Fluorescent 5-Pyrimidine and 8-Purine Nucleosides Modified with *N*-unsubstituted 1,2,3-Triazol-4-yl Moiety

Zhiwei Wen, Paloma R. Tuttle, A. Hasan Howlader, Anna Vasilyeva, Laura Gonzalez, Antonija Tangar, Ruipeng Lei, Eduardo E. Laverde, Yuan Liu, Jaroslava Miksovska, Stanislaw F. Wnuk*

Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199, USA

Corresponding authors: wnuk@fiu.edu

SUPPORTING INFORMATION

Contents

1. Mechanism for the formation of *N*-unsubstituted 1,2,3-triazoles

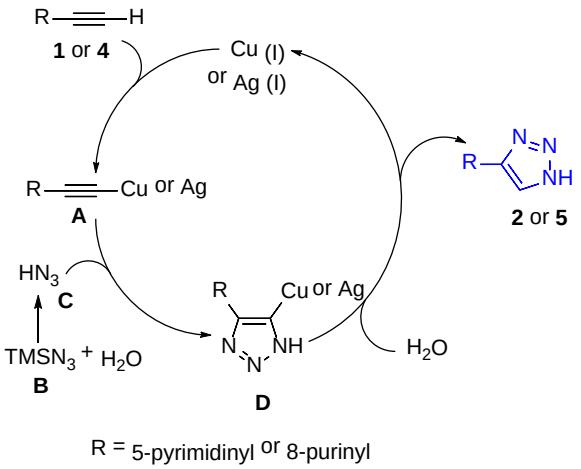


Figure S1. Mechanism for the formation of *N*-unsubstituted 1,2,3-triazoles

Likely terminal alkyne **1** or **4** reacts with Cu(I) or Ag(I) forming metal activated alkyne **A**, which further reacts with hydrazoic acid **C**, generated in-situ from $TMSN_3$ and H_2O, to produce intermediate **D**. Protonation of **D** yields the final *N*-unsubstituted 1,2,3-triazol-4-yl product **2** or **5** and releases Cu(I) or Ag(I) as catalyst.

2. Fluorescent Characterization

The fluorescent properties of the four *N*-unsubstituted triazolyl nucleosides (8-TrzdA **2a**, 8-TrzdG **2c**, 5-TrzdC **5b**, and 5-TrzdU **5d**) and their lipophilic protected analogs (3',5'-di-*O*-TBDMS-8-TrzdA **2b**, 3',5'-di-*O*-Ac-5-TrzdU **5c**, and 3',5'-di-*O*-5-TrzdC **5a**) were determined following the procedure reported by our group.[S1] Triazoles samples were tested with varying concentration in MeOH and other solvents specified in Table S2. The absorbance at the excitation wavelength did not exceed 0.1 absorbance units. For determination of quantum yield Φ_F, the absorbance of the sample solution was kept below 0.06. Quinine sulfate (Φ_F = 0.55) in 100 mM H_2SO_4 was used as reference standard to quantify the quantum yield. The test was performed in a 2 × 10 mm quartz cuvette at room temperature. Absorption spectra were measured using Cary 100Bio UV-Visible Spectrophotometer. Steady-state excitation and emission spectra were

investigated on a PC1 spectrofluorometer with bandwidth and slit width for ex/em set at 2 nm. Frequency-domain fluorescence lifetime were measured using a ChronosFD spectrofluorometer. Sample solutions were excited using a frequency modulated 280 nm LED. The emission was gathered with a 305 nm long-pass filter (Andover). 2,5-diphenyloxazole (τ = 1.4 ns) solution in EtOH was employed as a lifetime reference. A multiple-exponential decay model employing GlobalsWE software were used to fit the modulation phase data. The residual and $\chi 2$ parameter were employed as criteria for goodness of fit.

Table S1. Photophysical data for the protected triazolyl nucleoside analogs in MeOH

	2b	**5a**	**5c**
ε_{max} (M^{-1} cm^{-1})	14100	4150	10700
λ_{max} (abs) (nm)	285	293	291
λ_{max} (exc) (nm)	232	259	239
λ_{max} (exc) (nm)	292	312	294
λ_{max} (emi) (nm)	355	421	400
Stokes shift (nm)	63	109	109
Φ_F	0.48	0.02	0.003
τ_1 (ns)	1.01	0.46	0.05
τ_2 (ns)	2.82	4.48	1.59
$\tau_{average}$ (ns)	2.22	4.20	0.82
f_1 (%)	33	7	50
f_2 (%)	67	93	50

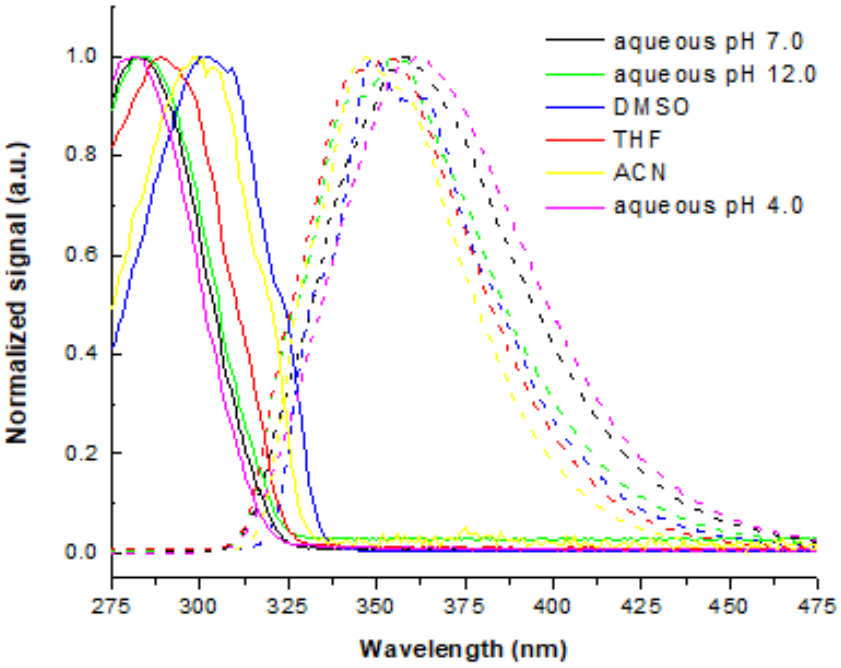


Figure S2. Absorption (solid line) and emission (dash line) spectra for compound **2a** in different solvents

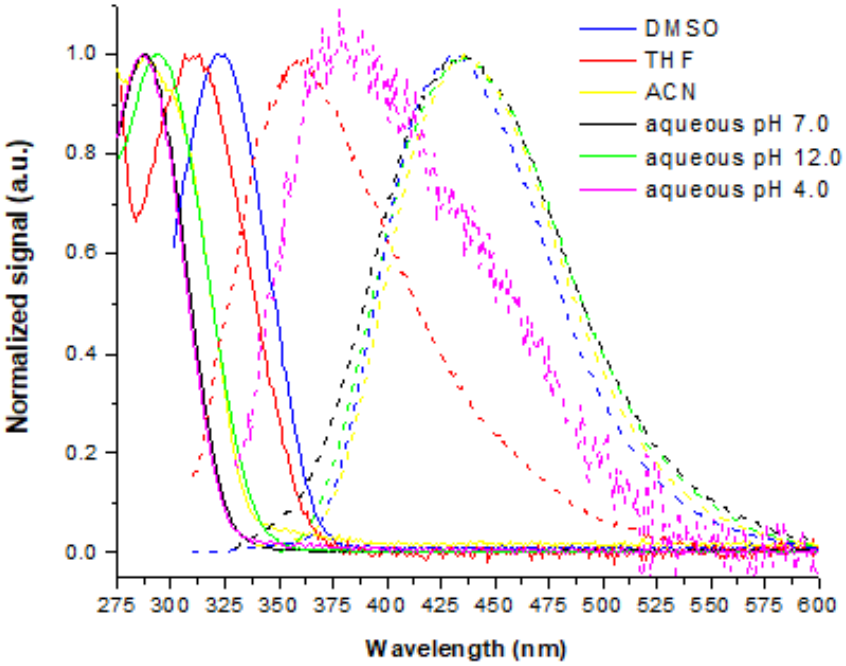


Figure S3. Absorption (solid line) and emission (dash line) spectra for compound **5b** in different solvents. Relatively high S/N ratio for **5b** in aqueous solution pH 4.0 is due to the low emission quantum yield.

Table S2. Photophysical data for **2a** and **5b** in various solvents

		5 mM Phosphate buffer			MeOH	DMSO	THF	ACN
		pH = 4.0	pH = 7.0	pH = 12.0				
2a	λ_{max}^{abs} (nm)	281	283	284	287	300	289	299
	λ_{max}^{em} (nm)	361	358	355	355	350	353	349
5b	λ_{max}^{abs} (nm)	287	289	293	295	322	289	300
	λ_{max}^{em} (nm)	378	435	433	407	432	359	435

3. Polymerase-catalyzed incorporation of 9 into DNA

Material for Enzymatic Reactions

All DNA oligonucleotide templates and primers were synthesized by Integrated DNA Technologies (IDT) (Coralville, IA). T4 polynucleotide kinase was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Radionucleotides [^{32}P] ATP (3000 Ci/mmol) was purchased from Perkin Elmer Inc. (Boston, MA, USA). Micro Bio-Spin TM 6 Columns were purchased from Bio-Rad (Hercules, CA). All other chemicals used were purchased from Sigma-Aldrich (St. Louis, MO) and Thermo Scientific (Pittsburgh, PA). Recombinant human DNA polymerase β (pol β) was expressed and purified as described previously.[S2]

Oligonucleotide Substrates

Table S3. Oligonucleotide Sequences

Oligonucleotide	**nt**	**Sequence (5'-3')**
Upstream	33	GCAGTCCTCTAGTCGTAGTAGCAGATCATCAAC
Downstream	37	GCCGGCATTAGGTGTAGTAGCTAGACTTACTCATTGC
Template strand	71	GCAATGAGTAAGTCTAGCTACTACACCTAATGCCGGC**T**GTTGATGATC TGCTACTACGACTAG AGGACTGC

The one nucleotide gap substrate was constructed by annealing the 5'-^{32}P-labeled upstream strand and the downstream strand containing a 5'-phosphate group with the template strand at a molar ratio of 1:2:2. The sequences of the oligos for constructing the substrate are listed in Table S3.

Enzymatic Activity Assay.

The incorporation of 8-TrzdATP **9** by human DNA polymerase was performed by incubating different concentrations of pol β with 5 nM ^{32}P-labeled substrates at 37 °C for 30 min as described previously.[S3-4] The enzymatic reactions were assembled in the presence of 8-TrzdATP (100 μM). The extension of an incorporated 8-TrzdATP was examined in the presence of both 100 μM 8-TrzdATP and 50 μM dGTP in the different concentrations of pol β. The experiments were repeated three times. The use of one nucleotide gap allow us to examine whether 8-TrzdATP can be incorporated into duplex DNA. The substrate was from pol β DNA synthesis product by a 15% urea-denaturing polyacrylamide gel through electrophoresis and detected by a Pharos FX Plus PhosphorImager (Bio-Rad Laboratory, Hercules, CA).

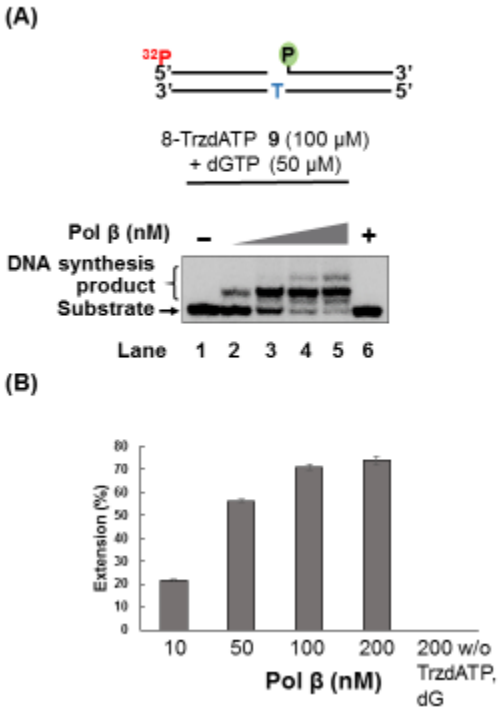
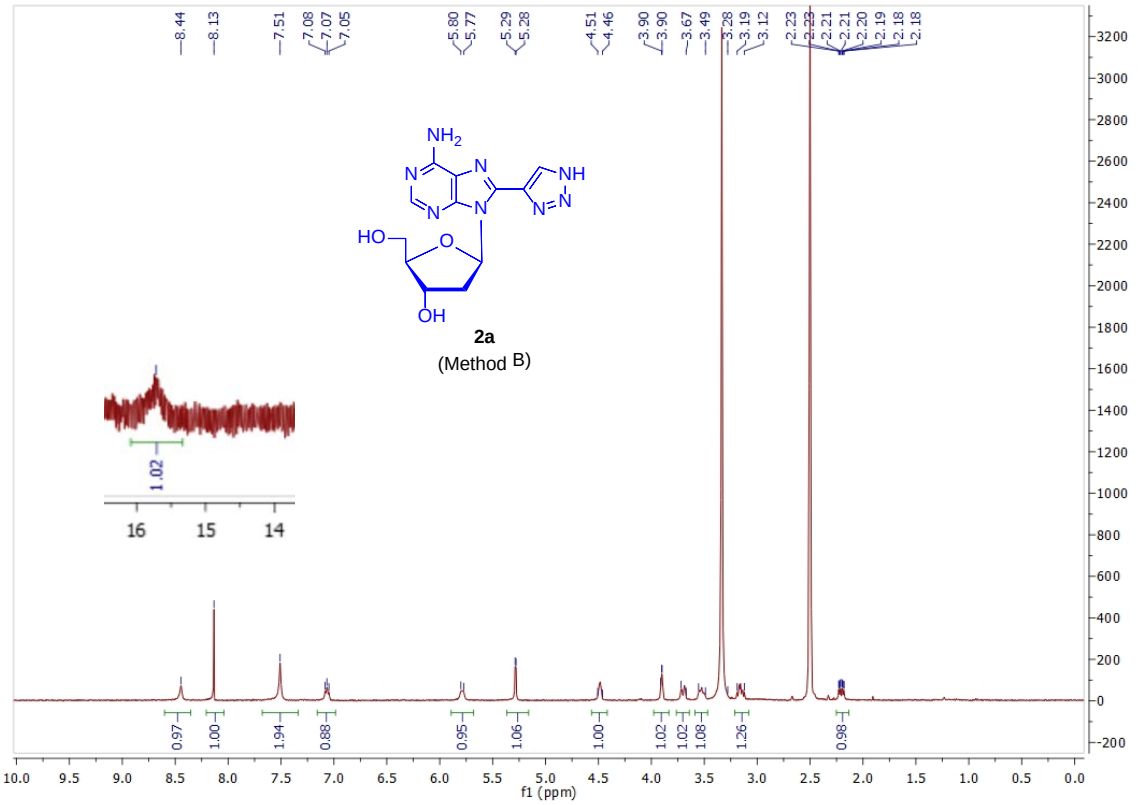
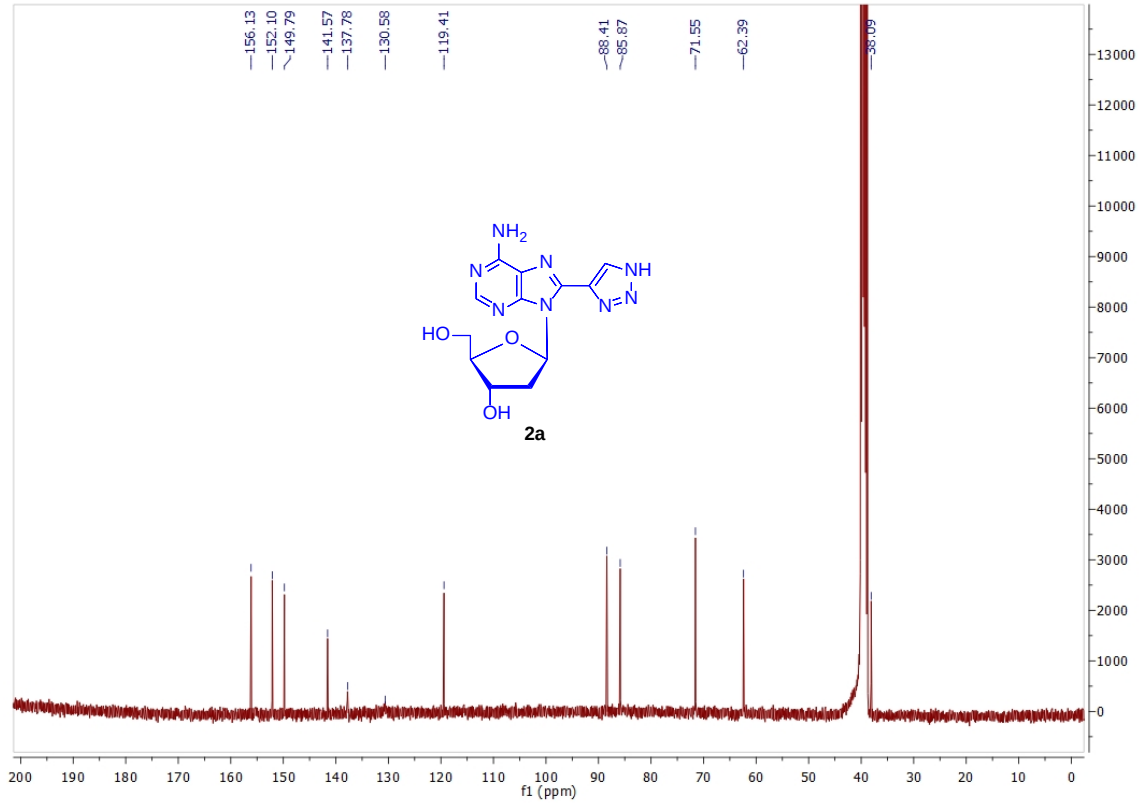
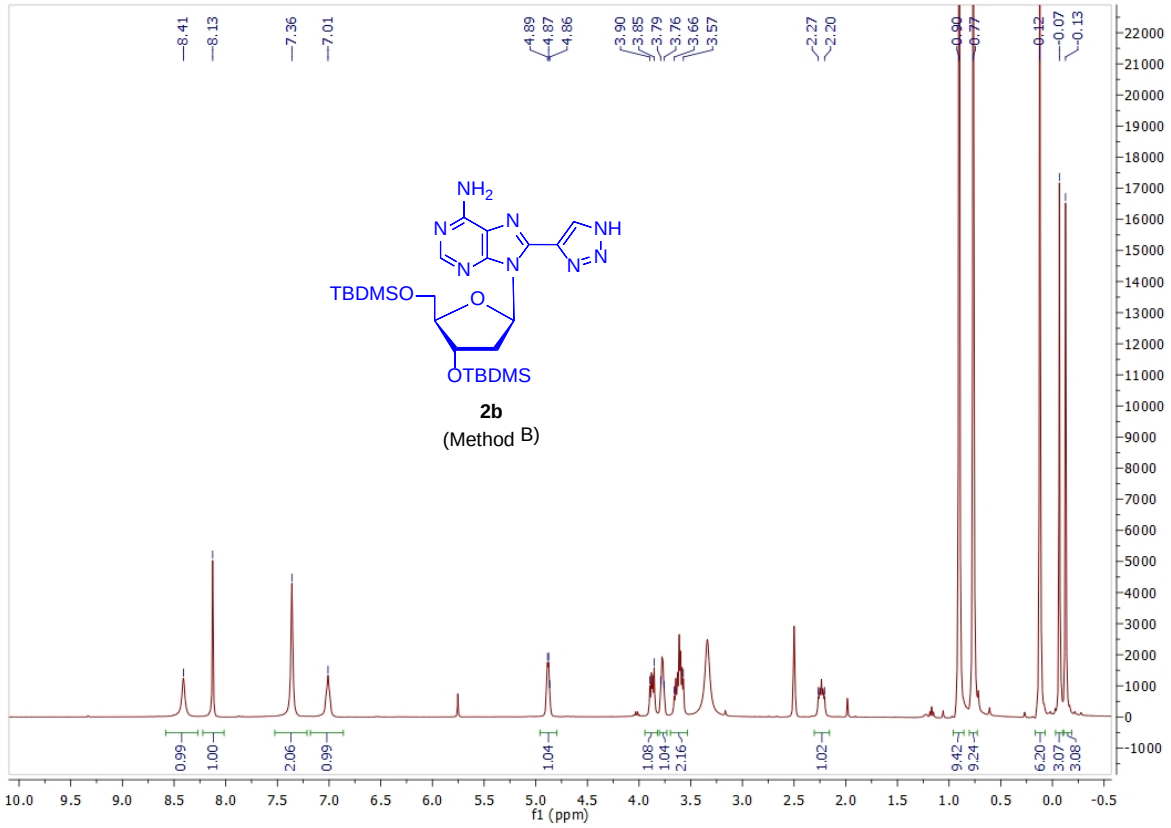


Figure S4. (**A**) The extension of an incorporated 8-TrzdATP **9** by pol β. The reaction was assembled in the reaction buffer containing the substrate, 100 μM 8-TrzdATP, and 50 μM dGTP along with different concentrations of pol β. Substrates was ^{32}P-labeled at the 5'-end of the upstream strand of the substrate as illustrated above the gel. Lane 1 indicates substrate only. Lanes 2-5 indicates DNA synthesis product at increasing concentrations of pol β (10 nM - 200 nM), respectively. Lane 6 indicates the reaction mixture with pol β without any nucleotides. (B) Bar chart illustrating the quantification of the pol β DNA synthesis product.

4. ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra

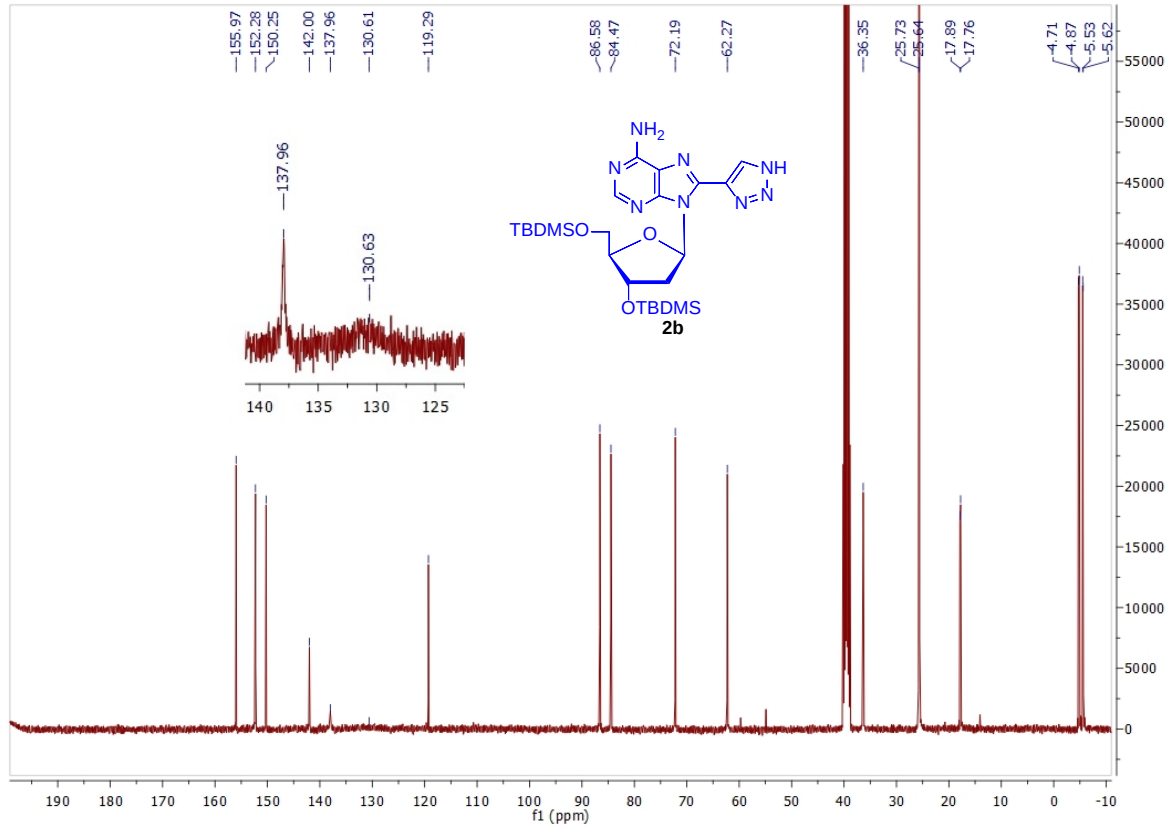




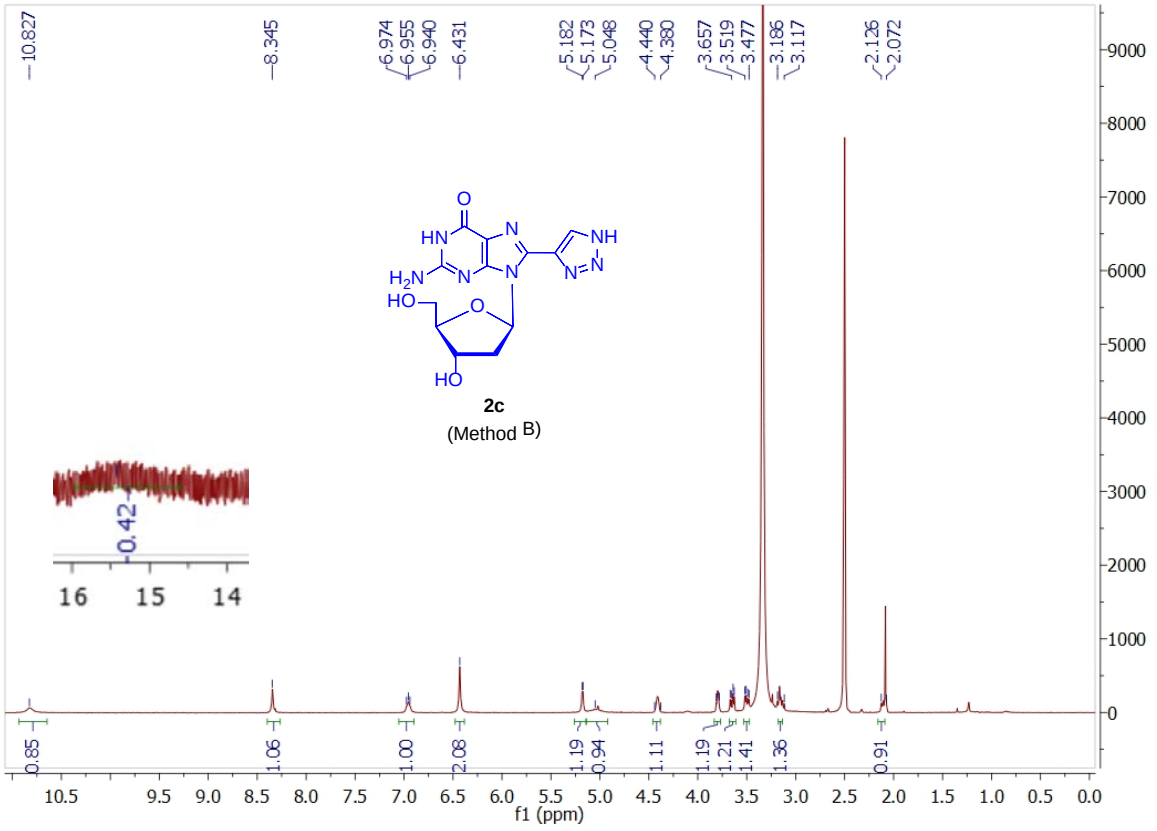


2b

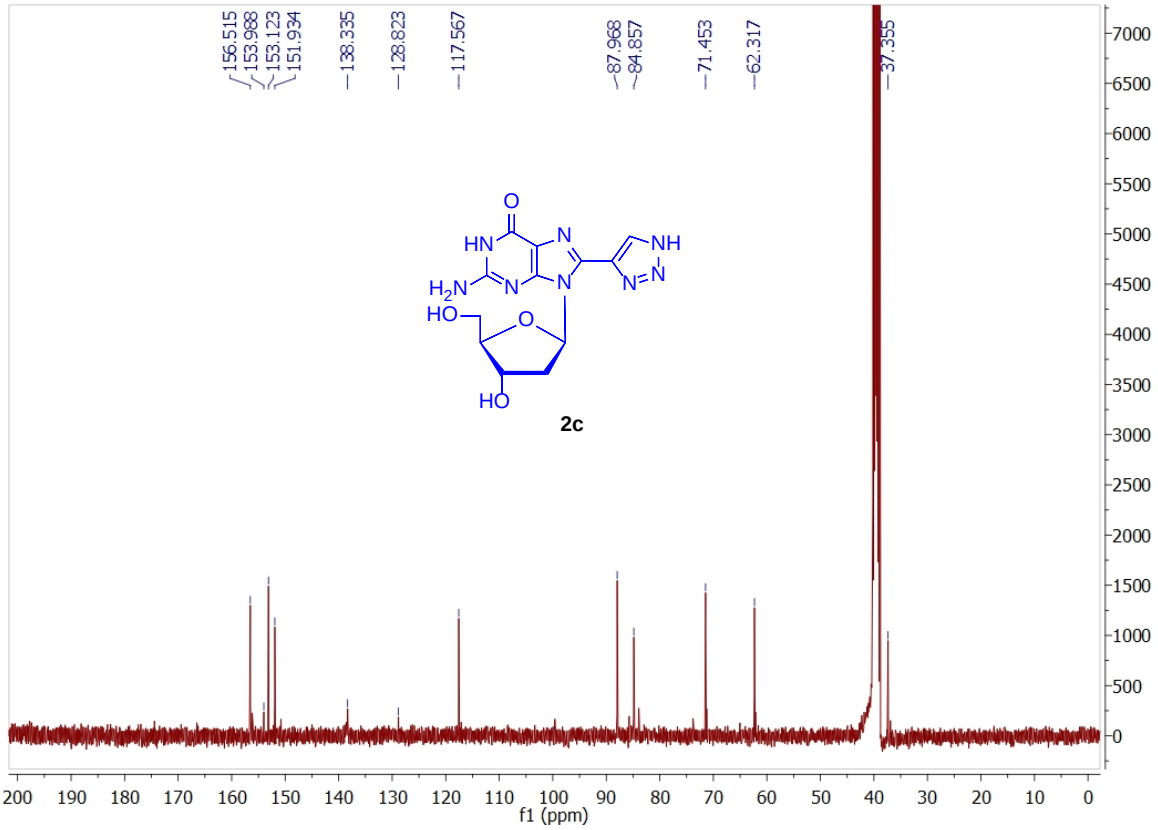
(Method B)



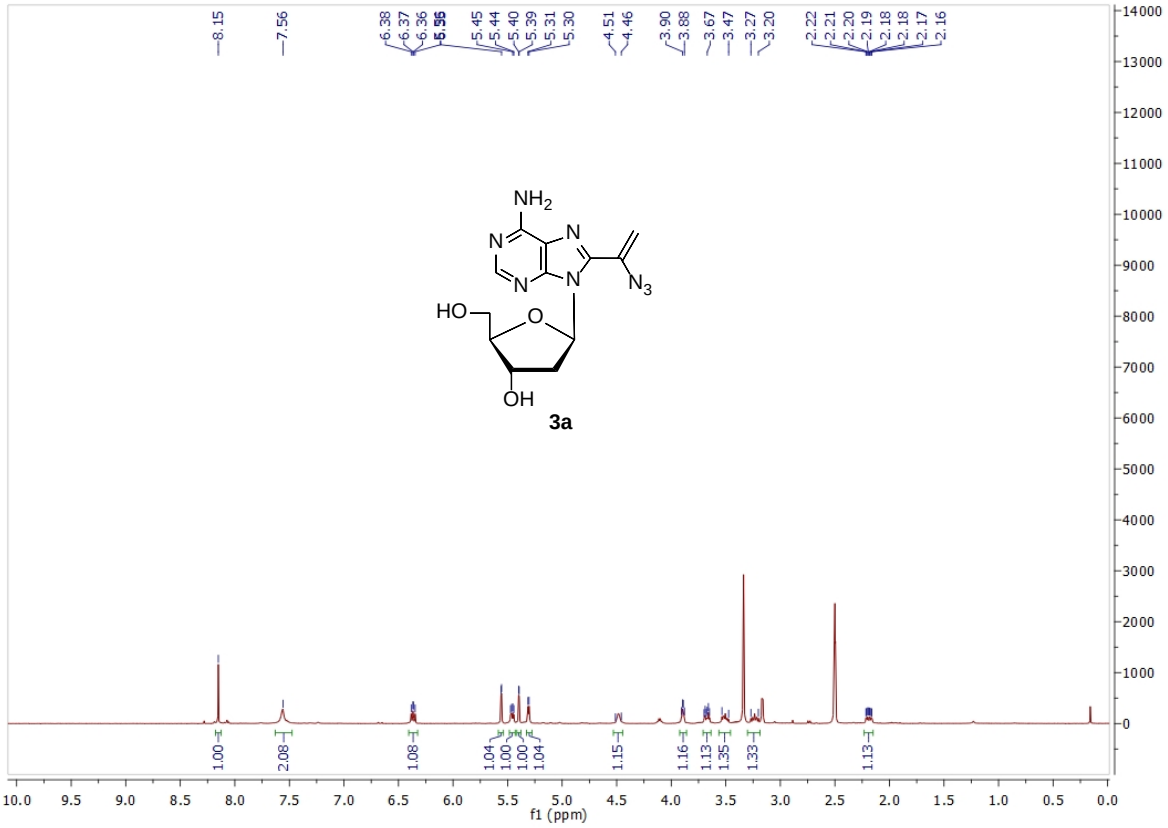
2b

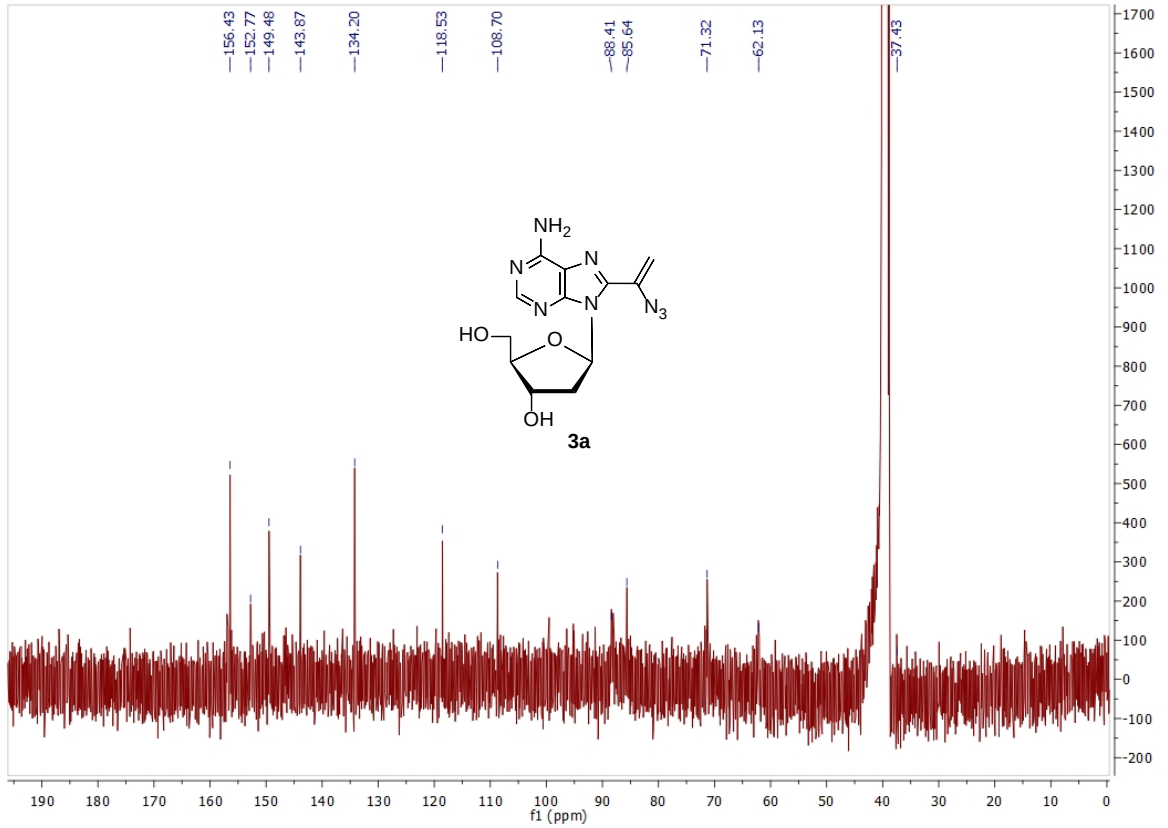


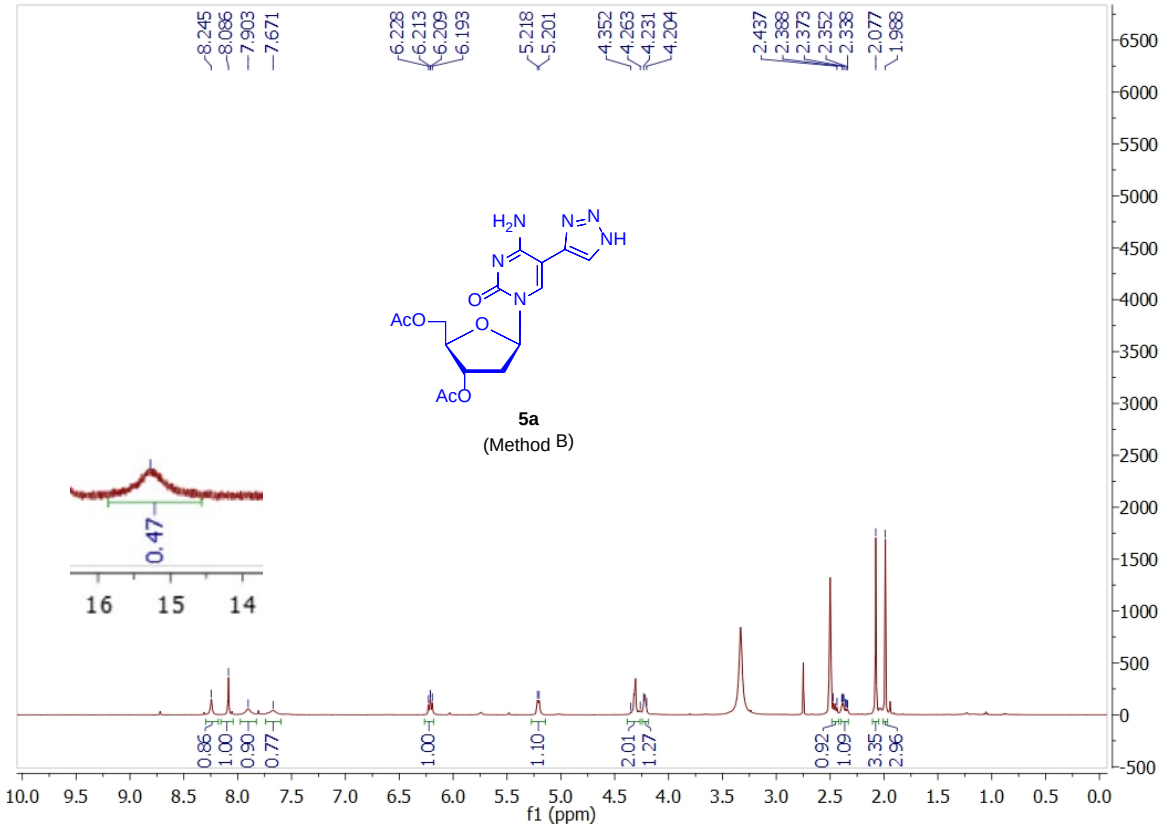
2c
(Method B)

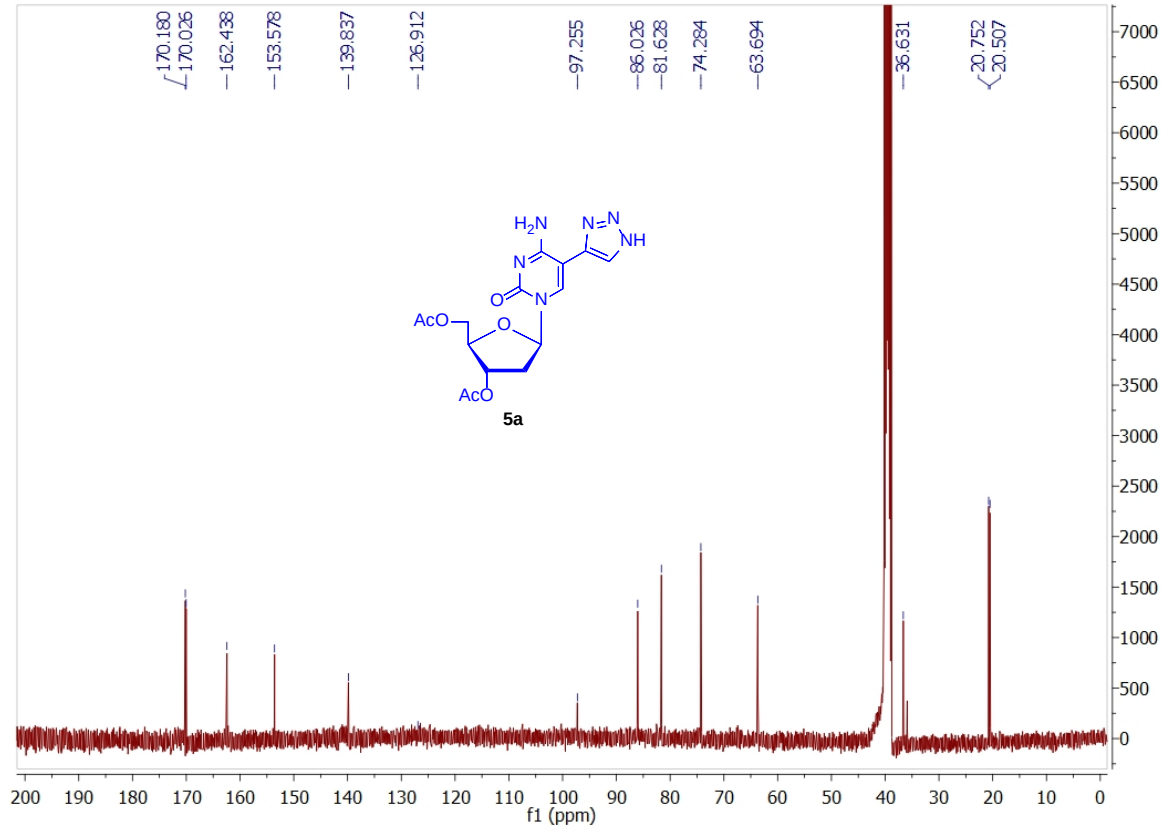


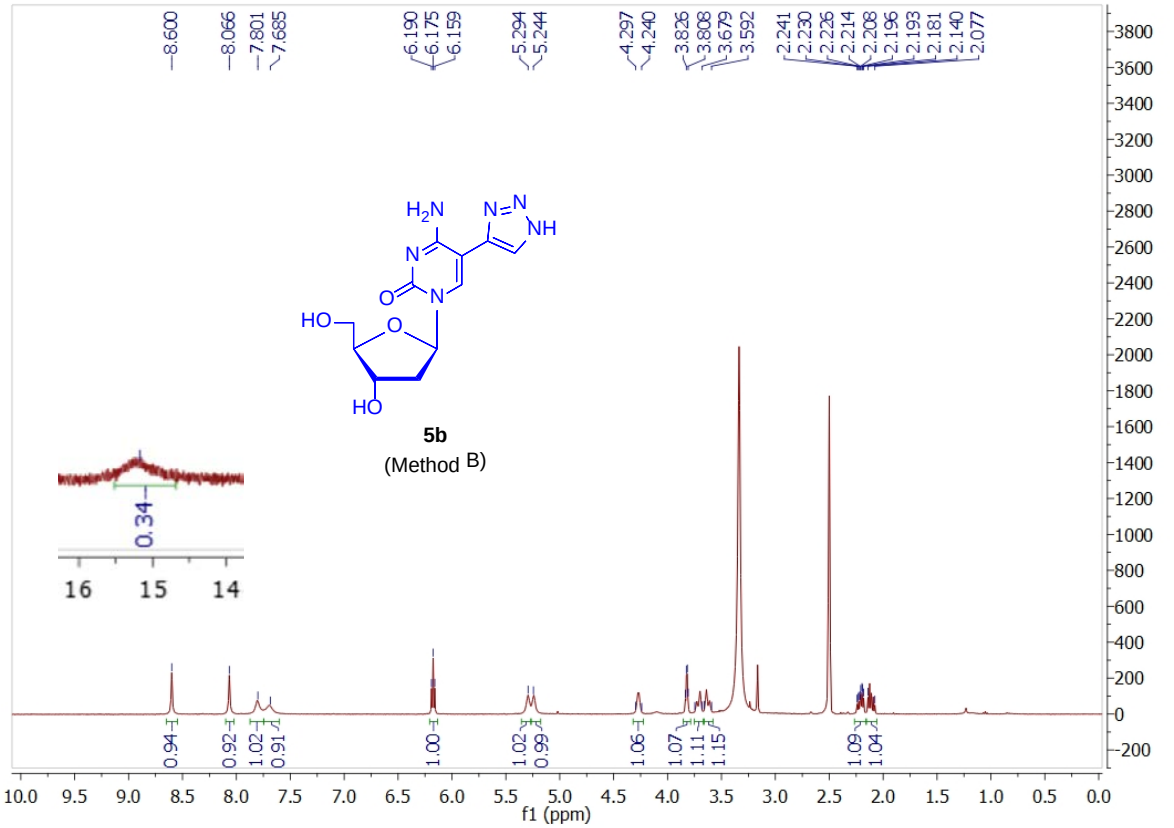
2c


8.15
7.56
6.38
6.37
6.36
5.55
5.45
5.44
5.40
5.39
5.31
5.30
4.51
4.46
3.90
3.88
3.67
3.47
3.27
3.20
2.22
2.21
2.20
2.19
2.18
2.17
2.16
NH2
N
N
N
N
N3
HO
O
OH
3a
1.00
2.08
1.08
1.04
1.00
1.00
1.04
1.15
1.16
1.13
1.35
1.33
1.13
f1 (ppm)


156.43
152.77
149.48
143.87
134.20
118.53
108.70
88.41
85.64
71.32
62.13
37.43
NH2
N
N
N
N
N3
HO
O
OH
3a
f1 (ppm)

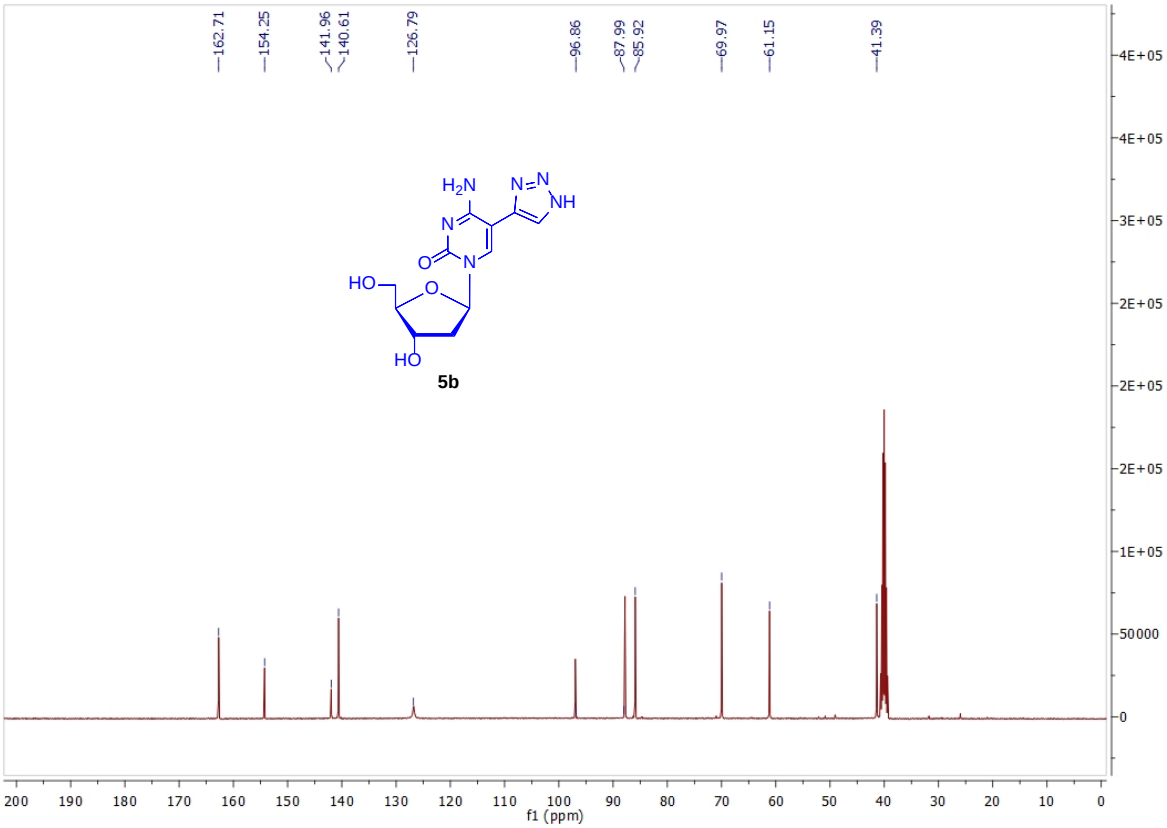

8.245
8.086
7.903
7.671
6.228
6.213
6.209
6.193
5.218
5.201
4.352
4.263
4.231
4.204
2.437
2.388
2.373
2.352
2.338
2.077
1.988
H2N
N=N
NH
N
O
N
AcO
O
AcO
5a
(Method B)
0.47
16
15
14
0.85
1.00
0.90
0.77
1.00
1.10
2.01
1.27
0.92
1.09
3.35
2.96
f1 (ppm)


170.180
170.026
162.438
153.578
139.837
126.912
97.255
86.026
81.628
74.284
63.694
36.631
20.752
20.507
H2N
N=N
NH
N
O
N
AcO
O
AcO
5a
f1 (ppm)

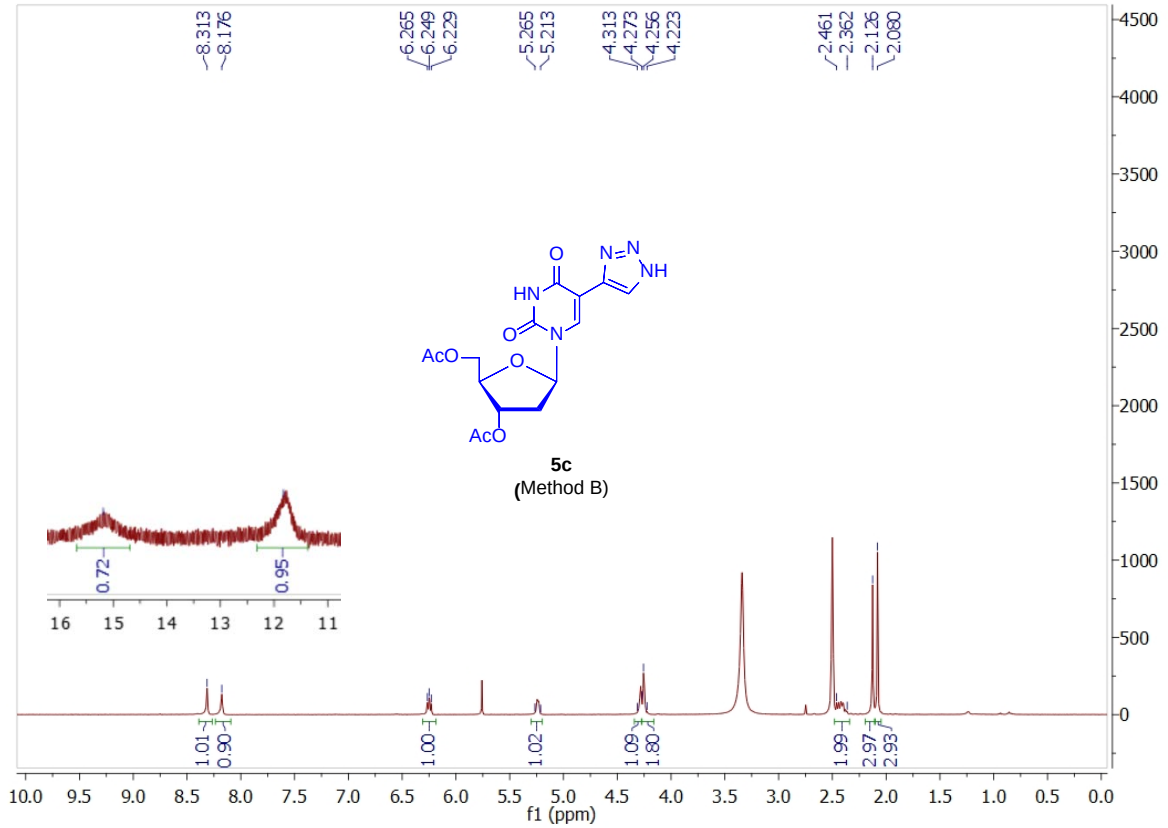


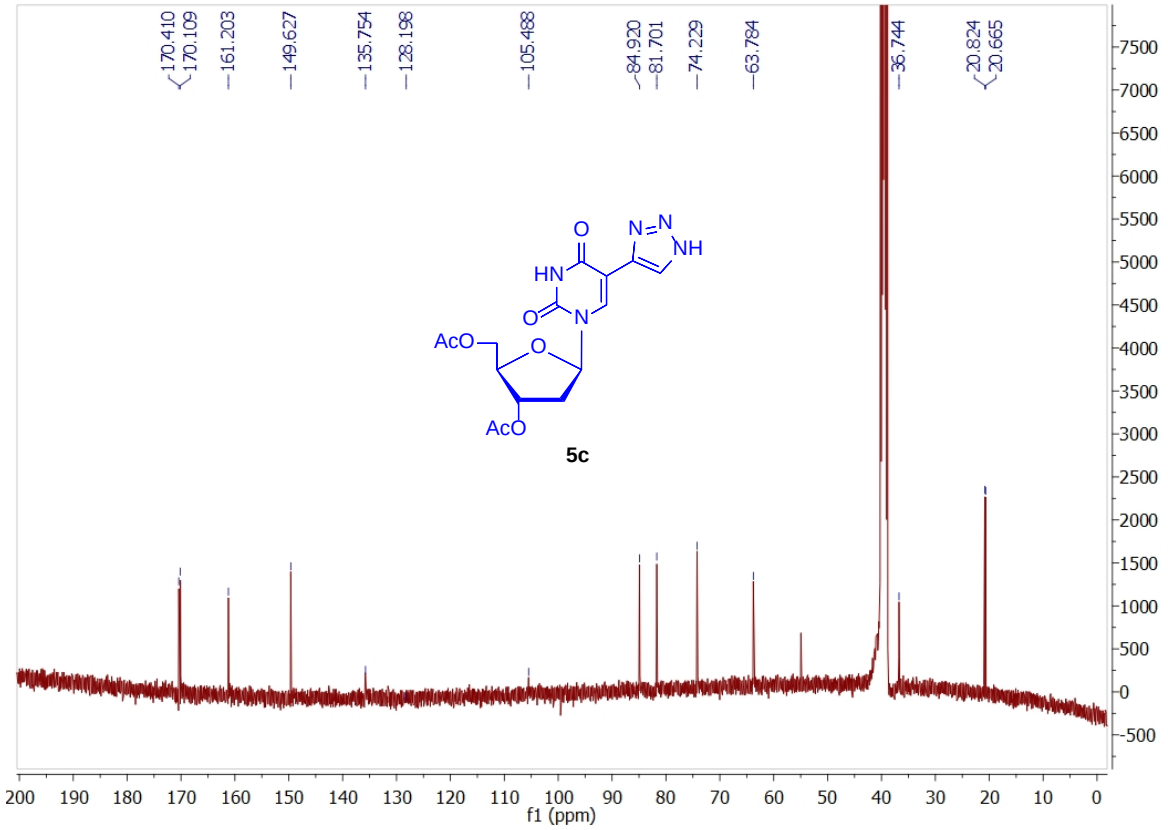
5b

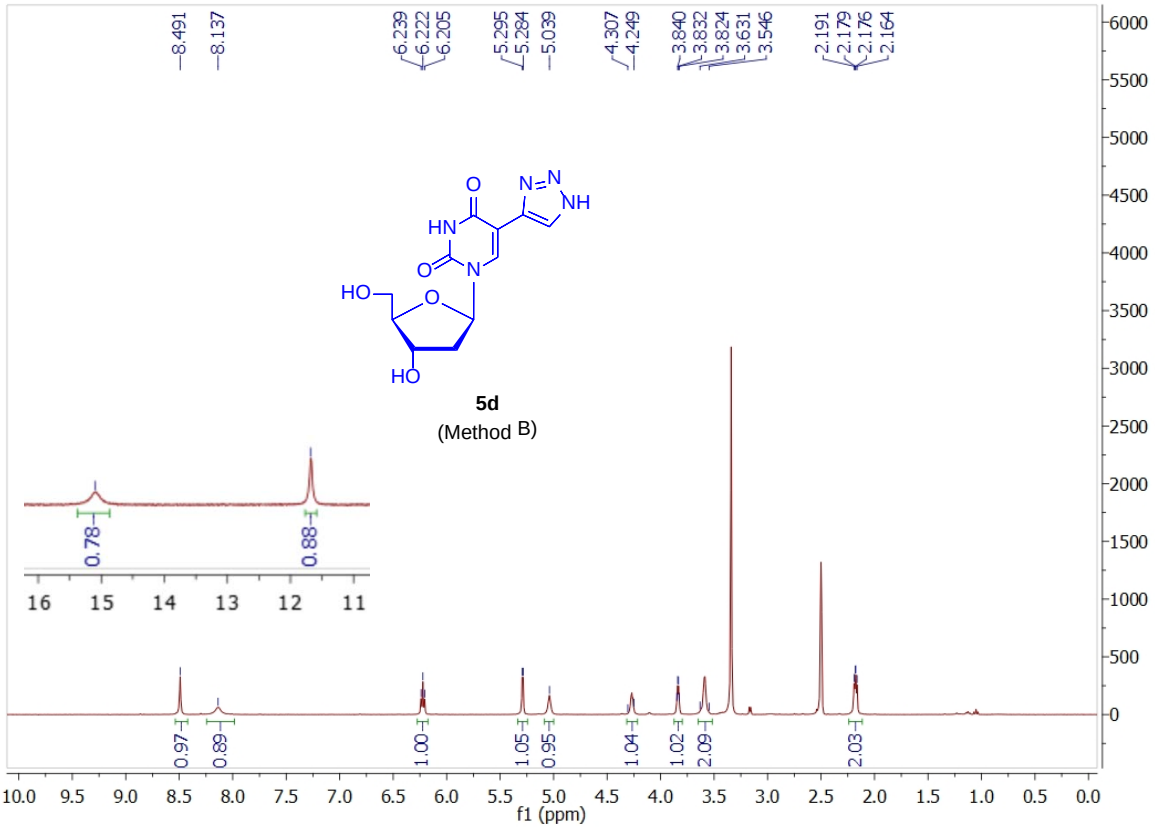
(Method B)

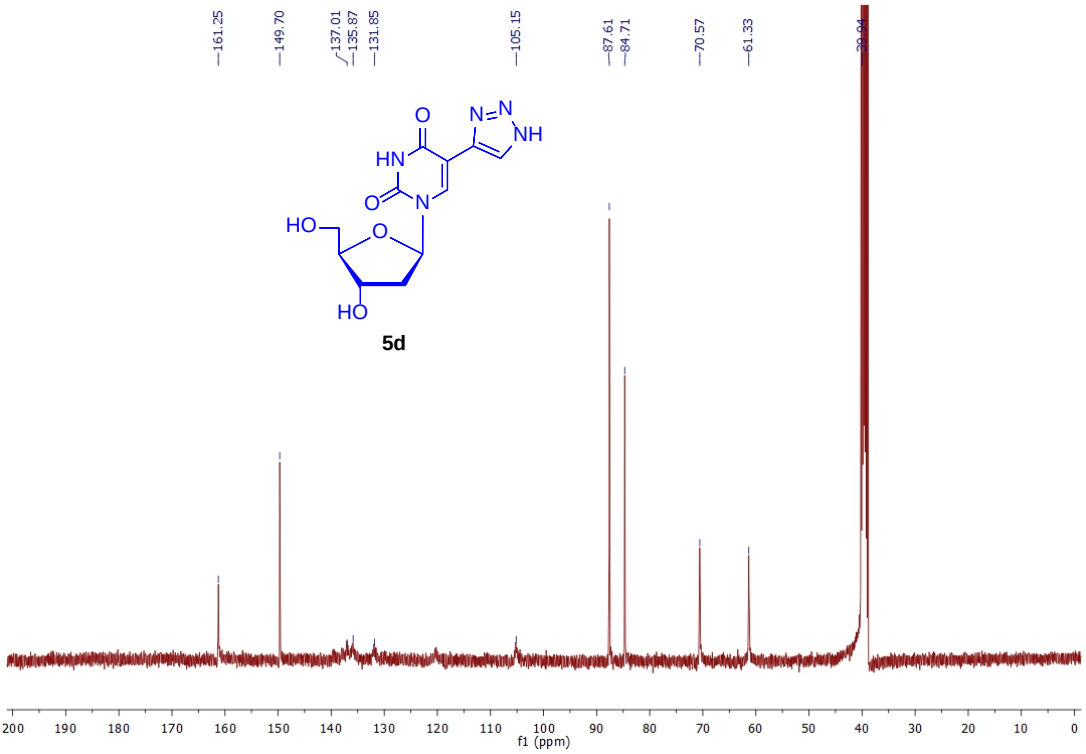


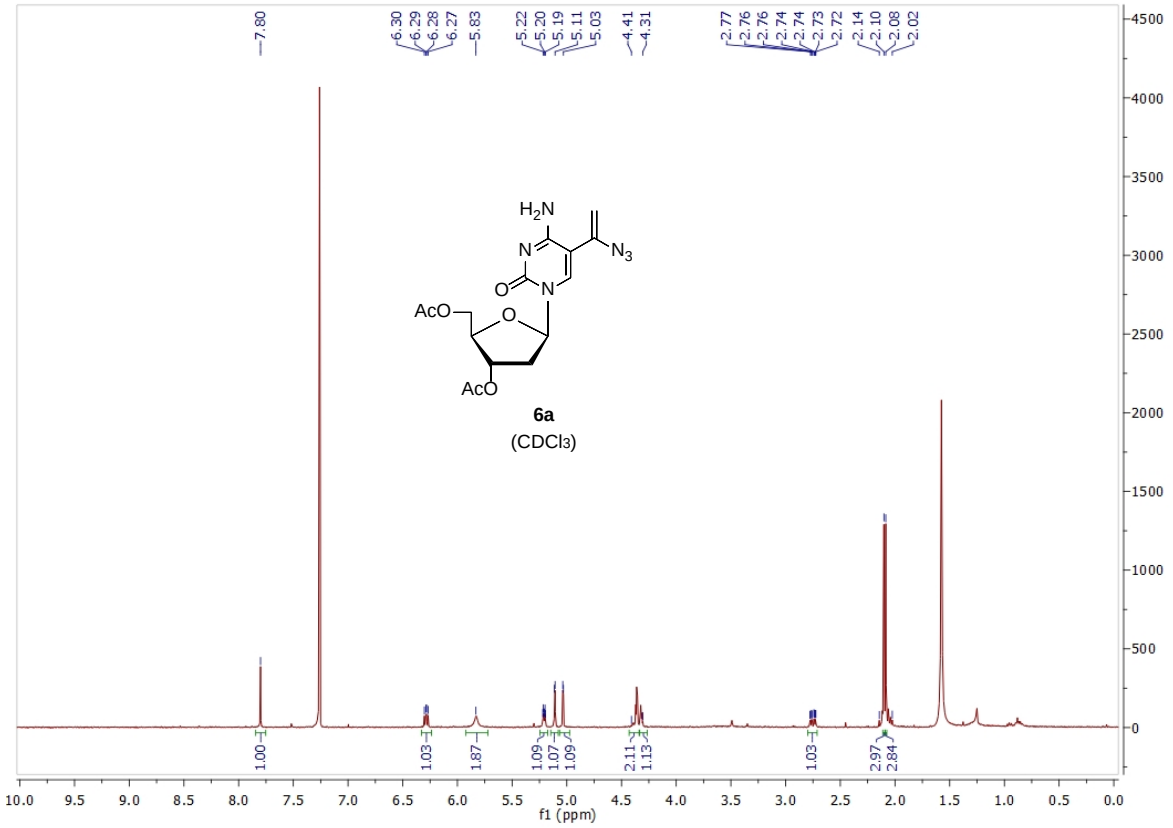
5b


8.313
8.176
6.265
6.249
6.229
5.265
5.213
4.313
4.273
4.256
4.223
2.461
2.362
2.126
2.080
O
N=N
NH
HN
O
N
AcO
O
AcO
5c
(Method B)
0.72
0.95
1.01
0.90
1.00
1.02
1.09
1.80
1.99
2.97
2.93
f1 (ppm)

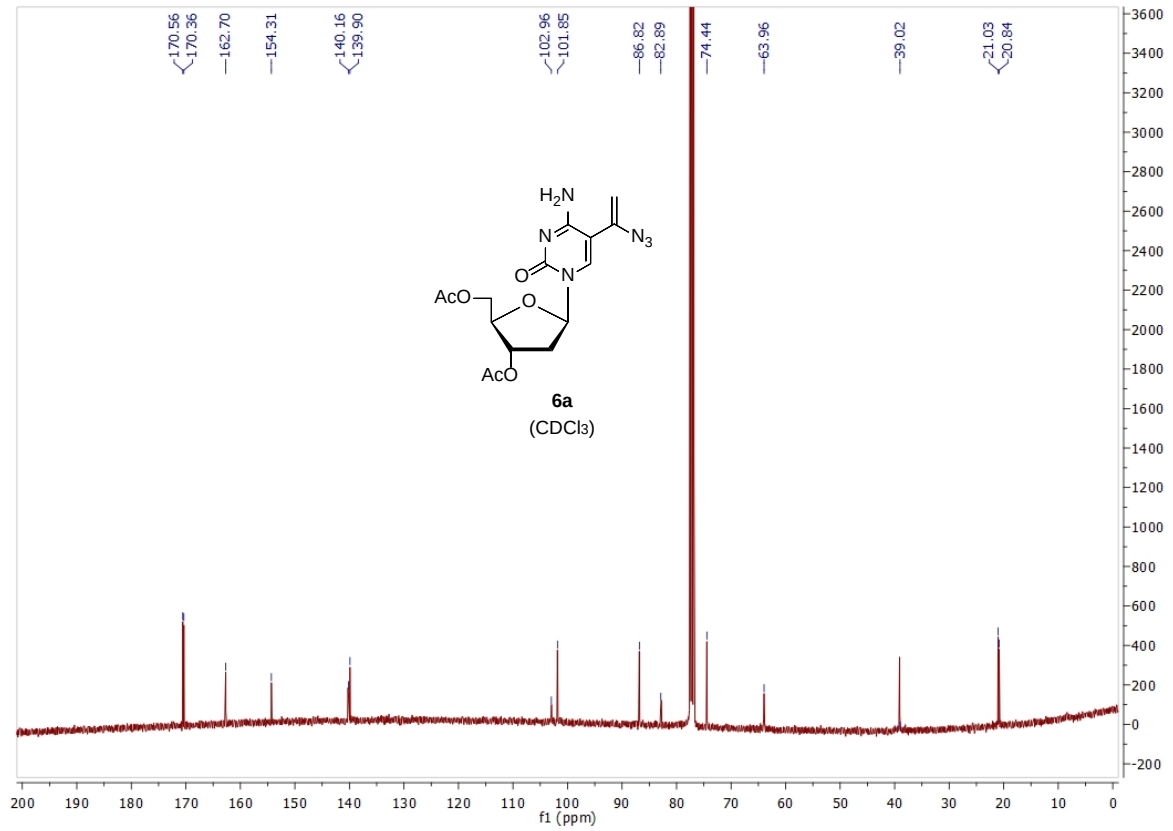

170.410
170.109
161.203
149.627
135.754
128.198
105.488
84.920
81.701
74.229
63.784
36.744
20.824
20.665
O
N=N
NH
HN
O
N
AcO
O
AcO
5c
f1 (ppm)


8.491
8.137
6.239
6.222
6.205
5.295
5.284
5.039
4.307
4.249
3.840
3.832
3.824
3.631
3.546
2.191
2.179
2.176
2.164
HO
HO
HN
O
N
O
N=N
NH
5d
(Method B)
0.78
0.88
0.97
0.89
1.00
1.05
0.95
1.04
1.02
2.09
2.03
f1 (ppm)

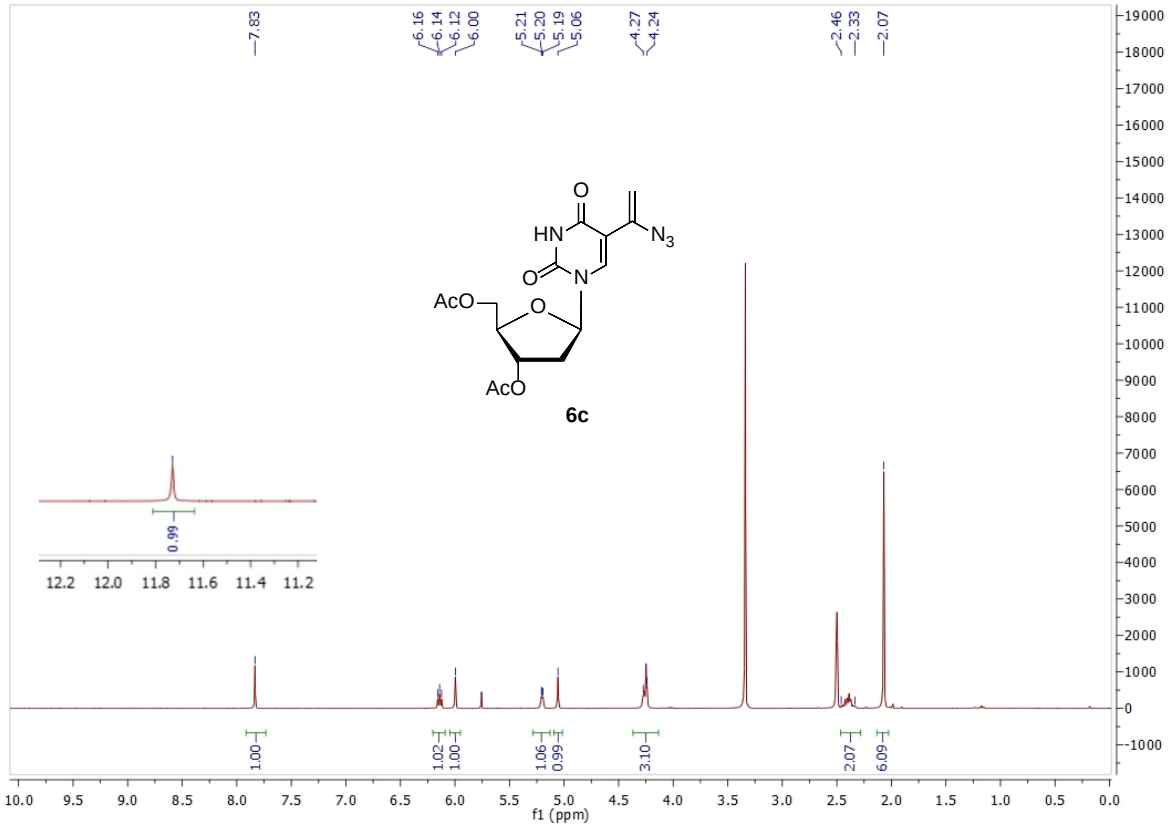

161.25
149.70
137.01
135.87
131.85
105.15
87.61
84.71
70.57
61.33
39.64
HO
HO
HN
O
N
O
N=N
NH
5d
f1 (ppm)

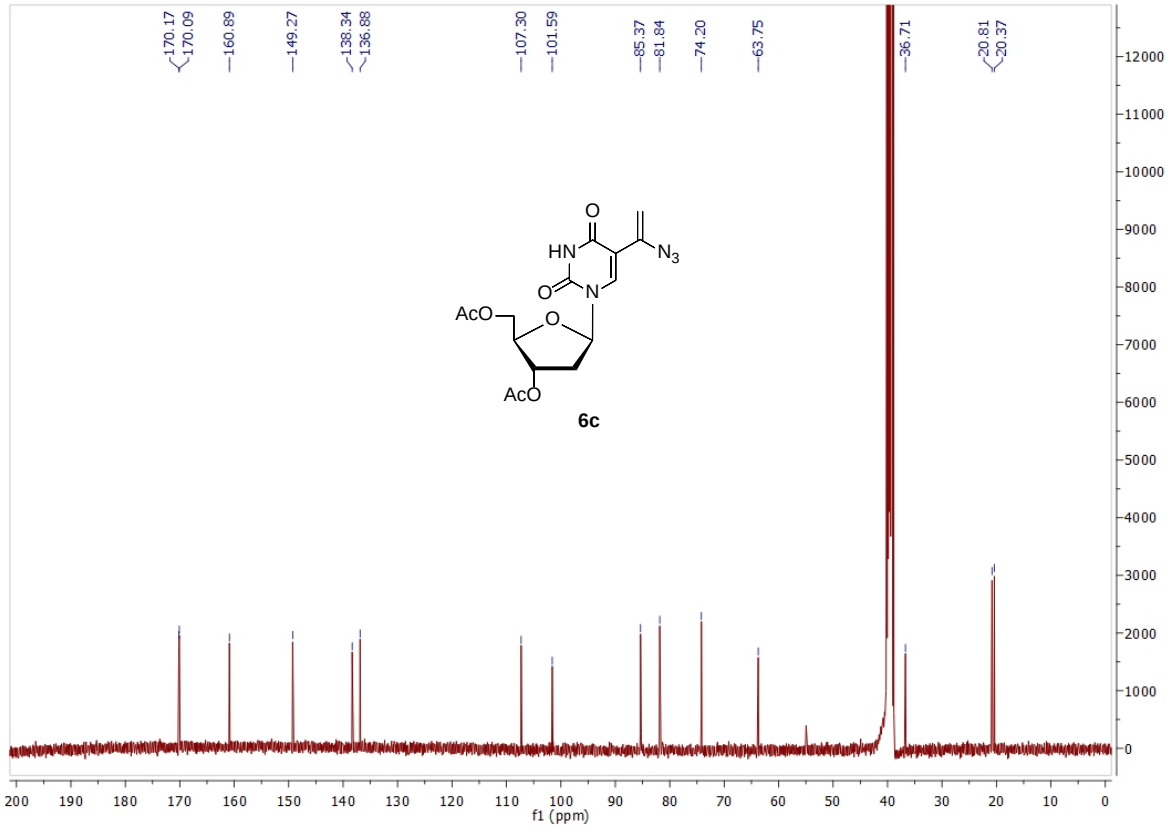


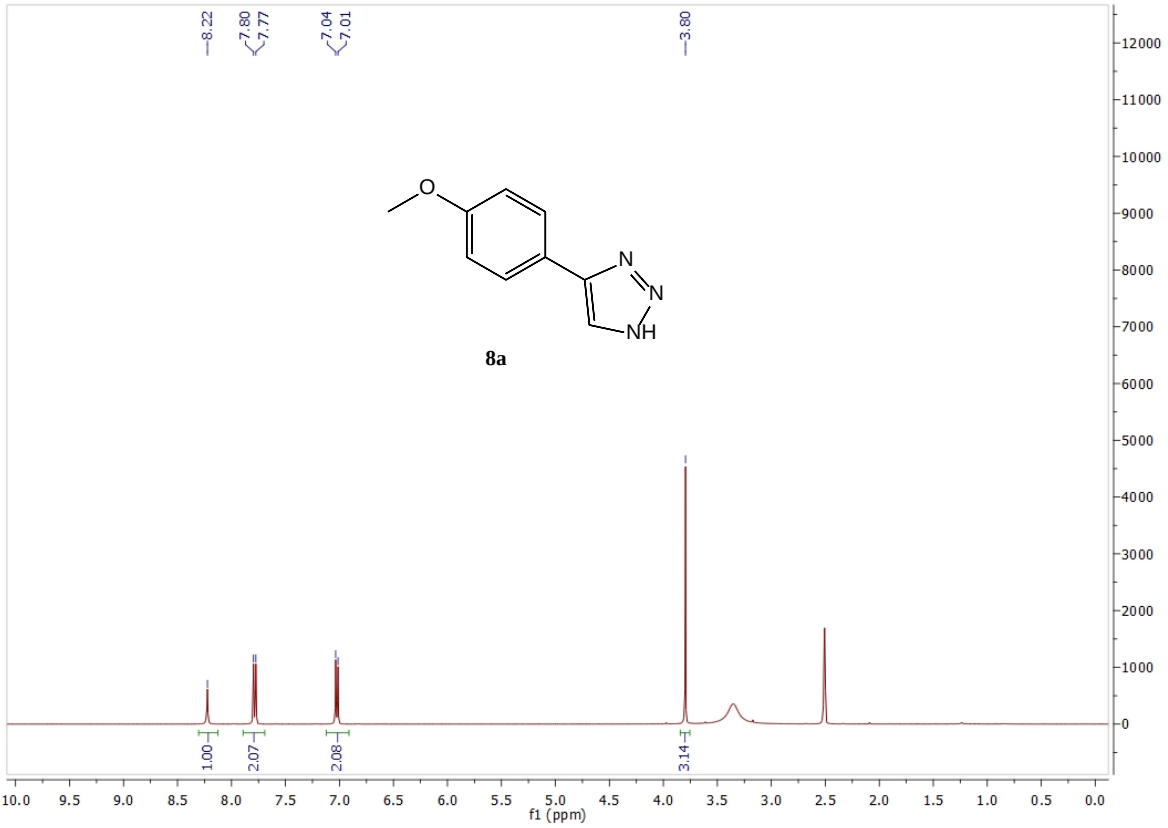
6a
($CDCl_3$)



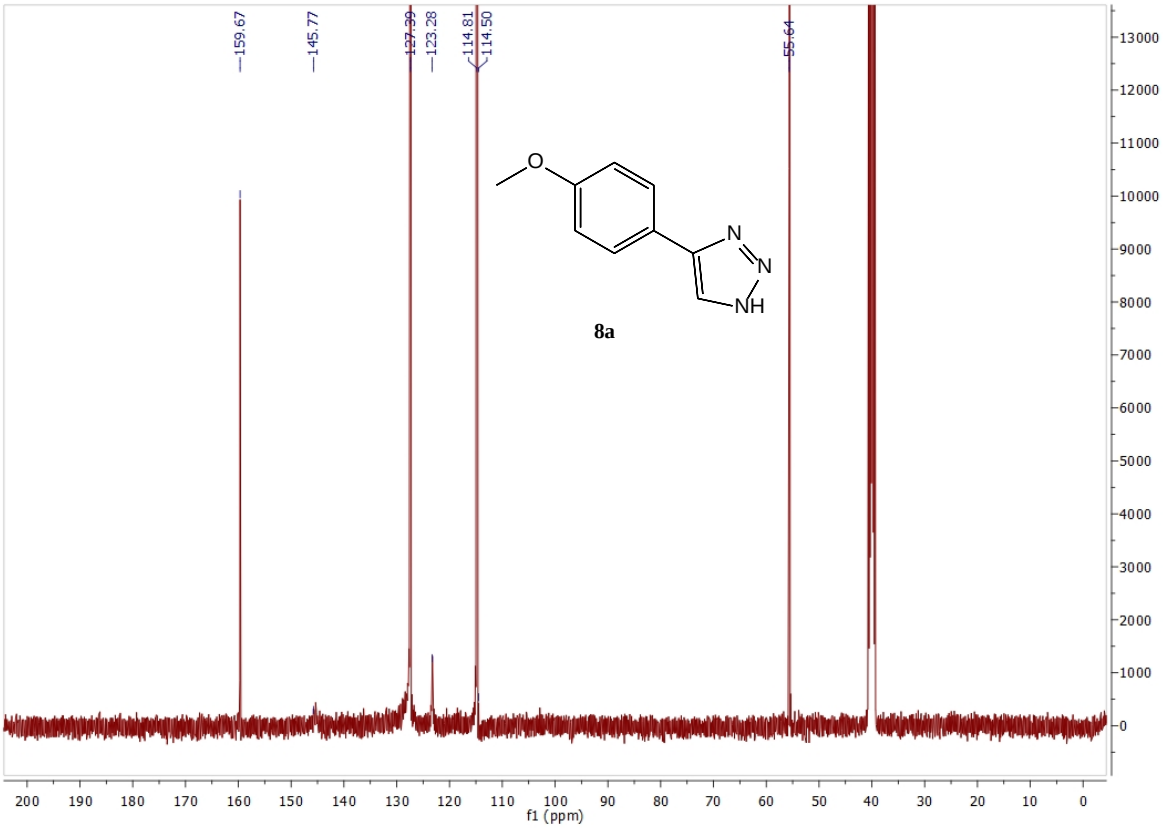
6a
($CDCl_3$)


7.83
6.16
6.14
6.12
6.00
5.21
5.20
5.19
5.06
4.27
4.24
2.46
2.33
2.07
HN
O
N
N3
AcO
AcO
6c
0.99
1.00
1.02
1.00
1.06
0.99
3.10
2.07
6.09
f1 (ppm)

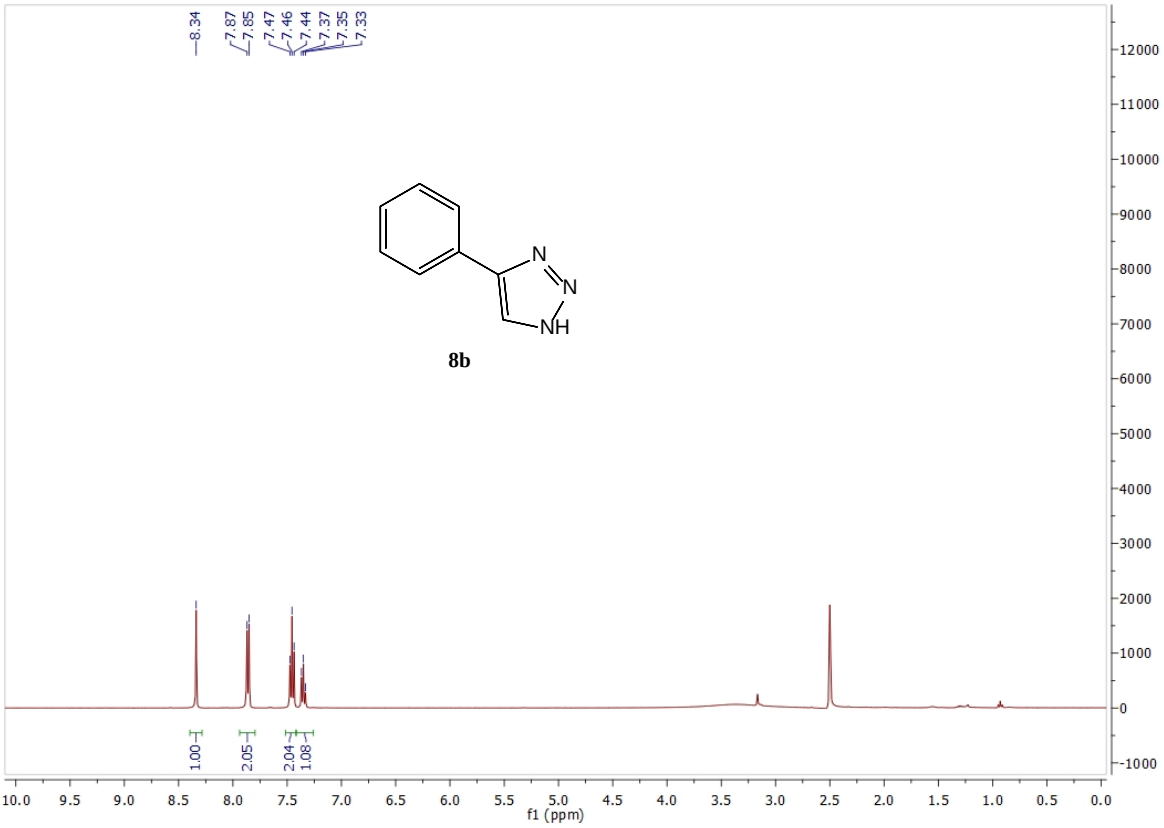

170.17
170.09
160.89
149.27
138.34
136.88
107.30
101.59
85.37
81.84
74.20
63.75
36.71
20.81
20.37
HN
O
N
N3
AcO
AcO
6c
f1 (ppm)


8.22
7.80
7.77
7.04
7.01
3.80
O
N
N
NH
1.00
2.07
2.08
3.14
f1 (ppm)

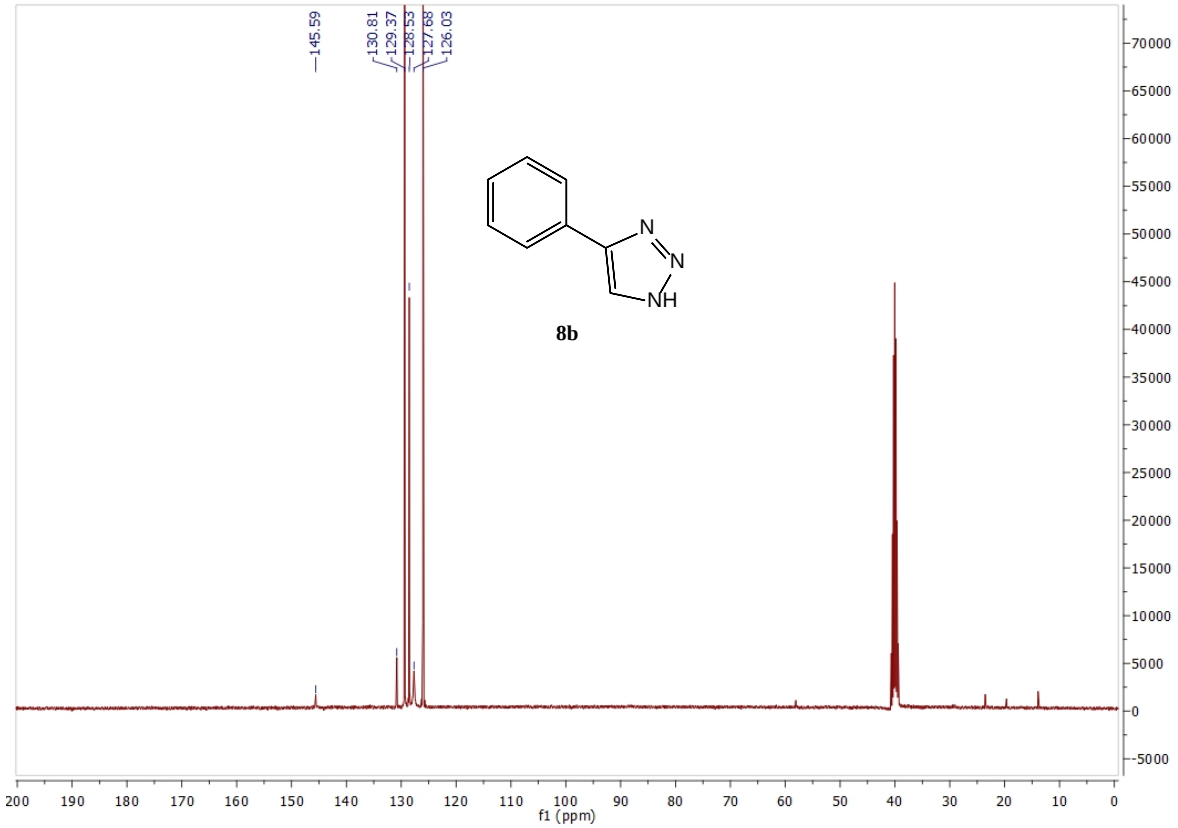
8a


159.67
145.77
127.39
123.28
114.81
114.50
55.64
O
N
N
NH
f1 (ppm)

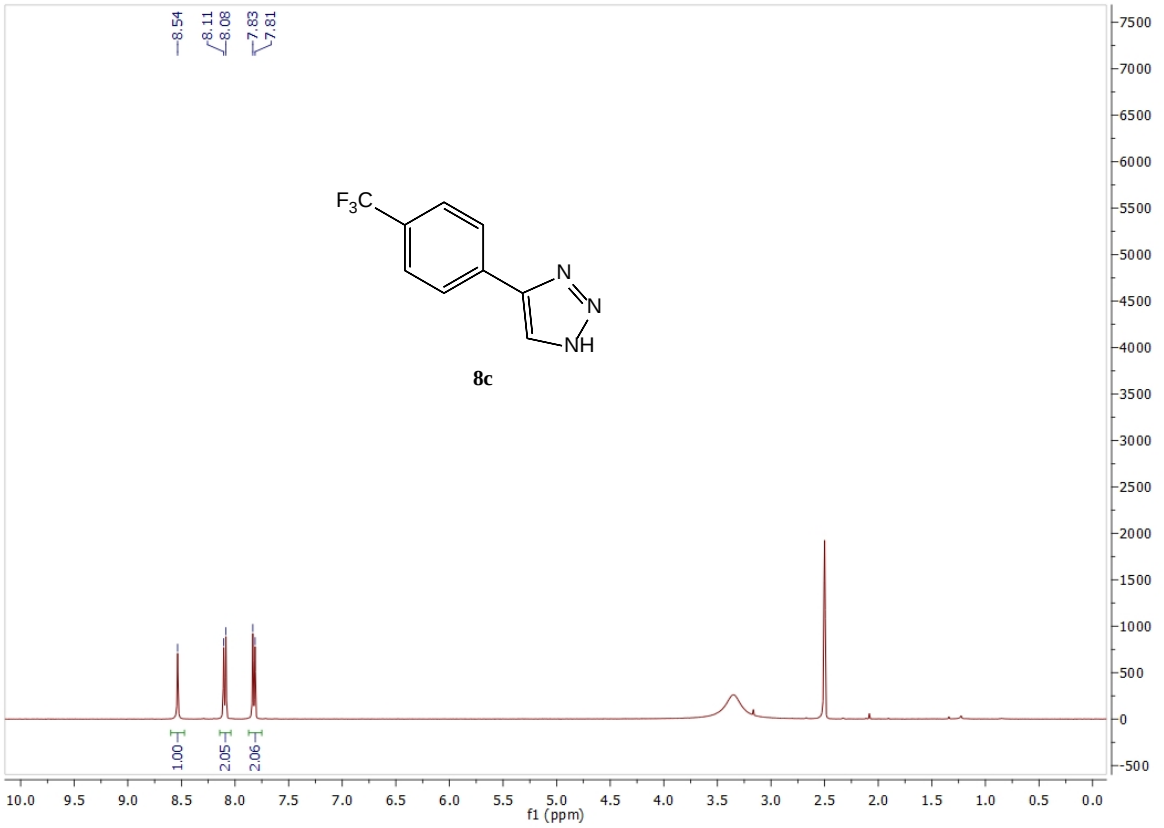
8a

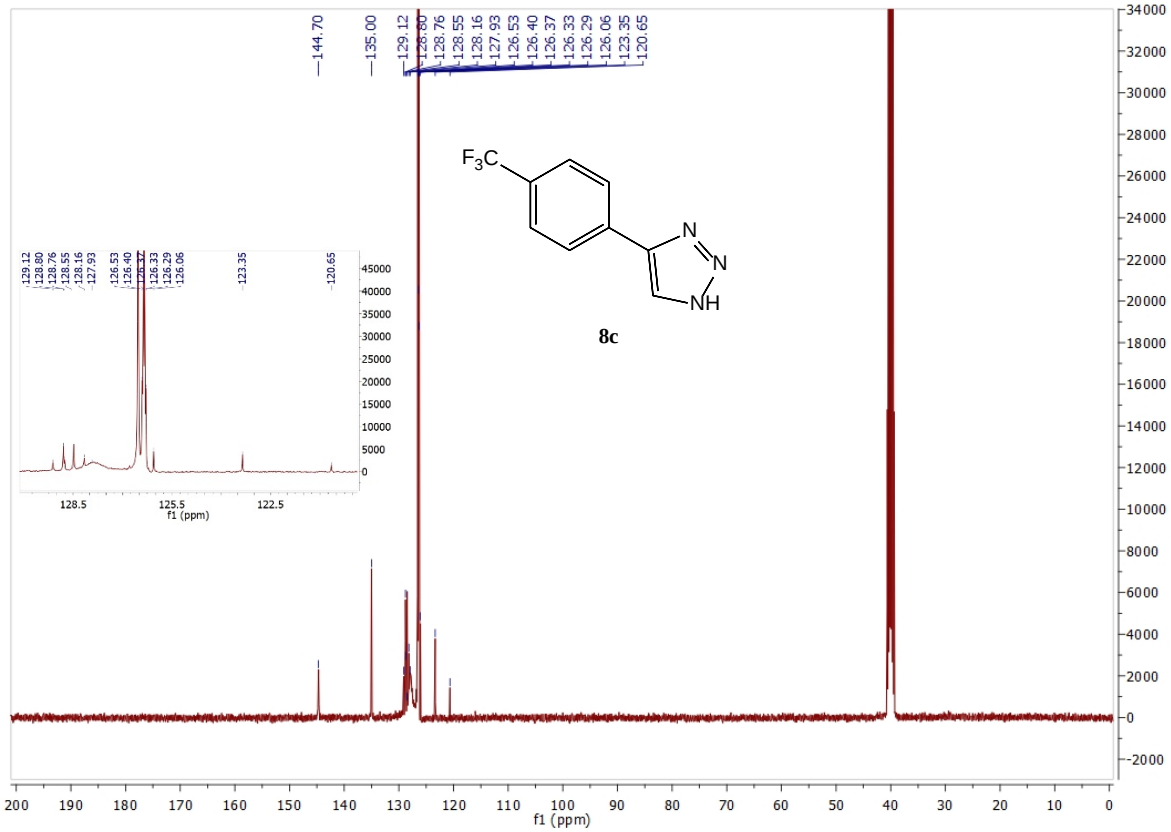

8.34
7.87
7.85
7.47
7.46
7.44
7.37
7.35
7.33
N
N
NH
1.00
2.05
2.04
1.08
f1 (ppm)

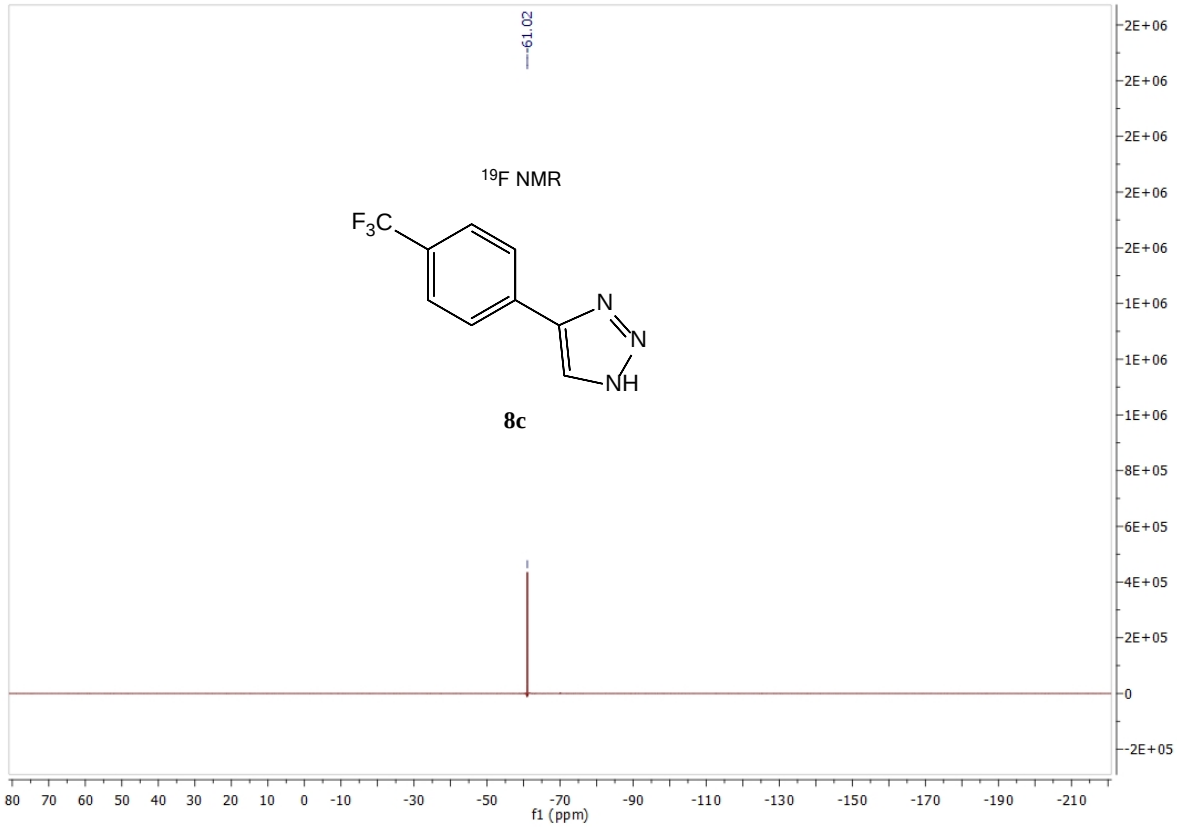
8b


145.59
130.81
129.37
128.53
127.68
126.03
N
N
NH
f1 (ppm)

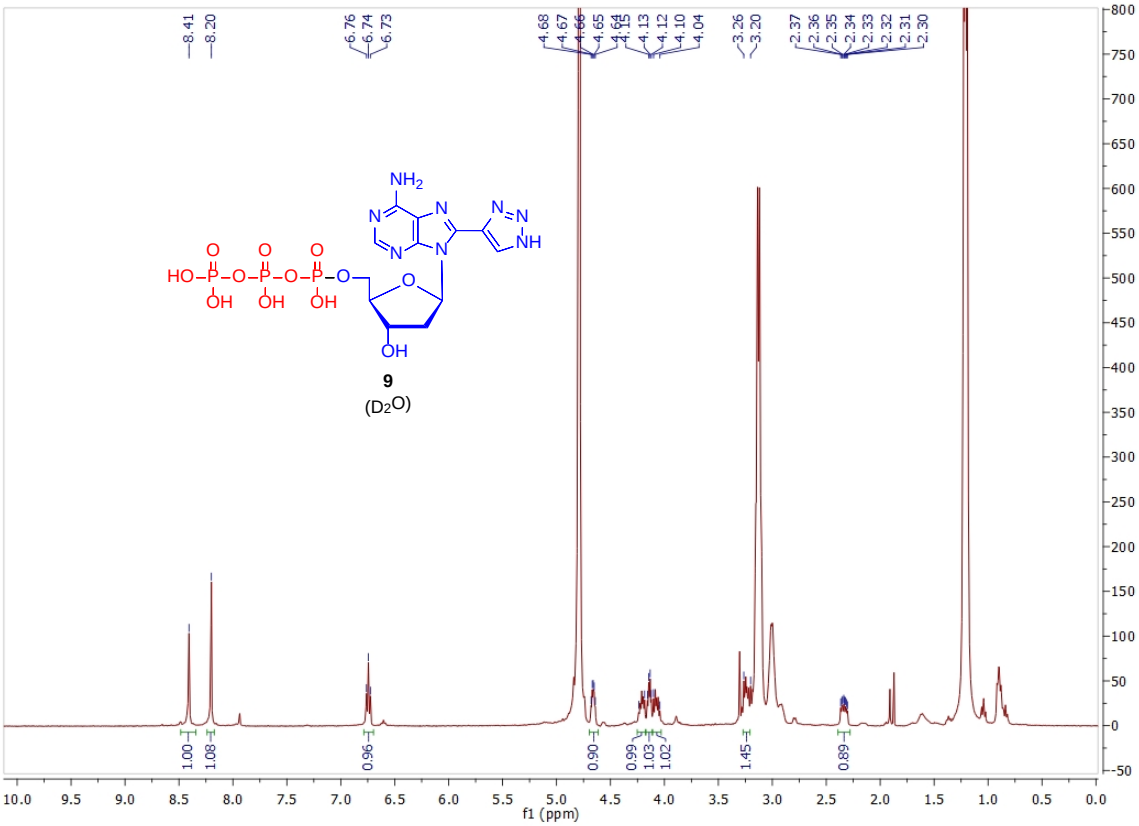
8b

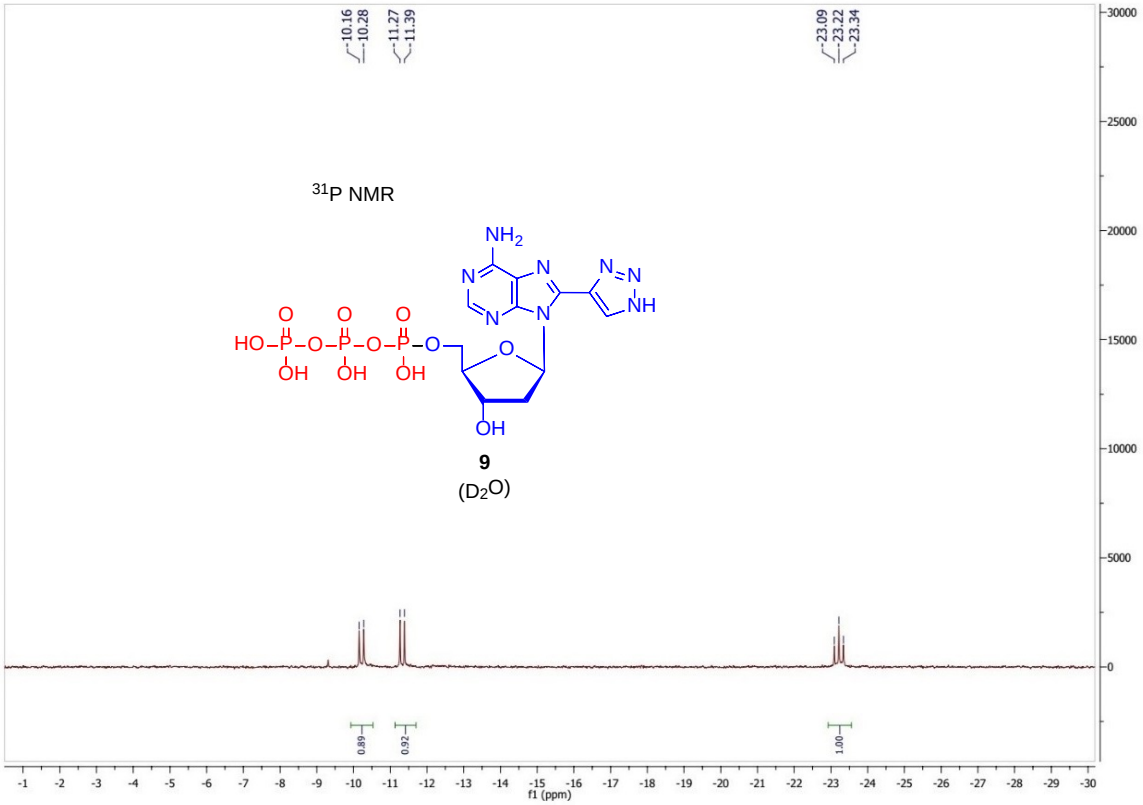

8.54
8.11
8.08
7.83
7.81
F3C
N
N
NH
8c
1.00
2.05
2.06
f1 (ppm)

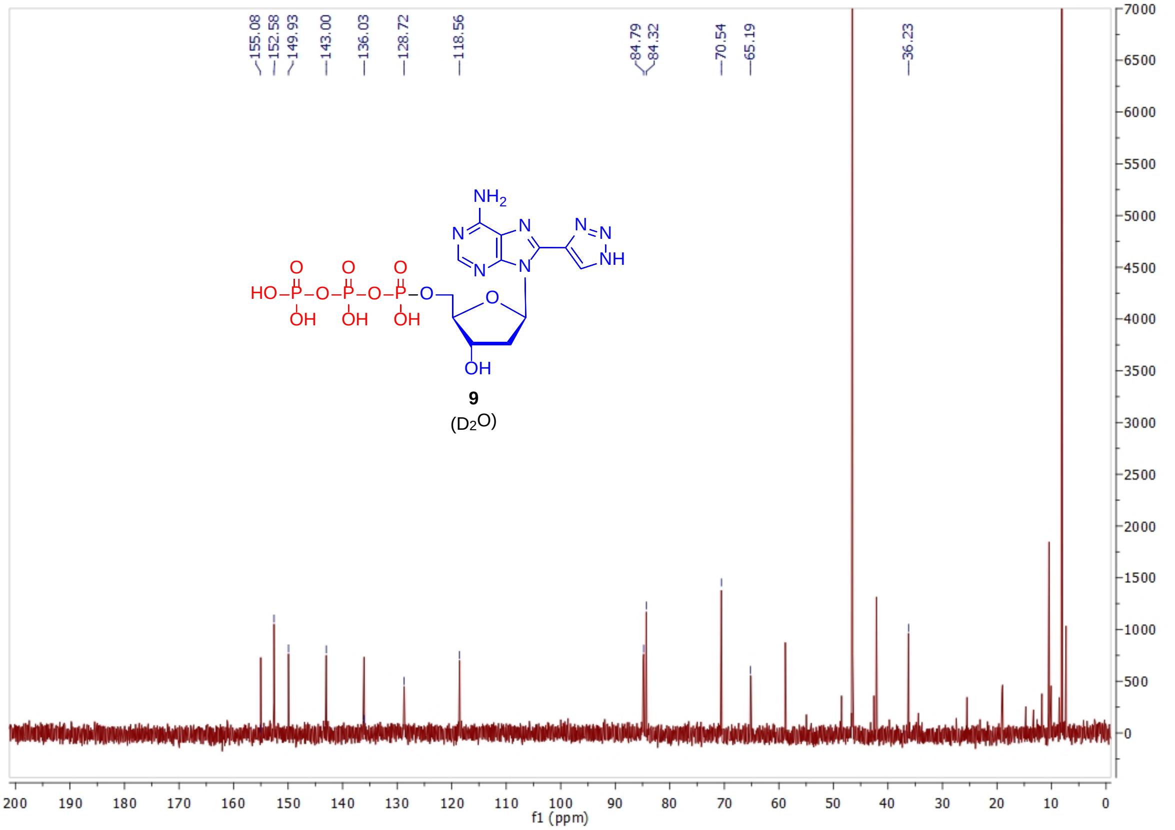

144.70
135.00
129.12
128.80
128.76
128.55
128.16
127.93
126.53
126.40
126.37
126.33
126.29
126.06
123.35
120.65
F3C
N
N
NH
8c
f1 (ppm)


-61.02
^{19}F NMR
F_3C
N
N
NH
2E+06
2E+06
2E+06
2E+06
2E+06
1E+06
1E+06
1E+06
8E+05
6E+05
4E+05
2E+05
0
-2E+05
80 70 60 50 40 30 20 10 0 -10 -30 -50 -70 -90 -110 -130 -150 -170 -190 -210
f1 (ppm)

8c


9
(D2O)
f1 (ppm)


31P NMR
9
(D2O)
f1 (ppm)


155.08
152.58
149.93
143.00
136.03
128.72
118.56
84.79
84.32
70.54
65.19
36.23
NH2
N
N
N
N
N
N
NH
O
HO
P
OH
9
(D2O)
f1 (ppm)

5. References

S1. Zayas, J.; Annoual, M.; Das, J. K.; Felty, Q.; Gonzalez, W. G.; Miksovska, J.; Sharifai, N.; Chiba, A.; Wnuk, S. F., Strain Promoted Click Chemistry of 2- or 8-Azidopurine and 5-Azidopyrimidine Nucleosides and 8-Azidoadenosine Triphosphate with Cyclooctynes. Application to Living Cell Fluorescent Imaging. *Bioconjug. Chem.* **2015,** *26*, 1519-1532.

S2. Ren, Y.; Lai, Y.; Laverde, E. E.; Lei, R.; Rein, H. L.; Liu, Y., Modulation of trinucleotide repeat instability by DNA polymerase β polymorphic variant R137Q. *PLoS One* **2017,** *12*, e0177299.

S3. Beaver, J. M.; Lai, Y.; Xu, M.; Casin, A. H.; Laverde, E. E.; Liu, Y., AP endonuclease 1 prevents trinucleotide repeat expansion via a novel mechanism during base excision repair. *Nucleic Acids Res.* **2015,** *43*, 5948-5960.

S4. Lai, Y.; Weizmann, Y.; Liu, Y., The deoxyribose phosphate lyase of DNA polymerase β suppresses a processive DNA synthesis to prevent trinucleotide repeat instability. *Nucleic Acids Res.* **2018,** *46*, 8940-8952.