# 2′β-Fluoro-Tricyclo Nucleic Acids (2′F-tc-ANA): Thermal Stability, Structural Studies, and RNase H activation

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## Supporting Information

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# 1. List of abbreviations

| | |
|---|---|
| **BSA** | bis(trimethylsilyl)acetamide |
| **Bz** | benzoyl |
| **CC** | column chromatography |
| **CEP-Cl** | chloro(2-cyanoethoxy)(diisopropylamino)phosphine |
| **CPG** | controlled pore glass |
| **DCM** | dichloromethane |
| **DMAP** | 4-dimethylaminopyridine |
| **DMF** | dimethylformamide |
| **DMTr-Cl** | 4,4′-dimethoxytrityl chloride |
| **DNA** | deoxyribonucleic acid |
| **ESI-MS** | electrospray ionisation mass spectrometry |
| **HPLC** | high-performance liquid chromatography |
| **MD** | molecular dynamics |
| **mRNA** | messenger RNA |
| **Py** | pyridine |
| **RHF** | restricted Hartree-Fock |
| **RNA** | ribonucleic acid |
| **RNase H** | ribonuclease Hr |
| **r.t.** | room temperature |
| **tc-DNA** | tricyclo-DNA |
| **THF** | tetrahydrofuran |
| **TLC** | thin layer chromatography |
| **$T_m$** | melting temperature |
| **Trizma** | 2-amino-2-hydroxyl-1,3-propanediol |
| **QM** | quantum mechanics |

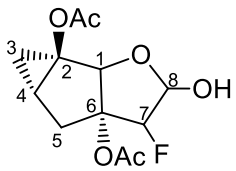
# 2. Synthesis of the nucleoside phosphoramidites

## Chemicals and characterization of the compounds

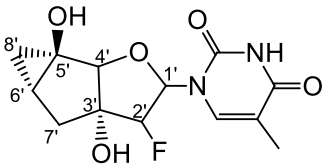
Chemicals for the synthesis of phosphoramidite building blocks were purchased from Sigma-Aldrich, ABCR, Fluka or TCI. Anhydrous solvents for reactions were obtained by filtration through activated alumina ($CH_3CN$, DCM, THF) or purchased from Sigma Aldrich (pyridine, DMF, dioxane). All reactions were performed under an atmosphere of argon in oven-dried glassware. Column chromatography (CC) was performed on silica gel (SiliCycle, 230−400 mesh) or silica gel (Fluka, 230−400 mesh, neutralized with 0.1% of w/Ca). All solvents for CC were of technical grade and distilled prior to use. Thin-layer chromatography (TLC) was performed on silica gel plates (Macherey–Nagel, 0.25 mm, UV254). Visualization was achieved either under UV light or by staining in dip solutions [Cer(IV)-sulfate (10.5 g), phosphormolybdenic acid (21 g), concentrated $H_2SO_4$ (60 ml), $H_2O$ (900 ml) or $KMnO_4$ (6 g), $K_2CO_3$ (40 g), 15% NaOH (3 ml) in $H_2O$ (800 ml)] followed by heating with a heat gun. NMR spectra were recorded on a Bruker Avance II 400 MHz, Bruker Avance III 300 MHz or Bruker Avance III 400 MHz spectrometers at 300 or 400 MHz ($^1H$), at 76 or 100 MHz ($^{13}C$), at 376.5 MHz ($^{19}F$) and at 122 MHz ($^{31}P$) in either $CDCl_3$ or $CD_3OD$. Chemical shifts (δ) are reported relative to the undeuterated residual solvent peak [$CHCl_3$: 7.26 ppm ($^1H$) and 77.16 ppm ($^{13}C$); $CHD_2OD$: 3.31 ppm ($^1H$) and 49.0 ppm ($^{13}C$)]. Signal assignments are based on DEPT or APT experiments, and on $^1H$,$^1H$ and $^1H$,$^{13}C$ correlation experiments. Chemical shifts for $^{31}P$ and $^{19}F$ NMR are reported relative to 85% $H_3PO_4$ and $CFCl_3$ as external standards, respectively. Electrospray ionization mass spectrometry in the positive mode (ion trap, $ESI^+$) was used for high resolution mass detection.

## Numbering of sugars and nucleosides

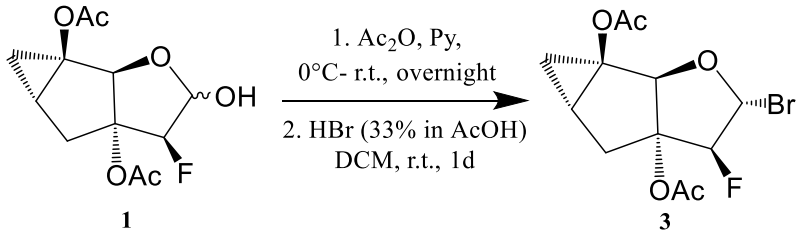
for sugar derivatives




for nucleosides



## 2.1 (1S,2R,4R,6S,7S,8R or S)-2,6,-diacetyloxy-8-bromo-7-fluoro-9-oxytricyclo-[4.3.0$^{1,6}$.0$^{2,4}$]nonane (3)




**1** **3**

A solution of **1** (0.66 g, 2.4 mmol, 1 eq.) in dry pyridine (12 ml) was cooled to 0°C and treated with $Ac_2O$ (3 ml, 31.8 mmol, 13 eq.). After 10 min, it was allowed to warm up to r.t. and stirred overnight. The resulting mixture was then concentrated, coevaporated with toluene (2 x 5 ml) to yield 0.78 g (>100%) of a brown oil, which was subjected to the next reaction without further purification.

A solution of crude triacetylated tricyclosugar (0.78 g, max 2.4 mmol, 1 eq.) in $CH_2Cl_2$ (9 ml, dry) and HBr (33% in acetic acid, 1.5 ml, 8.4 mmol, 3.5 eq.) were allowed to stir overnight at room temperature. The reaction mixture was washed with water (2 x 4 ml), saturated $NaHCO_3$ solution (2 x 4 ml), dried over $MgSO_4$, filtered and evaporated to yield the product **3** (0.818 g, >100%) as a brown oil.

**<u>Analytical data of 3:</u>**

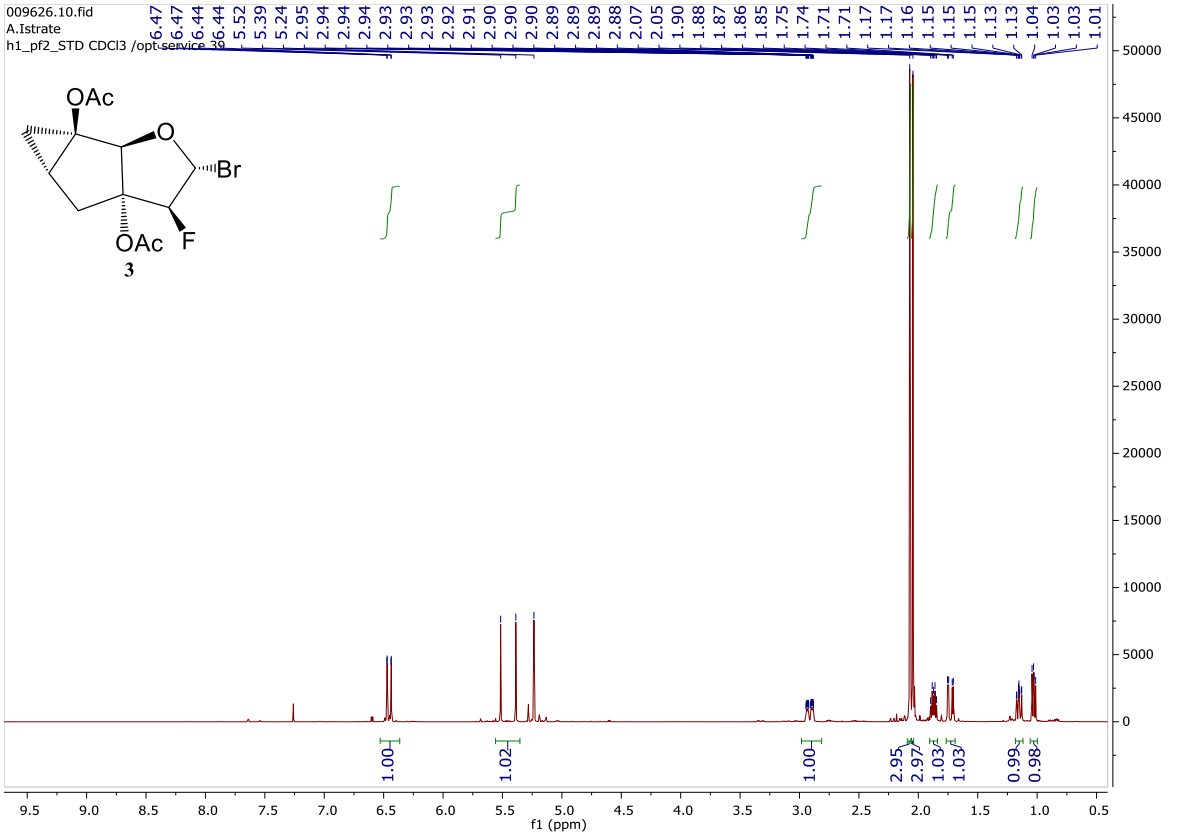
TLC (hexane/EtOAc 1:1) $R_f$ = 0.76.

$^1$H NMR ($CDCl_3$, 400 MHz): δ 6.45 (*dd*, *J* = 13.8 Hz, *J* = 0.9 Hz, 1H, H-C(8)), 5.45 (*d*, *J* = 51.1 Hz, 1H, H-C(7)), 5.24 (*s*, 1H, H-C(1)), 2.91 (*dddd*, *J* = 15.8 Hz, *J* = 5.6 Hz, *J* = 2.6 Hz, *J* = 1.3 Hz, 1H, H-C(5)), 2.07 (*s*, 3H, C*H*$_3$CO), 2.05 (*s*, 3H, C*H*$_3$CO), 1.90 – 1.84 (*m*, 1H, H-C(4)), 1.73 (*dd*, *J* = 15.8 Hz, *J* = 3.7 Hz, 1H, H-C(5)), 1.15 (*ddd*, *J* = 9.3 Hz, *J* = 7.0 Hz, *J* = 1.4 Hz, 1H, H-C(3)), 1.03 (*dd*, *J* = 7.0 Hz, *J* = 5.1 Hz, 1H, H-C(3)).

$^{13}$C NMR ($CDCl_3$, 100 MHz): δ 170.78 ($CH_3$*C*O), 169.75 ($CH_3$*C*O), 103.00 (*d*, *J* = 204.0 Hz, C(8)), 93.56 (*d*, *J* = 20.4 Hz, C(6)), 90.13 (C(1)), 89.24 (*d*, *J* = 36.9 Hz, C(7)), 63.53 (C(2)), 31.09 (*d*, *J* = 12.2 Hz, C(5)), 22.67 (C(4)), 21.52 (*C*$H_3$CO), 20.76 (*C*$H_3$CO), 17.29 (C(3)).

$^{19}$F NMR ($CDCl_3$, 376.5 MHz) δ -165.14 (*dd*, *J* = 51.1 Hz, *J* = 13.9 Hz).

ESI$^+$-HRMS: calculated for $C_{12}H_{14}O_5BrFNa$ ([M+Na]$^+$) 358.9889, found 358.9901.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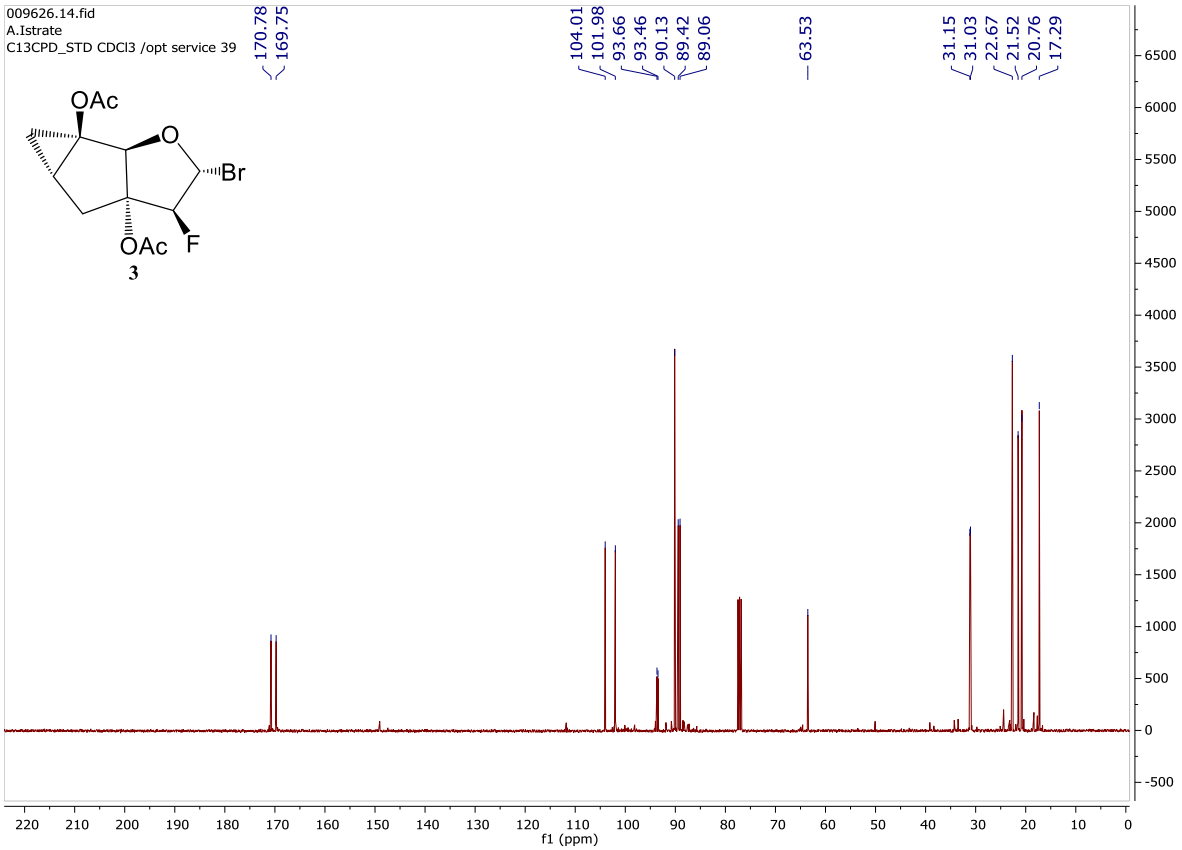
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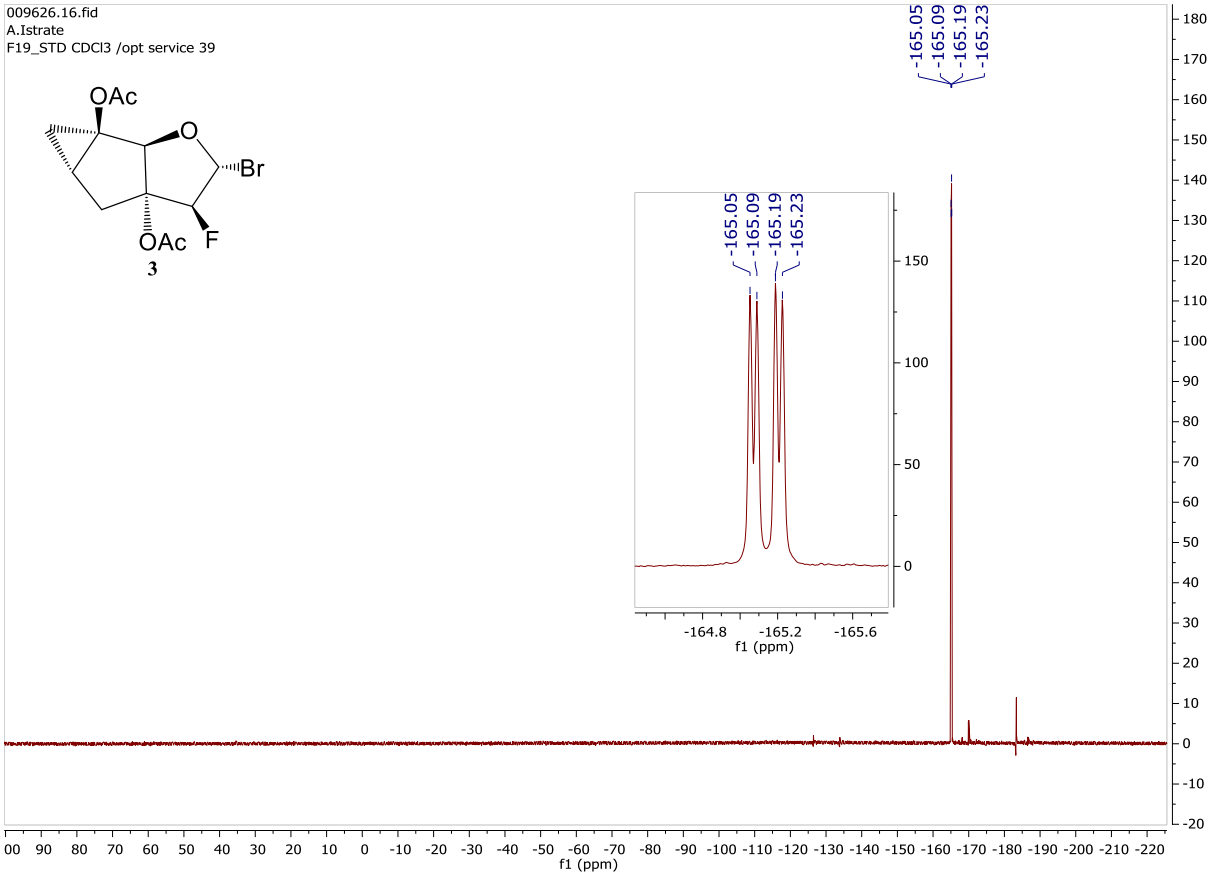
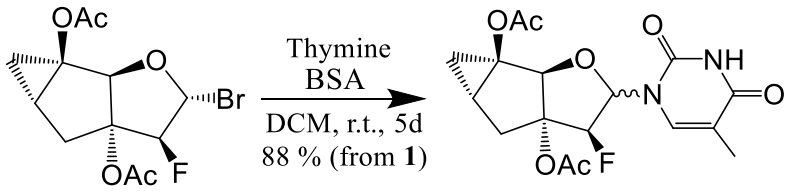



## 2.2 1-[(5'R,6'R)-3',5'-di-O-acetyl-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-β-D-arabinofuranosyl]thymine and 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-α-D-arabinofuranosyl]thymine (4)




**3** **4**

A suspension of thymine (0.6 g, 4.8 mmol, 2 eq.) in dry $CH_2Cl_2$ (20 ml) was treated with BSA (2.36 ml, 9.7 mmol, 4 eq.) and stirred at reflux for 30 min. The resulting clear solution was allowed to cool to room temperature. The compound **3** (0.810 g, 2.4 mmol, 1 eq.) in dry $CH_2Cl_2$ (8 ml) was added and the reaction mixture was stirred at r.t. for 5 days. Subsequently, the reaction mixture was washed with saturated $NaHCO_3$ solution (20 ml) and aqueous phase was extracted with EtOAc (3 x 20 ml). The combined organic phases were dried over $MgSO_4$, filtrated, evaporated and the crude product purified by CC (hexane/EtOAc 1:1) to give the product as a mixture of α and β anomers (811 mg, 88% from **1**) with an α/β ratio of ~1:2.5 according to NMR.

## Analytical data of 4:

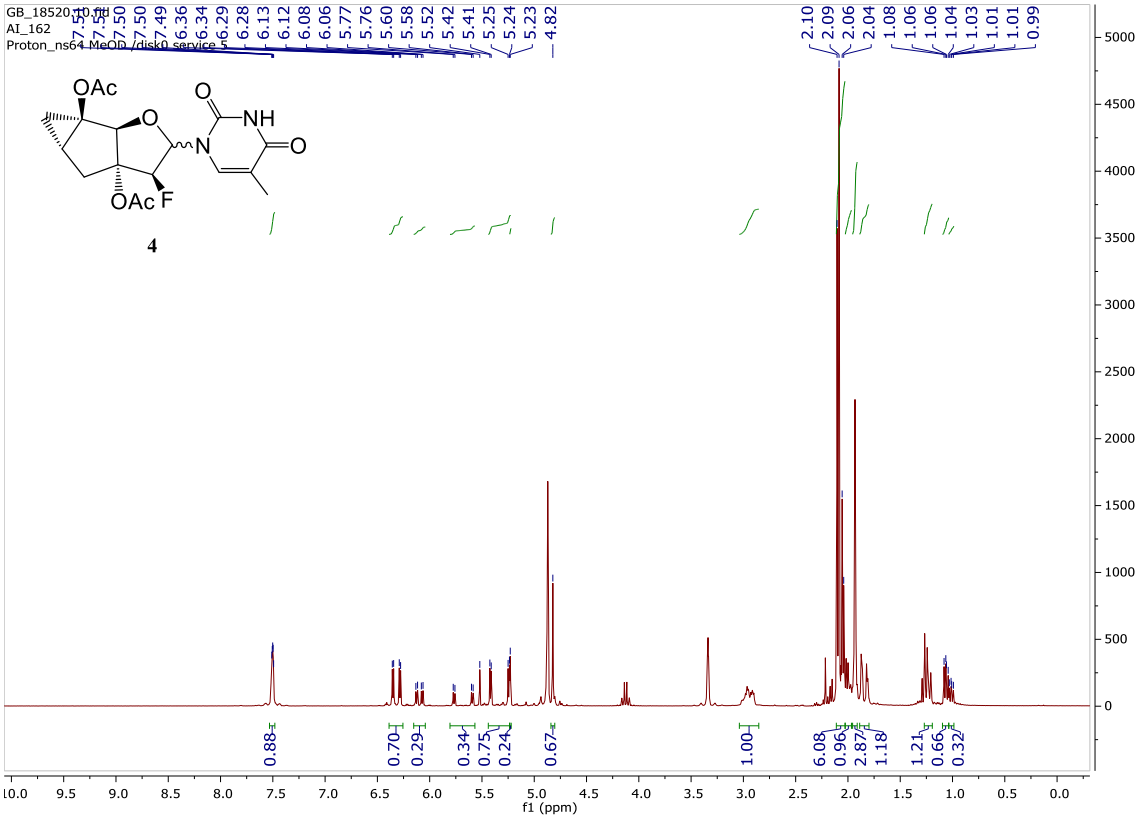
TLC (hexane/EtOAc 1:1) $R_f$ = 0.28.

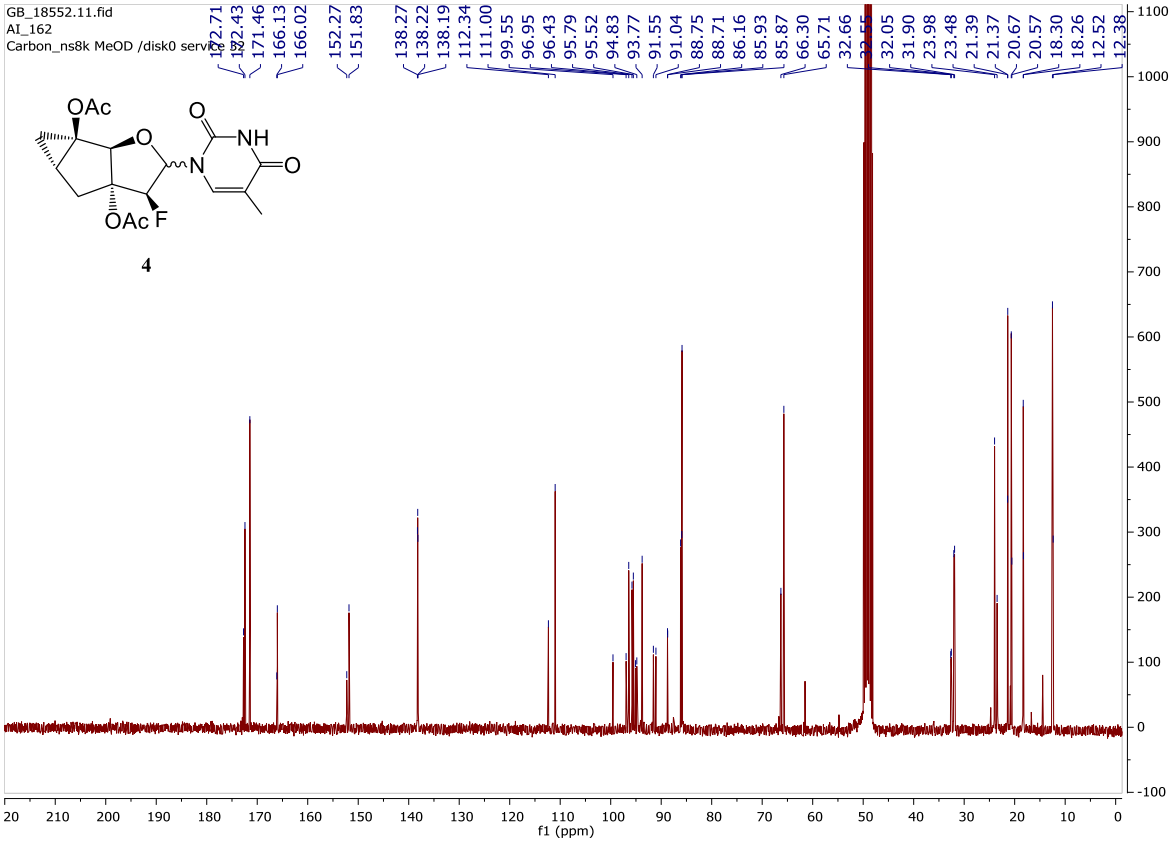
$^1$H NMR ($CD_3OD$, 300 MHz): δ 7.53 – 7.48 (*m*, 1H, H-C(6)), 6.32 (*dd*, *J* = 19.9 Hz, *J* = 3.8 Hz, 0.7H, H-C(1')), 6.10 (*dd*, *J* = 15.7 Hz, *J* = 4.9 Hz, 0.3H, H-C(1')), 5.68 (*dd*, *J* = 52.3 Hz, *J* = 4.8 Hz, 0.3H, H-C(2')), 5.33 (*dd*, *J* = 52.3 Hz, *J* = 3.9 Hz, 0.7H, H-C(2')), 5.23 (*s*, 0.3H, H-C(4')), 4.82 (*s*, 0.7H, H-C(4')), 3.03 – 2.88 (*m*, 1H, H-C(7')), 2.10, 2.09, 2.06, 2.04 (4*s*, 6H, C*H*$_3$CO), 1.95 – 1.92 (*m*, 3H, C*H*$_3$-C(5)), 2.04 – 1.97 (*m*, 1H, H-C(6')), 1.27 – 1.20 (*m*, 1H, H-C(7')), 1.06 (*dd*, *J* = 6.8 Hz, *J* = 5.1 Hz, 0.7H, H-C(8')), 1.01 (*dd*, *J* = 6.8 Hz, *J* = 5.0 Hz, 0.3H, H-C(8')).

$^{13}$C NMR ($CD_3OD$, 76 MHz): δ 172.71, 172.43, 171.46 ($CH_3CO$), 166.13, 166.02 (C(4)), 152.27, 151.83 (C(2)), 138.27, 138.22, 138.19 (C(6)), 112.34, 111.00 (C(5)), 98.25 (*d*, *J* = 195.9 Hz, C(2')), 95.65 (*d*, *J* = 20.5 Hz, C(3')), 95.10 (*d*, *J* = 200.3 Hz, C(2')), 94.95 (*d*, *J* = 18.9 Hz, C(3')), 91.29 (*d*, *J* = 38.1 Hz, C(1')), 88.73 (*d*, *J* = 2.9 Hz, C(4')), 86.04 (*d*, *J* = 17.7 Hz, C(1')), 85.87 (C(4')), 66.30, 65.71 (C(5')), 32.60 (*d*, *J* = 8.8 Hz, C(7')), 31.98 (*d*, *J* = 11.5 Hz, C(7')), 23.99, 23.48 (C(6')), 21.39, 21.37 (*C*H$_3$CO), 20.67, 20.57 (*C*H$_3$CO), 18.30, 18.26 (C(8')), 12.52, 12.38 (C(7)).

$^{19}$F NMR ($CD_3OD$, 376.5 MHz) δ -194.88 (*dd*, *J* = 52.3 Hz, *J* = 15.5 Hz), -183.72 (*dd*, *J* = 52.4 Hz, *J* = 19.7 Hz).

ESI$^+$-HRMS: calculated for $C_{17}H_{20}O_7N_2F$ ([M+H]$^+$) 383.1249, found 383.1248.

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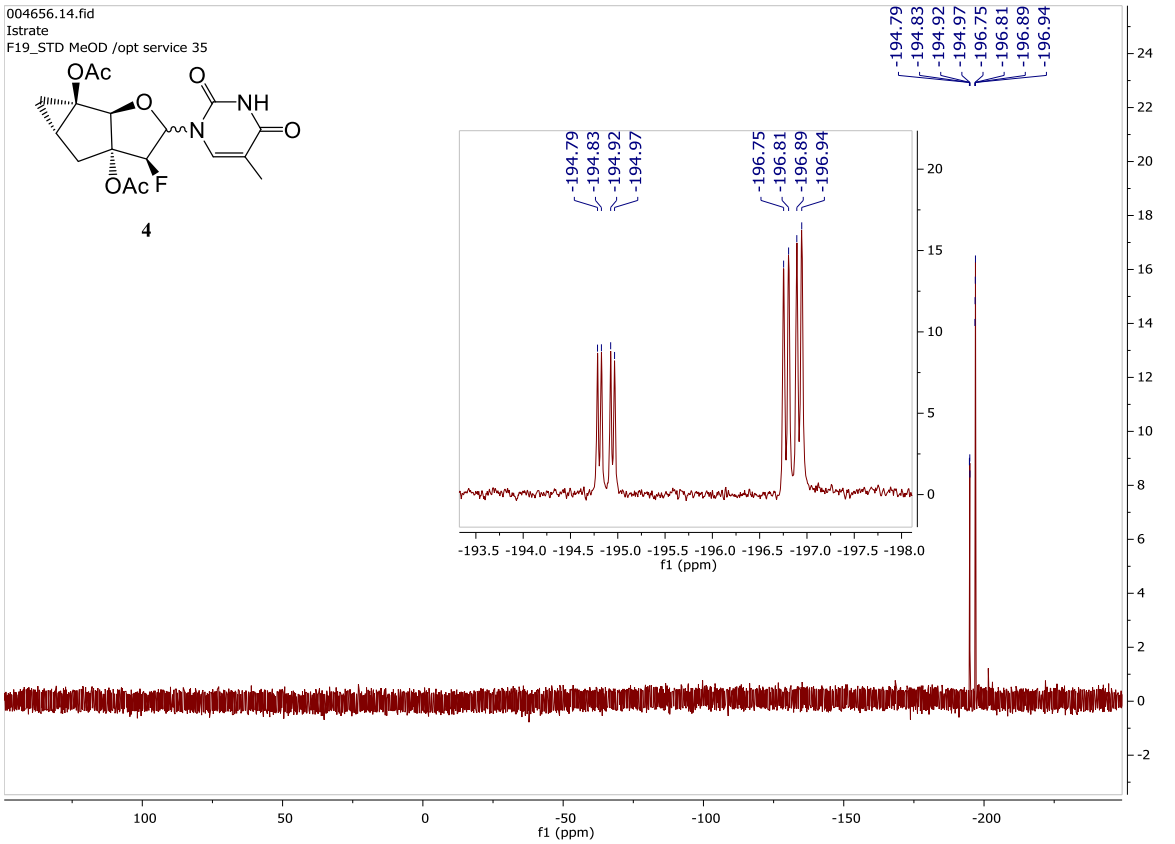
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O
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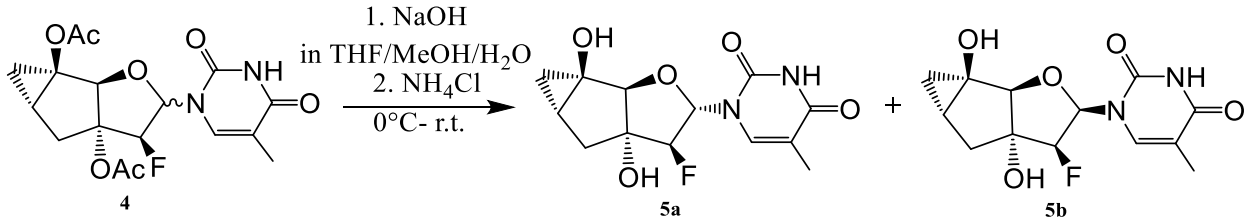
f1 (ppm)

## 2.3 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-α-D-arabinofuranosyl]thymine and 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-β-D-arabinofuranosyl]thymine (5a and 5b)




A solution of acetyl-protected nucleosides **4** (0.860 g, 2.25 mmol, 1 eq.) in 0.2 M NaOH in THF/MeOH/$H_2O$ 5:4:1 (45 ml, 9 mmol of NaOH, 4 eq.) was stirred at 0°C for 1 h. Then $NH_4Cl$ (0.6 g, 11.25 mmol, 5 eq.) was added and the reaction mixture was allowed to stir at r.t. for 20 min. Evaporation followed by purification of the adsorbed material by CC ($CH_2Cl_2$/MeOH 95:5) afforded free nucleosides **5a** (177 mg, 26%) and **5b** (445 mg, 66%) both as white foams.

**Analytical data of 5a:**

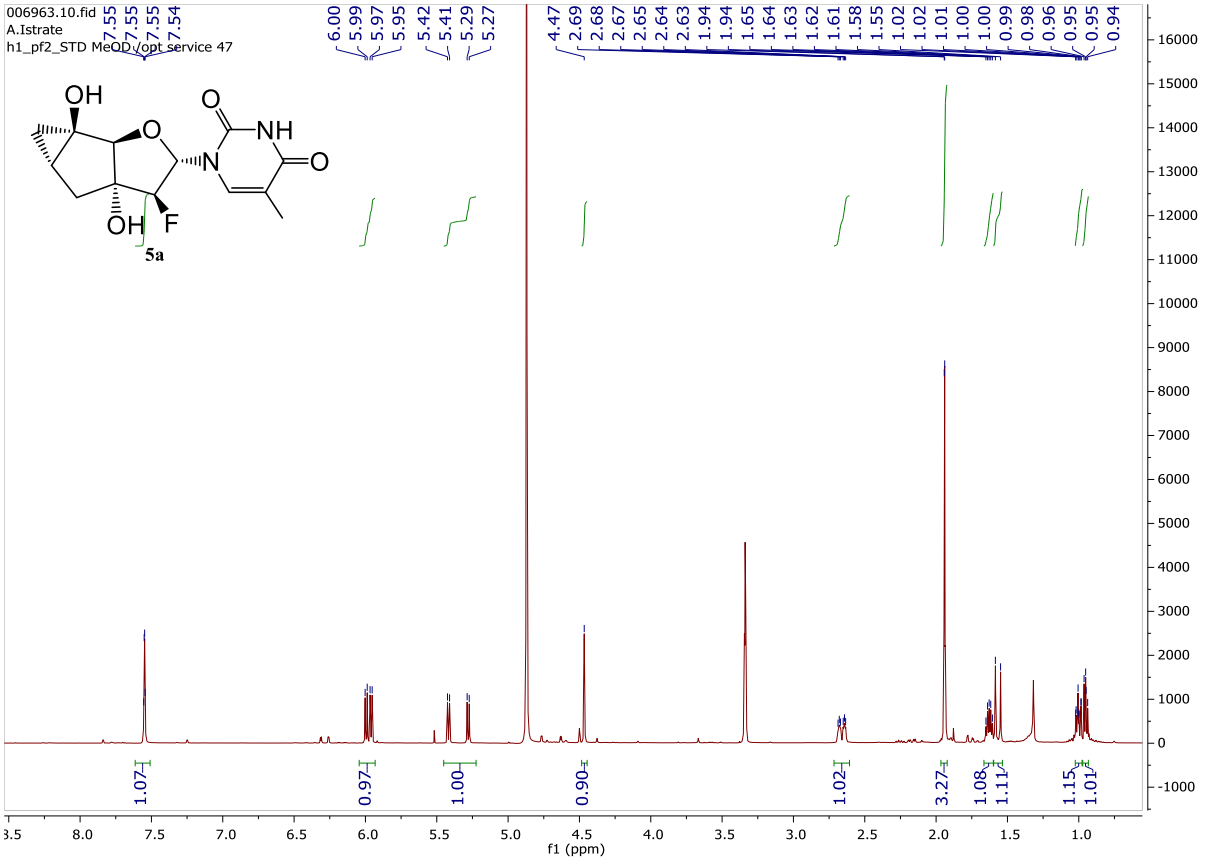
TLC (DCM/MeOH 95:5) $R_f$ = 0.15.

$^1$H NMR ($CD_3OD$, 400 MHz): δ 7.55 (*q*, *J* = 1.3 Hz, 1H, H-C(6)), 5.98 (*dd*, *J* = 13.9 Hz, *J* = 5.8 Hz, 1H, H-C(1')), 5.35 (*dd*, *J* = 55.2 Hz, *J* = 5.8 Hz, 1H, H-C(2')), 4.47 (*s*, 1H, H-C(4')), 2.66 (*dt*, *J* = 14.5 Hz, *J* = 3.3 Hz, 1H, H-C(7')), 1.94 (*d*, *J* = 1.2 Hz, 3H, C*H*$_3$-C(5)), 1.63 (*dt*, *J* = 9.2 Hz, *J* = 4.6 Hz, 1H, H-C(6')), 1.57 (*d*, *J* = 14.5 Hz, 1H, H-C(7')), 1.05 – 0.96 (*m*, 1H, H-C(8')), 0.95 (*dd*, *J* = 5.7 Hz, *J* = 4.4 Hz, 1H, H-C(8')).

$^{13}$C NMR ($CD_3OD$, 100 MHz): δ 166.26 (C(4)), 152.40 (C(2)), 138.74 (C(6)), 112.20 (C(5)), 101.84 (*d*, *J* = 194.0 Hz, C(2')), 89.98, 89.93, 89.86, 89.52 (C(4'), C(1')), 86.47 (*d*, *J* = 19.0 Hz, C(3')), 65.50 (C(5')), 35.20 (*d*, *J* = 7.2 Hz, C(7')), 24.78 (C(6')), 16.86 (C(8')), 12.33 (C(7)).

$^{19}$F NMR ($CD_3OD$, 376.5 MHz) δ -207.29 (*dd*, *J* = 55.1 Hz, *J* = 13.4 Hz).

ESI$^+$-HRMS: calculated for $C_{13}H_{16}O_5N_2F$ ($[M+H]^+$) 299.1038, found 299.1042.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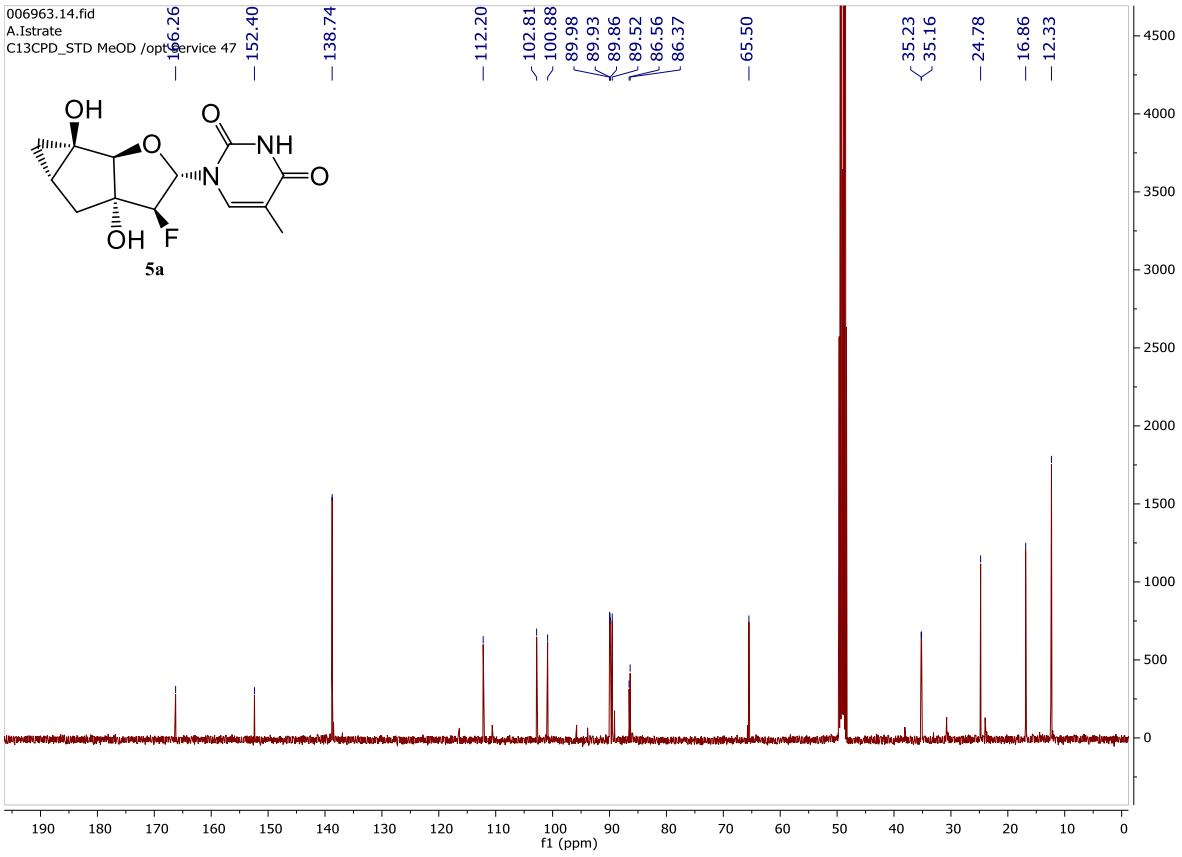
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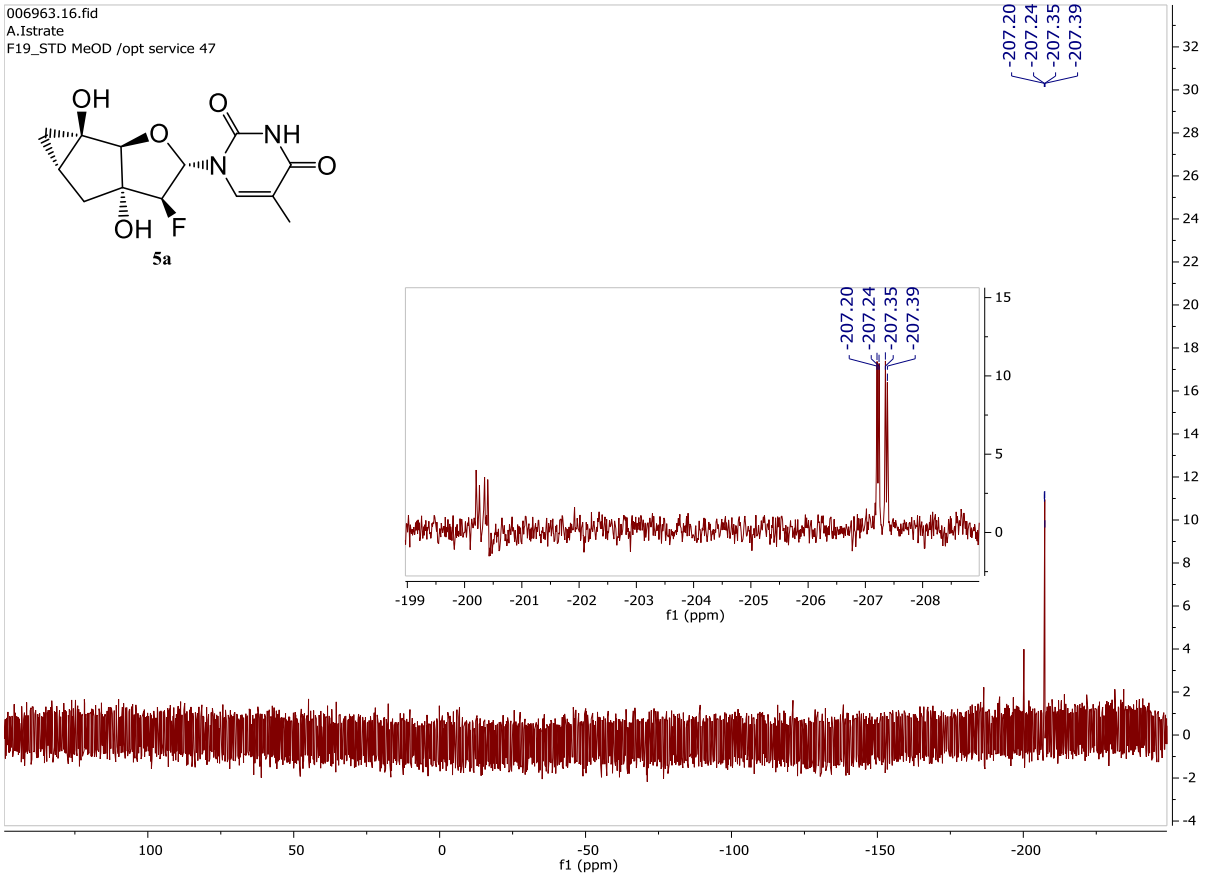



## Analytical data of 5b:

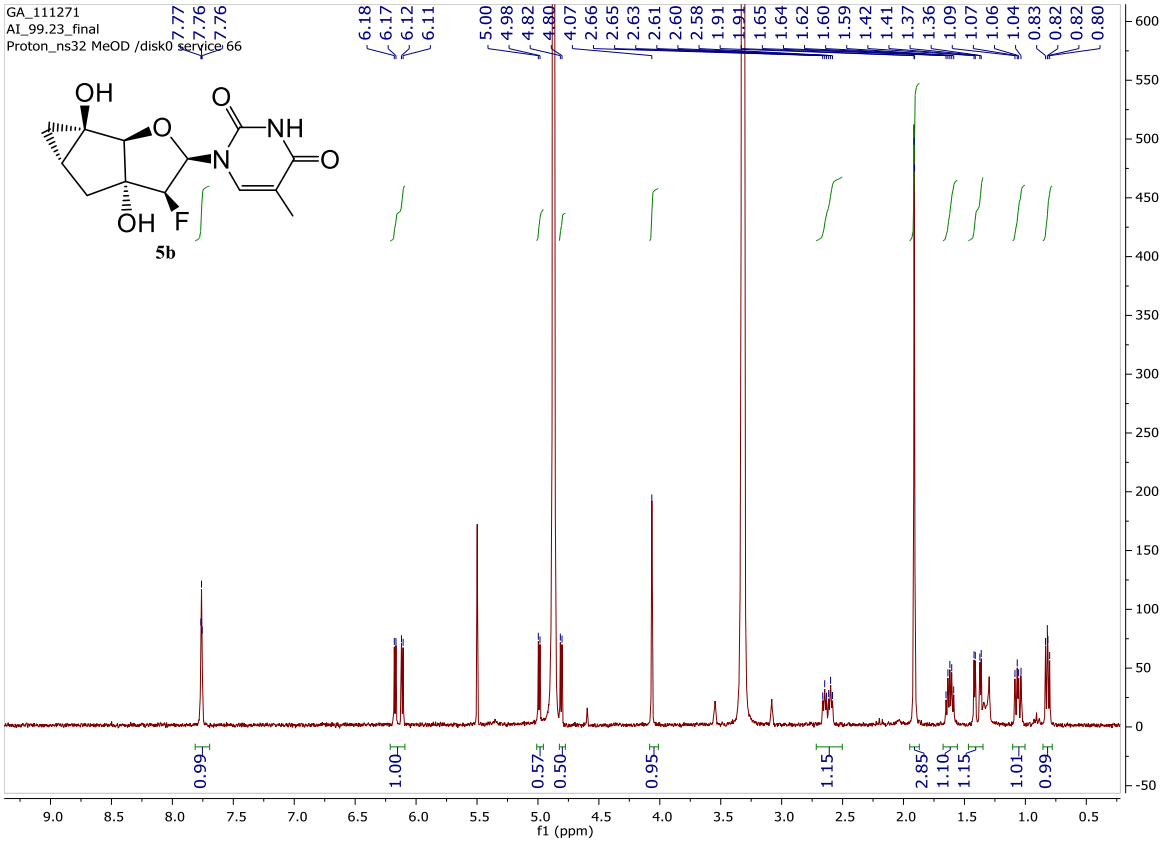
TLC (DCM/MeOH 95:5) $R_f$ = 0.13.

$^{1}$H NMR ($CD_3OD$, 300 MHz): δ 7.78 – 7.74 (*m*, 1H, H-C(6)), 6.14 (*dd*, *J* = 17.6 Hz, *J* = 4.1 Hz, 1H, H-C(1')), 4.90 (*dd*, *J* = 54.2 Hz, *J* = 4.1 Hz, 1H, H-C(2')), 4.07 (*s*, 1H, H-C(4')), 2.62 (*dt*, *J* = 14.6 Hz, *J* = 4.8 Hz, 1H, H-C(7')), 1.91 (*d*, *J* = 1.2 Hz, 3H, C*H*$_3$-C(5)), 1.62 (*dt*, *J* = 9.4 Hz, *J* = 4.8 Hz, 1H, H-C(6')), 1.39 (*dd*, *J* = 14.5 Hz, *J* = 3.1 Hz, 1H, H-C(7')), 1.06 (*dd*, *J* = 9.2 Hz, *J* = 5.8 Hz, 1H, H-C(8')), 0.82 (*dd*, *J* = 5.7 Hz, *J* = 4.4 Hz, 1H, H-C(8')).

$^{13}$C NMR ($CD_3OD$, 76 MHz): δ 166.33 (C(4)), 151.93 (C(2)), 138.75 (*d*, *J* = 2.5 Hz, C(6)), 110.54 (C(5)), 99.34 (*d*, *J* = 200.9 Hz, C(2')), 89.52 (C(4')), 89.11 (*d*, *J* = 19.3 Hz, C(3')), 85.79 (*d*, *J* = 17.9 Hz, C(1')), 64.10 (C(5')), 34.79 (*d*, *J* = 10.6 Hz, C(7')), 25.66 (C(6')), 18.42 (C(8')), 12.36 (C(7)).

$^{19}$F NMR ($CD_3OD$, 376.5 MHz) δ -198.17 (*dd*, *J* = 54.0 Hz, *J* = 17.7 Hz).

ESI$^+$-HRMS: calculated for $C_{13}H_{16}O_5N_2F$ ($[M+H]^+$) 299.1038, found 299.1044.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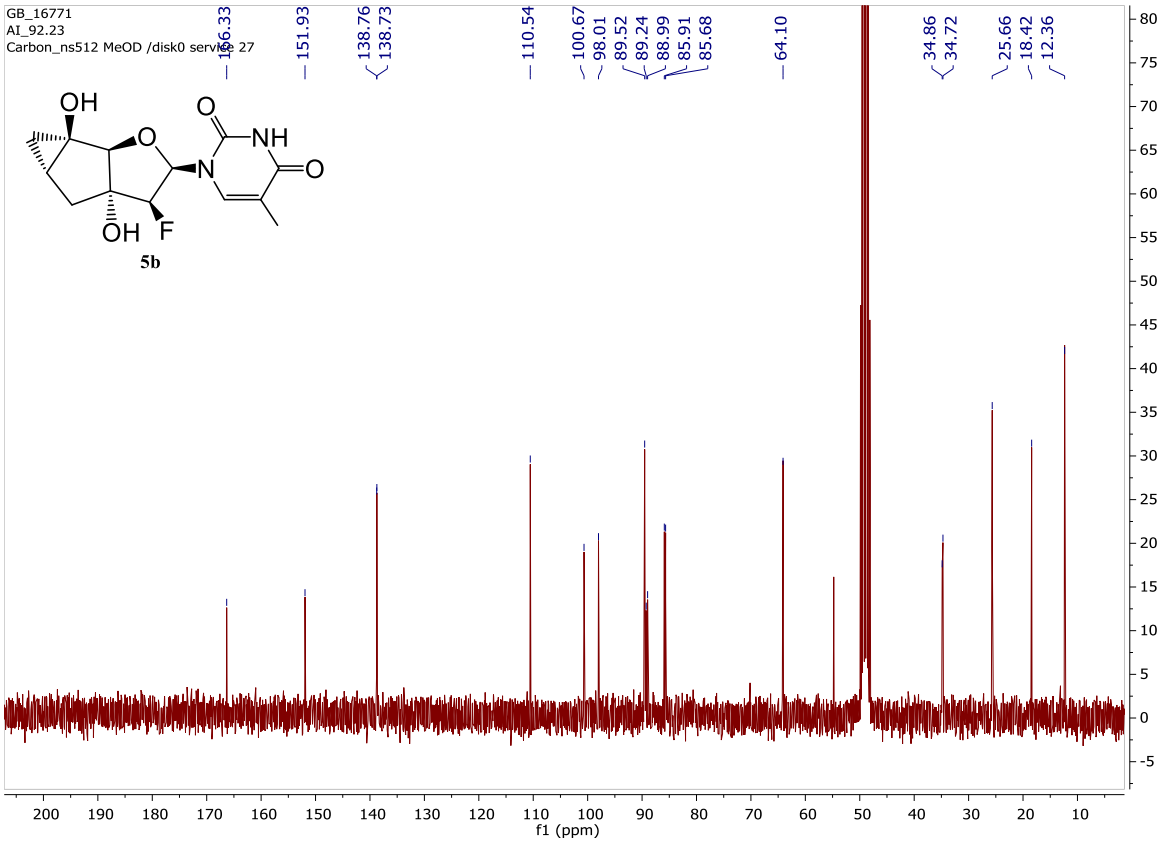
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O
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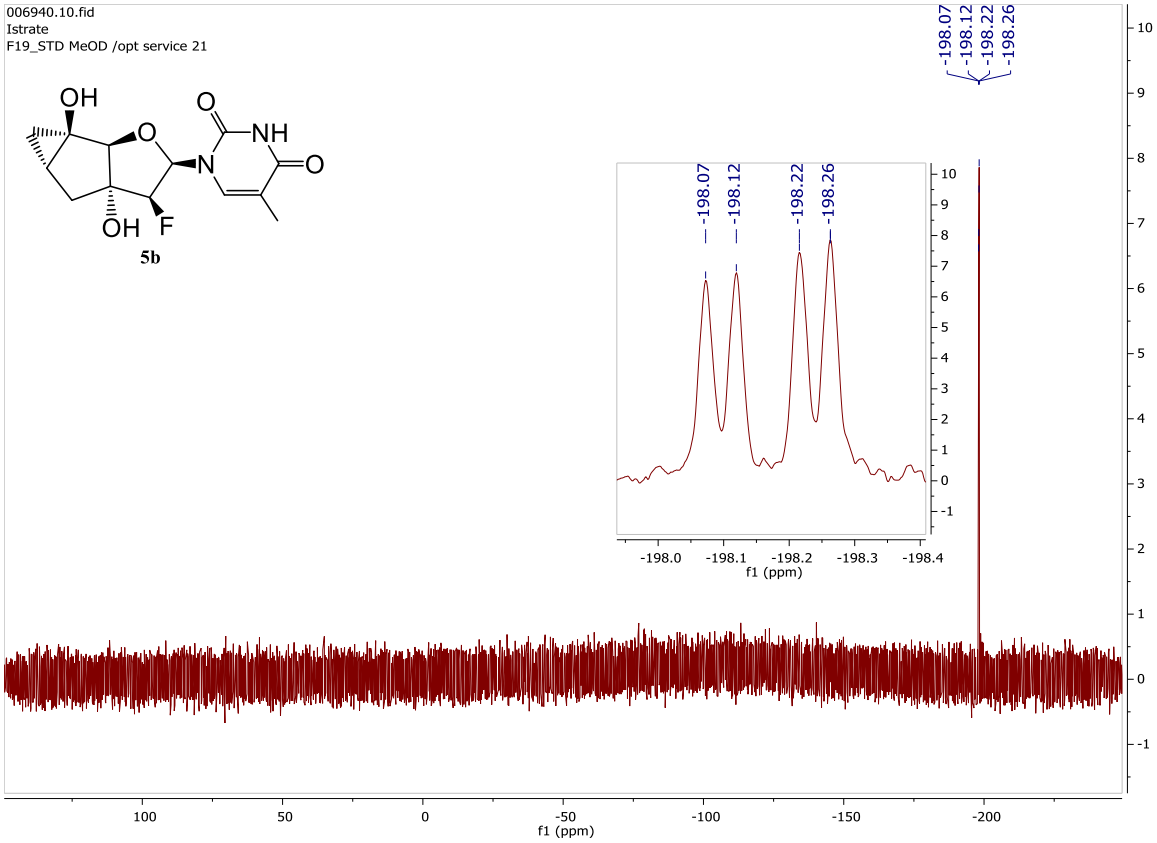
F
5b
f1 (ppm)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f1 (ppm)
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50
0
-50
-100
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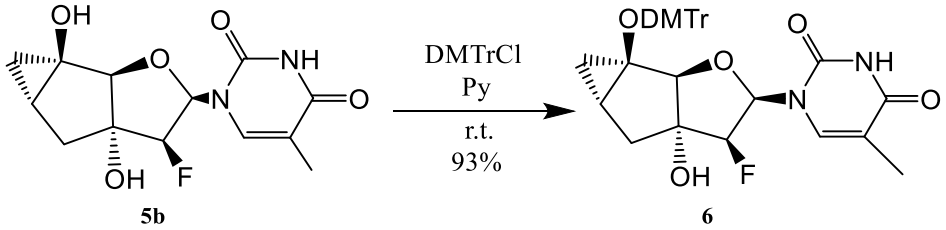
-200
f1 (ppm)

## 2.4 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-5'-O-((4,4'-dimethoxytriphenyl)methyl)-β-D-arabinofuranosyl]thymine (6)




**5b** **6**

To a solution of nucleoside **6b** (215 mg, 0.72 mmol, 1 eq.) in 3 ml dry pyridine DMTr-Cl (733 mg, 2.16 mmol, 3 eq.) was added in three equal portions over 5 h. The resulting orange solution was stirred overnight before washing with sat. $NaHCO_3$ solution (2 x 10 ml). Subsequently, the aqueous phase was extracted with $CH_2Cl_2$ (3 x 20 ml). The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude orange foam was purified by CC (hexane/EtOAc 1:1 +0.5% $Et_3N$, then hexane/EtOAc 1:4) to yield DMT-protected nucleoside **6** (403 mg, 93%) as a white foam.

## Analytical data of 6:

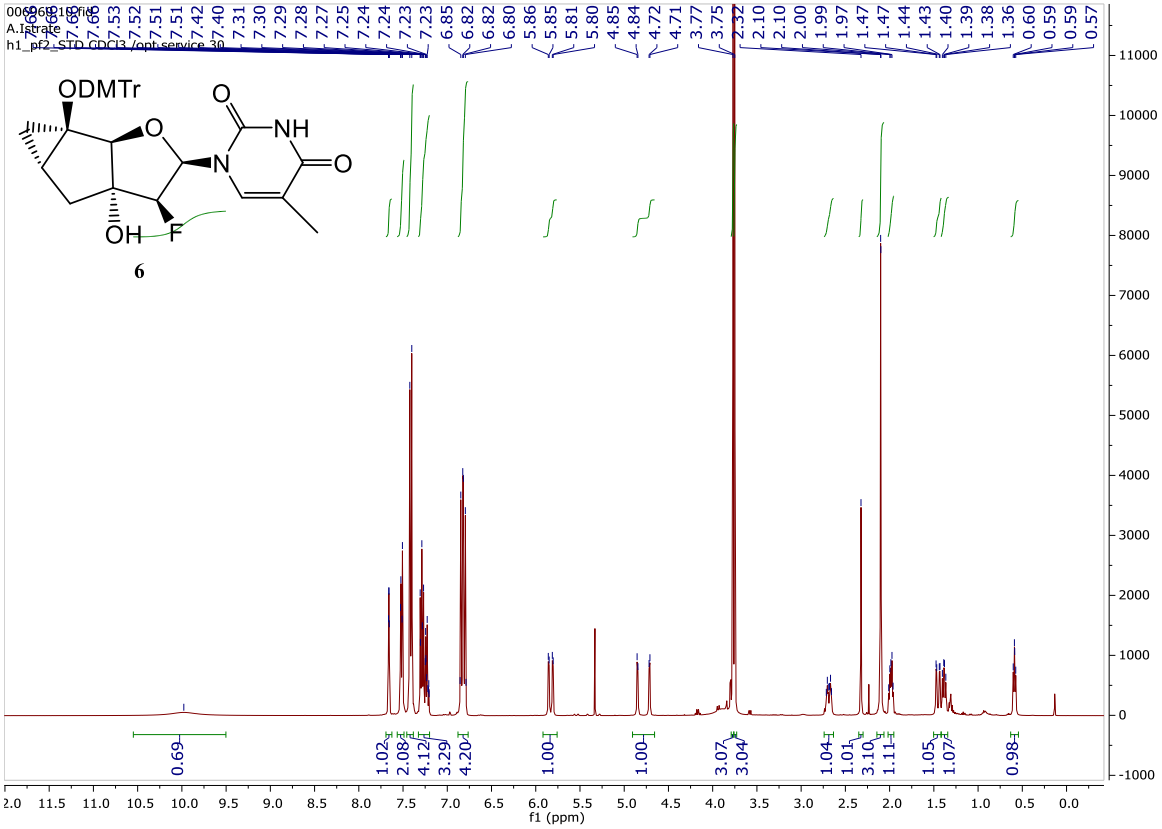
TLC (hexane/EtOAc 1:3) $R_f$ = 0.73.

$^1$H NMR ($CDCl_3$, 400 MHz): δ 9.98 (*br*, 1H, H-N(3)), 7.68 – 7.64 (*m*, 1H, H-C(6)), 7.56 – 7.48 (*m*, 2H, H-arom), 7.45 – 7.37 (*m*, 4H, H-arom), 7.33 – 7.20 (*m*, 3H, H-arom), 6.86 – 6.78 (*m*, 4H, H-arom), 5.83 (*dd*, *J* = 18.3 Hz, *J* = 3.8 Hz, 1H, H-C(1')), 4.78 (*d*, *J* = 54.2 Hz, *J* = 3.8 Hz, 1H, H-C(2')), 3.77, 3.75 (2*s*, 6H, *Me*O), 2.69 (*dt*, *J* = 14.5 Hz, *J* = 4.5 Hz, 1H, H-C(7')), 2.32 (*s*, 1H, H-C(4')), 2.10 (*d*, *J* = 0.9 Hz, 3H, C*H*$_3$-C(5)), 1.99 (*dt*, *J* = 9.9 Hz, *J* = 5.0 Hz, 1H, H-C(6')), 1.45 (*dd*, *J* = 14.7 Hz, *J* = 3.0 Hz, 1H, H-C(7')), 1.38 (*dd*, *J* = 9.3 Hz, *J* = 6.2 Hz, 1H, H-C(8')), 0.64 – 0.55 (*m*, 1H, H-C(8')).

$^{13}$C NMR ($CDCl_3$, 100 MHz): δ 164.04 (C(4)), 158.93, 158.86 (MeO-*C*-arom), 150.74 (C(2)), 146.45, 136.93, 136.88 (C-arom), 136.88 (C(6)), 131.20, 131.08, 128.55, 127.66, 126.98, 112.88, 112.80 (*C*H-arom), 110.73 (C(5)), 97.56 (*d*, *J* = 202.8 Hz, C(2')), 88.75 (*d*, *J* = 19.0 Hz, C(3')), 88.02 (C(4')), 87.83 (*C*(Ph)$_3$), 83.69 (*d*, *J* = 18.0 Hz, C(1')), 66.67 (C(5')), 55.38 (*Me*O-DMTr), 32.82 (*d*, *J* = 11.8 Hz, C(7')), 25.41 (C(6')), 18.24 (C(8')), 12.71 (C(7)).

$^{19}$F NMR ($CDCl_3$, 376.5 MHz) δ -194.66 (*dd*, *J* = 54.3 Hz, *J* = 18.3 Hz).

ESI$^+$-HRMS: calculated for $C_{34}H_{33}O_7N_2FNa$ ([M+Na]$^+$) 623.2164, found 623.2187.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N
O
OH
F
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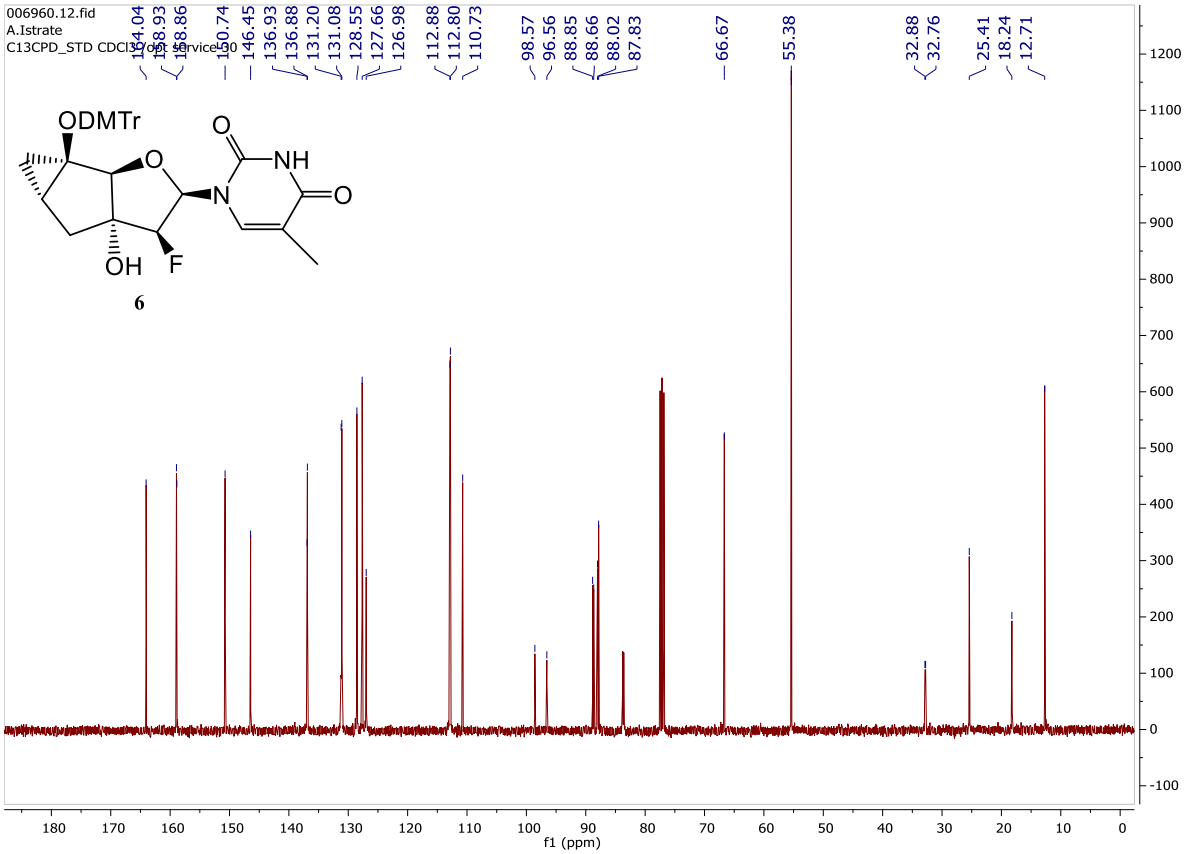
f1 (ppm)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NH
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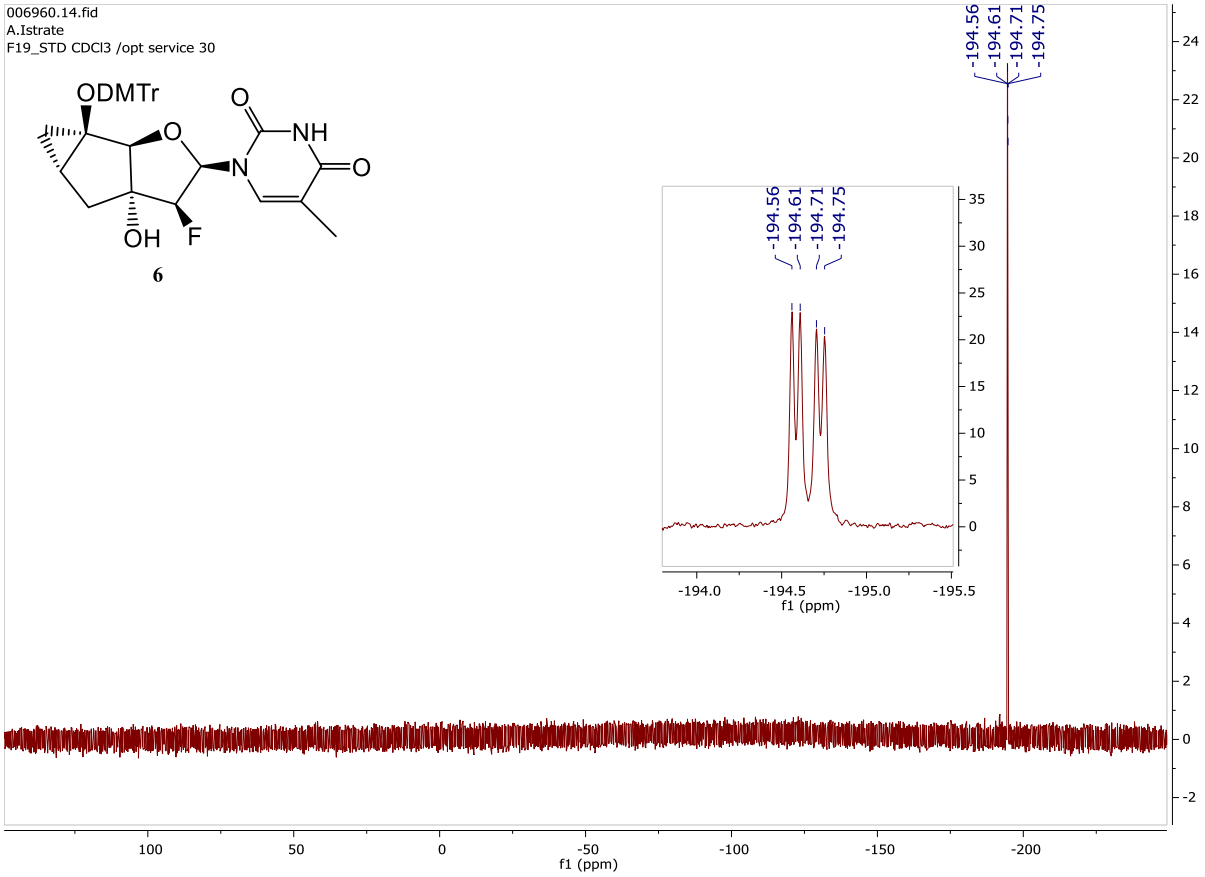
F
6
f1 (ppm)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NH
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O
OH
F
6
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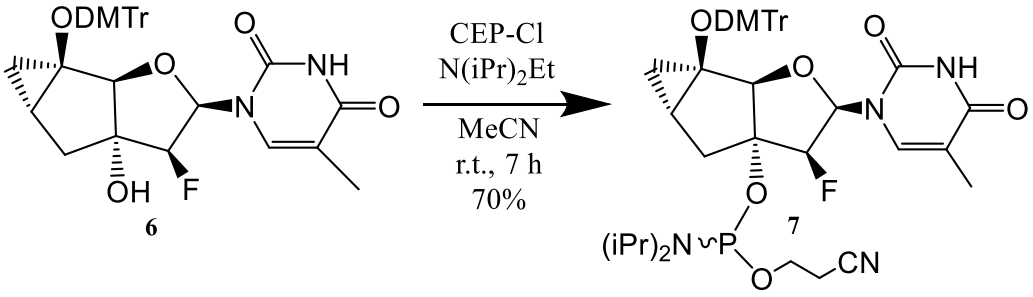
-194.61
-194.71
-194.75
f1 (ppm)
f1 (ppm)

## 2.5 1-[(5'R,6'R)-3'-O-(2-cyanoethoxy)-diisopropylaminophosphanyl-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-5'-O-((4,4'-dimethoxytriphenyl)methyl)-β-D-arabinofuranosyl]thymine (7)




To a solution of DMT-protected nucleoside **6** (313 mg, 0.52 mmol, 1 eq.) and N-ethyldiisopropylamine (0.36 ml, 2.08 mmol, 4 eq.) in dry $CH_3CN$ (10 ml) was slowly added CEP-Cl (0.23 ml, 1.04 mmol, 2 eq.) and the reaction mixture was allowed to stir at r.t. for 3 h. After that an additional portion of CEP-Cl (0.06 ml, 0.27 mmol, 0.5 eq.) was added. As monitored by TLC analysis, reaction was complete after 4 h at r.t. The reaction mixture was diluted with EtOAc (15 ml) then washed with sat. $NaHCO_3$ (2 x 15 ml). The aqueous phase was extracted with EtOAc (3 x 40 ml) and the combined organic phases were dried over $MgSO_4$, filtered and evaporated. The crude yellow oil was purified by CC using hexane/EtOAc (1:1 + 0.5% $Et_3N$) to afford the phosphoramidite **7** (292 mg of a white foam, 70%) as a mixture of isomers.

**Analytical data of 7:**

TLC (hexane/EtOAc 1:4) $R_f$ = 0.81.

$^1$H NMR ($CDCl_3$, 400 MHz): δ 9.91 (*br*, 1H, H-N(3)), 7.67 (*d*, *J* = 1.5 Hz, 1H, H-C(6)), 7.52 – 7.43 (*m*, 2H, H-arom), 7.43 – 7.33 (*m*, 4H, H-arom), 7.30 – 7.19 (*m*, 3H, H-arom), 6.86 – 6.75 (*m*, 4H, H-arom), 5.90 – 5.79 (*m*, 1H, H-C(1')), 5.26 – 5.09 (*m*, 1H, H-C(2')), 3.80, 3.79, 3.78 (3*s*, 6H, *Me*O), 3.76 – 3.67, 3.64 – 3.53 (2*m*, 2H, OC*H*$_2$CH$_2$CN), 3.53 – 3.42 (*m*, 2H, (Me$_2$C*H*)$_2$N), 2.69 (*d*, *J* = 3.0 Hz, 0.5H, H-C(4')), 2.67 – 2.53 (*m*, 2.5H, OCH$_2$C*H*$_2$CN, H-C(7')), 2.48 (*dt*, *J* = 14.7 Hz, *J* = 4.3 Hz, 0.5H, H-C(7')), 2.39 (*s*, 0.5H, H-C(4')), 2.11 – 2.03 (*m*, 0.5H, H-C(7')), 2.07 (*s*, 3H, C*H*$_3$-C(5)), 1.97 – 1.88 (*m*, 1H, H-C(6')), 1.70 (*dd*, *J* = 14.9 Hz, *J* = 1.4 Hz, 0.5H, H-C(7')), 1.34 (*dd*, *J* = 9.3 Hz, *J* = 6.3 Hz, 1H, H-C(8')), 1.16 – 1.04 (*m*, 12H, (*Me*$_2$CH)$_2$N), 0.56 (*t*, *J* = 5.7 Hz, 1H, H-C(8')).

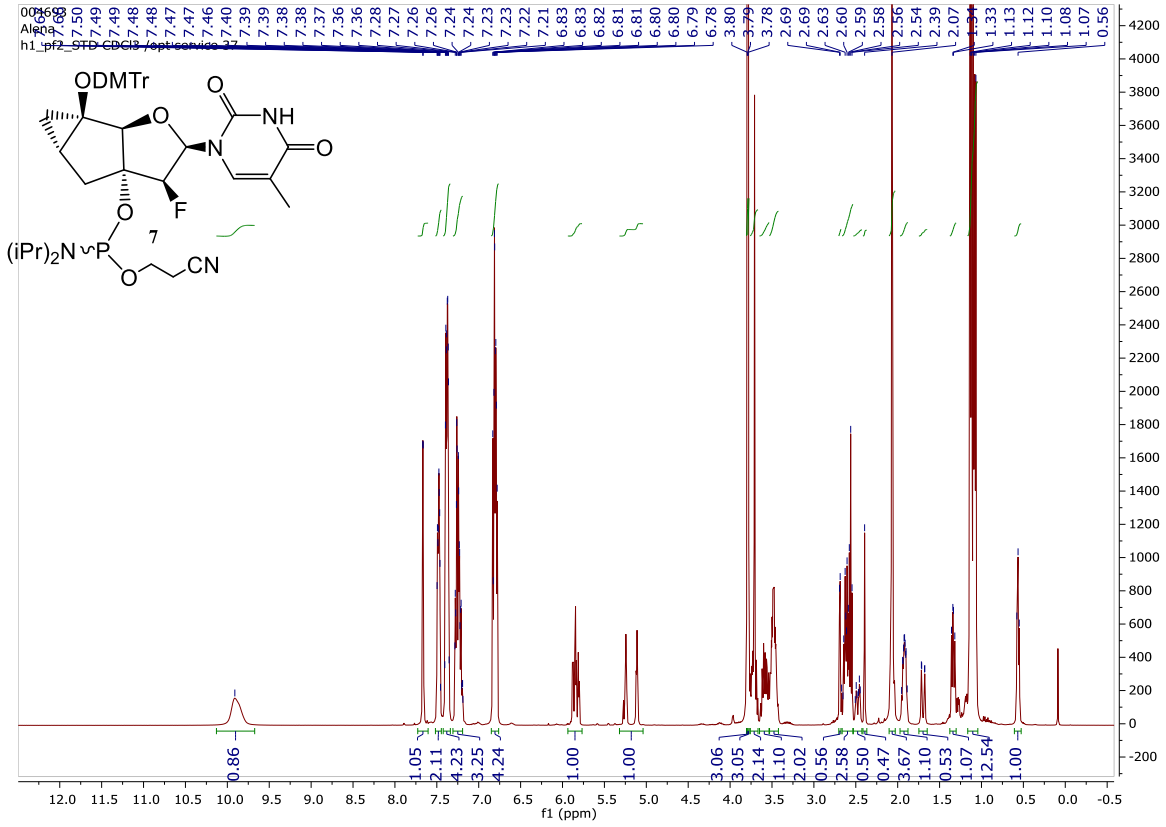
$^{13}$C NMR ($CDCl_3$, 100 MHz): δ 164.17 (C(4)), 158.83, 158.80, 158.77, 158.74 (MeO-*C*-arom), 150.54, 150.49 (C(2)), 146.22, 146.06, 136.84, 136.82, 139.79 (C-arom), 136.47, 136.30 (C(6)), 131.04, 130.98, 130.91, 130.84, 128.66, 128.55, 127.61, 127.04, 126.95 (*C*H-arom), 117.61, 117.49 (CN), 112.88, 112.84, 112.81 (*C*H-arom), 110.19 (C(5)), 95.66 (*dd*, *J*= 202.6 Hz, *J* = 5.4 Hz, C(2')), 95.41 (*dd*, *J*= 202.0 Hz, *J* = 7.0 Hz, C(2')), 92.37 (*dd*, *J* = 19.3 Hz, *J* = 3.8 Hz, C(3')), 91.56 (*dd*, *J* = 19.4 Hz, *J* = 3.9

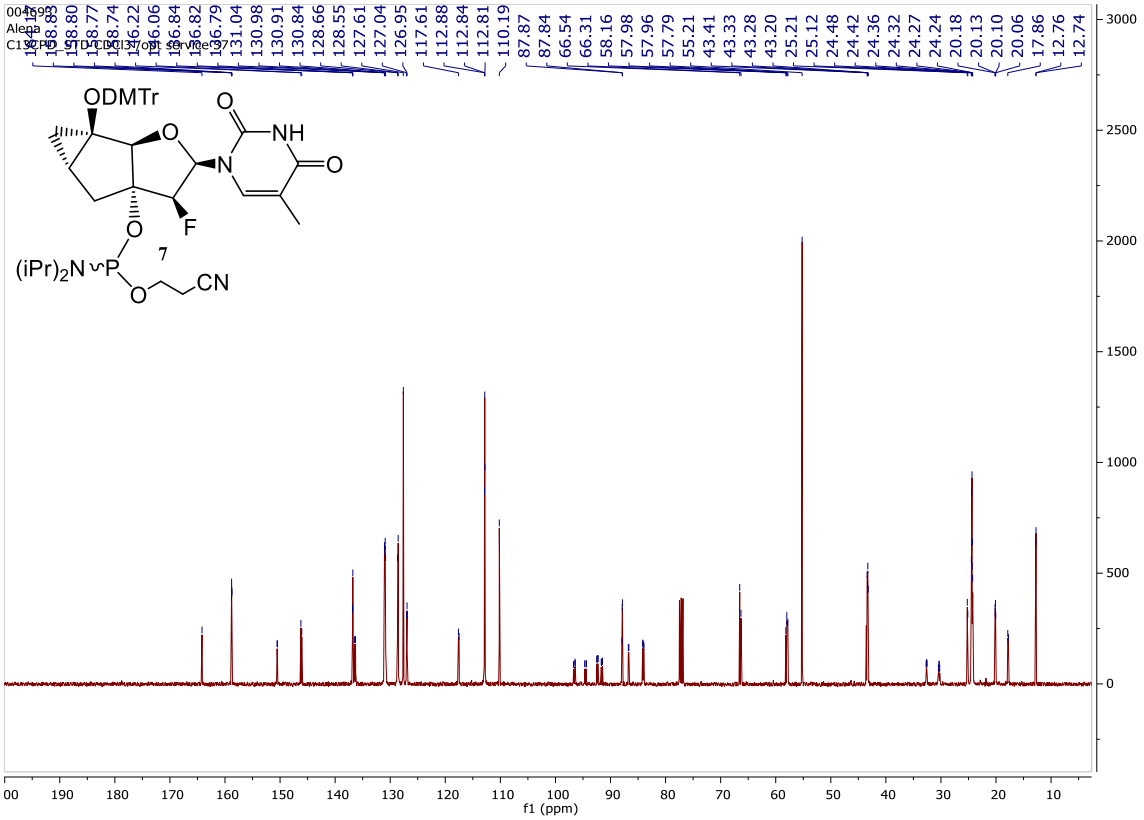
Hz, C(3')), 84.07 (*d*, *J* = 18.2 Hz, C(1')), 84.02 (*d*, *J* = 18.3 Hz, C(1')), 87.93 (C(4')), 87.87, 87.84 (*C*(Ph)$_3$), 86.71 (*d*, *J* = 9.5 Hz, C(4')), 66.54, 66.31 (C(5')), 58.16, 57.98, 57.96, 57.79 (O*C*H$_2$CH$_2$CN), 55.21 (*Me*O-DMTr), 43.34 (*d*, *J* = 12.7 Hz, (Me$_2$*C*H)$_2$N), 43.26 (*d*, *J* = 12.8 Hz, (Me$_2$*C*H)$_2$N), 32.61 (*dd*, *J* = 11.0 Hz, *J* = 6.0 Hz, C(7')), 30.57 – 30.12 (*m*, C(7')), 25.21, 25.12 (C(6')), 24.48, 24.42, 24.36, 24.32, 24.27, 24.24 ((*Me*$_2$CH)$_2$N), 20.14 (*d*, *J* = 8.0 Hz, OCH$_2$*C*H$_2$CN), 20.10 (*d*, *J* = 7.5 Hz, OCH$_2$*C*H$_2$CN), 17.86, 17.75 (C(8')), 12.76, 12.74 (C(7)).

$^{19}$F NMR (CDCl$_3$, 376.5 MHz) δ -195.23 (*dd*, *J* = 53.7 Hz, *J* = 15.2 Hz), -197.49 (*dd*, *J* = 52.7 Hz, *J* = 14.5 Hz).

$^{31}$P NMR (122 MHz, CDCl$_3$) δ 143.91 (*d*, *J* = 6.2 Hz), 142.88 (*d*, *J* = 3.9 Hz).

ESI$^+$-HRMS: calculated for C$_{43}$H$_{50}$O$_8$N$_4$FNaP ([M+Na]$^+$) 823.3243, found 823.3237.

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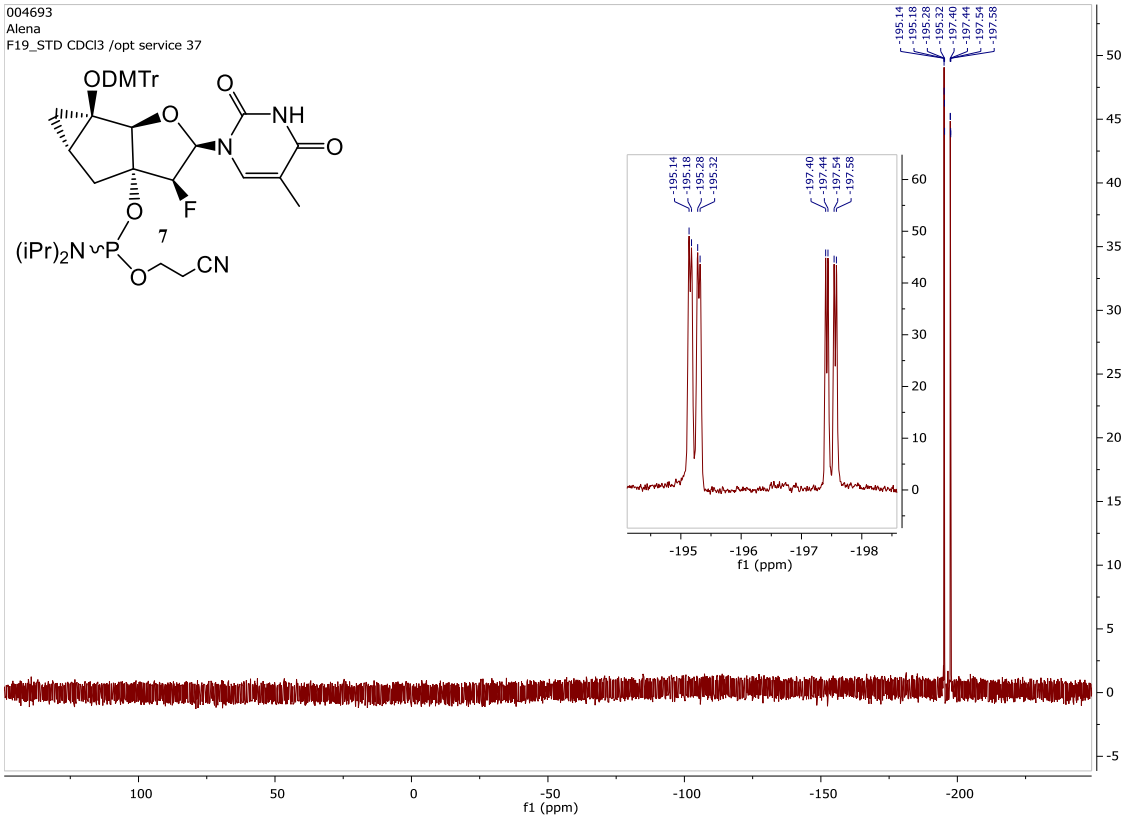
NH
N
O
O
F
O
7
(iPr)2N
P
O
CN
f1 (ppm)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O
O
F
O
7
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P
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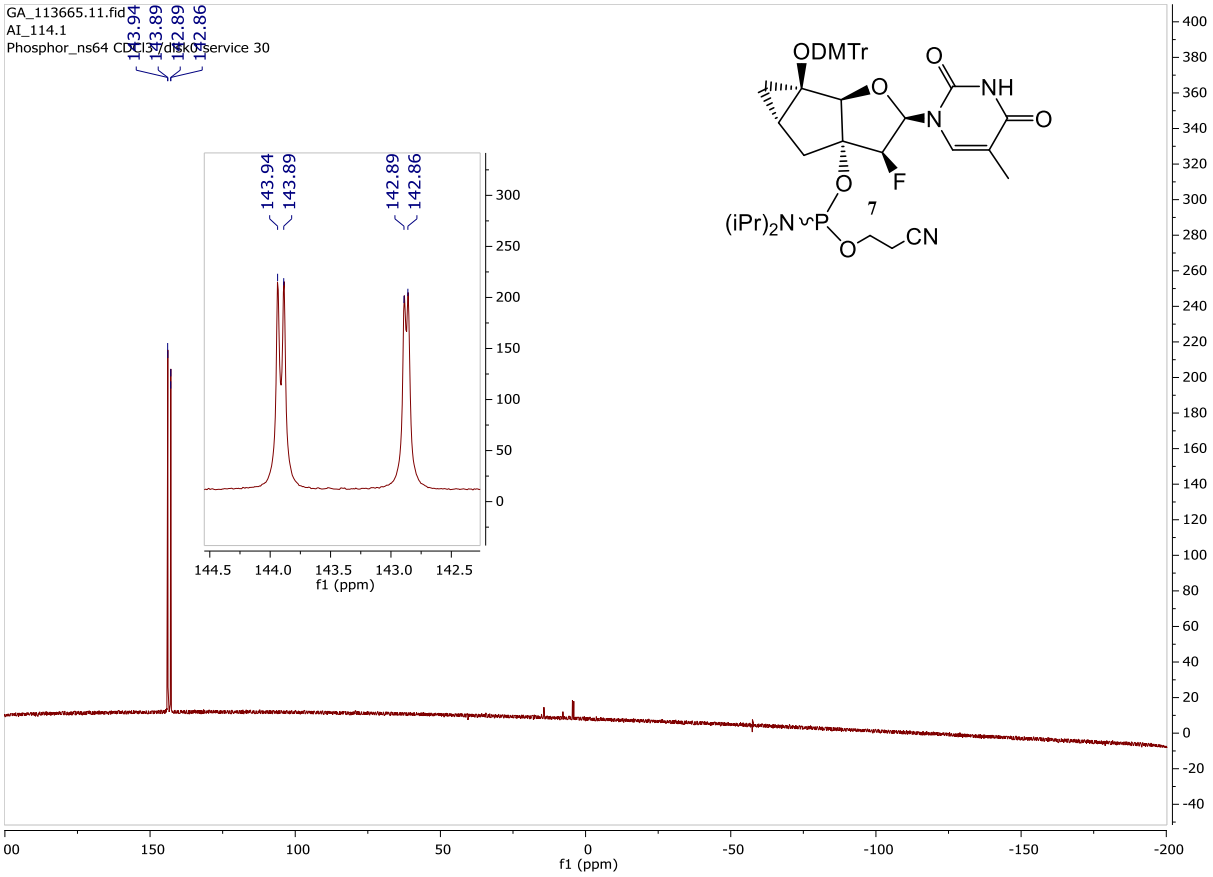
-197.58
f1 (ppm)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Phosphor_ns64 CDCl3 /dfs/service 30
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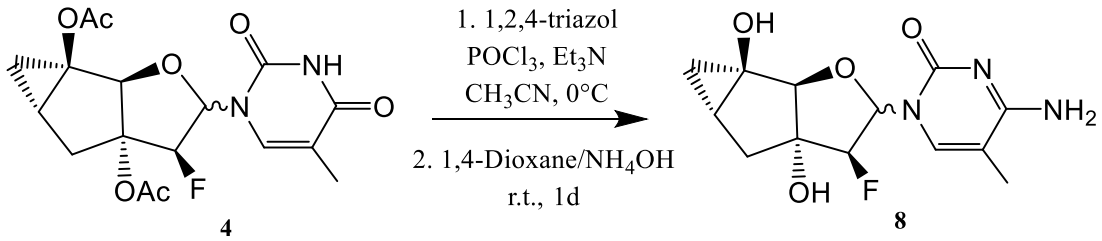
142.86
ODMTr
O
NH
N
O
O
F
O
P
7
(iPr)2N
O
CN
f1 (ppm)
f1 (ppm)

## 2.6 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-β-D-arabinofuranosyl]-5-methylcytosine and 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-α-D-arabinofuranosyl]-5-methylcytosine (8)




A solution of 1,2,4-triazol (0.77 g, 11.2 mmol, 22.5 eq) in 11 ml dry $CH_3CN$ was cooled to to 0°C and treated with $POCl_3$ (0.12 ml, 1.24 mmol, 2.5 eq). The resulting white suspension was stirred at 0°C for 15 min. After that $Et_3N$ (1.59 ml, 11.4 mmol, 23 eq) was added and the mixture was stirred for 1.5 h at the same temperature. A mixture of the nucleosides **4** (190 mg, 0.50 mmol, 1 eq) in dry $CH_3CN$ (3.5 ml) was added to the reaction mixture and the reaction mixture was stirred for 3.5 h at 0°C, then washed with sat. $NaHCO_3$ solution and sat. NaCl solution. The aqueous phases were extracted with EtOAc (3 x 20 ml). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated to yield 264 mg (>100%) of a white solid, which was subjected to the next reaction without further purification.

A solution of the crude nucleoside (264 mg), obtained as described above, in 1,4-dioxane (5 ml) was treated with 30-33% aqueous ammonium hydroxide solution (8 ml) at r.t. and the mixture was stirred for 7 h. After that the additional portion of ammonium hydroxide solution (3 ml) was added and the reaction mixture was stirred overnight. Next day the reaction mixture was concentrated and the crude product was purified by CC ($CH_2Cl_2$/MeOH 9:1 → 6:4) to afford free nucleosides **8** (132 mg, 90% (2 steps), mixture of 2 anomers) as a white foam.

**Analytical data of 8:**

TLC (DCM/MeOH 9:1) $R_f$ = 0.13.

$^1$H NMR ($CD_3OD$, 300 MHz): δ 7.81 – 7.78 (*m*, 0.7H, H-C(6)), 7.54 (*d*, *J* = 1.2 Hz, 0.3H, H-C(6)), 6.14 (*dd*, *J* = 17.8 Hz, *J* = 4.0 Hz, 0.7H, H-C(1')), 5.96 (*dd*, *J* = 13.9 Hz, *J* = 5.4 Hz, 0.3H, H-C(1')), 5.30 (*dd*, *J* = 55.2 Hz, *J* = 5.5 Hz, 0.3H, H-C(2')), 5.04 – 4.82 (*m*, 0.7H, H-C(2')), 4.46 (*s*, 0.3H, H-C(4')), 4.07 (*s*, 0.7H, H-C(4')), 2.71 – 2.56 (*m*, 1H, H-C(7')), 1.99 (*d*, *J* = 1.0 Hz, 3H, C*H*$_3$-C(5)), 1.62 (*dt*, *J* = 9.0 Hz, *J* = 4.5 Hz, 1H, H-C(6')), 1.52 (*d*, *J* = 14.5 Hz, 0.3H, H-C(7')), 1.38 (*dd*, *J* = 14.5 Hz, , *J* = 3.2 H, 0.7H, H-C(7')), 1.06 (*ddd*, *J* = 9.1 Hz, *J* = 5.7 Hz, *J* = 1.1 Hz, 0.7H, H-C(8')), 0.98 (*dd*, *J* = 9.0 Hz,

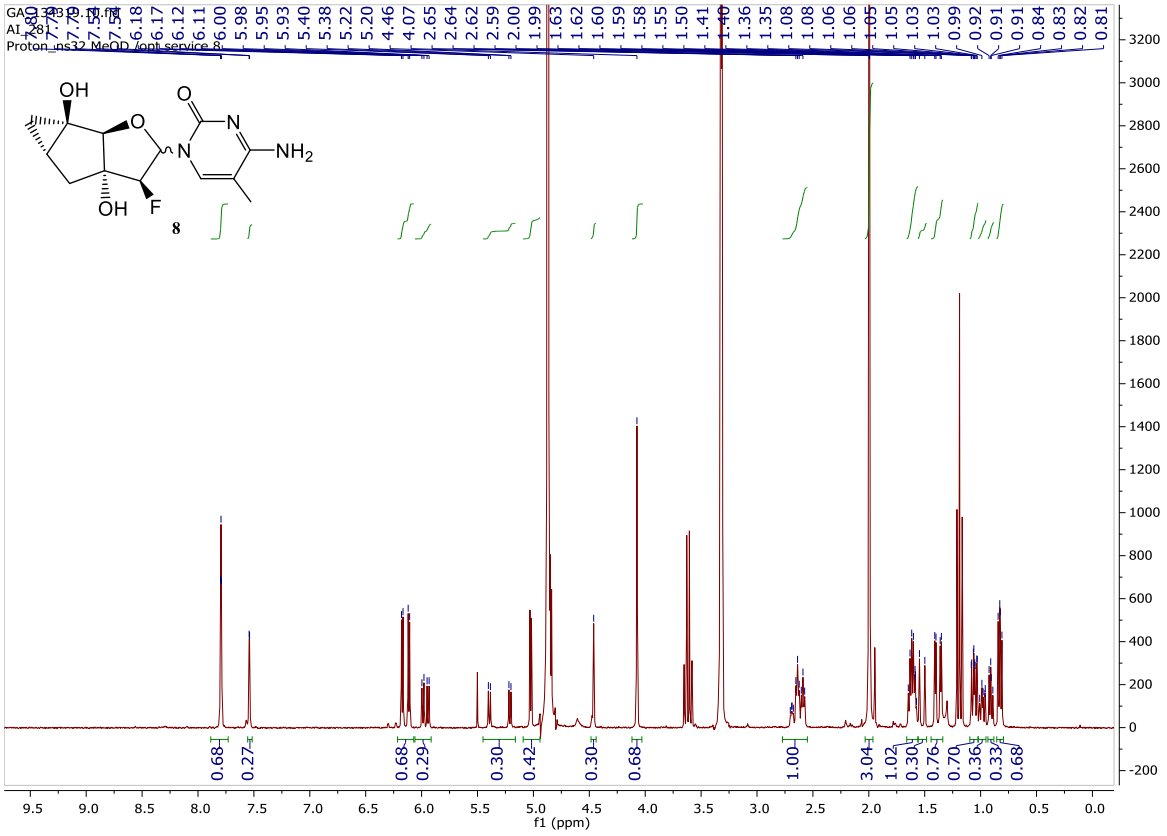
*J* = 5.8 Hz, 0.3H, H-C(8')), 0.91 (*dd*, *J* = 5.7 Hz, *J* = 4.3 Hz, 0.3H, H-C(8')), 0.83 (*dd*, *J* = 5.7 Hz, *J* = 4.4 Hz, 0.7H, H-C(8')).

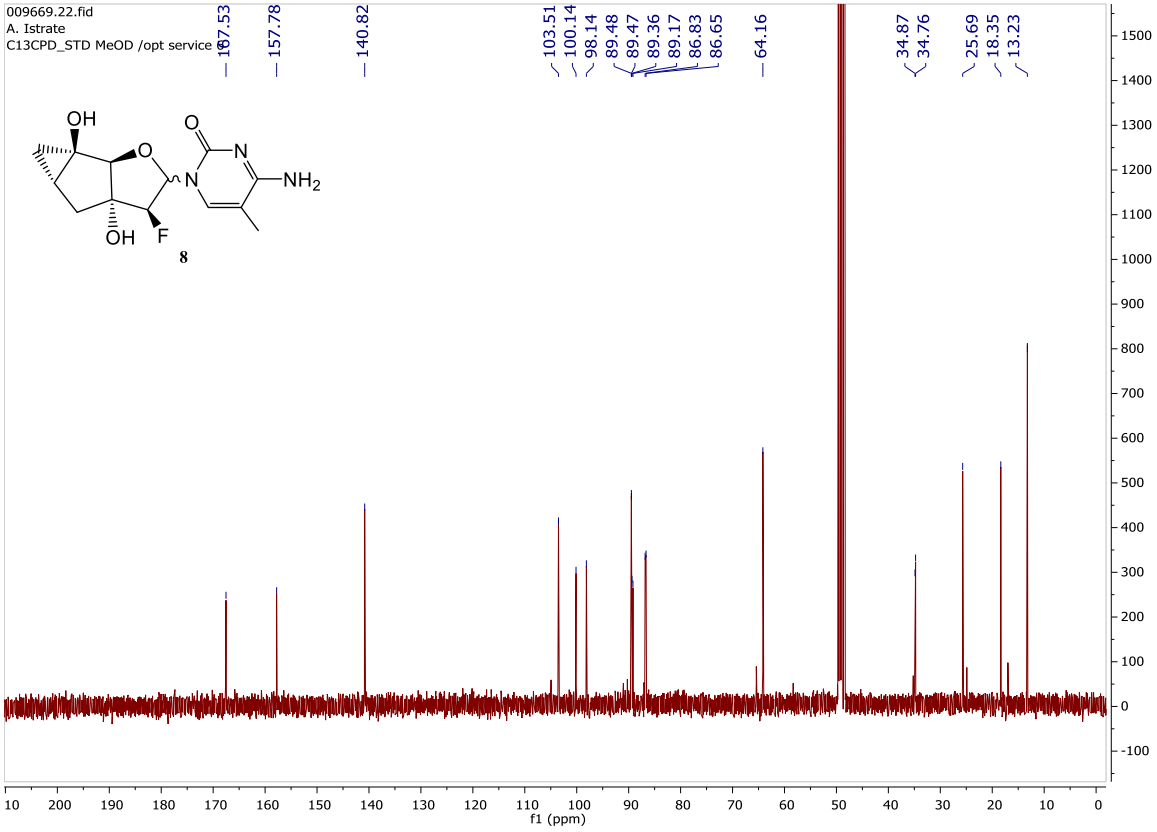
$^{1}$H NMR difference NOE (400 MHz, $CDCl_3$): δ 4.08 (H-C(4'), major isomer)→ 6.15 (2.0%, H-C(1')), 0.83 (2.0%, H-C(8')).

$^{13}$C NMR of the major isomer ($CD_3OD$, 100.6 MHz): δ 167.53 (C(2)), 157.78 (C(4)), 140.82 (C(6)), 103.51 (C(5)), 99.14 (*d*, *J* = 200.8 Hz, C(2')), 89.47 (*d*, *J* = 1.2 Hz, C(4')), 89.27 (*d*, *J* = 19.2 Hz, C(3')), 86.74 (*d*, *J* = 18.3 Hz, C(1')), 64.16 (C(5')), 34.81 (*d*, *J* = 10.9 Hz, C(7')), 25.69 (C(6')), 18.135 (C(8')), 13.23 (C(7)).

$^{19}$F NMR ($CD_3OD$, 376.5 MHz) δ -197.98 (*dd*, *J* = 54.3 Hz, *J* = 17.9 Hz).

ESI$^{+}$-HRMS: calculated for $C_{13}H_{17}O_4N_3F$ ([M+H]$^{+}$) 298.1198, found 298.1197.

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89.36
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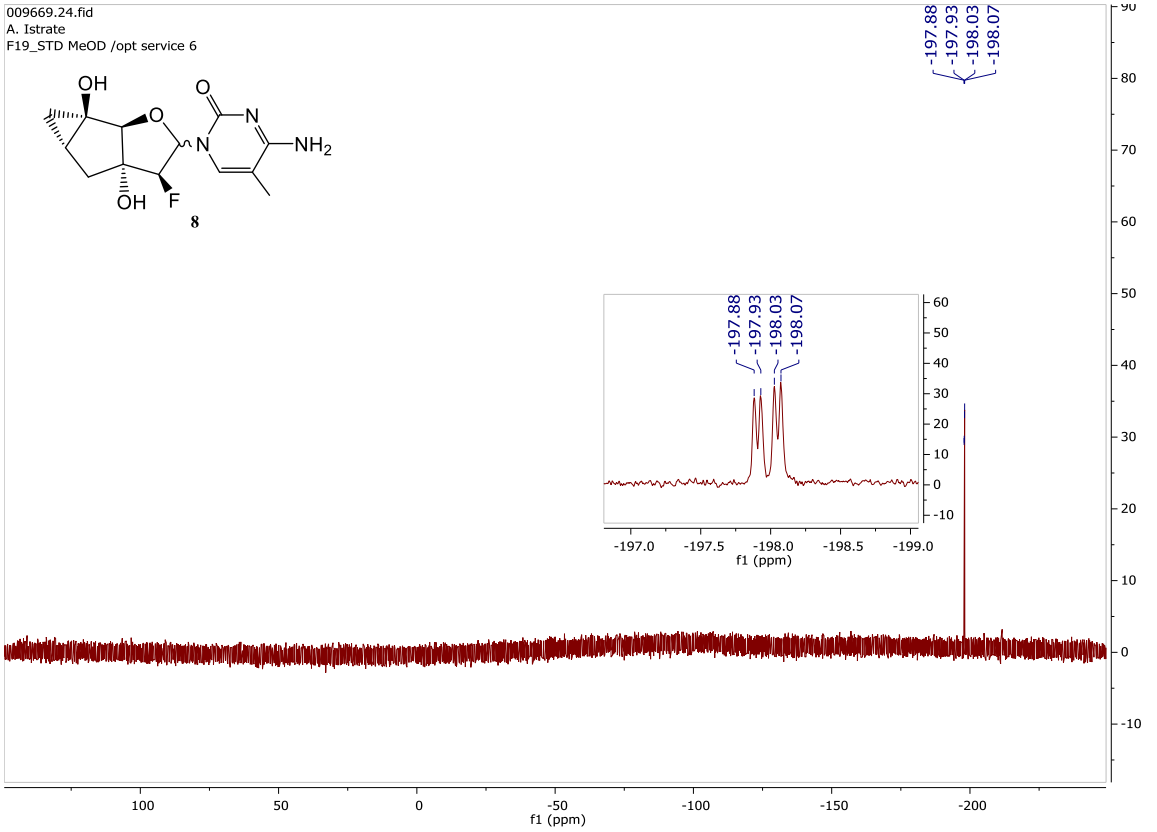
O
N
N
NH2
OH
F
8
f1 (ppm)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-197.93
-198.03
-198.07
OH
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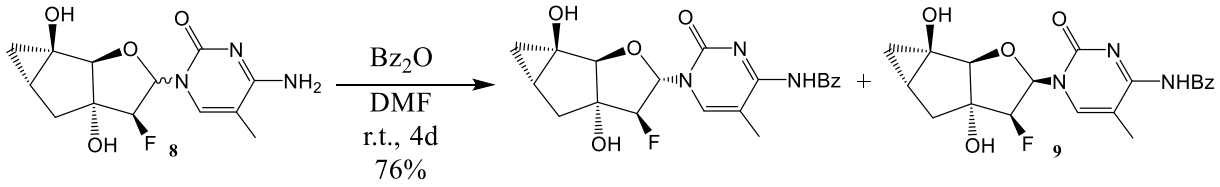
N
N
NH2
OH
F
8
f1 (ppm)

## 2.7 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-β-D-arabinofuranosyl]-4-N-benzoyl-5-methylcytosine (9) and 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-α-D-arabinofuranosyl]-4-N-benzoyl-5-methylcytosine




To a solution of **8** (0.129 g, 0.43 mmol, 1 eq) in 2 ml DMF was added benzoic anhydride (0.11 g, 0.49 mmol, 1.1 eq) and the resulting solution was stirred at r.t. for 3 days. On the third day additional portion of benzoic anhydride (40 mg, 0.18 mmol, 0.4 eq) was added and the reaction mixture was stirred overnight at r.t. After that the solvent was removed under reduced pressure, the residual oil was purified by CC (DCM/EtOH 99:1 → 96:4). During the CC first fractions with the product contained both α and β anomers while next fractions contained pure β anomer. Later fractions were combined and the solvent was removed to yield the compound **9** (0.09 g, 76%).

**Analytical data of 9:**

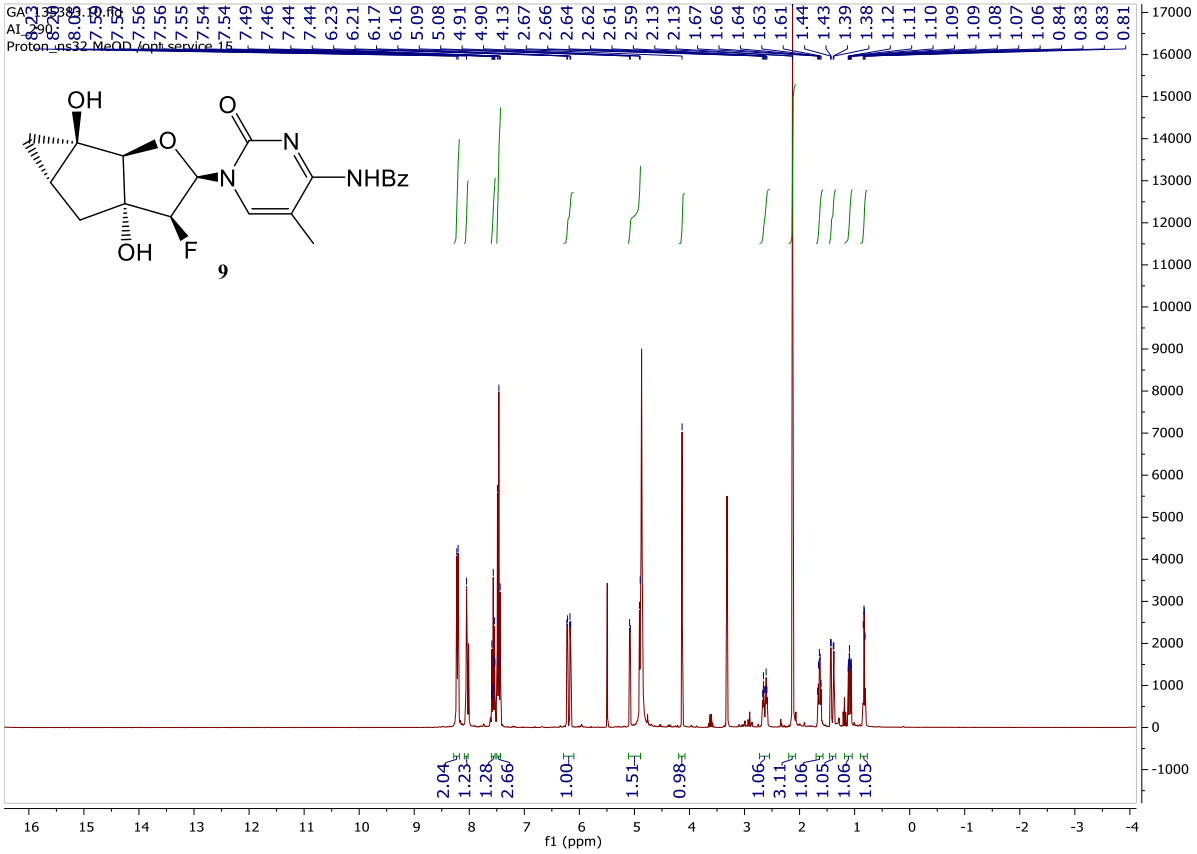
TLC (DCM/MeOH 95:5) $R_f$ (α anomer)= 0.41, $R_f$ (β anomer)= 0.33.

$^1$H NMR ($CD_3OD$, 300 MHz): δ 8.21 (*d*, *J* = 7.0 Hz, 2H, H-arom), 8.05 (*s*, 1H, H-C(6)), 7.60 – 7.53 (*m*, 1H, H-arom), 7.50 – 7.43 (*m*, 2H, H-arom), 6.19 (*dd*, *J* = 17.1 Hz, *J* = 4.0 Hz, 1H, H-C(1')), 5.00 (*dd*, *J* = 54.0 Hz, *J* = 3.95 Hz, 1H, H-C(2')), 4.13 (*s*, 1H, H-C(4')), 2.63 (*dt*, *J* = 14.4 Hz, *J* = 4.3 Hz, 1H, H-C(7')), 2.13 (*d*, *J* = 1.1 Hz, 3H, C*H*$_3$-C(5)), 1.64 (*dt*, *J* = 9.4 Hz, *J* = 4.8 Hz, 1H, H-C(6')), 1.41 (*dd*, *J* = 14.5 Hz, *J* = 3.3 Hz, 1H, H-C(7')), 1.09 (*ddd*, *J* = 9.2 Hz, *J* = 5.7 Hz, *J* = 1.1 Hz, 1H, H-C(8')), 0.83 (*dd*, *J* = 5.8 Hz, *J* = 4.4 Hz, 1H, H-C(8')).

$^{13}$C NMR ($CD_3OD$, 100 MHz): δ 169.90 (C(2)), 161.86 (C(4)), 150.76 (*C*OPh), 141.20 (C(6)), 137.57 (C-arom), 133.41, 130.32, 128.99 (*C*H-arom), 111.64 (C(5)), 99.04 (*d*, *J* = 201.0 Hz, C(2')), 89.90 (C(4')), 89.24 (*d*, *J* = 19.2 Hz, C(3')), 84.64 (*d*, *J* = 18.1 Hz, C(1')), 64.05 (C(5')), 34.91 (*d*, *J* = 10.5 Hz, C(7')), 25.77 (C(6')), 18.55 (C(8')), 13.76 (C(7)).

$^{19}$F NMR ($CD_3OD$, 376.5 MHz) δ -198.11 (*dd*, *J* = 54.0 Hz, *J* = 17.1 Hz).

ESI$^+$-HRMS: calculated for $C_{20}H_{20}O_5N_3FNa$ ($[M+Na]^+$) 424.1279, found 424.1272.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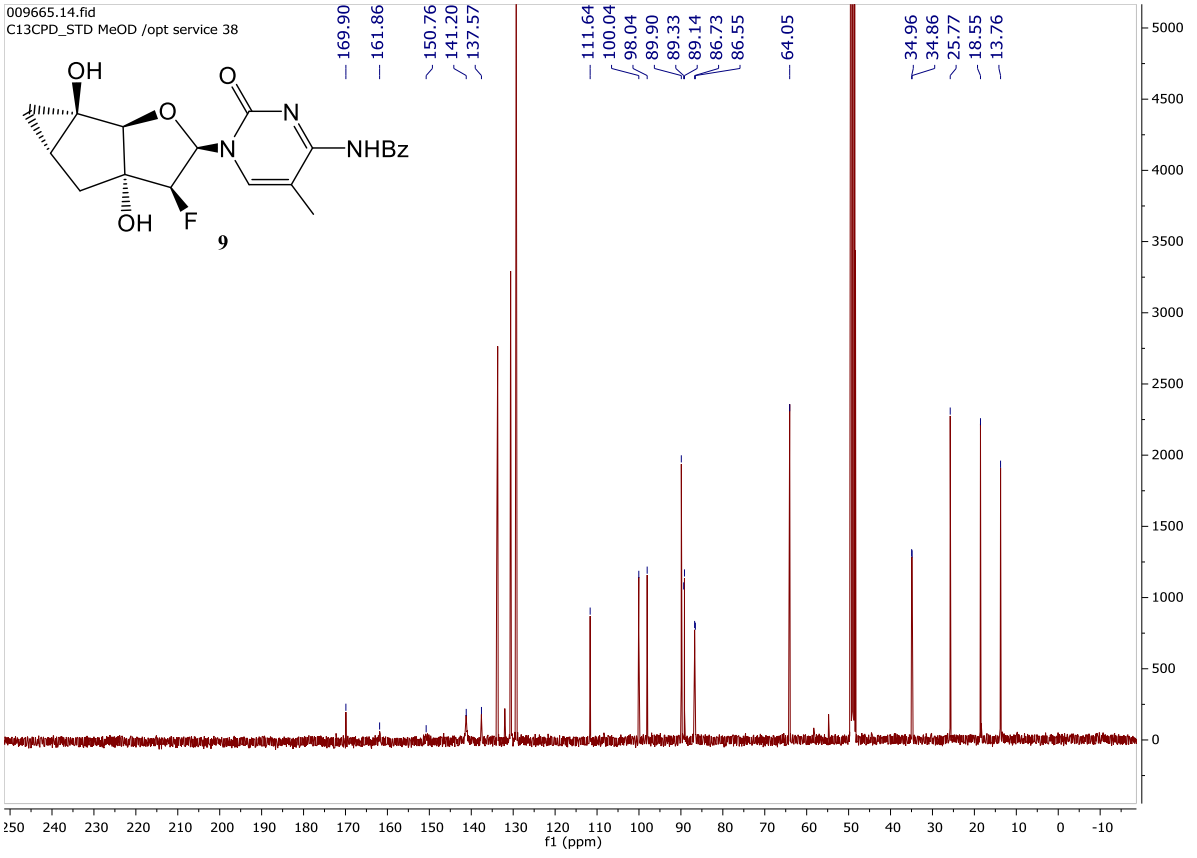
O
N
NHBz
OH
F
9
f1 (ppm)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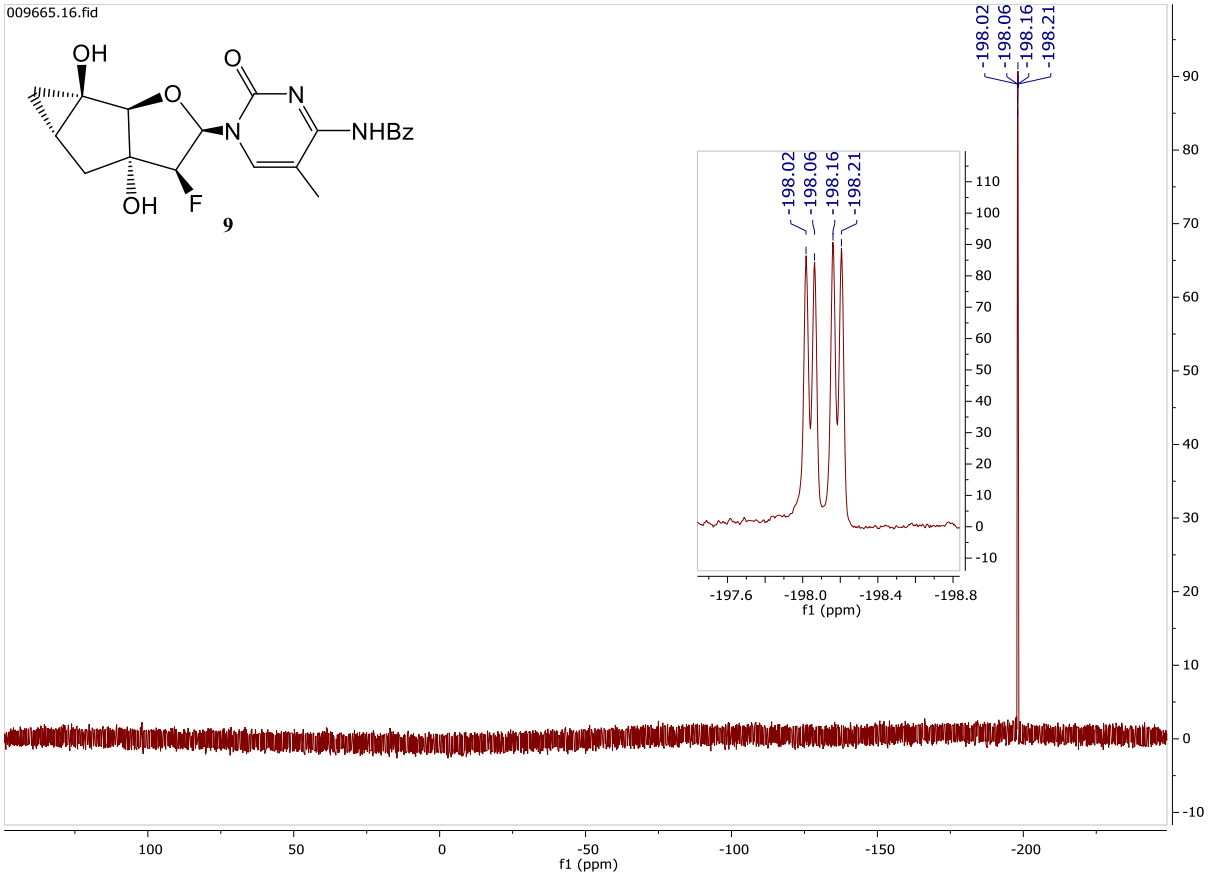
N
NHBz
OH
F
9
f1 (ppm)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N
NHBz
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F
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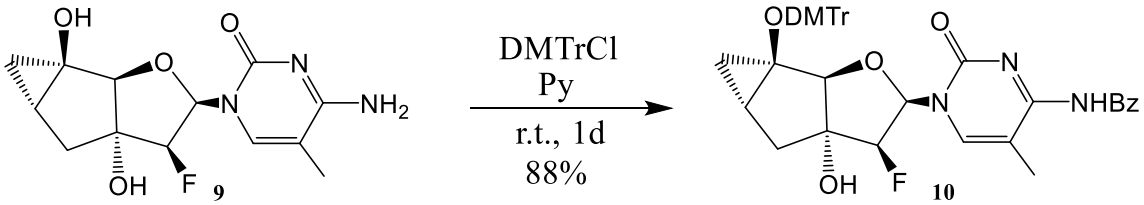
-198.02
-198.06
-198.16
-198.21
f1 (ppm)
f1 (ppm)

## 2.8 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-5'-O-((4,4'-dimethoxytriphenyl)methyl)-β-D-arabinofuranosyl]-4-N-benzoyl-5-methylcytosine (10)




To a solution of nucleoside **9** (0.09 g, 0.23 mmol, 1 eq.) in 0.9 ml dry pyridine DMTr-Cl (0.23 g, 0.69 mmol, 3 eq.) was added in three equal portions over 1 h. The resulting orange solution was stirred overnight before washing with sat. $NaHCO_3$ solution (2 x 15 ml). Subsequently, the aqueous phases were extracted with $CH_2Cl_2$ (3 x 20 ml). The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude orange foam was purified by CC (Hexane/EtOAc 3:1 +0.5 % $Et_3N$, then Hexane/EtOAc 3:1) to yield DMT-protected nucleoside **10** (0.14 g, 88%) as a white foam.

**Analytical data of 10:**

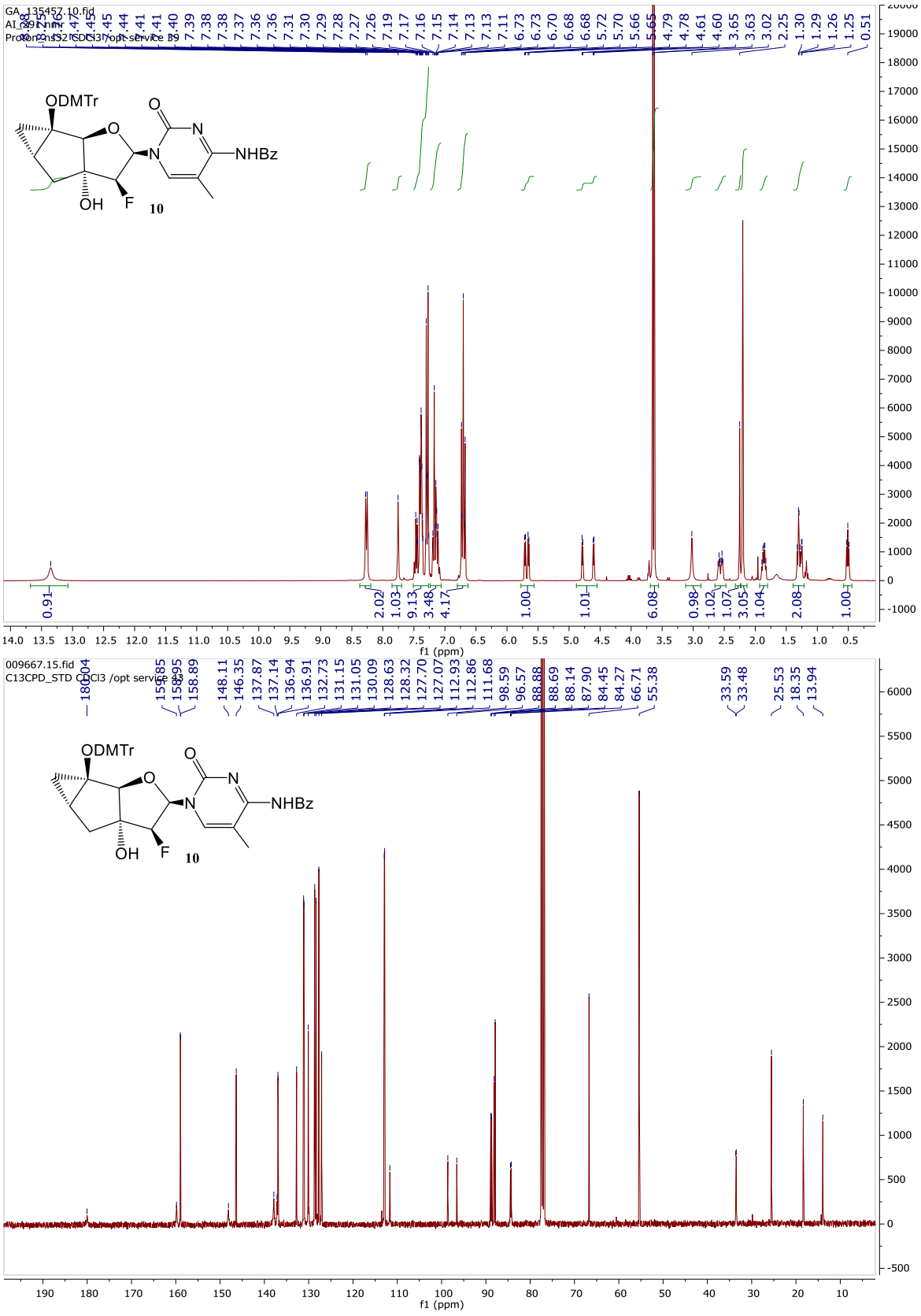
TLC (Hexane/EtOAc 1:1) $R_f$ = 0.56.

$^1$H NMR ($CDCl_3$, 300 MHz): δ 13.35 (*br*, 1H, N*H*), 8.27 (*d*, *J* = 7.1 Hz, 2H, H-arom), 7.76 (*s*, 1H, H-C(6)), 7.51 – 7.25 (*m*, 9H, H-arom), 7.22 – 7.07 (*m*, 3H, H-arom), 6.70 (*t*, J = 8.8 Hz, 4H, H-arom), 5.68 (*dd*, *J* = 16.7 Hz, *J* = 4.0 Hz, 1H, H-C(1')), 4.69 (*dd*, *J* = 54.0 Hz, 1H, H-C(2')), 3.65, 3.63 (2s, 6H, *Me*O), 3.02 (*br*, 1H, O*H*), 2.56 (*dt*, *J* = 14.6 Hz, *J* = 4.6 Hz, 1H, H-C(7')), 2.25 (*s*, 1H, H-C(4')), 2.20 (*s*, 3H, C*H*$_3$-C(5)), 1.86 (*dt*, *J* = 9.9 Hz, *J* = 5.1 Hz, 1H, H-C(6')), 1.36 – 1.23 (*m*, 2H, H-C(7'), H-C(8')), 0.51 (*t*, *J* = 5.8 Hz, 1H, H-C(8')).

$^{13}$C NMR ($CDCl_3$, 100 MHz): δ 180.04 (C(2)), 159.85 (C(4)), 158.95, 158.89 (MeO-*C*-arom), 148.11 (*C*OPh), 146.35 (C-arom), 137.87 (C(6)), 137.14, 136.94, 136.91 (C-arom), 132.73, 131.15, 131.05, 130.09, 128.63, 128.32, 127.70, 127.07, 112.93, 112.86 (*C*H-arom), 111.68 (C(5)), 97.58 (*d*, *J* = 203.1 Hz, C(2')), 88.79 (*d*, *J* = 19.3 Hz, C(3')), 88.14 (C(4')), 87.90 (*C*(Ph)$_3$), 84.36 (*d*, *J* = 17.9 Hz, C(1')), 66.71 (C(5')), 55.38 (*Me*O-DMTr), 33.54 (*d*, *J* = 11.0 Hz, C(7')), 25.53 (C(6')), 18.35 (C(8')), 13.94 (C(7)).

$^{19}$F NMR ($CDCl_3$, 376.5 MHz) δ -195.46 (*dd*, *J* = 55.0 Hz, *J* = 17.8 Hz).

ESI$^+$-HRMS: calculated for $C_{41}H_{39}O_7N_3F$ ([M+H]$^+$) 704.2767, found 704.2767.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F
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f1 (ppm)
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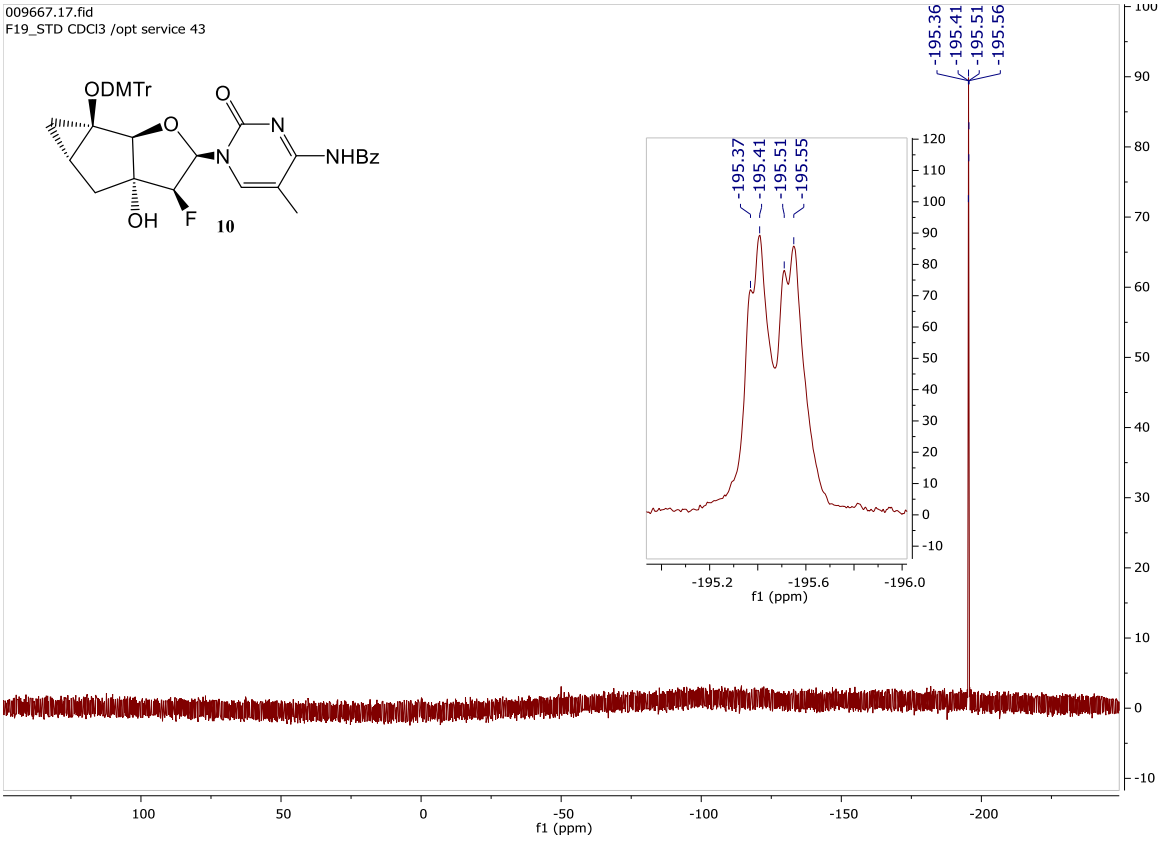
O
N
NHBz
OH
F
10
f1 (ppm)


009667.17.fid
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ODMTr
O
N
N
NHBz
OH
F
10
-195.36
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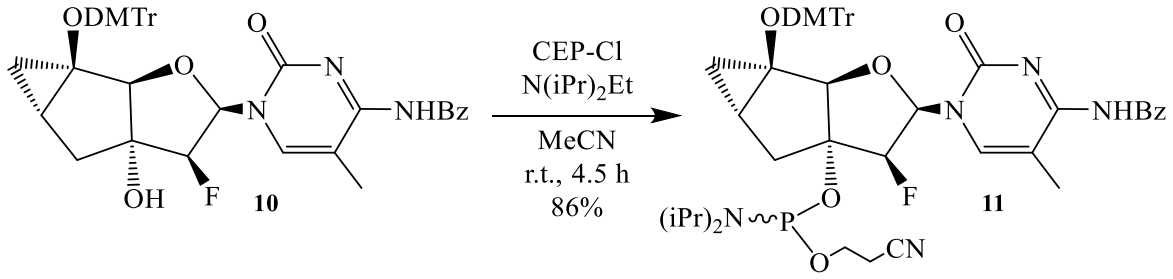
-195.56
-195.37
-195.41
-195.51
-195.55
-195.2
-195.6
-196.0
f1 (ppm)
100
50
0
-50
-100
-150
-200
f1 (ppm)

## 2.9 1-[(5'R,6'R)-3'-O-(2-cyanoethoxy)-diisopropylaminophosphanyl-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-5'-O-((4,4'-dimethoxytriphenyl)methyl)-β-D-arabinofuranosyl]-4-N-benzoyl-5-methylcytosine (11)




To a solution of DMT-protected nucleoside **10** (141 mg, 0.2 mmol, 1 eq.) and N-ethyldiisopropylamine (0.14 ml, 0.8 mmol, 4 eq.) in dry MeCN (1.2 ml) was slowly added CEP-Cl (0.09 ml, 0.4 mmol, 2 eq.) and the reaction mixture was stirred for 4.5 h. After that the reaction mixture was diluted with EtOAc and washed with sat. $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc and the combined organic phases were dried over $MgSO_4$, filtered and evaporated. The crude product was purified by CC using hexane/EtOAc 2:1 + 0.5 % $Et_3N$ to afford the phosphoramidite **11** (155 mg, 86%) as a mixture of isomers.

**Analytical data of 11:**

TLC (Hexane/EtOAc 3:1) $R_f$ = 0.57.

$^1$H NMR ($CDCl_3$, 400 MHz): δ 13.39 (*br*, 1H, N*H*), 8.35 (*d*, *J* = 7.6 Hz, 2H, H-arom), 7.88 – 7.81 (*m*, 1H, H-C(6)), 7.57 – 7.51 (*m*, 1H, H-arom), 7.51 – 7.43 (*m*, 4H, H-arom), 7.41 – 7.34 (*m*, 4H, H-arom), 7.30 – 7.19 (*m*, 3H, H-arom), 6.86 – 6.75 (*m*, 4H, H-arom), 5.92 – 5.81 (*m*, 1H, H-C(1')), 5.29 – 5.11 (*m*, 1H, H-C(2')), 3.80, 3.80, 3.79, 3.79 (4*s*, 6H, *Me*O), 3.76 – 3.65 (*m*, 1H, OC*H*$_2$CH$_2$CN), 3.64 – 3.53 (*m*, 1H, OC*H*$_2$CH$_2$CN), 3.53 – 3.41 (*m*, 2H, (Me$_2$C*H*)$_2$N), 2.65 (*d*, *J* = 2.7 Hz, 0.5H, H-C(4')), 2.63 – 2.51 (*m*, 2.5H, OCH$_2$C*H*$_2$CN, H-C(7')), 2.46 (*dt*, *J* = 15.2 Hz, *J* = 3.9 Hz, 0.5H, H-C(7')), 2.40 (*s*, 0.5H, H-C(4')), 2.26 (*s*, 3H, C*H*$_3$-C(5)), 2.09 (*dd*, *J* = 15.7 Hz, *J* = 5.7 Hz, 0.5H, H-C(7')), 1.93 (*tt*, *J* = 9.8 Hz, *J* = 5.1, 1H, H-C(6')), 1.74 (*d*, *J* = 15.1 Hz, 0.5H, H-C(7')), 1.35 (*dd*, *J* = 9.3 Hz, *J* = 6.4 Hz, 1H, H-C(8')), 1.16 – 1.03 (*m*, 12H, (*Me*$_2$CH)$_2$N), 0.61 – 0.54 (*m*, 1H, H-C(8')).

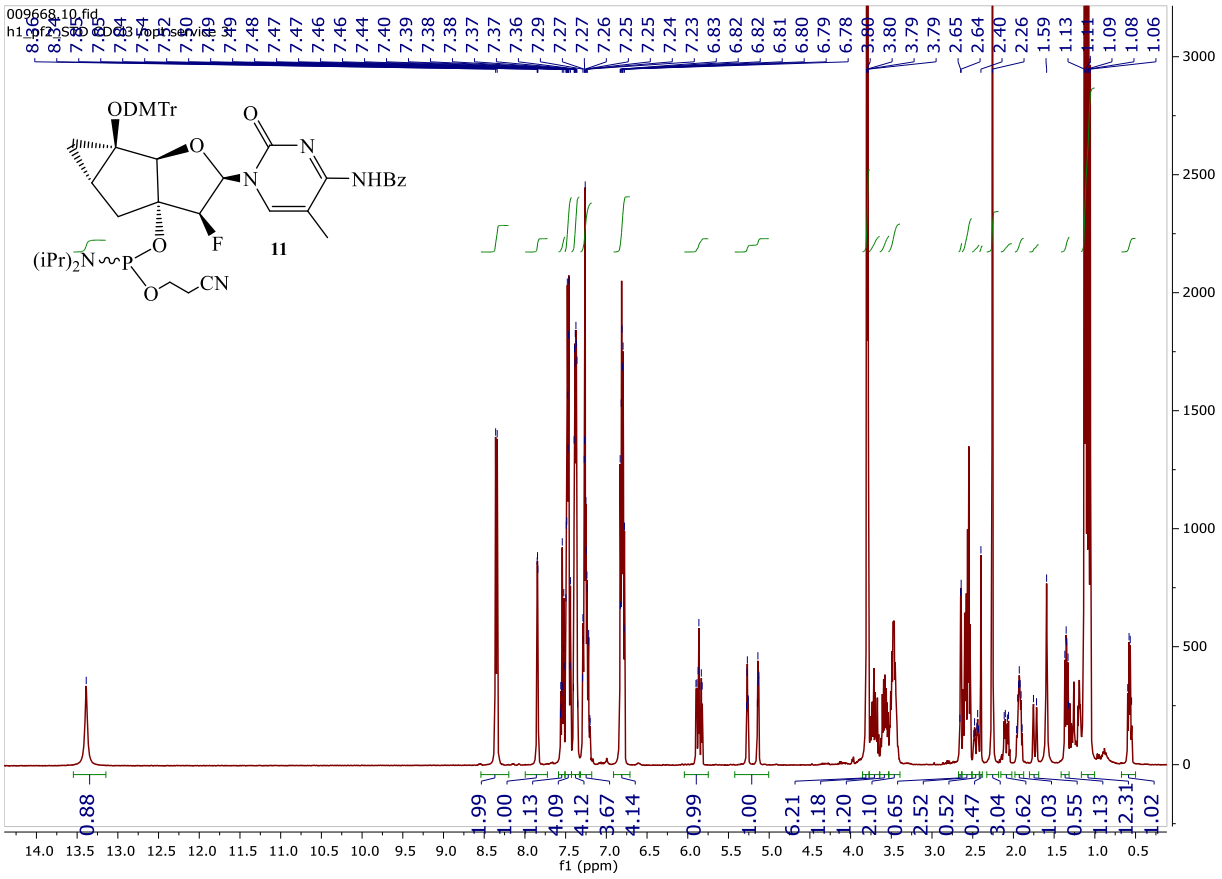
$^{13}$C NMR ($CDCl_3$, 100.6 MHz): δ 179.88 (C(2)), 159.93 (C(4)), 158.96, 158.92, 158.90, 158.86 (MeO-*C*-arom), 147.77 (*C*OPh), 146.26, 146.11 (C-arom), 137.76, 137.64 (C(6)), 137.34, 136.94, 136.88, 136.84 (C-arom), 132.59, 131.14, 131.10, 131.00, 130.94, 130.08, 128.77, 128.68, 128.27, 127.76, 127.18, 127.09 (*C*H-arom), 117.53, 117.47 (CN), 113.00, 112.96 (*C*H-arom), 111.25 (C(5)), 95.79 (*dd*, *J* = 203.0 Hz, *J* = 5.0 Hz, C(2')), 95.36 (*dd*, *J* = 202.7 Hz, *J* = 7.5 Hz, C(2')), 92.36 (*dd*, *J* = 19.5 Hz, *J*

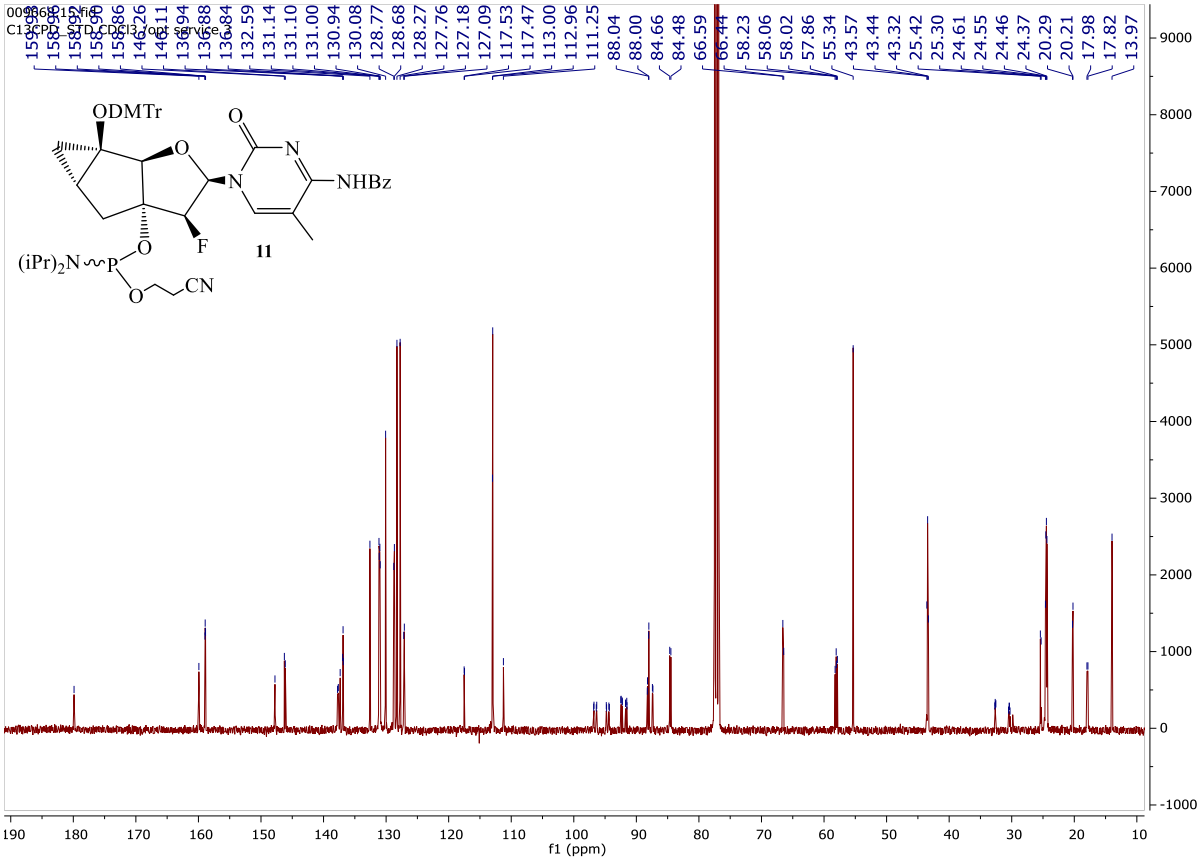
= 4.3 Hz, C(3')), 91.61 (*dd*, *J* = 19.4 Hz, *J* = 3.7 Hz, C(3')), 88.29 – 88.16 (*m*, C(4')), 88.04, 88.00 (*C*(Ph)$_3$), 87.40 (*d*, *J* = 8.0 Hz, C(4')), 95.57 (*d*, *J* = 18.3 Hz, C(1')), 66.59, 66.44 (C(5')), 58.04 (*dd*, *J* = 20.3 Hz, *J* = 16.6 Hz, O*C*H$_2$CH$_2$CN), 55.34 (*Me*O-DMTr), 43.62 – 43.27 (*m*, (Me$_2$*C*H)$_2$N), 32.62 (*dd*, *J* = 10.6 Hz, *J* = 6.7 Hz, C(7')), 30.39 (*dd*, *J* = 14.0 Hz, *J* = 10.4 Hz, C(7')), 25.42, 25.30 (C(6')), 24.61, 24.55, 24.46, 24.37 ((*Me*$_2$CH)$_2$N), 20.29, 20.21, (OCH$_2$*C*H$_2$CN), 17.98, 17.82 (C(8')), 13.97 (C(7)).

$^{19}$F NMR (CDCl$_3$, 376.5 MHz) δ -195.49 – -195.76 (*m*), -197.55 (*dd*, *J* = 52.3 Hz, *J* = 14.2 Hz).

$^{31}$P NMR (122 MHz, CDCl$_3$) δ 143.92 (*d*, *J* = 7.4 Hz), 142.75 (*d*, *J* = 4.4 Hz).

ESI$^+$-HRMS: calculated for $C_{50}H_{56}O_8N_5FP$ ([M+H]$^+$) 904.3845, found 904.3868.

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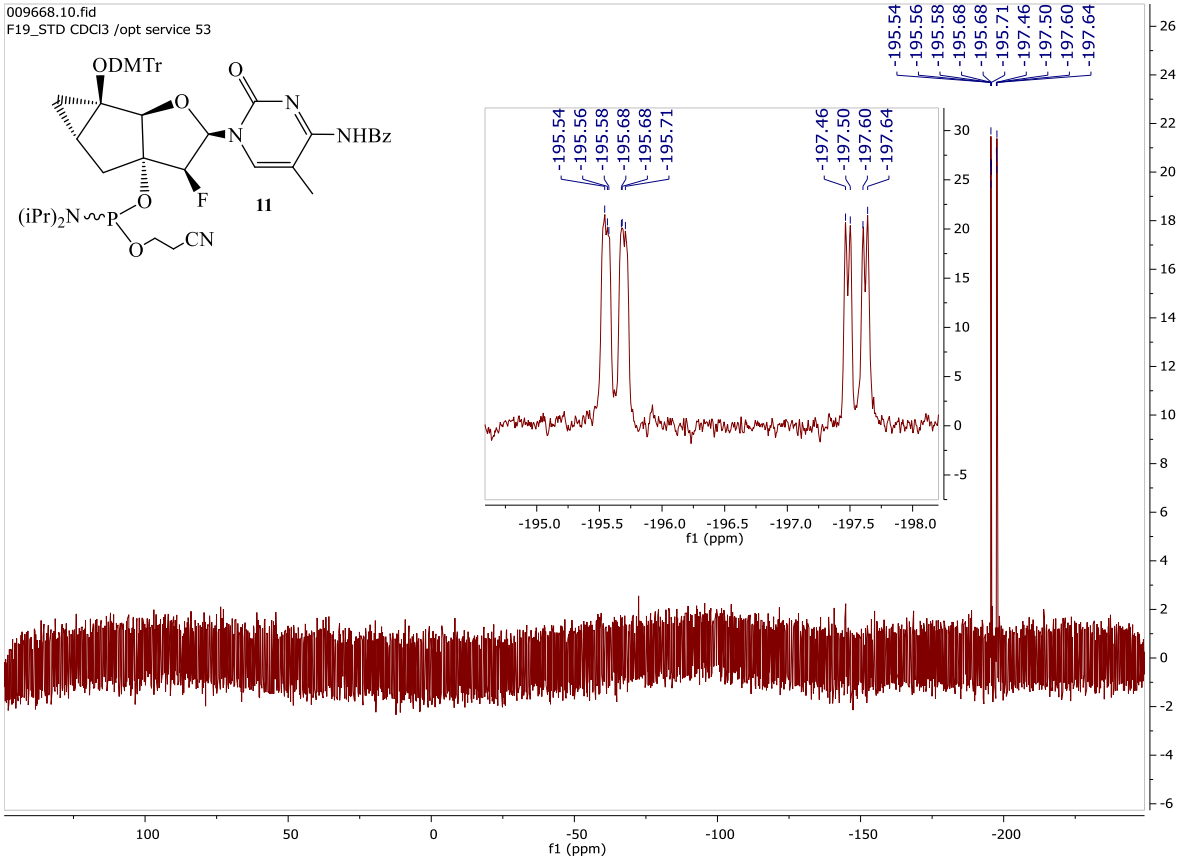
NHBz
11
f1 (ppm)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ODMTr
NHBz
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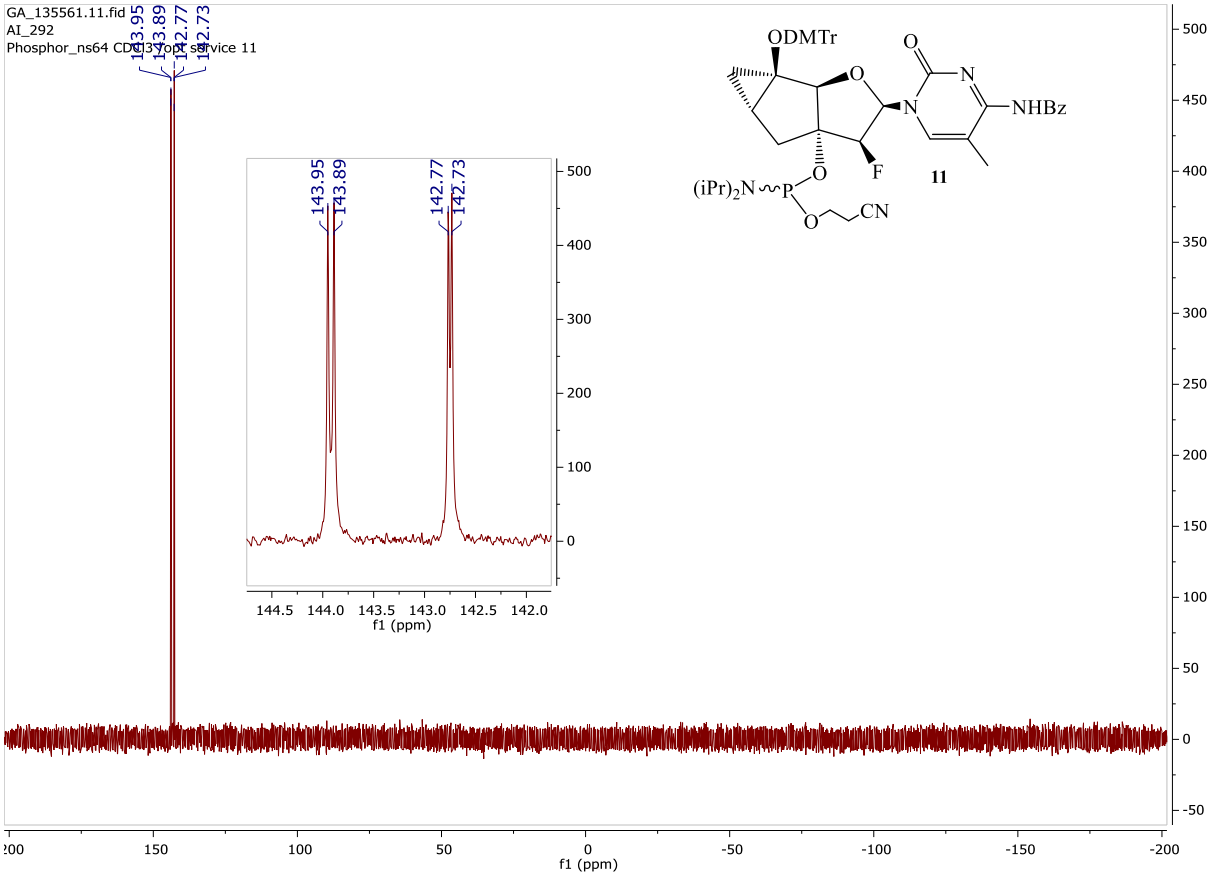
-197.50
-197.60
-197.64
f1 (ppm)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Phosphor_ns64 CDCl3 Top service 11
143.95
143.89
142.77
142.73
f1 (ppm)
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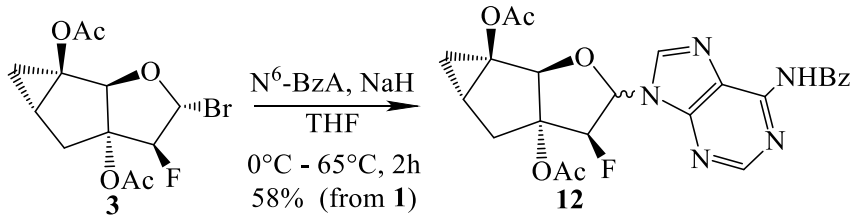
O
N
NHBz
O
N
F
(iPr)2N
P
O
CN
11

## 2.10 1-[(5'R,6'R)-3',5'-di-O-acetyl-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-β-D-arabinofuranosyl]-6-N-benzoyl-adenine and 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-α-D-arabinofuranosyl]-6-N-benzoyladenine (12)




A suspension of $N^6$-benzoyladenine (2.58 g, 10.8 mmol, 1.7 eq.) in dry THF (10 ml) at 0°C was treated with NaH (0.48 g, 12 mmol, 1.9 eq., 60% suspension in mineral oil), was allowed to warm up and stirred at r.t. for 1 h. After that the solution of **3** (max 2.14 g (crude), 6.35 mmol, 1 eq) in 10 ml THF was added and the reaction mixture was stirred at 65°C for 2 h. Subsequently, $H_2O$ was added, the reaction mixture was filtered through Celite® and the filter cake was washed with DCM. The filtrate was collected; the aqueous phase was extracted with DCM (3 x 20 ml). The combined organic phases were dried over $MgSO_4$, filtrated, evaporated and the crude product purified by CC (DCM/MeOH 99:1) to give the product as a mixture of α and β anomers (1.83 g, 58% from **1**) with an α/β ratio of ~1:5 according to NMR.

**Analytical data of 12:**

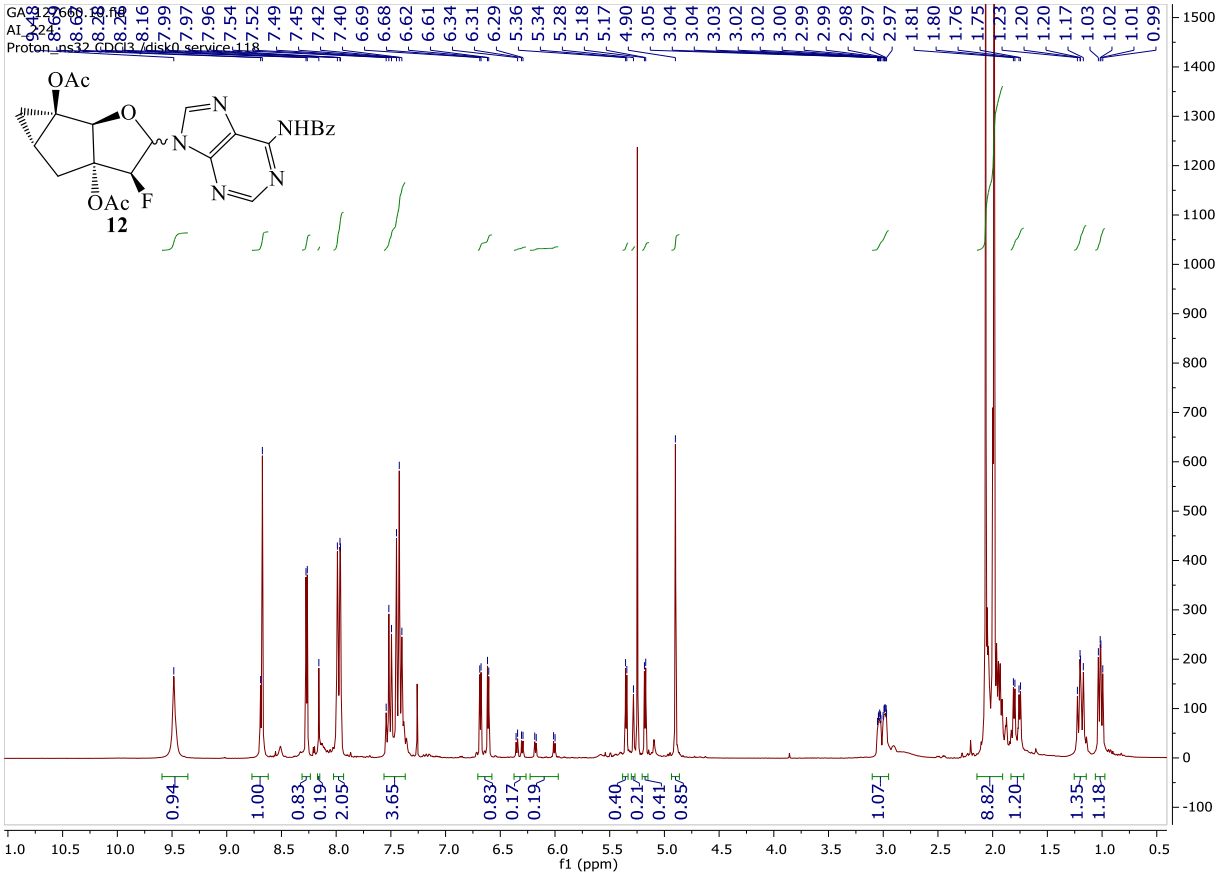
TLC (DCM/MeOH 97:3) $R_f$ = 0.32.

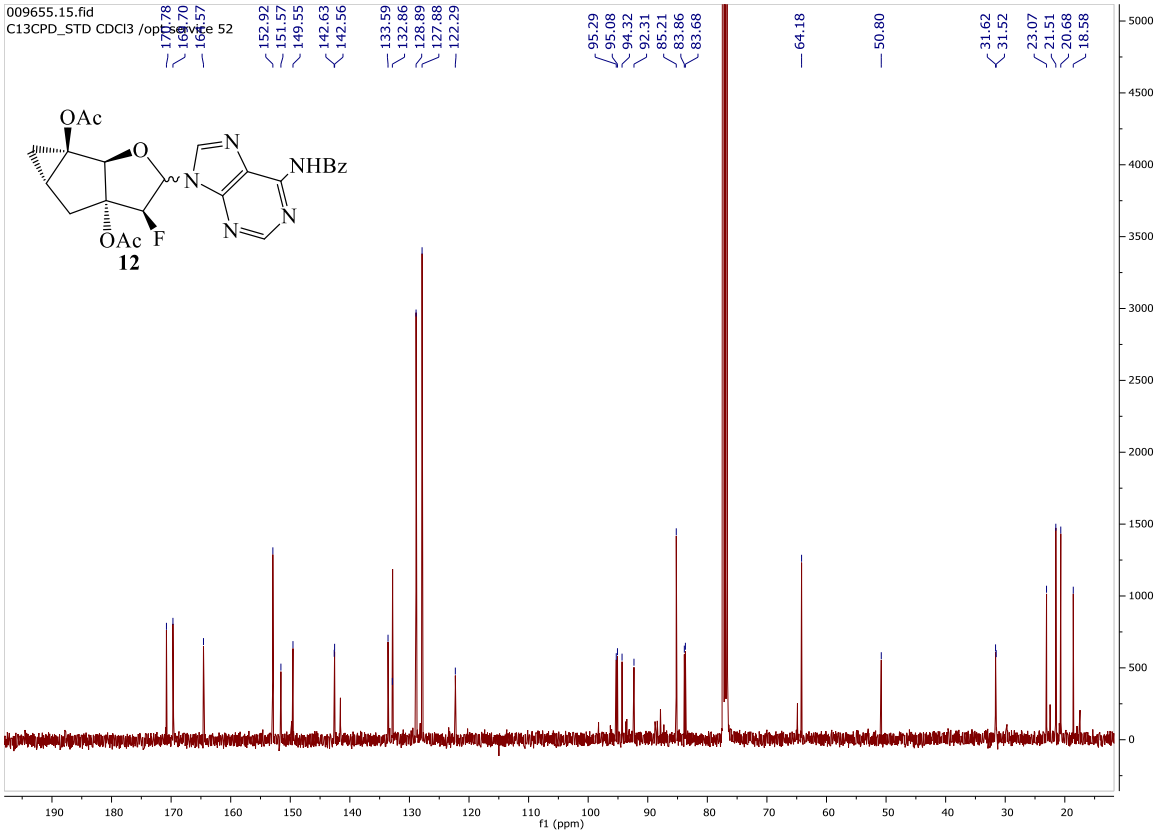
$^1$H NMR ($CDCl_3$, 300 MHz): δ 9.48 (*br*, 1H, N*H*), 8.69, 8.67 (2*s*, 1H, H-C(2)), 8.27 (*d*, *J* = 3.5 Hz, 0.83H, H-C(8)), 8.16 (*s*, 0.17H, H-C(8)), 8.01 – 7.94 (*m*, 2H, H-arom), 7.56 – 7.37 (*m*, 3H, H-arom), 6.65 (*dd*, *J* = 21.1 Hz, *J* = 3.5 Hz, 0.83H, H-C(1')), 6.32 (*dd*, *J* = 14.9 Hz, *J* = 4.1 Hz, 0.17H, H-C(1')), 6.09 (*dd*, *J* = 51.7 Hz, *J* = 4.1 Hz, 0.17H, H-C(2')), 5.26 (*dd*, *J* = 51.8 Hz, *J* = 3.5 Hz, 0.83H, H-C(2')), 5.28 (*s*, 0.17H, H-C(4')), 4.90 (*s*, 0.83H, H-C(4')), 3.07 – 2.95 (*m*, 1H, H-C(7')), 2.09 – 1.90 (*m*, 7H, 2 x C*H*$_3$CO, H-C(6')), 1.78 (*dd*, *J* = 15.4 Hz, *J* = 4.2 Hz, 1H, H-C(7')), 1.24 – 1.16 (*m*, 1H, H-C(8')), 1.01 (*dd*, *J* = 7.0 Hz, *J* = 5.1 Hz, 1H, H-C(8')).

$^{13}$C NMR ($CDCl_3$, 100 MHz, of the β anomer): δ 170.78, 169.70 ($CH_3CO$), 164.57 (*C*OPh), 152.92 (C(2)), 151.57 (C(4)), 149.55 (C(6)), 142.59 (*d*, *J* = 6.5 Hz, C(8)), 133.59 (C-arom), 132.86, 128.89, 127.88 (*C*H-arom), 122.29 (C(5)), 95.18 (*d*, *J* = 20.5 Hz, C(3')), 93.31 (*d*, *J* = 201.4 Hz, C(2')), 85.21 (C(4')), 83.77 (*d*, *J* = 18.1 Hz, C(1')), 64.18 (C(5')), 31.57 (*d*, *J* = 10.8 Hz, C(7')), 23.07 (C(6')), 21.51, 20.68 ($CH_3CO$), 18.58 (C(8')).

$^{19}$F NMR ($CDCl_3$, 376.5 MHz) δ -191.34 (*dd*, *J* = 52.0 Hz, *J* = 14.9 Hz), -192.97 (*dd*, *J* = 51.6 Hz, *J* = 21.1 Hz).

ESI$^+$-HRMS: calculated for $C_{24}H_{23}O_6N_5F$ ([M+H]$^+$) 496.1627, found 496.1616.

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169.57
152.92
151.57
149.55
142.63
142.56
133.59
132.86
128.89
127.88
122.29
95.29
95.08
94.32
92.31
85.21
83.86
83.68
64.18
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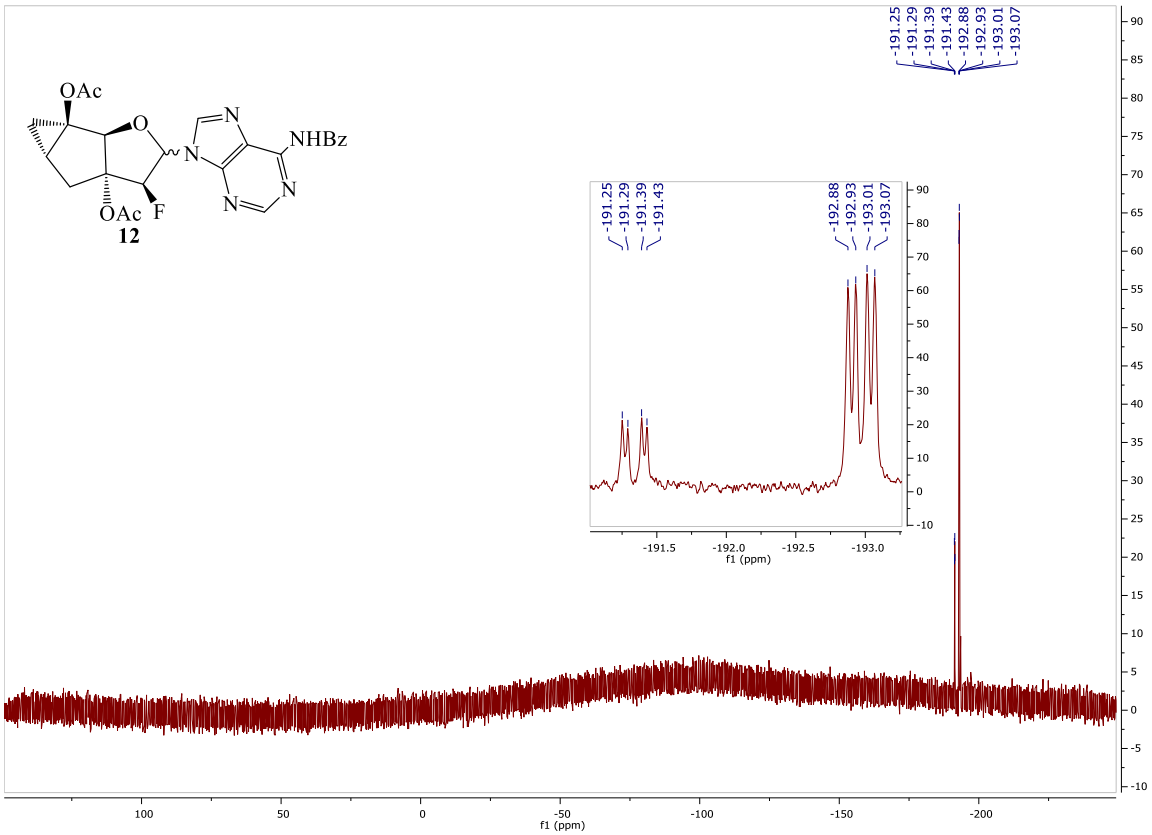
31.62
31.52
23.07
21.51
20.68
18.58
OAc
OAc
F
NHBz
12
f1 (ppm)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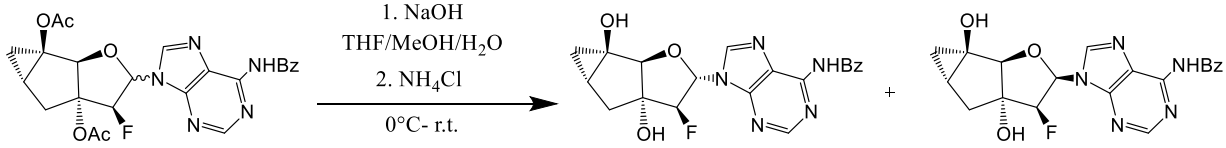
OAc
F
NHBz
12
f1 (ppm)

## 2.11 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-α-D-arabinofuranosyl]-6-N-benzoyladenine and 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-β-D-arabinofuranosyl]-6-N-benzoyladenine (13a and 13b)




**12** **13a** **13b**

A solution of acetyl-protected nucleosides **12** (1.83 g, 3.7 mmol, 1 eq.) in 0.2 M NaOH in THF/MeOH/$H_2O$ 5:4:1 (67 ml, 13 mmol of NaOH, 3.6 eq.) was stirred at 0°C for 20 min. Then $NH_4Cl$ (0.90 g, 16.8 mmol, 4.5 eq.) was added and the reaction mixture was allowed to stir at r.t. for 20 min. Evaporation followed by purification of the adsorbed material by CC (DCM/MeOH 97:3) afforded free nucleosides **13a** (159 mg, 63%) and **13b** (1.19 g, 94%).

**Analytical data of 13a:**

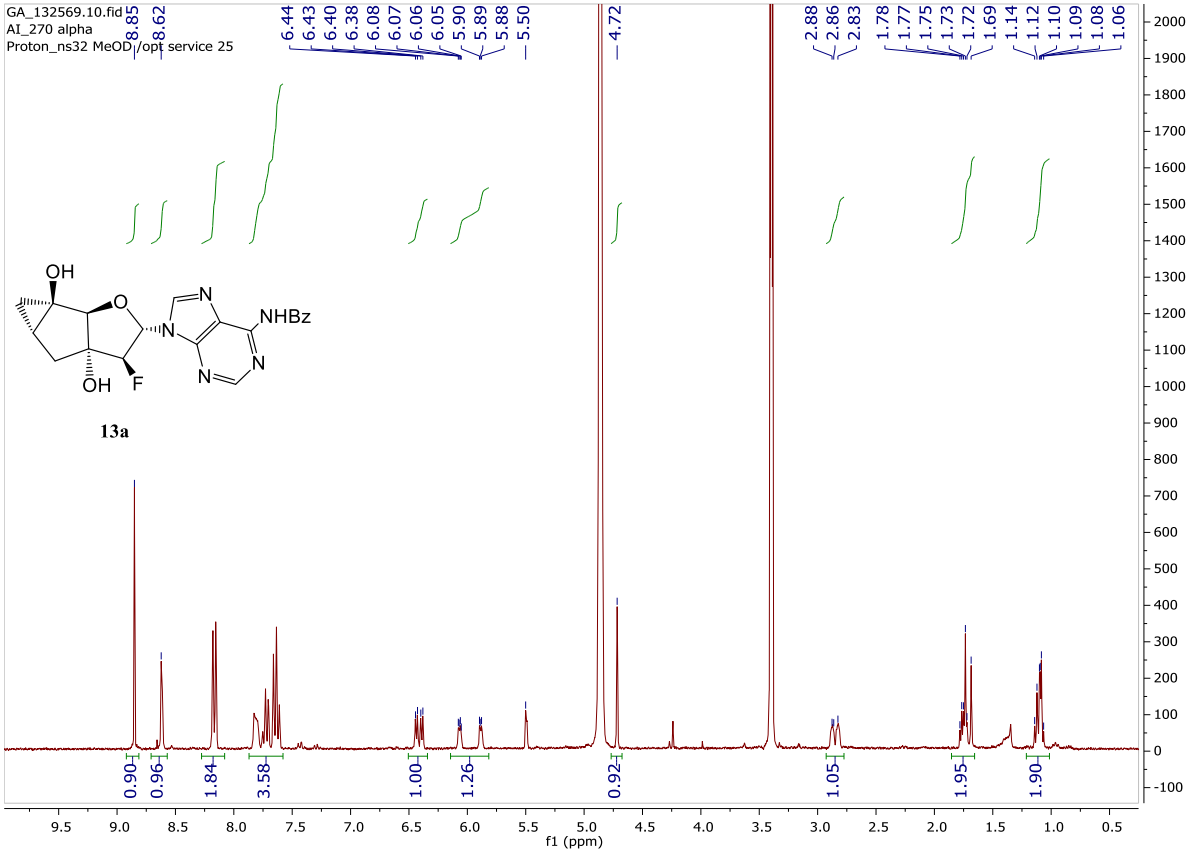
TLC (DCM/MeOH 97:3) $R_f$ = 0.48.

$^1$H NMR ($CD_3OD$, 300 MHz): δ 8.85 (*s*, 1H, H-C(2)), 8.62 (*s*, H-C(8)), 8.20 – 8.14 (*m*, 2H, H-arom), 7.84 – 7.60 (*m*, 3H, H-arom), 6.41 (*dd*, *J* = 13.7 Hz, *J* = 5.2 Hz, 1H, H-C(1')), 6.09 - 5.86 (*m*, 1H, H-C(2')), 4.72 (*s*, 1H, H-C(4')), 2.90 – 2.79 (*m*, 1H, H-C(7')), 1.80 – 1.67 (*m*, 2H, H-C(6'), H-C(7')), 1.15 – 1.05 (*m*, 2H, H-C(8')).

$^{13}$C NMR ($CD_3OD$, 100 MHz): δ 168.20 (*C*OPh), 153.44 (C(2)), 153.27 (C(4)), 151.41 (C(6)), 144.84 (C(8)), 134.94 (C-arom), 133.94, 129.77, 129.45 (*C*H-arom), 125.58 (C(5)), 102.07 (*d*, *J* = 195.3 Hz, C(2')), 89.83 (*d*, *J* = 4.9 Hz, C(4')), 88.16 (*d*, *J* = 34.1 Hz, C(1')), 86.89 (*d*, *J* = 18.8 Hz, C(3')), 65.43 (C(5')), 34.98 (*d*, *J* = 7.6 Hz, C(7')), 24.83 (C(6')), 16.85 (C(8')).

$^{19}$F NMR ($CD_3OD$, 376.5 MHz) δ -205.47 (*dd*, *J* = 54.5 Hz, *J* = 13.6 Hz).

ESI$^+$-HRMS: calculated for $C_{20}H_{19}O_4N_5F$ ($[M+H]^+$) 412.1416, found 412.1424.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6.43
6.40
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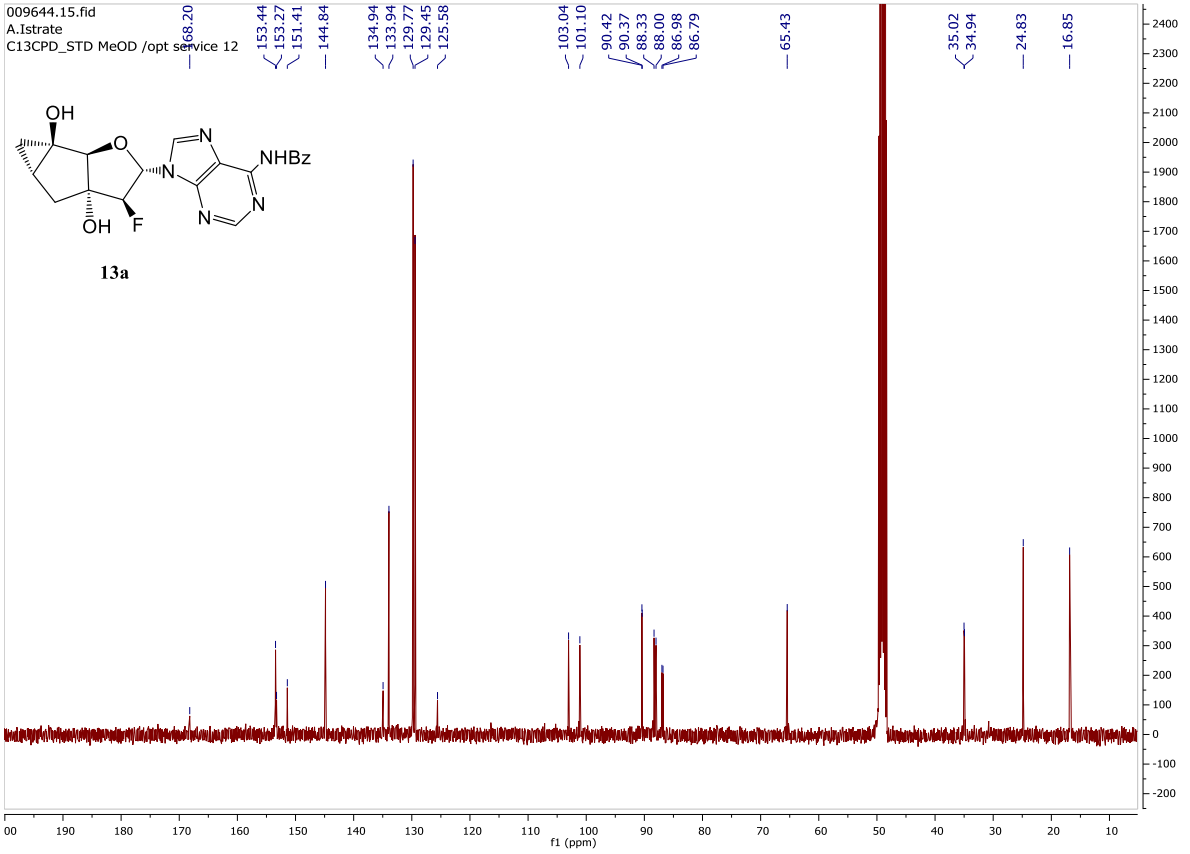
6.07
6.06
6.05
5.90
5.89
5.88
5.50
4.72
2.88
2.86
2.83
1.78
1.77
1.75
1.73
1.72
1.69
1.14
1.12
1.10
1.09
1.08
1.06
OH
O
N
N
NHBz
N
N
OH
F
13a
0.90
0.96
1.84
3.58
1.00
1.26
0.92
1.05
1.95
1.90
f1 (ppm)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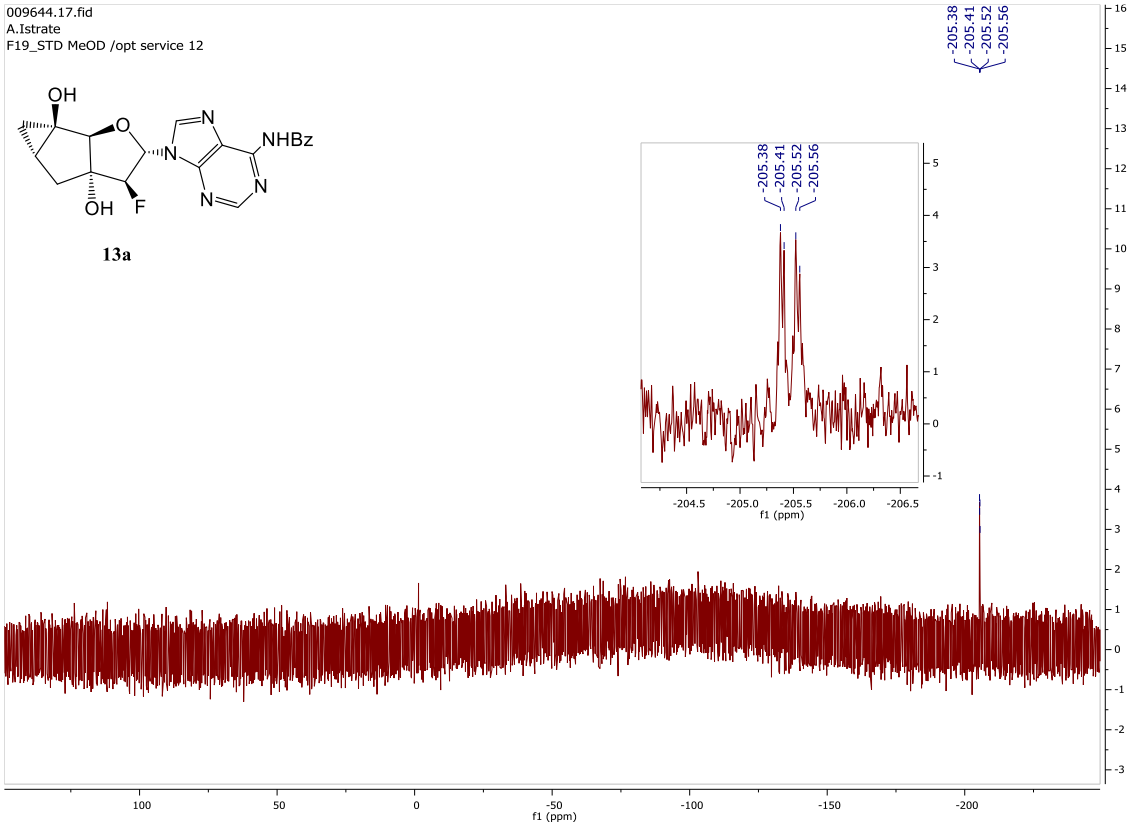
168.20
153.44
153.27
151.41
144.84
134.94
133.94
129.77
129.45
125.58
103.04
101.10
90.42
90.37
88.33
88.00
86.98
86.79
65.43
35.02
34.94
24.83
16.85
OH
O
N
N
NHBz
N
N
OH
F
13a
f1 (ppm)




## Analytical data of 13b:

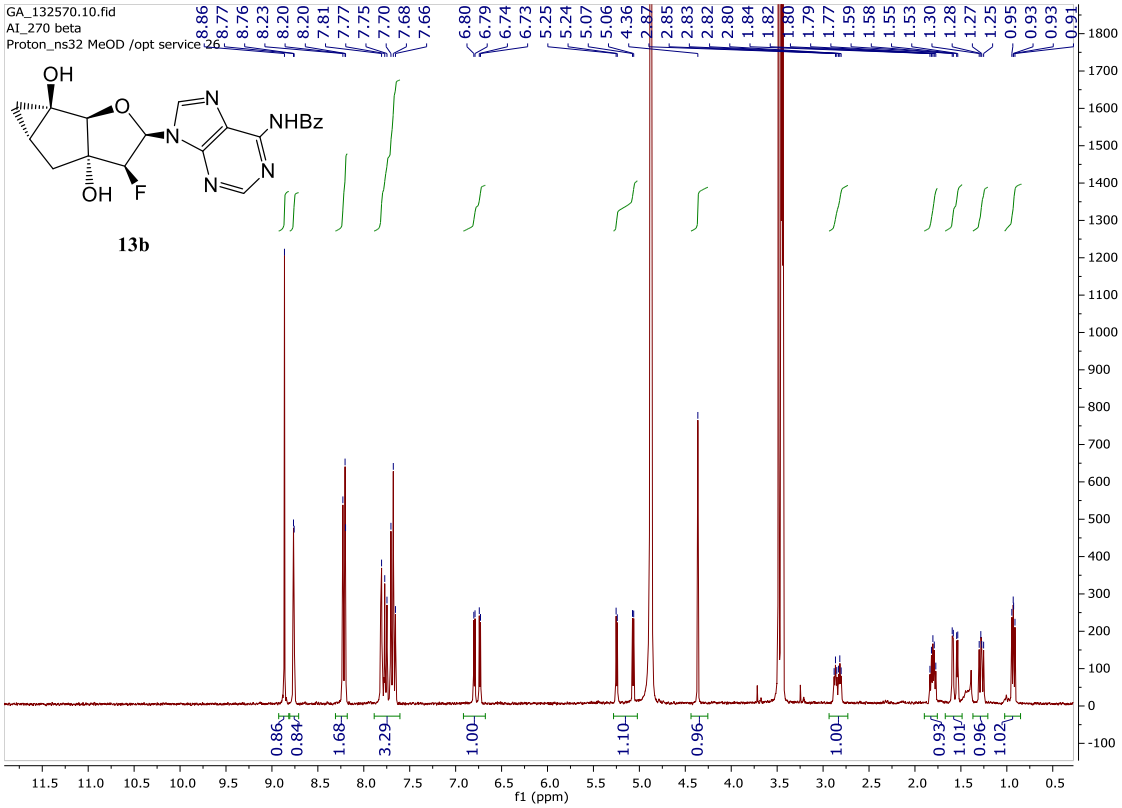
TLC (DCM/MeOH 97:3) $R_f$ = 0.43.

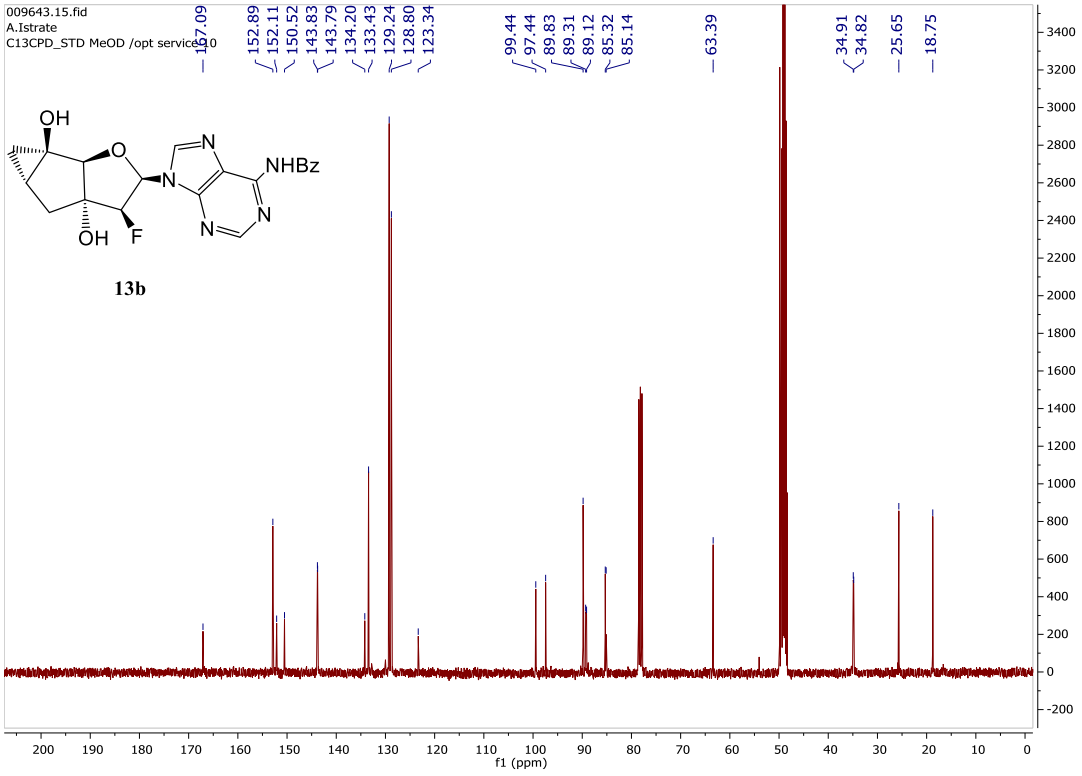
$^{1}$H NMR ($CD_3OD$, 300 MHz): δ 8.86 (*s*, 1H, H-C(2)), 8.76 (*d*, *J* = 2.1 Hz, 1H, H-C(8)), 8.25 – 8.18 (*m*, 2H, H-arom), 7.84 – 7.64 (*m*, 3H, H-arom), 6.77 (*dd*, *J* = 17.8 Hz, *J* = 3.9 Hz, 1H, H-C(1')), 5.16 (*dd*, *J* = 53.6 Hz, *J* = 3.9 Hz, 1H, H-C(2')), 4.36 (*s*, 1H, H-C(4')), 2.84 (*dt*, *J* = 14.3 Hz, *J* = 4.4 Hz, 1H, H-C(7')), 1.80 (*dt*, *J* = 9.5 Hz, *J* = 4.9 Hz, 1H, H-C(6')), 1.56 (*dd*, *J* = 14.5 Hz, *J* = 3.7 Hz, 1H, H-C(7')), 1.28 (*dd*, *J* = 8.8 Hz, *J* = 5.7 Hz, 1H, H-C(8')), 0.93 (*dd*, *J* = 5.8 Hz, *J* = 4.5 Hz, 1H, H-C(8')).

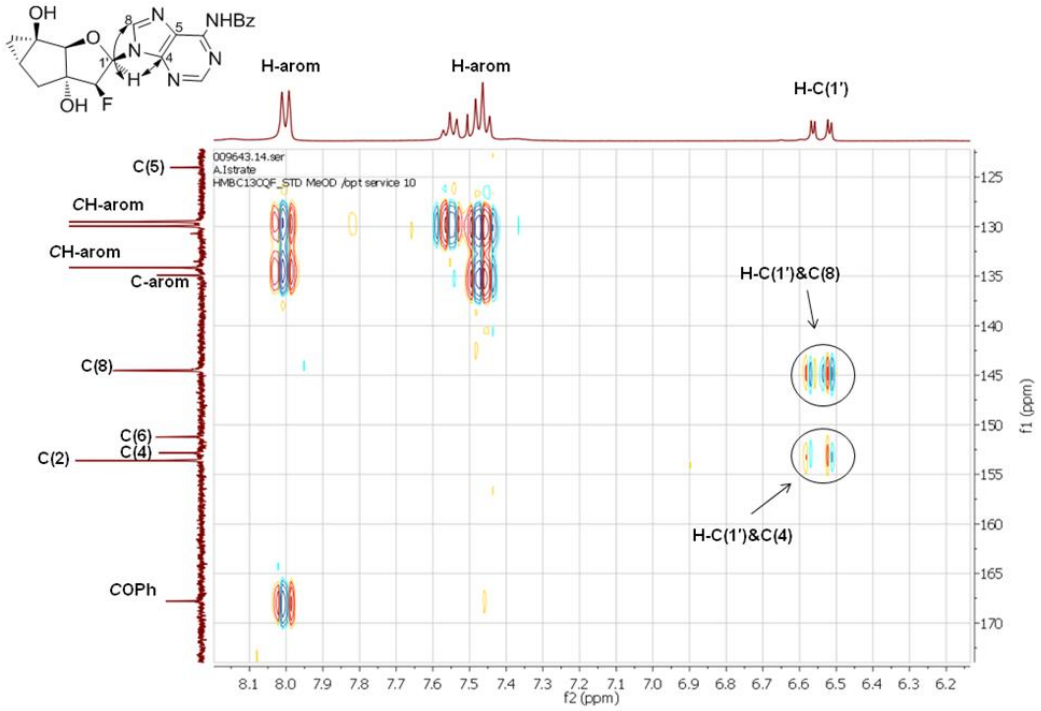
$^{13}$C NMR ($CD_3OD$, 100 MHz): δ 167.09 (*C*OPh), 152.89 (C(2)), 152.11 (C(4)), 150.22 (C(6)), 143.81 (*d*, *J* = 4.1 Hz, C(8)), 134.20 (C-arom), 133.43, 129.24, 128.80 (*C*H-arom), 123.34 (C(5)), 98.44 (*d*, *J* = 201.7 Hz, C(2')), 89.83 (C(4')), 89.22 (*d*, *J* = 18.9 Hz, C(3')), 85.23 (*d*, *J* = 18.1 Hz, C(1')), 63.99 (C(5')), 34.87 (*d*, *J* = 9.1 Hz, C(7')), 25.65 (C(6')), 18.75 (C(8')).

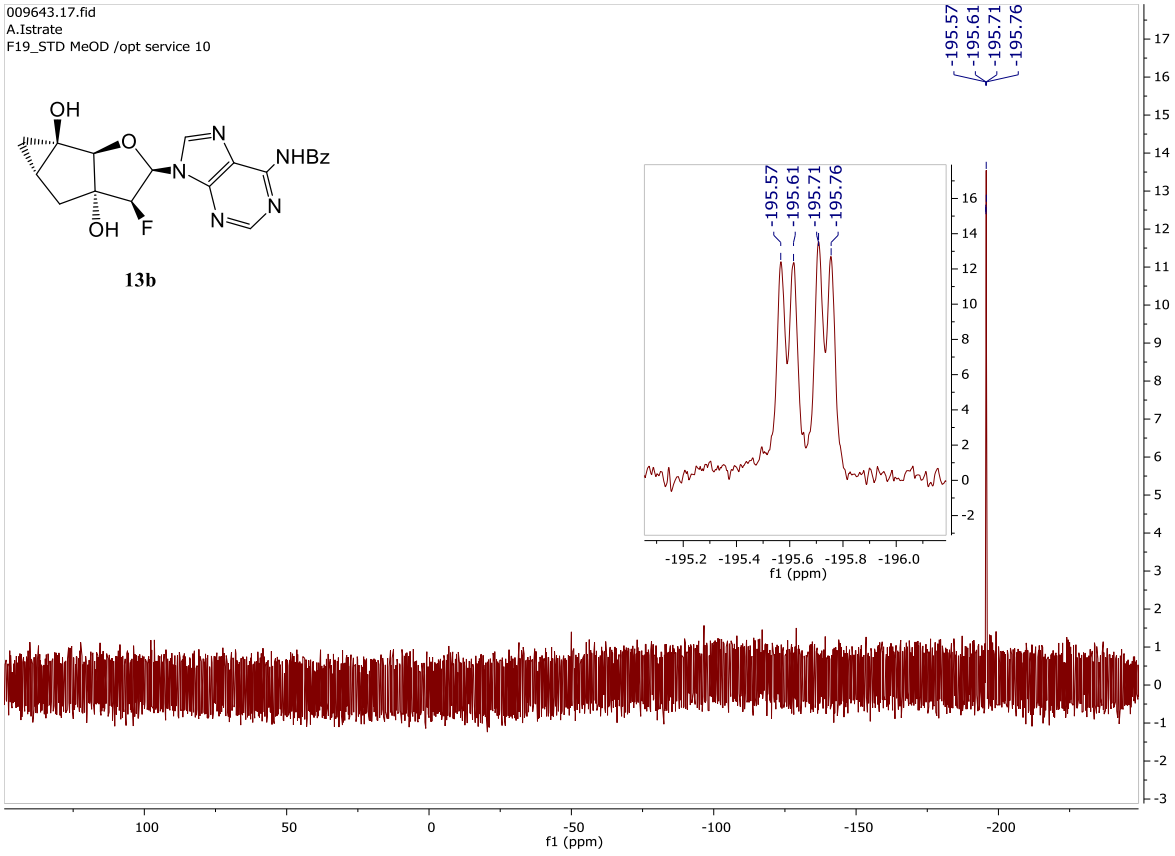
$^{19}$F NMR ($CD_3OD$, 376.5 MHz) δ -195.66 (*dd*, *J* = 53.3 Hz, *J* = 17.9 Hz).

ESI$^+$-HRMS: calculated for $C_{20}H_{19}O_4N_5F$ ($[M+H]^+$) 412.1416, found 412.1417.

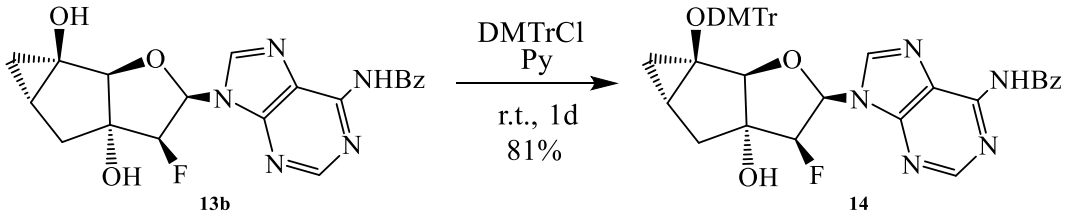

GA_132570.10.fid
AI_270 beta
Proton_ns32 MeOD /opt service 26
OH
O
N
N
NHBz
N
N
OH
F
13b
f1 (ppm)


009643.15.fid
A.Istrate
C13CPD_STD MeOD /opt service 10
OH
O
N
N
NHBz
N
N
OH
F
13b
f1 (ppm)


OH
NHBz
OH
F
H-arom
H-arom
H-C(1')
009643.14.ser
A.Istrate
HMBC13CQF_STD MeOD /opt service 10
C(5)
CH-arom
CH-arom
C-arom
C(8)
C(6)
C(4)
C(2)
COPh
H-C(1')&C(8)
H-C(1')&C(4)
f2 (ppm)
f1 (ppm)


009643.17.fid
A.Istrate
F19_STD MeOD /opt service 10
OH
NHBz
OH
F
13b
-195.57
-195.61
-195.71
-195.76
f1 (ppm)

## 2.12 1-[(5'R,6'R)-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-5'-O-((4,4'-dimethoxytriphenyl)methyl)-β-D-arabinofuranosyl]-6-N-benzoyladenine (14)




To a solution of nucleoside **13b** (725 mg, 1.76 mmol, 1 eq.) in 8 ml dry pyridine DMTr-Cl (1.79 g, 5.29 mmol, 3 eq.) was added in three equal portions over 2 h. The resulting orange solution was stirred overnight before washing with sat. $NaHCO_3$ solution (2 x 15 ml). Subsequently, the aqueous phase was extracted with $CH_2Cl_2$ (3 x 40 ml). The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude orange foam was purified by CC (DCM/MeOH 199:1 + 0.5% $Et_3N$, then DCM/MeOH 199:1 → 97:3) to yield DMT-protected nucleoside **14** (1.01 g, 81%) as a white foam.

**Analytical data of 14:**

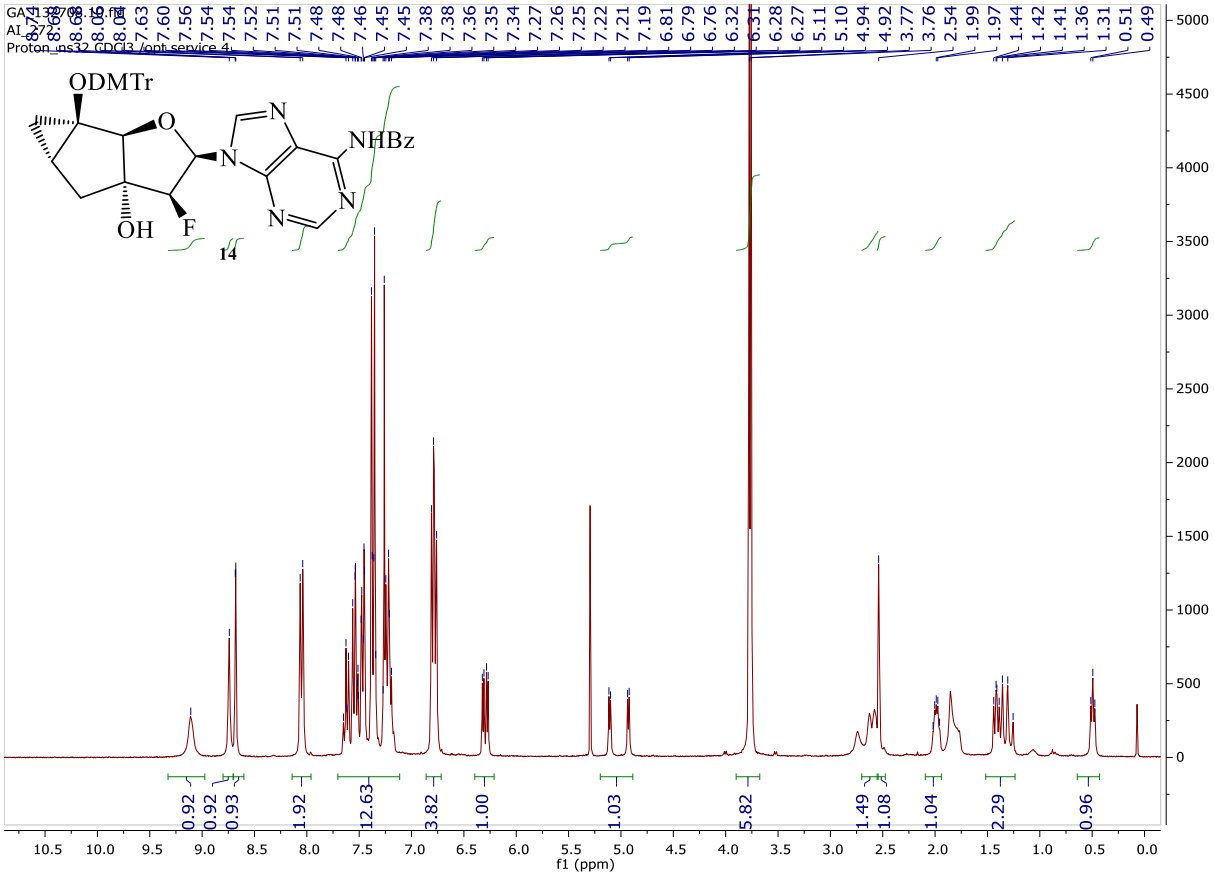
TLC (DCM/MeOH 95:5) $R_f$ = 0.26.

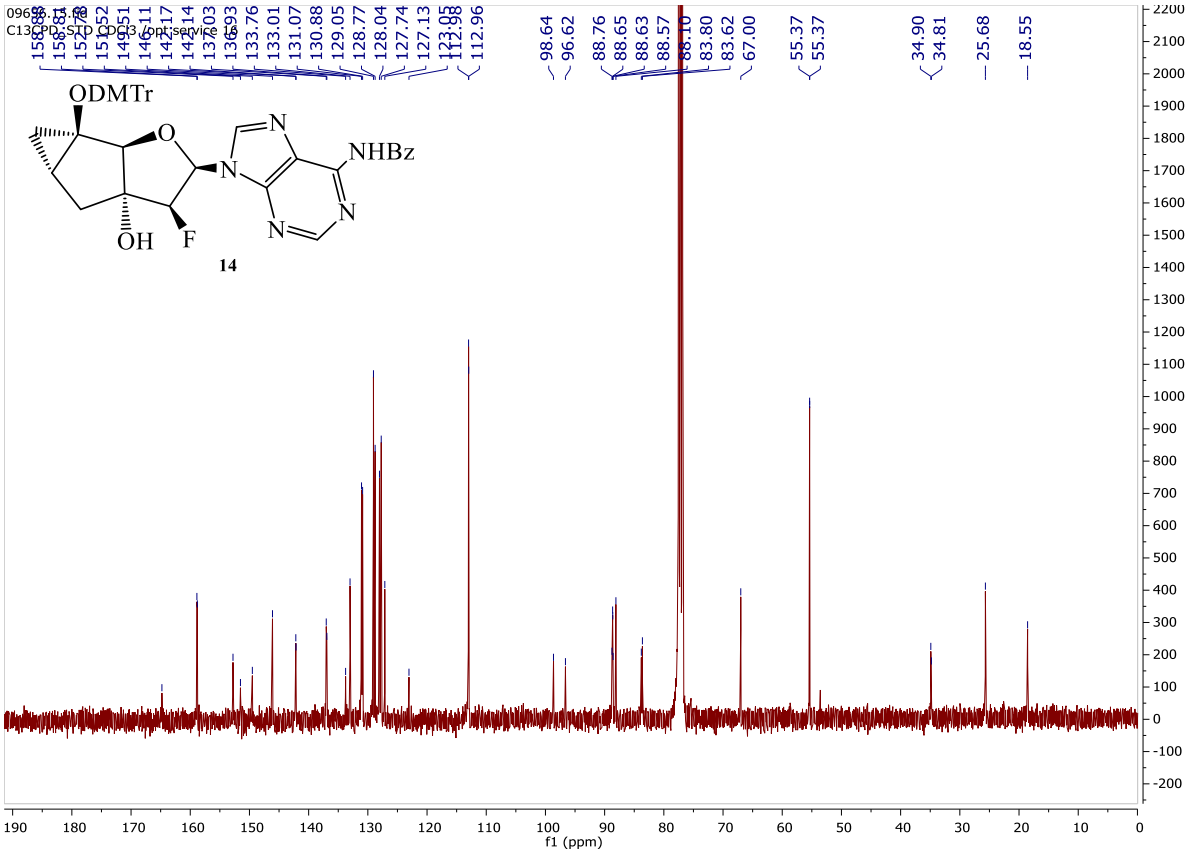
$^1$H NMR ($CDCl_3$, 300 MHz): δ 9.11 (*br*, 1H, H-N), 8.74 (*s*, 1H, H-C(2)), 8.68 (*d*, *J* = 1.1 Hz, 1H, H-C(8)), 8.05 (*d*, *J* = 7.4 Hz, 2H, H-arom), 7.67 – 7.14 (*m*, 12H, H-arom), 6.79 (*dd*, 4H, H-arom), 6.30 (*dd*, *J* = 12.0 Hz, *J* = 4.6 Hz, 1H, H-C(1')), 5.02 (*d*, *J* = 53.4 Hz, *J* = 4.6 Hz, 1H, H-C(2')), 3.77, 3.76 (2*s*, 6H, *Me*O), 2.67 – 2.54 (*m*, 1H, H-C(7')), 2.54 (*s*, 1H, H-C(4')), 1.99 (*dt*, *J* = 9.6 Hz, *J* = 4.8 Hz, 1H, H-C(6')), 1.47 – 1.22 (*m*, 2H, H-C(7'), H-C(8')), 0.53 – 0.46 (*m*, 1H, H-C(8')).

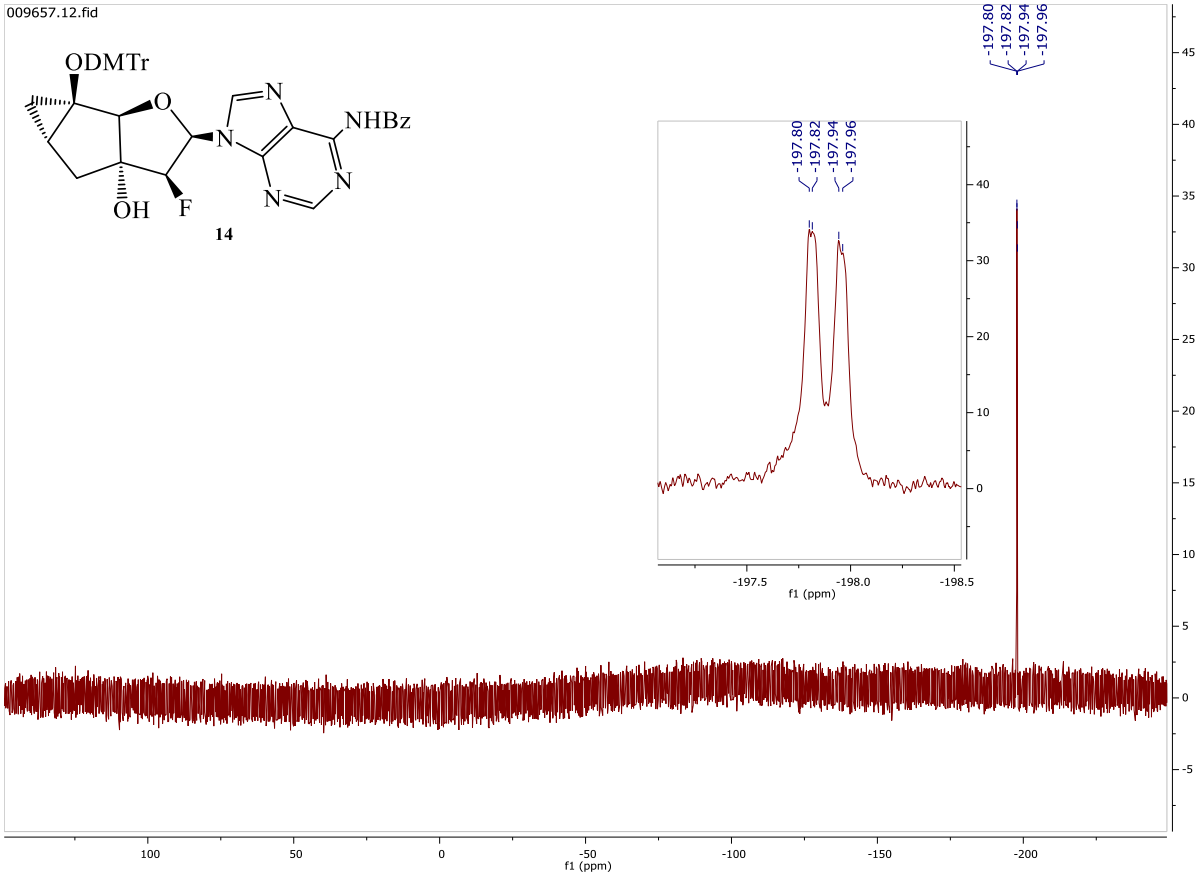
$^{13}$C NMR ($CDCl_3$, 100 MHz): δ 164.79 (*C*OPh), 158.88, 158.81 (MeO-*C*-arom), 152.78 (C(2)), 151.52 (C(4)), 149.51 (C(6)), 146.11 (C-arom), 142.15 (*d*, *J* = 2.7 Hz, C(8)), 137.03, 136.93, 133.76 (C-arom), 133.01, 131.07, 130.88, 129.05, 128.77, 128.04, 127.74, 127.13 (*C*H-arom), 123.05 (C(5)), 112.98, 112.96 (*C*H-arom), 97.63 (*d*, *J* = 203.5 Hz, C(2')), 88.82 – 88.54 (*m*, C(3'), C(4')), 88.10 (*C*(Ph)$_3$), 83.71 (*d*, *J* = 18.7 Hz, C(1')), 67.00 (C(5')), 55.37, 55.37 (*Me*O-DMTr), 34.85 (*d*, *J* = 9.5 Hz, C(7')), 25.68 (C(6')), 18.55 (C(8')).

$^{19}$F NMR ($CDCl_3$, 376.5 MHz) δ -197.73 – -198.00 (*m*).

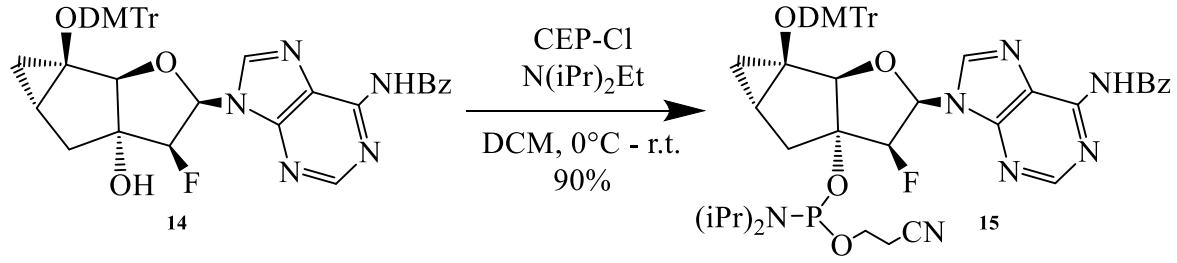
ESI$^+$-HRMS: calculated for $C_{41}H_{37}O_6N_5F$ ($[M+H]^+$) 714.2722, found 714.2714.


ODMTr
O
N
N
NHBz
N
N
OH
F
14
f1 (ppm)


ODMTr
O
N
N
NHBz
N
N
OH
F
14
f1 (ppm)


009657.12.fid
ODMTr
O
N
N
NHBz
N
N
OH
F
14
-197.80
-197.82
-197.94
-197.96
-197.80
-197.82
-197.94
-197.96
-197.5
-198.0
-198.5
f1 (ppm)
100
50
0
-50
-100
-150
-200
f1 (ppm)

## 2.13 1-[(5'R,6'R)-3'-O-(2-cyanoethoxy)-diisopropylaminophosphanyl-2'-deoxy-3',5'-ethano-2'-fluoro-5',6'-methano-5'-O-((4,4'-dimethoxytriphenyl)methyl)-β-D-arabinofuranosyl]-6-N-benzoyladenine (15)




To a solution of DMT-protected nucleoside **14** (1.01 g, 1.42 mmol, 1 eq.) and N-ethyldiisopropylamine (0.99 ml, 5.66 mmol, 4 eq.) in dry $CH_3CN$ (9 ml) at 0°C was slowly added CEP-Cl (0.63 ml, 2.83 mmol, 2 eq.) and the reaction mixture was allowed to stir at r.t. for 2 h. After that an additional portions of CEP-Cl (0.1 ml, 0.45 mmol, 0.3 eq.) and N-ethyldiisopropylamine (0.15 ml, 0.86 mmol, 0.6 eq.) were added. As monitored by TLC analysis, reaction was complete after 3.5 h at r.t. The reaction mixture was diluted with EtOAc and then washed with sat. $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc (3 x 30 ml) and the combined organic phases were dried over $MgSO_4$, filtered and evaporated. The crude yellow oil was purified by CC using hexane/EtOAc (2:3 + 0.5% $Et_3N$) → hexane/EtOAc (2:3) to afford the phosphoramidite **15** (1.17 g of a white foam, 90%) as a mixture of isomers.

**Analytical data of 15:**

TLC (hexane/EtOAc 1:3) $R_f$ = 0.71.

$^1$H NMR ($CDCl_3$, 400 MHz): δ 9.09, 9.08 (2*br*, 1H, H-N(3)), 8.80, 8.79 (2*s*, 1H, H-C(2)), 8.67 (*d*, *J* = 0.8 Hz, 0.5H, H-C(8)), 8.65 (*d*, *J* = 1.3 Hz, 0.5H, H-C(8)), 8.06 (*d*, *J* = 7.0 Hz, 2H, H-arom), 7.66 – 7.17 (*m*, 12H, H-arom), 6.86 – 6.73 (*m*, 4H, H-arom), 6.34 – 6.26 (*m*, 1H, H-C(1')), 5.47 – 5.30 (*m*, 1H, H-C(2')), 3.79, 3.79, 3.77 (3*s*, 6H, *Me*O), 3.75 – 3.67, 3.64 – 3.53 (2*m*, 2H, OC*H*$_2$CH$_2$CN), 3.55 – 3.42 (*m*, 2H, (Me$_2$C*H*)$_2$N), 2.79 (*d*, *J* = 2.5 Hz, 0.5H, H-C(4')), 2.63 – 2.48 (*m*, 3.0H, OCH$_2$C*H*$_2$CN, H-C(7'), H-C(4')), 2.40 (*dt*, *J* = 15.3 Hz, *J* = 4.2 Hz, 0.5H, H-C(7')), 2.09 (*dd*, *J* = 15.3 Hz, *J* = 5.8 Hz, 0.5H, H-C(7')), 2.04 – 1.94 (*m*, 1H, H-C(6')), 1.75 (*d*, *J* = 15.1 Hz, 0.5H, H-C(7')), 1.37 (*dd*, *J* = 9.3 Hz, *J* = 6.3 Hz, 1H, H-C(8')), 1.19 – 1.03 (*m*, 12H, (*Me*$_2$CH)$_2$N), 0.65 – 0.56 (*m*, 1H, H-C(8')).

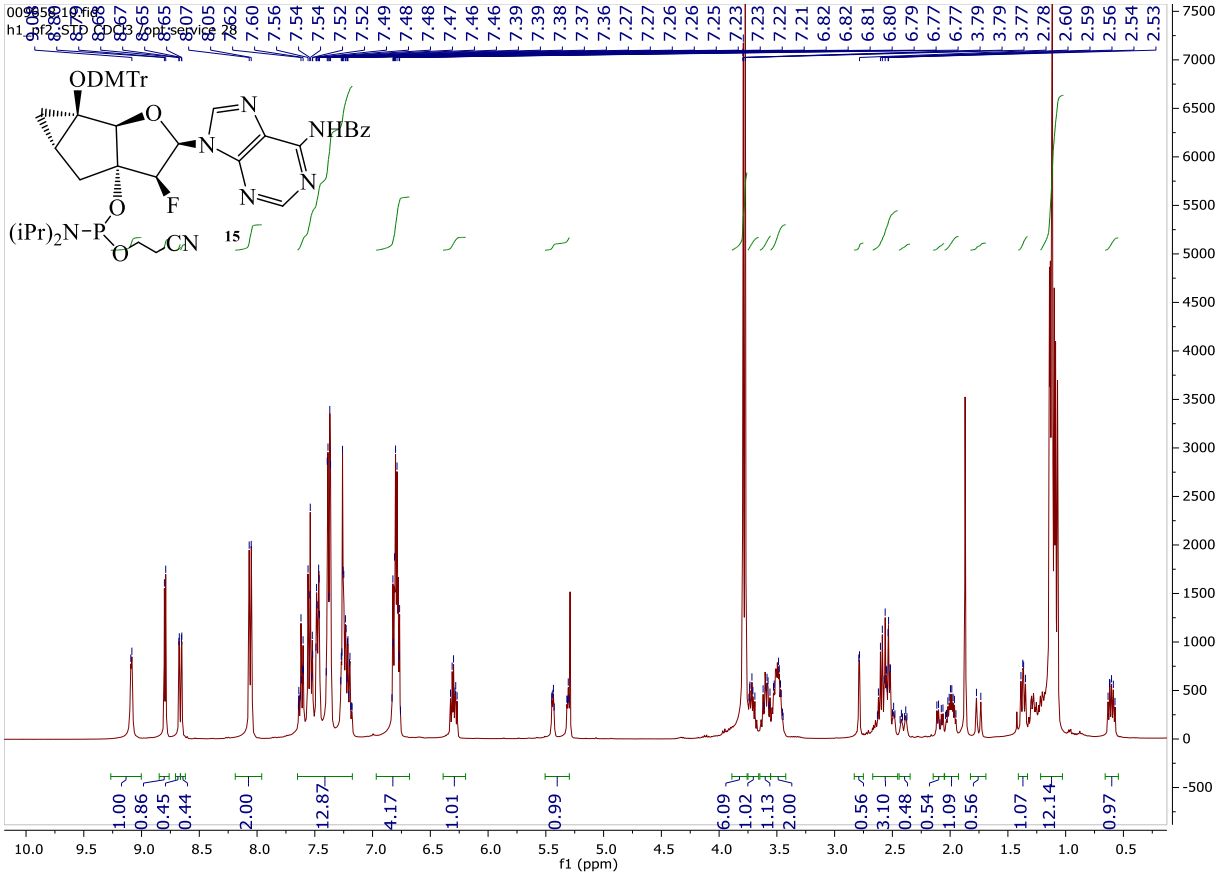
$^{13}$C NMR ($CDCl_3$, 100 MHz): δ 164.66 (*C*OPh), 158.90, 158.87, 158.81, 158.78 (MeO-*C*-arom), 152.85 (C(2)), 151.69, 151.63 (C(4)), 149.52, 149.49 (C(6)), 146.21, 146.09 (C-arom), 142.09 (*d*, *J* = 2.5 Hz, C(8)), 142.02 (*d*, *J* = 2.0 Hz, C(8)), 137.04, 137.01, 139.89, 133.84 (C-arom), 132.93, 131.16, 131.09, 130.92, 130.86, 129.04, 128.77, 128.70, 128.00, 127.77, 127.12, 127.03 (*C*H-arom), 123.20, 123.08

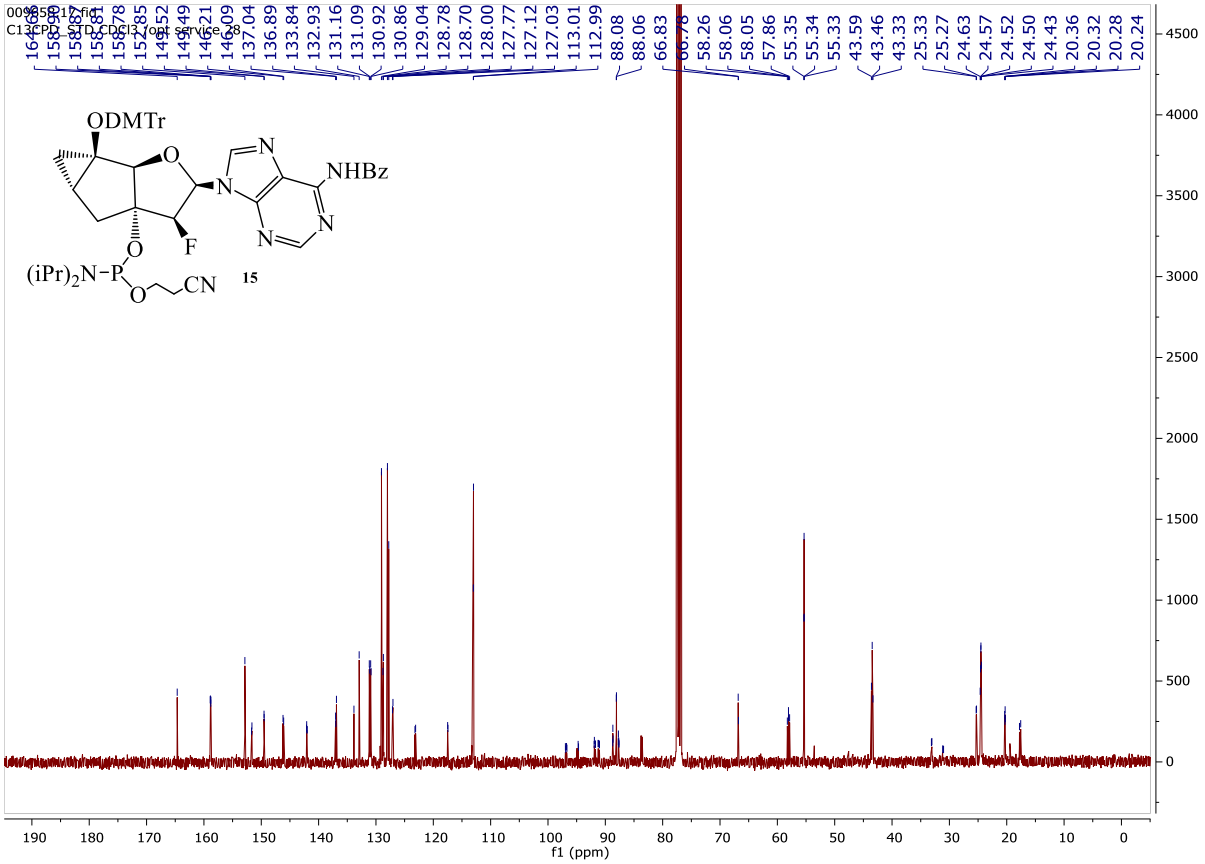
(C(5)), 117.50, 117.45 (CN), 113.01, 112.99 (*C*H-arom), 95.92 (*dd*, *J*= 204.7 Hz, *J* = 4.2 Hz, C(2')), 95.72 (*dd*, *J*= 203.6 Hz, *J* = 7.1 Hz, C(2')), 91.58 (*dd*, *J* = 19.3 Hz, *J* = 4.3 Hz, C(3')), 91.31 – 90.98 (*m*, C(3')), 88.74 – 88.63 (*m*, C(4')), 88.08, 88.06 (*C*$(Ph)_3$), 87.77 – 87.62 (*m*, C(4')), 83.73 (*d*, *J* = 19.0 Hz, C(1')), 83.64 (*d*, *J* = 18.7 Hz, C(1')), 66.83, 66.78 (C(5')), 58.26, 58.06, 58.05, 57.86 ($OCH_2CH_2CN$), 55.38 – 55.31 (*m*, *Me*O-DMTr), 43.62 – 43.30 (*m*, $(Me_2CH)_2N$), 33.2 – 33.0, 31.31 – 30.96 (2*m*, C(7')), 25.33, 25.27 (C(6')), 24.66 – 24.36 (*m*, $(Me_2CH)_2N$)), 20.38 – 20.21 (*m*, $OCH_2CH_2CN$), 17.78, 17.57 (C(8')).

$^{19}F$ NMR ($CDCl_3$, 376.5 MHz) δ -195.94 (*dt*, *J* = 52.4 Hz, *J* = 10.1 Hz), -198.08 (*d*, *J* = 52.0 Hz).

$^{31}P$ NMR (122 MHz, $CDCl_3$) δ 144.23 (*d*, *J* = 11.3 Hz), 142.94 (*d*, *J* = 6.6 Hz).

$ESI^+$-HRMS: calculated for $C_{50}H_{54}O_7N_7FP$ ($[M+H]^+$) 914.3801, found 914.3782.

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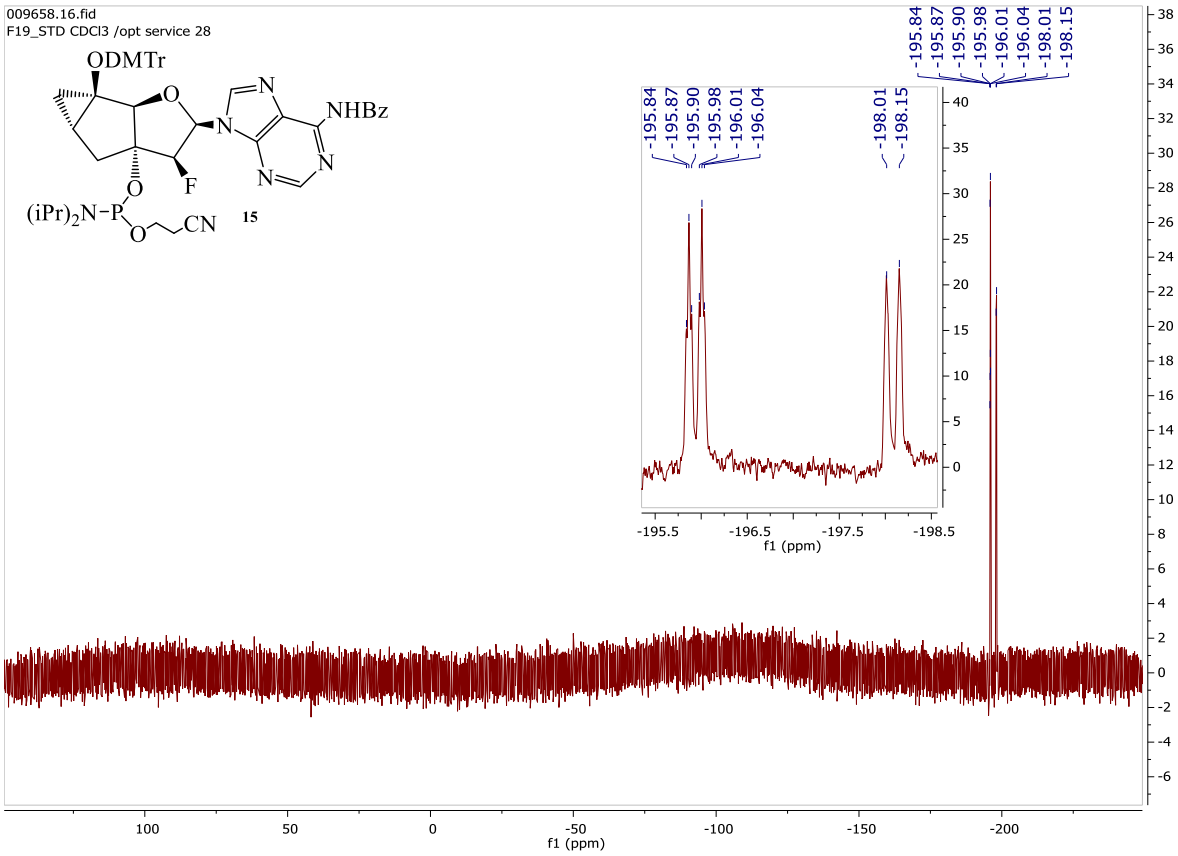
NHBz
F
(iPr)2N-P
CN
15
f1 (ppm)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F19_STD CDCl3 /opt service 28
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NHBz
F
(iPr)2N-P
CN
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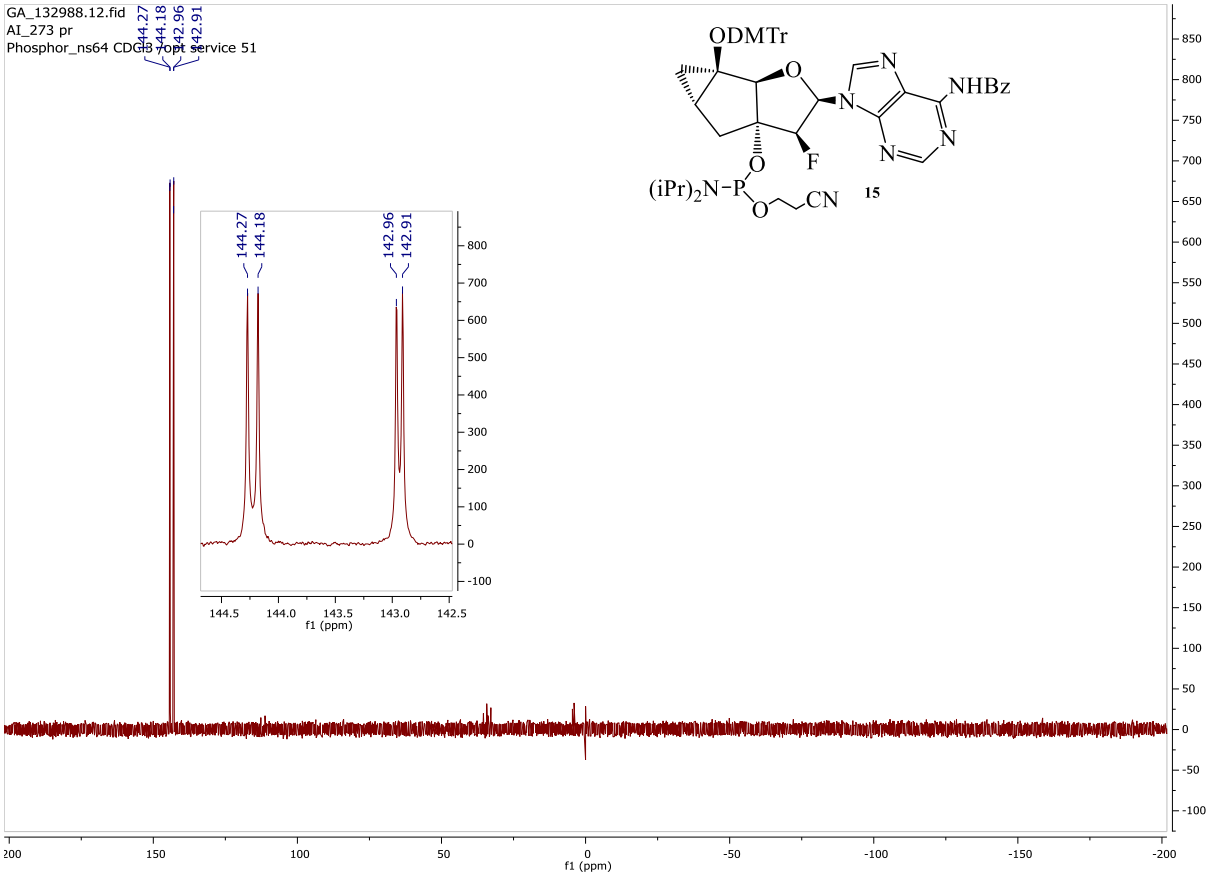
-196.04
-198.01
-198.15
f1 (ppm)


GA_132988.12.fid
AI_273 pr
Phosphor_ns64 CDCl3 root service 51
144.27
144.18
142.96
142.91
ODMTr
NHBz
(iPr)2N-P
CN
15
f1 (ppm)

## 3. X-ray structure analysis of the 2′F-tc-ANA-T nucleoside 5b

A colorless transparent crystal of $C_{13}H_{15}FN_2O_5$ •0.375 $H_2O$ (**5b**) was mounted in air and used for X-ray structure determination. All measurements were made on an *Oxford Diffraction SuperNova* area-detector diffractometer using mirror optics monochromated Mo *K*α radiation (λ = 0.71073 Å) and Al filtered (1,2). The unit cell constants and an orientation matrix for data collection were obtained from a least-squares refinement of the setting angles of reflections in the range $1.7° < \theta < 27.2°$. A total of 568 frames were collected using ω scans, with 7.5+7.5 seconds exposure time, a rotation angle of 1.0° per frame, a crystal-detector distance of 65 mm, at T = 173(2) K.

Data reduction was performed using the *CrysAlisPro* program. The intensities were corrected for Lorentz and polarization effects, and an absorption correction based on the multi-scan method using SCALE3 ABSPACK in *CrysAlisPro* was applied (1). Data collection and refinement parameters are given in **Table S1**.

The structure was solved by direct methods using *SHELXS-97*, which revealed the positions of all non-hydrogen atoms of the title compound (3). The non-hydrogen atoms were refined anisotropically. All H-atoms were placed in geometrically calculated positions and refined using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.5 Ueq of its parent atom.

The unit cell contains 4 independent molecules and 1.5 water molecules. One water molecule is disorder about a two-fold axis. Absolute configuration cannot be determined with this experiment.

Refinement of the structure was carried out on $F^2$ using full-matrix least-squares procedures, which minimized the function $\Sigma w(F_o^2 - F_c^2)^2$. The weighting scheme was based on counting statistics and included a factor to downweight the intense reflections.

All calculations were performed using the *SHELXL-97* program.

**Table S1. Crystal data and structure refinement for 5b.**

| | | |
|---|---|---|
| Identification code | shelx | |
| Empirical formula | C52 H62 F4 N8 O21 | |
| Formula weight | 1211.09 | |
| Temperature | 173(2) K | |
| Wavelength | 0.71069 Å | |
| Crystal system | Monoclinic | |
| Space group | C 2 | |
| Unit cell dimensions | a = 39.5150(10) Å | $\alpha$= 90°. |
| | b = 12.4690(2) Å | $\beta$= 113.266(2)°. |
| | c = 11.6820(2) Å | $\gamma$ = 90°. |
| Volume | 5287.8(2) $Å^3$ | |
| Z | 4 | |
| Density (calculated) | 1.521 $Mg/m^3$ | |
| Absorption coefficient | 0.127 $mm^{-1}$ | |
| F(000) | 2536 | |
| Crystal size | 0.3754 x 0.3368 x 0.2046 $mm^3$ | |
| Theta range for data collection | 1.727 to 27.283° | |
| Index ranges | -49<=h<=49, -15<=k<=15, -14<=l<=14 | |
| Reflections collected | 21257 | |
| Independent reflections | 10216 [R(int) = 0.0185] | |
| Completeness to theta = 25.000° | 99.7 % | |
| Absorption correction | Gaussian | |
| Max. and min. transmission | 1 and 0.575 | |
| Refinement method | Full-matrix least-squares on $F^2$ | |
| Data / restraints / parameters | 10216 / 7 / 788 | |
| Goodness-of-fit on $F^2$ | 0.981 | |
| Final R indices [I>2sigma(I)] | R1 = 0.0327, wR2 = 0.0757 | |
| R indices (all data) | R1 = 0.0361, wR2 = 0.0783 | |
| Absolute structure parameter | 0.3(2) | |
| Extinction coefficient | n/a | |
| Largest diff. peak and hole | 0.221 and -0.318 $e.Å^{-3}$ | |

**Table S2. Atomic coordinates ( x $10^4$) and equivalent isotropic displacement parameters ($Å^2$x $10^3$) for 5b. U(eq) is defined as one third of the trace of the orthogonalized $U^{ij}$ tensor.**

| | x | y | z | U(eq) |
|---|---|---|---|---|
| C(1) | 5412(1) | 5319(2) | 7743(2) | 22(1) |
| C(2) | 5442(1) | 4268(2) | 7276(2) | 23(1) |
| C(3) | 5472(1) | 4203(2) | 6168(2) | 23(1) |
| N(2) | 5469(1) | 5101(2) | 5471(2) | 20(1) |
| C(4) | 5458(1) | 6124(2) | 5898(2) | 21(1) |
| C(5) | 5442(1) | 3313(2) | 8049(3) | 33(1) |
| C(6) | 5556(1) | 5028(2) | 4370(2) | 21(1) |
| C(7) | 5963(1) | 5162(2) | 4651(2) | 24(1) |
| C(8) | 6004(1) | 4551(2) | 3560(2) | 21(1) |
| C(12) | 5626(1) | 3989(2) | 2921(2) | 20(1) |
| C(11) | 5703(1) | 2872(2) | 2607(2) | 25(1) |
| C(10) | 6113(1) | 2684(2) | 3208(3) | 31(1) |
| C(9) | 6298(1) | 3660(2) | 3954(3) | 28(1) |
| C(111) | 7960(1) | 802(2) | -1663(2) | 21(1) |
| C(112) | 8071(1) | 1851(2) | -994(2) | 20(1) |
| C(106) | 8222(1) | 2697(2) | 816(2) | 20(1) |
| C(103) | 8180(1) | 1826(2) | 2652(2) | 21(1) |
| C(102) | 8138(1) | 1835(2) | 3741(2) | 21(1) |
| C(105) | 8119(1) | 845(2) | 4437(2) | 25(1) |
| C(101) | 8103(1) | 2866(2) | 4258(2) | 21(1) |
| C(104) | 8161(1) | 3746(2) | 2455(2) | 20(1) |
| C(108) | 8500(1) | 1904(2) | -474(2) | 21(1) |
| C(107) | 8598(1) | 2418(2) | 827(2) | 25(1) |
| C(109) | 8628(1) | 732(2) | -458(2) | 25(1) |
| C(110) | 8300(1) | 129(2) | -1360(2) | 25(1) |
| C(205) | 5503(1) | 10356(2) | -928(3) | 31(1) |
| C(202) | 5487(1) | 9377(2) | -199(2) | 23(1) |
| C(203) | 5497(1) | 9415(2) | 963(2) | 23(1) |
| C(204) | 5468(1) | 7494(2) | 1131(2) | 23(1) |
| C(207) | 5925(1) | 8558(2) | 3849(2) | 31(1) |
| C(206) | 5523(1) | 8557(2) | 2910(2) | 24(1) |
| C(212) | 5496(1) | 9574(2) | 4452(2) | 22(1) |
| C(211) | 5503(1) | 10703(2) | 4906(2) | 22(1) |
| C(210) | 5891(1) | 11104(2) | 5408(2) | 25(1) |

| | | | | |
|---|---|---|---|---|
| C(209) | 6134(1) | 10251(2) | 5234(2) | 25(1) |
| C(208) | 5907(1) | 9210(2) | 4950(2) | 23(1) |
| C(201) | 5457(1) | 8337(2) | -786(2) | 23(1) |
| C(311) | 7181(1) | 3492(2) | -687(2) | 21(1) |
| C(310) | 6813(1) | 4038(2) | -1222(2) | 26(1) |
| C(312) | 7125(1) | 2339(2) | -430(2) | 20(1) |
| C(308) | 6703(1) | 2131(2) | -1069(2) | 22(1) |
| C(307) | 6631(1) | 1396(2) | -124(2) | 27(1) |
| C(306) | 7014(1) | 1246(2) | 902(2) | 23(1) |
| C(303) | 7124(1) | 1976(2) | 3006(2) | 22(1) |
| C(302) | 7150(1) | 1886(2) | 4185(2) | 21(1) |
| C(305) | 7264(1) | 2780(2) | 5117(2) | 27(1) |
| C(301) | 7071(1) | 850(2) | 4583(2) | 23(1) |
| C(304) | 6927(1) | 153(2) | 2474(2) | 25(1) |
| C(309) | 6519(1) | 3249(2) | -1263(3) | 27(1) |
| N(1) | 5410(1) | 6174(2) | 6996(2) | 22(1) |
| N(101) | 8105(1) | 3737(2) | 3539(2) | 23(1) |
| N(102) | 8191(1) | 2743(2) | 2013(2) | 20(1) |
| N(201) | 5438(1) | 7471(2) | -71(2) | 24(1) |
| N(202) | 5479(1) | 8504(2) | 1615(2) | 23(1) |
| N(301) | 6963(1) | 58(2) | 3689(2) | 26(1) |
| N(302) | 7025(1) | 1136(2) | 2174(2) | 24(1) |
| O(1) | 5384(1) | 5485(2) | 8747(2) | 30(1) |
| O(2) | 5489(1) | 6915(2) | 5334(2) | 28(1) |
| O(3) | 5469(1) | 3987(1) | 3852(2) | 22(1) |
| O(4) | 6084(1) | 5269(2) | 2757(2) | 29(1) |
| C(13) | 5923(1) | 2834(2) | 1821(3) | 33(1) |
| O(27) | 5442(1) | 2066(2) | 2440(2) | 28(1) |
| O(101) | 8183(1) | 4573(1) | 1935(2) | 26(1) |
| O(102) | 8074(1) | 2993(2) | 5257(2) | 28(1) |
| C(113) | 8109(1) | 611(2) | -2640(2) | 28(1) |
| O(103) | 7987(1) | 1865(1) | 94(2) | 20(1) |
| O(105) | 8638(1) | 2516(2) | -1214(2) | 30(1) |
| O(104) | 7619(1) | 361(2) | -1805(2) | 28(1) |
| C(213) | 5706(1) | 10836(2) | 6279(2) | 33(1) |
| O(202) | 5476(1) | 6674(2) | 1724(2) | 31(1) |
| O(201) | 5447(1) | 8187(2) | -1838(2) | 32(1) |
| O(203) | 5377(1) | 9542(1) | 3117(2) | 23(1) |

| | | | | |
|---|---|---|---|---|
| O(204) | 6022(1) | 8588(2) | 6058(2) | 31(1) |
| O(205) | 5203(1) | 11380(2) | 4271(2) | 28(1) |
| O(301) | 7094(1) | 662(2) | 5649(2) | 30(1) |
| O(302) | 6819(1) | -579(2) | 1722(2) | 36(1) |
| C(313) | 7021(1) | 3785(2) | -2038(2) | 30(1) |
| O(303) | 7212(1) | 2185(1) | 880(2) | 22(1) |
| O(304) | 6597(1) | 1659(2) | -2258(2) | 30(1) |
| O(305) | 7505(1) | 4008(2) | 61(2) | 27(1) |
| F(1) | 6162(1) | 4701(2) | 5804(1) | 39(1) |
| F(101) | 8776(1) | 1701(2) | 1804(1) | 36(1) |
| F(201) | 6142(1) | 9038(2) | 3307(2) | 49(1) |
| F(301) | 6401(1) | 1869(2) | 376(2) | 44(1) |
| O(1S) | 5000 | 2254(3) | 0 | 42(1) |
| O(2S) | 5000 | 4418(2) | 0 | 32(1) |

**Table S3. Bond lengths [Å] and angles [°] for 5b.**

| | |
|---|---|
| C(1)-O(1) | 1.237(3) |
| C(1)-N(1) | 1.376(3) |
| C(1)-C(2) | 1.443(4) |
| C(2)-C(3) | 1.346(4) |
| C(2)-C(5) | 1.494(4) |
| C(3)-N(2) | 1.382(3) |
| N(2)-C(4) | 1.376(3) |
| N(2)-C(6) | 1.460(3) |
| C(4)-O(2) | 1.219(3) |
| C(4)-N(1) | 1.371(3) |
| C(6)-O(3) | 1.416(3) |
| C(6)-C(7) | 1.518(4) |
| C(7)-F(1) | 1.389(3) |
| C(7)-C(8) | 1.548(4) |
| C(8)-O(4) | 1.417(3) |
| C(8)-C(9) | 1.540(4) |
| C(8)-C(12) | 1.551(4) |
| C(12)-O(3) | 1.449(3) |
| C(12)-C(11) | 1.501(4) |
| C(11)-O(27) | 1.396(3) |
| C(11)-C(13) | 1.494(4) |

| | |
|---|---|
| C(11)-C(10) | 1.510(4) |
| C(10)-C(13) | 1.504(4) |
| C(10)-C(9) | 1.508(4) |
| C(111)-O(104) | 1.403(3) |
| C(111)-C(113) | 1.496(4) |
| C(111)-C(112) | 1.498(3) |
| C(111)-C(110) | 1.501(4) |
| C(112)-O(103) | 1.436(3) |
| C(112)-C(108) | 1.561(4) |
| C(106)-O(103) | 1.425(3) |
| C(106)-N(102) | 1.452(3) |
| C(106)-C(107) | 1.522(4) |
| C(103)-C(102) | 1.346(4) |
| C(103)-N(102) | 1.376(3) |
| C(102)-C(101) | 1.449(3) |
| C(102)-C(105) | 1.497(4) |
| C(101)-O(102) | 1.228(3) |
| C(101)-N(101) | 1.375(3) |
| C(104)-O(101) | 1.217(3) |
| C(104)-N(101) | 1.367(3) |
| C(104)-N(102) | 1.376(3) |
| C(108)-O(105) | 1.414(3) |
| C(108)-C(109) | 1.543(4) |
| C(108)-C(107) | 1.551(3) |
| C(107)-F(101) | 1.401(3) |
| C(109)-C(110) | 1.511(4) |
| C(110)-C(113) | 1.508(4) |
| C(205)-C(202) | 1.504(4) |
| C(202)-C(203) | 1.343(4) |
| C(202)-C(201) | 1.450(4) |
| C(203)-N(202) | 1.385(3) |
| C(204)-O(202) | 1.229(3) |
| C(204)-N(201) | 1.362(3) |
| C(204)-N(202) | 1.374(3) |
| C(207)-F(201) | 1.387(4) |
| C(207)-C(206) | 1.531(4) |
| C(207)-C(208) | 1.547(4) |
| C(206)-O(203) | 1.418(3) |

| | |
|---|---|
| C(206)-N(202) | 1.454(3) |
| C(212)-O(203) | 1.440(3) |
| C(212)-C(211) | 1.502(4) |
| C(212)-C(208) | 1.559(4) |
| C(211)-O(205) | 1.404(3) |
| C(211)-C(213) | 1.493(3) |
| C(211)-C(210) | 1.495(4) |
| C(210)-C(209) | 1.500(4) |
| C(210)-C(213) | 1.505(4) |
| C(209)-C(208) | 1.539(4) |
| C(208)-O(204) | 1.421(3) |
| C(201)-O(201) | 1.229(3) |
| C(201)-N(201) | 1.384(3) |
| C(311)-O(305) | 1.391(3) |
| C(311)-C(313) | 1.495(3) |
| C(311)-C(310) | 1.501(4) |
| C(311)-C(312) | 1.502(4) |
| C(310)-C(309) | 1.507(4) |
| C(310)-C(313) | 1.518(4) |
| C(312)-O(303) | 1.444(3) |
| C(312)-C(308) | 1.555(4) |
| C(308)-O(304) | 1.412(3) |
| C(308)-C(307) | 1.547(4) |
| C(308)-C(309) | 1.547(4) |
| C(307)-F(301) | 1.389(3) |
| C(307)-C(306) | 1.528(4) |
| C(306)-O(303) | 1.412(3) |
| C(306)-N(302) | 1.476(3) |
| C(303)-C(302) | 1.345(3) |
| C(303)-N(302) | 1.377(3) |
| C(302)-C(301) | 1.448(4) |
| C(302)-C(305) | 1.497(4) |
| C(301)-O(301) | 1.235(3) |
| C(301)-N(301) | 1.377(3) |
| C(304)-O(302) | 1.220(3) |
| C(304)-N(302) | 1.372(3) |
| C(304)-N(301) | 1.375(3) |

| | |
|---|---|
| O(1)-C(1)-N(1) | 119.5(2) |
| O(1)-C(1)-C(2) | 124.2(2) |
| N(1)-C(1)-C(2) | 116.3(2) |
| C(3)-C(2)-C(1) | 118.0(2) |
| C(3)-C(2)-C(5) | 123.7(2) |
| C(1)-C(2)-C(5) | 118.3(2) |
| C(2)-C(3)-N(2) | 122.3(2) |
| C(4)-N(2)-C(3) | 122.1(2) |
| C(4)-N(2)-C(6) | 115.3(2) |
| C(3)-N(2)-C(6) | 121.3(2) |
| O(2)-C(4)-N(1) | 123.4(2) |
| O(2)-C(4)-N(2) | 122.1(2) |
| N(1)-C(4)-N(2) | 114.6(2) |
| O(3)-C(6)-N(2) | 109.3(2) |
| O(3)-C(6)-C(7) | 104.6(2) |
| N(2)-C(6)-C(7) | 113.6(2) |
| F(1)-C(7)-C(6) | 108.1(2) |
| F(1)-C(7)-C(8) | 113.0(2) |
| C(6)-C(7)-C(8) | 102.5(2) |
| O(4)-C(8)-C(9) | 108.4(2) |
| O(4)-C(8)-C(7) | 110.9(2) |
| C(9)-C(8)-C(7) | 114.9(2) |
| O(4)-C(8)-C(12) | 112.96(19) |
| C(9)-C(8)-C(12) | 106.7(2) |
| C(7)-C(8)-C(12) | 102.9(2) |
| O(3)-C(12)-C(11) | 111.7(2) |
| O(3)-C(12)-C(8) | 104.90(18) |
| C(11)-C(12)-C(8) | 106.7(2) |
| O(27)-C(11)-C(13) | 117.8(2) |
| O(27)-C(11)-C(12) | 119.5(2) |
| C(13)-C(11)-C(12) | 113.8(2) |
| O(27)-C(11)-C(10) | 123.4(2) |
| C(13)-C(11)-C(10) | 60.1(2) |
| C(12)-C(11)-C(10) | 108.4(2) |
| C(13)-C(10)-C(9) | 116.4(2) |
| C(13)-C(10)-C(11) | 59.42(19) |
| C(9)-C(10)-C(11) | 109.3(2) |
| C(10)-C(9)-C(8) | 105.9(2) |

| | |
|---|---|
| O(104)-C(111)-C(113) | 119.8(2) |
| O(104)-C(111)-C(112) | 118.3(2) |
| C(113)-C(111)-C(112) | 114.5(2) |
| O(104)-C(111)-C(110) | 122.4(2) |
| C(113)-C(111)-C(110) | 60.43(18) |
| C(112)-C(111)-C(110) | 108.3(2) |
| O(103)-C(112)-C(111) | 110.5(2) |
| O(103)-C(112)-C(108) | 104.54(18) |
| C(111)-C(112)-C(108) | 106.8(2) |
| O(103)-C(106)-N(102) | 108.4(2) |
| O(103)-C(106)-C(107) | 104.2(2) |
| N(102)-C(106)-C(107) | 116.9(2) |
| C(102)-C(103)-N(102) | 123.2(2) |
| C(103)-C(102)-C(101) | 118.0(2) |
| C(103)-C(102)-C(105) | 123.9(2) |
| C(101)-C(102)-C(105) | 118.2(2) |
| O(102)-C(101)-N(101) | 120.3(2) |
| O(102)-C(101)-C(102) | 124.8(2) |
| N(101)-C(101)-C(102) | 114.9(2) |
| O(101)-C(104)-N(101) | 122.5(2) |
| O(101)-C(104)-N(102) | 123.2(2) |
| N(101)-C(104)-N(102) | 114.2(2) |
| O(105)-C(108)-C(109) | 108.4(2) |
| O(105)-C(108)-C(107) | 111.6(2) |
| C(109)-C(108)-C(107) | 115.2(2) |
| O(105)-C(108)-C(112) | 113.9(2) |
| C(109)-C(108)-C(112) | 105.4(2) |
| C(107)-C(108)-C(112) | 102.2(2) |
| F(101)-C(107)-C(106) | 109.1(2) |
| F(101)-C(107)-C(108) | 112.6(2) |
| C(106)-C(107)-C(108) | 102.8(2) |
| C(110)-C(109)-C(108) | 106.0(2) |
| C(111)-C(110)-C(113) | 59.60(17) |
| C(111)-C(110)-C(109) | 109.2(2) |
| C(113)-C(110)-C(109) | 116.6(2) |
| C(203)-C(202)-C(201) | 118.3(2) |
| C(203)-C(202)-C(205) | 123.6(2) |
| C(201)-C(202)-C(205) | 118.1(2) |

| | |
|---|---|
| C(202)-C(203)-N(202) | 122.7(2) |
| O(202)-C(204)-N(201) | 122.4(2) |
| O(202)-C(204)-N(202) | 122.7(2) |
| N(201)-C(204)-N(202) | 114.9(2) |
| F(201)-C(207)-C(206) | 109.0(2) |
| F(201)-C(207)-C(208) | 113.1(2) |
| C(206)-C(207)-C(208) | 102.8(2) |
| O(203)-C(206)-N(202) | 109.1(2) |
| O(203)-C(206)-C(207) | 104.7(2) |
| N(202)-C(206)-C(207) | 114.2(2) |
| O(203)-C(212)-C(211) | 111.2(2) |
| O(203)-C(212)-C(208) | 104.1(2) |
| C(211)-C(212)-C(208) | 105.7(2) |
| O(205)-C(211)-C(213) | 119.0(2) |
| O(205)-C(211)-C(210) | 121.8(2) |
| C(213)-C(211)-C(210) | 60.50(18) |
| O(205)-C(211)-C(212) | 119.0(2) |
| C(213)-C(211)-C(212) | 114.3(2) |
| C(210)-C(211)-C(212) | 108.9(2) |
| C(211)-C(210)-C(209) | 108.8(2) |
| C(211)-C(210)-C(213) | 59.67(18) |
| C(209)-C(210)-C(213) | 116.3(2) |
| C(210)-C(209)-C(208) | 106.0(2) |
| O(204)-C(208)-C(212) | 114.2(2) |
| O(204)-C(208)-C(209) | 108.4(2) |
| C(212)-C(208)-C(209) | 105.5(2) |
| O(204)-C(208)-C(207) | 111.6(2) |
| C(212)-C(208)-C(207) | 103.0(2) |
| C(209)-C(208)-C(207) | 114.1(2) |
| O(201)-C(201)-N(201) | 119.8(2) |
| O(201)-C(201)-C(202) | 125.0(2) |
| N(201)-C(201)-C(202) | 115.2(2) |
| O(305)-C(311)-C(313) | 116.8(2) |
| O(305)-C(311)-C(310) | 123.6(2) |
| C(313)-C(311)-C(310) | 60.90(18) |
| O(305)-C(311)-C(312) | 119.2(2) |
| C(313)-C(311)-C(312) | 114.2(2) |
| C(310)-C(311)-C(312) | 108.8(2) |

| | |
|---|---|
| C(311)-C(310)-C(309) | 108.9(2) |
| C(311)-C(310)-C(313) | 59.36(17) |
| C(309)-C(310)-C(313) | 116.4(2) |
| O(303)-C(312)-C(311) | 110.30(19) |
| O(303)-C(312)-C(308) | 104.3(2) |
| C(311)-C(312)-C(308) | 106.1(2) |
| O(304)-C(308)-C(307) | 112.9(2) |
| O(304)-C(308)-C(309) | 107.0(2) |
| C(307)-C(308)-C(309) | 115.4(2) |
| O(304)-C(308)-C(312) | 112.7(2) |
| C(307)-C(308)-C(312) | 102.9(2) |
| C(309)-C(308)-C(312) | 105.9(2) |
| F(301)-C(307)-C(306) | 109.4(2) |
| F(301)-C(307)-C(308) | 112.6(2) |
| C(306)-C(307)-C(308) | 103.0(2) |
| O(303)-C(306)-N(302) | 107.4(2) |
| O(303)-C(306)-C(307) | 105.6(2) |
| N(302)-C(306)-C(307) | 115.2(2) |
| C(302)-C(303)-N(302) | 123.1(2) |
| C(303)-C(302)-C(301) | 117.7(2) |
| C(303)-C(302)-C(305) | 124.2(2) |
| C(301)-C(302)-C(305) | 118.1(2) |
| O(301)-C(301)-N(301) | 120.3(2) |
| O(301)-C(301)-C(302) | 123.7(2) |
| N(301)-C(301)-C(302) | 116.0(2) |
| O(302)-C(304)-N(302) | 122.5(2) |
| O(302)-C(304)-N(301) | 122.9(2) |
| N(302)-C(304)-N(301) | 114.6(2) |
| C(310)-C(309)-C(308) | 105.7(2) |
| C(4)-N(1)-C(1) | 126.3(2) |
| C(104)-N(101)-C(101) | 127.8(2) |
| C(104)-N(102)-C(103) | 121.6(2) |
| C(104)-N(102)-C(106) | 116.8(2) |
| C(103)-N(102)-C(106) | 121.5(2) |
| C(204)-N(201)-C(201) | 127.0(2) |
| C(204)-N(202)-C(203) | 121.6(2) |
| C(204)-N(202)-C(206) | 116.2(2) |
| C(203)-N(202)-C(206) | 121.6(2) |

| | |
|---|---|
| C(301)-N(301)-C(304) | 126.6(2) |
| C(304)-N(302)-C(303) | 122.0(2) |
| C(304)-N(302)-C(306) | 116.2(2) |
| C(303)-N(302)-C(306) | 121.8(2) |
| C(6)-O(3)-C(12) | 102.42(18) |
| C(11)-C(13)-C(10) | 60.47(19) |
| C(111)-C(113)-C(110) | 59.96(17) |
| C(106)-O(103)-C(112) | 101.96(18) |
| C(211)-C(213)-C(210) | 59.83(17) |
| C(206)-O(203)-C(212) | 103.11(18) |
| C(311)-C(313)-C(310) | 59.75(17) |
| C(306)-O(303)-C(312) | 103.15(18) |

Symmetry transformations used to generate equivalent atoms.

**Table S4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 5b. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2 a^{*2} U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$**

| | $U^{11}$ | $U^{22}$ | $U^{33}$ | $U^{23}$ | $U^{13}$ | $U^{12}$ |
|---|---|---|---|---|---|---|
| C(1) | 21(1) | 22(1) | 25(1) | -2(1) | 11(1) | -3(1) |
| C(2) | 24(1) | 23(1) | 26(1) | -3(1) | 14(1) | -2(1) |
| C(3) | 28(2) | 17(1) | 30(1) | -2(1) | 17(1) | -1(1) |
| N(2) | 23(1) | 19(1) | 22(1) | -2(1) | 12(1) | -2(1) |
| C(4) | 19(1) | 20(1) | 23(1) | -2(1) | 9(1) | -2(1) |
| C(5) | 54(2) | 22(1) | 37(2) | 1(1) | 32(2) | 1(1) |
| C(6) | 27(1) | 19(1) | 18(1) | 0(1) | 11(1) | 0(1) |
| C(7) | 28(1) | 24(1) | 25(1) | -1(1) | 13(1) | -4(1) |
| C(8) | 24(1) | 23(1) | 19(1) | 4(1) | 11(1) | 1(1) |
| C(12) | 24(1) | 23(1) | 18(1) | 2(1) | 11(1) | 1(1) |
| C(11) | 34(2) | 24(1) | 21(1) | -1(1) | 15(1) | -3(1) |
| C(10) | 35(2) | 24(1) | 37(2) | 1(1) | 20(1) | 5(1) |
| C(9) | 24(1) | 28(2) | 33(1) | 4(1) | 12(1) | 6(1) |
| C(111) | 27(1) | 22(1) | 17(1) | -1(1) | 11(1) | -4(1) |
| C(112) | 26(1) | 20(1) | 15(1) | 1(1) | 11(1) | 3(1) |
| C(106) | 23(1) | 20(1) | 17(1) | -3(1) | 10(1) | -2(1) |
| C(103) | 24(1) | 15(1) | 21(1) | -2(1) | 8(1) | 2(1) |
| C(102) | 22(1) | 21(1) | 19(1) | -3(1) | 8(1) | 0(1) |

| | | | | | | |
|---|---|---|---|---|---|---|
| C(105) | 33(2) | 24(1) | 22(1) | 0(1) | 14(1) | 4(1) |
| C(101) | 19(1) | 24(1) | 19(1) | -3(1) | 6(1) | 0(1) |
| C(104) | 21(1) | 19(1) | 19(1) | -3(1) | 4(1) | 1(1) |
| C(108) | 23(1) | 21(1) | 23(1) | 2(1) | 13(1) | 0(1) |
| C(107) | 24(1) | 25(1) | 24(1) | -4(1) | 8(1) | -2(1) |
| C(109) | 28(1) | 25(1) | 25(1) | 2(1) | 14(1) | 6(1) |
| C(110) | 34(2) | 19(1) | 25(1) | -1(1) | 15(1) | 3(1) |
| C(205) | 45(2) | 21(1) | 29(1) | -1(1) | 19(1) | -2(1) |
| C(202) | 26(1) | 20(1) | 23(1) | -3(1) | 9(1) | 1(1) |
| C(203) | 26(1) | 17(1) | 23(1) | -3(1) | 7(1) | 0(1) |
| C(204) | 22(1) | 21(1) | 24(1) | -2(1) | 7(1) | -1(1) |
| C(207) | 33(2) | 27(2) | 26(1) | -5(1) | 5(1) | 9(1) |
| C(206) | 32(2) | 18(1) | 20(1) | -2(1) | 7(1) | -1(1) |
| C(212) | 25(1) | 23(1) | 18(1) | 1(1) | 9(1) | -2(1) |
| C(211) | 27(1) | 23(1) | 19(1) | 1(1) | 11(1) | 3(1) |
| C(210) | 32(2) | 18(1) | 24(1) | -1(1) | 8(1) | 0(1) |
| C(209) | 25(1) | 22(1) | 25(1) | 0(1) | 7(1) | -1(1) |
| C(208) | 27(2) | 20(1) | 18(1) | 2(1) | 5(1) | 1(1) |
| C(201) | 23(1) | 21(1) | 25(1) | 0(1) | 10(1) | 2(1) |
| C(311) | 27(1) | 18(1) | 19(1) | 0(1) | 11(1) | -1(1) |
| C(310) | 33(2) | 18(1) | 25(1) | 2(1) | 11(1) | 4(1) |
| C(312) | 27(1) | 21(1) | 13(1) | 0(1) | 10(1) | 2(1) |
| C(308) | 26(1) | 20(1) | 19(1) | -3(1) | 7(1) | 1(1) |
| C(307) | 30(2) | 26(1) | 26(1) | -3(1) | 11(1) | -7(1) |
| C(306) | 31(2) | 21(1) | 21(1) | 1(1) | 14(1) | -1(1) |
| C(303) | 28(1) | 16(1) | 23(1) | 3(1) | 12(1) | -1(1) |
| C(302) | 22(1) | 20(1) | 21(1) | 2(1) | 9(1) | 0(1) |
| C(305) | 33(2) | 27(1) | 23(1) | -2(1) | 13(1) | -2(1) |
| C(301) | 22(1) | 25(1) | 23(1) | 4(1) | 9(1) | 2(1) |
| C(304) | 34(2) | 21(1) | 25(1) | 2(1) | 15(1) | 0(1) |
| C(309) | 25(2) | 25(1) | 28(1) | -3(1) | 7(1) | 5(1) |
| N(1) | 29(1) | 16(1) | 25(1) | -3(1) | 14(1) | 0(1) |
| N(101) | 30(1) | 18(1) | 23(1) | -6(1) | 12(1) | 2(1) |
| N(102) | 25(1) | 19(1) | 16(1) | -3(1) | 9(1) | 0(1) |
| N(201) | 30(1) | 18(1) | 25(1) | -6(1) | 12(1) | -1(1) |
| N(202) | 28(1) | 18(1) | 20(1) | -2(1) | 6(1) | -2(1) |
| N(301) | 40(1) | 18(1) | 26(1) | 3(1) | 18(1) | -4(1) |
| N(302) | 34(1) | 20(1) | 19(1) | 2(1) | 14(1) | -2(1) |

| | | | | | | |
|---|---|---|---|---|---|---|
| O(1) | 48(1) | 24(1) | 30(1) | -5(1) | 26(1) | -3(1) |
| O(2) | 39(1) | 21(1) | 26(1) | 0(1) | 15(1) | -1(1) |
| O(3) | 26(1) | 22(1) | 24(1) | -5(1) | 16(1) | -5(1) |
| O(4) | 30(1) | 32(1) | 32(1) | 11(1) | 19(1) | 2(1) |
| C(13) | 45(2) | 30(2) | 33(2) | -7(1) | 26(1) | -3(1) |
| O(27) | 39(1) | 25(1) | 26(1) | -1(1) | 18(1) | -8(1) |
| O(101) | 36(1) | 18(1) | 21(1) | -2(1) | 8(1) | 0(1) |
| O(102) | 39(1) | 28(1) | 23(1) | -7(1) | 18(1) | -1(1) |
| C(113) | 41(2) | 28(1) | 19(1) | -5(1) | 17(1) | -1(1) |
| O(103) | 22(1) | 23(1) | 16(1) | -4(1) | 10(1) | -2(1) |
| O(105) | 33(1) | 28(1) | 35(1) | 10(1) | 22(1) | 1(1) |
| O(104) | 28(1) | 33(1) | 19(1) | -1(1) | 6(1) | -9(1) |
| C(213) | 47(2) | 32(2) | 20(1) | -2(1) | 15(1) | 7(1) |
| O(202) | 41(1) | 19(1) | 32(1) | 2(1) | 14(1) | 1(1) |
| O(201) | 49(1) | 24(1) | 27(1) | -4(1) | 21(1) | 1(1) |
| O(203) | 24(1) | 22(1) | 19(1) | 0(1) | 5(1) | 3(1) |
| O(204) | 34(1) | 27(1) | 24(1) | 11(1) | 4(1) | -3(1) |
| O(205) | 30(1) | 29(1) | 27(1) | 4(1) | 16(1) | 8(1) |
| O(301) | 39(1) | 32(1) | 18(1) | 4(1) | 12(1) | -6(1) |
| O(302) | 60(2) | 22(1) | 31(1) | -4(1) | 25(1) | -10(1) |
| C(313) | 43(2) | 28(2) | 22(1) | 5(1) | 15(1) | 3(1) |
| O(303) | 26(1) | 24(1) | 16(1) | 3(1) | 8(1) | -4(1) |
| O(304) | 41(1) | 22(1) | 19(1) | -4(1) | 3(1) | 2(1) |
| O(305) | 27(1) | 26(1) | 33(1) | -6(1) | 16(1) | -6(1) |
| F(1) | 26(1) | 69(1) | 21(1) | 0(1) | 8(1) | 3(1) |
| F(101) | 29(1) | 53(1) | 21(1) | 1(1) | 4(1) | 14(1) |
| F(201) | 27(1) | 86(2) | 35(1) | -19(1) | 15(1) | -1(1) |
| F(301) | 32(1) | 65(1) | 43(1) | 9(1) | 24(1) | 8(1) |
| O(1S) | 59(2) | 30(2) | 36(2) | 0 | 15(2) | 0 |
| O(2S) | 42(2) | 29(2) | 31(2) | 0 | 20(1) | 0 |

# 4. Conformational analysis of 5b

## 4.1 Analysis of the $^{3}J_{H1',H2'}$ coupling constant of 5b

The generalized Karplus equation (4) was applied to calculate the $^{3}J_{H1',H2'}$ coupling constant vs the corresponding torsion angle for 2'F-tc-ANA-T nucleoside **5b**. This equation includes a correction for the inductive effect of the fluorine on the coupling constant. The **5b** crystal structure reveals that the torsion angle between protons H-C(1′) and H-C(2′) is in the 28.9° to 36.3° range, which is consistent with the observed $^{3}J_{H1',H2'}$ coupling constant of 4.1 Hz in the $^{1}$H-NMR of **5b** (**Fig. S1**). Thus, it is quite probable that the 2'F-tc-ANA nucleoside in solution also adopts the East sugar pucker.

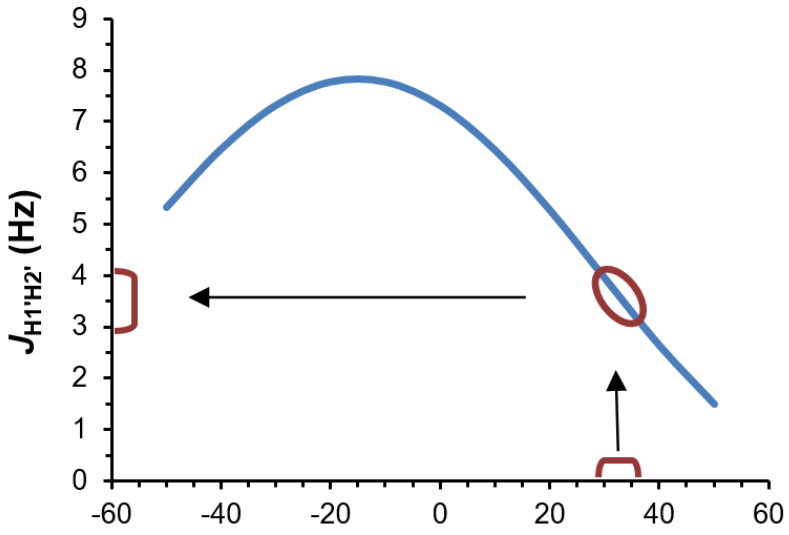



**Fig. S1. $^{3}J_{H1',H2'}$ versus H1′-C1′-C2′-H2′ torsion angle relation calculated for 5b from the generalized Karplus equation. The torsion angle values are limited by realistic -50 to 50° range.**

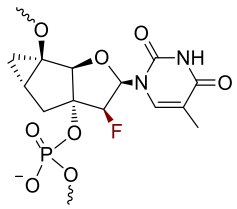
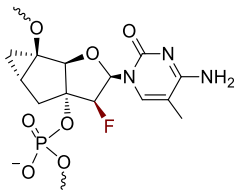
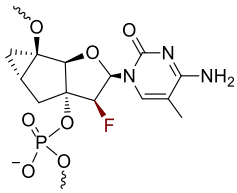
## 4.2 QM energy profiles

Potential energy profiles versus pseudorotation phase angle were obtained using quantum mechanical methods. The pseudorotation angle *P* was scanned in the 0-350˚ range with 10˚ step at a given $\nu_{max}$ (25˚, 30˚, 35˚, 40˚, 45˚). Each conformation was built by restraining the $\nu_2$ and $\nu_4$ dihedral angles; moreover, the β and ε angles were fixed to the values consistent with those adopted by tc-DNA in duplexes (90˚ and 150˚, respectively). All other parameters were optimized to their most favorable values with the Gaussian 09 software at the restricted Hartree-Fock (RHF) level of theory using 6-311G* basis set. The geometry optimization for each conformation was carried out in a separate Gaussian run. All calculations were performed on the UBELIX cluster. *Bash* and *awk* scripts were used to generate input files for Gaussian program, to control Gaussian job submission to the UBELIX cluster and to extract the calculated energies from the output files.

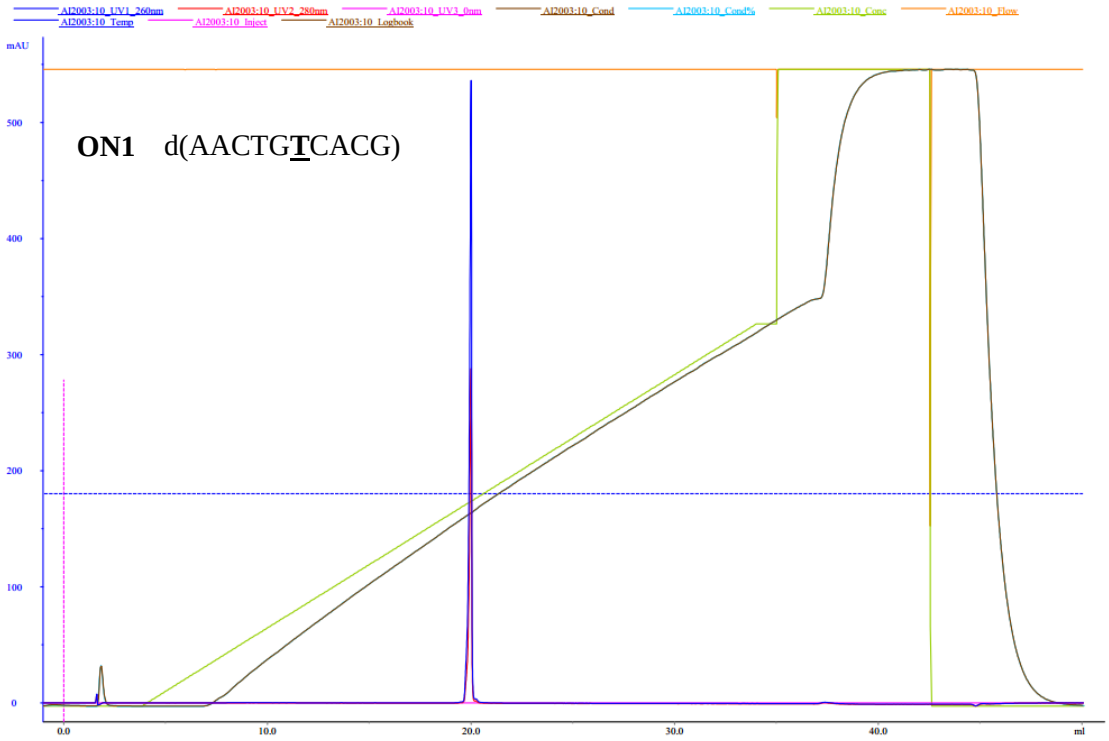
# 5. Oligonucleotide characterization

Sequences of synthesized oligonucleotides their characterization by ESI-MS are summarized in **Table S5**.

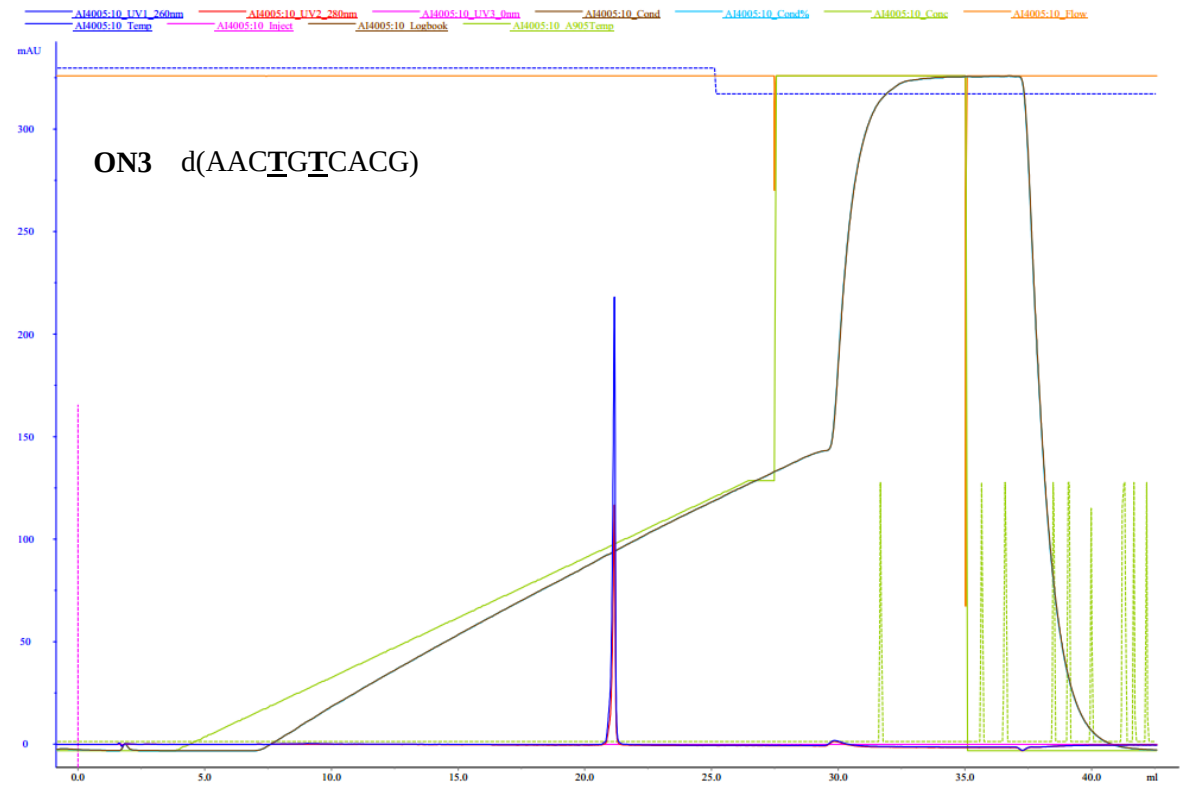
**Table S5. List of the synthesized oligonucleotides containing tc-DNA and 2'F-tc-ANA nucleotides and their characterization by ESI-MS. Capital letters: natural DNA nucleotides; lowercase letters: modified units. 2'F-ANA-tc-$^{5Me}$C and tc-$^{5Me}$C modifications are designated as C and *C*, respectively.**

| Entry | Sequence (5′→3′) | Modification | $MW_{calcd}$ | $MW_{found}$ |
|---|---|---|---|---|
| **ON1** | d(AACTG**T**CACG) |  | 3068,0 | 3067,5 |
| **ON2** | d(AAC**T**GTCACG) | | 3068,0 | 3067,5 |
| **ON3** | d(AAC**T**G**T**CACG) | | 3124,1 | 3123,6 |
| **ON4** | d(CCC**T**A**T**ACCC) | | 3020,0 | 3019,6 |
| **ON5** | d(CCCAA**TT**CCC) | | 3020,0 | 3019,6 |
| **ON6** | d(GCA**TTTTT**ACCG) | | 3891.6 | 3890.7 |
| | d(GCA**TTTTTT**ACCG) | | 4250.7 | 4251.7 |
| **ON7** | d(CCCT**A**TACCC) |  | 2964,0 | 2963,5 |
| **ON8** | d(CCCTAT**A**CCC) | | 2964,0 | 2963,5 |
| **ON9** | d(CCCT**A**T**A**CCC) | | 3020,0 | 3019,6 |
| **ON10** | d(CCC**AA**TTCCC) | | 3020,0 | 3019,6 |
| **ON11** | d(AACTGT**C**ACG) |  | 3082,1 | 3081,6 |
| **ON12** | d(AA**C**TGTCACG) | | 3082,1 | 3081,6 |
| **ON13** | d(AA**C**TGT**C**ACG) | | 3152,1 | 3151,6 |
| **ON14** | **CCCTATACCC** | **Fully modified 2'F-tc-ANA oligonucleotide** | 3632,4 | 3631,6 |
| **ON15** | ***CCCTATACCC*** | **Fully modified tc-DNA oligonucleotide** | 3453,1 | 3452,1 |

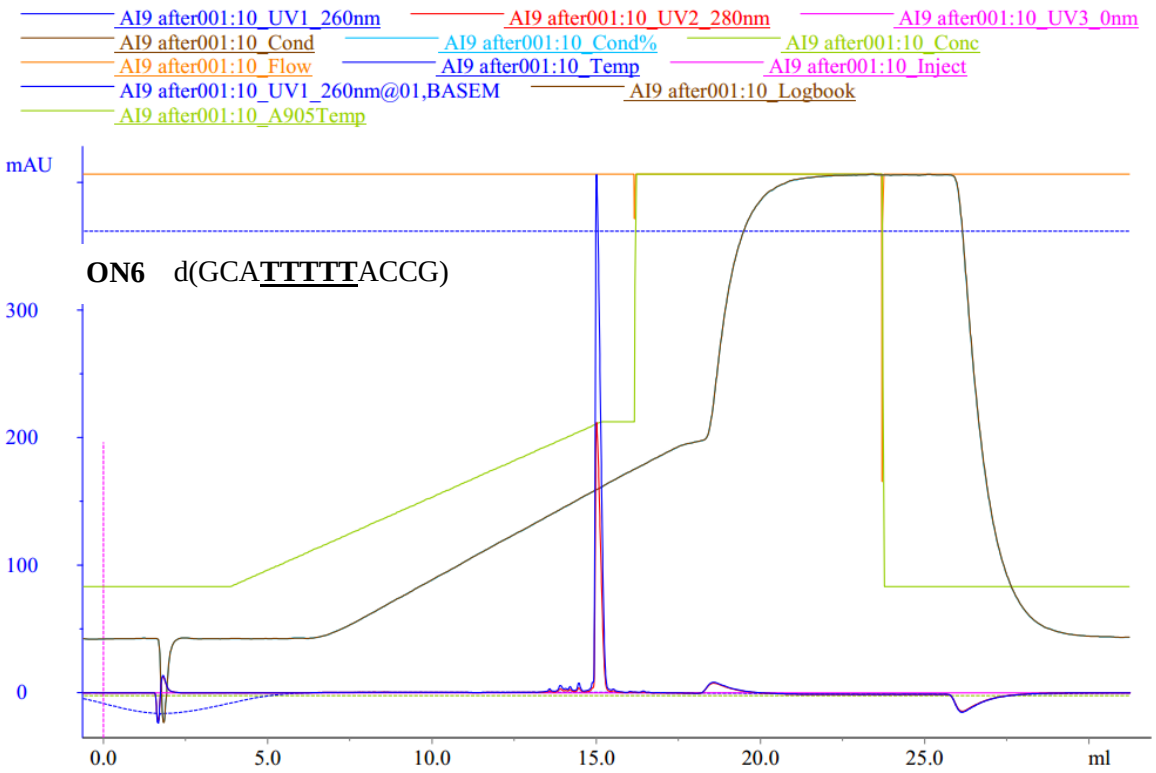
**HPLC data**



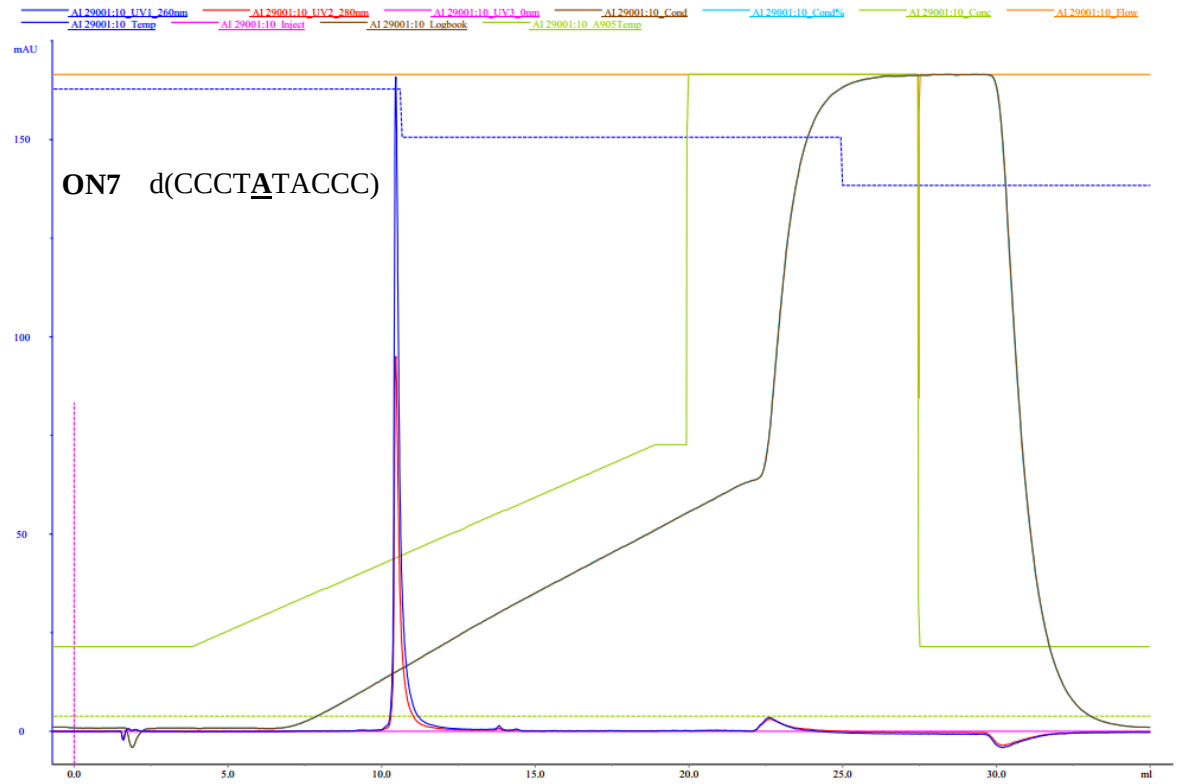

**ON1** (after purification). Conditions: Dionex DNA Pac-PA200; Buffer A: 25 mM Trizma in $H_2O$, buffer B 25 mM Trizma, 1.25 M NaCl in $H_2O$, pH 8.0, 0 to 60% B in A in 8 CV with 1 mL/min.



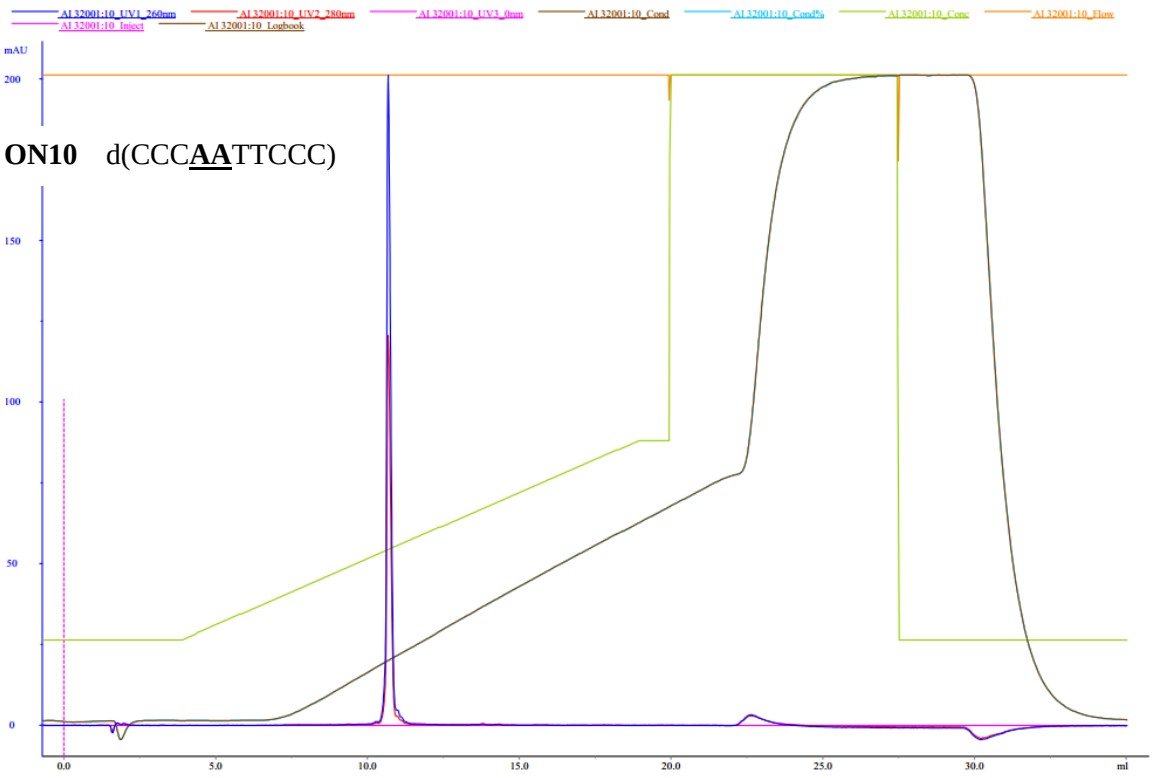

**ON3** (after purification). Conditions: Dionex DNA Pac-PA200; Buffer A: 25 mM Trizma in $H_2O$, buffer B 25 mM Trizma, 1.25 M NaCl in $H_2O$, pH 8.0, 0 to 40% B in A in 6 CV with 1 mL/min.



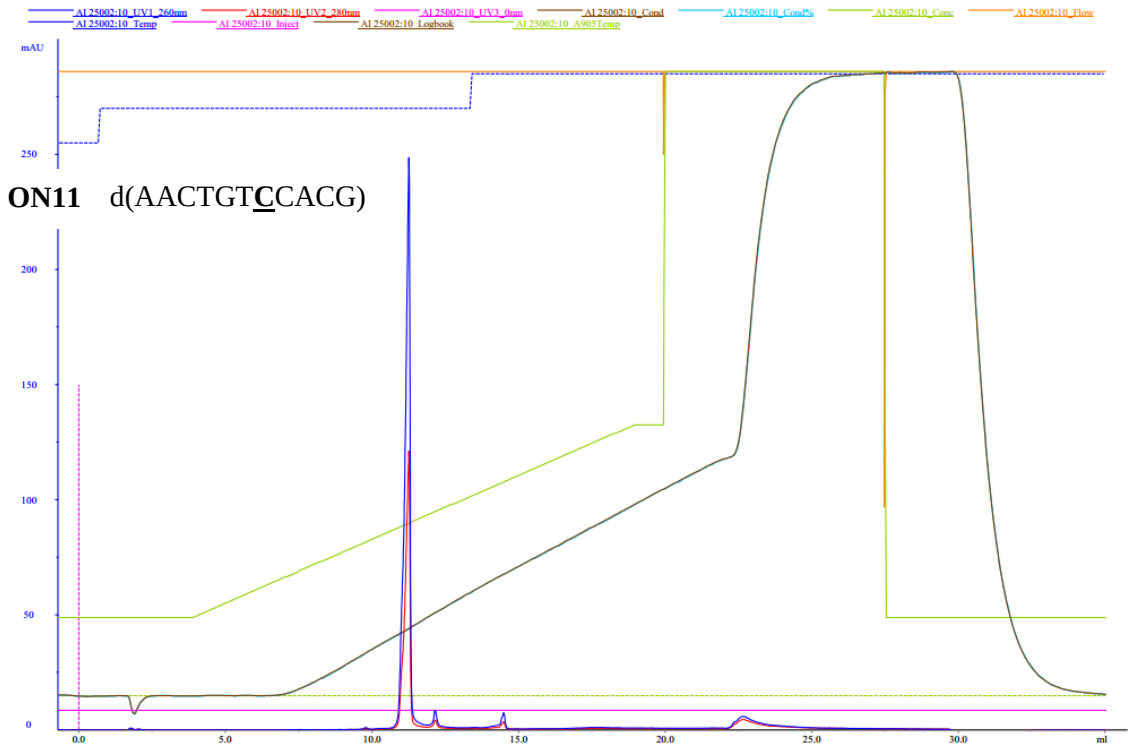

**ON6** (after purification). Conditions: Dionex DNA Pac-PA200; Buffer A: 25 mM Trizma in $H_2O$, buffer B 25 mM Trizma, 1.25 M NaCl in $H_2O$, pH 8.0, 25 to 55% B in A in 3 CV with 1 mL/min.



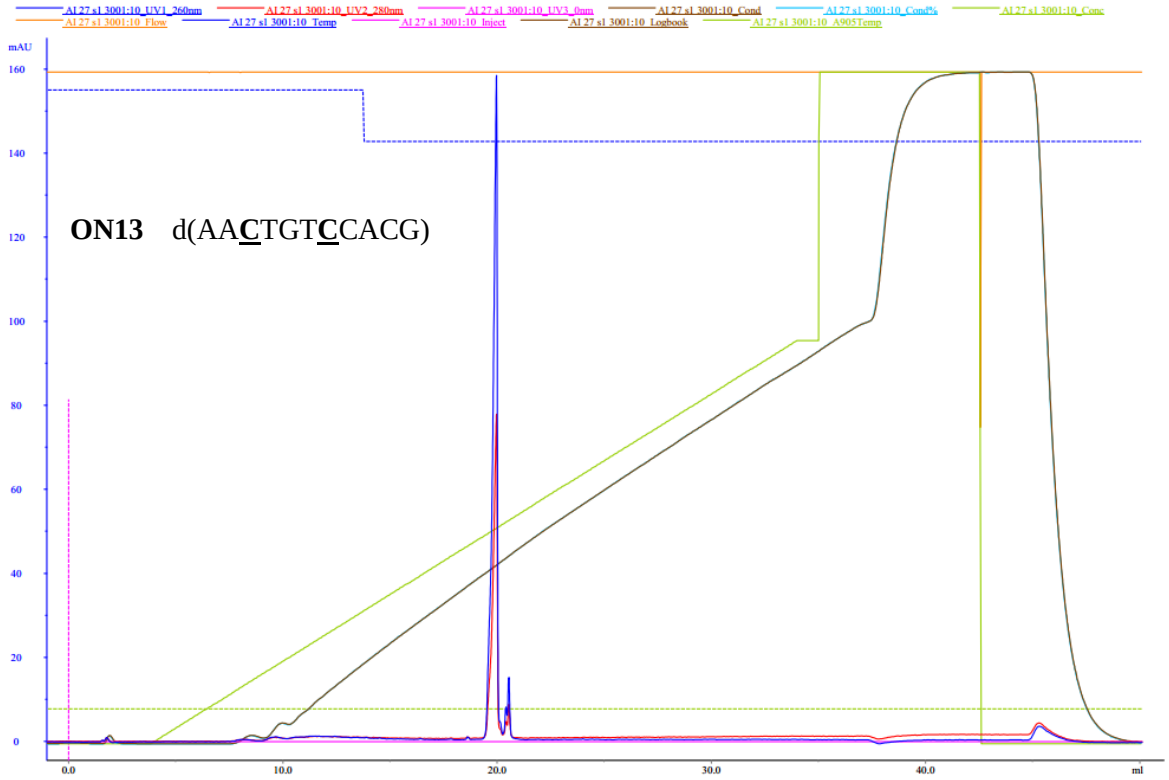

**ON7** (after purification). Conditions: Dionex DNA Pac-PA200; Buffer A: 25 mM Trizma in $H_2O$, buffer B 25 mM Trizma, 1.25 M NaCl in $H_2O$, pH 8.0, 15 to 45% B in A in 4 CV with 1 mL/min.



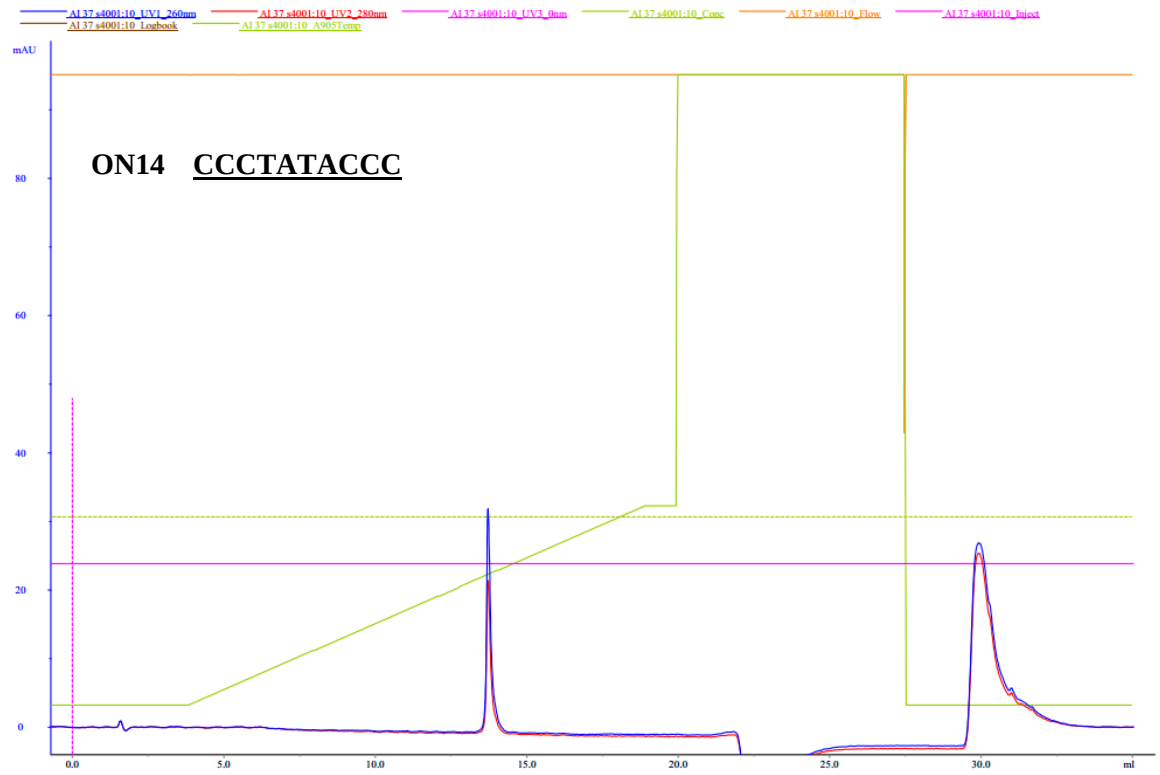

**ON10** (after purification). Conditions: Dionex DNA Pac-PA200; Buffer A: 25 mM Trizma in $H_2O$, buffer B 25 mM Trizma, 1.25 M NaCl in $H_2O$, pH 8.0, 15 to 45% B in A in 4 CV with 1 mL/min.




**ON11** (after purification). Conditions: Dionex DNA Pac-PA200; Buffer A: 25 mM Trizma in $H_2O$, buffer B 25 mM Trizma, 1.25 M NaCl in $H_2O$, pH 8.0, 15 to 45% B in A in 4 CV with 1 mL/min.




**ON13** (after purification). Conditions: Dionex DNA Pac-PA200; Buffer A: 25 mM Trizma in $H_2O$, buffer B 25 mM Trizma, 1.25 M NaCl in $H_2O$, pH 8.0, 0 to 60% B in A in 8 CV with 1 mL/min.




**ON14 (**after purification). Conditions: Dionex DNA Pac-PA200; Buffer A: 10 mM NaOH in $H_2O$, buffer B 10 mM NaOH, 1.5M NaCl in $H_2O$, pH 12, 5 to 35% B in A in 4 CV with 1 mL/min.

## 6. CD spectra of modified duplexes

To track structural changes upon duplex formation, the CD measurements were carried out at 90°C and 20°C (**Fig. S2**). The spectrum of the unmodified DNA duplex exhibits features characteristic for canonical B-form DNA duplexes. The spectrum of the corresponding 2′F-tc-ANA/DNA (**ON14/DNA**) duplex reveals a shallower negative band around 240 nm and a stronger positive band at 260 nm relative to the reference DNA/DNA duplex. The tc-DNA/DNA duplex shows a more intense positive band at 260 nm and a more pronounced negative band at 210 nm. These changes indicate a shift towards an A-like conformation concerning both modified duplexes compared to the natural duplex.

The CD spectra of tc-DNA/RNA and 2′F-tc-ANA/RNA duplexes are reminiscent to that of RNA/DNA reference spectra, indicating an A-like character of the modified duplexes. Specifically, all duplexes demonstrated a strong positive band around 260 nm, a strong negative band at 210 nm and very shallow negative band at 235 nm.

Besides this, either a clear shoulder at 270-290 nm (when paired with DNA) or an additional positive band at 280 nm (when paired with RNA) appeared in all modified duplexes. However, this feature is also visible for denatured strands (at 90°C) and may be due to the base compositions of the modified duplexes that are slightly different from that of the unmodified duplexes. More specifically, the reference DNA/DNA and DNA/RNA duplexes contained unmodified cytidines, whereas the modified duplexes contained 5-methyl-cytidines.

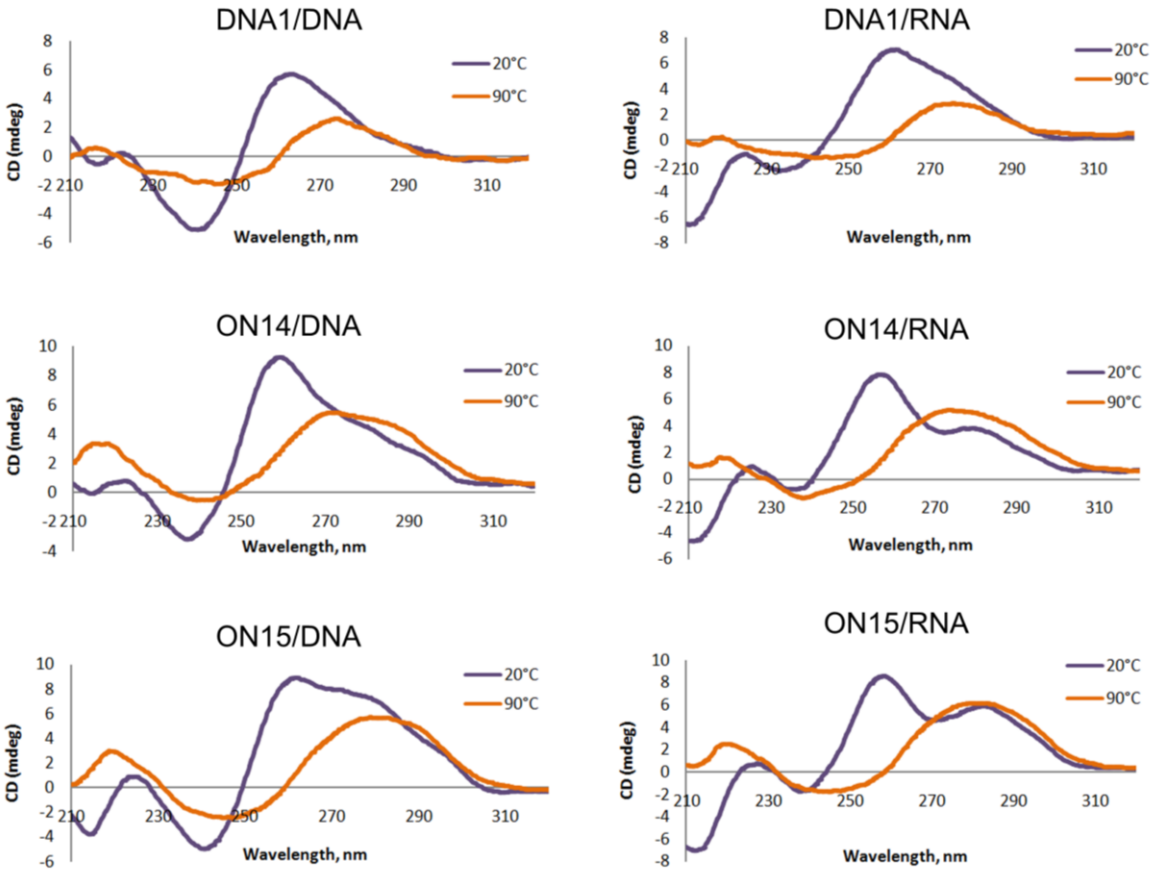



**Fig. S2. CD spectra (320-210 nm) of fully-modified 2′F-tc-ANA (ON14), tc-DNA (ON15), and the corresponding unmodified DNA (DNA1) oligonucleotides (5′-CCCTATACCC-3′) with complementary DNA and RNA. Conditions: 2 µM single strands in 150 mM NaCl and 10 mM $NaH_2PO_4$ at pH 7.0, 20 °C. Each spectrum is an average of at least three scans.**

## 7. RNase H degradation experiments

The sense strands, as well as the positive and negative antisense controls were all obtained from Microsynth AG. All these compounds were next re-purified by 20% polyacrylamide gel electrophoresis (acrylamide/bis acrylamide 29:1). Following purification, they were desalted with Sep-Pak C18 Classic Cartridge, 360 mg Sorbent per Cartridge, 55-105 µm Particle Size, 50/pk. Next, they were concentrated, re-dissolved in lower volumes of $dH_2O$, and quantified with witec ag NanoDrop ND-1000 Spectrophotometer. Additionally, after purification, the mass of all commercial oligomers was re-verified with ESI Q-TOF LCMS.

Next, 100 pmol of each sense strand was radiolabeled with 10 units of T4 Polynucleotide Kinase (EK0031, Thermo Scientific), $\gamma^{32}$P-ATP, 50 mM Tris-HCl (pH 7.6 at 25°C), 10 mM $MgCl_2$, 5 mM DTT, and 0.1 mM spermidine, 20 µL total volume, 37˚C, shaking for 1h. Following the reaction, Amicon Ultra- 0.5 mL centrifugal filters were applied to clean up the oligomers from unreacted $\gamma^{32}$P-ATP.

The actual RNAse H assays were carried out with: >100K cps labeled sense strand, 0.1 µM unlabeled sense strand, 2.5 µM antisense strand, 0.2 or 0.4 U/µL *E.Coli* RNase H (Thermo Scientific EN0201), 20 mM Tris-HCl (pH 7.8), 40 mM KCl, 8 mM MgCl2, 1 mM DTT, 20 µL total volume, 37˚C, shaking for 2 or 4h. The reactions were stopped by adding 20 µL Formamide Loading dye.

The reaction products heated to 95˚C for 5 min and 5 µL of each sample loaded onto a 1.5 cm wide lane of a 32cm x 42cm x 0.2 mm Polyacrylamide gel (20%, 29:1 acrylamide/bis acrylamide, 1 x TBE), Life Technologies, Inc. Model S2 BRL gel apparatus. Next, 1x TBE buffer was added to each chamber and the apparatus was connected to a Life Technologies GIBCO BRL PS 9009 Power Supply with the settings: ≤40 W, ≤2000V, ≤250 mA for 2-4 hours. Next, the opened gel was placed in a cassette along with a Fujifilm Imaging Plate, in a -20 ˚C OVER 48 h. Finally the plate was scanned with a Typhoon Phosphorimager at 50 pixels/$cm^2$.

# 8. References

(1) Oxford Diffraction (2010). *CrysAlisPro (Version 1.171.34.44).* Oxford Diffraction Ltd., Yarnton, Oxfordshire, UK.

(2) Macchi, P., et al. *Journal of Applied Crystallography* **2011**, ***44****(4)*, p. 763-771.

(3) Sheldrick, G.M. *Acta Crystallogr. A* **2008**, *64(Pt 1)*, p. 112-22.

(4) Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* 1980, *36*, 2783.