On the Double Bond Isostere of the Peptide Bond: Preparation of an Enkephalin Analogue

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Methodology for preparing dipeptide analogues in which a carbon–carbon double bond replaces the normal amide bond is described. Thus, the protected tyrosylglycine analogue, (*S*)-*trans*-5-t-butyloxycarbonylamino-6-(4-t-butoxyphenyl)hex-3-enoic acid has been synthesised and incorporated into the *Leu*-enkephalin analogue (3) by condensation with glycylphenylalanyl-leucine. The enkephalin analogue retained biological activity. The significance of this isosteric replacement of the amide group is discussed.

THE isolation of the natural opiates, the enkephalins (1) and (2), by Kosterlitz and his colleagues in 1975 [1] has stimulated many studies on structure–activity relationships of a range of related peptide derivatives [2] as well as

L - Tyr - Gly - Gly - L - Phe - L - Leu (1)

L - Tyr - Gly - Gly - L - Phe - L - Met (2)

structural comparisons with the exogenous morphine opiates.[3] Such studies have highlighted certain features of the natural substances, including their inability to cross the blood-brain barrier and their short lifetimes *in vivo* as a consequence of rapid enzymic degradations.[4] Various attempts to overcome the latter properties of the natural enkephalins have been made, amongst which are introduction of *N*-methyl groups into the amide bonds and incorporation of amino-acids of the (unnatural) D-configuration into the peptide sequence at the site of hydrolysis. Whilst some applications of these strategies have resulted in the preparation of active analogues, both approaches significantly modify the spatial characteristics of the region near the modified amino-acid and this may not be of benefit to the active-site conformation of the enkephalin analogue.

In our approach, described herein, it was considered that replacement of one of the labile peptide bonds with a stable group of similar spatial requirements might help to introduce the desired properties. Although isosteric replacements for the peptide bond have been investigated previously,[5] the simple substitution of an amide bond by a *trans*-carbon–carbon double bond has largely been ignored.[6] This is surprising when one realises the similarity in geometrical disposition of substituents attached to either of these functional groups (Figure). Thus, *X*-ray data show that amide bonds in peptides and proteins invariably adopt a planar configuration with the groups attached to the central bond in a *trans*-orientation. A comparison of typical parameters (Figure) shows that the distance X–Y in both structures is 3.8 Å, whilst the difference in the direction of bond approach is only about 5°; in such flexible molecules as peptides this small difference should be almost irrelevant to the overall geometry. Replacement of an amide bond by a *trans*-carbon–carbon double bond should not alter significantly the overall conformation of a peptide molecule, and hence its biological activity, provided that the replaced amide bond is not directly involved in either the secondary and tertiary structures of the peptide or the mechanism whereby the biological response is elicited. Conversely, one could argue that any such replacement in which a fundamental change of overall conformation or biological response *is* observed, indicates that the replaced amide bond has a more important role than that of a simple spacer unit.

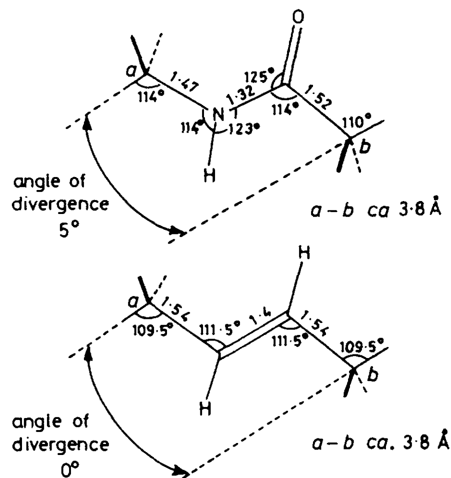
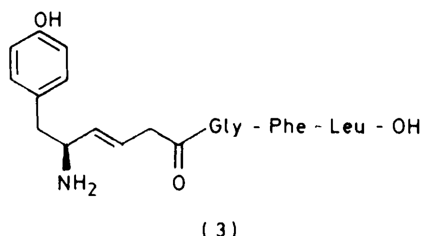


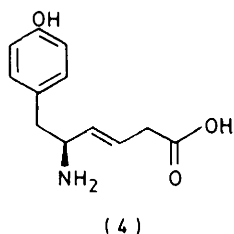
FIGURE Spatial disposition of amide and *trans*-olefin bonds (distances in Å)

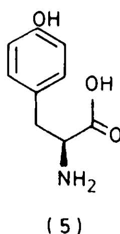
The target of our studies was the analogue (3) of the enkephalin (1). Replacement of the Tyr-Gly amide bond was selected because (i) hydrolysis of this bond, by an aminopeptidase, is one of the principal routes for the enzymic degradation of *Leu*-enkephalin,[4] (ii) the double bond should be more lipophilic than the amide bond and should enhance transport of the analogue (3) across fatty membranes, and (iii) this should help to resolve the controversy in the literature about the role of this amide bond in the secondary structure of the molecule, involving possible hydrogen bonding of the

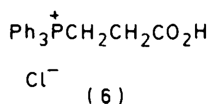
carbonyl group to the phenylalanine nitrogen *via* a β-bend.[3]

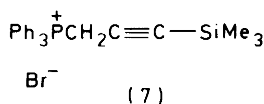
The synthesis of the isosteric *Leu*[5]-enkephalin analogue (3) was planned along the lines of a classical peptide synthesis involving the coupling of suitably protected units. Thus the tripeptide unit, H-Gly-L-Phe-L-Leu-OH

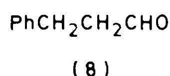


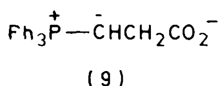


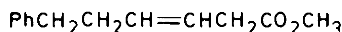












(10) *cis*
(11) *trans*

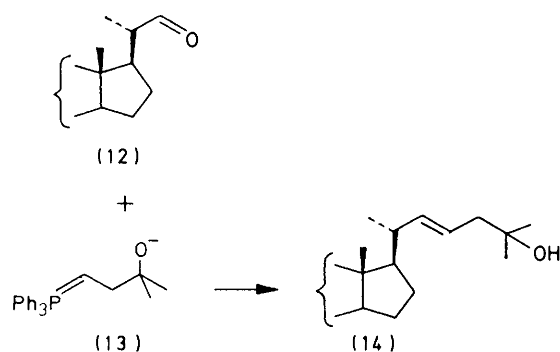
could be readily assembled in a protected form and then coupled to a protected form of the δ-amino-acid derivative (4). The synthesis of the latter amino-acid required careful attention to the selection of protecting groups so that final deprotection would not cause unwanted chemical transformations within this unit. Besides being a δ-amino acid, compound (4) also contains a phenolic group, and a *trans*-double bond in such a position that it is both an allylic amine and a βγ-unsaturated acid. Any synthesis should also result in the (*S*)-chiral form, in order to mimic the natural configuration of the Tyr[1]-Gly[2] unit, and for this reason we selected L-tyrosine (5) as the starting material.

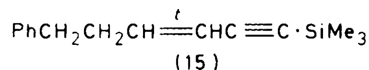
In order to synthesise the δ-amino-acid (4) from tyrosine (5), addition of a three-carbon unit and a change in the oxidation level of the latter is required. Several approaches to the desired three-carbon-synthon were examined, including use of the reagents (6)[7] and (7).[8] The acid (6) was reported to produce geometrical isomers in reactions with ketones,[7] so a study was made of its stereochemical behaviour with 3-phenylpropionaldehyde (8). Since the reagent (6) reacts *via* the unstabilised dianion (9), the product, after methylation, was expected to be the *cis*-isomer (10). Reaction between (8) and (9) afforded, in poor yield (20%), an acidic compound, which, after methylation, was identified, by ^1H n.m.r. spectroscopy, as the unexpected *trans*-isomer (11), containing only a small amount of the *cis*-isomer (*trans* : *cis* 95 : 5). The observed formation of the unexpected *trans*-isomer (11) requires some explanation. Modifications of the Wittig reaction have been reported in which the initially formed *erythro*-oxaphosphetan, derived from an unstabilised ylide and an aldehyde, is in equilibrium with the thermodynamically more stable *threo*-isomer.[9] In the reaction between the ylide (9) and phenylpropionaldehyde (8) it is assumed that the carboxylate anion acts as an intramolecular base to catalyse a similar transformation, resulting in the observed, predominant formation of the *trans*-isomer (11). A related explanation has been suggested by Salmond *et al.*[10] to rationalise formation of the *trans*-isomer (14) from reaction between the steroidal aldehyde (12) and the ylide (13).

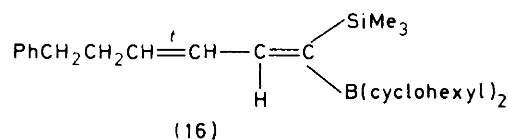
Reaction between the ylide from the salt (7) and phenylpropionaldehyde (8) afforded a good yield (68%) of the silylated *trans*-enyne (15). This could be oxidised to the corresponding βγ-unsaturated acid (11) by use of the method of Zweifel and Backlund.[11] Treatment of (15) when 1 equiv. of dicyclohexylborane yielded the intermediate (16), which was oxidised at 0 °C with 4 equiv. of alkaline hydrogen peroxide to yield the acid (11), shown to be identical with the product obtained from the ylide (9). Thus the *trans*-double bond in (15) maintains its stereochemical and positional integrity throughout these transformations.

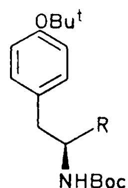
With these methods for introducing the three-carbon unit secured, attention was directed towards preparation of the dipeptide analogue (4), which required formation of a protected form of the amino-aldehyde corresponding to L-tyrosine. Preliminary experiments indicated that both amino and phenolic protection was necessary, the aldehyde of choice being the *N*-t-butoxycarbonyl-*O*-t-butyl-aldehyde (17). The aldehyde was itself obtained by reduction of the methyl ester (18) with di-isobutylaluminium hydride (DIBAL), under conditions similar to those described by Ito *et al.*[12] These authors showed that optically active amino-aldehydes are rapidly racemised by silica gel during chromatography and recommended purification *via* formation of the non-enolisable semicarbazones. Since the aldehyde (17) obtained from the reduction with DIBAL was sometimes contaminated by the alcohol (19), as an over-reduction product, it was purified and stored *via* its semicarbazone (20).

Preparation of the methyl ester (18) was achieved by a quicker and more efficient modification of the literature method[13] (Scheme 1). The use of boron trifluoride–ether as catalyst for the preparation of the t-butyl ether of tyrosine has not previously been reported; its use in this case seemed possible because of the known stability

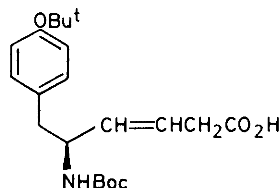




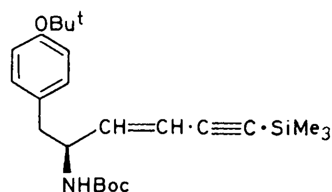




(17) R = CHO
(18) R = CO_2Me
(19) R = CH_2OH
(20) R = $CH{=}NNHCONH_2$



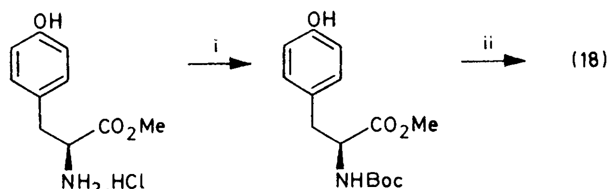
(21) *trans*
(22) *cis*



(23) *trans*
(24) *cis*

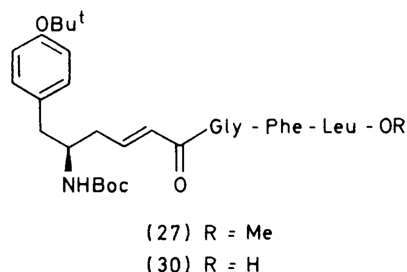
of the t-butoxycarbonyl group to the ether complex in dichloromethane at 0 °C.[14] The yield of the ester (18) from the methyl ester of tyrosine was 50%, an improvement of 20% over the longer literature method.[13]

Reaction of the aldehyde (17) with the ylide salt (9) proceeded in low yield (8%) to give a mixture of the corresponding *trans*- and *cis*-acids (21) and (22). A more efficient reaction occurred with the acetylenic ylide derived from (7). The product was a 13 : 1 mixture of the *trans*- and *cis*-enynes, (23) and (24). The less-polar *trans*-isomer (23) was isolated as an oil, whilst the more polar *cis*-isomer was isolated as a crystalline solid, m.p. 70°.

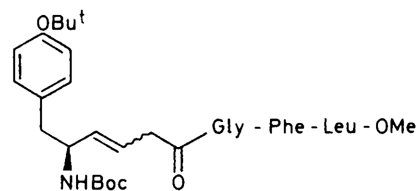


SCHEME 1 *Reagents:* i, Boc-azide *or* $(Boc)_2O$, Et_3N; ii, $Me_2C{=}CH_2$, CH_2Cl_2, $BF_3{\cdot}Et_2O$, 0 °C

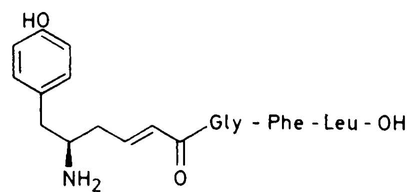
Treatment of the *trans*-enyne with dicyclohexylborane in tetrahydrofuran followed by oxidation with alkaline hydrogen peroxide yielded the required acid (21). Thus the functional groups did not interfere with the course of this reaction. Corresponding treatment of the minor, isomeric enyne (24) afforded the *cis*-isomer (22). Both acids (21) and (22) formed crystalline salts with dicyclohexylamine and repeated recrystallisation showed little change in the optical rotation of these salts, indicating little, if any, racemisation during their preparation.



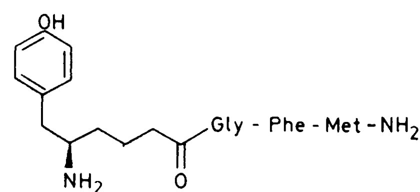
(27) R = Me
(30) R = H



(26) *trans*
(28) *cis*

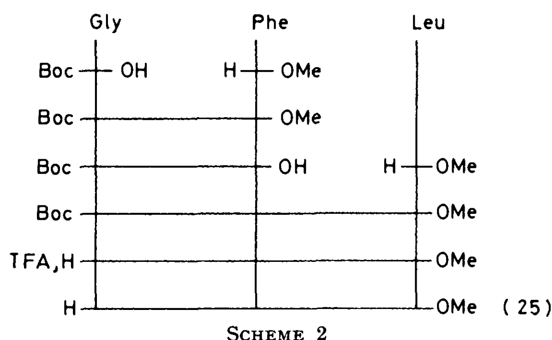


(29)



(31)

The acid (21) was coupled with the tripeptide unit (25), itself prepared by classical procedure (Scheme 2), by use of the dicyclohexylcarbodi-imide (DCC) and 1-hydroxybenzotriazole (HOBT) method. The fully protected peptide (26) was obtained in 68% yield, gave



SCHEME 2

correct analytical figures for $C_{39}H_{56}N_4O_8$, and showed the expected signals in its n.m.r. and i.r. spectra. Hydrolysis of the ester function was achieved with methanolic sodium hydroxide at pH 12, and removal of the Boc and Bu^t groups was effected with trifluoroacetic acid in dichloromethane, using anisole as a carbocation scavenger. The free amino-acid (3) was liberated by ion-exchange chromatography on Amberlite IR-45, as a white powder, m.p. 193—195°, $[\alpha]_D^{20}$ −18°, which was chromatographically homogeneous in both acidic and basic solvent systems. As anticipated, compound (3) was less polar than authentic [Leu^5]-enkephalin; for example, on t.l.c. ($CHCl_3$–MeOH–AcOH–H_2O 30 : 20 : 4 : 6) it showed R_F 0.8 (*cf.* 0.55 for the natural substance).[15]

Identical treatment of the *cis*-acid (22) with the tripeptide unit (25) did not proceed in an exactly analogous manner to that of the *trans*-acid (21) and two coupled products were formed. The more polar compound (13% yield), obtained as an oil, showed in its ^1H n.m.r. spectrum a new multiplet at δ 2.3, a shift of signal due to the vinylic protons of the double bond, and in its i.r. spectrum a double bond absorption at 980 cm^{-1}, characteristic of a conjugated *trans*-double bond, suggesting that the product was the *trans*-αβ-unsaturated compound (27), presumably formed by tautomeric shift of the βγ-bond. The less polar and major constituent of the coupled products was obtained as a white solid, m.p. 87—98° which showed a ^1H n.m.r. spectrum very similar to that of the *trans*-isomer (26); however the i.r. spectrum showed no absorption in the 950—1 000 cm^{-1} region but a strong peak at 770 cm^{-1}, indicating that this was the expected *cis*-βγ-unsaturated isomer (28). That the latter isomer was sensitive to rearrangement to give the more stable *trans*-αβ-unsaturated compound was further shown on attempted hydrolysis of the methyl ester group. Two products were formed from treatment of the ester (28) with methanolic sodium hydroxide. The less polar and minor product (7%), on treatment with an excess of ethereal diazomethane, gave back the starting ester (28), indicating that this was the desired *cis*-βγ-unsaturated compound. The more polar, major product (30) (37%), m.p. 100—104°, gave, on treatment with an excess of ethereal diazomethane, the *trans*-αβ-unsaturated ester (27), indicating that base-catalysed rearrangement of the *cis*-βγ-unsaturated system to the *trans*-αβ-unsaturated had occurred. Deprotection of the *trans*-αβ-unsaturated isomer (27) proceeded as expected to give the amino-acid (29), which proved useful for biological comparison with the target isostere (3). It has not proved possible, however, to prepare the fully deprotected *cis*-βγ-unsaturated compound.

Some preliminary biological results are reported in the Table. Shown are the results of receptor binding assays using rat brain homogenates at various temperatures with two different radioligands. A number of conclusions may be drawn from these results. The double bond isostere (3) of *Leu*^{5}-enkephalin exhibits activity virtually identical with that of the natural substrate, indicating that the isosteric replacement is consistent with full biological activity. Furthermore, when [^{3}H]-naloxone was used as the radioligand and incubation was carried out at 30 °C, a temperature at which the natural enkephalins are rapidly degraded by proteolytic enzymes, the analogue (3) again showed considerable binding indicating that, as anticipated, this analogue is more stable to proteolysis than is the natural substance (1). As a corollary to these observations it is apparent that the amide link between the Tyr^1-Gly^2 units of the enkephalins does not play an essential role in these molecules; neither intramolecular hydrogen bonding to the other amide groups, to form β-bends, nor intermolecular bonds, *e.g.* drug–receptor interaction, from this amide group are involved at the active site of the receptor, the link acting merely as a steric spacer. This is not necessarily true for all amide links and some evidence that the other amide bonds play a more significant role has been reported by Cox *et al.*,[16] who have recently also described the replacement of some of the other amide links in the enkephalins by double bonds. The isosteric replacement of a peptide link by a double bond thus helps to highlight the role of the bond in question.

The residual activity of the *trans*-αβ-unsaturated analogue (29) is of interest. It demonstrates that some flexibility of structure is permitted about this part of the enkephalin molecule. However, the steric constraints of the βγ-double bond are desirable for holding the substituents in the correct spatial relationship since

Receptor binding assays [a]

Compd. no.	Isostere structural features	$IC_{50}/\mu M$ [b] [^{3}H]*Leu*^{5}-Enkephalin	[^{3}H]Naloxone
(1)	*Leu*^{5}-Enk	0.003(1)	0.18
(2)	*Met*^{5}-Enk	0.001	⩾10
(3)	*trans*-βγ-*Leu*^{5}-Enk	0.004(6)	0.6 (at 30 °C)
(29)	*trans*-αβ-*Leu*^{5}-Enk	0.032	n.t.[c]

[a] Rat brain homogenates. [b] Concentration required to cause release of 50% of the bound radiolabelled substrate from the receptors, at 0 °C except where indicated. [c] n.t. = not tested.

the fully reduced Met[5]-analogue (31), described by Hudson *et al.*,[17] showed negligible opiate receptor binding.

EXPERIMENTAL

All m.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were recorded on a Perkin-Elmer 157G spectrometer [films (for oils), or Nujol mulls, chloroform solutions, or KBr discs for solids]. U.v. spectra were recorded for ethanolic solutions using a Pye–Unicam SP 800 instrument. ^{1}H N.m.r. spectra were recorded on either a JEOL MH-100 (100 MHz) or a Perkin-Elmer R12 (60 MHz) instrument. Samples were prepared as solutions in deuteriated solvents, as indicated, with tetramethylsilane as internal reference. Coupling constants are observed values. Mass spectra were obtained on A.E.I–Kratos instruments. Solvents were purified, as necessary, by distillation; tetrahydrofuran (THF) was distilled from lithium aluminium hydride immediately prior to use; 'petrol' refers to light petroleum of boiling range 40—60 °C. Extracts were dried over anhydrous sodium sulphate and evaporated *in vacuo* by use of a rotary evaporator. Reactions were generally carried out under dry nitrogen. Thin layer chromatography (t.l.c.) was carried out on Merck precoated plates of silica gel 60_{254} F, 0.1 mm thick; for preparative t.l.c. plates coated with 2 mm of silica gel 60_{254} F were used. Column chromatography was carried out using Merck silica gel G60, eluting under a slightly positive pressure of nitrogen and with loading ratios in the range 50—100 : 1.

2-Carboxyethyl(triphenyl)phosphonium Chloride (6).—3-Chloropropionic acid (7.42 g) and triphenylphosphine (18 g) were heated in xylene (100 ml) to reflux for 1.75 h, after which the xylene was removed by distillation and the residual glass crystallised from ethanol–ether to yield the title salt (6) as white crystals (17.5 g, 69%), m.p. 193—197 °C (lit.,[18] 196—198.5 °C).

3-Bromo-1-trimethylsilylprop-1-yne.—Prop-2-yn-1-ol (28 g) in THF (100 ml) was added, with cooling, to a solution of ethylmagnesium bromide [from magnesium turnings (24.4 g) and ethyl bromide (110 g)] in THF (200 ml) over 1.5 h. The mixture was stirred at room temperature for 0.75 h and then treated with trimethylsilyl chloride (112 g) added over 1.5 h. After stirring the mixture for 15 h, NH_4Cl(aq) was added and stirring continued until all the solid had dissolved. The layers were separated and the aqueous phase was extracted with ether (2 × 250 ml); the combined organic phase was concentrated *in vacuo*, dissolved in ethanol (200 ml), and stirred with ice (50 g) and concentrated HCl (1 ml) for 2 h. The mixture was diluted with H_2O (200 ml) and extracted with ether (2 × 500 ml); the extract was dried and the solvent removed *in vacuo*. Distillation under reduced pressure (N_2 atmos.) yielded 3-trimethylsilylprop-2-yn-1-ol (24 g, 38%), b.p. 60—62 °C at 12 mmHg (lit.,[19] 77—78 °C at 12 mmHg), δ($CDCl_3$) 0.15 (9 H, s), 2.1 (1 H, exch.), and 4.15 (2 H, s).

The alcohol (12 g) in dimethylformamide (50 ml) was added, with stirring, to a cooled (0 °C), freshly prepared solution of triphenylphosphine (60 g) and bromine (8.3 ml) in dimethylformamide (250 ml). After addition the temperature was allowed to rise to ambient, and stirring was continued for a further 18 h before extracting the reaction mixture with petrol (2 × 100 ml); the extract was dried and the solvent removed *in vacuo*. The residual oil was filtered through a SiO_2 pad (250 g), and washed with petrol (b.p. 60—80 °C); the solvent was removed *in vacuo* and the residue distilled to produce the title compound (4.9 g, 30%), b.p. 43 °C at 6 mmHg (lit.,[19] 44—45 °C at 2 mmHg), δ($CDCl_3$) 0.15 (9 H, s), and 3.85 (2 H, s).

Triphenyl-(3-trimethylsilylprop-2-ynyl)phosphonium Bromide (7).—3-Bromo-1-trimethylsilylprop-1-yne (4 g) and triphenylphosphine (7.2 g) were stirred in benzene (20 ml) in the dark at room temperature for 18 h. The precipitate was filtered off, washed with petrol, and dried *in vacuo* to yield the title compound (6.5 g, 70%), m.p. 155—159 °C (lit.,[19] 154—156 °C).

trans-*6-Phenyl-1-trimethylsilyhex-3-en-1-yne* (15).—The bromide salt (7) (0.45 g) in THF (5 ml) at −72 °C was treated with n-butyl-lithium (0.7 ml; 1.45M in hexane). After stirring for 0.75 h 3-phenylpropionaldehyde (8) (0.135 g) in THF (5 ml) was added. The mixture was stirred at −70 °C for 0.5 h, then allowed to warm to room temperature (0.5 h), poured into H_2O (50 ml), and extracted with ether (15 ml). The aqueous phase was re-extracted with petrol (2 × 50 ml), and the organic extracts were dried, concentrated *in vacuo* and purified by preparative t.l.c. [1 : 99 ether–petrol as eluant]. The major product was the title compound, isolated as a pale yellow oil (0.16 g, 68%), $\nu_{max.}$ (film) 2 120, 1 600, 950, 840, 750, and 700 cm^{-1}; δ($CDCl_3$) 0.2 (9 H, s), 2.4 (2 H, m), 2.65 (2 H, m), 5.48 (1 H, d, *J* 16 Hz), 6.18 (1 H, dd, *J* 16 and 6 Hz), and 7.12 (5 H, m).

Methyl trans-*6-Phenylhex-3-enoate* (11).—*Method A*. The phosphonium salt (6) (1.68 g), in THF (40 ml) at −75° was treated with n-butyl-lithium (6.32 ml; 1.0M in hexane). After 10 min 3-phenylpropionaldehyde (0.5 g) in THF (6 ml) was added dropwise to the dark red solution. The mixture was stirred for 1 h at −70 °C, at room temperature for 1.5 h, and then, briefly at 65 °C; it was then cooled to room temperature, poured into water (100 ml), and extracted with ether (2 × 50 ml). The aqueous phase was acidified with 2N-HCl and re-extracted with ether (3 × 50 ml). The acidic organic extract was dried, concentrated *in vacuo*, and treated with ethereal diazomethane until the yellow colour persisted. After 10 min the mixture was poured into water (50 ml) and extracted with chloroform (3 × 50 ml); the organic extract was dried, evaporated *in vacuo* and chromatographed (preparative t.l.c., 9 : 1 petrol–ether as eluant). The principal band yielded the title compound (11) as a pale yellow oil (0.105 g, 15%), $\nu_{max.}$ (CS_2) 1 735, 960, 740, and 695 cm^{-1}; δ($CDCl_3$) 2.4 (2 H, m), 2.72 (2 H, t, *J* 8 Hz), 3.04 (2 H, m), 3.69 (3 H, s), 5.58 (2 H, m, $W_{\frac{1}{2}}$ 20 Hz), and 7.2 (5 H, s) {on addition of the shift reagent [Eu(fod)$_3$] the peak at 5.58 shifted downfield and separated into two double triplets both with *J* 16 and 7 Hz, respectively}; *m/z* 204 (M^+, 56%), 172 (54), 145 (60), 130 (98), and 91 (100). A close inspection of the ^{1}H n.m.r. spectra from the Eu(fod)$_3$ experiment indicated the presence of a small quantity (<5%) of the *cis*-isomer, which was not separated.

Method B. Dicyclohexylborane was prepared in THF (5 ml) at 0 °C from 1M-borane–THF complex (10 ml) and cyclohexene (1.68 g). A sample (1 ml) of the resulting suspension was estimated by quenching with aqueous methanol and measuring the volume of hydrogen produced, which showed that the suspension was 0.625M with respect to the dialkylborane.

trans-6-Phenyl-1-trimethylsilylhex-3-en-1-yne (0.107 g) THF (5 ml) at 0 °C was treated with the above suspension (0.8 ml); after 30 min a further portion of dicyclohexylborane (0.5 ml) was added and stirring was continued for a

further 0.5 h at 0 °C. MeOH (2 ml) and 2N-NaOH (1 ml) were added, followed by 30% hydrogen peroxide (1.5 ml) at a rate which did not allow the temperature to rise above 10 °C. The mixture was stirred for 0.5 h, then poured into H_2O (2.5 ml) and extracted with ether (2 × 50 ml). The aqueous phase was acidified with 2N-HCl and re-extracted with ether (3 × 5 ml). The acidic ethereal extract was dried, concentrated *in vacuo*, and treated with an excess of ethereal diazomethane. After concentration *in vacuo* the residual oil was chromatographed by preparative t.l.c. to yield the title compound (11) (45 mg, 45%). Identity with the sample prepared by method A was confirmed by spectroscopic analysis, t.l.c., and g.l.c. (6 ft SE30 column, 130 °C, He carrier at 2 ml min^{-1}).

N-*t-Butoxycarbonyl-O-t-butyl-L-tyrosine Methyl Ester* (18).—*N*-Boc-L-tyrosine methyl ester (2.06 g), m.p. 101—105 °C, in dichloromethane (150 ml) at 0 °C was treated with boron trifluoride–ether (0.88 ml) and isobutene was bubbled through the solution for 4.5 h, during which the temperature was allowed to rise to 20 °C. The mixture was filtered and the filtrate washed with 2N-NaOH (2 × 150 ml), 1N-HCl (150 ml), and brine. The solution was dried and the solvent removed *in vacuo* to yield a pale yellow solid which was recrystallised from petrol to give the title compound (18) (1.36 g, 55%), m.p. 99—100 °C (lit.,[13] 99 °C), $[\alpha]_D$ −17° (*c* 0.5 in dimethylformamide) (lit.,[13] −17.4°, *c* 0.5 in dimethylformamide). The solid, which was filtered off, yielded, after purification, tyrosine methyl ester (300 mg, 22%).

(S)-2-*t-Butoxycarbonylamino*-3-(4-*t-butoxyphenyl*)*propanal* (17).—*N*-Boc-L-Tyr(OBu^t)OMe (18) (9.87 g) in toluene (500 ml) under nitrogen at −65 °C was treated with di-isobutylaluminium hydride (1.2M-solution in hexane; 47 ml) over 0.75 h. After stirring for 5 min, methanol (10 ml) was added, and the mixture was poured into a saturated solution of Rochelle salt (1 l) and vigorously stirred until the emulsion was broken (*ca.* 1.5 h). The layers were separated and the aqueous phase extracted with ether (2 × 250 ml). The combined organic extract was dried and the solvent removed *in vacuo* to yield an oil (9.5 g), $[\alpha]_D$ −18° (*c* 10.0 in MeOH); ^{1}H n.m.r. analysis indicated at least 80% of the required aldehyde, together with the starting ester (*ca.* 10%) and the fully reduced alcohol (19) (*ca.* 10%). (This aldehyde could be used directly for further transformations or it could be purified *via* the semicarbazone.) A purified sample of the aldehyde showed δ($CDCl_3$) 1.3 (9 H, s), 1.4 (9 H, s), 3.05 (2 H, d, *J* 6 Hz), 4.35 (1 H, m), 4.95 (1 H, br,s, exch.), 6.9 and 7.1 (4 H, 2d, *J* 8 Hz), and 9.6 (1 H, s).

Semicarbazone of the Aldehyde (17).—The crude reduction product (1.7 g) in 3 : 7 water–ethanol (50 ml) was treated with semicarbazide hydrochloride (4.84 g) and sodium acetate (10 g) and warmed to 80 °C for 10 min. The solvents were removed *in vacuo* and the residue was partitioned between ether and water. The ether extract was dried and the solvent removed *in vacuo* to yield an oil. Column chromatography through SiO_2 (300 g) (ethyl acetate–petrol mixtures as eluant) afforded, initially, unchanged ester (18) (60 mg, 10%), followed by the *semicarbazone* (20) (1.8 g, 80%), m.p. 75—80 °C; $[\alpha]_D^{20}$ +11.5° (*c* 1.0 in MeOH), $\nu_{max.}$ (KBr) 3 350 and 1 770 cm^{-1}; δ($CDCl_3$) 1.3 (9 H, s), 1.3 (9 H, s), 1.4 (9 H, s), 2.9 (2 H, d, *J* 8 Hz), 4.5 (1 H, br, s), 5.6 (2 H, br,s, exch.), 6.9 and 7.05 (4 H, 2d, *J* 8 Hz), 7.05 (1 H, s), and 9.5 (1 H, s, exch.) (Found: C, 60.0; H, 7.8; N, 14.6. $C_{19}H_{29}N_4O_4$ requires C, 60.45; H, 7.7; N, 14.6%).

The aldehyde (18) could be regenerated from the semicarbazone (20) as follows. The derivative (20) (0.77 g) in ethanol (20 ml) was treated with formalin (37%; 2 ml) and 0.5N-HCl (0.5 ml). After 2.25 h the mixture was poured into water (100 ml) and extracted with ether (3 × 75 ml). The extract was washed with brine and dried and the solvent removed *in vacuo* to yield an oil (0.70 g, 95%), shown by ^{1}H n.m.r. analysis to be the required aldehyde (18), $[\alpha]_D^{20}$ −23.0° (*c* 1.0 in MeOH).

Reaction of the Aldehyde (18) *with the Phosphonium Salt* (9).—The salt (9) (10 g) was suspended in THF (200 ml) at −75 °C and treated with n-butyl-lithium (1.6M; 13.7 ml, 22 mmol). After 20 min the aldehyde (18) (*ca.* 20) in THF (15 ml) was added and the mixture allowed to warm to room temperature. After 1 h, water (100 ml) was added and the mixture extracted with petrol (3 × 200 ml). The extract was dried and the solvent removed *in vacuo* to yield an oil which was overlaid with cold petrol, whereupon triphenylphosphine oxide began to crystallise. The mother liquors were chromatographed through silica gel (1 kg) using a Jobin–Yvon preparative h.p.l.c. apparatus and with ethyl acetate–light petroleum mixtures as eluant. The first fraction eluted was (S)-trans-2-*t-butoxycarbonylamino*-1-(4-*t-butoxyphenyl*)-6-*trimethylsilylhex*-3-*en*-5-*yne* (23) as an oil (6.3 g, 76%); $[\alpha]_D^{20}$ +6.0° (*c* 1.0 in MeOH); $\nu_{max.}$ (film) 3 350, 2 215, 1 700, 1 610, and 950 cm^{-1}; δ($CDCl_3$) 0.19 (9 H, s), 1.14 (9 H, s), 1.20 (9 H, s), 2.72 (2 H, d, *J* 6 Hz), 4.3—4.58 (2 H, m), 5.42 (1 H, d, *J* 16 Hz), 6.0 (1 H, dd, *J* 16 and 6 Hz), and 6.76 and 6.85 (4 H, 2d, *J* 8 Hz); *m/z* 415 (15%, M^+), 314 (17, M^+ − Boc), 303 (58), and 252 (100) (Found: M^+, 415.2531; $C_{24}H_{37}NO_3Si$ requires *M*, 415.2533).

The second fraction yielded (S)-cis-2-*t-butoxycarbonylamino*-1-(4-*t-butoxyphenyl*)-6-*trimethylsilylhex*-3-*en*-5-*yne* (24) as an oil (490 mg, 6.0%) which crystallised from aqueous ethanol, m.p. 70 °C; $[\alpha]_D^{20}$ +98.0° (*c* 1.0 in MeOH), $\lambda_{max.}$ 247 (ε 12 600), 236 (15 200), and 225 nm (15 500); $\nu_{max.}$ (film) 3 350, 2 140, 1 710, 1 610, 840, and 650 cm^{-1}; δ($CDCl_3$) 0.2 (9 H, s), 1.25 (9 H, s), 1.36 (9 H, s), 2.8 (2 H, d, *J* 6 Hz), 4.4—4.8 (2 H, m), 5.35 (1 H, d, *J* 11 Hz), 5.67 (1 H, dd, *J* 11 and 7 Hz), 6.68 and 6.88 (4 H, 2d, *J* 8 Hz), (Found: C, 69.6; H, 9.0; N, 3.3. $C_{24}H_{37}NO_3Si$ requires C, 69.4; H, 9.0; N, 3.4%).

(S)-trans-5-*t-Butoxycarbonylamino*-6-(4-t-*butoxyphenyl*)-*hex*-3-*enoic Acid* (21).—The silylated enyne (23) (1.66 g, 4 mmol) in THF (5 ml) was added to a suspension of dicyclohexylborane (14 mmol) in THF (40 ml) at 0 °C. After stirring for 0.5 h, methanol (5 ml) and 2N-NaOH (7.0 ml) were added, followed by hydrogen peroxide (30%; 4.5 ml); the latter was added at a rate which did not allow the temperature to rise above 10 °C. After stirring for a further 0.5 h at 0 °C the mixture was poured into water (100 ml) and 2N-NaOH (5 ml), and extracted with ether (3 × 50 ml). The aqueous phase was acidified with 2N-HCl and re-extracted with ether (3 × 100 ml). This ethereal extract was dried and the solvent removed *in vacuo* to yield *compound* (21) as a waxy solid (1.13 g, 75%); $[\alpha]_D^{20}$ −5° (*c* 1.0 in MeOH), $\nu_{max.}$ (film) 3 300br, 2 550br, 1 710, 1 610, 970, and 900 m^{-1}; δ($CDCl_3$) 1.28 (9 H, s), 1.36 (9 H, s), 2.7 (2 H, d. *J* 6 Hz), 3.0 (2 H, d, *J* 6 Hz), 4.28 (2 H, m), 5.19—5.81 (2 H, m), and 6.74 and 6.94 (4 H, 2d, *J* 8 Hz); δ[($^2H_8$)toluene; 62 °C] olefinic region: 5.1 (dd, *J* 16 and 5 Hz] and 5.5 (dt, *J* 16 and 6 Hz) (irradiation of the olefinic region collapses the doublet at 3.0 to a singlet); *m/z* 377 (8%, M^+), 321 (19, M^+ − Bu^t), 306 (19), 214 (100), 203 (30), 204 (46), 158 (>100), 145 (61), 115 (44), 114 (>100), 108 (>100), 107 (>100), 96 (44), and 57 (>100) (Found: M^+,

377.219 77. $C_{21}H_{31}NO_5$ requires *M*, 377.220 21). The *dicyclohexylammonium salt* had m.p. 140—145 °C (Found: C, 70.9; H, 9.7; N, 5.05. $C_{33}H_{54}N_2O_5$ requires C, 70.9; H, 9.7; N, 5.0%).

(S)-cis-5-*t-Butoxycarbonylamino*-6-(4-*t-butoxyphenyl*)*hex*-3-*enoic Acid* (22).—The silylated enyne (24) (0.45 g, 1.08 mmol) in THF (15 ml) at 0 °C was treated with a suspension of dicyclohexylborane (2.25 mmol) in THF (19 ml) in the manner described above. Oxidation with alkaline hydrogen peroxide [from methanol (2 ml), N-NaOH (3 ml), and 30% hydrogen peroxide (1 ml)] followed by work-up gave, after removal of the solvent, *compound* (22) as an oil (0.18 g, 44%), $[\alpha]_D^{20}$ +11.0° (*c* 1.0 in MeOH), $\nu_{max.}$ (film) 3 300br, 2 600br, 1 710, 1 610, 900, and 730 cm^{-1}; δ($CDCl_3$) 1.3 (9 H, s), 1.4 (9 H, s), 2.7—3.1 (4 H, m), 4.45 (1 H, m), 5.5 (2 H, m), 6.85 and 7.05 (4 H, 2d, *J* 8 Hz), and 8.6 (1 H, br, s, exch.); δ[($^2H_8$)toluene] olefinic region: 5.25 (dd, *J* 11 and 8 Hz) and 5.65 (dt, *J* 11 and 6.5 Hz). The *dicyclohexylammonium salt* had m.p. 126—131 °C (Found: C, 70.45; H, 9.7; N, 5.0. $C_{33}H_{54}N_2O_5$ requires C, 70.0; H, 9.7; N, 5.0%).

Condensation of the Aldehyde (17) *with the Salt* (6).—The salt (6) (1.4 g, 4 mmol) was suspended in THF (40 ml) at −75° and treated with n-butyl-lithium (2M; 4 ml, 8 mmol). The dark brown solution was stirred for 30 min and then added to a solution of the aldehyde (17) (2 mmol) in THF (40 ml) at −75 °C. The mixture was stirred while the temperature reached ambient level during 1 h, then heated for 5 min at 40 °C. The mixture was cooled to room temperature, poured into water (100 ml), and extracted with ether (3 × 50 ml). The aqueous phase was acidified with 2N-HCl and re-extracted with ether (3 × 50 ml). The acidic extract was dried and evaporated *in vacuo* and the residue purified by preparative t.l.c. (1 : 1 ethyl acetate–cyclohexane). The major fraction (55 mg, 8%) was shown, by further t.l.c. and spectral comparisons, to consist of a 1 : 1 mixture of the *cis*- and *trans*-isomers (21) and (22).

N-*t-Butoxycarbonylglycyl*-L-*phenylalanyl*-L-*leucine Methyl Ester*.—Ammonia was bubbled through a solution of phenylalanine methyl ester hydrochloride (33 g) in dichloromethane (250 ml) until precipitation of ammonium chloride ceased. The mixture was filtered and the filtrate rapidly concentrated *in vacuo*, to yield the free phenylalanine methyl ester, which was immediately added to *N*-t-butoxycarbonylglycine (27.5 g) in dichloromethane (400 ml) and treated with dicyclohexylcarbodi-imide (32.9 g) at room temperature for 16 h. The precipitated urea was filtered off and the filtrate washed with 2N-HCl (2 × 100 ml) and aqueous sodium hydrogen carbonate (100 ml), and dried before evaporation *in vacuo*. The resulting oil was dissolved in methanol (200 ml) and 2N-NaOH added, in portions, over 3 h, to maintain pH 12. The resultant solution was left for 12 h at 0 °C, then diluted with water (150 ml) and extracted with ether (200 ml), and the aqueous phase was then acidified with 2N-HCl and re-extracted with ether (2 × 300 ml). The acidic extract was dried and evaporated *in vacuo* and the solid residue recrystallised from dichloromethane–petrol, to give N-*t-butoxycarbonylglycyl*-L-*phenylalanine* as white crystals (40 g, 80%), m.p. 142—144 °C; $[\alpha]_D^{20}$ +30.7° (*c* 0.85 in MeOH) (Found: C, 61.2; H, 6.65; N, 8.3. $C_{17}H_{22}N_2O_5$ requires C, 61.1; H, 6.6; N, 8.4%).

The latter acid (9.7 g) in dichloromethane (400 ml) at 0 °C was treated, successively, with 1-hydroxybenzotriazole (4.05 g), leucine methyl ester [from the hydrochloride (5.5 g)], and dicyclohexylcarbodi-imide (6.4 g). After 16 h at room temperature the urea was filtered off and the filtrate washed with 2N-HCl and aqueous sodium hydrogen carbonate. Drying, evaporation *in vacuo*, and crystallisation from dichloromethane–petrol afforded white crystals of *the ester* (11.7 g, 87%), m.p. 118—120 °C, $[\alpha]_D^{20}$ −24.9° (*c* 1.0 in MeOH) (Found: C, 61.5; H, 7.9; N, 9.3. $C_{23}H_{35}N_3O_6$ requires C, 61.45; H, 7.85; N, 9.0%).

(S)-trans-5-*t-Butoxycarbonylamino*-6-(4-t-*butoxyphenyl*)-*hex*-3-*enoylglycyl*-L-*phenylalanyl*-L-*leucine Methyl Ester* (26).—*N*-Boc-Gly-L-Phe-L-Leu-OMe (1.12 g) was treated with 1 : 1 v/v dichloromethane–trifluoroacetic acid (20 ml) at room temperature for 20 min. The excess of reagent was rapidly removed in *vacuo*, keeping the temperature below 30 °C, the residue was dissolved in dichloromethane (50 ml), and ammonia gas was bubbled through. The ammonium trifluoroacetate was filtered off, the filtrate evaporated *in vacuo* and the crystalline residue added to a solution of the *trans*-βγ-unsaturated acid (21) (0.82 g) in dichloromethane (45 ml). The solution was cooled to 0 °C and 1-hydroxybenzotriazole (0.30 g) and dicyclohexylcarbodi-imide (0.515 g) were added. After 1.5 h at room temperature the mixture was filtered and the filtrate washed with aqueous citric acid and then aqueous sodium hydrogen carbonate. The organic phase was dried and the solvent removed *in vacuo*. The residue was then dissolved in ethyl acetate and subjected to column chromatography in SiO_2, using ethyl acetate as eluant. The major fractions were combined and dried to yield *compound* (26) (1.0 g, 68%) as an off-white solid, m.p. 95—98 °C, $[\alpha]_D^{20}$ −20.4° (*c* 0.8 in MeOH), $\nu_{max.}$ (KBr), 3 300, 1 750, 1 690, 1 645, 1 530, and 970 cm^{-1}; δ($CDCl_3$) 0.85 (6 H, m), 1.3 (9 H, s), 1.35 (3 H, br, m), 1.4 (9 H, s), 2.6—3.2 (6 H, m), 3.65 (3 H, s), 3.75 (2 H, m), 4.0—4.8 (4 H, m), 5.6 (2 H, m), 6.4—7.0 (3 H, exch.), 6.75 (4 H, m), and 7.22 (5 H, m) (Found: C, 66.0; H, 8.1; N, 7.8. $C_{39}H_{56}N_4O_8$ requires C, 66.1; H, 8.0; N, 7.9%).

(S)-trans-5-*Amino*-6-(4-*hydroxyphenyl*)*hex*-3-*enoylglycyl*-L-*phenylalanyl*-L-*leucine* (3).—The protected peptide (26) (0.735 g) was dissolved in methanol (15 ml) and the pH adjusted to 12 with 2N-NaOH. After 7 h, the solution was concentrated *in vacuo*, acidified with aqueous citric acid, and extracted with ether (100 ml) and ethyl acetate (2 × 100 ml). The combined organic extract was washed with brine, dried, and evaporated *in vacuo*. The resulting acid (620 mg, 86%) behaved as a chromatographically homogeneous product: R_F 0.9 (1 : 1 : 1 dichloromethane–ethyl acetate–methanol). A portion (0.40 g) was dissolved in trifluoroacetic acid–dichloromethane (3 : 1; 6 ml) and anisole (1 ml) at room temperature. After 15 min the solution was poured into ether (25 ml) and centrifuged. The solid was collected and re-washed with ether before dissolving in the minimum of trimethylamine–acetic acid buffer solution at pH 5.5 [from trimethylamine (2.5 ml), acetic acid (1.2 ml) and water (1 l)]. The solution was applied to an Amberlite IR 45 ion-exchange resin column and eluted with the same buffer. The major fraction was collected and evaporated *in vacuo*, and the residual solid (160 mg) recrystallised from ether–methanol followed by methanol to yield *compound* (3) as a white, chromatographically homogeneous powder (80 mg), m.p. 193—195 °C, $[\alpha]_D^{20}$ −18° (*c* 0.5 in dimethylformamide); R_F 0.8 (15 : 10 : 2 : 3 chloroform–methanol–acetic acid–water); $\nu_{max.}$ (KBr) 3 300, 2 500, 2 100br, 1 630br, 1 520br, 975, 830, 740, and 700 cm^{-1}; *m/z* (M^+ not observed) 318 (5%), 300 (12), 186 (52), 159 (57), 144 (26), 120 (56), 107 (25),

96 (22), and 91 (100) (Found: C, 61.4; H, 7.0; N, 9.8. $C_{29}H_{38}N_4O_6$,1.5H_2O requires C, 61.6; H, 7.3; N, 9.9%).

(R)-trans-5-*t-Butoxycarbonylamino*-6-(4-*t-butoxyphenyl*)-*hex*-2-*enoylglycyl*-L-*phenylalanyl*-L-*leucine Methyl Ester* (27) *and* (S)-cis-5-*t-butoxycarbonylamino*-6-(4-*t-butoxyphenyl*)*hex*-3-*enoylglycyl*-L-*leucine Methyl Ester* (28).—The *cis*-βγ-unsaturated acid (22) (85 mg) and H-Gly-L-Phe-L-Leu-OMe (70 mg) in dichloromethane (15 ml) were treated with 1-hydroxybenzotriazole (25 ml) and dicyclohexylcarbodi-imide (50 mg) at room temperature for 20 h. The mixture was filtered and the filtrate evaporated to dryness *in vacuo*. The residue was triturated with ethyl acetate (20 ml) and the mixture filtered. The filtrate was washed with N-HCl, aqueous sodium hydrogencarbonate, and water before drying and evaporating *in vacuo*. The residue was separated by preparative t.l.c., eluting with ethyl acetate. The more polar material (R_F 0.6) extracted from the plates afforded the trans-αβ-*unsaturated isomer* (27) (20 mg, 3%) as an oil, $\nu_{max.}$ (film) 3 300, 1 750, 1 700, 1 650, 1 530, 980, 900, 850, 730, and 700 cm^{-1}; δ($CDCl_3$–CD_3OD) 0.9 (6 H, d, *J* 5 Hz), 1.3 (9 H, s), 1.4 (9 H, s), 1.4 (3 H, m), 2.3 (2 H, m), 2.7 (2 H, d, *J* 7 Hz), 3.05 (2 H, d, *J* 6 Hz), 4.6 (3 H, m), 5.92 (1 H, d, *J* 15 Hz), 6.9 and 7.15 (4 H, 2d, *J* 9 Hz), 7.25 (5 H, s), and 7.4 (3 H, m, exch.).

The less polar fraction (R_F 0.7) yielded the cis-βγ-*unsaturated isomer* (28) as a white, crystalline solid (125 mg, 85%); m.p. 87—89 °C; $\nu_{max.}$ 3 300, 1 740, 1 690, 1 630, 1 540, 900, 840, 730, and 700 cm^{-1}; δ($CDCl_3$) 0.9 (6 H, m), 1.3 (9 H, s), 1.4 (9 H, s), 1.5 (3 H, br, m), 2.65 (4 H, m), 3.05 (2 H, d, *J* 6 Hz), 3.62 (3 H, s), 3.77 (2 H, d, *J* 6 Hz), 4.5 (4 H, m), 5.37 (2 H, m), 6.85 and 7.05 (4 H, 2d, *J* 9 Hz), 7.2 (5 H, s), and 6.3—7.5 (3 H, m, exch) (Found: C, 66.45; H, 8.1; N, 7.6. $C_{39}H_{56}N_4O_8$ requires C, 66.1; H, 8.0; N, 7.9%).

Attempted Deprotection of the cis-βγ-*Unsaturated Ester* (28).—The ester (125 mg) was dissolved in methanol (5 ml) and the pH adjusted to 11.0 with N-NaOH. After 3 h the mixture was diluted with water (50 ml) and extracted with ether (100 ml). The aqueous phase was acidified with 2N-HCl and re-extracted with ether (2 × 100 ml). The ether extract was dried and the solvents were removed *in vacuo* to yield a foam (100 mg). A small portion (5 mg) was treated with an excess of ethereal diazomethane and, after 15 min, the mixture was evaporated *in vacuo* to yield an oil, which behaved on t.l.c. as the *trans*-αβ-unsaturated ester (27). The remainder of the crude acid (95 mg) was purified by preparative t.l.c. using dichloromethane–ethanol–acetic acid (90 : 9.5 : 0.5) as eluant. The major band was isolated to give (*R*)-*trans*-5-t-butoxycarbonyl-amino-6-(4-t-butoxyphenyl)hex-2-enoylglycyl-L-phenyl-anyl-L-leucine (3) (45 mg, 37%), m.p. (dichloromethane) 100—104 °C, $\nu_{max.}$ (KBr) 3 300, 1 700, 1 650, 1 540, 980, 900, 730, and 700 cm^{-1}; δ(CD_3OD) 1.0 (6 H, m), 1.3 (9 H, s), 1.4 (9 H, s), 1.6 (3 H, m), 2.35 (2 H, t, *J* 6 Hz), 2.75 (2 H, d, *J* 8 Hz), 3.05 (2 H, d, *J* 8 Hz), 3.95 (2 H, s), 4.5 (3 H, m), 6.0 (1 H, d, *J* 15 Hz), 6.8 (1 H, m), 6.95 and 7.15 (4 H, 2d, *J* 9 Hz), and 7.35 (5 H, s). A slightly less polar band on the plates yielded an oil (5 mg) which, by ^1H n.m.r. analysis, appeared to be the *cis*-βγ-unsaturated isomer; treatment of this with diazomethane yielded one product, identical in its i.r. and t.l.c. properties with the *cis*-βγ-unsaturated ester (28).

(R)-trans-6-*Amino*-6-(4-*hydroxyphenyl*)*hex*-2-*enoylglycyl*-L-*phenylalanyl*-L-*leucine* (29).—The protected acid (30) (20 mg) was treated with 1 : 1 trifluoroacetic acid–dichloromethane (2 ml) containing anisole (0.1 mol) at room temperature for 20 min. The solvents were removed *in vacuo* and the residual salt was triturated with ether. The white powder that formed was dissolved in water (2 ml) and purified by elution through an Amberlite IR 45 column (OH^- form) using water as eluant. The main fraction was collected, the solution evaporated to dryness *in vacuo*, and the residue recrystallised from methanol to yield *compound* (29) (6 mg) as a white, chromatographically homogeneous solid, m.p. 203—206 °C (decomp.), $\nu_{max.}$ (KBr) 3 300, 2 600br, 1 700 1 660, 1 630, 1 520, 985, 835, 810, and 710 cm^{-1} (Found: C, 62.3; H, 7.5; N, 9.9. $C_{29}H_{38}N_4O_6$,H_2O requires C, 62.6; H, 7.5; N, 9.9%).

We thank Roussel Laboratories Ltd. and the S.R.C. for a CASE studentship (to M. M. H.). The skilled assistance of Mr. P. Hairsine for the h.p.l.c. separations and Mr. S. Clements-Jewery and his staff for the ligand binding studies is gratefully acknowledged.

[1/1182 *Received*, 27*th July*, 1981]

REFERENCES

[1] J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, and H. R. Morris, *Nature*, 1975, **258**, 577.
[2] D. Roemer and J. Pless, *Life Sci.*, 1979, **24**, 621; J. A. Barranger, S. I. Rapaport, W. R. Fredericks, P. G. Pentcher, K. D. MacDermot, J. K. Stensing, and R. O. Brady, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 481; B. von Graffenfriend, E. del Pozo, J. Roubick, E. Krebs, W. Poldinger, P. Burmeiser, and L. Kerp, *Nature*, 1978, **272**, 729.
[3] (*a*) G. Loew and S. K. Burt, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 7; (*b*) F. A. Momany, *Biochem. Biophys. Res. Commun.*, 1977, **75**, 1098; (*c*) A. F. Bradbury, D. G. Smyth and C. R. Snell, *Nature*, 1976, **260**, 165; (*d*) A. S. Horn and J.-R. Rodgers, *J. Pharmacol.*, 1977, **29**, 257; (*e*) C. R. Beddell, R. B. Clark, L. A. Lowe, S. Wilkinson, K.-J. Chang, P. Cuatrecasas and R. Miller, *Br. J. Pharmacol.*, 1977, **61**, 351; (*f*) P. S. Portoghese, *Acc. Chem. Res.*, 1978, **11**, 21.
[4] T. J. Hambrook, B. A. Morgan, M. J. Rance, and C. F. Smith, *Nature*, 1976, **262**, 782; M. Knight and W. A. Klee, *J. Biol. Chem.*, 1978, **253**, 3843; E. Hazum, K.-J. Chang, and P. Cuatrecasas, *Life Sci.*, 1978, **24**, 137.
[5] *Cf.* A. Korolkovas and J. H. Buckhalter, 'Essentials of Medicinal Chemistry,' Wiley, New York, 1976, pp. 23—26; C. W. Thornber, *Chem. Soc. Rev.*, 1980, **8**, 563.
[6] M. M. Hann, P. G. Sammes, P. D. Kennewell, and J. B. Taylor, *J. Chem. Soc., Chem. Commun.*, 1980, 234.
[7] H. S. Corey, J. R. D. McCormick, and W. E. Swenson, *J. Am. Chem. Soc.*, 1964, **86**, 1884.
[8] E. J. Corey and R. A. Ruden, *Tetrahedron Lett.*, 1973, 1495.
[9] M. Schlosser. G. Muller, and K. F. Christmann, *Angew. Chem., Int. Ed. Engl.*, 1966, **5**, 667.
[10] W. G. Salmond, M. A. Barta, and J. L. Havens, *J. Org. Chem.*, 1978, **48**, 710.
[11] G. Zweifel and S. J. Backlund, *J. Am. Chem. Soc.*, 1977, **99**, 3184.
[12] A. Ito, R. Takahashi, and Y. Baba, *Chem. Pharm. Bull.*, 1975, **23**, 3081.
[13] K. Jost, *Collect. Czech. Chem. Commun.*, 1971, **36**, 218.
[14] R. G. Huskey, L. M. Beacham, V. G. Matt, J. N. Smith, E. R. Williams, A. N. Thomas, and E. T. Wolters, *J. Org. Chem.*, 1971, **36**, 488.
[15] J. D. Bower, K. P. Guest, and B. A. Morgan, *J. Chem. Soc., Perkin Trans. 1*, 1976, 2488.
[16] M. T. Cox, D. W. Heaton, and J. Horbury, *J. Chem. Soc., Chem. Commun.*, 1980, 799; M. T. Cox, J. J. Gormley, C. F. Hayward, and N. G. Petter, *ibid.*, p. 808.
[17] D. Hudson, R. Sharpe, M. Szelke, I. MacIntyre, and G. Fink, U.S. Pat. Appl. G.B. 200 783A.
[18] D. B. Denny and L. C. Smith, *J. Org. Chem.*, 1962, **27**, 3404.
[19] M. Ahmed, G. C. Barley, M. W. Hearn, E. R. H. Jones, and V. Thaller, *J. Chem. Soc., Perkin Trans. 1*, 1974, 1981.

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