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SYNTHESIS OF A POTENT AND SELECTIVE NON-PEPTIDE CCK-B/GASTRIN RECEPTOR ANTAGONIST TRITIATED LIGAND.

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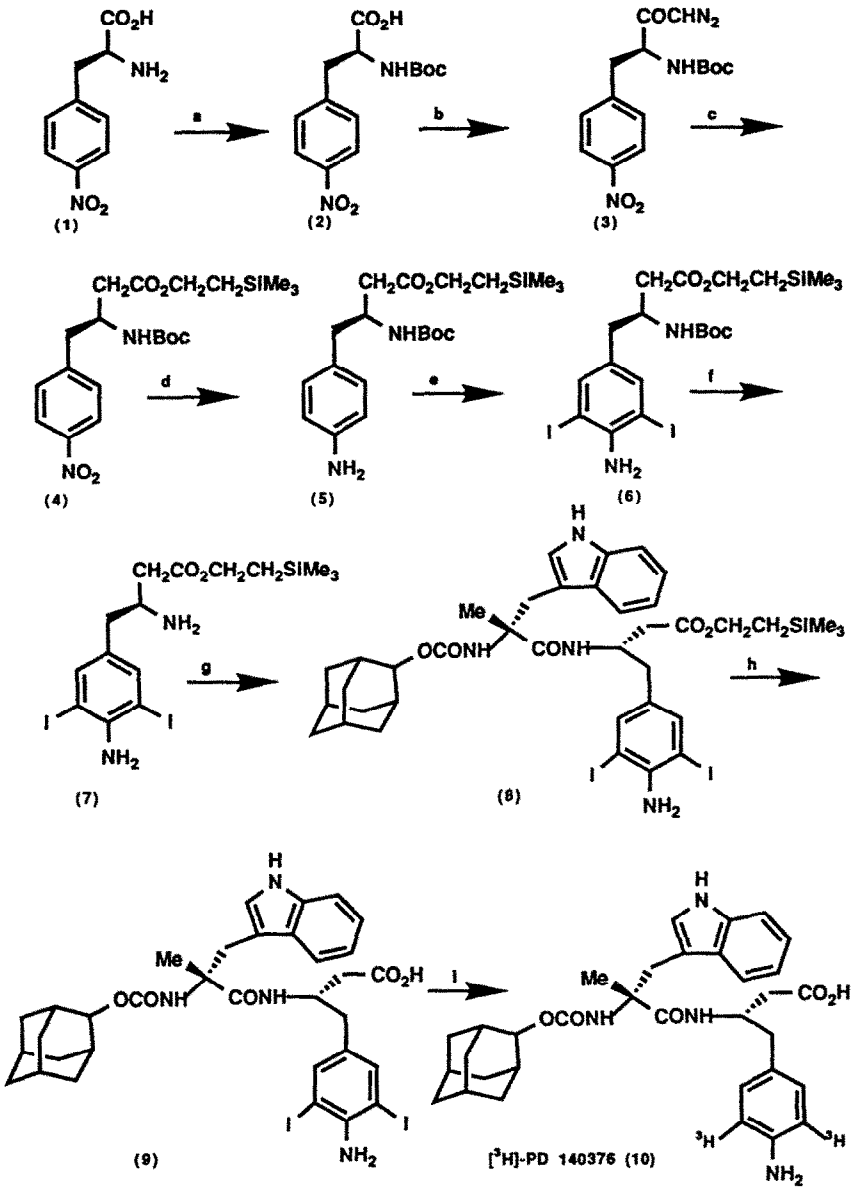
Abstract: The synthesis of a novel, potent and selective non-peptide gastrin/CCK-B receptor antagonist in a tritiated form is described, via a route which allows for introduction of the radiolabel in the final step.

In recent years there has been considerable interest in the neuropeptide cholecystokinin leading to the development of selective non-peptide antagonists of both the CCK-A and CCK-B receptor subtypes.[1] CCK-B and gastrin receptors are, at present, not clearly distinguishable and, to date, CCK-B receptor antagonists invariably exhibit gastrin antagonism at similar doses[1,3]. Potential therapeutic uses of CCK-B antagonists include treatment of gastro-intestinal disorders and anxiety.[2] We have recently described[3] a series of chemically novel, potent and selective CCK-B receptor antagonists, an example of which is CI-988 (Fig. 1, formerly known as PD 134308). To facilitate further biological investigation of the CCK-B receptor and evaluation of CCK-B receptor antagonists, a related compound, PD 140376 (**10**), was selected for radiolabelling. This compound offers several advantages over the alternative CCK-B/gastrin antagonist radioligand, [^{3}H]-L-365260, reported by Chang et al.[4] PD 140376 has higher affinity and selectivity for the CCK-B/gastrin receptor than L-365260, has a higher ratio of specific to non-specific binding in cortex and gastric mucosa and also has the potential for chemical cross-linking to the receptor via the aryl-NH_2 moiety.[5] The synthesis of radiolabelled PD 140376 and a brief account of its biological activity is described herein.



Scheme 1

Reagents: a) $NaHCO_3$, Boc_2O, aqueous dioxan; b) (i) $^tBuOCOCl$, N-Me-morpholine, THF; (ii) CH_2N_2, ether; c) $PhCO_2Ag$, NEt_3, $Me_3SiCH_2CH_2OH$; d) 10% Pd/C, H_2, EtOAc; e) NaI, chloramine T, AcOH; f) p-toluenesulphonic acid, CH_2Cl_2; g) 2-Adamantyloxycarbonyl-α-Me-Trp-OH, DCC, HOBt, EtOAc; h) TBAF, THF; i) 10% Pd/C, MeOH, $^iPr_2NEt$, $^3H_2$.

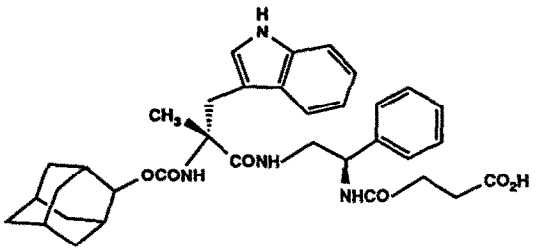


Fig. 1 CI-988

The synthesis of [^{3}H]-PD 140376 was achieved in nine steps from S-p-nitrophenylalanine **1** (Scheme 1) with the radiolabel conveniently introduced by catalytic tritiation with tritium gas in the final step. Protection of the amine and homologation of the acid via an Arndt-Eistert reaction gave the di-protected amino acid **4**, following previously described procedures.[6] Catalytic reduction of **4** over palladium on carbon gave **5** which was subsequently iodinated with two equivalents of sodium iodide and chloramine T in acetic acid[7] to give **6**. Deprotection of the amine, followed by coupling to 2-adamantyloxycarbonyl-α-methyl-tryptophan[3] with dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt), and deprotection of the acid with tetrabutylammonium fluoride (TBAF) gave the target compound precursor **9**. Hydrogenation of **9** over palladium on carbon gave the ^{1}H parent compond **10** (PD 140376).[8] Full experimental details of this procedure for analogous compounds have been described previously.[6] In homogenate binding studies using [^{125}I]-Bolton Hunter CCK-8 and mouse cerebral cortex[3], PD 140376 was found to have high affinity and selectivity for the CCK-B receptor (CCK-B K_i = 0.2nM; CCK-A K_i = 350nM). Furthermore this compound antagonised excitations of rat ventromedial hypothalamic neurones evoked by the CCK-B receptor selective agonist pentagastrin[9] (K_e = 1.4nM). Reduction of the aryl di-iodide, **9**, with tritium in the presence of di-isopropylethylamine and 10% palladium on carbon gave [^{3}H]-PD 140376 with a specific activity of 51.0 Ci/mmol and a radiochemical purity of 96% by TLC. [^{3}H]-PD 140376 bound saturably to membrane homogenates prepared from guinea pig cerebral cortex (B_{max}=119±15 fmol/mg protein), guinea pig gastric mucosa (B_{max}= 296±44 fmol/mg protein) and AR42J cells (B_{max}=31±4 fmol/10^5 cells) and with high affinity (K_D = 0.1-0.3nM depending on the tissue). Full experimental details will be reported elsewhere.[10] Binding of [^{3}H]-PD 140376 to guinea pig cortex was inhibited by a range of CCK-related peptides including CCK-8S (K_i = 0.65nM), pentagastrin (K_i = 22nM) and also by the CCK-B selective antagonists CI-988 (K_i = 0.98nM) and L-365,260 (K_i = 1.8nM). In contrast the potent

CCK-A receptor selective antagonist devazepide inhibited binding only at relatively high concentrations (K_i = 62nM).

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8. Compound **10**. mp 115-120°C; $[\alpha]_D^{20}$ = +11.2° (c=1, MeOH); H NMR (300MHz, d_6-DMSO) δ 1.25(s, 3H), 1.40-1.55(m, 2H), 1.60-1.85(m, 8H), 1.85-2.00(m, 4H), 2.21(dd, J=15.9,6.3Hz, 1H),2.32(dd, J=15.8,6.1Hz,1H), 2.5(obscured by DMSO, 1H), 2.61(dd, J=13.7,6.7Hz, 1H), 3.16(d, J=14.4Hz, 1H), 3.3(obscured by water, 1H), 4.10-4.20(m, 1H), 4.67(s, 1H), 6.46(d, J=8.3Hz, 2H), 6.66(s, 1H), 6.80(d, J=8.3Hz, 2H), 6.85-6.90(m, 2H), 7.01(t, J=7.3Hz, 1H), 7.29(d, J=8.0Hz, 1H), 7.45(d, J=7.8Hz, 1H), 7.55(d, J=8.1Hz, 1H), 10.80(s, 1H); Anal. Calc'd for $C_{33}H_{40}N_4O_5.H_2O$: C, 67.09; H, 7.17; N, 9.49. Found: C, 67.15; H, 7.42; N, 9.41.

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