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The Asymmetric Synthesis of Non-peptide CCK-A Receptor Agonists

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Abstract : The asymmetric synthesis, CCK receptor binding affinities and CCK-A agonist properties of a novel series of non-peptide CCK-A receptor selective ligands is reported.

INTRODUCTION

We have previously reported[1] on the design and synthesis of non-peptide CCK-A receptor selective ligands an example of which is compound (*RS*)-1 (see scheme). This series of compounds was prepared as putative non-peptide topographical mimetics of the endogenous ligand for CCK-A and CCK-B receptors, CCK-8 [AspTyr(SO$_3$H)MetGlyTrpMetAspPheNH$_2$].[2] The indole, phenyl and aryl urea moieties of (*RS*)-1 were appended off a central single carbon atom template (the α-carbon of the modified Trp residue) in an attempt to mimic the binding properties of the side chains of the important Trp, Phe and Tyr(SO$_3$H) residues respectively in CCK-8. [A more detailed description of this strategy and the reasons for targeting the Trp, Phe and Tyr(SO$_3$H) side chains can be found in our initial publication[1] on these compounds].

Since (*RS*)-1 is racemic, we embarked upon the asymmetric synthesis of both possible enantiomers [(*R*)-1 and (*S*)-1] of (*RS*)-1 in order to determine the CCK receptor affinities and CCK-A receptor functional activities of each enantiomer independently.

CHEMISTRY

The asymmetric synthesis of the enantiomer (*R*)-1 is summarised in the scheme below. Yields quoted in the scheme represent that achieved in the conversion of (S)-tryptophan methyl ester (*S*)-2 to the R-configured target molecule (*R*)-1 although, as one might expect, similar yields were obtained in the synthesis of the corresponding S antipode (*S*)-1. In the

interests of brevity this section will describe only the conversion of (*S*)-**2** to (*R*)-**1**.

Starting from the commercially available (S)-tryptophan methyl ester (*S*)-**2**, initial protection of the N^α nitrogen atom with benzylchloroformate followed by acid catalysed ring closure[3] of the urethane NH onto the 2-position of the indole, provided the hexahydro[2,3-b]pyrrolo indole (*S*)-**4** in an overall yield of 58% for the two steps. In agreement with literature precedent[3,4] the cyclisation gave exclusively the thermodynamically more stable trans isomer. Protection and stabalisation of (*S*)-**4** was accomplished by sodium carbonate-mediated reaction with benzylchloroformate to give the urethane (*S*)-**5** in 74% yield. Deprotonation of (*S*)-**5** with lithium bis(trimethylsilyl)amide at -78°C followed by quenching of the resulting enolate with benzyl-2-bromoacetate gave the desired product (*R*)-**6** with retention of stereochemistry as indicated by the characteristic upfield shift (δ 2.97) of the exo configured carboxymethyl protons.[4] The somewhat upfield chemical shift of the carboxymethyl protons is a consequence of its shielding by the diatropic ring current and thus serves as a useful indicator of the stereochemical outcome of the alkylation.[4] This alkylation step proceeded in 64% yield with complete diastereoselectivity from the exo face within the limits of 360MHz ^{1}HNMR detection. Subsequent ring opening of the tricycle (*R*)-**6** to the α,α-disubstituted tryptophan derivative (*R*)-**7** was achieved in good yield (84%) by stirring at ambient temperature with trifluoroacetic acid.[4] Removal of the three benzyloxycarbonyl protecting groups in (*R*)-**7** by catalytic hydrogenation followed by base promoted coupling of the N^α-nitrogen atom to 2-adamantylchloroformate[5] provided (*R*)-**8** in 53% yield over the two steps. Subsequent base-catalysed hydrolysis of the methyl ester group in (*R*)-**8** gave the key di-acid intermediate (*R*)-**9** in good yield (82%). The corresponding anhydride of the diacid (*R*)-**9**, formed in situ via initial formation of the pentafluorophenyl active ester, was taken up without purification in DMF at 0°C and the alkyl amine **11** added. The alkyl amine **11** was previously prepared by the dropwise addition of 1,4-diaminobutane to a solution of 2-chlorophenyl isocyanate in hexane at 0°C. The resulting ring opening occurred regiospecifically at the less hindered carbonyl moiety[6] to yield the required carboxylic acid (*R*)-**12** in moderate yield (42%). The structure of the acid (*R*)-**12**, and hence the regioselectivity of the anhydride ring opening reaction, was unequivocally confirmed on the basis of longe range ^{1}H/^{13}C NMR correlation studies (hmbc) which established connectivity between the methylene and amide protons of the -CH_2CONH- moiety. The target molecule[7] (*R*)-**1** was obtained upon coupling of the carboxylic acid derivative (*R*)-**12** to phenethylamine using conventional active ester methodology (51% yield).

Scheme
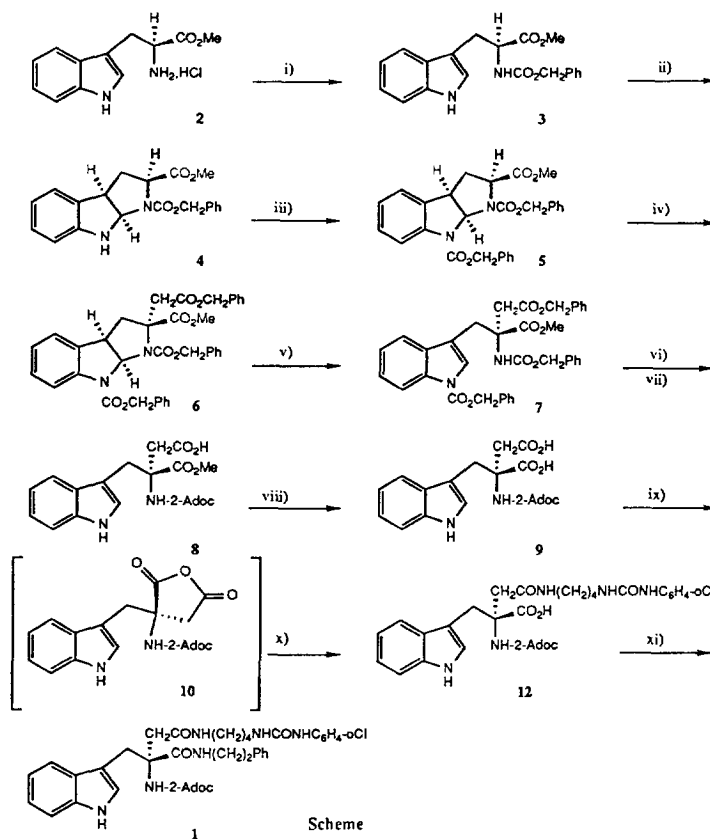
Reagents and conditions : i) Et$_3$N, ClCO$_2$CH$_2$Ph, EtOAc, -40 °C, N$_2$, 93% ; ii) TFA, 4 days, 62%; iii) Na$_2$CO$_3$, ClCO$_2$CH$_2$Ph, 1,4-Dioxane/H$_2$O, 74% ; iv) LHMDS, DMPU, BrCH$_2$CO$_2$CH$_2$Ph, THF, -78 °C, N$_2$, 60% ; v) TFA, 1.5 h, 84% ; vi) H$_2$, Pd/C 10% 45 psi, EtOH, 20 °C, 79% ; vii) Na$_2$CO$_3$, 2-AdocCl, 1,4-Dioxane/H$_2$O, 67% ; viii) LiOH, 20 h, reflux, THF, 82% ; ix) DCC, Pentafluorophenol, EtOAc, N$_2$, 0 °C ; x) H$_2$N(CH$_2$)$_4$NHCONHC$_6$H$_4$-oCl (11), DMF, 0 °C, 42% ; xi) 2-(1H-Benzo-triazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, Diisopropylethylamine, PhCH$_2$CH$_2$NH$_2$, DMF, 51%. (2-Adoc is 2-adamantyloxycarbonyl)
[Yields are quoted for the conversion of (S)-2 to (R)-1].

BIOLOGY

The CCK receptor affinities and CCK-A receptor functional activities of (R)-1 and (S)-1 are reported in the table. Somewhat surprisingly, the CCK-A receptor affinities of the pure enantiomers (R)-1 (IC$_{50}$=120nM) and (S)-1 (IC$_{50}$=150nM) were essentially equivalent, within experimental variation, to the racemic mixture (RS)-1 (IC$_{50}$=79nM). The finding that the two enantiomers exhibit the same receptor binding affinity is unusual but not without precedent.[8] Of particular interest, however, is that both (R)-1 and (S)-1 are, as hoped for, full efficacy agonists at the CCK-A receptor in rat pancreas albeit with reduced potencies in comparison to the endogenous agonist CCK-8 (see table).

Table.   CCK Receptor Affinities[9] and CCK-A Receptor Functional Activity[10] of α,α-Disubstituted Tryptophan Derivatives.

Compound	CCK Binding IC_{50} (μM)		Amylase Secretion
	CCK-A	CCK-B	EC_{50}(μM)
CCK-8	0.10nM	0.30nM	0.018nM
(*RS*)-1	0.079($\pm$0.015)	18($\pm$7.2)	-
(*R*)-1	0.12($\pm$0.020)	3.3($\pm$0.92)	5.0($\pm$4.0)
(*S*)-1	0.15($\pm$0.037)	3.6($\pm$0.78)	0.48($\pm$0.20)

CONCLUSIONS

In this paper we have demonstrated the feasibility of designing high efficacy, non-peptide agonists for peptide receptors based on the structure of the endogenous peptide agonist. In order to achieve this objective we have successfully completed the asymmetric synthesis of both possible enantiomers [(*R*)-1 and (*S*)-1] of the targeted CCK-8 mimetic, the α,α-disustituted tryptophan derivative (*RS*)-1.

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7. Physical data for compound (*R*)-1 : δ_H (300MHz, CDCl$_3$) 1.50-2.00 (18H, m), 2.56 (1H, d, J 14Hz), 2.73 (2H, t, J 7Hz), 2.92 (1H, d, J 14Hz), 3.22 (4H, m), 3.45 (3H, m), 3.60 (1H, d, J 14Hz), 4.77 (1H, s), 5.73 (1H, br.s), 6.36 (1H, br.s), 6.69 (1H, s), 6.84 (1H, s), 6.91   (1H, t, J 8Hz), 7.04-7.34 (11H, m), 7.49 (1H, d, J 8Hz), 7.95 (1H, s), 8.19 (1H,s); m/z (FAB) 767.3687 (M+H, C$_{43}$H$_{52}$N$_6$O$_5$Cl requires 767.3686); [α]$_D$ +13.0 (c 1, MeOH); mp. 109-114 C.
Physical data for compound (*S*)-1 : ^{1}HNMR and mp. as above; m/z (FAB) 789.3494 (M+Na, C$_{43}$H$_{51}$N$_6$O$_5$ClNa requires 789.3507); [α]$_D$ -13.7 (c 1, MeOH).
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