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THE SYNTHESIS OF A RADIOLIGAND WITH HIGH POTENCY AND SELECTIVITY FOR CCK_B/ GASTRIN RECEPTORS

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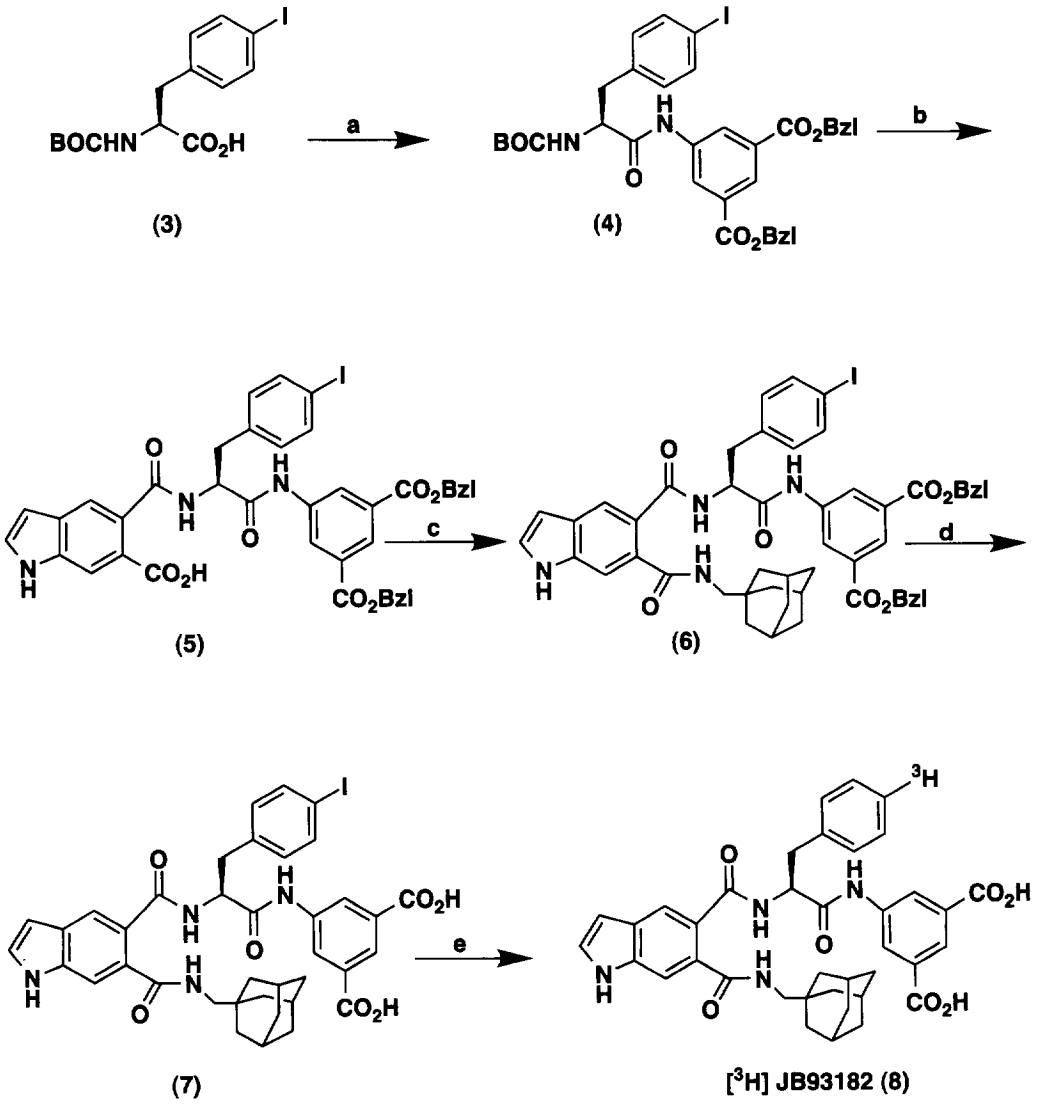
Abstract: The synthesis and characterisation of a non-peptide, tritiated radioligand, [^{3}H] JB93182, which has high affinity and selectivity for CCK_B/gastrin receptors, is described. Copyright © 1996 Elsevier Science Ltd

Gastrin and cholecystokinin are structurally related peptide hormones which are found both in the CNS and in gastrointestinal tissue.[1] Two classes of receptors for these hormones have been classified, namely CCK_A and CCK_B/gastrin, and selective non-peptide antagonists for each have been described.[2] We have recently reported on two new series of selective CCK_B/gastrin receptor antagonists, [3-5] which culminated in the identification of the indole derivative, JB93182, compound **1**,[6] as a ligand of particular interest. Some of the binding properties of this compound have already been disclosed.[5,7]

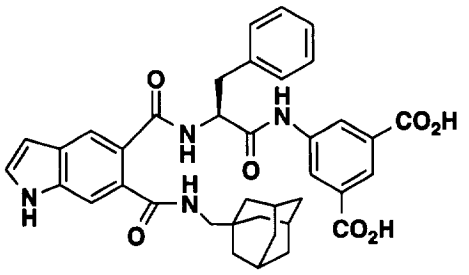
In order to help elucidate the possible plurality in CCK_B/gastrin binding sites as has been suggested,[8-10] we felt that it was important to examine various receptor containing tissues with a radiolabelled antagonist. The most commonly used radioligand for the CCK_B/gastrin receptor system is the agonist [^{125}I] Bolton Hunter CCK8S and, indeed, assays using this material had helped to identify JB93182.[5] Only a few examples of radiolabelled antagonists at CCK_B/gastrin receptors are available. [^{3}H]-L-365,260 has been reported by Chang *et al.* [11] but while this material has an adequate selectivity profile with respect to CCK_A receptors,[12] it has been shown to have a relatively poor specific to non-specific binding ratio.[13] Both a tritiated[13] version of the Parke-Davis ligand PD 140,376 (**2**) and an iodinated[14] analogue have also been described. However, in some assays PD compounds from this series have been shown to behave as partial agonists[15] at CCK_B/gastrin receptors. Our observations using the cold ligand suggest that this is a feature shared by PD 140,376,[16] which in some cases might lead to a lack of clarity in interpreting radioligand binding data.

This communication will describe the preparation and radiochemical characterisation of [^{3}H]-JB93182 (compound **8**).

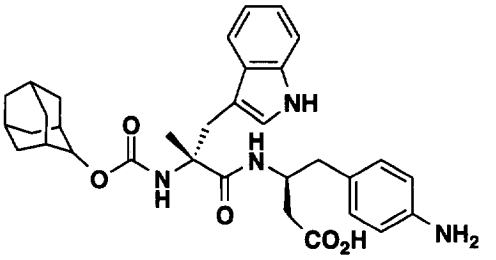


Scheme 1

Reagents: a) 3,5-bis(benzyloxycarbonyl)aniline, iPr_2NEt, PyBrOP®, DCM; b) (i) TFA, aq K_2CO_3; (ii) Indole-5,6-dicarboxylic acid anhydride, CH_3CN, Δ; c) 1-Adamantanemethylamine, iPr_2NEt, PyBOP®, DCM; d) LiOH, THF, H_2O, MeOH, Δ; e) T_2, 10% Pd on C, iPr_2NEt, EtOH.



JB93182 (1)



PD 140,376 (2)

The synthesis was carried out in five steps as shown in Scheme 1. The initial target, the iodo derivative **7**, was chosen as it allowed the possibility of introducing either an iodinated or a tritiated label in a final step. Thus the BOC derivative of 4-iodo-L-phenylalanine, **3**, was coupled with 3,5-bis(benzyloxycarbonyl)aniline[17] in dichloromethane (DCM) solution in the presence of the coupling reagent PyBrOP® [CAS reg. No. 132705-51-2, Novabiochem] and diisopropylethylamine. The reaction produced a 64% yield of the condensed product **4**. Removal of the BOC protection from this intermediate using TFA, revealed an amine which was reacted with indole-5,6-dicarboxylic acid anhydride,[5] in refluxing acetonitrile for 30 min. A mixture of compound **5** and the regioisomer with side-chains reversed was obtained in an overall yield of 40% and in an approximately 4:1 ratio. No attempt was made to separate the regioisomers at this stage, instead the mixture was reacted with 1-adamantanemethylamine in dichloromethane solution in the presence of the coupling reagent PyBOP® [CAS reg. No. 128625-52-5, Novabiochem] and diisopropylethylamine. After stirring overnight at room temperature, the desired less polar regioisomer **6** was isolated by column chromatography in 35% yield. This material was treated for about 1 min with a hot aqueous lithium hydroxide solution in a mixture of methanol-THF to give the crude diacid **7** which was purified by reverse phase HPLC.[18] In order to confirm that this key intermediate corresponded to the correct regioisomer of JB93182, a small amount was treated with hydrogen over 10% palladium on charcoal to remove the iodine. This gave a material that co-eluted on HPLC with an authentic sample of JB93182.

With compound **7** in hand, attempts were made to introduce a radiolabel. An iodine exchange reaction was carried out, but the radiolabelled material isolated proved to be too unstable for use. Instead tritiation was performed using tritium gas over 10% palladium on charcoal in a mixture of diisopropylethylamine and ethanol. This procedure incorporated a single tritium atom into the compound to leave the desired radiolabelled material, compound **8**, with a specific activity of 28 Ci/mmol and a radiochemical purity of at least 97% by HPLC. Saturation analysis indicated that compound **8** labelled a homogenous population of sites in rat cerebral cortical membranes. The B_{max} was 3.61± 0.65 pmol g^{-1} (original wet weight of tissue), and the pK_D was 9.48±0.08 (n=5 ± s.e. mean). A range of CCK_B/gastrin receptor ligands such as L-365,260 (pK_i 7.63± 0.07, Hill slope 1.17±0.13, n=8) competed with the binding of compound **8** to rat cortical membranes. A more

detailed analysis of the pharmacological behaviour of this labelled ligand and the significance of these results are presented elsewhere.[19,20]

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