Supporting Information

Synthesis, Gene Silencing and Molecular Modeling Studies of 4'-*C*-Aminomethyl-2'-*O*-Methyl Modified Small Interfering RNAs

Kiran R. Gore,[†] Ganesh N. Nawale,[†] S. Harikrishna,[†] Vinita G. Chittoor,[†] Sushil Kumar Pandey,[†] Claudia Höbartner,[§] Swati Patankar,[‡] P. I. Pradeepkumar[†]*

[†]Department of Chemistry, Indian Institute of Technology Bombay, Mumbai 400076, India

[§]Max Planck Institute for Biophysical Chemistry, Research Group Nucleic Acid Chemistry, Am Fassberg 11, 37077 Göttingen, Germany

[‡]Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

* To whom correspondence should be addressed. E-mail: pradeep@chem.iitb.ac.in

TABLE OF CONTENTS

UV thermal melting curves of siRNAs

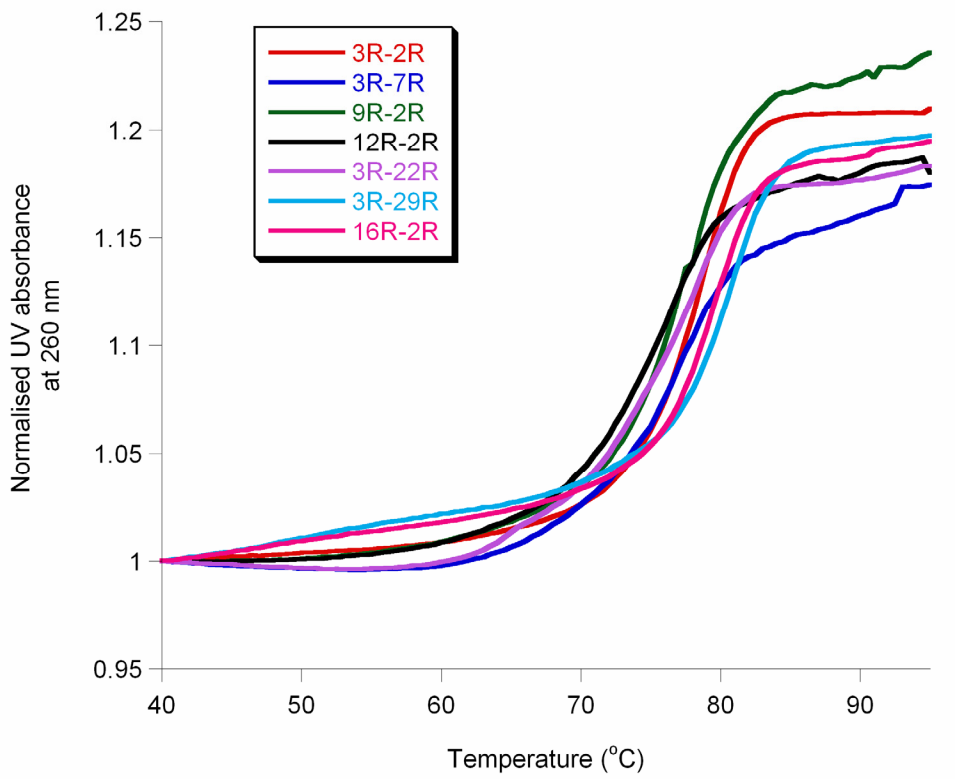
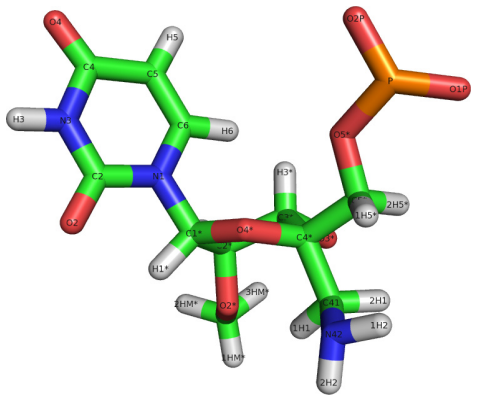


Figure S1. UV monitored thermal dissociation of unmodified and modified siRNA duplexes (1 μM) were obtained using buffer (100 mM NaCl, 10 mM Na_2PO_4, 0.1 mM EDTA, pH 7.4). Denaturation curves were obtained at 260 nm at a rate 0.5 ^{o}C/min. The T_m values reported are average of three measurements and summarised in Table 1 (main text).

Energy optimized structure of 4'-*C*-aminomethyl-2'-*O*-methyl uridine

Improper Dihedrals
C2 C6 N1 C1'; N1 N3 C2 O2
C2 C4 N3 H3; C5 N3 C4 O4
C4 C6 C5 H5; C5 H6 C6 N1
Dihedral angle
N*-NA-C -O
C -C -NA-H
CM-NA-C -O
C -CM-CM-HA
CM-H4-CM-N*
Angle
OS-CT-NT 70.165 112.025



Atom No.	Atom Type	Charge	Atom No.	Atom Type	Charge
P	P	1.087800	HM*2	H1	0.085100
O1P	O2	−0.766700	HM*3	H1	0.085100
O2P	O2	−0.766700	N42	NT	−1.032670
O5*	OS	−0.472500	H21	H	0.377970
C5*	CT	0.128900	H22	H	0.377970
C4*	CT	0.153200	C41	CT	0.186230
O4*	OS	−0.597900	H11	H1	0.057500
C3*	CT	0.069500	H12	H1	0.057500
O3*	OS	−0.588800	N1	N*	0.111000
C2*	CT	0.041500	C2	C	0.453900
O2*	OS	−0.327700	O2	O	−0.540700
C1*	CT	0.468600	N3	NA	−0.368100
H5*1	H1	0.084600	C4	C	0.602200
H5*2	H1	0.084600	O4	O	−0.565200
H3*	H1	0.146000	C5	CM	−0.313500
H2*	H1	0.090412	C6	CM	−0.232000
H1*	H1	0.041700	H5	HA	0.169700
CM2*	CT	−0.038000	H6	H4	0.255700
HM*1	H1	0.085100	N3H	H	0.308700

Figure S2. Energy optimized geometry and calculated RESP charges for 4'-*C*-aminomethyl-2'-*O*-methyl uridine using HF/6-31G* basis set in Gaussian03 program. New force field parameters are obtained using protocol developed by Aduri and co-workers (*J. Chem. Theory Comput.* **2007**, *3*, 1464–1475)

Final MD snapshot of siRNA duplexes after 20 ns

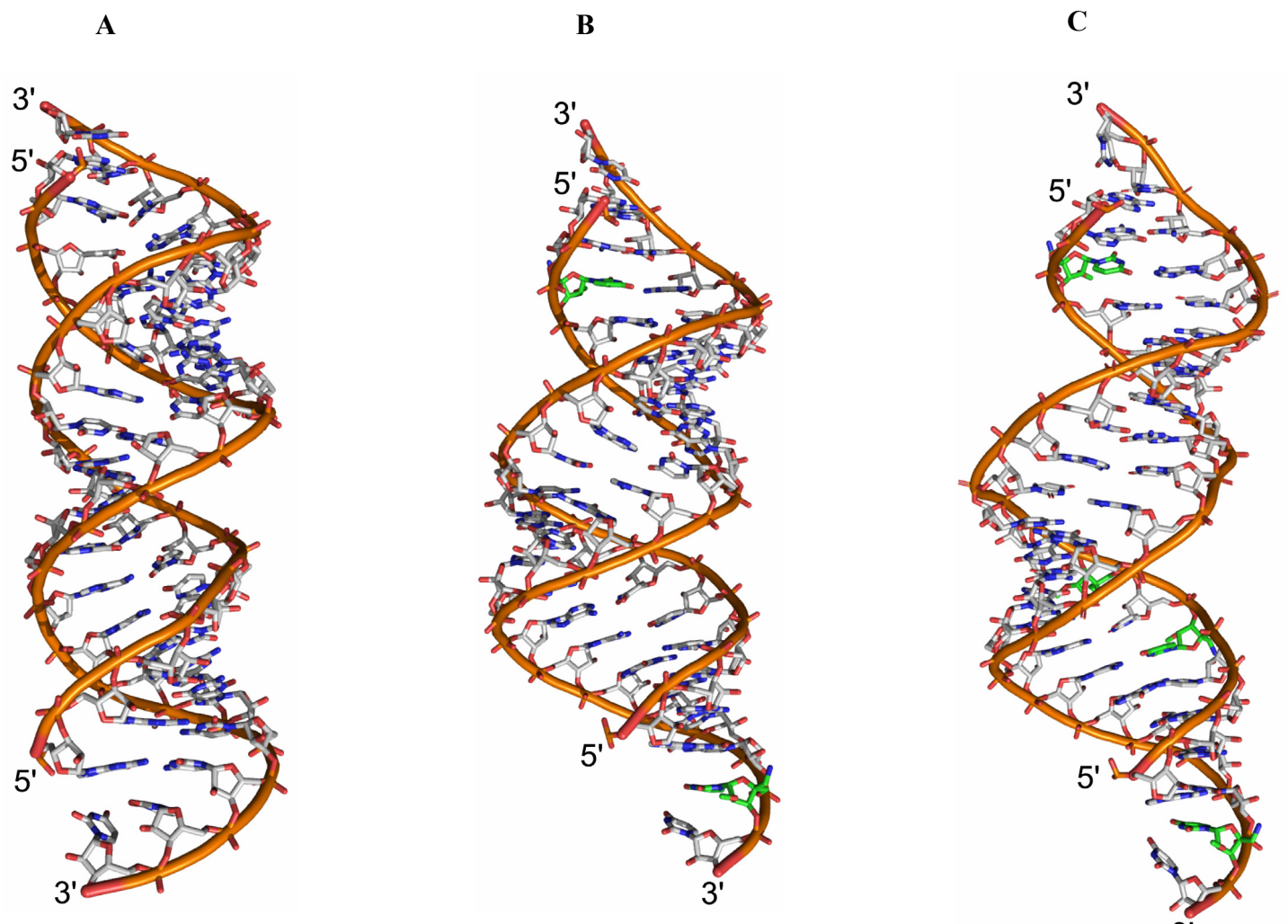
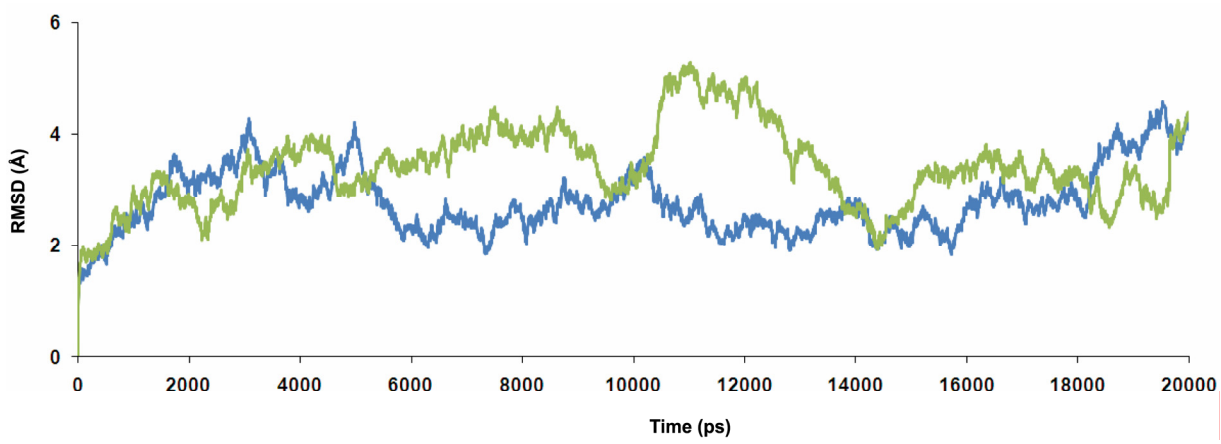


Figure S3. Final MD snapshot of modified and unmodified siRNA duplexes captured after 20 ns. (A) Unmodified siRNA duplex **3R-2R**. (B) Modified siRNA duplex **3R-7R**. (C) Modified siRNA duplex **6R-7R**. Modified nucleotides are coloured in green.

Time-dependent rmsd graphs of modified and unmodified siRNAs

A



B

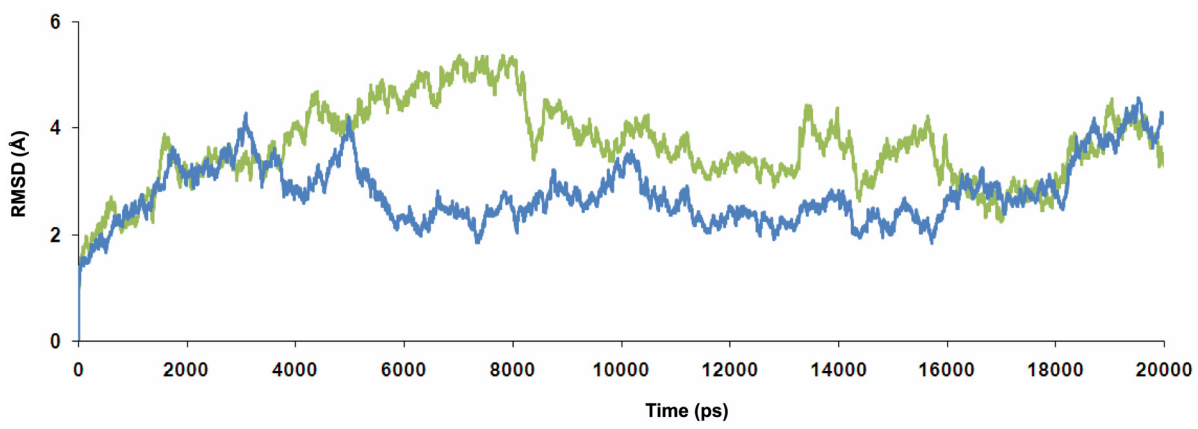
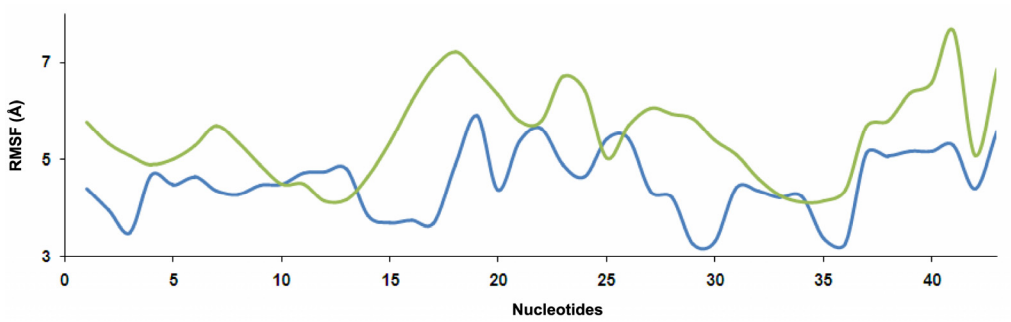


Figure S4. Time-dependent rmsd (Å) of the unmodified (blue) and modified RNA (green) heavy atoms over 20 ns of MD simulation. (A) Unmodified and modified siRNA duplexes **3R-2R**, and **3R-7R** respectively. (B) Unmodified and modified siRNA duplexes **3R-2R**, and **6R-7R** respectively. The average rmsd values for modified siRNA duplexes **3R-7R** and **6R-7R** was 3.0 Å (± 1.77) and 3.7 Å (± 1.91) respectively. In case of unmodified siRNA duplex **3R-2R**, the average rmsd was 2.5 Å (± 1.25).

Root mean square fluctuation (rmsf) of backbone heavy atoms of modified and unmodified siRNA

A



B

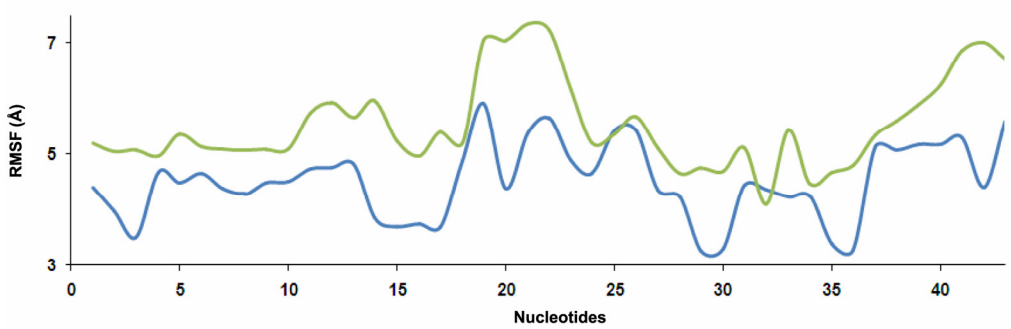
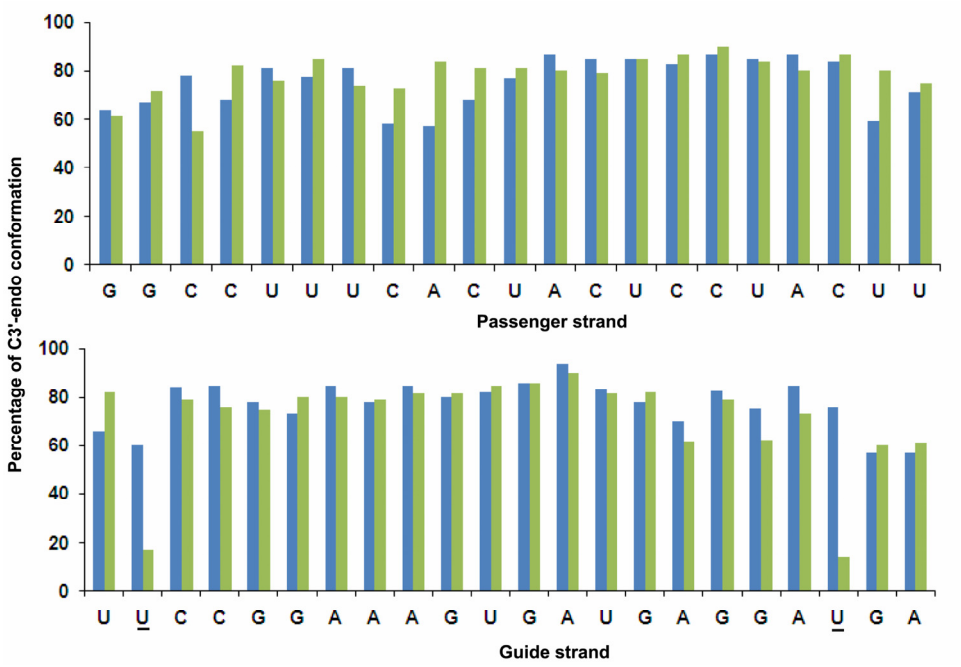


Figure S5. The root-mean-square fluctuations (rmsf) of backbone heavy atoms of unmodified (blue), and modified siRNA duplexes (green). (A) Modified siRNA duplex **3R-7R (**modified positions are 24 and 42**)**, and unmodified siRNA duplex **3R-2R**. (B) Modified siRNA duplex **6R-7R (**modified positions are 20, 22, 24 and 42**),** and unmodified siRNA duplex **3R-2R**.

Percentage of C3'-endo sugar puckering calculated from 20 ns MD

A



B

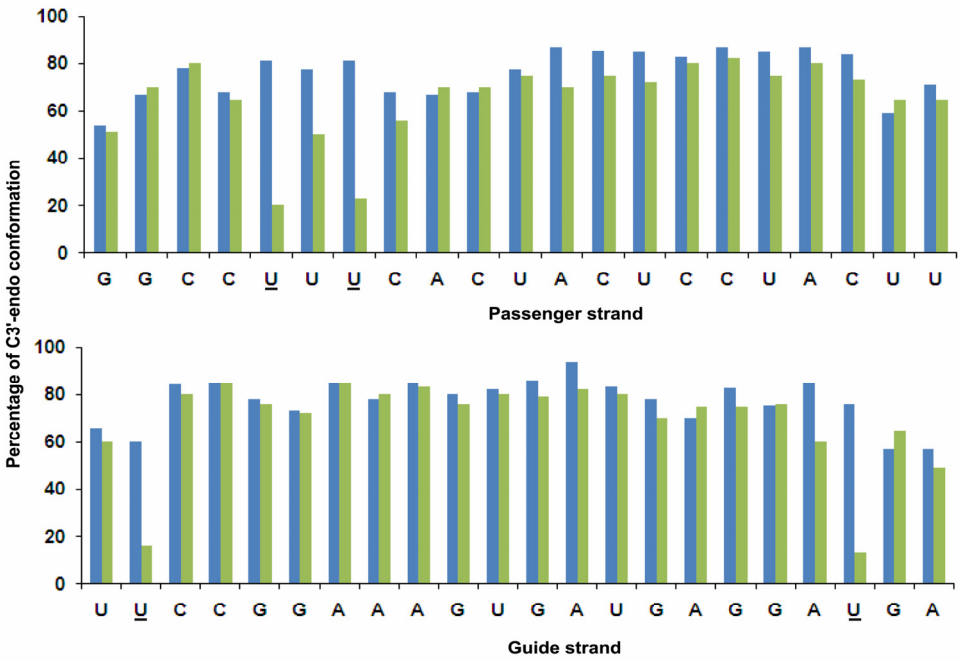
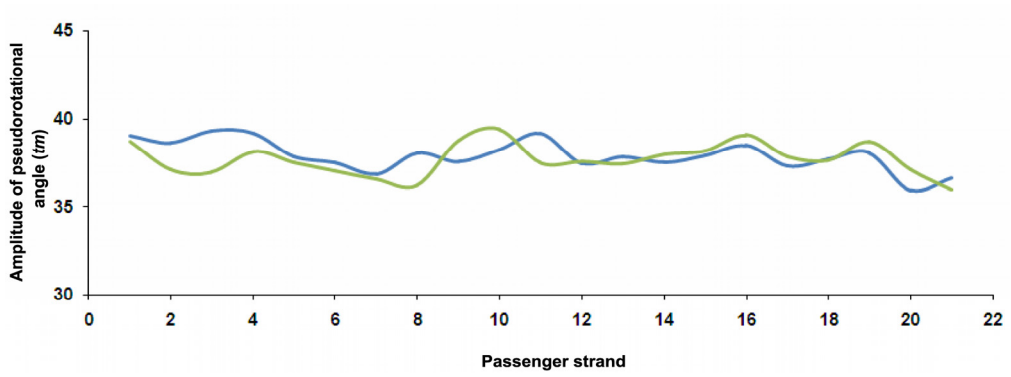


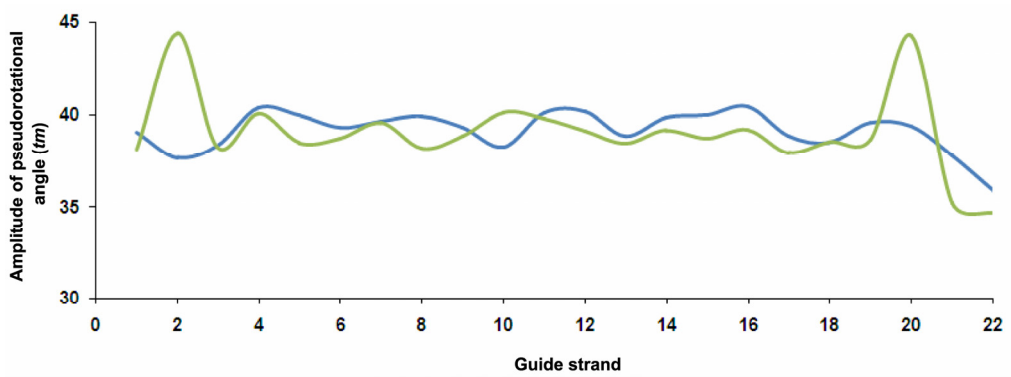
Figure S6. Percentage of C3'-endo sugar puckering of nucleotides in unmodified (blue) and modified (green) siRNA duplexes from 20 ns of MD simulations. (A) Sugar puckering of modified siRNA duplex **3R-7R,** and unmodified siRNA duplex **3R-2R**. (B) Sugar puckering of siRNA duplex **6R-7R**, and unmodified siRNA duplex **3R-2R**. Modified nucleotide positions are underlined.

Amplitude of pseudorotational angle (*tm*)

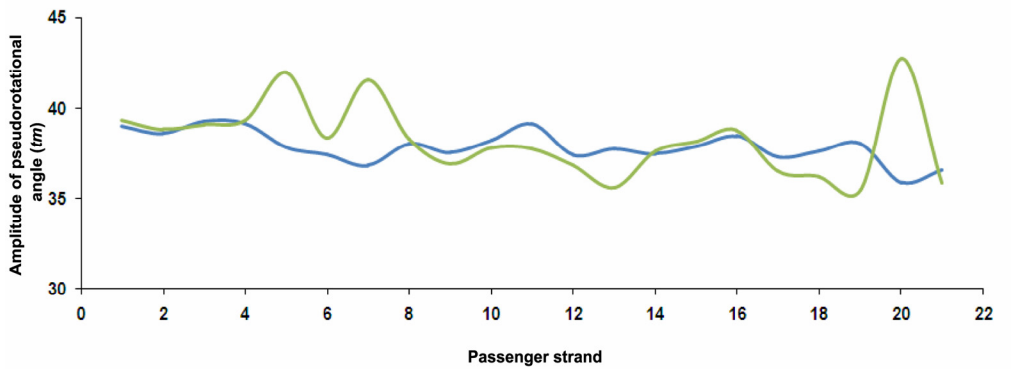
A



B



C



D

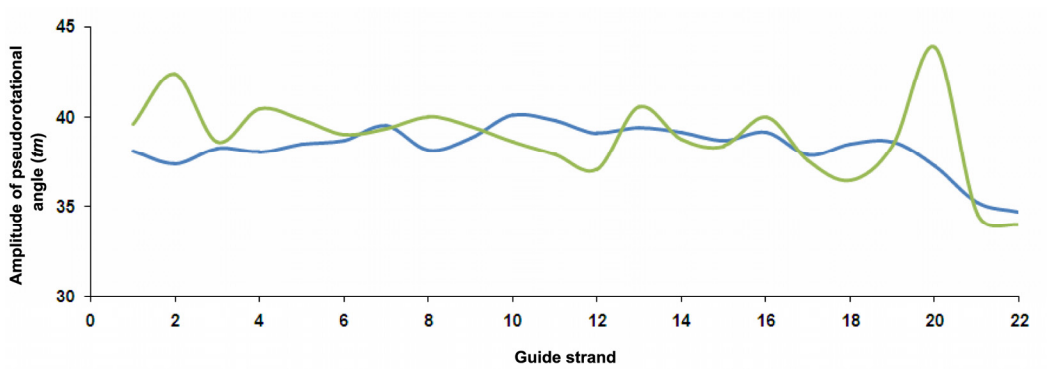
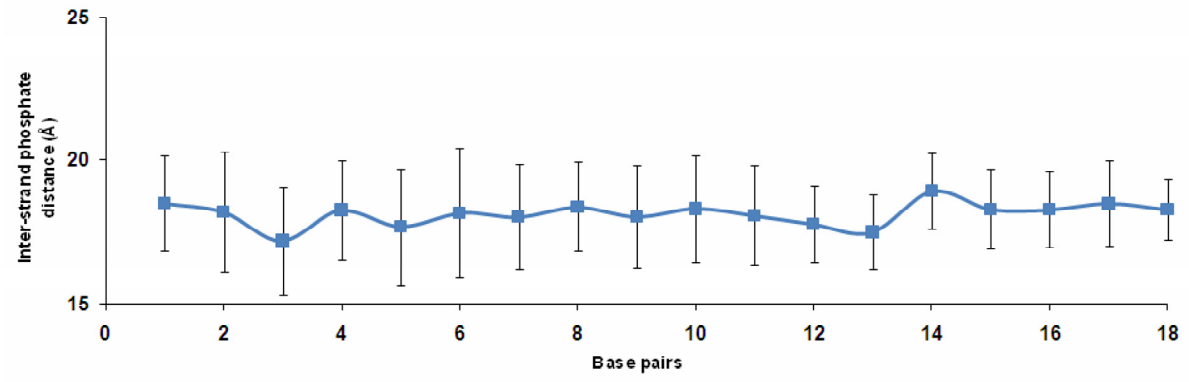


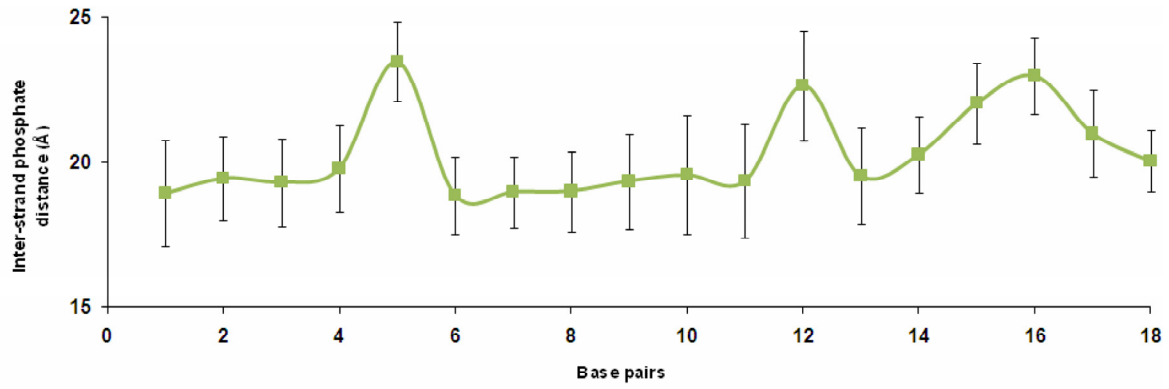
Figure S7. Amplitude of pseudo rotational angle (*tm*) of unmodified (blue) and modified (green) siRNA duplexes. (A) Passenger strand of modified siRNA duplex **3R-7R,** and unmodified siRNA duplex **3R-2R**. (B) Guide strand of modified siRNA duplex **3R-7R** (modified positions are 2 and 20), and unmodified siRNA duplex **3R-2R**. (C) Passenger strand of modified siRNA duplex **6R-7R** (modified positions are 5 and 7), and unmodified siRNA duplex **3R-2R**. (D) Guide strand of modified siRNA duplex **6R-7R** (modified positions are 2 and 20), and unmodified siRNA duplex **3R-2R**.

Interstrand phosphate distances calculated from 20 ns MD trajectory

A



B



C

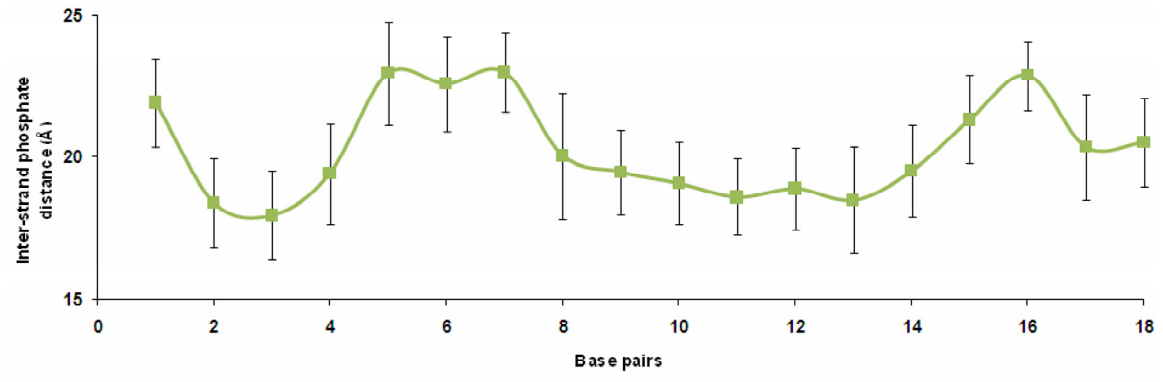
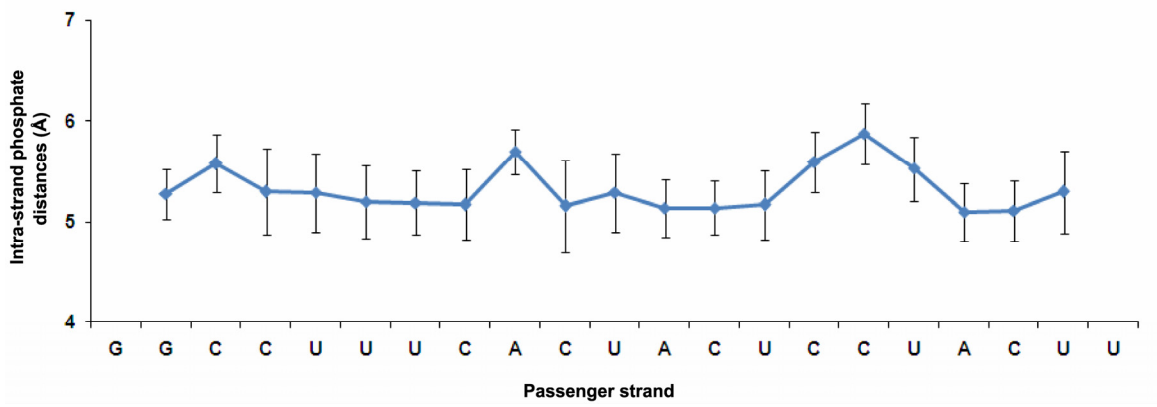


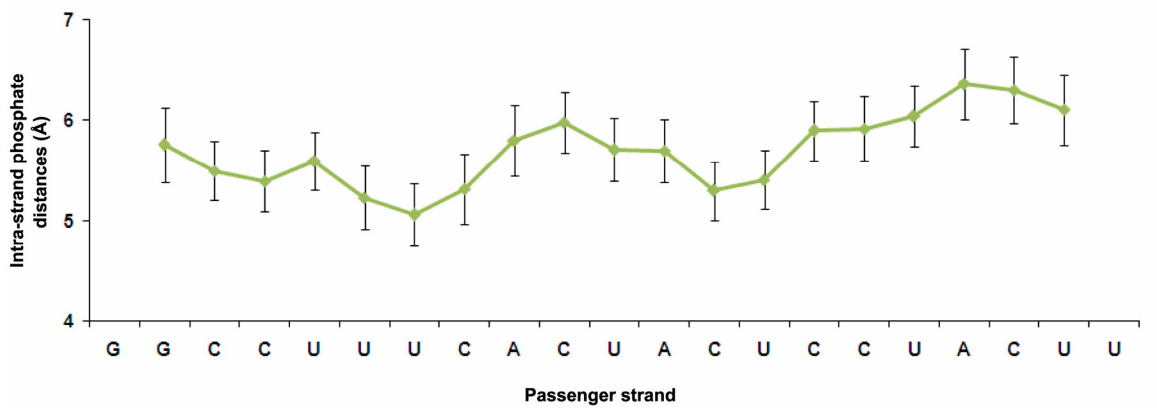
Figure S8. Interstrand phosphate distances with standard deviation (Å) of unmodified (blue) and modified (green) siRNA duplexes from 20 ns of MD simulation. (A) Unmodified siRNA duplex **3R-2R**. (B) Modified siRNA duplex **3R-7R** (modified position is 16). (C) Modified siRNA duplex **6R-7R** (modified positions are 5, 7 and 16).

Intrastrand phosphate distances calculated from 20 ns MD trajectory (passenger strand)

A



B



C

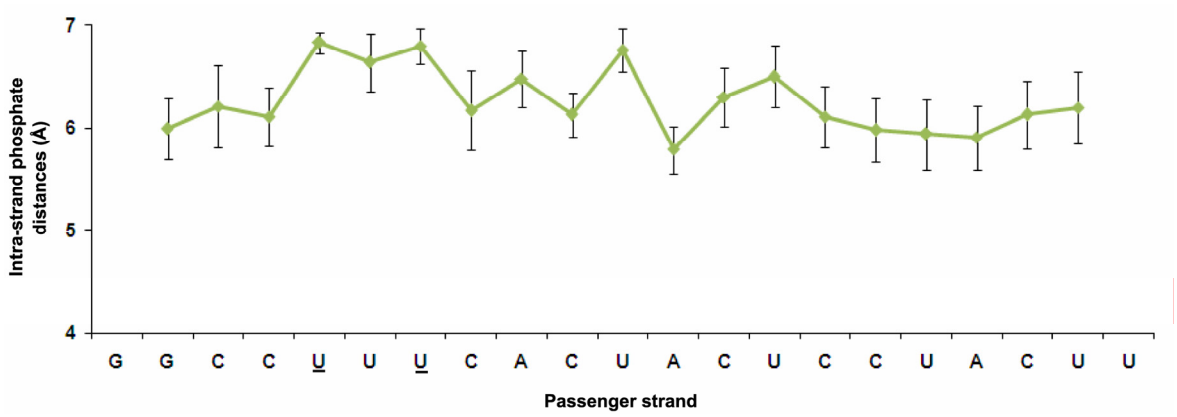
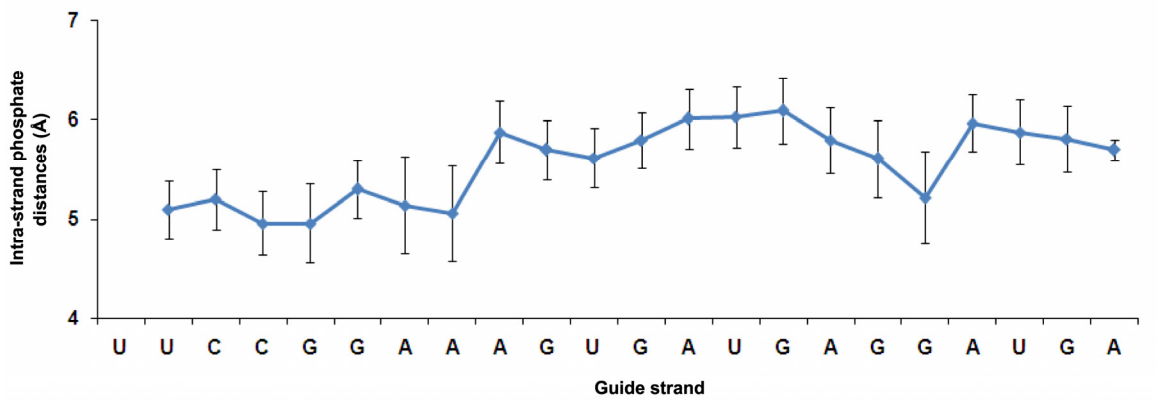


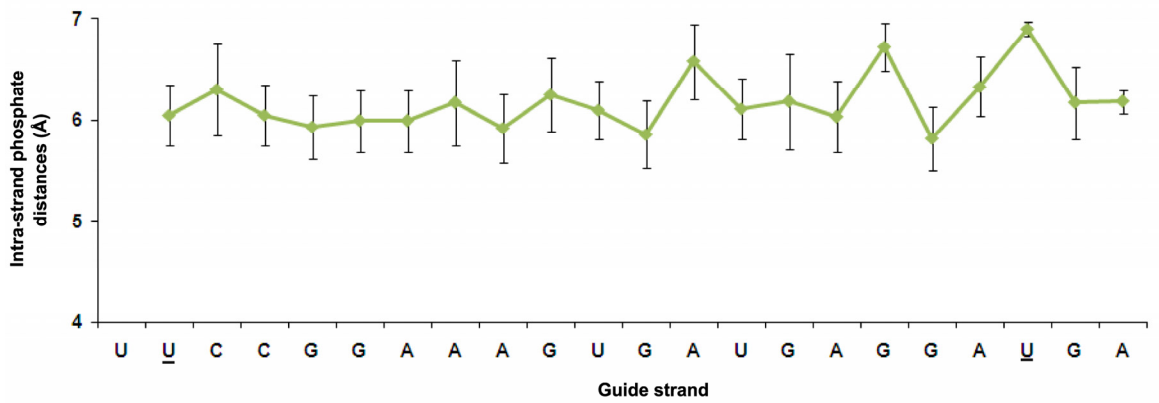
Figure S9. Average intrastrand phosphate distances with standard deviation (Å) of unmodified (blue) and modified (green) siRNA duplex from 20 ns of MD simulation. (A) Passenger strand of unmodified siRNA duplex **3R-2R**. (B) Passenger strand of modified siRNA duplex **3R-7R**. (C) Passenger strand of modified siRNA duplex **6R-7R**. Modified nucleotide positions are underlined.

Intrastrand phosphate distances calculated from 20 ns MD trajectory (guide strand)

A



B



C

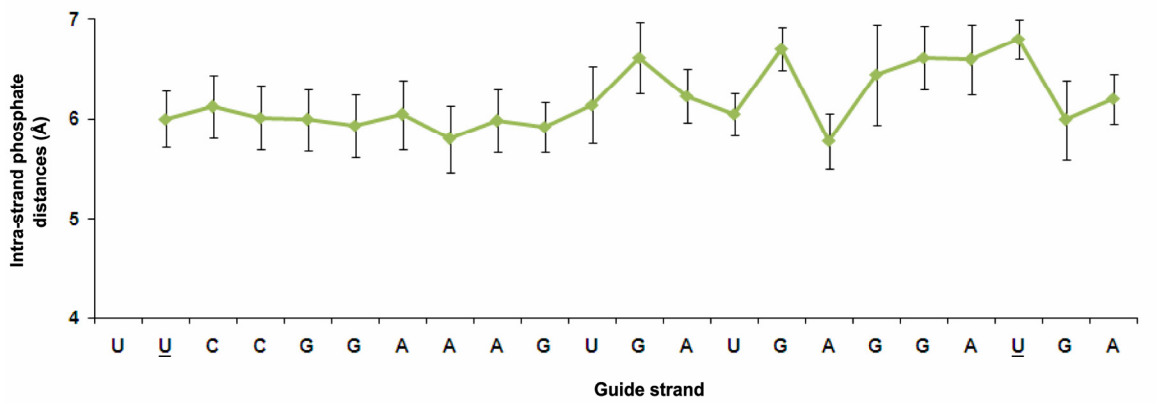


Figure S10. Average intrastrand phosphate distances with standard deviation (Å) of unmodified (blue) and modified (green) siRNA duplex from 20 ns of MD simulation. (A) Guide strand of unmodified siRNA duplex **3R-2R**. (B) Guide strand of modified siRNA duplex **3R-7R**. (C) Guide strand of modified siRNA duplex **6R-7R**. Modified nucleotide positions are underlined.

Snapshots of unmodified and modified siRNA nucleotides of the passenger strand

A **B**

Figure S11. Positions in the passenger strand of unmodified and modified (position 5 and 7) siRNAs captured after 20 ns of MD simulations. (A) Unmodified siRNA duplex **3R-2R** shows the proper intrastrand phosphate distances (5.8 Å) and stacking interaction. (B) Modifications (green) in the siRNA duplex **6R-7R** shows the disturbances in stacking interactions, and deviations in the intrastrand phosphate distances (6.9 Å). Black dotted line shown in the base represents the distance between two successive nucleotides. Modifications are shown in green color.

Water density in the minor groves of siRNA duplexes at different periods of simulation

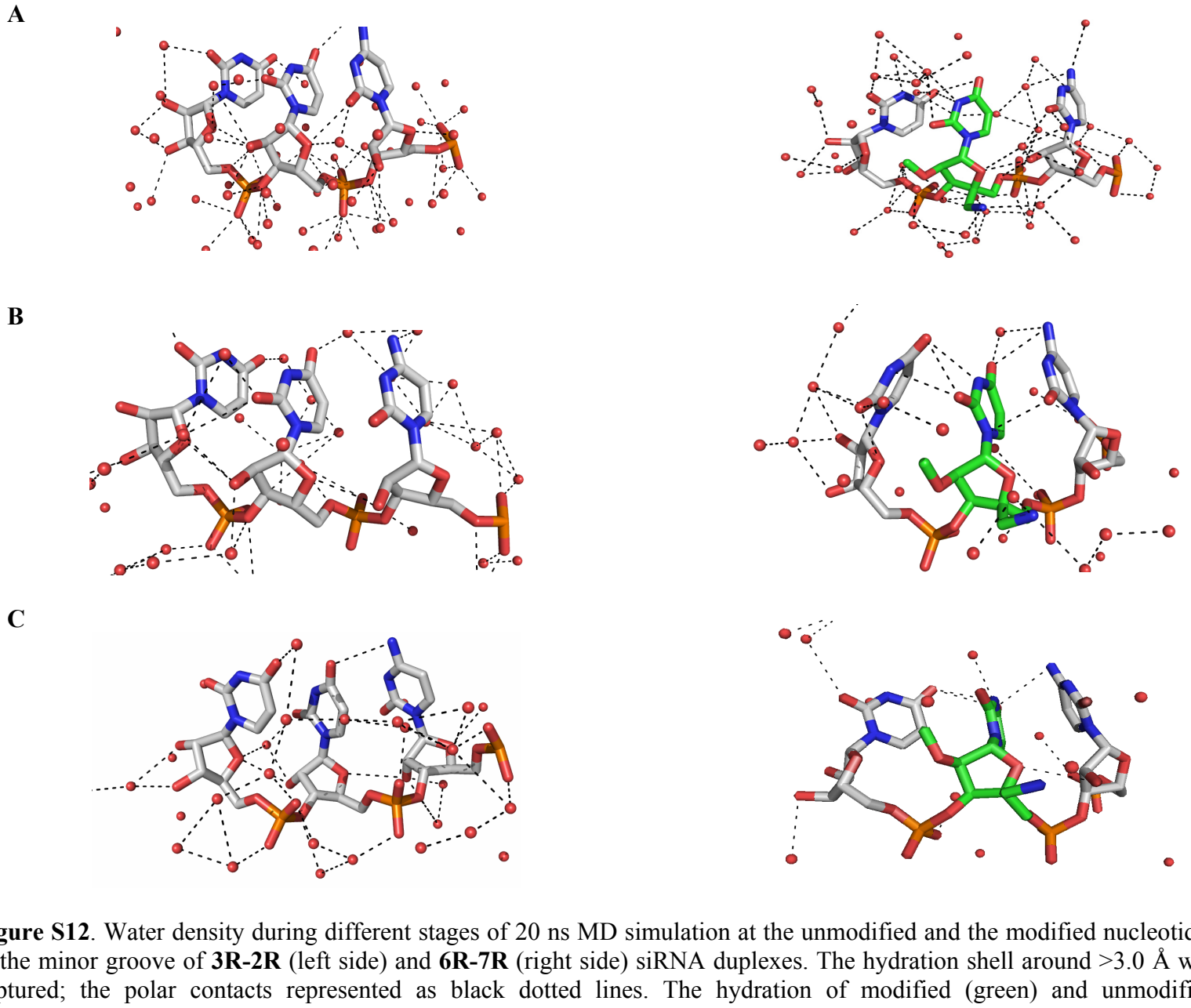


Figure S12. Water density during different stages of 20 ns MD simulation at the unmodified and the modified nucleotides in the minor groove of **3R-2R** (left side) and **6R-7R** (right side) siRNA duplexes. The hydration shell around >3.0 Å was captured; the polar contacts represented as black dotted lines. The hydration of modified (green) and unmodified nucleotides with one base on their either side is shown. (A) At 1 ns. (B) At 10 ns. (C) At 18 ns. Hydration shells were calculated using ptraj module in AMBER10.

Frequency distribution of χ angle

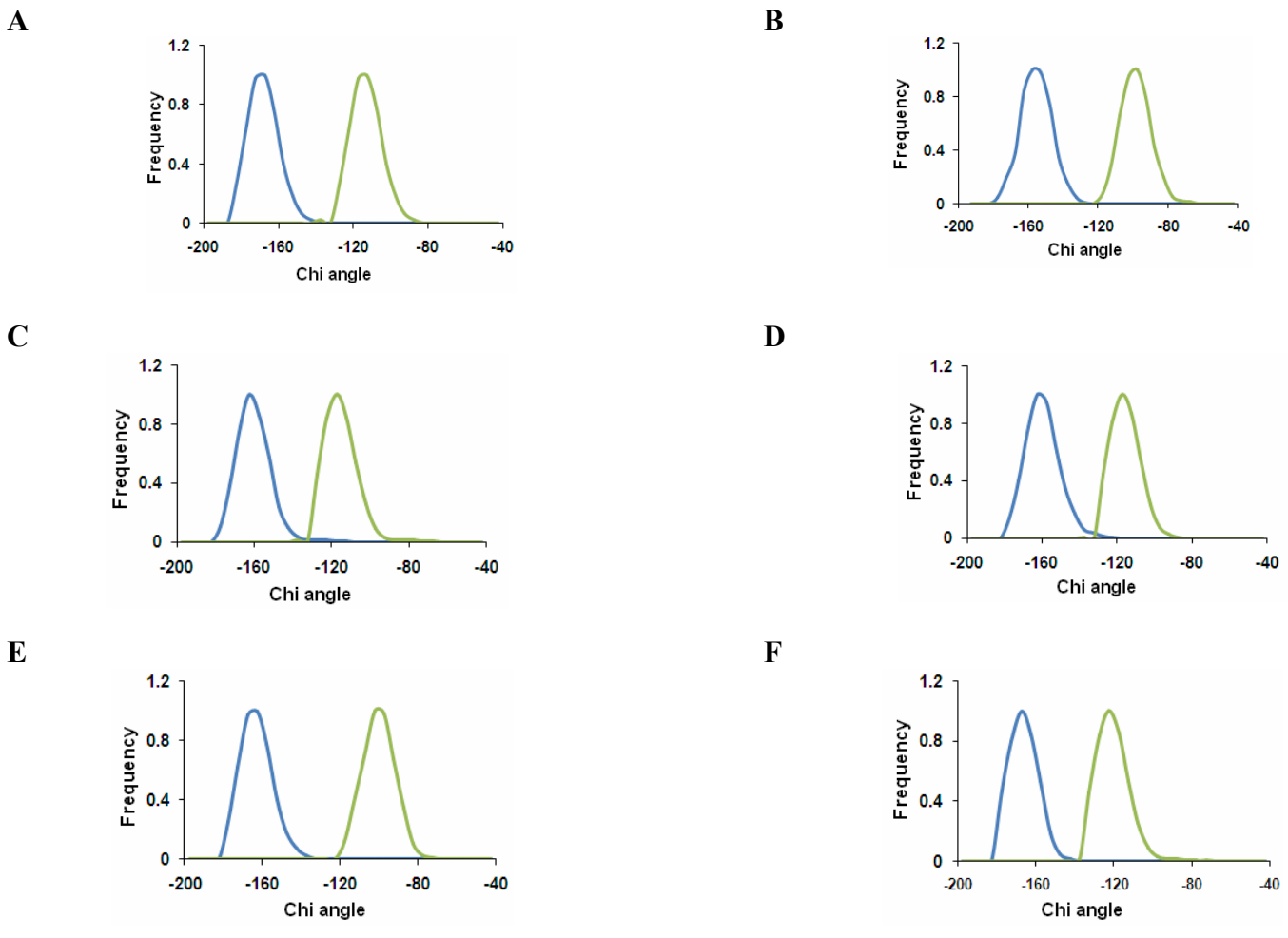


Figure S13. Frequency distribution of χ angle at the junction of sugar and base calculated over 20 ns MD simulation of unmodified nucleotide (blue) and modified nucleotides (green). (A) Modified nucleotide in the seed region of guide strand in the siRNA duplex **3R-7R**. (B) Modified nucleotide in the 3'-overhang of guide strand in the siRNA duplex **3R-7R**. (C) Modified nucleotide in the seed region of guide strand in the siRNA duplex **6R-7R**. (D) Modified nucleotide in the 3'-overhang of guide strand in the siRNA duplex **6R-7R**. (E) and (F) Two modified nucleotides in the 5'-end of the passenger strand of siRNA duplex **6R-7R**.

Representation of hydrogen bond, inter and intrastrand stacking of a general hexanucleotide system

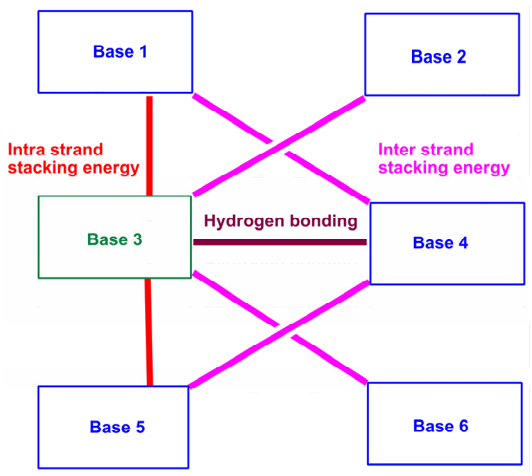


Figure S14. Representation of hydrogen bond, inter and intrastrand stacking of a general hexanucleotide system. Hydrogen bonding and stacking energies were calculated for the modified (green) and unmodified (blue) nucleotides containing siRNA duplexes. Bases 1, 3 and 5 are in one strand; Bases 2, 4 and 6 are in the other strand. Hydrogen bonding energy was calculated between the modified (Base 3) and unmodified (Base 4) nucleotide. Intrastrand stacking energy was calculated in between the trinucleotides of one strand (Bases 1, 3 and 5) in which the modified nucleotides are positioned in the middle. Interstrand stacking energy was calculated between the hexanucleotides (Bases 1-6) in which three nucleotides contributes from one strand along with their three complimentary bases in the other strand.

Average of positional rmsd (Å) at the modified regions

SiRNA strand and modification site	Unmodified	Modified	
	3R-2R	**3R-7R**	**6R-7R**
Guide strand (seed region)	1.99 ± 0.89	2.3 ± 1.42	3.5 ± 1.20
Guide strand (overhang)	2.7 ± 1.78	4.0 ± 0.95	5.1 ± 0.85
Passenger strand (position 1)	2.24 ± 1.66	-	3.7 ± 1.27
Passenger strand (position 2)	2.09 ± 1.04	-	4.4 ± 1.32

Table S1. The rmsd values were calculated for modified regions flanked by one nucleotide either side along with their complimentary bases. Positional rmsd was calculated over 20 ns MD simulation using ptraj module in AMBER10.

Hydration numbers in the grooves and the backbone regions of siRNA duplexes

Duplex	Major Groove	Minor Groove	Backbone (passenger strand)	Backbone (guide strand)
3R - 2R	19.33 ± 0.21	16.77 ± 0.34	40.59 ± 0.51	43.35 ± 0.56
3R - 7R	16.56 ± 0.12	10.75 ± 0.39	37.26 ± 0.55	39.65 ± 0.54
6R - 7R	16.20 ± 0.27	9.970 ± 0.25	35.30 ± 1.08	40.12 ± 0.65

Table S2. The hydration numbers were calculated based on number of water molecules occupied in each region over 20 ns MD simulation. The hydration numbers were calculated using ptraj module in AMBER 10.

Six local base pair parameters of unmodified siRNA duplex 3R-2R

Base pair	Shear	Stretch	Stagger	Buckle	Propeller	Open angle
G-C	−0.93	−0.18	−0.28	1.85	−21.32	8.45
G-C	−0.23	−0.03	−0.47	−17.51	−16.48	−0.61
C-G	0.14	−0.14	−0.23	−0.57	−12.65	−0.62
C-G	0.55	−0.27	0.18	4.73	−12.34	−1.37
U-A	−0.46	0.02	−0.23	2.49	−18.63	−2.30
U-A	−0.04	−0.02	0.32	−5.17	−2.650	−3.04
U-A	0.12	0.09	−0.23	11.46	−17.08	10.4
C-G	0.04	−0.14	−0.11	16.93	−22.29	−2.79
A-U	0.21	0.18	0.48	21.36	−21.50	−1.72
C-G	0.49	−0.09	−0.39	17.16	−24.93	0.51
U-A	−0.7	0.02	−0.91	9.13	−29.27	−4.41
A-U	0.12	−0.01	0.34	2.13	−24.35	0.17
C-G	−0.02	−0.24	0.5	3.26	−29.14	−2.46
U-A	−0.59	−0.14	0.65	13.55	−12.56	−6.22
C-G	0.1	−0.09	−0.61	25.62	−33.14	2.04
C-G	0.09	−0.15	−0.22	0.79	−14.60	3.00
U-A	−0.13	−0.24	0.84	−5.31	−7.910	2.02
A-U	−0.37	−0.2	−0.56	5.14	−16.35	−2.18
C-G	0.19	0.01	−0.03	13.17	−17.07	−2.03
U-A	0.47	0.19	0.69	45.31	7.26	17.51

Table S3. Six local base pair parameters for each nucleotide base pair were calculated from the averaged structure obtained from 20 ns MD simulation of unmodified siRNA duplex **3R-2R**. All the base pair parameter for each step was calculated using X3DNA program. All the values are reported in degrees.

Six local base pair parameters of modified siRNA duplex 3R - 7R

Base pair	Shear	Stretch	Stagger	Buckle	Propeller	Open angle
G-C	0.13	−0.04	−0.51	−19.65	17.85	−4.80
G-C	0.00	−0.11	0.44	10.46	1.92	−3.15
C-G	0.24	−0.02	−0.52	13.42	−15.45	4.17
C-G	−0.19	−0.12	−0.44	−10.98	−6.76	1.44
U-A	−0.15	0.06	0.3	−4.62	6.24	−7.13
U-A	−0.2	−0.02	−0.38	19.98	−7.23	3.69
U-A	0.09	−0.03	−0.77	20.92	−32.54	0.16
C-G	0.06	0.02	−0.27	16.64	−18.46	4.33
A-U	0.19	−0.1	0.18	3.98	−26.4	−7.67
C-G	0.31	−0.41	0.84	−3.21	−10.19	−5.50
U-A	0.10	−0.09	0.48	−1.08	−21.93	6.25
A-U	0.59	0.19	−0.63	−20.74	−19.65	−2.94
C-G	0.21	−0.09	0.11	5.32	−12.9	−3.45
U-A	0.22	−0.15	0.38	3.02	−14.49	4.94
C-G	0.18	−0.13	−0.45	5.35	−14.54	−0.34
C-G	0.33	0.04	−0.34	9.41	−17.54	6.11
U-A	−0.24	−0.07	0.13	6.78	−9.69	0.72
A-**U**	**0.31**	**0.89**	**−0.06**	**7.36**	**−14.45**	**−5.04**
C-G	0.5	0.14	−0.55	26.77	−19.5	4.19
U-A	0.05	0.15	−0.41	5.34	−1.53	3.95

Table S4. Six local base pair parameters for each nucleotide base pair were calculated from the averaged structure obtained from 20 ns MD simulation of modified siRNA duplex **3R-7R**. All the base pair parameters for each step were calculated using X3DNA program. Modified nucleotide position is highlighted by green underlined, and their corresponding values are highlighted in green colour. All the values are reported in degrees.

Six local base pair parameters of modified siRNA duplex 6R-7R

Base pair	Shear	Stretch	Stagger	Buckle	Propeller	Open angle
G-C	−0.33	0.01	−0.23	−19.39	−8.14	1.69
G-C	−0.34	−0.11	0.05	−6.69	−11.53	−1.66
C-G	0.03	−0.04	0.18	3.59	−14.47	−1.55
C-G	0.09	−0.15	0.01	−8.06	−23.62	−0.91
U-A	**0.11**	**−0.11**	**−0.09**	**−6.69**	**−22.83**	**−4.26**
U-A	0.39	−0.11	0.27	1.69	−0.10	0.87
U-A	**0.07**	**−0.01**	**−0.25**	**17.59**	**−17.21**	**−1.78**
C-G	0.12	−0.28	0.71	−4.58	−10.06	−2.61
A-U	0.08	−0.05	0.04	−0.94	−19.57	−1.87
C-G	−0.21	0.23	−0.12	2.82	−30.47	7.27
U-A	−0.13	0.13	−0.13	10.3	−2.32	−0.02
A-U	0.07	−0.05	0.16	17.89	−14.48	1.80
C-G	0.21	0.01	−0.56	6.90	−32.64	12.28
U-A	0.34	−0.11	0.41	−4.28	−14.82	−0.81
C-G	0.62	0.05	−0.43	4.51	−19.17	7.62
C-G	0.28	−0.26	0.10	1.63	−22.47	−0.62
U-A	−1.32	0.11	−0.46	9.17	−4.32	−4.60
A-**U**	**0.67**	**1.41**	**−0.58**	**10.89**	**−11.61**	**-2.29**
C-G	0.26	−0.06	−0.20	10.53	−26.04	−4.61
U-A	0.37	−0.01	0.63	−15.69	−29.21	−8.29

Table S5. Six local base pair parameters for each nucleotide base pair were calculated from the averaged structure obtained from 20 ns MD simulation of modified siRNA duplex **6R-7R**. All the base pair parameters for each step were calculated using X3DNA program. Modified nucleotide positions are highlighted by green underlined, and their corresponding values are highlighted in green colour. All the values are reported in degrees.

Shift, slide and rise parameters of each dinucleotide step

Base pair step	Shift			Slide			Rise		
	3R-2R	3R-7R	6R-7R	3R-2R	3R-7R	6R-7R	3R-2R	3R-7R	6R-7R
GG/CC	0.11	−0.08	−0.11	−1.40	−2.09	−2.04	3.47	3.20	3.28
GC/GC	0.18	−0.04	0.05	−1.83	−1.82	−1.88	3.07	3.20	3.16
CC/GG	0.13	0.06	0.00	−1.68	−1.93	−1.89	3.26	3.30	3.30
C**U**/AG	0.22	0.08	**−0.11**	−2.57	−1.83	**−1.73**	3.28	3.30	**3.38**
UU/AA	0.08	0.07	**0.88**	−2.33	−1.65	**−1.99**	2.88	3.25	**3.23**
U**U**/AA	0.36	0.06	**−0.29**	−2.14	−1.54	**−1.50**	2.95	3.30	**3.16**
UC/GA	−0.11	−0.02	**0.46**	−2.11	−1.65	**−1.71**	3.33	3.30	**3.35**
CA/UG	−0.19	0.03	0.33	−1.51	−1.71	−1.78	3.96	3.55	3.51
AC/GU	−0.36	−0.47	−0.13	−2.10	−1.85	−1.89	3.30	3.28	3.30
CU/AG	−0.14	0.02	0.22	−1.99	−1.69	−2.30	3.55	3.35	3.34
UA/UA	−0.28	−0.06	−0.18	−1.49	−1.45	−1.68	3.12	3.30	3.30
AC/GU	0.13	−0.14	0.44	−1.54	−1.55	−1.14	3.19	3.50	3.18
CU/AG	−0.14	0.09	−0.22	−1.58	−1.68	−2.27	3.35	3.67	3.42
UC/GA	−0.17	−0.10	0.23	−1.56	−1.61	−1.35	3.25	3.69	3.00
CC/GG	−0.11	0.04	−0.08	−1.87	−1.89	−1.84	3.48	3.50	3.55
CU/AG	0.01	0.03	−0.02	−1.69	−1.85	−1.87	3.38	3.70	3.37
UA/**U**A	−0.23	**−0.24**	**−0.56**	−1.45	**−1.67**	**−1.50**	3.31	**3.70**	**3.30**
AC/G**U**	−0.22	**1.13**	**0.20**	−1.55	**−2.19**	**−1.34**	3.25	**4.11**	**3.27**
CU/AG	−0.14	0.11	−0.33	−1.58	−1.78	−1.53	3.45	3.60	3.34

Table S6. Shift, slide and rise parameters for each di-nucleotide step were calculated using X3DNA program from the averaged structure obtained from 20 ns MD simulation of unmodified siRNA duplex **3R-2R** and modified siRNA duplexes **3R-7R** and **6R-7R**. Modified nucleotide positions are highlighted by green underlined, and their corresponding values are highlighted in green colour. All the values are reported in degrees.

Tilt, roll and twist parameters of each dinucleotide step

Base pair step	Tilt			Roll			Twist		
	3R-2R	3R-7R	6R-7R	3R-2R	3R-7R	6R-7R	3R-2R	3R-7R	6R-7R
GG/CC	1.31	−0.32	−0.21	4.50	5.70	6.16	23.50	27.36	29.97
GC/GC	−0.21	−0.08	0.22	2.05	2.49	2.30	28.27	30.75	29.05
CC/GG	1.33	1.19	0.86	7.73	8.46	8.01	34.68	29.69	30.05
C**U**/AG	1.53	0.87	**1.57**	11.01	9.21	**10.94**	27.45	28.09	**29.52**
UU/AA	1.57	1.39	**0.28**	12.32	8.08	**10.79**	27.79	27.87	**23.17**
U**U**/AA	−0.23	−0.58	**2.72**	7.87	8.65	**6.27**	28.86	29.30	**30.00**
UC/GA	0.66	-0.05	**−1.73**	9.81	7.89	**13.24**	31.39	29.72	**30.29**
CA/UG	−0.75	0.99	1.69	7.53	10.96	10.71	26.53	31.50	28.82
AC/GU	−1.53	−0.46	0.31	−1.40	4.78	4.28	26.77	28.59	31.30
CU/AG	1.18	−0.85	1.07	7.47	9.24	6.90	29.56	28.56	25.12
UA/UA	1.03	0.19	1.18	4.17	13.06	11.2	30.05	31.49	31.6
AC/GU	−0.97	0.34	0.47	8.40	4.94	5.77	29.25	29.85	32.08
CU/AG	0.80	−1.33	2.93	5.99	8.09	5.57	29.44	28.75	23.47
UC/GA	−1.59	1.52	0.62	10.56	5.91	5.82	31.30	30.50	30.18
CC/GG	−1.40	0.82	−0.82	9.80	5.91	11.72	29.49	31.75	31.06
CU/AG	−0.92	-1.17	−1.53	15.36	10.03	8.52	29.15	26.82	27.15
UA/**U**A	0.27	**−0.77**	**−0.13**	7.24	**7.26**	**16.41**	30.05	**44.85**	**38.11**
AC/G**U**	0.30	**1.77**	**0.14**	11.25	**13.61**	**8.49**	29.36	**30.25**	**31.00**
CU/AG	0.50	−0.24	2.25	8.20	2.10	8.36	28.55	31.06	29.39

Table S7. Tilt, roll and twist parameters for each di-nucleotide step were calculated using X3DNA program from the averaged structure obtained from 20 ns MD simulation of unmodified siRNA duplex **3R-2R**, and modified siRNA duplexes **3R-7R** and **6R-7R**. Modified nucleotide positions are highlighted by green underlined and their corresponding values are highlighted in green colour. All the values are reported in degrees.

Hydrogen bond occupancy of modified nucleotides over 20 ns MD simulation

SiRNA strand and modification site	Unmodified	Modified	
	3R - 2R	**3R - 7R**	**6R - 7R**
Guide strand (seed region)	99.30%	65%	61.80%
Passenger strand (position 1)	99.52%	-	60.27%
Passenger strand (position 2)	99.63%	-	63.89%

Table S8. The occupancy of two hydrogen bonds present between the modified nucleotide and their pairing base over 20 ns MD simulation calculated using ptraj module of AMBER 10.

Hydrogen bonding inter and intrastrand stacking energy calculated at MP2(full)/6-311G*level

SiRNA strand and modification site		3R-2R	3R-7R	6R-7R
Guide strand (seed region)	ΔE_{intra}	−25.9	−20.21	−18.57
	ΔE_{inter}	−6.31	−6.19	−3.66
	ΔE_{HB}	−12.4	−7.2	−6.9
	Total	**−44.61**	**−33.6**	**−29.13**
Passenger strand (position 1)	ΔE_{intra}	−26.31	-	−17.12
	ΔE_{inter}	−6.45	-	−3.58
	ΔE_{HB}	−12.5	-	−5.98
	Total	**−45.26**	-	**−26.68**
Passenger strand (position 2)	ΔE_{intra}	−25.68	-	−16.22
	ΔE_{inter}	−6.530	-	−4.27
	ΔE_{HB}	−12.67	-	−8.24
	Total	**−44.88**	-	**−26.73**

Table S9. Inter (ΔE_{inter}) and intra (ΔE_{intra}) strand stacking energy calculated for hexanucleotides including modified nucleotides as represented in Figure S14. Hydrogen bonding energy (ΔE_{HB}) calculated for only at modified base pair region. All the values are reported in kcal/mol.

ESI-MS data for RNA oligonucleotides

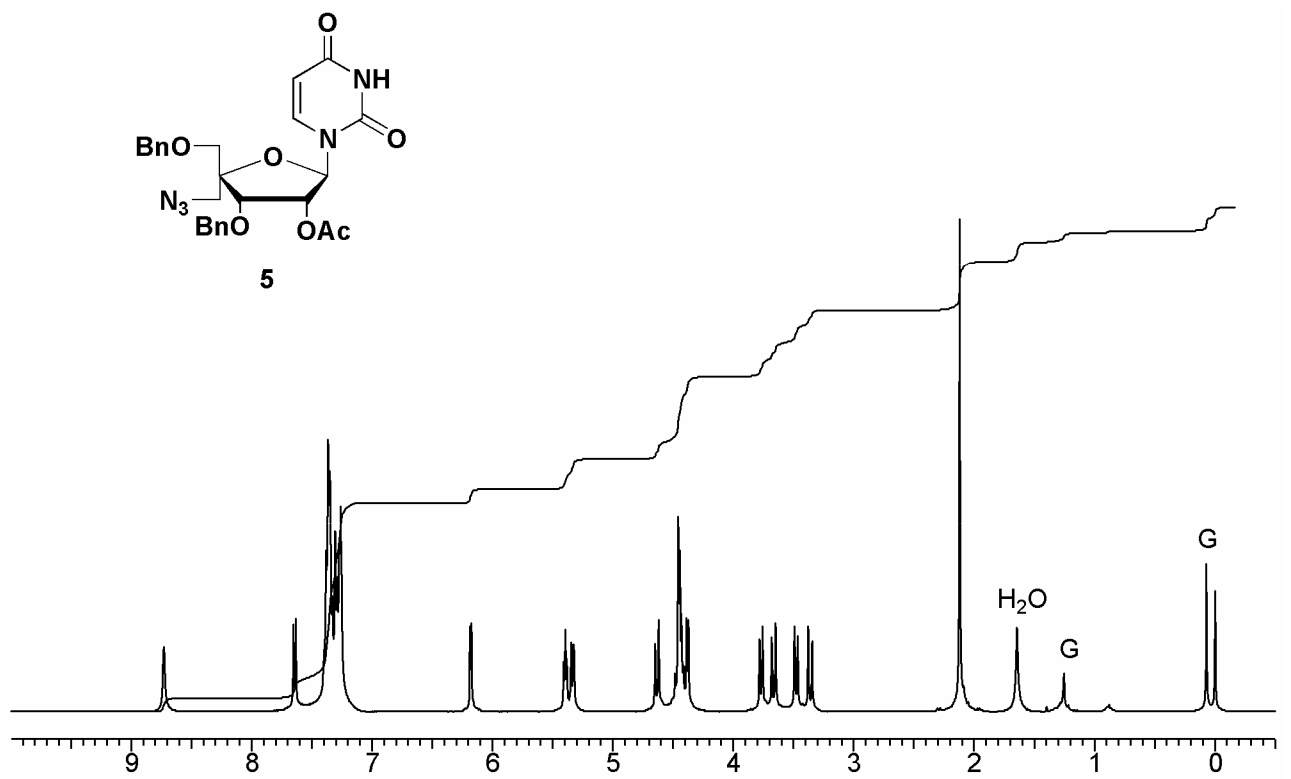
RNA	Sequence (5' - 3')	MW (calc)	MW (found)
3R	GGCCUUUCACUACUCCUACTT	6502.9	6502.4
2R	AGUAGGAGUAGUGAAAGGCCTT	7141.4	7141.9
6R	GGCCUUUCACUACUCCUACTT	6589	6589
7R	AGUAGGAGUAGUGAAAGGCCUT	7229	7229
9R	GGCCUUUCACUACUCCUACTT	6545.9	6546
12R	GGCCUUUCACUACUCCUACUT	6589	6613.7 ($1Na^+$)
16R	GGCCUUUCACUACUCCUACTT	6545.9	6546
22R	AGUAGGAGUAGUGAAAGGCCTT	7184.4	7228.4 ($2Na^+$)
29R	AGUAGGAGUAGUGAAAGGCCUT	7169.4	7171.3

Table S10. The molecular weights of unmodified and modified siRNA strands calculated using negative ion electrospray ionization (ESI) technique. 4'-*C*-aminomethyl-2'-*O*-methyl modification shown in green underlined, and 2'-*O*-methyl modification shown in red underlined.

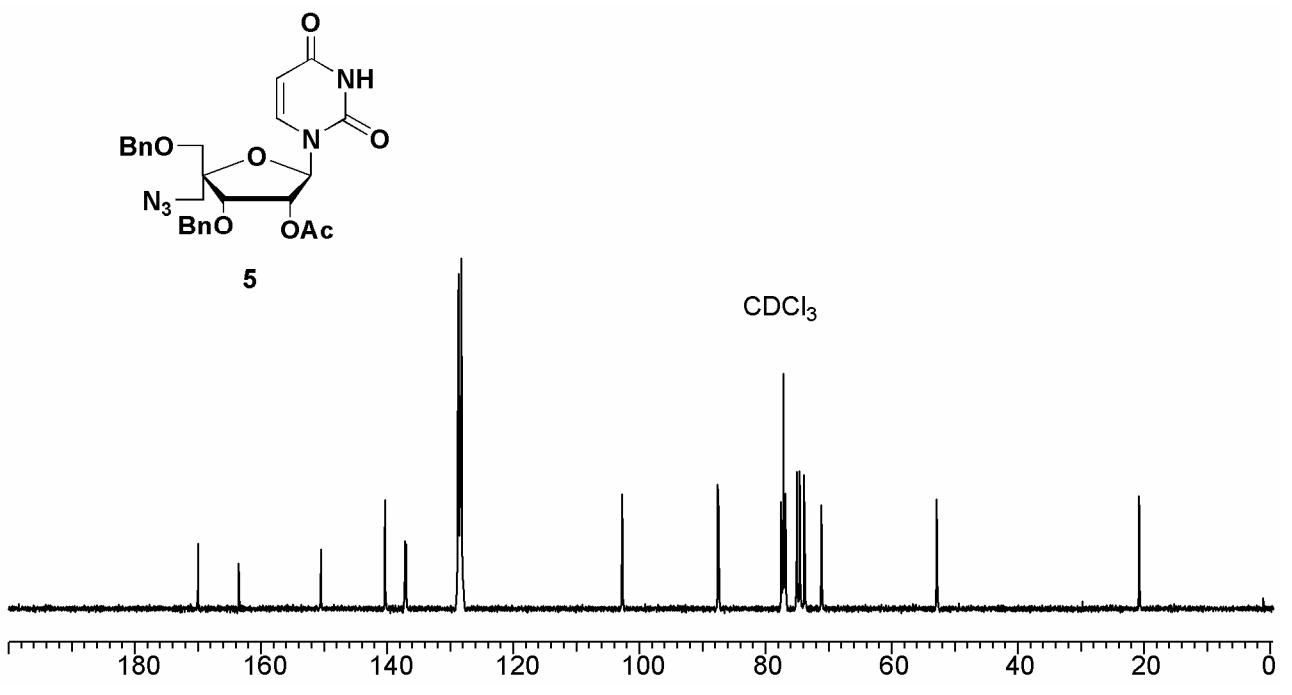
NMR spectra (^{1}H, ^{13}C, ^{31}P & ^{19}F)

(G- Grease, I- Impurity)

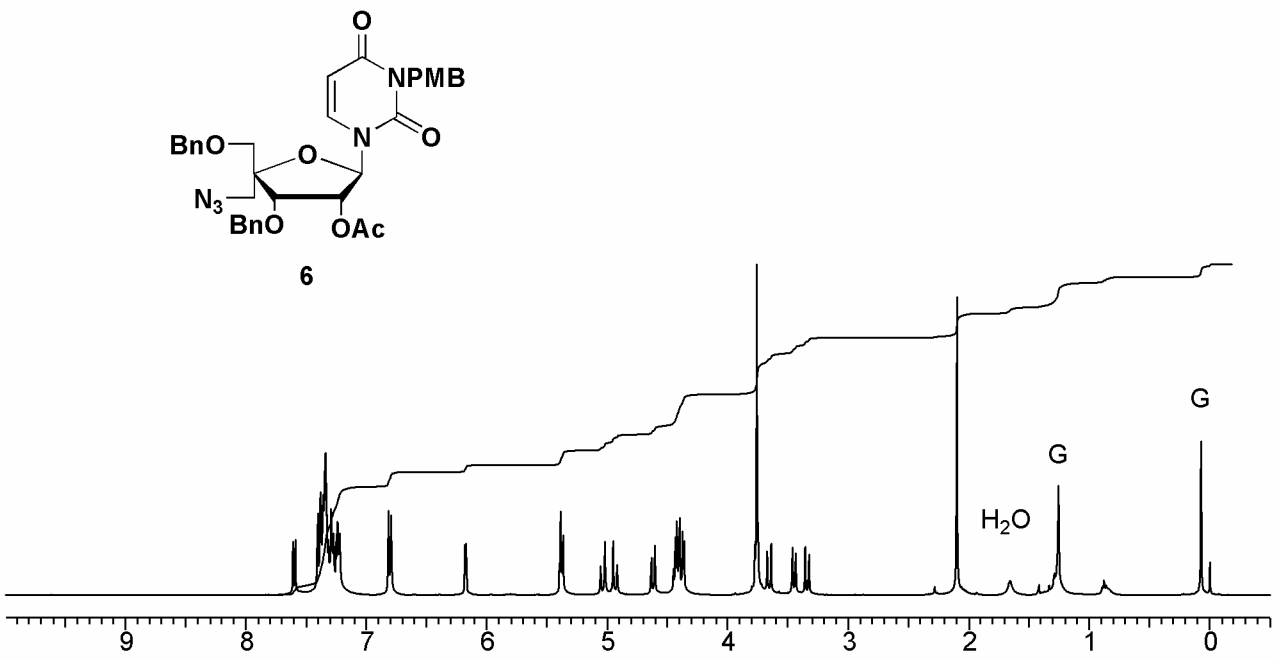
^{1}H NMR spectrum of compound **5**



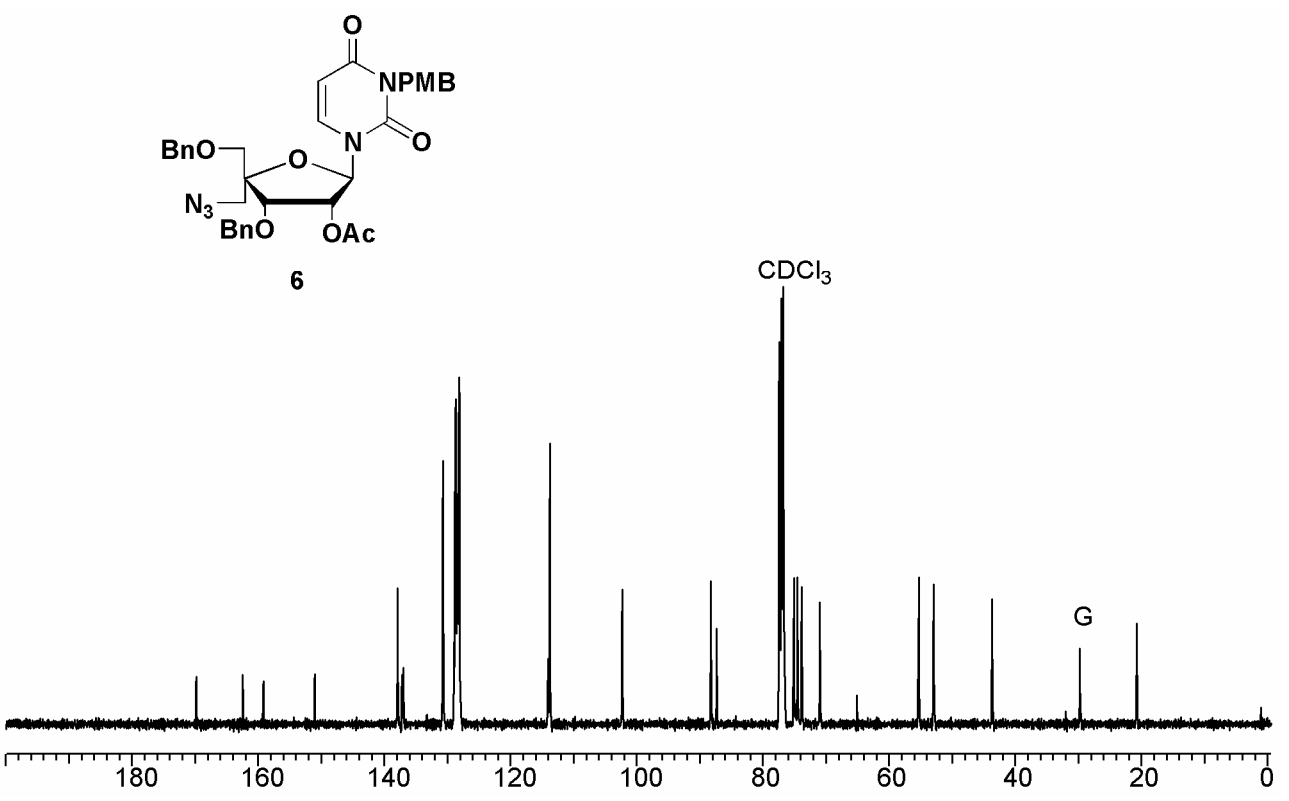
^{13}C NMR spectrum of compound **5**



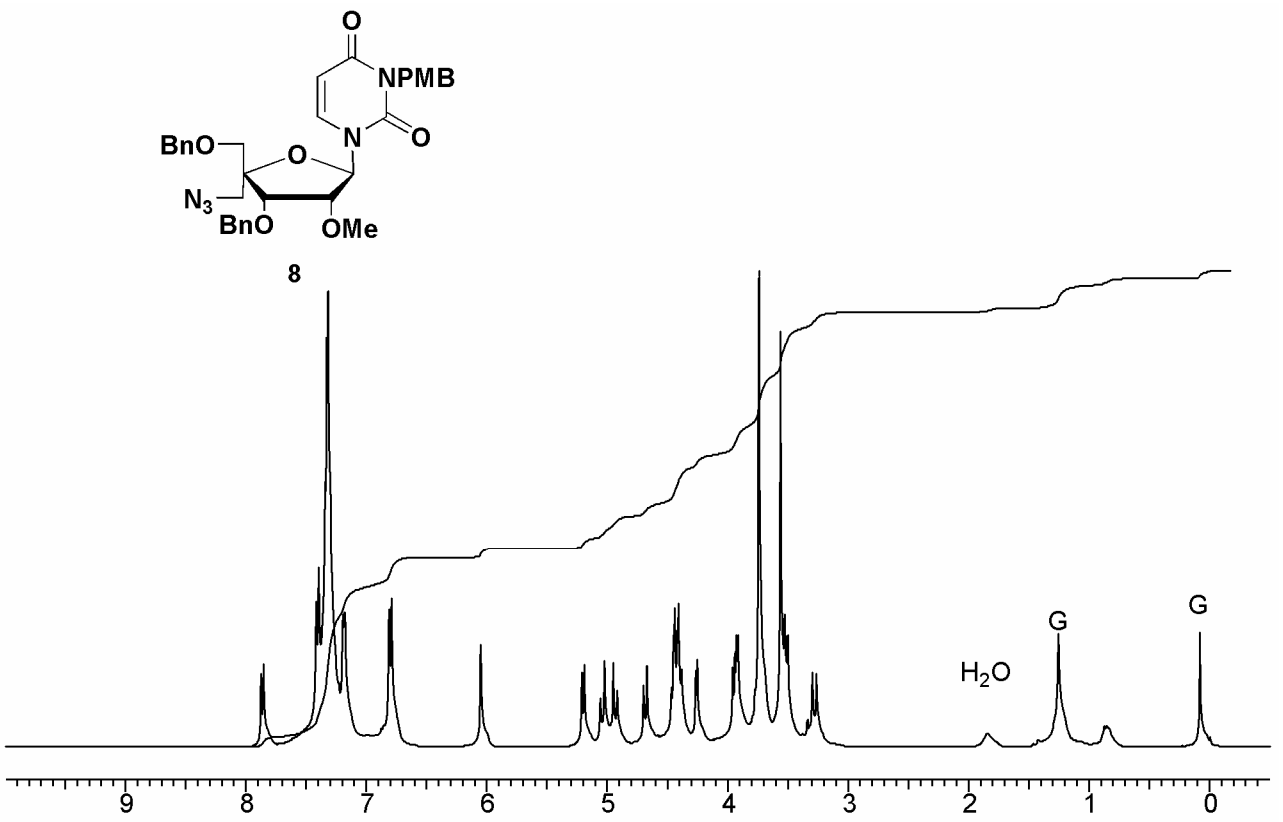
^{1}H NMR spectrum of compound **6**



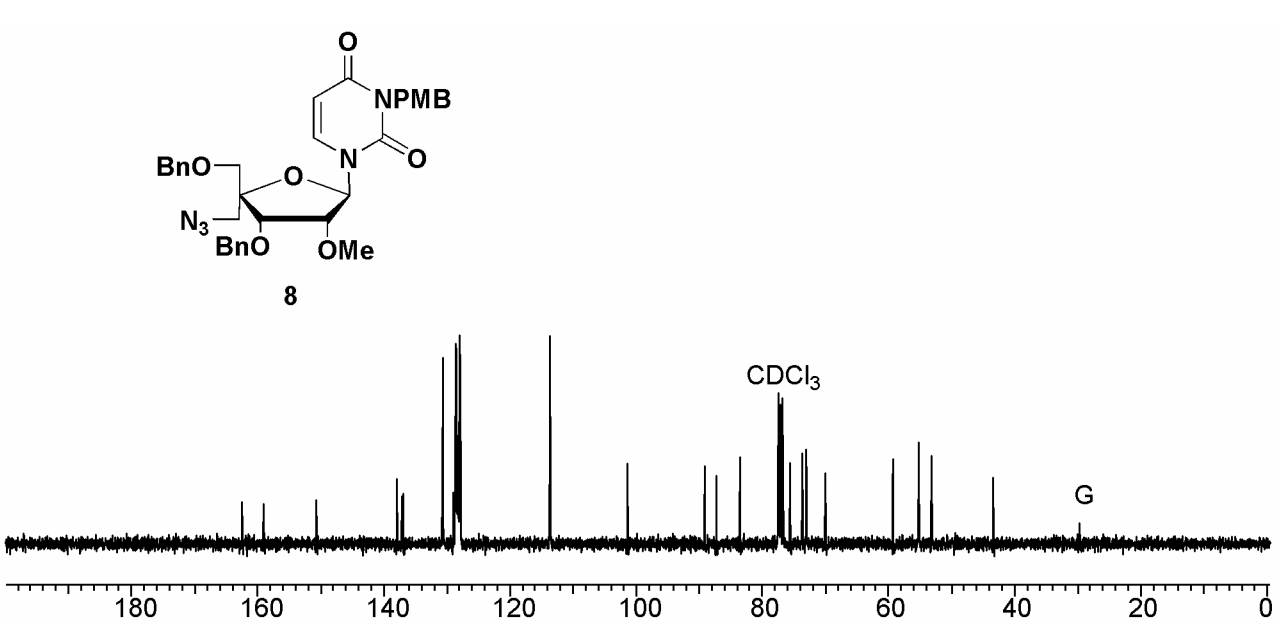
^{13}C NMR spectrum of compound **6**



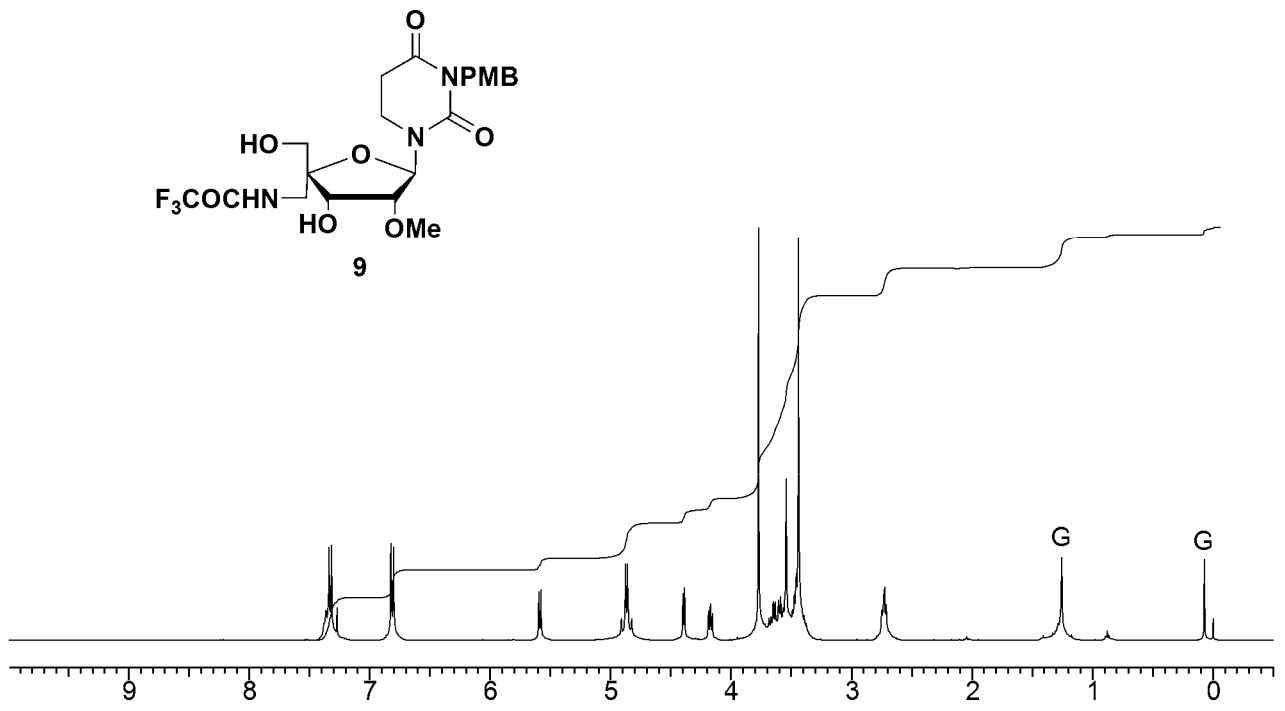
^{1}H NMR spectrum of compound **8**



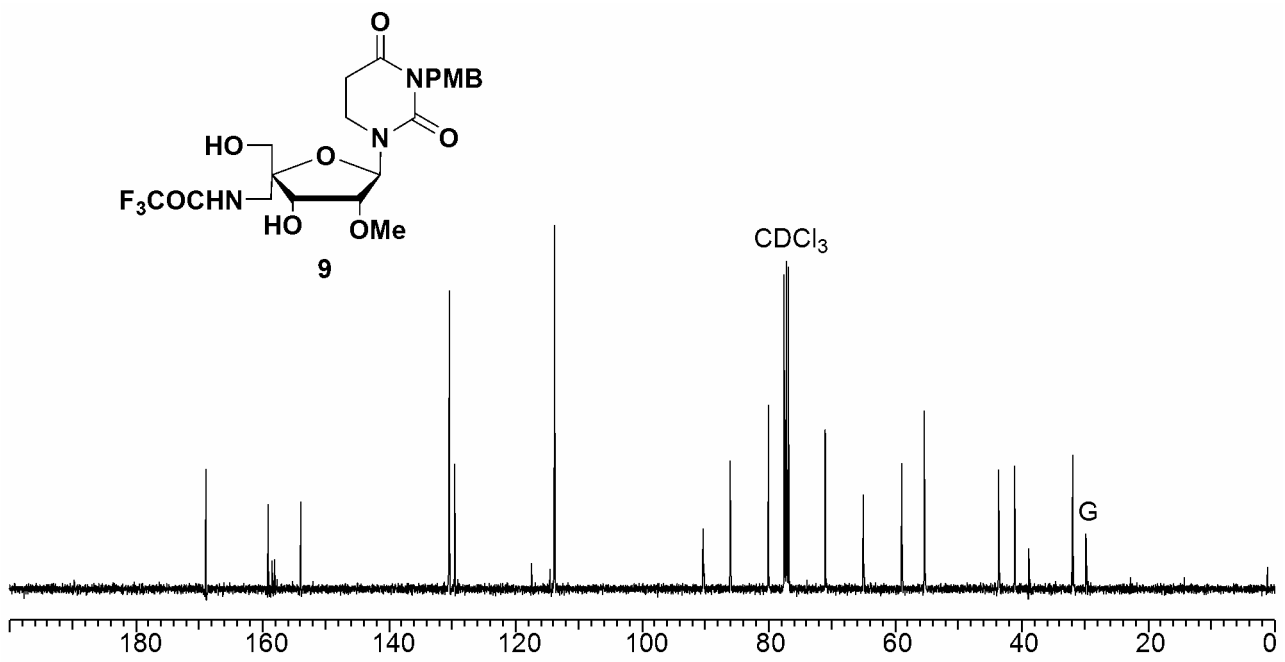
^{13}C NMR spectrum of compound **8**



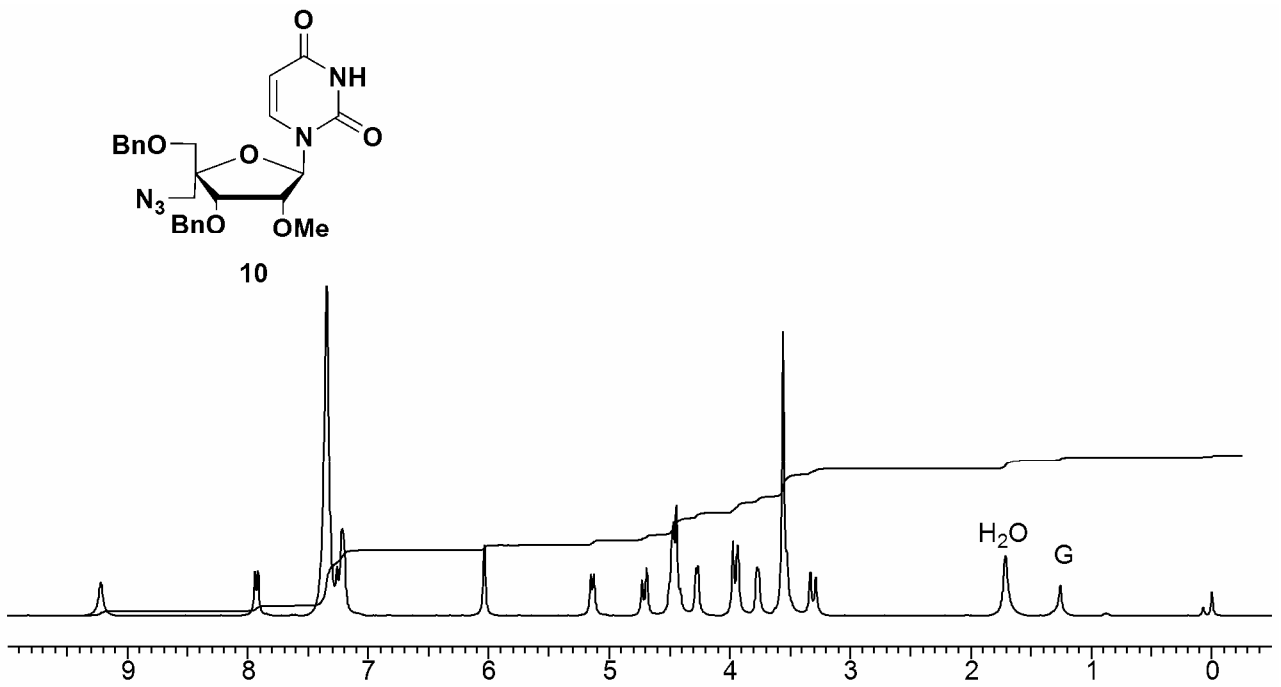
^{1}H NMR spectrum of compound **9**



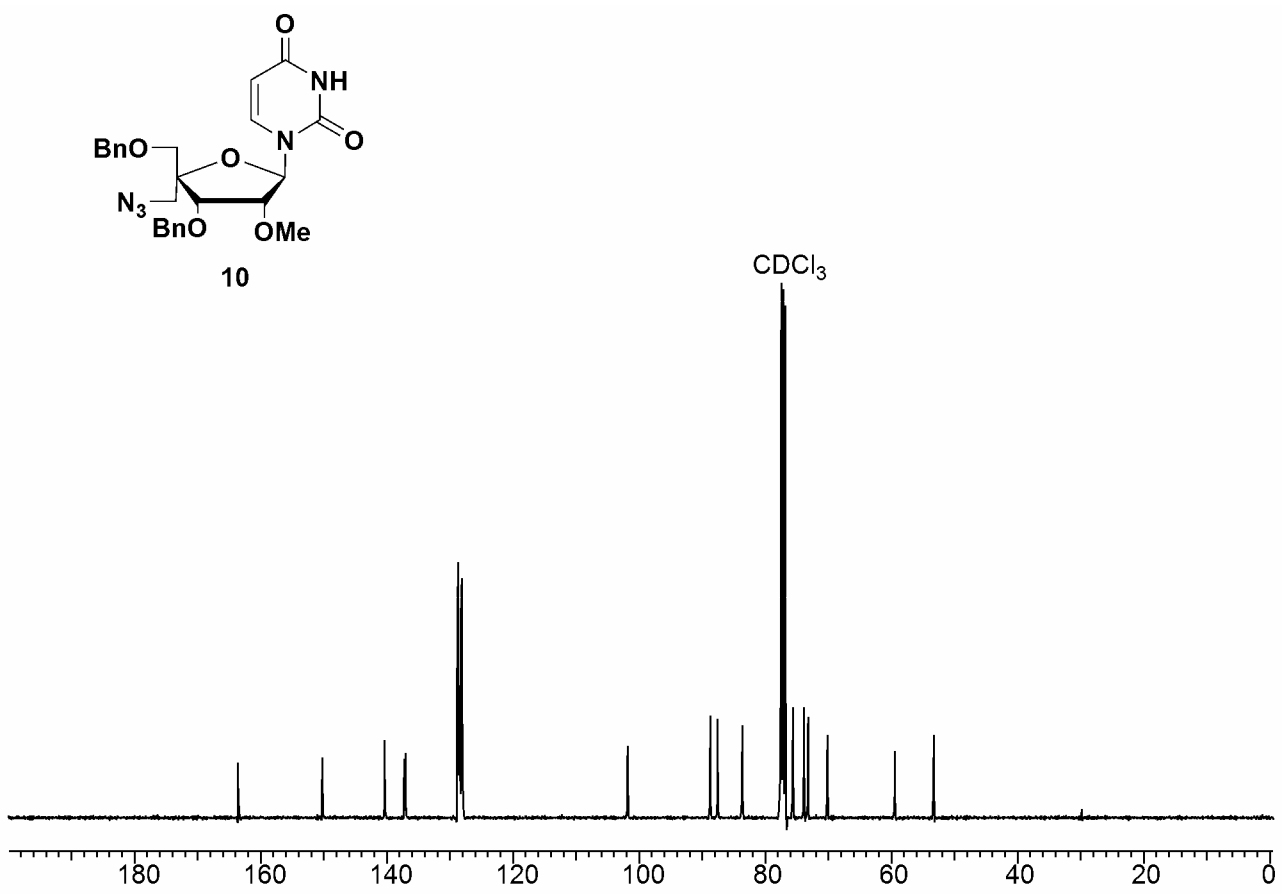
^{13}C NMR spectrum of compound **9**



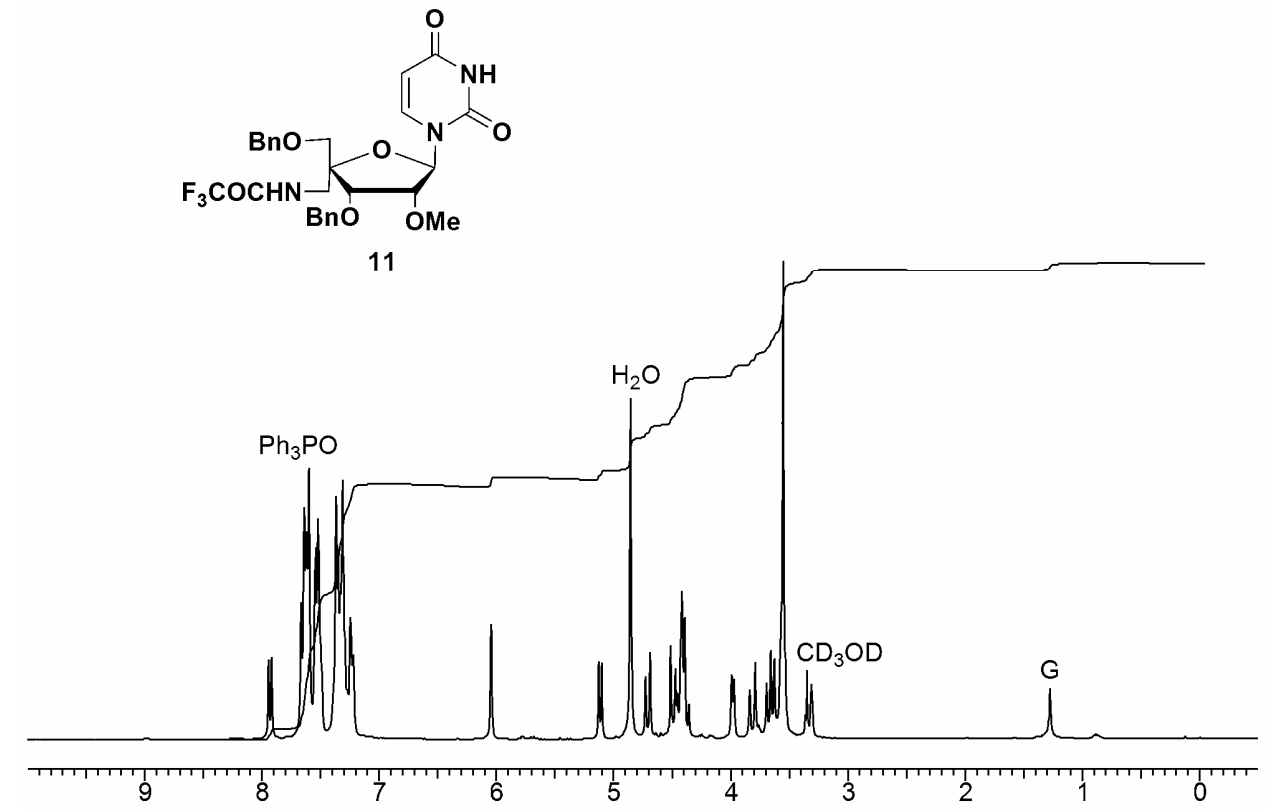
^{1}H NMR spectrum of compound **10**



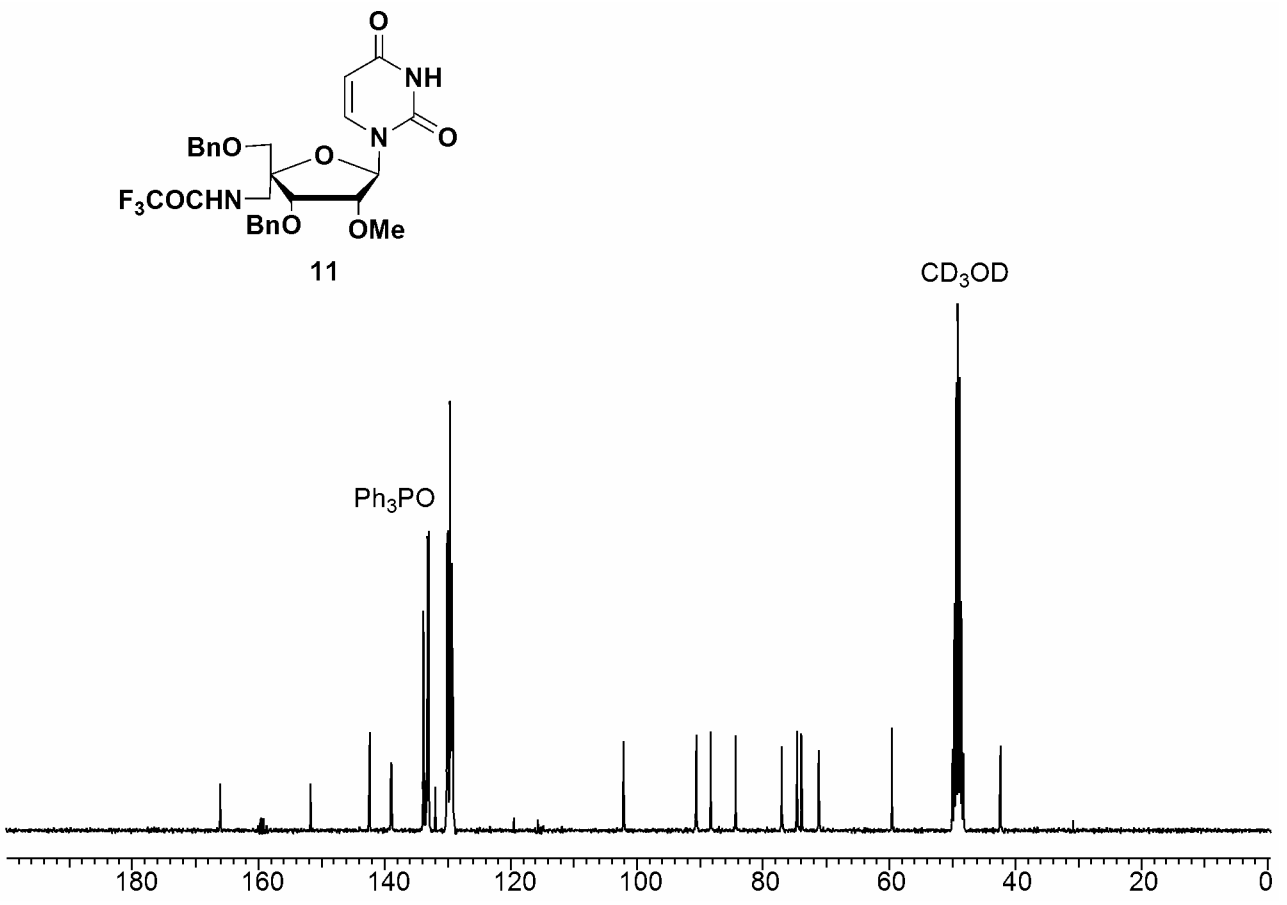
^{13}C NMR spectrum of compound **10**



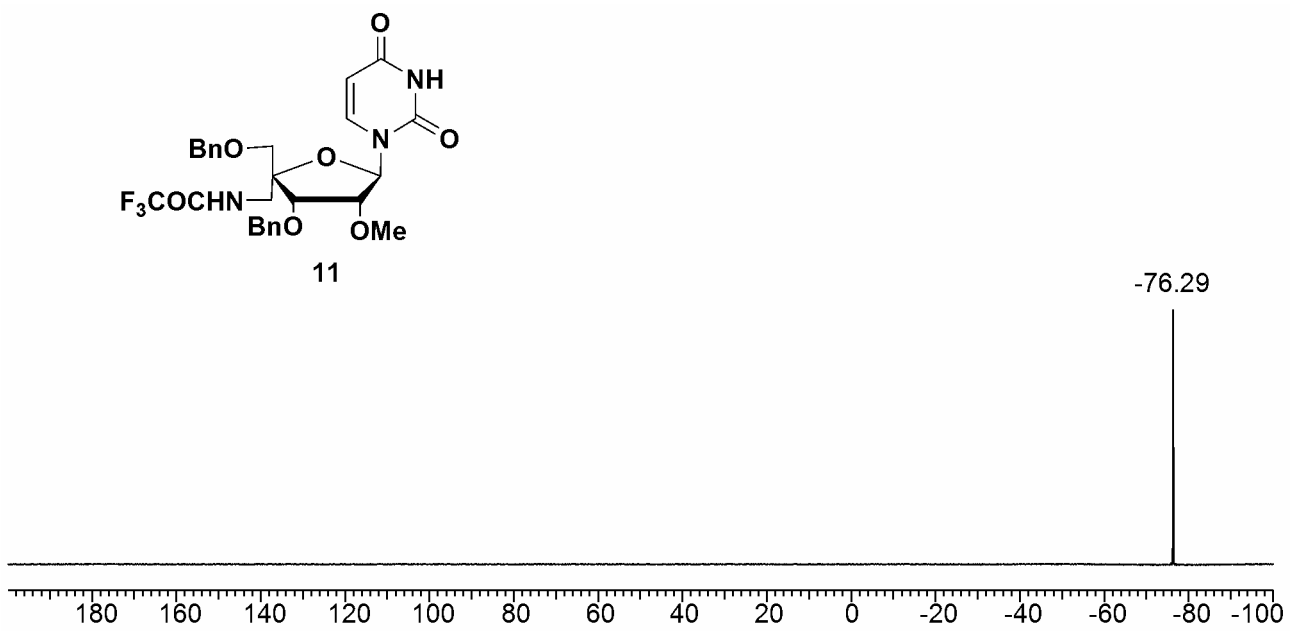
^{1}H NMR spectrum of compound **11**



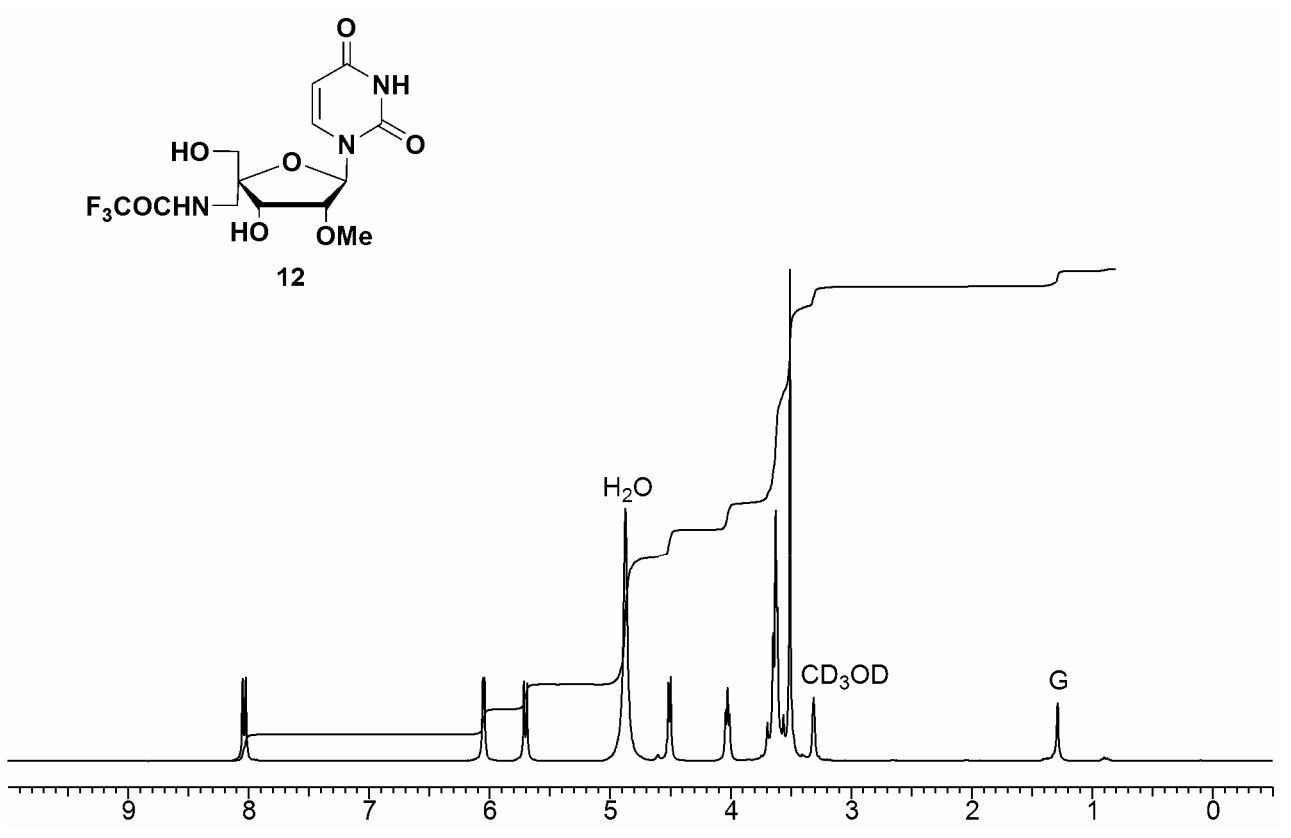
^{13}C NMR spectrum of compound **11**



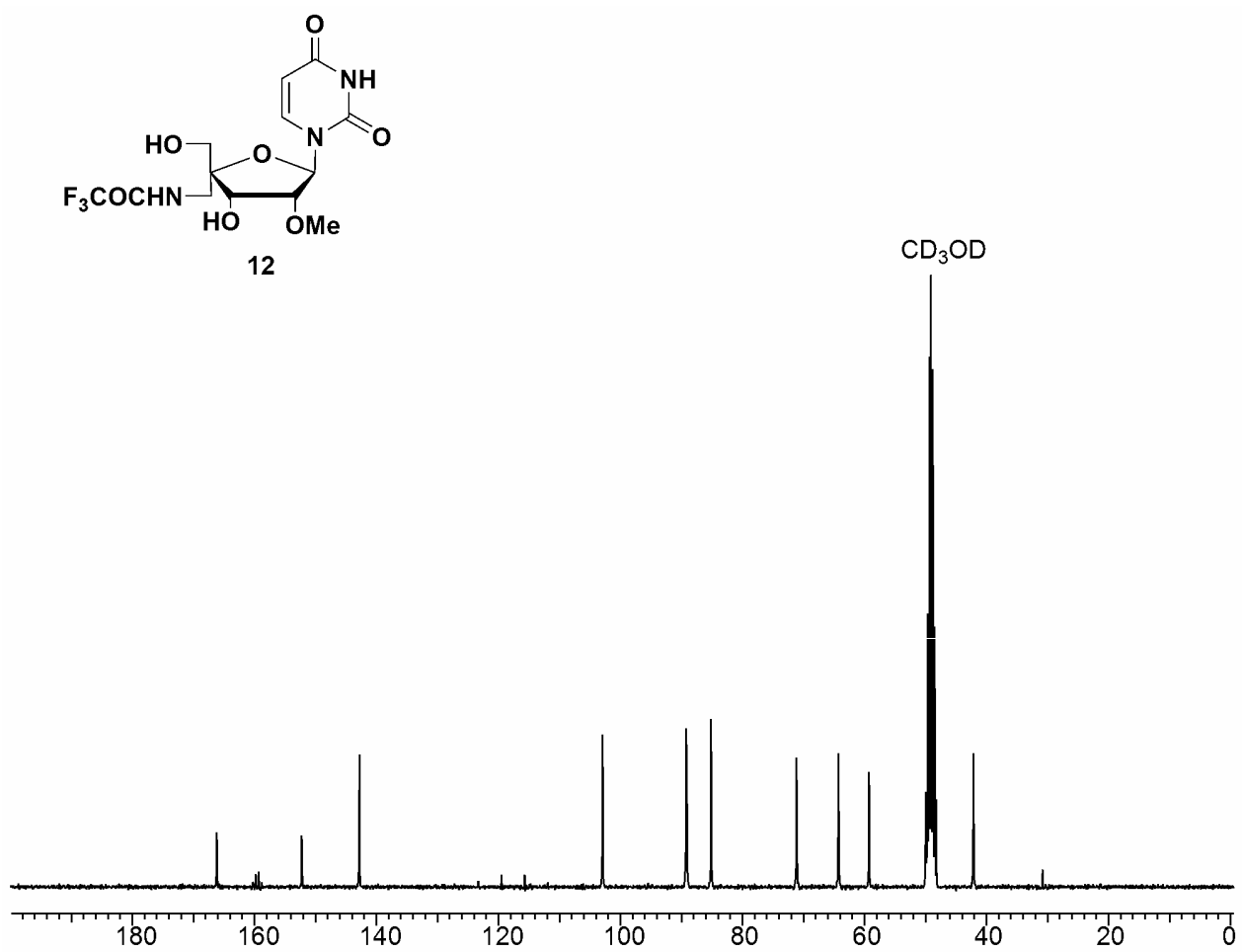
^{19}F NMR spectrum of compound **11**



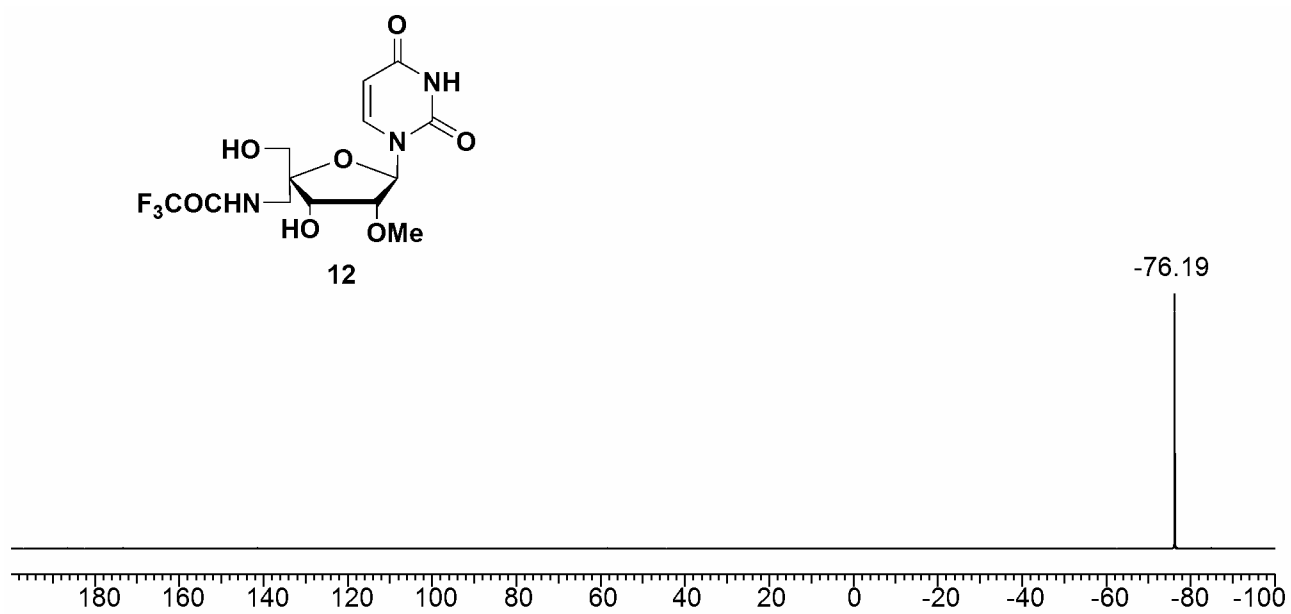
^{1}H NMR spectrum of compound **12**



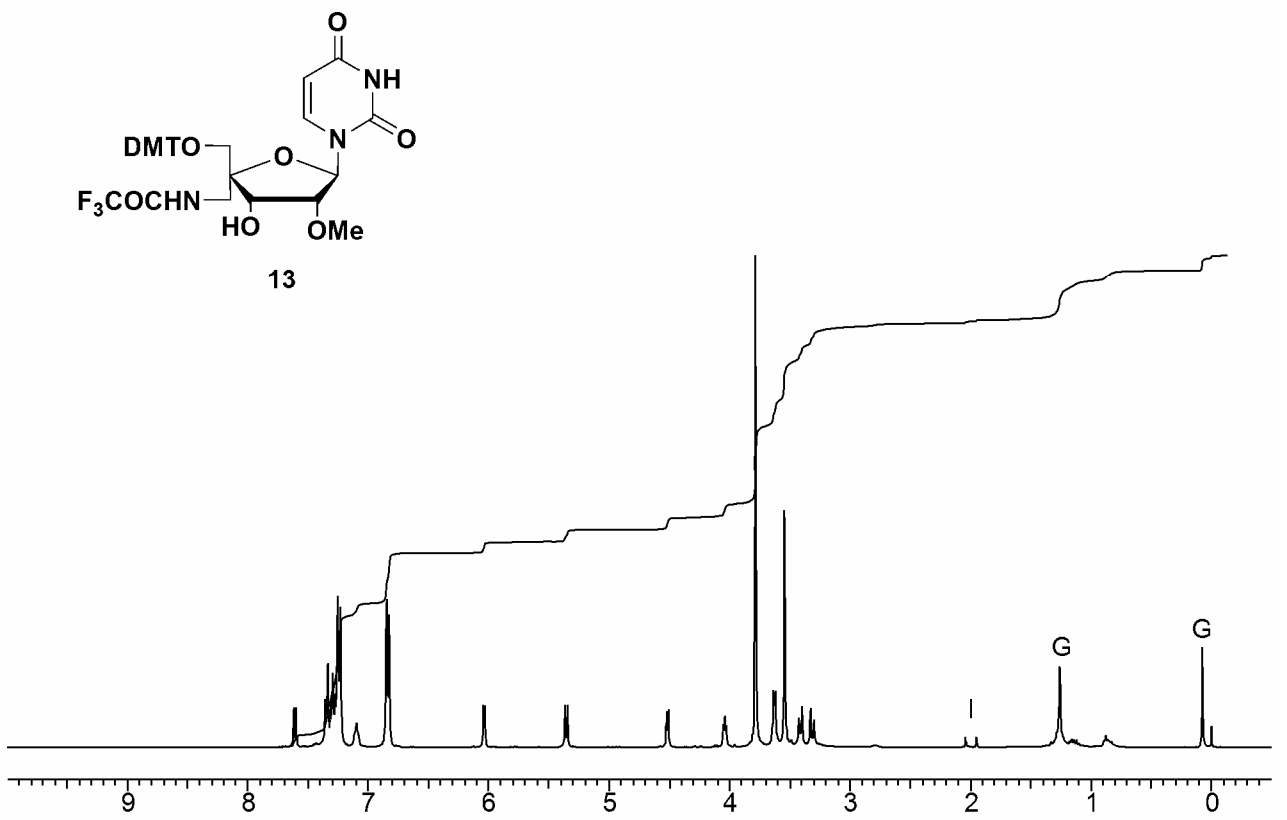
^{13}C NMR spectrum of compound **12**



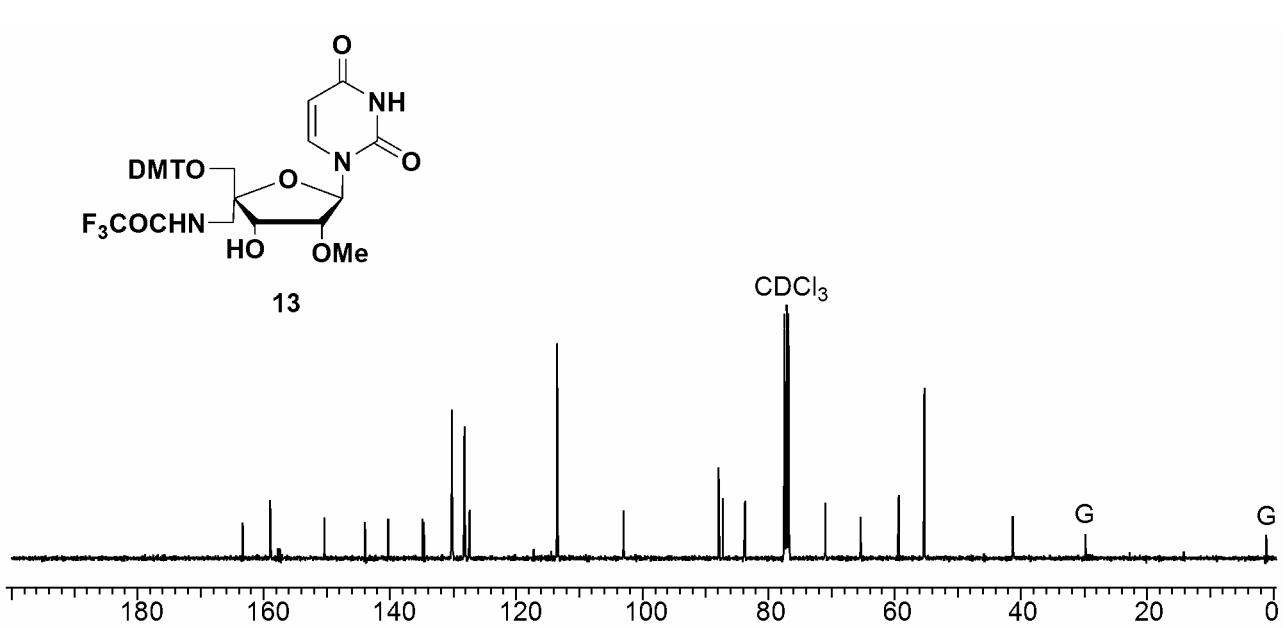
^{19}F NMR spectrum of compound **12**



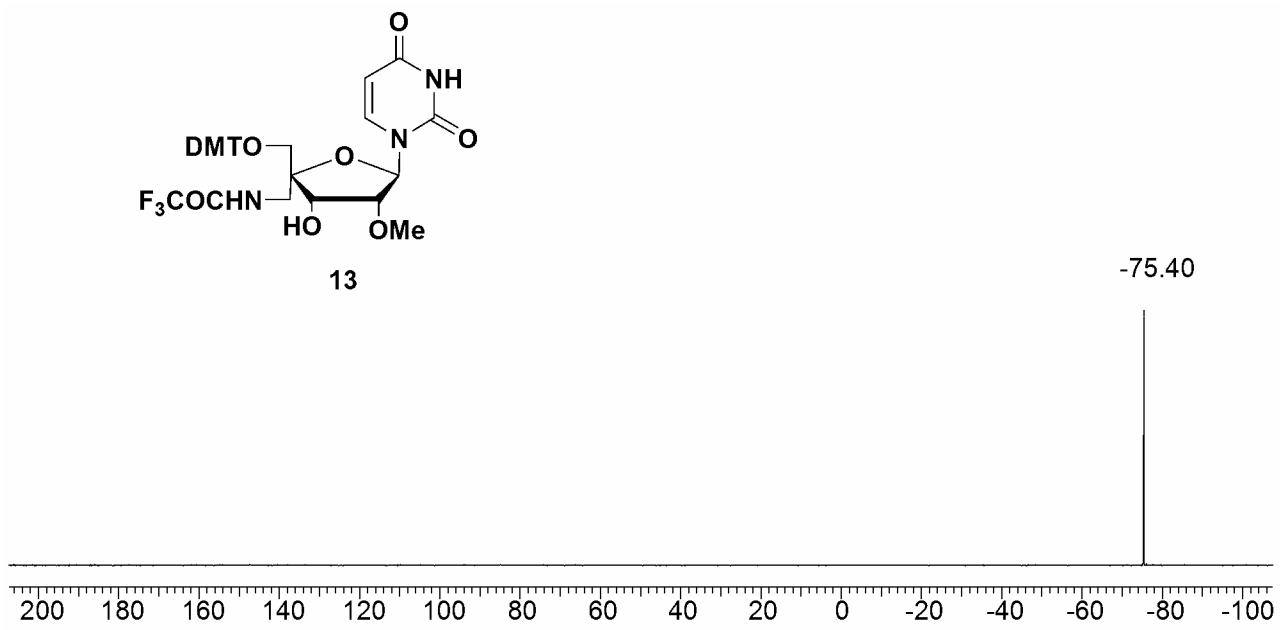
^{1}H NMR spectrum of compound **13**



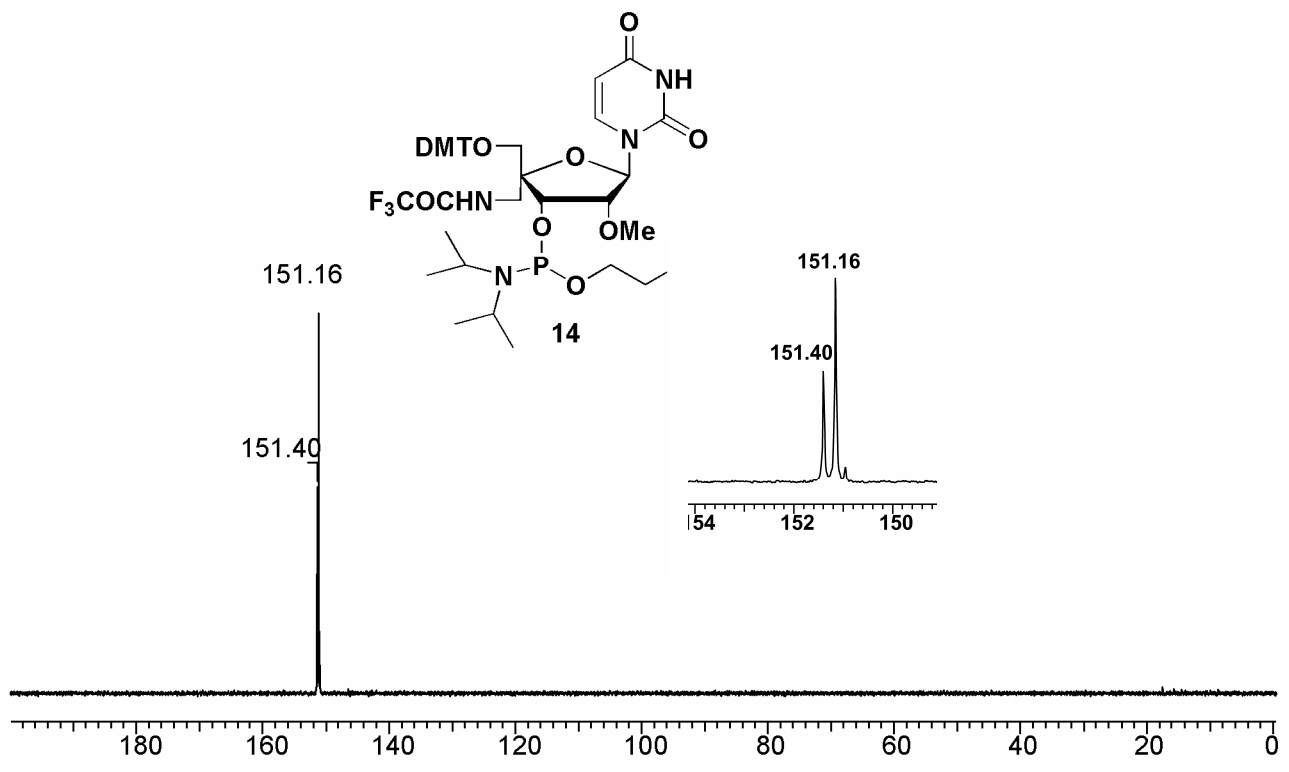
^{13}C NMR spectrum of compound **13**



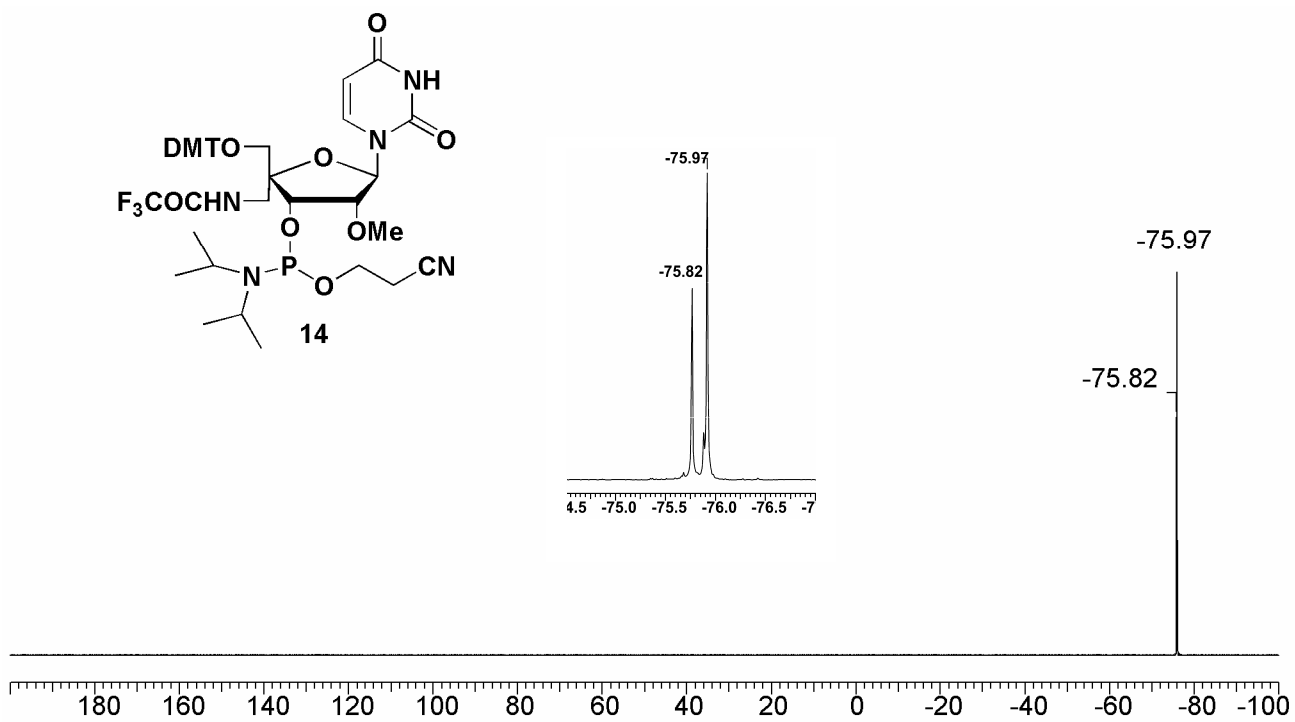
^{19}F NMR spectrum of compound **13**



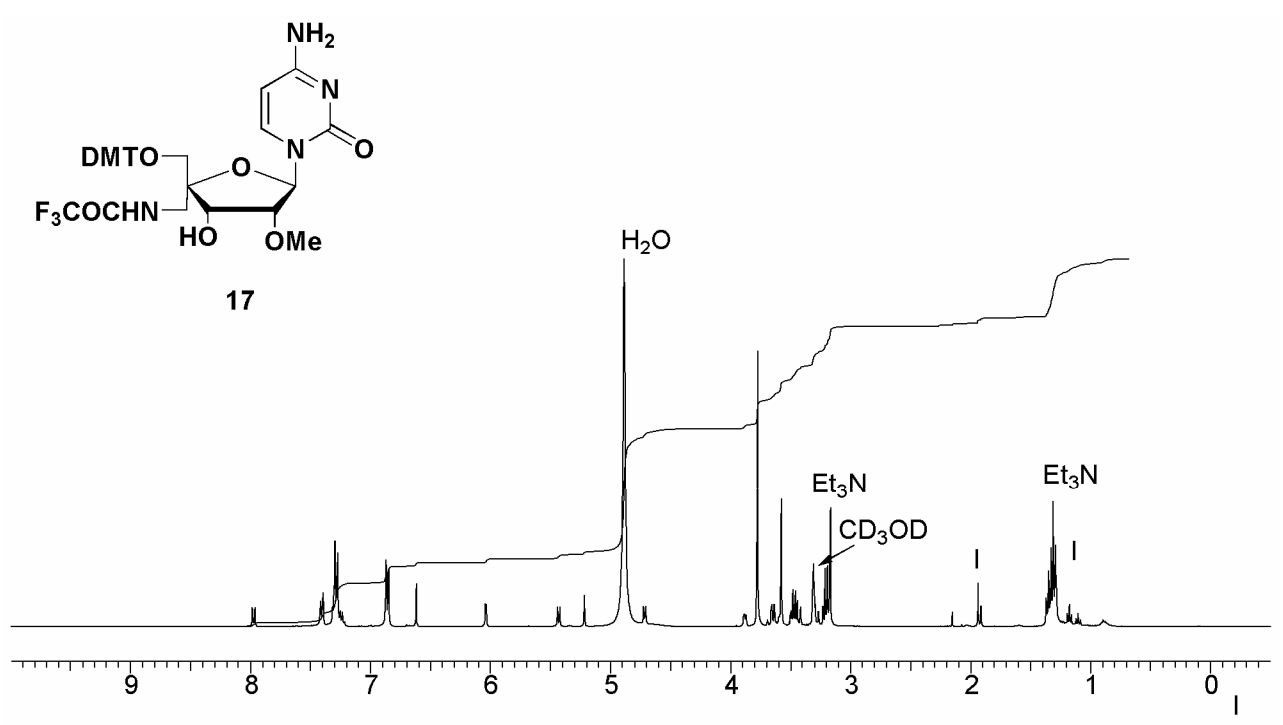
^{31}P NMR spectrum of compound **14**



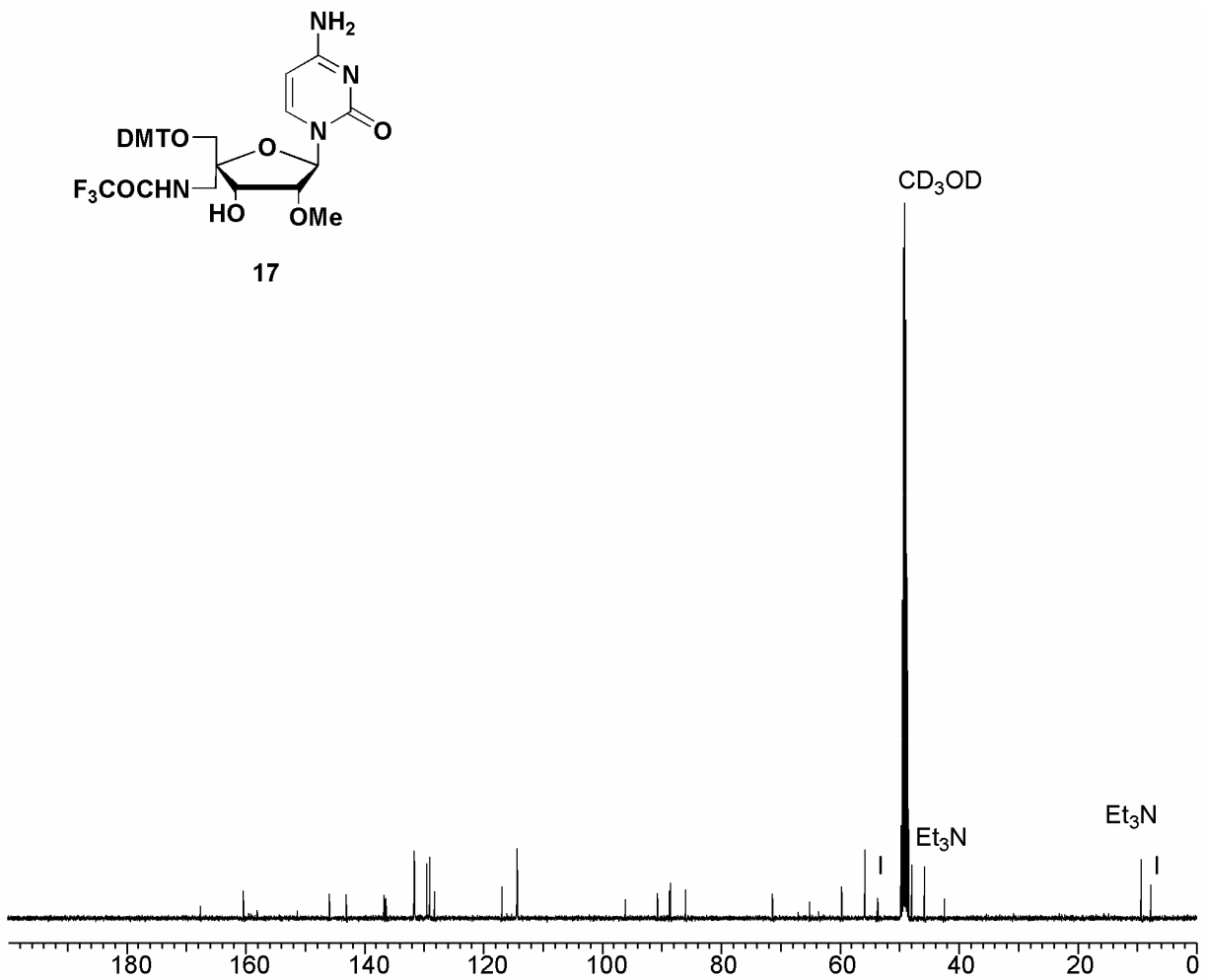
^{19}F NMR spectrum of compound **14**



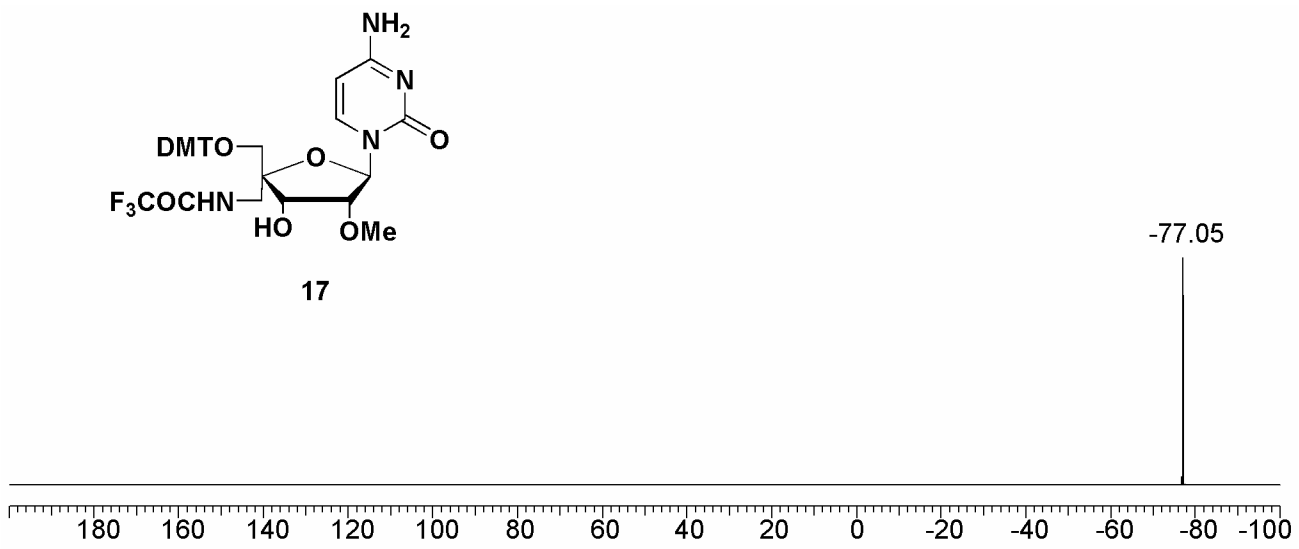
^{1}H NMR spectrum of compound **17**



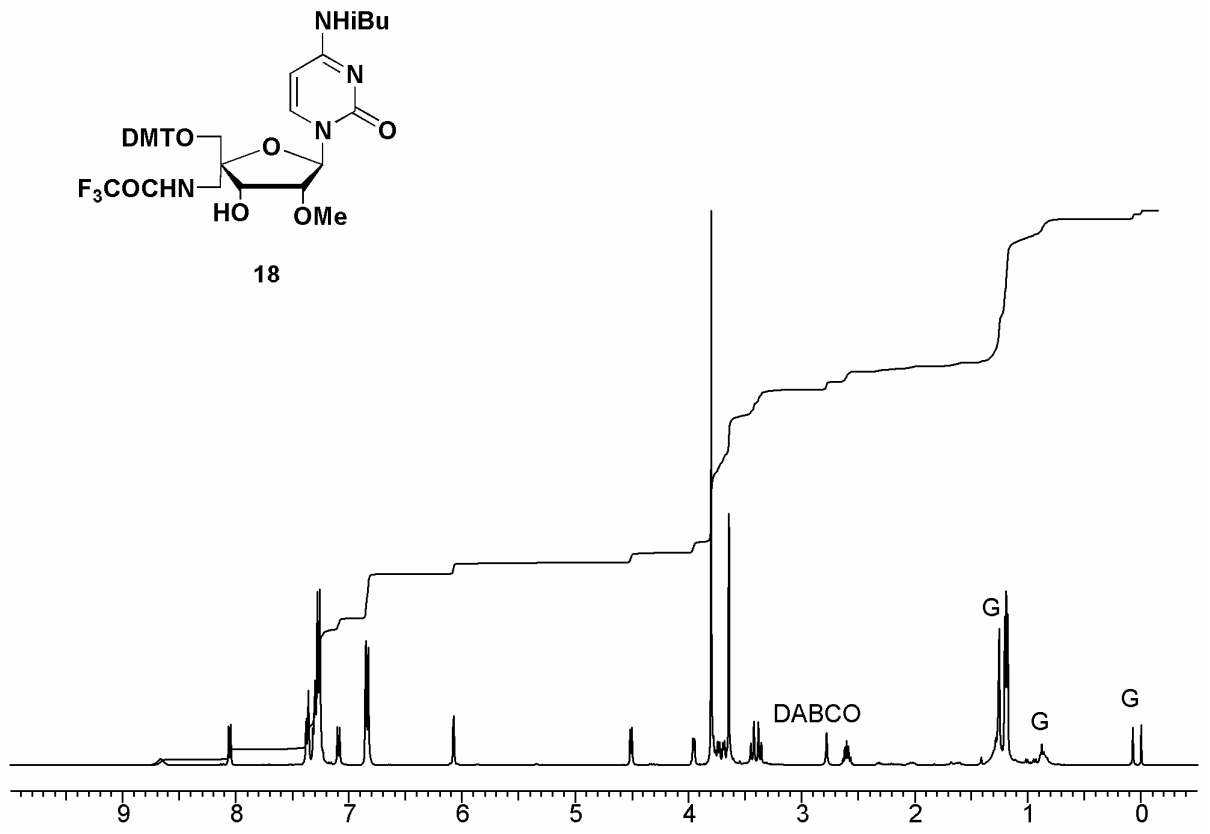
^{13}C NMR spectrum of compound **17**



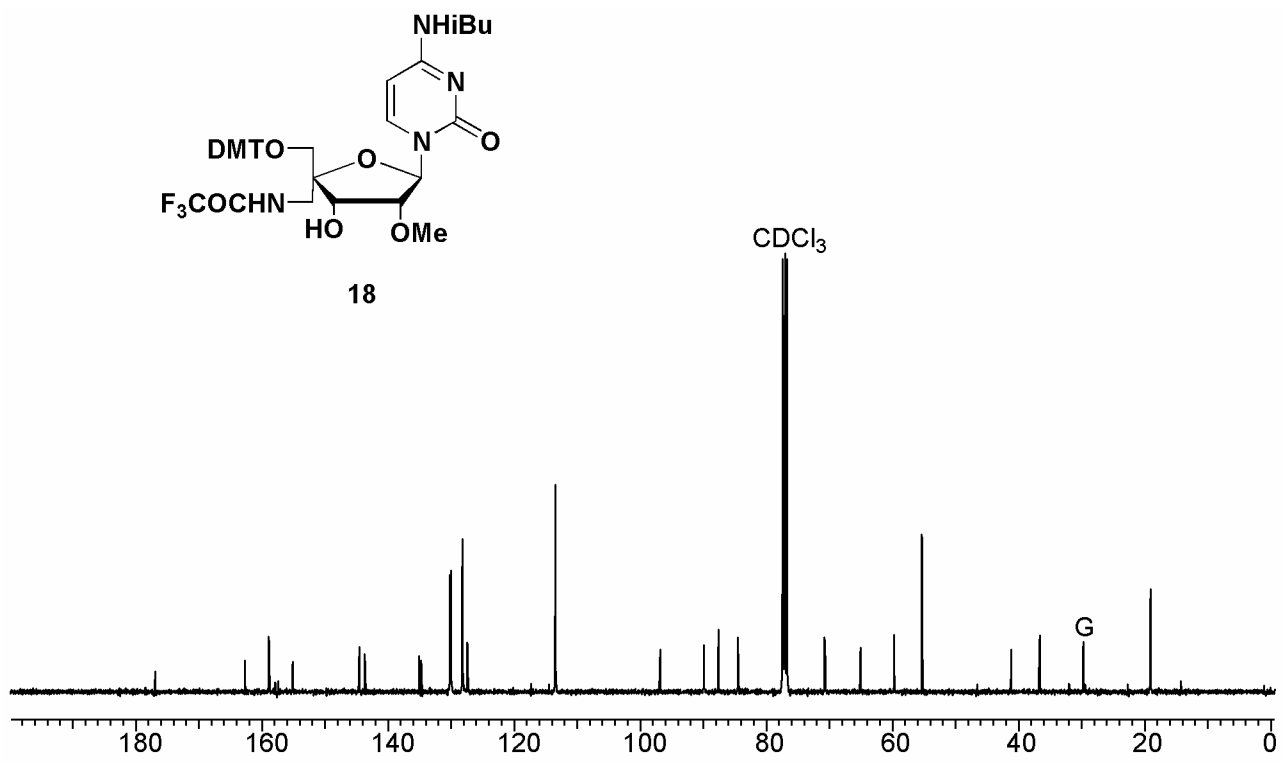
^{19}F NMR spectrum of compound **17**



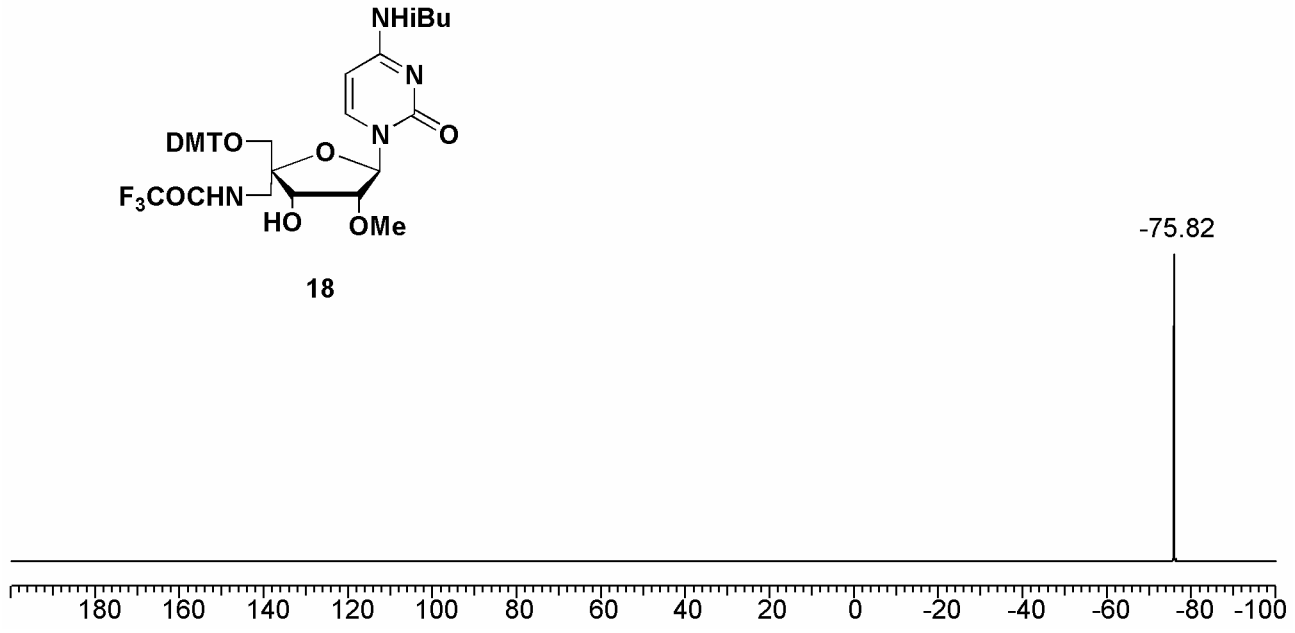
^{1}H NMR spectrum of compound **18**



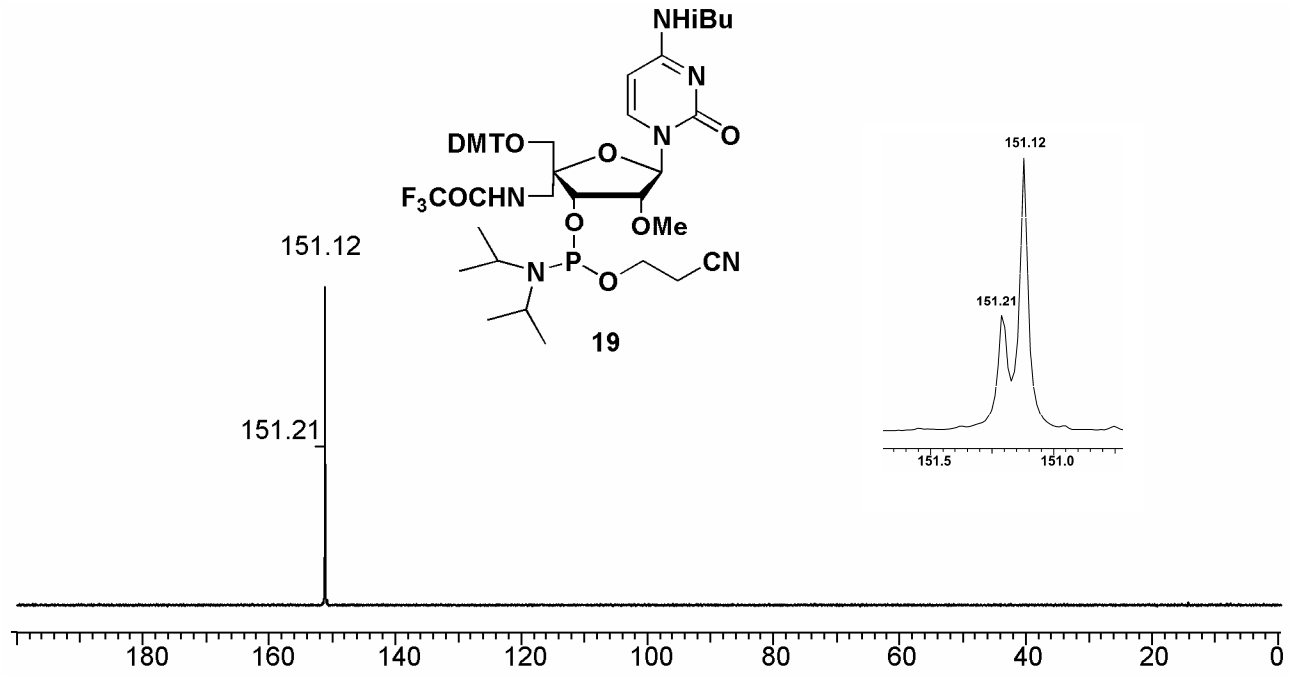
^{13}C NMR spectrum of compound **18**



^{19}F NMR spectrum of compound **18**



^{31}P NMR spectrum of compound **19**



^{19}F NMR spectrum of compound **19**