Incorporation of *trans*-Olefinic Dipeptide Isosteres into Enkephalin and Substance P Analogues

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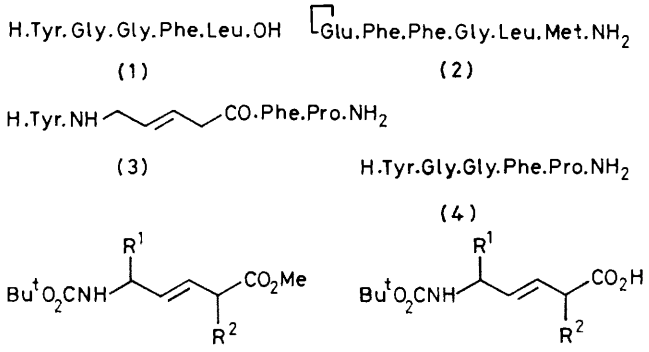
Summary Dipeptide isosteres are incorporated into hybrid polypeptide structures which possess biological potencies varying from 0·1 to 300% of those of the parent polypeptides

THE synthesis of some protected dipeptide isosteres is described in the preceding Communication [1] Here we report their incorporation into analogues of Leu-enkephalin (**1**) and the modified C-terminal hexapeptide (**2**) of substance P, which has been shown to possess similar biological potency to the parent undecapeptide in certain assay systems [2] By replacing various amide bonds in a polypeptide sequence with a *trans*-olefin, we hoped to maintain the geometry of the peptide backbone whilst investigating

whether such bonds were essential for biological activity, either for binding to the receptor or for maintaining a certain conformation by intramolecular hydrogen bonding. At the same time, hopefully, resistance to enzymic degradation and increased lipophilicity would lead to increased duration of action and passage through the blood-brain barrier, as outlined by Sammes *et al.*[3]

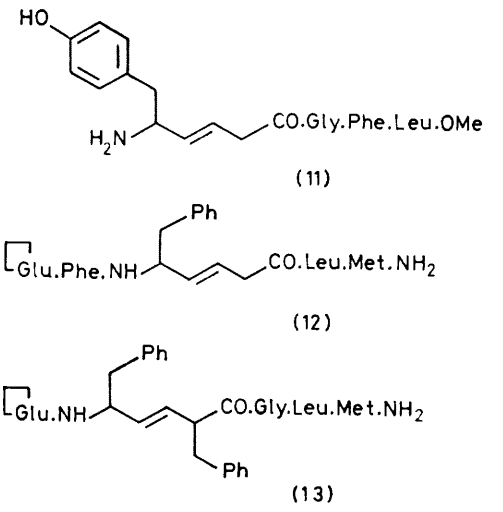


(**5**) $R^1=R^2=H$
(**6**) $R^1=CH_2C_6H_4OAc\text{-}p$, $R^2=H$
(**7**) $R^1=CH_2Ph$, $R^2=H$
(**8**) $R^1=R^2=CH_2Ph$
(**9**) $R^1=CH_2Ph$, $R^2=H$
(**10**) $R^1=R^2=CH_2Ph$

Firstly, the protected Gly.Gly isostere (**5**) was incorporated into an enkephalin analogue (**3**). Thus, (**5**) was coupled *via* its hydrazide with Phe.Pro.NH_2 and, after deprotection (HCl–AcOEt), the free amino group was coupled with BocTyr(Bu^t)OH using dicyclohexylcarbodiimide (DCCI) and 1-hydroxybenzotriazole. Further deprotection with HCl–MeOH led directly to the hydrochloride of (**3**). This compound exhibited 0·1% of the activity of the related pentapeptide (**4**) on the electrically stimulated guinea pig ileum preparation.

As another example of the isosteric replacement in the enkephalin structure, the peptide bond between Tyr^1 and Gly^2 was replaced using the Tyr.Gly isostere (**6**). Mild hydrolysis of (**6**) with 2N NaOH–MeOH gave the corresponding phenolic acid, which was coupled with H.Gly.Phe.-Leu.OMe using DCCI and 1-hydroxybenzotriazole. After chromatography (LH 20, MeOH and SiO_2, $CHCl_3$) the product was obtained as a homogeneous, white powder (80%). Deprotection was accomplished using aqueous trifluoroacetic acid in the presence of anisole and, after chromatography (SiO_2, $CHCl_3$–MeOH–H_2O 55:40:1), the product was freeze-dried from HCl–Bu^{t}OH to give the hydrochloride of (**11**) as a homogeneous, white powder (62%). This compound exhibited 300 and 24% of the activity of the Leu-enkephalin methyl ester on the electrically stimulated guinea pig ileum and mouse *vas deferens* preparations, respectively. Allowing for the fact that this compound is a racemate, these results compare favourably with those of Sammes *et al.*[3]

The hexapeptide analogues related to substance P were assembled using solid phase techniques. Thus, using DCCI, Met and Leu were sequentially coupled to a benzhydrylamine resin, followed by the acid (**9**), which was isolated as its dicyclohexylamine salt, m.p. 128–129 °C, after hydrolysis of (**7**) with 2N NaOH–MeOH. The synthesis was completed by coupling Phe with DCCI and pyroGlu as its trichlorophenyl ester. The hexapeptide unit was cleaved from the resin with HF giving the amide (**12**) which was purified by chromatography (AG 50, MeOH–H_2O, 70:30 and SiO_2, $CHCl_3 \rightarrow$ MeOH–$CHCl_3$ 1:9). This compound exhibited 24% of the activity of the related hexapeptide (**2**) on the isolated guinea pig ileum preparation.



For incorporation of the Phe.Phe isostere, the ester (**8**) was converted into the corresponding acid (**10**). Basic hydrolysis led to double bond migration and iodotrimethylsilane caused preferential urethane cleavage, so (**8**) was hydrolysed to the amino acid with concentrated HCl and reprotected with di-t-butyl carbonate in CH_2Cl_2 giving (**10**) (45%). The hexapeptide analogue (**13**) was constructed by sequential coupling to a benzhydrylamine resin using DCCI. Met, Leu, Gly, and (**10**) were added, followed by pyroGlu as its trichlorophenyl ester. Cleavage from the resin with HF gave the amide (**13**) which was purified by chromatography (AG 50, MeOH–H_2O 70:30 and SiO_2, $CHCl_3 \rightarrow$ MeOH–$CHCl_3$ 2:25). On the isolated guinea pig ileum preparation, (**13**) proved to be equipotent with both substance P and the hexapeptide (**2**).†

Thus we have demonstrated that the dipeptide isosteres may be incorporated into hybrid peptide structures either by classical coupling methods where yields are good, or by solid phase techniques where the efficiency of incorporation is of the order of 95%.

From these results and those of others[3] it can be seen that the replacement of an amide bond in a polypeptide backbone by a *trans*-olefin can give useful information about the necessity of that bond for biological activity. Clearly, the amide bond between Gly^2 and Gly^3 of the enkephalin derivatives is important for activity, whereas that between Tyr^1 and Gly^2 is not, although the specificity for different receptor types[4] may be altered by this isosteric replacement. The corresponding compounds, in which the amide bond is replaced by a saturated hydrocarbon linkage, have so far

† The corresponding saturated analogues of (**13**) have 0·35 and 40% of the activity of substance P for the *SS,RR*, and *SR,RS* enantiomers, respectively (R. H. B. Galt, N. N. Petter, and J. W. Growcott, unpublished observations).

been found to be less active than their olefinic counterparts,[5]† although these can still have appreciable activity This indicates that the *trans* geometry plays a large part in contributing to the biological activity

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