C5-Amino acid functionalized LNA: Positively poised for antisense applications

Dale C. Guenther, Pawan Kumar, Brooke A. Anderson and Patrick J. Hrdlicka*

Electronic Supplementary Information (ESI)

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General experimental section. Analytical grade solvents and reagents were purchased from commercial suppliers and used without further purification. Anhydrous solvents were either purchased (DMF) or dried with activated molecular sieves: CH_3CN (3 Å) and CH_2Cl_2/1,2-dichloroethane/*N*,*N*′-diisopropylethylamine (4 Å). Reactions using these solvents were conducted under an inert atmosphere (argon). All reactions were monitored by thin layer chromatography (TLC) using silica gel coated plates with a fluorescence indicator (SiO_2-60, F-254), which were visualized under UV light and/or by dipping in 5% conc. H_2SO_4 in abs. ethanol (v/v) followed by heating. Purification (>95% purity, assessed by one-dimensional NMR techniques) was accomplished using column chromatography (silica gel 60, particle size 0.040-0.063 mm) using moderate pressure (pressure ball). Evaporation of solvents was carried out under reduced pressure at temperatures below 40 °C. Chemical shifts are reported relative to deuterated solvents or other internal standards (trimethylsilane and 80% phosphoric acid for ^{1}H and ^{31}P NMR, respectively) or external standards (DMSO-d_6 and trifluorochloromethane for ^{13}C and ^{19}F NMR, respectively). Exchangeable protons were detected by disappearance of peaks upon D_2O addition. Assignments of NMR spectra are based on 2D spectra (COSY, HSQC) and DEPT. Quaternary carbons in ^{13}C NMR are not assigned, but their presence was verified by HSQC and DEPT spectra (absence of signals). Assignments of ^{1}H NMR signals of H5′/H5″/CH_2Ph and the corresponding ^{13}C NMR signals are interchangeable. MALDI-HRMS spectra were recorded on a Q-TOF mass spectrophotometer using 2,5-dihydroxybenzoic acid (DHB) as a matrix.

(1*R*,3*R*,4*R*,7*S*)-3-[5-(3-Aminopropyn-1-yl)uracil-1-yl]-1-(4,4′-dimethoxytrityloxymethyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptane (2).

Method A. Nucleoside **1**[S1] (1.28 g, 1.83 mmol) was dissolved in sat. methanolic ammonia (30 mL) and the mixture was stirred for 16 h at rt, at which point the solvents were evaporated. The resulting residue was purified by column chromatography (5-10% MeOH/CH_2Cl_2, v/v) to afford nucleoside **2** (1.11 g, 97%) as a brown foam, which was used in the next step without further purification. R_f = 0.5 (10% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 634.2146 ($[M+Na]^+$, $C_{34}H_{33}N_3O_8 \cdot Na^+$, Calcd 634.2160); ^{1}H NMR (DMSO-d_6, 500.1 MHz) δ 7.78 (s, 1H, H6), 7.43-7.46 (m, 2H, Ar), 7.30-7.36 (m, 6H, Ar), 7.23-7.27 (m, 1H, Ar), 6.91 (d, 4H, J = 9.0 Hz, Ar), 5.73 (br s, 1H, ex, 3′-OH), 5.42 (s, 1H, H1′), 4.25 (s, 1H, H2′), 4.07 (s, 1H, H3′), 3.78-3.80 (d, 1H, J = 8.0 Hz, H5″), 3.75-3.77 (d, 1H, J = 8.0 Hz, H5″), 3.75 (s, 6H, CH_3O), 3.48-3.52 (d, 1H, J = 11.5 Hz, H5′), 3.28-3.33 (m, 3H, 2 × CH_2NH_2, H5′ - partial overlap with H_2O); ^{13}C NMR (DMSO-d_6, 125.5 MHz) δ 161.8, 158.1, 149.0, 144.7, 141.1 (C6), 135.3, 135.1, 129.7 (Ar), 129.6 (Ar), 127.9 (Ar), 127.6 (Ar), 126.6 (Ar), 113.2 (Ar), 98.1, 93.6, 87.5, 86.9 (C1′), 85.6, 78.7 (C2′), 74.1, 71.3 (C5″), 69.5 (C3′), 58.9 (C5′), 55.0 (CH_3O), 31.1 (CH_2NH). Minor unidentified impurities were observed in the ^{13}C NMR spectrum below 40 ppm.

Method B. To a flame-dried round-bottomed flask was added 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-LNA uridine[S1] (2.00 g, 2.92 mmol), CuI (111 mg, 0.58 mmol), $Pd(PPh_3)_4$ (0.34 g, 0.29 mmol) and anhydrous DMF (30 mL). Several degas/argon cycles were performed, followed by addition of propargyl amine (0.47 mL, 7.31 mmol) and anhydrous Et_3N (1.80 mL, 12.90 mmol). The reaction mixture was stirred at room

temperature under argon atmosphere for 15.5 h, at which point the solvent was evaporated off at high vacuum. The resulting residue was diluted with CH_2Cl_2 (60 mL), washed with brine (2 × 100 mL), sat. aq. $NaHCO_3$ (100 mL), and H_2O (100 mL). The organic layer was evaporated to dryness and the resulting crude was purified via silica gel column chromatography (0-10% MeOH in CH_2Cl_2, v/v) to give nucleoside **2** (1.27 g, 71%) as a light brown foam.[S2]

Conjugation protocol for the synthesis of nucleosides 3x/3y/3z. Protected amino acids 2-(2,2,2-trifluoroacetamido)acetic acid and (*S*)-2,6-bis(2,2,2-trifluoroacetamido)hexanoic acid were prepared according to literature protocols.[S3] *S*-2-(2,2,2-Trifluoroacetamido)-4-methylpentanoic acid was also prepared essentially as described in the literature,[S4] except that sodium in methanol (0 °C), rather than potassium in methanol (40 °C), was used to generate methoxide. A solution of the appropriate protected amino acid, *O*-(*N*-succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU) and *N*,*N*′-diisopropylethylamine (DIPEA) in anhydrous DMF was stirred at rt for 30 min. After cooling the solution to 0 °C, nucleoside **2** was added and the reaction mixture was warmed to rt over 15 min. Upon completion of the reaction (reaction time specified below) the solvent was evaporated and the resulting residue dissolved in EtOAc (100 mL). The organic phase was sequentially washed with sat. aq. $NaHCO_3$ (2 × 50 mL) and brine (50 mL), dried (Na_2SO_4) and evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2, v/v) to afford desired nucleoside **3x/y/z** (quantities and yields specified below).

5-(TFA-glycyl-aminopropynyl)-5′-*O*-(4,4′-dimethoxytrityl) LNA uridine (3x). A solution of 2-(2,2,2-trifluoroacetamido)acetic acid (90 mg, 0.58 mmol), nucleoside **2** (0.30 g, 0.49 mmol), TSTU (190 mg, 0.63 mmol) and DIPEA (0.25 mL, 1.47 mmol) in anhydrous DMF (10 mL) was reacted (2 h), worked up and purified as described in the representative protocol to afford nucleoside **3x** (180 mg, 48%) as a slightly brown solid material. R_f = 0.4 (5% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 787.2200 ($[M + Na]^+$, $C_{38}H_{35}F_3N_4O_{10}{\cdot}Na^+$, Calcd 787.2203); ^{1}H NMR (DMSO-d_6, 500.1 MHz) δ 11.68 (s, 1H, ex, NH(U)), 9.62 (t, 1H, ex, J = 5.5 Hz, $NHCOCF_3$), 8.49 (t, 1H, ex, J = 5.2 Hz, $NHCH_2C{\equiv}C$), 7.78 (s, 1H, H6), 7.42-7.45 (m, 2H, Ar), 7.28-7.35 (m, 6H, Ar), 7.23-7.27 (m, 1H, Ar), 6.91-6.92 (2d, 4H, J = 9.0 Hz, Ar), 5.73 (d, 1H, ex, J = 5.0 Hz, 3′-OH), 5.43 (s, 1H, H1′), 4.25 (s, 1H, H2′), 4.04 (d, 1H, J = 5.0 Hz, H3′), 3.94-3.99 (dd, 1H, J = 17.7 Hz, 5.2 Hz, $CH_2C{\equiv}C$), 3.86-3.91 (dd, 2H, J = 17.7, 5.2 Hz, $CH_2C{\equiv}C$), 3.79-3.83 (m, 4H, H5″, $CH_2NHCOCF_3$), 3.75 (s, 6H, CH_3O), 3.55-3.58 (d, 1H, J = 11.0 Hz, H5′), 3.26-3.30 (d, 1H, J = 11.0 Hz, H5′, partial overlap with H_2O); ^{13}C NMR (DMSO-d_6, 125.5 MHz) δ 166.6, 161.7, 158.12, 158.08, 156.7 (q, J_{CF} = 36 Hz, $COCF_3$), 149.0, 144.7, 141.8 (C6), 135.4, 134.9, 129.8 (Ar), 129.6 (Ar), 127.9 (Ar), 127.5 (Ar), 126.7 (Ar), 115.9 (q, J_{CF} = 287 Hz, CF_3), 113.3 (Ar), 113.2 (Ar), 97.5, 88.8, 87.6, 86.9 (C1′), 85.6, 78.8 (C2′), 74.7, 71.4 (C5″), 69.6 (C3′), 59.1 (C5′), 55.0 (CH_3O), 41.7 ($CH_2NHCOCF_3$), 28.8 ($CH_2C{\equiv}C$); ^{19}F NMR (DMSO-d_6, 470.6 MHz) δ -74.8 (CF_3).

5-(TFA-leucyl-aminopropynyl)-5′-*O*-(4,4′-dimethoxytrityl) LNA uridine (3y). A solution of *S*-2-(2,2,2-trifluoroacetamido)-4-methylpentanoic acid (100 mg, 0.44 mmol), nucleoside **2** (0.25 g, 0.40 mmol), TSTU (160 mg, 0.53 mmol) and DIPEA (0.21 mL, 1.20 mmol) in anhydrous DMF (5 mL) was reacted (2 h), worked up and purified as

described in the representative protocol to afford nucleoside **3y** (170 mg, 49%) as a brown solid material. R_f = 0.5 (5% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 843.2797 ($[M + Na]^+$, $C_{42}H_{43}F_3N_4O_{10}{\cdot}Na^+$, Calcd 843.2829); ^{1}H NMR (DMSO-d_6, 500.1 MHz) δ 11.68 (s, 1H, ex, NH(U)), 9.54 (d, 1H, ex, J = 8.5 Hz, $NHCOCF_3$), 8.58-8.61 (m, 1H, ex, $NHCH_2C{\equiv}C$), 7.77 (s, 1H, H6), 7.42-7.45 (m, 2H, Ar), 7.28-7.35 (m, 6H, Ar), 7.22-7.26 (m, 1H, Ar), 6.89-6.93 (2d, 4H, J = 9.0 Hz, Ar), 5.71-5.74 (d, 1H, ex, J = 8.5 Hz, 3′-OH – partial overlap with CH_2Cl_2), 5.43 (s, 1H, H1′), 4.35-4.41 (m, 1H, $CHNHCOCF_3$), 4.25 (s, 1H, H2′), 4.02-4.05 (m, 1H, H3′), 3.85-3.99 (m, 2H, $CH_2C{\equiv}C$), 3.78-3.83 (m, 2H, H5″), 3.75 (s, 6H, CH_3O), 3.54-3.58 (d, 1H, J = 11.0 Hz, H5′), 3.26-3.30 (d, 1H, J = 11.0 Hz, H5′ - partial overlap with H_2O), 1.63-1.66 (m, 1H, CH_2-*i*Pr), 1.45-1.54 (m, 2H, CH_2-*i*Pr, $CH(CH_3)_2$), 0.81-0.89 (m, 6H, $(CH_3)_2CH$); ^{13}C NMR (DMSO-d_6, 125.5 MHz) 170.1, 161.7, 158.12, 158.08, 156.3 (q, $^2J_{CF}$ = 36 Hz, $COCF_3$), 149.0, 144.7, 141.8 (C6), 135.42, 135.40, 134.91, 134.87, 129.8 (Ar), 129.6 (Ar), 127.9 (Ar), 127.5 (Ar), 126.7 (Ar), 115.8 (q, $^1J_{CF}$ = 288 Hz, CF_3), 113.2 (Ar), 97.6, 88.9, 87.5, 86.9 (C1′), 85.6, 78.8 (C2′), 74.7, 71.4 (C5″), 69.6 (C3′), 59.1 (C5′), 55.0 (CH_3O), 51.5 ($CHNHCOCF_3$), 39.7 (CH_2-*i*Pr – overlap with DMSO-d_6), 28.9 ($CH_2C{\equiv}C$), 24.3 ($CHMe_2$), 22.9 (CH_3), 21.0 (CH_3); ^{19}F NMR (DMSO-d_6, 282.4 MHz) δ -74.3. An extra set of ^{13}C NMR signals are observed for some of the carbons (extra signals at 88.8, 74.8, 59.0, 24.2, 22.8, 20.9 ppm). We attribute these peaks to the presence of two different conformers, most likely rotamers – rather than scrambling of the chirality center in the amino acid residue – based on the observation that only one set of signals is observed when the spectrum is recorded in acetone-d_6.

5-(bis-TFA-lysyl-aminopropynyl)-5′-*O*-(4,4′-dimethoxytrityl) LNA uridine (3z). A solution of (*S*)-2,6-bis(2,2,2-trifluoroacetamido)hexanoic acid (0.27 g, 0.79 mmol), nucleoside **2** (0.50 g, 0.81 mmol), TSTU (0.32 g, 1.06 mmol) and DIPEA (0.42 mL, 2.40 mmol) in anhydrous DMF (10 mL) was reacted (3h), worked up and purified as described in the representative protocol to afford nucleoside **3z** (0.44 g, 58%) as a slightly brown solid material. R_f = 0.5 (5% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 954.2770 ([M + Na]$^+$, $C_{44}H_{43}F_6N_5O_{11}{\cdot}Na^+$, Calcd 954.2761); ^{1}H NMR (DMSO-d_6, 500.1 MHz) δ 11.68 (s, 1H, ex, NH), 9.50 (d, 1H, ex, J = 7.0 Hz, NHCH), 9.36 (br s, 1H, ex, NH(CF_3CO)CH_2), 8.55 (br s, 1H, ex, $NHCH_2C{\equiv}C$), 7.78 (s, 1H, H6), 7.42-7.45 (m, 2H, Ar), 7.28-7.35 (m, 6H, Ar), 7.22-7.26 (m, 1H, Ar), 6.88-6.93 (2d, 4H, J = 9.0 Hz, Ar), 5.72 (d, 1H, ex, J = 5.0 Hz, 3′-OH), 5.42 (ap d, 1H, J = 3.5 Hz, H1′), 4.27-4.32 (m, 1H, CHNH), 4.25 (s, 1H, H2′), 4.03 (ap t, 1H, J = 5.0 Hz, H3′), 3.78-3.98 (m, 4H, $CH_2C{\equiv}C$, H5″), 3.74 (s, 6H, CH_3O), 3.55-3.58 (d, 1H, J = 11.5 Hz, H5′), 3.26-3.29 (d, 1H, J = 11.5 Hz, H5′), 3.09-3.20 (m, 2H, $CH_2CH_2CH_2CH_2CHNH$), 1.65-1.74 (m, 2H, CH_2CHNH), 1.41-1.49 (m, 2H, $CH_2CH_2CH_2CHNH$), 1.18-1.32 (m, 2H, CH_2CH_2CHNH); ^{13}C NMR (DMSO-d_6, 125.5 MHz) δ 169.8, 161.7, 158.12, 158.08, 156.4 (2q, $^2J_{CF}$ = 36 Hz, 2×$COCF_3$), 149.0, 144.7, 141.80, 141.78 (C6), 135.42, 135.39, 134.91, 134.88, 129.8 (Ar), 129.6 (Ar), 127.9 (Ar), 127.5 (Ar), 126.6 (Ar), 115.9 (q, $^1J_{CF}$ = 286 Hz, CF_3), 115.7 (q, $^1J_{CF}$ = 290 Hz, CF_3), 113.2 (Ar), 97.54, 97.53, 88.81, 88.75, 87.5, 86.9 (C1′), 85.6, 78.8 (C2′), 74.84, 74.79, 71.4 (C5″), 69.6 (C3′), 59.1 (C5′), 59.0 (C5′), 54.9 (CH_3O), 53.0 (CHNH), 38.9 ($CH_2CH_2CH_2CH_2CHNH$), 30.5 (CH_2CHNH), 28.9 ($CH_2C{\equiv}C$), 27.6 ($CH_2CH_2CH_2CHNH$), 22.7 (CH_2CH_2CHNH); ^{19}F NMR (DMSO-d_6, 282.4 MHz) δ -74.3, -74.9. An extra set of ^{13}C NMR signals is observed for some of the carbons, which we

again attribute to the presence of two different conformers/rotamers (extra signals at ~ 141.8, 97.5, 88.8, 74.8 and 59.1 ppm – all belong to carbons in the (anticipated spatial) vicinity of the amino acid residue).

General phosphitylation protocol for the preparation of 4x/y/z. The appropriate nucleoside **3** was coevaporated with anhydrous 1,2-dichloroethane (2 × 10 mL) and dissolved in anhydrous CH_2Cl_2. DIPEA was added to this solution followed by dropwise addition of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (PCl reagent). The reaction was stirred at rt for 2 h, at which point ice cold ethanol (1 mL) was added and the solvents were evaporated. The resulting residue was purified by silica gel column chromatography (typically 0-5% MeOH/CH_2Cl_2, v/v) and subsequent trituration from CH_2Cl_2 and petroleum ether to provide phosphoramidites **4x/y/z**.

5-(TFA-glycyl-aminopropynyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl) LNA uridine (4x). A solution of nucleoside **3x** (146 mg, 0.19 mmol), DIPEA (137 μL, 0.78 mmol) and PCl reagent (66 μL, 0.29 mmol) in anhydrous CH_2Cl_2 (4 mL) was reacted and purified as described above to afford phosphoramidite **4x** (119 mg, 64%) as a white foam. R_f = 0.3 (5% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 987.3279 ($[M + Na]^+$, $C_{47}H_{52}F_3N_6O_{11}P{\cdot}Na^+$, Calcd 987.3282); ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 149.9, 148.8.

5-(TFA-leucyl-aminopropynyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl) LNA uridine (4y). A solution of

nucleoside **3y** (83 mg, 0.10 mmol), DIPEA (71 µL, 0.41 mmol) and PCl reagent (41 µL, 0.18 mmol) in anhydrous CH_2Cl_2 (4 mL) was reacted and purified as described above to afford phosphoramidite **4y** as a light yellow foam (38 mg, 37% yield). R_f = 0.3 (5% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 1043.3889 ($[M + Na]^+$, $C_{51}H_{60}F_3N_6O_{11}P{\cdot}Na^+$, Calcd 1043.3908); ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 149.8, 148.8.

5-(TFA-lysyl-aminopropynyl)-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl) LNA uridine (4z). A solution of nucleoside **3z** (154 mg, 0.16 mmol), DIPEA (112 µL, 0.65 mmol) and PCl-reagent (72 µL, 0.32 mmol) in anhydrous CH_2Cl_2 (4 mL) was reacted and purified as described above to afford phosphoramidite **4z** (83 mg, 45%) as a light yellow foam. R_f = 0.4 (5% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 1154.3789 ($[M + Na]^+$, $C_{53}H_{60}F_6N_7O_{12}P{\cdot}Na^+$, Calcd 1154.3840); ^{31}P NMR (DMSO-d_6, 121.5 MHz) δ 148.4, 147.9.

General protocol for the synthesis of modified ONs. **ASO L1** was obtained from a commercial vendor. All other modified ONs were synthesized on an automated DNA synthesizer (0.2 µmol scale) and using long-chain alkyl amine controlled pore glass (LCAA-CPG) solid support. Modified phosphoramidites (0.05 M in acetonitrile) were used to incorporate monomers **X**-**Z**. Extended hand couplings (15 min, 4,5-dicyanoimidazole), oxidation (60 s) and capping (30 s) were employed resulting in stepwise coupling yield of 99, 93, and 90% for phosphoramidites **4x**, **4y** and **4z**, respectively. ONs were deprotected and cleaved from solid support using ammonia (55 °C, 17 h), purified in the DMT-ON mode using reverse-phase ion-pair HPLC (0.05 M aq.

triethyl ammonium acetate / 25% water in CH_3CN), detritylated (80% aq. AcOH) and precipitated ($NaOAc/NaClO_4$/acetone, -18 °C for 12-16 h). Purity (>80%) and identity was verified by analytical HPLC and MALDI-TOF, respectively. Quantification of ONs was performed using extinction coefficients (OD_{260}/µmol) of 12.01 (G), 15.2 (A), 7.05 (C), and 8.40 (T).

Protocol – thermal denaturation studies. Thermal denaturation curves were recorded and analyzed as previously described. The two strands comprising a duplex were annealed (each at 1.0 µM, 85 °C, 2 min) in a medium salt phosphate buffer ($[Na^+]$ = 110 mM, $[Cl^-]$ = 100 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)), unless otherwise specified. A temperature ramp of 0.5 °C/min was used in all experiments. The reported T_m is the maximum of the first derivative curve, rounded to the nearest 0.5 °C, averaged from two experiments within 1.0 °C.

Table S1. MALDI-MS of synthesized ONs.[a]

ON	Sequence	Calculated *m/z* $[M]^+$	Observed *m/z* $[M]^+$
X1	5′-GTG A**X**A TGC	2876.5	2877.7
X2	3′-CAC **X**AT ACG	2805.5	2806.6
X3	3′-CAC TA**X** ACG	2805.5	2806.6
X4	3′-CAC **X**A**X** ACG	2929.6	2930.5
Y1	5′-GTG A**Y**A TGC	2932.6	2933.6
Y2	3′-CAC **Y**AT ACG	2861.6	2862.6
Y3	3′-CAC TA**Y** ACG	2861.6	2862.0
Y4	3′-CAC **Y**A**Y** ACG	3041.7	3042.1
Z1	5′-GTG A**Z**A TGC	2947.6	2948.7
Z2	3′-CAC **Z**AT ACG	2876.6	2877.8
Z3	3′-CAC TA**Z** ACG	2876.6	2877.7
Z4	3′-CAC **Z**A**Z** ACG	3071.7	3072.6
ASO Z1	5′- **Z**cg AAG TAC TCG GCG TAg g**Z**T	7309.8	7310.0

[a] Structures of monomers **X**/**Y**/**Z** are shown in Scheme 1 in the main text. Lower case letters denote canonical LNA monomers; underlined denotes phophorothioate backbone.

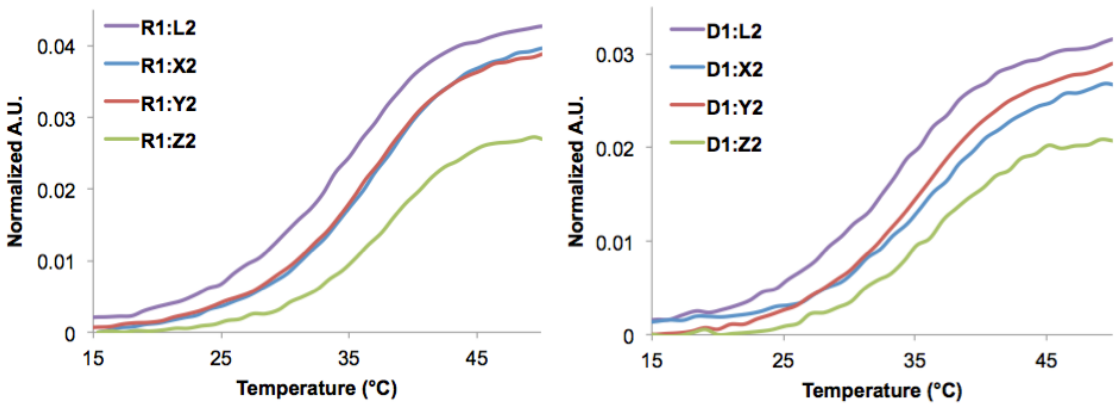

Figure S1. Representative thermal denaturation curves for the B2-series (3′-CAC TA**B** ACG). For monomer structures, see Scheme 1.

Table S2. T_m's of duplexes between **B1**-**B4** -series and complementary DNA targets.[a]

			T_m [ΔT_m/mod]/°C			
ON	**Duplex**	**B** =	**L**	**X**	**Y**	**Z**
B1	5′-GTG A**B**A TGC		36.0	37.5	36.5	38.5
D2	3′-CAC TAT ACG		[+6.5]	[+8.0]	[+7.0]	[+9.0]
D1	5′-GTG ATA TGC		34.0	36.0	36.5	39.0
B2	3′-CAC **B**AT ACG		[+4.5]	[+6.5]	[+7.0]	[+9.5]
D1	5′-GTG ATA TGC		36.5	38.0	37.0	37.0
B3	3′-CAC TA**B** ACG		[+7.0]	[+8.5]	[+7.5]	[+7.5]
D1	5′-GTG ATA TGC		39.0	46.0	44.5	49.0
B4	3′-CAC **B**A**B** ACG		[+4.8]	[+8.3]	[+7.5]	[+9.8]

[a] For monomer structures, see Scheme 1. ΔT_m = change in T_m relative to unmodified **D1**:**D2** duplex (29.5 °C).

Table S3. Thermodynamic parameters for duplex formation between **B1**-**B3**-series and complementary RNA or DNA.[a]

		complementary RNA			complementary DNA		
ON	**Sequence**	ΔG^{298} [$\Delta\Delta G^{298}$] (kJ/mol)	ΔH [$\Delta\Delta H$] (kJ/mol)	$-T^{298}\Delta S$ [$\Delta(-T^{298}\Delta S)$] (kJ/mol)	ΔG^{298} [$\Delta\Delta G^{293}$] (kJ/mol)	ΔH [$\Delta\Delta H$] (kJ/mol)	$-T^{298}\Delta S$ [$\Delta(-T^{298}\Delta S)$] (kJ/mol)
D1	5′-GTG ATA TGC	-36	-278	241	-42	-314	271
D2	3′-CAC TAT ACG	-39	-293	254	-42	-314	271
L1	5′-GTG A**L**A TGC	-49 [-13]	-309 [-31]	260 [+19]	-47 [-5]	-297 [+17]	250 [-21]
L2	3′-CAC **L**AT ACG	-47 [-8]	-331 [-38]	283 [+29]	-46 [-4]	-332 [-18]	286 [+15]
L3	3′-CAC TA**L** ACG	-50 [-11]	-340 [-47]	290 [+36]	-49 [-7]	-332 [-18]	283 [+12]
X1	5′-GTG A**X**A TGC	-55 [-19]	-385 [-107]	330 [+89]	-55 [-13]	-399 [-85]	344 [+73]
X2	3′-CAC **X**AT ACG	-47 [-8]	-386 [-93]	339 [+85]	-50 [-8]	-382 [-68]	332 [+61]
X3	3′-CAC TA**X** ACG	-53 [-14]	-409 [-116]	356 [+102]	-52 [-10]	-338 [-24]	285 [+14]
Y1	5′-GTG A**Y**A TGC	-46 [-10]	-310 [-32]	264 [+23]	-47 [-5]	-342 [-28]	295 [+24]
Y2	3′-CAC **Y**AT ACG	-54 [-15]	-480 [-187]	426 [+172]	-59 [-17]	-557 [-243]	499 [+228]
Y3	3′-CAC TA**Y** ACG	-53 [-14]	-490 [-197]	436 [+182]	-51 [-9]	-451 [-137]	400 [+129]
Z1	5′-GTG A**Z**A TGC	-59 [-23]	-426 [-148]	366 [+125]	-56 [-14]	-395 [-81]	339 [+68]
Z2	3′-CAC **Z**AT ACG	-51 [-12]	-427 [-134]	376 [+122]	-56 [-14]	-480 [-166]	423 [+152]
Z3	3′-CAC TA**Z** ACG	-59 [-20]	-428 [-135]	369 [+115]	-54 [-12]	-369 [-55]	315 [+44]

[a] Values were determined from thermal denaturation curves using the van't Hoff method and are reported as the average of two experiments. $\Delta\Delta G^{298}$, $\Delta\Delta H$ and $\Delta(T^{298}\Delta S)$ are calculated relative to reference duplexes **D1:D2**, **D1:R2** and **D2:R1**.

Table S4. Thermostaiblity of duplexes between **B1-B4** -series and complementary RNA at various ionic strengths.[a]

			complementary RNA (ΔT_m/mod)/°C		
ON	**Sequence**	**[Na$^+$] =**	**110 mM**	**40 mM**	**10 mM**
L1	5′-GTG A**L**A TGC		9.0	9.0	8.5
L2	3′-CAC **L**AT ACG		7.5	7.5	7.5
L3	3′-CAC TA**L** ACG		9.0	9.0	9.5
L4	3′-GCA **L**A**L** CAC		7.5	7.8	7.8
X1	5′-GTG A**X**A TGC		10.5	12.5	13.5
X2	3′-GCA **X**AT CAC		10.5	13.0	14.0
X3	3′-GCA TA**X** CAC		10.0	9.5	10.5
X4	3′-GCA **X**A**X** CAC		9.0	nd	nd
Y1	5′-GTG A**Y**A TGC		9.5	12.5	14.5
Y2	3′-GCA **Y**AT CAC		10.5	11.0	10.5
Y3	3′-GCA TA**Y** CAC		7.0	8.0	9.5
Y4	3′-GCA **Y**A**Y** CAC		9.3	10.8	11.8
Z1	5′-GTG A**Z**A TGC		12.5	16.5	18.0
Z2	3′-GCA **Z**AT CAC		11.0	12.5	14.5
Z3	3′-GCA TA**Z** CAC		14.0	17.5	19.5
Z4	3′-GCA **Z**A**Z** CAC		13.0	14.8	17.0

[a] Graphical representation shown in Figure 1 of main text. ΔT_m = change in T_m relative to matched duplex (**D1**:**R2** or **R1**:**D2**) in the corresponding buffer: **D1**:**R2** ($T_{m,110\ mM}$ = 28.0 °C, $T_{m,40\ mM}$ = 21.0 °C, $T_{m,10\ mM}$ = 11.5 °C); **R1**:**D2** ($T_{m,110\ mM}$ = 28.0 °C, $T_{m,40\ mM}$ = 22.0 °C, $T_{m,10\ mM}$ = 12.0 °C). Buffer conditions: ([Na$^+$] = 110 mM, [Cl$^-$] = 100 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)), ([Na$^+$] = 40 mM, [Cl$^-$] = 30 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)) or ([Na$^+$] = 10 mM, pH 7.0 (NaH_2PO_4/Na_2HPO_4)) for 110 mM Na$^+$, 40 mM Na$^+$, and 10 mM Na$^+$, respectively. nd = not determined.

Table S5. Thermostability of duplexes between **B1-B4** -series and complementary DNA at various ionic strengths.[a]

			complementary DNA ΔT_m/°C		
ON	**Sequence**	[Na^+] =	110 mM	40 mM	10 mM
L1	5′-GTG A**L**A TGC		6.5	7.0	6.0
L2	3′-CAC **L**AT ACG		4.5	4.0	4.5
L3	3′-CAC TA**L** ACG		7.0	6.5	6.5
L4	3′-GCA **L**A**L** CAC		5.0	5.5	5.0
X1	5′-GTG A**X**A TGC		10.5	nd	nd
X2	3′-GCA **X**AT CAC		6.5	6.5	7.0
X3	3′-GCA TA**X** CAC		8.5	9.5	10.0
X4	3′-GCA **X**A**X** CAC		8.0	nd	nd
Y1	5′-GTG A**Y**A TGC		7.0	9.0	9.5
Y2	3′-GCA **Y**AT CAC		7.0	5.5	6.5
Y3	3′-GCA TA**Y** CAC		7.5	9.5	8.5
Y4	3′-GCA **Y**A**Y** CAC		7.5	9.0	10.0
Z1	5′-GTG A**Z**A TGC		9.0	11.0	12.5
Z2	3′-GCA **Z**AT CAC		7.5	9.0	8.5
Z3	3′-GCA TA**Z** CAC		9.5	12.5	14.5
Z4	3′-GCA **Z**A**Z** CAC		10.0	11.0	14.0

[a] ΔT_m = change in T_m relative to matched duplex (**D1**:**D2**) in the corresponding buffer: $T_{m,110\ mM}$ = 29.5 °C, $T_{m,\ 40\ mM}$ = 23.5 °C, $T_{m,\ 10\ mM}$ = 14.0 °C. For buffers, see Table S4. nd = not determined.

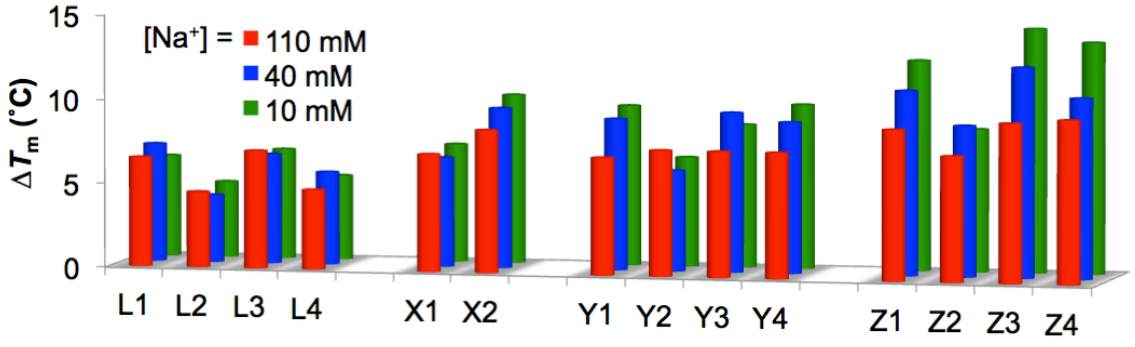

Figure S2. Thermostability of duplexes between **B1-B4** -series and complementary DNA at different ionic strengths. See Table S5 for conditions and raw data.

Table S6. Discrimination of mismatched DNA targets by **B1**-series and reference strands.[a]

			DNA: 3′-CAC T**M**T ACG			
			T_m/°C	Δ T_m/°C		
ON	**Sequence**	**M** =	A	C	G	T
D1	5′-GTG ATA TGC		**29.5**	-16.5	-8.0	-15.5
L1	5′-GTG A**L**A TGC		**34.5**	-18.0	-11.0	-16.0
X1	5′-GTG A**X**A TGC		**37.5**	-23.5	-14.5	-19.5
Y1	5′-GTG A**Y**A TGC		**36.5**	-18.0	-15.0	-17.5
Z1	5′-GTG A**Z**A TGC		**38.5**	-16.5	-12.5	-16.0

[a] For conditions of thermal denaturation experiments, see Table 1. T_m's of fully matched duplexes are shown in bold. ΔT_m = change in T_m relative to fully matched **D1**:**D2** duplex.

Table S7. Discrimination of mismatched RNA/DNA targets by **B4**-series and reference strands.[a]

			RNA: 5′-GUG A**M**A UGC				DNA: 5′-GTG A**M**A TGC			
			T_m/°C [°C]	ΔT_m/°C			T_m/°C [°C]	ΔT_m/°C		
ON	**Sequence**	**M**=	T	A	C	G	T	A	C	G
D2	3′-CAC TAT ACG		**28.0**	-17.0	-17.0	-12.0	**29.5**	<-19.5	-16.5	-7.5
L4	3′-CAC **L**A**L** ACG		**43.0**	-21.0	-16.5	-17.0	**40.0**	-17.0	-15.5	-19.5
X4	3′-CAC **X**A**X** ACG		**49.5**	-13.0	-15.5	-16.0	**46.0**	nd	nd	nd
Y4	3′-CAC **Y**A**Y** ACG		**46.5**	-17.0	-15.5	-16.0	**44.5**	-10.0	-13.0	-10.5
Z4	3′-CAC **Z**A**Z** ACG		**54.0**	-29.0	-20.0	-25.0	**49.0**	-4.0	-6.0	-6.0

[a] ΔT_m = change in T_m relative to fully matched duplex shown in bold (**R1**:**B4** or **D1**:**B4**). nd = not determined.

Table S8. Thermostability of duplexes between antisense ONs (ASO) and complementary targets.[a]

ON	Duplex	**b** =	T_m [ΔT_m/mod]/°C L	Z
ASO B1 R3	5′- **b**cg AAG TAC TCG GCG TAg g**b**T 3′- r(AGC UUC AUG UGC CGC AUC CA)		60.0	59.5
ASO B1 D3	5′- **b**cg AAG TAC TCG GCG TAg g**b**T 3′- d(AGC TTC ATG TGC CGC ATC CA)		61.0	57.5

[a] For monomer structures, see Scheme 1. Lower case letters denote canonical LNA; underlined denotes phophorothioate backbone.

References

S1. P. Kumar, M. Østergaard, B. Bharal, B. A. Anderson, D. C. Guenther, M. Kaura, D. J. Raible, P. K. Sharma and P. J. Hrdlicka, *J. Org. Chem.*, DOI:10.1021/jo500614a.

S2. Couplings between 5-iodo-5′-*O*-(4,4′-dimethoxytrityl)-LNA uridine and Fmoc-protected amino acids were unsuccessful in our hands.

S3. R. B. C. Jagt, R. F. Gómez-Biagi, and M. Nitz, *Angew. Chem. Int. Ed.*, 2009, **48**, 1995.

S4. P. A. Jass, V. W. Rosso, S. Racha, N. Soundararajan, J. J. Venit, A. Rusowicz, S. Swaminathan, J. Livshitz, and E. J. Delaney, *Tetrahedron*, 2003, **59**, 9019.

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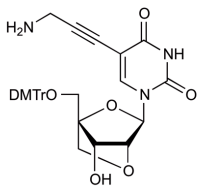
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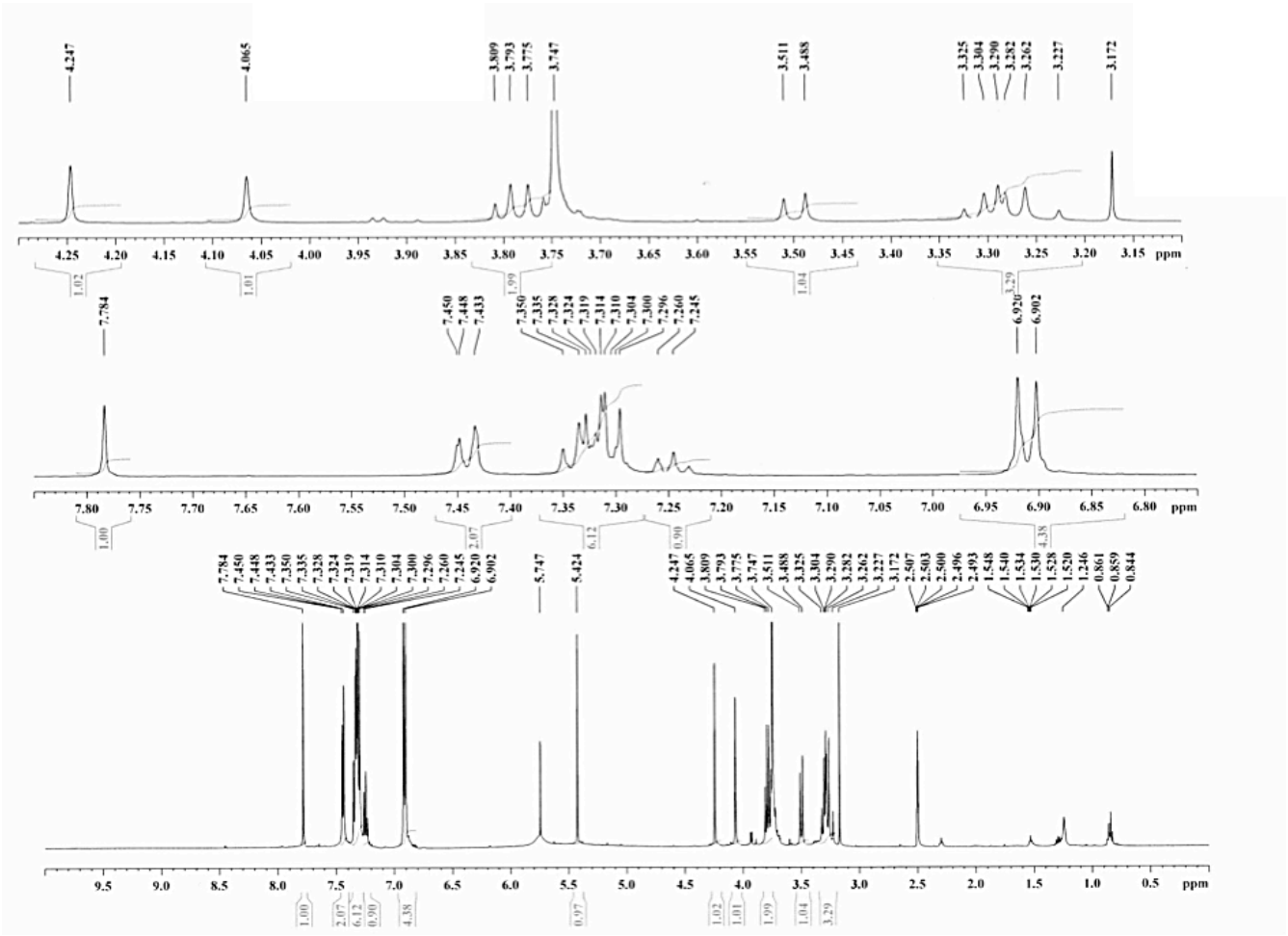
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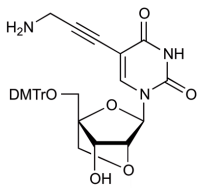
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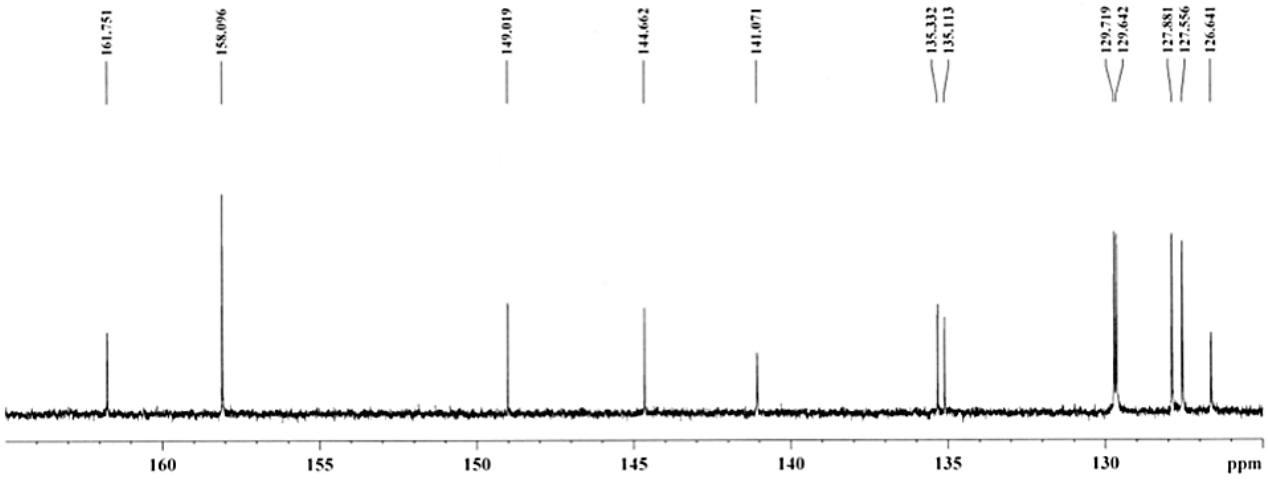
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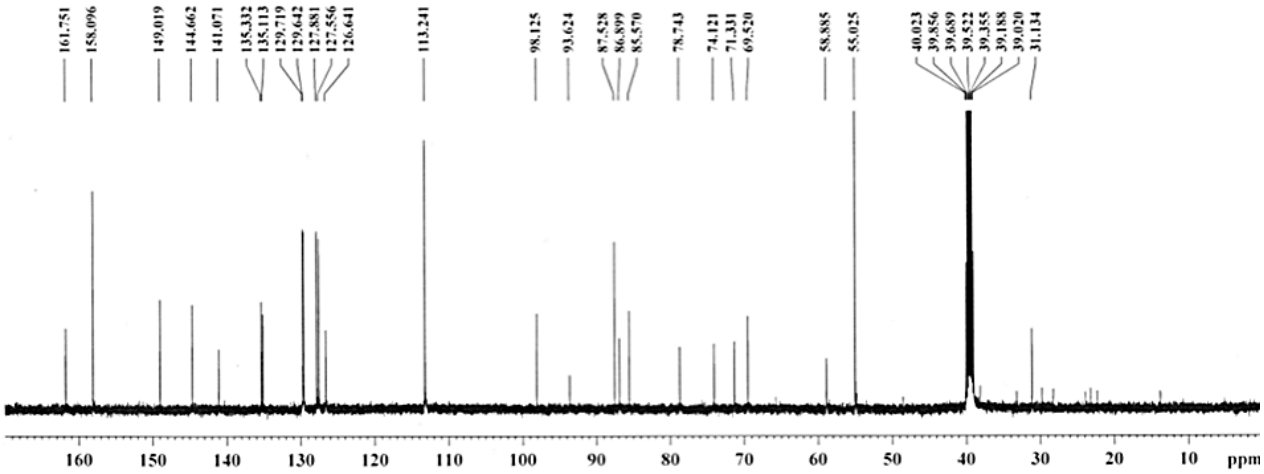
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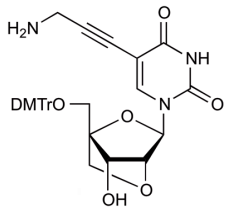
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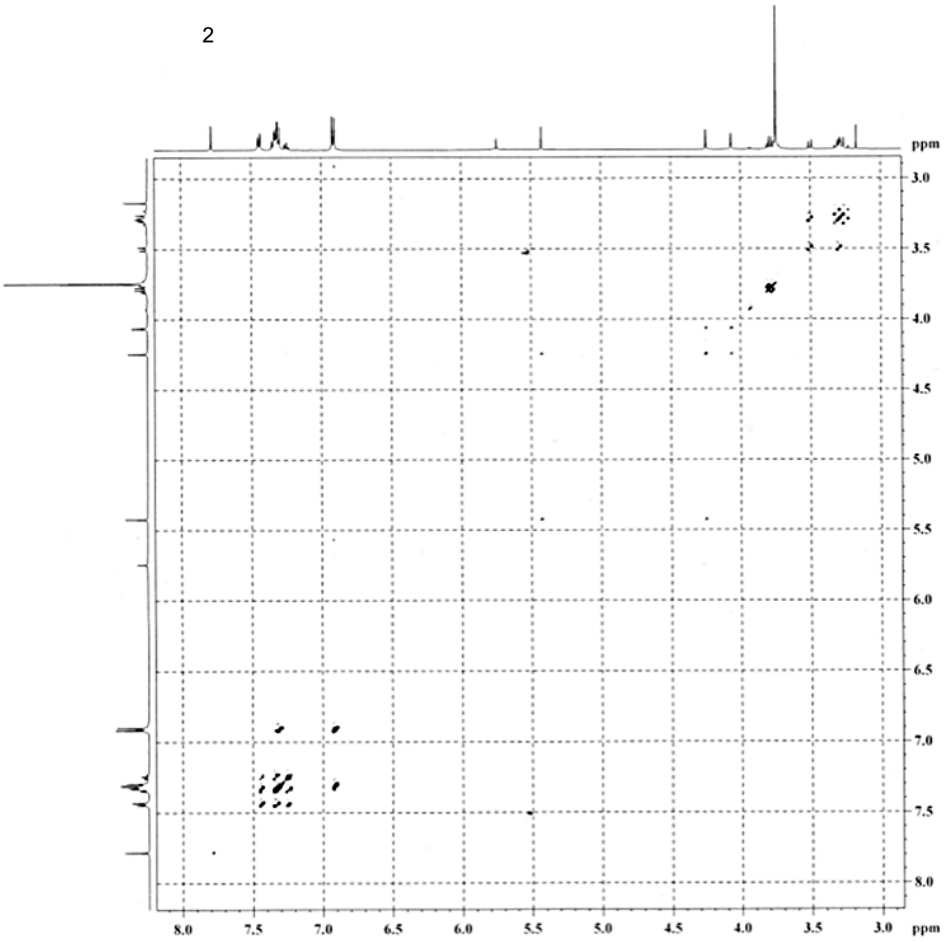
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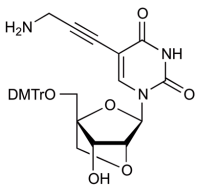
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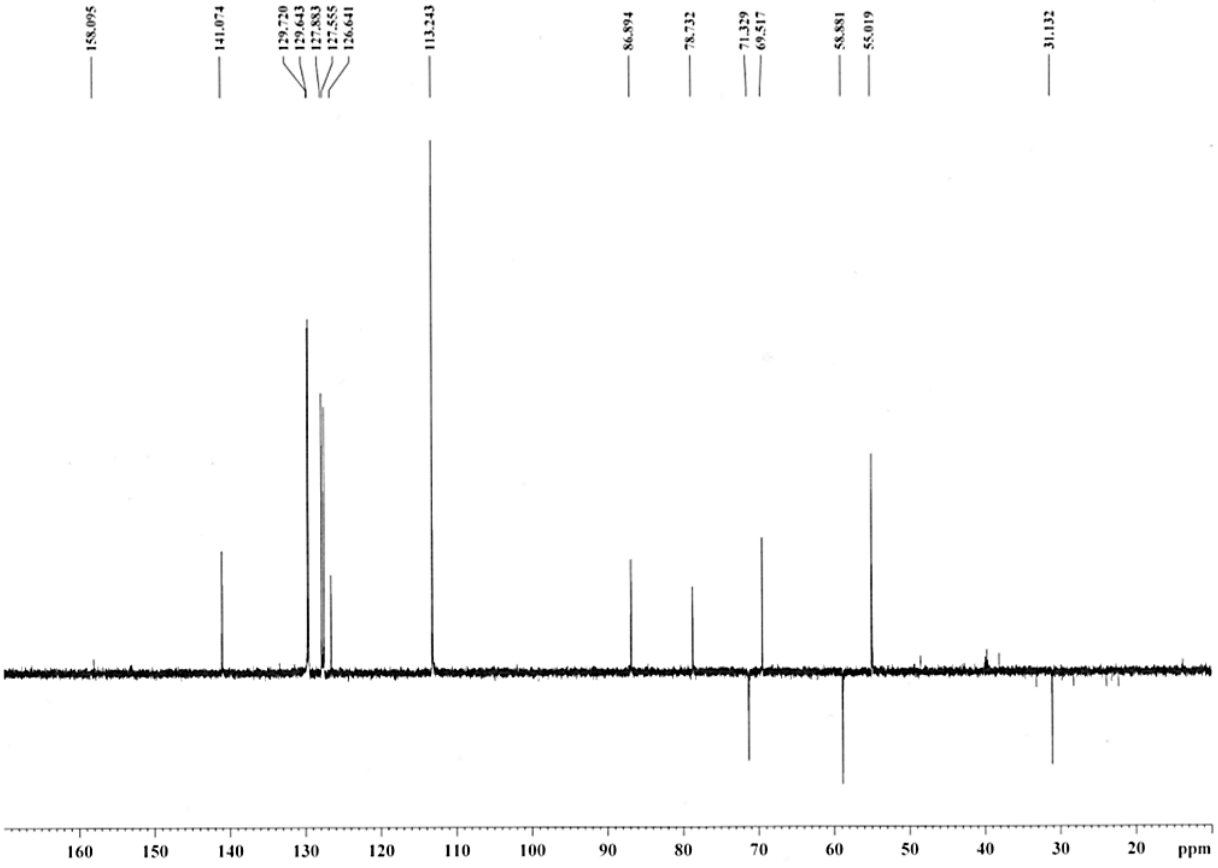
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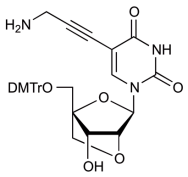
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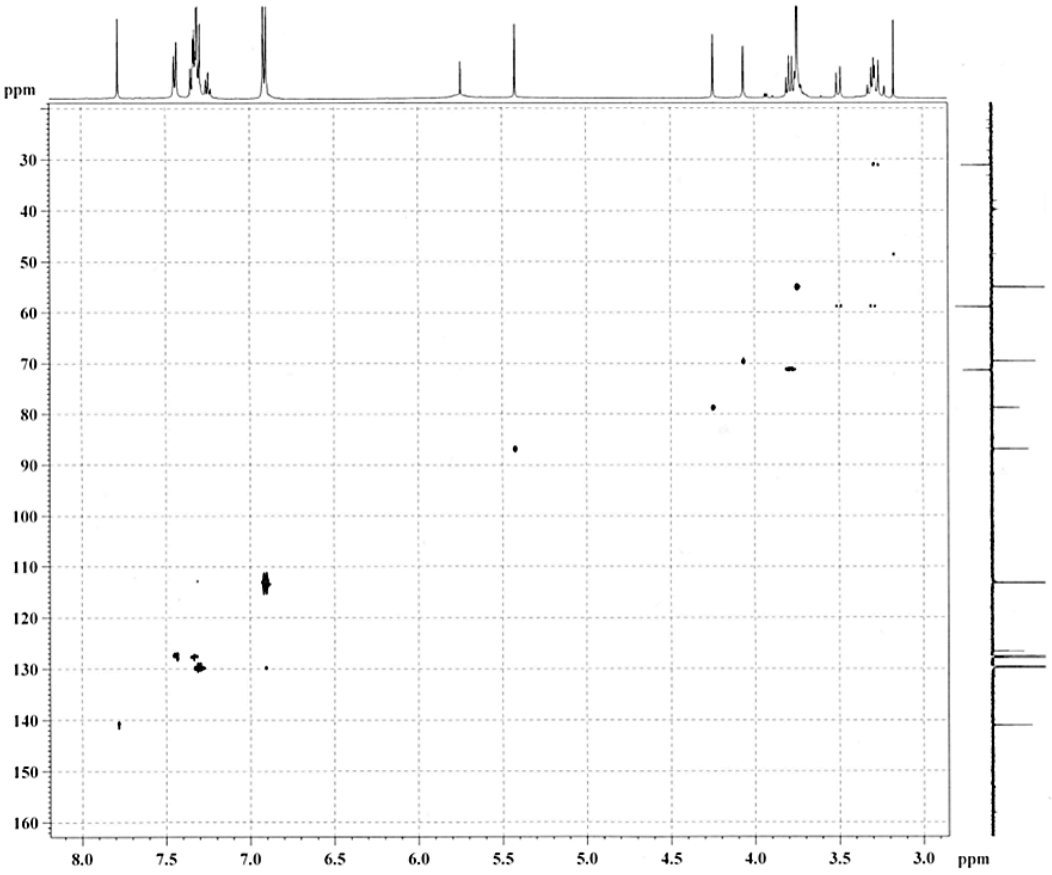
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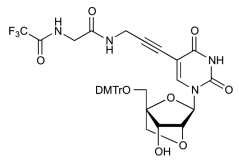
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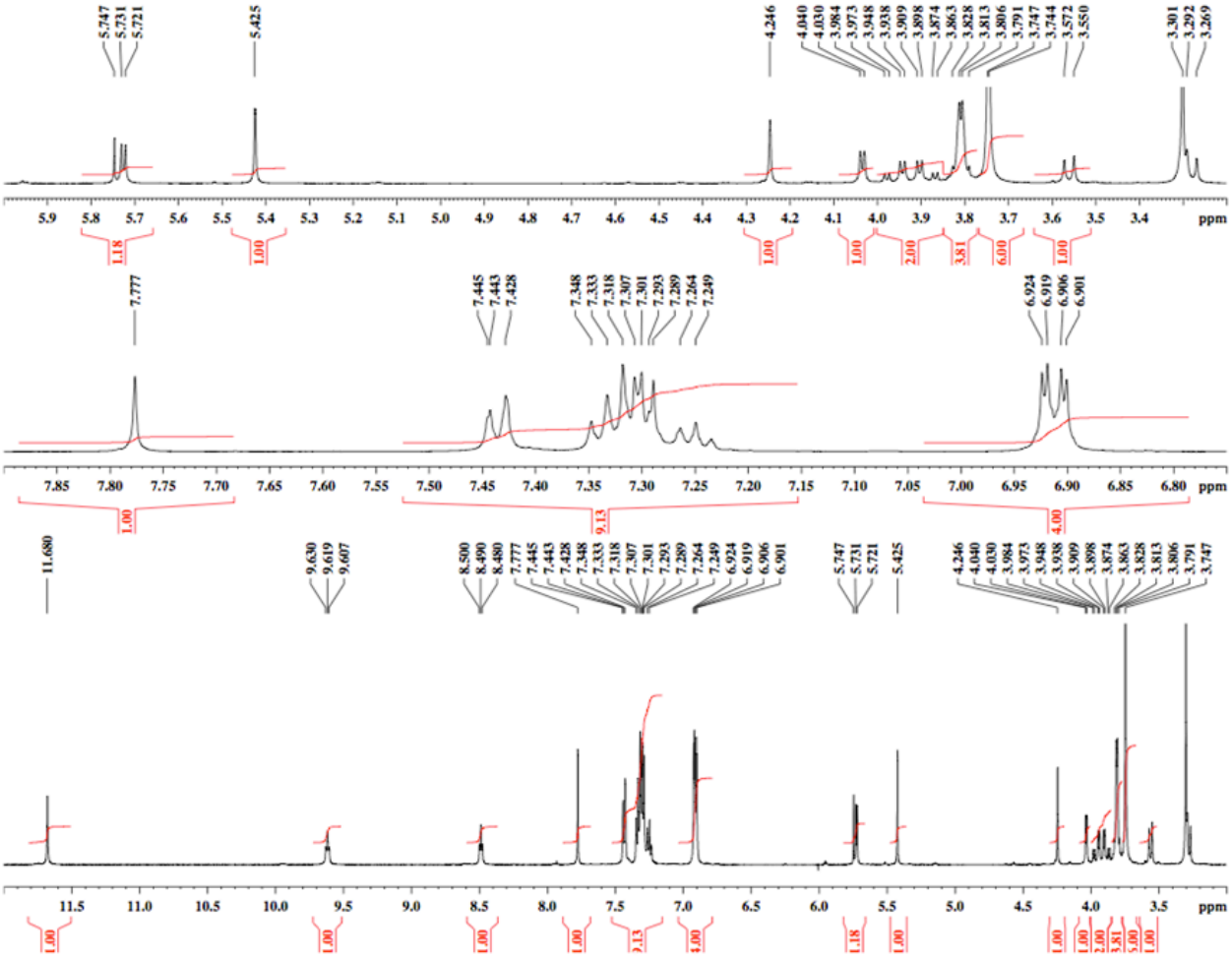
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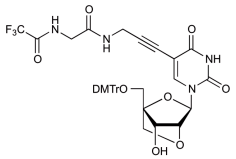
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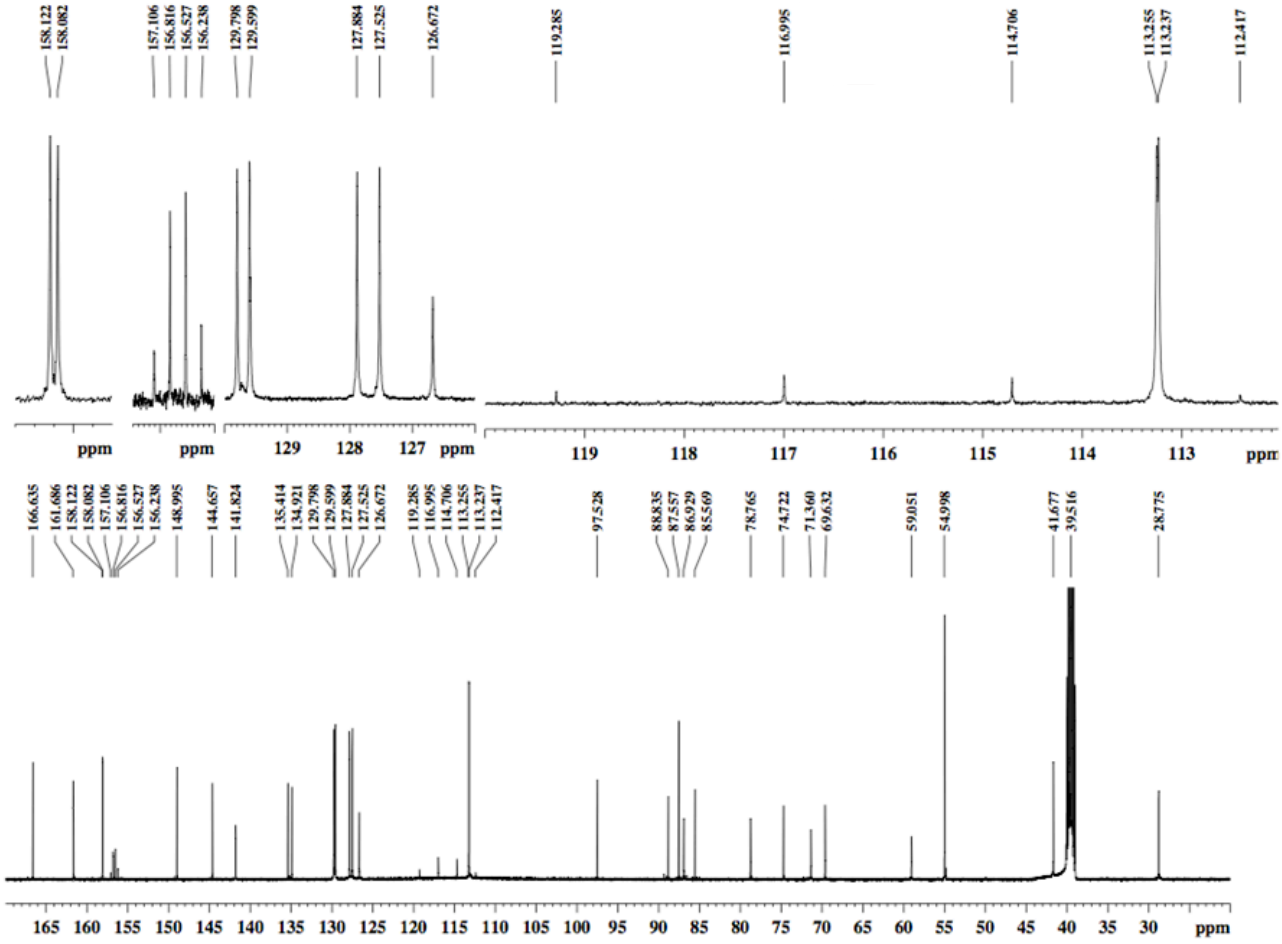
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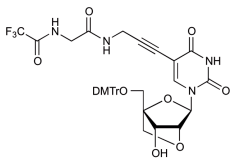
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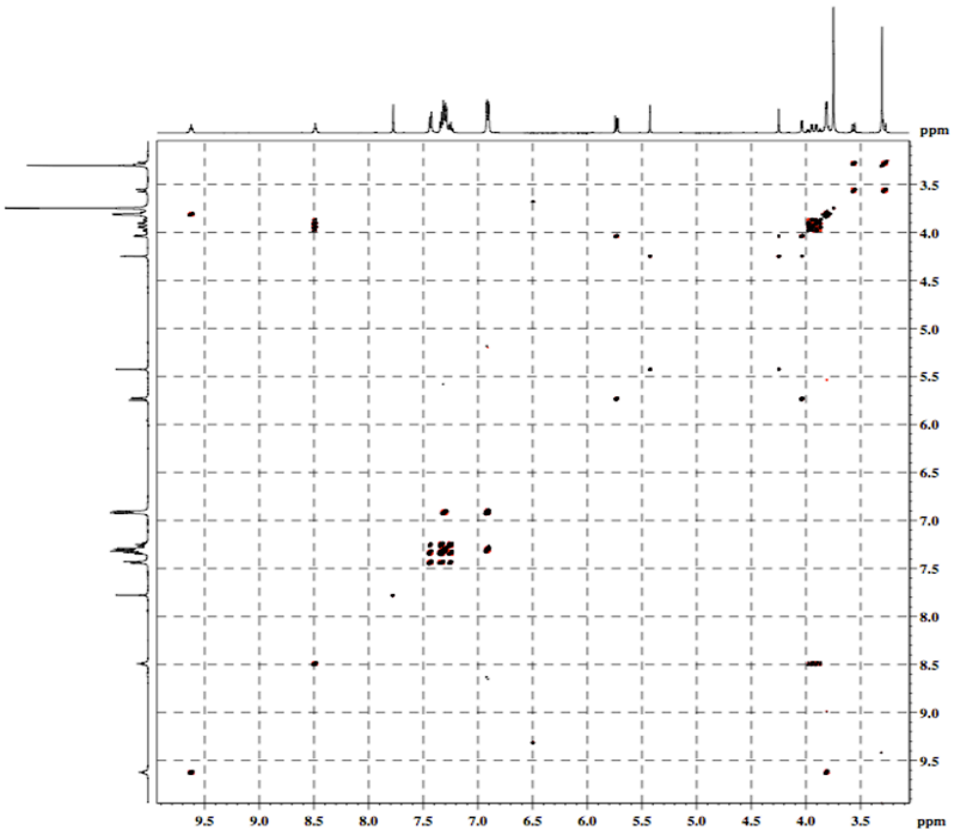
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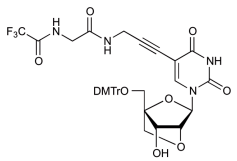

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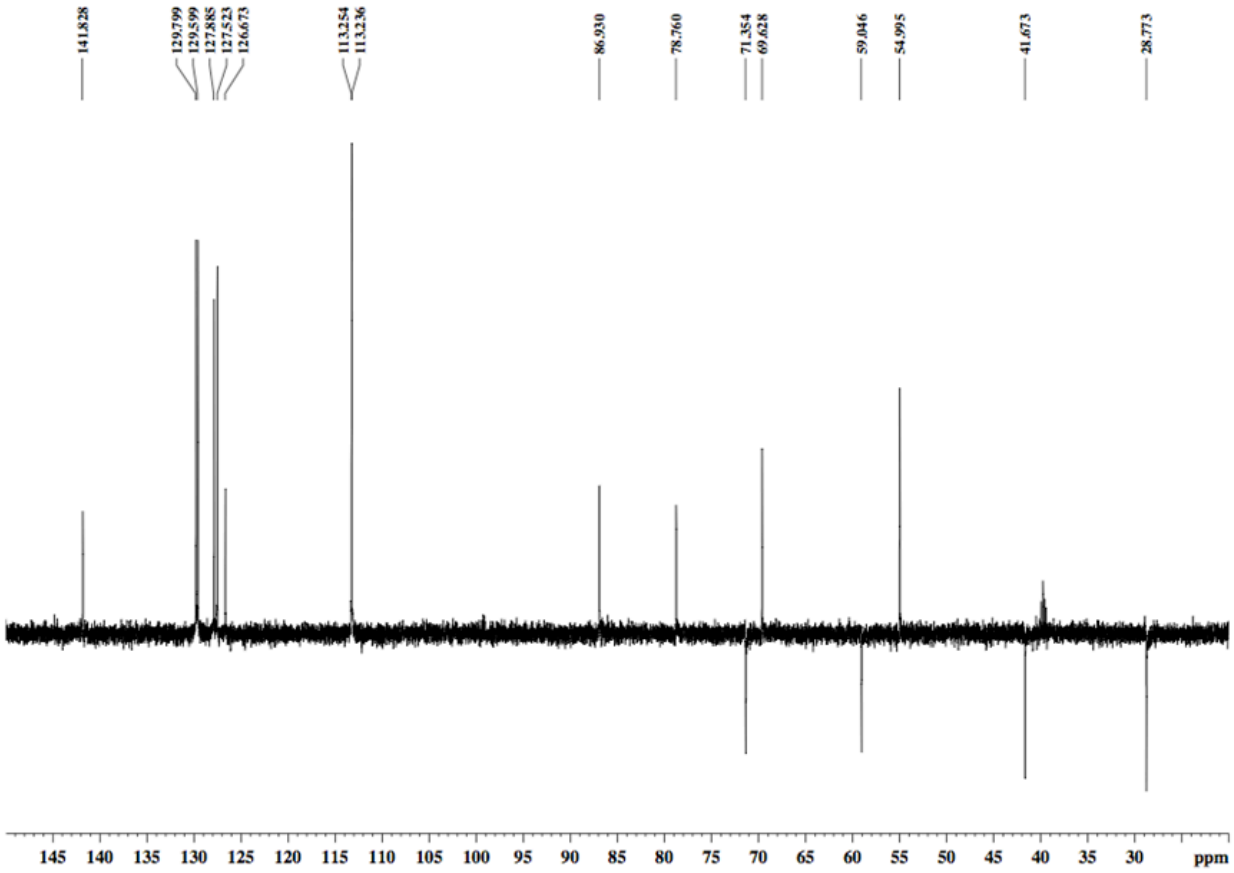
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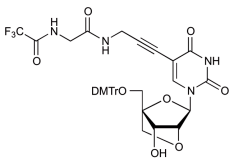

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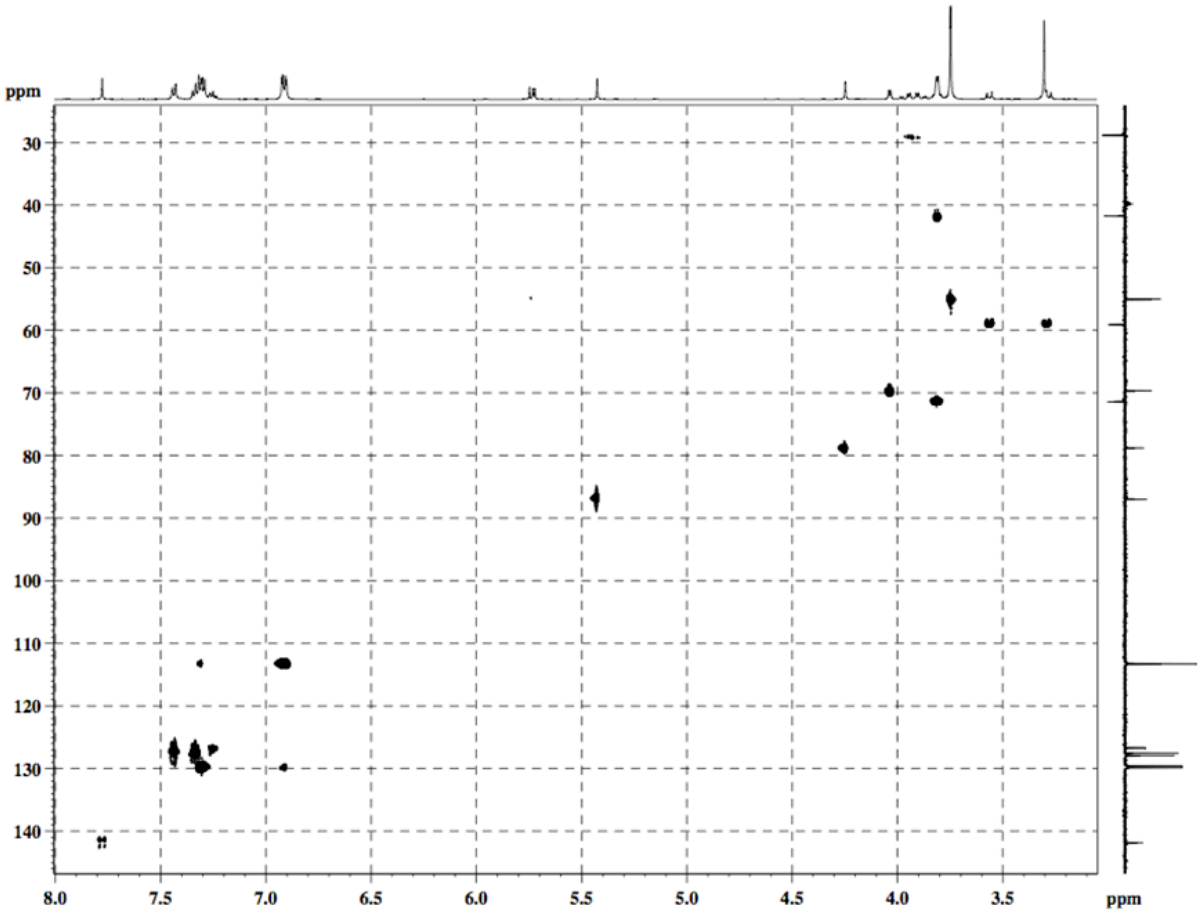
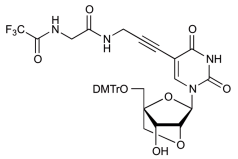
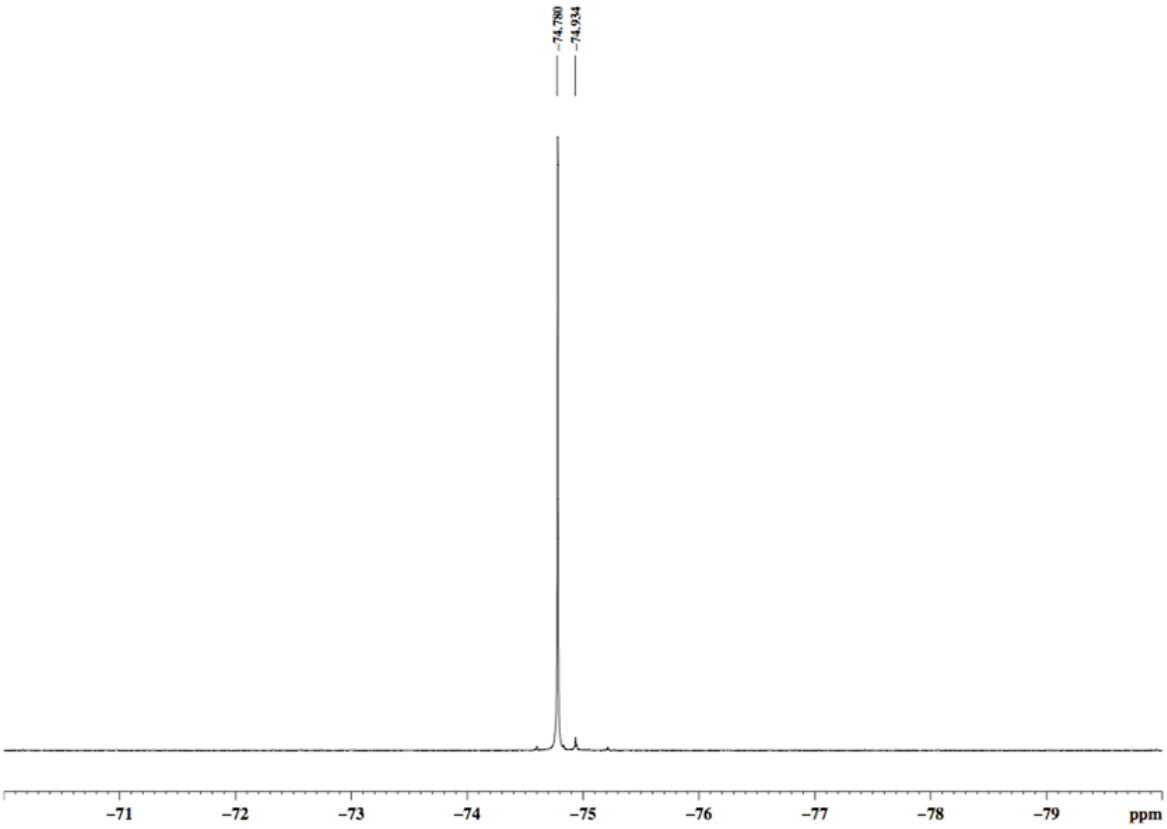

ppm
30
40
50
60
70
80
90
100
110
120
130
140
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm

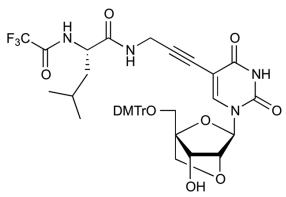
^{19}F



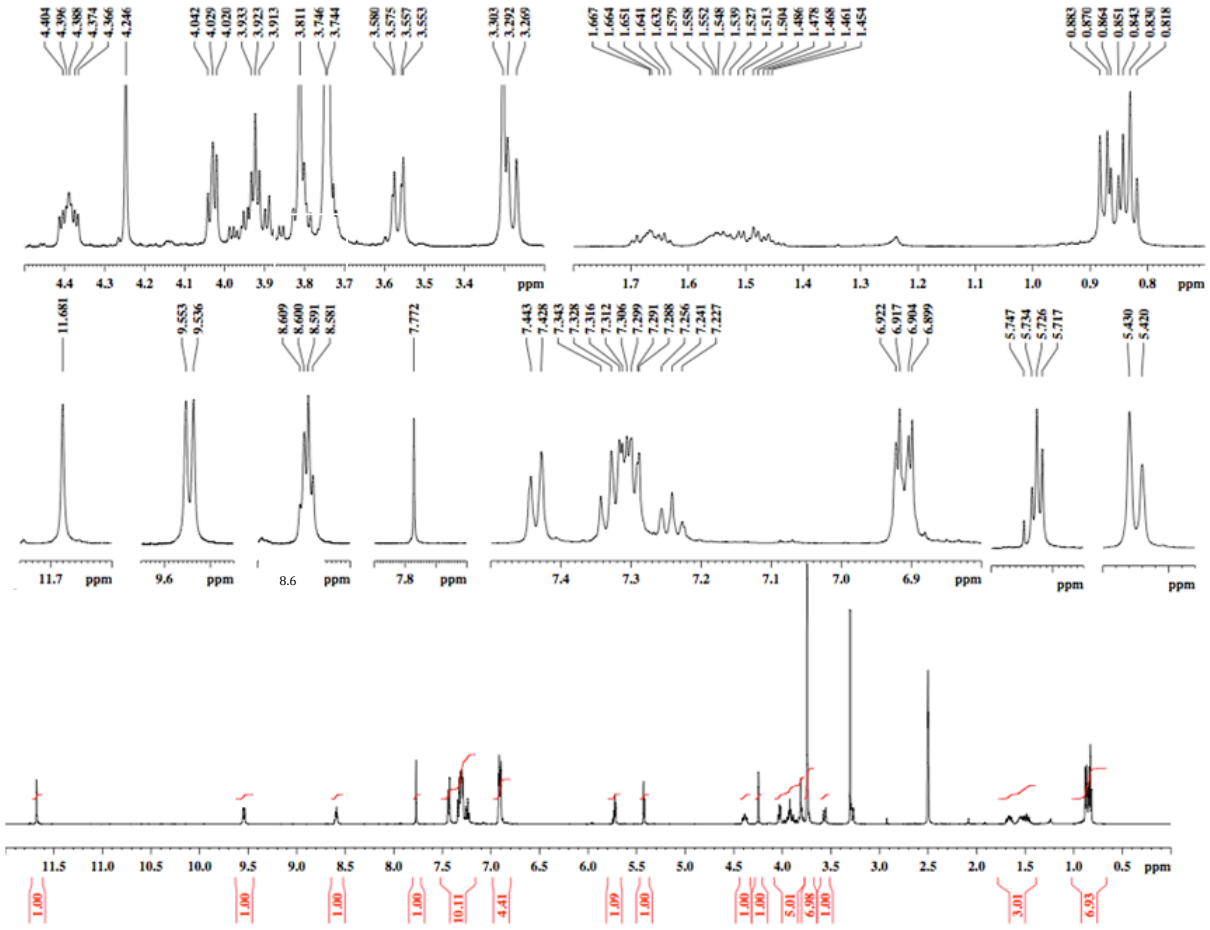
3x



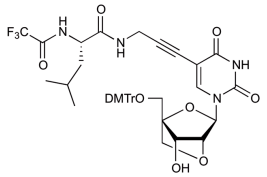
^{1}H



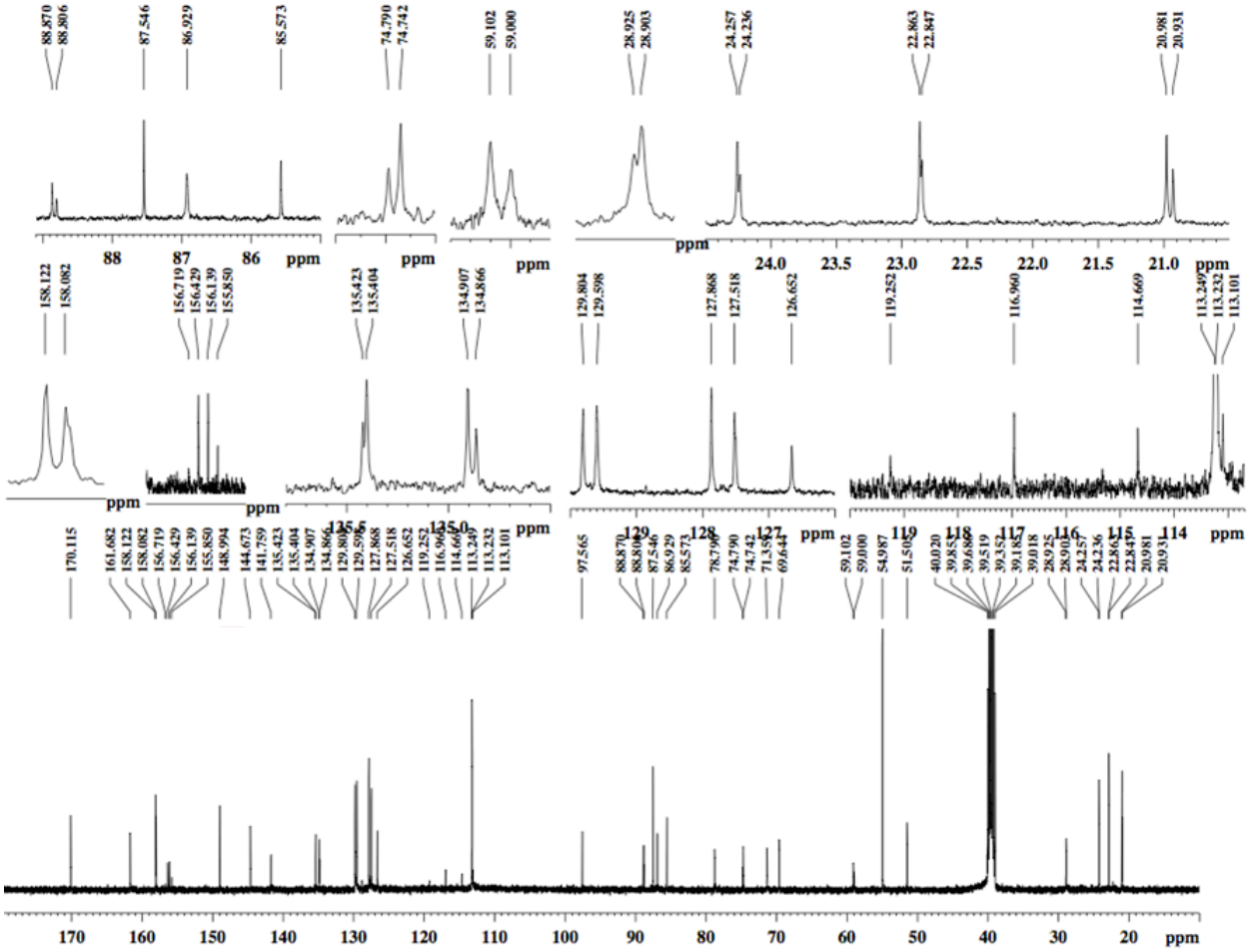
3y



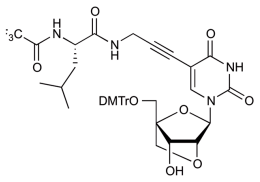
^{13}C


F3C
O
H
N
O
H
N
O
NH
O
N
DMTrO
O
OH
O

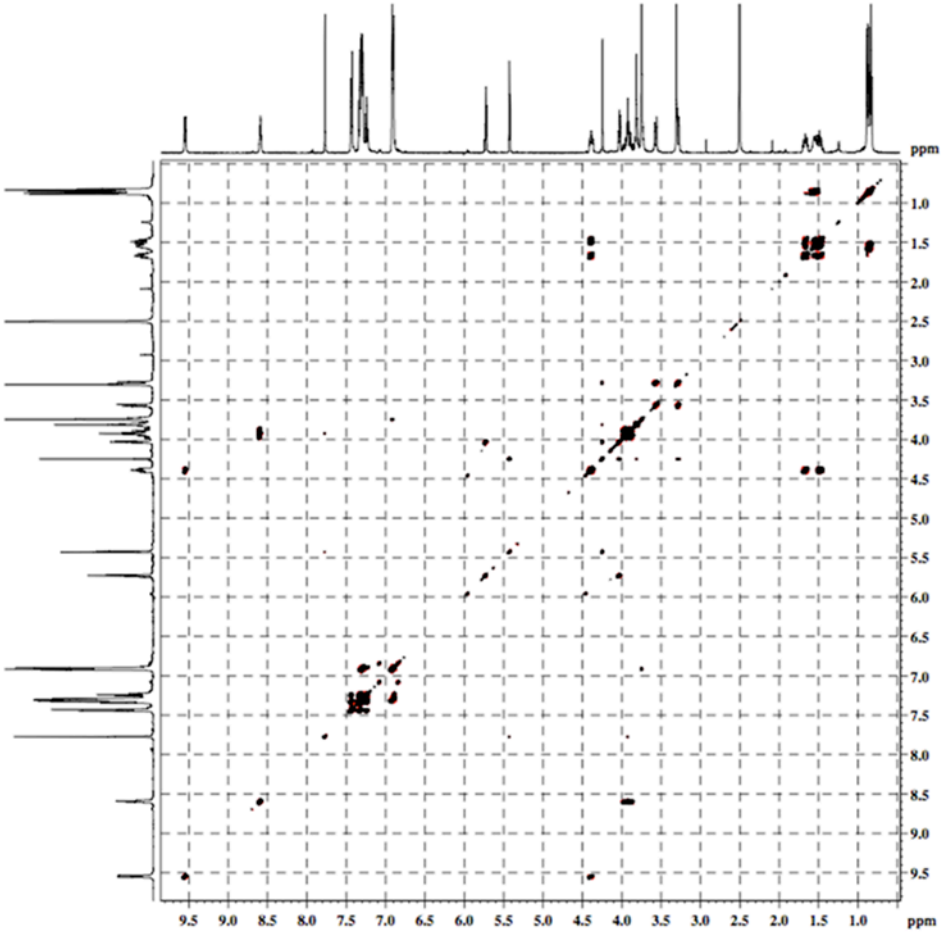
3y


88.870
88.806
87.546
86.929
85.573
74.790
74.742
59.102
59.000
28.925
28.903
24.257
24.236
22.863
22.847
20.981
20.931
158.122
158.082
156.719
156.429
156.139
155.850
135.423
135.404
134.907
134.866
129.804
129.598
127.868
127.518
126.652
119.252
116.960
114.669
113.249
113.232
113.101
ppm
170.115
161.682
158.122
158.082
156.719
156.429
156.139
155.850
148.994
144.673
141.759
135.423
135.404
134.907
134.866
129.804
129.598
127.868
127.518
126.652
119.252
116.960
114.669
113.249
113.232
113.101
97.565
88.870
88.806
87.546
86.929
85.573
78.790
74.790
74.742
71.358
69.644
59.102
59.000
54.987
51.508
40.020
39.858
39.686
39.519
39.352
39.185
39.018
28.925
28.903
24.257
24.236
22.863
22.847
20.981
20.931

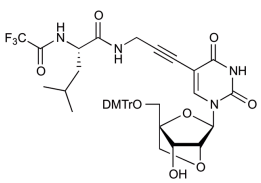
COSY


DMTrO
NH
OH

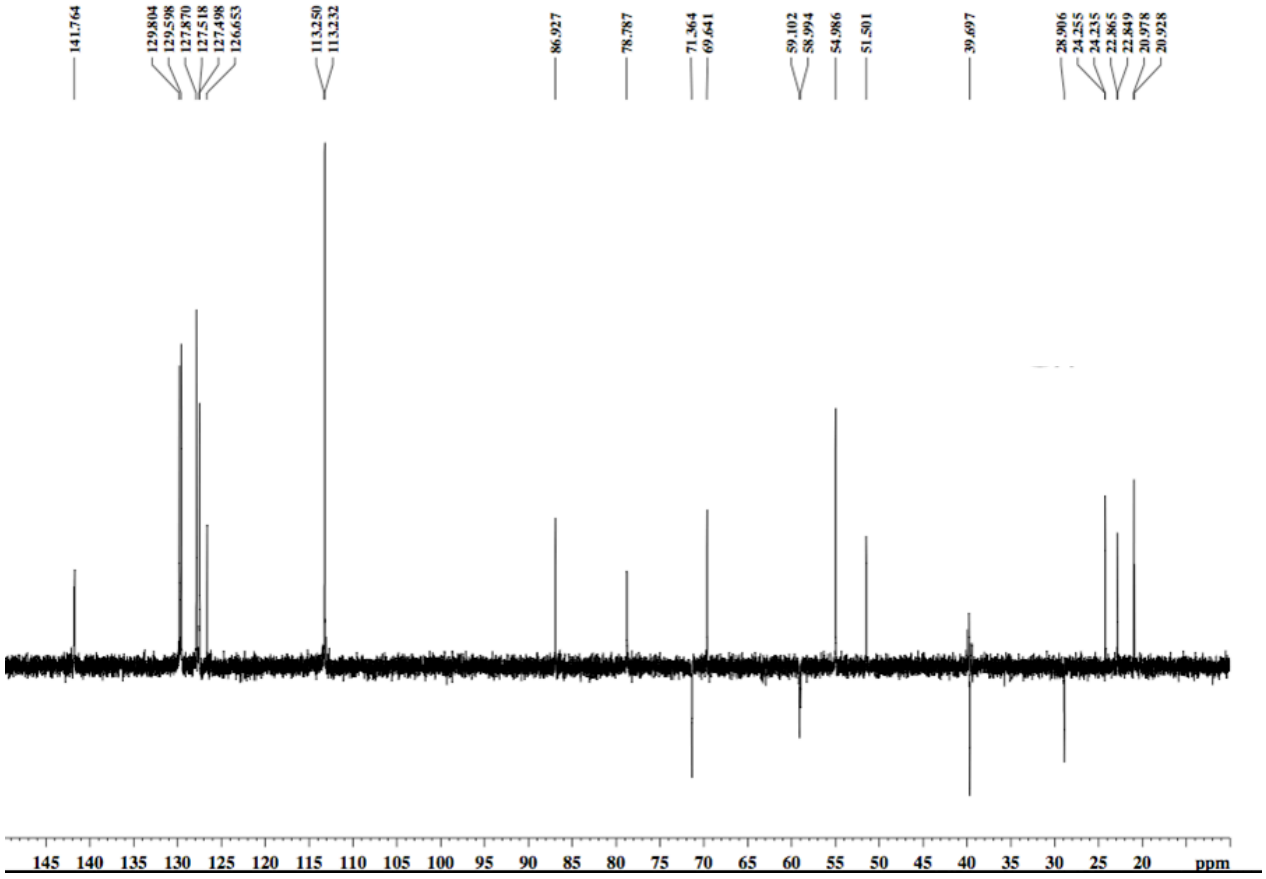
3y


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
ppm

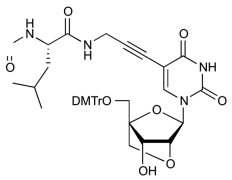
DEPT



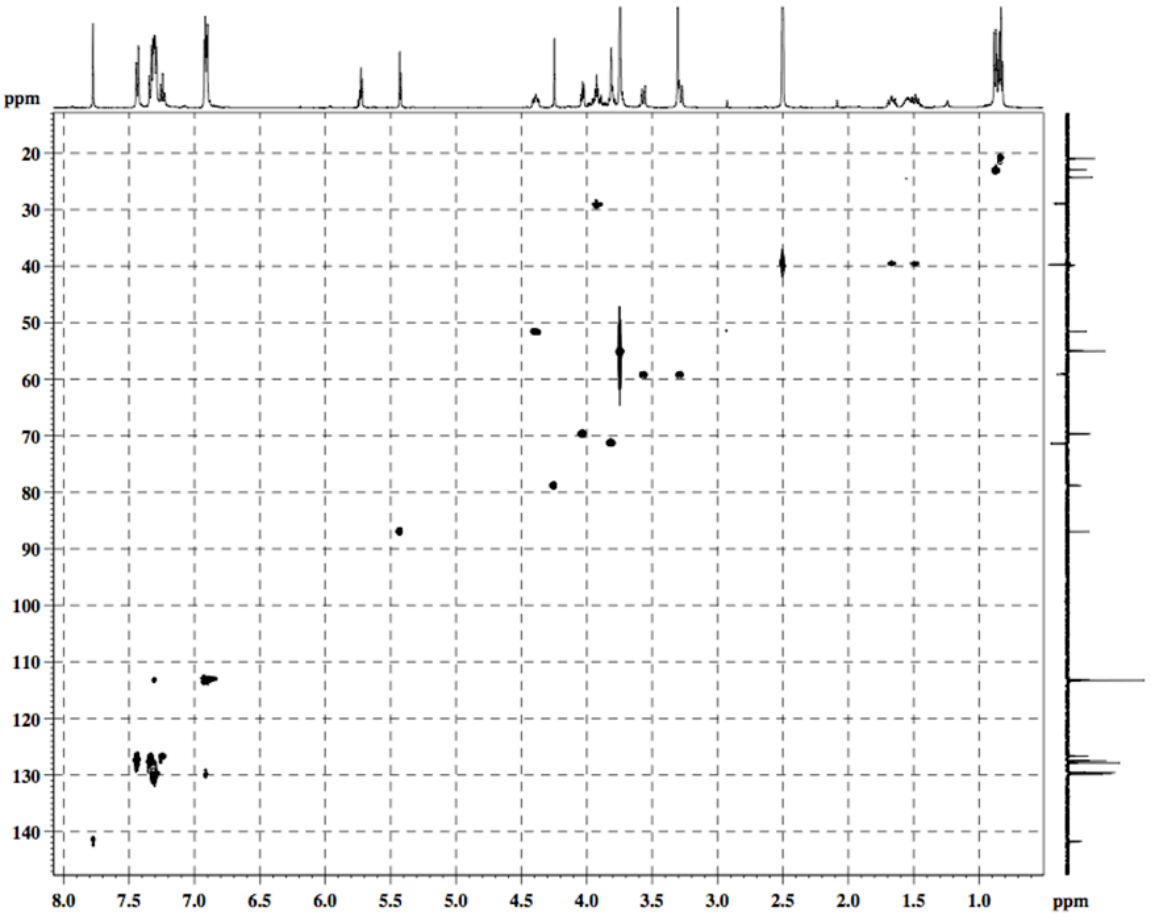
3y


141.764
129.804
129.598
127.870
127.518
127.498
126.653
113.250
113.232
86.927
78.787
71.364
69.641
59.102
58.994
54.986
51.501
39.697
28.906
24.255
24.235
22.865
22.849
20.978
20.928
145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 ppm

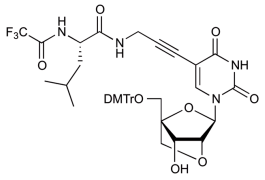
HSQC


DMTrO
OH
NH

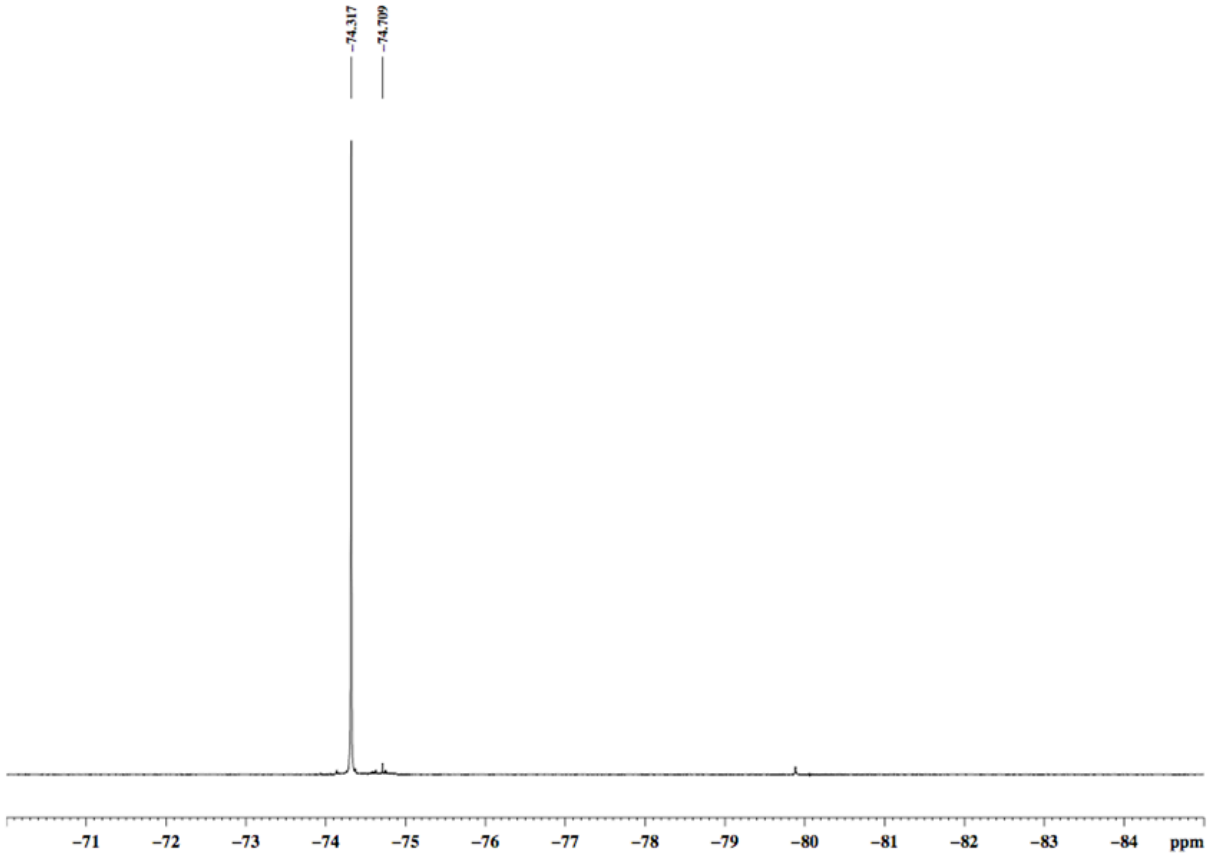
3y


ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

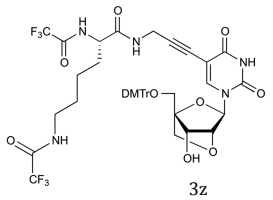
^{19}F



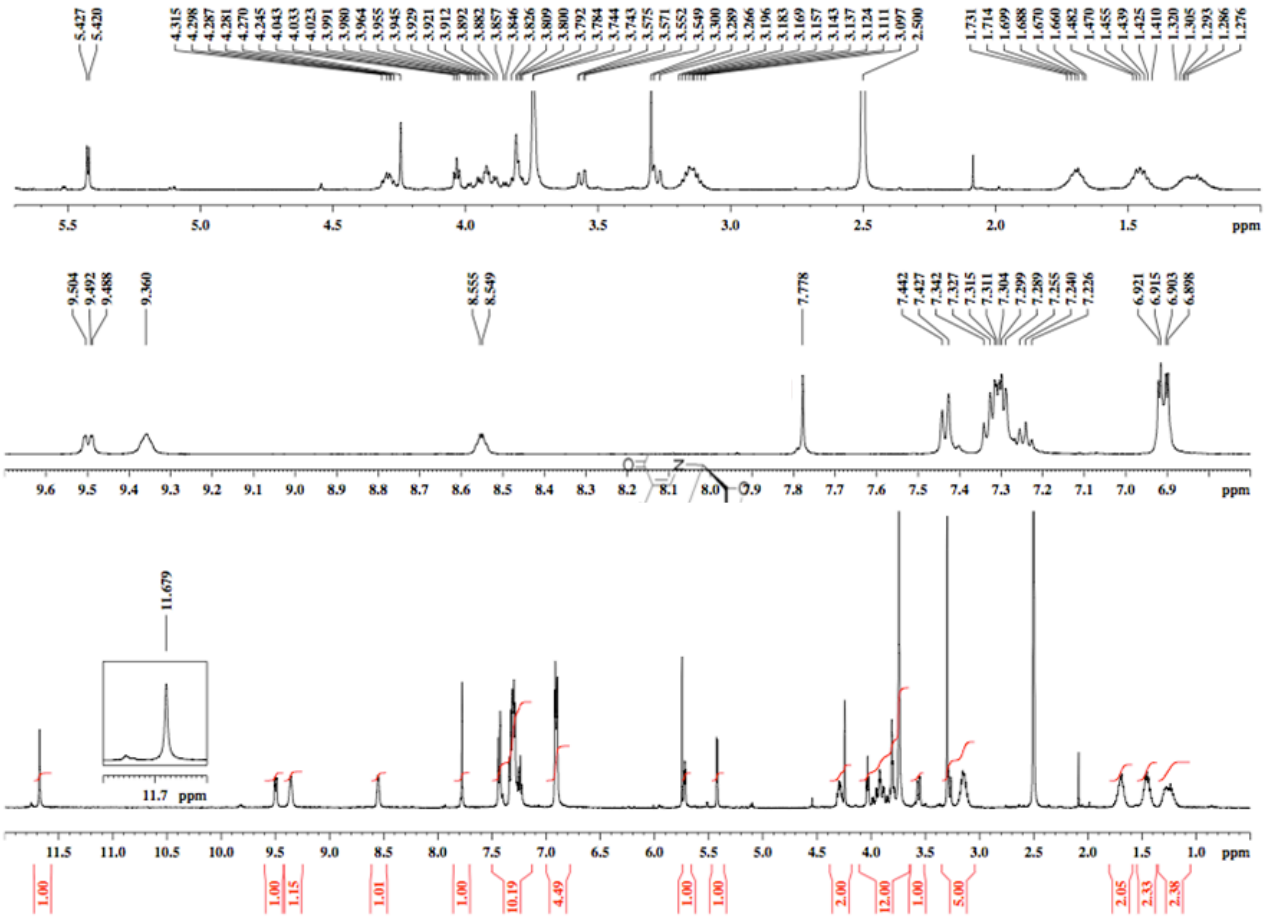
3y



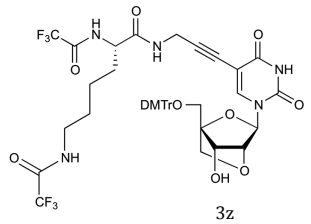
^{1}H


F3C
O
NH
O
NH
O
NH
O
DMTrO
O
N
O
OH
O
NH
O
CF3

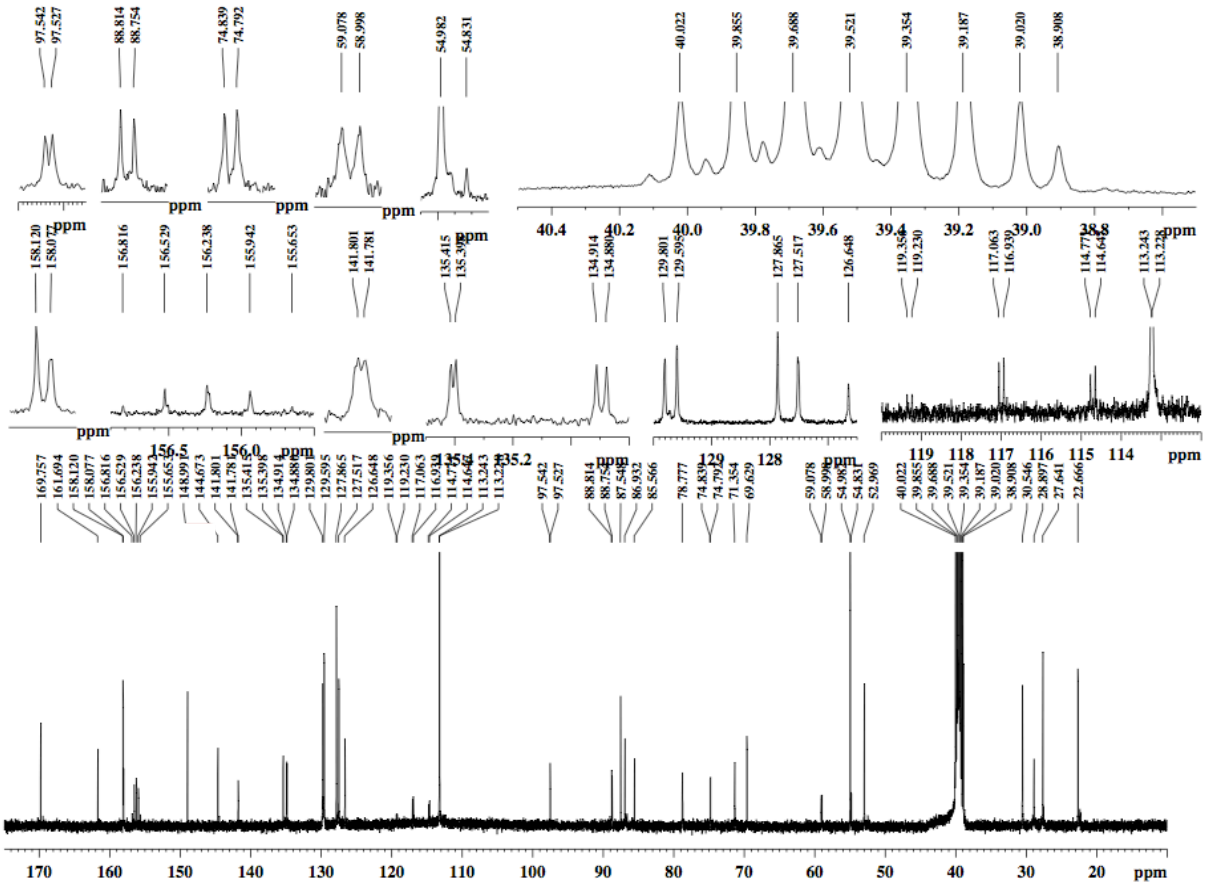
3z


5.427
5.420
4.315
4.298
4.287
4.281
4.270
4.245
4.043
4.033
4.023
3.991
3.980
3.964
3.955
3.945
3.929
3.921
3.912
3.892
3.882
3.857
3.846
3.826
3.809
3.800
3.792
3.784
3.744
3.743
3.575
3.571
3.552
3.549
3.300
3.289
3.266
3.196
3.183
3.169
3.157
3.143
3.137
3.124
3.111
3.097
2.500
1.731
1.714
1.699
1.688
1.670
1.660
1.482
1.470
1.455
1.439
1.425
1.410
1.320
1.305
1.293
1.286
1.276
ppm
9.504
9.492
9.488
9.360
8.555
8.549
7.778
7.442
7.427
7.342
7.327
7.315
7.311
7.304
7.299
7.289
7.255
7.240
7.226
6.921
6.915
6.903
6.898
ppm
11.679
11.7 ppm
ppm
1.00
1.00
1.15
1.01
1.00
10.19
4.49
1.00
1.00
2.00
12.00
1.00
5.00
2.05
2.33
2.38

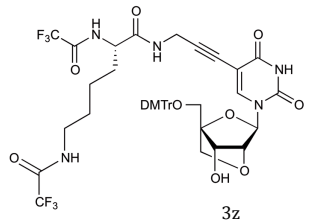
^{13}C



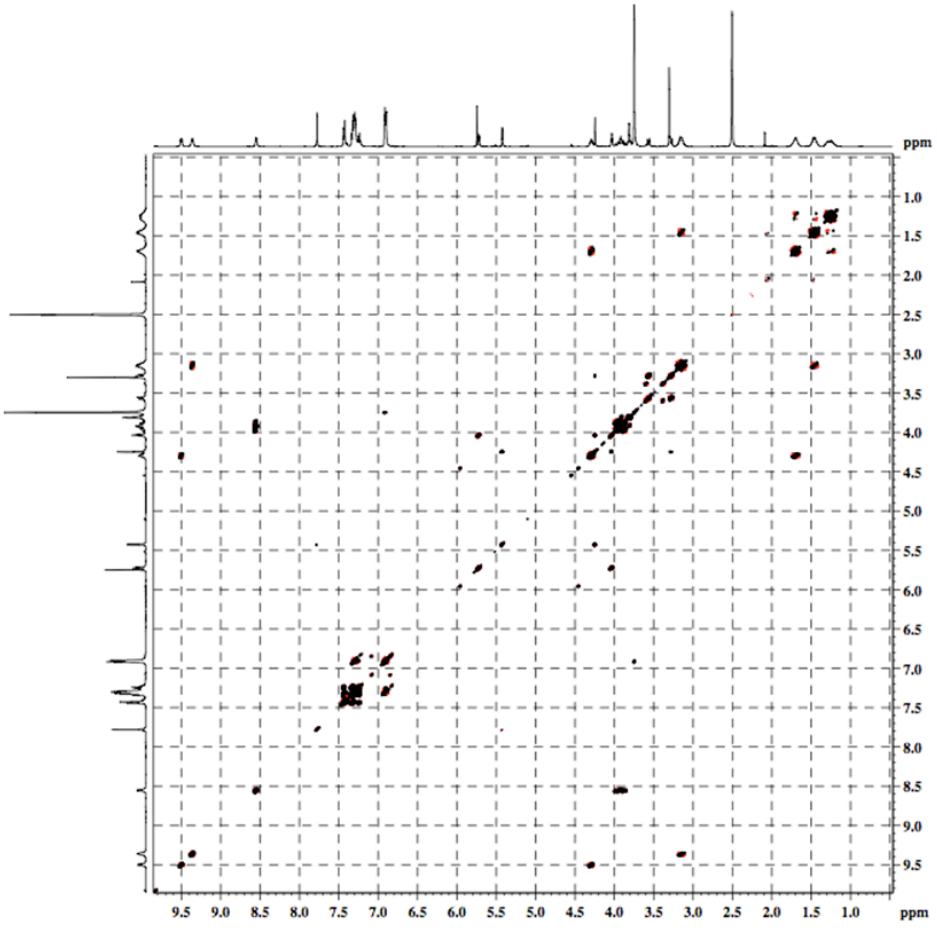
3z



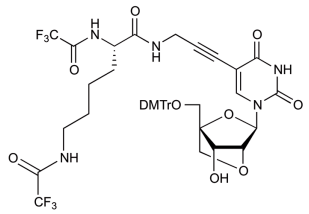
COSY


F3C
O
H
N
O
H
N
O
NH
O
DMTrO
O
N
O
O
OH
O
NH
O
CF3

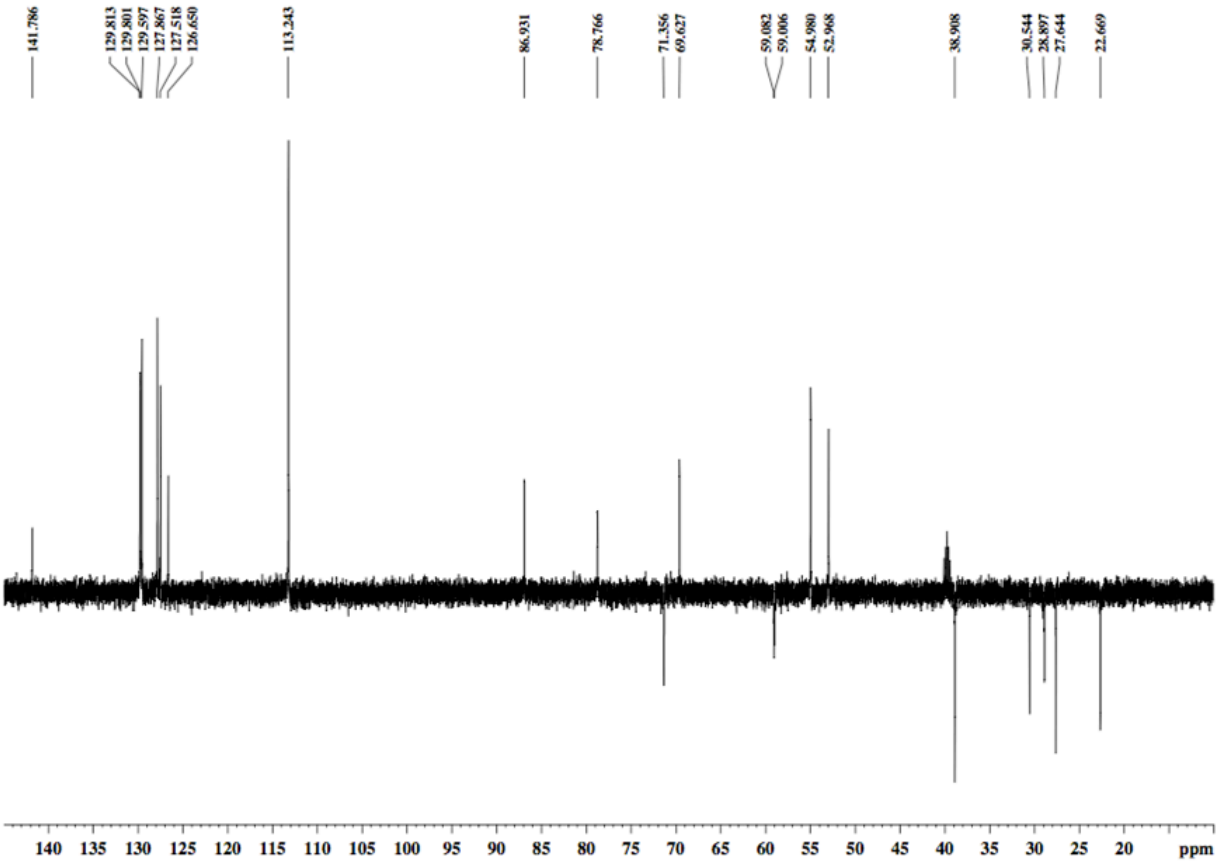
3z


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm

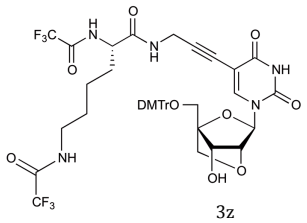
DEPT



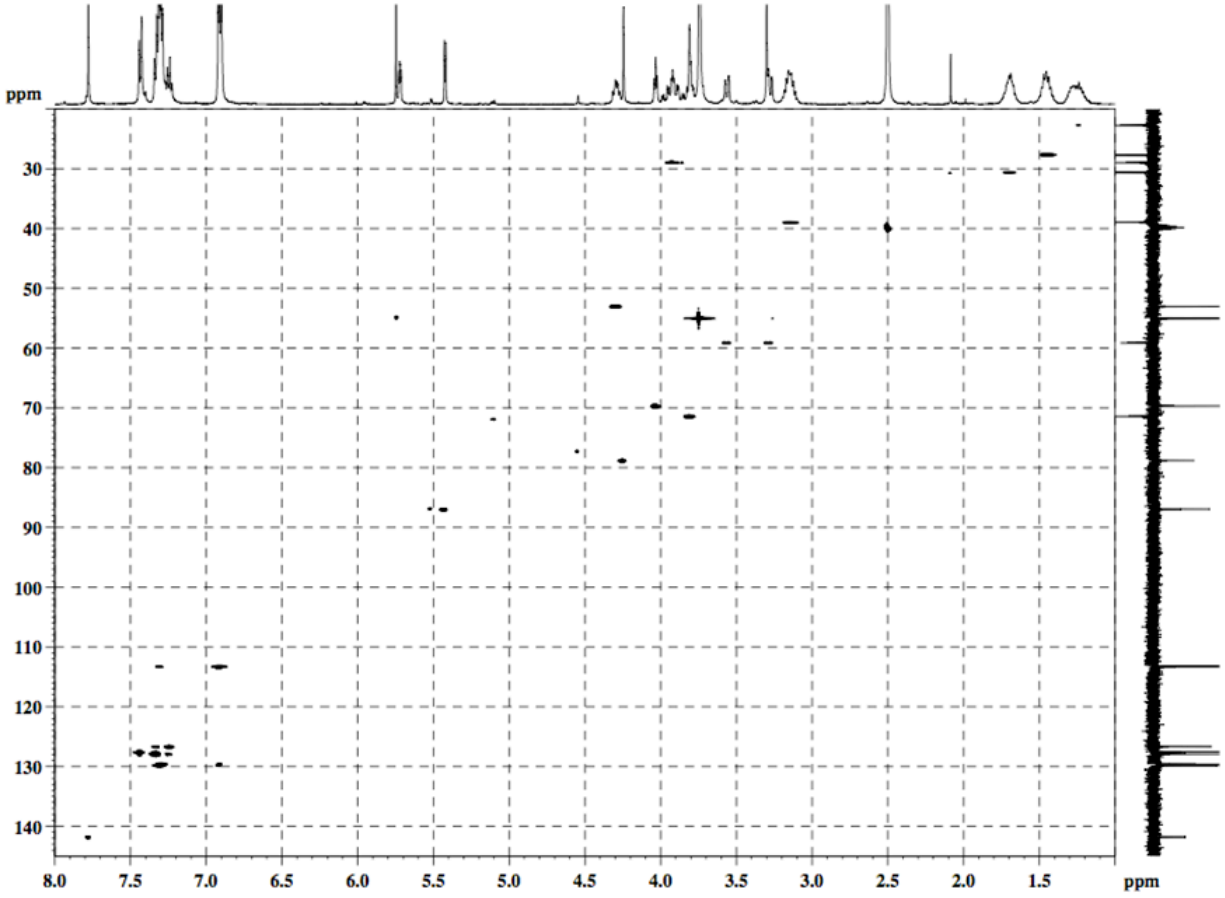
3z


141.786
129.813
129.801
129.597
127.867
127.518
126.650
113.343
86.931
78.766
71.356
69.627
59.082
59.006
54.980
52.968
38.908
30.544
28.897
27.644
22.669
140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 ppm

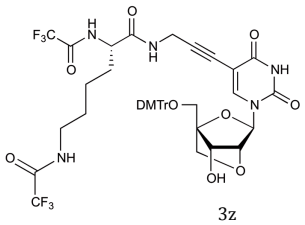
HSQC


F3C
O
H
N
O
H
N
O
NH
O
DMTrO
O
N
O
OH
O
NH
O
CF3

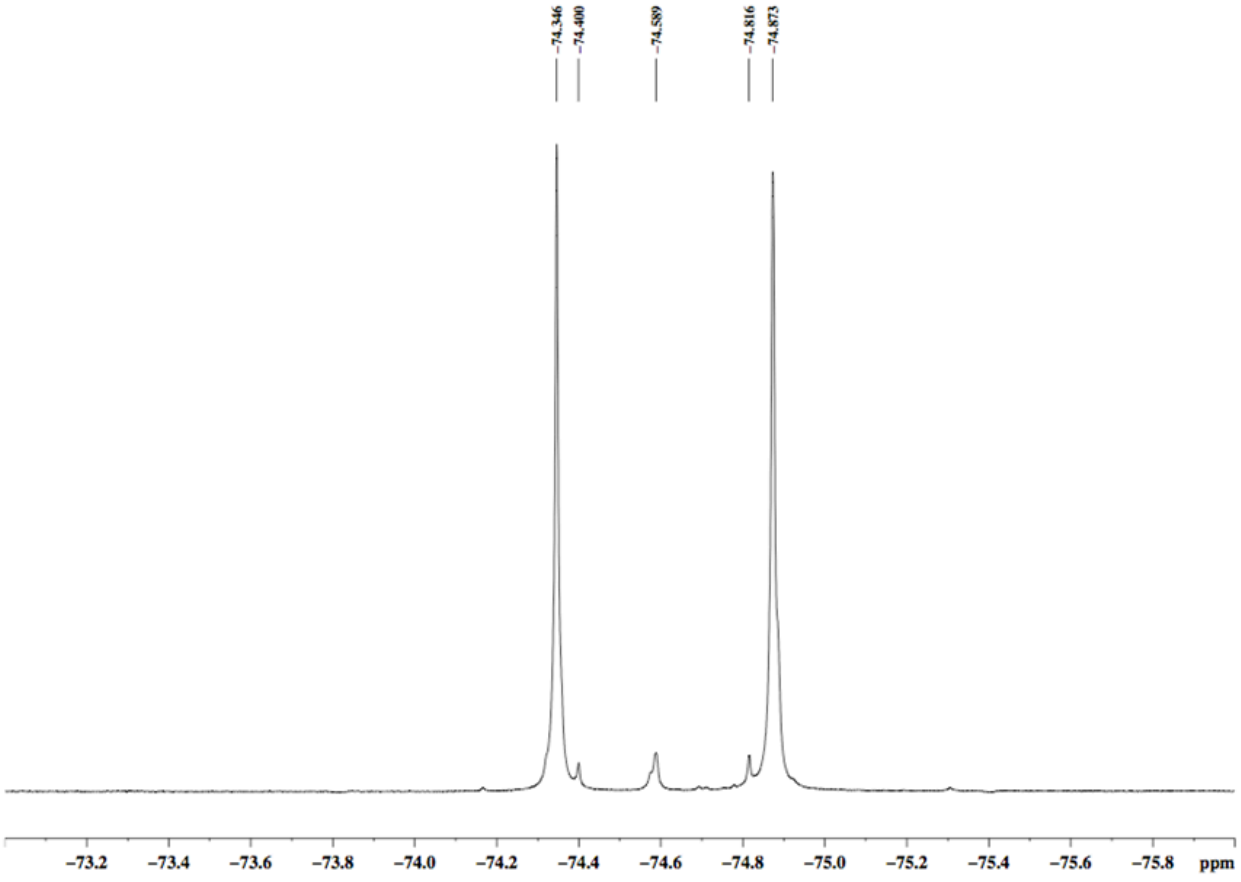
3z


ppm
30
40
50
60
70
80
90
100
110
120
130
140
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm

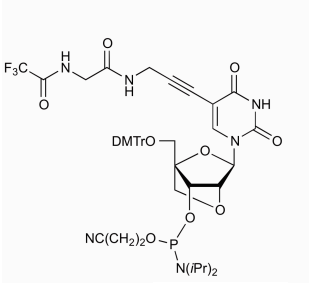
^{19}F



3z

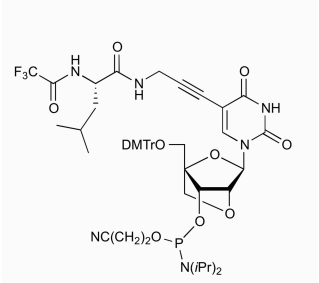

−74.346
−74.400
−74.589
−74.816
−74.873
−73.2
−73.4
−73.6
−73.8
−74.0
−74.2
−74.4
−74.6
−74.8
−75.0
−75.2
−75.4
−75.6
−75.8
ppm

^{31}P

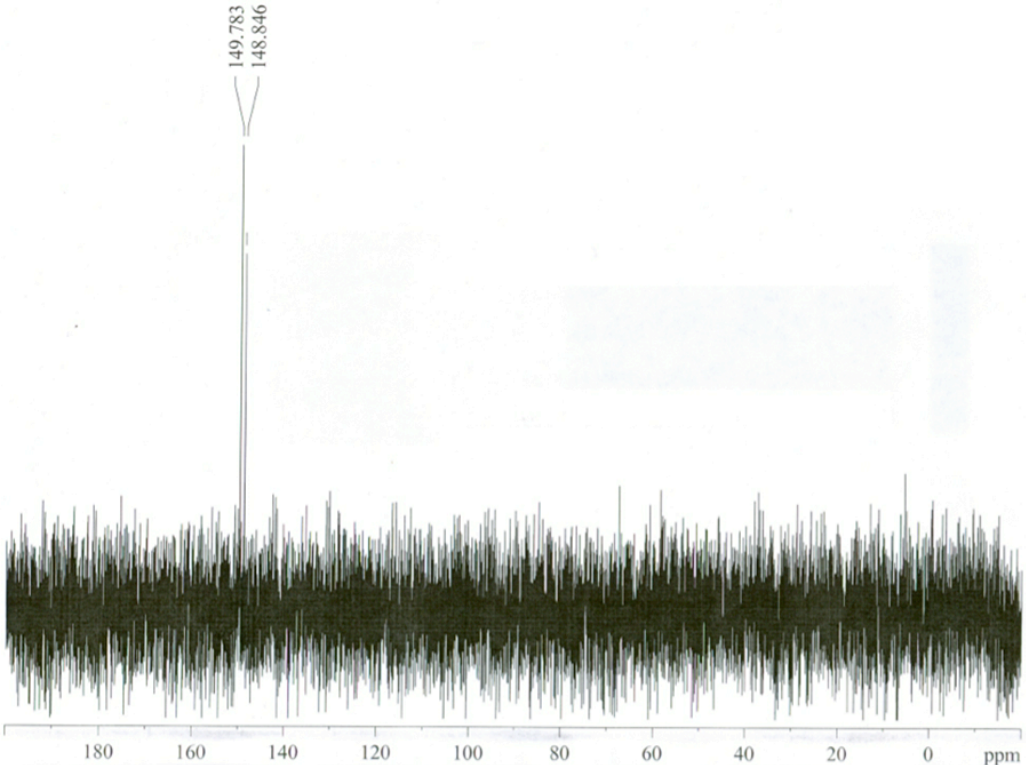


4x

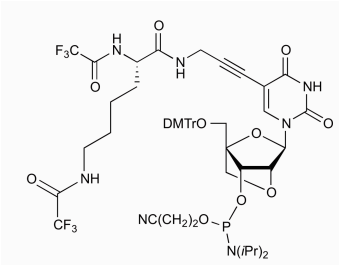
^{31}P


F3C
O
H
N
O
N
H
O
NH
N
O
DMTrO
O
O
O
NC(CH2)2O
P
N(iPr)2

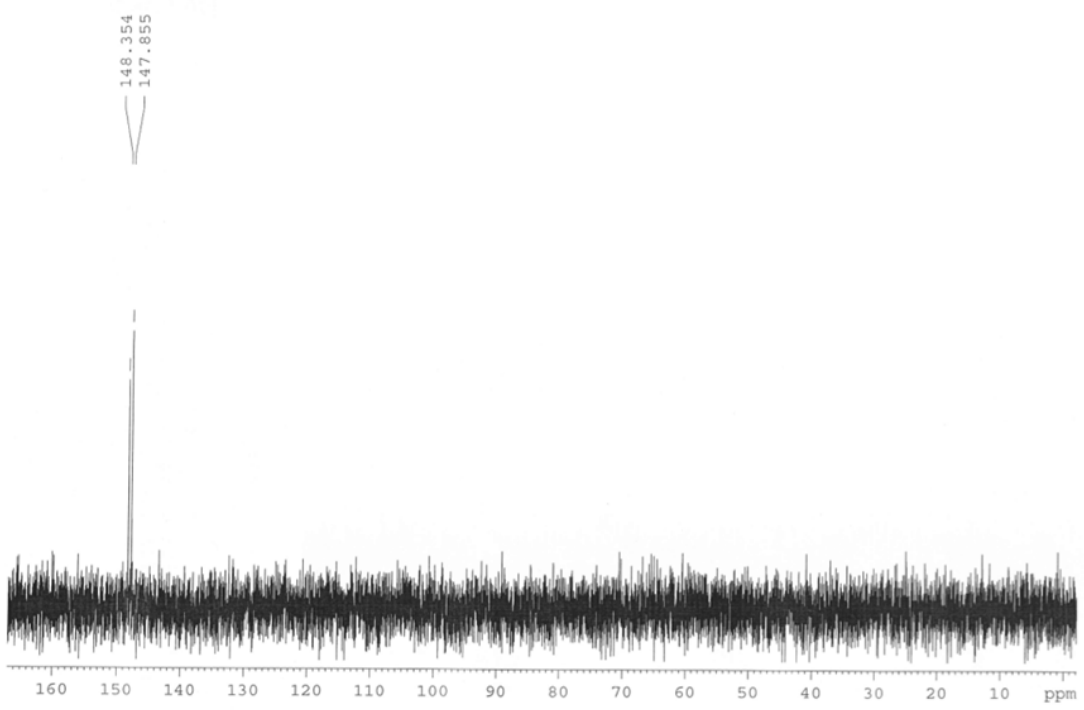
4y


149.783
148.846
180
160
140
120
100
80
60
40
20
0
ppm

^{31}P


F3C
O
H
N
O
N
H
O
NH
O
N
O
DMTrO
O
O
O
NH
O
CF3
NC(CH2)2O
P
N(iPr)2

4z


148.354
147.855
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm