Bioorganic & Medicinal Chemistry Letters, Vol.3, No.5, pp. 831-834, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

A NEW APPROACH TO ENHANCE BIOAVAILABILITY OF BIOLOGICALLY ACTIVE PEPTIDES: CONJUGATION OF A δ OPIOID AGONIST TO β-CYCLODEXTRIN

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(*Received* 10 *June* 1992; *accepted* 20 *July* 1992)

Abstract: The cyclic δ opioid agonist [p-I-Phe^4]DPDPE, **1**, was conjugated to mono-6-amino-permethyl-β-cyclodextrin at the C-terminus to improve the bioavailability of **1**. In the rat brain binding assay, the conjugate **8** showed an IC_{50} = 134 nM vs. a δ ligand and IC_{50} > 10 μM at the μ receptor, making it less potent and selective than **1**. However, **8** shows antinociceptive properties (i.v.) in the mouse tail flick test and prolonged activity.

In spite of excellent progress in the field of peptide design[1] which has led to molecules of extraordinary biological potencies and selectivities in receptor binding and bioassay studies, examples of peptide pharmaceuticals still are relatively rare.

Metabolic resistance against proteases can be increased by incorporation of modified peptide bonds,[1,2] D-amino acids, conformational constraint and retro-inverso analogues.[1,3] In vivo solubility and bioavailability (e.g. the blood-brain barrier) can, in principle, be improved by coupling peptides to carrier systems such as glucose transporters.[4]

The highly δ opioid receptor selective cyclic peptide [p-I-Phe^4]DPDPE,[5] has been a lead compound in our efforts to synthesize a potent and selective δ opioid analgesic. Due to its cyclic nature and presence of two D-amino acids, DPDPE has excellent metabolic stability, but bioavailability still remains to be fully optimized. Since enkephalin coupled to monosacharides retains its biological activity,[6] we decided to conjugate [p-I-Phe^4]DPDPE, **1**, to permethylated β-cyclodextrin (β-CD, **2**, Figure 1), potentially an excellent drug delivery system, and determine the opioid activity profile of the conjugate **8**. Analogue **1** was chosen for conjugation

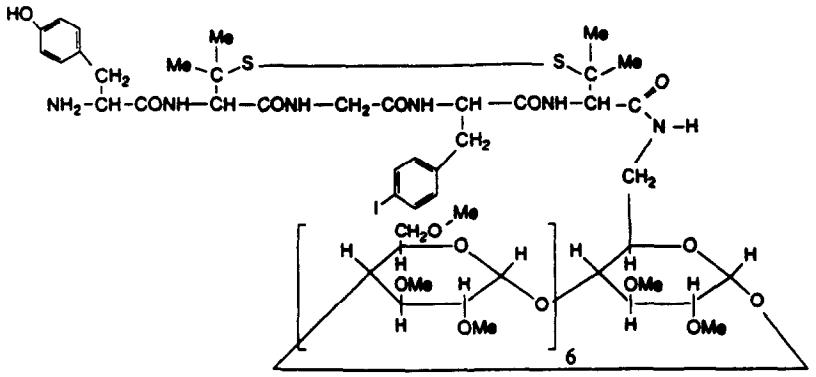


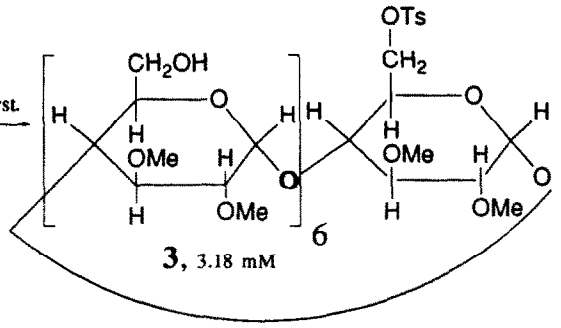
Figure 1

Scheme I

A

β–CD + TsCl $\xrightarrow[40\ C,\ 4\ hrs]{\text{pyridine (150 ml)}}$ $\xrightarrow{\text{2x recryst.}}$ **3**, 3.18 mM

2, 22mM 22mM



$\xrightarrow[90\ C,\ 4\ hr]{NaN_3,\ 27\ ml\ H_2O}$ N_3-β–CD $\xrightarrow[RT,\ overnight]{Me_2SO_4\ 22.5\ ml;\ aliquat\ 0.2\ g,\ KOH\ 7.25g}$ $\xrightarrow{NH_3\ (aq),\ 5\ ml}$

$\xrightarrow[Cellulox\ Ester]{dialysis}$ N_3-β–CD(per-OMe) **4**, 0.4g $\xrightarrow[overnight]{PtO_2,\ H_2\ ,\ HCl\ aq}$ HCl.NH_2-β-CD(per-OMe) **5**, 69.5%

B

[p-I-Phe^4] DPDPE **1** $\xrightarrow[0^\circ C \rightarrow RT,\ overnight]{\text{di-t-butyl-dicarbonate (1.1 eq), DIEA}}$ Boc-[p-I-Phe^4]DPDPE **6**

C

6 + **5** $\xrightarrow[DIEA,\ 4\text{-NMP, DCM/DMF}]{DCC\ (1.1eq),\ HOBt\ (2eq)}$ Boc-[p-I-Phe^4]DPDPE-NH-β–CD(per-OMe) **7** $\xrightarrow[30\ min,\ RT]{25\%\ TFA/DCM}$

[p-I-Phe^4]DPDPE-NH-β–CD(per-OMe)

8

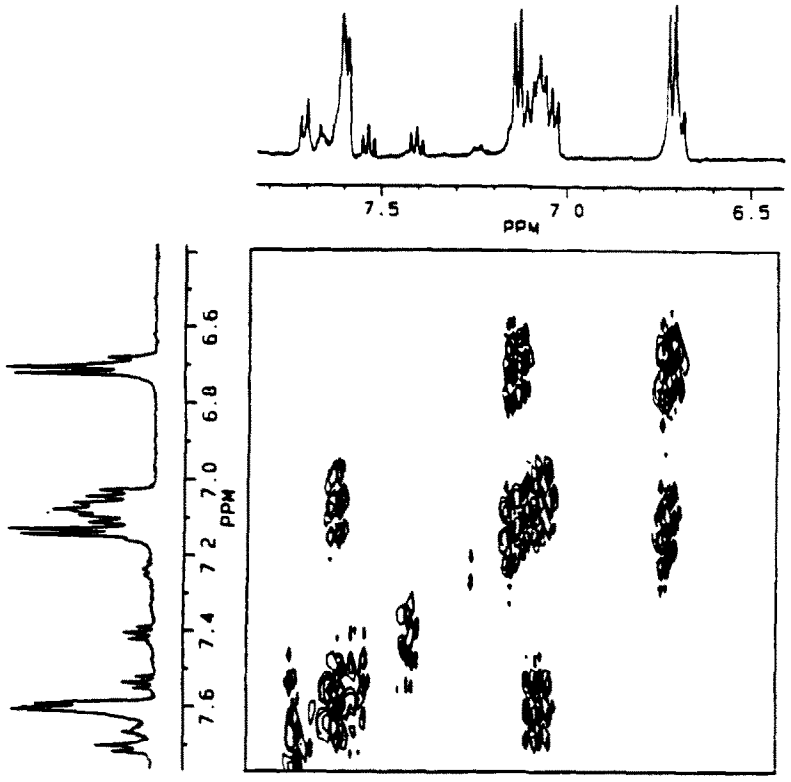


Figure 2. A portion of the 2D-NMR DQF-COSY revealing the AA'XX' systems of p-I-Phe^4 and Tyr^1 in the [p-I-Phe^4]DPDPE-cyclodextrin compound **8**.

because of its potent and exceptionally selective[5] δ opioid receptor biological activities. This paper complements one of Parrot-Lopez et al.,[7] which did not report any biological data for their compound.

We synthesized the conjugate **8** by coupling Boc-[p-I-Phe^4]DPDPE, **6**, and HCl·NH_2-β-CD(per-OMe) **5** according to the methods outlined in Scheme I. The substrate **6** was obtained by N^{α}-Boc protection of [p-I-

Table I Opioid receptor binding activities and in vitro GPI and MVD bioassay potencies of **1** and **8**.

Compound	IC_{50}(nM) [^{3}H]pClDPDPE	IC_{50}(nM) [^{3}H]CTOP	Ratio IC_{50}(CTOP)/ IC_{50}pClDPDPE	A_{50}(nM) MVD	A_{50}(nM) GPI	Ratio A_{50}(GPI)/ A_{50}(MVD)
1	1.6	609	380	4.1	7300	1780
8	145±8	>10μM	>75	100	7500	75

Phe[4]]DPDPE synthesized by a combination of solid and solution phase peptide methods. To obtain **5**, we have monotosylated[8] β-CD, and converted the tosylate **3** to an azide, followed by permethylation of all free hydroxyl groups of the saccharide moiety. The crude product was extensively dialyzed, affording **4** in modest yield. Catalytic reduction of **4** resulted in the hydrochloride salt of amino-β-CD(per-OMe) (**5**) in good yield. The conjugate **8** was extensively characterized by ^{1}H NMR (d_6-DMSO), HPLC and quantitative amino acid analysis.[9] The aromatic region DQF-NOESY spectrum of **8** is shown in Figure 2, and reveals two sets of off-diagonal cross peaks for the AA'XX' systems of Tyr[1] and p-I-Phe[4] residues, as expected for the desired compound **8**.

We have examined the biological properties of **8** using both its binding properties to rat brain homogenates at δ (vs [^{3}H]pClDPDPE) and μ (vs [^{3}H]CTOP) opioid receptors as well as its classical in vitro bioactivity in the MVD (δ) and GPI (μ) bioassay systems (Table 1). The data demonstrate that, while the potency of **8** in comparison to **1** has decreased, **8** exhibits surprisingly potent antinociceptive properties (about 50% that for **1**, data not shown) given i.c.v. in the mouse tail flick test, and is active given i.v. as well, and showed no toxicity. Furthermore, the compound has a very prolonged action in both the MVD and GPI assays ($t_{½}$ > 3 hours, data not shown) that could not be reversed even after repeated washings. An examination of the reverse phase HPLC chromatograms of **8** and **1** (data not shown), clearly reveals an increased lipophilicity of the conjugate in comparison to **1**. The reduced affinity of **8** relative to **1** may be a result of adverse steric effects at the δ receptor and/or to the loss of a negative charge at the C-terminus. As for the loss of binding potency of **8** at the μ receptor but its almost equivalent potency to **1** in the GPI assay, this may be due to the heterogeneity of opioid receptors. At present we are evaluating the abilities of **8** and related analogues to cross the blood-brain barrier both in vitro and in vivo.

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9. Satisfactory analytical data were obtained for all compounds reported in this paper.