Supporting Information

PNA-Based Multivalent Nanoscaffolds Activate the Dopamine D_2 Receptor

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Materials and Instrumentation

Commercial-grade reagents and solvents were used without further purification except as indicated. Boc-protected *aeg*PNA monomers were purchased from PolyOrg, Inc. (Leominster, MA, USA). HMBA Resin, 100-200 mesh, 1% DVB was obtained from Advanced Chemtech (Louisville, KY, USA). Boc-mini-PEG was purchased from Peptides International (Louisville, KY, USA). ^{L}Kγ-PNA thymine monomer was synthesized according to published procedures.[1,2] All other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). PNA oligomer synthesis was performed on an Applied BioSystems 433A Automated Peptide Synthesizer. Purification of PNA oligomers was carried out using a X-Bridge Prep BEH 130 C18 5 µm (10 x 250 mm) column on an Agilent 1200s HPLC. The typical flow rate was 4 mL/min. HPLC solvents consisted of HPLC grade ACN:MilliQ H_2O (9:1) and 0.10% aq. TFA. Wavelengths 220 nm, 260 nm, and 315 nm were monitored. High-resolution mass spectra (HRMS) were obtained on a LC/MSD TOF (Agilent Technologies, Santa Clara, CA, USA). UV quantification of PNA was performed using an Agilent 8453 UV-Vis Spectrophotometer.

Abbreviations

ACN, acetonitrile; Boc, *tert*-butoxycarbonyl; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; ESI-MS, electrospray ionization mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HPLC, high performance liquid chromatography; MBHA resin, 4-methylbenz-hydrylamine resin; NMP, *N*-methyl-2-pyrrolidinone; mini-PEG, 8-amino-3,6-dioxaoctanoic acid; PPHT, 2-(*N*-phenethyl-*N*-propyl)amino-5-hydroxytetralin; phosphate buffered saline; PNA, peptide nucleic acid; TFA, trifluoroacetic acid; TfOH, trifluoromethanesulfonic acid;

General Synthesis and Purification Procedures

Preparation of PNA Oligomers. MBHA Resin (0.3 mmol/g) was prepared by swelling in CH_2Cl_2 and downloading the resin with Boc protected *N,N*-dimethyl-L-lysine to 0.1 mmol/g capacity. PNA oligomers were made via solid-phase peptide synthesis in accordance with literature procedures on 5 mol scale.[1-3] PNA oligomers were prepared using solid-phase peptide synthesis on an ABI443i peptide synthesizer. Boc-PNA monomers were used. A standard cycle included deprotection, activation, coupling (30 min), washing, and capping. The first step, and any step incorporating the ^{L}Kγ-PNA monomer were modified to allow for two deprotection steps and a longer coupling time (90 min). Monomers following the incorporation of ^{L}Kγ-PNA monomer were also modified to double-couplings, in addition to three deprotection sequences and longer coupling time (90 min). After the PNA 12-mer was synthesized, a global Boc-deprotection, capping (acetylation), and washing was performed. Here, the Fmoc sidechain on the ^{L}Kγ-PNA monomer(s) were removed. Using a similar double-coupling procedure, the mini-PEG side chains were installed. Standard solutions of TFA (5% *m*-cresol) and piperidine (20% in DMF) were used to remove Boc and Fmoc protecting groups, respectively.

Sequences. Sequence used for PPHT-conjugated PNA: AGT-AGA-TCA-CTG. Complementary antiparallel sequence (cPNA): CAG-TGA-TCT-ACT. Longer cPNA were prepared as previously described.[4]

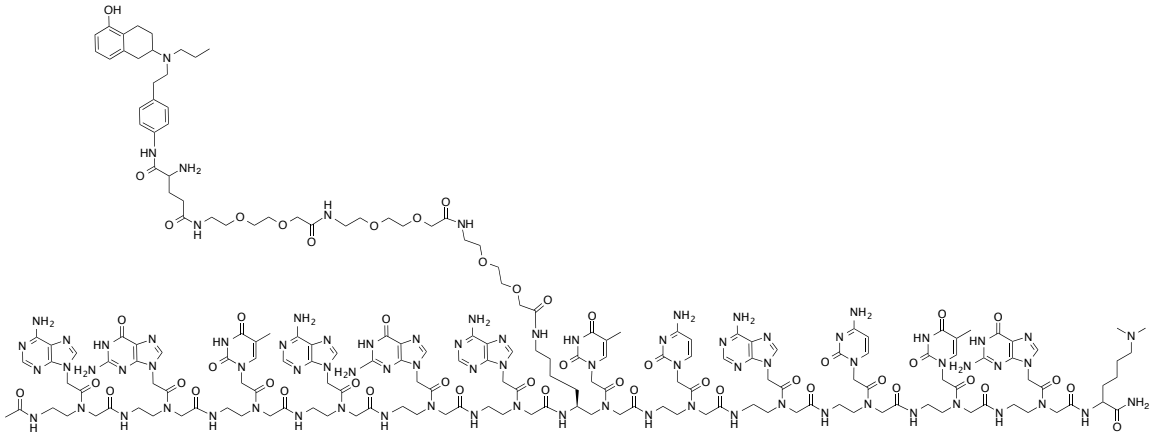
General Resin Cleavage. Upon completion of PNA synthesis or solid phase coupling, the PNA-bound resin was transferred to a glass reaction vessel and washed with CH_2Cl_2, then TFA. The resin was swelled in TFA. The solvent was removed and a solution of *m*-cresol (150 μL), thioanisole (150 μL), TfOH (300 μL), and TFA (900 μL) was added and allowed to stand on the resin for 60 min. The solution was drained into a scintillation vial. This was repeated for a total of 3 washes, each time collecting the eluent in the scintillation vial. The combined eluent was concentrated, transferred to microfuge tubes, and precipitated using Et_2O at a ratio of 1:10. The resulting flaky off-white solid was washed 3 times with Et_2O and dried under vacuum. The resulting residue was diluted with H_2O:ACN (2:1) and purified by reversed phase HPLC.

General Conjugation Procedures. The (±)-PPHT ligand (±)-2-(*N*-phenethyl-*N*-propyl)amino-5-hydroxytetralin was synthesized according to literature protocol and coupled to glutamic acid prior to conjugating to the free amino moiety on the PNA oligomer scaffold via mini-PEG.[5]

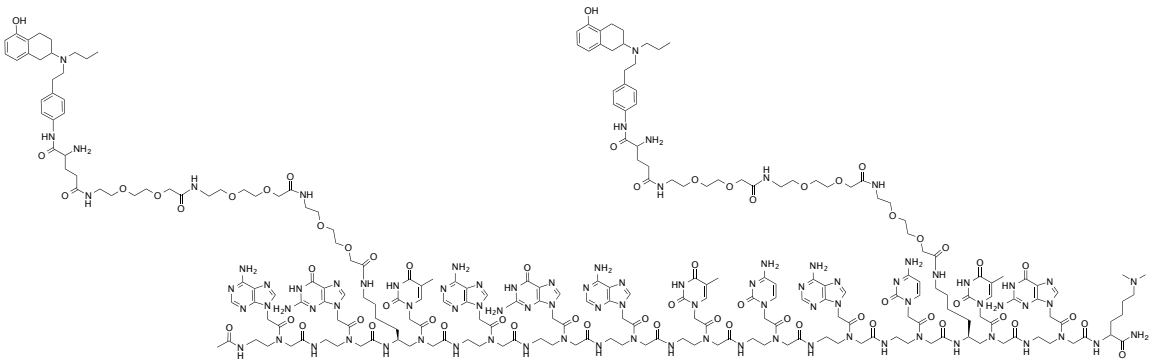
General HPLC Purification. PNA and L-PNA residues were purified by reversed-phase HPLC using a 10 x 250 mm Waters XBridge prep BEH130 C18 5μm reversed phase column on an Agilent 1200s HPLC. Wavelengths 220 nm and 260 nm were monitored. HPLC solvents consisted of *A* 0.1% aqueous TFA and *B* ACN:water (9:1). All L-PNA residues were purified using the following method. With the column thermostat set at 35°C and a flow rate at 3mL/min, the initial gradient was held at 0% ACN 0-1.9 min while increasing the flow rate to 4mL/min. The gradient was increased to 10% *B* at 2 min, and set to reach 29% *B* at 23 minutes. The column was then washed with 100% *B* for 5 min and re-equilibrated at 0% *B* for 5 min.

PNA Oligomer Structures and Characterization Data.

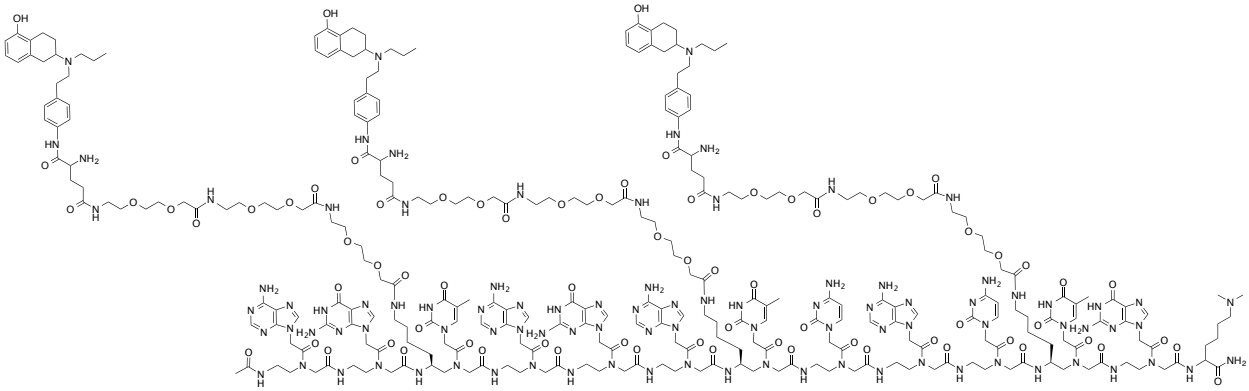
The individual L-PNAs are named in accordance with the nomenclature set out in the main text.



PNA A1. The PNA was cleaved from the resin upon completion of synthesis and purified by HPLC eluting at 17.2 min. MS (ESI) *m/z* calcd for $C_{188}H_{255}N_{81}O_{49}$ 4433.63, found 4433.00.



PNA B1. The PNA was cleaved from the resin upon completion of synthesis and purified by HPLC eluting at 20.9 min. Note that the HPLC method was modified and the thermostat was set at 40 °C. MS (ESI) *m/z* calcd for $C_{236}H_{330}N_{88}O_{61}$ 5375.79, found 5375.55.



PNA C1. The PNA was cleaved from the resin upon completion of synthesis and purified by HPLC eluting at 23.6 min. Note that the HPLC method was modified and the thermostat was set at 45 °C. MS calcd for $C_{284}H_{405}N_{95}O_{73}$ 6317.79, found 6317.98.

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