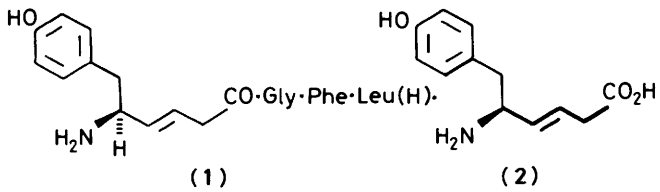
On the Double Bond Isostere of the Peptide Bond: Preparation of Modified Di- and Tri-peptides incorporating Proline and Alanine Analogues

Nicholas J. Miles and Peter G. Sammes *,†
Department of Organic Chemistry, The University, Leeds LS2 9JT
Peter D. Kennewell and Robert Westwood
Roussel Laboratories Ltd., Kingfisher Drive, Covingham, Swindon, Wilts, SN3 5BZ

The preparation of some analogues of representative peptides that incorporate double bond isosteres of the sequences, Pro-Gly, Pro-Leu, Pro-Phe, Ala-Gly, and Ala-Ala, are described. The method of synthesis involves selective hydroboronation and oxidation of conjugated enynes, to generate βγ-unsaturated acids, followed by selective α-alkylation in order to introduce a second substituent into the 'peptide' backbone.

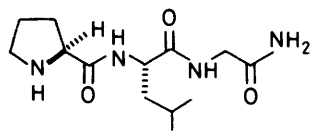
In a previous paper[1] we have described the preparation of the *Leu*-enkephalin analogue (**1**), which possesses opiate properties characteristic of the natural peptide. In compound (**1**) the dipeptide analogue prepared, the tyrosine-glycine isostere (**2**), only possesses one side-chain, the 4-hydroxybenzyl substituent of the tyrosine mimic. Herein we describe a process for the introduction of a second substituent by direct alkylation.

Two targets were initially chosen for these studies. The first was the melanocyte stimulating hormone release inhibiting factor (MIF), which has been assigned structure (**3**).[2] One of the problems encountered in attempts to assay the efficacy of MIF *in vivo* is the concurrency of degradation, for example, by enzymic hydrolysis; leucine amino-peptidase reportedly degrades over 90% of MIF within 4 h,[3] major hydrolysis of the proline-leucine bond being observed. A carbon–carbon double bond analogue of MIF (**3**) would be the derivative (**4**). Provided the replaced amide group is not involved in binding at the receptor site, for example, by aiding solvation, hydrogen bonding or because of dipolar characteristics, the analogue (**4**)

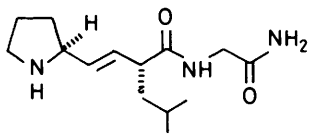


(**1**)

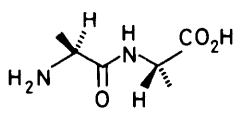
(**2**)



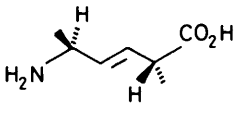
(**3**)



(**4**)



(**5**)

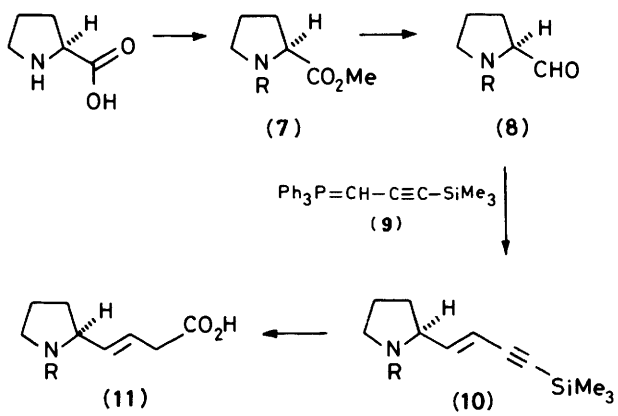


(**6**)

might be expected to retain some biological activity as well as being inert to peptidase attack.

The second target aimed at was the analogue of the dipeptide D-Ala-D-Ala (**5**).[4] This peptide unit is utilised in bacterial cell wall synthesis and it was hoped that the double bond mimic (**6**) might act as a competitive inhibitor.

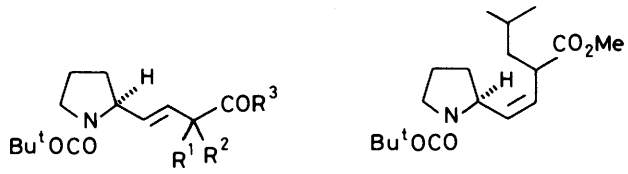
The approach to the analogue (**4**) commenced with L-proline (Scheme 1). Formation of the methyl ester followed by *N*-protection with the t-butoxycarbonyl group gave the ester (**7**). Selective reduction of the ester group with di-isobutylaluminium hydride produced the corresponding aldehyde (**8**), which could be treated with the Wittig reagent (**9**) to form the enyne (**10**). The major product was the required *trans*-isomer but, in this series, the product was contaminated by the less-polar *cis*-isomer, 10:1 *trans* : *cis* ratio. Separation of the *trans*-isomer (**10**) was achieved by silica gel chromatography but the yield obtained was always low (30—40%). Selective hydroboration and oxidation of the enyne (**10**) afforded the required *N*-protected acid (**11**). In order to introduce the isobutyl group corresponding to the leucine side-chain, selective α-alkylation was investigated. Previous work with simple unsaturated acids shows that α-alkylation might be expected. Thus enolate anions from αβ-unsaturated esters react in this manner[5] and, in a series of experiments, Schlessinger *et al.* illustrated this tendency by reaction of the lithium enolate of ethyl crotonate with a range of alkylating agents.[6] The question of the stereochemistry of the resulting α-substituted αβ-unsaturated ester was attended to by Kende and Toder[7] who recommended use of the methoxyethoxymethyl (MEM) esters. Although the direct alkylation of carboxylic acids had been reported,[8] in view of the presence of



Scheme 1.

† *Present address*: Smith, Kline and French Research Ltd., The Frythe, Welwyn, Herts AL6 9AR

the carbonate protecting group it was decided to proceed *via* an ester of the acid (**11**). The MEM ester (**12**) was initially prepared and alkylated with isobutyl iodide using lithium di-isopropylamide as base and in the presence of hexamethylphosphoric triamide (HMPA). A low yield (28%) of the mono-alkylated compound (**13**) was isolated accompanied by a small amount (8%) of the dialkylated material (**14**). The use of isobutyl bromide in place of the iodide gave poorer yields as did other variations. Subsequent attempts at deprotection of the alkylated ester (**13**), using anhydrous zinc bromide as catalyst,[9] was also inefficient, the best yield of liberated acid being less than 50%. Since both the alkylation and deprotection reactions on the MEM ester gave poor yields, use of the simpler methyl ester (**15**) as a protecting group was studied. This has the advantage of being both easily applied and removed and offers minimal steric hindrance to the alkylation step. In this series alkylation with isobutyl iodide, under the conditions described above, proceeded selectively and in moderate yield (50%) to give the mono-alkylated product (**16**). Little dialkylation was observed, although a small quantity of the *cis*-compound (**20**) (7%) was obtained, probably arising from contamination of the starting *trans*-ester (**15**). Alkylation was also achieved with benzyl bromide to produce the protected proline-phenylalanine analogue (**17**) in 56% yield.



(**20**)

(**12**) $R^1 = R^2 = H$, $R^3 = OCH_2OCH_2CH_2OMe$

(**13**) $R^1 = Bu^i$, $R^2 = H$, $R^3 = OCH_2OCH_2CH_2OMe$

(**14**) $R^1 = R^2 = Bu^i$, $R^3 = OCH_2OCH_2CH_2OMe$

(**15**) $R^1 = R^2 = H$, $R^3 = OMe$

(**16**) $R^1 = Bu^i$, $R^2 = H$, $R^3 = OMe$

(**17**) $R^1 = PhCH_2$, $R^2 = H$, $R^3 = OMe$

(**18**) $R^1 = Bu^t$, $R^2 = R^3 = OH$

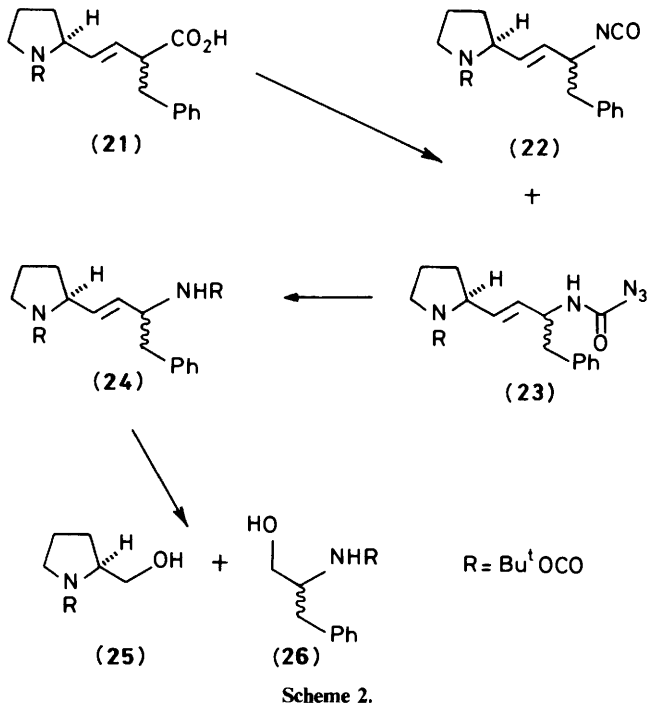
(**19**) $R^1 = Bu^t$, $R^2 = H$, $R^3 = NHCH_2CONH_2$

Although the alkylation products were expected to behave as a mixture of diastereoisomers, the alkylated materials, (**16**) and (**17**), could not be separated by normal chromatographic methods. Furthermore, the n.m.r. spectra of these products were complicated owing to the presence, in solution, of slowly equilibrating rotamers of the t-butoxycarbonyl group which lead to line broadening of key signals.[10] In order to investigate the chirality about the alkylated centre and to check the stereochemical integrity about the proline residue a chemical degradation procedure was employed. In this method (Scheme 2) the phenylalanine analogue (**17**) was initially hydrolysed to the acid (**21**) before a modified Curtius rearrangement using the Yamada method was carried out.[11] All reports on the Curtius reaction indicate that rearrangement proceeds with retention of configuration at the adjacent centre. Reaction of the acid (**21**) with diphenylphosphoryl azide and triethylamine in toluene at reflux afforded, as the principal product, the isocyanate (**22**) (45% yield), accompanied by the azido-amide (**23**) (20% yield). Heating either compound, or the mixture, with t-butyl alcohol produced the corresponding t-butoxycarbamate (**24**). Ozonolysis in dichloromethane followed by reduction of the

Table[a]

	$[\alpha]_{D1}$ Reference	$[\alpha]_D$ from (**24**)
N-BOC–prolinol	−47.2°	−45.3°
N-BOC–phenylalaninol	−29.2°	−0.9°

[a] Rotations obtained at 25 °C at *c* 1% in MeOH; average of 3 determinations quoted.



Scheme 2.

product with sodium borohydride in ethanol afforded two main products identified as *N*-t-butoxycarbonylproline (**25**) and *N*-t-butoxycarbonylphenylalaninol (**26**). The optical rotations of these isolates were compared with those of authentic samples prepared by reduction of their respective methyl esters with di-isobutylaluminium hydride (see Table). The L-proline derivative (**25**) serves as an internal reference and its close optical rotation compared with that of the reference specimen indicates substantially complete retention of configuration at this centre during the formation of the analogue (**21**) and its subsequent degradation. Of interest was the lack of optical rotation associated with the phenylalaninol portion (**26**). This indicates that no chiral induction occurs during alkylation, despite the existence of the adjacent chiral centre associated with the proline residue. The analogue (**19**) and the methyl ester (**17**) must, therefore, exist as a mixture of diastereoisomers. Although these mixtures could not be separated by chromatographic means, subsequent low temperature (−50 °C) examination at 400 MHz was sufficient to freeze out the rotamers and reveal the t-butyl group as a 4-peak signal (two diastereoisomers, each existing in two rotamer forms). The leucine analogue (**16**) gave similar results.

Completion of the synthesis of the MIF analogue (**3**) was continued with the diastereoisomeric intermediate (**16**). Alkaline hydrolysis gave the acid (**18**). This acid was next coupled with glycinamide using a modified procedure originally reported by Cash.[12] Removal of the t-butoxycarbonyl group from the amide (**19**) was effected either with trimethylsilyl iodide, produced *in situ* in acetonitrile from sodium iodide and

trimethylsilyl chloride[13] or by use of trifluoroacetic acid in trifluoroethanol.[14] The latter method was more efficient, giving an almost quantitative yield of the tripeptide analogue.

The above methodology was also applied to the preparation of the D-Ala-Ala analogue (**6**) (Scheme 3). In this series, also, alkylation of the intermediate with methyl iodide showed no stereoselectivity and the product was isolated as a mixture of diastereoisomers.

The above discussion details methods for the preparation of the double bond isosteres of protected units of Pro-Gly, Pro-Leu, Pro-Phe, Ala-Gly, and Ala-Ala.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. Specific rotations $[\alpha]_D^{20}$ were measured at the sodium D line frequency at 20 °C using a NPL Automatic Polarimeter, Type 243. I.r. spectra were recorded on a Perkin-Elmer 297 spectrophotometer for samples prepared as thin films unless otherwise stated. U.v. spectra were recorded for ethanolic solutions in quartz cells with a Pye Unicam SP800 spectrophotometer. ^{1}H N.m.r. spectra were recorded on a Jeol FX90Q (90 MHz), a Perkin-Elmer R32 (90 MHz), or a Varian EM (60 MHz) instrument. The 400 MHz spectra were kindly provided by Dr. B. E. Mann and his staff at the University of Sheffield. Samples were prepared as solutions in deuteriochloroform, unless otherwise stated, using tetramethylsilane as internal reference. Mass spectral data were recorded on a Kratos MS25 instrument and accurate mass measurements were made on an AEI/Kratos MS9/50 spectrometer. Solvents were generally distilled before use. Where appropriate, solvents were stored over 4A molecular sieves. Light petroleum refers to the fraction of boiling range 40—60 °C. Solutions were dried over anhydrous magnesium sulphate then filtered and evaporated under reduced pressure using a Büchi rotary evaporator. Thin layer chromatography (t.l.c.) was carried out on Merck plates coated with a 0.2 mm layer of Kieselgel 60F_{254} silica. Preparative layer chromatography (p.l.c.) was carried out on 20 × 20 cm plates coated with 2 mm of Kieselgel GF_{254} silica. In the nomenclature of compounds all stereocentres of known configuration are defined. Where a reaction produces a compound bearing a new chiral centre of unknown or mixed configuration, the centre is left undefined.

N-t-Butoxycarbonyl-L-proline. Methyl Ester (**7**).—Thionyl chloride (70 ml, 0.96 mol) was added, dropwise, to a solution of L-proline (100 g, 0.87 mol) in methanol at 5 °C. The solution was then heated to reflux for 16 h after which the solvent was removed by distillation and the crude methyl ester hydrochloride (156 g) dissolved in dimethylformamide (1.5 l). Triethylamine (175 g, 1.7 mol) was slowly added and the solution stirred for 20 min before the precipitate of triethylamine hydrochloride was filtered off. Di-t-butyl dicarbonate (200 g, 0.89 mol) was added to the solution and stirring was continued for a further 4 h. Solvent was removed, under reduced pressure, and the residue partitioned between brine (0.5 l) and ether (1 l). The aqueous phase was extracted with ether (2 × 0.5 l) and the combined organic extracts were washed with dilute HCl (2 × 250 ml), aqueous sodium hydrogen carbonate (250 ml) and finally water (250 ml) before being dried and evaporated to give the *title ester* as an oil (184.7 g, 91%), $[\alpha]_D^{20}$ −63° (*c* 1.0, MeOH); $\nu_{max.}$ 1 750 and 1 705 cm^{-1}; δ_H 1.37 (9 H, s, Me_3C), 1.7—2.1 (4 H, br m), 3.5 (2 H, m, CH_2), 3.75 (3 H, s, MeO), and 4.3 (1 H, m, NCH) (Found: C, 57.4; H, 8.2; N, 6.0. $C_{11}H_{19}NO_4$ requires C, 57.6; H, 8.0; N, 6.1%).

(2′*S*,3*E*)-4-(1-t-*Butoxycarbonylpyrrolidin-2-yl*)-1-*trimethylsilylbut-3-en-1-yne* (**10**).—The methyl ester (**7**) (74 g, 0.32 ml) in toluene (1.5 l) under nitrogen at −60 °C was treated dropwise with a solution of di-isobutylaluminium hydride in hexane (1M; 780 ml, 0.78 mol) over 1.5 h after which the mixture was stirred for a further 30 min at −60 °C. The reaction mixture was quenched by the continuous addition of methanol (100 ml) and the solution poured into 1M-aqueous potassium sodium tartrate (2.5 l). The mixture was stirred for 2 h after which the phases were separated and the aqueous phase extracted with ether (3 × 500 ml). The combined extracts were dried and evaporated to afford the crude aldehyde (**8**) as a viscous oil (68.2 g), δ_H 1.45 (9 H, s, Me_3C), 1.95 (4 H, m), 3.55 (2 H, m, NCH_2), 4.15 (1 H, br m, NCH), and 9.65 (1 H, d, *J* 2 Hz, CHO).

Triphenyl(3-trimethylsilylprop-2-ynyl)phosphonium bromide (**9**)[15] (146 g, 0.32 mol) was stirred under N_2 in THF (1.5 l) at −60 °C whilst a solution of butyl-lithium in hexane (1.55 M, 255 ml, 0.39 mol) was added over 20 min. The mixture was stirred for a further 30 min at −60 °C before the slow addition of a solution of the freshly prepared aldehyde (68.2 g) in THF (200 ml). The solution was allowed to warm to room temperature over 30 min and, after a further 1 h, brine (650 ml) was added and the mixture extracted with light petroleum (3 × 650 ml). The combined organic extracts were dried and the solvent removed under reduced pressure. The residue was triturated with light petroleum (100 ml) and the precipitate of triphenylphosphine oxide removed by filtration. The filtrate afforded a brown oil (79.3 g) which was first filtered through a short column of SiO_2, using ethyl acetate–light petroleum (1:10) as eluant, followed by chromatography through SiO_2 60G (2 kg) using a Jobin-Yvon h.p.l.c. apparatus with ethyl acetate–light petroleum (7:93) as solvent. The major fraction was the trans-*isomer* (**10**) (30.2 g, 32%), isolated as a viscous oil, $[\alpha]_D^{20}$ −122° (*c* 0.07, MeOH); $\nu_{max.}$ 2 135, 1 700, 1 480, 1 455, 1 390, 1 368, 1 250, 1 170, 1 115, 1 080, 955, 850, and 760 cm^{-1}; $\lambda_{max.}$ 255 nm (ε 1 330); δ_H 0.18 (9 H, s, Me_3Si), 1.45 (9 H, s, Me_3C), 1.6—2.2 (4 H, br m), 3.38 (2 H, m, NCH_2), 4.35 (1 H, m, NCH), 5.6 (1 H, d, *J* 16.7 Hz, 3-H), and 6.1 (1 H, dd, *J* 6.4, 16.7 Hz, 4-H) (Found: C, 65.8; H, 9.6; H, 9.6; N, 5.0. $C_{16}H_{27}NO_2Si$ requires C, 65.5; H, 9.3; N, 4.8%).

As a minor, more polar fraction, was isolated the corresponding cis-*isomer* (3.3 g, 3.5%), δ 0.19 (9 H, s, Me_3Si), 1.44 (9 H, s, Me_3C), 1.7—2.2 (4 H, m), 3.42 (2 H, m, NCH_2), 4.20 (1 H, m, NCH), 5.46 (1 H, d, *J* 11 Hz, 3-H), and 5.9 (1 H, dd, *J* 7, 11 Hz, 4-H) (Found: *m/z* 293.18099. $C_{16}H_{27}NO_2Si$ requires 293.18110).

(2′*S*,3*E*)-4-(1-t-*Butoxycarbonylpyrrolidin-2-yl*)*but-3-enoic Acid* (**11**).—Borane·THF complex (1M; 210 ml, 210 mmol) in THF (220 ml) at 0 °C under N_2 was treated with cyclohexane (37.5 g, 457 mmol), added dropwise, the temperature being maintained below 5 °C. The mixture was stirred for a further 1 h when a precipitate of dicyclohexylborane formed. To the suspension was then added, at 0 °C, a solution of the enyne (**10**) (20 g, 68 mmol) dissolved in THF (55 ml). The turbidity was gradually dissipated over a period of 2 h. Methanol (75 ml) was added followed by aqueous potassium hydroxide (1M; 220 ml), and then, with care, a solution of hydrogen peroxide (100 vol; 58 ml), the temperature of the reaction mixture being kept below 20 °C throughout these operations. The reaction mixture was then stirred at room temperature for 1.5 h, poured into water (900 ml), and extracted with ether (2 × 350 ml). The aqueous phase was acidified with phosphoric acid and extracted with ether (3 × 300 ml). The combined organic extracts were dried and evaporated under reduced pressure to yield the *title acid* as a waxy solid (13.1 g, 75%), m.p. 68—72 °C; $[\alpha]_D^{20}$ −16.8° (*c* 1.0, MeOH); $\nu_{max.}$ (Nujol) 3 700—2 500 and 1 750—1 650 cm^{-1}; δ_H 1.43 (9 H, s, Me_3C), 1.7—2.1 (4 H, m), 3.08 (2 H, d, *J* 6 Hz, CH_2CO), 3.40 (2 H, m, NCH_2), 4.25 (1 H, m, NCH), 5.55 (2 H, m, vinylic H), and 9.0 (1 H, br s, exch. D_2O, CO_2H) (Found: C,

61.1; H, 8.3; N, 5.2. $C_{13}H_{21}NO_4$ requires C, 61.2; H, 8.5; N, 5.5%).

(2'S,3E)-*Methoxyethoxymethyl* (1-*Butoxycarbonylpyrrolidine*-2-*yl*)*but*-3-*enoate* (**12**).—Methoxyethoxymethyl chloride (1.75 ml, 15 mmol) was added to a solution of triethylamine (1.52 g, 15 mmol) in THF (40 ml) under N_2. To the suspension thus formed was added a solution of the acid (**11**) (1.90 g, 7.5 mmol) in THF (30 ml) and the mixture was stirred at room temperature for 3.5 h. The solution was poured into an aqueous buffer solution (pH 3) (100 ml) and extracted with ether (2 × 100 ml). The combined extracts were dried and the solvent removed under reduced pressure to yield the title ester as an oil (2.12 g, 83%), $\nu_{max.}$ 2 980, 1 740, and 1 695 cm^{-1}; δ 1.45 (9 H, s, Me_3C), 1.6—2.1 (4 H, m), 3.1 (2 H, d, *J* 6 Hz, CH_2CO), 3.4 (3 H, s, MeO), 3.5 (2 H, br m, NCH_2), 3.5—3.9 (4 H, m, OCH_2CH_2O), 4.3 (1 H, m, NCH), 5.4 (2 H, s, OCH_2O), and 5.65 (2 H, m, vinylic H) (Found: *m*/*z* 343.199 46. $C_{17}H_{29}NO_6$ requires M^+, 343.199 47).

Alkylation of the MEM Ester (**12**) *with Isobutyl Iodide*.—To a solution of isopropylamine (4.4 ml, 32 mmol) and hexamethylphosphoric triamide (HMPA) in THF (200 ml) at −78 °C under N_2 was added a solution of butyl-lithium in hexane (1.5M; 202 ml, 30 mmol); the solution was stirred for 30 min at the same temperature, before a solution of the MEM ester (**12**) (4.45 g, 13.0 mmol) in THF (40 ml) was added. The reaction mixture was stirred for 45 min before isobutyl iodide (10.9 g, 60 mmol) was added and the solution stirred at −60 °C for a further 50 min; the reaction was then quenched with brine (100 ml). The mixture was separated and the aqueous phase extracted with ether (2 × 200 ml). The combined organic extracts were dried and the solvent removed under reduced pressure to yield an oil which was fractionated through SiO_2 60G (600 g) using ethyl acetate–light petroleum (1:1) as eluant. Three products were separated and identified; in order of elution these were:

(2'S,3E)-*Methoxyethoxymethyl* 4-(1-*t*-*Butoxycarbonylpyrrolidin*-2-*yl*)-2-*isobutylbut*-3-*enoate* (**13**) (1.46 g, 28%), isolated as an oil, $[\alpha]_D^{20}$ −15.3° (*c* 1.1, MeOH); $\nu_{max.}$ 1 740 and 1 695 cm^{-1}; $\delta_H(C_6D_6)$ 0.91 (6 H, 2 × d, *J* 5 Hz, Me_2CH), 1.54 (9 H, s, Me_3C), 1.4—1.85 (7 H, m), 2.8 (1 H, m, CHCO), 3.16 (3 H, s, MeO), 3.3—3.4 (4 H, m, OCH_2CH_2O), 3.7 (2 H, m, NCH_2), 4.2 (1 H, m, NCH), 5.3 (2 H, s, OCH_2O), and 5.55 (2 H, m, vinylic H) (Found: C, 63.6; H, 9.6; N, 3.6%; *m*/*z* 399.262 57. $C_{21}H_{37}NO_6$ requires C, 63.1; H, 9.3; N, 3.5%; *M*, 399.262 07).

(2'S,3Z)-*Methoxyethoxymethyl* 4-(1-*t*-*Butoxycarbonylpyrrolidin*-2-*yl*)-2-*isobutylbut*-3-*enoate*, as an oil (0.3 g, 5.8%), $[\alpha]_D^{20}$ −5.7° (*c* 1.0, MeOH); $\nu_{max.}$ 1 740 and 1 695 cm^{-1}; δ_H 0.9 (6 H, d, *J* 6 Hz, Me_2CH), 1.5 (9 H, s, Me_3C), 1.2—2.0 (7 H, m), 2.8 (1 H, m, CHCO), 3.0 (3 H, s, MeO), 3.3—3.5 (4 H, m, OCH_2CH_2O), 3.8 (2 H, m, NCH_2), 4.5 (1 H, m, NCH), 5.4 (2 H, s, OCH_2O), and 5.5 (2 H, m, vinylic H) (Found: *m*/*z* 399.262 45. $C_{21}H_{27}NO_6$ requires 399.262 07).

(2'S,3E)-*Methoxyethoxymethyl* 4-(1-*Butoxycarbonylpyrrolidin*-2-*yl*)-2,2-*di*-*isobutylbut*-3-*enoate* (**14**), as an oil (0.51 g, 8.6%), $[\alpha]_D^{20}$ −31.7° (*c* 1.0, MeOH); $\nu_{max.}$ 1 735 and 1 695 cm^{-1}; $\delta(C_6D_6)$ 0.90 (12 H, d, *J* 6 Hz, 2 × Me_2CH), 1.50 (9 H, s, Me_3C), 1.2—2.0 (10 H, m), 3.1 (3 H, s, MeO), 3.2—3.5 (4 H, m, OCH_2CH_2O), 3.7 (2 H, m, NCH_2), 4.3 (1 H, m, NCH), 5.3 (2 H, s, OCH_2O), and 5.6 (2 H, m, vinylic H) (Found: *m*/*z* 455.323 80. $C_{25}H_{46}NO_6$ requires 455.324 67).

Preparation of the Methyl Ester (**15**).—The acid (**11**) (5.6 g, 22 mmol) was esterified with an ethereal solution of diazomethane at room temperature. The crude ester was chromatographed through SiO_2 60G (100 g), using 1:1 ethyl acetate–light petroleum ether as eluant. The *ester* (**15**) was obtained as an oil (4.88 g, 82%), $[\alpha]_D^{20}$ −21.4° (*c* 1.0, MeOH); $\nu_{max.}$ 1 745 and 1 695 cm^{-1}; δ_H 1.44 (9 H, s, Me_3C), 1.7—2.1 (4 H, m), 3.07 (2 H, d, *J* 6 Hz, CH_2CO), 3.40 (2 H, m, NCH_2), 3.69 (3 H, s, MeO), 4.27 (1 H, m, NCH), and 5.57 (2 H, m, vinylic H) (Found: C, 62.6; H, 8.6; N, 5.1. $C_{14}H_{23}NO_4$ requires C, 62.4; H, 8.6; N, 5.2%).

Alkylations of the Methyl Ester (**15**).—(a) *Isobutyl iodide*. In a manner similar to that described for the MEM ester, the methyl ester (1.33 g, 4.9 mmol) was treated with lithium di-isopropylamide (2 equiv.) in the presence of HMPA (5 equiv.) in THF (100 ml). Isobutyl iodide (5.7 ml, 49 mmol) was added and the reaction mixture stirred at −78 °C for 1 h before being quenched with brine (100 ml). The mixture was extracted with ether (2 × 100 ml), and the combined extracts were dried and the solvent removed under reduced pressure to yield an oil (1.65 g). Chromatography through SiO_2 60G (100 g), using ethyl acetate–petroleum (1:3) as eluant gave (2'S,3E)-*methyl*-4-(1-*t*-*butoxycarbonylpyrrolidin*-2-*yl*)-2-*isobutylbut*-3-*enoate* (**16**) as an oil (0.74 g, 50%); $[\alpha]_D^{20}$ −20° (*c* 1.2, MeOH); $\nu_{max.}$ 1 740 and 1 695 cm^{-1}; δ_H (400 MHz) 0.86 (3 H, d, *J* 6 Hz, Me), 0.90 (3 H, d, *J* 6 Hz, Me), 1.43 (9 H, s, Me_3C), 1.5—2.0 (7 H, m), 3.09 (1 H, m, CHCO), 3.38 (2 H, m, NCH_2), 3.66 (3 H, two non-equivalent s, MeO), 4.25 (1 H, m, NCH), and 5.45 (2 H, m, vinylic H). At −50 °C the methoxy signal splits into a three-peak pattern; the NCH peak at δ 4.25 showed a downfield component at δ 4.37 of *ca.* 20% of the total peak integral; the t-butyl peak at 1.43 separates into three peaks at δ 1.42, 1.43, and 1.47. The patterns are explained by the existence of two diastereoisomers in solution and at the lower temperature, freezing out of the different conformers about the t-butoxycarbonyl group (Found: C, 66.7; H, 9.8; N, 4.3. $C_{18}H_{31}NO_4$ requires C, 66.4; H, 9.6; N, 4.3%)

The minor, cis-*monoalkylated ester* (**20**) was also isolated (0.11 g, 7%), as an oil, $[\alpha]_D^{20}$ −1.1° (*c* 1.0, MeOH); $\nu_{max.}$ 1 730 and 1 695 cm^{-1}; δ_H 1.0 (6 H, m, Me_2CH), 1.49 (9 H, s, Me_3C), 1.5—2.1 (7 H, m), 3.25 (1 H, m, CHCO), 3.55 (2 H, m, NCH_2), 3.72 (3 H, s, MeO), 4.6 (1 H, m, NCH), and 5.5 (2 H, m, vinylic H) (Found: *m*/*z* 325.224 71. $C_{18}H_{32}NO_4$ requires *M*, 325.225 30).

A small quantity (56 mg, 3.6%) of a material, identified as the dialkylated product, was also recovered from the chromatography.

(b) *Benzyl bromide*. In a similar manner, the methyl ester (**15**) (0.75 g, 2.8 mmol) was treated with lithium di-isopropylamide (1.1 equiv.) and then benzyl bromide (1.2 ml, 10 mmol). Work-up, including chromatography through SiO_2 (230—400 mesh; 100 g), using ethyl acetate–light petroleum (1:3) as eluant afforded (2'S,3E)-*methyl* 2-*benzyl*-4-(1-*t*-*butoxycarbonylpyrrolidin*-2-*yl*)*but*-3-*enoate* (**17**) as an oil (0.56 g, 56%), $[\alpha]_D^{20}$ −50.6° (*c* 1.0, MeOH); $\nu_{max.}$ 1 740 and 1 690 cm^{-1}; δ (400 MHz) 1.43 (9 H, s, Me_3C), 1.5—2.0 (4 H, m), 2.8 (1 H, m, PhC*H*), 3.1 and 3.33 (2 H, m, NCH_2), 3.33 (2 H, m, $PhCH_2$), 3.62 (3 H, s, MeO), 4.2 and 4.35 (1 H, m, NCH), 5.36 (1 H, dd, *J* 7, 16 Hz, vinylic H), 5.50 (1 H, dd, *J* 8, 16 Hz, vinylic H), and 7.2 (5 H, m, ArH) (Found: C, 70.2; H, 8.2; *m*/*z* 359.209 49. $C_{21}H_{29}NO_4$ requires C, 70.2; H, 8.1%; M^+, 359.209 64).

(2'S,3E)-2-*Benzyl*-4-(1-*t*-*butoxycarbonylpyrrolidin*-2-*yl*)*but*-3-*enoic Acid* (**21**).—The methyl ester (**17**) (0.6 g, 1.67 mmol) in methanol (7.5 ml) was stirred with 1M-potassium hydroxide (3 ml, 3 mmol) at room temperature for 16 h. The solution was acidified using aqueous phosphoric acid and extracted with ether (2 × 50 ml). The combined organic extracts were dried and the solvent removed under reduced pressure to yield the title acid as an oil (0.53 g, 88%), $[\alpha]_D^{20}$ −54.1° (*c* 1.0, MeOH); $\nu_{max.}$ 3 400, 1 730, and 1 690 cm^{-1}; δ_H 1.42 (9 H, s, Me_3C), 1.5—2.0 (4 H, m), 2.8 (1 H, m, $PhCH_2CH$), 3.0—3.3 (4 H, m, $PhCH_2$ and NCH_2), 4.2 (1 H, m, NCH), 5.4 (2 H, m, vinylic H), 7.2 (5 H,

m, ArH) (Found: C, 69.8; H, 7.9; *m/z* 345.193 16. $C_{20}H_{27}NO_4$ requires C, 69.6; H, 7.8%; M^+, 345.194 00).

Preparation of (2'S,1E)-1-*Benzyl*-1-*t-butoxycarbonylamino*-3-(*t-butoxycarbonylpyrrolidin*-2-*yl*)*prop*-2-*ene* (**24**).—A solution of the acid (**21**) (0.22 g, 0.7 mmol), triethylamine (76 mg, 0.75 mmol), and diphenylphosphoryl azide (0.19 g, 0.7 mmol) in toluene (10 ml) was heated to reflux for 7 h. The solvent was removed under reduced pressure and the residual oil chromatographed through SiO_2 (230—400 mesh; 30 g) using ethyl acetate–light petroleum (1:4) as eluant. The main fraction was the isocyanate (**22**) (0.11 g, 45%), $\nu_{max.}$ 2 260 and 1 760 cm^{-1}; δ_H 1.45 (9 H, s, Me_3C), 1.5—2.1 (4 H, m), 2.88 (2 H, d, *J* 7 Hz, PhCH_2), 3.4 (2 H, m, NCH_2), 4.31 (1 H, m, NCH), 5.55 (2 H, m, vinylic H), and 7.29 (5 H, m, ArH).

The minor fraction (49 mg, 20%) was the azido amide (**23**), $\nu_{max.}$ 3 280, 1 140, and 1 695 cm^{-1} (Found: *m/z* 385.211 97. $C_{20}H_{27}N_5O_3$ requires *M*, 385.211 38).

The isocyanate (0.10 g, 0.26 mmol) was heated under reflux in t-butyl alcohol (6 ml) for 2 days after which the solvent was removed and the residue filtered and through SiO_2, using ethyl acetate—light petroleum (1:4) as solvent to give the *title carbamate* (92 mg, 77%) as an oil, $[\alpha]_D^{20}$ −43.5° (*c* 1.0, MeOH); $\nu_{max.}$ 3 340 and 1 695 cm^{-1}; δ 1.45 (18 H, s, 2 × Me_3C), 1.5—2.0 (4 H, m), 2.85 (2 H, d, *J* 6 Hz, PhCH_2), 3.37 (2 H, m, NCH_2), 4.5 (1 H, m, NCH), 5.43 (2 H, m, vinylic H), and 7.25 (5 H, m, ArH) (Found: C, 69.5; H, 8.8; N, 6.5. $C_{24}H_{36}N_2O_4$ requires C, 69.2; H, 8.7; N, 6.7%).

Treatment of the crude Curtius reaction product with t-butyl alcohol also afforded the title carbamate.

Reductive Ozonolysis of the Carbamate (**24**).—The carbamate (0.11 g, 0.27 mmol) in dichloromethane (15 ml) at —15 °C was treated with a stream of ozonised oxygen for 8 min. The solution was flushed with nitrogen and the solvent removed under reduced pressure. The residue was dissolved in dry ethanol (5 ml) and treated with sodium borohydride (52 mg, 5 equiv.) at 0 °C with stirring. After 1.5 h at 0 °C the reaction mixture was quenched with dilute phosphoric acid and the solution extracted with chloroform (3 × 15 ml). The combined organic extracts were dried, the solvent removed under reduced pressure, and the residue chromatographed through SiO_2 (230—400 mesh; 10 g) using ethyl acetate–light petroleum (1:1) as eluant. Two major fractions were obtained. *N*-t-Butoxycarbonylprolinol (**25**) was isolated as an oil (14 mg, 25%), $[\alpha]_D^{20}$ −45.3° (*c* 1.0, MeOH), identical in its chromatographic and spectroscopic properties with an authentic sample (*vide infra*). N-Butoxycarbonylphenylalaninol (**26**) crystallised from ethyl acetate–light petroleum as colourless crystals (9 mg, 13%), m.p. 83—84 °C; $[\alpha]_D^{20}$ −0.9° (*c* 1.0, MeOH); $\nu_{max.}$ 3 500—3 100, 3 350, and 1 682 cm^{-1} (Found: C, 59.8; H, 9.6; N, 6.8. $C_{10}H_{19}NO_2$ requires C, 59.7; H, 9.5; N, 7.0%). The ^1H n.m.r. and i.r. spectra of this material were superimposible on those of an authentic sample of *N*-Boc-L-phenylalaninol (*vide infra*).

N-*t-Butoxycarbonyl*-L-*prolinol* (**25**).—N-t-Butoxycarbonyl-L-proline methyl ester (**7**) (1.38 g, 6.0 mmol) in toluene (30 ml) at −78 °C was treated with a solution of di-isobutylaluminium hydride in hexane (1.2M; 12 ml, 2.4 equiv.). After being stirred at −78 °C for 30 min the reaction mixture was warmed to room temperature over 3 h and then quenched with methanol (2 ml). The mixture was poured into aqueous potassium sodium tartrate (1M; 40 ml) and extracted with ether (2 × 35 ml). The combined organic layers were dried and the solvent removed under reduced pressure to yield an oil (1.1 g) which was chromatographed through SiO_2 (230—400 mesh; 80 g) using ethyl acetate–light petroleum (1:1) as eluant. The *title alcohol* was isolated as an oil (0.43 g, 36%), $[\alpha]_D^{20}$ −47.2° (*c* 1.0, MeOH); $\nu_{max.}$ 3 420, 1 690, and 1 670 cm^{-1}; δ_H 1.50 (9 H, s, Me_3C) 1.6—2.2 (4 H, m), 3.53 (2 H, m, NCH_2), 3.73 (2 H, d, *J* 7 Hz, CH_2O), 4.0 (1 H, m, NCH), and 4.64 (1 H, br s, OH) (Found: C, 59.8; H, 9.6; N, 6.8. $C_{10}H_{19}NO_3$ requires C, 59.7; H, 9.5; N, 7.0%).

N-*t-Butoxycarbonyl*-L-*phenylalaninol*.—N-t-Butoxycarbonyl-L-phenylalanine methyl ester (0.7 g, 2.5 mmol) in toluene (40 ml) was reduced with di-isobutylaluminium hydride (1.2M solution in hexane; 9 ml, 10.8 mmol) in a manner similar to that described above. After chromatography the *title alcohol* was isolated as a crystalline solid (0.44 g, 70%), m.p. 94.5—95.5 °C; $[\alpha]_D^{20}$ −29.2° (*c* 1.0, MeOH); $\nu_{max.}$ 3 500—3 100 and 1 683 cm^{-1}; δ_H 1.45 (9 H, s, Me_3C), 2.5 (1 H, br s, OH), 2.85 (2 H, d, *J* 7 Hz, PhCH_2), 3.7 (1 H, m, CH_2O), 3.85 (1 H, m, NCH), 4.9 (1 H, br s, NH), and 7.33 (5 H, s, ArH) (Found: C, 67.0; H, 8.6; N, 5.3. $C_{14}H_{21}NO_3$ requires C, 66.9; H, 8.4; N, 5.6%).

Preparation of the Glycinamide Adduct (**3**).—The methyl ester (**16**) (0.69 g, 2.1 mmol) was hydrolysed with 1M-potassium hydroxide (3 ml) in methanol (10 ml) at room temperature for 13 h. The mixture was then acidified with phosphoric acid, diluted with brine and extracted with ethyl acetate (2 × 20 ml); the organic extract was dried and the solvent removed under reduced pressure to afford the *free acid* (**18**) (0.59 g, 90%), as an oil, $[\alpha]_D^{20}$ −20° (*c* 1.0, MeOH); $\nu_{max.}$ 3 700—3 000, 1 730, and 1 690 cm^{-1} (Found: *m/z* 311.209 79. $C_{17}H_{19}NO_4$ requires *M*, 311.209 65).

N-Methylmorpholine (0.36 g, 3.6 mmol) was added to the free acid (**18**) (0.5 g) dissolved in THF (10 ml) and the solution cooled to 0 °C before isobutyl chloroformate (0.39 g, 2.4 mmol) was added. The solution was then stirred for 1 h at 0 °C before a solution of glycinamide hydrochloride (1.0 g, 9.0 mmol) and *N*-methylmorpholine (1.2 g, 12 mmol) in THF–water (1:1) (6 ml) was added. The solution was stirred for 16 h without further cooling and the solvents removed under reduced pressure. The residue was chromatographed through SiO_2 (60G; 100 g) using methanol–chloroform (1:9) as eluant to afford (2'S,3E)-4-(1-*t-butoxycarbonylpyrrolidin*-2-*yl*)-2-*isobutylbut*-3-*enoylglycinamide* (**19**) (0.73 g, 94%) as an oil, $[\alpha]_D^{20}$ −32° (*c* 1.0, MeOH); $\nu_{max.}$ 3 700—3 000 and 1 750—1 600 cm^{-1}; δ_H 1.43 (9 H, s, Me_3C), 1.1—2.1 (7 H, m), 2.95 (1 H, br s, NH), 3.39 (2 H, m, NCH_2), 3.9 (2 H, d, *J* 6 Hz, NCH_2CO), 4.2 (1 H, m, NCH), 5.5 (2 H, m, vinylic H), 5.7 (1 H, br s, NH), and 6.5 (1 H, br s, NH) (Found: C, 62.2; H, 9.2; N, 11.8. $C_{19}H_{33}N_3O_4$ requires C, 62.1; H, 9.1; N, 11.4%).

(2'S,3E)-2-*Isobutyl*-4-*pyrrolidin*-2-*yl*)*but*-3-*enoylglycinamide Hydrochloride* (**27**).—The *N*-Boc-glycinamide (**19**) (0.31 g, 1 mmol) was dissolved in 2,2,2-trifluoroethanol (5.85 ml) at room temperature under N_2 and trifluoroacetic acid (0.31 ml, 4 mmol) was added; the mixture was then stirred for 24 h. Solvents were removed under reduced pressure and concentrated HCl (0.5 ml) added to the residue before removal of the water and excess of acid under reduced pressure to afford the *title salt* as a solid foam (0.25 g, 97%), δ 0.9 (6 H, d, *J* 6 Hz, Me_2CH), 1.1—2.1 (7 H, m), 3.35 (2 H, m, NCH_2), 3.9 (2 H, d, *J* 7 Hz, NCH_2CO), 4.2 (1 H, m, NCH), and 5.85 (2 H, m, vinylic H) [Found: (M^+ − HCl) 267.194 31. $C_{14}H_{25}N_3O_2$ requires 267.194 67].

Methyl N-*t-Butoxycarbonyl*-D-*alaninate*.—A solution of methyl D-alaninate hydrochloride (7.8 g, 56 mmol) in dimethylformamide (100 ml) at 0 °C was treated with triethylamine (11.3 g, 0.11 mol) and then di-t-butyl dicarbonate (13.5 g, 62 mmol) in dimethylformamide (50 ml). The solution was stirred at room temperature for 8 h, filtered, and the solvent removed under reduced pressure. The residue was dissolved in chloroform (75 ml) and washed with dilute HCl (50 ml),

followed by aqueous sodium hydrogen carbonate (20 ml) and water. The organic phase was dried and the solvent removed under reduced pressure to yield the *title ester* as an oil (8.5 g, 75%), $[\alpha]_D^{20}$ + 45.1° (*c* 1.0 MeOH); $\nu_{max.}$ 3 360, 1 740, and 1 700 cm^{-1}; δ 1.40 (3 H, d, *J* 7 Hz, *Me*CH), 1.47 (9 H, s, Me_3C), 3.77 (3 H, s, MeO), 4.4 (1 H, q, *J* 7 Hz, MeC*H*), and 5.2 (1 H, br s, NH) (Found: C, 53.2; H, 8.6; N, 6.9. $C_9H_{17}NO_4$ requires C, 53.2; H, 8.4; N, 6.9%).

(5R,3E)-5-*t*-*Butoxycarbonylamino*-1-*trimethylsilylhex*-3-*en*-1-*yne* (**28**).—N-t-Butoxycarbonyl-D-alanine methyl ester (5 g, 25 mmol) in toluene (100 ml) under N_2 at −78 °C was stirred with di-isobutylaluminium hydride (1.2M solution in hexane; 49 ml, 2.4 equiv.) for 1 h before quenching of the reaction mixture with methanol (5 ml). The solution was warmed to room temperature and poured into a stirred solution of potassium sodium tartrate (1M; 500 ml). After 2.5 h the mixture was extracted with ether (3 × 250 ml) and the combined organic extracts dried, and the solvent removed under reduced pressure to yield the crude aldehyde, which was immediately processed further.

A suspension of triphenyl(3-trimethylsilylprop-2-ynyl)phosphonium bromide (**9**) (11.2 g, 25 mmol) in THF (150 ml) at −78 °C under N_2 was treated with butyl-lithium (1.54M in hexane; 25 mmol) over 40 min to generate a deep red solution. A solution of the freshly prepared, crude aldehyde (4.9 g) in THF (10 ml) was added, with stirring, at −78 °C over 10 min and the reaction mixture was then allowed to slowly warm to room temperature. After 40 min saturated aqueous ammonium chloride (150 ml) was added and the phases separated. The aqueous layer was re-extracted with light petroleum (2 × 150 ml) and the organic extracts combined, dried, and the solvent removed under reduced pressure. Trituration of the residue with light petroleum at 0 °C afforded a precipitate of triphenylphosphine oxide which was filtered off and the filtrate chromatographed through SiO_2 (230—400 mesh; 250 g), using ethyl acetate–light petroleum (1:9) as eluant. The major product was the *title olefin* (2.2 g, 33%), as an oil, $[\alpha]_D^{20}$ + 66.5° (*c* 1.0, MeOH); $\nu_{max.}$ 3 420, 2 160, and 1 695 cm^{-1}; δ_H 0.17 (9 H, s, Me_3Si), 1.20 (3 H, d, *J* 7 Hz, *Me*CH), 1.43 (9 H, s, Me_3C), 4.27 (1 H, m, MeC*H*), 4.50 (1 H, br s, NH), 5.59 (1 H, dd, *J* 1, 16 Hz, CH=CH-C≡), and 6.12 (1 H, dd, *J* 6, 16 Hz, C*H*=CH-C≡) (Found: C, 63.3; H, 9.6; N, 4.9. $C_{14}H_{25}NO_2Si$ requires C, 62.9; H, 9.4; N, 5.2%).

Methyl (5R,3E)-5-*t*-*Butoxycarbonylaminohex*-3-*enoate*.—A solution of the silylated acetylene (**28**) (2 g, 7.5 mmol) in THF (5 ml) was added to a freshly prepared solution of dicyclohexylborane in THF (20 ml) (*ex.* hexane, 20 mmol) at 0 °C and the mixture was stirred at this temperature for 1.5 h; methanol (5 ml) and aqueous potassium hydroxide (1M; 20 ml) were then added followed by hydrogen peroxide (100 vol, 6 ml) (added with care), the reaction temperature being kept below 5 °C. The reaction mixture was stirred for a further 1 h and then worked up by partitioning between saturated aqueous sodium hydrogen carbonate (30 ml) and ether (40 ml). The organic layer was re-extracted with more base and the combined aqueous phases were acidified with phosphoric acid and extracted with ether (2 × 200 ml). The organic extract was dried and solvent removed under reduced pressure to yield (5*R*,3*E*)-5-t-butoxycarbonylaminohex-3-enoic acid as an oil (0.43 g, 28%), $[\alpha]_D^{20}$ + 22° (*c* 1.0, MeOH); $\nu_{max.}$ 3 380 and 1 700 cm^{-1}. The acid was esterified with diazomethane in ether to afford the *title ester* (88%), $[\alpha]_D^{20}$ +21.1° (*c* 1.0, MeOH); $\nu_{max.}$ 3 340, 1 740, and 1 690 cm^{-1}; δ_H 1.23 (3 H, d, *J* 7 Hz, *Me*CH), 1.45 (9 H, s, Me_3C), 3.07 (2 H, d, *J* 6 Hz, CH_2CO_2), 3.7 (3 H, s, MeO), 4.2 (1 H, m, NCH), 4.5 (1 H, br s, NH), and 5.6 (2 H, m, olefinic H) (Found: C, 58.9; H, 8.8; N, 5.6. $C_{13}H_{23}NO_4$ requires C, 59.3; H, 8.6; N, 5.8%).

Methyl (5R,3E)-5-*t*-*Butoxycarbonylamino*-2-*methylhex*-3-*enoate*.—The ester (**29**) (0.41 g, 1.6 mmol) in THF (10 ml) was added to a freshly prepared solution of lithium di-isopropylamide (1.7 mmol) in THF (40 ml) containing hexamethylphosphoric triamide (1.35 ml, 7.8 mmol) at −78 °C. After 15 min iodomethane (0.48 ml, 7.8 mmol) was added and the solution stirred at −78 °C for 45 min before quenching of the reaction mixture with aqueous ammonium chloride (25 ml). The aqueous layer was extracted with ether (3 × 25 ml) and the combined organic extracts were dried and the solvent removed under reduced pressure. The residual oil was chromatographed through SiO_2 (70—230 mesh; 50 g) using ethyl acetate–light petroleum (1:4) as eluant to yield the *title ester* as an oil (0.26 g, 61%), $[\alpha]_D^{20}$ +25° (*c* 1.0, MeOH); $\nu_{max.}$ 3 370, 1 740, and 1 695 cm^{-1}; δ_H 1.19 (3 H, d, *J* 7 Hz, *Me*CH), 1.25 (3 H, d, *J* 7 Hz, *Me*C*H*), 1.44 (9 H, s, Me_3C), 3.15 (1 H, m, MeC*H*CO), 3.66 (3 H, s, MeO), 4.20 (1 H, m, NCH), 4.50 (1 H, m, NH), and 5.57 (2 H, m, vinylic H) (Found: *m/z* 257.163 37. $C_{13}H_{23}NO_4$ requires *M*, 257.162 70).

(5R,3E)-5-*Amino*-2-*methylhexan*-3-*oic Acid* (**6**).—The ester (**30**) (0.17 g, 0.67 mmol) in methanol (3.3 ml) was hydrolysed with aqueous potassium hydroxide (1M; 1.3 ml) at room temperature for 15 h before acidification with phosphoric acid and extraction into chloroform (3 × 15 ml). The combined extracts were dried and evaporated to give an oil (0.15 g) which was passed through SiO_2 (70—130 mesh; 20 g) using ethyl acetate–light petroleum (1:3) containing 0.1% acetic acid as eluant. The main fraction contained (5R,3E)-5-*t*-*butoxycarbonylamino*-2-*methylhexan*-3-*oic acid* (70 mg, 43%), $[\alpha]_D^{20}$ +24.1° (*c* 1.0, MeOH); $\nu_{max.}$ 3 330 and 1 700 cm^{-1}; δ_H 1.25 (6 H, m, 2 × *Me*CH), 1.45 (9 H, s, Me_3C), 3.15 (1 H, m, MeC*H*CO), 3.6 (1 H, br s, OH), 4.2 (1 H, m, NCH), 4.6 (1 H, br s, NH), and 5.6 (2 H, m, vinylic H) (Found: C, 59.3; H, 8.5; N, 5.7. $C_{12}H_{21}NO_4$ requires C, 59.3; H, 8.6; N, 5.8%).

This acid (60 mg, 0.25 mmol) was dissolved in 2,2,2-trifluoroethanol (1 ml) and trifluoroacetic acid (0.04 ml) at room temperature. After 24 h the solvent was removed under reduced pressure to yield an oil which was dissolved in water (10 ml) and extracted with dichloromethane (2 × 10 ml). The aqueous phase was separated and the solvent removed under reduced pressure to yield the title amino acid as an amorphous solid, $\nu_{max.}$ 3 800—2 500 and 1 675 cm^{-1}; δ_H 1.21 (3 H, d, *J* 8 Hz, *Me*CH), 1.33 (3 H, d, *J* 7 Hz, *Me*CH), 3.24 (1 H, m, MeC*H*CO), 3.9 (1 H, m, NeC*H*N), 5.61 (1 H, dd, *J* 7, 15 Hz, C*H*=CH), and 5.93 (1 H, dd, *J* 7, 15 Hz, C*H*=CH). The compound gave no parent ion in its mass spectrum.

Acknowledgements

We thank the S.E.R.C. and Roussel Laboratories Ltd., for a CASE award (to N. J. M.).

References

1 M. M. Hann, P. G. Sammes, P. D. Kennewell, and J. B. Taylor, *J. Chem. Soc., Perkin Trans 1*, 1982, 307.
2 R. G. M. Nair, A. J. Kasten, and A. V. Schally, *Biochem. Biophys. Rev. Commun.*, 1971, **43**, 1376.
3 N. Marks and R. Walter, *Proc. Soc. Exp. Biol. Med.*, 1972, **140**, 673.
4 *Cf.* D. J. Tipper and J. L. Strominger, *Proc. Natl. Sci., U.S.A.*, 1965, **54**, 1133.
5 M. W. Rathke and D. Sullivan, *Tetrahedron Lett.*, 1972, 4249.
6 J. L. Herrmann, G. R. Kierczykowski, and R. H. Schelssinger, *Tetrahedron Lett.*, 1973, 2433.
7 A. S. Kende and B. H. Toder, *J. Org. Chem.*, 1982, **47**, 167.

8 P. L. Creger, *J. Am. Chem. Soc.*, 1967, **89**, 2500.
9 E. J. Corey, J. L. Gras, and P. Ulrich, *Tetrahedron Lett.*, 1976, 809.
10 C. M. Deber, F. A. Bovey, J. A. Carver, and E. R. Blout, *J. Am. Chem. Soc.*, 1970, **92**, 6191.
11 K. Ninomiya, T. Shioiri, and S. Yamada, *Tetrahedron*, 1974, 2151.
12 W. D. Cash, *J. Org. Chem.*, 1961, **26**, 2136.
13 G. A. Olah, S. C. Narang, B. G. B. Gupta, and R. Malhotra, *J. Org. Chem.*, 1717, **44**, 1247; R. S. Lott, V. S. Chauhan, and C. H. Stammer, *J. Chem. Soc., Chem. Commun.*, 1979, 495; S. W. King and C. H. Stammer, *J. Org. Chem.*, 1891, **46**, 4789.
14 A. J. Parker, U. Mayer, R. Schmidt, and V. Gutmann, *J. Org. Chem.*, 1978, **43**, 1843; D. J. Raber, W. C. Neal, M. D. Dukes, J. M. Harris, and D. L. Mount, *J. Am. Chem. Soc.*, 1978, **100**, 8137.
15 M. Ahmed, G. C. Barley, M. T. W. Hearn, E. R. H. Jones, and V. Thaller, *J. Chem. Soc., Perkin Trans. 1*, 1974, 1981.

Received 7th February 1985; *Paper* 5/221