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Adamantyl Amino Acid as γ-Turn Inducer for Peptide

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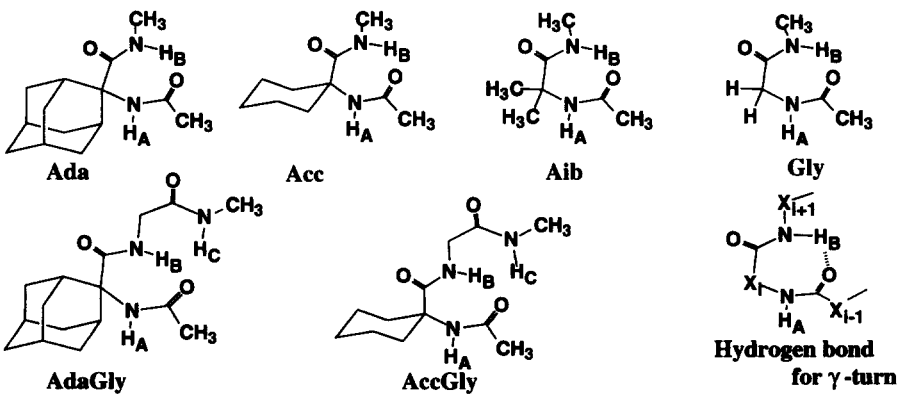
Abstract *; The structures of six peptide mimics having different bulkiness and/or rigidity of the amino acids were investigated spectroscopically. Comparison of ^{1}H NMR, IR spectra and H-D exchange rate of the amide protons reveals that 2-amino-2-carboxyadamantane induces the high population of γ-turn conformation in the room temperature region and may be utilized as a promising γ-turn inducer for synthetic peptides.* © 1997 Elsevier Science Ltd.

Syntheses of artificial proteins are now one of the most interesting and promising areas in chemistry for the construction of bioactive and/or biomimetic molecules.[1] The typical structural motifs of natural proteins such as α-helix, β-sheet and β-turn provide clear bases for the design of synthetic proteins. Among these structural motifs of proteins, the β-turns having the ten membered ring structure are the important motif for folding proteins to produce their compact structures and their structural mimics have been widely investigated. Recently, the γ-turn consisted of a seven membered ring attracts significant attentions as another turn element which may give a more compact and rigid structure of the protein compared with the β-turn.[2] In this paper we report unique characteristics of 2-amino-2-carboxyadamantane[3] which dominantly induce a γ-turn in the peptide chain.

In order to compare the structural effects of 2-amino-2-carboxyadamantane with those of other amino acids, we prepared following six model peptides. [4] In this series, we choose the four different amino acids which



are expected to have different bulkiness at the α-position and structural rigidity. First we examined ^{1}H NMR spectra of the AcNH-X-CONHMe type of diamide compounds, **Ada**, **Acc**, **Aib** and **Gly**. The empirical order of bulkiness is of the order of **Ada > Acc > Aib > Gly**. The spectra exhibiting the signals for the two amide protons, H_A and H_B, of these diamides are shown in Figure 1a - d. [5] These spectra demonstrate a clear

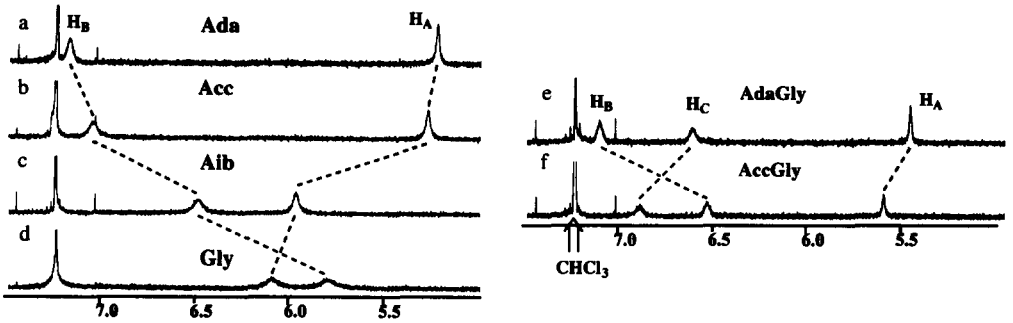


Figure 1. The 500 MHz ^1H NMR spectra of the peptide mimics (2 mM) in $CDCl_3$ at 323 K. The dotted lines indicate correlations of each proton, H_A, H_B and H_C.

tendency for large downfield shifts of H_B with increasing bulkiness of the amino acids. The observation suggests that the rigidity and/or bulkiness of the amino acids enhances stereochemistry to form a hydrogen bond of H_B in $CHCl_3$. Furthermore, H_A and H_B protons of **Ada** show quite contrast chemical shift changes on addition of methanol, i.e., by changing the solvent from $CDCl_3$ to the 1 : 1 mixture of $CDCl_3$ and CD_3OD, the chemical shift of H_A changes from δ 5.217 to δ 7.015 and, in contrast, H_B from δ 7.215 to δ 7.177. This characteristic behavior is consistent with the intramolecular hydrogen bond formation of H_B and the intermolecular hydrogen bond formation of H_A with methanol. The IR spectra shown in Figure 2a - c also support these conclusions. Based on the peak assignments for **Aib** reported by Aubry at al.,[6] the spectra of **Acc** and **Ada** are unambiguously analyzed as summarized in Table 1. Noteworthy is the total disappearance of the free NH_B stretching absorption for **Ada**, which is in contrast to the strong or significant absorptions of the corresponding NH_B for **Aib** or **Acc** observed in the 3460 cm^{-1} region. The results strongly indicate that the adamantyl moiety has the clear ability to enforce the γ-turn conformation of the peptide through hydrogen bond

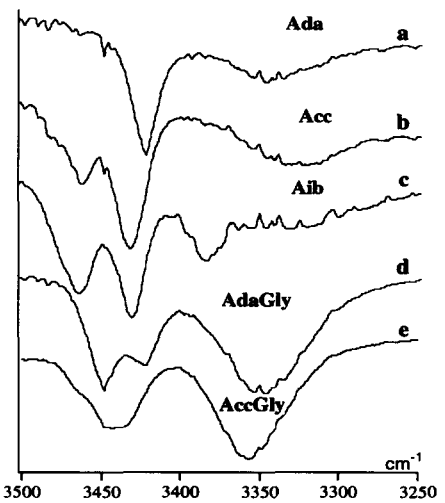


Figure 2. Infra-red spectra of the peptide mimics in CH_2Cl_2 at 293 K. The assignments for the absorptions are given in Table 1.

Table 1 The NH region of IR spectra of synthetic peptides.[a]

peptides	Ada	Acc	Aib [c]	AdaGly	AccGly
			cm^{-1}		
free H_A	3421	3430	3429 [d]	3423	3437
free H_B	n.o.[b]	3461	3464	n.o.[b]	3462 [e]
free H_C	____	____	____	3448	3448
hydrogen bonded NH	~ 3340	~ 3330	~ 3330	~ 3350	~ 3350

[a] At 293 K, in CH_2Cl_2. [b] Not observed. [c] The assignments are taken from ref. 6. [d] Another N-HA vibration assigned to that of hydrogen bonded HA with the carbonyl oxygen of the amino acid itself is observed at 3384 cm^{-1}, see ref. 6. [e] The value is obtained by computational peak separation of the shoulder peak shown in Figure 2e.

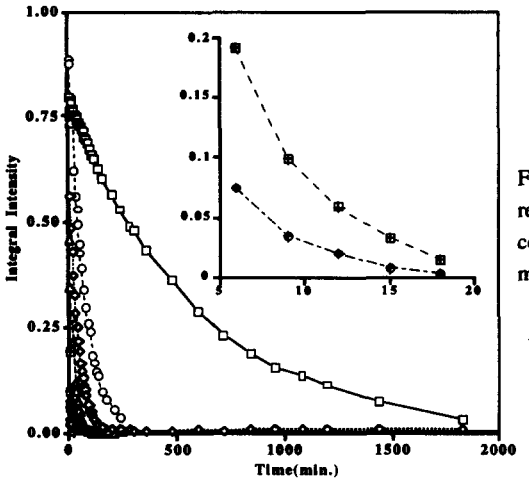


Figure 3. The time courses of the H - D exchange reaction of the peptide mimics. The data were collected by ^{1}H NMR integration in the 1 : 1 mixture of CD_3Cl and CD_3OD at 298 K.
—□— H_B / **Ada**, —◇— H_A / **Ada**,
—○— H_B / **Acc**, —△— H_A / **Acc**,
—⊞— H_B / **Aib**, —⊕— H_A / **Aib**

formation of H_B. The existence ratio of γ-turn conformation is increasing again with the increasing bulkiness of the amino acids, **Ada** > **Acc** > **Aib**, judging from the relative intensities of the NH stretching absorptions for free H_A and free H_B.

The rates of proton-deuterium exchange for H_A and H_B observed in the 1 : 1 mixture of $CDCl_3$ and CD_3OD give further insight into stability of these hydrogen bonds. The time courses of ^{1}H NMR integration intensities for these protons in **Ada**, **Acc** and **Aib** are summarized in Figure 3, where all the exchange reactions follow the clear first-order rate law for the substrates. The rate constants for H_B of **Ada** and **Acc** are determined to be 1.7×10^{-3} and 14×10^{-3} s^{-1}, respectively, and the other rate constants are much faster than the latter. [7] The remarkably small rate constant for H_B of **Ada** means that the hydrogen bond involving this proton is stable over the order of several hours. All of the data indicate that the adamantyl moiety dominantly induces the γ-turn conformation of **Ada** which is both statistically and dynamically stable. The computational grid search analyses of **Ada** and **Acc** also suggest that the most stable conformation of **Ada** contains γ-turn conformation which is 0.8 kcal/mol more stable than the most stable $\alpha/3_{10}$ helix type conformer, while the most stable conformer of **Acc** is shown to be $\alpha/3_{10}$ helix type which is 0.4 kcal/mole more stable than γ-turn conformer. [8] The latter conclusion shows general agreement with the results reported for **Acc** previously. [9] Based on these observations, we further examined ^{1}H NMR and IR spectra of dipeptides, **AdaGly** and **AccGly**. Since these dipeptides have two NH protons which are available for β-turn (H_C) and γ-turn (H_B) as shown in Figure 1, the data are expected to provide the direct information on the stabilities of these two different kinds of turns in these dipeptides. The ^{1}H NMR data shown in Figure 1e and f reveal the largest downfield shifts of H_B of **AdaGly** and H_C of **AccGly** suggesting dominant existence of γ-turn in the former and β-turn in the latter at 323 K. The situation for **AdaGly**, however, is not so simple, because of the strong temperature dependent character of the chemical shift of H_C in **AdaGly**. On lowering temperature, the chemical shifts of H_C and H_B become identical (δ 7.008) at 263 K and finally the H_C proton shows the largest downfield shift to appear at δ 7.268 at 223 K, while the H_B proton is observed at δ 6.938 at this temperature. The observations suggest that the perticipation of the hydrogen bond of H_C in **AdaGly** becomes much more significant in the low temperature region, where the hydrogen bond of H_B also remains. In contrast, the H_C proton of **AccGly** always shows the largest downfield shift in the present temperature range from 223 K to 323 K. The IR data shown in Figure 2d - e and Table 1 again show the dominant role of the hydrogen bonded H_B of **AdaGly** at

room temperature, i.e., the free NH_B stretching absorption which is expected to appear in 3460 cm^{-1} region seems to be negligibly weak and the significant amount of the H_C proton still remains free to show the strong absorption at 3448 cm^{-1}. The situation is quite different for **AccGly** where the free NH_B stretching absorption is clearly observed at 3460 cm^{-1} as a shoulder peak. Thus, it is concluded that the adamantyl amino acid induced the high population of γ-turn conformation in the room temperature region and may be utilized as a promising γ-turn inducer for synthetic peptides.[10] However, it should be stated in fairness that the general methods for peptide syntheses could not be applicable for 2-amino-2-carboxyadamantane due to its unusually low reactivity. Although the present model peptides are prepared via the oxazolone intermediate,[11] it is clear that the more general methods should be developed for the more general utilization of this amino acid.

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(4) Experimental details for the preparation of these peptides are given in the supporting information.

(5) The peak assignments for H_A and H_B protons were carried out by using data of COSY or decoupling experiments.

(6) Aubry, A.; Protas, J.; Boussard, G.; Marraud, M. *Biopolymers*, **1978**, *17*, 1693.

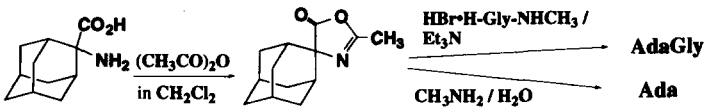
(7) Other exchange rate constants ($k \times 10^{-3}$ s^{-1}) are 25 for H_A of **Ada**, 52 for H_A of **Acc**, 280 for H_A of **Aib** and 210 for H_B of **Aib**.

(8) The grid search analyses are performed on Sybyl (Tripos Inc.) using Tripos force field.

(9) Paul, P. C. K.; Sukumar, M.; Piazzesi, A. M.; Valle, G.; Tonilo, C.; Balaram, P. *J. Am. Chem. Soc.* **1986**, *108*, 6363.

(10) Single hydrogen bonding systems are generally expected to be destroyed in polar solvents having competitive hydrogen bonding abilities. Therefore, the present unique ability of the adamantyl amino acid should be utilized as one of cooperative interactions for controlling the conformation of the peptide working under aqueous conditions.

(11) The synthetic scheme is as follows:



The experimental procedures and spectroscopic data for **Ada** and **AdaGly** are available on request.

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