Supporting information
To accompany

Oligodeoxynuclotides Incorporating Structurally Simple 5-Alkynyl-2'-deoxyuridines Fluorimetrically Respond to Hybridization.

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General

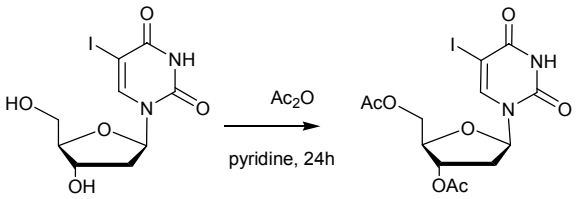
^{1}H NMR, ^{13}C NMR and spectra ^{31}P NMR were recorded on either a Varian Mercury Plus operating at 400 MHz (^{1}H) or 100.6 MHz (^{13}C), on a Varian Inova operating at either 600 MHz (^{1}H), 150.9 MHz (^{13}C) or 242.9 MHz (^{31}P). Chemical shifts are reported in parts per million (δ) and are referenced to the solvent, *i.e.* 7.26/77.1 for $CDCl_3$, 2.49/39.5 for DMSO-D_6 and 2.05/29.8 acetone-D_6. Phosphorus chemical shifts are reported relative to phosphoric acid (δ 0.0 ppm). Multiplicities are described as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or app (apparent). Coupling constants (*J*) are reported in Hertz (Hz). High-resolution mass spectra for modified nucleotides were recorded on a Finnigan MAT 8200 mass spectrometer or by ESI-TOF using a Micromass LCT. Thin layer chromatography (TLC) was performed on Merck Kieselgel 60 TLC plates. Flash column chromatography was performed on Merck Kieselgel 60, 230-400 mesh silica gel. Purity and homogeneity of all materials was determined from TLC, ^{1}H NMR, and ^{13}C NMR. Anhydrous solvents were obtained by passing through activated alumina columns (Innovative Technologies, Newburyport, MA). All moisture sensitive reactions were performed under a static nitrogen atmosphere.

Oligonucleotide Chemistry

Syntheses of DNA oligomers were performed on an ABI 392 DNA synthesizer with commercially available reagents from Glen Research (Sterling VA) using manufacturer-supplied cycles and conditions. The coupling time was extended to 180 seconds for all phosphoramidites. DNA oligomers were concomitantly cleaved from the support and deprotected by 16 h treatment with concentrated aqueous ammonia at 60 ºC. The crude oligomers were lyophilized and then purified by C18-RP-HPLC (100 mM NEt_3•HOAc, pH 6.5), firstly with "DMT-on" and then with "DMT-off", desalted by size exclusion chromatography and identified by MALDI-TOF MS.

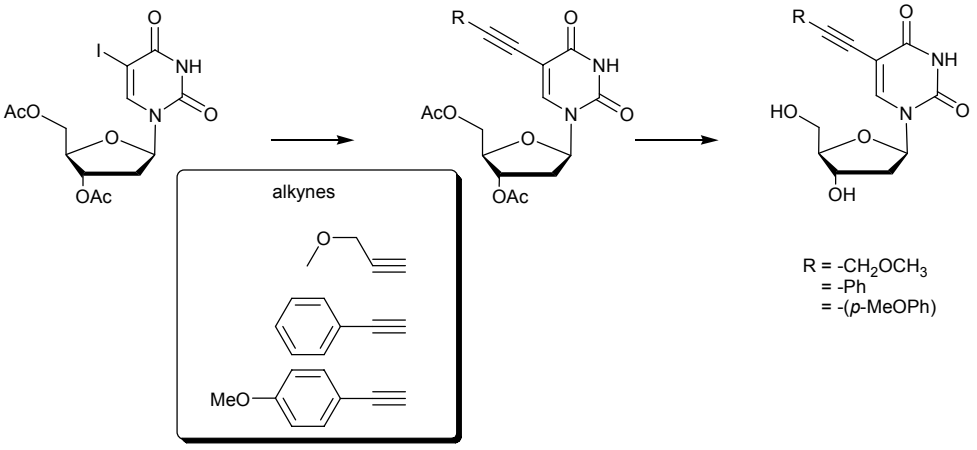
The concentrations of oligonucleotides solutions were determined in one of the two following ways: the concentration of unmodified oligonucleotides was estimated

according to the nearest neighbor approximation;[i] while solutions of modified oligonucleotides were measured at 80 ºC and the extinction coefficient for the oligomer was calculated as the sum of the individual nucleosides. UV-vis spectral measurements were made on a Varian Cary III fitted with a thermoelectric cell holder. Fluorescence spectra were measured on a Photon Technologies International Quanta Master 7/2005 spectrophotometer also fitted with a thermoelectric cell holder for temperature control.



A mixture of 5-iodo-2′-deoxyuridine (2.0 g, 5.65 mmol), pyridine (30 mL) and acetic anhydride (18 mL) was stirred for 24 h at room temperature. The solvent was removed under vacuum. The residue was dissolved in dichloromethane (100 mL) and washed with dilute aqueous $NaHCO_3$. The organic phase was separated and dried over anhydrous Na_2SO_4 and then filtered. The filtrate was evaporated and 3′,5′-di-*O*-acetyl-5-iodo-2′-deoxyuridine was achieved as a white powder in 89 % (2.2 g).

3′,5′-di-*O*-acetyl-5-iodo-2′-deoxyuridine: mp 164-166 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.35 (br, 1H), 7.96 (s, 1H), 6.29 (dd, *J* = 8.1, 5.6 Hz, 1H), 5.23 (m, 1H), 4.31-4.42 (m, 2H), 4.29–4.30 (m, 1H), 2.51-2.56 (m, 1H), 2.12–2.18 (m, 1H), 2.20 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (100.6 MHz): δ 170.6 and 170.3, 159.9, 150.1, 144.0, 85.7, 82.9, 74.3, 69.2, 64.2, 38.4, 21.3 and 21.1; HRMS (ESI-TOF) found: 437.9931 (M^+); calcd 437.9924 for $C_{13}H_{15}IN_2O_7$.



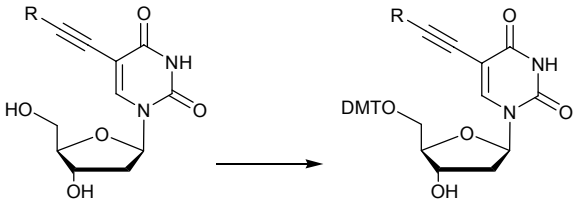
A round bottom flask was charged with 3′,5′-diacetyl-5-iodo-2′-deoxyuridine (0.4386 g, 1 mmol), one of methyl propargyl ether, phenylacetylene or ethynylanisole (3 mmol), Et_3N (0.28 mL, 2 mmol) and DMF (7 mL). This mixture was deoxygenated by purging with with N_2, then $Pd(PPh_3)_4$ (0.1550 g, 0.1 mmol) and CuI (0.038g, 0.2 mmol) were added. The mixture was stirred for 24 h in the dark and at room temperature after which the reaction mixture was dissolved in CH_2Cl_2 (50 mL) and washed by saturated EDTA (2 × 100 mL). The organic phase was separated, dried over Na_2SO_4 and filtered. After removal of the solvent, the residue was purified by silica gel column chromatography using gradient elution with hexanes:acetone(7.5:2.5 to 5:5, v/v). The 3′,5′-di-*O*-acetyl-5-alkynyl-2'-deoxyuridines were obtained as white to off-white solids. Each of the 3′, 5′-di-*O*-acetyl-5-alkynyl-2′-deoxyuridines were separately dissolved in pyridine:ethanol (1:5 v/v, 50 mL total volume) cooled to 0-5 ºC. As the temperature was maintained below 5 ºC, 1 M NaOH (5 mL) was added and the mixture was stirred until completion of reaction, as determined by TLC analysis. Once the hydrolysis was complete, the reaction mixture was neutralized with Dowex 50W-X8 resin (H^+ form) and then filtered. After removal of the solvent the residue was purified by precipitation in hexanes and acetone obtain pure 5-alkynyl-2'-deoxyuridines.

5-(methoxymethylethynyl)-2′-deoxyuridine: white solid (first step 71 %; second step 91 %). mp 173-174 °C; ^{1}H NMR (400 MHz, DMSO-D_6): δ 11.63 (s, 1H), 8.25 (s, 1H), 6.09 (app t, *J* = 6.7 Hz, 1H), 5.24 (d, *J* = 4.3 Hz, 1H), 5.12 (t, *J* = 5.0 Hz, 1H), 4.24 (s,

2H), 4.20-4.22 (m, 1H) 3.76-3.79 (m, 1H), 3.52-3.63 (m, 2H), 3.27 (s, 3H) 2.10-2.13 (m, 2H); ^{13}C NMR (100.6 MHz, DMSO-D_6): δ 162.2, 150.1, 144.7, 98.4, 89.2, 88.3, 85.5, 79.4, 70.7, 61.6, 60.2, 55.9, 31.4; HRMS (EI): found 296.1016 (M^+), calcd 296.1008 for $C_{13}H_{16}N_2O_6$.

5-phenylethynyl-2′-deoxyuridine: off-white solid (first step 90 %; second step 86 %). mp 177-178 °C; ^{1}H NMR (400 MHz, DMSO-D_6): δ 11.69 (s, 1H), 8.36 (s, 1H), 7.37-7.45 (m, 5H), 6.10 (m, 1H), 5.25 (br, 1H), 5.19 (br, 1H), 4.22 (m, 1H), 3.77 (m, 1H), 3.54-3.63 (m, 2H), 2.13 (m, 2H); HRMS (ESI-TOF): found 329.1131 $(M+H)^+$, calcd 329.1132 for $C_{17}H_{16}N_2O_5$.

5-(4-methoxyphenylethynyl)-2′-deoxyuridine: off-white solid (first step 83%; second step 68 %). mp 179-181 °C; ^{1}H NMR (400 MHz, DMSO-D_6): δ 11.66 (s, 1H), 8.31 (s, 1H), 7.41 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 6.64 (t, *J* = 6.6 Hz, 1H), 5.25 (d, *J* = 4.3 Hz, 1H), 5.14-5.16 (m, 1H), 4.22-4.26 (m, 1H), 3.79 (m, 1H), 3.76 (s, 3H), 3.54-3.66 (m, 2H), 2.08-2.19 (m, 2H); ^{13}C NMR (400 MHz, DMSO-D_6): δ 162.2, 160.1, 150.1, 144.0, 133.4, 115.0, 115.0, 99.2, 92.5, 88.3, 85.4, 81.5, 70.7, 61.5, 55.9. HRMS (ESI-TOF): found 381.1063 $(M+Na)^+$, calcd 381.1062 for $C_{18}H_{18}N_2NaO_6$



R = -CH_2OCH_3
= -Ph
= -(*p*-MeOPh)

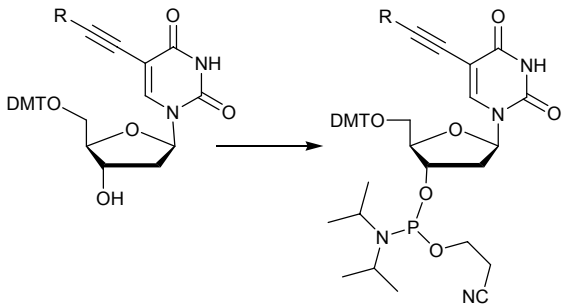
4,4′-Dimethoxytritylchloride (0.44 g, 1.3 mmol) was added to a solution of one of the 5-alkynyl-2′-deoxyuridines (1.0 mmol) and Et_3N (1 mL) in dry pyridine (10 mL). The reaction mixture was stirred at room temperature for 5 h and then was quenched by the addition of methanol (2 mL). The resultant mixture was dissolved in CH_2Cl_2 and washed with a 0.5 M aqueous solution of $NaHCO_3$ (50 mL). The organic phase was separated and dried over Na_2SO_4 (2 g) and then the solvent was removed under vacuum with gentle heating (< 40 ºC). The residue after evaporation of the solvent was purified by silica gel column chromatography using gradient elution with hexanes:acetone:Et_3N (80:15:5 to 40:55:5, v/v).

5-(methoxymethylethynyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine: White foam (56 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (s, 1H), 7.18-7.43 (m, 10H), 6.83 (d, J = 8.6 Hz, 4H), 6.30 (app t, J = 6.3 Hz, 1H), 4.53 (m, 1H), 4.10 (m, 1H), 3.92-4.02 (m, 2H), 3.77 (s, 6H), 3.31-3.39 (m, 2H), 3.13 (s, 3H), 2.49-2.55 (m, 1H), 2.24-2.30 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 161.7, 158.8, 149.5, 144.7, 143.1, 135.7, 135.6, 130.2, 128.2, 128.1, 127.2, 113.5, 100.0, 90.1, 87.2, 86.7, 86.0, 72.5, 63.7, 60.3, 57.6, 55.5, 41.7. HRMS (ESI-TOF): found 621.2440 $(M+H)^+$, calcd 621.2207 for $C_{34}H_{34}N_2O_8Na$.

5-phenylethynyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine: White foam (83 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.04-7.45 (m, 15H), 6.75-6.79 (m, 4H), 6.35 (dd, J = 8.0, 6.0 Hz, 1H), 4.55 (m, 1H), 4.13-4.15 (m, 2H), 3.68 (s, 3H), 3.67 (s, 3H), 3.30-3.44 (m, 2H), 2.52-2.58 (m, 1H), 2.27-2.34 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 162.2, 158.8, 149.9, 144.6, 142.2, 135.8, 131.9, 130.2, 130.1, 128.4, 128.3, 128.2, 128.1,

127.2, 122.7, 113.6, 100.8, 93.9, 87.3, 86.9, 86.09, 72.59, 63.8, 55.4, 41.9. HRMS (ESI-TOF): found: 631.2440 $(M+H)^+$, calcd 631.2439 for $C_{38}H_{35}N_2O_7$.

5-(4-methoxyphenylethynyl)-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)uridine: White foam (77 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11(s, 1H), 7.12-7.44 (m, 10H), 6.98 (d, J = 8.8 Hz, 2H), 6.75-6.79 (m, 4H), 6.67 (d, J = 8.8 Hz, 2H), 6.34 (dd, J = 8.1, 6.0 Hz, 1H), 4.54-4.55 (m, 1H), 4.11-4.13 (m, 1H), 3.76-3.77 (m, 1H), 3.75 (s, 3H), 3.68 (s, 3H), 3.67 (s, 3H), 3.66 (s, 3H), 3.22-3.42 (m, 2H), 2.52-2.57 (m, 1H), 2.25-2.32 (m, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 161.9, 159.8, 158.8, 149.6, 144.63, 141.8, 135.8, 135.7, 133.4, 130.2, 130.1, 128.2, 128.1, 127.2, 114.8, 113.8, 113.5, 101.1, 94.0, 87.2, 86.9, 86.1, 72.6, 63.8, 55.5, 55.4, 41.8. HRMS (ESI-TOF): found 661.2552 $(M+H)^+$, calcd 661.2544 for $C_{39}H_{37}N_2O_8$.



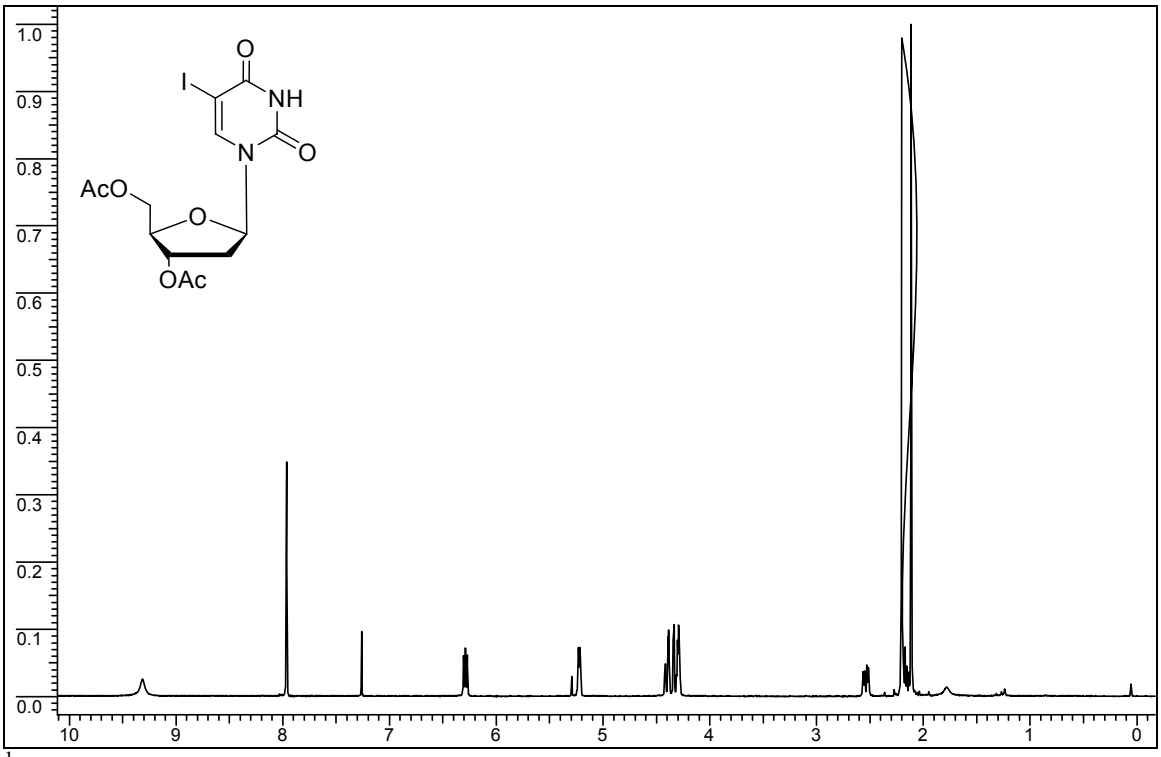
1a, R = -CH_2OCH_3
1b, R = -Ph
1c, R = -(*p*-MeOPh)

2-Cyanoethyldiisopropylphosphoramidochloridite (0.475 g, 2.0 mmol) was added to solution of a 5-alkynyl-2′-deoxy-5′-*O*-(4,4´-dimethoxytrityl)uridine (1.0 mmol) and Et_3N (2 mL) in dry dichloromethane (15 mL). The reaction mixture was stirred at room temperature and under a static N_2 atmosphere for 2 h. The reaction was then quenched by the addition of methanol (2 mL) and then extracted against 0.5 M $NaHCO_3$ (50 mL). The organic phase was separated, dried over Na_2SO_4 (1 g) and the solvent was removed under vacuum. The residue was purified by column chromatography using gradient elution with hexanes:acetone:Et_3N (80:15:5 to 40:55:5, v/v).

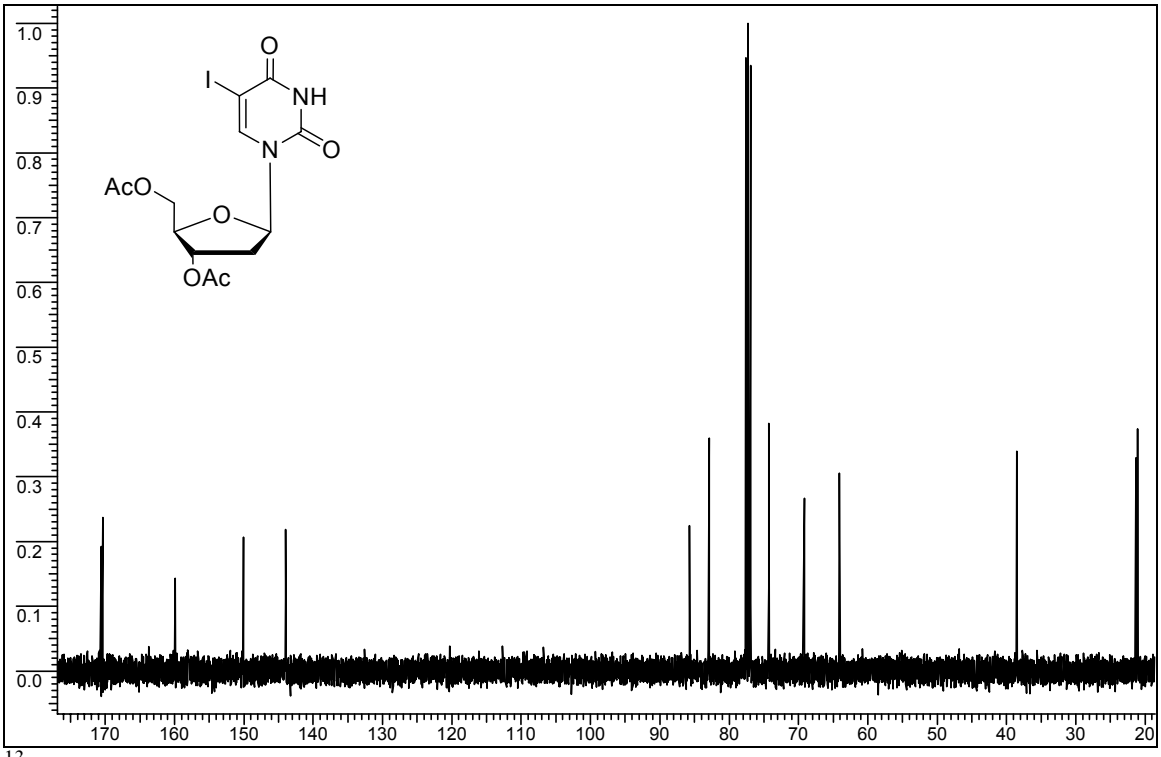
5-(methoxymethylethynyl)-2′-deoxy-3′-(2-cyanoethyldiisopropylphosphoramidite)-5′-*O*-(4,4´-dimethoxytrityl)uridine (**1a**): White foam (89 %). Two diastereomers: ^{1}H NMR (600MHz, acetone-D_6): δ 8.11 and 8.07 (2s, 1H), 7.20-7.43 (m, 10H), 6.81-6.84 (m, 4H), 6.25-6.30 (2m, 1H), 4.58-4.61 (2m, 1H), 4.16 and 4.21 (2m, 1H), 3.92-4.02 (2m, 2H), 3.77 (s, 3H), 3.88 (s, 3H), 3.52-3.67 (m, 2H), 3.23-3.44 (m, 2H), 3.08-311 (m, 3H), 2.63 (m, 2H) 2.53-2.58 (m, 1H), 2.29-2.32 (m, 1H), 1.05-1.24 (m, 14H); ^{13}C NMR (150.8 MHz $CDCl_3$): δ 161.7, 161.6, 158.8 (br), 144.7, 144.6, 143.0 (br), 135.7, 135.6 (two signals), 135.5, 130.1-130.2 (m), 128.2, 128.1 (two signals), 128.0, 127.1 (br), 113.6, 113.5, 100.0, 99.9, 90.0, 89.9, 87.2 (br), 86.0, 85.9, 74.1, 73.9, 73.6, 73.5, 69.7 (br), 63.4, 63.3, 60.2 (two signals), 58.6, 58.5, 57.5 (two signals), 55.5, 55.4, 54.0 (br), 43.6, 43.5, 43.4, 43.3, 40.8-40.9 (m), 31.9, 24.7-24.8 (m), 22.7 (br), 20.6, 20.5, 20.4, 20.3. ^{31}P NMR (242.9 MHz, acetone-D_6): δ two diastereomeric peaks at 150.1 and 149.7 (0.47/0.53). HRMS (ESI-TOF): found 821.3286 $(M+Na)^+$, calcd. 821.3291 for $C_{43}H_{51}N_4NaO_9P$.

5-(phenylethynyl)-2′-deoxy-3′-(2-cyanoethyldiisopropylphosphoramididite)-5′-*O*-(4,4′-dimethoxytrityl)uridine (**1b**): White foam (84 %). Two diastereomers: ^{1}H NMR (600 MHz, acetone-D_6): δ 8.21 and 8.17 (2s, 1H), 6.96-7.46 (m, 15H), 6.75-6.79 (m, 4H), 6.31-6.36 (2m, 1H), 4.60-4.64 (2m, 1H), 4.24 and 4.19 (2m, 1H), 3.68 (s, 3H), 3.66 (s, 3H), 3.56-3.64 (m, 2H), 3.28-3.50 (m, 2H), 2.61-2.73 (m, 2H), 2.56-2.60 (m, 1H), 2.30-2.38 (m, 1H), 1.05-1.25 (m, 14H). ^{13}C NMR (150.8 MHz, $CDCl_3$): δ 161.8, 161.7, 158.8, 153.8, 149.7 (two signals), 144.5 (two signals), 142.2 (br), 135.7, 135.7, 135.6, 135.5, 130.2 (two signals), 130.1 (two signals), 128.0-128.4 (m), 127.2 (br), 122.7 (br), 113.6, 113.5, 100.8 (two signals), 93.9, 93.8, 87.2 (br), 85.9-86.1 (m), 80.2 (two signals), 74.2, 74.1, 73.8, 73.7, 63.5, 63.3, 60.2 (two signals), 58.3-58.8 (m), 55.4, 55.3, 43.6, 43.5, 43.4, 43.3, 40.8-40.9 (m), 24.7-24.8 (m), 22.7 (br), 20.8, 20.6, 20.5, 20.4. ^{31}P NMR (242.9 MHz, acetone-D_6): δ two diastereomeric peaks at 150.0 and 149.6 (0.48/0.52). HRMS (ESI-TOF) found 853.3381 $(M+Na)^+$, calcd 853.3342 for $C_{47}H_{51}N_4NaO_8P$.

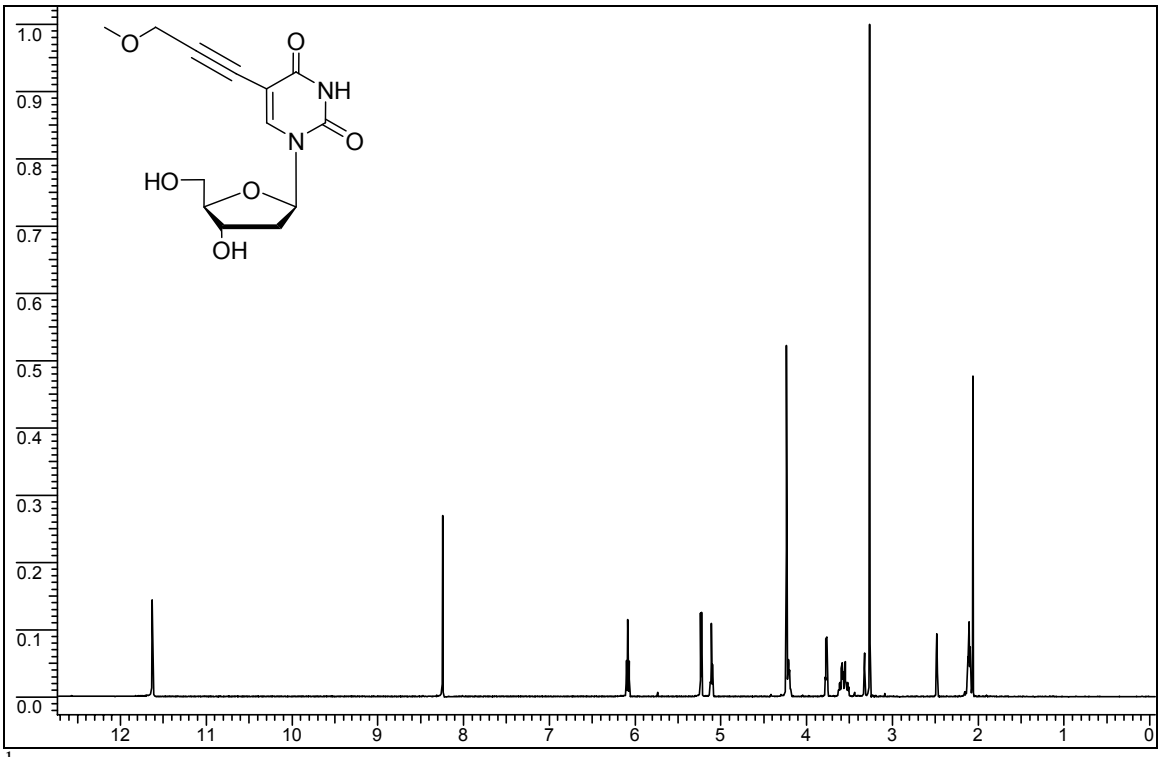
5-(4-methoxyphenylethynyl)-2′-deoxy-3′-(2-cyanoethyldiisopropylphosphoramididite)-5′-*O*-(4,4′-dimethoxytrityl)uridine (**1c**): Off-white to slightly yellow foam (82 %). Two diastereomers: ^{1}H NMR (600 MHz, acetone-D_6): δ 8.16 and 8.11 (2s, 1H), 7.14-7.45 (m, 10H), 6.91-6.97 (m, 2H), 6.64-6.79 (m, 6H), 6.30-6.39 (2m, 1H), 4.58-4.63 (2m, 1H), 4.17 and 4.20 (2m, 1H), 3.76 (2s, 3), 3.53 (s, H), 3.66 (s, 3H), 3.52-3.67 (m, 2H), 3.25-3.43 (m, 2H), 2.57-2.65 (m, 2H), 2.53-2.57 (m, 1H), 2.29-2.36 (m, 1H), 1.06-1.28 (m, 14H). ^{13}C NMR (150.8 MHz, $CDCl_3$): δ 161.5, 161.4, 159.7 (br), 158.8 (two signals), 149.3, 149.2, 144.6, 144.4, 141.8 (br), 135.8, 135.7, 135.6, 135.5, 130.3, 130.2 (two signals), 130.1, 128.1-128.3 (m), 127.2 (br), 114.8 (br), 113.7 (two signals), 113.6, 113.5, 101.2, 101.1, 94.0 (two signals), 87.3, 85.9-86.1 (m), 74.1, 73.9, 73.7, 73.6, 63.5, 63.4, 58.7-58.1 (m), 55.4, 55.3, 43.6, 43.5, 43.4, 43.3, 40.8-40.9 (m), 29.5, 24.7-24.9 (m), 22.6 (br), 20.3, 20.9. ^{31}P NMR (242.9 MHz, acetone-D_6): δ two diastereomeric peaks at 150.0 and 149.60 (0.52/0.48). HRMS (ESI-TOF) found 883.3416 $(M+Na)^+$, calcd 883.3348 for $C_{48}H_{53}N_4NaO_9P$.



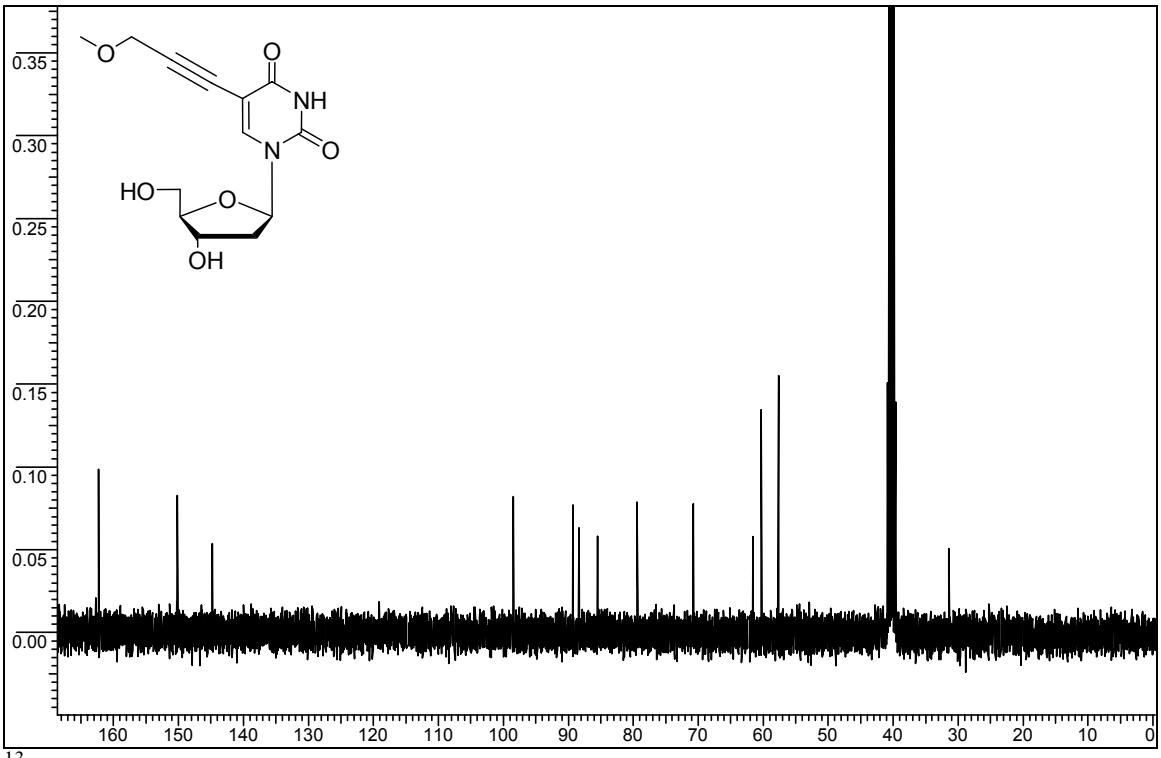
^{1}H NMR spectrum: **3′,5′-di-*O*-acetyl-5-iodo-2′-deoxyuridine**



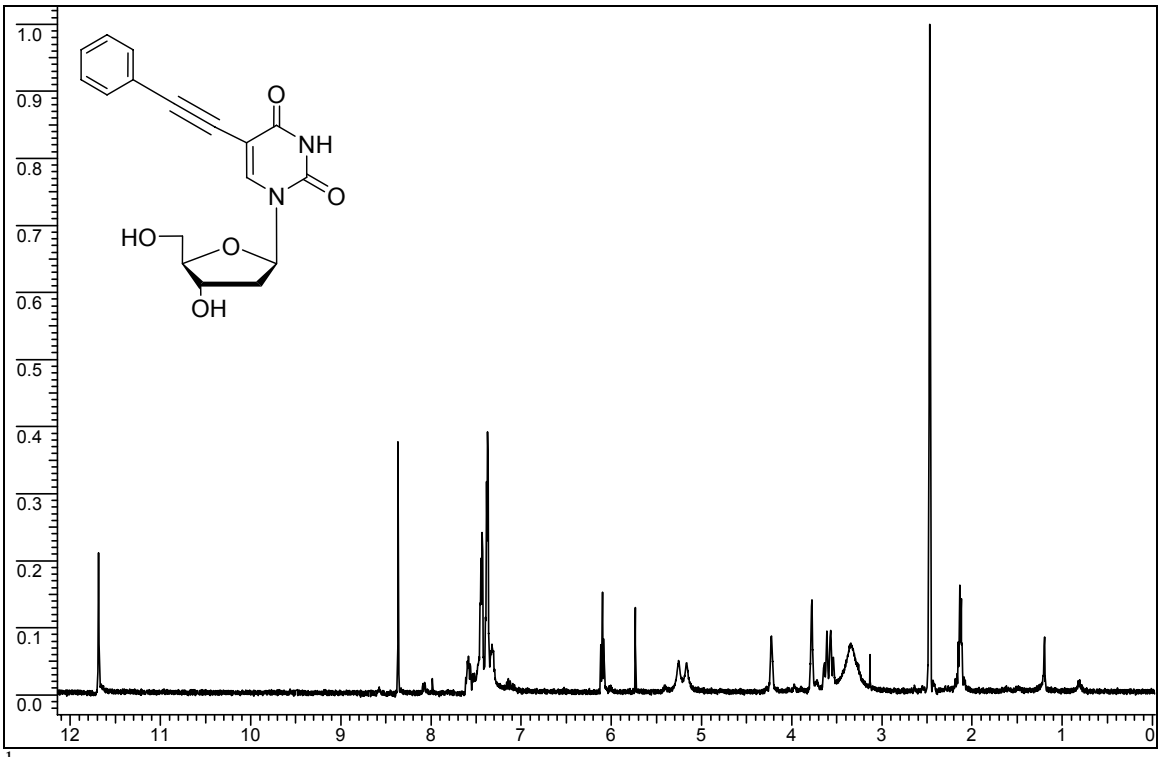
^{13}C NMR spectrum: **3′,5′-di-*O*-acetyl-5-iodo-2′-deoxyuridine**



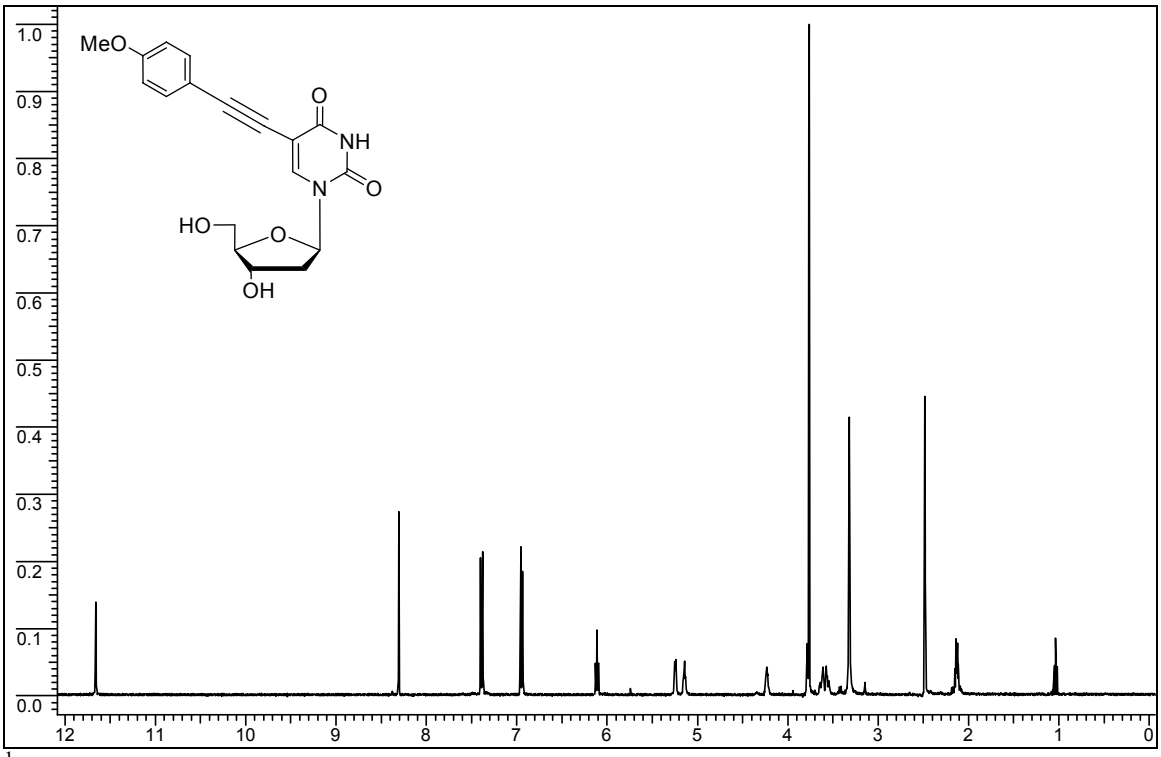
^{1}H NMR spectrum: **5-(methoxymethylethynyl)-2′-deoxyuridine**



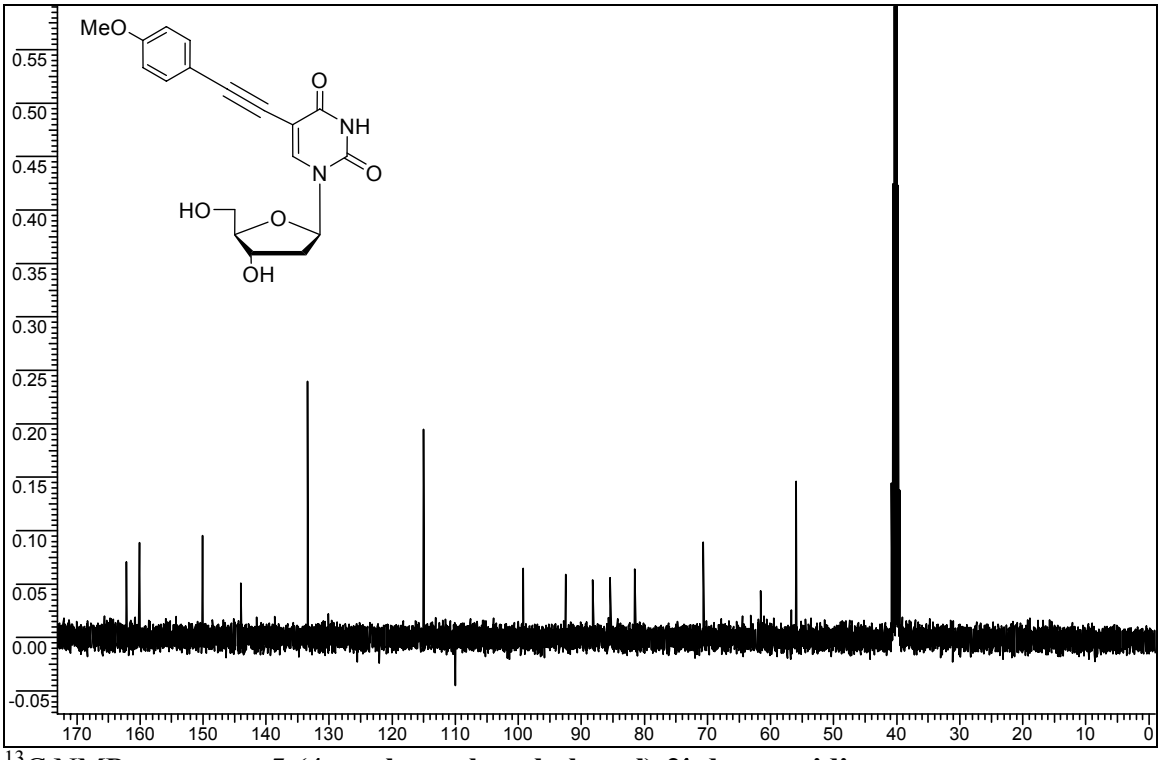
^{13}C NMR spectrum: **5-(methoxymethylethynyl)-2′-deoxyuridine**



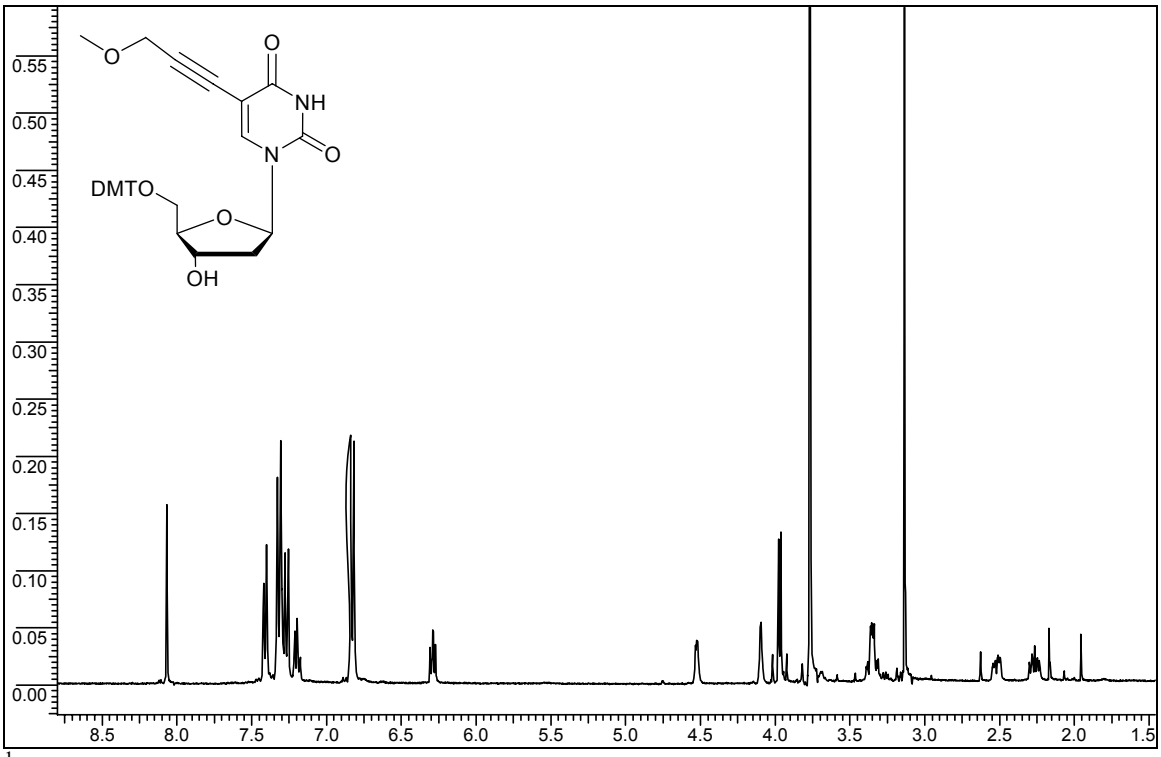
^{1}H NMR spectrum: **5-phenylethynyl-2′-deoxyuridine**



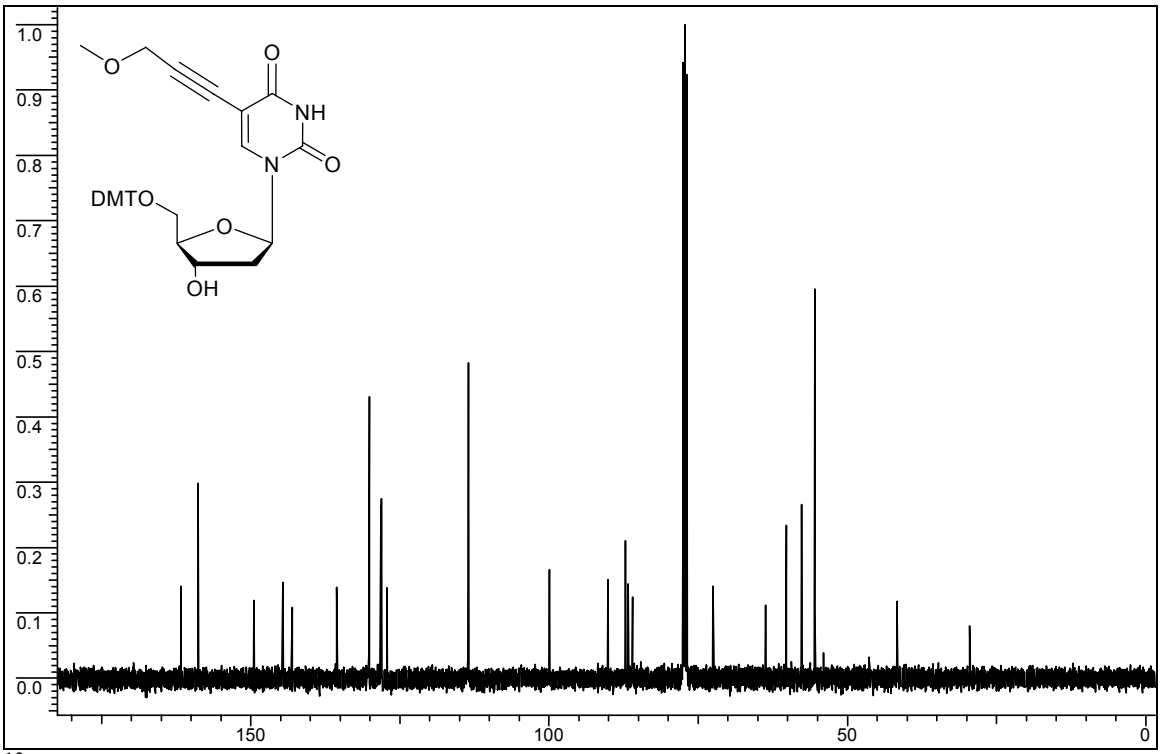
^{1}H NMR spectrum: **5-(4-methoxyphenylethynyl)-2′-deoxyuridine**



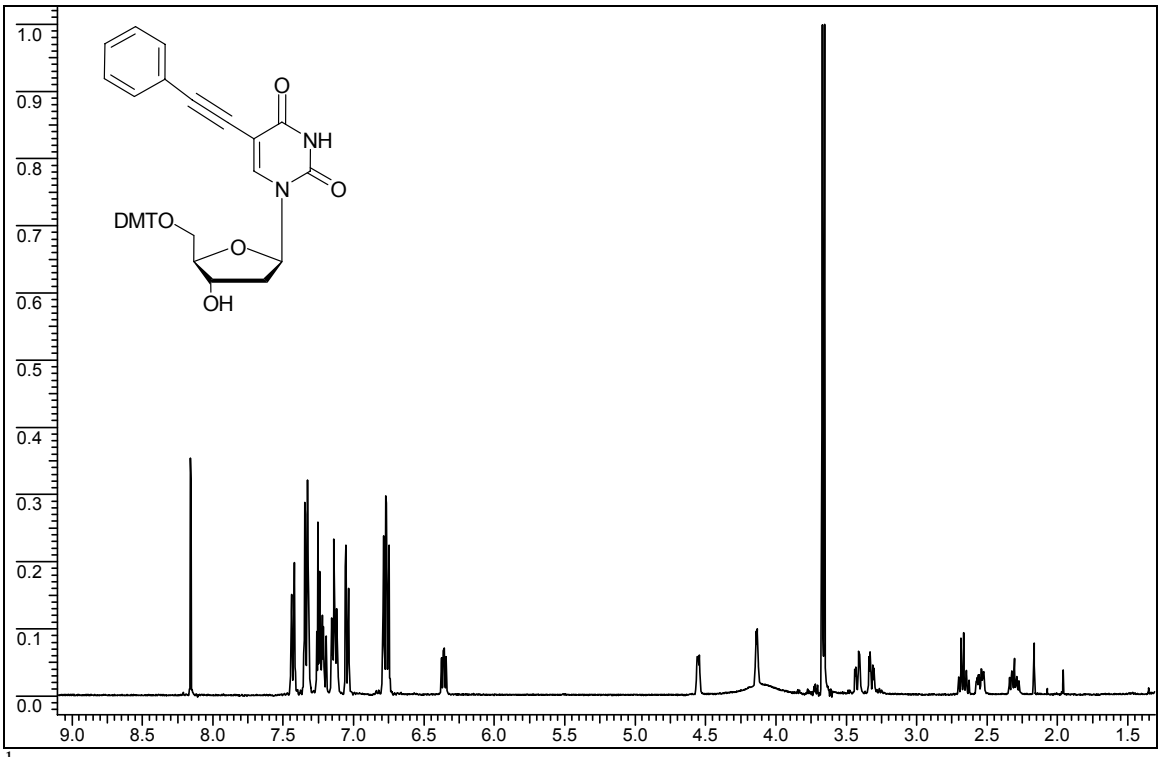
^{13}C NMR spectrum: **5-(4-methoxyphenylethynyl)-2′-deoxyuridine**



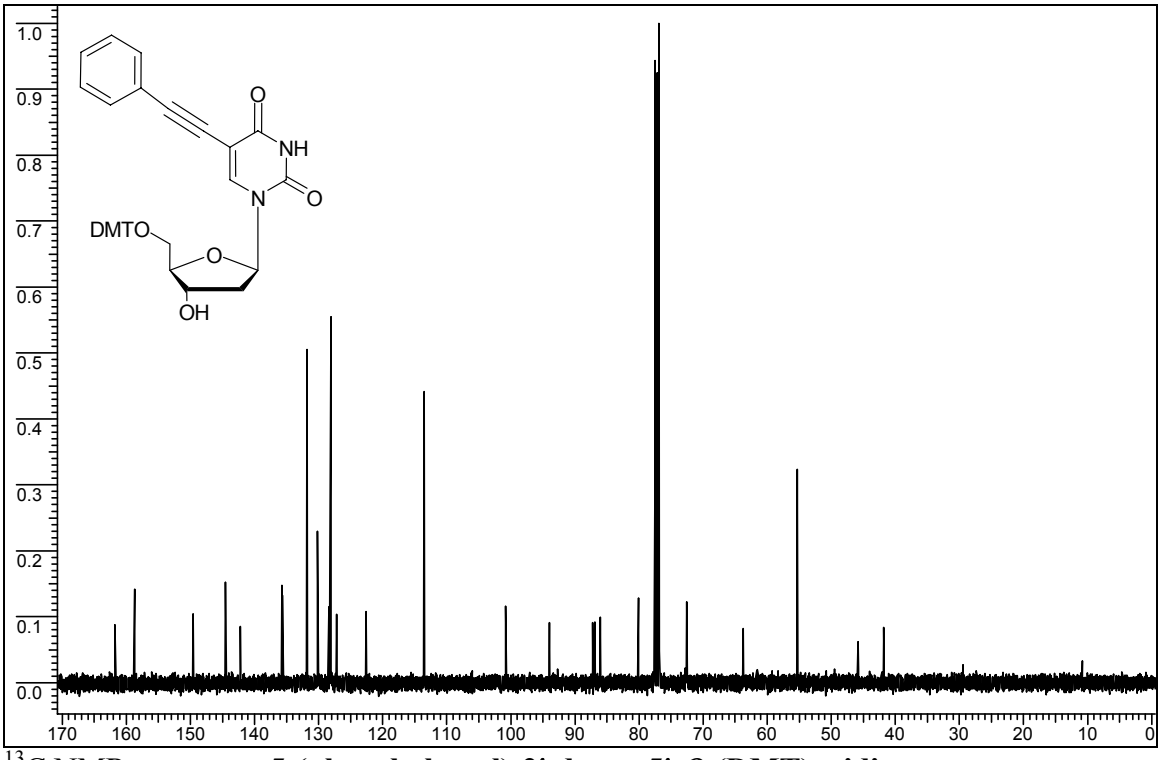
^{1}H NMR spectrum: **5-(methoxymethylethynyl)-2′-deoxy-5′-*O*-(4,4´-dimethoxytrityl)-uridine**



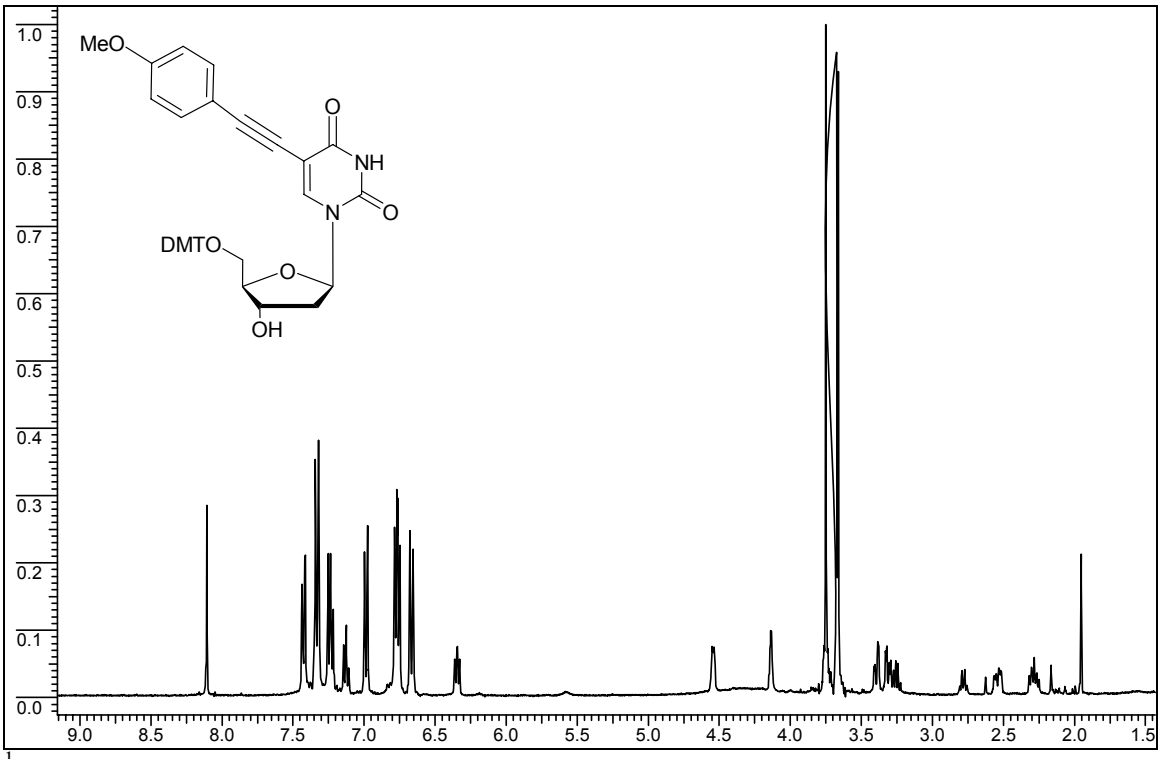
^{13}C NMR spectrum: **5-(methoxymethylethynyl)-2′-deoxy-5′-*O*-(4,4´-dimethoxytrityl)-uridine**



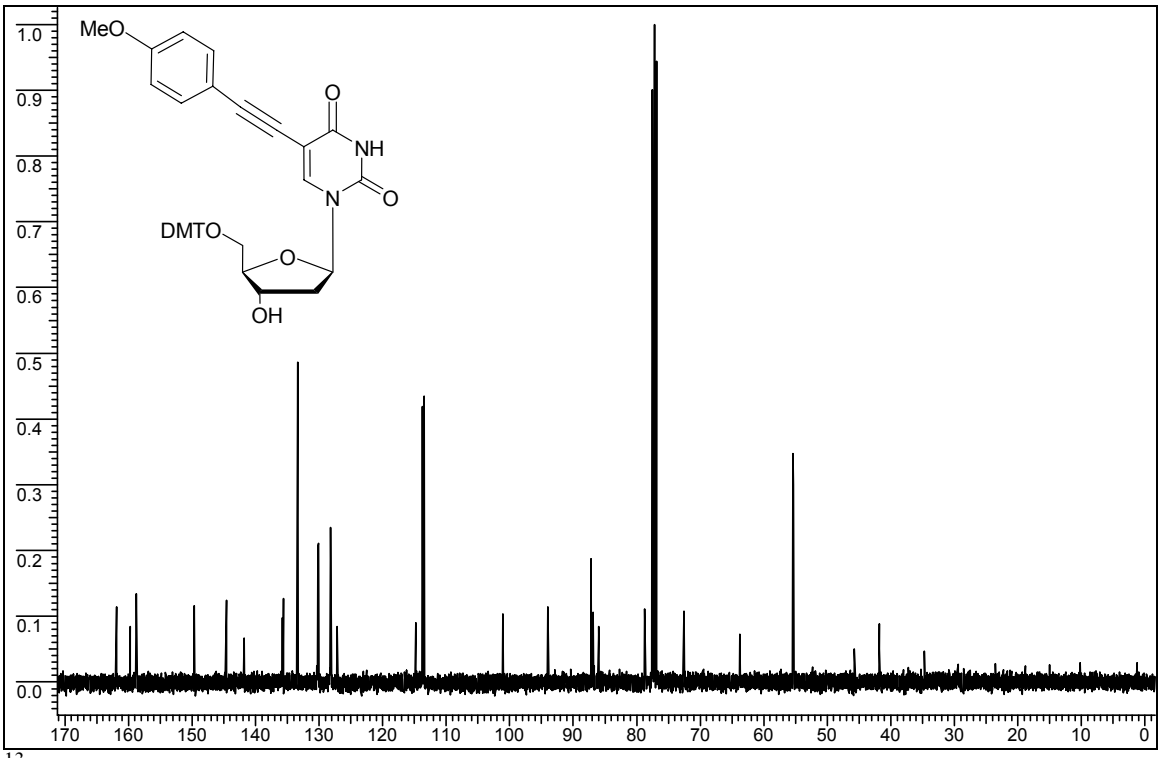
^{1}H NMR spectrum: **5-(phenylethynyl)-2′-deoxy-5′-*O*-(DMT)uridine**



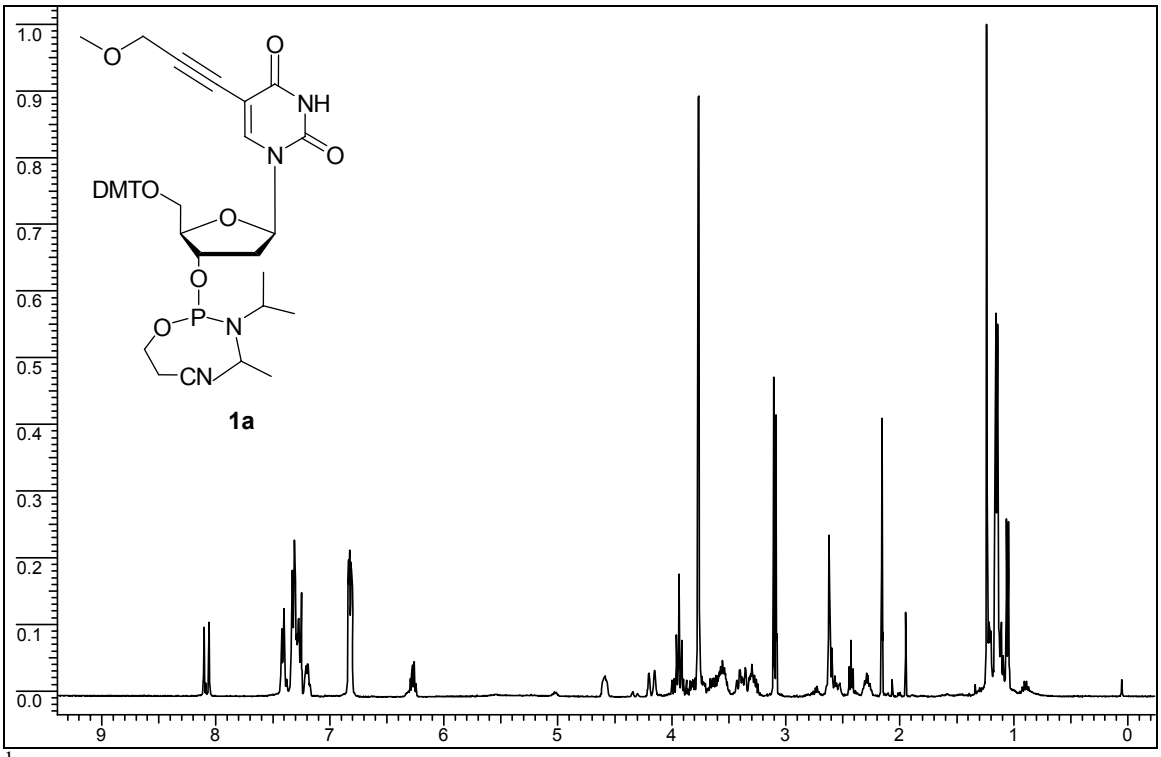
^{13}C NMR spectrum: **5-(phenylethynyl)-2′-deoxy-5′-*O*-(DMT)uridine**



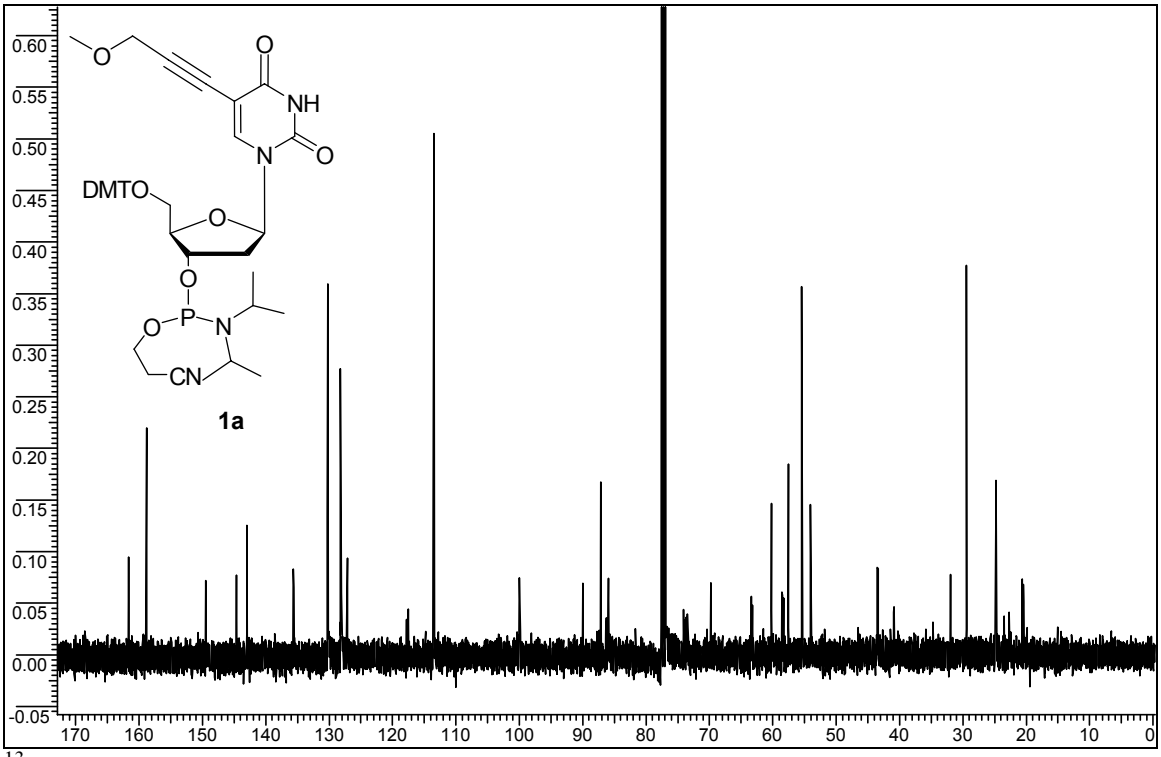
^{1}H NMR spectrum: **5-(4-methoxyphenylethynyl)-2′-deoxy-5′-*O*-(DMT)uridine**



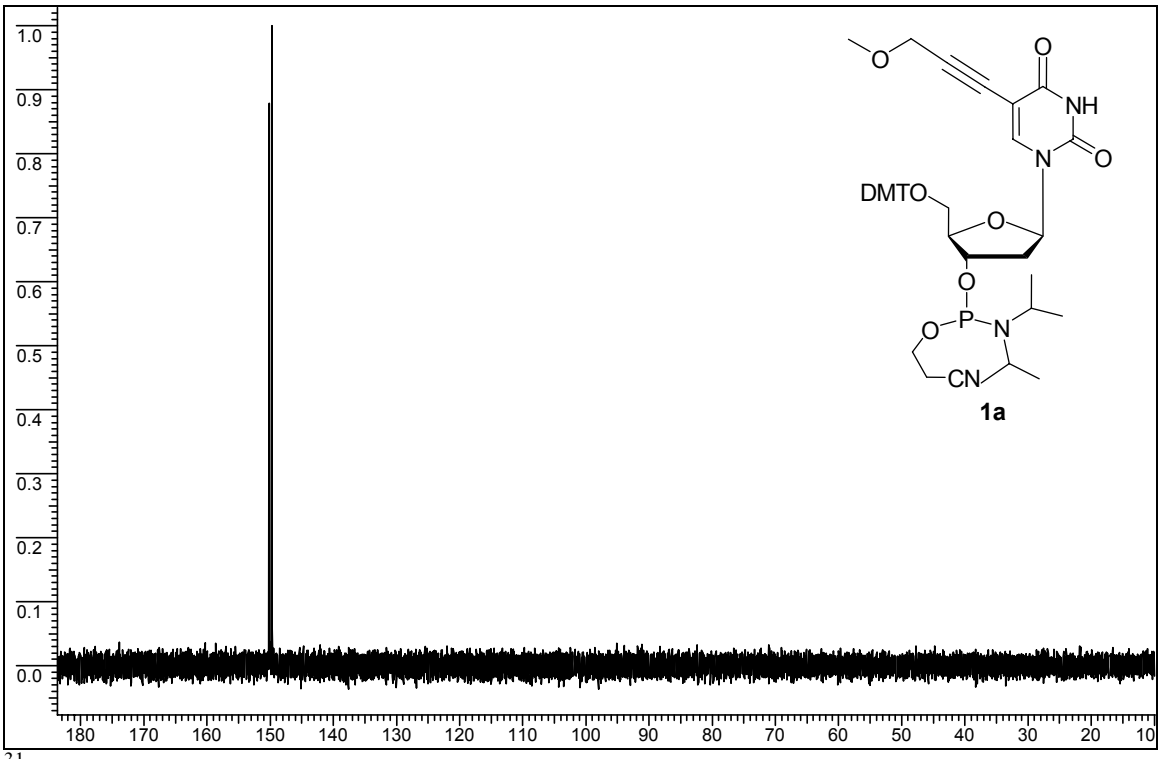
^{13}C NMR spectrum: **5-(4-methoxyphenylethynyl)-2′-deoxy-5′-*O*-(DMT)uridine**



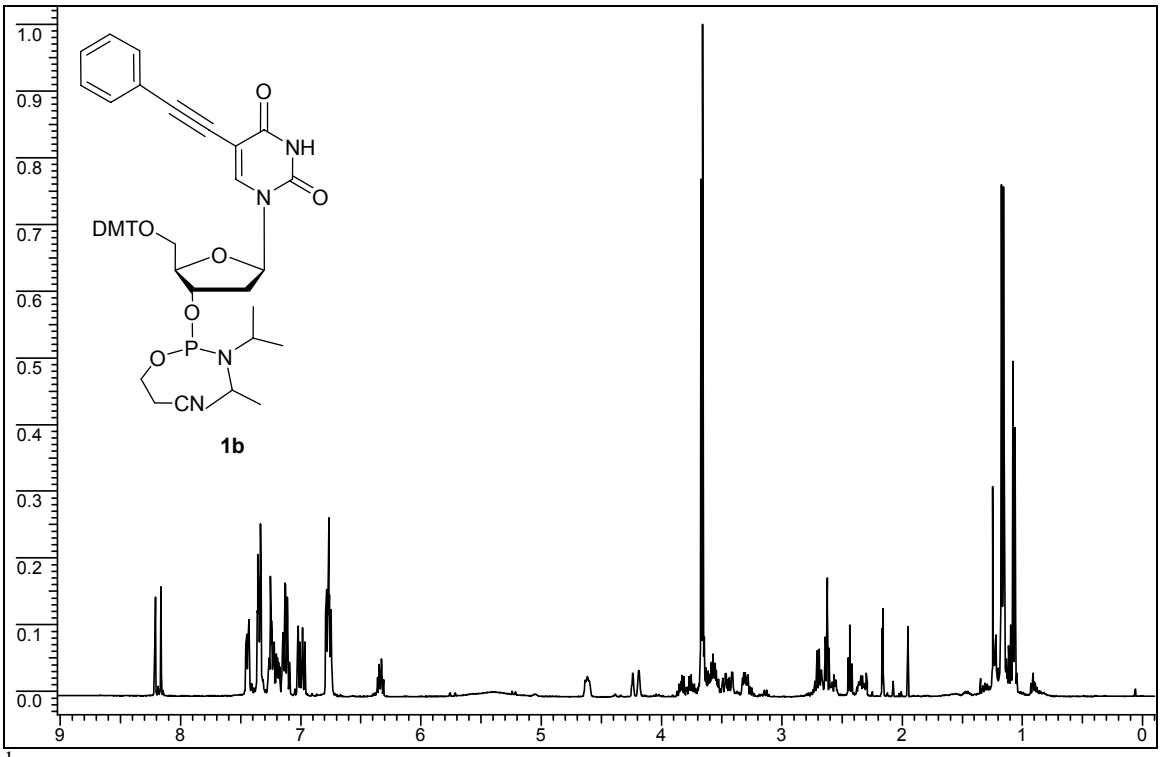
^{1}H NMR spectrum: **5-(methoxymethylethynyl)-2′-deoxy-3′-(2-cyanoethyldiisopropyl-phosphoramidite)-5′-*O*-(4,4′-dimethoxytrityl)uridine**



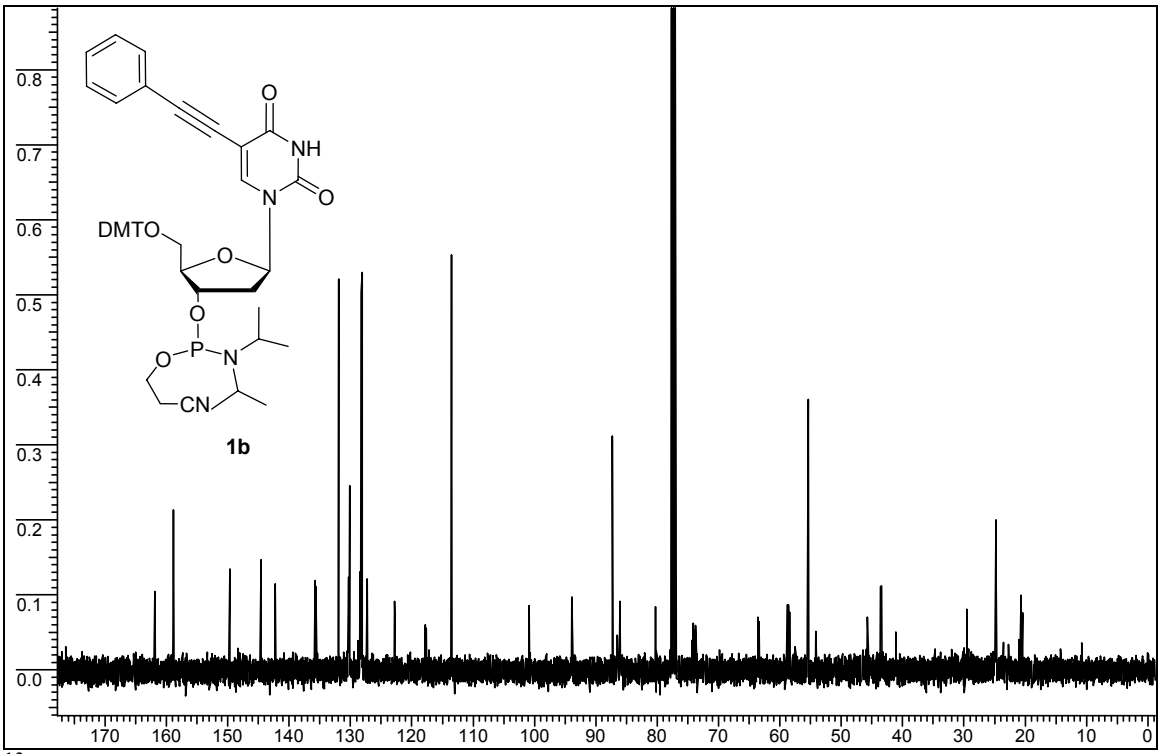
^{13}C NMR spectrum: **5-(methoxymethylethynyl)-2′-deoxy-3′-(2-cyanoethyldiisopropyl-phosphoramidite)-5′-*O*-(4,4′-dimethoxytrityl)uridine**



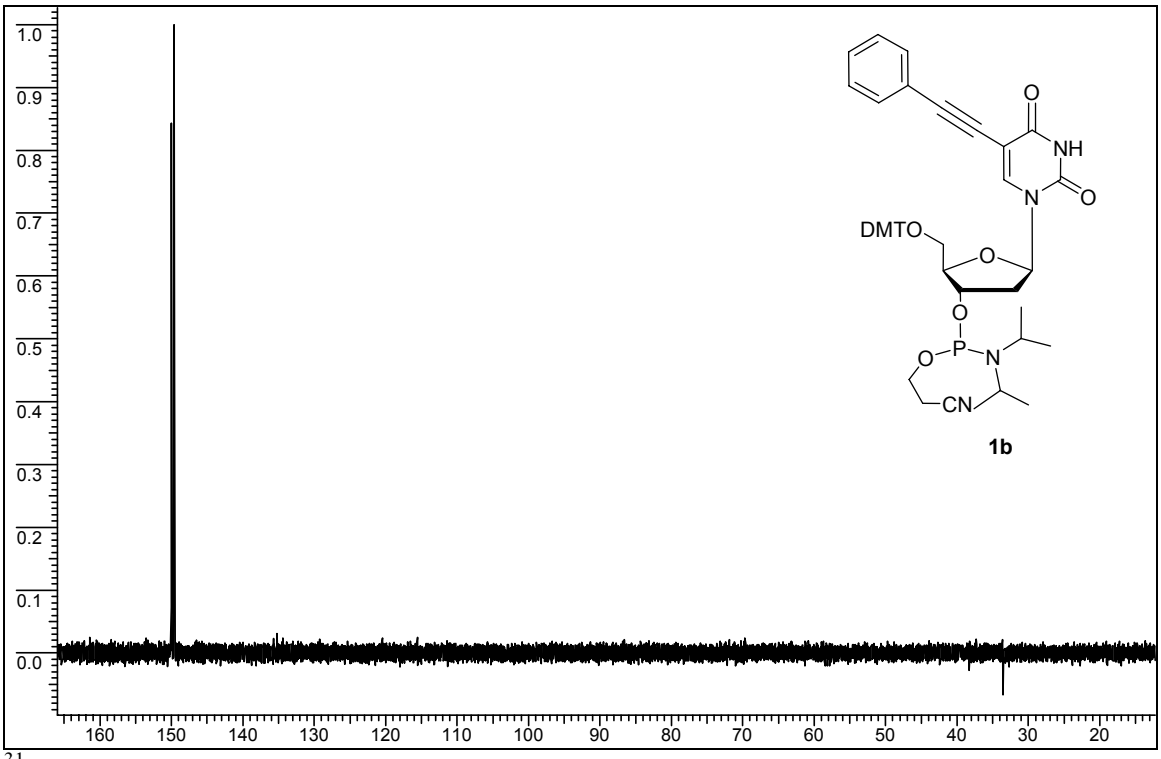
^{31}P NMR spectrum: **5-(methoxymethylethynyl)-2′-deoxy-3′-(2-cyanoethyldiisopropyl-phosphoramidite)-5′-*O*-(4,4′-dimethoxytrityl)uridine**



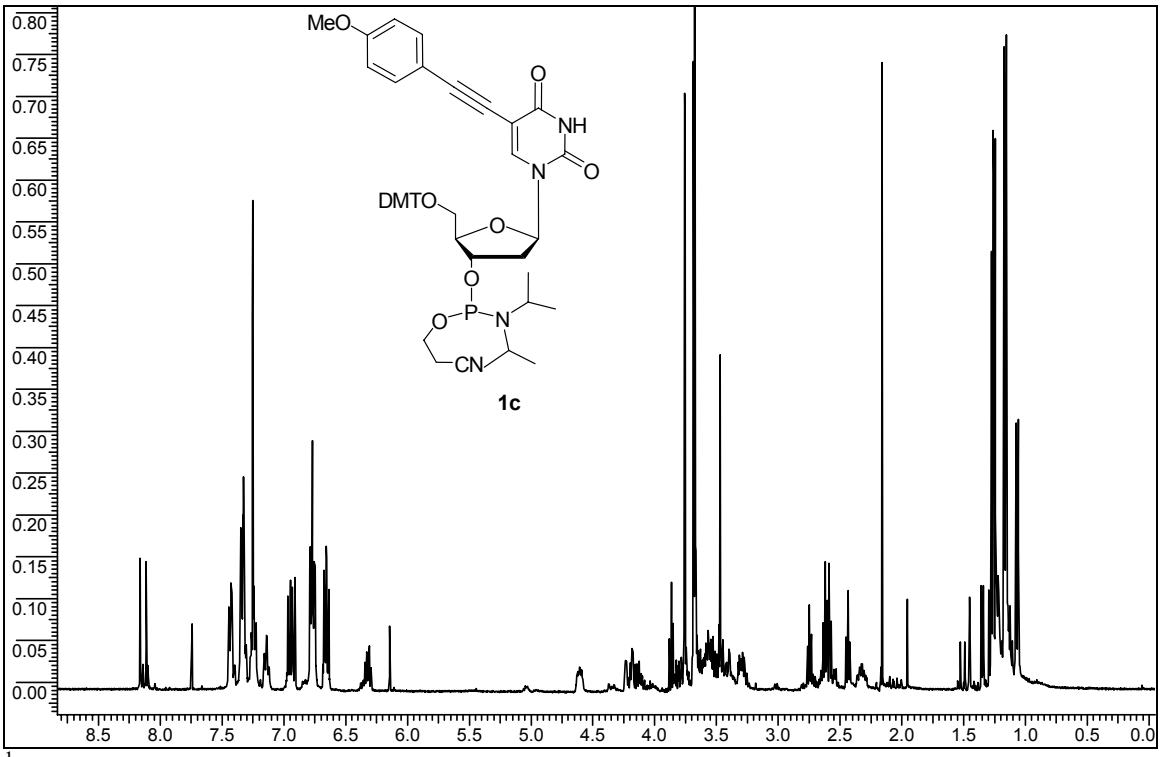
^{1}H NMR spectrum: **5-(phenylethynyl)-2′-deoxy-3′-(2-cyanoethyldiisopropylphosphoramididite)-5′-*O*-(4,4´-dimethoxytrityl)uridine**



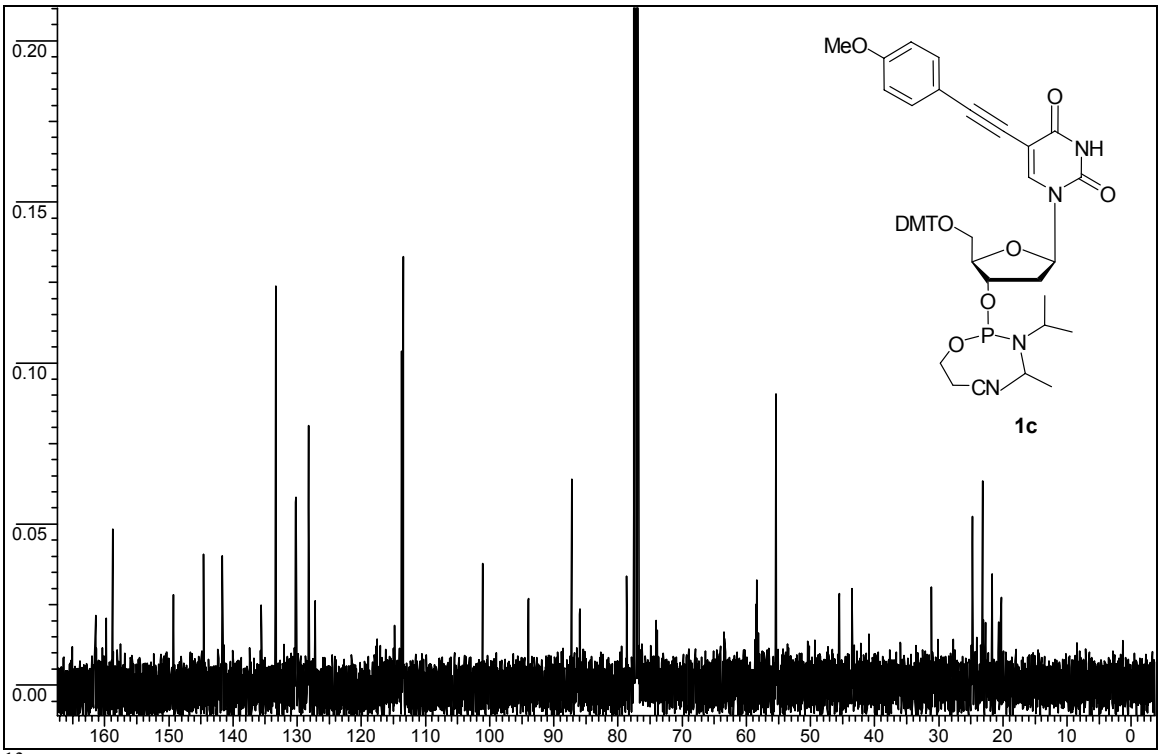
^{13}C NMR spectrum: **5-(phenylethynyl)-2′-deoxy-3′-(2-cyanoethyldiisopropylphosphoramididite)-5′-*O*-(4,4´-dimethoxytrityl)uridine**



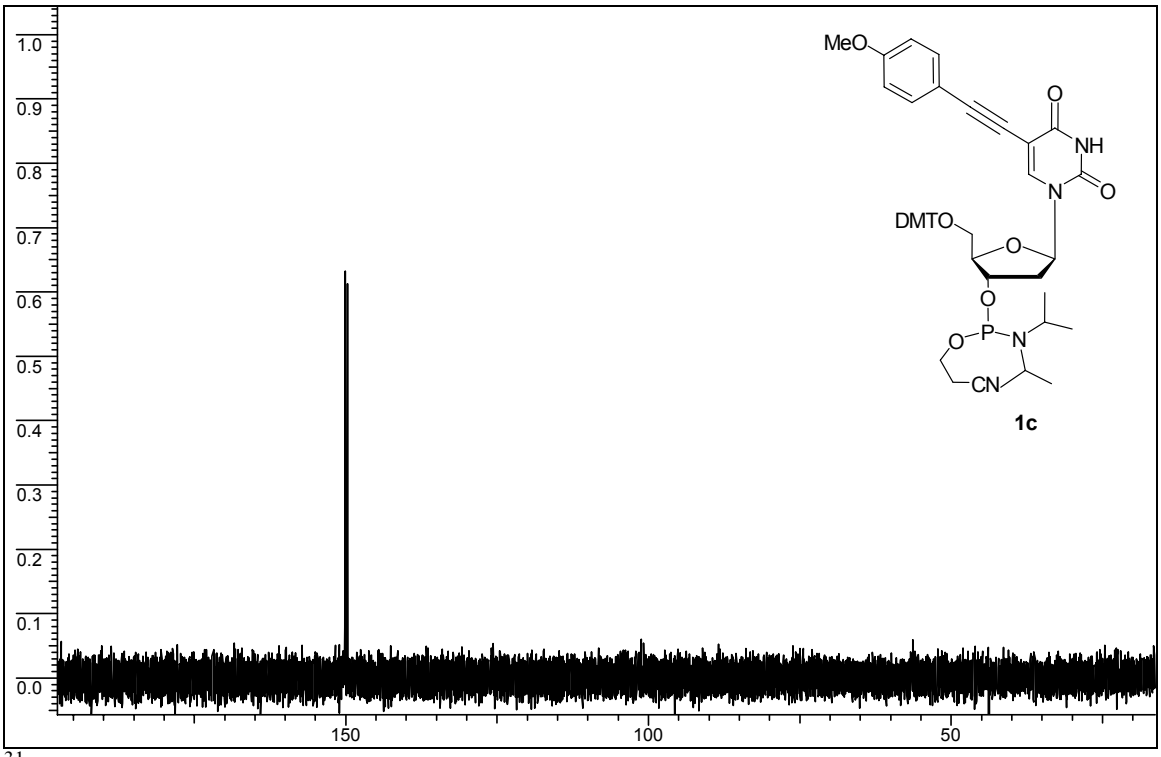
^{31}P NMR spectrum : **5-(phenylethynyl)-2′-deoxy-3′-(2-cyanoethyldiisopropyl-phosphoramididite)-5′-*O*-(4,4´-dimethoxytrityl)uridine**



^{1}H NMR spectrum: **5-(4-methoxyphenylethynyl)-2′-deoxy-3′-(2-cyanoethyldi-isopropylphosphoramididite)-5′-*O*-(4,4′-dimethoxytrityl)uridine**



^{13}C NMR spectrum: **5-(4-methoxyphenylethynyl)-2′-deoxy-3′-(2-cyanoethyldi-isopropylphosphoramididite)-5′-*O*-(4,4′-dimethoxytrityl)uridine**



^{31}P NMR spectrum: **5-(4-methoxyphenylethynyl)-2′-deoxy-3′-(2-cyanoethyldi-isopropylphosphoramididite)-5′-*O*-(4,4′-dimethoxytrityl)uridine**

Table 1 ESI-TOF Mass Spectral Data for Modified Oligodeoxynucleotides*

Oligomer	Sequence 5'→ 3'	Formula	Calculated Average Mass (Daltons)	Observed Average Mass (Daltons)
3	CGC-AAT-^{MME}**U**-TAA-CGC	$C_{129}H_{162}N_{48}O_{76}P_{12}$	3972.63	3972.57
4	CGC-AAT-^{Ph}**U**-TAA-CGC'	$C_{133}H_{162}N_{48}O_{75}P_{12}$	4004.67	4004.60
5	CGC-AAT-^{MeOPh}**U**-TAA-CGC	$C_{134}H_{164}N_{48}O_{76}P_{12}$	4034.70	4034.67
11	^{MME}**U**-CCA-GCG-CAA-C	$C_{108}H_{136}N_{42}O_{63}P_{10}$	3340.22	3340.17
12	^{Ph}**U**-CCA-GCG-CAA-C	$C_{112}H_{136}N_{42}O_{62}P_{10}$	3372.26	3372.27
13	^{MeOPh}**U**-CCA-GCG-CAA-C	$C_{113}H_{138}N_{42}O_{63}P_{10}$	3402.29	3402.32
15	GCC-TAA-CTT-CCG-GAG-ATG-^{MME}**U**	$C_{188}H_{236}N_{70}O_{114}P_{18}$	5857.81	5857.51
16	GCC-TAA-CTT-CCG-GAG-ATG-^{Ph}**U**	$C_{192}H_{236}N_{70}O_{113}P_{18}$	5889.86	5890.52
17	GCC-TAA-CTT-CCG-GAG-ATG-^{MeOPh}**U**	$C_{193}H_{238}N_{70}O_{114}P_{18}$	5919.88	5920.71

* Modified nucleoside in bold print. ^{MMe}U = (2'-deoxy-β-D-*ribo*pentofuranosyl)-5-methoxymethyl-ethynyluridine. ^{Ph}U = (2'-deoxy-β-D-*ribo*pentofuranosyl)-5-phenylethynyluridine. ^{MeOPh}U = (2'-deoxy-β-D-*ribo*pentofuranosyl)-5-(*para*-methoxy)phenylethynyluridine

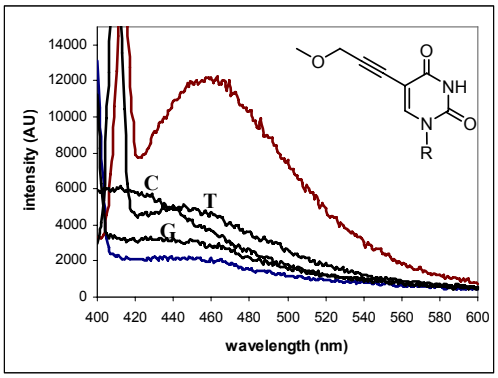
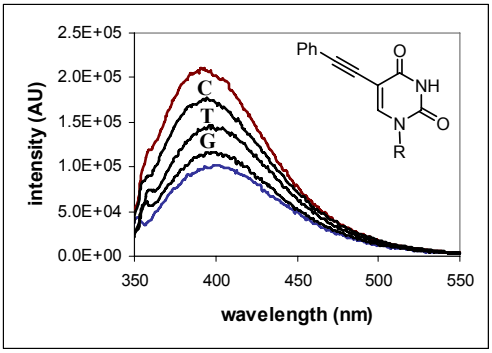


Fig 1 Room temperature steady state fluorescence emission of oligomer **3** alone (blue trace) or in the presence of complementary oligonucleotide **6** (magenta), or mismatched sequences **7** (G), **8** (C), **9** (T) (black traces). The spike in the spectrum between 400-415 nm is the Raman signal due to water.

A



B

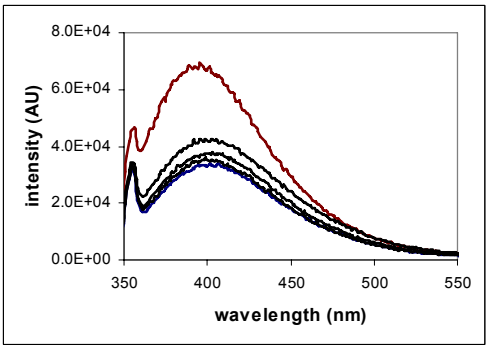


Fig 2 **A.** Room temperature steady state fluorescence emission of oligomer **4** alone (blue trace) or in the presence of complementary oligonucleotide **6** (magenta), or mismatched sequences **7** (G), **8** (C), **9** (T), black traces. **B.** Fluorescence emission of oligomer **4** alone (blue trace) or in the presence of complementary oligonucleotide **6** (magenta), or mismatched sequences **7** (G), **8** (C), **9** (T), black traces, at 43 °C. The weak, relatively sharp band at approximately 355 nm is the due to solvent (Raman band of water).

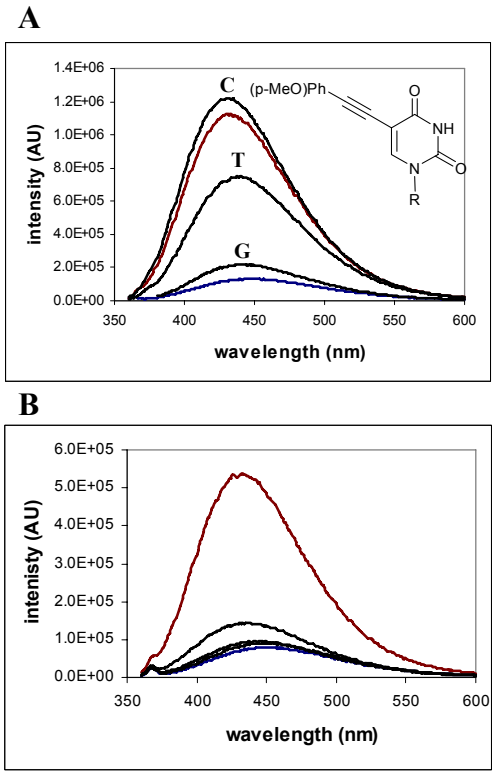


Fig 3 **A.** Room temperature fluorescence emission of oligomer **5** alone (blue trace) or in the presence of complementary oligonucleotide **6** (magenta), or mismatched sequences **7** (G), **8** (C), **9** (T). **B.** Fluorescence emission of oligomer **5** alone (blue trace) or in the presence of complementary oligonucleotide **6** (magenta), or mismatched sequences **7** (G), **8** (C), **9** (T) (black traces) at 43 °C.

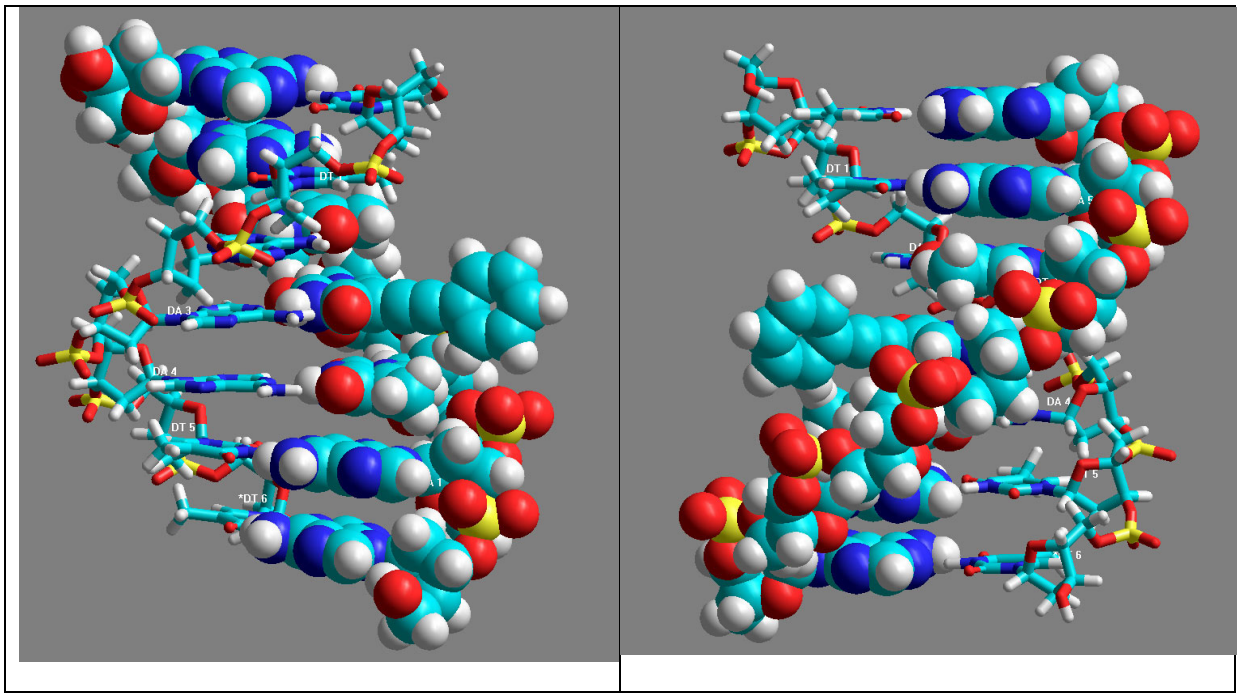


Fig. 4. Two views of the core of sequence **4**, which show the 5-phenylethynyl substituent protruding into the major groove, asymmetrically disposed toward the modified strand (rendered as space filling model).

Molecular modeling for the purpose of visualization was done using HyperChem™ ver. 7.5 utilizing the nucleic acids build function. Seven central nucleotides of sequence **4** were constructed in standard B-form and then the 5-phenylethynyl substituent was built. The substituent converged during geometry optimization (substituent only, MM+ force field) to the conformation shown from several different starting structures. The structures illustrate that a substantial portion of the fluorophore (the nucleobase)[2] is contained in the hydrophobic base-stack while the phenyl group experiences an environment that prevents free rotation.

References

1. J.D. Puglisi, I. Tinoco, Jr., Methods Enzymol., RNA Process. Pt. A, 1989, 180, 304.
2. R. H. E. Hudson, J. M. Moszynski, Synlett, 2006, 18, 2997.