Supplementary Data

Synthesis of Folate Derivative Phosphoramidites for siRNA Incorporation

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Procedures

General materials and methods

All starting reagents and solvents were obtained from commercial sources and used without additional purification, unless otherwise indicated. Anhydrous CH_2Cl_2 and Et_3N were purchased from Sigma-Aldrich and kept dry using a PureSolv 400 Solvent Purification System.

Compound and oligonucleotide characterization

^{1}H, ^{13}C and ^{31}P NMRs were recorded in DMSO-d6, $CDCl_3$ or CD_3OD using a Bruker Avance III NMR spectrometer. NMR spectra were processed with ACD/NMR Processor. High-performance liquid chromatography (HPLC) was performed on a Waters 1525 binary HPLC pump with a Waters 2489 UV/Vis detector, using a C18 4.6 mm x 150 mm reverse-phase column and eluting from 5 to 100% acetonitrile in a triethylamine-acetic acid (TEAA) buffer (pH 7.00) over 30 minutes. LC/MS chromatograms were acquired on an Agilent 6545 QTOF-MS with Agilent 1260 Infinity Binary Pump HPLC using a ZORBAX Eclipse Plus C18 2.1x100mm 1.8-Micron Agilent column and a mobile phase of 5 mM ammonium acetate buffer (pH 7)/acetonitrile (95:5). Oligonucleotide samples were prepared at a concentration of 0.01 O.D/µL with an injection volume of 20 µL. Data were analyzed using Agilent Technologies MassHunter Workstation Qualitative Analysis Software (Qual. 10.0).

Statistical analysis

The half-maximal inhibitory concentration (IC_{50}) values were calculated after siRNA transfection in HeLa cells using GraphPad Prism 9 Software. Modified siRNAs were tested at ten concentrations following the carrier-free transfection protocol. Prism's variable slope (four-parameter) model was used for the analysis, and the resulting dose-response curves can be found in Supplementary Figure S3.

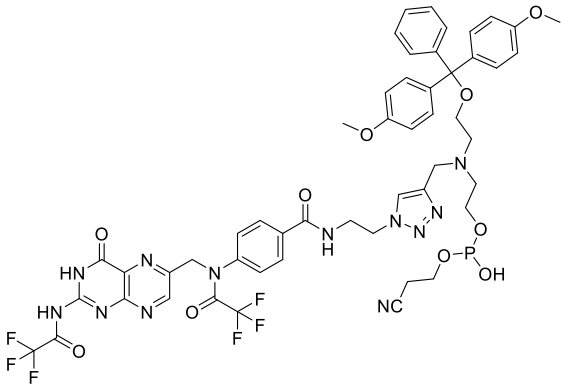
Tables

Table S1 Sequences and mass spectrometry data of modified oligonucleotide strands

Code	*Sequence*	*Mass (predicted)*	*Mass (found)*
F1	5’ CUU ACG CUG AGU ACU UCG A**F** 3’	6580.9985	6580.4427
F2	5’ CUU ACG CUG AGU ACU **F**CG ATT 3’	6886.3612	6886.4511
F3	5’ CUU ACG CUG **F**GU ACU UCG ATT 3’	6859.0428	6858.7023
F4	5’ CUU ACG CU**F** AGU ACU UCG ATT 3’	6844.0431	6844.3508

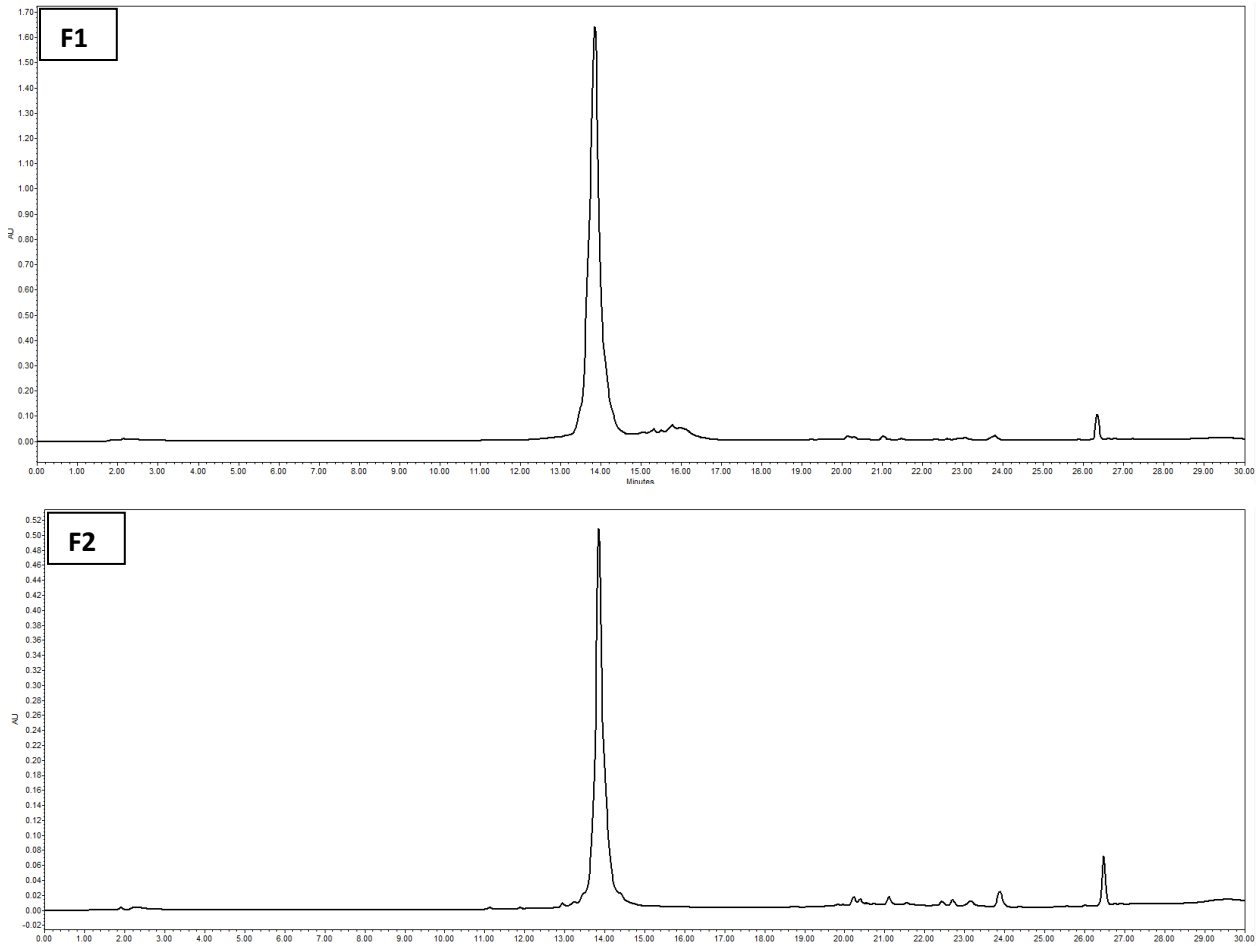
All strands code for firefly luciferase and correspond to the sense strand. **F** indicates the position of the folate modification.

Figures



Exact Mass: 1134.3336
Molecular Weight: 1134.9912

Figure S1 Structure of hydrolyzed product of compound 3≠



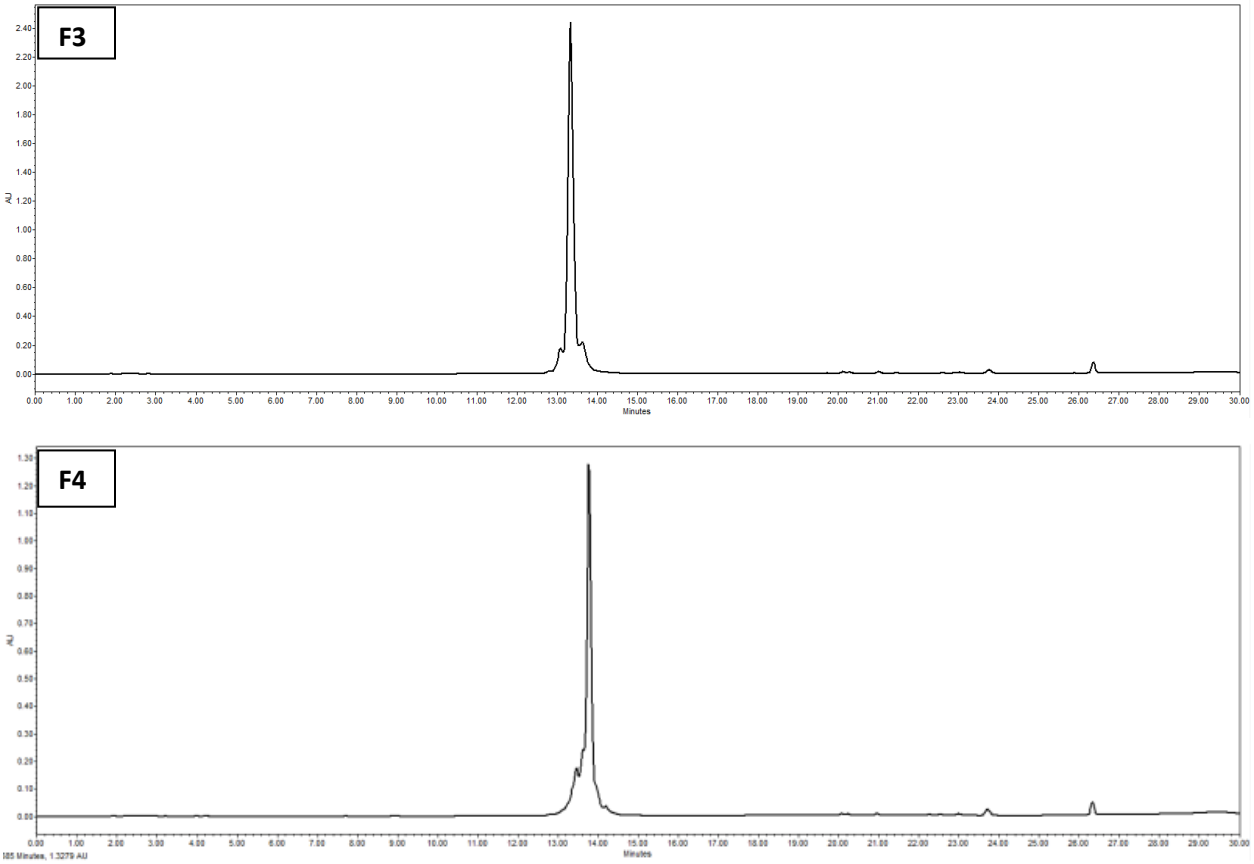


Figure S2 Analytical HPLC spectra of folate-modified oligonucleotide sense strands. Corresponding sequences can be found in Table S1. Eluting from 5% to 95% ACN in 0.1 M TEAA buffer (pH 7.0) over 30 minutes.

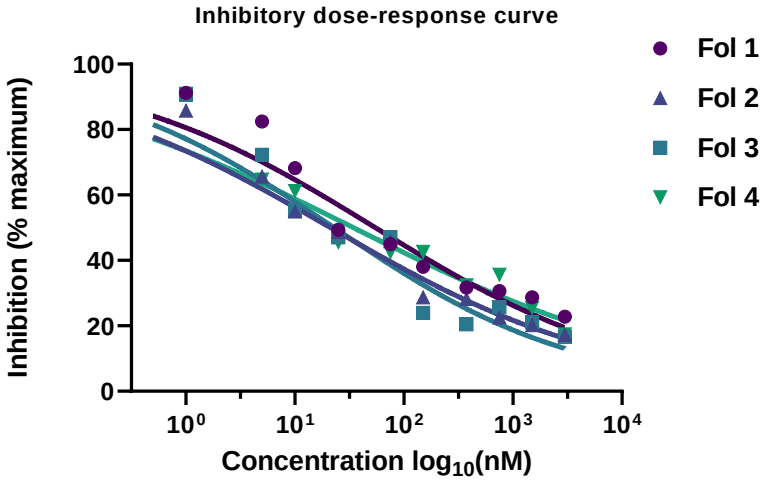


Figure S3 Inhibitory dose-response curves for modified siRNAs in HeLa cells, following a carrier-free transfection protocol.

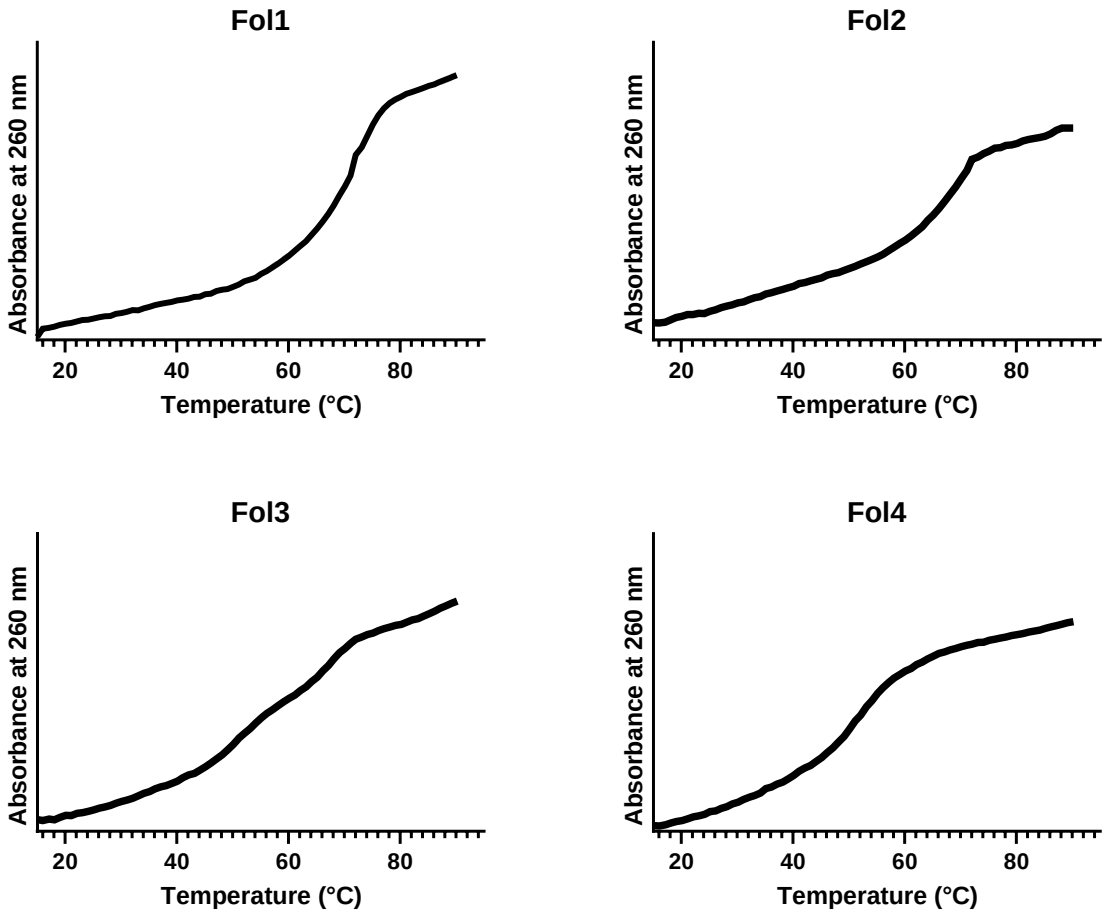
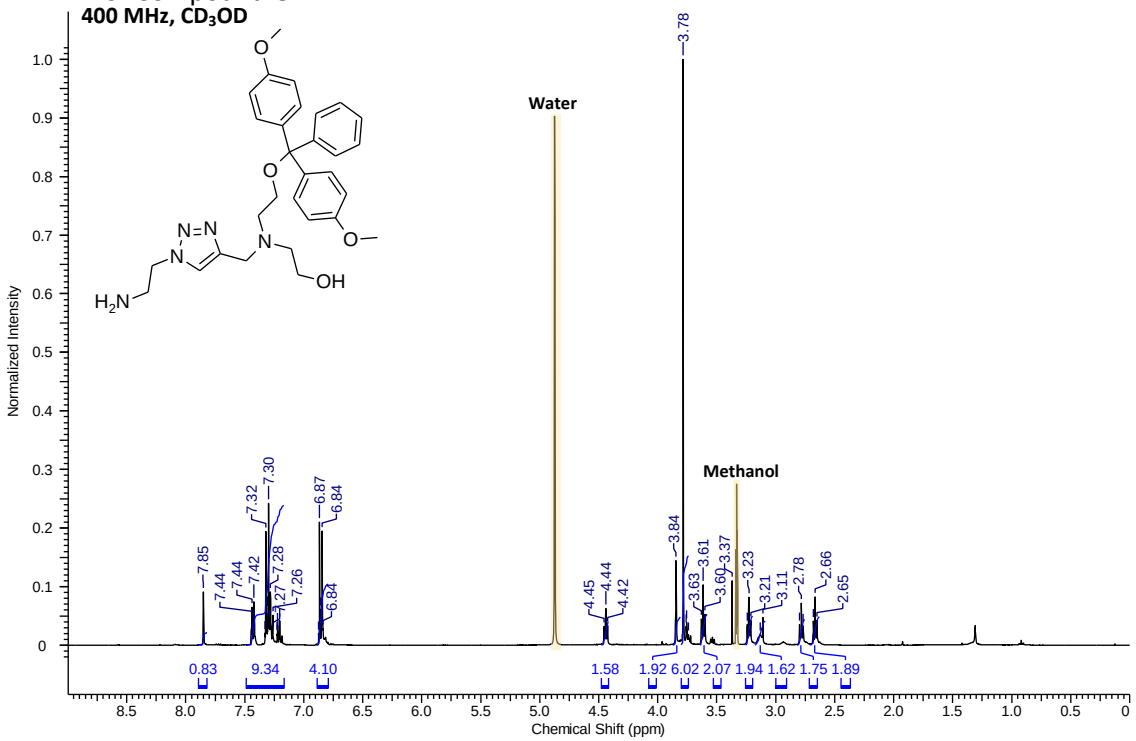


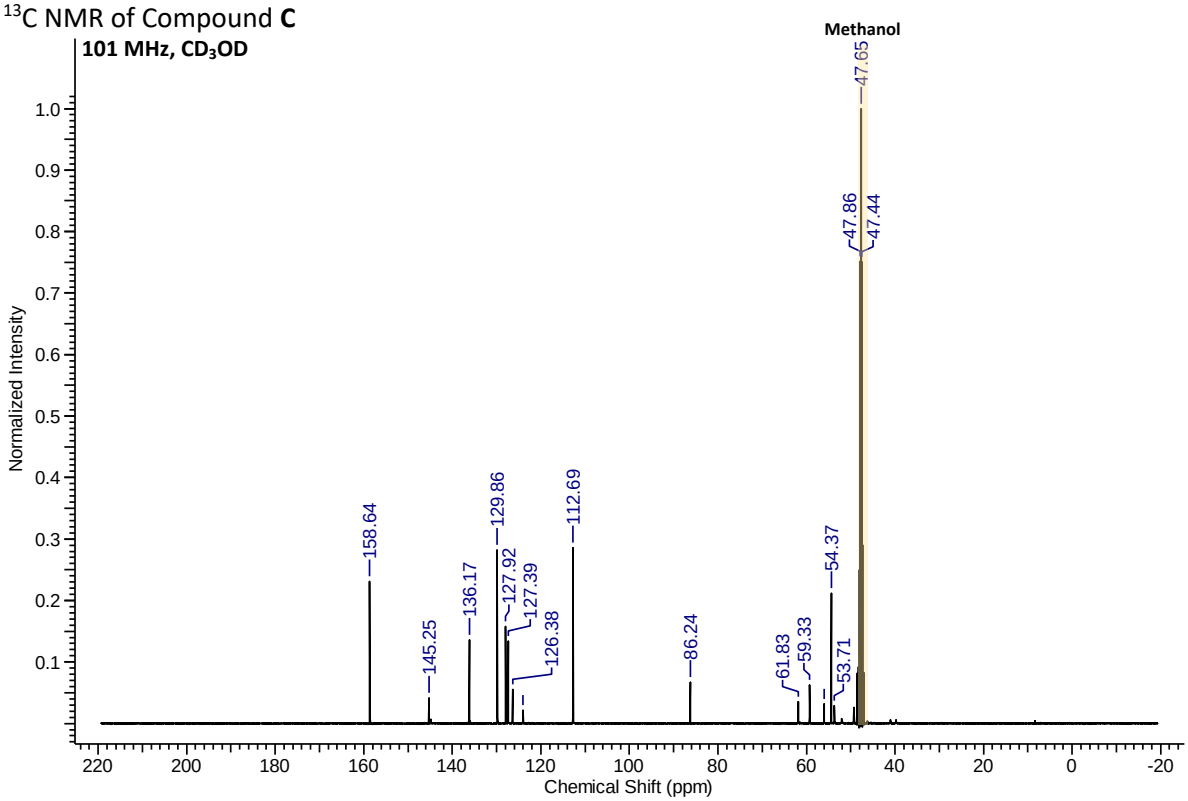
Figure S4 Melting temperature curve of modified siRNAs (Fol1-Fol4)

NMR Data

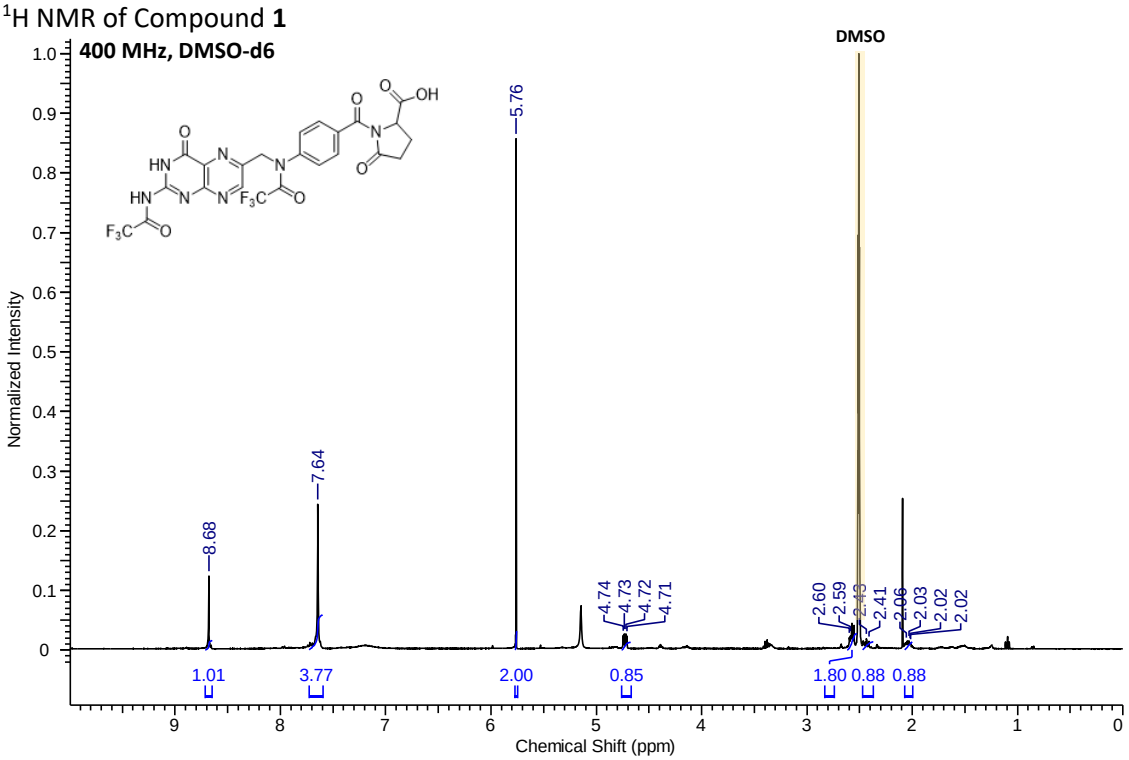
^{1}H NMR of Compound **C**



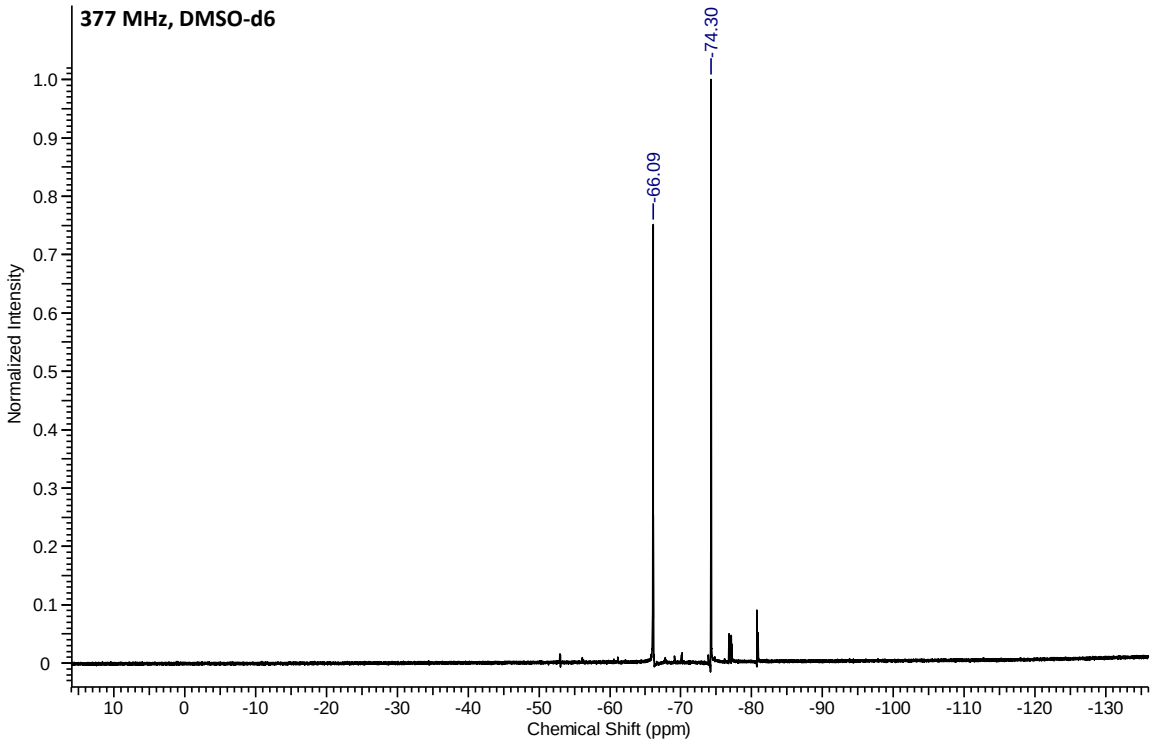
^{13}C NMR of Compound **C**



^{1}H NMR of Compound **1**



^{19}F NMR of Compound **1**



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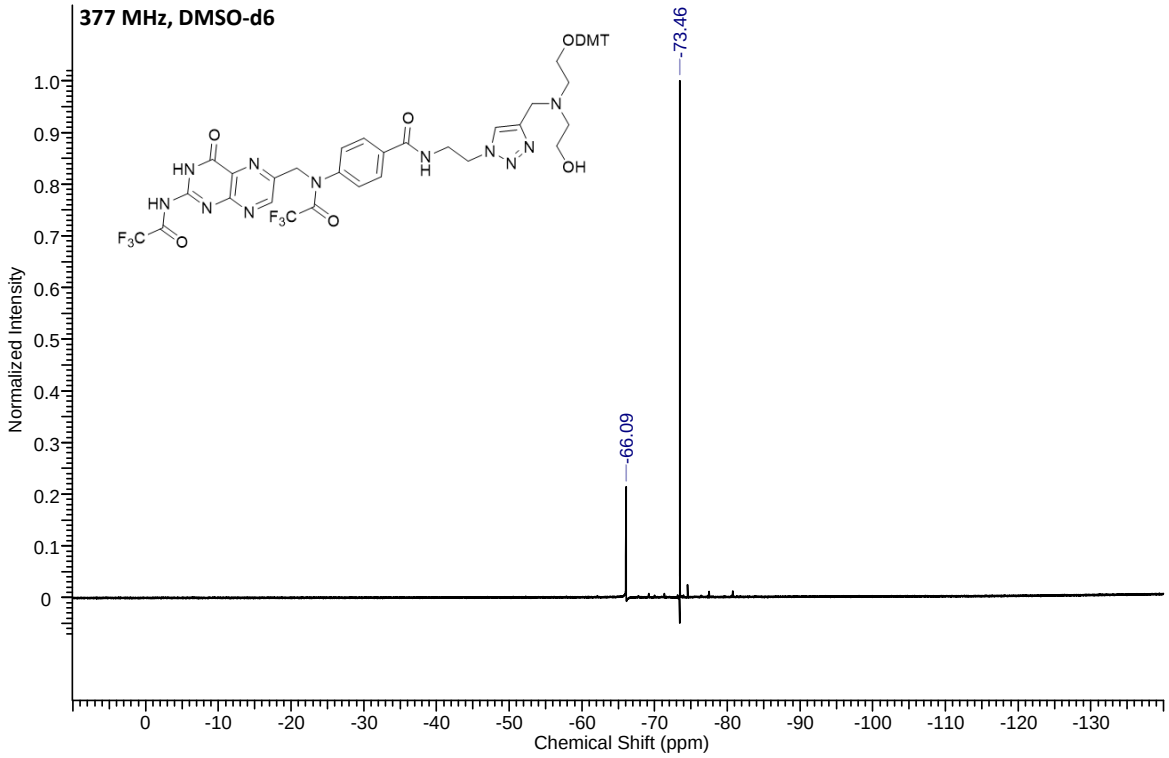
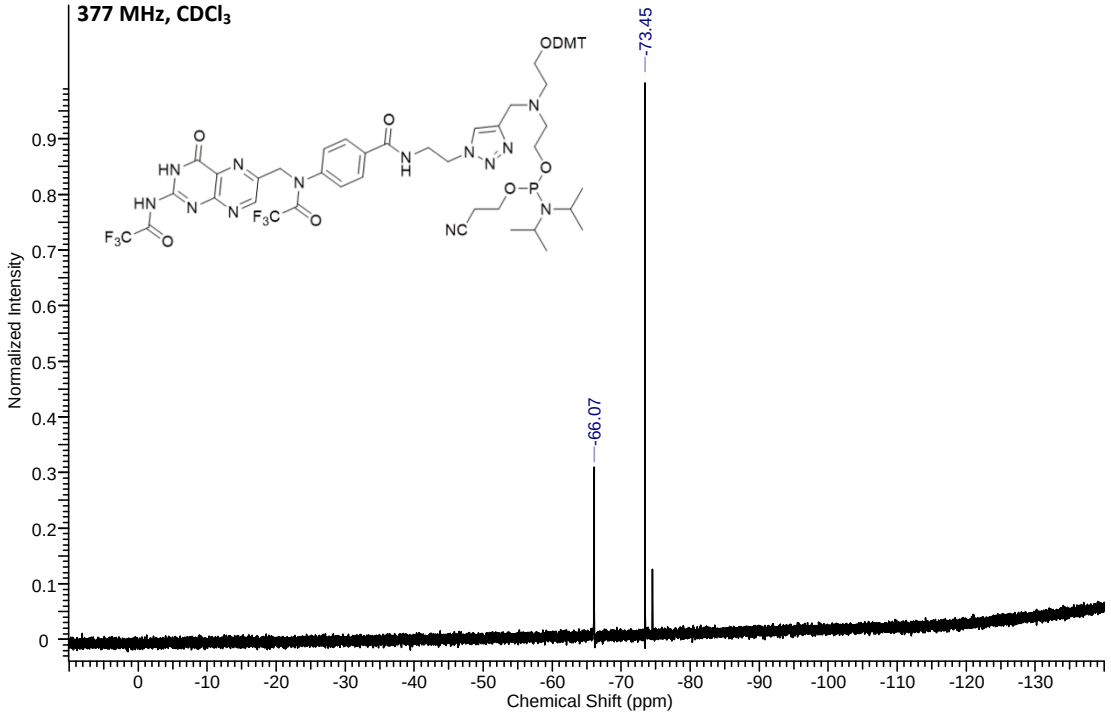


^{19}F NMR of Compound **3≠**



^{31}P NMR of Compound **3≠**