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DIASTEREOSELECTIVE SYNTHESIS OF CYCLOPROPYL PHENYLALANINES AND THEIR INCORPORATION INTO DIPEPTIDES

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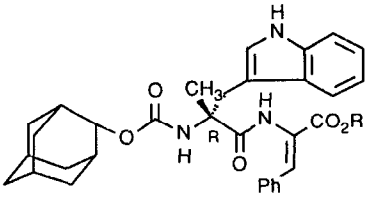
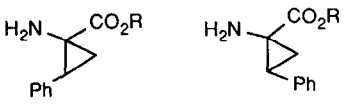
Abstract: *2,3-Methanophenylalanine methyl esters have been prepared diastereoselectively and incorporated into dipeptoid analogues of the tetrapeptide cholecystokinin, CCK(30-33). (Z)-(1S, 3S, 6S)- and (E)-(1R, 3S, 6S)-7(H)-6-isopropyl- 5-methoxy-1-phenyl-4,7-diazaspiro[2,5]oct-4-en-8-ones (**12a,b**) have been hydrolysed to afford the (Z)- and (E)- ∇Phe-Val-OMe dipeptides (**16a,b**), respectively.*

The dehydropeptides, N^{α}-(2-Adoc)-R-α-Me-Trp-Δ^{Z}Phe-OR (**1**, R=Me); and (**2**, R=H)[1] have recently been identified as dipeptoid mimetics of CCK-4 (CCK(30-33)).[2a,b] The carboxylic acid (**2**) was found to have 60 and 54nm affinity for the CCK-A and CCK-B receptors respectively (see Table 1). The corresponding E-isomers were not obtainable.



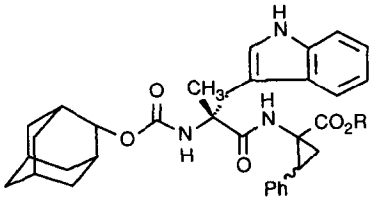
(**1**, R= Me; **2**, R= H)

Consequently, it was decided to synthesise the cyclopropyl analogues of (**1**) and (**2**) via the replacement of the dehydrophenylalanine moiety with each of the (Z)- and (E)-2,3-methanophenylalanines (**3**) to give the cyclopropyl dipeptides (**4**) and (**5**), in order further to probe requirements for affinity at the CCK-A and CCK-B receptors.



Z- isomer E-isomer

(**3**, R= Me)

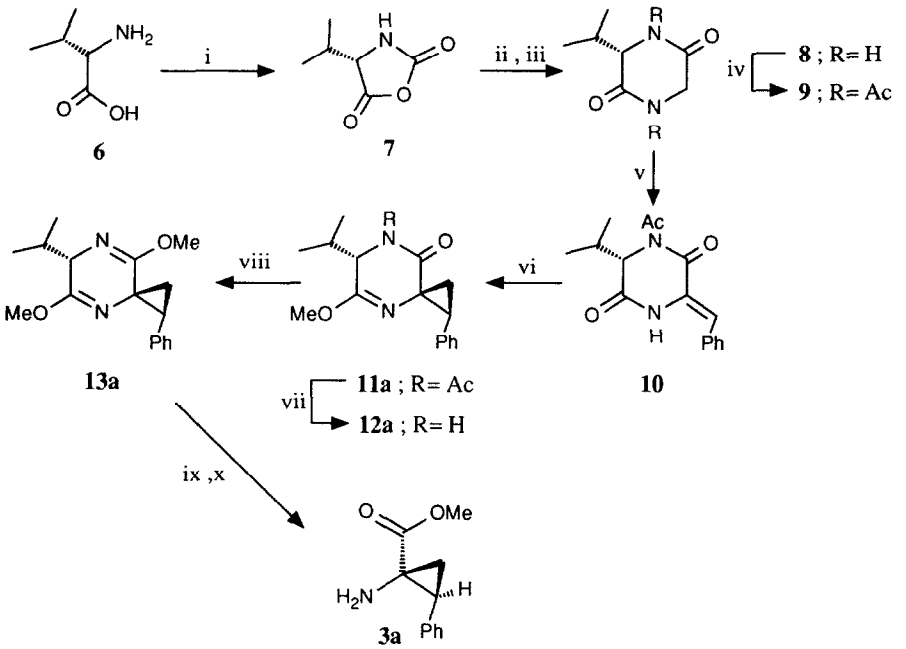


(**4**, R= Me; **5**, R= H)

In this communication we describe the synthesis of 2,3-methanophenylalanine methyl esters (**3**), the preparation and binding affinities of the cyclopropyl dipeptides (**4**) and (**5**), together with the preparation of other cyclopropyl dipeptides.

In the synthesis of 2,3-methanophenylalanine methyl esters (**3**) our methodology was based on a modification of Schöllkopf's bislactim ether approach[3], 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**)[4] were easily separated by flash chromatography and served as intermediate to both (Z)- and (E)-2,3-methanophenylalanine methyl esters (**3a,b**). The relative stereochemistry of each benzylidene isomer of (**10**) was ascertained by nmr experiments (n.O.e.). The (Z)-benzylidene (**10a**) was then treated with diazomethane to give directly a 4:1 mix of diasteromeric 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 to the bislactim ether (**13a**) (70%) with Meerwein's reagent and hydrolysed under mild conditions to generate the desired ester (**3a**) as a colourless oil in 58% yield.

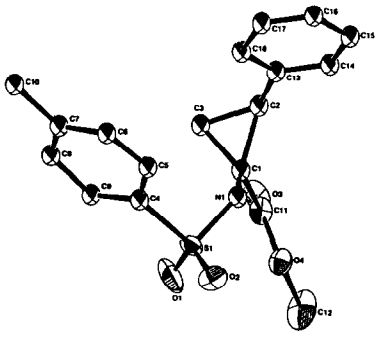
Scheme 1



Reagents and conditions : i, phosgene, tetrahydrofuran (THF), 40°C, 4h; ii, glycine ethyl ester hydrochloride, Et_3N, $CHCl_3$, -78°C, 1.5h, 7, THF, -78°C, 3h; iii, $PhCH_3$, reflux, 12h; iv, Ac_2O, 110°C, 7h; v, 9, benzaldehyde, DMF, 0°C, t-BuOK, THF, 0°C, 12h; vi, CH_2N_2, ether, room temp., 48h; vii, K_2CO_3, CH_3OH, 0°C, 15 mins.; viii, Me_3OBF_4, CH_2Cl_2, room temp., 24h; ix, 0.25M HCl, ether 0°C, 12h; x, NH_4OH, ether, 0°C.

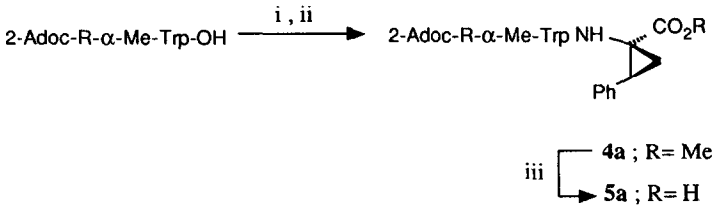
The stereochemistry of (**3a**) was determined via obtaining a crystal structure of the N-tosyl derivative (**14a**). It was found to be (2S,3S) as expected (Figure 1).

Figure 1. X-ray Crystal Structure of 14a



This procedure was repeated for E-benzylidene (**10b**) giving similar yields to generate the (E)-2,3-methanophenylalanine methyl ester (**3b**) as a colourless oil in 60% yield. The cyclopropyl dipeptides (**4**) and (**5**) were prepared via Stammer's procedure[5] of coupling 2,3- methanophenylalanine methyl esters (Scheme 2). The N^{α}-(2-Adoc)-R-α-methyl-tryptophan (**15**) was treated with *iso*-butyl chloroformate and N-methylmorpholine followed by a solution of (**3a**) to generate the desired product N^{α}-(2-Adoc)-R-α-Me-Trp-(2S,3S)∇^{Z}Phe-OMe (**4a**) in 45% yield. The ester (**4a**) was then hydrolysed under mild basic conditions to afford the desired N^{α}-(2-Adoc)-R-α-Me-Trp-(2S,3S)∇^{Z}Phe-OH (**5a**) (50%).

Scheme 2



Abbreviation : 2-Adoc = 2-Adamantyloxycarbonyl; NMM = N-methylmorpholine.
Reagents and conditions . i, isobutyl chloroformate, NMM, THF, room temp., 1h; ii, (2S,3S)-(Z)-2,3-methano-phenylalanine methyl ester, THF, room temp., 24h; iii, 0.1M NaOH, EtOH, reflux, 3h.

Table 1. CCK Receptor Binding Affinities[a]

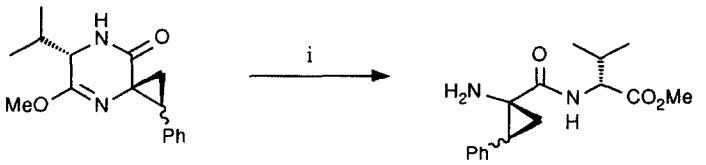
No.	Compound	R	IC_{50},nM CCK-A	IC_{50},nM CCK-B	A/B ratio
1	N^{α}-(2-Adoc)-R-α-Me-Trp-Δ^{Z}Phe-OR	Me	-[b]	270	-
2	"	H	60	54	1.1
4a	N^{α}-(2-Adoc)-R-α-Me-Trp-(2S,3S)∇^{Z}Phe-OR	Me	720	600	1.2
5a	"	H	84	140	0.6
4b	N^{α}-(2-Adoc)-R-α-Me-Trp-(2S,3R)∇^{E}Phe-OR	Me	26	3.9	6.7
	CCK(26-33) (Sulphated)	-	0.1	0.3	0.3

[a]IC_{50} represents the concentration (nM) producing half-maximal inhibition of specific binding of [^{125}I] Bolton Hunter CCK-8 to CCK receptors in the mouse cerebral cortex (CCK-B) or the rat pancreas (CCK-A). At the concentration of the iodinated radioligand used (35pm), in relation to its K_D (350pm), the Cheng and Prussoff correction was insignificant and the IC_{50} was taken as a measure of the apparent binding affinity (K_i) of the compounds.
[b]Not measured.

The IC_{50} values given in Table 1 illustrate the importance of stereochemistry of the cyclopropane in determining CCK-A/CCK-B ratio, and that the preferred stereochemistry in **4b** gives a compound with an IC_{50}=3.9nM at the CCK-B receptor, indicating an 100-fold increase in affinity over its diastereomeric form (**4a**).

The tetrahydropyrazinones (**12a**) and (**12b**) were partially hydrolysed with 0.1M hydrochloric acid to give the dipeptides ∇^{Z}Phe-Val-OMe (**16a**) and ∇^{E}Phe-Val-OMe (**16b**) respectively in quantitative yields (Scheme 3).[6]

Scheme 3



12a,b **16a,b**

Reagents and conditions : i, 0.1M HCl, CH_3CN, room temp., 24h.

We have therefore developed a new diastereoselective synthesis of cyclopropyl amino acids and derived dipeptides which complement other recent procedures.[7-9]

References and Notes:

1. The symbols ∇ and Δ are as defined by Stammer, C.H. *Tetrahedron*, **1990**, 46, 2231, which states:- "The symbol ∇^{Z} or ∇^{E} prefixed to the abbreviation for an amino acid residue as in ∇^{Z}Phe, means the Z-diasteromer of 2,3-methano- or cyclopropane-phenylalanine. It is used here only when the methanoamino acid appears in a peptide chain. The Δ^{Z} symbol indicates a dehydroamino acid as a Δ^{Z}Phe, meaning (Z)-2,3-dehydrophenylalanine."
2. (a) Horwell, D.C. EP 405 537/1991 : C.A., **115**, 50307; (b) Peptoid: as defined by Horwell, D.C.; Birchmore, B.R.; Boden, P.R.; Higginbottom, M.; Ping Ho, Y.; Hughes, J.; Hunter J.C.; Richardson, R.S. *Eur. J. Med. Chem.*, **1990**, 25, 53.
3. (a) Schöllkopf, U. *Tetrahedron*, **1983**, 39, 2085; (b) Schöllkopf, U. *Pure Appl. Chem.*, **1983**, 55, 1799; (c) Schöllkopf, U. *Top. Curr. Chem.*, **1983**, 109, 65.
4. **a** refers to Z-configuration, and **b** refers to E-configuration.
5. Mapelli, C.; Kimura H.; Stammer, C.H. *Int J. Pept. Protein Res.*, **1986**, 28, 347.
6. Schöllkopf, U. DE 3 831 716/1988 : C.A., **113**, 115879.
7. Williams, R.M.; Fegley, G.J. *J. Am. Chem. Soc.*, **1991**, 113, 8796.
8. Fernández, D.; de Frutos, P.; Marco, J.L.; Fernández-Alvarez, E.; Bernabé, M. *Tetrahedron Lett.*, **1989**, 30, 3101.
9. Alcaraz, C.; Herrero, A.; Marco, J.L.; Fernández-Alvarez, E.; Bernabé, M. *Tetrahedron Lett.*, **1992**, 33, 5605.