# Supporting Information

## Nitroazole Universal Bases in Peptide Nucleic Acids

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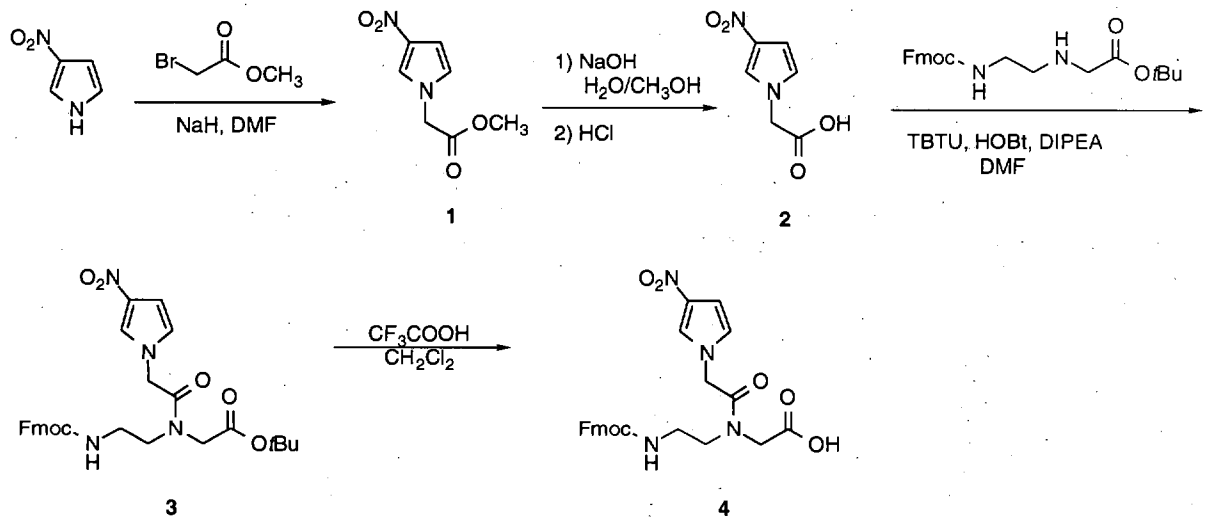
**General Experimental:** All solvents and reagents were purchased from commercial sources except as noted below. DMF was dried over and distilled from $CaH_2$ prior to use; all other materials were used as received from the vendor. $^1H$ NMR spectra were recorded at 360 MHz using a Brüker AM-360 Spectrometer. Unless otherwise noted, spectra were recorded in $CDCl_3$ with 0.05% tetramethylsilane as an internal reference. UV/Vis absorbance measurements were performed using a Pharmacia Ultrospec 2000 spectrophotometer. HPLC was performed using a Beckman 125 pump and a Beckman 168 photodiode array detector.

### Synthesis of 3-nitropyrrole PNA monomer:



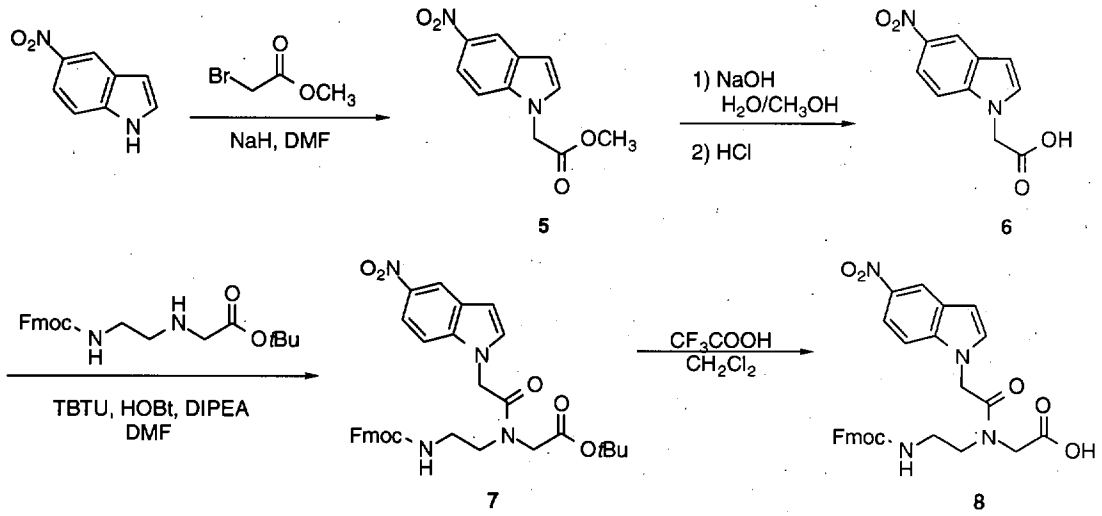
**1-Methoxycarbonylmethyl-3-nitropyrrole (1).** To a solution of 407 mg (3.63 mmol) of 3-nitropyrrole[1] in 15 mL of anhydrous DMF was added 95.6 mg (3.98 mmol, 1.10 eq) of sodium hydride. After the evolution of gas subsided, the resulting green solution was treated with a solution of 2.00 mL of methyl bromoacetate in 2 mL of DMF. The resulting mixture was stirred at rt under nitrogen for 26 h and was then poured into 250 mL of diethyl ether. This mixture was washed with water and brine and was dried over anhydrous $Na_2SO_4$. After filtration and concentration *in vacuo*, the residue was purified using flash column chromatography (silica gel, 1:1 hexanes/ethyl acetate), producing 452 mg (67.5%) of the desired product as a yellow solid. $^1$H NMR: δ 7.56 (dd, 1H), 6.76 (dd, 1H), 6.60 (dd, 1H), 4.68 (s, 2H), 3.80 (s, 3H).

**1-Carboxymethyl-3-nitropyrrole (2).** Methyl ester **1** (438 mg) was dissolved in 100 mL of methanol, and 40 mL of 5 M aq NaOH solution was added. After stirring for 16 h at rt, the mixture was acidified to pH 2 by the addition of conc. HCl solution, and the solution was extracted with diethyl ether. The combined organic extracts were extracted with sat. aq $NaHCO_3$; the combined aqueous extracts were then acidified by the careful addition of HCl. The mixture was then extracted with diethyl ether, and the combined organic phases were dried over anhydrous $Na_2SO_4$, filtered, and concentrated *in vacuo* to produce the desired product as a light brown solid (313 mg, 77.2%). $^1$H NMR (DMSO-$d_6$): δ 13.17 (br s, 1H), 7.92 (dd, 1H), 6.90 (dd, 1H), 6.63 (dd, 1H), 4.82 (s, 1H).

***tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(3-nitropyrrole-1-yl)acetyl] glycinate (3).** To a solution of *tert*-butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-glycinate[2] (451 mg, 1.14 mmol) in anhydrous DMF (35 mL) was added 3-nitropyrroleacetic acid **2** (208 mg, 1.22 mmol), TBTU (*O* -benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium tetrafluoroborate, 458 mg, 1.43 mmol), HOBt (1-hydroxybenzotriazole, 193 mg, 1.43 mmol), and DIPEA (diisopropylethylamine, 221 mg, 1.71 mmol). The reaction mixture was stirred at rt for 5.5 h and then was concentrated *in vacuo*. The residue was brought up in $CH_2Cl_2$ and

washed with brine. The aqueous phase was back-extracted with $CH_2Cl_2$. The combined organic phases were washed with 0.1 N aq. HCl solution, saturated aq $NaHCO_3$ solution, and brine. The organic phase was dried over anhydrous $Na_2SO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel, 3:1 $CH_2Cl_2$/EtOAc), producing the desired product as a brittle white foam (463 mg, 72%). Rf = 0.43 (3:1 $CH_2Cl_2$/EtOAc). HRMS (FAB, MeOH/NBA) 548.2257 ($M^+$), calcd 548.2271 $^1H$ NMR ($CDCl_3$) δ 7.79 (d, 2H), 7.61 (t, 2H), 7.56-7.29 (m, 5H), 6.85-6.45 (m, 2H), 5.90/5.39 (rotomer t, 1H), 4.69-3.30 (m, 11H), 1.50/1.47 (rotomer s, 9H).

**Synthesis of 5-nitroindole PNA monomer:**




**1-Methoxycarbonylmethyl-5-nitroindole (5).** To a solution of 669 mg (4.24 mmol) of 5-nitroindole (Aldrich) in 15 mL of anhydrous DMF was added 143 mg (5.96 mmol, 1.40 eq) of NaH. After 30 min, the resulting red solution was treated with 2.40 mL (25.4 mmol, 6.04 eq) of methyl bromoacetate. The resulting mixture was stirred at rt under nitrogen for 16 h and was then poured into 250 mL of diethyl ether. This mixture was washed with water and brine and was dried over anhydrous $Na_2SO_4$. Filtration and concentration *in vacuo* produced 994 mg

(89.4%) of the desired product as a yellow solid. $^1$H NMR: δ 8.60 (d, 1H), 8.12 (dd, 1H), 7.25 (m, 2H), 6.74 (d, 1H), 4.92 (s, 2H), 3.79 (s, 3H).

**1-Carboxymethyl-5-nitroindole (6).** Methyl ester **5** (1.85 g) was dissolved in 300 mL of methanol, and 120 mL of 5 M aq NaOH solution was added. After stirring for 16 h at rt, the mixture was acidified to pH 2 by the addition of conc. HCl solution, and the solution was extracted with diethyl ether. The combined organic extracts were extracted with sat. aq $NaHCO_3$; the combined aqueous extracts were then acidified by the careful addition of HCl. The mixture was then extracted with diethyl ether, and the combined organic phases were dried over anhydrous $Na_2SO_4$, filtered, and concentrated *in vacuo* to produce the desired product as a yellow solid (1.47 g, 84.2%). $^1$H NMR (DMSO-$d_6$): δ 13.07 (br s, 1H), 8.58 (d, 1H), 8.03 (dd, 1H), 7.61 (m, 2H), 6.75 (d, 1H), 5.12 (s, 1H).

***tert*-Butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-*N*-[(5-nitroindole-1-yl)acetyl]-glycinate (7).** To a solution of *tert*-butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl]-glycinate[2] (482 mg, 1.22 mmol) in anhydrous DMF was added 5-nitroindoleacetic acid (320 mg, 1.22 mmol), TBTU (485 mg, 1.51 mmol), HOBt (207 mg, 1.43 mmol), and DIPEA (236 mg, 1.83 mmol). The reaction mixture was stirred at rt for 6 h and then was concentrated *in vacuo*. The residue was brought up in $CH_2Cl_2$ and washed with brine. The aqueous phase was back-extracted with $CH_2Cl_2$. The combined organic phases were washed with 0.1 N aq. HCl solution, saturated aq $NaHCO_3$ solution, and brine. The organic phase was dried over anhydrous $Na_2SO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (silica gel, 1:1 hexanes/EtOAc), producing the desired product as a brittle yellow foam (498 mg, 73%). Rf = 0.33 (1:1 hexanes/EtOAc). HRMS (FAB, MeOH/NBA) 599.2586 $(M+H)^+$, calcd 599.2506 $^1$H NMR ($CDCl_3$) δ 8.11/8.05 (rotomer d, 1H), 7.77-7.11 (m, 11H), 6.75/6.69 (rotomer d, 1H), 4.91-3.41 (m, 11H), 1.50/1.47 (rotomer s, 9H).

**General Procedure for Preparation of PNA Monomers for Solid Phase Synthesis:**

A portion (50-100 mg) of the PNA monomer *t*-butyl ester (**3** or **7**) was treated with 3 mL of a 1:1 (v/v) mixture of trifluoroacetic acid and dichloromethane. The resulting solution was stirred at room temperature for 2 h and was then co-evaporated with toluene three times. The resulting solid was dissolved in the appropriate volume of anhydrous N-methylpyrrolidinone to make a 0.2 M solution.

**Preparation of PNA and DNA Oligomers**: PNA was synthesized on a PE Biosystems Expedite 8909 DNA Synthesizer with the PNA synthesis software. Oligomers were synthesized on a 2 μmol scale using the protocol provided by the manufacturer[3] except that the nitroazole monomers were coupled manually to avoid waste involved with priming of the instrument. Deprotection and cleavage from the solid support was accomplished by treatment twice with 4:1 trifluoroacetic acid/*m*-cresol for 5 min. PNA was precipitated from the combined solutions by the addition of 1.5 volumes of diethyl ether. The residue was purified by HPLC using a YMC AQ $C_{18}$ column at 60 °C. MALDI-TOF mass spectrometry was performed at the Core Laboratory for Protein Microsequencing and Mass Spectrometry of the University of Massachusetts Medical School-Worchester Foundation Campus. Experiments were performed using a sinnapinic acid matrix: **PNA 1** (H-TGT ACG $N_p$ CAC AAC TA-$NH_2$) m/z 4033.5 $[M+H]^+$, calcd 4033.6; **PNA 2** (H-TGT ACG $N_i$ CAC AAC TA-$NH_2$) m/z 4083.9 $[M+H]^+$, calcd 4084.3.

Oligodeoxynucleotides were obtained from Integrated DNA Technologies, Inc., as cartridge-purified materials. HPLC analysis showed these oligodeoxynucleotides to be homogeneous; therefore, they were used without further purification.

Stock solutions of PNA and DNA oligomers in water were quantitated by measuring the absorbance at 260 nm using the following values for the extinction coefficients: A, 15,400; C, 7,300; G, 11,700; T, 8,800; 5-nitroindole, 18,100; 3-nitropyrrole, 5,300 $mol^{-1}cm^{-1}$. PNA

solutions were quantitated at 55 °C to melt out secondary structures of single-stranded oligomers that would cause hypochromicity in the absorbance measurements.[3]

**General Procedure for $T_m$ Experiments**: Thermal denaturation experiments were performed using solutions of PNA and complementary DNA (4 μM in each strand) in buffer containing 10 mM sodium phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0. All melting curves were performed using a Pharmacia Ultrospec 2000 Spectrophotometer fitted with a Peltier-heated cell holder and a computer-driven temperature controller. Samples were heated at a rate of 0.5 °C/min.

**Analysis of Thermal Denaturation Data**: From the absorbance vs. temperature data obtained from the $T_m$ experiment, a first derivative plot ($\partial A/\partial T$ vs. T) was produced. The maximum ($T_{max}$) of each derivative curve was determined; the use of this plot rather than ($\partial A/\partial 1/T$ vs. 1/T) is reported to lead to less than a 3% error.[4] At the maximum of the derivative plot, the fraction of strands in the double helical state,α, is 0.414. To determine the $T_m$'s ($\alpha = 0.5$), each $T_{max}$ value was multiplied by [0.167 / 0.172].[5]

Thermodynamic parameters were estimated using the method of Gralla and Crothers:[4]

$$\Delta H = (-18.28\ \text{J/mol•K})/(1/T_{max} - 1/T_{3/4}), \qquad (1)$$

where $T_{3/4}$ is the half-height of the high temperature side of the differential melting curve. ΔG at $T_{max}$ was determined by the equation:

$$\Delta G_{tmax} = -RT_{max} \ln(K), \qquad (2)$$

where K at $T_{max}$ ($\alpha = 0.414$) is:

$$K_{tmax} = 2\alpha / (1-\alpha)^2 C_t = 2.411 / C_t. \qquad (3)$$

$C_t$ is the total concentration of DNA and PNA strands.

Entropy was calculated using the equation:

$$\Delta S = (\Delta G_{tmax} - \Delta H) / T_{max}; \quad (4)$$

entropy and enthalpy were assumed to be invariant with temperature. All reported data was determined from the average of three experiments.

**References**:

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