SYNTHESIS OF 5-(2-PYREN-1-YL-ETHYLENYL)-2'-dU AS A FLUORESCENT PROBE FOR STUDYING ELECTRON TRANSFER IN DNA

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Abstract : This paper describes the synthesis of 5-(2-(pyren-1-yl-ethylenyl)-2'-deoxyuridine (PEdU) based on complete, selective reduction of the ethynyl linker in 5-(2-(pyren-1-yl-ethynyl)-2'-deoxyuridine. It also describes a reliable route for making the 1-ethynylpyrenyl intermediate based on a number of attempts to use previously reported procedures.

Introduction. Because of their long fluorescence lifetimes and reversible redox properties, pyrene and its derivatives have frequently been used as fluorescent probes particularly in biological systems.¹⁴ Previous work on the pyrenyllabeled nucleoside 5-(1-carboxypyrenyl)-2'-deoxyuridine (PcodU, Figure 1) showed that photoinduced electron transfer (ET) occurred in <30 ps to form the pyrene**/dU* charge transfer (CT) product. However, the average lifetime of this product's back reaction was 67 ps in methanol.⁵ This was too fast to permit its use in studies of Subsequent photophysical studies on the nucleoside N-(1-pyrenylmethyl)-2'electron migration in DNA. deoxyuridine-5-carboxamide (PMAdU, Figure 1) showed that ET still occurred rapidly (<100 ps). However, in this case the average lifetime of the CT product's back reaction was 430 ps.⁶ This rate of back ET was nearly slow enough to permit its use in studies of ET in DNA, but a nucleoside conjugate with a longer ET product lifetime was still desirable. Computational studies on pyrenyl-labeled nucleoside models demonstrated that a carbonyl-linking group at the C-5 position of uracil subunit made the free energy of ET products in pyrenyl-labeled nucleosides sensitive to the angle between the carbonyl group and the uracil plane.^{6,7} As a consequence in the nucleosides discussed above, there were a variety of conformers with a variety of ET product lifetimes. To lessen the free energy variation among conformers of pyrenyl-labeled nucleosides, we elected to synthesize the 5-(2-(pyren-1-yl-ethylenyl)-2'-deoxyuridine nucleoside (PEdU, see Scheme 1). This new nucleoside eliminated the carbonyl linker between the pyrenyl and uracil subunits, but still maintained a short connection between two subunits to ensure rapid formation of the pyrene*/dU* CT product from the pyrenyl (π,π^*) local excited state.

Results and Discussion. Scheme 1 shows that our strategy for making PEdU, 7, was based on the complete reduction of the ethynyl linker in 5. This route to PEdU was appealing, since a synthesis of 5 based on Sonogashira Pd(0) cross-

coupling of 3 and 4 had been reported earlier.⁸ The two challenges in this strategy turned out to be finding a practical route to 3 and developing selective triple-bond hydrogenation conditions for the highly conjugated compound 5.



Figure-1: Structures of two pyrenyl-dU nucleosides with short linkers between the pyrenyl and uracil subunits.

The acetylenic bond in 3 can be formed in several different ways. One of the most practical methods is based on the elimination of HX from haloalkenes with a strong base such as ^tBuOK, KOH, or *n*-butyllithium. Haloalkenes can be made from an aldehydic group using the Wittig reaction. This method has been reported by Baumgartel and Retzlav who synthesized 3 by the reaction of 1-formylpyrene, 1, with (bromomethyl)triphenylphosphonium bromide in the presence of three equivalents of ^tBuOK in dry THF.⁹ However, in our hands this method did not make 3. Specifically, the ¹H NMR of the crude product mixture in CDCl₃ did not show an acetylenic proton. Changing the order of addition of reagents and the reaction temperature also did not produce 3. Additionally, using (chloromethyl)-triphenylphosphonium chloride in place of (bromomethyl)triphenyl-phosphonium bromide also failed to make 3. The failure of this reaction was perhaps not too surprising, as Schottenberger *et al.* have reported that the enyne dimerized during their synthesis of ethynylferrocene using bromophosphonium.¹⁰

We also tried to synthesize 3 according to a procedure used to synthesize 4-ethynylpyrene from 4-acetylpyrene.¹¹ In to this procedure, 1-acetylpyrene, freshly prepared lithium di-isopropyl amide, and diethyl chlorophosphate were reacted under a N₂ gas atmosphere at -78 °C. However, in our hands this method too did not produce 3. Finally, we produced 3 in two steps based on modification of a procedure reported by Corey and Fuchs.¹² In the first step, 1 was added to a mixture of 4 equivalents of triphenylphosphine (PPh₃) and two equivalents of tetrabromomethane previously stirred in dry CH₂Cl₂ at 0 °C for 10 min. The reaction mixture was further stirred at room temperature for 15 min to afford 2 in 92% yield. In the second step, 2 was lithiated with 2.5 equivalents of *n*-butyllithium added dropwise over 15 min at -78 °C, and then the reaction mixture was stirred for 2 – 3 h at the same temperature. After warming and stirring the mixture for 1 h at room temperature, the reaction was quenched with 5% aqueous HCl (cold) to afford 3 in 90% yield.

Compound 5a was synthesized in 86% yield as expected by Pd(0) catalyzed cross-coupling of compounds 3 and 4a.⁸ Because the reaction of 3 with 4 was sensitive to the reaction conditions, the reagent ratios were carefully adjusted to those described in the experimental section below. When this was not done, the yield of the cross-coupling product was low, because homocoupling of 4 competed with cross-coupling. Choice of *N*,*N*-dimethyl-formamide (DMF) over methanol (MeOH) as solvent was important in increasing product yield, because it improved the solubility of the Pd(0) catalyst. Compound 5b was synthesized in 68% yield using the same-procedure used to make 5a.

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Scheme-1: (a) $P(Ph)_3$, CBr_4 , CH_2Cl_2 , 0 C; (b) 2.5 equiv. *n*-butyllithium, ether, -78 C; (c) 5% aqueous HCl, 0 °C; (d) PdL₄, Cul, Et₃N, 4, DMF; (e) H₂ (50 psi), Pd/C, MeOH; (f) NH₄OH, MeOH.

The most critical step in making the ethylenylpyrenyl-dU nucleoside 6 was the hydrogenation of 5. Heterogeneous catalysis was the most common way to hydrogenate fully a carbon-carbon triple bond.¹³ Although heterogeneous catalysts were simple to use, they lacked selectivity and there were no criteria for choosing which one to use. The synthetic literature reported hydrogenation of structures similar to 5 using Pd/C and hydrogen in methanol, ethanol, and water.¹⁴ A difficulty arose in this case, however, because the reported reactions were for nonconjugated triple bonds. We did not, of course, want to hydrogenate the easily reducible pyrenyl ring, uracil's double bond, or either of uracil's carbonyl groups. We found that temperature and hydrogen gas pressure had pronounced effects on the rate of carbon-carbon triple bond hydrogenation as well as on hydrogenation selectivity. In particular, increasing the reaction

temperature or the hydrogen gas pressure beyond its optimal value increased the reaction rate but decreased reaction selectivity.

We hydrogenated 5 in a PVC-coated glass, pressure-reaction vessel, usually called a Fischer Porter bottle. The reaction vessel was fitted with an intake valve, a high-pressure gauge, and a safety valve and held Pd/C (10% by wt.), a suitable amount of dry MeOH (20 mL), and a stirring bar under N₂ gas to avoid ignition of the Pd powder. After adding compound 5 dissolved in MeOH, the vessel was sealed and charged with H₂ gas in a three-step cycle: charging to 50 psi, depressurizing, and then degassing with an aspirator. This charging cycle was repeated 5 – 6 times. Finally, the vessel was charged with H₂ gas to 50 psi and stirred for 24 h at room temperature. Reaction progress was checked by UV-vis absorbance after 24 h, and more catalyst suspended in MeOH was added. The vessel was recharged again with H₂ gas (50 psi) as described above, and the reaction mixture was stirred for another 24 h. After 48 h all starting material was consumed. The reaction mixture was filtered; the solvent was removed *in vacuo*; and the crude material was purified by column chromatography to afford 6: 6a in 81% yield and 6b in 65% yield.

The bisacetate groups of **6b** were removed by dissolving the compound in MeOH and stirring it in an aqueous solution of ammonium hydroxide at room temperature overnight to afford compound **7** in 93% yield. The 5'-DMT protected nucleoside **6a** was used to make the corresponding phosphoramidite reagent and then incorporated into DNA oligomers via automated solid-state DNA synthesis.

All reactions were performed under a dry N2 gas (boil-off from liquid N2) atmosphere. Experimental. Tetrakis(triphenylphosphine)palladium(0) (PdL₄) was prepared according to a reported procedure¹⁵ and was stored in the freezer (-20 °C) of a Vacuum Atmospheres M040-2 glove-box pressurized with dry N₂ gas. PdL₄ coupling reactions were assembled in the glove-box and then run under a N₂ gas atmosphere outside of the box. Flash column chromatography was carried out on a Biotage Flash-40™ system using either prepackaged KP-Sil™ cartridges, or Flash-40[™] cartridge housings repacked with Whatman[™] flash silica (60Å pore, 230 – 400 mesh). Silica gel used for gravity column chromatography was Whatman[™] flash silica (60Å pore, 230 – 400 mesh). The following solvents were dried and redistilled in continuous circulation distillation apparati: ether (dried with benzophenone/Na⁰), triethylamine (Et₃N, dried with CaH₂), CH₂Cl₂ (dried with CaH₂), methanol (MeOH, dried with magnesium turnings), and N,N-dimethylformamide (DMF, dried with CaH₂). Copper(I) iodide and Pd/C (10% by wt.) were obtained from Strem Chemicals (Newburyport). 5-Iodo-2'-deoxyuridine (IdU), n-butyllithium, 4,4'-dimethoxytrityl chloride (DMTCl), PPh₃, tetrabromomethane, ¹BuOK, and 1-formylpyrene were obtained from Aldrich Chemicals Co. (Milwaukee, WI) and were used as received without further purification. IdU was coevaporated three times with dry pyridine prior to use. Compounds $4a^{16}$ and $4b^{17}$ in Scheme 1 were synthesized according to reported procedures. n-Butyllithium was standardized by titration with 2-butanol in dry toluene using 1,10-phenanthroline as indicator prior to use. Other reagents, chemicals, and solvents were obtained from common suppliers and were usually used without further purification. Mass spectrometry was performed at the Georgia Institute of Technology. ¹H and ¹³C NMR spectra were recorded at GSU on a Varian Unity+300 spectrometer operating, respectively, at 300- and 75-MHz frequencies. The chemical shifts of the ¹H NMR were referenced to TMS, while those for ¹³C were referenced to peaks of the solvent used for the measurement.

Synthesis of 1-(2,2-Dibromovinyl)pyrene, 2. In a round-bottom flask, PPh₃ (20.98 g, 79.98 mmol) and tetrabromomethane (13.26 g, 39.98 mmol) were dissolved in 40 mL of dry CH₂Cl₂ and stirred for 5 - 10 min at 0 °C. 1-Formylpyrene (4.60 g, 20 mmol) dissolved in 10 mL of dry CH₂Cl₂ was transferred at 0 °C to the mixture of PPh₃ and tetrabromomethane via a canuula. The reaction mixture was stirred further for 15 – 20 min at 0 °C. The solvent was removed *in vacuo*, and the solid material was crystallized from 9:1 (v/v) hexane/CH₂Cl₂ to give 2 (7.10 g, 92% yield). ¹H NMR (CDCl₃, 300 MHz) δ : 7.98 – 8.22 (m, pyrenyl aromatic and vinylic H). ¹³C NMR (CDCl₃, 75 MHz) δ : 93.14, 123.49, 124.44, 124.48, 124.58, 125.48, 125.59, 126.12, 126.32, 127.24, 127.93, 128.07, 128.26, 130.22, 130.77, 131.16, 131.29, 136.35. HRMS (EI) *m/z* for C₁₈H₁₀⁷⁹Br₂ (M)⁺: calcd. 383.9149, found 383.9144.

Preparation of 1-Ethynylpyrene, 3. In a round-bottom flask, *n*-butyllithium (1.60 M solution in hexane, 3.12 mL, 5.00 mmol) in 15 mL of dry ether was cooled to -78 °C using a dry-ice/acetone bath. In another round-bottom flask, 2 (0.77 g, 1.99 mmol) was dissolved in 45 mL of dry ether and also cooled to -78 °C. *n*-Butyllithium was added dropwise to the solution of 2 over 20 – 30 min, while the temperature was maintained at -78 °C. The reaction mixture was then stirred at -78 °C for 2 h and left stirring until the temperature of the dry-ice/acetone bath warmed to 25 °C (ca. 3 h). The reaction mixture was quenched with 100 mL of 5% HCl in ice water and stirred for 15 min at 0 °C. The alkyne 3 was extracted with chloroform (3 x 50 mL) and dried over anhydrous Na₂SO₄. The solvent was removed *in vacuo*, and the c rude p roduct was chromatographed on a silica g el c olumn e luted with h exanes. E vaporating the solvent gave 0.41 g of 3 as a yellow solid (90% yield) whose analytical data were identical to those previously reported in the literature.^{9,18}

Synthesis of 5'-*O*-4,4'-Dimethoxytrityl-5-(pyren-1-yl-ethynyl)-2'-deoxyuridine, 5a. The protected nucleoside 4a was dried by coevaporation over dry THF three times prior to reaction. To a solution of 4a (1.18 g, 1.80 mmol) in 3 mL of dry DMF inside the glove-box, were added successively Cul (0.034 g, 0.18 mmol), PdL₄ (0.104 g, 0.09 mmol), and Et₃N (0.6 mL, 3.6 mmol) as neat compounds, and 3 (0.53 g, 2.3 mmol) as a solution in DMF (7 mL). The sealed flask was brought outside the glove-box and stirred for 5 h at 60 °C. The solvent was removed *in vacuo*. The spent Pd catalyst was removed using silica gel, pad filtration eluting with 1:1 (v/v) chloroform/ethyl acetate, and the solution was evaporated to dryness. The crude material was purified on a flash silica column that was preequilibrated with 100 mL of 1% (v/v) pyridine in CH₂Cl₂ prior to loading the crude material. The solvent polarity was increased gradually with MeOH starting with 100% CH₂Cl₂. The column was finally eluted with 4% (v/v) MeOH in CH₂Cl₂ to afford 5a as a yellow foam (1.18 g, 86% yield). ¹H NMR (CDCl₃, 300 MHz) &: 2.37 (1H, quintet, J = 6.0 Hz, $H_{2'a}$), 2.66 (1H, dd, J = 6.0, 9.5 Hz, $H_{2'B}$), 3.31 (1H, dd, J = 3.0, 10.0 Hz, $H_{5'a}$), 3.42 (3H, s, CH_{3(DMT)}), 3.44 (3H, s, CH_{3(DMT)}), 3.46 (1H, m, $H_{5'B}$), 4.22 (1H, m, H_4), 4.59 (1H, m, $H_{3'}$), 6.45 (1H, t, J = 6.0 Hz, H_1), 6.69 (4H, q, J = 5.0 Hz, Ar), 7.03 (1H, t, J = 7.0 Hz, Ar), 7.2 (2H, t, J = 7.7 Hz, Ar), 7.34 (4H, d, J = 9.0 Hz, Ar), 7.45 (2H, d, J = 7.7 Hz, Ar), 7.58 (1H, d, J = 7.7 Hz, Ar), 7.85 – 8.11 (7H, m, Ar), 8.31 (1H, s, H_6), 8.45 (1H, d, J = 9.0 Hz, Ar). ¹³C NMR (CDCl₃, 75 MHz) δ : 41.633, 54.88, 63.57, 72.47, 85.73, 86.12, 86.86, 86.96, 93.36, 100.96, 113.23, 117.02, 124.02, 124.08, 125.30,

125.59, 126.04, 1 26.91, 1 27.05, 1 27.88, 1 27.97, 1 28.06, 128.39, 129.42, 1 29.85, 1 29.94, 1 30.92, 1 31.00, 1 31.13, 131.81, 135.38, 135.50, 141.70, 144.46, 149.69, 158.40, 158.42, 161.90. HRMS (FAB) m/z for C₄₈H₃₈N₂O₇ (M)⁺: calcd. 754.2679, found 754.2685. Compound **5b** was synthesized in exactly the same manner as **5a** (data not shown).

Synthesis of 5'-O-4,4'-Dimethoxytrityl-5-(2-pyren-1-yl-ethylenyl)-2'-deoxyuridine, 6a. A suspension of 0.35 g of Pd/C (10% by wt.) in 50 mL of dry MeOH was activated at room temperature by stirring under H₂ (50 psi) for 20 min. Compound 5a (0.53 g, 0.7 mmol) was dissolved in 150 mL of dry MeOH then added to a suspension of the previously activated Pd/C catalyst. The reaction mixture was stirred overnight at room temperature under H₂ (50 psi). The next day an additional one-half of the amount of the initially used catalyst was added, and the mixture was stirred further under H₂ (50 psi) until complete consumption of the starting material (total reaction time 2 days). The solution was then filtered, reduced in volume, and chromatographed on a silica gel column preequilibrated with 100 mL of 1% (v/v) pyridine in CH₂Cl₂. The column was eluted with 10:0, 9.9:0.1, 9.7:0.3, 9.5:0.5, and 9.4:0.6 CH₂Cl₂/MeOH (v/v). Compound 6a was obtained as pale yellow foam (0.43 g, 81% yield). ¹H NMR (CDCl₃, 300 MHz) 5: 1.73 (1H, quintet, J = 6.6 Hz, $H_{2'\alpha}$), 2.10 (1H, dd, J = 3.0, 10.8 Hz, $H_{2'\beta}$), 2.33 (1H, m, $CH_{2(linker)}$), 2.47 (1H, m, $CH_{(linker)}$), 3.13 $(1H, dd, J = 3.0, 10.5 Hz, H_{5'\alpha}), 3.21 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.21 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.32 (1H, dd, J = 3.0, 10.5 Hz, H_{5'B}), 3.49 (1H, m, CH_{(linker)}), 3.49 (1H, m, CH_{(linker)})), 3.49 (1H, m, CH_{(linker)}), 3.49 (1H, m, CH_{(linker)}), 3.49 (1H, m, CH_{(linker)}), 3.49 (1H, m, CH_{(linker)}), 3.49 (1H, m, CH_{(linker)})), 3.49 (1H, m, CH_{(linker)}), 3.49 (1H, m, CH_{(linker)})), 3.49 (1H, m, CH_{(linker)}), 3.49 (1H, m, CH_{(linker)})), 3.49 (1H, m, CH_{(linker)}))), 3.49 (1H, m, CH_{(linker)}))), 3.49 (1H, m, CH_{(linker)})))$ 3.57 (6H, s, (CH₃)_{2(DMT)}), 3.89 (2H, m, H₃·/H₄·), 6.28 (1H, t, J = 6.6 Hz, H₁·), 6.73 (4H, dd, J = 3.0, 9.0 Hz, Ar), 7.13 - 10.027.28 (8H, m, Ar), 7.40 (3H, dd, J = 5.3, 7.5 Hz, Ar), 7.90 - 7.97 (5H, m, Ar), 8.10 (2H, t, J = 7.5 Hz, Ar), 8.17 (1H, d, J = 9.0 Hz, Ar), 9.15 (1H, s, H_{N3}). The CH₂CH₂ linkage assignment was confirmed by a COSY experiment. ¹³C NMR (CDCl₃, 75 MHz)δ: 29.49, 32.33, 37.66, 40.67, 55.04, 63.42, 71.91, 84.49, 85.62, 86.72, 113.22, 114.52, 123.60, 124.61, 124.70, 124.76, 124.81, 125.81, 126.59, 127.15, 127.38, 127.98, 128.20, 128.76, 129.71, 130.07, 130.08, 130.80, 131.19, 135.33, 135.38, 135.58, 135.92, 144.19, 150.34, 158.61, 163.48. HRMS (FAB) m/z for $C_{48}H_{42}N_2O_7 (M)^+$: calcd. 758.2992, found 758.2993.

Synthesis of 3',5'-Di-O-acetyl-5-(2-pyren-1-yl-ethylenyl)-2'-deoxyuridine, 6b. Compound 5b (0.50 g, 0.93 mmol) was dissolved in 130 mL of dry MeOH and added to a suspension of Pd/C catalyst (10% by wt., 0.25 g) in dry MeOH (50 mL) that was previously activated as above for 6a. The reaction mixture was stirred under H₂ (50 psi) at room temperature overnight. The next day an additional one-half of the amount of the initially used catalyst was added, and the mixture was stirred further under H₂ (50 psi) until complete consumption of the starting material (total reaction time 2 days). The solution was filtered, reduced in volume to 0.5 mL, and then chromatographed on a silica gel column eluted with 10:0, 9.9:0.1, 9.8:0.2, 9.7:0.3, and 9.6:0.4 MeOH in CH₂Cl₂ (v/v). The product 6b was obtained as yellow powder (0.33 g, 65% yield). ¹H NMR (CDCl₃, 300 MHz) δ : 1.14 (1H, m, H_{2'a}), 1.77 (3H, s, CH₃), 1.93 (1H, dd, J = 5.4, 14.0 Hz, H_{2'b}), 2.01 (3H, s, CH₃), 2.76 (1H, m, CH_(linker)), 2.95 (1H, m, CH_(linker)), 3.56 – 3.61 (3H, m, H_{5'a}/CH₂(linker)), 3.79 (1H, dd, J = 4.7, 12.0 Hz, H_{5'b}), 3.89 (1H, m, H_{4'}), 4.61 (1H, m, H_{3'}), 6.01 (1H, dd, J = 5.4, 8.8 Hz, H_{1'}), 6.55 (1H, s, H₆), 7.79 (1H, d, J = 7.6 Hz, Ar), 7.95 – 8.18 (7H, m, Ar), 8.28 (1H, d, J = 9.3 Hz, Ar), 8.45 (1H, br, H_{N3}). The CH₂CH₂ linker assignment was confirmed by a COSY experiment. ¹³C NMR (CDCl₃, 75 MHz) δ : 20.45, 20.80, 25.58, 29.76, 32.12, 36.71, 63.27, 67.93, 73.87, 81.89, 84.39, 114.10, 123.27, 124.80, 124.95, 125.09, 126.01, 126.94, 127.32, 127.62, 127.76, 128.89, 129.97, 130.75, 131.25, 135.10, 135.22, 150.09, 163.17, 169.77, 170.13. HRMS (FAB) m/z for C₃₁H₂₈N₂O₇ (M)⁺: calcd. 540.1897, found 540.1905.

Synthesis of 5-(2-Pyren-1-yl-ethylenyl)-2'-deoxyuridine, 7. To a solution of bisacetate protected 6b (0.10 g, 0.18 mmol) in 5 mL of MeOH was added 1.2 mL of 30% aqueous ammonia. The reaction mixture was stirred at room temperature overnight, and the solvent was removed *in vacuo*. The residue was suspended in water (10 mL), filtered, washed with water, dried under vacuum, and purified by flash column chromatography using 1:1 (v/v) CH₂Cl₂/ethyl acetate to afford 7 as a yellow solid (0.08 g, 93% yield). ¹H NMR (DMSO-d₆, 300 MHz,) δ : 1.79 – 1.95 (2H, m, H_{2'a}/H_{2'β}), 2.59 – 2.78 (2H, m, CH_{2(linker)}), 3.44 – 3.48 (4H, m, H_{5'a}/H_{5'β}/CH_{2(linker)}), 3.71 (1H, m, H_{4'}), 4.10 (1H, m, H_{3'}), 5.00 (1H, t, *J* = 4.8 Hz, H_{5'OH}), 5.17 (1H, d, *J* = 4.2 Hz, H_{3'-OH}), 6.10 (1H, t, *J* = 7.2 Hz, H_{1'}), 7.67 (1H, s, H₆), 7.94 (1H, d, *J* = 7.8 Hz, Ar), 8.05 – 8.28 (7H, m, Ar), 8.44 (1H, d, *J* = 9.3 Hz, Ar), 11.37 (1H, br, H_{N3}). The CH₂CH₂ of the linker assignment was confirmed by a COSY experiment. ¹³C NMR (DMSO-d₆, 75 MHz) δ : 29.03, 32.00, 39.35, 61.21, 70.31, 83.88, 87.32, 112.92, 123.47, 124.13, 124.20, 124.81, 124.97, 126.13, 126.56, 127.29, 127.44, 128.29, 129.39, 130.41, 130.87, 135.95, 136.71, 150.32, 163.56. MS (FAB) *m/z* for C₂₇H₂₅N₂O₅ (M+H)⁺: calcd. 457.1763, found 457.1776.

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