Supporting Information

Peptoid Helicity Modulation: Precise Control of Peptoid Secondary St ructures via Position-Specific Placement of Chiral Monomers.

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Table of contents

1. General methods S2

2. Amine submonomers S2

3. Peptoid synthesis and purification S2 – S3

4. Circular dichroism spectroscopy S4

5. HPLC data of **1** – **30** S5 – S9

6. ESI-MS data of **1** - **30** S10

7. Interpretation of CD spectra S11

1. General methods

Solvents and reagents purchased from commercial sources (Sigmaaldrich, Acros Organics, Novabiochem, and Merck) were used without further purification. Peptide synthesis grade DMF (Acros Organics, NJ, USA) was used for peptoid synthesis. *N,N′*-Diisopropylcarbodiimide was purchased from Advanced ChemTech, KY, USA. Abbreviations for reagents are as follows: 9-fluorenylmethoxycarbonyl (Fmoc); trifluoroacetic acid (TFA); triisopropylsilane (TIS); dichloromethane (DCM); *N*,*N*'-dimethylformamide (DMF); *N*,*N*'-diisopropylcarbodiimide (DIC); acetonitrile (ACN); and 1-Methyl-2-pyrrolidinone (NMP).

2. Amine submonomers

(*S*)-*N*-(1-phenylethyl)glycine (or *N*spe, (*S*)-1-phenylethylamine), (*R*)-*N*-(1-phenylethyl)glycine (or *N*rpe, (*R*)-1-phenylethylamine), and *N*-(1-phenylmethyl)glycine (or *N*pm, benzylamine) were obtained from Sigmaaldrich (Milwaukee, WI, USA) at a purity >99%. Structures of the peptoid side chains derived from these amine submonomers are shown in Table 1.

3. Peptoid synthesis and purification

Peptoids **1 - 30** were synthesized using microwave-assisted solid-phase submonomer synthesis methods[1] on an Fmoc-Rink amide MBHA resin. A CEM MARS multimodal microwave reactor equipped with a fiber-optics temperature probe and magnetic stirrer was used (CEM Corp., Matthews, NC, USA). The fiber-optics temperature probe was positioned in the reaction mixture, and a solution was stirred and irradiated at different reaction conditions as described below. Fmoc-Rink amide MBHA resin (0.59 mmol/g, 102 mg, 0.06 mmol) was swelled in DMF for 20 minutes and drained. The resin was treated with 20% piperidine in DMF (5 mL) at room temperature for 60 seconds and at 80 ºC (microwave, 800W max power, 80% power, ramp 2 min, hold 2 minutes, stirring level 3) in the microwave reactor. The resin was washed (5 mL each time) with DMF (3x), methanol (3x), and DCM (2x). The deprotected Rink amide resin (0.06 mmol) was suspended in DMF (2 mL) and drained. Acylation reaction was performed by the addition of bromoacetic acid (1 mL of 1.2 M bromoacetic acid stock solution, 1.2 mmol), followed by DIC (0.19 mL of 0.152 g, 1.2 mmol). Reaction mixture was stirred at 35 ºC (microwave, 400W max power, 15% power, hold 2 minutes, stirring level 3) in the microwave reactor. The resin was washed (5 mL each time) with DMF (3x) and DCM (2x). Displacement reaction was perfor

med by addition of primary amine as 1.5 M solution in NMP (1.6 ml, 1.5 M primary amine in NMP st ock solution, 2.4 mmol), followed by stirring at 95 ºC (microwave, 400 W max power, 75% power, ra mp 2 minutes, hold 90 seconds, stirring level 3) in the microwave reactor. The resin was washed with DMF (3x) and DCM (2x). Acylation and displacement reaction were performed until desired sequenc e is obtained. The peptoids were cleaved from the resin by the addition of cleavage solution (95:2.5:2. 5 (v/v/v) TFA/water/TIS, 1 mL). The reaction mixture was stirred for 10 - 20 minutes at room temper ature. The cleavage solution was filtered by solid-phase extraction (SPE) cartridges with 20 μ PE frit (Applied Separations, Allentown, PA, USA), diluted by the addition of excess water, and the volatiles were removed by lyophilizer. The final product was dissolved in ACN. Crude peptoid oligomers were analyzed on a C18 analytical HPLC column at room temperature (SunFire C18, 4.6 x 250 mm, 5 μm). Analytical HPLC was performed on a Waters HPLC system (Waters 2489 UV/Visible Detector, Wate rs 1525 Binary HPLC Pump, Waters 2707 Autosampler, and Waters 5CH column oven). A linear gra dient of 5-100% ACN/water (0.1% TFA) over 30 min was used with a flow rate of 1 mL/min. Crude p eptoid oligomers were purified using a preparative HPLC system (Waters prepLC system, Waters 248 9 UV/Visible Detector, Waters fraction collector III). Preparative HPLC was performed on a C18 colu mn (SunFire, 5 μm, 19 mm x 150 mm) with a linear gradient of 5-100% ACN/water (0.1% TFA) over 30 min with a flow rate of 14 mL/min. Sample elution was monitored at 220 and 254 nm by absorban ce. The purity of the product fractions were confirmed by analytical HPLC. Each fraction was further analyzed by LC/MS performed on an Agilent 1100 liquid chromatography system with an Agilent 61 30 single quadrupole mass spectrometer (Applied Biosystems). Fractions containing pure product (>9 7% purity) were collected, lyophilized, and stored at -80 °C.

4. Circular dichroism spectroscopy

CD measurements were performed on a Jasco model 810 spectropolarimeter (Jasco, Inc., Easton, MD, USA). CD sample solutions (typically 50 μM) were made in 1.5 mL microtube by dilution of stock so lutions (1 mM). Stock solutions (1 mM) were prepared in 12 mL scintillation vial by a precise weighi ng of at least 1 mg of lyophilized peptoid powder and then adding appropriate volume of solvent. CD spectra were acquired in a quartz cell with a path length of 1 mm, employing a scan rate of 20 nm/min . CD spectra reported here represent the average of 3 successive spectral accumulations. Data are expr essed in terms of per-residue molar ellipticity (deg cm^2/dmol), as calculated per mole of amide groups present and normalized by the molar concentration of peptoids.

5. HPLC data of 1 - 30

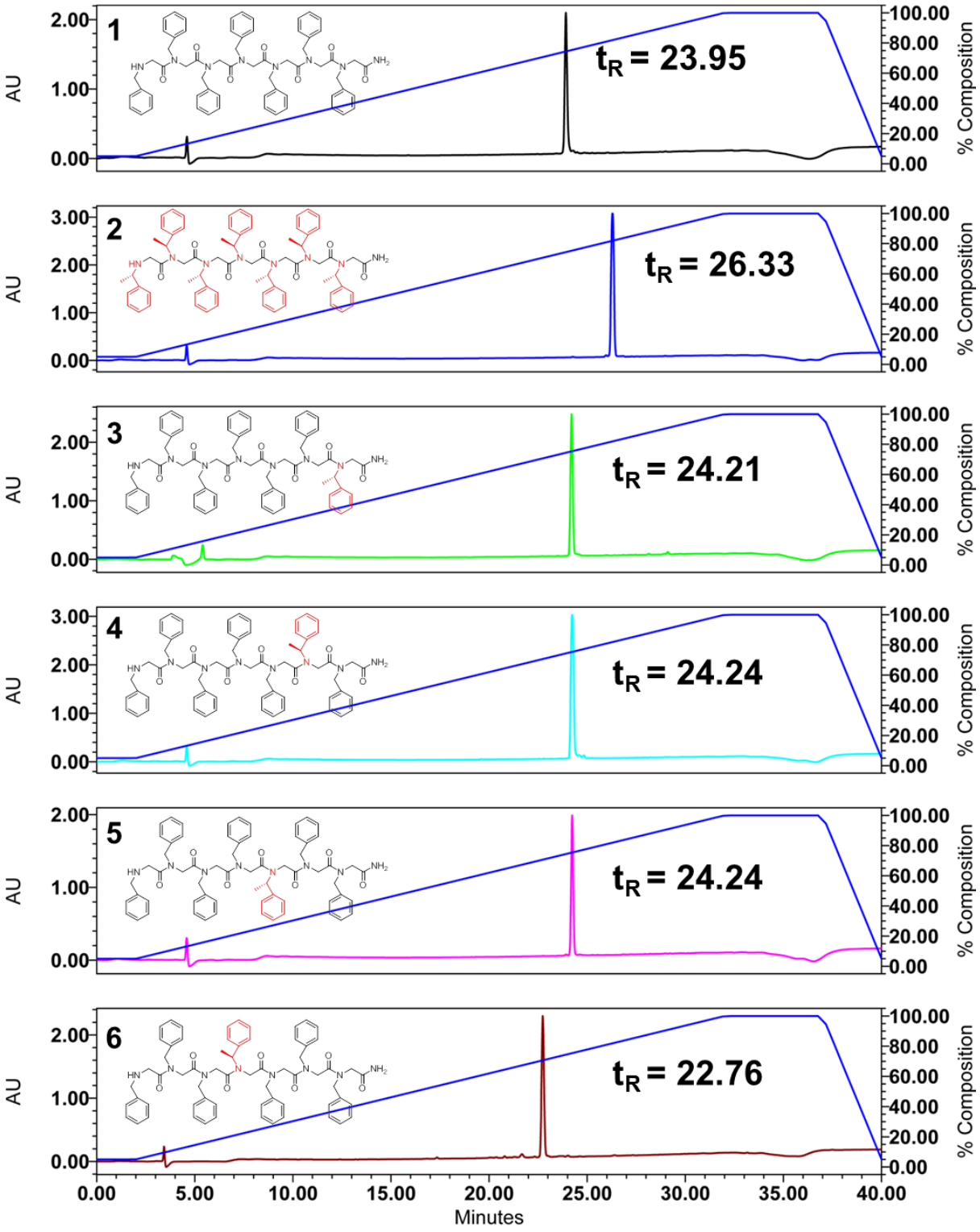

Fig. S1. HPLC chromatograms of **1** - **6** with UV detection at 220 nm.

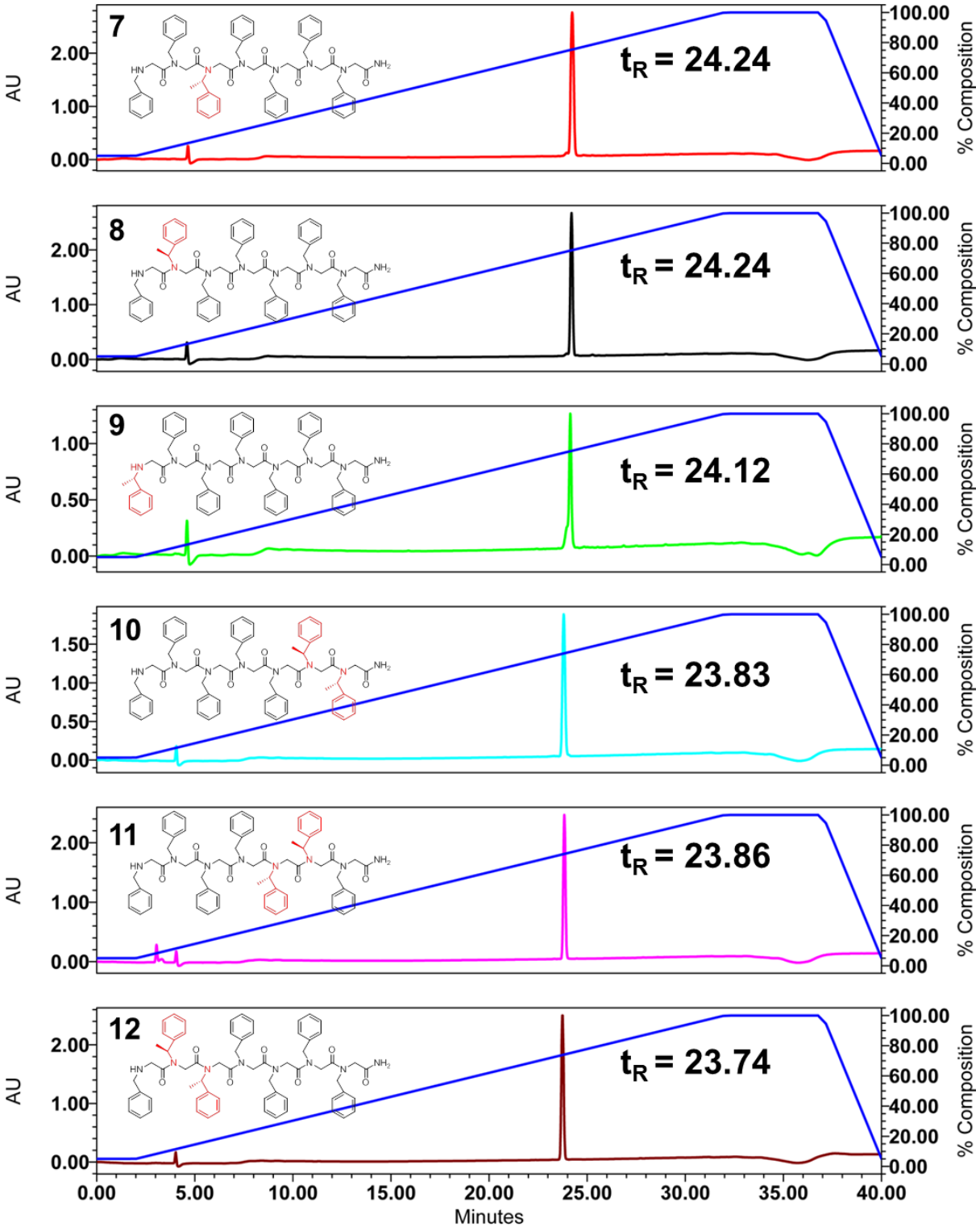

Fig. S2. HPLC chromatograms of **7** - **12** with UV detection at 220 nm.

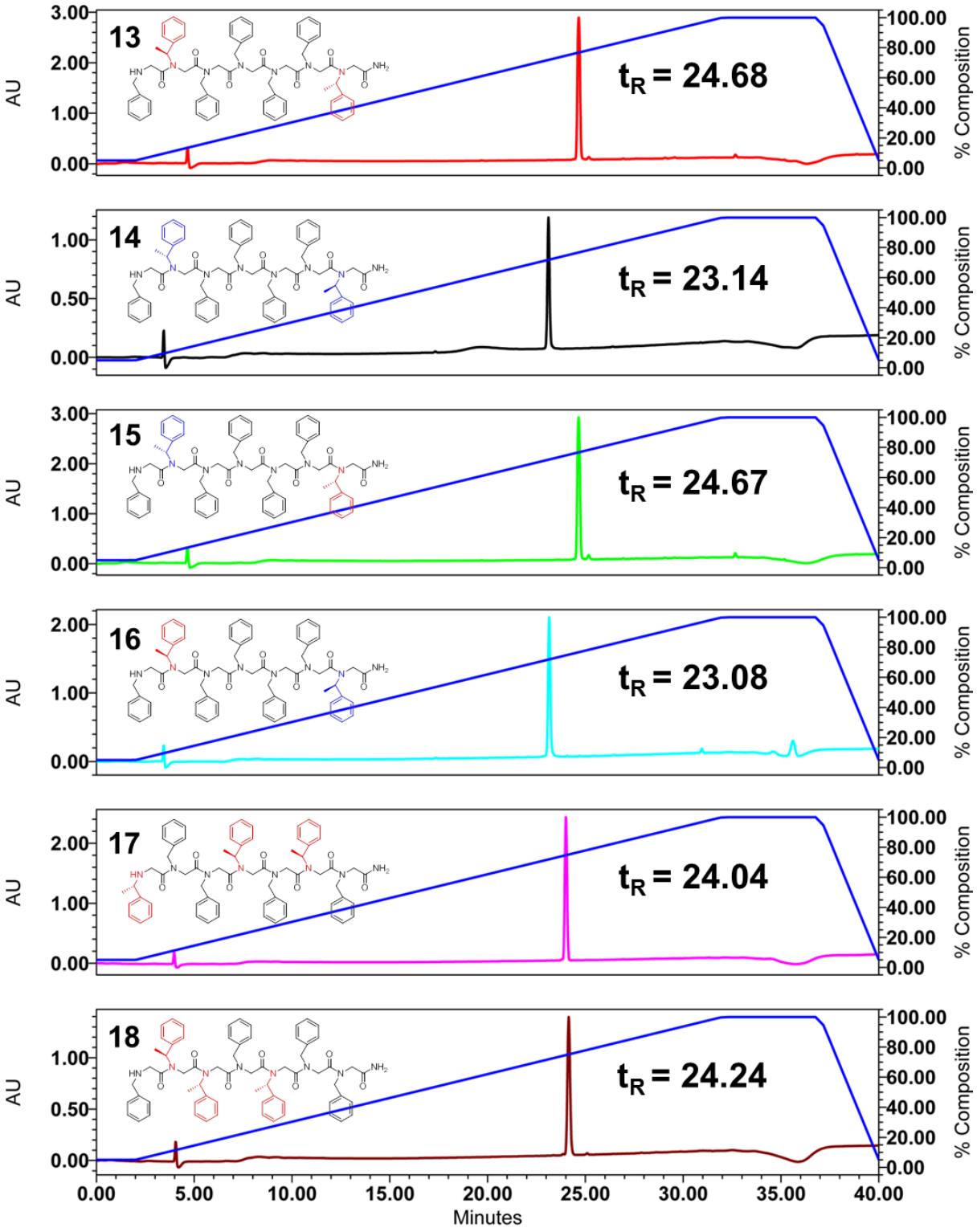

Fig. S3. HPLC chromatograms of **13** - **18** with UV detection at 220 nm.

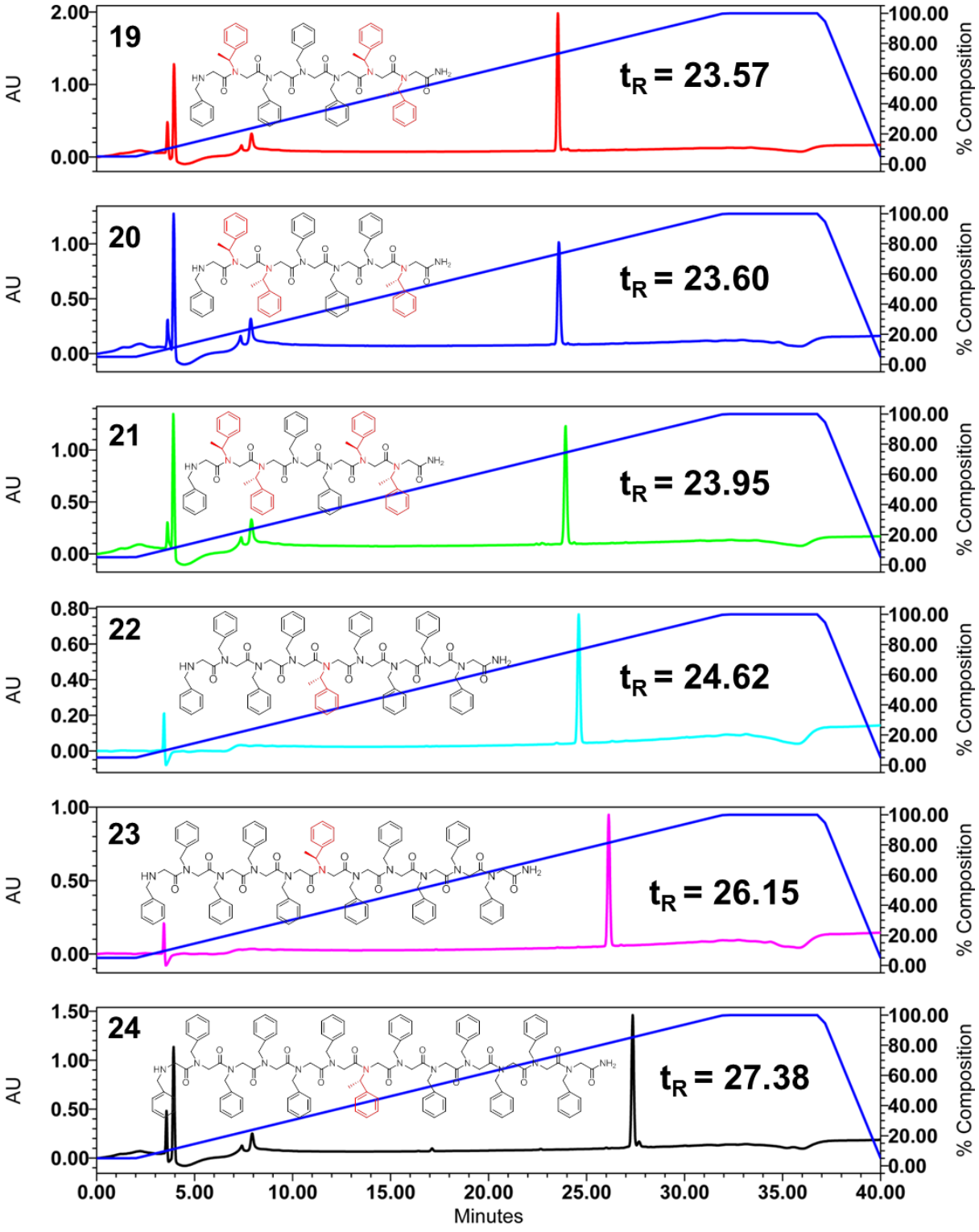

Fig. S4. HPLC chromatograms of **19** - **24** with UV detection at 220 nm.

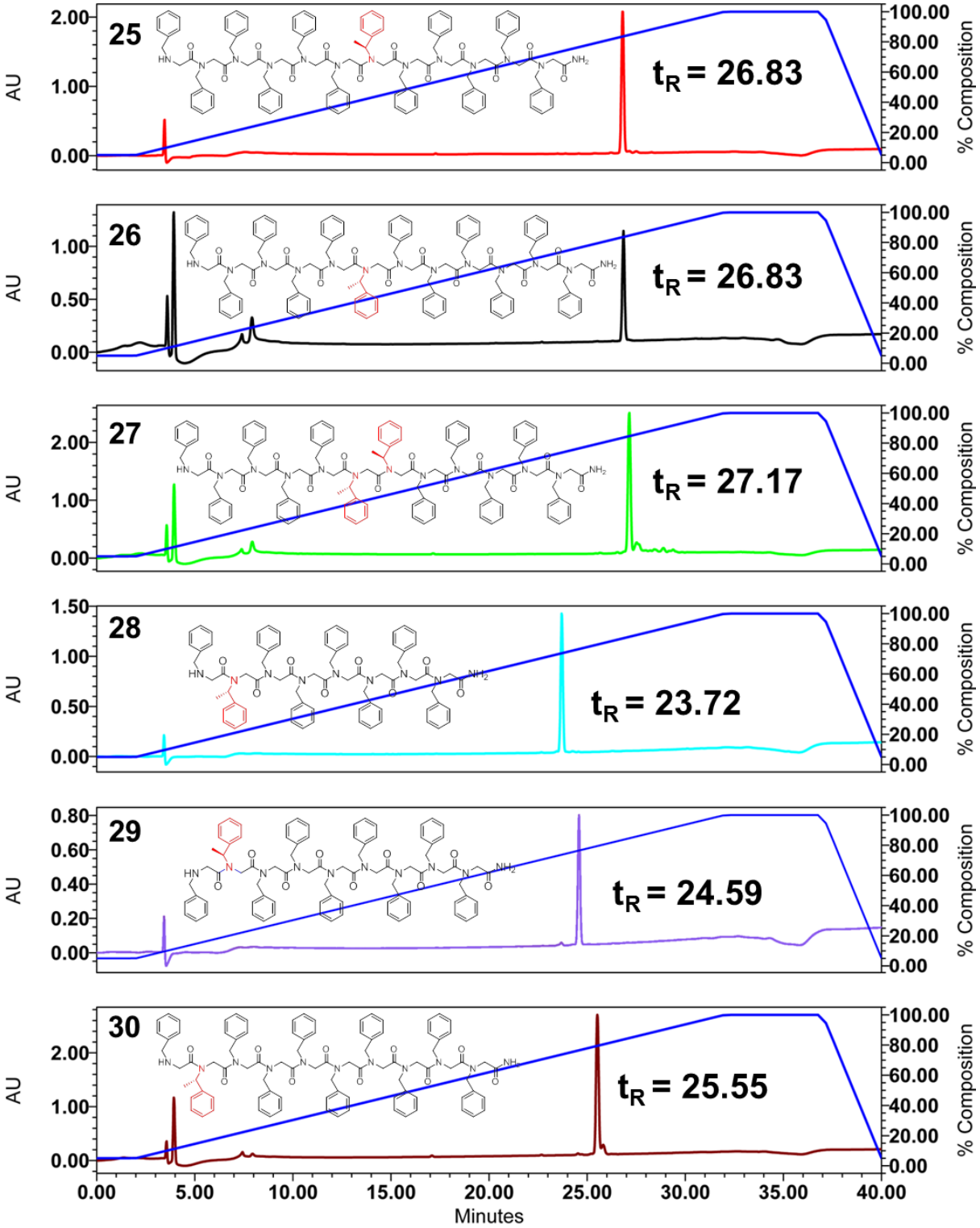

Fig. S5. HPLC chromatograms of **25** - **30** with UV detection at 220 nm.

6. ESI-MS data of 1 - 30

Table S1. ESI-MS data of **1** - **30**.

Compounds	Mass calculated	Mass observed[a]
1	1048.3	1048.8 (H^+)
2	1146.4	1147.0 (H^+)
3	1062.3	1062.7 (H^+)
4	1062.3	1062.8 (H^+)
5	1062.3	1062.8 (H^+)
6[b]	1062.3	542.0 (Na^++H^+)
7	1062.3	1062.8 (H^+)
8[b]	1062.3	542.0 (Na^++H^+)
9[b]	1062.3	542.0 (Na^++H^+)
10	1076.3	1076.9 (H^+)
11	1076.3	1076.6 (H^+)
12	1076.3	1076.9 (H^+)
13	1076.3	1077.0 (H^+)
14	1076.3	1077.0 (H^+)
15	1076.3	1077.0 (H^+)
16	1076.3	1076.6 (H^+)
17	1090.3	1091.0 (H^+)
18	1090.3	1091.0 (H^+)
19	1090.3	1091.5 (H^+)
20	1090.3	1091.5 (H^+)
21	1104.4	1104.3 (H^+)
22	1355.7	1355.3 (H^+)
23	1649.8	1649.4 (H^+)
24[b]	1942.9	981.1 (Na^++H^+)
25	1798.2	1798.6 (H^+)
26	1798.2	1798.7 (H^+)
27	1812.2	1812.7 (H^+)
28	1208.6	1208.5 (H^+)
29	1355.7	1355.1 (H^+)
30	1503.8	1503.9 (H^+)

[a]Observed in ESI-MS. [b] The observed parent peaks are doubly charged (Na^++H^+) peaks, not fragments.

7. Interpretation of CD spectra

(1) Peptoid **15** and **16**.

Since it was demonstrated that two positions, the second from N-terminus and the first from C-termin us, contributed more significantly on the peptoid structure formation, peptoids **15 - 16** were prepared t o investigate the relationship between two α-chiral monomers with the opposite chirality (Fig. S6). Th e CD spectrum of **15** with *N*spe on the first position from the C-terminus and *N*rpe on the second posit ion from the N-terminus showed uncharacteristic CD signature, but negative Cotton effect at 195 nm was observed probably due to the influence of C-terminal *N*spe. Peptoid **16**, which has switched positi ons of *N*spe and *N*rpe of **15**, showed a symmetrical CD signature, and again the positive Cotton effect at 195 nm was possibly induced by C-termnial *N*rpe. These results agree with the conclusions reached by Lee and coworkers in their helical polyisocyanate studies; the chirality of the monomer at the begi nning of the polymer chain growth played a critical role in determining the overall handedness of the chain.[2] In addition, we confirmed the importance of the placement of an α-chiral, aromatic residue on the carboxy terminus as Barron and coworkers noted.[3]

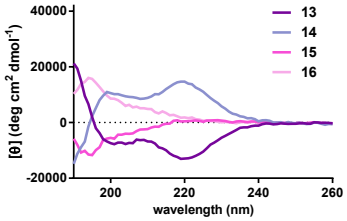

Fig. S6. CD spectra of peptoid heptamers **13**-**16** (50 μM in acetonitrile) were recorded as per-residue molar ellipticity, or [θ]. Data were acquired at 20 ºC.

(2) Peptoid **22**-**30**.

To compare with peptoids **22**-**24** that include an odd number of monomer units, even numbered peptoi d dodecamers with one *N*spe (**25** and **26**) and two *N*spe's (**27**) at the middle positions were prepared. Again, non-helical peptoid CD signature was obtained with **25** and **26**, but slightly increased but weak Cotton effect was observed with **27**. Peptoid nonamers, **22** and **29**, showed similar CD signatures. Bot h peptoid nonamers appear to have an elevated population of threaded loop conformations; however, more in-depth study is required to provide more conclusive evidences on this assumption.

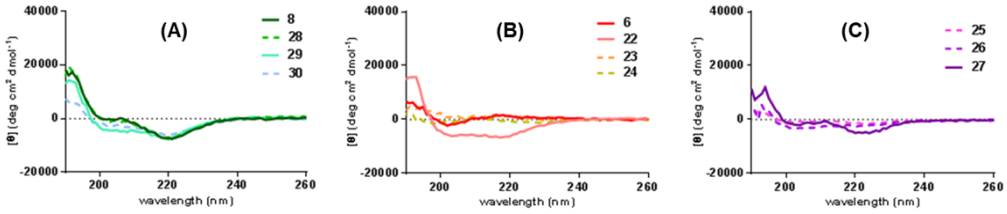

Fig. S7. CD spectra of longer peptoids (50 μM in acetonitrile) were recorded as per-residue molar elli pticity, or [θ]. Data were acquired at 20 ºC. Single *N*spe incorporation (A) at the second position from N-terminus and (B) at the middle position of the sequence. (C) CD spectra of **25**-**27**.

(3) Additive effect of **13** and synergistic effects of **20**, **21**, and **2**.

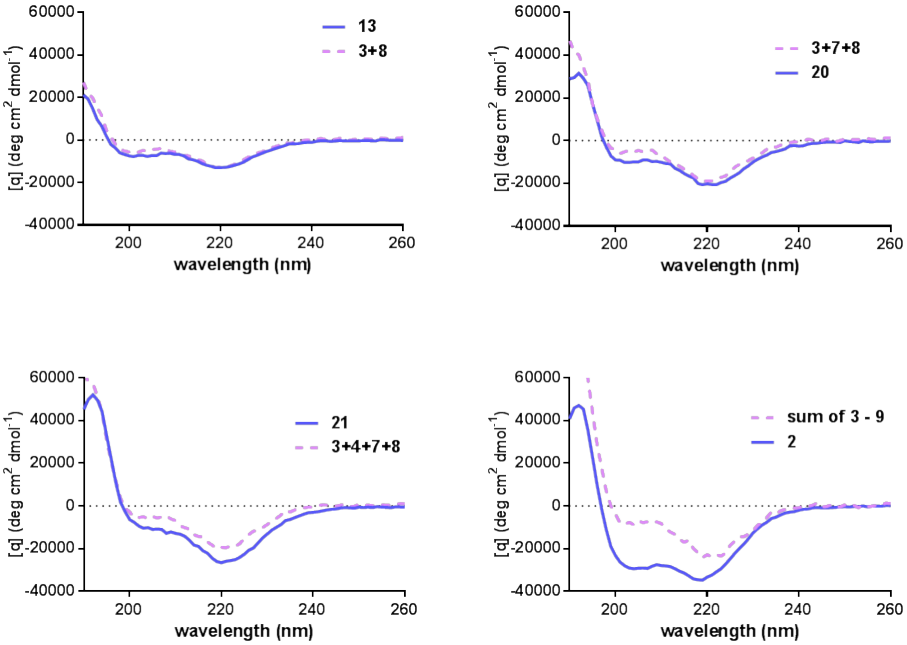

Fig. S8. CD spectra of peptoids **13**, **20**, **21**, and **2** (blue) in comparison to the sum of CD spectra of cor responding one *N*spe containing peptoids.

Reference

(1) R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, *J. Am. Chem. Soc.* 1992, **114**, 10646-1 0647.
(2) P. N. Shah, J. Min, J. Lee, *Chem. Comm.* 2012, **48**, 826-828.
(3) C. W. Wu, T. J. Sanborn, K. Huang, R. N. Zuckermann, A. E. Barron, *J. Am. Chem. Soc.* 2001, **12 3**, 6778-6784.