ORGANIC LETTERS

2010
Vol. 12, No. 7
1588−1591

Oligooxopiperazines as Nonpeptidic α-Helix Mimetics

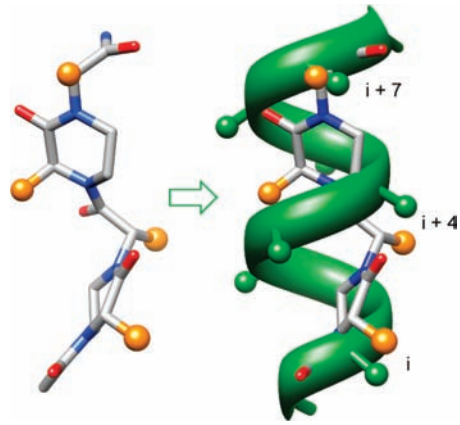
Petra Tošovská and Paramjit S. Arora*

Department of Chemistry, New York University, 100 Washington Square East, New York, New York 10003

arora@nyu.edu

Received February 5, 2010

ABSTRACT



A new class of nonpeptidic α-helix mimetics derived from α-amino acids and featuring chiral backbones is described. NMR and circular dichroism spectroscopies, in combination with molecular modeling studies, provide compelling evidence that oligooxopiperazine dimers adopt stable conformations that reproduce the arrangement of *i*, *i*+4, and *i*+7 residues on an α-helix.

α-Helices play fundamental roles in mediating protein−protein interactions. Several approaches for stabilizing peptides in helical conformations or mimicking this conformation with nonnatural oligomers have been described.[1] Examination of complexes of proteins with other biomolecules reveals that often one face of the helix featuring the *i*, *i*+4 and *i*+7 residues is involved in binding. Synthetic scaffolds that display protein-like functionality and reproduce the arrangement of key side chains on an α-helix would be invaluable as inhibitors of selective protein interactions. In elegant reports, Hamilton and others have successfully demonstrated the utility of nonpeptidic helix mimetics based on aromatic scaffolds.[2] Here, we report a complementary approach that affords nonaromatic helix mimetics which feature a chiral backbone and are easily synthesized from α-amino acids. We hypothesized that scaffolds that present chiral backbones, as compared to the aromatic templates, may be more effective in discriminating between chiral protein pockets.[3] Molecular modeling studies, 2D NMR, and circular dichroism spectroscopies provide strong support for our design features.

(1) (a) Henchey, L. K.; Jochim, A. L.; Arora, P. S. *Curr. Opin. Chem. Biol.* **2008**, *12*, 692–697. (b) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399–1408. (c) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366–1375. (d) Patgiri, A.; Jochim, A. L.; Arora, P. S. *Acc. Chem. Res.* **2008**, *41*, 1289–1300. (e) Garner, J.; Harding, M. M. *Org. Biomol. Chem.* **2007**, *5*, 3577–3585. (f) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252–262. (g) Chin, J. W.; Schepartz, A. *J. Am. Chem. Soc.* **2001**, *123*, 2929–2930.

(2) (a) Shaginian, A.; Whitby, L. R.; Hong, S.; Hwang, I.; Farooqi, B.; Searcey, M.; Chen, J.; Vogt, P. K.; Boger, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 5564–5572. (b) Plante, J. P.; Burnley, T.; Malkova, B.; Webb, M. E.; Warriner, S. L.; Edwards, T. A.; Wilson, A. J. *Chem. Commun.* **2009**, 5091–5093. (c) Restorp, P.; Rebek, J., Jr. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5909–5911. (d) Ahn, J.-M.; Han, S.-Y. *Tetrahedron Lett.* **2007**, *48*, 3543–3547. (e) Davis, J. M.; Tsou, L. K.; Hamilton, A. D. *Chem. Soc. Rev.* **2007**, *36*, 326–334. (f) Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4130–4163.

(3) Maity, P.; Konig, B. *Org. Lett.* **2008**, *10*, 1473–1476.

10.1021/ol1003143
Published on Web 03/02/2010

We were attracted to the piperazine skeleton because it is considered a privileged scaffold for peptidomimetic research and drug discovery.[4] Specifically, the 2-oxopiperazine and the diketopiperazines have a rich history in medicinal chemistry and are considered to be "drug-like" scaffolds.[5] Although, the potential of oxopiperazine oligomers has not been explored, we were intrigued by our computational studies that predicted stable structures in these oligomers due to the conformational constraints inherent in the system. Molecular modeling studies indicate that an oxopiperazine dimer spans the length of an 8mer α-helix and superimposes amino acid functionality onto the *i*, *i*+4, and *i*+7 residues of the helix (Figure 1). We began our analysis of oligooxopiperazines by searching the Cambridge Structural Database for examples of oxopiperazine derivatives. This search resulted in five hits (CSD codes: KEMXUV, ZOZTUD, ZARZOH, FOBFEH, and KEMXUV) of single piperazine ring systems relevant to our system. Although this is a narrow set to base hypotheses upon, these hits provided invaluable insights regarding the ϕ and φ dihedral angles favored in the amino acid residue linking two piperazine rings and corroborated our molecular modeling calculations (Figure 2). A full discussion of the accessible ϕ and φ dihedral angles in oxopiperazine dimers is included in the Supporting Information.

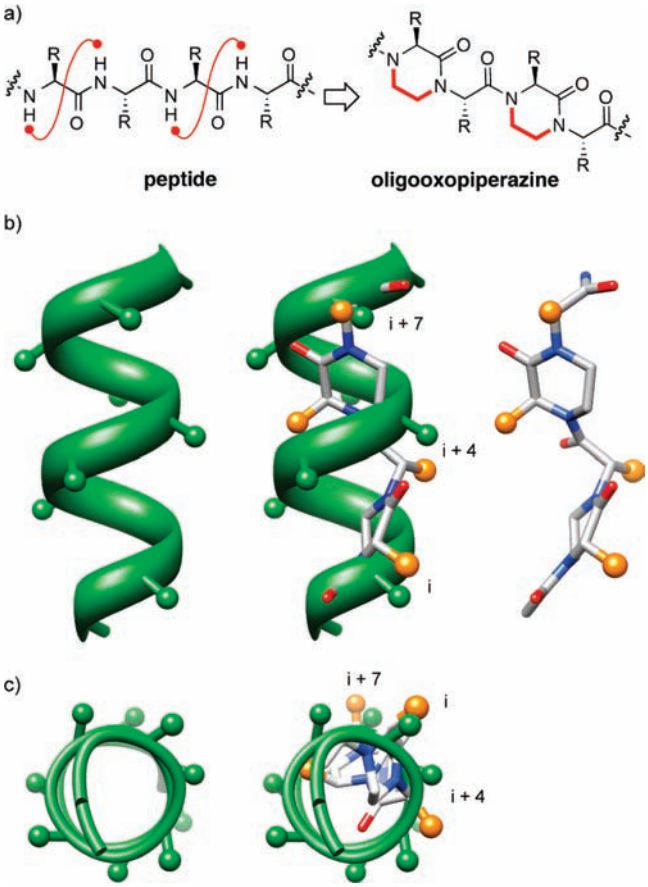


Figure 1. (a) Design of amino acid-derived oligooxopiperazines. (b) An 8mer canonical α helix with side chain residues depicted as green spheres (left). Predicted structure of an oxopiperazine dimer with side chain residues depicted as orange spheres (right) and overlay of the piperazine dimer and the α-helix (center). (c) Top-down view of a canonical α-helix (left) and overlay of the *i*, *i*+4 and *i*+7 residues of the α-helix and side chain residues of oxopiperazine dimer (right).

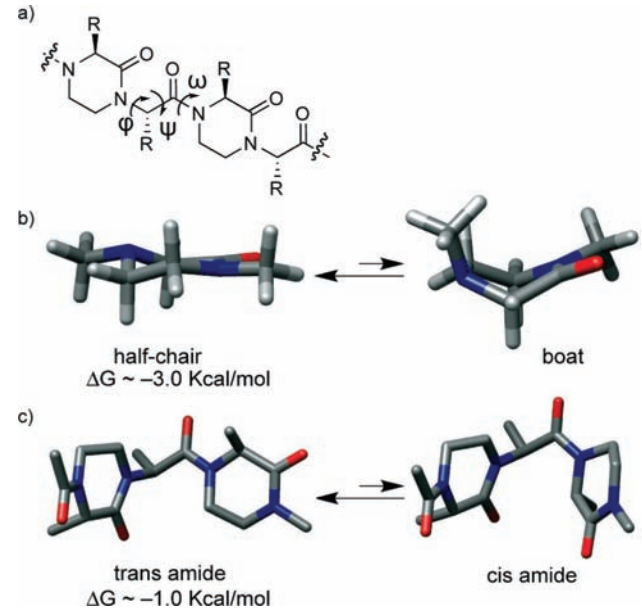


Figure 2. (a) Rotatable bonds in oxopiperazine dimer. (b) Favored chair and (c) amide bond geometries; values were calculated with Macromodel MMFF force field in chloroform.

Molecular modeling studies of oxopiperazine dimers and trimers composed of alanine residues, and examination of the Cambridge Structural Database, suggest that piperazine rings are well-defined and the overall folding of the oligomer is determined by the geometry of the amide bond (ω dihedral angle) that links piperazine rings (Figure 2). An oxopiperazine dimer features a tertiary amide bond that would be expected to adopt trans and cis geometeries, as observed in polyproline peptides. A trans-amide geometry would place side chain groups on the dimer such as to reproduce the arrangement of *i*, *i*+4, and *i*+7 residues on an α-helix. Only constructs composed of alanine residues were modeled with the expectation that flexible side chains would overcome slight differences in backbone geometries. Modeling studies, performed with Macromodel MMFF force field in chloroform,[6] suggest that the trans conformation would be favored by roughly 1 kcal/mol in oxopiperazine dimers composed of alanine residues. The trans to cis ratio is expected to increase in dimers built from bulkier amino acid residues. Several synthetic routes to piperazines are known, which we anticipated would allow rapid synthesis and evaluation of the desired compounds.[7]

In these initial studies, we report the design and synthesis of three oxopiperazine dimers (Figure 3a), and evaluate their potential to adopt a stable conformation in solution by NMR and circular dichroism spectroscopies.

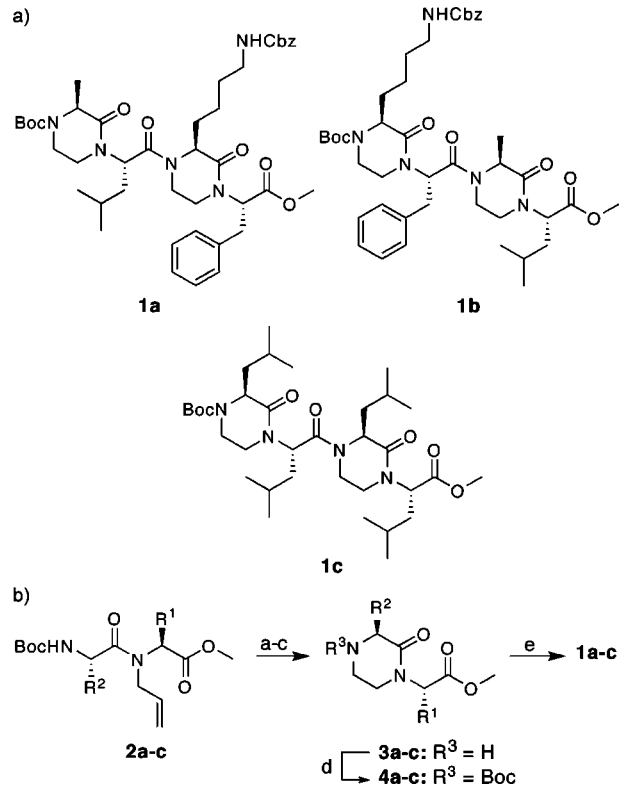


Figure 3. (a) Oxopiperazine helix mimetics designed for the current study. (b) Synthesis of dimers **1a**−**c**: (a) O_3, (b) Me_2S, (c) TFA and triethylsilane. Combined yield for steps a−c: **3a**, 81%; **3b**, 80%; **3c**, 85%; (d) Boc_2O: **4a**, 98%; **4b**, 94%; **4c**, 97%; (e) LiOH **3**, DCC, HOBt: **1a**, 73%; **1b**, 70%; **1c**, 71%. a: $R^1 = CH_2CH(CH_3)_2$, $R^2 = CH_3$. b: $R^1 = CH_2Ph$, $R^2 = (CH_2)_4NHCbz$. c: $R^1 = CH_2CH(CH_3)_2$, $R^2 = CH_2CH(CH_3)_2$.

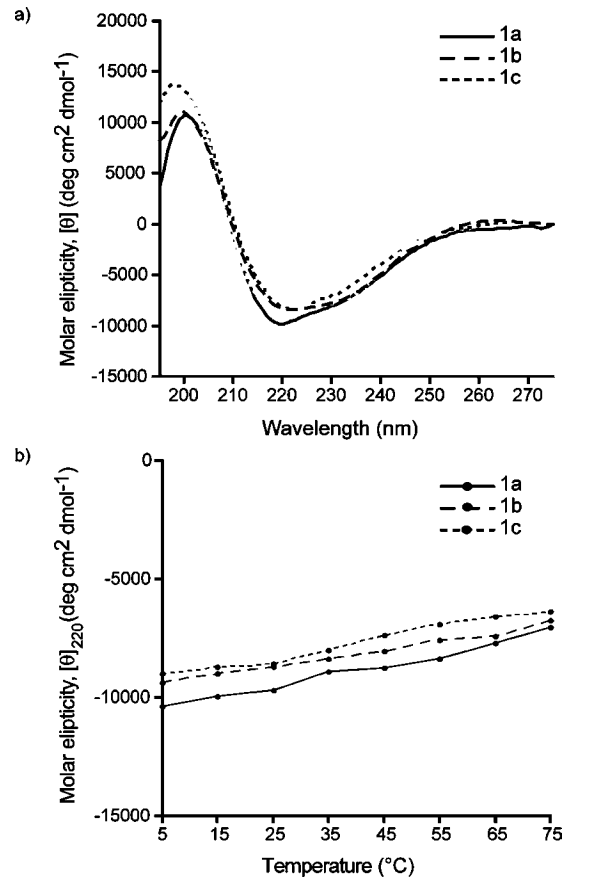


Figure 4. (a) Circular dichroism spectra of oxopiperazines **1a**−**c** in acetonitrile. (b) Effect of temperature on the stability of **1a**−**c**. CD spectra obtained in methanol are shown in the Supporting Information.

Compounds **1a**−**c** were designed to test the impact of different side chain combinations on the stability of the oxopiperazine dimer conformation. We evaluated several routes for the synthesis of these compounds and eventually found the reductive amination route described by Moeller and co-workers to afford short oligomers in respectable yields (Figure 3b).[7b]

The solution conformation of dimers **1a**−**c** was investigated by circular dichroism spectroscopy in methanol and acetonitrile solutions. Figure 4 shows CD spectra in acetonitrile; spectra in methanol are included in the Supporting Information. The CD spectra of **1a**−**c** display double minima near 220 and 230 nm and maxima at 200 nm. Surprisingly, the overall shape is reminiscent of CD spectra of α-helices; although, the maxima and minima are red-shifted by 10 nm. Although CD spectra of artificial systems are often difficult to interpret,[8] the spectra of **1a**−**c** indicate a high degree of preorganization. The thermal stabilities of **1a**−**c** were investigated by monitoring the temperature-dependent change in the intensity of the 220 nm bands in the CD spectra (Figure 4b). We observe a gradual increase in the signal intensity at 220 nm with temperature, but the dimers retain over 70% of their room-temperature elipticity at 75 °C. Similar noncooperative denaturation behavior has been observed with other conformationally defined oligomers.[9] Overall, the CD studies demonstrate that helix mimetics **1a**−**c** adopt stable conformations confirming our molecular modeling analysis.

We next utilized 2D NMR spectroscopy to analyze the conformations adopted by **1a** as a model oxopiperazine helix mimetic, specifically we wanted to determine the geometry

(4) Patchett, A. A.; Nargund, R. P. *Annu. Rep. Med. Chem.* **2000**, *35*, 289–298.

(5) (a) Herrero, S.; Garcia-Lopez, M. T.; Latorre, M.; Cenarruzabeitia, E.; Del Rio, J.; Herranz, R. *J. Org. Chem.* **2002**, *67*, 3866–3873. (b) Kitamura, S.; Fukushi, H.; Miyawaki, T.; Kawamura, M.; Konishi, N.; Terashita, Z.; Naka, T. *J. Med. Chem.* **2001**, *44*, 2438–2450. (c) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699–1720. (d) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed.* **1993**, *32*, 1244–1267.

(6) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.

(7) (a) Franceschini, N.; Sonnet, P.; Guillaume, D. *Org. Biomol. Chem.* **2005**, *3*, 787–793. (b) Tong, Y. S.; Fobian, Y. M.; Wu, M. Y.; Boyd, N. D.; Moeller, K. D. *J. Org. Chem.* **2000**, *65*, 2484–2493. (c) Sugihara, H.; Fukushi, H.; Miyawaki, T.; Imai, Y.; Terashita, Z.; Kawamura, M.; Fujisawa, Y.; Kita, S. *J. Med. Chem.* **1998**, *41*, 489–502.

(8) Driver, R. W.; Hoang, H. N.; Abbenante, G.; Fairlie, D. P. *Org. Lett.* **2009**, *11*, 3092–3095.

(9) (a) Saludes, J. P.; Ames, J. B.; Gervay-Hague, J. *J. Am. Chem. Soc.* **2009**, *131*, 5495–5505. (b) Wang, D.; Chen, K.; Dimartino, G.; Arora, P. S. *Org. Biomol. Chem.* **2006**, *4*, 4074–4081.

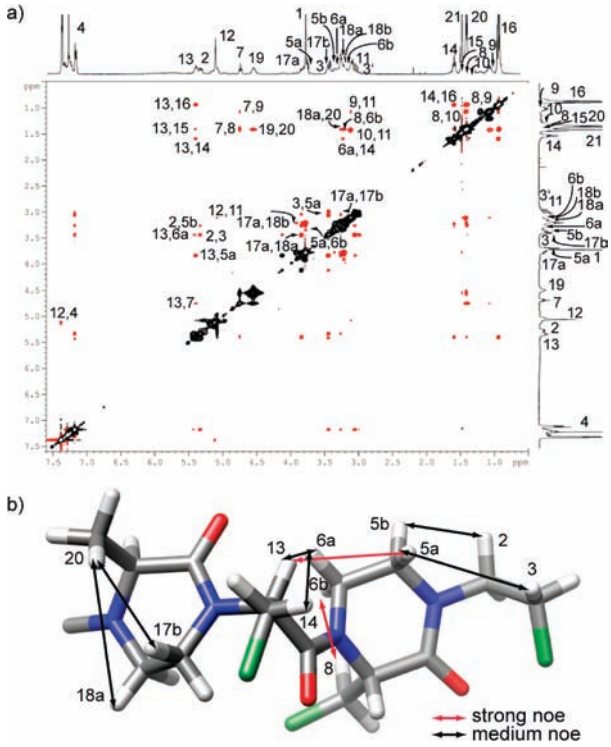


Figure 5. (a) Cross-section of the NOESY spectra of **1a** in $CDCl_3$. (b) Overlay of key NOEs on the predicted oligooxopiperazine conformation. (Side chain groups not shown for clarity.)

adopted by the tertiary amide bond linking two piperazine rings. A combination of COSY and NOESY spectroscopy was used to assign ^{1}H NMR resonances for **1a**. The NOESY spectrum reveals several NOEs in the two-ring system, which would be expected from a *trans*-amide geometry in **1a** but not from the cis-amide conformation (Figure 5). We do not observe NOE crosspeaks between protons on neighboring piperazine rings. This absence of NOEs is expected based on the proposed low energy conformation in which these protons lie outside the 5 Å distance typically required to observe the nuclear Overhouser effect. Thus, the NOESY studies strongly corroborate our modeling analysis. Significantly, the NMR spectra did not display peaks indicative of a minor cis-amide isomer, suggesting that the trans conformation is substantially more stable than the cis analog.

In summary, through rational design and synthesis, we have developed a new class of nonpeptidic α-helix mimetics. NMR and circular dichroism spectroscopies provide compelling evidence that oligooxopiperazine dimers adopt stable conformations that reproduce the arrangement of *i*, *i*+4, and *i*+7 residues on an α-helix. Given the importance of the helix conformation in protein−protein interactions,[10] and the potential of nonpeptidic scaffolds that mimic this conformation, we believe that oxopiperazine scaffolds will offer attractive new tools for chemical biology.[11] In ongoing studies, we are examining the potential of oxopiperazine helix mimetics to disrupt chosen protein−protein interactions. The results of these studies will be reported in due course.

Acknowledgment. We are grateful for financial support from the NSF (CHE-0848410).

Supporting Information Available: Experimental procedures and structural characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL1003143

(10) (a) Jochim, A. L.; Arora, P. S. *Mol. BioSyst.* **2009**, *5*, 924–926. (b) Jones, S.; Thornton, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 13–20.
(11) (a) Wells, J. A.; McClendon, C. L. *Nature* **2007**, *450*, 1001–1009. (b) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. *Nat. Chem. Biol.* **2007**, *3*, 252–262.