Supporting Information

A new unnatural base pair system between fluorophore and quencher base analogues for nucleic acid-based imaging technology

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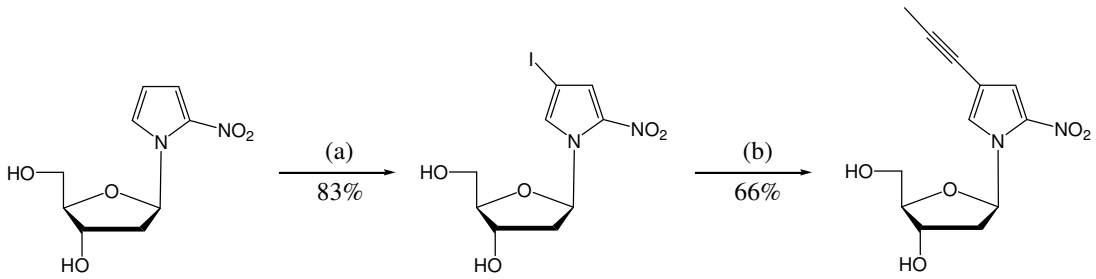
1. Chemical synthesis of the Px nucleoside



Conditions: (a) NIS, CH_3CN (b) $Pd(PPh_3)_2Cl_2$, DMF

General methods and materials

Reagents and solvents were purchased from standard suppliers and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm silica gel 60 plates impregnated with 254 nm fluorescent indicator (Merck). ^1H NMR, and ^{13}C NMR spectra were recorded on a Bruker (300-AVM) magnetic

resonance spectrometer. Nucleoside purification was performed on a Gilson HPLC system with a preparative C18 column (Waters μ-BONDASPHERE, 150 × 19 mm). High resolution mass spectra (HRMS) were recorded on a JEOL JM 700 mass spectrometer.

1-(2-Deoxy-β-D-ribofuranosyl)-4-iodo-2-nitropyrrole

A mixture of 1-(2-deoxy-β-D-ribofuranosyl)-2-nitropyrrole (456 mg, 2 mmol) and *N*-iodosuccinimide (900 mg, 4 mmol) in CH_3CN (8 ml) was stirred at room temperature for 14 h. The mixture was separated by ethyl acetate and water. The organic phase was dried with Na_2SO_4, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography and RP-HPLC to give 1-(2-deoxy-β-D-ribofuranosyl)-4-iodo-2-nitropyrrole (587 mg, 1.66 mmol, 83%).

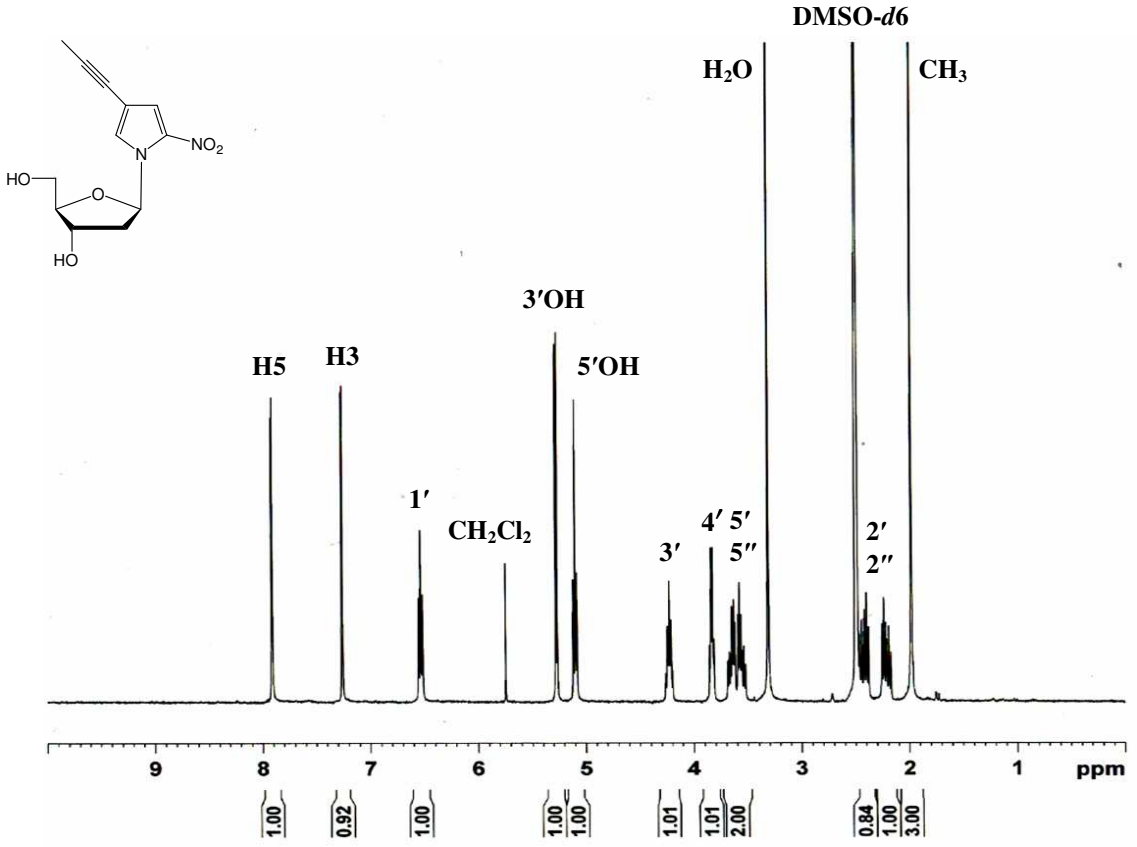
^{1}H NMR (300 MHz, DMSO-*d*6) δ 7.90 (d, 1H, *J* = 2.0 Hz), 7.40 (d, 1H, *J* = 2.0 Hz), 6.54 (t, 1H, *J* = 5.6 Hz), 5.27 (d, 1H, *J* = 4.3 Hz), 5.10 (t, 1H, *J* = 4.9 Hz), 4.23 (m, 1H), 3.83 (m, 1H), 3.53-3.85 (m, 2H), 2.18-2.45 (m, 2H). UV-vis spectral data in EtOH λ_{max} = 350 nm (ε 10500). HRMS (FAB, 3-NBA matrix) for $C_9H_{12}IN_2O_5$ $(M+H)^+$ calcd 354.9791, found 354.9784.

1-(2-Deoxy-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole

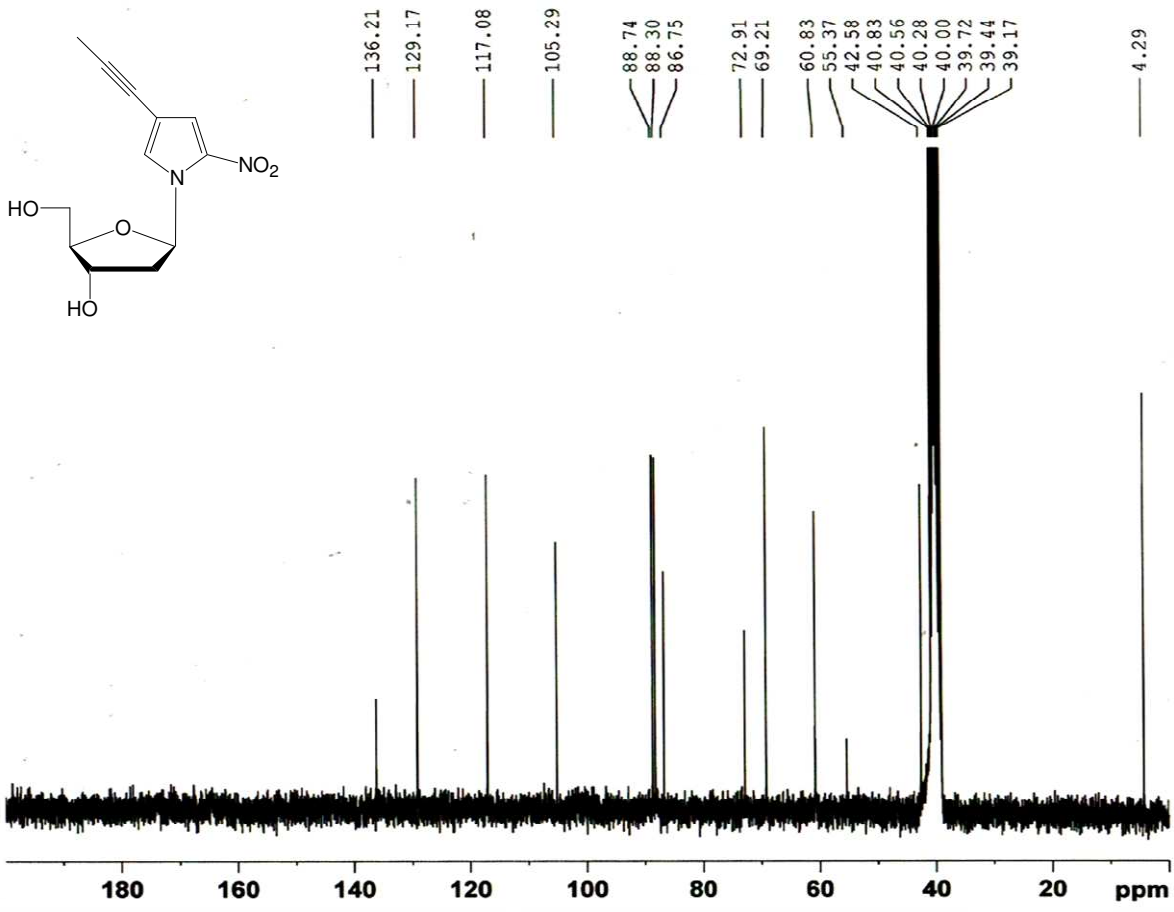
To a solution of 1-(2-deoxy-β-D-ribofuranosyl)-4-iodo-2-nitropyrrole (354 mg, 1.0 mmol), dichloro bis(triphenylphosphine)palladium (35 mg, 0.05 mmol) in DMF (5 ml) was added tributyl(1-propynyl)tin (987 mg, 3.00 mmol). The mixture was heated at 100 °C for 30 min. The reaction mixture was separated by ethyl acetate and water. The organic phase was dried with Na_2SO_4, and evaporated *in vacuo*. The residue was

purified by silica gel column chromatography and RP-HPLC to give 1-(2-deoxy-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole (177 mg, 0.66 mmol, 66%).

^{1}H NMR (300 MHz, DMSO-*d*6) δ 7.92 (d, 1H, *J* = 2.2 Hz), 7.27 (d, 1H, *J* = 2.2 Hz), 6.55 (t, 1H, *J* = 5.7 Hz), 5.28 (d, 1H, *J* = 4.5 Hz), 5.11 (t, 1H, *J* = 5.2 Hz), 4.24 (m, 1H), 3.85 (m, 1H), 3.53-3.70 (m, 2H), 2.45 (m, 1H), 2.22 (m, 1H), 1.99 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 136.21, 129.17, 117.08, 105.29, 88.74, 88.30, 86.75, 72.91, 69.21, 60.83, 42.58, 4.29. UV-vis spectral data in EtOH λ_{max} = 226 nm (ε 12700), 362 nm (ε 7450). HRMS (FAB, 3-NBA matrix) for $C_{12}H_{15}N_2O_5$ $(M+H)^+$ calcd 267.0981, found 267.0991.



^{1}H NMR (300 MHz, DMSO-*d*6) spectrum of 1-(2-deoxy-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole (Px nucleoside).



^{13}C NMR (75 MHz, DMSO-*d*6) spectrum of 1-(2-deoxy-β-D-ribofuranosyl)-4-propynyl-2-nitropyrrole (Px nucleoside)

2. Quenching ability of 2-nitropyrrole derivatives to fluorescent Dss

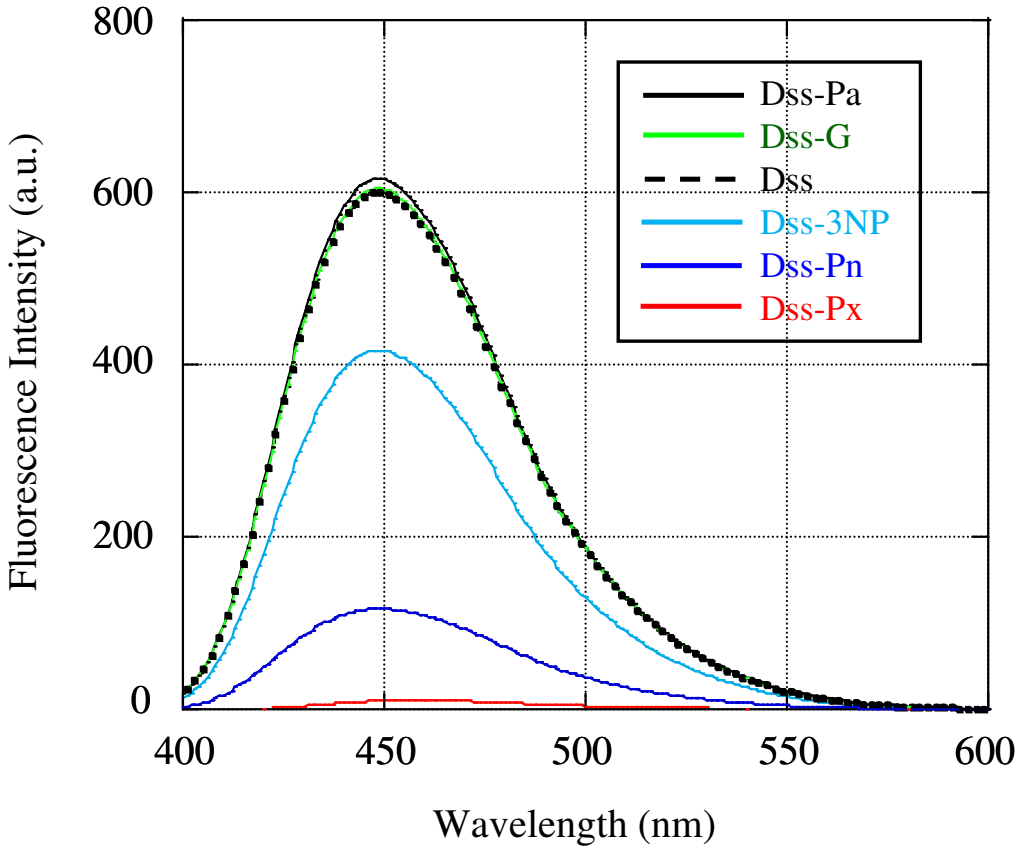


Figure S1. Quenching ability of the 2′-deoxyribonucleosides of 2-nitropyrrole derivatives to the fluorescent Dss deoxyribonucleoside.

The emission spectra of the 2′-deoxyribonucleoside of **Dss** (5 μM) with **Pn**, **Px**, **Pa**, 3-nitropyrrole (3NP) or G (5 mM each) was monitored in a solution containing 10% CH_3CN, 40% H_2O, and 50% EtOH at the excitation wavelength of 385 nm at 25 °C.