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# 2,3-SUBSTITUTED 2-AZANORBORNANES AS POLAR β-TURN MIMETICS

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**Abstract** - The design and synthesis of *N*-(3-indolylmethyl)-3-benzyl-2-azabicyclo[2.2.1]heptane-2-carboxamide (**3**) as a conformationally constrained non-peptide β-turn mimetic is described. Compound **3** is shown to mimic the Trp and Phe side chain conformation and the overall dipole moment of MEN10627 (**2**), a cyclic hexapeptide with a high NK-2 affinity. The tachykinin receptor affinities of **3** are evaluated and compared to those of MEN10627 and previously reported Trp-Phe mimetic **1**. Copyright © 1996 Elsevier Science Ltd

β-Turn conformations have been found in a large number of both naturally occurring and synthetic peptides,[1] and the number of well-documented examples of β-turns is still growing. There is evidence that cyclic somatostatin and neurokinin A analogues bind to their receptors in a β-turn conformation.[2,3] We are interested in designing non-peptide drug candidates for neuropeptide receptors and therefore view β-turn mimetics as an important area of research.

We recently reported the design and synthesis of a series of 1-acyl-3-alkyl substituted pyrrolidines as conformationally constrained, non-peptide β-turn mimetics.[4] Computer modelling studies showed that pyrrolidine **1** (figure 1) overlaid very closely with MEN10627 (**2**) (figure 2),[3] a constrained cyclic hexapeptide with high affinity for the NK-2 receptor. Dipeptide mimetic **1**, however, did not display a significant affinity for this receptor. Computer-assisted calculations of the dipole moments of **1** and **2** indicated that the two dipole moments are nearly at right angles (see table 1). A possible explanation for the lack of affinity shown by pyrrolidine **1** for the NK-2 receptor is therefore that the direction of its dipole moment may cause it to interact unfavourably with the NK-2 receptor.

**Figure 1**: The pyrrolidine-based Trp-Phe mimetic **1**.

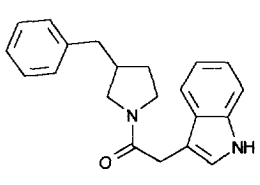
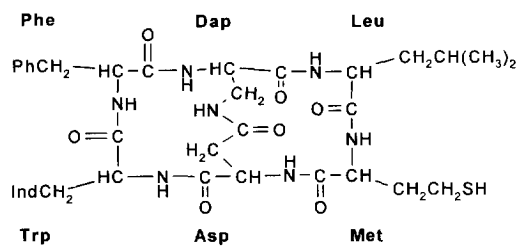


**Figure 2**: NK-2 antagonist MEN10627 (**2**).

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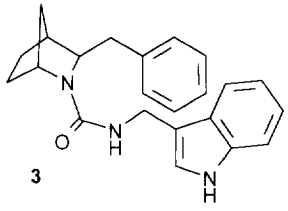
To investigate the role of the dipole moment in the NK-2 affinity of **1** we set out to design a Trp-Phe mimetic that would overlay closely with the Trp-Phe β-turn in MEN10627 and with β-turn mimetic **1**, and that would also possess a dipole moment that is similar in size and direction to that of MEN10627. We thereby assumed that the overall dipole moment of MEN10627 would be optimal for binding to the NK-2 receptor.

Computer-assisted molecular modelling identified 2-azanorbornane **3** (figures 3 and 4) as a mimetic that fulfilled our requirements.[5,6] A comparison of the dipole moments of **3** and MEN10627 (as calculated by the molecular modelling program) is shown in table 1 (see also figure 4).

**Figure 3**: Trp-Phe mimetic **3**.



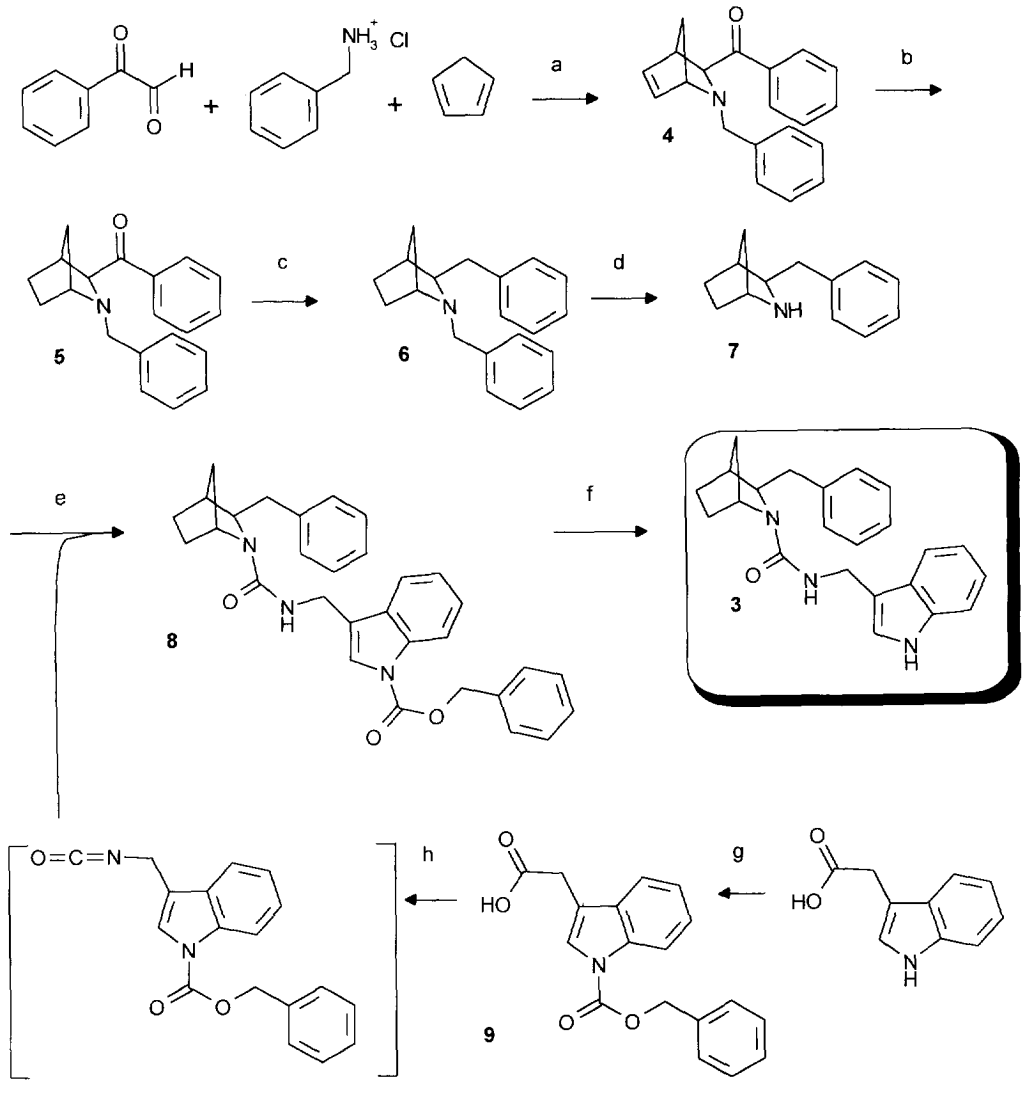

**3**

**Table 1**: A comparison of the dipole moments of **1**, **2**(MEN10627) and **3**.

| No. | dipole *x*-component (D) | dipole *y*-component (D) | dipole *z*-component (D) | total dipole moment (D) |
|---|---|---|---|---|
| **1** | -0.35 | 3.39 | -0.23 | 3.42 |
| **2** | 5.59 | -0.77 | 2.17 | 6.05 |
| **3** | 5.16 | -0.40 | 1.94 | 5.53 |

**Figure 4**: An overlay of Trp-Phe mimetic **3** (Carbon atoms are shown in white, oxygen atoms in red and nitrogen atoms in blue) on MEN10627 (**2**) (in green). The arrows represent the dipole moments. The displayed conformation of MEN10627 was determined by X-ray crystallography.[3] The displayed conformation of **3** is 6.0 kcal/mol above its lowest energy conformation.[6]

**Scheme 1**: The synthesis of β-turn mimetic **3**.




Reagents and conditions: (a) $H_2O$, 20 h (95%); (b) $H_2$ (43 psi), Pd/C, EtOAc, 1 h (95%); (c) (i) $AlCl_3$ (3.5 equiv.), $Et_2O$, 5 min., (ii) $LiAlH_4$ (1.75 equiv.), reflux, 5 min., (iii) add **4** in THF, reflux, 2 h (85%); (d) $H_2$ (42 psi), $Pd(OH)_2$, MeOH, 5 h (94%); (e) add **7** in $CH_2Cl_2$ to the crude product of step h in $CH_2Cl_2$, 1 h (75%); (f) $H_2$ (42 psi), $Pd(OH)_2$, MeOH, 3 h (82%); (g) (i) LHMDS (2 equiv.), THF, -78 °C, 0.5 h, (ii) BnOCOCl, -78 °C, 1 h (73%); (h) (i) DPPA, $Et_3N$, toluene, reflux, 2 h, (ii) remove volatiles *in vacuo*.

The synthesis of the 2-azanorbornane based mimetic **3** is outlined in scheme 1. All compounds are racemates, but only the 3-*exo*-substituted regio isomers of compounds **3** to **7** were obtained. The 2-azanorbornene **4** was obtained *via* an aza-Diels-Alder reaction, following the procedure of Grieco *et al.*[7] In our hands this procedure yielded exclusively 3-*exo*-benzyl substituted product. We envisaged that ketone **4** could be converted into amine **6** in one step via an acid catalysed hydrogenation under pressure. However, hydrogenation in the presence of palladium and TFA under mild conditions yielded mainly ketone **5**, whereas more vigorous conditions ($CH_3COOH/HClO_4$, 50 °C) caused decomposition of the azanorbornane skeleton. A two-step conversion of unsaturated ketone **4** to amine **6** was subsequently investigated. Saturation of the double bond by hydrogenation over palladium under neutral conditions proceeded in excellent yield. The resulting ketone **5** could be reduced to amine **6** in a yield of 85% using a $AlCl_3/LiAlH_4$ mixture. Interestingly, it was found that the ketone **5** was only reduced to a methylene if the $AlCl_3/LiAlH_4$ mixture in $Et_2O$ was heated to reflux before the ketone was added. If the reagent mixture was not preheated, selective reduction of the carbonyl group to an alcohol resulted. More conventional methods for the removal of the carbonyl group, such as hydrogenolysis or conversion to a hydrazone, followed by reduction, were found to be unsuitable for this conversion. Amine **6** was debenzylated by hydrogenation over Pearlman's catalyst and the resulting amine **7** coupled to the isocyanate formed *in situ* from *N*-protected indoleacetic acid **9** *via* a Curtius rearrangement to give urea **8**. Removal of the protecting group furnished the desired urea **3**.[8]

**Table 2**: Tachykinin receptor affinity data for compounds **1**, **2** (MEN10627), **3** and **8**.

| No. | NK-1 binding[a] ($IC_{50}$, μM) | NK-2 binding[b] ($IC_{50}$, μM) | NK-3 binding[c] ($IC_{50}$, μM) |
|---|---|---|---|
| **1** | 3.7 | 14% at 10 μM | 3.5 |
| **2** | 0.8[3] | 0.000079[3] | inactive[3] |
| **3** | 6.7 | 31% at 10 μM | 35% at 10 μM |
| **8** | 55% at 10 μM | 18% at 10 μM | 10 |

a) Human IM-9 cells, $^{125}$I-BH-SP.[9a]; b) Hamster urinary bladder, $^{125}$I-NKA.[9b]; c) Human receptor stably expressed in CHO cells, $^{125}$I-[MePhe$^7$]-NKB.[9c]

The tachykinin receptor affinities of the Trp-Phe mimetics **3** and **8** were evaluated. The results are shown in table 2. The tachykinin receptor affinities of 2-azanorbornane **3** were compared to those of Trp-Phe mimetic **1**. The most remarkable difference is that azanorbornane **3** shows no affinity for the NK-3 receptor, whereas **1** does. It is possible that the NK-3 affinity of pyrrolidine **1** may be due to a conformation that azanorbornane **3** cannot adopt.

A comparison of the tachykinin affinities of **3** and the structure it mimics, MEN10627, shows that shedding two-thirds of the hexapeptide results in a very significant loss of NK-2 affinity, but only a small loss in NK-1 affinity. This suggests that the NK-1 affinity of MEN10627 resides in the Trp-Phe portion of the peptide. The selectivity of the hexapeptide for the NK-2 receptor is likely to come from an interaction with the Dap-Leu-Met-Asp section. Mimetic **3**'s lack of NK-2 affinity compared to MEN10627 may be due to one or several of the reasons stated below:

1) The key binding groups for MEN10627 are not Trp and Phe. Given the fact that the NK-2 selectivity seems dependent on the Dap-Leu-Met-Asp portion of the hexapeptide, this is likely to be a factor in the dramatic loss of binding.

2) MEN10627's binding conformation for interaction with the NK-2 receptor is not accessible to mimetic **3**. The angle space available to **3** was found to be much smaller than that of MEN10627 and the conformation of the hexapeptide determined by X-ray (figure 4) is not necessarily the binding conformation.

3) The azanorbornane core of **3** interacts unfavourably with the receptor. This is unlikely to be the main cause because the azanorbornane skeleton is almost entirely contained within the space occupied by MEN10627.

The affinities obtained for the 2-azanorbornane-based β-turn mimetics **3** and **8** were compared to those of Z-Trp-Phe-$NH_2$, an N-protected dipeptide lead molecule with micromolar affinity for the NK-1 and NK-2 receptors (NK-1 binding, $IC_{50}$ = 9.3 μM; NK-2 binding, $IC_{50}$ = 5.2 μM).[9b] The β-turn mimetics **3** and **8** show an NK-2 receptor affinity inferior to that of Z-Trp-Phe-$NH_2$. This indicates either that Z-Trp-Phe-$NH_2$ does not bind in a β-turn conformation or that the benzyloxycarbonyl group and/or the two amides of the dipeptide are important for binding to the NK-2 receptor. However, mimetic **3** does show an affinity for the NK-1 receptor comparable to that of Z-Trp-Phe-$NH_2$.

In summary we report synthetic methodology for the construction of a conformationally restrained Trp-Phe mimetic which we believe to be the first compound designed to mimic the size and direction of the dipole moment as well as the orientation of the two central amino acid side chains in a β-turn.

**References and Notes**

1. G. D. Rose, L. M. Gierasch, J. A. Smith, *Adv. Protein Chem.*, **1985**, *37*, 1-109.
2. a) D. F. Veber, *Proc. 12th Am. Pept. Symp.*,ed. J. A. Smith, J. E. Rivier, Escom, Leiden, 1992, 1-14. b) Y.-B. He, Z. Huang, K. Raynor, T. Reisine, M. Goodman, *J. Am. Chem. Soc.*, **1993**, *115*, 8066-8072.
3. V. Pavone, A. Lombardi, F. Nastri, M. Saviano, O. Maglio, G. D'Auria, L. Quartara, C. A. Maggi, C. Pedone, *J. Chem. Soc., Perkin Trans. 2*, **1995**, 987-993.
4. D. C. Horwell, W. Howson, D. Naylor, H. M. G. Willems, *BioMed. Chem. Lett.*, **1995**, *5*, 1445-1450.
5. Dipole moment calculations (in Debyes) were based on the point charge distribution in the molecule. The point charge distribution for each molecule was calculated using the Gasteiger-Hückel method. The dipoles were displayed using SYBYL-Basic.

6. Computer molecular modelling was performed using the SYBYL program (Version 6.0) supplied by Tripos Associates, 1699 South Hanley Road, Suite 303, St. Louis, Missouri 63144, USA. The lowest energy state of **3** (figure 4) was found using 1000 iterations of the RANDOMSEARCH algorithm. Geometry optimisation at each iteration was effected by minimisation of the energy (Tripos force field, no electrostatic charges) by MAXIMIN-II. Energy minimisation was terminated when the rms energy gradient fell below 0.02 $kcal.mol^{-1}.A^{-1}$. The lowest energy conformer found was overlayed by the method of least squares with MEN10627. The molecules were aligned by superimposing the Cαs and Cβs of the Trp and Phe residues of MEN10627 on the corresponding atoms of **3**. The side chains appended to the azanorbornane template were subsequently rotated to overlay with the Trp and Phe side chains of the peptide. The energy of the resulting conformation of **3** was calculated and compared with the lowest energy value. The rms distance over 6 points (i.e. between the Cαs, Cβs and Cγs of MEN10627's Trp and Phe residues and the corresponding atoms on **3**) of the template in this conformation to the peptide was 0.36 Å.

7. P. A. Grieco, S. D. Larsen, W. F. Fobare, *Tetrahedron Lett.*, **1986**, *27*, 1975-1978.

8. All new compounds gave satisfactory CHN's and spectroscopic data. The relative conformation within bicyclic compound **8** was determined by nOe experiments. Selected data for key compounds:
Compound **3**: m.p. 69-70 °C; IR (film) 3428, 3388, 2969, 1652, 1515 and 1355 $cm^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) 8.04 (1H, br s), 7.70 (1H, d, *J* 8), 7.40 (1H, d, *J* 8), 7.30-7.10 (8H, m), 4.61 (2H, m), 4.33 (1H, br s), 3.92 (1H, s, C(1)H), 3.52 (1H, s, C(3)H), 3.18 (1H, br d, *J* 12), 2.48 (1H, dd, *J* 13 and 10), 2.30 (1H, s, C(4)H), 1.91 (1H, d, *J* 10, C(7)H), 1.65-1.50 (3H, m, $CH_2$ + C<u>H</u>H) and 1.26 (2H, m, C(7)H + CH<u>H</u>); *m/z* (CI) 360 ($M+ H^+$, 12%). Compound **6**: m.p. 46-47 °C; IR (film) 3410, 3026, 2958, 1604, 1494 and 1453 $cm^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) 7.35-7.10 (10H, m), 3.66 (1H, d, *J* 10), 3.55 (1H, d, *J* 10), 3.18 (1H, s, C(1)H), 2.45 (2H, d, *J* 7), 2.23 (1H, t, *J* 7, C(3)H), 2.11 (1H, d, *J* 4, C(4)H), 1.98 (1H, m, C<u>H</u>H-CHH), 1.79 (1H, dd, *J* 2 and 10, C(7)H), 1.55 (1H, m, CH<u>H</u>-CHH), 1.25 (2H, m, CHH-C<u>H</u>$_2$) and 1.18 (1H, dd, *J* 2 and 10, C(7)H); *m/z* (CI) 278 ($M+ H^+$, 16%).

9. a) S. Boyle, S. Guard, M. Higginbottom, D. C. Horwell, W. Howson, A.T. McKnight, K. Martin, M. C. Pritchard, J. O'Toole, J. Raphy, D. C. Rees, E. Roberts, K. J. Watling, G. N. Woodruff, J. Hughes, *BioMed. Chem.*, **1994,** *2*, 357-370.

b) S. Boyle, S. Guard, J. Hodgson, D. C. Horwell, W. Howson, J. Hughes, A. McKnight, K. Martin, M. C. Pritchard, K. J. Watling, G. N. Woodruff, *BioMed. Chem.*, **1994**, *2*, 101-113.

c) P. Boden, J. M. Eden, J. Hodgson, D. C. Horwell, W. Howson, J. Hughes, A. T. McKnight, K. Meecham, M. C. Pritchard, J. Raphy, G. S. Ratcliffe, N. Suman-Chauhan, G. N. Woodruff, *BioMed. Chem.Lett.*, **1994**, *4*, 1679-1684.

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