Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
This journal is © The Royal Society of Chemistry 2016

Electronic Supplementary Information

2'-*O*-Methyl- and 2'-*O*-propargyl-5-methylisocytidine: synthesis, properties and impact on the $isoC_d$ – dG and the $isoC_d$ – $isoG_d$ base pairing in nucleic acids with parallel and antiparallel strand orientation

Sunit K. Jana[a], Peter Leonard[a], Sachin A. Ingale[a] and Frank Seela*[a,b]

[a]Laboratory of Bioorganic Chemistry and Chemical Biology, Center for Nanotechnology, Heisenbergstrasse 11, 48149 Münster, Germany and [b]Laboratorium für Organische und Bioorganische Chemie, Institut für Chemie neuer Materialien, Universität Osnabrück, Barbarastrasse 7, 49069 Osnabrück, Germany

Corresponding author: Prof. Dr. Frank Seela

Phone: +49 (0)251 53 406 500; Fax: +49 (0)251 53 406 857

E-mail: Frank.Seela@uni-osnabrueck.de

Homepage: www.seela.net

Table of Contents

Table S1. ^{13}C NMR data of 2'-*O*-alkylated 5-methylpyrimidine nucleosides…….....3

Table S2. Selected ^{1}H-^{13}C coupling constants of 2'-*O*-5-dimethylpyrimidine derivatives….....4

Fig. S1 MALDI-TOF spectra of selected oligonucleotides…….……5-6

Fig. S2 Reversed-phase RP-18 HPLC profile of the acidic hydrolysis of dA…..…….……6

Fig. S3 Anticipated mechanism for the fragmentation of oligonucleotides containing $^{Me}iC_d$ (**1b**)……..7

References…….…….7

Fig. S4 Reversed-phase HPLC profiles of purified oligonucleotides…….……8-9

Fig. S5 Melting profiles of duplexes…….……..10-13

Fig. S6-S82 NMR spectra……..……...…...14-52

Table S1. ^{13}C NMR data of 2'-*O*-alkylated 5-methylpyrimidine nucleosides [a,b]

	C(2)	C(4)	C(5)	C(6)	C(1')	C(2')[c]	C(3')	C(4')[c]	C(5')	C≡C	OCH_3 OCH_2 qC	N=CH	CH_3 NCH_3
3	154.5	170.3	114.4	134.7	90.0	81.4	68.3	86.3	60.8	-	57.5	-	13.6
4	154.1	169.7	114.0	134.6	89.6	78.1	67.9	86.0	60.5	77.6 79.4	56.7	-	13.2
5	150.6	163.7	109.4	136.1	85.6	82.4	68.3	85.1	60.6	-	57.4	-	12.3
6	150.4	163.6	110.2	136.7	87.8	78.5	75.5	78.4	68.3	-	57.8	-	12.1 21.1 21.2
7	150.4	163.3	109.9	135.4	85.0	79.2	77.0	82.5	60.1	-	57.4	-	12.0 20.9
8	150.1	163.4	109.7	135.5	86.5	81.1	68.0	80.6	69.5	-	57.4	-	11.8 20.9
9	156.5	170.8	117.2	138.6	96.7	87.5	71.5	86.0	73.9	-	57.9	-	12.8
10	150.4	163.7	109.6	135.5	86.4	82.9	68.7	82.3	63.1	-	57.7 85.9	-	11.7
11	150.5	163.6	110.2	135.5	86.3	80.5	70.2	79.7	62.8	-	55.0 58.2 86.2	-	11.7 20.6
12	150.7	163.6	110.1	135.7	85.4	80.3	70.8	82.9	60.8	-	58.1	-	12.3 20.7
13	150.2	163.3	110.0	135.7	86.8	79.0	69.6	78.4	69.1	-	57.9	-	11.9 20.8
14	156.6	169.7	117.6	138.7	96.5	84.9	73.5	84.4	73.8	-	58.7	-	13.1 20.5
1d	154.2	170.0	113.8	134.4	91.2	72.5	69.6	85.4	60.5	-	-	-	13.3
17	157.1	170.1	116.0	133.7	87.1	83.2	68.0	84.4	60.2	-	57.5	158.0	13.7 34.6 40.6
18	157.0	170.2	115.9	133.7	87.1	80.2	68.0	84.4	60.0	77.3 79.6		158.0	13.6
19	157.1	170.0	116.3	133.1	87.6	82.5	68.6	83.1	62.8	-	54.8 57.8 85.7	158.0	13.1 34.6 40.6
20	157.3	170.2	116.3	133.2	87.3	79.7	68.7	83.1	63.0	77.6 79.8	56.9 55.0 86.0	158.2	13.0
23	154.4	[d]	114.2	134.7	89.9	79.3	68.3	86.1	62.8	-	52.4	-	13.4
24	[d]	[d]	[d]	134.4	90.0	79.3	68.3	86.1	62.8	-	52.4	-	13.6

[a]Measured in DMSO-d_6 at 298 K. [b] Pyrimidine numbering. [c] Tentative. [d] Not detected.

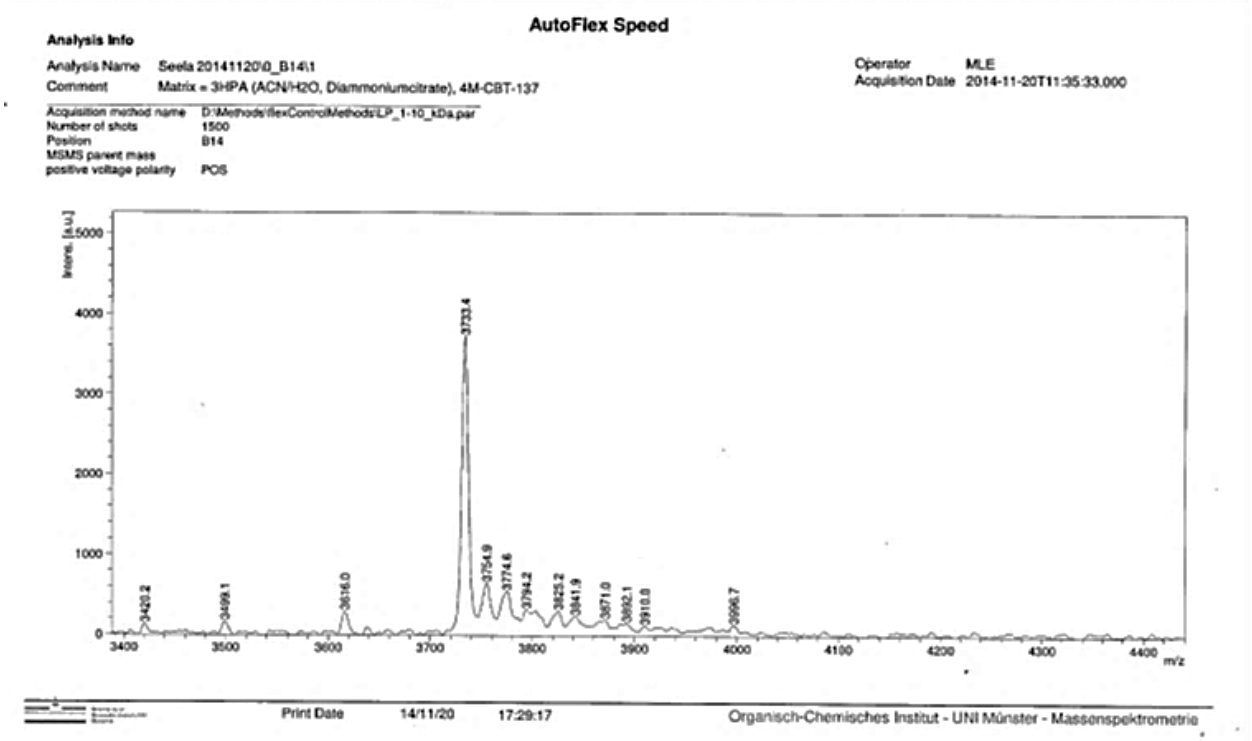
Table S2. Selected ^{1}H-^{13}C coupling constants (Hz) of 2'-*O*-alkylated 5-methylpyrimidine nucleosides. [a,b]

^{1}H-^{13}C Coupling Constants	**3**	**4**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**17**	**18**	**19**	**20**	**23**	**24**
^{1}J(C6, H-C6)	180	179	181	182	174	183	180	180	181	180	183	180	185	173	179	177	183
^{3}J(C6, H-C1')	4.9	n.d.	5.7	4.8	5.8	5.8	n.d.	5.3	6.0	n.d.	4.9	n.d.	5.7	n.d.	n.d.	n.d.	n.d.
^{1}J(C1', H-C1')	164	163	167	168	167	170	168	166	166	164	171	171	170	171	171	163	163
^{1}J(C2', H-C2') [c]	146	151	153	155	150	153	148	151	150	170	152	149	150	155	148	147	145
^{1}J(C3', H-C3')	151	151	159	163	150	151	148	157	159	157	158	146	148	143	147	150	151
^{1}J(C4', H-C4') [c]	148	149	153	151	151	152 [c]	147	150	149	150	156	147	147	145	146	148	149
^{1}J(C5', H-C5')	141	140	153	141	152	151	142	145	141	152	153	141	141	143	143	145	145
^{1}J (N=CH)	-	-	-	-	-	-	-	-	-	-	-	181	182	172	181	-	-

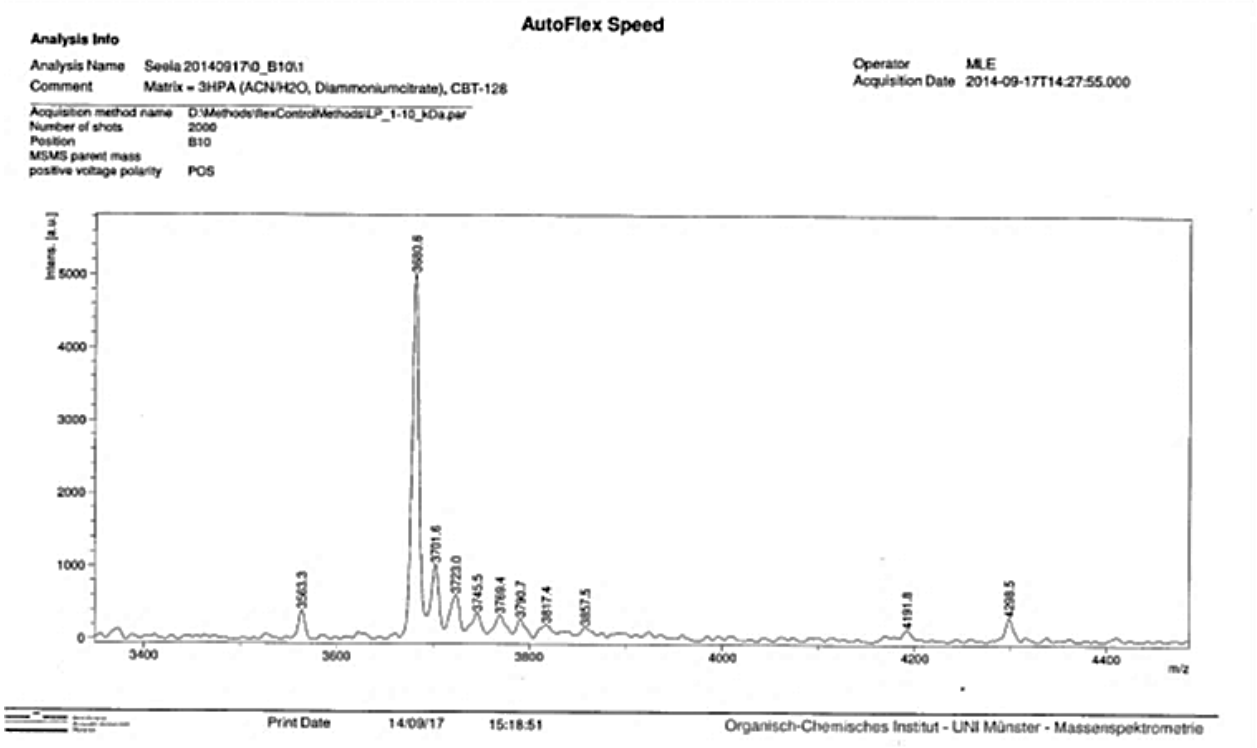
[a] Measured in DMSO-d_6 at 298 K. [b] Pyrimidine numbering. [c] ^{1}J(C2', H-C2') or ^{1}J(C4', H-C4'). n.d.: not detected.

Selected MALDI-TOF mass spectra of oligonucleotides

a)



b)



c)

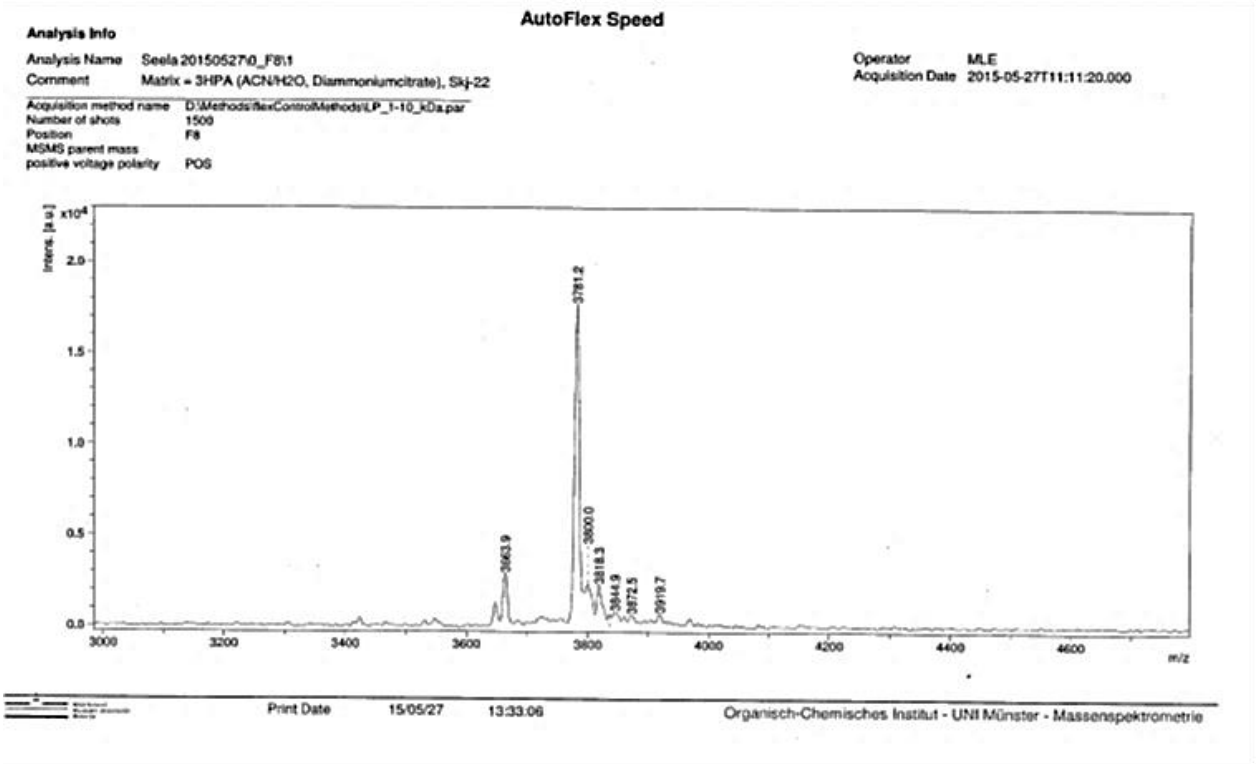


Fig. S1 MALDI-TOF spectra of a) **ODN-5**, b) 5'-d(AGTATTGAiC_FiC_FTA)[1] and c) **ODN-9** measured in the linear positive mode.

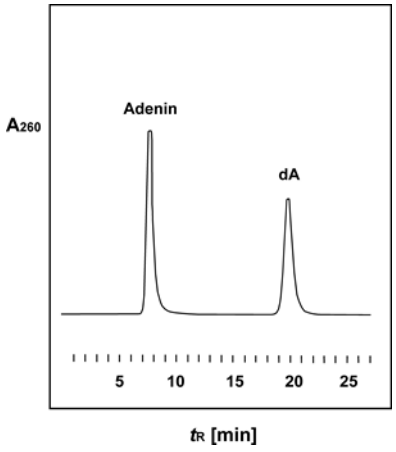


Fig. S2 Reversed-phase HPLC elution profiles of dA after treatment (240 min) with 0.1 N HCL at rt, monitored at 260 nm using an isocratic mixture of 0.1 M TEAA/MeCN 97:3.

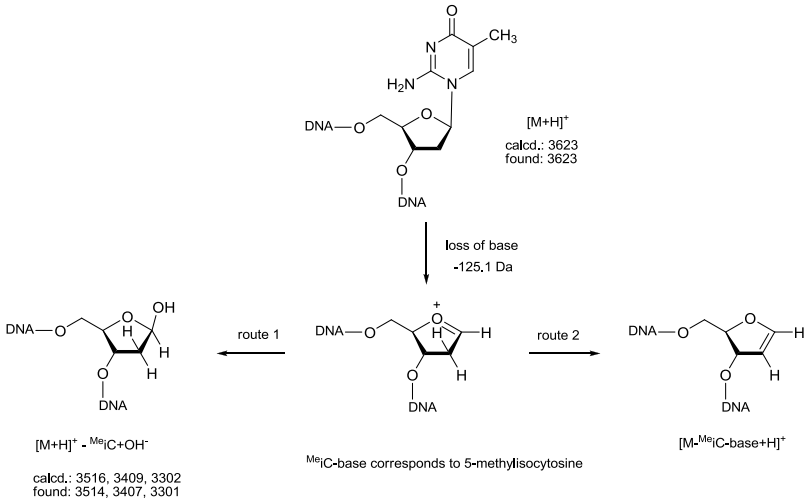


Fig. S3 Anticipated mechanism for the fragmentation of oligonucleotides containing $^{Me}iC_d$ (**1b**).[2,3]

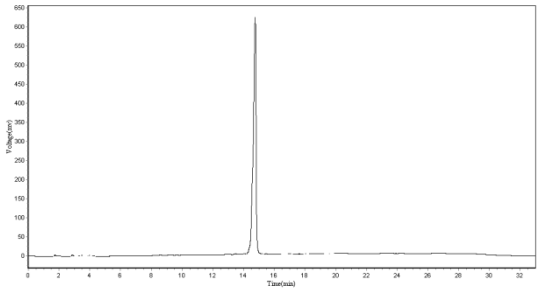
References

(1) S. A. Ingale, P. Leonard, Q. N. Tran and F. Seela, *J. Org. Chem.*, 2015, **80**, 3124-3138.

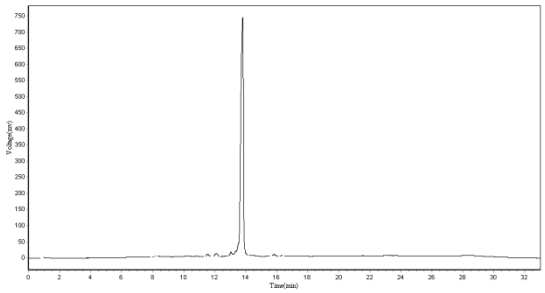
(2) J. L. York, *J. Org. Chem.*, 1981, **46**, 2171-2173.

(3) N. N. Kochetkov and E. I. Budovski, Organic Chemistry of Nucleic Acids, Part A, Chap. 3, Plenum: New York, 2002.

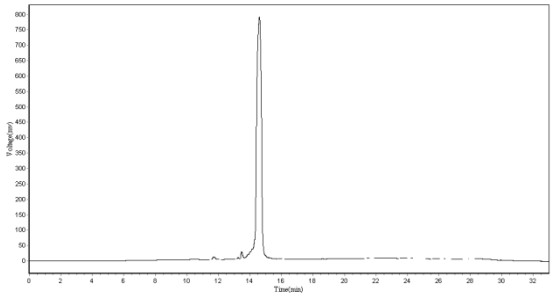
Reversed-phase HPLC-profiles of purified oligonucleotides



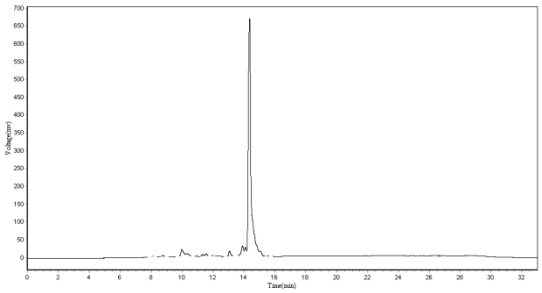
(a)



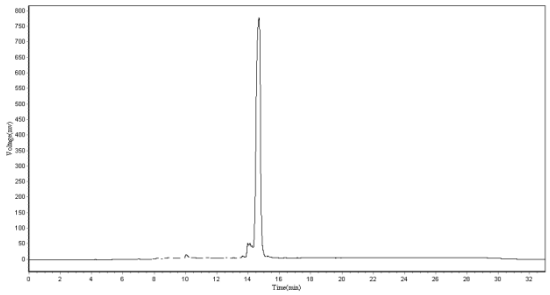
(b)



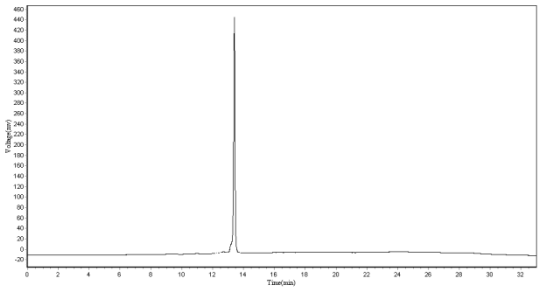
(c)



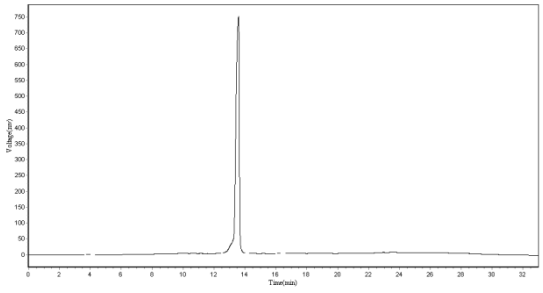
(d)



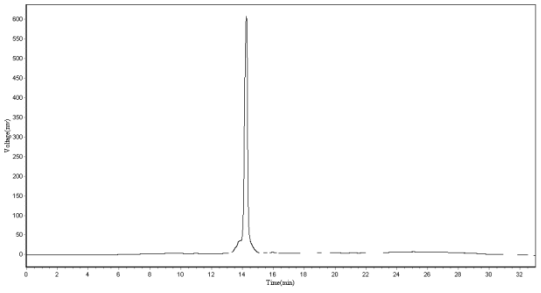
(e)



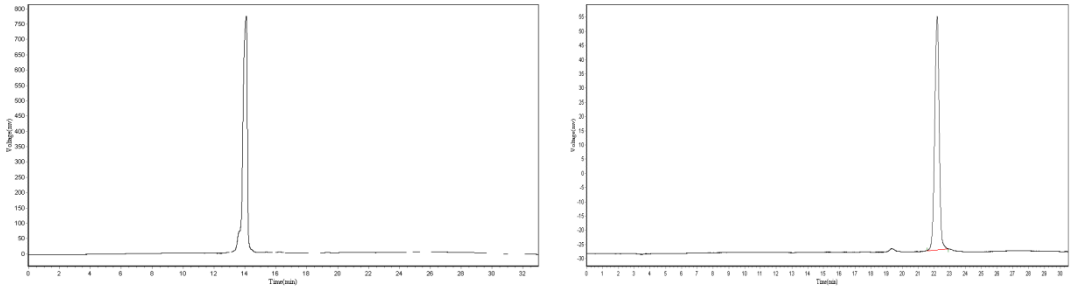
(f)



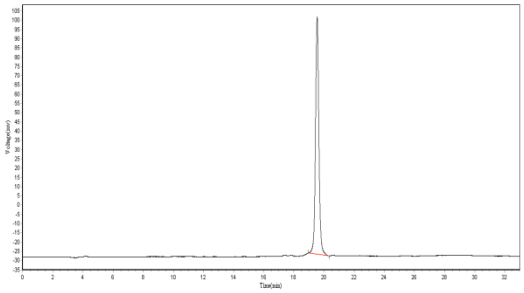
(g)



(h)



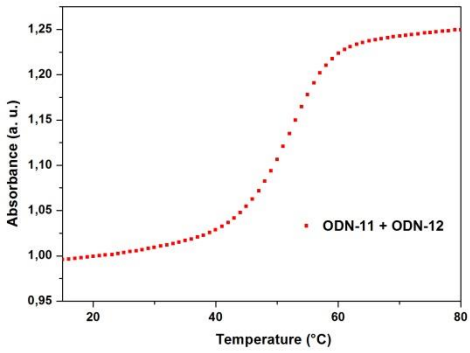
(i) (j)



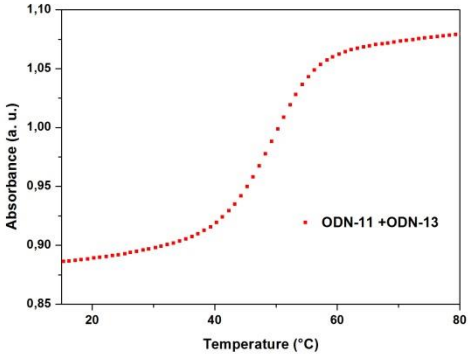
(k)

Fig. S4 Selected reversed-phase HPLC profiles of purified oligonucleotides: (a) **ODN-14**, (b) **ODN-11**, (c) **ODN-4**, (d) **ODN-16**, (e) **ODN-15**, (f) **ODN-6**, (g) **ODN-12**, (h) **ODN-8**, (i) **ODN-9**, (j) **ODN-17**, (k) **ODN-18**. HPLC (RP-18) profiles of oligonucleotides were monitored at 260 nm. For elution the following solvent system was used: MeCN (A) and 0.1 M $(Et_3NH)OAc$ (pH 7.0)/MeCN, 95:5 (B). Gradient I: 0-25 min 0-20% A in B, flow rate 0.7 mL min^{-1}. For ODN-**17** and ODN-**18** the following gradient system was used: 0-30 min 0-20% A in B, flow rate 0.7 mL $min^{-1.}$

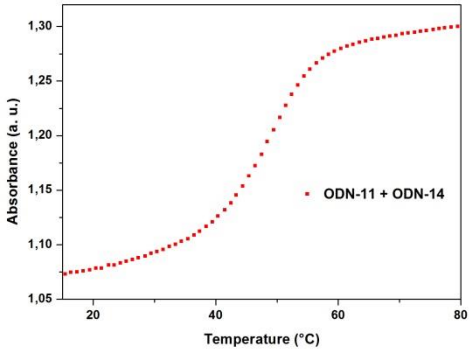
Melting profiles of duplexes



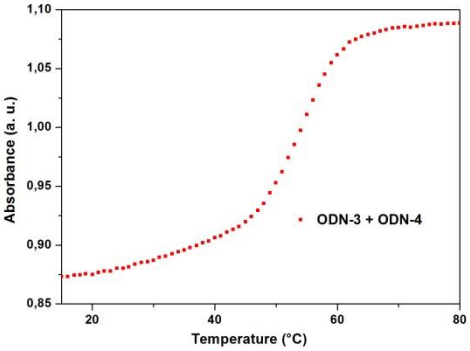
(a)



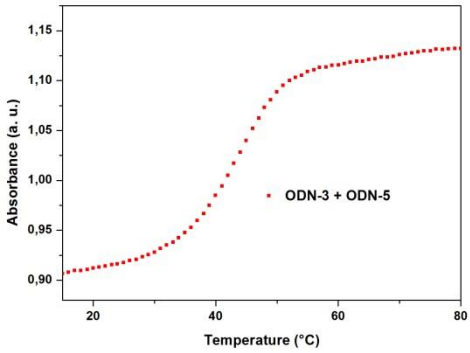
(b)



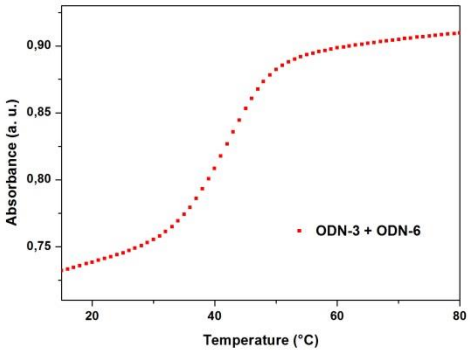
(c)



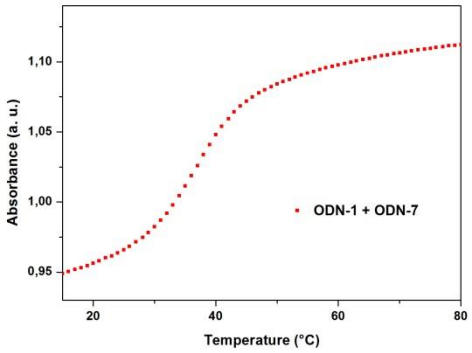
(d)



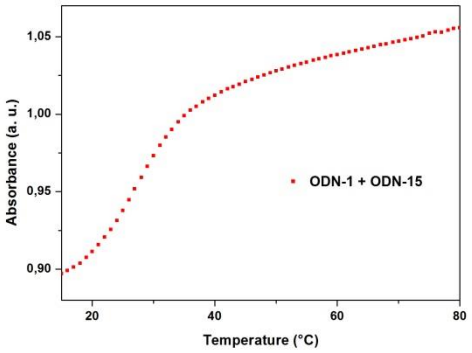
(e)



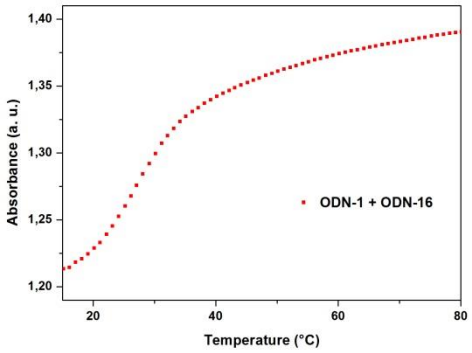
(f)


Absorbance (a. u.)
1,10
1,05
1,00
0,95
ODN-1 + ODN-7
20
40
60
80
Temperature (°C)

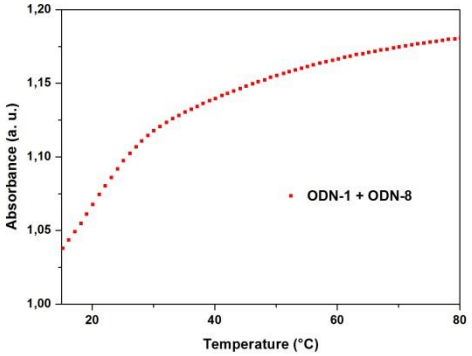
(g)


Absorbance (a. u.)
1,05
1,00
0,95
0,90
ODN-1 + ODN-15
20
40
60
80
Temperature (°C)

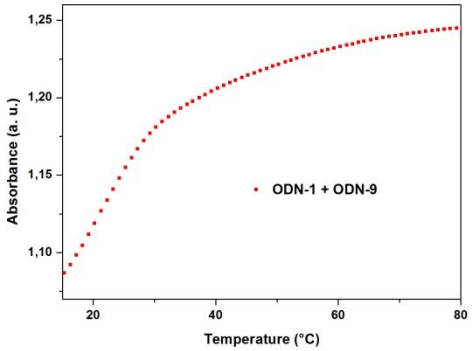
(h)


Absorbance (a. u.)
1,40
1,35
1,30
1,25
1,20
ODN-1 + ODN-16
20
40
60
80
Temperature (°C)

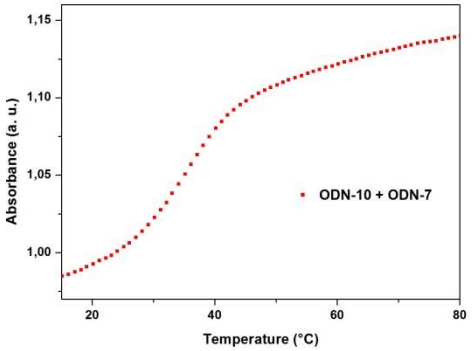
(i)


Absorbance (a. u.)
1,20
1,15
1,10
1,05
1,00
ODN-1 + ODN-8
20
40
60
80
Temperature (°C)

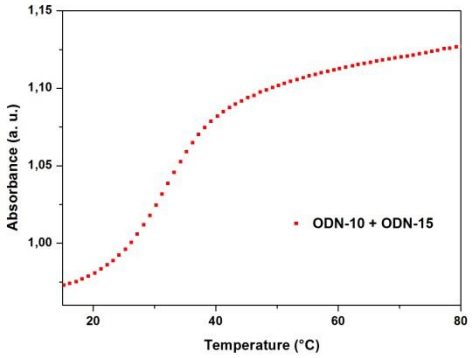
j)


Absorbance (a. u.)
1,25
1,20
1,15
1,10
ODN-1 + ODN-9
20
40
60
80
Temperature (°C)

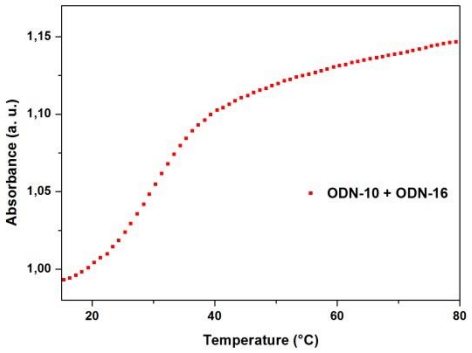
(k)


Absorbance (a. u.)
1,15
1,10
1,05
1,00
ODN-10 + ODN-7
20
40
60
80
Temperature (°C)

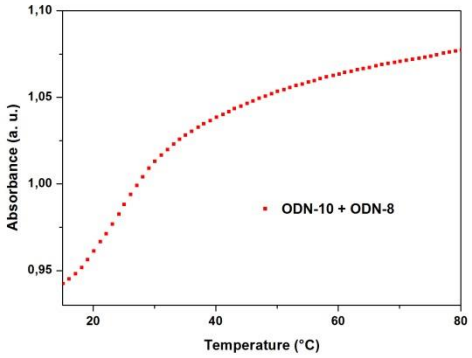
(l)


Absorbance (a. u.)
Temperature (°C)
ODN-10 + ODN-15

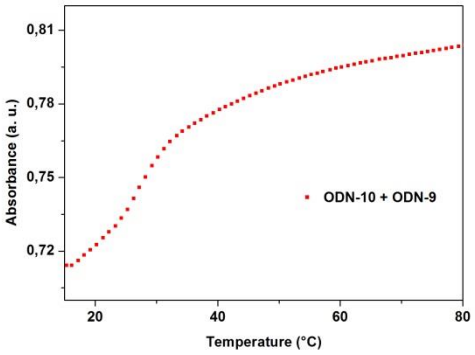
(m)


Absorbance (a. u.)
Temperature (°C)
ODN-10 + ODN-16

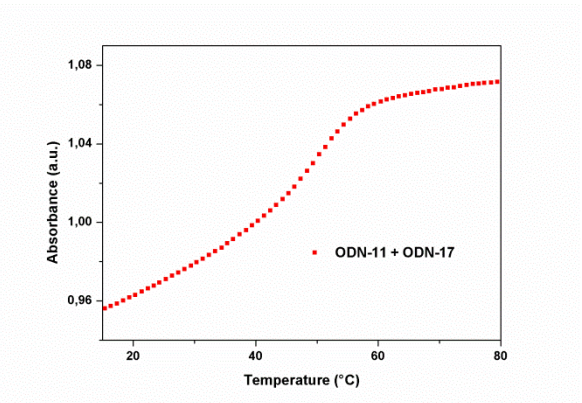
(n)


Absorbance (a. u.)
Temperature (°C)
ODN-10 + ODN-8

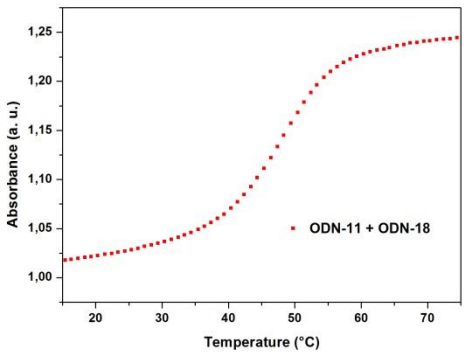
(o)


Absorbance (a. u.)
Temperature (°C)
ODN-10 + ODN-9

(p)


Absorbance (a.u.)
Temperature (°C)
ODN-11 + ODN-17

(q)


Absorbance (a. u.)
Temperature (°C)
ODN-11 + ODN-18

(r)

Fig. S5 Original melting curves of duplexes obtained from heating experiments determined at 260 nm with a single-strand concentration of 5 µM + 5 µM in 0.1 M NaCl, 10 mM $MgCl_2$, 10 mM Na-cacodylate (pH 7.0). (a) **ODN-11 • ODN-12**, (b) **ODN-11 • ODN-13**, (c) **ODN-11 • ODN-14**, (d) **ODN-3 • ODN-4**, (e) **ODN-3 • ODN-5**, (f) **ODN-3 • ODN-6**, (g) **ODN-1 • ODN-7**, (h) **ODN-1 • ODN-15**, (i) **ODN-1 • ODN-16**, (j) **ODN-1 • ODN-8**, (k) **ODN-1 • ODN-9**, (l) **ODN-10 • ODN-7**, (m) **ODN-10 • ODN-15**, (n) **ODN-10 • ODN-16**, (o) **ODN-10 • ODN-8**, (p) **ODN-10 • ODN-9**, (q) azido-functionalized duplex **ODN-11 • ODN-17**, (r) cross-linked duplex **ODN-11 • ODN-18**.

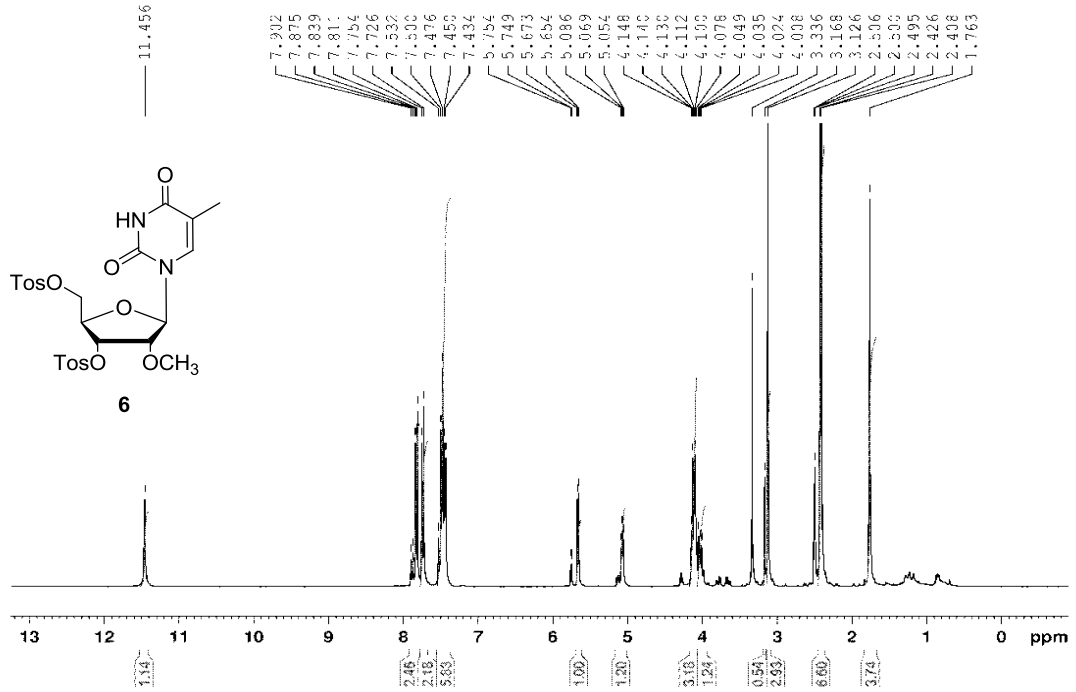


Fig. S6 ^{1}H-NMR spectrum of compound **6.**

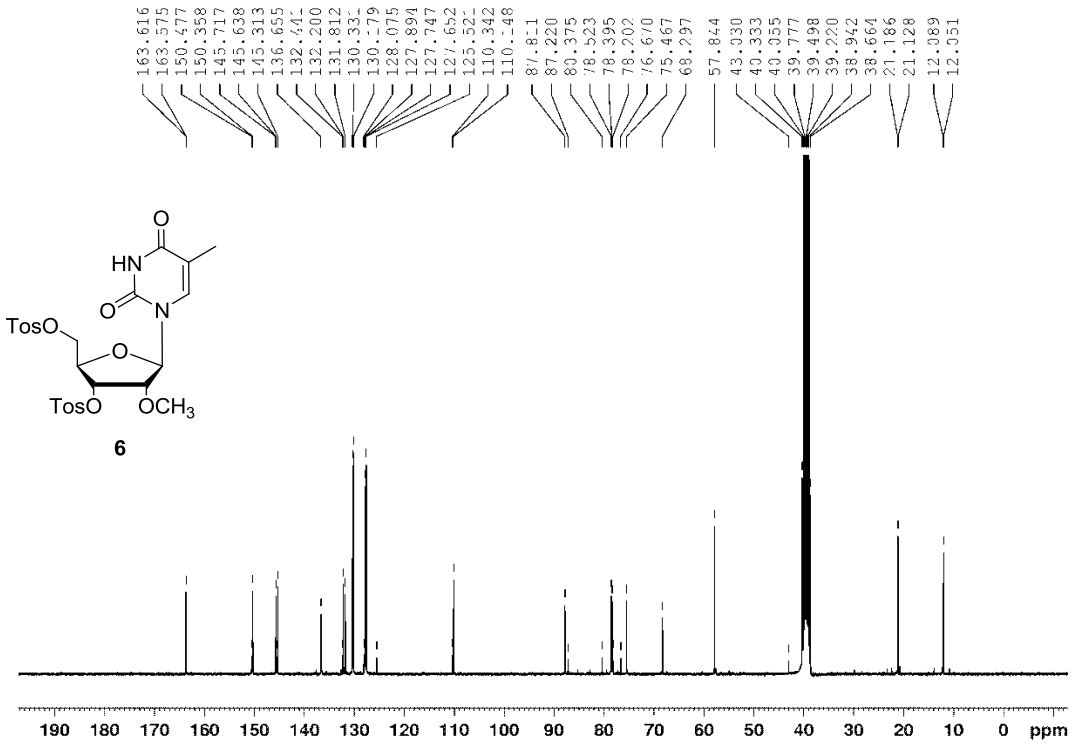


Fig. S7 ^{13}C-NMR spectrum of compound **6**.

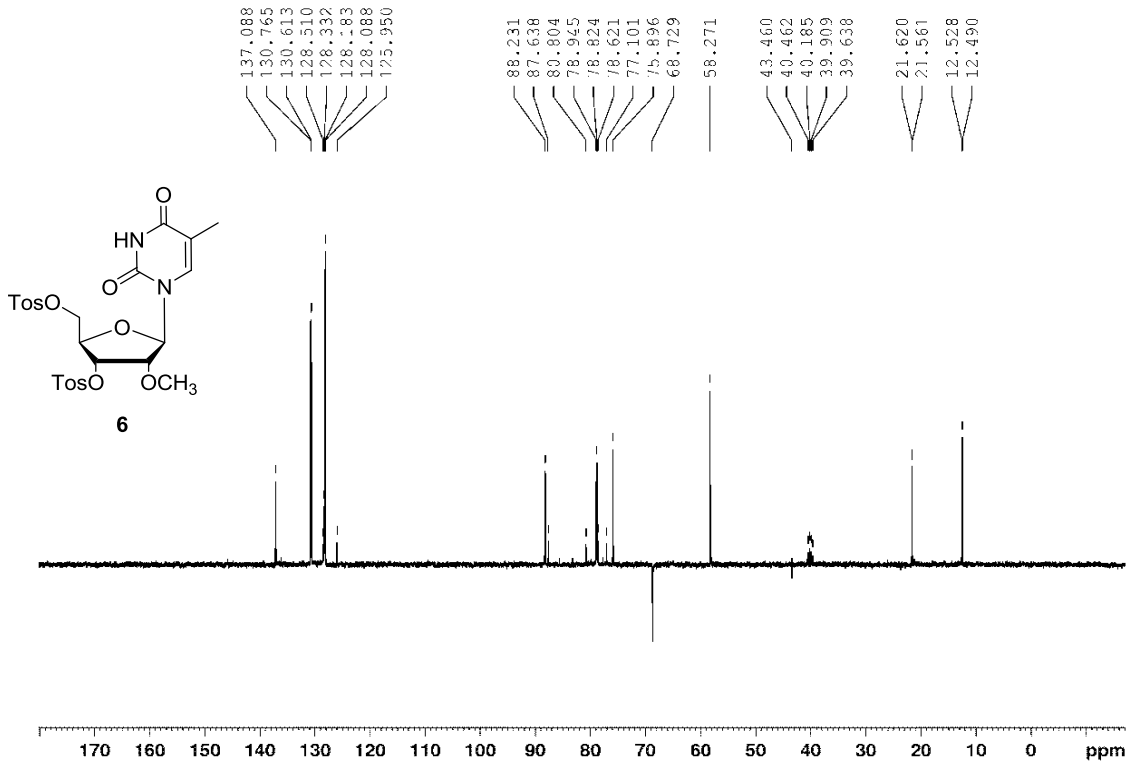


Fig. S8 Dept-135 NMR spectrum of compound **6**.

Fig. S9 ^{1}H-^{13}C gated-decoupled spectrum of compound **6**.

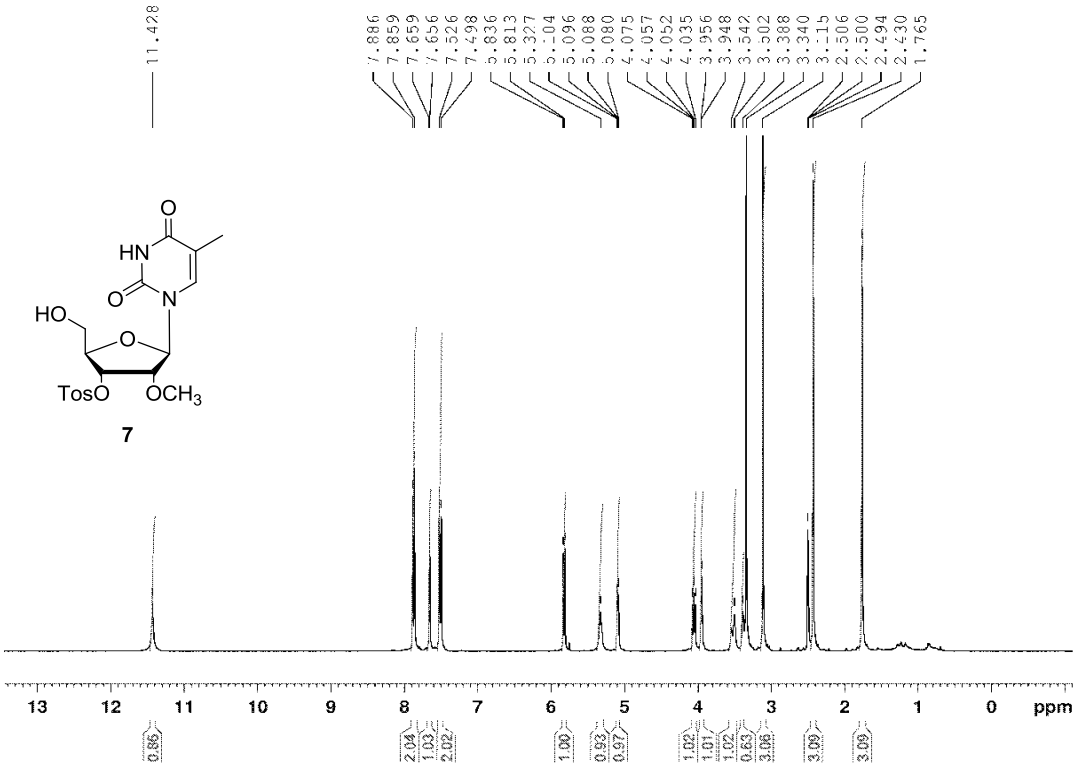


Fig. S10 ^{1}H-NMR spectrum of compound **7**.

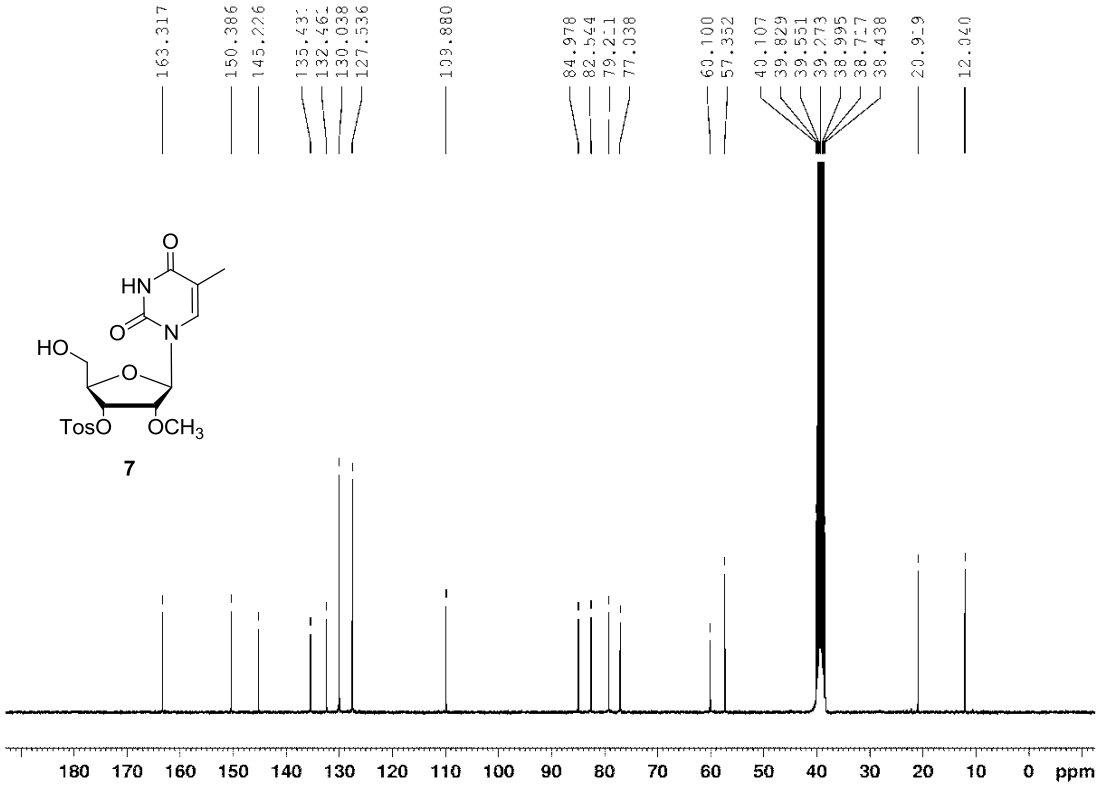


Fig. S11 ^{13}C-NMR spectrum of compound **7**.

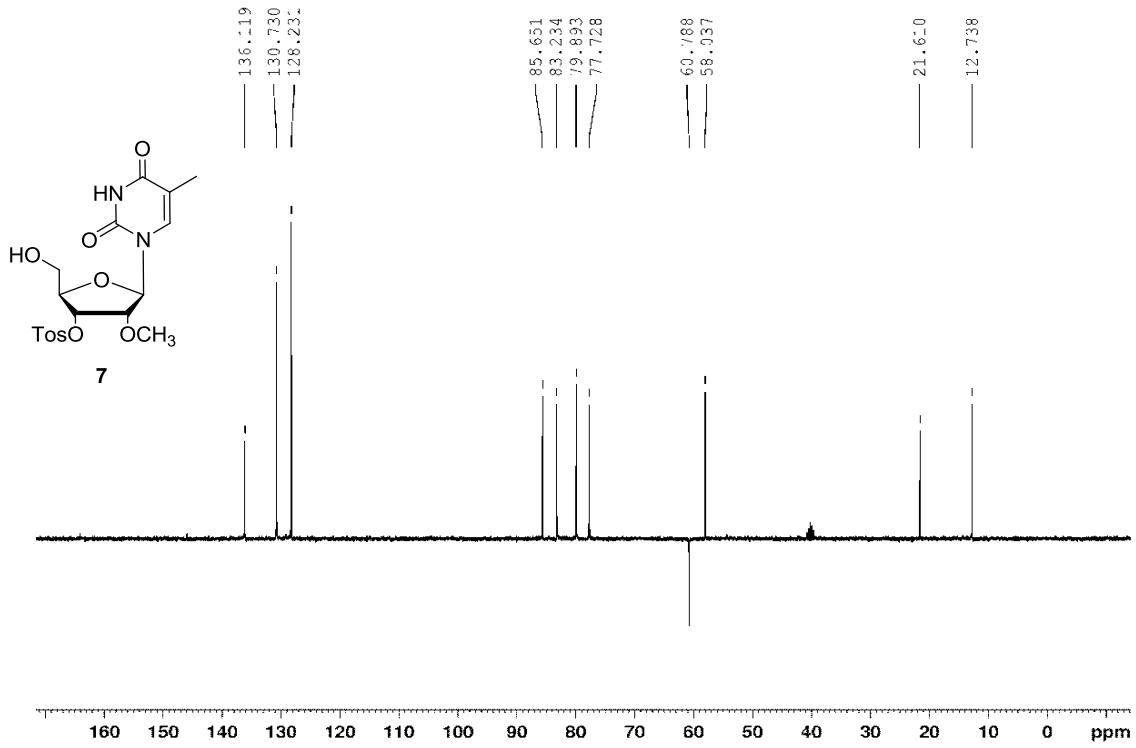


Fig. S12 DEPT-135 NMR spectrum of compound **7**.

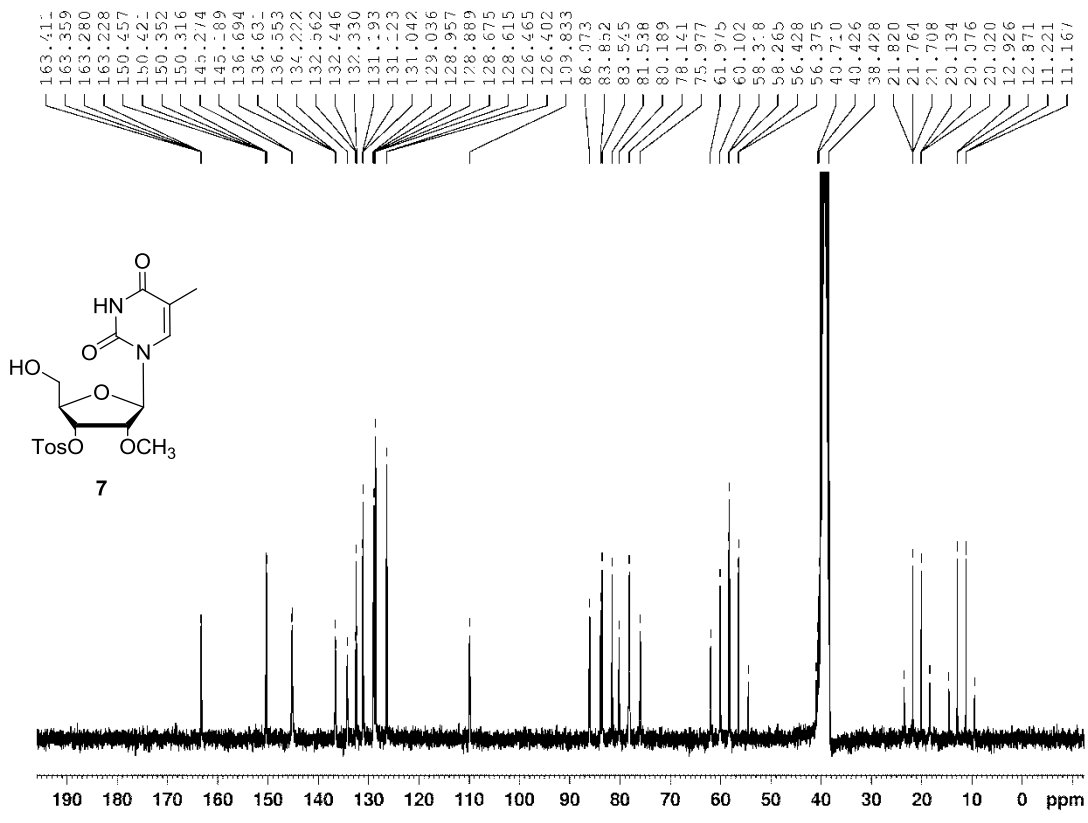


Fig. S13 ^{1}H-^{13}C gated-decoupled spectrum of compound **7**.

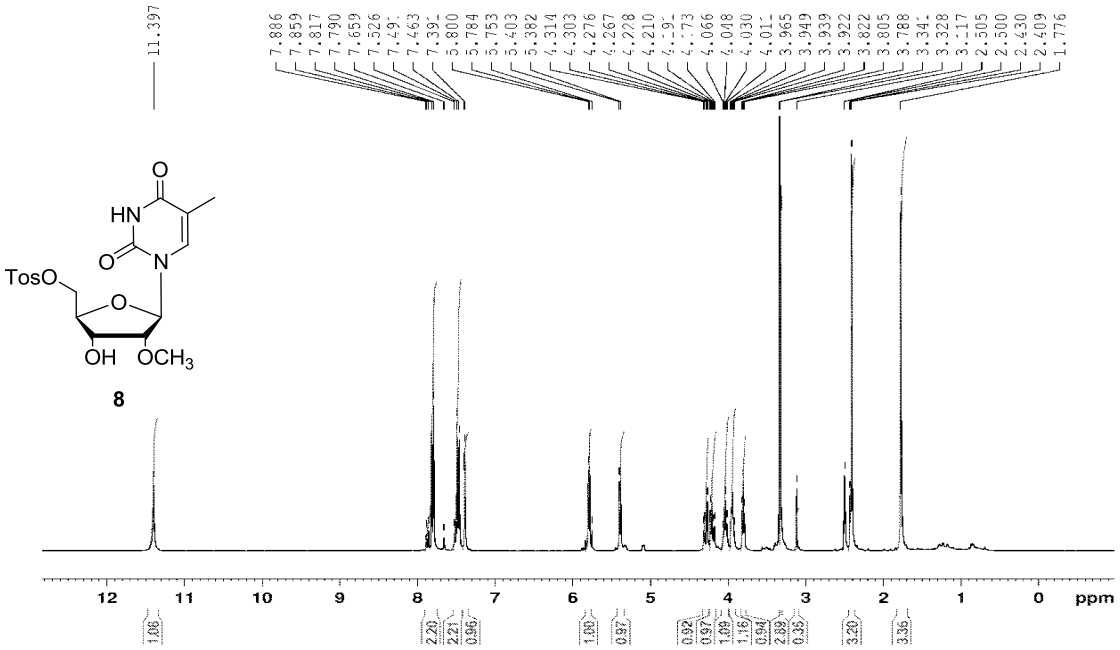


Fig. S14 ^{1}H-NMR spectrum of compound **8**.

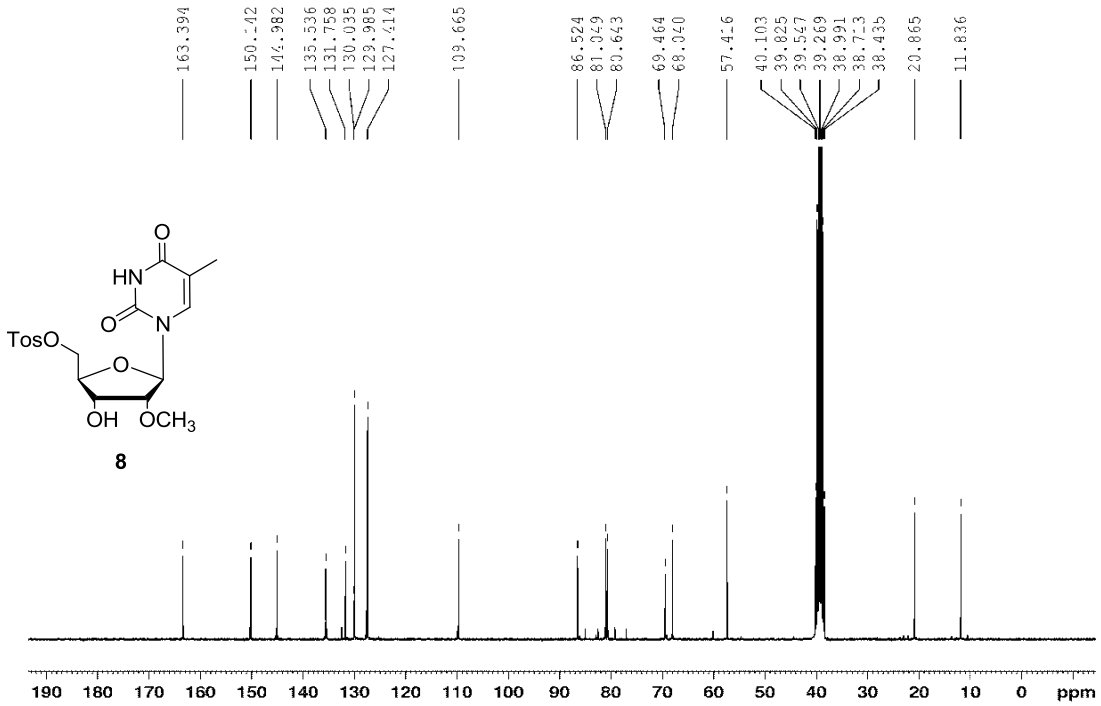


Fig. S15 ^{13}C-NMR spectrum of compound **8**.

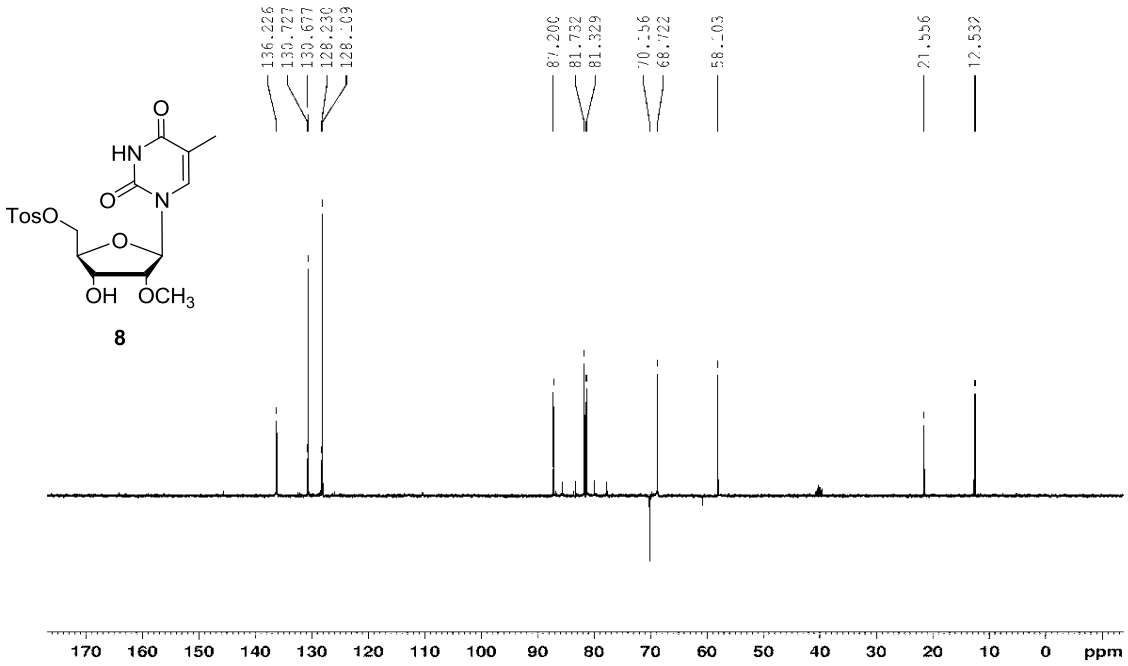


Fig. S16 DEPT-135 NMR spectrum of compound **8**.

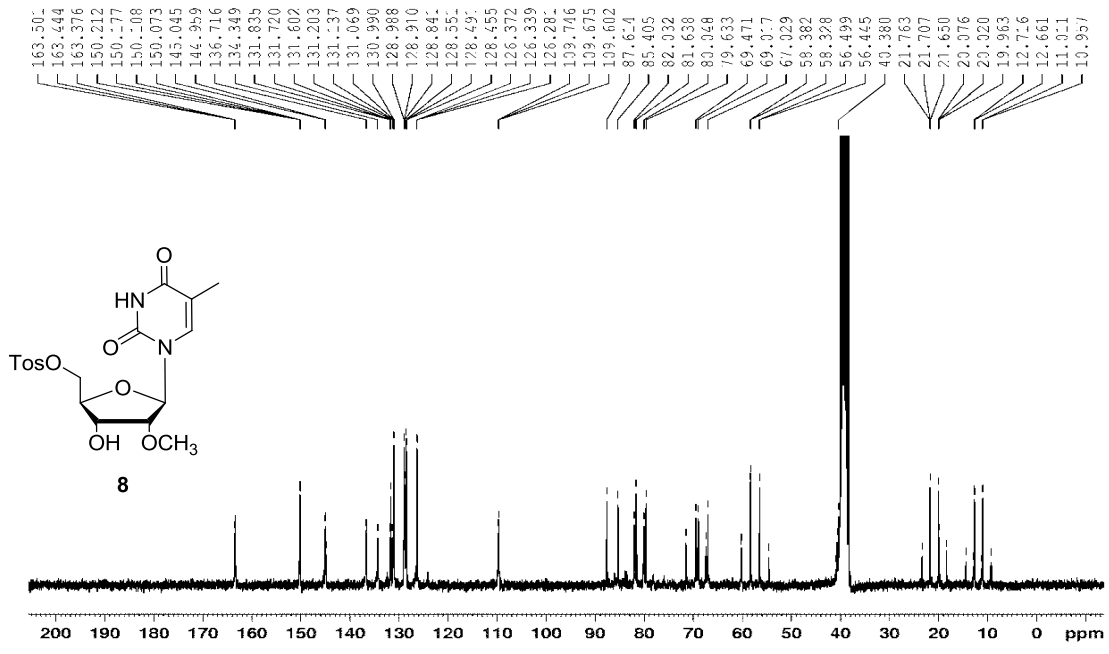


Fig. S17 ^{1}H-^{13}C gated-decoupled spectrum of compound **8**.

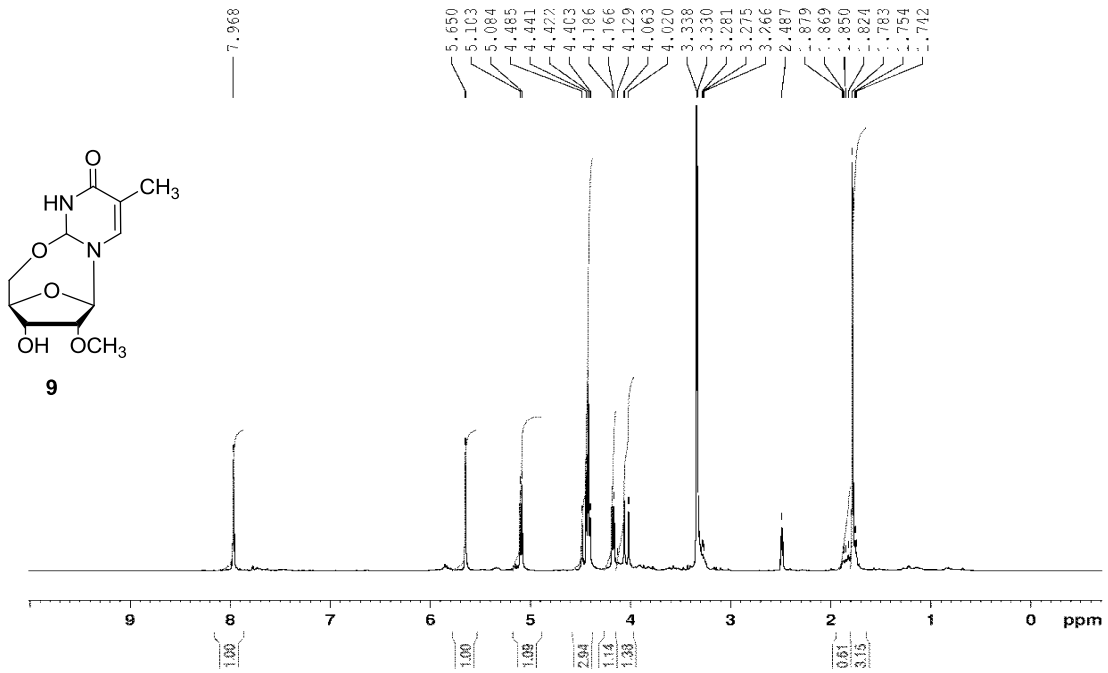


Fig. S18 ^{1}H-NMR spectrum of compound **9**.

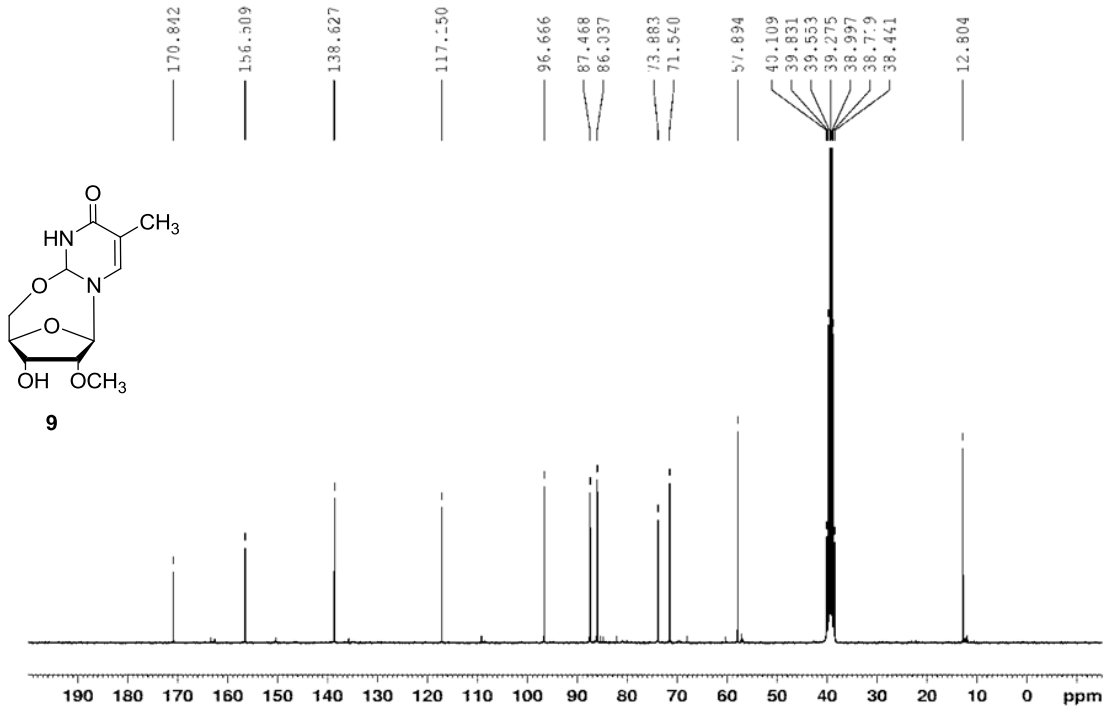


Fig. S19 ^{13}C-NMR spectrum of compound **9**.

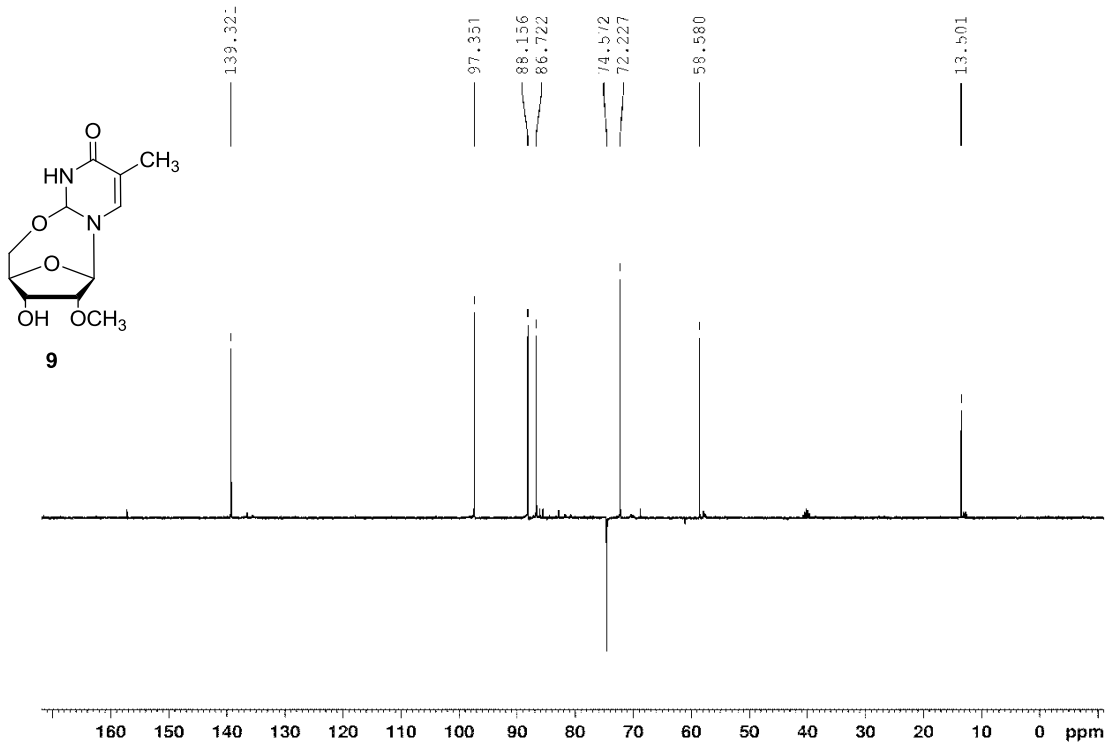


Fig. S20 DEPT-135 NMR spectrum of compound **9**.

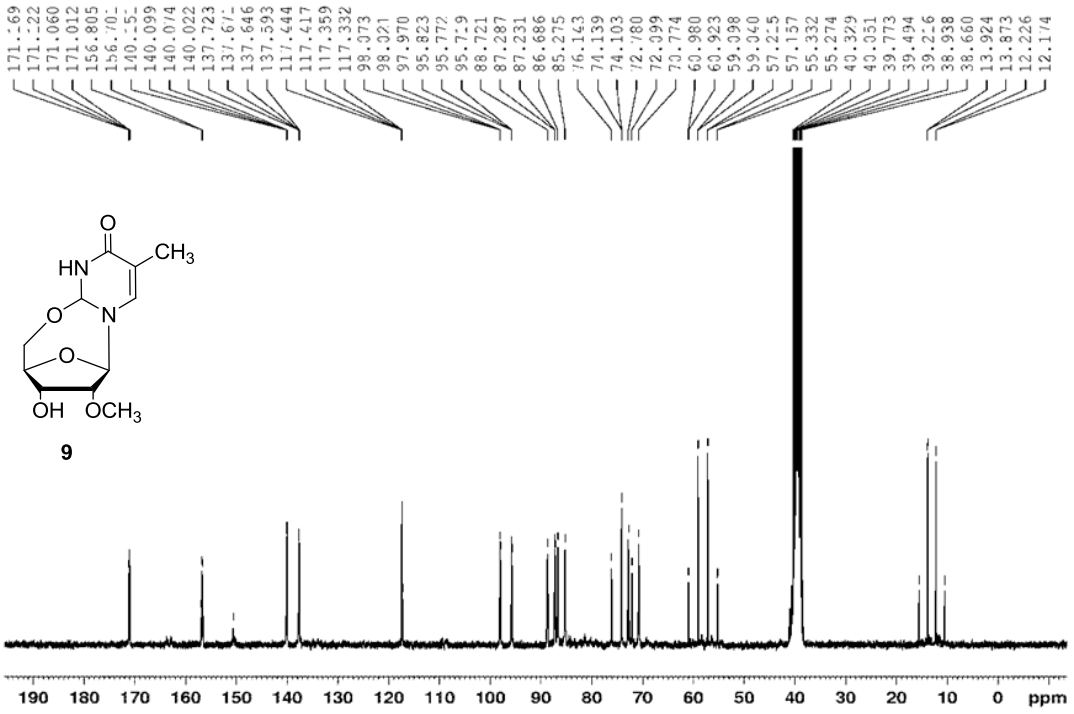


Fig. S21 ^{1}H-^{13}C gated-decoupled spectrum of compound **9**.

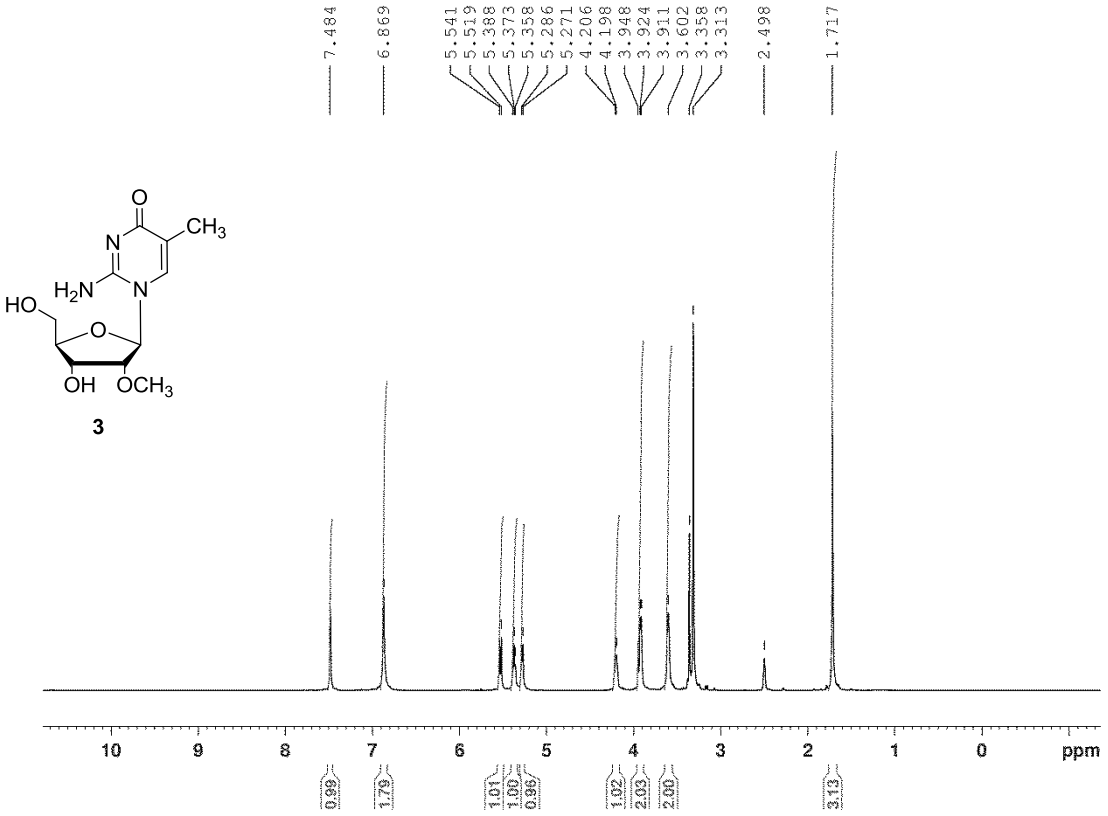


Fig. S22 ^{1}H-NMR spectrum of compound **3**.

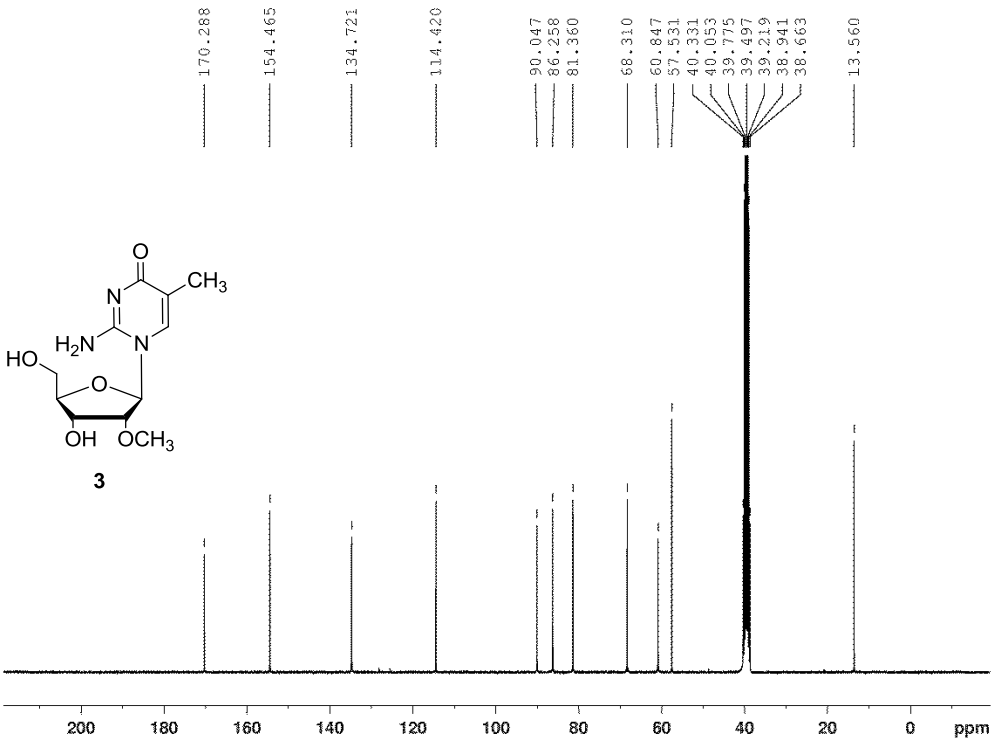


Fig. S23 ^{13}C-NMR spectrum of compound **3**.

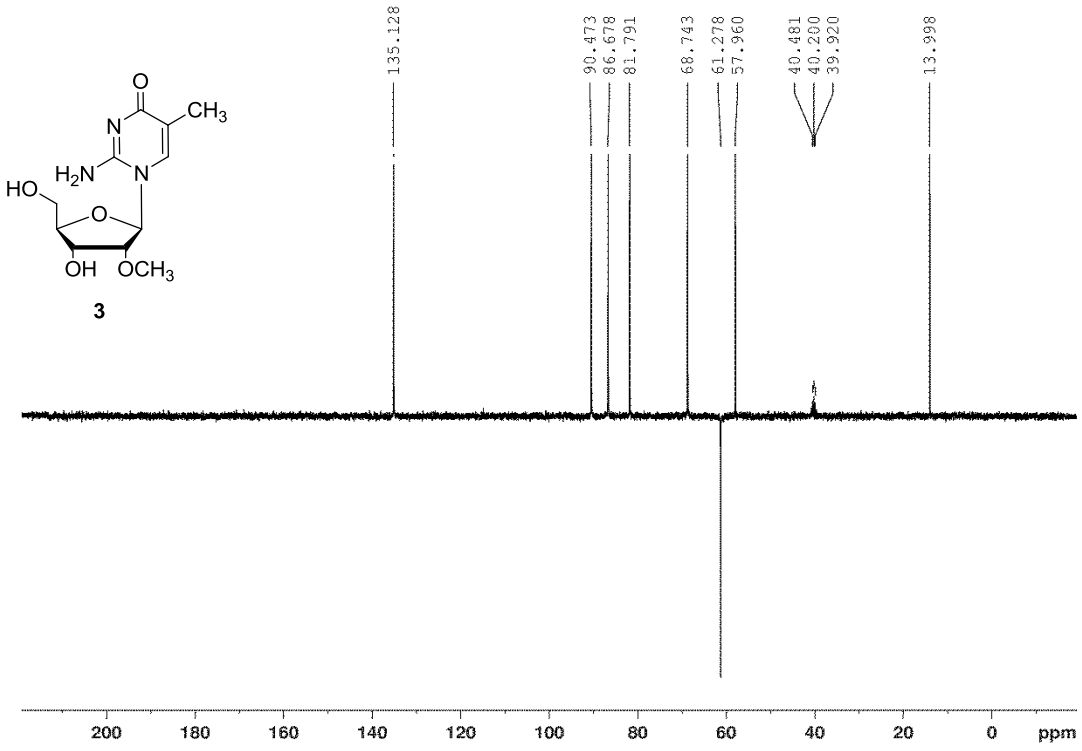


Fig. S24 DEPT-135 NMR spectrum of compound **3**.

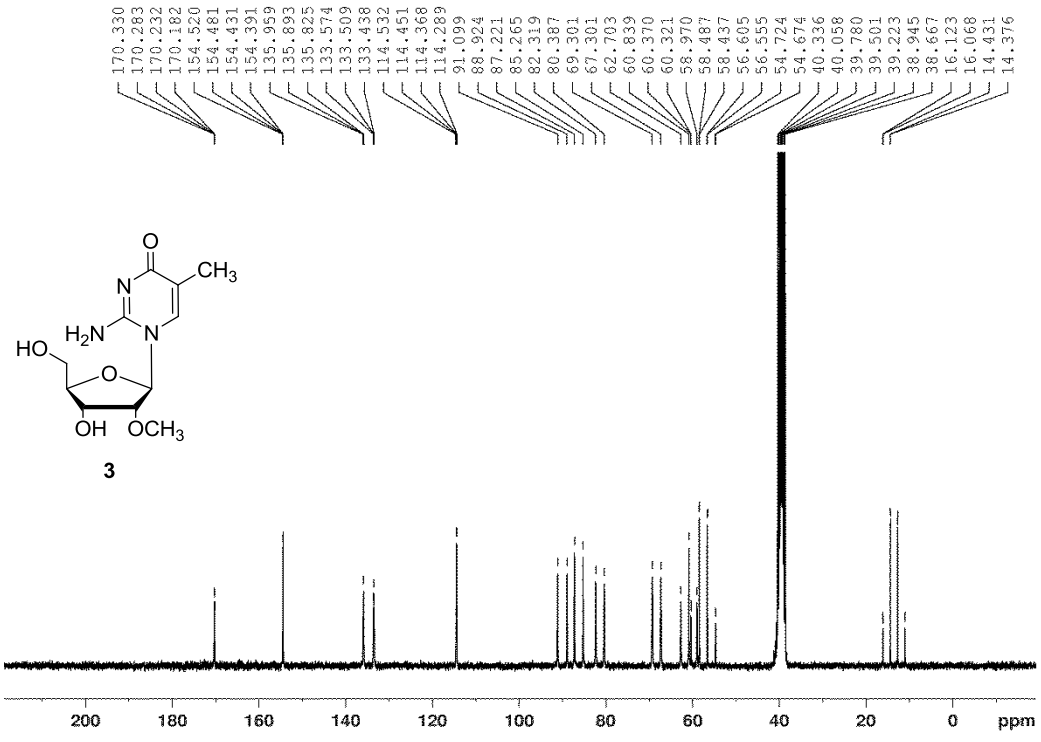


Fig. S25 ^{1}H-^{13}C gated-decoupled spectrum of compound **3** .

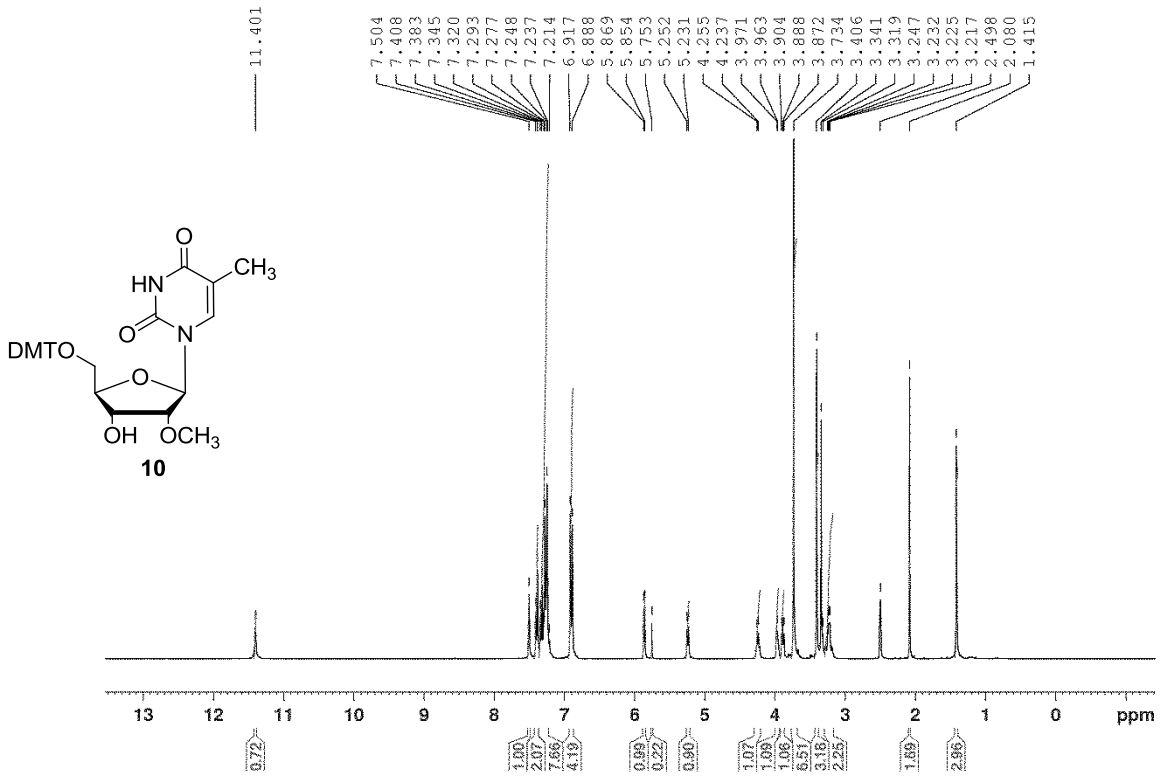


Fig. S26 ^{1}H-NMR spectrum of compound **10**.

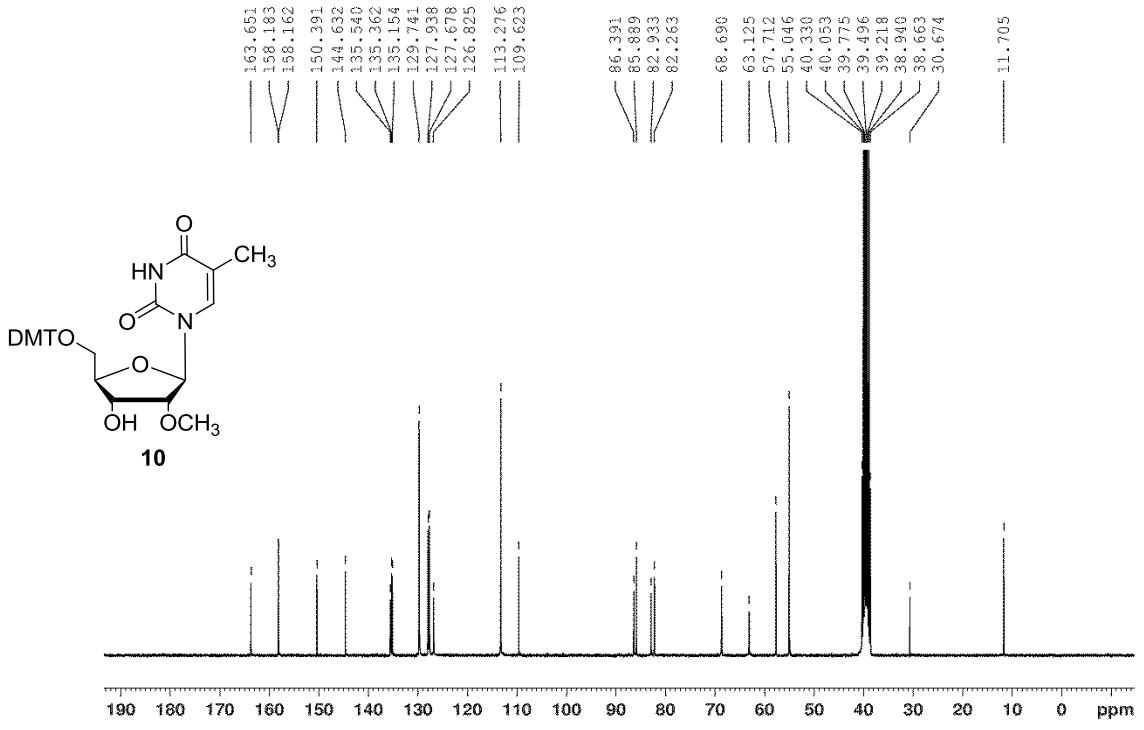


Fig. S27 ^{13}C-NMR spectrum of compound **10**.

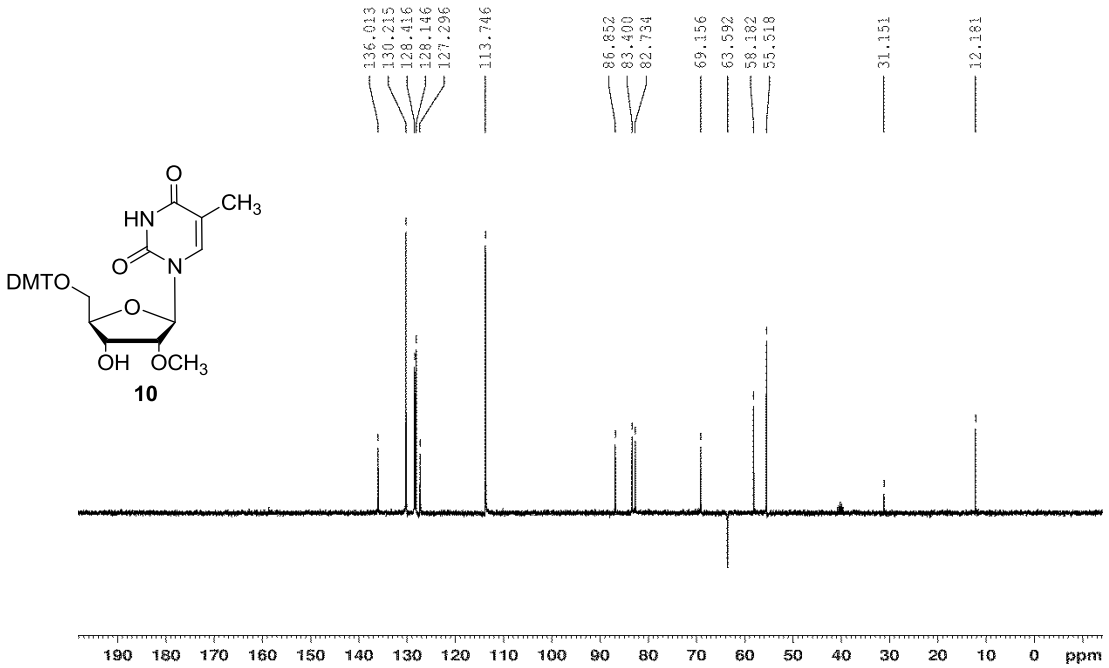


Fig. S28 DEPT-135 NMR spectrum of compound **10**.

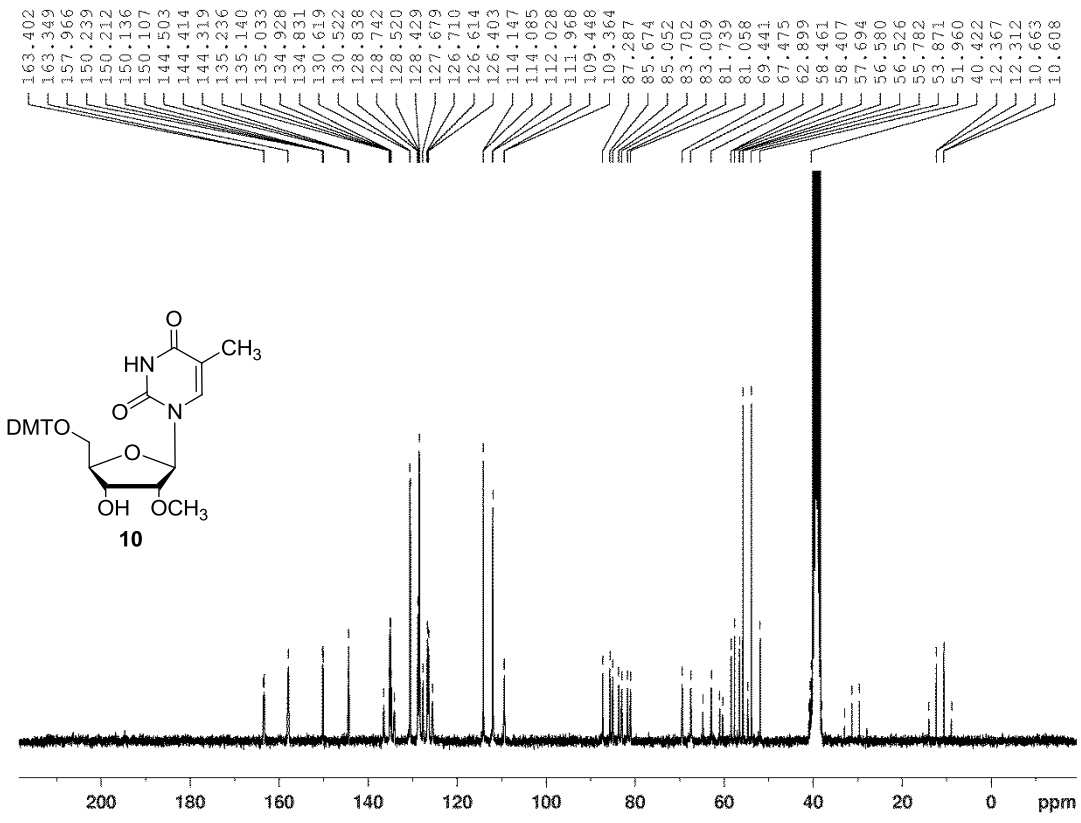


Fig. S29 ^{1}H-^{13}C gated-decoupled spectrum of compound **10**.

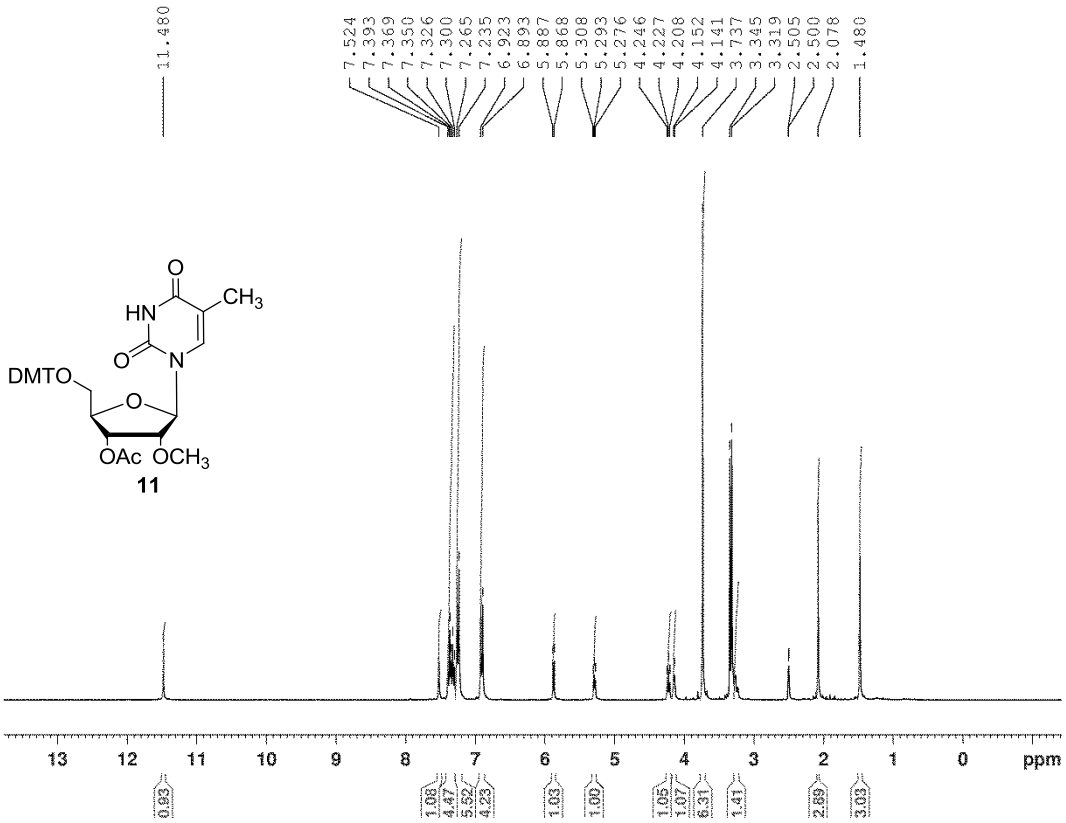


Fig. S30 ^{1}H-NMR spectrum of compound **11**.

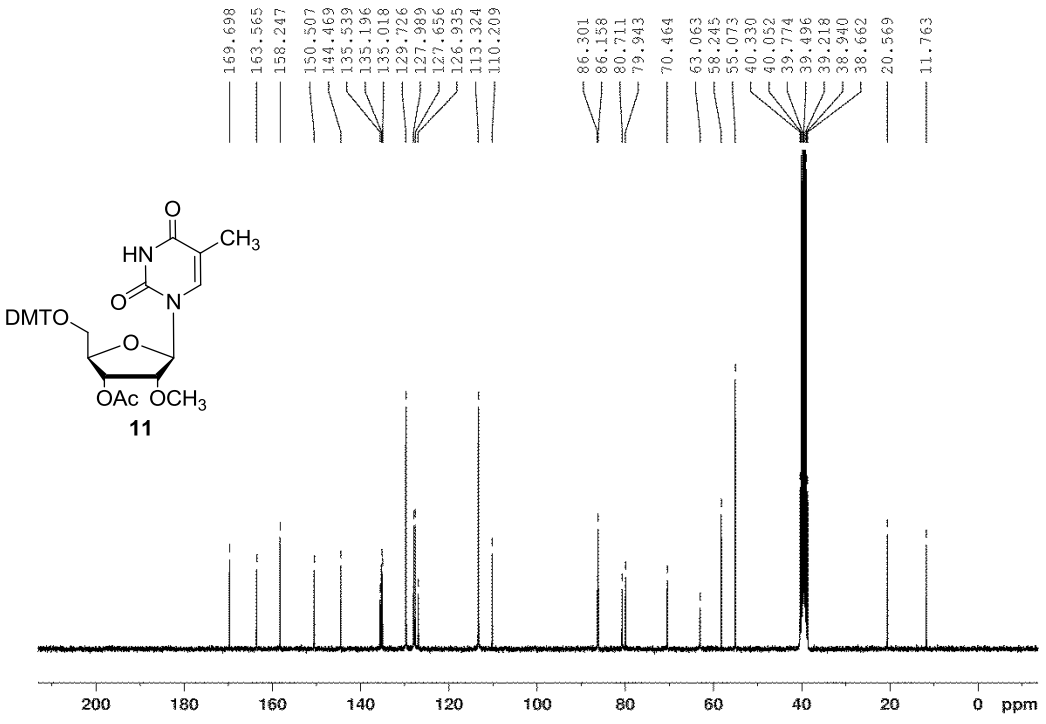


Fig. S31 ^{13}C-NMR spectrum of compound **11**.

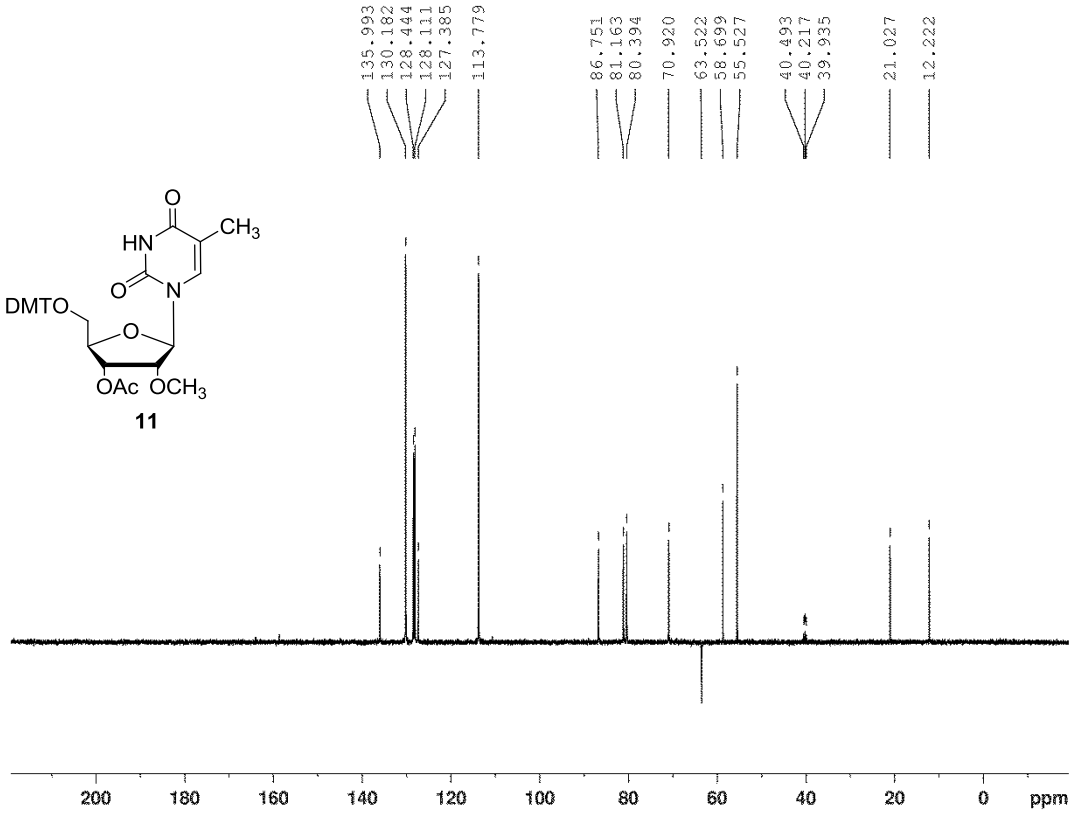


Fig. S32 DEPT-135 NMR spectrum of compound **11**.

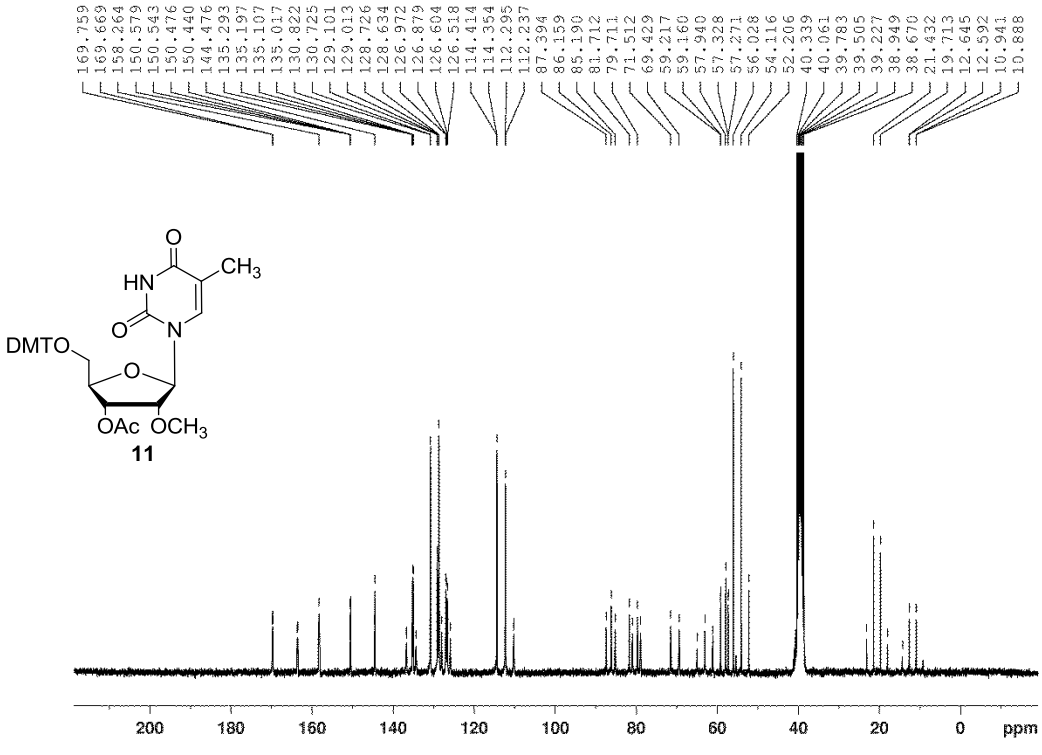


Fig. S33 ^{1}H-^{13}C gated-decoupled spectrum of compound **11**.

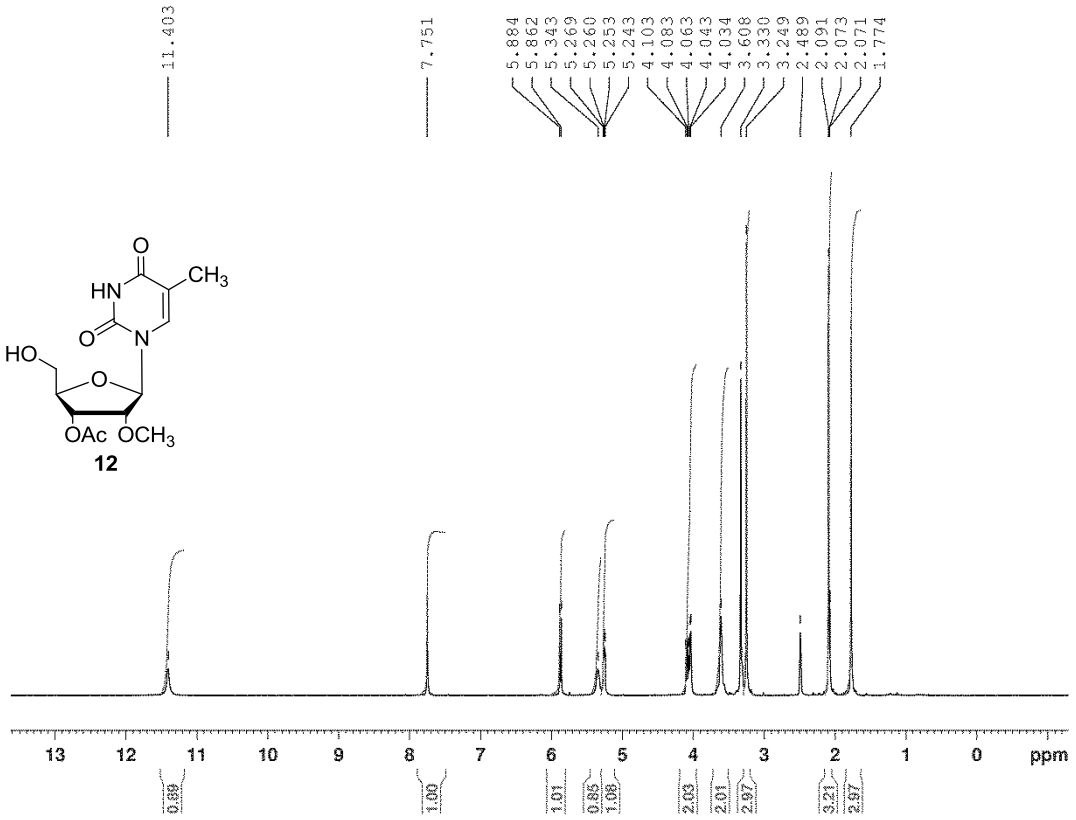


Fig. S34 ^{1}H-NMR spectrum of compound **12**.

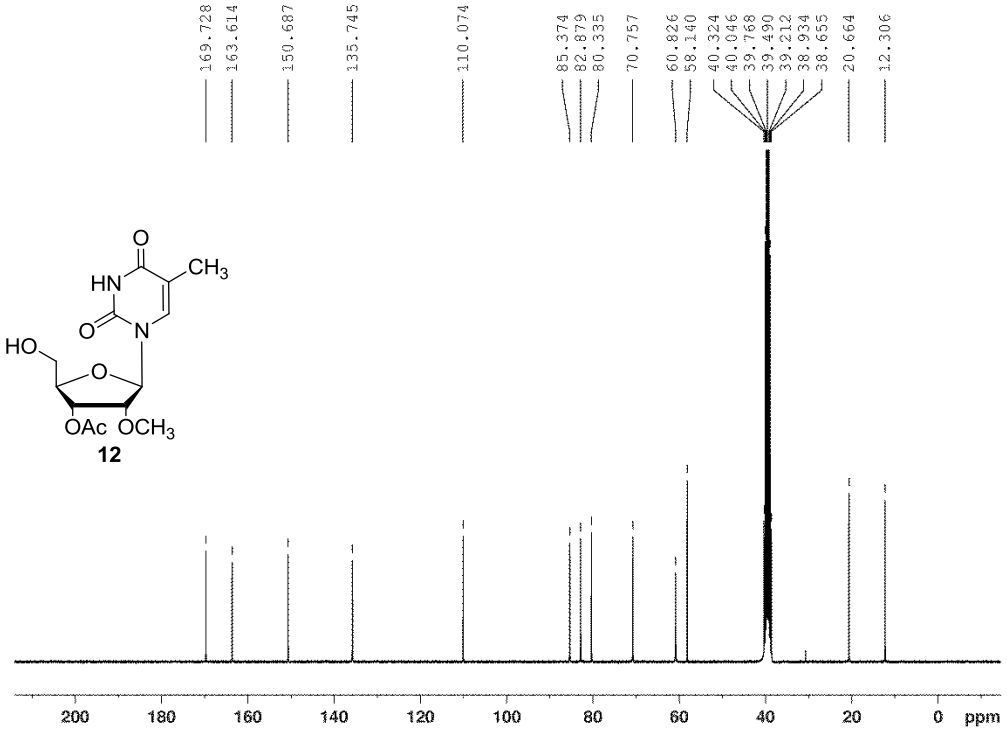


Fig. S35 ^{13}C-NMR spectrum of compound **12**.

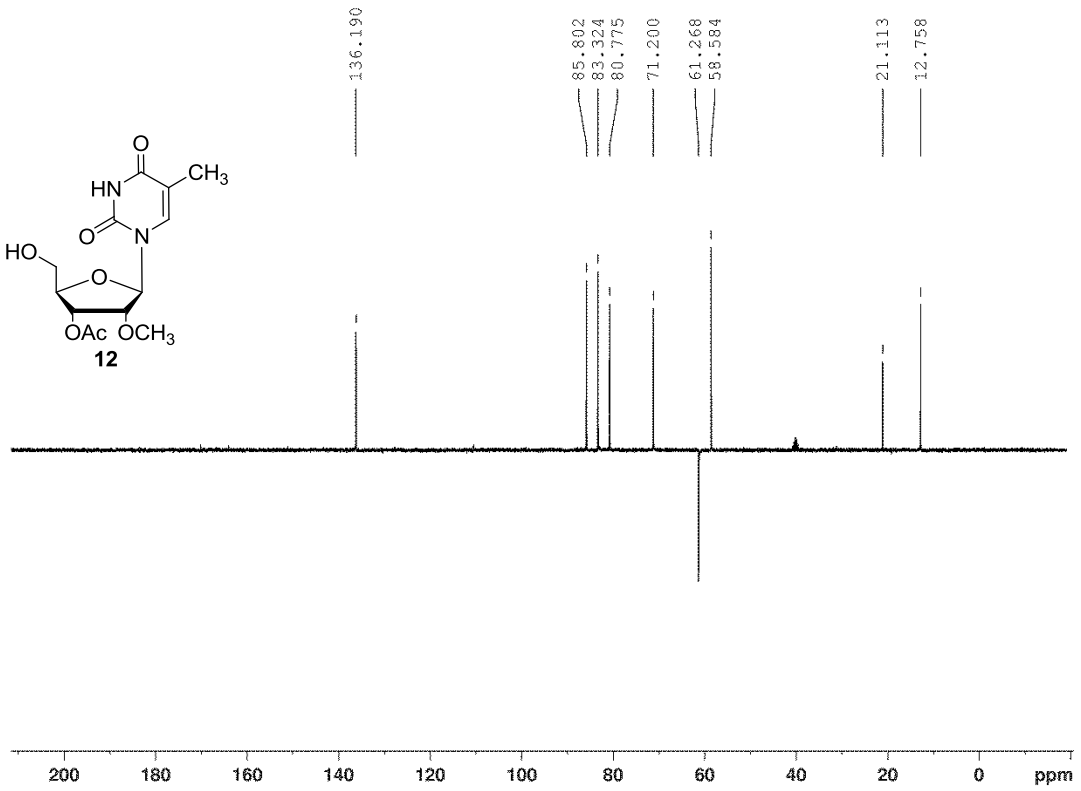


Fig. S36 DEPT-135 NMR spectrum of compound **12**.

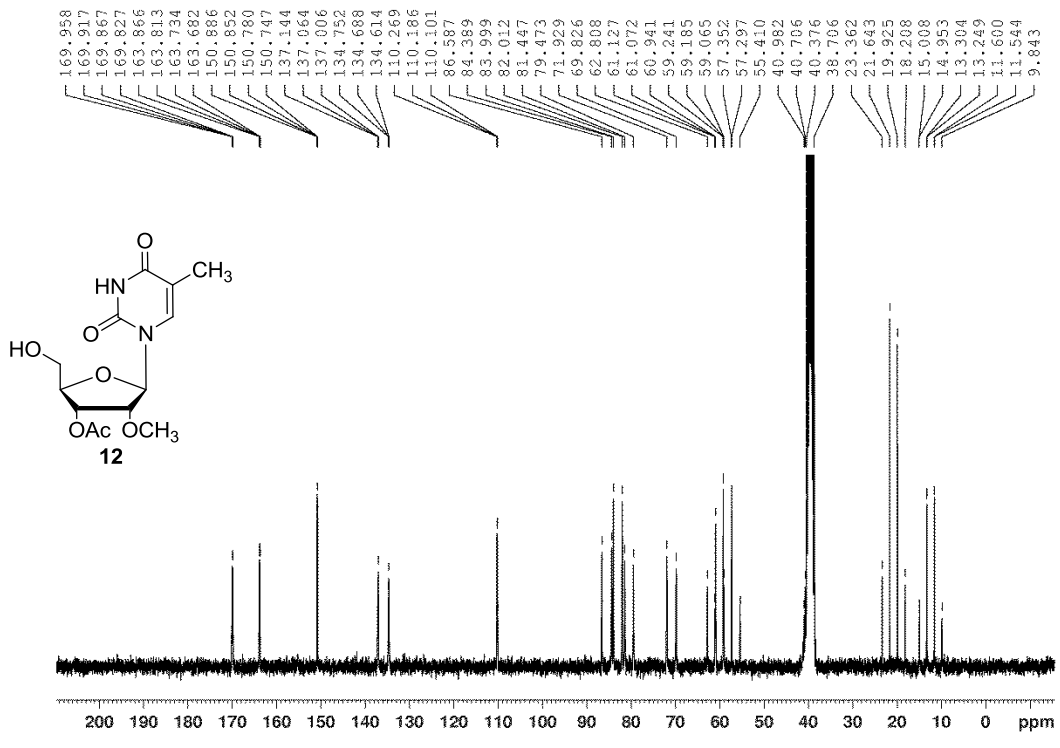


Fig. S37 ^{1}H-^{13}C gated-decoupled spectrum of compound **12**.

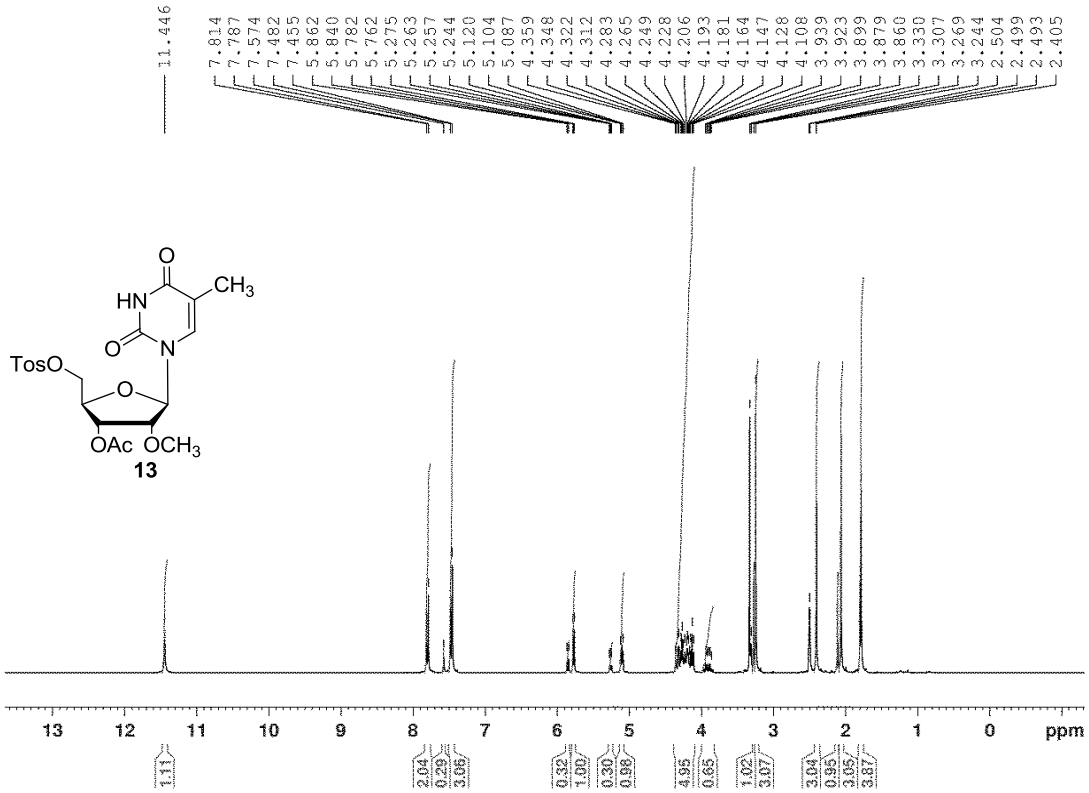


Fig. S38 ^{1}H-NMR spectrum of compound **13**.

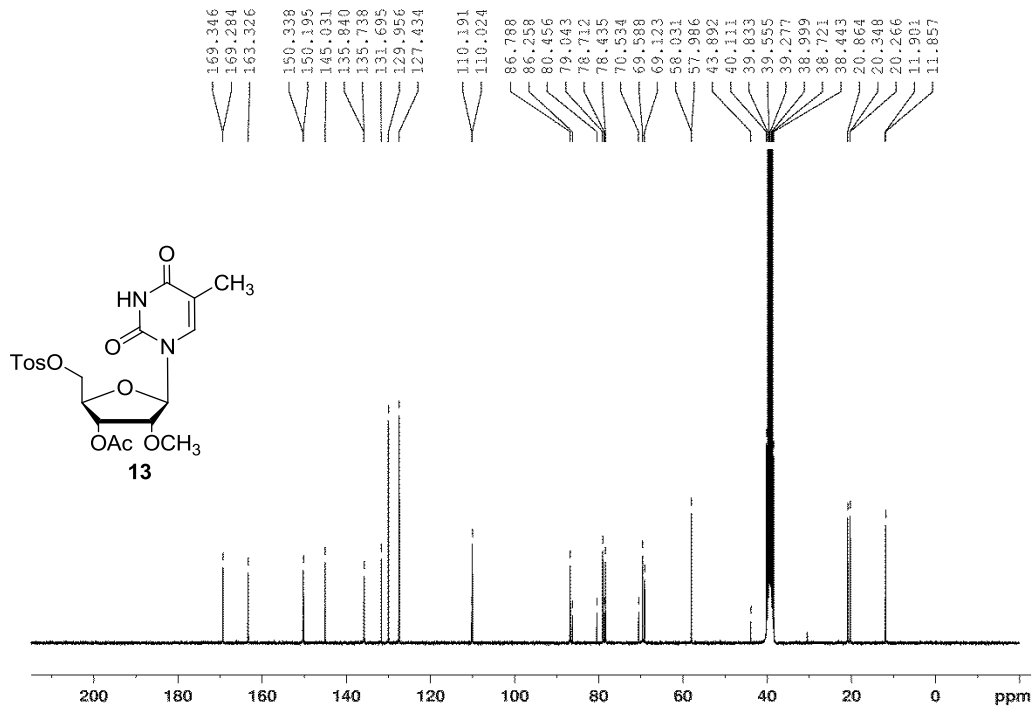


Fig. S39 ^{13}C-NMR spectrum of compound **13**.

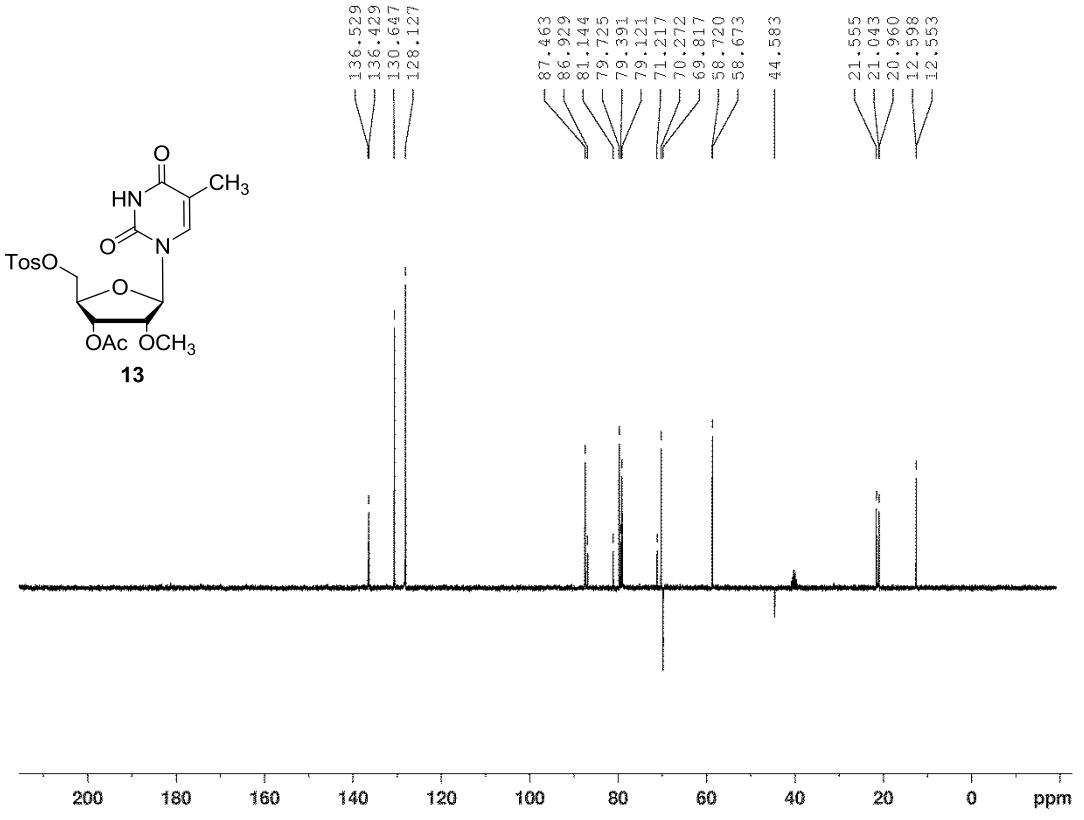


Fig. S40 DEPT-135 NMR spectrum of compound **13**.

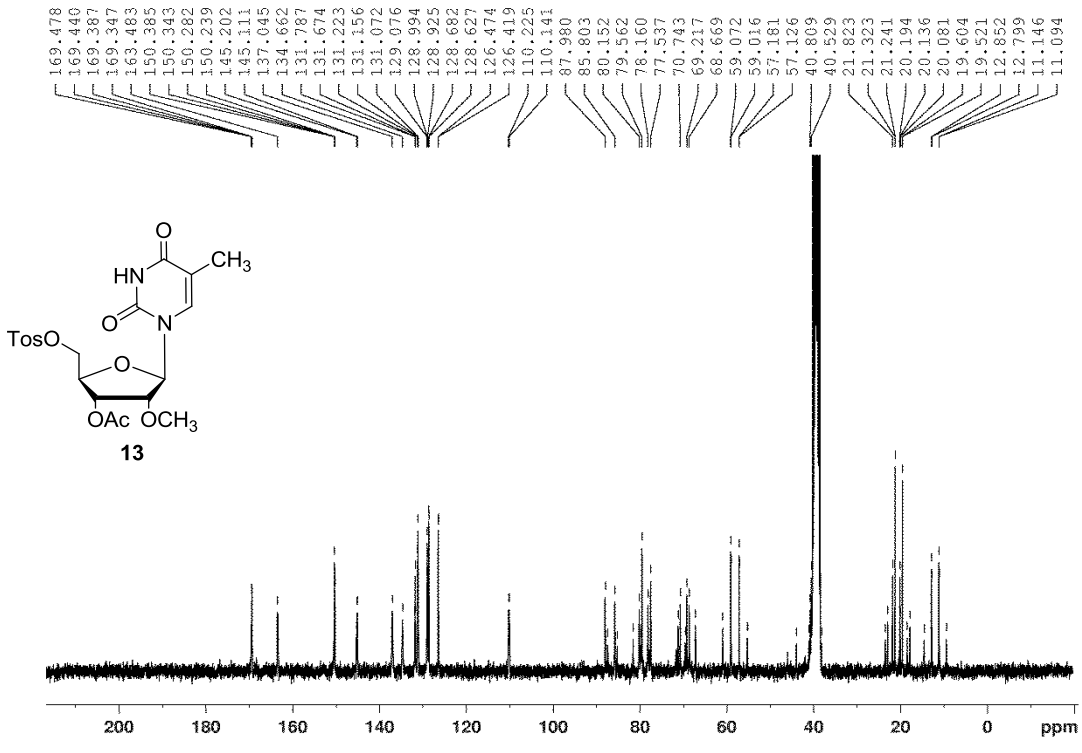


Fig. S41 ^{1}H-^{13}C gated-decoupled spectrum of compound **13**.

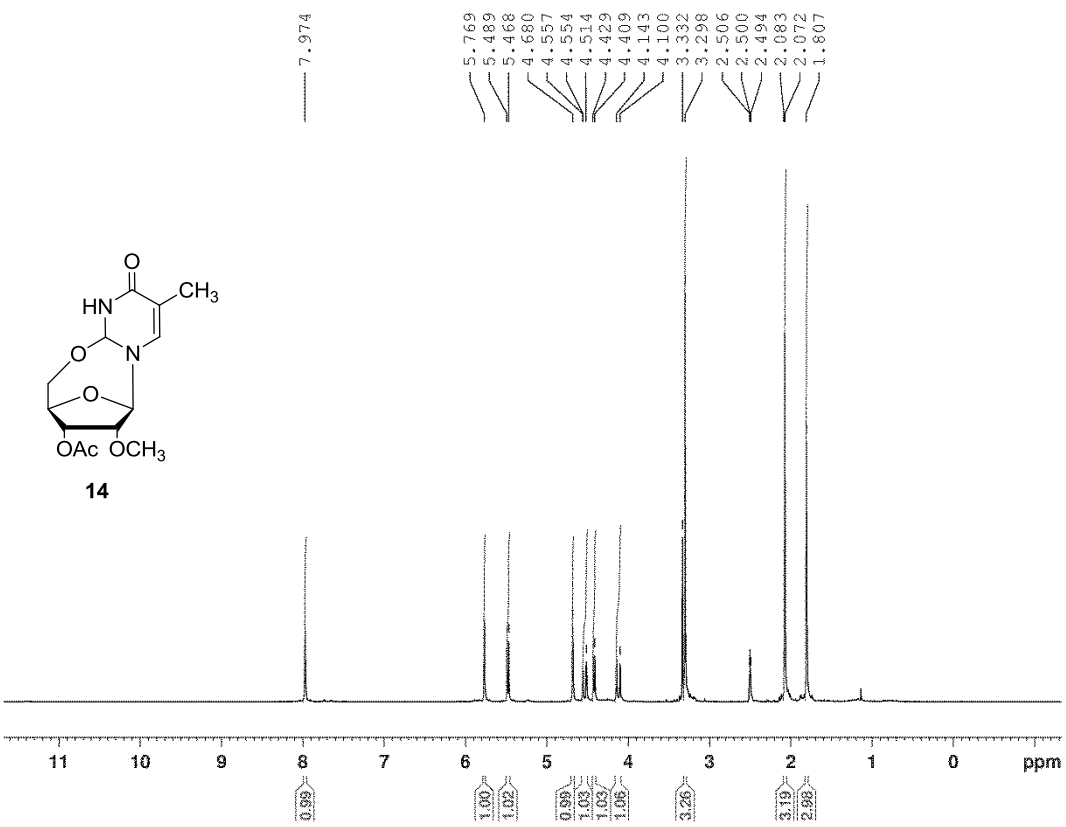


Fig. S42 ^{1}H-NMR spectrum of compound **14**.

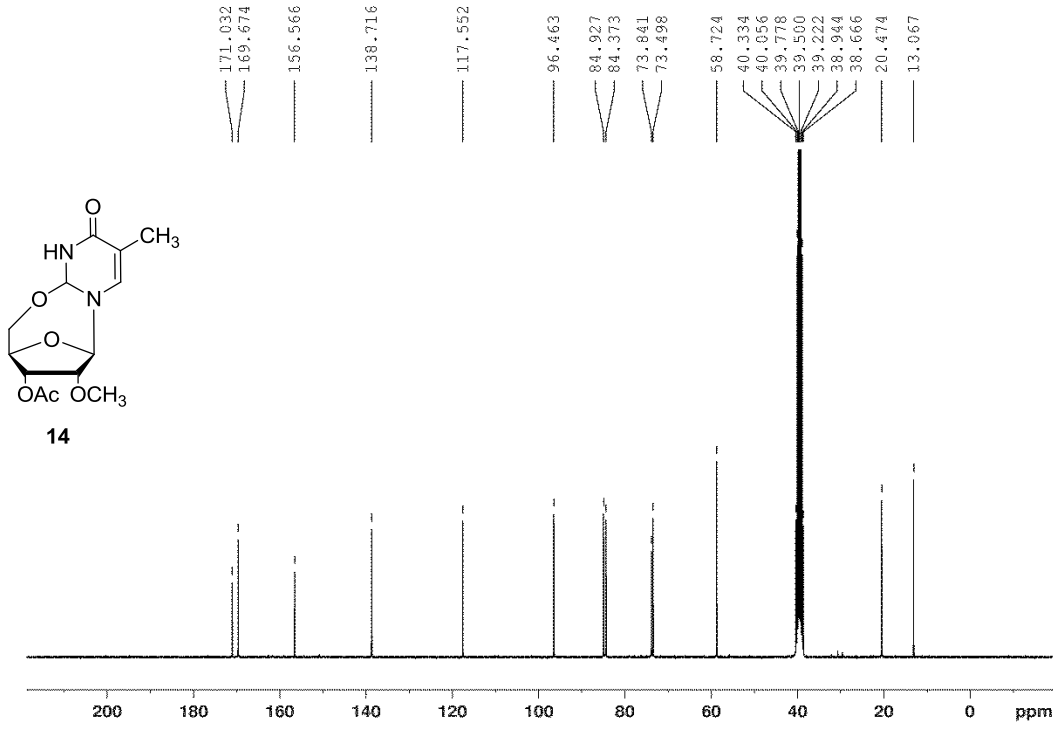


Fig. S43 ^{13}C-NMR spectrum of compound **14**.

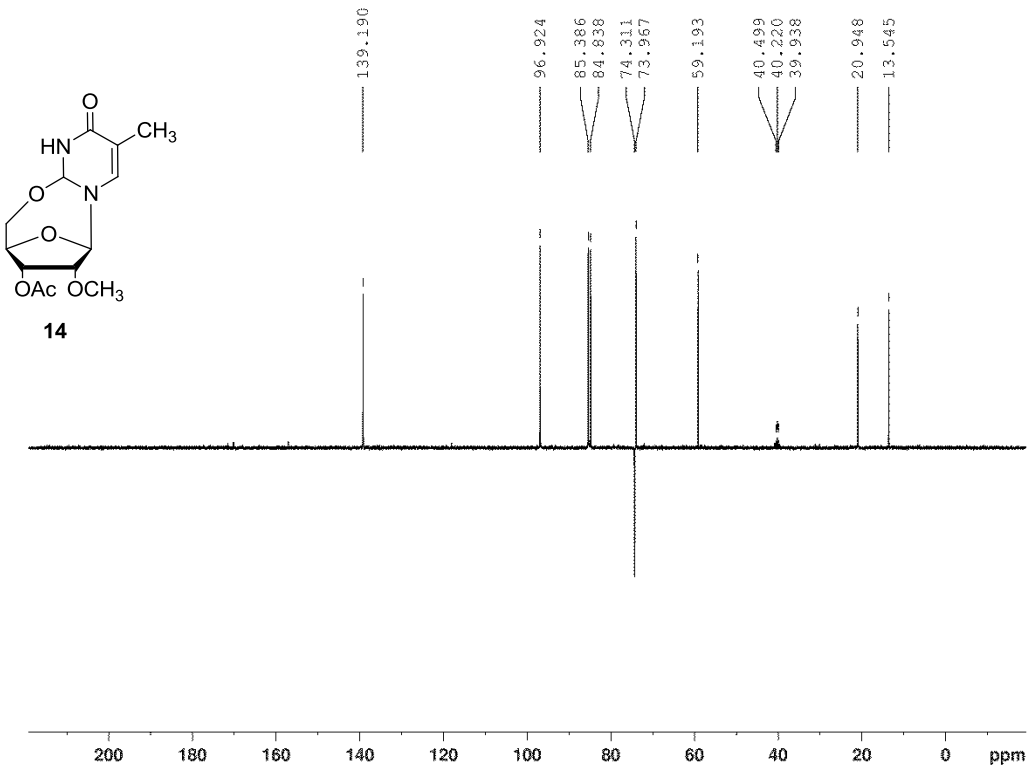


Fig. S44 DEPT-135 NMR spectrum of compound **14**.

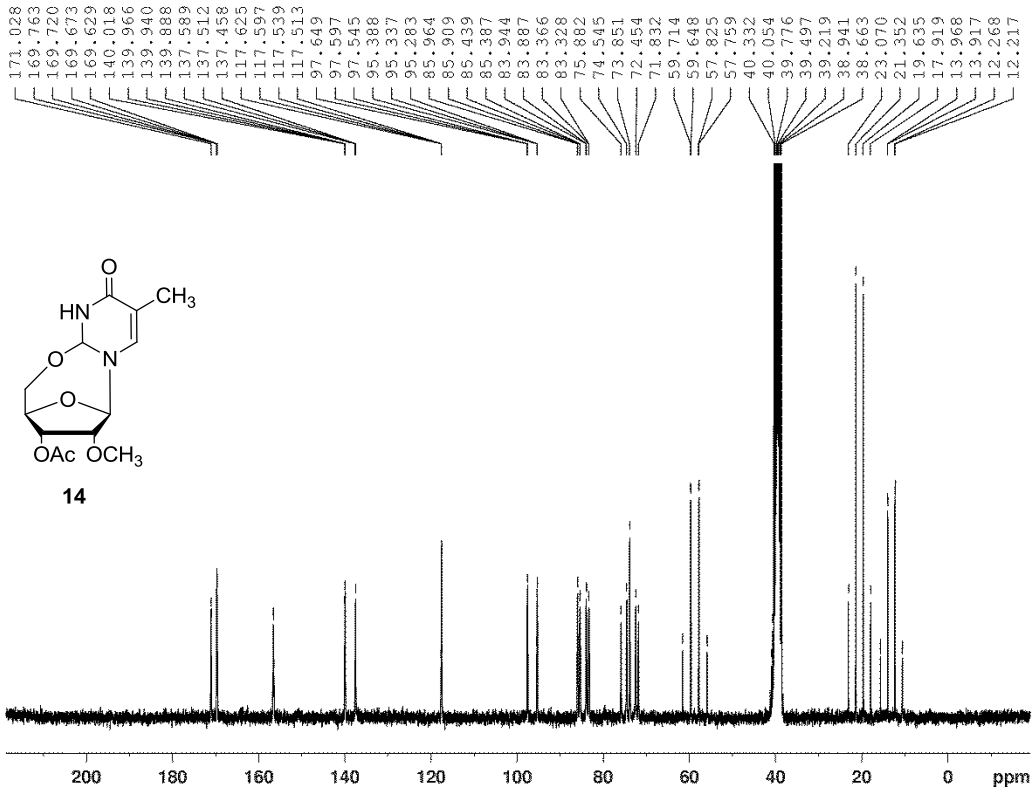


Fig. S45 ^{1}H-^{13}C gated-decoupled spectrum of compound **14**.

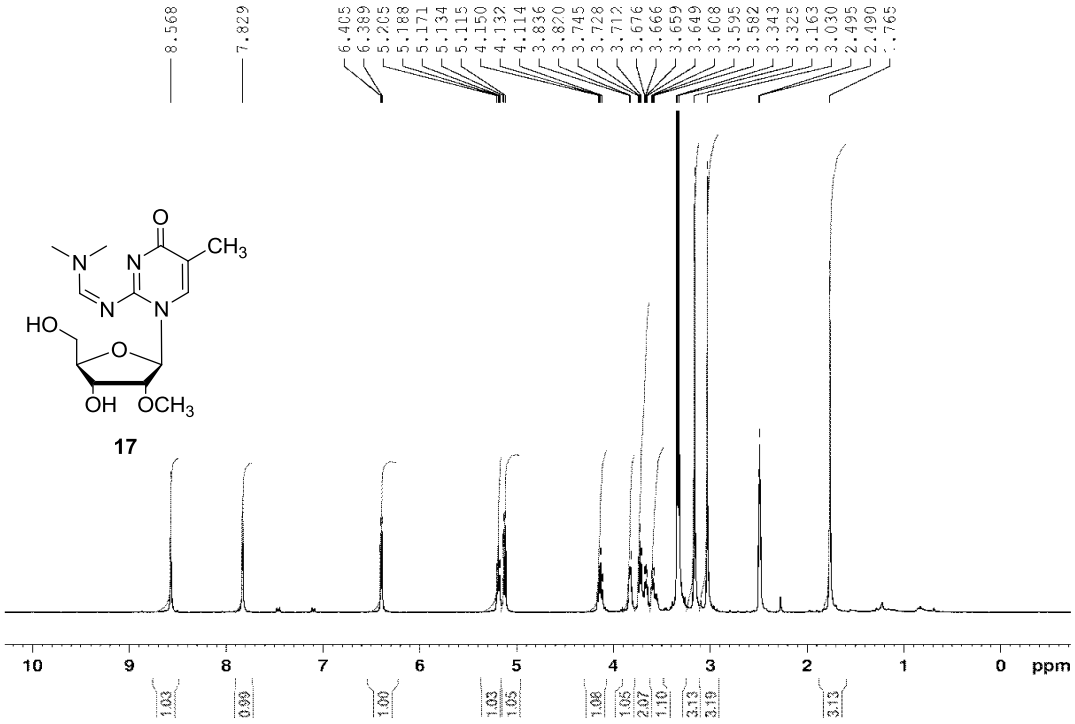


Fig. S46 ^{1}H-NMR spectrum of compound **17**.

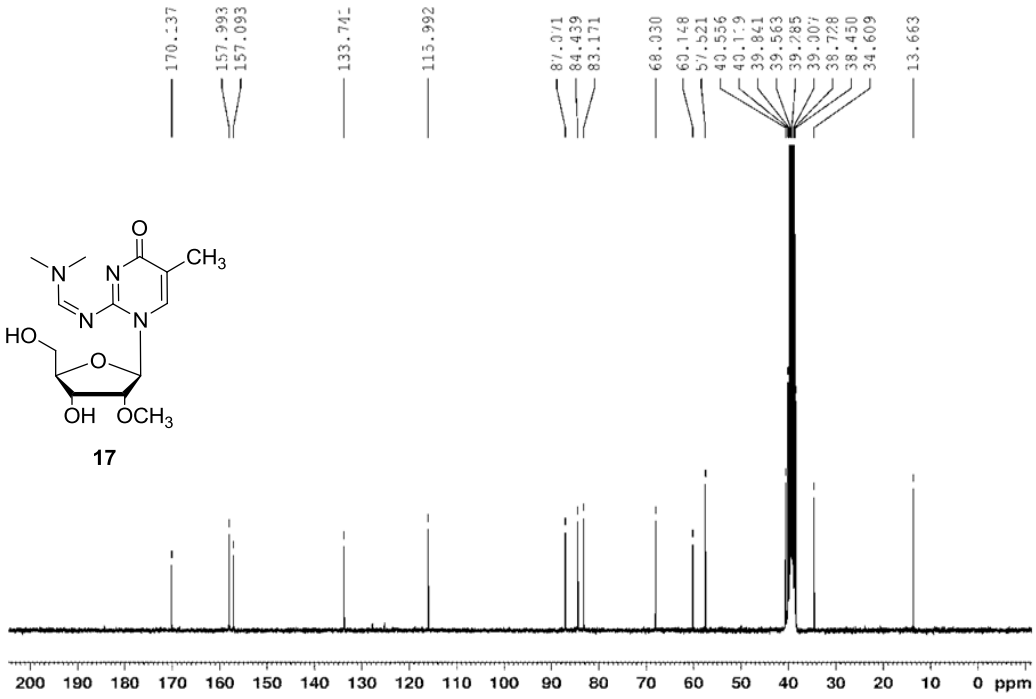


Fig. S47 ^{13}C-NMR spectrum of compound **17**.

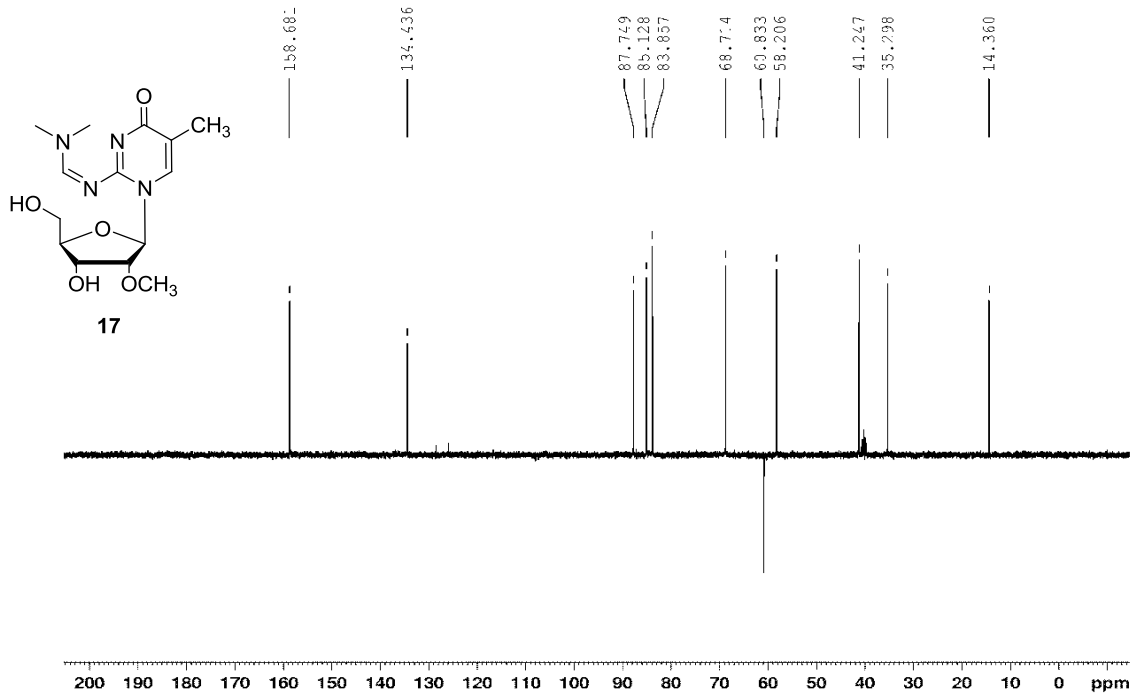


Fig. S48 DEPT-135 NMR spectrum of compound **17**.

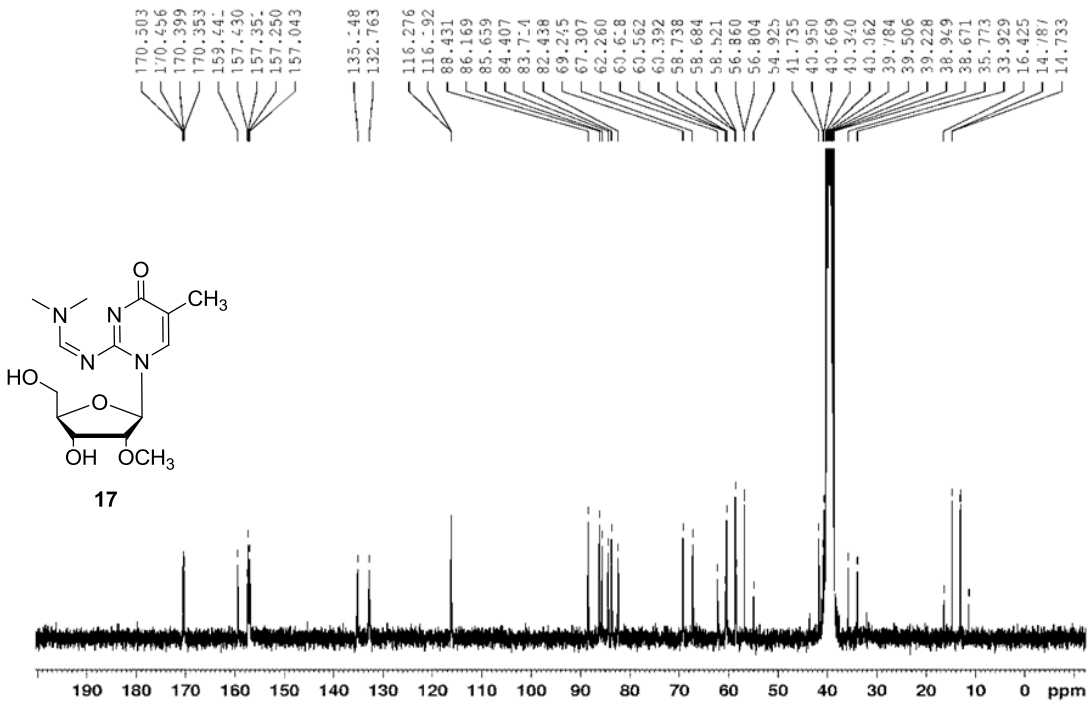


Fig. S49 ^{1}H-^{13}C gated-decoupled spectrum of compound **17**.

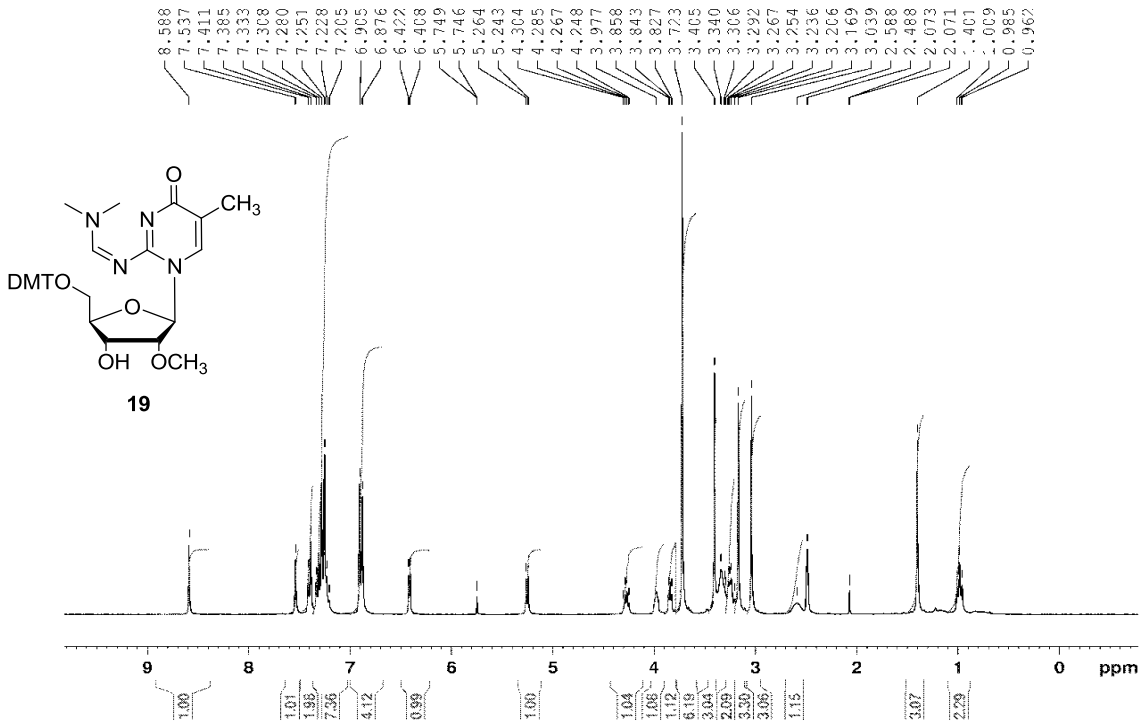


Fig. S50 ^{1}H-NMR spectrum of compound **19**.

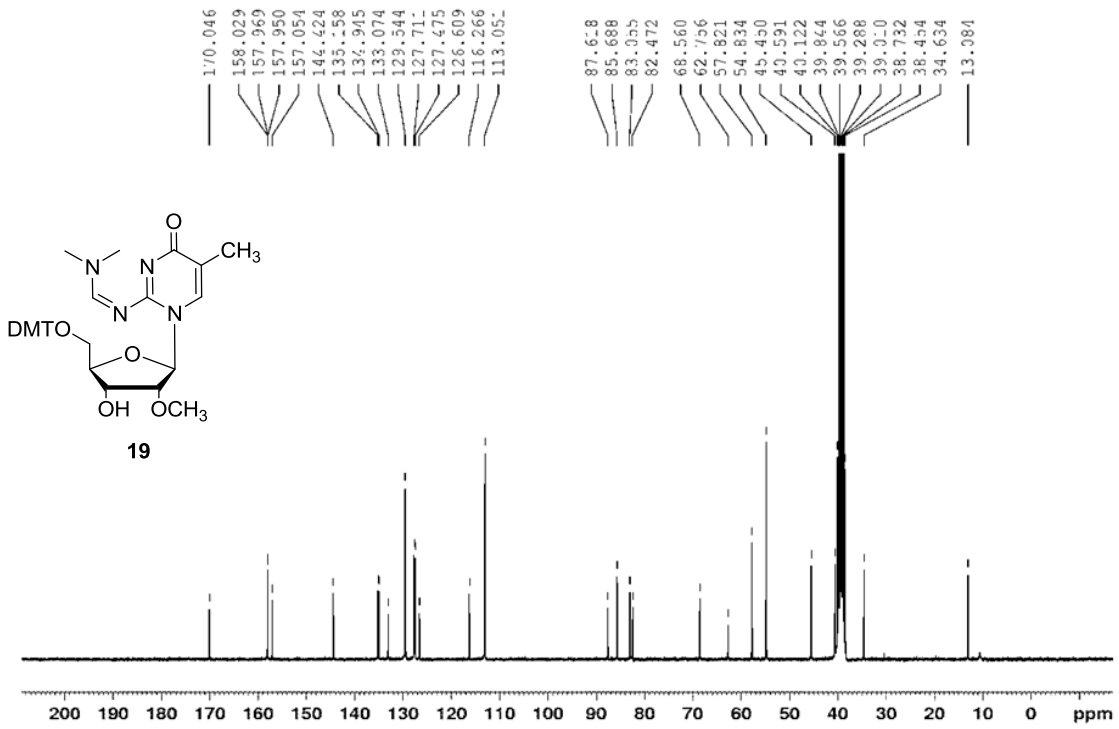


Fig. S51 ^{13}C-NMR spectrum of compound **19**.

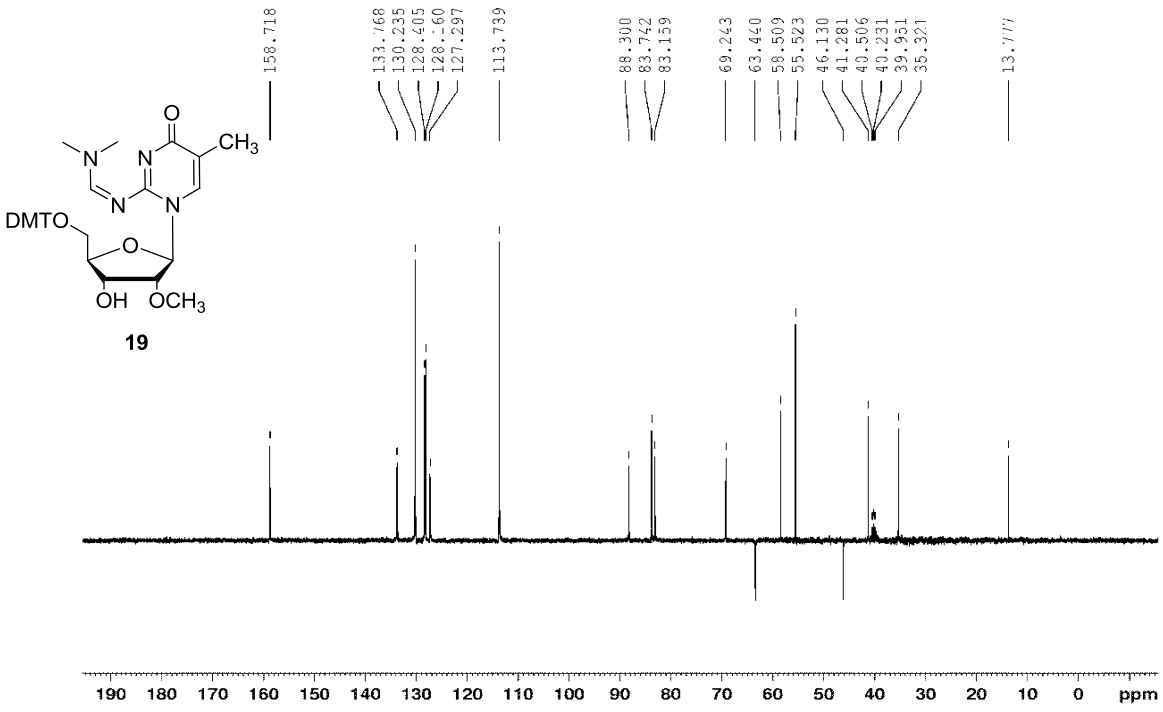


Fig. S52 DEPT-135 spectrum of compound **19**.

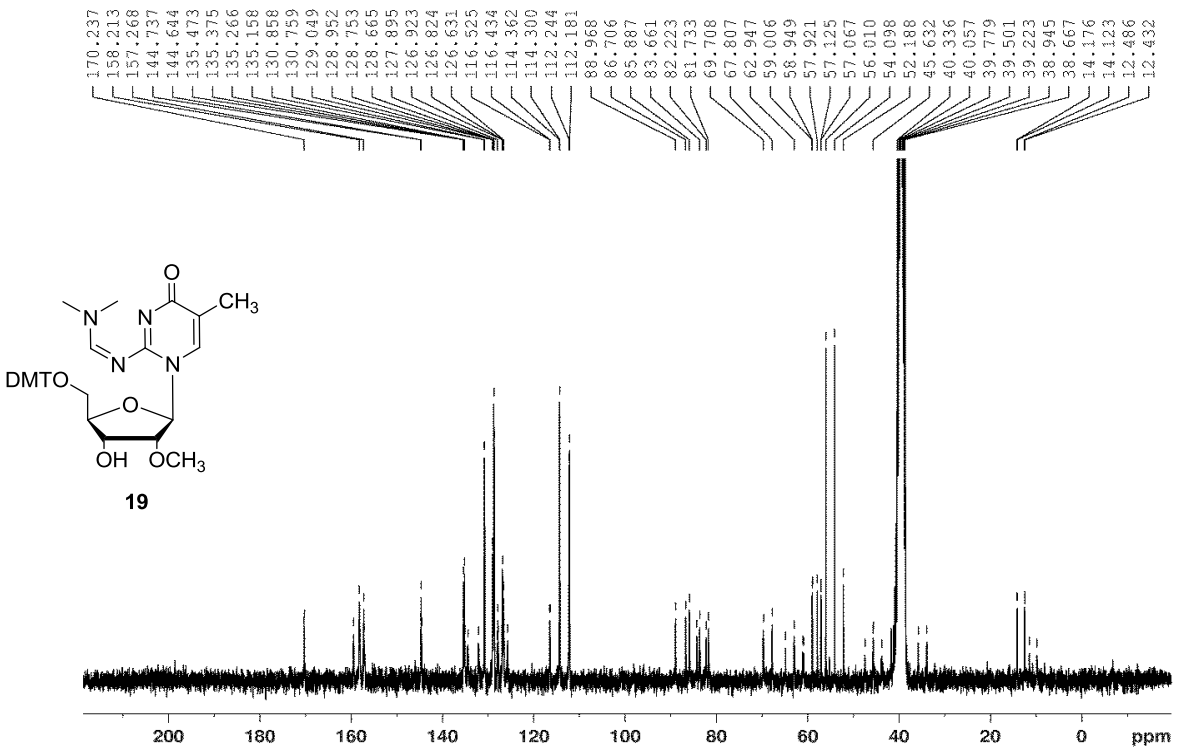


Fig. S53 ^{1}H-^{13}C gated-decoupled spectrum of compound **19**.

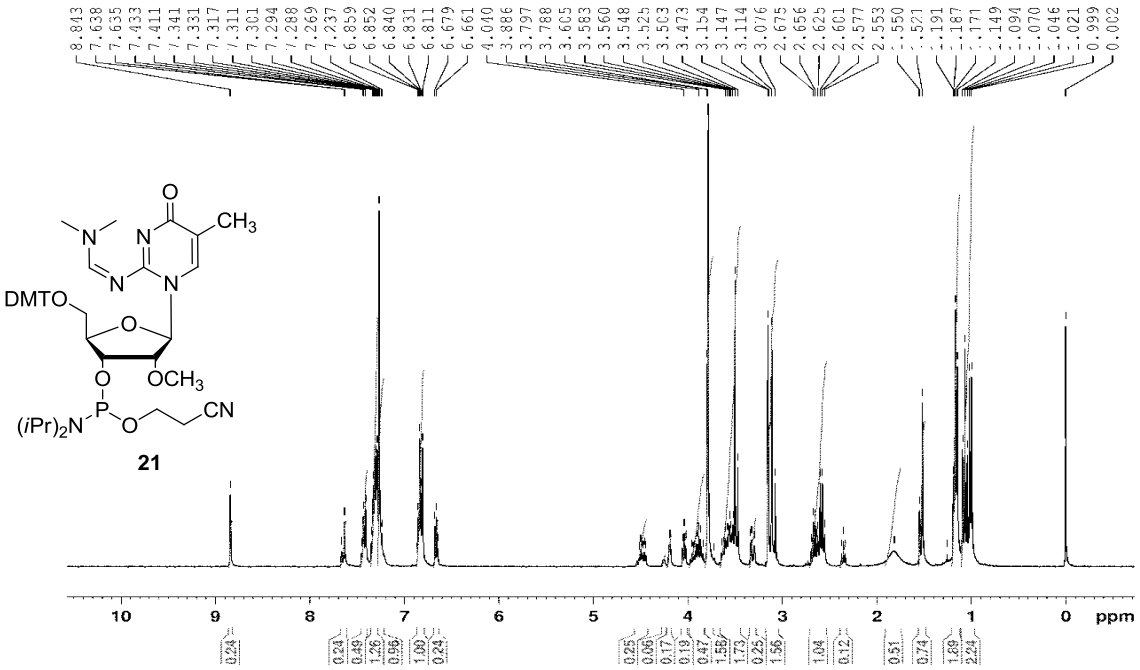


Fig. S54 ^{1}H-NMR spectrum of compound **21**.

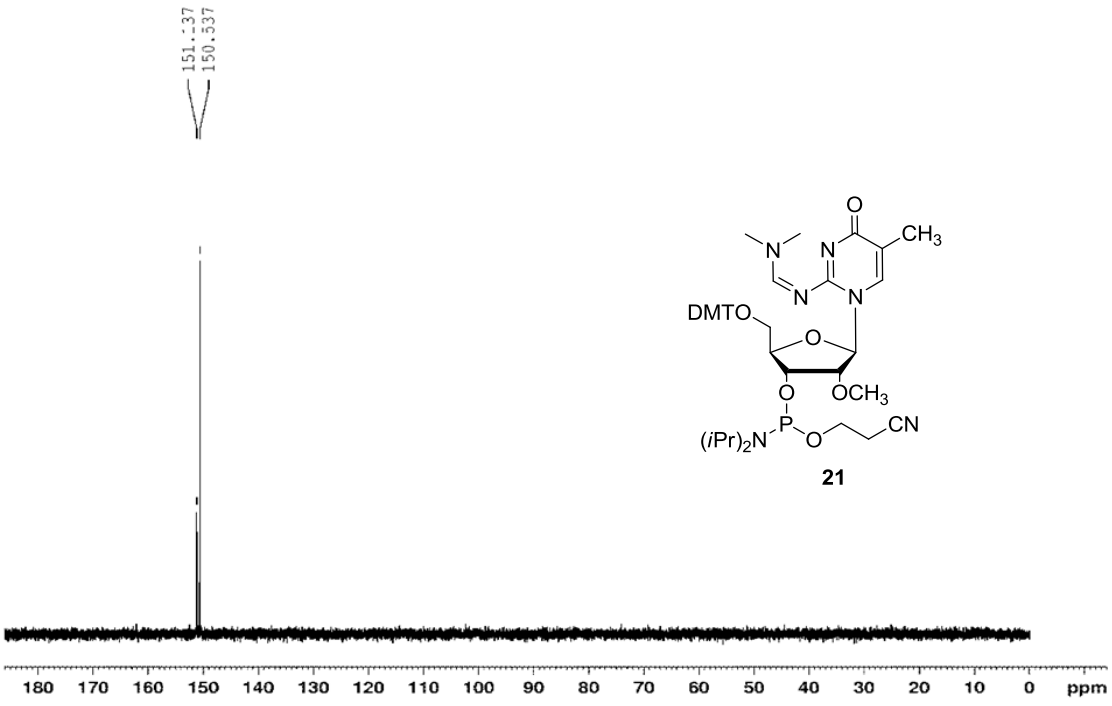


Fig. S55 ^{31}P-NMR spectrum of compound **21**.

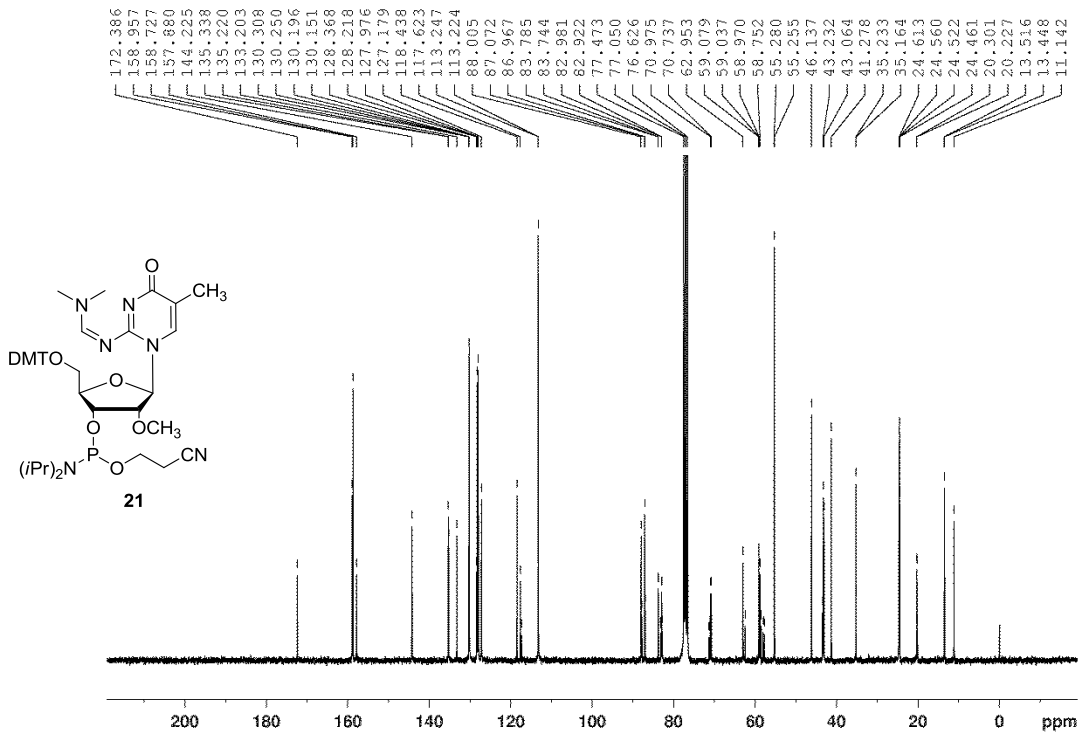


Fig. S56 ^{13}C-NMR spectrum of compound **21**

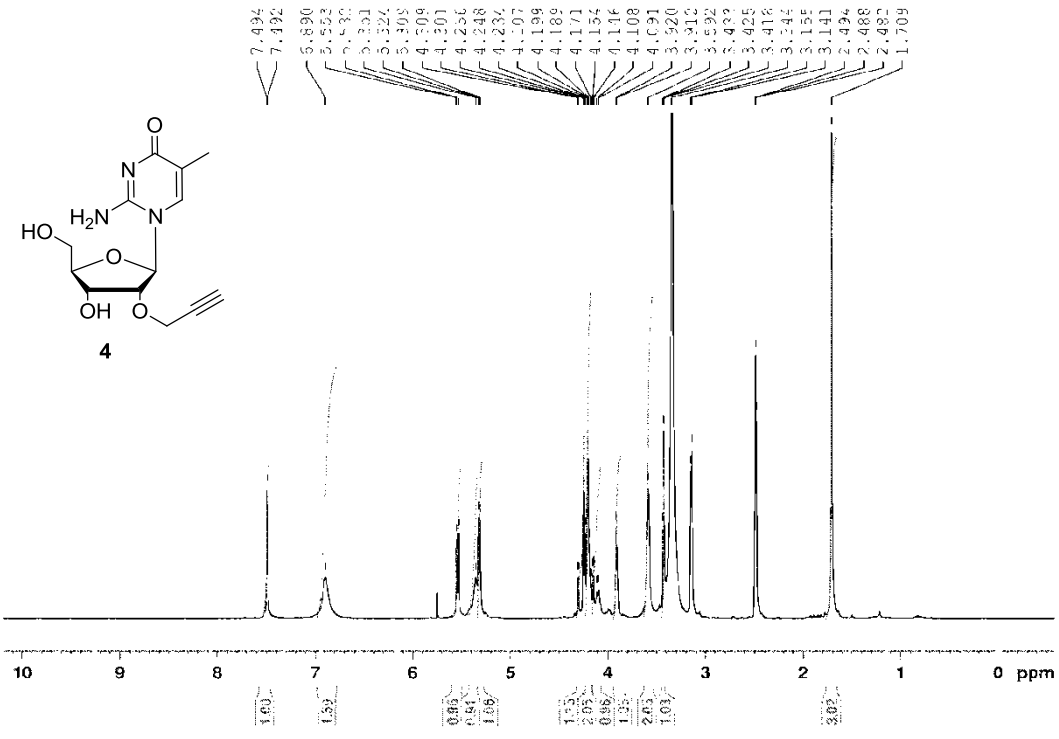


Fig. S57 ^{1}H-NMR spectrum of compound **4**.

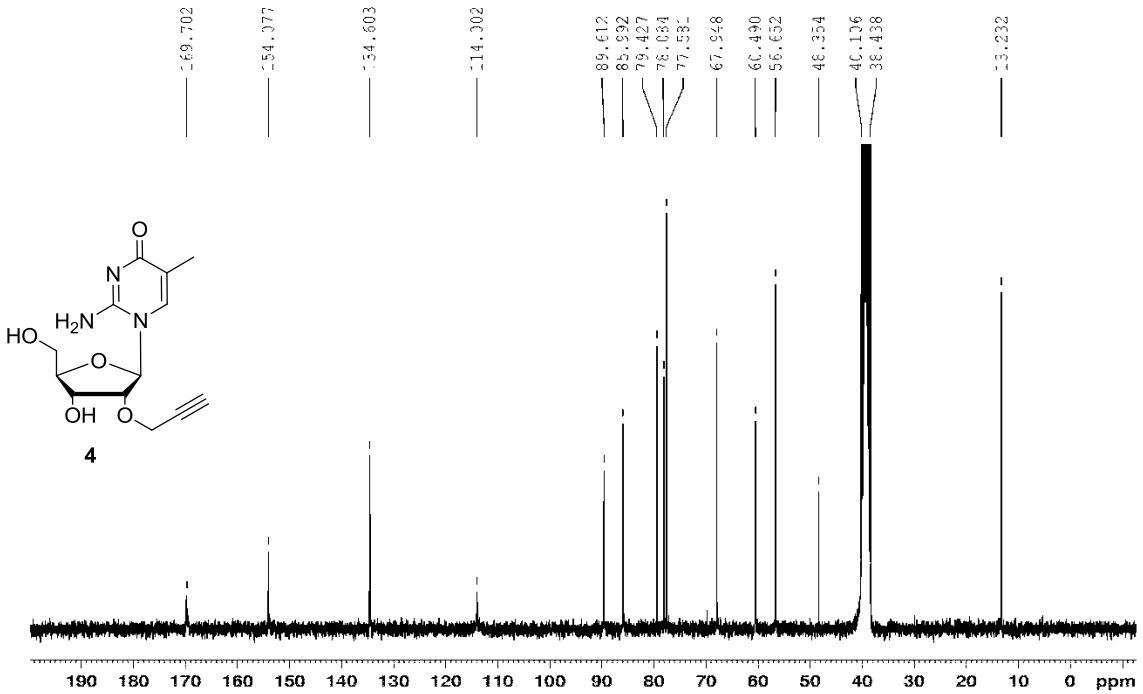


Fig. S58 ^{13}C-NMR spectrum of compound **4**.

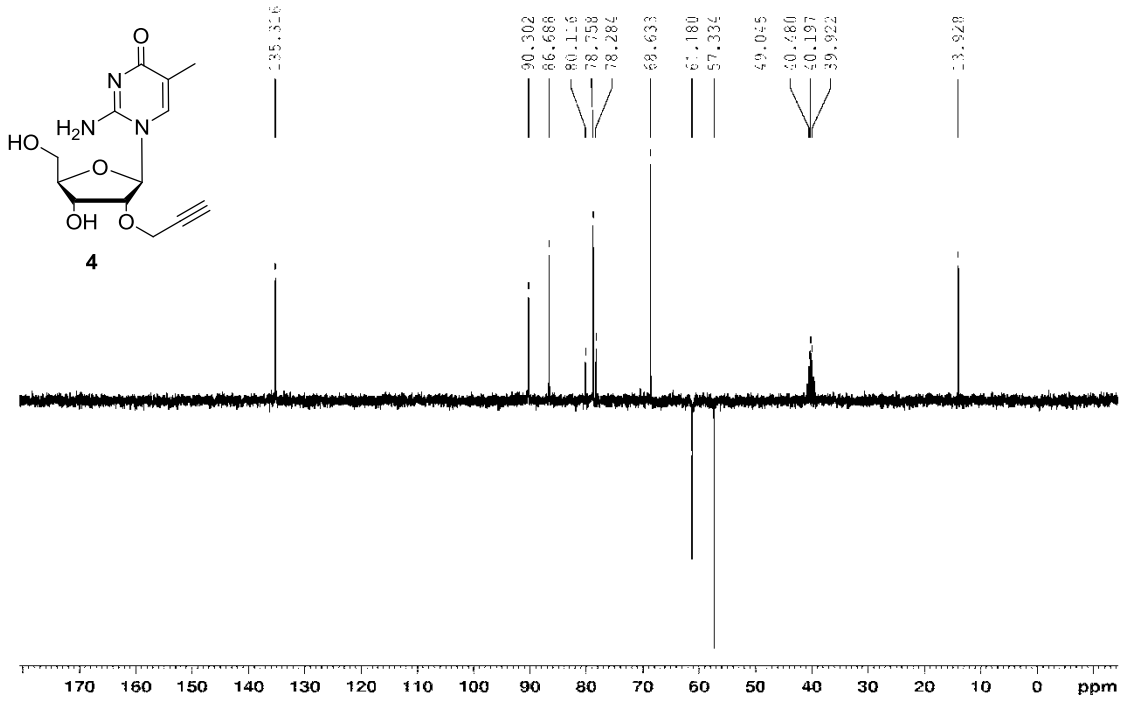


Fig. S59 DEPT-135 spectrum of compound **4**.

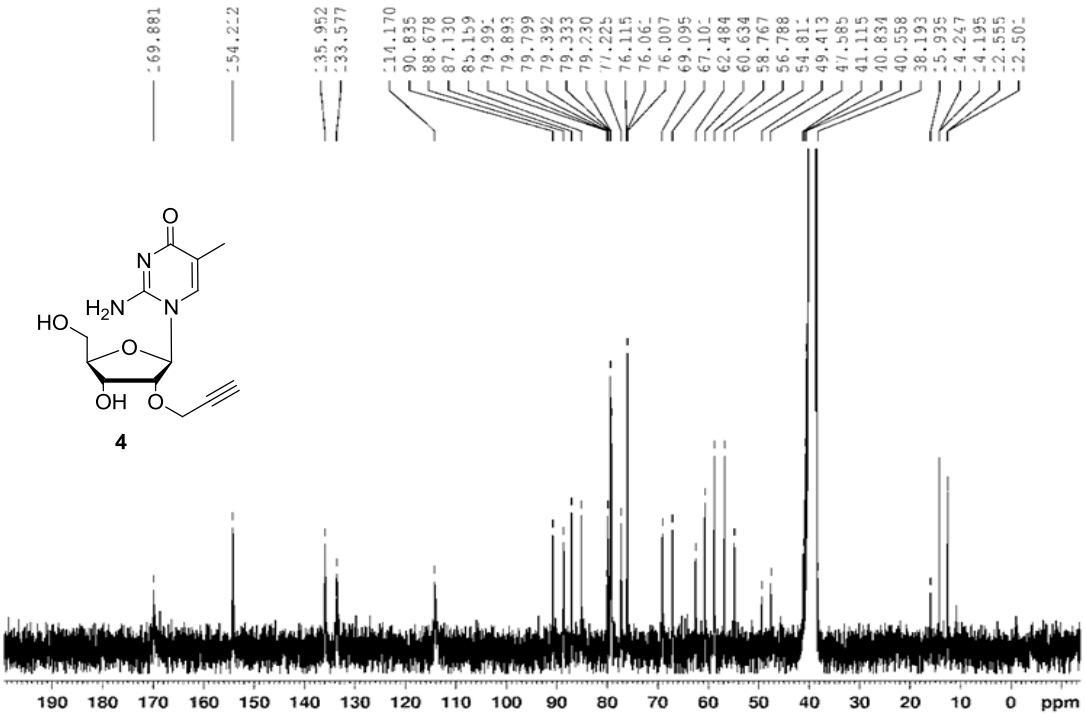


Fig. S60 ^{1}H-^{13}C gated decoupled spectrum of compound **4**.

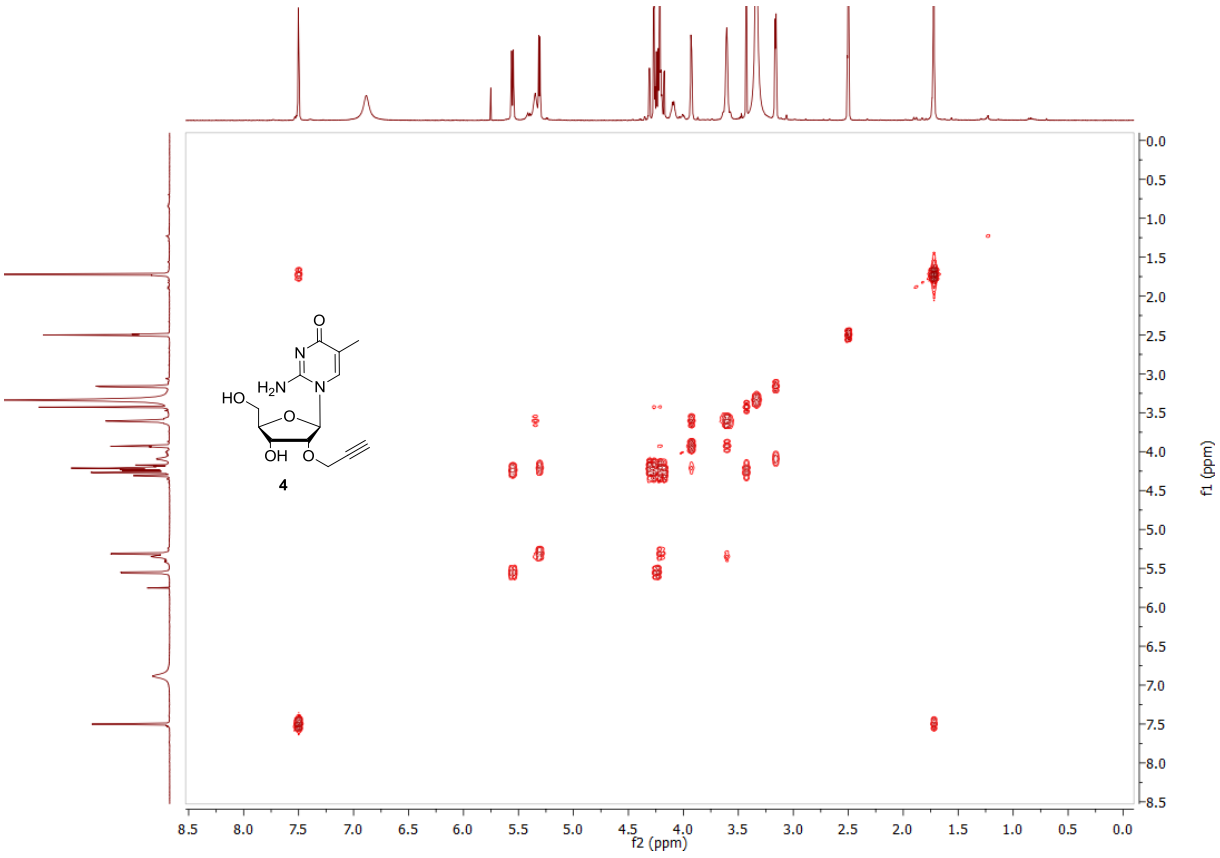


Fig. S61 ^{1}H-^{1}H Cosy spectrum of compound **4**.

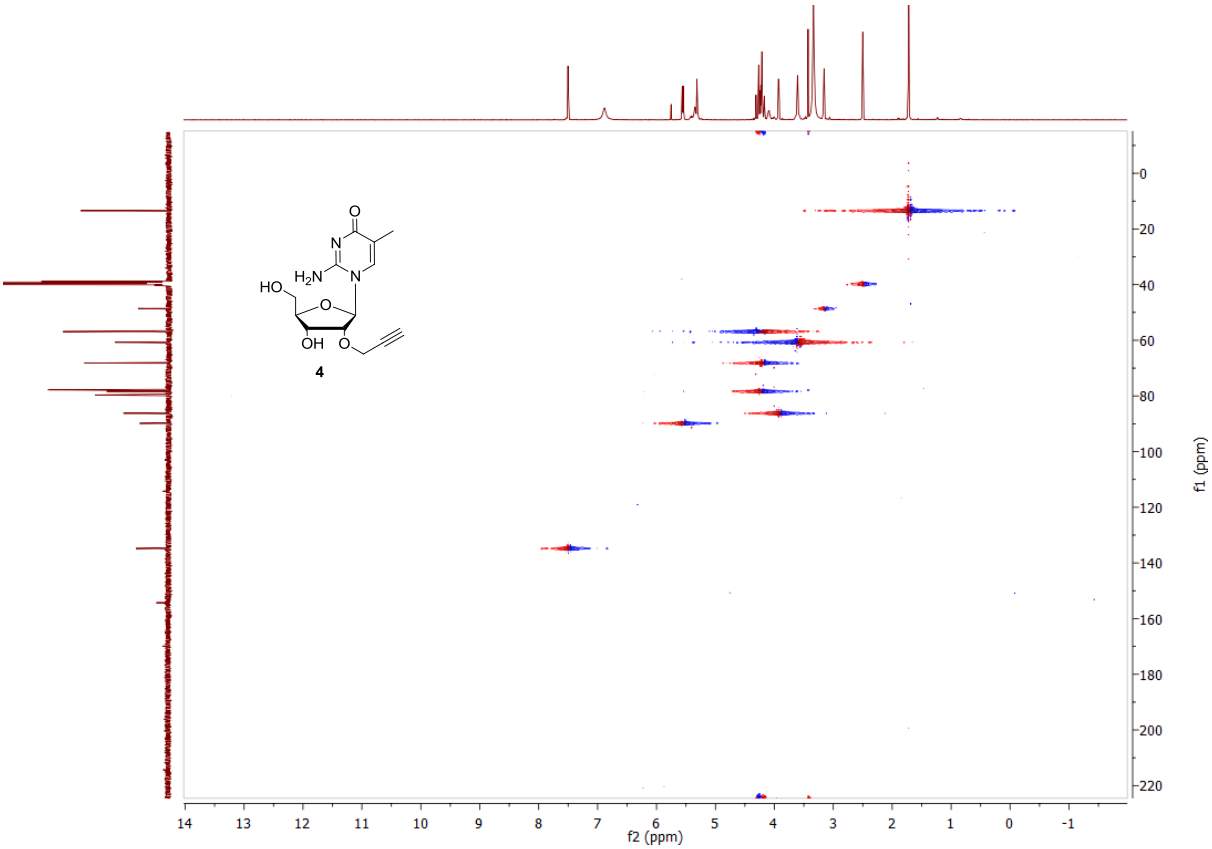


Fig. S62 ^{1}H-^{13}C HSQC spectrum of compound **4**.

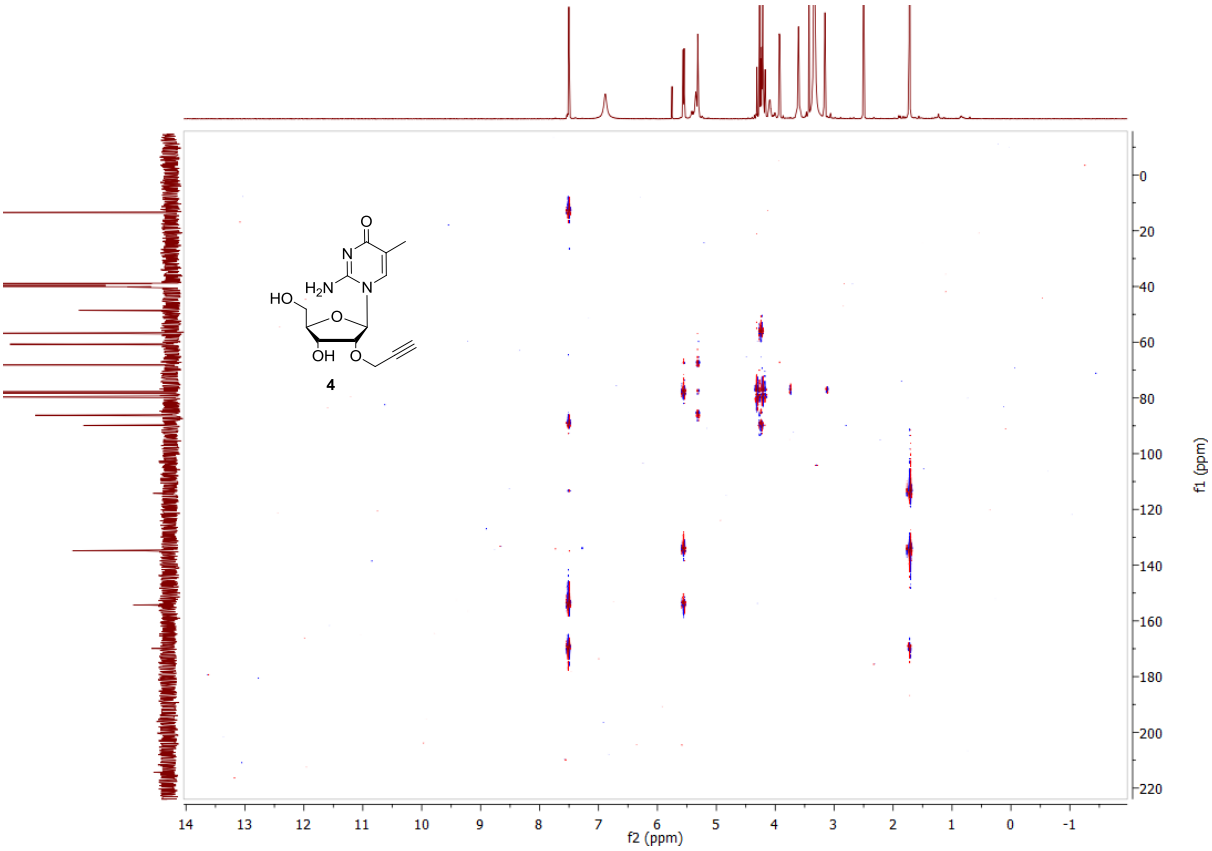


Fig. S63 ^{1}H-^{13}C HMBC spectrum of compound **4**.

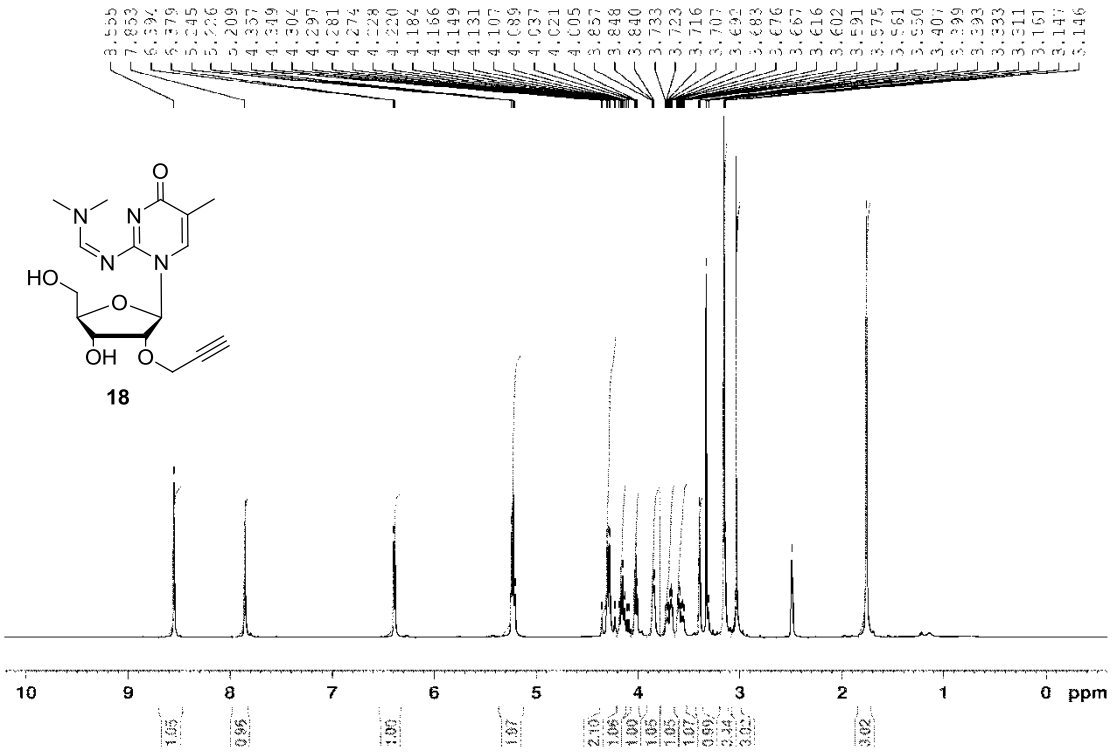


Fig. S64 ^{1}H-NMR spectrum of compound **18**.

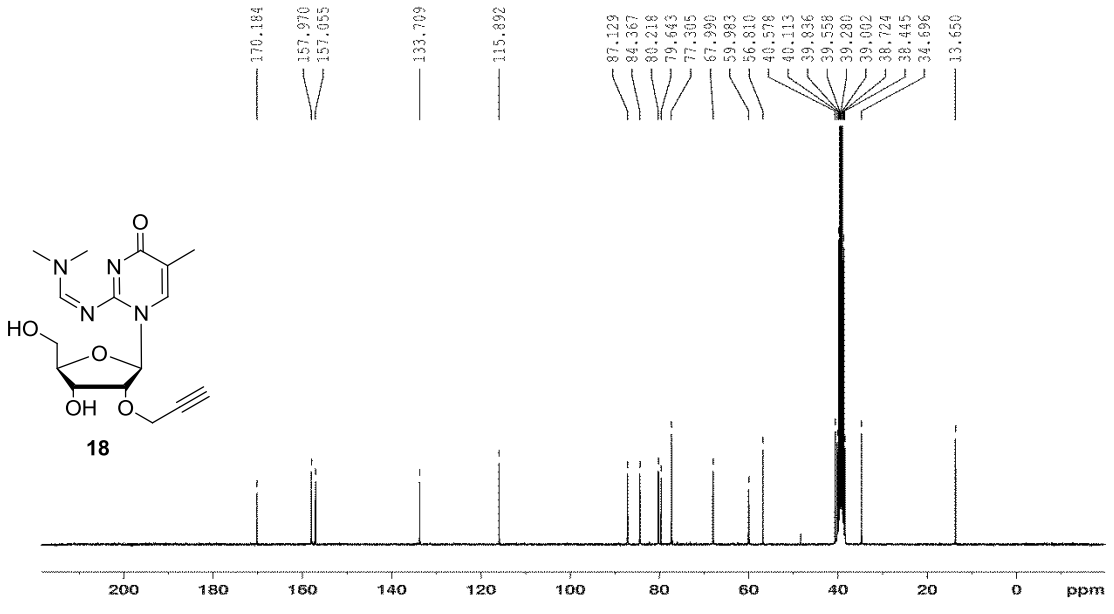


Fig. S65 ^{13}C-NMR spectrum of compound **18.**

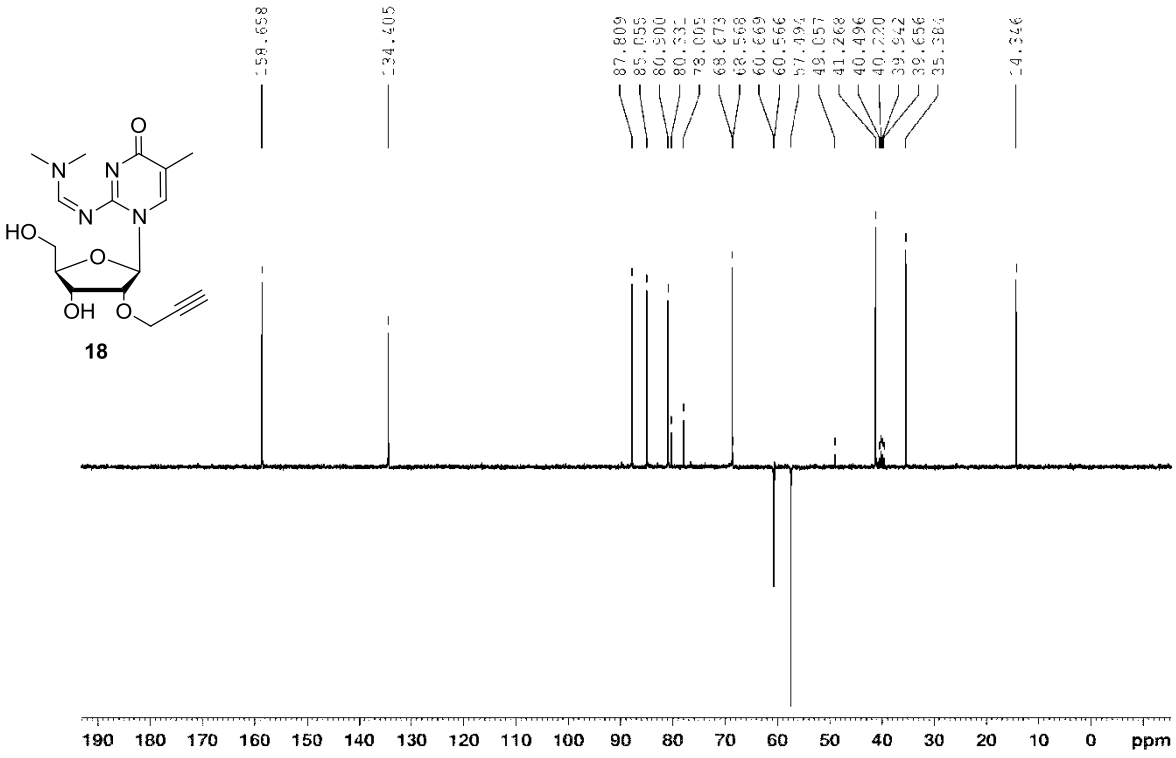


Fig. S66 DEPT-135 NMR spectrum of compound **18.**

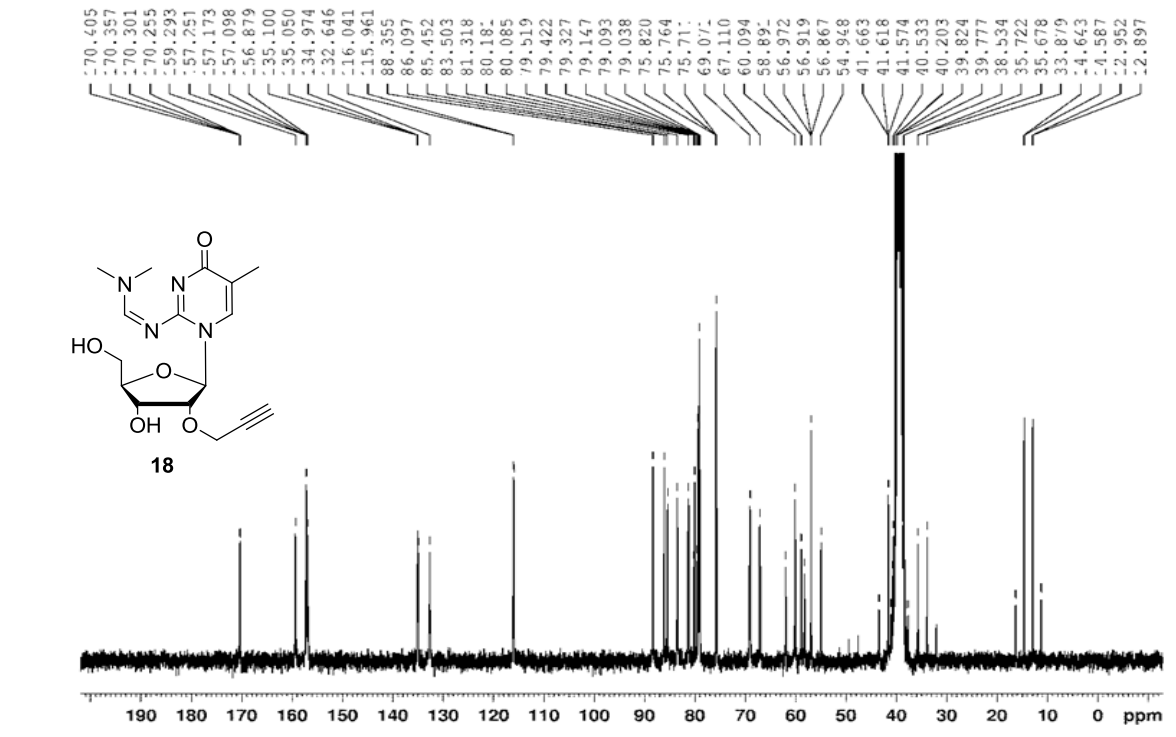


Fig. S67 ^{1}H-^{13}C gated-decoupled spectrum of compound **18**.

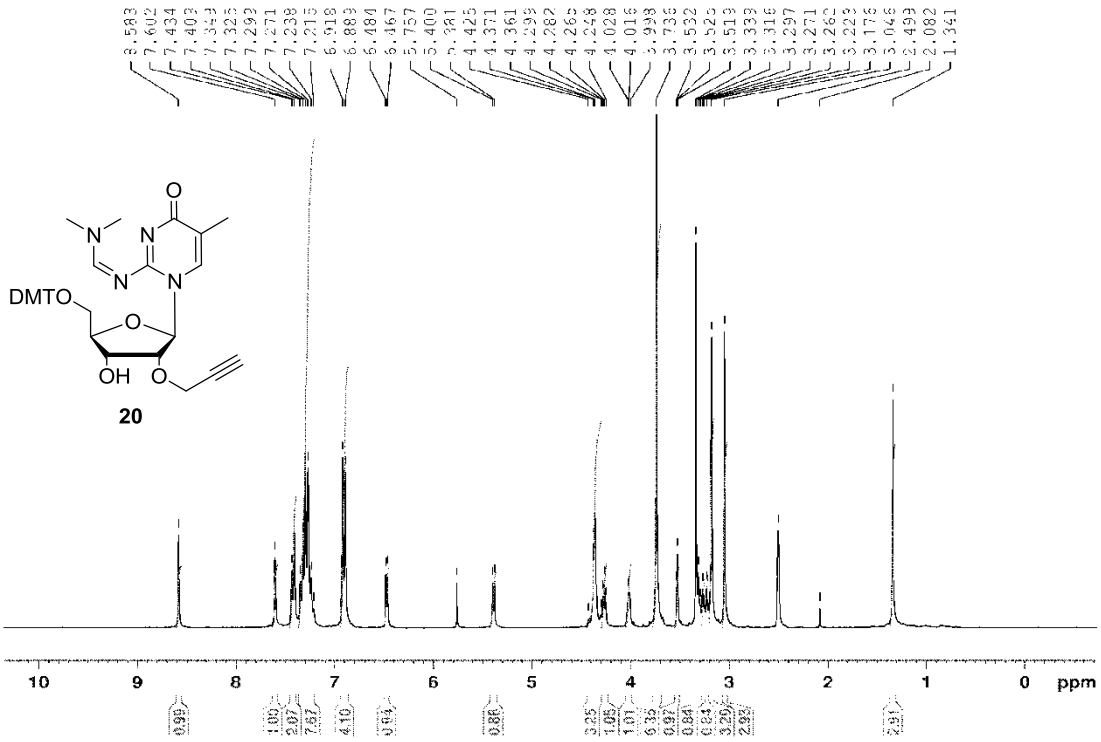


Fig. S68 ^{1}H-NMR spectrum of compound **20**.

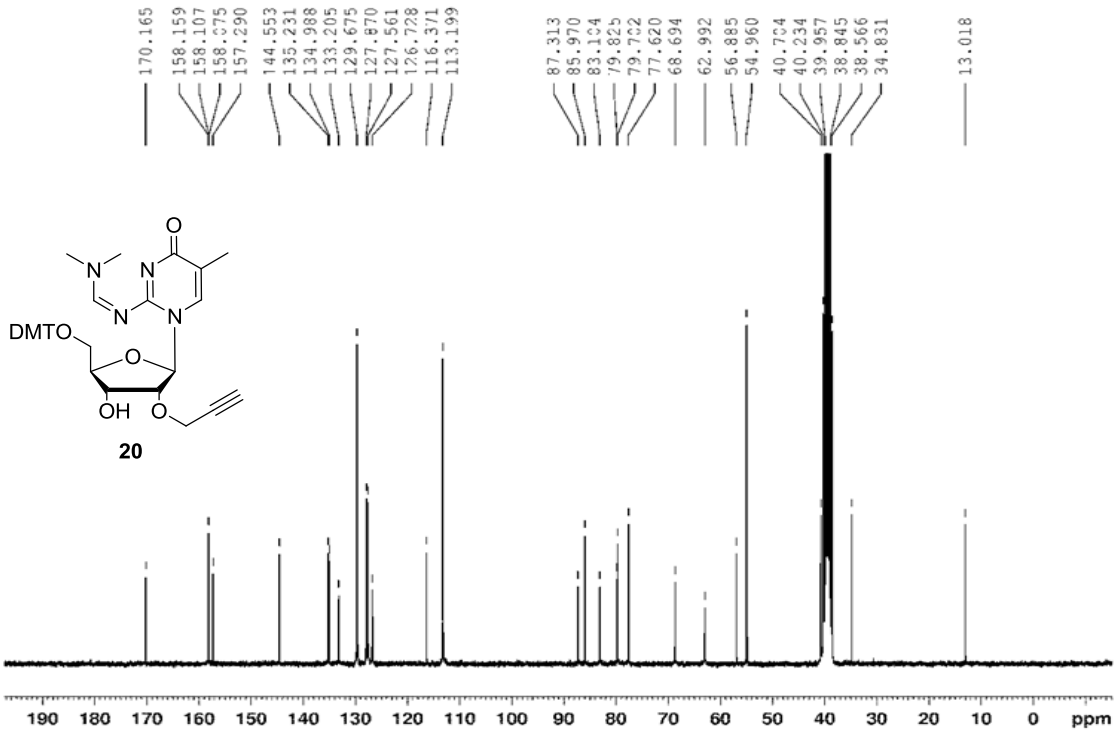


Fig. S69 ^{13}C-NMR spectrum of compound **20.**

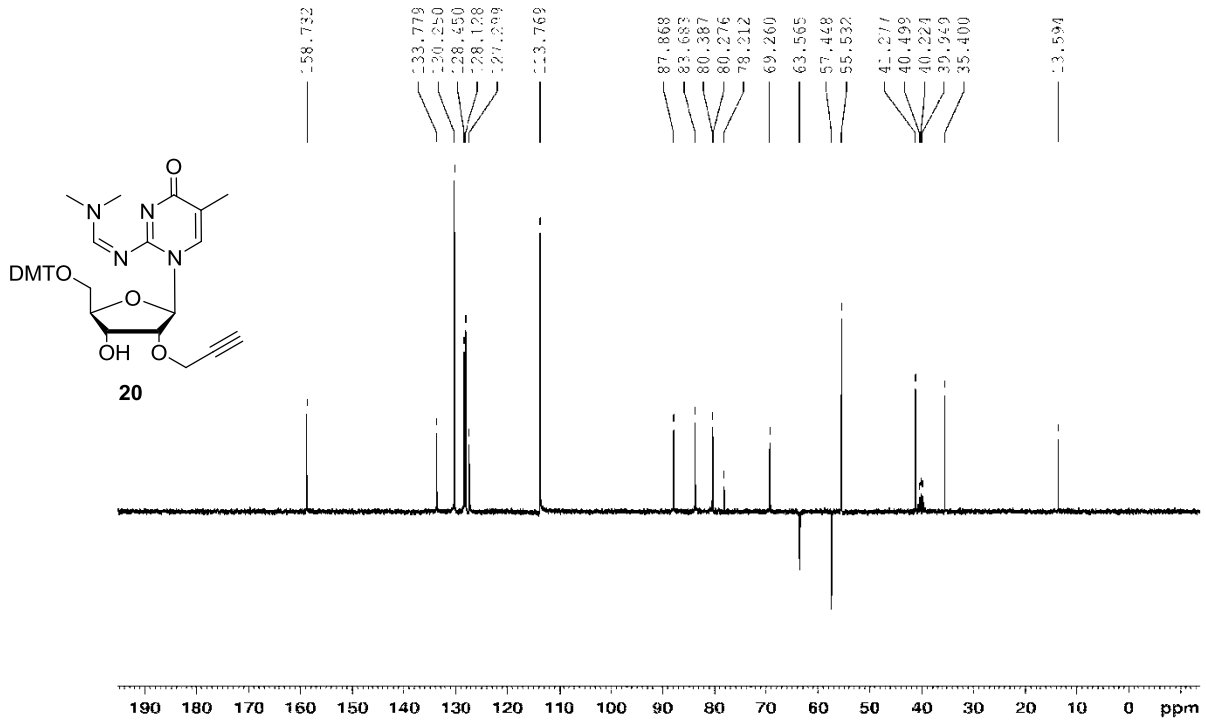


Fig. S70 DEPT-135 NMR spectrum of compound **20.**

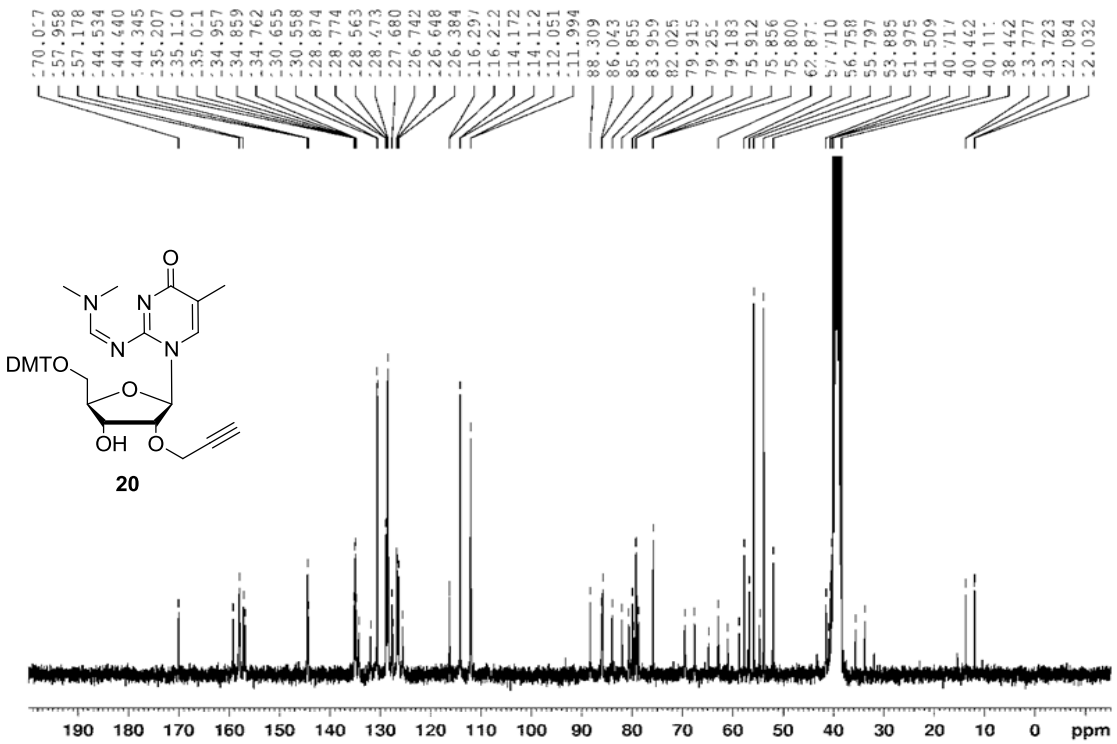


Fig. S71 ^{1}H-^{13}C gated-decoupled spectrum of compound **20**.

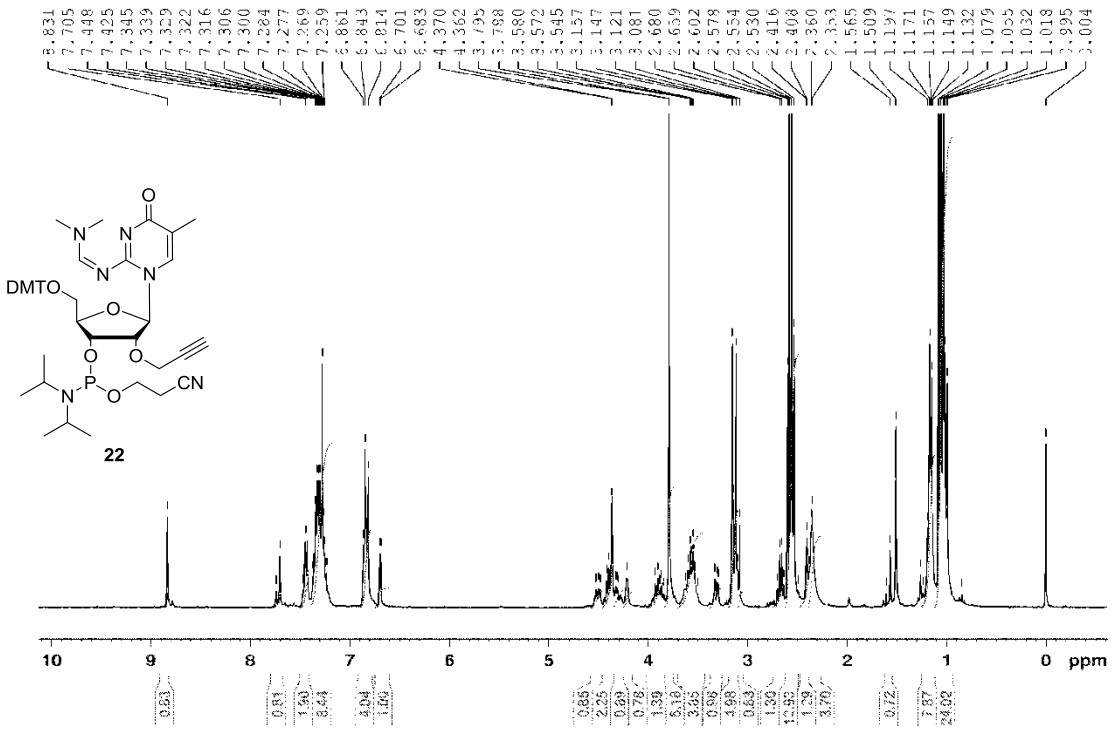


Fig. S72 ^{1}H-NMR spectrum of compound **22.**

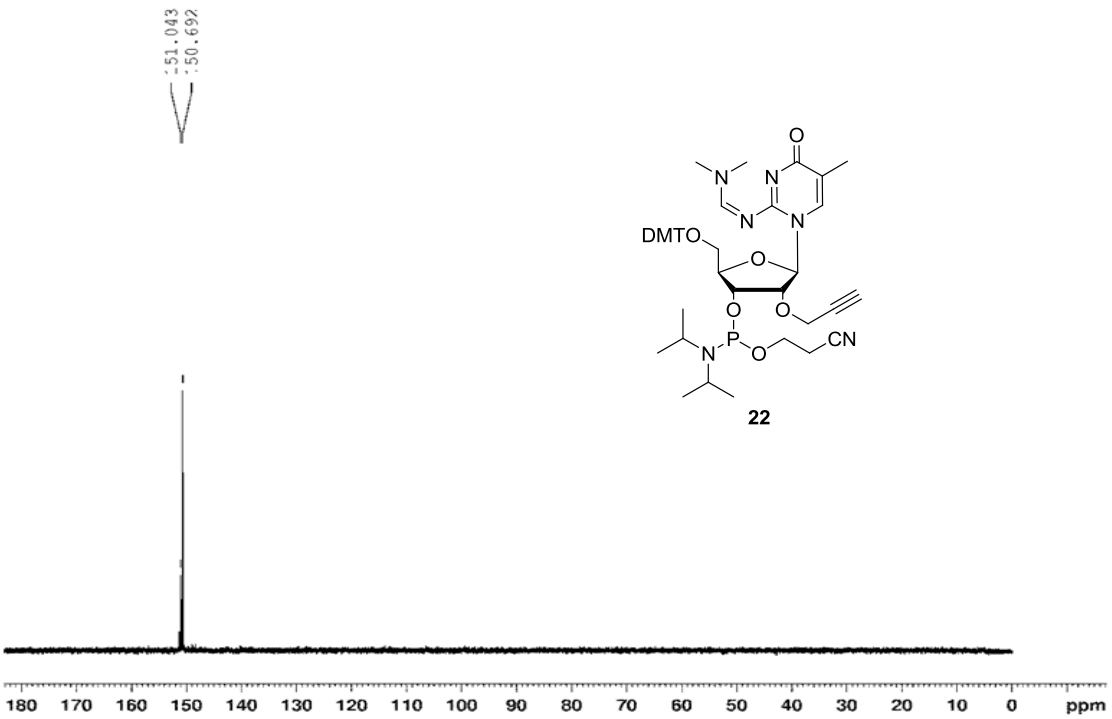


Fig. S73 ^{31}P-NMR spectrum of compound **22.**

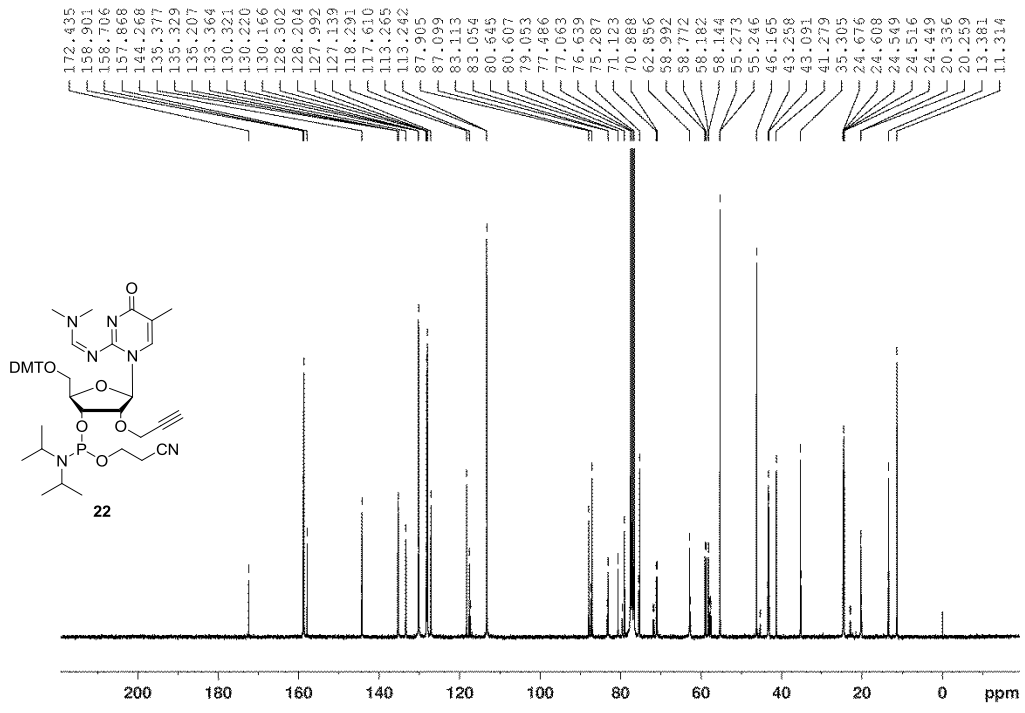


Fig. S74 ^{13}C-NMR spectrum of compound **22.**

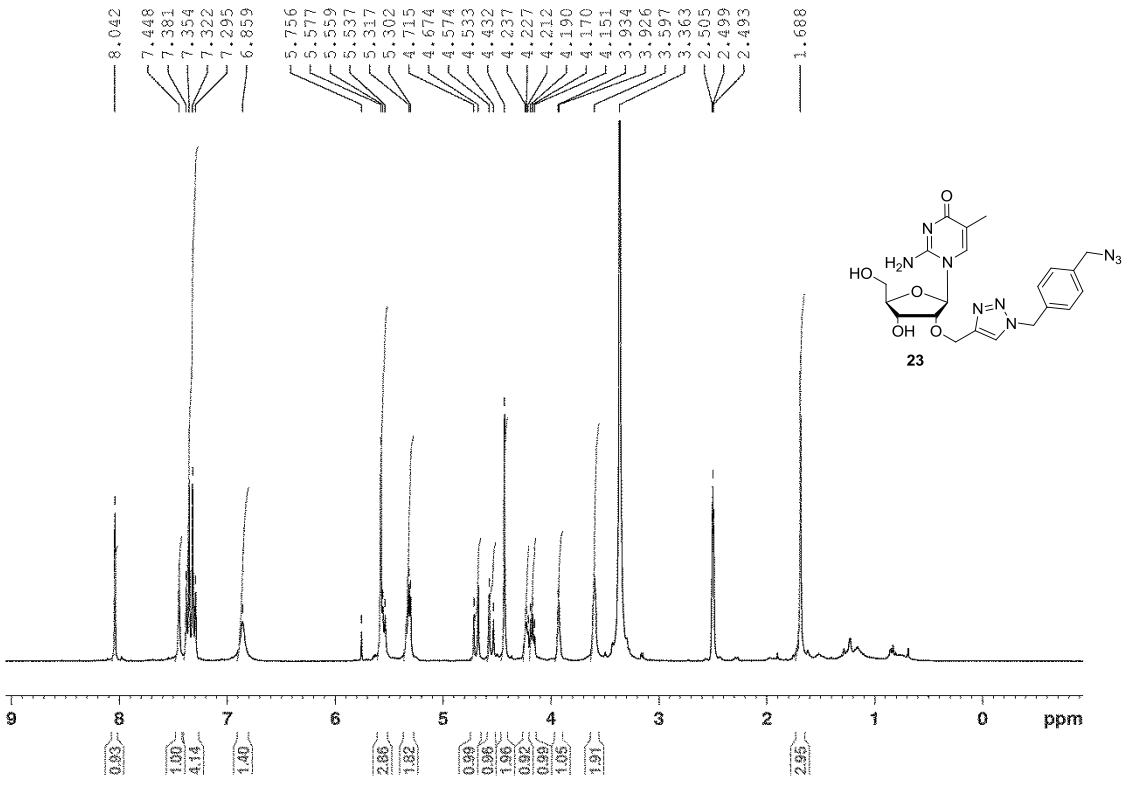


Fig. S75 ^{1}H-NMR spectrum of compound **23**.

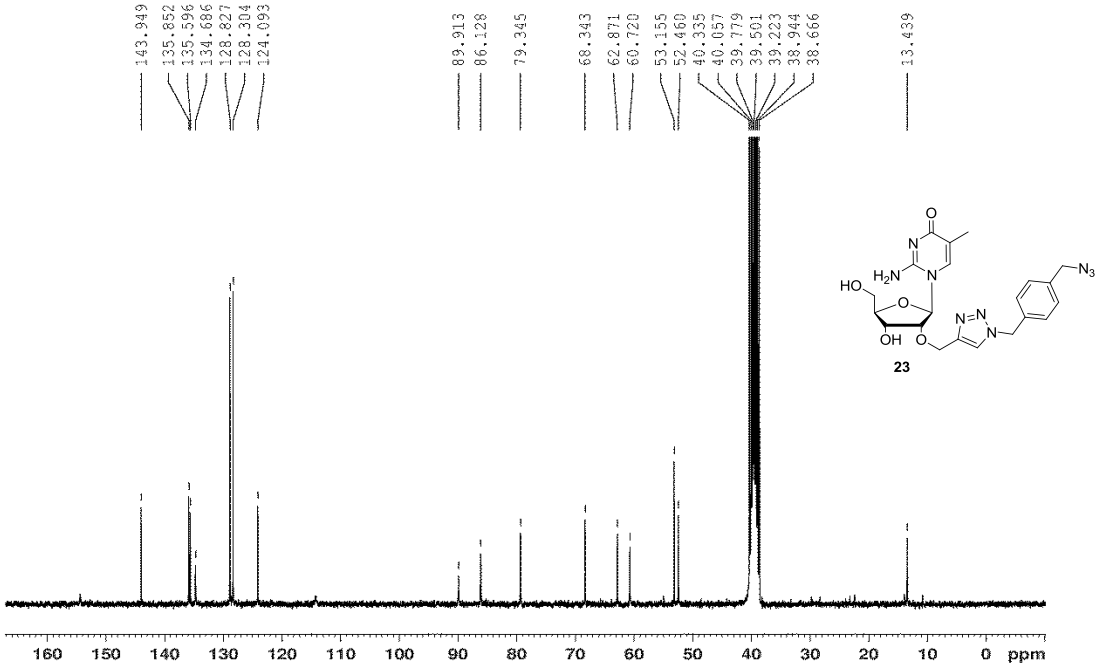


Fig. S76 ^{13}C-NMR spectrum of compound **23.**

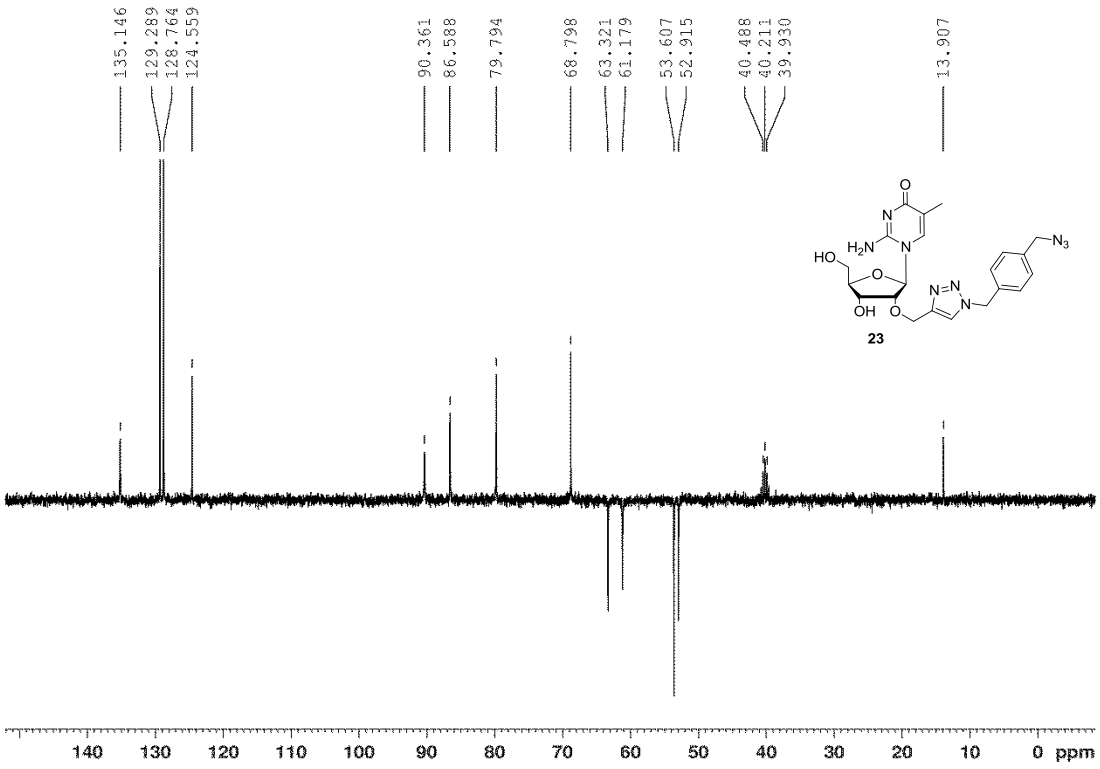


Fig. S77 DEPT-135 NMR spectrum of compound **23.**

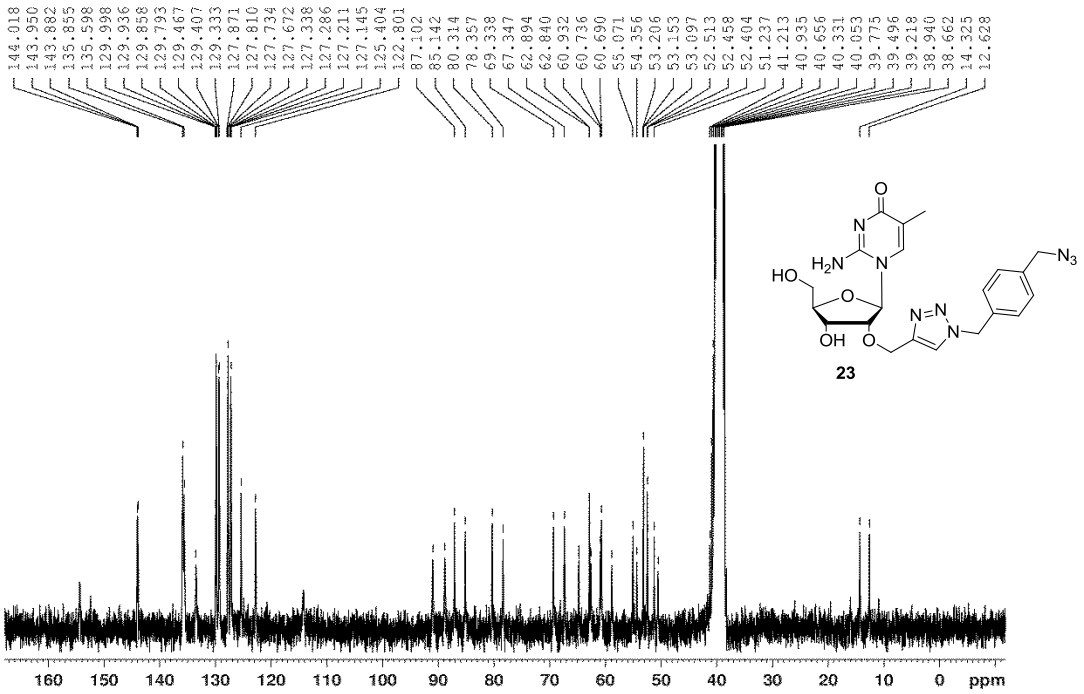


Fig. S78 ^{1}H-^{13}C gated-decoupled spectrum of compound **23**.

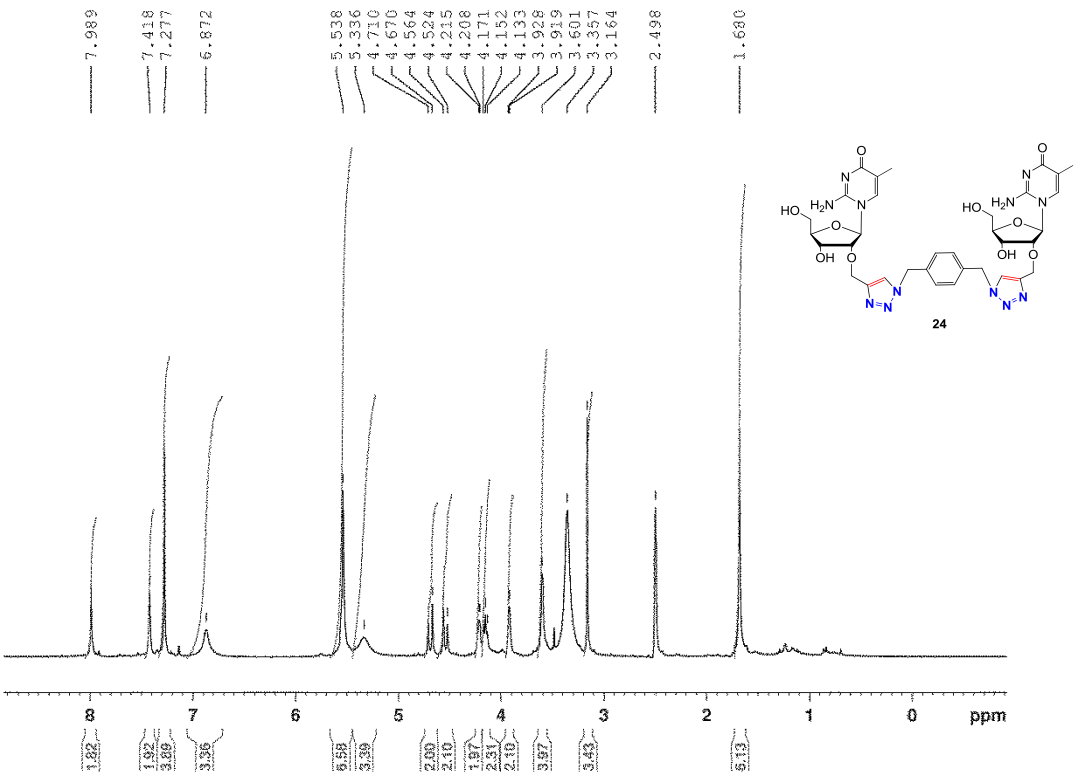


Fig. S79 ^{1}H-NMR spectrum of compound **24**.

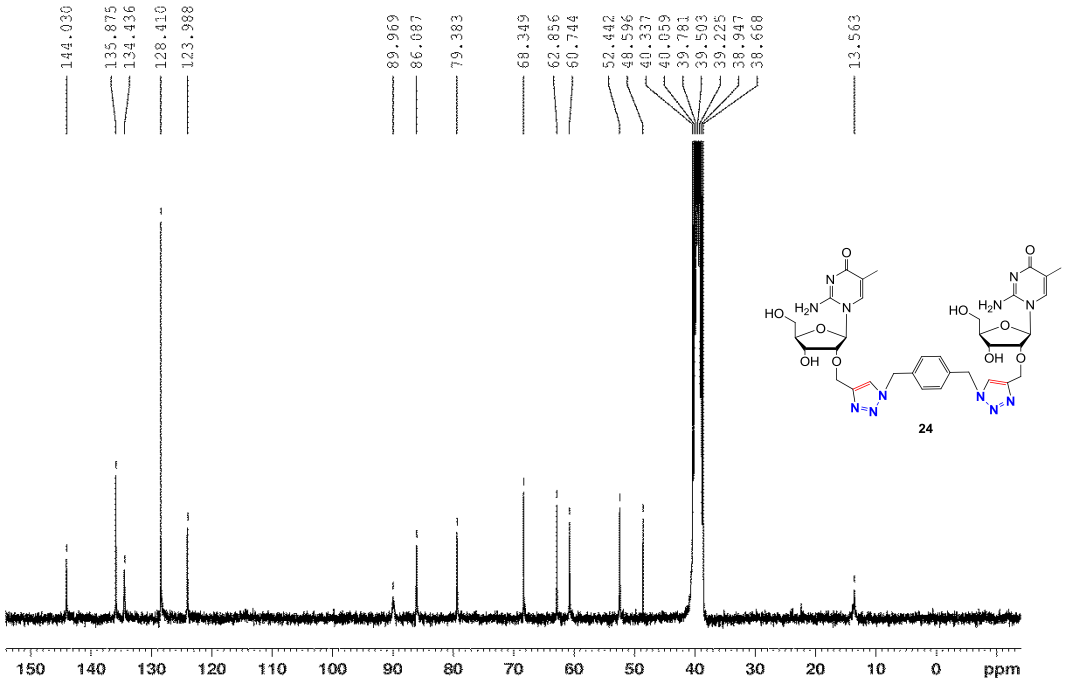


Fig. S80 ^{13}C-NMR spectrum of compound **27.**

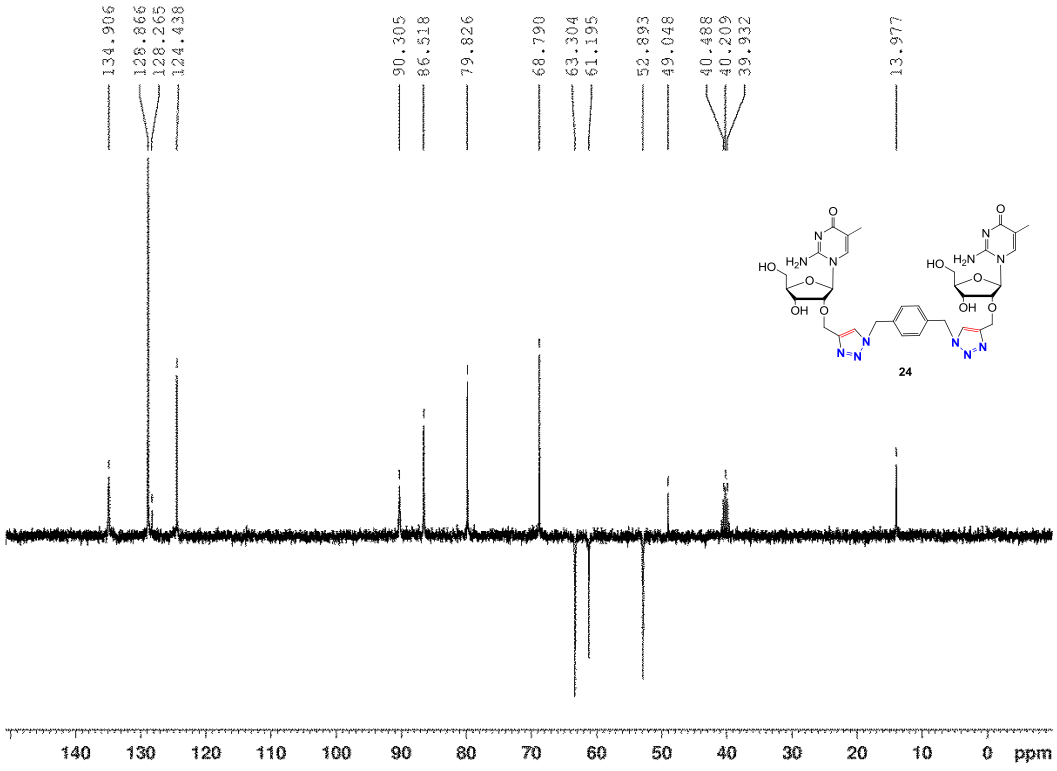


Fig. S81 DEPT-135 NMR spectrum of compound **24.**

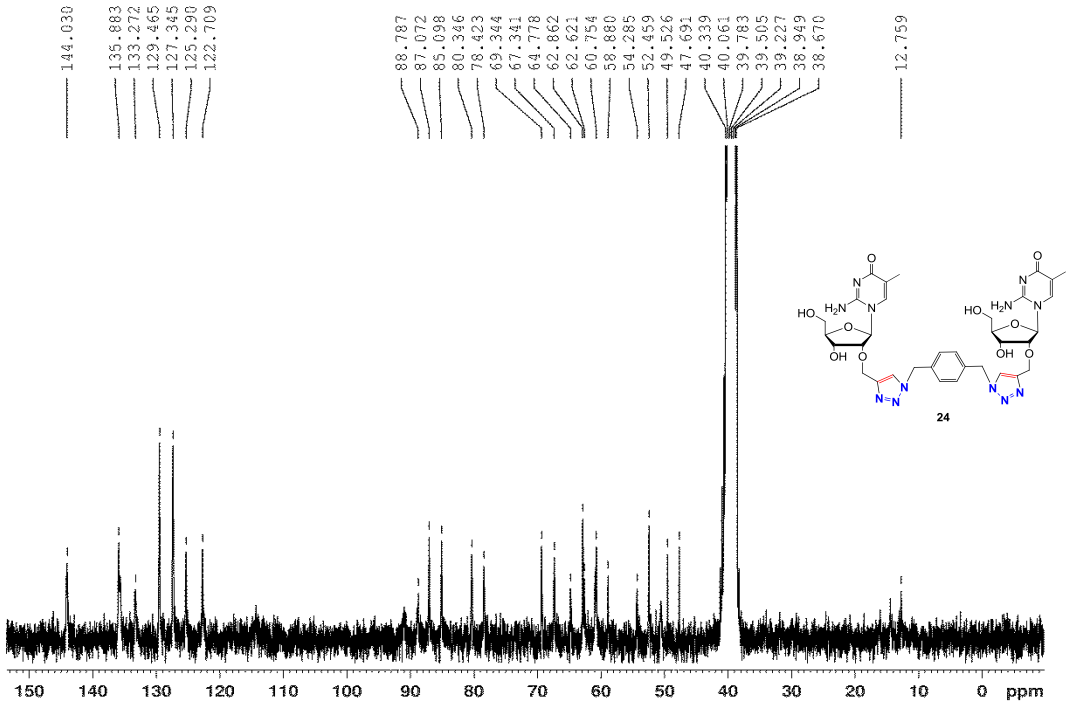


Fig. S82 ^{1}H-^{13}C gated-decoupled spectrum of compound **24**.