Supplementary Information

Gram-scale enzymatic synthesis of 2'-deoxyribonucleoside analogues using nucleoside transglycosylase

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1. General information

1.1 Reagents and Solvents

All reagents and solvents were used as supplied from commercial sources and used without any further purification unless otherwise specified. Solvents were all HPLC grade and used without any further purification, unless otherwise specified. Thin layer chromatography (TLC) was performed using Merck silica plates coated with fluorescent indicator UV254. TLC plates were analysed under 254 nm UV light or developed in p-Anisaldehyde. Normal-phase column chromatography was carried out using Fluorochem Silicagel 60 Å 40-63 µm. Normal phase auto column chromatography was carried out using Silicycle silicagel on an interchim puriflash XS520 plus.

1.2 NMR Spectroscopy

NMR spectroscopy was carried out using a Bruker 400 UltraShieldTM "Avance I" spectrometer. All chemical shifts (δ) in CDCl3 were referenced at 7.26 ppm (1H) and 77.16 ppm (13C) and in DMSO-d_6 at 2.50 ppm (1H) and 39.52 ppm (13C). Chemical shifts are reported in parts per million (ppm) and coupling constants are quoted in hertz (Hz). Abbreviations for splitting patterns are s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). App (apparent) denotes signals in which similar *J* values have resulted in false equivalence. All NMR data was processed using MestRenova 11.0.3 software.

1.3 Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS was carried out on an Agilent 1200 series HPLC instrument in conjunction with an Agilent Quadrupole mass detector 9 (HPLC Agilent Technologies 6130 Quadrupole), using an agilent Infinity Lab Poroshell 120, 4.6 x 100 mm, 2.7 µ C18 column. A combination of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) was used in all cases (MM-ES+APCI). The solvent system used was Acetonitrile/Water with 5 mM Ammonium Acetate buffer.

1.4 High-Pressure Liquid Chromatography (HPLC)

HPLC analysis was performed on a Dionex Ultimate 300 instrument utilising the VWD3400 variable wavelength detector. Analytical reversed-phase HPLC (RP-HPLC) was carried out on

a Shimadzu Prominence instrument utilising a PDA Detector scanning from 190-600 nm. Semi-preparative RP-HPLC purification was carried out on a Dionex Ultimate 3000 series instrument using a 150 x 21.2 mm Kinetex 5 µm C18 column.

2. General experimental techniques and procedures

2.1 Preparation of stock solutions

100 mM Phosphate buffer solution pH 6

1.84 g (13.7 mM) of $Na_2HPO_4 \cdot 7H_2O$ and 5.96 g (86.3 mM) of $NaH_2PO_4 \cdot H_2O$ were dissolved in 500 mL of mQ H_2O, adjusted using 0.2 mL of 1M HCL obtaining a final pH of 6.

Sugar donor stock solution

727 mg (3 mmol) of thymidine were dissolved in 30 mL of mQ H_2O to prepare a 100 mM solution. Solution was shaken by hand for 1 min to ensure full solubility of the thymidine.
682 mg (3 mmol) of deoxycytidine (**8**) were dissolved in 30 mL of mQ H_2O to prepare a 100 mM solution. Solution was shaken by hand for 1 min to ensure full solubility of the deoxycytidine (**8**). Sugar donor stock solution is stored at rt.

Nucleobase stock solutions

As an example, 11.5 mg of nucleobase 10 was dissolved using sonication in 1768 µL of mQ H_2O to form a 50 mM solution. Nucleobase solution is stored at rt.

NDT enzyme solution

Lactobacillus Leichmanii NDT (*Ll*NDT, N2665 Sigma-Aldrich, expressed in *E. coli*) stock solution was prepared by dissolving 0.921 mg of powder enzyme in 0.921 mL of 100 mM Na_2HPO_4 and 20% glycerol (1 mg/mL). Solution stored in −18 ˚C. One unit of enzyme produces 1 µM of hypoxanthine in 1 minute at 40°C, pH 6.0 (1.22 units/mg solid).

2.2 General Procedures

General Enzymatic Nucleoside Transglycosylation Procedure A

A 50 mM nucleobase stock solution in mQ H_2O (10.0 μmol, 200 μL, 1 equiv) was mixed with a 100 mM thymidine or deoxycytidine solution dissolved in mQ H_2O (50.0 μmol, 500 μL, 5 equiv) and 298 μL extra of mQ H_2O to afford a final volume of 1 mL. Next, 2 μL of NDT stock solution (2 μg/mL) was added and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. Then, an aliquot of 100 μL of reaction mixture was transferred to a 1 mL HPLC vial, quenched with 100 μL of HFIP and diluted with 800 μL of 100 mM Na_2HPO_4 buffer. The crude was analysed by analytical RP-HPLC, and conversion of the target nucleoside was calculated by monitoring the ratio of the peak area of starting material to peak area of product. Reactions were carried out in triplicate and conversions were expressed as the average with the corresponding standard deviation.

2.3 Analytical RP-HPLC method parameters

Method A

Column specification: Luna C18 Polar Omega 3 μ (100 x 4.6 μm)
Column temperature: 40 °C
Mobile phase A: 0.1% v/v TFA in water
Mobile phase B: 0.1% v/v TFA in Acetonitrile
Flow rate 1.5 mL/min
Gradient profile:

Time (min)	% A	% B
0	99	1
0.5	99	1
4	88	12
6.5	50	50
7	5	95
8	5	95
9	99	1
11.30	99	1

Detection wavelength of 254 nm.

Method B

Column specification: Luna C18 Polar Omega 3 μ (100 x 4.6 μm)
Column temperature: 40 °C
Mobile phase A: 0.1% v/v TFA in water

Mobile phase D: Acetonitrile

Flow rate 1.5 mL/min

Gradient profile:

Time (min)	% A	% D
0	95	5
8	40	60
8.5	5	95
11	5	95
11.5	95	5
14	95	5

Detection wavelength of 254 nm.

Method C

Column specification: Phenomenex Luna C18 3 µ phenyl-hexyl (150 x 4.6 mm)

Column temperature: 40 °C

Mobile phase A: 0.1% v/v TFA in water

Mobile phase B: 0.1% v/v TFA in Acetonitrile

Flow rate 1.2 mL/min

Gradient profile:

Time (min)	% A	% B
0	99	1
8	70	30
9	5	95
11	5	95
11	99	1
13	99	1

Detection wavelength of 254 nm.

2.4 RP-HPLC semi preparatory method parameters:

Method D

Column specification: Kinetex 5 µm XB-C18 100 Å, 150 x 21.2 mm Å

Column temperature: 40 °C

Mobile phase A: Water

Mobile phase B: Acetonitrile

Flow rate: 12 mL/min

Time (min)	% A	% B
0	95	5
18	82	18
19	5	95
23	5	95
24	95	5
28	95	5

Detection wavelength of 254 nm.

3. Optimisation of the reaction conditions

3.1 Co-solvent screening

Nucleobases **9** (purine) and **10** (pyrimidine) were prepared and analysed using the general enzymatic nucleoside transglycosylation procedure. The 50 mM nucleobase stock solution was dissolved in the various solvents investigated.

Table S1 – Co-solvent screening with purine **9** and pyrimidine **10** The standard error was calculated through the standard deviation, N = 3.

Entry	Co-Solvent (20% v/v)	Nucleobase **9** Conversion (%)	Nucleobase **10** Conversion (%)
1	H_2O	98 ± 1	48 ± 1
2	PBS	98 ± 1	79 ± 1
3	ACN	20 ± 6	9 ± 6
4	DMSO	98 ± 1	78 ± 1
5	MeOH	98 ± 1	80 ± 1
6	EtOH	98 ± 1	84 ± 3
7	IPA	97 ± 1	85 ± 2
8	Acetone	97 ± 1	81 ± 5

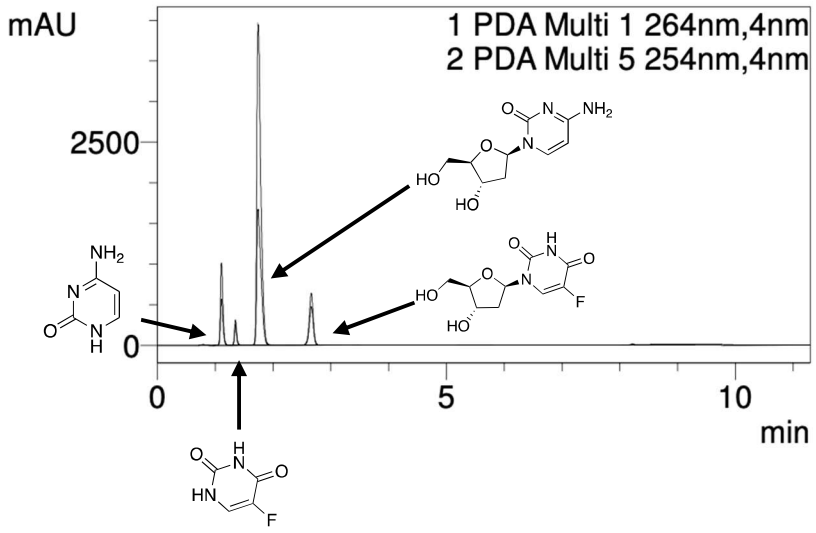


Figure S1 - Representative example of the reaction forming nucleoside **12** in 20% MeOH v/v corresponding to entry 5 Table S1.

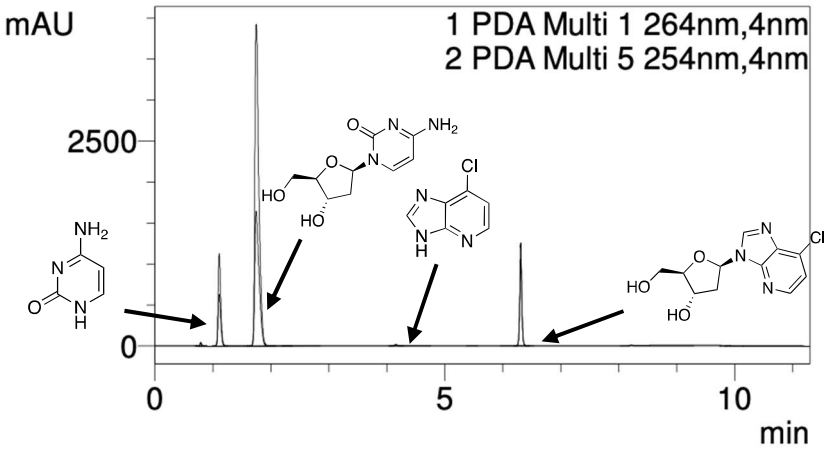
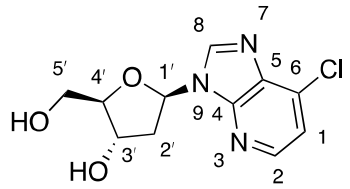


Figure S2 - Representative example of the reaction forming nucleoside **11** in 20% MeOH v/v corresponding to entry 5 Table S1.

2'-Deoxy-deaza-6-chloropurine (**11**)



Prepared using the general enzymatic nucleoside transglycosylation procedure A. A 50 mM nucleobase stock solution (12 mg dissolved in 1563 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-deaza-6-chloropurine (**11**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.77 (s, 1H, H^8), 8.33 (d, J = 5.3 Hz, 1H, H^1 or H^2), 7.48 (d, J = 5.3 Hz, 1H, H^1 or H^2), 6.50 (dd, J = 7.3, 6.2 Hz, 1H, $H^{1'}$), 5.33 (d, J = 4.2 Hz, 1H, O$H^{3'}$), 5.01 (*app* t, J = 5.6 Hz, 1H, O$H^{5'}$), 4.44 (*app* dq, J = 7.0, 3.5 Hz, 1H, $H^{3'}$), 3.89 (*app* td, J = 4.5, 2.9 Hz, 1H, $H^{4'}$), 3.62 (*app* dt, J = 11.7, 5.0 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.53 (ddd, J = 11.8, 5.9, 4.5 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.76 (ddd, J = 13.2, 7.4, 5.9 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.34 (ddd, J = 13.2, 6.2, 3.4 Hz, 1H, $H^{2'}$ or $H^{2''}$).

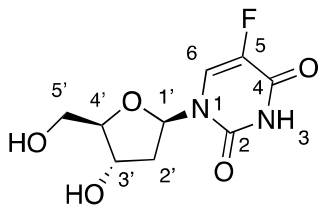
^{1}H NMR in agreement with literature values[1]

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 147.1 (C^4), 144.5 (C^1 or C^2), 143.7 (C^8), 133.2 (C^4 or C^5), 133.0 (C^4 or C^5), 118.7 (C^1 or C^2), 87.9 ($C^{4'}$), 83.9 ($C^{1'}$), 70.6 ($C^{3'}$), 61.6 ($C^{5'}$). $C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

RP-HPLC (Method A): t_R = 6.38 min, 98% conversion (std = 0.2)

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{11}H_{13}ClN_3O_3$ 270.0640; found, 270.0632.

2'-Deoxy-5-fluorouracil (12)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (11.5 mg dissolved in 1768 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (227 mg in 10 mL), which was dissolved in mQ H_2O (100.0 µmol, 500 µL, 10 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-5-fluorouracil **(12)** for characterisation.

RP-HPLC (Method A): t_R = 2.62 min, 78 % conversion (std = 1).

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the large-scale characterisation.

3.2 2'-Deoxy-5-ethynyluridine (3) optimisation

2'-Deoxy-5-ethynyluridine (**3**) optimisation reactions were prepared and analysed using the general enzymatic nucleoside transglycosylation procedure A. The 50 mM nucleobase stock solution was dissolved in ethanol (12 mg dissolved in 1763 µL of ethanol) and the 100 mM deoxycytidine solution was adjusted to match the equivalents used in the optimisation study. 10 equivalents of deoxycytidine used a 200 mM stock solution, 20 equivalents of deoxycytidine used a 400 mM stock solution. The stock solutions were dissolved in mQ H_2O (for 100 mM stock, 50.0 µmol, 500 µL, 5 equiv).

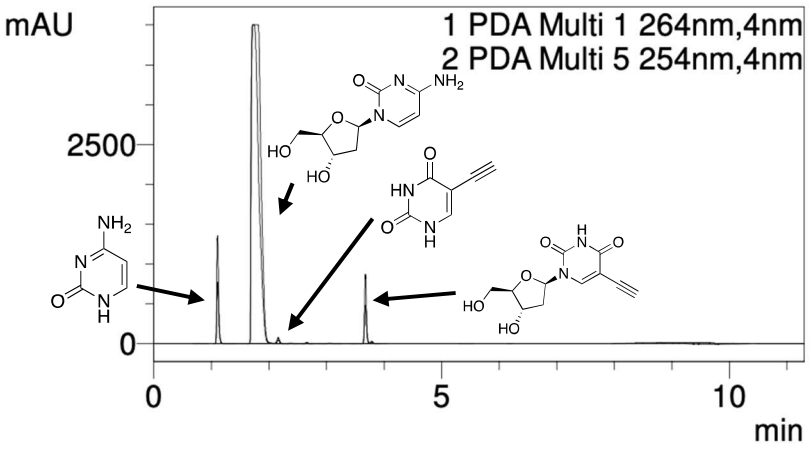
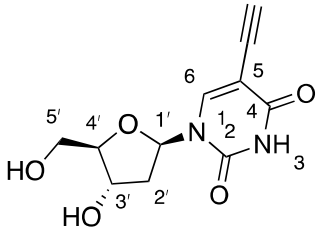


Figure S3 - Representative HPLC example of entry 8 Figure 2 (C) forming nucleoside **3.**

2'-Deoxy-5-ethynyl-2'-deoxyuridine (**3**)



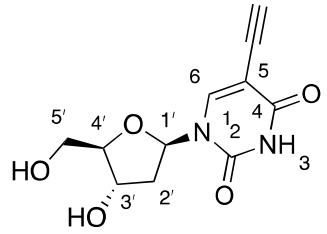
Compound **3** was prepared using the general enzymatic nucleoside transglycosylation procedure A. 50 mM nucleobase stock solution (10.7 mg dissolved in 1683 µL) in EtOH (10.0 µmol, 200 µL, 1 equiv) was mixed with a 400mM deoxycytidine solution (908 mg in 10 mL), which was dissolved in mQ H_2O (200.0 µmol, 500 µL, 20 equiv). Next, 296 µL mQ H_2O solution was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was analysed by RP-HPLC and purified by preparative HPLC (method D) affording nucleoside **3** for characterisation.

RP-HPLC (Method A): t_R = 3.60 min, 90 % conversion (std = 1).

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the large-scale characterisation.

3.3 Scale up of pyrimidines.

2'-Deoxy-5-ethynyluridine (**3**)



Deoxycytidine (2.27 g, 10 mmol, 10 equiv) and 5-ethynyluracil (**13**) (136 mg, 1 mmol, 1 equiv) were dissolved in 100 mL of mQ H_2O. The solution was left stirring for an hour at 40 °C before 400 µL of enzyme stock were added. The reaction was stirred for 24 h at 40 °C. The solvent was evaporated *in vacuo*, dry loaded onto silica and purified by flash column chromatography (silica gel, 0-40% CH_2Cl_2/MeOH) to obtain nucleoside **3** in 52% yield (132 mg, 0.52 mmol) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 11.60 (s, 1H, N*H*), 8.28 (s, 1H, H^6), 6.10 (*app* t, *J* = 6.5 Hz, 1H, $H^{1'}$), 5.23 (d, *J* = 4.3 Hz, 1H, O$H^{3'}$), 5.11 (*app* t, *J* = 4.9 Hz, 1H, O$H^{5'}$), 4.26 – 4.21 (m, 1H, $H^{3'}$), 4.08 (s, 1H, C^{5}CC*H)*), 3.79 (*app* q, *J* = 3.3 Hz, 1H, $H^{4'}$), 3.62 (ddd, *J* = 11.9, 5.0, 3.3 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.56 (ddd, *J* = 11.8, 4.9, 3.4 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.17-2.10 (*m*, 2H, $H^{2'}$ and $H^{2''}$).

^{1}H NMR in agreement with literature values[2]

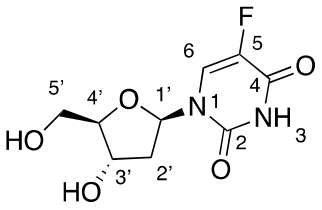
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 161.6 (C^4), 149.4 (C^2), 144.5(C^6), 97.6 (C^5C), 87.6($C^{4'}$), 84.8($C^{1'}$), 83.5(C^{5}C*C*H), 76.4(C^5), 70.0($C^{3'}$), 60.8($C^{5'}$), 40.2($C^{2'}$).

RP-HPLC (Method A): t_R = 3.60 min, 82% conversion

IR ν_{max} (cm^{-1}): 3247(C≡C-H stretch), 1680 (C=O stretch)

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{11}H_{12}N_2O_5Na$ 275.0638; found, 275.0636

2'-Deoxy-5-fluorouridine (**12**)



Deoxycytidine (1.14 g, 5 mmol, 5 equiv) and 5-fluorouracil (130 mg, 1 mmol, 1 equiv) were dissolved in 100 mL of mQ H_2O. The solution was left stirring for an hour at 40 °C before 200 µL of enzyme were added. The reaction was stirred for 24 h at 40 °C. The crude residue was concentrated *in vacuo*, dry loaded onto silica and purified by flash column chromatography (silica gel, 0-40% CH_2Cl_2/MeOH) to obtain nucleoside (**12**) in 56% yield as a white solid (137 mg, 1.0 mmol).

^{1}H NMR (400 MHz, DMSO-*d_6,* ppm) δ 11.80 (s, 1H, N*H*), 8.21 (d, *J* = 7.2 Hz, 1H, *H*^{6}), 6.12 (*app* td, *J* = 6.6, 2.0 Hz, 1H, *H*$^{1'}$), 5.23 (d, *J* = 4.3 Hz, 1H, O*H*$^{3'}$), 5.13 (*app* t, *J* = 5.0 Hz, 1H, O*H*$^{5'}$), 4.23 (*app* dt, *J* = 7.7, 3.8 Hz, 1H, *H*$^{3'}$), 3.78 (*app* q, *J* = 3.3 Hz, 1H, *H*$^{4'}$), 3.62 (ddd, *J* = 12.6, 5.4, 3.8 Hz, 1H, *H*$^{5'}$ or *H*$^{5''}$), 3.56 (ddd, *J* = 11.9, 4.9, 3.5 Hz, 1H, *H*$^{5'}$ or *H*$^{5''}$), 2.14-2.06 (*m,* 2H, *H*$^{2'}$ and *H*$^{2''}$).

^{1}H NMR in agreement with literature values[3]

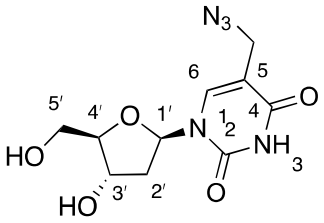
^{13}C{^{1}H}-NMR (101 MHz, DMSO-*d_6*, ppm) δ 157.0 (d, *J* = 26.3 Hz, *C*^{4}), 149 (*C*^{2}*),* 139.9 (d, *J* = 230.0 Hz *C*^{5}), 124.7 (d, *J* = 34.3 Hz, *C*^{6}), 87.48 (*C*$^{4'}$), 84.52 (*C*$^{1'}$), 70.11 (*C*$^{3'}$), 61.00(*C*$^{5'}$).
C$^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

^{19}F NMR (471 MHz, DMSO, ppm) δ −167.2.

RP-HPpLC (Method A): t_R = 2.62 min, 80% conversion.

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_9H_{12}FN_2O_5$. 269.0544; found, 269.0536.

2'-Deoxy-5-Azidomethyluridine (**15**)

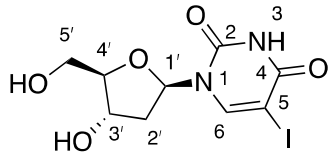


Deoxycytidine (1.36 g, 5.98 mmol, 10 equiv) and 5-(azidomethyl)pyrimidine-2,4(1*H*,3*H*)-dione (100 mg, 0.6 mmol, 1 equiv) were dissolved in 50 mL of 2 mM Na_2HPO_4 pH 6. The solution was left stirring for an hour at 40 °C before 200 µL of enzyme were added. The reaction was stirried for 24 h at 40 °C. The crude residue was concentrated *in vacuo*, dry loaded onto silica

and purified by flash column chromatography (silica gel, 0-12% CH_2Cl_2/MeOH) to obtain the target compound (**15**) in 31% yield (53 mg, 0.6 mmol) as a white solid.

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the small-scale characterisation.

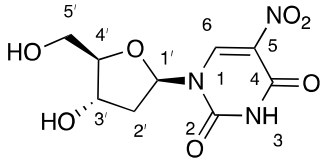
2'-Deoxy-5-iodouridine (**14**)



Deoxycytidine (2.86 g, 12.6 mmol, 3 equiv) and 5-iodouracil (1.00 g, 4.2 mmol, 1 equiv) were dissolved in 300 mL of mQ H_2O. The solution was left stirring for an hour at rt before 600 µL of enzyme were added. The reaction was stirred for 24 h at rt. The crude residue was concentrated *in vacuo*, loaded onto silica and purified by flash column chromatography (silica gel, 0-20% CH_2Cl_2/MeOH) to obtain the target compound (**14**) in 56% yield (840 mg, 4.2 mmol) as a white solid.

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the small-scale characterisation.

2'-Deoxy-5-nitrouridine (20)



Deoxycytidine (788 mg, 3.18 mmole, 5 equiv) and 5-nitrouracil (100 mg, 0.64 mmol, 1 equiv) were dissolved in 64 mL of mQ H_2O.The solution was left stirring for an hour at 40 °C before 128 µL of enzyme were added. The reaction was stirred for 24 h at 40 °C. The crude residue was concentrated *in vacuo*, loaded onto silica, and purified by flash column chromatography (silica gel, 0-40% (CH_2Cl_2/MeOH) to obtain to obtain compound 5-nitrouridine (**20**) in 22% yield as a white solid (37 mg, 0.14 mmol).

^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 12.01 (s, 1H, N*H*), 9.49 (s, 1H, *C*^{6}), 6.06 (*app* t, *J* = 5.9 Hz, 1H, *H*$^{1'}$), 5.29 (d, *J* = 4.5 Hz, O*H*$^{3'}$), 5.24 (*app* t, *J* = 4.4 Hz,1H, O*H*$^{5'}$), 4.31 – 4.20 (m, 1H,

H$^{3'}$), 3.89 (*app* q, *J* = 3.4 Hz, 1H, *H*$^{4'}$) 3.69 (ddd, J = 11.8, 4.5, 3.2 Hz, 1H, *H*$^{5'}$ or *H*$^{5''}$), 3.60 (ddd, *J* = 11.9, 4.5, 3.1 Hz, 1H, *H*$^{5'}$ or *H*$^{5''}$), 2.30 - 2.24 (*m*, 2H, *H*$^{2'}$ and *H*$^{2''}$).

^{1}H NMR in agreement with literature values[4]

^{13}C{^{1}H}-NMR (101 MHz, DMSO-*d*$_6$, ppm): δ 154.8 (*C*^{4}), 148.7 (*C*^{2}), 145.4 (*C*^{6}), 125.4 (*C*^{5}), 88.0 (*C*$^{4'}$), 86.5 (*C*$^{1'}$), 69.1 (*C*$^{3'}$), 60.1 (*C*$^{5'}$), 40.8 (*C*$^{2'}$).

RP-HPLC (Method A): t_R = 3.28 min, 38% conversion

IR ν_{max} (cm^{-1}): 1717 (N=O stretch).

HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_9H_{11}O_7N_3Na$, 296.0489; found, 296.0488.

4. Protein expression and Purification

4.1 Expression

The coding sequence for the wild type *Lactobacillus leichmannii* NDT (Uniprot: Q9R5V5) with an N-terminal 6-His tag, was synthesized by GenScript (with codon optimization for *Escherichia coli*) and subcloned into a pET29a+ plasmid. The plasmids were received from GenScript in the lyophilised form. Plasmid DNA was resuspended in nuclease -free water (6 µL) according to manufacturer protocol.

Amino acid sequence of protein used in this work:
GSSHHHHHHSSGLEVLFQGPAMPKKTIYFGAGWFTDRQNKAYKEAMEALKENPTIDLENSY
VPLDNQYKGIRVDEHPEYLHDKVWATATYNNDLNGIKTNDIMLGVYIPDEEDVGLGMELGYA
LSQGKYVLLVIPDEDYGKPINLMSWGVSDNVIKMSQLKDFNFNKPRFDFYEGAVY*

Plasmid (0.8 µL) containing target gene pET29a+-NDT was transformed into BL21 (DE3) *E. coli* competent cells (Invitrogen) using the heat shock method . Transformants harbouring the plasmid were plated on LB agar containing 50 µg/ mL kanamycin and incubated at 37 °C overnight. Colonies were inoculated into 10 mL LB media containing 50 µg/ mL kanamycin and grown overnight at 37 °C while shaking gently at 200 rpm. Overnight transformants were used to inoculate 450 mL of LB media supplemented with 50 µg/ mL kanamyc into an OD of 0.01 and incubated at 37 °C, 200 rpm until reaching an OD of ~0.4. When OD reached ~0.4

cells were induced with IPTG (0.5 mM,2.5 mL) solution for 3 hours.. The cells were harvested by centrifugation (10,000 x g for 20 min at 4 °C) and the supernatant was discarded. The cell pellet was resuspended in 10 mM sodium phosphate buffer pH 7 (~10 mL buffer per 1 g of cell pellet). The resuspended cell pellets were lysed using a French press. The resulting cell lysate was separated by centrifugation (10,000 g, 30 mins, 4 °C) and the supernatant collected.

4.2 Purification

Binding Buffer (Buffer A): 10 mM sodium phosphate, 10 mM imidazole, 100 mM NaCl, pH 7.0
Binding Buffer (Buffer B): 10 mM sodium phosphate, 500 mM imidazole, 100 mM NaCl, pH 7.0.

The collected supernatant was filtered through a 0.45 μM PES filter and the proteins were purified by affinity chromatography. A 5 mL Histrap FF column (GE Healthcare 17525501) was fitted on an Akta Pure protein purification system and equilibrated with 5 column volumes (CV) of binding buffer A before the supernatant of the cell lysate was loaded on to the column. The flow-through was collected as the non-absorbed fraction (NAF). The column was then washed with binding buffer A for 13 CV and when the UV absorbance was stable the protein was eluted with a gradient of imidazole (Buffer B 0-100%) over 17 CV. The fractions were analysed by SDS-PAGE.

General SDS-PAGE conditions involved mixing the sample with appropriate amount of 4X SDS dye and loaded into wells of NovexTM 4-20% Tris-Glycine Mini-Gels 50:50. Gels were run in 1x running buffer for 60 minutes at 140 V. SDS-PAGE gels were stained in Coomassie Blue for 1 hour prior to de-staining overnight at room temperature, on a shaking-platform.

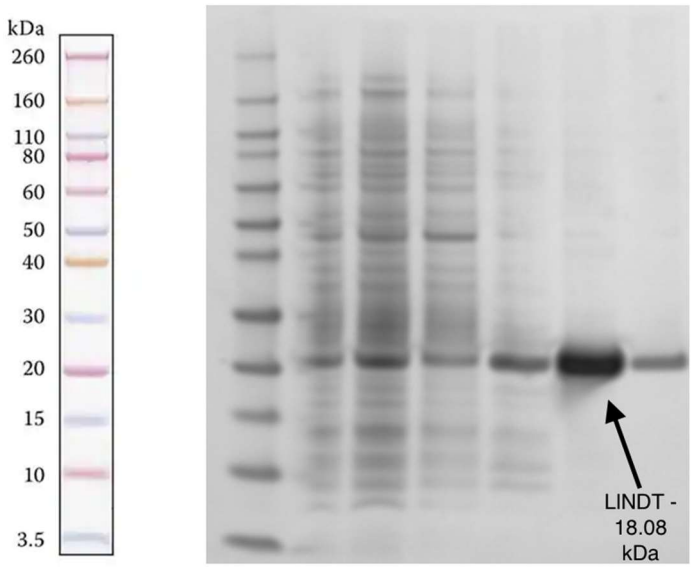


Figure S4 – HisTRAP FF SDS PAGE gel – lane order, Marker, flow through, flow through, column wash, pure NDT fraction, pure NDT fraction, post fraction wash out.

Appropriate fractions were pooled together, and 200 μL (400 units) of HRV 3C protease was added and dialysed over 48 hours at 4 °C into 20 mM sodium phosphate, 100 mM NaCl, pH 7.0 for histag removal. Histag cleavage was monitored and analysed by SDS-PAGE. Once his cleavage was complete, fractions were pooled and loaded onto a 5 mL Histrap FF column (GE Healthcare 17525501) using a Akta Pure protein purification system. The system was once again equilibrated with 5 column volumes of binding buffer A before loading of the pooled fractions. The flow-through was collected as the non-absorbed fraction containing the cleaved LlNDT protein. This was confirmed once again by SDS-PAGE, before appropriate fractions were applied to a 5,000 MW Amicon Centrifugal filter unit to be concentrated (10 mg/mL) and calculated by nanodrop.

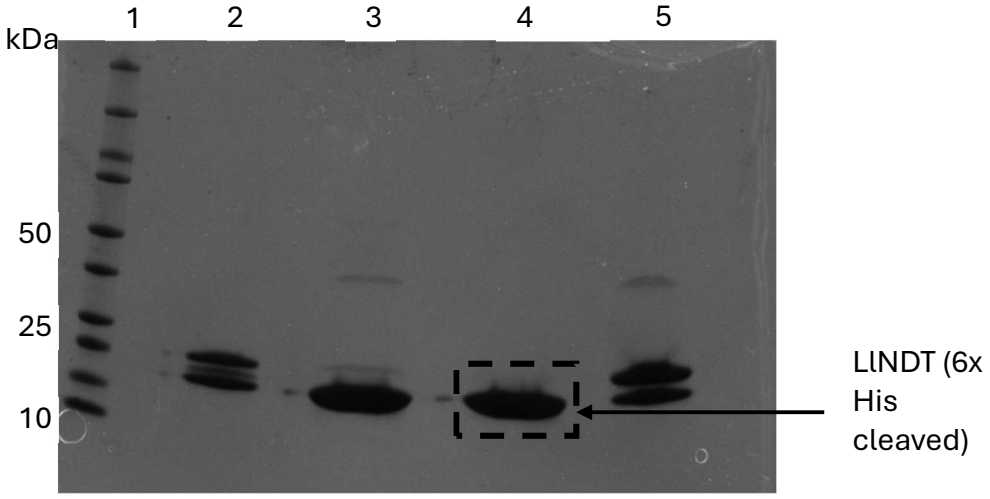


Figure S5 – SDS PAGE gel – lane order: 1 = ladder, 2 = HRV 3C protease histag removal after 24 hours, 3 = HRV 3C protease histag removal after 48 hours, 4 = pooled fractions after the second IMAC following histag removal, 5= HRV 3C protease histag removal after 30 hours.

For crystallization studies, pooled fractions were additionally purified by SEC using the following protocol (SEC buffer: 20 mM sodium phosphate, 100 mM NaCl, pH 7.0). A Superdex 200 prep grade 16/60 Size Exclusion chromatography (SEC) column was equilibrated with SEC buffer and loaded with 2.5 mL aliquots of the HisTrap concentrate (10 mg/mL). The column was eluted with SEC buffer with 1 mL fractions collected and analysed by SDS-PAGE. Fractions containing NDT were concentrated using a 5,000 MW Amicon Centrifugal filter unit to 1.5 mL at a concentration of 21 mg/mL, with 0.75 mL in 10 % glycerol (20 mM sodium

phosphate, 100 mM NaCl, pH 7.0) and an alternative 0.75 mL in 20 mM sodium phosphate, 100 mM NaCl, pH 7.0. This was flash frozen with N_2 (l) and stored at 20°C.

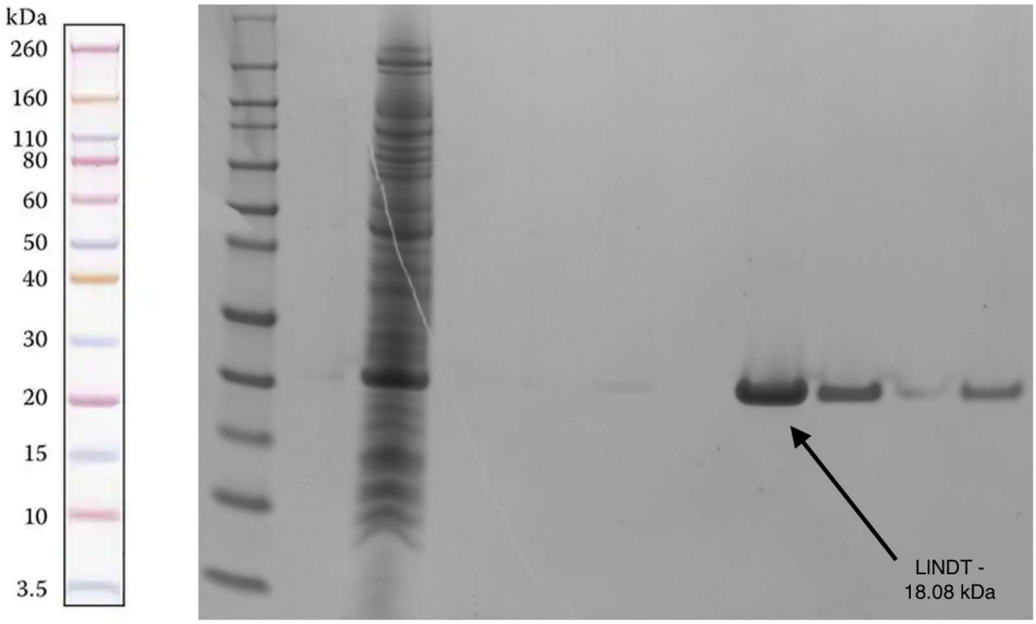


Figure S6 - Size exclusion chromatography (SEC) SDS PAGE gel – lane order, blank, HisTrap flow through, blank, HisTrap elution, blank, SEC pure fraction, SEC pure fraction, blank, SEC pure fraction.

4.3 Crystallisation

Purified LLNDT was concentrated to 10 mg mL^{-1} and this was screened for crystallisation in 96 well-plate format against a number of commercially available crystallization screens using a Mosquito robot (SPT Labtech), which was programmed to deliver drop volumes of 150 nL protein plus 150 nL precipitant solution. Crystals were obtained in several different conditions. Crystals for Dataset #1 (*apo*-) were obtained from drops containing 20 mM NaH_2PO_4 buffer at pH 7.0 with 0.2 M sodium citrate and 20% (w/v) PEG 3350. Crystals for Dataset #2 (compound **3**) were obtained from conditions containing 35% tascimate, again at pH 7.0. Crystals for Dataset #3 (compound **50**) were obtained from conditions containing 0.2 M sodium citrate tribasic dehydrate and 20% (w/v) PEG 3350 again at pH 7.0. Substrate soaks for crystals yielding Datasets #2 and #3 were obtained by incubating crystals for 4 h in their mother liquor containing 10 mM ligand, which had in each case been derived from a 100 mM stock in DMSO.

4.4 *Data Collection and Refinement*

The datasets described were collected at the Diamond Light Source, Didcot, Oxfordshire U.K. on beamline I03. Data were processed and integrated using XDS[5] and scaled using SCALA[6] included in the Xia2 processing system[7]. Data collection statistics are provided in **Table S2**. Crystals for all Datasets were obtained in space group $I3_12$, with approximately the same cell dimensions and with two molecules in the asymmetric unit that constituted one third of the LLNDT hexamer. The solvent content in the crystals was approximately 67% in each case. The structures of LLNDT were solved by molecular replacement using MOLREP[8] with the monomer of LLNDT (PDB code 1F8Y[9]) as the model. The structures were built and refined using iterative cycles in Coot[10] and REFMAC[11], employing local NCS restraints in the refinement cycles. Following building and refinement of the protein and water molecules in the structural complexes obtained from Datasets #2 and #3, residual density was observed in the omit maps at the active sites. For Dataset #2, this could be modelled as the deoxyribosyl intermediate covalently bonded to E98; for dataset #3 density in subunit A was modelled as 2-deoxycytidine (**8**); in subunit B it was modelled as the deoxyribosyl intermediate covalently bonded to E98 and also two overlapping molecules of 7-Bromo-1H-imidazo[4,5-b]pyridine (**17**).

The final structures obtained from Datasets #1, #2 and #3 exhibited R_{cryst} / R_{free} values of 0.18/0.23, 0.20/0.23 and 0.19/0.21 respectively. Refinement statistics for the structures are presented in **Table S2.** The coordinates and structure factors for Datasets #1, #2 and #3 have been deposited in the Protein Databank as ***apo*-LLNDT (PDB code 9EZK**), LLNDT-deoxyribose (**PDB code 9F08**) and LLNDT-deoxycytidine-deoxyribose-7-Bromo-1H-imidazo[4,5-b]pyridine, respectively (**PDB code 9F09**).

Table S2 - Data collection and refinement statistics for LLNDT. Numbers in brackets refer to data for highest resolution shells.

	Dataset #1 ***apo*-LLNDT**	Dataset #2 **LLNDT with deoxyribose (16)**	Dataset #3 **LLNDT with deoxyxcytidine, deoxyribose and 7-Bromo-1H-imidazo[4,5-b]pyridine (17)**
Beamline	I03	I03	I03
Wavelength (Å)	0.976277	0.976277	0.976269
Resolution (Å)	60.94-2.79 (2.94-2.79)	60.61 – 2.37 (2.46-2.37)	60.78 – 2.37 (2.46-2.37)

Space Group	$I3_12$	$I3_12$	$I3_12$
Unit cell (Å)	a = b = c =149.27; α = β = γ = 90.00˚	a = b = c =148.46; α = β = γ = 90.00˚	a = b = c =148.88; α = β = γ = 90.00˚
No. of molecules in the asymmetric unit	2	2	2
Unique reflections	13954 (2028)	22224 (2312)	22441 (2337)
Completeness (%)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
R_{merge} (%)	0.15 (2.20)	0.06 (0.96)	0.09 (1.48)
$R_{p.i.m.}$	0.03 (0.49)	0.01 (0.22)	0.02 (0.32)
Multiplicity	40.8 (40.0)	41.2 (38.5)	41.7 (43.5)
$<I/\sigma(I)>$	22.6 (2.3)	42.5 (5.1)	33.3 (3.6)
Overall *B* from Wilson plot ($Å_2$)	75	61	56
$CC_{1/2}$	1.00 (0.82)	1.00 (0.95)	1.00 (0.91)
R_{cryst}/ R_{free} (%)	0.18/0.23	0.20/0.23	0.19/0.21
r.m.s.d 1-2 bonds (Å)	0.008	0.007	0.008
r.m.s.d 1-3 angles (°)	1.820	1.646	1.695
Avge main chain B ($Å^2$)	78	64	58
Avge side chain B ($Å^2$)	84	69	67
Avge waters B ($Å^2$)	66	59	58
Avge Ligand B ($Å^2$)	-	62	68

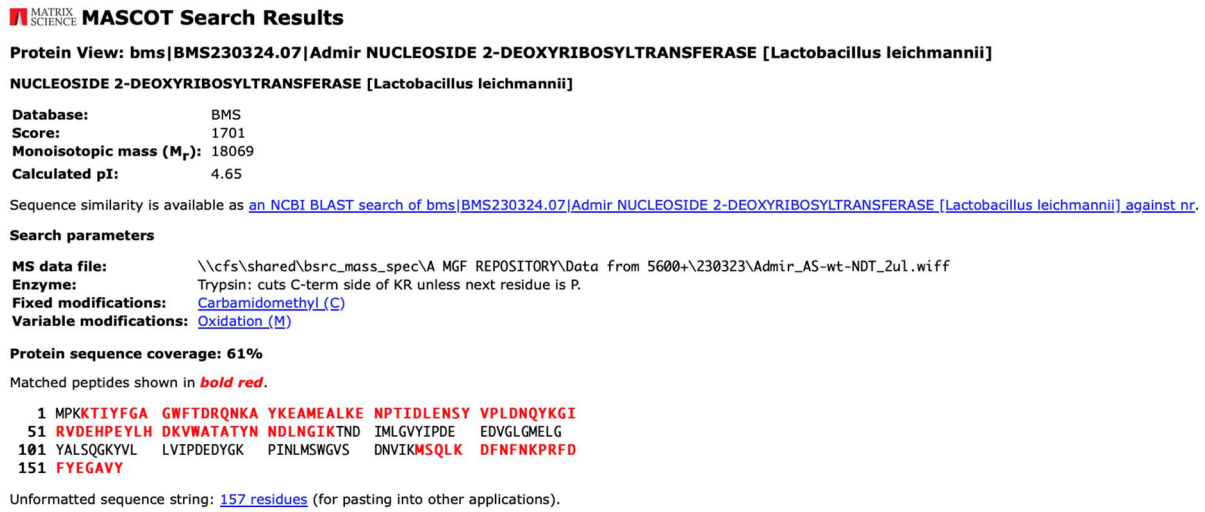
4.5 **Protease (tryspin) digestion**

SDS gel bands corresponding to the expressed WT NDT and the commercial NDT (Sigma-Aldrich N2665) were excised at the MW marker of 18 kDa corresponding to the single monomer mass of NDT. Trypsin digestion and resulting peptide analysis was carried out by the mass spectrometry and proteomics facility at The University of St Andrews.

The results obtained from the trypsin digestion were contrasted against the amino acid sequence from LINDT.

MPKKTIYFGAGWFTDRQNKAYKEAMEALKENPTIDLENSYVPLDNQYKGIRVDEHPEYLHD
KVWATATYNNDLNGIKTNDIMLGVYIPDEEDVGLGMELGYALSQGKYVLLVIPDEDYGKPINL
MSWGVSDNVIKMSQLKDFNFNKPRFDFYEGAVY.

Both gel bands matched the same amino acid sequence corresponding with the provided LINDT.



MATRIX SCIENCE **MASCOT Search Results**

Protein View: bms|BMS230324.07|Admir NUCLEOSIDE 2-DEOXYRIBOSYLTRANSFERASE [Lactobacillus leichmannii]

NUCLEOSIDE 2-DEOXYRIBOSYLTRANSFERASE [Lactobacillus leichmannii]

Database: BMS
Score: 1701
Monoisotopic mass (M_r): 18069
Calculated pI: 4.65

Sequence similarity is available as an NCBI BLAST search of bms|BMS230324.07|Admir NUCLEOSIDE 2-DEOXYRIBOSYLTRANSFERASE [Lactobacillus leichmannii] against nr.

Search parameters

MS data file: \\cfs\shared\bsrc_mass_spec\A MGF REPOSITORY\Data from 5600+\230323\Admir_AS-wt-NDT_2ul.wiff
Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)

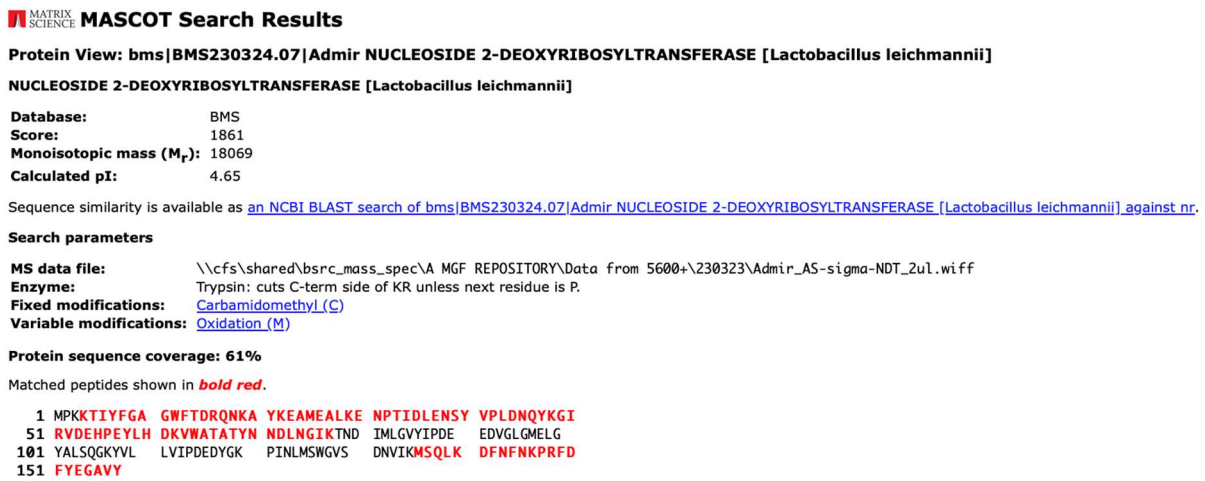
Protein sequence coverage: 61%

Matched peptides shown in ***bold red***.

```
  1 MPKKTIYFGA  GWFTDRQNKA  YKEAMEALKE  NPTIDLENSY  VPLDNQYKGI
 51 RVDEHPEYLH  DKVWATATYN  NDLNGIKTND  IMLGVYIPDE   EDVGLGMELG
101 YALSQGKYVL   LVIPDEDYGK   PINLMSWGVS   DNVIKMSQLK  DFNFNKPRFD
151 FYEGAVY
```

Unformatted sequence string: 157 residues (for pasting into other applications).

Figure S7 - Sequence coverage of 61% for the WT *LI*NDT (expressed using IPTG) (emPAI score of 89.39).



MATRIX SCIENCE **MASCOT Search Results**

Protein View: bms|BMS230324.07|Admir NUCLEOSIDE 2-DEOXYRIBOSYLTRANSFERASE [Lactobacillus leichmannii]

NUCLEOSIDE 2-DEOXYRIBOSYLTRANSFERASE [Lactobacillus leichmannii]

Database: BMS
Score: 1861
Monoisotopic mass (M_r): 18069
Calculated pI: 4.65

Sequence similarity is available as an NCBI BLAST search of bms|BMS230324.07|Admir NUCLEOSIDE 2-DEOXYRIBOSYLTRANSFERASE [Lactobacillus leichmannii] against nr.

Search parameters

MS data file: \\cfs\shared\bsrc_mass_spec\A MGF REPOSITORY\Data from 5600+\230323\Admir_AS-sigma-NDT_2ul.wiff
Enzyme: Trypsin: cuts C-term side of KR unless next residue is P.
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)

Protein sequence coverage: 61%

Matched peptides shown in ***bold red***.

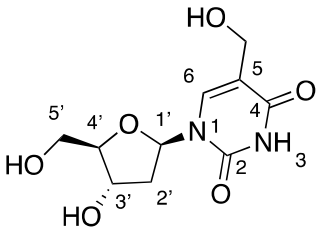
```
  1 MPKKTIYFGA  GWFTDRQNKA  YKEAMEALKE  NPTIDLENSY  VPLDNQYKGI
 51 RVDEHPEYLH  DKVWATATYN  NDLNGIKTND  IMLGVYIPDE   EDVGLGMELG
101 YALSQGKYVL   LVIPDEDYGK   PINLMSWGVS   DNVIKMSQLK  DFNFNKPRFD
151 FYEGAVY
```

Figure S8 - Sequence coverage of 61% for the *LI*NDT (Sigma-Aldrich) (emPAI score of 82.68).

5. Scope Screening

5.1 Pyrimidine SAR

2'-Deoxy-5-hydroxymethyluridine (18)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (28.8 mg dissolved in 4.05 mL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100 mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording nucleoside 2'-deoxy-5-hydroxymethyluridine (**18**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 11.30 (s, 1H, N*H*), 7.72 (d, *J* = 1.3 Hz, 1H, H^6), 6.19 (*app* t, *J* = 6.9 Hz, 1H, $H^{1'}$), 5.24 (*br* s, 1H, O$H^{3'}$), 4.95 (*br* s, 1H, O$H^{5'}$), 4.89 (s, 1H, C^5CH_2OH), 4.23 (*app* dt, *J* = 6.5, 3.3 Hz, 1H, $H^{3'}$), 4.13 (s, 2H, C^5CH_2OH), 3.77 (*app* q, *J* = 4.0 Hz, 1H, $H^{4'}$), 3.61-3.49 (*m*, 2H, $H^{5'}$ and $H^{5''}$), 2.18 – 1.95 (m, 2H, $H^{2'}$ and $H^{2''}$).

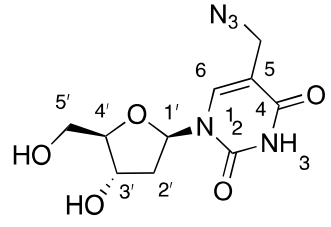
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 162.6(C^4), 150.3(C^2), 136.7(C^6), 114.2(C^5), 87.3($C^{4'}$), 83.9($C^{1'}$), 70.5($C^{3'}$), 61.4($C^{5'}$), 56.0 (C^5C). *$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

NMR values in agreement with literature[12]

RP-HPLC (Method A): t_R = 2.48 min, 60% conversion (std =5).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{10}H_{14}N_2O_6Na$ 281.0774; found, 281.0738

2'-Deoxy-5-Azidomethyluridine (15)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (10.5 mg dissolved in 1257 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 200 mM deoxycytidine solution (454 mg in 10 mL), which was dissolved in mQ H_2O (100.0 µmol, 500 µL, 10 equiv). Next, 296 µL 30 mM Na_2HPO_4 pH 6 solution was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at rt for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-5-azidomethyluridine (**15**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 11.55 (s, 1H, N*H*), 8.04 (s, 1H, H^6), 6.15 (*app* t, *J* = 6.7 Hz, 1H, $H^{1'}$), 5.25 (d, *J* = 4.2 Hz, 1H, O$H^{3'}$), 5.03 (*app* t, *J* = 5.2 Hz, 1H, O$H^{5'}$), 4.28-4.18 (m, 1H, $H^{3'}$), 4.07 (d, *J* = 1.5 Hz, 2H, CH_2), 3.79 (*app* q, *J* = 3.7 Hz, 1H, $H^{4'}$), 3.61 (ddd, *J* = 11.8, 5.4, 4.0 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.55 (ddd, *J* = 11.8, 5.2, 4.1 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.14 – 2.08 (m, 2H, $H^{2'}$ *and* $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 162.8(C^4), 150.2(C^2), 139.8(C^6), 108.2(C^5), 87.4($C^{4'}$), 84.2($C^{1'}$), 70.2($C^{3'}$), 61.2($C^{5'}$), 46.9(C^5CH_2), 45.7($C^{2'}$).

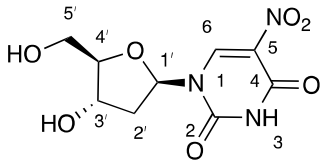
NMR values in agreement with literature[12]

RP-HPLC (Method A): t_R = 4.12 min, 60% conversion (std = 2).

IR ν_{max} (cm^{-1}): 2110 (N=N=N stretch), 1672 (C=O).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{10}H_{13}N_5O_5Na$ 306.0809; found, 306.0804.

2'-Deoxy-5-nitrouridine (20)



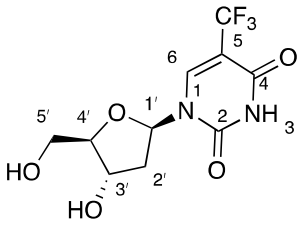
Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (6 mg dissolved in 769 µL) in DMSO (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (227 mg in 10 mL), which was dissolved in mQ H_2O (80.0 µmol, 792 µL, 8 equiv). Next, 8 µL of NDT stock solution was added to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24

h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-5-nitrouridine (**20**) for characterisation.

RP-HPLC (Method A): t_R = 5.09 min, 51% conversion (std = 1).

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the small-scale characterisation.

2'-Deoxy-5-trifluorouridine (21)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (17.4 mg dissolved in 1.9 mL) in DMSO (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100 mM deoxycytidine solution (682 mg in 30 mL) dissolved in 100 mM Na_2HPO_4 buffer solution (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL 100 mM Na_2HPO_4 buffer solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-5-trifluorouridine (**21**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 11.82 (br s, 1H, *NH*), 8.67 (s, 1H, H^6), 6.09 (*app* t, *J* = 6.1 Hz, 1H, $H^{1'}$), 5.24 (br s, 1H, O$H^{3'}$), 5.18 (br s, 1H, O$H^{5'}$), 4.24 (*app* q, *J* = 5.0 Hz, 1H, $H^{3'}$), 3.82 (*app* q, *J* = 3.2 Hz, 1H, $H^{4'}$), 3.69 – 3.61 (m, 1H, $H^{5'}$ *or* $H^{5''}$), 3.61 – 3.54 (m, 1H, $H^{5'}$ *or* $H^{5''}$), 2.22 - 2.15 *(m,* 2H, $H^{2'}$ *and* $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 159.5 (C^4), 149.9 (C^2), 142.0 (C^6), 102.8, 102.5, 87.6($C^{4'}$), 85.4($C^{1'}$), 69.4($C^{3'}$), 60.3($C^{5'}$), 40.6($C^{2'}$). CF^3 carbon missing.

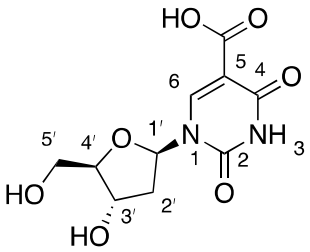
^{19}F NMR (376 MHz, DMSO, ppm) δ −61.5.

NMR values in agreement with literature[13]

RP-HPLC (Method A): t_R = 4.77 min, 71% conversion (std = 1).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{10}H_{11}F_3N_2O_5Na$, 292.0459; found, 292.0462.

2'-Deoxy-5-carboxyuridine (22)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (22.3 mg dissolved in 2857 µL) in DMSO (10.0 µmol, 200 µL, 1 equiv) was mixed with a 200 mM deoxycytidine solution (454 mg in 10 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 10 equiv). Next, 296 µL 100 mM Na_2HPO_4 buffer solution was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-5-carboxyuridine (**22**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 12.84 (br s, 1H, COO*H*), 12.15 (br s, 1H, N*H*), 8.84 (s, 1H, H^6), 6.09 (*app* t, *J* = 6.3 Hz, 1H, $H^{1'}$), 5.27 (d, *J* = 4.3 Hz, 1H, O$H^{3'}$), 5.08 (t, *J* = 4.6 Hz, 1H, O$H^{5'}$), 4.23 (*app* dq, *J* = 5.9, 3.9 Hz, 1H, $H^{3'}$), 3.87 (*app* q, *J* = 3.5 Hz, 1H, $H^{4'}$), 3.62 (ddd, *J* = 11.7, 4.7, 3.6 Hz, 1H, $H^{5'}$ *or* $H^{5''}$), 3.56 (ddd, *J* = 11.7, 4.6, 3.6 Hz, 1H, $H^{5'}$ *or* $H^{5''}$), 2.28 – 2.12 (m, 2H, $H^{2'}$ *and* $H^{2''}$).

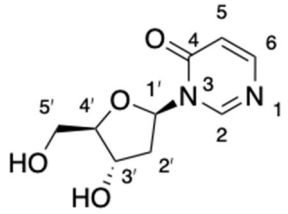
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 164.2(*C*OOH), 163.3 (C^2 or C^4), 149.4 (C^2 or C^4), 148.0 (C^6), 102.3(C^5), 88.0($C^{4'}$), 86.0($C^{1'}$), 70.0($C^{3'}$), 60.8($C^{5'}$), 40.5($C^{2'}$).

NMR values in agreement with literature[14]

RP-HPLC (Method A): t_R = 3.34 min, 26% conversion (std = 1).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{10}H_{12}N_2O_7Na$, 295.0537; found, 295.0527.

3-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-4(1*H*)-one (23).[15]



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (28 mg dissolved in 5828 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100 mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 3-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-4(1*H*)-one (**23**).

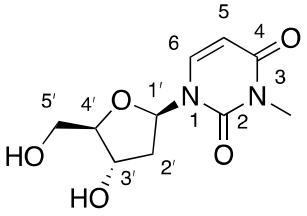
^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.73 (s, 1H, H^2), 7.93 (dd, *J* = 6.6, 0.6 Hz, 1H, H^6), 6.37 (dd, *J* = 6.6, 1.0 Hz, 1H, H^5), 6.25 (*app* t, *J* = 6.5 Hz, 1H $H^{1'}$), 5.28 (d, *J* = 4.3 Hz, 1H, O$H^{3'}$), 5.08 (*app* t, *J* = 5.1 Hz, 1H, O$H^{5'}$), 4.27 (*app* dq, *J* = 5.9, 3.8 Hz, 1H, $H^{3'}$), 3.88 (*app* q, *J* = 3.6 Hz, 1H, $H^{4'}$), 3.65 (ddd, *J* = 11.9, 5.2, 3.6 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.58 (ddd, *J* = 11.9, 5.0, 3.8 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.38-2.27 (m, 1H, $H^{2'}$ or $H^{2''}$), 2.12 (ddd, *J* = 13.2, 6.8, 6.0 Hz, 1H, $H^{2'}$ or $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 159.6 (C^4), 153.2 (C^6), 148.8 (C^2), 114.4 (C^5), 88.0 ($C^{4'}$), 84.7 ($C^{1'}$), 70.0 (C^3), 60.8 ($C^{5'}$), 41.1($C^{2'}$).

RP-HPLC (Method A): t_R = 2.80 min, 57% conversion (std = 1)

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_9H_{12}N_2O_4Na$ 235.0689; found, 235.0687.

2'-Deoxy-*N*-3-methyluridine (24)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (16.0 mg dissolved in 2537.2261 µL) in DMSO (10.0 µmol, 200 µL, 1 equiv) was mixed with a 200mM deoxycytidine solution (454 mg in 10 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 10 equiv). Next, 296 µL 100 mM Na_2HPO_4 buffer solution was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-*N*-3-methyluridine (**24**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 7.92 (d, *J* = 8.1 Hz, 1H*, H^5* or *H^6*), 6.19 (dd, *J* = 7.3, 6.1 Hz, 1H, *H$^{1'}$*), 5.77 (d, *J* = 8.0 Hz, 1H, *H^5* or *H^6*), 5.26 (d, *J* = 4.2 Hz, 1H*,* O*H$^{3'}$*), 5.02 (t, *J* = 5.1 Hz, 1H*,* O*H$^{5'}$*), 4.24 (*app* dq, *J* = 6.6, 3.3 Hz, 1H, *H$^{3'}$*), 3.81 (*app* q, *J* = 3.6 Hz, 1H, *H$^{4'}$*), 3.63 – 3.57 (m, 1H, *H$^{5'}$ or H$^{5''}$*), 3.54 (*app* dd, J = 8.0, 3.9 Hz, 1H, *H$^{5'}$ or H$^{5''}$*), 3.16 (s, 3H, N^{3}C*H^3*), 2.20 – 2.04 (m, 2H, *H$^{2'}$ and H$^{2''}$*).

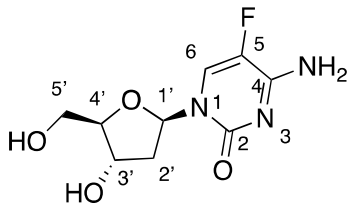
^{1}H NMR in agreement with literature[16]

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 162.1(*C^2 or C^4*), 150.6(*C^2 or C^4*), 138.7(*C^{6}or C^5)*, 100.7(*C^{6}or C^5)*, 87.5*(C$^{4'}$*), 85.3(*C$^{1'}$*), 70.2*(C$^{3'}$*), 61.2*(C$^{5'}$*), 27.1(N^3*C*H^3). *H$^{2'}$* carbon is in the DMSO peak.

RP-HPLC (Method A): t_R = 4.04 min, 22% conversion (std = 1).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{10}H_{14}N_2O_5Na$, 265.0795; found, 265.0791.

2'-Deoxy-5-fluorocytidine (25)[17]



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (16 mg dissolved in 2479 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 200mM thymidine solution (1454 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 10 equiv). Next, 296 µL mQ H_2O solution was added alongside

4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-5-fluorocytidine (**25**)**.**

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.08 (d, J = 7.2 Hz, 1H, H^6), 7.71 (s, 1H, NH), 7.48 (s, 1H, NH), 6.10 (ddd, J = 8.1, 6.1, 2.2 Hz, 1H, $H^{1'}$), 5.18 (d, J = 4.2 Hz, 1H, O$H^{3'}$), 5.07 (*app* t, J = 5.1 Hz, 1H, O$H^{5'}$), 4.21 (*app* dq, J = 7.0, 3.7 Hz, 1H, $H^{3'}$), 3.77 (*app* q, J = 3.4 Hz, 1H, $H^{4'}$), 3.61 (ddd, J = 11.9, 5.1, 3.5 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.55 (ddd, J = 11.9, 5.0, 3.6 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.11 (ddd, J = 13.2, 6.0, 3.5 Hz, 1H, $H^{2'}$ or $H^{2''}$), 1.97 (ddd, J = 13.1, 7.2, 6.0 Hz, 1H, $H^{2'}$ or $H^{2''}$).

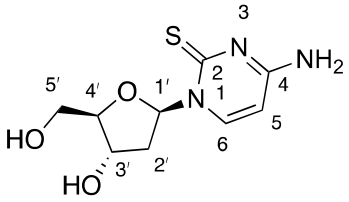
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 205.0, 180.6, 153.2, 125.3 (d, J = 31.9 Hz, C^6), 87.3($C^{4'}$), 85.1($C^{1'}$), 70.1($C^{3'}$), 61.0($C^{5'}$), 40.4($C^{2'}$).

^{19}F NMR (376 MHz, DMSO) δ -73.4.

RP-HPLC (Method A): t_R = 2.03 min, 62% conversion (std = 16).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_9H_{12}FN_3O_4Na$ 268.0704; found, 268.0703.

2'-Deoxy-2-thiocytidine (26)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (10.7 mg dissolved in 1683 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 200mM deoxycytidine solution (454 mg in 10 mL), which was dissolved in mQ H_2O (100.0 µmol, 500 µL, 10 equiv). Next, 296 µL mQ H_2O solution was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-2-thiocytidine (**26**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.09 (d, J = 7.5 Hz, 1H, H^5), 7.65 (s, 1H, NH*)*, 7.51 (s, 1H, *NH*), 6.95 (*app* t, J = 6.4 Hz, 1H, $H^{1'}$), 6.07 (d, J = 7.5 Hz, 1H, H^6), 5.21 (d, J = 4.1 Hz, 1H, O$H^{3'}$), 5.05 (*app* t, J = 5.2 Hz, 1H, O$H^{5'}$), 4.20 (*app* dq, J = 6.1, 3.7 Hz, 1H, $H^{3'}$), 3.83 (*app* q, J = 3.6 Hz, 1H, $H^{4'}$), 3.63 (ddd, J = 11.8, 5.2, 3.5 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.61 – 3.54 (m, 1H, $H^{5'}$

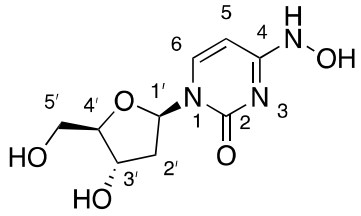
or $H^{5''}$), 2.36 (ddd, J = 13.3, 6.0, 3.7 Hz, 1H, $H^{2'}$ or $H^{2''}$), 1.88 (*app* dt, J = 13.1, 6.4 Hz, 1H, $H^{2'}$ or $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 179.3(C^4 or C^2), 160.3(C^4 or C^2), 141.2(C^5), 97.8(C^6), 89.8($C^{1'}$), 87.8($C^{4'}$), 69.9 ($C^{3'}$), 60.9($C^{5'}$), 40.8 ($C^{2'}$).

RP-HPLC (Method A): t_R = 3.61 min, 52% conversion (std = 1).

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_9H_{14}N_3O_3S$ 244.0750; found, 244.0750.

2'-Deoxy-4-hydroxyaminocytidine (27)[18]



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (10.1 mg dissolved in 1589 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM thymidine solution (727 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 10 equiv). Next, 296 µL 100 mM Na_2HPO_4 buffer solution was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-4-hydroxyaminocytidine (**27**).

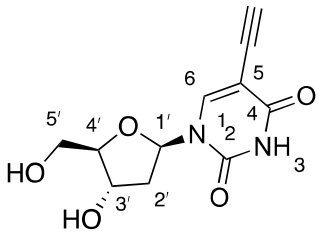
^{1}H NMR (600 MHz, DMSO) δ 7.16 (d, J = 8.2 Hz, 1H, H^6), 6.14 (dd, J = 8.0, 6.1 Hz, 1H, $H^{1'}$), 5.61 (d, J = 8.2 Hz, 1H, H^5), 4.20 (*app* dt, J = 5.8, 2.9 Hz, 1H, $H^{3'}$), 3.73 (*app* td, J = 3.9, 2.6 Hz, 1H, $H^{4'}$), 3.57 – 3.47 (m, 2H, $H^{5'}$ *and* $H^{5''}$), 2.10 – 1.94 (m, 2H, $H^{2'}$ *and* $H^{2''}$).

^{13}C NMR (151 MHz, DMSO) δ 174.4, 149.4(C^2), 145.1(C^4), 131.7(C^6), 98.0(C^5), 87.4($C^{4'}$), 83.9($C^{1'}$), 71.1($C^{3'}$), 62.0($C^{5'}$), 39.2($C^{2'}$).

RP-HPLC (Method A): t_R = 1.9 min, 46% conversion (std = 4).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_9H_{13}N_3O_5Na$ 266.07474; found, 266.0745.

2'-Deoxy-5-ethynyluridine (3)

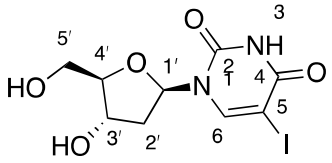


Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (10.7 mg dissolved in 1683 µL) in EtOH (10.0 µmol, 200 µL, 1 equiv) was mixed with a 400mM deoxycytidine solution (908 mg in 10 mL), which was dissolved in mQ H_2O (200.0 µmol, 500 µL, 20 equiv). Next, 296 µL mQ H_2O solution was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-5-ethynyluridine (**3**) for characterisation.

RP-HPLC (Method A): t_R = 3.60 min, 90 % conversion (std = 1).

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the large-scale characterisation.

2'-Deoxy-5-iodouridine (14)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (21.2 mg dissolved in 1782 µL) in EtOH (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-5-iodouridine (**14**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 11.65 (s, 1H, N*H*), 8.39 (s, 1H, H^6), 6.09 (*app* t, *J* = 6.6 Hz, 1H, $H^{1'}$), 5.23 (br s, 1H, O$H^{3'}$), 5.13 (br s, 1H, O$H^{5'}$), 4.24 (*app* q, 4.3 Hz 1H, $H^{3'}$), 3.79 (*app*

q, *J* = 3.3 Hz, 1H, *H*$^{4'}$), 3.63 (*app* dd, *J* = 11.9, 3.3 Hz, 1H, *H*$^{5'}$ or *H*$^{5''}$), 3.56 (*app* dd, *J* = 11.8, 3.3 Hz, 1H, *H*$^{5'}$ or *H*$^{5''}$), 2.15 – 2.09 (m, 2H, *H*$^{2'}$ *and H*$^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 160.5(*C*^{4} *or C*^{5}), 150.1(*C*^{4} *or C*^{5}), 145.0(*C*^{6}), 87.5(*C*$^{4'}$), 84.6(*C*$^{1'}$), 70.0(*C*$^{3'}$), 69.2(*C*^{2}), 60.8(*C*$^{5'}$), 30.7(*C*$^{2'}$).

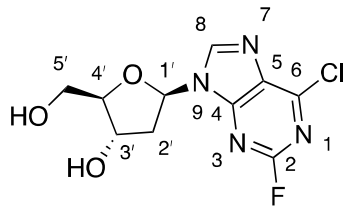
NMR in agreement with literature[19]

RP-HPLC (Method A): t_R = 4.10 min, 88% conversion (std = 4).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_9H_{11}IN_2O_5Na$ 376.0905; found, 376.9599.

5.2 Purine SAR

2'-Deoxy-2-fluoro-6-chloropurine (32)

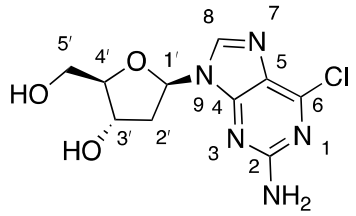


Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (32.2 mg dissolved in 3798 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-2-fluoro-6-chloro-purine (**32**) for characterisation.

RP-HPLC (Method A): t_R = 6.33 min, 89% conversion (std =1).

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the large-scale characterisation.

2'-Deoxy-6-chloroguanosine (33)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (32.2 mg dissolved in 3798 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-6-chloroguanosine (**33**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.34 (s, 1H, H^8), 6.94 (s, 2H, NH), 6.22 (dd, J = 7.4, 6.1 Hz, 1H, $H^{1'}$), 5.28 (d, J = 4.1 Hz, 1H O$H^{3'}$), 4.92 (*app* t, J = 5.5 Hz, 1H, O$H^{5'}$), 4.37 (*app* dq, J = 6.6, 3.4 Hz, 1H, $H^{3'}$), 3.83 (*app* td, J = 4.6, 2.9 Hz, 1H, $H^{4'}$), 3.58 (*app* dt, J = 11.8, 5.1 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.51 (*app* dt, J = 11.7, 5.0 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.61 (ddd, J = 13.2, 7.5, 5.8 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.25 (ddd, J = 13.2, 6.2, 3.4 Hz, 1H, $H^{2'}$ or $H^{2''}$).

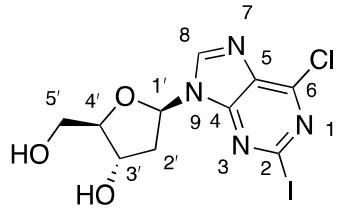
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 159.7 (C^6), 153.6 (C^4), 149.4 (C^2), 141.0 (C^8), 123.5 (C^5), 87.7($C^{4'}$), 83.0($C^{1'}$), 70.5($C^{3'}$), 61.5($C^{5'}$).
$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

NMR values in agreement with literature[20]

RP-HPLC (Method A): t_R = 5.30 min, 97% conversion (std = 1)

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_{13}ClN_5O_3$. 286.0712 ; found, 286.0696.

2'-Deoxy-2-iodo-6-chloropurine (34)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (17.9 mg dissolved in 1277 µL) in EtOH (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-2-iodo-6-chloropurine (**34**).

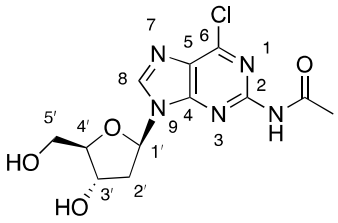
^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.87 (s, 1H, H^8), 6.35 (*app* t, J = 6.4 Hz, 1H, $H^{1'}$), 5.35 (d, J = 4.4 Hz, 1H, $OH^{3'}$), 4.92 (*app* t, J = 5.5 Hz, 1H, $OH^{5'}$), 4.42 (*app* dq, J = 6.1, 4.1 Hz, 1H, $H^{3'}$), 3.88 (*app* q, J = 4.4 Hz, 1H, $H^{4'}$), 3.61 (ddd, J = 11.8, 5.5, 4.5 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.52 (ddd, J = 11.8, 5.5, 4.5 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.70 (*app* dt, J = 13.6, 6.3 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.43-2.29 (m, 1H, $H^{2'}$ or $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 150.8(C^6 or C^2), 148.8(C^5 or C^4), 145.6(C^8), 138.3(C^5 or C^4), 123.7(C^6 or C^2), 88.1($C^{4'}$), 84.2($C^{1'}$), 70.2 ($C^{3'}$), 61.1($C^{5'}$).
*$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

RP-HPLC (Method A): t_R = 6.80 min, 92% conversion (std = 1)

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_{10}ClN_4O_3$ 396.9559; found, 396.9555.

2'-Deoxy-4-acetamide-6chloropurine (35)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (19.4 mg dissolved in 1833 µL) in EtOH (10.0 µmol, 200 µL, 1 equiv) was mixed with a 200 mM deoxycytidine solution (454 mg in 10 mL) dissolved in mQ H_2O (100.0 µmol, 500 µL, 10 equiv). Next, 296 µL mQ H_2O was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-4-acetamide-6chloropurine (**35**).

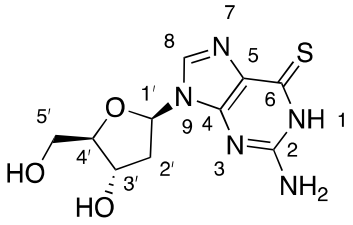
^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 10.85 (s, 1H, H^{8}), 8.70 (s, 1H, N*H*), 6.35 (*app* t, *J* = 6.7 Hz, 1H, $H^{1'}$), 5.33 (br s, 1H, O$H^{3'}$), 4.88 (br s, 1H, O$H^{5'}$) , 4.46 (*app* dt, *J* = 6.4, 3.4 Hz, 1H, $H^{3'}$), 3.87 (*app* td, *J* = 4.7, 3.1 Hz, 1H, $H^{4'}$), 3.61 (*app* dd, *J* = 11.8, 4.7 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.54 (*app* dd, *J* = 11.8, 4.8 Hz, 1H $H^{5'}$ or $H^{5''}$), 2.77 (*app* dt, *J* = 13.1, 6.5 Hz 1H $H^{2'}$ or $H^{2''}$), 2.32 (ddd, *J* = 13.2, 6.4, 3.7 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.18 (s, 3H, COCH^{3}).

^{13}C{1H}-NMR (101 MHz, DMSO-d_6, ppm) δ 168.5 (*C*O), 152.3, 152.0, 149.0, 144.7, 127.6 (C^{2}), 88.1($C^{4'}$), 83.8($C^{1'}$), 70.5($C^{3'}$), 61.5($C^{5'}$), 45.8, 24.5(COCH^{3}).
*$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

RP-HPLC (Method A): t_R = 5.34 min, 82.1% conversion (std = 3).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{12}H_{14}ClN_5O_4Na$, 350.0627; found, 350.0630.

2'-Deoxy-6-thioguanosine (36)

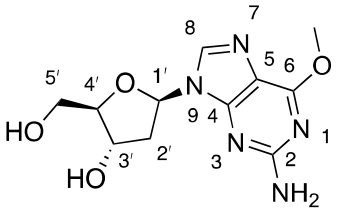


Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (7.5 mg dissolved in 897 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-6-thioguanosine (**36**) for characterisation.

RP-HPLC (Method A): t_R = 3.7 min, 97% conversion (std = 1)

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the large-scale characterisation.

2'-Deoxy-6-O-methylguanosine (37)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (10 mg dissolved in 1211 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-6-O-methylguanosine (**37**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.08 (s, 1H, H^8), 6.43 (s, 2H, NH), 6.21 (dd, J = 7.9, 6.0 Hz, 1H, $H^{1'}$), 5.25 (br s, 1H, O$H^{3'}$), 4.97 (br s, 1H, $OH^{5'}$), 4.35 (*app* dd, J = 5.8, 2.9 Hz, 1H, $H^{3'}$), 3.96 (s, 3H, C^{6}OCH_3), 3.82 (*app* d, J = 2.8 Hz, 1H, $H^{4'}$), 3.57 (*app* dd, J = 11.7, 4.7 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.50 (*app* dd, J = 11.7, 4.5 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.63 – 2.53 (m, 1H, $H^{2'}$ or $H^{2''}$), 2.21 (ddd, J = 13.1, 6.0, 3.0 Hz, 1H, $H^{2'}$ or $H^{2''}$).

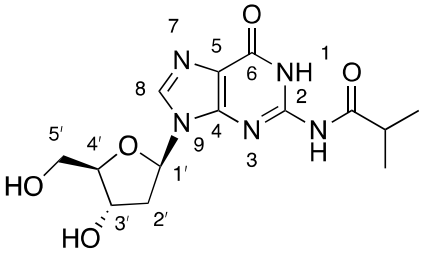
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 160.6, 159.7, 153.7, 137.7, 113.9(C^5), 87.6($C^{4'}$), 82.7($C^{1'}$), 70.7($C^{3'}$), 63.2($C^{5'}$), 53.1(C^{6}OCH$_3$). *$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

NMR in agreement with literature[21]

RP-HPLC (Method A): t_R = 4.95 min, 98% conversion (std = 1)

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{11}H_{16}N_5O_4$. 282.1197; found, 282.1197.

2'-Deoxy-N2-Isobutyrylguanosine (38)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (17.5 mg dissolved in 1582 µL) in EtOH (10.0 µmol, 200 µL, 1 equiv) was mixed with a 200 mM deoxycytidine solution (454 mg in 10 mL) dissolved in mQ H_2O (100.0 µmol, 500 µL, 10 equiv). Next, 296 µL mQ H_2O was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-N2-Isobutyrylguanosine (**38**).

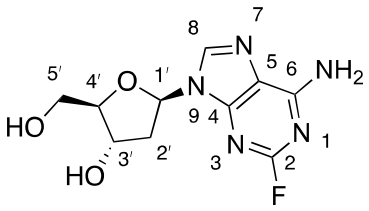
^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 12.17 (s, 1H, N*H*), 11.55 (s, 1H, N*H*), 8.49 (s, 1H, H^{8}), 6.52 (*app* t, *J* = 6.5 Hz, 1H, $H^{1'}$), 5.29 (d, *J* = 4.2 Hz, 1H, O$H^{3'}$), 4.95 (*app* t, *J* = 5.4 Hz, 1H, O$H^{5'}$), 4.32 (*app* dq, *J* = 7.2, 3.8 Hz, 1H, $H^{3'}$), 3.86 (*app* q, *J* = 4.2 Hz, 1H, $H^{4'}$), 3.61 (*app* dt, *J* = 11.8, 4.9 Hz, 1H, , $H^{5'}$ or $H^{5''}$), 3.56 – 3.49 (*app* m, 1H, , $H^{5'}$ or $H^{5''}$), 2.80 – 2.70 (m, 1H, NHCOC*H*), 2.38 – 2.29 (m, 1H, $H^{2'}$ or $H^{2''}$), 1.12 (d, *J* = 6.8 Hz, 6H, NHCOCHCH_3CH_3). The other $H^{2'}$ or $H^{2''}$is in the DMSO peak.

^{1}H NMR in agreement with literature[22]

RP-HPLC (Method A): t_R = 6.39 min, 47.1% conversion (std = 18).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{14}H_{19}N_5O_5Na$ 360.1278; found, 360.1281.

2'-Deoxy-2-fluoroadenosine (39)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (13 mg dissolved in 1698 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM thymidine solution (727 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-2-fluoroadenosine **(39).**

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.31 (s, 1H, H^{8}), 7.82 (s, 2H, NH), 6.23 (dd, J = 7.5, 6.2 Hz, 1H, $H^{1'}$), 5.29 (d, J = 4.2 Hz, 1H, O$H^{3'}$), 4.94 (*app* t, J = 5.6 Hz, 1H, O$H^{5'}$), 4.38 (*app* dq, J = 6.4, 3.3 Hz, 1H, $H^{3'}$), 3.85 (*app* td, J = 4.6, 2.8 Hz, 1H, $H^{4'}$), 3.64 – 3.55 (m, 1H, $H^{5'}$ or $H^{5''}$), 3.50 (ddd, J = 11.7, 5.9, 4.5 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.65 (ddd, J = 13.4, 7.5, 5.8 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.26 (ddd, J = 13.3, 6.2, 3.3 Hz, 1H, $H^{2'}$ or $H^{2''}$).

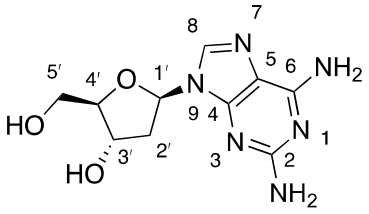
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 159.5, 157.7 (d, $^{3}J_{C-F}$ = 21.4 Hz, C^{4} or C^{6}), 150.2 (d, $^{3}J_{C-F}$ = 20.0 Hz, C^{4} or C^{6})139.7(C^{8}), 117.5(C^{5}), 87.9($C^{4'}$), 83.5($C^{1'}$), 70.7($C^{3'}$), 61.6($C^{5'}$). $C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR. C2 missing from the carbon spectrum.

^{19}F NMR (376 MHz, DMSO, ppm) δ −52.1.

RP-HPLC (Method A): t_R = 4.68 min, 98% conversion (std = 1).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{10}H_{12}FN_5O_3Na$ 292.0816; found, 292.0807.

2'-Deoxy-2-aminoadenosine (40)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (5.4 mg dissolved in 719 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-2-aminoadenosine (**40**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 7.91 (s, 1H, H^{8}), 6.82 (s, 2H, NH), 6.16 (dd, J = 8.2, 5.9 Hz, 1H, $H^{1'}$), 5.84 – 5.71 (m, 2H, NH), 5.22 (d, J = 3.9 Hz, 2H, O$H^{3'}$ and O$H^{5'}$), 4.35 (*app* dq, J = 5.9, 2.8 Hz 1H, $H^{3'}$), 3.83 (*app* td, J = 4.3, 2.3 Hz, 1H, $H^{4'}$), 3.58 (*app* dd, J = 11.8, 4.4 Hz,

1H, $H^{5'}$ or $H^{5''}$), 3.50 (*app* dd, *J* = 11.8, 4.2 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.59 (ddd, *J* = 13.5, 8.3, 5.6 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.16 (ddd, *J* = 13.1, 5.9, 2.6 Hz, 1H, $H^{2'}$ or $H^{2''}$).

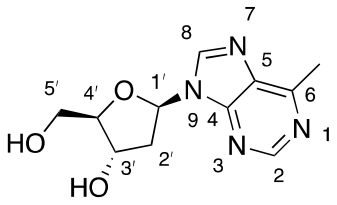
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 160.1, 156.2, 151.2(C^5), 135.9(C^8), 113.4(C^6), 87.7($C^{4'}$), 83.1($C^{1'}$), 71.0 ($C^{3'}$), 62.0($C^{5'}$).
$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

NMR in agreement with literature[23]

RP-HPLC (Method A): t_R = 3.75 min, 98% conversion (std = 1).

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_{15}N_6O_3$. 267.1200; found, 267.1210.

2'-Deoxy-6-methylpurine (41)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (10.5 mg dissolved in 1565.5 µL) in EtOH (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL) dissolved in 100 mM Na_2HPO_4 buffer solution (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL 100 mM Na_2HPO_4 buffer solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-6-methylpurine (41).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.78 (s, 1H, H^8 *or* H^2), 8.71 (s, 1H, H^8 *or* H^2), 6.45 (dd, *J* = 7.3, 6.3 Hz, 1H, $H^{1'}$), 5.33 (d, *J* = 4.2 Hz, 1H, O$H^{3'}$), 4.98 (*app* t, *J* = 5.6 Hz, 1H, O$H^{5'}$), 4.44 (*app* dq, *J* = 6.8, 3.5 Hz, 1H, $H^{3'}$), 3.89 (*app* td, *J* = 4.6, 2.9 Hz, 1H, $H^{4'}$), 3.62 (*app* dt, *J* = 11.8, 4.9 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.52 (ddd, *J* = 11.7, 5.6, 4.5 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.77 (ddd, *J* = 13.3, 7.3, 5.9 Hz, 1H, $H^{2'}$ *or* $H^{2''}$), 2.72 (s, 3H, C^6 CH_3), 2.33 (ddd, *J* = 13.3, 6.3, 3.5 Hz, 1H, $H^{2'}$ or $H^{2''}$).

^{13}C{1H}-NMR (101 MHz, DMSO-d_6, ppm) δ 158.2 (C^6 *or* C^5), 151.6 (C^8 or C^2), 149.8 (C^4), 143.9 (C^8 or C^2), 132.9 (C^6 *or* C^5), 88.0($C^{4'}$), 83.7 ($C^{1'}$), 70.7 ($C^{3'}$), 61.6 ($C^{5'}$) 19.1 (C^6 CH$_3$).

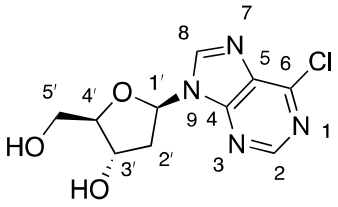
$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

NMR in agreement with literature[24]

RP-HPLC (Method A): t_R = 3.60 min, 98% conversion (std = 1)

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{11}H_{14}N_4O_3Na$, 273.0958; found, 273.0960.

2'-Deoxy-6-chloropurine (42)[25]



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (22.3 mg dissolved in 2886 µL) in EtOH (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM thymidine solution (727 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-6-chloropurine (**42**).

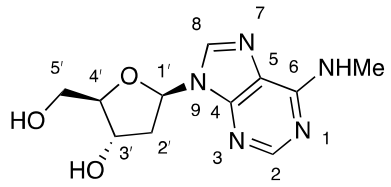
^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.89 (s, 1H, H^8), 8.80 (s, 1H, H^2), 6.47 (*app* td, *J* = 6.6, 1.8 Hz, 1H, $H^{1'}$), 5.45-5.31 (m, 1H, O$H^{3'}$), 4.95 (br s, 1H, O$H^{5'}$),4.45 (*app* d, *J* = 5.3 Hz, 1H, $H^{3'}$), 3.96 – 3.86 (m, 1H, $H^{4'}$), 3.63 (*app* dd, *J* = 11.7, 4.4 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.54 (*app* dd, *J* = 11.9, 4.4 Hz 1H $H^{5'}$ or $H^{5''}$), 2.83 – 2.72 (m, 1H, $H^{2'}$ or $H^{2''}$), 2.38 (ddd, *J* = 13.4, 6.4, 3.9 Hz, 1H, $H^{2'}$ or $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 151.6 (C^4 *or* C^5), 151.3 (C^2), 149.2 (C^4 *or* C^5), 145.8 (C^8), 131.4 (C^6), 88.1($C^{4'}$),84.2($C^{1'}$), 70.4($C^{3'}$), 61.3($C^{5'}$).
$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

RP-HPLC (Method A): t_R = 5.40 min, 85% conversion (std = 1).

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_{12}ClN_4O_3$ 271.0592; found, 271.0586.

2'-Deoxy-6-methyladenosine (43)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (12.8 mg dissolved in 1716 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM deoxycytidine solution (682 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-6-methyladenosine (**43**).

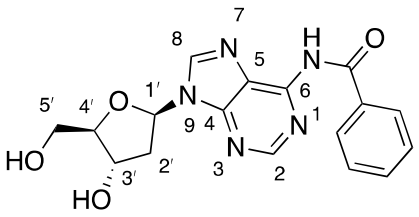
^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.31 (s, 1H, H^2 or H^8), 8.21 (s, 1H, H^2 or H^8), 7.76 (s, 1H, N*H*), 6.35 (dd, *J* = 7.9, 6.0 Hz, 1H, $H^{1'}$), 5.29 (d, *J* = 4.0 Hz, 1H, O$H^{3'}$), 5.22 (dd, *J* = 6.7, 4.9 Hz, 1H, O$H^{5'}$), 4.48 – 4.36 (m, 1H, $H^{4'}$), 3.88 (*app* td, *J* = 4.3, 2.5 Hz, 1H, $H^{3'}$), 3.62 (*app* dt, *J* = 11.9, 4.6 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.52 (ddd, *J* = 11.8, 6.7, 4.2 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.95 (s, 3H, C^{6}NHCH_3), 2.72 (ddd, *J* = 13.4, 8.0, 5.8 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.25 (ddd, *J* = 13.2, 6.1, 2.9 Hz, 1H, $H^{2'}$ or $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 152.4(C^2 or C^8),139.2(C^2 or C^8) 119.7, 88.0 ($C^{4'}$), 83.9 ($C^{1'}$), 71.0 ($C^{3'}$), 61.9($C^{5'}$).
$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

RP-HPLC (Method A): t_R = 3.82 min, 98% conversion (std = 1)

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{11}H_{16}N_5O_3$ 266.1248; found, 266.1249.

2'-Deoxy-N6-benzoyladenosine (44)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (10.5 mg dissolved in 823 µL) in mq H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM thymidine solution (727 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-N6-benzoyladenosine (**44**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 11.18 (s, 1H, N*H*), 8.76 (s, 1H, H^2 or H^8), 8.69 (s, 1H, H^2 or H^8), 8.09 – 8.00 (m, 2H, C^6bzH^{ortho}), 7.70 – 7.61 (m, 1H, C^6bzH^{para}), 7.61 – 7.52 (m, 2H, C^6bzH^{meta}), 6.49 (dd, *J* = 7.3, 6.3 Hz, 1H, $H^{1'}$), 5.36 (d, *J* = 4.2 Hz, 1H, O$H^{3'}$), 5.01 (*app* t, *J* = 5.6 Hz, 1H, O$H^{5'}$), 4.46 (*app* dt, *J* = 6.7, 3.3 Hz, 1H, $H^{3'}$), 3.91 (*app* td, *J* = 4.6, 2.9 Hz, 1H, $H^{4'}$), 3.65 (*app* dt, *J* = 11.7, 4.9 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.55 (*app* dt, *J* = 11.8, 5.0, 5.0 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.81 (ddd, *J* = 13.3, 7.4, 5.9 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.37 (ddd, *J* = 13.2, 6.3, 3.4 Hz, 1H $H^{2'}$ or $H^{2''}$).

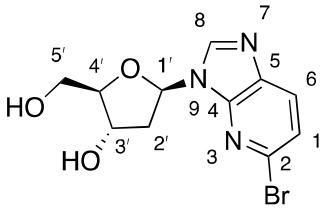
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 150.3(C^2 or C^8), 143.0(C^2 or C^8), 132.4(C^6bzC^{meta}or C^6bzC^{para}) 128.4(C^6bzC^{meta}or C^6bzC^{para} or C^6bzC^{ortho}),125.9(C^6bzC^{meta}or C^6bzC^{para} or C^6bzC^{ortho}), 88.0($C^{4'}$), 83.7($C^{1'}$), 70.7($C^{3'}$), 61.6($C^{5'}$).
$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

NMR in agreement with literature[26]

RP-HPLC (Method A): t_R = 6.54 min, 62% conversion (std = 1).

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{17}H_{18}N_5O_4$ 356.1353; found, 356.1363.

2'-Deoxy-deaza-2-bromopurine (45)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (23 mg dissolved in 2323 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM thymidine solution (727 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-deaza-2-bromopurine (**45**).

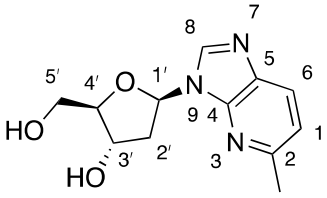
^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.71 (s, 1H, H^8), 8.09 (d, J = 8.3 Hz, 1H, H^1 or H^6), 7.51 (d, J = 8.4 Hz, 1H, H^1 or H^6), 6.44 (dd, J = 7.4, 6.2 Hz, 1H, $H^{1'}$), 5.33 (d, J = 4.3 Hz, 1H, O$H^{3'}$), 4.90 (*app* t, J = 5.6 Hz, 1H, O$H^{5'}$), 4.51 – 4.31 (m, 1H, $H^{3'}$), 3.88 (*app* td, J = 4.7, 2.9 Hz, 1H, $H^{4'}$), 3.61 (*app* dt, J = 11.7, 5.2 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.52 (*app* dt, J = 11.7, 5.2 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.73 (ddd, J = 13.3, 7.4, 5.9 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.34 (ddd, J = 13.3, 6.3, 3.5 Hz, 1H, $H^{2'}$ or $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 144.2(C^2), 134.7(C^5), 134.6 (C^4), 130.6(C^1 or C^6), 122.1(C^1 or C^6), 87.9($C^{4'}$), 83.4 ($C^{1'}$), 70.7($C^{3'}$), 61.6($C^{5'}$).
$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

RP-HPLC (Method A): t_R = 6.50 min, 97% conversion (std = 1)

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{11}H_{13}BrN_3O_3$. 314.0135; found, 314.0135.

2'-Deoxy-deaza-2-methylpurine (46)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (11.4 mg dissolved in 1712 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM thymidine solution (727 mg in 30 mL) dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-deaza-2-methylpurine (**46**).

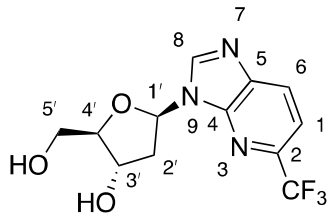
^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.56 (s, 1H, H^8), 7.98 (d, J = 8.1 Hz, 1H, H^1 or H^6), 7.18 (d, J = 8.1 Hz, 1H, H^1 or H^6), 6.48 (dd, J = 8.1, 6.0 Hz, 1H, $H^{1'}$), 5.30 (d, J = 4.0 Hz, 1H, O$H^{3'}$), 5.19 (dd, J = 6.7, 4.8 Hz, 1H, O$H^{5'}$), 4.43 (*app* dq, J = 5.9, 2.7 Hz Hz, 1H, $H^{3'}$), 3.90 (*app* td, J = 4.3, 2.4 Hz, 1H, $H^{4'}$), 3.64 (*app* dt, J = 11.8, 4.6 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.54 (ddd, J = 11.5, 6.7, 4.2 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.76 (ddd, J = 13.5, 8.1, 5.7 Hz, 1H, $H^{2'}$ *or* $H^{2''}$), 2.57 (s, 3H, C^2 CH_3), 2.28 (ddd, J = 13.1, 6.0, 2.8 Hz, 1H, $H^{2'}$ *or* $H^{2''}$).

^{13}C{1H}-NMR (101 MHz, DMSO-d_6, ppm) δ 152.57 (C^2), 145.76 (C^4), 143.03(C^8), 133.54 (C^5), 127.89 (C^1 or C^6), 118.27 (C^1 or C^6), 87.91 ($C^{4'}$), 83.63 ($C^{1'}$), 71.09 ($C^{3'}$), 61.96 ($C^{5'}$), 23.77 (C^2 CH_3).
$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

RP-HPLC (Method A): t_R = 3.60 min, 98% conversion (std = 1)

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{12}H_{16}N_3O_3$ 250.1186; found, 250.1178.

2'-Deoxy-deaza-2-trifluoropurine (47)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (11 mg dissolved in 1176 µL) in EtOH (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM thymidine solution (727 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was

shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-deaza-2-trifluoropurine (**47**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.94 (s, 1H, H^8), 8.38 (d, J = 8.2 Hz, 1H, H^6), 7.81 (d, J = 8.3 Hz, 1H, H^1), 6.53 (dd, J = 7.3, 6.3 Hz, 1H, $H^{1'}$), 5.36 (d, J = 4.3 Hz, 1H, O$H^{3'}$), 4.89 (*app* t, J = 5.6 Hz, 1H, O$H^{5'}$), 4.46 (*app* dt, J = 6.3, 3.4 Hz, 1H, $H^{3'}$), 3.89 (*app* td, J = 4.9, 2.9 Hz, 1H, $H^{4'}$), 3.62 (*app* dt, J = 11.7, 5.2 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.52 (*app* dt, J = 11.7, 5.1 Hz,, 1H, $H^{5'}$ or $H^{5''}$), 2.81 (ddd, J = 13.3, 7.4, 5.9 Hz, 1H, $H^{2'}$), 2.37 (ddd, J = 13.3, 6.3, 3.5 Hz, 1H, $H^{2''}$).

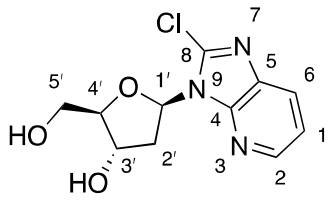
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 147.3(C^4), 145.9 (C^8), 140.3 (q, $^{2}J_{C–F}$ = 34.2 Hz, C^2) 137.8 (C^5), 129.0(C^6), 122.1 (q, $^{1}J_{C–F}$ = 273.5 Hz, CF_3) 115.2 (q, $^{3}J_{C–F}$ = 3.4 Hz, C^1), 88.0($C^{4'}$), 83.5($C^{1'}$), 70.7($C^{3'}$), 61.6($C^{5'}$). $C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

^{19}F NMR (376 MHz, DMSO, ppm) δ −64.4.

RP-HPLC (Method A): t_R = 7.00 min, 88% conversion (std = 2)

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{12}H_{12}F_3N_3O_3Na$ 326.0723; found, 326.0725.

2'-Deoxy-deaza-8-chloropurine (48)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (11.8 mg dissolved in 1537 µL) in DMSO (10.0 µmol, 200 µL, 1 equiv) was mixed with a 200 mM deoxycytidine solution (454 mg in 10 mL) dissolved in mQ H_2O (100.0 µmol, 500 µL, 10 equiv). Next, 296 µL 100 mM pH 6 Na_2HPO_4 was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-deaza-8-chloropurine (**48**).

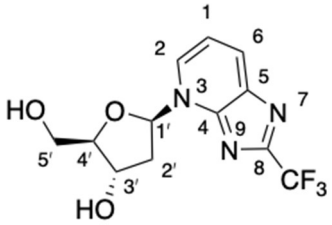
^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.38 (dd, J = 4.9, 1.5 Hz, 1H, H^{2}), 8.11 (dd, J = 8.1, 1.5 Hz, 1H H^{6}), 7.39 (dd, J = 8.1, 4.9 Hz, 1H, H^{1}), 6.46 (*app* t, J = 7.1 Hz, 1H, $H^{1'}$), 5.36 (d, J = 4.4 Hz, 1H, O$H^{3'}$), 5.00 (dd, J = 6.8, 5.1 Hz, 1H, O$H^{5'}$), 4.54 (ddd, J = 7.6, 6.4, 3.4 Hz, 1H, $H^{3'}$), 3.89 (*app* td, J = 5.3, 3.1 Hz, 1H, $H^{4'}$), 3.66 (*app* dt, J = 11.7, 5.1 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.48 (ddd, J = 11.9, 6.8, 5.5 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.38 (ddd, J = 13.6, 7.7, 6.3 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.27 (ddd, J = 13.3, 6.8, 3.3 Hz, 1H, $H^{2'}$ or $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 146.6(C^{5}), 143.8(C^{2}), 142.0(C^{4} *or* C^{8}), 134.1(C^{4} *or* C^{8}), 127.2(C^{6}), 119.4(C^{1}), 88.0($H^{4'}$), 84.9($C^{1'}$), 71.0($H^{3'}$), 61.9($C^{5'}$), 36.5($C^{2'}$).

RP-HPLC (Method A): t_R = 6.59 min, 66% conversion (std = 0.2).

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{11}H_{13}ClN_3O_3$ 270.0640; found, 270.0628.

2'-Deoxy-deaza-8-trifluoropurine (49)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (15 mg dissolved in 1603 µL) in DMSO (10.0 µmol, 200 µL, 1 equiv) was mixed with a 200 mM deoxycytidine solution (454 mg in 10 mL) dissolved in mQ H_2O (100.0 µmol, 500 µL, 10 equiv). Next, 296 µL mQ H_2O was added alongside 4 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-deaza-8-trifluoropurine (**49**).

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.90 (dd, J = 6.4, 1.1 Hz, 1H, H^{2}), 8.62 (dd, J = 7.8, 1.0 Hz, 1H, H^{6}), 7.54 (dd, J = 7.8, 6.4 Hz, 1H, H^{1}), 6.91 (*app* t, J = 6.2 Hz, 1H, $H^{1'}$), 5.44 (*app* s, 1H, $OH^{3'}$), 5.25 (*app* s, 1H, $OH^{5'}$), 4.44 – 4.28 (*app* m, 1H, $H^{3'}$), 4.09 (*app* q, J = 3.7 Hz, 1H, $H^{4'}$), 3.76 (*app* dd, J = 12.0, 3.6 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.69 (*app* dd, J = 12.1, 4.0 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.68 (ddd, J = 13.6, 6.4, 4.1 Hz, 1H, $H^{2'}$), 2.43 (*app* dt, J = 13.6, 6.0 Hz, 1H, $H^{2''}$).

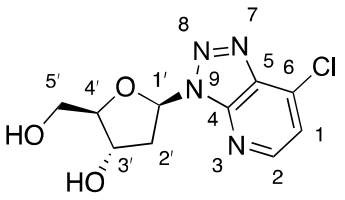
^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 149.3 (C^{4} or C^{5}), 142.0 (C^{4} or C^{5}), 133.8 (C^{6}), 130.4 (C^{2}), 122.1 (CF_3), 114.9 (C^{1}), 90.6 ($C^{1'}$), 89.1($C^{4'}$), 69.8($C^{3'}$), 60.7($C^{5'}$), 41.7($C^{2'}$). C^{8} not observed.

^{19}F NMR (376 MHz, DMSO) δ −63.6.

RP-HPLC (Method A): t_R = 5.87 min, 26% conversion.

HRMS (ESI) *m/z*: [M+Na] Calcd for $C_{12}H_{12}F_3N_3O_3Na$ 326.0723; found, 326.0720.

2'-Deoxy-triazolo-6-chloropurine (50)



Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (17.2 mg dissolved in 2225.7 µL) in mq H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM thymidine solution (727 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-triazolo-6-chloropurine (**50**).

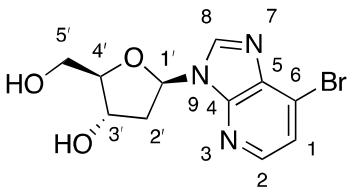
^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.74 (d, *J* = 5.0 Hz, 1H, H^1 or H^2), 7.74 (d, *J* = 5.0 Hz, 1H, H^1 or H^2), 6.82 (dd, *J* = 7.0, 5.4 Hz, 1H, $H^{1'}$), 5.42 (d, *J* = 4.6 Hz, 1H, $OH^{3'}$), 4.72 (*app* t, *J* = 5.7 Hz, 1H, $OH^{5'}$), 4.61 (*app* dq, *J* = 6.4, 4.7 Hz, 1H, $H^{3'}$), 3.92 (*app* td, *J* = 5.7, 4.2 Hz, 1H, $H^{4'}$), 3.56 (*app* dt, *J* = 11.0, 5.4 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.39 (*app* dt, *J* = 11.7, 5.9 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.13 (ddd, *J* = 13.6, 6.2, 5.4 Hz, 1H, $H^{2'}$ or $H^{2''}$).*Other $H^{2'}$ or $H^{2''}$ is in the DMSO peak.

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 151.6(C^1 *or* C^2), 146.2 (C^6), 135.0(C^4 *or* C^5), 134.8(C^4 *or* C^5), 120.7(C^1 *or* C^2), 88.4($C^{4'}$), 85.7($C^{1'}$), 70.6($C^{3'}$), 61.8($C^{5'}$), 38.1($C^{2'}$).

RP-HPLC (Method A): t_R = 6.57 min, 12% conversion (std = 4).

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{10}H_{11}ClN_4O_3Na$ 293.0412; found, 293.0410.

2'-Deoxy-deaza-6-bromopurine (51)



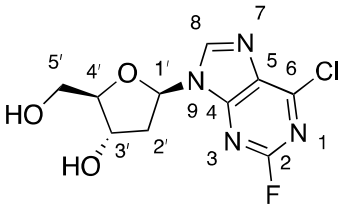
Prepared using the general enzymatic nucleoside transglycosylation procedure. A 50 mM nucleobase stock solution (14 mg dissolved in 1414 µL) in mQ H_2O (10.0 µmol, 200 µL, 1 equiv) was mixed with a 100mM thymidine solution (727 mg in 30 mL), which was dissolved in mQ H_2O (50.0 µmol, 500 µL, 5 equiv). Next, 298 µL mQ H_2O solution was added alongside 2 µL of NDT stock solution to afford a final volume of 1 mL and the resulting reaction mixture was shaken at 950 rpm at 40 °C for 24 h. The crude reaction mixture was purified by preparative HPLC (method D) affording 2'-deoxy-deaza-6-bromopurine (**51**) for characterisation.

RP-HPLC (Method B): t_R = 4.49 min, 90% conversion (std=4).

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the large-scale characterisation.

5.3 Scale up of Purines

2'-Deoxy-4-fluoro-6-chloropurine (32)



Deoxycytidine (658 mg, 2.9 mmol, 5 equiv) and 6-chloro-2-fluoro-9H-purine (100 mg, 0.6 mmol, 1 equiv) were dissolved in 59 mL of mQ H_2O. The solution was left stirring for an hour at 40 °C before 116 µL of enzyme was added, while stirring for 24 h at 40 °C. The crude residue was reduced in vacuo, loaded onto silica and purified by flash column chromatography (silica gel, 0-30% MeOH/CH_2Cl_2) to obtain 2'-deoxy-4-fluoro-6-chloropurine **(32)** in 54% yield (90 mg, 0.6 mmol) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.88 (s, 1H,H^8), 6.36 (t, J = 6.4 Hz, 1H, $H^{1'}$), 5.36 (d, J = 4.4 Hz, 1H, $OH^{3'}$), 4.93 (*app* t, J = 5.5 Hz, 1H, $OH^{5'}$), 4.43 (*app* dq, J = 6.0, 4.0 Hz, 1H, $H^{3'}$), 3.89 (*app* td, J = 4.4, 3.3 Hz, 1H, $H^{4'}$), 3.62 (ddd, J = 11.9, 5.4, 4.5 Hz, 1H, , $H^{5'}$ or $H^{5''}$), 3.53

(ddd, *J* = 11.8, 5.4, 4.5 Hz, 1H, , $H^{5'}$ or $H^{5''}$), 2.71 (*app* dt, *J* = 13.5, 6.3 Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.36 (ddd, *J* = 13.4, 6.4, 4.2 Hz, 1H, $H^{2'}$ or $H^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 156.3 (d, $^{1}J_{C–F}$ = 214.0 Hz, C^{2}), 153.4 (d, $^{3}J_{C–F}$ = 17.5 Hz C^{4}), 150.6 (d, $^{3}J_{C–F}$ = 18.1 Hz C^{6}), 146.7 (d, $^{5}J_{C–F}$ = 3.3 Hz C^{8}), 130.7 (d, $^{4}J_{C–F}$ = 4.6 Hz C^{5}), 88.1($C^{4'}$), 84.3($C^{1'}$), 70.1($C^{3'}$), 61.1($C^{5'}$). $C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

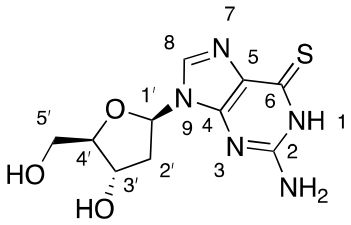
^{19}F NMR (376 MHz, DMSO, ppm) δ −51.7.

NMR in agreement with literature[27]

RP-HPLC (Method A): t_R = 6.33 min, 90% conversion.

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{10}H_{11}ClFN_4O_3$. 289.0498; found, 289.0495.

2'-Deoxy-6-thioguanosine (36)



Deoxycytidine (1.14 g, 5 mmol, 5 equiv) and 6 thioguanine (167.2 mg, 1 mmol, 1 equiv) were dissolved in 100 mL of mQ H_2O to give a final concentration of 50 mM and 10 mM respectively. The solution was left stirring for an hour at 40 °C before 200 µL of enzyme were added while stirring for 24 h and heating at 40 °C. The crude residue was allowed to cool to rt, with yellow crystals precipitating out in the solution, crystals were then dried, obtaining 2'-deoxy-6-thioguanosine (**36**) in 57% yield (163 mg, 1 mmol) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 11.92 (s, 1H, N*H*), 8.10 (s, 1H, H^{8}), 6.79 (s, 2H, *NH*), 6.11 (dd, *J* = 7.6, 6.1 Hz, 1H, $H^{1'}$), 5.26 (d, *J* = 4.0 Hz, 1H, O$H^{3'}$), 4.92 (*app* t, *J* = 5.4 Hz, 1H, O$H^{5'}$), 4.34 (*app* dq, *J* = 6.4, 3.2 Hz, 1H, $H^{3'}$), 3.81 (*app* td, *J*= 4.5, 2.7 Hz, 1H, $H^{4'}$), 3.56 (*app* dt, *J* = 11.6, 5.1 Hz, 1H, $H^{5'}$ or $H^{5''}$), 3.50 (*app* dt, *J* = 11.7, 5.0 Hz, 1H, $H^{5'}$ or $H^{5''}$), 2.21 (ddd, *J* = 13.1, 6.1, 3.3 Hz, 1H, $H^{2'}$ or $H^{2''}$). The other 2 $H^{2''}$ is under the solvent peak.

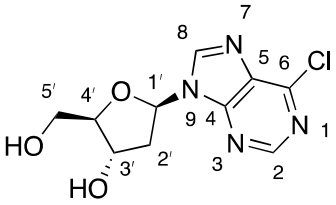
^{13}C{1H}-NMR (101 MHz, DMSO-d_6, ppm) δ 175.0 (C^6), 153.0 (C^2), 147.4(C^4), 138.2(C^8), 128.4(C^5), 87.6($C^{4'}$), 82.7($C^{1'}$), 70.6($C^{3'}$), 61.5($C^{5'}$).
$C^{2'}$ peak hidden by DMSO peak confirmed through 2D NMR.

NMR in agreement with literature[28]

RP-HPLC (Method A): t_R = 3.7 min, 97% conversion.

HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{10}H_{13}N_5O_3SNa$ 306.0631; found, 306.0645.

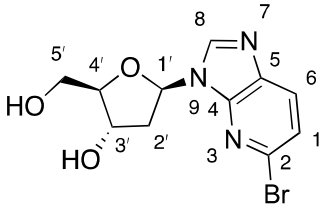
2'-Deoxy-6-chloropurine (42)



Prepared using the general scale-up enzymatic nucleoside transglycosylation procedure. Deoxycytidine (7.35 g, 32.4 mmol, 5 equiv) and 6-chloropurine (1.00 g, 6.47 mmol, 1 equiv) were transferred to a flask with 300 mL mQ H_2O and 600 µL NDT. The mixture was stirred at 40 ℃ for 24 h. The crude reaction mixture was freeze dried and purified by column chromatography (silica gel, 0-20% MeOH/CH_2Cl_2) obtain 2'-deoxy-6-chloropurine (**42**) with a 78% yield (1.37 g, 6.47 mmol) as a white solid.

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the small-scale characterisation.

2'-Deoxy-deaza-2-bromopurine (45)

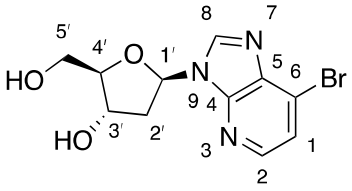


Deoxycytidine (551 mg, 2.42 mmol, 3 equiv) and 5-bromo-3*H*-imidazo[4,5-*b*]pyridine (160 mg, 808 µmol, 1 equiv) were dissolved in 80 mL of mQ H_2O. Solution was left stirring for an hour

at 40 °C before 160 µL of enzyme were added while stirring for 24 h and heating to 40 °C. The crude residue was reduced *in vacuo*, loaded onto silica and purified by flash column chromatography (silica gel, 0-30% MeOH/CH_2Cl_2) to obtain 2'-deoxy-deaza-2-bromopurine (**45**) with an 82% yield (208 mg, 808 µmol) as a white solid.

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the small-scale characterisation.

2'-Deoxy-deaza-6-bromopurine (51)



Deoxycytidine (551 mg, 2.42 mmol, 3 equiv) and 7-bromo-3H-imidazo[4,5-b]pyridine (160 mg, 808 µmol, 1 equiv) were dissolved in 80 mL of mQ H_2O. Solution was left stirring for an hour at 40 °C before 160 µL of enzyme were added while stirring for 24 h and heating to 40 °C. The crude residue was reduced *in vacuo*, loaded onto silica and purified by flash column chromatography (silica gel, 0-30% MeOH/CH_2Cl_2) to obtain 2'-deoxy-deaza-6-bromopurine (**51**) with a 93% yield (236 mg, 808 µmol) as an orange solid.

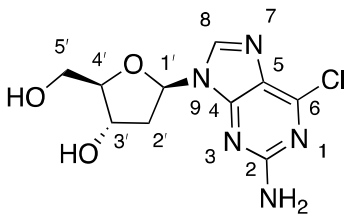
^{1}H NMR (400 MHz, MeOD) δ 8.73 (s, 1H, *H*^{8}), 8.23 (d, *J* = 5.3 Hz, 1H, *H*^{2}), 7.60 (d, *J* = 5.3 Hz, 1H, *H*^{1}), 6.59 (dd, *J* = 7.5, 6.1 Hz, 1H, *H*$^{1'}$), 4.63 (*app* dt, *J* = 6.0, 3.0 Hz, 1H, *H*$^{3'}$), 4.11 (*app* q, *J* = 3.3 Hz, 1H, *H*$^{4'}$), 3.88 (*app* dd, *J* = 12.2, 3.3 Hz, 1H, *H*$^{5'}$) 3.78 (*app* dd *J* = 12.2, 3.7 Hz, 1H, *H*$^{5''}$), 2.88 (ddd, *J* = 13.5, 7.5, 6.0 Hz, 1H, *H*$^{2'}$), 2.50 (ddd, *J* = 13.5, 6.2, 3.2 Hz, 1H, *H*$^{2''}$).

^{13}C{^{1}H}-NMR (101 MHz, MeOD) δ 147.1 (*C*^{4}), 145.7 (*C*^{2}), 145.6 (*C*^{8}), 136.5 (*C*^{5}), 125.0 (*C*^{6}), 123.6 (*C*^{1}), 89.7 (*C*$^{4'}$), 87.1 (*C*$^{1'}$), 72.8 (*C*$^{3'}$), 63.4 (*C*$^{5'}$), 41.4 (*C*$^{2'}$).

RP-HPLC (Method B): t_R = 4.49 min, 89% conversion.

HRMS (ESI) *m/z*: $[M+Na]^+$ calculated for $C_{11}H_{12}BrN_3O_3Na$, 335.9954; found 335.9952.

2'-Deoxy-6-chloroguanosine (33)

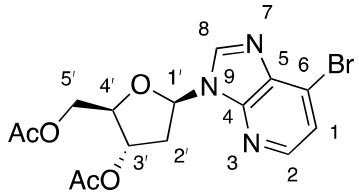


Deoxycytidine (322 mg, 1.40 mmol, 3 equiv) and 6-chloro-9*H*-purin-2-amine (80 mg, 0.47 mmol, 1 equiv) were dissolved in 40 mL of mQ H_2O. The solution was left stirring for an hour at 40 °C before 200 µL of enzyme was added, while stirring for 24 h at RT. The crude residue was reduced in vacuo, loaded onto silica and purified by flash column chromatography (silica gel, 0-30% MeOH/CH_2Cl_2) to obtain 2'-deoxy-6-chloroguanosine **(33)** in 88% yield (118 mg, 0.47 mmol) as a white solid.

Characterisation of the target nucleoside was confirmed through ^{1}H NMR in comparison with the small-scale characterisation.

5.4 ^{15}N Labelling

3',5'-tri-O-acetyl-deaza-6-bromopurine (57)



An oven-dried vial under an argon atmosphere was charged with nucleoside **51** (100 mg, 318 µmol) and 4-Dimethylaminopyridine (4 mg, 33 µmol, 0.1 equiv). The vial was capped with a rubber septum, and a solution of acetic anhydride (300 µL, 3.2 mmol, 10 equiv) and pyridine (3 mL) were added and stirred at rt for 18 h. The crude mixture was diluted with ice cold water (10 mL) and extracted three times with ethyl acetate (10 mL x 3). The combined organic layers was washed once with $NaHCO_3$ (10 mL) and twice with brine (10 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated *in vacuo*, to obtain 2',5'-tri-O-acetyl-deaza-6-bromopurine (**57**) in (114 mg, 90% yield) as an orange oil.

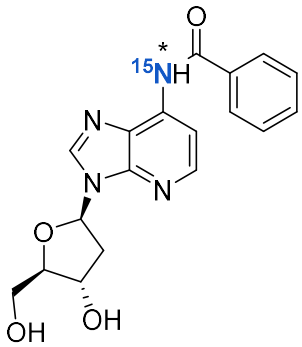
^{1}H NMR (500 MHz, DMSO-d_6, ppm) δ 8.80 (s, 1H, H^8), 8.28 (d, J = 5.2 Hz, 1H, H^2), 7.67 (d, J = 5.2 Hz, 1H, H^1), 6.53 (*app* t, J = 7.0 Hz, 1H, $H^{1'}$), 5.48 – 5.42 (m, 1H, $H^{3'}$), 4.37 – 4.27 (m, 2H, $H^{5'}$ or $H^{5''}$ and $H^{4'}$), 4.23 (*app* dd, J = 11.0, 5.4 Hz, 1H $H^{5'}$ or $H^{5''}$), 3.22 (*app* dt, J = 14.3, 7.2

Hz, 1H, $H^{2'}$ or $H^{2''}$), 2.12 (s, 3H, $C^{3'}$ or $C^{5'}OCOCH^3$), 2.01 (s, 3H, $C^{3'}$ or $C^{5'}OCOCH^3$). Other $H^{2'}$ or $H^{2''}$ is under the DMSO peak.

^{13}C NMR (126 MHz, DMSO-d_6, ppm) δ 170.6 ($C^{3'}$ or $C^{5'}OC$), 170.5 ($C^{3'}$ or $C^{5'}OC$), 147.8 (*C^4 or C^5*), 146.7 (*C^4 or C^5*), 145.1(*C^2 or C^8)*, 145.0 (*C^2 or C^8)*, 123.7 (*C^6*), 122.5 (*C^1*), 84.5 (*$C^{1'}$*), 82.2 (*$C^{4'}$*), 74.8 (*$C^{3'}$*), 64.0 (*$C^{5'}$*), 35.8 (*$C^{2'}$*), 21.3 ($C^{3'}$ or $C^{5'}OCOC$), 21.0 ($C^{3'}$ or $C^{5'}OCOC$).

HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{15}H_{17}BrN_3O_5$ 398.03461; found, 398.0349.

2'-Deoxy-deaza-6-^{15}N-benzamidepurine (58)



An oven-dried vial under an argon atmosphere was charged with $Pd_2dba_3{\cdot}CHCl_3$ (15 mg, 15 μmol, 5 mol%) and 1,1′-bis-(diphenylphosphino) ferrocene (dppf) (25 mg, 45 μmol, 15 mol%). The vial was capped with a rubber septum, and a solution of acetate protected nucleoside **52** (120 mg, 301 μmol, 1 equiv.) in toluene (1.3 mL) was added dropwise. Then, benzamide (44 mg, 362 μmol, 1.2 equiv.) and Cs_2CO_3 (137 mg, 422 μmol, 1.40 equiv.) were added and the reaction mixture was stirred at 80 °C for 4 h. After complete consumption of the starting nucleoside (determined by thin-layer chromatography), the resulting brown suspension was allowed to cool to rt, diluted in MeOH, filtered using a syringe filter and concentrated in *vacuo*. The residue was loaded onto silica gel and purified by flash column chromatography (silica gel, 0-6% Toluene / MeOH) to obtain 2'-deoxy-deaza-6-^{15}N-benzamidepurine (**58**) (48 mg, 45%) as an off white solid.

^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 10.40 (d, $J^{H\text{-}15N}$ = 91.3 Hz, 1H, N*H*), 8.65 (s, 1H, H^8), 8.33 (d, *J* = 5.5 Hz, 1H, *H^1 or H^2*), 8.06 – 8.02 (m, 3H, *H^1 or H^2 and C^6bzH^{meta} or C^6bzH^{ortho}*), 7.69 – 7.63 (m, 1H, *C^6bzH^{para}*), 7.61 – 7.54 (m, 2H, *C^6bzH^{meta} or C^6bzH^{ortho}*), 6.52 (dd, *J* = 7.8, 6.0 Hz, 2H, *$H^{1'}$*), 5.33 (d, *J* = 4.1 Hz, 1H, O*$H^{3'}$*), 5.16 (dd, *J* = 6.4, 5.0 Hz, 1H, O*$H^{5'}$*), 4.50 – 4.43 (*app* m, 1H, *$H^{3'}$*), 3.92 (*app* td, *J* = 4.4, 2.7 Hz, 1H, *$H^{4'}$*), 3.66 (*app* dt, *J* = 11.8, 4.8 Hz, 1H, *$H^{5'}$* or *$H^{5''}$*), 3.55 (ddd, *J* = 11.8, 6.4, 4.4 Hz, 1H, *$H^{5'}$* or *$H^{5''}$*), 2.82 (ddd, *J* = 13.3, 7.7, 5.8 Hz, 1H, *$H^{2'}$* or *$H^{2''}$*), 2.33 (*app* ddt, *J* = 9.3, 6.2, 3.1 Hz, 1H, *$H^{2'}$* or *$H^{2''}$*).

^{13}C{^{1}H}-NMR (101 MHz, DMSO-d_6, ppm) δ 166.0(*C*O), 147.0(*C^4 or C^5*), 144.7(*C^1 or C^2)*, 142.2(*C^8)*, 137.2 (d, *J^{15N} = 15.3 Hz, C^6)*, 132.2(*C^6bzC^{para}*), 128.5(*C^6bzC^{meta}* or *C^6bzC^{ortho}*) 127.9(*C^6bzC^{meta}* or *C^6bzC^{ortho}*) 126.8(*C^4 or C^5*), 109.1(*C^1 or C^2*), 87.9(*$C^{4'}$*), 83.9(*$C^{1'}$*), 70.9(*$C^{3'}$*), 61.8 *($C^{5'}$).*$C^{2'}$* carbon is in the DMSO peak.

HRMS (ESI) *m/z*: [M+H] Calcd for $C_{18}H_{19}N_3{}^{15}NO_4$ 354.12147; found, 354.1227.

6. References

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7. Analytical Section

2'-Deoxy-deaza-6-chloropurine (**11**)

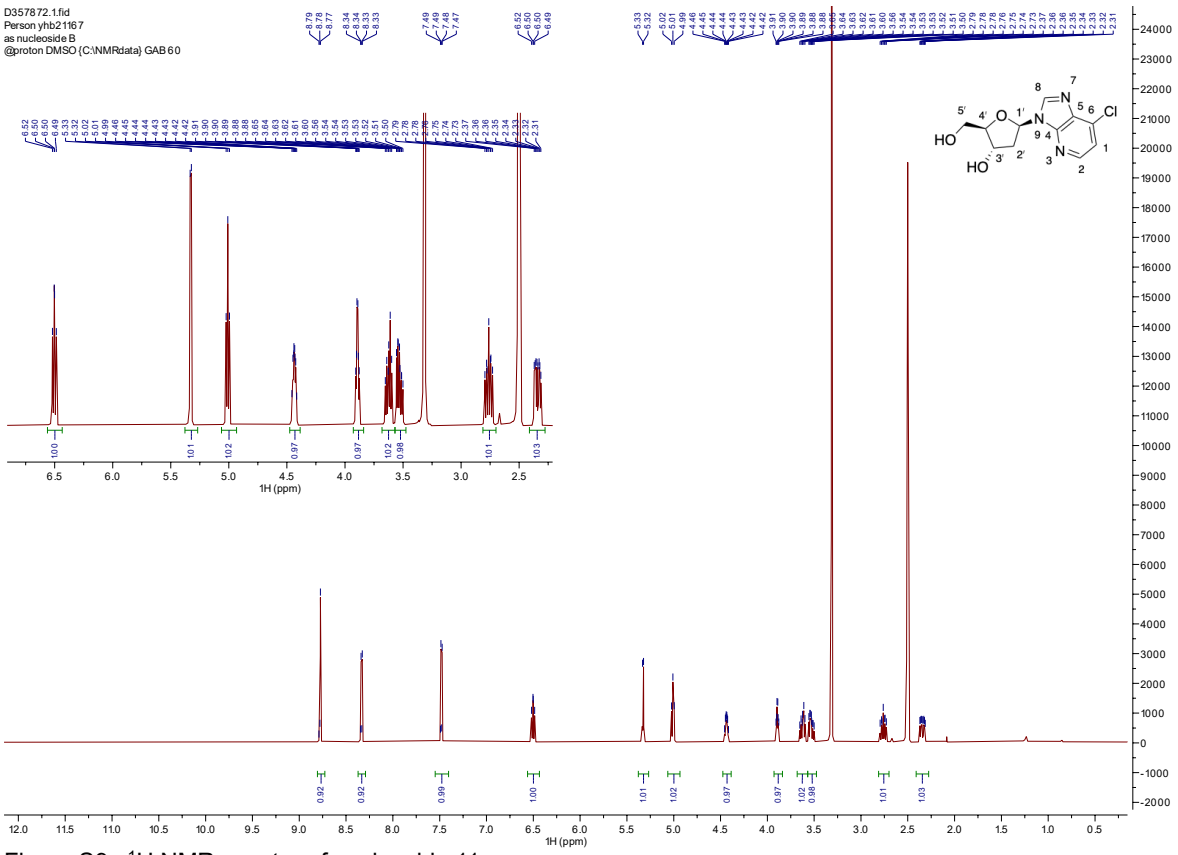

Figure S9 - ^{1}H NMR spectra of nucleoside 11

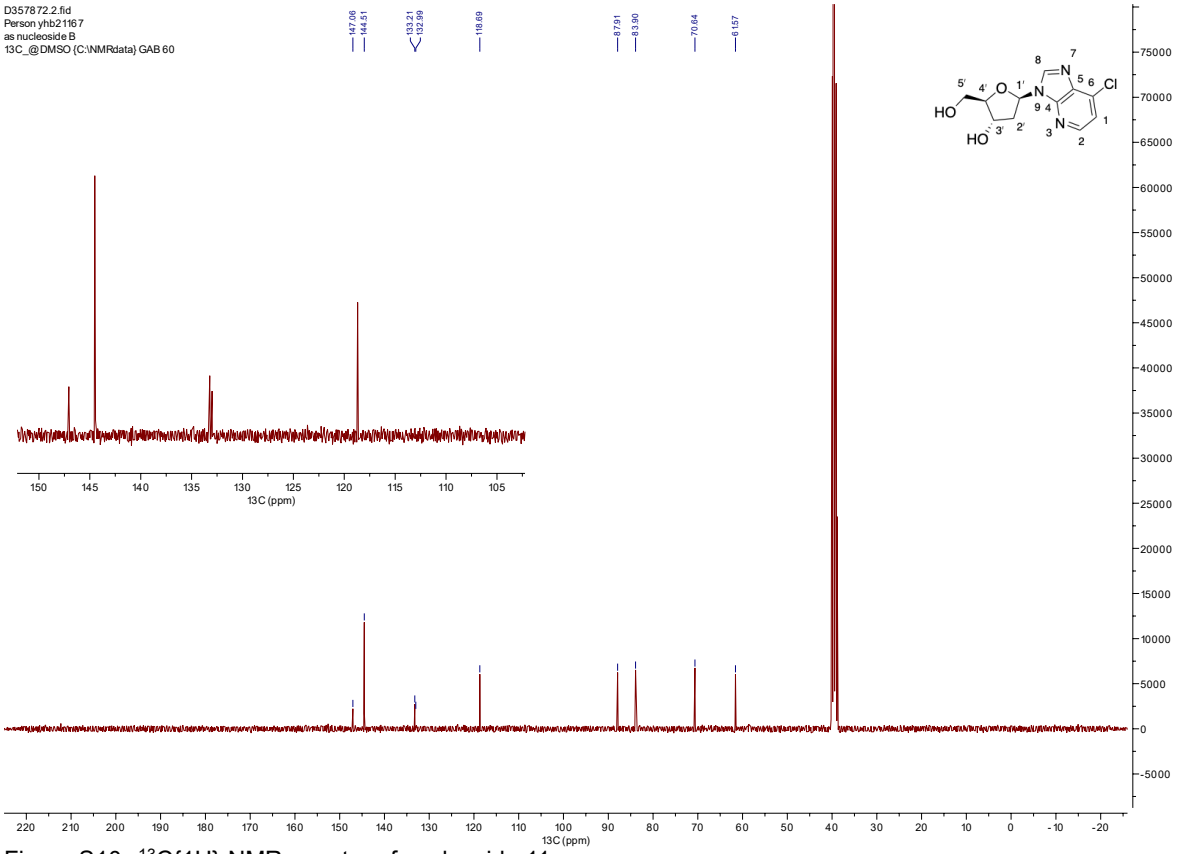

Figure S10 - $^{13}C\{1H\}$-NMR spectra of nucleoside 11

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Sample ID :
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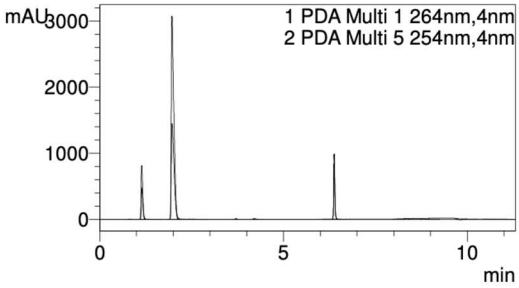
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : REACTIONS.lcb
Vial # : 1-24
Injection Volume : 10 uL
Date Acquired : 24/02/2022 18:00:28
Date Processed : 24/02/2022 18:11:48

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

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4	1.964	14967389	3049604	
5	2.441	3082	942	
6	2.547	5133	1451	
7	3.703	22347	8586	
8	4.202	40872	10019	
9	6.088	1292	277	
10	6.375	1804083	818195	
11	8.300	43018	6738	
12	8.635	43211	2317	
13	8.736	6000	1863	

Figure S11 - HPLC trace from the reaction used to obtain Nucleoside 11. R.T- 1.14 = cytosine nucleobase released, 1.96 = nucleoside dC, 6.38 = target nucleoside 11.

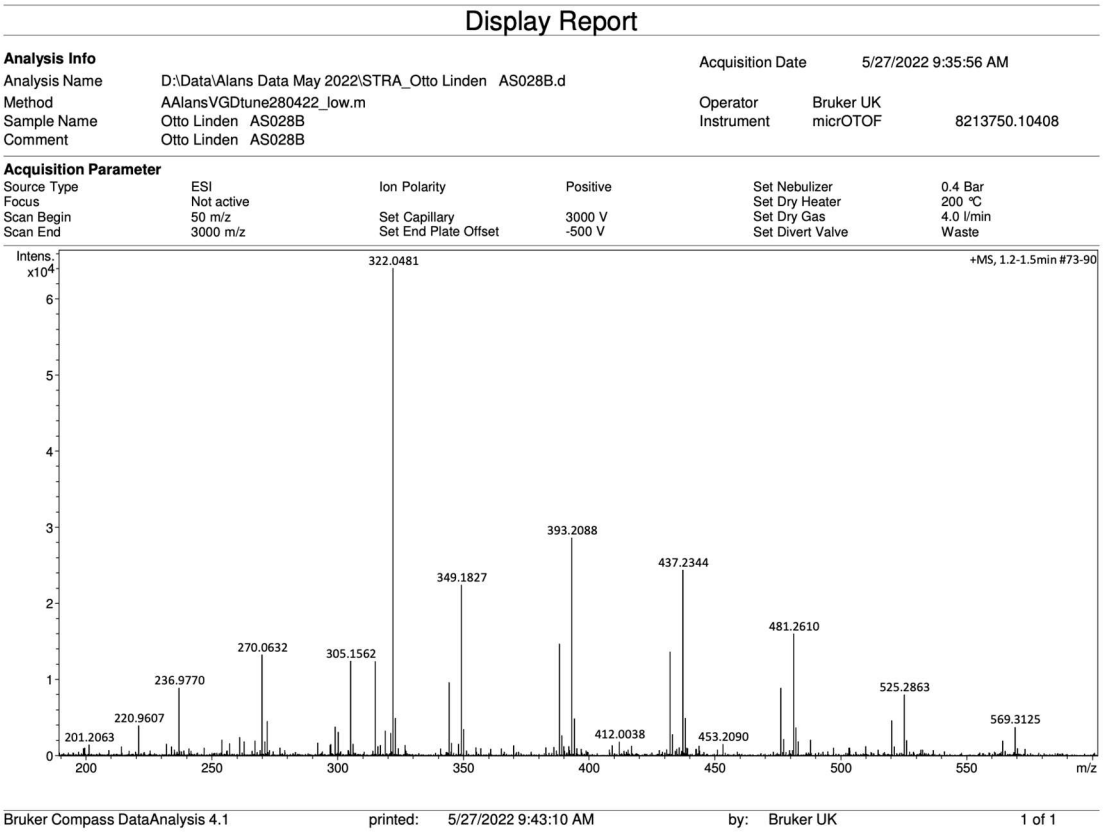

Figure S12 - HRMS for nucleoside 11

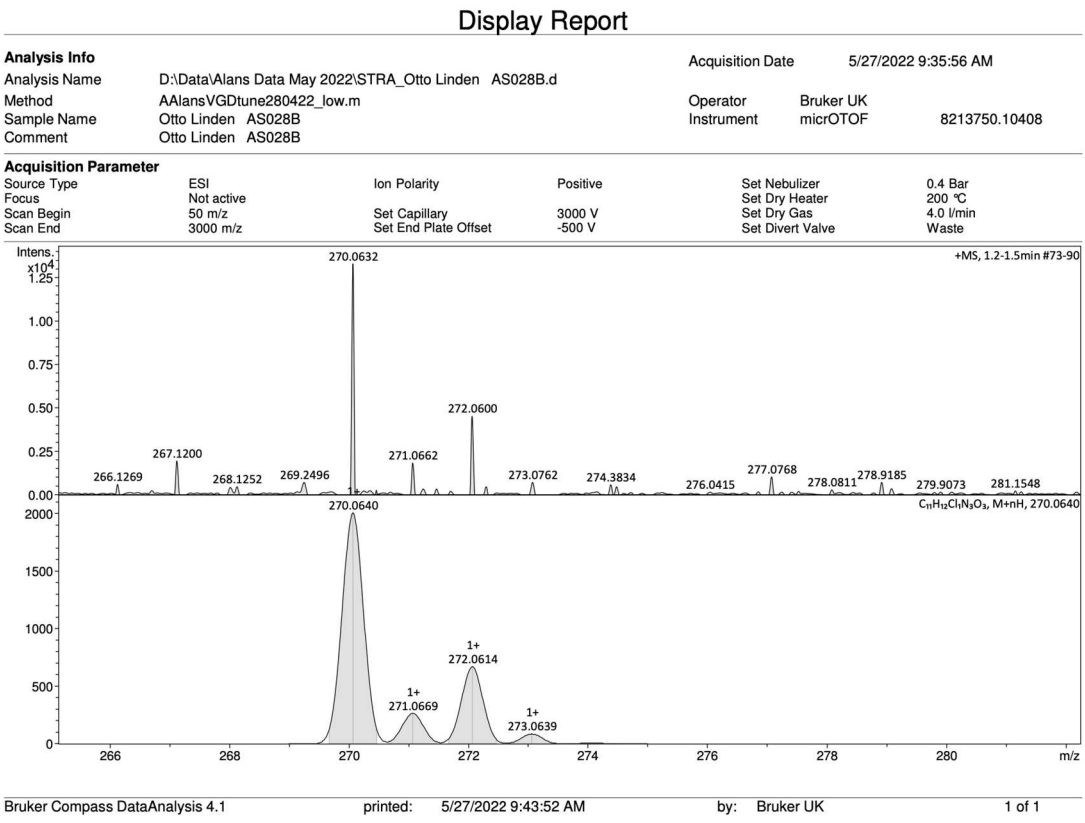

Figure S13 - HRMS for nucleoside 11

2'-Deoxy-5-fluorouracil (12)

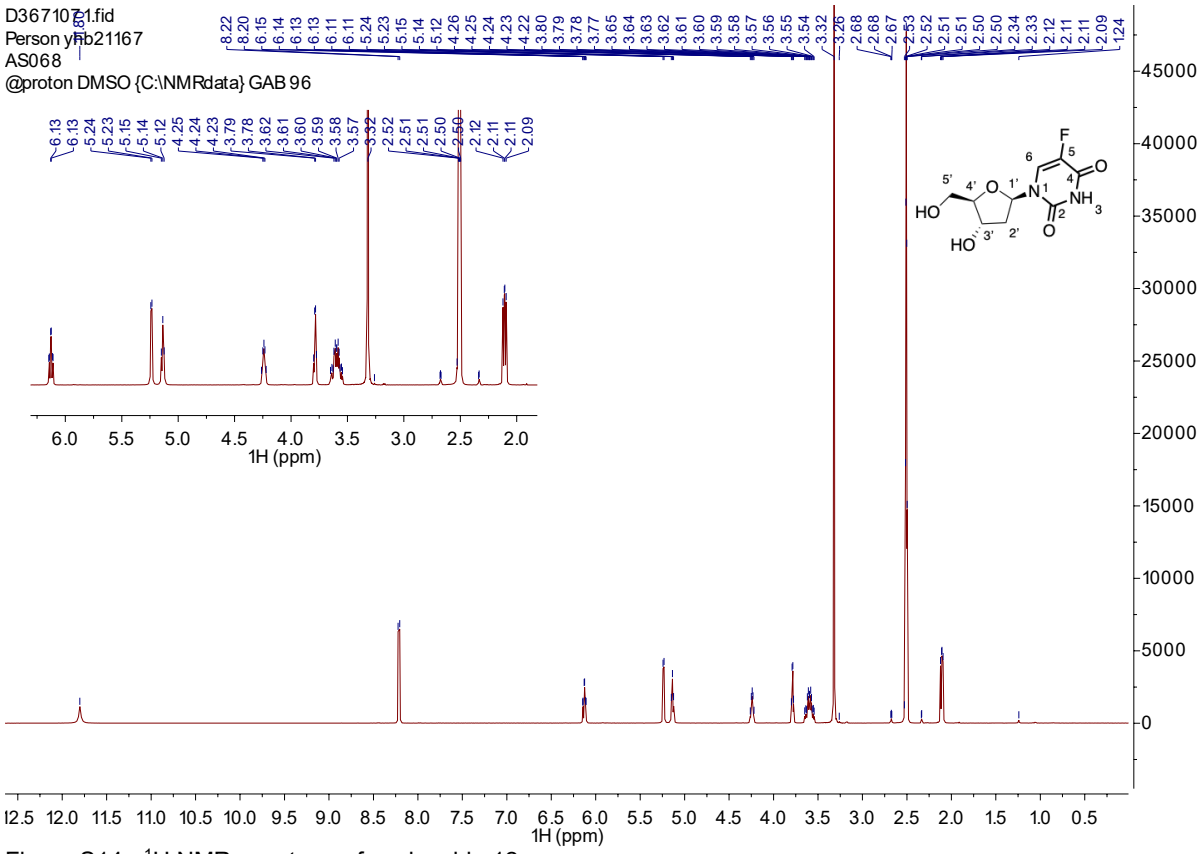

Figure S14 - ^{1}H NMR spectrum of nucleoside 12

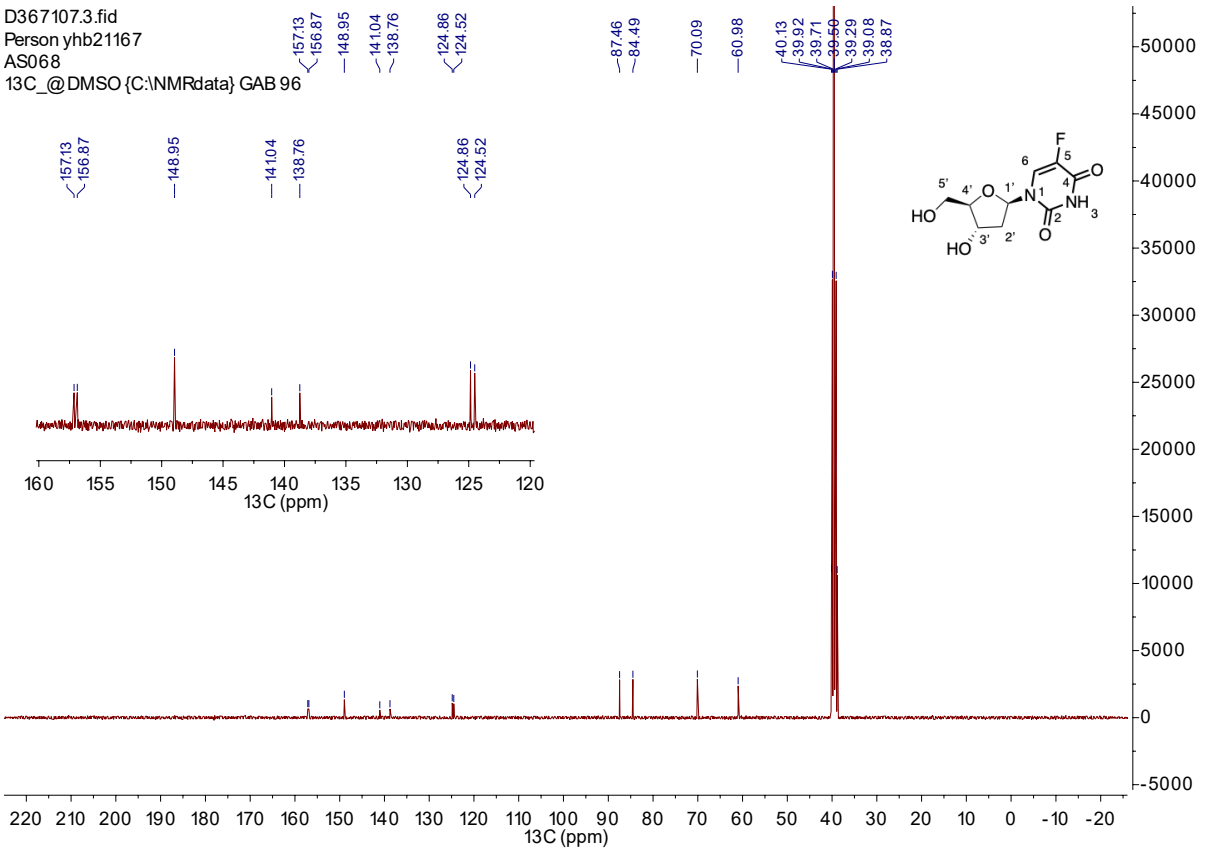

Figure S15 - ^{13}C NMR spectrum of nucleoside 11

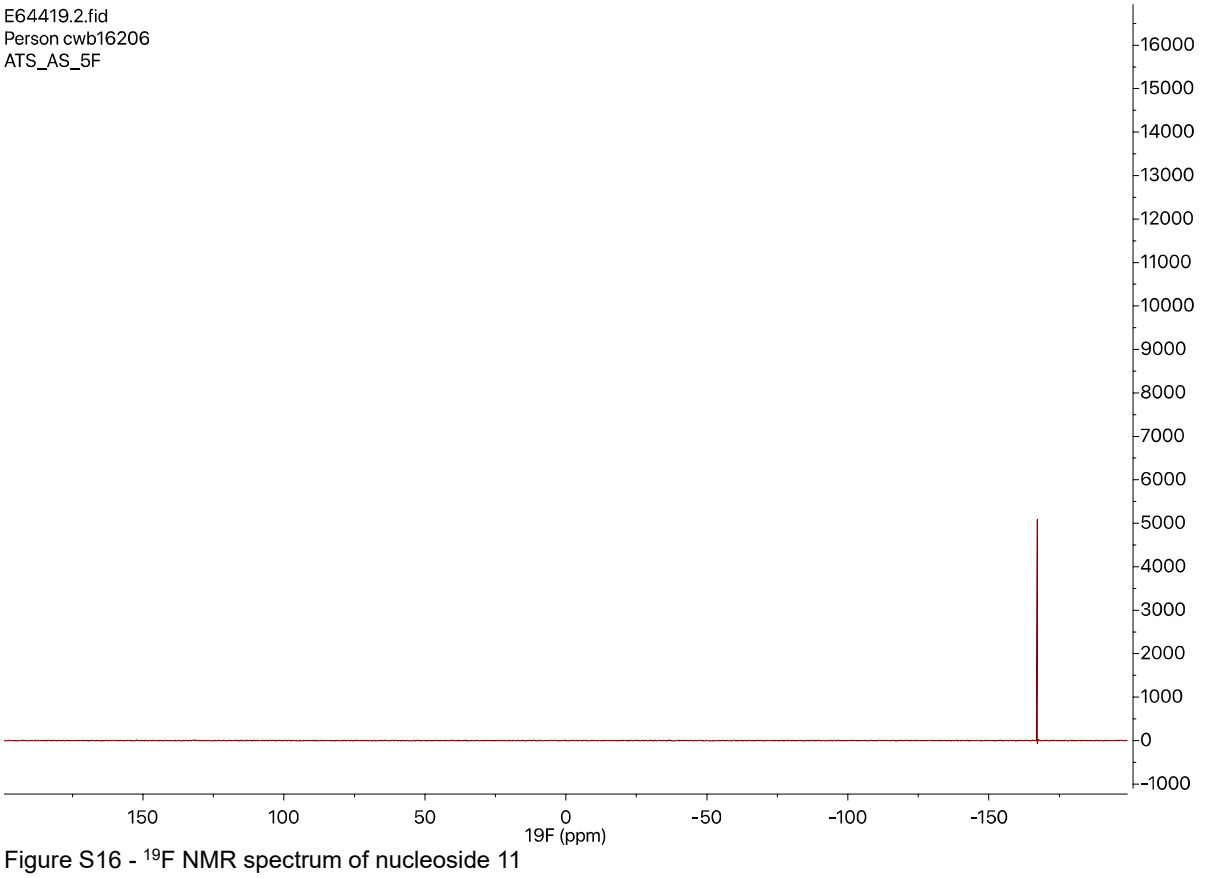

Figure S16 - ^{19}F NMR spectrum of nucleoside 11

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Method Filename : Admir Trans glyco Run.lcm
Batch Filename : AS068 - 5FU.lcb
Vial # : 1-3
Injection Volume : 10 uL
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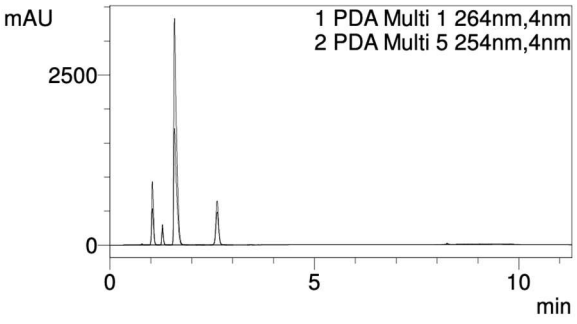
Date Processed : 01/11/2022 16:36:54

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

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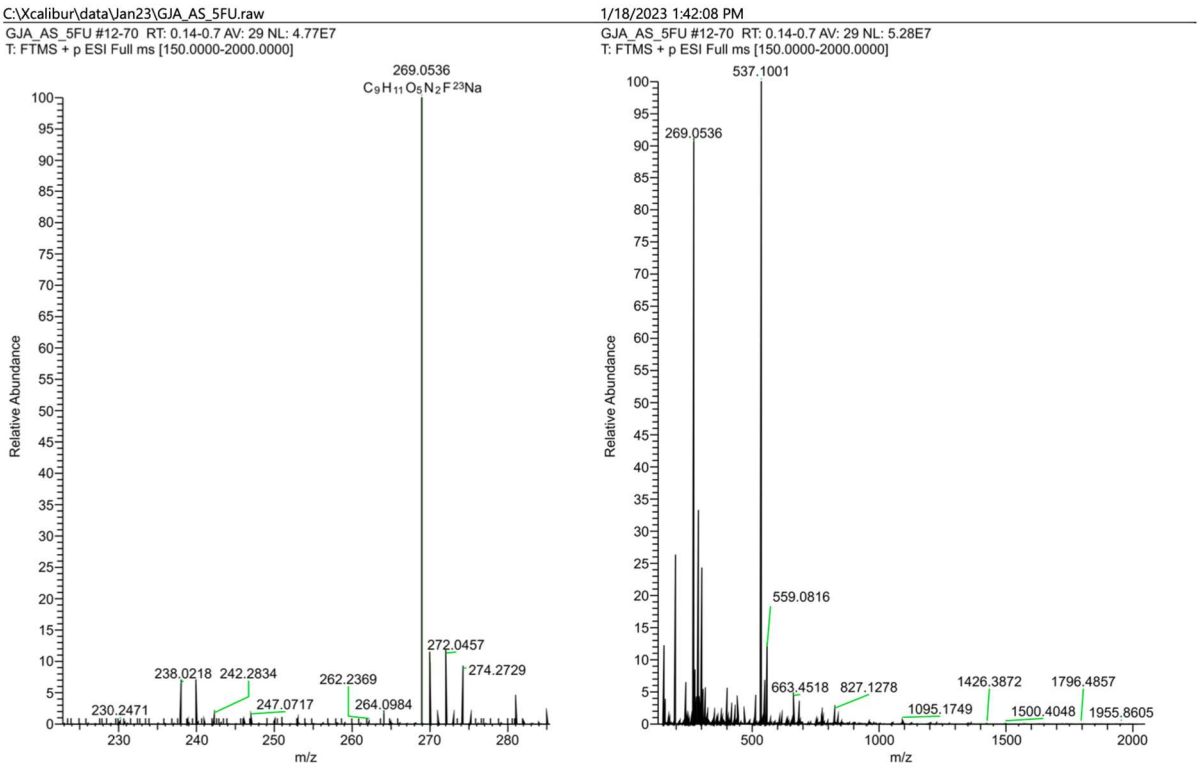
5	2.189	7311	1805	
6	2.624	2937292	642168	
7	3.447	2185	754	
8	8.056	1156	144	
9	8.248	48781	17513	
10	8.436	3236	639	
11	8.600	7724	1286	
12	8.696	3400	1021	

Figure S17 - HPLC spectrum. R.T = 1.04 = nucleobase cytosine, 1.29 = nucleobase , 1.58 = nucleoside dC, 2.62 = nucleoside **11**



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
269.0536	$C_9H_{11}O_5N_2F^2{}^3Na$	22.31616296 02772	4.50	-3.10	269.05442	1	79.14	4	1	82.3	99.24	(Collection)

Figure S18 - HRMS spectrum of 11

2'-Deoxy-5-ethynyluridine (3)

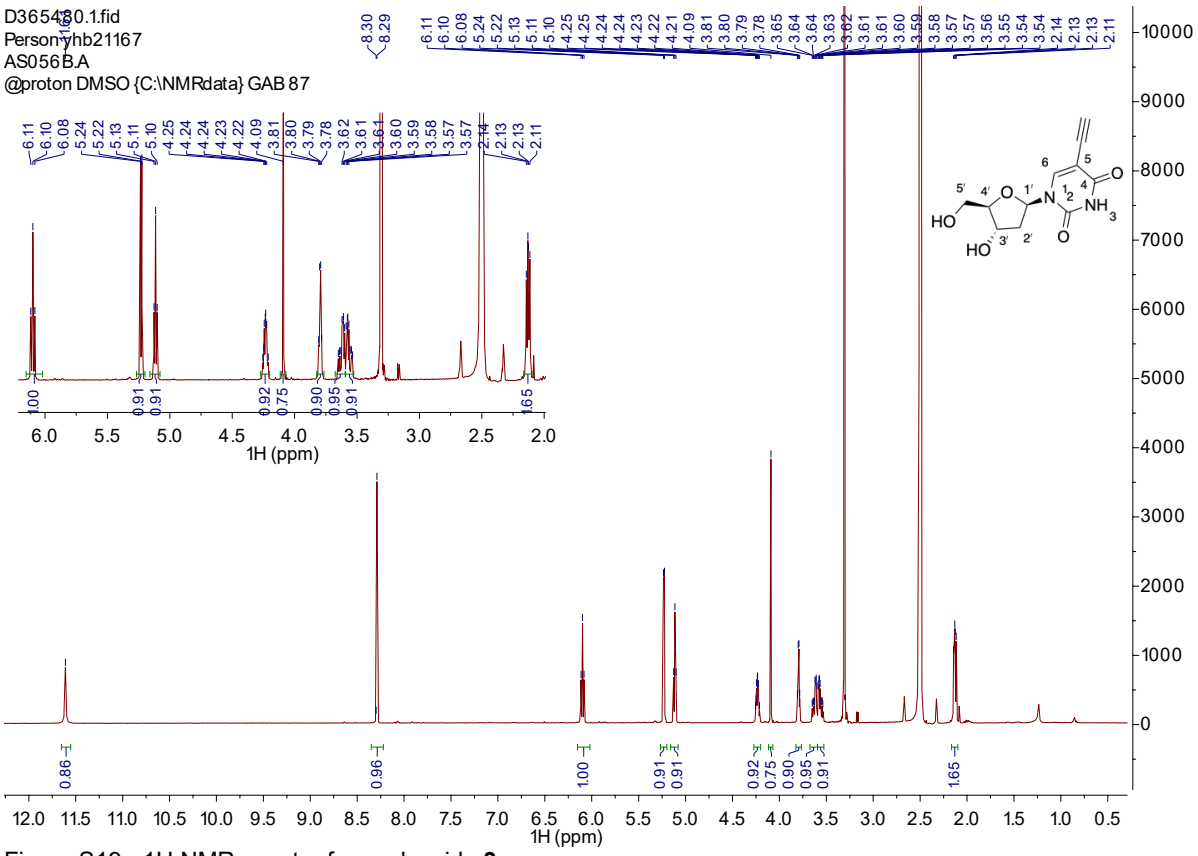


Figure S19 - 1H NMR spectra for nucleoside **3**

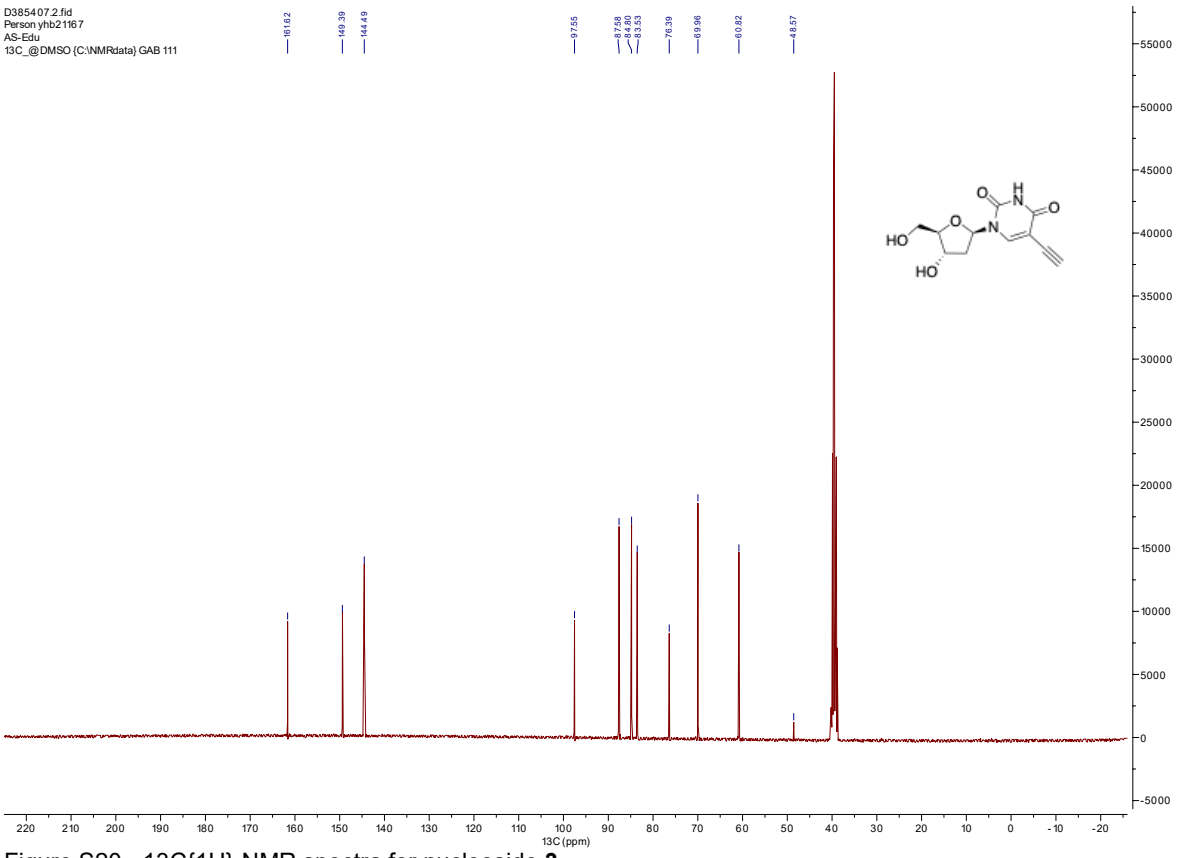


Figure S20 - 13C{1H}-NMR spectra for nucleoside **3**

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Batch Filename : 1 RXNS.lcb
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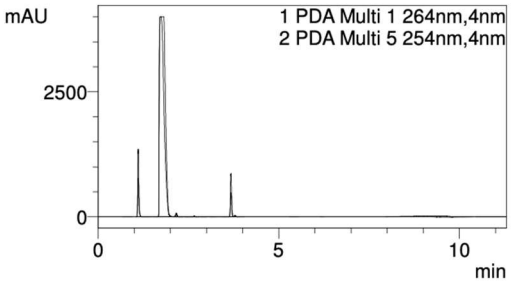
Sample Type : Unknown

Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

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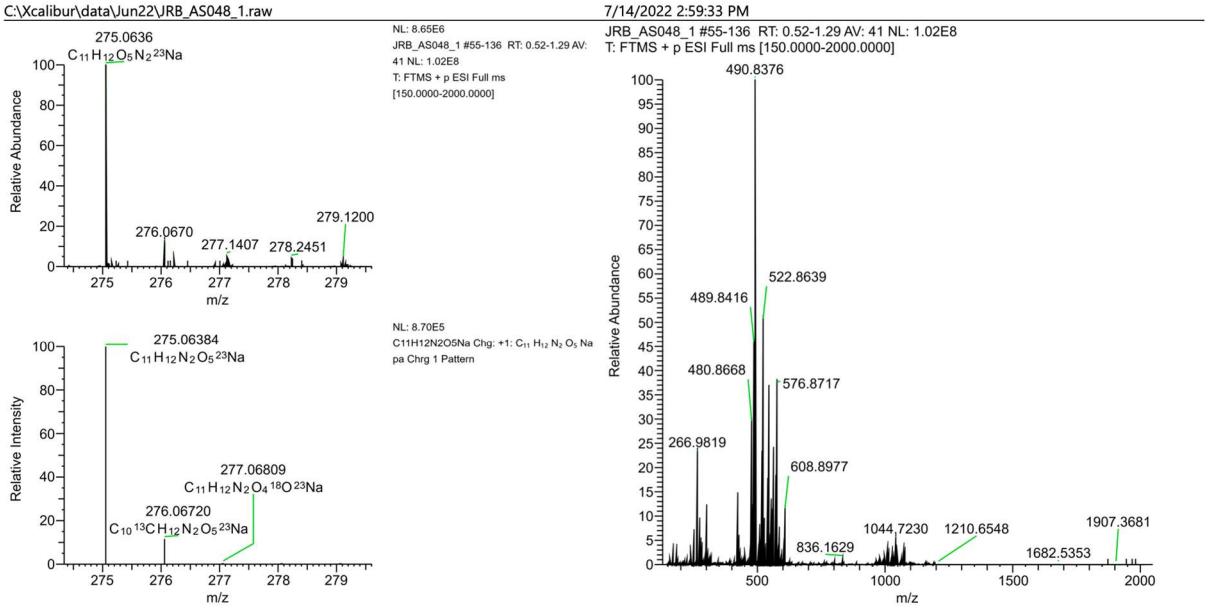
6	2.164	215611	72594	
7	2.380	20804	6303	
8	2.661	45634	13927	
9	2.899	2026	630	
10	3.054	29344	6052	
11	3.493	1007	347	
12	3.678	1973171	858740	
13	3.790	82075	31685	
14	3.952	1861	557	
15	5.397	2669	370	
16	6.185	1903	828	
17	8.266	36439	3513	
18	8.330	16576	4110	
19	8.447	31445	4791	
20	8.604	55315	5234	
21	8.704	17751	5352	
22	8.760	41330	5579	
23	8.910	50155	5585	
24	9.064	35299	5666	
25	9.152	42071	5843	
26	9.280	49131	6010	
27	9.424	35684	6209	
28	9.536	90301	6418	
29	10.074	58658	2393	
30	10.694	13008	519	
Total		51269097	6399083	

Figure S21 - HPLC trace for the reaction used to obtain nucleoside **3**. R.T = 1.11 = nucleobase cytosine, 1.8 = nucleoside dC, 2.16 = nucleobase 1, 3.67 = nucleoside **3**



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
275.0636	$C_{11}H_{12}O_5N_2{}^{23}Na$	44.82596841 79682	6.50	-1.00	275.06384	1	79.8	4	0	81.75	99.88	(Collection)
275.0636	$C_8H_{11}O_7N_4$	5.385700161 61853	5.50	4.88	275.06223	3	68.71	4	1	72.23	89.17	(Collection)
275.0636	$C_9H_7O_3N_8$	24.60494466 8071	10.50	0.02	275.06356	2	77.97	3	1	80.93	96.64	(Collection)

Figure S22 - HRMS spectra for nucleoside **3**

2'-Deoxy-5-iodouridine (14)

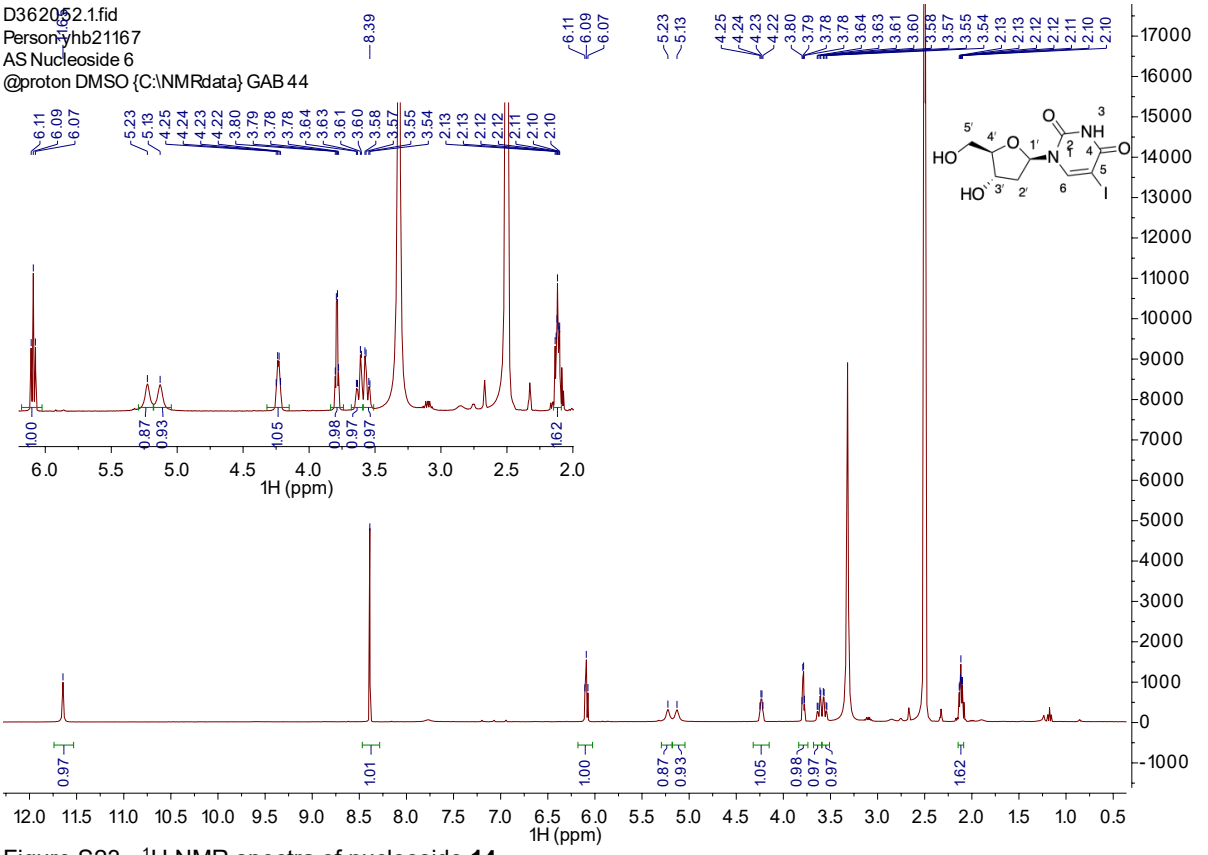


Figure S23 - ^{1}H NMR spectra of nucleoside **14**

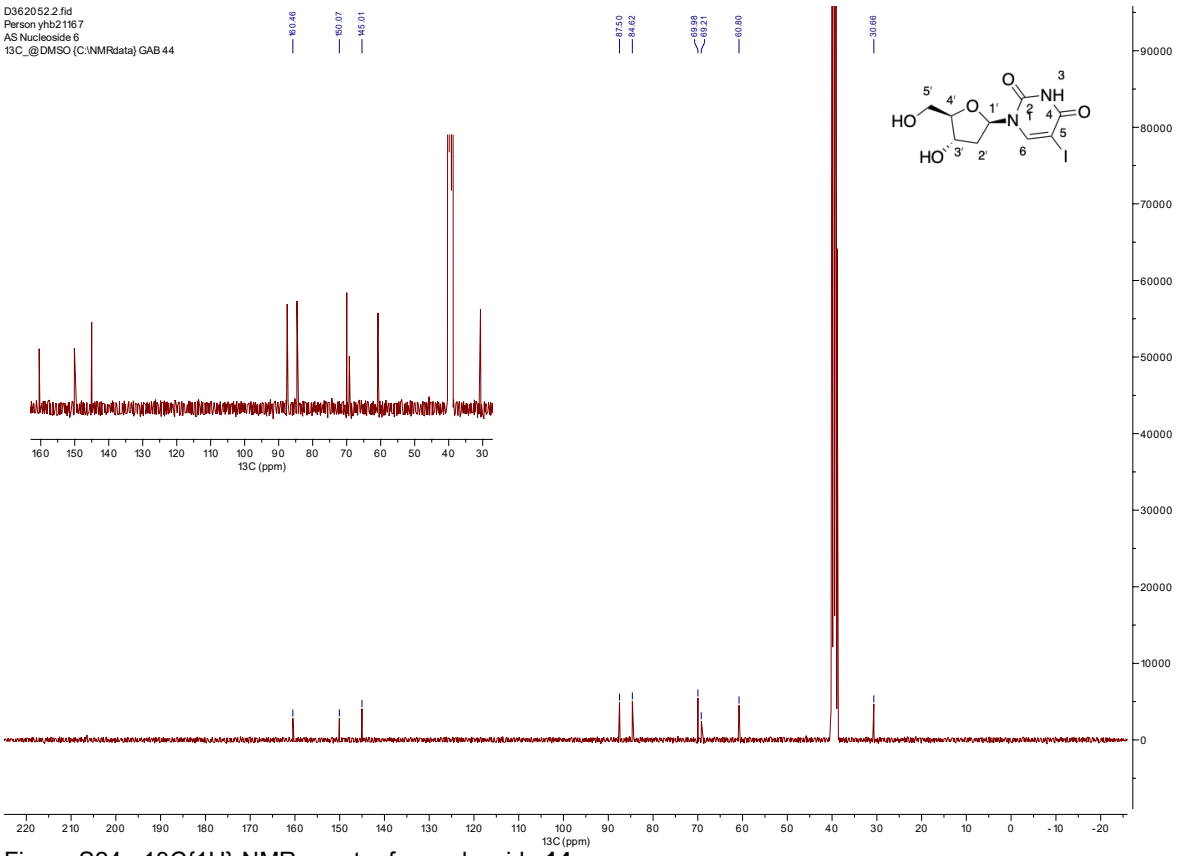
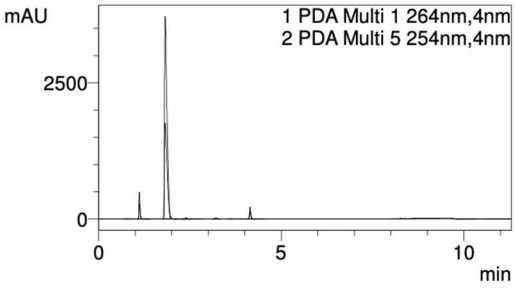


Figure S24 - 13C{1H}-NMR spectra for nucleoside **14**

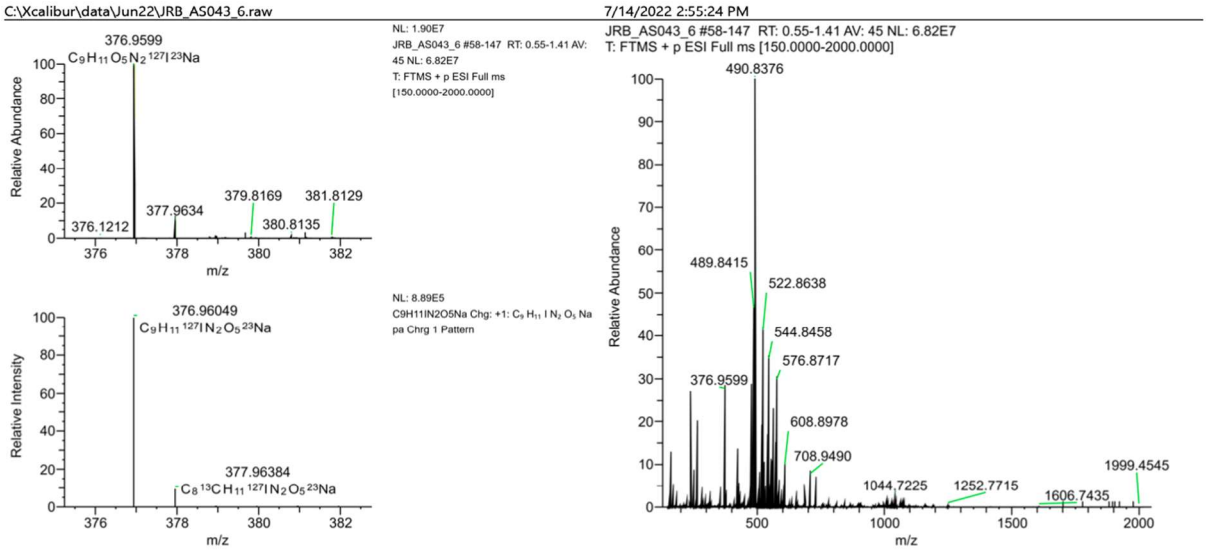
Sample Name	: 6 RXN		
Sample ID	:		
Data Filename	: RXNS_16052022_005.lcd		
Method Filename	: Admir Trans glyco Run.lcm		
Batch Filename	: RXNS.lcb		
Vial #	: 1-6	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 16/05/2022 11:37:43	Acquired by	: Shimadzu
Date Processed	: 16/05/2022 11:49:03	Processed by	: Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.791	8609	3877	
2	0.851	10616	4774	
3	1.117	1023365	482375	
4	1.297	5778	1965	
5	1.824	17508738	3674741	
6	2.394	62612	17925	
7	3.218	59850	20838	
8	3.597	21030	8806	
9	3.925	1508	580	
10	4.147	549528	213511	
11	8.268	47587	8646	
12	8.482	27702	2538	
13	8.616	12479	2319	
14	8.704	7288	1999	
15	8.768	10658	1840	
16	8.920	14689	1293	
17	9.152	4611	545	
18	10.077	62372	2489	
19	10.710	11082	435	
Total		19450102	4451498	

Figure S25 - HPLC trace for the reaction used to obtain nucleoside 14. R.T = 1.11 = nucleobase cytosine, 1.82 = nucleoside dC, 4.15 =.nucleoside **14**



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
376.9599	$C_9H_{11}O_5N_2^{127}I^{23}Na$	46.7356598009499	4.50	-1.43	376.96049	1	94.02	4	0	96.65	99.9	(Collection)
376.9599	$C_7H_6O_9N_8^{127}I$	35.0355946383476	8.50	-0.69	376.96021	2	92.35	3	0	95.53	99.18	(Collection)
376.9599	$C_9H_6O_{15}^{23}Na$	28.9691114361368	6.50	0.14	376.95989	3	92.03	4	0	95.53	96.72	(Collection)
376.9599	$C_7HO_{13}N_6$	25.0423348843379	10.50	0.89	376.95961	4	91.82	4	0	95.53	97.16	(Collection)
376.9599	$C_{10}H_2O_{11}N_4^{2}{}^{3}Na$	20.7792007970678	11.50	-3.40	376.96123	5	91.6	4	0	95.53	97.38	(Collection)
376.9599	$C_{10}H_7ON_6^{127}I^{23}Na$	19.4774227325355	9.50	-4.98	376.96182	6	91.53	3	0	95.53	99.35	(Collection)
376.9599	$C_6H_{10}O_7N_4^{12}{}^{7}I$	15.0830730609195	3.50	2.86	376.95887	7	83.24	3	0	87.02	92.09	(Collection)

Figure S26 - HRMS for nucleoside **14**

2'-Deoxy-5-Azidomethyluridine (**15**)

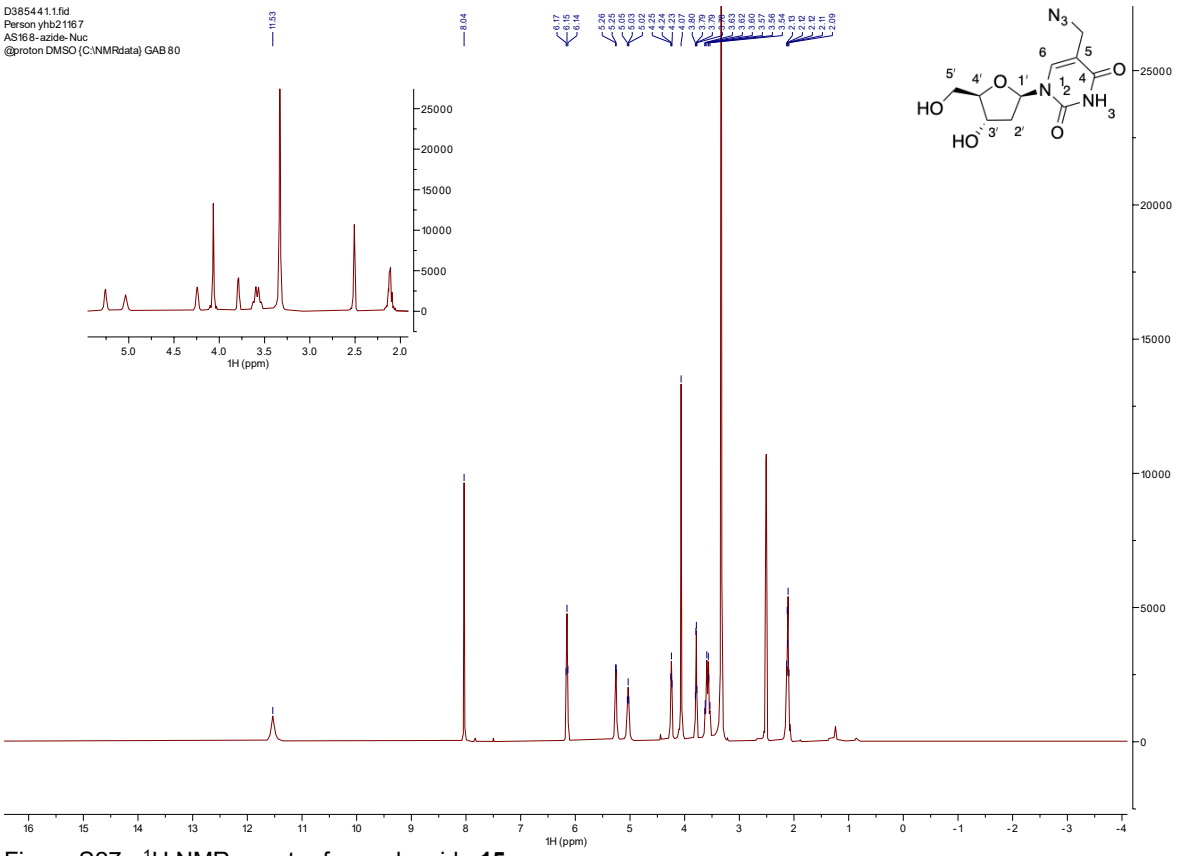


Figure S27 - ^{1}H NMR spectra for nucleoside **15**

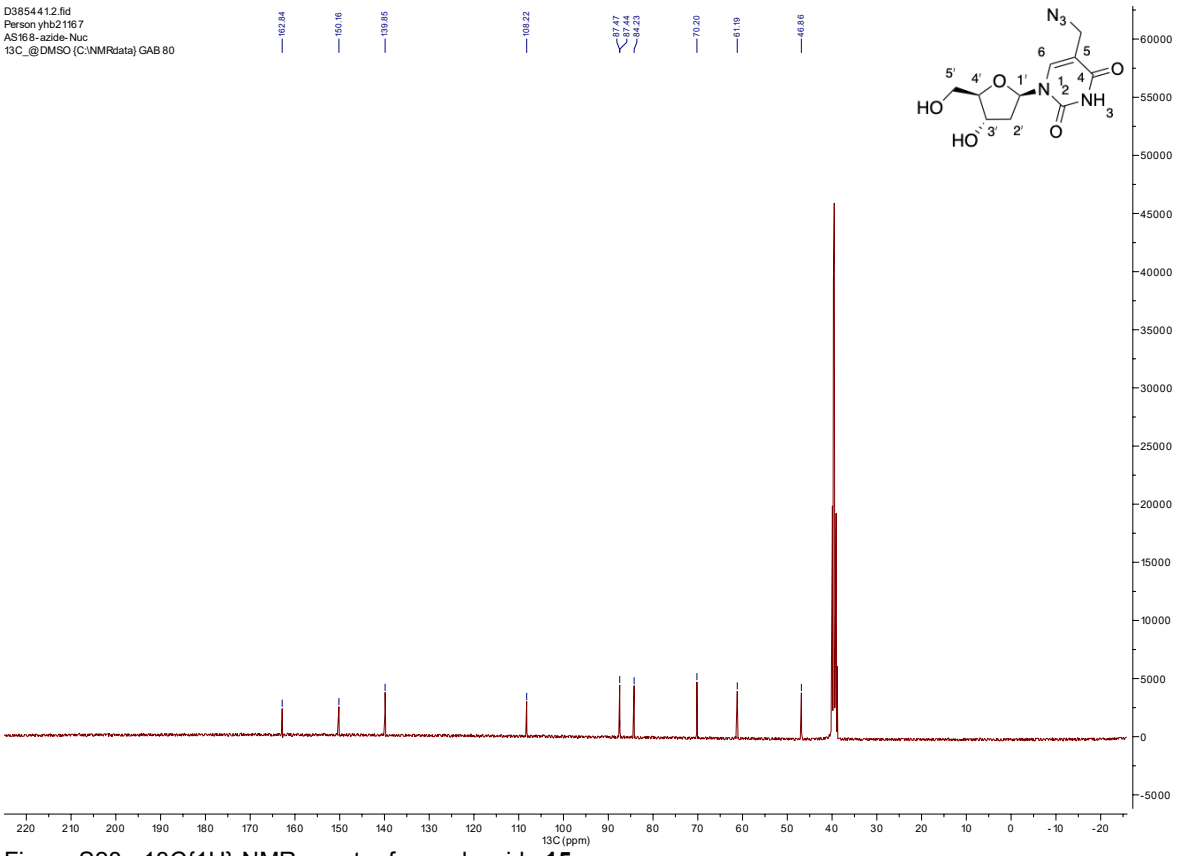
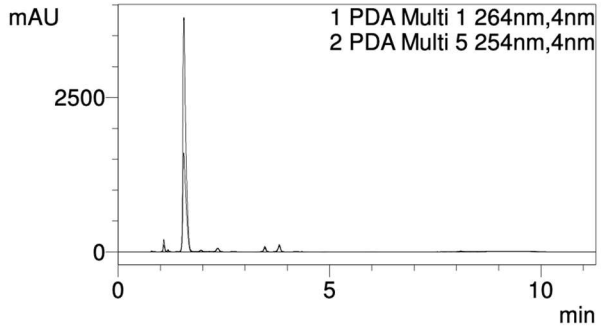


Figure S28 - 13C{1H}-NMR spectra for nucleoside **15**

Sample Name : x10 0.4
Sample ID :
Data Filename : 0.4_07022024_004.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : 0.4.lcb
Vial # : 1-19
Injection Volume : 10 uL
Date Acquired : 07/02/2024 13:54:19
Date Processed : 07/02/2024 14:05:40

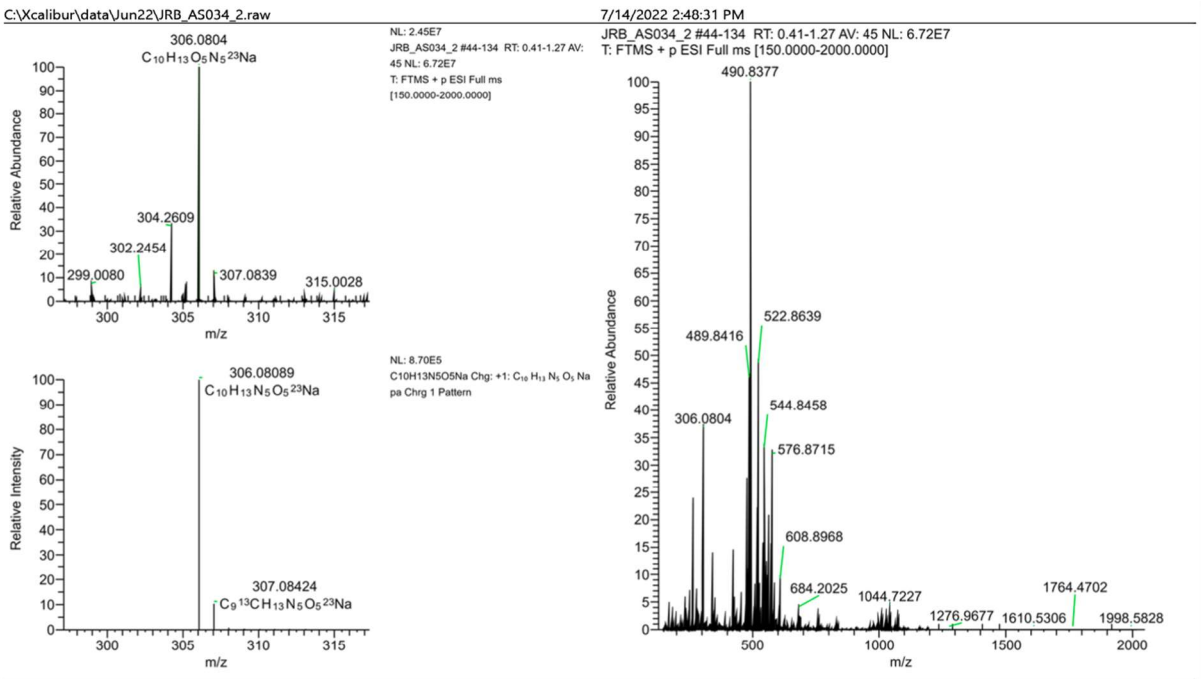
Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.796	14288	10580	
2	0.858	5503	2483	
3	1.083	480695	190150	
4	1.183	70638	32692	
5	1.391	11365	3826	
6	1.556	18298506	3781769	
7	1.962	124826	26110	
8	2.163	30957	5652	
9	2.358	282466	58706	
10	2.739	16937	2814	
11	3.473	319135	83683	
12	3.814	477530	116089	
13	4.052	1053	254	
14	4.204	18693	3835	
15	4.350	3265	864	
16	4.892	1713	373	
17	6.038	1342	294	
18	7.742	1152	304	
19	8.103	43458	11963	
20	8.224	5498	1448	
21	8.424	3049	418	
22	8.640	1930	197	

Figure S29 - HPLC trace for the reaction used to obtain nucleoside **15**. R.T = 1.08 = released nucleobase cytosine, 1.27 = nucleobase, 1.55 = nucleoside dC, 2.3 = nucleobase, 3.47 = nucleoside **15**



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
306.0804	$C_{10}H_{13}O_5N_5{}^{23}Na$	44.0410963897038	6.50	-1.50	306.08089	1	91.49	3	1	94.12	99.89	(Collection)
306.0804	$C_9H_{17}O_8N^{23}Na$	23.0864445267396	1.50	2.87	306.07955	2	89.46	3	1	93.15	97.97	(Collection)
306.0804	$C_7H_{12}O_7N_7$	7.41142834995913	5.50	3.78	306.07927	3	80.08	3	2	84.11	89.06	(Collection)
306.0804	$C_{11}H_{16}O_9N$	6.48639426340963	4.50	-4.99	306.08196	4	80.03	4	0	84.11	87.14	(Collection)

Figure S30 - HRMS for nucleoside **15**

2'-Deoxy-5-hydroxymethyluridine (18)

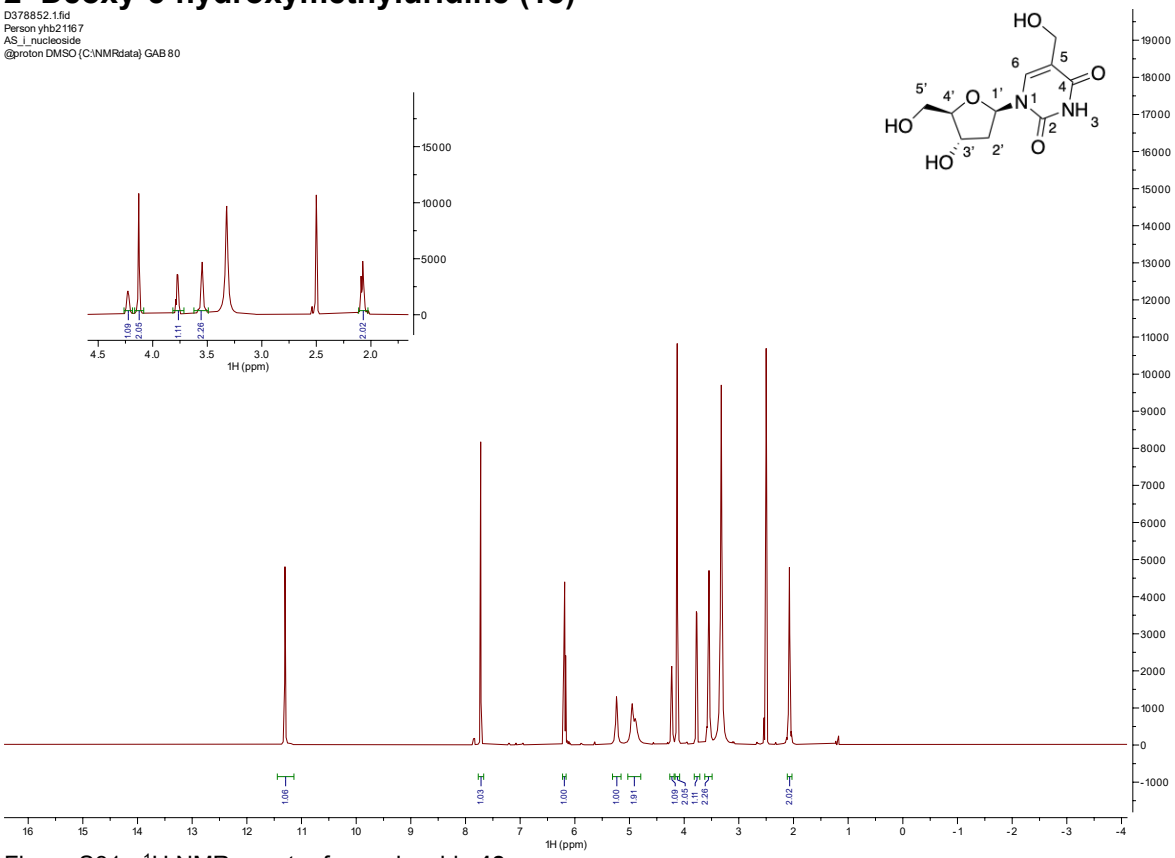


Figure S31 - ^{1}H NMR spectra for nucleoside **18**

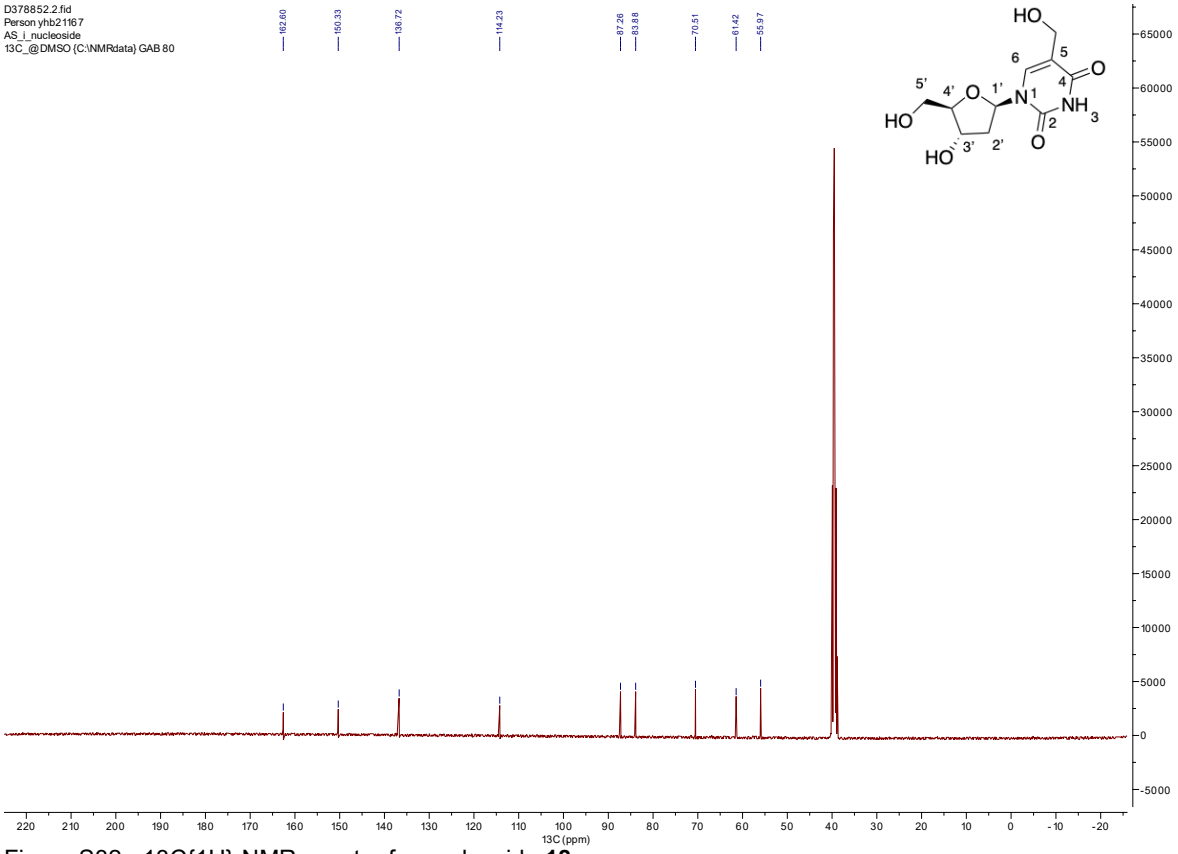
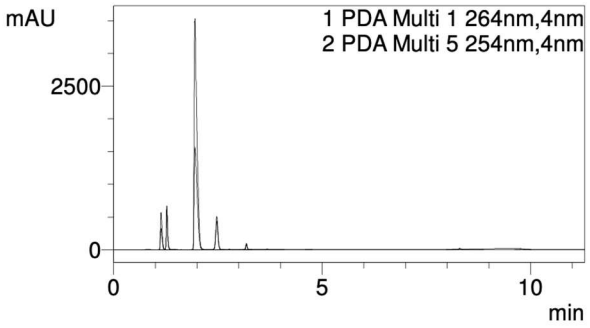


Figure S32 - 13C{1H}-NMR spectra for nucleoside **18**

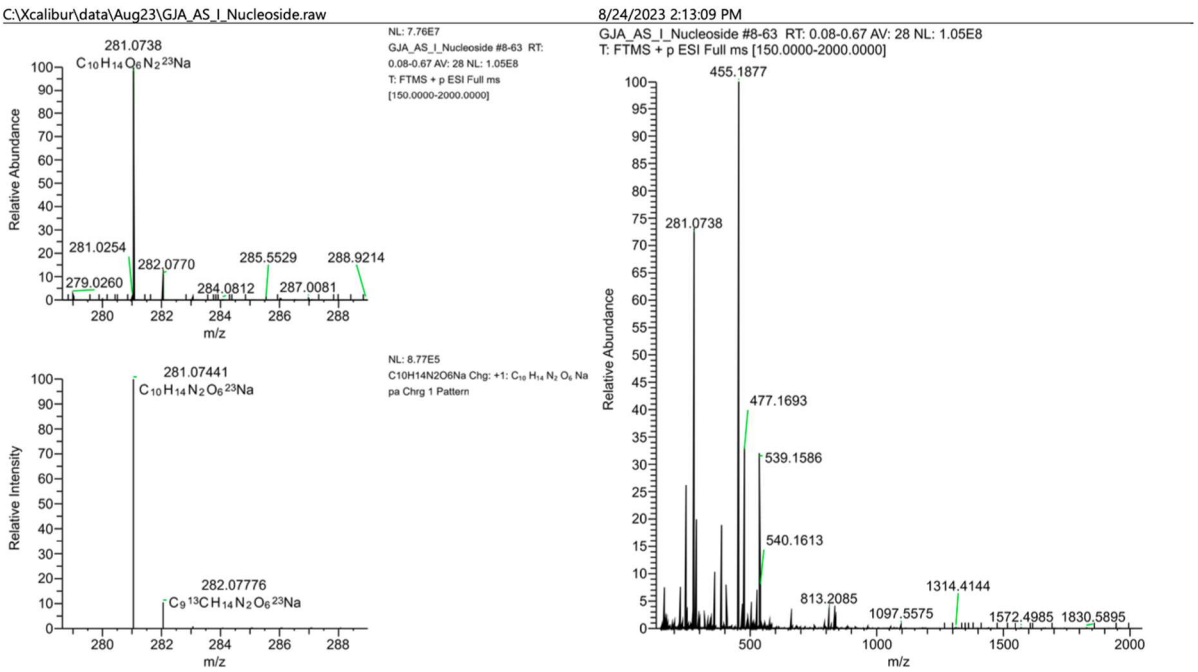
Sample Name	: BASE I RXN		
Sample ID	:		
Data Filename	: BASE I RXN_17032022_003.lcd		
Method Filename	: Admir Trans glyco Run.lcm		
Batch Filename	: BASE I RXN.lcb		
Vial #	: 1-54	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 17/03/2022 19:31:14	Acquired by	: Shimadzu
Date Processed	: 17/03/2022 19:42:35	Processed by	: Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.784	5393	2737	
2	0.848	1880	693	
3	1.139	1609034	558849	
4	1.278	1437412	659572	
5	1.952	16217450	3488901	
6	2.476	1868923	504713	
7	2.778	23560	5532	
8	2.902	2530	480	
9	3.186	237327	93416	
10	3.484	6131	2638	
11	3.604	1122	434	
12	3.685	24094	9335	
13	3.825	12741	3754	
14	3.905	12954	3589	
15	4.029	1114	365	

Figure S33 - HPLC trace for the reaction used to obtain nucleoside **18**. R.T = 1.1 = released nucleobase cytosine, 1.27 = nucleobase, 1.95 = nucleoside dC, 2.4 = nucleoside **18**



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
281.0738	$C_{10}H_{14}O_6N_2{}^2{}^3Na$	54.6030982305484	4.50	-1.99	281.07441	1	97.25	4	0	99.62	100	(Collection)
281.0738	$C_7H_{13}O_8N_4$	16.8056303288766	3.50	3.76	281.07279	3	84.87	4	1	88.65	89.96	(Collection)
281.0738	$C_8H_9O_4N_8$	22.2137193798215	8.50	-1.00	281.07413	2	94.29	3	1	98.29	96.46	(Collection)

Figure S34 - HRMS for nucleoside **18**

2'-Deoxy-5-nitrouridine (20)

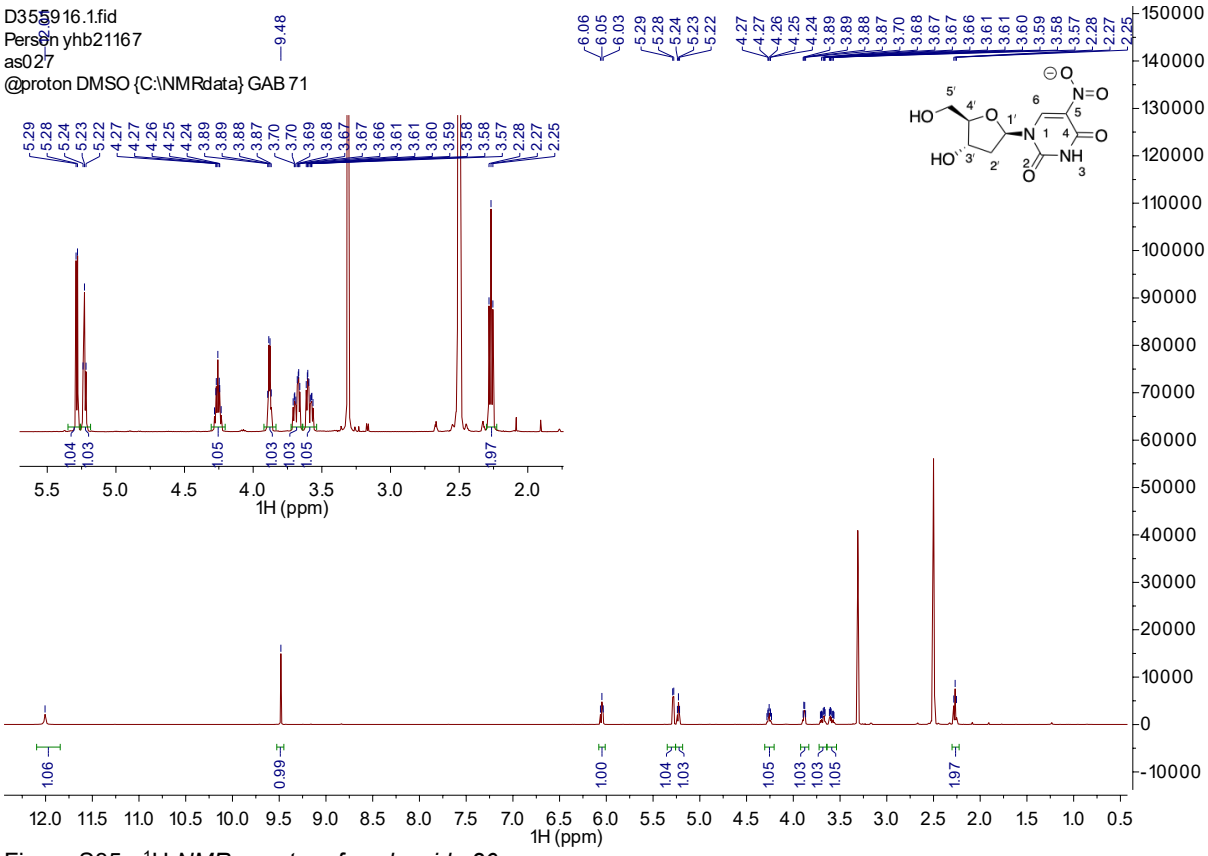


Figure S35 - ^{1}H *NMR spectra of nucleoside 20*

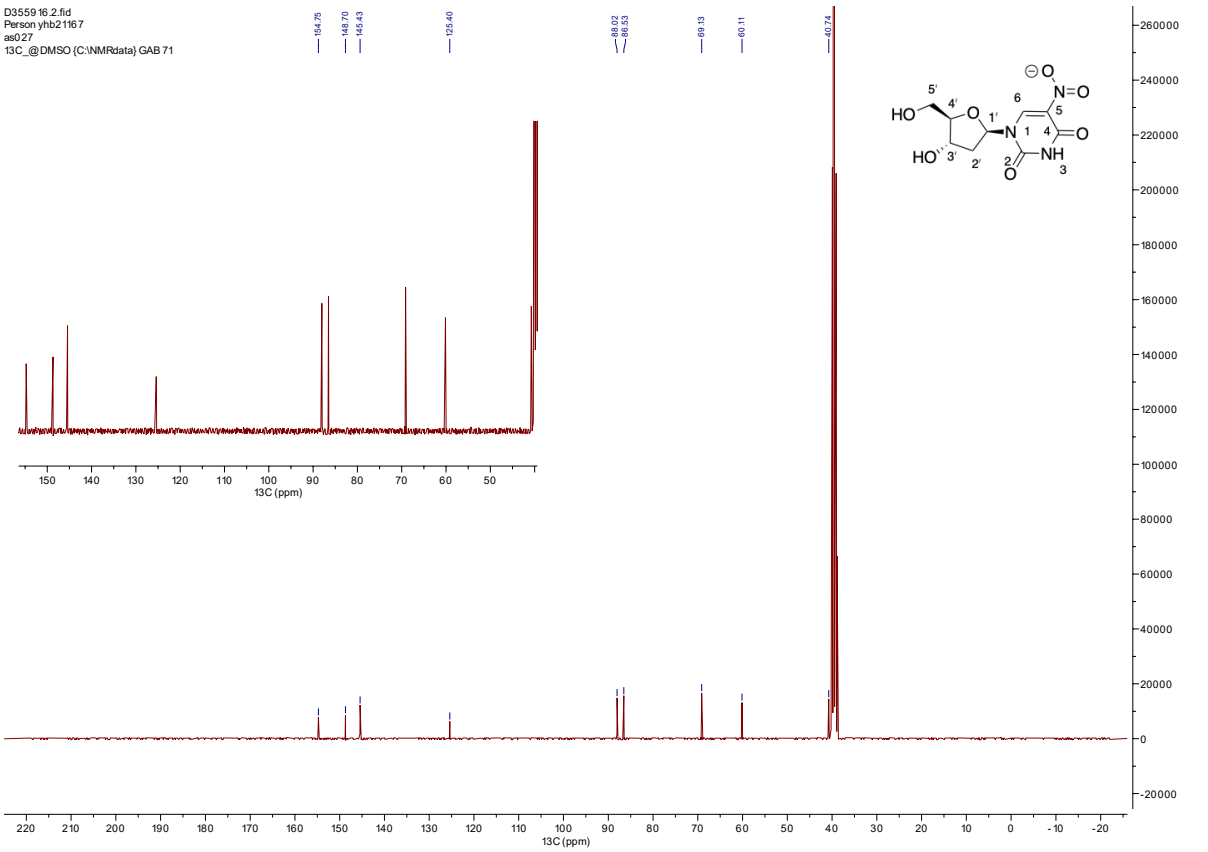
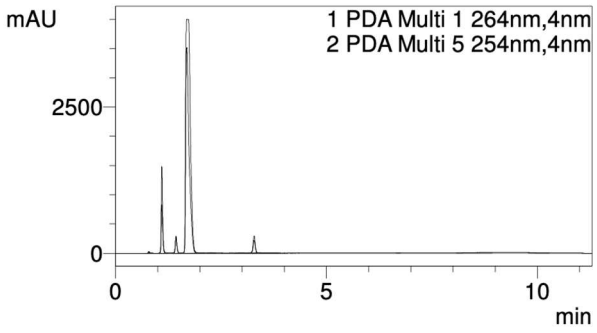


Figure S36 - 13C{1H}-NMR spectra of nucleoside 20

Sample Name : a1 rxn
Sample ID :
Data Filename : RXNS + STDS_03022023_005.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : RXNS + STDS.lcb
Vial # : 1-19
Injection Volume : 10 uL
Date Acquired : 03/02/2023 11:58:50
Date Processed : 03/02/2023 12:10:11

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.793	43851	23810	
2	1.100	3365440	1456292	
3	1.286	11205	2399	
4	1.437	772064	284463	
5	1.721	31227025	4000141	
6	2.268	20920	4883	
7	2.988	1042	153	
8	3.288	768419	213461	
9	3.573	3422	863	
10	3.667	3439	898	
11	3.792	1470	428	

Figure S37 - HPLC trace for the reaction used to obtain nucleoside **20**. R.T = 1.1 = released nucleobase cytosine, 1.43 = nucleobase, 1.72 = nucleoside dC, 3.28 = nucleoside **20**

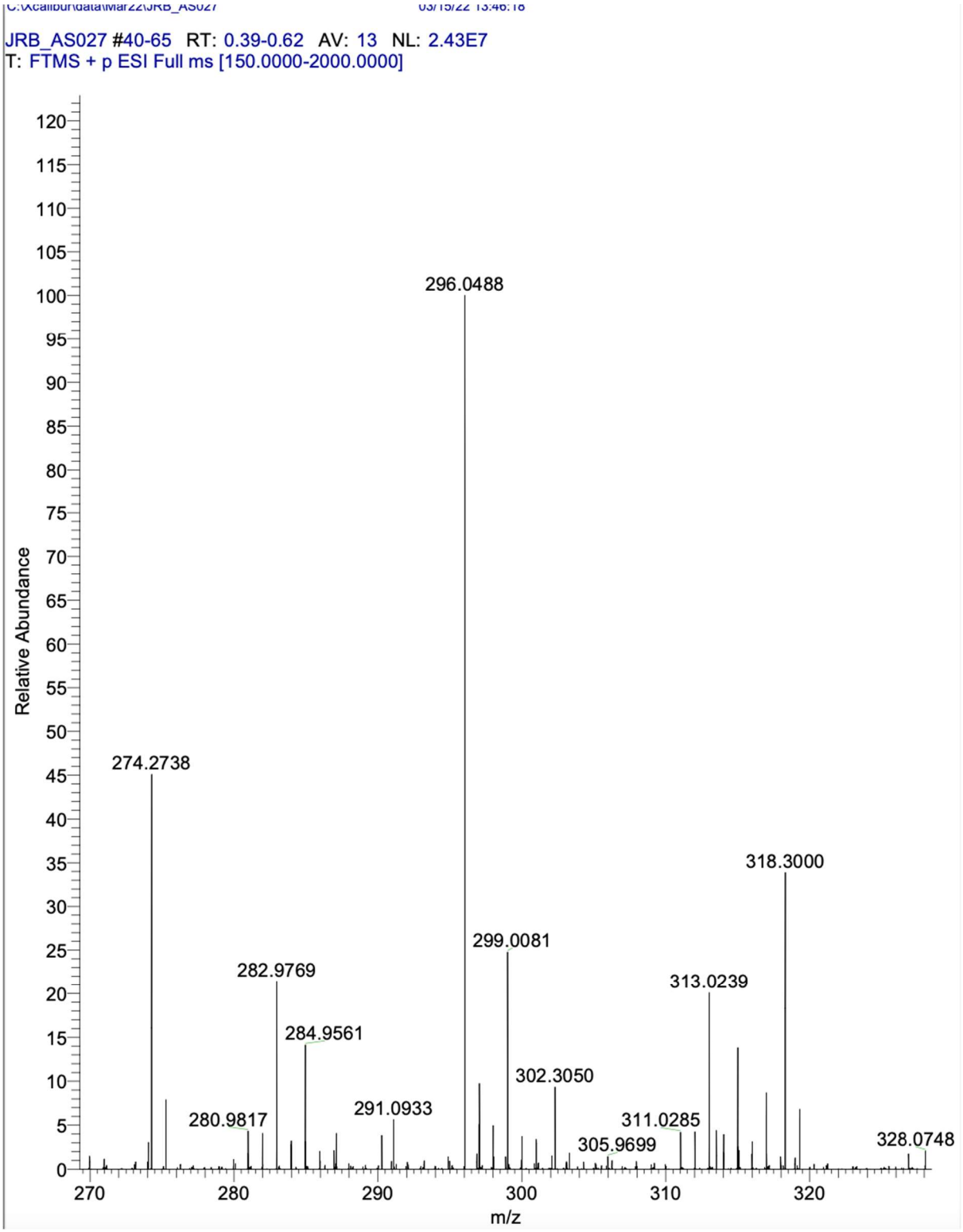


Figure S38 - HRMS for nucleoside 20

2'-Deoxy-5-trifluorouridine (21)

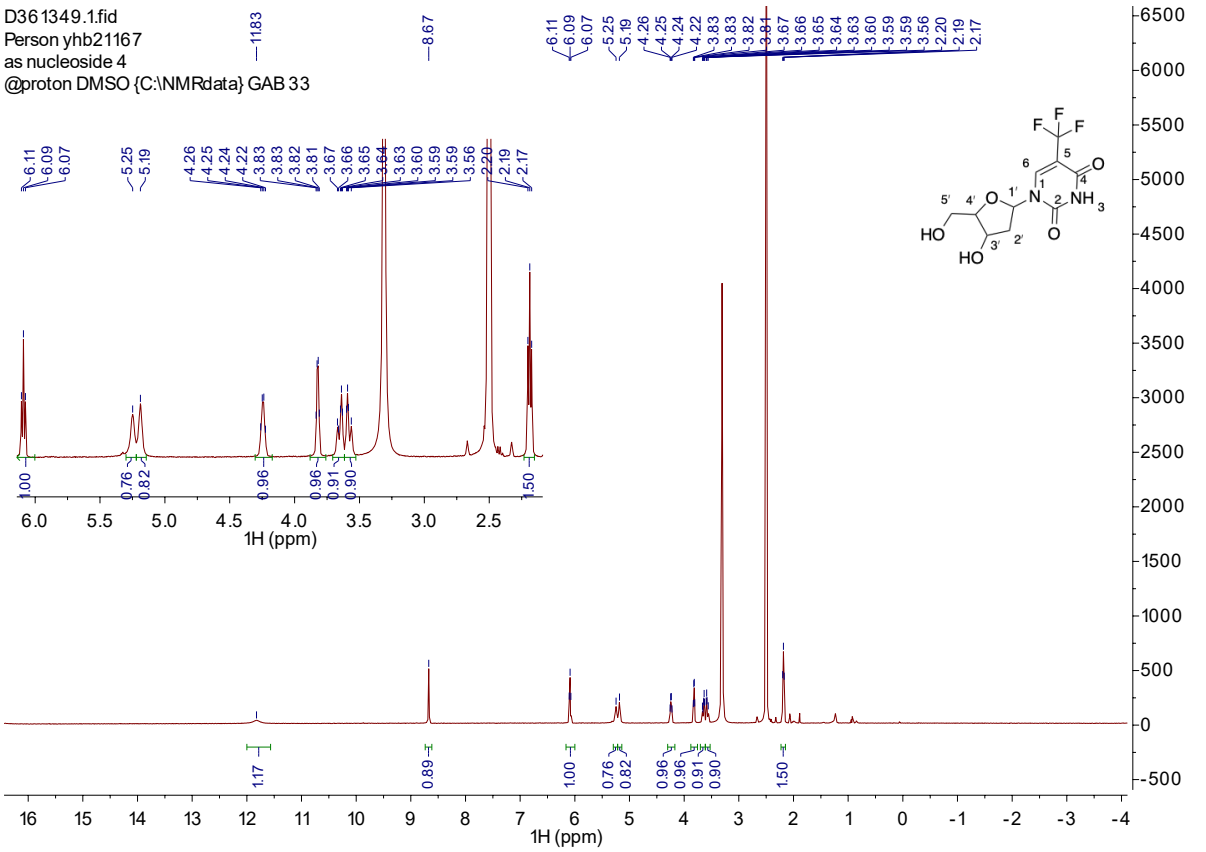


Figure S39 - 1H NMR spectra of nucleoside 21

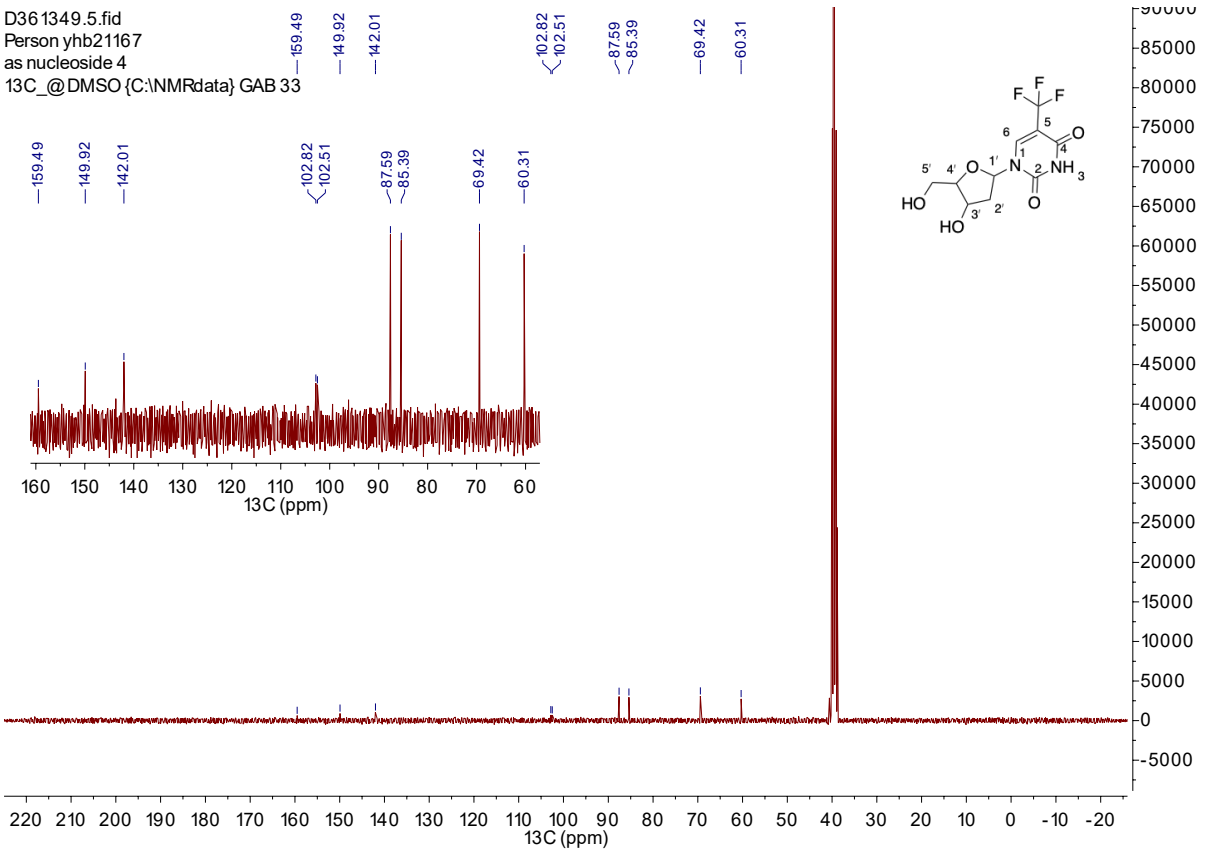


Figure S40 - 13C{1H}-NMR spectra of nucleoside 21

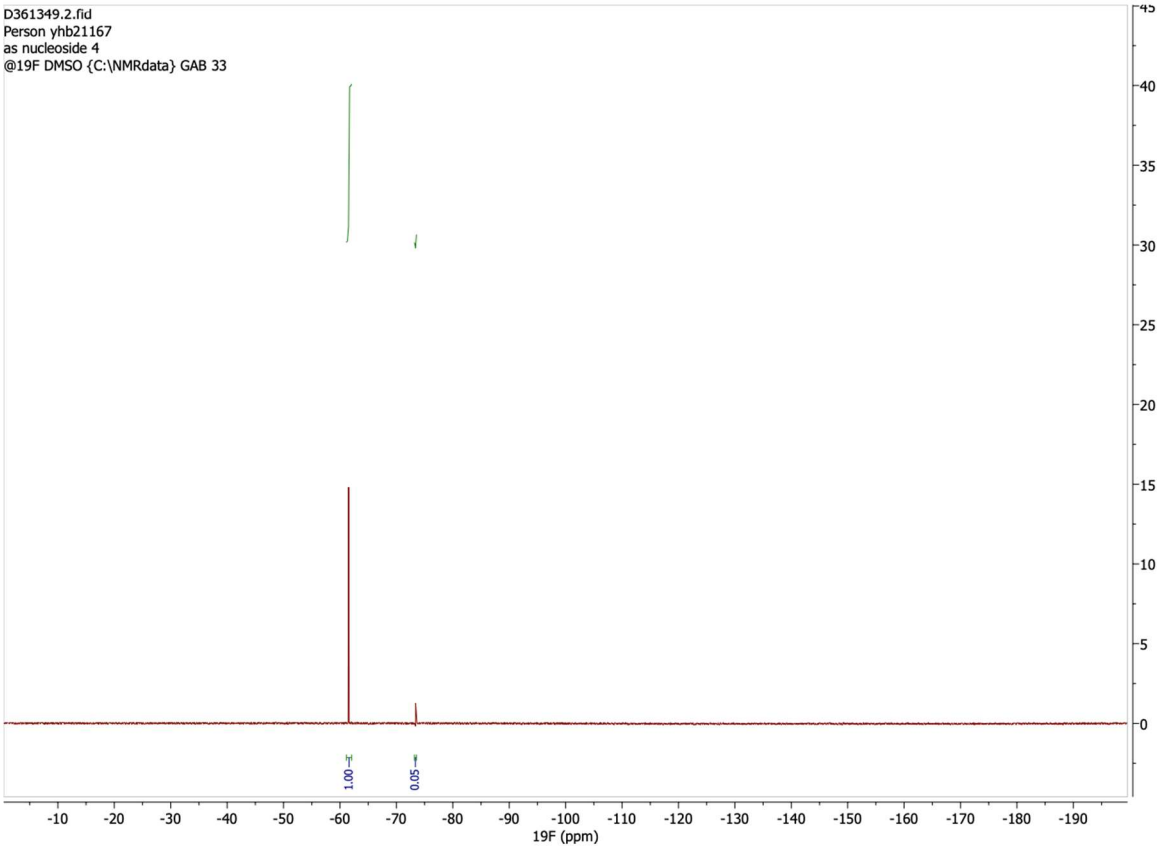
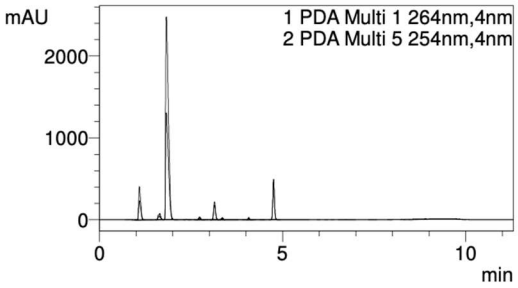


Figure S41 - ^{19}F NMR spectra for nucleoside 21

Sample Name : 4 rxn
Sample ID :
Data Filename : rest of the rxns_15062022_008.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : rest of the rxns.lcb
Vial # : 1-18
Injection Volume : 10 uL
Date Acquired : 15/06/2022 15:50:38
Date Processed : 15/06/2022 16:01:59

Sample Type : Unknown

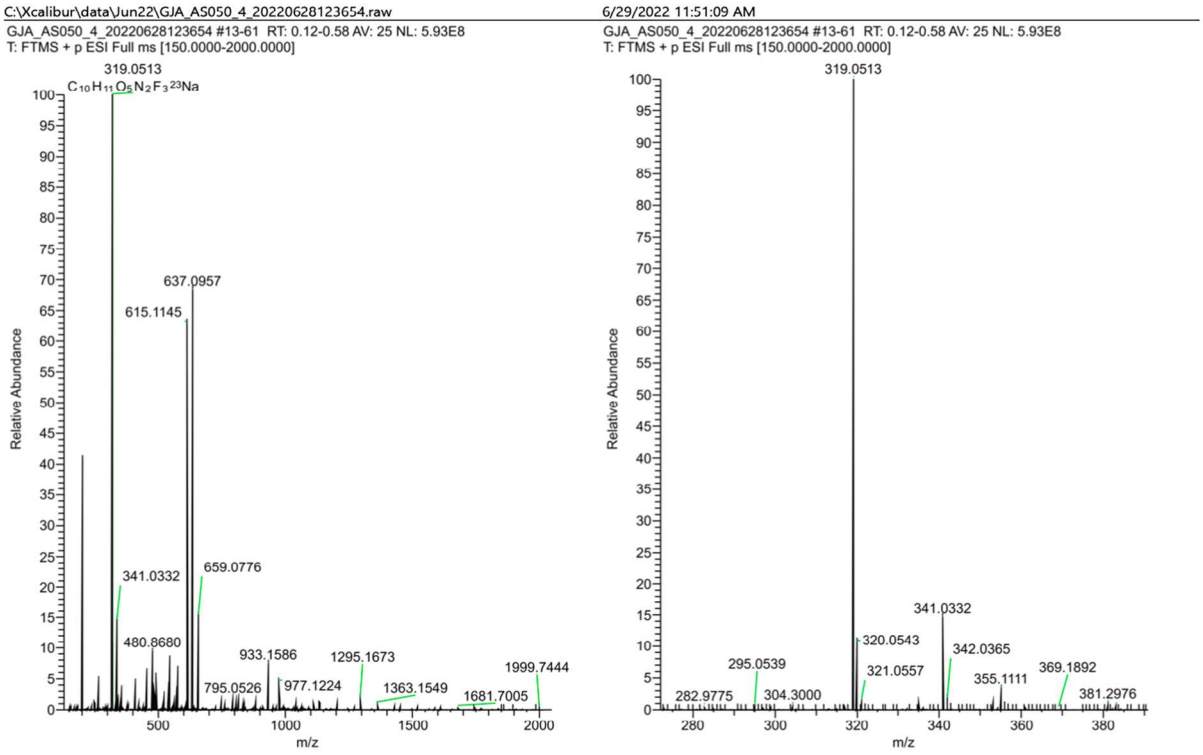
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.787	7400	2755	
2	1.091	1561616	397981	
3	1.299	13166	2562	
4	1.648	358738	73850	
5	1.829	12969229	2469692	
6	2.382	24125	5950	
7	2.733	129162	32877	
8	3.143	638386	176476	
9	3.357	101110	26751	
10	3.495	4198	1245	
11	3.640	1831	407	
12	4.076	78410	29411	
13	4.413	1477	322	
14	4.755	1545418	487774	
15	5.165	1686	315	
16	5.329	1540	415	
17	5.411	1423	347	
18	5.635	1511	420	
19	5.978	1204	544	
20	6.175	1900	577	
21	6.347	1289	397	
22	6.554	1684	670	
23	6.725	6932	2656	
24	6.886	3337	623	
25	7.519	1010	233	
26	7.908	1541	641	
27	8.256	7609	3341	
28	8.327	2488	883	
29	8.469	2005	730	
30	8.595	4710	807	
31	8.696	7133	1125	
32	8.896	2094	684	
33	9.809	10482	1543	
Total		17495844	3725004	

Figure S42 - HPLC trace of the reaction to obtain nucleoside 21. R.T = 1.1 = nucleobase cytosine, 1.6 = nucleoside dC, 4.76 = nucleoside 21



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
319.0513	$C_{10}H_{11}O_5N_2F_3{}^{23}Na$	72.4012349652965	4.50	0.37	319.05123	1	98.38	4	0	99.82	100	(Collection)
319.0513	$C_{13}H_{10}O_4N_2F_2{}^{23}Na$	49.1233746901087	8.50	3.96	319.05008	2	97.16	4	0	99.82	100	(Collection)

Figure S43 - HRMS spectra of the nucleoside 21

2'-Deoxy-5-carboxyuridine (22)

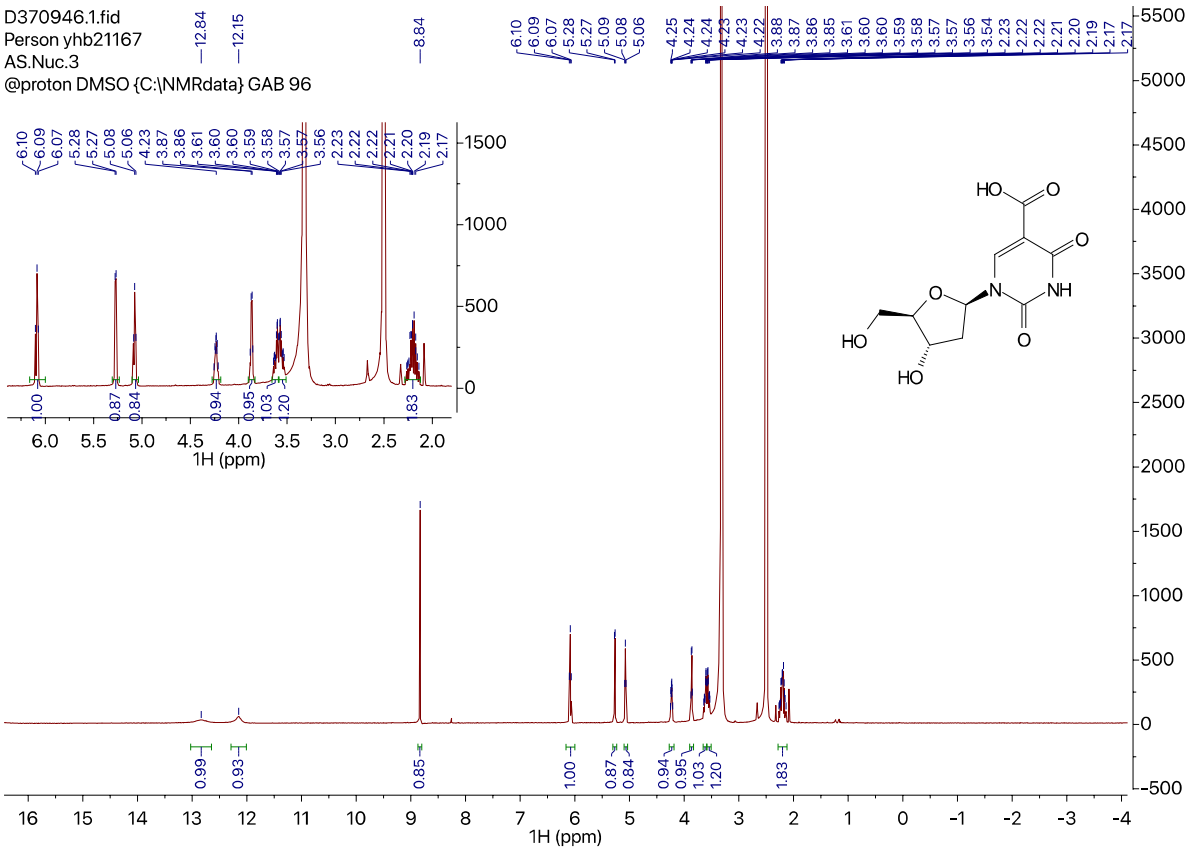


Figure S44 - 1H NMR spectrum of Nucleoside 22

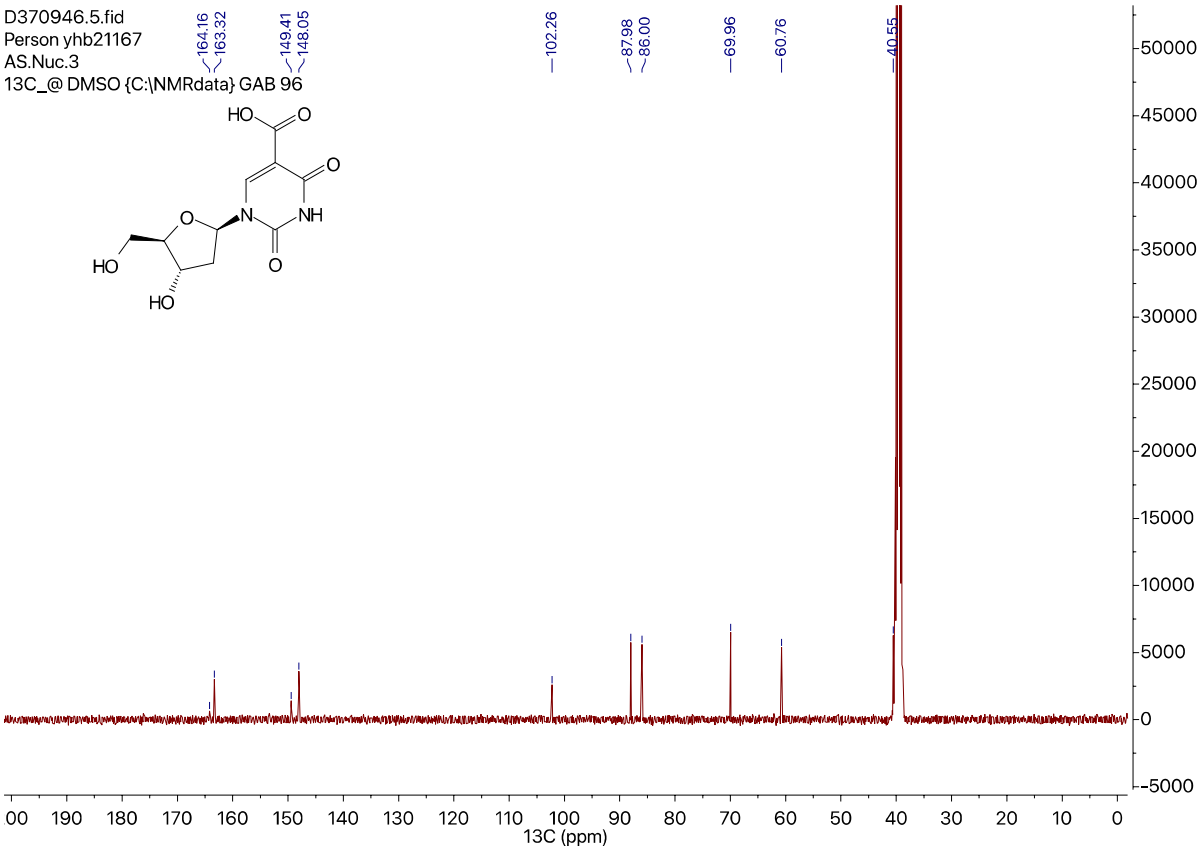
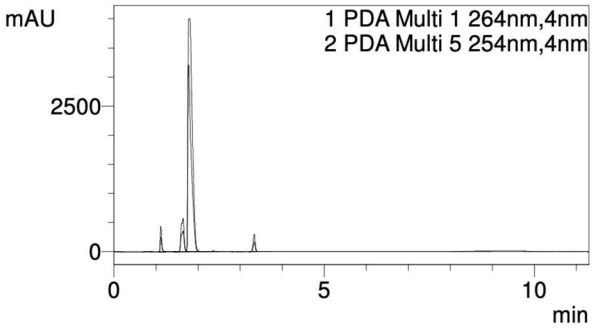


Figure S45 - 13C NMR of Nucleoside 22

Sample Name : 3
Sample ID :
Data Filename : rest of rxns_21062022_012.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : rest of rxns.lcb
Vial # : 1-27
Injection Volume : 10 uL
Date Acquired : 21/06/2022 21:59:58
Date Processed : 21/06/2022 22:11:19

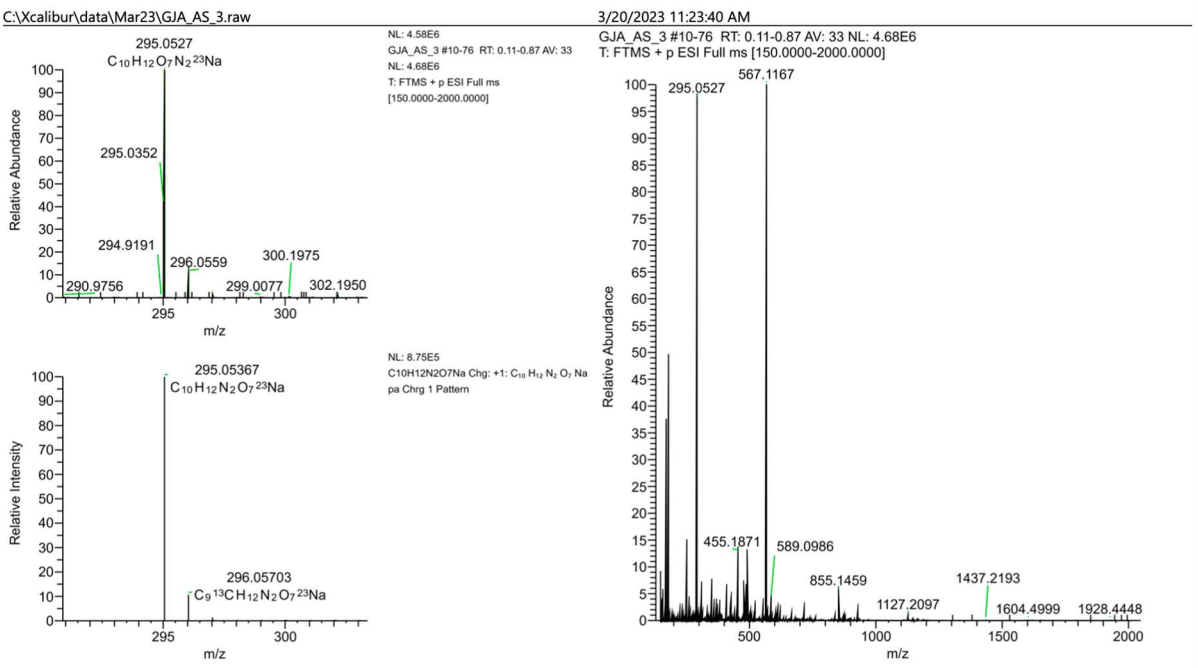
Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.801	6268	2225	
2	0.885	1073	436	
3	1.116	997568	427625	
4	1.305	12191	1715	
5	1.645	2809996	569637	
6	1.793	29333782	4000097	
7	2.365	53923	14259	
8	3.339	997625	297370	
9	3.627	2646	984	
10	8.252	11717	2031	
11	8.323	7906	1731	
12	8.462	9791	1375	

Figure S46 - *HPLC spectrum of Nucleoside 22. R.t of 1.1 min = cytosine released, 1.79 min = dC, 3.339 min = Nucleoside 22, 1.645 min = nucleobase starting material.*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
295.0527	$C_{10}H_{12}O_7N_2^{2}{}^{3}Na$	42.28884306 60264	5.50	-3.41	295.05367	1	96.06	4	0	99.05	100	(Collection)
295.0527	$C_{22}H_8{}^{23}Na$	11.06284581 17435	18.50	2.86	295.05182	4	83.7	4	0	87.74	78.91	(Collection)
295.0527	$C_7H_{11}O_9N_4$	39.67389013 2808	4.50	2.07	295.05205	2	86.68	4	0	89.29	92.78	(Collection)
295.0527	$C_6H_7O_5N_8$	11.66907437 75202	9.50	-2.46	295.05339	3	83.74	3	1	87.74	88.37	(Collection)

Figure S47- HRMS spectrum of Nucleoside 22

3-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-4(1*H*)-one (23).

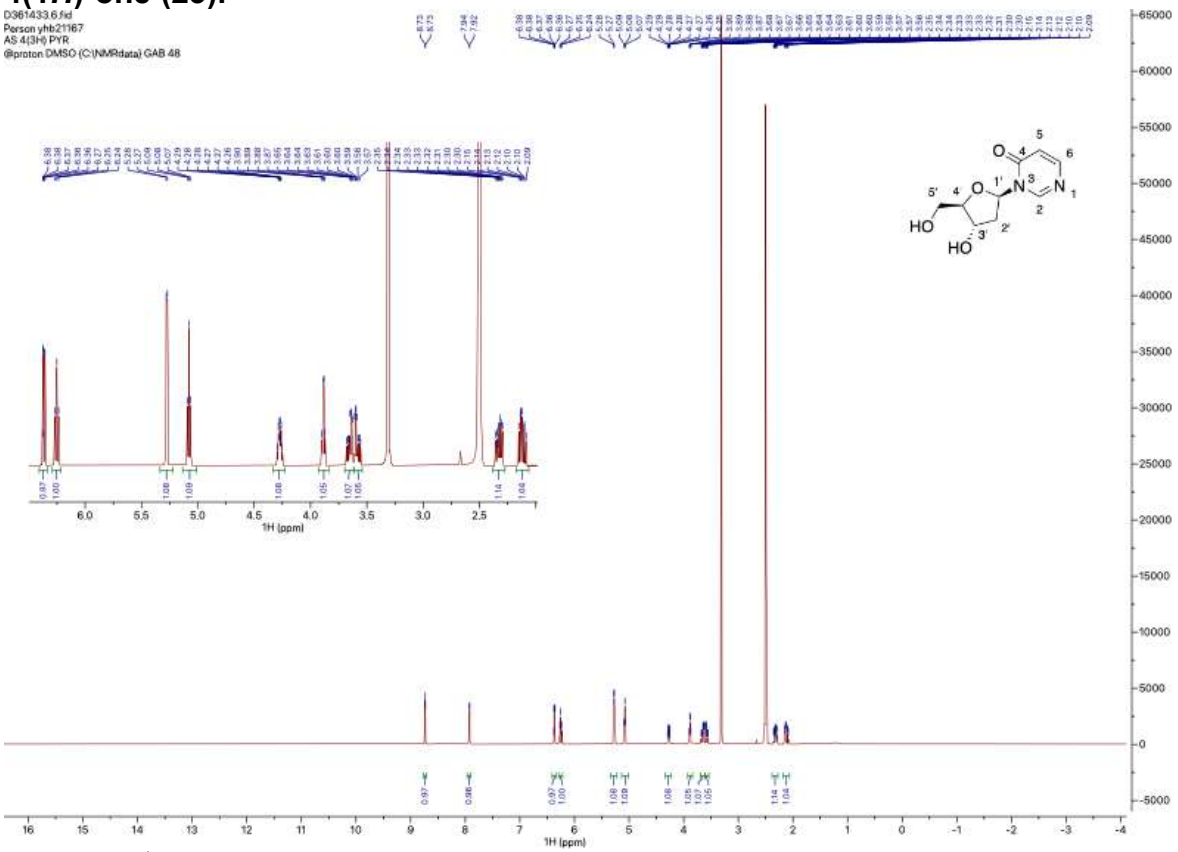


Figure S48 - ^{1}H NMR spectra of Nucleoside **23**

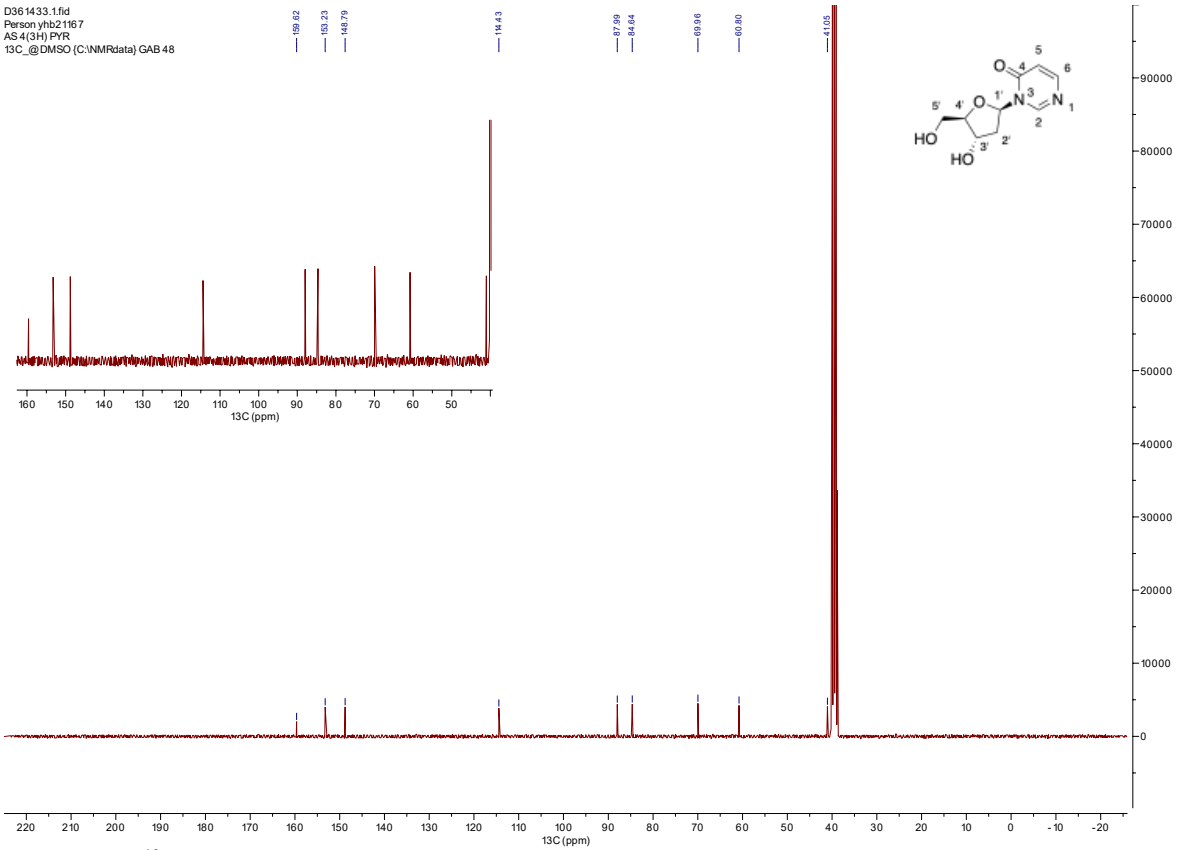
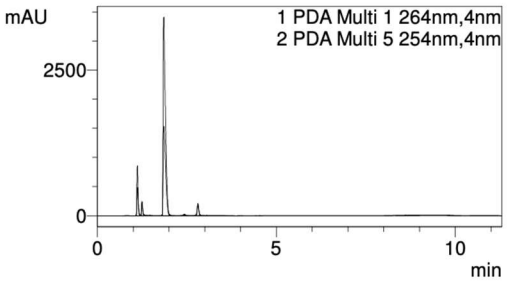


Figure S49 - ^{13}C{1H}-NMR spectra of Nucleoside **23**

Sample Name : 4 PYRIMIDINONE RXN
Sample ID :
Data Filename : RXNS AND STDS_22042022_020.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : RXNS AND STDS.lcb
Vial # : 1-80
Injection Volume : 10 uL
Date Acquired : 22/04/2022 14:40:03
Date Processed : 22/04/2022 14:51:24

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



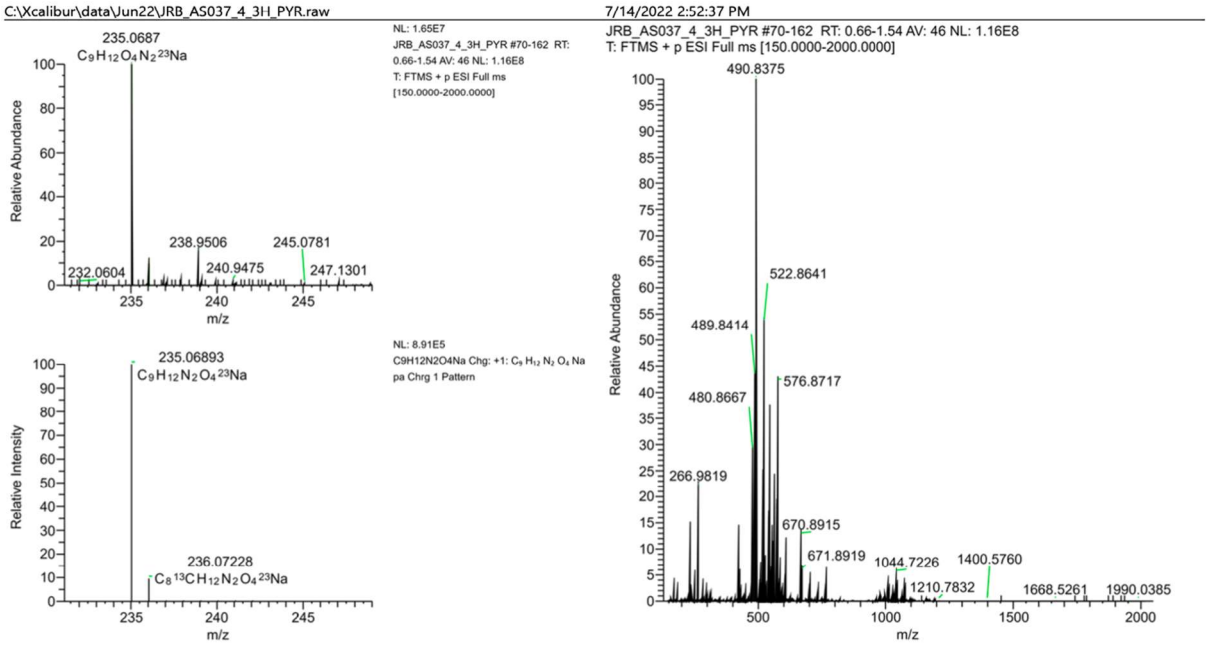
PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.784	7989	2884	
2	1.119	1972913	838866	
3	1.249	522358	201293	
4	1.382	3489	1704	
5	1.463	3431	1652	
6	1.856	15384031	3361927	
7	2.434	82037	23463	
8	2.811	684939	206266	
9	3.063	14910	4115	
10	3.264	12284	790	
11	3.617	5927	2466	
12	8.184	2046	246	
13	8.352	1385	620	
14	8.622	6414	857	
15	8.719	3901	1038	
16	8.776	3660	784	
17	8.933	1657	410	
18	9.834	18151	2815	
19	10.670	14929	608	
Total		18746450	4652803	

PDA Ch5 254nm

Peak#	Ret. Time	Area	Height	ID#
1	1.119	1125290	477253	
2	1.249	545285	225850	
3	1.858	7414817	1511895	
4	2.434	74241	21752	
5	2.811	573732	175084	
Total		9733365	2411834	

Figure S50 - *HPLC trace of the reaction used to obtain Nucleoside 23. R.T = 1.2 = nucleobase cytosine, 1.25 = nucleobase x, 1.86 = nucleoside dC, 2.81= Nucleoside 23*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
235.0687	$C_9H_{12}O_4N_2^{23}Na$	62.7537339124268	4.50	-0.79	235.06893	1	79.21	4	0	80.12	100	(Collection)
235.0687	$C_7H_7O_2N_8$	10.5207389060421	8.50	0.40	235.06865	2	68.4	3	2	71.61	89.83	(Collection)
235.0687	$C_7H_{15}ON_4^{32}S_2$	5.69244491721778	2.50	2.40	235.06818	3	68.14	4	4	71.61	83.1	(Collection)
235.0687	$C_2H_{12}O_2N_8^{23}Na^{32}S$	5.32555156401635	0.50	-3.70	235.06961	4	68.12	3	4	71.61	90.36	(Collection)

Figure S51 - *HRMS of Nucleoside 23 sodium adduct.*

2'-Deoxy-*N*-3-methyluridine (24)

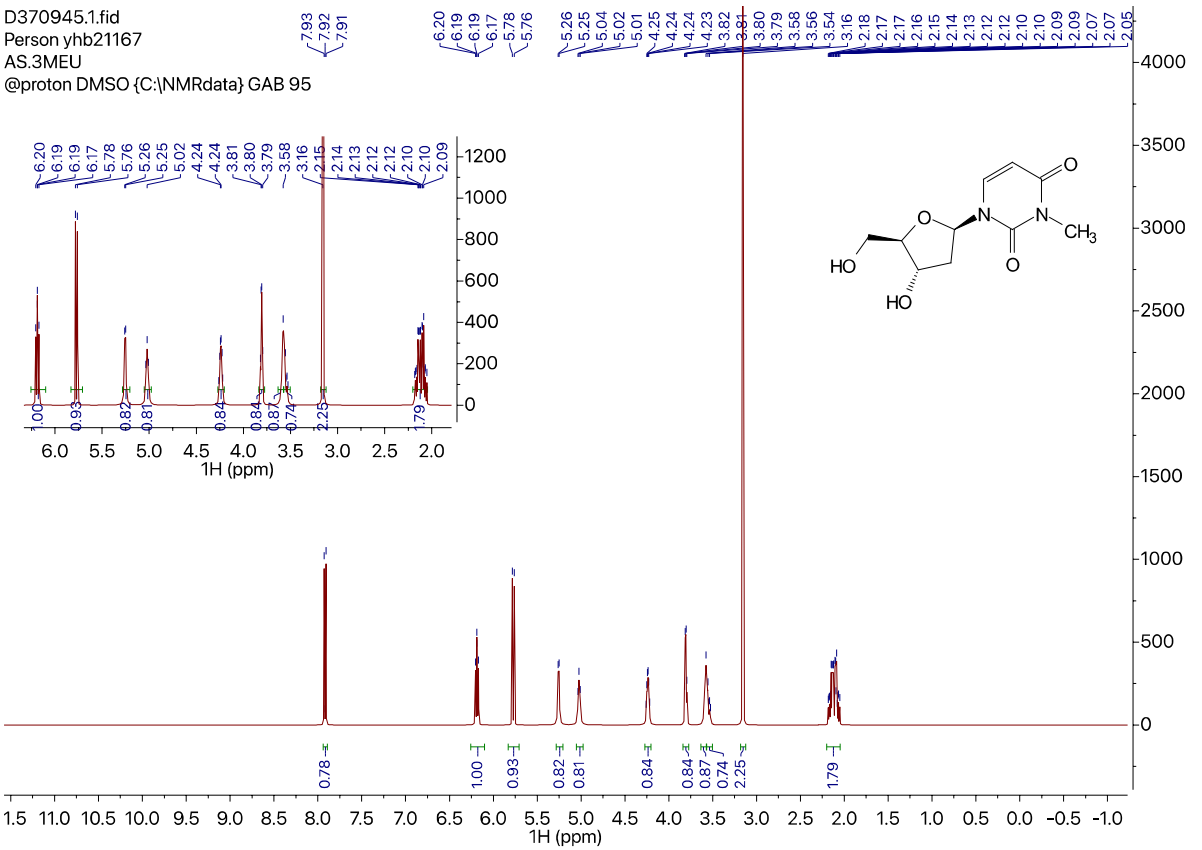


Figure S52 - *^{1}H NMR spectra of nucleoside 24*

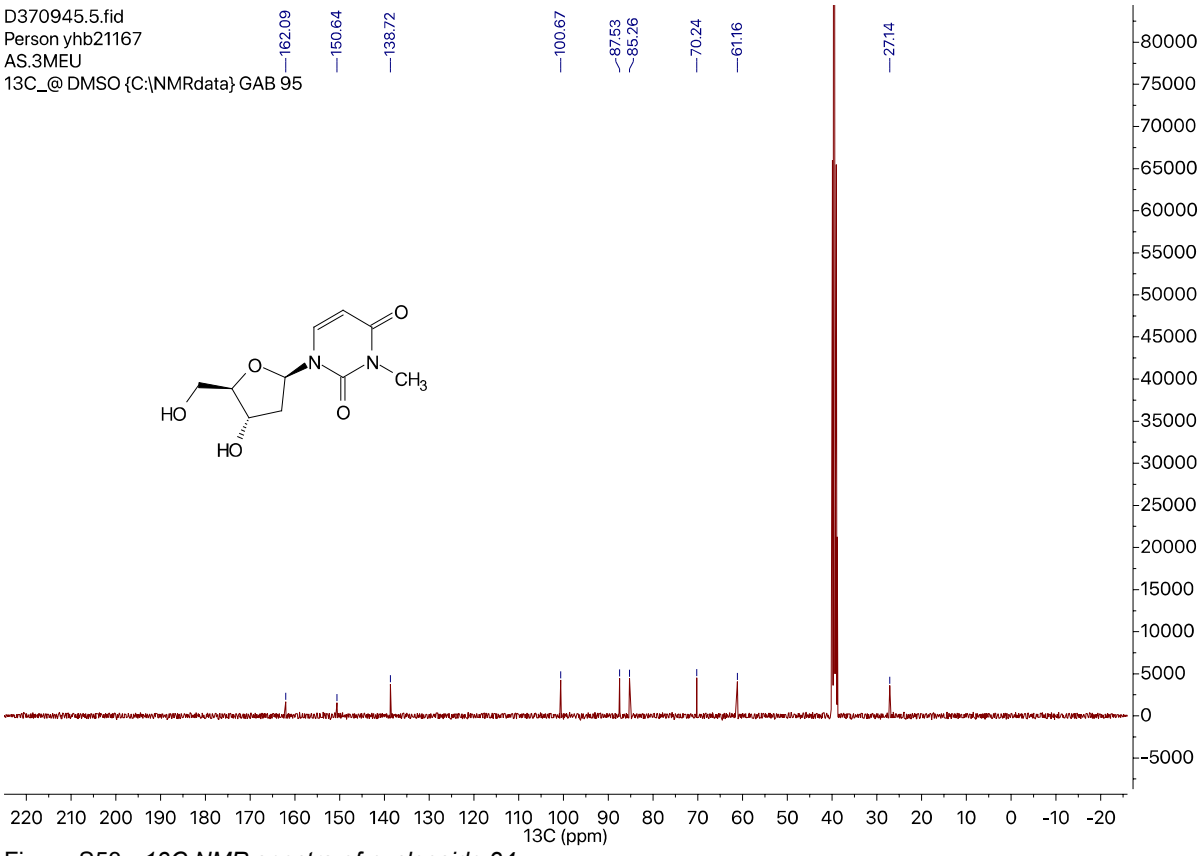
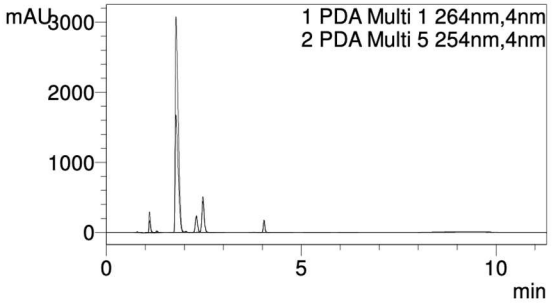


Figure S53 - *13C NMR spectra of nucleoside 24*

Sample Name : 1.4c 3meu
Sample ID :
Data Filename : 1.;4c_12102022_001.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : 1.;4c.lcb
Vial # : 1-4
Injection Volume : 10 uL
Date Acquired : 12/10/2022 11:41:51
Date Processed : 12/10/2022 11:53:12

Sample Type : Unknown

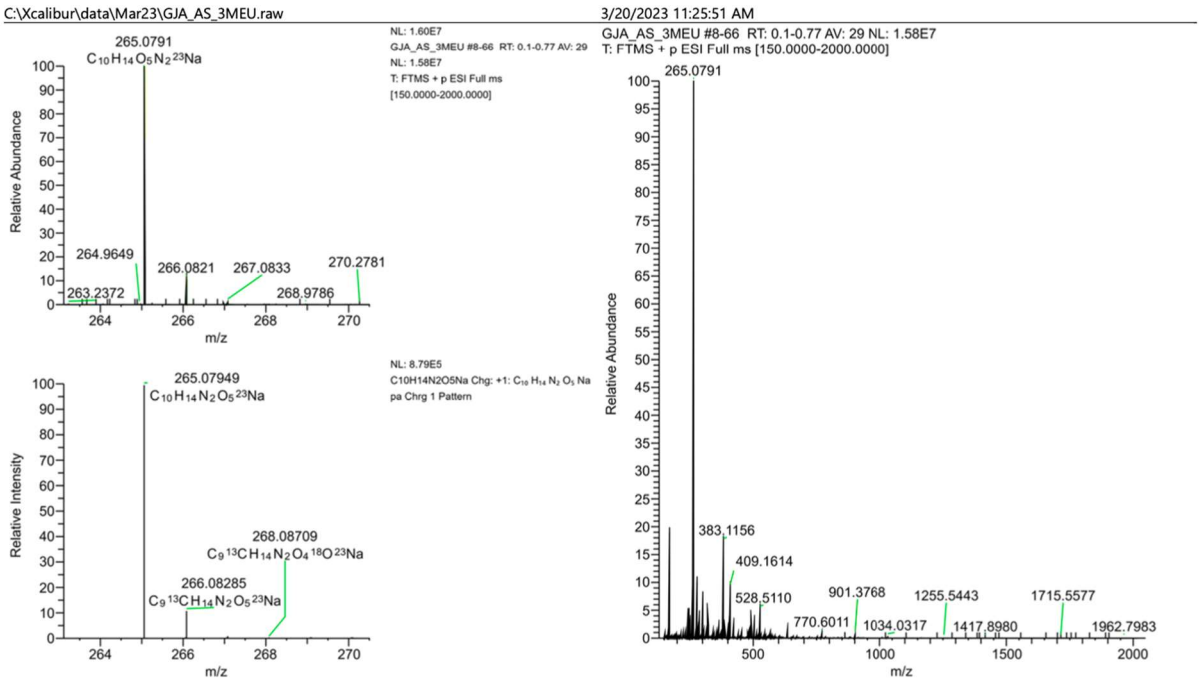
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.795	23069	11990	
2	1.109	765781	291807	
3	1.296	63683	21959	
4	1.789	16583029	3069034	
5	2.040	41034	13461	
6	2.311	916751	232495	
7	2.479	1862804	444172	
8	3.781	18588	5604	
9	4.048	513801	173697	
10	4.448	1459	145	
11	4.665	2842	231	
12	5.234	3687	465	
13	5.375	3029	282	
14	5.698	1476	412	
15	5.987	1722	496	

Figure S54 - *HPLC spectra of nucleoside 24. R.T = cytosine released = 1.1min, dC = 1.78 min, nucleobase starting material r.t = 2.48 min, product nucleoside 24 r.t = 4.05 min*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
265.0791	$C_{10}H_{14}O_5N_2{}^2{}^3Na$	28.4819213813393	4.50	-1.53	265.07949	1	93.35	4	1	96.96	99.23	(Collection)
265.0791	$C_7H_{13}O_7N_4$	16.8275882958357	3.50	4.57	265.07788	3	82.81	4	1	86.48	90.2	(Collection)
265.0791	$C_8H_9O_3N_8$	21.6078234376168	8.50	-0.47	265.07921	2	92.05	3	1	95.96	96.68	(Collection)

Figure S55 - *HRMS of nucleoside 24*

2'-Deoxy-5-fluorocytidine (25)

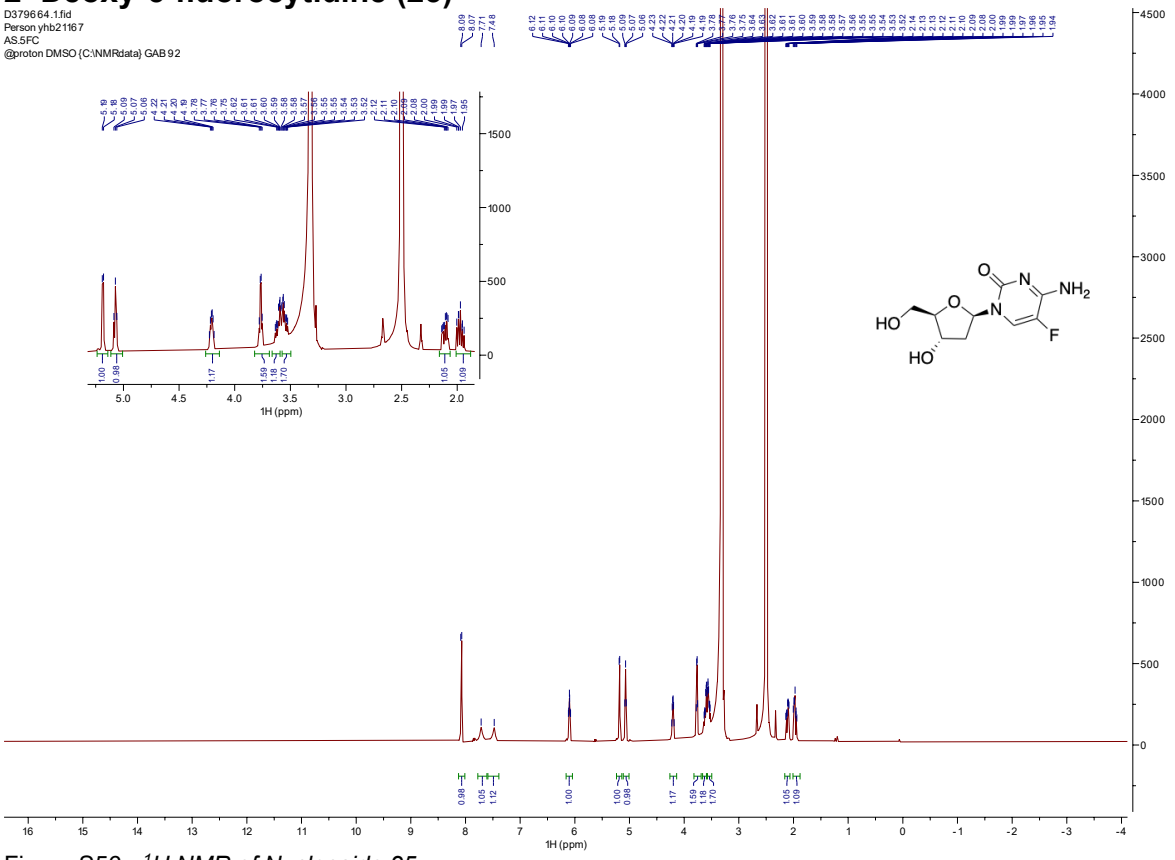


Figure S56 - *[1]H NMR of Nucleoside 25*

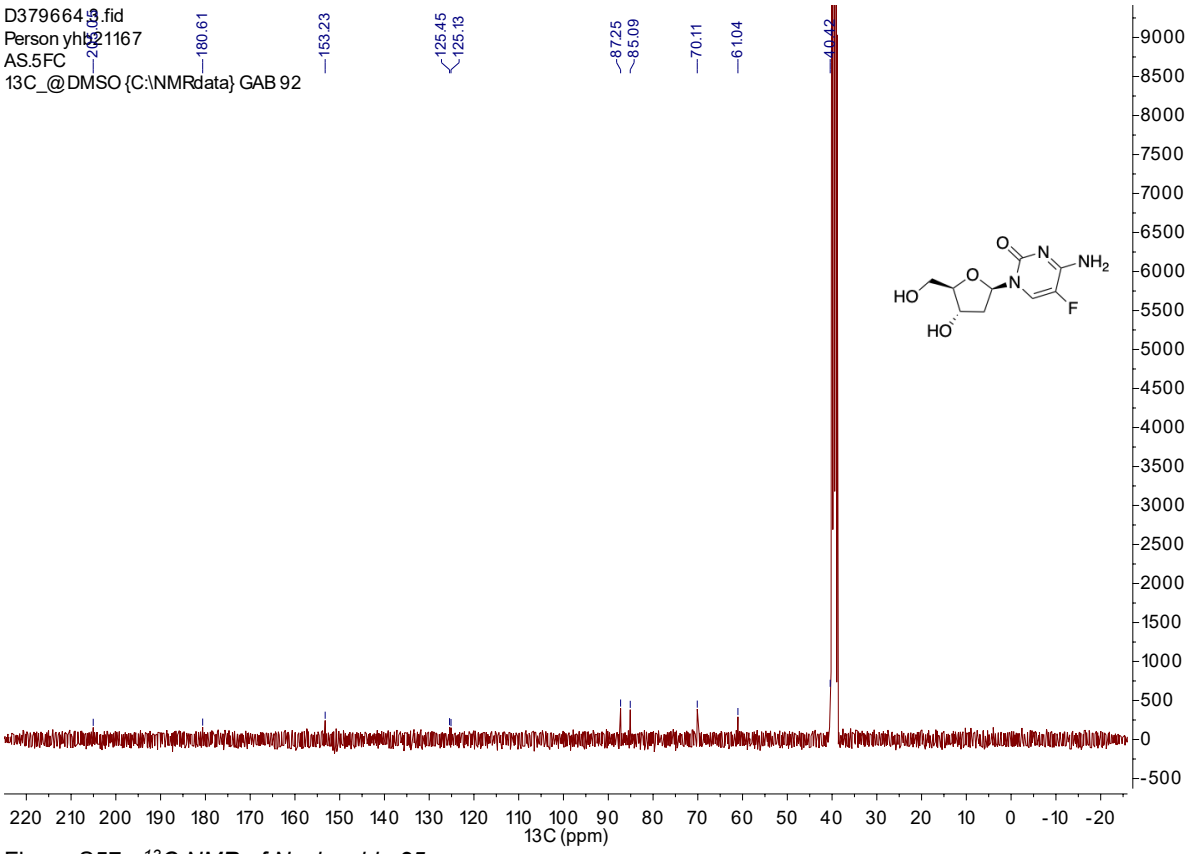


Figure S57 - *[13]C NMR of Nucleoside 25*

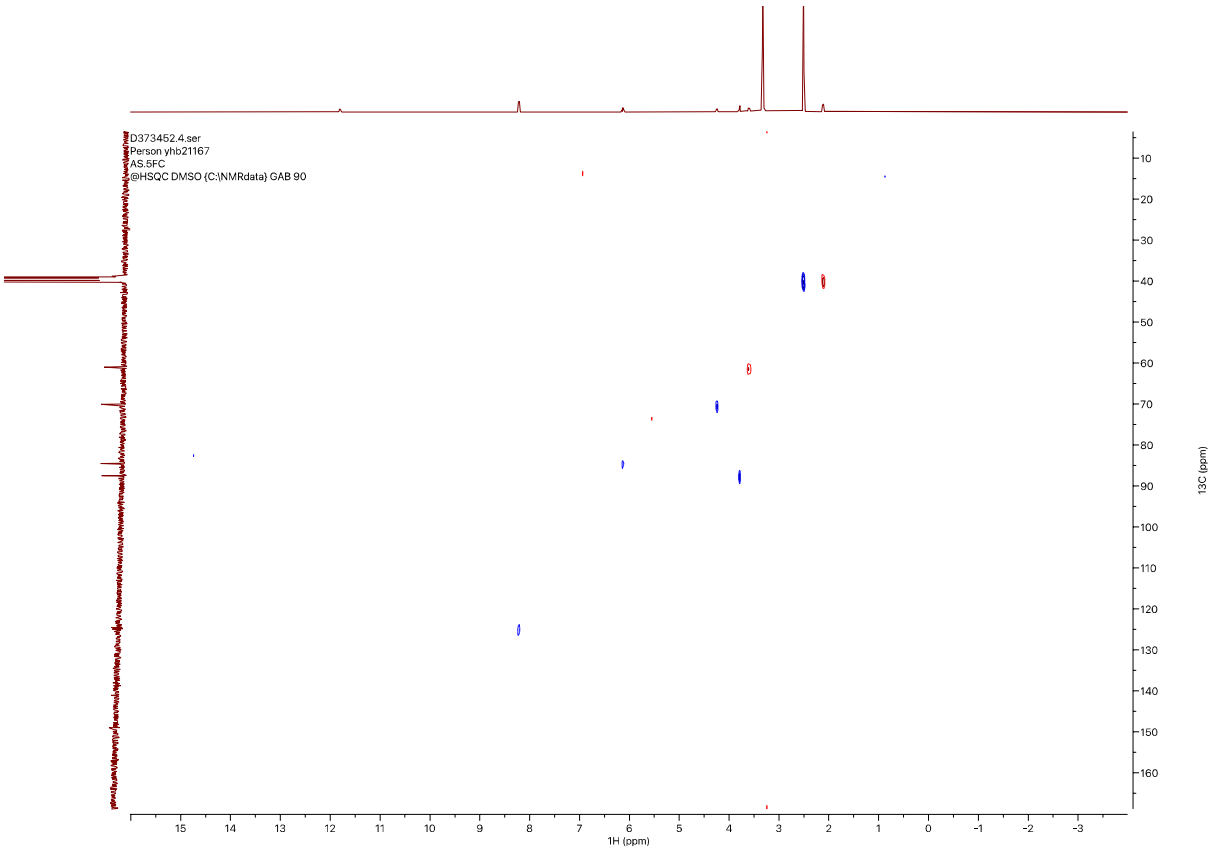


Figure S58 - *HSQC NMR of Nucleoside 25*

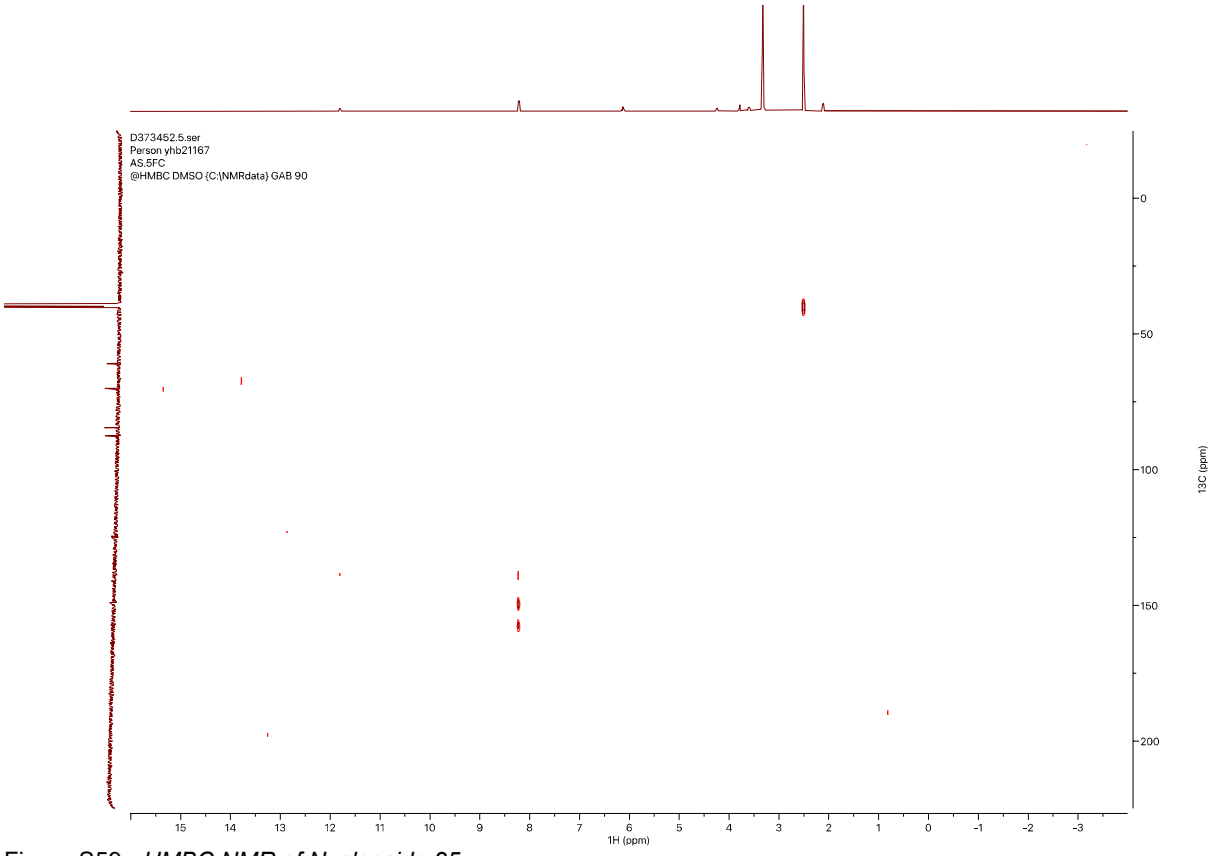


Figure S59 - *HMBC NMR of Nucleoside 25*

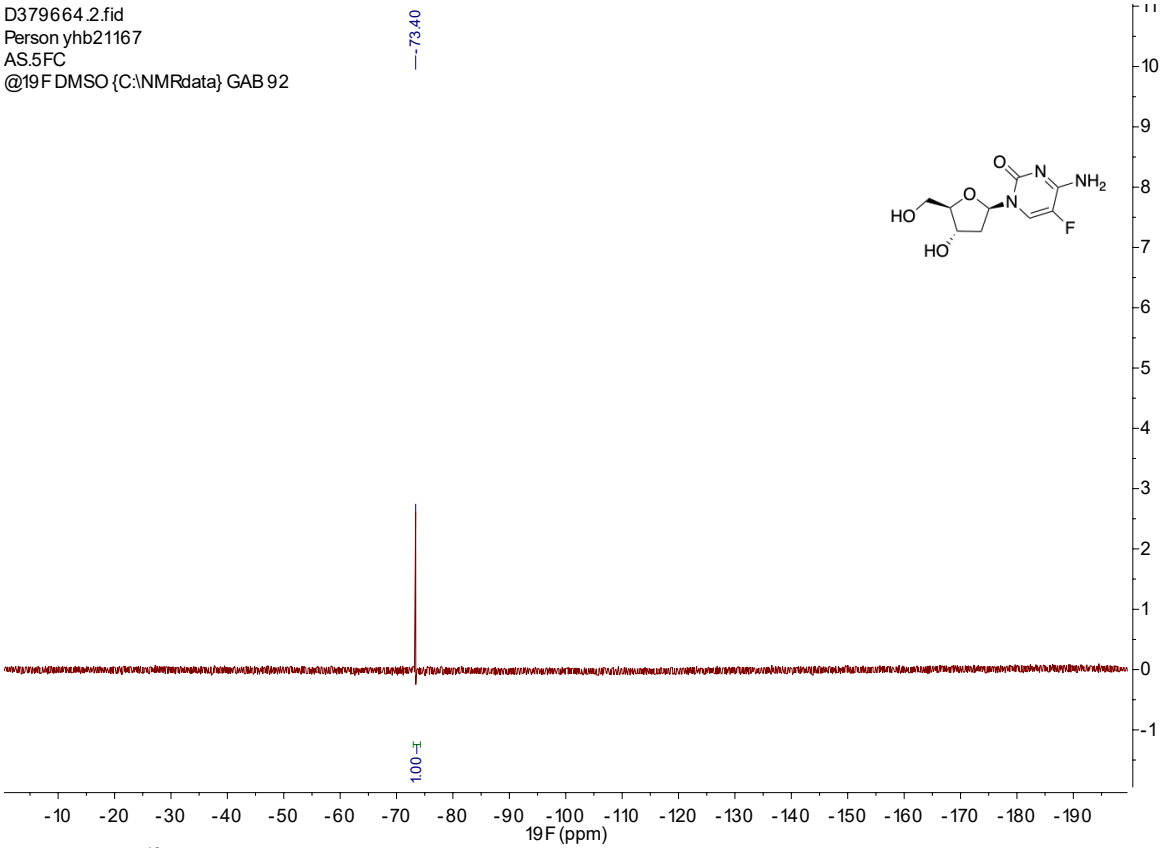
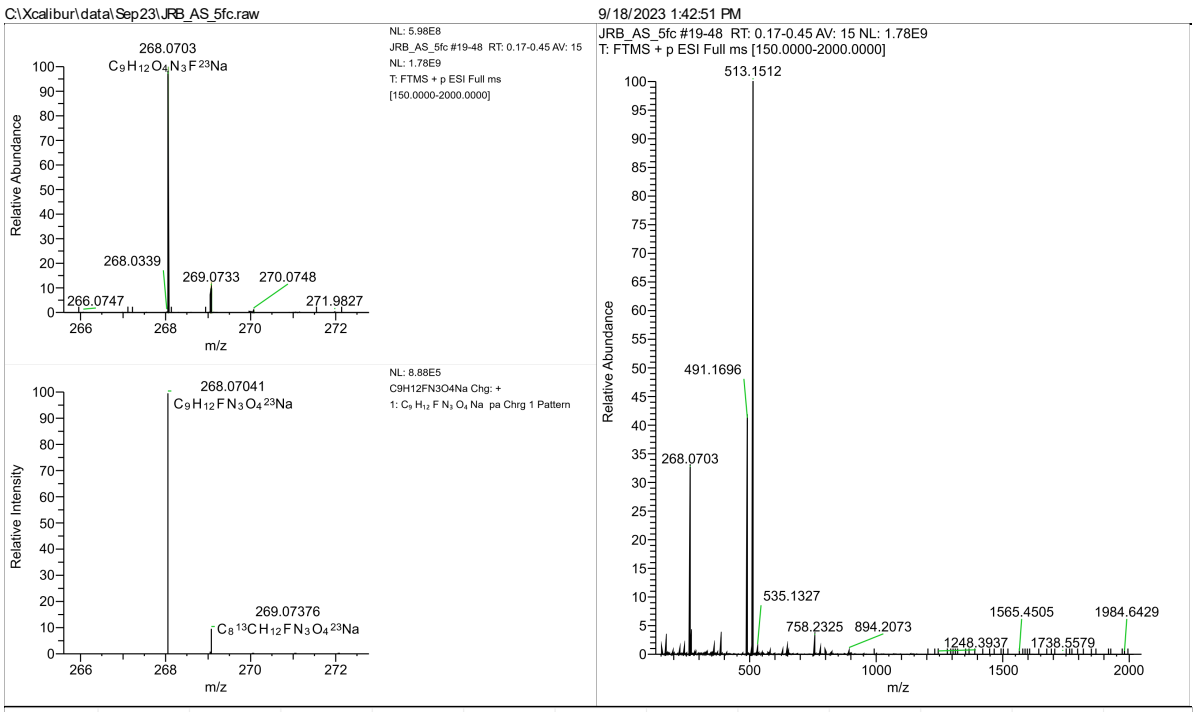


Figure S60 - *^{19}F NMR of Nucleoside 25*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
268.0703	$C_9H_{12}O_4N_3F^{23}Na$	44.95617385 75759	4.50	-0.33	268.07041	1	96	3	1	98.83	98.99	(Collection)
268.0703	$C_{12}H_{11}O_3N_3^{23}Na$	31.25472405 46759	8.50	3.94	268.06926	2	95.28	3	1	98.83	99.01	(Collection)
268.0703	$C_{10}H_6ON_9$	13.34367085 00413	12.50	4.98	268.06898	3	93.56	3	2	98.01	96.22	(Collection)
268.0703	$C_7H_7O_2N_9F$	13.24836931 83193	8.50	0.72	268.07013	4	84.97	3	1	88.96	89.64	(Collection)

Figure S61 - *HRMS of Nucleoside 25*

2'-Deoxy-2-thiocytidine (26)

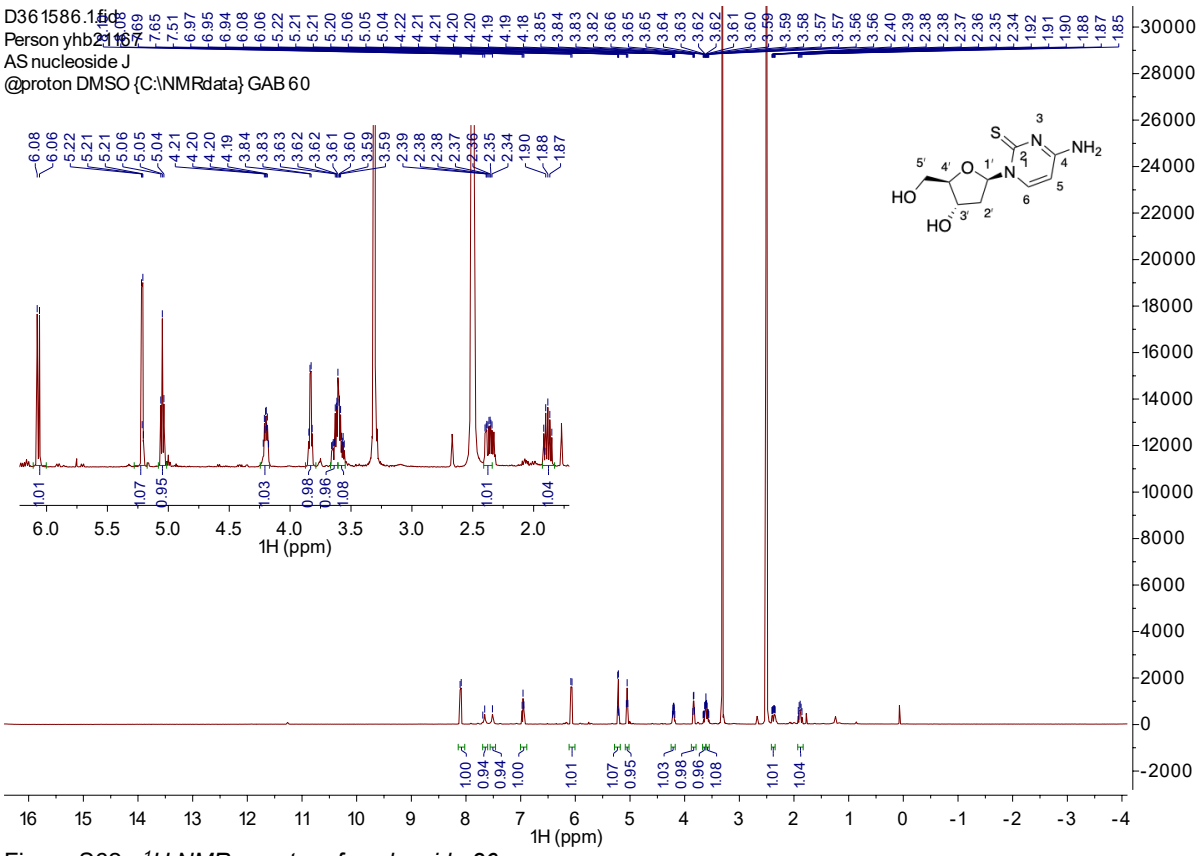


Figure S62 - *^{1}H NMR spectra of nucleoside 26*

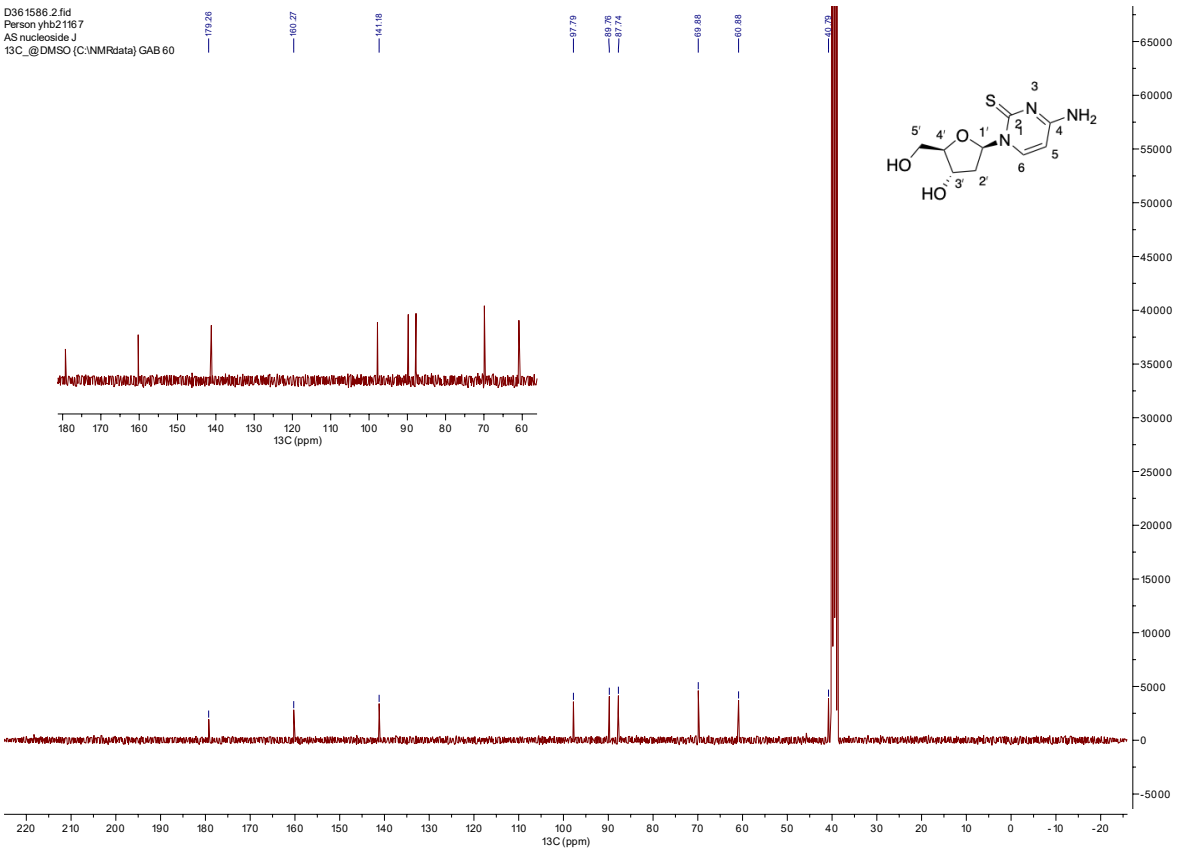
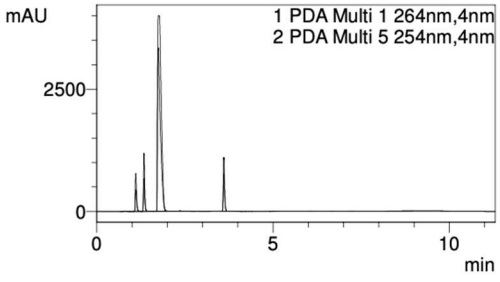


Figure S63 - *13C{1H}-NMR spectra of nucleoside 26*

Sample Name : J
Sample ID :
Data Filename : RXNS_15062022_018.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : RXNS.lcb
Vial # : 1-78
Injection Volume : 10 uL
Date Acquired : 15/06/2022 22:19:55
Date Processed : 15/06/2022 22:31:16

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



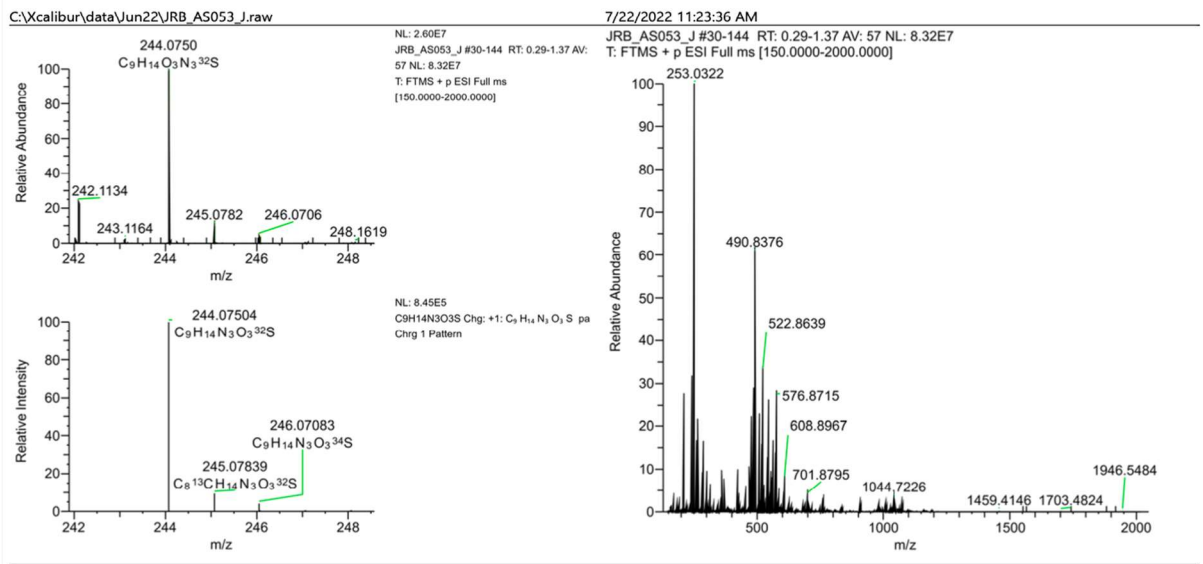
PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.784	5282	2134	
2	1.109	1916986	769980	
3	1.343	2751862	1169326	
4	1.761	29410270	3999974	
5	2.367	37554	11510	
6	2.574	6928	1891	
7	3.606	2890797	1097609	
8	4.992	19077	4146	
9	6.033	1909	589	
10	6.648	1735	816	
11	7.218	1126	440	
12	8.260	3970	1638	
13	8.333	1727	650	
14	8.472	1814	610	
15	8.600	5725	783	
16	8.700	2965	982	
17	8.752	3110	699	
18	8.899	1673	477	
19	9.813	10815	1551	
Total		37075326	7065804	

PDA Ch5 254nm

Peak#	Ret. Time	Area	Height	ID#
1	1.109	1098514	439959	
2	1.343	1552086	657837	
3	1.748	18657295	3332580	
4	2.366	35491	10838	
5	3.606	2010206	762680	
6	4.992	25757	5984	
Total		23379349	5209878	

Figure S64 - *HPLC trace of the reaction used to obtain nucleoside 26. R.T = 1.11 = nucleobase cytosine, 1.34 = nucleobase J, 1.76 = nucleoside dC, 3.61 = nucleoside 26*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
244.0750	$C_9H_{14}O_3N_3{}^{32}S$	40.8994723005338	4.50	-0.28	244.07504	1	92.97	5	1	95.86	98.08	(Collection)
244.0750	$C_{11}H_{11}N_5P$	20.0633362098386	9.50	1.28	244.07466	2	87.9	3	2	91.67	97.63	(Collection)
244.0750	$C_3H_{15}O_2N_7P^3{}^2S$	11.0880202231583	0.50	3.95	244.07401	3	83.21	4	3	87.21	93.79	(Collection)
244.0750	$C_3H_{17}N_7P_3$	17.6965468840072	0.50	-1.27	244.07528	4	79.9	1	2	83.35	94.46	(Collection)
244.0750	$CH_{10}O_6N_9$	13.3338231646019	1.50	0.47	244.07486	5	79.67	2	2	83.35	94.46	(Collection)
244.0750	$C_{17}H_{10}ON$	10.9182442379281	13.50	-2.95	244.07569	6	79.54	3	1	83.35	83.02	(Collection)
244.0750	$C_2H_{14}ON_9{}^{32}S_2$	5.19220499549998	0.50	-3.09	244.07572	7	79.24	4	4	83.35	86.01	(Collection)
244.0750	$C_4H_{15}ON_7{}^{35}Cl^{32}S$	5.07914737475869	0.50	3.22	244.07418	8	79.23	4	4	83.35	68.11	(Collection)

Figure S65 - *HRMS of nucleoside 26*

2'-Deoxy-4-hydroxyaminocytidine (27)

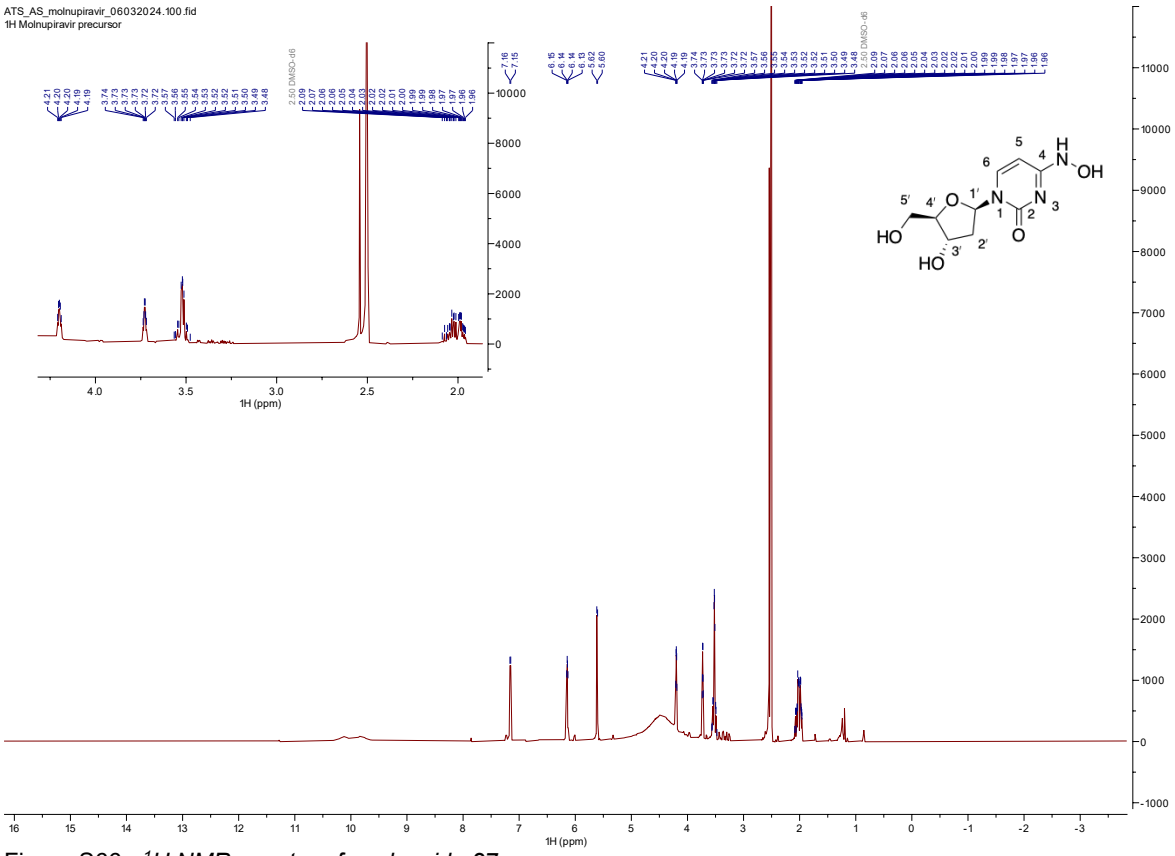


Figure S66 - *^{1}H NMR spectra of nucleoside 27*

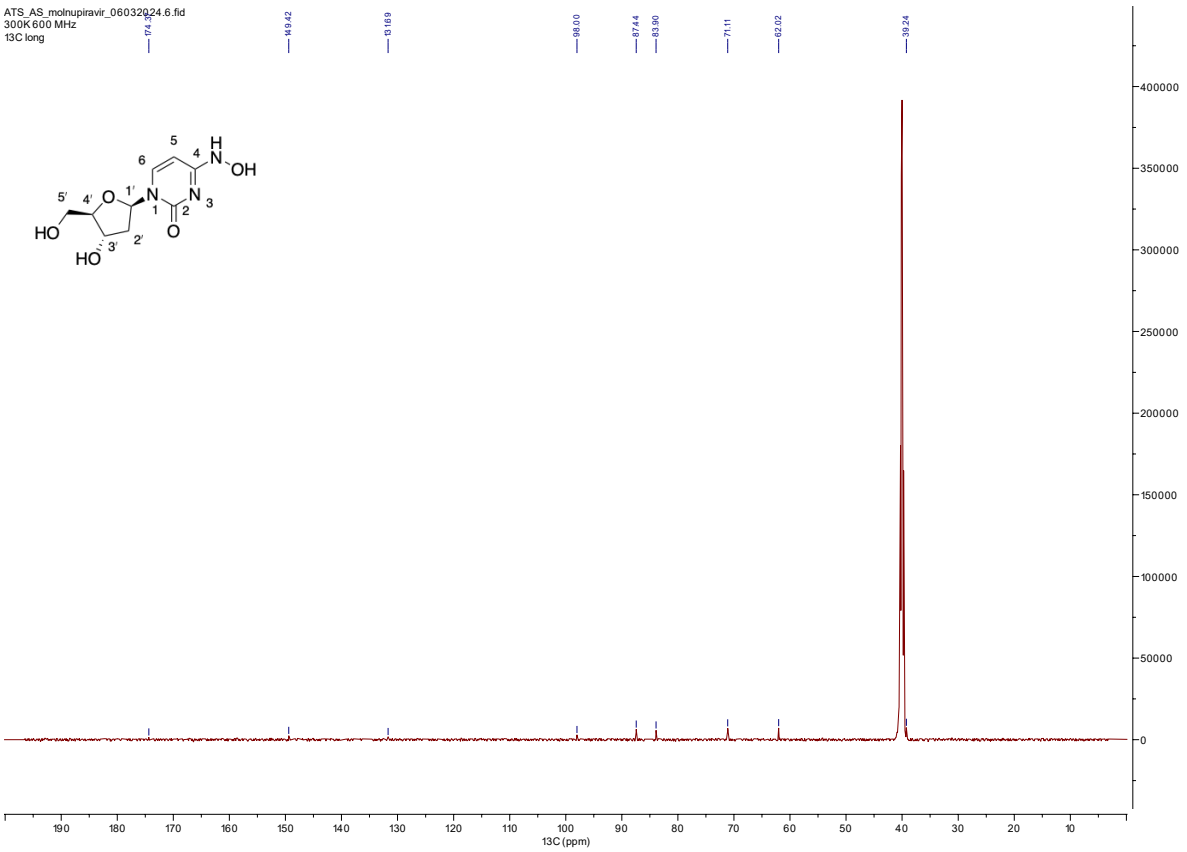


Figure S67 - *13C{1H}-NMR spectra of nucleoside 27*

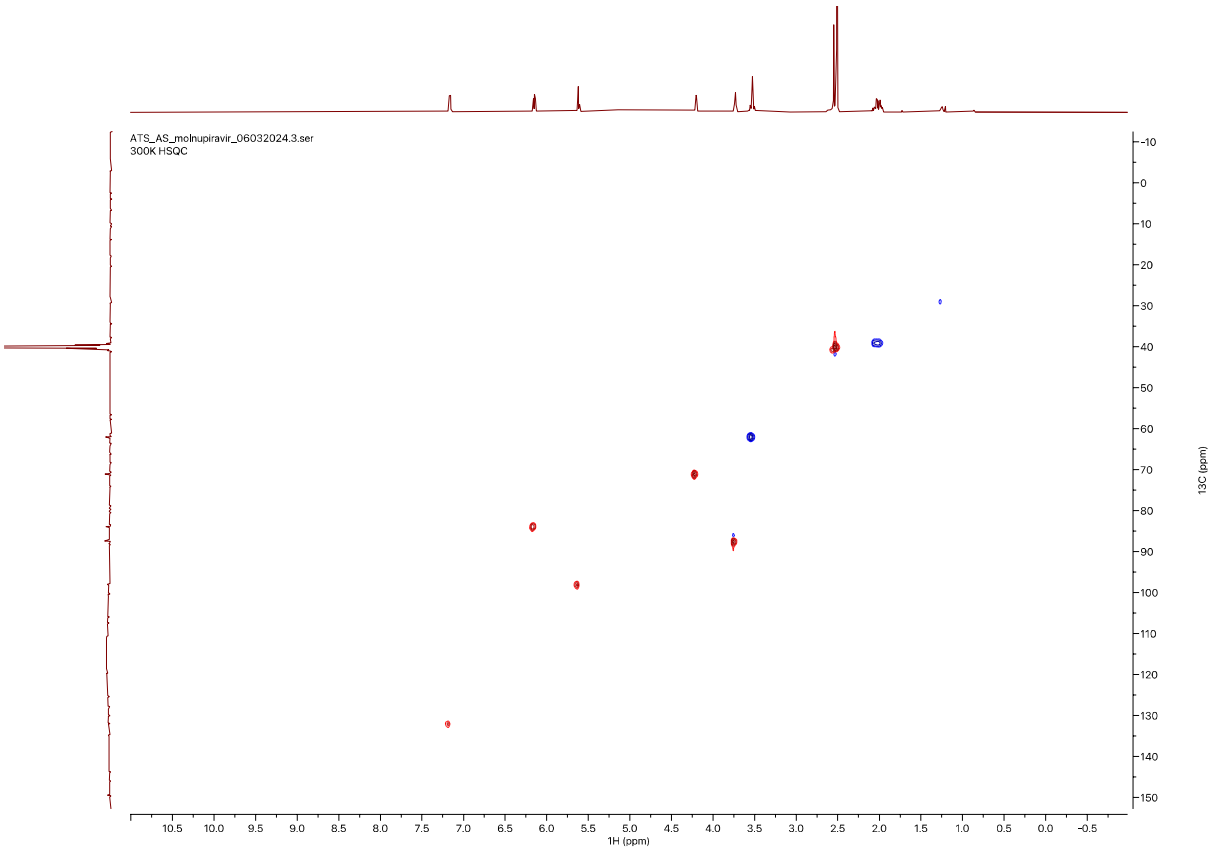


Figure S68 – HSQC *NMR spectra of nucleoside 27*

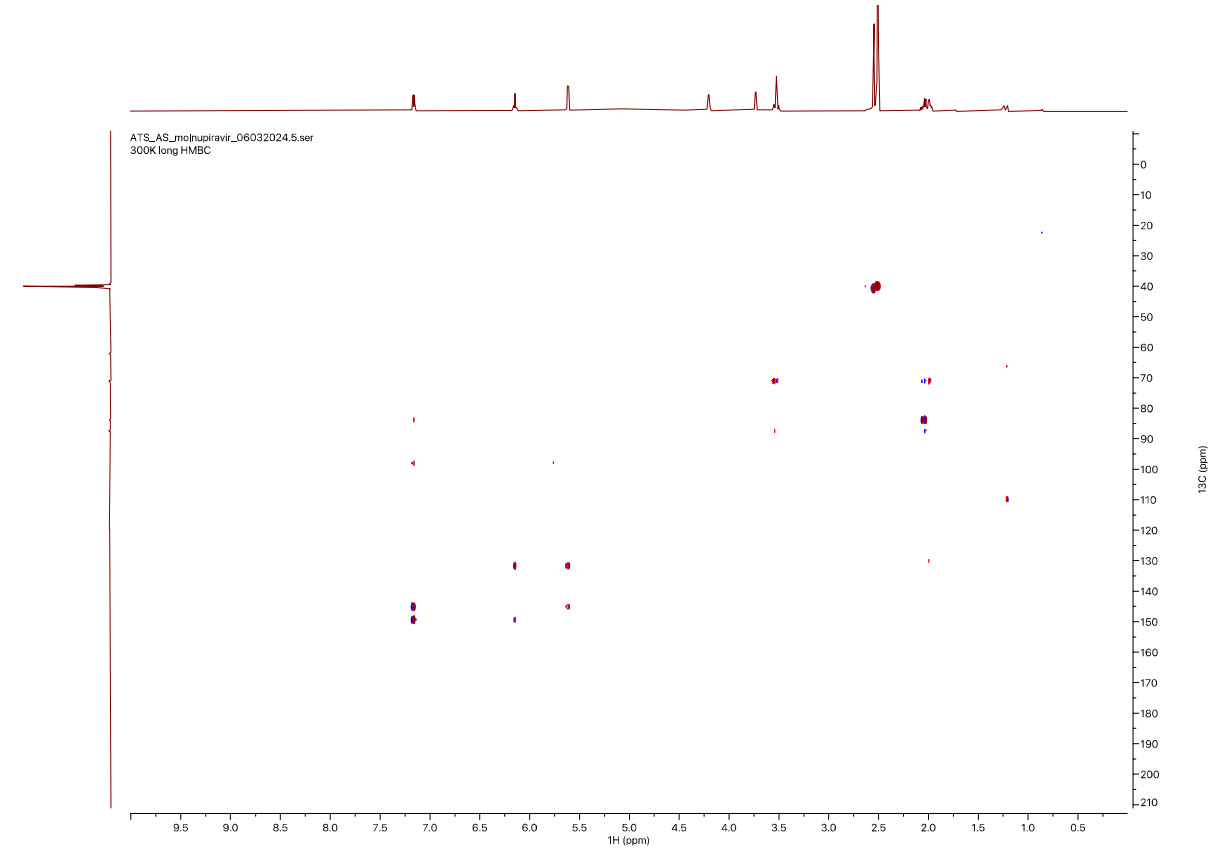
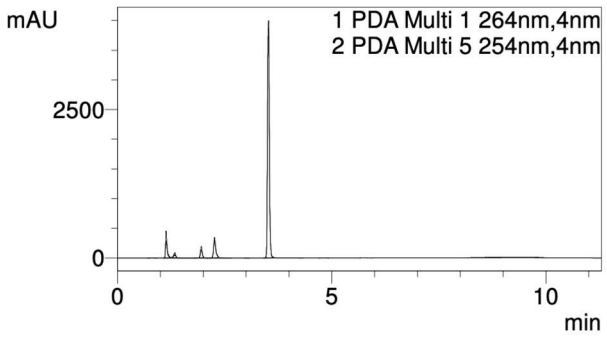


Figure S69 – HMBC *NMR spectra of nucleoside 27*

Sample Name : 1.4
Sample ID :
Data Filename : 1.4_08092022_003.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : 1.4.lcb
Vial # : 1-21
Injection Volume : 10 uL
Date Acquired : 08/09/2022 17:14:05
Date Processed : 08/09/2022 17:25:26

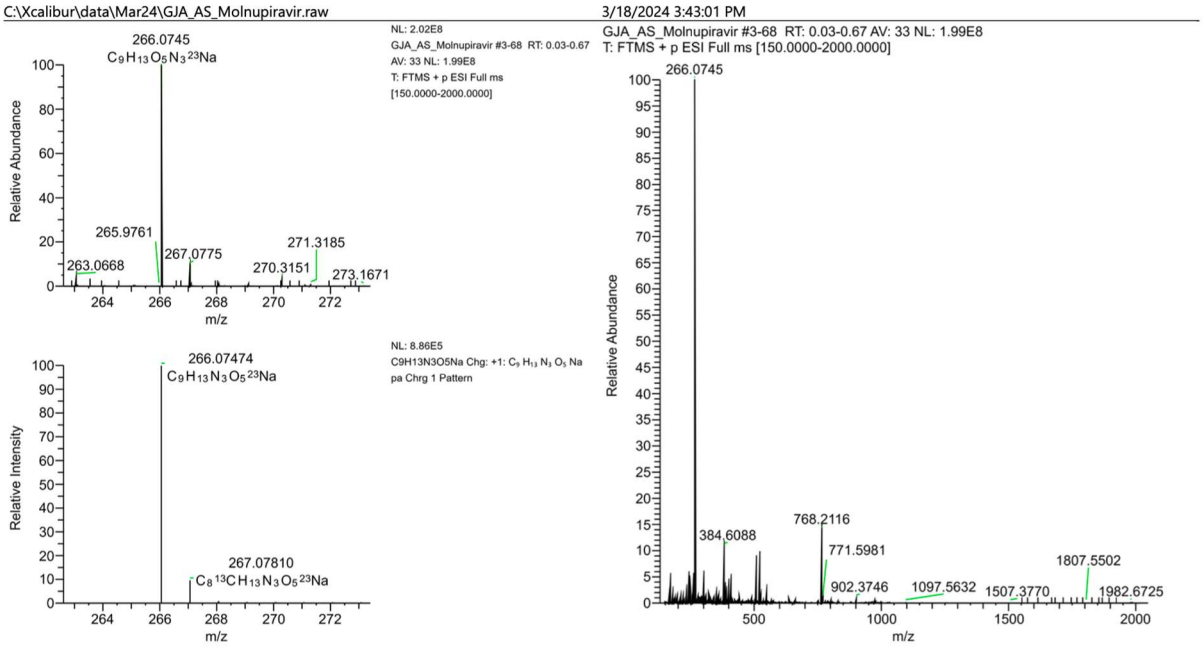
Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.789	5648	2616	
2	1.137	1229812	439020	
3	1.340	305768	86342	
4	1.957	576722	191071	
5	2.267	1230115	343993	
6	2.994	1055	280	
7	3.523	12256196	3833717	
8	4.805	2176	410	
9	8.257	4008	785	
10	8.336	1835	651	
11	8.480	2593	541	
12	8.599	4379	1057	
13	8.694	4183	1430	
14	8.752	4239	1069	
15	8.898	2083	586	
16	9.807	11844	2214	
Total		15642656	4905780	

Figure S70 - *HPLC trace of the reaction used to obtain nucleoside 27. R.T = 1.13min = nucleobase, 1.95. = nucleoside 27, 2.26 = thymine, 3.52 = dT.*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
266.0745	$C_9H_{13}O_5N_3^{23}Na$	40.3827117059813	4.50	-0.98	266.07474	1	95.14	4	1	98.19	99	(Collection)
266.0745	$C_8H_{14}O_8N_2$	21.5149249763985	3.00	0.06	266.07447	2	93.15	4	1	97.13	97.6	(Collection)
266.0745	$C_8H_7N_{10}^{23}Na$	19.9857759164232	10.00	-0.96	266.07474	3	93.07	2	3	97.13	96.15	(Collection)
266.0745	$C_9H_{10}O_4N_6$	6.54028595480959	8.00	-4.97	266.07580	4	84.12	3	1	88.43	88.37	(Collection)

Figure S71- *HRMS of nucleoside 27*

2'-Deoxy-2-fluoro-6-chloropurine (32)

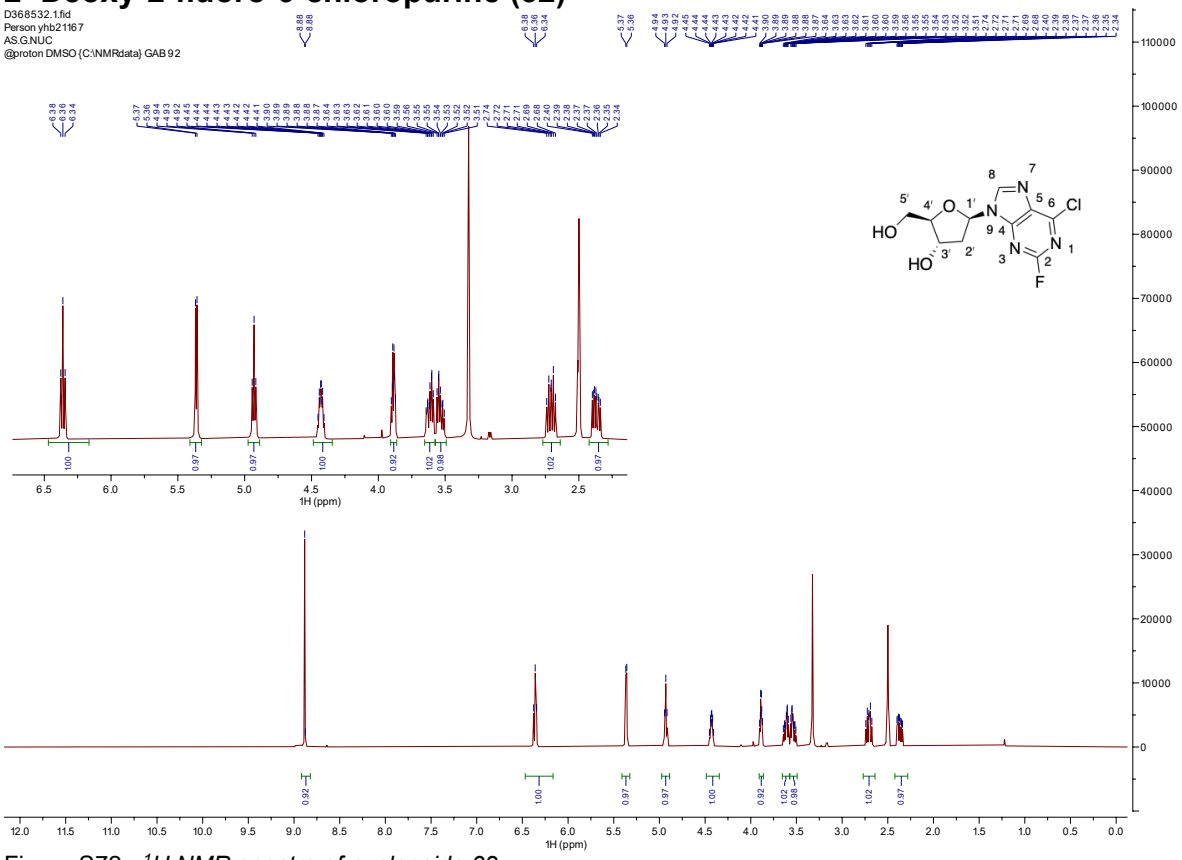


Figure S72 - *^{1}H NMR spectra of nucleoside 32.*

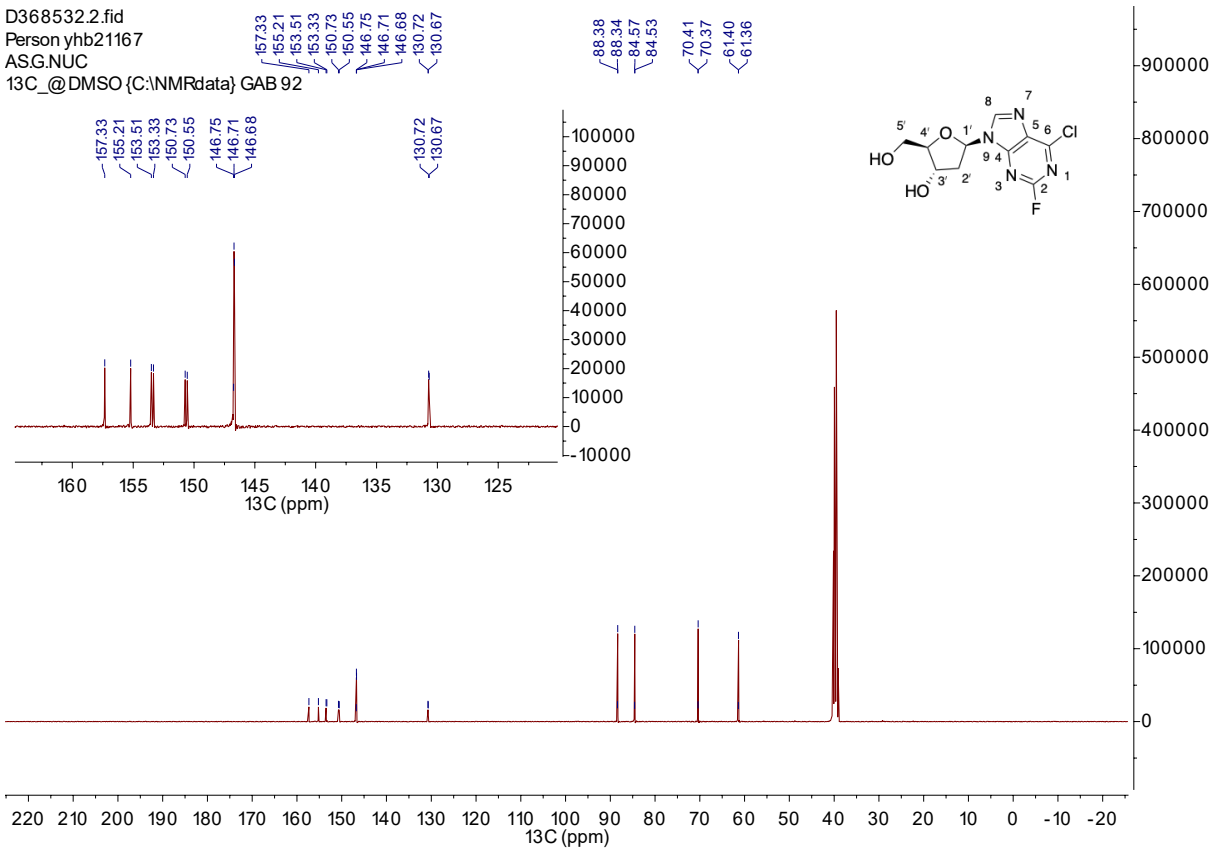


Figure S73 - *13C{1H}-NMR spectra of nucleoside 32.*

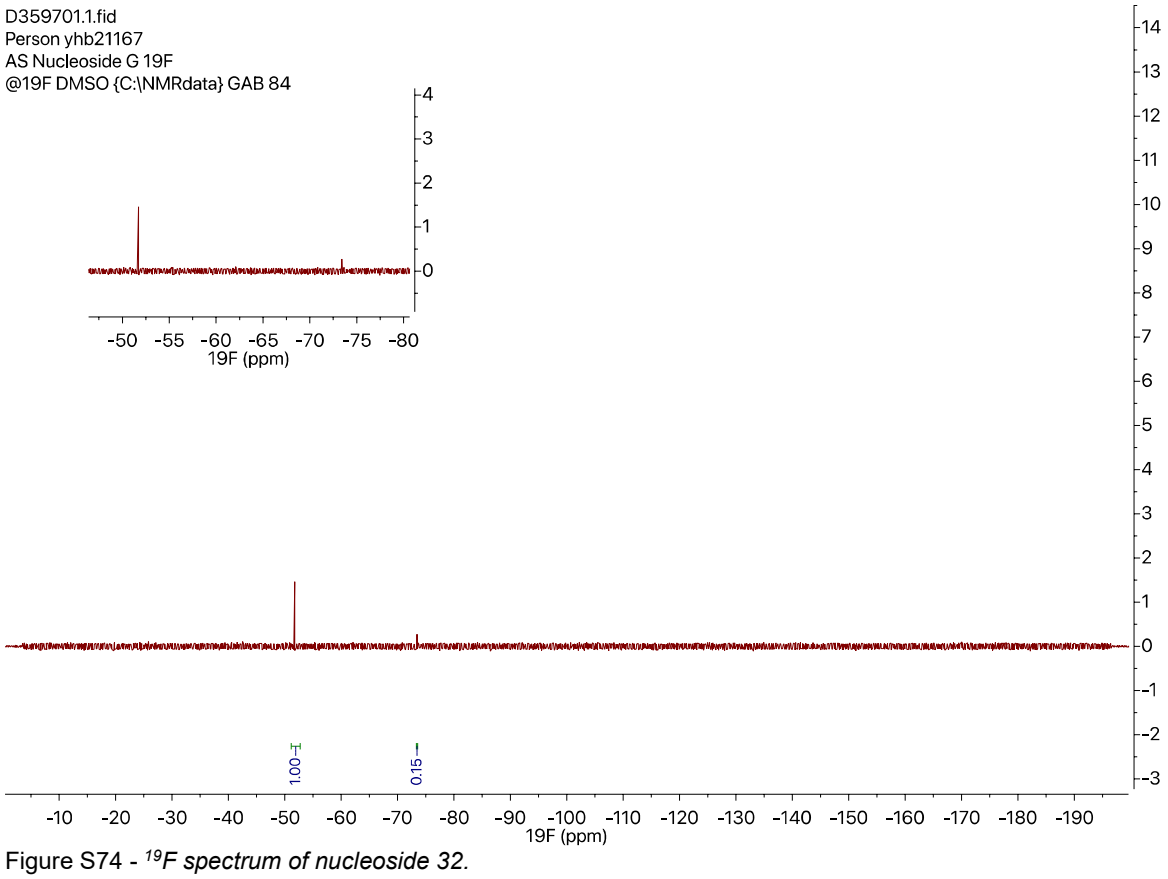
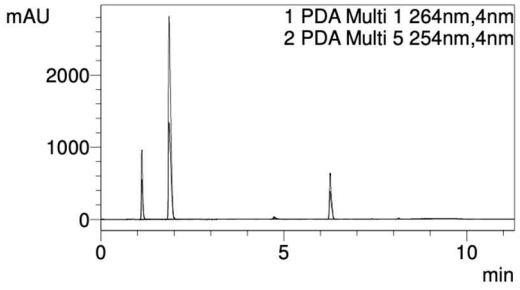


Figure S74 - *^{19}F spectrum of nucleoside 32.*

Injection Volume : 10 uL
Date Acquired : 22/04/2022 16:02:36 Acquired by : Shimadzu
Date Processed : 22/04/2022 16:13:57 Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.784	5531	2451	
2	1.120	2410264	932236	
3	1.316	1143	638	
4	1.865	12055751	2756412	
5	2.429	5875	1833	
6	3.486	1740	764	
7	3.616	1507	520	
8	4.249	1243	439	
9	4.736	243300	32785	
10	5.389	1148	264	
11	6.267	2408413	633560	
12	6.676	10845	3573	
13	6.946	1450	491	
14	7.202	1041	293	

Figure S75 - *HPLC trace of the reaction used to obtain nucleoside 32. R.T = 1.1 = nucleobase cytosine released. 1.87 = Nucleoside dC. 4.74= Nucleobase left over. 6.27 = nucleoside 32.*

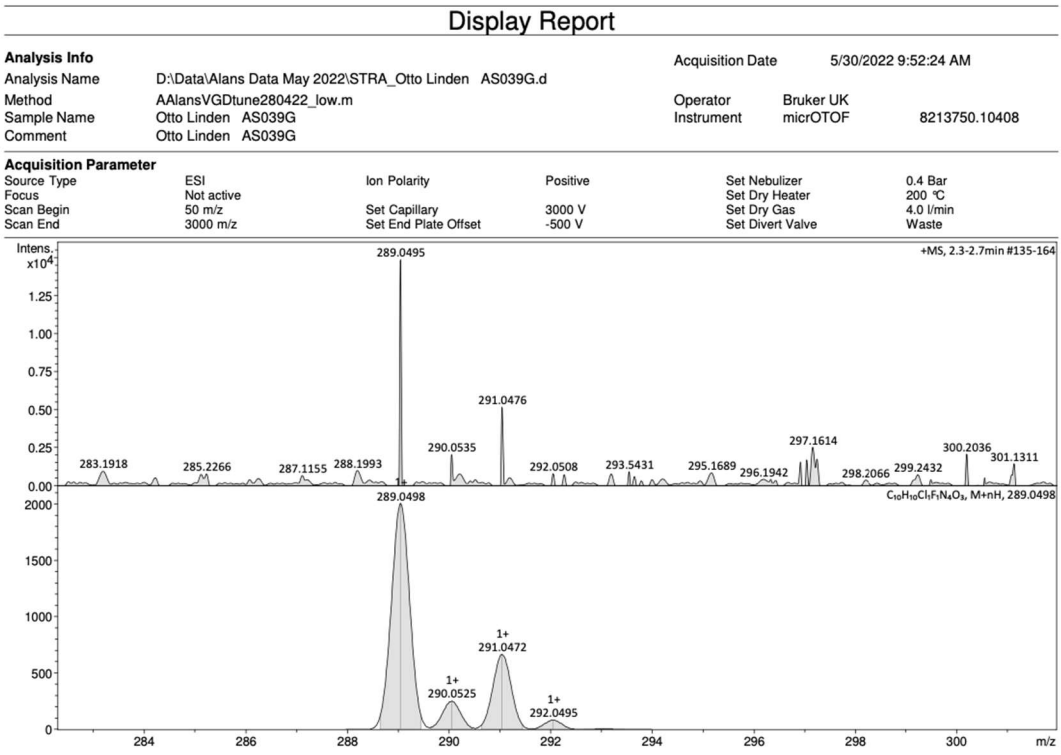


Figure S76 - *HRMS of nucleoside 32.*

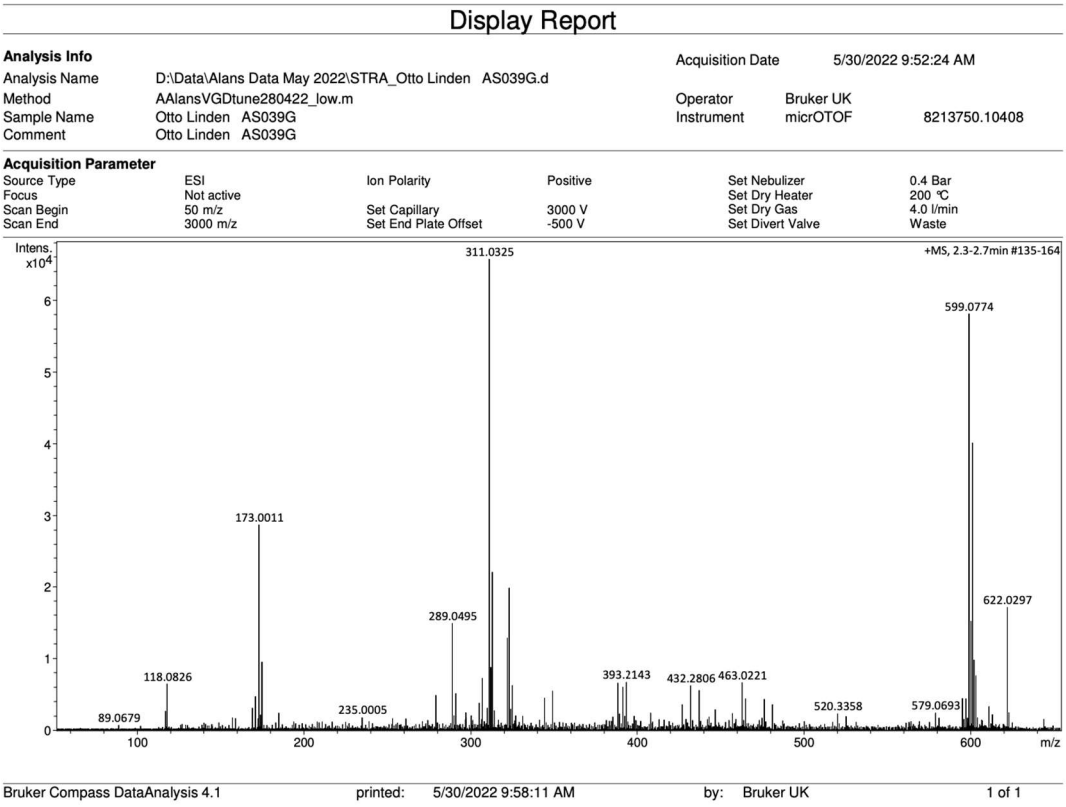


Figure S77 - *HRMS of nucleoside 32.*

2'-Deoxy-6-chloroguanosine (33)

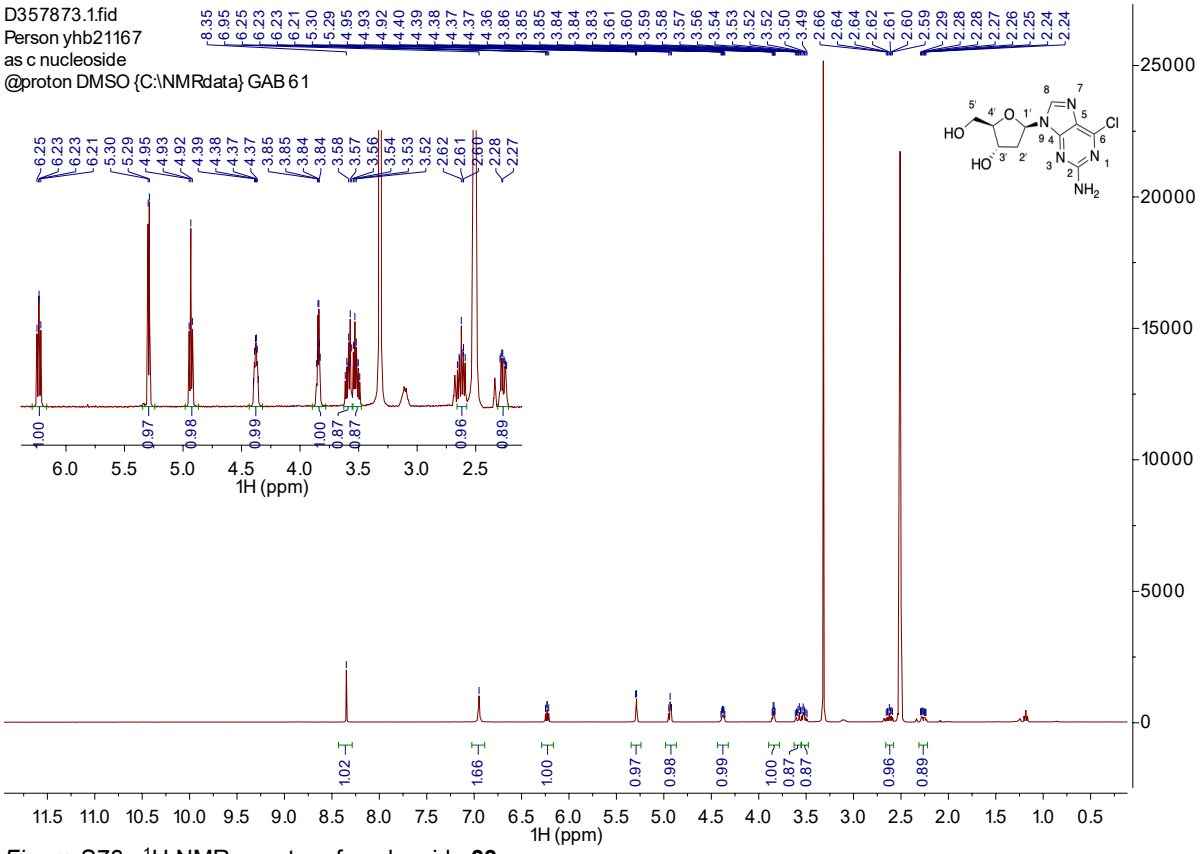


Figure S78 - ^{1}H NMR spectra of nucleoside **33**

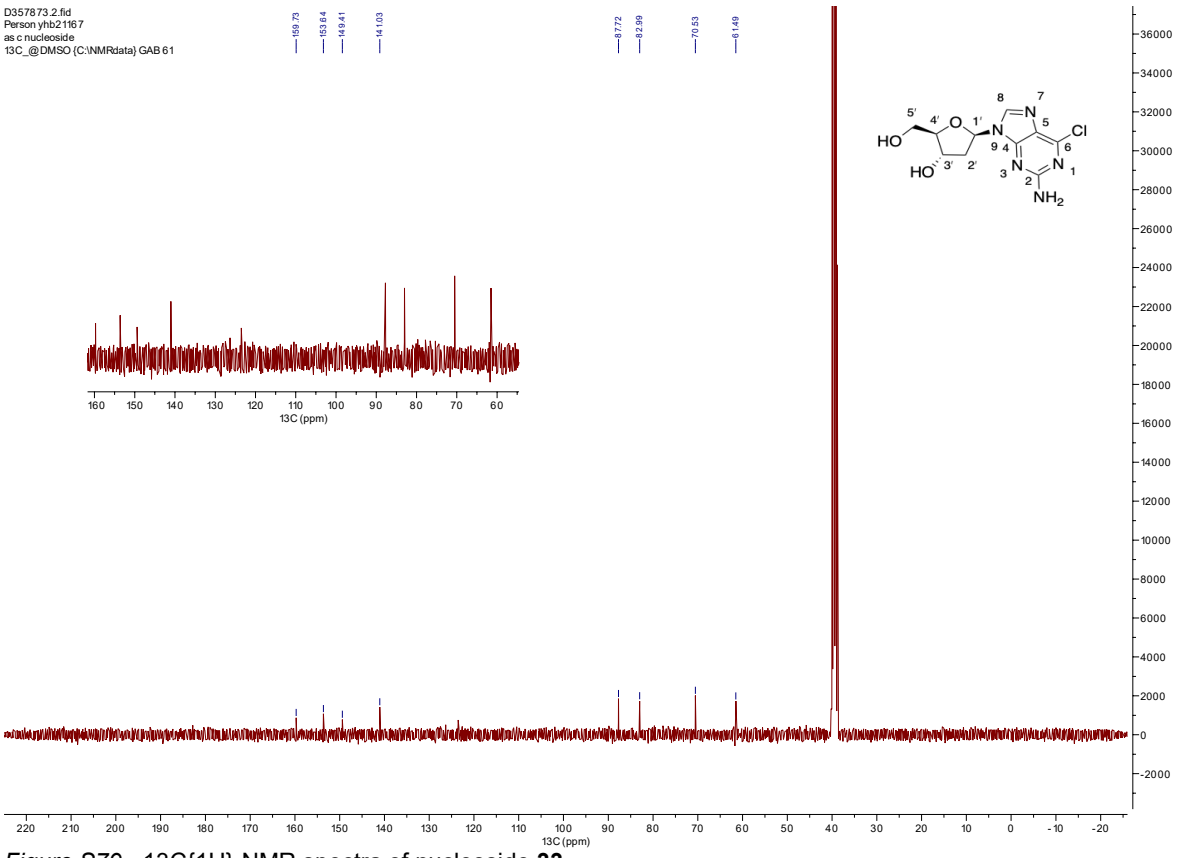
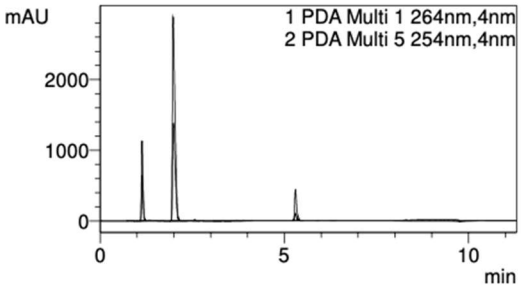


Figure S79 - 13C{1H}-NMR spectra of nucleoside **33**

Sample Name : C AS028
Sample ID :
Data Filename : REACTIONS_24022022_010.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : REACTIONS.lcb
Vial # : 1-18
Injection Volume : 10 uL
Date Acquired : 24/02/2022 16:49:48
Date Processed : 24/02/2022 17:01:09

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.798	4214	2127	
2	0.878	2271	571	
3	1.007	2844	693	
4	1.140	3250098	1121268	
5	1.348	34698	8632	
6	1.681	5918	704	
7	1.984	13869902	2842731	
8	2.459	4203	1282	
9	2.571	45613	12291	
10	3.096	1728	136	
11	3.329	2547	793	
12	3.587	6290	2071	
13	3.711	19967	7920	
14	3.894	10641	3480	
15	4.022	1284	449	
16	5.303	392110	102252	
17	5.890	1299	483	

Figure S80 - HPLC trace for the reaction used to obtain nucleoside **33.** R.T = 1.14 = nucleobase released cytosine, 1.98 = nucleoside dC, 5.30 = target nucleoside **33**

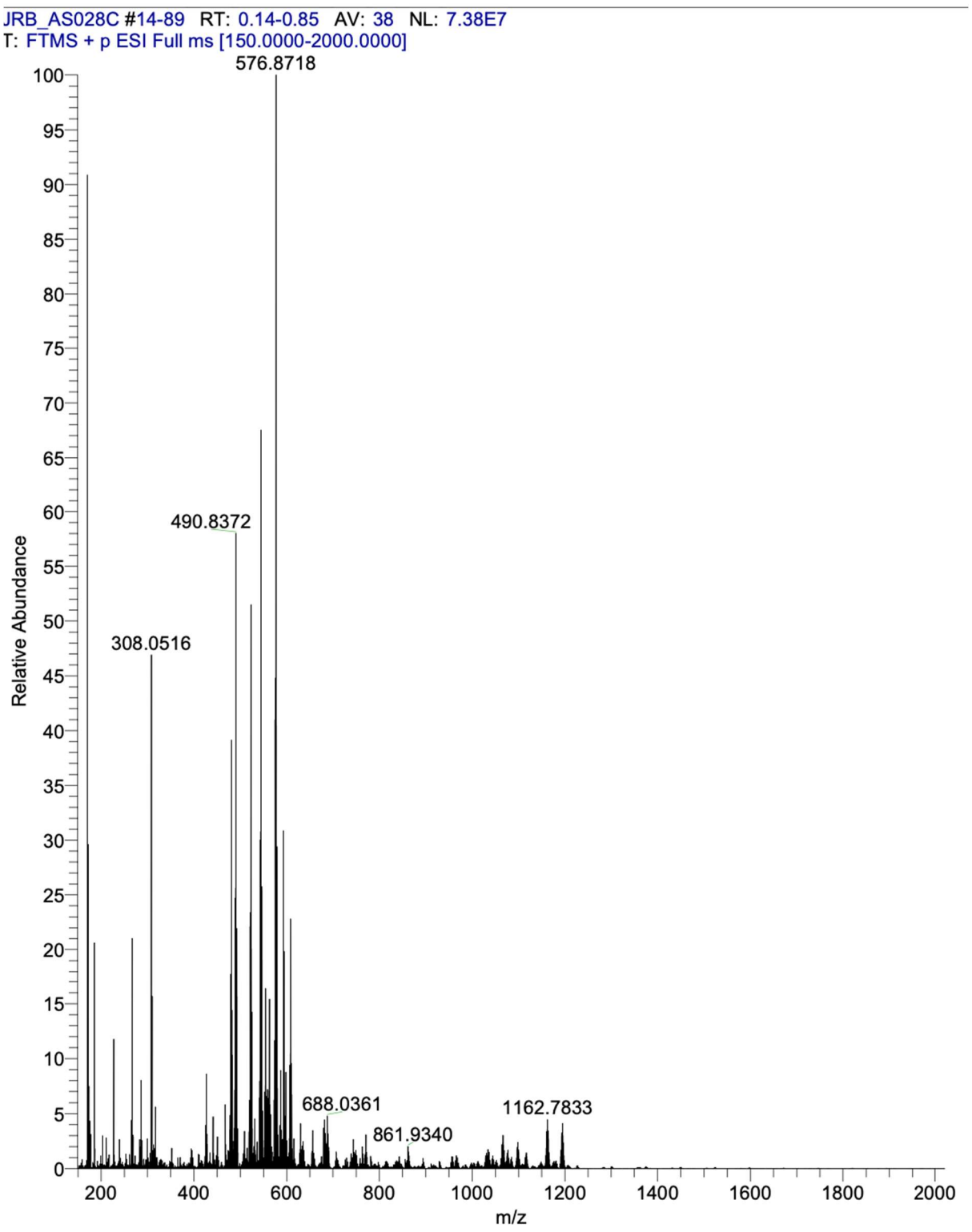


Figure S81 - HRMS of nucleoside **33**

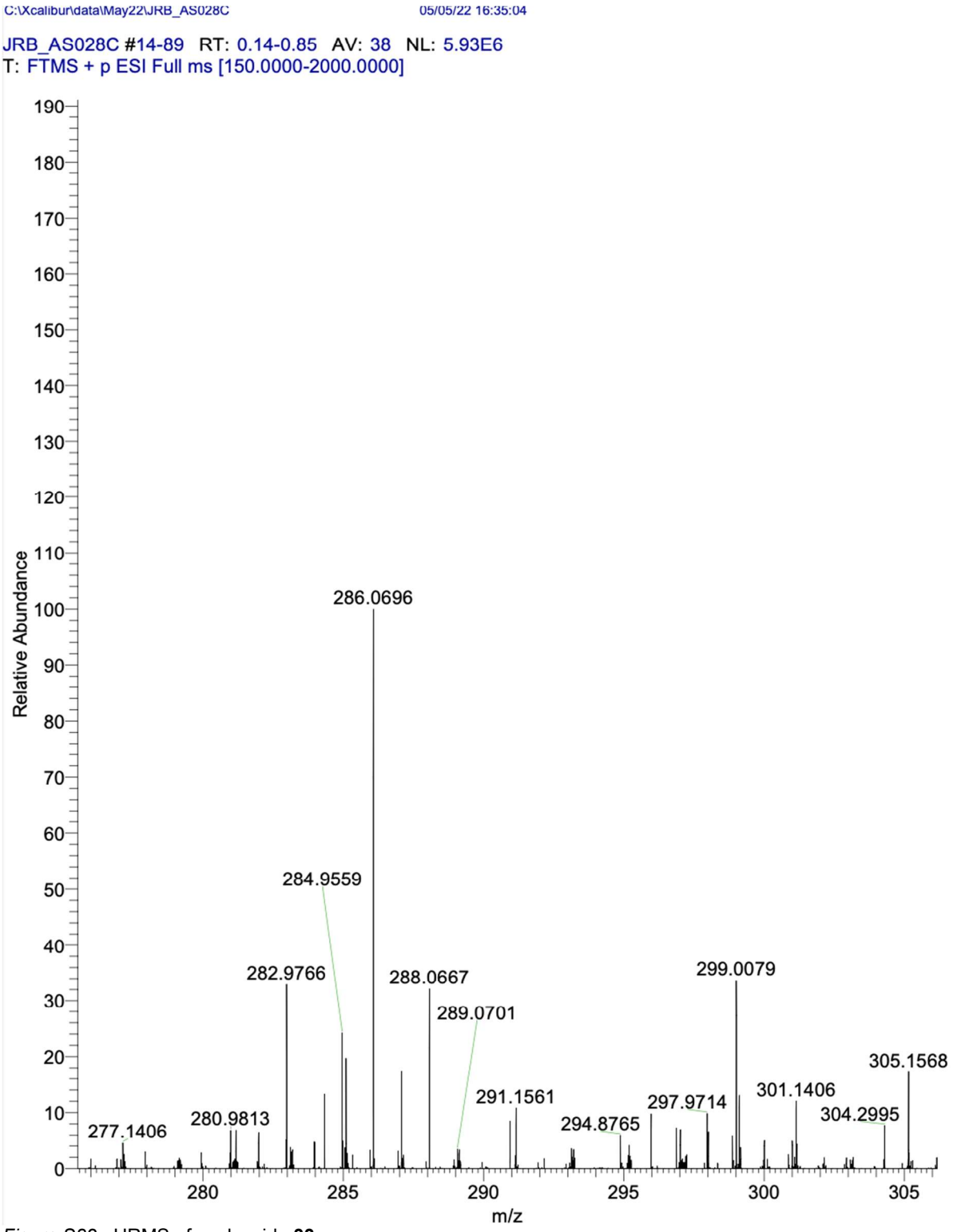


Figure S82 - HRMS of nucleoside **33**

2'-Deoxy-2-iodo-6-chloropurine (34)

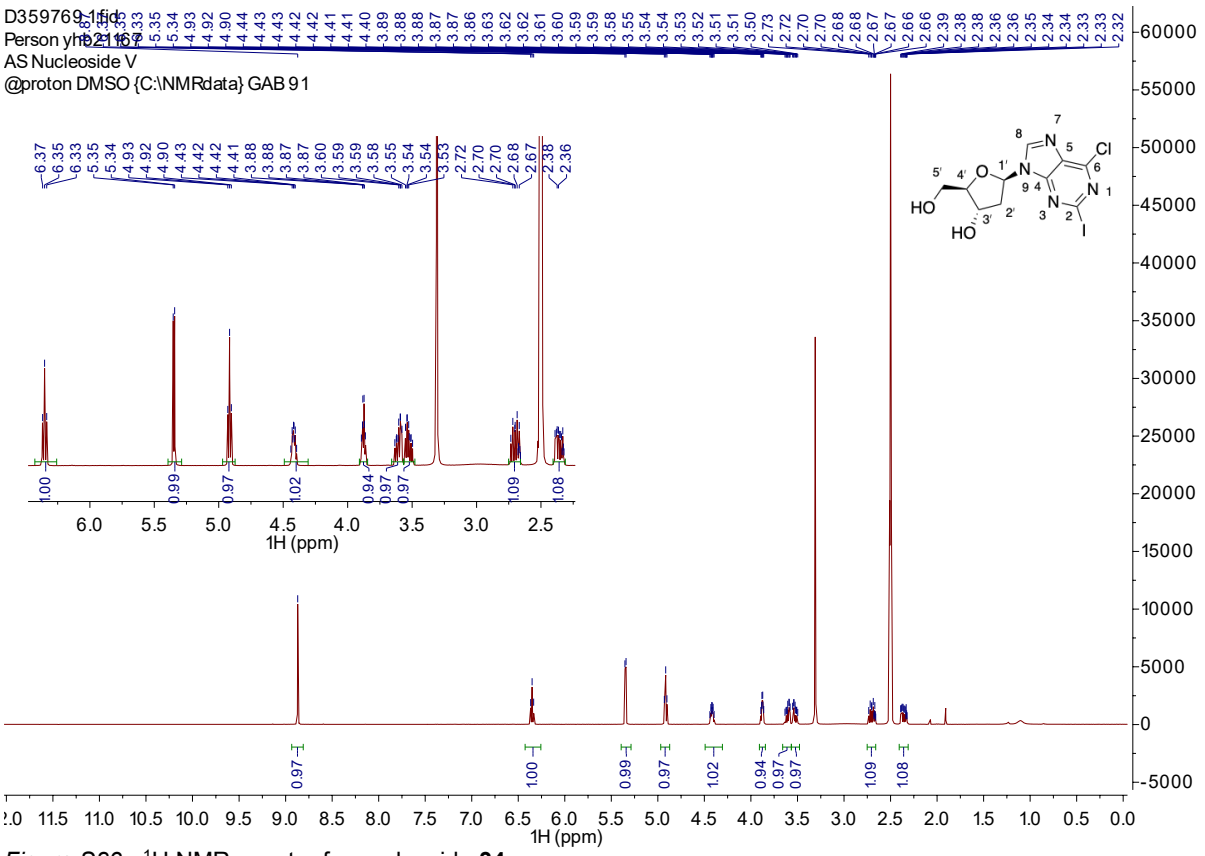


Figure S83 - ^{1}H NMR spectra for nucleoside **34.**

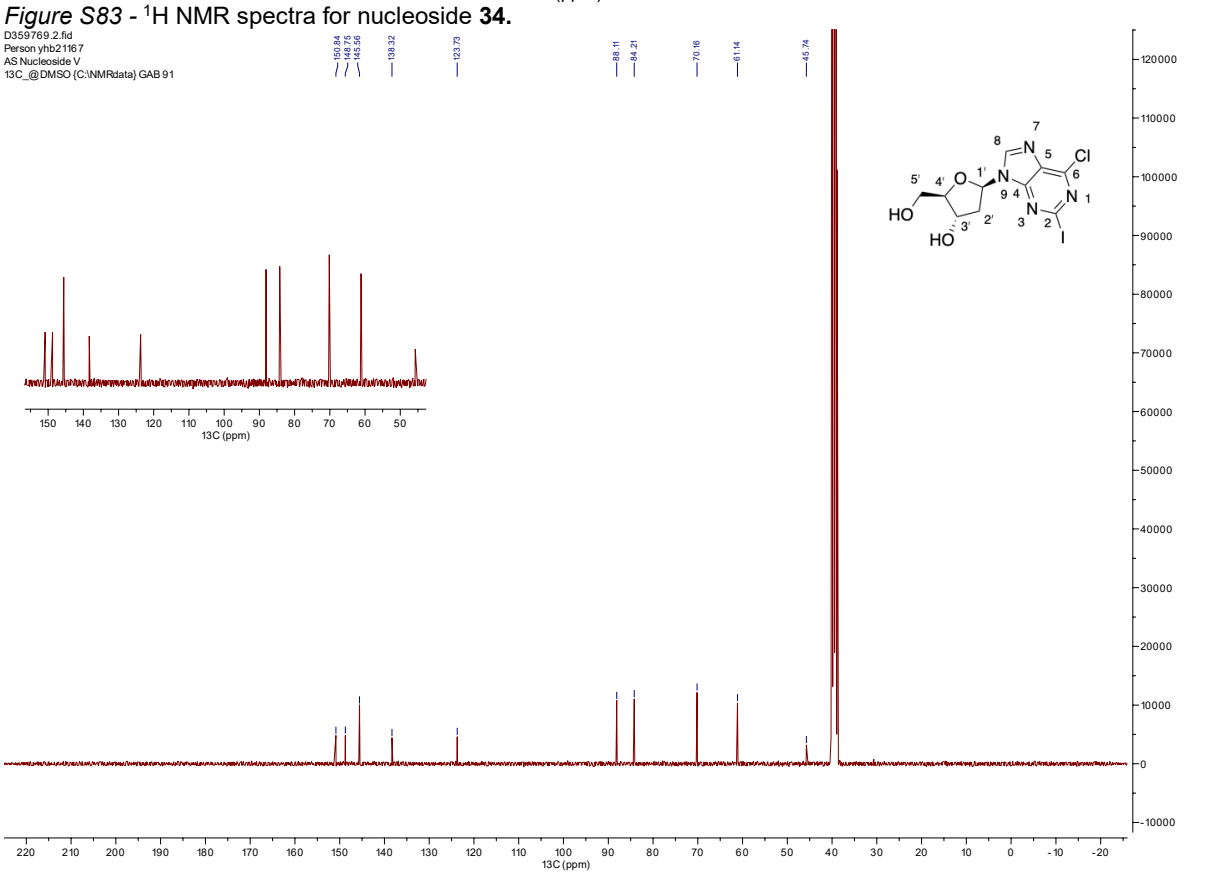
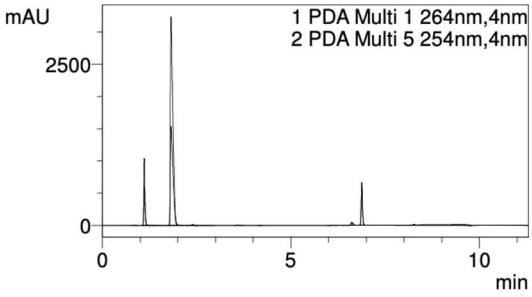


Figure S84 - 13C{1H}-NMR spectra for nucleoside **34.**

Sample Name : V2 RXN
Sample ID :
Data Filename : V2 RXN_12052022_003.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : V2 RXN.lcb
Vial # : 1-11
Injection Volume : 10 uL
Date Acquired : 12/05/2022 12:12:21
Date Processed : 12/05/2022 12:23:42

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.797	5683	2717	
2	0.876	1255	534	
3	1.112	2269177	999098	
4	1.298	8464	2957	
5	1.824	14849851	3195860	
6	2.396	39746	10805	
7	3.588	16828	7091	
8	4.154	1226	352	
9	6.102	3083	570	
10	6.431	1963	503	
11	6.614	182796	47545	
12	6.883	1550750	650555	
13	7.150	1605	485	
14	7.504	1058	227	
15	7.575	2435	479	

Figure S85 - HPLC trace for the reaction used to obtain nucleoside **34.**. R.T = 1.11 = nucleobase cytosine, 1.82 = dC, 6.61 = nucleobase V, 6.88 = nucleoside **34.**

Display Report

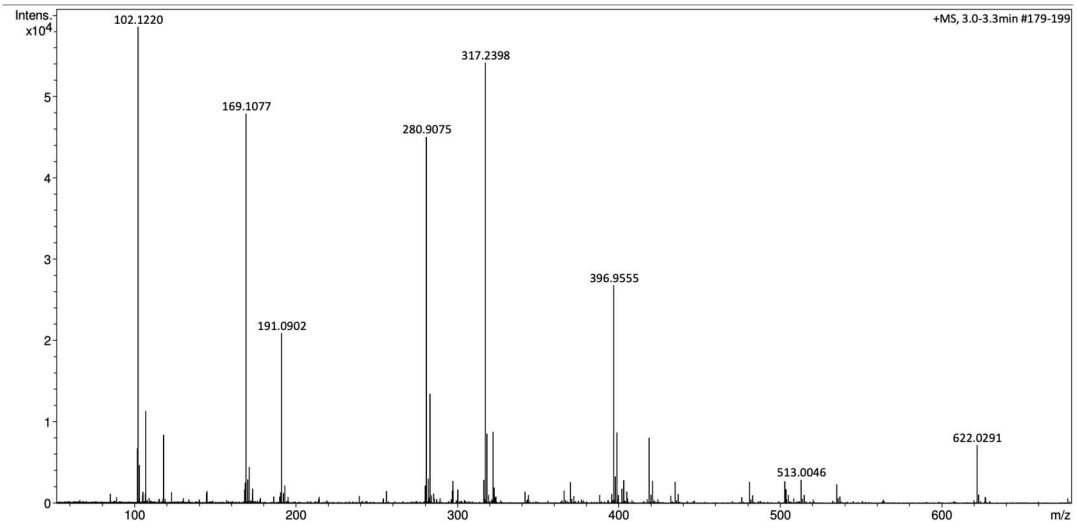
Analysis Info

Analysis Name D:\Data\Alans Data May 2022\STRA_Otto Linden AS042V.d
Method AAlansVGDtune280422_low.m
Sample Name Otto Linden AS042V
Comment Otto Linden AS042V

Acquisition Date 5/30/2022 10:36:46 AM
Operator Bruker UK
Instrument micrOTOF 8213750.10408

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	200 °C
Scan Begin	50 m/z	Set Capillary	3000 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Bruker Compass DataAnalysis 4.1 printed: 5/30/2022 10:42:28 AM by: Bruker UK 1 of 1

Figure S86 - HRMS of nucleoside **34.**

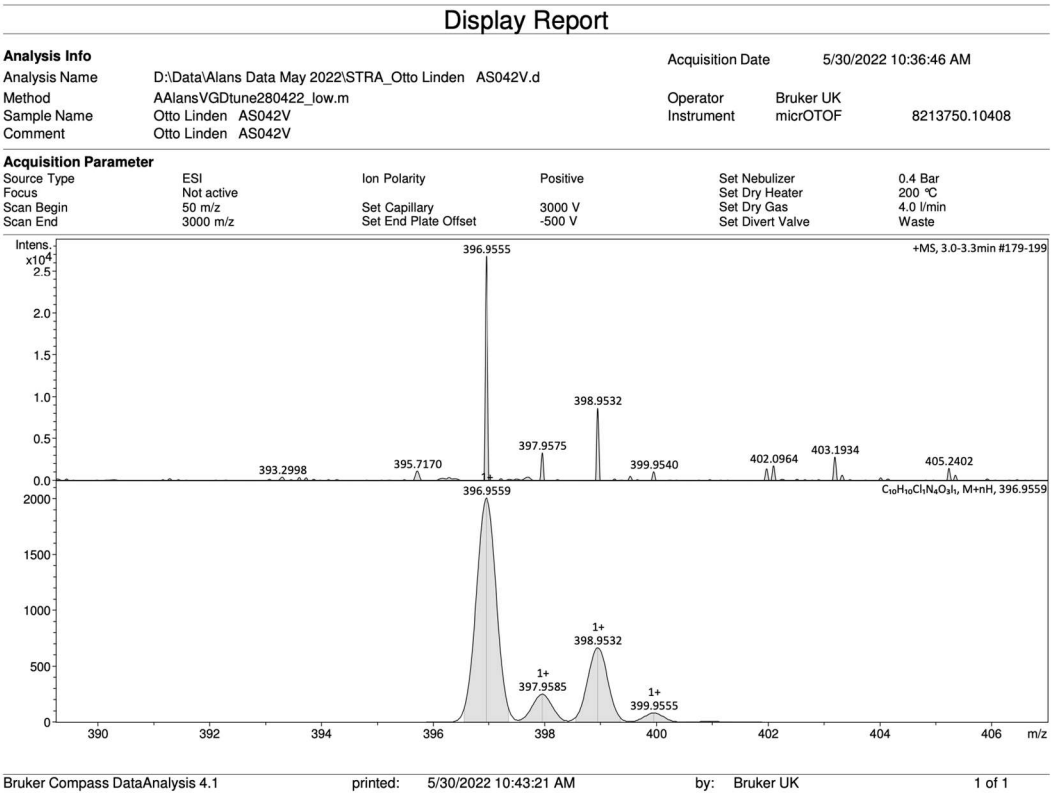


Figure S87 - HRMS of nucleoside **34.**

2'-Deoxy-4-acetamide-6chloropurine (35)

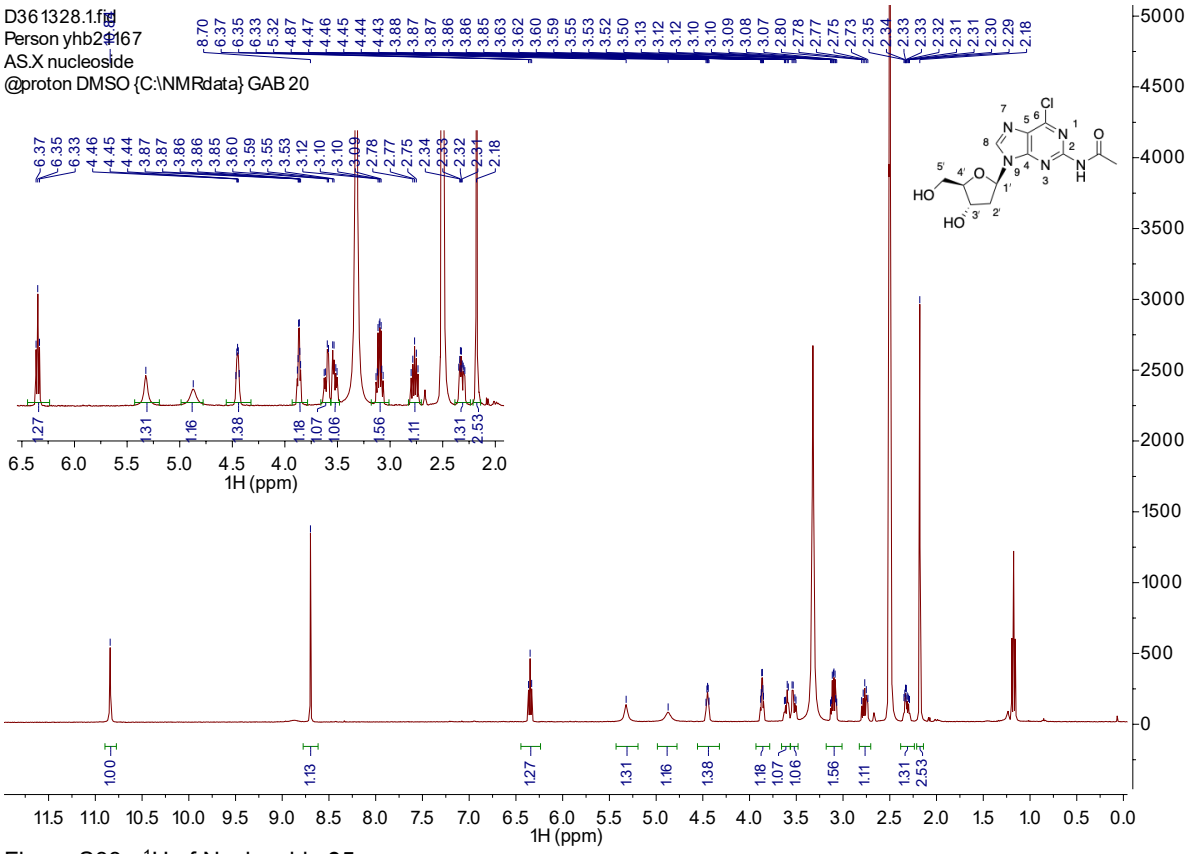


Figure S88 - ^{1}H of Nucleoside **35**

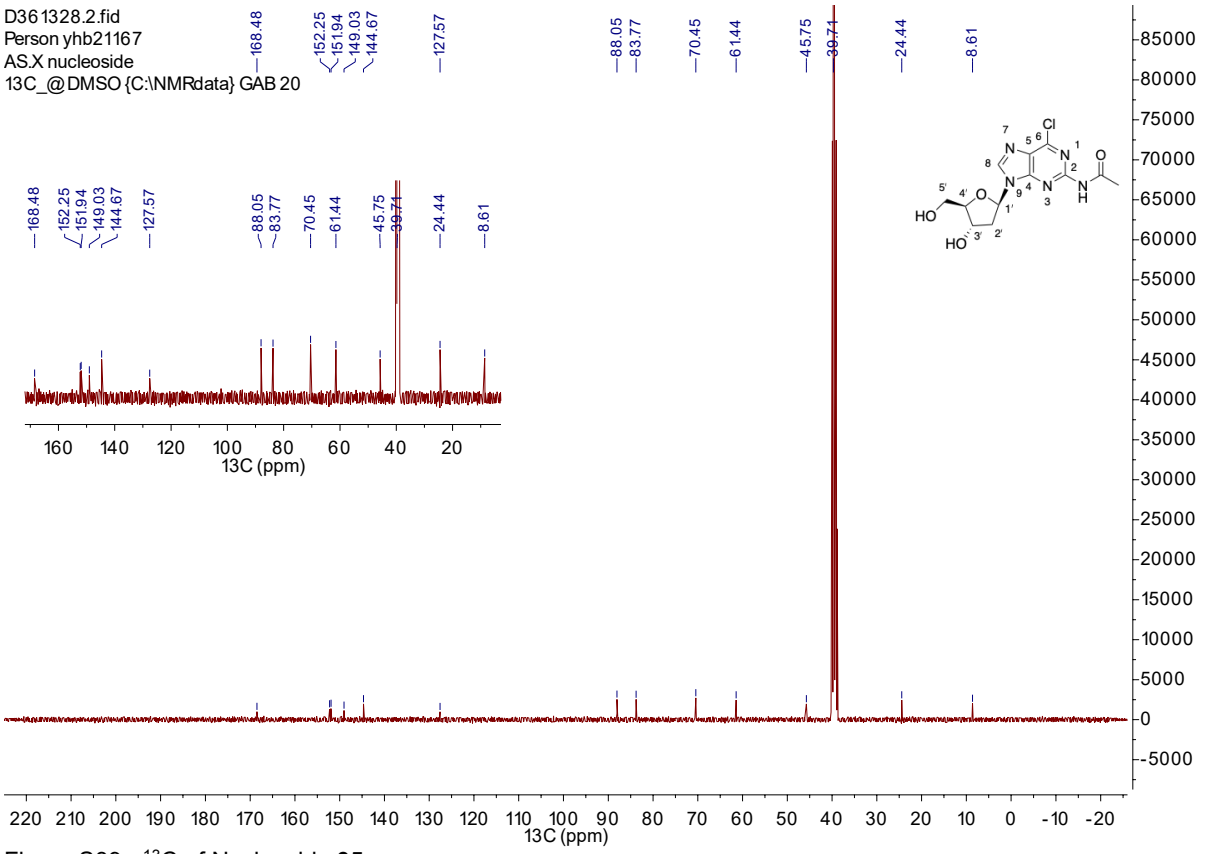
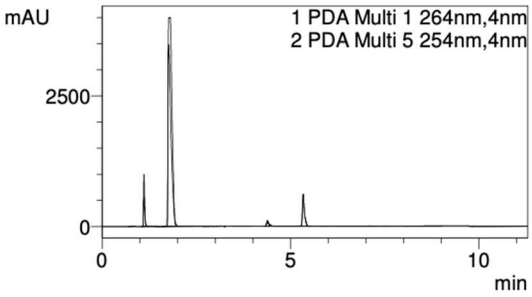


Figure S89 - ^{13}C of Nucleoside **35**

Sample Name : X RXN
Sample ID :
Data Filename : RXNS_09062022_004.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : RXNS.lcb
Vial # : 1-49
Injection Volume : 10 uL
Date Acquired : 09/06/2022 17:41:21
Date Processed : 09/06/2022 17:52:42

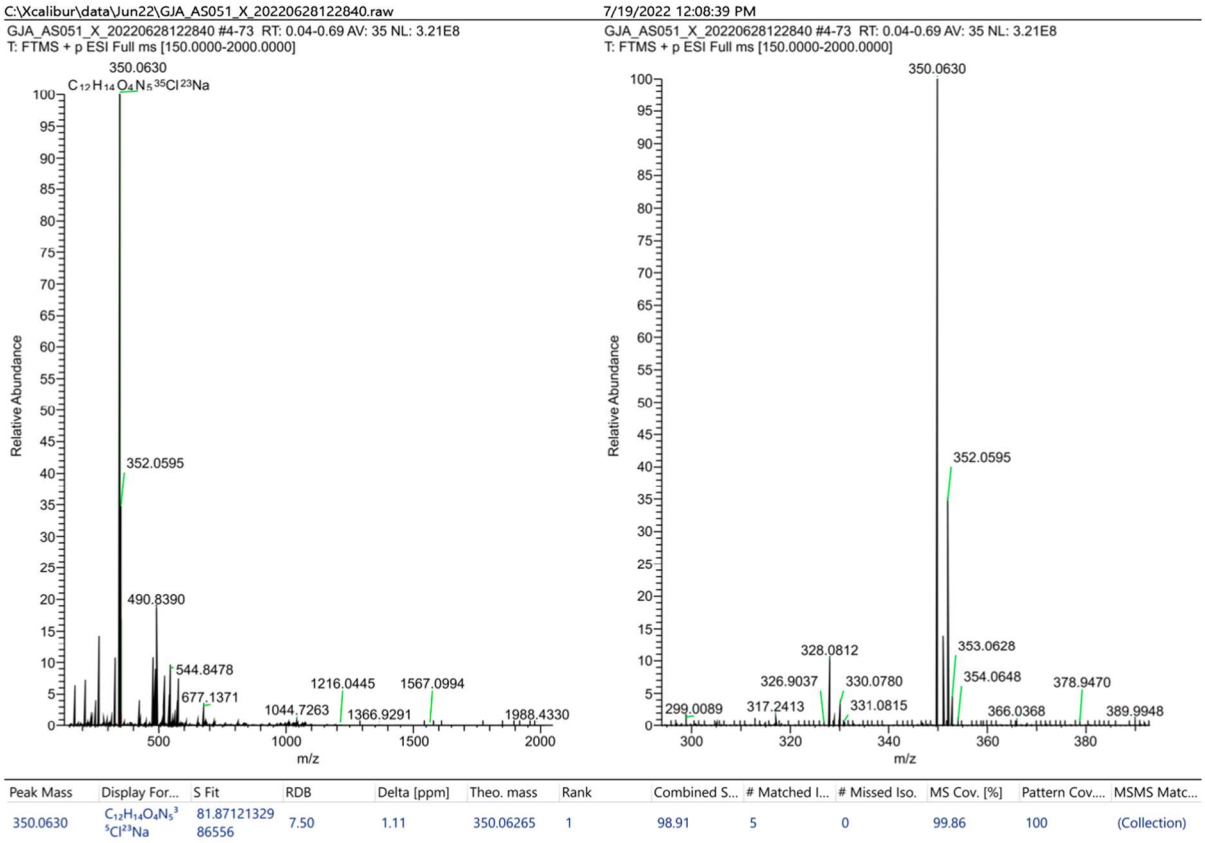
Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.790	5700	2498	
2	1.115	2115202	957360	
3	1.801	29559025	3999980	
4	2.344	5948	1885	
5	3.173	3017	1237	
6	3.554	3549	1521	
7	3.643	1346	517	
8	3.880	1308	505	
9	4.393	434955	86075	
10	4.695	10366	3101	
11	4.853	3230	1147	
12	4.963	1075	235	
13	5.152	1039	349	
14	5.342	1936233	578142	
15	5.861	9592	2650	
16	6.027	2565	1093	
17	6.283	5097	1299	
18	6.517	7727	3323	

Figure S90 - HPLC trace of the reaction to obtain Nucleoside **35.** R.T = 1.12 = nucleobase cytosine, 1.80 = dC, 4.39 = nucleobase, 5.34 = Nucleoside **35**



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
350.0630	$C_{12}H_{14}O_4N_5{}^3{}^5Cl^{23}Na$	81.87121329 86556	7.50	1.11	350.06265	1	98.91	5	0	99.86	100	(Collection)

Figure S91 - HRMS spectra of the Nucleoside **35** sodium adduct.

2'-Deoxy-6-thioguanosine (36)

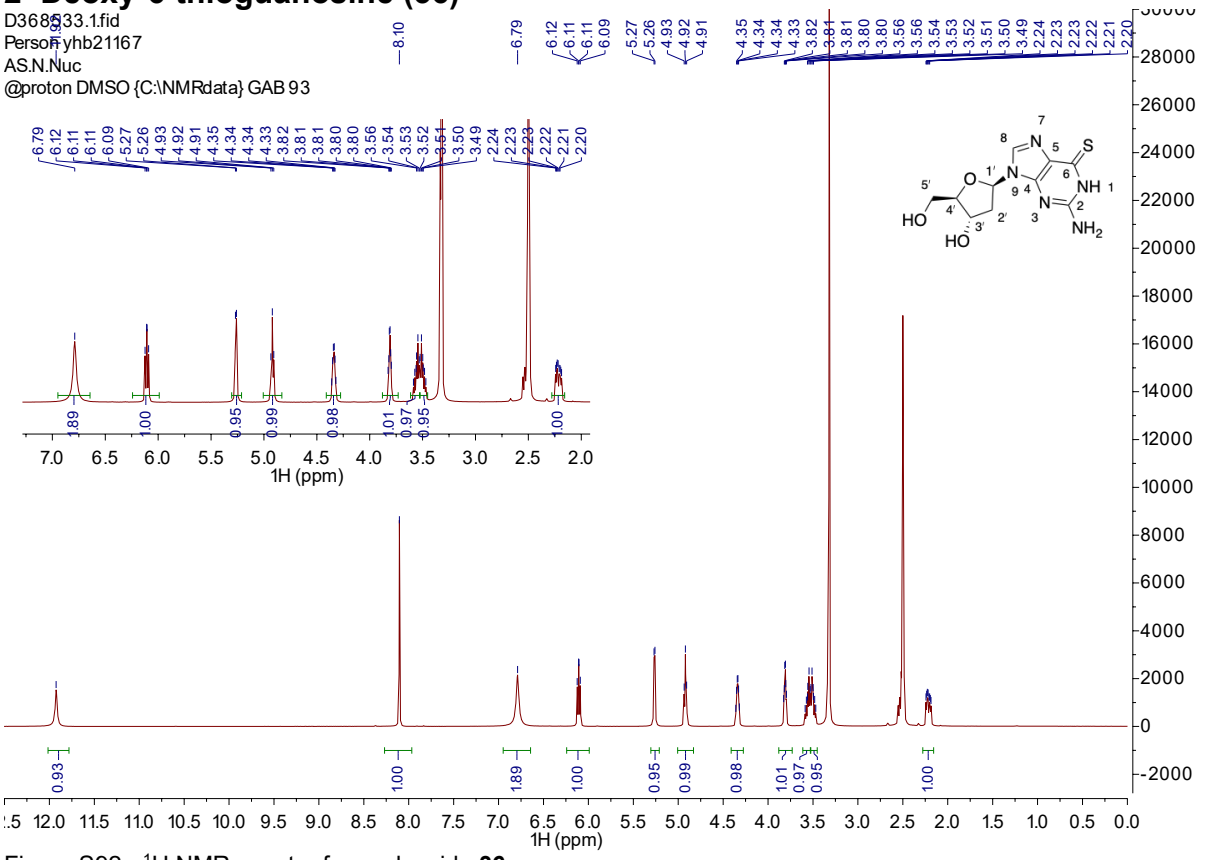


Figure S92 - ^{1}H NMR spectra for nucleoside **36**

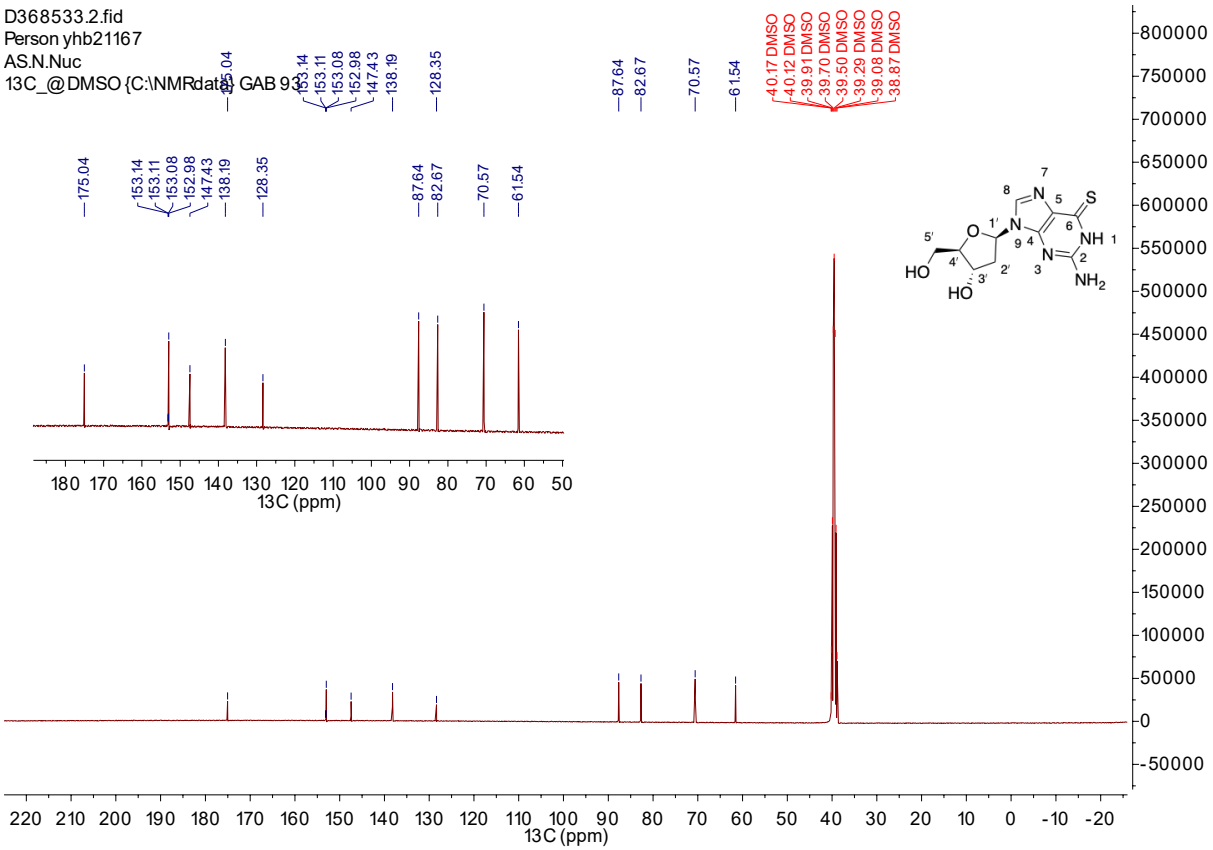
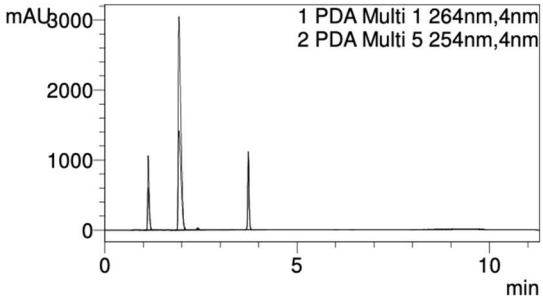


Figure S93 - 13C{1H}-NMR spectra for nucleoside **36**

Sample Name	: as029 N		
Sample ID	:		
Data Filename	: BASE N_02032022_003.lcd		
Method Filename	: Admir Trans glyco Run.lcm		
Batch Filename	: BASE N.lcb		
Vial #	: 1-25	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 02/03/2022 16:53:58	Acquired by	: Shimadzu
Date Processed	: 02/03/2022 17:05:19	Processed by	: Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.784	5403	2603	
2	1.132	2993036	1046734	
3	1.344	2157	1084	
4	1.929	14367319	2997447	
5	2.420	96347	26915	
6	2.523	8834	2680	
7	3.216	1314	157	
8	3.304	16763	6567	
9	3.623	3654	1729	
10	3.734	2696139	1107304	
11	4.115	3268	937	
12	5.997	3492	1629	
13	6.440	3392	1460	
14	8.186	3002	371	

Figure S94 - HPLC trace for the reaction used to obtain nucleoside **36**. R.T 1.13 = released cytosine nucleobase, 1.93 = nucleoside donor dC, 2.42 = nucleobase N, 3.73 = target nucleoside **36**

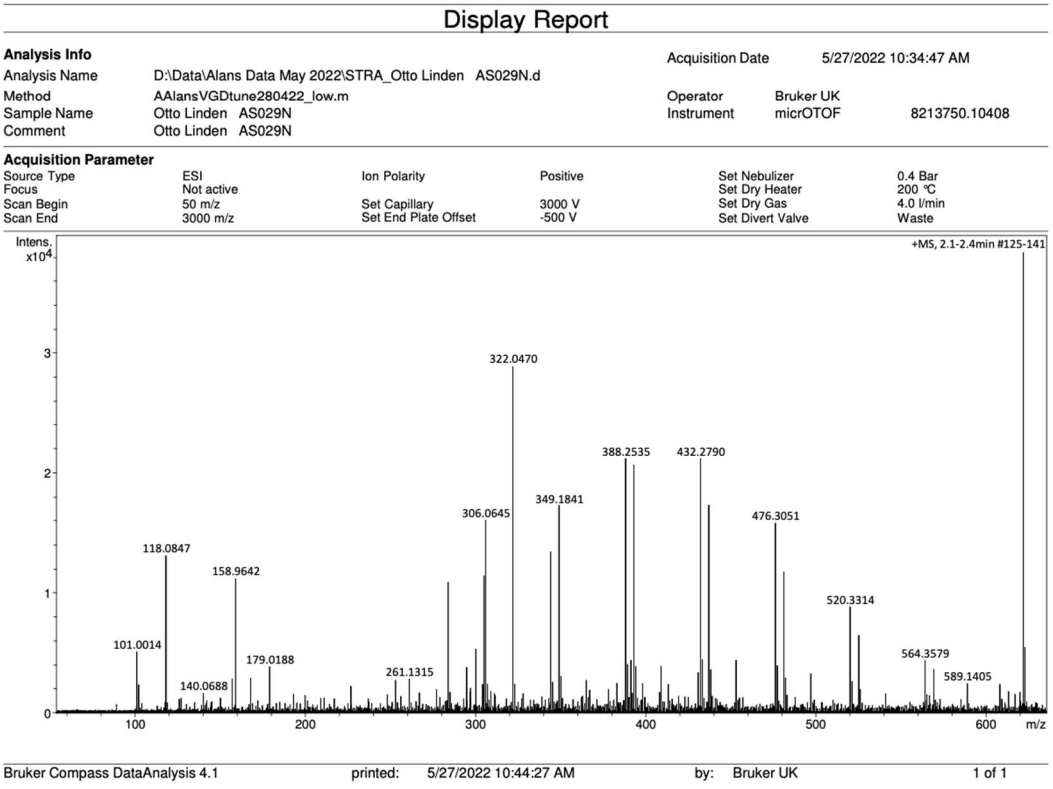


Figure S95 - HRMS for nucleoside **36**

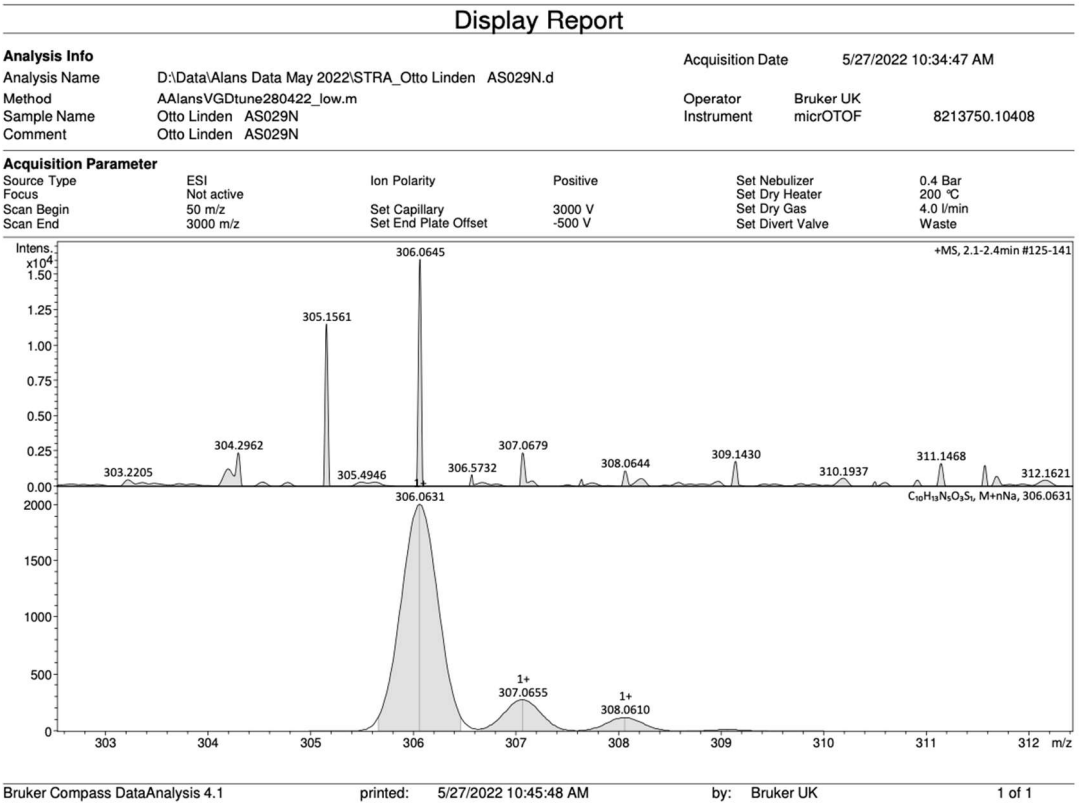


Figure S96 - HRMS for nucleoside **36**

2'-Deoxy-6-O-methylguanosine (37)

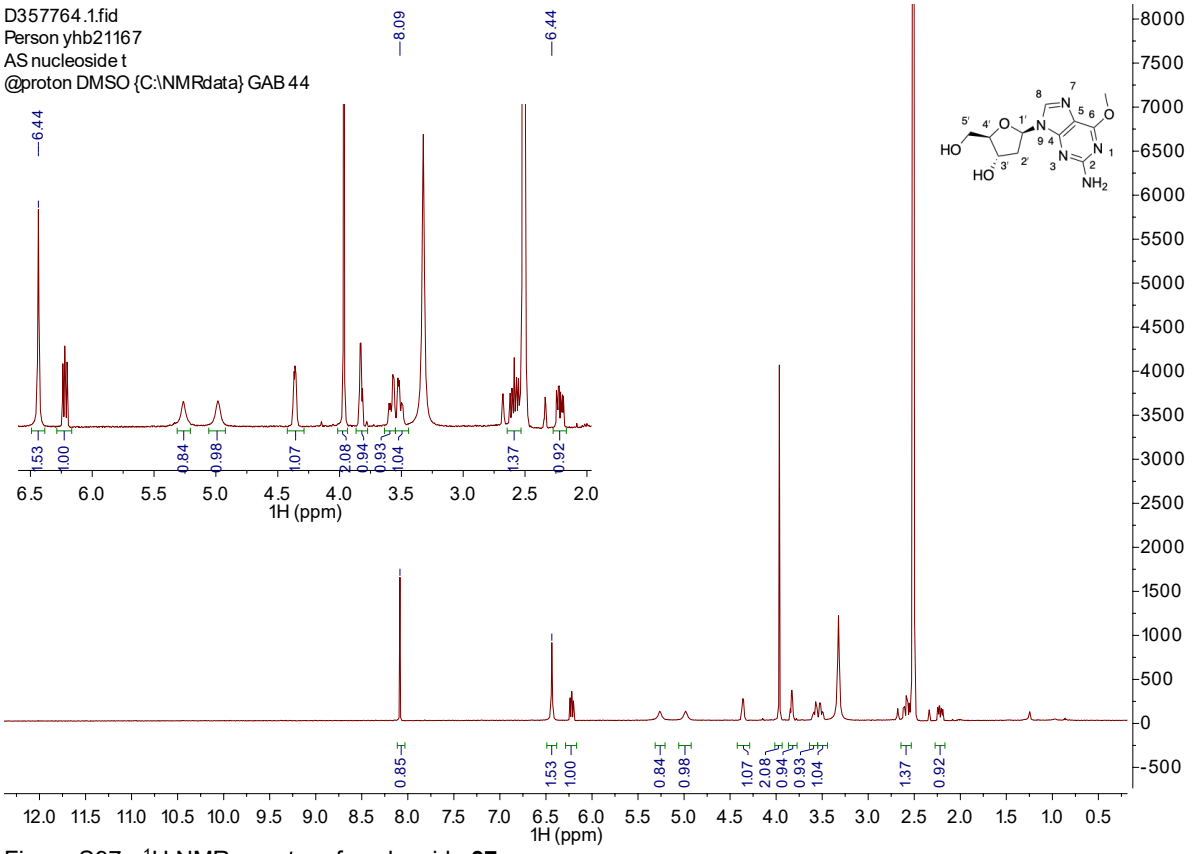


Figure S97 - ^{1}H NMR spectra of nucleoside **37**

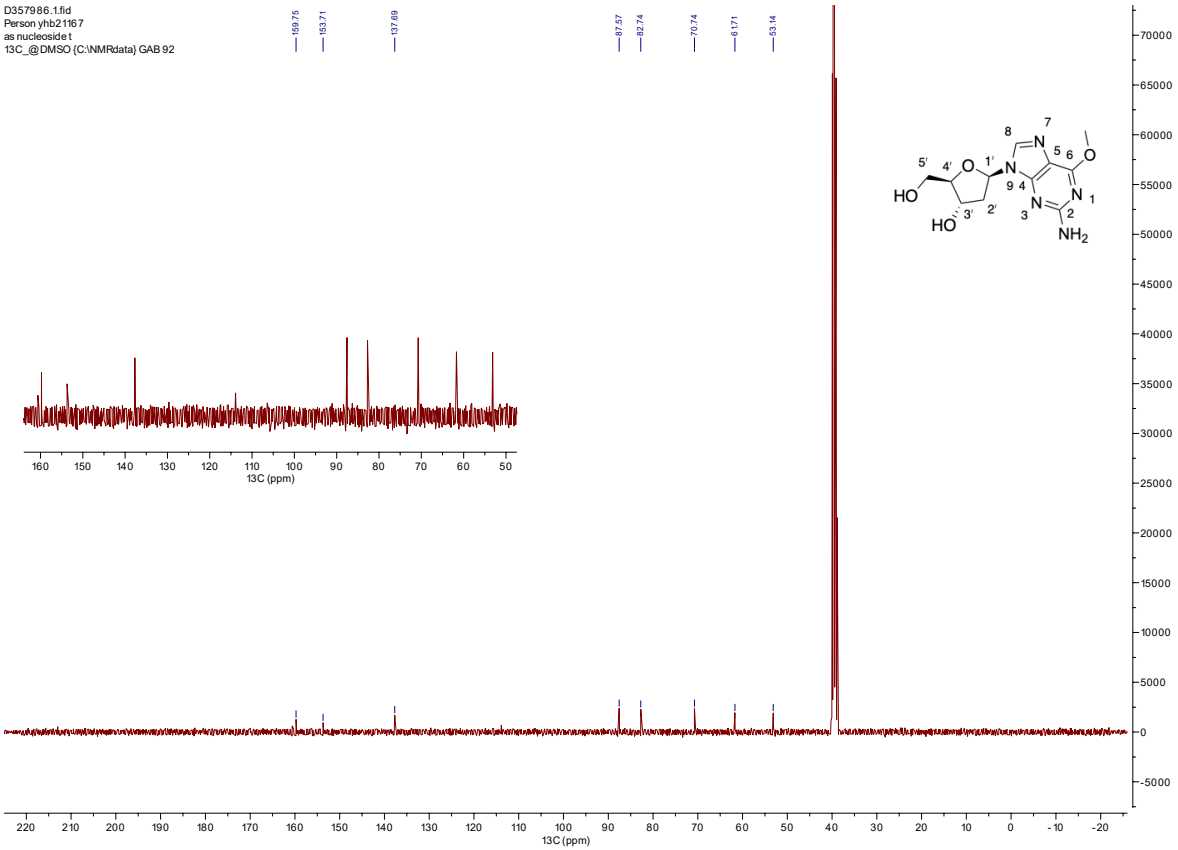
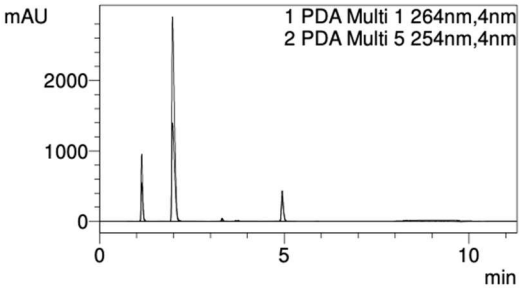


Figure S98 - 13C{1H}-NMR spectra of nucleoside **37**

Sample Name : T AS028
Sample ID :
Data Filename : REACTIONS_24022022_005.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : REACTIONS.lcb
Vial # : 1-5
Injection Volume : 10 uL
Date Acquired : 24/02/2022 15:50:55
Date Processed : 24/02/2022 16:02:15

Sample Type : Unknown

Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.798	4149	2047	
2	0.878	2254	567	
3	1.008	2252	611	
4	1.141	2723781	946705	
5	1.664	1469	570	
6	1.977	14264332	2855972	
7	2.350	5755	1311	
8	2.446	2427	852	
9	2.559	3016	933	
10	3.321	92089	35213	
11	3.688	21600	8461	
12	3.758	29275	10814	
13	3.896	2170	471	
14	3.984	2376	438	
15	4.079	2820	598	
16	4.205	2499	571	
17	4.798	3121	1137	
18	4.947	1330403	351701	
19	5.280	1110	218	
20	5.902	1151	495	
21	8.304	79905	8581	
22	8.635	100360	6104	
23	8.736	20694	6196	
24	8.792	60690	6364	

Figure S99 - HPLC spectra of the reaction used to obtain nucleoside **37.** R.T = 1.1 = nucleobase cytosine, 1.98 = nucleoside dC, 4.95 = Target nucleoside **37.**

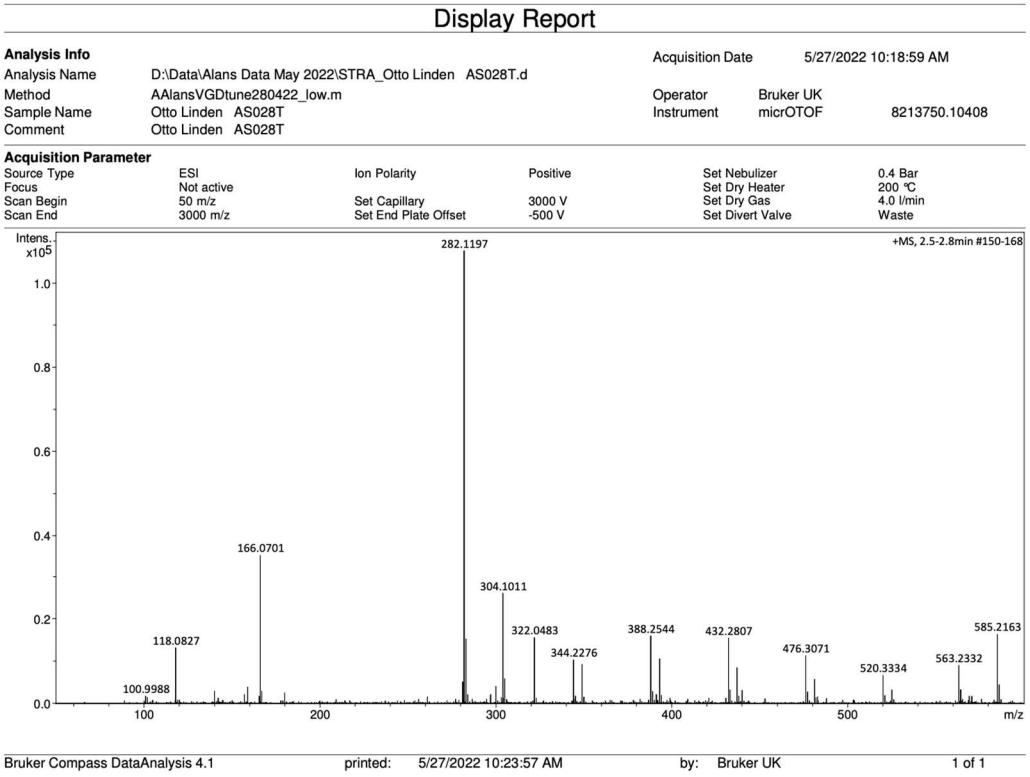


Figure S100 - HRMS of nucleoside **37**

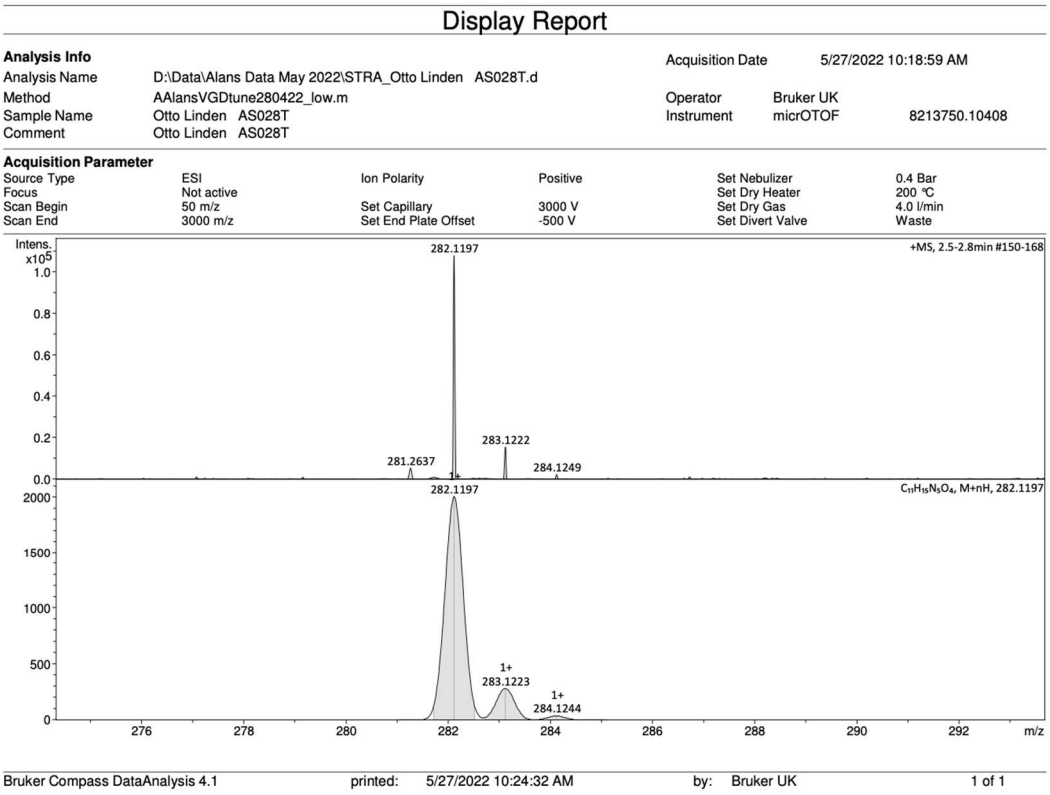


Figure S101 - HRMS to nucleoside **37**

2'-Deoxy-N2-Isobutyrylguanosine (38)

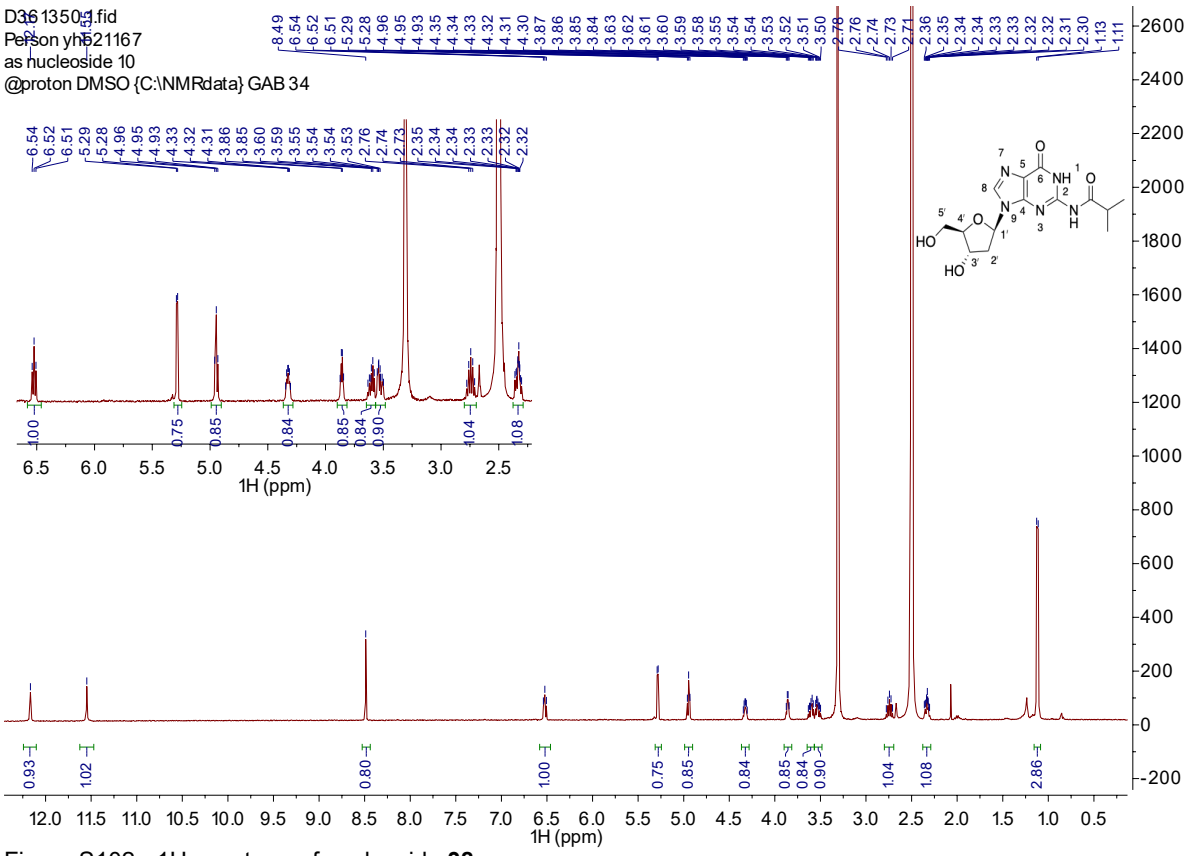
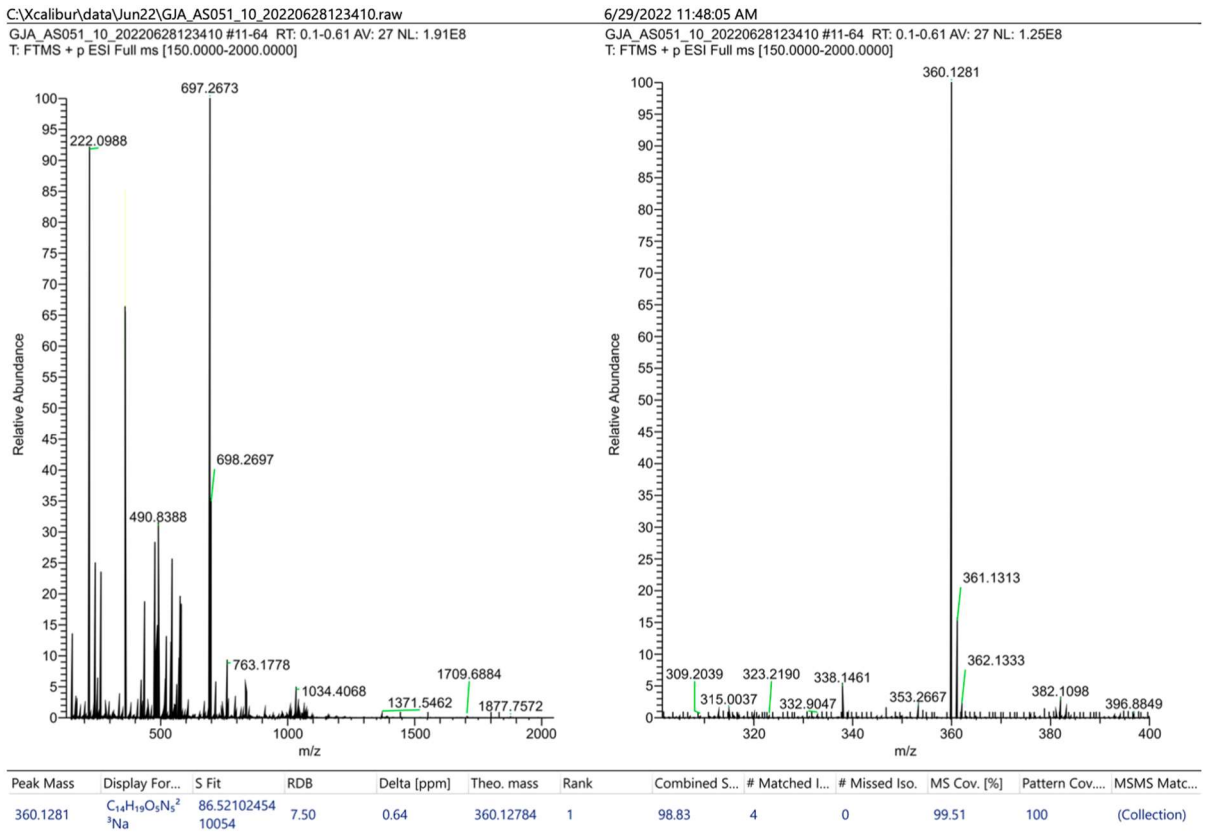


Figure S102 - 1H spectrum of nucleoside **38**

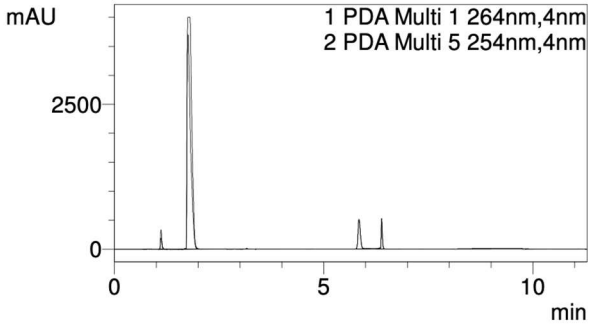


Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
360.1281	$C_{14}H_{19}O_5N_5^2{}^3Na$	86.52102454 10054	7.50	0.64	360.12784	1	98.83	4	0	99.51	100	(Collection)

Figure S103 - HRMS spectrum of nucleoside **38**

Sample Name : 10 RXN
Sample ID :
Data Filename : RXNS_09062022_012.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : RXNS.lcb
Vial # : 1-57
Injection Volume : 10 uL
Date Acquired : 09/06/2022 19:15:35
Date Processed : 09/06/2022 19:26:55

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.788	5718	2457	
2	1.114	757140	319844	
3	1.563	1327	621	
4	1.793	31369464	4000024	
5	2.334	13859	4381	
6	2.543	3464	1068	
7	3.161	21225	8228	
8	3.541	2969	1306	
9	3.774	2048	776	
10	3.869	3328	1472	
11	4.638	1591	479	
12	5.846	2106096	507846	
13	6.057	1199	524	
14	6.388	1157235	514468	
15	6.570	1148	338	

Figure S104 - HPLC spectrum of nucleoside **38.** R.T = 1.11 = released nucleobase cytosine 1.79 = nucleoside deoxycytidine dC, 5.84 = nucleobase 10, 6.39 = nucleoside **38**

2'-Deoxy-2-fluoroadenosine (39)

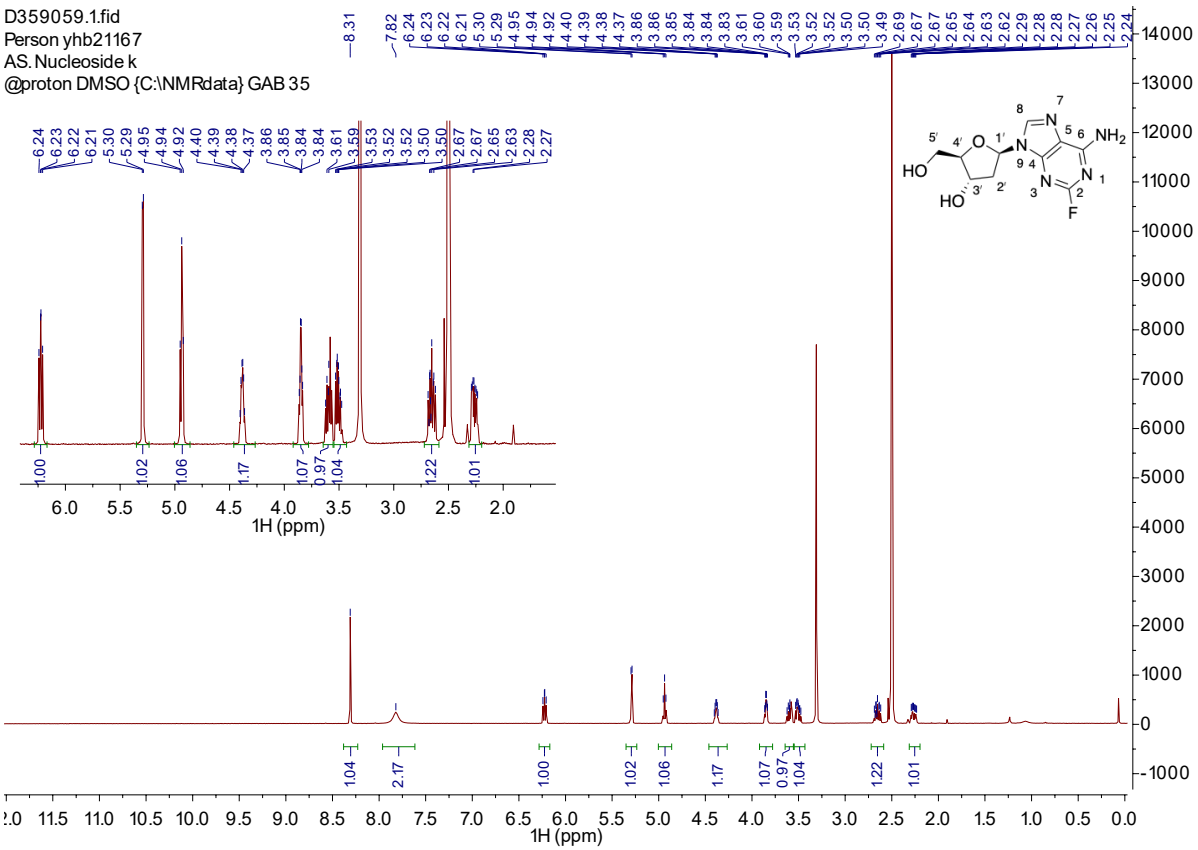


Figure S105 - ^{1}H NMR spectra of nucleoside 39

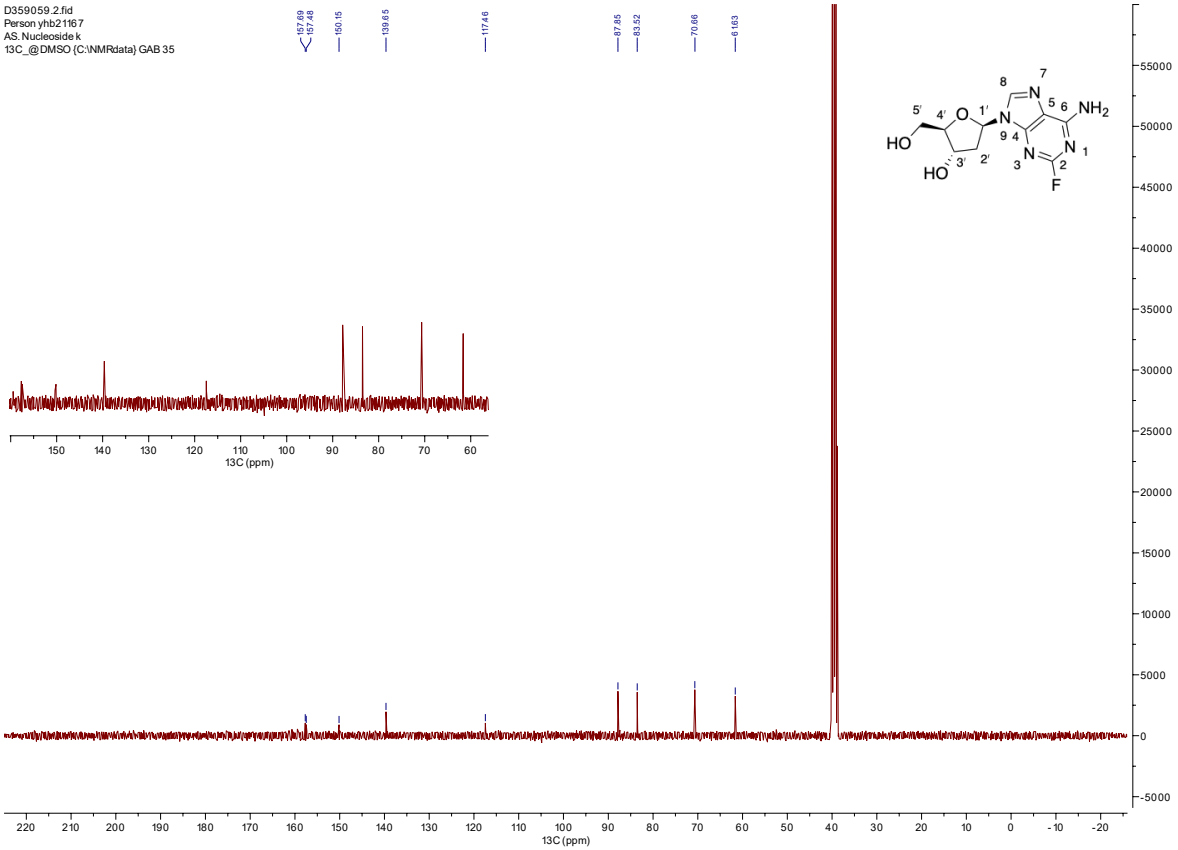


Figure S106 - 13C{1H}-NMR spectra of nucleoside 39

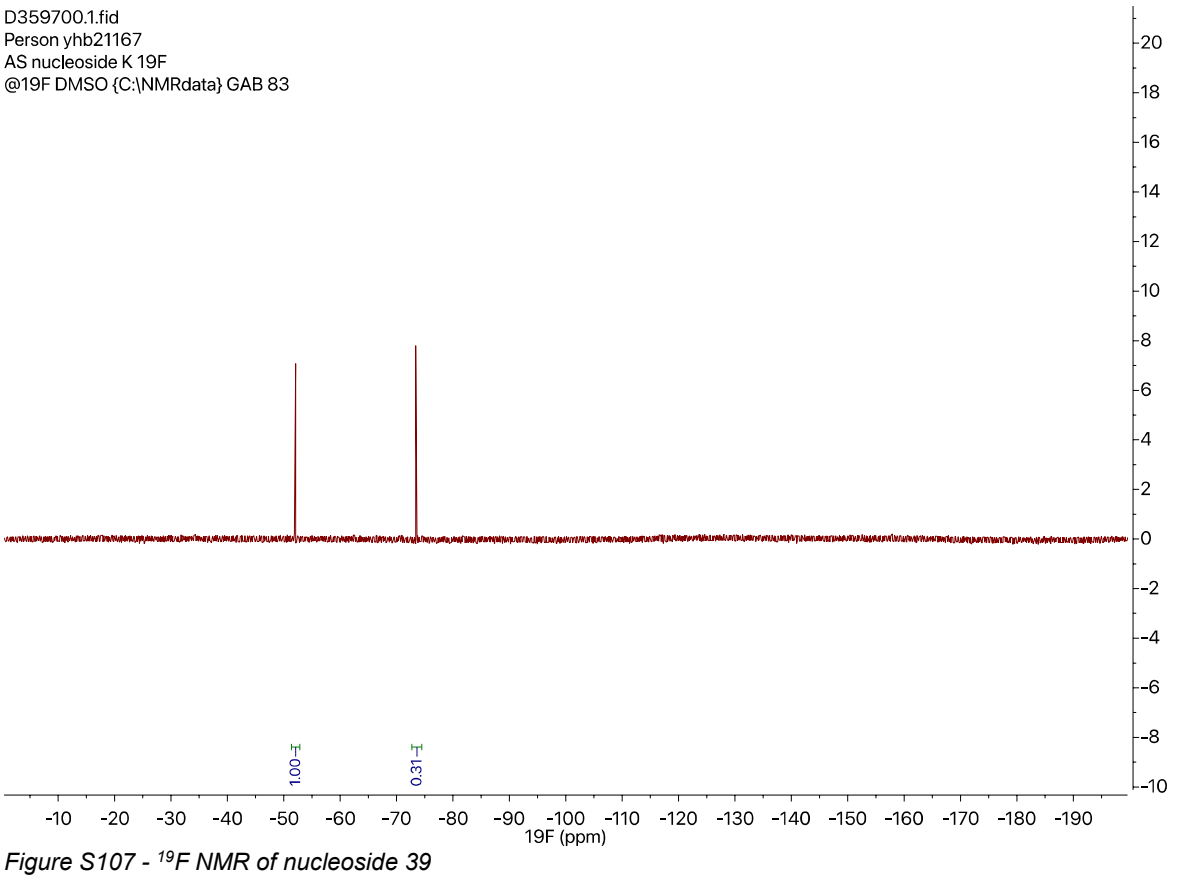
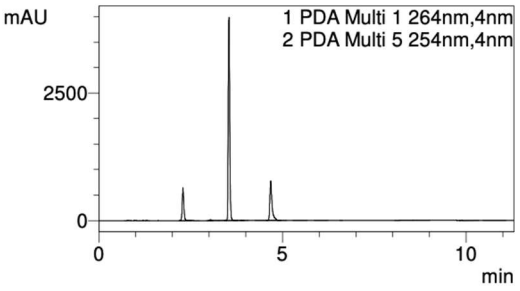


Figure S107 - ^{19}F NMR of nucleoside 39

Sample Name : K RXN
Sample ID :
Data Filename : AS050 RXNS_09062022_003.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : AS050 RXNS.lcb
Vial # : 1-18
Injection Volume : 10 uL
Date Acquired : 09/06/2022 13:22:05
Date Processed : 09/06/2022 13:33:26

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.790	5915	2640	
2	0.979	9420	3723	
3	1.210	1886	818	
4	1.303	2340	996	
5	2.292	2010451	634396	
6	3.040	58111	18609	
7	3.127	8566	2700	
8	3.545	11979555	3955552	
9	3.887	10235	4237	
10	4.355	9782	2312	
11	4.682	3249450	766165	
12	5.353	1175	330	
13	5.527	10391	2734	
14	5.900	1302	502	

Figure S108 - HPLC trace for the reaction used to obtain nucleoside 39. R.T = 2.29 = nucleobase released thymine, 3.55 = nucleoside dT, 4.68 = nucleoside 39

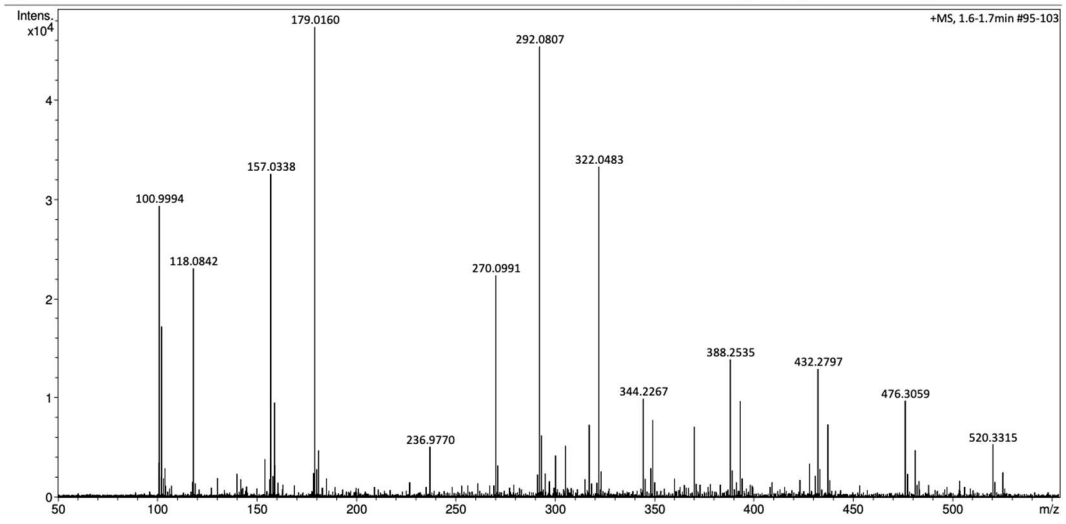
Display Report

Analysis Info

Analysis Name	D:\Data\Alans Data May 2022\STRA_Otto Linden AS033K.d	Acquisition Date	5/27/2022 9:50:16 AM
Method	AAlansVGDtune280422_low.m	Operator	Bruker UK
Sample Name	Otto Linden AS033K	Instrument	micrOTOF 8213750.10408
Comment	Otto Linden AS033K		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	200 °C
Scan Begin	50 m/z	Set Capillary	3000 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Bruker Compass DataAnalysis 4.1 printed: 5/27/2022 9:57:38 AM by: Bruker UK 1 of 1

Figure S109 - HRMS of nucleoside 39

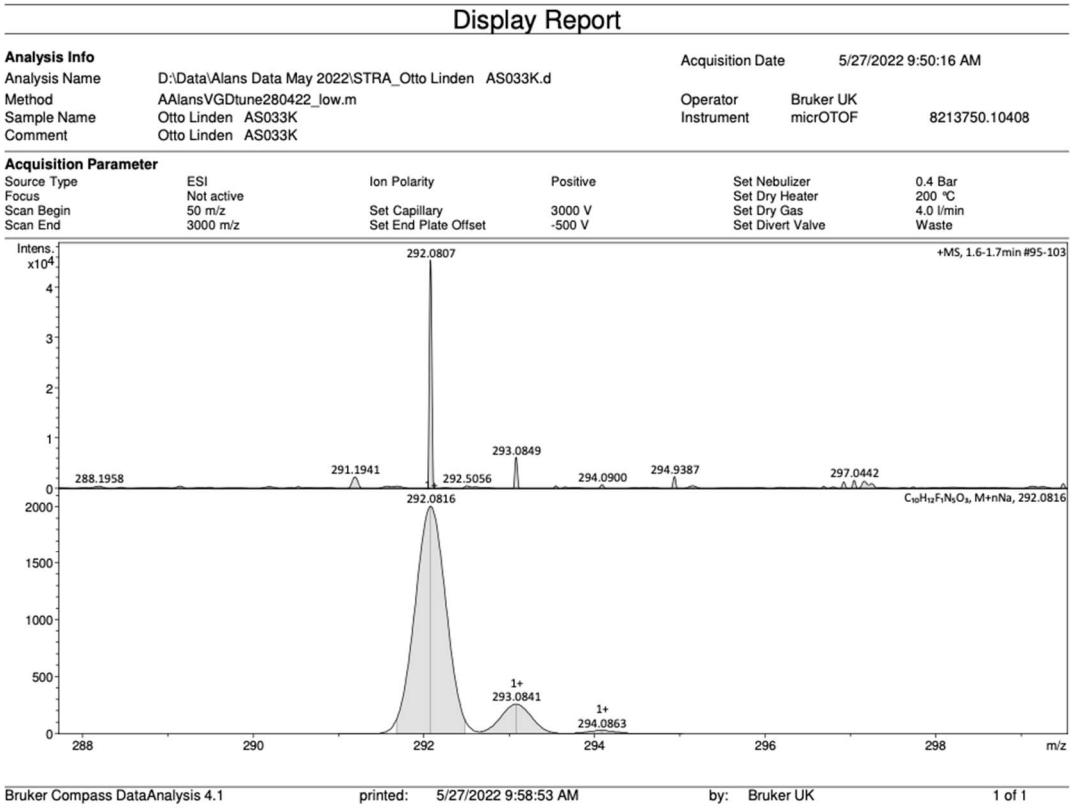


Figure S110 - HRMS of nucleoside 39

2'-Deoxy-2-aminoadenosine (40)

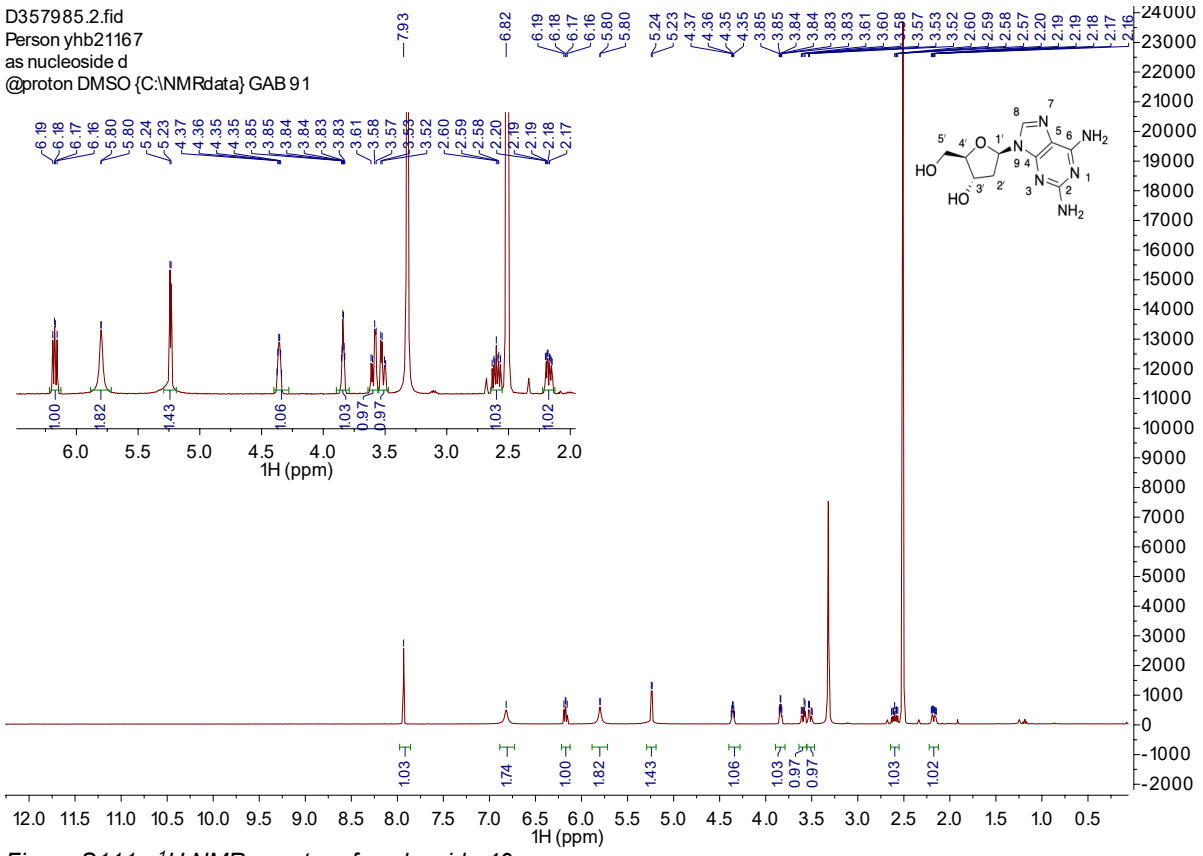


Figure S111 - ^{1}H NMR spectra of nucleoside 40

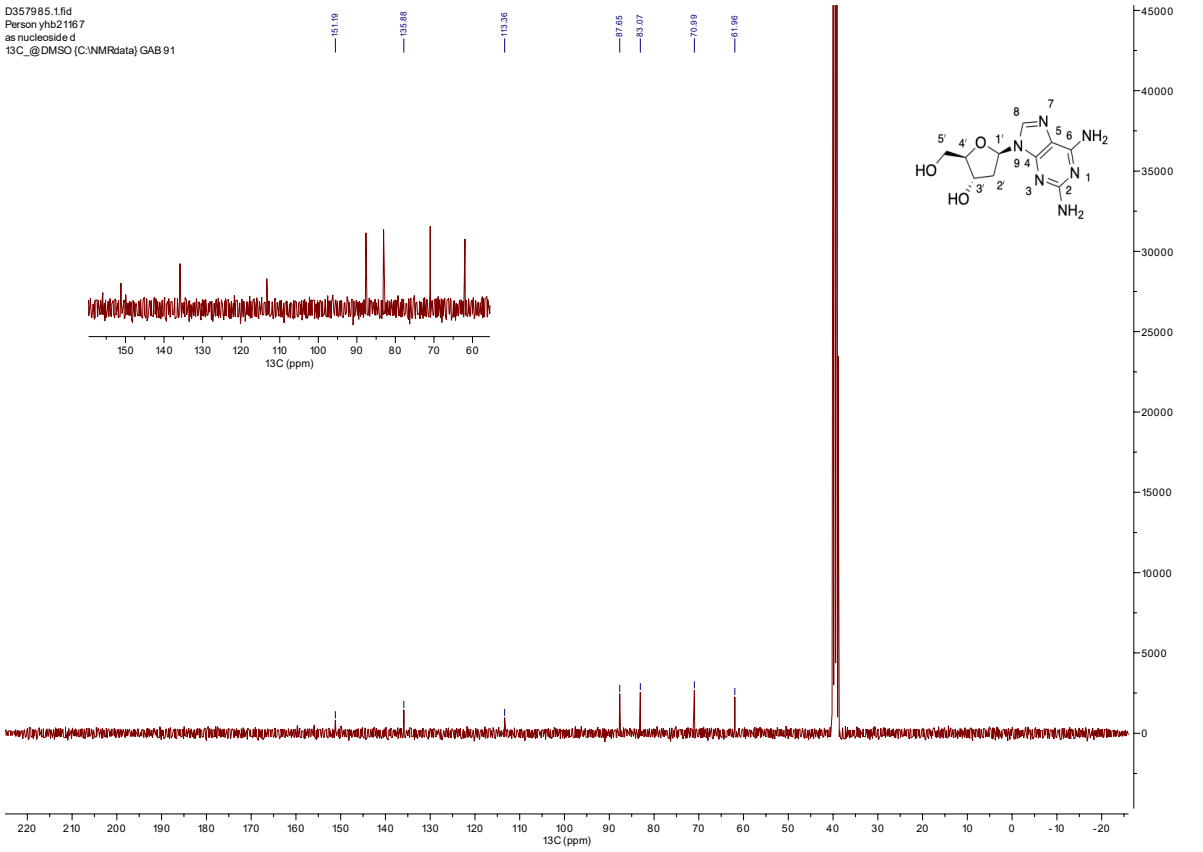
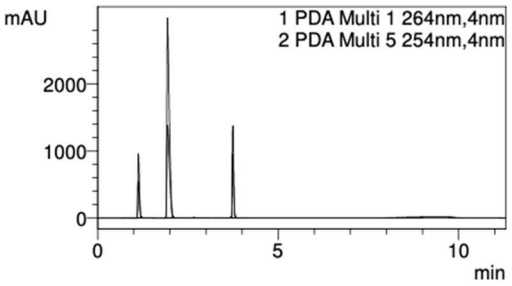


Figure S112 - 13C{1H}-NMR spectra of nucleoside 40

Sample Name : as029 D
Sample ID :
Data Filename : BASE D_02032022_003.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : BASE D.lcb
Vial # : 1-4 Sample Type : Unknown
Injection Volume : 10 uL
Date Acquired : 02/03/2022 12:46:14 Acquired by : Shimadzu
Date Processed : 02/03/2022 12:57:35 Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.785	6131	3369	
2	0.847	2208	815	
3	1.132	2846846	950125	
4	1.337	3101	1473	
5	1.939	13998177	2950758	
6	2.432	2969	951	
7	2.532	11510	3366	
8	2.673	30045	8007	
9	3.058	1122	387	
10	3.313	3476	1259	
11	3.532	4330	740	
12	3.747	2518323	937818	
13	8.163	2513	291	
14	8.309	8774	3216	

Figure S113 - *HPLC trace of the reaction used to obtain nucleoside 40. R.T - 1.1 = nucleobase released cytosine, 1.9 = nucleoside dC, 3.75 = nucleoside 40*

Display Report

Analysis Info
Analysis Name D:\Data\Alans Data May 2022\STRA_Otto Linden AS029D.d
Method AAlansVGDtune280422_low.m
Sample Name Otto Linden AS029D
Comment Otto Linden AS029D
Acquisition Date 5/26/2022 4:19:26 PM
Operator Bruker UK
Instrument micrOTOF 8213750.10408

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	200 °C
Scan Begin	50 m/z	Set Capillary	3000 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste

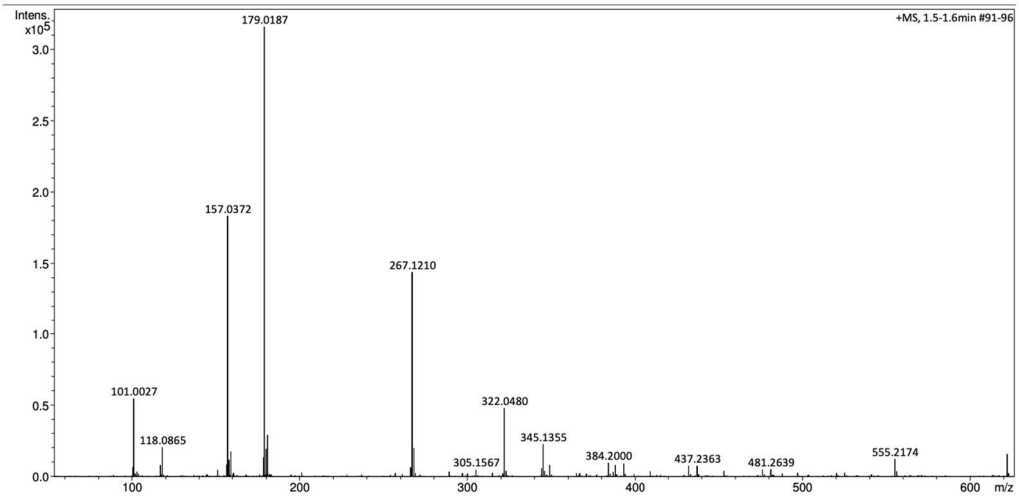


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Figure S114 - *HRMS of nucleoside 40*

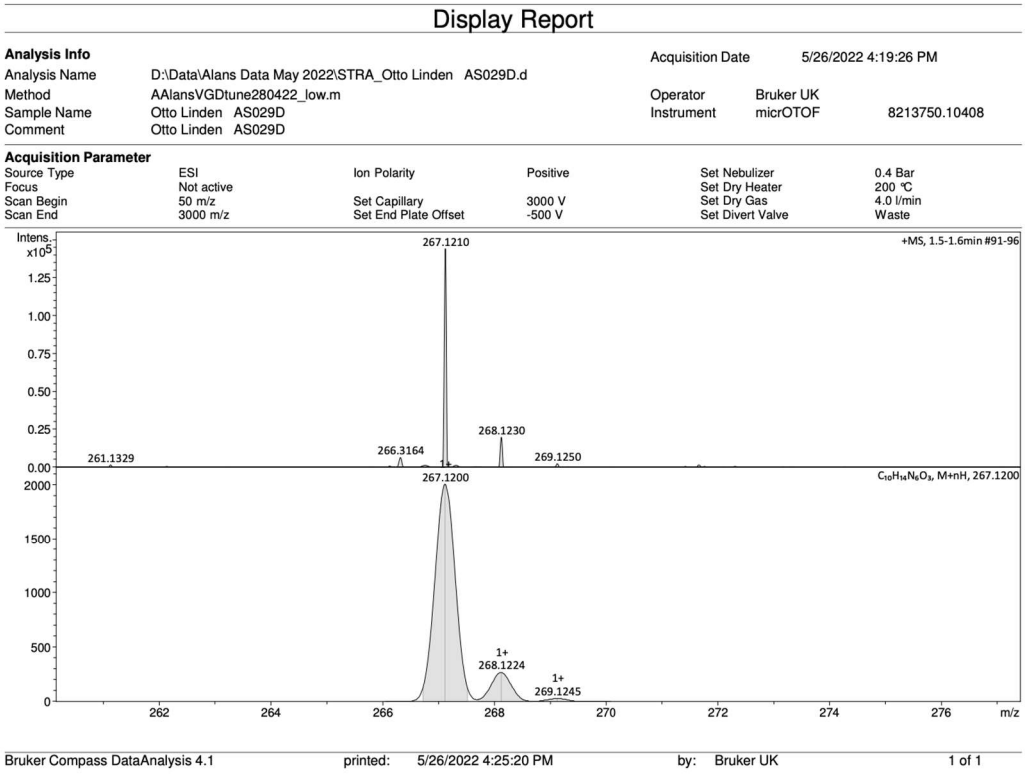


Figure S115 - *HRMS of nucleoside 40*

2'-Deoxy-6-methylpurine (41)

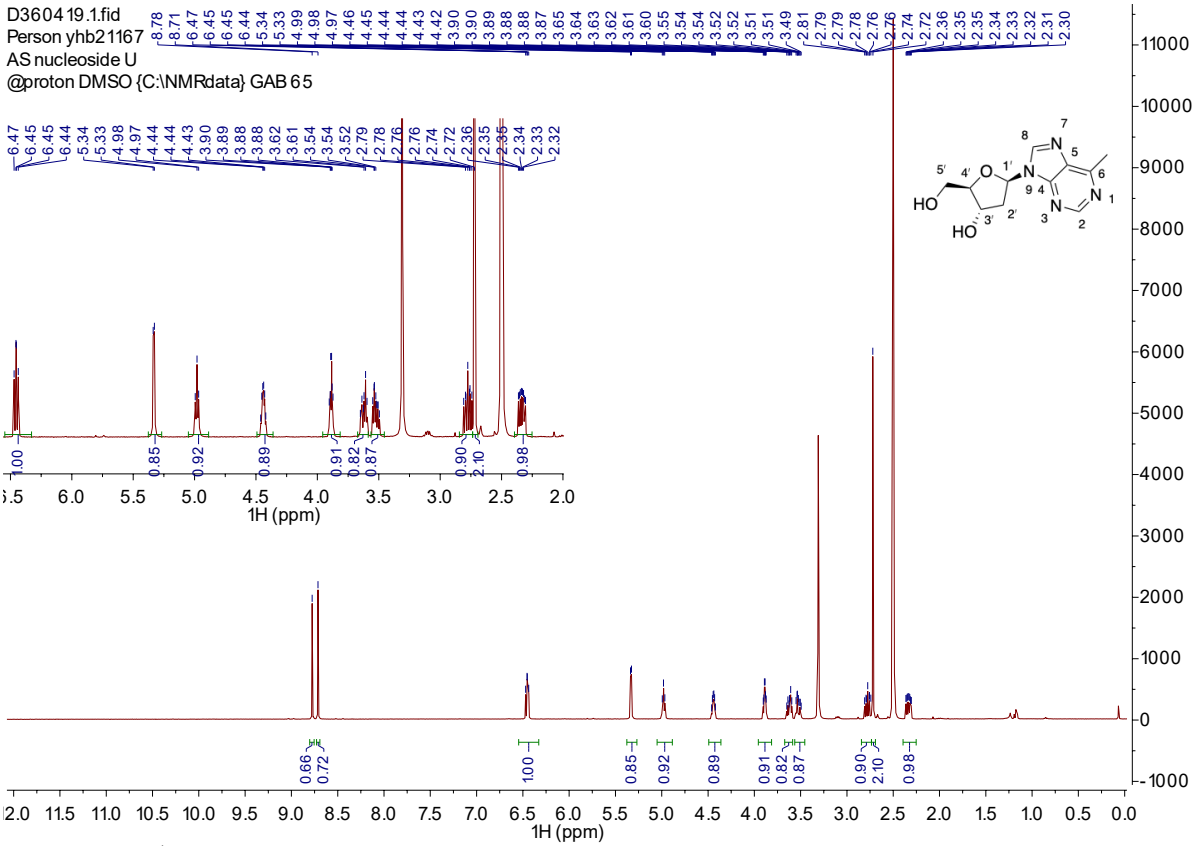


Figure S116 - *[1]H of nucleoside 41*

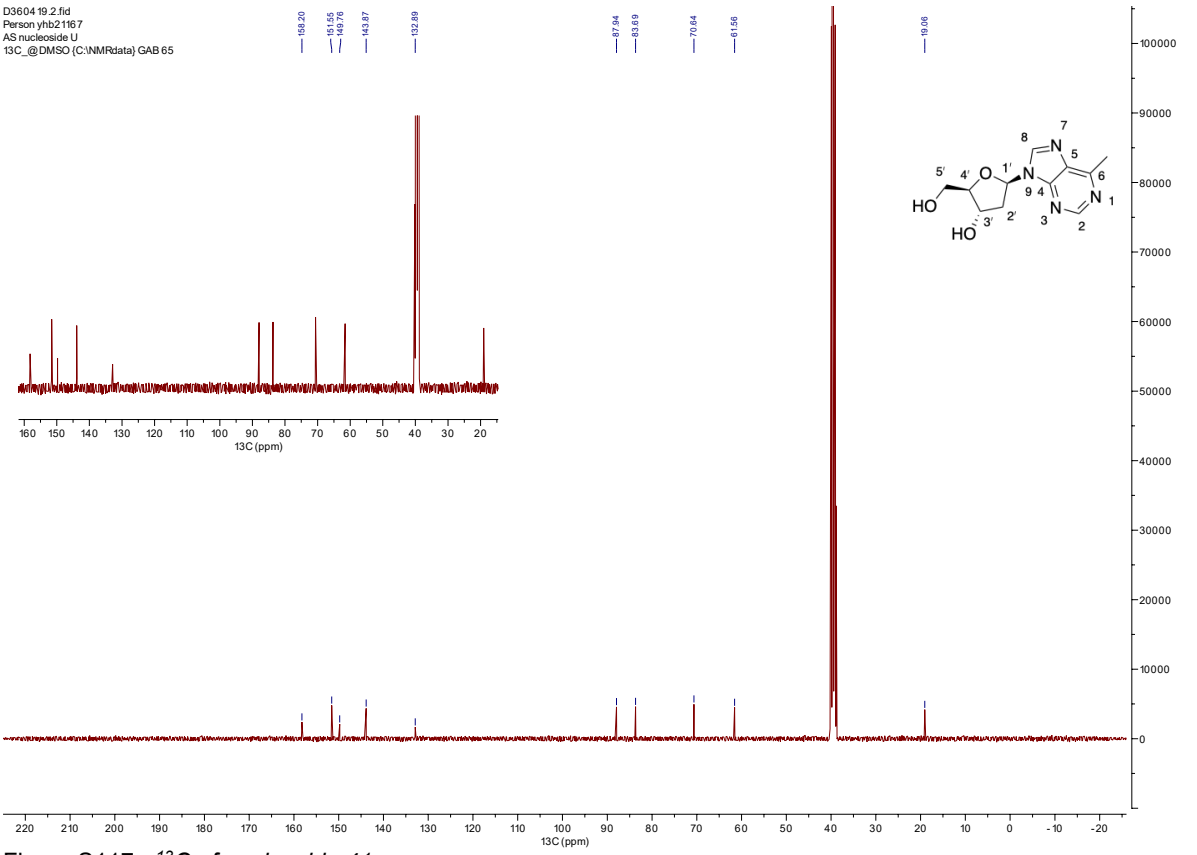
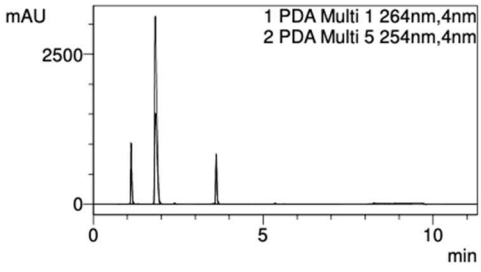


Figure S117 - *[13]C of nucleoside 41*

Sample Name : U RXN
Sample ID :
Data Filename : U RXN_12052022_002.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : U RXN.lcb
Vial # : 1-13
Injection Volume : 10 uL
Date Acquired : 12/05/2022 12:36:00
Date Processed : 12/05/2022 12:47:20

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.797	6153	2861	
2	0.876	1451	612	
3	1.116	2523698	996498	
4	1.312	7654	3718	
5	1.384	2007	953	
6	1.494	1842	759	
7	1.585	2071	395	
8	1.824	14036019	3095295	
9	2.395	44486	11092	
10	2.560	1284	225	
11	3.108	1240	208	
12	3.214	4115	846	
13	3.338	2769	662	
14	3.506	19654	7273	
15	3.621	1877739	823609	
16	4.072	5334	1250	
17	4.466	5755	1215	
18	4.842	1404	335	
19	4.944	1144	178	
20	5.166	2982	328	
21	5.351	46774	10025	
22	5.633	7324	1652	
23	5.955	3658	1485	
24	6.043	1471	420	
25	6.135	1483	468	
26	6.281	9954	2656	

Figure S118 - *HPLC trace of the reaction to obtain nucleoside 41. R.T = 1.12 = Nucleobase cytosine, 1.82 = nucleoside dC, 3.62 = nucleoside 41*

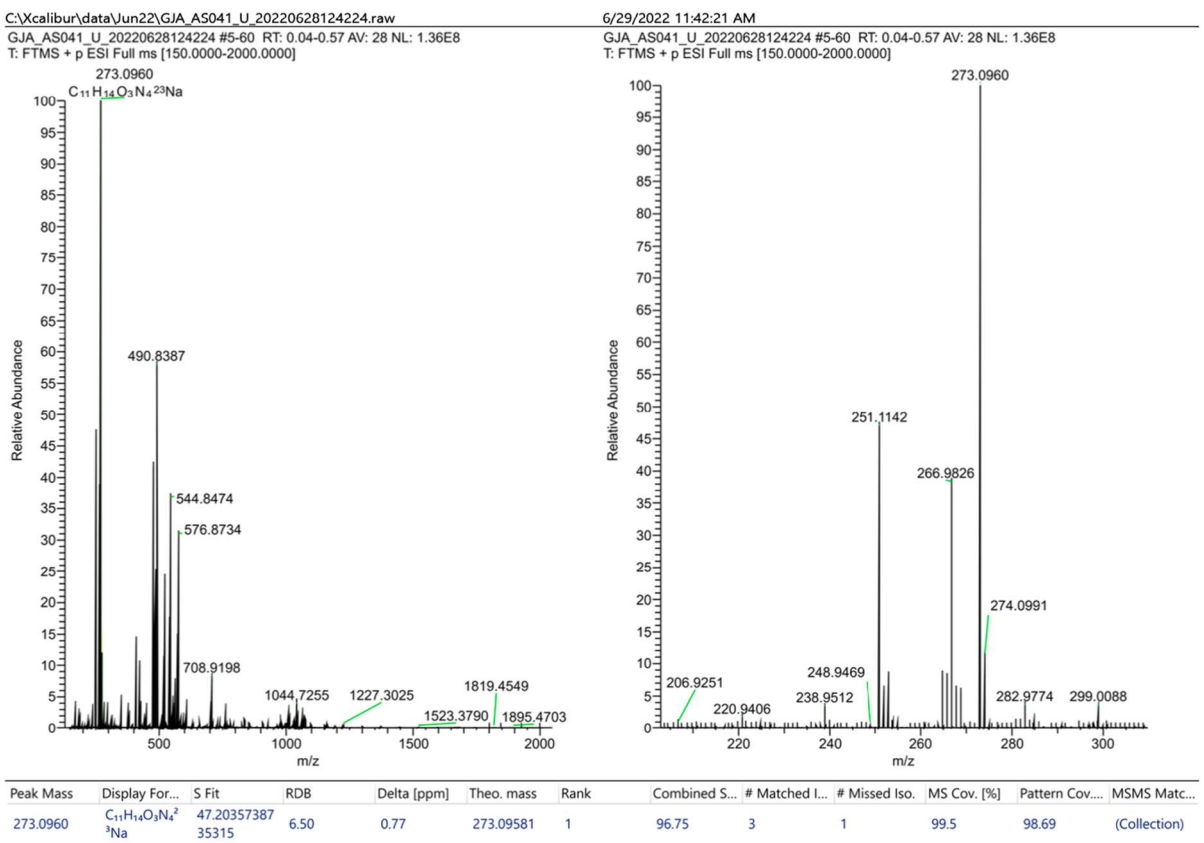


Figure S119 - *HRMS trace of the nucleoside 41 sodium adduct.*

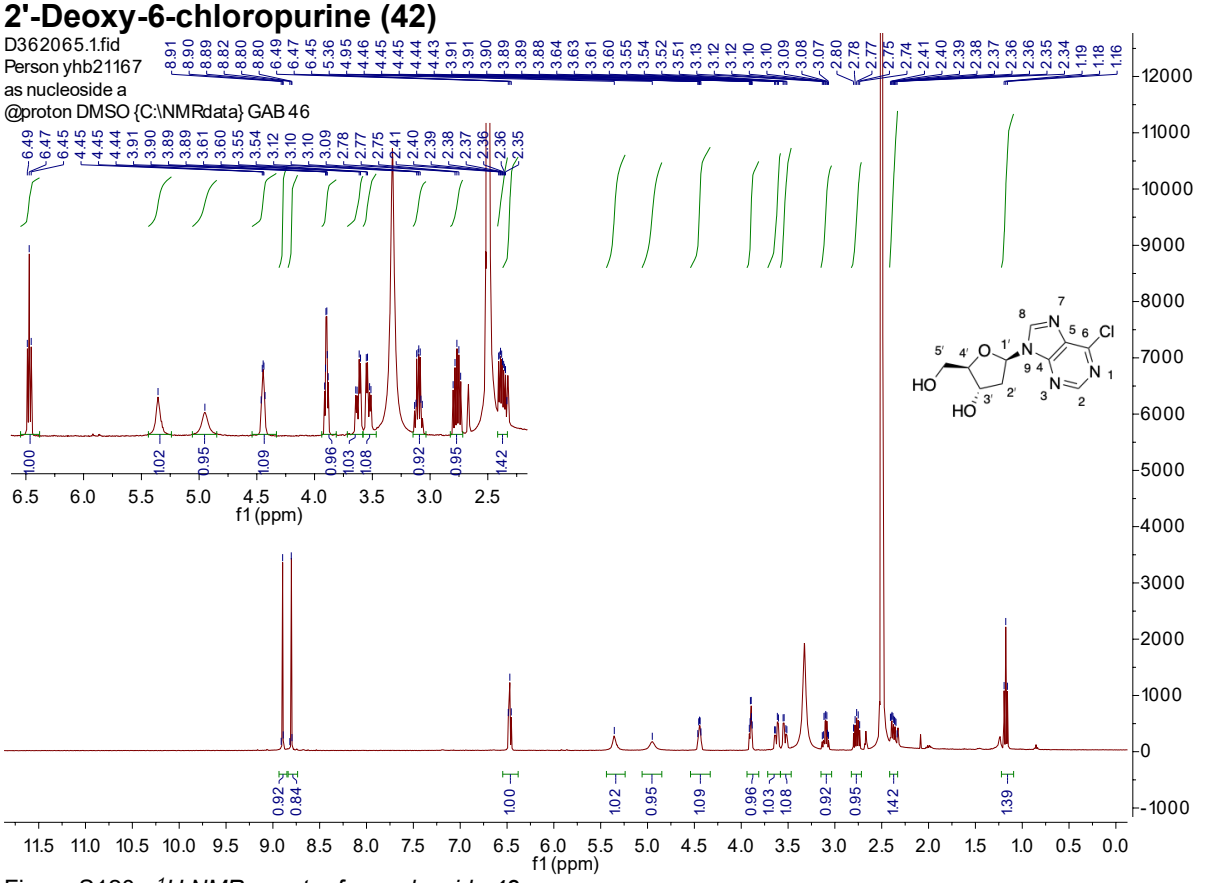


Figure S120 - *^{1}H NMR spectra for nucleoside 42.*

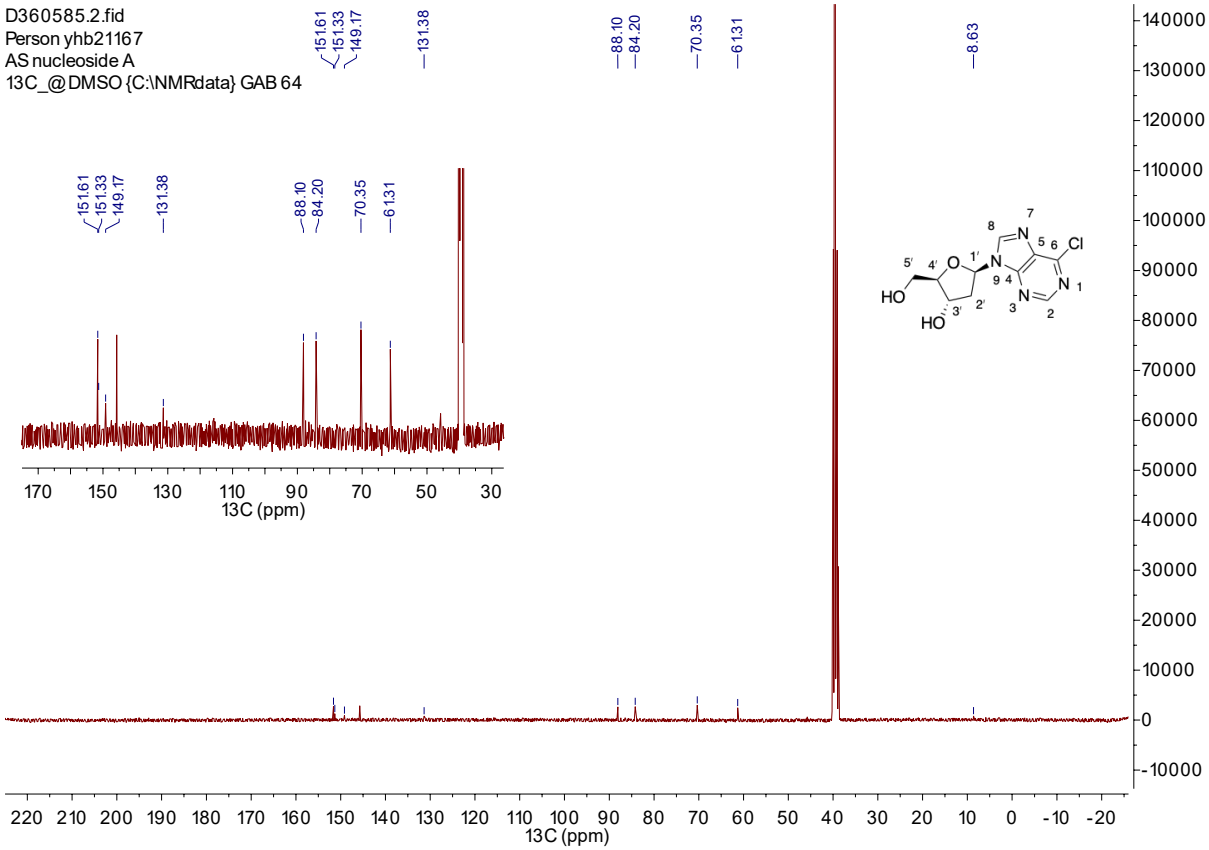
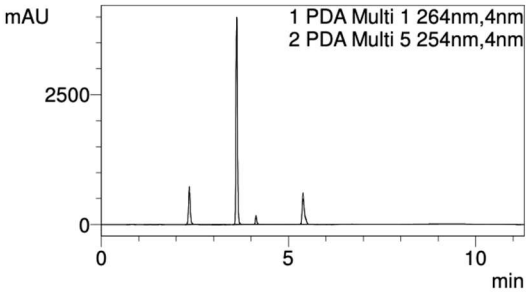


Figure S121 - *13C{1H}-NMR spectra for nucleoside 42.*

Sample Name	: A RXN		
Sample ID	:		
Data Filename	: RXNS AND STDS_22042022_004.lcd		
Method Filename	: Admir Trans glyco Run.lcm		
Batch Filename	: RXNS AND STDS.lcb		
Vial #	: 1-64	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 22/04/2022 11:31:32	Acquired by	: Shimadzu
Date Processed	: 22/04/2022 11:42:53	Processed by	: Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.783	6260	3089	
2	0.847	3993	1516	
3	1.163	1192	409	
4	1.312	2406	856	
5	1.568	6575	2508	
6	2.354	2397463	716717	
7	3.100	1458	245	
8	3.254	7162	2537	
9	3.619	11422241	3986016	
10	4.134	475763	173524	
11	4.504	14594	1167	
12	4.869	11012	904	
13	5.033	4402	851	
14	5.391	2622815	599142	
15	5.610	1526	655	
16	6.051	8765	3657	

Figure S122 - *HPLC trace used in the reaction where nucleoside 42 is obtained. R.T = 2.35 = thymine, 3.62 = dT, 4.13 = starting material nucleobase A, 5.39 = nucleoside 42.*

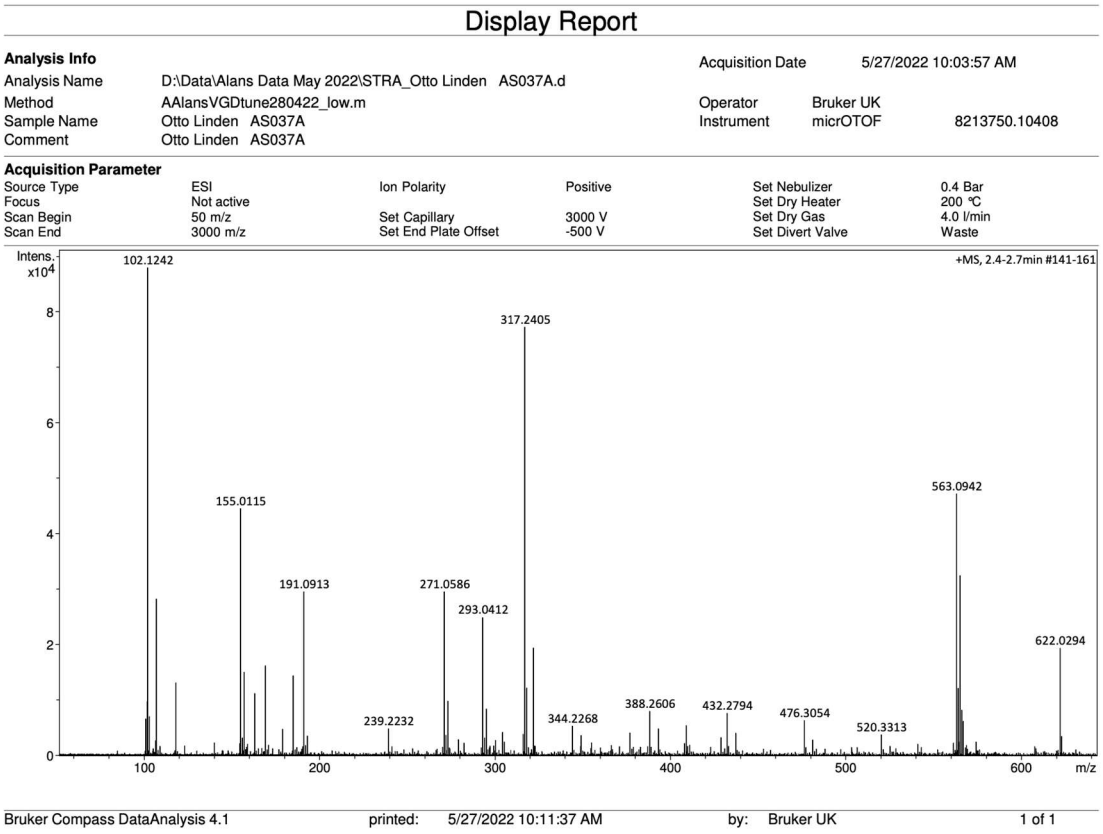


Figure S123 - *HRMS of nucleoside 42.*

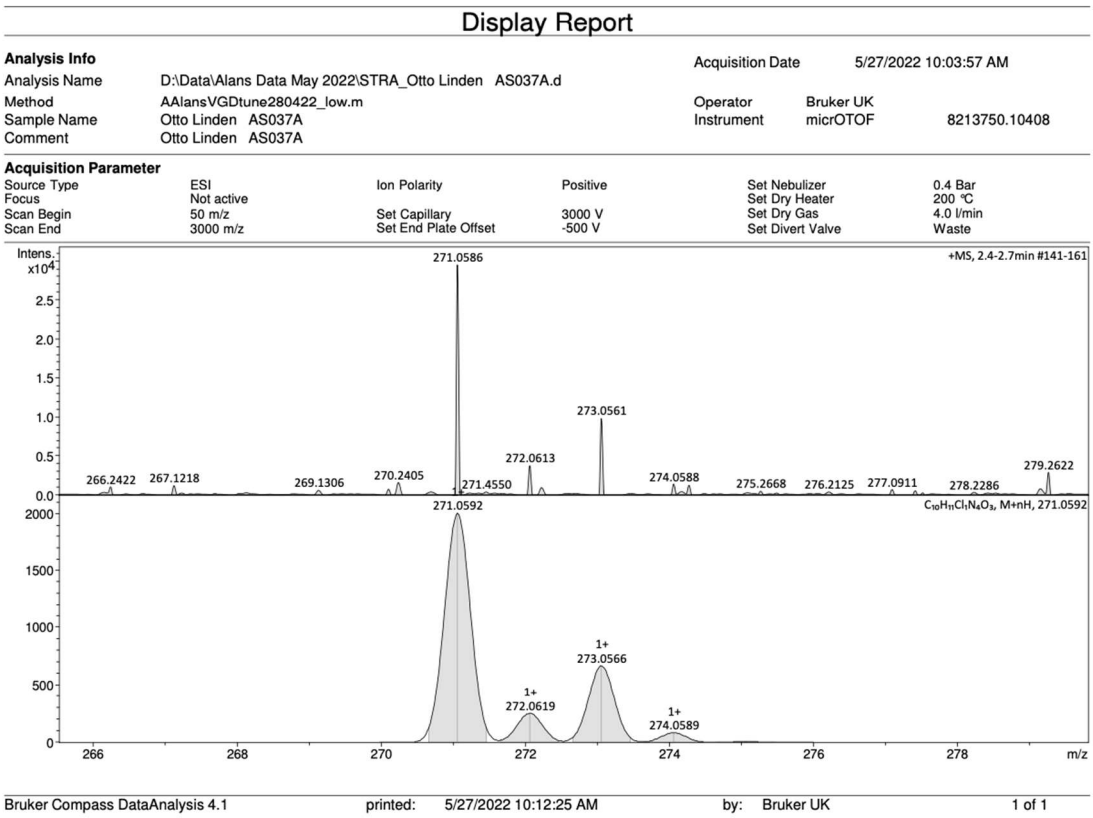


Figure S124 - *HRMS of nucleoside 42.*

2'-Deoxy-6-methyladenosine (43)

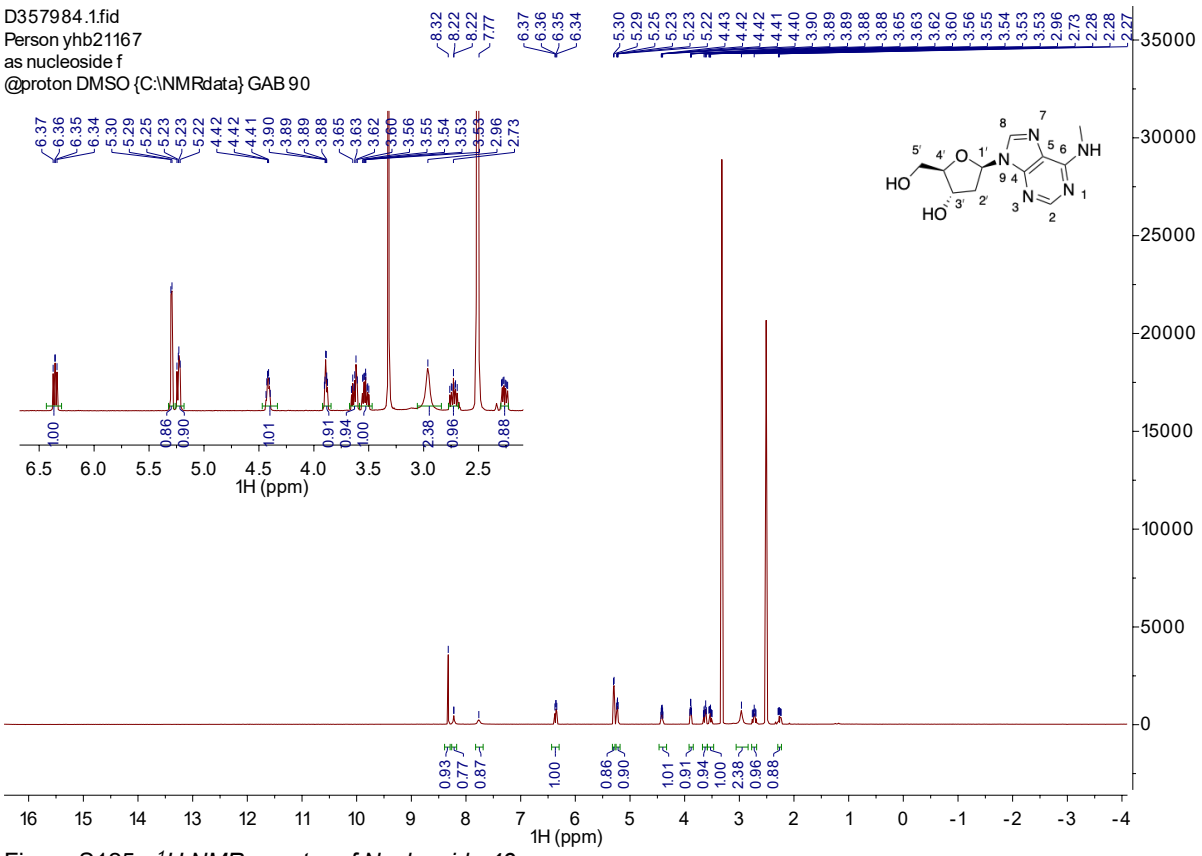


Figure S125 - *^{1}H NMR spectra of Nucleoside 43*

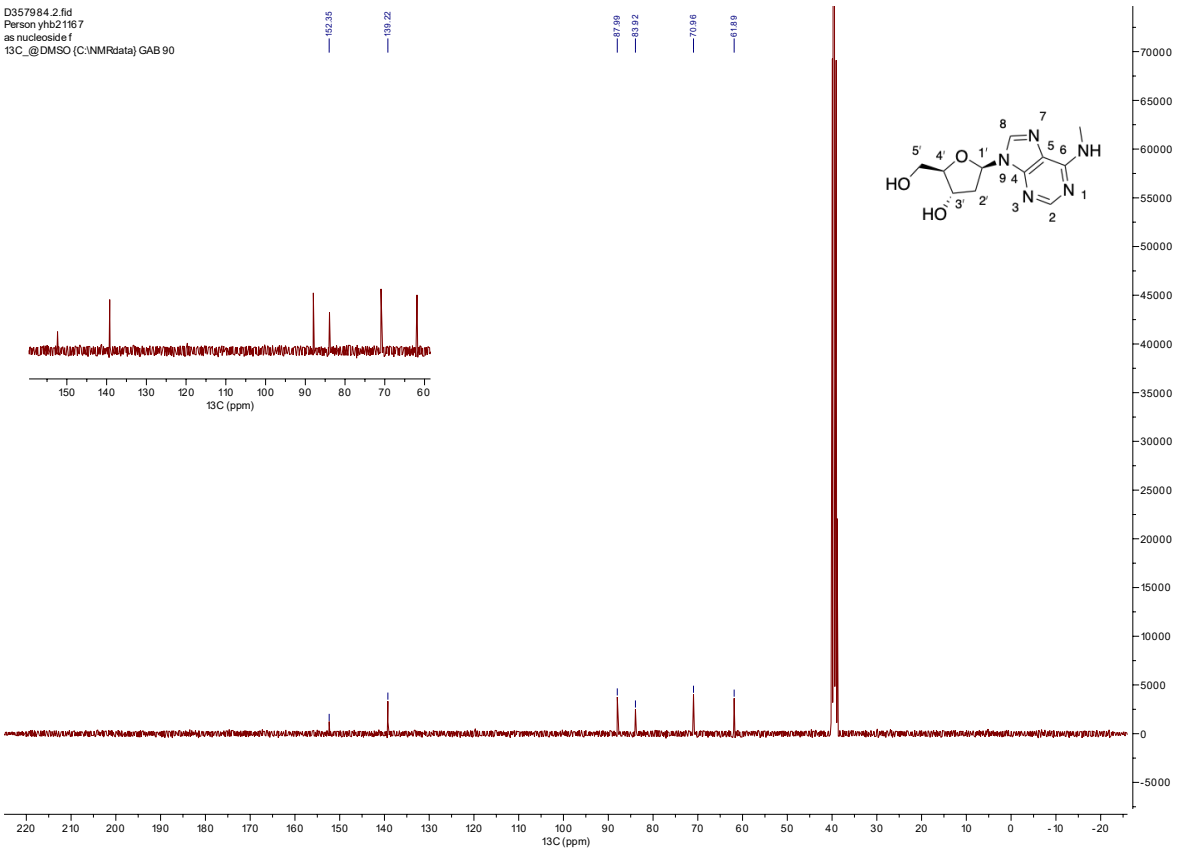
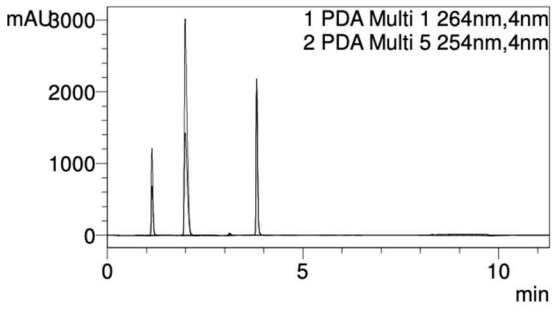


Figure S126- *13C{1H}-NMR spectra of Nucleoside 43*

Sample Name : F AS028
Sample ID :
Data Filename : REACTIONS_24022022_004.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : REACTIONS.lcb
Vial # : 1-4
Injection Volume : 10 uL
Date Acquired : 24/02/2022 15:39:08
Date Processed : 24/02/2022 15:50:28

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.798	4296	2170	
2	0.878	2284	576	
3	1.008	2535	649	
4	1.142	3333757	1194309	
5	1.991	14345579	2990956	
6	2.458	4410	1354	
7	2.568	4627	1327	
8	3.129	109524	32941	
9	3.719	26537	8597	
10	3.821	6109263	2167997	
11	4.495	1334	350	
12	4.712	18284	5320	
13	5.499	12696	2787	

Figure S127 - *HPLC trace for the reaction used to obtain Nucleoside 43. R.T = 1.1 = nucleobase cytosine released, 1.99 = nucleoside dC, 3.13 = nucleobase F, 3.82 = Nucleoside 43*

Display Report

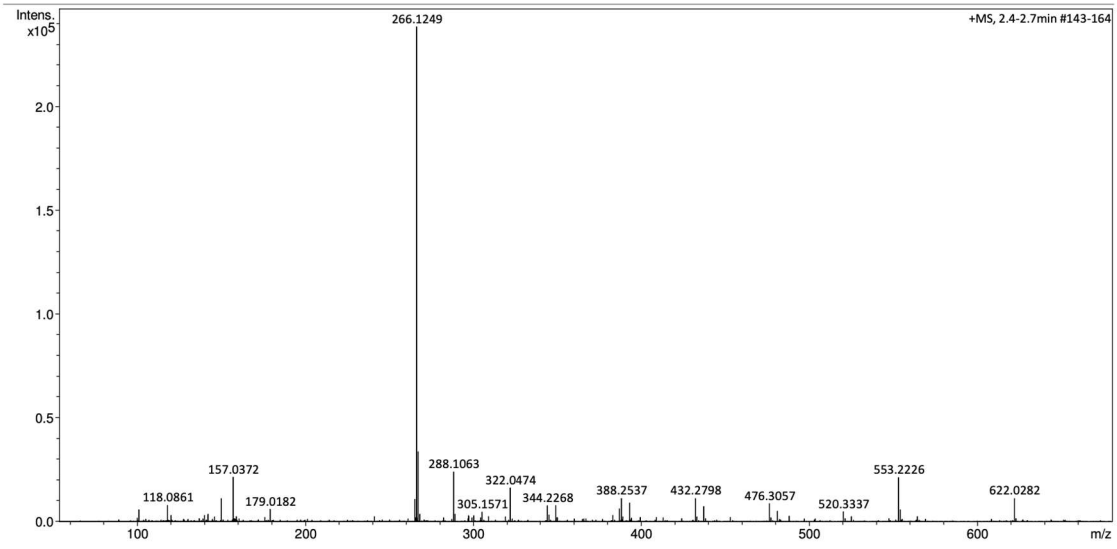
Analysis Info

Analysis Name D:\Data\Alans Data May 2022\STRA_Otto Linden AS028F.d
Method AAlansVGDtune280422_low.m
Sample Name Otto Linden AS028F
Comment Otto Linden AS028F

Acquisition Date 5/26/2022 4:06:32 PM
Operator Bruker UK
Instrument micrOTOF 8213750.10408

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	200 °C
Scan Begin	50 m/z	Set Capillary	3000 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Bruker Compass DataAnalysis 4.1 printed: 5/26/2022 4:11:22 PM by: Bruker UK 1 of 1

Figure S128 - *HRMS of Nucleoside 43*

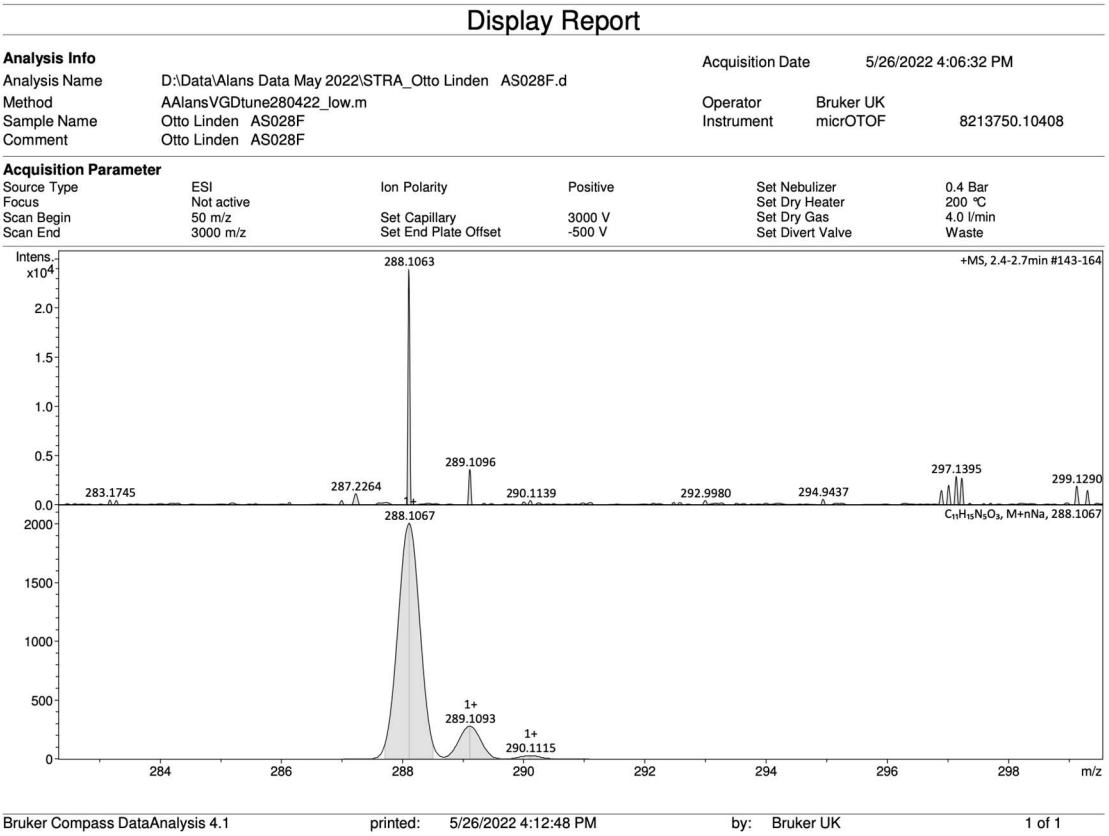


Figure S129 - *HRMS of Nucleoside 43*

2'-Deoxy-N6-benzoyladenosine (44)

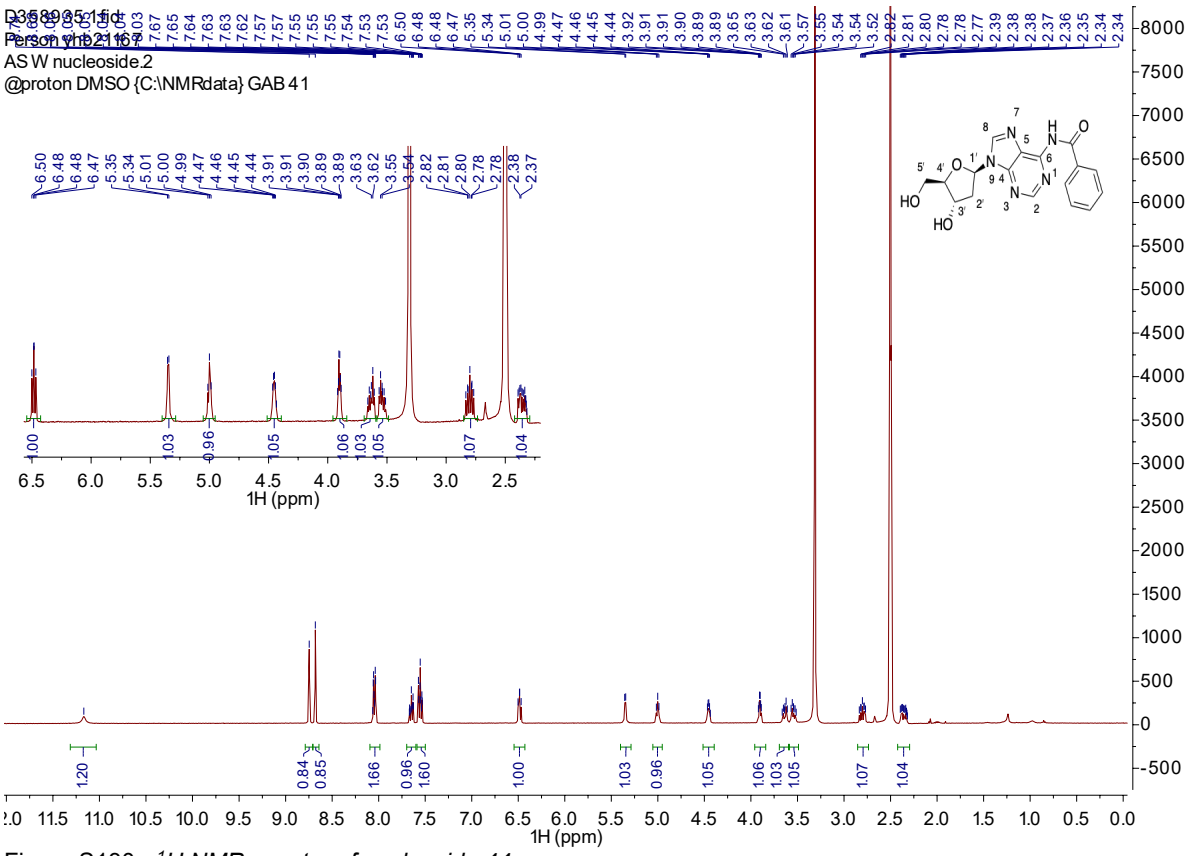


Figure S130 - *^{1}H NMR spectra of nucleoside 44*

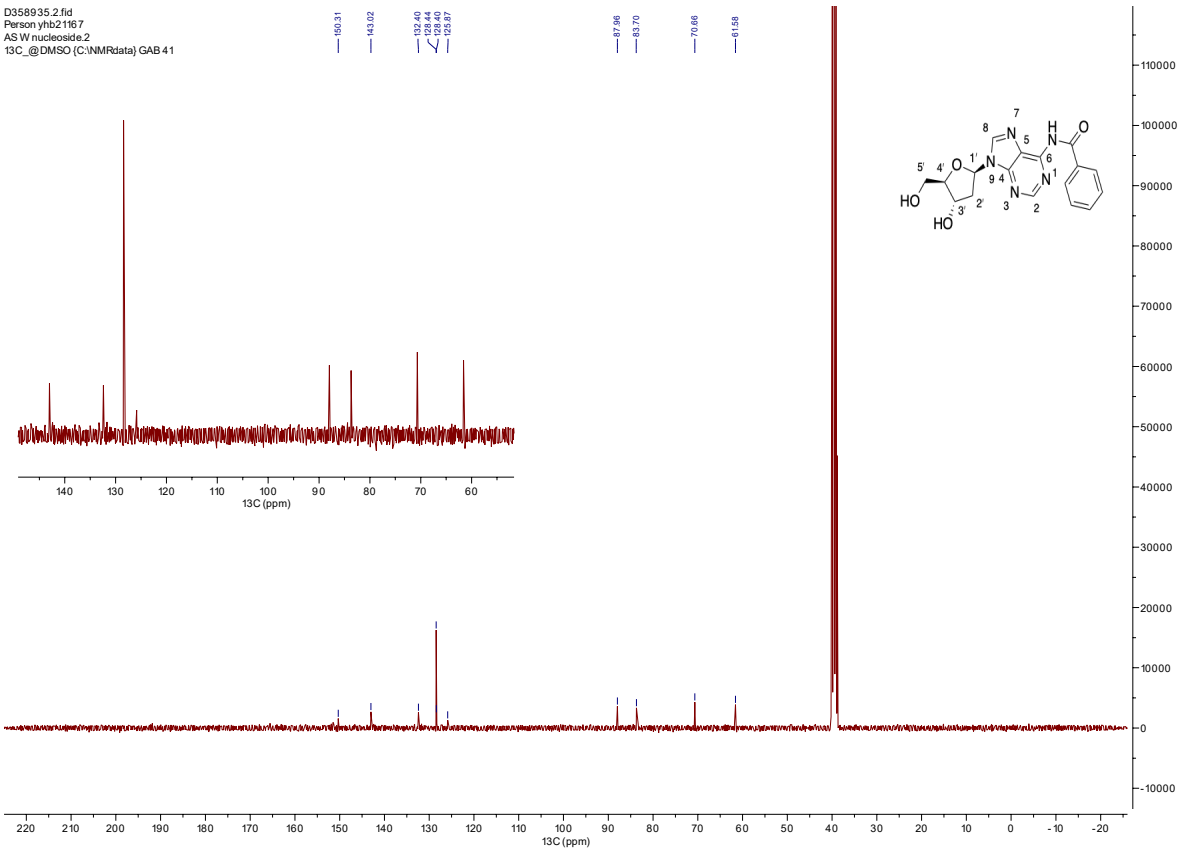
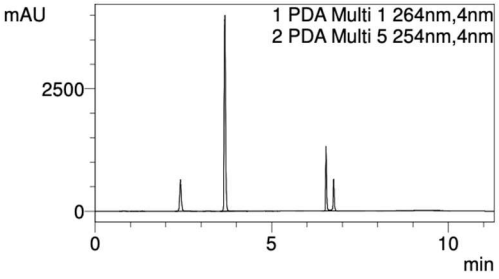


Figure S131 - *13C{1H}-NMR spectra of nucleoside 44*

Sample Name : as029 w
Sample ID :
Data Filename : base w_02032022_003.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : base w.lcb
Vial # : 1-22 Sample Type : Unknown
Injection Volume : 10 uL
Date Acquired : 02/03/2022 16:18:36 Acquired by : Shimadzu
Date Processed : 02/03/2022 16:29:56 Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.784	6728	3005	
2	1.134	1583	153	
3	1.336	1203	447	
4	2.419	2175811	643698	
5	3.212	12804	4390	
6	3.677	11938343	3995239	
7	4.911	1534	279	
8	5.770	1969	298	
9	6.540	2640750	1299609	
10	6.757	1578028	647973	
11	8.085	1973	834	

Figure S132 - *HPLC trace of the reaction used to obtain nucleoside 44. R.T = 2.42 = nucleobase thymine, 3.68 = dT, 6.54= nucleoside 44, 6.76 = starting material nucleobase*

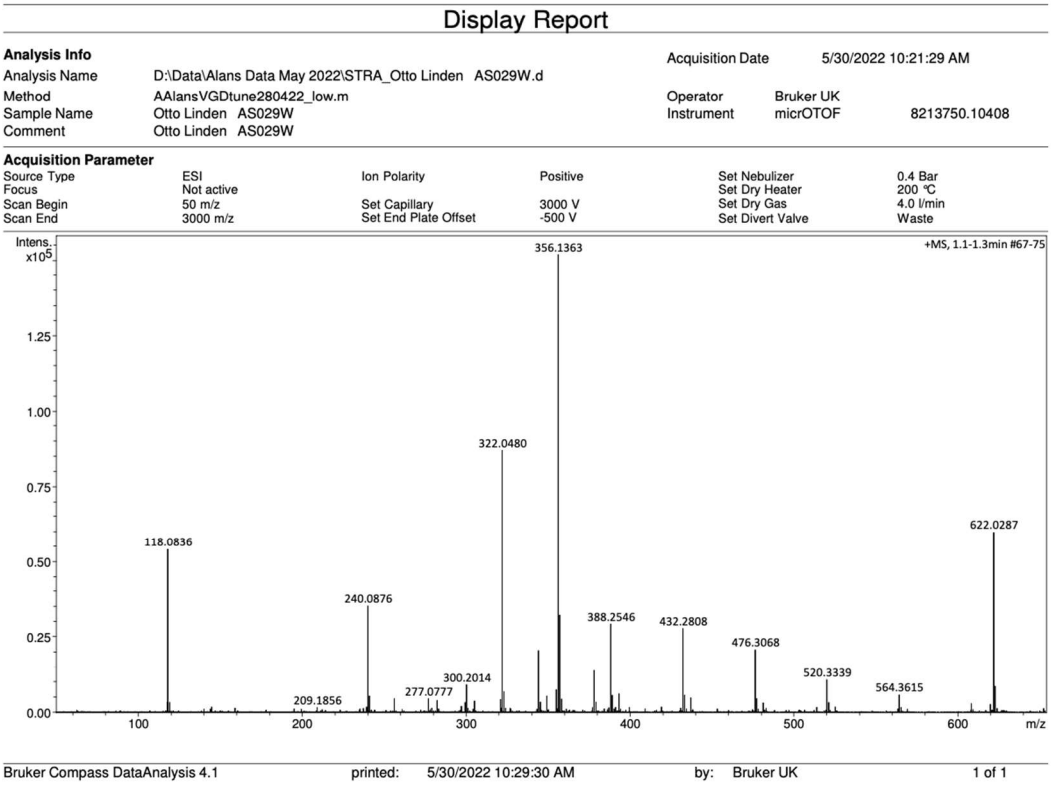


Figure S133 - *HRMS of nucleoside 44*

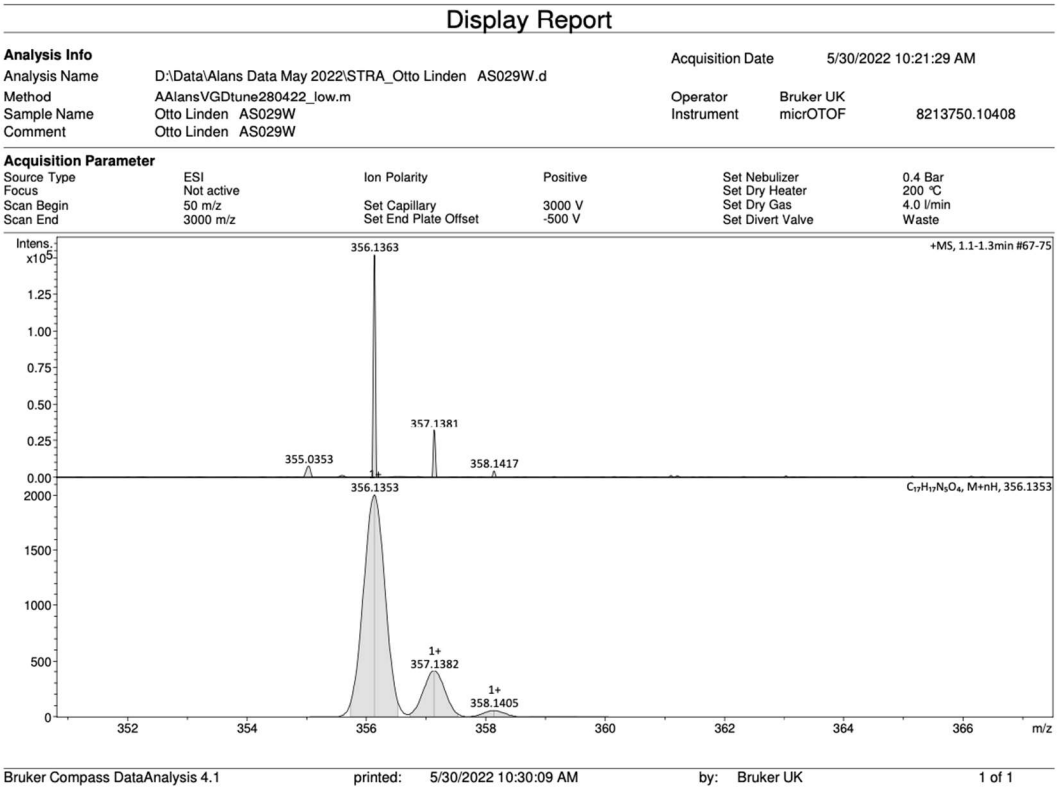


Figure S134 - *HRMS of nucleoside 44*

2'-Deoxy-deaza-2-bromopurine (45)

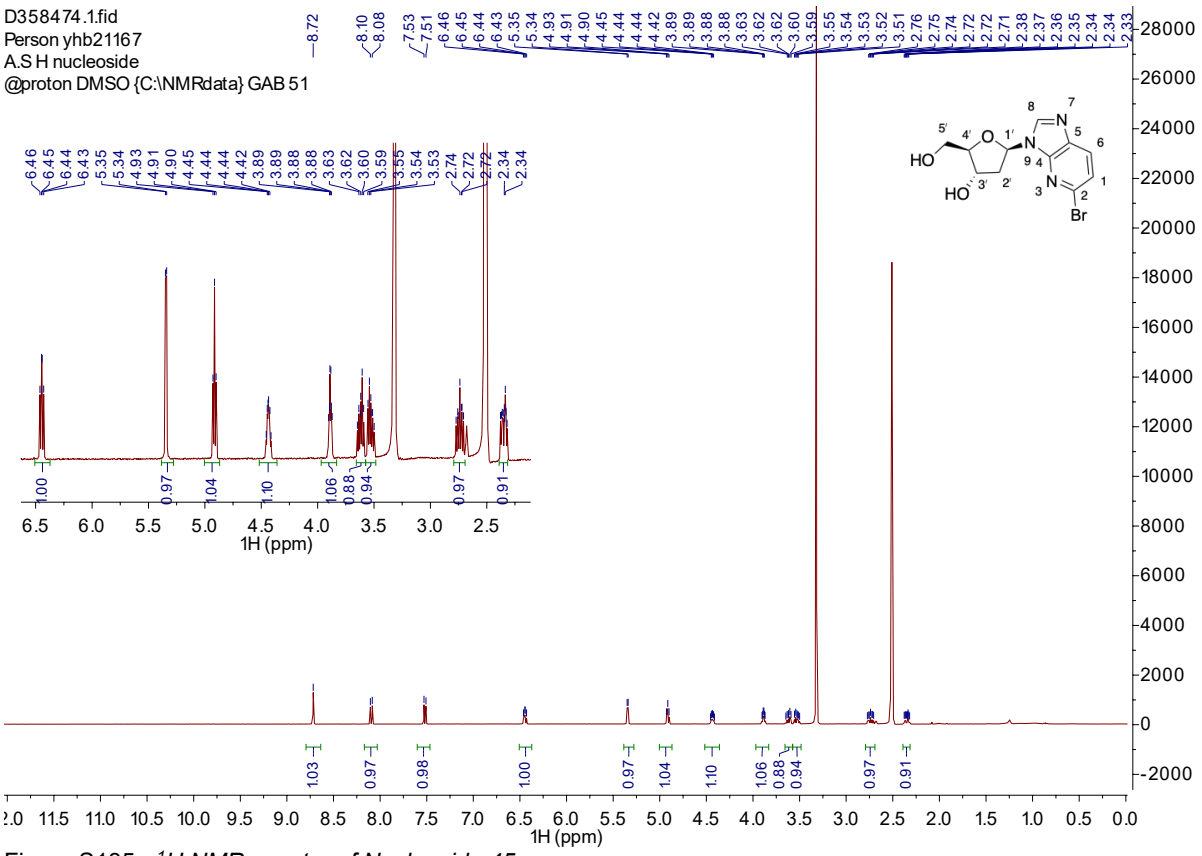


Figure S135 - *^{1}H NMR spectra of Nucleoside 45*

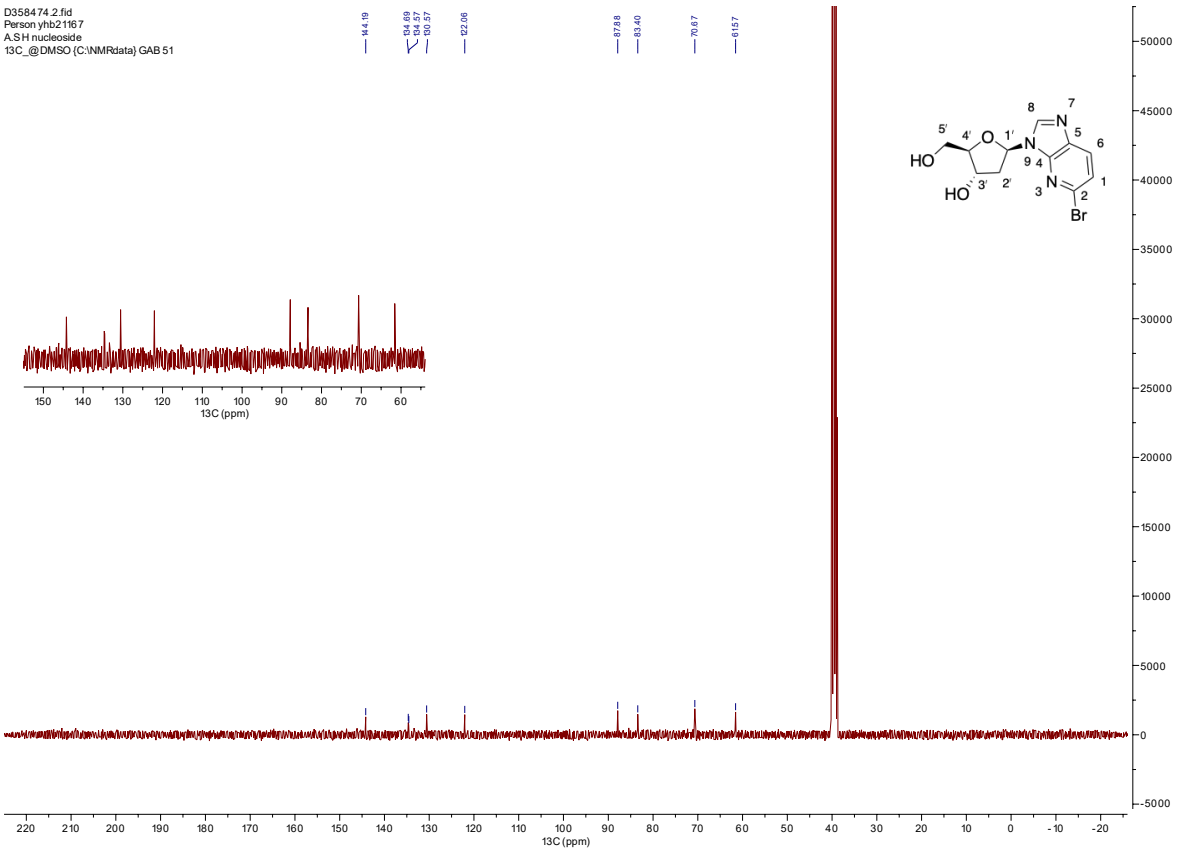


Figure S136 - *13C{1H}-NMR spectra of Nucleoside 45*

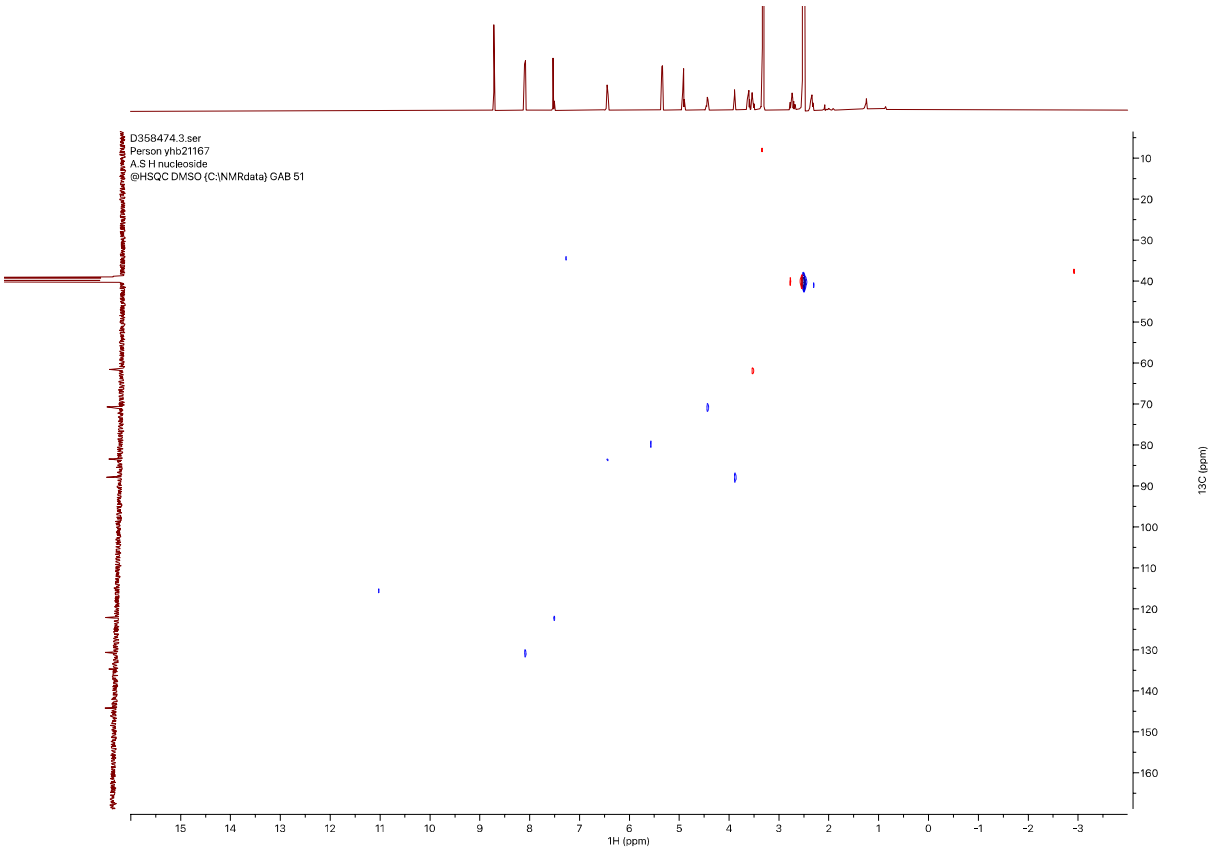


Figure S137 – *HSQC NMR spectra of Nucleoside 45*

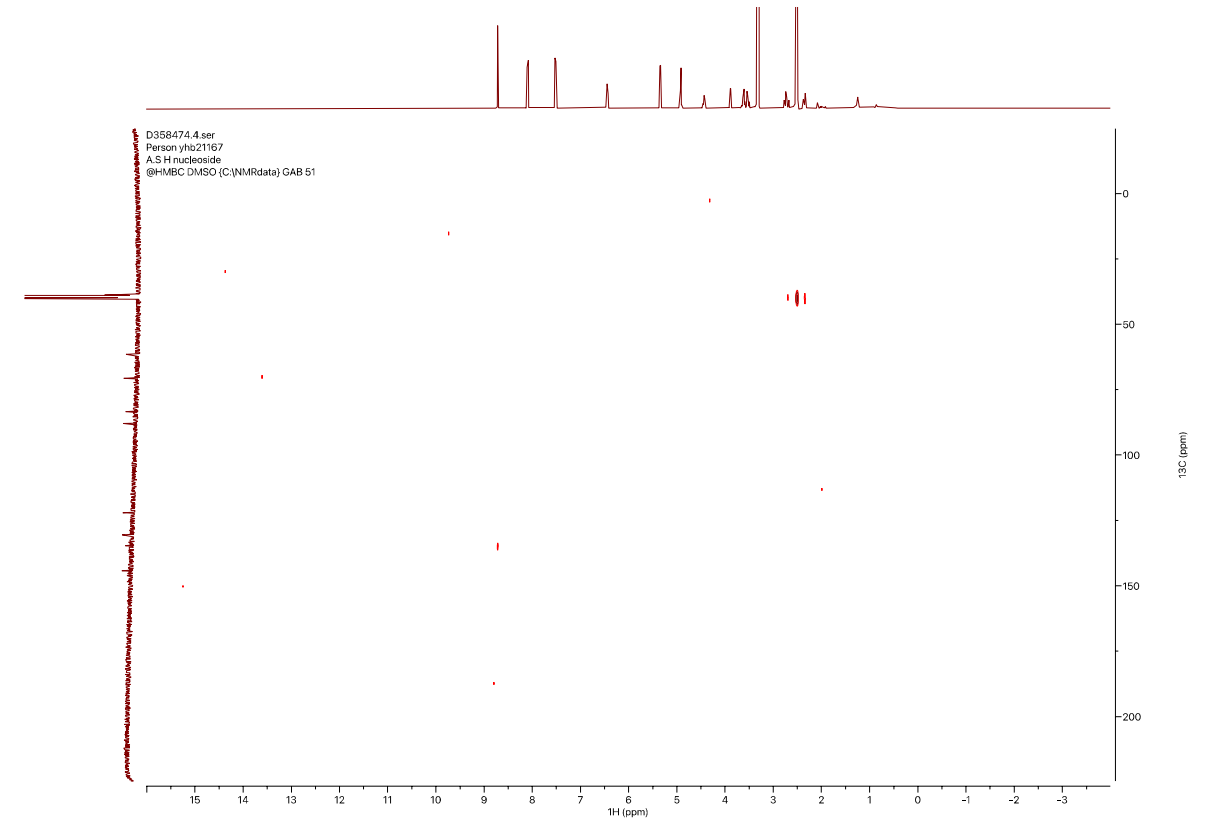
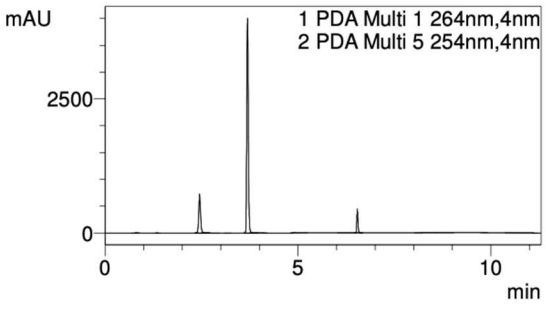


Figure S138 – *HMBC NMR spectra of Nucleoside 45*



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.792	4497	1626	
2	1.352	1866	607	
3	2.453	2493808	721506	
4	3.187	1387	324	
5	3.696	11693104	3962055	
6	4.920	48099	9236	
7	5.184	1283	163	
8	6.135	4644	1052	
9	6.239	2779	601	
10	6.336	4030	983	
11	6.540	952779	440731	
12	8.229	5381	618	
13	8.309	4724	1621	

Figure S139 - *HPLC trace of the reaction used to obtain Nucleoside 45. R.T =2.4 = nucleobase released thymine, 3.7 = nucleoside dT, 6.54= Target Nucleoside 45*

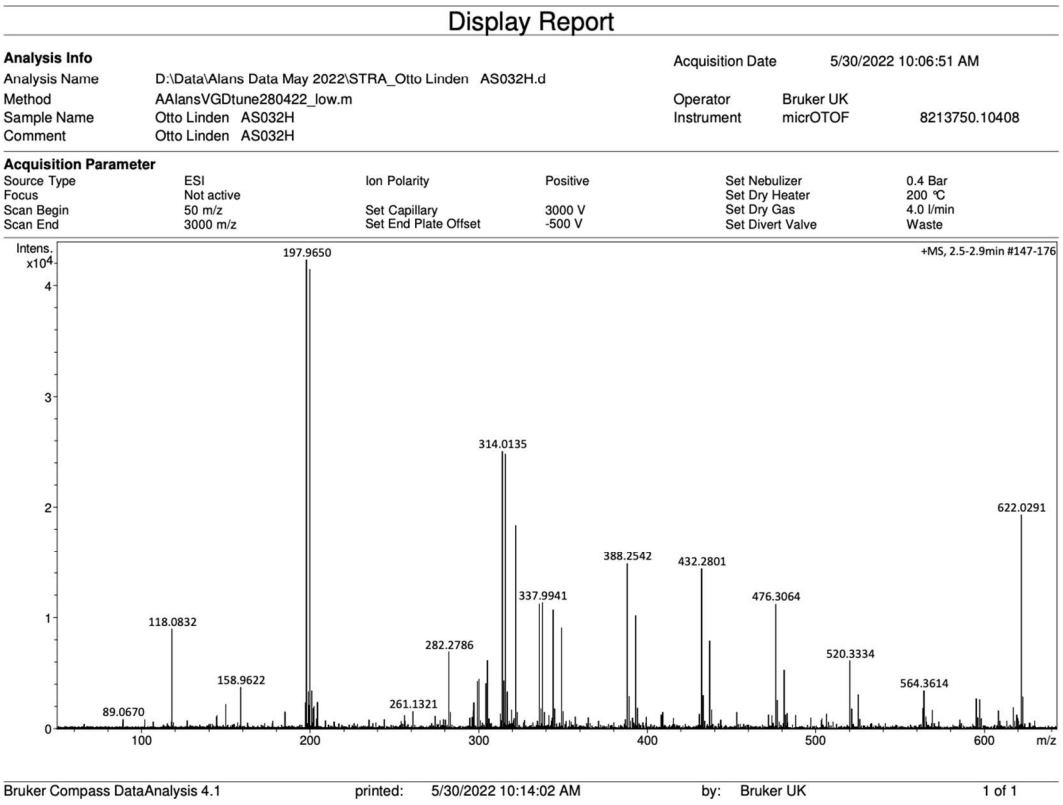


Figure S140 - *HRMS of Nucleoside 45*

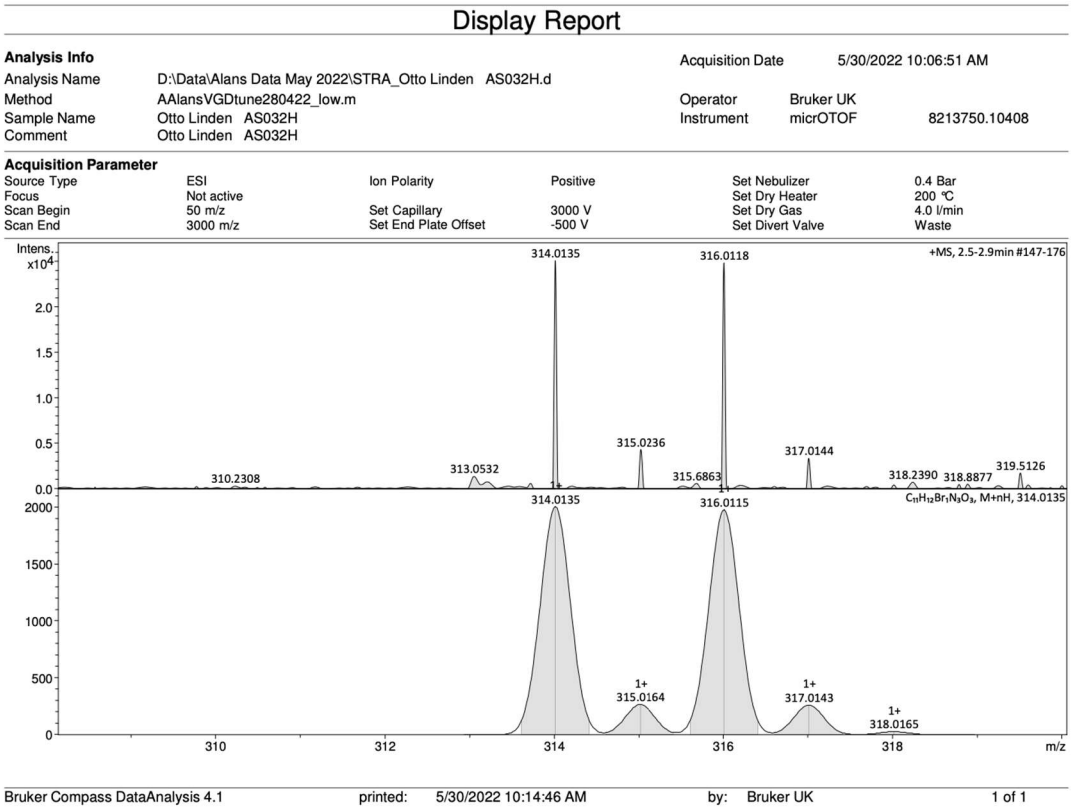


Figure S141 - *HRMS of Nucleoside 45*

2'-Deoxy-deaza-2-methylpurine (46)

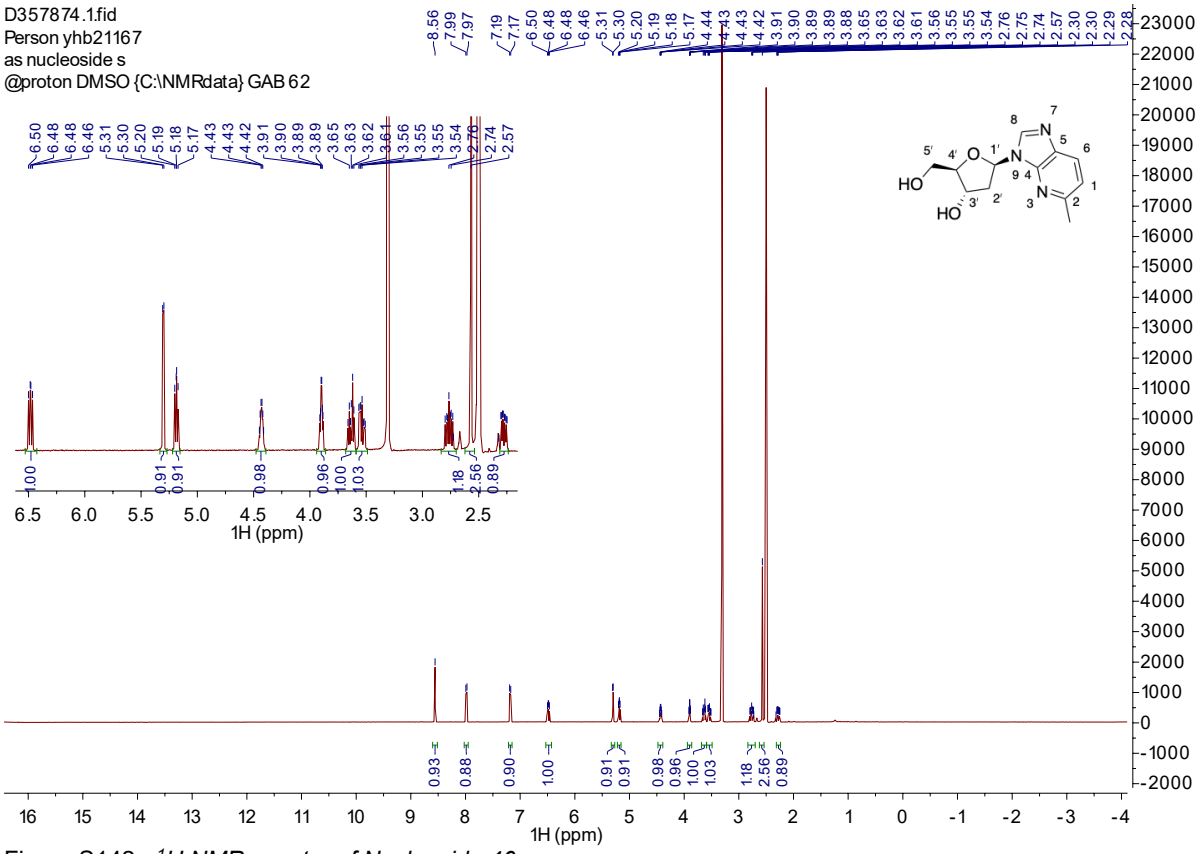


Figure S142 - *^{1}H NMR spectra of Nucleoside 46*

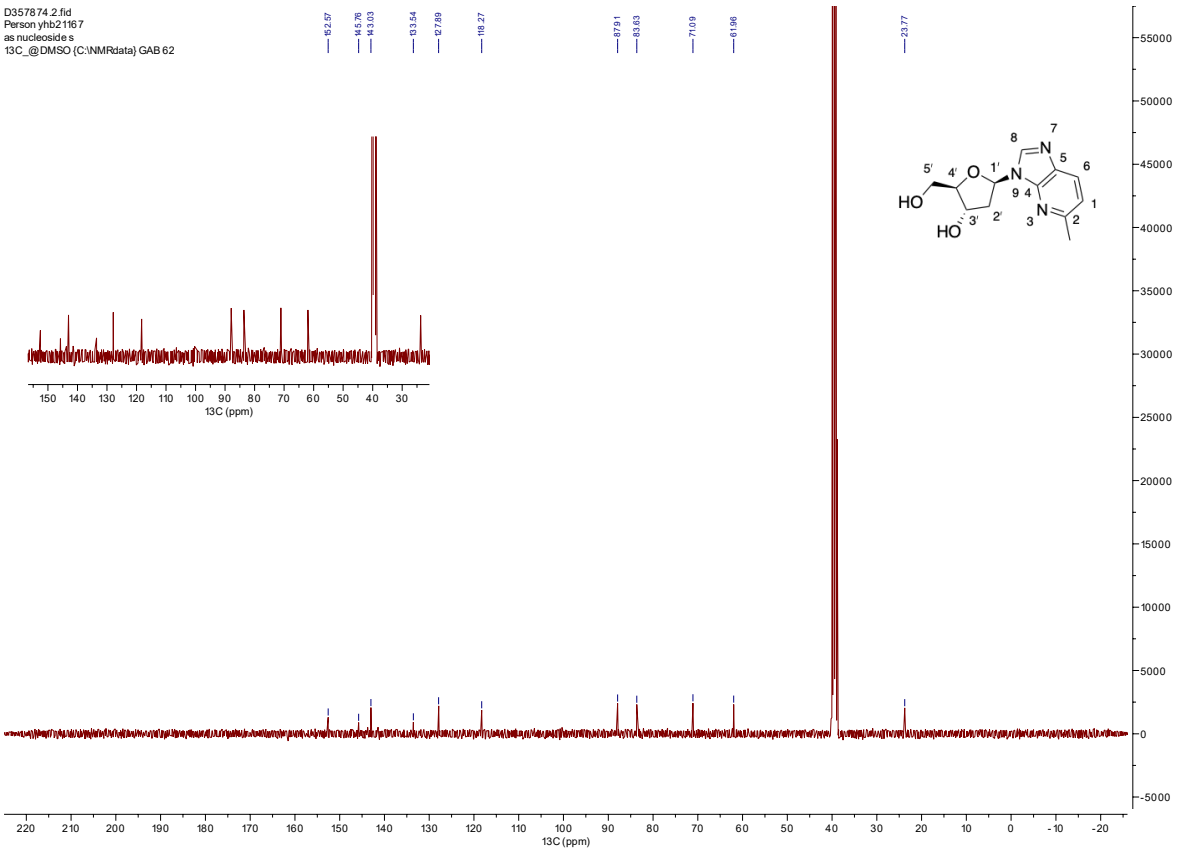
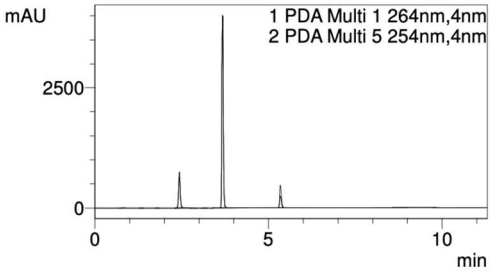


Figure S143 - *13C{1H}-NMR spectra of Nucleoside 46*

Sample Name : AS032 S
Sample ID :
Data Filename : BASE S RXN_08032022_002.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : BASE S RXN.lcb
Vial # : 1-85
Injection Volume : 10 uL
Date Acquired : 08/03/2022 15:17:38
Date Processed : 08/03/2022 15:28:59

Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.787	4320	1544	
2	1.342	1694	545	
3	1.789	1175	391	
4	2.435	2499073	739466	
5	3.170	1380	335	
6	3.440	22812	6483	
7	3.680	11889360	3974722	
8	4.919	4383	502	
9	5.128	4582	453	
10	5.343	1645117	465451	
11	5.972	1048	256	
12	8.233	5566	650	

Figure S144 - *HPLC trace of the final reaction to obtain Nucleoside 46. R.T = 2.44 = thymine nucleobase, 3.68 = nucleoside thymidine, 5.34 = Nucleoside 46*

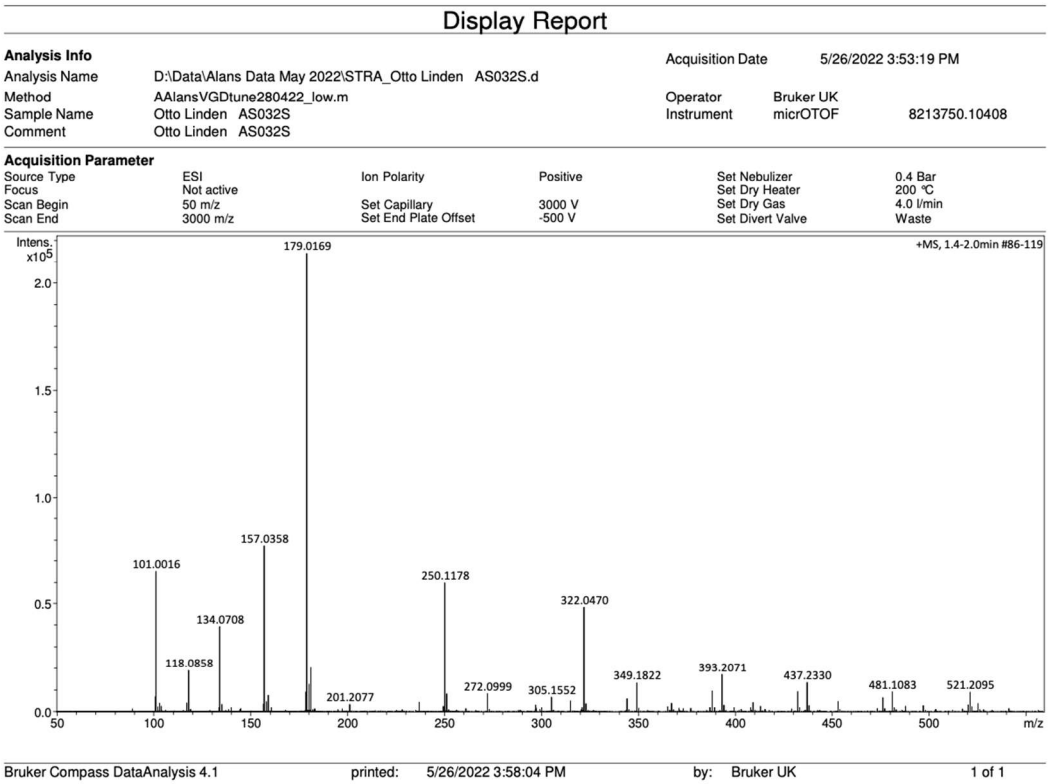


Figure S145 - *HRMS of Nucleoside 46*

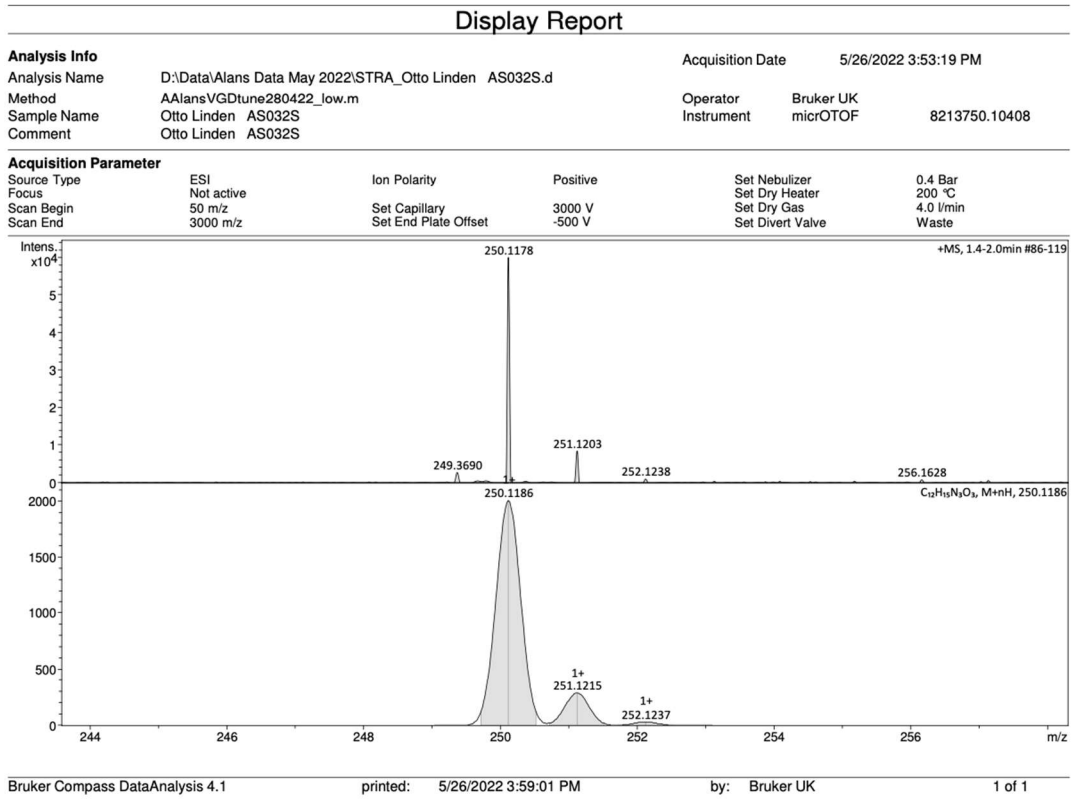


Figure S146 - *HRMS of Nucleoside 46*

2'-Deoxy-deaza-2-trifluoropurine (47)

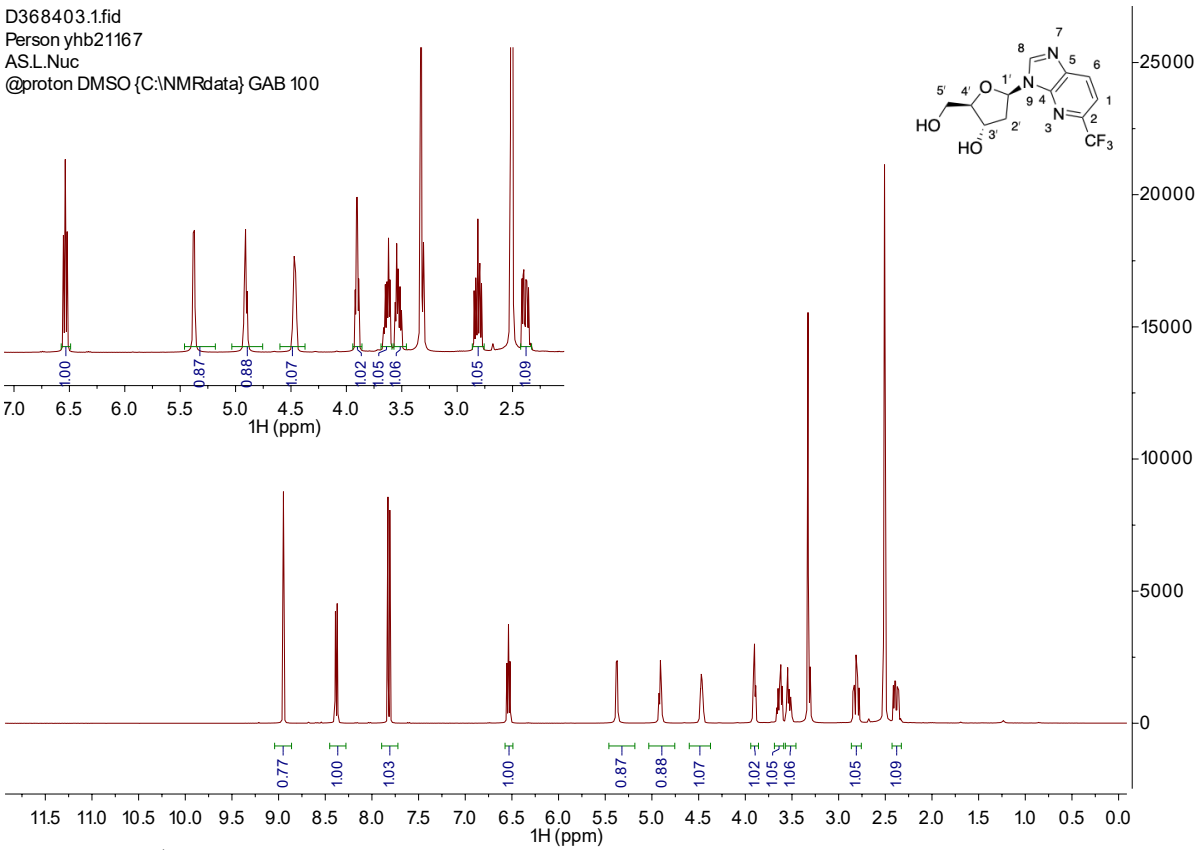


Figure S147 - *^{1}H NMR spectra of nucleoside 47*

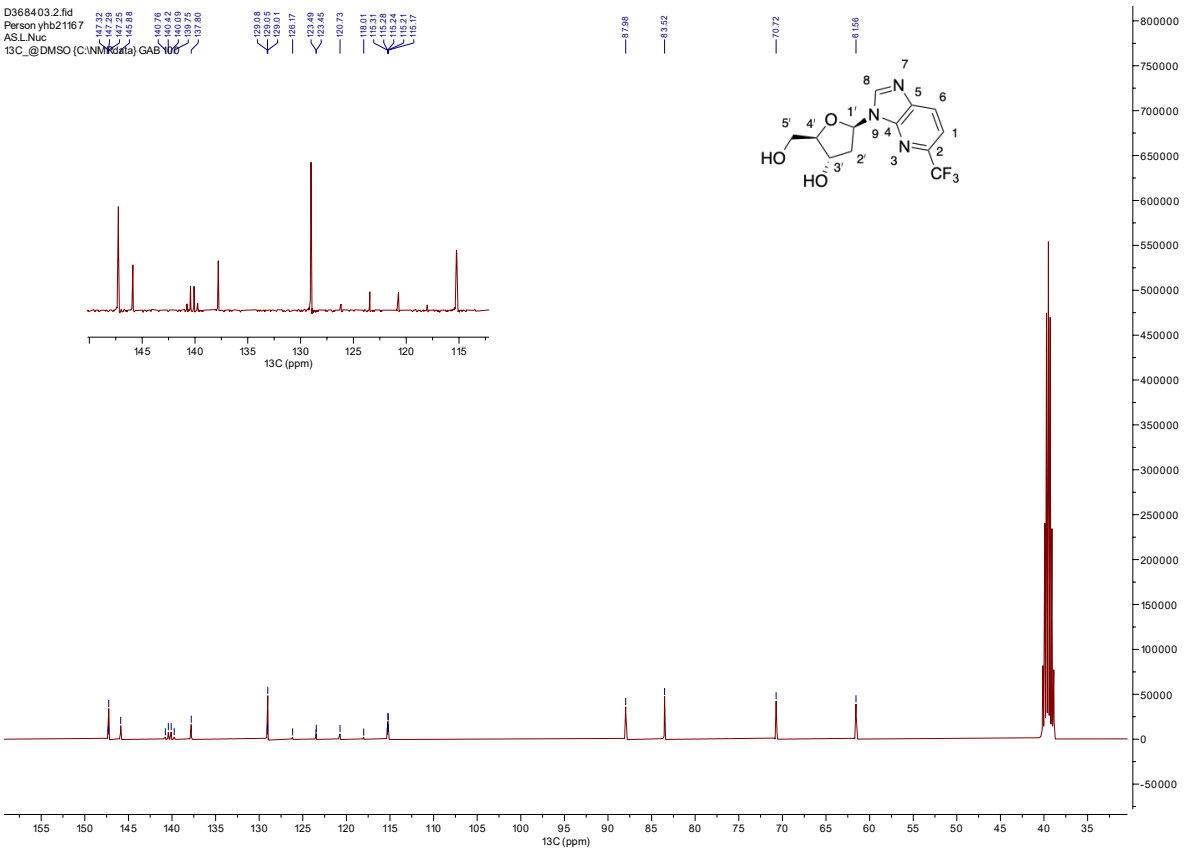


Figure S148 -*13C{1H}-NMR spectra of nucleoside 47*

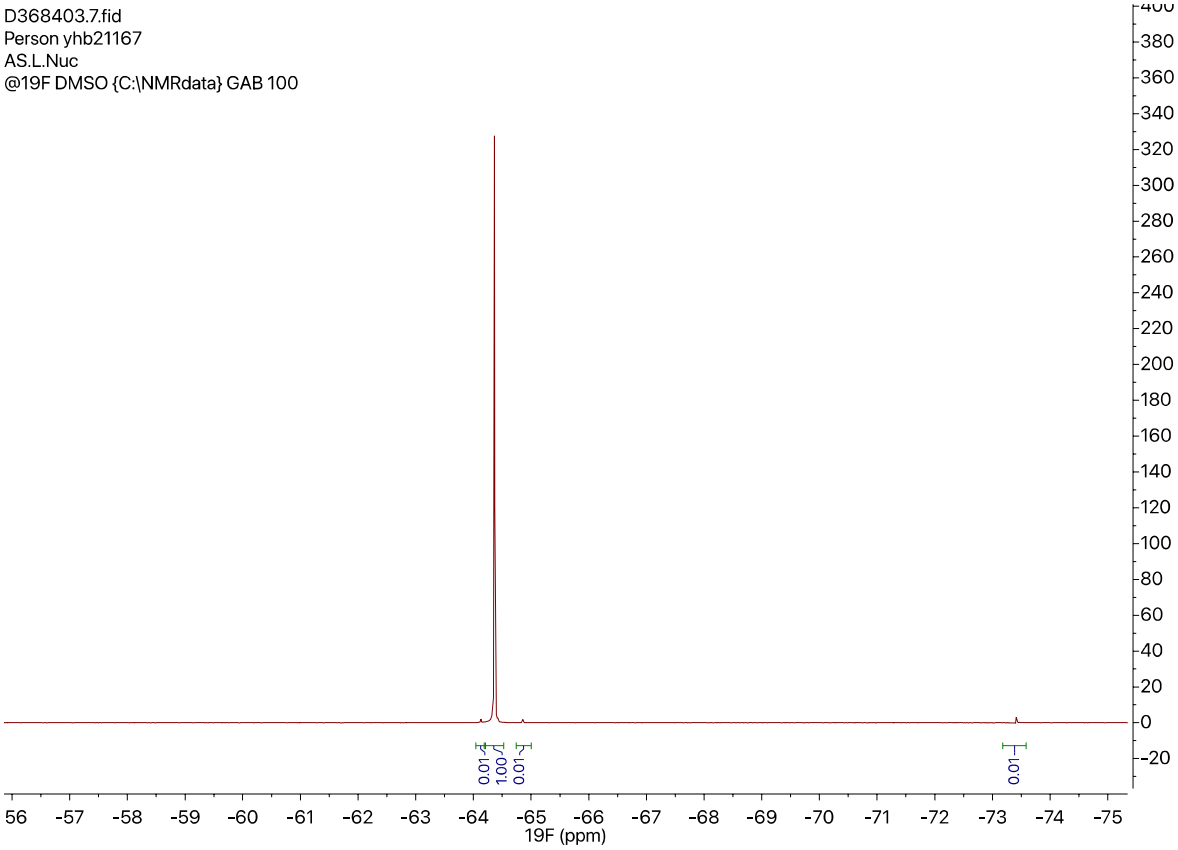
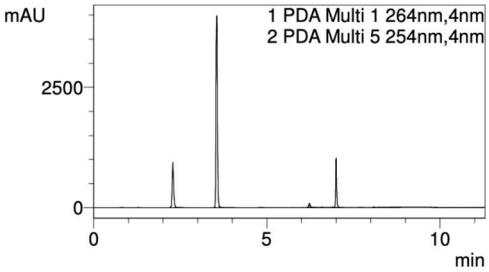


Figure S149 - *19F NMR spectra for nucleoside 47*

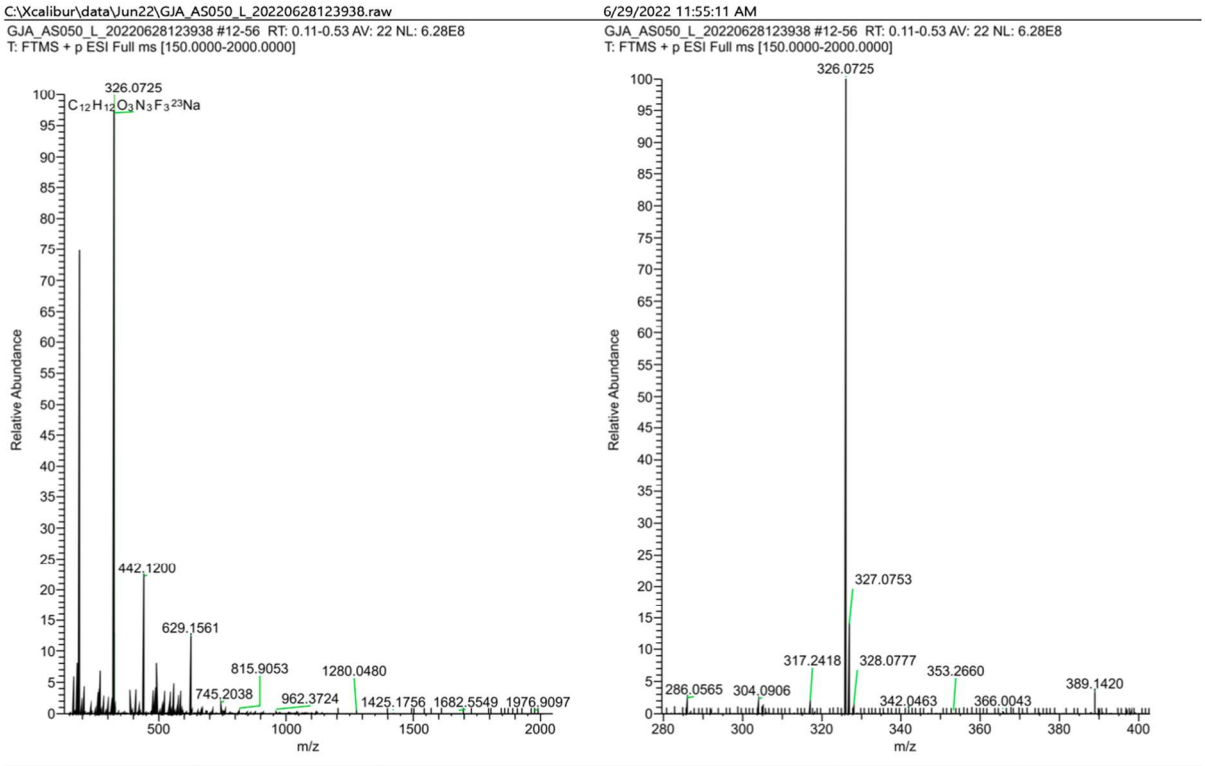
Sample Name	: L.2 RXN		
Sample ID	:		
Data Filename	: AS050 RXNS_09062022_012.lcd		
Method Filename	: Admir Trans glyco Run.lcm		
Batch Filename	: AS050 RXNS.lcb		
Vial #	: 1-27	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 09/06/2022 15:08:08	Acquired by	: Shimadzu
Date Processed	: 09/06/2022 15:19:29	Processed by	: Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.789	5645	2499	
2	1.117	1392	161	
3	1.287	3089	1023	
4	2.287	2999723	925809	
5	3.552	11738995	3925434	
6	4.813	2265	423	
7	6.228	341675	90028	
8	6.593	57570	5945	
9	7.000	2106961	972883	
10	7.695	5197	2495	
11	8.087	14627	7413	

Figure S150 - *HPLC trace for the reaction used to obtain nucleoside 47. R.T- 2.29 = thymine, 3.5 = thymidine, 6.23 = left over nucleobase L, 7.0 = nucleoside 47.*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
326.0725	$C_{12}H_{12}O_3N_3F_3{}^{23}Na$	85.08998321 64638	6.50	0.66	326.07230	1	98.99	3	0	99.77	100	(Collection)
326.0725	$C_{15}H_{11}O_2N_3F_2{}^{23}Na$	28.89309220 14385	10.50	4.16	326.07115	2	96.04	4	0	99.77	99.91	(Collection)

Figure S151 - *HRMS of nucleoside 47*

2'-Deoxy-deaza-8-chloropurine (48)

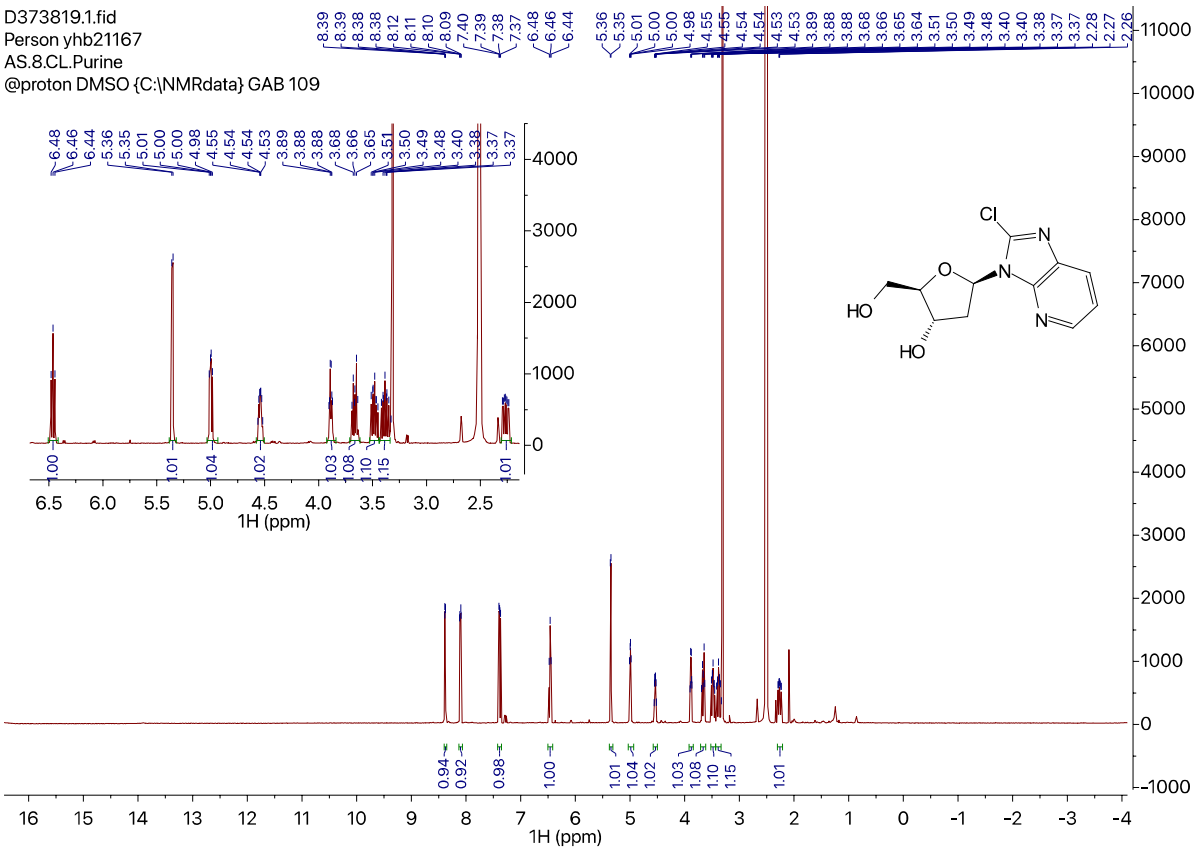


Figure S152 - *1H NMR of nucleoside 48*

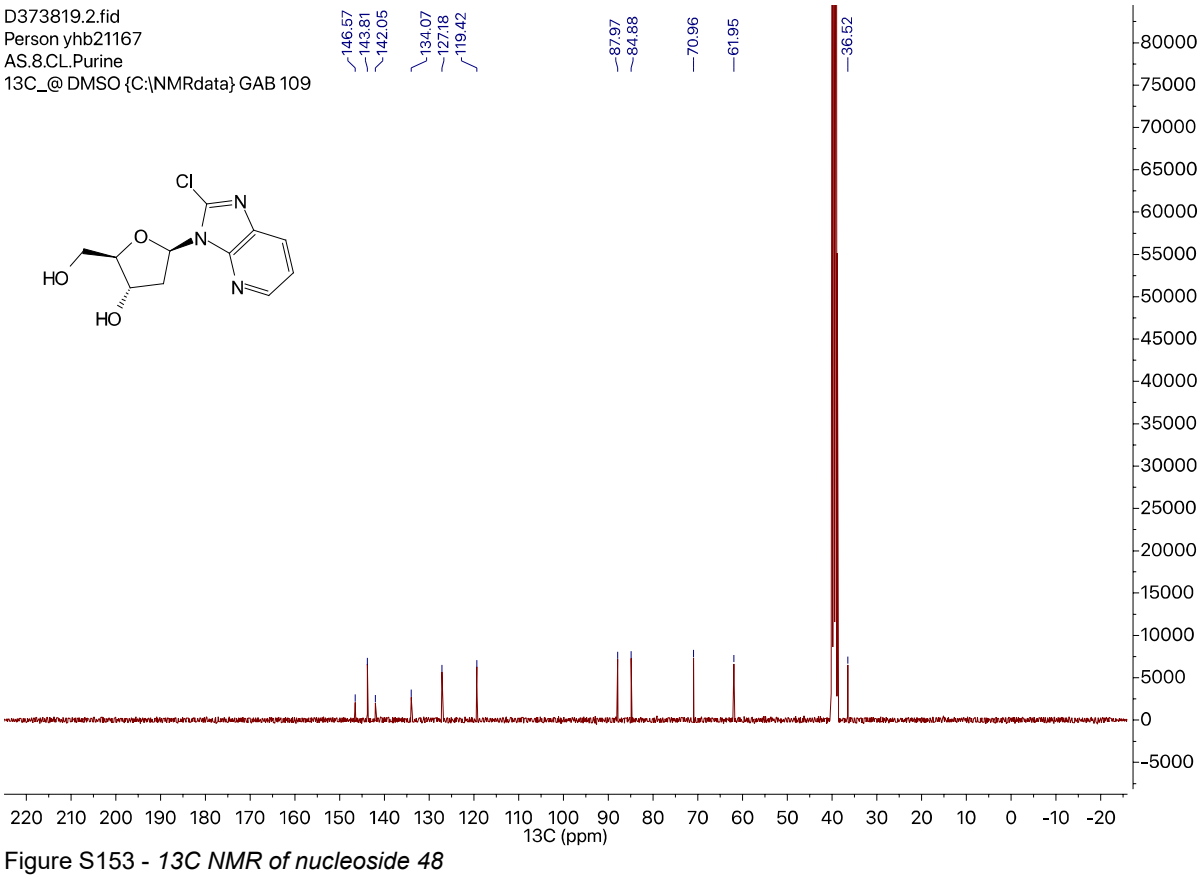
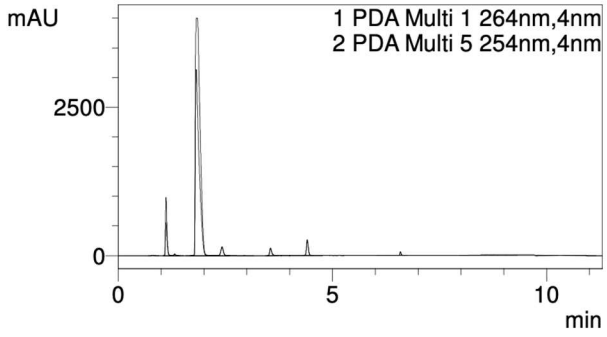


Figure S153 - *13C NMR of nucleoside 48*

Sample Name : 1.2
Sample ID :
Data Filename : rxns 1.2,1.4,1.5_20092022_001.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : rxns 1.2,1.4,1.5.lcb
Vial # : 1-16
Injection Volume : 10 uL
Date Acquired : 20/09/2022 12:10:11
Date Processed : 20/09/2022 12:21:32

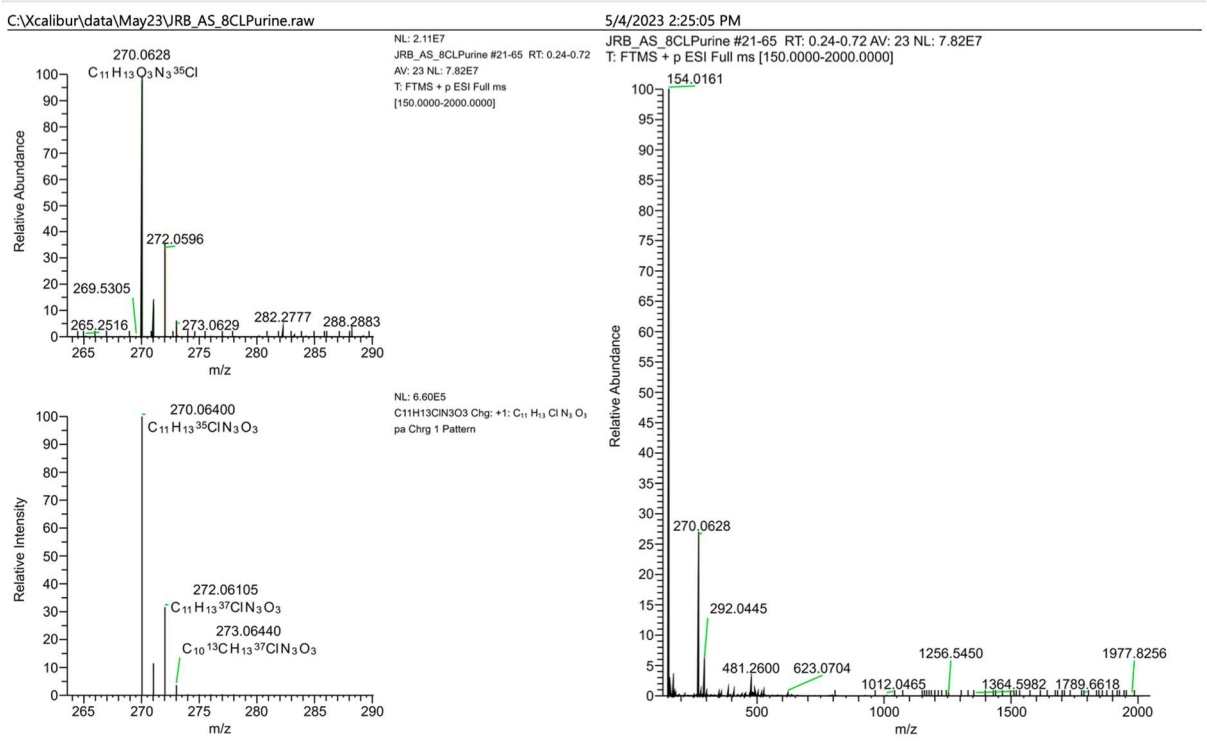
Sample Type : Unknown
Acquired by : Shimadzu
Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.792	9662	4698	
2	1.117	2426984	967378	
3	1.319	68226	23949	
4	1.833	30298234	4000096	
5	2.424	611590	146754	
6	3.556	466491	127865	
7	3.880	42488	3659	
8	4.413	900178	268008	
9	6.280	3359	340	
10	6.588	138583	63653	
11	8.053	2460	499	
12	8.319	31093	3854	
13	8.472	27770	3308	

Figure S154 - *HPLC spectrum of the reaction. R.T = 1.11 = released cytosine, 1.83 = dc, 3.56 = nucleobase 4.4 = nucleoside 48*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
270.0628	$C_{12}H_8O_3N_5$	25.41828821 25836	11.50	2.53	270.06217	1	71.16	3	1	73.7	97.42	(Collection)
270.0628	$C_{11}H_{13}O_3N_3{}^3{}^5Cl$	7.402979891 57069	6.50	-4.24	270.06400	2	70.21	8	0	73.7	74.34	(Collection)

Figure S155 - *HRMS of the nucleoside 48*

2'-Deoxy-deaza-8-trifluoropurine (49)

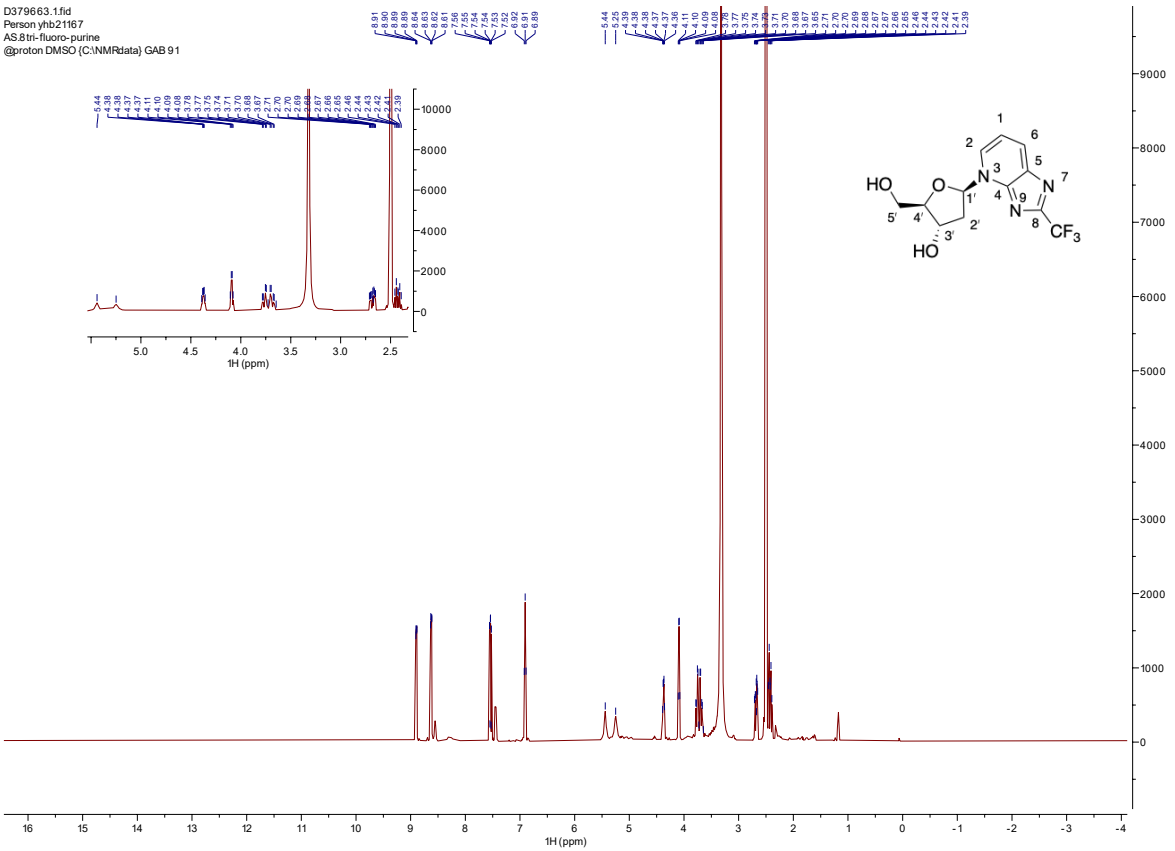


Figure S156 - *[1]H NMR of Nucleoside 49*

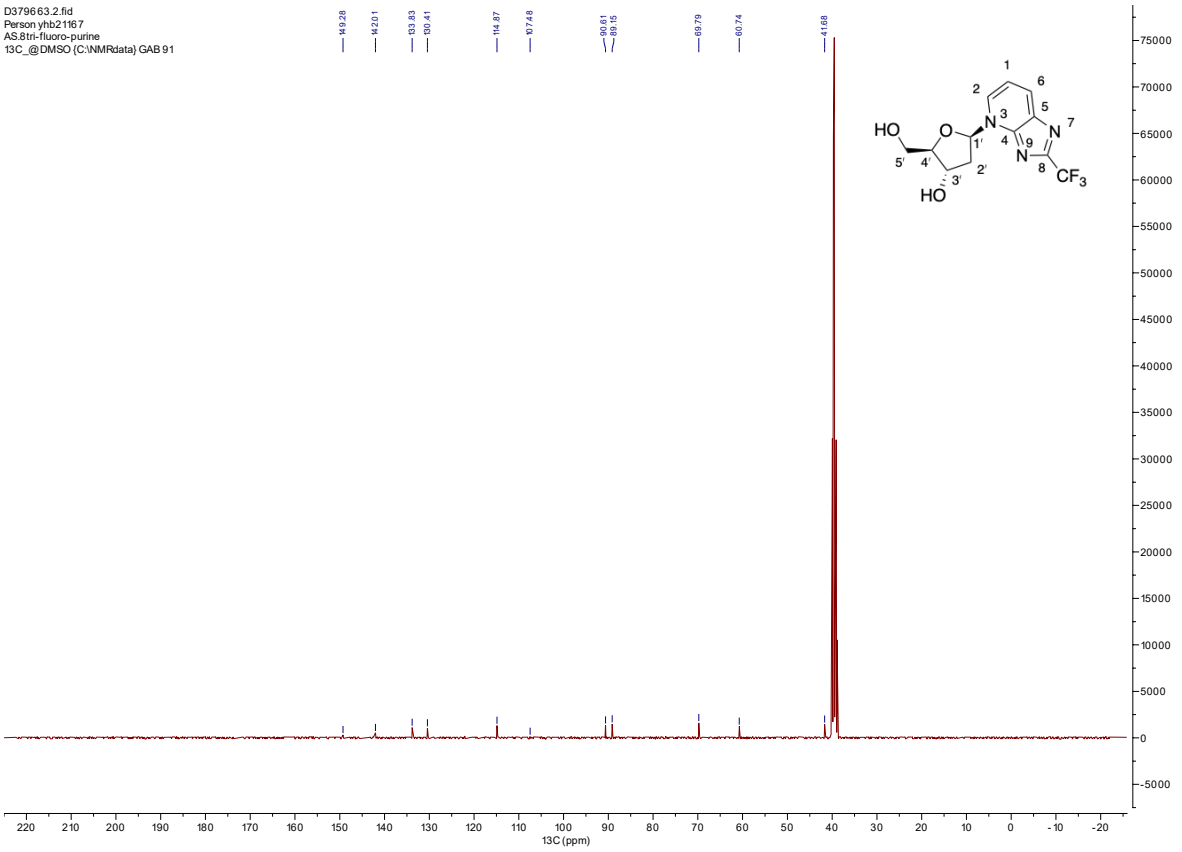


Figure S157 - *[13]C NMR of Nucleoside 49*

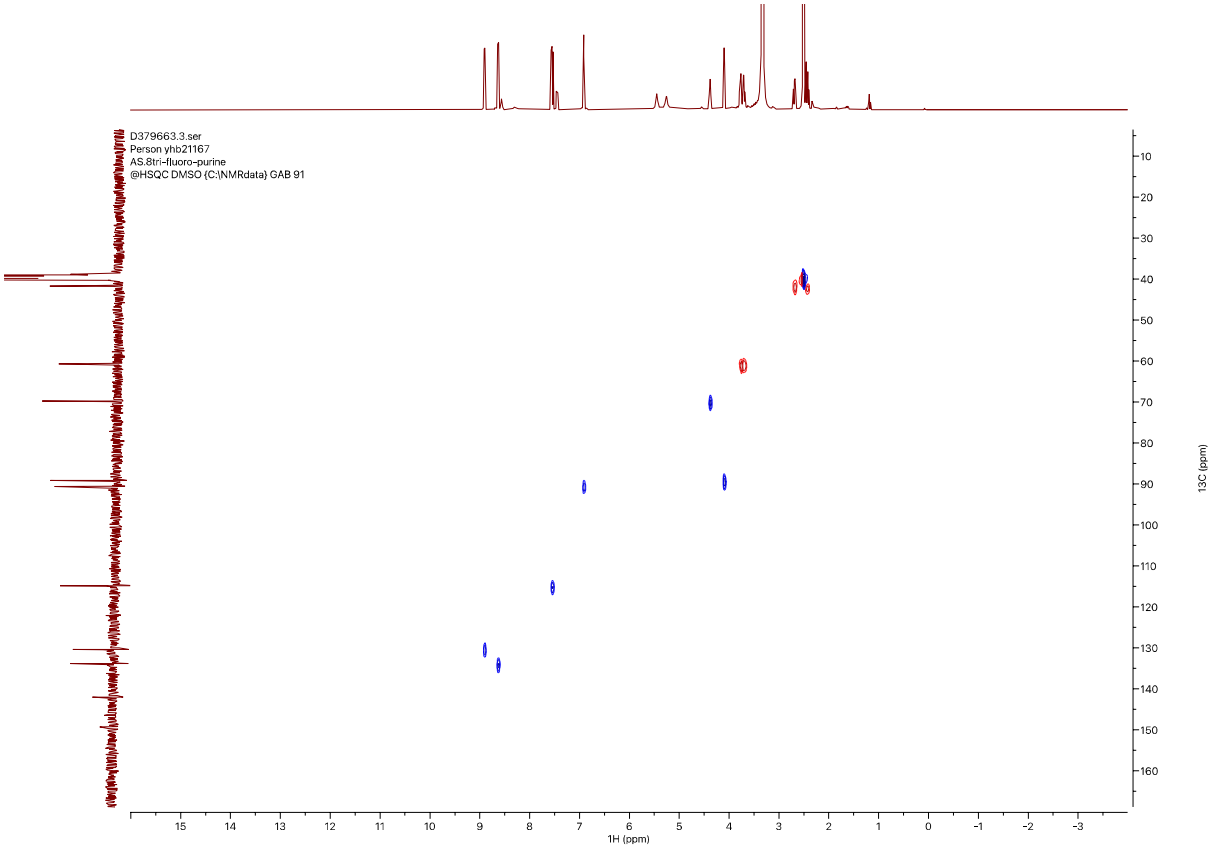


Figure S158 - HSQC *NMR of Nucleoside 49*

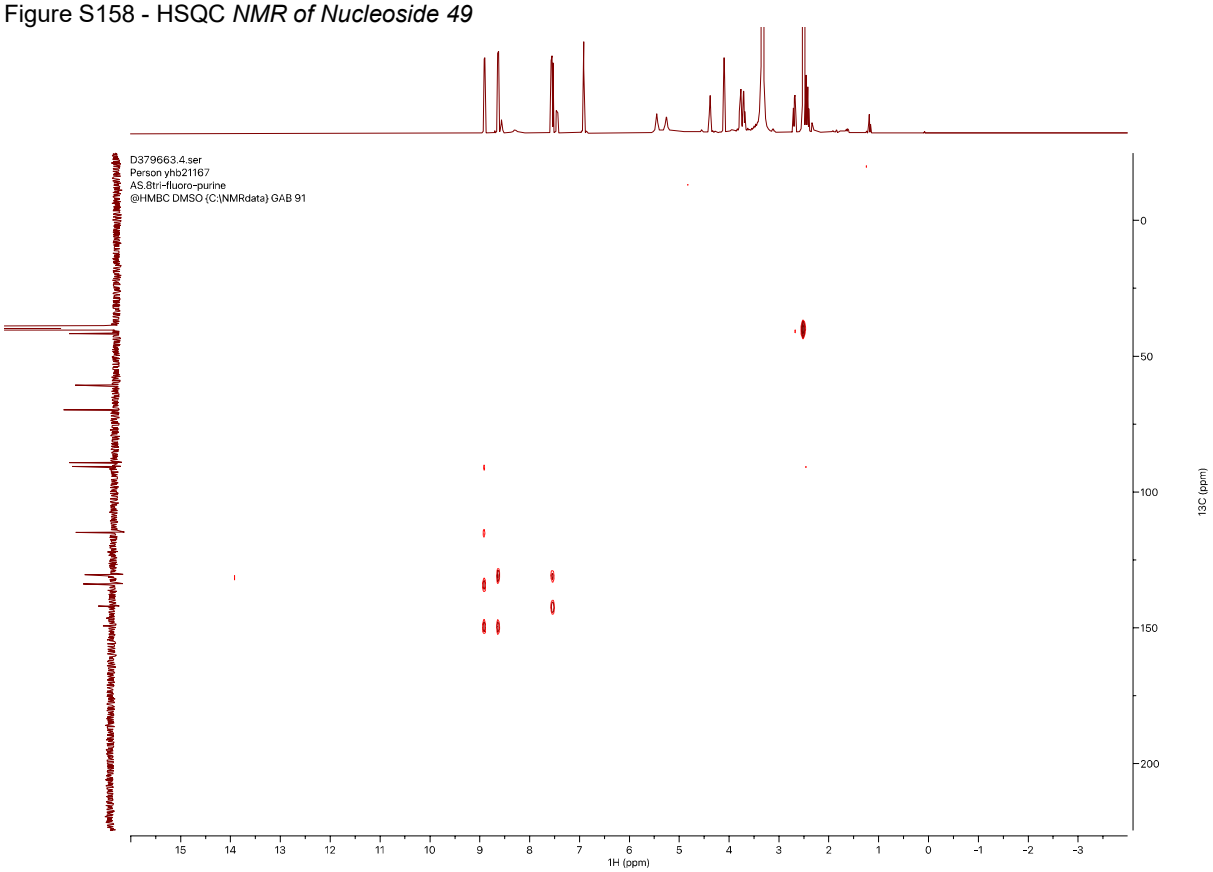


Figure S159 - HMBC *NMR of Nucleoside 49*

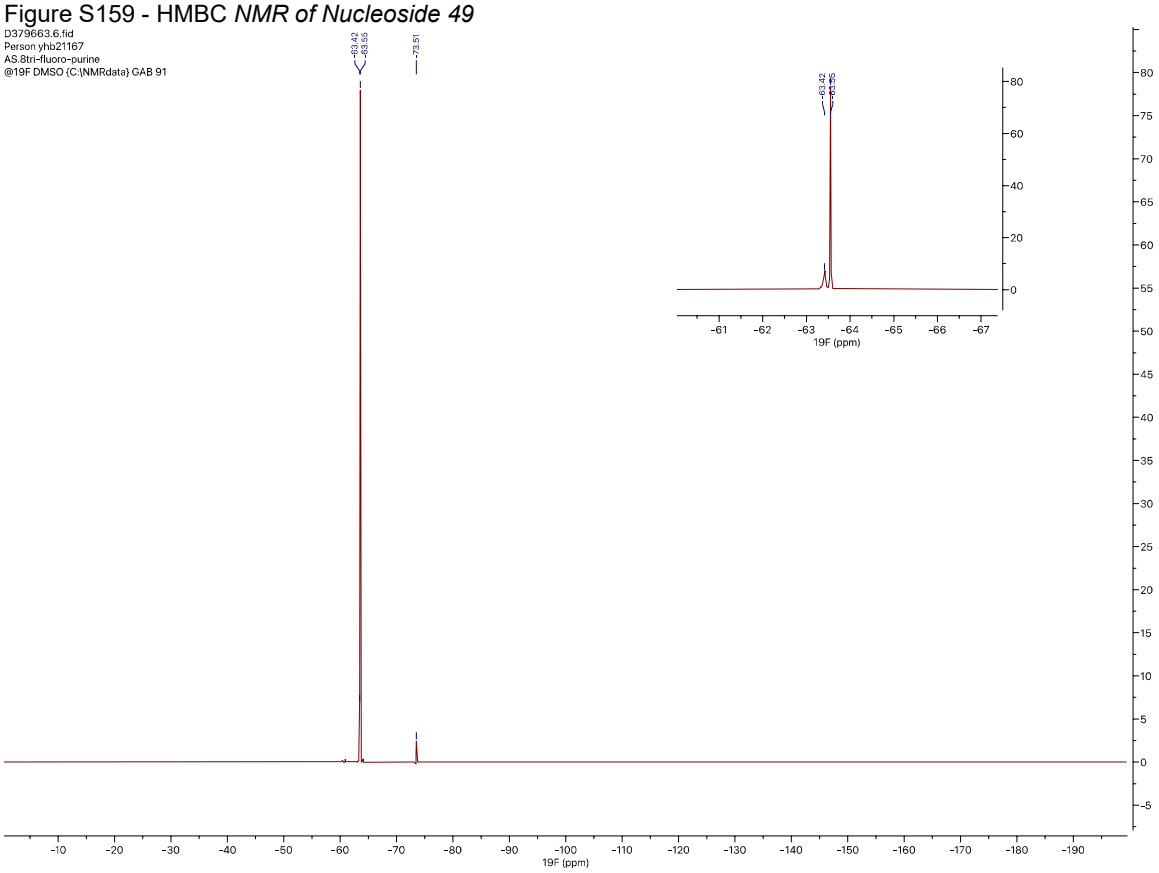
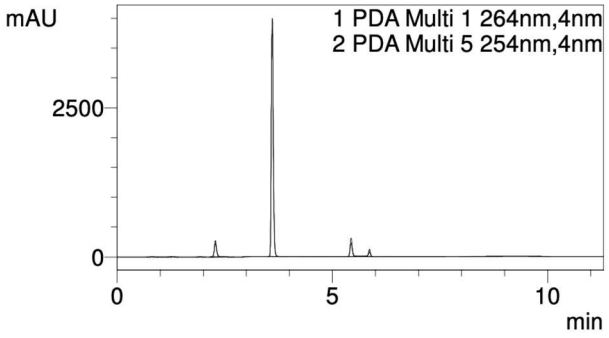


Figure S160 - 19F NMR of nucleoside **49**

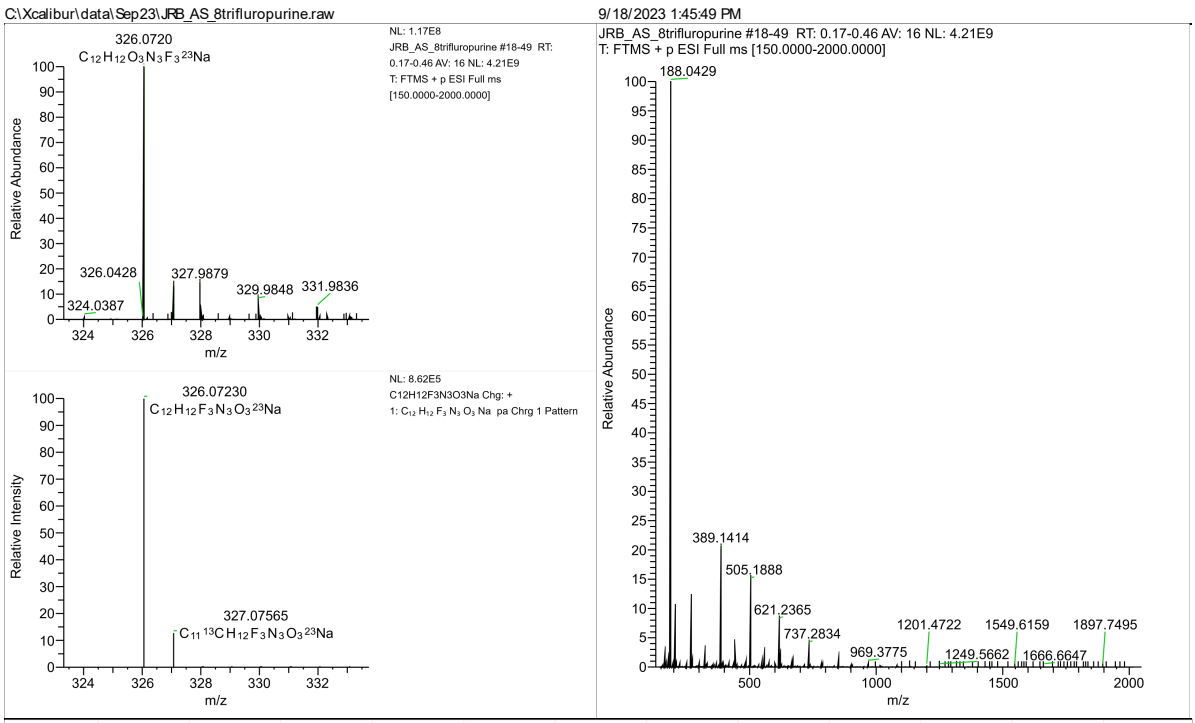
Sample Name : trifluoro
Sample ID :
Data Filename : runs day after 24hr part 2_22092022_006.lcd
Method Filename : Admir Trans glyco Run.lcm
Batch Filename : runs day after 24hr part 2.lcb
Vial # : 1-101 Sample Type : Unknown
Injection Volume : 10 uL
Date Acquired : 22/09/2022 14:31:26 Acquired by : Shimadzu
Date Processed : 22/09/2022 14:42:46 Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.789	6573	3856	
2	1.149	1478	327	
3	1.222	2310	1420	
4	1.296	2746	559	
5	1.923	1468	336	
6	2.283	1025025	269894	
7	3.024	1995	417	
8	3.604	13072395	3802622	
9	4.464	4947	632	
10	4.662	8833	755	
11	4.947	2888	614	
12	5.222	10737	1468	
13	5.437	1161118	312940	
14	5.590	49819	10559	
15	5.684	45789	10917	
16	5.862	479643	123977	
17	5.968	9568	2497	
18	6.114	12805	1679	
19	6.270	23392	2852	
20	6.502	8055	1670	
21	6.581	6557	1222	
22	6.702	4373	944	
23	6.903	8679	1041	

Figure S161 - HPLC spectrum – R.T = 2.28 = thymine released, 3.60 = T, 5.44 = nucleobase, 5.86 = nucleoside 49.



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
326.0720	$C_{12}H_{12}O_3N_3F_3{}^{23}Na$	73.2865415590522	6.50	-0.78	326.07230	1	82.53	3	0	83.05	100	(Collection)
326.0720	$C_{10}H_7ON_9F_3$	36.4160024107731	10.50	0.08	326.07202	2	79.88	3	0	82.29	99.39	(Collection)
326.0720	$C_9H_{11}O_5N_5F_3$	8.49136601032539	5.50	4.18	326.07068	3	69.41	3	1	72.8	89.72	(Collection)

Figure S162 - HRMS of nucleoside 49

2'-Deoxy-triazolo-6-chloropurine (50)

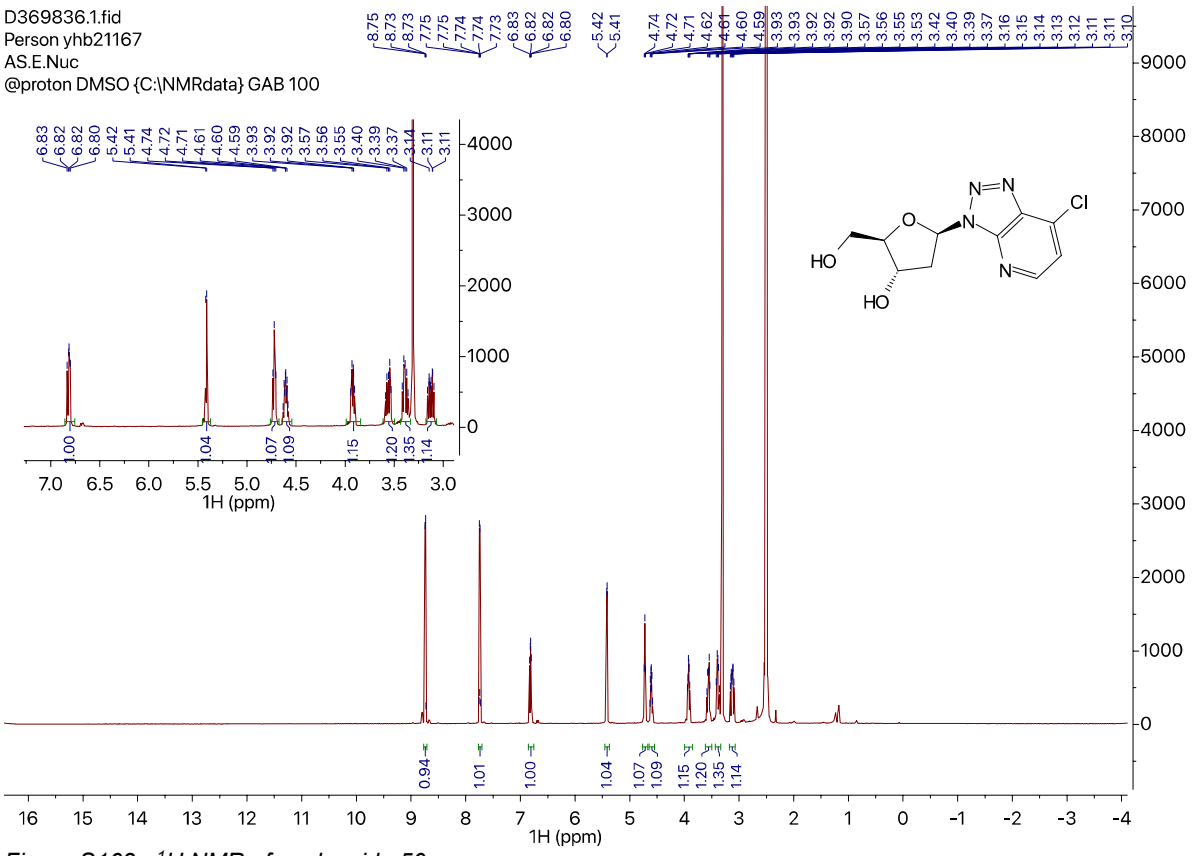


Figure S163 - ^{1}H NMR of nucleoside 50

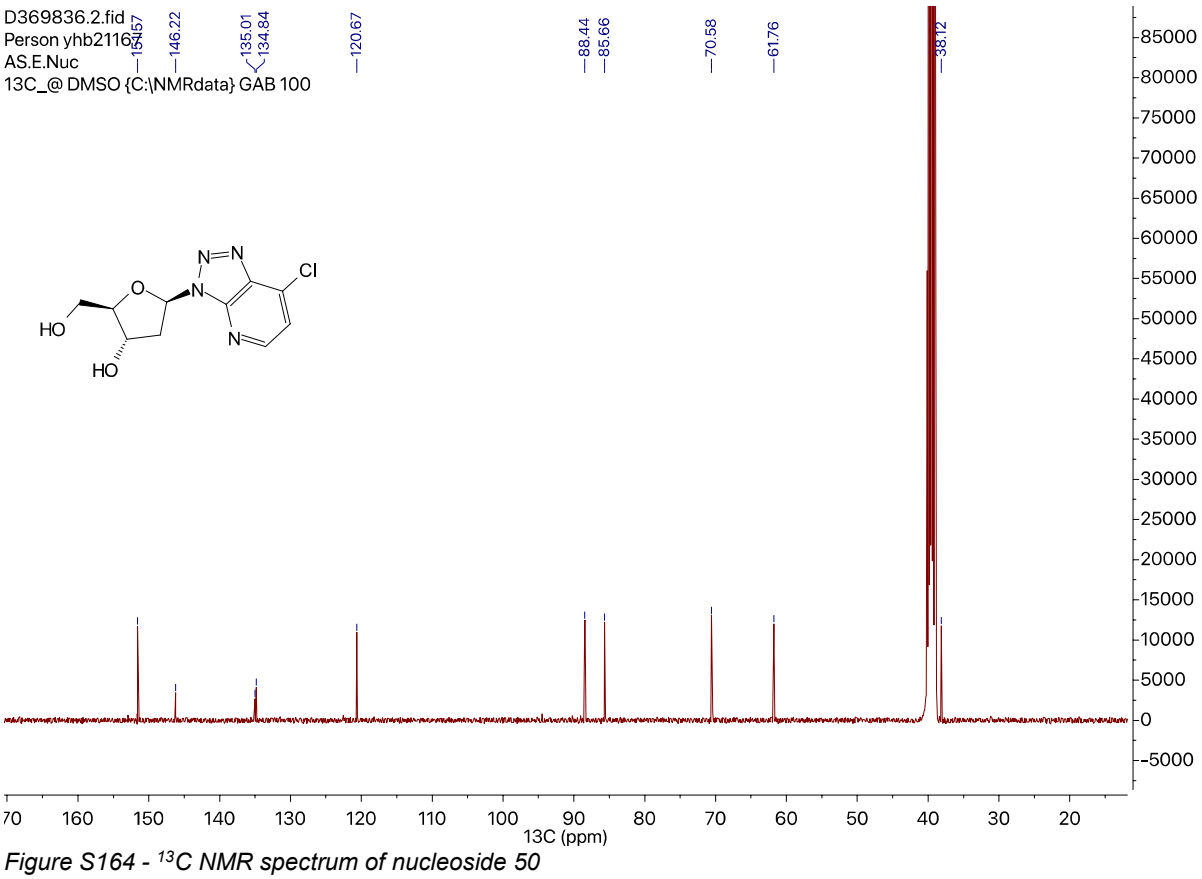
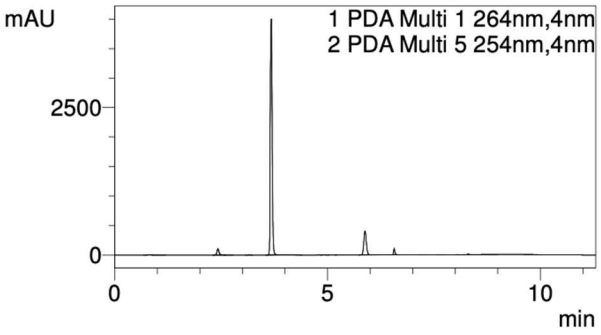


Figure S164 - ^{13}C NMR spectrum of nucleoside 50

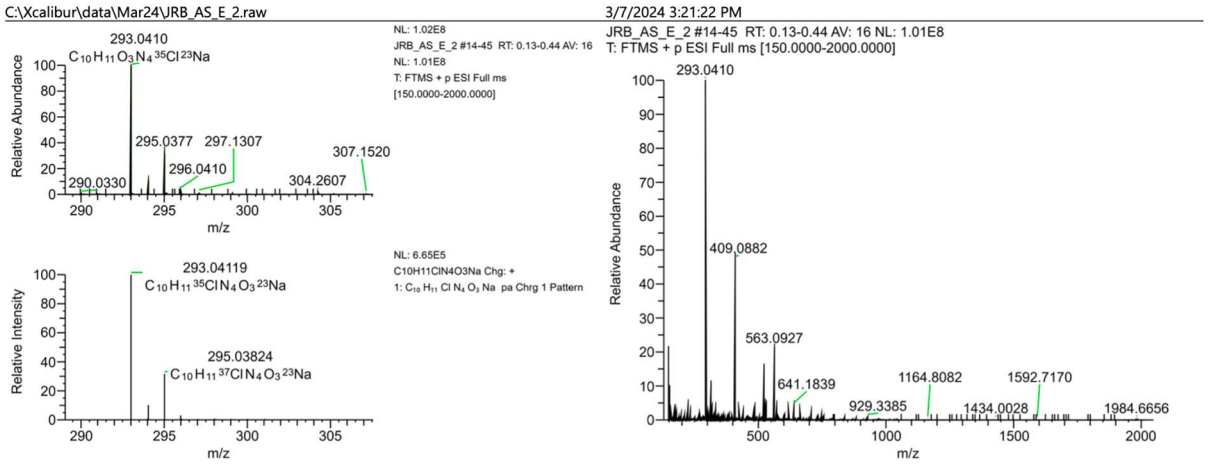
Sample Name	: BASE E RXN		
Sample ID	:		
Data Filename	: BASE E RXN_17032022_001.lcd		
Method Filename	: Admir Trans glyco Run.lcm		
Batch Filename	: BASE E RXN.lcb		
Vial #	: 1-37	Sample Type	: Unknown
Injection Volume	: 10 uL		
Date Acquired	: 17/03/2022 16:10:52	Acquired by	: Shimadzu
Date Processed	: 17/03/2022 16:22:13	Processed by	: Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.785	8256	2726	
2	1.024	1992	213	
3	2.426	375090	107166	
4	3.167	1920	488	
5	3.680	12870173	3996706	
6	3.983	6306	2325	
7	4.916	1534	308	
8	5.882	1636722	397847	
9	6.232	2040	722	
10	6.566	217494	90376	
11	7.235	1252	474	
12	7.714	1697	289	
13	8.059	8568	3301	
14	8.216	3632	545	

Figure S165 - HPLC spectrum of nucleoside 50. R.T = 2.4 = thymine released, 3.68 = thymidine, 5.88 = nucleobase, 6.57 = nucleoside 50



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
293.0410	$C_{10}H_{11}O_3N_4{}^{35}Cl^{23}Na$	52.7699880087094	6.50	-0.71	293.04119	1	94.31	5	1	96.61	99.34	(Collection)
293.0410	$C_9H_{12}O_6N_3{}^{35}Cl$	40.6054176780013	5.00	0.23	293.04091	2	93.5	5	1	96.43	98.65	(Collection)
293.0410	$C_9H_{15}O_7{}^{35}Cl^{23}Na$	31.7308858117625	1.50	3.86	293.03985	3	93.03	5	1	96.43	98.49	(Collection)
293.0410	$C_8H_9O_2N_7{}^{35}Cl^{23}Na$	21.5690226030449	7.00	3.88	293.03985	4	92.49	5	2	96.43	97.48	(Collection)
293.0410	$C_8H_6ON_{10}{}^{35}Cl$	20.9982487284048	10.50	0.25	293.04091	5	92.46	5	4	96.43	96.19	(Collection)
293.0410	$C_7H_{10}O_5N_6{}^{35}Cl$	9.66607099982584	5.50	4.81	293.03957	6	83.17	5	2	87.26	90.6	(Collection)
293.0410	$C_{11}H_3O_2N_9$	25.7787361296112	15.00	1.91	293.04042	7	70.18	2	2	72.65	96.29	(Collection)
293.0410	$C_{12}H_9O_7N_2$	23.3019701113375	9.50	1.89	293.04043	8	70.05	2	2	72.65	98.18	(Collection)
293.0410	$C_{13}H_8O_4N_3{}^{23}Na$	22.3320579314074	11.00	0.96	293.04070	9	70	2	2	72.65	98.62	(Collection)
293.0410	$C_{10}H_8O_2N_7{}^{35}Cl$	7.40243068081283	10.00	-4.33	293.04225	10	69.21	5	3	72.65	73.44	(Collection)

Figure S166 - HRMS spectrum of Nucleoside **50**

2'-Deoxy-deaza-6-bromopurine (51)

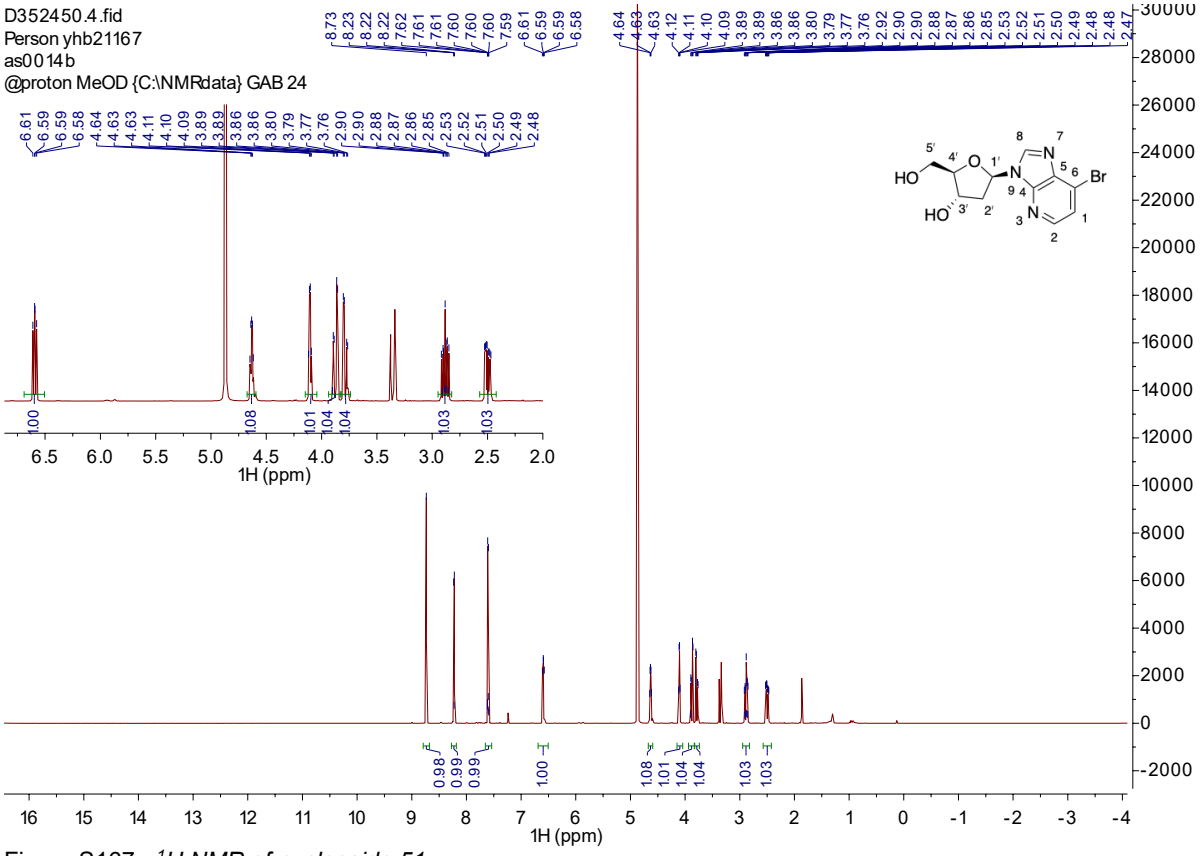


Figure S167 - *^{1}H NMR of nucleoside 51*

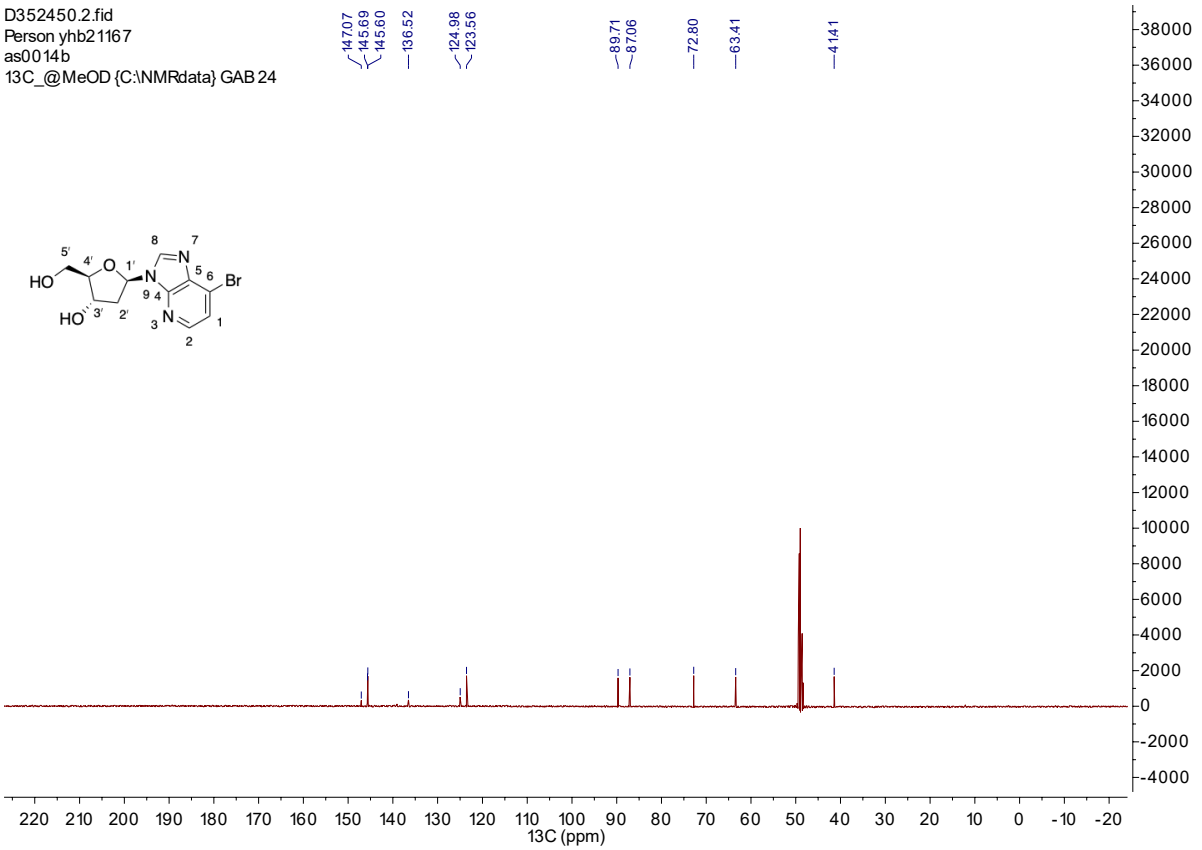
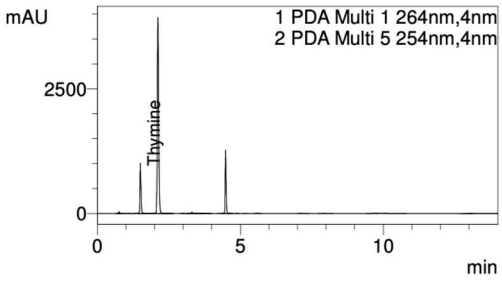


Figure S168 - *13C{1H}-NMR spectra of nucleoside 51*

Sample Name : AS014 24HOURS
Sample ID :
Data Filename : AS014 FINAL RUN_17112021_002.lcd
Method Filename : JMW NDT method with Thymine calibration.lcm
Batch Filename : AS014 FINAL RUN.lcb
Vial # : 1-99 Sample Type : Unknown
Injection Volume : 10 uL
Date Acquired : 17/11/2021 10:47:39 Acquired by : Shimadzu
Date Processed : 17/11/2021 11:01:41 Processed by : Shimadzu



PDA Ch1 264nm

Peak#	Ret. Time	Area	Height	ID#
1	0.762	56587	29925	
2	0.864	1947	629	
3	0.946	1548	485	
4	1.084	5680	1747	
5	1.168	2433	359	
6	1.281	2802	835	
7	1.365	6761	2292	
8	1.505	2573951	974049	
9	2.118	13477936	3913571	
10	2.445	3802	1277	
11	2.974	4013	852	
12	3.088	1540	493	
13	3.224	2354	668	
14	3.303	68894	21786	
15	3.649	2354	789	
16	4.292	6714	2155	
17	4.485	2515277	1117390	
18	4.719	1218	458	
19	4.857	2549	878	
20	5.106	1731	477	
21	5.556	7400	2413	
22	5.617	7424	2331	
23	7.109	6302	1462	
24	7.908	9182	1584	
25	9.709	15593	1466	
26	9.817	47309	1690	
27	12.901	41663	2262	
Total		18874961	6084319	

Figure S169 - *HPLC trace used to obtain nucleoside 51. R.T = 1.5 = nucleobase thymine, 2.12 = nucleoside dT, 4.49 = nucleoside 51 formed.*

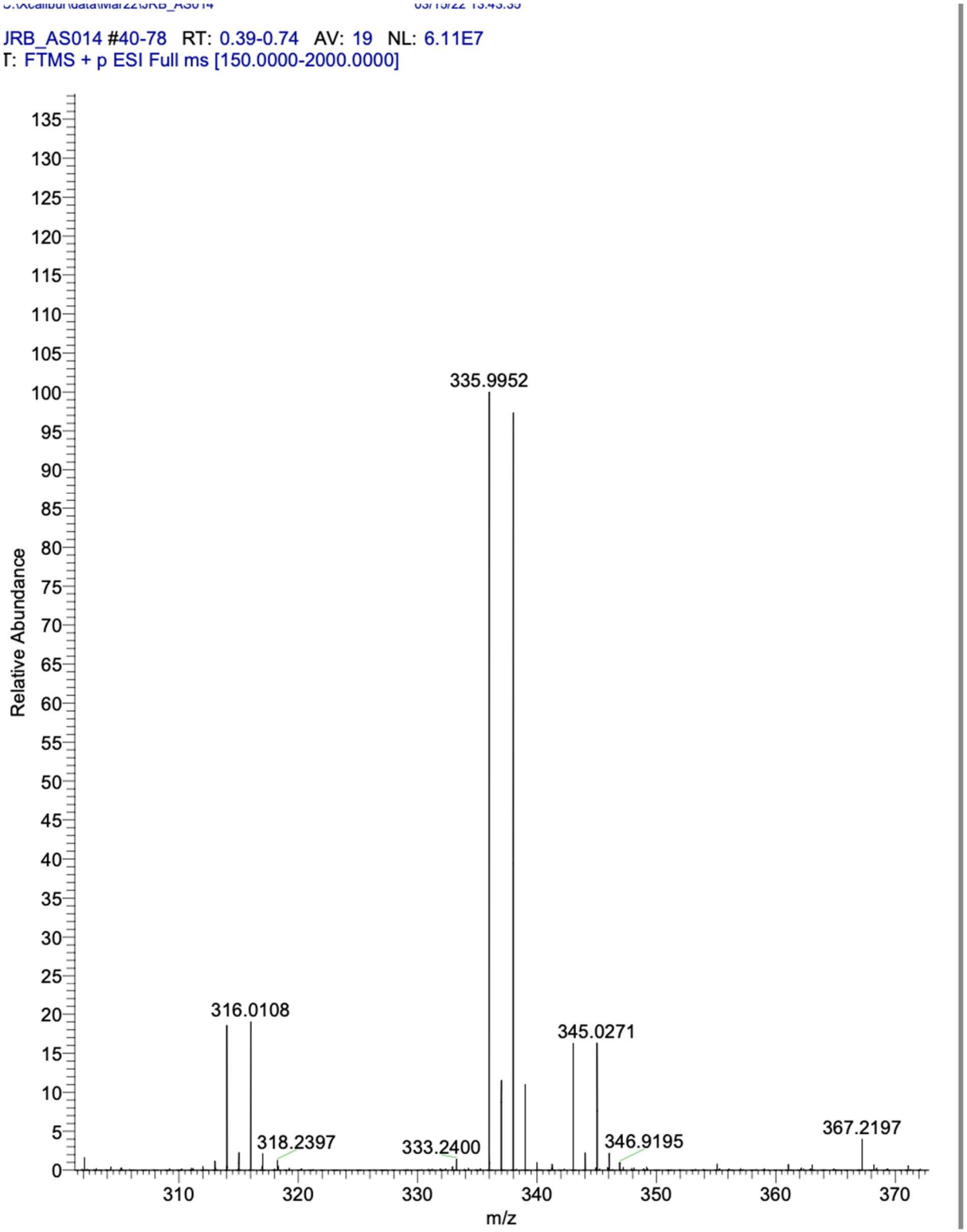


Figure S170 - *HRMS spectra of nucleoside 51*

3',5'-tri-O-acetyl-deaza-6-bromopurine (57)

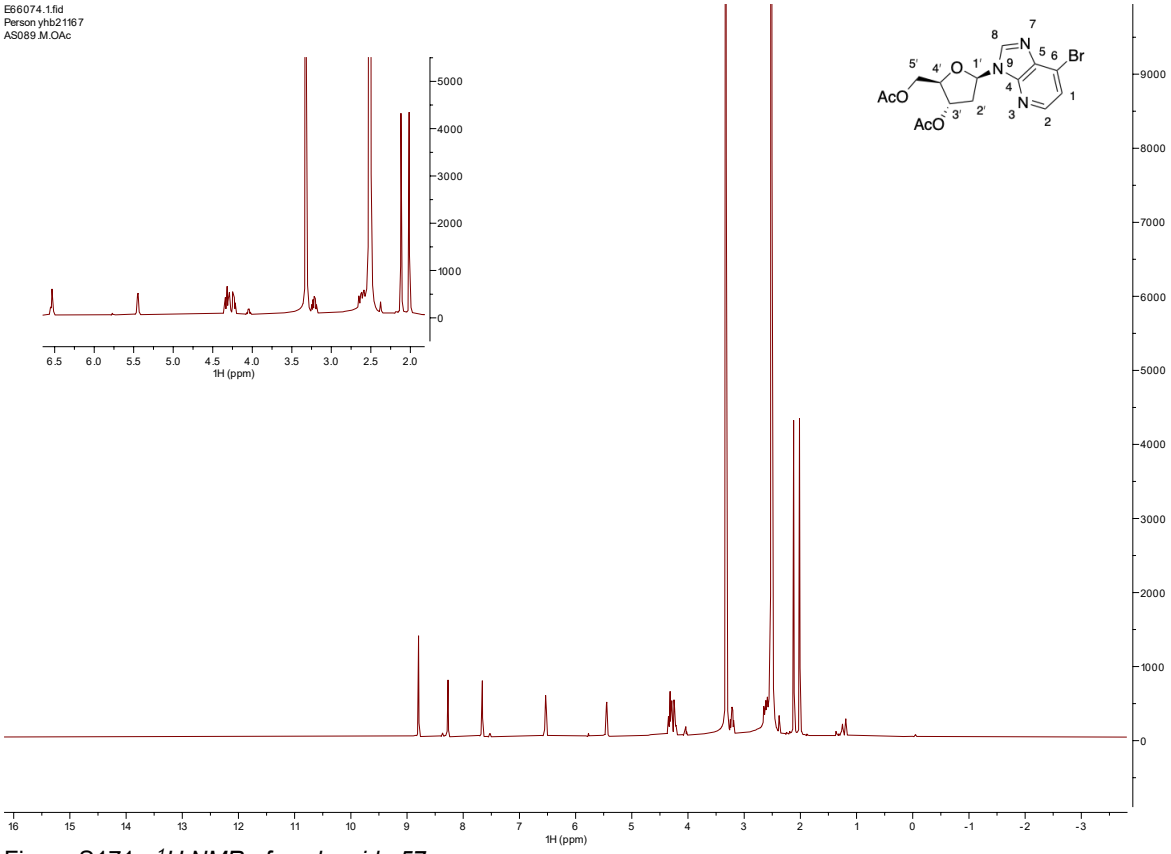


Figure S171 - *^{1}H NMR of nucleoside 57*

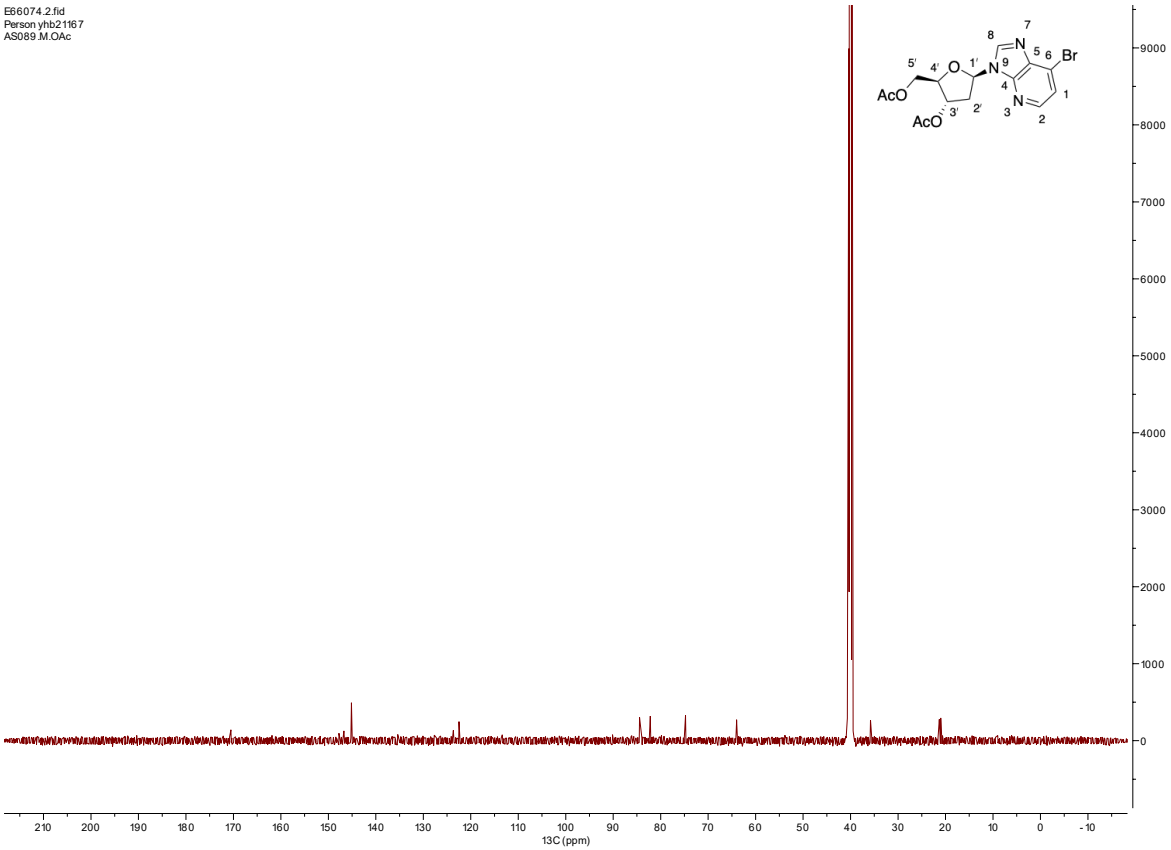
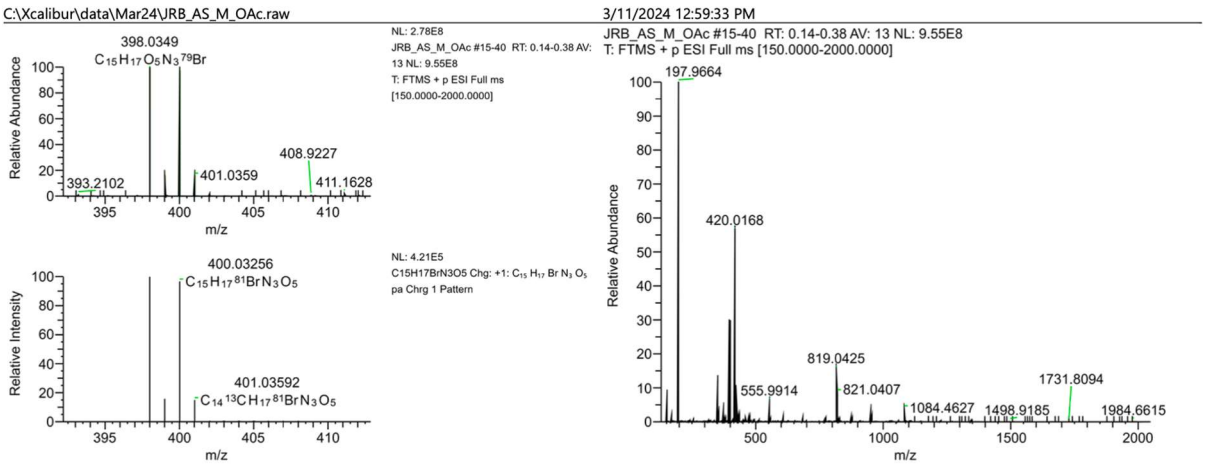


Figure S172 - *13C{1H}-NMR spectra of nucleoside 57*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
398.0349	$C_{15}H_{17}O_5N_3{}^{79}Br$	76.74632812 40714	8.50	0.80	398.03461	1	98.33	6	0	99.53	100	(Collection)
398.0349	$C_{13}H_{15}O_4N_6{}^{79}Br$	42.72774283 39578	9.00	4.17	398.03327	2	96.54	6	0	99.53	100	(Collection)
398.0349	$C_{14}H_{11}N_{10}{}^{79}Br$	53.68974568 94709	14.00	0.81	398.03460	3	96.22	5	0	98.59	99.5	(Collection)
398.0349	$C_{16}H_{13}ON_7{}^{79}Br$	31.80131168 36724	13.50	-2.56	398.03595	4	95.07	6	0	98.59	99.26	(Collection)
398.0349	$C_{17}H_{19}O_6{}^{79}Br$	29.22286456 30406	8.00	-2.57	398.03595	5	94.94	6	0	98.59	98.78	(Collection)
398.0349	$C_{14}H_6O_7N_8$	28.57148981 40074	16.00	-1.17	398.03540	6	48.69	2	2	49.81	97.64	(Collection)
398.0349	$C_{15}H_{12}O_{12}N$	28.06826795 5912	10.50	-1.19	398.03540	7	48.66	2	2	49.81	96.68	(Collection)
398.0349	$C_{13}H_{10}O_{11}N_4$	25.02068370 39156	11.00	2.19	398.03406	8	48.5	2	2	49.81	97.06	(Collection)
398.0349	$C_{16}H_8O_8N_5$	15.53671146 19261	15.50	-4.55	398.03674	9	48	2	2	49.81	97.24	(Collection)
398.0349	$C_{28}H_4ON_3$	13.39448354 04418	28.50	0.10	398.03489	10	41.33	2	2	42.88	73.72	(Collection)

Figure S173 - *HRMS spectra of nucleoside 57*

2'-Deoxy-deaza-6-^{15}N-benzamidepurine (58)

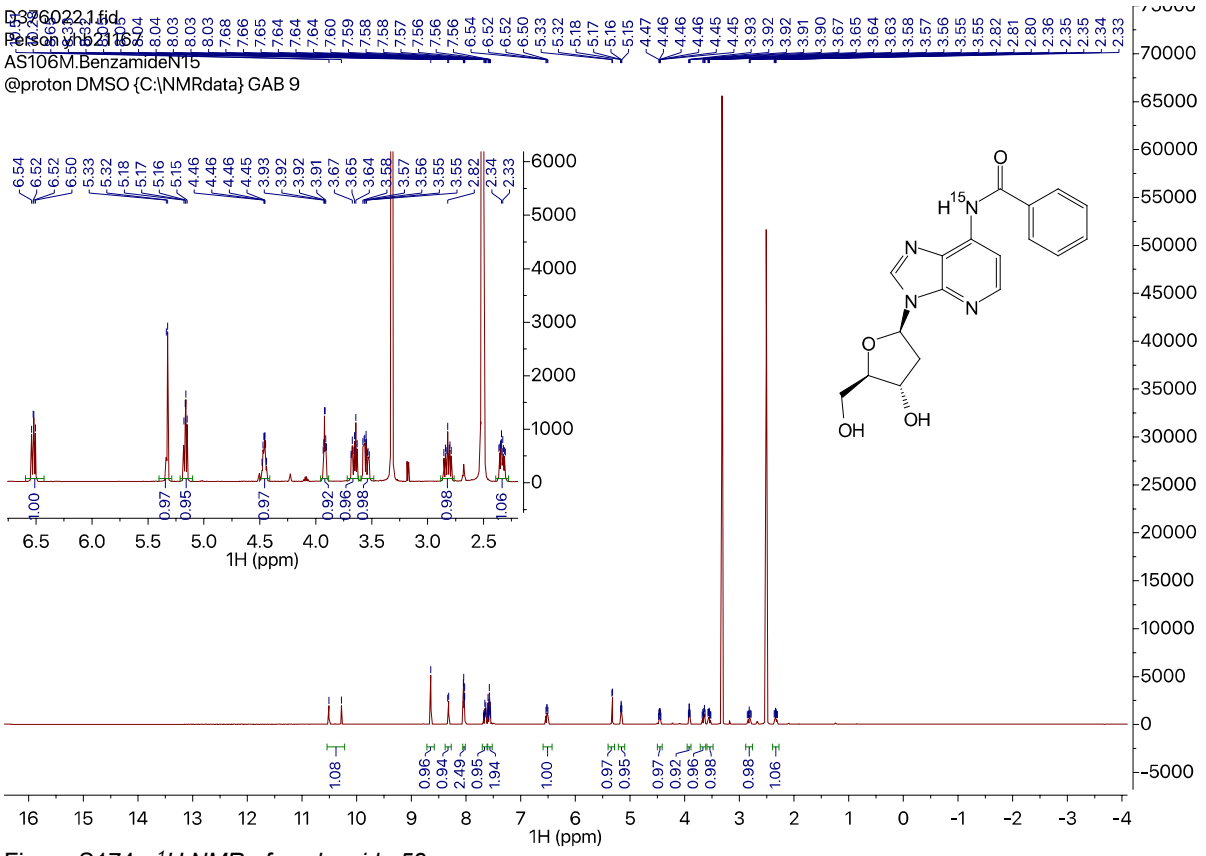


Figure S174 - *[1]H NMR of nucleoside 58*

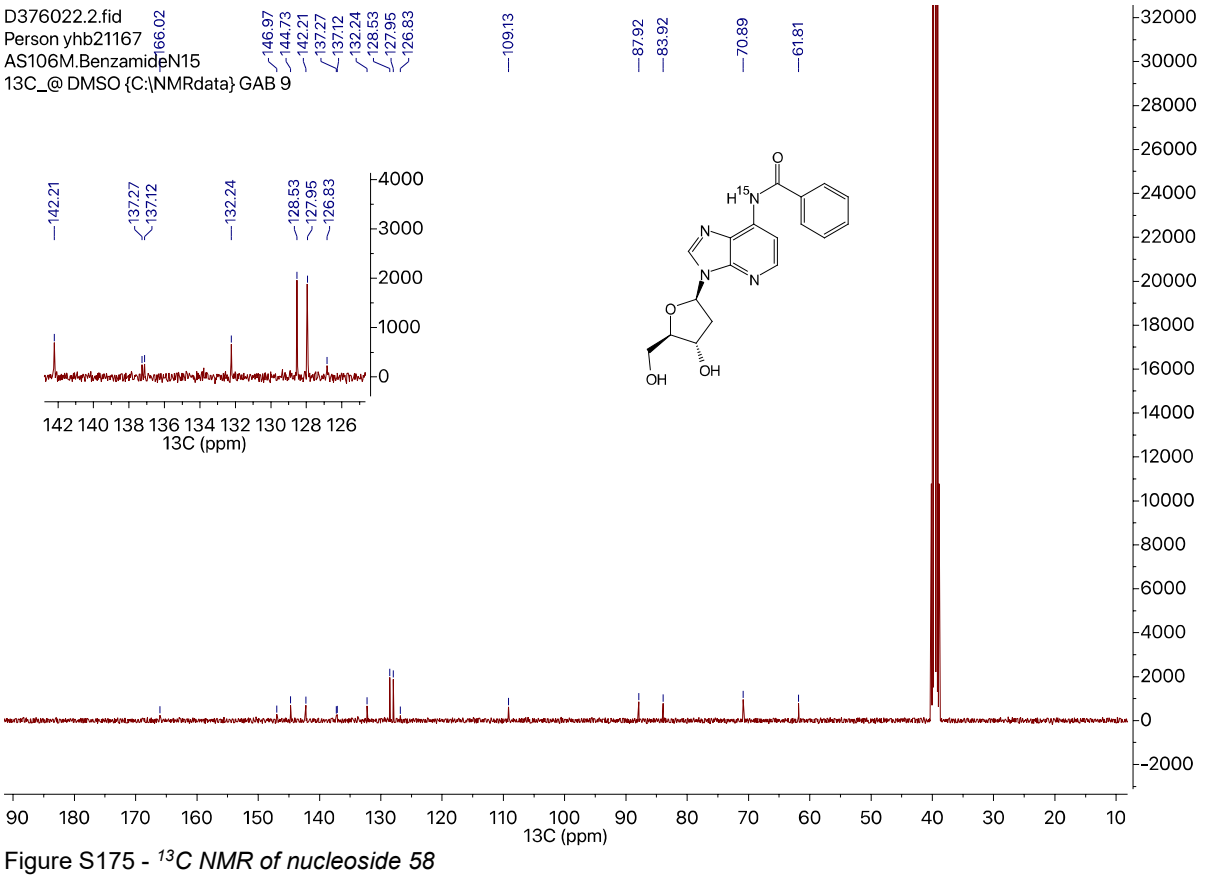
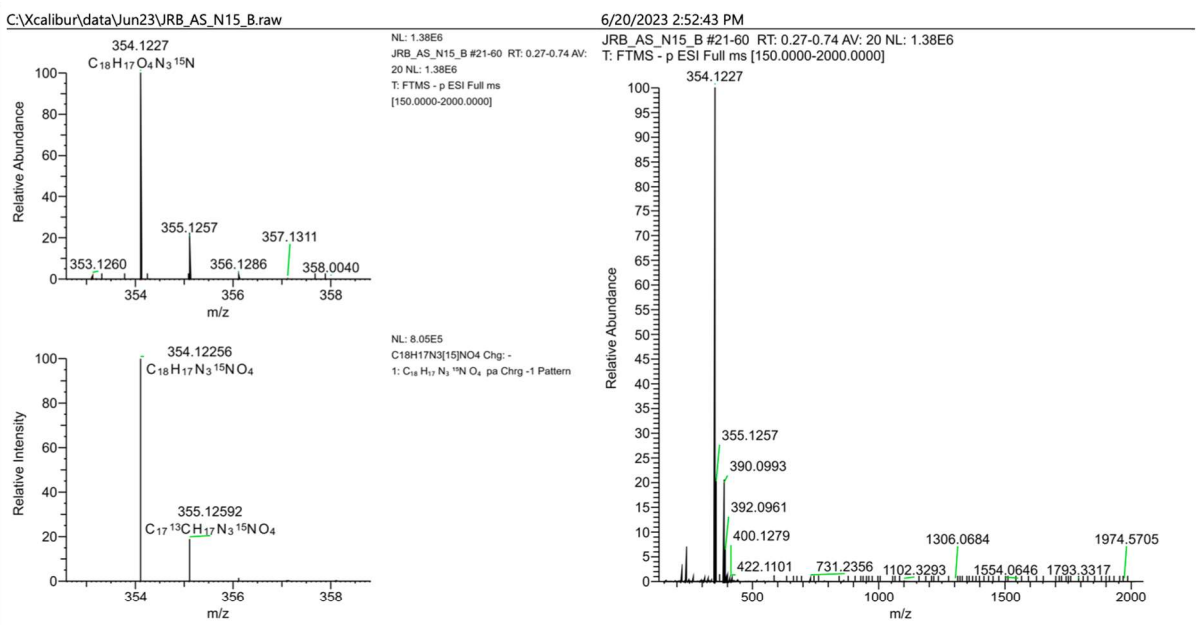


Figure S175 - *[13]C NMR of nucleoside 58*



Peak Mass	Display For...	S Fit	RDB	Delta [ppm]	Theo. mass	Rank	Combined S...	# Matched I...	# Missed Iso.	MS Cov. [%]	Pattern Cov....	MSMS Matc...
354.1227	$C_{18}H_{12}N_9$	28.00503217 41993	17.50	4.76	354.12102	1	94.45	4	0	98.14	99.91	(Collection)
354.1227	$C_{22}H_{16}O_2N_3$	18.84156337 7564	16.50	-2.82	354.12370	2	92.33	4	0	96.41	97.41	(Collection)
354.1227	$C_5H_{20}O_{11}N_7$	10.27946244 09353	-0.50	3.32	354.12153	3	77	3	1	80.71	92.09	(Collection)
354.1227	$C_{19}H_{13}N_7{}^{15}N$	0	17.50	-0.28	354.12280	4	0	1	3	0	0	(Collection)
354.1227	$C_6H_{21}O_{11}N_5{}^{15}N$	0	-0.50	-1.73	354.12332	5	0	1	3	0	0	(Collection)
354.1227	$C_{18}H_{17}O_4N_3{}^{15}N$	0	12.50	3.50	354.12147	6	0	1	3	0	0	(Collection)

Figure S176 - *HRMS of nucleoside 58.*