JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by ISTANBUL TEKNIK UNIV

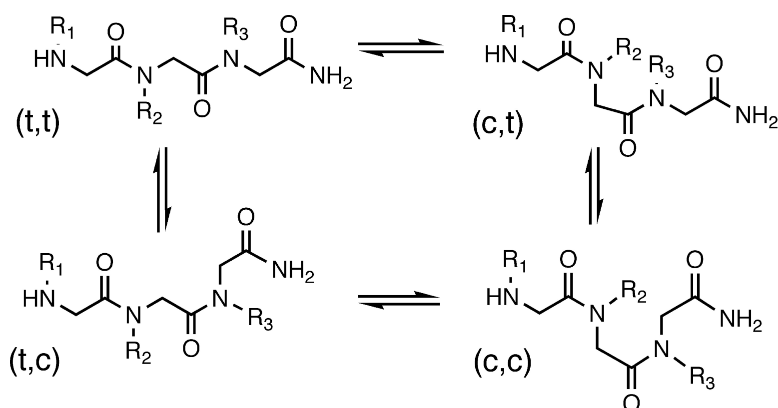
Article

Kinetics and Equilibria of Cis/Trans Isomerization of Backbone Amide Bonds in Peptoids

Qiang Sui, Dan Borchardt, and Dallas L. Rabenstein

J. Am. Chem. Soc., **2007**, 129 (39), 12042-12048• DOI: 10.1021/ja0740925 • Publication Date (Web): 07 September 2007

Downloaded from http://pubs.acs.org on February 14, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
ARTICLES
Published on Web 09/07/2007

Kinetics and Equilibria of Cis/Trans Isomerization of Backbone Amide Bonds in Peptoids

Qiang Sui, Dan Borchardt, and Dallas L. Rabenstein*

Contribution from the Department of Chemistry, University of California, Riverside, California 92521

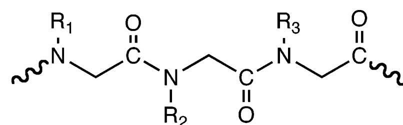
Received June 5, 2007; E-mail: dallas.rabenstein@ucr.edu

Abstract: The biological activities of N-substituted glycine oligomers (peptoids) have been the subject of extensive research. As compared to peptides, both the cis and trans conformations of the backbone amide bonds of peptoids can be significantly populated. Thus, peptoids are mixtures of configurational isomers, with the number of isomers increasing by a factor of 2 with each additional monomer residue. Here we report the results of a study of the kinetics and equilibria of cis/trans isomerization of the amide bonds of N-acetylated peptoid monomers, dipeptoids, and tripeptoids by NMR spectroscopy. Resonance intensities indicate the cis conformation of the backbone amide bonds of the peptoids studied is more populated than is generally the case for the analogous secondary amide bond to proline residues in acyclic peptides. Rate constants were measured by inversion−magnetization transfer techniques over a range of temperatures, and activation parameters were derived from the temperature dependence of the rate constants. The rate of cis/trans isomerization by rotation around the amide bonds in the peptoids studied is generally slower than that around amide bonds to proline residues and takes place on the NMR inversion−magnetization transfer time scale only by rotation around the amide bond to the C-terminal peptoid residue.

Introduction

Although peptides have a wide range of biological activities, they are generally poor drug candidates because they are prone to degradation by proteases, have low oral bioavailability, and are frequently immunogenic.[1] These characteristics have inspired the design of non-natural peptide mimics with the fundamental molecular features of peptides,[2] including N-substituted glycine oligomers (peptoids),[3,4] β-peptides,[5−8] γ-peptides,[8−11] and peptide nucleic acids.[12]

Peptoids were among the first reported peptide backbone-modified peptidomimetics.[2−4,13] They consist of an achiral polyglycine backbone, with the side chains moved from the C_α carbon to the adjacent amide nitrogen. Peptoids are generally



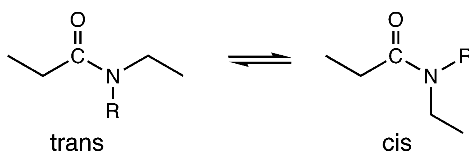
synthesized by the highly versatile solid-phase "submonomer" methodology, in which each monomer is added in two steps, first the acylation of a resin-bound secondary amine with bromoacetic acid followed by nucleophilic displacement of the bromide with a primary amine.[4] The high efficiency for the addition of each monomer unit together with the wide variety of primary amines that have been used has enabled the automated synthesis of long-chain peptoids (up to 50mers) with diverse side chain chemistries.[14,15] Peptoids are highly resistant to degradation by proteases[16] and are more biocompatible than peptides.[17] Thus they have been extensively studied as potential pharmaceuticals.[2,13]

One consequence of the peptoid side chains being on the backbone nitrogens is that the cis conformation of the amide

(1) Latham, P. W. *Nat. Biotechnol.* **1999**, *17*, 755−757.
(2) Patch, J. A.; Barron, A. E. *Curr. Opin. Chem. Biol.* **2002**, *6*, 872−877.
(3) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367−9371.
(4) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646−10647.
(5) Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinoni, B. *Helv. Chim. Acta* **1996**, *79*, 913−941.
(6) Appela, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071−13072.
(7) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219−3232.
(8) Guichard, G. In *Pseudo-Peptides in Drug Discovery*; Nielson, P. E., Ed.; Wiley-VCH Verlag GmbH & Co.: Weinheim, 2004; pp 33−120.
(9) Hintermann, T.; Gademann, K.; Jaun, B.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 983−1002.
(10) Brenner, M.; Seebach, D. *Helv. Chim. Acta* **2001**, *84*, 2155−2166.
(11) Hannessian, S.; Luo, X.; Schaum, R.; Michnick, S. *J. Am. Chem. Soc.* **1998**, *120*, 8569−8570.
(12) Nielsen, P. E.; Koppelhus, U.; Beck, F. In *Pseudo-Peptides in Drug Discovery*; Nielson, P. E., Ed.; Wiley-VCH Verlag GmbH & Co.: Weinheim 2004; pp 153−191.
(13) Patch, J. A.; Kirshenbaum, K.; Seurynck, S. L.; Zuckermann, R. N. In *Pseudo-Peptides in Drug Discovery*; Nielson, P. E., Ed.; Wiley-VCH Verlag GmbH & Co.: Weinheim, 2004; pp 1−31.
(14) Kirshenbaum, K.; Barron, A. E.; Goldsmith, R. A.; Armand, R. A.; Bradley, P.; Troung, E. K.; Dill, K. T. V.; Cohen, F. E.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4305−4308.
(15) Murphy, J. E.; Uno, T.; Hamer, J. D.; Dwarki, F. E.; Cohen, F. E.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1517−1522.
(16) (a) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Biorg. Med. Chem. Lett.* **1994**, *4*, 2657−2662. (b) Miller, S. M.; Simon, R. J.; Ng, S.; Zuckermann, R. N.; Kerr, J. M.; Moos, W. H. *Drug Dev. Res.* **1995**, *35*, 20−32.
(17) Gibbons, J. A.; Hancock, A. A; Vitt, C. R.; Knepper, S.; Buckner, S. A; Brune, M. E.; Milicic, I.; Kerwin, J. F., Jr.; Richter, L. S.; Taylor, E. W.; Spear, K. L.; Zuckermann, R. N.; Spellmeyer, D. C.; Braeckman, R. A.; Moos, W. H. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 885−899.

10.1021/ja0740925 CCC: $37.00 © 2007 American Chemical Society

bonds linking the monomer residues can be more highly populated than is the case for the backbone amide bonds of peptides, with the exception of those to the nitrogen of proline.[18] Thus, in solution a peptoid comprised of *n* monomers can exist as an equilibrium mixture of 2^{n-1} configurational isomers, or 2^n if the N-terminal amino group is acetylated. Depending on



the size of the peptoid and the sidechains on the backbone nitrogens, peptoids can fold into helical secondary structures.[14] The helices are stabilized predominantly by side chain−backbone steric interactions rather than the backbone hydrogen bonds that stabilize polypeptide helices;[14,19−21] peptoids as small as 5mers with side chains branched at the carbon immediately adjacent to the backbone amide nitrogen (the side chain C_α carbon) can form stable helices with cis-amide bonds.[22]

Much of the interest in peptoids as potential pharmaceuticals has focused on smaller peptoids. As examples, tripeptoid ligands for the opiate and α_1-adrenergic receptors and high-affinity di- and tripeptoid ligands for G-protein coupled receptors were identified by screening combinatorial peptoid libraries,[17,23] and tripeptoid libraries have been screened for antimicrobial activity.[24,25] However, because of the high specificity of ligand−receptor binding, not all configurational isomers of a di- or tripeptoid are expected to be biologically active. Thus, the distribution and kinetics of interchange among peptoid configurational isomers are of interest.

In the research reported here, we have sought to understand better the conformational properties of peptoids in solution, in particular the kinetics and equilibria of cis/trans isomerization by rotation around the amide bonds of peptoids. Because interchange between the cis and trans isomers of amide bonds is generally slow on the NMR time scale, the NMR spectrum of a peptoid is the composite of the NMR spectra of its 2^{n-1} configurational isomers, or 2^n isomers if the peptoid is acetylated. Additionally, their NMR spectra are highly degenerate at longer chain lengths, making peptoids complicated systems to study by NMR.[22] We report here on the kinetics and equilibria of cis/trans isomerization of the model peptoids in Table 1;[26] to facilitate spectral resolution and assignment, peptoids with N-α-chiral side chains were not included in this study. The ^{1}H NMR spectrum of each configurational isomer in the mixture of isomers for each peptoid was assigned, and the isomer populations were determined from integrated resonance intensities. The kinetics of interchange between configurational isomers were characterized by inversion−magnetization transfer NMR experiments.

Table 1. Peptoids Synthesized and Studied in This Research[a]

peptoid	peptoid sequence	monoisotopic mass; theoretical (found)
1	Ac−N(butyl)−NH_2	172.2 (172.1)
2	Ac−N(benzyl)−NH_2	206.2 (206.2)
3	Ac−N(butyl)−N(benzyl)−NH_2	319.4 (319.3)
4	Ac−N(methyl)−N(benzyl)−NH_2	277.3(277.1)
5	Ac−N(butyl)−N(methyl)−NH_2	243.3 (243.2)
6	N(butyl)−N(methyl)−N(benzyl)−NH_2	348.2 (348.2)
7	Ac−N(butyl)−N(methyl)−N(benzyl)−NH_2	390.2 (390.3)

[a] N(butyl) is N-butylglycine, N(benzyl) is N-benzylglycine, and N(methyl) is N-methylglycine.

Experimental Section

Materials. *N*-Butylamine, benzylamine, 35% methylamine in water, bromoacetic acid, and *N*,*N*′-diisopropylcarbodiimide (DIPCDI) were obtained from Sigma-Aldrich. Rink amide 4-methylbenzhydrylamine (MBHA) resin and piperidine were obtained from NovaBiochem. Sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TMSP) and D_2O (99.9%) were obtained from Cambridge Isotope Laboratories.

Peptoid Synthesis and Purification. Peptoids were synthesized on 0.4 g of Fmoc-Rink amide resin with a substitution level of 0.45 mmol/g using the submonomer methodology developed by Zuckermann et al.[4] The peptoid was cleaved from the resin with a solution of 95% trifluoroacetic acid (TFA) and 5% water, frozen with dry ice, and then lyophilized to a dry powder.

Peptoids were purified by reversed-phase HPLC on a Vydac 10 mm × 250 mm C18 semi-prep column (5 μm particles, 300 Å pore size) using a Bio-Rad 2800 HPLC Solvent Delivery System equipped with a Linear model 205 dual-wavelength UV detector. The detector was set at 215 nm to monitor the amide bond. Mobile phase A was water with 0.1% TFA; mobile phase B was acetonitrile with 0.1% TFA. Peptoids were eluted using a linear gradient of 5%−60% mobile phase B over 30 min at a 4 mL/min flow rate and then equilibrated with 5% mobile phase B for 10 min before the next injection. Depending on the hydrophobicity and length of the peptoids, they were typically eluted between 12 and 20 min.

MALDI-MS was used to confirm peptoid identities; the molecular weights obtained by MALDI-MS are reported in Table 1. The purity of each peptoid, as determined by HPLC and ^{1}H NMR, was greater than 99%.

NMR Samples. NMR samples were prepared by dissolving peptoid in 100% D_2O at 3−10 mM concentrations with TMSP added as a chemical shift reference. 0.1 M DCl and 0.1 M NaOD were used to

(18) The amide bonds to 19 of the 20 gene-coded amino acids exist almost exclusively in the trans conformation. The exception is proline; amide bonds to proline typically exist up to 10% in the cis conformation in acyclic peptides. (a) Wüthrich, K.; Grathwohl, C. *FEBS Lett.* **1974**, *43*, 337−340. (b) Fischer, G. *Chem. Soc. Rev.* **2000**, *29*, 119−127.
(19) Armand, P.; Kirshenbaum, K.; Falicov, A.; Dunbrack, R. L., Jr.; Dill, K. A.; Zuckermann, R. N.; Cohen, F. E. *Folding Des.* **1997**, *2*, 369−375.
(20) Wu, C. W.; Sanborn, T. J.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2001**, *123*, 2958−2963.
(21) Wu, C. W.; Sanborn, T. J.; Huang, K.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2001**, *123*, 6778−6784.
(22) Armand, P.; Kirshenbaum, K.; Goldsmith, R. A.; Farr-Jones, S.; Barron, A. E.; Troung, K. T. V.; Dill, K. A.; Mierke, D. F.; Cohen, F. E.; Zuckermann, R. N.; Bradley, E. K. *Proc. Natl. Acad. Sci. U.S.A.*, **1998**, *95*, 4309−4314.
(23) Zuckermann, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R.; Kerr, J. M.; Figliozzi, G. M.; Goff, D. A.; Siani, M. A.; Simon, R. J.; Banville, S. C.; Brown, E. G.; Wang, L.; Richter, L. S.; Moos, W. H. *J. Med. Chem.* **1994**, *37*, 2678−2685.
(24) Ng, S.; Goodson, B.; Ehrhardt, A.; Moos, W. H.; Siani, M.; Winter, J. *Biorg. Med. Chem.* **1999**, *7*, 1781−1785.
(25) Larger peptoids have also been studied as drug candidates: as analogues of lung surfactant protein B (Seurynck, S.; Patch, J. A.; Barron, A. E. *Chem. Biol.* **2005**, *12*, 77−88); as agents to prevent HIV-Tat/Tar interactions (Hamy, F.; Felder, E. R.; Heizmann, G. N.; Lazdins, J.; Aboul-Ela, F.; Varani, G.; Karn, J.; Klimkait, T. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3548−3553; Kesavan, V.; Tamilarasu, N.; Cao, H.; Rana, T. M. *Bioconjugate Chem.* **2002**, *13*, 1171−1175; Klimkait, T.; Felder, E. R.; Albrecht, G.; Hamy, F. *Biotechnol. Bioeng.* **1999**, *61*, 155−164; Daelemans, D.; Schols, D.; Witvrouw, M.; Pannecouque, C.; Hates, S.; Dooren, S. V.; Hamy, S. V.; Klimkait, T.; Clercq, E.; VanDamme, A. M. *Mol. Pharmacol.* **2000**, *57*, 116−124.) and as agents to complex with and deliver nucleic acids to cells (Murphy, J. E.; Uno, T.; Hamer, J. D.; Cohen, F. E.; Dwarki, V.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1517−1522.)

(26) The dipeptoids studied here were N-acetylated to prevent formation of cyclic diketopiperazines, as has been reported by others (Taylor, W. E.; Gibbons, J. A.; Baeckman, R. A. *Pharm. Res.* **1997**, *14*, 572−577) and was also observed in this research.

adjust the pD to ~3.0. pD values were obtained by using the equation pD = pH reading + 0.40 to correct for the deuterium isotope effect.[27]

NMR Spectroscopy. One- and two-dimensional ^{1}H NMR spectra were measured on a 500 MHz Varian Unity-Inova spectrometer. The resonance from residual water in the samples was suppressed in 1D and 2D experiments with a water presaturation pulse. Two-dimensional total correlation spectroscopy (TOCSY) and rotating frame Overhauser effect spectroscopy (ROESY) spectra were measured with the following parameters: the spectral window was 5500 Hz in both dimensions, 8196 or 16 384 points were collected in the acquisition (F2) dimension, 64 dummy scans were run prior to acquisition, the number of t_1 increments was either 64 or 128, the number of transients was 64, the transmitter power was 63dB, and the mixing time was 120−300 ms.

The kinetics of interchange between configurational isomers were studied using two 1D inversion−magnetization transfer experiments. In the first experiment, selective inversion of a resonance for a specific isomer was achieved with a selective Dante pulse ($(\text{p1-d2})_n$).[28] The Dante inversion pulse was followed by a delay time t, during which magnetization transfer takes place by chemical exchange, and a $90°_{\pm x,\pm y}$ observation pulse. Typical values for parameters in the Dante inversion−magnetization transfer experiment were as follows: the delay time was arrayed from 0.0001 to 24 s, the number of transients was 4−32, and 16K data points were collected. For the Dante pulse, p1 = 1 μs, d2 = 0.488 ms, and the pulse power was decreased to 43 dB so that the number of pulses (n) in the DANTE pulse was 160−230 (determined by calibration to obtain a 180° inversion of the selected resonance).

In the second experiment (a two-site inversion−magnetization transfer experiment), selective inversion of the trans resonance of a cis/trans pair of resonances was achieved with the first two 90° pulses of the pulse sequence: $90°_x$-τ-$90°_x$-t-$90°_{\pm x,\ \pm y}$, where τ is a delay set equal to $1/(2|\nu_{\text{trans}} - \nu_{\text{cis}}|)$, and t is a variable delay during which magnetization transfer takes place by chemical exchange.[29−32] Typical values used for the parameters were as follows: the delay time t was arrayed from 0.001 s to at least 5 times the longest T_1 of the resonances used, the number of transients was 16−128, the transmitter was set on the trans resonance, and 16K data points were collected.

Rate constants were obtained from the inversion−magnetization transfer data using method one in ref 32. The procedure involved fitting the intensity vs time data by nonlinear least-squares methods to an equation which expresses the resonance intensity in terms of the spin−lattice relaxation times, the intensity of the exchanging resonances at equilibrium, and the rate constant.[32] The nonlinear least-squares fits were performed with the program Scientist (Micromath).

Results

Assignment of NMR Spectra. The rate of interchange between configurational isomers by rotation around their amide bonds is slow on the chemical shift time scale for the peptoids listed in Table 1, with the result that the ^{1}H NMR spectrum of each peptoid is a composite of the spectra of all its isomers. The procedure used to assign their ^{1}H NMR spectra is illustrated by assignment of the spectrum of peptoid **3** in Table 1. In solution, peptoid **3** is a mixture of the four configurational isomers shown in Figure 1. The four isomers are labeled (t,t), etc, where the first letter designates the conformation across the amide bond between the acetyl group and the N(butyl) residue and the second letter designates the conformation across the amide bond between the N(butyl) and N(benzyl) residues. Portions of the NMR spectrum of **3** are shown in Figure 2. Four singlets are observed in the region from 1.9 to 2.2 ppm for the acetyl protons (labeled H_1 in Figure 1), and four triplets are observed between 3.20 and 3.45 ppm for the butyl side chain $C_\alpha H_2$ protons (H_{10}), confirming that four isomers are present. Singlet resonances are observed in the 4.0−4.7 ppm region for the H_4, H_7, and H_{14} protons. For the H_4, H_7, and H_{14} protons, 12 resonances should be observed; eight are observed between 4.0 and 4.5 ppm, and two pairs of overlapping resonances are observed in the 4.6−4.7 ppm region. The intensities of the latter resonances are reduced due to the water presaturation pulse. Cross-peaks between the resonances in the 4.6−4.7 ppm region and resonances for the aromatic protons in the TOCSY spectrum indicate the 4.6−4.7 ppm resonances are for the H_{14} protons and that there are two H_{14} resonances at ~4.70 ppm and two at ~4.61 ppm (see Supporting Information). With these four resonances, the expected 12 resonances for the H_4, H_7, and H_{14} protons are accounted for.

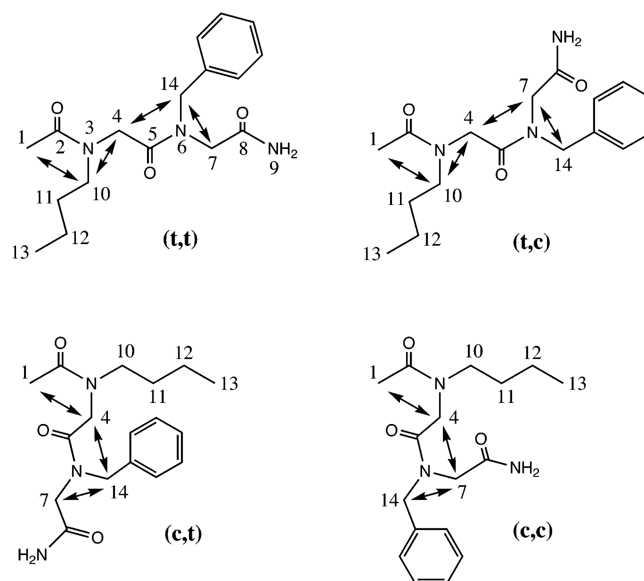


Figure 1. Four configurational isomers of peptoid **3**. The first letter in the label for each isomer indicates the conformation across the amide bond that links the acetyl group to the N(butyl) residue, and the second letter, the amide bond that links the N(butyl) and N(benzyl) residues; e.g., (t,t) indicates the configurational isomer in which the acetyl methyl carbon and the N(butyl) C_α carbon are trans and the N(butyl) and N(benzyl) backbone CH_2 carbons are trans across the respective amide bonds. The arrows indicate the dipolar cross-peaks in the ROESY spectrum of **3** used to assign the ^{1}H NMR spectrum for each configurational isomer.

Resonances were assigned to the four isomers of **3** using dipolar cross-peaks in ROESY spectra (see Supporting Information). The dipolar connectivities used to assign the resonances are indicated in Figure 1. According to the isomer conformations in Figure 1, cross-peaks between the acetyl (H_1) and H_4 protons are possible only for the (c,t) and (c,c) isomers. Cross-peaks in the ROESY spectrum indicate the resonances at 4.389 and 4.418 ppm are for H_4(c,c) and H_4(c,t); the resonance at 4.389 ppm was assigned to H_4(c,c) on the basis of a cross-peak to an H_7 resonance at 4.147 ppm. Thus, the H_4(c,c) resonance has a chemical shift of 4.389 ppm, the H_4(c,t) resonance is at 4.418 ppm, and the H_7(c,c) resonance is at 4.147 ppm. The H_1(c,t) resonance has a chemical shift of 1.941 ppm, and the H_1(c,c) resonance, a chemical shift of 2.013 ppm. Cross-peaks between

(27) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188−190.
(28) Morris, G. A.; Freeman, R. *J. Magn. Reson.* **1978**, *29*, 433−462.
(29) Robinson, G.; Chapman, B. E.; Kuchel, P. W. *Eur. J. Biochem.* **1984**, *143*, 643−649.
(30) Robinson, G.; Kuchel, P. W.; Chapman, B. E.; Doddrell, D. M.; Irving, M. G. *J. Magn. Reson.* **1985**, *63*, 314−319.
(31) King, G. F.; Middlehurst, C. R.; Kuchel, P. W. *Biochemistry* **1986**, *25*, 1054−1062.
(32) Mariappan, S. V. S.; Rabenstein, D. L. *J. Magn. Reson.* **1992**, *100*, 183−188.

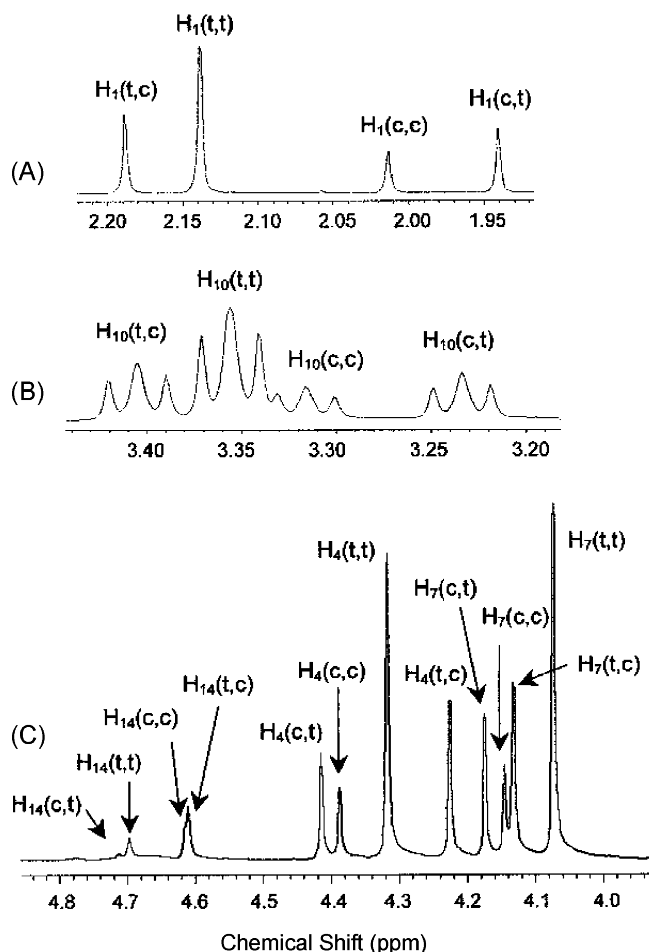


Figure 2. Resonance assignments for the acetyl methyl protons (A), $C_\alpha H_2$ protons of the N(butyl) side chain (B), and backbone CH_2 protons and CH_2 protons of the benzyl side chain (C) of peptoid **3**.

the H_{14} resonance at 4.717 ppm and the resonances at 4.418 and 4.177 ppm indicate the resonances at 4.717 and 4.177 ppm are for H_{14}(c,t) and H_7(c,t), respectively. Cross-peaks between the resonance at 4.62 ppm and the H_7(c,c) resonance at 4.147 ppm indicate the resonance at 4.62 ppm is for the H_{14}(c,c) protons. The H_{10}(c,c) and H_{10}(c,t) resonances were assigned using cross-peaks to the H_4(c,c) and H_4(c,t) resonances, respectively. In the same way, resonances for the (t,t) and (t,c) isomers were assigned based on $H_1 \rightarrow H_{10} \rightarrow H_4 \rightarrow H_{14} \rightarrow H_7$ and $H_1 \rightarrow H_{10} \rightarrow H_4 \rightarrow H_7 \rightarrow H_{14}$ connectivities, respectively. The chemical shifts of the H_{11}, H_{12}, and H_{13} protons of the *n*-butyl side chains of the four isomers were determined from cross-peaks to the assigned H_{10} resonances in the TOCSY spectrum (see Supporting Information for two-dimensional spectra and a chemical shift table).

Peptoids that contain one, two and three amide bonds can exist as two, four and eight isomers, respectively, and that is what is observed, as illustrated by the N-acetyl resonances for peptoids **1**, **4** and **7** in Figure 3.

Equilibrium Constants for Cis/Trans Isomerization. Peptoids **1** and **2**, peptoids **3**−**6**, and peptoid **7** exist in solution as equilibrium mixtures of two, four, and eight configurational isomers, respectively. The percentage of each isomer, as determined from integrated resonance intensities, is reported in Table 2; equilibrium constants for pairs of isomers related by rotation around a single amide bond are reported in Table 3.

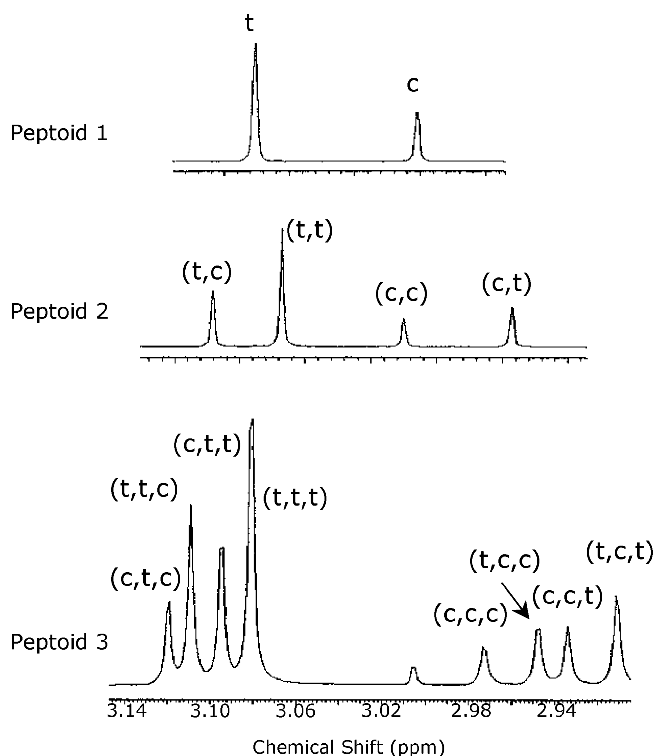


Figure 3. Resonance assignments for the acetyl methyl protons of peptoids **1** and **4** and the N(methyl) protons of peptoid **7**.

Table 2. Populations of the Configurational Isomers of the Peptoids in Table 1[a]

peptoid	%c	%t
1	28.6	71.4
2	37.7	62.3

peptoid	%cc	%ct	%tc	%tt
3	12.1	17.3	24.7	45.9
4	12.9	17.6	24.1	45.4
5	8.6	16.5	20.2	54.7
6	9.6	12.7	29.1	48.6

peptoid	%ccc	%cct	%ctc	%tcc	%ctt	%tct	%ttc	%ttt
7	4.8	7.2	8.7	7.2	15.1	10.9	15.8	30.3

[a] The first letter indicates the conformation of the first amide bond, etc.

Table 3. Equilibrium Constants for Cis/Trans Isomerization of the Peptoids in Table 1 by Rotation around One Amide Bond[a,b]

peptoid	$K_{c/t}$					
1	2.49					
2	1.65					
peptoid	$K_{tc/tt}$	$K_{ct/tt}$	$K_{cc/ct}$	$K_{cc/tc}$		
3	1.86	2.65	1.43	2.04		
4	1.88	2.58	1.36	1.87		
5	2.71	3.32	1.92	2.35		
6	1.67	3.82	1.33	3.03		
peptoid	$K_{ttc/ttt}$	$K_{tct/ttt}$	$K_{ctt/ttt}$	$K_{ctc/ctt}$	$K_{cct/ctt}$	$K_{cct/tct}$
7	1.92	2.78	2.01	1.74	2.10	1.52
peptoid	$K_{tcc/tct}$	$K_{ctc/ttc}$	$K_{tcc/ttc}$	$K_{ccc/cct}$	$K_{ccc/ctc}$	$K_{ccc/tcc}$
7	1.51	1.82	2.19	1.50	1.81	1.50

[a] 25 °C. [b] Equilibrium constants are for cis-to-trans interchange; e.g., $K_{ctc/ctt}$ = [c,t,t]/[c,t,c] is for (c,t,c) → (c,t,t).

Kinetics of Cis/Trans Isomerization. Exchange among the configurational isomers of peptoids **1** and **2**, peptoids **3**−**6**, and peptoid **7** can take place by rotation around one, two, and three amide bonds, respectively. The kinetics of exchange were

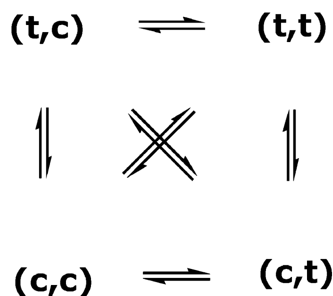


Figure 4. Possible pathways for interchange among the four configurational isomers of peptoid **3**. Interchange takes place only by the (t,c) ↔ (t,t) and (c,c) ↔ (c,t) pathways on the NMR inversion−magnetization transfer time scale at temperatures ≤ 60 °C.

studied by inversion−magnetization transfer NMR experiments. The experimental procedure is illustrated using peptoid **3**. Exchange by rotation around the two amide bonds of the four configurational isomers of peptoid **3** can take place by the pathways shown in Figure 4. Exchange is possible, in principle, by both single and double amide bond rotations, for example (t,t) ↔ (t,c) and (t,c) ↔ (c,t). The Dante inversion−magnetization transfer experiment was used to identify the pathways by which exchange takes place on the NMR inversion−magnetization transfer time scale.[33,34] The resonance for one isomer was selectively inverted, and the integrated intensities of the resonances for the same protons of the other three isomers were measured as a function of the delay time *t*. To illustrate, Figure 5 shows the intensities of resonances for the acetyl protons of the four isomers of **3** at several delay times after selectively inverting the resonance for the (c,t) isomer at 55 °C. The resonance for the (c,c) isomer first decreases in intensity due to magnetization transfer by chemical exchange as the delay time *t* is increased and then increases in intensity and ultimately returns to its equilibrium intensity due to T_1 relaxation, while the intensities of resonances for the (t,t) and (t,c) isomers do not change. When the resonance for (c,c) was selectively inverted, the resonance for the (c,t) isomer decreased in intensity, while the intensities of resonances for the (t,c) and (t,t) isomers remained constant. Taken together, these results indicate that, on the time scale of the inversion−magnetization transfer experiment, exchange takes place between (c,t) and (c,c), but not between (c,t) and (t,t) or (t,c) or between (c,c) and (t,t) or (t,c) at ≤55 °C.When the acetyl resonances for (t,c) and (t,t) were each selectively inverted, similar results were obtained, i.e., magnetization transfer was observed between (t,t) and (t,c) but not between (t,t) and (c,c) or (c,t) or between (t,c) and (c,c) or (c,t) (see Supporting Information). The temperature was varied from 35 to 65 °C at 5 °C intervals. The same behavior was observed for temperatures ≤ 60 °C. At 65 °C, a small decrease was also observed in the intensity of the resonances for (c,c) and (c,t) when the resonance for (t,t) was selectively inverted, which suggests a small amount of exchange between

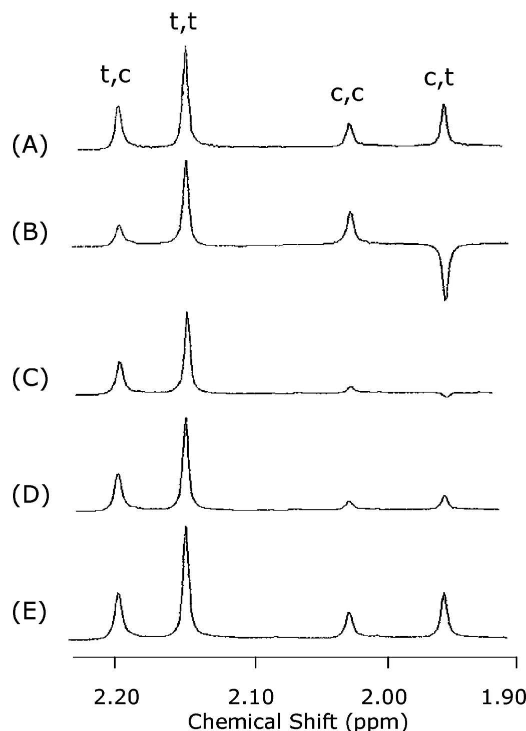


Figure 5. Acetyl (H_1) methyl resonances of peptoid **3** at 55 °C. Spectrum (A) is the normal 1D spectrum. Spectra (B−E) were measured with the DANTE inversion−magnetization transfer pulse sequence with selective inversion of the acetyl resonance of the (c,t) isomer with delay times t = 0.0001, 0.25, 0.55, and 24 s, respectively.

(t,t) and (c,c) and between (t,t) and (c,t) on the inversion−magnetization time scale at temperatures ≥ 65 °C. However, at temperatures ≤ 60 °C, exchange is between (c,c) and (c,t) and between (t,c) and (t,t) on the inversion−magnetization transfer time scale; i.e., at temperatures ≤ 60 °C, the system can be treated as two separate and independent two-site exchange processes in which exchange takes place only by rotation around the amide bond between the N(butyl) and N(benzyl) residues. Because the (t,t) ↔ (t,c) and (c,t) ↔ (c,c) exchange processes are independent, their exchange kinetics were studied by the simpler two-site inversion−magnetization transfer experiment. Inversion−magnetization transfer experiments were performed at 5 °C intervals over the 35−60 °C temperature range on the (c,c)/(c,t) and (t,c)/(t,t) pairs of isomers. To illustrate, the H_1(c,c) and H_1(c,t) resonances are plotted in Figure 6 as a function of delay time following inversion of the H_1(c,t) resonance. The rate constant for (c,c) to (c,t) exchange, $k_{cc/ct}$, was derived from the inversion−magnetization transfer data by a nonlinear least-squares fit of the inversion−magnetization transfer data to an equation that expresses the resonance intensity as a function of delay time in terms of known and adjustable parameters, including the rate constant $k_{cc/ct}$ and T_1 relaxation times.[32] The fit of inversion−magnetization transfer data for the H_1(c,c) resonance to the model equation is shown in Figure 7. A value of 0.24 ± 0.02 s^{-1} was obtained for $k_{cc/ct}$ from the fit. Rate constant $k_{cc/ct}$ was measured over the temperature range 35−60 °C. Likewise, rate constant $k_{tc/tt}$ was measured over the same temperature range using the two-site inversion−magnetization transfer experiment with selective inversion of the H_1(t,t) resonance. Activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for (c,c) → (c,t) exchange and for (t,c) → (t,t) exchange

(33) Chemical exchange pathways were identified using the 1D Dante inversion−magnetization transfer experiment rather than 2D EXSY experiments because of the small chemical shift differences between resonances of some isomers and more accurate and convenient measurement of peak intensity changes in 1D spectra of the former experiment than the peak volume in the 2D EXSY experiment.

(34) To study chemical exchange by the inversion−magnetization transfer experiment, exchange must be slow enough that separate resonances are observed for the exchange partners but fast enough that the first-order rate constant for exchange, k_{ex}, is $\geq 1/T_1$, where T_1 is the spin-lattice relaxation time of the reporter nucleus.

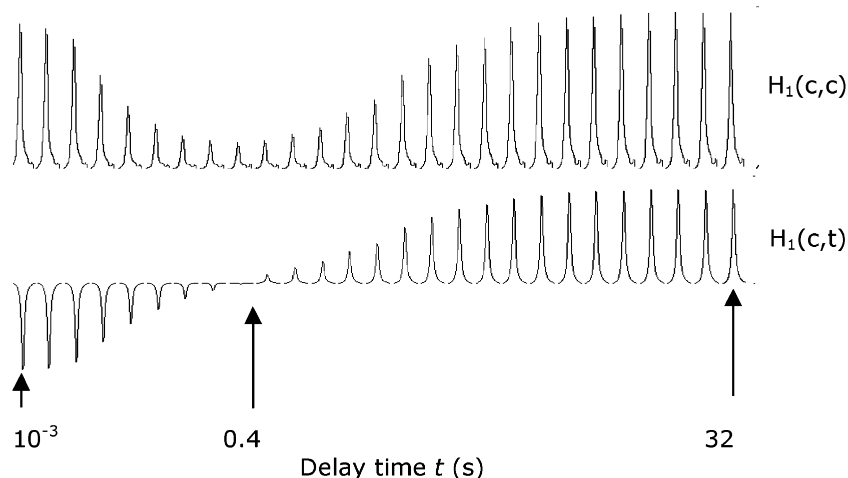


Figure 6. Resonances for the acetyl (H_1) methyl protons of the (c,c) and (c,t) isomers of peptoid **3** as a function of the delay time t after inversion of the acetyl methyl resonance of the (c,t) isomer with the two-site inversion−magnetization transfer pulse sequence. The temperature was 55 °C, and the carrier was set on the acetyl resonance for (c,t). The delay times were as follows: 0.0001, 0.001, 0.01, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, 0.6, 0.8, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 10, 12, 15, 20, and 24 s.

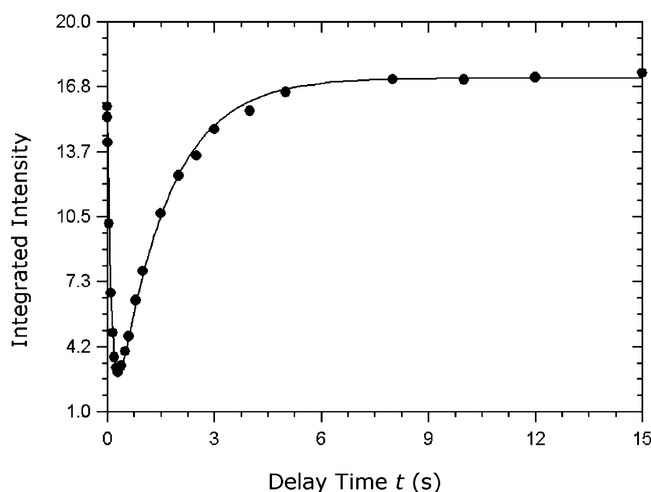


Figure 7. Integrated intensities of the resonance for the acetyl methyl protons of the (c,c) isomer of peptoid **3** as a function of the delay time t in the two-site inversion−magnetization transfer experiment at 55 °C when the acetyl methyl resonance of the (c,t) isomer was selectively inverted. The smooth curve through the data points was obtained by nonlinear least-squares analysis of the data, as described in the Experimental Section.

were determined from the dependence of $k_{cc/ct}$ and $k_{tc/tt}$ on temperature, respectively, with the Eyring equation:

$$\ln(k/T) = \ln(k_B/h) + \Delta S^{\ddagger}/R - \Delta H^{\ddagger}/RT \qquad (1)$$

where k is $k_{cc/ct}$ or $k_{tc/tt}$, respectively, k_B is the Boltzmann constant, h is Planck's constant, and R is the gas constant. Plots of $\ln(k/T) - \ln(k_B/h)$ vs $1/T$ were linear for both (c,c) → (c,t) and (t,c) → (t,t) exchanges (see Supporting Information). Rate constants for (c,c) → (c,t) and (t,c) → (t,t) exchanges at 25 °C were calculated using the activation parameters and eq 1. Rate constants for the reverse reactions were calculated using these rate constants and the equilibrium constants for the exchange reactions. The rate constants at 25 °C are reported in Table 4; activation parameters are reported in the Supporting Information.

The same procedure was applied to peptoids **1**, **2**, and **4**−**7**. Peptoids **1** and **2** are two-site exchange systems, and the same two-site exchange processes described above for **3** are applicable to peptoids **4**−**6**. Peptoid **7** is peptoid **6** with the N-terminus acetylated, thus doubling the number of isomers to eight. Application of the Dante inversion−magnetization transfer sequence to **7** showed that only C-terminal amide bond cis/trans isomerization was observable over the temperature range studied and that the following exchange reactions could be treated as independent two-site exchange processes: (t,t,c) ↔ (t,t,t), (t,c,c) ↔ (t,c,t), (c,c,c) ↔ (c,c,t), and (c,t,c) ↔ (c,t,t). Rate constants at 25 °C for all exchange processes characterized are reported in Table 4. Activation parameters for all rate constants reported in Table 4 and the temperature range over which the kinetics were studied are reported in the Supporting Information.

Table 4. Rate Constants for Cis/Trans Isomerization by Rotation around a Single Amide Bond[a,b]

peptoid	cis → *trans*		trans → *cis*	
1	$k_{c/t}$	0.074 ± 0.017	$k_{t/c}$	0.030 ± 0.007
2	$k_{c/t}$	0.084 ± 0.007	$k_{t/c}$	0.052 ± 0.004
3	$k_{tc/tt}$	0.22 ± 0.01	$k_{tt/tc}$	0.12 ± 0.01
	$k_{cc/ct}$	0.24 ± 0.02	$k_{ct/cc}$	0.17 ± 0.01
4	$k_{tc/tt}$	0.19 ± 0.01	$k_{tt/tc}$	0.10 ± 0.01
	$k_{cc/ct}$	0.27 ± 0.01	$k_{ct/cc}$	0.20 ± 0.01
5[c]	$k_{cc/ct}$	0.049 ± 0.01	$k_{ct/cc}$	0.021 ± 0.004
6	$k_{tc/tt}$	0.24 ± 0.02	$k_{tt/tc}$	0.15 ± 0.01
	$k_{cc/ct}$	0.26 ± 0.04	$k_{ct/cc}$	0.20 ± 0.03
7	$k_{ttc/ttt}$	0.28 ± 0.04	$k_{ttt/ttc}$	0.15 ± 0.02
	$k_{ctc/ctt}$	0.14 ± 0.02	$k_{ctt/ctc}$	0.079 ± 0.003
	$k_{ccc/cct}$	0.37 ± 0.04	$k_{cct/ccc}$	0.27 ± 0.04
	$k_{tcc/tct}$	0.21 ± 0.03	$k_{tct/tcc}$	0.13 ± 0.03

[a] Rate constants have units of s^{-1}; T = 25 °C. [b] Activation parameters are reported for all rate constants in the Supporting Information. [c] Due to resonance overlap, rate constants were not determined for the (t,c) ↔ (t,t) exchange reaction.

Discussion

As noted above, much of the research on peptoids as drugs has focused on di- and tripeptoids;[17,23−25] the goal of the present study was to characterize the kinetics and equilibria of cis/trans isomerization of the backbone amide bonds in several model di- and tripeptoids. Thus, it was necessary that there be resolved and assignable resonances for each of their 2^{n-1} isomers or 2^n isomers for the N-acetylated peptoids.[26] The peptoids studied were chosen to meet these criteria; their ^{1}H NMR spectra were completely assigned, even for peptoid **7** which has eight

configurational isomers, and the well-resolved singlet resonances for its N-acetyl and N-methyl protons served as reporter resonances for studying the kinetics and equilibria of exchange among the eight isomers.[35]

An unexpected finding is that the rate of exchange between cis and trans configurations is strongly dependent on the location of the amide bond in the peptoid and not on the monomer residues linked by the bond. Specifically, cis/trans exchange was observed with the inversion−magnetization transfer experiment only for the amide bond to the C-terminal residue at temperatures ≤ 60 °C.[36] For example, cis/trans exchange was observed for the amide bond linking the N(butyl) and N(methyl) residues of peptoid **5** but not for the amide bond linking the same two residues in peptoids **6** and **7**. Likewise, cis/trans exchange was observed for the amide bond linking the N-acetyl and N(butyl) residues of **1** but not for the amide bond linking the same two residues in peptoids **3**, **5** and **7**. The rate of cis/trans exchange for the amide bond linking the N(methyl) and N(benzyl) residues in **7** also is dependent on the conformations of the other amide bonds in the peptoid; the rate constants for cis-to-trans and trans-to-cis exchanges each vary by a factor of ∼3, depending on the conformations of the amide bonds linking the N-acetyl and N(butyl) residues and the N(butyl) and N(methyl) residues of **7**.

Exchange between cis and trans isomers takes place by a shift of the lone pair of electrons on the amide nitrogen from a p_z orbital to an sp^3 orbital followed by rotation around the C−N bond.[18b,37] By analogy to amide bonds to proline in peptides, it is expected that cis/trans isomerization will be accelerated by factors that stabilize the transition state, including intramolecular hydrogen bonding to the lone pair of electrons on the pyramidalized amide nitrogen in the transition state.[18b] Molecular models suggest that one of the two protons on the C-terminal amide group can hydrogen bond to the nitrogen of the amide bond to the C-terminal residue. A similar hydrogen bond between the hydrogen of the amide bond linking the Pro and Xaa residues and the transition state sp^3-hybridized proline nitrogen in cyclic disulfide-bridged peptides of the sequence Ac-Cys-Pro-Xaa-Cys-NH_2 has been proposed to account for the accelerated rate of cis/trans isomerization of their Cys-Pro amide bonds.[38] The rate constants for cis/trans isomerization of the amide bonds to the C-terminal residues of the peptoids studied are similar to those for cis/trans isomerization of the Cys-Pro peptide bond in the cyclic disulfide-bridged peptides and significantly faster than rates for the same amide bonds in the acyclic dithiol forms of the peptides.[38]

The trans conformation of the backbone amide bonds in the peptoids studied is more populated than the cis conformation (Table 2), as is also generally the case for proline amide bonds in acyclic peptides. However, the population of the cis conformation for the peptoids is on average 35%, as compared to <10% for proline amide bonds in acyclic peptides.[18b,38] As a consequence, all possible peptoid isomers are relatively highly populated, even the (c,c,c) isomer of peptoid **7** at 4.8%.

The results in Table 3 indicate the equilibrium constant for cis-to-trans isomerization for a given peptoid amide bond is also strongly dependent on the conformations of the other amide bonds. For example, the ratio of equilibrium constants for cis-to-trans isomerization of the amide bond to the C-terminal residue of peptoids **3**−**6** ($K_{tc/tt}/K_{cc/ct}$) is 1.30, 1.38, 1.41, and 1.26, respectively; i.e., the trans conformation is favored even more when the other amide bond also has the trans conformation. Likewise, the ratio $K_{ct/tt}/K_{cc/tc}$ is 1.30, 1.38, 1.41, and 1.27 for peptoids **3**−**6**, respectively, indicating this is also the case for the other amide bonds.

Conclusions

Peptoids were among the first reported chemically diverse peptide mimics for the development of pharmaceuticals. However, in contrast to peptides, in which the backbone amide bonds to 19 of the 20 gene-coded amino acids are almost exclusively in the trans conformation, both the cis and trans conformations of each backbone amide bond in peptoids are populated. Thus, peptoids are a mixture of 2^{n-1} configurational isomers, or 2^n if the peptoid is N-acetylated.

Because of the specificity of ligand−receptor binding, it is likely that not all configurational isomers will be biologically active. Thus, not only is the population of the various isomers important, but also the rate constants for exchange among the isomers; e.g., is the rate of exchange among the isomers fast relative to the on/off rates for ligand−receptor binding? The rate constants in Table 4 indicate that the rate of cis/trans exchange for even the most labile of the amide bonds, the amide bonds between the two C-terminal residues, is rather slow. For example, the half-lives of the (t,t,c), (c,t,c), (c,c,c), and (t,c,c) isomers of **7**, before rotation around the C-terminal amide bond to form the (t,t,t), (c,t,t), (c,c,t), and (t,c,t) isomers, respectively, are 2.5, 5.0, 1.9, and 3.3 s. The half-lives before rotation around the other amide bonds are even longer. The dynamics of di- and tripeptoid binding to receptor molecules have not been characterized; however, it seems likely that the on/off rates are faster than the rates of interchange among the configurational isomers. It will be important in future research to identify specific isomers of di- and tripeptoids that bind to receptor molecules and to characterize the on/off kinetics of binding.

Supporting Information Available: NMR spectra, resonance assignments, magnetization transfer data and Eyring plots for peptoid **3**, magnetization transfer data for peptoid **7**, and activation parameters for cis-to-trans exchange for peptoids **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

JA0740925

(35) The NMR spectrum of the major configurational isomer of a pentapeptoid has been assigned by a strategy described by Bradley; however it was labor intensive as it involved the sequential ^{13}C-labeling of each peptoid unit, and thus the use of five ^{13}C-labelled peptoids, to overcome the degeneracy problem. Bradley, E. K. *J. Magn. Res., Ser. B* **1996**, *110*, 195−197.

(36) The finding that exchange is fast enough to be observed by the inversion−magnetization transfer method only for the amide bond to the C-terminal residue is in contrast to the rate of cis/trans isomerization for amide bonds linking amino acids to the nitrogen of proline, where cis/trans exchange is fast enough to be observed for prolines located in the interior of peptides.[38]

(37) Fischer, S.; Dunbrack, R. L., Jr.; Karplus, M. *J. Am. Chem. Soc.* **1994**, *116*, 11931−11937.

(38) Shi, T.; Spain, S. M.; Rabenstein, D. L. *J. Am. Chem. Soc.* **2004**, *126*, 790−796.