Conformations of \(\beta\)-Amino Acid Residues in Peptides: X-Ray Diffraction Studies of Peptides Containing the Achiral Residue 1-Aminocyclohexaneacetic Acid, \(\beta^{3,3}\)Ac6c

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INTRODUCTION

There has been an explosive upsurge of interest in the structural properties of polypeptides containing \(\beta\)- and \(\gamma\)-amino acid residues.1–4 Research in this area has been fuelled by the observation of novel folded structures, stabilized by diverse intramolecular hydrogen bonds in oligo \(\beta\) and \(\gamma\) peptides and also in hybrid sequences containing \(\alpha\)-amino acid and their backbone homologated counterparts.5–14 Conformational diversity may be anticipated on backbone homologation of amino acid residues since the available torsional space for the residues is enhanced. In the case of \(\beta\)-residues the number of torsional variables is three \((\phi, \theta, \psi)\) while it is four \((\phi, \theta_1, \theta_2, \psi)\) in the case of \(\gamma\)-residues. The accommodation of \(\beta\) and \(\gamma\) residues into hydrogen bonded folded peptide structures was determined in early studies of oligo \(\beta\)-peptides and hybrid sequences containing \(\alpha\)-amino acid and their backbone homologated counterparts.5–14

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conformational properties of peptides containing the achiral \( \beta,\beta \)-disubstituted \( \beta \)-residue, 1-aminocyclohexanecarboxylic acid (\( \beta^{3,3}\)Ac\(_6\)c, Figure 1). \( \beta^{3,3}\)Ac\(_6\)c can be considered as a homologue of the well-studied \( \alpha \)-residue 1-aminocyclohexane-1-carboxylic acid\(^{15–17} \) (Ac\(_6\)c) and \( \gamma \)-residue, 1-amino-methylcyclohexanecarboxylic acid (gabapentin).\(^{18,19} \) Preliminary structural studies have been reported by Seebach et al. on the isomeric residue, 1-amino-methylcyclohexanecarboxylic acid (\( \beta^{2,2}\)Ac\(_6\)c).\(^{20} \)

**RESULTS**

Table I lists the backbone torsion angles determined in crystals for the free amino acid, \( \beta^{3,3}\)Ac\(_6\)c, protected derivatives and di- and tri-peptides containing this residue. In an overwhelming majority of the structures the amino group occupies an axial orientation with respect to the cyclohexane ring, while the CH\(_2\)—CO group adopts the equatorial position. The only exceptions are the free amino acid (1), amino acid hydrochloride (2) and the C-terminus residue in the dipeptide, Boc-\( \beta^{3,3}\)Ac\(_6\)c-\( \beta^{3,3}\)Ac\(_6\)c-NHMe (10). In these three cases an intramolecular 6-atom (\( \gamma_{\phi,\psi} \)) N—H · · · OC hydrogen bond is observed. Figure 2 illustrates the observed molecular conformation in these three examples. The C\(_{\gamma}\) hydrogen bond in a \( \beta \)-residue may be considered as a formal backbone expansion of the C\(_{\gamma}\) hydrogen bond proposed in the fully extended structure of \( \alpha \)-peptides.\(^{21} \) It may be noted that the free \( \alpha \)-amino acid analogue Ac\(_6\)c has the amino group in an equatorial orientation.\(^{17} \) Interestingly, a survey of the peptides containing Ac\(_6\)c residue reveals that the amino group adopts an axial orientation in almost all cases, an observation similar to that reported here for the \( \beta^{3,3}\)Ac\(_6\)c residue.

The structure of the model sequence Piv-Pro-\( \beta^{3,3}\)Ac\(_6\)c-NHMe has been shown to form a C\(_{11}\) hydrogen bond in which the \( \alpha\beta \)-turn is an expanded version of the Type II \( \beta \)-turn in an \( \alpha\alpha \)-segment.\(^{22} \) This unit has been used to generate a \( \beta \)-hairpin structure with an \( \alpha\beta \) hybrid nucleating turn. The peptide Boc-Aib-Pro-\( \beta^{3,3}\)Ac\(_6\)c-NHMe (11) was synthesized in order to test the competing possibilities of -Aib-Pro- C\(_{10}\) hydrogen bond formation versus -Pro-\( \beta^{3,3}\)Ac\(_6\)c- C\(_{11}\) hydrogen bond formation. Figure 3 illustrates the observed molecular conformation of the protected tripeptide Boc-Aib-Pro-\( \beta^{3,3}\)Ac\(_6\)c-NHMe (11) in crystals. Peptide 11 forms an Alb-Pro Type III \( \beta \)-turn stabilized by a single C\(_{10}\) (4 \( \rightarrow \) 1) intramolecular hydrogen bond. The peptide Boc-\( \beta \)Pro-\( \beta^{3,3}\)Ac\(_6\)c-OMe, (12) containing two \( \beta \)-residues, adopts an extended conformation devoid of intramolecular hydrogen bonds. In this case the \( \beta \)Pro residue adopts a trans conformation about the

**FIGURE 1** Chemical structures of Ac\(_6\)c, Gpn and \( \beta\)Ac\(_6\)c residues.
Table I  Backbone Torsion Angles (deg) and Orientation of Substituents on the β³,³Ac₆c Residue Determined in Crystal Structures

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ϕ</th>
<th>θ</th>
<th>ψ</th>
<th>ω</th>
<th>Amino group</th>
</tr>
</thead>
<tbody>
<tr>
<td>β³,³Ac₆c 1</td>
<td>60.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Equatorial</td>
</tr>
<tr>
<td>β³,³Ac₆c.HCl 2</td>
<td>55.4</td>
<td>168.1</td>
<td>—</td>
<td>—</td>
<td>Equatorial</td>
</tr>
<tr>
<td>Ac-β³,³Ac₆c-OH 3</td>
<td>—61.8</td>
<td>—60</td>
<td>—161.7</td>
<td>—</td>
<td>Axial</td>
</tr>
<tr>
<td>Piv-β³,³Ac₆c-OH 4 Mol 1</td>
<td>61.9</td>
<td>65.2</td>
<td>161.0</td>
<td>—</td>
<td>Axial</td>
</tr>
<tr>
<td>Mol 2</td>
<td>—60.1</td>
<td>—64.1</td>
<td>—158.5</td>
<td>—</td>
<td>Axial</td>
</tr>
<tr>
<td>Boc-β³,³Ac₆c-OH 5</td>
<td>60.3</td>
<td>44.8</td>
<td>82.5 (−96.3)</td>
<td>—</td>
<td>Axial</td>
</tr>
<tr>
<td>Boc-β³,³Ac₆c-NHMe 6</td>
<td>49.5</td>
<td>43.2</td>
<td>—145.1</td>
<td>176.6</td>
<td>Axial</td>
</tr>
<tr>
<td>Boc-β³,³Ac₆c-Aib-OMe 7 Mol 1</td>
<td>—63.7</td>
<td>—63.6</td>
<td>—105.5</td>
<td>170.3</td>
<td>Axial</td>
</tr>
<tr>
<td>Mol 2</td>
<td>—58.1</td>
<td>—70.2</td>
<td>—117.9</td>
<td>173.9</td>
<td>Axial</td>
</tr>
<tr>
<td>Boc-Aib-β³,³Ac₆c-OMe 8</td>
<td>—59.8</td>
<td>—63.2</td>
<td>91.9</td>
<td>—177.0</td>
<td>Axial</td>
</tr>
<tr>
<td>Boc-β³,³Ac₆c-β³,³Ac₆c-OMe 9 (β³,³Ac₆c1)</td>
<td>—60.8</td>
<td>—176.2</td>
<td>106.6</td>
<td>169.8</td>
<td>Axial</td>
</tr>
<tr>
<td>(β³,³Ac₆c2)</td>
<td>62.1</td>
<td>53.6</td>
<td>82.7</td>
<td>178.3</td>
<td>Axial</td>
</tr>
<tr>
<td>Boc-β³,³Ac₆c-β³,³Ac₆c-NHMe 10 (β³,³Ac₆c1)</td>
<td>71.9</td>
<td>72.0</td>
<td>—73.6</td>
<td>176.6</td>
<td>Axial</td>
</tr>
<tr>
<td>(β³,³Ac₆c2)</td>
<td>—168.7</td>
<td>—58.0</td>
<td>—118.0</td>
<td>—179.7</td>
<td>Equatorial</td>
</tr>
<tr>
<td>Boc-Aib-Pro-β³,³Ac₆c-NHMe 11</td>
<td>62.0</td>
<td>58.7</td>
<td>107.7</td>
<td>176.3</td>
<td>Axial</td>
</tr>
<tr>
<td>Boc-Pro-β³,³Ac₆c-OMe 12</td>
<td>60.2</td>
<td>62.6</td>
<td>—43.4 (59.6)</td>
<td>171.7 (−167.7)</td>
<td>Axial</td>
</tr>
</tbody>
</table>

a The values given in parentheses correspond to the alternate conformation observed at the disordered C-terminus.

C²—C⁶ bond (θ = 180°) resulting in a stretched peptide backbone. The N-terminal urethane blocking group adopts the less frequent cis conformation (ω = −0.12°).23 The structures of two dipeptide esters containing β³,³Ac₆c are illustrated in Figure 4. No intramolecular hydrogen bonds are observed in these structures. Interestingly, in both the cases there is close approach of a carbonyl oxygen to an axial C—H bond on a cyclohexane ring. The observed parameters H ⋅ ⋅ ⋅ O = 2.70–2.75 Å, C ⋅ ⋅ ⋅ O = 3.65–3.70 Å, C—H ⋅ ⋅ ⋅ O = 168°–170° are suggestive of a potentially stabilizing interaction, although the C—H groups are not polarized by the presence of electronegative substituents. Figure 5 illustrates the observed conformations in five derivatives and peptides containing β³,³Ac₆c. In all the cases the structures do not possess any intramolecular hydrogen bonds. In two cases, Boc-β³,³Ac₆c-OMe 5 and the dipeptide Boc-β³,³Ac₆c-β³,³Ac₆c-NHMe 10, the N-terminal urethane group adopts a cis geometry (ω = 14.5° for 5; ω = −9.1° for 10). Inspection of the crystal packing arrangement in both cases reveals registry of facing urethane groups through the formation of a pair of hydrogen bonds between molecules related by a centre of inversion (see Figure 6).

DISCUSSION

Conformations of the β³,³Ac₆c Residue in Peptides

The sterically allowed regions of conformational space available to amino acid residues can be significantly reduced by substitution at backbone carbon atoms. In the case of α-amino acid residues the reduction in accessible conformational space is best illustrated by comparing the Ramachandran map for glycyl, alanyl, and α-aminoisobutyryl (Aib) residues, in which substituents are progressively introduced at the tetrahedral C² carbon atom.24 By extension, limitations of allowed conformations can also be imposed in the case of β-amino acid residues by substitution at the C² and C⁶ atoms. The seminal work of Seebach et al. has provided an entry to studies of the structural chemistry of substituted β-residues.1,2 In an alternative approach, Geissman and collaborators have introduced conformational constraints in the β-residues by covalently bridging the C² and C⁶ positions, as exemplified by their studies of 2-aminoacyclonacoxaloxycarboxylic acids.7–9 In the present study we have systematically determined the solid state conformations of several derivatives and peptides containing the β,β-disubstituted achiral residue, β³,³Ac₆c. This residue can be compared to both its lower and higher homologues Ac₆c and gabapentin (Gpn), respectively. In the case of the α-residue, Ac₆c, constraints are imposed on both the ϕ and ψ torsion angles, limiting the residue to helical regions of conformational space (ϕ = ±60°, ψ = ±30°).15–17 In the case of the γ-residue, Gpn, the symmetrically positioned β,β-dialkyl substituents restricts the torsion angle θ₁ (C²—C⁶—C⁸) and θ₂ (C⁶—C⁸—C²) to gauche conformations, resulting in the stabilization of several unique intramolecularly hydrogen bonded conformations.19
In $\beta^{3.3}\text{Ac}_{6}c$, the dialkyl substituents impose restrictions on the torsion angles $\phi$ and $\theta$. Interestingly, in many of the structures determined, no intramolecular hydrogen bonds are observed. The exceptions are the dipeptide Boc-$\beta^{3.3}\text{Ac}_{6}c-\beta^{3.3}\text{Ac}_{6}c$-NHMe, (10) in which residue 2 adopts a $C_6$ hydrogen bonded conformation. A $C_{11}$ hydrogen bonded expanded analogue of a $\beta$-turn structure is also observed in the hybrid peptide Piv-Pro-$\beta^{3.3}\text{Ac}_{6}c$-NHMe. Figure 7 illustrates the observed backbone hydrogen bonded conformations involving $\beta^{3.3}\text{Ac}_{6}c$, and also provides for comparison, hydrogen bonded conformations observed in the isomeric residue, $\beta^{2.2}\text{Ac}_{6}c$ studied by Seebach et al.\textsuperscript{20} The $C_6$ conformation has been observed in both $\beta^{2.2}\text{Ac}_{6}c$ and $\beta^{3.3}\text{Ac}_{6}c$ residues. Inspection of the torsion angles in the two illustrated examples suggests that the hydrogen bond interaction can be obtained with a gauche conformation about the $C^2-C^\beta$ bond ($\theta$) and with one of the other dihedral angles, $\phi$ or $\psi$ in fully extended conformation ($\phi \sim 180^\circ$, $\psi \sim 180^\circ$). Similar hydrogen bond interaction is also observed in the case of the free amino acid (1) and hydrochloride salt (2). The $C_6$ conformation in model peptides has also been shown to be energetically favorable by quantum mechanical calculations, despite the poor geometry of the hydrogen bond.\textsuperscript{25} The $C_6$ conformation of $\beta$-residues may be viewed as expanded analogue of the $C_5$ conformation.

![Diagram of molecular conformations](image_url)

**FIGURE 2** Molecular conformation in crystals of (a) the free amino acid $\beta^{3.3}\text{Ac}_{6}c$ 1, (b) $\beta^{3.3}\text{Ac}_{6}c$ hydrochloride 2 and (c) the dipeptide Boc-$\beta^{3.3}\text{Ac}_{6}c-\beta^{3.3}\text{Ac}_{6}c$-NHMe 10. $C_6$ hydrogen bond parameters, 1, $N \cdots O = 2.72 \text{ Å}$; $H \cdots O = 1.94 \text{ Å}; <N-H \cdots O = 137.8^\circ$; 2, $N \cdots O = 2.80 \text{ Å}; H \cdots O = 2.24 \text{ Å}; <N-H \cdots O = 118.6^\circ$; 10, $N \cdots O = 2.77 \text{ Å}; H \cdots O = 2.07 \text{ Å}; <N-H \cdots O = 139.2^\circ$.
observed in fully extended α-peptides as exemplified in the crystal structures of homo oligomers of α,α-diethyl and dipropyl glycines. In α-residues another well-characterized intramolecularly hydrogen bonded structure is the C7 of γ-turn structure. In this case a 7 atom hydrogen bonded ring is formed by interaction of C=O\(_{i-1}\)···HN\(_{i+1}\). The β-residue analogue of this structure would be a C\(_8\) conformation. At present the C\(_8\) structure (φ = −111°, θ = 69.6°, ψ =

![FIGURE 3](image)

**FIGURE 3** Molecular conformation in crystals of (a) Boc-Aib-Pro-β\(_{3,3}\)Ac\(_6\)-NHMe 11 and (b) Boc-βPro-β\(_{3,3}\)Ac\(_6\)-OMe 12. The C=O(OMe) group is disordered over two positions with refined occupancy ratio 0.59:0.41.

![FIGURE 4](image)

**FIGURE 4** Conformation in crystals of (a) Boc-Aib-β\(_{3,3}\)Ac\(_6\)-OMe 8 and (b) Boc-β\(_{3,3}\)Ac\(_6\)-β\(_{3,3}\)Ac\(_6\)-OMe 9.
4.5°) has been observed in the solid state only in crystal structures of β2,2Ac6c residues and has not been reported either in β2,2Ac6c or β3,3Ac6c residues. The C8 conformation has been advanced for the β2,2Ac6c residue in a solution NMR study of a chemotactic tripeptide analogue. The C8 structure has also been postulated on the basis of NMR analysis of cyclic pentapeptide containing β-residues.

In the crystal structure of the protected dipeptide ester 9, there is no intramolecular hydrogen bond observed for the β3,3Ac6c-β3,3Ac6c segment (see Figure 4). In contrast, in the
FIGURE 6 Packing of molecules in Boc-\(\beta^3\)-Ac-OH 5 (top) and Boc-\(\beta^3\)-Ac-\(\beta^3\)-Ac-NHMe 10 (bottom) crystals.
case of the tripeptide Boc-β<sup>2,2</sup>Ac<sub>6</sub>C<sub>b</sub>-β<sup>2,2</sup>Ac<sub>6</sub>C<sub>b</sub>-β<sup>2,2</sup>Ac<sub>6</sub>C<sub>b</sub>-OMe, residues 2 and 3 form a C<sub>10</sub> hydrogen bond with reversed polarity, i.e., NH<sub>(i)</sub>/C<sub>1</sub>/C<sub>1</sub>/C<sub>1</sub>OC<sub>(i+1)</sub>. In the dipeptide Piv-Pro-β<sup>3,3</sup>Ac<sub>6</sub>C<sub>b</sub>-NHMe, the β<sup>3,3</sup>Ac<sub>6</sub>C<sub>b</sub> residue adopts <i>i</i> + 2 position of an expanded version of type II β-turn. All the three backbone conformational angles are approximately in the region for gauche conformation. This type of hybrid αβ turn is positioned to nucleate a peptide hairpin. Indeed, solution NMR studies support a β-hairpin in the octapeptide, Boc-Leu-Phe-Val-[Pro-β<sup>3,3</sup>Ac<sub>6</sub>C<sub>b</sub>-Leu-Phe-Val-OMe. These examples suggest that β<sup>2,2</sup>Ac<sub>6</sub>C<sub>b</sub> residue can be positioned in specific intramolecularly hydrogen bonded structures.

**Backbone Conformations of β-Residues in Peptides**

The determination of the crystal structures of several peptides containing the β<sup>3,3</sup>Ac<sub>6</sub>C<sub>b</sub> residue provides an opportunity to analyze torsion angle preferences for β-residues.
dues. An understanding of the conformational biases imposed by backbone substitution patterns is essential for using β-residues, in a rational manner, in the design of specifically folded peptides. The conformational analysis of z-amino acid containing peptides has benefited enormously by the use of the Ramachandran map, which allows a simple representation of individual residue conformations in two-dimensional φ-ψ space. The introduction of an additional torsional variable θ in the case of β-residues necessitates the use of three-dimensional maps for a complete description. To simplify the problem of representation, we have used two-dimensional φ-ψ plots for fixed values of θ, which is largely constrained to either gauche (θ ~ 60°) or trans (θ ~ 180°) conformations. Figure 8 shows a two-dimensional scatter plot in φ-ψ space for θ = +60°. All the experimentally observed β\(^{3,3}\)Ac\(_{6}\)c residues are marked. In addition, the observed φ-ψ distributions for various types of intramolecularly hydrogen bonded structures observed in the case of all reported β-residues are indicated. The clusters marked correspond to specific hydrogen bond types. The C\(_{6}\) and C\(_{8}\) structures are formed by a single β-residue and differ in hydrogen bond directionality. The C\(_{12}\) structure corresponds to a ββ segment with conventional hydrogen bond polarity, which is an expanded analogue of the type III turn in z-peptides.\(^{30}\) The C\(_{10}\) and C\(_{14}\) structures are formed by ββ and βββ segments with reversed hydrogen bond polarity. The helices arising from repetition of C\(_{12}\) and C\(_{14}\) hydrogen bond type were initially characterized crystallographically by Gellman and collaborators.\(^{7–9}\) The C\(_{11}\) and mixed clusters correspond to hybrid sequences containing both β and z residues. For example, a C\(_{11}\) hydrogen bond can be formed in a zβ segment as exemplified by the structure of peptide Piv-Pro-β\(^{3,3}\)Ac\(_{6}\)c-NHMe (see Figure 7). Larger hydrogenbonded rings C\(_{13}\), C\(_{14}\), C\(_{15}\), and C\(_{16}\) can also be formed in β\(_{1}\), zββ, zββ, and ββζ segments, respectively, as exemplified in the crystal structures of Boc-Leu-Aib-Val-βGly-yAbu-Leu-Aib-Val-Ala-Leu-Aib-OMe,\(^{11}\) Boc-Leu-Aib-Val-βGly-yAbu-Leu-Aib-Val-OMe,\(^{11}\) Boc-Leu-Phe-Val-Aib-βPhe-Val-Phe-Phe-Val-OMe,\(^{31}\) and Boc-Val-Ala-Phe-Aib-βVal-βPhe-Aib-Val-Ala-Phe-Aib-OMe.\(^{32}\) The following features of the scatter plot in Figure 8 merit mention:

1. For β\(^{3,3}\)Ac\(_{6}\)c, both φ and θ values are restricted to the region of ±60° and have the same sign. In contrast, the formation of a C\(_{12}\) helix requires that φ and θ are close to the gauche conformation, but have opposite signs. This clearly suggests that the β\(^{3,3}\)Ac\(_{6}\)c residue is precluded from forming many of the intramolecularly hydrogen bonded structures observed in oligo β-peptides.

2. A noteworthy feature is the characterization of two C\(_{11}\) conformations in zβ-segments, which results in an expanded analogue of the conventional β-turns. These two structures correspond to a change of ~180° at the φ value of the β-residue, which arise due to a flip of the central peptide unit linking the z and β amino acids. A similar flip of the central linking peptide unit relates the type I and type II β-turn conformations in zβ segments.\(^{30,33}\) This feature is also observed for two residue turns in hybrid β\(_{1}\)ζ segments (C\(_{13}\) hydrogen bond). In Figure 8, the point in the upper left quarter labeled as a β\(_{1}\)ζ turn corresponds to the C\(_{13}\) turn observed in Boc-βPhe-Gpn-Phe-OMe.\(^{19}\) This is a non-helical turn analogous to the type II β-turn in zζ sequences. The helical β\(_{1}\)ζ turns cluster in the lower left quadrant.

3. The ββ C\(_{10}\) turn observed in the -β\(^{2,2}\)Ac\(_{6}\)c-β\(^{2,2}\)Ac\(_{6}\)c-segment by Seebach et al.,\(^{20}\) which has inverted hydrogen bond polarity, requires distinct conformations at the two β-residues, that differ in ψ values by ~180°.

The unsubstituted parent β residue β-glycine (βhGly, also referred in the earlier literature as the β-alanyl residue)\(^{2}\) may be taken as the starting point for analyzing conformational space accessible to amino acids with homologated backbones. Experimentally determined crystal structures have captured the βhGly residue in extended conformations in β-strands and in folded conformation in turns and helices.\(^{34}\) Conformational limitations can be imposed by introducing substituents at the z and β positions. Substitution results in creating chiral centers adding another dimension to the conformational problem. The introduction of symmetrical substituents at the 2 and 3 positions as in β\(^{2,2}\)Ac\(_{6}\)c and β\(^{3,3}\)Ac\(_{6}\)c results in achiral residues in which two of the three conformational angles are restricted to a limited range of values. Figure 9 illustrates examples of some of the chiral and multiply substituted β-residues whose conformations have been determined in several distinct peptide crystal structures. Constraints introduced by backbone cyclization provide an entry into β-residues with well-defined conformational preferences as exemplified by 2-aminocyclopentanecarboxylic acid (ACPC), 2-aminocyclohexanecarboxylic acid (ACHC) and nipecotic acid, which have been investigated by Gellman and coworkers.\(^{4}\)

The use of β, γ, and higher ω amino acids in the design of hybrid peptide sequences provides an opportunity to mimic the folded structures observed in proteins and natural poly-
peptides. Unnatural synthetic backbones can provide stability in biological situations. The creation of novel hydrogen bonded structures and folding patterns which have no analogues in the world of natural polypeptides is also an enticing possibility. The development and applications of $\beta$ and higher $\omega$ amino acids with well-characterized conformational propensities will advance the area of hybrid peptide structures.

**FIGURE 9** Experimentally determined torsion angles for chiral and multiply substituted $\beta$-residues in specific backbone conformations. The torsion angles listed are averaged over the observed structures and the e.s.d.s are shown in parentheses. The number of structures used are $\beta^3$hVal$^{5,32,35-37}$ 8, $\beta^3$hLeu$^{5,36,37}$ 5, $\beta^3$hPhe($\theta$ gauche)$^{31,32,35}$ 4, $\beta^3$hPhe ($\theta$ trans)$^{38-40}$ 10, $\beta^3$hAla$^{5,35}$ 5, (S)-Nipecotic acid$^{41,42}$ 3, (S,S)-trans ACPC$^{12-14}$ 27 (C$_{11}$), 4 (C$_{14}$)/C$_{15}$, (R,R)-trans ACPC$^{9}$ 12.
MATERIALS AND METHODS

Synthesis of $\beta^{3,3}$Ac6c (Scheme 1)

1-aminocyclohexane-1-carboxylic acid (Ac6c) was reduced to the amino alcohol \((\text{i})\) following the procedure of McKennon et al.\(^{43}\) 7.15 g (50 mmol) of 1-aminocyclohexane-1-carboxylic acid (Ac6c), 3.78 g NaBH\(_4\) (100 mmol) and 100 ml dry THF were placed in a two necked round bottom flask. The flask was cooled to 0°C in an ice bath. A solution of 12.7 g (50 mmol) of iodine in 25 ml of dry THF was added drop wise over 30 min under stirring, resulting in vigorous evolution of hydrogen. After addition of the iodine was complete and gas evolution had ceased, the flask was heated to reflux for 24 h and then cooled to room temperature and methanol was added until the mixture became clear. After 30 min stirring, the solvent was removed in vacuo yielding a white paste, which was dissolved by addition of 75 ml of 20% aqueous KOH. The solution was stirred for 8 h and extracted with 4 x 75 ml of dichloromethane. The organic extracts were dried over anhydrous Na\(_2\)SO\(_4\) and concentrated under vacuum, yielded 5.93 g of the amino alcohol \((\text{i})\) as a white gum, which was directly used for further reaction.

Boc-derivative \((\text{ii})\): 5.8 g (45 mmol) of compound \((\text{i})\) was prepared as Boc derivative by the treatment of \((\text{i})\) with di-tert-butyldicarbonate in 1, 4-dioxane-water (1:1) in presence of 2N NaOH. Standard work up yielded 8.75 g of solid \((\text{ii})\), Yield 85%.

Tosyl derivative \((\text{iii})\): 8.0 g (35 mmol) of Boc protected amino alcohol \((\text{ii})\) was dissolved in freshly distilled 50 ml of pyridine and the solution was stirred at 0°C, while 13.3 g (70 mmol) of p-toluenesulfonyl chloride was slowly added. The reaction mixture was stored at 4°C for 5 days and 100 ml of ice cold water was added and extracted with 3 x 10 ml of ethyl acetate. Organic layer was discarded and aqueous layer was neutralized with ammonia solution, until alkaline. The solution was concentrated. On cooling, crystals of $\beta^{3,3}$Ac6c (1) were obtained, which gave ninhydrin positive test (Yield 2.1 g).

Peptide Synthesis

Peptides were synthesized by classical solution phase methods. The N-terminal was protected by either tert-butyloxycarbonyl (Boc) or Pivaloyl (Piv) group, while C-terminal was protected as a methyl ester (OMe). The peptide-NHMe was prepared by passing methylene gas in solution of peptide-OMe in methanol under dry condition. Deprotection of N- and C- terminus was achieved with 98% formic acid and 2N NaOH/MeOH, respectively. Couplings were mediated by using either DCC/HOBt or mix anhydride procedure. Peptides were purified by medium pressure liquid chromatography (MPLC) on a C18 reverse phase column-using methanol-water gradient. The homogeneity of the peptides was checked by analytical RP-HPLC. The peptides were further characterized by \(^1\)H NMR recorded on a Bruker AMX-400 MHz spectrometer and also through electrospray ionisation mass spectrometry (ESI-MS), which were recorded on HP-1100 Mass Spectrometer.

Structure Solution and Refinement

Single crystals suitable for X-ray diffraction were obtained by slow evaporation of concentrated solutions in aqueous/organic solvents. Table II summarizes the unit cell parameters and the final R-values for all the 12 compounds. X-ray data were collected at room temperature on a Bruker AXS SMART APEX CCD diffractometer, using Mo K\(\alpha\) radiation \((\lambda = 0.71073\AA)\). \(\omega\)-Scan type was used. Structures were solved by direct methods in SHELXL-97\(^{44}\) or by Patterson heavy atom method in DIRDIF\(^{45}\) (for 2). Refinement was carried out against \(F^2\) with full matrix least square methods in SHELXL-
Table II  Details of the Unit Cell and Final R-Factors for Compounds 1-12

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Empirical formula</th>
<th>Unit cell</th>
<th>Space group</th>
<th>Final R(%)</th>
<th>wR2(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,3Ac6c</td>
<td>C8H15N1O2</td>
<td>a = 7.543 (1) Å, b = 11.301 (7) Å, c = 7.543 (1) Å, $\beta = 90.0 (1)^\circ$</td>
<td>P2₁2₁2₁</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3,3Ac6c·HCl</td>
<td>C8H18NO3 Cl·H₂O</td>
<td>a = 7.886 (1) Å, b = 6.481 (1) Å, c = 22.108 (3) Å, $\beta = 112.5 (1)^\circ$</td>
<td>P2₁/c</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>3,3Ac6c-OH</td>
<td>C10H17N1O3</td>
<td>a = 10.934 (2) Å, b = 7.961 (1) Å, c = 13.146 (2) Å, $\beta = 112.6 (1)^\circ$</td>
<td>P2₁/c</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>3,3Ac6c-OH</td>
<td>C10H17N1O3</td>
<td>a = 12.159 (1) Å, b = 12.881 (1) Å, c = 75.8 (1)^\circ</td>
<td>P2₁/c</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>3,3Ac6c·Boc-</td>
<td>C13H24N1O4</td>
<td>a = 10.075 (7) Å, b = 11.297 (7) Å, c = 10.075 (7) Å, $\beta = 96.4 (1)^\circ$</td>
<td>P2₁/c</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>3,3Ac6c·NHMe</td>
<td>C22H38N2O5</td>
<td>a = 12.721 (8) Å, b = 12.875 (7) Å, c = 12.721 (8) Å, $\beta = 99.3 (1)^\circ$</td>
<td>P2₁/c</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>3,3Ac6c·OMe</td>
<td>C22H39N3O4</td>
<td>a = 11.769 (7) Å, b = 11.287 (7) Å, c = 11.769 (7) Å, $\beta = 99.3 (1)^\circ$</td>
<td>P2₁/c</td>
<td>8</td>
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</tr>
<tr>
<td>3,3Ac6c-OMe</td>
<td>C23H42N4O5 H₂O</td>
<td>a = 11.081 (1) Å, b = 11.283 (1) Å, c = 11.081 (1) Å, $\beta = 90.0 (1)^\circ$</td>
<td>P2₁/c</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES