On Double Bond Isosteres of the Peptide Bond; an Enkephalin Analogue

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Summary A route to peptide analogues incorporating the replacement of an amide bond with a *trans*-carbon–carbon double bond is presented and applied to the synthesis of a Leu^5–enkephalin analogue.

ALTHOUGH isosteric replacements for the peptide bond have been investigated[1] the simple replacement of an amide bond by a *trans*-carbon–carbon double bond seems to have been largely ignored. This is surprising when comparing the similar geometrical disposition of substituents attached to either of these functional groups (Figure) but is understandable in view of the lack of suitable synthetic methods for effecting such a substitution. Herein we report a method for the generation of the system (**1**, $R^2 = H$), which may be compared to the normal dipeptide structure (**2**). The substitution has been exemplified by preparation of the analogue (**3**) of Leu^5–enkephalin (**4**), a ligand chosen because of current interest in its mode of action and, especially because of its conformation at the receptor site compared with exogenous opiates such as morphine.[2]

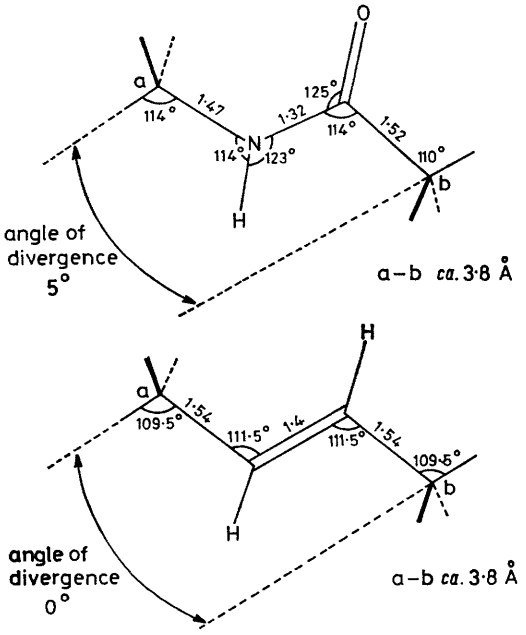
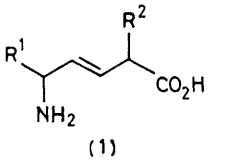
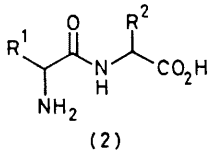


FIGURE. Spatial disposition of amide and *trans*-olefin bonds (distances in Å).

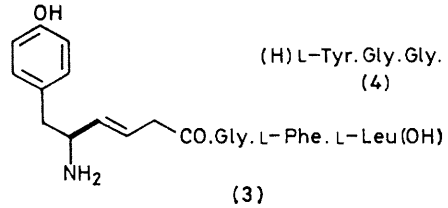
The enkephalins are rapidly hydrolysed *in vivo* and a principal process by which this occurs is believed to involve an amino-peptidase which cleaves the Tyr^1–Gly^2 bond.[3] The substitution of this bond should prevent this degradation procedure. Furthermore, the substitution of an amide bond by a double bond should increase the lipophilicity of the peptide and thus facilitate its passage through the blood–brain barrier.



(1)



(2)



(H) L-Tyr. Gly. Gly. L-Phe. L-Leu(OH)
(4)

(3)

Bearing in mind the requirement to maintain the stereochemical integrity about the tyrosyl residue in the analogue (**3**), we selected L-tyrosine as one of the starting materials. Tyrosine methyl ester, protected as the *O*-*t*-butyl-*N*-Boc

derivative (**5**) was reduced with di-isobutylaluminium hydride (2 equiv.) in toluene at −65 °C for 45 min. The resulting aldehyde (**6**) $[\alpha]_D$ −18° (*c* 1·0, MeOH) was obtained as an oil after purification *via* its semicarbazide by the method of Ito *et al.*[4] Protection of the phenolic group was essential since use of the free phenol gave lower yields of aldehyde and problems with the next stage of the synthesis. Condensation at −70 °C of the freshly prepared aldehyde (**6**) with the ylide (**7**), itself prepared from prop-2-ynyl alcohol,[5] afforded the *trans*-enyne (**8**) (42%), $[\alpha]_D$ +6·0° (*c* 1·0, MeOH), together with a small quantity (6·5%) of the crystalline *cis*-isomer, m.p. 70 °C, $[\alpha]_D$ +98·0° (*c* 1·0, MeOH).

Hydroboration of the *trans*-enyne with dicyclohexylborane (2·2 equiv.) followed by oxidation with alkaline hydrogen peroxide (7 equiv.) according to the method of Zweifel and Backlund[6] produced the desired $\beta\gamma$-unsaturated acid (**9**), (75%), characterised as its dicyclohexylammonium salt, m.p. 140—145 °C.

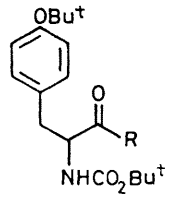
Coupling of the acid (**9**) to the tripeptide unit, (**10**) was achieved by using dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole as the activating agent. The protected pentapeptide analogue (**11**) was obtained after rapid column chromatography (SiO_2, EtOAc) as a solid (68%), m.p. 95—98 °C, $[\alpha]_D$ −20·4° (*c* 0·8, MeOH). Deprotection, using KOH in methanol, followed by trifluoroacetic acid in the presence of anisole and purification through Amberlite IR45 ion exchange resin yielded the analogue (**3**) as a homogeneous white powder, m.p. 193—195 °C, $[\alpha]_D$ −18° (*c* 0·5, dimethylformamide), *Rf* 0·8 (SiO_2, $CHCl_3$–, MeOH–, AcOH–, H_2O, 30:20:4:6). Leu^5-enkephalin was a more polar material under these conditions, R_f 0·6.

Biological assays on the derivative (**3**) showed an IC_{50} value of 600 nM against [^{3}H]naloxone bound to rat brain homogenates at 30 °C as against a value of 176 nM for [^{3}H]-Leu^5-enkephalin at 0 °C. Against bound [^{3}H]-Leu^5-enkephalin the IC_{50} values at 0 °C were 4·6 nM for (**3**) as against 3·1 for Leu^5-enkephalin (**4**).

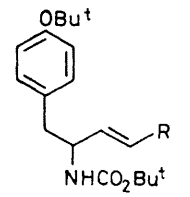
The high activity of the analogue (**3**) indicates that the presence of the Tyr^1–Gly^2 amide group is not essential for opiate activity. Thus the attainment of the receptor conformation and production of the biological response are not dependent upon hydrogen bonding to this region of the molecule in the form of either intramolecular bonds, *e.g.* β-loops, or intermolecular bonds, *e.g.* drug–receptor interactions. However, the observation of Hudson *et al.* that the fully reduced hydrocarbon analogue (**12**) shows negligible opiate receptor binding indicates that the *trans*-disposition of the substituents to the Tyr^1–Gly^2 bond is essential for activity.[7]

The use of the olefinic isosteric transformation offers an alternative strategy for the design of peptide analogues. It does not rely on the incorporation of D-amino acids and thus does not alter the spatial disposition of the α-substituents of a natural peptide.

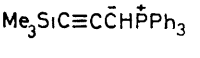
The possibility that either the analogue (**3**) or its precursors can participate as an inhibitor of peptidases or as a suicidal inhibitor[8] of certain amine oxidases remains to be established.



(5) R = OMe
(6) R = H

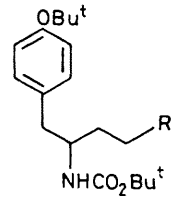


(8) R = C≡CSiMe$_3$
(9) R = CH_2CO_2H
(11) R = CH_2CO Gly L-Phe L-Leu(OMe)



(7)

(H)Gly L-Phe L-Leu(OMe)
(10)



(12) R = CH_2CO Gly L-Phe L-Leu(OMe)

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