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Design, Synthesis and Structural Analysis of D,L-Mixed Polypyrrolinones 1: From Nonpeptide Peptidomimetics to Nanotubes

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Abstract

To expand the potential conformational space available to the polypyrrolone structural motif, an open chain, D,L-alternating hexapyrrolinone was designed and synthesized. Structural studies, including solution NMR and X-Ray crystallographic analysis, revealed that the hexapyrrolinone adopts a turn conformation both in solution and in the solid state, with aggregation in solution and a nanotube-like quaternary structure in the crystal.

A major achievement in the field of non-peptide peptidomimetics would comprise the design and synthesis of biologically relevant mimics, capable of adopting conformations similar to the three principal secondary structures accessible to peptides and proteins (e.g., β -turns, β -strands, or α -helices), with conformational control manifest simply via structural modification of the side-chains and/or the stereogenicity of the constituent monomers. To date, only a limited number of conformationally versatile nonpeptide peptidomimetics have been reported.¹

In 1992, the Hirschmann-Smith collaboration reported the design and synthesis of a new class of peptidomimetics based on the pyrrolinone scaffold (Figure 1).² Computational studies suggested that oligomers based on this repeating vinylogous amide motif held the promise of adopting structures similar, but not identical to, the three secondary structures of peptides and proteins.^{2,3} As such, the polypyrrolinones constituted early examples of foldamers as defined by Gellman.⁴ 1a,b and elegantly demonstrated for β -peptides both by Seebach⁵ 1c and Gellman.⁴ 1a,b Pleasingly, initial studies demonstrated that homochiral polypyrrolinones (e.g., all D),⁶ such as (–)-**1**, can adopt extended parallel (**1a**, Figure 1) or antiparallel (**1b**) β -strand/sheet mimics, depending on the presence or absence of a Boc C-terminus.^{2,3,7} This observation led to the design and synthesis of a series of potent pyrrolinone-based enzyme inhibitors,⁸ requiring the β -strand/-sheet motif, including metalloproteases,⁹ and orally bioavailable HIV-1 protease inhibitors,¹⁰ as well as a peptide-pyrrolinone hybrid ligand for the class II MHC protein HLA-DR1.¹¹

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Supporting Information Available Experimental procedures and spectroscopic data for all new compounds are available.

With these initial achievements, a central theme of the pyrrolinone-based peptidomimetic program became the expansion, by design, of the diverse conformational space accessible to the polypyrrolinone structural motif for peptide/protein mimicry. We recognized that the ability to control the various possible conformations, via simple modulation of the side-chain structure and/or α -stereogenicity, would significantly enhance the opportunities for the polypyrrolinone construct in biologically relevant mimics.

The well recognized principle that D-amino acids stabilize β -turns,¹² in conjunction with the pioneering reports by Karle¹³ and De Santis,¹⁴ later extended by the Lorenzi,¹⁵ Ghadiri,¹⁶ and Ciufolini¹⁷ laboratories, demonstrating that peptides embodying alternating D- and L-amino acids can adopt turn conformations, and in some cases assemble into nanotubes, suggested that a sequence of alternating D,L-linked pyrrolinones might also preferentially adopt a turn structure. That this scenario proved correct was demonstrated by the synthesis of the alternating D,L-*tetra*-pyrrolinone (–)-**2**, shown by NMR studies, in conjunction with molecular modeling, to adopt a low-energy turn conformation in solution.^{3c} Importantly, control over the conformation of (–)-**2** was achieved by alternating the building block configuration along the sequence. An equally important issue concerned the biological relevance of such polypyrrolinone turn mimics. Towards this end, we designed and synthesized several mimics [cf. (–)-**3**] of the neuropeptide hormone somatostatin-14 (SRIF), known to incorporate a β -turn as the pharmacophore structural element.¹⁸ Importantly, a heterochiral tetrapyrrolinone proved to be a functional mimic of SRIF.¹⁹

Having achieved both bioactive β -strand and β -turn mimics with designed polypyrrolinones, we turned to larger congeners, with an eye towards the possibility of helix mimics.^{3b} We selected first alternating D,L-hexapyrrolinone (–)-**4** (Figure 1) and carried out a 10,000-step Monte Carlo conformational search²⁰ employing the MMFFS force field.²¹ The calculations rendered a set of low energy conformations, typified by a turn motif with four intramolecular hydrogen bonds among neighboring residues and one hydrogen bond spanning the cleft between the terminal residues (Figure 2a).

The computationally-predicted ability of the pyrrolinone-derived turn to draw the termini of the hexapyrrolinone oligomer close together confirmed our interest in **4** as a synthetic target. To access **4**, we turned to our iterative pyrrolinone synthetic protocol, beginning with aldehyde (–)-**5** (Figure 2c) and building the pyrrolinone chain in the C to N direction (see Supporting Information). Yields for the two-step iterative sequences were in general good.

To establish the solution conformation of (–)-**4**, a series of ROESY NMR experiments in CHCl_3 [1 mM in (–)-**4**], similar to those exploited with (–)-**23c** were conducted. Ten critical long-range NOEs were observed (Figure 2d). The ROE NMR data was then employed as a constraint file in a 10,000-step Monte Carlo conformational search; the result revealed (–)-**4** to exist in solution in a flat, G-shaped turn conformation similar to that previously reported for (–)-**2** (Figure 2b).^{3c}

Crystallization of (–)-**4** from CHCl_3 via vapor-phase equilibration with hexane furnished single crystals (m.p. 195–197 °C) suitable for X-ray analysis.²² Pleasingly, the derived ORTEP diagram (Figure 3a) reveals a flat G-shaped structure similar to the low energy conformation observed in solution. Importantly, overlay of the solution structure with the crystal structure provided excellent atom-to-atom correspondence (Figure 3b; root mean-square value of 0.508 Å). Of equal importance, the unit cell structure possesses four (–)-**4** monomers self-assemble into a nanotube-like quaternary structure (Figure 3c–e), with the monomers arrayed in an antiparallel fashion.

A crystallographic three-fold rotation axis, parallel to the *c* axis passes through the center of the best molecular plane. The tubular structure is best revealed by removing the internal and

external side-chains (Figure 3e). In this way, the intermolecular hydrogen bonds, which stabilize the molecular assembly, are more clearly visible. To ascertain the possibility of self-assembly of (–)-**4** in solution, we performed a variable concentration ¹H NMR analysis to define the lowest concentration at which intermolecular interactions occur (Figure 4). A significant change in the proton resonance at δ 5.50 ppm (1×10^{-4} M), highly indicative of solvent exposure, was observed with increasing concentration (ca. 1×10^{-4} M to 3×10^{-2} M). We therefore assigned this resonance to the N-terminal pyrrolinone N-H(6), the only pyrrolinone N-H that cannot form an intramolecular hydrogen bond with the carbonyl oxygen of an adjacent pyrrolinone. The internal pyrrolinone N-Hs [NH(1–5)], which resonate in the hydrogen bonded N-H region (ca. δ 6.95 to 8.00 ppm), displayed modest shifts (0.06 – 0.41 ppm, 3.5×10^{-3} M to 2.6×10^{-2} M) consistent with self-assembly.

Taken together these results suggest that the lowest concentration at which intermolecular hydrogen bonding occurs is ca. 10^{-3} M.

In summary, the design, synthesis and structural analysis of an alternating D,L-hexapyrrolinone (–)-**4** has been achieved. Structural analysis, involving solution NMR studies and X-Ray crystallography, reveals that (–)-**4** adopts a turn conformation both in solution and in the solid state, with aggregation occurring in solution and nanotube-like structures in the solid-state. Further extension of the D,L-alternating pyrrolinone motif holds the promise of additional novel chemical and structural properties (e.g., helices). Studies to this end continue in our Laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. (a) Gellman SH. *Acc Chem Res.* 1998; 31:173. (b) Horne SW, Gellman SH. *Acc Chem Res.* 2008; 41:1399. [PubMed: 18590282] (c) Seebach D, Hook DF, Glattli A. *Biopolymers (Peptide Science)*. 2006; 84:23. [PubMed: 16235225] (d) Stigers KD, Soth MJ, Nowick JS. *Curr Opin Chem Biol.* 1999; 3:714. [PubMed: 10600726] (e) Hill DJ, Mio MJ, Prince RB, Hughes TS, Moore JS. *Chem Rev.* 2001; 101:3893. [PubMed: 11740924] (f) Hecht, S.; Huc, I., editors. *Foldamers: Structure, Properties and Applications*. Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim, Germany: 2007. (g) Angelo NG, Arora PS. *J Am Chem Soc.* 2005; 127:17134. [PubMed: 16332031] (h) Goodman CM, Choi S, Shandler S, DeGrado WF. *Nature Chem Biol.* 2007; 3:252. and references cited therein. [PubMed: 17438550]
2. Smith AB III, Keenan TP, Holcomb RC, Sprengler PA, Guzman MC, Wood JL, Carrol PJ, Hirschmann R. *J Am Chem Soc.* 1992; 114:10672.
3. (a) Smith AB III, Guzman MC, Sprengler PA, Keenan TP, Holcomb RC, Wood JL, Carrol PJ, Hirschmann R. *J Am Chem Soc.* 1994; 116:9947. (b) Smith AB III, Favor DA, Sprengler PA, Guzman MC, Carrol PJ, Furst GT, Hirschmann R. *Bioorg Med Chem.* 1999; 7:9. [PubMed: 10199652] (c) Smith AB III, Wang W, Sprengler PA, Hirschmann R. *J Am Chem Soc.* 2000; 122:11037.
4. (a) Dado GP, Gellman SH. *J Am Chem Soc.* 1994; 116:1054. (b) Appella DH, Christianson LA, Karle IL, Powell DR, Gellman SH. *J Am Chem Soc.* 1996; 118:13071. see also ref. 1.

5. (a) Seebach D, Overhand M, Kühnle FNM, Marinoni B, Oberer L, Hommel U, Widmer H. *Helv Chim Acta*. 1996; 79:913. (b) Seebach D, Ciceri PE, Overhand M, Jaun B, Rigo D, Oberer L, Hommel U, Amstutz R, Widmer H. *Helv Chim Acta*. 1996; 79:2043. (c) Seebach D, Matthews JL. *Chem Commun*. 1997:2015. see also ref. 1.
6. While D, L descriptors are not directly applicable to the pyrrolinone units, their use simplifies comparison with peptidal structures.
7. (a) Smith AB III, Holcomb RC, Guzman MC, Keenan TP, Sprengeler PA, Hirschmann R. *Tetrahedron Lett*. 1993; 34:63. Guzman, MC. PhD Thesis. University of Pennsylvania; 1995.
8. Smith AB III, Akaishi R, Jones DR, Keenan TP, Guzman MC, Holcomb RC, Sprengeler PA, Wood J, Hirschmann R, Holloway MK. *Biopolymers (Peptide Science)*. 1995; 37:29. [PubMed: 7880965]
9. Smith AB III, Nittoli T, Sprengeler PA, Duan JW, Liu RQ, Hirschmann R. *Org Lett*. 2000; 2:3809. [PubMed: 11101425]
10. (a) Smith AB III, Cantin L-D, Pasternak A, Guise-Zawacki L, Yao WQ, Charnley AK, Barbosa J, Sprengeler PA, Hirschmann R, Munshi S, Olsen DB, Schleif WA, Kuo LC. *J Med Chem*. 2003; 46:1831. and references cited therein. [PubMed: 12723947] (b) Smith AB III, Charnley AK, Harada H, Beiger JJ, Cantin LD, Kenesky CS, Hirschmann R, Munshi S, Olsen DB, Stahlhut MW, Schleif WA, Kuo LC. *Bioorg Med Chem Lett*. 2006; 16:859. [PubMed: 16298527]
11. Lee KH, Olson GL, Bolin DR, Benowitz AB, Sprengeler PA, Smith AB III, Hirschmann RF, Wiley DC. *J Am Chem Soc*. 2000; 122:8370. and references cited therein.
12. Nutt RF, Veber DF, Saperstein R, Hirschmann R. *Int J Peptide Protein Res*. 1983; 21:66. [PubMed: 6131045]
13. (a) Karle IL, Handa BK, Hassall CH. *Acta Cryst*. 1975; B31:555. (b) Chiang CC, Karle IL. *Int J Peptide Protein Res*. 1982; 20:133. [PubMed: 7118435]
14. De Santis P, Morosetti S, Rizzo R. *Macromolecules*. 1974; 7:52. [PubMed: 4837982]
15. (a) Tomasic L, Lorenzi GP. *Helv Chim Acta*. 1987; 70:1012. (b) Pavone V, Benedetti E, Di Blasio B, Lombardi A, Pedone C, Tomasich L, Lorenzi GP. *Biopolymers*. 1989; 28:215. [PubMed: 2720106] (c) Saviano M, Zaccaro L, Lombardi A, Pedone C, Di Blasio B, Sun X, Lorenzi GP. *J Incl Phenom*. 1994; 18:27. (d) Sun X, Lorenzi GP. *Helv Chim Acta*. 1994; 77:1520.
16. (a) Ghadiri MR, Granja JR, Milligan RA, McRee DE, Khazanovich N. *Nature*. 1993; 366:324. [PubMed: 8247126] (b) Ghadiri MR, Kobayashi K, Granja JR, Chadha RK, McRee DE. *Angew Chem Int Ed Engl*. 1995; 34:93. (c) Hartgerink JD, Granja JR, Milligan RA, Ghadiri MR. *J Am Chem Soc*. 1996; 118:43. (d) Clark TD, Buriak JM, Kobayashi K, Isler MP, McRee DE, Ghadiri MR. *J Am Chem Soc*. 1998; 120:8949.
17. Xi N, Alemany LB, Ciufolini MA. *J Am Chem Soc*. 1998; 120:80.
18. (a) Nutt, RF.; Colton, CD.; Saperstein, R.; Veber, DF. *Somatostatin*. Reichlin, S., editor. Plenum; New York, NY: 1987. p. 83. (b) Veber, DF. *Peptides-Chemistry and Biology: Proceedings of the 12th American Peptide Symposium*. Smith, JA.; Rivier, JE., editors. ESCOM; Leiden: 1992. p. 3
19. Smith AB III, Charnley AK, Mesaros EF, Kikuchi O, Wang W, Benowitz A, Chu CL, Feng JJ, Chen KH, Lin A, Cheng FC, Taylor L, Hirschmann R. *Org Lett*. 2005; 7:399. [PubMed: 15673249]
20. Chang G, Guida WC, Still WC. *J Am Chem Soc*. 1989; 111:4379.
21. (a) Halgren TA. *J Comput Chem*. 1996; 17:490. (b) Halgren TA. *J Comput Chem*. 1996; 17:520. (c) Halgren TA. *J Comput Chem*. 1996; 17:553. (d) Halgren TA, Nachbar RB. *J Comput Chem*. 1996; 17:587. (e) Halgren TA. *J Comput Chem*. 1996; 17:615. (f) Halgren TA. *J Comput Chem*. 1999; 20:720. (g) Halgren TA. *J Comput Chem*. 1999; 20:730.
22. For crystallographic data of hexapyrrolinone (–)-4 see Supporting Information

Figure 1. Designed β-strand/β-sheet tris-pyrrolinones **(-)-1a,b**; β-turn tetrapyrrolinones **(-)-2** and **(-)-3**; hexapyrrolinone **(-)-4**.

Figure 2.

a) A computational model of (-)-4 in CHCl_3 arising from a 10,000-step Monte Carlo conformational search. b) The solution structure of the alternating D,L-hexapyrrolinone (-)-4. c) Synthesis of hexapyrrolinone (-)-4. d) Ten critical long-range NOEs, color coded by their interactions with the prenyl side-chain.

Figure 3.

a) ORTEP diagram of the D,L-mixed hexapyrrolinone (-)-4; b) Overlay of the crystal structure (blue) and the solution structure (red) of (-)-4; c) and d) Stereoviews illustrating the nanotube-like assembly of (-)-4 in the crystal packing diagram, side view and top view, respectively, with intermolecular hydrogen bonding illustrated; and e) Stereoview with side-chains removed to highlight the nanotube-like assembly.

Figure 4. Concentration dependence of the N-H Chemical Shifts in CDCl₃ for the D,L-mixed Hexapyrrolinone (–)-4. N-terminal N-H(6) (○); Internal N-Hs (NH(1–5) (△◇□▲■)).