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Preparation of Dipeptoid Mimetics for the Tetrapeptide Cholecystokinin, CCK(30-33)

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Abstract

The diastereoselective synthesis of 2,3-methanophenylalanine methyl esters (**5**) has been achieved in 58% yield. The preparation of the dehydropeptides (**1**, R = Me; 2, R = H) and the cyclopropylpeptides (**3**, R = Me; 4, R = H) possessing good binding affinities for the CCK-A and CCK-B receptors is described. Conformational studies of the dipeptide esters **1** and **3** indicated the presence of a β-turn within the peptide backbone, although there was no preference in type. The Phe and Trp moieties, however, did prefer to be situated on the same side of the peptide turn which is favourable for receptor binding.

Cholecystokinin (CCK) is a peptide hormone which is found to exist in several biologically active forms. In the periphery, the prevalent forms are CCK-58, CCK-33, CCK-8 (H-Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2) and CCK-4 (H-Trp-Met-Asp-Phe-NH_2), whilst in the central nervous system (CNS) they exist predominantly as CCK-8 (sulphated). The function of CCK within these peripheral sites is as a regulator of gut function, digestion and feeding (Dockray 1989) whereas in the CNS it functions as a neurotransmitter and neuromodulator (Crawley 1988; Altar 1989). There are two types of CCK receptor known, namely, the CCK-A (alimentary) and CCK-B (brain) receptors. The CCK-A receptors predominate in the periphery, although they have been identified in several discrete brain regions within the CNS (Hill & Woodruff 1990) where their role is proposed to be in the modulation of dopaminergic function (Vickory et al 1989; O'Neill et al 1991). In contrast, the CCK-B receptors are found to predominate in the CNS where their role is thought to be in the modulation of pain (Baber et al 1989), anxiety (Ravard & Dourish 1990), cognition (Itoh 1990) and perhaps the regulation of certain feeding behaviour (Dourish et al 1989). More recently, both the CCK-A (Wank et al 1992) and CCK-B/gastrin (Kopin et al 1992) receptors have been cloned. The latter study (Kopin et al 1992) revealed a high homogeneity between CCK-B and gastrin receptors within canines.

Recent advances in the development of CCK antagonists have included the benzodiazepine derivatives devazepide (MK-329, **I**) which has an affinity for the CCK-A receptor comparable to CCK itself ($IC50 = 10^{-10}$ M) in a variety of animal models (Lotti et al 1987), and the potent CCK-B/gastrin selective compound (R)-L-365,260 (**II**) (Bock et al 1989). Several research groups have used CCK-4 as the basis for further exploration of high affinity CCK-B antagonists. Horwell et al (1990) initially evolved a series of dipeptoids represented by the tryptophan analogue **III** with moderate affinity (IC50~1–10 μM) for the CCK-B receptor. This has led on to the discovery of the novel dipeptoids PD-134308 (Cl-988, **IV**) possessing a greatly enhanced potency at the CCK-B receptor (IC50 = 1·7 nM) and its closely related analogue PD-135138 (**V**) (Horwell et al 1991).

As a consequence of this the dehydrophenylalanine (ΔPhe) and cyclopropylphenylalanine (∇Phe)† dipeptide analogues N^{α}-(2-Adoc)-*R*-α-Me-Trp-Δ^{Z}Phe-OR (1, R = Me; 2, R = H) and N^{α}-(2-Adoc)-*R*-α-Me-Trp-∇Phe-OR (3, R = Me; 4, R = H) were prepared (Campbell et al 1993) owing to the conformationally restrictive nature that these ΔPhe and ∇Phe residues have been found to induce upon the peptide backbone within several bioactive peptides (Schmidt et al 1988; Kaur et al 1992; Mapelli et al 1986).

Materials and Methods

Preparation of 2,3-methanophenylalanine methyl esters (∇Phe-OMe) (**5**)

Firstly a synthesis of 2,3-methanophenylalanine methyl esters (**5**) was established. Our methodology was based on a modification of Schollkopf's bislactim ether approach (Schollkopf 1983a-c), utilizing a chiral diketopiperazine template (**8**) (Scheme 1). Diketopiperazine (**8**) was bisacylated to generate **9** in 60% yield. Treatment of **9** with potassium *t*-butoxide and benzaldehyde afforded mainly the monoacylated (*Z*)-benzylidene (**10a**) (Z:E, 9 : 1) in excellent yield (90%). The two geometrical isomers (**10a,b**)‡ were easily separated by flash chromatography and served as intermediates to both (*Z*)- and (*E*)-2,3-methanophenylalanine methyl esters (**5a,b**). The relative stereochemistry of each benzylidene isomer of **10** was ascertained by NMR (N.O.E). The (*Z*)-benzylidene (**10a**) was then treated with

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† The symbols ∇ and Δ are as defined by Stammer (1990) who states: "The symbol ∇^{Z} and Δ^{E} prefixed to the abbreviation for an amino acid residue as in ∇^{Z} Phe, means the *Z*-diastereomer of 2,3-methano- or cyclopropanephenylalanine. It is used here only when the methanoamino acid appears in a peptide chain. The Δ^{Z} symbol indicates a dehydroamino acid as a ∇^{Z}Phe, meaning (*Z*)-2,3-dehydrophenylalanine.

‡ **a** refers to *Z*-configuration, and **b** refers to *E*-configuration.

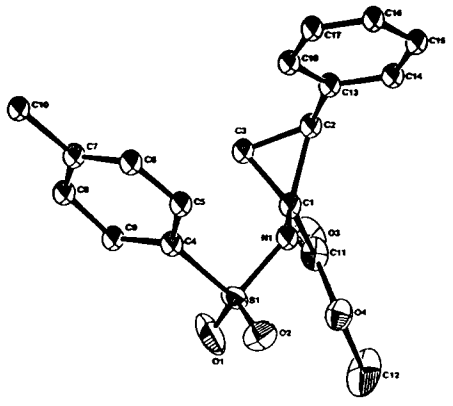


FIG. 1. X-ray crystal structure of **14a**.

diazomethane to give directly a 4 : 1 mix of diastereomeric spirocyclopropanes (**11**), without any isolation of the pyrazoline intermediate, but in low yield (20%). These diastereomers were separable by flash chromatography and the major component (**11a**) was deacylated by treatment with potassium carbonate to give **12a** quantitatively. Spirocyclopropane (**12a**) was then converted into the bislactim ether (**13a**) (70%) with Meerwein's reagent (Me_3OBF_4)and hydrolysed under mild conditions to generate the desired ester **5a** as a colourless oil in 58% yield. The stereochemistry of **5a** was determined by obtaining a crystal structure of the *N*-tosyl derivative (**14a**). It was found to be (2*S*,3*S*) as expected (Fig. 1).

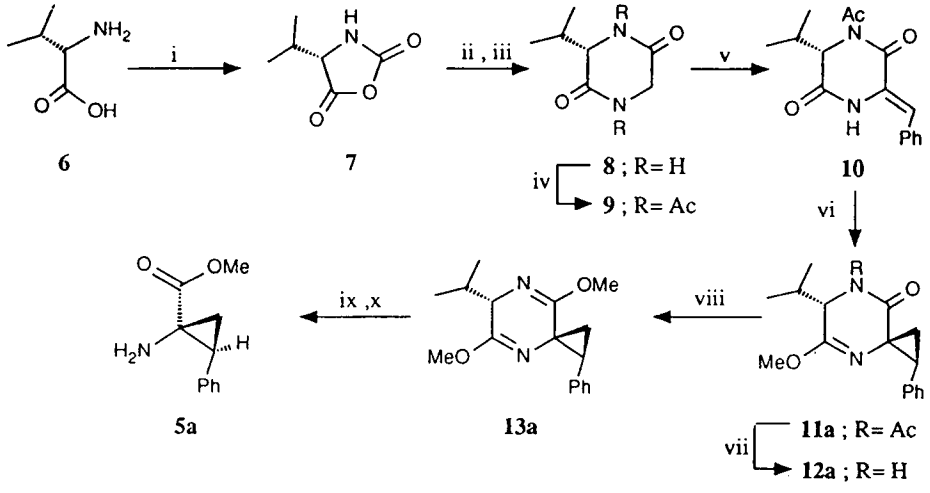
This procedure was repeated for the (*E*)-benzylidene (**10b**) giving similar yields to generate the (*E*)-2,3-methanophenylalanine methyl ester (**5b**) as a colourless oil in 60% yield.

Preparation of the cyclopropyl dipeptides (**3**, *R* = *Me*; **4**, *R* = *H*)

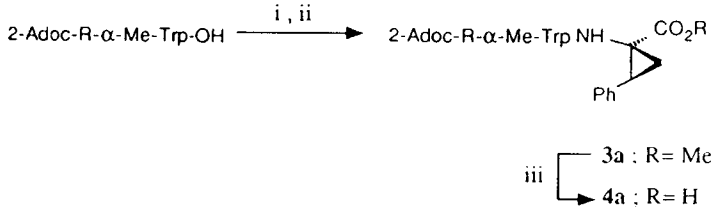
The cyclopropyl dipeptides (**3**) and (**4**) were prepared via Stammer's procedure (Mapelli et al 1986) of coupling 2,3-methanophenylalanine methyl esters (Scheme 2). The N^{α}-(2-Adoc)-*R*-α-methyl-tryptophan was treated with *iso*-butyl chloroformate and *N*-methylmorpholine followed by a solution of **5a** to generate the desired product N^{α}-(2-Adoc)-*R*-α-Me-Trp-(2S,3S)∇^{Z} Phe-OMe (**3a**) in 45% yield. The ester (**3a**) was then hydrolysed under mild basic conditions to afford the desired N^{α}-(2-Adoc)-*R*-α-Me-Trp-(2S,3S)∇^{Z}Phe-OH (**4a**) (50%). The corresponding dipeptide ester **3b** was prepared in the same manner.

Preparation of the dehydropeptides (**1**, *R* = *Me*; **2**, *R* = *H*)

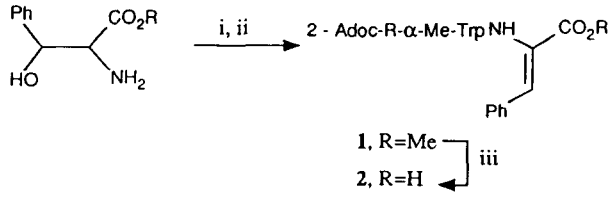
The dehydropeptides (**1**) and (**2**) were prepared via firstly coupling (*RS*)-*threo*-3-phenylserine methyl ester with N^{α}-(2-Adoc)-*R*-α-methyl-tryptophan by standard DCC/HOBt conditions, followed by dehydration using disuccinimiydl carbonate (DSC) (Ogura et al 1981) to yield solely the



Scheme 1. Reagents and conditions: (i) phosgene, tetrahydrofuran (THF), 40°C, 4 h; (ii) glycine ethyl ester hydrochloride, Et_3N, $CHCl_3$, −78°C, 1·5 h, **7**, THF, -78°C, 3h; (iii) $PhCH_3$,Δ, 12 h; (iv) Ac_2O, 110°C, 7 h; (v) **9**, benzaldehyde, DMF, 0°C, *t*-BuOK, THF, 0°C, 12 h; (vi) CH_2N_2, Et_2O, room temp., 48 h; (vii) K_2CO_3, CH_3OH, 0°C, 15 min.; (viii) Me_3OBF_4, CH_2Cl_2, room temp., 24 h; (ix) 0.25 M HCl, Et_2O, 0°C, 12 h; (x) NH_4OH, Et_2O, 0°C.



SCHEME 2. Abbreviations: 2-Adoc = 2-Adamantyloxycarbonyl; NMM = *N*-methylmorpholine. Reagents and conditions: (i) isobutyl chloroformate, NMM, THF, room temp., 1 h; (ii) (2*S*,3*S*)-(*Z*)-2,3-methanophenylalanine methyl ester, THF, room temp., 24 h; (iii) 0·1 M NaOH, EtOH, Δ, 3 h.



Scheme 3. Reagents and conditions: (i) DCC, HOBt, EtOAc, 2-Adoc-*R*-α-Me-Trp-OH, EtOAc; (ii) DSC, CH_3CN, room temp.; (iii) 0·1 M NaOH, EtOH,Δ.

(*Z*)-stereoisomer (**1**) in 49% yield. The ester (**1**) was then hydrolysed to the carboxylic acid (**2**) under dilute basic conditions in 40% yield (Scheme 3).

Results and Discussion

From the CCK binding affinities, given in Table 1, the presence of either a dehydrophenylalanine (ΔPhe) or cyclopropylphenylalanine (∇Phe) moiety within the dipeptide gives favourable receptor binding at both CCK-A and CCK-B sites in comparison to CCK-4. In particular, the preferred stereochemistry in (*E*)-(2*S*,3*R*)-∇Phe dipeptide ester (**3b**) gives a compound with an excellent binding affinity for the CCK-B receptor, IC50 = 3·9 nM indicating a 60-fold increase in affinity over the ΔPhe analogue (**1**) and a 100-fold increase over its diastereomeric form (**3a**).

Conformational studies on the (*Z*)-ΔPhe dipeptide ester (**1**, R = Me), and (*Z*)- and (*E*)-∇Phe dipeptide esters (**3a** and **3b**, R = Me) indicated the presence of a β-turn, although there was no preference in type (Toniolo C et al 1993a, b); Pantano et al 1993; Aubry et al 1994; Valle et al 1993; Formaggio et al 1993).

The phenyl and indole rings of the Phe and Trp moieties respectively, however, did prefer to be situated on the same side of the β-turn, thus giving the molecule a suitable conformation for receptor binding. Examples of low energy conformations of the three dipeptides are presented in Figs. 2–4.

In each case the β-turn type stated represents the initial conformation of the dipeptide before minimisation studies were applied, and not necessarily the resultant dipeptide conformation which is defined by the φ, ψ and χ values. Fig. 5 shows the (φ,ψ) energy map of the model system *N*-Ac-α-Me-*R*-Trp-*N*-Me

In conclusion, we have prepared both dehydroPhe and cyclopropylPhe dipeptoid analogues of CCK-4 which have been shown to possess good binding affinities for both the CCK-A and CCK-B receptors. Conformational studies of the dipeptide esters (**1** and **3**) indicated the presence of a β-turn within the peptide backbone, although no preference in type; which is favourable for receptor binding

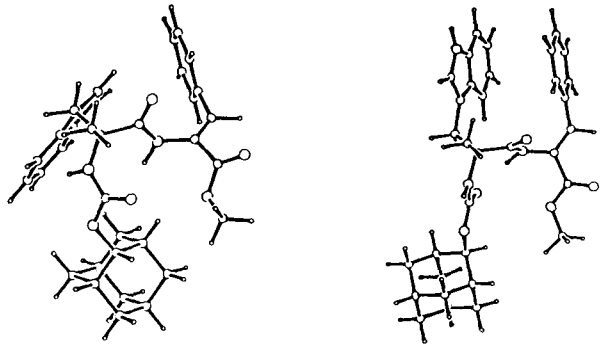


FIG. 2. Two minimum energy conformations of N^{α}-(2-Adoc)-*R*-α-Me-Trp-Δ^{Z}Phe-OR (**1**, R = Me).

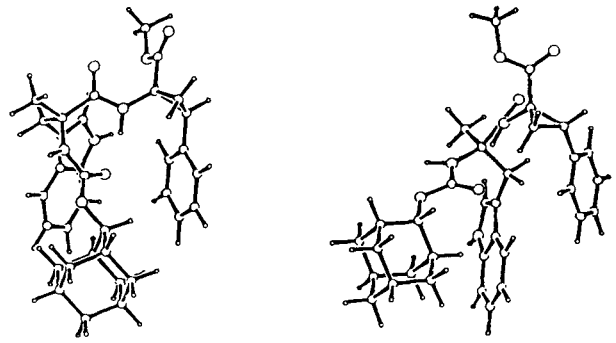


FIG. 3. Two minimum energy conformations of N^{α}-(2-Adoc)-*R*-α-Me-Trp-∇^{Z}Phe-OR (**3a**, R = Me).

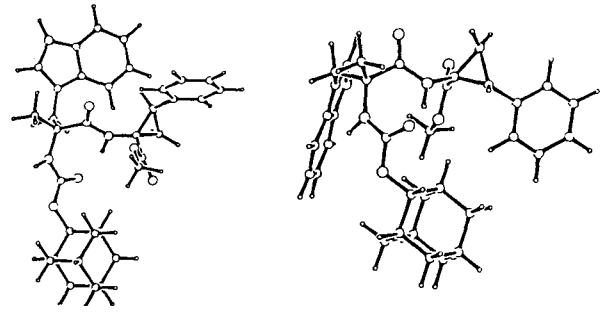


FIG. 4. Two minimum energy conformations of N^{α}-(2-Adoc)-*R*-α-Me-Trp-∇^{E}Phe-OR (**3b**, R = Me).

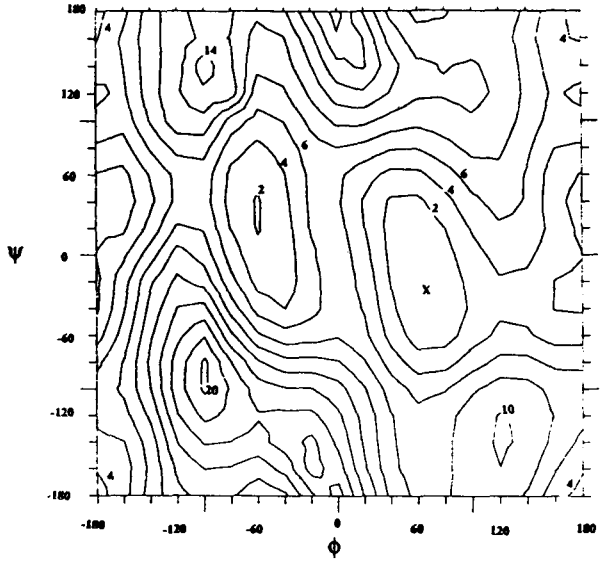


FIG. 5. A (φ,ψ) energy contour map of a model system *N*-Ac-α-Me-*R*-Trp-N-Me.

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