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SYNTHESIS AND CHARACTERIZATION OF AN OLIGONUCLEOTIDE CONTAINING THE BIFURCATED NUCLEOBASE ω -ADENYLPROPYL URACIL

Christopher Switzer,* Thazha P. Prakash, and Yoonmo Ahn

Department of Chemistry, University of California, Riverside, CA 92521

Abstract. A novel bifurcated nucleoside that bears an adenine group trimethylene linked to the 5-position of uracil, 5-(omega-adenylpropyl)-2'-deoxyuridine, has been synthesized and incorporated into a DNA dodecamer. This nucleoside is designed to both intercalate and hydrogen bond when incorporated into an oligonucleotide helix. Initial results of its influence on helix properties are reported. Copyright © 1996 Elsevier Science Ltd

Intercalators distort the structures of nucleic acid helices and compensate for this distortion by way of favorable interactions.¹ One means for increasing the stability of intercalated complexes is to reduce or eliminate the entropic cost of bimolecular binding. In the past this has been accomplished through bis-intercalators² and anchoring an intercalator directly to an oligonucleotide.³ We seek to examine the effect of incorporating a group into an oligonucleotide that is at once capable of both hydrogen bonding to an opposite strand base and intercalation. Towards this end, the synthesis of a uridine derivative bearing a trimethylene linked adenine at the 5-position is reported, along with its incorporation into a DNA dodecamer. The design of this nucleoside is based upon the work of Leonard et al.⁴ detailing physical interactions of pyrimidine and purine dimers connected by way of hydrocarbon chains.

Due to synthetic expedience, 2'-deoxyuridylate was chosen to bear the tethered base. Adenine was chosen as the base to be tethered because of its superior ability to π -stack. The trimethylene linker was decided upon because of its tendency to promote optimal stacking when it separates two bases.⁴ The synthesis of 5-(ω -adenylpropyl)-2'-deoxyuridine in a form suitable for oligonucleotide synthesis is shown in Figures 1 and 2. N⁹-Propynyl adenine was prepared by the method of Joshi and Zemlicka.⁵ This material was converted into its N⁶-dibenzoyl derivative **3**⁶ rather than a monobenzoyl one because when the latter was employed, solubility problems were later encountered. The coupling⁷ of dibenzoyl propynyl adenine **3** to 5-iodo-2'-deoxyuridine (**4**) was performed using a slight modification⁸ of the method reported by Hobbs.⁹ Transformation of nucleoside **6**¹⁰ bearing a trimethylene linker into phosphoramidite **8** was accomplished by standard methods.¹¹

The following dodecanucleotide containing a single 5-(ω -adenylpropyl)-2'-deoxyuridylate was synthesized: 5'-d-AAAAAAUAAAAA-3' (**9**). After automated synthesis, deprotection of the oligomer was performed using standard conditions of concentrated ammonium hydroxide at 55 °C for 15 hours. Purification of **9** was effected by reverse-phase HPLC. Incorporation of the intact modified base was shown by MALDI-TOF mass spectrometry, where the correct parent ion was observed (Figure 3). Purity of the oligomer was assessed as greater than 95% based on end-labelling followed by PAGE. An examination of the duplex between oligomer **9** and 5'-d-T₅AT₆ gave a $T_m = 33$ °C which was essentially identical (within 1 °C) to that of the all natural duplex counterpart having thymine in place of the bifurcated base.

In an appropriate context, oligonucleotides bearing bifurcated bases could find use in the stabilization of nucleic acid complexes through intercalation and/or hydrogen-bonding. Alternatively, tethered extrahelical bases might enable novel supermolecular interactions.

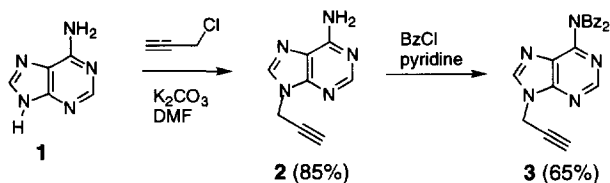


Figure 1.

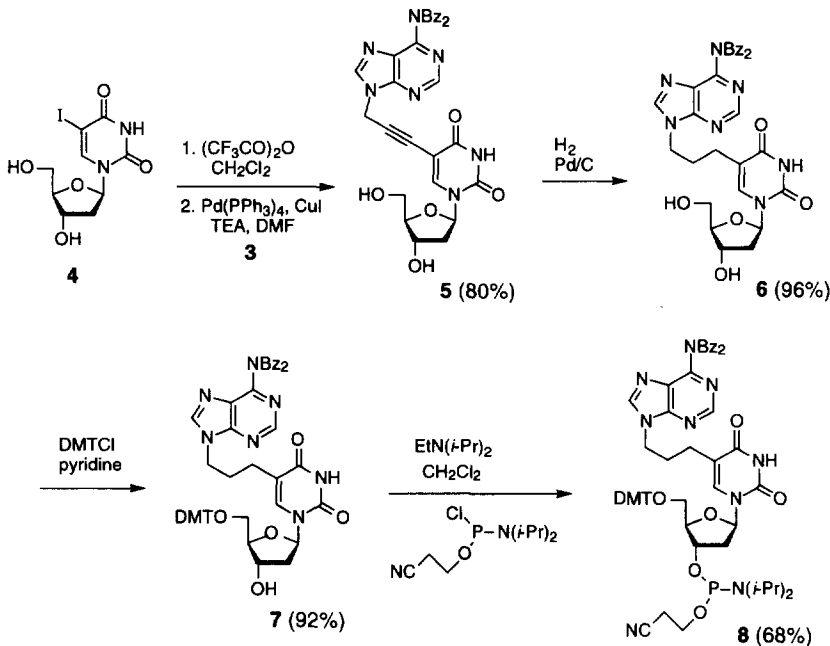


Figure 2.

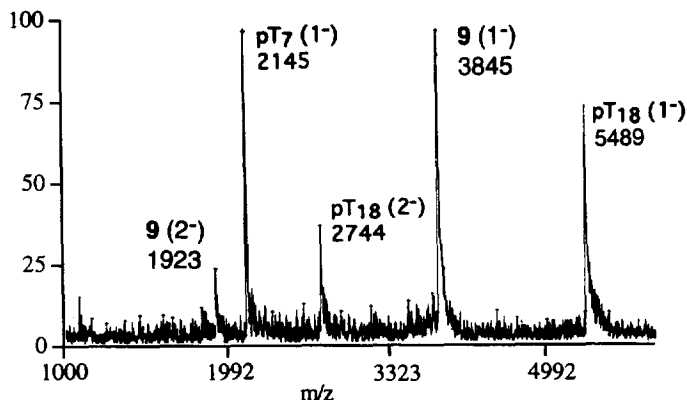


Figure 3. Laser desorption mass spectrum of 5'-d-AAAAAAUAAAAA-3' (**9**); U = 5-(ω -adenylpropyl)-2'-deoxyuridylylate. Calculated masses ($[M-H]^-$) are (**9**): 3848; pT7: 2146; and pT18: 5493.

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6. 6-N,N-Dibenzoyl-9-propargyladenine (**3**). To a solution of 9-Propargyladenine⁵ **2** (460 mg, 2.67 mmol) in pyridine at 0°C was added benzoyl chloride (1.5 mL, 10.7 mmol). The mixture was warmed to RT and allowed to stir for 6 h. The pyridine was removed by evaporation, the residue dissolved in CH₂Cl₂, and subsequently washed with 5% aq. NaHCO₃. The organic phase was dried over Na₂SO₄ and evaporated to give a residue, which was chromatographed on SiO₂ with 3% MeOH/CH₂Cl₂ to yield 840 mg of **3** (65%): ¹H NMR (CDCl₃) δ : 2.57 (s, 1 H), 5.04 (s, 2 H), 7.33-7.51 (m, 6 H), 7.86 (d, J = 7.8 Hz, 4 H), 8.28 (s, 1 H), 8.68 (s, 1 H). ¹³C

NMR (CDCl₃) δ : 33.36, 75.30, 75.57, 127.18, 128.67, 129.36, 132.96, 133.97, 144.09, 151.74, 152.25, 152.61, 172.21. HRMS (FAB⁺) calcd for C₂₂H₁₆N₅O₂ 382.1304 (MH⁺), found 382.1293.

7. 5-[ω -(6-N,N-Dibenzoyladen-9-yl)propynyl]-2'-deoxyuridine (**5**). To a suspension of 5-iodo-2'-deoxyuridine (**4**) (708 mg, 2.00 mmol) in CH₂Cl₂ (12 mL) was added trifluoroacetic anhydride (2.54 mL, 18.0 mmol) at room temperature. The mixture was stirred overnight. After concentration of the mixture, the residue was dried in vacuo at room temperature to give 1.09 g of 3',5'-di-O-trifluoroacetyl-5-iodo-2'-deoxyuridine as a solid foam, which was used without further purification in the next step. To this material, along with **3** (2.288 g, 6.00 mmol), tetrakis(triphenylphosphine)palladium (0) (462 mg, 0.40 mmol), and copper (I) iodide (152 mg, 0.80 mmol) were added dry DMF (20 mL) and Et₃N (0.558 mL, 4.00 mmol). The mixture was stirred at RT for 48 h, and then concentrated under a vacuum. The residue was purified by chromatography (SiO₂, 5%-10% MeOH/CH₂Cl₂). The fraction containing **5** was concentrated and treated with anion exchange resin (AG1X8, HCO₃⁻, 1.71 g, 3.0 eq.) in 20 mL of 1/1, CH₂Cl₂/MeOH at room temperature for 30 min. Evaporation of the solvent gave **5** (977 mg, 80%): ¹H NMR (DMSO-*d*₆) δ : 2.15 (m, 2 H), 3.60 (m, 2 H), 3.81 (m, 1 H), 4.24 (m, 1 H), 5.11 (t, *J* = 5.0 Hz, 1 H), 5.27 (d, *J* = 4.2 Hz, 1 H), 5.43 (s, 2 H), 6.11 (t, *J* = 6.5 Hz, 1 H), 7.47 (m, 4 H), 7.60 (m, 2 H), 7.81 (d, *J* = 7.5 Hz, 4 H), 8.29 (s, 1 H), 8.73 (s, 1 H), 8.74 (s, 1 H), 11.70 (s, 1 H). ¹³C NMR (DMSO-*d*₆) δ : 34.03, 60.90, 70.02, 78.11, 84.91, 85.55, 87.65, 97.12, 126.61, 129.04, 133.39, 133.46, 144.68, 146.42, 149.38, 150.81, 151.90, 152.50, 161.52, 172.07. MS (FAB⁺) *m/z*: 608 (MH⁺). HRMS (FAB⁺) calcd for C₃₁H₂₆N₇O₇ 608.1894 (MH⁺), found 608.1904.

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10. 5-[ω -(6-N,N-Dibenzoyladen-9-yl)propyl]-2'-deoxyuridine (**6**). To compound **5** (182 mg, 0.30 mmol) was added CH₂Cl₂/MeOH (1/1, 6 mL), and the mixture was heated gently until a solution was obtained. 10% Pd/C (60 mg) was added, and the resulting mixture was stirred under H₂ pressure (50 psi) at RT for 48 h. The mixture was filtered through celite and concentrated. Chromatography (SiO₂, 5%-10% MeOH/CH₂Cl₂) gave **6** (177 mg, 96%) as a white solid: ¹H NMR (DMSO-*d*₆) δ : 2.0-2.3 (m, 6 H), 3.57 (m, 2 H), 3.76 (m, 1 H), 4.2-4.3 (m, 3 H), 5.03 (t, *J* = 5.1 Hz, 1 H), 5.24 (d, *J* = 4.2 Hz, 1 H), 6.17 (t, *J* = 6.6 Hz, 1 H), 7.46 (m, 4 H), 7.59 (m, 2 H), 7.75 (s, 1 H), 7.77 (d, *J* = 7.5 Hz, 4 H), 8.63 (s, 1 H), 8.67 (s, 1 H), 11.33 (s, 1 H). ¹³C NMR (DMSO-*d*₆) δ : 23.63, 27.92, 43.22, 61.23, 70.33, 83.99, 87.31, 112.19, 126.79, 128.77, 128.99, 133.31, 133.55, 136.71, 147.20, 150.33, 150.61, 151.53, 153.07, 163.35, 172.07. MS (FAB⁺) *m/z*: 612 (MH⁺). HRMS (FAB⁺) calcd for C₃₁H₃₀N₇O₇ 612.2207 (MH⁺), found 612.2227.

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