Supplementary Information

Probing the Molecular Determinants of Fluorinase Specificity

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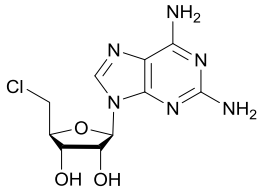
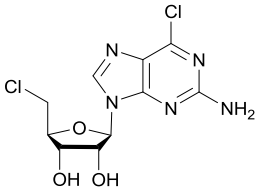
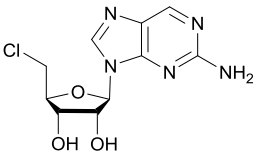
Supplementary Results

Table S1. % product yield and fold improvement (Fold imp) of fluorinases over FlA1 for substrates **5**, **6** and **8** at 37°C. Reaction conditions: Synthesized substrate (0.2 mM), L-methionine (0.1 mM), NaF (80 mM), and fluorinase (50 μM) at a single time point of 4 h. % yield (mean of triplicates) = (concentration of product generated / concentration of substrate) x 100%. Fold imp = % yield of evolved variants / % yield of FlA1.

Fluorinase (Mutation changes)	 **5**		 **6**		 **8**	
	% yield	**Fold imp**	**% yield**	**Fold imp**	**% yield**	**Fold imp**
FlA1	28.8 ± 0.3	-	0	-	4.5 ± 0.1	-
fah2019 (F213Y)	35.4 ± 0.5	1.2	0.2 ± 0*	-	9.8 ± 0.2	2.2
fah2047 (A279L)	38.0 ± 0.2	1.3	0.6 ± 0*	-	11.9 ± 0.3	2.7
frh2066 (Y77W)	31.6 ± 0.4	1.1	0	-	0.1 ± 0.1	0
fah2081 (A279Y)	37.7 ± 0.2	1.3	0.1 ± 0*	-	2.2 ± 0.1	0.5
fah2114 (F213Y, A279L)	37.6 ± 0.5	1.3	0.9 ± 0*	-	5.4 ± 0	1.2

[*] Standard deviation less than 0.1

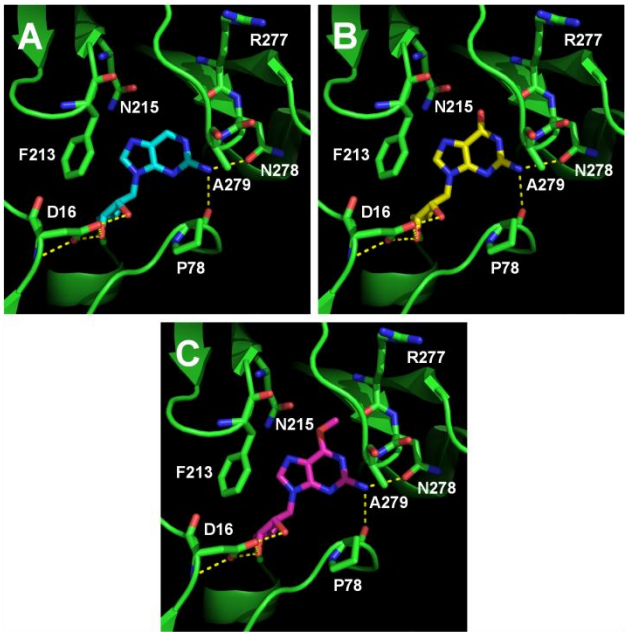


Figure S1. Novel substrate binding to wild-type FlA1 showing the loss of hydrogen bonds interaction with the removal of R^2 substituent of amino group. A. Substrate **8** (in light blue). B. Substrate **11** (in yellow). C. Substrate **10** (in pink).

Experimental Methods

Fluorinase expression in *E. coli* and purification

Purified protein of FlA1 and the evolved variants were prepared as previously described.[1]

Enzymatic reactions and measurement of substrate/product stability

Reaction conditions for measurement of substrate/product stability: Synthesized substrate/product (0.2 mM) in sodium phosphate buffer (50 mM, pH 7.8 with 10% glycerol) in a final volume of 0.1 mL for 4 h or 24 h.

Single time point enzymatic reaction conditions (in triplicates): Synthesized substrate (0.2 mM), L-methionine (0.1 mM), NaF (80 mM), and fluorinase (50 μM) in sodium phosphate buffer (50 mM, pH 7.8 with 10% glycerol) in a final volume of 0.1 mL for 4 h or 24 h.

Time-course reaction conditions (in triplicates): Substrate **1**, **2** or **5** (0.2 mM), L-methionine (0.1 mM), NaF (80 mM), and fluorinase (50 μM) in sodium phosphate buffer (50 mM, pH 7.8 with 10% glycerol) in a final volume of 0.7 mL. An aliquot of 0.1 mL was removed for samplings at 30 min (substrate **5** only), 1 h, 3 h, 5 h, 7 h and 24 h.

Negative control reactions (for substrate **1**) to exclude the background conversion of **1a** from endogenous SAM were carried out in the same reaction conditions (as above) except without addition of **1** and L-methionine. Reactions were run in 1.5 mL Eppendorf tube at 37°C in shaking incubator (New Brunswick™ Innova) at 250 rpm. Reactions were stopped by heating the samples at 95°C for 1 min (using a PCR machine). The precipitated protein was then removed by centrifugation (20,238 x *g* for 10 min). 10 μL of the reaction mixture was used for HPLC-UV analysis.

HPLC-UV analysis of enzymatic reactions

HPLC analysis was carried out using Shimadzu Prominence system UFLC. Mobile phase A: 0.1% formic acid in water; B: 0.1% formic acid in methanol. The reaction mixture was analyzed on Phenomenex, Kinetex® 2.6 µm Biphenyl 100 Å, LC Column 150 x 4.6 mm, 0.6 mL min^{-1} flow rate, gradient elution, 5 – 95% B for 15 min. The UV wavelength and retention time of the products were summarized in the table below. The % yield was calculated using a dilution of synthesized product standards.

Product	***UV wavelength* / nm**	***Retention time* / min**
5'-FDA, **1a**	258	8.42
FDEA, **2a**	258	12.09
3a	258	12.55
4a	258	13.92
5a	291	8.16
6a	308	12.29
7a	258	13.40
8a	310	8.45
9a	258	10.76
10a	280	12.39
11a	258	7.83

Structural modeling of enzyme variants

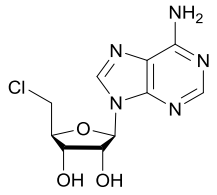
The recently solved crystal structure of FlA1 (PDB code 5B6I) was used as the basis for modeling all mutants, which were generated as previously described.[1] For the substrate coordinates, the crystal structure of the *Streptomyces cattleya* fluorinase trimer complexed with substrate **2** (PDB code 4CQJ)[2] was superposed onto the FlA1 trimer to place the substrate into the active sites (RMSD = 0.38Å across 759 C-alpha atoms). The coordinates were then modified to generate the various model substrates using Discovery Studio software (BIOVIA), re-docked to the enzyme active site and subjected to 1000 steps of energy minimization using AMBER[3]. The resulting models were visualized using PyMOL.[4]

Synthesis and Characterization of Compounds

General considerations

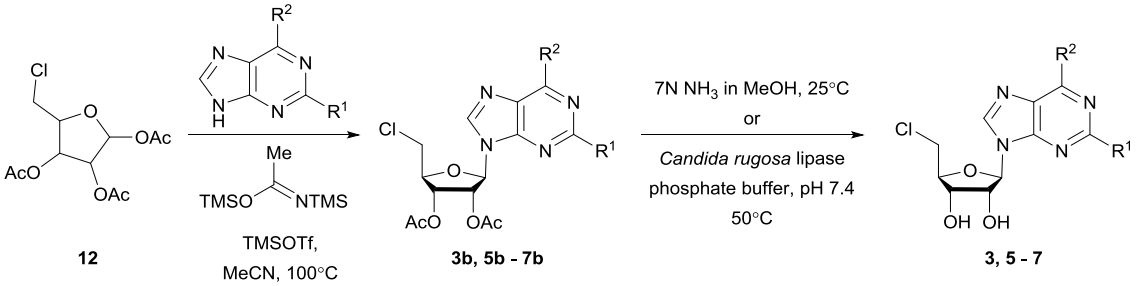
All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF), acetonitrile (MeCN), *N*,*N*′-dimethylformamide (DMF), and methylene chloride (CH_2Cl_2) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent. E. Merck silica gel (60, particle size 0.040 – 0.063 mm) was used for flash column chromatography. Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25 or 0.50 mm E. Merck silica gel plates (60F-254). NMR spectra were recorded on DRX-600 or DRX-400 instruments and calibrated using residual undeuterated solvent ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.16 ppm, methanol-d_4: δ_H = 3.31 ppm, δ_C = 49.00 ppm; DMSO-d_6: δ_H = 2.50 ppm, δ_C = 39.52 ppm) as an internal reference. Data for ^{1}H NMR spectra are reported as follows: chemical shift δ_H ppm (multiplicity, coupling constant (Hz), integration). The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad. High resolution mass spectra (HRMS) were recorded on an Agilent 6210 Series 1969A ESI-TOF (time of flight) mass spectrometer using EI (electron ionization) or ESI (electrospray ionization).

Preparation of Substrates



1

Compound **1** was prepared in accordance to our previously reported method.[1]



12 **3b, 5b - 7b** **3, 5 - 7**

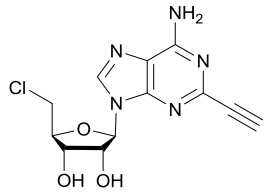
General procedure A

12[5] (147 mg, 0.5 mmol) and the respective purine (1 mmol) was dissolved in MeCN (3 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (0.375 mL, 1.5 mmol) was added followed by TMSOTf (27 µL, 0.15 mmol). Reaction was stirred at 100°C for 4 h. Solvent was removed, diluted with EtOAc, washed with $NaHCO_3$ and dried with Na_2SO_4. The crude was then purified using

column chromatography to give the Ac-intermediate (**3b, 5b** – **6b**) that was stirred in 7N NH_3 in MeOH (3 mL) for 1 h. Solvent was concentrated in *vacuo* to yield the desired product (**3, 5** – **6**).

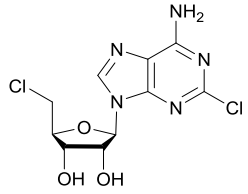
General procedure B

12[5] (147 mg, 0.5 mmol) and the respective purine (1 mmol) was dissolved in MeCN (3 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (0.375 mL, 1.5 mmol) was added followed by TMSOTf (27 µL, 0.15 mmol). Reaction was stirred at 100°C for 4 h. Solvent was removed, diluted with EtOAc, washed with $NaHCO_3$ and dried with Na_2SO_4. The crude was then purified using column chromatography to give Ac-intermediate (**7b**) that was then stirred with *Candida rugosa* lipase (50 mg) in 0.1 M sodium phosphate buffer pH 7.4 (5 mL) at 50°C for 3 h. Reaction was centrifuged and decanted. Solvent was removed and crude was purified using column chromatography to yield the desired product (**7**).



2

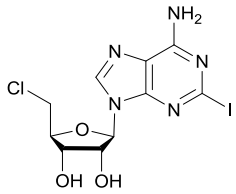
(2R,3R,4S,5S)-2-(6-amino-2-ethynyl-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (2).[2] Compound **4** (41 mg, 0.1 mmol) was added to a solution of $Pd(PPh_3)_2Cl_2$ (7 mg, 0.01 mmol, 0.1 eq.) and CuI (2 mg, 0.01 mmol, 0.1 eq.) in DMF (3 mL) and triethylamine (21 µL, 0.15 mmol, 1.5 eq.) and ethynyltrimethylsilane (29 µL, 0.2 mmol, 2.0 eq.) were slowly added. The mixture was heated to 85°C for 90 min, after which time the volatile components were removed. Crude reaction was stirred in 7N NH_3 in MeOH (2 mL) for 1 h. Solvent was removed and crude purified using column chromatography (CH_2Cl_2: MeOH 100:3) to yield **2** (18 mg, 58%) over two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.41 (s, 1 H), 7.48 (s, 2 H), 5.91 (d, *J* = 5.7 Hz, 1 H), 5.51 (d, *J* = 57.6 Hz, 2 H), 4.70 (d, *J* = 4.6 Hz, 1 H), 4.20 (s, 1 H), 4.10 (ddd, *J* = 6.2, 5.0, 3.7 Hz, 1 H), 3.98–3.81 (m, 2 H), 1.91 (s, 1 H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.9, 155.9, 149.3, 144.8, 140.6, 118.9, 87.3, 83.8, 83.4, 74.8, 72.8, 71.2, 44.7 ppm. HRMS (ESI) calc. for $C_{12}H_{13}ClN_5O_3^+$ $(M+H)^+$: 310.0701 found: 310.0715.



3

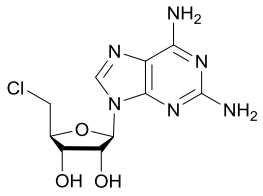
(2R,3R,4S,5S)-2-(6-amino-2-chloro-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (3). Following general procedure A, **12** (147 mg, 0.5 mmol) and 2-chloro,6-aminopurine (169 mg, 1 mmol) resulted in a crude mixture which was purified by column chromatography (CH_2Cl_2:MeOH 100:4) to yield **3** (121 mg, 76%) over the two steps. ^{1}H NMR (600 MHz, methanol-d_4) δ = 8.23 (d, *J* = 0.8 Hz, 1 H), 5.96 (d, *J* = 5.0 Hz, 1 H), 4.75 (t, *J* = 5.1 Hz, 1 H), 4.39 (dd, *J* = 5.3, 4.4 Hz, 1 H), 4.26 (q, *J* = 4.8 Hz, 1 H), 3.95 (ddd, *J* = 11.9, 5.1, 0.8 Hz, 1 H), 3.85 (ddd, *J* = 11.9, 5.1, 0.9 Hz, 1 H) ppm. ^{13}C NMR (151 MHz, methanol-d_4) δ = 155.6,

151.9, 141.7, 124.2, 119.7, 90.5, 85.5, 75.0, 72.8, 45.3 ppm. HRMS (ESI) calc. for $C_{10}H_{12}Cl_2N_5O_3^+$ $(M+H)^+$: 320.0312, found: 320.0317.



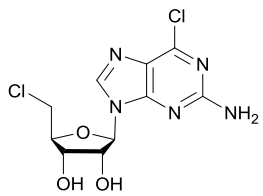
4

(2R,3R,4S,5S)-2-(6-amino-2-iodo-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (4). Compound **6** (120 mg, 0.3 mmol) was added to a solution of CuI (106 mg, 0.6 mmol) and I_2 (76 mg, 0.3 mmol) in THF (4 mL). CH_2I_2 (0.24 mL, 3 mmol) and isoamyl nitrite (0.12 mL, 0.9 mmol) was added slowly and reaction was refluxed for 3 h, cooled to room temperature and filtered through celite. Solvent was removed and the crude was purified using column chromatography (DCM:MeOH 100:5). The purified intermediate was dissolved in 7N NH_3 in MeOH (4 mL) and heated to 60°C for 2 h in the microwave reactor. Solvent was removed and crude was purified using column chromatography (CH_2Cl_2:MeOH 100:3) to yield **4** (30 mg, 24%) over the two steps. ^{1}H NMR (400 MHz, methanol-d_4) δ = 8.14 (s, 1 H), 5.95 (d, J = 5.1 Hz, 1 H), 4.76 (d, J = 5.0 Hz, 1 H), 4.39 (t, J = 4.8 Hz, 1 H), 4.26 (q, J = 5.0 Hz, 1 H), 3.99–3.77 (m, 2 H) ppm. ^{13}C NMR (101 MHz, methanol-d_4) δ 157.3, 151.1, 141.3, 120.8, 120.7, 90.7, 85.7, 75.0, 72.9, 45.2 ppm. HRMS (ESI) calc. for $C_{10}H_{12}ICIN_5O_3^+$ $(M+H)^+$: 411.9673, found: 411.967.



5

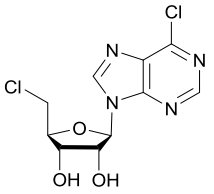
(2S,3S,4R,5R)-2-(chloromethyl)-5-(2,6-diamino-9H-purin-9-yl)tetrahydrofuran-3,4-diol (5). Following general procedure A, **12** (147 mg, 0.5 mmol) and 2,6 diaminopurine (150 mg, 1 mmol) resulted in a crude mixture which was purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH 100:4:0.1) to yield **5** (124 mg, 83%) over the two steps. ^{1}H NMR (600 MHz, methanol-d_4) δ = 7.93 (s, 1 H), 5.87 (d, J = 5.2 Hz, 1 H), 4.72 (t, J = 5.3 Hz, 1 H), 4.35 (dd, J = 5.3, 4.3 Hz, 1 H), 4.23 (q, J = 4.8 Hz, 1 H), 3.93 (dd, J = 11.9, 5.0 Hz, 1 H), 3.83 (dd, J = 11.9, 4.9 Hz, 1 H) ppm. ^{13}C NMR (151 MHz, methanol-d_4) δ = 162.1, 157.7, 153.1, 138.1, 114.7, 89.7, 85.3, 74.9, 72.9, 45.5 ppm. HRMS (ESI) calc. for $C_{10}H_{14}ClN_6O_3^+$ $(M+H)^+$: 301.0816, found: 301.0825.



6

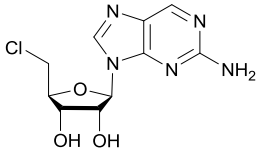
(2R,3R,4S,5S)-2-(2-amino-6-chloro-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (6). Following general procedure A, **12** (147 mg, 0.5 mmol) and 2-amino,6-chloropurine

(169 mg, 1 mmol) resulted in a crude mixture which was purified by column chromatography (CH_2Cl_2:MeOH 100:4) to yield **6** (129 mg, 81%) over the two steps. ^{1}H NMR (400 MHz, methanol-d_4) δ = 8.20 (s, 1 H), 5.94 (d, J = 5.1 Hz, 1 H), 4.81 (d, J = 5.4 Hz, 1 H), 4.48–4.35 (m, 1 H), 4.25 (d, J = 4.7 Hz, 1 H), 3.91 (d, J = 5.1 Hz, 1 H), 3.84 (d, J = 5.0 Hz, 1 H) ppm. ^{13}C NMR (101 MHz, methanol-d_4) δ = 161.7, 161.7, 155.0, 151.9, 143.1, 125.5, 90.2, 85.4, 74.7, 72.8, 45.4 ppm. HRMS (ESI) calc. for $C_{10}H_{12}Cl_2N_5O_3^+$ $(M+H)^+$: 320.0312, found: 320.0317.



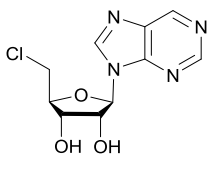
7

(2R,3R,4S,5S)-2-(6-chloro-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (7). Following general procedure B, **12** (147 mg, 0.5 mmol) and 6-chloropurine (154 mg, 1 mmol) resulted in a crude mixture which was purified by column chromatography (CH_2Cl_2:MeOH 100:5) to yield **7** (40 mg, 27%) over the two steps. ^{1}H NMR (600 MHz, methanol-d_4) δ = 8.84–8.62 (m, 2 H), 6.16 (dt, J = 8.7, 4.8 Hz, 1 H), 4.64–4.52 (m, 1 H), 4.44 (dt, J = 9.7, 4.6 Hz, 1 H), 4.36–4.25 (m, 1 H), 4.04–3.81 (m, 2 H) ppm. ^{13}C NMR (151 MHz, methanol-d_4) δ = 153.3, 153.1, 151.8, 147.1, 133.2, 91.0, 85.7, 75.0, 72.8, 45.3 ppm. HRMS (ESI) calc. for $C_{10}H_{11}Cl_2N_4O_3^+$ $(M+H)^+$: 305.0208, found: 305.021.



8

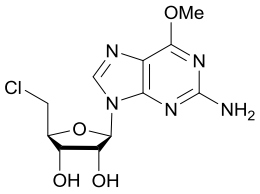
(2R,3R,4S,5S)-2-(2-amino-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (8). Et_3N (0.5 mL) was added to compound **6** (50 mg) and Pd/C (10 mg) in THF (5 mL). The reaction mixture was stirred under a hydrogen balloon overnight. The reaction mixture was stirred under a hydrogen balloon overnight. Reaction mixture was filtered and solvent removed; the crude was purified using column chromatography (CH_2Cl_2:MeOH 100:4) to yield **8** (34 mg, 77%). ^{1}H NMR (600 MHz, methanol-d_4) δ = 8.57 (s, 1 H), 8.21 (s, 1 H), 5.97 (d, J = 5.3 Hz, 1 H), 4.84 (d, J = 5.3 Hz, 1 H), 4.40 (dd, J = 5.3, 4.2 Hz, 1 H), 4.27–4.18 (m, 1 H), 3.94 (dd, J = 11.9, 5.2 Hz, 1 H), 3.83 (dd, J = 11.9, 5.1 Hz, 1 H) ppm. ^{13}C NMR (151 MHz, methanol-d_4) δ = 162.2, 154.6, 150.2, 143.5, 128.7, 89.9, 85.5, 74.6, 72.9, 45.4 ppm. HRMS (ESI) calc. for $C_{10}H_{13}ClN_5O_3^+$ $(M+H)^+$: 286.0707, found: 286.0714.



9

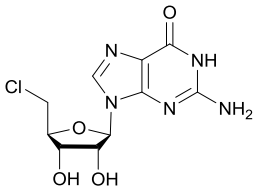
(2S,3S,4R,5R)-2-(chloromethyl)-5-(9H-purin-9-yl)tetrahydrofuran-3,4-diol (9). Et_3N (0.5 mL) was added to compound **7** (50 mg) and Pd/C (10 mg) in THF (5 mL). The reaction mixture was stirred under a hydrogen balloon overnight. Reaction mixture was filtered and

solvent removed; the crude was purified using column chromatography (CH_2Cl_2:MeOH 100:5) to yield **9** (31 mg, 69%). ^{1}H NMR (400 MHz, methanol-d_4) δ = 9.12 (s, 1 H), 8.96 (s, 1 H), 8.68 (s, 1 H), 6.18 (d, *J* = 5.0 Hz, 1 H), 4.91 (t, *J* = 5.1 Hz, 1 H), 4.45 (t, *J* = 4.9 Hz, 1 H), 4.31 (q, *J* = 4.8 Hz, 1 H), 3.96 (dd, *J* = 11.9, 5.0 Hz, 1 H), 3.86 (dd, *J* = 11.9, 5.0 Hz, 1 H) ppm. ^{13}C NMR (101 MHz, methanol-d_4) δ = 153.7, 152.7, 149.2, 147.2, 135.8, 90.5, 85.6, 75.0, 72.9, 45.3 ppm. HRMS (ESI) calc. for $C_{10}H_{12}ClN_4O_3^+$ $(M+H)^+$: 271.0598, found: 271.0606.



10

(2R,3R,4S,5S)-2-(2-amino-6-methoxy-9H-purin-9-yl)-5-(chloromethyl)tetrahydrofuran-3,4-diol (10). 12[5] (147 mg, 0.5 mmol) and 2-amino,6-chloropurine (169 mg, 1 mmol) was dissolved in MeCN (3 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (0.375 mL, 1.5 mmol) was added followed by TMSOTf (27 µL, 0.15 mmol). Reaction was stirred at 100°C for 4 h. Solvent was removed, diluted with EtOAc, washed with $NaHCO_3$ and dried with Na_2SO_4. The crude was then purified using column chromatography (CH_2Cl_2:MeOH 100:2) to give an Ac-intermediate that was stirred with NaOMe (108 mg, 2 mmol) in MeOH (5 mL) at 50°C for 2 h. Solvent was removed and the resulting crude purified by column chromatography (CH_2Cl_2:MeOH 100:4) to yield **10** (65 mg, 41%) over the two steps. ^{1}H NMR (400 MHz, methanol-d_4) δ = 7.98 (s, 1 H), 5.92 (d, *J* = 5.1 Hz, 1 H), 4.76 (d, *J* = 5.2 Hz, 1 H), 4.38 (d, *J* = 1.0 Hz, 1 H), 4.24 (d, *J* = 4.7 Hz, 1 H), 4.04 (s, 3 H), 3.91 (d, *J* = 5.0 Hz, 1 H), 3.87–3.78 (m, 1 H) ppm. ^{13}C NMR (101 MHz, methanol-d_4) δ = 162.8, 162.0, 155.0, 139.6, 115.7, 89.9, 85.3, 74.8, 72.9, 54.3, 45.4 ppm. HRMS (ESI) calc. for $C_{11}H_{15}ClN_5O_4^+$ $(M+H)^+$: 316.0813, found: 316.082.

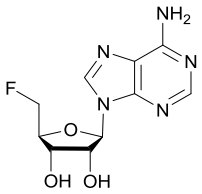


11

2-amino-9-((2R,3R,4S,5S)-5-(chloromethyl)-3,4-dihydroxytetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one (11). 12[5] (147 mg, 0.5 mmol) and N2-acetylguanine (193 mg, 1 mmol) was dissolved in MeCN (3 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (0.375 mL, 1.5 mmol) was added followed by TMSOTf (27 µL, 0.15 mmol). Reaction was stirred at 100°C for 4 h. Solvent was removed, diluted with EtOAc, washed with $NaHCO_3$ and dried with Na_2SO_4. The crude was then purified using column chromatography (CH_2Cl_2:MeOH 100:2) to give an Ac-intermediate that was stirred with NaOH (1M in H_2O, 3mL) in 70°C for 3 h. Solvent was removed and the resulting crude purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH 100:5:0.1) to yield **11** (32 mg, 21%) as a mixture of anomers over the two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.22 (s, 1 H), 7.88 (s, 1 H), 6.49 (s, 2 H), 6.19 (s, 2 H), 6.01 (d, *J* = 6.1 Hz, 1 H), 5.73 (d, *J* = 6.0 Hz, 1 H), 5.50 (dd, *J* = 21.3, 6.1 Hz, 2 H), 5.35 (dd, *J* = 5.0, 1.6 Hz, 2 H), 4.59 (dq, *J* = 11.5, 5.8 Hz, 2 H), 4.20–3.99 (m, 4 H), 3.92 (td,

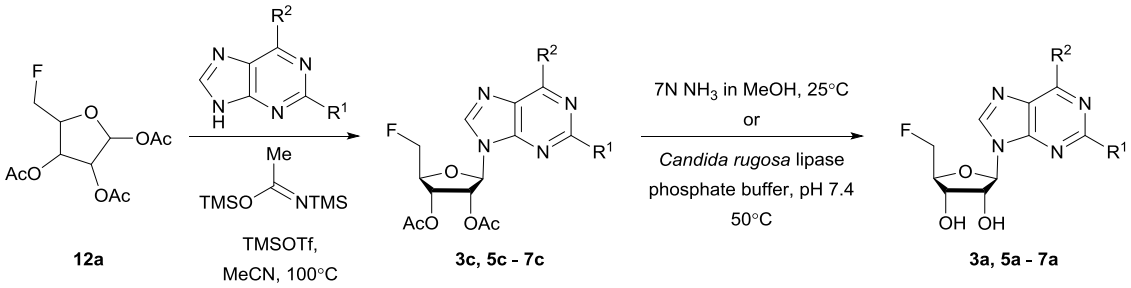
J = 11.2, 5.4 Hz, 2 H), 3.87–3.68 (m, 2 H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 160.7, 156.7, 154.1, 153.7, 152.9, 151.3, 142.7, 135.6, 116.8, 107.7, 89.3, 86.5, 83.7, 83.5, 73.4, 72.6, 71.2, 71.1, 44.8, 44.6 ppm. HRMS (ESI) calc. for $C_{10}H_{13}ClN_5O_4^+$ $(M+H)^+$: 302.0651, found: 302.0658.

Preparation of Products



1a

Compound **1a** was prepared in accordance to our previously reported method.[1]

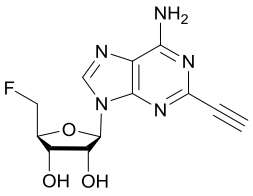


General procedure C

12a[6] (28 mg, 0.1 mmol) and the respective purine (0.2 mmol) was dissolved in MeCN (2 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (70 µL, 0.3 mmol) was added followed by TMSOTf (5 µL, 0.03 mmol). Reaction was stirred at 100°C for 4 h. Solvent was removed, diluted with EtOAc, washed with $NaHCO_3$ and dried with Na_2SO_4. The crude was then purified using column chromatography to give Ac-intermediate (**3c, 5c** – **6c**) that was stirred in 7N NH_3 in MeOH (1 mL) for 1 h. Solvent was removed to yield the desired product (**3a, 5a** – **6a**).

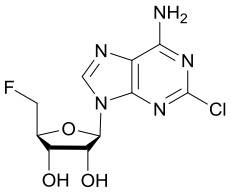
General procedure D

12a[6] (28 mg, 0.1 mmol) and the respective purine (0.2 mmol) was dissolved in MeCN (2 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (70 µL, 0.3 mmol) was added followed by TMSOTf (5 µL, 0.03 mmol). Reaction was stirred at 100°C for 4 h. Solvent was removed, diluted with EtOAc, washed with $NaHCO_3$ and dried with Na_2SO_4. The crude was then purified using column chromatography to give Ac-intermediate (**7c**) that was stirred with *Candida rugosa* lipase (10 mg) in 0.1 M sodium phosphate buffer pH 7.4 (2 mL) at 50°C for 3 h. Reaction mixture was then centrifuged and decanted. Solvent was removed and crude was purified using column chromatography to yield the desired product (**7a**).



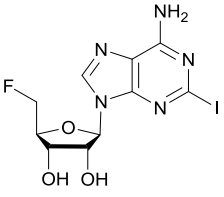
2a

(2R,3R,4S,5S)-2-(6-amino-2-ethynyl-9H-purin-9-yl)-5-(fluoromethyl)tetrahydrofuran-3,4-diol (2a).[2] Compound **4a** (40 mg, 0.1 mmol) was added to a solution of $Pd(PPh_3)_2Cl_2$ (7 mg, 0.01 mmol, 0.1 eq.) and CuI (2 mg, 0.01 mmol, 0.1 eq.) in DMF (3 mL) and triethylamine (21 µL, 0.15 mmol, 1.5 eq.) and ethynyltrimethylsilane (29 µL, 0.2 mmol, 2.0 eq.) were slowly added. The mixture was heated to 85°C for 90 min, after which time the volatile components were removed. Crude reaction was stirred in 7N NH_3 in MeOH (2 mL) for 1 h. Solvent was removed and crude purified using column chromatography (CH_2Cl_2: MeOH 100:3) to yield **2a** (12 mg, 41%) over two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.32 (s, 1 H), 7.48 (s, 2 H), 5.92 (d, *J* = 5.0 Hz, 1 H), 5.60 (s, 1 H), 5.40 (s, 1 H), 4.74–4.68 (m, 1 H), 4.58 (ddd, *J* = 16.6, 6.8, 3.7 Hz, 2 H), 4.23 (t, *J* = 4.9 Hz, 1 H), 4.13 (dtd, *J* = 23.8, 4.8, 3.5 Hz, 1 H), 1.90 (s, 1 H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 155.8, 149.2, 144.8, 140.1, 118.8, 87.5, 83.6, 83.3, 82.4 (d, *J* = 18.4 Hz), 82.0, 73.95 (d, *J* = 162.8 Hz), 69.4 (d, *J* = 5.9 Hz) ppm. ^{19}F NMR (376 MHz, methanol-d_4) δ = -232.53 ppm. HRMS (ESI) calc. for $C_{12}H_{13}FN_5O_3^+$ $(M+H)^+$: 294.0997 found: 294.1008.



3a

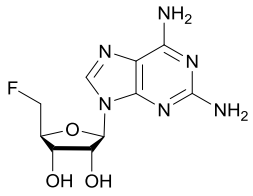
(2R,3R,4S,5S)-2-(6-amino-2-chloro-9H-purin-9-yl)-5-(fluoromethyl)tetrahydrofuran-3,4-diol (3a). Following general procedure C, **12a** (28 mg, 0.1 mmol) and 2-chloro,6-aminopurine (34 mg, 0.2 mmol) resulted in a crude mixture which was purified by column chromatography (CH_2Cl_2:MeOH 100:4) to yield **3a** (24 mg, 81%) over the two steps. ^{1}H NMR (600 MHz, methanol-d_4) δ = 8.17 (s, 1 H), 6.00 (d, *J* = 4.2 Hz, 1 H), 4.80–4.62 (m, 2 H), 4.57 (t, *J* = 4.4 Hz, 1 H), 4.39 (t, *J* = 5.2 Hz, 1 H), 4.23 (ddt, *J* = 27.4, 5.8, 3.1 Hz, 1 H) ppm. ^{13}C NMR (151 MHz, methanol-d_4) δ = 158.3, 155.6, 151.9, 145.9, 141.0, 90.5, 84.64 (d, *J* = 18.5 Hz), 84.6, 84.3, 83.1, 75.8, 71.07 (d, *J* = 5.1 Hz) ppm. ^{19}F NMR (565 MHz, methanol-d_4) δ = –232.33 ppm. HRMS (ESI) calc. for $C_{10}H_{12}FClN_5O_3^+$ $(M+H)^+$: 304.0613, found: 304.0617.



4a

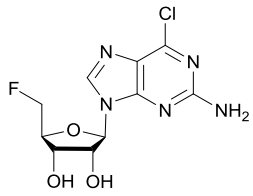
(2R,3R,4S,5S)-2-(6-amino-2-iodo-9H-purin-9-yl)-5-(fluoromethyl)tetrahydrofuran-3,4-diol (4a). Compound **6a** (40 mg, 0.1 mmol) was added to a solution of CuI (38 mg, 0.2 mmol) and I_2 (26 mg, 0.1 mmol) in THF (4 mL). CH_2I_2 (80 µL, 1 mmol) and isoamyl nitrite (40 µL, 0.3 mmol) was added slowly and reaction was refluxed for 3 h, cooled to room

temperature and filtered through celite. Solvent was removed and the crude was purified using column chromatography (CH_2Cl_2:MeOH 100:5). The purified intermediate was dissolved in 7N NH_3 in MeOH (2 mL) and heated to 60°C for 2 h in the microwave reactor. Solvent was removed and the resulting crude was purified using column chromatography (CH_2Cl_2:MeOH 100:3) to yield **4a** (12 mg, 31%) over the two steps. ^{1}H NMR (400 MHz, methanol-d_4) δ = 8.08 (s, 1 H), 5.99 (d, J = 4.2 Hz, 1 H), 4.75–4.59 (m, 2 H), 4.57 (ddd, J = 5.2, 4.2, 1.1 Hz, 1 H), 4.39 (t, J = 5.2 Hz, 1 H), 4.23 (ddt, J = 26.7, 5.3, 3.5 Hz, 1 H) ppm. ^{13}C NMR (101 MHz, methanol-d_4) δ = 157.3, 151.1, 140.5 (d, J = 4.5 Hz), 120.9, 120.5, 90.6, 84.6 (d, J = 2.0 Hz), 83.8 (d, J = 191 Hz), 75.8 (d, J = 1.8 Hz), 71.1 (d, J = 5.4 Hz) ppm. ^{19}F NMR (376 MHz, methanol-d_4) δ = –232.02 ppm. HRMS (ESI) calc. for $C_{10}H_{12}FIN_5O_3^+$ $(M+H)^+$: 395.9969, found: 395.9976.



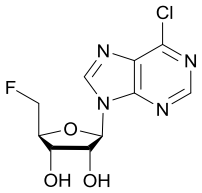
5a

(2R,3R,4S,5S)-2-(2,6-diamino-9H-purin-9-yl)-5-(fluoromethyl)tetrahydrofuran-3,4-diol (5a). Following general procedure C, **12a** (28 mg, 0.1 mmol) and 2,6 diaminopurine (30 mg, 0.2 mmol) resulted in a crude mixture which was purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH 100:4:0.1) to yield **5a** (25 mg, 88%) over the two steps. ^{1}H NMR (600 MHz, methanol-d_4) δ = 7.87 (s, 1 H), 5.91 (d, J = 4.5 Hz, 1 H), 4.78–4.60 (m, 2 H), 4.52 (ddd, J = 5.4, 4.7, 1.2 Hz, 1 H), 4.37 (t, J = 5.1 Hz, 1 H), 4.20 (ddt, J = 28.0, 5.1, 3.1 Hz, 1 H) ppm. ^{13}C NMR (151 MHz, methanol-d_4) δ = 162.1, 157.8, 153.0, 137.5, 114.6, 89.7, 84.4, 83.9 (d, J = 187.2 Hz), 75.7, 71.2 (d, J = 5.3 Hz) ppm. ^{19}F NMR (565 MHz, methanol-d_4) δ = –232.41 ppm. HRMS (ESI) calc. for $C_{10}H_{14}FN_6O_3^+$ $(M+H)^+$: 285.1111, found: 285.1115.



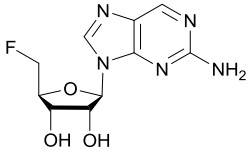
6a

(2R,3R,4S,5S)-2-(2-amino-6-chloro-9H-purin-9-yl)-5-(fluoromethyl)tetrahydrofuran-3,4-diol (6a). Following general procedure C, **12a** (28 mg, 0.1 mmol) and 2-amino,6-chloropurine (34 mg, 0.2 mmol) resulted in a crude mixture which was purified by column chromatography (CH_2Cl_2:MeOH 100:4) to yield **6a** (22.4 mg, 74%) over the two steps. ^{1}H NMR (400 MHz, methanol-d_4) δ = 8.14 (s, 1 H), 5.98 (d, J = 4.4 Hz, 1 H), 4.74–4.64 (m, 1 H), 4.64–4.58 (m, 2H), 4.39 (t, J = 5.1 Hz, 1 H), 4.27–4.14 (m, 1 H) ppm. ^{13}C NMR (101 MHz, methanol-d_4) δ = 161.8, 155.1, 151.9, 142.5 (d, J = 4.3 Hz), 125.4, 90.2, 84.6 (d, J = 5.8 Hz), 83.9 (d, J = 183.8 Hz), 75.5, 71.3 (d, J = 5.3 Hz) ppm.^{19}F NMR (565 MHz, methanol-d_4) δ = –232.11 ppm. HRMS (ESI) calc. for $C_{10}H_{12}FClN_5O_3^+$ $(M+H)^+$: 304.0613, found: 304.0611.



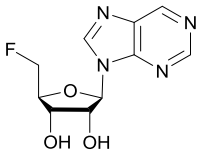
7a

(2R,3R,4S,5S)-2-(6-chloro-9H-purin-9-yl)-5-(fluoromethyl)tetrahydrofuran-3,4-diol (7a). Following general procedure D, **12a** (28 mg, 0.1 mmol) and 6-chloropurine (31 mg, 0.2 mmol) resulted in a crude mixture which was purified by column chromatography (CH_2Cl_2:MeOH 100:5) to yield **7a** (9 mg, 31%) over the two steps. ^{1}H NMR (600 MHz, methanol-d_4) δ = 8.76 (s, 1 H), 8.64 (s, 1 H), 6.20 (d, J = 4.2 Hz, 1 H), 4.82–4.63 (m, 3 H), 4.45 (t, J = 5.2 Hz, 1 H), 4.31–4.23 (m, 1 H) ppm. ^{13}C NMR (151 MHz, methanol-d_4) δ = 153.3, 153.0, 151.7, 146.5, 133.1, 91.0, 84.9 (d, J = 18.7 Hz), 83.7 (d, J = 170.3 Hz), 75.8, 71.1 (d, J = 5.3 Hz) ppm. ^{19}F NMR (565 MHz, methanol-d_4) δ = –232.50 ppm. HRMS (ESI) calc. for $C_{10}H_{11}FClN_4O_3^+$ $(M+H)^+$: 289.0514, found: 289.0507.



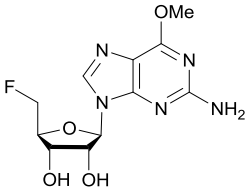
8a

(2R,3R,4S,5S)-2-(2-amino-9H-purin-9-yl)-5-(fluoromethyl)tetrahydrofuran-3,4-diol (8a). Et_3N (0.1 mL) was added to compound **6a** (10 mg) and Pd/C (2 mg) in THF (2 mL). The reaction mixture was stirred under a hydrogen balloon overnight. Reaction mixture was filtered and solvent removed; the crude was purified using column chromatography (CH_2Cl_2:MeOH 100:4) to yield **8a** (5.5 mg, 61%). ^{1}H NMR (600 MHz, methanol-d_4) δ = 8.57 (s, 1 H), 8.15 (s, 1 H), 6.01 (d, J = 4.6 Hz, 1 H), 4.78–4.66 (m, 2 H), 4.64 (td, J = 4.9, 1.2 Hz, 2 H), 4.22 (dddd, J = 27.3, 5.1, 3.7, 2.8 Hz, 1 H) ppm. ^{13}C NMR (151 MHz, methanol-d_4) δ = 162.2, 154.6, 150.2, 142.8, 128.6, 89.8, 84.6 (d, J = 18.5 Hz), 83.8 (d, J = 170.2 Hz), 75.4, 71.3 (d, J = 5.4 Hz) ppm. ^{19}F NMR (565 MHz, methanol-d_4) δ = –232.13 ppm. HRMS (ESI) calc. for $C_{10}H_{13}FN_5O_3^+$ $(M+H)^+$: 270.1002, found: 270.1007.



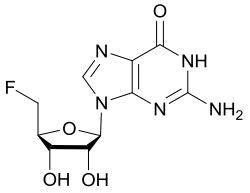
9a

(2S,3S,4R,5R)-2-(fluoromethyl)-5-(9H-purin-9-yl)tetrahydrofuran-3,4-diol (9a). Et_3N (0.1 mL) was added to compound **7a** (10 mg) and Pd/C (5 mg) in THF (3 mL). The reaction mixture was stirred under a hydrogen balloon overnight. Reaction mixture was filtered and solvent removed; the crude was purified using column chromatography (CH_2Cl_2:MeOH 100:5) to yield **9a** (3.4 mg, 43%). ^{1}H NMR (400 MHz, methanol-d_4) δ = 9.11 (s, 1 H), 8.95 (s, 1 H), 8.60 (s, 1 H), 6.22 (d, J = 4.3 Hz, 1 H), 4.77–4.71 (m, 2 H), 4.71–4.60 (m, 1 H), 4.46 (t, J = 5.1 Hz, 1 H), 4.35–4.18 (m, 1 H) ppm. ^{13}C NMR (101 MHz, methanol-d_4) δ = 153.7, 152.7, 149.1, 146.6 (d, J = 4.5 Hz), 135.6, 90.5, 84.9 (d, J = 18.4 Hz), 83.7 (d, J = 170.6 Hz), 75.7 (d, J = 2.0 Hz), 71.2 (d, J = 5.3 Hz) ppm. ^{19}F NMR (565 MHz, methanol-d_4) δ = –232.24 ppm. HRMS (ESI) calc. for $C_{10}H_{12}FN_4O_3^+$ $(M+H)^+$: 255.0893, found: 255.0901.



10a

(2R,3R,4S,5S)-2-(2-amino-6-methoxy-9H-purin-9-yl)-5-(fluoromethyl)tetrahydrofuran-3,4-diol (10a). 12a[6] (28 mg, 0.1 mmol) and 2-amino,6-chloropurine (34 mg, 0.2 mmol) was dissolved in MeCN (2 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (70 µL, 0.3 mmol) was added followed by TMSOTf (5 µL, 0.03 mmol). Reaction was stirred at 100°C for 4 h. Solvent was removed, diluted with EtOAc, washed with $NaHCO_3$ and dried with Na_2SO_4. The crude was then purified using column chromatography (DCM:MeOH 100:2) to give Ac-intermediate that was stirred with NaOMe (11 mg, 0.2 mmol) in MeOH (2 mL) at 50°C for 2 h. Solvent was removed and the resulting crude was purified by column chromatography (CH_2Cl_2:MeOH 100:4) to yield **10a** (15 mg, 50%) over the two steps. ^{1}H NMR (400 MHz, methanol-d_4) δ = 7.92 (s, 1 H), 5.95 (d, *J* = 4.5 Hz, 1 H), 4.77–4.59 (m, 2 H), 4.58–4.55 (m, 1 H), 4.39 (t, *J* = 5.1 Hz, 1 H), 4.26–4.14 (m, 1 H), 4.05 (s, 3 H) ppm. ^{13}C NMR (101 MHz, methanol-d_4) δ = 162.8, 162.1, 155.0, 139.0 (d, *J* = 4.2 Hz), 115.6, 89.9, 84.6 (d, *J* = 8.1 Hz), 83.7 (d, *J* = 143.9 Hz), 75.6 (d, *J* = 2.0 Hz), 71.3 (d, *J* = 5.2 Hz), 54.3 ppm. ^{19}F NMR (376 MHz, methanol-d_4) δ = –232.12 ppm. HRMS (ESI) calc. for $C_{11}H_{15}FN_5O_4^+$ $(M+H)^+$: 300.1108, found: 300.1111.



11a

2-amino-9-((2R,3R,4S,5S)-5-(fluoromethyl)-3,4-dihydroxytetrahydrofuran-2-yl)-1,9-dihydro-6H-purin-6-one (11a). 12a[6] (28 mg, 0.1 mmol) and N2-acetylguanine (38 mg, 0.2 mmol) was dissolved in MeCN (2 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (70 µL, 0.3 mmol) was added followed by TMSOTf (5 µL, 0.03 mmol). Solvent was removed, diluted with EtOAc, washed with $NaHCO_3$ and dried with Na_2SO_4. The crude was then purified using column chromatography (CH_2Cl_2:MeOH 100:2) to give an Ac-intermediate that was stirred with NaOH (1M in H_2O, 3mL) in 70°C for 3 h. Solvent was removed and the resulting crude purified by column chromatography (CH_2Cl_2:MeOH:NH_4OH 100:5:0.1) to yield **11a** as a mixture of anomers (6 mg, 21%) over the two steps. First anomer: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.09 (s, 1 H), 6.33 (s, 2 H), 6.05 (d, *J* = 5.1 Hz, 1 H), 4.85–4.63 (m, 1 H), 4.57 (qd, *J* = 10.4, 4.2 Hz, 1 H), 4.42 (t, *J* = 4.7 Hz, 1 H), 4.07 (dtd, *J* = 20.0, 4.8, 3.3 Hz, 2 H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = ^{13}C NMR (101 MHz, DMSO) δ = 171.3, 160.6, 155.1, 153.7, 141.5, 107.7, 89.5, 83.7, 82.3, 82.1 (d, *J* = 14.3 Hz), 73.9, 69.2 (d, *J* = 5.8 Hz) ppm. ^{19}F NMR (376 MHz, DMSO-d_6) δ = –226.69 ppm. Second anomer: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.80 (s, 1 H), 6.48 (s, 2 H), 5.73 (d, *J* = 5.2 Hz, 1 H), 5.54 (d, *J* = 5.6 Hz, 1 H), 5.32 (s, 1 H), 4.79–4.62 (m, 1 H), 4.60–4.49 (m, 1 H), 4.42 (q, *J* = 4.7 Hz, 1 H), 4.15 (s, 1 H), 4.06 (ddt, *J* = 23.8, 4.7, 2.4 Hz, 1 H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.3, 156.7, 153.7, 151.2, 135.2, 116.7, 86.7, 83.8, 82.2 (d, *J* = 18.6 Hz), 73.1, 69.5 (d, *J* = 5.6 Hz) ppm.

^{19}F NMR (376 MHz, DMSO-d_6) δ = –227.01 ppm. HRMS (ESI) calc. for $C_{10}H_{13}FN_5O_4^+$ $(M+H)^+$: 286.0946, found: 286.0958.

^{1}H, ^{13}C and ^{19}F NMR Spectra of Compounds

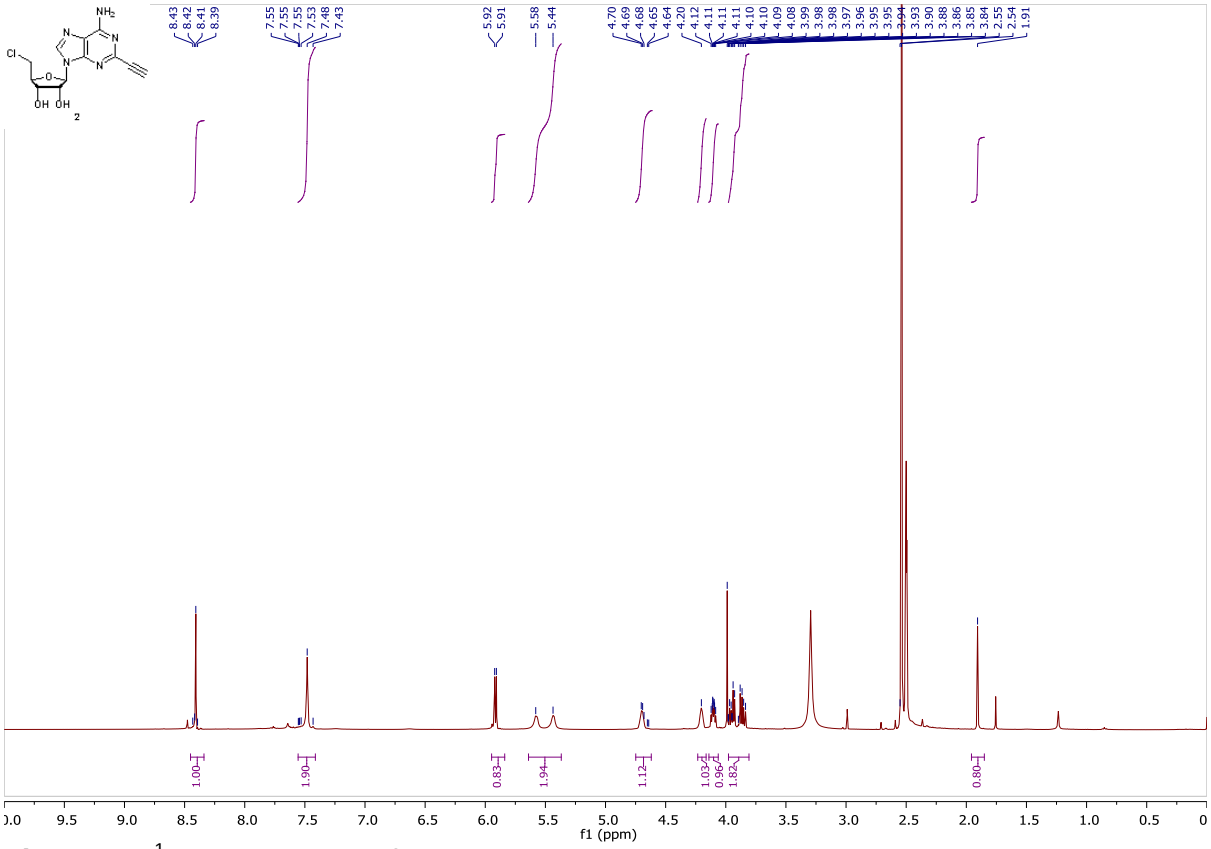


Figure S2. ^{1}H NMR spectra of **2**.

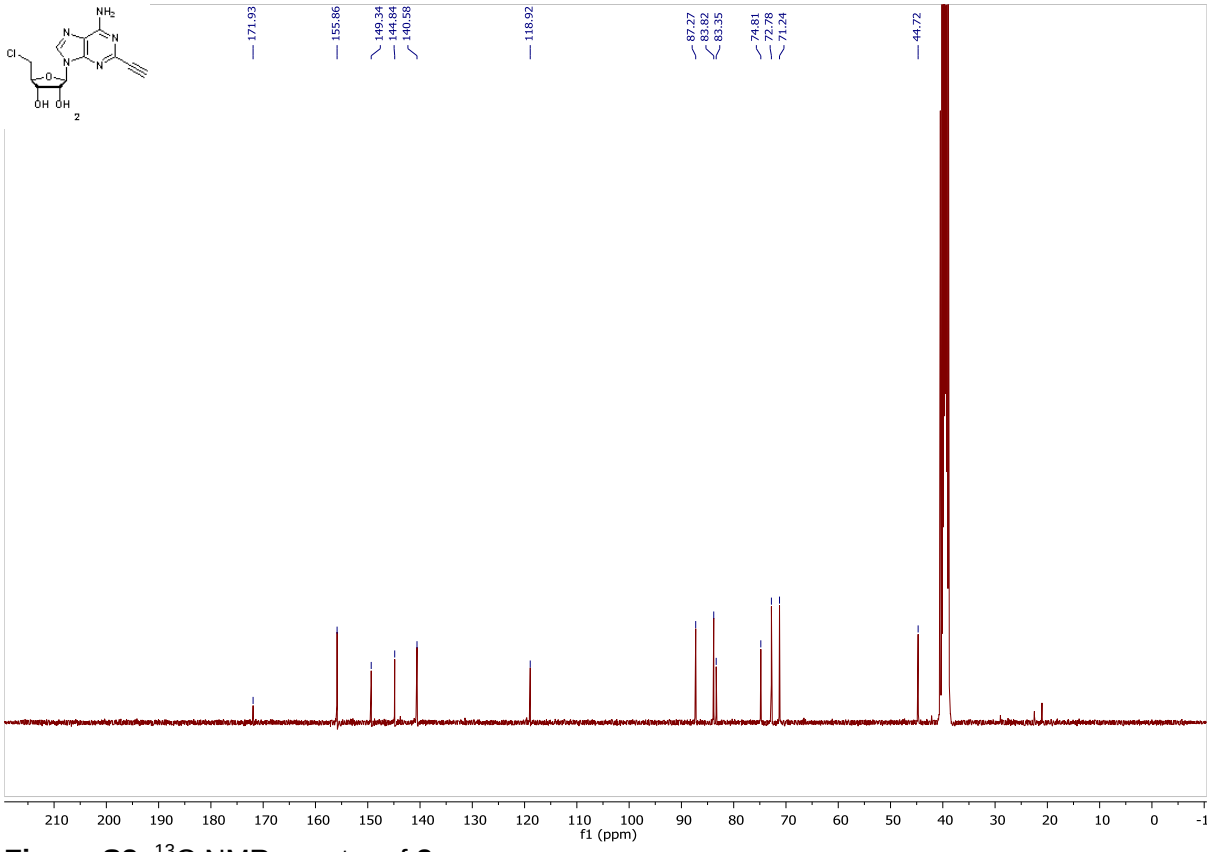


Figure S3. ^{13}C NMR spectra of **2**.

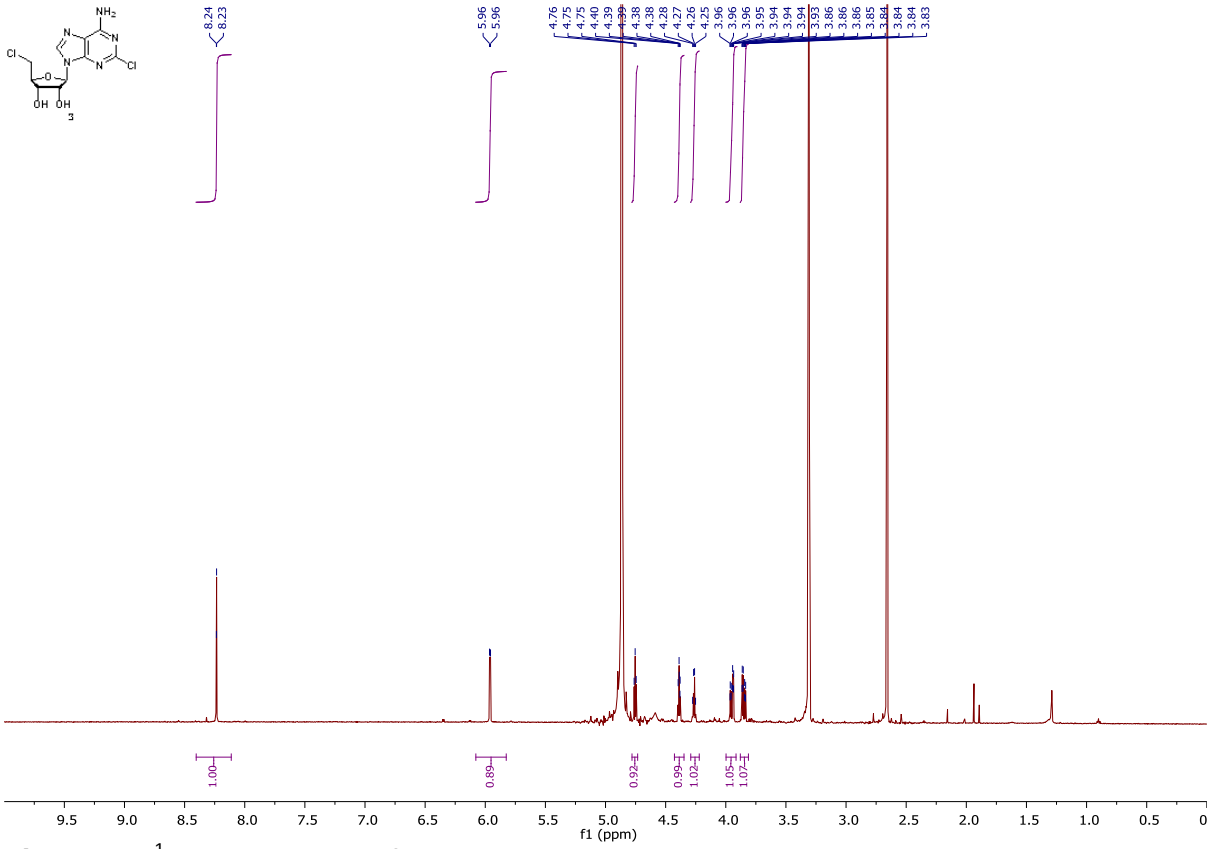


Figure S4. ^{1}H NMR spectra of **3**.

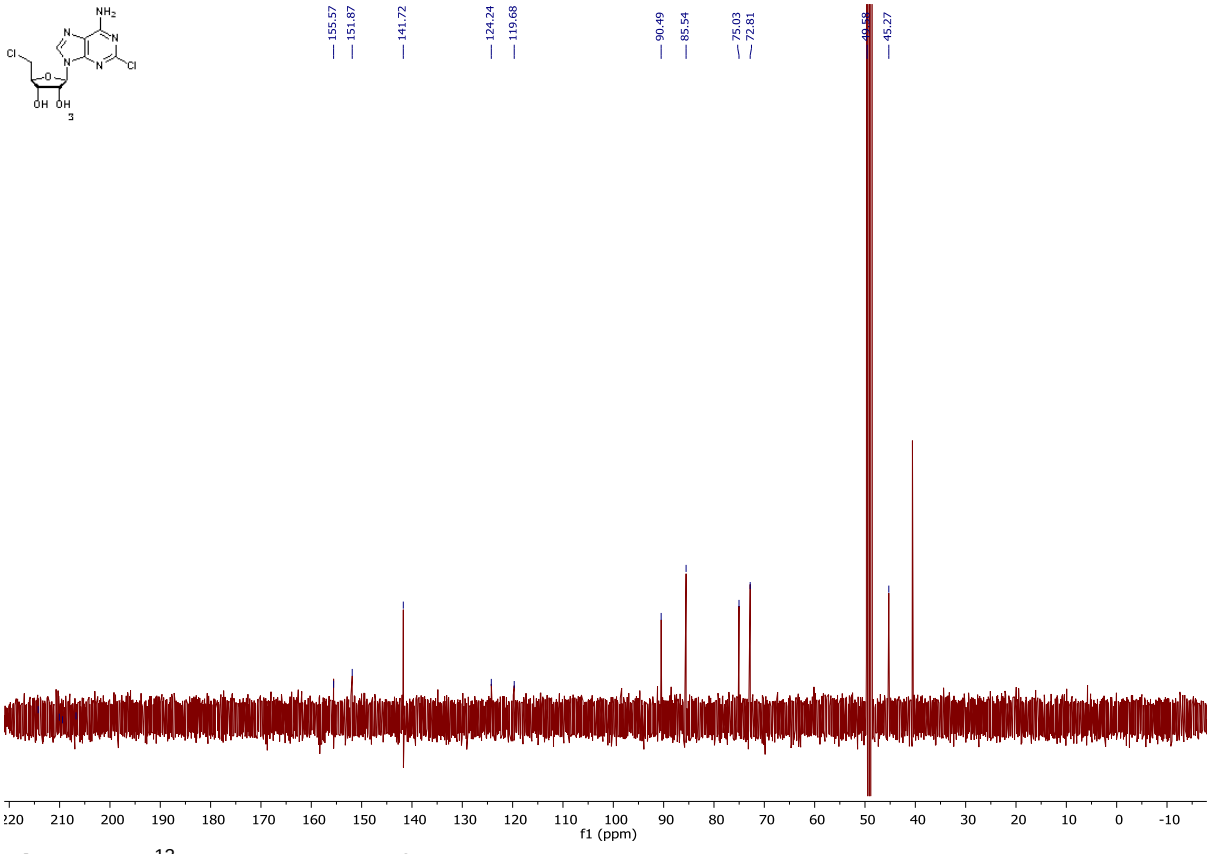


Figure S5. ^{13}C NMR spectra of **3**.

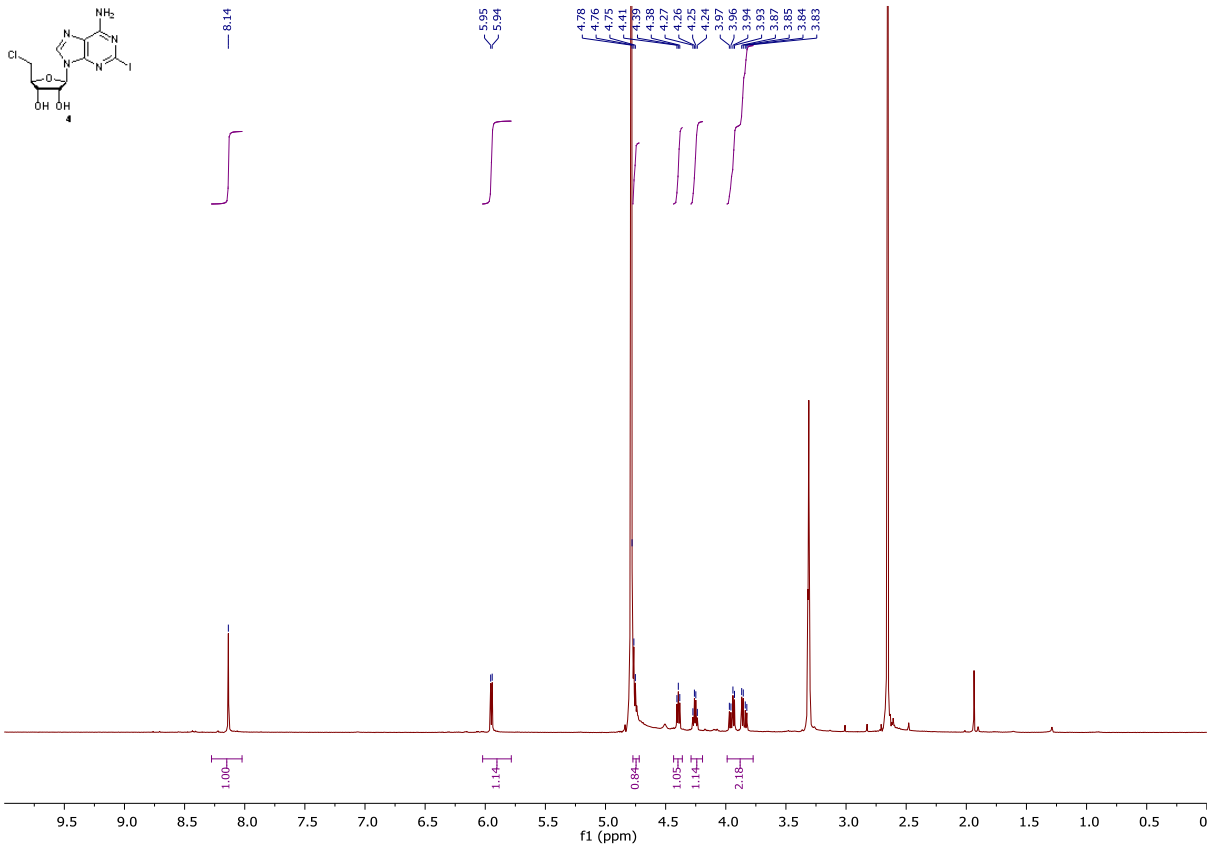


Figure S6. ^{1}H NMR spectra of **4**.

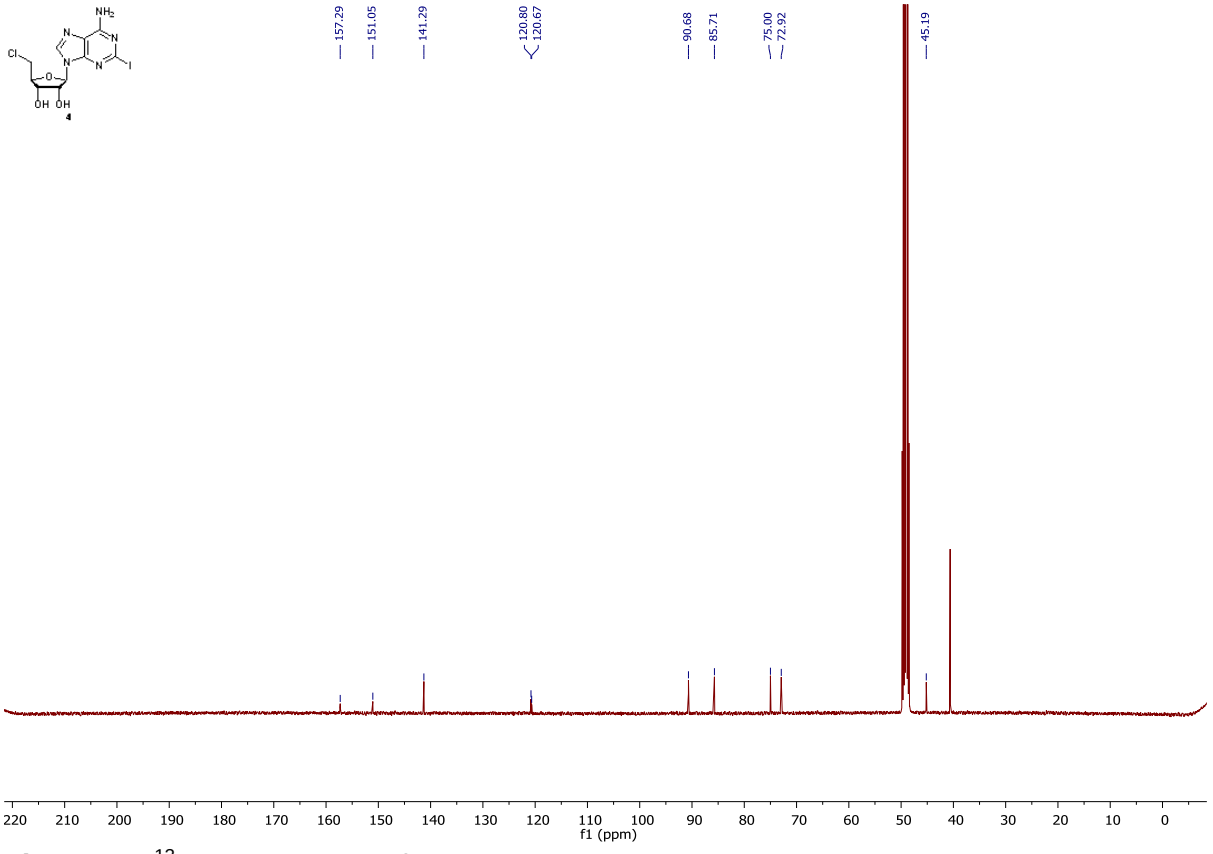


Figure S7. ^{13}C NMR spectra of **4**.

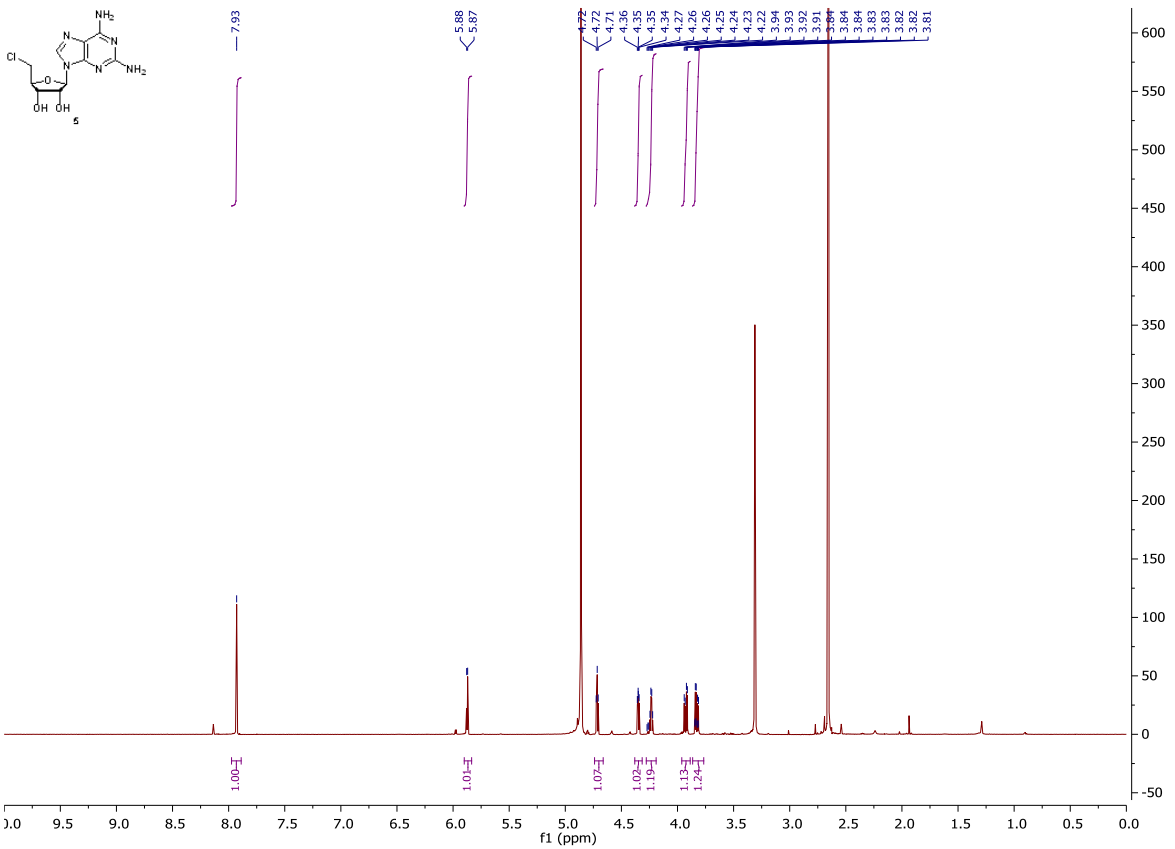


Figure S8. ^{1}H NMR spectra of **5**.

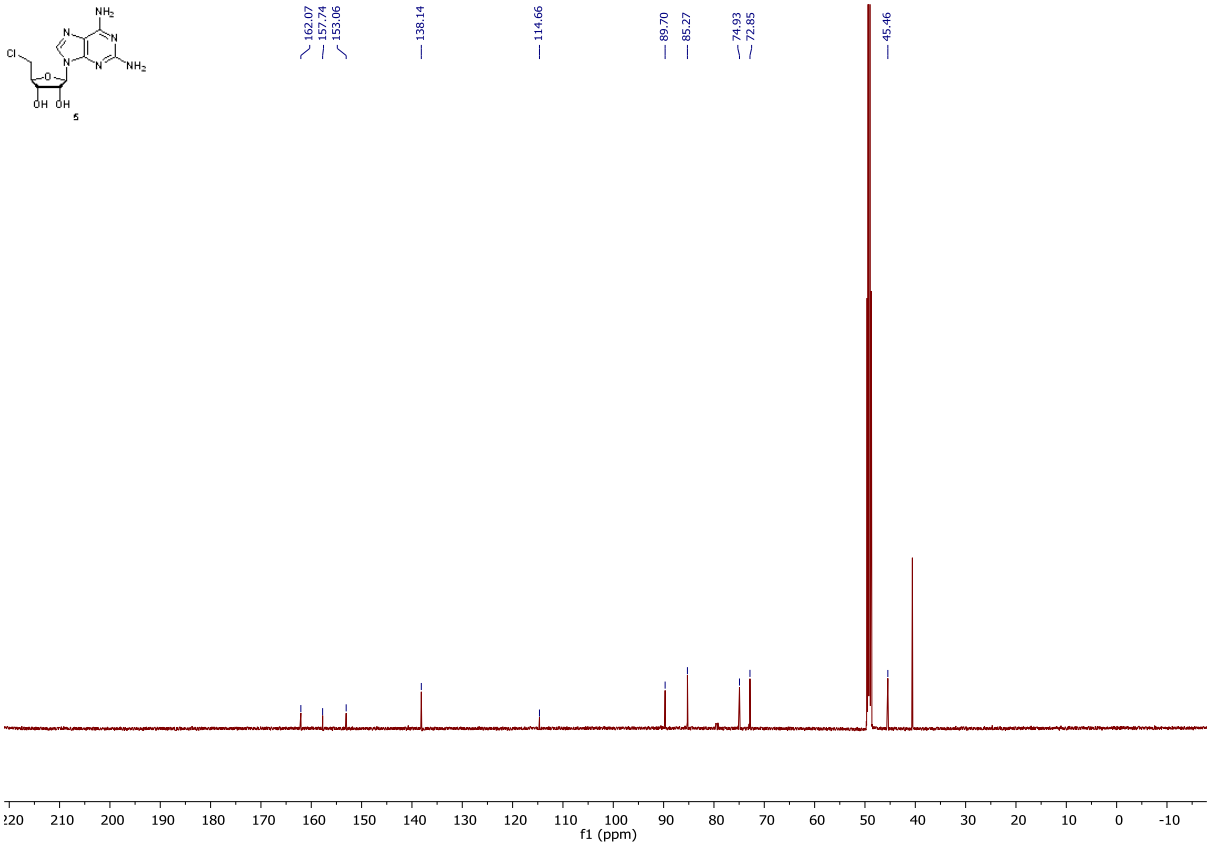


Figure S9. ^{13}C NMR spectra of **5**.

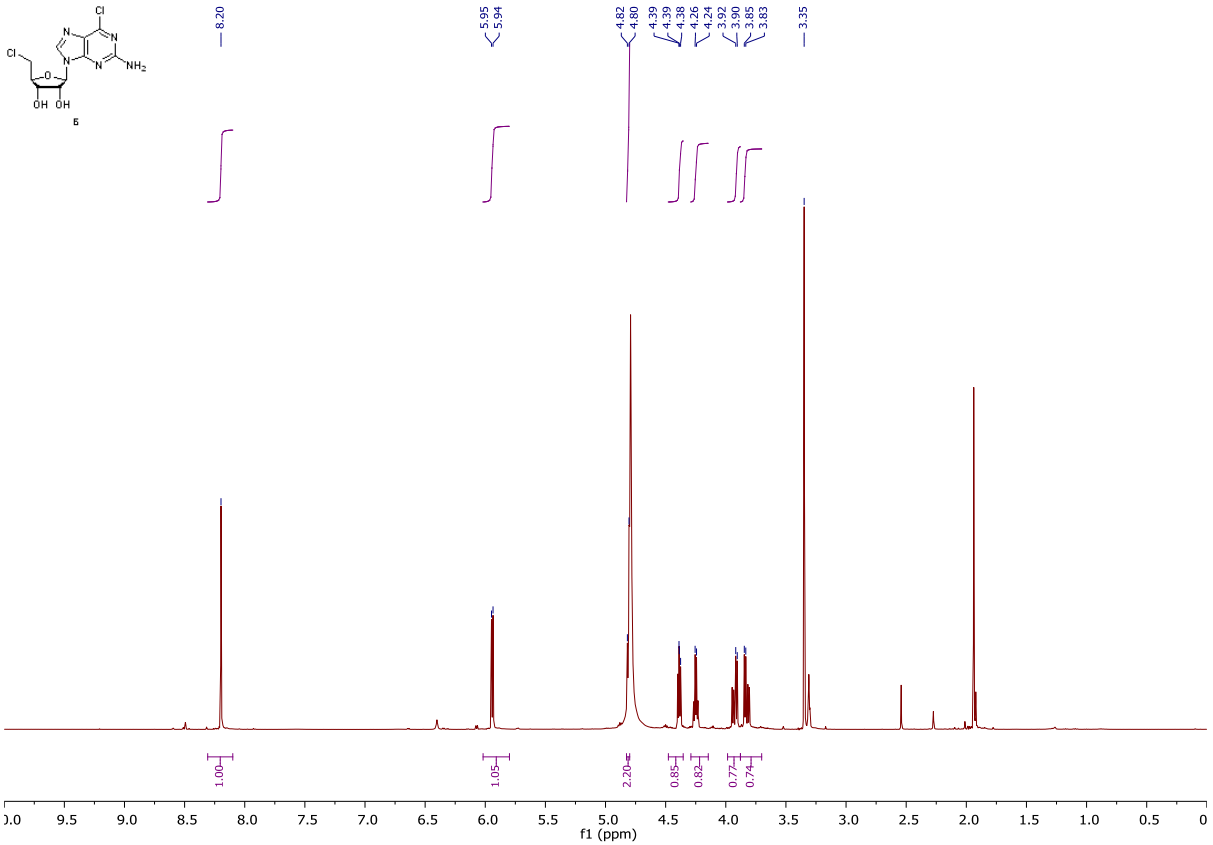


Figure S10. ^{1}H NMR spectra of **6**.

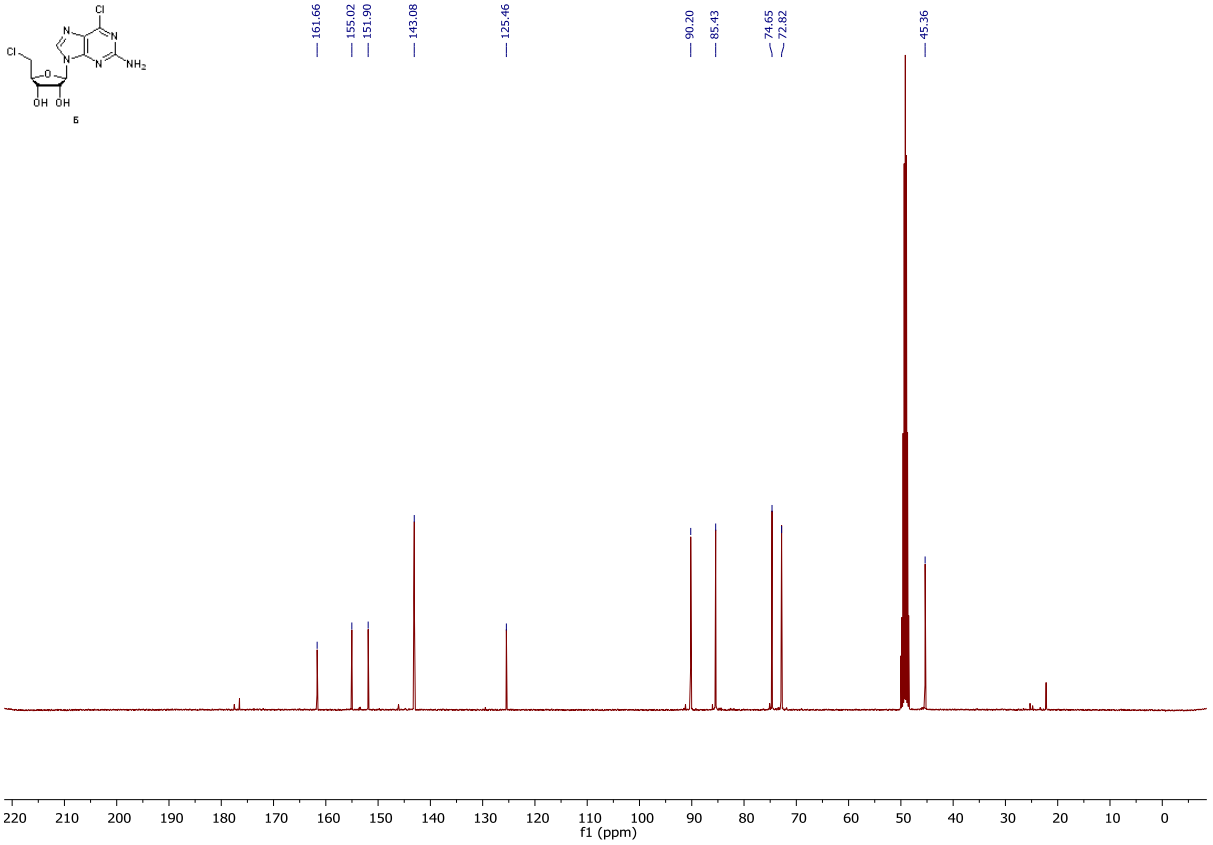


Figure S11. ^{13}C NMR spectra of **6**.

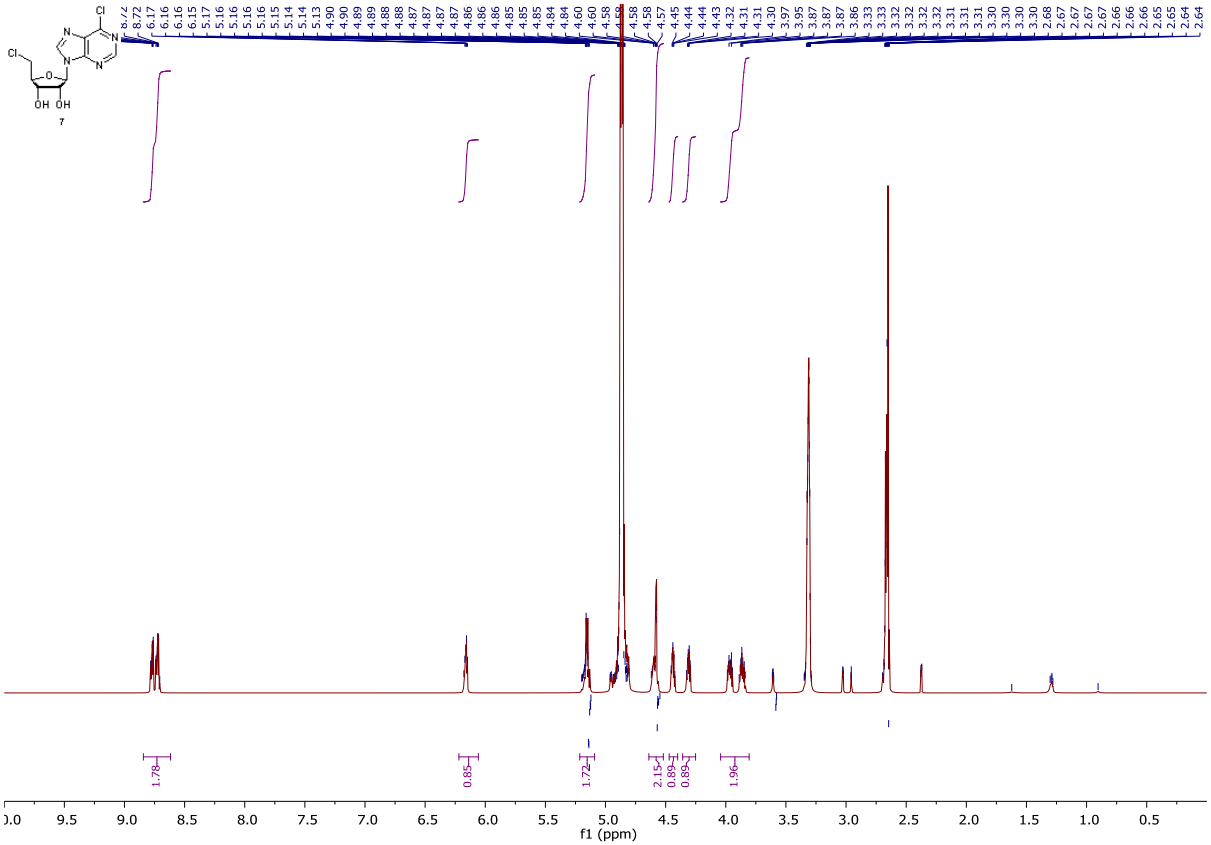


Figure S12. ^{1}H NMR spectra of **7**.

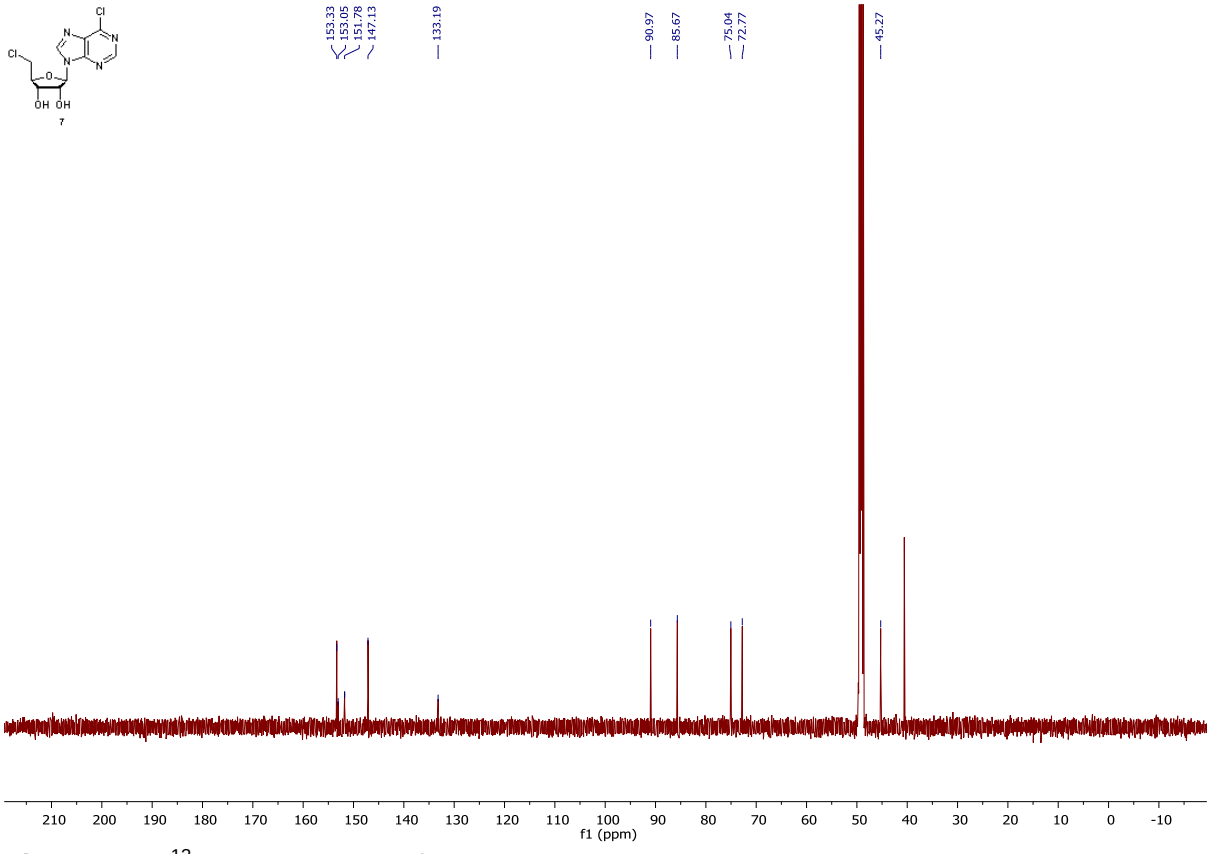


Figure S13. ^{13}C NMR spectra of **7**.

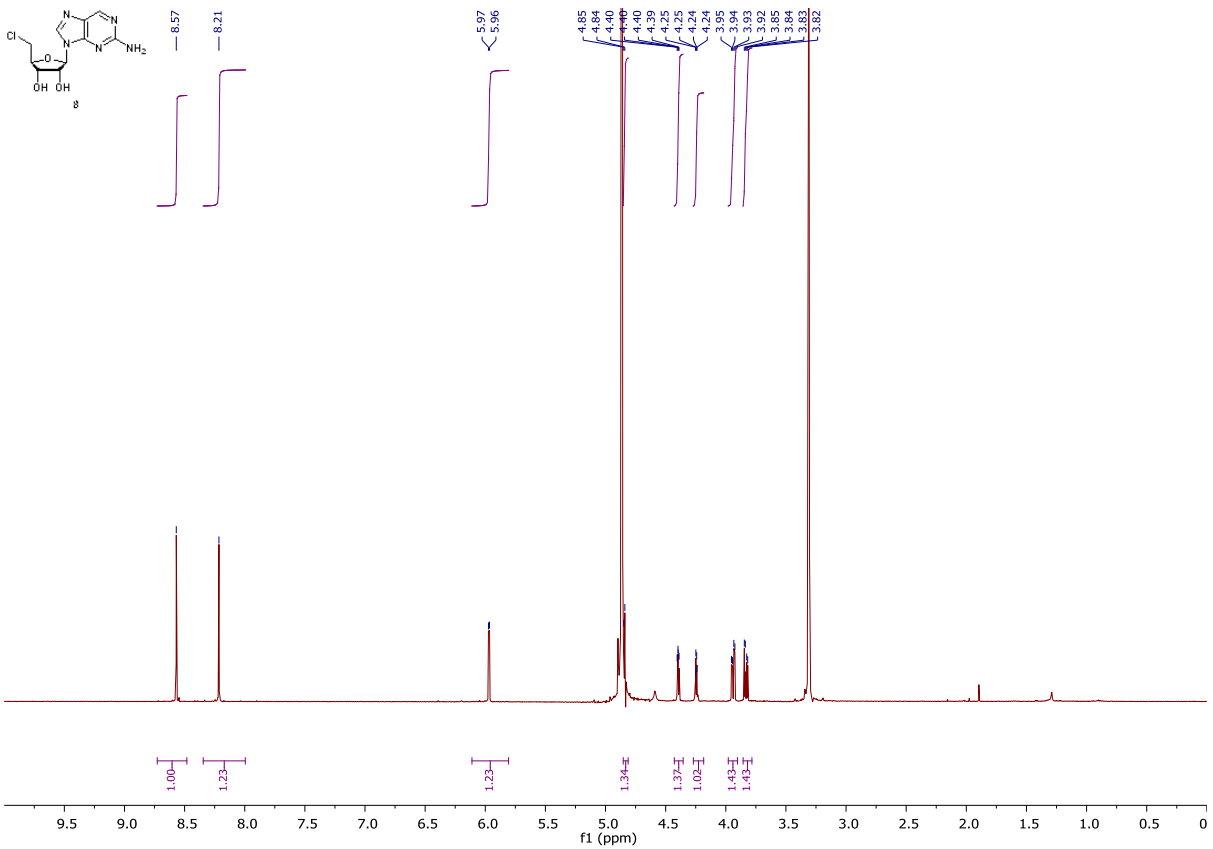


Figure S14. ^{1}H NMR spectra of **8**.

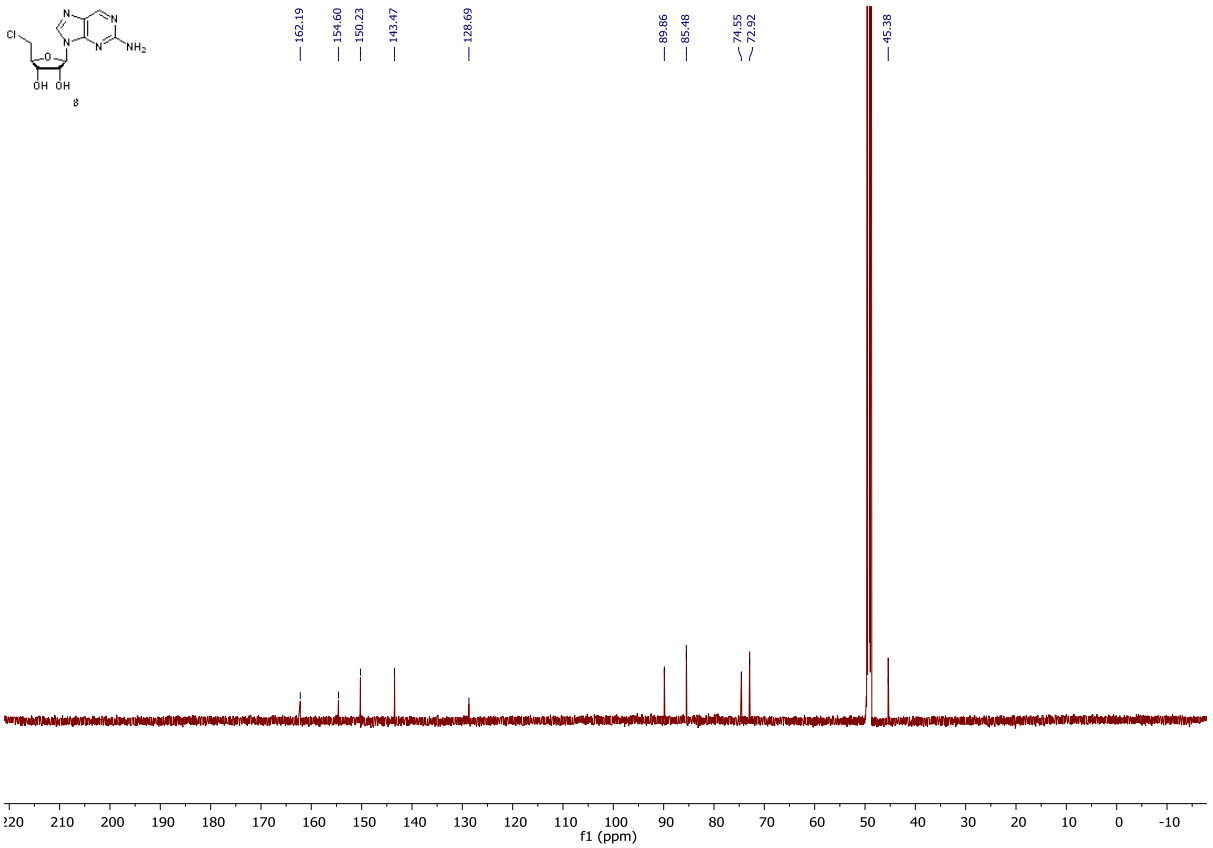


Figure S15. ^{13}C NMR spectra of **8**.

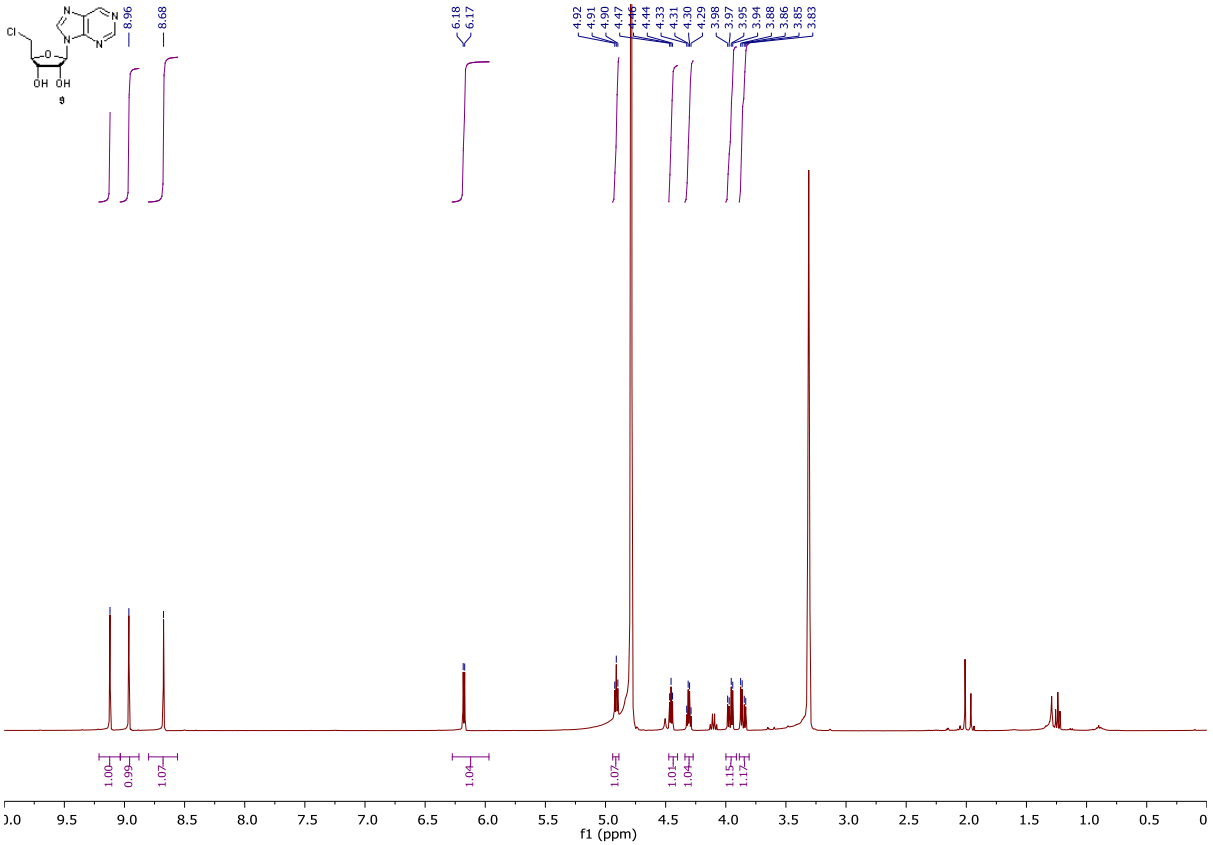


Figure S16. ^{1}H NMR spectra of **9**.

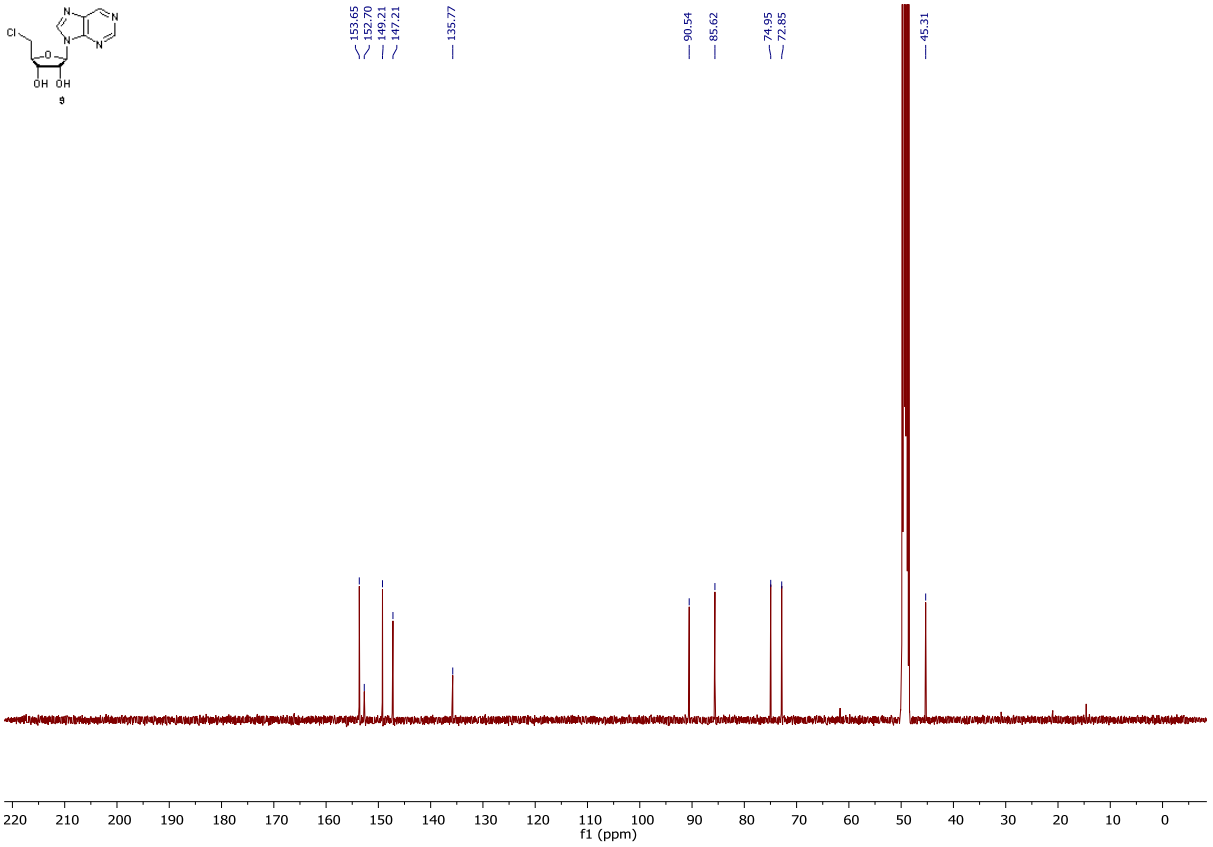


Figure S17. ^{13}C NMR spectra of **9**.

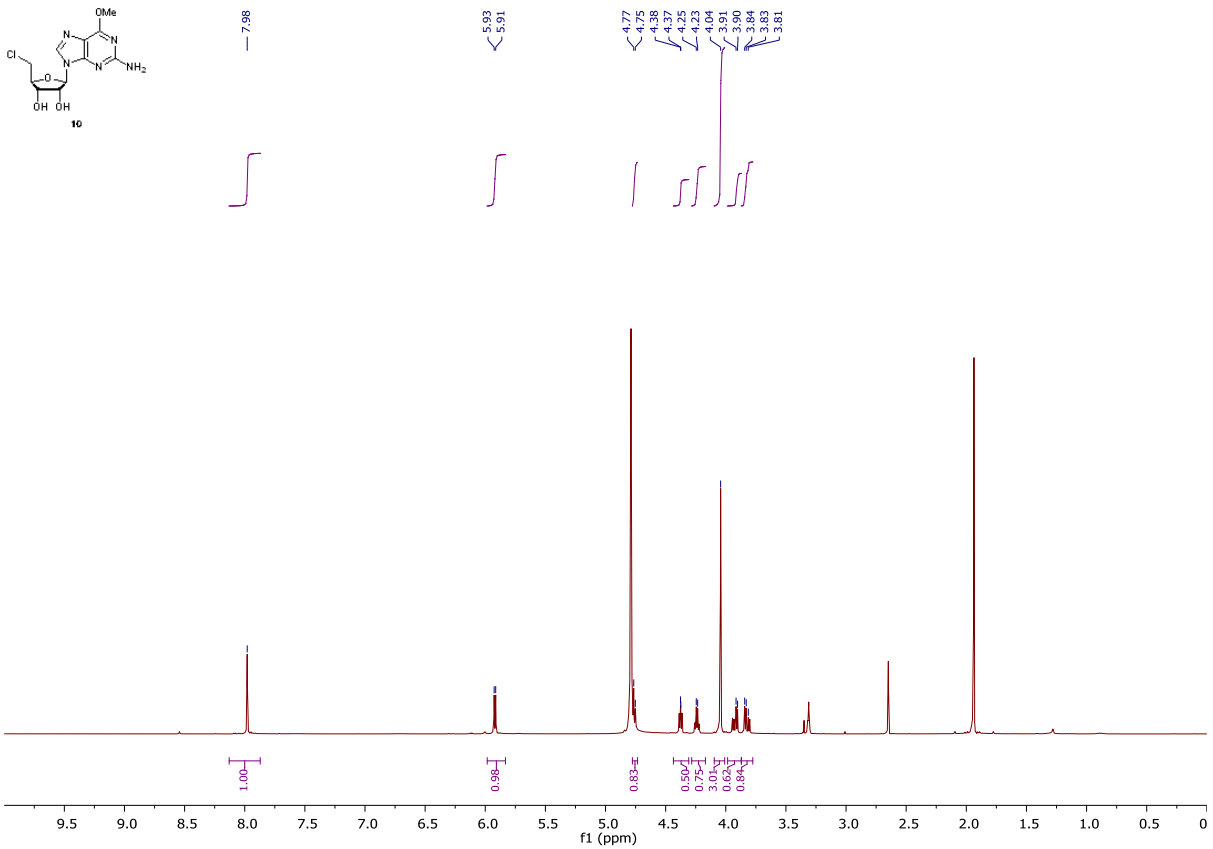


Figure S18. ^{1}H NMR spectra of **10**.

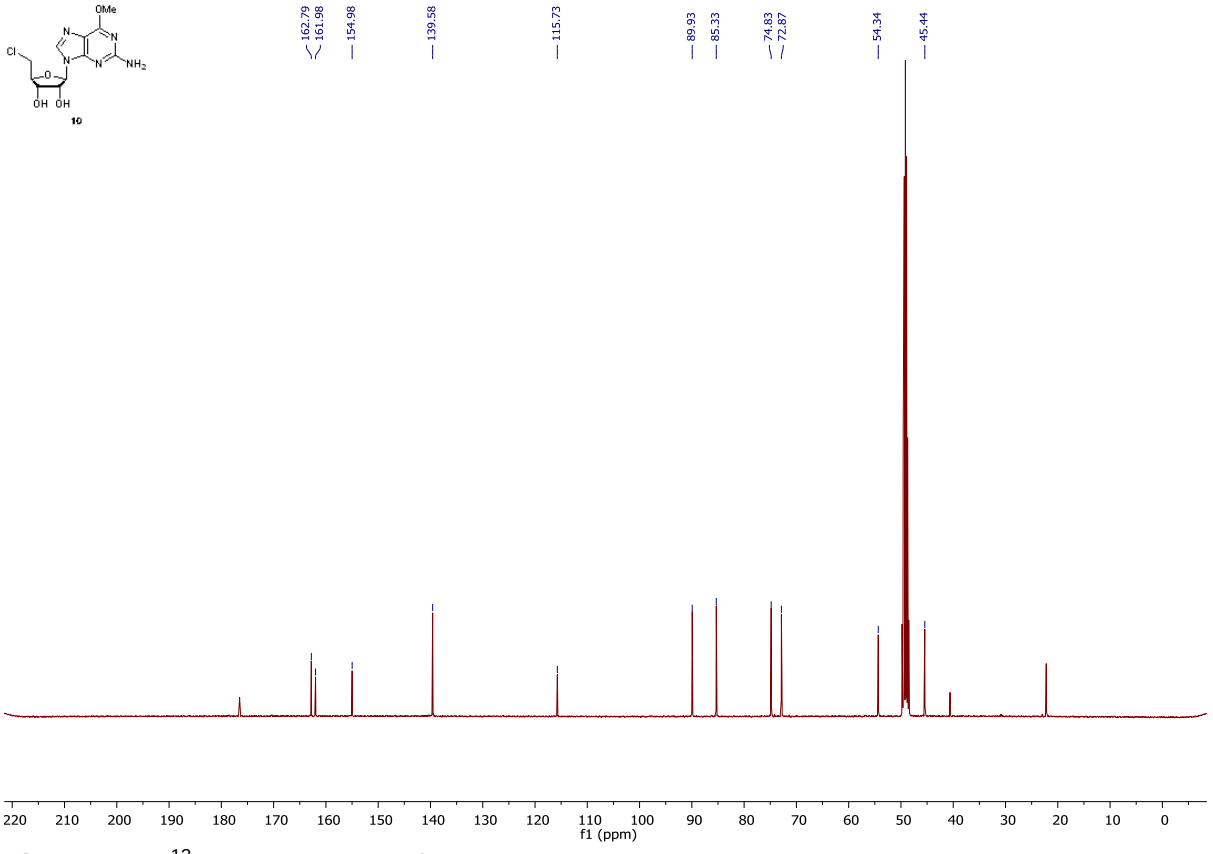


Figure S19. ^{13}C NMR spectra of **10**.

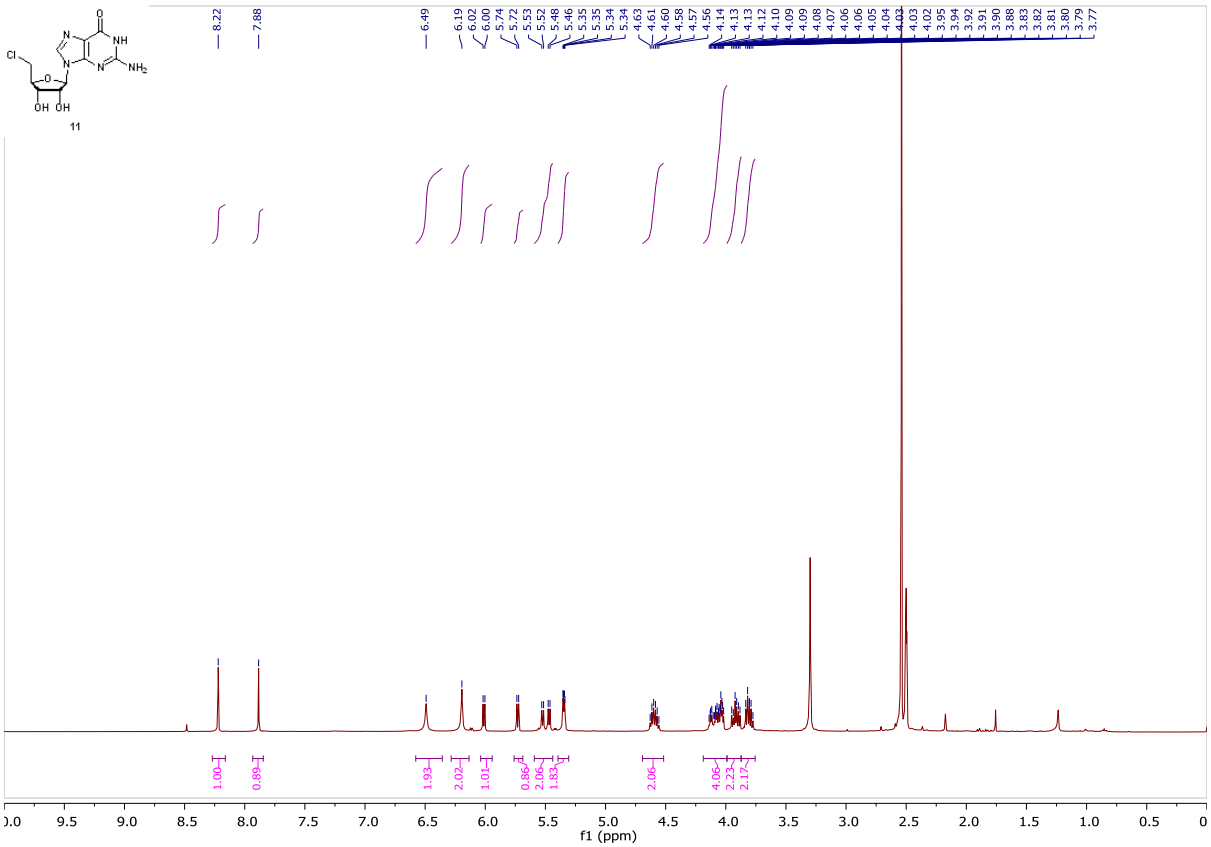


Figure S20. ^{1}H NMR spectra of **11**.

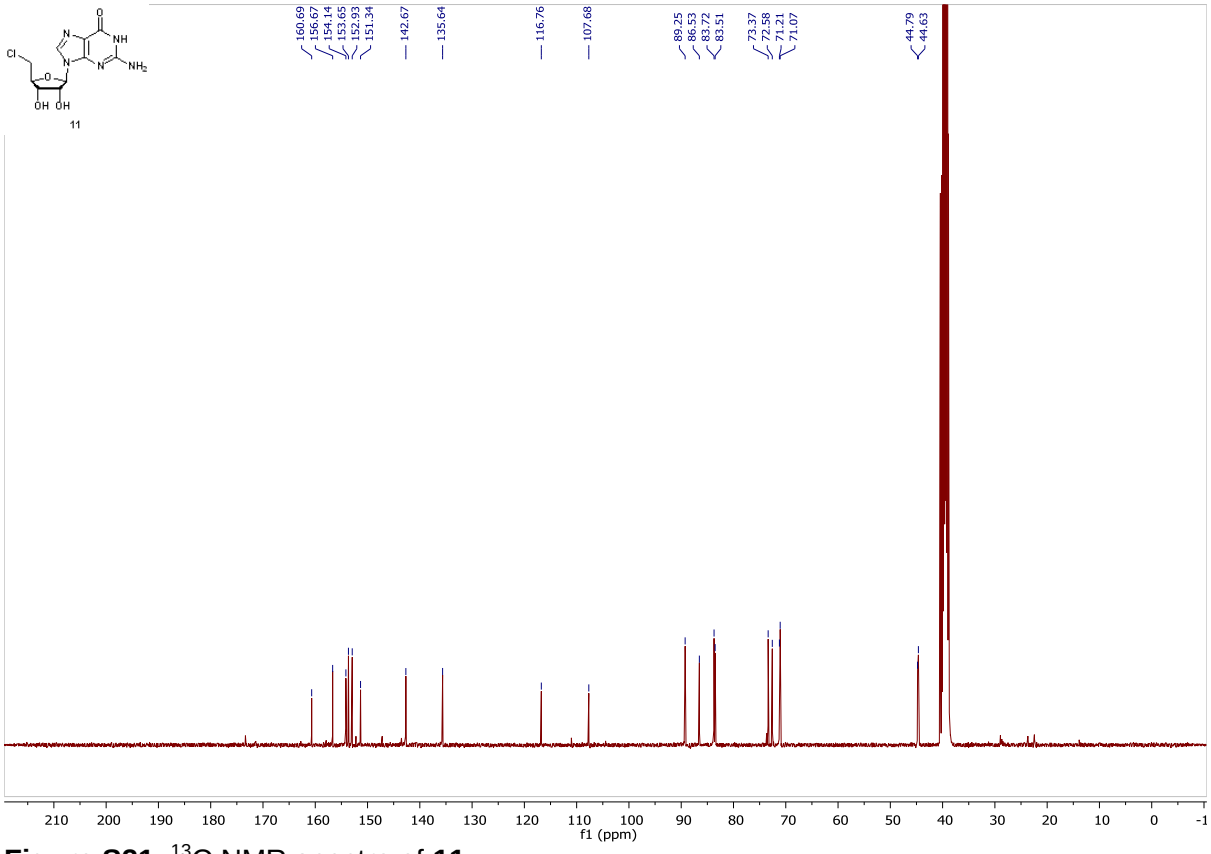


Figure S21. ^{13}C NMR spectra of **11**.

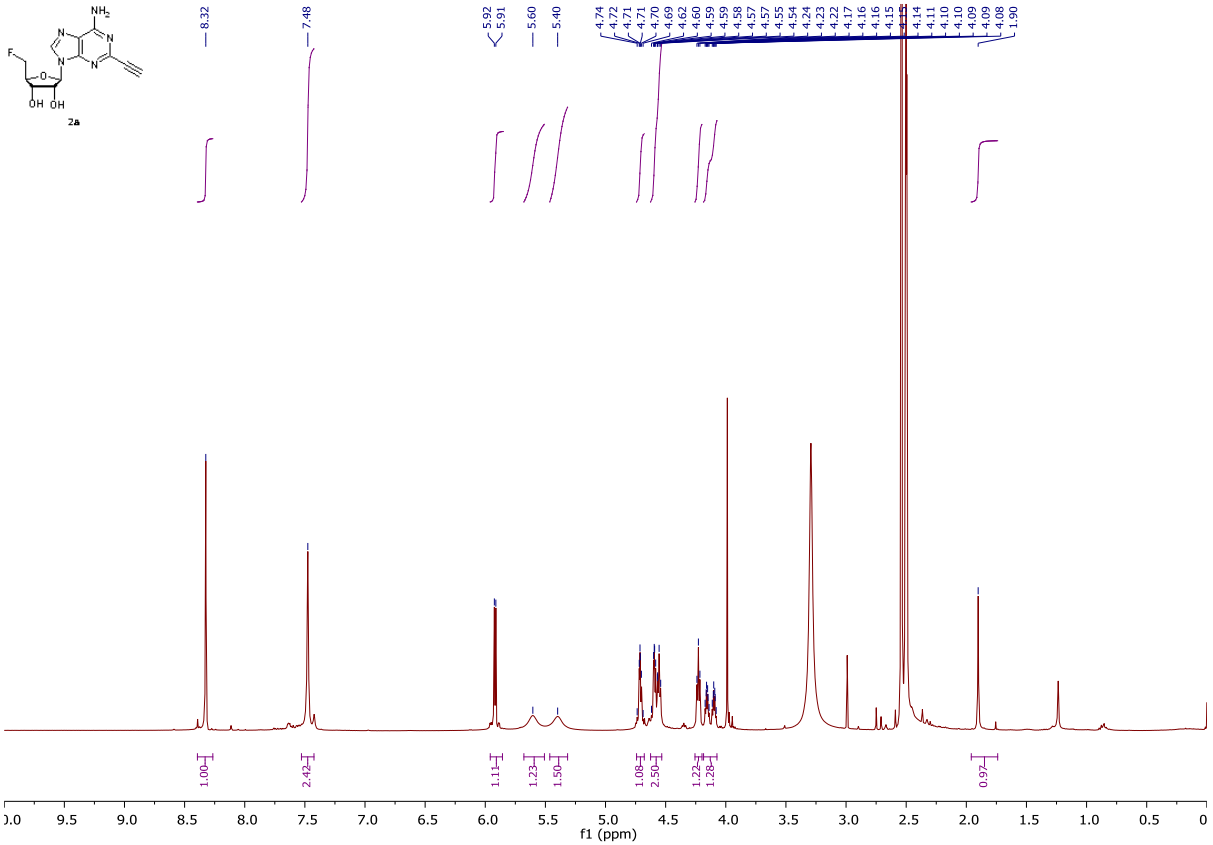


Figure S22. ^{1}H NMR spectra of **2a**.

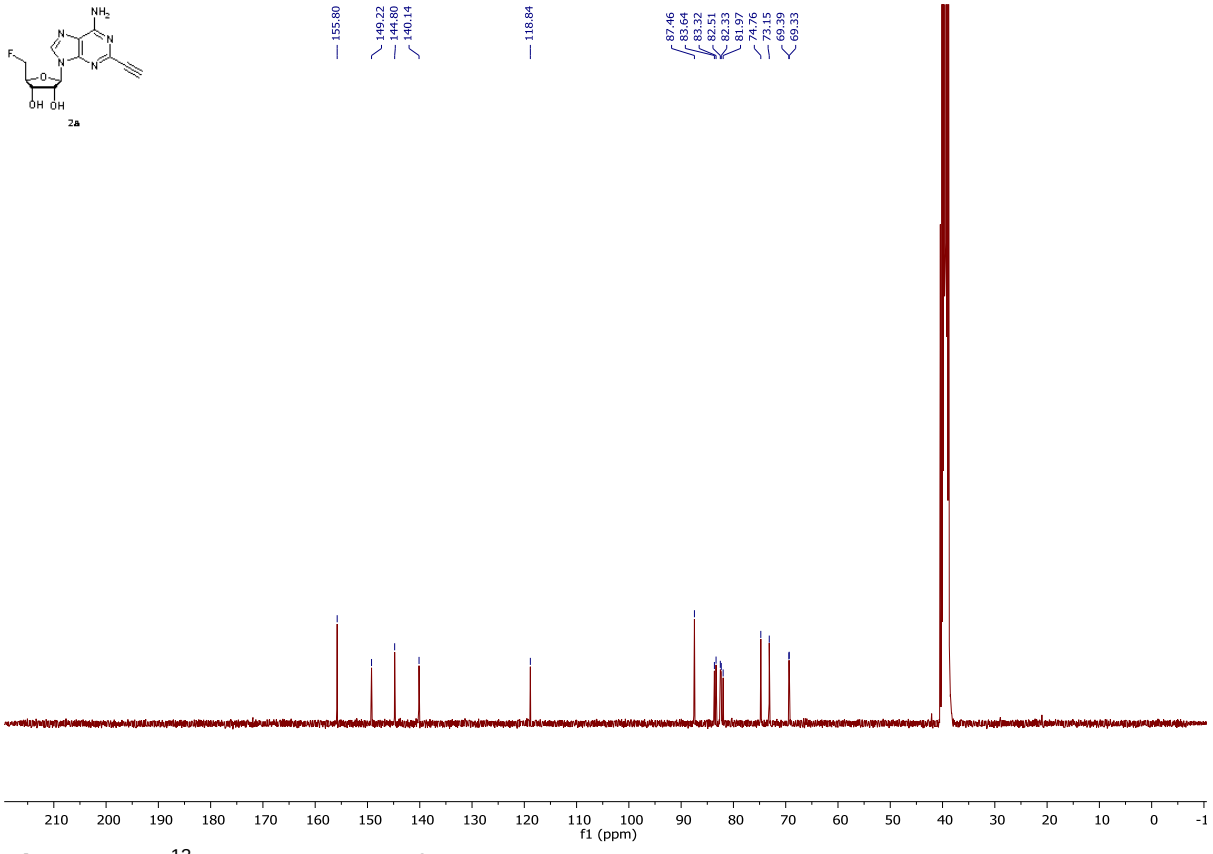


Figure S23. ^{13}C NMR spectra of **2a**.

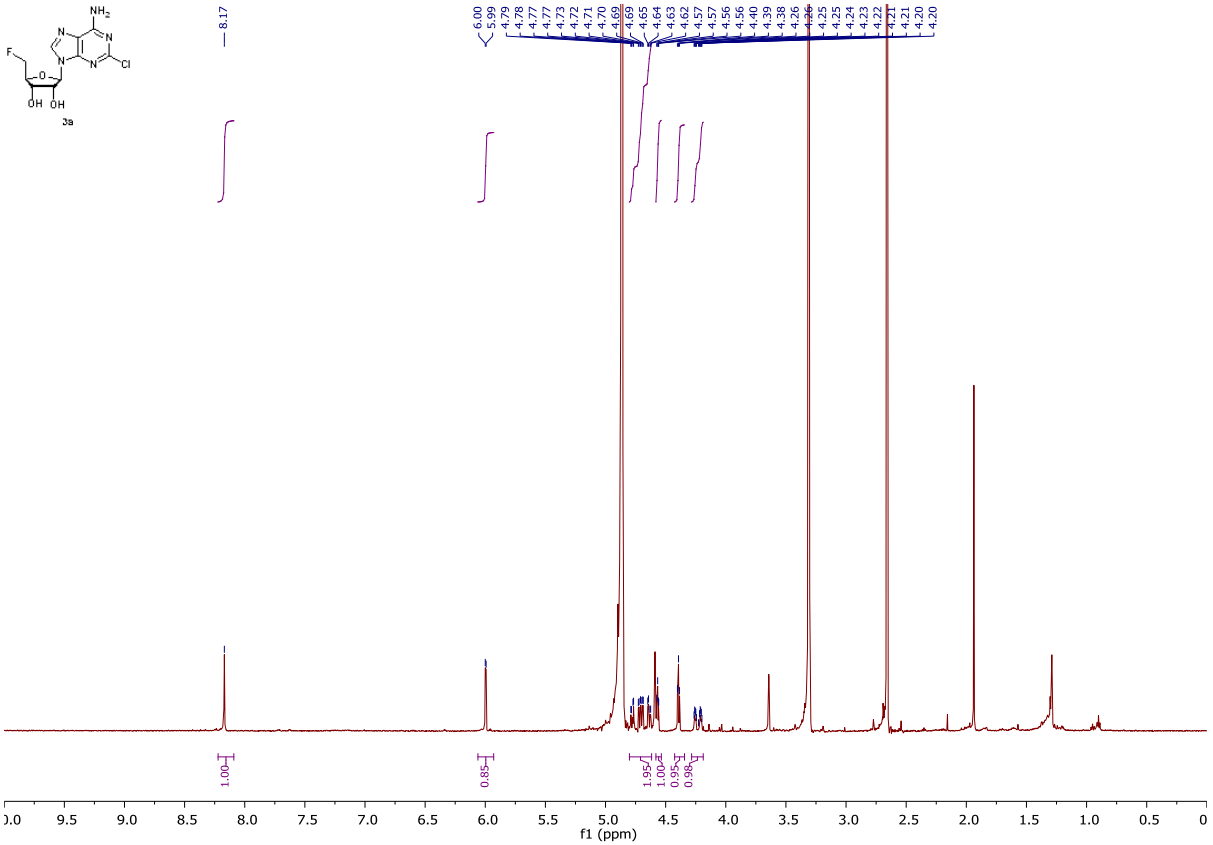


Figure S24. ^{1}H NMR spectra of **3a**.

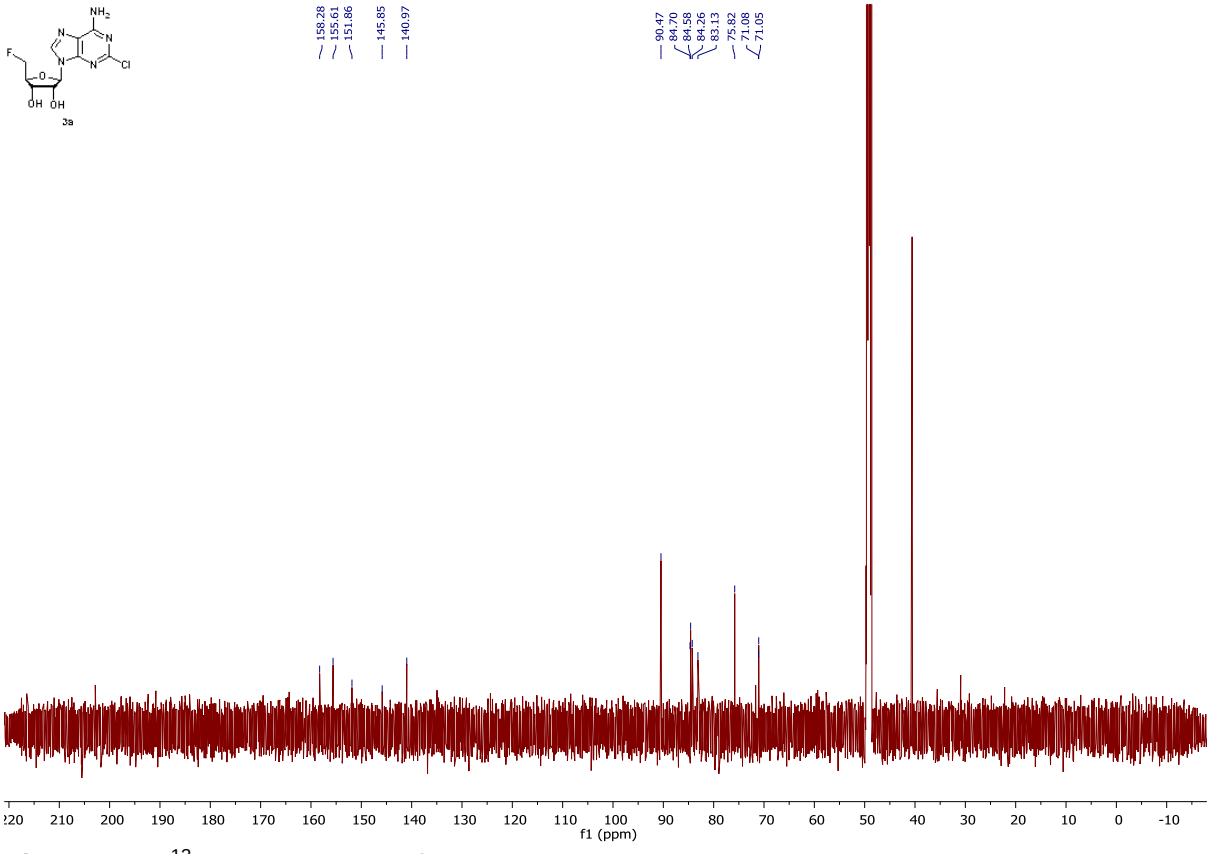


Figure S25. ^{13}C NMR spectra of **3a**.

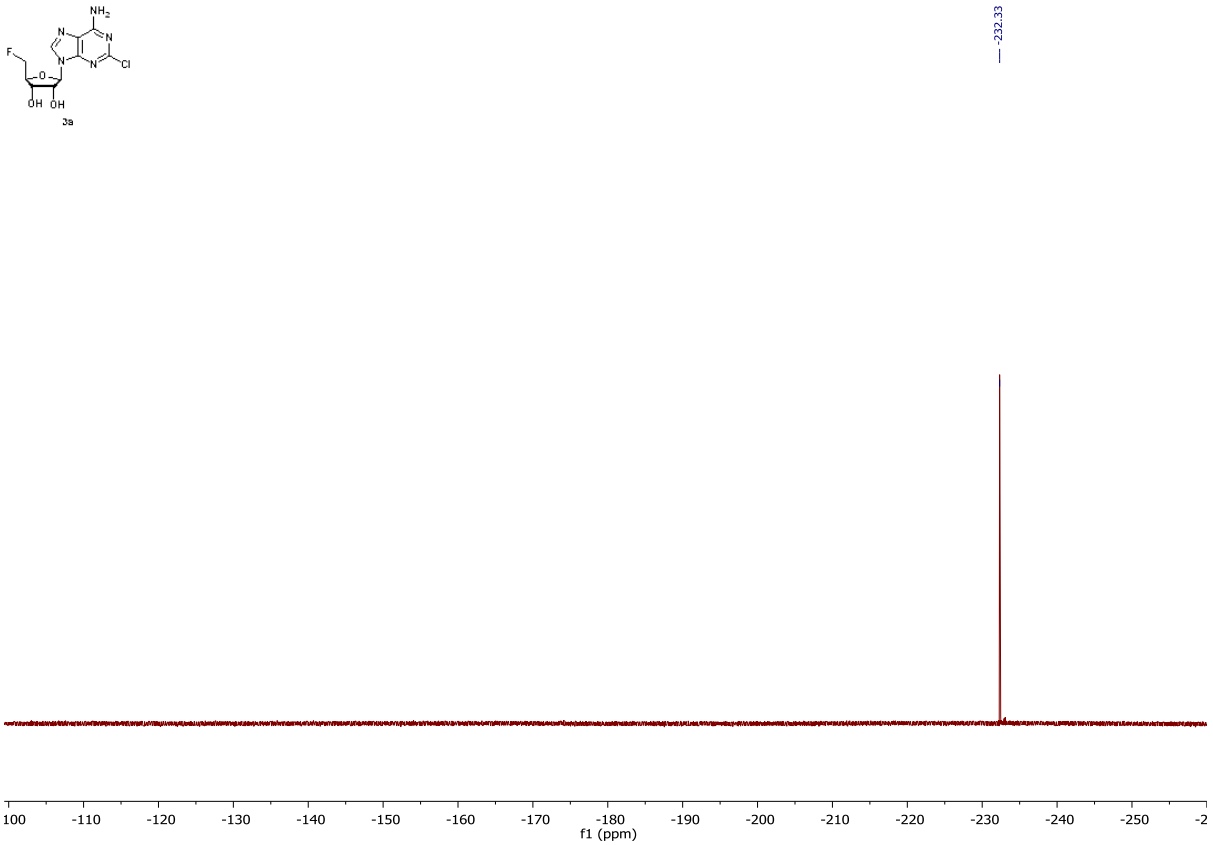


Figure S26. ^{19}F NMR spectra of **3a**.

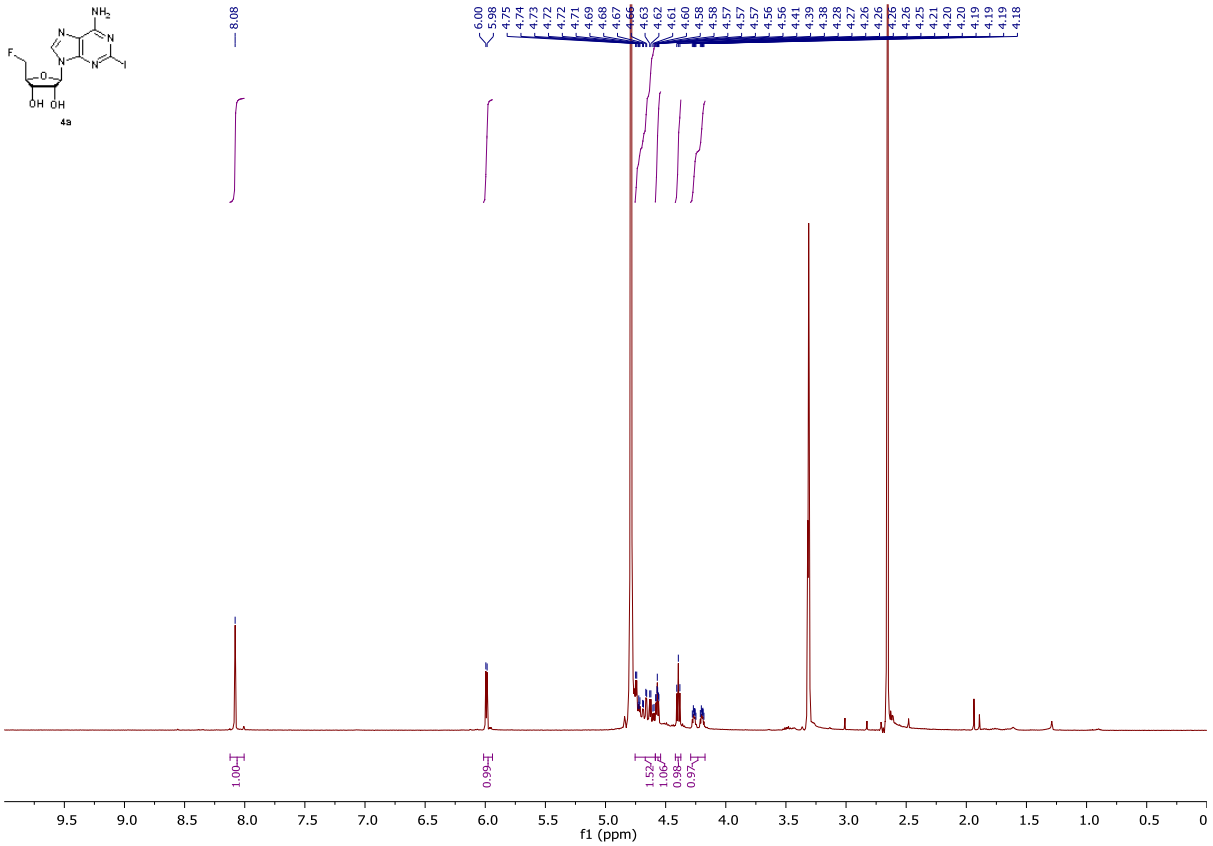


Figure S27. ^{1}H NMR spectra of **4a**.

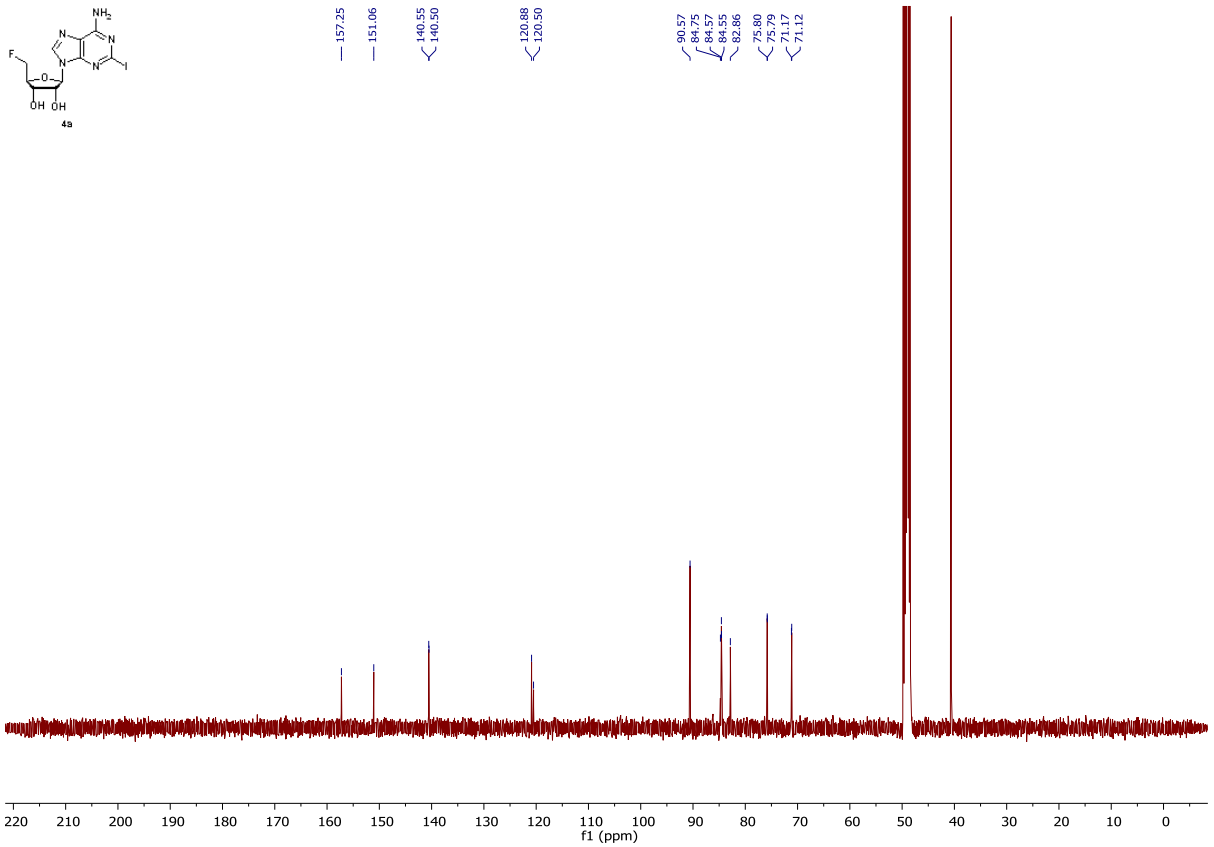


Figure S28. ^{13}C NMR spectra of **4a**.

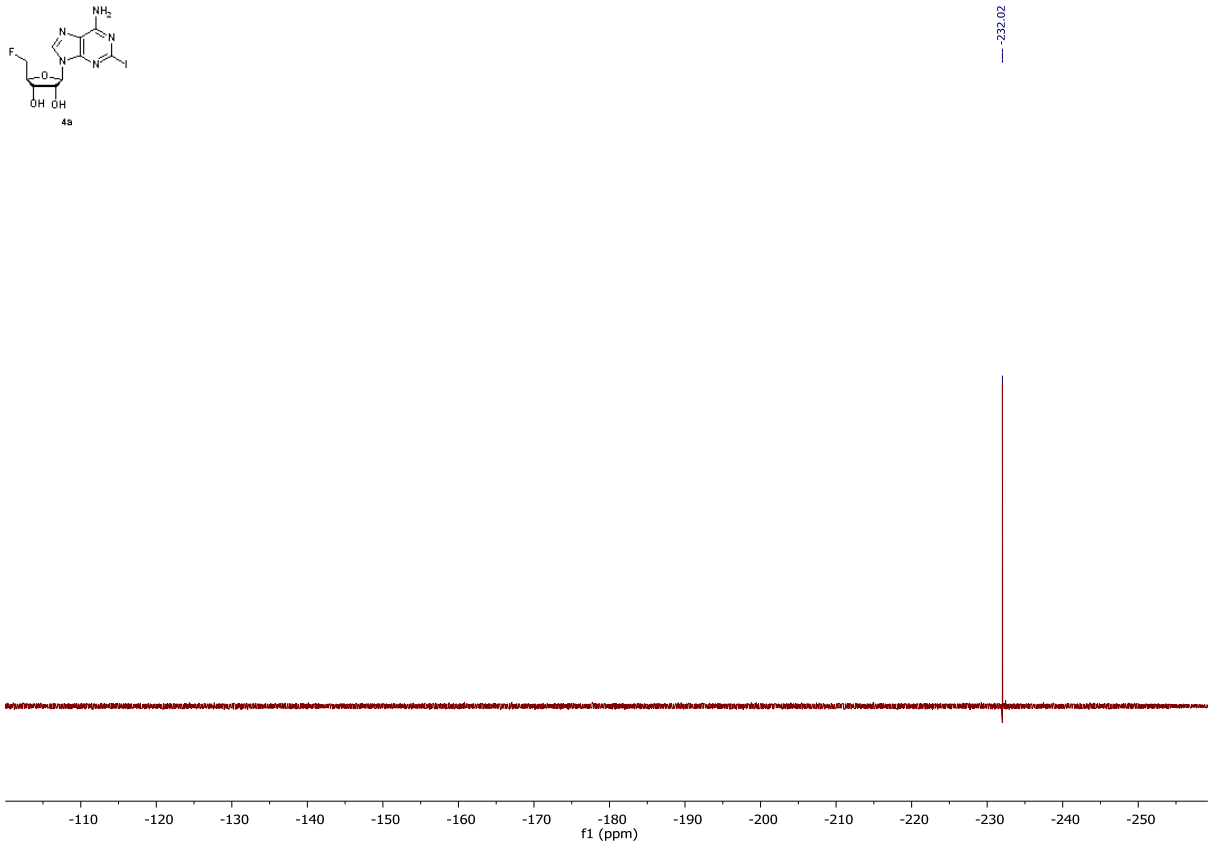


Figure S29. ^{19}F NMR spectra of **4a**.

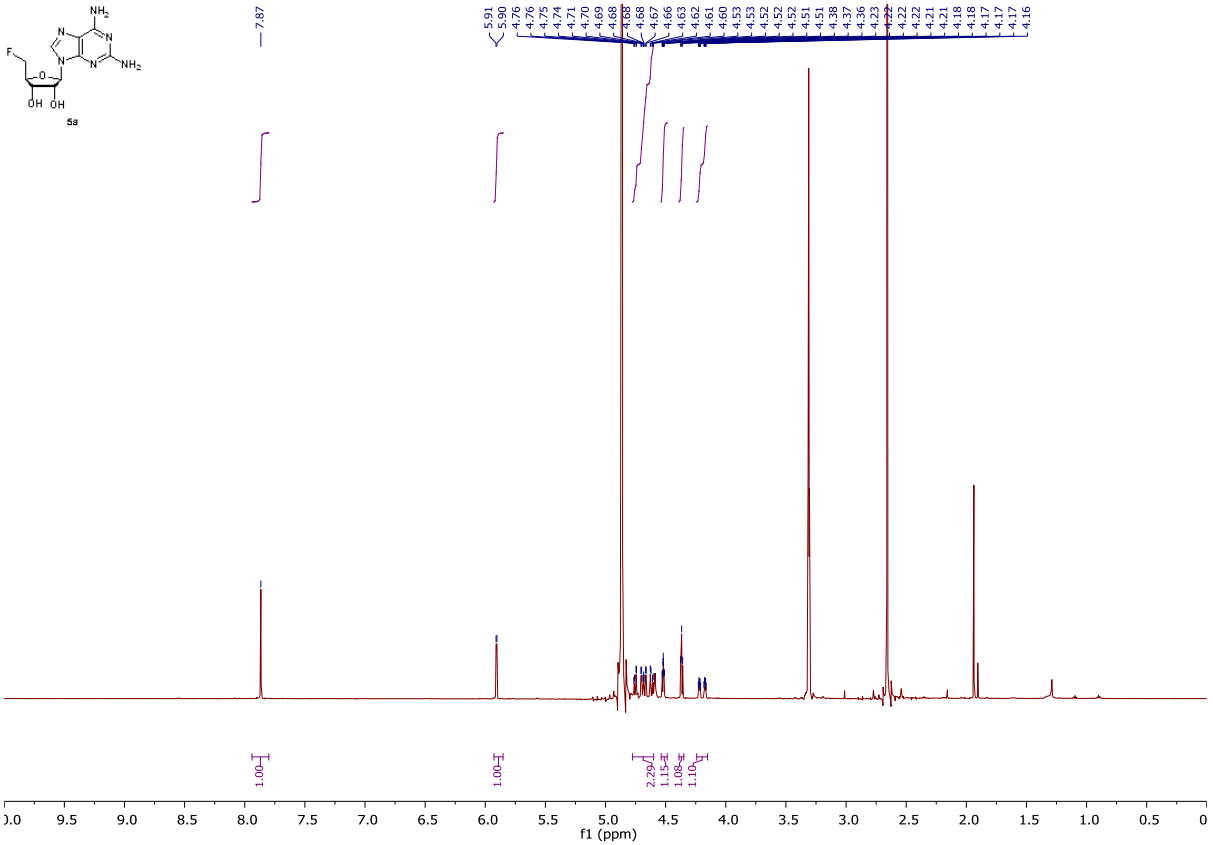


Figure S30. ^{1}H NMR spectra of **5a**.

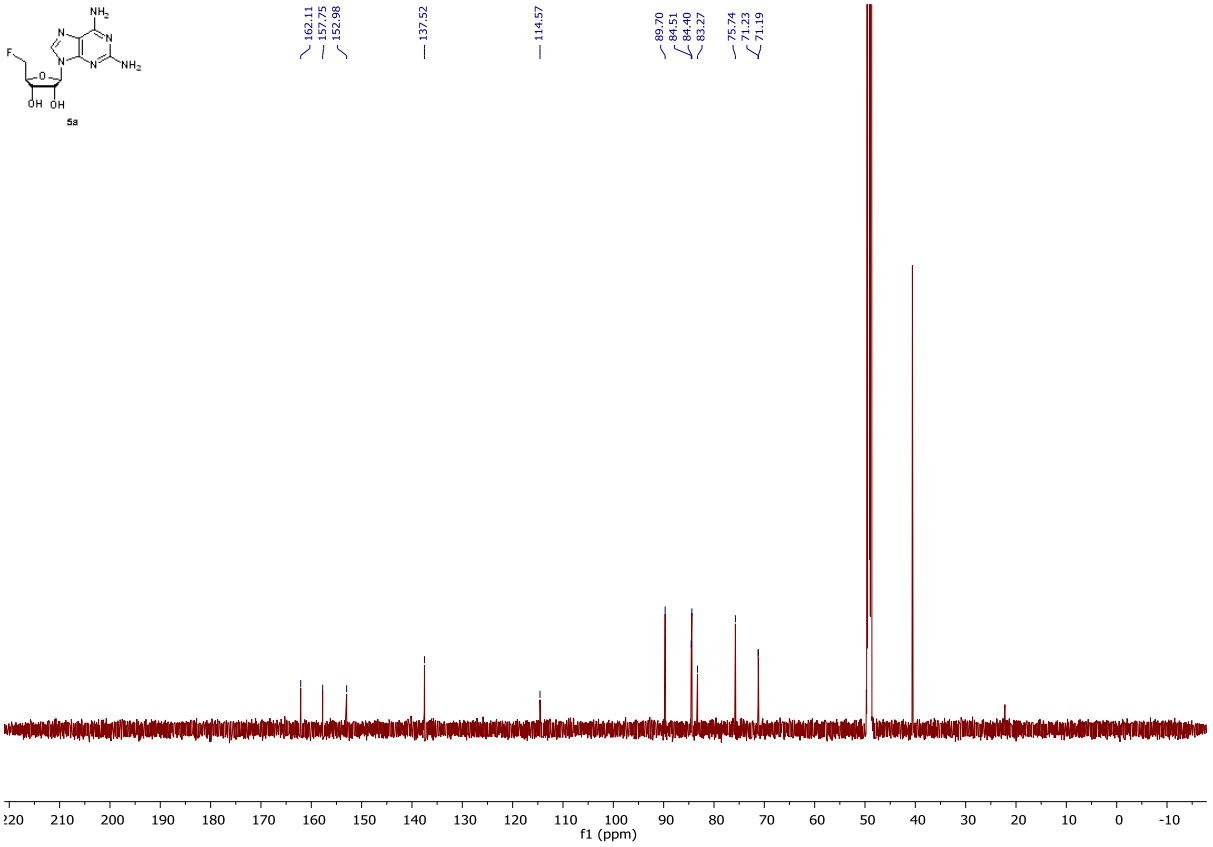


Figure S31. ^{13}C NMR spectra of **5a**.

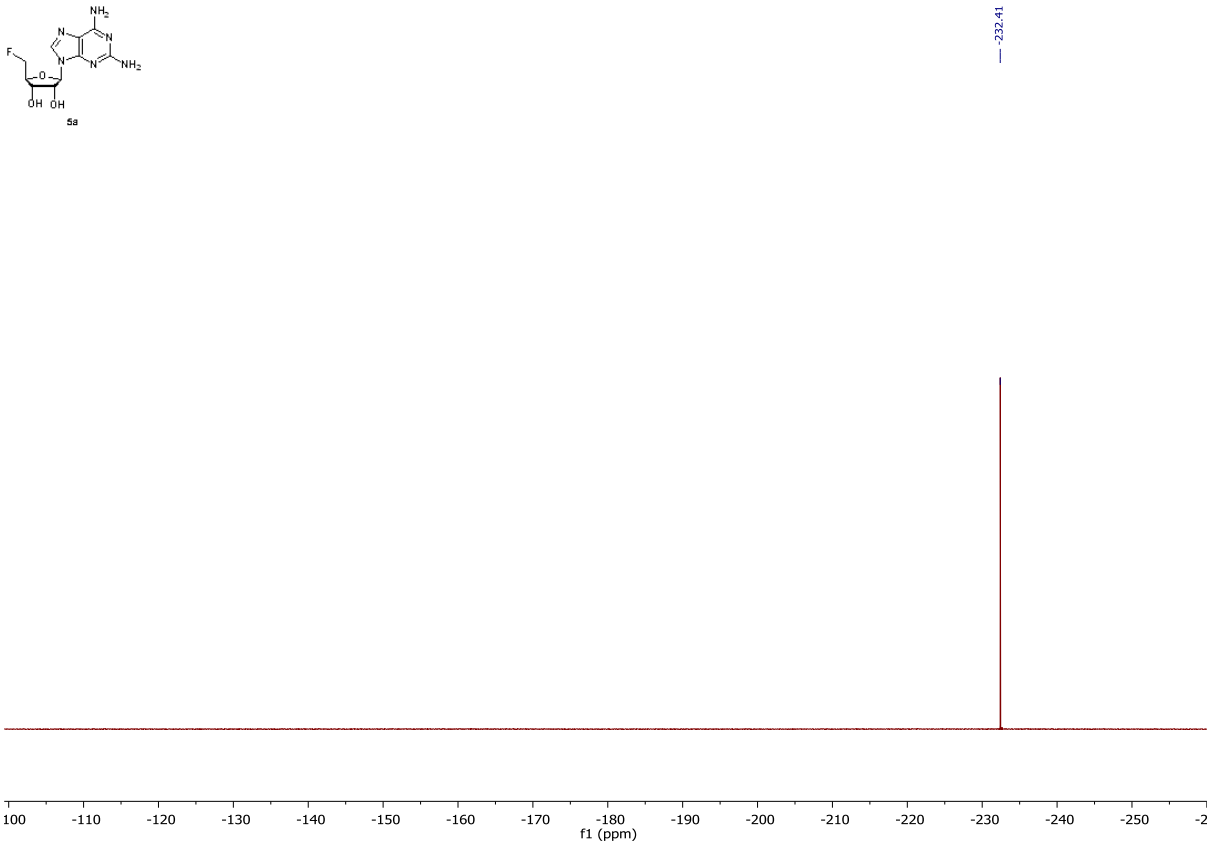


Figure S32. ^{19}F NMR spectra of **5a**.

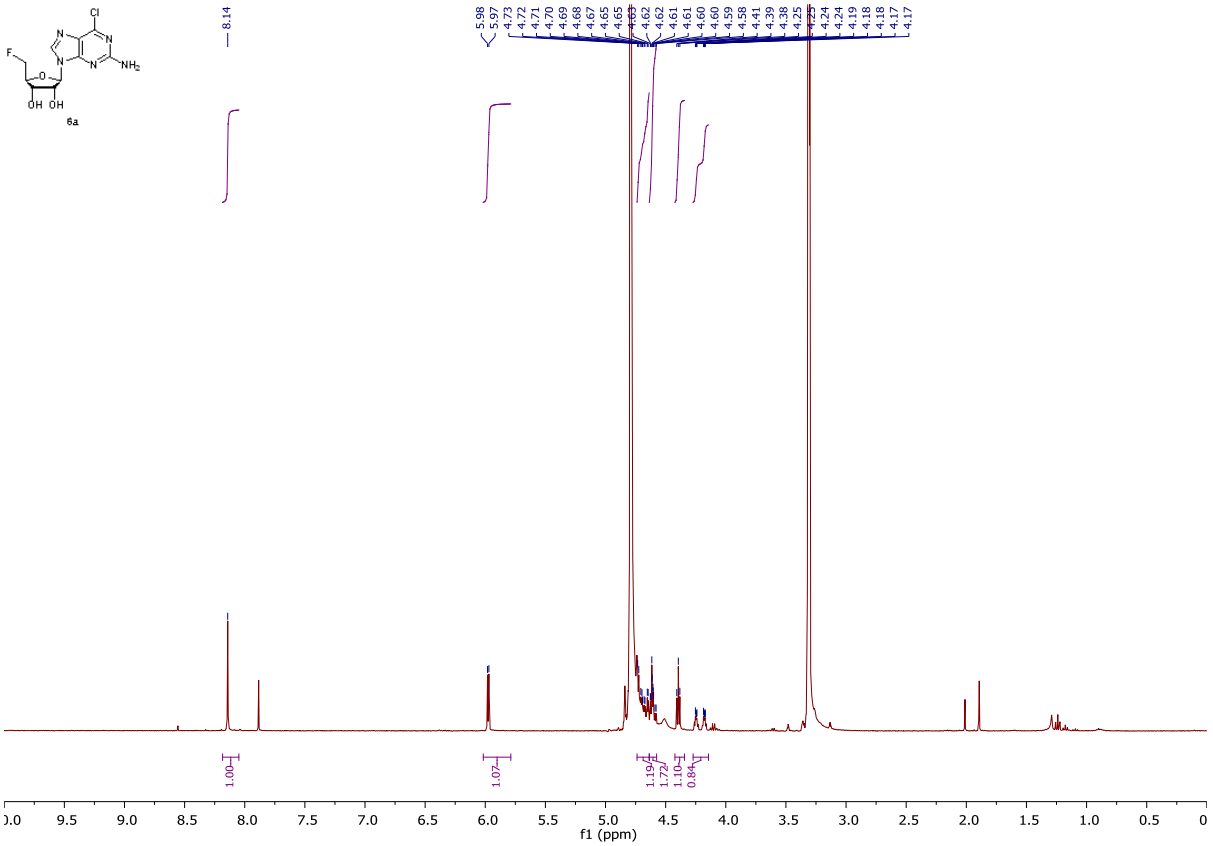


Figure S33. ^{1}H NMR spectra of **6a**.

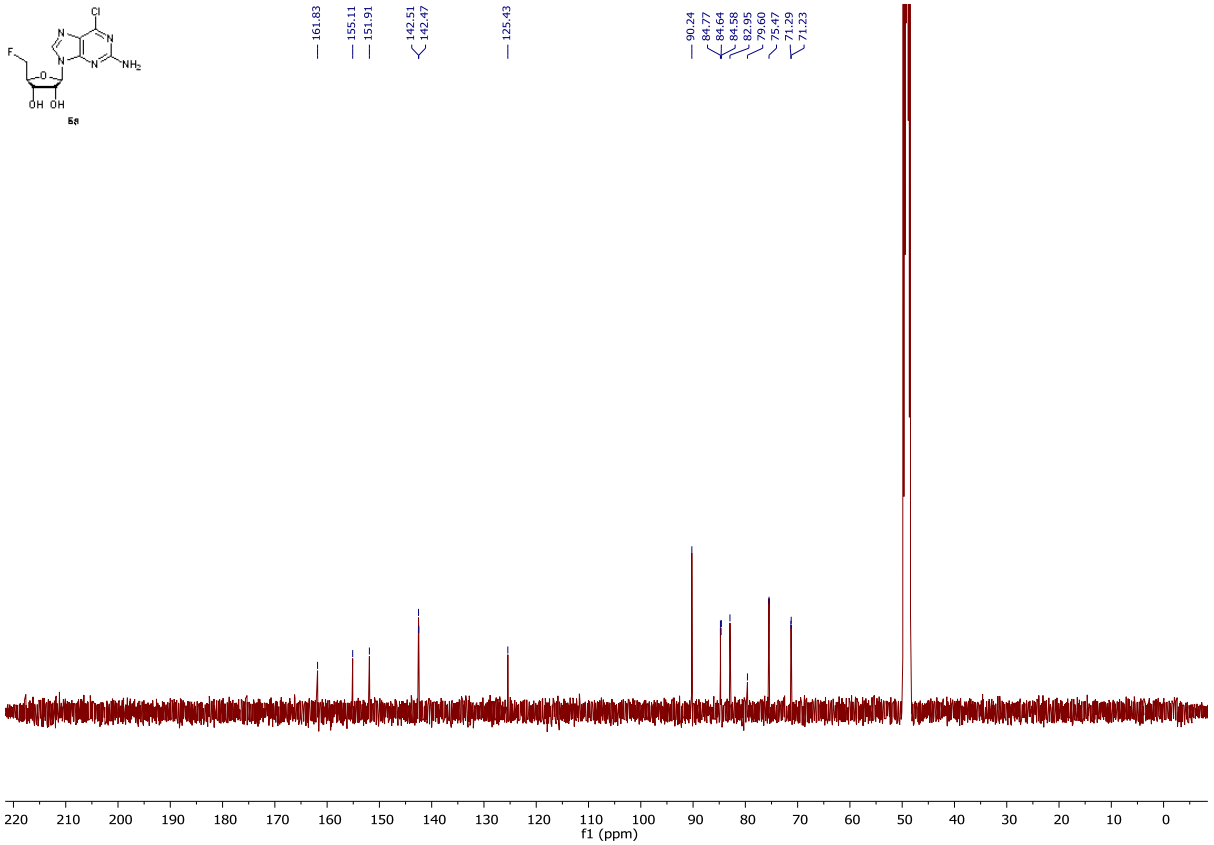


Figure S34. ^{13}C NMR spectra of **6a**.

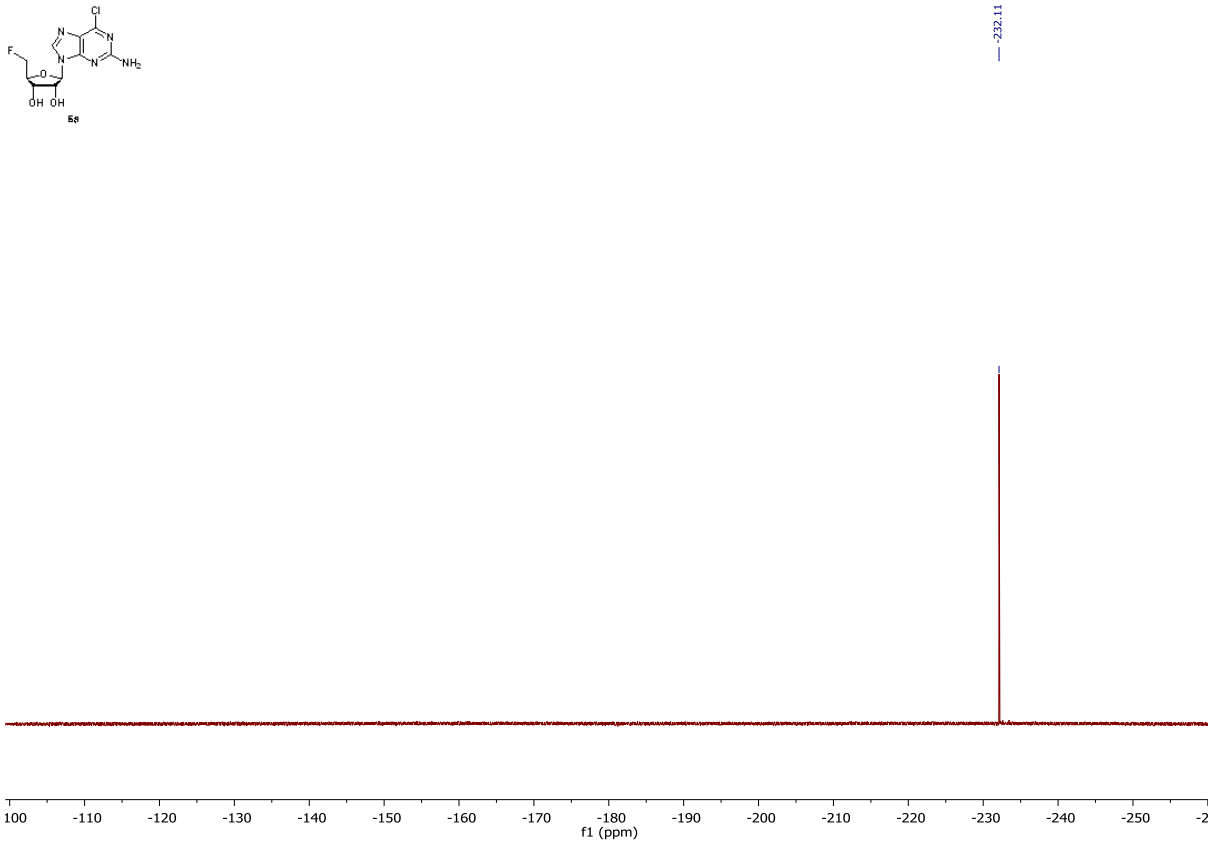


Figure S35. ^{19}F NMR spectra of **6a**.

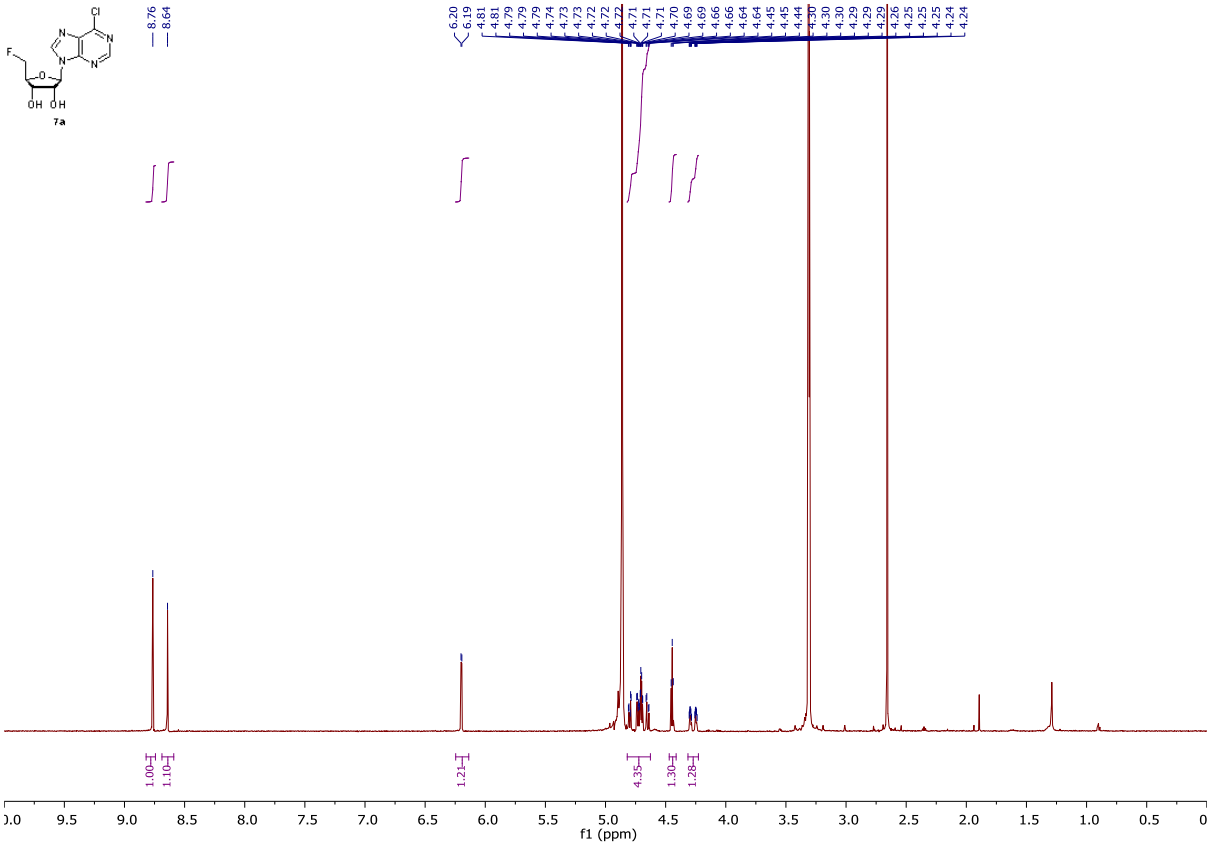


Figure S36. ^{1}H NMR spectra of **7a**.

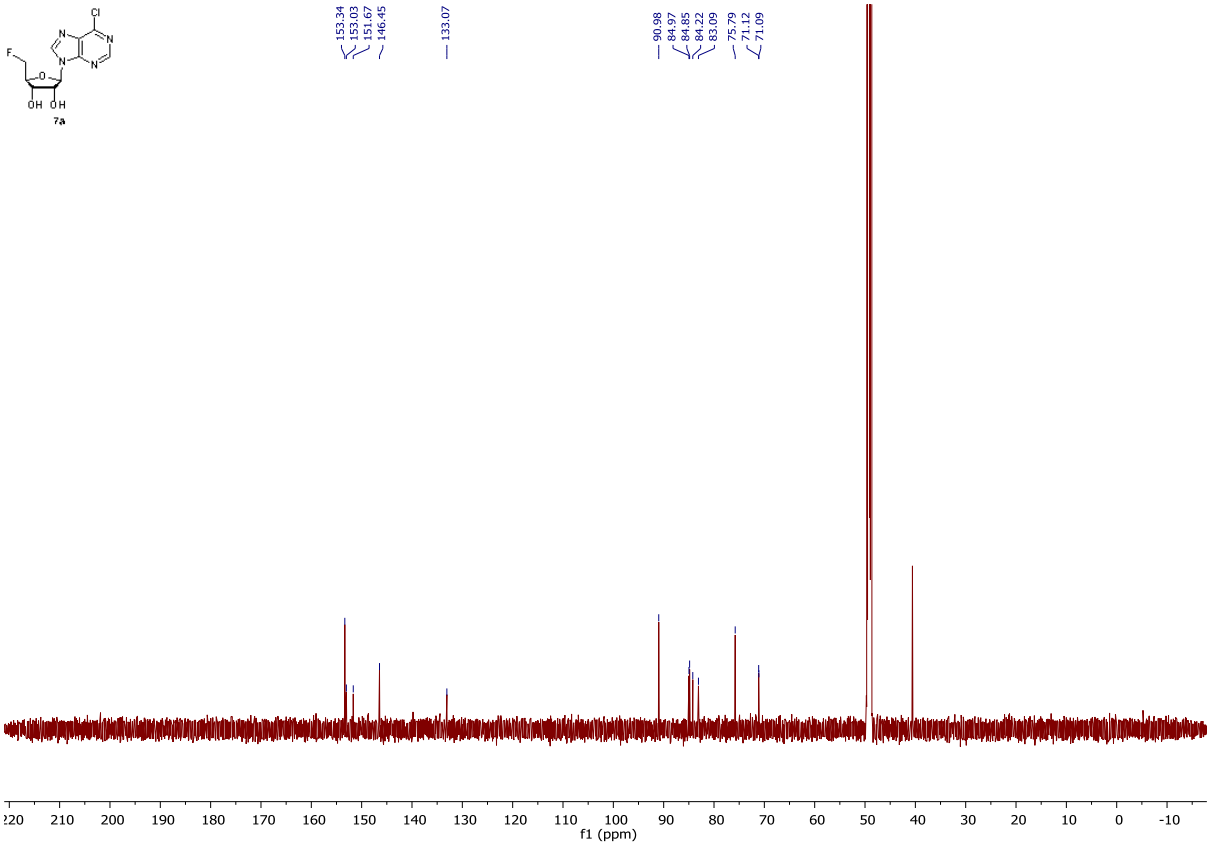


Figure S37. ^{13}C NMR spectra of **7a**.

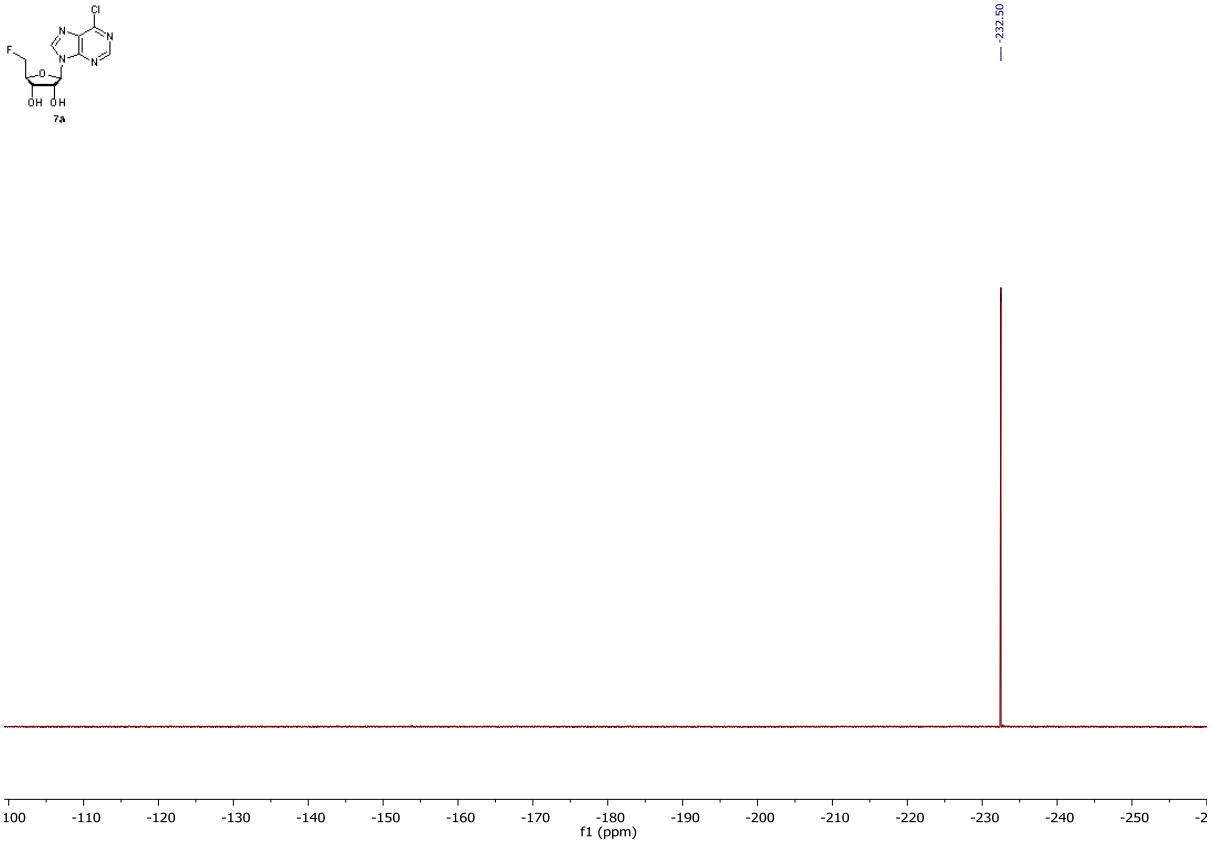


Figure S38. ^{19}F NMR spectra of **7a**.

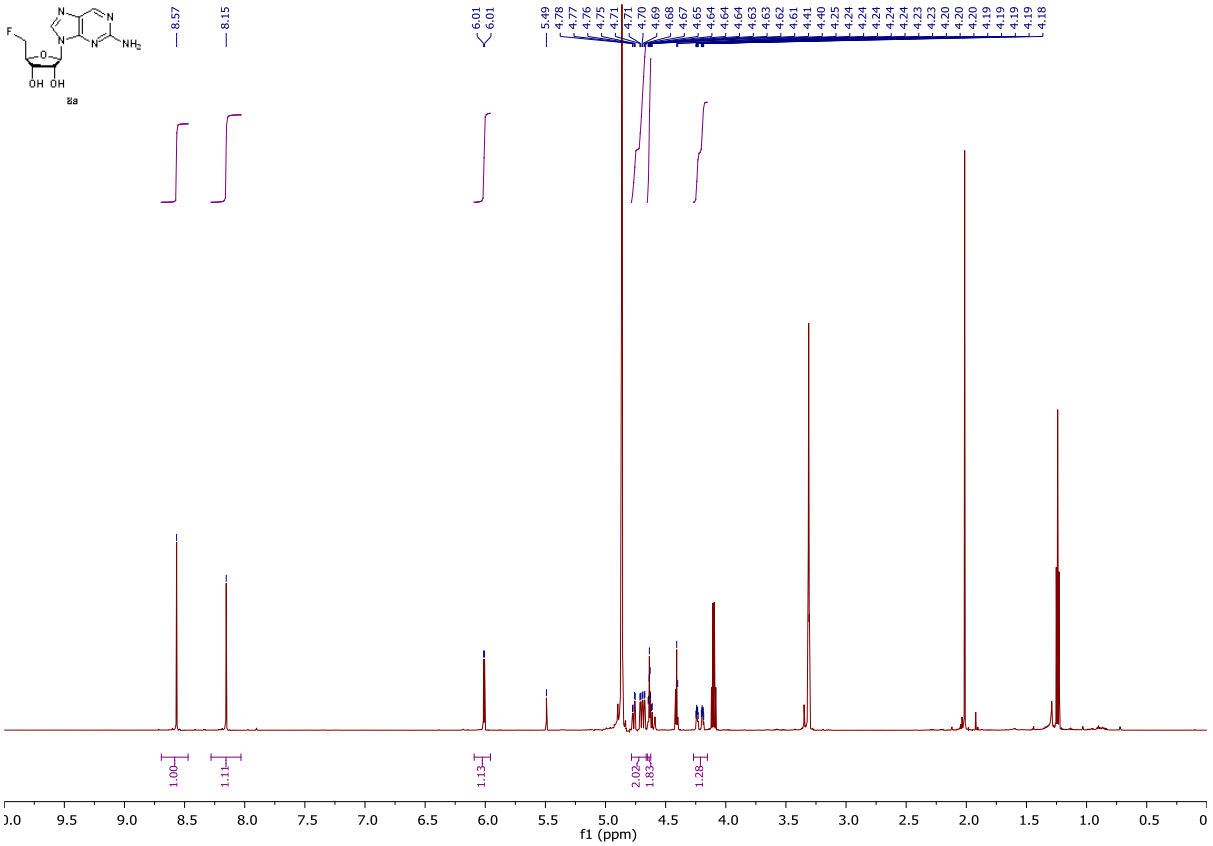


Figure S39. ^{1}H NMR spectra of **8a**.

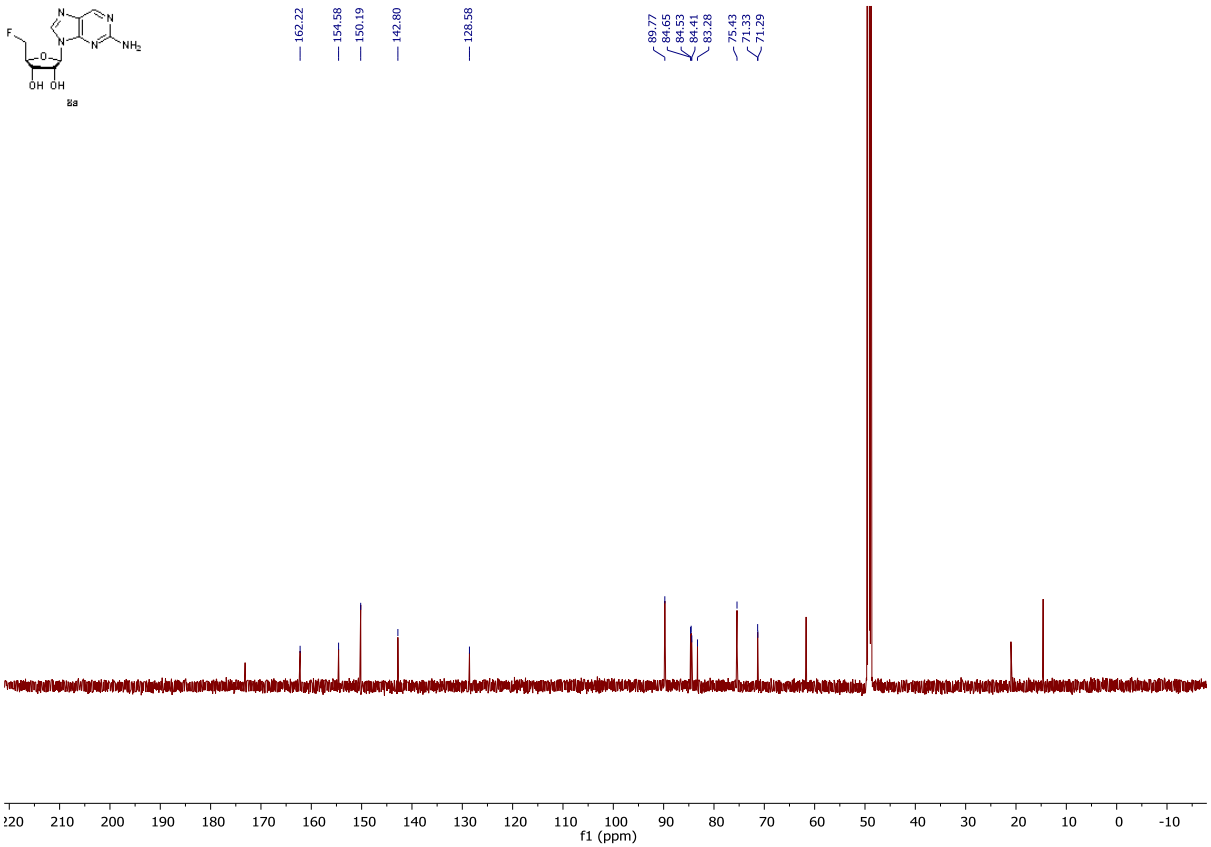


Figure S40. ^{13}C NMR spectra of **8a**.

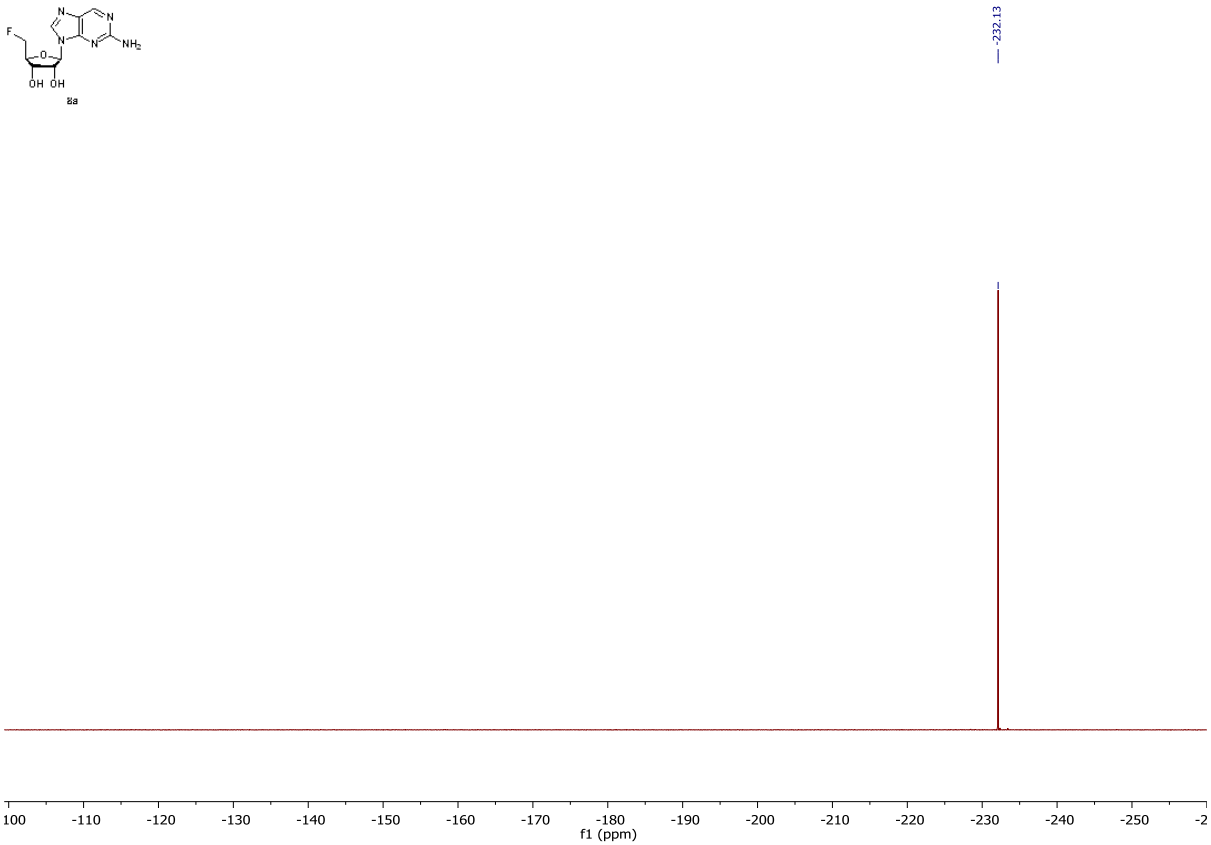


Figure S41. ^{19}F NMR spectra of **8a**.

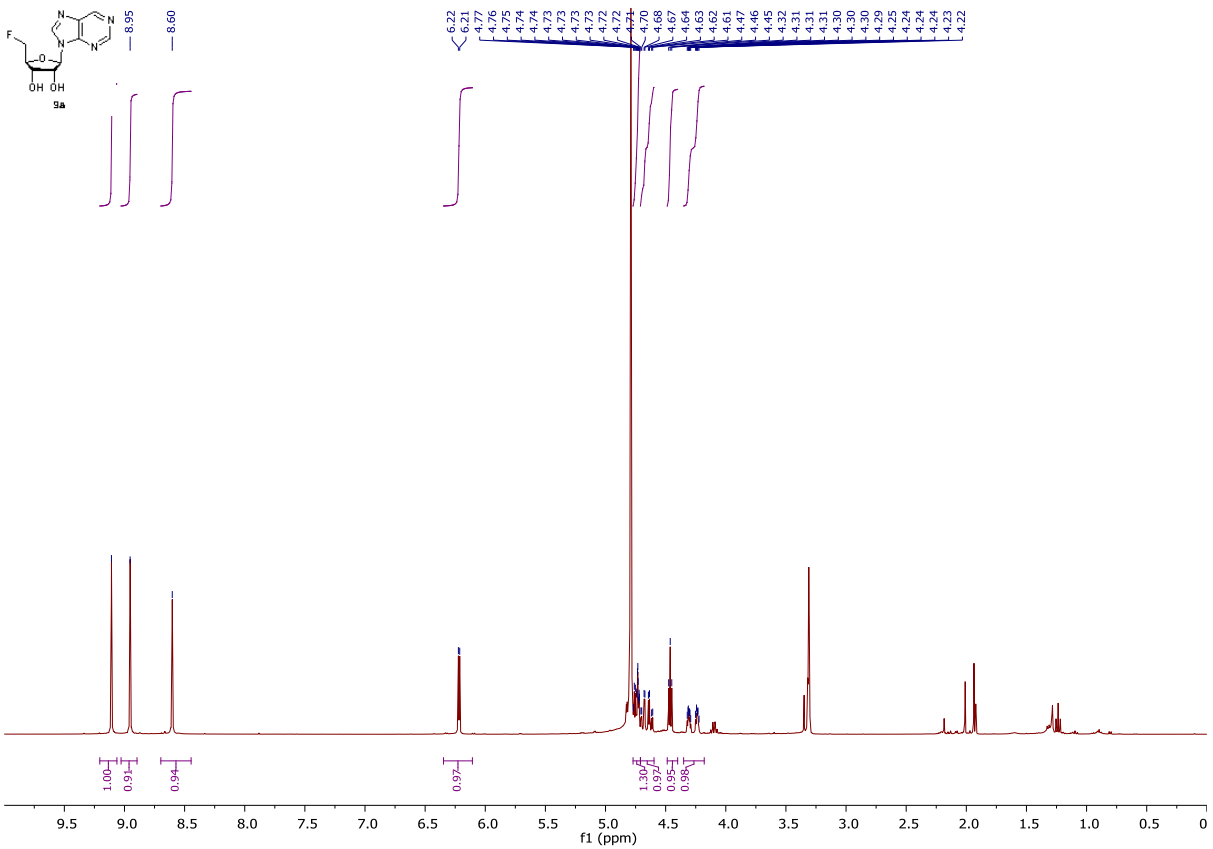


Figure S42. ^{1}H NMR spectra of **9a**.

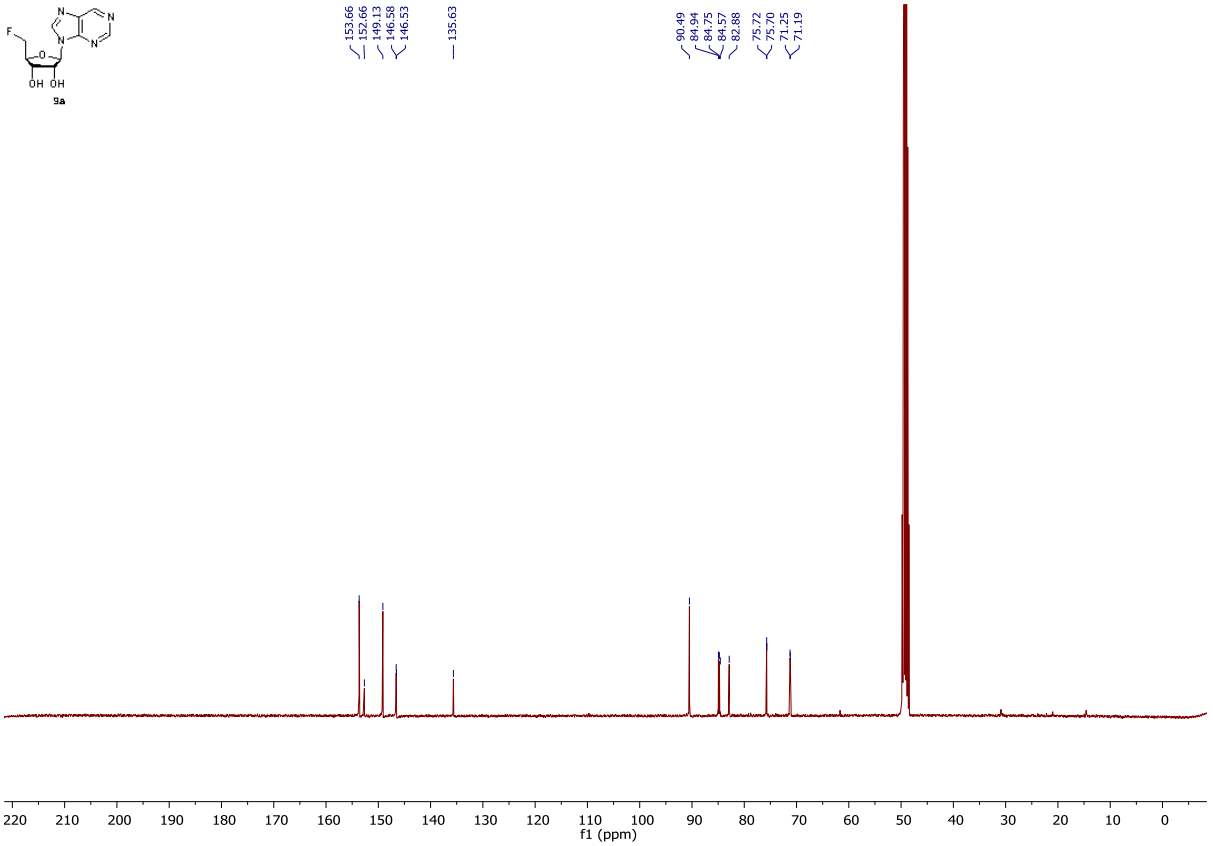


Figure S43. ^{13}C NMR spectra of **9a**.

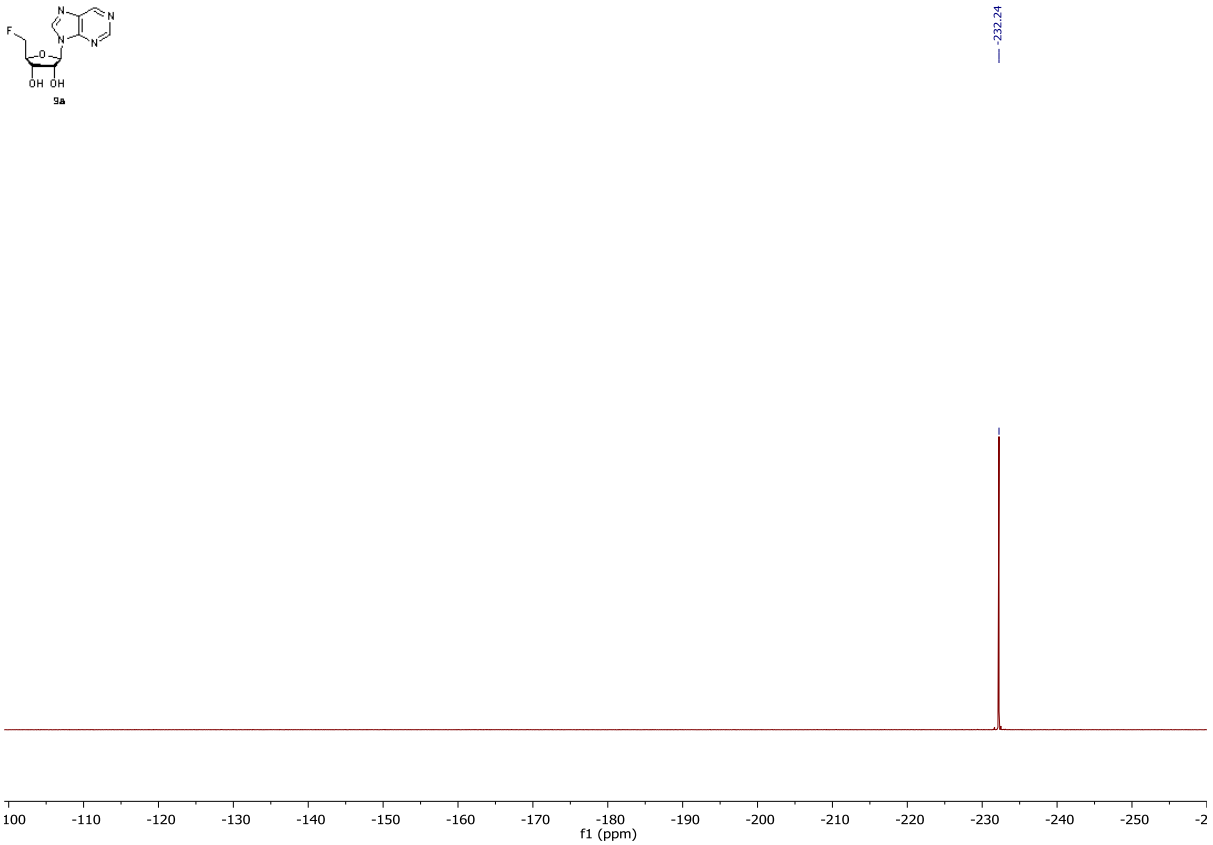


Figure S44. ^{19}F NMR spectra of **9a**.

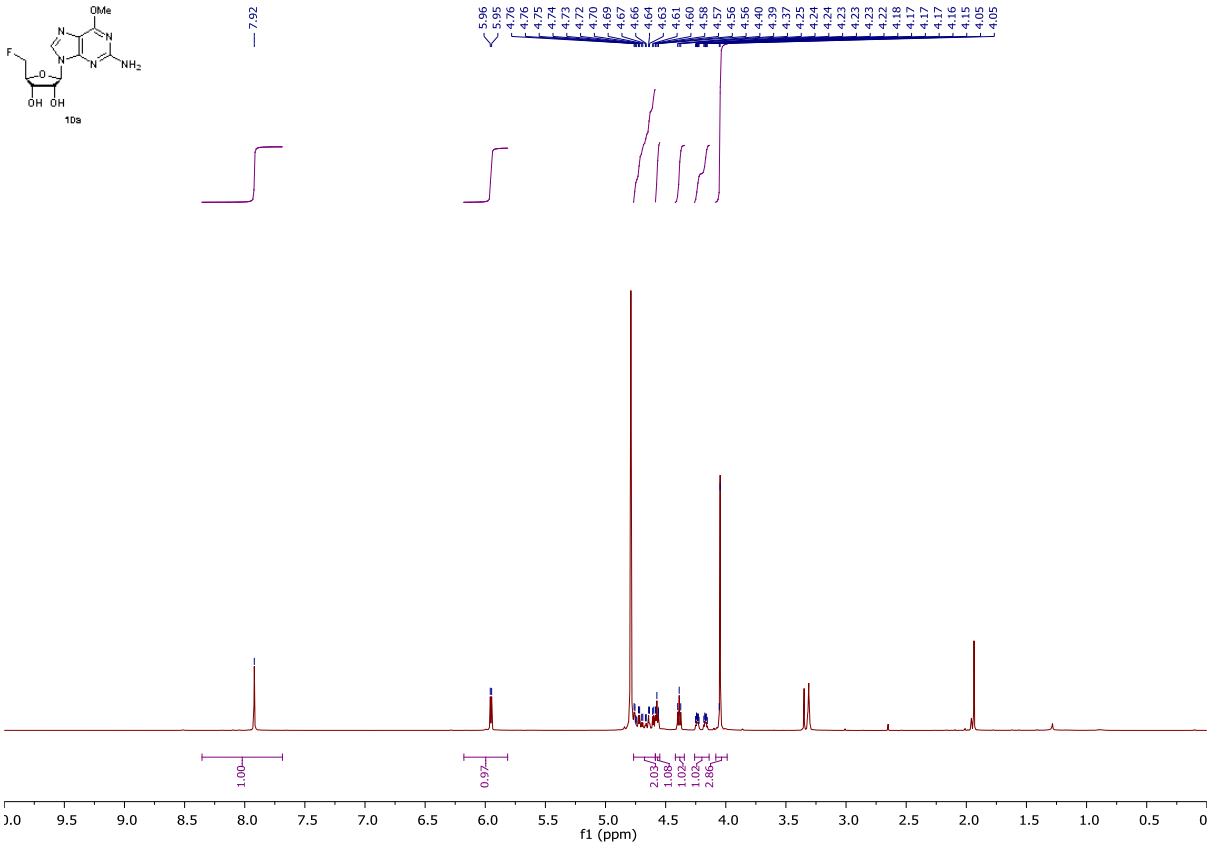


Figure S45. ^{1}H NMR spectra of **10a**.

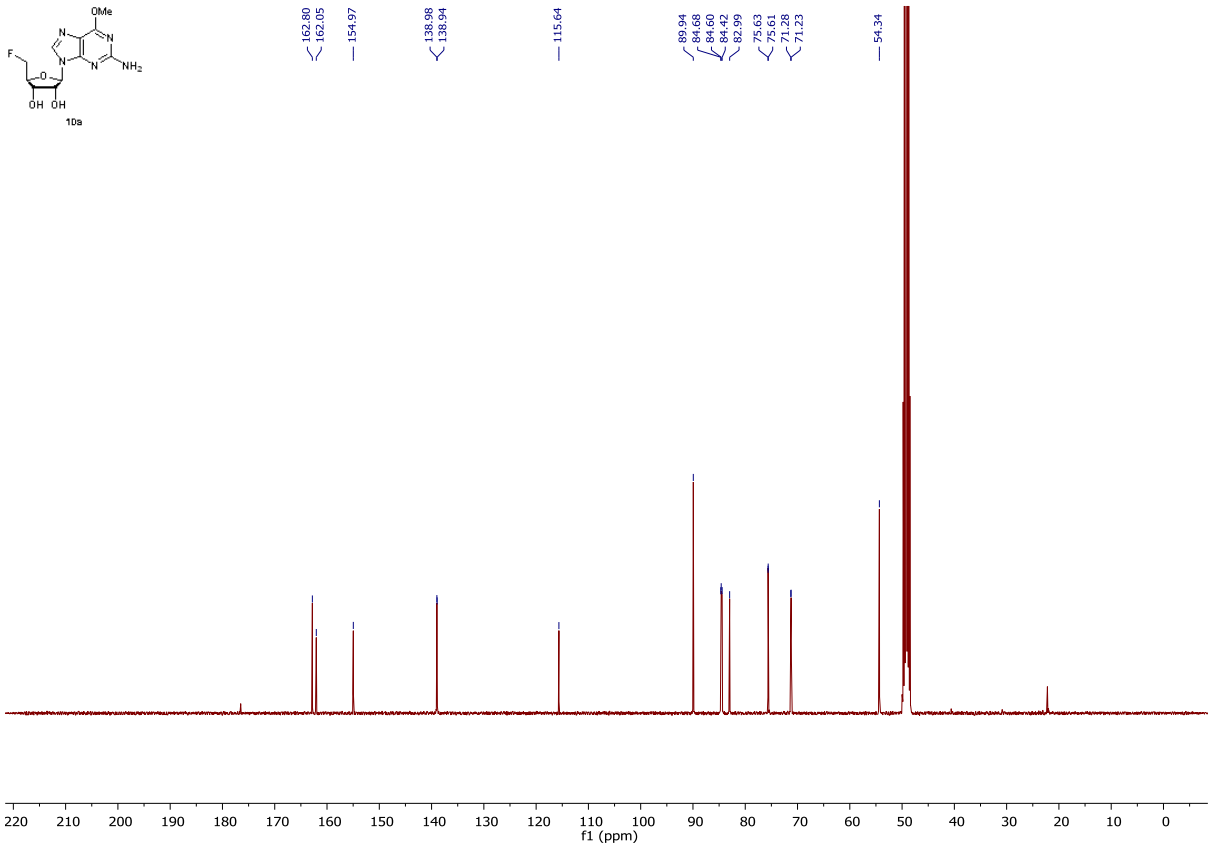


Figure S46. ^{13}C NMR spectra of **10a**.

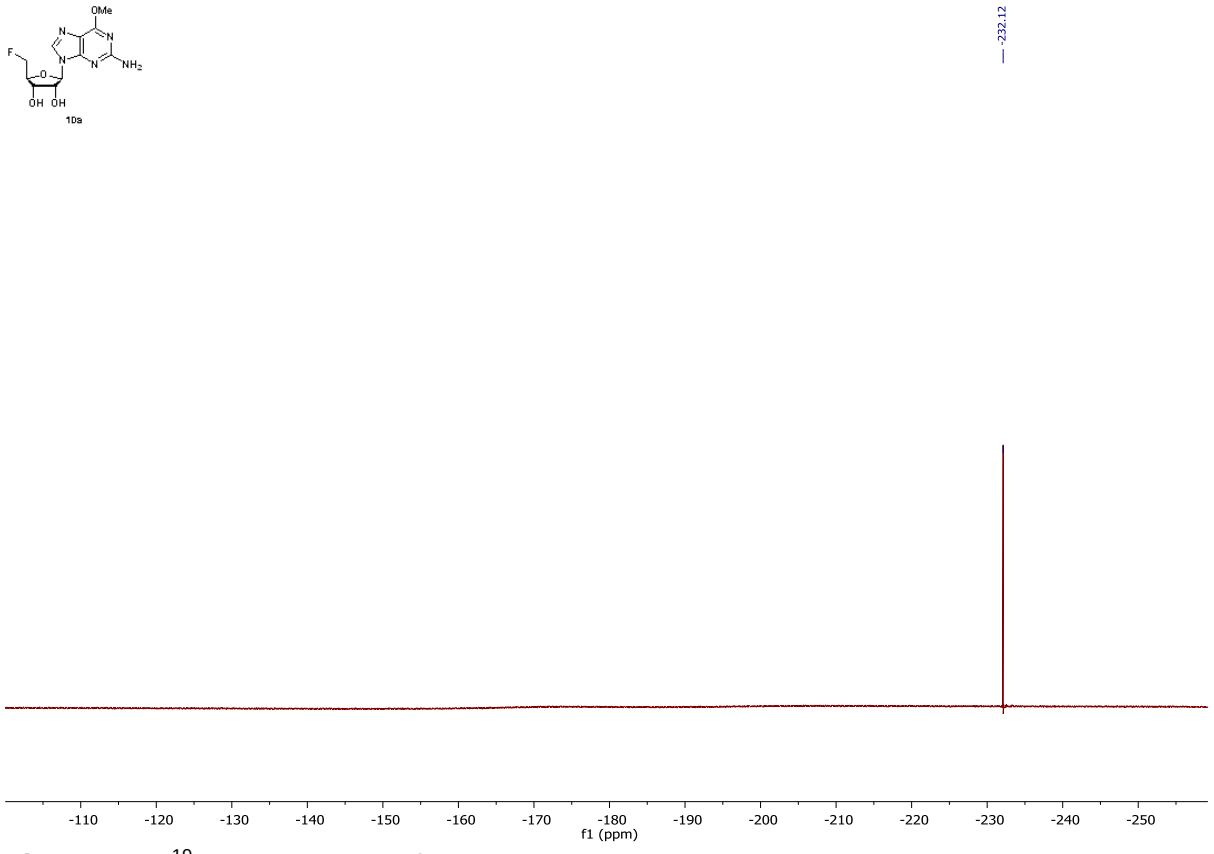


Figure S47. ^{19}F NMR spectra of **10a**.

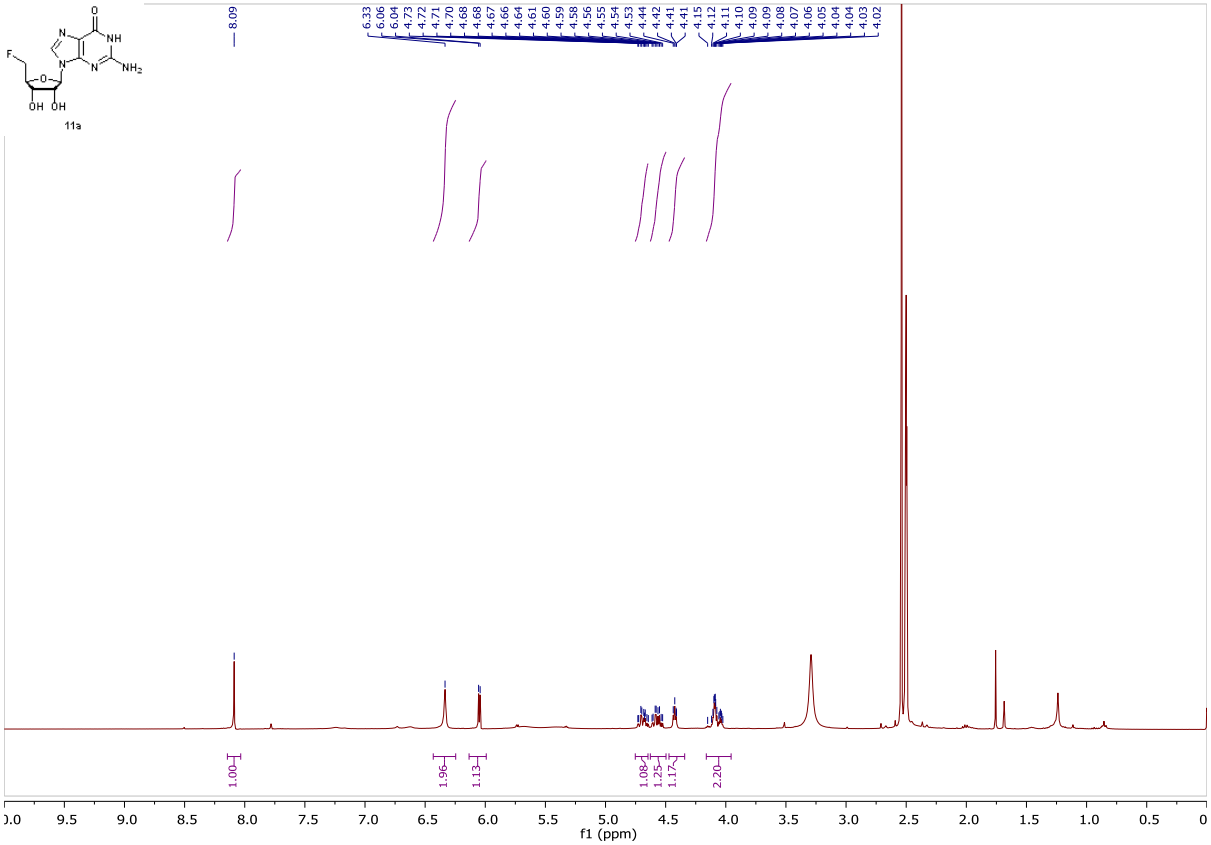


Figure S48. ^{1}H NMR spectra of **11a (first anomer)**.

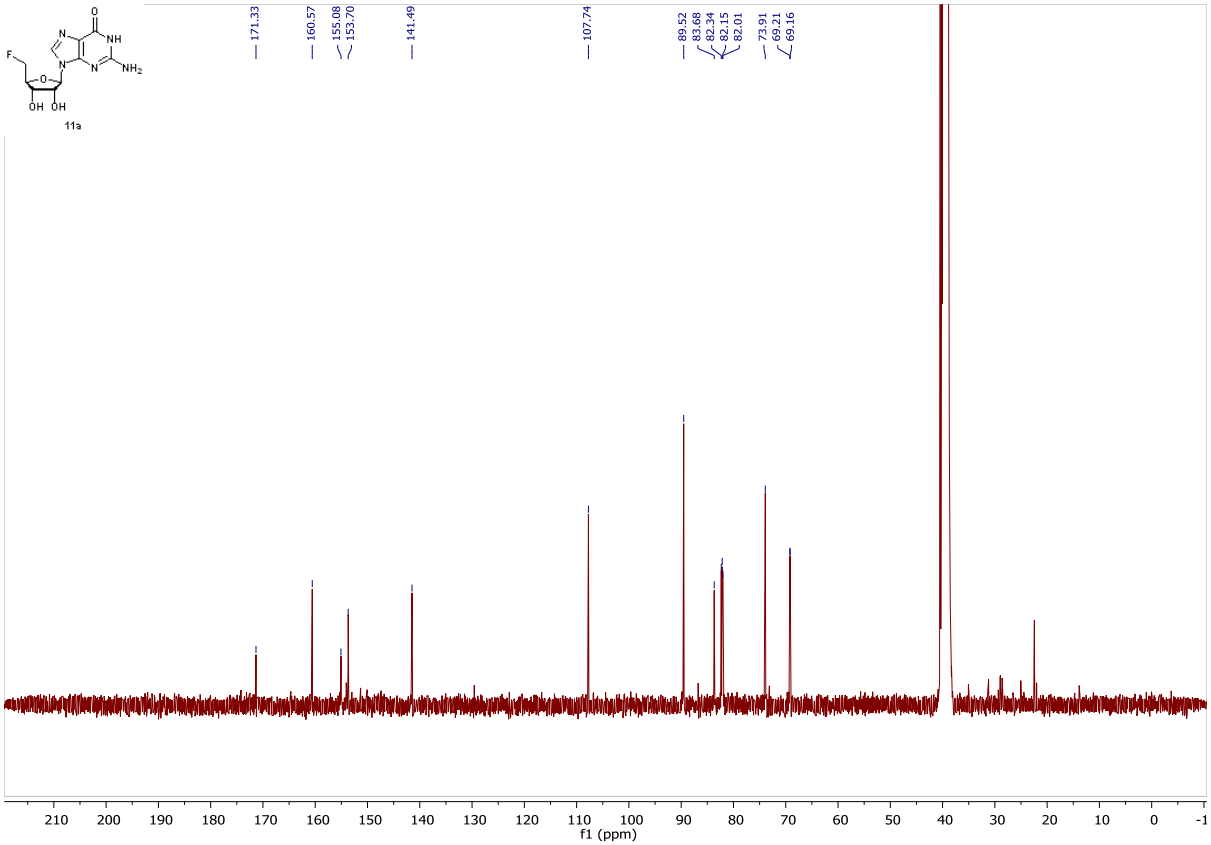


Figure S49. ^{13}C NMR spectra of **11a (first anomer)**.

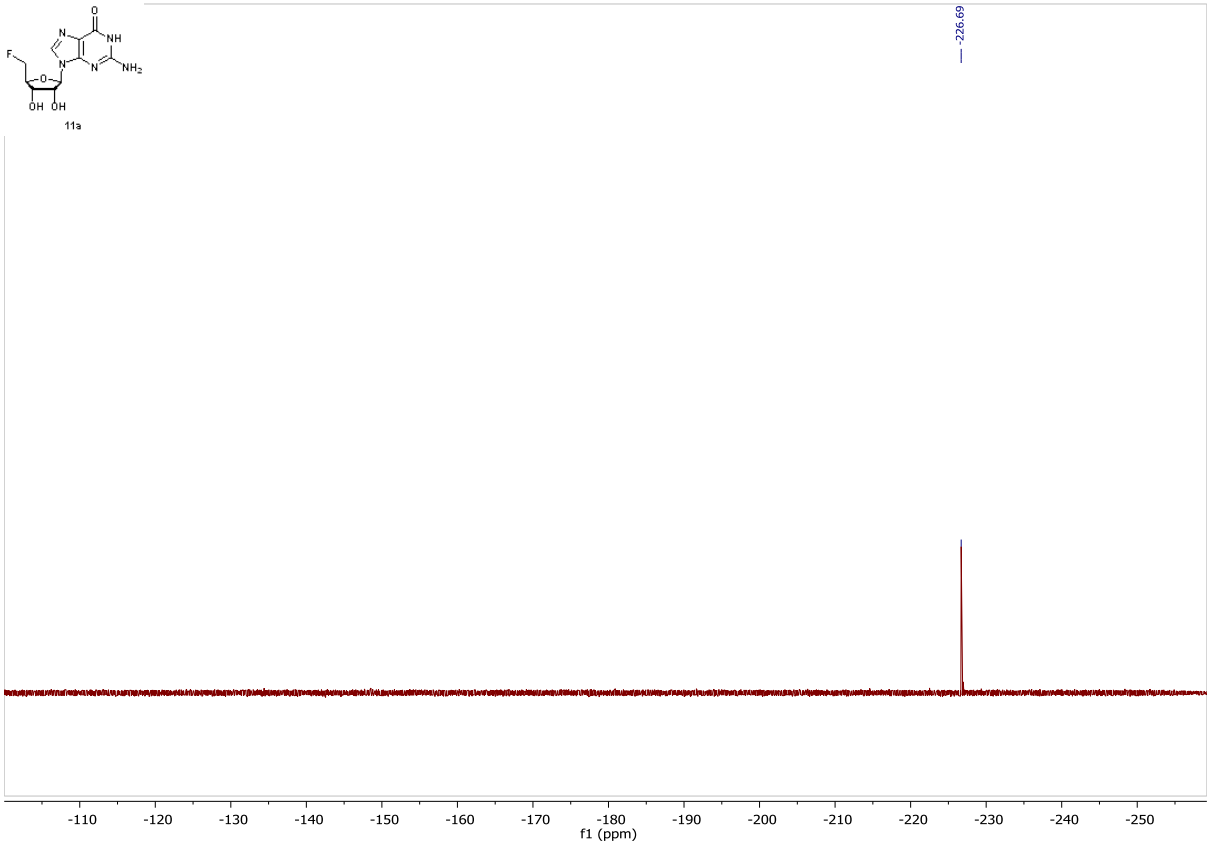


Figure S50. ^{19}F NMR spectra of **11a (first anomer)**.

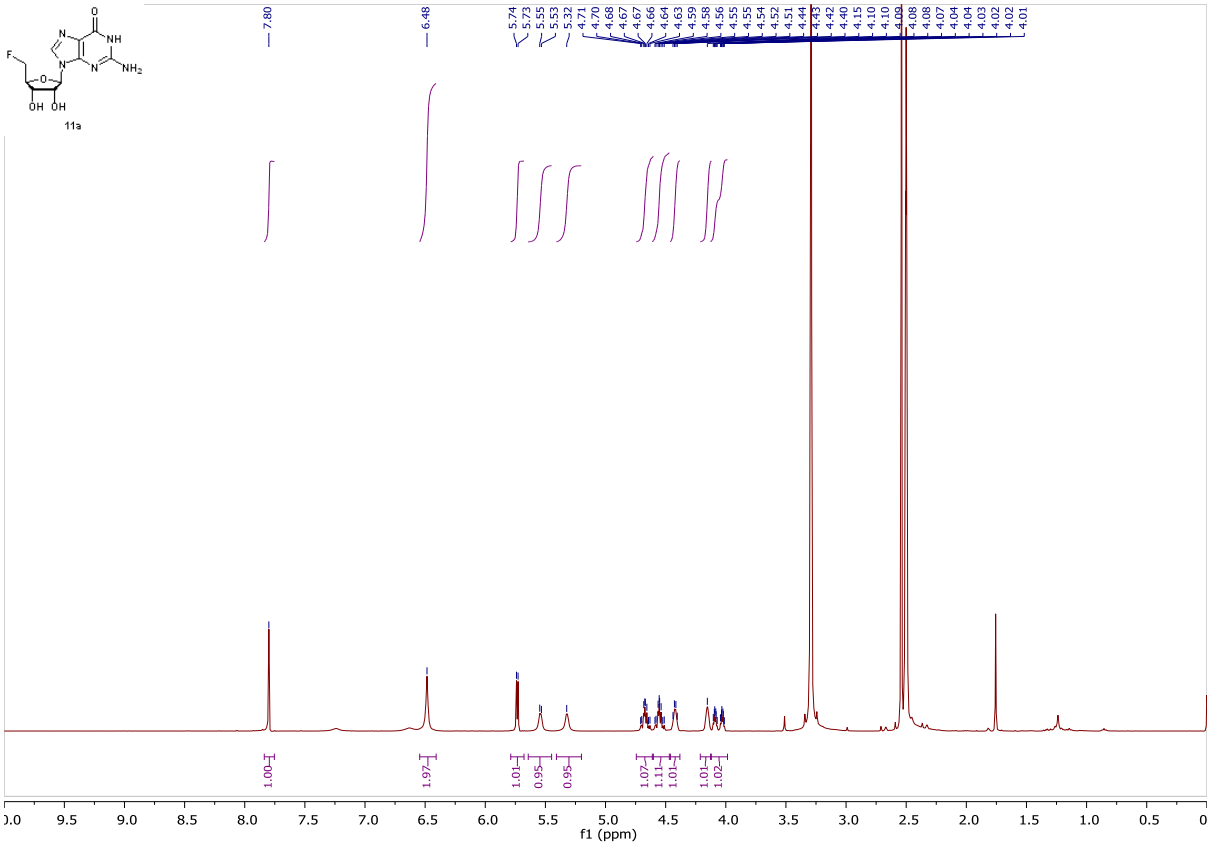


Figure S51. ^{1}H NMR spectra of **11a (second anomer)**.

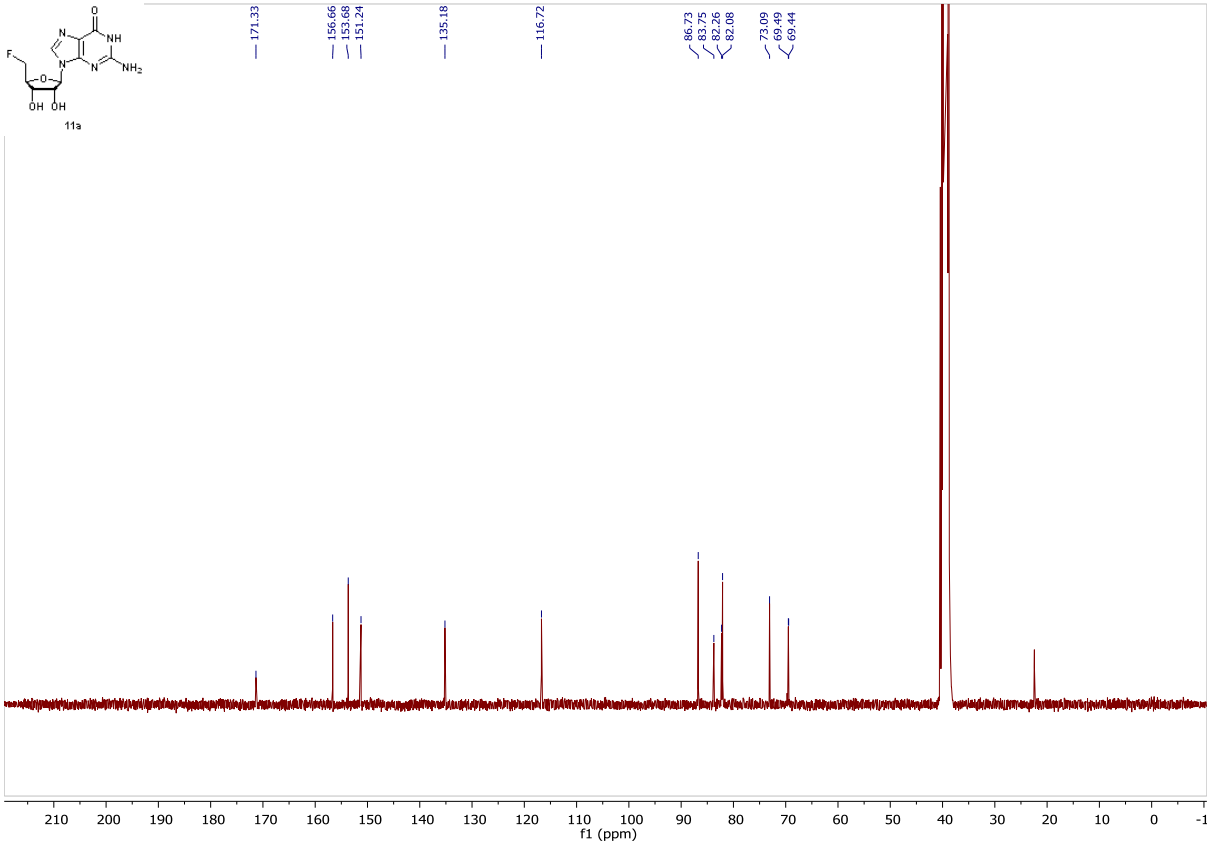


Figure S52. ^{13}C NMR spectra of **11a (second anomer)**.

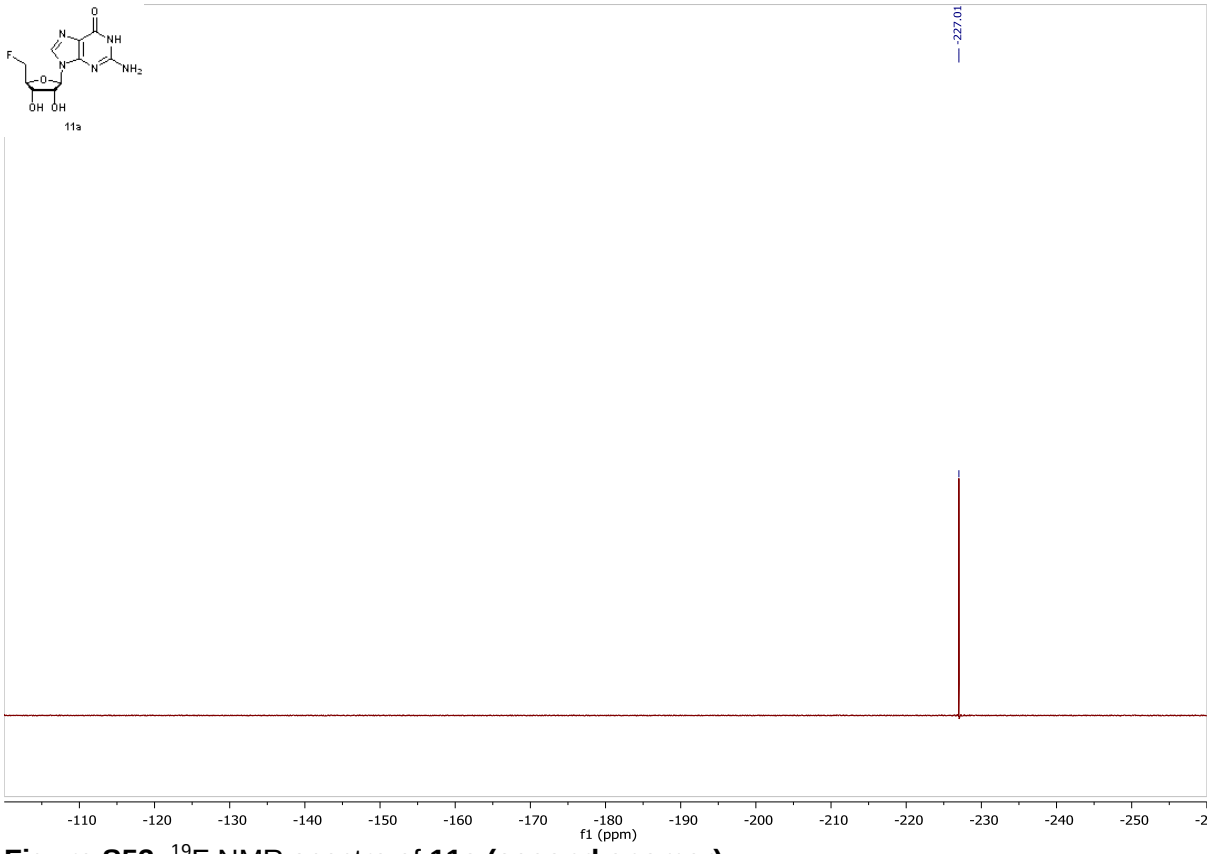


Figure S53. ^{19}F NMR spectra of **11a (second anomer)**.

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