# Stereochemistry of gabapentin and several derivatives: Solid state conformations and solution equilibria*

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**Gabapentin (1-(aminomethyl)cycloheaxaneacetic acid; Gpn) is a widely used anti-epileptic drug. The target site of action of Gpn remains controversial. Gpn can exist in two isomeric chair forms. The crystal structures of Gpn 1 and eight derivatives, Gpn hydrochloride 2, Gpn lactam 3, Boc-Gpn-OH 4, Ac-Gpn-OH 5, Piv-Gpn-OH 6, Tosyl-Gpn-OH 7, Boc-Gpn-OSu 8 and Boc-Gpn-NHMe 9, are described. The aminomethyl group occupies an axial position in 1, 3, 6 and 7, while it lies in an equatorial orientation in 2, 4, 5 and 8. The structure of Boc-Gpn-NHMe 9 reveals that the crystals contain both chair forms of the derivative in the ratio 0.7 : 0.3, favouring the aminomethyl group in an axial position. In all cases, the torsional angles about the $C^a$–$C^b$ ($\theta_1$) and $C^b$–$C^g$ ($\theta_2$) bonds of the $\textbf{\textit{g}}$-amino acid residue are characteristic of a *gauche, gauche* ($g$, $g$) conformation. In solution, NMR studies establish rapid conformational exchange, as anticipated, at room temperature. Low temperature NMR studies permit conformational freezing and determination of the free-energy difference between the two 1,1-disubstituted cyclohexane conformers. The largest free-energy difference is observed in the free amino acid (0.38 kcal mol$^{-1}$), with the most stable conformer having the aminomethyl group in the equatorial position. The free-energy difference between the two forms is significantly reduced in the protected derivatives, with almost equal populations observed in solution for the fully protected neutral derivatives, Boc-Gpn-NHMe and Gpn lactam.**

THE role of $\textbf{\textit{g}}$-aminobutyric acid (GABA) as an inhibitory neurotransmitter has stimulated an enormous amount of activity on the synthesis of GABA analogues as potential central nervous system agents[1]. Gabapentin (1-(amino-methyl) cyclohexaneacetic acid; Gpn) has been introduced as an anti-epileptic drug which is orally active[2]. More recently, Gpn (Neurontin[®]) has been advanced for the treatment of neuropathic pain[3]. The mechanism of action of Gpn remains a subject of considerable discussion[4]. $\textbf{\textit{g}}$-Aminobutyric acid type-B receptors have been suggested as sites for Gpn action[5]. However, subsequent work led to the conclusion that Gpn has no agonist-like activity at GABA$_B$ receptor heterodimers[6,7]. A high affinity Gpn binding site has been demonstrated on the $\textbf{\textit{a}}_2\textbf{\textit{d}}$ subunit of a calcium channel[8]. Both Gpn and its analogue pregabalin, (S-(+)-3-isobutyl GABA), have been shown to inhibit neuronal Ca$^{2+}$ influx in the human motor cortex, suggesting that the site of action of the drug may be a voltage-dependent Ca$^{2+}$ channel[9,10]. Gpn has also been shown to have modest inhibitory activity on branched chain amino acid trans-ferases (BCAT), with distinctly different effects on various isozymes[11]. The branched chain amino acid metabolic pathways have been implicated in several neurological disorders[12].

Despite its structural simplicity, Gpn can exist in two distinct conformations corresponding to the two inter-convertible chair forms of the cyclohexane ring. Figure 1 schematically illustrates the consequences of the confor-mational interconversion for interaction at a potential receptor site. In principle, three major elements of inter-action can be considered; two involving electrostatic anchor-ing of the charged alkylamino and carboxylate groups, while the third would be mediated by apolar (hydro-phobic) contacts between the hydrocarbon ring and a potential nonpolar group on the receptor. In this simplistic, three-point attachment model, originally formulated by Ogston[13], any chiral receptor site would be readily able to distinguish between two possible chair conformations, suggesting that a specific biological activity may be medi-ated by a single conformation. The conformational require-ments for Gpn biological activity have been probed using 3-methyl substituted derivatives, specifically the *cis* and *trans* isomers of (3R)-(1-aminomethyl-3-methylcyclohexyl)-acetic acid[14,15].

Despite its widespread clinical use, the only publicly available structures of Gpn are those reported in single crystals by Ibers, who described both the free amino acid and its monohydrate[16]. We have undertaken a systematic study of the structures of Gpn and its derivatives in order to probe the factors which determine the conformational distributions in this class of 1,1-disubstituted cyclo-hexanes[14]. We discuss in this article, crystal structures of the free amino acid Gpn **1** and eight derivatives, namely Gpn hydrochloride **2**, Gpn lactam **3**, Boc-Gpn-OH (*t*-

butyloxycarbonyl-gabapentin-OH) **4**, Ac-Gpn-OH (acetyl-gabapentin-OH) **5**, Piv-Gpn-OH (pivaloyl-gabapentin-OH) **6**, Tosyl-Gpn-OH **7**, Boc-Gpn-OSu (Boc-gabapentin-*N*-hydroxysuccinimide ester) **8** and Boc-Gpn-NHMe **9**. NMR evidence for the conformational interconversion and estimated free-energy differences between the two forms in Gpn and its derivatives are also reported. The stereochemistry of Gpn and its derivatives is also specially relevant in view of the growing interest in the use of **g**amino acid residues in the design of peptides of structural and biological importance[17–20].

## Experimental procedure

### *Synthesis*

Gpn **1**, Gpn hydrochloride **2** and Gpn lactam **3** were the products of Hikal Ltd, Bangalore, India. The N-protected derivatives were prepared by standard procedures and purified.

### *Boc-Gpn-OH 4*

This was obtained as a white crystalline solid by treatment of the free amino acid with di-tert-butyldicarbonate [(Boc)$_2$O] using a reported procedure[21]. Yield 90%; m.p. 127–128°C.

### *Ac-Gpn-OH 5/Piv-Gpn-OH 6*

Gpn-OH (0.65 g, 5 mmol) was dissolved in 2N NaOH (10 ml) and cooled in an ice-bath. Ac-chloride/Piv-chloride (6 mmol) was added over a period of 30 min maintaining pH 8–9. After the addition was complete, the reaction mix-

ture was slowly allowed to come to room temperature and stirring was continued for another 2 h. The reaction mixture was diluted with water (10 ml) and washed with ether, the aqueous layer was cooled and acidified with 6N HCl and then extracted with ethyl acetate. The organic layer was dried over Na$_2$SO$_4$ and evaporated. Recrystallization using ethyl acetate/petroleum ether gave Ac-Gpn-OH/Piv-Gpn-OH as white crystalline solids. Ac-Gpn-OH: Yield 850 mg (80%); m.p. 125–126°C. Piv-Gpn-OH: Yield 1.10 g (87%); m.p. 89–90°C.

### *Tosyl-Gpn-OH 7*

This was prepared by treatment of the free amino acid with tosyl chloride using a reported procedure[21], yielding a white crystalline solid. Yield 82%; m.p. 115–116°C.

### *Boc-Gpn-OSu 8*

To a stirred, ice-cold solution of Boc-Gpn-OH (1.35 g, 5 mmol) and *N*-hydroxysuccinimide (0.69 g, 6 mmol) in dry ethyl acetate (20 ml), dicyclohexyl carbodiimide (1.03 g, 5 mmol) was added and the reaction mixture was allowed to attain room temperature. After 12 h, dicyclohexyl urea was filtered-off and the filtrate was washed with 1N HCl, 1M Na$_2$CO$_3$ and brine solution. The organic layer was dried over Na$_2$SO$_4$ and evaporated. Recrystallization using ethanol gave Boc-Gpn-OSu as a white crystalline solid. Yield 1.65 g, (89%); m.p. 171–172°C.

### *Boc-Gpn-NHMe 9*

Boc-Gpn-OH (0.54 g, 2 mmol) was dissolved in dry THF (5 ml) and cooled to 0°C in an ice-salt bath. TEA (0.3 ml, 2 mmol) was added followed by ethyl chloroformate (0.26 ml, 2 mmol). After 10 min, 10 ml of dry THF saturated with methylamine gas was added. After 2 h, the reaction mixture was filtered and the filtrate evaporated under vacuum. The residue was dissolved in ethyl acetate and washed with 1N HCl, 1N Na$_2$CO$_3$ and water. Evaporation of ethyl acetate gave Boc-Gpn-NHMe as a white solid, which was purified by medium pressure liquid chromatography over a reverse-phase C$_{18}$ column (40–60 μm). Yield 480 mg (84%); m.p. 87–88°C.

### *X-ray diffraction*

Single crystals suitable for X-ray diffraction were obtained by slow evaporation of concentrated solutions in aqueous/organic solvents. Table 1 summarizes the crystal and diffraction data for the compounds **1** to **9**. X-ray data were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer using Mo K$_a$ radiation (**l** = 0.71073 Å). **w**-Scan type was use. In the case of Boc-Gpn-NHMe, diffraction data were collected at – 73°C to address the issue of disorder. All the structures were obtained by direct methods using SHELXS-97 (ref. 22). Refinement was carried out against $F^2$ with full matrix
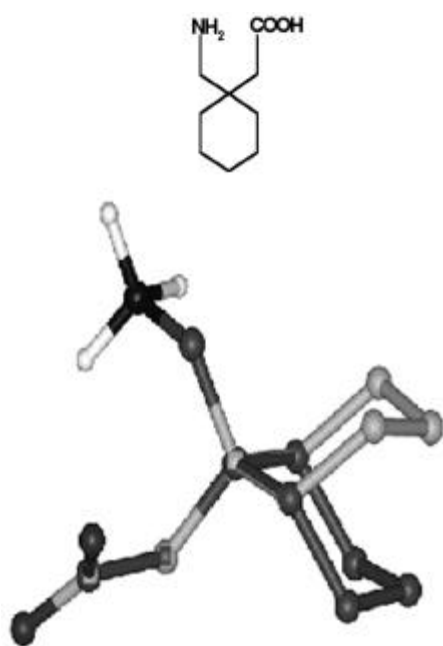


**Figure 1.** (Top) Gabapentin, 1-(aminomethyl)cycloheaxaneacetic acid. (Bottom) Superposition of the two possible chair forms of the 1,1-disubstituted cyclohexane.

**Table 1.** Crystal and diffraction parameters

| | Gabapentin **1** | Gabapentin HCl **2** | Gabapentin lactam **3** | Boc-Gpn-OH **4** | Ac-Gpn-OH **5** |
|---|---|---|---|---|---|
| Empirical formula | $C_9 H_{17} N_1 O_2$ | $C_9 H_{17} N_1 O_2$ HCl 1/2 $H_2O$ | $C_9 H_{15} N_1 O_1$ | $C_{14} H_{25} N_1 O_4$ | $C_{11} H_{19} N_1 O_3$ |
| Crystal habit | Clear, rod | Clear, rectangular | Clear, thin plates | Clear, rectangular | Clear, rod |
| Crystal size (mm) | $0.6 \times 0.2 \times 0.08$ | $0.6 \times 0.36 \times 0.35$ | $0.52 \times 0.1 \times 0.04$ | $0.4 \times 0.2 \times 0.1$ | $0.46 \times 0.18 \times 0.18$ |
| Crystallizing solvent | Water | Water | Hexane/ethyl acetate | Ethyl acetate | Methanol |
| Space group | $P 2_1/c$ | $C 2/c$ | $C 2/c$ | $P 2_1/c$ | $P 2_1/c$ |
| Cell parameters | | | | | |
| $a$ (Å) | 5.9034(16) | 27.831(6) | 22.243(7) | 12.061(3) | 7.877(4) |
| $b$ (Å) | 6.9193(19) | 6.537(15) | 6.187(19) | 11.0628(3) | 7.440(3) |
| $c$ (Å) | 22.476(6) | 13.369(3) | 13.352(4) | 12.026(3) | 19.875(9) |
| $b$ (deg) | 90.062(4) | 111.911(3) | 108.513(8) | 107.907(4) | 93.087(7) |
| Volume (Å$^3$) | 918.1(4) | 2256.6(9) | 1742.4(9) | 1526.9(7) | 1163.2(9) |
| $Z$ | 4 | 8 | 8 | 4 | 4 |
| Molecules/asymmetric unit | 1 | 1 | 1 | 1 | 1 |
| Cocrystallized solvent | None | $H_2O$ | None | None | None |
| Molecular weight | 171.24 | 216.70 | 153.22 | 271.35 | 213.27 |
| Density (g/cm$^3$)(cal) | 1.239 | 1.276 | 1.168 | 1.18 | 1.218 |
| F (000) | 376 | 936 | 672 | 592 | 464 |
| Radiation | Mo K$_a$ | Mo K$_a$ | Mo K$_a$ | Mo K$_a$ | Mo K$_a$ |
| Temperature (°C) | 21 | 21 | 21 | 21 | 21 |
| $2q$ max. (°) | 53.72 | 53.84 | 55.22 | 55.5 | 53.1 |
| Measured reflections | 9210 | 8142 | 8774 | 12172 | 8045 |
| $R_{int}$ | 0.1566 | 0.0279 | 0.0366 | 0.0266 | 0.0179 |
| Independent reflections | 1934 | 2265 | 1888 | 3316 | 2264 |
| Observed reflections [$|F| > 4 s (F)$] | 1778 | 2052 | 1501 | 2318 | 1930 |
| Final $R(\%)/wR2(\%)$ | 5.35/14.5 | 3.26/9.03 | 5.97/15.52 | 5.70/14.14 | 4.56/12.90 |
| Goodness-of-fit ($S$) | 1.075 | 1.043 | 1.070 | 1.002 | 1.045 |
| $\Delta r$ max (e Å$^{-3}$) / $\Delta r$ min (e Å$^{-3}$) | 0.74/– 0.21 | 0.30/– 0.23 | 0.25/– 0.14 | 0.28/– 0.13 | 0.25/– 0.16 |
| No. of restraints/parameters | 0/177 | 0/200 | 0/160 | 0/272 | 0/212 |
| Data-to-parameter ratio | 10 : 1 | 10.3 : 1 | 9.4 : 1 | 8.5 : 1 | 9.1 : 1 |

| | Piv-Gpn-OH **6** | Tosyl-Gpn-OH **7** | Boc-Gpn-OSu **8** | Boc-Gpn-NHMe **9a** | Boc-Gpn-NHMe[a] **9b** |
|---|---|---|---|---|---|
| Empirical formula | $C_{14} H_{25} N_1 O_3$ | $C_{16} H_{23} N_1 O_4 S_1$ | $C_{18} H_{28} N_2 O_6$ | $C_{15} H_{28} N_2 O_3$ | $C_{15} H_{28} N_2 O_3$ |
| Crystal habit | Clear, thin plates | Clear, rod | Clear, cubic | Clear, thin plates | Clear, rod |
| Crystal size (mm) | $0.47 \times 0.11 \times 0.002$ | $0.5 \times 0.3 \times 0.03$ | $0.35 \times 0.25 \times 0.25$ | $0.5 \times 0.3 \times 0.02$ | $0.4 \times 0.1 \times 0.08$ |
| Crystallizing solvent | Methanol/water | Ethyl acetate/ petroleum ether | Ethyl acetate | Methanol | Methanol |
| Space group | $P 2_1/c$ | $P na2_1$ | $P 2_1/c$ | Pbcn | Pbcn |
| Cell parameters | | | | | |
| $a$ (Å) | 18.985(8) | 24.676(7) | 15.068(14) | 30.040(5) | 29.781(13) |
| $b$ (Å) | 9.144(4) | 11.681(3) | 12.396(12) | 11.274(2) | 11.203(5) |
| $c$ (Å) | 8.632(4) | 24.015(6) | 11.373(11) | 9.821(17) | 9.792(4) |
| $b$ (deg) | 96.750(7) | 90.0 | 112.170(2) | 90 | 90 |
| Volume (Å$^3$) | 1488.1(10) | 6922.0(3) | 1967.0(3) | 3326.1(10) | 3267.0(2) |
| $Z$ | 4 | 16 | 4 | 8 | 8 |
| Molecules/asymmetric unit | 1 | 4 | 1 | 1 | 1 |
| Cocrystallized solvent | None | None | None | None | None |
| Molecular weight | 255.35 | 325.41 | 368.42 | 284.39 | 284.39 |
| Density (g/cm$^3$)(cal) | 1.140 | 1.249 | 1.244 | 1.136 | 1.156 |
| F (000) | 560 | 2784 | 792 | 1248 | 1284 |
| Radiation | Mo K$_a$ | Mo K$_a$ | Mo K$_a$ | Mo K$_a$ | Mo K$_a$ |
| Temperature (°C) | 21 | 21 | 21 | 21 | – 73 |
| $2q$ max. (°) | 53.8 | 53.68 | 52.9 | 54.08 | 52.86 |
| Measured reflections | 10698 | 69275 | 8818 | 23959 | 21811 |
| $R_{int}$ | 0.027 | 0.0250 | 0.1432 | 0.0371 | 0.088 |
| Independent reflections | 2931 | 13909 | 3515 | 3383 | 3140 |
| Observed reflections [$|F| > 4 s (F)$] | 2048 | 10043 | 2404 | 2487 | 1975 |
| Final $R(\%)/wR2(\%)$ | 6.15/14.18 | 5.04/13.33 | 7.32/17.81 | 8.9/19.30 | 6.8/18.83 |
| Goodness-of-fit ($S$) | 1.137 | 1.017 | 1.135 | 1.180 | 1.06 |
| $\Delta r$ max (e Å$^{-3}$)/$\Delta r$ min (e Å$^{-3}$) | 0.25/– 0.14 | 0.25/– 0.19 | 0.27/– 0.27 | 0.41/– 0.34 | 0.55/– 0.54 |
| No. of restraints/parameters | 0/263 | 3/819 | 0/347 | 1/281 | 6/283 |
| Data-to-parameter ratio | 7.8 : 1 | 12.3 : 1 | 6.9 : 1 | 8.8 : 1 | 7 : 1 |

[a]Data for the low-temperature structure.

least squares methods using SHELXL-97 (ref. 23). The hydrogen atoms were located from difference Fourier maps, except in the case of the disordered cyclohexane ring in Boc-Gpn-NHMe and Tosyl-Gpn-OH, where the hydrogen atoms were geometrically fixed. The crystallographic coordinates and temperature factors for the structures are deposited at the Cambridge Crystallographic Data Centre with deposition numbers **1**, CCDC 217734; **2**, CCDC 217735; **3**, CCDC 217736; **4**, CCDC 217737; **5**, CCDC 217738; **6**, CCDC 217739; **7**, CCDC 217740; **8**, CCDC 217741; **9a**, CCDC 217742 and **9b**, CCDC 217743. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; Fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

*NMR spectroscopy*

The $^1$H NMR spectra were recorded on a BRUKER AMX 400 MHz spectrometer. Spectra were recorded in methanol (CD$_3$OD) solution and are referenced with respect to internal tetramethylsilane. Equilibration times of about 15–20 min were used in the low-temperature spectra.

## Results and discussion

Figures 2 and 3 show views of the molecular conformation of Gpn **1**, Gpn hydrochloride **2**, Gpn lactam **3**, Boc-Gpn-OH **4**, Ac-Gpn-OH **5**, Piv-Gpn-OH **6**, Tosyl-Gpn-OH **7**, Boc-Gpn-OSu **8**, Boc-Gpn-NHMe **9a**, and Boc-Gpn-NHMe (at – 73°C) **9b**. In the case of **7**, there are four independent molecules in the asymmetric unit. Two positions with an occupancy of 0.7 : 0.3 are observed for the terminal carboxylic acid groups in molecules **7A** and **7B**, corresponding to rotation about the $C^a$–CO ($\psi$) bond (Table 2). Table 2 lists the orientation of the aminomethyl and carboxymethyl substituents on the cyclohexane ring. In Boc-Gpn-NHMe both conformations of the cyclohexane ring are observed, with partial occupancy. The torsion angles $f$, $q_1$, $q_2$ and $\psi$, which are important in considering the use of Gpn in peptide design, are also listed in Table 2. Intramolecular hydrogen bonds are observed in four structures, the hydrochloride **2** and the three *N*-protected gabapentin derivatives **4**, **5** and **6**. In Gpn hydrochloride, a seven-membered N–H···O hydrogen bond between the protonated amino group and the carbonyl oxygen of the protonated carboxylic acid group is observed. The conformations of the three *N*-protected derivatives **4**, **5** and **6** are of interest in considering the nature of local folding patterns when the Gpn residue is introduced into peptide chains. In Boc-Gpn-OH **4** and Piv-Gpn-OH **6**, a seven-membered N–H···O hydrogen bond between the Gpn N-H group and the carbonyl group of the terminal carboxylic acid group is observed. In the cases of **2**, **4** and **6**, the carboxylic acid group adopts the expected *syn* conformation. In Ac-Gpn-OH **5**,

a nine-membered intramolecular hydrogen bond is formed between the acetyl C=O group and the OH moiety of the terminal carboxylic acid. Interestingly, the carboxylic acid adopts the infrequently observed *anti* conformation. A similar local conformation for a C-terminus Gpn residue has been observed in the N-protected dipeptides Piv-Pro-Gpn-OH and Boc-Aib-Gpn-OH, with the terminal COOH group adopting an *anti* conformation[20].

## Cyclohexane conformation

In all the nine structures, the cyclohexane ring adopts an almost perfect chair conformation. The average endocyclic torsion angles and their estimated standard deviations (in parentheses) in Gpn and its derivatives are: **1** 53.7° (3.18); **2** 53.9° (1.53); **3** 54.7° (2.13); **4** 54.3° (1.83); **5** 53.9° (1.97); **6** 53.7° (1.59); **7A** 53.3° (2.18); **7B** 53.5° (2.44); **7C** 53.5° (3.59); **7D** 53.2° (4.37); **8** 53.8° (1.36); **9b** (aminomethyl axial) 58.8° (4.22); **9b** (aminomethyl equatorial) 41.3° (6.41). The mean value obtained by averaging over all the Gpn derivative structures is 53.3° (1.86). Classical studies on cyclohexane by electron diffraction yield a value of 55.9° (ref. 24), while X-ray diffraction studies on cyclohexane derivatives resulted in values of 55.4° (axial substitution)[25] and 55.0° (equatorial substitution)[25].

The structure determined for the free amino acid Gpn **1**, is practically identical to that reported by Ibers[16], who also described the structure of its hydrate. Gpn polymorphs have been discussed in the patent literature and characterized by powder X-ray diffraction data[26]. However, no single-crystal X-ray diffraction structure of a polymorph is reported. During the course of our study, we made several attempts to obtain single crystals of polymorphic forms using a variety of crystallization conditions, but were unsuccessful; always recovering the same form as **1**. This is unsurprising, as the literature has several well-studied examples of recalcitrant polymorphs[27].

## Orientation of substituents

The conformation in crystals of Gpn **1** has the aminomethyl group in an axial position. The structure of the hydrochloride salt **2** which crystallizes as a hemihydrate, yields the alternative conformation, which has the carboxymethyl group axial. Gpn lactam **3**, is a derivative which has been reported to be neuroprotective in retinal ischemia[28]. The crystal structure of the lactam reveals a conformation in which the aminomethyl group is axial. The torsion angle about the amide bond in the lactam, – 0.3°, is indicative of a perfectly planar *cis* amide conformation. Boc-Gpn-OH **4** and Ac-Gpn-OH **5** crystallize in the conformation with the carboxymethyl group in the axial orientation. Piv-Gpn-OH **6** has an axial aminomethyl group in the crystal structure. All the four independent molecules observed in the crystal structure of Tosyl-Gpn-OH **7**, adopt a conformation with the aminomethyl group in the axial position. The
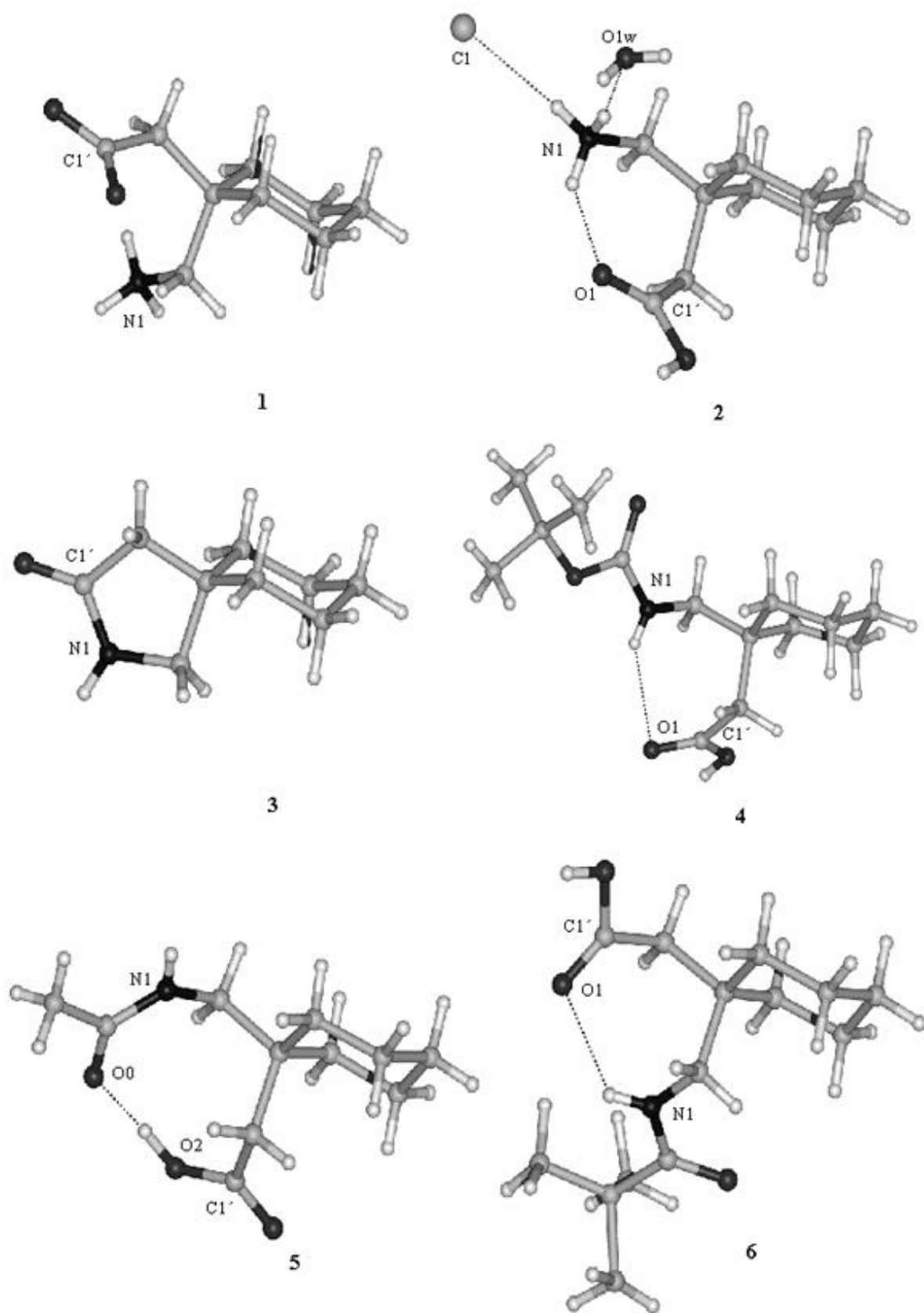
**Figure 2.** Molecular conformation in crystals observed for Gpn **1**, Gpn hydrochloride **2**, Gpn lactam **3**, Boc-Gpn-OH **4**, Ac-Gpn-OH **5** and Piv-Gpn-OH **6**. Dotted lines indicate hydrogen bonds.
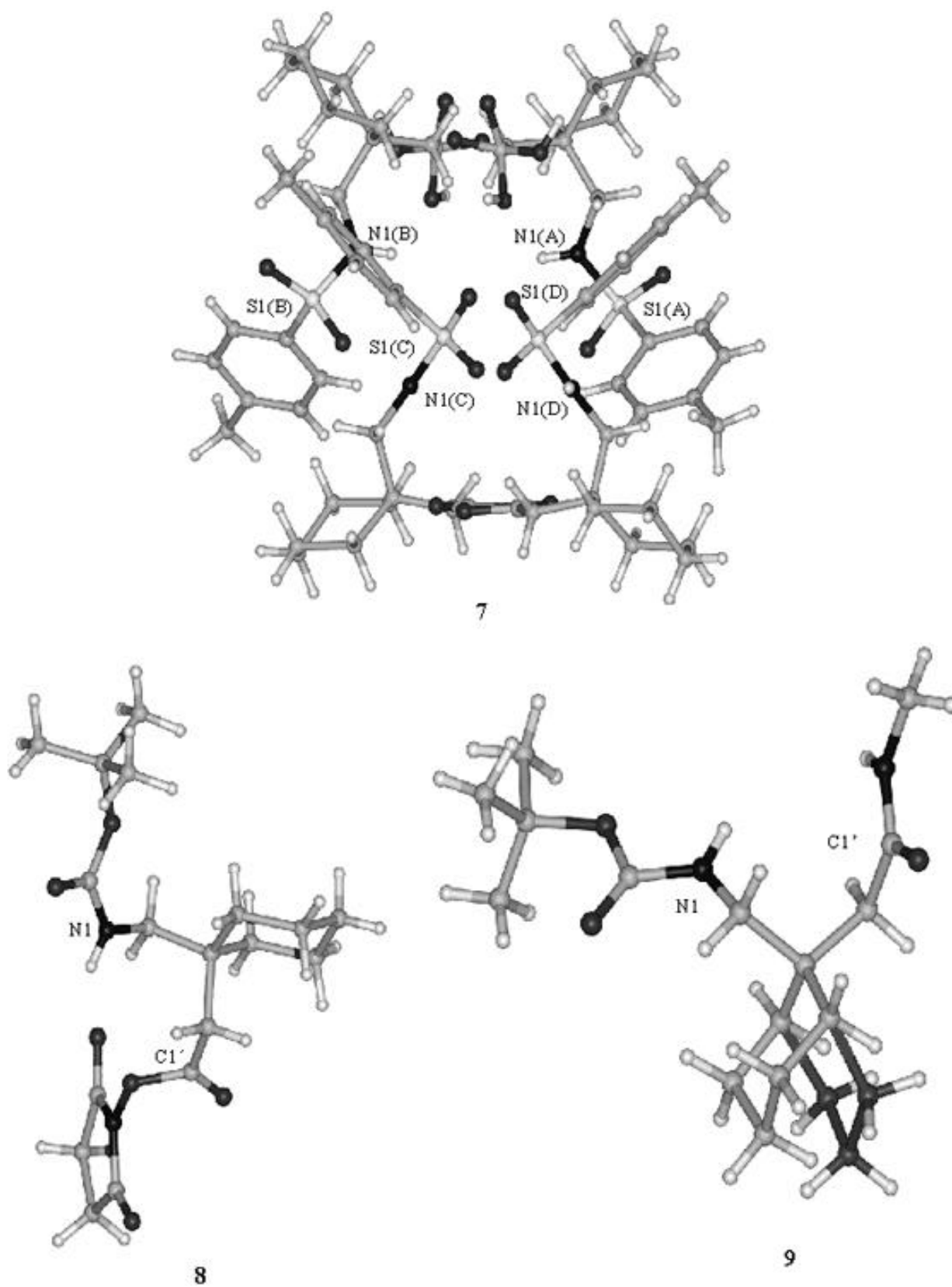
**Figure 3.** Molecular conformation in crystals observed for Tosyl-Gpn-OH **7**, Boc-Gpn-OSu **8** and Boc-Gpn-NHMe **9**. Note that for **9**, structure determination carried out at room temperature and at – 73°C yielded identical structures. Both possible chair forms are present in the crystal with an occupancy ratio of 0.7 : 0.3 (axial: equatorial aminomethyl group).

fully-protected derivative, Boc-Gpn-OSu **8** crystallizes with carboxymethyl group in the axial position. The other fully-protected derivative Boc-Gpn-NHMe **9** reveals an interesting disorder in the crystals with both forms, amino-methyl axial and carboxymethyl axial being present in the crystals, with an occupancy of 0.7 : 0.3, respectively. This form presents a frozen view of the distribution in solution with the two conformations present in the solid state. X-ray diffraction data determined at low temperature (– 73°C) yielded a structure practically identical to that at room temperature.

The observation of both possible chair conformations in crystals of Gpn derivatives suggests that the two forms may not be significantly different in energy, resulting in an appreciable population of both structures in solution. Crystallization may be kinetically controlled by poorly-understood nucleation events, with the added possibility that packing forces can determine the conformation selected in crystals.

## Crystal packing

In view of the large number of crystal structures presented, we describe only a selected set of four examples which illustrate specific features of molecular packing in crystals (Figures 4 *a*, *b*). On going from Gpn **1** to the hydrochloride hemihydrate **2**, there is an inversion of the cyclohexane ring. Inspection of the packing diagram reveals that an intra-molecular hydrogen bond is formed between one of the amino hydrogens and the carboxyl group of the carboxylic acid. Molecules in the crystal are linked together by bridging

chloride ions and a single water molecule. The water molecule occupies a special position (Figure 4 *a*). The packing arrangement in Gpn lactam **3**, consists of a closed dimeric motif in which the *cis* amide group forms a pair of hydrogen bonds. In the packing of *cis* amides, as in dioxopiperazines, two forms of packing arrangements have been observed, namely the closed dimeric motif and the more common displaced arrangement, where a continuous network of hydrogen bonds stabilizes the crystal[29]. The packing diagrams for one example each of a protected Gpn derivative with the aminomethyl group in the axial (Piv-Gpn-OH **6**) and equatorial (Boc-Gpn-OH **4**) orienta-tions are illustrated in Figure 4 *b*. The two molecules differ by only the single oxygen atom of the urethane (Boc) group, which is absent in the corresponding pivaloyl deri-vative. Both molecules adopt similar conformations sta-bilized by a single intramolecular hydrogen bond between the NH and CO group of Gpn. Interestingly, in both cases a single intermolecular OH···O hydrogen bond between the carbonyl group of the Piv or Boc moieties and the Gpn carboxylic acid O–H group stabilizes the crystal. It appears that the orientation of the substituents on the cyclohexane ring is not a function of crystal pack-ing forces; rather the observed conformations in crystals may be selected by nucleating factors.

## Solution equilibria: NMR studies

Figure 5 shows the temperature dependence of the methy-lene proton resonances of the aminomethyl (2.7–3.1 *δ*) and carboxymethyl (2.3–2.5 *δ*) groups in Gpn. The

**Table 2.** Conformational parameters in the gabapentin residue[a]

| Compounds | | $f$ (deg) | $q_1$ (deg) | $q_2$ (deg) | $y$ (deg) | Aminomethyl group |
|---|---|---|---|---|---|---|
| Gabapentin **1** | | – | 59.7 | 51.1 | | Axial |
| Gabapentin HCl[b] **2** | | – | 66.4 | – 83.2 | – 124.5 | Equatorial |
| Gabapentin lactam **3** | | | 21.6 | – 21.9 | | Axial |
| Boc-Gpn-OH[b] **4** | | 119.6 | 57.4 | 40.0 | 79.7 | Equatorial |
| Ac-Gpn-OH[b] **5** | | – 101.3 | 59.0 | 75.9 | – 79.4 | Equatorial |
| Piv-Gpn-OH[b] **6** | | 99.8 | 45.3 | 53.1 | 100.9 | Axial |
| Tosyl-Gpn-OH **7** (four molecules) | **A** | 144.6 | 66.7 | 63.8 | – 86.8 (– 5.1)[c] | Axial |
| | **B** | 146.9 | 69.0 | 65.3 | – 88.4 (– 4.0)[c] | Axial |
| | **C** | – 124.7 | 59.9 | 61.7 | – 171.1 | Axial |
| | **D** | – 124.1 | 59.6 | 61.0 | – 169.4 | Axial |
| Boc-Gpn-OSu **8** | | 102.4 | 61.1 | 68.2 | – 94.6 | Equatorial |
| Boc-Gpn-NHMe[d] **9b** | | 125.5 | 68.7 | 52.8 | – 118.5 | [e] |

[a]All derivatives are achiral and crystallize in centrosymmetric space groups. One sign of the dihedral angle is chosen arbitrarily. The dihedral angles are defined as $f$ = C′–N–C$^g$–C$^b$, $q_1$ = N–C$^g$–C$^b$–C$^a$, $q_2$ = C$^g$–C$^b$–C$^a$–C′ and $y$ = C$^b$–C$^a$–C′–O (ref. 30).
[b]Intramolecular hydrogen bonds are observed in these structures: **2** (N1···O1 = 2.76 Å, H···O1 = 2.05 Å, ∠N1–H···O1 = 136.7°), **4** (N1···O1 = 3.08 Å, H···O1 = 2.50 Å, ∠N1–H···O1 = 133.3°), **5** (O0···O2 = 2.54 Å, O0···H = 1.55 Å, ∠O0···H–O2 = 173.3°), **6** (N1···O1 = 2.97 Å, H···O1 = 2.37 Å, ∠N1–H···O1 = 129.0°).
[c]The terminal carboxylic acid group has a disorder with an occupancy ratio of 0.7 : 0.3 and the corresponding $y$ values are given in parentheses.
[d]Values are listed only for the structure obtained at low temperature.
[e]The two conformations, aminomethyl group axial and equatorial, coexist with an occupancy ratio of 0.7 : 0.3.
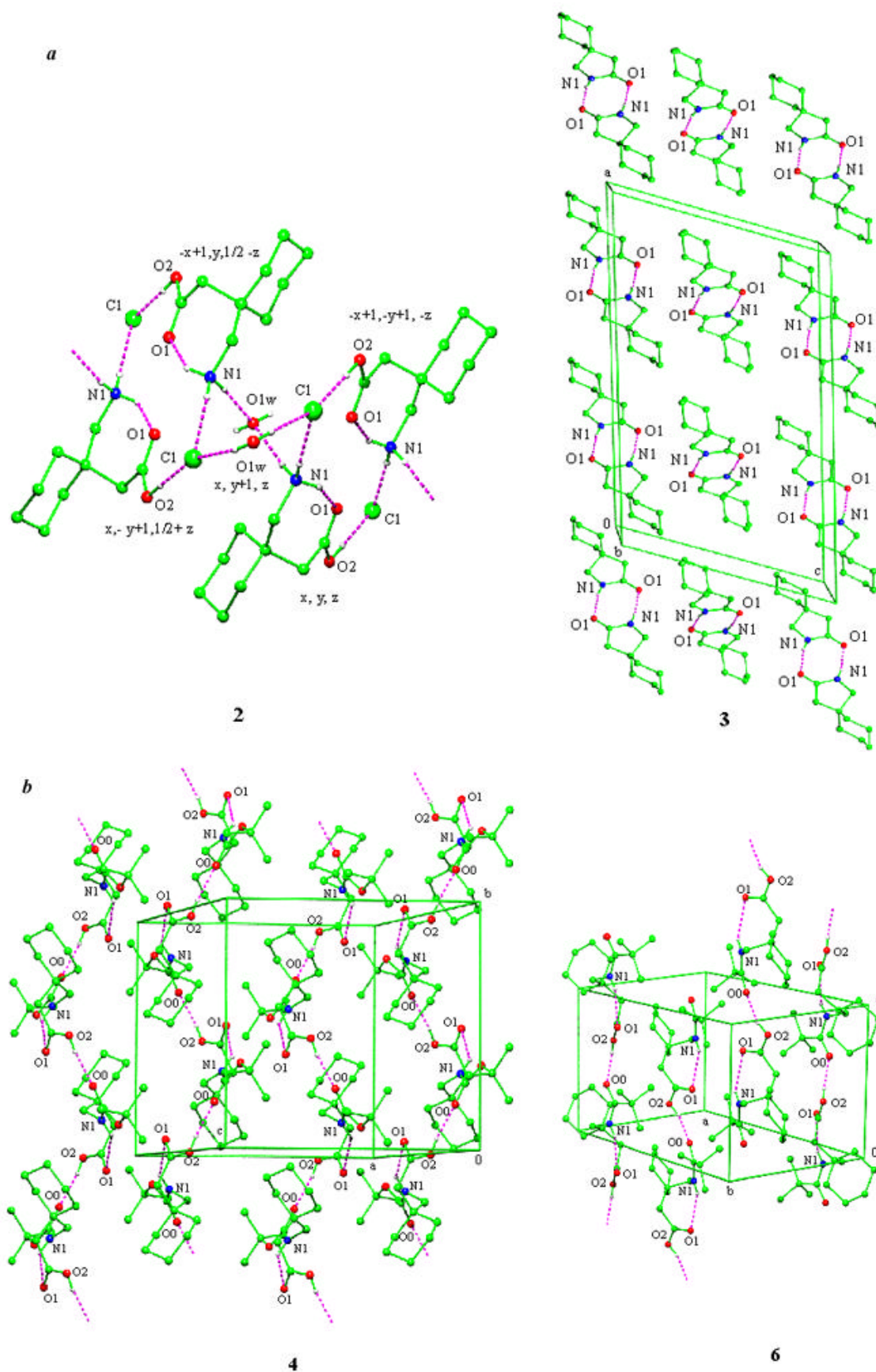
**Figure 4.** View of packing in crystals of (*a*) Gpn hydrochloride **2** and gabapentin lactam **3**; and (*b*) Boc-Gpn-OH **4** and Piv-Gpn-OH **6**.

resonances appear as a sharp singlet at ~ 30°C, suggesting rapid interconversion between the two possible chair conformations of the cyclohexane ring. Upon cooling to – 80°C, two distinct sets of resonances can be observed for each of the methylene groups. The more intense pair of resonances corresponds to the conformation with the
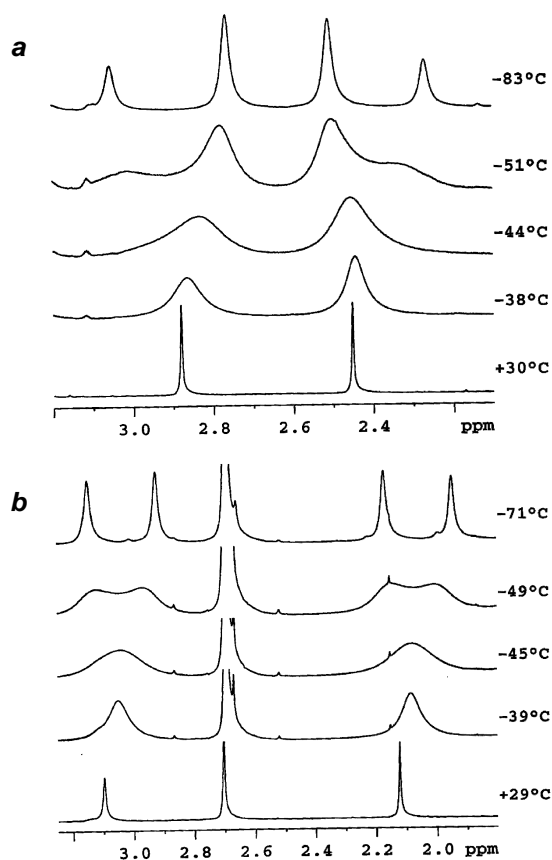


**Figure 5.** Partial 400 MHz ¹H NMR spectra in CD₃OD at various temperatures. Only the CH₂–N and CH₂–CO proton resonances are shown. *a*, Gpn; *b*, Boc-Gpn-NHMe.

aminomethyl group in an equatorial position. The unambiguous assignment of stereochemistry was made possible by the analysis of the spectra of a constrained Gpn analogue, which is incapable of conformational interconversion (unpublished). Interestingly, the conformation observed for Gpn in crystals (reported above and in ref. 12) corresponds to the *major* conformation observed in solution, i.e. aminomethyl group is equatorial. The results of an identical set of NMR experiments carried out on the fully protected derivative Boc-Gpn-NHMe are also shown in Figure 5. Once again, at – 71°C two sets of resonances corresponding to two possible chair conformations of the cyclohexane ring are observed. In this case, the two chair conformations are more equally populated. Interestingly, in crystals, as noted earlier, both conformations are observed with an occupancy of 0.7 : 0.3; the form with the aminomethyl group axial being predominant. Table 3 summarizes the population ratios for the two cyclohexane ring conformations determined for Gpn and its eight derivatives. The free energy difference between the two forms is slightly larger in the case of Gpn **1** and Gpn hydrochloride **2**, compared to the other derivatives. Indeed, protection of the amino and carboxyl groups appears to result in diminishing the free-energy difference between the two forms. Almost equal populations are observed in Boc-Gpn-NHMe **9**. The crystallographic investigation on Gpn and several of its derivatives suggests that two possible chair conformations of the 1,1-disubstituted cyclohexane ring can be observed in the solid state. Figure 6 shows a superposition of nine independent Gpn units determined in this study. Three structures, Gpn hydrochloride, Gpn lactam and Boc-Gpn-NHMe are excluded. Two conformational families are clearly identifiable. Evidently, the nature of the conformation captured in crystals may be determined by crystal packing forces and also by kinetic nucleation events. It is noteworthy that both forms are present in the crystals of Boc-Gpn-NHMe **9**, resulting in static disorder

**Table 3.** Conformational distribution and NMR parameters for Gpn and derivatives

| Compound | $P_{ax} : P_{eq}$ (CH₂NH)[a] | $\Delta G$ (kcal mol⁻¹) | $T_c$[b] (°C) | $d$ (ppm)[c] | | | |
|---|---|---|---|---|---|---|---|
| | | | | $C^{\beta}H_{ax}$ | $C^{\beta}H_{eq}$ | $C^{a}H_{ax}$ | $C^{a}H_{eq}$ |
| Gpn **1** | 0.27 : 0.73 | 0.38 | – 44, – 44 | 3.05 | 2.76 | 2.51 | 2.27 |
| Gpn HCl **2** | 0.29 : 0.71 | 0.33 | – 46, – 46 | 3.22 | 2.93 | 2.54 | 2.32 |
| Gpn lactam **3** | 0.48 : 0.52 | 0.02 | – 39, – 41 | 3.25 | 3.09 | 2.26 | 2.12 |
| Boc-Gpn-OH **4** | 0.38 : 0.62 | 0.19 | – 50, – 50 | 3.21 | 3.00 | 2.34 | 2.08 |
| Ac-Gpn-OH **5** | 0.47 : 0.53 | 0.05 | – 47, – 47 | 3.42 | 3.17 | 2.37 | 2.13 |
| Piv-Gpn-OH **6** | 0.40 : 0.60 | 0.15 | – 49, – 49 | 3.34 | 3.13 | 2.35 | 2.13 |
| Tosyl-Gpn-OH **7** | 0.39 : 0.61 | 0.17 | – 40, – 50 | 3.81 | 3.63 | 2.86 | 2.62 |
| Boc-Gpn-OSu **8** | 0.41 : 0.59 | 0.14 | – 51, – 47 | 3.23 | 3.03 | 2.70 | 2.40 |
| Boc-Gpn-NHMe **9** | 0.49 : 0.51 | 0.01 | – 45, – 45 | 3.15 | 2.92 | 2.17 | 1.95 |

[a]Ratio of the forms with the aminomethyl group axial or equatorial determined under conditions of slow exchange at – 70 to – 86°C in methanol (CD₃OD). Peak areas were determined manually.
[b]Coalescence temperature estimated from the two sets of resonances (–CH₂NH– and –CH₂–COOH). For Tosyl-Gpn-OH the frequency difference between the two forms is much larger for the –CH₂–COOH group compared to the –CH₂–NH₂ group, resulting in significantly different coalescence temperatures for the two sets of resonances.
[c]Chemical shifts of the methylene protons of the –CH₂–NH– (C^{β}H₂) and –CH₂–COOH (C^{a}H₂) groups determined under conditions of slow exchange at – 70 to –86°C.
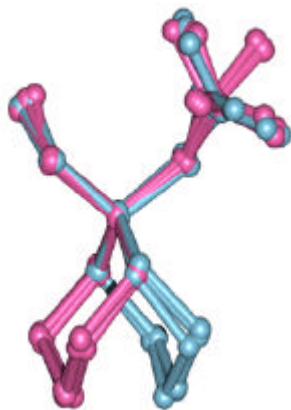
**Figure 6.** Superposition of nine Gpn moieties determined in crystal structures. Seven atoms, N, C$^g$, C$^b$, C$^a$, C′ C$^{b1}$ and C$^{b2}$, are used for the superposition. (RMSD = 1.0358 Å).

involving the cyclohexane ring. NMR studies confirm that relatively small free energy differences separate the two possible chair conformations, resulting in an appreciable population of both forms in solution. In view of the widespread of use of Gpn in diverse clinical applications, it may be of some interest to further probe the conformational requirements of the pharmacological receptors for this molecule.

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