Electronic Supplementary Material (ESI) for RSC Advances

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Supporting Information

S1. Materials and methods: All the CD experiments were performed in Milli-Q water by Jasco J-815 instrument, HPLC studies were carried out by Shimadzu CT0-20AC and UV experiments were performed by Varian Cary 300 BIO instrument. CD experiments were performed with 1 mm or 10 mm path length quartz cuvettes. All the CD data were collected at a scans rate 100 nm/min at 1 nm data intervals and are presented as an average of three successive scans. CD spectra were baseline subtracted and smoothed via a five point adjacent averaging algorithm. Hydrochloric acid or phosphoric was used to decrease the pH and sodium hydroxide solution was used for increasing the pH of the unbuffered solution. Apparent pka of A-motif was determined by citrate buffer. For thermodynamic studies, acetate buffer was used. CD and UV-melting studies were carried out by increasing temperature at a rate of 1 ^{o}C per minute. Melting temperature (T_m) was determined by first derivatives of melting curves using Origin software. Experiments were repeated three times and average values were taken for calculating reported parameters. For pH-induced conformational changes, first pH titration was standardized on pH meter outside the CD instrument and pH values were taken to draw the curve against CD ellipticity at wavelength 217 nm or 265 nm.

S2. Synthesis

(*R*)-2-Bromosuccinic acid (2): To a stirred solution of sodium bromide (60 g, 588 mmol) in 6 *N* sulfuric acid (25 ml), 20 g of (*R*)-Aspartic acid (150 mmol) was added and cooled at 0 ^{o}C. Sodium nitrite (12.4 g, 180 mmol) was added slowly for time period of 30 min under nitrogen atmosphere and temperature was maintained 0^{o}C. Reaction mixture was stirred for next 3 hr. Urea (4.6 g) was added and stirred for 20 min. Compound was extracted with ether (3x100ml). Organic layer was dried over sodium sulphate and concentrated gave pale yellow solid gave 60% yield. $[\alpha]_D^{25}$ = +5.9 (*c* = 1 in methanol).

^{1}H NMR (500MHz, DMSO) δ 4.51 (dd, *J* = 6.3, 8.8 Hz, 1H), 3.07 (dd, *J* = 8.5, 17.3 Hz, 1H), 2.91 - 2.84 (m, 1H). ^{13}C NMR (125 MHz, DMSO) δ 171.46, 170.59, 41.01, 40.03.

(*R*)-2-Bromobutane-1,4-diol (3): To a stirred solution of (*R*)-2-Bromosuccinic acid **2** (16.0 g, 81.6 mmol) in dry THF, 2M solution of borane dimethyl sulfide complex in THF (2.7 equiv) was added drop by drop at 0 ^{o}C. Reaction was allowed to stir for 5 hr at room temperature. After completion, reaction was quenched with methanol drop by drop and solvent was removed by rotavapour. Three times co evaporation was carried out with methanol gave pale yellow oil as crude product. Purification was carried out by silica gel chromatography with (5 to 10% methanol/chloroform) to afford colorless oil (12.1 g, 87.7 %) yield. $[\alpha]_D^{25}$ = +2.9 (*c* = 1 in methanol).

^{1}H NMR (500MHz, $CDCl_3$) δ 5.88 (br s, 1H), 4.34 - 4.29 (m, 1H), 3.90 - 3.86 (m, 1H), 3.85 - 3.84 (m, 2H), 3.82 - 3.77 (m, 1H), 2.18 - 2.05 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 67.22, 60.17, 55.03, 37.93.

(*S*)-2-(oxiran-2-yl)ethanol (4): Dry dichloromethane was added to (*R*)-bromodiol **3** (7.3 g, 4.43 mmol) and stirred for 5 min. 1.8 equiv cesium carbonate(25.2 g, 77.3 mmol) was added under argon condition. Reaction was stirred for 72 hours at room temp. After completion, reaction mixture was filtered and washed with dichloromethane. Filtrate was dried over magnesium sulphate and organic solvent was removed by vacuum to gave slightly pale yellow oily liquid (*S*)-epoxide as (2.4 g, 64.9%) yield. Next step was carried out without further purification. $[\alpha]_D^{25}$ = -2.6 (*c* = 1 in methanol).

^{1}H NMR (500MHz, $CDCl_3$) δ 3.85 - 3.78 (m, 2H), 3.12 - 3.08 (m, 1H), 2.81 - 2.80 (m, 1H), 2.59 (d, *J* = 1.9 Hz, 1H), 2.02 - 1.96 (m, 1H), 1.77-1.70 (m, 1H). ^{13}C NMR (125 MHz $CDCl_3$) δ 60.09, 50.64, 46.67, 34.75.

(*S*)-2-(2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)oxirane (5): (*S*)-Epoxide **4** (3.0 g, 34 mmol) was dissolved in dry dichloromethane followed by the addition of triethylamine (8.6 g, 85 mmol) and dimethoxy trityl chloride (13.8 g) at room temp under nitrogen condition. Reaction was allowed to stir for overnight. After completion of reaction product was filtered and filtrate was poured in saturated aqueous sodium bicarbonate solution and washed with dichloromethane. Organic layer was collected and washed with brine solution and dried over sodium sulphate and concentrated to afford crude yellow color paste mass. Compound was purified by silica gel chromatography (2% ethyl acetate/ hexane with 1.0% triethylamine) gave (7.5 g, 60% yield) as pasty mass. $[\alpha]_D^{25}$ = -0.5 (*c* = 1 in dichloroethane).

^{1}H NMR (500MHz ,$CDCl_3$) δ 7.51-7.49 (m, 2H), 7.40 - 7.37 (m, 4H), 7.35-7.32 (m, 2H), 7.28 - 7.20 (m, 1H), 6.90 - 6.87 (m, 4H), 3.82(s, 6H), 3.34-3.25 (m, 2H), 3.17 - 3.13 (m, 1H), 2.84-2.82 (m, 1H), 2.56 (dd, *J* = 2.5, 5.0 Hz, 1H), 1.89-1.85 (m, 2H); ^{13}C NMR (125 MHz $CDCl_3$) δ 158.34, 145.05, 136.25, 129.91, 128.05, 127.69, 126.60, 113.00, 85.96, 60.36, 55.15, 55.05, 50.26, 47.13, 33.32; HRMS calcd for $C_{25}H_{26}O_4Na$ $[M+Na]^+$ 413.1729, found $[M+Na]^+$ 413.1728.

(*S*)-1-(4-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxybutyl)-5-methylpyrimidine-2,4(1H,3H)-dione (6): To a stirred solution of thymine (500 mg, 3.96 mmol) in dry DMF, 0.3 equiv NaH was added and reaction mixture was stirred for one hour at room temperature. DMT epoxide (1.3 g, 3.3 mmol) dissolved in dry DMF was added and reaction mixture was stirred for 12 hours at 110 ^{o}C. Solvent was removed under vacuum and ethyl acetate was added and washed with saturated bicarbonate solution. Organic layer was washed with brine and dried over sodium sulphate. Organic layer was concentrated and loaded over silica gel column. Compound was eluted with 5 to 50% ethyl acetate/hexane with 1% triethylamine to afford (800 mg, 45%) as white foam product. $[\alpha]_D^{25}$ = +22.9 (*c* = 1 in dichloroethane).

^{1}H NMR (500MHz ,$CDCl_3$) δ 7.33 - 7.30 (m, 2H), 7.24 - 7.19 (m, 6H), 7.16 - 7.12 (m, 1H), 7.08 (s, 1H), 6.78 - 6.74 (m, 4H), 4.01 - 3.94 (m, 1H), 3.85 (dd, *J* = 2.5, 13.9 Hz, 1H), 3.72 (s, 6H), 3.40 (dd, *J* = 8.2, 13.9Hz, 1H), 3.37-3.34 (m, 1H), 3.25 - 3.19 (m, 1H), 1.83 (s, 3H), 1.70 - 1.65 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.11, 158.60, 151.20, 144.40, 142.12, 135.52, 129.90, 127.99, 127.93, 126.95, 113.27, 109.75, 87.11, 70.33, 62.14, 55.22, 53.67, 33.77, 12.26; HRMS calcd for $C_{30}H_{32}N_2O_6Na$ $[M+Na]^+$ 539.2158, found $[M+Na]^+$ 539.2156.

Thymine Phosphoramidite (7): Compound **6** (703 mg, 1.4 mmol) was dried under high vacuum and anhydrous dichloromethane was added followed by the addition of diisopropyl ammonium tetrazolide (120 mg, 0.5 mmol) was added. 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite (633 mg, 1.5 mmol) was added and reaction was allowed to stir

at room temperature. After 3 hours, the reaction was diluted with dichloromethane and poured in saturated aqueous sodium bicarbonate. Product was extracted in dichloromethane and dried over magnesium sulphate. The product was purified by slica gel column with dichloromethane/hexanes (1 to 80%) with 1%triethylamine to afford (766 mg, 73%) product as white foam solid.

^{31}P NMR (121 MHz, $CDCl_3$) δ 149.119, 148.406; HRMS calcd for $C_{39}H_{49}N_4O_7PNa$ $[M+Na]^+$ 739.3237, found $[M+Na]^+$ 739.3234.

(*S*)-1-(6-amino-9H-purin-9-yl)-4-(bis(4-methoxyphenyl)(phenyl)methoxy)butan-2-ol (8): Adenine (2.1 g, 15.5 mmol) was dissolved in dry DMF. 0.3 equiv NaH was added and reaction mixture was stirred for one hour at room temperature. DMT epoxide (6.0g, 15.4mmol) dissolved in dry DMF was added and reaction mixture was stirred for 12 hours at 110 ^oC. The solvent was removed by vacuum and ethyl acetate was added followed by washing with saturated bicarbonate solution. The organic layer was washed with brine and dried over sodium sulphate. The solvent was concentrated and compound was loaded over silica gel column. Compound was eluted with 20 to 80 % ethyl acetate/hexane with 1% triethylamine to afford (4.0 g, 45%) as product. $[\alpha]_D^{25}$ = +2.7 (*c* = 1 in dichloroethane).

^{1}H NMR (500MHz , $CDCl_3$) δ 8.32 (s, 1H), 7.83 (s, 1H), 7.42 - 7.39 (m, 2H), 7.33 - 7.28 (m, 6 H), 7.25 - 7.21 (m, 1H), 6.86 - 6.82 (m, 4H), 5.88 (br , 2H), 4.32 (dd, *J* = 2.5, 14.2 Hz, 1H), 4.26 - 4.19 (m, 1H), 4.16 - 4.10 (m, 1H), 3.80 (s, 6H), 3.44-3.40 (m, 1H), 3.32 - 3.28 (m, 1H), 1.84 - 1.69 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.69, 155.59, 152.86, 144.70, 141.87, 135.99, 135.88, 130.04, 128.15, 127.05, 119.48, 113.37, 86.98, 69.78, 61.60, 60.53, 55.36, 50.25, 34.19; HRMS calcd for $C_{30}H_{31}N_5O_4$ $[M]^+$ 526.2454 , found $[M]^+$ 526.2452.

(*S*)-N-(9-(4-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxybutyl)-9H-purin-6-yl)benzamide (9): Starting material **7** (500 mg, 0.95 mmol) was dried well and dissolved in anhydrous pyridine under nitrogen condition. TMS-Cl (412.8 mg, 3.8 mmol) was added at 0^oC and stirred for 30 min and next to room temperature for next 4 hours. Benzoyl chloride (200 mg, 1.42 mmol) was added drop by drop at 0 ^oC and reaction was stirred for 4 hr at room temperature. After completion of reaction, water was added to stop the reaction and aqueous ammonia was added at 0^oC and stirred for 1 hr. Compound was extracted with dichloromethane. Organic layer was dried over sodium sulphate and concentrated gave yellow oily paste. Resulting oily paste was dissolved in THF and 1.2 equiv tetrabutylammonium fluoride was added and stirred for 30 min at room temp. Dichloromethane was added to the reaction mixture and washed with water and organic layer was washed with brine and dried over sodium sulphate. Organic layer was concentrated and loaded over silica gel column. Column was done with (20 to 90%

ethyl acetate/ hexane) with 1% triethylamine to afford white color foam with (400 mg) as 60% yield. $[\alpha]_D^{25}$ = +12.2 (*c* = 1 in dichloroethane).

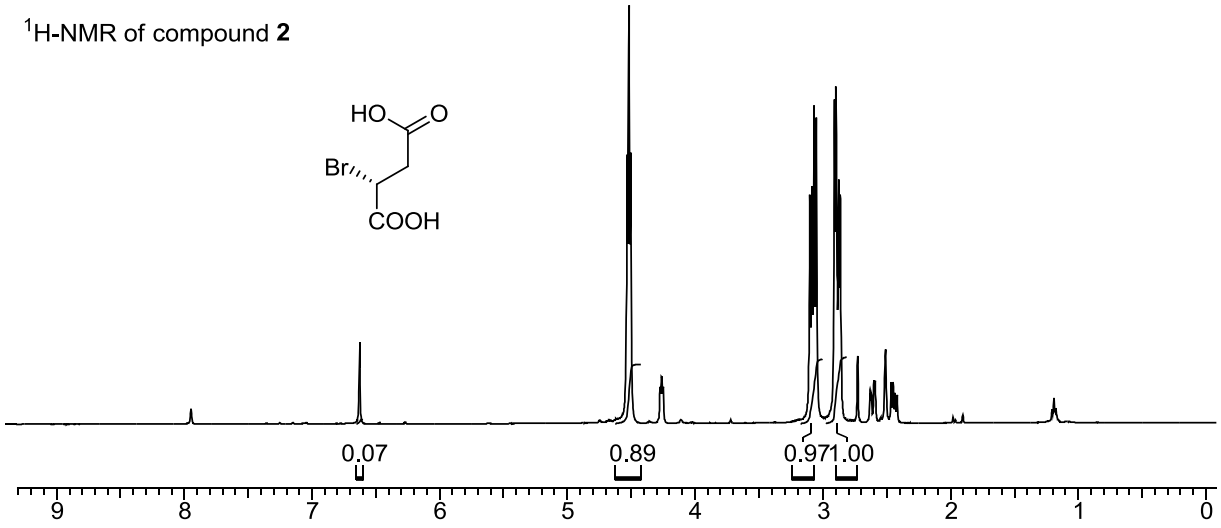
^{1}H NMR (500MHz , $CDCl_3$) δ 9.17 (br s, 1H), 8.65 (s, 1H), 7.99 (s, 1H), 7.94 (d, *J* = 7 Hz, 2H), 7.52 - 7.47 (m, 1H), 7.44 - 7.38 (m, 2H), 7.33 - 7.28 (m, 2H), 7.23 - 7.16 (m, 6H), 7.15 - 7.09 (m, 1H), 6.76 - 6.70 (m, 4H), 4.29 (dd, *J* = 2.5, 13.9Hz, 1H), 4.18 - 4.03 (m, 3H), 3.69 (s, 6H), 3.36 - 3.30 (m, 1H), 3.25-3.21 (m, 1H), 1.76 - 1.67 (m, 1H), 1.66 - 1.56 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.94, 158.79, 152.59, 152.39, 149.56, 144.72, 144.56, 135.98, 135.85, 133.96, 132.90, 130.11, 129.01, 128.18, 128.12, 127.14, 122.76, 113.47, 87.16, 69.85, 61.76, 55.43, 50.00, 34.20; HRMS calcd for $C_{37}H_{36}N_5O_6$ $[M+H]^+$ 630.2716, found $[M+H]^+$ 630.2725.

Adenine Phosphoramidite (10): Compound **9** (629 mg, 1 mmol) was dried under high vacuum and anhydrous dichloromethane was added followed by the addition of diisopropyl ammonium tetrazolide (85 mg, 0.5 mmol) under nitrogen condition. After 1 min 2-cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite (376mg, 1.25mmol) was added and reaction was allowed to stir at room temperature. After 3 hours reaction was diluted with dichloromethane and poured in saturated aqueous sodium bicarbonate. Product was extracted in dichloromethane and dried over magnesium sulphate. Product was purified by slica gel column by dichloromethane/hexanes (20 to 30%) with 1% triethylamine to afford product as white foam (560 mg, 67%).

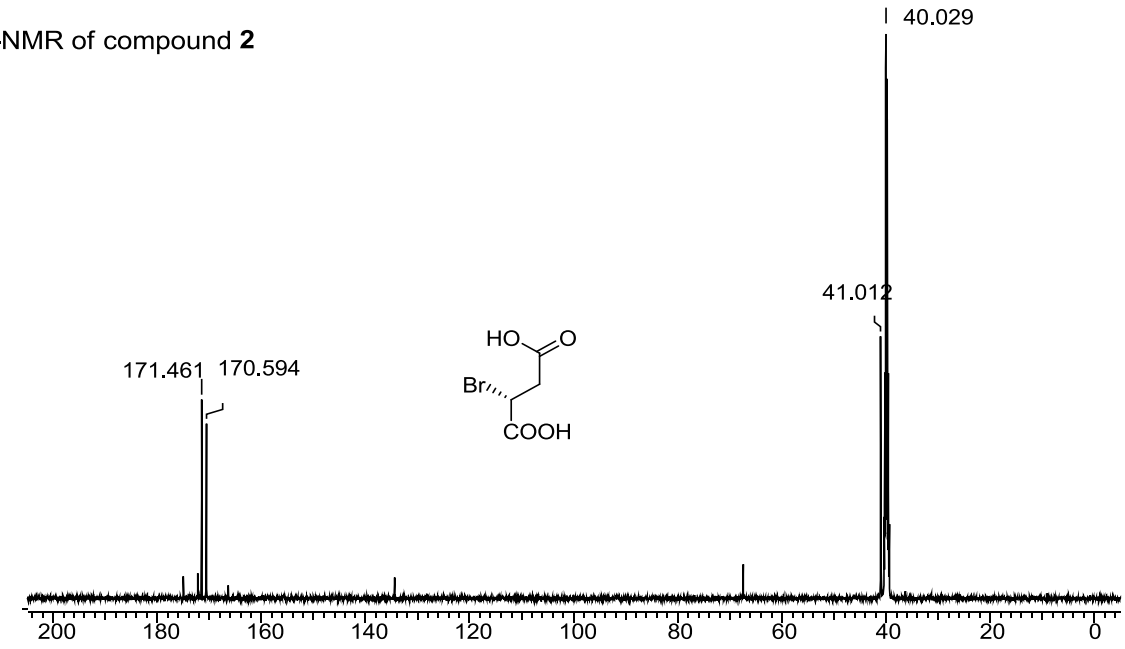
^{31}P NMR (121 MHz, $CDCl_3$) δ 149.119, 148.406; HRMS calcd for $C_{46}H_{52}N_7O_6P$ $[M+H]^+$ 830.3795, found $[M+H]^+$ 830.3796.

S3. NMR Spectra

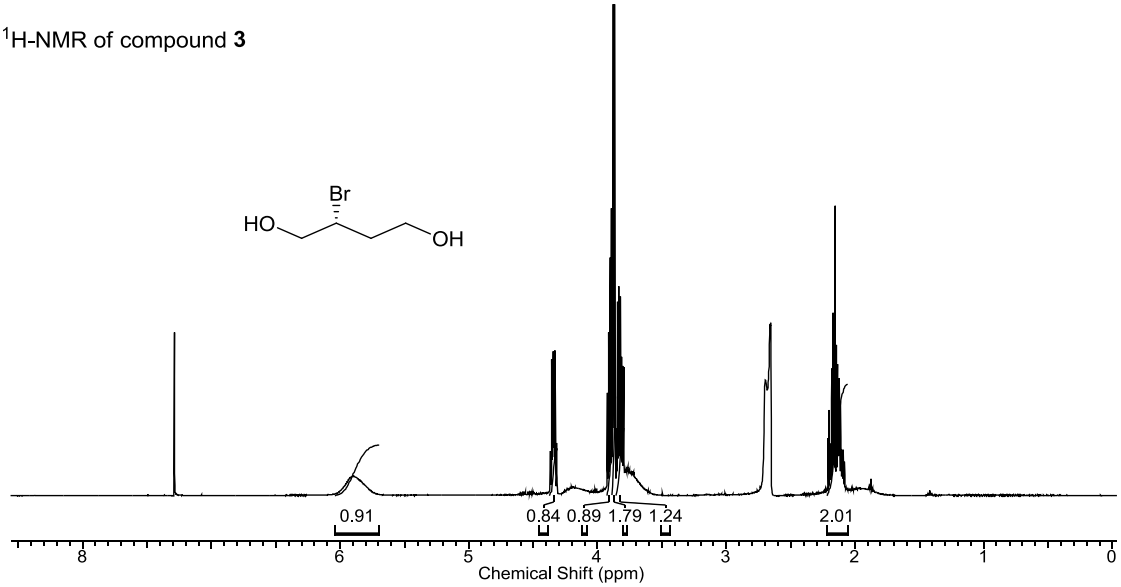
^{1}H-NMR of compound **2**



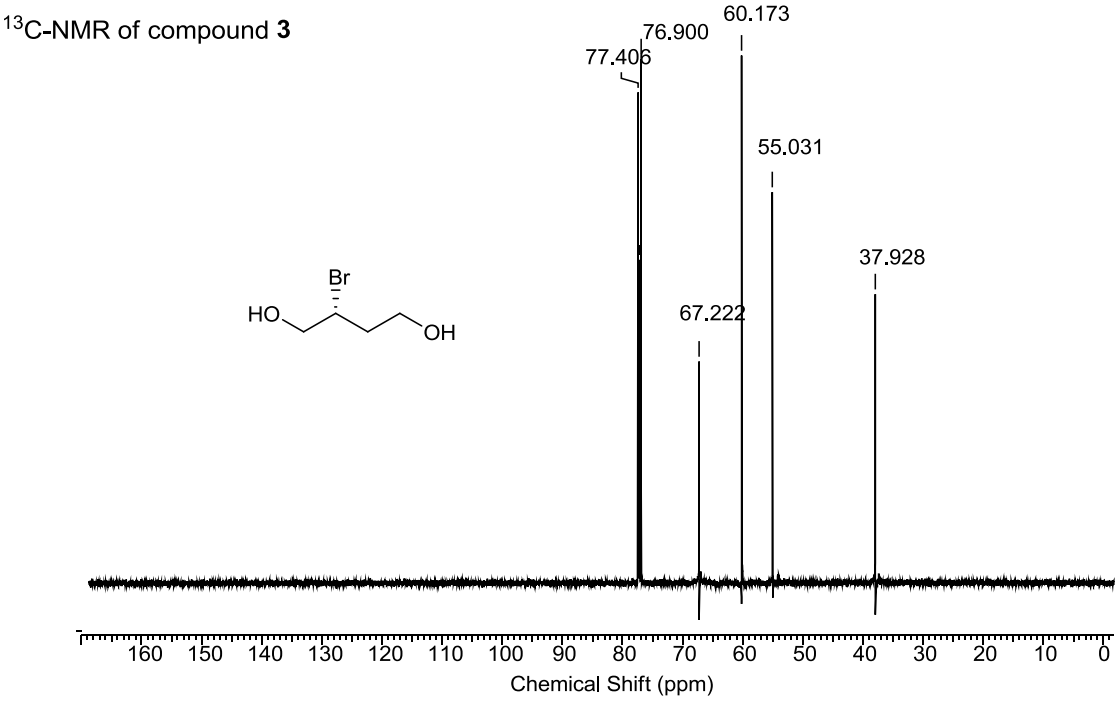
^{13}C-NMR of compound **2**



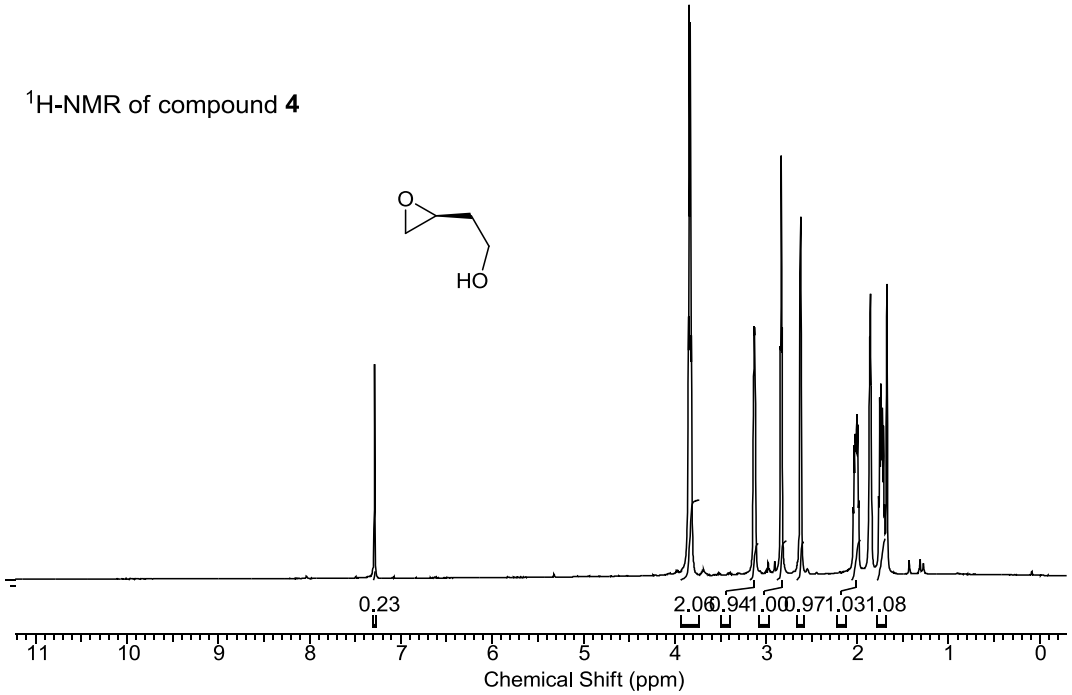
^{1}H-NMR of compound **3**



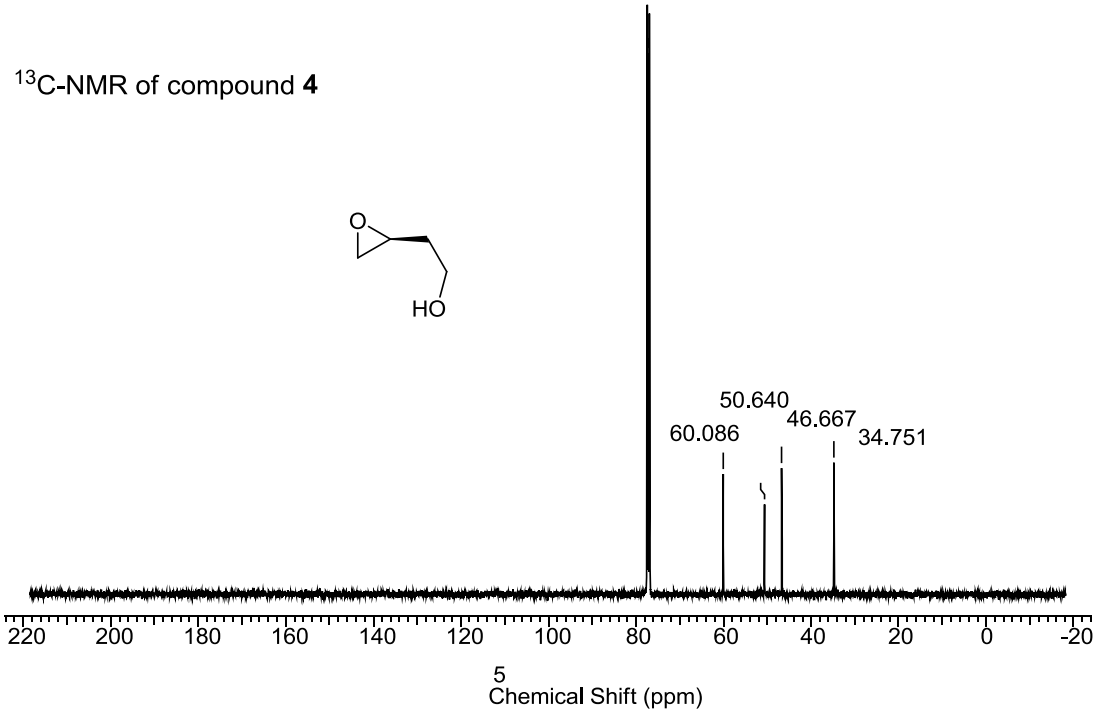
^{13}C-NMR of compound **3**



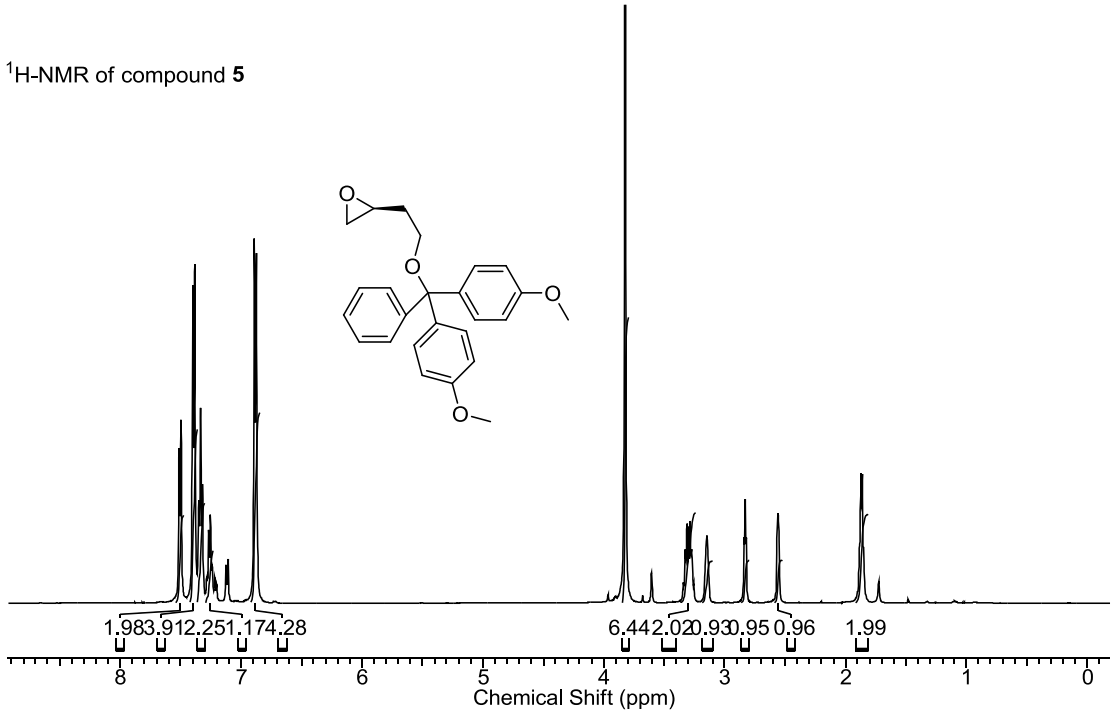
^{1}H-NMR of compound **4**



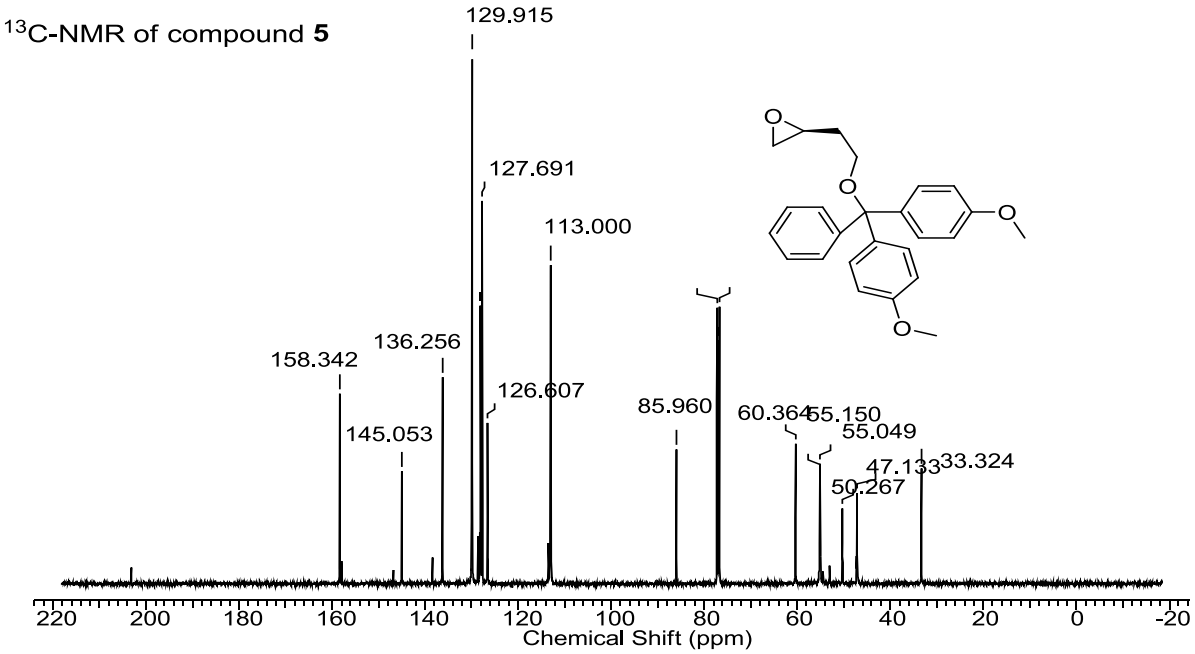
^{13}C-NMR of compound **4**



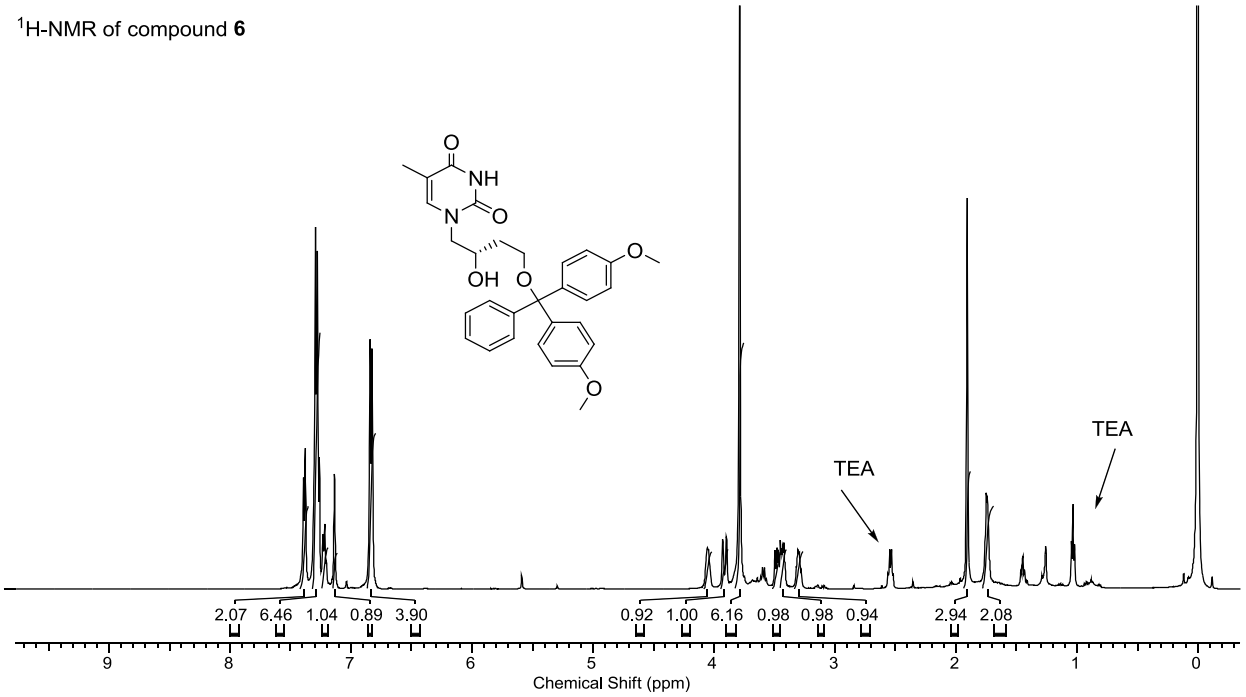
^{1}H-NMR of compound **5**



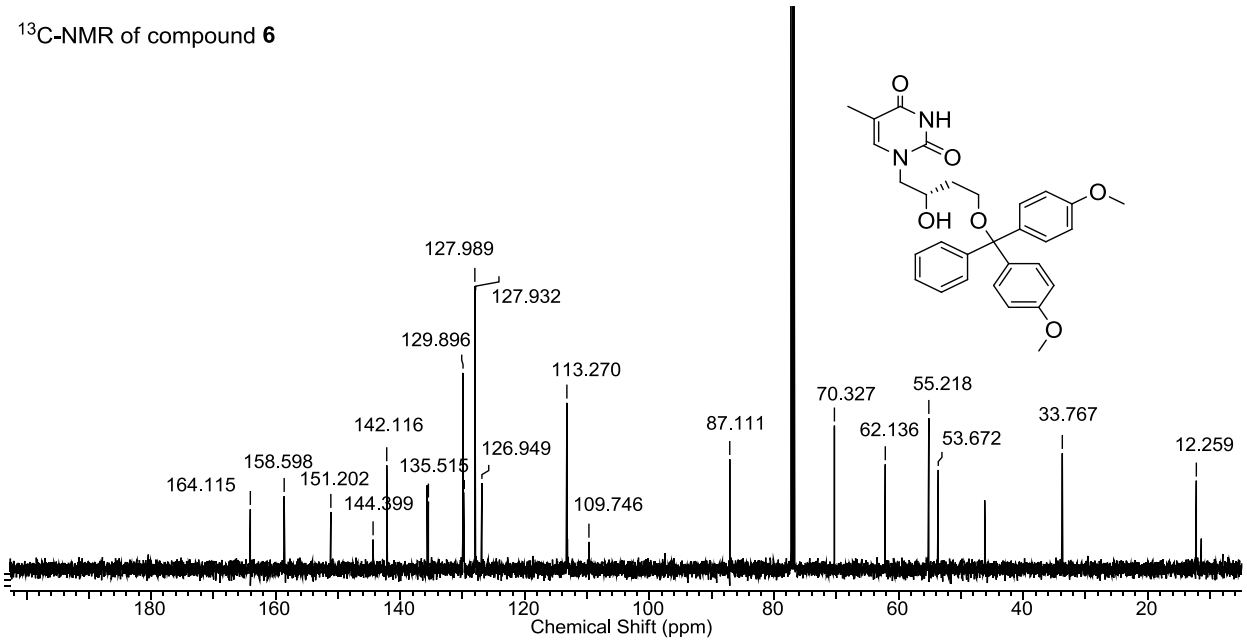
^{13}C-NMR of compound **5**



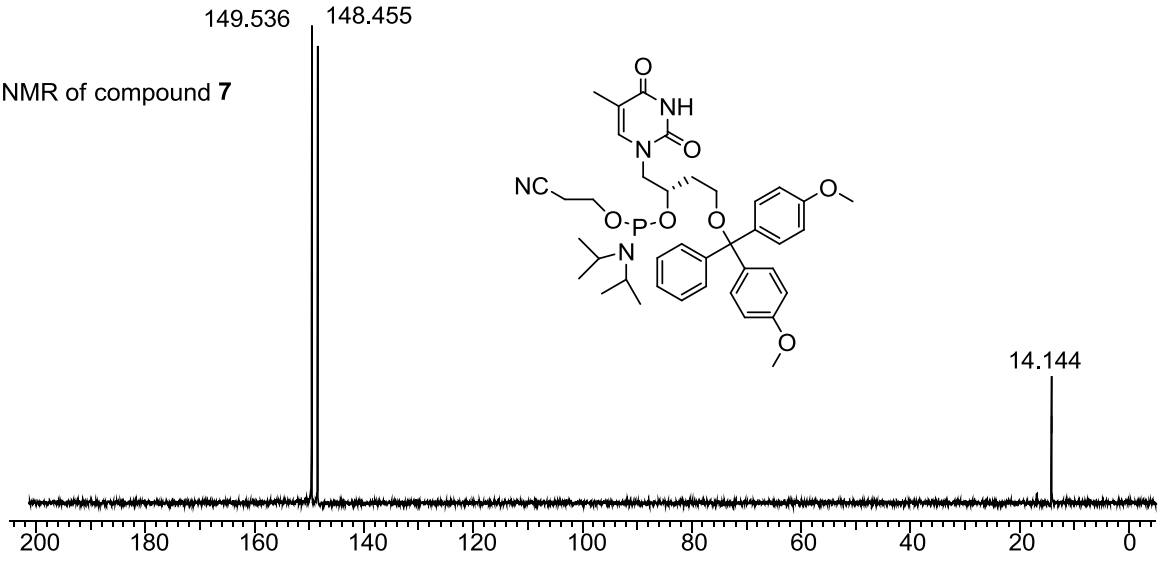
^{1}H-NMR of compound **6**



^{13}C-NMR of compound **6**



^{31}P-NMR of compound **7**



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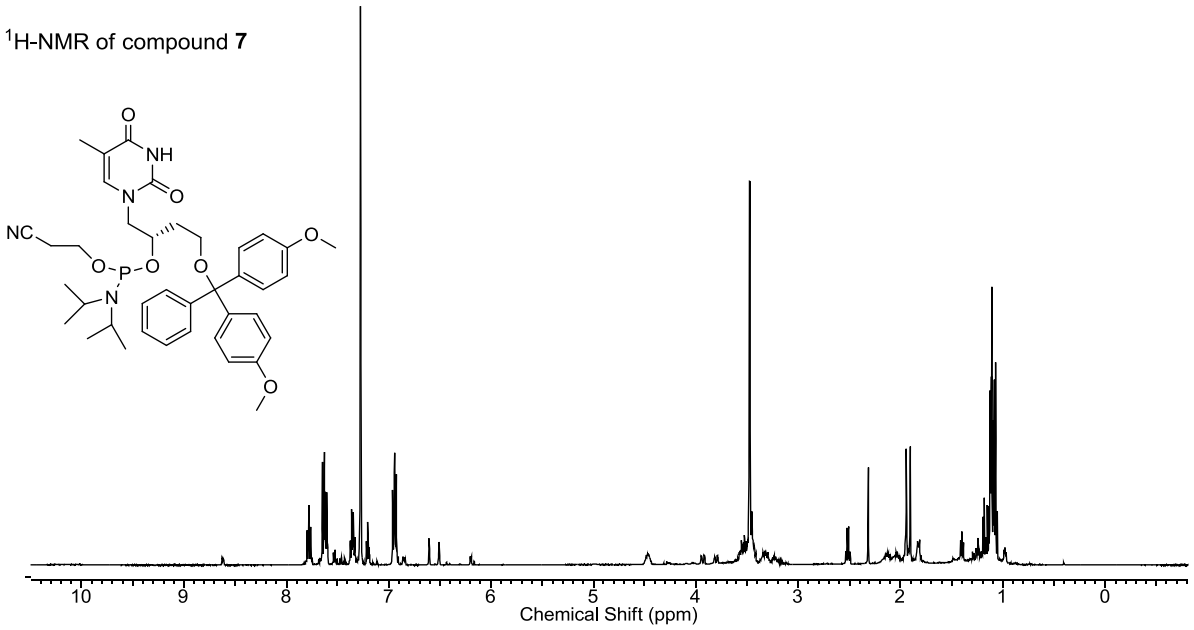
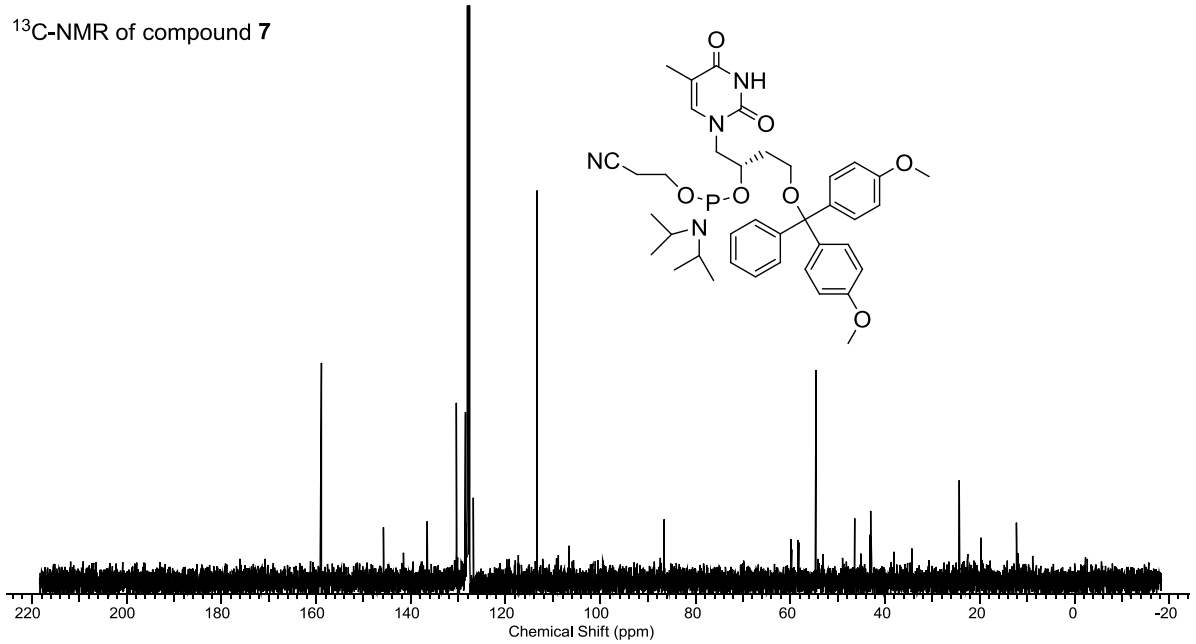
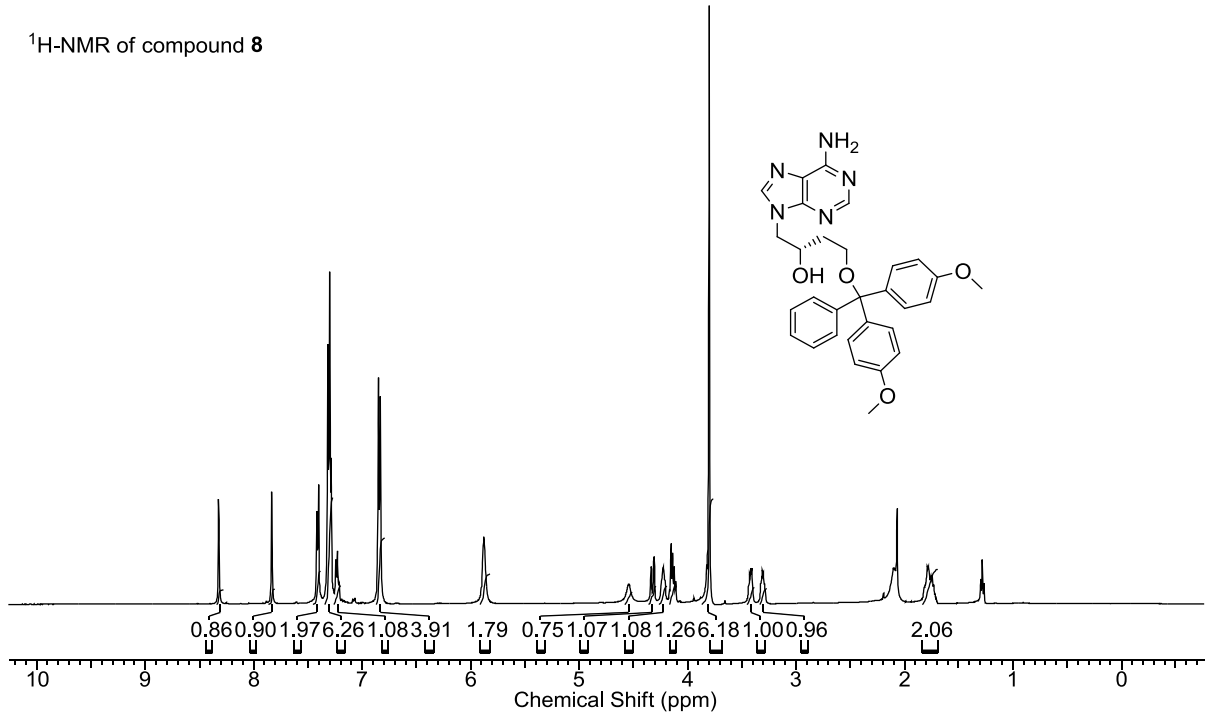


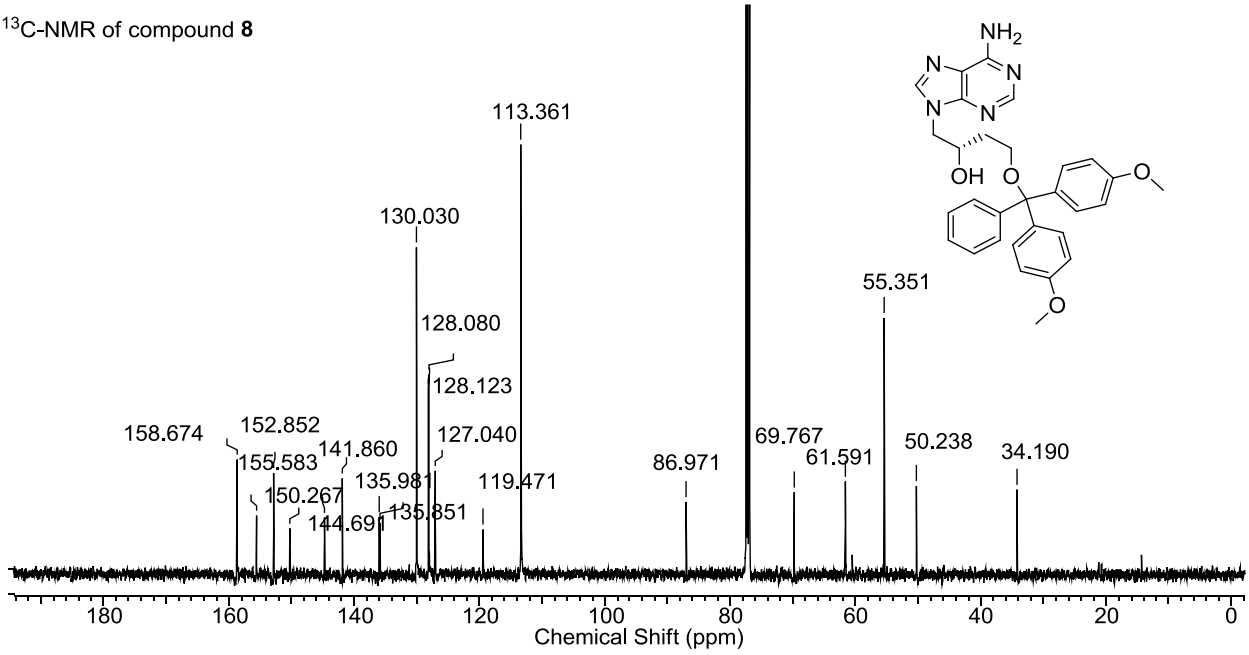
^{13}C-NMR of compound **7**



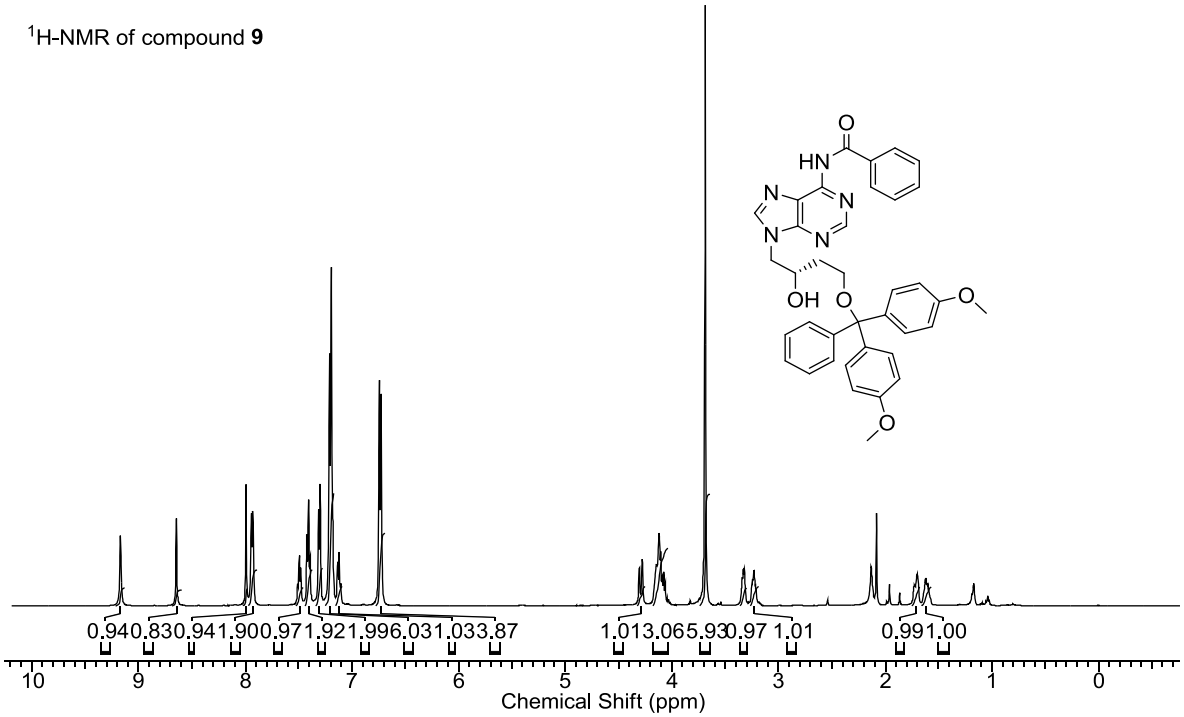
^{1}H-NMR of compound **8**



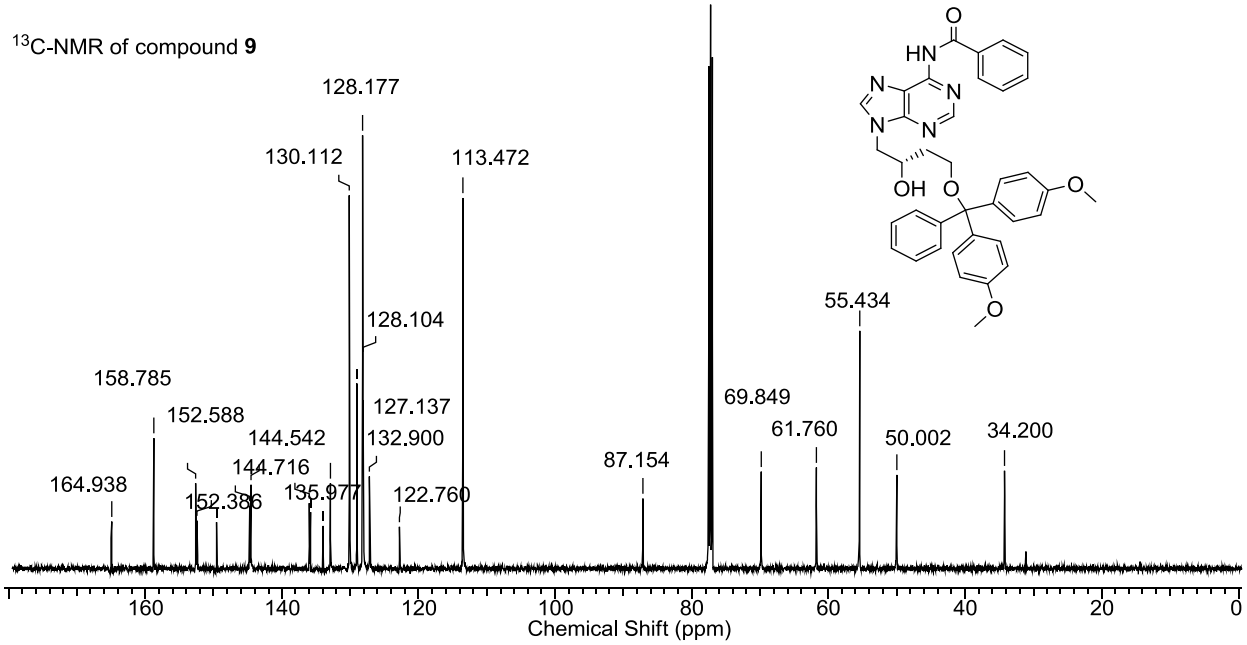
^{13}C-NMR of compound **8**



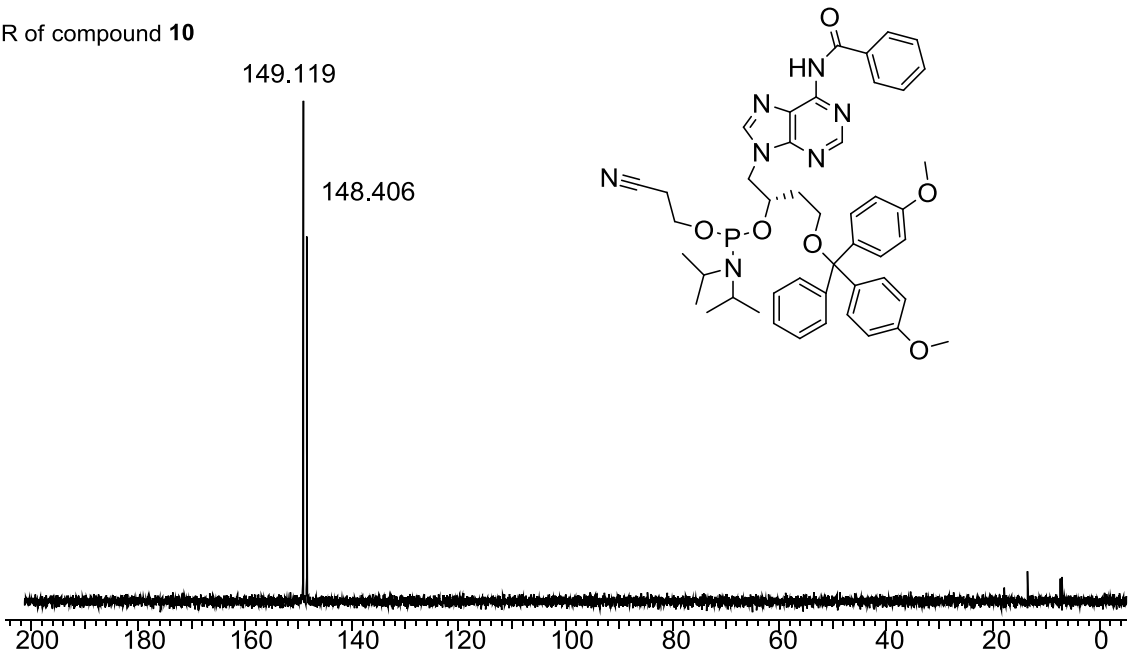
^{1}H-NMR of compound **9**



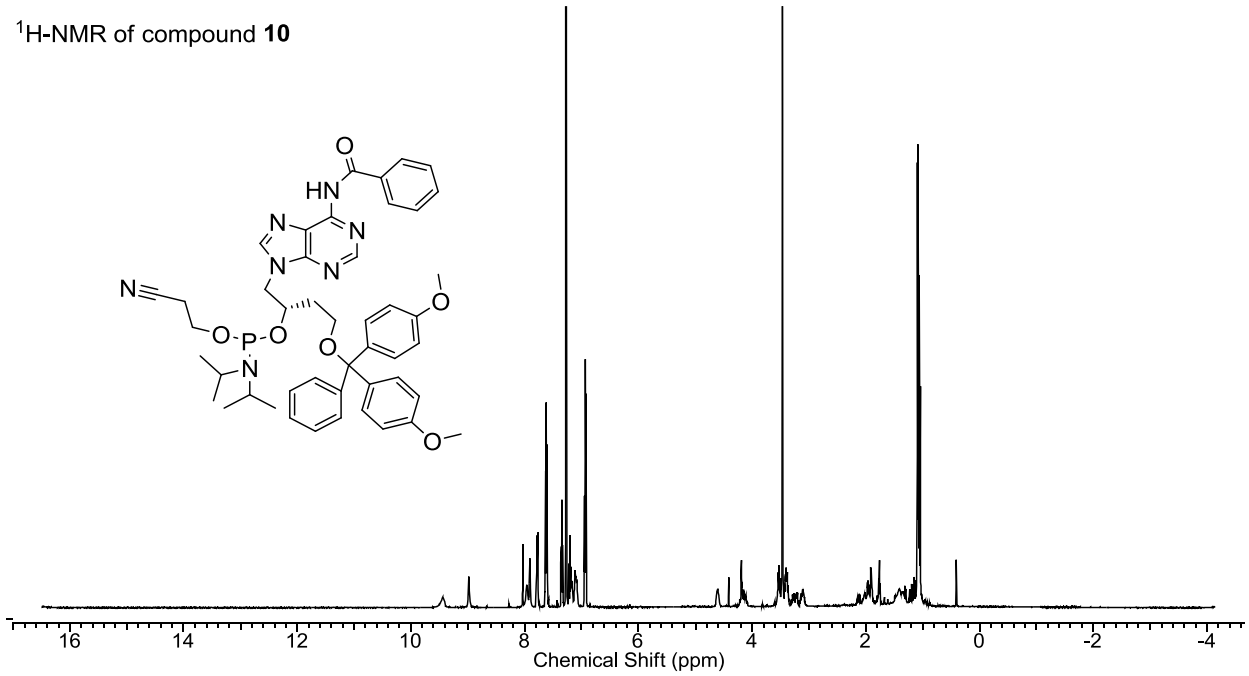
^{13}C-NMR of compound **9**



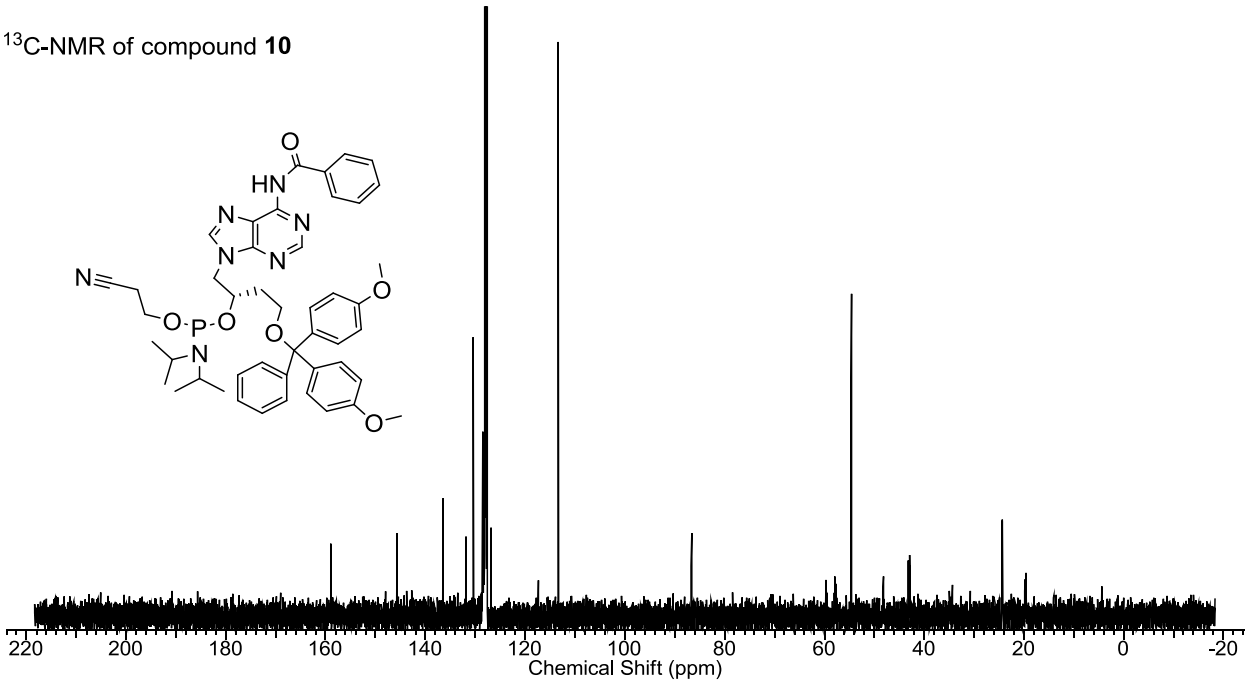
^{31}P-NMR of compound **10**



^{1}H-NMR of compound **10**



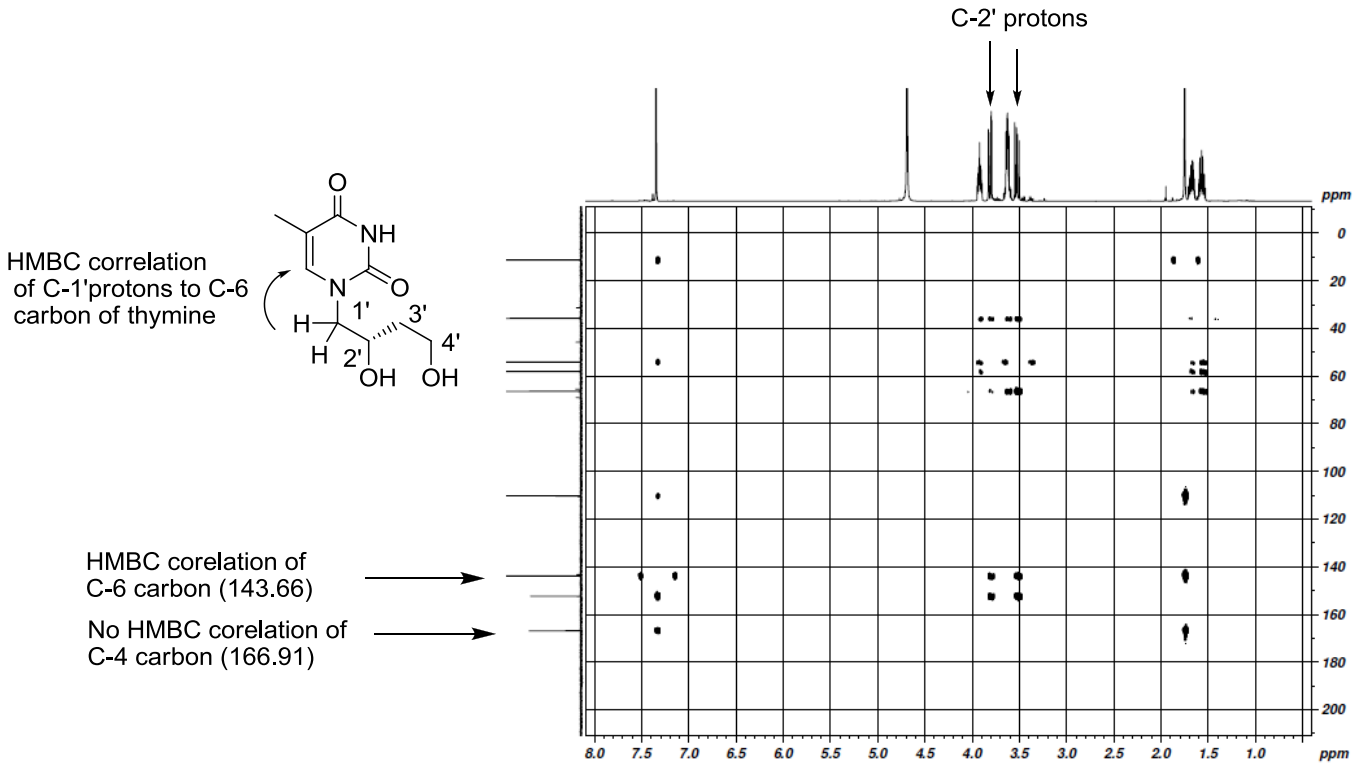
^{13}C-NMR of compound **10**



S4. Elucidation of regioselectivity of N-alkylation of nucleobases

Acyclic Thymine Nucleoside

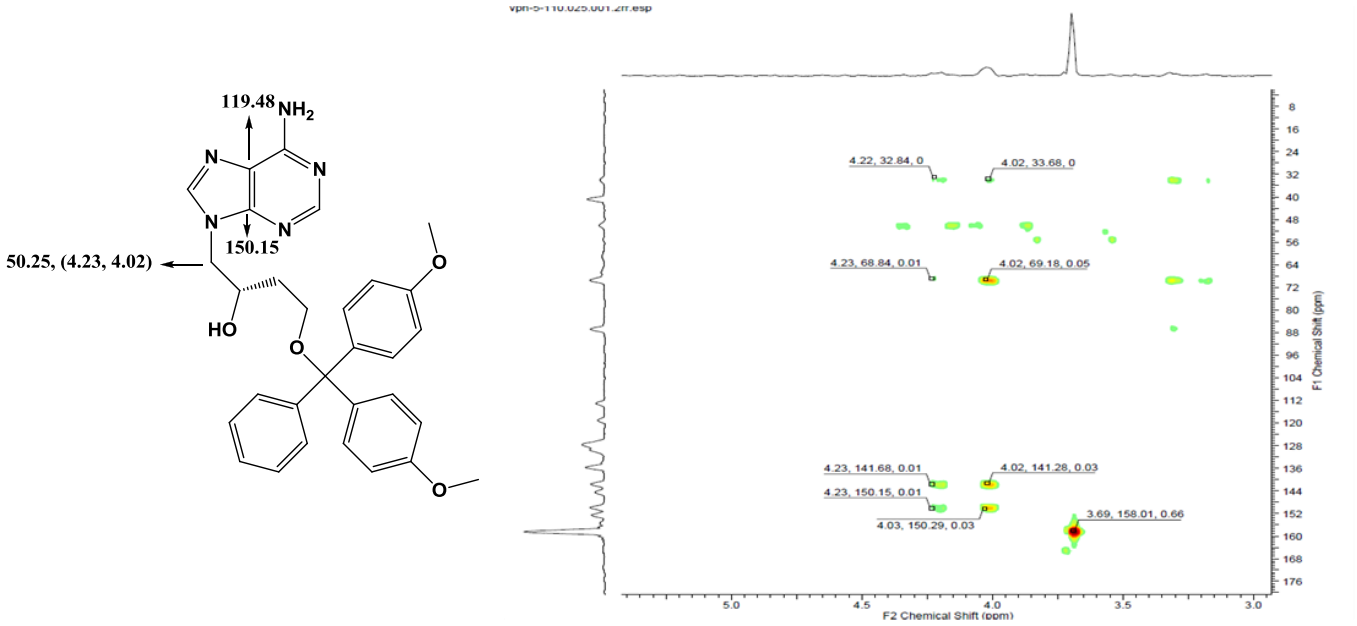
Regioselectivity of C-N bond can be elucidated by the HMBC. Three bonds coupling as shown in figure S10a clearly indicate that diol is attached at N-1 position of thymine ring. On the other hand no coupling was observed of C-1' protons with C-3 of the ring, which rule out the N-3 alkylation of thymine.



S4a. HMBC

Acyclic DMT-adenine Nucleoside

C-9 alkylation of adenine was also confirmed by HMBC. Figure S10b clearly indicate that C-1' protons show coupling with C-4 carbon of the ring. No coupling was observed with C-5 of the adenine. This result clearly indicates that regioselectivity of the N-9 alkylation of adenine, which rule out the N-7 alkylation of adenine.



S4b. HMBC

S5. MALDI-TOF-MS

The MALDI matrix was 3-hydroxypicolinic acid (HPA), 1:1 acetonitrile/aq. ammonium citrate solution. On a spot of matrix, 1 µL of oligonucleotide sample was placed on MALDI plate and dried at room temperature. MALDI-TOF-MS was analyzed on positive mode. For analysis of A-motif, ON-1 and ON-3 were dissolved in 10 mM citrate buffer at pH 3.

Oligonucleotides	Sequences	Calc mass	Observed mass
ON-1	4'-a a a a a a a a a a a a a a A- 3'	4244.33	4243.692
ON-2	4'-t t t t t t t t t t t t t t T-	4108.43	4112.345
ON-3	4'-a a a a a a a a t t t t t t t T- 3'	4457.07	4460.590
ON-4	4'-a t a t a t a t a t a t a t a T-3'	4457.07	4455.859
ON-6	5'-AAAAAAAaAAAAAAA- 3'	4608.20	4605.818
ON-7	5'-TTTTTTTtTTTTTTT-3'	4472.30	4476.363

ON-5 and ON-8 (main text) were purchased from Sigma Aldrich.

S6. pH-induced structural transition of ON-3

At 20 ^{o}C pH based titration was carried out for ON-3 and pH was increased by addition of 2 µl of 0.1 M sodium hydroxide solution. Figure S6 demonstrate that changes in CD are very sensitive by slight increase in pH.

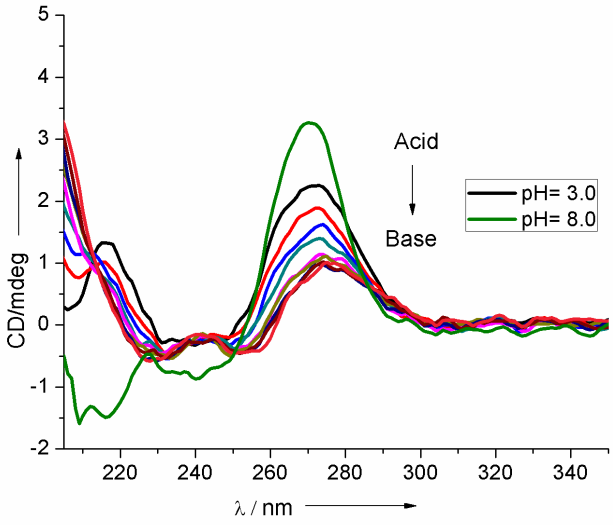


Figure S6. pH titration of ON-3 (4µM) at 20 ^{o}C.

S7. Reversibility of A-switch (ON-1):

In order to demonstrate the reversibility of BuNA-based A-switch, the pH of 1.5 µM solution of ON-1 in unbuffered solution at pH 2 was increased with gradual addition of 2 µl of 0.1 N sodium hydroxide solution till pH 9.0 and structural transition was visualized by circular dichroism (Figure S7). To demonstrate its reversibility, pH was decreased with addition of 1 N hydrochloric acid, which was found to be highly reversible transition. This indicates the highly reversibility of A-switch.

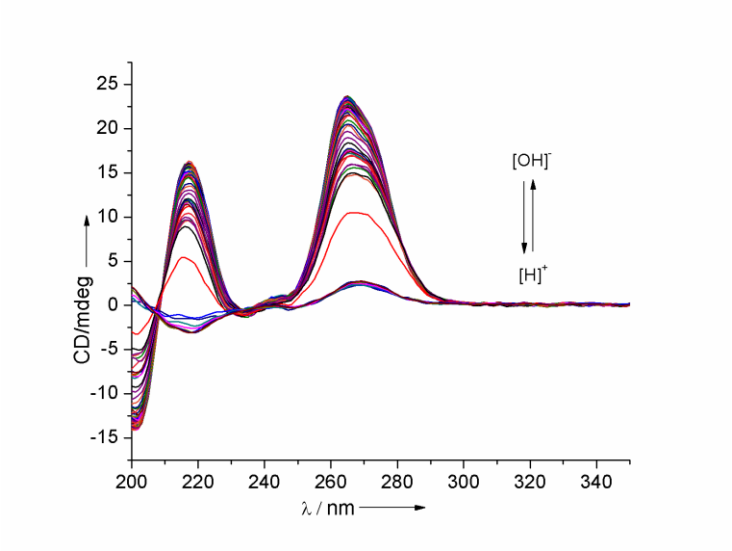


Figure S7. pH-induced reversibility of A-switch.

S8. UV-melting

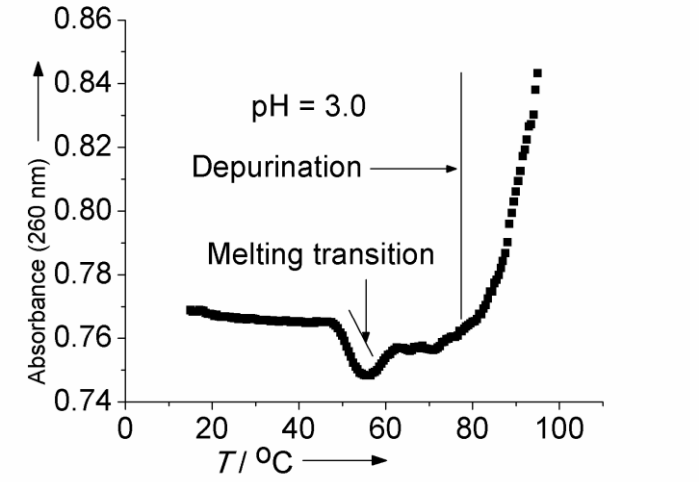


Figure S8. UV-melting at pH 3.0 with 4 µM of poly d$(A)_{15}$ (ON-5) DNA. T_m (51 °C).

S9. MALDI-TOF-MS of ON-1 and ON-3 at neutral and acidic pH.

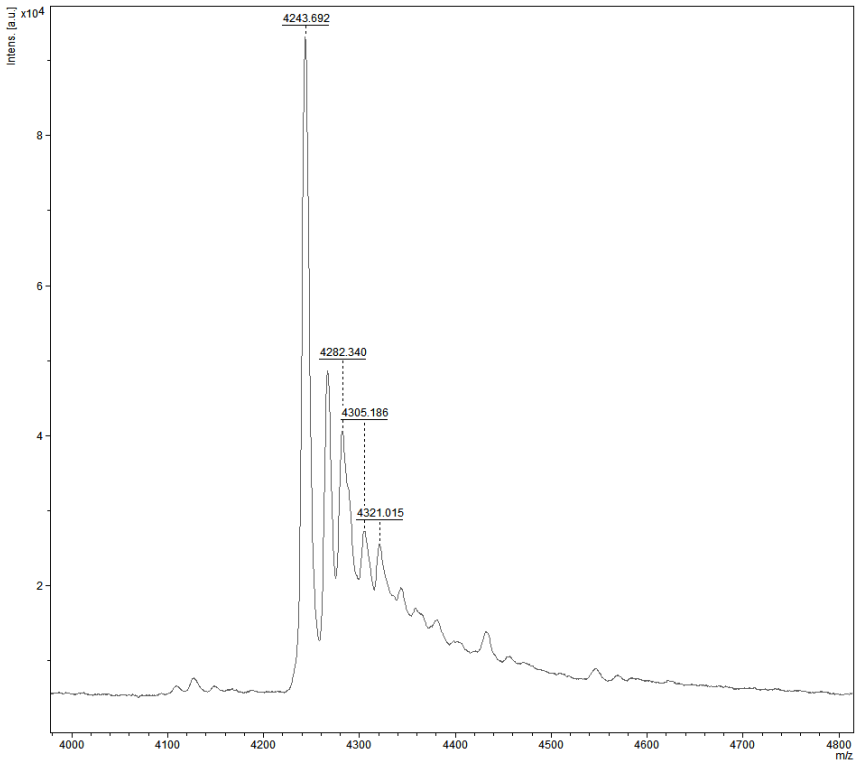


Figure S9a. MALDI-TOF-MS of ON-1 at neutral pH

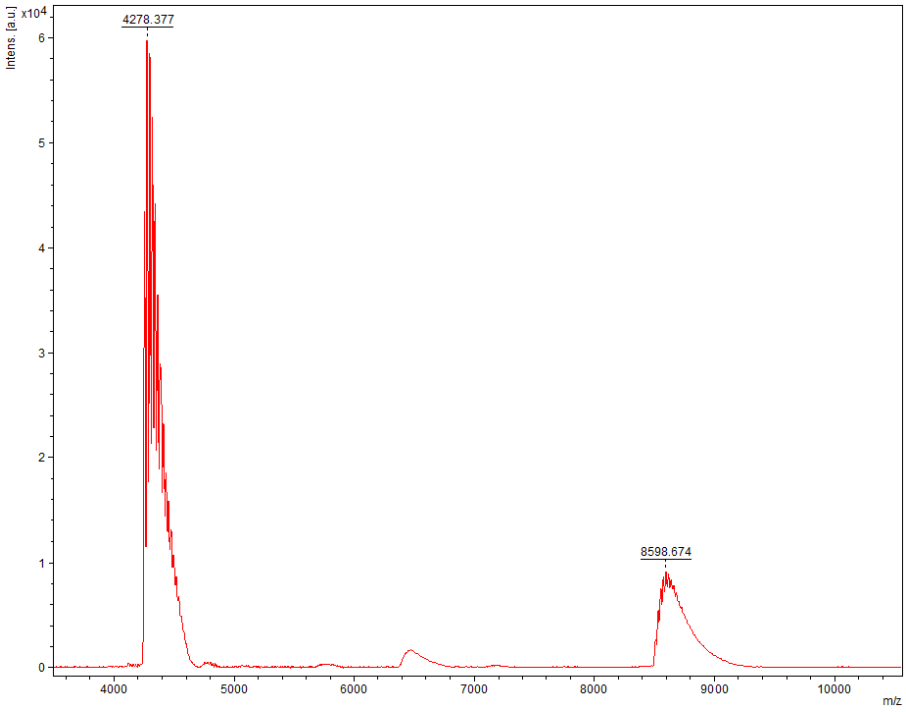


Figure S9b. MALDI-TOF-MS of ON-1 at pH 3

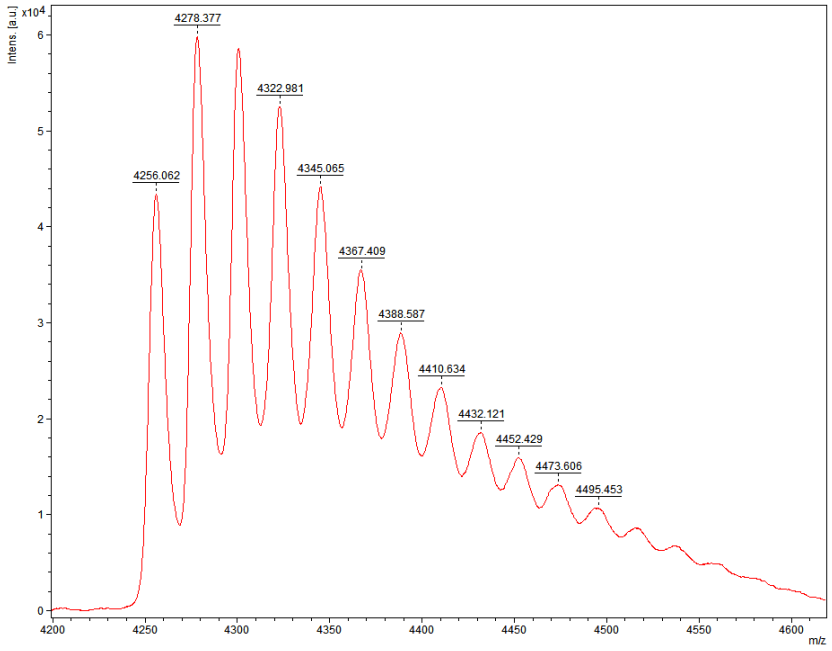


Figure S9c. MALDI-TOF-MS of ON-1 at pH 3

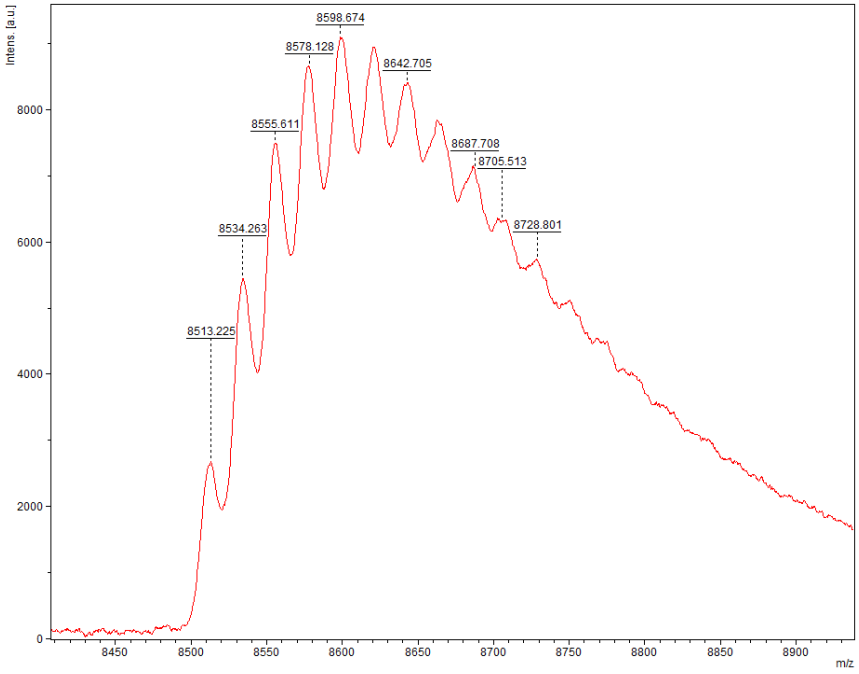


Figure S9d. MALDI-TOF-MS of ON-1 at pH 3

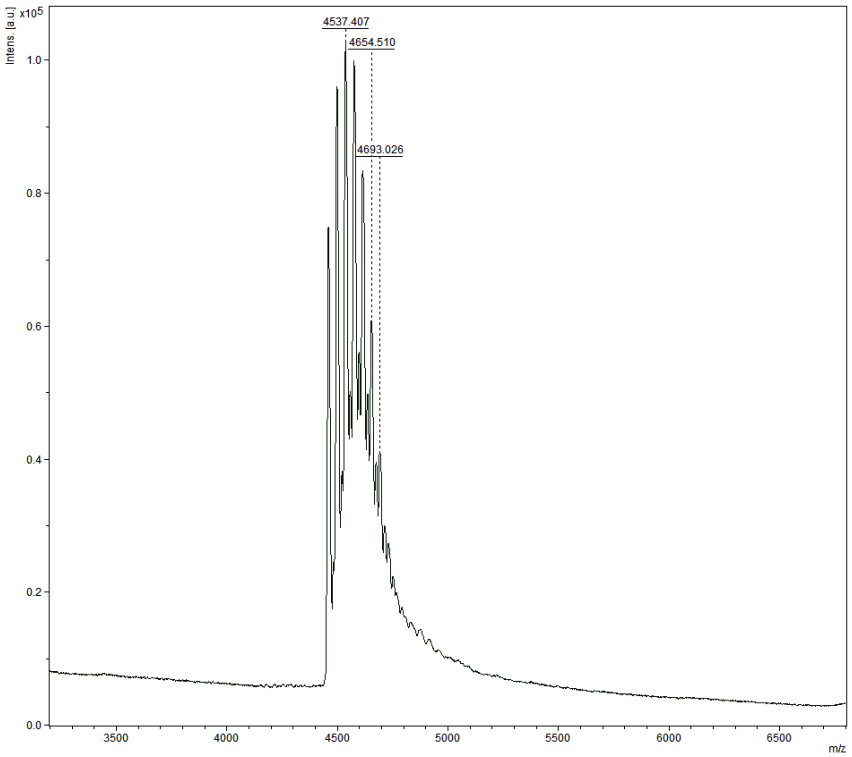


Figure S9e. MALDI-TOF-MS of ON-3 neutral pH

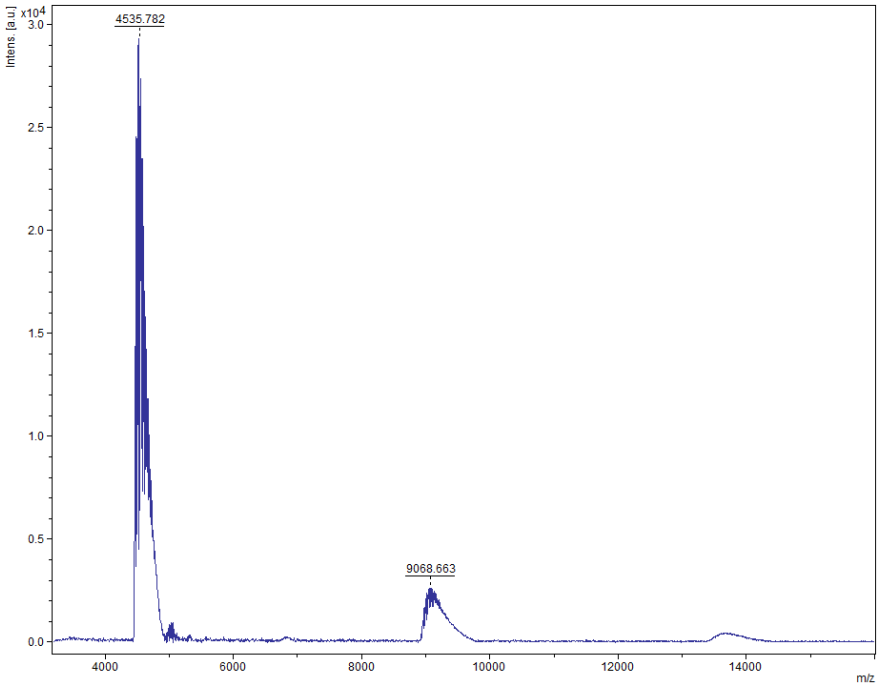


Figure S9f. MALDI-TOF-MS of ON-3 at pH 3

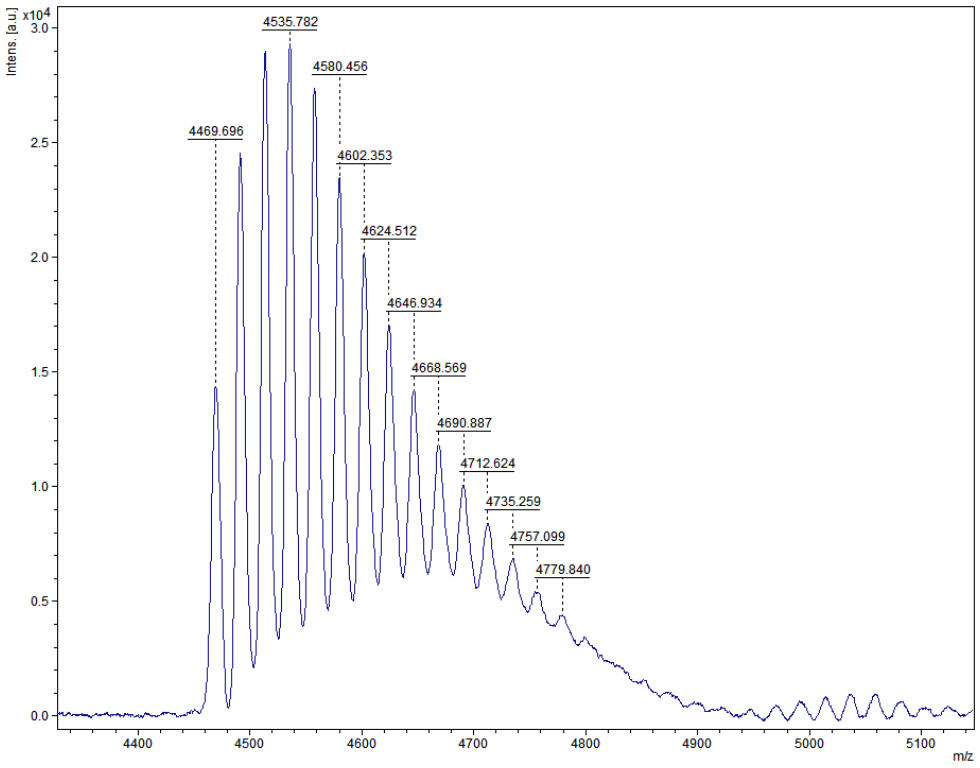


Figure S9g. MALDI-TOF-MS of ON-3 at pH 3

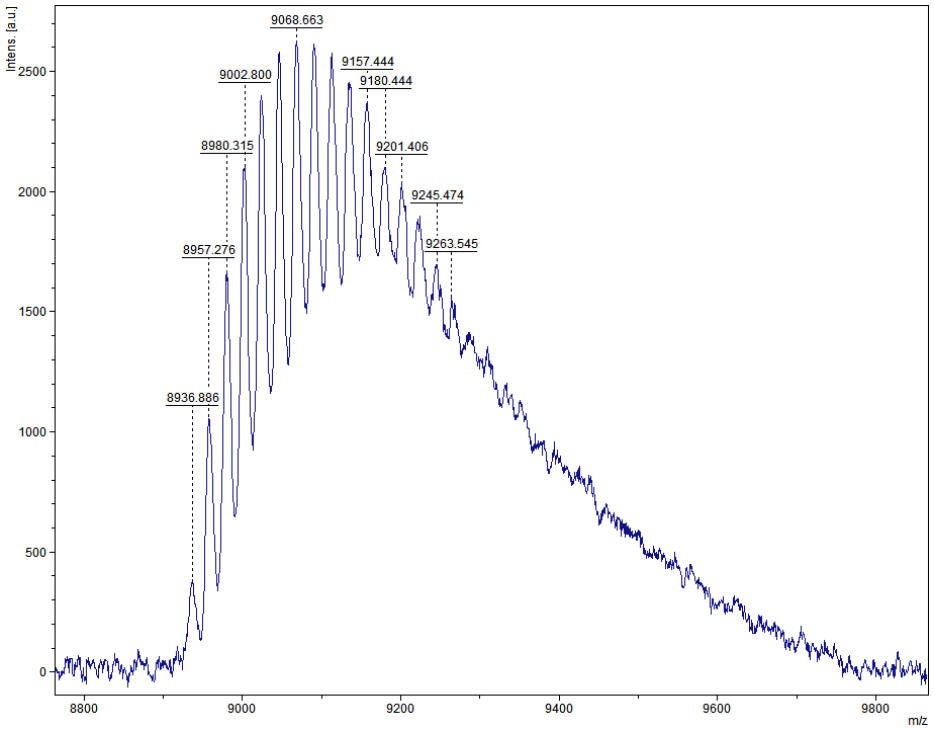


Figure S9h. MALDI-TOF-MS of ON-3 at pH 3

S10. pH-induced structural transition of ON-4

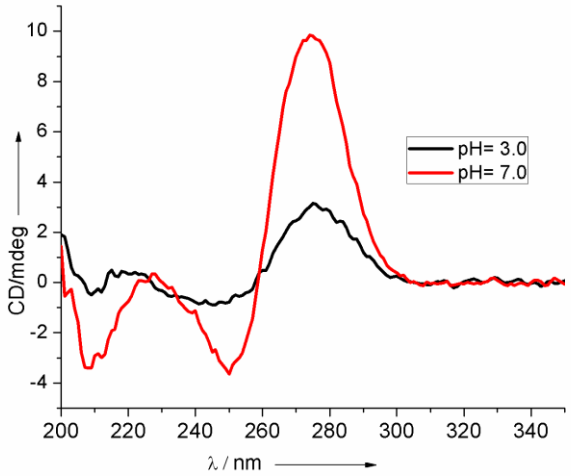


Figure S10. Effect of pH on ON-4. Condition: 4 µM oligonucleotide concentration at pH 3.0 in unbuffered solution.

S11. HPLC profile of ON-3 oligonucleotide

Oligonucleotides were heated at 90 ^{o}C for 20 min at pH 3. No degradation was observed of ON-3 as shown in figure S10a and S10b.

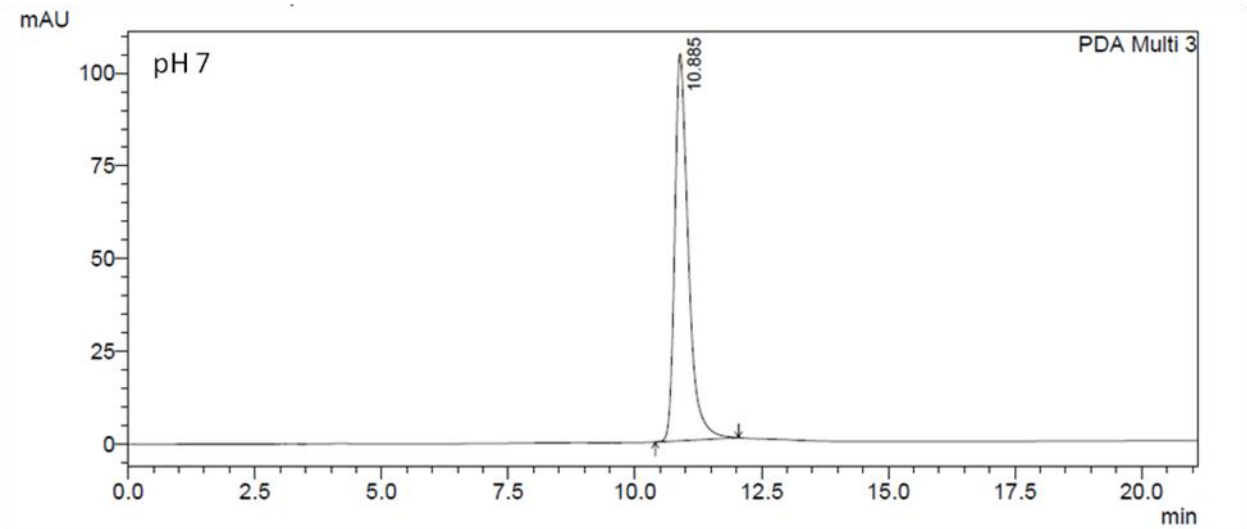


Figure S11a. HPLC profile of ON-3

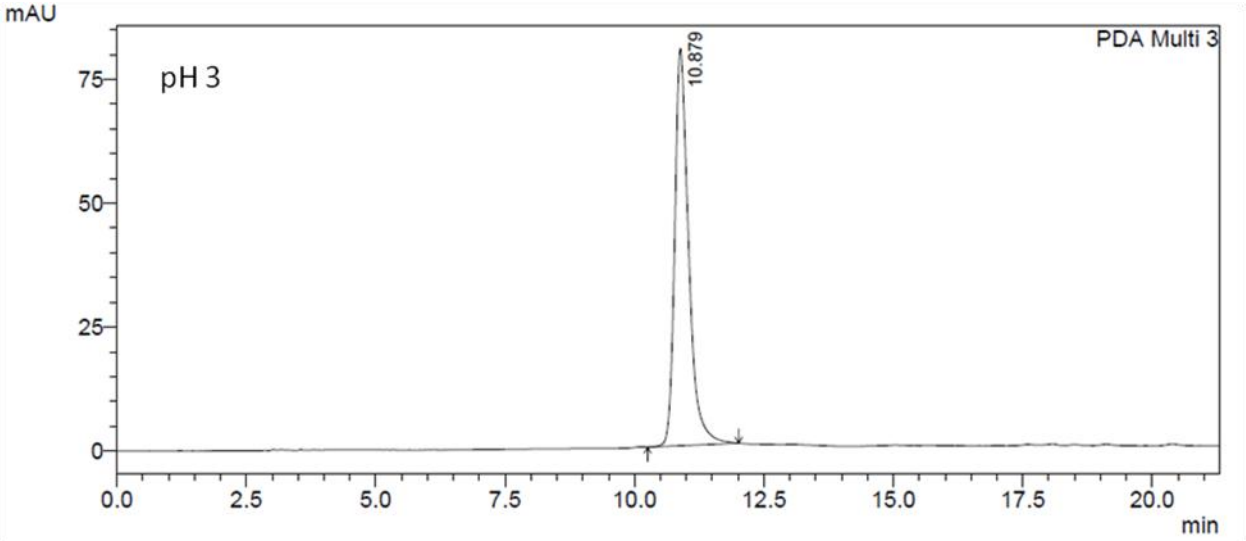


Figure S11b. HPLC profile of ON-3 heated at pH 3.