*Butoxycarbonyl*)*glycyl*]*hydrazino*}*sulfonyl*-L-*proline Benzyl Ester* **42**.—A solution of (*tert*-butoxycarbonyl)glycine hydrazide (0.380 g, 2.01 mmol), chlorosulfonyl-L-proline benzyl ester **27** (0.609 g, 2.01 mmol) and triethylamine (0.30 cm^3, 2.15 mmol) in dichloromethane (10 cm^3) was heated under reflux for 48 h. Evaporation and flash chromatography gave the *title azasulfonamidotripeptide* (0.570 g, 62%) as a crisp foam, $[\alpha]_D^{20}$ −29.0 (*c* 1.1, $CHCl_3$) (Found: C, 50.1; H, 5.9; N, 12.0; S, 6.8%; *m/z*, DCI 474, < 1%, MNH_4^+ and 457, < 1%, MH^+. $C_{19}H_{28}N_4O_7S$ requires C, 50.0; H, 6.2; N, 12.3; S, 7.0%; M, 456).

X-Ray Structure Analysis of Compound **16**.—*Crystal data*. $C_{12}H_{17}N_3O_5S$, M_r = 315.34, triclinic, $P\bar{1}$ (No. 2), *a* = 7.542(2), *b* = 8.925(4), *c* = 11.471(5) Å, α = 98.21(4), β = 95.87(3), γ = 97.44(3)° (from least-squares fitting of setting angles for 25 reflections 27.5 ⩽ θ ⩽ 70.2°), *V* = 752 Å^3, *Z* = 2, D_x = 1.393 g cm^{-3}, Cu-Kα radiation, needle-like crystal, 0.6 × 0.2 × 0.2 mm, μ = 21.04 cm^{-1}.

Data collection and processing. Data were collected on a CAD-4F diffractometer in the $\omega:2\theta$ mode, $0 < 2\theta \leqslant 144°$ ($-2 \leqslant h \leqslant 9$, $-11 \leqslant k \leqslant 11$, $-14 \leqslant l \leqslant 14$). 3747 Reflections measured, 2951 unique ($R_{merge} = 0.033$) of which 2259 were observed [$I \geqslant 3\sigma(I)$]. No significant variation in intensity of 3 check reflections was observed. Data were corrected for Lorentz and polarisation effects[19] and extinction.

Structure solution and refinement. The structure was solved by direct methods[20] which yielded coordinates for all non-H atoms. Refinement of the model was undertaken using the CRYSTALS program package.[19] Full-matrix least-squares refinement based on F of positional and anisotropic thermal parameters for all non-H atoms and isotropic thermal parameters for each H-atom type was continued until convergence, and the H-atom coordinates were geometrically calculated. An empirical absorption correction[21] based on θ was applied (min. 0.622 and max. corrections 1.321) and a 3-term Chebychev polynomial weighting scheme was employed. At convergence $R = 0.047$, $R_w = 0.061$ for 210 parameters, min. and max. residual electron density -0.36 and 0.33 e Å^{-3}.

X-Ray Structure Analysis of Compound **28**.—*Crystal data.* $C_{18}H_{27}N_3O_6S$, $M_r = 413.488$, trigonal, $P3_1$ (No. 144), $a = 10.708(1)$, $c = 16.833(2)$ Å. $\gamma = 120°$ (from least-squares fitting of setting angles for 25 reflections $30.6 \leqslant \theta \leqslant 47.2°$), $V = 1671.5$ Å^3, $Z = 3$, $D_x = 1.232$ g cm^{-3}, Cu-Kα radiation, triangular prismatic crystal, $0.4 \times 0.4 \times 0.8$ mm, $\mu = 15.64$ cm^{-1}.

Data collection and processing. Data were collected on a CAD-4F diffractometer in the $\omega:2\theta$ mode, $0 < 2\theta \leqslant 144°$ ($-13 \leqslant h \leqslant 13$, $-1 \leqslant k \leqslant 13$, $-1 \leqslant l \leqslant 20$). 8206 Reflections measured, 4334 unique ($R_{merge} = 0.038$; Friedel pairs were not merged) of which 3569 were observed [$I \geqslant 3\sigma(I)$]. No significant variation in intensity of 3 check reflections was observed.

Structure solution and refinement. The structure was solved by direct methods[20] which yielded coordinates for all non-H atoms. Refinement of the model was undertaken using the CRYSTALS program package.[19] On examination, it was clear that the three methylene groups of the five-membered ring were affected to varying degrees by disorder. A number of different models were tried to fit this region of the electron density. The most satisfactory model employed anisotropic thermal parameters for C(22) and C(24), while two fractionally occupied isotropic atoms were included for 'C(23)', *viz.* C(231) and C(232), with occupancies of 60.8(8) and 39.2(8)% respectively. Positional and anisotropic thermal parameters were refined for all non-H atoms except the disordered sites of 'C(23)'. An extinction parameter was refined and an empirical absorption correction[21] based on θ was applied (min. and max. corrections 0.621 and 1.852, respectively). A 3-term Chebychev polynomial weighting scheme was employed. A Flack enantiopole[22] was included in the refinement, and converged to a value of 0.03(2), confirming the absolute configuration to be as shown. At convergence $R = 0.045$, $R_w = 0.055$ for 259 parameters.

Calculated hydrogen atom coordinates, fractional non-H-atom coordinates, anisotropic thermal parameters and full lists of bonded dimensions for both compounds **16** and **28** are available from the Cambridge Crystallographic Data Centre.*

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* For full details of the deposition scheme, see 'Instructions for Authors (1994)', *J. Chem. Soc., Perkin Trans. 1*, Issue 1.

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