Stereochemical Implications on Diversity in β-Turn Peptidomimetic Libraries

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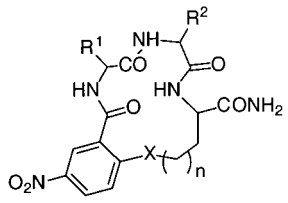
Stereoisomers of compound **1** were prepared, and their preferred solution-phase conformations (in DMSO) were determined by a combination of NMR, CD, and molecular simulation approaches. It was shown that the stereochemistry of the *pseudo i* + 2 residue is the predominant consideration.

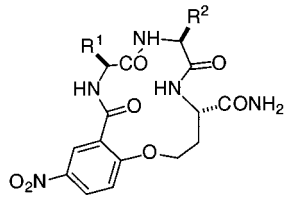
Discussions relating to efficient "coverage of diversity space" in large libraries tend to focus on issues relating to molecular mass, lipophilicity, hydrogen bonds, and pharmacophore types, i.e., physical properties of the constituents.[1,2] This paper addresses different parameters that have received less attention: stereochemistry and conformation.

The circumstances that led us to be interested in stereochemical diversity are as follows. Our group has developed solid-phase S_NAr macrocyclization methodology for syntheses of libraries of compounds such as **I,** which mimic the turn regions of proteins.[3−5] The first target protein that we are interested in, the nerve growth factor (NGF), presents an interesting problem for the following reason. Despite the fact that there is good pharmacological evidence that turn regions of NGF are critical for binding to its high affinity receptor (TrkA), it is unclear which type of turn conformations are involved.[6,7] Crystallographic studies exist, but they are of limited value in this regard. In the two crystal structures reported for NGF, the critical turn between residues 92−96 adopts a different turn type in the two crystals that were studied.[8,9] Libraries of peptidomimetics designed to mimic the NGF turn regions should therefore maximize conformational diversity to enhance the probability of identifying a lead compound.

The simplest and cheapest way to prepare a small library of structures **I** is to use L-amino acids throughout. However, this is a poor approach to test different conformations if the preferred shapes of these macrocycles are not particularly dependent upon the substituents R^1 and R^2. For the compounds **1** this appears to be the case, because CD spectra of **1a**−**d** (Figure 1a) have very similar profiles. Changes in the endocyclic heteroatom from O to N in compounds **2a**−**c** have an effect, but the CD spectra of these materials are very similar to each other and are not very different from those of compounds **1**. The CD spectra shown in Figure 1 can be loosely regarded as being indicative of type-I-like turns,[10] and for compounds **2** this assertion is supported by extensive NMR measurements interfaced with molecular simulations.[3−5]

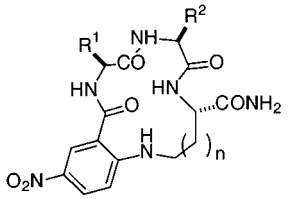


I



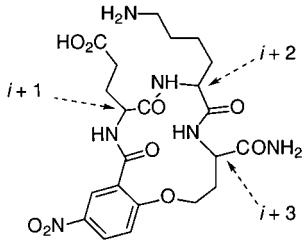
1

	R^1	R^2
a	CH_2CO_2H	CHMeEt
b	CHMeEt	$(CH_2)_4NH_2$
c	CH_2CONH_2	$(CH_2)_4NH_2$
d	CH_2OH	CH_2CHMe_2



2

	R^1	R^2	n
a	CHMeEt	$(CH_2)_4NH_2$	2
b	CH_2OH	$(CH_2)_4NH_2$	2
c	CHMeEt	CHMeOH	3



3

	configuration		
	i + 1	*i* + 2	*i* + 3
a	L	L	L
b	L	D	L
c	D	L	L
d	L	L	D

The data outlined above implies that it is necessary to incorporate D-amino acids into focused library **3** to obtain significant conformational diversity. However, appropriately protected D-amino acids are always expensive and sometimes commercially unavailable; hence this strategy

(1) Martin, E. J.; Blaney, J. M.; Siani, M. A.; Spellmeyer, D. C.; Wong, A. K.; Moos, W. H. *J. Med. Chem.* **1995**, *38*, 1431−6.
(2) Sadowski, J.; Wagener, M.; Gasteiger, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2674−7.
(3) Feng, Y.; Wang, Z.; Jin, S.; Burgess, K. *J. Am. Chem. Soc.* **1998**, *120*, 10768−9.
(4) Feng, Y.; Burgess, K. *Chem.−Eur. J.* **1999**, in press.
(5) Feng, Y.; Wang, Z.; Jin, S.; Burgess, K. *Chem.− Eur. J.* **1999**, in press.
(6) LeSauteur, L.; Wei, L.; Gibbs, B.; Saragovi, H. U. *J. Biol. Chem.* **1995**, *270*, 6564−9.
(7) Beglova, N.; LeSauteur, L.; Ekiel, I.; Saragovi, H. U.; Gehring, K. *J. Biol. Chem.* **1998**, *273*, 23652−8.
(8) McDonald, N. Q.; Lapatto, R.; Murray-Rust, J.; Gunning, J.; Wlodawer, A.; Blundell, T. L. *Nature* **1991**, *345*, 411−4.
(9) Holland, D. R.; Cousens, L. S.; Meng, W.; Matthews, B. W. *J. Mol. Biol.* **1994**, *239*, 385−400.
(10) Perczel, A.; Hollosi, M. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: New York and London, 1996; pp 362−364.

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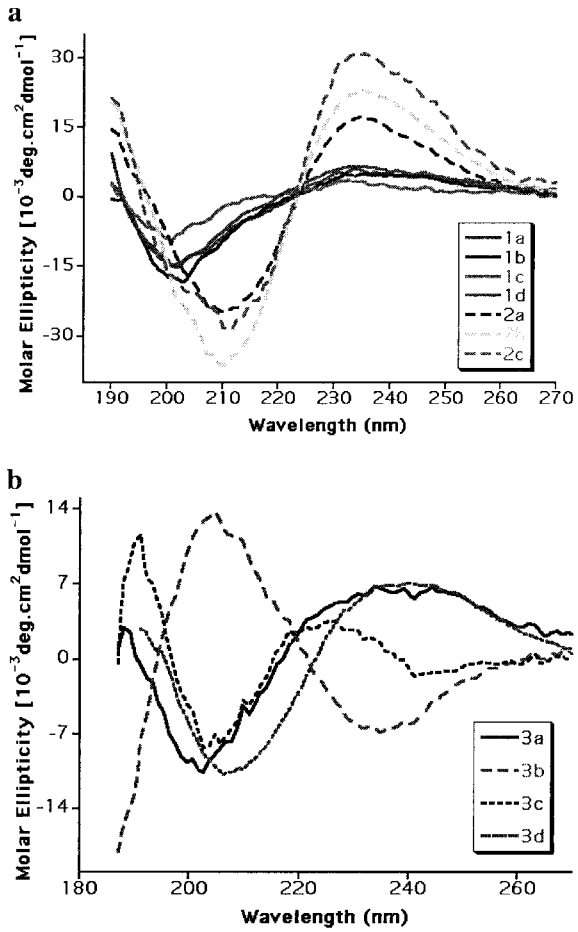

Figure 1. CD spectra of (a) compounds **1** and **2** and (b) compounds **3**, in 20% MeOH/H_2O.

should be used conservatively. It therefore became pertinent to ascertain at which stereocenter in structure **3** (labeled here i +1, i + 2, and i + 3, by analogy with a β-turn) would incorporation of a D-amino acid have maximal effect on the conformation. Derivatives **3a**−**d** were therefore prepared to test this point.

Extensive conformational analyses were carried out for compounds **3a**−**d**. Full details of these analyses are given in the Experimental Section, and a brief summary of the approach used is as follows. Complete ^{1}H NMR assignments were obtained for each compounds via a combination of COSY and ROESY spectroscopies (DMSO solvent).[11] Close contacts identified from the ROE data, coupling constants, temperature coefficients for N*H* protons,[12,13] and D/H exchange rates for N*H* protons[14] were compared with groups of low-energy conformations predicted using the quenched molecular dynamics technique.[15,16] Excellent agreement was obtained for compounds **3a**−**c**, indicating they tend to adopt the conformations shown in Figure 2. Correspondence between the

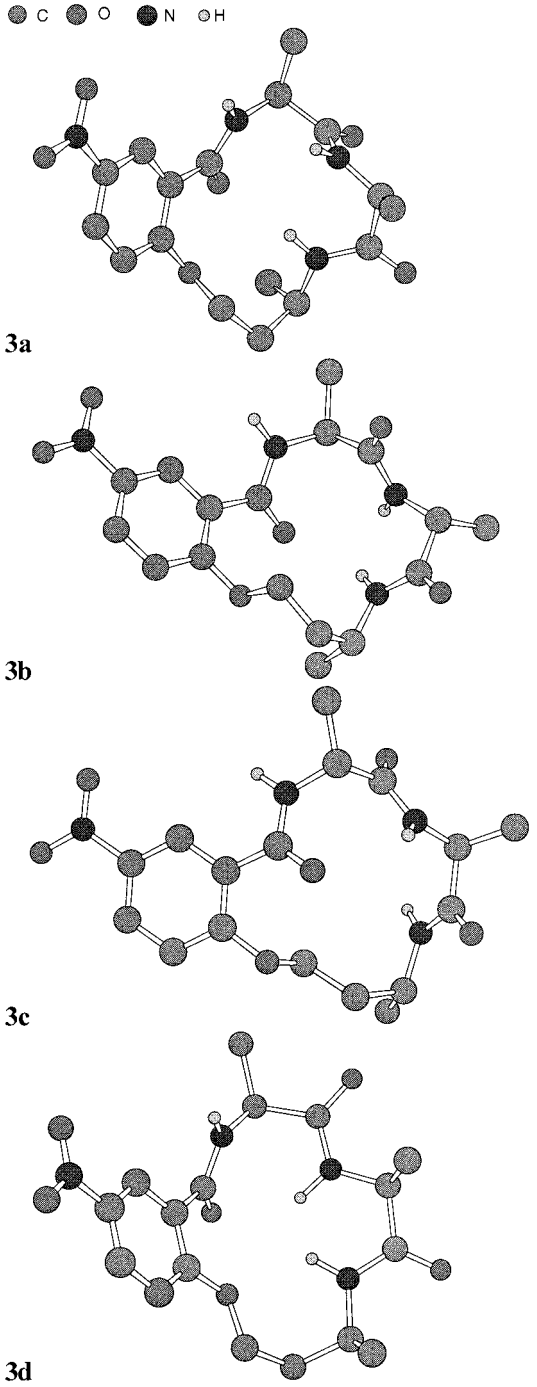

Figure 2. Preferred low-energy conformers of compounds **3a**−**d** from molecular simulations. Only the backbone atoms are shown for clarity.

simulated conformer and the spectroscopic data was poor for compound **3d**, indicating this molecule may be undergoing rapid exchange between two significantly populated conformational states in solution. The low energy conformers of **3a**−**c** shown in Figure 2 are probably useful guidelines, whereas the one shown for **3d** is less relevant. Throughout, we were not able to detect preferred orientations of the side chains, just as would be expected for a small molecule with limited conformational constraints.

CD spectra for compounds **3a**−**d** were also recorded, and these data (Figure 1b) form a useful basis for discussion of conformational trends in the series. The LLL derivative **3a** (i.e., having L configuration at i +1, i + 2,

(11) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
(12) Ohnishi, M.; Urry, D. W. *Biochem. Biophys. Res. Commun.* **1969**, *36*, 194−202.
(13) Kopple, K. D.; Ohnishi, M.; Go, A. *J. Am. Chem. Soc.* **1969**, *91*, 4264−72.
(14) Englander, S. W.; Downer, N. W.; Teitelbaum, H. *Annu. Rev. Biochem.* **1972**, *41*, 903−24.
(15) O'Connor, S. D.; Smith, P. E.; Al-Obeidi, F.; Pettitt, B. M. *J. Med. Chem.* **1992**, *35*, 2870−81.
(16) Pettitt, B. M.; Matsunaga, T.; Al-Obeidi, F.; Gehrig, C.; Hruby, V. J.; Karplus, M. *Biophy. J. Biophys. Soc.* **1991**, *60*, 1540−4.

and $i + 3$) has a CD profile consistent with the type-I turn shown for **3a** in Figure 2. Type-I turn character also dominates for the DLL compound **3c**, though its CD spectrum (and molecular simulations for this compound, see Supporting Information) shows evidence that a small fraction of the molecules adopts type-II conformations. It is the LDL configuration in **3b** that appears to induce a markedly different conformational preference; all of the data, including the large positive CD maximum ellipticity at approximately 206 nm, indicate that this particular compound predominantly adopts a type-II-like conformation. Finally, like **3a** and **3c**, the LLD-configured compound **3d** has a CD that resembles an ideal type-I spectrum more closely than type-II, though the ellipticity minimum is shifted to a higher wavelength.

The overall conclusion of these studies is that stereochemical variations at the $i + 2$ position in the peptidomimetics **3** cause maximal effects on the conformational diversity covered in libraries of these compounds. This may be because the $i + 2$ amino acid is central in the tripeptide string, and that sequence contains the only stereocenters in these macrocycles. The detailed conformational studies presented here may eventually be valuable for interpreting the pharmacological activities of these compounds.

Experimental Procedure

NMR Studies. NMR spectra were recorded using the same procedure as previously described[5] except that 16 transients and 3-s acquisition times were used to obtain one-dimensional ^{1}H NMR spectra. The temperature range used to measure the temperature coefficients of the amide protons was 20−50 °C. DQF−COSY and ROESY spectra were recorded using 16 scans per t_1 increment.

Molecular Simulations. The molecular simulations were performed as previously described,[5] except that 1000 steps of steepest descent minimization were used throughout. Cluster analyses were performed using threshold cutoff values 0.65−0.75 Å.

CD Studies. CD measurements were obtained on an Aviv (model 62 DS) spectrometer. The cyclic peptidomimetics were dissolved in H_2O/MeOH (80:20 v/v) (c = 0.1 mg/mL, 0.1 cm path length). The CD spectra were recorded at 25 °C.

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Supporting Information Available: Experimental procedures for the preparation of compounds **3** and data from their NMR/molecular simulation studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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