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INVESTIGATION INTO THE PREFERRED CONFORMATION OF GABAPENTIN FOR INTERACTION WITH ITS BINDING SITE ON THE $\alpha_2\delta$ SUBUNIT OF A CALCIUM CHANNEL

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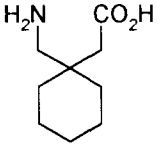
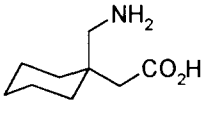
Abstract: A series of conformationally restricted Gabapentin analogues has been synthesised and used to propose the preferred conformation of Gabapentin for it to bind to the $\alpha_2\delta$ subunit of a calcium channel.

Gabapentin (Neurontin®) (1) is an anti-convulsant with, as yet, an undefined mechanism of action.[1,2] It does not interact directly with any of the enzymes in the metabolic pathways of glutamate and GABA at any significant concentrations, nor does it bind to any of the GABA receptors, despite being designed as a lipophilic GABA analogue.[3] The discovery of a high affinity Gabapentin binding site located on the $\alpha_2\delta$ subunit of a calcium channel has recently been reported[4], and it is proposed that Gabapentin acts via an interaction at this site.



(1)



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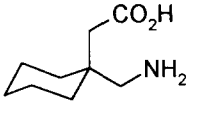

(3)

Gabapentin can exist in a number of low energy conformations, the two lowest of which have the cyclohexane ring in a chair conformation and the aminomethyl moiety axial (2) or equatorial (3). As part of a programme to improve the binding affinity of Gabapentin we wished to determine whether (2) or (3) was the favoured conformation for binding to the $\alpha_2\delta$ subunit. Several analogues were therefore synthesised which had a methyl group appended to the cyclohexane ring to lock the ring with the aminomethyl moiety in either the axial or equatorial conformation. *Trans* and *cis* 4-methyl gabapentin (4 and 5 respectively) were synthesised (in 13% and 15% overall yields repectively from 4-methylcyclohexanone) by the routes outlined in Schemes I and II[5] respectively.[6,7] Similarly, the *trans* and *cis* isomers of (3R,5S)-3,5-dimethyl gabapentin (6) and (7) were synthesised[6,7] in 10% and 11% overall yield respectively from *cis*-3,5-dimethylcyclohexanone. In Scheme I the

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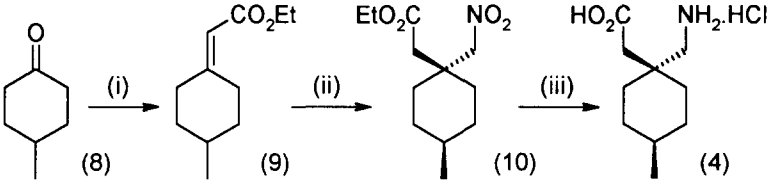
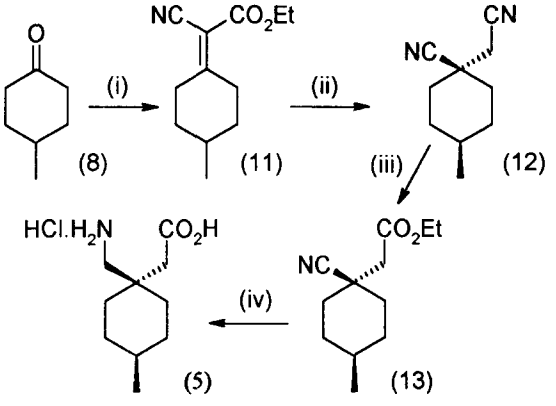
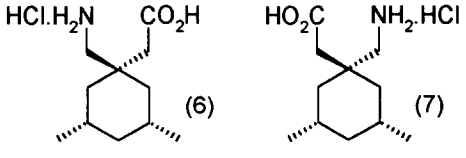
nitromethane undergoes Michael addition from the least hindered equatorial direction giving rise to the isomers where the aminomethyl moiety ends up in an equatorial position. In Scheme II, the attack of cyanide is reversible,[8] and so the reaction proceeds to minimise the 1,3-diaxial interactions between the axial protons on C-3 and C-5 and the axial group on C-1 by placing the cyanide moiety on C-1 in the axial position as opposed to the bulkier ethyl acetate moiety. This gives rise to isomers where the aminomethyl moiety is in the axial frame.



Scheme I: (i) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF; (ii) $MeNO_2$, $Bu_4N^+F^-$, THF, 70°C; (iii) (a) Raney Ni, H_2, MeOH, 30°C; (b) 1,4-Dioxane, 6N HCl, reflux.



Scheme II: (i) $NCCH_2CO_2Et$, NH4+AcO-, PhMe, azeotrope; (ii) KCN, EtOH, H_2O, reflux; (iii) EtOH, HCl, 0°C then H_3O^+; (iv) (a) H_2, Raney Ni, MeOH, 30°C; (b) 1,4-Dioxane, 6N HCl, reflux.

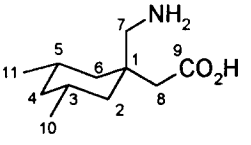


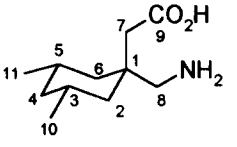
The conformations of (4), (5), (6) and (7) were determined by NMR spectroscopy in d^6-DMSO. The ^1H and ^{13}C chemical shifts of compounds (4) to (7) were assigned using ^1H-^1H double quantum filtered COSY, and ^1H observed heteronuclear multiple quantum coherence (HMQC) for the ^{13}C resonance's. The relative

stereochemistries of the alkyl side-chains to the aminomethyl and acetyl groups were determined by nOe difference. In all compounds the methyl moieties occupy equatorial positions, as expected, shown by the large coupling of the adjacent proton to nearby axial protons. The chemical shifts for *trans* and *cis* (3R,5S)-3,5-dimethyl gabapentin (6) and (7) are shown in Table 1 below.

Irradiation of the methylene resonance at 2.83ppm (CH_2NH_2) of compound (7) enhanced the signals at 1.51 ppm (1.8%), and 0.78ppm (4.4%) which are the equatorial and axial protons at C2 and C6 respectively. This is consistent with the aminomethyl moiety occupying an equatorial position. Irradiation of the methylene resonance at 2.45 ppm (CH_2CO_2H) enhanced the signals at 1.61(7.6%) and 1.51ppm (2.1%) which are the axial protons at C3 and C5 (also the equatorial proton at C4), and the equatorial protons at C2 and C6 respectively. This is characteristic of the acetyl moiety occupying the axial position. Consistent nOe's were observed for compounds (4), (5) and (6).

Table 1: NMR data for (3R,5S)-3,5-Dimethylgabapentins (6) and (7).

(6) 



(7)

		Compound (6)		Compound (7)	
		$\delta^{13}C$	$\delta^{1}H$	$\delta^{13}C$	$\delta^{1}H$
C1		35.8		37.1	
C2,C6	Hax,Heq	40.9	0.80,1.60	40.6	0.78,1.51
C3,C5	Hax	26.5	1.60	27.2	1.61
C4	Hax,Heq	43.2	0.43,1.60	43.6	0.44,1.64
C7	CH_2N	41.7	2.99	48.3	2.83
C8	CH_2CO_2H	43.0	2.31	36.4	2.45
C9	CO_2H	172.5		173.4	
C10,C11	$2xCH_3$	22.4	0.82	23.0	0.84
	NH_3^+		7.98		7.97
	CO_2H		12.32		12.00

Gabapentin (1) and the four methyl analogues (4), (5), (6) and (7) were evaluated in the $\alpha_2\delta$ binding assay.[4] The results are summarised in Table 2.

As can be seen from Table 2 the two conformers which contain the aminomethyl moiety in the axial position (5) and (6) are much lower affinity than the corresponding isomers containing an equatorial aminomethyl group (4) and (7) respectively.

Table 2: Summary of binding data at the $\alpha_2\delta$ subunit.

Compound No.	IC_{50} μM
(1)	0.14
(4)	0.42
(5)	17% @ 10μM
(6)	38% @ 10μM
(7)	0.143

We can therefore infer from these data that, for optimal binding to the $\alpha_2\delta$ subunit, Gabapentin also adopts an equatorial aminomethyl moiety. Thus the equatorial aminomethylene conformer (3) appears to be the preferred binding conformation of Gabapentin. We are currently evaluating these alkylated analogues of Gabapentin in animal models of epilepsy, the results of which will be published elsewhere.

Acknowledgement.

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References and Notes.

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6. All intermediates and final compounds gave satisfactory ^{1}H NMR, infra-red and mass spectra, as well as a satisfactory elemental analysis.
7. Full experimental details will be published elsewhere.
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