Supplementary Material (ESI) for Chemical Communications

Characterizing the Structure and Dynamics of Folded Oligomers: Pulsed ESR Studies of Peptoid Helices

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Materials:
4-Amino-2,2,6,6-tetramethylpiperidine 1-Oxyl, 2-methoxyethylamine, benzyl amine and piperidine were purchased from TCI (Portland, Or). Bromoacetic acid and ammonia in methanol (7 N) were purchased from Aldrich (Milwaukee, WI). N,N'-diisopropylcarbodiimide was purchased from Chem-Impex International (Wood Dale, Il). Trifluoroacetic acid was purchased from Acros (Belgium). Fmoc-protected rink amide polystyrene resin (0.57 mmol/g loading) was purchased from Nova Biochem (San Diego, CA).

Peptoid Synthesis:
Peptoid syntheses were performed on an Illiad 2 automated synthesizer (Charybdis Technologies, San Diego CA), following the 2-step, "submonomer" approach described by Zuckermann et al (JACS, 1992). Acylation procedure: to the deprotected amine of the rink amide resin was added 1.2 M bromoacetic acid (20 equivalents) in DMF followed by neat N,N'-diisopropylcarbodiimide (22 equivalents) at room temp. The slurry was agitated for 20 min, drained and then washed five times with DMF. Displacement procedure: to the resin bound bromoacetamide was added 1.0 M amine (20 equivalents) in NMP at room temp. The slurry was agitated for 20 min, drained and then washed five times with DMF. Cleavage procedure: the peptoid product was cleaved from the resin in 95:5 trifluoroacetic acid/water (v/v) (40 mL/g) for 15 min with gentle stirring. The cleavage slurry was separated by filtration and the filtrate was condensed to an oil under rotary evaporation. Procedure for regeneration of the nitroxide free-radical: to the crude cleavage product was added 1:9 water/7N methanolic ammonia (v/v) with vigorous stirring for 4 h. The solution was then condensed to an oil under rotary evaporation, dissolved in 3:7 acetonitrile/water (v/v) and lyophilized.

Analytical HPLC:
The purity of the peptoid synthesis products was evaluated by HPLC on a Beckman Coulter System Gold. Lyophilized peptoid product was dissolved in 3:7 acetonitrile/water (v/v) to a concentration of 1 g/L and 5 μL was injected on to a C_4 column (Duragel G, 3um, 300 A, 0.2 x 5.0 cm). The compounds were analyzed at 0.5 mL/min with a linear gradient of 5% to 95% solvent B in solvent A over 30 min (solvent A is 0.1% TFA in water and solvent B is 0.1% TFA in acetonitrile). Analytes were quantified by UV absorbance at 214 nm (Figure S1).

HPLC/MS:
Molecular weights were verified (Table S1) using a liquid-chromatography-coupled electrospray mass spectrometer (Agilent 1100 series LC/MSD electrospray trap XCT). LC was performed with the same solvent system and column materials as described above. Mass values were calibrated by co-injecting a cocktail of the unknown peptoid

along with three mass standards including ampicilin (MW = 350.4 g/mol) and two peptoids of known molecular mass (542.7 g/mol and 805.04 g/mol) and performing a linear fit with an R^2 of 1.00.

Circular Dichrosim:
CD spectra (cf. Fig. 1 in main text, Fig. S3 in supplemental) were recorded on AVIV 202SF CD spectrometer, in a 1mm path length cell at room temperature. Compounds were dissolved in acetonitrile to a concentration of 100 μM (determined by weight). For similar peptoid sequences, the double minimum spectrum between 220 and 200 nm is characteristic of a polyproline-I helix type secondary structure.

NMR:
In order to further confirm the identity of the major product after ammonia treatment, we conducted NMR studies on compound **7**. The ammonia-treated crude peptoid product was purified by semi-preparative HPLC and lyophilized. Protons of **7** were NMR-silent using standard pulse sequences, as expected, due to their enhanced relaxation in the presence of the unpaired spin of the free radical. Upon treatment of the same sample with ascorbic acid, which has been reported to reduce the nitroxide free radical to a non-paramagnetic state[S1], we observed a typical NMR signal showing the anticipated resonances of the peptoid. The spectrum was obtained on a 250 MHz Varian at 16 mM in CD_3OD with a tetramethylsilane internal standard 0.03% (v/v) ^1H NMR (CD_3OD, 500 MHz) δ 1.45 (d, J = 5.5 Hz, 6H), 1.52 (d, J = 6.0 Hz, 6H), 1.95-2.05 (m, 2H), 2.10-2.20 (m, 2H), 3.26 (t, J = 6.3 Hz, 2H), 3.42 (s, 3H), 3.67 (t, J = 6.0 Hz, 2H), 3.93 (d, J = 5.5 Hz, 2H), 4.15 (s, 2H), 4.44 (d, J = 8.3 Hz, 2H), 4.60 (s, 2H), 4.73(s, 1H), 7.30-7.40 (m, 5H);

DQC-ESR:
Signals were recorded using Ku-band FT-ESR spectrometer[5c] working in its DQC mode at the RF frequency of 17.4 GHz. The 6-pulse DQC sequence (cf. Fig. S2) with π/2 pulses of 3.2 ns and π pulses of 6.2 ns, corresponding to magnetic component of RF field (B_1) in the rotating frame of reference of ca. 30G, has been used in all cases.
The 0.25 mM wet (1% H_2O) methanolic solutions of peptoids were placed into 2 mm i.d. sample tubes and vitrified by flash freezing in liquid nitrogen. The samples were then transferred into the dielectric resonator probe of the spectrometer residing in the CF935 helium flow ESR cryostat (Oxford Instruments, Inc.). DQC signals were recorded at the temperature of 70K. These signals after linear baseline correction[5a] are shown in Fig. 2 in the main text. They were inverted by Tikhonov regularization to produce Fig. 3 in the main text.

References

S1 Battiste, J.; Wagner, G. Biochemistry 2000, 39, 5355-5365

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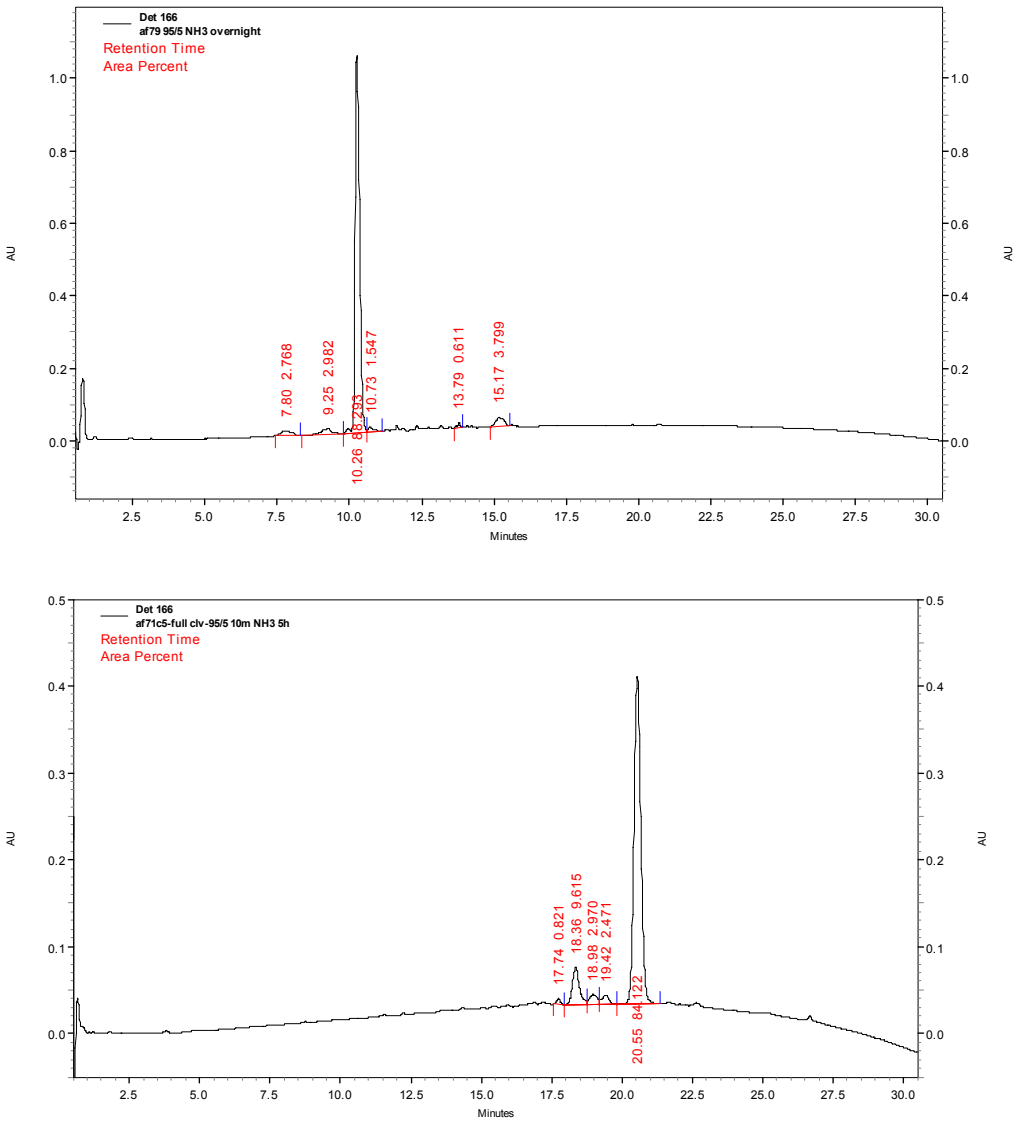

Figure S1. Representative HPLC showing chromatograms for crude products **1** and **2** (UV absorbance at 214 nm) after aqueous ammonia treatment.

HPLC and LCMS characterization data for peptoid products					
Compound	HPLC retention time (min)	% Purity crude product by HPLC	*m/z* $[M+H]^+$ observed (daltons)	*m/z* $[M+H]^+$ calculated (daltons)	
1	20.50	94	1246.7	1246.8	
2	20.64	99	1246.7	1246.8	
3	20.57	92	1246.7	1246.8	
4	20.49	99	1246.7	1246.8	
5	20.55	93	1246.7	1246.8	
6	9.50*	80	1176.5	1176.8	
7	10.26	96	491.7	491.7	

Table S1. Analytical HPLC was performed on a C_{18} column running 5-95% B in 30 mins (* with exception of compound **6** which was eluted with 5-95% B in 10 min). All crude products were subsequently purified by preparative HPLC prior to ESR studies.

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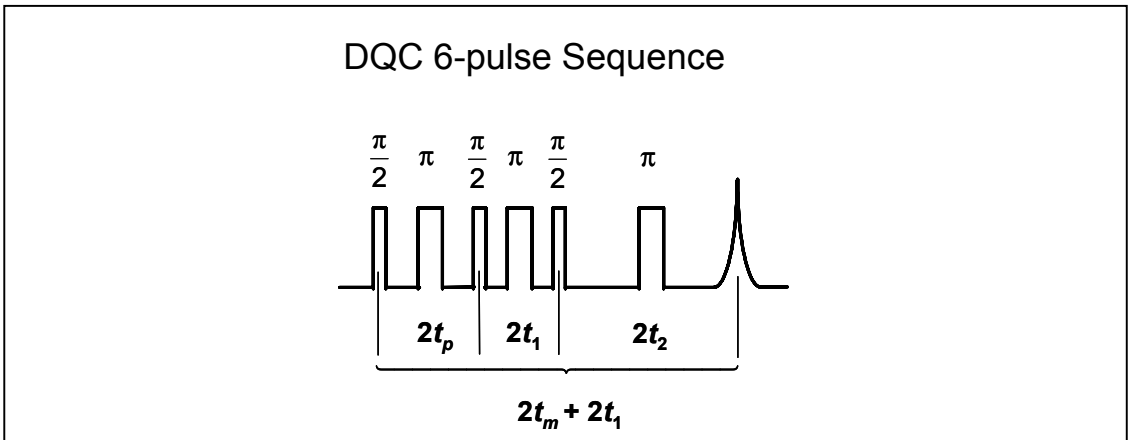

Figure S2.
DQC six-pulse sequence is shown. The distance $2t_m + 2t_1$ from the first $\pi/2$ pulse to the echo is kept constant. The time t_1 of DQC filter that includes 4th π pulse is also kept constant and the signal is recorded vs. the time variable $t_\xi = t_m - 2t_p$. The 256-step phase cycle is used to suppress all coherence pathways that are not affected by dipole-dipole coupling. In other words, the signal obtained from this pulse sequence originates only from the evolution of coherence caused by dipolar coupling between two spins.

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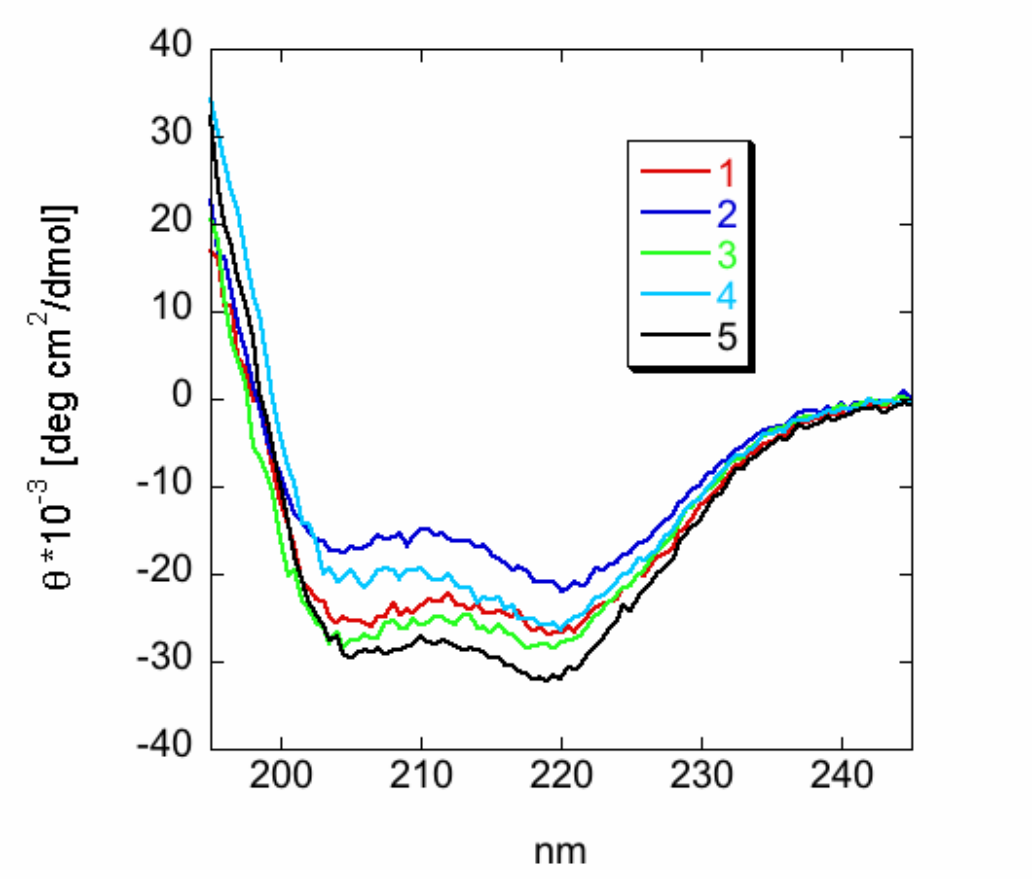
Figure S3: Circular Dichroism



CD spectra for double spin-labeled oligomers **1-5** in acetonitrile (100 μM, 1mm path length).