Supplementary Information

Bicyclic and tricyclic C-C mismatch-binding ligands bind to CCG trinucleotide repeat DNAs

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Contents

Experimental section

General: Reagents and solvents were purchased from standard suppliers and used without purification. DNAs were purchased from Life Technologies and Gene Design Inc.

***T*$_m$ measurements:** Thermal denaturation profiles were recorded on a UV-2700 spectrophotometer (Shimadzu) equipped with a TMSPC-8 temperature controller and a 10 mm path-length cell. The absorbance of DNA duplex (5 μM) with ligand (10 μM) in phosphate buffer (10 mM, pH 7.0) containing NaCl (100 mM) was monitored at 260 nm from 2 to 100 °C (1 °Cmin^{-1}). T_m was calculated by using the median method. The thermal denaturation profiles of repeat DNAs (4 μM) in the absence and presence of ligand (20 μM) were measured in phosphate buffer (10 mM, pH 7.0) containing NaCl (100 mM) and 5% DMSO.

Absorbance spectra measurements: UV spectra were measured in phosphate buffer (10 mM, pH 7.0) containing NaCl (100 mM) without and with **Am-BzND** (10–50 μM) by using a BECKMAN COULTER DU800 UV/Vis spectrophotometer.

ESI-TOF-MS measurements: Samples were prepared by mixing DNAs (10 μM hairpin DNA or 20 μM repeat DNA) and **Am-BzND** (20–40 μM for hairpin DNA or 80–160 μM for repeat DNA) in 50% methanol in water containing 100 mM ammonium acetate. Mass spectra were obtained with JEOL JMS-T100LP AccuTOF LC-plus 4G mass spectrometer in negative mode. Spray temperature was fixed at –10 °C with a sample flow rate of 20 μL min^{-1}.

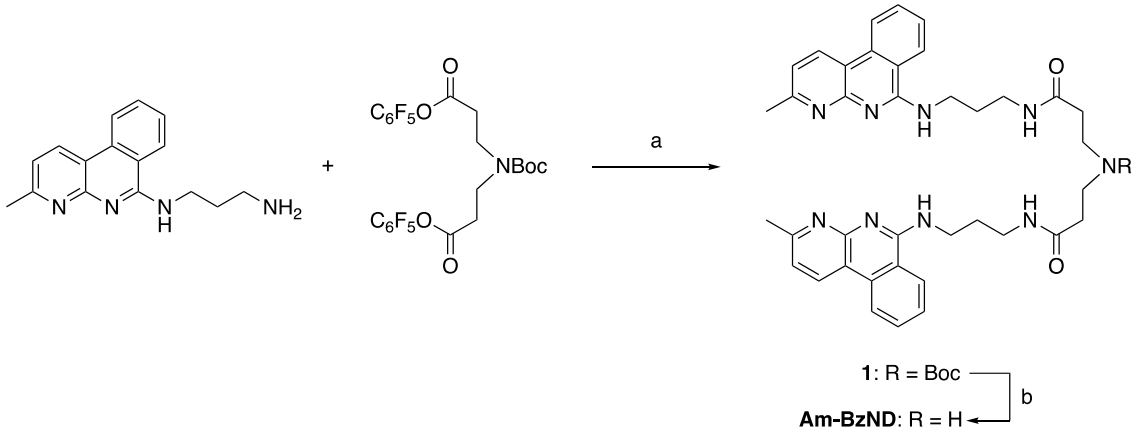
CD measurements: CD experiments were carried out on a J-725 CD spectrometer (JASCO) using a 10 mm path length cell. CD spectra of DNAs (5 μM DNA duplex or 4 μM repeat DNA) in the absence and presence of ligand (10 μM for DNA duplex or 20 μM for repeat DNA) were measured in phosphate buffer (10 mM, pH 7.0) containing NaCl (100 mM) without (for DNA duplex) and with (for repeat DNA) 5% DMSO.

SPR measurements: A streptavidin-coated SA sensor chip (GE Health care) was washed with HBS-EP+ buffer (HEPES (10 mM, pH 7.4), NaCl (150 mM), EDTA (3 mM), surfactant P20 (0.005%, *v*/*v*)) for 6 min then activated with three consecutive 1 min injections of activation mixture (30 mL; NaOH (50 mM) and NaCl (1 M)). 5'-Biotinylated trinucleotide repeat DNAs were diluted to 0.2 μM with HBS-EP+ buffer and allowed to flow onto the SA chip. d(CGG)$_9$,

$d(CCG)_9$, $d(CAG)_9$, and $d(CTG)_9$ were immobilized on the chip at 490, 539, 584, 580 response units, respectively. Binding of ligands to the immobilized DNA was analyzed by using a BIAcore T200 SPR system (GE Healthcare). Ligand (5, 10, 20, 40, 80 nM in HBS-EP+ buffer) was injected over the flow cells on the sensor surface in single-cycle mode.

Simulation of ligand-bound structures: Molecular modeling studies were carried out using the Maestro 10.0 molecular modeling package. Initial structures of ligand-DNA complexes were manually constructed. The ligand-DNA complexes were subjected to energy minimization using the AMBER* force field and Polak–Ribier Conjugate Gradient method with a convergence threshold of 0.05. The obtained structure was subjected to the further refinement by manual change of the ligand position and subsequent energy minimization. These refinements were done several times.

Scheme S1[a]



[a]Reagent and condition: (a) DIEPA, THF-DMF, 97% (b) 4N HCl/AcOEt, $CHCl_3$, 83%.

Bis-{2-[3-(3-methylbenzo[*c*][1,8]naphthyridin-6-ylamino)-propylcarbamoyl]-ethyl}-carbamic Acid *tert*-Butyl Ester (1)

A mixture of *N*-(3-methylbenzo[*c*][1,8]naphthyridin-6-yl)propane-1,3-diamine (40 mg, 150 μmol) and *N*-(*tert*-butoxycarbonyl)imino-3,3'-bis-(pentafluorophenyl propionate)[1] (25 mg, 42 μmol) was stirred in THF/DMF = 2:1 (3 mL). Then *N*,*N*-diisopropylethylamine (115 μL, 660 μmol) was added to the mixture. The mixture was stirred at room temperature overnight. The solvent was evaporated to dryness. The resulting mixture was dissolved in chloroform, washed with saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel chromatography to give **1** (31 mg, 97%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47 (d, 2 H, *J* = 8 Hz), 8.38 (d, 2 H, *J* = 8.4 Hz), 8.06 (br 2 H), 7.74 (t, 2 H, *J* = 7.2 Hz), 7.61 (t, 2 H, *J* = 7.2 Hz), 7.11 (d, 2 H, *J* = 8 Hz), 3.84 (m, 4 H), 3.67 (m, 4 H), 3.33 (m, 4 H), 2.71 (t, 4 H, *J* = 6.4 Hz), 2.69 (s, 6H), 1.80 (quin, 4 H, *J* = 6.0 Hz), 1.43 (s, 9 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.4, 159.8, 156.5, 156.0, 154.7, 133.8, 131.1, 130.7, 127.5, 123.0, 122.5, 119.2, 118.0, 112.6, 80.3, 70.6, 44.5, 37.4, 36.2, 29.8, 28.4, 25.0; HRMS (ESI) *m*/*z*: calcd. for $[C_{43}H_{51}N_9O_4+H]^+$ 758.4137; found 758.4139.

3,3'-azanediylbis{*N*-[3-(3-methylbenzo[*c*][1,8]naphthyridin-6-ylamino)-propyl]-propanamide} (Am-BzND)

To a $CHCl_3$ solution (1.5 mL) of **1** (9 mg, 24.6 μmol) was added ethyl acetate containing 4 N HCl (1 mL). The mixture was stirred at room temperature for 1 h. Solvent was evaporated to dryness

to give **Am-BzND** as hydrochloric salt. ^{1}H NMR (CD_3OD, 700 MHz) δ 8.97 (d, 2 H, J = 8.4 Hz), 8.41 (d, 2 H, J = 7.8 Hz), 8.35 (d, 2 H, J = 8.4 Hz), 7.89 (t, 2 H, J = 7.8 Hz), 7.81 (t, 2 H, J = 7.2 Hz), 7.36 (d, 2 H, J = 7.8 Hz), 3.71 (t, 4 H, J = 6.6 Hz), 3.37 (t, 4 H, J = 6.0 Hz), 3.33 (t, 4 H, J = 6.6 Hz), 2.80 (t, 4 H, J = 6.0 Hz), 2.76 (s, 6H), 1.94 (quin, 4 H, J = 6.6 Hz); ^{13}C NMR (CD_3OD, 175 MHz) δ 173.1, 160.1, 153.3, 150.7, 140.0, 133.8, 132.2, 131.2, 125.2, 124.2, 120.6, 118.7, 117.3, 45.1, 40.3, 37.9, 31.5, 29.7, 19.6; HRMS (ESI) m/z: calcd. for $[C_{38}H_{43}N_9O_2+H]^+$ 658.3612; found 658.3613.

The product was further purified by HPLC to give **Am-BzND** (6.5 mg, 83%).

^{1}H NMR (CD_3OD, 600 MHz) δ 8.57 (d, 2 H, J = 7.8 Hz), 8.41 (d, 2 H, J = 7.2 Hz), 8.14 (d, 2 H, J = 8.4 Hz), 7.72 (t, 2 H, J = 7.2 Hz), 7.59 (t, 2 H, J = 7.2 Hz), 7.12 (d, 2 H, J = 7.8 Hz), 3.72 (t, 4 H, J = 6.6 Hz), 3.27 (t, 4 H, J = 6.0 Hz), 3.12 (t, 4 H, J = 6 Hz), 2.61 (t, 4 H, J = 6.6 Hz), 2.60 (s, 6H), 1.87 (quin, 4 H, J = 6.6 Hz)

Reference

1. K. Nakatani, S. Sando, H. Kumasawa, J. Kikuchi, I. Saito, *J. Am. Chem. Soc.*, 2001, **123**, 12650–12657.

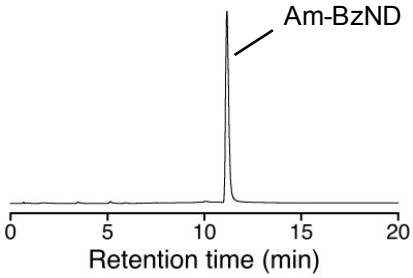


Figure S1. HPLC chart of **Am-BzND**. Reserved-phase HPLC was performed on COSMOSIL 5-C18-MS-II. Elution with 1–21% acetonitrile in 0.1% acetic acid.

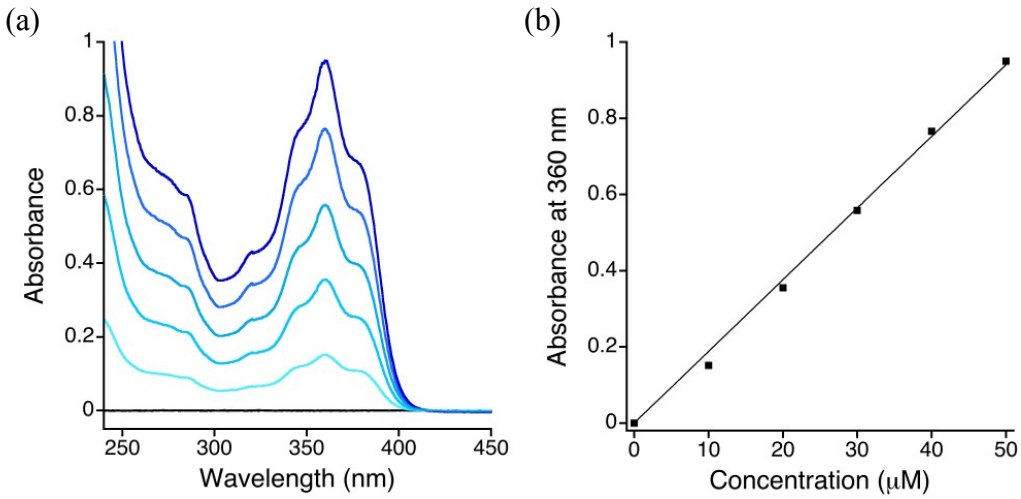


Figure S2. (a) Absorption spectra of **Am-BzND** in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl. **Am-BzND** concentration was 0, 10, 20, 30, 40, and 50 μM. (b) Plot of absorbance at 360 nm vs. **Am-BzND** concentration (0, 10, 20, 30, 40, and 50 μM).

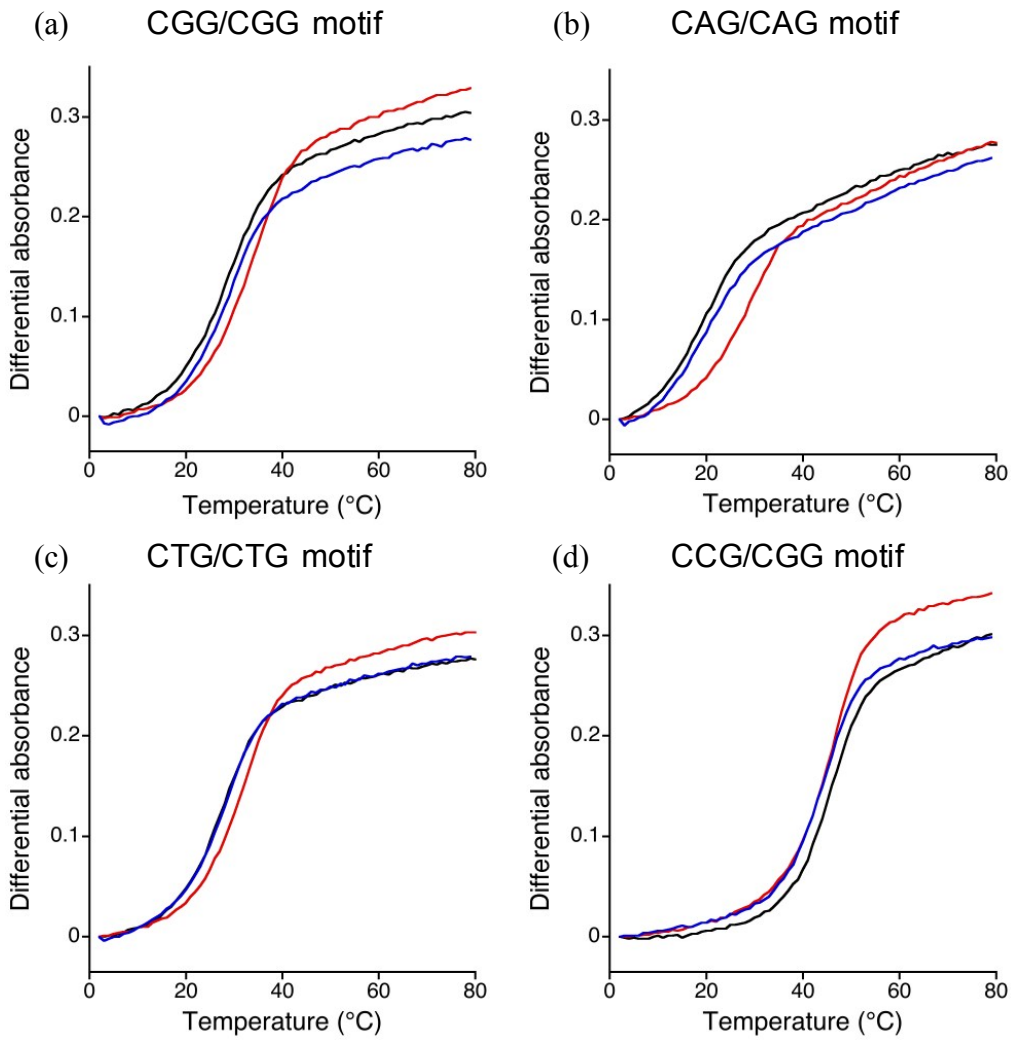


Figure S3. Thermal melting curves of CXG/CYG motif-containing DNA duplexes (5 μM), d(CTAA CXG AATG)/d(CATT CYG TTAG), without (black) and with **Am-BzND** (red) and **Am-ND** (blue) in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl. Key: (a) CGG/CGG, (b) CAG/CAG, (c) CTG/CTG and (d) CCG/CGG motif-containing DNA duplexes. Ligand concentration was 10 μM.

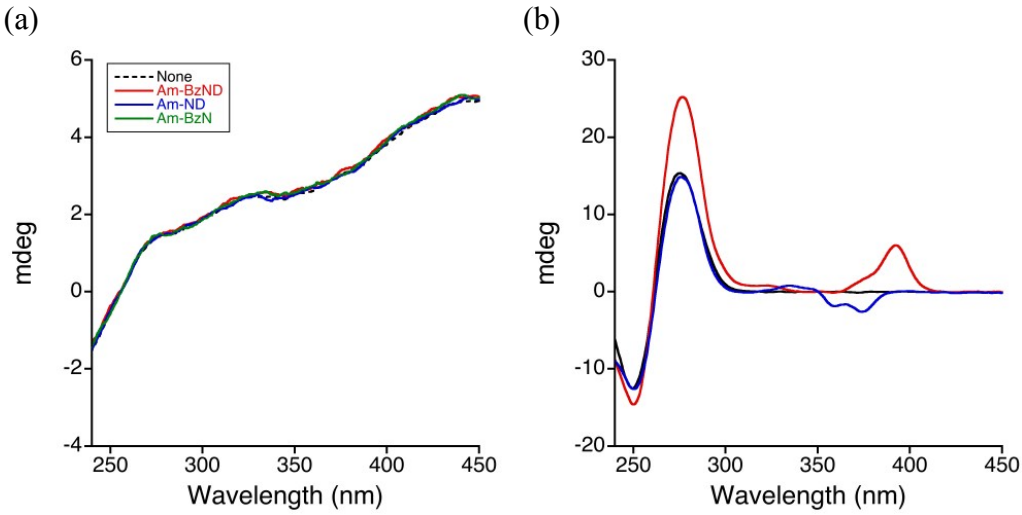


Figure S4. (a) CD spectra of 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl without (dotted line) and with **Am-BzND** (red), **Am-ND** (blue), and **Am-BzN** (green). Ligand concentration was 20 μM. (b) CD spectra of 5'-d(CTAA CCG AATG)-3'/5'-d(CATT CCG TTAG)-3' (black) in the presence of **Am-BzND** (red) and **Am-ND** (blue). DNA and ligand concentrations were 5 μM and 20 μM, respectively.

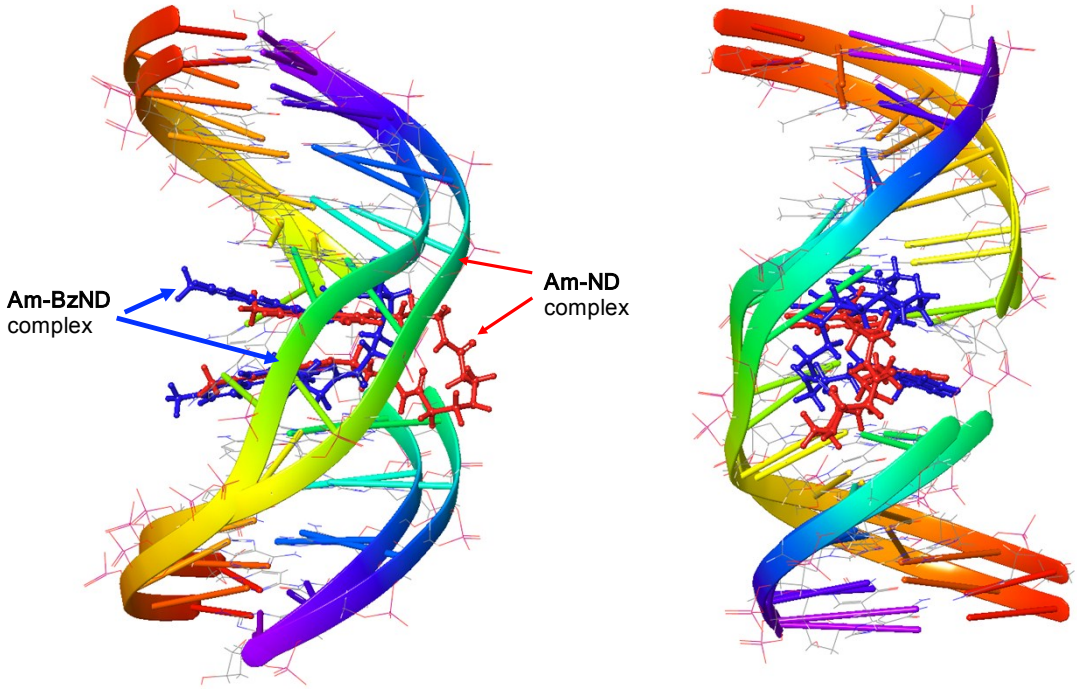


Figure S5. Molecular modelling structures of the 1:1 complexes consisting of CCG/CCG motif-containing DNA duplex and **Am-BzND** (blue) and **Am-ND** (red). The backbone was shown in a ribbon presentation. The backbone of the **Am-BzND** bound DNA (indicated by a blue arrow) was significantly distorted more than that in the **Am-ND** complex.

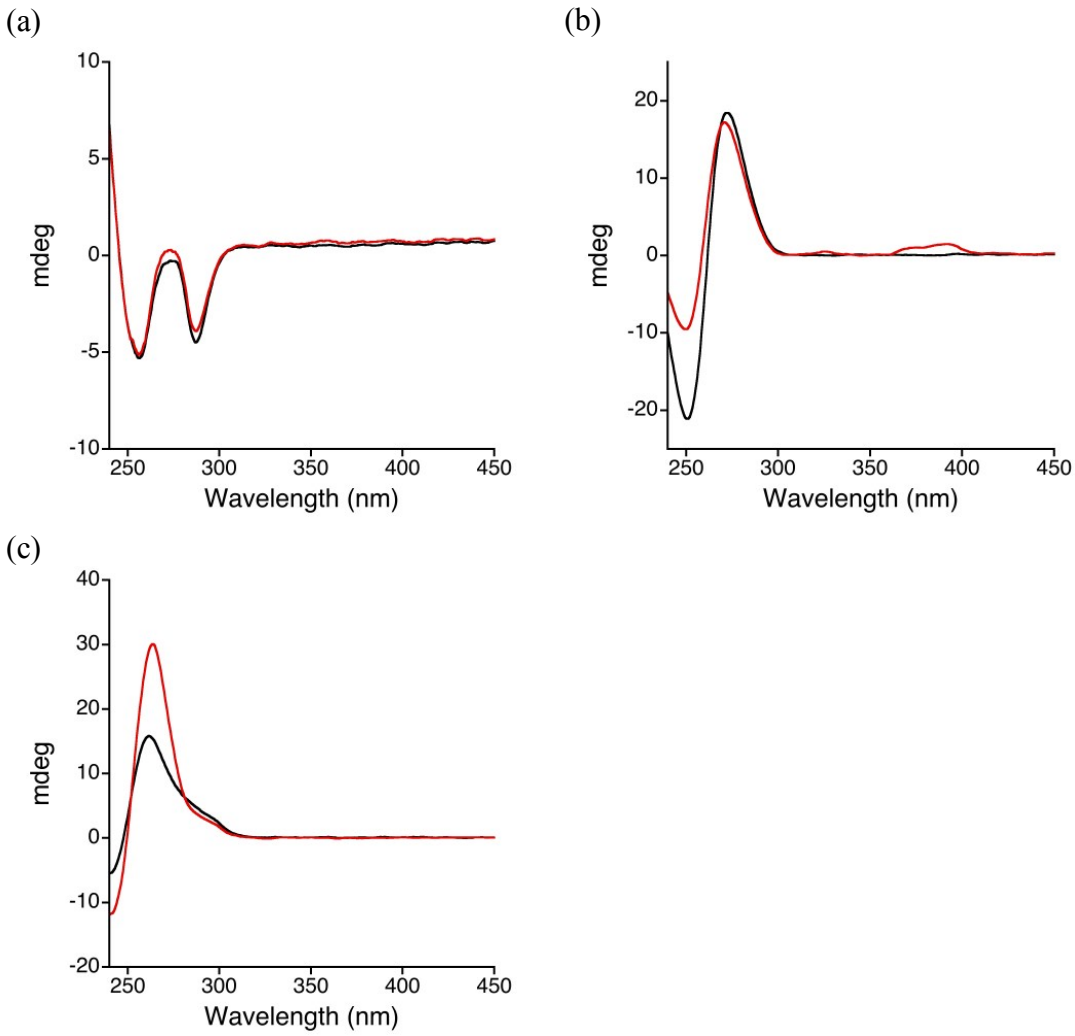


Figure S6. CD spectra of (a) $d(GAA)_9$, (b) $d(ATT)_9$, and (c) $d(TGG)_9$ without (black) and with **Am-BzND** (red). DNA and **Am-BzND** concentrations were 4 μM and 20 μM, respectively.

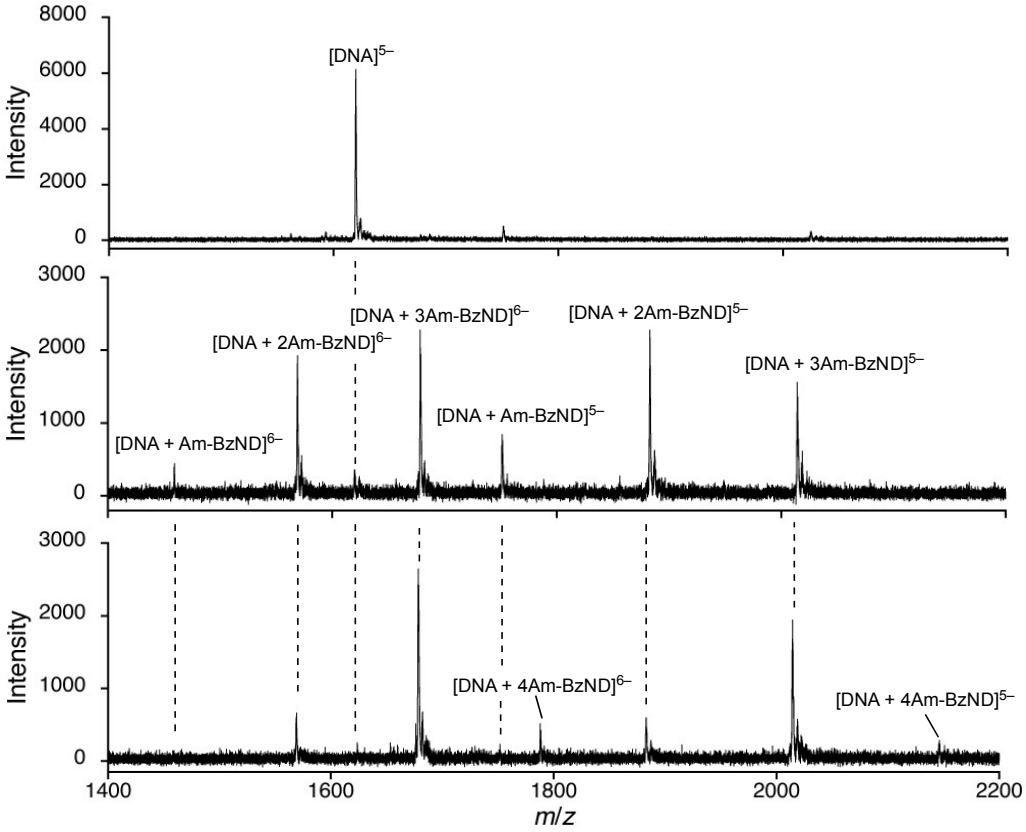
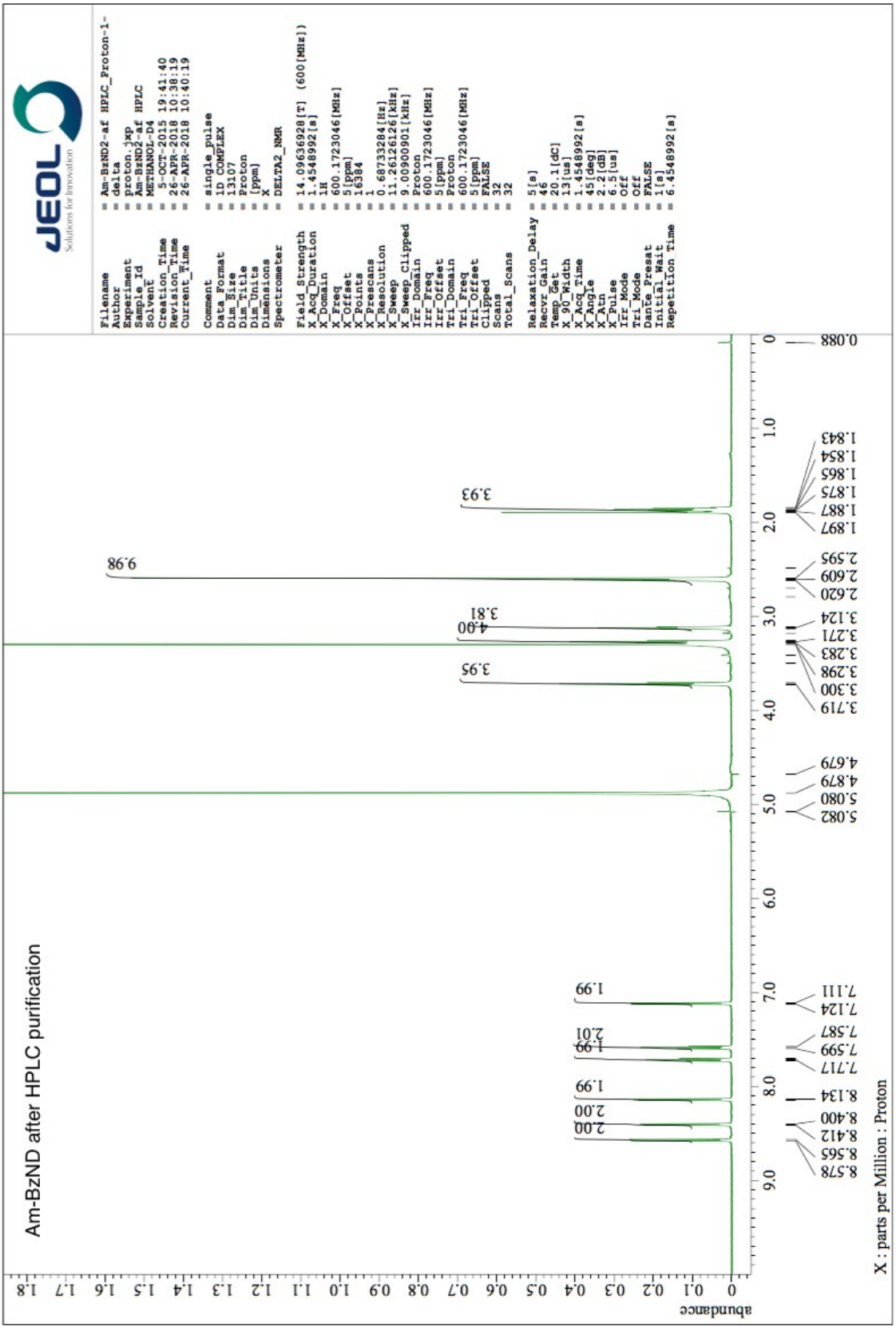


Figure S7. ESI-TOF-MS spectra of d(CCG)$_9$ (20 μM) (top) in the presence of 80 μM (middle) and 160 μM (bottom) **Am-BzND**. Dotted lines are eye-guides showing the same ion in three plots.


Am-BzND after HPLC purification
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Solutions for Innovation
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