

Supplementary materials

Antisense gapmers with LNA-wings and (*S*)-5'-C-aminopropyl-2'-arabino-fluoro-nucleosides could efficiently suppress the expression of *KNTC2*

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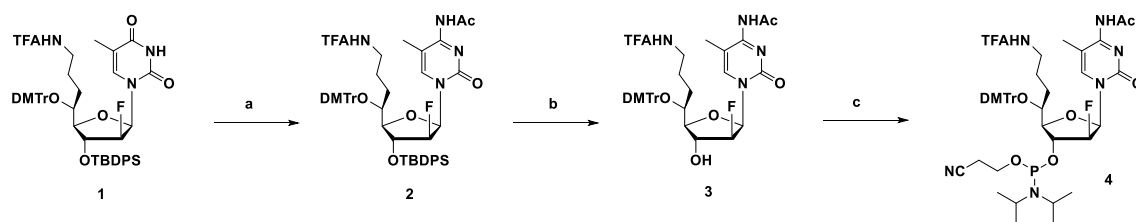
Scheme S1. Novel synthesis of (*S*)-5'-*C*-aminopropyl-2'-arabino fluoro-5-methyl-cytidine phosphoramidites.

General remark and details of the synthesis of compounds **2-4**.

Figure S1. Thermal Stability of Duplexes: the UV melting profiles of duplex composed of each KN5ara gapmer and cRNA-1.

Table S1. The sequences of all oligonucleotides used in this research.

¹H-, ¹³C- and ¹⁹F-NMR spectra of compounds **2-4** and ³¹P-NMR spectra of compound **4**.



Scheme S1. Synthesis of (*S*)-5'-*C*-aminopropyl-2'-arabino-5-fluoro-2-methylcytidine phosphoramidite **4**. ^aReagents and conditions: (a)(i) TPSCl, DMAP, Et₃N, MeCN, r.t., 3 h; (ii) 28% NH₃ aq., r.t., 3 h; (iii) Ac₂O, pyridine, r.t., 2 h; **2**: 60%; (b) 1M TBAF-THF solution, THF, r.t., 3 h, **3**: 76%; (c) DIPEA, CEPCl, THF, r.t., 1.5 h, **4**: 74%.

General Remark.

All chemicals and dry solvents (DCE, DCM, DMF, MeCN, THF, and pyridine) were obtained from commercial sources and used without any further purification. Thin layer chromatography (TLC) was performed on silica gel plates precoated with fluorescent indicator with visualization by UV light or by dipping into a solution of 5% (v/v) concentrated H₂SO₄ in a mixture of *p*-anisaldehyde and methanol, and then heating. Silica gel (63-210 mesh) was used for column chromatography. ¹H NMR (400 or 500 M Hz), ¹³C {¹H} NMR (101 M Hz), ¹⁹F NMR (376 M Hz), ³¹P NMR (162 M Hz) were recorded on 400 or 500 M Hz NMR equipment. Acetonitrile-*d*₃, CDCl₃, or DMSO-*d*₆ was used as a solvent for obtaining NMR spectra. Chemical shifts (δ) are given in parts per million (ppm) from CDCl₃ (7.26 ppm) for ¹H NMR spectra and CDCl₃ (77.2 ppm) for ¹³C NMR spectra. The abbreviations s, d, t, q, and m signify singlet, doublet, triplet, quadruplet, and multiplet, respectively. High resolution mass spectra (HRMS) were obtained in positive ion electrospray ionization (ESI-TOF) mode.

***N*-Acetyl-2'-arabino fluoro-(*S*)-5'-*O*-[bis(4-methoxyphenyl)phenylmethyl]- 3'-*O*-[(1,1-dimethylethyl)diphenylsilyl]- 5-methyl-5'-*C*-trifluoroacetylaminopropyl-**cytidine (2)**. 4-Dimethylaminopyridine (0.10 g, 0.80 mmol), triethylamine (0.36 mL, 2.58 mmol), 2,4,6-triisopropylbenzenesulfonyl chloride (0.31 g, 1.04 mmol) were added into the solution of compound **1** in MeCN (5.0 mL) under argon atmosphere. The reactant was stirred for 3 h at room temperature, and then the 28% aqueous ammonia solution (2.08 mL) was dropwised. The mixture was stirred for more 3 h at room temperature and extracted with ethyl acetate and water; the organic layer was washed with brine, dried by Na₂SO₄, filtered and concentrated. The residue was dissolved with pyridine (5.0 mL) and acetic anhydride (0.10 mL, 1.04 mmol) was added. After being stirred for 2 h at room temperature, the reactant was extracted with ethyl acetate and water; the organic layer was washed with brine, dried by Na₂SO₄, filtered and concentrated. The crude material was purified by column chromatography (50% ethyl acetate in hexane) to afford desired product **2** (0.31 g, 60%). ¹H NMR (400 M Hz, CDCl₃) δ: 0.73-0.77 (m, 1H), 0.93 (s, 9H), 1.15-1.19 (m, 2H), 1.35-1.43 (m, 1H), 1.78 (s, 3H), 2.28 (s, 3H), 2.76-2.79 (m, 2H), 3.08 (d, *J* = 9.6 Hz, 1H), 3.71 (s, 3H), 3.72 (s, 3H), 4.10 (d, *J* = 3.2 Hz, 1H), 4.66 (dd, *J* = 4.6, 25.6 Hz, 1H), 5.24 (dd, *J* = 4.1, 53.2 Hz, 1H), 6.24 (dd, *J* = 4.1, 17.4 Hz, 1H), 6.73 (dd, *J* = 9.2, 17.8 Hz, 4H), 7.03-7.55 (m, 19H), 7.91 (s, 1H), 9.24 (t, *J* = 5.7 Hz, 1H), 9.91 (s, 1H); ¹³C{¹H} NMR (101 M Hz, CDCl₃) δ: 13.80, 14.10, 24.27, 24.95, 26.49, 28.09, 54.97, 55.00, 72.54, 76.28, 76.55, 79.18, 83.87, 84.25, 85.60, 94.69, 96.59, 105.34, 111.58, 112.91, 114.45, 117.32, 126.62, 127.53, 127.87, 129.67, 129.80, 130.21, 131.62, 131.78, 135.12, 135.20, 135.80, 136.24, 142.05, 146.12, 153.50, 155.76, 156.11, 156.42, 158.03, 158.10, 162.76, 170.63; ¹⁹F NMR (376 M Hz, CDCl₃) δ: -121.88 (d, *J* = 73.76 Hz); HRMS (ESI-TOF) *m/z* Calcd for C₅₄H₅₈F₄N₄NaO₈Si [M + Na]⁺, 994.4000, found 994.3989.**

***N*-Acetyl-2'-arabino fluoro-(*S*)-5'-*O*-[bis(4-methoxyphenyl)phenylmethyl]-5-methyl-5'-*C*-trifluoroacetylaminopropyl-cytidine (**3**).** Tetrabutylammonium fluoride (TBAF, 1.45 mL of a 1M solution in THF) was added in a solution of compound **2** (0.51 g, 0.68 mmol) in THF (14.5 mL) under argon atmosphere, and the reactant was stirred for 3 h at room temperature. The reactant was extracted with ethyl acetate and saturated NaHCO₃; the organic layer was washed with brine, dried by Na₂SO₄, filtered and concentrated. The crude material was purified by column chromatography (83% ethyl acetate in hexane) to afford desired product **3** as a white solid (0.39 g, 76%). ¹H NMR (400 M Hz, DMSO-*d*₆) δ: 1.05-1.34 (m, 4H), 1.85 (s, 3H), 2.28 (s, 3H), 2.81-2.83 (m, 2H), 3.37-4.40 (m, 1H), 3.86-3.88 (m, 1H), 4.29 (dd, *J* = 5.6, 21.6 Hz, 1H), 5.09 (dd, *J* = 2.4, 55.2 Hz, 1H), 5.87 (d, *J* = 5.2 Hz, 1H), 6.09 (dd, *J* = 4, 16.8 Hz, 1H), 6.84-6.89 (m, 4H), 7.19-7.35 (m, 7H), 7.46 (d, *J* = 7.2 Hz, 2H), 7.73 (s, 1H), 9.25 (t, *J* = 5.5 Hz, 1H), 9.91 (bs, 1H); ¹³C{¹H} NMR (101 M Hz, CDCl₃) δ: 13.80, 13.95, 20.05, 23.98, 27.87, 55.01, 55.06, 72.45, 74.06, 79.18, 83.15, 85.94, 113.04, 126.69, 127.65, 127.83, 130.10, 136.30, 136.51, 146.38, 158.16; ¹⁹F NMR (376 M Hz, CDCl₃) δ: -119.40 (s); HRMS (ESI-TOF) *m/z* Calcd for C₃₈H₄₀F₄N₄NaO₈ [M + Na]⁺, 779.2680, found 779.2663.

***N*-Acetyl-2'-arabino fluoro-3'-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-(*S*)-5'-*O*-[bis(4-methoxyphenyl)phenylmethyl]- 5-methyl-5'-*C*-trifluoroacetylaminopropyl-cytidine (**4**).** N,N-diisopropylethylamine (DIPEA, 0.45 mL, 2.55 mmol) and 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (CEPCI, 0.23 mL, 1.02 mmol) were added in a solution of compound **3** (0.39 g, 0.51 mmol) in

THF (4.00 mL) in turn under argon atmosphere. The reactant was stirred for 1.5 h at room temperature, and then extracted with ethyl acetate and saturated NaHCO₃; the organic layer was washed with brine, dried by Na₂SO₄, filtered and concentrated. The crude material was purified by column chromatography (78% ethyl acetate in hexane) to afford desired product **4** as a white solid (0.36 g, 74%). ¹⁹F NMR (376 M Hz, CDCl₃) δ: -119.89 (d, *J* = 184.24 Hz); ³¹P NMR (162 M Hz, CDCl₃) δ: 151.64, 151.96. HRMS (ESI-TOF) *m/z* Calcd for C₄₇H₅₇F₄N₆NaO₉P [M + Na]⁺, 956.3900, found 956.3917.

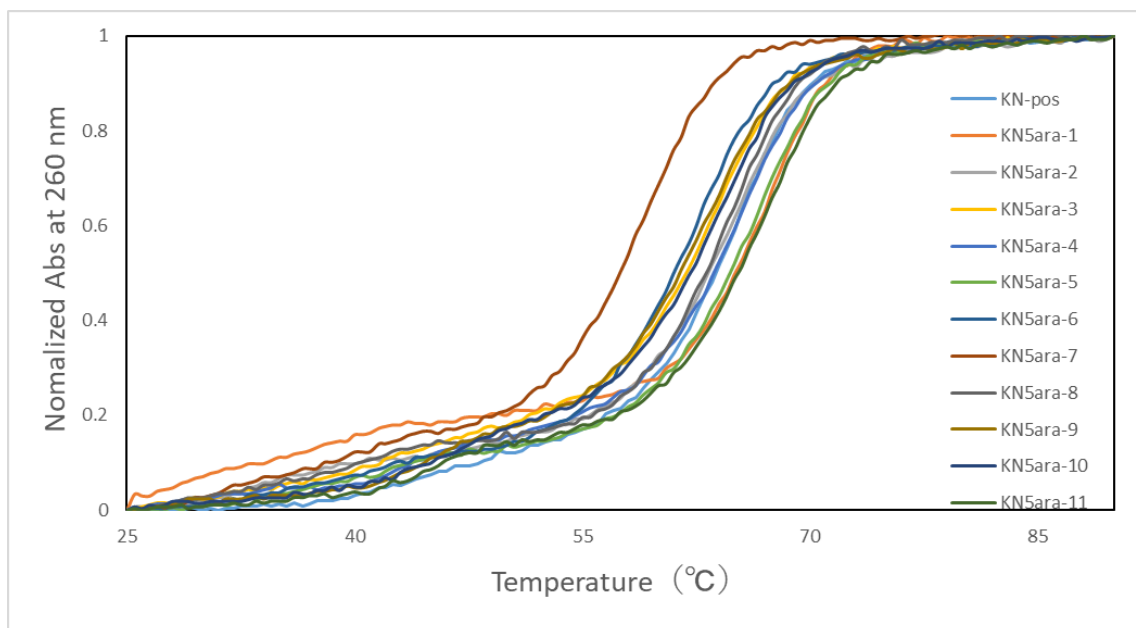


Figure S1. Thermal Stability of Duplexes: the UV melting profiles of duplex composed of each KN5ara gapmer and cRNA-1.

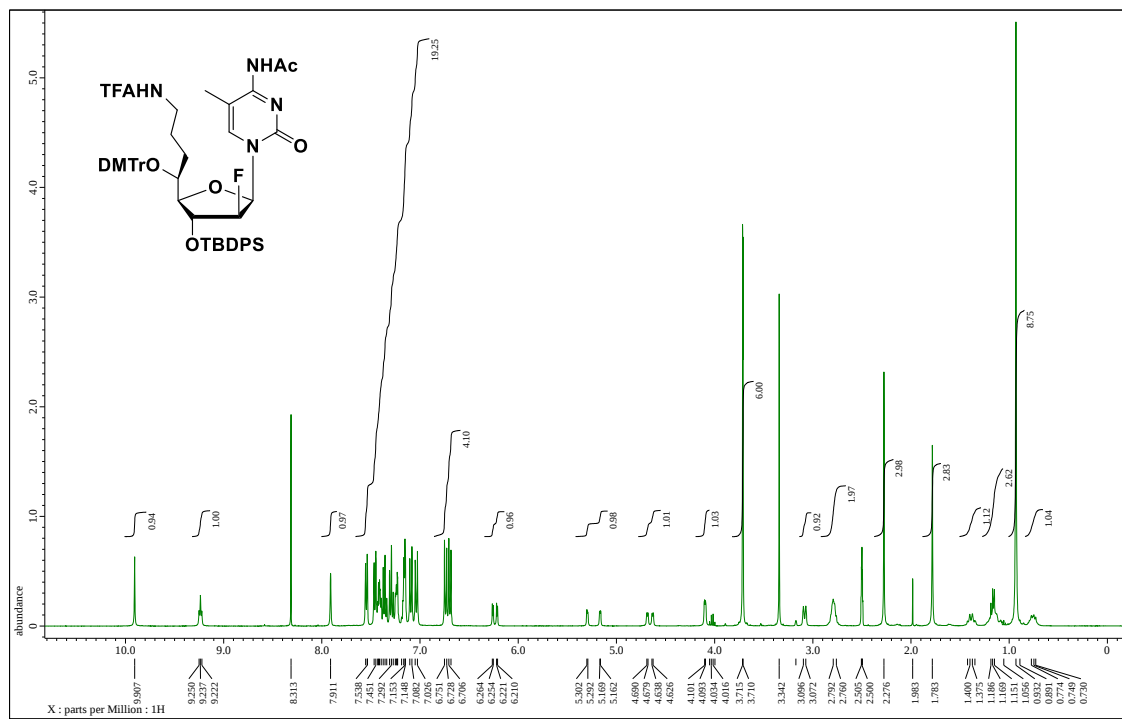
Table S1. The sequences of all oligonucleotides used in this research.

Abbreviation of oligonucleotides	Sequence ^a
KN-pos	5' - <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G·C·T·T·T)· <u>T·G·G</u> - 3'
KN5ara-1	5' - T · <u>A·^{Me}C</u> ·d(A·T·G·G·A·G·C·T·T·T)· <u>T·G·G</u> - 3'
KN5ara-2	5' - <u>T·A</u> · ^{Me}C ·d(A·T·G·G·A·G·C·T·T·T)· <u>T·G·G</u> - 3'
KN5ara-3	5' - <u>T·A·^{Me}C</u> ·d(A· T ·G·G·A·G·C·T·T·T)· <u>T·G·G</u> - 3'
KN5ara-4	5' - <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G· ^{Me}C ·T·T·T)· <u>T·G·G</u> - 3'
KN5ara-5	5' - <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G·C· T ·T·T)· <u>T·G·G</u> - 3'
KN5ara-6	5' - <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G·C·T· T ·T)· <u>T·G·G</u> - 3'
KN5ara-7	5' - <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G·C·T·T· T)· <u>T·G·G</u> - 3'
KN5ara-8	5' - <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G·C·T·T·T)· T · <u>G·G</u> - 3'
KN5ara-9	5' - <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G·C·T·T· T)· T · <u>G·G</u> - 3'
KN5ara-10	5' - <u>T·A·^{Me}C</u> ·d(A T G·G·A·G·C·T·T·T)· <u>T·G·G</u> - 3'
KN5ara-11	5' - <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G·C· TTT)· <u>T·G·G</u> - 3'
KN-pos-F	5' -F- <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G·C·T·T·T)· <u>T·G·G</u> - 3'
KN5ara-6-F	5' -F- <u>T·A·^{Me}C</u> ·d(A·T·G·G·A·G·C·T· T ·T)· <u>T·G·G</u> - 3'
KN5ara-10-F	5' -F- <u>T·A·^{Me}C</u> ·d(A T G·G·A·G·C·T·T·T)· <u>T·G·G</u> - 3'
KN5ara-11-F	5' -F- <u>A·^{Me}C</u> ·d(A·T·G·G·A·G·C· TTT)· <u>T·G·G</u> - 3'
KN5ara-12-F	5' -F- <u>A·^{Me}C</u> ·d(A·T·G·G·A·G·C·TTT)· <u>T·G·G</u> - 3'
cRNA-1	5' - r(CCAAAAGCUCCAUGUA) - 3'
cRNA-2	5' -F- r(CCAAAAGCUCCAUGUA) - 3'

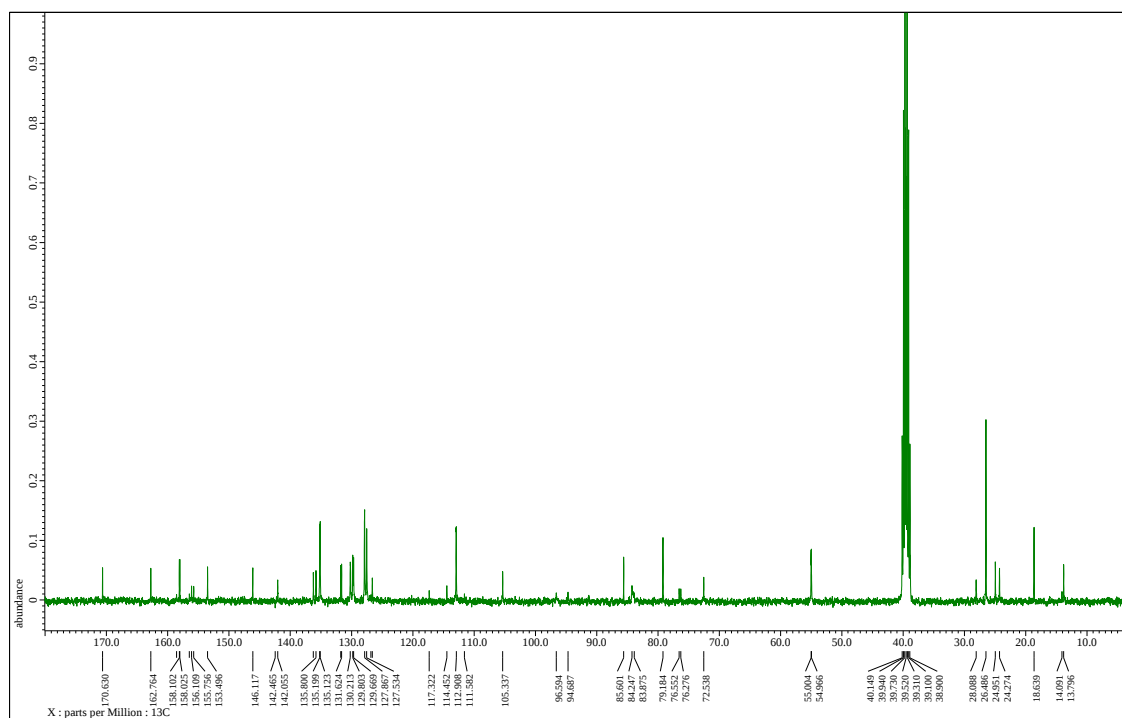
^aT and ^{Me}C in red denote (*S*)-5'-*C*-Aminopropyl-2'-arabino fluoro-thymidine (**5ara-T**) and (*S*)-5'-*C*-Aminopropyl-2'-arabino fluoro-5-methyl-cytidine (**5ara-^{Me}C**), respectively. ACGT with underline denote the corresponding LNAs. Black dots denote the phosphorothioate (PS) linkages. F denotes fluorescein.

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

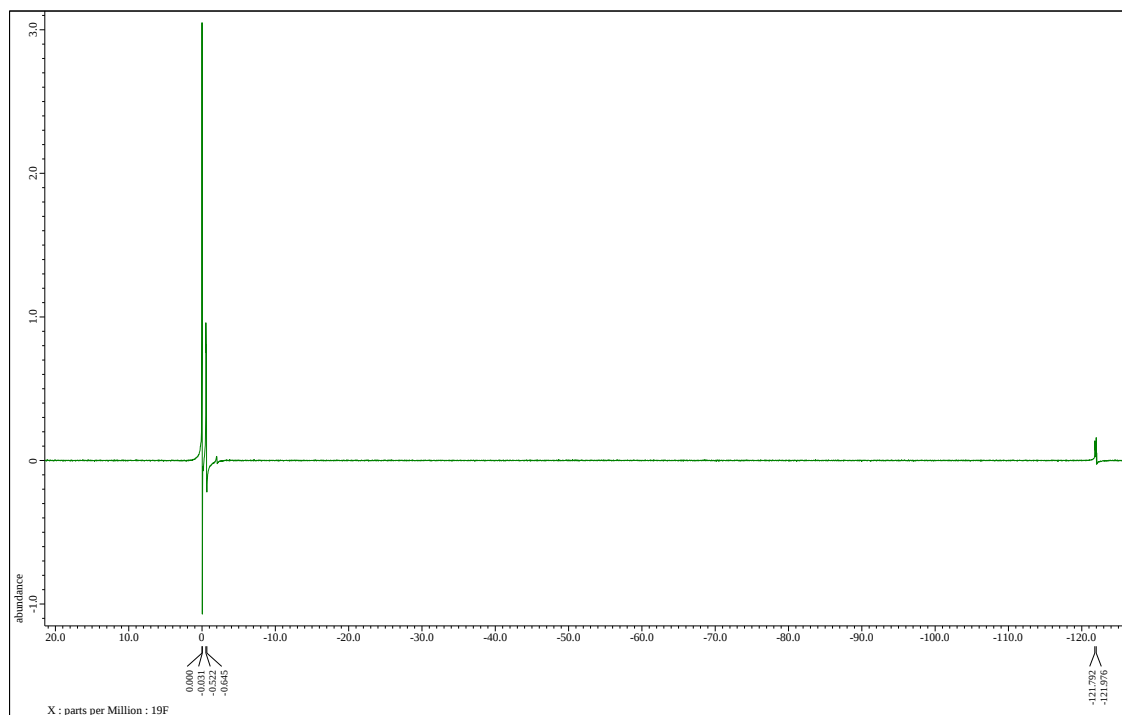
^1H NMR spectrum of compound **2**



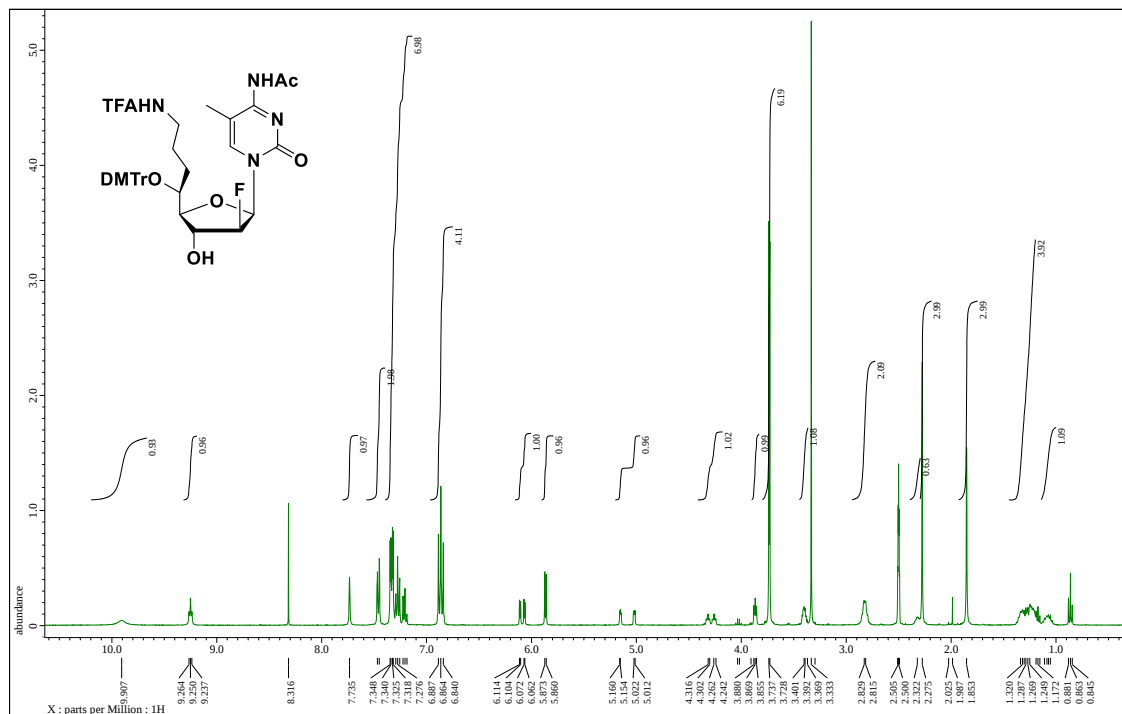
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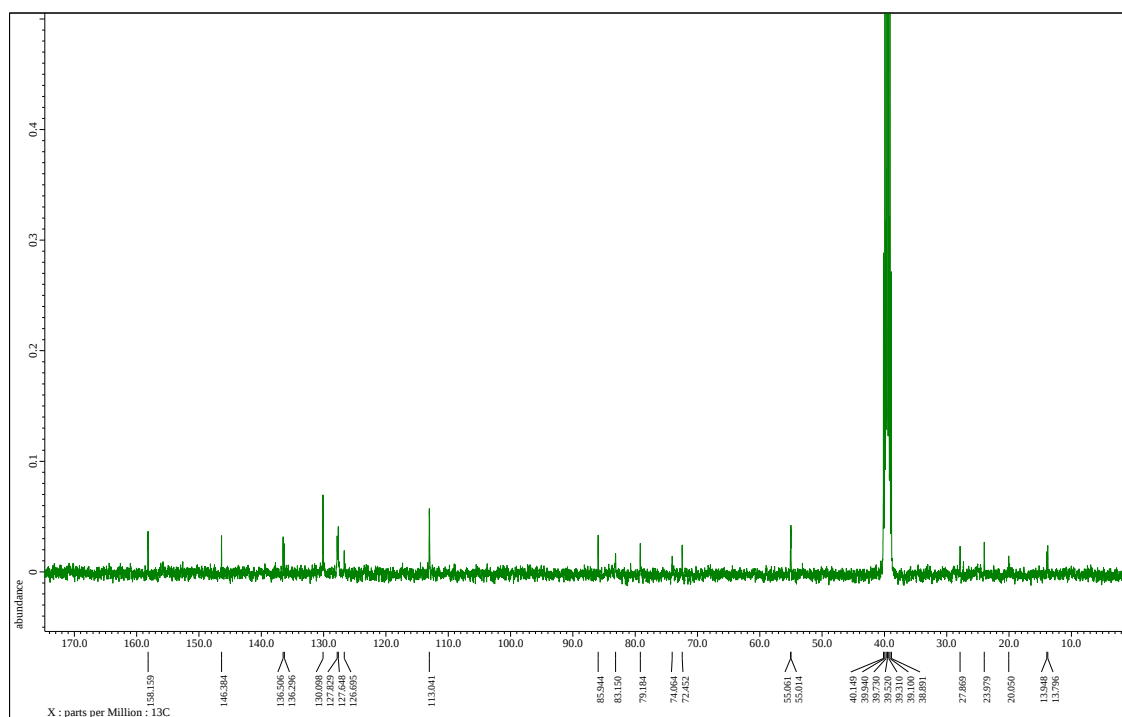
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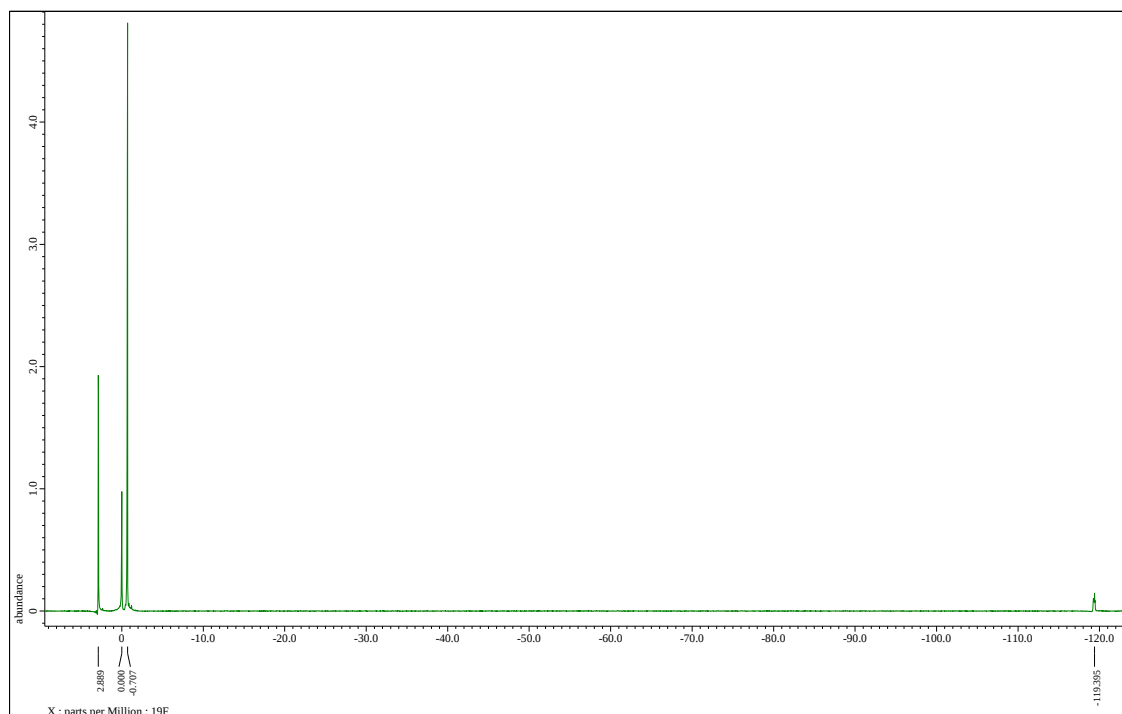
^1H NMR spectrum of compound **3**



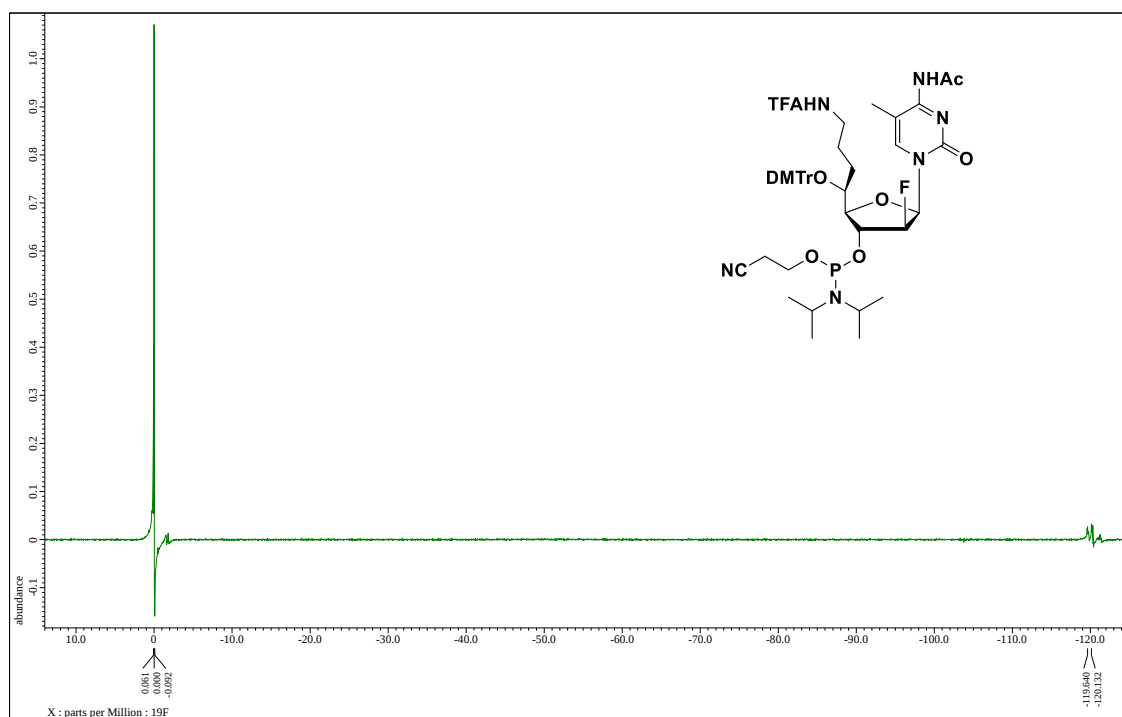
^{13}C NMR spectrum of compound **3**



^{19}F NMR spectrum of compound **3**



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



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

