J|A|C|S
ARTICLES
Published on Web 11/04/2006

N-Methylated Cyclic Pentaalanine Peptides as Template Structures

Jayanta Chatterjee,[†] Dale Mierke,[‡] and Horst Kessler*,[†]

Contribution from the Department Chemie, Lehrstuhl II für Organische Chemie, Technische Universität München, Lichtenbergstrasse 4, Garching D-85747, Germany, and Department of Molecular Pharmacology, Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912

Received May 4, 2006; E-mail: Kessler@ch.tum.de

Abstract: The *N*-methylation of cyclic peptides can be used to modify the activity and/or selectivity of biologically active peptides. As *N*-methylation introduces different flexibility and lipophilicity, it can also improve the bioavailability (the ADMET profile). To search for conformationally constrained cyclic peptides, a library of 30 different *N*-methylated peptides with the basic sequence *cyclo*(-D-Ala-L-Ala_4-) was synthesized. Based on the NMR analysis, seven of these peptides exhibited single conformations (>98%). The structural features of these peptides were determined by a combination of NMR and distance geometry and then further refined by molecular dynamics simulations in an explicit DMSO solvent box. The structures provided from these efforts can now serve as templates for the rational design of cyclic pentapeptides with a distinct backbone conformation or for "spatial screening" to explore the bioactive conformation of medically important peptide systems.

Introduction

The conformation of cyclic peptides of ring size 3−7 is relatively well understood.[1a−f] However, peptidomimetics[2a−e] such as peptide bond analogues,[3a−d] peptoids,[4a−e] sugar amino acids,[5a−g] stereoisomers,[6] and turn mimetics[7a−e] usually introduce unpredictable conformational changes; e.g., β-turn mimetics in cyclic peptides often do not appear in the expected turn position.[8] Another synthetic modification causing conformational changes is *N*-methylation,[9a−c] which is also found in natural cyclic peptides including Cyclosporin A[10a−c] and Omphalotin.[11a−c] We are interested in those *N*-methylated cyclic

[†] Technische Universität München.

[‡] Brown University.

(1) (a) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512−523. (b) Kessler, H.; Bermel, W.; Friedrich, A.; Krack, G.; Hull, W. E. *J. Am. Chem. Soc.* **1982**, *104*, 6297−6304. (c) Mierke, D. F.; Kurz, M.; Kessler, H. *J. Am. Chem. Soc.* **1994**, *116*, 1042−1049. (d) Matter, H.; Kessler, H. *J. Am. Chem. Soc.* **1995**, *117*, 3347−3359. (e) Morita, H.; Kayashita, T.; Takeya, K.; Itokawa, H.; Shiro, M. *Tetrahedron* **1995**, *51*, 12539−12548. (f) Loiseau, N.; Gomis, J. M.; Santolini, J.; Delaforge, M.; Andre, F. *Biopolymers* **2003**, *69*, 363−385.

(2) (a) Hirschmann, R. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1278−1301. (b) Giannis, A. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267. (c) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699−1720. (d) Hruby, V. J.; Li, G.; Haskel-Luevano, C.; Shenderovich, M. *Biopolymers* **1997**, *43*, 219−266. (e) Voyer, N. *Bioorg. Chem.* **1997**, *184*, 1−37.

(3) (a) Kazmierski, W.; Wire, W. S.; Lui, G. K.; Knapp, R. J.; Shook, J. E.; Burks, T. F.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **1988**, *31*, 2170−2177. (b) Hu, B.; Finsinger, D.; Peter, K.; Guttenberg, Z.; Barmann, M.; Kessler, I.; Escherich, A.; Moroder, L.; Bohm, J.; Baumeister, W.; Sui, S. F.; Sackmann, E. *Biochemistry* **2000**, *39*, 12284−12294. (c) Michielin, O.; Zoete, V.; Gierasch, T. M.; Eckstein, J.; Napper, A.; Verdine, G.; Karplus, M. *J. Am. Chem. Soc.* **2002**, *124*, 11131−11141. (d) Chierici, S.; Jourdan, M.; Figuet, M.; Dumy, P. *Org. Biomol. Chem.* **2004**, *2*, 2437−2441.

(4) (a) Simon, R. J., et al. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367−9371. (b) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 543−544. (c) Armand, P.; Kirshenbaum, K.; Goldsmith, R. A.; Farr-Jones, S.; Barron, A. E.; Truong, 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. (d) Tran, T. A.; Mattern, R. H.; Afargan, M.; Amitay, O.; Ziv, O.; Morgan, B. A.; Taylor, J. E.; Hoyer, D.; Goodman, M. *J. Med. Chem.* **1998**, *41*, 2679−2685. (e) Mattern, R. H.; Tran, T. A.; Goodman, M. *J. Pept. Sci.* **1999**, *5*, 161−175.

(5) (a) von Roedern, E. G.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 687−689. (b) von Roedern, E. G.; Lohof, E.; Hessler, G.; Hoffmann, M.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 10156−10167. (c) Chakraborty, T. K.; Jayaprakash, S.; Diwan, P. V.; Nagaraj, R.; Jampani, S. R. B.; Kunwar, A. C. *J. Am. Chem. Soc.* **1998**, *120*, 12962−12963. (d) Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S.; Sharma, J. A. R. P.; Ravikanth, V.; Diwan, P. V.; Nagaraj, R.; Kunwar, A. C. *J. Org. Chem.* **2000**, *65*, 6441−6457. (e) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491−514. (f) van Well, R. M.; Marinelli, L.; Altona, C.; Erkelens, K.; Siegal, G.; van Raaij, M.; Llamas-Saiz, A. L.; Kessler, H.; Novellino, E.; Lavecchia, A.; van Boom, J. H.; Overhand, M. *J. Am. Chem. Soc.* **2003**, *125*, 10822−10829. (g) Jensen, K. J.; Brask, J. *Biopolymers* **2005**, *80*, 747−761.

(6) Wermuth, J.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1997**, *119*, 1328−1335.

(7) (a) Schumann, F.; Müller, A.; Koksch, M.; Müller, G.; Sewald, N. *J. Am. Chem. Soc.* **2000**, *122*, 12009−12010. (b) Han, Y. L.; Giragossian, C.; Mierke, D. F.; Chorev, M. *J. Org. Chem.* **2002**, *67*, 5085−5097. (c) Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Fairlie, D. P. *Chem. Rev.* **2004**, *104*, 6085−6117. (d) Gutierrez-Rodriguez, M.; Martin-Martinez, M.; Garcia-Lopez, M. T.; Herranz, R.; Cuevas, F.; Polanco, C.; Rodriguez-Campos, I.; Manzanares, I.; Cardenas, F.; Feliz, M.; Lloyd-Williams, P.; Giralt, E. *J. Med. Chem.* **2004**, *47*, 5700−5712. (e) Cluzeau, J.; Lubell, W. D. *Biopolymers* **2005**, *80*, 98−150.

(8) (a) Etzkorn, F. A.; Guo, T.; Lipton, M. A.; Goldberg, S. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1994**, *116*, 10412−10425. (b) Haubner, R.; Schmitt, W.; Hölzemann, G.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7881−7891.

(9) (a) Veber, D. F.; et al. *Life Sci.* **1984**, *34*, 1371−1378. (b) Gilon, C.; Dechantsreiter, M. A.; Burkhart, F.; Friedler, A.; Kessler, H. In H*ouben-Weyl Methods of Organic Chemistry. Vol E22c. Synthesis of Peptides and Peptidomimetics*; Goodman, M., Felix, A., Moroder, L., Tonolio, C., Eds.; Georg Thieme Verlag: Stuttgart, Germany, 2002; pp 215−291 and references cited therein. (c) Teixido, M.; Albericio, F.; Giralt, E. *J. Pept. Res.* **2005**, *65*, 153−166.

(10) (a) Ruegger, A.; Kuhn, M.; Lichti, H.; Loosli, H. R.; Huguenin, R.; Quiquerez, C.; Wartburg, A. V. *Helv. Chim. Acta* **1976**, *59*, 1075−1092. (b) Angell, Y. M.; Thomas, T. L.; Flentke, G. R.; Rich, D. H. *J. Am. Chem. Soc.* **1995**, *117*, 7279−7280. (c) Klages, J.; Neubauer, C.; Coles, M.; Kessler, H.; Luy, B. *ChemBioChem* **2005**, *6*, 1672−1678.

(11) (a) Sterner, O.; Etzel, W.; Mayer, A.; Anke, H. *Nat. Prod. Lett.* **1997**, *10*, 33−38. (b) Thern, B.; Rudolph, J.; Jung, G. *Angew. Chem., Int. Ed.* **2002**, *41*, 2307−2309. (c) Sewald, N. *Angew. Chem., Int. Ed.* **2002**, *41*, 4661−4663.

 10.1021/ja063123d CCC: $33.50

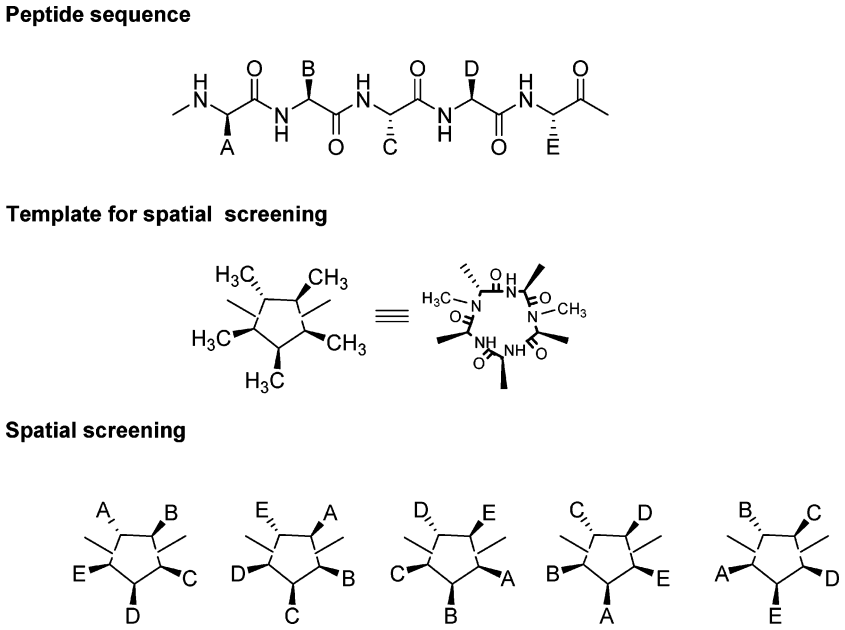


Figure 1. A peptide with pharmacophoric groups A, B, C, D, and E can be screened for the spatial orientation in the bioactive conformation, by synthesis of the five position-shifted cyclic isomers. In the absence of *N*-methylation, the five isomers would have an identical constitution but present pharmacophores differently. In this example, however, the five di-*N*-methylated compounds with shifted *N*-methylated peptide bonds are constitutional isomers.

peptides which have a preferred conformation and may even be orally available.[12a−c] For rational design[1d,13a−c] and spatial screening of distinct bioactive conformations,[14a−b] it is desirable to explore cyclic peptides which (i) are small enough to exhibit conformational rigidity or at least adopt a preferred conformation[5f] and (ii) simultaneously allow the introduction of sufficient functionality (pharmacophores) to achieve the desired biological activity. Conformational restriction seems to be an important prerequisite for oral availability.[15] Cyclic pentapeptides (which are the smallest peptides having strain-free all-trans peptide bonds) or cyclic hexapeptides are well suited for this purpose.[1a,16,17]

Conformations of cyclic peptides are mainly dictated by the chiralities of constituent amino acids. An especially strong influence on conformation is delivered by D- and L-proline.[1b,1c,18] Glycine, on the other hand, can adopt the position of a D- or an L-amino acid, allowing greater flexibility to the system.[19] A systematic screening of the conformational space for bioactive conformations in cyclic pentapeptides has been carried out for thymopentin,[20] RGD-peptides,[14b,21,22] LDT-peptides,[23] and CXCR4.[24] This procedure, known as "spatial screening" (Figure 1),[14a,22] is based on the fact that a cyclic pentapeptide containing one D- and four L-amino acid residues prefer a βII′(γ) conformation, with the D- residue at the $i + 1$ position of the βII′ turn,[25] which is also valid for *cyclo*(-D-Ala-L-Ala$_4$-). Recently a reinvestigation of the conformation of *cyclo*(-D-Pro-L-Ala$_4$-) with modern NMR techniques in solid state and in solution with extended MD simulation also established this structure.[25b] *N*-Methylation of the peptide bond introduces another dimensionality in the cyclopeptide conformation. The effect of *N*-methylation first of all reduces the energy difference between the cis and trans isomer,[26] and at the *N*-methylated site a cis peptide bond is often formed.[9b,17,27] In addition steric hindrances and the lack of hydrogen bond donor ability influence the conformation, making it difficult to predict the conformation of multiply *N*-methylated peptides. However, a systematic shift of the position of *N*-methylation and the single D- residue allows presenting the side chains (pharmacophore) in different spatial positions without changing the constitution (connectivity). We are well aware that here we have selected the structures which

(12) (a) Wenger, R. M. *Helv. Chim. Acta* **1984**, *67*, 502−525. (b) Wenger, R. M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 77−85. (c) Ankersen, M.; Johansen, N. L.; Madsen, K.; Hansen, B. S.; Raun, K.; Nielsen, K. K.; Thorgersen, H.; Hansen, T. K.; Peschke, B.; Lau, J.; Lundt, B. F.; Andersen, P. H. *J. Med. Chem.* **1998**, *41*, 3699−3704.
(13) (a) Bartlett, P. A.; Lauri, G.; Morgan, B. P.; Etzkorn, F. A.; Guo, T. *J. Cell. Biochem.* **1993**, 202−202. (b) Lauri, G.; Bartlett, P. A. *J. Comput.-Aided Mol. Des.* **1994**, *8*, 51−66. (c) Bartlett, P. A.; Lauri, G. *Abstr. Pap. Am. Chem. Soc.* **1996**, *211*, 14.
(14) (a) Kessler, H.; Gratias, R.; Hessler, G.; Gurrath, M.; Müller, G. *Pure Appl. Chem.* **1996**, *68*, 1201−1205. (b) Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461−7472.
(15) Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615−2623.
(16) (a) Kessler, H.; Matter, H.; Gemmecker, G.; Kling, A.; Kottenhahn, M. *J. Am. Chem. Soc.* **1991**, *113*, 7550−7563. (b) Che, Y.; Marshall, G. R. *J. Med. Chem.* **2006**, *49*, 111−124.
(17) Manavalan, P.; Momany, F. A. *Biopolymers* **1980**, *19*, 1943−1973.
(18) (a) Kessler, H.; Müller, A. *Liebigs Ann. Chem.* **1986**, 1687−1704. (b) Kessler, H.; Bats, J. W.; Griesinger, C.; Koll, S.; Will, M.; Wagner, K. *J. Am. Chem. Soc.* **1988**, *110*, 1033−1049. (c) Kessler, H.; Klein, M.; Wagner, K. *Int. J. Pept. Protein Res.* **1988**, *31*, 481−498. (d) Bean, J. W.; Kopple, K. D.; Peishoff, C. E. *J. Am. Chem. Soc.* **1992**, *114*, 5328−5334. (e) Spath, J.; Stuart, F.; Jiang, L.; Robinson, J. A. *Helv. Chim. Acta* **1998**, *81*, 1726−1738. (f) Nikiforovich, G. V.; Kover, K. E.; Zhang, W. J.; Marshall, G. R. *J. Am. Chem. Soc.* **2000**, *122*, 3262−3273. (g) Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev.* **2001**, *101*, 3131−3152.
(19) Kurz, M. Dissertation, Technical University Munich, 1991.
(20) Kessler, H.; Kutscher, B.; Klein, A. *Liebigs Ann. Chem.***1986**, 893−913.
(21) Gurrath, M.; Müller, G.; Kessler, H.; Aumailley, M.; Timpl, R. *Eur. J. Biochem.* **1992**, *210*, 911−921.
(22) Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1375−1389.
(23) Boer, J.; Gottschling, D.; Schuster, A.; Semmrich, M.; Holzmann, B.; Kessler, H. *J. Med. Chem.* **2001**, *44*, 2586−2592.
(24) (a) Fujii, N.; Oishi, S.; Hiramatsu, K.; Araki, T.; Ueda, S.; Tamamura, H.; Otaka, A.; Kusano, S.; Terakubo, S.; Nakashima, H.; Broach, J. A.; Trent, J. O.; Wang, Z. X.; Peiper, S. C. *Angew. Chem., Int. Ed.* **2003**, *42*, 3251−3253. (b) Tamamura, H.; Mizumoto, M.; Hiramatsu, K.; Kusano, S.; Terakubo, S.; Yamamoto, N.; Trent, J. O.; Wang, Z. X.; Peiper, S. C.; Nakashima, H.; Otaka, A.; Fujii, N. *Org. Biomol. Chem.* **2004**, *2*, 1255−1257.
(25) (a) Dechantsreiter, M. A.; Planker, E.; Mathä, B.; Lohof, E.; Hölzemann, G.; Jonczyk, A.; Goodman, S. L.; Kessler, H. *J. Med. Chem.* **1999**, *42*, 3033−3040. (b) Heller, M.; Sukopp, M.; Tsomaia, N.; John, M.; Mierke, D. F.; Reif, B.; Kessler, H. *J. Am. Chem. Soc.* **2006**, *128*, 13806−13814.
(26) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1970**, *9*, 219−235.
(27) Kessler, H.; Anders, U.; Schudok, M. *J. Am. Chem. Soc.* **1990**, *112*, 5908−5916.

only consist of one D- and four L- residues. For a broader variation, the systematic alteration of chiralities adds another dimension into this procedure.

Here we present the synthesis and results from the conformational study of multiply *N*-methylated cyclic alanine peptides of the general formula *cyclo*(-D-Ala-Ala_4-) with different patterns of mono-, di-, tri-, and tetra-*N*-methylated peptide bonds. Our aim is to identify cyclic *N*-methylated alanine peptides which prefer a single conformation in solution and therefore can be used as a template for biologically active peptides (assuming that the substitution of the alanine with other functional side chains will in the first approximation not change the backbone conformation). With the examination of the entire series of these peptides, we can further elucidate the influence of *N*-methylation of peptide bonds on the conformation of cyclic pentapeptides.

General Methods

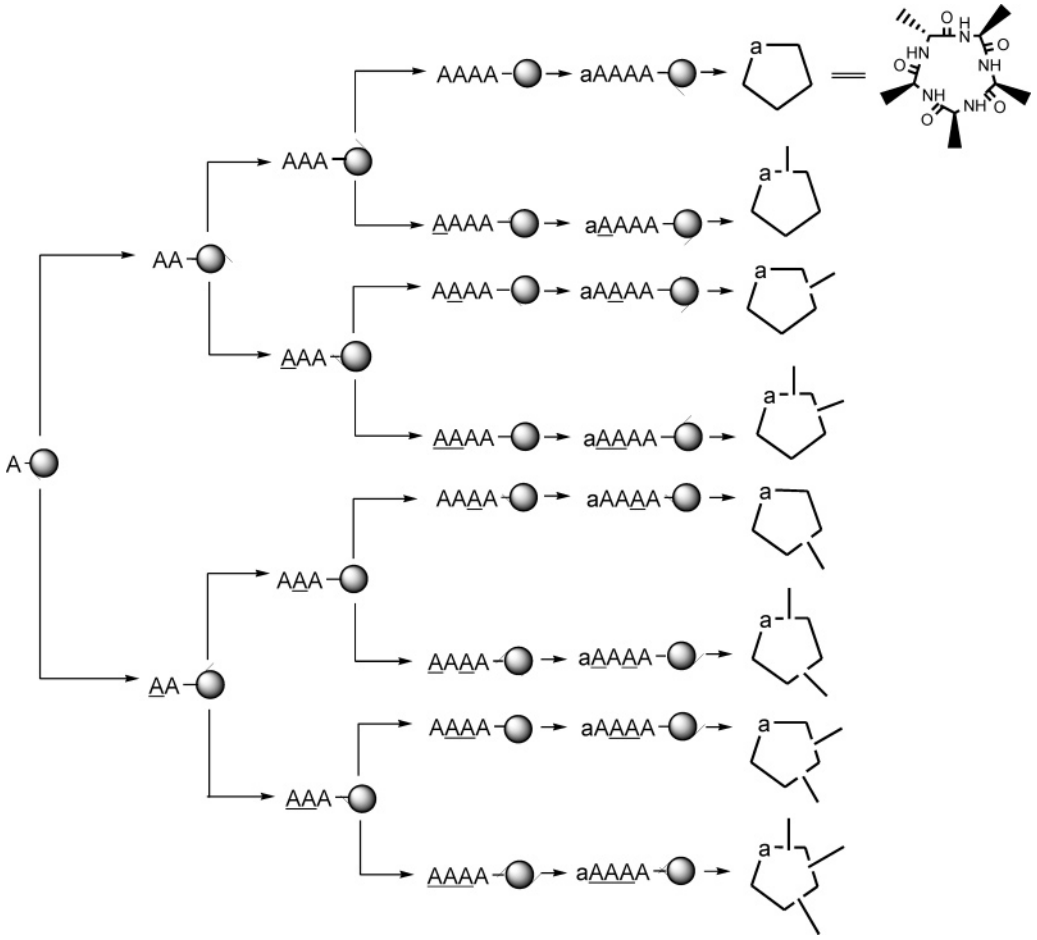
Synthesis of the Cyclic Peptides. *N*-Methyl alanine was synthesized as described by Freidinger et al.[28] All linear peptides were synthesized using standard Fmoc solid-phase strategy using the *o*-chlorotrityl chloride resin.[29,30] Nonmethylated or *N*-methylated alanine was taken as the C-terminal amino acid. However, the yields were lower in the case of a C-terminal *N*-methylated alanine because of endopeptolysis by diketopiperazine formation,[9c,31] which occurs when *N*-methylated amino acids or proline are in position one from the resin. One other observation was the cleavage of the sequence Ala^2-D/L(Me)Ala^1 from the resin, when a D/L(Me)Ala was tried to couple with it. Fmoc-deprotection was achieved with 20 vol % piperidine in NMP, and the other amino acids (2 equiv each) were sequentially coupled with 2 equiv of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)[32] and 1-hydroxybenzotriazole (HOBt) in 1-methyl-2-pyrrolidinone (NMP) as solvent. *N*,*N*-Diisopropylethylamine (DIEA) was used to adjust the pH to 8. However, in the case of coupling to a *N*-methylated residue, 2 equiv each of *N*-methyl alanine or alanine, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate (HATU),[33,34] and 1-hydroxy-7-azabenzotriazole (HOAt)[35] were used along with DIEA to maintain a pH of 8 in NMP as solvent. Due to HOBt/HOAt and HBF_4 formation, the pH drops while the reaction proceeds, leading to reduced nucleophilicity of the amino group. Therefore, in the case of insufficient couplings as monitored with the Kaiser test,[36] additional base was added, but the pH was not allowed to exceed a value of 8.5. The coupling time ranged from 20 to 45 min. The *o*-chlorotrityl linker allows cleaving off the linear peptide with a mild treatment of an acetic acid/2,2,2-trifluoroethanol (TFE) mixture in dichloromethane (DCM) without affecting the peptide bonds. The head-to-tail cyclization was performed with diphenylphosphorylacid azide (DPPA),[37,38] applying the solid base method using $NaHCO_3$ in *N*,*N*-dimethylformamide (DMF).[39] After the completion of cyclization, which was monitored by ESI mass spectroscopy, DMF was evaporated and the crude peptide was redissolved in a minimum amount of dry acetonitrile, leaving behind the cyclization reagents. The pure compound was obtained by reversed-phase high-performance liquid chromatography (RP-HPLC) purification. The peptides were characterized by ESI mass spectroscopy and various NMR techniques. A representative synthesis is shown in Scheme 1, where a small "a" stands for D-Ala and "A" stands for L-Ala; three further schemes are provided in the Supporting Information illustrating the synthesis of all 30 peptides. The underlined letters a or A indicate *N*-methylation. After each coupling the resin was divided into two halves, one for the coupling with nonmethylated and the other for the coupling with methylated alanine.

NMR Spectroscopy. All spectra were recorded at 297 K on a 500 MHz Bruker DMX spectrometer (Bruker, Karlsruhe, Germany), in DMSO-d_6 (^1H, 2.49 ppm; ^{13}C 39.5 ppm) and were processed using XWINNMR (Bruker) and analyzed with either XWINNMR or SPARKY.[40] The assignment of all proton and carbon resonances followed the standard strategy as previously described.[41] Sequential assignment was accomplished by through-bond connectivities from heteronuclear multiple bond correlation (HMBC)[42] spectra. The *N*-methyl group was taken as the reference for the sequential assignment, the protons of which show a correlation with the ^{13}C atom of the intraresidual β-protons by a four-bond coupling in the HMBC spectrum. The β-protons further show a correlation with the ^{13}C shift of intraresidual carbonyl by a three-bond coupling, and this carbonyl also correlates with the H^α shift of the same residue and the H^N shift of the adjacent residue (if not *N*-methylated) by a strong two-bond coupling. In this way the full sequence assignment was accomplished. TOCSY spectra were recorded with a mixing time of 60 ms, and ROESY spectra, with a mixing time of 150 ms. Temperature coefficients for the amide protons of each peptide were determined from one-dimensional spectra in the range from 297 to 327 K with a step size of 5 K. Many of these compounds show more than one conformation in slow exchange on the NMR time scale of chemical shift separation at room temperature. Chemical exchange was confirmed by exchange peaks in ROESY spectra, which show an inverted sign compared to signals caused by ROEs.[43]

Computational Methods. Proton distances were calculated according to the isolated two-spin approximation from volume integrals of rotating frame nuclear Overhauser enhancement (ROESY)[44,45] spectra. The integrated volumes of the ROE cross-peaks were offset corrected[46] and converted to proton−proton distances employing the cross-peak intensity of $H^\alpha-H^\beta$ of alanine as reference (2.19 Å). Restraints were obtained by adding and subtracting 10% to the calculated experimental distances, accounting for errors from the two-spin approximation and cross-peak integration. Metric matrix DG calculations were carried out with a home-written program utilizing random metrization.[47] Experimental distance constraints which were more restrictive than the geometric distance bounds (holonomic restraints) were used to create the final distance matrix. The structures were first embedded in four dimensions and then partially minimized using conjugate gradients followed by distance and angle driven dynamics (DADD),[48,49] wherein

(28) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77−81.
(29) Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiu, G.; Sotiriu, P.; Yao, W. Q.; Schäfer, W. *Tetrahedron Lett.* **1989**, *30*, 3943−3946.
(30) Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513−520.
(31) Anteunis, M. J. O.; Vanderauwera, C. *Int. J. Pept. Protein Res.* **1988**, *31*, 301−310.
(32) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927−1930.
(33) Carpino, L. A.; Elfaham, A.; Minor, C. A.; Albericio, F. *Chem. Commun.* **1994**, 201−203.
(34) Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678−9683.
(35) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.
(36) Kaiser, E.; Colescot, R. I.; Bossinge, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598.
(37) Shioiri, T.; Yamada, S.; Ninomiya, K. *J. Am. Chem. Soc.* **1972**, *94*, 6203−6205.
(38) Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. *J. Org. Chem.* **1979**, *44*, 3101−3105.
(39) Brady, S. F. P., W. J.; Arison, B. H.; Freidinger, R. M.; Nutt, R. F.; Veber, D. F. *8th Annual Peptide Symposium*; 1983; pp 127−130.
(40) Kneller, D. G.; Kuntz, I. D. *J. Cell. Biochem.* **1993**, 254−254.
(41) Kessler, H.; Schmitt, W. In *Encyclopedia of Nuclear Magnetic Resonance*; Grant, D. M., Harris, R. K., Eds.; J. Wiley & Sons: New York, 1995.
(42) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.
(43) Kessler, H.; Gehrke, M.; Griesinger, C. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 490−536.
(44) Bothnerby, A. A.; Stephens, R. L.; Lee, J. M.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811−813.
(45) Kessler, H.; Griesinger, C.; Kerssebaum, R.; Wagner, K.; Ernst, R. R. *J. Am. Chem. Soc.* **1987**, *109*, 607−609.
(46) (a) Griesinger, C.; Ernst, R. R. *J. Magn. Reson.* **1987**, *75*, 261−271. (b) Bax, A. *J. Magn. Reson.* **1988**, *77*, 134−147.
(47) Havel, T. F. *Prog. Biophys. Mol. Biol.* **1991**, *56*, 43−78.
(48) Mierke, D. F.; Scheek, R. M.; Kessler, H. *Biopolymers* **1994**, *34*, 559−563.

Scheme 1. Schematic Approach Showing the Combinatorial Approach Adapted to Obtain the Library of Cyclic Peptides Starting with L-Alanine Loaded to the Resin



only distance constraints were used. The DADD simulation was carried out at 1000 K for 50 ps with a gradual reduction in temperature over the next 30 ps. The DADD procedure utilized holonomic and experimental distance constraints plus a chiral penalty function for the generation of the violation "energy" and forces. A distance matrix was calculated from each structure, and the EMBED algorithm was used to calculate coordinates in three dimensions. About 95−100 structures were calculated for each peptide, and >90% of the structures of every peptide did not show any significant violation. The MD calculations were carried out with the program DISCOVER using the CVFF force field.[50] The structure resulting from the DG calculation was placed in a cubic box of length 25 Å and soaked with DMSO,[51] and a restrained MD simulation was carried out. After energy minimization using the steepest descent and conjugate gradient, the system was heated gradually, starting from 10 K and increasing to 50, 100, 150, 200, 250, and 300 K in 2 ps steps, each by direct scaling of velocities. The system was equilibrated for 50 ps with temperature bath coupling (300 K). Configurations were saved every 100 fs for another 150 ps. Finally a 150 ps free MD simulation at 300 K was carried out to prove that the stability of the calculated conformation in the solvent is similar to the structure obtained from experimental restraints.

Results

A combinatorial approach was adopted for the synthesis of the library of 30 compounds shown in Scheme 1. Most of the pure cyclic peptides exhibit two or more conformations in different amounts, in slow exchange on the NMR time scale; the conformational abundance of all compounds is listed in Figure 2A. However here we describe only those compounds which are conformationally homogeneous on the NMR time scale. Out of the 30 peptides only 7 (Figure 2B) were found to prefer one conformer (>97%). Thus these cyclic peptides can serve as useful templates for designing non-Ala peptides. Cyclic peptides which exhibit an abundance of 85% may also be useful candidates, and the structural characterization is currently underway.

Conformational Preference and Constitution. Six out of seven compounds that preferred a single conformation had the D-residue *N*-methylated (Figure 2B). This resembles the old observation that a D-Pro (bearing an *N*-alkyl amide bond) has a very strong conformation determining effect.[1a,16,17] In cyclic peptides of the type *cyclo*(-D-Pro-L-Xaa$_4$-), proline always occupies the $i + 1$ position of a βII′ turn.[25b] However, it seems that the cyclic five-membered ring of proline, which fixes the Φ angle only between $\pm 60°$,[52] is not optimal for steric reasons. In compound **1**, Φ is close to $+120°$, allowing maximal distance between the *N*-methyl group and the β-protons of the adjacent D-Ala[1] and Ala[5]. In the series of five compounds bearing a single *N*-methylation (Figure 2A), only **1** shows a conformational abundance of >98% on the NMR time scale. Compound **1** shows a tendency to form a (βII′/γ) turn structure. However, a

(49) Mierke, D. F.; Geyer, A.; Kessler, H. *Int. J. Pept. Protein Res.* **1994**, *44*, 325−331.
(50) Hagler, A. T.; Lifson, S.; Dauber, P. *J. Am. Chem. Soc.* **1979**, *101*, 5122−5130.
(51) Mierke, D. F.; Kessler, H. *J. Am. Chem. Soc.* **1991**, *113*, 9466−9470.
(52) Hutchinson, E. G.; Thornton, J. M. *Protein Sci.* **1994**, *3*, 2207−2216.

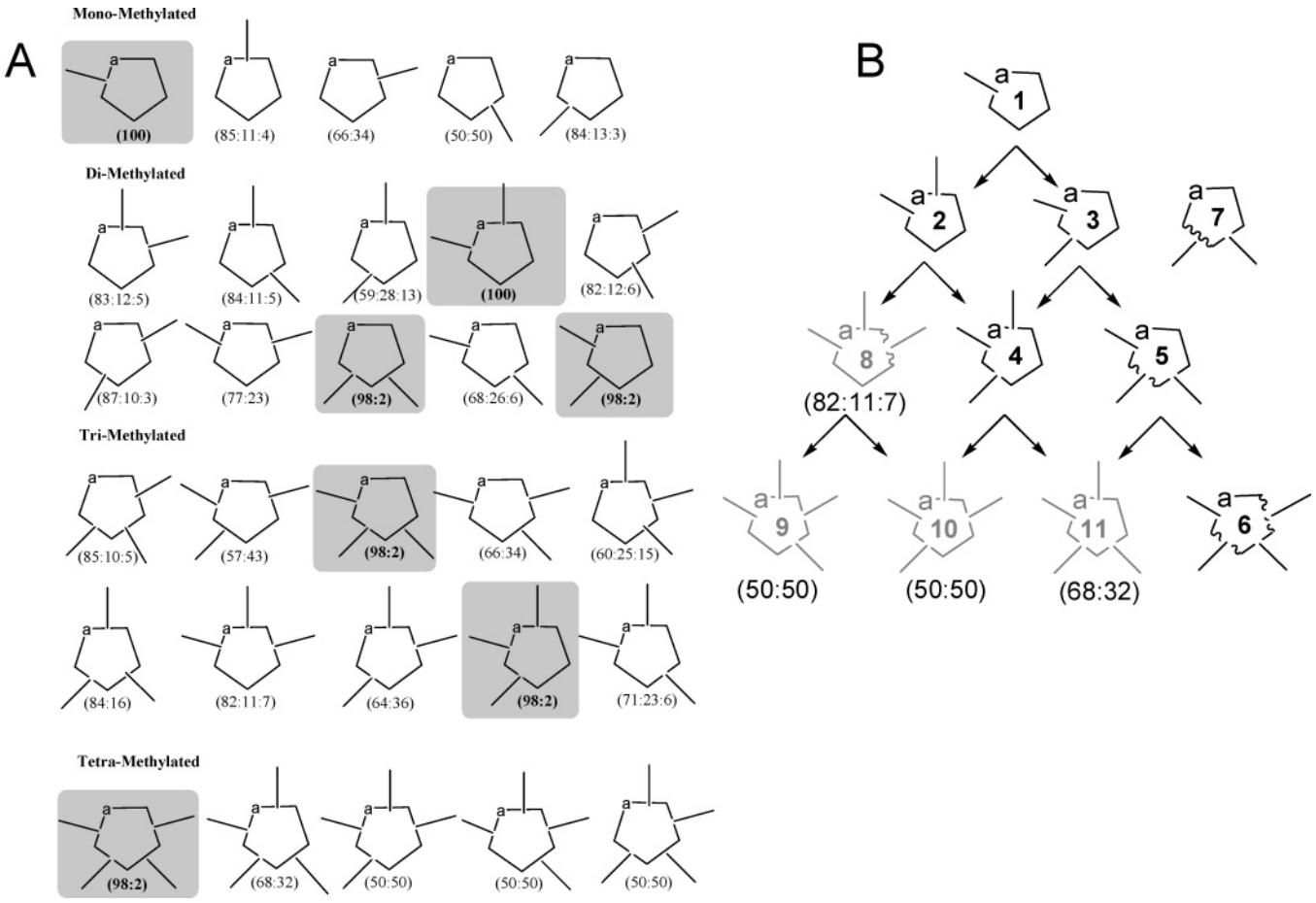


Figure 2. (A) Library of all the synthesized *N*-methylated peptides with their conformational abundance on the NMR time scale of chemical shift separation. The values in parentheses denote the ratio of all the conformers calculated from a ^{1}H spectrum. (B) Schematic diagram illustrating the numbering of the compounds and the correlation between the location and number of *N*-methylated sites and the compounds preferring a single conformation. Conformationally pure peptides are shown in black, and those having multiple conformations on the NMR time scale are shown in gray, with the trans/cis abundance given in the parentheses. The three tetra-*N*-methylated compounds are shown to complete the pattern. The wavy bonds in **5**, **6**, **7**, and **8** denote a cis peptide bond.

standard βII′-turn[53,54] is not possible, because the *N*-methyl group sterically interferes with the β-protons of Ala^5 and D-Ala^1. It is remarkable that *N*-methyl D-Ala induces a similar conformation as D-Pro, but it seems that *N*-methyl D-Ala provides an even stronger force,[55] as Φ is not constrained to about $\pm 60°$ as in proline. Out of the 10 di-*N*-methylated compounds only **2**, **3**, and **7** reveal to prefer a single conformation. Out of these, two compounds have an *N*-methylation on either side of the monomethylated parent compound **1**. Further, out of 10 tri-*N*-methylated compounds only **4** and **5** prefer a single conformation, which are the compounds arising from *N*-methylation on either side of compound **3**. The third tri-*N*-methylated compound **8** in Figure 2B, resulting from further *N*-methylation of compound **2**, shows three conformations in 82%, 11%, and 7% abundance and will not be discussed here. Four of the conformationally homogeneous peptides (**1**−**4**) have all-trans amide bonds, whereas compounds **5**, **6**, and **7** have a cis peptide bond between amino acids Ala^4 and Ala^5; the tetra-*N*-methylated peptide **6** has even a second cis bond between Ala^2 and Ala^3.

Conformation of the Seven Cyclic Peptides. *cyclo*(-D(Me)Ala^1-Ala^2-Ala^3-Ala^4-Ala^5) (1). The ROESY spectrum of the compound did not show the presence of a single $H^{\alpha}(i)-H^{\alpha}(i+1)$ cross-peak,[27] so all the peptide bonds have to be in a trans conformation. This peptide is the simple *N*-methylated analogue of the parent peptide *cyclo*(-aA$_4$-), which prefers a βII′ on one side with D-Ala at the $i+1$ position of the βII′ turn and an equilibrium of a closed or open γ type conformation about Ala^4. The spectrum reveals strong cross-peaks between the H^NAla^2−H^{α}D-Ala^1 and H^NAla^2−H^NAla^3. The conformation of **1** (Figure 3) in a way resembles that of the parent peptide *cyclo*(-aA$_4$-) without the *N*-methyl group.

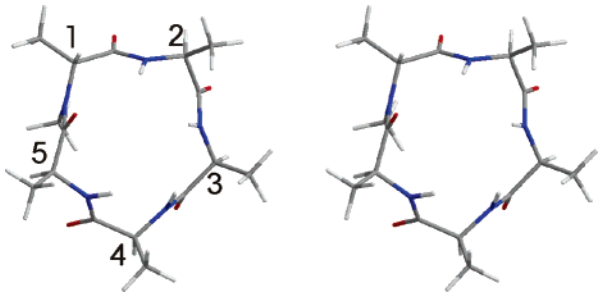


Figure 3. Stereo pictures of **1**, resulting from energy minimization of 200 steps of steepest descent of the average structure after 150 ps of restrained and free MD simulation in DMSO solvent box.

In *cyclo*(-aA$_4$-) a βII′ turn about a^{1}A^2 is the most characteristic feature; in this βII′ turn both H^N's of Ala^3 and Ala^2 are directed

(53) Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109.
(54) Nagarajaram, H. A.; Ramakrishnan, C. *J. Bioscience* **1995**, *20*, 591−611.
(55) Takeuchi, Y.; Marshall, G. R. *J. Am. Chem. Soc.* **1998**, *120* (22), 5363−5372.

Table 1. Table Depicting the Values of Temperature Gradients of NH Protons ($-\Delta\delta/\Delta T$) in ppb/K; the Values Shown in Parentheses Are the Respective ^3J (H^N-H^a) Coupling Constants in Hz

compound	Ala[1]	Ala[2]	Ala[3]	Ala[4]	Ala[5]
1		2.5 (9.1)	2.7 (8.1)	3.4 (6.9)	2.3 (8.3)
2			3.7 (7.4)	0.6 (7.3)	5.5 (8.2)
3		1.5 (9.4)	2.3 (6.2)	1.1 (7.9)	
4			4.7 (5.7)	−1.3 (7.8)	
5		6.0 (6.9)	0.4 (7.5)		
6		5.1 (9.4)			
7	5.5 (6.2)	5.8 (6.7)	2.3 (7.6)		

above the ring plane and have a short distance. The carbonyl group of Ala[5] forms a bifurcated hydrogen bond to both NH protons of Ala[2] and Ala[3]. In **1** the peptide bond between Ala[5] and D(Me)Ala[1] is turned by about −60°, resulting in the "disruption" of the hydrogen bond to Ala[3] H^N. The conformational change obviously is a consequence of the steric hindrance of the *N*-methyl group with the β-protons of Ala[5] and D-Ala[1]. It is well accepted that internal hydrogen bonding in cyclic peptides does not lead to strong energetic stabilization, and instead stereoelectronic and steric effects are more important in determining the conformation of cyclic peptides.[56] In addition to the steric effect of methyl groups, the carbonyl group of Ala[5] is now ideally syn-oriented to the $C^\alpha H$ bond of D-Ala[1] (see below in the discussion). The conformation about residues Ala[3]-Ala[5] is similar to that in *cyclo*(-aA$_4$-); however, it may also form a rapid equilibrium between a γ-turn and the conformation in which both the NH bonds point in the same direction as in the case of *cyclo*(-pA$_4$-).[4] We also observe in **1** ROEs between the NH protons; however due to a relatively low number of data we cannot exclude the participation of a γ-turn conformation in rapid equilibrium with the conformation shown in Figure 3. The coupling constants (Table 1) are in good agreement with the proposed conformation, and the Φ angles of Ala[3] and Ala[5] are close to −120° and that of Ala[2] close to −100°, leading to an antiperiplanar arrangement of NH to the $C^\alpha H$ bond and large coupling constants. An exception is Ala[4], which may be involved partially in a γ-turn structure by a twist of the peptide bond Ala[3]CO−Ala[4]NH, resulting in the changes of Ψ and Φ angles which are in fast equilibrium[51] and therefore exhibiting a smaller H^NH^α coupling constant.

Only slight deviations between calculated (from the trajectory average) and observed (from the ROESY spectrum) distances are observed, with a single exception; the H^NAla[4] and H^αAla[4] distance is too short by about 0.4 Å, again giving evidence for a participation of the γ-turn about this residue, similar to the case in the non-*N*-methylated cyclic pentapeptides.

***cyclo*(-D(Me)Ala[1]-(Me)Ala[2]-Ala[3]-Ala[4]-Ala[5]) (2).** This compound can be obtained by a further substitution of the H^N of Ala[2] with an *N*-methyl group with respect to compound **1**. Sterically it is indeed possible; however, some steric hindrance with the β-protons of Ala[2] and H^N of Ala[3] leads to further deviation from the βII′ turn which is present in the nonmethylated stem peptide *cyclo*(-aA$_4$-). The structure exhibits all carbonyl groups syn oriented to the $C^\alpha H$ bond of the following residue (Figure 4). As this is an energetically preferred orientation, **2** exhibits only a single conformation on the NMR time scale of chemical shift separation (spectral details can be found in the Supporting Information).

(56) Snyder, J. P. *J. Am. Chem. Soc.* **1984**, *106*, 2393−2400.

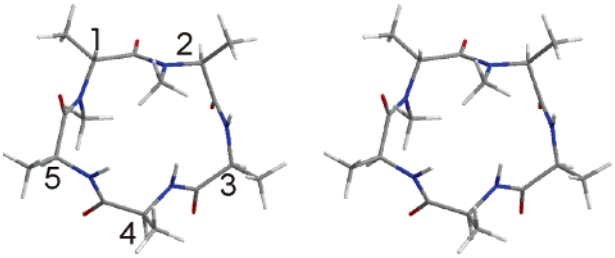


Figure 4. Stereo pictures of **2** (for details see Figure 3).

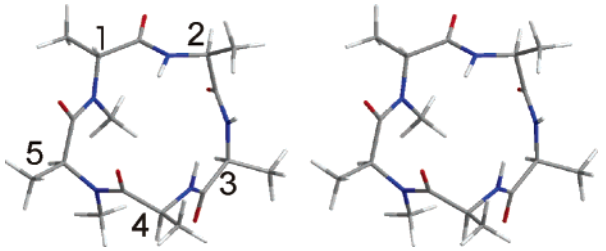


Figure 5. Stereo pictures of **3** (for details see Figure 3).

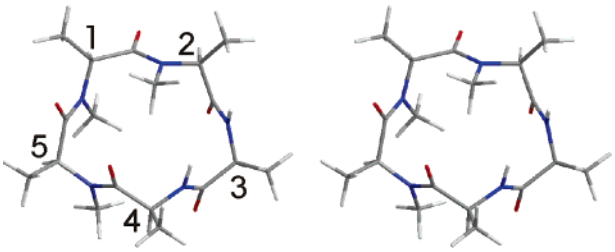


Figure 6. Stereo pictures of **4** (for details see Figure 4).

***cyclo*(-D(Me)Ala[1]-Ala[2]-Ala[3]-Ala[4]-(Me)Ala[5]) (3).** The structure of **3** (Figure 5) can be created by shifting the *N*-methyl groups from residue Ala[2] in **2** to Ala[5]. The steric interference of the *N*-methyl group of Ala[5] with β-protons of Ala[5] results in the rotation of the peptide bond plane by about 170° in comparison to the Ala[4]-Ala[5] peptide bond plane in **2**. The rest of the molecule is almost identical to the fragment in **2**.

***cyclo*(-D(Me)Ala[1]-(Me)Ala[2]-Ala[3]-Ala[4]-(Me)Ala[5]) (4).** Compound **4** (Figure 6) results from the *N*-methylation of residue Ala[5] of **2** or the residue Ala[2] of **3**. This compound shows structural similarity with both **2** and **3**. The structure of the fragment from Ala[3] to Ala[1] is almost identical with the same fragment in **3**, and the one from Ala[5] to Ala[4] is identical to that of **2**.

***cyclo*(-D(Me)Ala[1]-Ala[2]-Ala[3]-(Me)Ala[4]-(Me)Ala[5]) (5).** This compound results from the shifting of the *N*-methyl group of residue Ala[2] in **4** to residue Ala[4]. The *N*-methylation of residue Ala[4] results in the complete rotation of the peptide bond plane between Ala[3] and Ala[4] by 170° in comparison to the previously described structures. This is because of the strong steric clash between the *N*-methyl of Ala[4] and β-protons of Ala[4] and Ala[3] (Figure 7). In addition **5** shows a cis peptide bond between the residues Ala[4] and Ala[5], which might result from the steric clash between the *N*-methyl groups of Ala[4] and Ala[5], as the *N*-methyl of Ala[5] would project below the plane, if the Ala[4]-Ala[5] peptide bond were trans. The fragment of **5** from D-Ala[1]H^α to Ala[3]H^N resembles a βII′ turn; however, the Ala[5]-Ala[1] peptide bond has also undergone a flip of 80° in comparison to Ala[5]-Ala[1] in **4**. This twist in the peptide bond is caused by steric interaction, which the *N*-methyl group would encounter with the β-protons

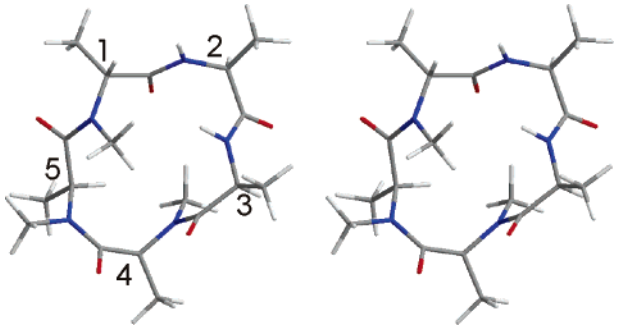


Figure 7. Stereo pictures of **5** (for details see Figure 3).

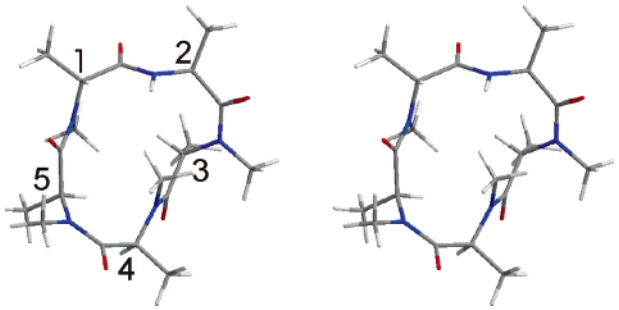


Figure 8. Stereo pictures of **6** (for details see Figure 3).

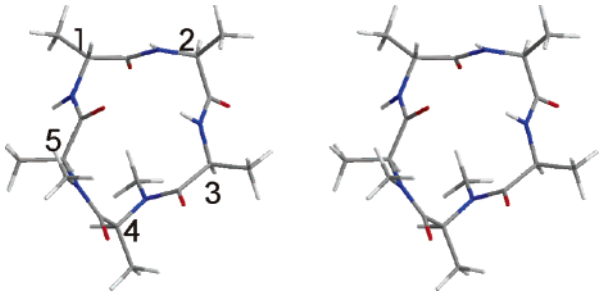


Figure 9. Stereo pictures of **7** (for details see Figure 3).

of Ala^5, as it has been forced to project down the plane by the cis peptide bond.

***cyclo*(-D(Me)Ala^1-Ala^2-(Me)Ala^3-(Me)Ala^4-(Me)Ala^5) (6).** Compound **6** can be obtained by further *N*-methylation of the Ala^3 residue of **5**. To our surprise this compound exhibited two cis peptide bonds, one about Ala^2-Ala^3 and the other about Ala^4-Ala^5 (Figure 8). This is the only compound, which has an *N*-methylation at residue Ala^3, which results in the formation of an Ala^2-Ala^3 cis peptide bond to accommodate the bulky methyl group. The compound shows a structural similarity with **5**, in the region from Ala^4 to Ala^1; however, it is interesting to note the orientations of the *N*-methyl groups of D-Ala^1 and Ala^4

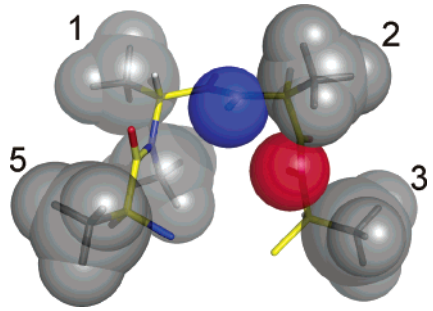


Figure 10. Compound **1** showing the regions having similar structural elements to those of peptides **2**, **3**, and **4**. The site where a methyl group can be incorporated is given in blue, and the disallowed region is shown in red. The residue numbers are denoted.

with respect to their orientation in **5**. Both the Ala^3-Ala^4 and Ala^5-D-Ala^1 peptide bonds have undergone a flip of about 160° about their adjacent Φ and Ψ angles, and thus the *N*-methyl groups always tend to project in opposite directions of the plane to minimize their mutual steric interaction. The driving force to orient the Ala^4 *N*-methyl group above the plane is the Ala^2-Ala^3 cis peptide bond which forces the β-protons of Ala^3 to project down the plane, which eventually reorients the *N*-methyl group projecting it above the plane.

***cyclo*(-DAla^1-Ala^2-Ala^3-(Me)Ala^4-(Me)Ala^5) (7).** This is the only compound (Figure 9) in the homogeneous series in which the D-Ala^1 is not *N*-methylated. The compound resembles **5**, in the region from Ala^2 to Ala^5 revealing the tendency of the peptides to form a cis peptide bond when the *N*-methylation is at Ala^4 and Ala^5. The upper part of the molecule resembles a βII′ turn with the D-Ala^1 at the $i + 1$ position of the turn, as in the case of *cyclo*(-aA$_4$-); however the Ψ of Ala^2 (Table 2), i.e., of the ($i + 2$) residue, deviates more than that of the ($i + 1$) residue from the standard angle of −120° and 0° for the ($i + 1$) and ($i + 2$) residue.[52−54]

Discussion

Synthesis of all different *N*-methylated derivatives of the cyclic pentapeptide *cyclo*(-D-Ala-Ala$_4$-) except the pentamethylated compound has been achieved. Whereas cyclization turned out not to be a crucial step, synthesis of some linear precursors (when the sequence Ala^2-D/L(Me)Ala^1 is linked to the resin) did not work. This problem can be overcome by changing the sequence, which, after cyclization, leads to the desired peptide (Scheme 4 in the Supporting Information).

Our NMR analysis showed that only 7 out of the 30 compounds prefer a single conformation on the NMR time scale of chemical shift separation, whereas the others indicate

Table 2. Table with the Φ and Ψ Values of Peptides **1**−**7** in Degrees[a,b]

	D-Ala^1			Ala^2			Ala^3			Ala^4			Ala^5		
	Φ	Ψ	Ω	Φ	Ψ	Ω	Φ	Ψ	Ω	Φ	Ψ	Ω	Φ	Ψ	Ω
1	117	−62	t	−82	−63	t	−131	−56	t	−73	−56	t	−133	83	t
2	129	−76	t	−97	−76	t	−77	−44	t	−120	−67	t	−117	77	t
3	138	−56	t	−88	−87	t	−119	−16	t	−105	88	t	53	63	t
4	126	−69	t	−105	−83	t	−81	−53	t	−108	102	t	57	70	t
5	−89	−116	t	−65	−25	t	−126	−69	t	59	78	t	−124	62	c
6	134	−84	t	−142	7	t	−130	57	c	−142	56	t	−117	146	c
7	84	−108	t	−66	−42	t	−144	78	t	56	66	t	−109	173	c

[a] The Ω values are summarized as cis and trans peptide bond, denoted as c and t, respectively. The ideal Φ and Ψ of the ($i + 1$) residue in a βII′ turn are 60° and −120° and of the ($i + 2$) residue are −80° and 0°, respectively, and in a γ turn the values range from 70° to 80° for Φ($i + 1$) and −60° to −70° for Ψ($i + 1$). [b] The temperature gradient of Ala^{3}H^N is in agreement with its solvent shielded nature.

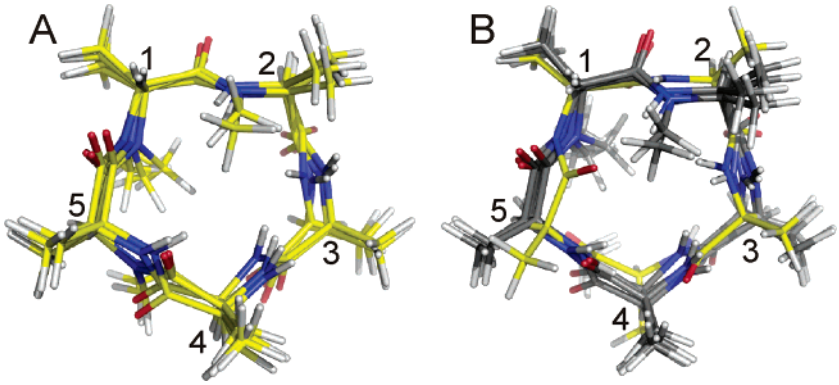


Figure 11. (A) Overlay of **1**, **2**, **3**, and **4**, showing the similar structural elements. Note the flip in the Ala[4]-Ala[5] peptide bond owing to *N*-methylation. It can clearly be seen that the peptide bond between Ala[4] and Ala[5] is twisted in the two compounds **3** and **4** about the adjacent Φ and Ψ angles to allow the *N*-methyl group to fit sterically. (B) Overlay of the parent peptide *cyclo*(-aA$_4$-) (yellow) and peptides **1**, **2**, **3**, and **4** (gray).

equilibrium between two or more conformations in slow exchange. The seven "homogeneous" peptides were studied in detail by ROE-restrained molecular dynamics and finally by free dynamics in explicit solvent.

The seven compounds show that most of the Φ-angles are close to 120°, which orients the carbonyl group syn to the C$^\alpha$H bond. This is a good indication that the structures are energetically relaxed, and this may be the reason for preferring a single conformation. However, a single conformation does not mean that there is rigidity on the fast NMR time scale. The structures which are related to the stem peptide (**1**, **2**, **3**, and **4**) give evidence for a rapid reorientation of the peptide bond Ala[3]-Ala[4].[25b,57]

It is evident that molecules with the sequence Ala[5]-D(Me)Ala[1] are strongly fixed in a conformation in which all three methyl groups avoid steric clash. Any hydrogen bond in a βII′ turnlike structure which is found in the nonmethylated stem peptide *cyclo*(-aA$_4$-) is removed by the twist of the Ala[5]-D(Me)Ala[1] peptide bond about the adjacent phi and psi angles. The conformation of the higher *N*-methylated compounds can be directly derived from the structure of **1**. If there is enough space (indicated in blue in Figure 10), the incorporation of an N-Me group instead of the NH group introduces only minor conformational changes (see **2**, **3**, and **4**).

In contrast, introduction of the N-Me group at the Ala[2]-Ala[3] peptide bond (indicated in red in Figure 10) is not sterically allowed, and the conformation changes into a cis peptide bond (see **6**). This is similar to a substitution of NH of the Ala[3]-Ala[4] peptide bond by N-Me: this introduces a cis peptide bond between the Ala[4] and Ala[5] residues. Thus we can classify these seven compounds into two families, one with **1**, **2**, **3**, and **4**, in which all the peptide bonds are trans showing a structural similarity in the region from Ala[5]C$^\alpha$-Ala[4]C$^\alpha$ (Figure 11), and the other consisting of **5**, **6**, and **7**, which share an Ala[4]-Ala[5] cis peptide bond.

An overlay of all the four peptides **1**, **2**, **3**, and **4** with the parent peptide *cyclo*(-aA$_4$-) (Figure 11), clearly depicts the influence of *N*-methylation on the peptide structure with respect to the parent peptide. The *N*-methylation of Ala[1] forces the peptide bond outward, nullifying the chances of hydrogen bonding with Ala[2] and/or Ala[3]NH. The *N*-methylation of Ala[2], however, results in an inward twist of the peptide bond to avoid any steric clash with Ala[2] β-protons. Finally, the *N*-methylation of Ala[5] leads to the twist of the peptide bond plane by about 170° with respect to the nonmethylated peptide bond.

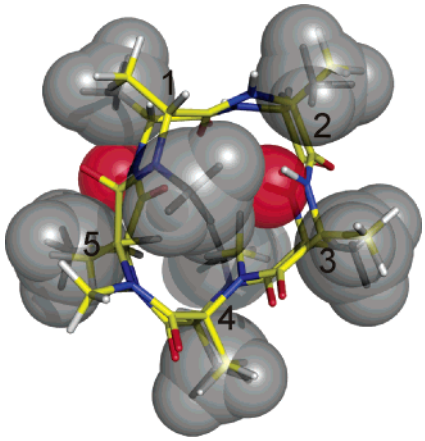


Figure 12. An overlay of **5** and **7**, clearly depicting the rotation of the Ala[5]-D-Ala[1] peptide bond plane as a consequence of the substitution by an *N*-methyl group. The red spheres denote the NHs which, on methylation, lead to conformational changes. Only the spheres of the β-protons of **7** are shown.

The compounds **5** and **7** are almost structurally identical except for the twist of the Ala[5]-D-Ala[1] peptide bond plane, owing to steric hindrance (Figure 12). The structural features of **6** differ greatly in conformation in the region of D-Ala[1]-Ala[3] from **5** and **7**, owing to the Ala[2]-Ala[3] cis peptide bond.

The further *N*-methylation of the conformationally homogeneous peptides though does not guarantee the existence of a preferred conformer on the NMR time scale; however, there is a certain consistency in the conformational orientation of the cyclic peptides. The major conformer of peptide **8**, obtained by extending the *N*-methylation of **2**, reveals a cis peptide bond between Ala[2] and Ala[3], similarly both **9** and **10** also exhibit this characteristic cis peptide bond. One of the conformers of **9** is structurally identical to **8**; however, the other conformer surprisingly exhibits a trans Ala[2]-Ala[3] peptide bond and a cis Ala[3]-Ala[4] peptide bond instead. In compound **10**, both conformers contain the Ala[2]-Ala[3] cis peptide bond, while only one of the conformers also exhibits an Ala[4]-Ala[5] cis peptide bond. Finally, the major conformer of peptide **11** shows structural similarities with **5**, revealing an Ala[4]-Ala[5] cis peptide bond, and the minor is an all trans peptide resembling **4**.

These findings now allow for the rational design of a smaller sequence of amino acids into a specific conformation. Especially

(57) Mierke, D. F.; Kurz, M.; Kessler, H. *J. Am. Chem. Soc.* **1994**, *116*, 1042−1049.

the above-mentioned conformation of the Xaa-D(Me)Yaa will certainly be found in any pentapeptide regardless of the chemical nature of the amino acids, Xaa or Yaa. On the other hand, the structures presented here can be taken for "spatial screening" as mentioned above (see Figure 1). We were actually surprised to find so few different structures in these "homogeneous" cyclic pentapeptides. Perhaps some of the other structures, which do not prefer a single conformation (e.g., a pattern of cis/trans isomers) but still show a predominant conformation, can also be used for the design. The examination of these compounds as structural templates of preferred conformation is currently underway.

Conclusion

Our results show that though *N*-methylation introduces considerable flexibility to the backbone of cyclic pentapeptides, still some patterns of *N*-methylation help in directing the molecule to a specified structure. Although most of the multiply *N*-methylated pentapeptides of the structural type *cyclo*(-aA$_4$-) exhibit equilibrium of various conformations in slow interconversion; however, 7 out of the 30 *N*-methylated peptides are conformationally homogeneous on the NMR time scale of chemical shift separation. But we have to realize that although the compounds exhibit conformational preferences they still can be flexible (with dynamics fast on the NMR time scale). Especially a flip of the plane of the peptide bond Ala^3-Ala^4, which has been previously proven for *cyclo*(-aA$_4$-), is observed. In addition to the seven "homogeneous" structures examined here, 10 more structures show the tendency to adopt a preferred conformation. We believe that the substitution of the amino acid residues and retaining chiralities with the same pattern of *N*-methylation will give rise to the same backbone conformation described above. One significant observation is that, out of the seven identified structures, six have the D-residue *N*-methylated. Thus one might speculate that incorporation of more D-residues may direct the molecule to adopt a preferred conformation. One other important observation is that multiple *N*-methylation, i.e., tri- and tetra-*N*-methylation, introduces conformational equilibria between a number of conformations. Thus most likely, for cyclic pentapeptides, the promising templates are the ones with mono- and di-*N*-methylation.

Acknowledgment. The authors gratefully acknowledge technical assistance from Burkhard Cordes and Guido Clever of the Carell group at LMU (München) for measuring the high resolution mass spectra. We thank the Humboldt Foundation for the generous support via the Max-Planck-Forschungspreis.

Supporting Information Available: Experimental details, with ^{1}H-chemical shift data, ESI-HRMS, TOCSY, ROESY spectra of peptides **1**−**7** and ROE violation lists are available along with the complete reference of all coauthors of refs 4a and 9a. This material is available free of charge via the Internet at http://pubs.acs.org.

JA063123D