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THE USE OF A PROLINE RING AS A CONFORMATIONAL RESTRAINT IN CCK-B RECEPTOR "DIPEPTOIDS".

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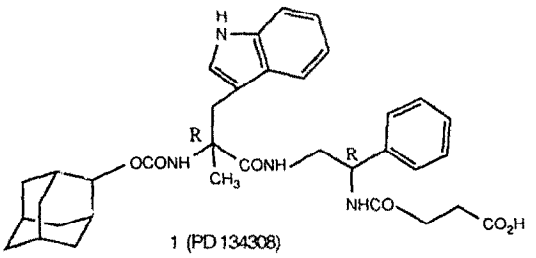
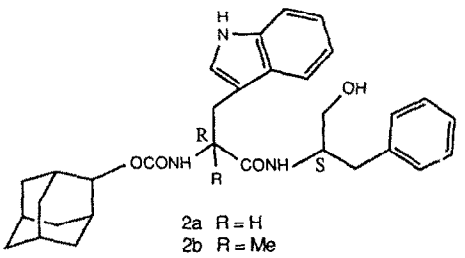
Abstract : Examination of molecular dynamics simulations and an X-ray crystal structure of a selective CCK-B receptor dipeptoid Trp derivative led to the synthesis of a conformationally restrained Pro derivative. The CCK receptor binding of this target compound (7) is described.

The design of "dipeptoid" analogues of cholecystokinin (CCK) has aroused much interest since the disclosure by Horwell et al. in 1991 that α-methyltryptophan derivatives [e.g. **1** (PD 134308) and **2b**] are highly selective CCK-B receptor antagonists.[1] The α-methyl group on the Trp moiety is characteristic of this series of compounds and enhances their activity. The purpose of this study was to investigate what conformational effects this substituent has on the ϕ,Ψ (peptide backbone) angles of the modified Trp.

A molecular dynamics study of **2b** and the less active non α-methyl derivative **2a**[2] is shown in the figure. Models of compounds **2a**, **2b** and **7** were studied with Sybyl[3] using the crystal structure of **1** as a starting point.[4] Point charges were calculated by the Gasteiger-Hückel method.[5] A distant dependent dielectric constant was employed throughout.[6] The molecules were energy minimized with respect to the Tripos force field[7] using MAXIMIN II.[8] Molecular dynamics calculations were performed with the Sybyl dynamics option using the force field outlined above. For each model 500 ps of dynamics were performed with 1fs increments at a temperature of 500 K. The conformation of each molecule was recorded every 1 ps. The figure shows the conformation of the Trp amide backbone angles ϕ,Ψ at these 1 ps intervals throughout the 500 ps dynamics runs. These results indicate that some of the conformations

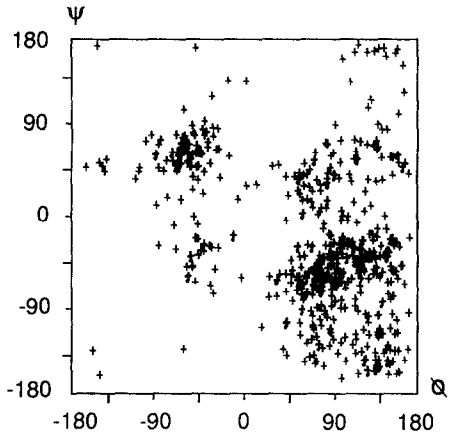


1 (PD 134308)

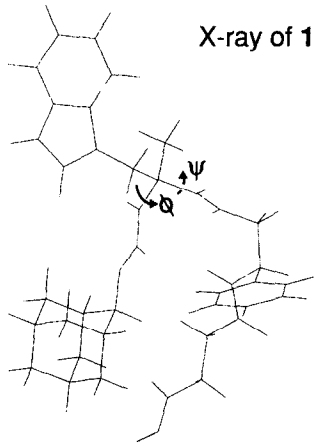


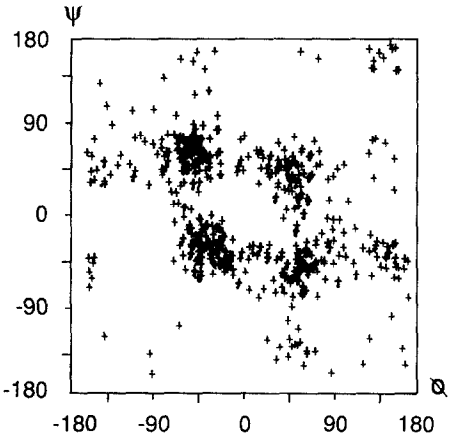
2a R = H
2b R = Me

Figure: X-ray structure of **1** and ϕ,ψ angles of **2a**, **2b** and **7** determined by molecular dynamics

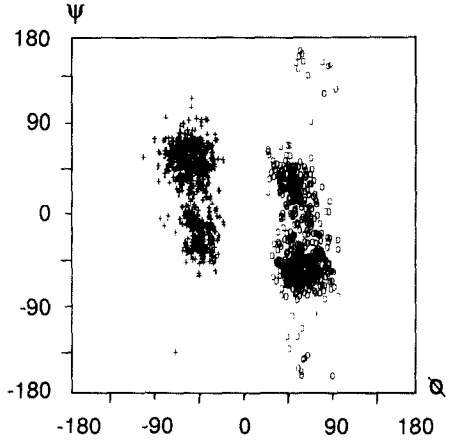


Compound **2a**





Compound **2b**

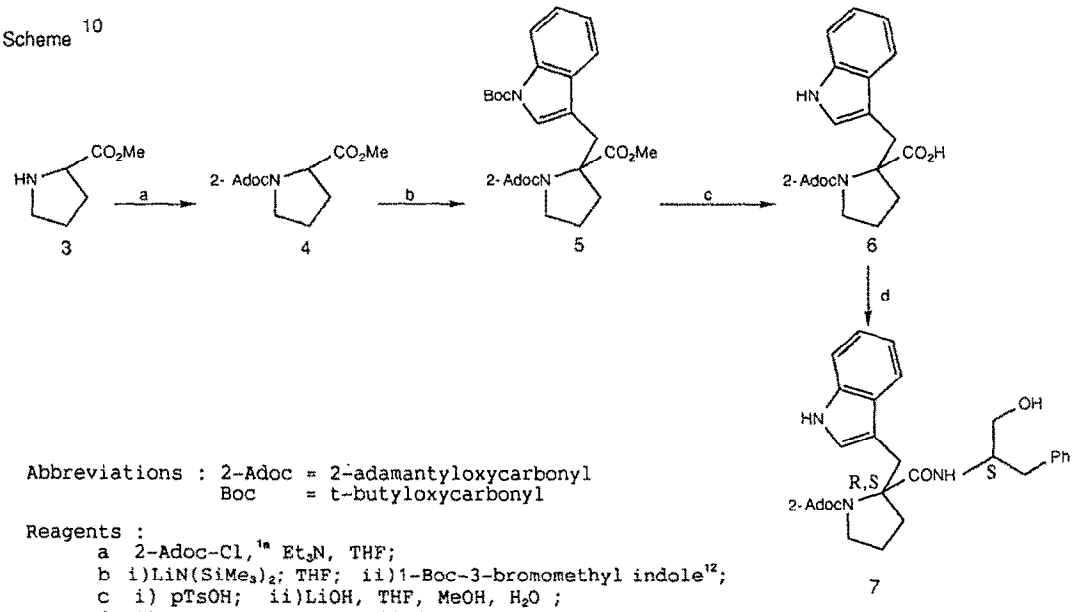


Compound **7** + R-stereochemistry
o S-stereochemistry

identified for **2a** are not accessed by the α-methyl analogue **2b** in this dynamics run. This is consistent with other observations made with α,α-dialkyl substituted amino acids.[9] However, the proline derivative **7** localises the conformations of the amide backbone into a subset of conformations accessible to both **2a** and **2b** as shown by the molecular dynamics study (fig). Furthermore, in the conformation of the X-ray crystal structure of compound **1**[4] the α-methyl and the carbamate NH proton are in an eclipsed orientation. It was noted that this conformation could be mimicked by using the corresponding proline derivative **7** in which a five membered ring replaces the eclipsed bonds.

This led us to synthesise compound **7** (scheme). The indole moiety was introduced by alkylation of the protected proline **4** and the acid **6** was converted to the final product by coupling with phenylalaninol.

Scheme [10]



Abbreviations : 2-Adoc = 2-adamantyloxycarbonyl
Boc = t-butyloxycarbonyl

Reagents :
a 2-Adoc-Cl,[13a] Et_3N, THF;
b i)$LiN(SiMe_3)_2$; THF; ii)1-Boc-3-bromomethyl indole[12];
c i) pTsOH; ii)LiOH, THF, MeOH, H_2O ;
d i)HOBT, DCCI, THF; ii)(S)-2-amino-3-phenyl-1-propanol.

The proline derivative **7** was found to have significantly weaker affinity than **2a** in an <u>in vitro</u> CCK-B receptor binding assay (see table) indicating that effects other than the amide backbone conformation are involved in this molecular recognition. This decreased affinity may reflect a role for the carbamate NH group or it may be caused by an undesired steric interaction between the receptor and the new five membered ring. The relative lack of change in the CCK-A affinity indicates that this weaker interaction is much less affected by these conformational constraints.

Table 1. Receptor Binding Affinity (IC_{50} nM)[11]

Compound	CCK-B	CCK-A	A/B ratio
1	1.7(1.3-2.7)	4300(1200-8500)	2500
2a	6.3(4.2-8.9)	780(690-850)	120
2b	852(365-1590)	1080(770-2020)	1.3
7	2080 (1790-2700)	1050(743-1710)	0.50

Although the conformational effects of proline in polypeptides has been studied elsewhere[13] this is the first example to our knowledge of incorporating an α-alkylated proline ring into a small peptide derivative in order to probe the conformational effects of a desirable α-methyl substituent.

Acknowledgement

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References and Notes

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3. SYBYL version 5.4 supplied by Tripos Associates of 1699 South Hanley Road, Suite 303, St Louis, Missori 63144, USA.
4. The angles in the X-ray crystal structure of compound 1 are phi = -49°, psi = -51°. Debaerdemaeker, T.; Heigl, T.; Steiner, K. Acta Cryst. manuscript submitted for publication.
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10. The chemical structures of all previously unreported compounds were confirmed by NMR, IR, MS and elemental analyses. Compound **7** was obtained as a mixture of diastereoisomers : mp 91-96°C; IR 3520-3160 br, 1670cm^{-1}; $C_{34}H_{41}N_3O_4$. $0.75H_2O$ requires C,71.75; H,7.5; N,7.4; found C,71.9; H,7.35; N,7.3% MS (CI) 566 (M^+ + H), 135.
11. CCK receptor binding assays were performed with homogenized rat pancreas (CCK-A) or mouse cerebral cortex (CCK-B) using [^{125}I]Bolton Hunter CCK26-33 as previously described.[1] Values given are the geometric mean and the ranges from at least three different experiments.
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