



Article

Solution State Conformational Preferences of Dipeptides Derived from N-Aminoazetidinecarboxylic Acid: An Assessment of the Hydrazino Turn

Amandine ALTMAYER-HENZIEN, Valerie DECLERCK, Denis Merlet, Jean-Pierre BALTAZE, Jonathan FARJON, Régis Guillot, and David J. Aitken

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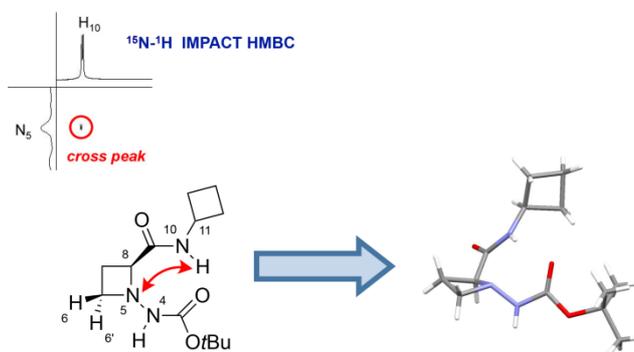
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3 **Solution State Conformational Preferences of Dipeptides Derived from**
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5 ***N*-Aminoazetidinecarboxylic Acid: An Assessment of the Hydrazino Turn**
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10 Amandine Altmayer-Henzien,[§] Valérie Declerck,[§] Denis Merlet,[†] Jean-Pierre Baltaze,[‡]
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12 Jonathan Farjon,[†] Régis Guillot[‡] and David J. Aitken^{*,§}
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17 [§]Laboratoire de Synthèse Organique et Méthodologie, [†]Laboratoire de RMN en Milieu
18 Orienté, and [‡]Services Communs, ICMMO (UMR CNRS8182), Université Paris Sud, 15 Rue
19 Georges Clemenceau, 91405 Orsay cedex, France
20
21
22
23
24
25

26
27 david.aitken@u-psud.fr
28
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32 **Table of Contents**
33



50 **Abstract**

51 Four model compounds and four dipeptides containing *N*-aminoazetidinecarboxylic acid
52 (AAzC) and a particular stereoisomer of 2-aminocyclobutanecarboxylic acid (ACBC) were
53 studied to establish their solution-state conformational preferences, particularly regarding the
54 ability of AAzC to induce a three-center hydrogen bonded folding feature known as a
55 “hydrazino turn”. On the basis of IR and NMR experiments, supported by molecular
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3 modelling, the AAzC residue adopted a *trans* configuration amenable to the formation of a
4 cyclic 8-membered hydrogen bond conformation in solution, in all cases studied. The
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6 implication of the heterocyclic nitrogen atom of AAzC in the *trans*-like structure was
7
8 demonstrated via a refined ^1H - ^{15}N HMBC experiment giving exploitable data at natural ^{15}N
9
10 isotopic abundance, providing unprecedented evidence for the solution-state hydrazino turn
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12 conformation. The predominance of this secondary structural feature depended on the
13
14 configuration of the neighbouring ACBC residue in the dipeptides: while the *trans*-ACBC
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16 derivatives prefer the hydrazino turn, the *cis*-ACBC derivatives may also populate low energy
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18 10-membered hydrogen bonded ring structures. X-Ray diffraction analysis of three
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20 compounds confirmed the presence of a solid-state hydrazino turn in two cases, with
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22 geometries similar to those deduced from the solution state studies, but in the third compound
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24 no intramolecular hydrogen bonding feature was in evidence.
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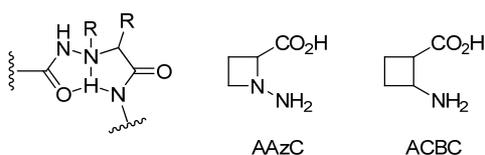
34 Introduction

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36 The specific functions performed by natural peptides are the result of their well-defined
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38 architectures, which depend on locally-ordered secondary structural features. Significant
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40 efforts have been made to understand and control the factors which determine the
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42 conformational preferences of peptides and a key contemporary challenge at the interface of
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44 chemistry and biology is the rational design of specifically-folded peptidomimetic materials.
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46 A fruitful approach has been to examine the behavior of short oligomer sequences assembled
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48 from monomer building blocks which are analogs of Nature's amino acids.¹⁻⁵ In this respect,
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50 peptides which contain β -amino acids are of considerable importance, in part because they
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52 can behave as biologically active peptidomimetics⁶⁻⁸ but also because they have emerged as
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54 self-structuring manifolds for the construction of foldamers.⁹⁻¹³
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3 In the peptidomimetics area, hydrazino peptides have attracted particular attention. These
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5 compounds are obtained when one or more of the α -amino acids in a peptide sequence are
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7 replaced by an α -hydrazino acid. Conceptually, an α -hydrazino acid is an *aza*-analog of a β -
8
9 amino acid, whose β -carbon has been replaced by a nitrogen atom.¹⁴ This strategy for the
10
11 backbone modification of α -peptides has been known for some time,¹⁵ and has provided
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13 peptidomimetic materials showing biologically activity¹⁶⁻¹⁸ or resistance to proteolysis.¹⁹ One
14
15 of the specific features of hydrazino peptides is their capacity to adopt an intramolecular 8-
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17 membered ring hydrogen bond, a conformational preference which is also known for
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19 oligomers of α -aminoxy acids.²⁰⁻²³ On the basis of a number of X-ray crystal structures,²⁴⁻²⁸ it
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21 was suggested that the additional backbone nitrogen in a hydrazino peptide might participate
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23 in a 3-center hydrogen bond, called a hydrazino turn, which stabilizes the 8-membered ring
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25 secondary structure (Figure 1). The significance of the hydrazino turn in solution has been
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27 more difficult to demonstrate, although some linear and cyclic *aza*- β^3 -peptides do appear to
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29 have accessible 8-membered ring conformations.^{19,29-36} Nonetheless, theoretical studies have
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31 not confirmed a decisive role for the 3-center hydrogen bond in determining the folding
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33 properties of hydrazino peptides.³⁷ Only one study of an alternating mixed peptide constructed
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35 from a cyclic β -amino acid (2-aminocyclopentanecarboxylic acid) and its *aza*-analogue has
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37 been carried out, revealing that the adoption of an 8-ring pseudocyclic network was
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39 configuration-dependent and did not appear to involve the 3-center hydrazino turn.³⁸
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42 Further examination of the conformational behavior of building blocks which combine a
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44 cyclic molecular structure with α -hydrazino acid is thus warranted, and these criteria are met
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46 by *N*-aminoazetidinecarboxylic acid (AAzC; Figure 1).^{39,40} This compound is an *aza*-analogue
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48 of 2-aminocyclobutanecarboxylic acid (ACBC; Figure 1) whose *trans* configuration is
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50 reported to stabilize 8-membered hydrogen bond ring structures in short peptides^{41,42} while
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52 the *cis* configuration forms only intra-residue 6-membered hydrogen bonds.⁴³ In this work, we
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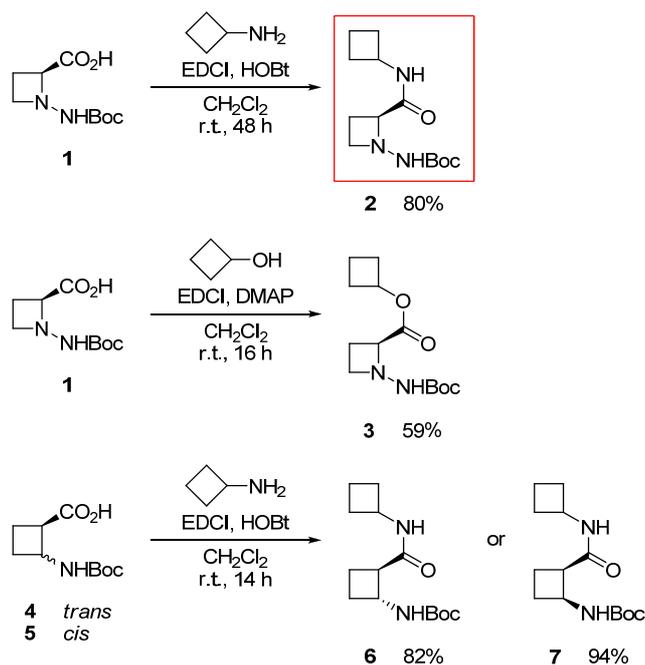
report on the detailed structural analysis of dipeptide derivatives of AAzC, leading to direct solution state evidence for the bifurcated hydrogen bond of a hydrazino turn which stabilizes an 8-membered hydrogen bond ring.

Figure 1. The hydrazinoturn bonding pattern and the molecular structures of AAzC and ACBC.



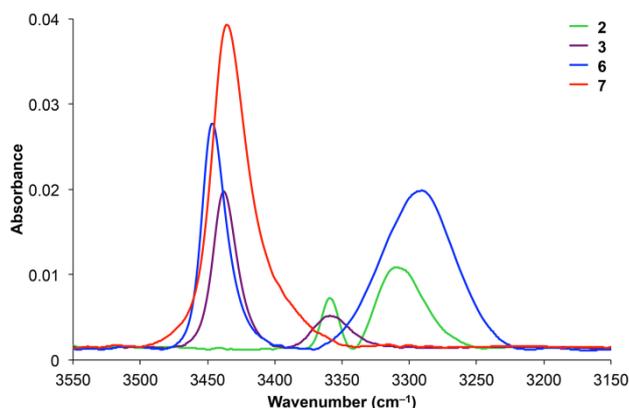
Results and discussion

To begin our studies, a model amide of (*S*)-AAzC was prepared. The Boc-protected hydrazino acid **1**, prepared from 6-azauracil according to the published procedure,³⁹ was condensed with cyclobutylamine to give amide **2**. For comparison purposes, the cyclobutyl ester **3** and the cyclobutylamides of *N*-Boc *trans*- and *cis*-ACBCs, **6** and **7** respectively, were also prepared. The ACBC precursors **4** and **5** were readily available following the published protocols.⁴⁴ The syntheses of compounds **2**, **3**, **6** and **7** are outlined in Scheme 1.

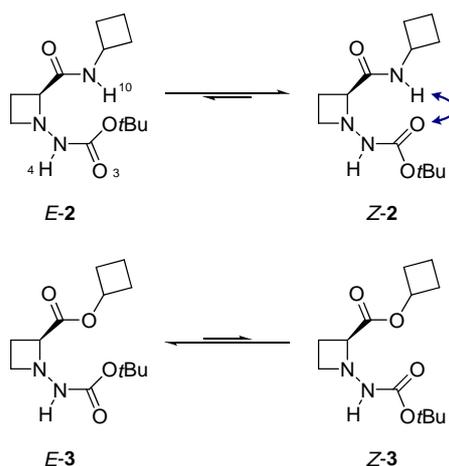
Scheme 1. Preparation of model compound amide **2** and related derivatives **3**, **6** and **7**.

Solution state FTIR absorption spectroscopic analysis of amide **2** was carried out in chloroform at a concentration of 10 mM (Figure 2). The NH stretching frequency domain generally shows a free amide absorption above 3400 cm^{-1} , whereas this band is shifted to lower frequencies (between 3350 and 3250 cm^{-1}) when the NH is involved in a hydrogen bond. The IR absorption spectrum of **2** revealed a stretching band at ca. 3310 cm^{-1} , attributed to the hydrogen bonded amide $\text{NH}_{(10)}$ and a second band at ca. 3360 cm^{-1} , corresponding to the free carbamate $\text{NH}_{(4)}$ with a *Z* geometry.³¹ Although the *Z* conformer is usually less favoured, the formation of an intramolecular hydrogen bond between $\text{C}=\text{O}_{(3)}\cdots\text{H}_{(10)}\text{N}$ stabilizes this conformer over the *E* conformer (Scheme 2). This behaviour contrasts with that of the cyclobutyl ester **3**. The solution state IR absorption spectrum of **3** showed a strong absorption band at ca. 3440 cm^{-1} and a weak band at ca. 3360 cm^{-1} . In this case, no intramolecular hydrogen bond involving $\text{C}=\text{O}_{(3)}$ is possible, so ester **3** exists as a mixture of *E* and *Z* conformers in which the former predominates.

Figure 2. FTIR absorption spectra of compounds 2, 3, 6 and 7 (10 mM solution in CHCl_3).



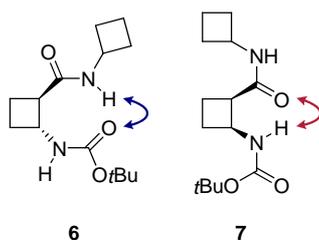
Scheme 2. Equilibria for *E* and *Z* conformers of amide 2 and ester 3.



In comparison, the absorption spectrum of the reference *trans*-ACBC amide **6**, expected to adopt the 8-membered hydrogen bond ring, showed a band at ca. 3445 cm^{-1} corresponding to the carbamate NH and another band at ca. 3290 cm^{-1} assigned to the hydrogen bonded amide NH. In contrast, *cis*-ACBC amide **7** showed a single absorption band at ca. 3435 cm^{-1} assigned to both the free amide NH and the hydrogen bonded carbamate NH. The small red shift (10 cm^{-1}) observed for the carbamate NH suggests that the hydrogen bond interaction is of only moderate strength. These observations are in agreement with the formation of an 8-

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3 membered hydrogen bond ring for **6** and a 6-membered hydrogen bond ring for **7** (Figure 3),
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6 which in turn supports the 8-membered hydrogen bond ring conformer proposed for AAzC
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8 amide **2** in the previous paragraph.
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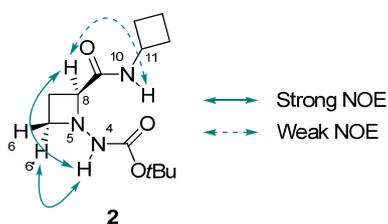
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13 **Figure 3. Structures of reference amides 6 and 7.**



NMR spectroscopic analysis of the AAzC amide **2** was carried out in CDCl₃ solution. Complete attribution of all ¹H and ¹³C signals was achieved by means of 1D selective TOCSY and 2D HSQC experiments. The ¹H NMR spectrum showed a strongly deshielded signal for NH₍₁₀₎ (8.46 ppm) suggesting that this proton is implicated in a strong hydrogen bond. The intramolecular nature of this hydrogen bonding was confirmed by the absence of significant NH chemical shift variation with concentration in the range 5–60 mM. In an isotopic exchange experiment (addition of methanol-*d*₄ to a 60 mM solution in CDCl₃), only the NH₍₄₎ signal moved to lower field and disappeared rapidly (See Supporting Information). These results are in good agreement with an 8-membered hydrogen bond ring conformation. A series of NOESY experiments was performed on amide **2** in order to extract interatomic distances for application in molecular modeling studies. The main correlations are illustrated in Figure 4. A hybrid Monte Carlo molecular mechanics (MCMM) conformational search was carried out on **2** in vacuum using SPARTAN'06 software⁴⁵ and the MMFF94 force field without restraints. Four low-energy conformer families emerged and each was subjected to a DFT geometrical optimization using B3LYP 6-31G** basis sets. After refinement, the four conformer families were still accessible. Each family of conformers was assessed for its

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3 compatibility with the NOESY-derived interatomic distances using the DYNAMO molecular
4 modelling facility in NMR PIPE.⁴⁶ The lowest energy family correlated perfectly with the
5 NMR data, and corresponded to an 8-membered hydrogen bond ring with the cyclobutyl
6 group facing the *t*-butyl group. The minimum energy conformers are shown in Figure 5 and
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8 important structural parameters are presented in Table 1.
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17 **Figure 4. NOE contacts observed for amide 2.**



31 **Figure 5. Lowest energy conformer of amide 2.**

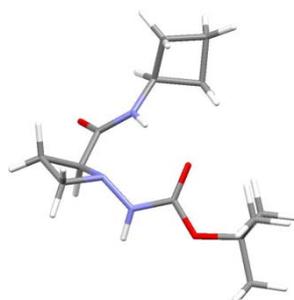
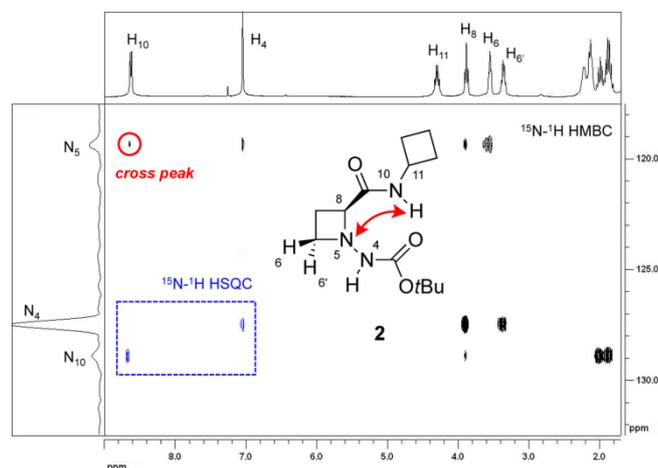


Table 1. Significant calculated parameters for compounds 2 and 12-15 in solution state

	Distances (Å)			H bond				Torsion
	H ₍₄₎ ⁻ H ₍₆₎ [']	H ₍₄₎ ⁻ H ₍₈₎ [']	H ₍₈₎ ⁻ H ₍₁₀₎	Hydrazino turn		10-Membered ring		
				C=O ₍₃₎ ⋯H ₍₁₀₎ N	N ₍₅₎ ⋯H ₍₁₀₎ N	C=O ₍₁₅₎ ⋯H ₍₄₎ N	C=O ₍₁₅₎ ⋯H ₍₁₀₎ N	
2	2.58	2.69	3.36	2.18	2.29			-96.2°
12	2.59	2.67	3.36	2.15	2.29			-96.7°
13a	2.61	2.80	3.34	2.11	2.32			-96.3°
13b	2.56	2.84	3.32	2.09	2.33			-96.7°
13c	3.66	3.53	3.36			2.22	2.46	-91.9°
13d	3.63	3.57	3.35			2.50	2.30	-92.3°
14	2.60	2.66	3.36	2.14	2.26			-96.2°
15a	2.62	2.71	3.37	2.21	2.26			-96.3°
15b	2.59	2.65	3.39	2.27	2.31			-97.1°

The potential implication of nitrogen N₍₅₎ in the stabilization of the 8-membered hydrogen bond ring in compound **2** was evaluated through a newly developed ¹H-¹⁵N heteronuclear NMR experiment. IMPACT HMBC (IMProved and Accelerated Constant-Time Heteronuclear Multiple-Bond Correlation) NMR spectroscopy is a variation of the 2D HMBC technique which provides a high quality 2D spectrum with minimal artifacts and enhanced sensitivity, thus improving the detection of weak interaction such as hydrogen bonds which involve nuclei (such as ¹⁵N) which have low natural abundance.^{47,48} Firstly, unambiguous assignment of each of the three ¹⁵N NMR signals of amide **2** was achieved *via* a ¹H-¹⁵N HSQC experiment. The IMPACT HMBC experiment was then carried out, and showed a correlation peak between N₍₅₎ and H₍₁₀₎ which was interpreted as the result of a hydrogen bond between these two atoms. These studies provide convincing evidence for the formation of a hydrazino turn in solution (Figure 6).

Figure 6. ^1H - ^{15}N IMPACT HMBC NMR for compound **2** in CDCl_3 with a superimposition of a part of the ^1H - ^{15}N HSQC (in blue) allowing the attribution of the different nitrogen atoms at 263 K.

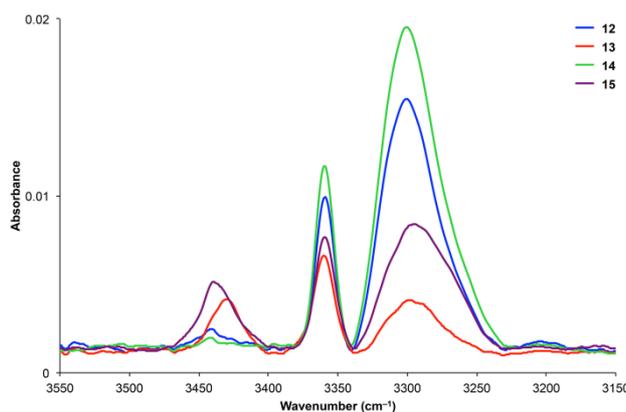
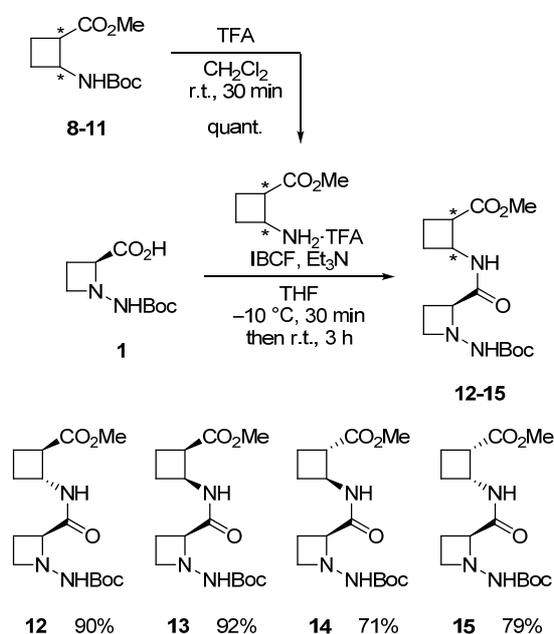


The ensemble of evidence presented above suggests that the AAzC residue presents its own preferred structural pattern based on a hydrazino turn stabilized 8-membered hydrogen bond. To establish the stability of this secondary structural feature, we decided to study dipeptides combining an AAzC unit and each of the stereoisomers of ACBC, which are also known to possess a pronounced local folding potential.

Four dipeptides combining (*S*)-AAzC with a designated stereoisomer of ACBC were prepared. Dipeptides **12-15** were synthesized in good yield by coupling the Boc-hydrazino acid **1** with the methyl ester of each stereoisomer of ACBC (obtained by deprotection of compounds **8-11**, which in turn were obtained by esterification of the appropriate ACBC stereoisomer) as shown in Scheme 3. The four dipeptides **12-15** were fully characterized using the same suite of structural analyses (IR, NMR, molecular modelling) as described above for the AAzC amide **2**.

FTIR analysis of the two dipeptides containing a *trans*-ACBC residue **12** and **14** gave results similar to those obtained for the model amide **2**, *i.e.* a stretching band at ca. 3360 cm^{-1}

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3 assigned to the carbazate NH vibration and a strong band at ca. 3300 cm⁻¹ from the hydrogen
4 bonded amide NH (Figure 7). Thus, these two dipeptides appear to adopt the cyclic 8-
5 membered hydrogen bond conformation. The case of the two *cis* derivatives **13** and **15** is less
6 clear cut, since three absorption bands are present: a small stretching band at ca. 3435 cm⁻¹
7 associated to a free amide NH and/or to the carbazate NH vibration of the *E* conformer, a
8 second absorption band at ca. 3360 cm⁻¹ assigned to the carbazate NH vibration in the *Z*
9 conformation, and a third band at ca. 3300 cm⁻¹ corresponding to the hydrogen bonded amide
10 NH. Thus, dipeptides **13** and **15** exist as a mixture of conformers. While these observations
11 can be in part explained by the presence of a hydrazino turn conformer, they also indicate the
12 presence of a conformation in which the amide NH₍₁₀₎ is non-hydrogen bonded or in which
13 NH₍₄₎ and C=O₍₃₎ are not involved in a hydrogen bond, leaving the carbazate function to adopt
14 its preferred *E* geometry. While this additional conformer is of interest, it was not possible at
15 this stage to identify it unambiguously. If a longer-distance intramolecular non-covalent
16 interaction were implicated, such as a 10-membered ring, the carbazate moiety could be in
17 either a *E* or *Z* geometry but NH₍₄₎ would be hydrogen-bonded, giving an absorption at ca.
18 3300 cm⁻¹, while the non-bonded NH₍₁₀₎ would appear at ca. 3440 cm⁻¹. On the other hand, an
19 extended conformation would imply a free NH₍₄₎ and an “unrestrained” carbazate geometry
20 (*i.e.* *E* > *Z*), as long as a non-bonded NH₍₁₀₎ giving rise to an intense absorption signal at 3440
21 cm⁻¹ and a low absorption at 3360 cm⁻¹.
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Figure 7. FTIR absorption spectra of dipeptides **12-15** (10 mM solution in CHCl₃).Scheme 3. Preparation of dipeptides **12-15**.

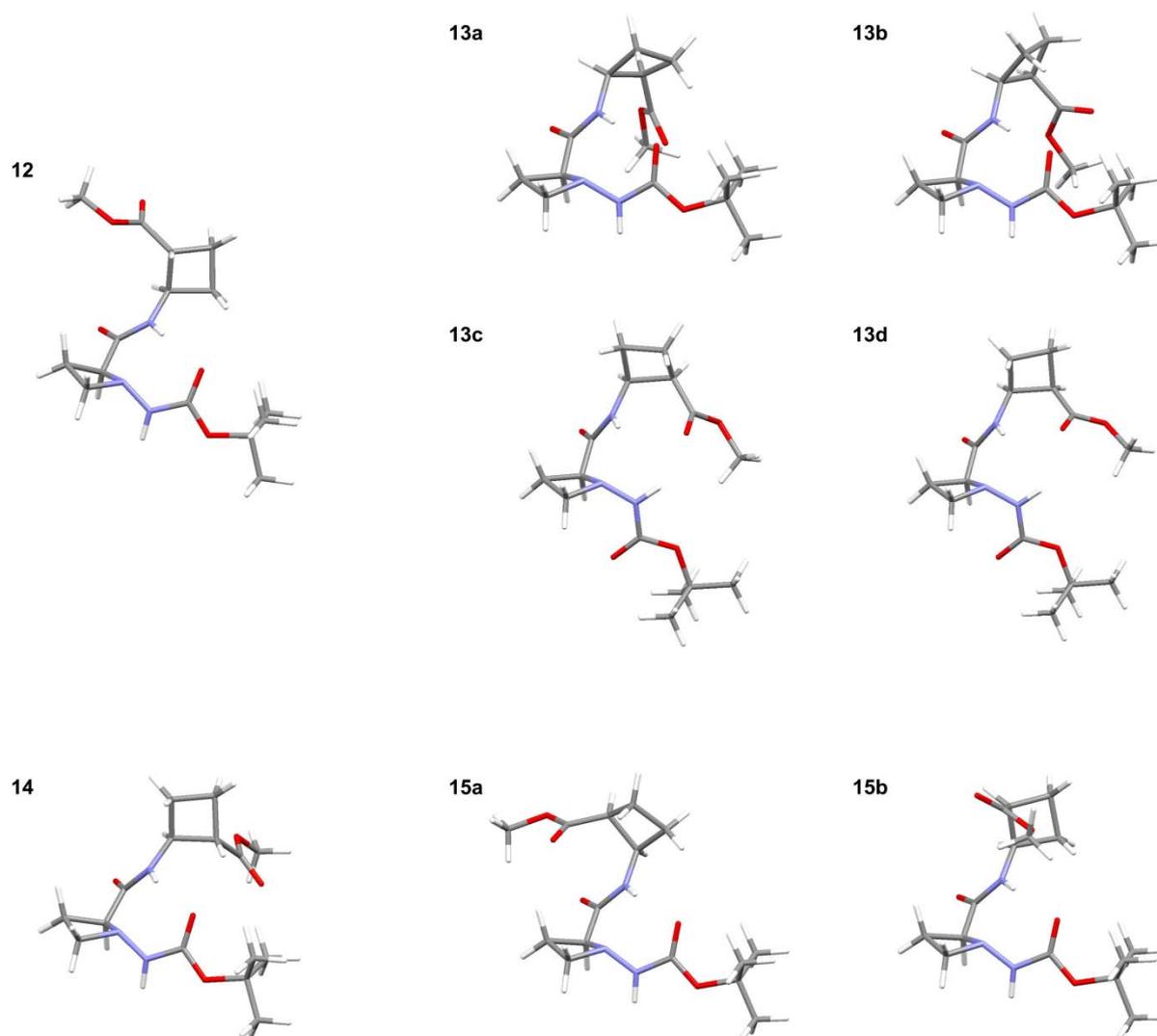
After complete attribution of all ¹H and ¹³C signals, 1D NMR experiments (concentration dependence, isotopic exchange) showed that dipeptides **12-15** behaved in a similar fashion to the model amide **2**: a low-field, non concentration-dependent chemical shift for NH₍₁₀₎ and an isotopic exchange sensitive NH₍₄₎ signal again indicated a cyclic 8-membered hydrogen bond conformer in all four cases.

NOESY experiments on dipeptides **12-15** provided correlation patterns similar to that observed for the model amide **2** and were used to extract interatomic distances (See

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3 Supporting information). A MCMM conformational search followed by DFT geometry
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5 optimization was carried out as described above for amide **2** (Data are collected in Table 1).
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7 For the two dipeptides containing a *trans*-ACBC residue, **12** and **14**, the conformational
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9 search gave respectively two and three low-energy conformer families which remained after
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11 DFT geometry optimization. In both cases, the lowest potential energy corresponded to the
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13 hydrazino turn conformation and fitted perfectly with the NOESY NMR data (Figure 8). The
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15 situation was more complex for the two *cis*-ACBC peptides **13** and **15**. The conformational
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17 search gave nine and eight families of conformers, respectively. Conformational refinement
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19 reduced the number of accessible families of conformers to six and five, respectively. The
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21 large number of conformer families is partly due to “family twins” arising from the two
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23 conformations that a *cis*-ACBC unit can adopt, each with one substituent pseudo-axial and the
24
25 other pseudo-equatorial. For dipeptide **15**, the two hydrazino turn conformations **15a** and **15b**
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27 emerged as the lowest energy families, and appear to be populated equally. In the case of
28
29 dipeptide **13**, the hydrazino turn was relinquished in favor of a 10-membered hydrogen bond
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31 ring in the two lowest energy conformers, **13c** and **13d**, although this observation alone does
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33 not explain the FTIR and 1D NMR isotopic exchange data described above. The four low-
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35 energy conformer families were therefore subjected to an ab initio geometrical optimization at
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37 the B3LYP 6-31G** level of theory in chloroform (implicit method) using GAUSSIAN’09
38
39 software.⁴⁹ The four low energy conformer families - two hydrazino turn families **13a** and
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41 **13b** and two 10-membered hydrogen bond ring families **13c** and **13d** - emerged with very
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43 similar energies suggesting roughly equal populations. This situation best explains the FTIR
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45 results, and is consistent with the NOESY NMR data.
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Figure 8. Lowest energy conformers of compounds 12-15.

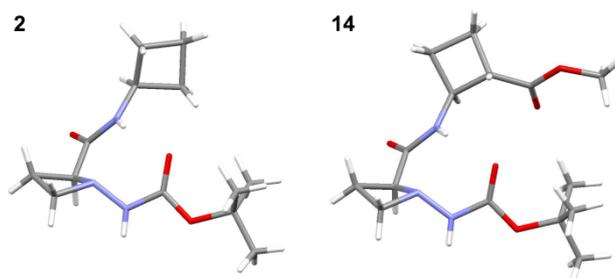


The four dipeptides **12-15** were analysed by the ^1H - ^{15}N IMPACT HMBC NMR experiment in CDCl_3 to search for an interaction between $\text{NH}_{(10)}$ and $\text{N}_{(5)}$. The sensitivity of all ^1H - ^{15}N correlations was enhanced by a factor in the range 2 to 8 compared to the standard ^1H - ^{15}N HMBC experiment, and a specific correlation between $\text{NH}_{(10)}$ and $\text{N}_{(5)}$ was observed in all four cases. These observations provide convincing evidence for the presence of the bifurcated hydrogen bonding system of a hydrazino turn in solution.

We were able to deduce the coupling constants ($^1J_{\text{NH}}$) through the hydrogen bond between $\text{NH}_{(10)}$ and $\text{N}_{(5)}$ then use the values to evaluate the distance between the two atoms. For each

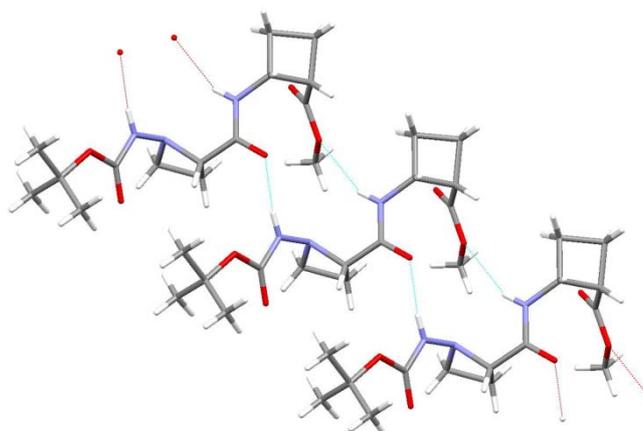
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3 peptide **12-15**, a deconvolution procedure allowed the comparison of a reconstructed ^1H
4 spectrum with the extracted rows from the ^1H - ^{15}N IMPACT HMBC spectra.⁵⁰ The
5 reconstructed spectra which showed the best fit with the extracted spectra provided the value
6 of the $^{\text{h1}}J_{\text{NH}}$ coupling constant. These coupling constants were then applied in an empirical
7 equation which had previously been developed to assess the distance between an amide NH
8 and an imidazole N in proteins.⁵¹ In the event, the $^{\text{h1}}J_{\text{NH}}$ coupling constants for dipeptides **12-**
9 **15** were in the range 2.8 to 3.4 Hz, which corresponds to a $\text{N}_{(5)}\cdots\text{H}_{(10)}$ distance of 2.8-2.9 Å.
10 The same analysis conducted on the model amide **2** provided a $^{\text{h1}}J_{\text{NH}}$ value of 3.2 Hz which
11 corresponds to a $\text{N}_{(5)}\cdots\text{H}_{(10)}$ distance of 2.9 Å. The interatomic distances deduced by this
12 method are slightly higher than those obtained from the molecular modelling studies (Table
13 1), but still constitute a reasonable approximation considering that the equation used is purely
14 empirical.

15
16 While the principle objective of this work was to study AAzC derivatives in solution state, it
17 was instructive to examine and compare the results with solid state data when available.
18 Single crystals of model AAzC amide **2** and dipeptide **14** were amenable to X-ray diffraction
19 analysis and the crystal structure conformations of these compounds are shown in Figure 9.
20 Each compound adopts a conformation very similar to its lowest-energy solution state
21 structure with a hydrazino turn evidently contributing to the 8-membered hydrogen bond ring.
22 In both cases, the AAzC unit adopts a *trans*-like configuration, with a backbone torsion angle
23 of -97.3° for **2** and -93.9° for **14**. This latter value is slightly lower than those calculated from
24 solution state studies (Table 1) which might be the result of packing effects in the solid state.
25 In each case, $\text{H}_{(10)}$ participates in a three-center hydrogen bond with $\text{C}=\text{O}_{(3)}$ and $\text{N}_{(5)}$ for which
26 distances are in the range 2.2-2.3 Å (Table 2).
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Figure 9. X-Ray structures of amide 2 and dipeptide 14.**Table 2. Significant parameters for crystal structure of compounds 2 and 14.**

	Distances (Å)			H bond		Torsion
	H ₍₄₎ -H ₍₆₎	H ₍₄₎ -H ₍₈₎	H ₍₈₎ -H ₍₁₀₎	C=O ₍₃₎ ⋯H ₍₁₀₎ N	N ₍₅₎ ⋯H ₍₁₀₎ N	(N ₍₄₎ -N ₍₅₎) [∧] (C ₍₈₎ -C ₍₉₎)
2	2.51	2.66	3.20	2.22	2.25	-97.3°
14	2.48	2.62	3.22	2.29	2.29	-93.9°

Intriguingly, single crystal analysis of dipeptide **15** showed an extended molecular structure in which no intramolecular interactions were present (Figure 10). The off-set head-to-head alignment of the molecules in the crystal facilitates the formation of a network of intermolecular C=O₍₉₎⋯H₍₄₎N hydrogen bonds. N₍₅₎ is not involved in any non-covalent interaction.

Figure 10. X-Ray structure of dipeptide 15.

Conclusion

This first detailed structural study of AAzC derivatives in solution indicates that the title compound conveniently combines the rigidity of a cyclic molecular skeleton with a hydrazino acid function in order to facilitate the formation of a hydrazino turn conformer in small peptides. NMR data, in particular, provided strong evidence for a non-covalent interaction between the AAzC ring-nitrogen and the amide NH of the adjacent residue, consistent with the tenet that a bifurcated hydrogen bond is a central feature of the cyclic 8-membered hydrazino turn feature. Nonetheless, low energy conformations structured around a cyclic 10-membered ring hydrogen bond also appears accessible, which suggests that AAzC might be able to adapt its local structure to accommodate the demands of neighboring residues. AAzC thus emerges as a potentially useful building block for inclusion in peptides with predictable yet malleable conformational behavior.

Experimental section

All reagents and solvents were of commercial grade and were used without further purification. Dichloromethane was dried over activated alumina and THF was distilled from sodium/benzophenone. Flash chromatography was performed with SDS silica gel (35-70 μm). Analytical thin-layer chromatography was performed with 0.25 mm commercial silica gel plates (EMD, Silica Gel 60F₂₅₄). TLC plates were visualised by UV fluorescence at 254 nm then revealed using a phosphomolybdic acid solution (10% in EtOH) or a ninhydrin solution (0.3% in *n*-BuOH). Retention factors (R_f) are given for such analyses. Routine nuclear magnetic resonance (NMR) data were acquired on a spectrometer operating at 360 MHz for ¹H and at 90 MHz for ¹³C. Chemical shifts (δ) are reported in ppm from tetramethylsilane. Splitting patterns for ¹H NMR signals are designated as: s (singlet), d (doublet), t (triplet), q

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3 (quadruplet), quint (quintuplet), bs (broad singlet) or m (multiplet). Coupling constants (nJ)
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5 are reported in Hz. High resolution mass spectrometry (HRMS) data were recorded using the
6
7 electrospray ionization technique in positive mode (ESI+) with a tandem Q-TOF analyzer.
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9 Samples for Fourier-transform infrared spectroscopy (IR) were prepared as solution in CHCl_3
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11 (10 mM) in a NaCl solution cell. Maximum absorbances (ν) are given for significant bands in
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13 cm^{-1} . Melting points were obtained in open capillary tubes and are uncorrected. Optical
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15 rotations were measured using a 10 cm quartz cell. Values for $[\alpha]_D^T$ were obtained with the D-
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17 line of sodium at the indicated temperature T, using solutions of concentration (c) in units of
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19 $\text{g}\cdot 100\text{ mL}^{-1}$.
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25 **General procedure for the preparation of cyclobutylamides derivatives.** To an ice cold
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27 solution of Boc-hydrazino acid **1** or Boc-amino acid **4** or **5** (1.0 mmol, 1 eq.) in dry CH_2Cl_2
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29 (20 mL) were added 1-hydroxybenzotriazole monohydrate (189.2 mg, 1.4 mmol, 1.4 eq.) and
30
31 cyclobutylamine (94 μL , 1.1 mmol, 1.1 eq.). The mixture was stirred for 10 min at 0 °C then
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33 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (287.6 mg, 1.5 mmol, 1.5 eq.)
34
35 was added. The resulting mixture was then stirred for 14 h at room temperature, then washed
36
37 successively with 1 M aqueous KHSO_4 (10 mL) and saturated NaHCO_3 (10 mL). The organic
38
39 layer was dried over MgSO_4 , filtered and evaporated under reduced pressure. Flash
40
41 chromatography ($\text{CH}_2\text{Cl}_2/\text{EtOAc}$) gave the cyclobutylamide derivative **2**, **6** or **7**.
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46 **(S)-N-Cyclobutyl-1-(t-butyloxycarbonylamino)azetidione-2-carboxamide (2).** Coupling of
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48 Boc-(S)-AAzC **1** with cyclobutylamine according to the general procedure followed by flash
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50 chromatography ($\text{CH}_2\text{Cl}_2/\text{AcOEt} = 50/50$) gave the cyclobutylamide (–)-**2** (216.2 mg, 80%) as
51
52 a white solid. Mp 190.5-191.1 °C (dec.); R_f 0.22 ($\text{CH}_2\text{Cl}_2/\text{AcOEt} = 50/50$); $[\alpha]_D^{24} -53$ (c 0.50,
53
54 CHCl_3); IR (CHCl_3) ν 3359, 3310, 3020, 3006, 2980, 2940, 2896, 2873, 1714, 1652, 1544,
55
56 1497; $^1\text{H NMR}$ (360 MHz, CDCl_3) δ 1.43 (s, 9H), 1.58-1.78 (m, 2H), 1.86-2.10 (m, 3H),
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58 2.13-2.38 (m, 3H), 3.32 (pseudo dd, 1H, $^2J = 15.7$ Hz, $^3J = 8.8$ Hz), 3.61-3.68 (m, 1H), 3.88
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(pseudo t, 1H, $^3J = 8.8$ Hz), 4.30-4.43 (m, 1H), 6.15 (bs, 1H), 8.46 (bs, 1H); ^{13}C NMR (90 MHz, CDCl_3) δ 15.3, 19.8, 28.3, 30.7, 31.0, 44.2, 54.7, 72.2, 81.1, 155.6, 170.4; HRMS (ESI+) Calcd for $\text{C}_{13}\text{H}_{23}\text{N}_3\text{NaO}_3$ $[\text{M}+\text{Na}]^+$: 292.1632, found 292.1622.

***N*-Cyclobutyl-(1*R*,2*R*)-2-(*t*-butyloxycarbonylamino)cyclobutanecarboxamide (6).**

Coupling of Boc-(1*R*,2*R*)-ACBC **4** with cyclobutylamine according to the general procedure followed by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{AcOEt} = 70/30$) gave the cyclobutylamide (–)-**6** (219.9 mg, 82%) as a white solid. Mp 225-226 °C (dec.); R_f 0.26 ($\text{CH}_2\text{Cl}_2/\text{AcOEt} = 70/30$); $[\alpha]_D^{23} -9$ (c 0.50, CHCl_3); IR (CHCl_3) ν 3446, 3290, 3019, 2981, 2938, 2897, 2874, 1694, 1644, 1554, 1501; ^1H NMR (360 MHz, CDCl_3) δ 1.46 (s, 9H), 1.60-1.79 (m, 3H), 1.80-2.03 (m, 3H), 2.03-2.22 (m, 2H), 2.22-2.39 (m, 2H), 2.85 (pseudo dd, 1H, $^2J = 18.2$ Hz, $^3J = 9.2$ Hz), 4.05-4.17 (m, 1H), 4.28-4.44 (m, 1H), 4.93 (bs, 1H), 8.17 (bs, 1H); ^{13}C NMR (90 MHz, CDCl_3) δ 15.4, 18.7, 24.9, 28.5, 30.9, 31.2, 44.7, 48.8, 50.4, 80.6, 156.4, 172.0; HRMS (ESI+) Calcd for $\text{C}_{14}\text{H}_{24}\text{N}_2\text{NaO}_3$ $[\text{M}+\text{Na}]^+$: 291.1679, found 291.1667.

***N*-Cyclobutyl-(1*R*,2*S*)-2-(*t*-butyloxycarbonylamino)cyclobutanecarboxamide (7).**

Coupling of (1*R*,2*S*)-ACBC **5** with cyclobutylamine according to the general procedure followed by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{AcOEt} = 80/20$) gave the cyclobutylamide (–)-**7** (253.5 mg, 94%) as a white solid. Mp 174-175 °C (dec.); R_f 0.50 ($\text{CH}_2\text{Cl}_2/\text{AcOEt} = 70/30$); $[\alpha]_D^{24} -108$ (c 0.50, CHCl_3); IR (CHCl_3) ν 3436, 3021, 2980, 2951, 2897, 1699, 1657, 1502; ^1H NMR (360 MHz, CDCl_3) δ 1.40 (s, 9H), 1.63-1.74 (m, 2H), 1.75-1.91 (m, 3H), 2.01-2.11 (m, 1H), 2.13-2.37 (m, 4H), 3.11 (pseudo dt, 1H, $^3J = 8.2$ Hz, $^3J = 2.6$ Hz), 3.29-3.42 (m, 2H), 5.29 (bs, 1H), 5.73 (bs, 1H); ^{13}C NMR (90 MHz, CDCl_3) δ 15.3, 18.3, 25.5, 29.2, 31.2, 31.4, 44.9, 46.5, 79.4, 155.4, 171.9; HRMS (ESI+) Calcd for $\text{C}_{14}\text{H}_{24}\text{N}_2\text{NaO}_3$ $[\text{M}+\text{Na}]^+$: 291.1679, found 291.1674.

Cyclobutyl (*S*)-1-(*t*-butyloxycarbonylamino)azetidine-2-carboxylate (3). To a solution of Boc-(*S*)-AAzC **1** (46.5 mg, 0.22 mmol, 1 eq.) in CH_2Cl_2 (2.5 mL) were added successively

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3 DMAP (39.4 mg, 0.32 mmol, 1.5 eq.) and cyclobutanol (19 μ L, 0.24 mmol, 1.1 eq.). The
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5 mixture was then cooled to 0 $^{\circ}$ C, and EDCI·HCl (61.8 mg, 0.32 mmol, 1.1 eq.) was added.
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7 The solution was stirred for 5 min at 0 $^{\circ}$ C then 15 h at room temperature, at which point the
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9 solvent was evaporated under reduced pressure. Flash chromatography (petroleum
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11 ether/EtOAc = 90/10) gave the methyl ester (–)-**3** (34.3 mg, 59%) as a colorless oil. R_f 0.25
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13 (petroleum ether/EtOAc = 80/20); $[\alpha]_D^{20}$ –110 (c 0.50, CHCl₃); IR (CHCl₃) ν 3438, 3359,
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15 3019, 2984, 2891, 1728, 1717, 1477, 1467; ¹H NMR (360 MHz, CDCl₃) δ 1.42 (s, 9H), 1.51-
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17 1.66 (m, 1H), 1.70-1.83 (m, 1H), 1.92-2.15 (m, 3H), 2.16-2.38 (m, 3H), 3.54 (ddd, 1H, ² J =
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19 14.1 Hz, ³ J = 5.9 Hz, ³ J = 4.5 Hz), 3.99 (pseudo dd, 1H, ² J = 14.1 Hz, ³ J = 7.7 Hz), 4.72
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21 (pseudo t, 1H, ³ J = 7.5 Hz), 5.01 (pseudo quint., 1H, ³ J = 7.6 Hz), 6.36 (bs, 1H); ¹³C NMR (90
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23 MHz, CDCl₃) δ 13.5, 18.6, 28.4, 30.3, 53.8, 66.4, 69.2, 80.4, 154.8, 171.8; HRMS (ESI+)
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25 Calcd for C₁₃H₂₂N₂NaO₄ [M+Na]⁺: 293.1472, found 293.1457.
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32 **General procedure for the preparation of methyl esters.** To a solution of Boc-amino acid **4**
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34 or **5** (215.3 mg, 1.0 mmol, 1 eq.) in CH₂Cl₂ (11 mL) were added successively DMAP (12.2
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36 mg, 0.1 mmol, 0.1 eq.) and MeOH (0.13 mL, 13 mmol, 13 eq.). The mixture was then cooled
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38 to 0 $^{\circ}$ C, and EDCI·HCl (210.9 mg, 1.1 mmol, 1.1 eq.) was added. The solution was stirred for
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40 5 min at 0 $^{\circ}$ C then 15 h at room temperature, at which point the solvent was evaporated under
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42 reduced pressure. Flash chromatography (petroleum ether/EtOAc = 90/10) gave the methyl
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44 ester **8-11**.
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48 **Methyl (1R,2R)-2-(*t*-butyloxycarbonylamino)cyclobutanecarboxylate (8).** Coupling of
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50 Boc-(1R,2R)-ACBC **4** with methanol according to the general procedure followed by flash
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52 chromatography (Petroleum Ether/AcOEt = 90/10) gave the methyl ester (–)-**8** (192.6 mg,
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54 84%) as a white solid. R_f 0.32 (Petroleum Ether/AcOEt = 80/20); $[\alpha]_D^{19}$ –53 (c 1.08, CHCl₃);
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56 ¹H NMR (360 MHz, CDCl₃) δ 1.37 (s, 9H), 1.78-1.96 (m, 3H), 2.11-2.25 (m, 1H), 2.87-3.06
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(m, 1H), 3.63 (s, 3H), 4.08-4.24 (m, 1H), 5.02 (bs, 1H); ^{13}C NMR (90 MHz, CDCl_3) δ 18.2, 27.3, 28.4, 46.8, 48.9, 51.7, 79.5, 154.7, 173.5.

Methyl (1R,2S)-2-(*t*-butyloxycarbonylamino)cyclobutanecarboxylate (9). Coupling of Boc-(1R,2S)-ACBC **5** with methanol according to the general procedure followed by flash chromatography (Petroleum Ether/AcOEt = 90/10) gave the methyl ester (–)-**9** (174.4 mg, 79%) as a white solid. R_f 0.44 (Petroleum Ether/AcOEt = 80/20); $[\alpha]_D^{20}$ –130 (c 1.09, CHCl_3); ^1H NMR (360 MHz, CDCl_3) δ 1.38 (s, 9H), 1.84-2.03 (m, 2H), 2.11-2.37 (m, 2H), 3.29-3.40 (m, 1H), 3.66 (s, 3H), 4.43-4.49 (m, 1H), 5.33 (bs, 1H); ^{13}C NMR (90 MHz, CDCl_3) δ 18.5, 28.4, 29.5, 45.3, 45.9, 51.7, 79.4, 154.8, 174.7.

Methyl (1S,2S)-2-(*t*-butyloxycarbonylamino)cyclobutanecarboxylate (10). Coupling of Boc-(1S,2S)-ACBC *ent*-**4** with methanol according to the general procedure followed by flash chromatography (Petroleum Ether/AcOEt = 90/10) gave the methyl ester (+)-**10** (190.3 mg, 83%) as a white solid. R_f 0.32 (Petroleum Ether/AcOEt = 80/20); $[\alpha]_D^{18}$ +58 (c 1.08, CHCl_3); ^1H NMR (360 MHz, CDCl_3) δ 1.38 (s, 9H), 1.41 (s, 9H), 1.81-2.00 (m, 3H), 2.17-2.28 (m, 1H), 2.89-3.05 (m, 1H), 3.67 (s, 3H), 4.13-4.26 (m, 1H), 4.84 (bs, 1H); ^{13}C NMR (90 MHz, CDCl_3) δ 18.3, 27.4, 28.4, 47.0, 49.0, 51.8, 79.7, 154.8, 173.5.

Methyl (1S,2R)-2-(*t*-butyloxycarbonylamino)cyclobutanecarboxylate (11). Coupling of Boc-(1S,2R)-ACBC *ent*-**5** with methanol according to the general procedure followed by flash chromatography (Petroleum Ether/AcOEt = 90/10) gave the methyl ester (+)-**11** (167.4 mg, 73%) as a white solid. R_f 0.44 (Petroleum Ether/AcOEt = 80/20); $[\alpha]_D^{20}$ +124 (c 1.09, CHCl_3); ^1H NMR (360 MHz, CDCl_3) δ 1.37 (s, 9H), 1.83-2.02 (m, 2H), 2.10-2.38 (m, 2H), 3.28-3.40 (m, 1H), 3.65 (s, 3H), 4.32-4.49 (m, 1H), 5.34 (bs, 1H); ^{13}C NMR (90 MHz, CDCl_3) δ 18.5, 28.4, 29.5, 45.3, 45.9, 51.7, 79.4, 154.8, 174.7.

General procedure for the preparation of dipeptides. TFA (0.69 mL, 9 mmol, 30 eq.) was added dropwise to a solution of Boc-amino ester **8-11** (68.8 mg, 0.3 mmol, 1 eq.) in CH_2Cl_2

(2.1 mL). The mixture was stirred for 30 min at room temperature, then the solvent and the excess TFA were evaporated under reduced pressure to give an oil (c. 110 mg). The resulting TFA salt **TFA·H₂N-ACBC-OMe** was used directly in the next reaction.

To a cold (−10 °C) solution of Boc-(*S*)-AAzC **1** (64.9 mg, 0.3 mmol, 1 eq.) in dry THF (0.3 mL) were added successively Et₃N (132 μL, 0.95 mmol, 3.2 eq.) and IBCF (39 μL, 0.3 mmol, 1 eq.). The solution was stirred for 5 min at −10 °C, then a solution of **TFA·H₂N-ACBC-OMe** in dry THF (0.3 mL) was added. Residual salts were taken up by rinsing with dry THF (0.3 mL) and added to the cold reaction mixture. The resulting mixture was stirred for 30 min at −10 °C and then for 3 h at room temperature, at which point the solution was filtered and evaporated under reduced pressure. Flash chromatography (CH₂Cl₂/EtOAc = 50/50) of the residue gave the dipeptide **12-15**.

(1*R*,2*R*)-Methyl 2-((*S*)-1-(*t*-butyloxycarbonylamino)azetidine-2-carboxamido)cyclobutane carboxylate (12). Coupling of Boc-(*S*)-AAzC **1** with **TFA·H₂N-(1*R*,2*R*)-*t*-ACBC-OMe** according to the general procedure followed by flash chromatography (CH₂Cl₂/AcOEt = 50/50) gave the dipeptide (−)-**12** (87.9 mg, 90%) as a white solid. Mp 110 °C; *R_f* 0.16 (CH₂Cl₂/AcOEt = 50/50); [α]_D²⁶ −82 (c 0.50, CHCl₃); IR (CHCl₃) ν 3359, 3301, 3019, 2982, 2870, 1724, 1661, 1544, 1498; ¹H NMR (360 MHz, CDCl₃) δ 1.40 (s, 9H), 1.82-2.06 (m, 4H), 2.11-2.28 (m, 2H), 3.09-3.20 (m, 1H), 3.34 (pseudo dd, 1H, ²*J* = 15.9 Hz, ³*J* = 8.5 Hz), 3.56-3.72 (m, 1H), 3.64 (s, 3H), 3.86 (pseudo t, 1H, ³*J* = 8.8 Hz), 4.51 (pseudo quint, 1H, ³*J* = 8.3 Hz), 6.32 (bs, 1H), 8.67 (bs, 1H); ¹³C NMR (90 MHz, CDCl₃) δ 18.7, 19.8, 26.8, 28.3, 46.4, 46.7, 51.8, 54.5, 72.0, 81.0, 155.7, 170.7, 173.5; HRMS (ESI+) Calcd for C₁₅H₂₅N₃NaO₅ [M+Na]⁺: 350.1686, found 350.1685.

(1*R*,2*S*)-Methyl 2-((*S*)-1-(*t*-butyloxycarbonylamino)azetidine-2-carboxamido)cyclobutane carboxylate (13). Coupling of Boc-(*S*)-AAzC **1** with **TFA·H₂N-(1*R*,2*S*)-*c*-ACBC-OMe** according to the general procedure followed by flash chromatography

(CH₂Cl₂/AcOEt = 50/50) gave the dipeptide (-)-**13** (90.4 mg, 92%) as a white solid. Mp 153 °C; *R_f* 0.15 (CH₂Cl₂/AcOEt = 50/50); [α]_D²⁵ -166 (*c* 0.50, CHCl₃); IR (CHCl₃) ν 3430, 3360, 3299, 3012, 2979, 2894, 1723, 1661, 1527, 1501; ¹H NMR (360 MHz, CDCl₃) δ 1.43 (s, 9H), 1.82-2.01 (m, 2H), 2.11-2.40 (m, 4H), 3.37 (pseudo dd, 1H, ³*J* = 11.7 Hz, ³*J* = 8.2 Hz), 3.51 (pseudo dd, 1H, ²*J* = 15.6 Hz, ³*J* = 8.6 Hz), 3.56-3.64 (m, 1H), 3.58 (s, 3H), 4.07 (pseudo t, 1H, ³*J* = 8.8 Hz), 4.62 (pseudo quint, 1H, ³*J* = 8.2 Hz), 6.22 (bs, 1H), 8.30 (bs, 1H); ¹³C NMR (90 MHz, CDCl₃) δ 19.2, 19.7, 27.7, 28.4, 44.6, 45.3, 51.5, 54.3, 70.4, 81.0, 155.3, 171.5, 173.4; HRMS (ESI+) Calcd for C₁₅H₂₅N₃NaO₅ [M+Na]⁺: 350.1686, found 350.1679.

(1*S*,2*S*)-Methyl 2-((*S*)-1-(*t*-butyloxycarbonylamino)azetidine-2-carboxamido)cyclobutane carboxylate (14**).** Coupling of Boc-(*S*)-AAzC **1** with TFA·H₂N-(1*S*,2*S*)-*t*-ACBC-OMe according to the general procedure followed by flash chromatography (CH₂Cl₂/AcOEt = 50/50) gave the dipeptide (+)-**14** (69.7 mg, 71%) as a white solid. Mp 120 °C; *R_f* 0.17 (CH₂Cl₂/AcOEt = 50/50); [α]_D²⁴ +4 (*c* 0.50, CHCl₃); IR (CHCl₃) ν 3360, 3301, 3020, 2981, 2955, 2934, 2871, 1725, 1661, 1542, 1497; ¹H NMR (360 MHz, CDCl₃) δ 1.36 (s, 9H), 1.81-2.02 (m, 4H), 2.11-2.27 (m, 2H), 2.98-3.08 (m, 1H), 3.35 (pseudo dd, 1H, ²*J* = 15.7 Hz, ³*J* = 8.8 Hz), 3.55 (s, 3H), 3.53-3.62 (m, 1H), 3.88 (pseudo t, 1H, ³*J* = 8.8 Hz), 4.47 (pseudo quint, 1H, ³*J* = 8.1 Hz), 6.68 (bs, 1H), 8.71 (bs, 1H); ¹³C NMR (90 MHz, CDCl₃) δ 18.8, 19.6, 27.1, 28.2, 46.0, 46.6, 51.6, 54.3, 71.7, 80.8, 155.8, 170.9, 173.5; HRMS (ESI+) Calcd for C₁₅H₂₅N₃NaO₅ [M+Na]⁺: 350.1686, found 350.1694.

(1*S*,2*R*)-Methyl 2-((*S*)-1-(*t*-butyloxycarbonylamino)azetidine-2-carboxamido)cyclobutane carboxylate (15**).** Coupling of Boc-(*S*)-AAzC **1** with TFA·H₂N-(1*S*,2*R*)-*c*-ACBC-OMe according to the general procedure followed by flash chromatography (CH₂Cl₂/AcOEt = 50/50) gave the dipeptide (+)-**15** (77.7 mg, 79%) as a white solid. Mp 109 °C; *R_f* 0.19 (CH₂Cl₂/AcOEt = 50/50); [α]_D²⁶ +40 (*c* 0.50, CHCl₃); IR (CHCl₃) ν 3439, 3359, 3295, 3019, 2979, 2955, 2895, 2871, 1726, 1660, 1531, 1496; ¹H NMR (360 MHz, CDCl₃) δ

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2
3 1.39 (s, 9H), 1.76-2.00 (m, 2H), 2.13-2.41 (m, 4H), 3.34-3.45 (m, 2H), 3.55-3.64 (m, 1H),
4
5 3.72 (s, 3H), 3.94 (pseudo t, 1H, $^3J = 8.7$ Hz), 4.68 (pseudo quint, 1H, $^3J = 8.3$ Hz), 6.15 (bs,
6
7 1H), 8.59 (bs, 1H); ^{13}C NMR (90 MHz, CDCl_3) δ 18.7, 19.9, 27.7, 28.3, 44.6, 45.3, 51.7,
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9 54.5, 71.5, 80.7, 155.1, 171.1, 173.3; HRMS (ESI+) Calcd for $\text{C}_{15}\text{H}_{25}\text{N}_3\text{NaO}_5$ $[\text{M}+\text{Na}]^+$:
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11 350.1686, found 350.1690.
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28 Supporting Information

29
30 General information, copies of ^1H and ^{13}C NMR spectra for all new compounds, ^1H - ^{15}N
31
32 IMPACT HMBC NMR spectra and X-ray diffraction data for compounds **2**, **14** and **15** in cif
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34 format. This material is available free of charge via the Internet at <http://pubs.acs.org>.
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