OL | Organic Letters

Letter

Cite This: Org. Lett. 2019, 21, 1963−1967

pubs.acs.org/OrgLett

Synthesis and Biophysical Characterization of RNAs Containing 2′-Fluorinated *Northern* Methanocarbacyclic Nucleotides

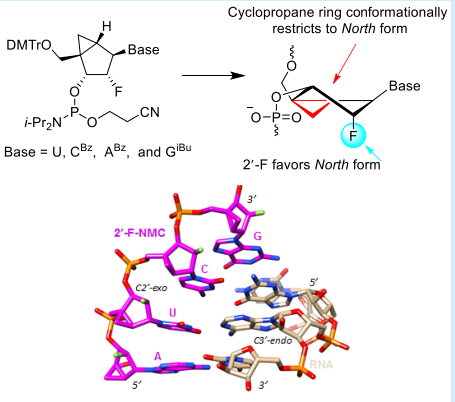
Masaaki Akabane-Nakata,[†] Pawan Kumar,[†] Rajat S. Das,[†] Namrata D. Erande,[†] Shigeo Matsuda,[†] Martin Egli,[‡] and Muthiah Manoharan*[,†]

[†]Alnylam Pharmaceuticals, 300 Third Street, Cambridge, Massachusetts 02142, United States

[‡]Department of Biochemistry, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232, United States

Supporting Information

ABSTRACT: 2′-Fluorinated *Northern* methanocarbacyclic (2′-F-NMC) nucleosides and phosphoramidites, based on a bicyclo[3.1.0]hexane scaffold bearing all four natural nucleobases (U, C, A, and G), were synthesized to enable exploration of this novel nucleotide modification related to the clinically validated 2′-deoxy-2′-fluororibonucleotides (2′-F-RNA). Biophysical properties of the 2′-F-NMC-containing oligonucleotides were evaluated. A duplex of 2′-F-NMC-modified oligonucleotide with RNA exhibited thermal stability similar to that of the parent RNA duplex, 2′-F-NMC-modified oligonucleotides had higher stability against 5′- and 3′-exonucleolytic degradation than the corresponding oligonucleotides modified with 2′-F-RNA, and 2′-F-NMC-modified oligonucleotides exhibited higher lipophilicity than the corresponding RNA oligonucleotides as well as those modified with 2′-F-RNA.



Therapeutics based on RNA interference (RNAi) have great potential for treating human diseases. Both US FDA and EMA have recently approved the first small interfering RNA (siRNA) drug, ONPATTRO (patisiran), for treatment of polyneuropathy caused by hereditary transthyretin-mediated amyloidosis.[1] The oligonucleotides used in oligonucleotides based therapeutics require use of unnatural nucleotide building blocks to stabilize the agents against nuclease degradation, to enhance cell-membrane permeability, and to limit immune responses.[2] The 2′-deoxy-2′-fluororibonucleotide (2′-F-RNA, Figure 1A) and 2′-*O*-methyl (2′-*O*Me) modifications have been used to modify siRNAs,[3−5] antisense oligonucleotides,[6,7] aptamers,[8] microRNAs,[9] and ribozymes.[10] Incorporation of 2′-F-RNA and 2′-*O*-Me residues stabilizes an A-form RNA duplex.[4,11] Specifically, 2′-F ribo-substitution preorganizes the sugar into a C3′-*endo* or *North* conformation and hence reduces the entropic penalty for the formation of the A-form duplex, and increases base stacking and Watson−Crick hydrogen-bond stabilities due to its electron-withdrawing power.[11] Furthermore, 2′-F-RNA-modified siRNAs have reduced immune stimulation and improved activity *in vitro* and *in vivo* compared to unmodified siRNA,[4] and are in clinical development. The 2′-F-RNA-modified oligonucleotides are, however, more sensitive to nucleolytic degradation than other 2′-modified oligonucleotides; moreover, these monomers are recognized, albeit poorly, by human RNA polymerases at high concentrations.[12,13] Hence, chemically modified building blocks that retain the advantages of 2′-F-RNA with additional features to overcome these limitations have the potential to improve pharmacological properties of oligonucleotides. A number of fluorine-containing building blocks have been

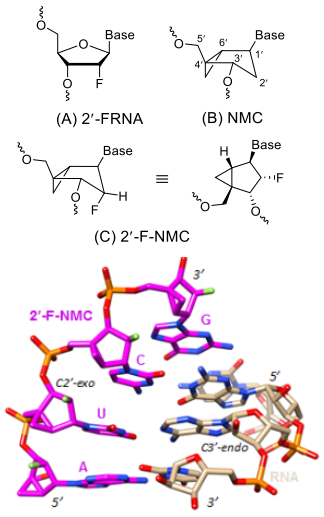


Figure 1. (A−C) Structures of (A) 2′-F-RNA, (B) NMC, and (C) 2′-F-NMC. (D) Model of a hybrid duplex between 2′-F-NMC and RNA constructed using the program UCSF Chimera; 2′-F-NMC residues have an idealized C2′-*exo* pucker with an axial 2′-fluorine.

Received: December 31, 2018
Published: March 20, 2019

DOI: 10.1021/acs.orglett.8b04153
Org. Lett. 2019, 21, 1963−1967

synthesized and evaluated in the context of oligonucleotide-based therapeutics.[14−29] However, to the best of our knowledge, none of these modifications have been made with all four nucleobases. As nucleotide pharmacology in a therapeutic oligonucleotide like siRNA depends on both sequence and position, it is necessary to have all four nucleosides with same ribosugar modification to evaluate its therapeutic potential.[30]

Conformationally restricted nucleic acids bearing bicyclic or tricyclic scaffolds that exhibit high affinity for complementary RNA are also being widely explored as potential therapeutic oligonucleotide modifications.[31,32] Nucleosides with a carbocyclic bicyclo[3.1.0]hexane system, here referred to as *Northern* methanocarbacyclic (NMC) nucleosides, are constrained to an RNA-like sugar pucker (Figure 1B).[33−38] The bicyclic NMC sugar is predicted to adopt a pseudoboat C2′-*exo* conformation due to the methylene bridge between the C4′ and C6′ positions. Consequently, NMC-modified oligonucleotides form more stable duplexes with RNA than do unmodified DNA.

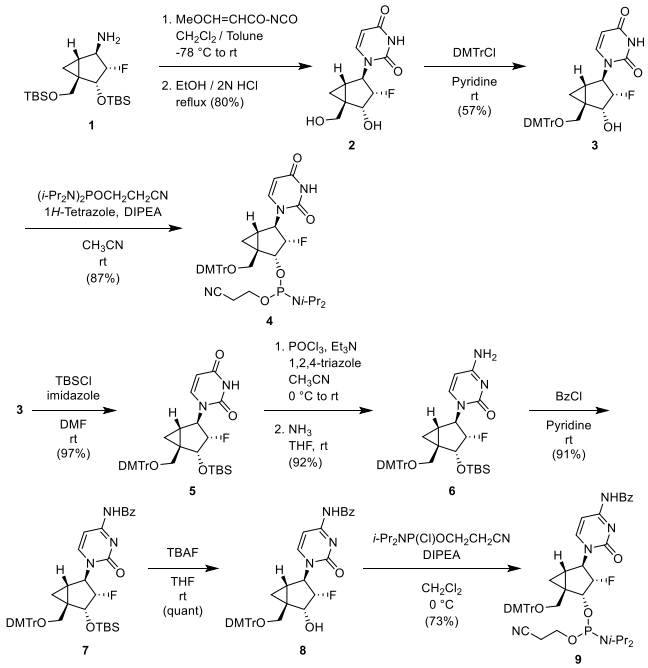
Jung and co-workers recently synthesized thymidine analogs 2′-F-NMC T (Figure 1C) and *ara*-2′-F-NMC T and oligonucleotides containing these building blocks.[18] Deoxyoligonucleotides containing 2′-F-NMC T have higher RNA binding affinity than oligonucleotides containing 2′-deoxy-2′-fluoro uridine or nonfluorinated NMC thymidine.[19] This duplex stabilization is presumed to result from stabilization of Watson−Crick hydrogen-bonding and base-stacking interactions due to the 2′-fluoro incorporation and demonstrates the potential of 2′-F-NMC analogs in oligonucleotide-based therapeutics.

Our laboratory has systematically evaluated the role of chemical modifications in siRNA activity. To assess 2′-F-NMC analogs in terms of their sequence- and position-dependent RNAi activity, we required all RNA nucleobase analogs. Here, we report the synthesis of 2′-F-NMC phosphoramidites bearing the four natural RNA nucleobases (i.e., A, U, G, C) from a common starting material in a convergent approach. We also report the binding affinities to a target RNA and susceptibilities to exonuclease degradation of oligonucleotides containing 2′-F-NMC.

The 2′-F-NMC U and 2′-F-NMC C phosphoramidites were synthesized as depicted in Scheme 1. The starting amine **1** was prepared according to the procedure reported by Jung et al.[18] and was coupled with 3-methoxyacryloyl isocyanate and cyclized under acidic conditions to afford the uridine derivative **2**.[39,40] The 5′-OH group of the nucleoside **2** was protected with 4,4′-dimethoxytriphenylmethyl chloride (DMTrCl) to give compound **3**. Subsequent phosphitylation of the 3′-OH group of **3** gave the desired phosphoramidite **4**. For the synthesis of 2′-F-NMC C, fully protected nucleoside **5** was obtained by *tert*-butyldimethylsilyl (TBS) protection of **3**. Compound **5** was then converted into the cytidine derivative **6** by reacting with 1,2,4-triazole in the presence of Et_3N and $POCl_3$, followed by treatment with aqueous NH_3. The exocyclic amine of **6** was benzoylated using benzoyl chloride (BzCl), and the resulting protected cytidine derivative **7** was treated with tetra-*n*-butylammonium fluoride (TBAF) to obtain alcohol **8**. Phosphitylation of **8** gave the desired phosphoramidite **9**.

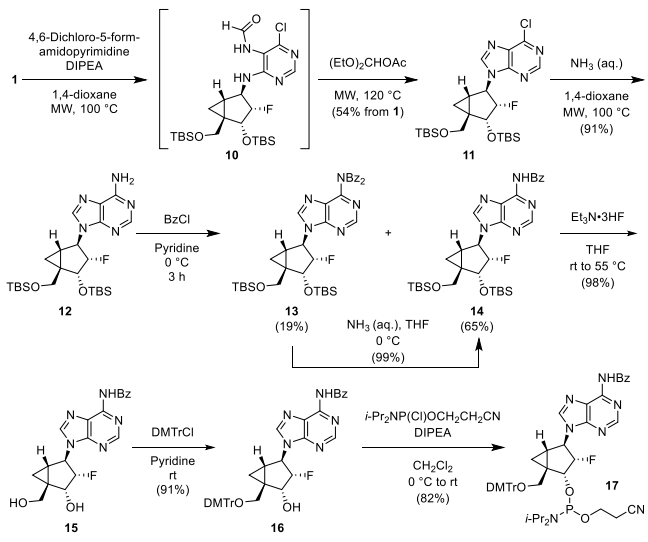
To synthesize the purine analogs, Jung et al. attempted to introduce the *N*-benzoyladenine base into the unsubstituted enone derivative of the NMC scaffold by 1,4-addition. The

Scheme 1. Synthesis of 2′-F-NMC U and C Amidites



yield of the reaction for this adenine precursor was low, and synthesis of 2′-F-NMC purine analogs was not pursued.[18] This prompted us to explore alternate routes. Nencka et al. reported a one-pot build-up procedure leading to 6-chloro- or 2-amino-6-chloropurines, which are, respectively, adenosine and guanosine precursors, from a primary amine.[41] Marquez et al. also achieved excellent yields of bicyclo[3.1.0]hexane carbocyclic nucleosides, which involve 6-chloropurines on a primary amine, using a microwave reactor.[42] Therefore, we evaluated the synthesis of the 2′-F-NMC purine phosphoramidites from the starting amine **1**.[18] As shown in Scheme 2, the reaction of **1** with 4,6-dichloro-5-formamidopyrimidine followed by cyclization of the formamido intermediate **10** in the presence of diethoxymethyl acetate gave the 6-chloropurine derivative **11** in 54% yield over two steps. Ammonolysis of the compound **11** using a microwave reactor

Scheme 2. Synthesis of 2′-F-NMC A Amidite

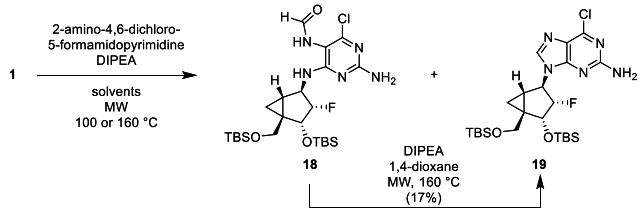


DOI: 10.1021/acs.orglett.8b04153
Org. Lett. 2019, 21, 1963−1967

produced the desired adenine nucleoside **12** in 91% yield. The amino group was then protected with BzCl to furnish the dibenzoyl derivative **13** (19%) and monobenzoyl derivative **14** (65%). Compound **13** was readily converted to **14** by treatment with aqueous NH_3 in THF. The silyl protection was then removed by treating **14** with $Et_3N{\cdot}3HF$ at 55 °C, to obtain diol **15** in 98% yield. Subsequent dimethoxytritylation of the 5′-OH followed by phosphitylation of **16** yielded the desired phosphoramidite **17** (82%).

For the synthesis of 2′-F-NMC G, various conditions were evaluated for the preparation of the 2-amino-6-chloropurine intermediate **19** using the microwave-assisted reaction of the amine **1** with 2-amino-4,6-dichloro-5-formamidopyrimidine (Table 1). The reaction in 1,4-dioxane at 100 °C led to only

Table 1. Construction of 2′-F-NMC G Precursor 19

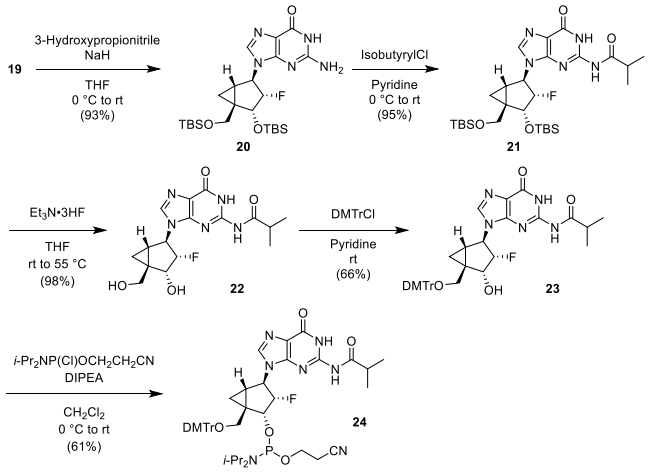


				yield (%)	
entry	solvent	temp (°C)	time (h)	**18**	**19**
1	1,4-dioxane	100	12	88	trace
2	toluene	160	2	70	24
3	*n*-BuOH	160	2	59	40

the intermediate **18** as a mixture of rotamers (entry 1). By heating at 160 °C in toluene, the intermediate **18** and the desired product **19** were obtained in 70% and 24% yield, respectively (entry 2). The yield of **19** was improved to 40% by changing the solvent to *n*-BuOH (entry 3). However, longer reaction times resulted only in substitution of 6-Cl groups by *n*-BuOH. Attempts to cyclize intermediate **18** in 1,4-dioxane by heating under microwave conditions resulted in disappointingly low yields (17%). The presence of the bulky 5′-*O*-TBS group may hinder imidazole ring closure, resulting in poor yields. Attempts to use diethoxymethyl acetate for the cyclization yielded a complex mixture, including **19**, as shown by LC-MS (data not shown). 2′-F-NMC G was eventually synthesized from the 2-amino-6-chloropurine derivative **19** by reacting with 3-hydroxypropionitrile in the presence of NaH (Scheme 3). Standard protection of exocyclic amine using isobutyryl chloride afforded protected nucleoside **21** (95%). Desilylation using $Et_3N{\cdot}3HF$ gave diol **22** in 98% yield. Dimethoxytritylation gave DMTr-protected compound **23** (66%), which was converted to the corresponding phosphoramidite **24** in 61% yield.

To gauge the effect of modified nucleotides on RNA affinity, the 2′-F-NMC modifications were incorporated into 10-mer and 12-mer oligoribonucleotides via standard solid-phase synthesis (see Supporting Information). The 2′-F-NMC-modified oligonucleotides were mixed with complementary RNA in PBS buffer, and the melting temperatures (T_m) were determined (Table 2). Modified duplexes containing a single 2′-F-NMC nucleotide at the center showed a decrease of at most 1.3 °C in the melting temperature compared to the unmodified duplex (Table 2, Entry 1−4). In a similar context, incorporation of 2′-F-RNA-modified nucleotides resulted in

Scheme 3. Synthesis of 2′-F-NMC G Amidite



slight improvements in the thermal stability. These experiments demonstrate that the 2′-F-NMC modification is well tolerated in an RNA duplex. The slight loss in thermal stability may prove advantageous in RNAi-based applications where high binding affinities in the seed region can induce undesirable off-target effects.[43] Importantly, a 10-mer duplex with six 2′-F-NMC nucleotides in the center displayed thermal stability comparable to that of the unmodified dsRNA (entry 5) suggesting that this modification is very well accommodated in an RNA:RNA duplex.

Table 2. UV Melting Temperatures of Modified Duplexes

entry	duplex	T_m[a] (ΔT_m) (°C)[b] F-NMC	2′-F
1	5′-UACAG**U**CUAUGU 3′-AUGUCAGAUACA	53.4 (−0.2)	54.1 (0.5)
2	5′-UACAGUCUAUGU 3′-AUGUC**A**GAUACA	53.4 (−0.2)	54.2 (0.6)
3	5′-UACAGU**C**UAUGU 3′-AUGUCAGAUACA	52.3 (−1.3)	54.3 (0.7)
4	5′-UACAGUCUAUGU 3′-AUGUCA**G**AUACA	53.1 (−0.5)	54.5 (0.9)
5[c]	5′-GC**GAUCUC**AC 3′-CGCUAGAGUG	57.4 (−0.2)	65.3 (7.7)

[a] T_m values were obtained in PBS (pH 7.4) using 2.0 μM concentrations of each strand. Red letters indicate sites of modification. [b] ΔT_m is the difference in melting temperature between the duplex with the modified strand and the unmodified reference duplex (5′-UACAGUCUAUGU-3′:3′-AUGUCAGAUACA-5′, T_m = 53.6 °C). [c] Unmodified reference duplex for entry 5 is 5′-GCGAUCUCAC-3′:3′-CGCUAGAGUG-5′ with T_m = 57.6 °C.

Next, the thermal stability of duplexes carrying 2′-F-NMC base pairs was determined (Table S2). Interestingly, in this context the $U^{F\text{-}NMC}$–$A^{F\text{-}NMC}$ base pair was better accommodated (a drop of only 0.4 °C compared to unmodified RNA) than a $C^{F\text{-}NMC}$–$G^{F\text{-}NMC}$ base pair (a drop of 2.2 °C compared to unmodified RNA).

The global conformations of RNA duplexes with one or two 2′-F-NMC nucleotides were evaluated using circular dichroism (CD) spectroscopy. The CD spectra of modified duplexes were comparable to that of the unmodified RNA and featured a strong positive band at around 260 nm and a negative band

DOI: 10.1021/acs.orglett.8b04153
Org. Lett. 2019, 21, 1963−1967

at around 210 nm characteristic of an A-form duplex (Figure 2). This indicates that the 2′-F-NMC modification does not significantly distort the global geometry of an RNA:RNA duplex.

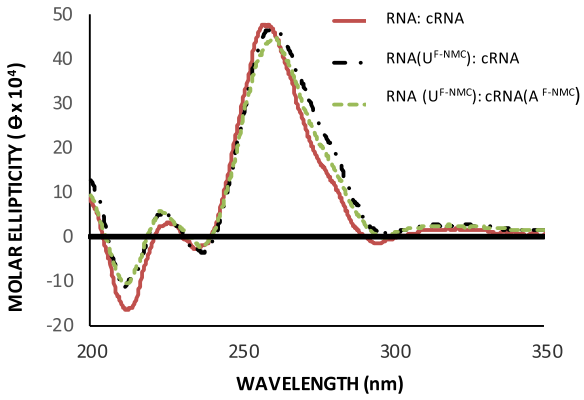


Figure 2. CD spectra of 2′-F-NMC-modified RNA and complementary RNA (cRNA) duplex at 15 °C in PBS (pH 7.4). See Table S3 for the details of the duplexes.

To determine the effect of the 2′-F-NMC modification on degradation of oligonucleotides by exonucleases, we incorporated 2′-F-NMC C ($C^{F\text{-}NMC}$) or 2′-F-RNA C (C^{F}) at the terminus or at the penultimate position of a dT oligonucleotide. The oligonucleotide modified at the 3′ end with 2′-F-NMC ($dT_{19}C^{F\text{-}NMC}$) was more resistant to degradation by snake venom phosphodiesterase (SVPD) than was the oligonucleotide modified with 2′-F-RNA (Figure 3A). The

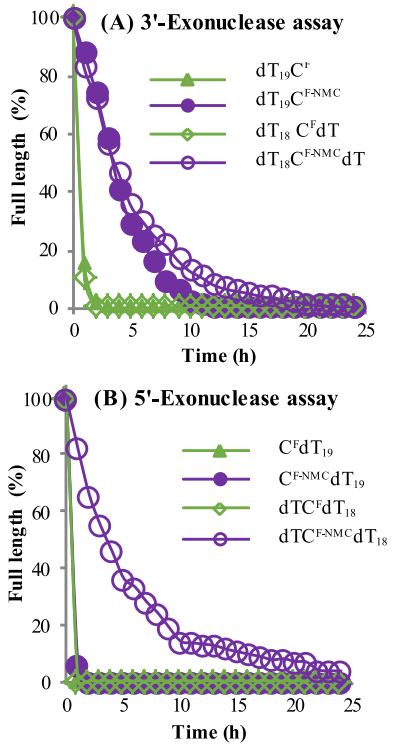


Figure 3. HPLC quantification of indicated full-length oligonucleotide after incubation with (A) SVPD and (B) PDE-II as a function of time. For $dT_{18}C^{F\text{-}NMC}dT$, the percentage of 19-mer $dT_{18}C^{F\text{-}NMC}$ remaining is plotted.

half-life of the 2′-F-NMC-modified oligonucleotide was around 90 min, whereas the half-life of $dT_{19}C^{F}$ was 15 min. The 3′-terminal dT in the oligonucleotide with the 2′-F-NMC residue at the penultimate position relative to the 3′ end ($dT_{18}C^{F\text{-}NMC}dT$) was lost within an hour; however, $dT_{18}C^{F\text{-}NMC}$ had a half-life of 170 min, whereas $dT_{18}C^{F}dT$ and $dT_{19}C^{F}$ had half-lives of around 15 min.

Oligonucleotides modified at the 5′ ends were incubated with the 5′-exonuclease phosphodiesterase II (PDE-II). Unlike the 3′-end-modified $dT_{18}C^{F\text{-}NMC}dT$, removal of the 5′-terminal dT was not observed when $dTC^{F\text{-}NMC}dT_{18}$ was incubated with PDE-II. Rather, the full-length 20-mer oligonucleotide with a penultimate $C^{F\text{-}NMC}$ was very stable with a half-life of 5 h (Figure 3B). Surprisingly, $C^{F\text{-}NMC}dT_{19}$ was degraded rapidly with a half-life of 12 min. This indicates that 2′-F-NMC modification improves stability at the penultimate position. Nevertheless, the 2′-F-NMC-modified oligonucleotides were more stable than 2′-F-RNA-modified oligonucleotides, as $C^{F}dT_{19}$ and $dTC^{F}dT_{18}$ were completely degraded by the first time point (1 h) after addition of PDE-II.

In summary, we present efficient routes for convergent synthesis of the four 2′-F-NMC ribonucleosides. 2′-F-NMC residues are well accommodated in double-stranded RNAs and do not alter the global structure of the duplex. 2′-F-NMC oligonucleotides are more resistant to nuclease degradation than are oligonucleotides modified with 2′-F-RNA. The higher lipophilicity of an oligonucleotide containing 2′-F-NMC residues compared to that with 2′-F-RNA residues (Figure S2) may result in improved cellular uptake and endosomal release of RNAs.[44] Having access to all four phosphoramidites will allow exploration of the full potential of 2′-F-NMC modification in therapeutic oligonucleotides such as siRNAs. These studies are ongoing in our laboratories.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b04153.

Experimental, compound characterization, and assays (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: mmanoharan@alnylam.com.

ORCID

Pawan Kumar: 0000-0001-7936-4613
Rajat S. Das: 0000-0003-3169-6218
Martin Egli: 0000-0003-4145-356X
Muthiah Manoharan: 0000-0002-7931-1172

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We dedicate this work to our long-time advisor, Professor Michael E. Jung (UCLA), for his numerous contributions to chemistry, medicinal chemistry, and nucleic acid chemistry. We are grateful to the Alnylam Research Group for their support, Drs. Ivan Zlatev and K.G. Rajeev for critical reading of the manuscript, Dr. Anne Noronha (Concordia University) for

DOI: 10.1021/acs.orglett.8b04153
Org. Lett. 2019, 21, 1963−1967

CD spectral measurements, and Ms. Nikki M. Ramos for excellent administrative help.

REFERENCES

(1) https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm616518.htm.
(2) Bumcrot, D.; Manoharan, M.; Koteliansky, V.; Sah, D. W. *Nat. Chem. Biol.* **2006**, *2*, 711.
(3) Allerson, C. R.; Sioufi, N.; Jarres, R.; Prakash, T. P.; Naik, N.; Berdeja, A.; Wanders, L.; Griffey, R. H.; Swayze, E. E.; Bhat, B. *J. Med. Chem.* **2005**, *48*, 901.
(4) Manoharan, M.; Akinc, A.; Pandey, R. K.; Qin, J.; Hadwiger, P.; John, M.; Mills, K.; Charisse, K.; Maier, M. A.; Nechev, L.; Greene, E. M.; Pallan, P. S.; Rozners, E.; Rajeev, K. G.; Egli, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 2284.
(5) Pallan, P. S.; Greene, E. M.; Jicman, P. A.; Pandey, R. K.; Manoharan, M.; Rozners, E.; Egli, M. *Nucleic Acids Res.* **2011**, *39*, 3482.
(6) Kawasaki, A. M.; Casper, M. D.; Freier, S. M.; Lesnik, E. A.; Zounes, M. C.; Cummins, L. L.; Gonzalez, C.; Cook, P. D. *J. Med. Chem.* **1993**, *36*, 831.
(7) Monia, B. P.; Lesnik, E. A.; Gonzalez, C.; Lima, W. F.; McGee, D.; Guinosso, C. J.; Kawasaki, A. M.; Cook, P. D.; Freier, S. M. *J. Biol. Chem.* **1993**, *268*, 14514.
(8) Ng, E. W.; Shima, D. T.; Calias, P.; Cunningham, E. T., Jr.; Guyer, D. R.; Adamis, A. P. *Nat. Rev. Drug Discovery* **2006**, *5*, 123.
(9) Davis, S.; Propp, S.; Freier, S. M.; Jones, L. E.; Serra, M. J.; Kinberger, G.; Bhat, B.; Swayze, E. E.; Bennett, C. F.; Esau, C. *Nucleic Acids Res.* **2009**, *37*, 70.
(10) Pieken, W. A.; Olsen, D. B.; Benseler, F.; Aurup, H.; Eckstein, F. *Science* **1991**, *253*, 314.
(11) Patra, A.; Paolillo, M.; Charisse, K.; Manoharan, M.; Rozners, E.; Egli, M. *Angew. Chem., Int. Ed.* **2012**, *51*, 11863.
(12) Peng, C. G.; Damha, M. J. *Can. J. Chem.* **2008**, *86*, 881.
(13) Arnold, J. J.; Sharma, S. D.; Feng, J. Y.; Ray, A. S.; Smidansky, E. D.; Kireeva, M. L.; Cho, A.; Perry, J.; Vela, J. E.; Park, Y.; Xu, Y.; Tian, Y.; Babusis, D.; Barauskus, O.; Peterson, B. R.; Gnatt, A.; Kashlev, M.; Zhong, W.; Cameron, C. E. *PLoS Pathog.* **2012**, *8*, No. e1003030.
(14) Egli, M.; Pallan, P. S.; Allerson, C. R.; Prakash, T. P.; Berdeja, A.; Yu, J.; Lee, S.; Watt, A.; Gaus, H.; Bhat, B.; Swayze, E. E.; Seth, P. P. *J. Am. Chem. Soc.* **2011**, *133*, 16642.
(15) Seth, P. P.; Yu, J.; Jazayeri, A.; Pallan, P. S.; Allerson, C. R.; Ostergaard, M. E.; Liu, F.; Herdewijn, P.; Egli, M.; Swayze, E. E. *J. Org. Chem.* **2012**, *77*, 5074.
(16) Pallan, P. S.; Yu, J.; Allerson, C. R.; Swayze, E. E.; Seth, P.; Egli, M. *Biochemistry* **2012**, *51*, 7.
(17) Seth, P. P.; Pallan, P. S.; Swayze, E. E.; Egli, M. *ChemBioChem* **2013**, *14*, 58.
(18) Jung, M. E.; Dwight, T. A.; Vigant, F.; Ostergaard, M. E.; Swayze, E. E.; Seth, P. P. *Angew. Chem., Int. Ed.* **2014**, *53*, 9893.
(19) Ostergaard, M. E.; Dwight, T.; Berdeja, A.; Swayze, E. E.; Jung, M. E.; Seth, P. P. *J. Org. Chem.* **2014**, *79*, 8877.
(20) Istrate, A.; Medvecky, M.; Leumann, C. J. *Org. Lett.* **2015**, *17*, 1950.
(21) Martinez-Montero, S.; Deleavey, G. F.; Dierker-Viik, A.; Lindovska, P.; Ilina, T.; Portella, G.; Orozco, M.; Parniak, M. A.; Gonzalez, C.; Damha, M. J. *J. Org. Chem.* **2015**, *80*, 3083.
(22) Martinez-Montero, S.; Deleavey, G. F.; Martin-Pintado, N.; Fakhoury, J. F.; Gonzalez, C.; Damha, M. J. *ACS Chem. Biol.* **2015**, *10*, 2016.
(23) Kel'in, A. V.; Zlatev, I.; Harp, J.; Jayaraman, M.; Bisbe, A.; O'Shea, J.; Taneja, N.; Manoharan, R. M.; Khan, S.; Charisse, K.; Maier, M. A.; Egli, M.; Rajeev, K. G.; Manoharan, M. *J. Org. Chem.* **2016**, *81*, 2261.
(24) Ostergaard, M. E.; Nichols, J.; Dwight, T. A.; Lima, W.; Jung, M. E.; Swayze, E. E.; Seth, P. P. *Mol. Ther.–Nucleic Acids* **2017**, *7*, 20.
(25) Istrate, A.; Katolik, A.; Istrate, A.; Leumann, C. J. *Chem. - Eur. J.* **2017**, *23*, 10310.
(26) Malek-Adamian, E.; Guenther, D. C.; Matsuda, S.; Martinez-Montero, S.; Zlatev, I.; Harp, J.; Burai Patrascu, M.; Foster, D. J.; Fakhoury, J.; Perkins, L.; Moitessier, N.; Manoharan, R. M.; Taneja, N.; Bisbe, A.; Charisse, K.; Maier, M.; Rajeev, K. G.; Egli, M.; Manoharan, M.; Damha, M. J. *J. Am. Chem. Soc.* **2017**, *139*, 14542.
(27) Harp, J. M.; Guenther, D. C.; Bisbe, A.; Perkins, L.; Matsuda, S.; Bommineni, G. R.; Zlatev, I.; Foster, D. J.; Taneja, N.; Charisse, K.; Maier, M. A.; Rajeev, K. G.; Manoharan, M.; Egli, M. *Nucleic Acids Res.* **2018**, *46*, 8090.
(28) Malek-Adamian, E.; Patrascu, M. B.; Jana, S. K.; Martínez-Montero, S.; Moitessier, N.; Damha, M. J. *J. Org. Chem.* **2018**, *83*, 9839.
(29) Frei, S.; Istrate, A.; Leumann, C. J. *Beilstein J. Org. Chem.* **2018**, *14*, 3088.
(30) Schlegel, M. K.; Foster, D. J.; Kel'in, A. V.; Zlatev, I.; Bisbe, A.; Jayaraman, M.; Lackey, J. G.; Rajeev, K. G.; Charisse, K.; Harp, J.; Pallan, P. S.; Maier, M. A.; Egli, M.; Manoharan, M. *J. Am. Chem. Soc.* **2017**, *139*, 8537.
(31) Goyenvalle, A.; Leumann, C.; Garcia, L. *J. Neuromuscul. Dis.* **2016**, *3*, 157.
(32) Shen, X.; Corey, D. R. *Nucleic Acids Res.* **2018**, *46*, 1584.
(33) Altmann, K.-H.; Kesselring, R.; Francotte, E.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 2331.
(34) Siddiqui, M. A.; Ford, H.; George, C.; Marquez, V. E. *Nucleosides Nucleotides* **1996**, *15*, 235.
(35) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J.; Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739.
(36) Maier, M. A.; Choi, Y.; Gaus, H.; Barchi, J. J., Jr.; Marquez, V. E.; Manoharan, M. *Nucleic Acids Res.* **2004**, *32*, 3642.
(37) Terrazas, M.; Ocampo, S. M.; Perales, J. C.; Marquez, V. E.; Eritja, R. *ChemBioChem* **2011**, *12*, 1056.
(38) Pallan, P. S.; Marquez, V. E.; Egli, M. *Biochemistry* **2012**, *51*, 2639.
(39) Shaw, G.; Warrener, R. N. *J. Chem. Soc.* **1958**, 157.
(40) Umemiya, H.; Kagechika, H.; Hashimoto, Y.; Shudo, K. *Nucleosides Nucleotides* **1996**, *15*, 465.
(41) Dejmek, M.; Kovačková, S.; Zborníková, E.; Hřebabecký, H.; Šála, M.; Dračínský, M.; Nencka, R. *RSC Adv.* **2012**, *2*, 6970.
(42) Saneyoshi, H.; Deschamps, J. R.; Marquez, V. E. *J. Org. Chem.* **2010**, *75*, 7659.
(43) Janas, M. M.; Schlegel, M. K.; Harbison, C. E.; Yilmaz, V. O.; Jiang, Y.; Parmar, R.; Zlatev, I.; Castoreno, A.; Xu, H.; Shulga-Morskaya, S.; Rajeev, K. G.; Manoharan, M.; Keirstead, N. D.; Maier, M. A.; Jadhav, V. *Nat. Commun.* **2018**, *9*, 723.
(44) Wolfrum, C.; Shi, S.; Jayaprakash, K. N.; Jayaraman, M.; Wang, G.; Pandey, R. K.; Rajeev, K. G.; Nakayama, T.; Charrise, K.; Ndungo, E. M.; Zimmermann, T.; Koteliansky, V.; Manoharan, M.; Stoffel, M. *Nat. Biotechnol.* **2007**, *25*, 1149.

DOI: 10.1021/acs.orglett.8b04153
Org. Lett. 2019, 21, 1963−1967

Synthesis and Biophysical Characterization of RNAs Containing 2′-Fluorinated *Northern* Methanocarbacyclic Nucleotides

Masaaki Akabane-Nakata, Pawan Kumar, Rajat S. Das, Namrata D. Erande, Shigeo Matsuda, Martin Egli,[†] and Muthiah Manoharan*

Alnylam Pharmaceuticals, 300 Third Street, Cambridge, Massachusetts 02142, United States
[†]Department of Biochemistry, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232, United States
*email: mmanoharan@alnylam.com

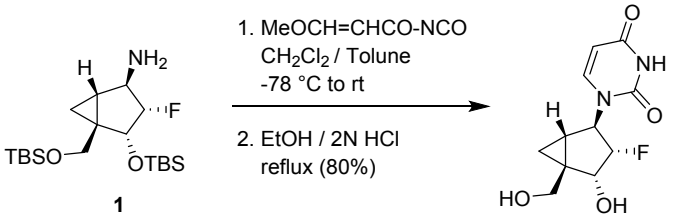
Table of Contents

Synthetic procedures and compound characterization

General conditions

TLC was performed on Merck silica gel 60 plates coated with F_{254}. Compounds were visualized under UV light (254 nm) or after spraying with the *p*-anisaldehyde staining solution followed by heating. Flash column chromatography was performed using a Teledyne ISCO Combi Flash system with pre-packed RediSep Teledyne ISCO silica gel cartridges. All moisture-sensitive reactions were carried out under anhydrous conditions using dry glassware, anhydrous solvents, and argon atmosphere. The microwave reactions were performed using a Discover® SP microwave system (CEM corporation) and heated in sealed glass tubes at 200 W with a 30 sec premixing time. The reaction temperature was monitored with an internal infrared probe. All commercially available reagents and solvents were purchased from Sigma-Aldrich unless otherwise stated and were used as received. ESI-MS spectra were recorded on a Waters Qtof Premier instrument using the direct flow injection mode. ^{1}H NMR spectra were recorded at 400 or 500 MHz. ^{13}C NMR spectra were recorded at 101 or 126 MHz. ^{19}F NMR spectra were recorded at 470 MHz. ^{31}P NMR spectra were recorded at 202 MHz. Chemical shifts are given in ppm referenced to the solvent residual peak (DMSO-d_6 – ^{1}H: δ at 2.50 ppm and ^{13}C δ at 39.5 ppm; acetone-d_6 – ^{1}H: δ at 2.05 ppm and ^{13}C δ at 29.8 and 206.3 ppm; CD_3CN – ^{1}H: δ at 1.94 ppm and ^{13}C δ at 1.32 and 118.3 ppm). Coupling constants are given in Hertz. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), septet (sept), broad signal (brs), or multiplet (m).

Synthesis of compound 2

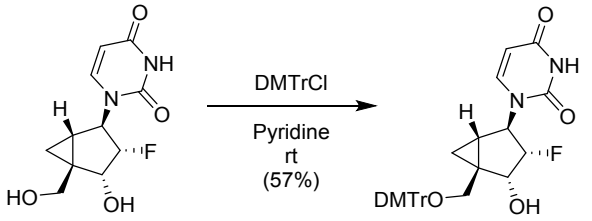


1 **2**

3′-Methoxy acrylic acid (1.07 g, 10.5 mmol) was dissolved in CH_2Cl_2 (15 mL). It was cooled to 0 °C in an ice bath, and oxalyl chloride (4.43 mL, 52.3 mmol) was added to the solution dropwise. The solution was allowed to warm to room temperature and was stirred for 16 h. Solvent was removed, and the residual solid was kept under high vacuum for 10 min. This acid chloride was dissolved in toluene (30 mL). To the solution was added silver cyanate (1.88 g, 12.6 mmol) followed by refluxing for 2 h. After cooling to room temperature, the liquid was carefully filtered through 0.2-µm syringe filter and then cooled to -78 °C. Compound **1**, which was made following Jung et al.,[1] (1.36 g, 3.49 mmol) in CH_2Cl_2 (10 mL) was added dropwise to this solution over 5 min. The solution was then warmed to room temperature and stirred for 18 h. The reaction was quenched by adding EtOH. The mixture was filtered and evaporated to a syrup. The residue was dissolved in 2 N HCl (8 mL) and MeOH (25 mL) and was refluxed at 90 °C for 16 hours. After excess solvent was removed, the crude residue was purified by column chromatography on silica gel (0–15% MeOH in CH_2Cl_2) to provide compound **2** as white powder (710 mg, 80%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 5.55 (dd, J = 8.0 and 1.8 Hz, 1H), 4.98 (brs, 2H), 4.75 (d, J = 17.6 Hz, 1H), 4.41–4.58 (m, 2H), 4.01 (d, J = 11.4 Hz, 1H), 3.08 (d, J = 11.5 Hz, 1H), 1.33 (dd, J = 8.9 and 3.6 Hz, 1H), 0.95–0.97 (m, 1H), 0.65-0.69 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.2, 150.8, 141.4, 101.4, 95.3 (d, J = 190.3 Hz), 69.9 (d, J = 16.4 Hz), 61.31, 59.9 (d, J = 27.7 Hz), 36.2, 20.6, 10.9 (d, J = 3.8 Hz). ^{19}F NMR (470 MHz, DMSO-d_6) δ -187.57 – -187.37 (m). HRMS calc. for $C_{11}H_{14}FN_2O_4$ $[M + H]^+$ 257.0932, found 257.0948.

Synthesis of compound 3



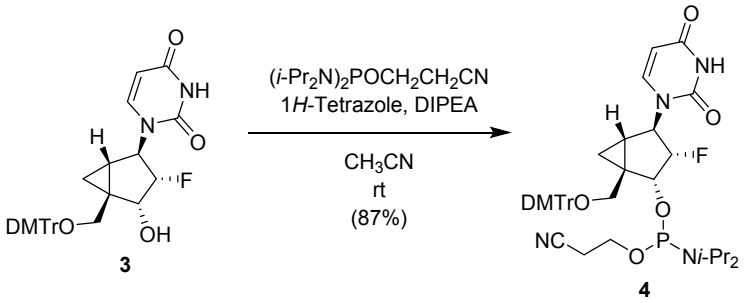
2 **3**

To a solution of compound **2** (0.858 g, 3.35 mmol) in pyridine (5 mL), 4,4′-dimethoxytrityl chloride (1.48 g, 4.36 mmol) was added. The mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of MeOH. After excess solvent was removed, the residue was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$ (aq.) and brine. The organic layer was dried with Na_2SO_4 and purified by column chromatography on silica gel (30–80 % ethyl acetate in hexanes). Compound **3** was obtained as white foam (1.07 g, 57%).

^{1}H NMR (400 MHz, acetone-d_6) δ 10.07 (s, 1H), 8.11 (d, J = 8.1 Hz, 1H), 7.48–7.50 (m, 2H), 7.24–7.38 (m, 7H), 6.91 (d, J = 8.5 Hz, 4H), 5.26 (d, J = 8.0 Hz, 1H), 4.96–5.06 (m, 2H), 4.74

(dd, J = 50.5 and 5.4 Hz, 1H), 4.01–4.15 (m, 2H), 3.79 (s, 6H), 2.76 (d, J = 10.1 Hz, 1H), 1.39 (dd, J = 8.9 and 3.7 Hz, 1H), 1.13–1.16 (m, 1H), 0.60–0.65 (m, 1H). ^{13}C NMR (126 MHz, acetone-d_6) δ 163.6, 159.8, 159.7, 151.7, 150.7, 146.2, 142.2, 136.9, 136.6, 131.1, 131.0, 129.0, 128.8, 127.8, 124.6, 114.1, 114.1, 102.8, 96.3 (d, J = 190.3 Hz), 87.4, 72.5 (d, J = 16.4 Hz), 65.0, 61.1 (d, J = 26.5 Hz), 55.6, 35.6, 22.9, 11.5 (d, J = 7.6 Hz). ^{19}F NMR (470 MHz, acetone-d_6) δ -187.37 (ddd, J = 52.5, 19.4 and 19.4 Hz). HRMS calc. for $C_{32}H_{31}FN_2NaO_6$ $[M + Na]^+$ 581.2064, found 581.2062.

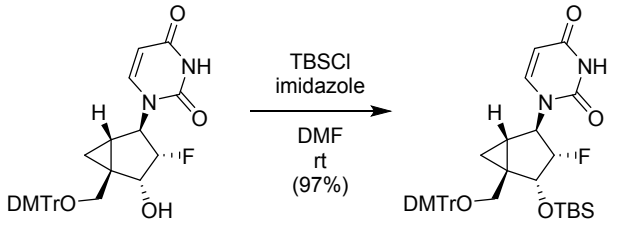
Synthesis of compound 4



To a solution of compound **3** (1.12 g, 2.00 mmol) in CH_3CN (20 mL) were added DIPEA (0.418 mL, 2.40 mmol), 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (0.669 mL, 3.00 mmol) and 1*H*-tetrazole (0.45 M in CH_3CN, 4.88 mL, 2.20 mmol). The solution was stirred at room temperature for 3 h. The reaction was quenched with MeOH. The solvents were removed under reduced pressure, and the residue was dissolved in CH_2Cl_2, washed with saturated $NaHCO_3$ (aq.), and dried with Na_2SO_4. The volume of CH_2Cl_2 was reduced to 5 mL, and the mixture was added dropwise to 300 mL stirring hexanes. The precipitated white solid was collected and dried under high vacuum. Compound **4** was obtained as white powder (1.31 g, 87%).

^{1}H NMR (500 MHz, CD_3CN) δ 9.29 (brs, 1H), 7.98–8.03 (m, 1H), 7.42–7.47 (m, 2H), 7.25–7.35 (m, 7H), 6.86–6.89 (m, 4H), 5.13–5.16 (m, 1H), 4.89–5.09 (m, 2H), 4.67–4.80 (m, 1H), 3.57–3.93 (m, 11H), 2.74 (d, J = 10.3 Hz, 0.5H), 2.68 (d, J = 10.2 Hz, 0.5H), 2.65 (t, J = 6.0 Hz, 1H), 2.55 (t, J = 6.0 Hz, 1H), 1.22–1.26 (m, 1H), 1.08–1.20 (m, 13H), 0.61–0.66 (m, 1H). ^{13}C NMR (101 MHz, CD_3CN) δ 164.1, 164.1, 159.8, 159.8, 151.9, 151.9, 146.1, 146.1, 142.8, 142.6, 136.7, 136.7, 136.5, 131.3, 131.2, 129.1, 129.1, 129.0, 129.0, 128.1, 128.0, 119.8, 119.5, 114.2, 114.2, 102.9, 97.0, 96.0, 95.1, 94.1, 87.5, 87.5, 74.3, 74.2, 74.1, 74.0, 73.5, 73.4, 73.2, 64.2, 61.9, 61.6, 59.9, 59.7, 59.2, 59.0, 56.0, 56.0, 44.3, 44.2, 44.1, 44.1, 44.0, 43.9, 35.5, 35.4, 35.4, 35.3, 25.2, 25.1, 25.1, 25.1, 25.0, 24.9, 24.9, 22.7, 21.2, 21.2, 21.1, 21.1, 12.1, 12.1, 12.1, 12.0. ^{19}F NMR (470 MHz, CD_3CN) δ -186.32 – -186.10 (m), -185.78 – -185.57 (m). ^{31}P NMR (202 MHz, CD_3CN) δ 151.49 (d, J = 12.1 Hz), 151.37 (d, J = 8.1 Hz). HRMS calc. for $C_{41}H_{49}FN_4O_7P$ $[M + H]^+$ 759.3317, found 759.3344.

Synthesis of compound 5

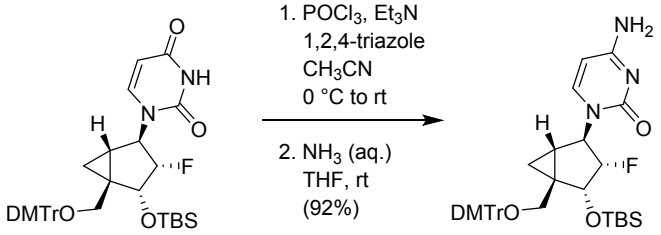


3 **5**

To a solution of compound **3** (1.70 g, 3.04 mmol) in DMF (30 mL) were added *tert*-butyldimethylsilyl chloride (688 mg, 4.56 mmol) and imidazole (622 mg, 9.13 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was quenched with water and then diluted with diethyl ether. The organic layer was washed with water and brine, dried with Na_2SO_4, and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–50% ethyl acetate in hexanes). Compound **5** was isolated as a white foam (1.98 g, 97%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.40 (s, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.17–7.33 (m, 9H), 6.87–6.90 (m, 4H), 5.12 (d, J = 8.0 Hz, 1H), 4.67–4.85 (m, 3H), 3.72 (s, 6H), 3.61 (d, J = 10.1 Hz, 1H), 2.78 (d, J = 10.1 Hz, 1H), 1.41–1.44 (m, 1H), 0.93–0.95 (m, 1H), 0.73 (s, 9H), 0.62–0.67 (m, 1H), -0.02 (s, 3H), -0.08 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.2, 158.2, 158.2, 150.8, 144.5, 141.4, 135.1, 134.9, 129.9, 129.7, 127.9, 127.7, 126.9, 113.2, 113.1, 101.4, 93.9 (d, J = 193.9 Hz), 85.7, 71.6 (d, J = 16.2 Hz), 63.2, 60.3 (d, J = 26.3 Hz), 55.0, 34.7, 25.5, 21.0, 17.7, 10.8 (d, J = 7.1 Hz), -4.86, -5.37. ^{19}F NMR (470 MHz, DMSO-d_6) δ -187.66 (ddd, J = 50.0, 18.0 and 18.0 Hz). HRMS calc. for $C_{38}H_{45}FN_2NaO_6Si$ $[M + Na]^+$ 695.2923, found 695.2959.

Synthesis of compound 6



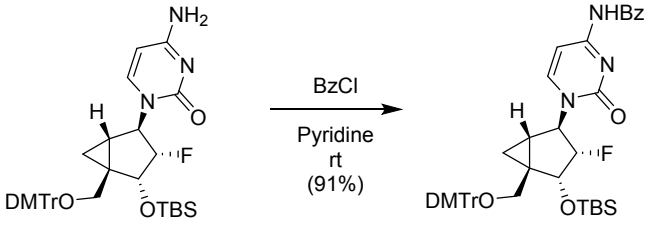
5 **6**

Phosphorus oxychloride (0.353 mL, 3.79 mmol) was added dropwise to a solution of 1,2,4-triazole (2.09 g, 30.3 mmol) and triethylamine (4.23 mL, 30.3 mmol) in CH_3CN (10 mL) maintained in an ice bath. After stirring for 30 min, a solution of compound **5** (850 mg, 1.26 mmol) in CH_3CN (5 mL) was added dropwise to the reaction. After stirring for 10 min at 0 °C, the reaction was stirred at room temperature for 4 h. The solvent was then removed under vacuum, and the residue was partitioned between ethyl acetate and water. The organic layer was washed with saturated $NaHCO_3$ (aq.) and brine, dried (Na_2SO_4), and concentrated under vacuum. The crude residue was dissolved in THF (10 mL), and NH_3 (aq.) (2.5 mL) was added. The reaction mixture was stirred at room temperature overnight and diluted with ethyl acetate. The solution was sequentially washed with water and brine, dried (Na_2SO_4), and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–10% MeOH in CH_2Cl_2) to give compound **6** as a white foam (779 mg, 92%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (d, J = 7.4 Hz, 1H), 7.17–7.34 (m, 11H), 6.88–6.90 (m, 4H), 5.54 (d, J = 7.4 Hz, 1H), 4.94 (d, J = 18.2 Hz, 1H), 4.82 (dd, J = 18.2 and 5.7 Hz, 1H), 4.51

(dd, J = 50.6 and 5.7 Hz, 1H), 3.73 (s, 6H), 3.54 (d, J = 10.1 Hz, 1H), 2.78 (d, J = 10.1 Hz, 1H), 1.41–1.44 (m, 1H), 0.92–0.96 (m, 1H), 0.60–0.71 (m, 10H), -0.04 (s, 3H), -0.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.5, 158.2, 155.2, 144.5, 141.7, 135.2, 129.8, 129.7, 127.9, 127.7, 126.9, 113.3, 113.2, 94.4 (d, J = 194.9 Hz), 94.1, 85.6, 71.5 (d, J = 15.2 Hz), 63.4, 60.6 (d, J = 25.3 Hz), 55.0, 34.6, 25.5, 21.4, 17.7, 10.9 (d, J = 7.1 Hz), -4.86, -5.37. ^{19}F NMR (470 MHz, DMSO-d_6) δ -186.59 (ddd, J = 50.4, 18.4 and 18.4 Hz). HRMS calc. for $C_{38}H_{47}FN_3O_5Si$ $[M + H]^+$ 672.3264, found 672.3278.

Synthesis of compound 7

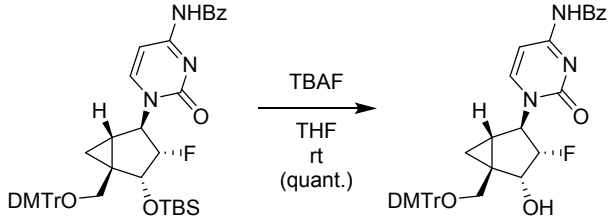


6 **7**

To a solution of compound **6** (650 mg, 0.967 mmol) in pyridine (10 mL) at 0 °C was added dropwise benzoyl chloride (0.168 mL, 1.45 mmol). The mixture was stirred at 0 °C for 3 h. The reaction mixture was diluted with ethyl acetate and washed with saturated $NaHCO_3$ (aq.), water, and brine, dried (Na_2SO_4), and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (25–75% ethyl acetate in hexanes) to give compound **7** as a white foam (663 mg, 88%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1H), 8.60 (d, J = 7.6 Hz, 1H), 7.99–8.01 (m, 2H), 7.49–7.64 (m, 3H), 7.22–7.36 (m, 10H), 6.90–6.92 (m, 4H), 5.02 (d, J = 17.6 Hz, 1H), 4.88 (dd, J = 5.8 and 17.6 Hz, 1H), 4.68 (dd, J = 5.8 and 50.6 Hz, 1H), 3.75 (s, 6H), 3.61 (d, J = 10.0 Hz, 1H), 2.81 (d, J = 10.0 Hz, 1H), 1.63–1.66 (m, 1H), 1.01–1.04 (m, 1H), 0.68–0.71 (m, 10H), -0.03 (s, 3H), -0.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.7, 163.2, 158.7, 158.7, 155.3, 150.8, 146.5, 144.5, 135.7, 135.65, 133.5, 133.2, 130.2, 128.9, 128.8, 128.4, 128.4, 127.4, 113.8, 113.7, 95.9 (d, J = 219.2 Hz), 93.3, 86.2, 71.8 (d, J = 13.1 Hz), 63.8, 62.1 (d, J = 21.2 Hz), 55.4, 35.3, 26.0, 21.4, 18.2, 11.5 (d, J = 6.1 Hz), -4.37, -4.94. ^{19}F NMR (470 MHz, DMSO-d_6) δ -187.76 (ddd, J = 50.7, 17.8 and 17.8 Hz). HRMS calc. for $C_{45}H_{51}FN_3O_6Si$ $[M + H]^+$ 776.3526, found 776.3508.

Synthesis of compound 8

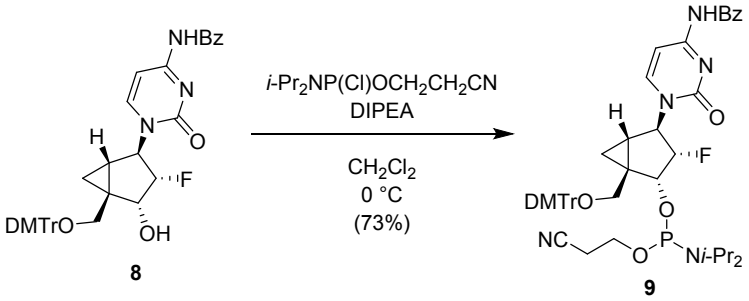


7 **8**

To a solution of compound **7** (570 mg, 0.735 mmol) in THF (10 mL) at 0 °C was added dropwise tetra-*n*-butylammonium fluoride (1 M in THF, 1.10 mL, 1.10 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum. The crude

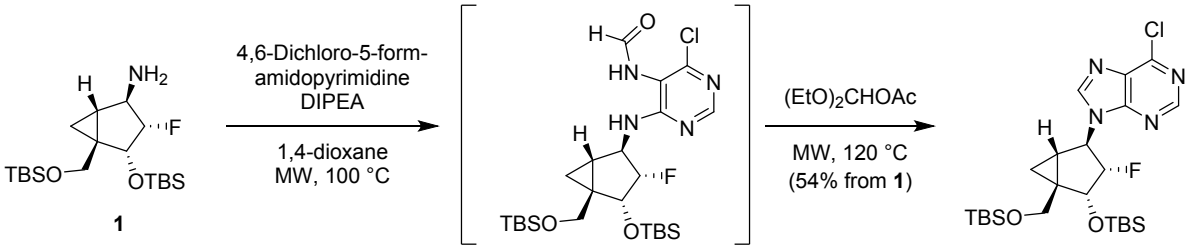
residue was purified by column chromatography on silica gel (50-100% ethyl acetate in hexanes) to give compound **8** as a white foam (485 mg, quant.).
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 8.50 (d, J = 7.5 Hz, 1H), 8.00–8.02 (m, 2H), 7.23–7.64 (m, 13H), 6.90– 6.94 (m, 4H), 5.09 (d, J = 8.1 Hz, 1H), 4.99 (d, J = 17.6 Hz, 1H), 4.79 (ddd, J = 8.1, 8.1 and 22.2 Hz, 1H), 4.60 (dd, J = .1 and 50.4 Hz, 1H), 3.76–4.81 (m, 7H), 2.62 (d, J = 10.1 Hz, 1H), 1.47– 1.49 (m, 1H), 1.04–1.07 (m, 1H), 0.59–0.61 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.8, 163.3, 158.6, 158.6, 155.2, 146.4, 144.9, 136.3, 136.0, 133.6, 133.2, 130.2, 130.0, 128.9, 128.9, 128.4, 128.3, 127.3, 113.8, 97.1, 95.0 (d, J = 191.9 Hz), 86.3, 70.7 (d, J = 17.2 Hz), 64.4, 61.8 (d, J = 27.3 Hz, 1H), 55.4 (d, J = 2.0 Hz), 34.6, 21.86, 11.5 (d, J = 7.1 Hz). ^{19}F NMR (470 MHz, DMSO-d_6) δ -187.19 (ddd, J = 50.1, 18.8 and 18.8 Hz). HRMS calc. for $C_{39}H_{37}FN_3O_6$ $[M + H]^+$ 662.2661, found 662.2690.

Synthesis of compound 9



To a solution of compound **8** (500 mg, 0.756 mmol) and DIPEA (0.395 mL, 2.27 mmol) in CH_2Cl_2 (9 mL) at 0 °C was added dropwise 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.202 mL, 0.907 mmol) and the mixture was stirred at 0 °C for 1 h. The reaction mixture was diluted with ethyl acetate and washed with saturated $NaHCO_3$ (aq.), water, and brine, dried (Na_2SO_4), and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (50–65% ethyl acetate in hexanes) to give compound **9** as a white foam (479 mg, 73%).
^{1}H NMR (400 MHz, CD_3CN) δ 9.31 (brs, 1H), 8.55–8.59 (m, 1H), 7.95–7.97 (m, 2H), 7.18–7.64 (m, 13H), 6.89–6.92 (m, 4H), 4.98–5.14 (m, 2H), 4.66–4.80 (m, 1H), 3.90–3.96 (m, 1H), 3.59–3.87 (m, 10H), 2.74 (d, J = 8.2 Hz, 0.4H), 2.69 (d, J = 8.2 Hz, 0.6H), 2.62 (t, J = 4.6 Hz, 0.8H), 2.53 (t, J = 4.6 Hz, 1.2H), 1.38–1.41 (m, 1H), 1.07–1.19 (m, 13H), 0.65–0.66 (m, 1H). ^{13}C NMR (101 MHz, CD_3CN) δ 168.20, 163.43, 163.36, 159.74, 156.11, 147.39, 147.24, 145.55, 145.50, 136.99, 136.84, 136.81, 136.78, 134.43, 133.85, 133.83, 131.07, 131.04, 131.00, 130.97, 129.61, 129.26, 129.19, 129.06, 129.04, 129.02, 128.04, 128.01, 119.67, 119.45, 114.21, 114.20, 97.76, 96.62, 95.57, 95.54, 94.69, 93.63, 93.61, 87.49, 87.43, 74.16, 74.03, 74.00, 73.88, 73.23, 73.08, 64.46, 63.28, 63.23, 63.01, 62.96, 59.77, 59.57, 59.09, 58.88, 55.91, 55.88, 44.18, 44.06, 44.01, 43.89, 35.48, 35.43, 35.35, 25.10, 25.06, 25.03, 24.99, 24.92, 24.84, 24.78, 22.78, 22.68, 21.08, 21.01, 12.34, 12.28, 12.21. ^{19}F NMR (470 MHz, CD_3CN) δ -186.47 – -186.24 (m), -185.74 – -185.53 (m). ^{31}P NMR (202 MHz, CD_3CN) δ 151.61(d, J = 13.9 Hz), 151.48 (d, J = 6.9 Hz). HRMS calc. for $C_{48}H_{54}FN_5O_7P$ $[M + H]^+$ 862.3739, found 862.3726.

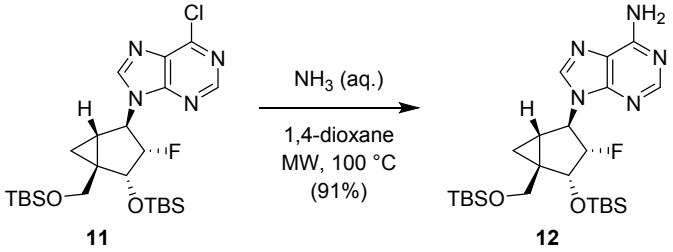
Synthesis of compound 11



To a solution of compound **1** (1.80 g, 4.62 mmol) and DIPEA (2.41 mL, 13.9 mmol) in 1,4-dioxane (20 mL) was added 4,6-dichloro-5-formamidopyrimidine (1.06 g, 5.54 mmol). The reaction mixture was stirred and heated in microwave reactor at 100 ºC for 6 h. The reaction was cooled to room temperature and concentrated under vacuum. The residue containing compound **10** was dissolved in diethoxymethyl acetate (10 mL), and the solution was stirred and heated in a microwave reactor at 120 ºC for 2 h. After concentration under vacuum, the crude residue was purified by column chromatography on silica gel (0–15% ethyl acetate in hexanes) to afford compound **11** as a yellow foam (1.32 g, 54%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (s, 1H), 8.76 (s, 1H), 5.12 (d, J = 15.6 Hz, 1H), 4.89 (brs, 1H), 4.80 (dd, J = 32.4 and 5.4 Hz, 1H), 4.19 (d, J = 10.8 Hz, 1H), 3.36 (d, J = 10.8 Hz, 1H), 1.79 (dd, J = 8.8 and 3.9 Hz, 1H), 1.15–1.18 (m, 1H), 0.93 (s, 9H), 0.85-0.90 (m, 10H), 0.10 (s, 6H), 0.06 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 151.5, 151.5, 158.2, 148.9, 144.5, 131.1, 93.4 (d, J = 192.8 Hz), 70.7 (d, J = 15.1 Hz), 63.3, 59.0 (d, J = 27.7 Hz), 35.9, 25.8, 25.6, 22.0, 17.9, 17.8, 10.8 (d, J = 7.6 Hz), -4.88, -5.27, -5.59, -5.66. ^{19}F NMR (470 MHz, DMSO-d_6) δ -189.95 – -189.76 (m). HRMS calc. for $C_{24}H_{41}ClFN_4O_2Si_2$ $[M + H]^+$ 527.2435, found 527.2441.

Synthesis of compound 12

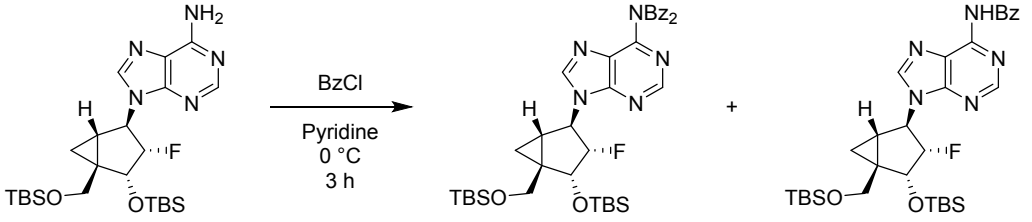


A mixture of compound **11** (100 mg, 0.190 mmol), ammonium hydroxide solution (2 mL), and 1,4-dioxane (2 mL) was stirred and heated in a microwave reactor at 100 ºC for 6 h. After the mixture was concentrated under vacuum, the crude residue was purified by column chromatography on silica gel (50–100% ethyl acetate in hexanes). Compound **12** was isolated as a white solid (87.3 mg, 91%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (s, 1H), 8.15 (s, 1H), 7.28 (brs, 2H), 4.98 (d, J = 16.3 Hz, 1H), 4.87 (dd, J = 19.9 and 5.1 Hz, 1H), 4.82 (dd, J = 50.7 and 5.1 Hz, 1H), 4.17 (d, J = 10.8 Hz, 1H), 3.33 (d, J = 10.8 Hz, 1H), 1.67 (dd, J = 8.4 and 3.9 Hz, 1H), 1.12 (dd, J = 3.9 and 3.9 Hz, 1H), 0.92 (s, 9H), 0.82– 0.86 (m, 10H), 0.09 (s, 6H), 0.07 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.0, 152.6, 149.0, 137.8, 118.7, 94.1 (d, J = 194.0 Hz), 70.7 (d, J = 16.4 Hz), 63.4, 58.1 (d, J = 26.5 Hz), 35.8, 25.8, 25.6, 22.3, 18.0, 17.9, 10.8 (d, J = 7.6 Hz), -4.85, -

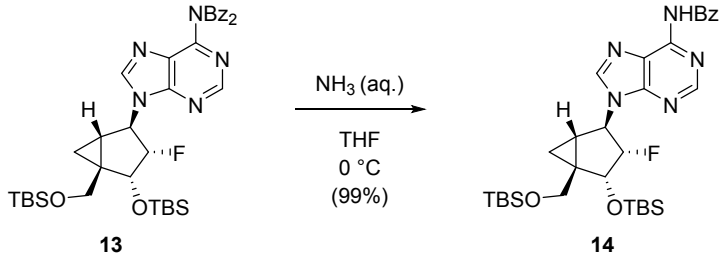
5.21, -5.50, -5.60. ^{19}F NMR (470 MHz, DMSO-d_6) δ -188.53 – -188.34 (ddd, J = 50.4, 18.4 and 18.4 Hz). HRMS calc. for $C_{24}H_{43}FN_5O_2Si_2$ $[M + H]^+$ 508.2934, found 508.2929.

Syntheses of compounds 13 and 14



To a solution of compound **12** (1.00 g, 1.97 mmol) in pyridine (20 mL) was added dropwise benzoyl chloride (0.274 mL, 2.36 mmol) at 0 °C, and the mixture was stirred at 0 °C for 3 h. The reaction was quenched with MeOH and concentrated under vacuum. The residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ (aq.), water, and brine, dried (Na_2SO_4), and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–100% ethyl acetate in hexanes). Compound **13** was isolated as a white foam (270 mg, 19%), and compound **14** was isolated as a white foam (786 mg, 65%).

Conversion of 13 to 14



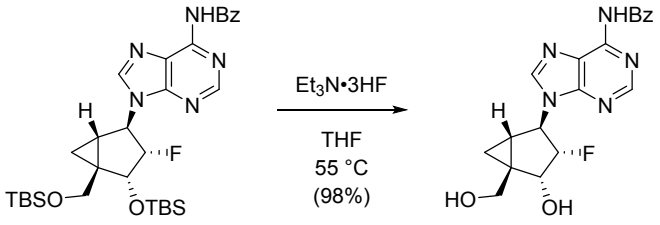
A mixture of compound **13** (180 mg, 0.251 mmol), ammonium hydroxide solution (2 mL), and THF (2 mL) was stirred at 0 °C for 3 h. The reaction mixture was diluted with ethyl acetate and washed with water and brine, dried (Na_2SO_4), and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (20–50% ethyl acetate in hexanes). The desired compound **14** was isolated as a white foam (152 mg, 99%).

Compound **13**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (s, 1H), 8.62 (s, 1H), 7.76 (d, J = 7.3 Hz, 4H), 7.57 (dd, J = 7.3 and 7.3 Hz, 2H), 7.44 (dd, J = 7.3 and 7.3 Hz, 4H), 5.10 (d, J = 16.1 Hz, 1H), 4.93 (dd, J = 19.8 and 5.6 Hz, 1H), 4.68 (dd, J = 14.0 and 5.6 Hz, 1H), 4.12 (d, J = 10.8 Hz, 1H), 3.38 (d, J = 10.8 Hz, 1H), 1.69 (dd, J = 8.9 and 3.9 Hz, 1H), 1.11–1.13 (m, 1H), 0.87 (s, 9H), 0.84–0.86 (m, 10H), 0.09 (s, 3H), 0.04 (s, 6H), 0.02 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.0, 152.5, 151.6, 150.7, 144.5, 133.6, 133.2, 128.9, 128.9, 127.0, 93.8 (d, J = 194.9 Hz), 70.9 (d, J = 16.2 Hz), 63.2, 59.2 (d, J = 26.3 Hz), 35.9, 25.8, 25.6, 22.1, 17.9, 17.8, 10.9 (d, J = 7.1 Hz), -4.85, -5.17, -5.51, -5.63. ^{19}F NMR (470 MHz, DMSO-d_6) δ -187.76 (dddd, J = 51.2, 39.5, 39.5 and 4.7 Hz). HRMS calc. for $C_{38}H_{51}FN_5O_4Si_2$ $[M + H]^+$ 716.3458, found 716.3480.

Compound **14**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.19 (s, 1H), 8.75 (s, 1H), 8.53 (s, 1H), 8.03 (d, J = 7.4 Hz, 2H), 7.64 (dd, J = 7.4 and 7.4 Hz, 1H), 7.54 (dd, J = 7.4 and 7.4 Hz, 2H), 5.13 (d, J = 16.0 Hz, 1H), 4.78–4.92 (m, 2H), 4.18 (d, J = 10.9 Hz, 1H), 3.38 (d, J = 10.9 Hz, 1H), 1.76

(dd, J = 8.8 and 3.7 Hz, 1H), 1.14–1.16 (m, 1H), 0.91 (s, 9H), 0.86–0.87 (m, 10H), 0.09 (s, 6H), 0.08 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.6, 152.0, 151.5, 150.2, 141.7, 133.5, 132.4, 128.5, 128.4, 125.5, 93.9 (d, J = 194.0 Hz), 70.8 (d, J = 15.1 Hz), 63.4, 58.6 (d, J = 26.5 Hz), 36.0, 25.9, 25.6, 22.0, 18.0, 17.8, 10.9 (d, J = 7.6 Hz), -4.83, -5.20, -5.46, -5.56. ^{19}F NMR (470 MHz, DMSO-d_6) δ -188.93 – -188.73 (m). HRMS calc. for $C_{31}H_{47}FN_5O_3Si_2$ [M + H]$^+$ 612.3196, found 612.3206.

Synthesis of compound 15

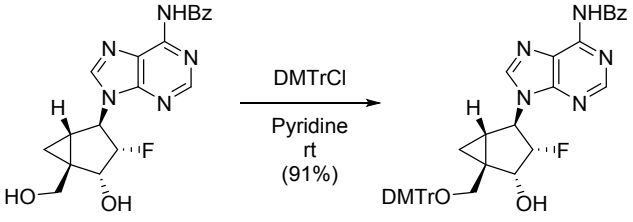


14 **15**

To a solution of compound **14** (750 mg, 1.23 mmol) in THF (12 mL) was added dropwise triethylamine trihydrofluoride (0.999 mL, 6.13 mmol) at room temperature, and the mixture was stirred at 55 °C for 6 h. The reaction mixture was concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–10% MeOH in CH_2Cl_2). Compound **15** was isolated as a white foam (463 mg, 98%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.18 (s, 1H), 8.75–8.76 (m, 2H), 8.03 (d, J = 7.4, 2H), 7.63 (dd, J = 7.4 and 7.4, 1H), 7.54 (dd, J = 7.4 and 7.4, 2H), 5.10–5.15 (m, 3H), 4.63–4.74 (m, 2H), 4.11 (dd, J = 11.5 and 5.4 Hz, 1H), 4.60 (dd, J = 11.5 and 4.7 Hz, 1H), 1.71 (dd, , J = 8.7 and 3.7 Hz, 1H), 1.15–1.17 (m, 1H), 0.79–0.82 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.6, 151.8, 151.5, 150.3, 142.2, 133.4, 132.4, 128.5, 128.4, 125.4, 94.9 (d, J = 191.5 Hz), 70.0 (d, J = 16.4 Hz), 61.5, 58.6 (d, J = 26.5 Hz, 1H), 35.8, 21.9, 11.2 (d, J = 7.6 Hz). ^{19}F NMR (470 MHz, DMSO-d_6) δ -188.79 – -188.59 (m). HRMS calc. for $C_{19}H_{19}FN_5O_3$ [M + H]$^+$ 384.1466, found 384.1471.

Synthesis of compound 16



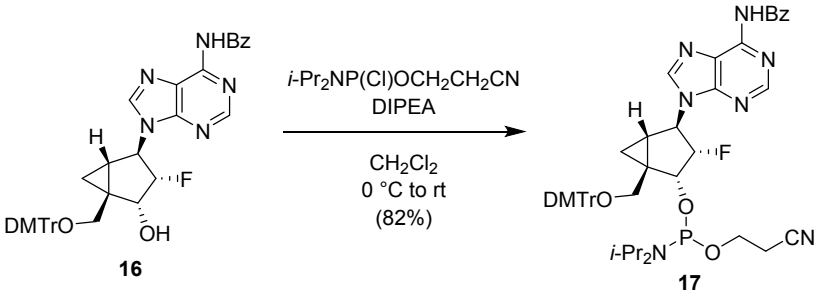
15 **16**

To a solution of compound **15** (400 mg, 1.04 mmol) in pyridine (10 mL) was added 4,4′-dimethoxytrityl chloride (424 mg, 1.25 mmol), and the mixture was stirred at room temperature overnight. The reaction was quenched with dry MeOH and concentrated under vacuum. The residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ (aq.), water, and brine, dried (Na_2SO_4), and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (50–100% ethyl acetate in hexanes) to give compound **16** as a white foam (650 mg, 91%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.22 (s, 1H), 8.71 (s, 1H), 8.61 (s, 1H), 8.04 (d, J = 7.3 Hz, 1H), 7.64 (dd, J = 7.3 and 7.3 Hz, 1H), 7.54 (dd, J = 7.3 and 7.3 Hz, 1H), 7.37–7.39 (m, 2H),

7.19–7.30 (m, 7H), 6.85–6.87 (m, 4H), 5.12–5.16 (m, 2H), 4.86–5.02 (m, 2H), 3.69–3.71 (m, 7H), 2.83 (d, J = 9.9 Hz, 1H), 1.67– 1.69 (m, 1H), 1.12–1.14 (m, 1H), 0.74–0.78 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.6, 158.0, 158.0, 151.9, 151.5, 150.3, 144.7, 142.3, 135.8, 135.6, 133.4, 132.4, 129.7, 129.6, 128.5, 128.4, 127.9, 127.7, 126.7, 125.6, 113.2, 94.7 (d, J = 190.3 Hz), 85.5, 70.9 (d, J = 16.4 Hz), 63.4, 59.2 (d, J = 27.7 Hz, 1H), 33.9, 22.5, 10.8 (d, J = 6.3 Hz). ^{19}F NMR (470 MHz, DMSO-d_6) δ -188.07 – -187.87 (m). HRMS calc. for $C_{40}H_{37}FN_5O_5$ [M + H]$^+$ 686.2773, found 686.2790.

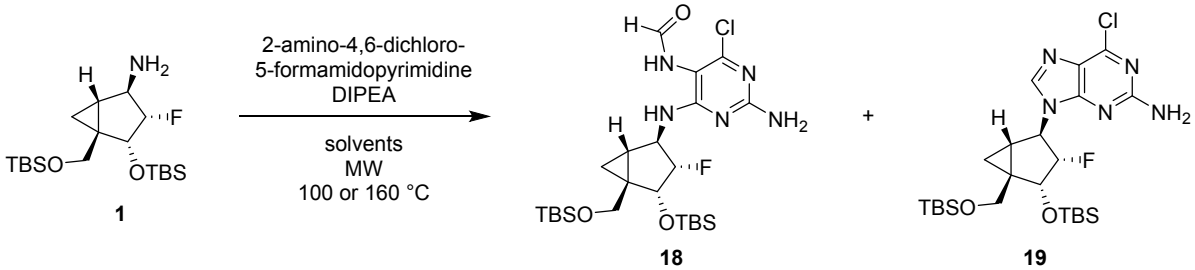
Synthesis of compound 17



To a solution of compound **16** (560 mg, 0.817 mmol) and DIPEA (0.427 mL, 2.45 mmol) in CH_2Cl_2 (8 mL) was added dropwise 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.219 mL, 0.980 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with saturated $NaHCO_3$ (aq.) and washed with saturated $NaHCO_3$ (aq.), water, and brine, dried (Na_2SO_4), and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (50% ethyl acetate in hexanes) to give compound **17** as a white foam (593 mg, 82%).

^{1}H NMR (400 MHz, CD_3CN) δ 9.51 (brs, 1H), 8.61–8.63 (m, 1H), 8.49–8.53 (m, 1H), 8.00 (d, J = 7.3 Hz, 2H), 7.61–7.65 (m, 1H), 7.53 (dd, J = 7.3 and 7.3 Hz, 2H), 7.41–7.47 (m, 2H), 7.19–7.34 (m, 7H), 6.82–6.86 (m, 4H), 5.15–5.37 (m, 2H), 5.02 (dd, J = 50.3 and 5.8 Hz, 1H), 3.55–3.86 (m, 11H), 2.94 (d, J = 10.0 Hz, 0.3H), 2.89 (d, J = 10.1 Hz, 0.7H), 2.57 (t, J = 5.9 Hz, 0.6H), 2.51 (t, J = 6.1 Hz, 1.4H), 1.63–1.69 (m, 1H), 1.20–1.23 (m, 1H), 1.07–1.18 (m, 12H), 0.79–0.87 (m, 1H). ^{13}C NMR (101 MHz, CD_3CN) δ 159.7, 159.7, 153.1, 152.7, 151.0, 146.1, 143.4, 143.3, 136.9, 136.9, 135.1, 133.6, 131.2, 131.1, 129.7, 129.2, 129.1, 129.0, 128.0, 127.9, 125.6, 119.6, 119.5, 114.2, 96.7, 96.0, 95.9, 94.8, 94.0, 94.0, 87.2, 74.7, 74.6, 74.4, 73.9, 73.8, 73.6, 64.2, 64.1, 61.3, 61.3, 61.1, 61.0, 59.9, 59.7, 59.4, 59.2, 56.0, 56.0, 44.2, 44.1, 44.0, 35.3, 35.3, 35.2, 25.2, 25.1, 25.1, 25.1, 25.0, 25.0, 24.9, 23.9, 23.9, 21.1, 21.0, 12.1, 12.0, 12.0. ^{19}F NMR (470 MHz, CD_3CN) δ -186.55 – -186.34 (m), -187.18 – -186.96 (m). ^{31}P NMR (202 MHz, CD_3CN) δ 151.47 (d, J = 12.1 Hz), 151.19 (d, J = 16.0 Hz). HRMS calc. for $C_{49}H_{54}FN_7O_6P$ [M + H]$^+$ 886.3852, found 886.3837.

Synthesis of compound 18 and 19

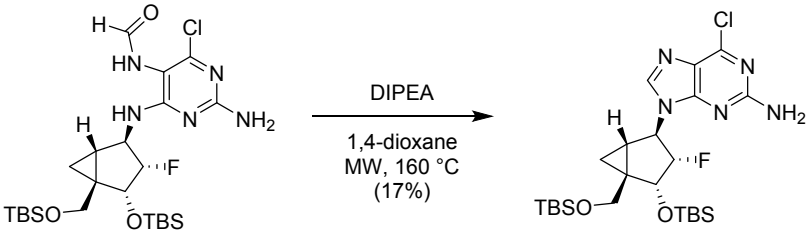


Reaction using 1,4-dioxane: To a solution of compound **1** (100 mg, 0.257 mmol) and DIPEA (0.134 mL, 0.770 mmol) in 1,4-dioxane (3 mL) was added 2-amino-4,6-dichloro-5-formamidopyrimidine (79.7 mg, 0.385 mmol). The reaction mixture was stirred and heated in a microwave reactor at 100 ºC for 12 h. The reaction was cooled to room temperature, and the solution was concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–50% ethyl acetate in hexanes). Compound **18** was isolated as a yellow solid (127 mg, 88%).

Reaction using toluene: To a solution of compound **1** (100 mg, 0.257 mmol) and DIPEA (0.134 mL, 0.770 mmol) in toluene (3 mL) was added 2-amino-4,6-dichloro-5-formamidopyrimidine (79.7 mg, 0.385 mmol). The reaction mixture was stirred and heated in microwave reactor at 160 ºC for 2 h. The reaction was cooled to room temperature, and the solution was concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (20–50% ethyl acetate in hexanes). Compound **18** was isolated as a yellow solid (100.8 mg, 70%), and compound **19** was isolated as a white solid (33.0 mg, 24%).

Reaction using n-BuOH: To a solution of compound **1** (100 mg, 0.257 mmol) and DIPEA (0.134 mL, 0.770 mmol) in *n*-butanol (3 mL) was added 2-amino-4,6-dichloro-5-formamidopyrimidine (79.7 mg, 0.385 mmol). The reaction mixture was stirred and heated in microwave reactor at 160 ºC for 2 h. The reaction was cooled to room temperature and the solution was concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (20–50% ethyl acetate in hexanes). Compound **18** was isolated as a yellow solid (84.2 mg, 59%), and compound **19** was isolated as a white solid (55.9 mg, 40%).

Conversion of 18 to 19



18 **19**

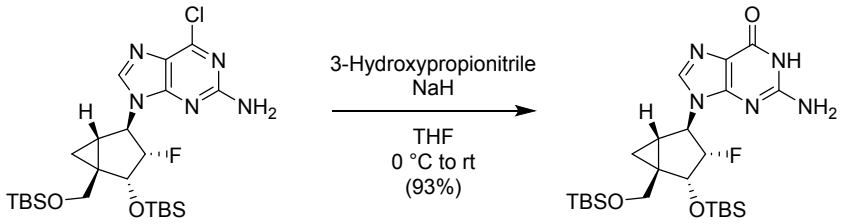
A solution of compound **18** (84.0 mg, 0.150 mmol) and DIPEA (52.2 μL, 0.300 mmol) in 1,4-dioxane (2 mL) was stirred and heated in microwave reactor at 160 ºC for 48 h. The reaction was cooled to room temperature and the solution was concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (0–50% ethyl acetate in hexanes). Compound **19** was isolated as a white solid (14.0 mg, 17%), and starting material **18** was collected as a yellow solid (54.6 mg, 65%).

Compound **18** (mixture of rotamers): ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.11 (brs, 0.8H), 8.51 (d, J = 11.4 Hz, 0.2H), 8.15 (brs, 0.8H), 7.79 (d, J = 11.4 Hz, 0.2H), 6.59–6.67 (m, 2.2H), 4.30–4.66 (m, 3H), 3.84–3.87 (m, 1H), 3.41–3.45 (m, 1H), 1.11–1.19 (m, 1H), 4.79–4.87 (m, 2H), 0.86–0.89 (m, 19H), 0.62–0.68 (m, 1H), 0.08–0.09 (m, 3H), 0.05–0.06 (m, 3H), -0.01–0.01 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.6, 160.9, 160.7, 160.7, 160.2, 159.3, 156.9, 155.6, 101.9, 101.3, 95.3 (d, J = 193.9 Hz), 95.1 (d, J = 195.9 Hz), 71.8 (d, J = 19.2 Hz), 71.7 (d, J = 16.2 Hz), 63.1, 56.3 (d, J = 25.3 Hz), 55.9 (d, J = 25.3 Hz), 34.9, 34.7, 25.8, 25.8, 25.6, 22.6, 22.3, 18.0,

18.0, 17.9, 10.6 (d, J = 6.1 Hz), 10.4 (d, J = 6.1 Hz), -4.73, -4.79, -4.96, -4.99, -5.28, -5.32, -5.45. ^{19}F NMR (470 MHz, DMSO-d_6) δ -188.06 – -187.84 (m). HRMS calc. for $C_{24}H_{44}ClFN_5O_3Si_2$ [M + H]$^+$ 560.2650, found 560.2647.

Compound **19**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 (s, 1H), 6.99 (s, 1H), 4.79–4.87 (m, 2H), 4.69 (dd, J = 50.6 and 5.5 Hz, 1H), 4.17 (d, J = 10.8 Hz, 1H), 3.32 (d, J = 10.8 Hz, 1H), 1.68 (dd, J = 8.5 and 3.1 Hz, 1H), 1.10–1.12 (m, 1H), 0.93 (s, 9H), 0.80-0.86 (m, 10H), 0.10 (s, 6H), 0.07 (s, 3H), 0.01 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.8, 153.6, 149.4, 139.9, 123.4, 93.6 (d, J = 192.9 Hz), 70.6 (d, J = 16.2 Hz), 63.4, 58.0 (d, J = 27.3 Hz), 35.8, 25.8, 25.6, 22.1, 18.0, 17.8, 10.7 (d, J = 8.1 Hz), -4.84, -5.26, -5.57, -5.65. ^{19}F NMR (470 MHz, DMSO-d_6) δ -189.95 – -189.06 (m). HRMS calc. for $C_{24}H_{42}ClFN_5O_2Si_2$ [M + H]$^+$ 542.2544, found 542.2551.

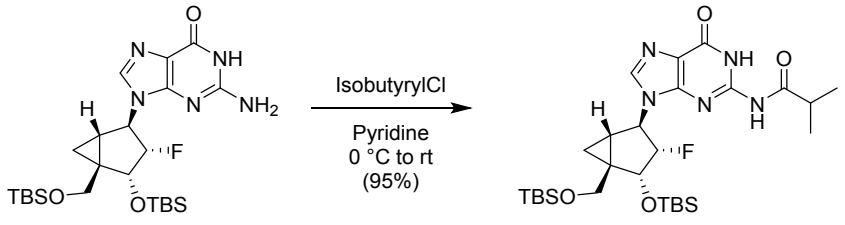
Synthesis of compound 20



19 **20**

3-Hydroxypropionitrile (0.272 mL, 3.98 mmol) was dissolved in THF (5 mL) and cooled to 0 °C. Sodium hydride (60% in mineral oil, 159 mg, 3.98 mmol) was added in portions, and the mixture was stirred at room temperature for 30 min and cooled to 0 °C. A solution of compound **19** (480 mg, 0.885 mmol) in THF (5 mL) was added dropwise at 0 °C, and the mixture was stirred at room temperature. After 16 h, the reaction was quenched by addition of saturated NH_4Cl (aq.). The reaction mixture was extracted with CH_2Cl_2 and ethyl acetate. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–10% MeOH in CH_2Cl_2) to give compound **20** as a white solid (430 mg, 93%).
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (brs, 1H), 7.90 (s, 1H), 6.52 (brs, 2H), 4.73–4.83 (m, 2H), 4.57 (dd, J = 50.6 and 5.2 Hz, 1H), 4.15 (d, J = 11.0 Hz, 1H), 3.28–3.31 (m, 1H), 1.67 (dd, J = 8.8 and 3.8 Hz, 1H), 1.06–1.08 (m, 1H), 0.92 (s, 9H), 0.77–0.86 (m, 10H), 0.08 (s, 6H), 0.07 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.8, 153.7, 150.6, 134.3, 116.5, 94.2 (d, J = 192.8 Hz), 70.7 (d, J = 16.4 Hz), 63.3, 57.7 (d, J = 26.5 Hz), 35.7, 25.8, 25.6, 22.4, 17.9, 17.8, 10.7 (d, J = 7.6 Hz), -4.85, -5.21, -5.58, -5.66. ^{19}F NMR (470 MHz, DMSO-d_6) δ -188.37 – -188.18 (m). HRMS calc. for $C_{24}H_{43}FN_5O_3Si_2$ [M + H]$^+$ 524.2883, found 524.2894.

Synthesis of compound 21

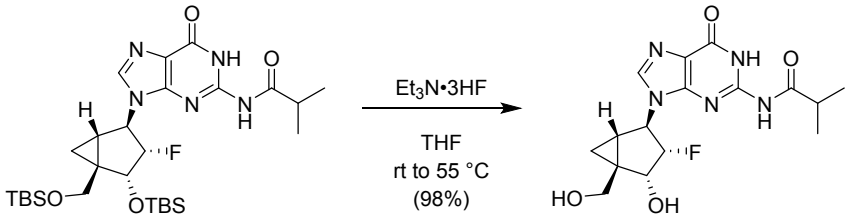


20 **21**

To a solution of compound **20** (430 mg, 0.821 mmol) in pyridine (8 mL) was added dropwise isobutyryl chloride (0.103 mL, 0.985 mmol) at 0 °C, and the mixture was stirred at room

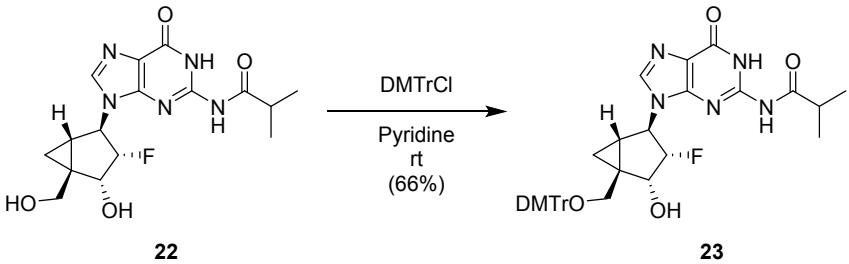
temperature overnight. The reaction was quenched with MeOH and concentrated under vacuum. The residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ (aq.). The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–50% ethyl acetate in hexanes). Compound **21** was isolated as a white foam (464 mg, 95%).
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.09 (s, 1H), 11.72 (s, 1H), 8.19 (s, 1H), 4.79–4.87 (m, 2H), 4.67 (dd, J = 50.5 and 5.3 Hz, 1H), 4.17 (d, J = 10.8 Hz, 1H), 3.31–3.32 (m, 1H), 2.78 (sept, J = 6.8 Hz, 1H), 1.68–1.70 (m, 1H), 1.10–1.12 (m, 7H), 0.94 (s, 9H), 0.84–0.88 (m, 10H), 0.10 (s, 6H), 0.07 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.2, 154.9, 148.2, 148.0, 136.7, 120.1, 93.9 (d, J = 194.0 Hz), 706 (d, J = 15.1 Hz), 63.3, 58.2 (d, J = 26.5 Hz), 35.8, 34.7, 25.8, 25.6, 22.4, 18.9, 18.8, 18.0, 17.8, 10.8 (d, J = 7.6 Hz), -4.83, -5.24, -5.58, -5.65. ^{19}F NMR (470 MHz, DMSO-d_6) δ -188.86 – -188.67 (m). HRMS calc. for $C_{28}H_{49}FN_5O_4Si_2$ $[M + H]^+$ 594.3302, found 594.3312.

Synthesis of compound 22



To a solution of compound **21** (300 mg, 0.505 mmol) in THF (5 mL) was added dropwise triethylamine trihydrofluoride (0.247 mL, 1.52 mmol) at room temperature and the mixture was stirred at 55 °C for 6 hours. The reaction mixture was concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (0–10% MeOH in CH_2Cl_2). Compound **22** was isolated as a white solid (181 mg, 98%).
^{1}H NMR (500 MHz, DMSO-d_6) δ 12.08 (brs, 1H), 11.70 (brs, 1H), 8.29 (s, 1H), 5.13 (t, J = 5.0 Hz, 1H), 5.07 (d, J = 7.2 Hz, 1H), 4.83 (d, J = 15.9 Hz, 1H), 4.53–4.67 (m, 2H), 4.09 (dd, J = 11.5 and 5.0 Hz, 1H), 3.15 (dd, J = 11.5 and 5.0 Hz, 1H), 2.78 (sept, , J = 6.8 Hz, 1H), 1.62 (dd, J = 8.6 and 3.7 Hz, 1H), 1.08–1.12 (m, 7H), 0.73–0.77 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.2, 154.9, 148.1, 148.0, 137.2, 120.1, 95.0 (d, J = 190.3 Hz), 69.7 (d, J = 16.4 Hz), 61.4, 58.3 (d, J = 26.5 Hz, 1H), 35.7, 34.7, 21.9, 18.9, 18.8, 11.1 (d, J = 7.6 Hz). ^{19}F NMR (470 MHz, DMSO-d_6) δ -188.50 – -188.30 (m). HRMS calc. for $C_{16}H_{21}FN_5O_4$ $[M + H]^+$ 366.1572, found 366.1562.

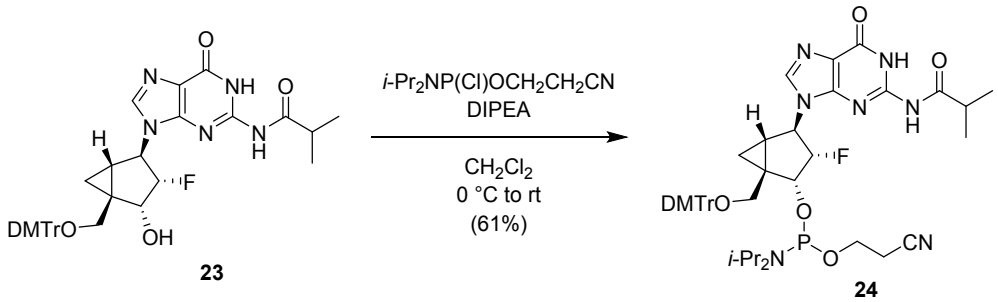
Synthesis of compound 23



To a solution of compound **22** (380 mg, 1.04 mmol) in pyridine (10 mL) was added 4,4′-dimethoxytrityl chloride (423 mg, 1.25 mmol) and the mixture was stirred at room temperature overnight. The reaction was quenched with dry MeOH and concentrated *in vacuo*. The residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$ (aq.), water, and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (50–100% ethyl acetate in hexanes). Compound **23** was isolated as a white foam (455 mg, 66%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.12 (brs, 1H), 11.70 (brs, 1H), 8.18 (s, 1H), 7.36 (d, J = 7.5 Hz, 2H), 7.29 (dd, J = 7.5 and 7.5 Hz, 2H), 7.21–7.25 (m, 5H), 6.85–6.87 (m, 4H), 5.11 (d, J = 8.0 Hz, 1H), 4.85–4.95 (m, 2H), 4.74 (dd, J = 50.4 and 5.7 Hz, 1H), 3.71–3.73 (m, 7H), 2.74–2.79 (m, 2H), 1.58 (d, J = 9.0 and 3.8 Hz, 1H), 1.10 (t, J = 6.5 Hz, 6H), 1.04– 1.06 (m, 1H), 0.67–0.71 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 180.2, 158.0, 154.9, 148.2, 148.0, 144.9, 137.0, 135.6, 135.4, 129.7, 129.7, 127.8, 127.6, 126.7, 120.2, 113.2, 94.7 (d, J = 190.3 Hz), 85.6, 70.7 (d, J = 16.4 Hz), 63.3, 58.7 (d, J = 26.5 Hz, 1H), 34.7, 33.8, 22.7, 18.8, 18.8, 10.7 (d, J = 7.6 Hz). ^{19}F NMR (470 MHz, DMSO-d_6) δ -187.75 – -187.55 (m). HRMS calc. for $C_{16}H_{21}FN_5O_4$ [M + H]$^+$ 668.2879, found 668.2894.

Synthesis of compound 24

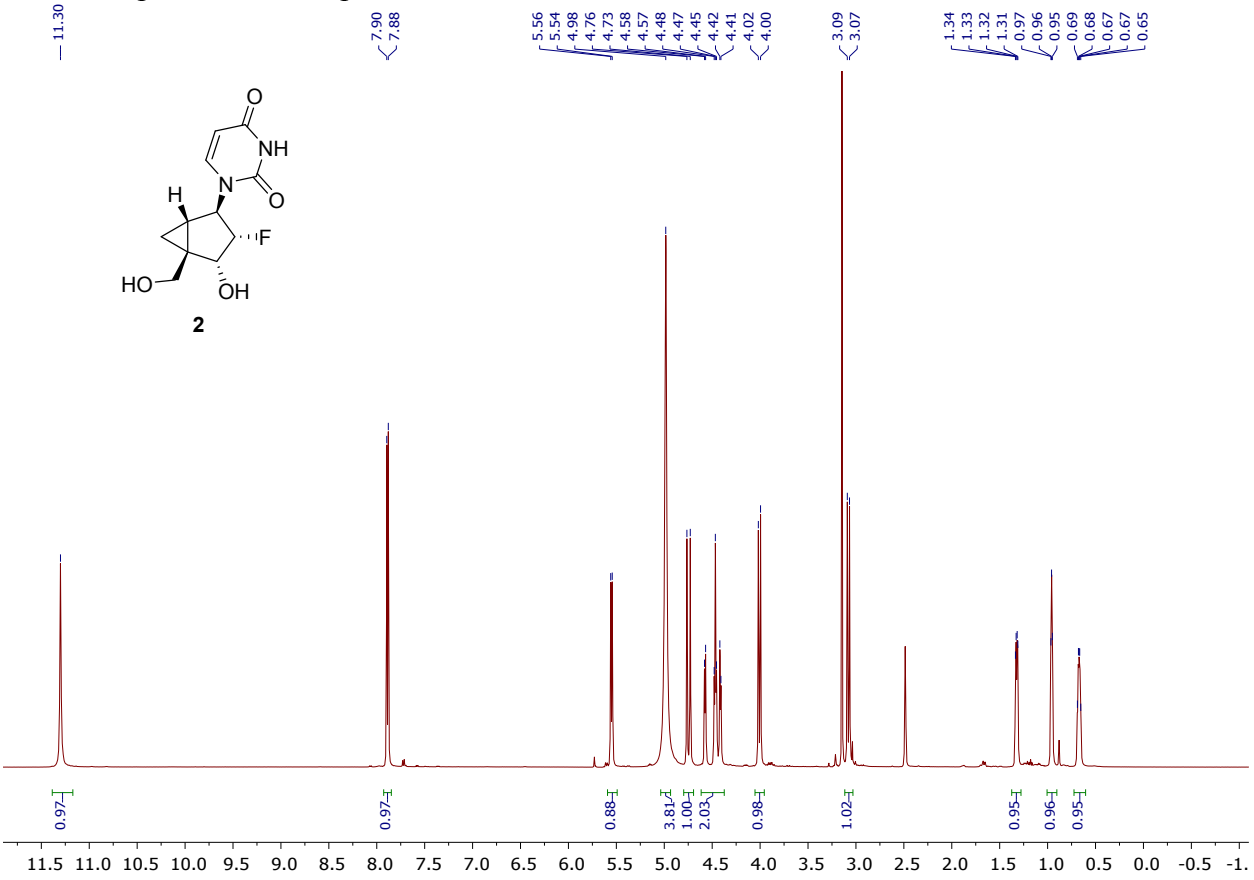


To a solution of compound **23** (450 mg, 0.674 mmol) and DIPEA (0.352 mL, 2.02 mmol) in CH_2Cl_2 (7 mL) was added dropwise 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.180 mL, 0.809 mmol) at 0 °C and the mixture was stirred at room temperature overnight. The reaction mixture was quenched with saturated $NaHCO_3$ (aq.). The organic layer was washed with water and brine, dried (Na_2SO_4) and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (60% ethyl acetate in hexanes) to give compound **24** as a white foam (354 mg, 61%).

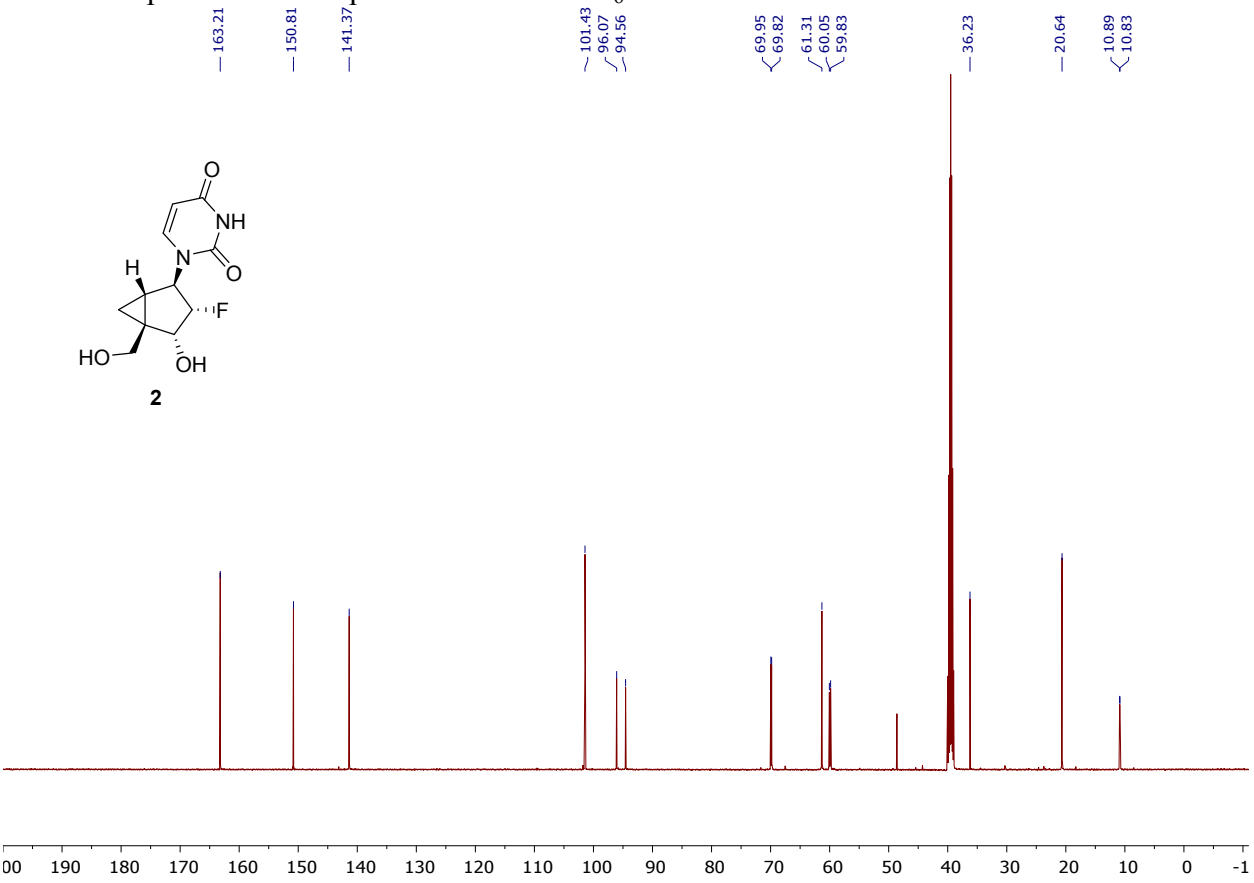
^{1}H NMR (500 MHz, CD_3CN) δ 8.15 (s, 0.8H), 8.11 (s, 0.2H), 7.45–7.48 (m, 2H), 7.23–7.37 (m, 7H), 6.84–6.87 (m, 1H), 5.13–5.19 (m, 0.8H), 5.02–5.08 (m, 0.2H), 4.79–4.92 (m, 2H), 3.75–3.86 (m, 7H), 3.55–3.67 (m, 4H), 2.84–2.88 (m, 1H), 2.49–2.58 (m, 3H), 1.61–1.64 (m, 1H), 1.05–1.19 (m, 19H), 0.78–0.81 (m, 1H). ^{13}C NMR (126 MHz, CD_3CN) δ 180.4, 180.4, 159.3, 159.2, 156.0, 149.0, 148.6, 148.5, 145.7, 145.7, 137.9, 136.3, 136.3, 136.3, 136.3, 130.7, 130.7, 130.7, 130.6, 128.6, 128.6, 128.5, 128.5, 127.5, 127.5, 121.6, 121.6, 119.1, 119.0, 113.7, 113.7, 113.7, 96.2, 95.5, 95.5, 94.7, 94.0, 93.9, 86.7, 86.7, 73.7, 73.6, 73.6, 73.5, 73.1, 73.0, 72.9, 63.8, 63.7, 60.2, 60.2, 60.0, 59.9, 59.2, 59.0, 58.7, 58.6, 55.5, 55.4, 43.7, 43.6, 43.6, 43.5, 36.2, 36.2, 34.7, 34.7, 34.5, 34.5, 24.7, 24.6, 24.6, 24.5, 24.5, 24.5, 24.4, 24.3, 23.2, 23.2, 20.6, 20.6, 20.6, 20.5, 18.7, 18.7, 11.9, 11.8, 11.7, 11.6. ^{19}F NMR (470 MHz, CD_3CN) δ -186.46 – -186.25 (m), -186.92 – -186.70 (m). ^{31}P NMR (202 MHz, CD_3CN) δ 151.31 (d, J = 12.1 Hz), 151.31 (d, J = 12.1 Hz). HRMS calc. for $C_{46}H_{56}FN_7O_7P$ [M + H]$^+$ 868.3957, found 868.3962.

^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra for the new compounds

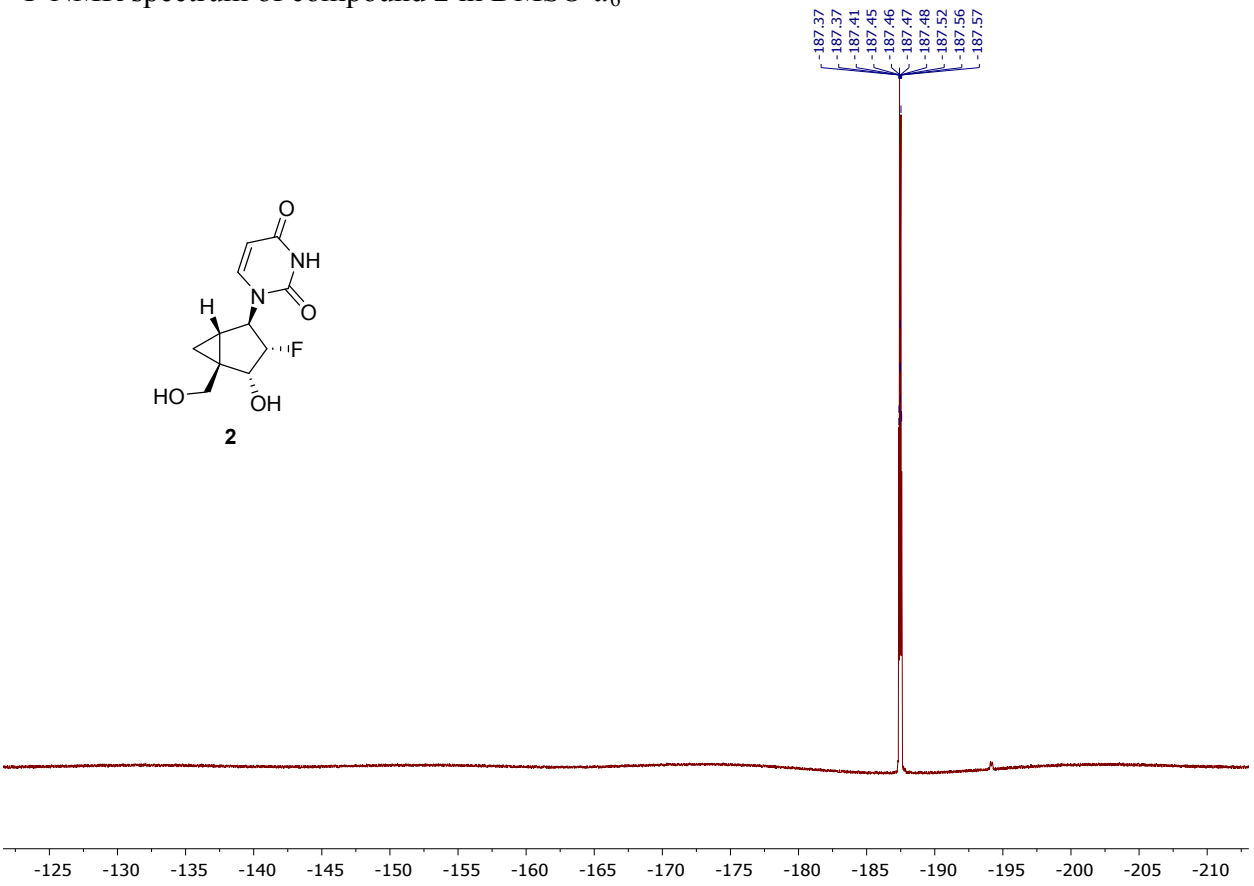
^{1}H NMR spectrum of compound **2** in DMSO-d_6



^{13}C NMR spectrum of compound **2** in DMSO-d_6

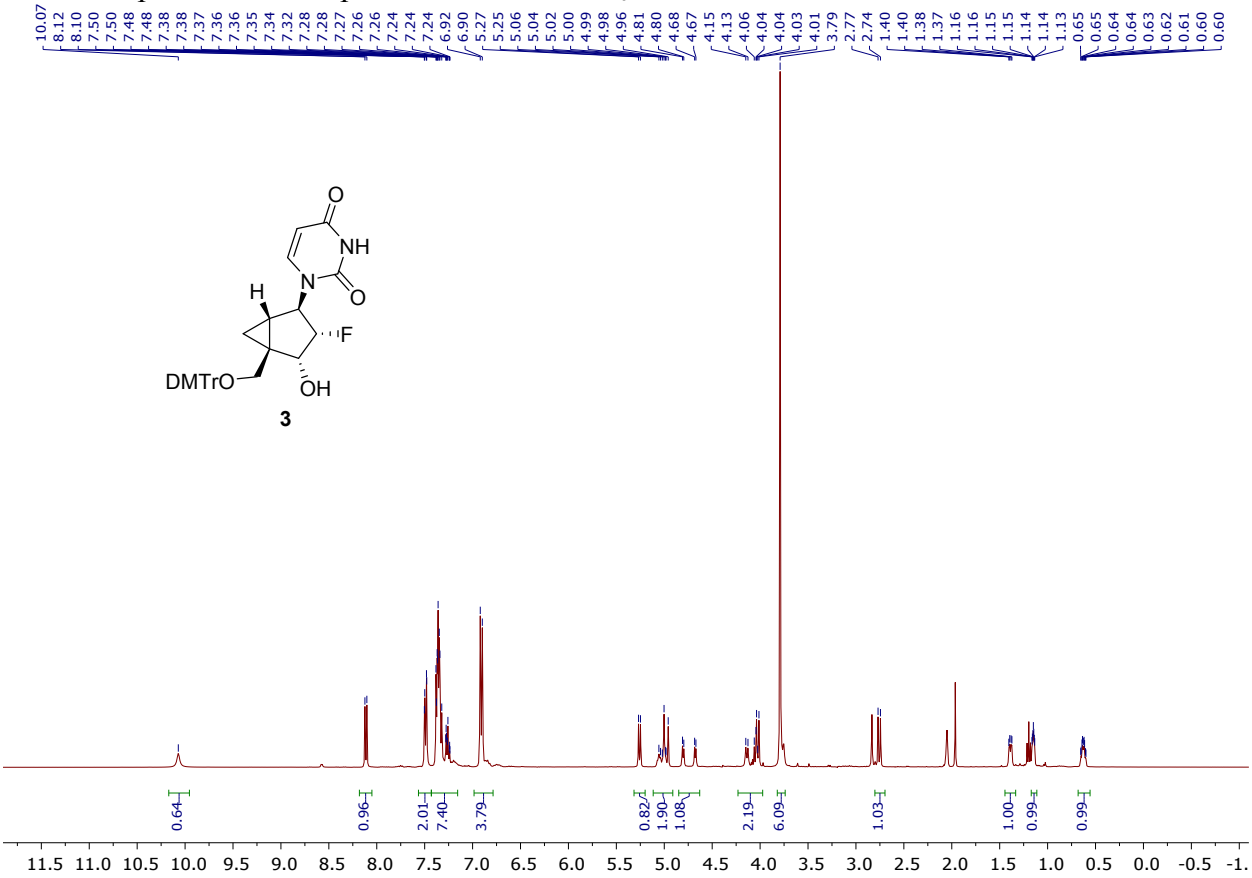


^{19}F NMR spectrum of compound **2** in DMSO-d_6

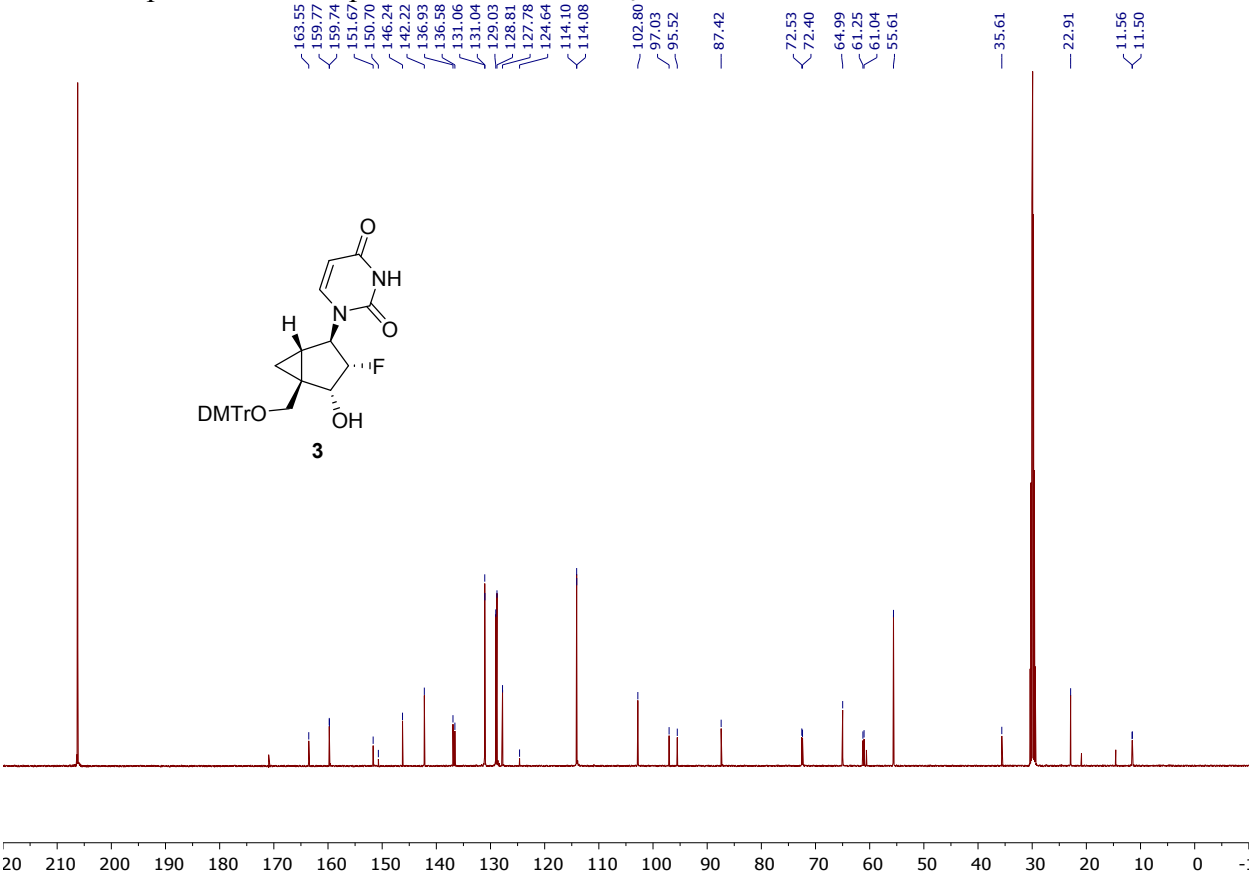


2

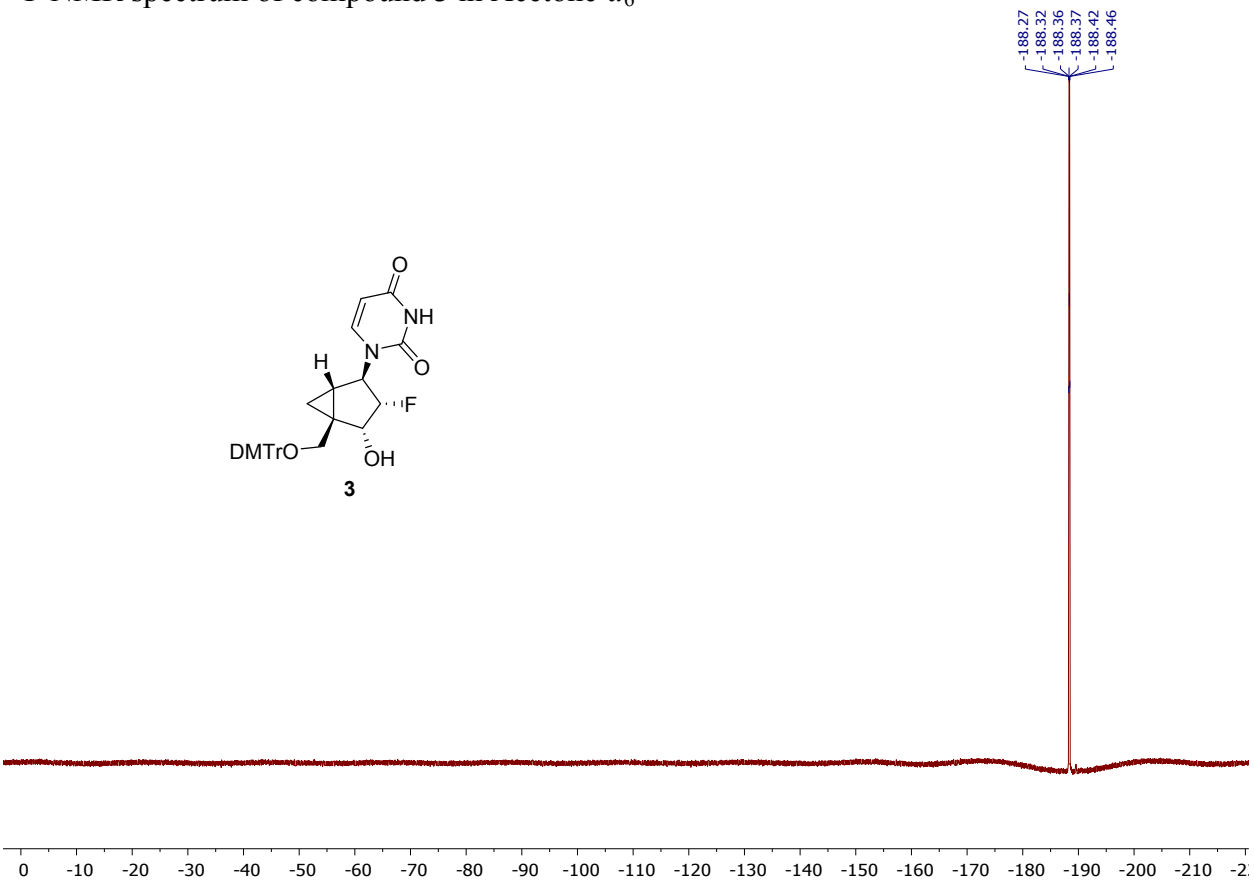
^{1}H NMR spectrum of compound **3** in Acetone-d_6



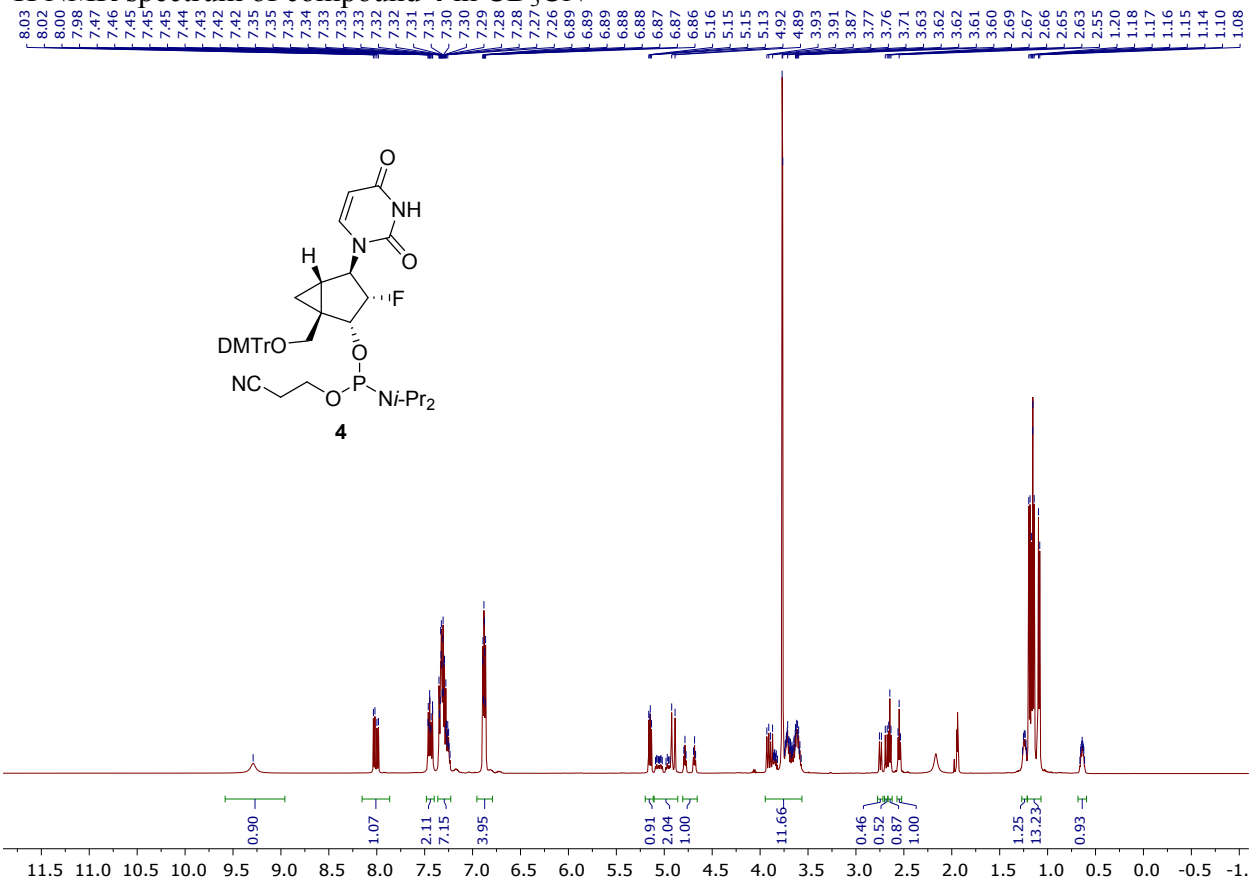
^{13}C NMR spectrum of compound **3** in Acetone-d_6



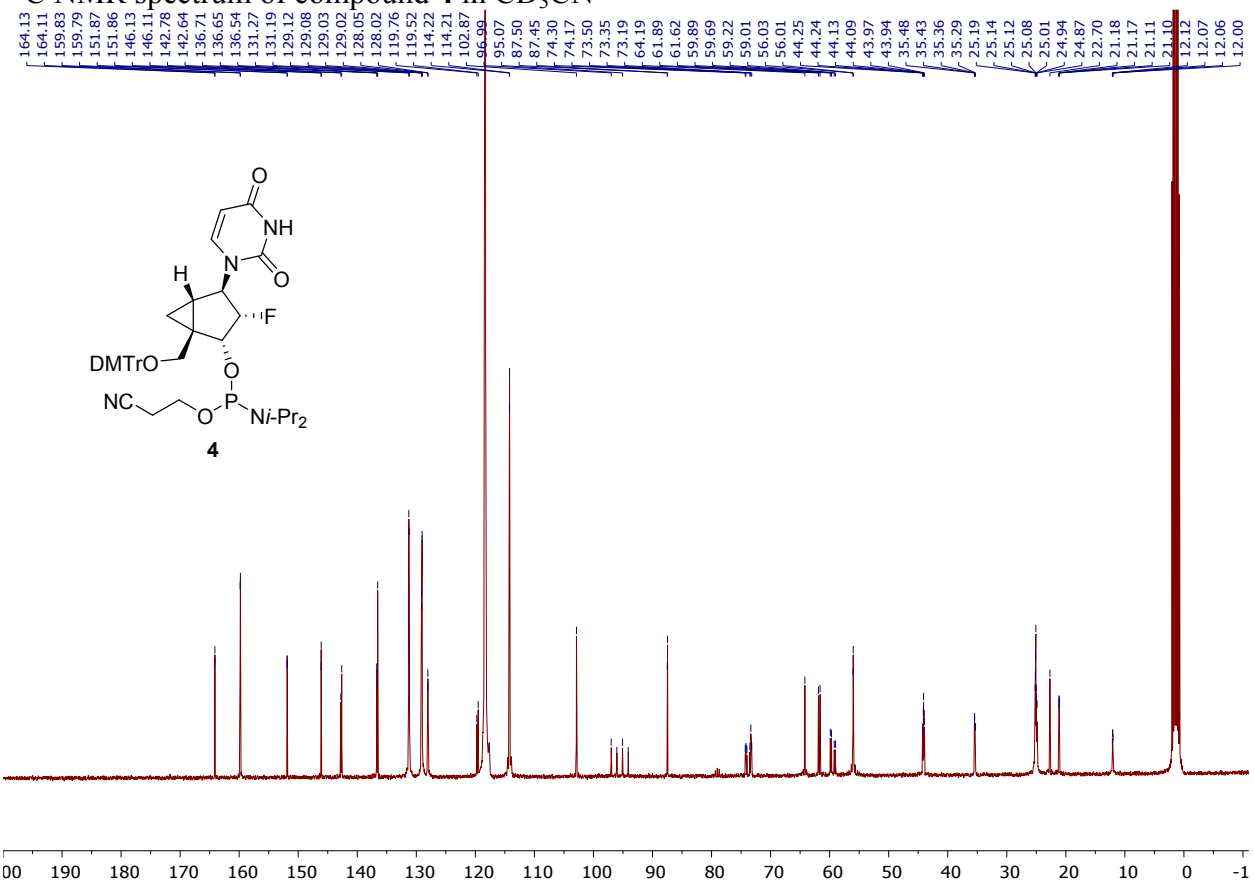
^{19}F NMR spectrum of compound **3** in Acetone-d_6



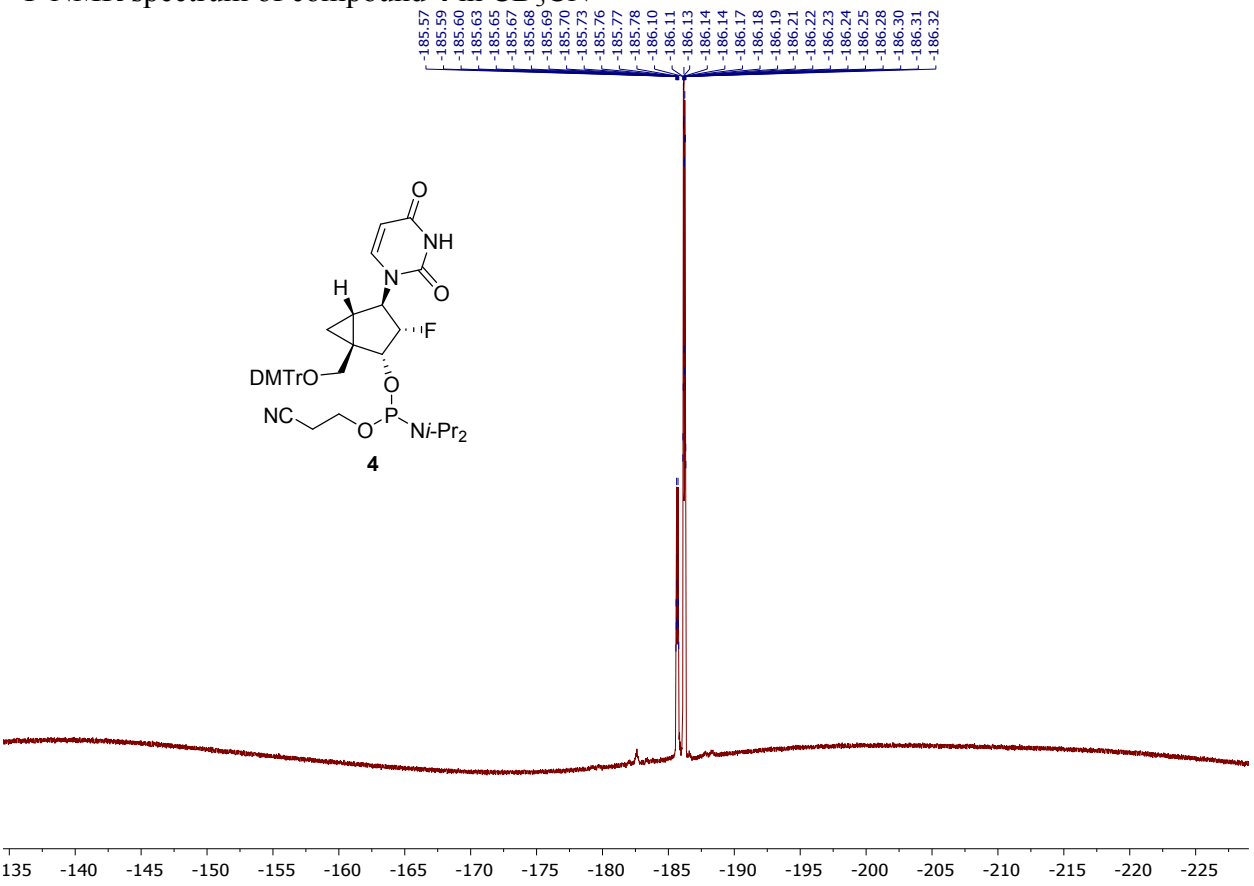
^{1}H NMR spectrum of compound **4** in CD_3CN



^{13}C NMR spectrum of compound **4** in CD_3CN

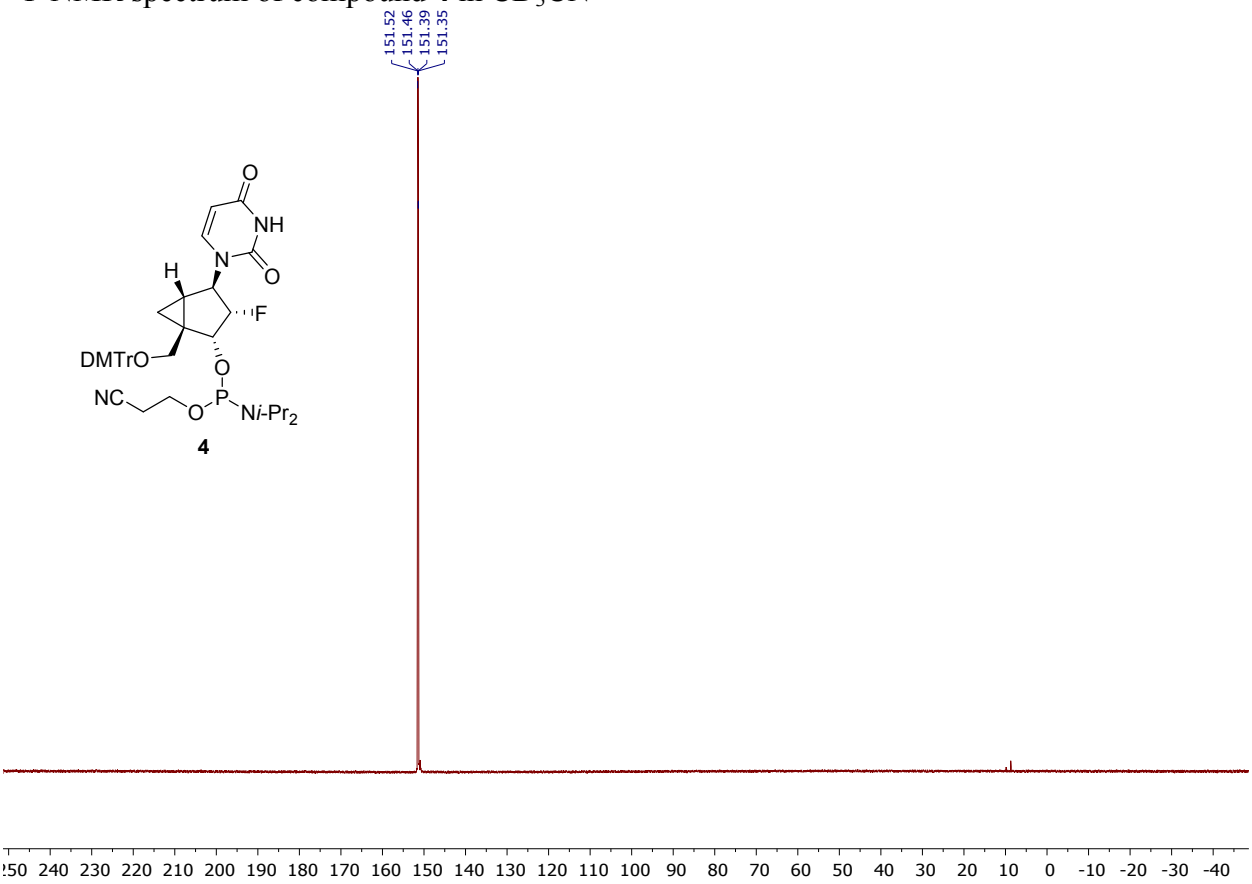


^{19}F NMR spectrum of compound **4** in CD_3CN



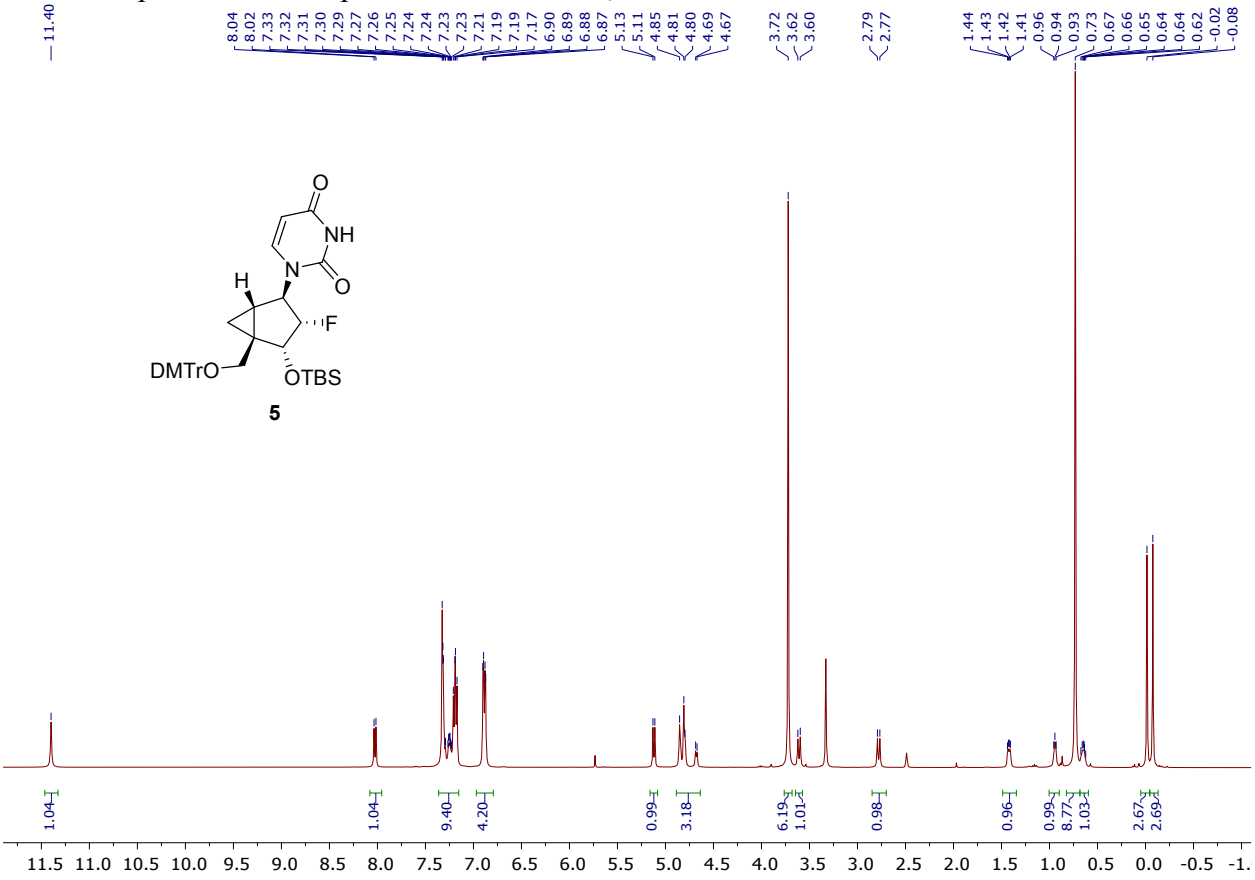
4

^{31}P NMR spectrum of compound **4** in CD_3CN

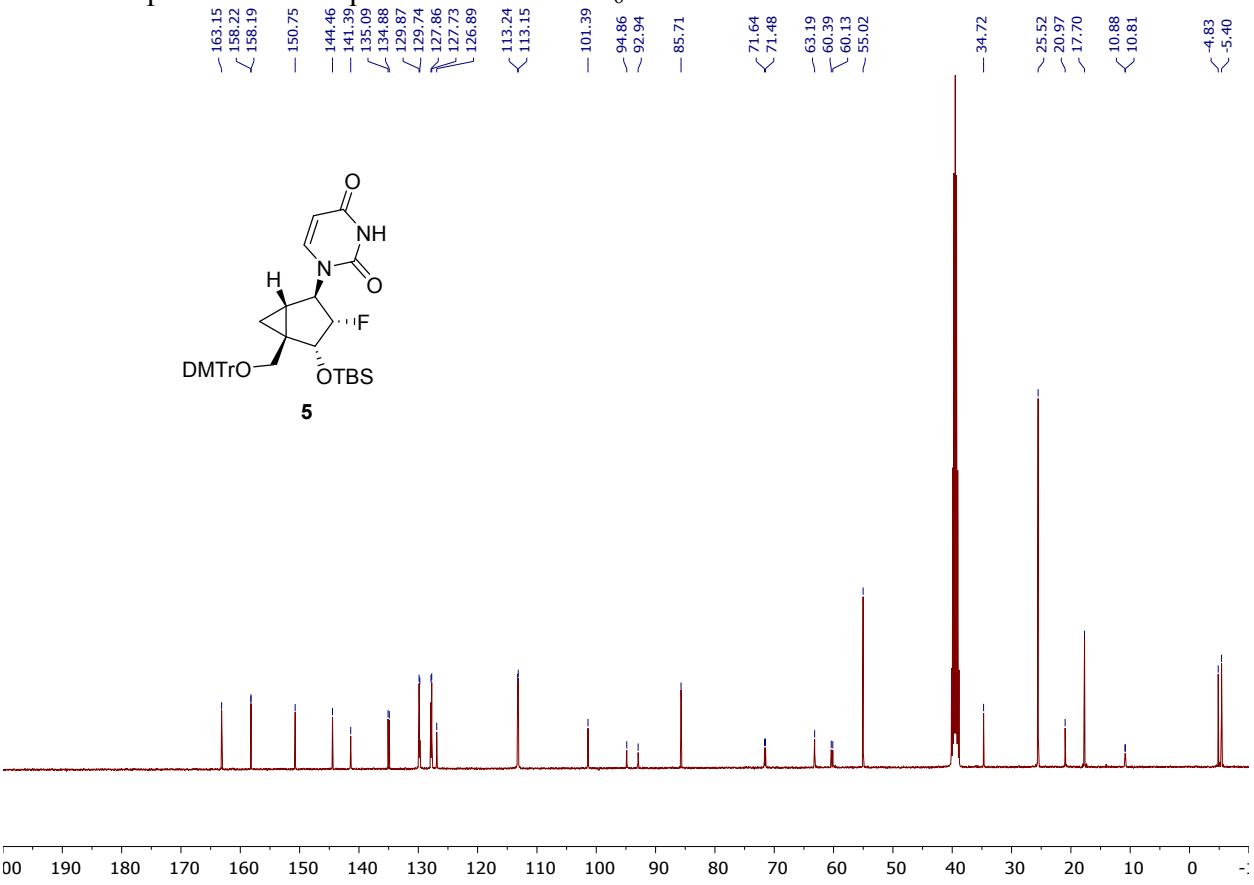


4

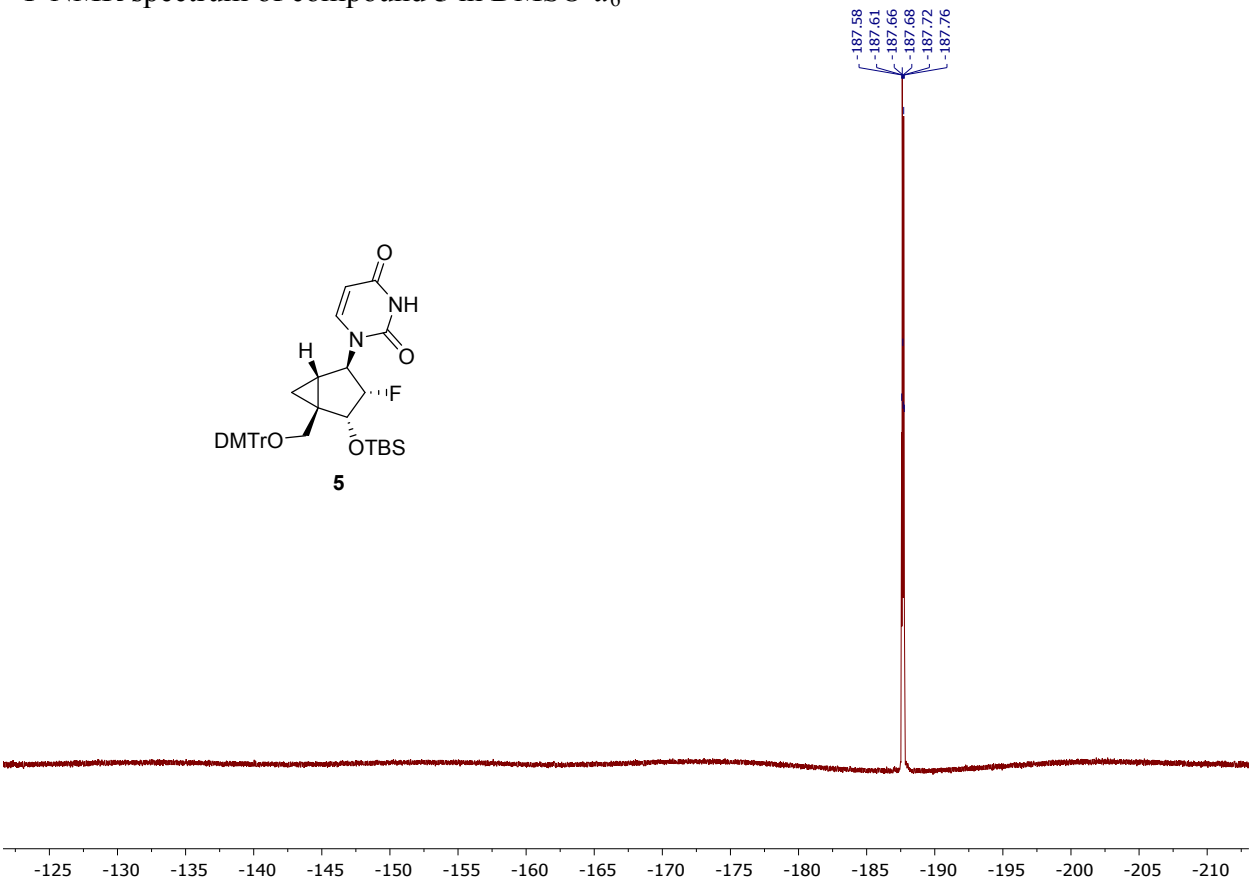
^{1}H NMR spectrum of compound **5** in DMSO-d_6



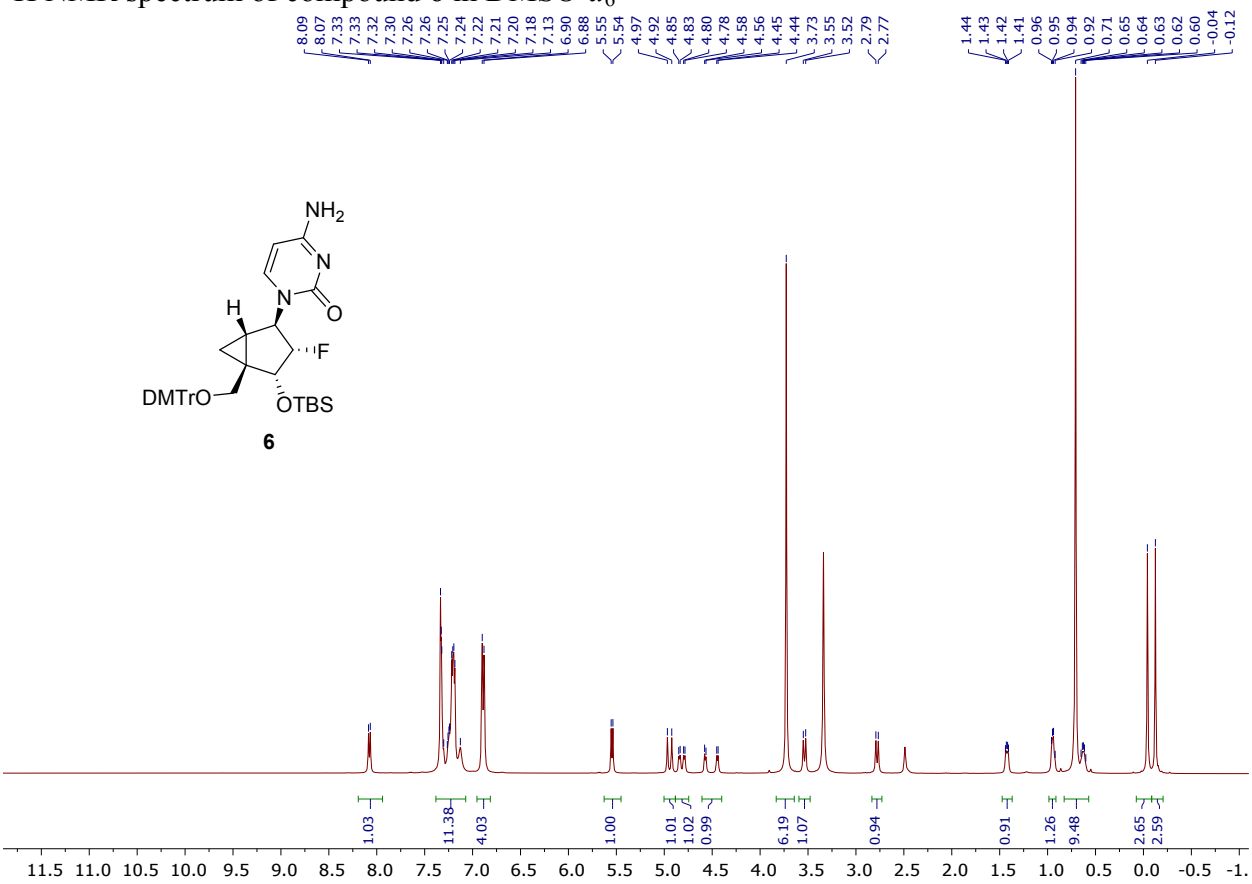
^{13}C NMR spectrum of compound **5** in DMSO-d_6



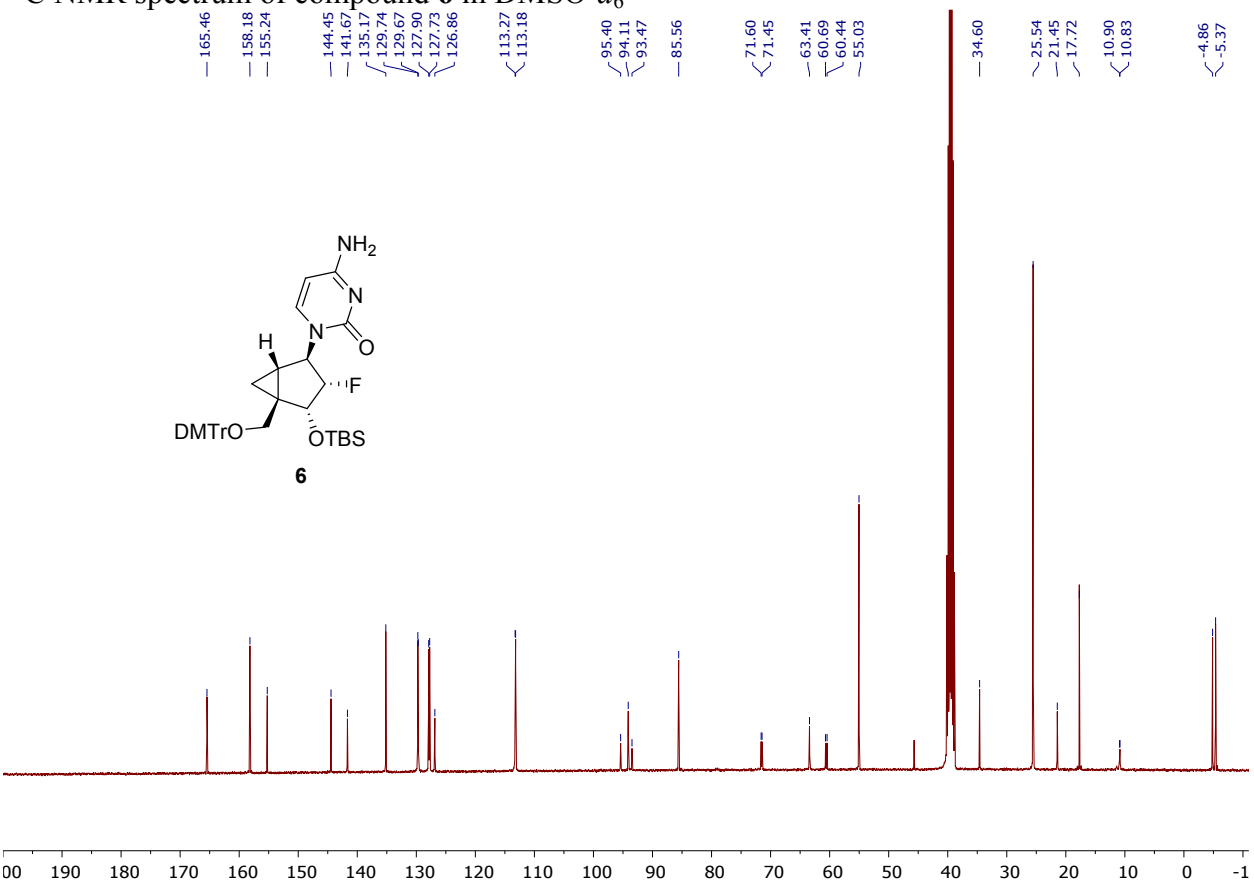
^{19}F NMR spectrum of compound **5** in DMSO-d_6



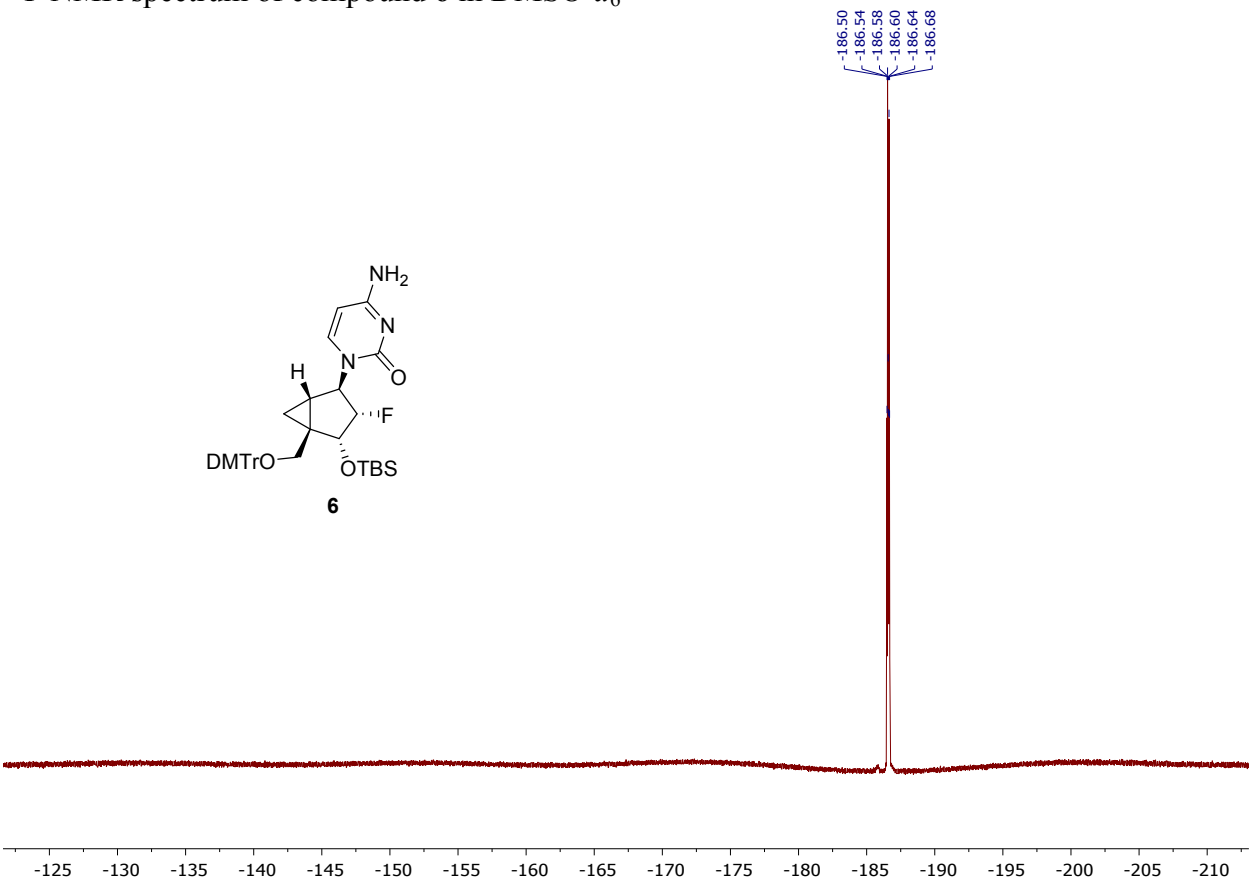
^{1}H NMR spectrum of compound **6** in DMSO-d_6



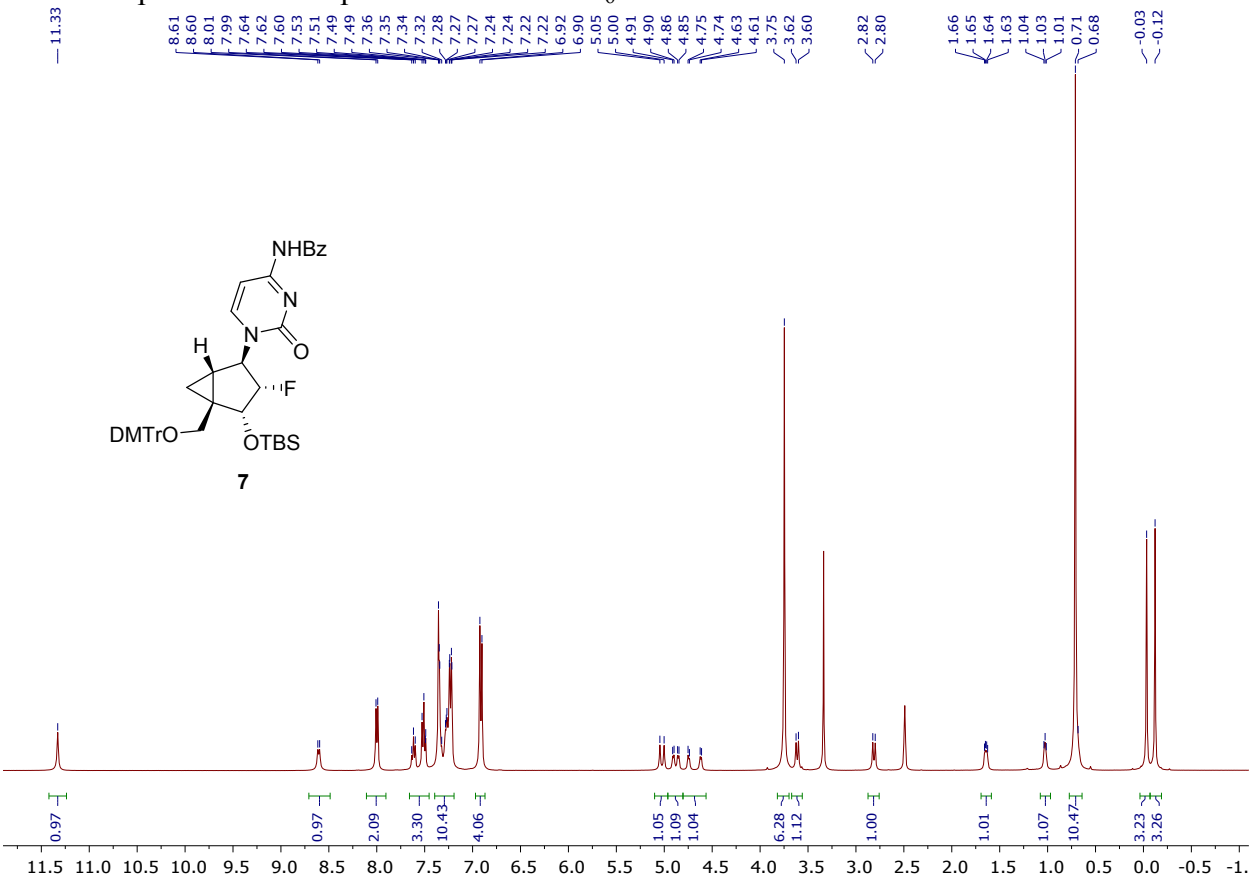
^{13}C NMR spectrum of compound **6** in DMSO-d_6



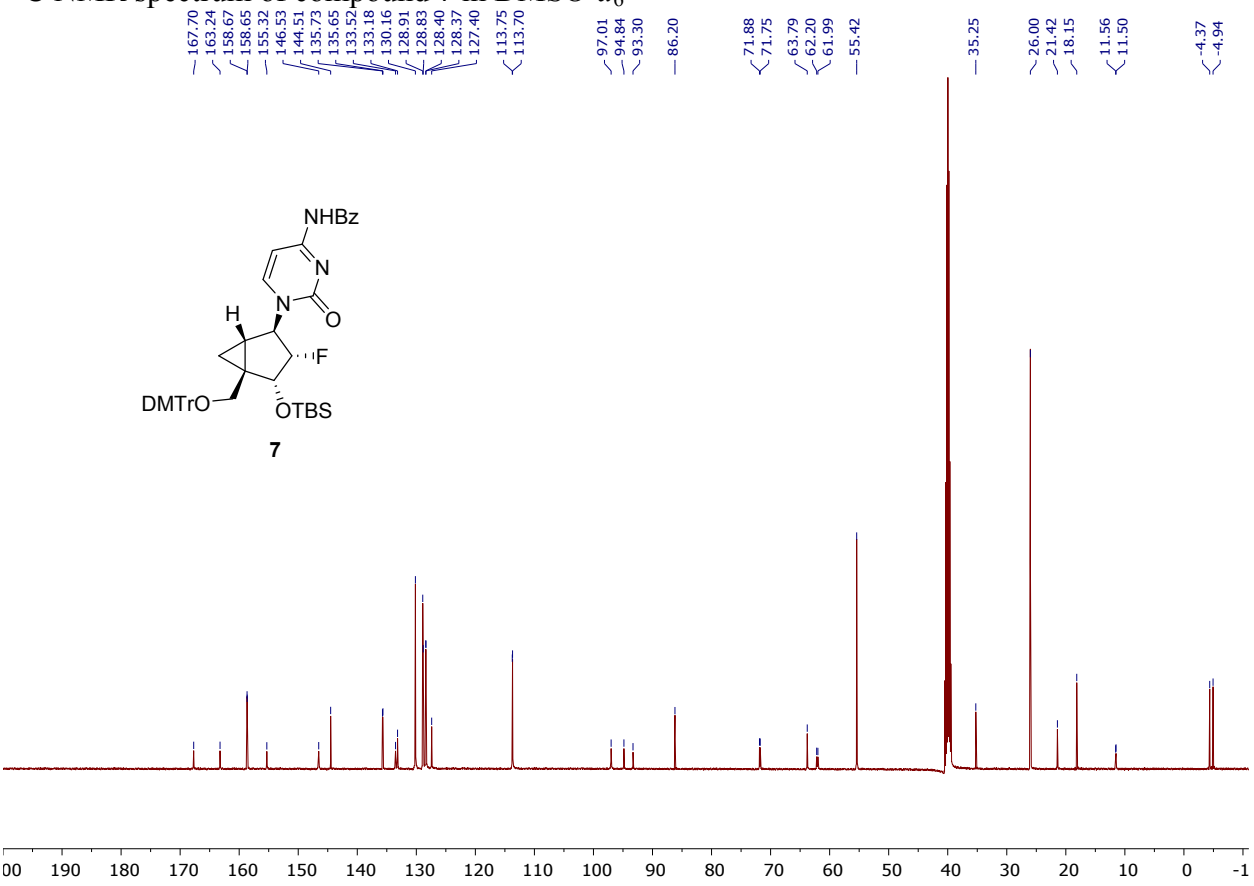
^{19}F NMR spectrum of compound **6** in DMSO-d_6



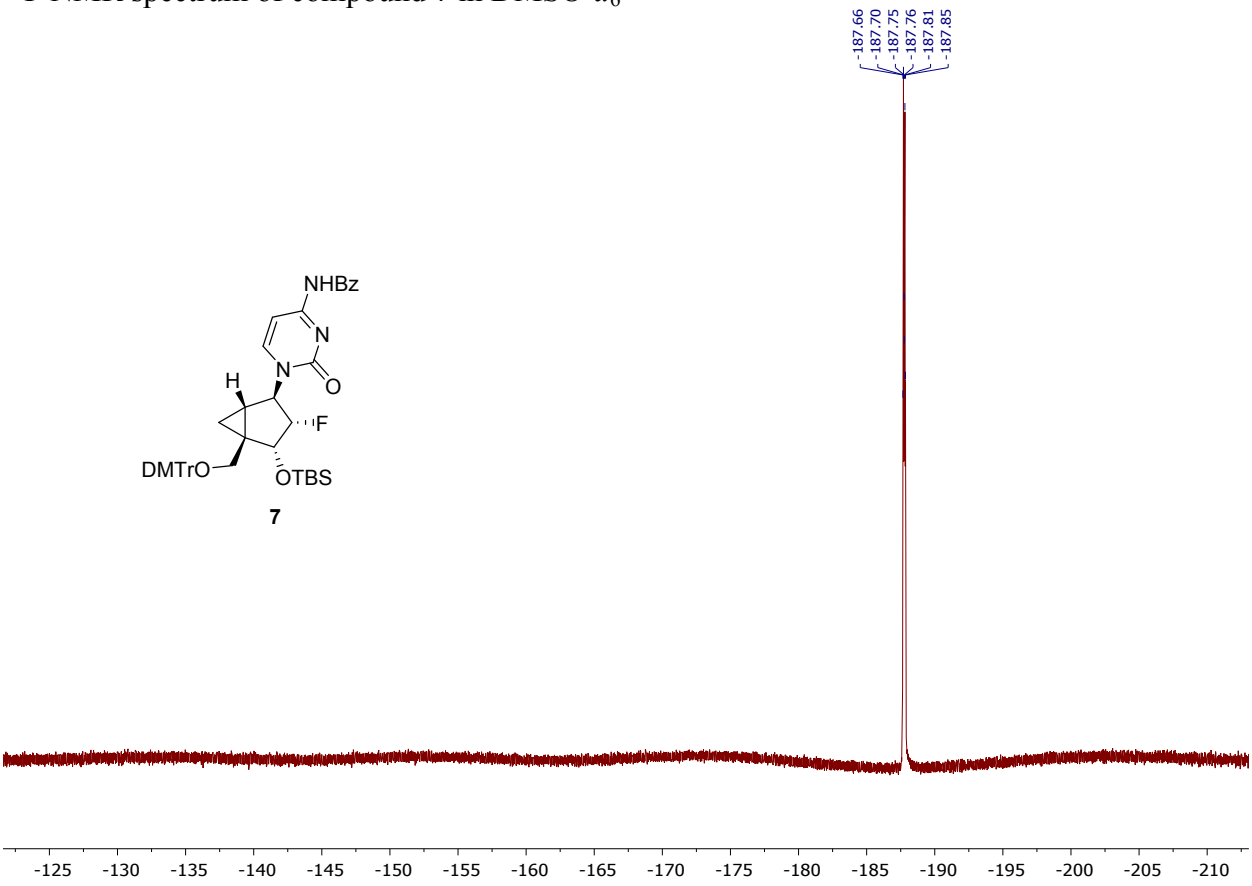
^{1}H NMR spectrum of compound **7** in DMSO-d_6



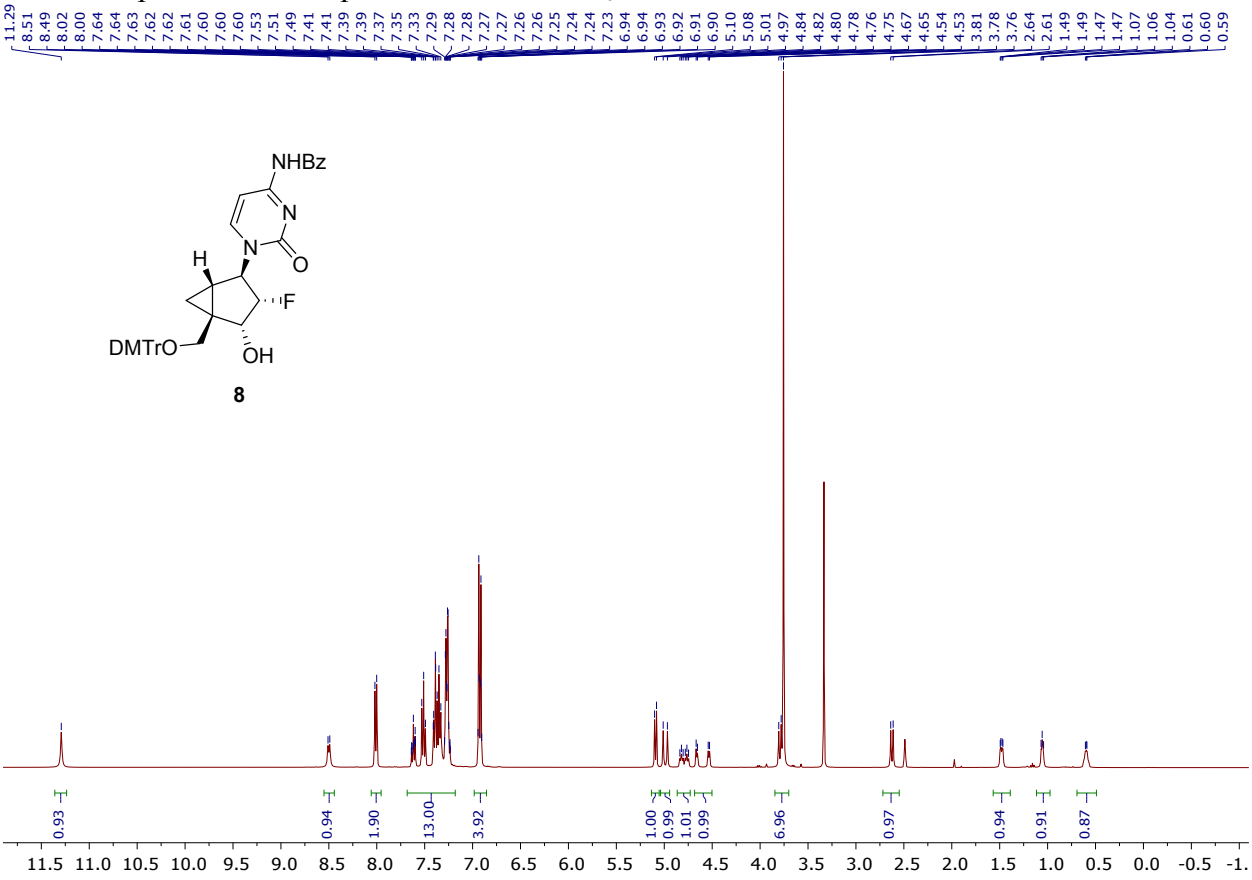
^{13}C NMR spectrum of compound **7** in DMSO-d_6



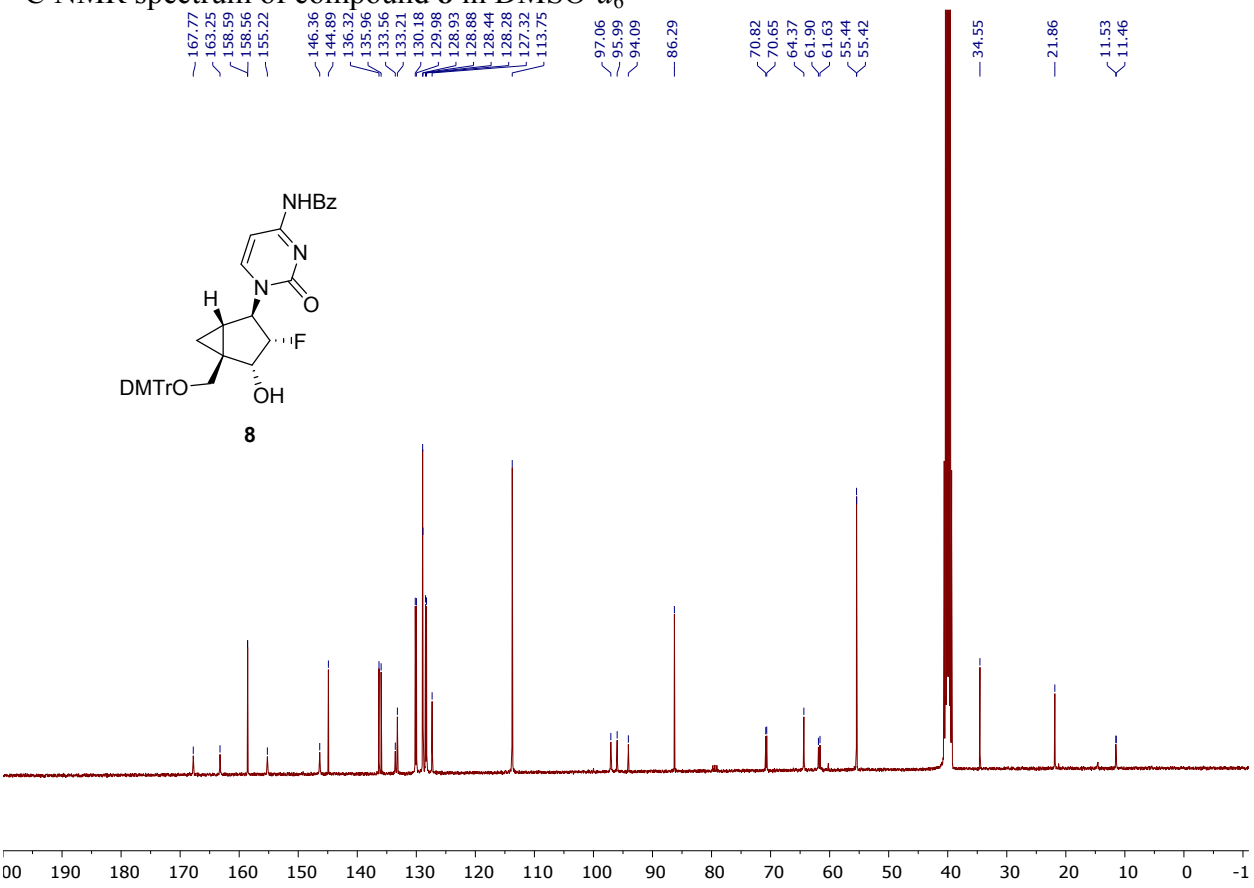
^{19}F NMR spectrum of compound **7** in DMSO-d_6



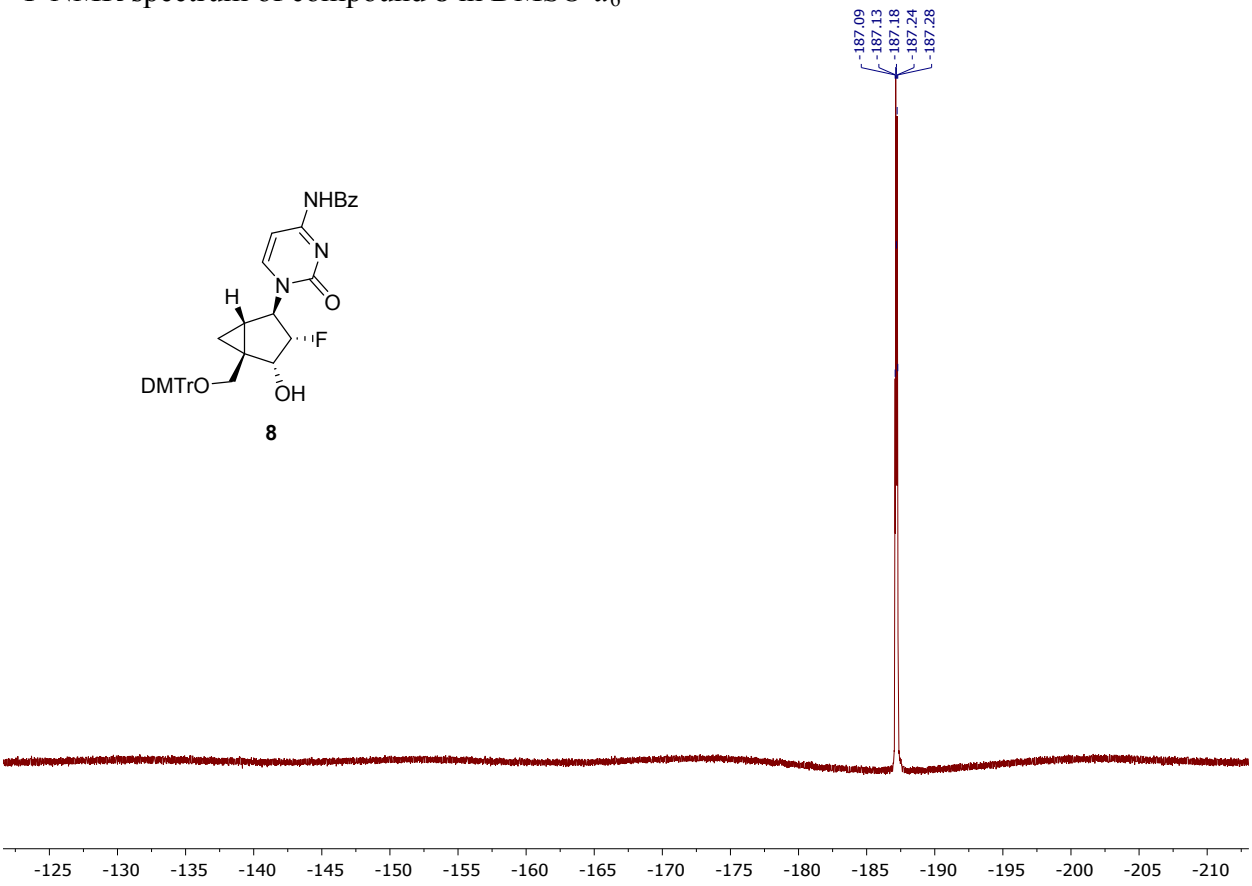
^{1}H NMR spectrum of compound **8** in DMSO-d_6



^{13}C NMR spectrum of compound **8** in DMSO-d_6

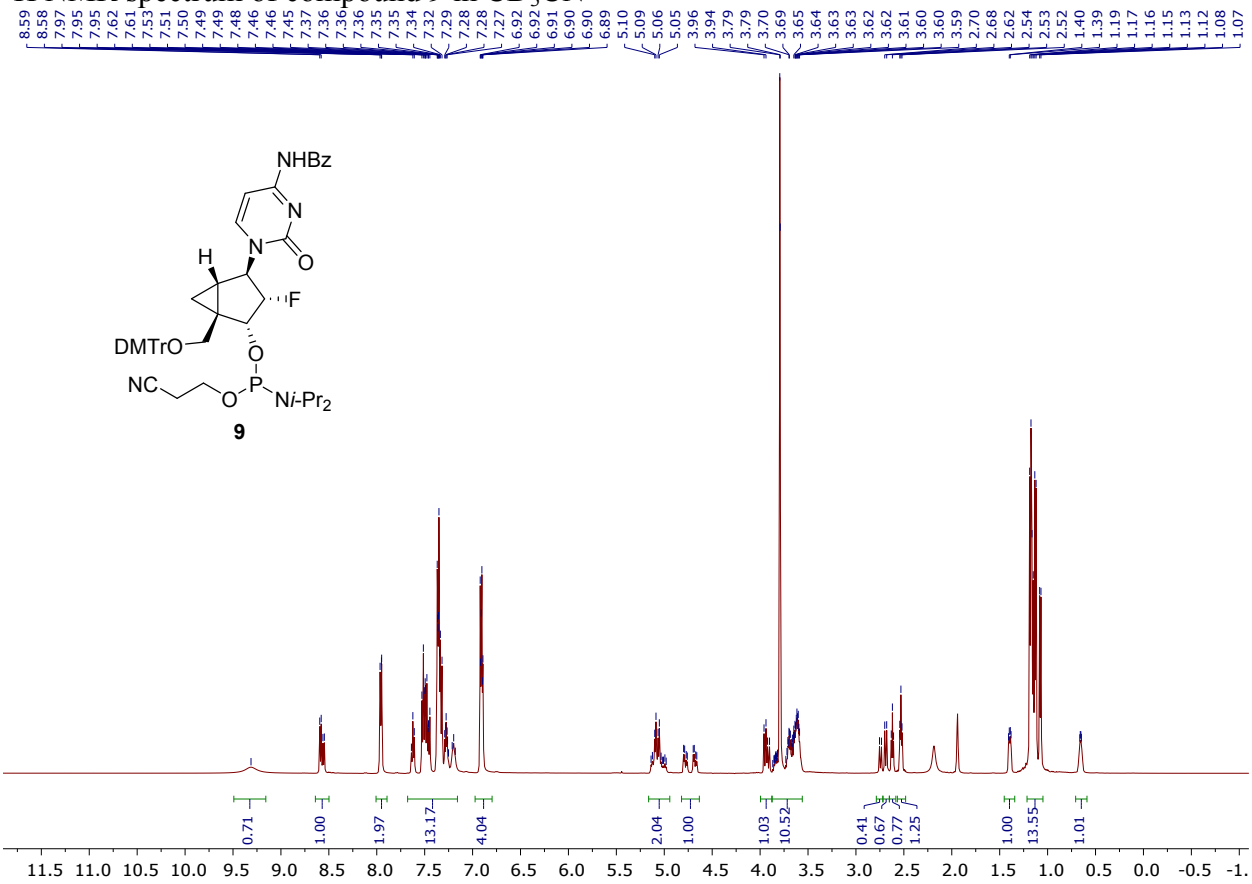


^{19}F NMR spectrum of compound **8** in DMSO-d_6

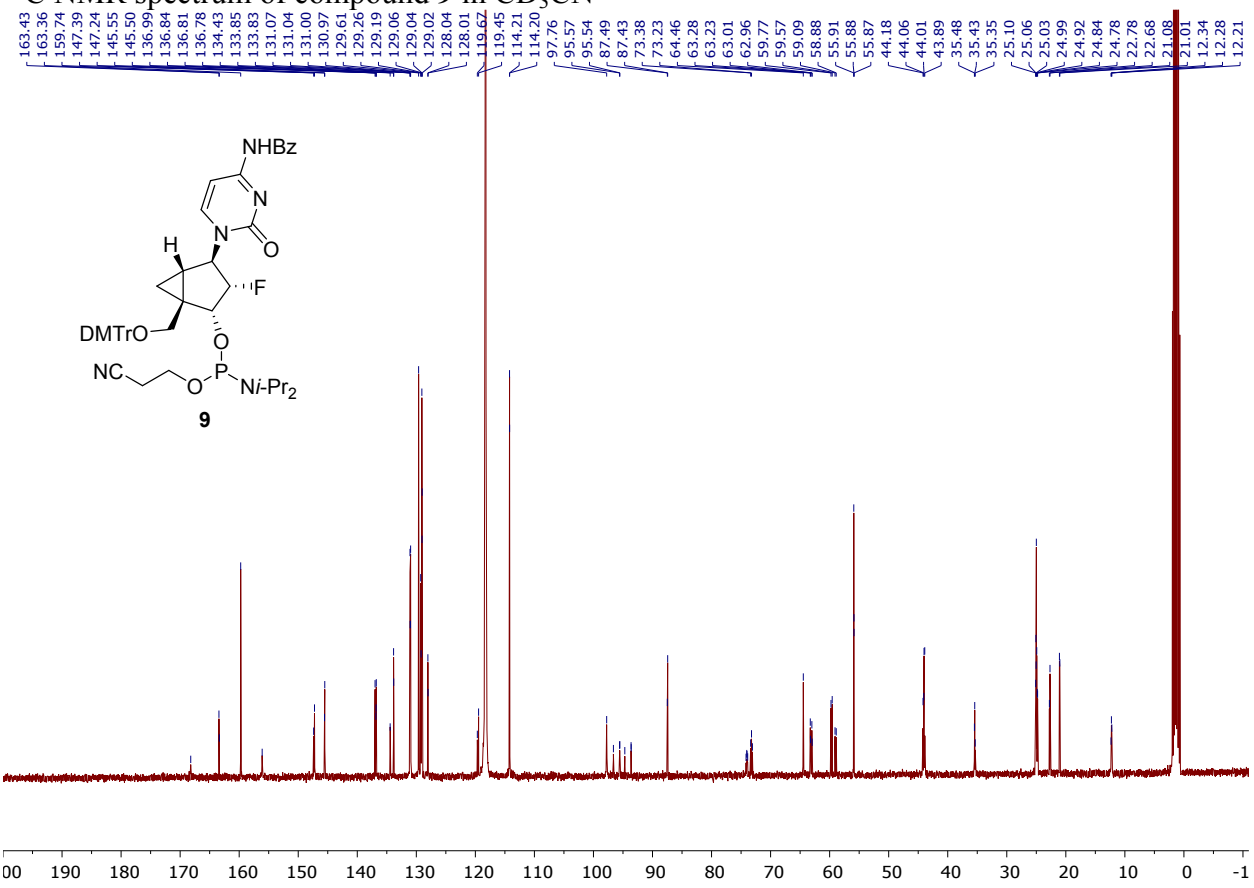


8

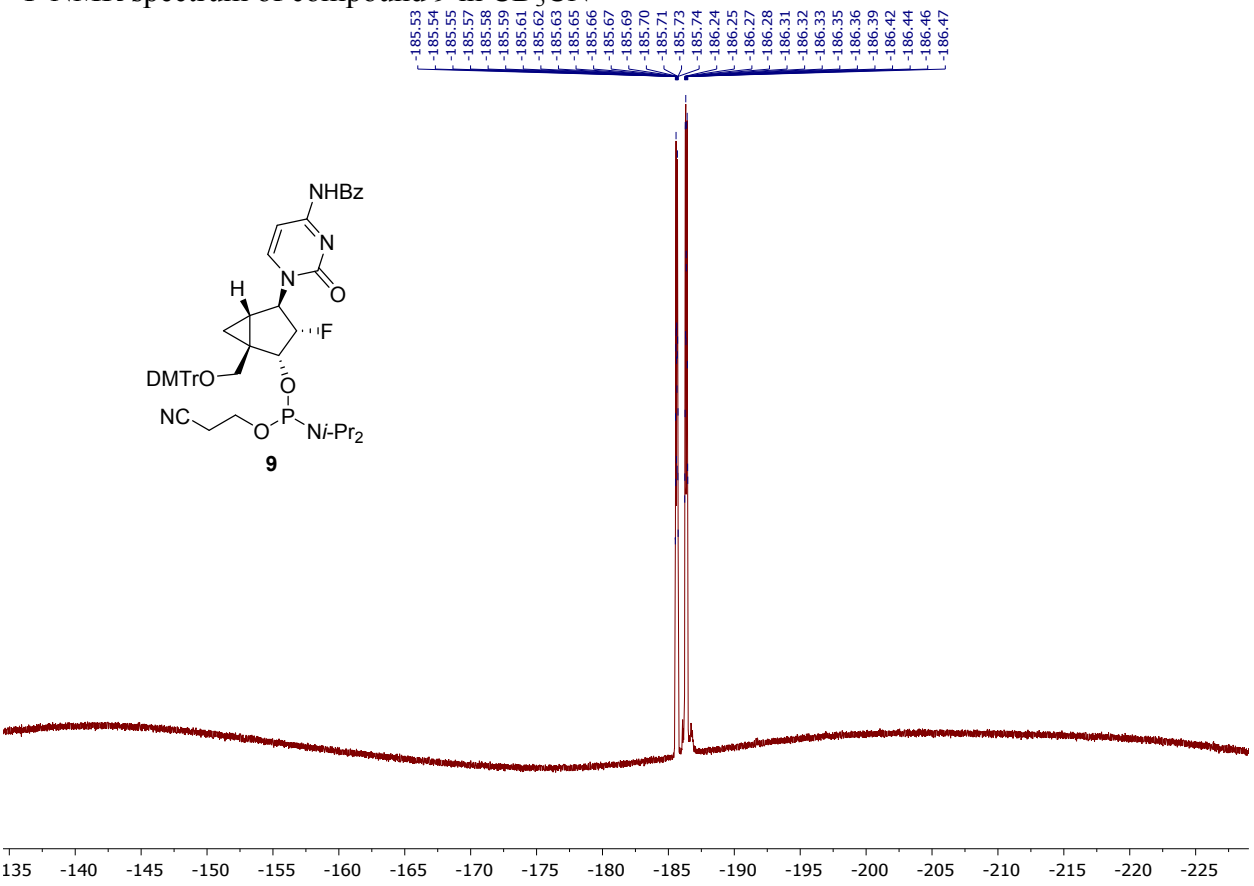
^{1}H NMR spectrum of compound **9** in CD_3CN



^{13}C NMR spectrum of compound **9** in CD_3CN

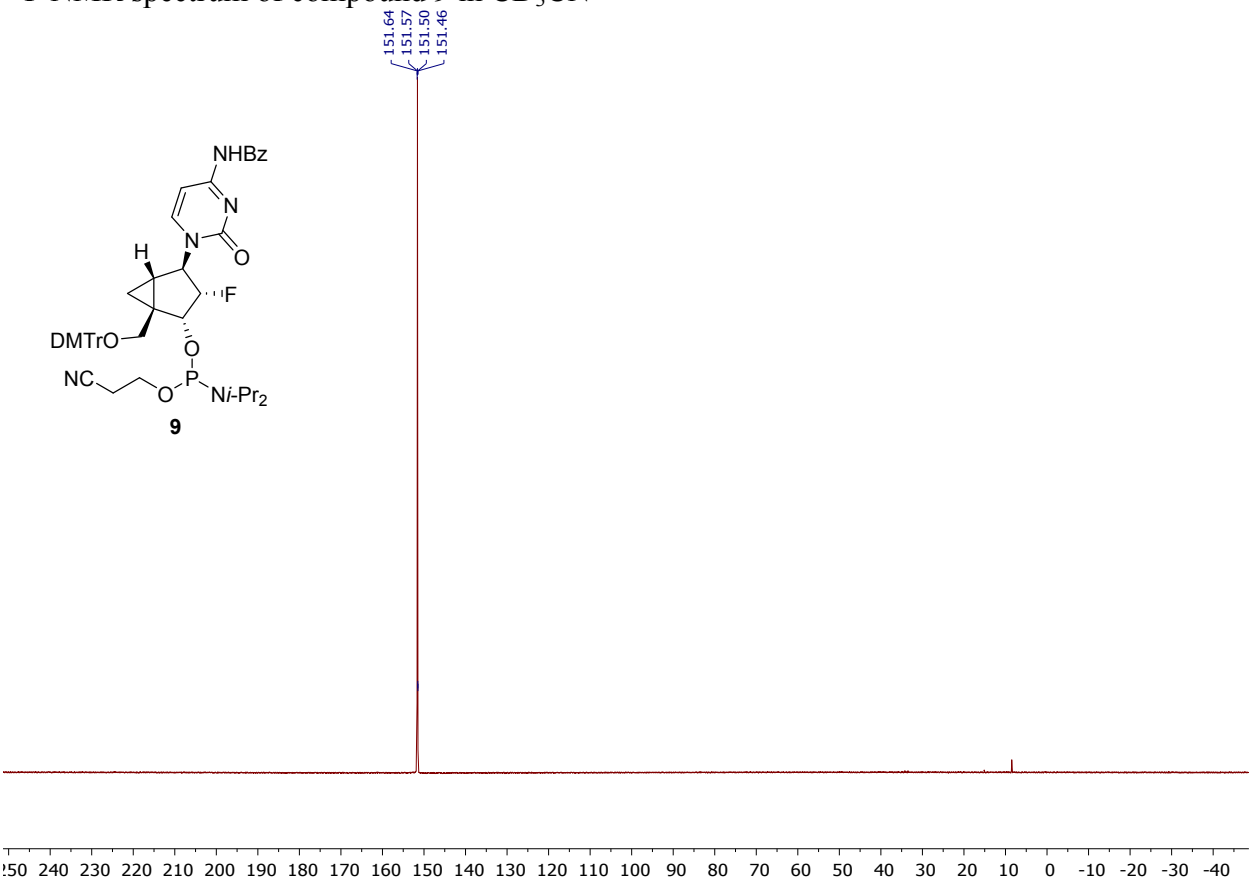


^{19}F NMR spectrum of compound **9** in CD_3CN



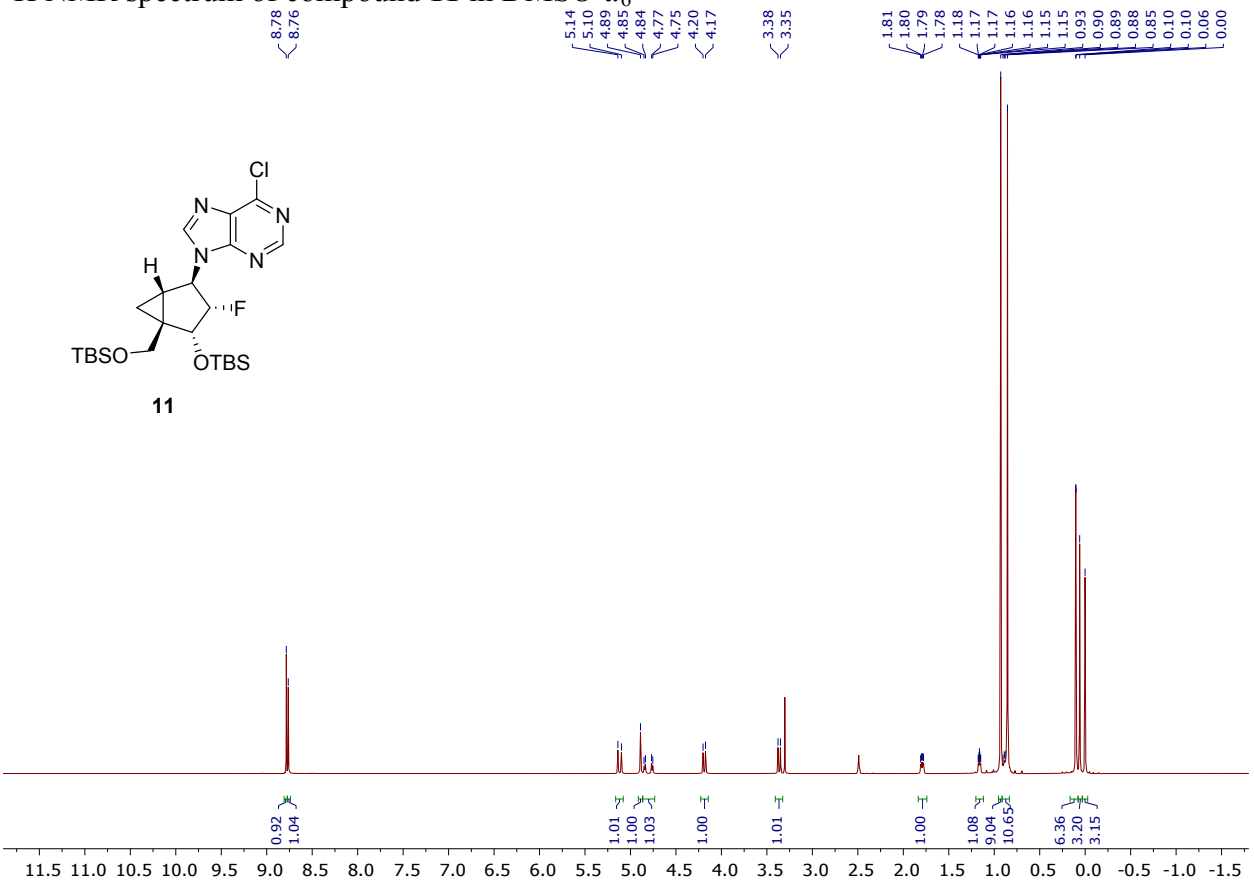
9

^{31}P NMR spectrum of compound **9** in CD_3CN

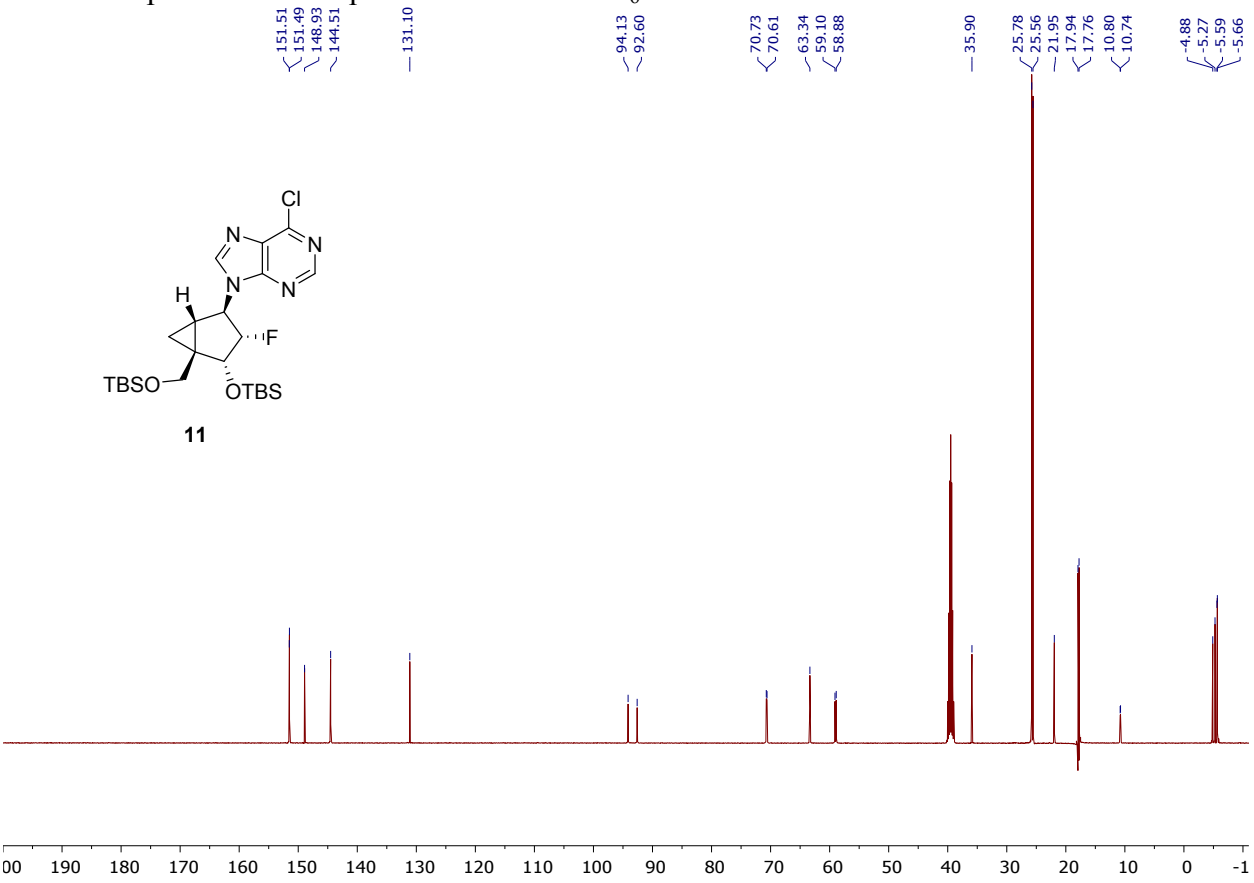


9

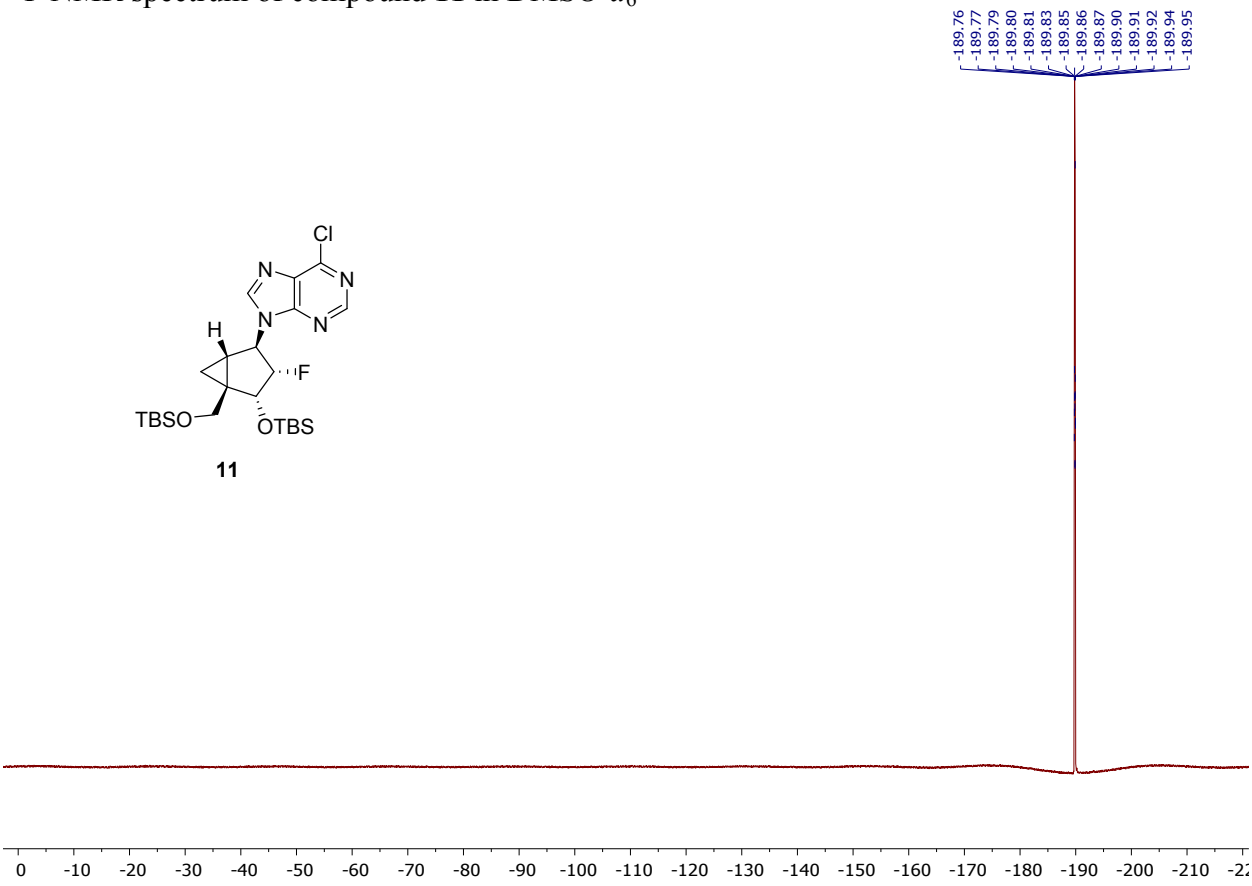
^{1}H NMR spectrum of compound **11** in DMSO-d_6



^{13}C NMR spectrum of compound **11** in DMSO-d_6

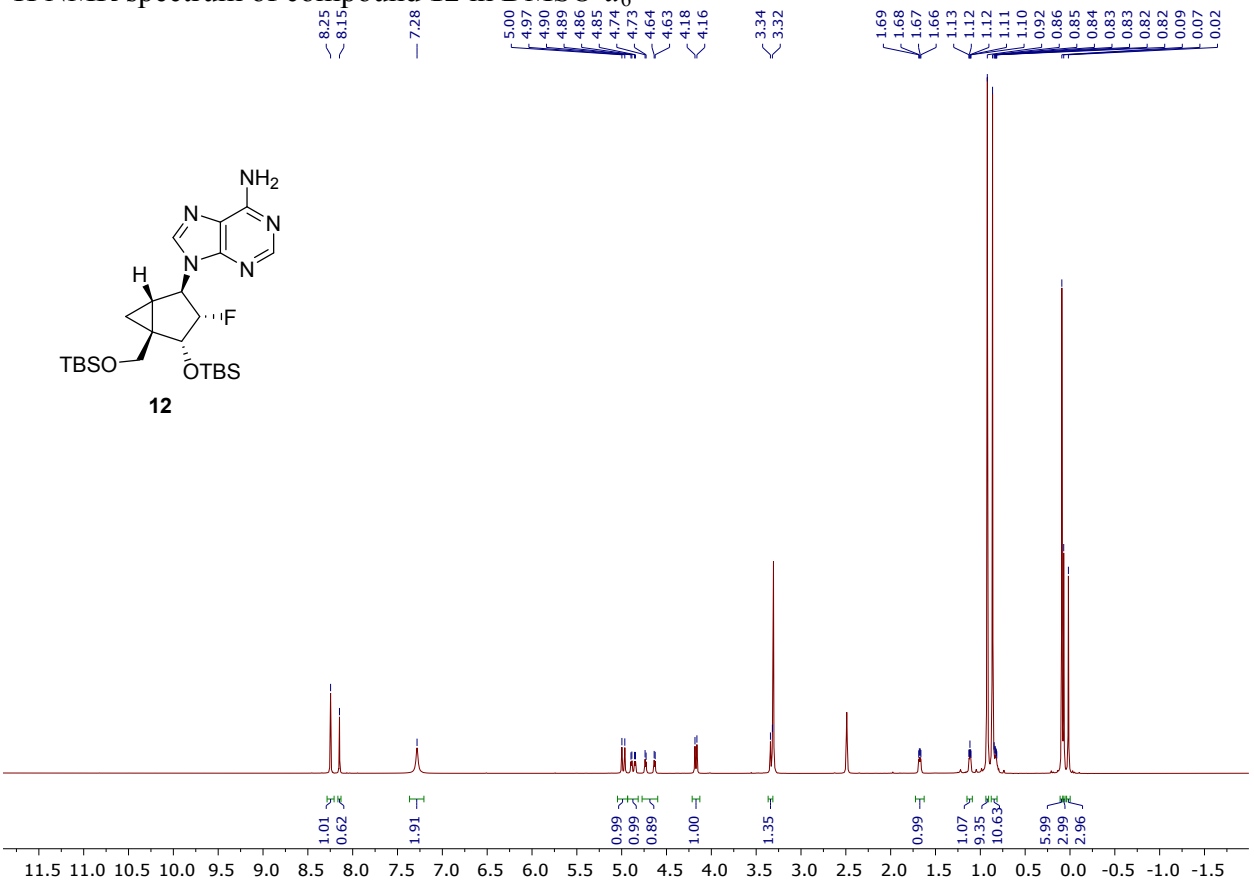


^{19}F NMR spectrum of compound **11** in DMSO-d_6

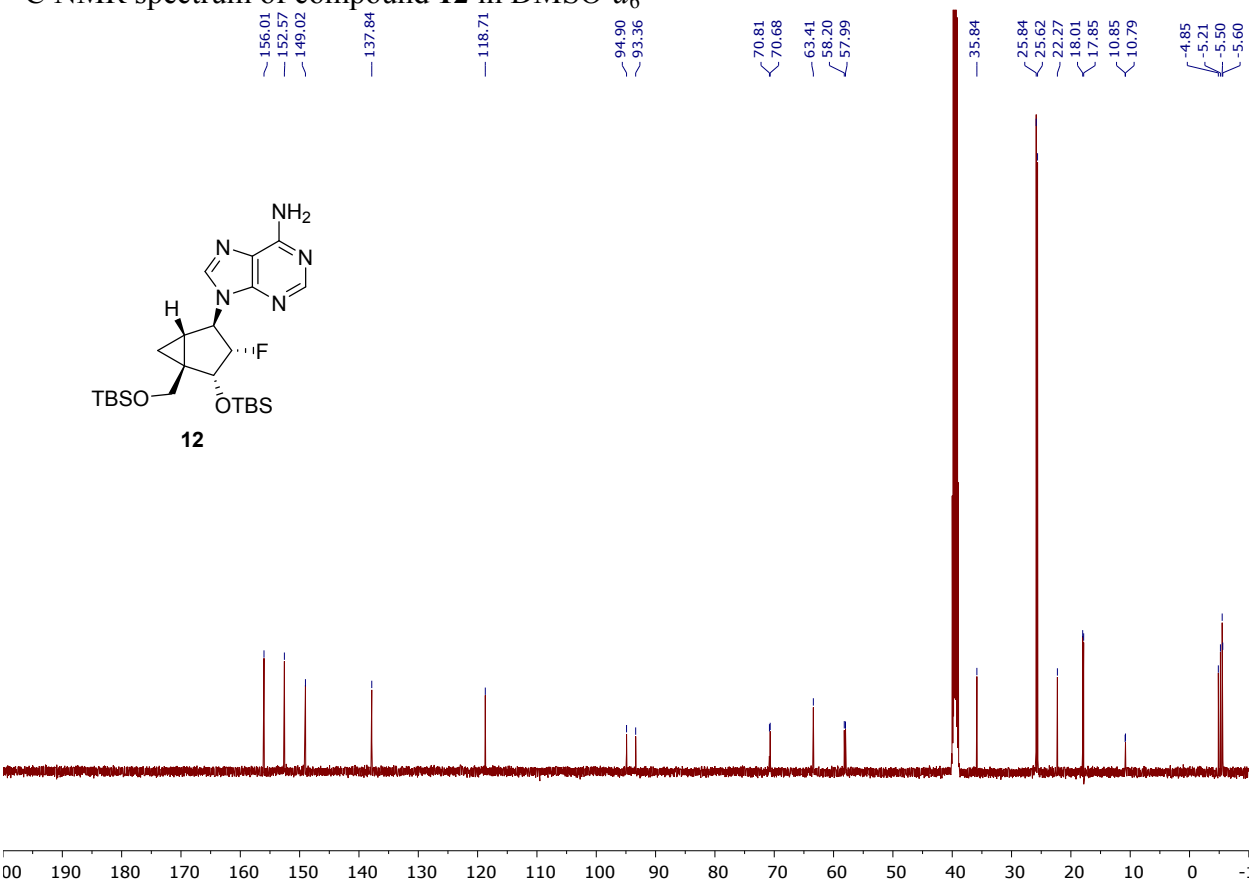


11

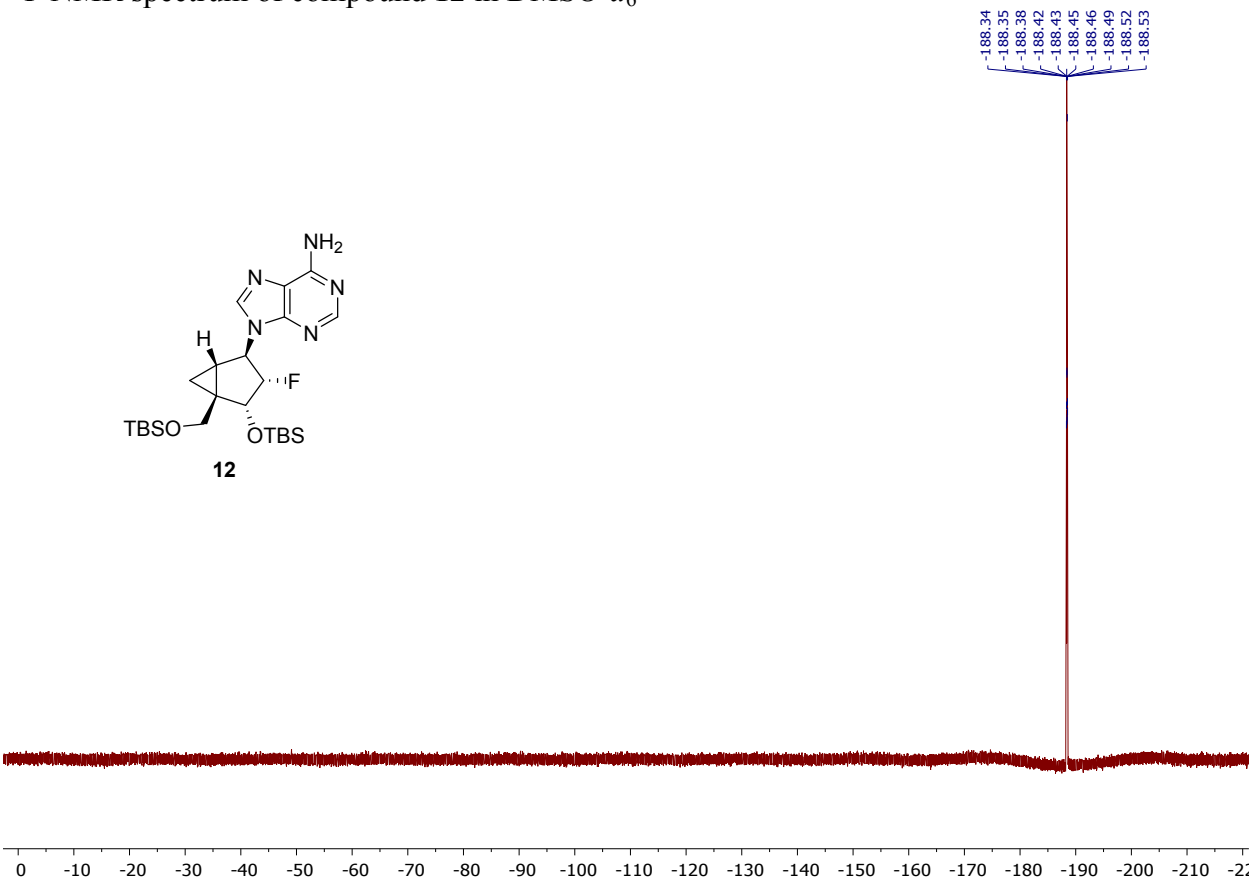
^{1}H NMR spectrum of compound **12** in DMSO-d_6



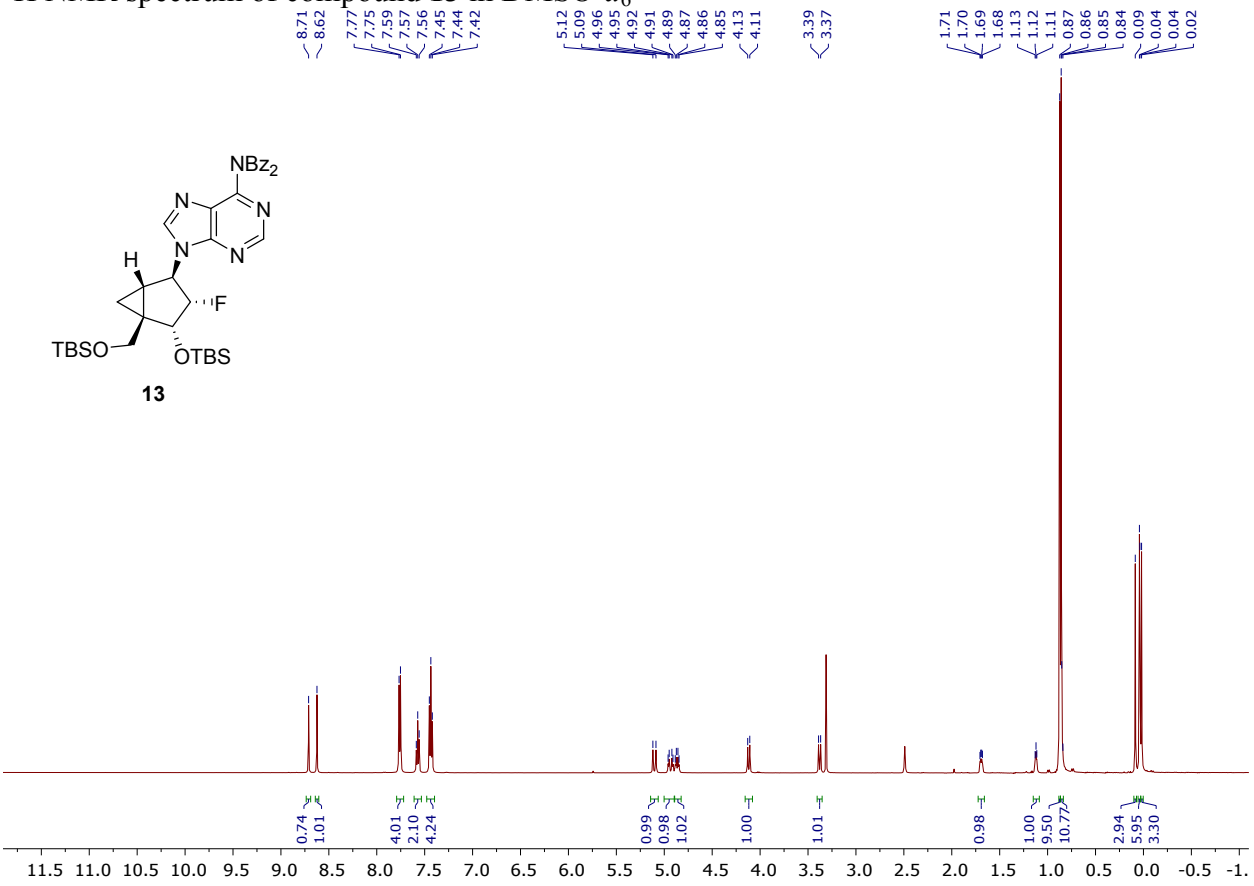
^{13}C NMR spectrum of compound **12** in DMSO-d_6



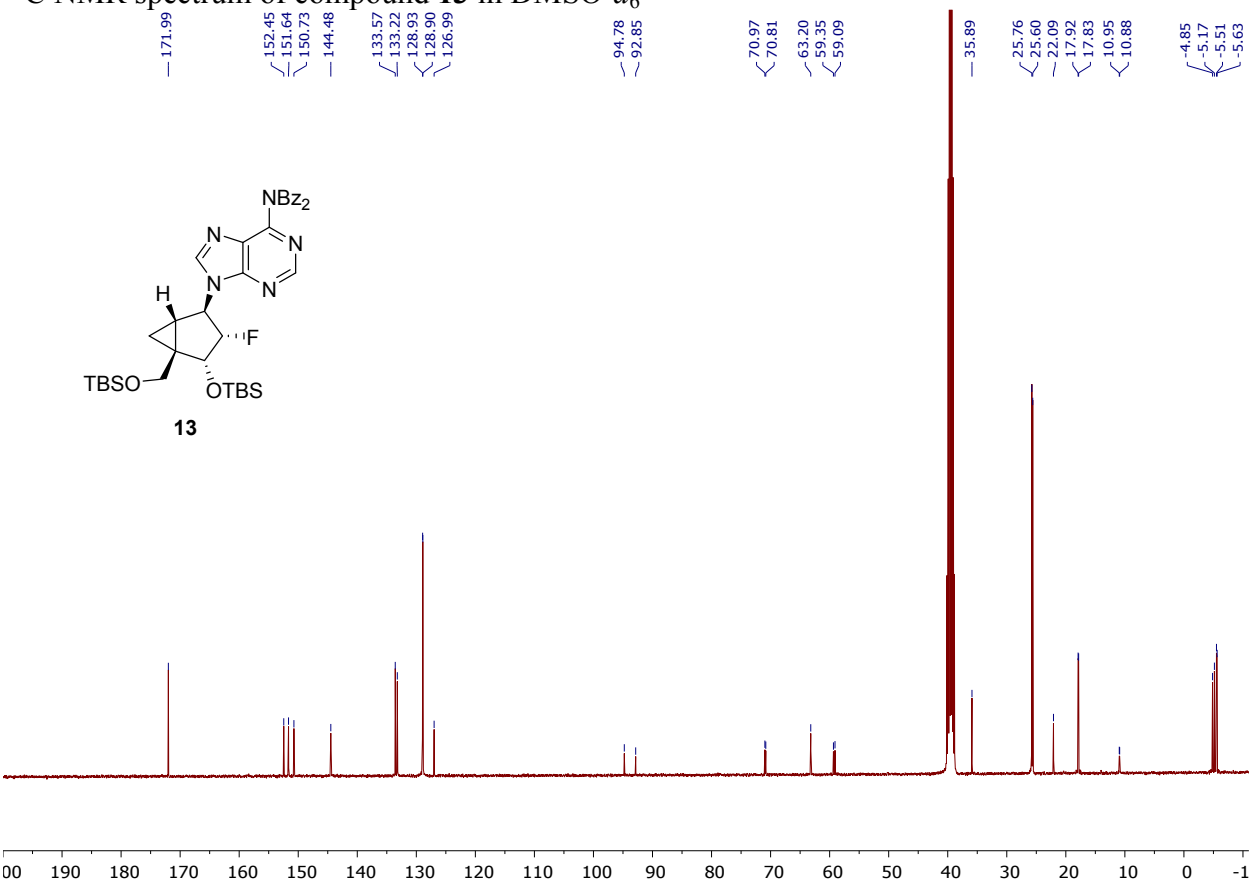
^{19}F NMR spectrum of compound **12** in DMSO-d_6



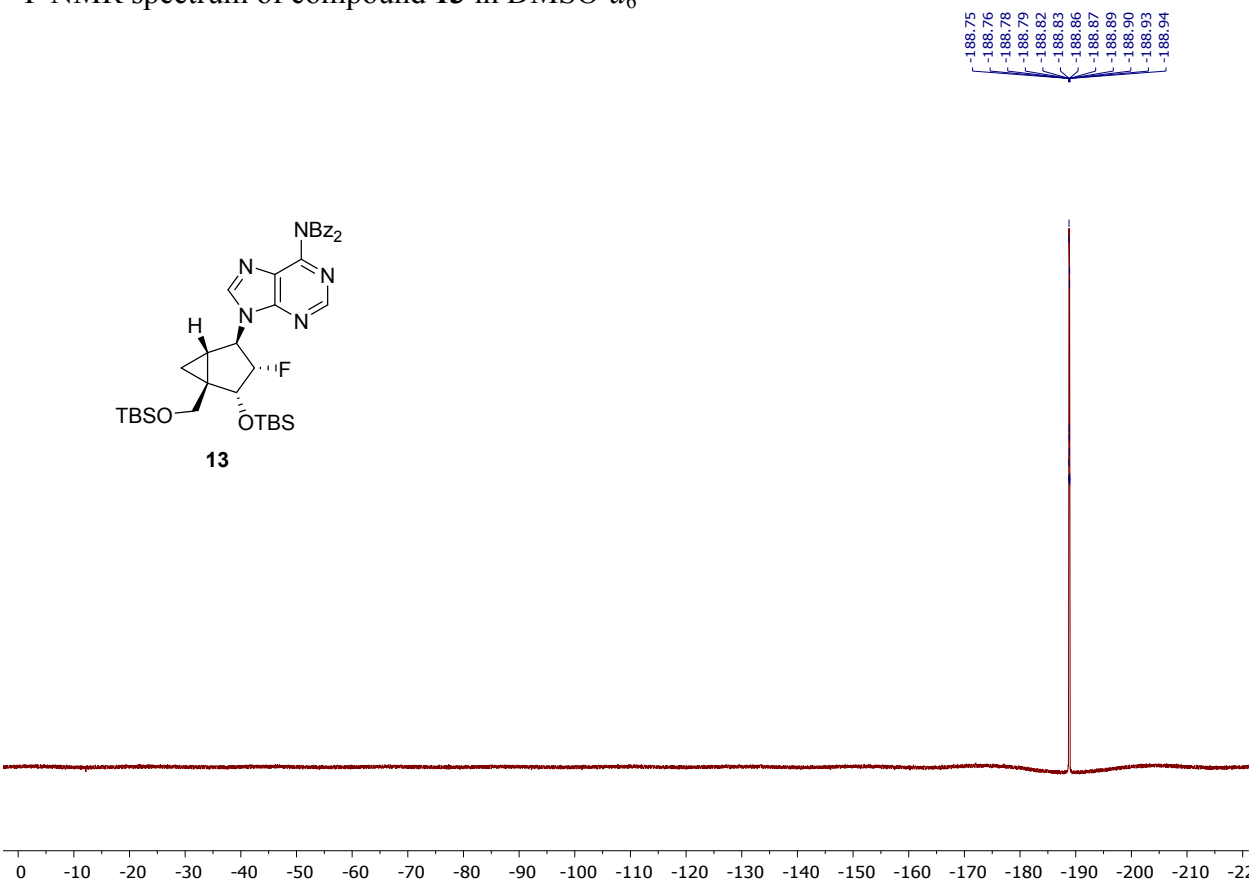
^{1}H NMR spectrum of compound **13** in DMSO-d_6



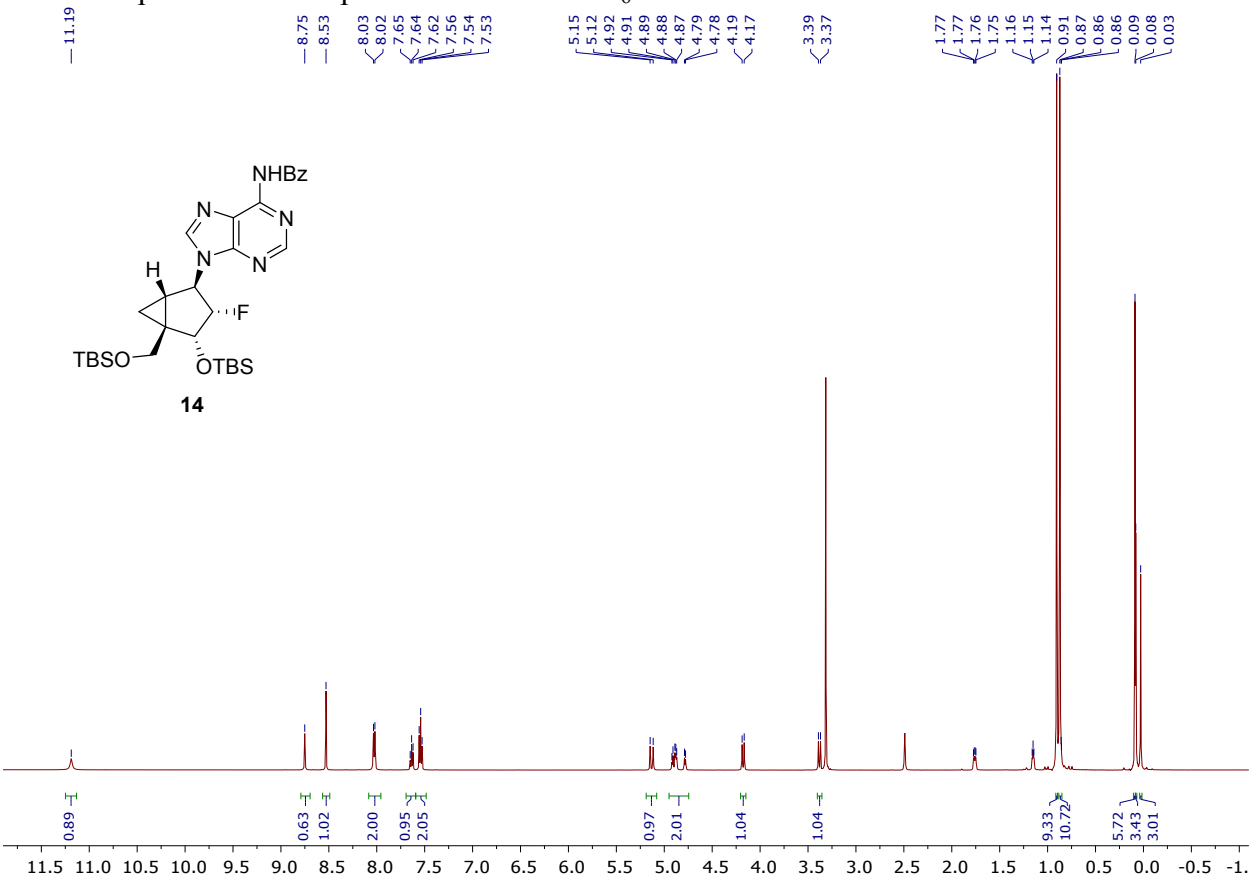
^{13}C NMR spectrum of compound **13** in DMSO-d_6



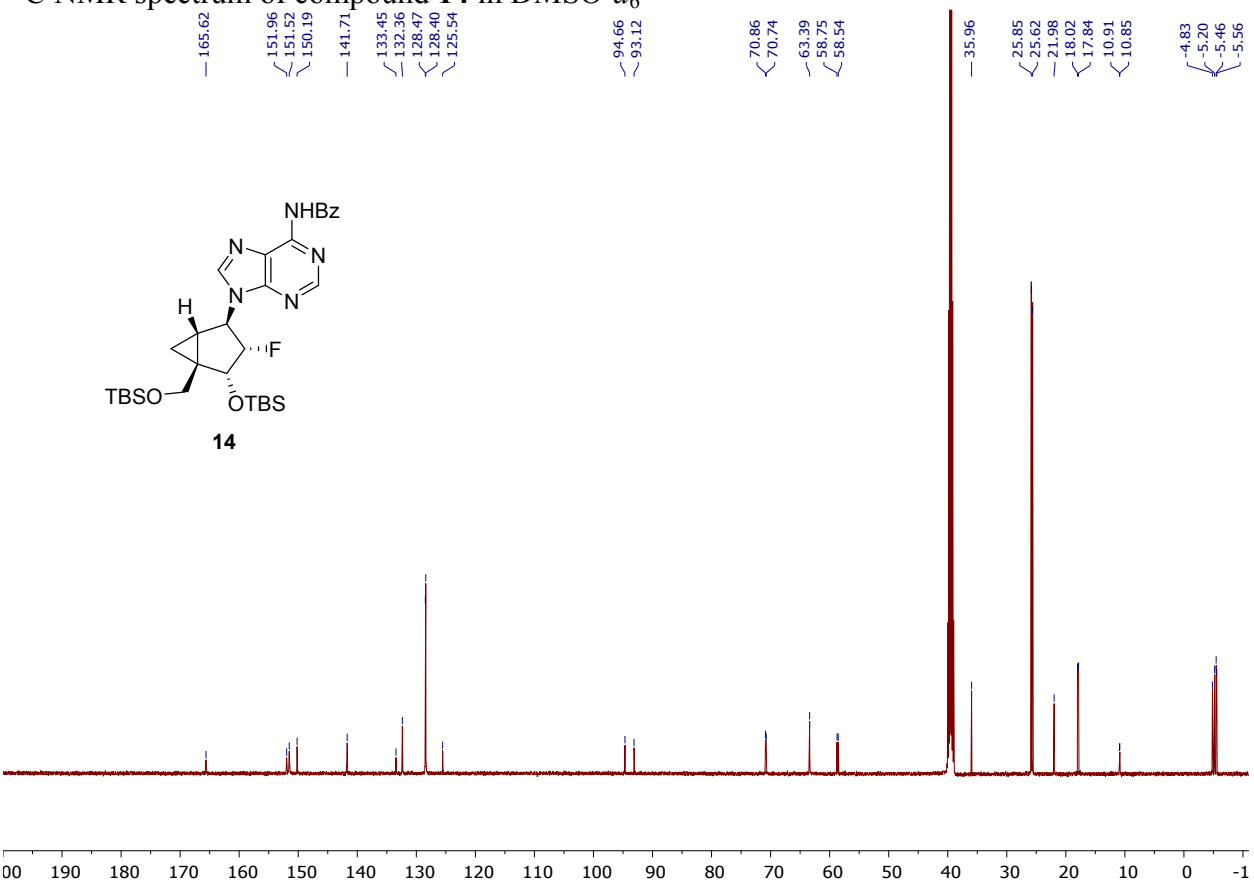
^{19}F NMR spectrum of compound **13** in DMSO-d_6



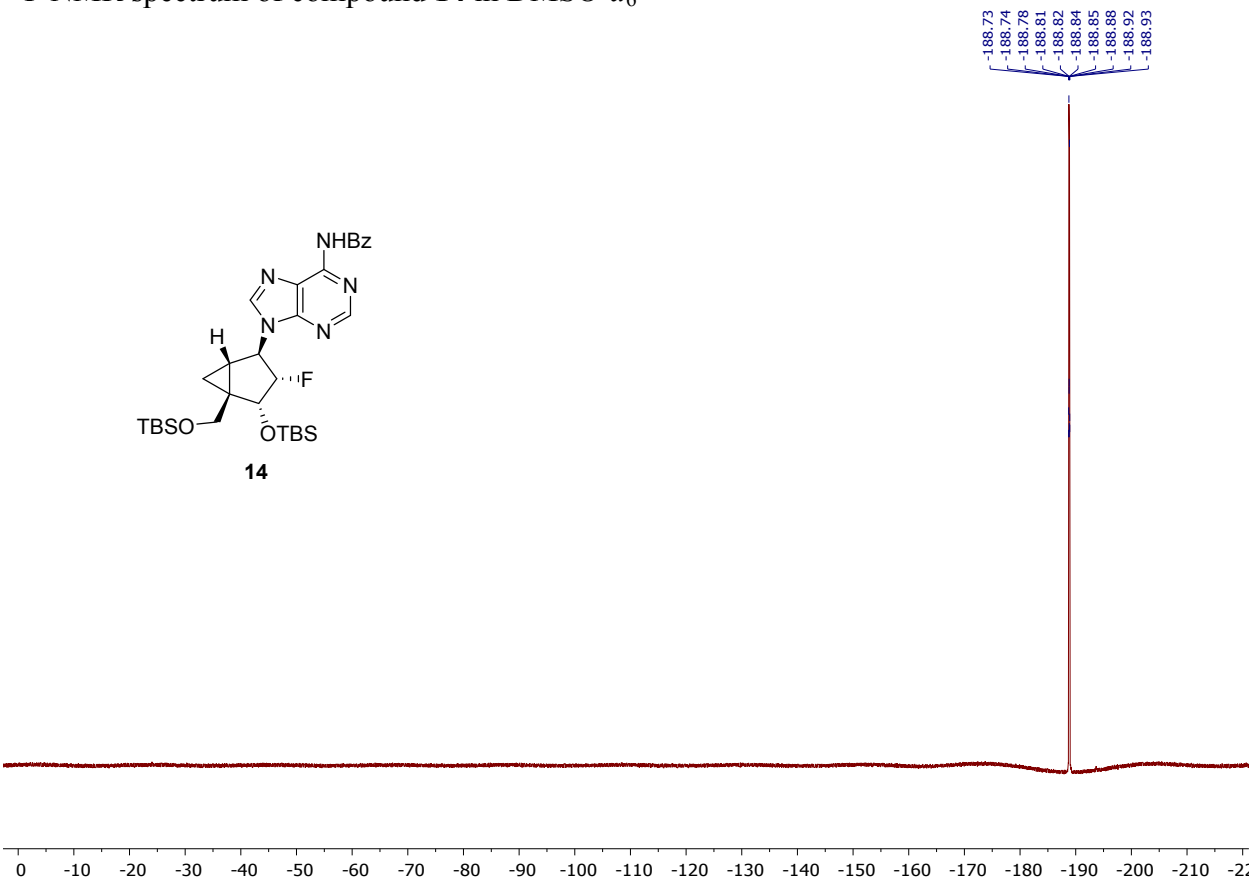
^{1}H NMR spectrum of compound **14** in DMSO-d_6



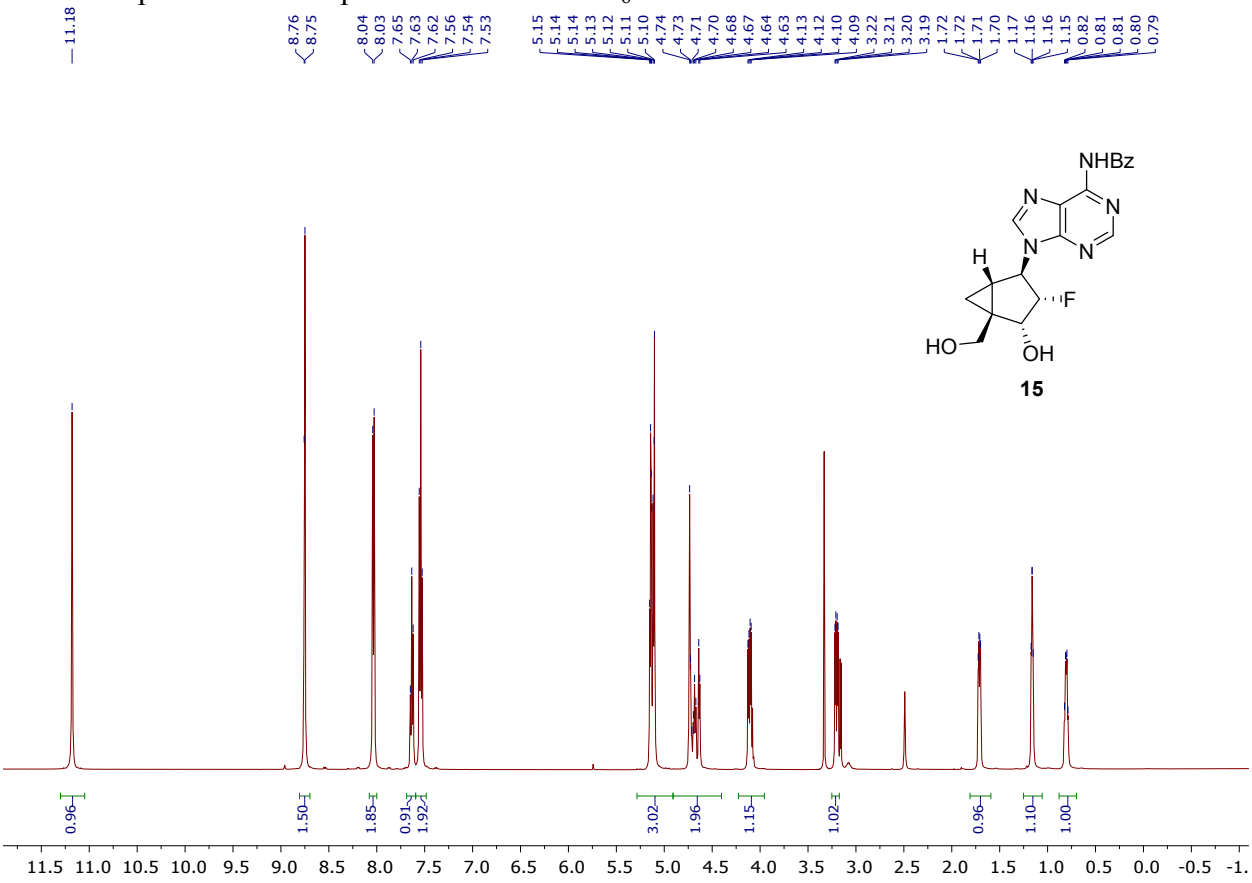
^{13}C NMR spectrum of compound **14** in DMSO-d_6



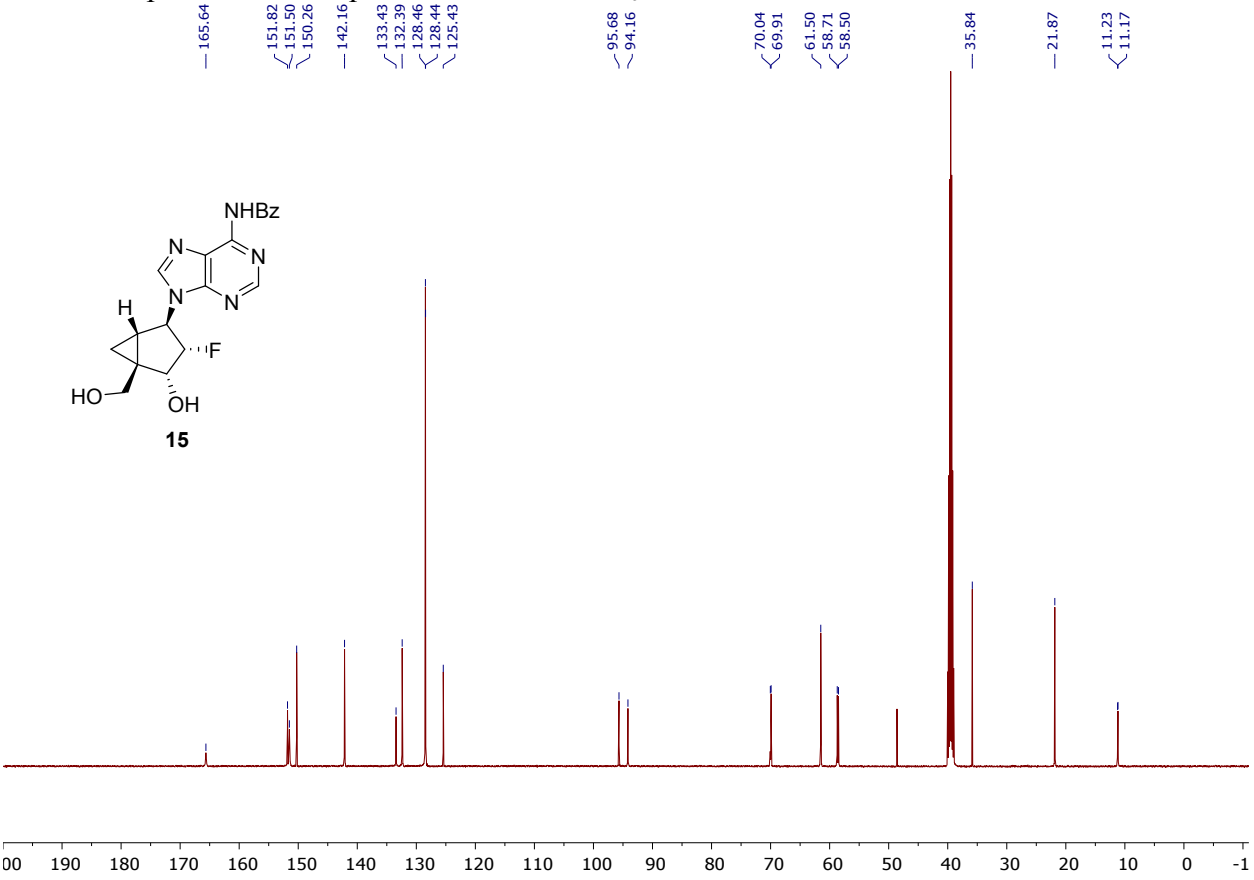
^{19}F NMR spectrum of compound **14** in DMSO-d_6



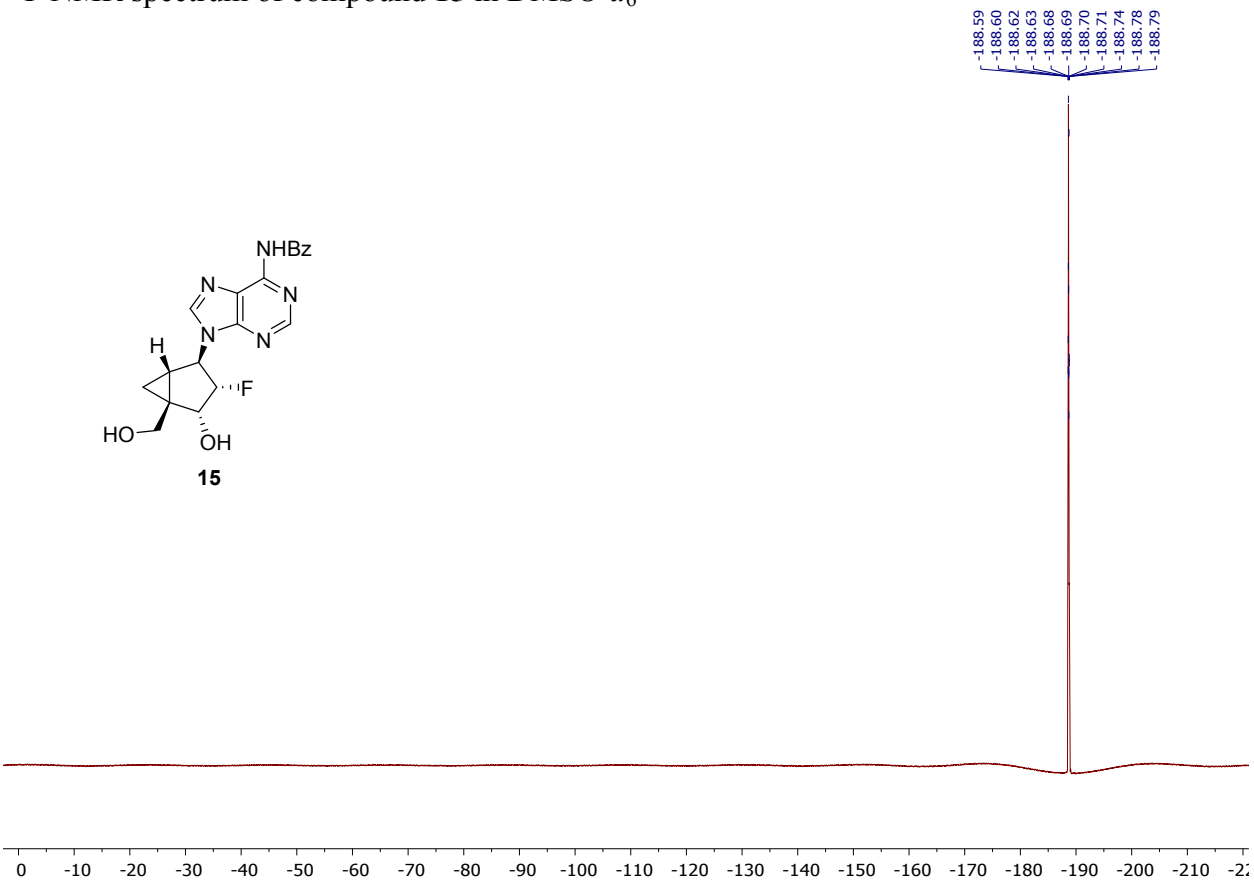
^{1}H NMR spectrum of compound **15** in DMSO-d_6



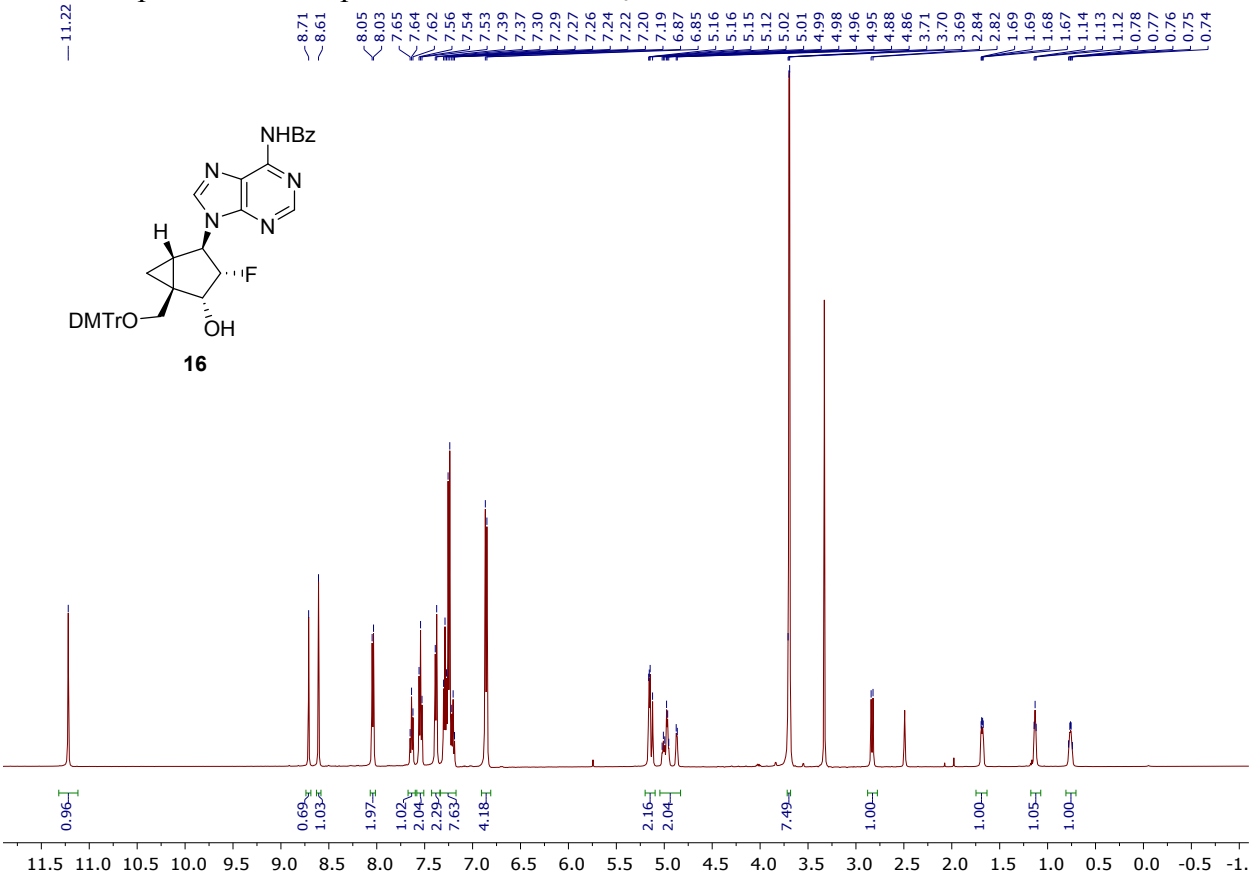
^{13}C NMR spectrum of compound **15** in DMSO-d_6



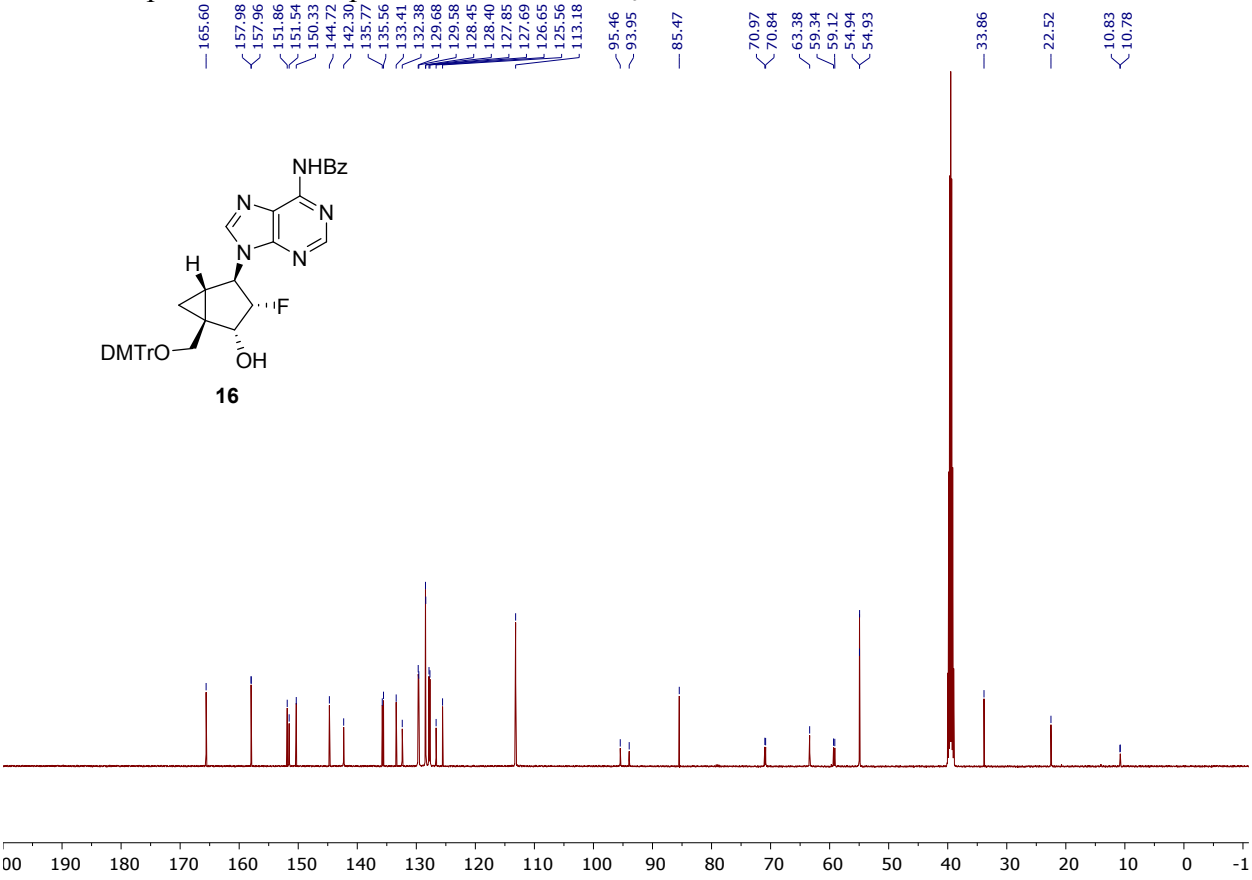
^{19}F NMR spectrum of compound **15** in DMSO-d_6



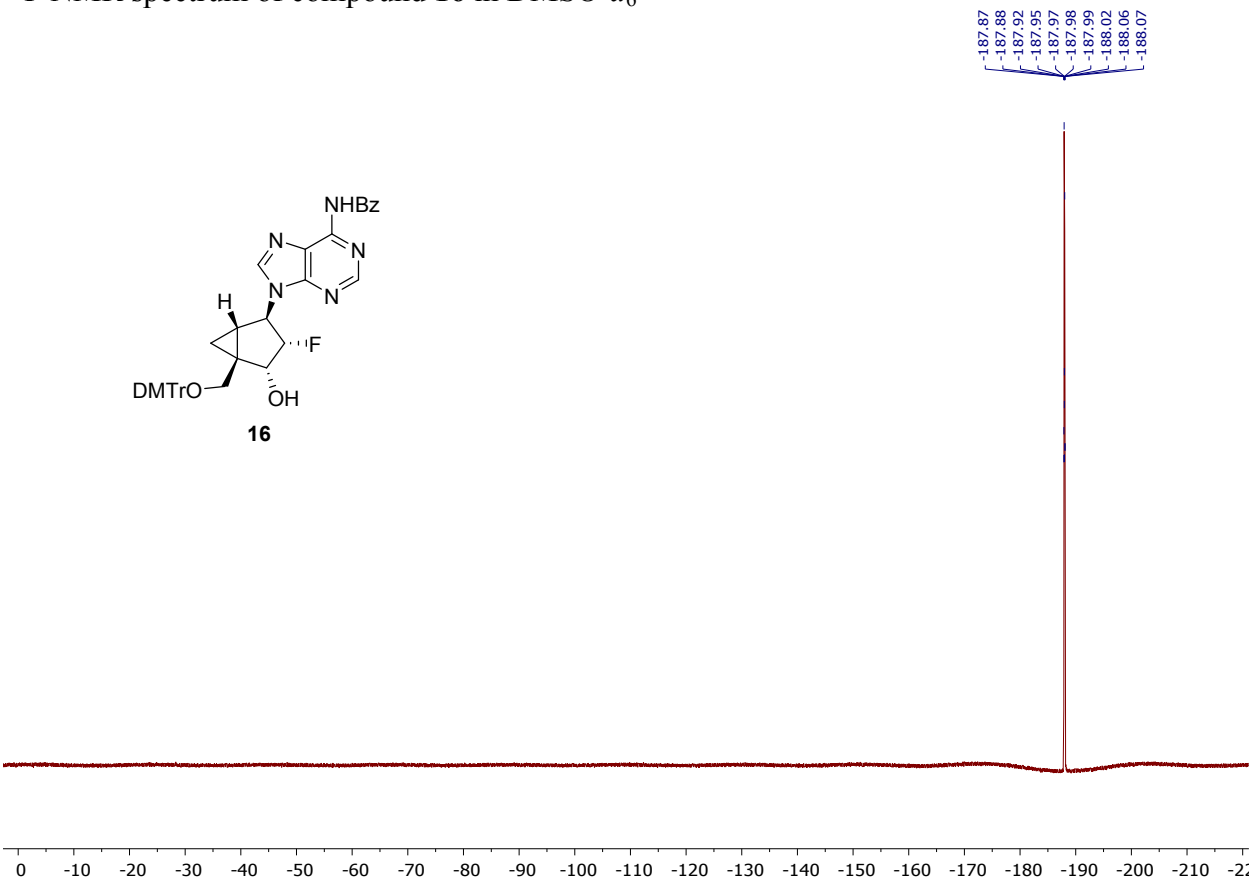
^{1}H NMR spectrum of compound **16** in DMSO-d_6



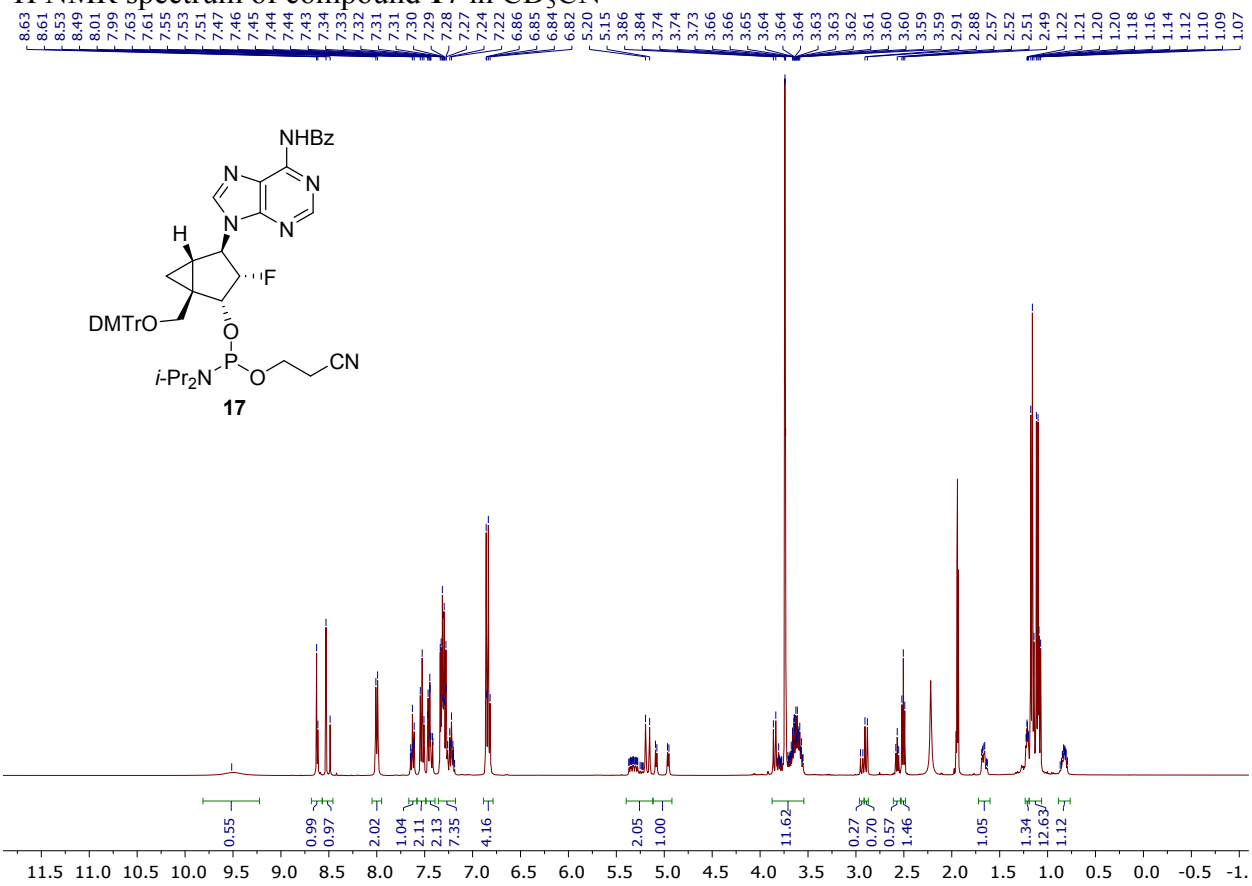
^{13}C NMR spectrum of compound **16** in DMSO-d_6



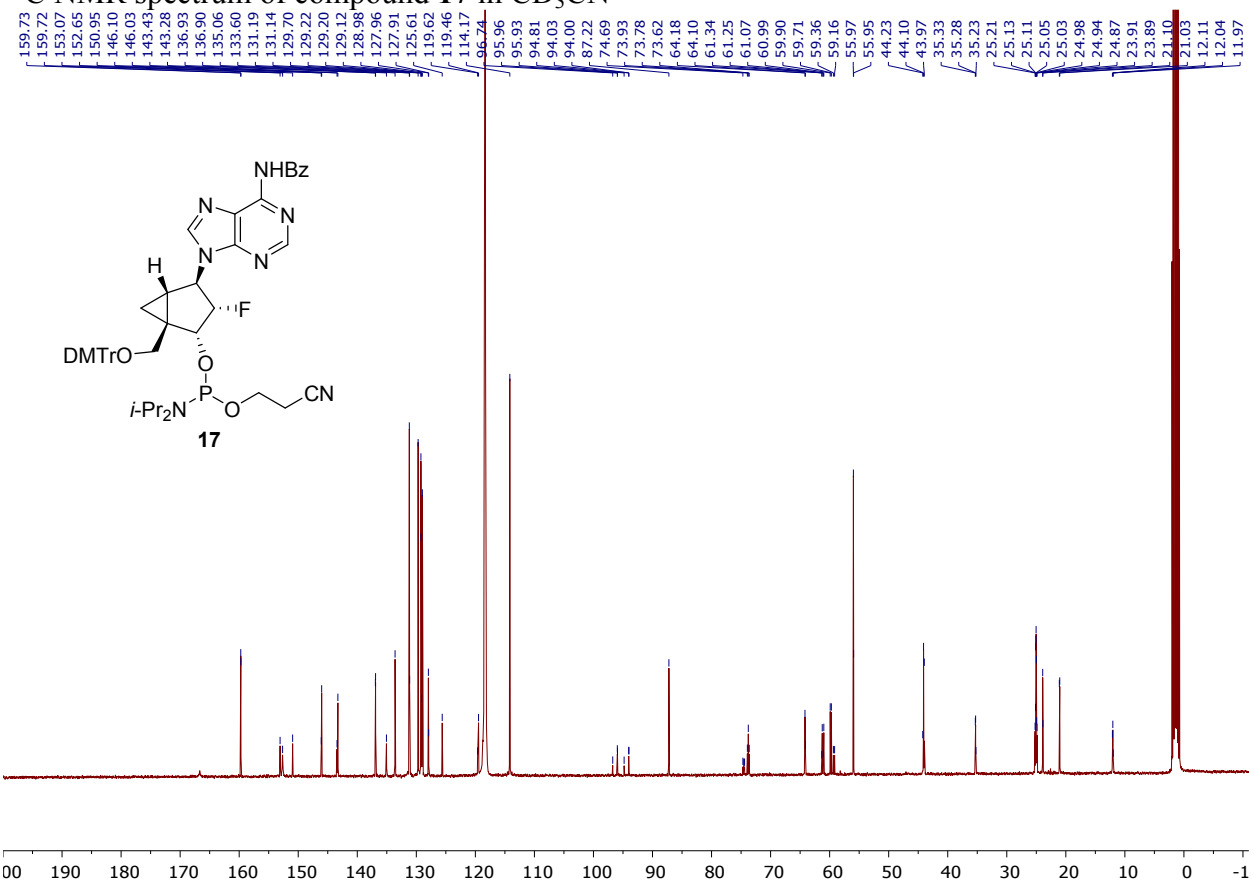
^{19}F NMR spectrum of compound **16** in DMSO-d_6



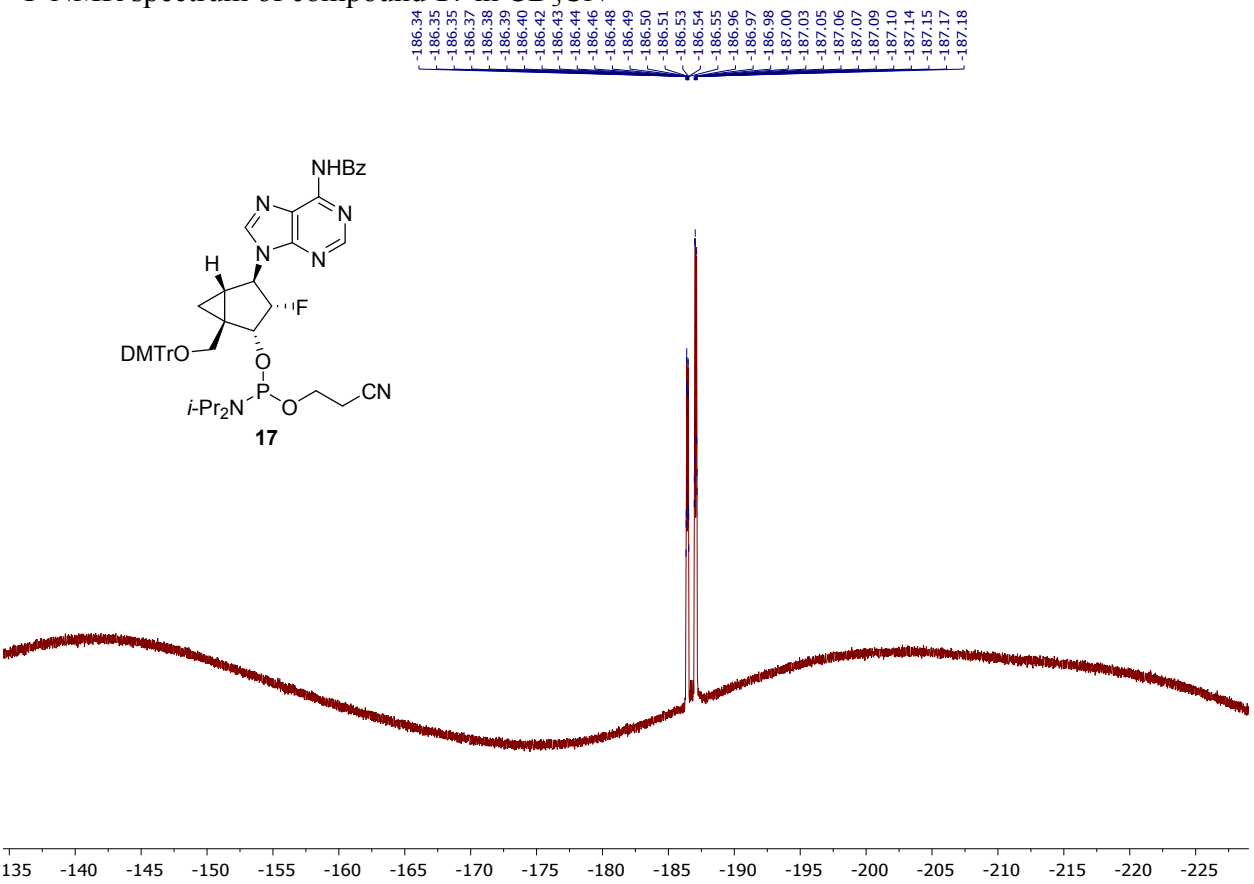
^{1}H NMR spectrum of compound **17** in CD_3CN



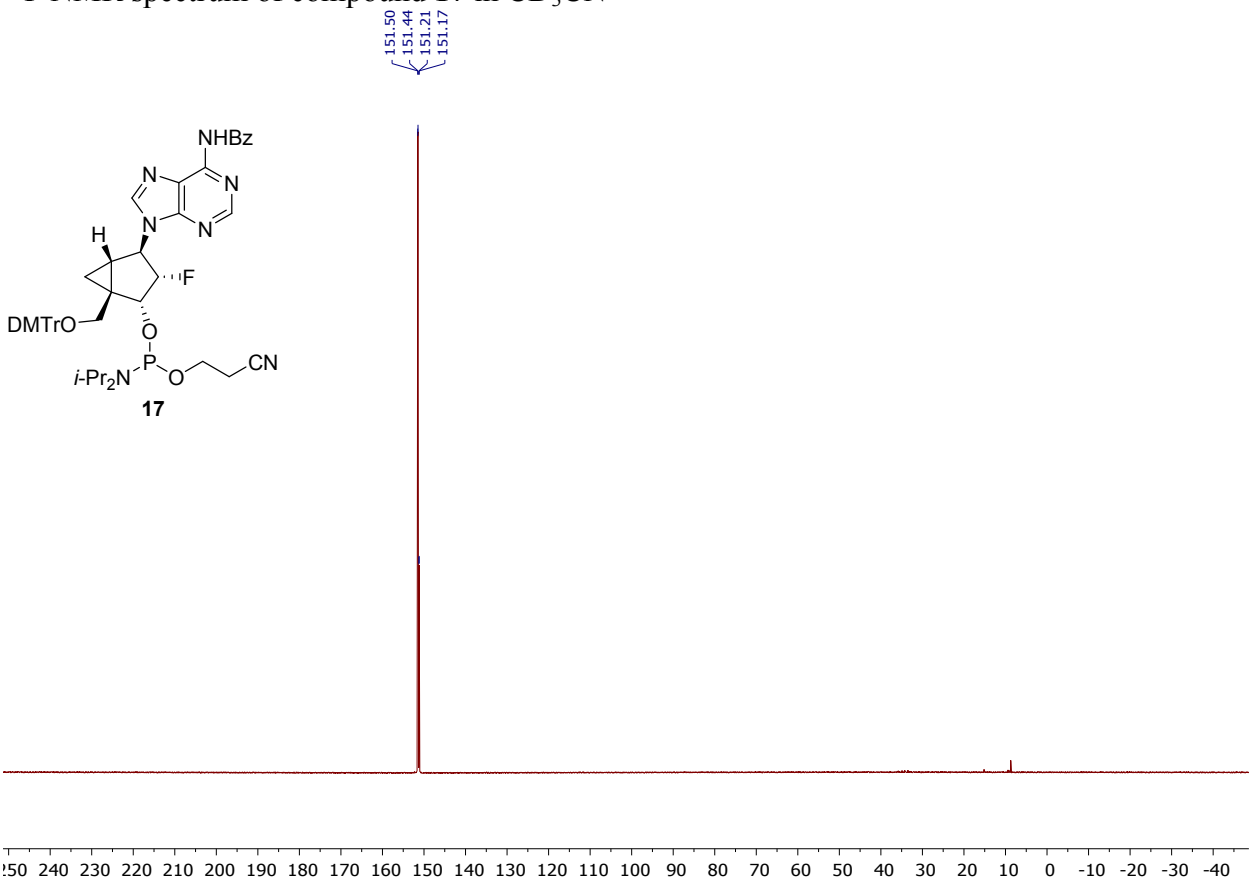
^{13}C NMR spectrum of compound **17** in CD_3CN



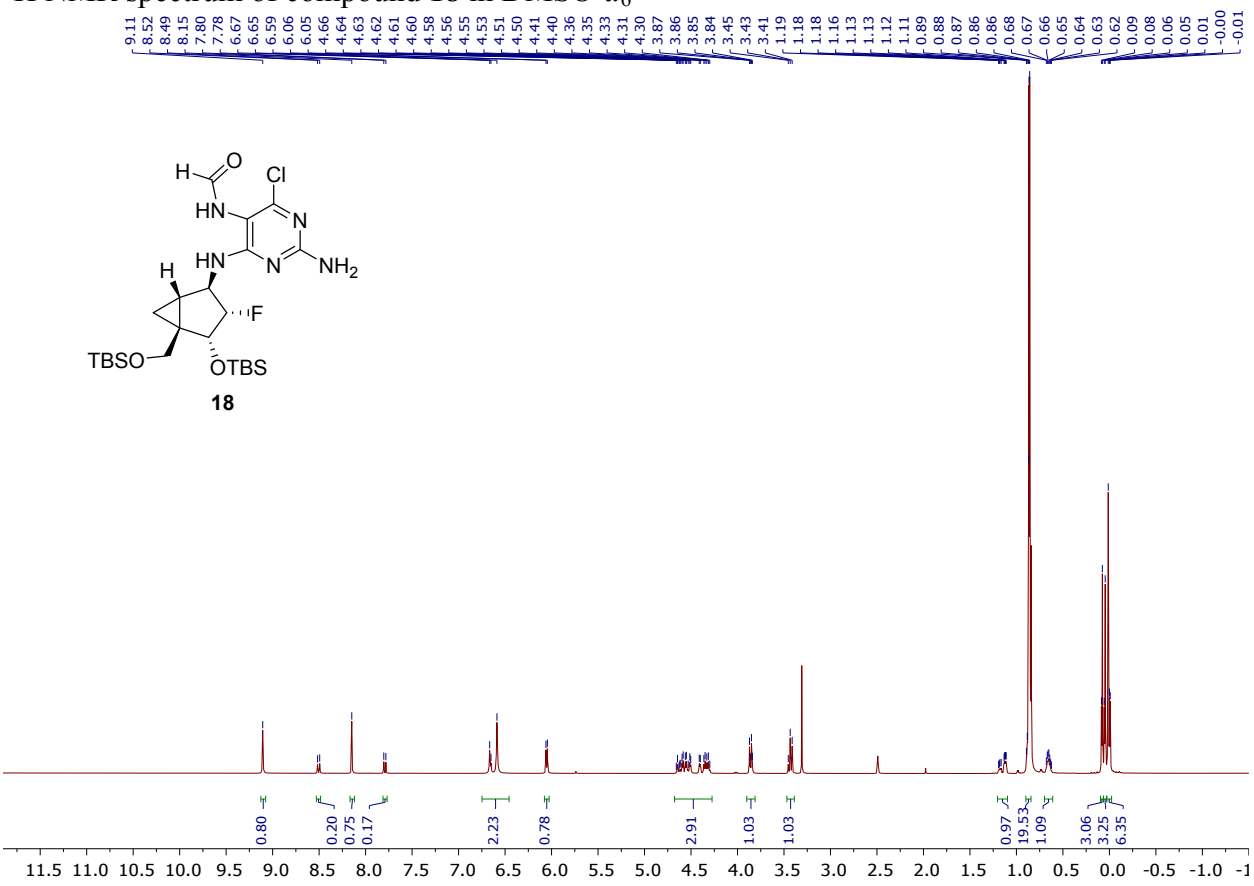
^{19}F NMR spectrum of compound **17** in CD_3CN



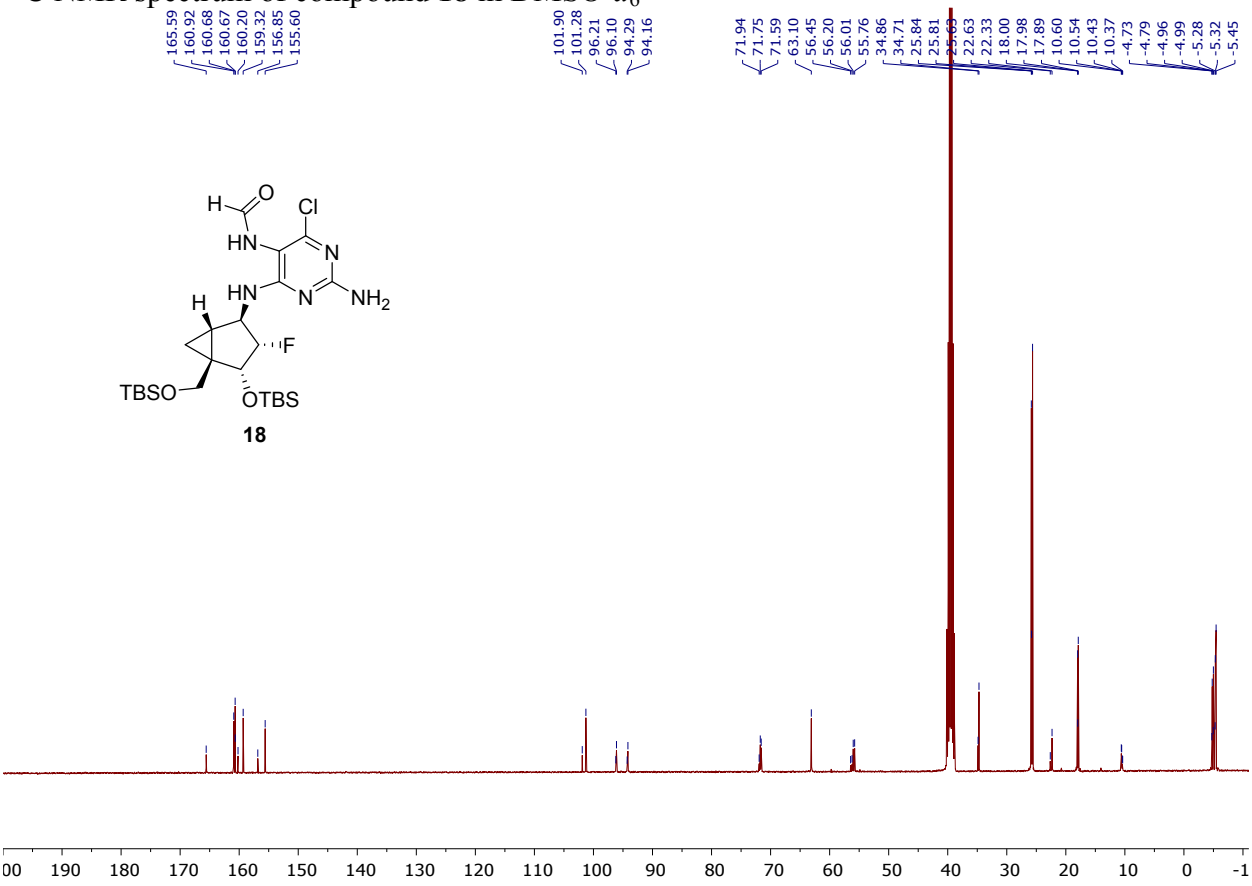
^{31}P NMR spectrum of compound **17** in CD_3CN



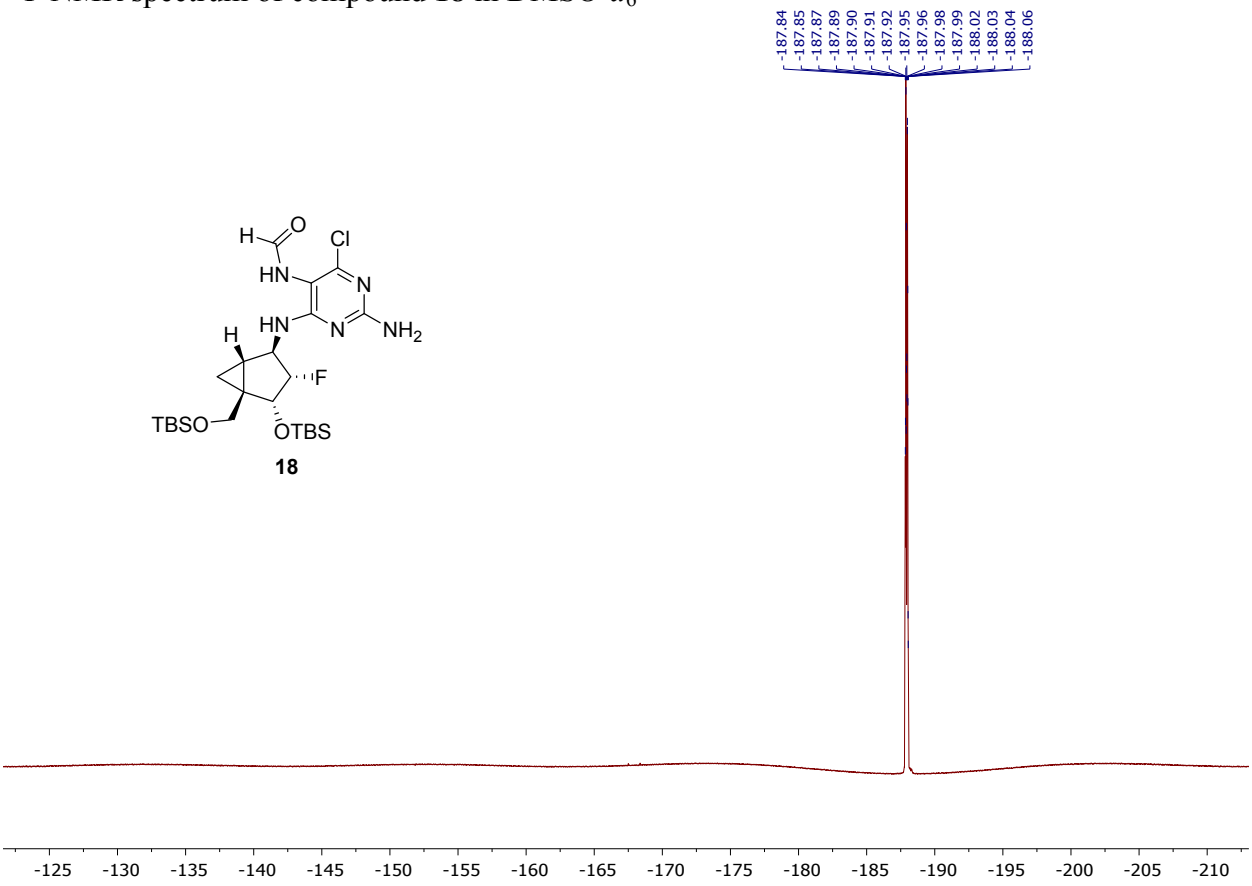
^{1}H NMR spectrum of compound **18** in DMSO-d_6



^{13}C NMR spectrum of compound **18** in DMSO-d_6

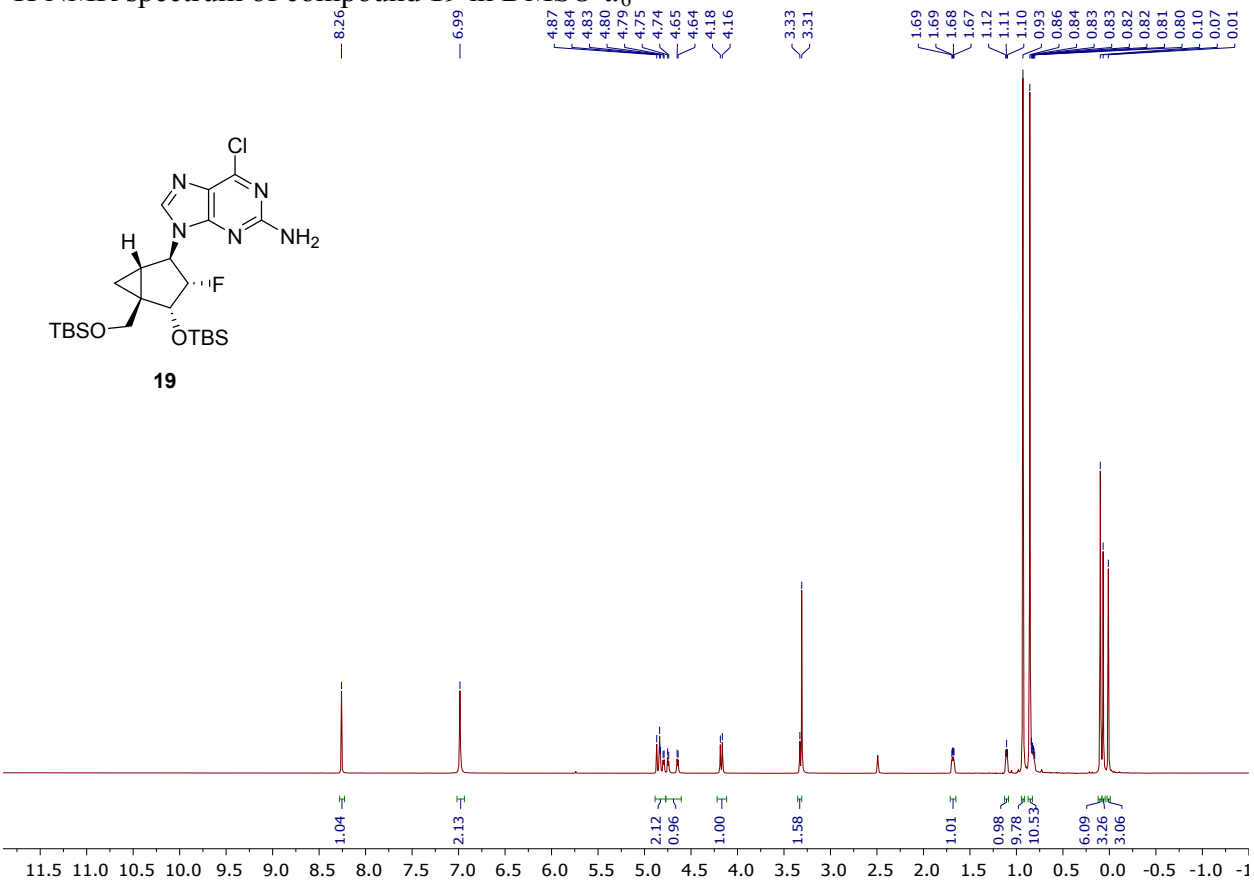


^{19}F NMR spectrum of compound **18** in DMSO-d_6

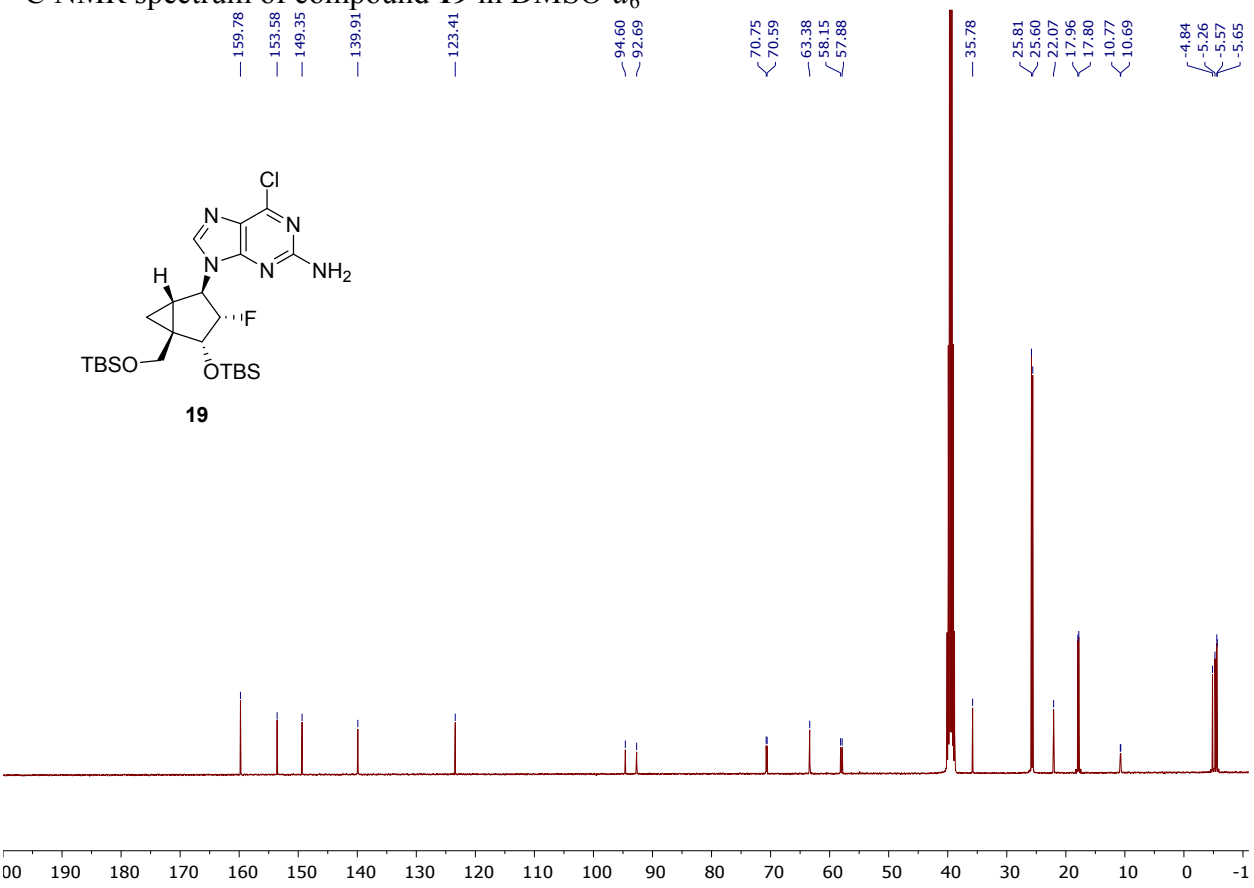


18

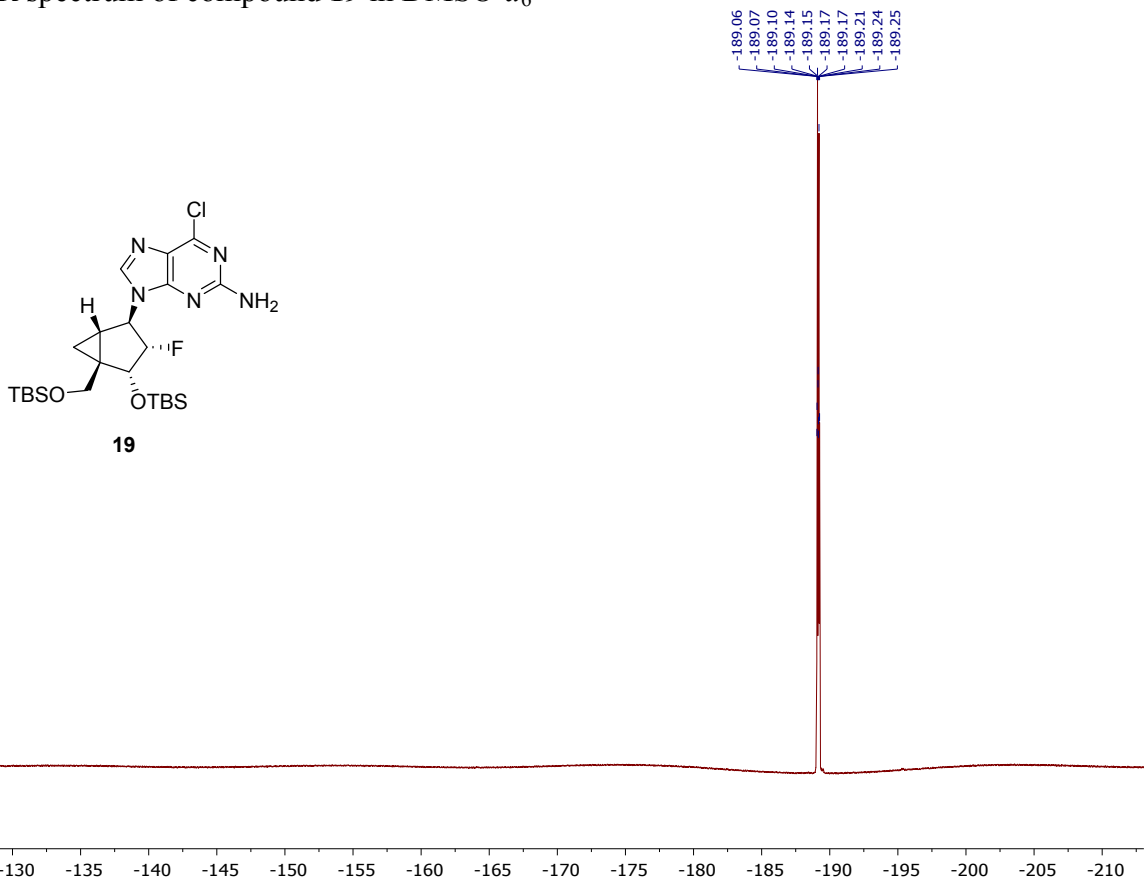
^{1}H NMR spectrum of compound **19** in DMSO-d_6



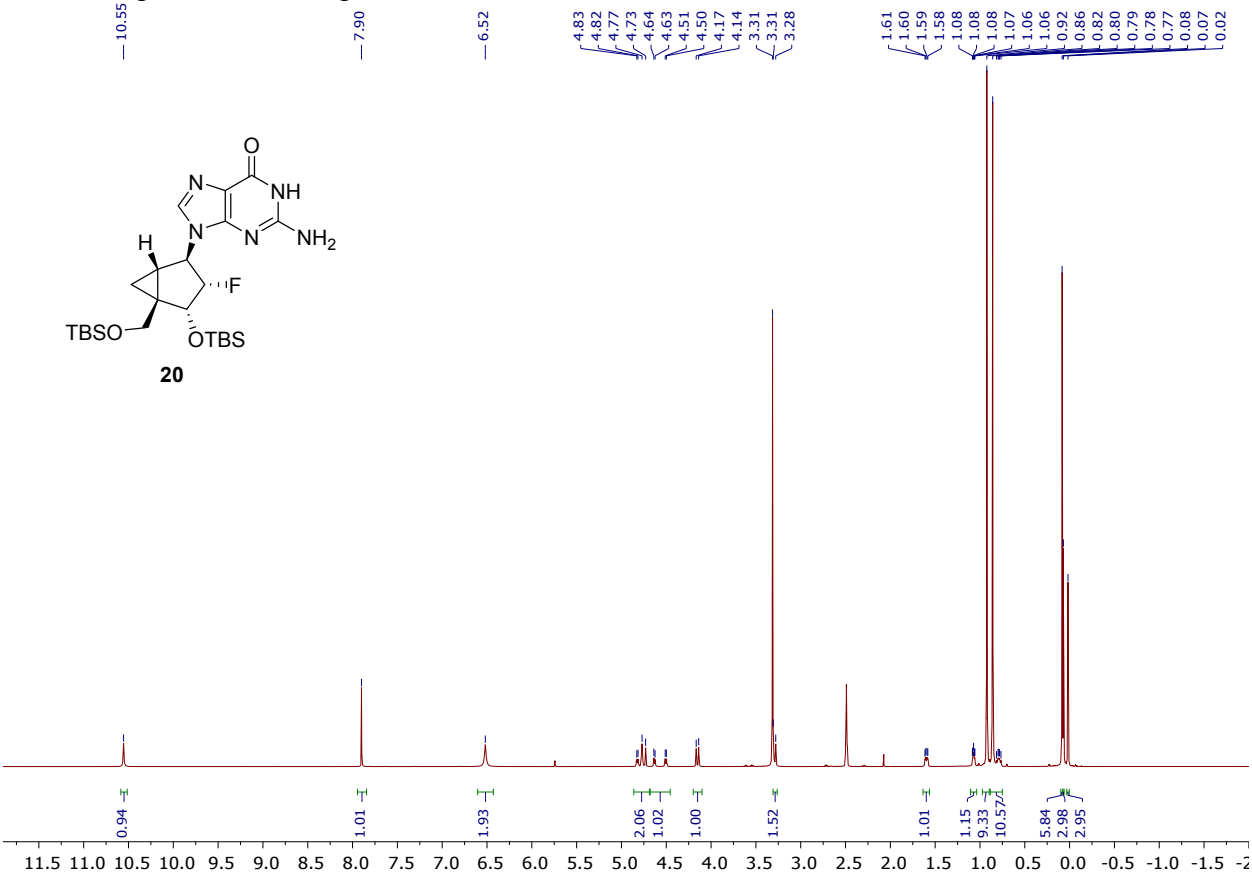
^{13}C NMR spectrum of compound **19** in DMSO-d_6



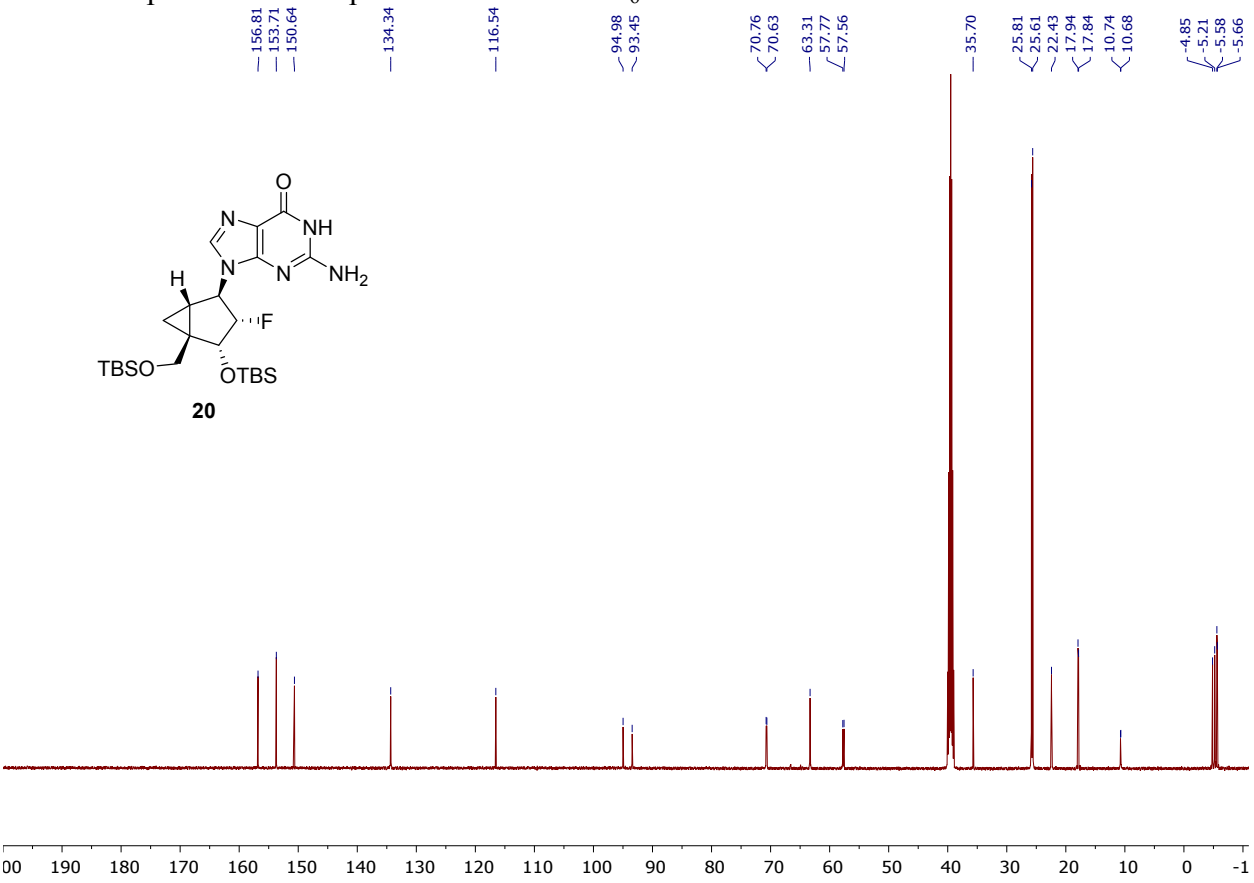
^{19}F NMR spectrum of compound **19** in DMSO-d_6



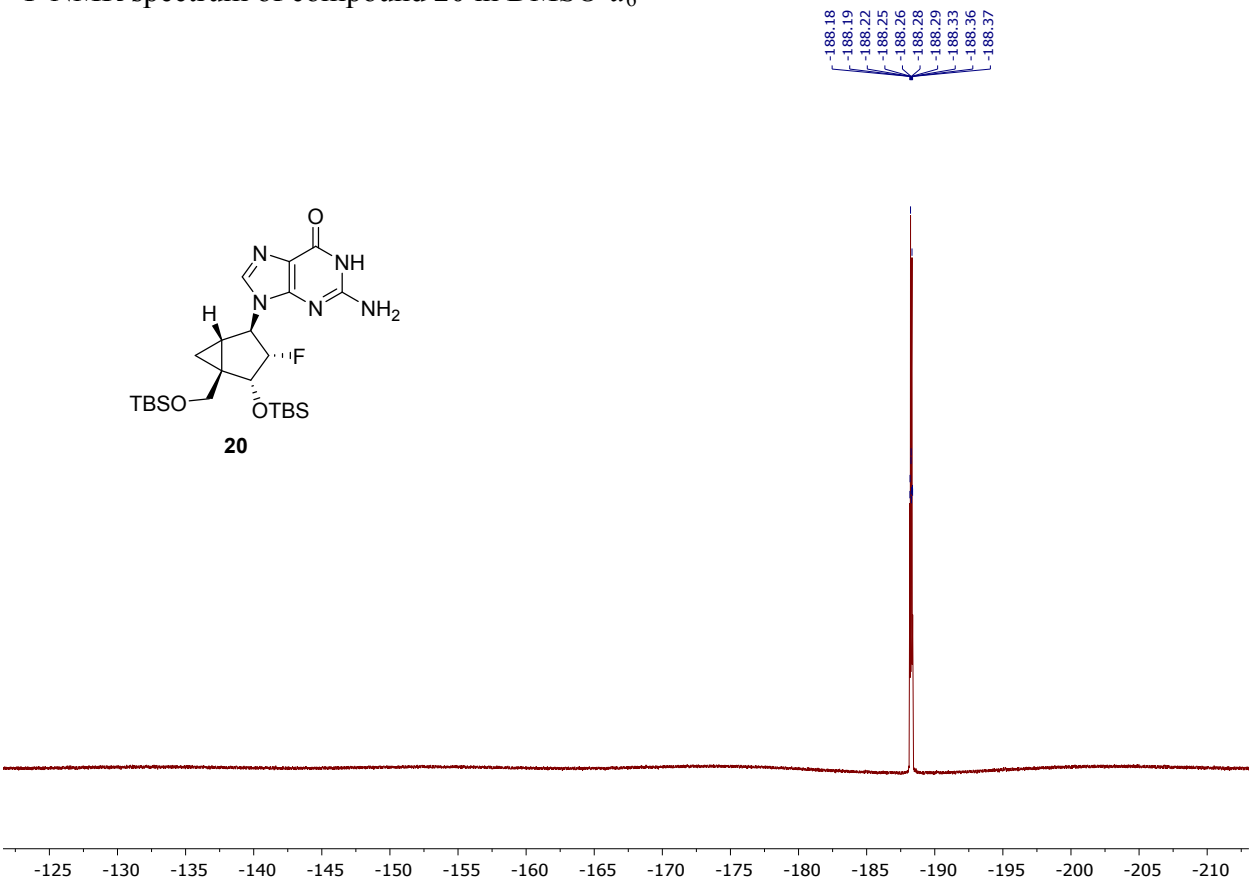
^{1}H NMR spectrum of compound **20** in DMSO-d_6



^{13}C NMR spectrum of compound **20** in DMSO-d_6

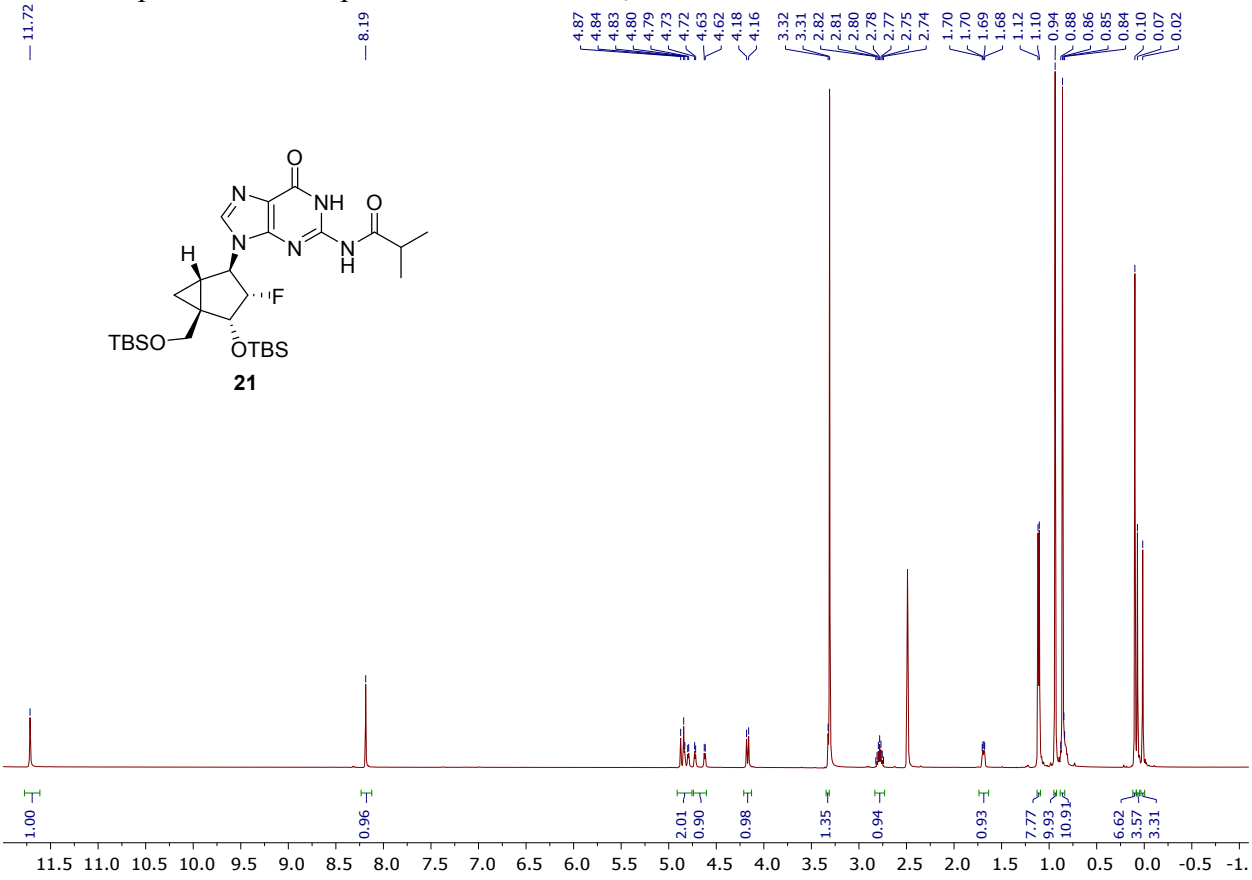


^{19}F NMR spectrum of compound **20** in DMSO-d_6

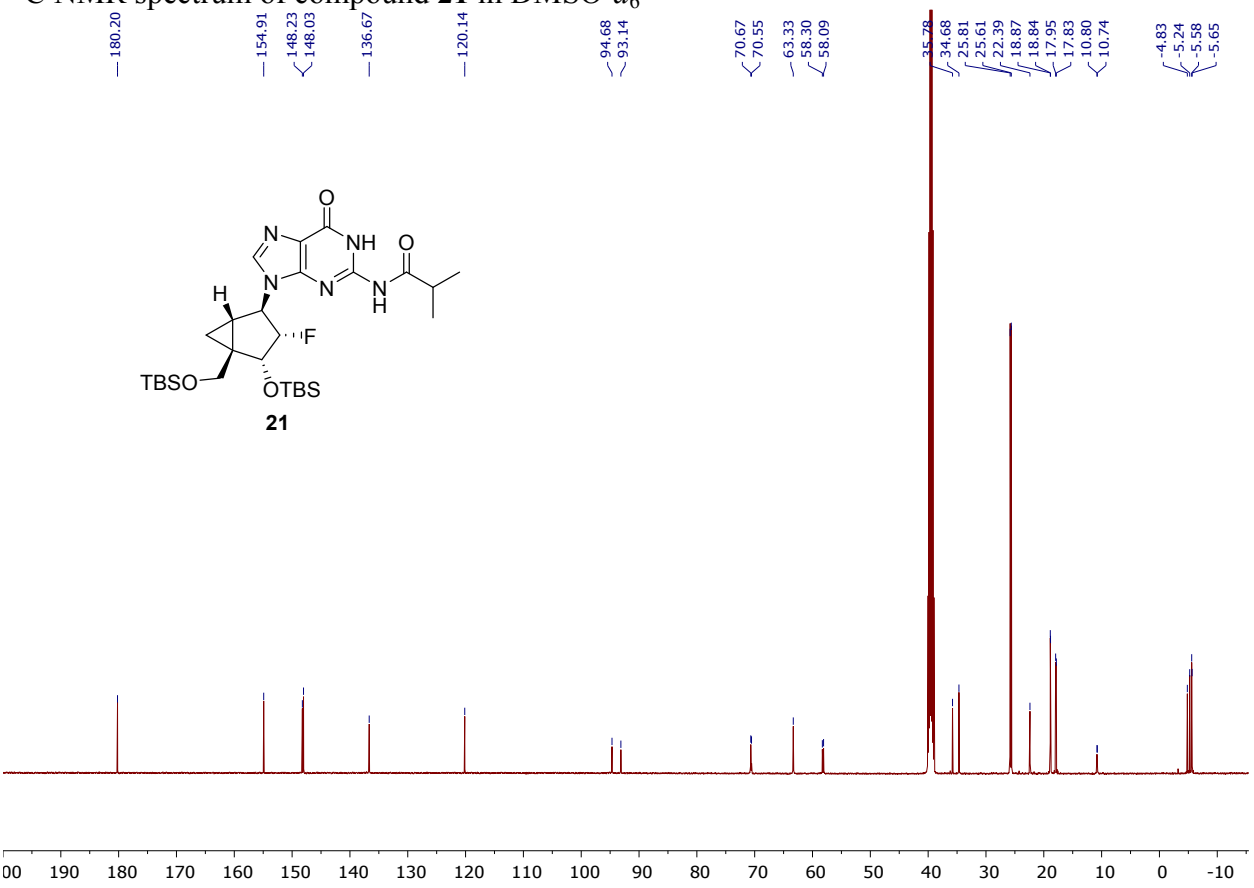


20

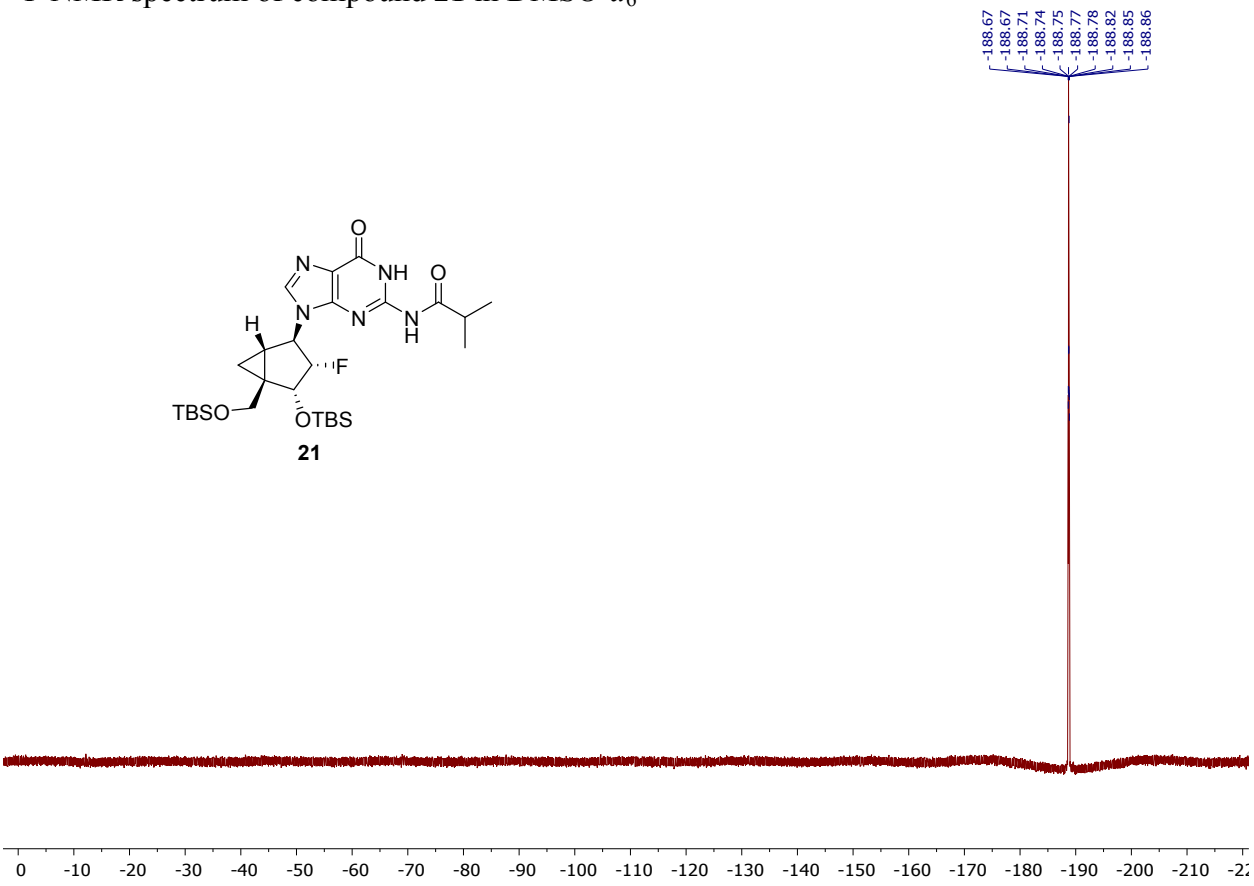
^{1}H NMR spectrum of compound **21** in DMSO-d_6



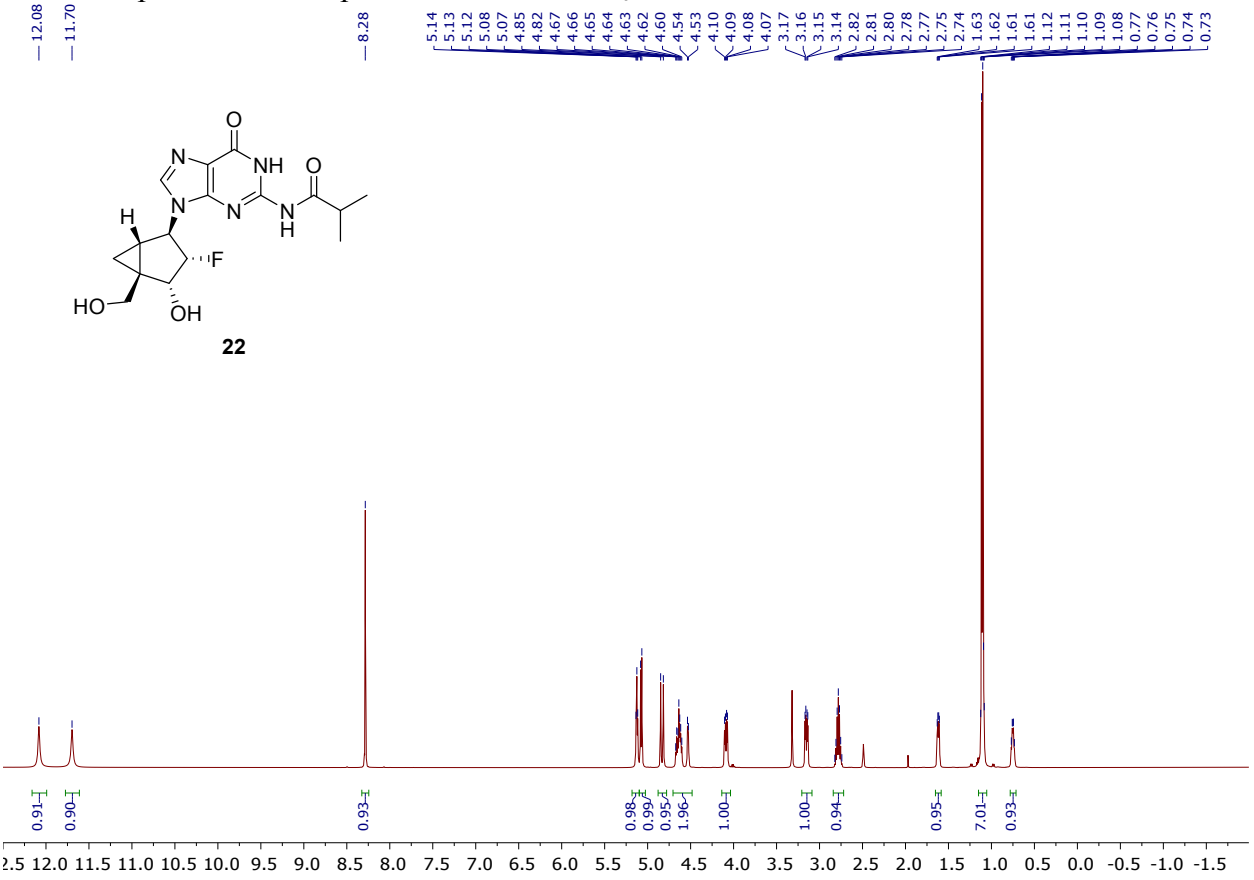
^{13}C NMR spectrum of compound **21** in DMSO-d_6



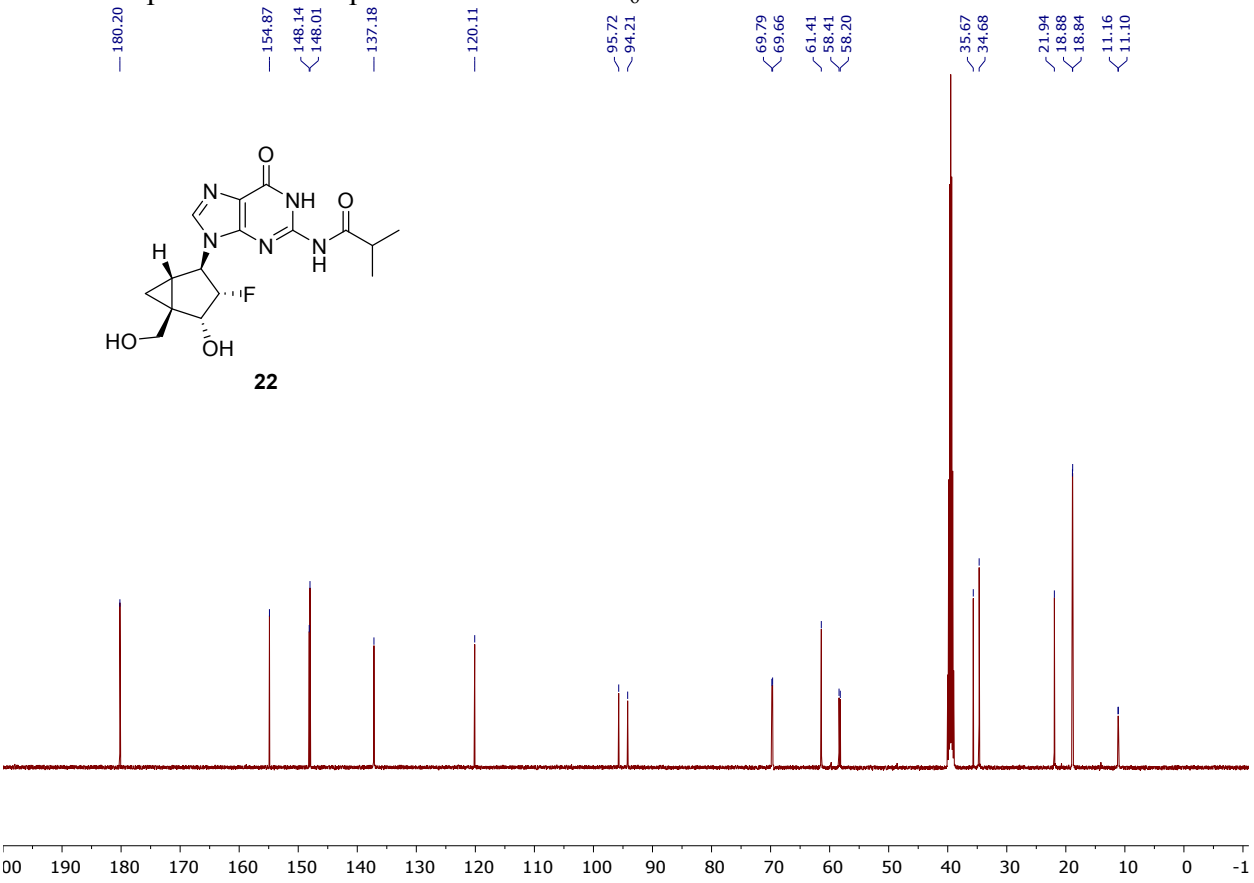
^{19}F NMR spectrum of compound **21** in DMSO-d_6



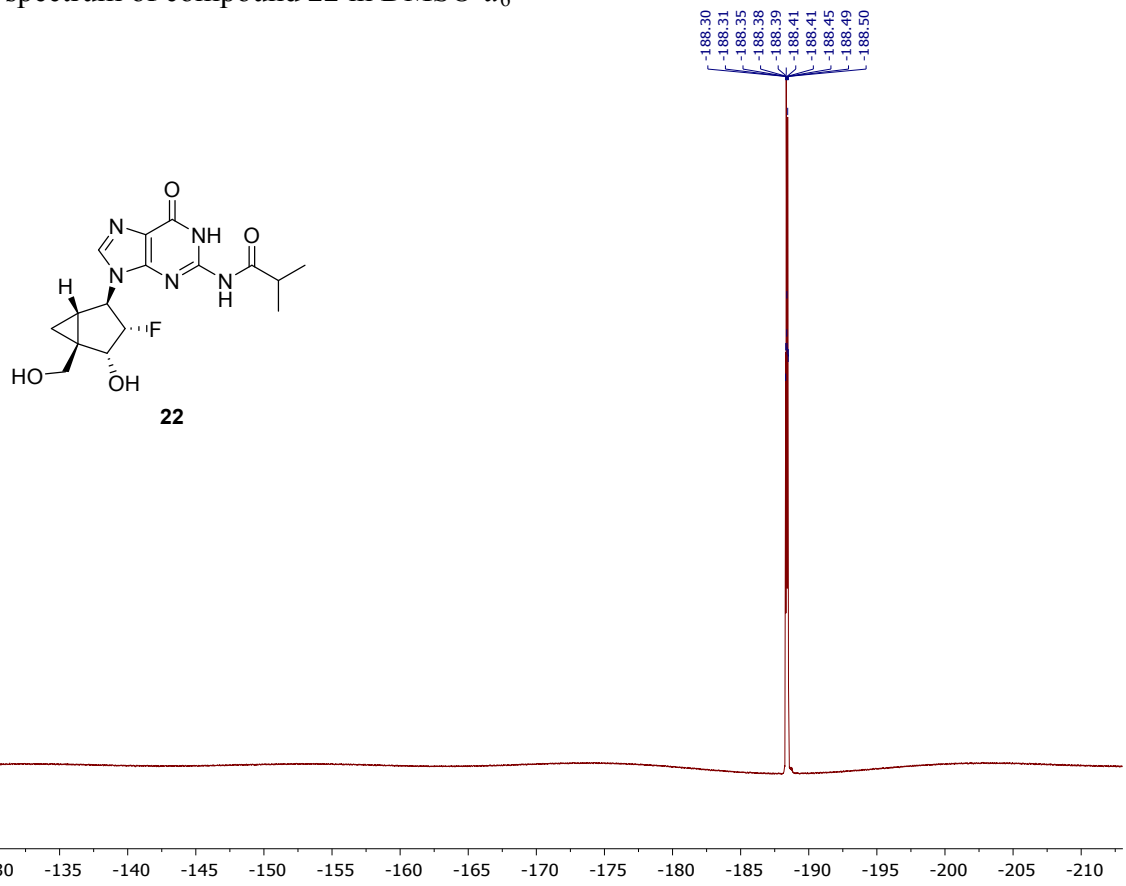
^{1}H NMR spectrum of compound **22** in DMSO-d_6



^{13}C NMR spectrum of compound **22** in DMSO-d_6

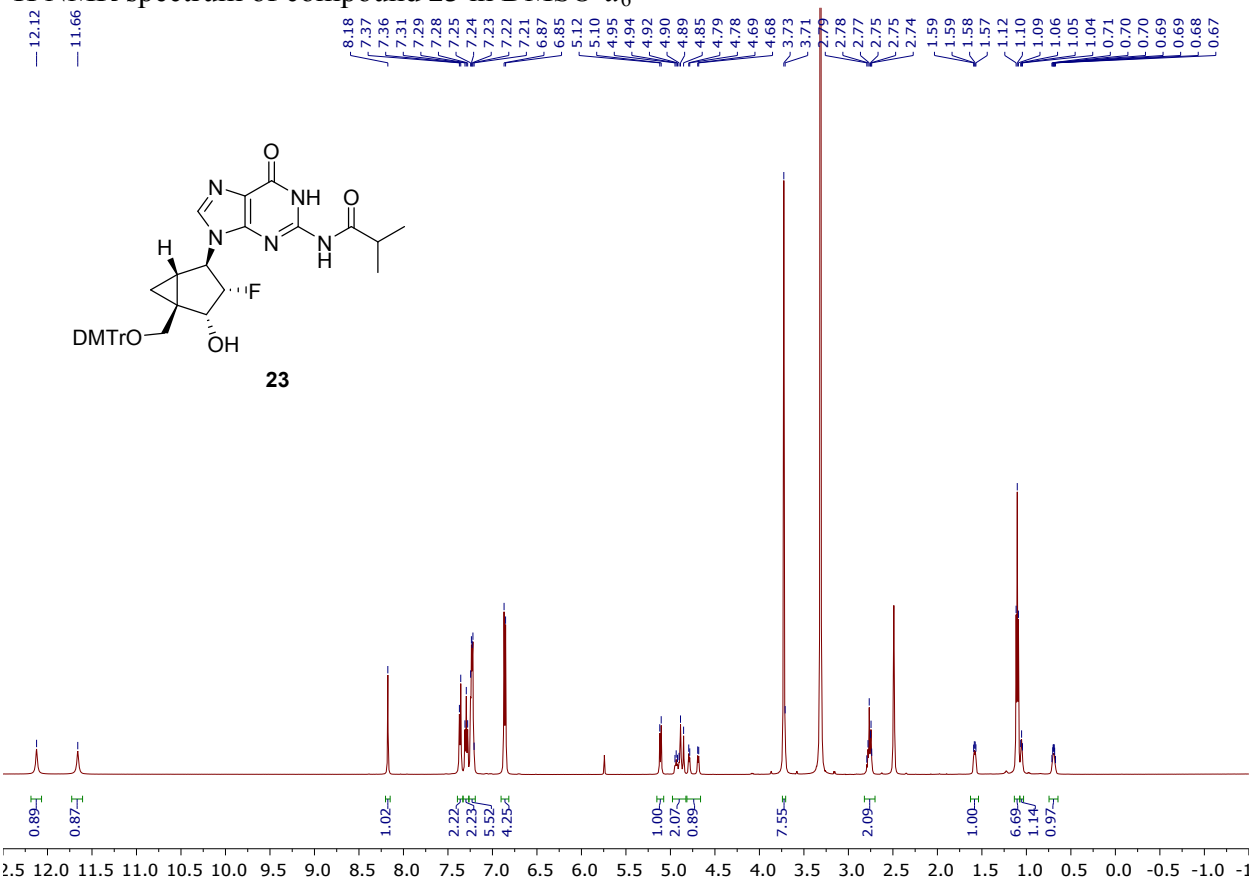


^{19}F NMR spectrum of compound **22** in DMSO-d_6

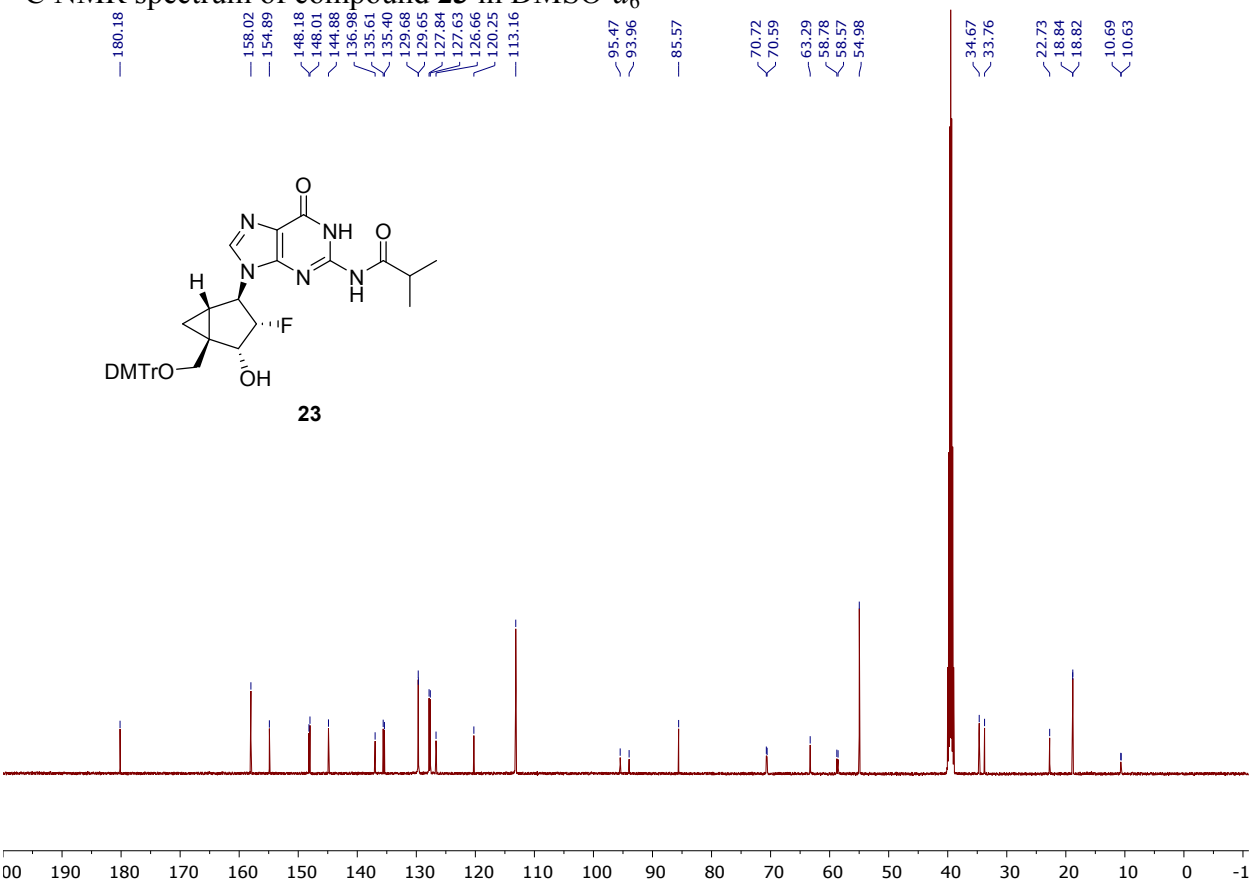


22

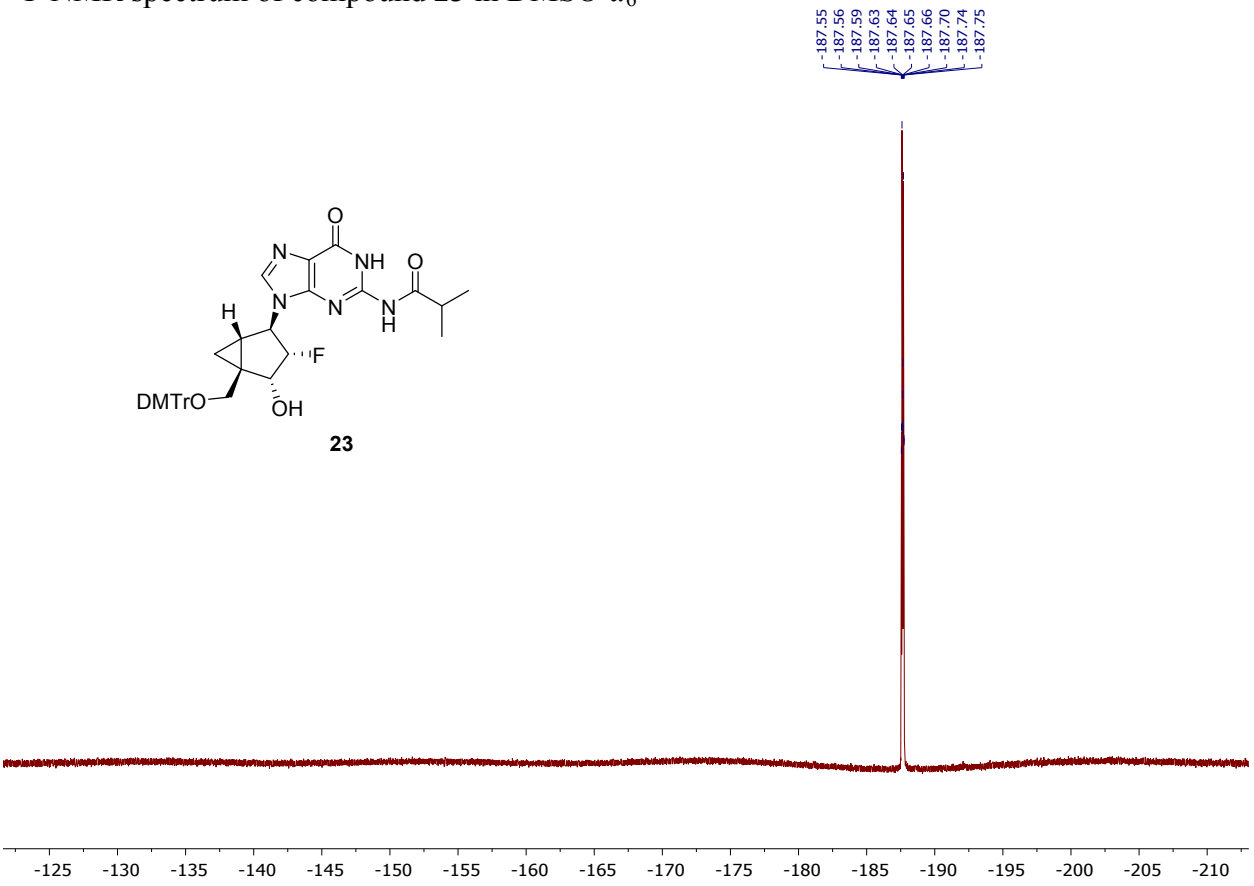
^{1}H NMR spectrum of compound **23** in DMSO-d_6



^{13}C NMR spectrum of compound **23** in DMSO-d_6

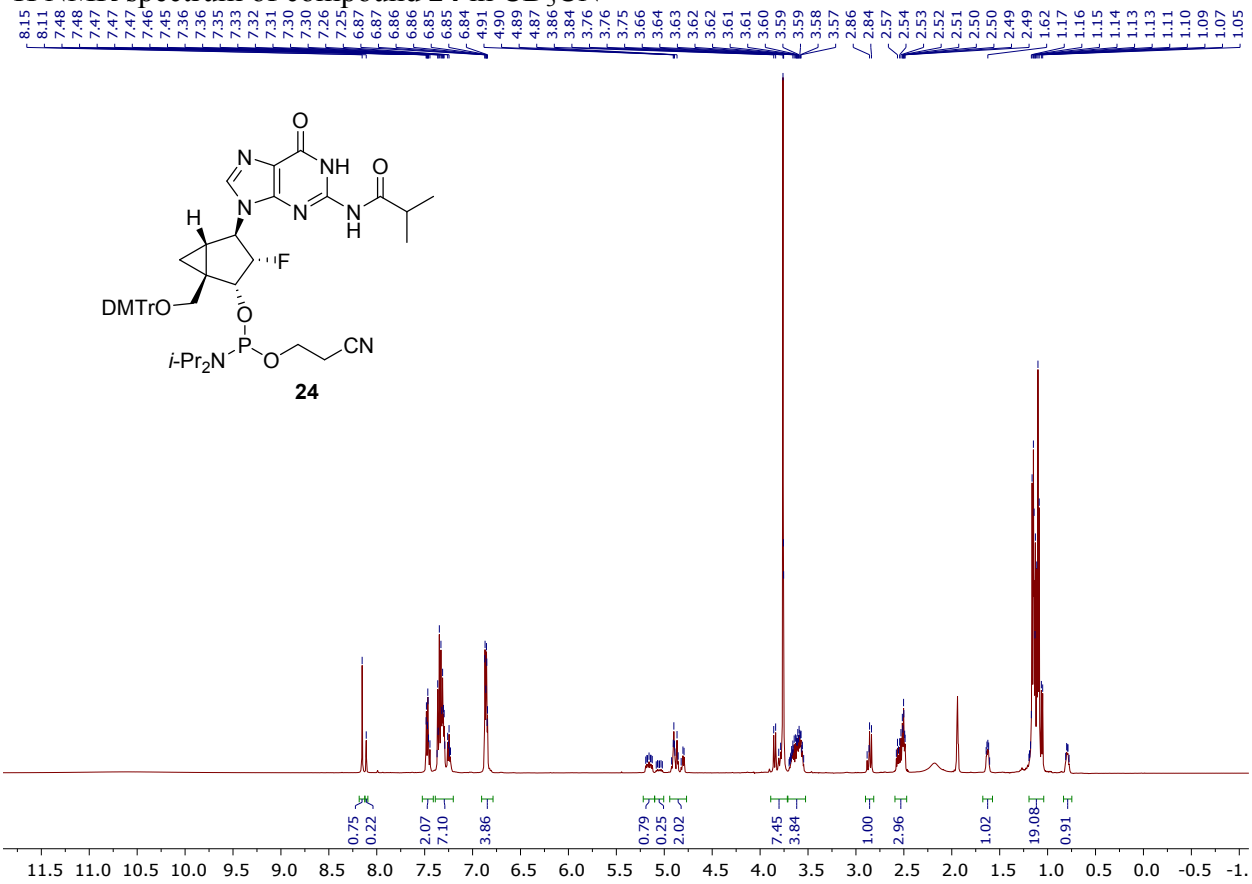


^{19}F NMR spectrum of compound **23** in DMSO-d_6

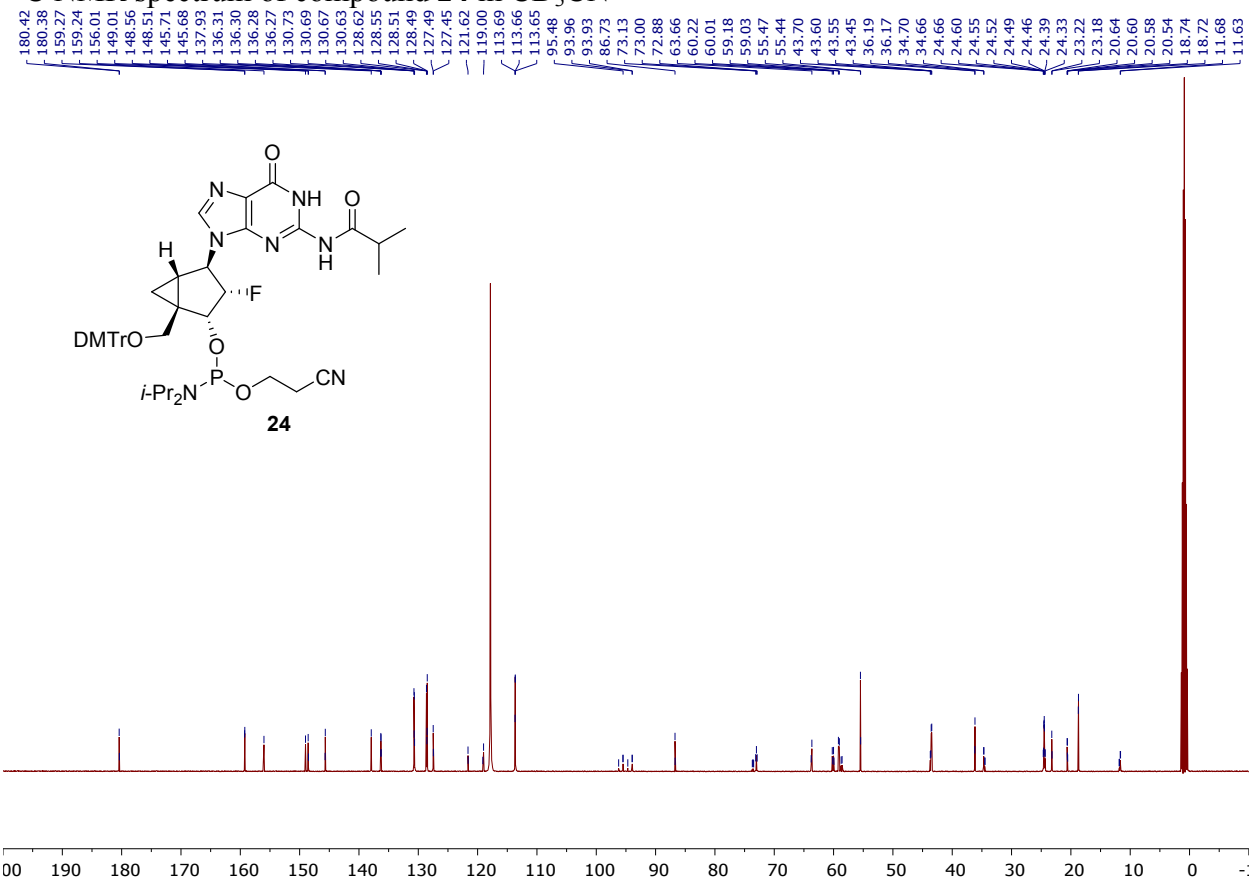


23

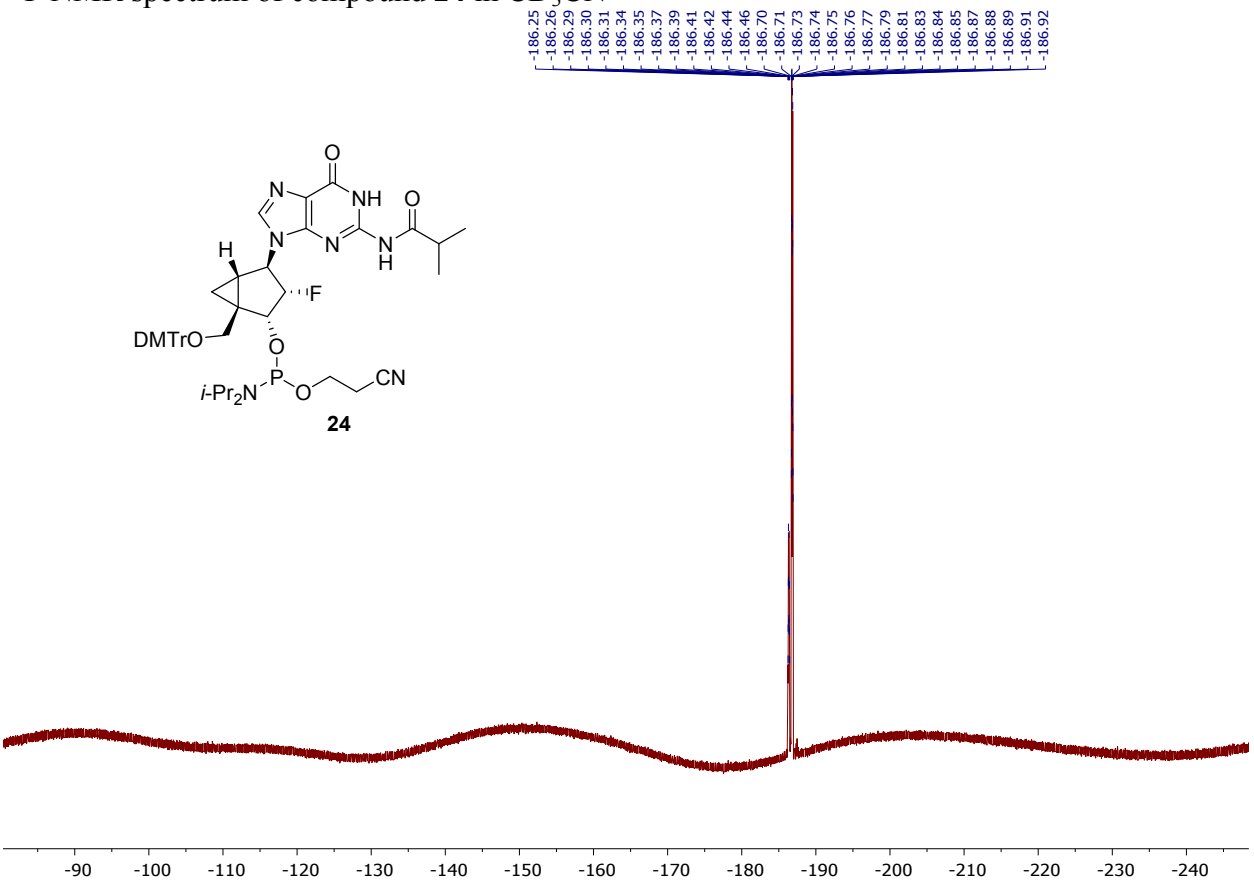
^{1}H NMR spectrum of compound **24** in CD_3CN



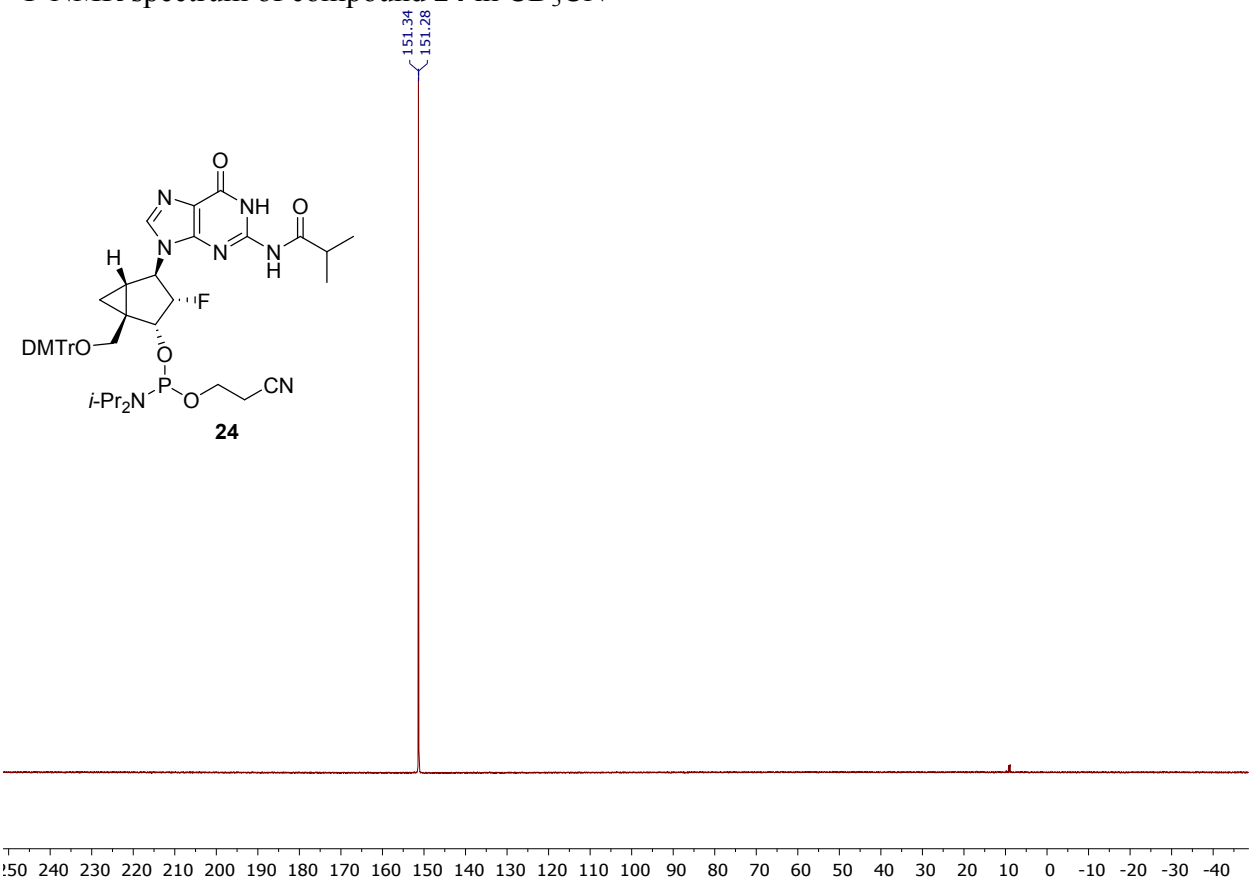
^{13}C NMR spectrum of compound **24** in CD_3CN



^{19}F NMR spectrum of compound **24** in CD_3CN



^{31}P NMR spectrum of compound **24** in CD_3CN



24

Oligonucleotide synthesis and characterization

Oligonucleotides were synthesized on an ABI-394 DNA/RNA synthesizer using standard solid-phase synthesis and deprotection protocols. A solution of 0.25 M 5-(*S*-ethylthio)-1*H*-tetrazole in acetonitrile (CH_3CN) was used as the activator. The phosphoramidite solutions (commercially available 2′-F-RNA amidites and standard RNA and DNA amidites and synthesized 2′-F-NMC amidities) were prepared at 0.15 M in anhydrous CH_3CN. The oxidizing reagent was 0.02 M I_2 in THF/pyridine/H_2O. The detritylation reagent was 3% dichloroacetic acid (DCA) in CH_2Cl_2. After completion of the automated synthesis, the oligonucleotides were manually released from support and deprotected using 30% NH_4OH and 5% diethylamine for 6 h at 55 °C. After filtration through a nylon syringe filter (0.45 μm), oligonucleotides were either stored until purification or, in the case of RNA, the 2′-hydroxyl groups were deprotected by treating with $Et_3N{\cdot}3HF$ at 60 °C for 10 min. Oligonucleotides were purified using anion-exchange high-performance liquid chromatography (IEX-HPLC) using an appropriate gradient of mobile phase (0.15 M NaCl, 10% CH_3CN and 1.0 M NaBr, 10% CH_3CN) and desalted using size-exclusion chromatography with water as an eluent. Oligonucleotides were then quantified by measuring the absorbance at 260 nm using the following extinction coefficients: (A, 13.86 L/mol/cm; T/U, 7.92 L/mol/cm; C, 6.57 L/mol/cm; and G, 10.53 L/mol/cm). The purities and identities of modified oligonucleotides were verified by analytical anion exchange chromatography and mass spectrometry, respectively (Table S1).

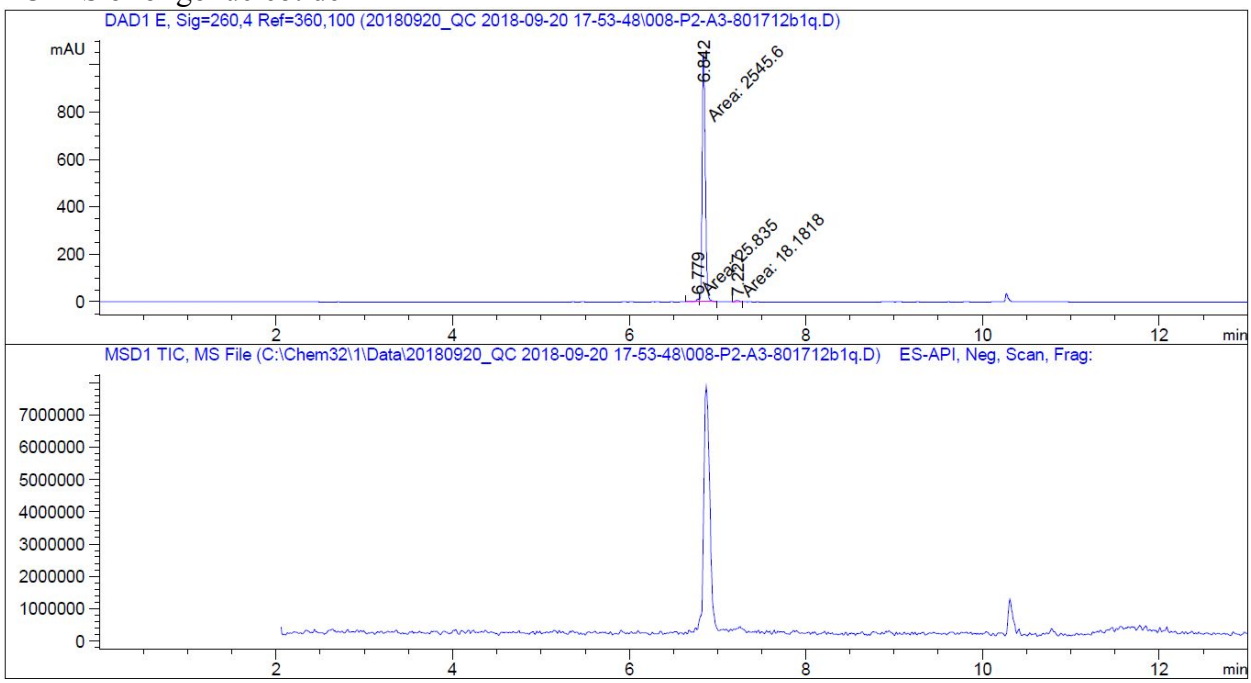
Table S1. MS (*m/z*) analysis of modified oligonucleotides [a]

oligonucleotide no.	sequence (5′-3′)	mass (m/z) calc.	mass (m/z) obs.
1	rUACAGU$^{F\text{-}NMC}$CUAUGU	3769.3	3768.1
2	rUACAGUC$^{F\text{-}NMC}$UAUGU	3769.3	3768.3
3	rACAUAG$^{F\text{-}NMC}$ACUGUA	3815.4	3814.3
4	rACAUAGA$^{F\text{-}NMC}$CUGUA	3815.4	3814.6
5	rGCG$^{F\text{-}NMC}$A$^{F\text{-}NMC}$U$^{F\text{-}NMC}$C$^{F\text{-}NMC}$U$^{F\text{-}NMC}$C$^{F\text{-}NMC}$AC	3192.1	3191.2
6	rGCG^{F}A^{F}U^{F}C^{F}U^{F}C^{F}AC	3131.9	3130.9
7	dTTTTTTTTTTTTTTTTTTC$^{F\text{-}NMC}$	6034.9	6033.6
8	dTTTTTTTTTTTTTTTTTTC$^{F\text{-}NMC}$T	6034.9	6033.6
9	dC$^{F\text{-}NMC}$TTTTTTTTTTTTTTTTTT	6034.9	6033.5
10	dTC$^{F\text{-}NMC}$TTTTTTTTTTTTTTTTT	6034.9	6033.6
11	dTTTTTTTTTTTTTTTTTTC^{F}	6024.9	6023.6
12	dTTTTTTTTTTTTTTTTTTC^{F}T	6024.9	6023.6
13	dC^{F}TTTTTTTTTTTTTTTTTT	6024.9	6023.6
14	dTC^{F}TTTTTTTTTTTTTTTTT	6024.9	6023.5

[a] Prefix r indicates ribonucleotide, prefix d indicates deoxyribonucleotide, superscript F indicates 2′-F-RNA, superscript F-NMC indicates 2′-F-NMC.

LC-MS profiles of modified oligonucleotides

LC-MS of oligonucleotide **1**

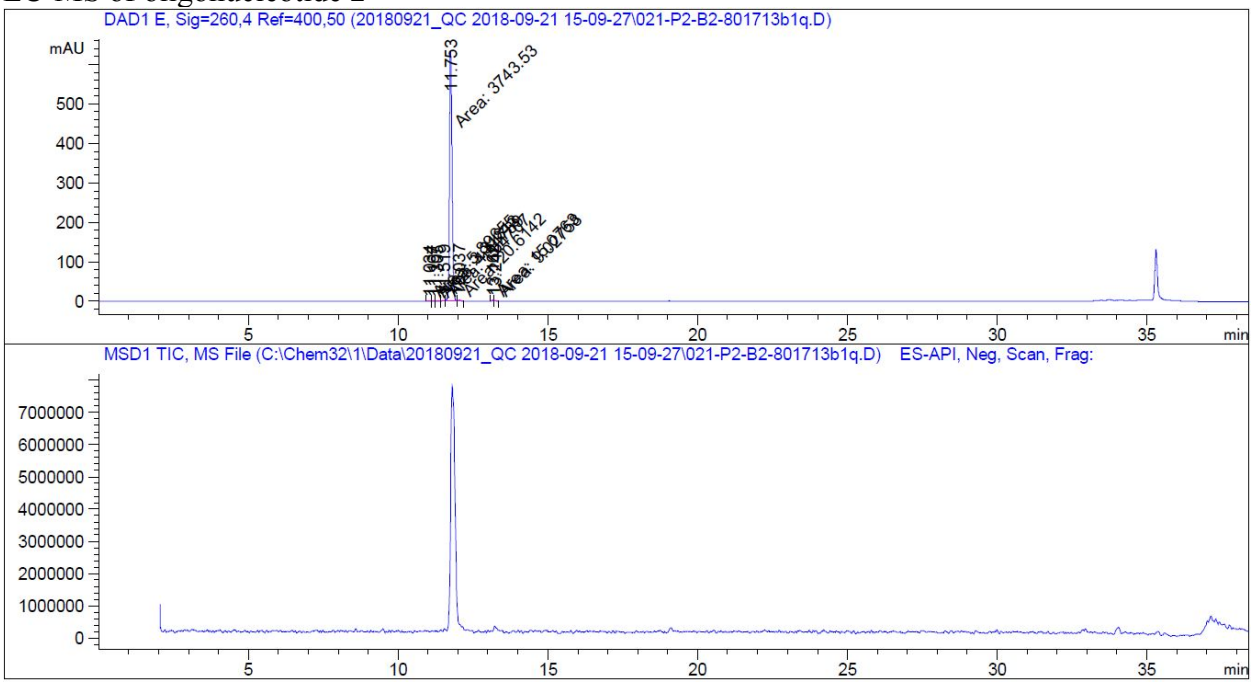


Signal 1: DAD1 E, Sig=260,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.779	MF	0.0372	25.83503	11.56033	0.9976
2	6.842	FM	0.0403	2545.59912	1052.68103	98.3003
3	7.227	MM	0.0570	18.18183	5.31740	0.7021
Totals :				2589.61598	1069.55877	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	3768.07	193519	100.00
B	2512.05	99297	51.31
C	3790.73	24010	12.41
D	4521.15	11440	5.91
E	3806.24	11093	5.73
F	1886.66	2866	1.48

LC-MS of oligonucleotide **2**

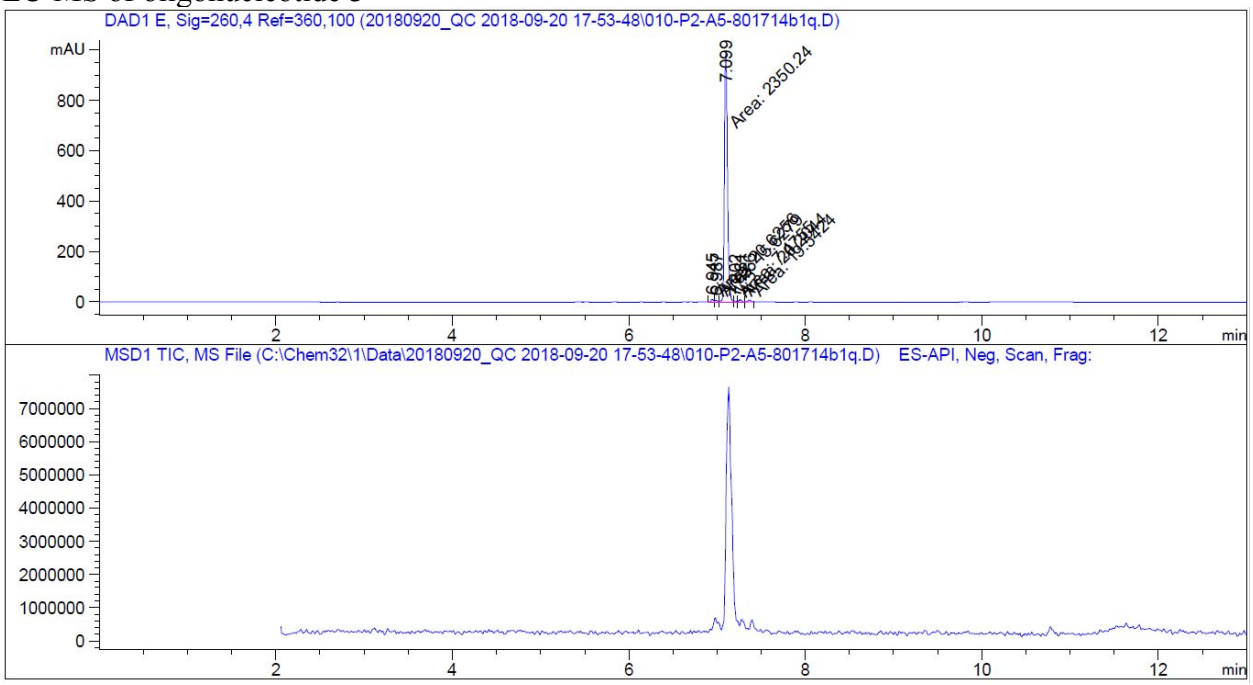


Signal 1: DAD1 E, Sig=260,4 Ref=400,50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.034	MF	0.1273	5.89655	7.72295e-1	0.1545
2	11.167	FM	0.0995	3.31813	5.55868e-1	0.0869
3	11.305	MF	0.1199	4.14789	5.76448e-1	0.1087
4	11.519	MF	0.1168	15.47070	2.20845	0.4053
5	11.753	MF	0.0986	3743.53467	632.88232	98.0731
6	12.037	FM	0.1223	20.61416	2.81020	0.5400
7	13.162	MF	0.0776	15.07630	3.23634	0.3950
8	13.248	FM	0.0823	9.02758	1.82825	0.2365
Totals :				3817.08598	644.87017	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	3768.33	180907	100.00
B	3789.17	21956	12.14
C	3806.07	8885	4.91
D	4522.13	8146	4.50
E	7559.83	6072	3.36
F	1913.41	3013	1.67

LC-MS of oligonucleotide **3**

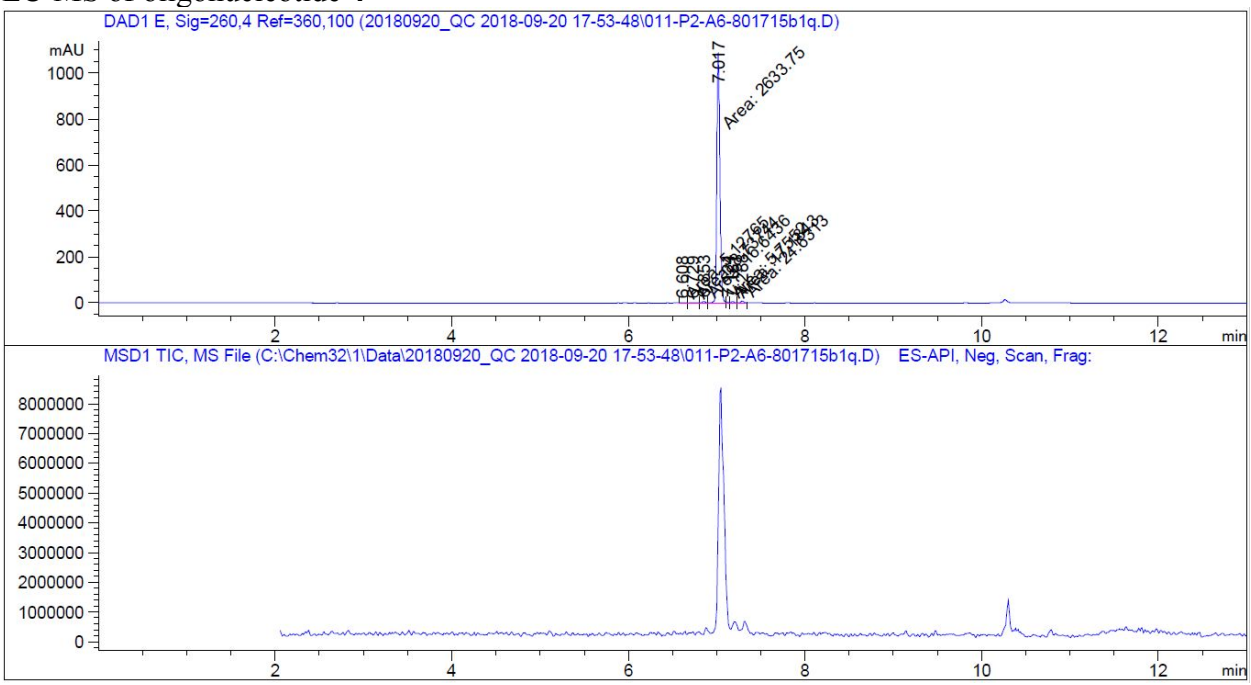


Signal 1: DAD1 E, Sig=260,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.945	MF	0.0349	20.62556	9.84293	0.8475
2	6.987	FM	0.0387	15.62788	6.72436	0.6421
3	7.099	FM	0.0395	2350.24292	991.69318	96.5700
4	7.202	FM	0.0328	7.47550	3.79936	0.3072
5	7.261	FM	0.0395	20.20444	8.52857	0.8302
6	7.366	FM	0.0421	19.54237	7.73297	0.8030
Totals :				2433.71867	1028.32136	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	3814.28	240861	100.00
B	3836.38	28399	11.79
C	3852.29	10562	4.39
D	1911.86	8272	3.43
E	7648.09	4892	2.03
F	1915.94	2916	1.21

LC-MS of oligonucleotide **4**

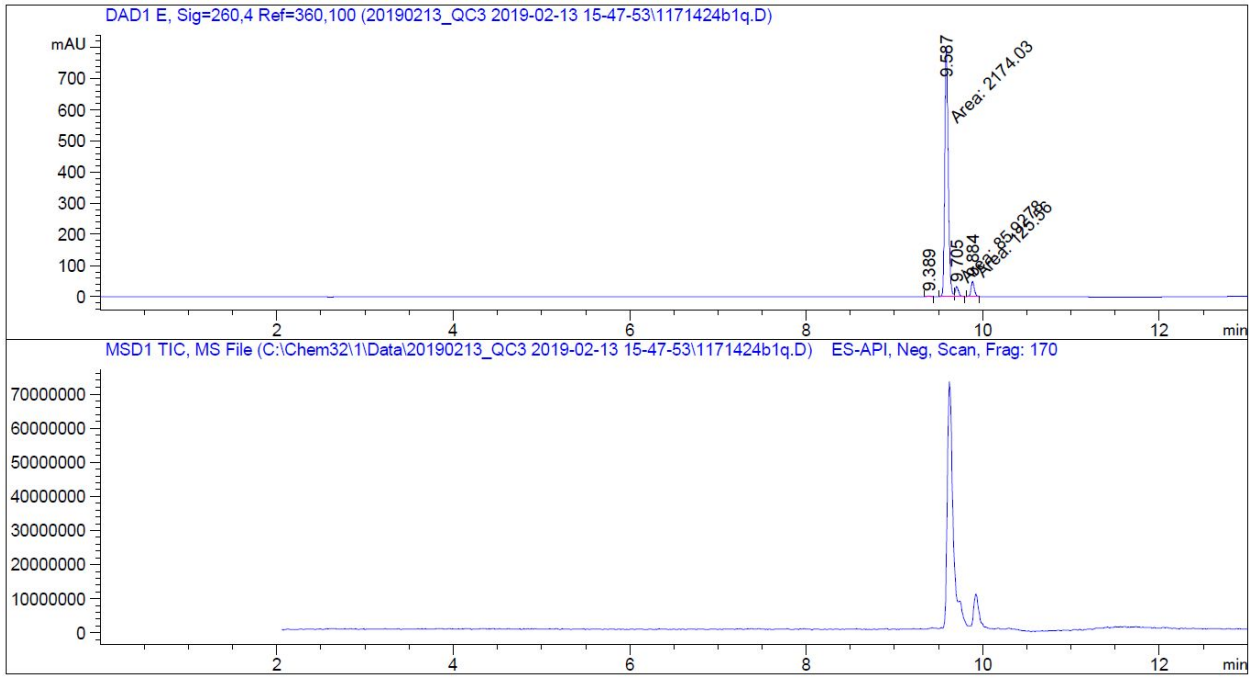


Signal 1: DAD1 E, Sig=260,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.608	MF	0.0406	5.12765	6.76424	0.1891
2	6.729	FM	0.0658	8.73744	1087.47449	0.3222
3	6.853	FM	0.0410	16.64358	6.76424	0.6137
4	7.017	FM	0.0404	2633.75317	1087.47449	97.1208
5	7.124	FM	0.0317	5.75520	3.02896	0.2122
6	7.181	FM	0.0440	17.18426	6.50296	0.6337
7	7.288	FM	0.0460	24.63130	8.92478	0.9083
Totals :				2711.83259	1117.01522	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	3814.58	261723	100.00
B	3836.27	28580	10.92
C	4577.05	16613	6.35
D	3851.86	12774	4.88
E	7650.64	9363	3.58
F	2179.16	4006	1.53

LC-MS of oligonucleotide **5**

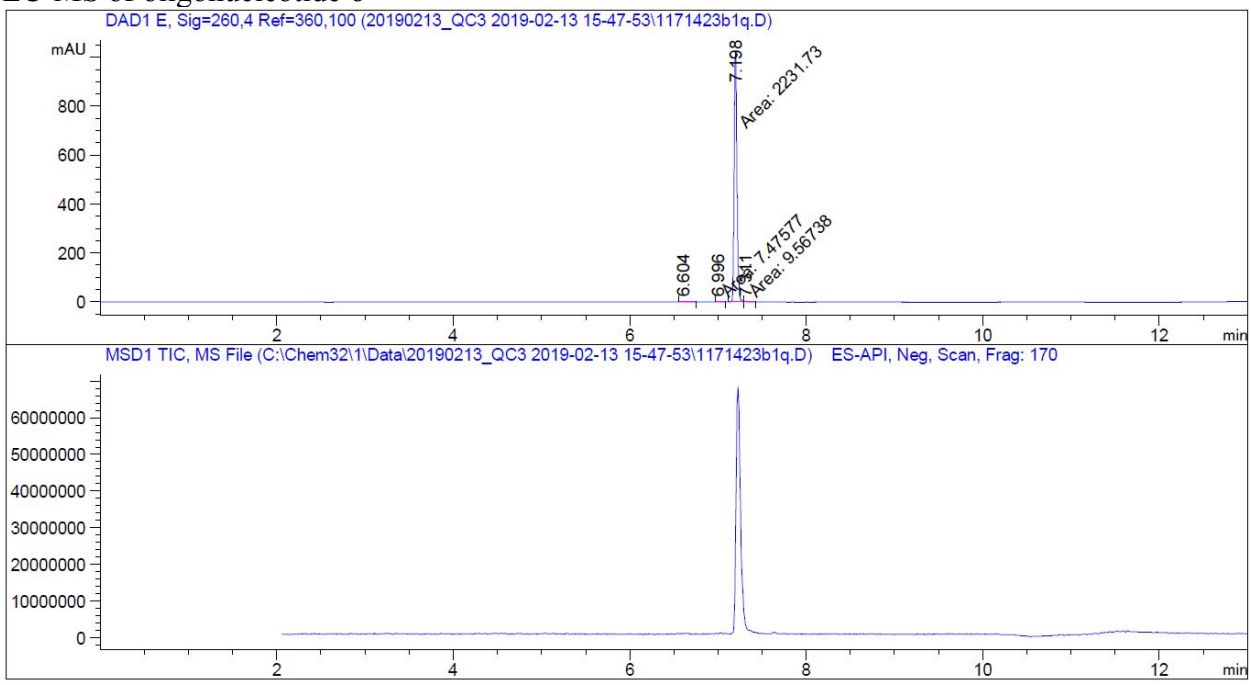


Signal 1: DAD1 E, Sig=260,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.389	BB	0.0405	5.62434	2.18961	0.2352
2	9.587	MF	0.0452	2174.02734	801.21161	90.9201
3	9.705	FM	0.0456	85.92779	31.39135	3.5936
4	9.884	MM	0.0435	125.56005	48.09347	5.2511
Totals :				2391.13953	882.88603	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	3191.15	3290006	100.00
B	3213.04	261456	7.95
C	1506.31	116857	3.55
D	870.98	101509	3.09
E	2165.62	86278	2.62
F	9121.79	69561	2.11
G	3228.90	57053	1.73
H	6404.45	48157	1.46
I	1189.01	35256	1.07

LC-MS of oligonucleotide **6**

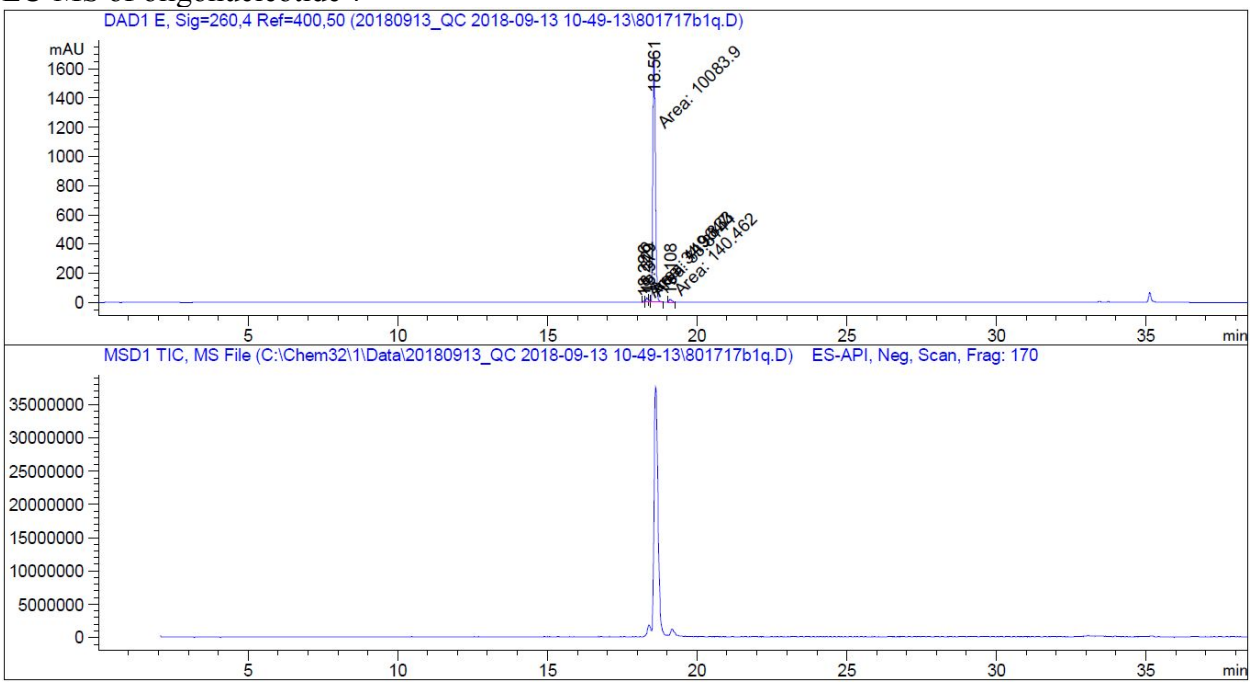


Signal 1: DAD1 E, Sig=260,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.604	BB	0.0348	5.30078	2.35100	0.2352
2	6.996	MM	0.0519	7.47577	2.40067	0.3317
3	7.198	MF	0.0364	2231.72876	1021.93787	99.0087
4	7.311	FM	0.0698	9.56738	2.28580	0.4244
Totals :				2254.07269	1028.97534	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	3130.89	2925074	100.00
B	3152.93	220007	7.52
C	3168.87	45694	1.56
D	6283.79	42364	1.45

LC-MS of oligonucleotide **7**

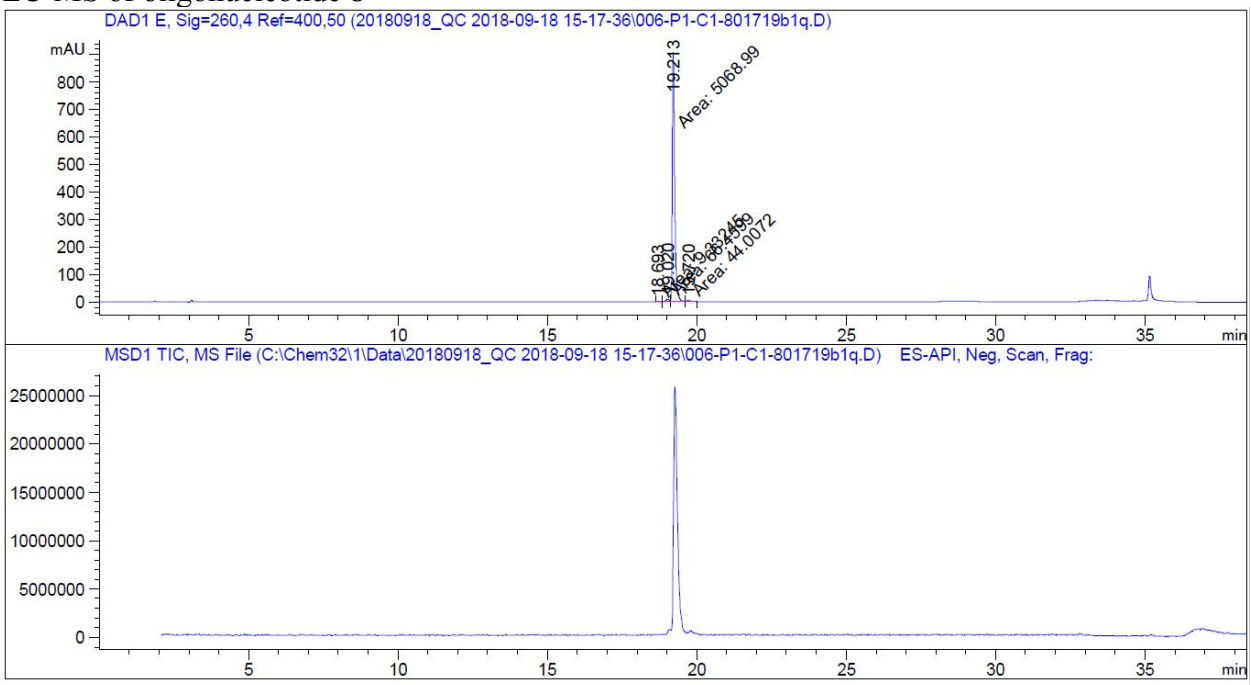


Signal 1: DAD1 E, Sig=260,4 Ref=400,50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.226	MF	0.0648	34.96468	8.99796	0.3342
2	18.322	MF	0.0780	149.83298	32.03236	1.4320
3	18.379	FM	0.0536	53.81436	16.73637	0.5143
4	18.561	FM	0.0982	1.00839e4	1711.43762	96.3770
5	19.108	MM	0.1116	140.46207	20.97394	1.3425
Totals :				1.04630e4	1790.17825	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	6033.57	1068765	100.00
B	6055.79	48914	4.58
C	6070.96	21501	2.01

LC-MS of oligonucleotide **8**

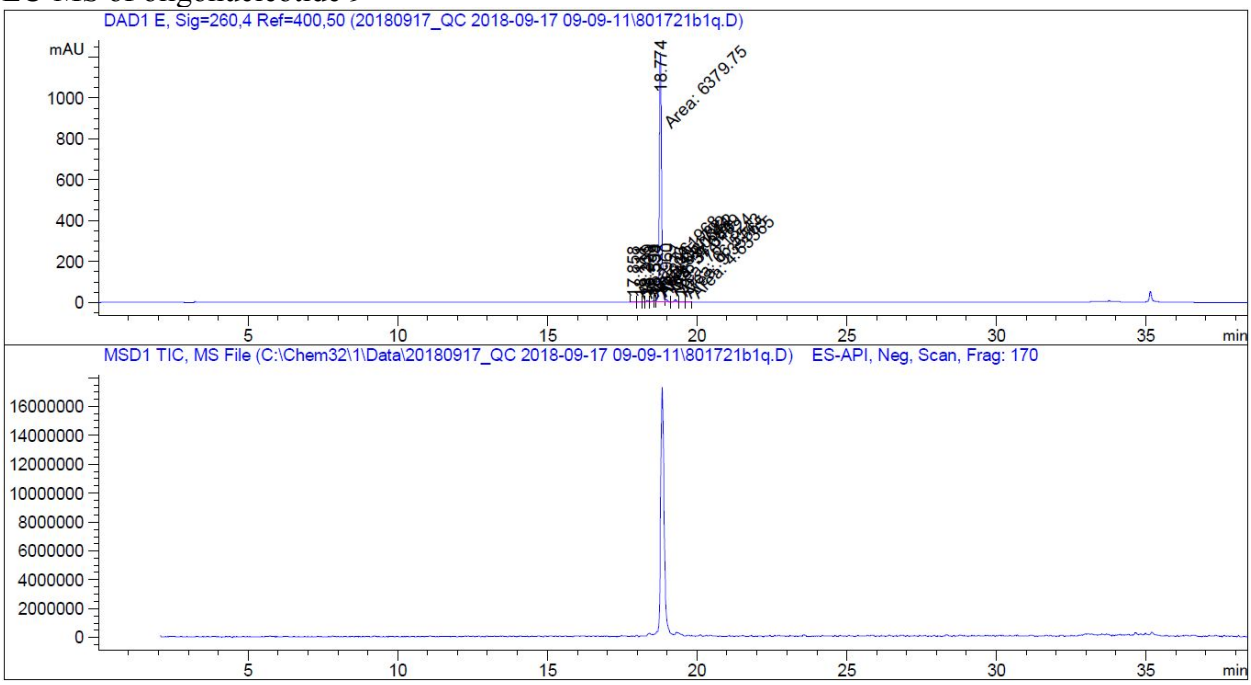


Signal 1: DAD1 E, Sig=260,4 Ref=400,50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.693	MF	0.1279	9.33245	1.21628	0.1799
2	19.020	FM	0.1154	66.45992	9.59986	1.2808
3	19.213	FM	0.0932	5068.98975	906.52277	97.6912
4	19.720	FM	0.1172	44.00722	6.25764	0.8481
Totals :				5188.78933	923.59654	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	6033.59	572332	100.00
B	6055.43	75402	13.17
C	6076.11	7580	1.32

LC-MS of oligonucleotide **9**

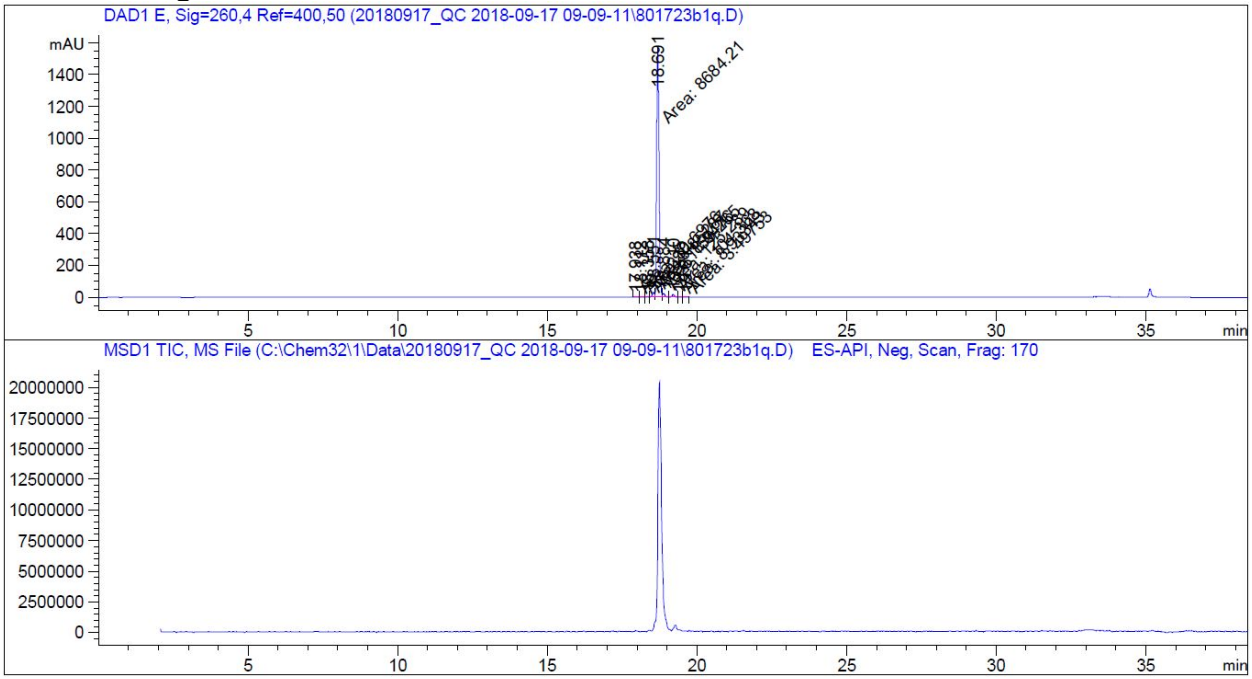


Signal 1: DAD1 E, Sig=260,4 Ref=400,50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.858	MF	0.0841	7.61968	1.51046	0.1142
2	18.111	MF	0.1028	4.51722	7.32179e-1	0.0677
3	18.266	MF	0.0907	6.80644	1.25035	0.1021
4	18.339	MF	0.0844	45.13523	8.90776	0.6767
5	18.559	MF	0.0773	31.68493	6.83555	0.4751
6	18.594	FM	0.0684	37.34602	9.09903	0.5599
7	18.774	MF	0.0869	6379.74902	1222.99255	95.6544
8	18.960	FM	0.0894	76.18241	14.19998	1.1422
9	19.277	MF	0.1035	66.55429	10.71249	0.9979
10	19.436	MF	0.1190	9.35565	1.31053	0.1403
11	19.677	FM	0.1457	4.63365	5.29995e-1	0.0695
Totals :				6669.58454	1278.08089	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	6033.54	395110	100.00
B	6055.67	24989	6.32

LC-MS of oligonucleotide **10**

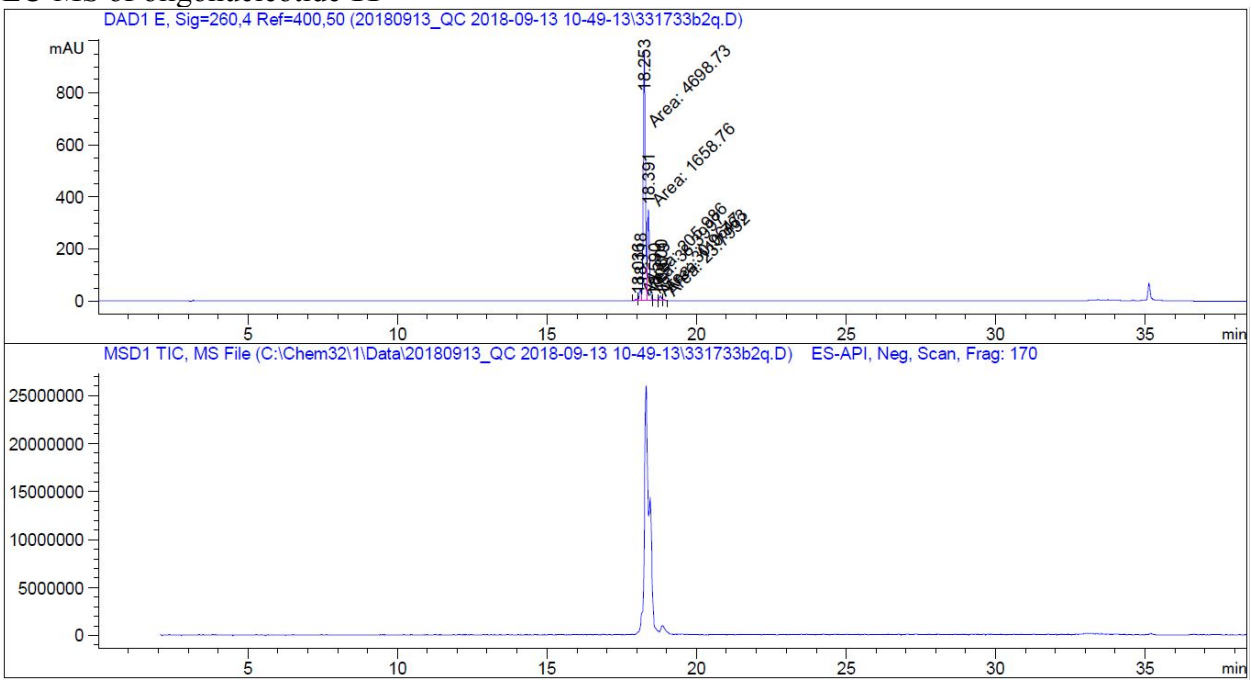


Signal 1: DAD1 E, Sig=260,4 Ref=400,50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.938	MF	0.0753	10.69759	2.36644	0.1173
2	18.112	FM	0.1420	3.85287	4.52311e-1	0.0423
3	18.358	FM	0.0946	18.94757	3.33801	0.2078
4	18.551	MF	0.0870	159.26486	30.52726	1.7465
5	18.691	FM	0.0922	8684.20703	1570.49268	95.2314
6	18.884	FM	0.0865	125.28930	24.12666	1.3739
7	19.210	FM	0.0998	104.36846	17.43545	1.1445
8	19.385	FM	0.0817	8.93343	1.82279	0.0980
9	19.612	FM	0.1197	3.49753	4.86936e-1	0.0384
Totals :				9119.05863	1651.04852	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	6033.62	503790	100.00
B	6057.73	20133	4.00

LC-MS of oligonucleotide **11**

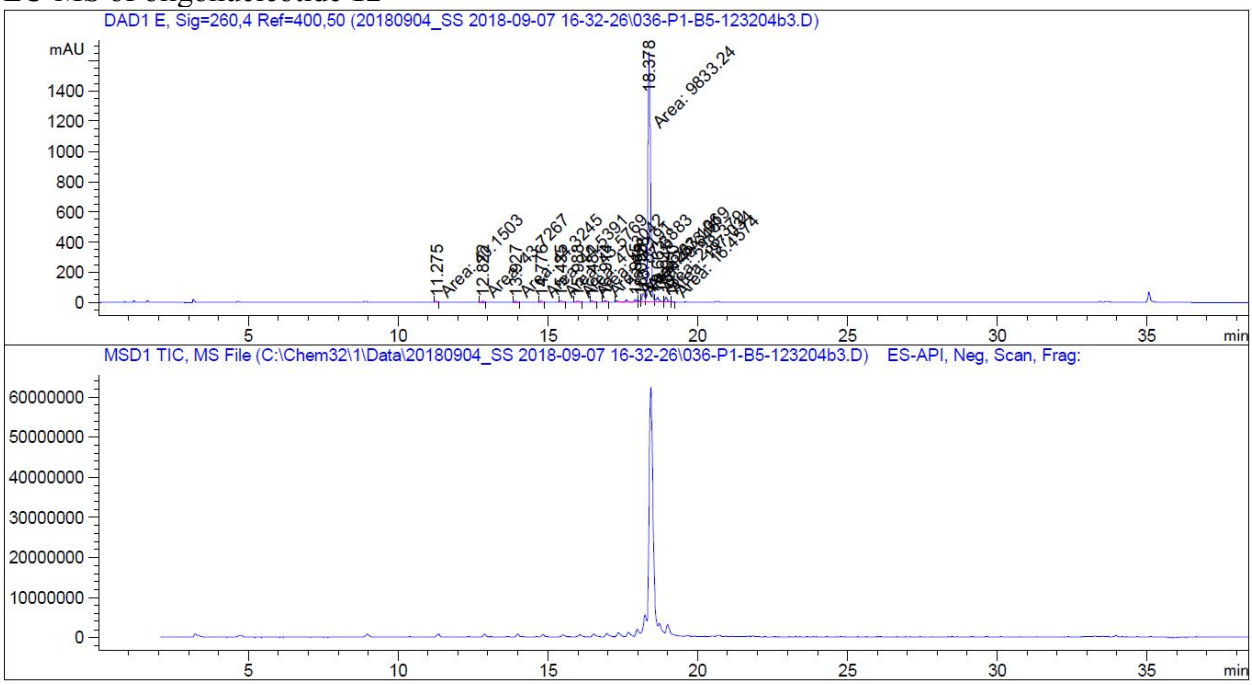


Signal 1: DAD1 E, Sig=260,4 Ref=400,50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.036	MF	0.0917	38.39970	6.97909	0.5674
2	18.118	FM	0.0756	205.98582	45.39040	3.0438
3	18.253	MF	0.0817	4698.72803	958.45923	69.4319
4	18.391	FM	0.0789	1658.76025	350.57336	24.5111
5	18.590	MF	0.0991	30.96471	5.20635	0.4576
6	18.800	MF	0.0962	110.76262	19.19070	1.6367
7	18.879	FM	0.0730	23.79322	5.43442	0.3516
Totals :				6767.39435	1391.23356	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	6023.56	680634	100.00
B	6047.94	43498	6.39
C	6061.89	17038	2.50

LC-MS of oligonucleotide **12**

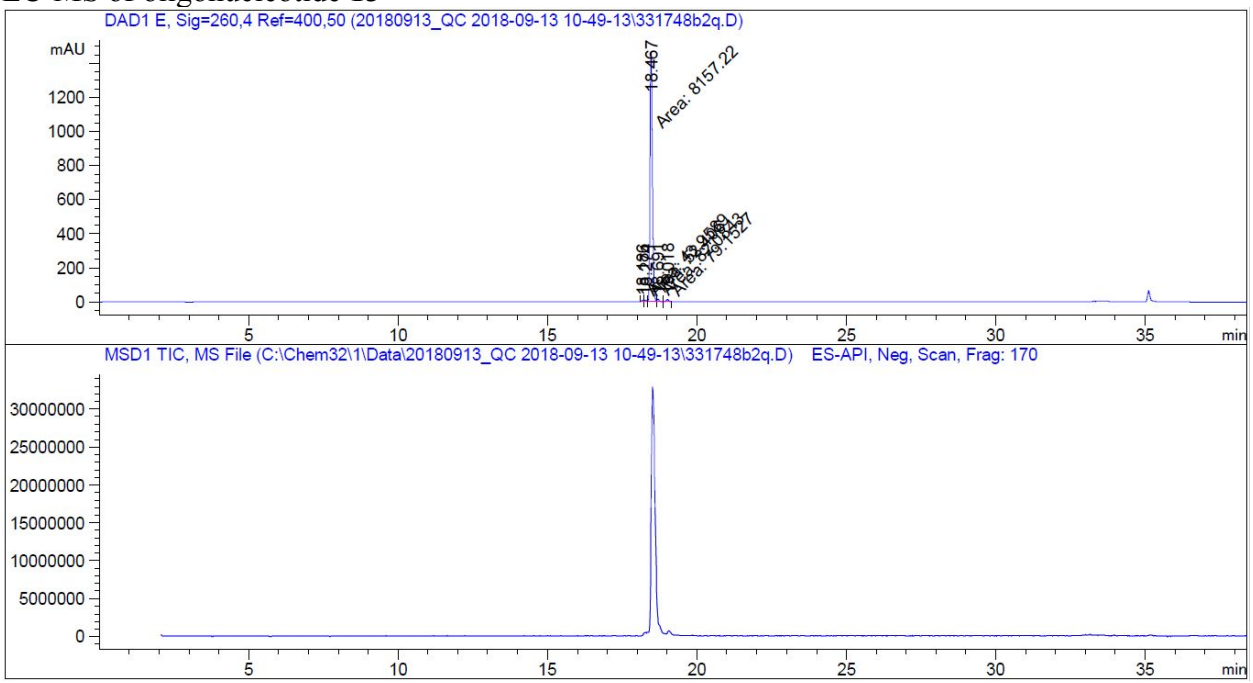


Signal 1: DAD1 E, Sig=260,4 Ref=400,50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.275	MM	0.0909	40.15033	7.36323	0.3542
2	12.822	MM	0.0892	43.72666	8.17193	0.3857
3	13.927	MM	0.0858	44.32454	8.61037	0.3910
4	14.776	MM	0.0830	44.32454	8.94593	0.3929
5	15.435	MM	0.0789	43.57689	9.20867	0.3844
6	15.988	MM	0.0901	47.30423	8.75251	0.4173
7	16.482	MM	0.0857	44.19102	8.59180	0.3898
8	16.914	MM	0.0892	57.38834	10.72132	0.5062
9	17.915	MF	0.2234	263.10620	19.62890	2.3208
10	18.093	FM	0.0661	49.64504	12.51488	0.4379
11	18.178	FM	0.1075	373.91949	57.99459	3.2982
12	18.378	FM	0.0991	9833.24023	1654.43323	86.7360
13	18.663	FM	0.1265	238.37929	31.41087	2.1027
14	18.945	FM	0.1033	197.03368	31.80303	1.7380
15	19.160	FM	0.1217	16.45735	2.25354	0.1452
Totals :				1.13370e4	1880.40481	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	6023.58	1424615	100.00
B	6045.66	72458	5.09
C	6061.21	33996	2.39

LC-MS of oligonucleotide **13**

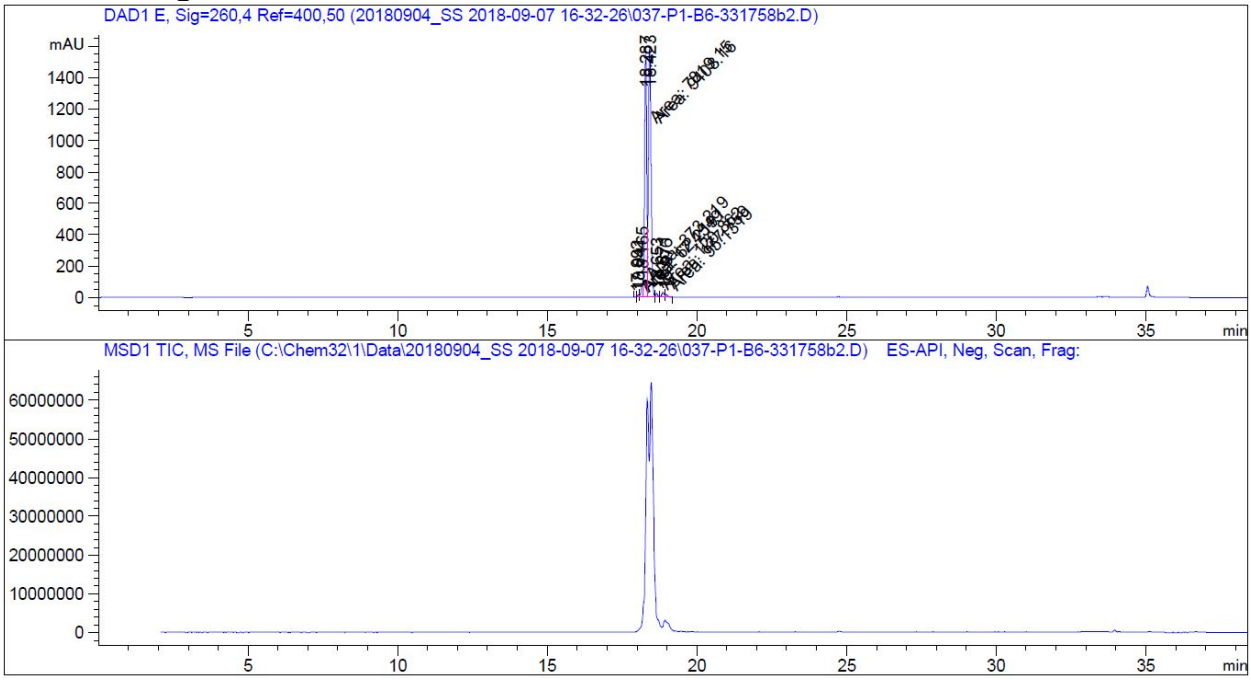


Signal 1: DAD1 E, Sig=260,4 Ref=400,50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.186	MF	0.0665	43.95890	11.02158	0.5224
2	18.274	FM	0.0884	52.40604	9.87683	0.6228
3	18.467	FM	0.0929	8157.22119	1462.82361	96.9387
4	18.691	FM	0.0845	82.08432	16.18163	0.9755
5	19.018	FM	0.1049	79.15268	12.57681	0.9406
Totals :				8414.82313	1512.48045	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	6023.57	846699	100.00
B	6045.22	37876	4.47
C	6062.31	17979	2.12
D	2019.25	11223	1.33

LC-MS of oligonucleotide **14**



Signal 1: DAD1 E, Sig=260,4 Ref=400,50

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.992	MF	0.0564	17.45490	5.16025	0.0966
2	18.041	MF	0.0753	62.21910	13.77304	0.3442
3	18.165	FM	0.0689	373.21851	90.24255	2.0650
4	18.287	MF	0.0841	7819.15283	1549.55908	43.2620
5	18.423	FM	0.0985	9408.15918	1592.34009	52.0537
6	18.653	MF	0.0874	120.86180	23.05918	0.6687
7	18.871	MF	0.0941	177.75880	31.49580	0.9835
8	18.976	FM	0.0939	95.13191	16.88994	0.5263
Totals :				1.80740e4	3322.51994	

Component	Molecular Weight	Absolute Abundance	Relative Abundance
A	6023.52	2267977	100.00
B	6044.57	89491	3.95
C	6061.12	55432	2.44
D	2418.85	15687	0.69

Thermal melting studies

UV melting curves were recorded using a Cary 4000 Scan UV-Visible Spectrophotometer. The concentration of oligonucleotide was 2 µM, and samples were prepared in PBS buffer (137 mM sodium chloride, 2.7 mM potassium chloride, 8 mM sodium phosphate dibasic, and 2 mM potassium phosphate monobasic, pH 7.4). Samples were annealed by heating to 85 °C and then slowly cooled to 10 °C. Samples were then heated to 85 °C at a gradient of 1 °C/min, and the change in UV absorbance at 260 nm was recorded. The melting temperature was calculated from the first derivative of the melting curve.

Table S2. UV melting temperatures of duplexes containing 2′-F-NMC : 2′-F-NMC base pairs and their comparison to those carrying 2′-F-RNA : 2′-F-RNA base pairs.

entry	duplex	T_m [a] (ΔT_m) (°C) [b]	
		2′-F-NMC	2′-F
1	5′-UACAG**U**CUAUGU 3′-AUGUC**A**GAUACA	53.2 (−0.4)	55.0 (1.4)
2	5′-UACAGU**C**UAUGU 3′-AUGUCA**G**AUACA	51.4 (−2.2)	55.6 (2.0)

[a] T_m values were obtained in PBS (pH 7.4) using 2.0 µM concentrations of each strand. [b] ΔT_m is the difference in melting temperature between the duplex with the modified strand and the unmodified reference duplex (5′-UACAGUCUAUGU-3′:3′-AUGUCAGAUACA-5′, T_m = 53.6 °C).

Table S3. Duplexes analyzed for T_m studies [a]

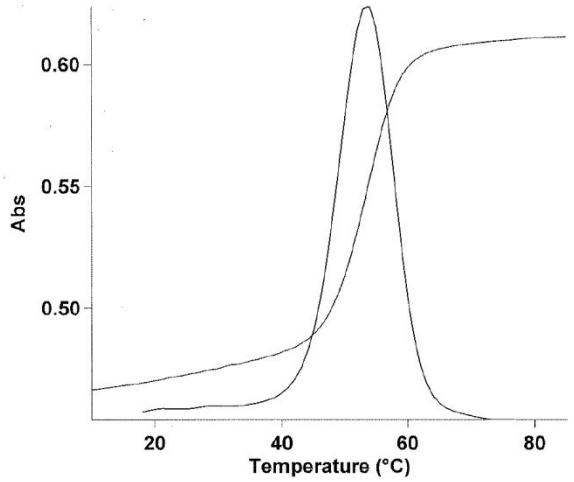
duplex no.	duplex type [b]	duplex [c]
1	RNA[1]: cRNA[1]	5′-UACAGUCUAUGU 3′-AUGUCAGAUACA
2	RNA(U$^{F\text{-}NMC}$): cRNA[1]	5′-UACAGU$^{F\text{-}NMC}$CUAUGU (**1**) 3′-AUGUCAGAUACA
3	RNA(C$^{F\text{-}NMC}$): cRNA[1]	5′-UACAGUC$^{F\text{-}NMC}$UAUGU (**2**) 3′-AUGUCAGAUACA
4	RNA[1]: cRNA(G$^{F\text{-}NMC}$)	5′-UACAGUCUAUGU 3′-AUGUCA$^{F\text{-}NMC}$GAUACA (**3**)
5	RNA[1]: cRNA(A$^{F\text{-}NMC}$)	5′-UACAGUCUAUGU 3′-AUGUCAG$^{F\text{-}NMC}$AUACA (**4**)
6	RNA(U^{F}): cRNA[1]	5′-UACAGU^{F}CUAUGU 3′-AUGUCAGAUACA
7	RNA(C^{F}): cRNA[1]	5′-UACAGUC^{F}UAUGU 3′-AUGUCAGAUACA
8	RNA[1]: cRNA(G^{F})	5′-UACAGUCUAUGU 3′-AUGUCA^{F}GAUACA
9	RNA[1]: cRNA(A^{F})	5′-UACAGUCUAUGU 3′-AUGUCAG^{F}AUACA

10	RNA($U^{F\text{-}NMC}$): cRNA($A^{F\text{-}NMC}$)	5′-UACAG$U^{F\text{-}NMC}$CUAUGU (**1**) 3′-AUGUC$A^{F\text{-}NMC}$GAUACA (**4**)
11	RNA($C^{F\text{-}NMC}$): cRNA($G^{F\text{-}NMC}$)	5′-UACAGU$C^{F\text{-}NMC}$UAUGU (**2**) 3′-AUGUCA$G^{F\text{-}NMC}$AUACA (**3**)
12	RNA(U^{F}): cRNA(A^{F})	5′-UACAGU^{F}CUAUGU 3′-AUGUCA^{F}GAUACA
13	RNA(C^{F}): cRNA(G^{F})	5′-UACAGUC^{F}UAUGU 3′-AUGUCAG^{F}AUACA
14	RNA^2: $cRNA^2$	5′-GCGAUCUCAC 3′-CGCUAGAGUG
15	RNA($B^{F\text{-}NMC}$): $cRNA^2$	5′-GC$G^{F\text{-}NMC}A^{F\text{-}NMC}U^{F\text{-}NMC}C^{F\text{-}NMC}U^{F\text{-}NMC}C^{F\text{-}NMC}$AC (**5**) 3′-CGCUAGAGUG
16	RNA(B^{F}): $cRNA^2$	5′-GC$G^{F}A^{F}U^{F}C^{F}U^{F}C^{F}$AC (**6**) 3′-CGCUAGAGUG

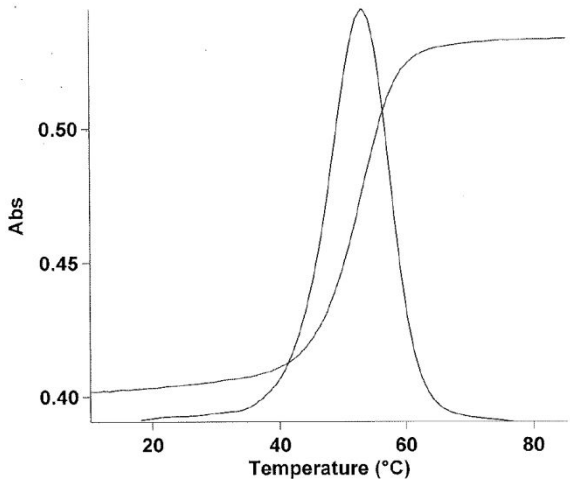
[a] Superscript F indicates 2′-F-RNA, superscript F-NMC indicates 2′-F-NMC. [b] lower case c in each duplex type, (e.g. cRNA) refers to the complementary strand. [c] Numbers in parentheses indicate numbers from Table S1.

Figure S1. T_m profiles of the duplexes

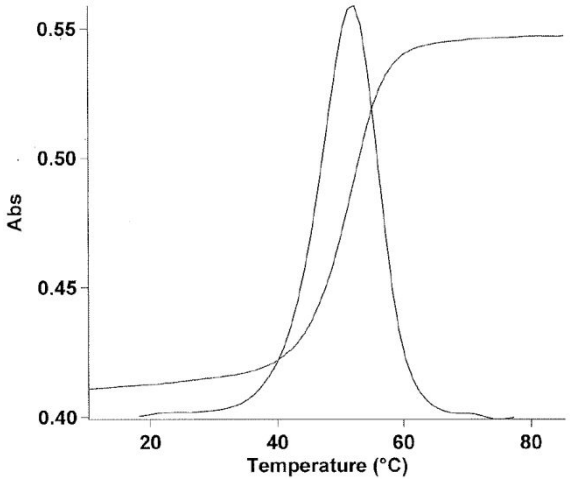
RNA[1]: cRNA[1]



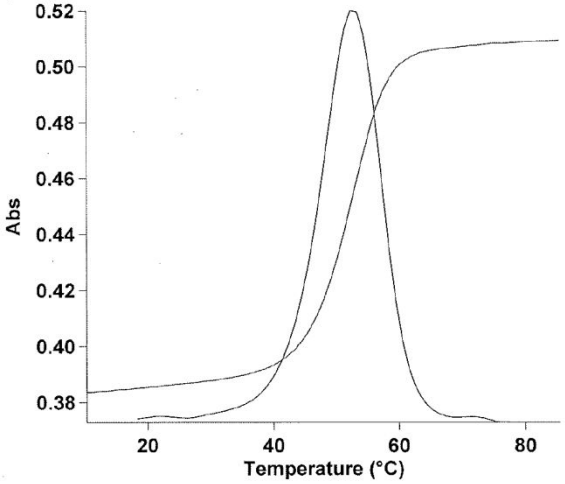
RNA(U[F-NMC]): cRNA[1]



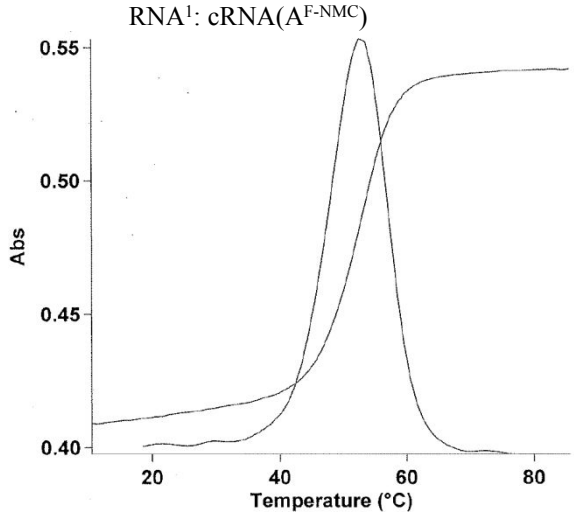
RNA(C[F-NMC]): cRNA[1]



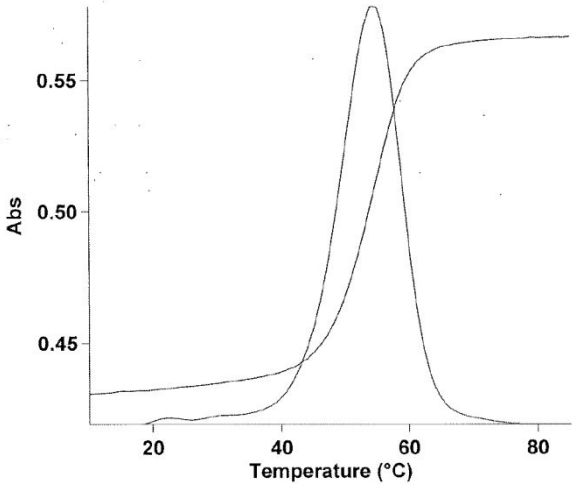
RNA[1]: cRNA(G[F-NMC])



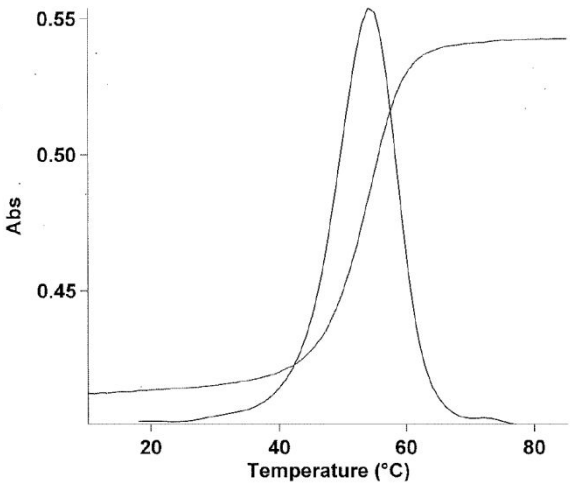
RNA[1]: cRNA(A[F-NMC])



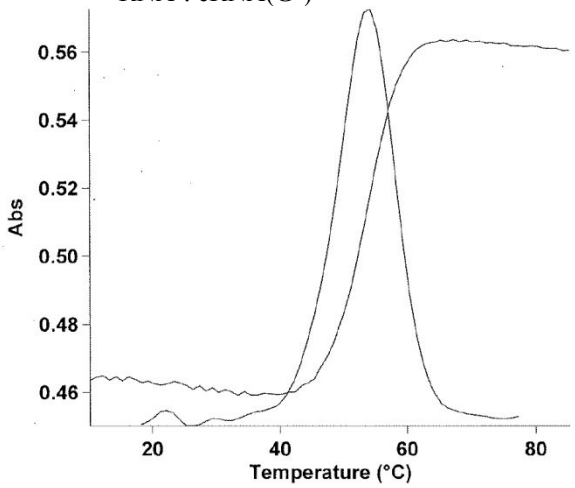
RNA(U^F): $cRNA^1$



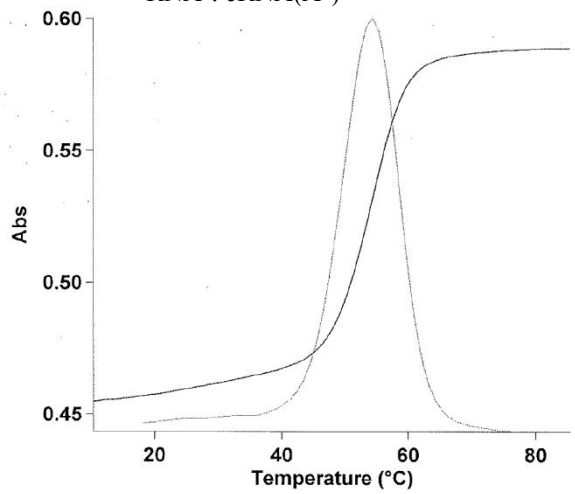
RNA(C^F): $cRNA^1$



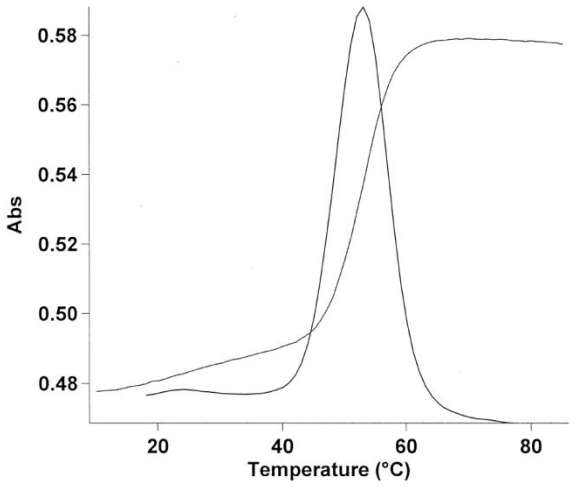
RNA^1: cRNA(G^F)



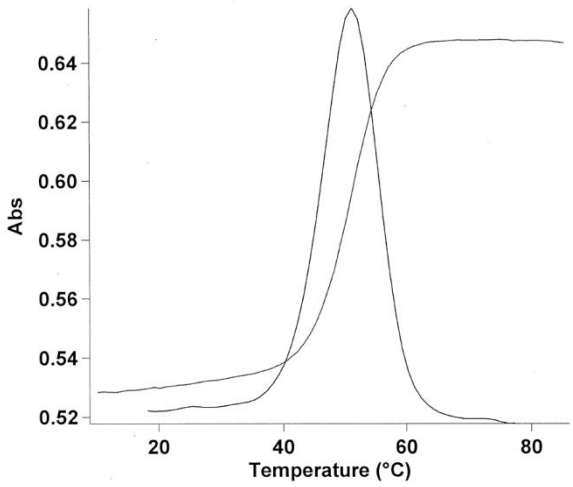
RNA^1: cRNA(A^F)



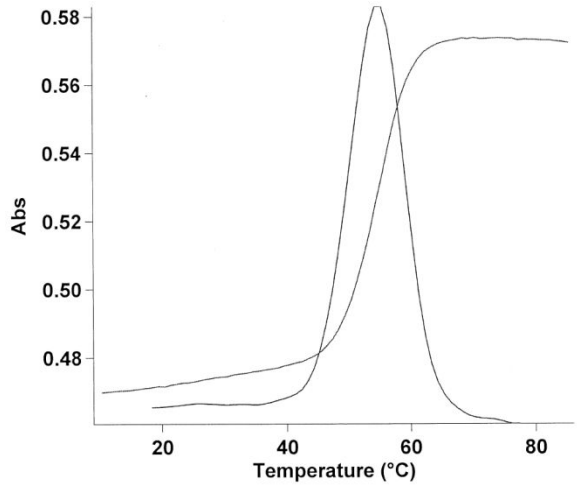
RNA($U^{F\text{-}NMC}$): cRNA($A^{F\text{-}NMC}$)



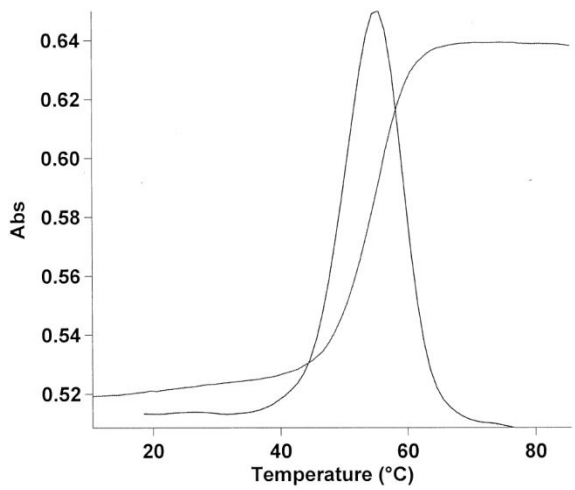
RNA($C^{F\text{-}NMC}$): cRNA($G^{F\text{-}NMC}$)



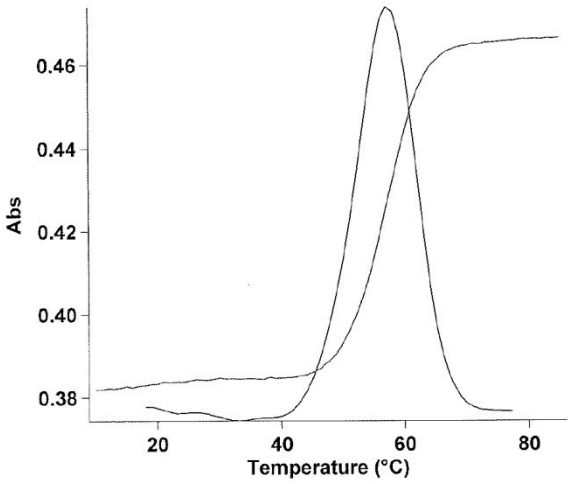
RNA(U^{F}): cRNA(A^{F})



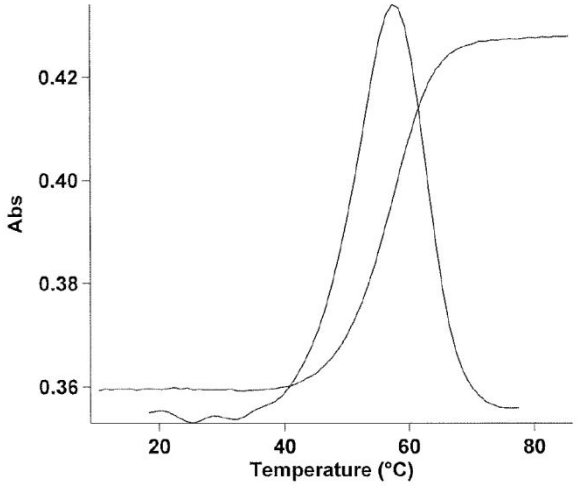
RNA(C^{F}): cRNA(G^{F})



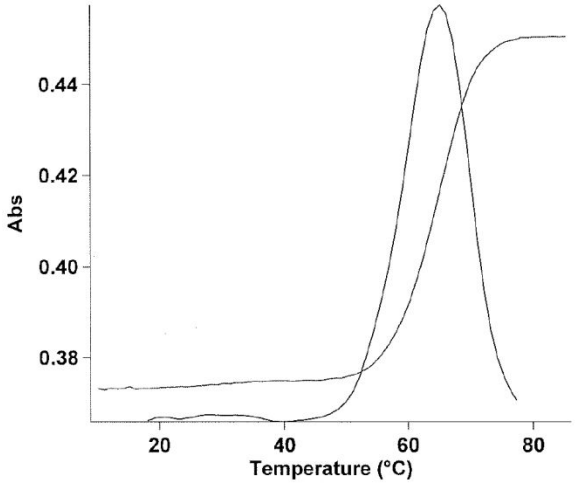
RNA^{2}: cRNA^{2}



RNA(B$^{F\text{-}NMC}$): cRNA^{2}



RNA(B^{F}): cRNA^{2}



Circular dichroism spectroscopy

The circular dichroism (CD) spectra were obtained on a Jasco J-815 spectropolarimeter equipped with a Julabo F25 circulating bath. The sample was allowed to equilibrate for 5 min at 15 ºC in PBS (137 mM sodium chloride, 2.7 mM potassium chloride, 8 mM sodium phosphate dibasic, and 2 mM potassium phosphate monobasic, pH 7.4) at a final concentration of 2.61 μM of duplex. The spectrum was an average of 5 scans. Spectra were collected at a rate of 50 nm/min with a bandwidth of 1 nm and at a sampling wavelength of 0.2 nm using fused quartz cells (Starna 29-Q-10) at a temperature of 15 ºC. The CD spectra were recorded from 350 to 200 nm. The molar ellipticity was calculated from the equation $[\theta] = \theta/10Cl$, where θ is the ellipticity (mdeg), C is the molar concentration of oligonucleotides (M), and l is the path length of the cell (cm). The data were processed using software supplied by JASCO and were transferred into Microsoft Excel for presentation.

Table S4. Modified duplexes for CD spectroscopy [a]

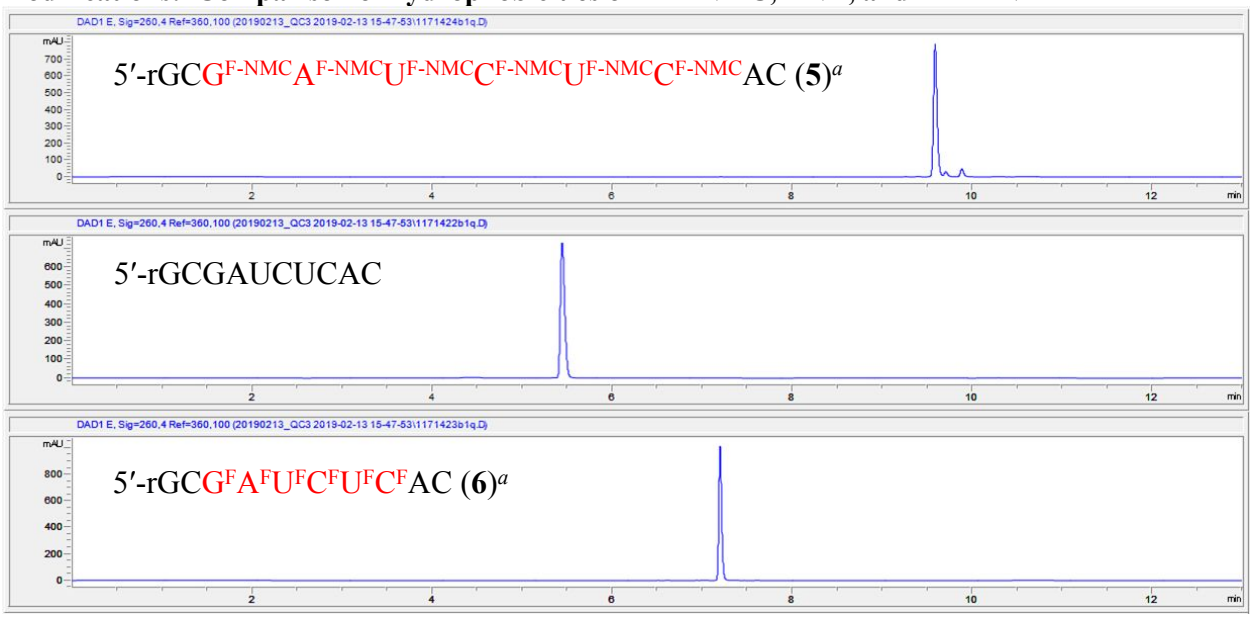
duplex no.	duplex type	duplex [b]
1	RNA: cRNA	5′-UACAGUCUAUGU 3′-AUGUCAGAUACA
2	RNA($U^{F\text{-}NMC}$): cRNA	5′-UACAG$U^{F\text{-}NMC}$CUAUGU (**1**) 3′-AUGUCAGAUACA
3	RNA($U^{F\text{-}NMC}$): cRNA($A^{F\text{-}NMC}$)	5′-UACAG$U^{F\text{-}NMC}$CUAUGU (**1**) 3′-AUGUC$A^{F\text{-}NMC}$GAUACA (**4**)

[a] Superscript F-NMC indicates 2′-F-NMC. [b] Numbers in parentheses indicate numbers from Table S1.

Exonuclease assays

Oligonucleotides were prepared at a final concentration of 0.1 mg/mL in either 50 mM Tris (pH 7.2) and 10 mM $MgCl_2$ or 50 mM sodium acetate (pH 6.5) and 10 mM $MgCl_2$ for assays in the presence of 3′-specific or 5′-specific exonucleases, respectively. The exonuclease (75 mU/mL SVPD or 500 mU/mL PDE-II) was added to oligonucleotide solution immediately prior to the first injection onto the HPLC column, and enzymatic degradation kinetics were monitored for 24 h at 25 °C. Samples collected over 24 h were injected directly onto a Dionex DNAPac PA200 analytical column at 30 °C column temperature. The gradient was from 37% to 52% 1 M NaBr, 10% CH_3CN, 20 mM sodium phosphate buffer at pH 11 over 10 min with a flow rate of 1 mL/min. The amount of 20-mer or 19-mer (for experiment with $dT_{18}C^{F\text{-}NMC}dT$) was determined as the area under the curve of the peak detected at A_{260}. Percent full-length ON was calculated by dividing the area under the curve at the first time point and multiplying by 100. Activity of enzyme was verified for each experiment by including a oligodeoxythymidylate with a terminal phosphorothioate linkage dT_{20} in each experiment. Each aliquot of enzyme was thawed just prior to the experiment. The half-life was determined by fitting to first-order kinetics. Each degradation experiment was run in duplicate.

Figure S2. UV trace from HPLC data of HPLC/mass spectra for oligonucleotides carrying multiple modifications: Comparison of hydrophobicities of 2′-F-NMC, RNA, and 2′-F-RNA



[a] Superscript F-NMC indicates 2′-F-NMC. Superscript F indicates 2′-F-RNA. Numbers in parentheses indicate numbers from Table S1. Conditions: The analysis was performed on C8 columns using buffer A (95 mM hexafluoroisopropanol (HFIP)-16 mM TEA buffer) and buffer B (methanol).

Modeling study

The model shown in Figure 1 was built using the program UCSF Chimera[2] starting from an RNA duplex, riboses in one strand were replaced by 2′-F-NMC sugars with a C2′-*exo* pucker without altering backbone or glycosidic torsion angles.

References

(1) Jung, M. E.; Dwight, T. A.; Vigant, F.; Ostergaard, M. E.; Swayze, E. E.; Seth, P. P. *Angew. Chem. Int. Ed.* **2014**, *53*, 9893.

(2) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605.