



Supporting Information

Photoaffinity Capture Compounds to Profile the Magic Spot Nucleotide Interactomes

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Abbreviations

DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DTT	Dithiothreitol
EDC	1-Ethyl-3-carbodiimide
Et ₂ O	Diethyl ether
ETT	5-(Ethylthio)-1 <i>H</i> -tetrazole
Fm	Fluorenylmethyl
Fmoc	Fluorenylmethyloxycarbonyl
<i>m</i> CPBA	<i>meta</i> -Chloroperoxybenzoic acid
MeCN	Acetonitrile
HMDA	Hexamethylenediamine
HPLC	Reverse phase high-performance liquid chromatography
HRMS	High resolution mass spectrometry
MSN	Magic spot nucleotides
NHS	N-Hydroxysuccinimide
PEG	Polyethylene glycol
pGp	Guanosine-3',5'-bisphosphate
pGpp	Guanosine-3'-diphosphate-5'-phosphate
ppApp	Adenosine-3',5'-bisdiphosphate
ppGp	Guanosine-3'-phosphate-5'-diphosphate
ppGpp	Guanosine-3',5'-bisdiphosphate
pppGpp	Guanosine-3'-diphosphate-5'-triphosphate
SAX	Strong anion exchange
TBA	Tetrabutylammonium
TEAA	Triethylammonium acetate

1. General synthetic remarks

Reactions were carried out using glassware magnetically stirred, unless noted otherwise. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel canula.

Reagents were purchased from commercial suppliers (Acros, Aldrich, Fluka, TCI) and used without further purification, unless noted otherwise.

Solvents were obtained in analytical grade and used as received for extractions, precipitation and solid washing.

Dry solvents for reactions were purchased in a dry form from Sigma and stored over molecular sieves as well as under the atmosphere of dry N₂.

Deuterated solvents for NMR and reactions were obtained from Armar Chemicals, Switzerland and euriso-top, Germany, in the indicated purity grade and used as received for NMR spectroscopy.

Strong ion-exchange chromatography was performed using an automated Äkta® – system. Q-Sepharose was purchased from Aldrich. Buffer solutions were produced manually using milliQ H₂O.

Sulfo-SBED biotin label transfer reagent was purchased from Thermofisher®.

TBA-salt preparations were performed by either using DowexH⁺ followed by TBA(OH) addition or Chelex®100 (preloaded with TBA). In both cases, the TBA salts were obtained after lyophilization.

Lyophilizations were done with Christ Freeze Dryer Alpha 1-4 LDplus and Christ Freeze Dryer Alpha 1-2 LDplus.

Ribonuclease T2 from *Aspergillus oryzae* (50 ku) was purchased from Worthington Biochemical corporation as lyophilized powder and dissolved in a storage buffer [glycerol / NaH_2PO_4 (10 mM, pH 6.8), 1 / 1]. The stock solution was stored at $-20\text{ }^\circ\text{C}$.

^1H -NMR spectra were recorded on Bruker 300 MHz spectrometers, Bruker 400 MHz (with cryoprobe) and Bruker 500 MHz spectrometers in the indicated deuterated solvent. Data are reported as follows: chemical shift (δ , ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br. s, broad signal), coupling constant(s) (J , Hz), integration. All signals were referenced to the internal solvent signal as standard (D_2O , δ 4.79). After NaClO_4 – purification acetone residues were present in the products. These were also considered for yield determination.

$^{13}\text{C}\{^1\text{H}\}$ -NMR spectra were recorded with ^1H -decoupling on Bruker 126 MHz, Bruker 101 MHz (with cryoprobe) spectrometers at 298K in the indicated deuterated solvent. If possible, signals were referred to the internal solvent signal as standard.

$^{31}\text{P}\{^1\text{H}\}$ -NMR spectra and ^{31}P -NMR spectra were recorded with ^1H -decoupling or ^1H coupling, respectively, on Bruker 202 MHz, 162 MHz (with cryoprobe) and Bruker 122 MHz spectrometers in the indicated deuterated solvent. All signals were referenced to an internal standard (PPP).

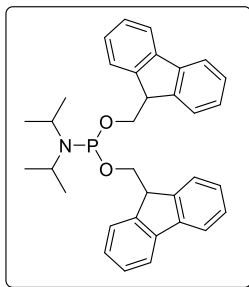
Mass spectra were recorded by C. Warth (Mass spectrometry service of the University of Freiburg) on a Thermo LCQ Advantage [spray voltage: 2.5 – 4.0 kV, spray current: 5 μA , ion transfer tube: 250 (150) $^\circ\text{C}$, evaporation temperature: 50 – 400 $^\circ\text{C}$].

Capture compounds were stored at $-20\text{ }^\circ\text{C}$ under light exclusion.

2. Synthesis of MSN capture compounds

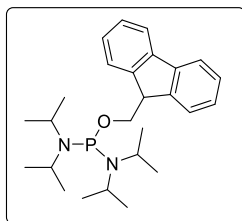
2.1. Syntheses adapted from literature

Synthesis of (FmO)₂P-N(*i*Pr)₂ (SI-1)



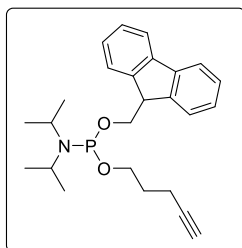
The compound was synthesized in two steps as reported previously starting from PCl₃. Analytical data were identical to literature.^[1] The compound was stored at −20°C.

Synthesis of (FmO)P-[N(*i*Pr)₂]₂ (SI-2)



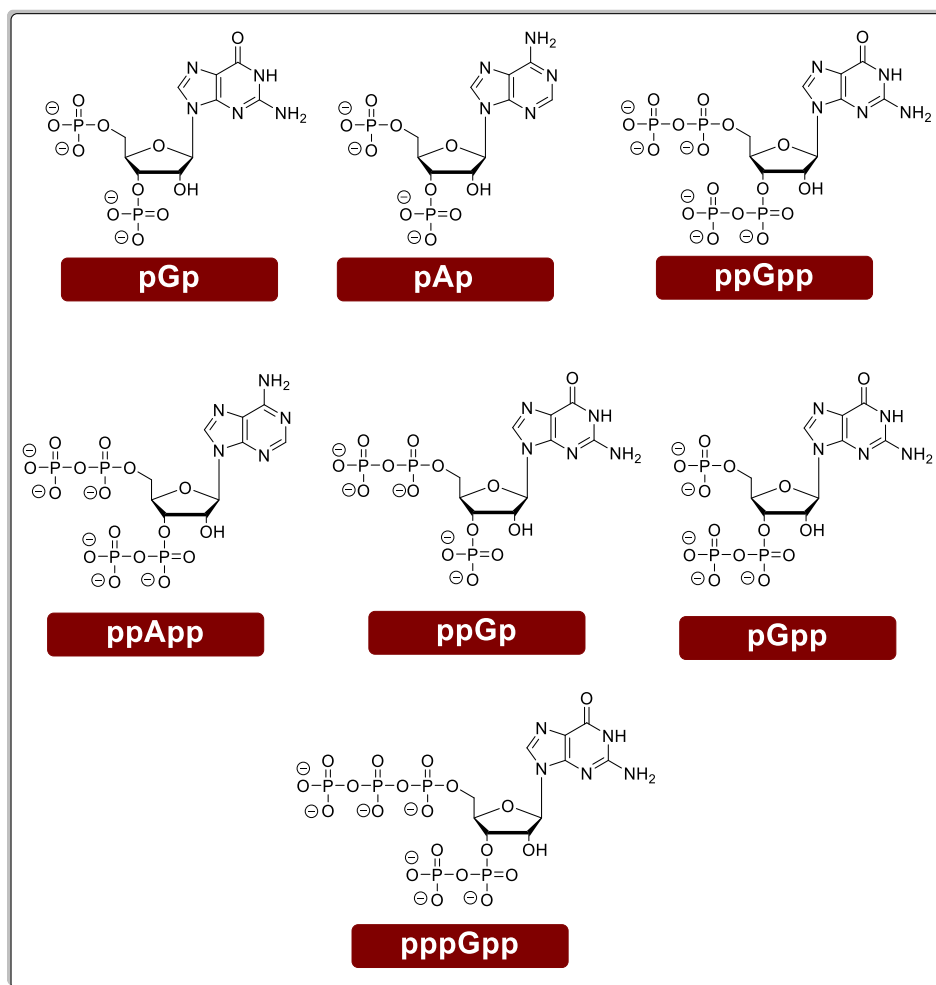
The compound was synthesized as reported previously. Analytical data were identical to literature.^[2] The compound was stored at −20°C.

Synthesis of (Pentynyl)(FmO)P-N(*i*Pr)₂ (SI-3)



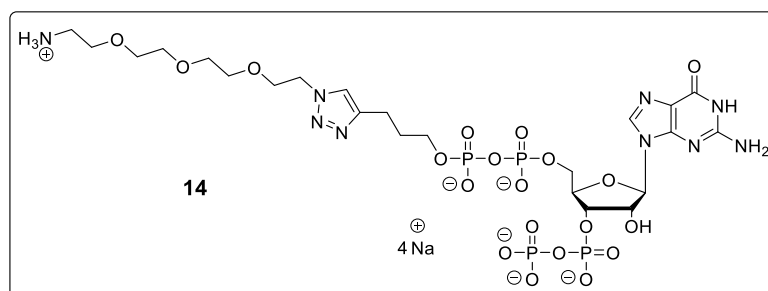
The compound was synthesized as reported previously. Analytical data were identical to literature.^[3] The compound was stored at −20°C.

Synthesis of natural MSN



pGp, ppGpp, pppGpp, ppGp, pGpp and ppApp were synthesized according to Jessen et al.^[3] The analytical data was in accordance with literature.

Synthesis of amino-ppGpp (14)



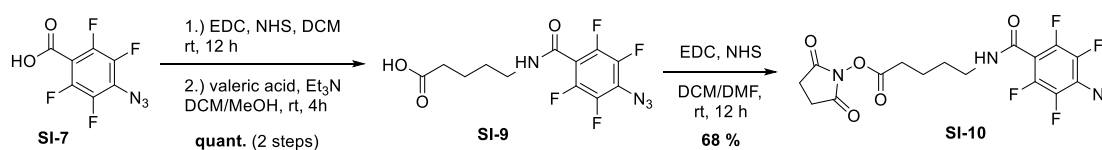
Amino-ppGpp **14** was synthesized according to Jessen et al.^[3] The analytical data was in accordance with literature.

2.2. Synthesis of Pull-down linker 2

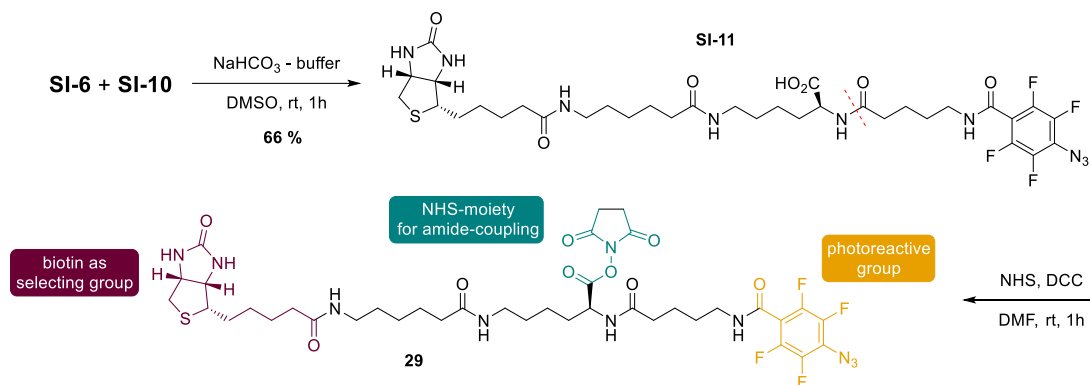
Part 1: Synthesis of the biotine-lysine moiety



Part 2: Synthesis of fluorophenylazide-NHS moiety:

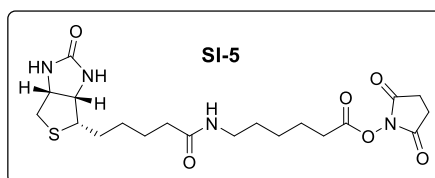


Part 3: Coupling of moieties and NHS-ester synthesis



Supporting figure 1: Synthesis of fluorophenylazide linker **29** from commercial precursors **SI-4** and **SI-7** based on sequential, chemoselective NHS-ester couplings.

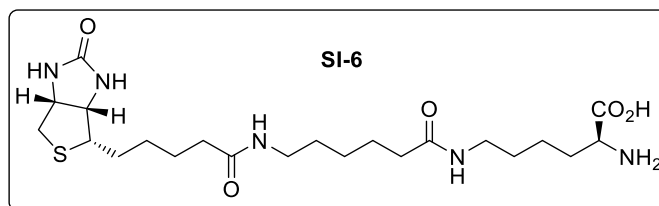
Synthesis of Biotin-NHS-ester SI-5



To a solution of N-(+)-Biotinyl-6-aminoheptanoic acid (**SI-4**, 400 mg, 1.12 mmol) in dry DMF (20 ml), EDC·HCl (535 mg, 2.80 mmol, 2.50 eq.) and NHS (322 mg, 2.80 mmol, 2.50 eq.) were added. The solution was stirred overnight at 37 °C. Then the solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (CH₂Cl₂/MeOH, 15:1). The product **SI-5** was obtained as white solid (462 mg, 1.02 mmol, 90%).

¹H-NMR (400 MHz, DMSO-*d*₆, δ/ppm): 7.73 (t, *J* = 5.6 Hz, 1H), 6.41 (s, 1H), 6.34 (s, 1H), 4.30 (ddd, *J* = 7.4, 5.1, 0.9 Hz, 1H), 4.12 (ddd, *J* = 7.8, 4.4, 1.9 Hz, 1H), 3.09 (ddd, *J* = 8.6, 6.1, 4.4 Hz, 1H), 3.01 (ddd, *J* = 6.5, 6.5, 6.5 Hz, 2H), 2.85 – 2.75 (m, 5H), 2.70 – 2.61 (m, 2H), 2.61 – 2.53 (m, 1H), 2.04 (t, *J* = 7.4 Hz, 2H), 1.69 – 1.55 (m, 3H), 1.55 – 1.23 (m, 9H). **¹³C{¹H}-NMR** (101 MHz, DMSO-*d*₆, δ/ppm): 171.81, 170.26, 168.94, 162.69, 61.04, 59.18, 55.44, 54.92, 38.06, 35.23, 30.15, 28.64, 28.23, 28.05, 25.45, 25.31, 23.96. **HRMS** (ESI) *m/z* for C₂₀H₃₁O₆N₄S [M-H]⁺: calcd. 455.1960, found 455.1959. **R_f** (SiO₂, CH₂Cl₂/MeOH, 15:1): 0.31.

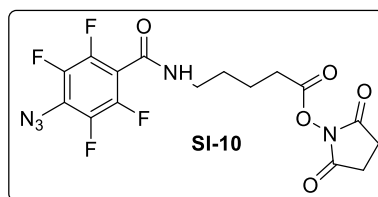
Synthesis of Biotin-lysine derivative SI-6



To a solution of compound **SI-5** (60.0 mg, 132 μmol) in acetone (6 ml) and NaHCO₃-solution (0.1 M, 3 ml) was added Fmoc-Lysine (0.146 mg, 356 μmol, 3.00 eq.). The solution was stirred overnight at room temperature. The reaction mixture was diluted with CH₂Cl₂ (10 ml) and extracted with H₂O (2 x 10ml). The combined aqueous layers were diluted with H₂O (10 ml) and the solution was acidified to pH 2.0 using HCl-solution (0.1 M). The precipitate was separated by centrifugation, washed with cold HCl-solution (0.1 M, 15 ml) and lyophilized. The resulting solid was dissolved in MeOH (2 ml) and piperidine (300 μl) was added. The solution was stirred at room temperature for 1 h. The crude product was precipitated by adding Et₂O (10 ml), separated by centrifugation, washed with Et₂O (10 ml) and dried under high vac. The solid was redissolved in DMSO (1 ml) reprecipitated with Et₂O, washed with Et₂O (2 x 10 ml) and dried under high vac. The resulting solid was dissolved in H₂O (2 ml) and lyophilized. The product **SI-6** was obtained as white solid (24.8 mg, 49.8 μmol, 39%).

¹H-NMR (400 MHz, D₂O, δ/ppm): 4.62 (ddd, *J* = 8.0, 5.0, 0.9 Hz, 1H), 4.43 (dd, *J* = 7.9, 4.4 Hz, 1H), 3.69 (dd, *J* = 6.6, 5.7 Hz, 1H), 3.35 (dt, *J* = 9.8, 5.1 Hz, 1H), 3.19 (dtd, *J* = 6.7, 4.5, 2.3 Hz, 4H), 3.05 – 2.97 (m, 1H), 2.79 (d, *J* = 13.1 Hz, 1H), 2.25 (td, *J* = 7.3, 4.2 Hz, 4H), 1.91 – 1.80 (m, 2H), 1.79 – 1.27 (m, 16H). **¹³C{¹H}-NMR** (101 MHz, D₂O, δ/ppm): 176.82, 176.63, 174.81, 165.35, 62.07, 60.24, 55.36, 54.68, 39.67, 39.05, 38.87, 35.66, 35.49, 30.12, 28.06, 27.99, 27.80, 27.64, 25.50, 25.17, 25.00, 21.80. **HRMS** (ESI) *m/z* for C₂₂H₃₈O₅N₅S [M-H]⁻: calcd. 484.2599, found 484.2598.

Synthesis of Fluorophenylazide SI-10



To a solution of 4-azidotetrafluorobenzoic acid (**SI-7**, 400 mg, 1.70 mmol) in dry CH_2Cl_2 (9.6 ml), was added EDC·HCl (390 mg, 2.04 mmol, 1.20 eq.) in one portion and the mixture was purged with argon for 5 min. Afterwards NHS (235 mg, 2.04 mmol, 1.20 eq.) was added and the solution was stirred overnight at room temperature in a foil covered flask. Afterwards the solution was washed with H_2O (3 x 10 ml) and the aqueous layer was extracted with CH_2Cl_2 (20 ml). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure.

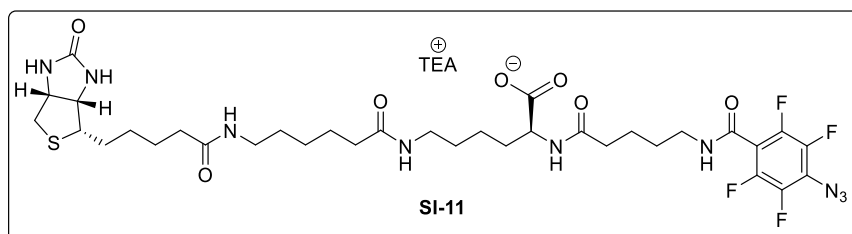
The resulting solid (**SI-8**) was dissolved in a mixture of dry CH_2Cl_2 and MeOH (1/1, 20 ml). Subsequently, 5-aminovaleric acid (212 mg, 1.82 mmol, 1.10 eq.) and NEt_3 (228 μl , 167 mg, 1.65 mmol, 1.00 eq.) were added. The solution was stirred at room temperature in a foil covered flask. After 90 min further 5-Aminoverleric acid (39.0 mg, 0.330 mmol, 0.20 eq.) was added. The solution was stirred at room temperature for additional 3 h. The solution was diluted with H_2O (15 ml) and the aqueous layer was extracted with CH_2Cl_2 (2 x 10 ml). The combined organics were washed with HCl (1 M, 20 ml) and sat.-aq. NaCl-solution (15 ml), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (AcOEt/HOAc, 99:1).

The resulting intermediate product (**SI-9**) was dissolved in in dry CH_2Cl_2 (20 ml) was added EDC·HCl (441 mg, 2.31 mmol, 1.50 eq.) in one portion and the mixture was purged with argon for 5 min. Afterwards NHS (265 mg, 2.31 mmol, 1.50 eq.) and DMF (1.4 ml) were added. The solution was stirred overnight at room temperature in a foil covered flask. The solution was extracted with water (8 x 20 ml). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product (**SI-10**) was isolated as light brown, sticky oil (499 mg, 1.16 mmol, 68%, 3 steps).

^1H -NMR (400 MHz, D_2O , δ/ppm): 6.23 (s, 1H), 3.51 (td, $J = 6.5, 6.5$ Hz, 2H), 2.84 (s, 4H), 2.69 (t, $J = 6.8$ Hz, 2H), 1.93 – 1.82 (m, 2H), 1.81 – 1.71 (m, 2H). **$^{13}\text{C}\{^1\text{H}\}$ -NMR** (101 MHz, D_2O , δ/ppm): 169.32, 168.56, 39.64, 30.80, 28.22, 25.73, 21.92.* **^{19}F -NMR** (377 MHz, CDCl_3 , δ/ppm): -140.73 - -140.92 (m), -150.49 - -150.67 (m). **HRMS** (ESI) m/z for $\text{C}_{16}\text{H}_{14}\text{O}_5\text{N}_3\text{F}_4$

$[M-H]^+$: calcd. 432.0926, found 432.0926. R_f (SiO₂, AcOEt/HOAc, 99:1): 0.88. *5 carbon signals are not detectable due to ¹³C-¹⁹F-couplings.

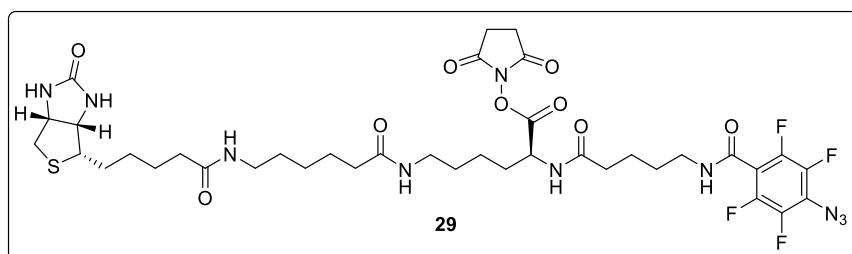
Synthesis of linker structure SI-11



Compound **SI-6** (18.2 mg, 0.038 μ mol) was dissolved in NaHCO₃-solution (0.1 M, 2.75 ml) and compound **SI-10** (18.0 mg, 0.038 μ mol, 1.00 eq.) was dissolved in DMSO (306 μ l). The two solutions were combined and stirred for 1 h at room temperature. The solution was directly applied to an automated MPLC (Interchim) system (AQ-column). Elution was performed with a MeCN/H₂O (10 mM TEAA) – gradient. The product containing fractions were combined and lyophilized. The product (**SI-11**, 20.0 mg, 0.022 μ mol, 66%) was isolated as white solid.

¹H-NMR (400 MHz, DMSO-*d*₆, δ /ppm): 8.89 (t, J = 5.6 Hz, 1H), 7.86 (s, 1H), 7.80 (s, 1H), 7.73 (t, J = 5.6 Hz, 1H), 6.44 (s, 1H), 6.35 (s, 1H), 4.30 (dd, J = 7.8, 5.2 Hz, 1H), 4.15 – 4.08 (m, 1H), 4.08 – 4.02 (m, 1H), 3.24 (q, J = 6.4 Hz, 3H), 3.12 – 3.06 (m, 1H), 3.03 – 2.94 (m, 4H), 2.82 (dd, J = 12.4, 5.1 Hz, 1H), 2.59 – 2.55 (m, 1H), 2.52 (d, J = 1.9 Hz, 3H), 2.17 – 2.11 (m, 2H), 2.02 (dt, J = 9.2, 7.5 Hz, 4H), 1.72 – 1.15 (m, 20H). **¹⁹F-NMR** (377 MHz, DMSO-*d*₆, δ /ppm): –143.05 – –143.20 (m), –151.49 – –151.64 (m). **HRMS** (ESI) m/z for C₃₄H₄₈O₇N₉F₄S $[M-H]^+$: calcd. 802.3328, found 802.3326.

Activation of acid SI-11 as NHS-ester

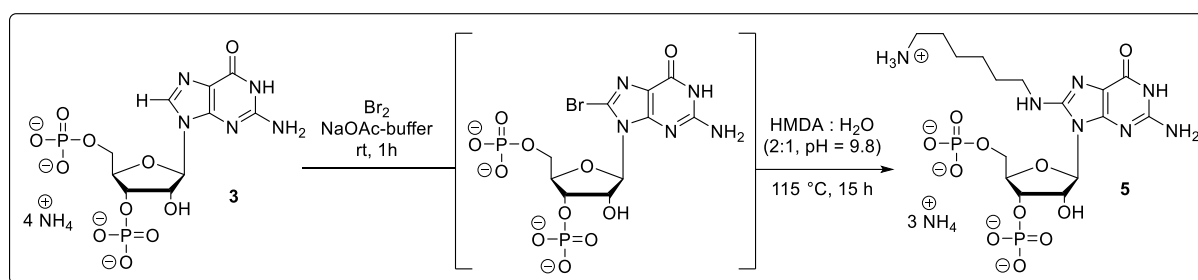


Carboxylic acid **SI-11** (20.0 mg, 24.1 μ mol) and NHS (5.54 mg, 48.2 μ mol, 2.0 eq.) were dissolved in DMF (1.0 ml) and DCC (14.9 mg, 72.3 μ mol, 3.0 eq.) was added. The reaction mixture was stirred for 2 h and the product was isolated by precipitation with Et₂O (10 ml) and drying under high vacuum. The isolated material was a mixture of starting material and

product (29) as rapidly analyzed by HPLC. (see attachment) It was subjected to follow-up reactions immediately and without further purification.

2.3. Syntheses of base-modified amino-ppGpp

Synthesis of amino-pGp (5)



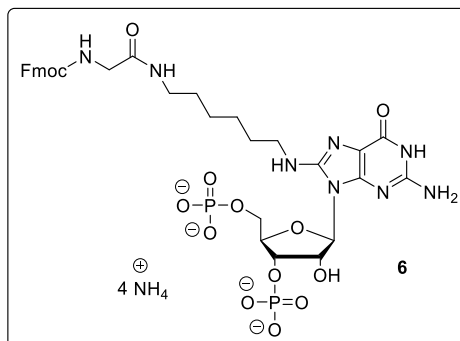
pGp x 4 NH₄ (**3**, 600 mg, 1.17 mmol) was dissolved in NaOAc – buffer (500 mM, pH 4.0, 22.0 ml). A solution of Br₂ (211 µl, 658 mg, 4.11 mmol, 3.5 eq.) in H₂O (13.6 ml) was added. The resulting mixture was stirred for 1 h at rt. Afterwards NaS₂O₃ – buffer was added and the solution was stirred for 20 min at rt. The product was precipitated by adding EtOH (300 mL). After incubating the suspension at 0°C for 30 min, the precipitate was separated by centrifugation and washed with EtOH (2 x 80 ml) and dried under high vacuum.

The resulting solid was dissolved in mixture of hexamethylenediamine and water (2:1, pH 9.8, 7.5 ml). The resulting solution was stirred in a pressure vessel at 115°C for 15 hours. The solution was cooled down to rt and the product was precipitated with EtOH (200 ml). After incubating the suspension at 0°C for 30 min, the precipitate was separated by centrifugation and washed with EtOH (2 x 40 ml). The crude product was dried under high vacuum and purified by automated SAX (Äkta pure system, Q-Sepharose®). Elution was performed with NH₄HCO₃ – buffer (300 mM). Product containing fractions were combined and lyophilized. The product (**5**, 534 mg, 876 µmol, 75 %) was isolated as white solid.

¹H-NMR (400 MHz, D₂O, δ/ppm): 5.91 (d, *J* = 7.6 Hz, 1H), 4.88 – 4.83 (m, 2H), 4.54 – 4.44 (m, 1H), 4.22 (dd, *J* = 11.8, 3.0 Hz, 1H), 4.11 (dd, *J* = 11.8, 2.6 Hz, 1H), 3.51 – 3.34 (m, 2H), 2.99 (dd, *J* = 7.4, 7.4 Hz, 2H), 1.76 – 1.61 (m, 5H), 1.49 – 1.35 (m, 5H). **¹³C{¹H}-NMR** (101 MHz, D₂O, δ/ppm): 156.52, 152.64, 151.46, 150.36, 111.09, 86.17, 83.58 (dd, *J* = 8.4, 3.7 Hz), 72.98 (d, *J* = 4.8 Hz), 70.03 (d, *J* = 4.4 Hz), 64.36 (d, *J* = 4.6 Hz), 42.15, 39.33, 27.79,

26.43, 25.21, 25.04. $^{31}\text{P}\{^1\text{H}\}$ -NMR (162 MHz, D_2O , δ/ppm): 0.35, -0.10. HRMS (ESI) m/z for $\text{C}_{16}\text{H}_{30}\text{N}_7\text{O}_{11}\text{P}_2$ $[\text{M}+\text{H}]^+$: calcd. 558.1473, found 558.1473.

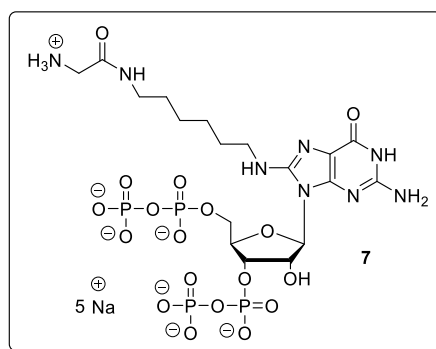
Synthesis of Fmoc-Gly-HDMA-pGp (**6**)



Fmoc-Gly-NHS (482 mg, 1.22 mmol, 2.0 eq.) was dissolved in DMSO (14 ml) before a solution of HMDA-pGp x 1.8 TBA (**5**, 605 mg, 612 μmol) in H_2O (4.0 ml) was added. The resulting solution was stirred for 4 h at rt before further Fmoc-Gly-NHS (241 mg, 612 μmol , 1.0 eq.) was added. The resulting solution was stirred for 12 hours at rt. Afterwards H_2O (65 ml) was added and the precipitate was separated by centrifugation. The supernatant was applied to automated SAX (Äkta, Q-Sepharose, NH_4HCO_3 – buffer). The product containing fractions were combined and lyophilized. The product (**6**, 357 mg, 395 μmol , 65 %) isolated as white solid.

^1H -NMR (400 MHz, D_2O , δ/ppm): 7.91 (d, $J = 7.5$ Hz, 1H), 7.77 (d, $J = 7.7$ Hz, 1H), 7.74 – 7.70 (m, 1H), 7.56 (dd, $J = 8.2, 8.2$ Hz, 1H), 7.50 (dd, $J = 7.2, 7.2$ Hz, 1H), 7.45 – 7.37 (m, 2H), 7.35 – 7.28 (m, 1H), 5.69 (d, $J = 6.9$ Hz, 1H), 4.55 (d, $J = 5.8$ Hz, 1H), 4.47 – 4.39 (m, 3H), 4.34 (t, $J = 6.0$ Hz, 1H), 4.27 – 4.14 (m, 2H), 4.11 (s, 1H), 3.72 (s, 1H), 3.61 (s, 1H), 3.36 – 3.04 (m, 4H), 1.62 – 1.43 (m, 4H), 1.39 – 1.24 (m, 4H). $^{31}\text{P}\{^1\text{H}\}$ -NMR (162 MHz, D_2O , δ/ppm): 3.52 (s), 2.06 (d). HRMS (ESI) m/z for $\text{C}_{33}\text{H}_{43}\text{N}_8\text{O}_{14}\text{P}_2$ $[\text{M}+\text{H}]^+$: calcd. 837.2368, found 837.2367.

Synthesis of Gly-HDMA-ppGpp (**7**)

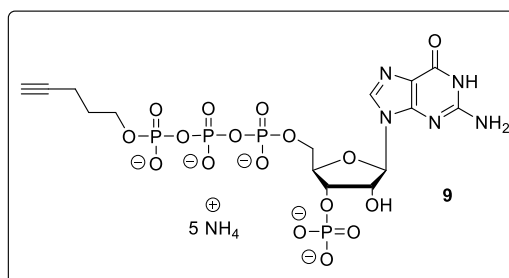


Fmoc-Gly-HDMA-pGp x 1.8 TBA (**6**, 100 mg, 78.9 μmol) was dissolved in DMF (2.0 mL) and ETT (51.3 mg, 394 μmol , 5.0 eq.) was added. Afterwards a solution of $(\text{FmO})_2\text{P-NiPr}_2$ (137 mg, 237 μmol , 3.0 eq.) in DMF (2.0 mL) was added. The resulting solution was stirred for 15 min at room temperature. The solution was cooled down to -20°C and *m*CPBA (77 %, 52.9 mg, 237 μmol , 3.0 eq.) was added. The solution was stirred for 10 min at -20°C and warmed to 0°C before DBU (400 μl) was added. The resulting solution was stirred for 30 min at rt. before the product was precipitated by the addition of Et_2O (40 mL). The precipitate was separated by centrifugation, washed with Et_2O (2 x 10 mL) and dried under high vacuum. The crude product was purified by automated SAX (Äkta, Q-Sepharose, NaClO_4 – buffer). The product containing fractions were combined and precipitated using an 8-fold volume of NaClO_4 - solution (-20°C , 500 mM in acetone). The resulting solid was separated by centrifugation, washed with acetone (-20°C , 2 x 3.0 mL) and dried under high vacuum for 2 h. The product (**7**, 23.8 mg, 26.9 μmol , 34 %) was isolated as white solid.

^1H -NMR (400 MHz, D_2O , δ/ppm): 5.89 (d, $J = 6.8$ Hz, 1H), 5.08 – 5.03 (m, 1H), 4.99 – 4.93 (m, 1H), 4.48 (ddd, $J = 4.0, 3.8, 3.8$ Hz, 1H), 4.28 – 4.22 (m, 2H), 3.65 (s, 2H), 3.47 – 3.34 (m, 2H), 3.24 (ddd, $J = 6.5, 6.4, 2.9$ Hz, 2H), 1.72 – 1.63 (m, 2H), 1.57 – 1.51 (m, 2H), 1.46 – 1.34 (m, 4H). **$^{13}\text{C}\{^1\text{H}\}$ -NMR** (101 MHz, D_2O , δ/ppm): 156.96, 152.41, 151.87, 151.25, 151.16, 112.41, 86.38, 83.14 (m), 74.12 (m), 69.97 (m), 65.14 (m), 42.18, 41.23, 39.19, 27.99, 27.85, 25.53, 25.44. **$^{31}\text{P}\{^1\text{H}\}$ -NMR** (162 MHz, D_2O , δ/ppm): -5.76 (d, $J = 22.6$ Hz, 1P), -6.26 (d, $J = 21.3$ Hz, 1P), -10.79 (d, $J = 21.4$ Hz, 2P). **HRMS** (ESI) m/z for $\text{C}_{18}\text{H}_{34}\text{N}_8\text{O}_{18}\text{NaP}_2$ $[\text{M}+\text{Na}]^+$: calcd. 797.0834, found 797.0835.

2.4. Synthesis of pentynyl-substituted MSN

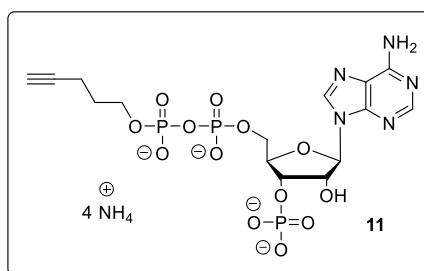
Synthesis of pentynyl-pppGp (**9**)



ppGp x 2.5 TBA (**8**, 200 mg, 178 μ mol) was dissolved in DMF (2.5 ml) and ETT (82.9 mg, 623 μ mol, 3.5 eq.) was added. Afterwards a solution of P-Amidite **SI-3** (90%, 202 mg, 445 μ mol, 2.5 eq.) in DMF (2.5 ml) was added and the resulting solution was stirred for 15 min at rt. The solution was cooled to -20°C and *m*CPBA (77%, 99.5 mg, 445 μ mol, 2.5 eq.) was added. After stirring for 10 min at -20°C , precipitation was induced by adding Et₂O (40 ml). The resulting solid was washed with ether (2 x 15 ml) and dried under high vacuum before being dissolved in MeOH (8.0 ml). The resulting solution was stirred for 5 h at 37°C . The solvent was removed under reduced pressure and the residue was dissolved in DMF (5 ml) and piperidine (500 μ l) was added at rt. After stirring the solution at rt for 30 min, precipitation was induced by the addition of Et₂O (40 ml). The resulting precipitate was washed with Et₂O (2 x 20 ml) and dried under high vacuum. The solid was redissolved in H₂O (20 ml) and acidified with HCl to pH 5.3. Afterwards RNase T2 (50 μ l) was added and the solution was incubated at 37°C overnight. The solution was directly applied to automated SAX (Äkta pure system, Q-Sepharose[®], NH₄HCO₃ - buffer). The product containing fractions were combined and lyophilized. The product (**9**, 64.0 mg, 82.2 μ mol, 46 %) was isolated as white solid.

¹H-NMR (400 MHz, D₂O, δ /ppm): 8.15 (s, 1H), 5.97 (d, J = 7.1 Hz, 1H), 4.98 – 4.91 (m, 1H), 4.88 – 4.81 (m, 1H), 4.59 – 4.52 (m, 1H), 4.32 – 4.21 (m, 2H), 3.98 (ddd, J = 6.5, 6.5, 6.5 Hz, 2H), 2.26 (dd, J = 2.7 Hz, 1H), 2.21 (ddd, J = 7.3, 7.3, 2.7 Hz, 2H), 1.73 (dddd, J = 6.9, 6.8, 6.8 Hz, 2H). **¹³C{¹H}-NMR** (101 MHz, D₂O, δ /ppm): 159.05, 153.93, 152.05, 137.89, 116.37, 86.34, 84.93, 83.41 (dd, J = 9.3, 3.6 Hz), 74.25 (d, J = 5.0 Hz), 72.74 (d, J = 4.9 Hz), 69.11, 65.45 (d, J = 5.4 Hz), 65.24 (d, J = 6.0 Hz), 28.79 (d, J = 7.3 Hz), 14.12. **³¹P{¹H}-NMR** (162 MHz, D₂O, δ /ppm): 0.44, -11.05 (d, J = 19.3 Hz), -11.56 (d, J = 18.5 Hz), -23.25 (t, J = 19.1 Hz). **HRMS** (ESI) m/z for C₁₅H₂₁O₁₇N₅P₄ [M-H₂]²⁻ calcd 333.4947 found 333.4947.

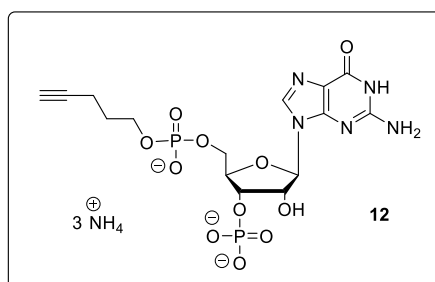
Synthesis of pentynyl-ppAp (**11**)



pAp x 1.87 TBA (**4**, 80.0 mg, 91.2 μmol) was dissolved in DMF (1.0 ml) and ETT (47.4 mg, 365 μmol , 4.0 eq.) was added. Afterwards a solution of P-Amidite **SI-3** (90%, 104 mg, 228 μmol , 2.5 eq.) in DMF (2.0 ml) was added and the resulting solution was stirred for 15 min at rt. The solution was cooled to -20°C and *m*CPBA (77%, 61.1 mg, 273 μmol , 3.0 eq.) was added. After stirring for 10 min at -20°C , precipitation was induced by Et_2O (40 ml). The resulting solid was washed with Et_2O (2 x 15 ml) and dried under high vacuum before being dissolved in MeOH (3.0 ml). The resulting solution was stirred for 3 h at 37°C . The solvent was removed under reduced pressure and the residue was dissolved in DMF (2.5 ml) before piperidine (250 μl) was added at rt. After stirring the solution at rt for 30 min, precipitation was induced by the addition of Et_2O (20 ml). The resulting precipitate was washed with ether (2 x 10 ml) and dried under high vacuum. The solid was redissolved in H_2O (10 ml) and acidified with HCl to pH 5.3. Afterwards RNase T2 (50 μl) was added and the solution was incubated at 37°C overnight. The solution was directly applied to automated SAX (Äkta pure system, Q-Sepharose[®], NH_4HCO_3 - buffer). The product containing fractions were combined and lyophilized. The product (**11**, 43.0 mg, 67.1 μmol , 74 %) was isolated as white solid.

^1H -NMR (400 MHz, D_2O , δ/ppm): 8.61 (s, 1H), 8.33 (s, 1H), 6.21 (d, $J = 6.6$ Hz, 1H), 4.94 – 4.88 (m, 1H), 4.88 – 4.83 (m, 1H), 4.61 (dddd, $J = 2.7, 2.7, 2.7$ Hz, 1H), 4.28 – 4.22 (m, 2H), 3.95 (ddd, $J = 6.5, 6.5, 6.5$ Hz, 2H), 2.25 – 2.16 (m, 3H), 1.73 (dddd, $J = 6.8, 6.7, 6.7$ Hz, 2H). **$^{13}\text{C}\{^1\text{H}\}$ -NMR** (101 MHz, D_2O , δ/ppm): 153.95, 150.46, 149.12, 140.63, 118.63, 86.52, 84.82, 83.51 (dd, $J = 9.2, 3.6$ Hz), 74.23 (d, $J = 5.1$ Hz), 73.67 (d, $J = 5.1$ Hz), 69.15, 65.27 – 65.07 (m, 2C), 28.75 (d, $J = 7.3$ Hz), 14.07. **$^{31}\text{P}\{^1\text{H}\}$ -NMR** (162 MHz, D_2O , δ/ppm): -0.14, -10.92 (d, $J = 21.1$ Hz), -11.55 (d, $J = 21.1$ Hz). **HRMS** (ESI) m/z for $\text{C}_{15}\text{H}_{21}\text{O}_{13}\text{N}_5\text{P}_3$ $[\text{M}-\text{H}]^-$ calcd 572.0354 found 572.0350.

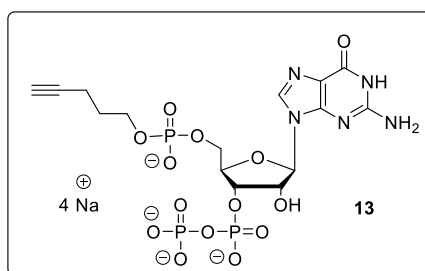
Synthesis of pentynyl-pGp (**12**)



Guanosine dihydrate (**1**, 50.0 mg, 157 μmol), ETT (81.5 mg, 627 μmol , 4.0 eq.) and P-Amidite **SI-3** (121 mg, 266 μmol , 1.7 eq.) were coevaporated separately with dry MeCN (3 x 3.0 ml). Afterwards, a solution of ETT in DMSO (400 μl) and DMF (100 μl) was added to Guanosine and the resulting solution was cooled to 3 $^{\circ}\text{C}$. Subsequently, a solution of P-Diamidite **SI-2** in DMF (3 $^{\circ}\text{C}$, 500 μl) was added. The reaction mixture was stirred for 1 h at 0 $^{\circ}\text{C}$ before (FmO)P-(NiPr₂)₂ was added and the solution was stirred for 45 min at rt. The solution was cooled to -20 $^{\circ}\text{C}$ and TBHP (5.5 M in decanes, 94.0 μl , 517 μmol , 3.3 eq.) was added dropwise. The solution was warmed to rt and stirred for 1 h. Afterwards, piperidine (90.0 μl) was added and the solution was stirred for 30 min at rt. The intermediate product was precipitated by the addition of Et₂O (10 ml). The precipitate was separated by centrifugation, Et₂O (3 x 5 ml) and dried under high vacuum. The solid was dissolved in H₂O (5 ml) and RNase T2 (50 μl) was added. The solution was incubated for 12 h at 37 $^{\circ}\text{C}$ and directly applied to automated SAX (Äkta pure system, Q-Sepharose®, NH₄HCO₃ - buffer). The product containing fractions were combined and lyophilized. The product (**12**, 38.0 mg, 67.8 μmol , 43 %) was isolated as white solid.

¹H-NMR (400 MHz, D₂O, δ /ppm): 8.09 (s, 1H), 5.92 (d, J = 6.2 Hz, 1H), 4.90 (dd, J = 5.7, 5.7 Hz, 1H), 4.86 – 4.79 (m, 1H), 4.48 – 4.42 (m, 1H), 4.11 – 4.00 (m, 2H), 3.81 – 3.68 (m, 2H), 2.16 (dd, J = 2.7, 2.7 Hz, 1H), 2.11 – 2.01 (m, 2H), 1.61 (dddd, J = 6.6 Hz, 2H). **¹³C{¹H}-NMR** (101 MHz, D₂O, δ /ppm): 158.84, 153.98, 151.91, 137.57, 116.10, 86.78, 84.48, 83.13 (dd, J = 8.8, 3.7 Hz), 73.93 (d, J = 5.1 Hz), 72.58 (d, J = 4.9 Hz), 69.18, 64.86 (d, J = 5.3 Hz), 64.66 (d, J = 5.7 Hz), 28.60 (d, J = 7.6 Hz), 13.99. **³¹P{¹H}-NMR** (162 MHz, D₂O, δ /ppm): 0.24, -0.16. **HRMS** (ESI) m/z for C₁₅H₂₀O₁₁N₅P₂ [M-H]⁻ calcd 508.0640 found 508.0644.

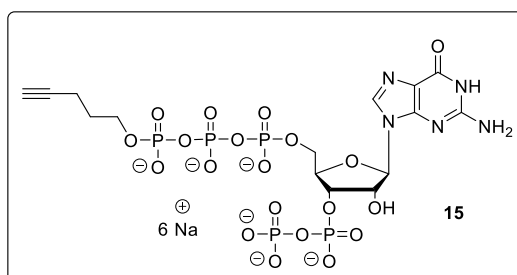
Synthesis of pentynyl-pGpp (**13**)



Pentynyl-pGp x 1.75 TBA (**12**, 35.0 mg, 37.6 μmol) was dissolved in DMF (1.0 ml) and ETT (14.6 mg, 113 μmol , 3.0 eq.) was added. Afterwards a solution of $(\text{FmO})_2\text{P-NiPr}_2$ (**SI-1**, 90%, 32.6 mg, 56.3 μmol , 1.5 eq.) in DMF (2.5 ml) was added and the resulting solution was stirred for 15 min at rt. The solution was cooled to -20°C and *m*CPBA (77%, 12.6 mg, 56.3 μmol , 1.5 eq.) was added. After stirring for 10 min at -20°C , precipitation was induced by adding Et_2O (40 ml). The resulting solid was washed with Et_2O (2 x 15 ml) and dried under high vacuum. The crude product was purified by automated SAX (Äkta pure system, Q-Sepharose®, NaClO_4 - buffer). The product containing fractions were precipitated by NaClO_4 - solution (0.5 M in acetone, -20°C , 40 ml), and the precipitate was separated by centrifugation, washed with acetone (-20°C , 3 x 10 ml) and dried under high vacuum. The product (**13**, 18.0 mg, 26.7 μmol , 71%) was isolated as white solid.

^1H -NMR (400 MHz, D_2O , δ/ppm): 8.10 (s, 1H), 5.99 (d, $J = 5.4$ Hz, 1H), 5.03 (ddq, $J = 28.3$, 9.0, 4.6 Hz, 1H), 4.95 (dd, $J = 5.3$, 5.3 Hz, 1H), 4.49 (br.s, $J = 4.1$ Hz, 1H), 4.18 – 4.08 (m, 2H), 3.83 – 3.71 (m, 2H), 2.12 – 2.05 (m, 2H), 1.69 – 1.59 (m, 2H). **$^{13}\text{C}\{^1\text{H}\}$ -NMR** (101 MHz, D_2O , δ/ppm): 159.10, 153.91, 151.97, 137.91, 116.48, 87.35, 83.09 – 82.87 (m), 74.43 (d, $J = 5.2$ Hz), 72.65 (d, $J = 3.6$ Hz), 65.11 (d, $J = 5.1$ Hz), 64.63 (d, $J = 5.6$ Hz), 28.60 (d, $J = 7.4$ Hz), 13.94. **$^{31}\text{P}\{^1\text{H}\}$ -NMR** (162 MHz, D_2O , δ/ppm): 0.24, -5.90 (d, $J = 22.7$ Hz), -11.08 (d, $J = 22.8$ Hz). **HRMS** (ESI) m/z for $\text{C}_{15}\text{H}_{21}\text{O}_{14}\text{N}_5\text{P}_3$ $[\text{M-H}]^-$ calcd 588.0303 found 588.0300.

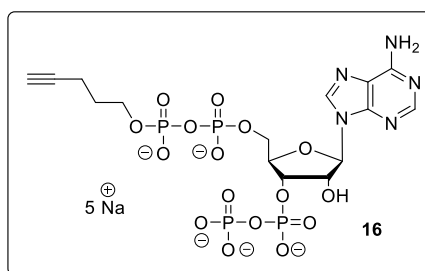
Synthesis of pentynyl-pppGpp (15)



Pentynyl-pppGp x 3.2 TBA (**9**, 90.0 mg, 62.6 μmol) was dissolved in DMF (1.0 ml) and ETT (24.4 mg, 187 μmol , 3.0 eq.) was added. Afterwards a solution of $(\text{FmO})_2\text{P-NiPr}_2$ (**SI-1**, 90%, 54.3 mg, 93.9 μmol , 1.5 eq.) in DMF (2.5 ml) was added and the resulting solution was stirred for 15 min at rt. The solution was cooled to -20°C and *m*CPBA (21.0 mg, 93.9 μmol , 1.5 eq.) was added. After stirring for 10 min at -20°C , precipitation was induced by Et_2O (40 ml). The resulting solid was washed with ether (2 x 15 ml) and dried under high vacuum. The crude product was purified by automated SAX (Äkta pure system, Q-Sepharose®, NaClO_4 - buffer). The product containing fractions were precipitated by NaClO_4 – solution (0.5 M in acetone, -20°C , 40 ml), and the precipitate was separated by centrifugation, washed with acetone (-20°C , 3 x 10 ml) and dried under high vacuum. The product (**15**, 41.0 mg, 46.5 μmol , 74%) was isolated as white solid.

$^1\text{H-NMR}$ (400 MHz, D_2O , δ/ppm): 8.14 (s, 1H), 6.00 (d, $J = 6.3$ Hz, 1H), 5.01 – 4.93 (m, 1H), 4.89 (dd, $J = 5.7, 5.7$ Hz, 1H), 4.58 – 4.49 (m, 1H), 4.36 – 4.18 (m, 2H), 3.95 (ddd, $J = 6.5, 6.5, 6.5$ Hz, 2H), 2.26 (dd, $J = 2.7, 2.7$ Hz, 1H), 2.18 (ddd, $J = 7.3, 7.3, 2.7$ Hz, 2H), 1.69 (dddd, $J = 7.9, 7.9, 7.4, 7.4$ Hz, 2H). **$^{13}\text{C}\{^1\text{H}\}\text{-NMR}$** (101 MHz, D_2O , δ/ppm): 159.14, 153.95, 151.98, 137.98, 116.38, 86.88, 83.27 (dd, $J = 9.2, 4.3$ Hz), 74.86 (d, $J = 5.2$ Hz), 72.99 (d, $J = 4.2$ Hz), 65.57 (d, $J = 5.3$ Hz), 65.22 (d, $J = 6.0$ Hz), 28.78 (d, $J = 7.4$ Hz), 14.07. **$^{31}\text{P}\{^1\text{H}\}\text{-NMR}$** (162 MHz, D_2O , δ/ppm): -5.72 (d, $J = 22.2$ Hz), -10.73 – -11.00 (m, 2P), -11.34 (d, $J = 17.9$ Hz), -22.84 (dd, $J = 18.5$ Hz). **HRMS** (ESI) for $\text{C}_{15}\text{H}_{23}\text{O}_{20}\text{N}_5\text{P}_5$ $[\text{M-H}]^-$ calcd 747.9630 found 747.9636.

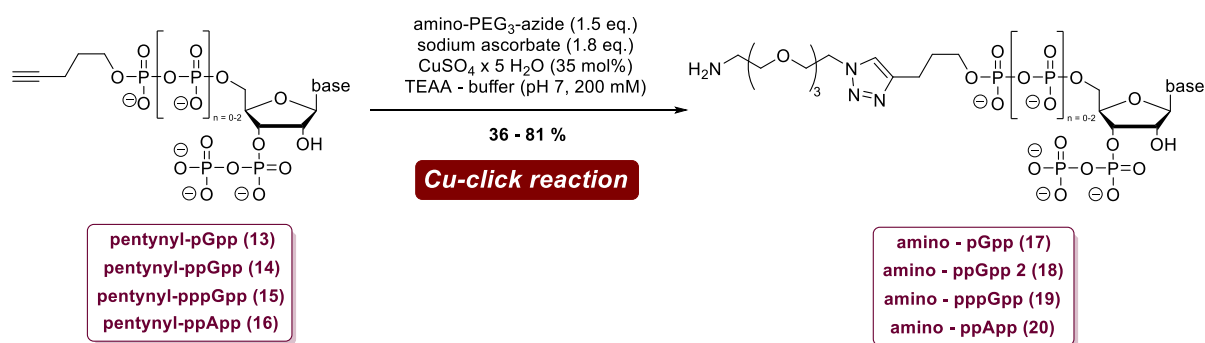
Synthesis of pentynyl-ppApp (16)



Pentynyl-ppAp x 1.89 TBA (**11**, 45.0 mg, 43.8 μmol) was dissolved in DMF (1.0 ml) and ETT (20.0 mg, 154 μmol , 3.5 eq.) was added. Afterwards a solution of $(\text{FmO})_2\text{P-NiPr}_2$ (**SI-1**, 80%, 42.8 mg, 65.8 μmol , 1.5 eq.) in DMF (2.0 ml) was added and the resulting solution was stirred for 15 min at rt. The solution was cooled to -20°C and *m*CPBA (77%, 19.6 mg, 87.7 μmol , 2.0 eq.) was added. After stirring for 10 min at -20°C , precipitation was induced by Et_2O (40 ml). The resulting solid was washed with ether (2 x 15 ml) and dried under high vacuum. The crude product was purified by automated SAX (Äkta pure system, Q-Sepharose®, NaClO_4 - buffer). The product containing fractions were precipitated by NaClO_4 - solution (0.5 M in acetone, -20°C , 40 ml) and the precipitate was separated by centrifugation, washed with acetone (-20°C , 3 x 10 ml) and dried under high vacuum. The product (**16**, 28.3 mg, 37.1 μmol , 84%) was isolated as white solid.

^1H -NMR (400 MHz, D_2O , δ/ppm): 8.56 (s, 1H), 8.28 (s, 1H), 6.21 (d, $J = 6.2$ Hz, 1H), 4.99 – 4.92 (m, 1H), 4.88 (dd, $J = 5.6, 5.6$ Hz, 1H), 4.61 – 4.54 (m, 1H), 4.30 – 4.18 (m, 2H), 3.86 (ddd, $J = 6.5, 6.3, 6.3$ Hz, 2H), 2.19 (dd, $J = 2.7, 2.7$ Hz, 1H), 2.15 – 2.09 (m, 2H), 1.63 (dddd, $J = 6.8, 6.7, 6.7$ Hz, 3H). **$^{13}\text{C}\{^1\text{H}\}$ -NMR** (101 MHz, D_2O , δ/ppm): δ 155.68, 152.93, 149.35, 139.97, 118.68, 86.66, 84.75, 83.36 (dd, $J = 9.4, 4.1$ Hz), 74.72 (d, $J = 5.1$ Hz), 73.56 (d, $J = 4.3$ Hz), 69.04, 65.31 (d, $J = 5.7$ Hz), 65.03 (d, $J = 6.0$ Hz), 28.72 (d, $J = 7.4$ Hz), 14.01. **$^{31}\text{P}\{^1\text{H}\}$ -NMR** (162 MHz, D_2O , δ/ppm): -5.85 (d, $J = 22.6$ Hz), -10.58 – -11.14 (m, 2P), -11.55 (d, $J = 20.5$ Hz). **HRMS** (ESI, m/z) for $\text{C}_{15}\text{H}_{21}\text{O}_{16}\text{N}_5\text{P}_4$ $[\text{M}-\text{H}_2]^{2-}$ calcd 325.4972 found 325.4973.

2.5. Synthesis of phosphate-modified amino-MSN



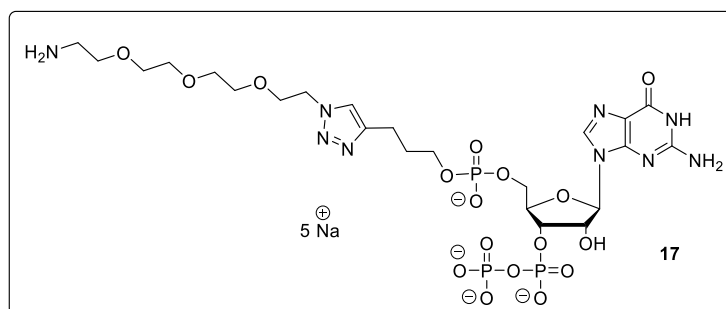
Supporting figure 2: Synthesis of phosphate-modified amino-MSN from pentynyl-MSN by Cu-catalyzed cycloaddition reactions.

General procedure A for Cu-click reactions towards amino-MSN 17-20:

Pentynyl-MSN (10 – 25 μ mol) was dissolved in TEAA-buffer (200 mM, 1.5 ml). Amino-PEG₃-azide (1.5 eq.) and sodium ascorbate (1.8 eq.) were added and argon was flown through the solution for 20 min at rt. Afterwards CuSO₄ x 5 H₂O (0.35 eq.) was added and the solution was stirred over argon atmosphere for 2-3 h. Afterwards H₂O (17 ml) was added and the solution was directly applied to automated SAX (Äkta pure system, Q-Sepharose®, NaClO₄ - buffer). The product containing fractions were precipitated by NaClO₄ – solution (0.5 M in acetone, -20°C, 40 ml) and the solid was separated by centrifugation, washed with acetone (-20°C, 3 x 10 ml) and dried under high vacuum.

As the products still contained residual amounts of Cu^{II}-ions, NMR-analysis was not possible at this stage. The Cu^{II}-impurities were removed in subsequent steps allowing NMR-characterization of the final capture compounds.

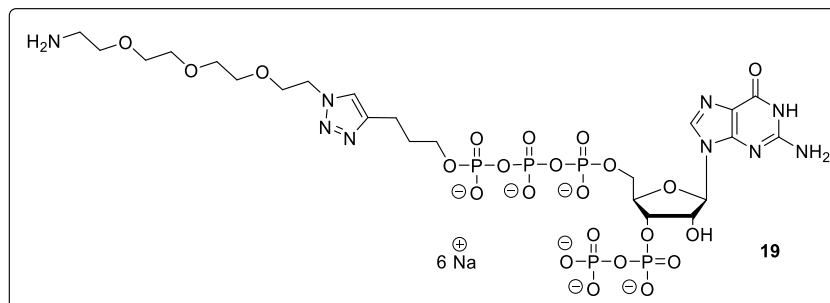
Synthesis of amino-pGpp (17)



Pentynyl-pGpp (**13**, 10.0 mg, 14.7 μ mol) was subjected to the general **procedure A**. The product (**17**, 4.80 mg, 5.36 μ mol, 36%) was isolated as white solid.

HRMS (ESI) m/z for $C_{23}H_{41}N_9O_{17}P_3$ $[M+H]^+$: calcd 808.1828, found 808.1816. **HPLC-UV** analysis (see SI, chapter 9) was performed.

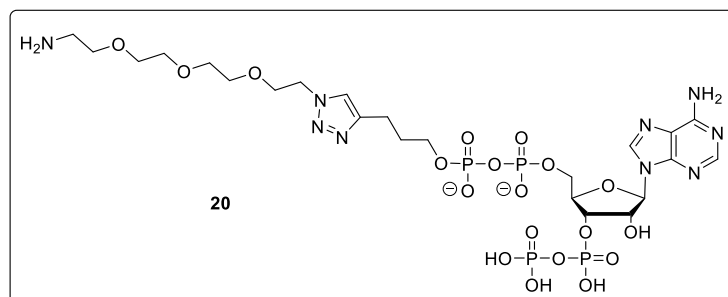
Synthesis of amino-pppGpp (**19**)



Pentynyl-pppGpp (**15**, 15.0 mg, 17.0 μmol) was subjected to the general **procedure A**. The product (**19**, 15.2 mg, 13.8 μmol , 81%) was isolated as white solid.

HRMS (ESI, m/z) m/z for $C_{23}H_{40}N_9O_{23}P_5$ $[M-H_2]^{2-}$: calcd 482.5468, found 482.5468. **HPLC-UV** analysis (see appendix) was performed.

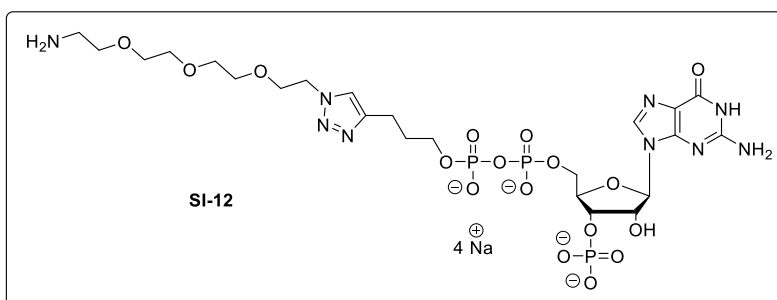
Synthesis of amino-ppApp (**20**)



Pentynyl-ppApp (**16**, 10.0 mg, 13.1 μmol) was subjected to the general **procedure A**. The product (**20**, 5.30 mg, 5.40 μmol , 41%) was isolated as white solid.

HRMS (ESI) m/z for $C_{23}H_{39}N_9O_{19}P_4$ $[M-H_2]^{2-}$: calcd 434.5662, found 434.5671. **HPLC-UV** analysis (see SI, chapter 9) was performed.

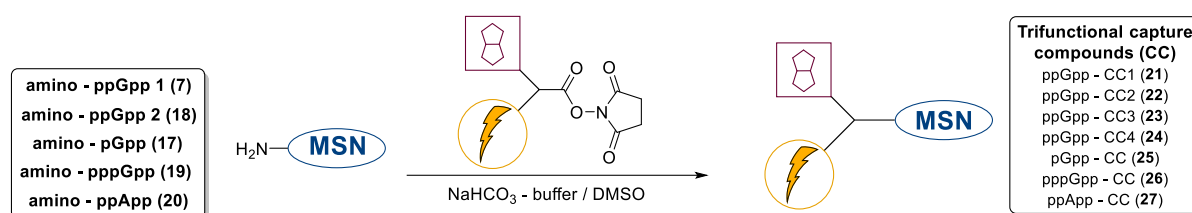
Synthesis of amino-ppGp (SI-12)



Pentynyl-ppGp (**10**, 15.0 mg, 22.2 μmol) was subjected to the general **procedure A**. The product (**SI-12**, 15.3 mg, 17.0 μmol , 77%) was isolated as white solid.

HRMS (ESI) m/z for $\text{C}_{23}\text{H}_{39}\text{N}_9\text{O}_{17}\text{P}_3$ $[\text{M-H}]^-$: calcd 806.1682, found 806.1686. **HPLC-UV** analysis (see SI, chapter 9) was performed.

2.6. Syntheses of MSN-capture compounds

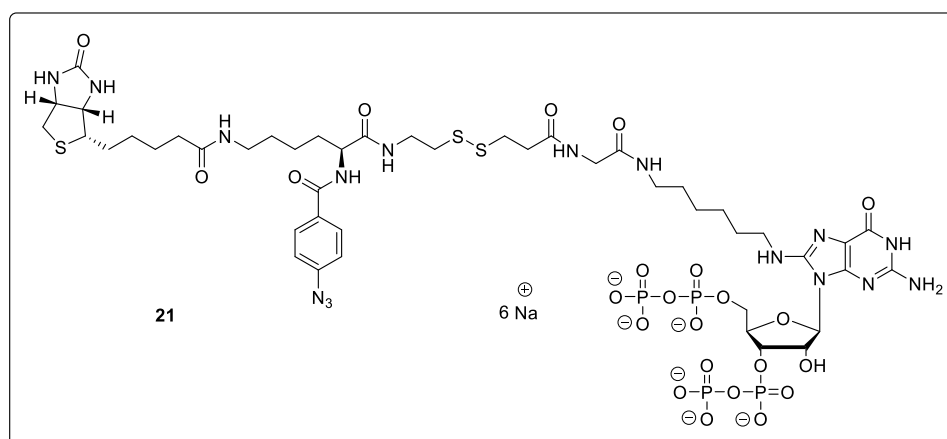


Supporting figure 3: Synthesis of trifunctional MSN-capture compounds from amino-MSN and NHS-esters by chemoselective amide forming reactions.

General procedure B for NHS – Ester coupling towards MSN-capture compounds 21-27:

Amino – MSN (0.7 – 3.4 μmol) were dissolved in NaHCO_3 – buffer (200 mM) in a concentration of 10 $\mu\text{g}/\mu\text{l}$. Sulfo-NHS – Ester (1.2 – 1.5 eq.) were dissolved in H_2O in a concentration of 10 $\mu\text{g}/\mu\text{l}$. The solutions were mixed and the resulting reaction mixture was incubated for 30 min at rt under light exclusion. Reaction progress was monitored by HPLC. Afterwards the mixture was diluted with H_2O (6.0 ml) and directly applied to automated SAX (Äkta pure system, Q-Sepharose®, NaClO_4 – buffer, VIS-detection at 600 nm). The product containing fractions were determined by HPLC and precipitated by NaClO_4 – solution (0.5 M in acetone, -20°C , 40 ml). The solid was separated by centrifugation, washed with acetone (-20°C , 3 x 10 ml) and dried under high vacuum. In contrast to Sulfo-NHS esters, NHS-esters were dissolved in DMSO before mixing.

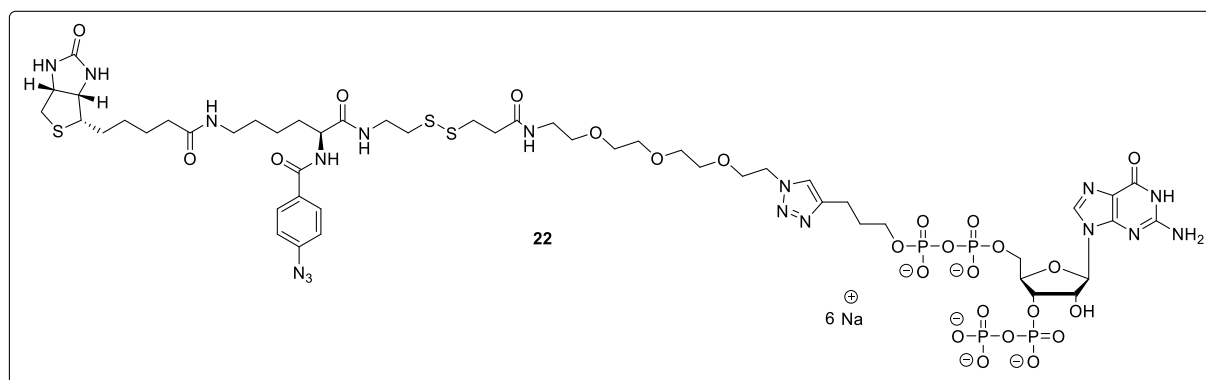
Synthesis of ppGpp – CC1 (21)



CC2 was synthesized from 8'-amino-ppGpp (**7**, 1.21 mg, 1.36 μmol) and Sulfo-SBED according to the general **procedure B**. The product (**21**, 1.30 mg, 829 nmol, 61%) was isolated as white solid.

¹H-NMR (400 MHz, D₂O, δ/ppm): 7.99 – 7.92 (m, 2H), 7.33 – 7.27 (m, 2H), 5.96 (d, *J* = 6.5 Hz, 1H), 4.86 – 4.80 (m, 1H), 4.58 (dd, *J* = 9.3, 5.8 Hz, 1H), 4.52 – 4.30 (m, 2H), 3.97 (s, 2H), 3.69 (dd, *J* = 12.9, 6.9 Hz, 2H), 3.47 (d, *J* = 11.3 Hz, 2H), 3.41 – 3.23 (m, 6H), 3.06 (ddd, *J* = 14.1, 10.4, 5.3 Hz, 6H), 2.94 – 2.77 (m, 3H), 2.35 (dd, *J* = 4.4 Hz, 2H), 2.30 (dd, *J* = 7.3, 7.3 Hz, 2H), 2.11 – 1.91 (m, 2H), 1.89 – 1.35 (m, 17H). **³¹P{¹H}-NMR** (162 MHz, D₂O, δ/ppm): -5.75 (br. s), -10.61 (br.s). **HRMS** (ESI) *m/z* for C₄₆H₇₃N₁₆O₂₃P₄S₃ [M+H]⁺: calcd 1437.3142, found 1437.3147. **HPLC-UV** analysis was performed (see SI, chapter 9).

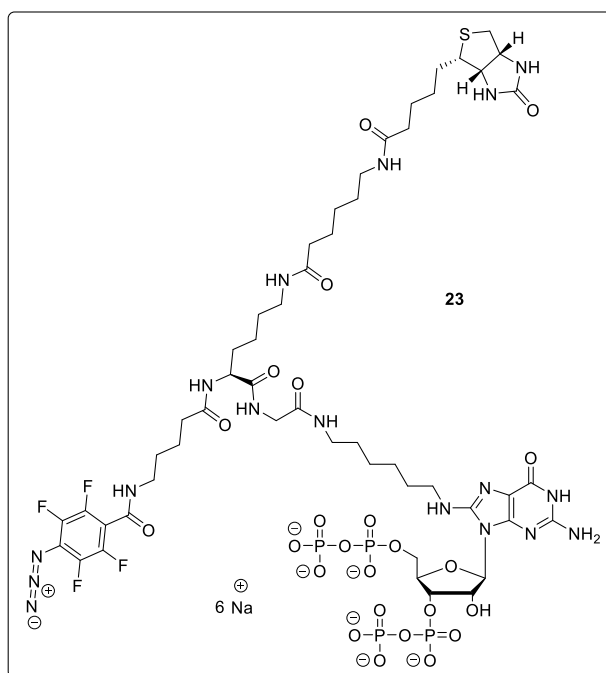
Synthesis of ppGpp – CC2 (22)



CC2 was synthesized from amino-ppGpp (**18**, 770 μg, 772 nmol) and Sulfo-SBED according to the general **procedure B**. The product (**22**, 370 μg, 223 nmol, 29%) was isolated as white solid.

¹H-NMR (400 MHz, D₂O, δ/ppm): 8.10 (s, 1H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.67 (s, 1H), 7.17 (d, *J* = 8.5 Hz, 2H), 5.94 (d, *J* = 6.1 Hz, 1H), 5.05 – 4.93 (m, 3H), 4.61 – 4.49 (m, 4H), 4.45 (dd, *J* = 8.9, 5.8 Hz, 1H), 4.37 – 4.19 (m, 3H), 3.92 (dd, *J* = 5.1, 5.1 Hz, 2H), 3.87 (ddd, *J* = 6.4, 6.4, 6.4 Hz, 2H), 3.65 – 3.51 (m, 12H), 3.36 (dd, *J* = 5.3, 5.3 Hz, 2H), 3.21 (q, *J* = 4.8 Hz, 3H), 2.97 – 2.84 (m, 5H), 2.73 (d, *J* = 13.0 Hz, 1H), 2.66 – 2.52 (m, 4H), 2.18 (t, *J* = 7.2 Hz, 2H), 1.98 – 1.84 (m, 2H), 1.78 (p, *J* = 7.2 Hz, 2H), 1.70 – 1.37 (m, 8H), 1.36 – 1.20 (m, 2H). **³¹P{¹H}-NMR** (162 MHz, D₂O, δ/ppm): -5.80 (d, *J* = 20.8 Hz), -10.71 – -11.11 (m), -11.57 (d, *J* = 20.9 Hz). **HRMS** (ESI) *m/z* for C₅₁H₇₇N₁₇O₂₅P₄S₃ [M-H]₂²⁻: calcd 773.6700, found 773.6704. **HPLC-UV** analysis was performed (see attachment).

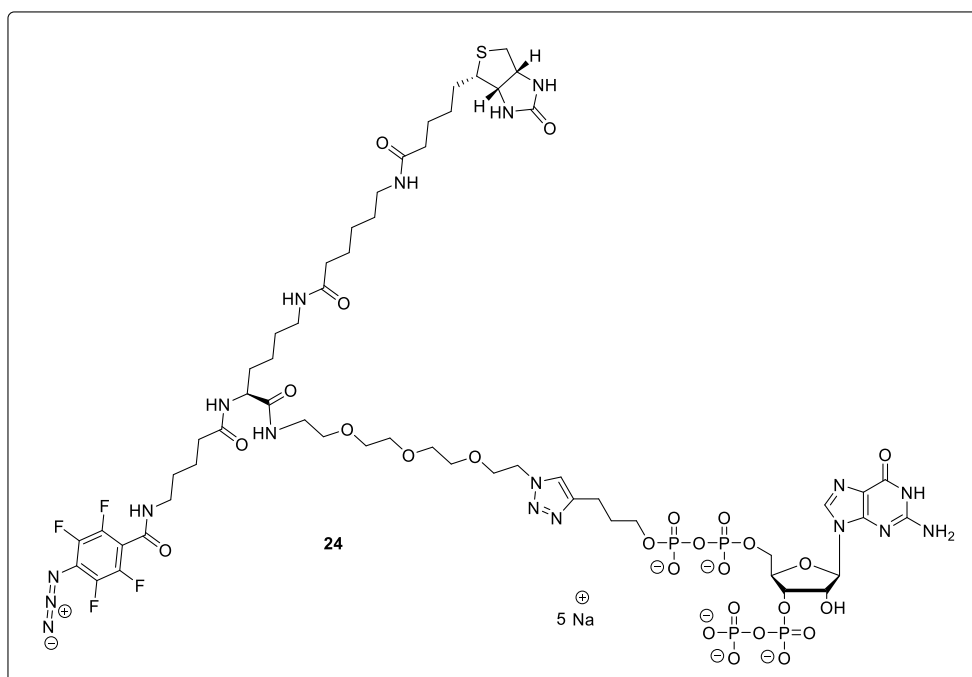
Synthesis of ppGpp – CC3 (23)



ppGpp – CC5 was synthesized from 8-amino-ppGpp (**7**, 3.00 mg, 3.39 μ mol) and the synthesized Fluorophenylazide-NHS - linker **29** according to the general **procedure B**. The product (**23**, 1.10 mg, 650 nmol, 19%) was isolated as white solid.

^1H -NMR (400 MHz, D_2O , δ/ppm): 5.85 (d, $J = 6.8$ Hz, 1H), 5.05 (s, 1H), 4.59 (dd, $J = 8.0$, 4.8 Hz, 1H), 4.47 (t, $J = 3.6$ Hz, 1H), 4.39 (dd, $J = 8.0$, 4.4 Hz, 1H), 4.33 – 4.15 (m, 3H), 3.87 (d, $J = 3.7$ Hz, 2H), 3.48 – 3.08 (m, 12H), 2.98 (dd, $J = 13.1$, 5.0 Hz, 1H), 2.77 (d, $J = 13.0$ Hz, 1H), 2.37 (dd, $J = 7.3$, 7.3 Hz, 2H), 2.27 – 2.18 (m, 2H), 1.80 – 1.23 (m, 32H). **$^{19}\text{F}\{^1\text{H}\}$ -NMR** (377 MHz, δ/ppm): -142.95 – -143.21 (m, 2F), -150.98 – -151.26 (m, 2F). **$^{31}\text{P}\{^1\text{H}\}$ -NMR** (162 MHz, D_2O , δ/ppm): -5.79 (d, $J = 22.2$ Hz, 2P), -10.81 (d, $J = 20.4$ Hz, 2P). **HRMS** (ESI) m/z for $\text{C}_{52}\text{H}_{80}\text{N}_{17}\text{O}_{24}\text{F}_4\text{P}_4\text{S}$ $[\text{M}+\text{H}]^+$: calcd 1558.4164, found 1558.4139. **HPLC-UV** analysis was performed (see SI, chapter 9).

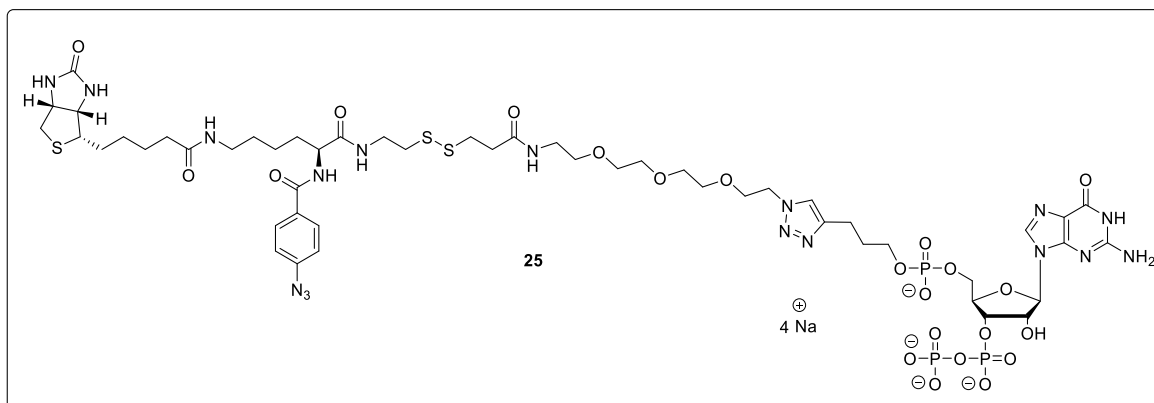
Synthesis of ppGpp - CC4 (24)



ppGpp – CC3 was synthesized from amino-ppGpp (**18**, 3.00 mg, 3.00 μmol) and the synthesized Fluorophenylazide-NHS - linker **29** according to the general **procedure B**. The product (**24**, 2.20 mg, 1.24 μmol , 41%) was isolated as white solid.

^1H -NMR (400 MHz, D_2O , δ/ppm): ^1H NMR (400 MHz, D_2O) δ 8.11 (s, 1H), 7.70 (s, 1H), 5.95 (d, $J = 6.2$ Hz, 1H), 4.63 – 4.58 (m, 1H), 4.59 – 4.48 (m, 2H), 4.46 – 4.34 (m, 1H), 4.33 – 4.15 (m, 3H), 4.00 – 3.79 (m, 4H), 3.74 – 3.48 (m, 12H), 3.48 – 3.35 (m, 4H), 3.35 – 3.26 (m, 1H), 3.24 – 3.06 (m, 4H), 2.98 (dd, $J = 13.0, 5.0$ Hz, 1H), 2.77 (d, $J = 13.0$ Hz, 1H), 2.59 (dd, $J = 8.0, 8.0$ Hz, 2H), 2.45 – 2.30 (m, 3H), 1.92 – 1.19 (m, 28H). **$^{19}\text{F}\{^1\text{H}\}$ -NMR** (377 MHz, δ/ppm): -143.09 – -143.27 (m), -151.02 – -151.18 (m). **$^{31}\text{P}\{^1\text{H}\}$ -NMR** (162 MHz, D_2O , δ/ppm): -6.06 (br.s), -10.66 – -11.24 (m), -11.61 (d, $J = 21.0$ Hz). **HRMS** (ESI) m/z for $\text{C}_{57}\text{H}_{83}\text{N}_{18}\text{O}_{26}\text{F}_4\text{P}_4\text{S}$ $[\text{M}-\text{H}_3]^{3-}$: calcd 555.8117, found 555.8113. **HPLC-UV** analysis was performed (see SI, chapter 9).

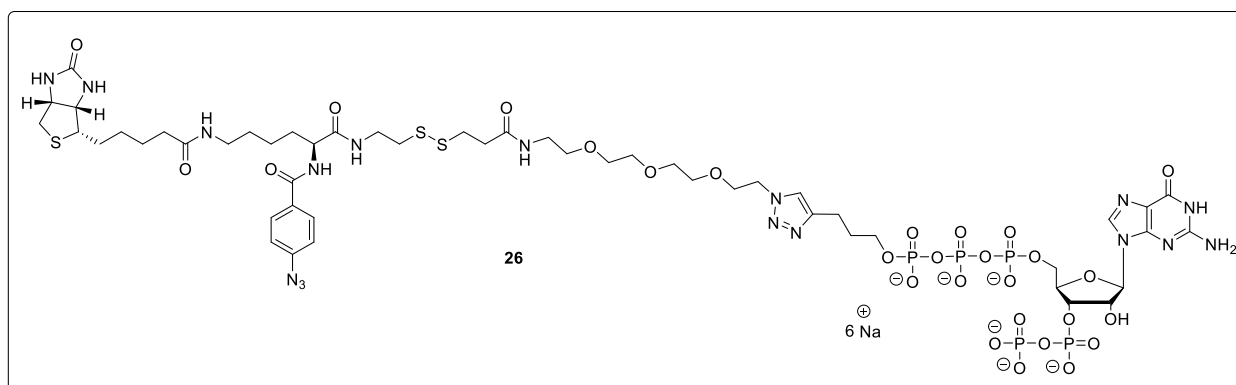
Synthesis of pGpp – CC (25)



pGpp – CC5 was synthesized from amino-pGpp (**17**, 2.00 mg, 2.23 μ mol) and Sulfo-SBED according to the general **procedure B**. The product (**25**, 1.35 mg, 867 nmol, 39%) was isolated as white solid.

¹H-NMR (400 MHz, D₂O, δ /ppm): 8.03 (s, 1H), 7.77 (d, J = 8.3 Hz, 2H), 7.61 (s, 1H), 7.12 (d, J = 8.5 Hz, 2H), 5.91 (d, J = 5.4 Hz, 1H), 4.98 (s, 1H), 4.57 – 4.37 (m, 4H), 4.34 – 4.24 (m, 1H), 4.18 – 4.04 (m, 2H), 3.89 (dd, J = 5.1, 5.1 Hz, 2H), 3.80 – 3.65 (m, 2H), 3.63 – 3.46 (m, 13H), 3.32 (dd, J = 5.3, 5.3 Hz, 2H), 3.23 – 3.12 (m, 3H), 2.94 – 2.80 (m, 5H), 2.70 (d, J = 13.1 Hz, 1H), 2.58 (dd, J = 6.7, 6.7 Hz, 2H), 2.56 – 2.43 (m, 2H), 2.15 (dd, J = 7.2, 7.2 Hz, 2H), 1.96 – 1.79 (m, 2H), 1.74 (dd, J = 7.8, 7.8 Hz, 2H), 1.66 – 1.34 (m, 9H), 1.34 – 1.15 (m, 2H). **³¹P{¹H}-NMR** (162 MHz, D₂O, δ /ppm): 0.28, -5.90 (br. s), -11.08 (br. s). **HRMS** (ESI) m/z for C₅₁H₇₅N₁₇O₂₂P₃S₃ [M-H]³⁻: calcd 488.7888, found 488.7890. **HPLC-UV** analysis was performed (see SI, chapter 9).

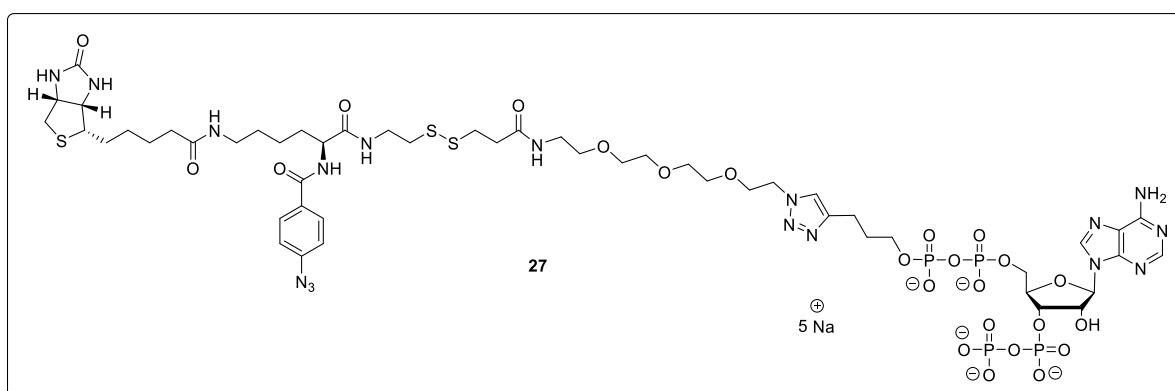
Synthesis of pppGpp – CC (26)



pppGpp – CC7 was synthesized from amino-pppGpp (**19**, 2.10 mg, 1.91 μmol) and Sulfo-SBED according to the general **procedure B**. The product (**26**, 1.56 mg, 886 nmol, 46%) was isolated as white solid.

^1H -NMR (400 MHz, D_2O , δ/ppm): 8.10 (s, 1H), 7.82 (d, $J = 8.4$ Hz, 2H), 7.71 (s, 1H), 7.18 (d, $J = 8.4$ Hz, 2H), 5.94 (d, $J = 6.4$ Hz, 1H), 4.96 (t, $J = 6.7$ Hz, 1H), 4.61 – 4.49 (m, 4H), 4.45 (dd, $J = 8.8, 5.8$ Hz, 1H), 4.38 – 4.21 (m, 3H), 3.99 – 3.86 (m, 4H), 3.69 – 3.49 (m, 13H), 3.36 (dd, $J = 5.3, 5.3$ Hz, 2H), 3.28 – 3.17 (m, 3H), 3.00 – 2.83 (m, 5H), 2.73 (d, $J = 13.0$ Hz, 1H), 2.68 – 2.57 (m, 4H), 2.18 (dd, $J = 7.2, 7.2$ Hz, 2H), 1.99 – 1.85 (m, 2H), 1.85 – 1.74 (m, 2H), 1.69 – 1.37 (m, 8H), 1.36 – 1.21 (m, 2H). **$^{31}\text{P}\{^1\text{H}\}$ -NMR** (162 MHz, D_2O , δ/ppm): -5.72 (d, $J = 22.4$ Hz), -10.90 (d, $J = 22.3$ Hz), -10.97 (d, $J = 19.2$ Hz), -11.37 (d, $J = 17.4$ Hz), -22.89 (dd, $J = 18.5$ Hz). **HRMS** (ESI) m/z for $\text{C}_{51}\text{H}_{77}\text{N}_{17}\text{O}_{28}\text{P}_5\text{S}_3$ $[\text{M}-\text{H}]^{3-}$: calcd 542.0997, found 542.1000. **HPLC-UV** analysis was performed (see SI, chapter 9).

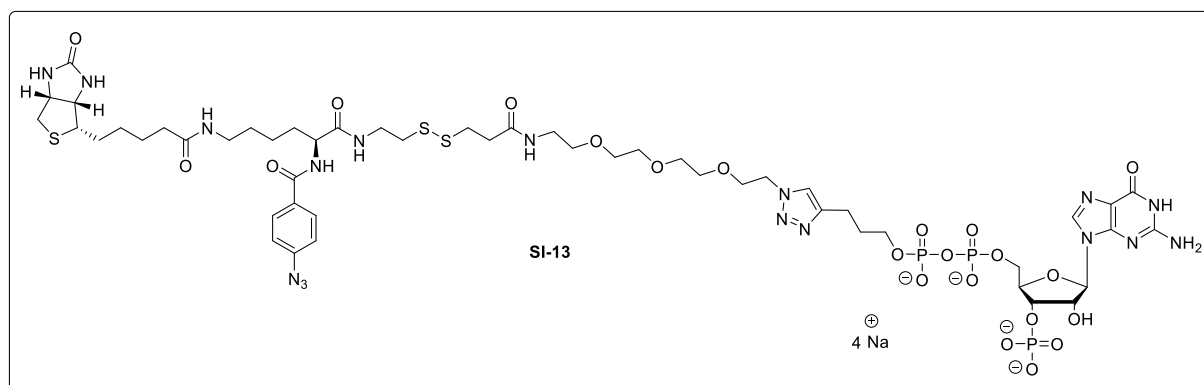
Synthesis of ppApp – CC (27)



ppApp – CC8 was synthesized from amino-ppApp (**20**, 2.00 mg, 2.04 μmol) and Sulfo-SBED according to the general **procedure B**. The product (**27**, 2.02 mg, 1.21 μmol , 60%) was isolated as white solid.

¹H-NMR (400 MHz, D₂O, δ/ppm): 8.52 (s, 1H), 8.18 (d, *J* = 2.8 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.59 (s, 1H), 7.15 (dd, *J* = 8.7, 2.8 Hz, 2H), 6.14 (d, *J* = 6.1 Hz, 1H), 4.96 (br.s, 1H), 4.78 – 4.75 (m, 1H), 4.62 – 4.51 (m, 2H), 4.51 – 4.38 (m, 2H), 4.38 – 4.17 (m, 3H), 3.95 – 3.79 (m, 4H), 3.66 – 3.47 (m, 13H), 3.35 (dd, *J* = 5.3, 5.3 Hz, 2H), 3.25 – 3.15 (m, 3H), 2.98 – 2.82 (m, 5H), 2.73 (d, *J* = 13.0 Hz, 1H), 2.61 (dd, *J* = 6.7, 6.7 Hz, 2H), 2.57 – 2.44 (m, 2H), 2.17 (dd, *J* = 7.2, 7.2 Hz, 2H), 1.98 – 1.80 (m, 2H), 1.78 – 1.67 (m, 2H), 1.67 – 1.35 (m, 8H), 1.34 – 1.20 (m, 2H). **³¹P{¹H}-NMR** (162 MHz, D₂O, δ/ppm): -5.79 (d, *J* = 22.2 Hz), -10.90 (d, *J* = 19.7 Hz), -11.56 (d, *J* = 20.4 Hz). **HRMS** (ESI) *m/z* for C₅₁H₈₀N₁₇O₂₄NaP₄S₃ [M+H]²⁺: calcd 778.6781, found 778.6774. **HPLC-UV** analysis was performed (see SI, chapter 9).

Synthesis of ppGp – CC (SI-13)



ppGp – CC6 was synthesized from amino-ppGp (**SI-12**, 2.00 mg, 2.23 μmol) and Sulfo-SBED according to the general **procedure B**. The product (**SI-13**, 1.45 mg, 931 nmol, 42%) was isolated as white solid.

¹H-NMR (400 MHz, D₂O, δ/ppm): 8.13 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.67 (s, 1H), 7.15 (d, *J* = 8.5 Hz, 2H), 5.91 (d, *J* = 7.0 Hz, 1H), 4.76 (s, 1H), 4.60 – 4.50 (m, 3H), 4.45 (dd, *J* = 8.9, 5.8 Hz, 1H), 4.37 – 4.28 (m, 1H), 4.23 (br. s, *J* = 4.2 Hz, 2H), 3.99 – 3.82 (m, 4H), 3.68 – 3.48 (m, 13H), 3.36 (dd, *J* = 5.3, 5.3 Hz, 2H), 3.28 – 3.14 (m, 3H), 2.99 – 2.83 (m, 5H), 2.73 (d, *J* = 13.1 Hz, 1H), 2.69 – 2.51 (m, 4H), 2.18 (dd, *J* = 7.2, 7.2 Hz, 2H), 1.99 – 1.84 (m, 2H), 1.84 – 1.74 (m, 2H), 1.68 – 1.35 (m, 8H), 1.34 – 1.22 (m, 2H). **³¹P{¹H}-NMR** (162 MHz, D₂O, δ/ppm): 3.89 (s), -10.97 (d, *J* = 21.0 Hz), -11.52 (d, *J* = 20.7 Hz). **HRMS** (ESI) *m/z* for C₅₁H₇₅N₁₇O₂₂P₃S₃ [M-H]³⁻: calcd 488.7888, found 488.7889. **HPLC-UV** analysis was performed (see SI, chapter 9).

3. Procedural remarks, pull-down experiments

The pull-down experiments were performed exactly according to the method published by Laventie, Glatter and Jenal in 2017 (“Pull-Down with a c-di-GMP-Specific Capture Compound Coupled to Mass Spectrometry as a Powerful Tool to Identify Novel Effector Proteins”).^[4,5] In contrast to the example described by Laventie et al. *E. coli* and *S. typhimurium* were used as organisms.

The following methodical steps were performed exactly according to Laventie et al. including the application of identical materials (e.g.: buffers, solvents, reagents, magnetic beads, UV-cross linker...):

- 1.) Lysate Preparation
- 2.) Removal of Free Nucleotides (soluble fraction only)
- 3.) Membrane Resuspension and Solubilization (membrane fraction only)
- 4.) Protein concentration measurement
- 5.) Capture
- 6.) Washing steps
- 7.) MS Sample Preparation
- 8.) LC-MS/MS analysis
- 9.) Database Search
- 10.) Label-Free Quantification

Important: no reducing agents (e.g.: DTT) were used in buffers before the trypsin digest, as some of the capture compounds bear a disulfide bridge.

Notably, typical reaction set-ups differed slightly from Laventie et al. and are summarized in the following tables:

MSN – pull-down experiments in *E. coli*:

Supporting table 1: MSN – pull down experiment set-up using *E. coli* extracts. Usually, the protein concentration was above 10 mg/ml so the extract was diluted accordingly before the capture experiment. Both the capture experiment as well as the competition control were performed in triplicates. MSN – competitors were unmodified ppGpp, pppGpp, pGpp, ppApp or ppGp.

	Capture Experiment	Competition Control
<i>E. coli</i> extract ($c_{\text{protein}} = 10 \text{ mg/mL}$)	30 μl	30 μl
MSN – competitor ($c = 20 \text{ mM}$)	-	10 μl
Capture buffer (5x)	20 μl	20 μl
ddH₂O	40 μl	30 μl
30 min incubation at 4 °C		
MSN – capture compound ($c = 200 \text{ }\mu\text{M}$)	10 μl	10 μl
2 h incubation at 4 °C, then UV-crosslinking		
final volume	100 μl	100 μl

MSN – pull-down experiments in *S. typhimurium*:

Supporting table 2: MSN – pull down experiment set-up using *S. typhimurium* extracts. In this case the protein concentration of the soluble fraction was below 10 mg/ml. Consequently, the applied volume was increased to keep the protein roughly constant compared to the *E. coli* experiments. Both the capture experiment as well as the competition control were performed in triplicates. In the case of *S. typhimurium*, ppGpp-CC1&2 were applied as mixture.

	Capture Experiment	Competition Control
<i>S. typhimurium</i> extract ($c_{\text{protein}} = 4.7 \text{ mg/mL}$)	60 μl	60 μl
ppGpp (c = 20 mM)	-	10 μl
Capture buffer (5x)	20 μl	20 μl
ddH₂O	10 μl	-
30 min incubation at 4 °C		
ppGpp – CC1+2 - mix ($c = 100 \text{ }\mu\text{M}$ each)	10 μl	10 μl
2 h incubation at 4 °C, then UV-crosslinking		
final volume	100 μl	100 μl

3.1. Data-analysis: methods of quantification and statistical methods

Acquired raw-files were imported into the Progenesis QI software (v2.0, Nonlinear Dynamics Limited), which was used to extract peptide precursor ion intensities across all samples applying the default parameters. MS/MS data was exported directly from Progenesis in mgf format and searched using Mascot against an Escherichia coli database (consisting of 8700 forward and reverse protein sequences downloaded from Uniprot and 391 commonly observed contaminants) or a Salmonella database (consisting of 8868 forward and reverse protein sequences downloaded from Uniprot and 385 commonly observed contaminants), respectively. Results from the database search were imported into Progenesis and a list containing all quantified peptides exported. The quantitative data was processed using the SafeQuant R package v.2.3.2 [PMID: 27345528], <https://github.com/eahrne/SafeQuant/>. The database search results were filtered using the ion score to set the false discovery rate (FDR) to 1% on the peptide and protein level, respectively, based on the number of reverse protein sequence hits in the dataset. Furthermore, the analysis included global data normalization by equalizing the total peak areas across all LC-MS runs, data imputation using the knn algorithm, summation of peak areas per protein and LC-MS/MS run, followed by calculation of protein abundance ratios. Only isoform specific peptide ion signals were considered for quantification. The summarized protein expression values were used for statistical testing of between condition differentially abundant proteins. Here, empirical Bayes moderated t-Tests were applied, as implemented in the R/Bioconductor limma package (<http://bioconductor.org/packages/release/bioc/html/limma.html>). The resulting per protein and condition comparison p-values were adjusted for multiple testing using the Benjamini-Hochberg method. To meet additional assumptions (normality and homoscedasticity) underlying the use of linear regression models and t-Tests, MS-intensity signals were transformed from the linear to the log-scale.

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD032009 and 10.6019/PXD032009.

4. Pull-down results: enrichment tables and hit-maps

Legend:

- Table entries marked in **green** are known ppGpp – receptors.^[6]
- Table entries marked in **yellow** were already captured by Laub et al.^[7]
- *Threshold 1*: $\log_2(\text{enrichment}) \geq 2.0$
- *Threshold 2*: $q\text{-value} \leq 0.05$

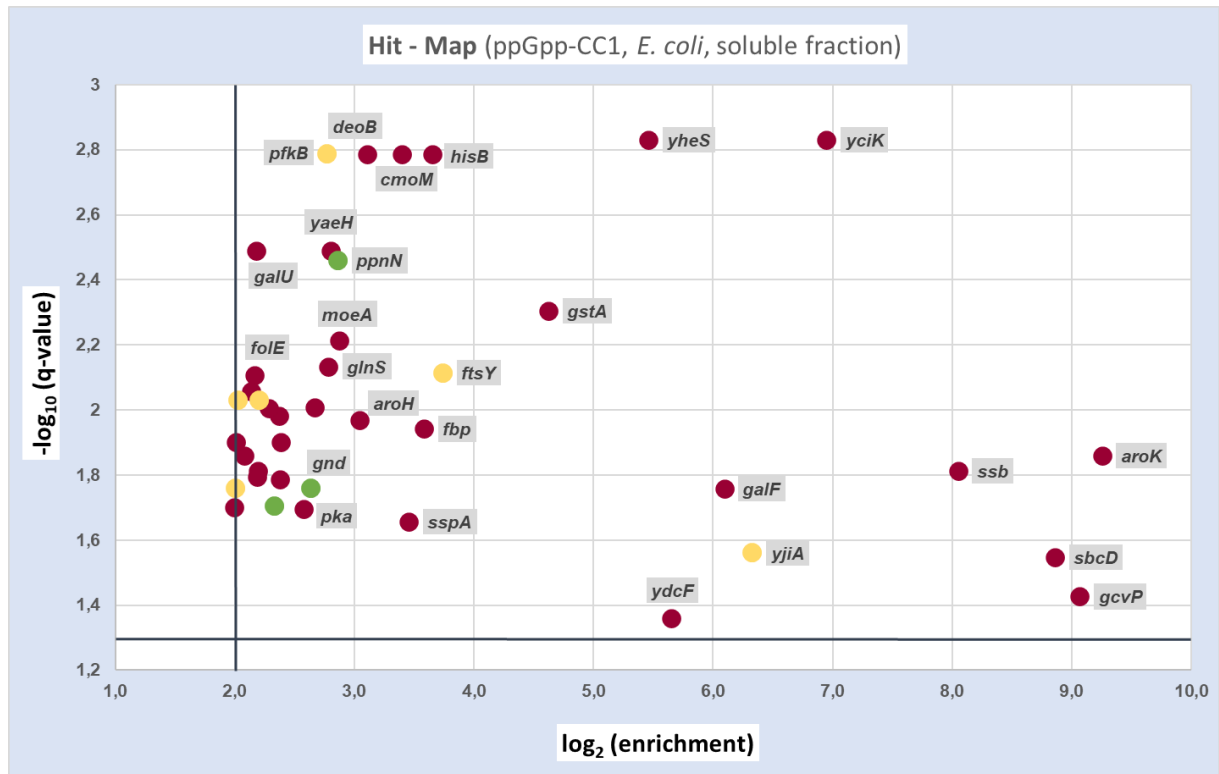
4.1. *E. coli* (soluble fraction)

4.1.1. ppGpp-CC1 (soluble fraction)

Supporting table 3: putative ppGpp receptors captured by ppGpp-CC1 from the soluble fraction of E. coli cell lysate.

Nr.	ac	gene	protein	\log_2 (enrichment)	q-value
1	P0A6D7	<i>aroK</i>	Shikimate kinase 1	9,3	0,01
2	P33195	<i>gcvP</i>	Glycine dehydrogenase	9,1	0,04
3	P0AG76	<i>sbcD</i>	Nuclease SbcCD subunit D	8,9	0,03
4	P0AGE0	<i>ssb</i>	Single-stranded DNA-binding protein	8,0	0,02
5	P31808	<i>yciK</i>	Uncharacterized oxidoreductase	6,9	0,00
6	P24203	<i>yjiA</i>	P-loop guanosine triphosphatase	6,3	0,03
7	P0AAB6	<i>galF</i>	UTP--glucose-1-phosphate uridylyltransferase	6,1	0,02
8	P34209	<i>ydcF</i>	uncharacterized protein	5,6	0,04
9	P63389	<i>yheS</i>	Uncharacterized ABC transporter ATP- binding protein	5,5	0,00
10	P0A9D2	<i>gstA</i>	Glutathione S-transferase	4,6	0,00
11	P10121	<i>ftsY</i>	Signal recognition particle receptor	3,7	0,01
12	P06987	<i>hisB</i>	Histidine biosynthesis bifunctional protein	3,7	0,00
13	P0A993	<i>fbp</i>	Fructose-1,6-bisphosphatase class 1	3,6	0,01
14	P0ACA3	<i>sspA</i>	Stringent starvation protein A	3,5	0,02
15	P36566	<i>cmo</i> <i>M</i>	tRNA 5-carboxymethoxyuridine methyltransferase	3,4	0,00
16	P0A6K6	<i>deoB</i>	Phosphopentomutase	3,1	0,00
17	P00887	<i>aroH</i>	Phospho-2-dehydro-3-deoxyheptonate aldolase	3,0	0,01
18	P12281	<i>moe</i> <i>A</i>	Molybdopterin molybdenumtransferase	2,9	0,01
19	P0ADR8	<i>ppnN</i>	Pyrimidine/purine nucleotide 5'- monophosphate nucleosidase	2,9	0,00
20	P62768	<i>yaeH</i>	uncharacterized protein	2,8	0,00
21	P00962	<i>glnS</i>	Glutamine--tRNA ligase	2,8	0,01
22	P06999	<i>pfkB</i>	ATP-dependent 6-phosphofructokinase isozyme 2	2,8	0,00

23	P0AF20	<i>nagC</i>	N-acetylglucosamine repressor	2,7	0,01
24	P00350	<i>gnd</i>	6-phosphogluconate dehydrogenase, decarboxylating	2,6	0,02
25	P76594	<i>pka</i>	Protein lysine acetyltransferase	2,6	0,02
26	P0A7B8	<i>hslV</i>	ATP-dependent protease subunit	2,4	0,01
27	P32132	<i>typA</i>	GTP-binding protein TypA/BipA	2,4	0,02
28	P60906	<i>hisS</i>	Histidine--tRNA ligase	2,4	0,01
29	P0A9M5	<i>gpt</i>	Xanthine phosphoribosyltransferase	2,3	0,02
30	P0AEE5	<i>mglB</i>	D-galactose-binding periplasmic protein	2,3	0,01
31	P14081	<i>selB</i>	Selenocysteine-specific elongation factor	2,2	0,01
32	P08203	<i>araD</i>	L-ribulose-5-phosphate 4-epimerase	2,2	0,02
33	P0A850	<i>tig</i>	Trigger factor	2,2	0,02
34	P0AEP3	<i>galU</i>	UTP--glucose-1-phosphate uridylyltransferase	2,2	0,00
35	P0A6T5	<i>folE</i>	GTP cyclohydrolase 1	2,2	0,01
36	P0A6M8	<i>fusA</i>	Elongation factor G	2,1	0,01
37	P21165	<i>pepQ</i>	Xaa-Pro dipeptidase	2,1	0,01
38	P0A796	<i>pfkA</i>	ATP-dependent 6-phosphofructokinase isozyme 1	2,0	0,01
39	P0A7B5	<i>proB</i>	Glutamate 5-kinase	2,0	0,01
40	P17117	<i>nfsA</i>	Oxygen-insensitive NADPH nitroreductase	2,0	0,02
41	P0A7A5	<i>pcm</i>	Protein-L-isoaspartate O-methyltransferase	2,0	0,02



Supporting figure 4: graphic representation of proteins captured by ppGpp-CC1 from the *E. coli* soluble fraction. Only hits complying the threshold criteria are shown in the plot.

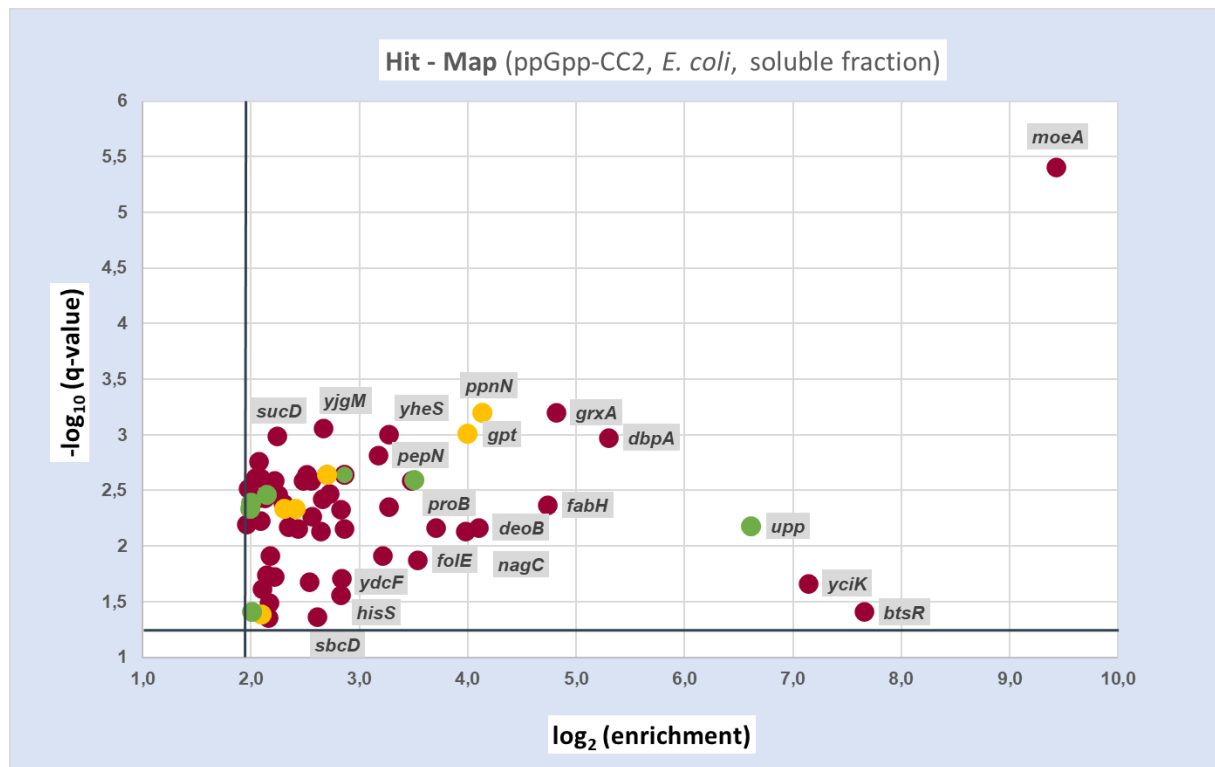
4.1.2. ppGpp-CC2 (soluble fraction)

Supporting table 4: putative ppGpp receptors captured by ppGpp-CC2 from the soluble fraction of *E. coli* cell lysate. An extract of this table was already shown in the main part of the manuscript.

Nr.	ac	gene	protein	log ₂ (enrichment)	q-value
1	P12281	<i>moeA</i>	Molybdopterin molybdenumtransferase	9,4	0,00
2	P0AFT5	<i>btsR</i>	Transcriptional regulatory protein	7,7	0,04
3	P31808	<i>yciK</i>	Uncharacterized oxidoreductase YciK	7,1	0,02
4	P0A8F0	<i>upp</i>	Uracil phosphoribosyltransferase	6,6	0,01
5	P21693	<i>dbpA</i>	ATP-dependent RNA helicase	5,3	0,00
6	P68688	<i>grxA</i>	Glutaredoxin 1	4,8	0,00
7	P0A6R0	<i>fabH</i>	3-oxoacyl-[acyl-carrier-protein] synthase 3	4,7	0,00
8	P0ADR8	<i>ppnN</i>	Pyrimidine/purine nucleotide 5'-monophosphate nucleosidase	4,1	0,00
9	P0A6K6	<i>deoB</i>	Phosphopentomutase	4,1	0,01
10	P0A9M5	<i>gpt</i>	Xanthine phosphoribosyltransferase	4,0	0,00
11	P0AF20	<i>nagC</i>	N-acetylglucosamine repressor	4,0	0,01
12	P0A7B5	<i>proB</i>	Glutamate 5-kinase	3,7	0,01
13	P0A6T5	<i>folE</i>	GTP cyclohydrolase 1	3,5	0,01

14	P76594	<i>pka</i>	Protein lysine acetyltransferase	3,5	0,00
15	P0A7D7	<i>purC</i>	Phosphoribosylaminoimidazole-succinocarboxamide synthase	3,5	0,00
16	P63389	<i>yheS</i>	Uncharacterized ABC transporter ATP-binding protein	3,3	0,00
17	P0AEE5	<i>mglB</i>	D-galactose-binding periplasmic protein	3,3	0,00
18	P30749	<i>moaE</i>	Molybdopterin synthase catalytic subunit	3,2	0,01
19	P04825	<i>pepN</i>	Aminopeptidase N	3,2	0,00
20	P00350	<i>gnd</i>	6-phosphogluconate dehydrogenase, decarboxylating	2,9	0,00
21	P38489	<i>nfsB</i>	Oxygen-insensitive NAD(P)H nitroreductase	2,9	0,01
22	P34209	<i>ydcF</i>	Uncharacterized protein	2,8	0,02
23	P60906	<i>hisS</i>	Histidine--tRNA ligase	2,8	0,03
24	P0AC59	<i>grxB</i>	Glutaredoxin 2	2,8	0,00
25	P0A761	<i>nanE</i>	Putative N-acetylmannosamine-6-phosphate 2-epimerase	2,7	0,00
26	P31120	<i>glmM</i>	Phosphoglucosamine mutase	2,7	0,00
27	P61949	<i>fldA</i>	Flavodoxin 1	2,7	0,00
28	P39337	<i>yjgM</i>	Uncharacterized N-acetyltransferase	2,7	0,00
29	P31060	<i>modF</i>	ABC transporter ATP-binding protein	2,7	0,00
30	P05637	<i>apaH</i>	Bis(5'-nucleosyl)-tetrakisphosphatase	2,6	0,01
31	P0AG76	<i>sbcD</i>	Nuclease	2,6	0,04
32	P00887	<i>aroH</i>	Phospho-2-dehydro-3-deoxyheptonate aldolase, Trp-sensitive	2,6	0,01
33	P0AEP3	<i>galU</i>	UTP--glucose-1-phosphate uridylyltransferase	2,6	0,00
34	P36566	<i>cmoM</i>	tRNA 5-carboxymethoxyuridine methyltransferase	2,5	0,02
35	P68206	<i>yjbJ</i>	Uncharacterized protein	2,5	0,00
36	P52061	<i>rdgB</i>	dITP/XTP pyrophosphatase	2,5	0,00
37	P0A870	<i>talB</i>	Transaldolase B	2,4	0,01
38	P14081	<i>selB</i>	Selenocysteine-specific elongation factor	2,4	0,00
39	P0A796	<i>pfkA</i>	ATP-dependent 6-phosphofructokinase	2,4	0,00
40	P0A7B8	<i>hslV</i>	ATP-dependent protease subunit	2,3	0,01
41	P25665	<i>metE</i>	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	2,3	0,00
42	P0AEZ9	<i>moaB</i>	Molybdenum cofactor biosynthesis protein B	2,3	0,00
43	P00934	<i>thrC</i>	Threonine synthase	2,2	0,00
44	P0AGE9	<i>sucD</i>	Succinate--CoA ligase subunit alpha	2,2	0,00
45	P07118	<i>valS</i>	Valine--tRNA ligase	2,2	0,02
46	P37330	<i>glcB</i>	Malate synthase G	2,2	0,00
47	P37744	<i>rfaA</i>	Glucose-1-phosphate thymidyltransferase	2,2	0,01
48	P0A6T3	<i>galK</i>	Galactokinase	2,2	0,03

49	P0A6N8	<i>yeiP</i>	Elongation factor P-like protein	2,2	0,04
50	P0A799	<i>pgk</i>	Phosphoglycerate kinase	2,1	0,00
51	P0A6M8	<i>fusA</i>	Elongation factor G	2,1	0,02
52	Q46829	<i>bglA</i>	6-phospho-beta-glucosidase	2,1	0,00
53	P0ACW6	<i>ydcH</i>	Uncharacterized protein	2,1	0,02
54	P0A9J0	<i>rng</i>	Ribonuclease G	2,1	0,04
55	P0A6P9	<i>eno</i>	Enolase	2,1	0,01
56	P08997	<i>aceB</i>	Malate synthase A	2,1	0,00
57	P0A7E5	<i>pyrG</i>	CTP synthase	2,1	0,00
58	P32132	<i>typA</i>	GTP-binding protein TypA/BipA	2,1	0,00
59	P06987	<i>hisB</i>	Histidine biosynthesis bifunctional protein	2,0	0,00
60	P69503	<i>apt</i>	Adenine phosphoribosyltransferase	2,0	0,04
61	P62768	<i>yaeH</i>	UPF0325 protein YaeH	2,0	0,00
62	P0A7D4	<i>purA</i>	Adenylosuccinate synthetase	2,0	0,00
63	P0AB89	<i>purB</i>	Adenylosuccinate lyase	2,0	0,00
64	P0AE12	<i>amn</i>	AMP nucleosidase	2,0	0,01

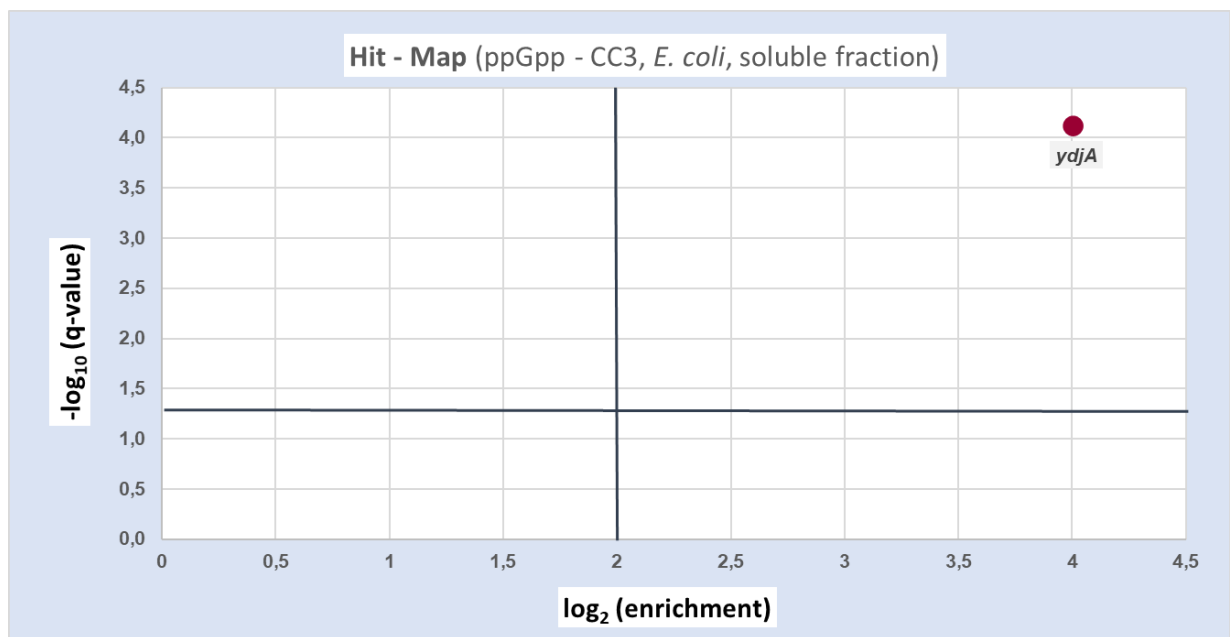


Supporting figure 5: graphic representation of proteins captured by ppGpp-CC2 from the *E. coli* soluble fraction. Only hits complying the threshold criteria are shown in the plot.

4.1.3. ppGpp-CC3 (soluble fraction)

Supporting table 5: putative ppGpp receptors captured by ppGpp-CC3 from the soluble fraction of *E. coli* cell lysate.

Nr.	ac	gene	protein	\log_2 (enrichment)	q-value
1	P0ACY1	<i>ydjA</i>	Putative NAD(P)H nitroreductase	4,0	0,00

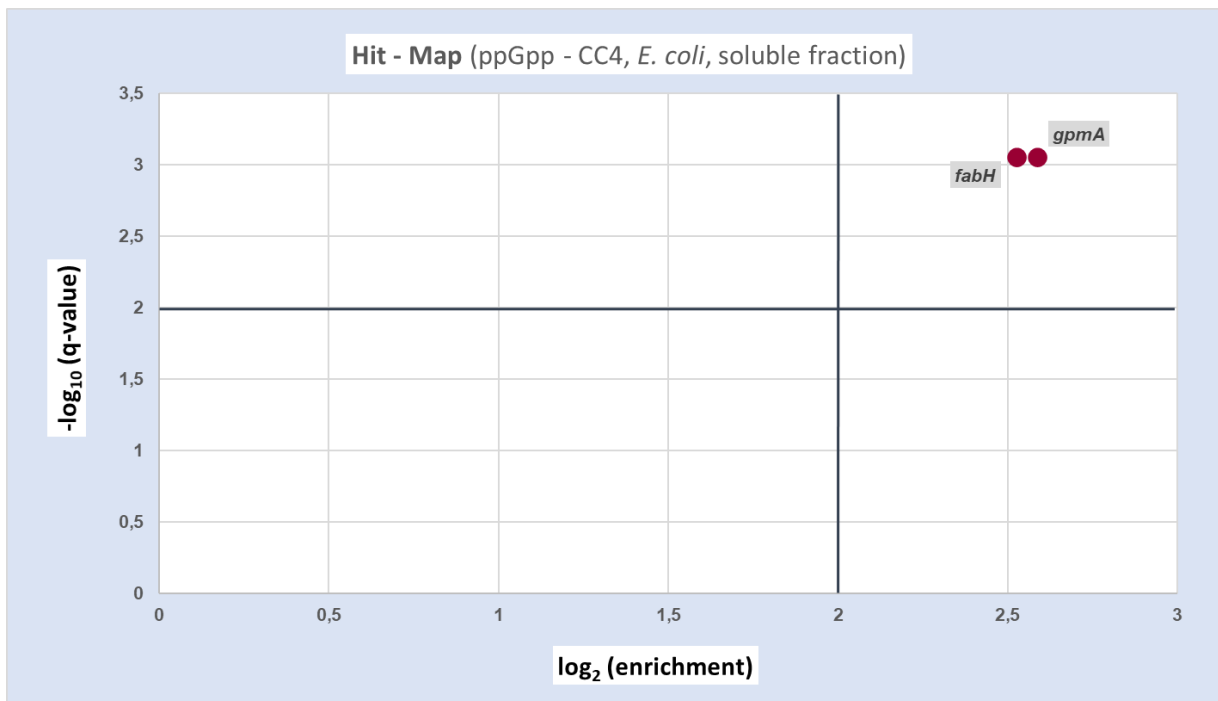


Supporting figure 6: graphic representation of proteins captured by ppGpp-CC3 from the *E. coli* soluble fraction. Only hits complying the threshold criteria are shown in the plot.

4.1.4. ppGpp-CC4 (soluble fraction)

Supporting table 6: putative ppGpp receptors captured by ppGpp-CC4 from the soluble fraction of *E. coli* cell lysate.

Nr.	ac	gene	protein	\log_2 (enrichment)	q-value
1	P62707	<i>gpmA</i>	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	2,6	0,00
2	P0A6R0	<i>fabH</i>	3-oxoacyl-[acyl-carrier-protein] synthase 3	2,5	0,00

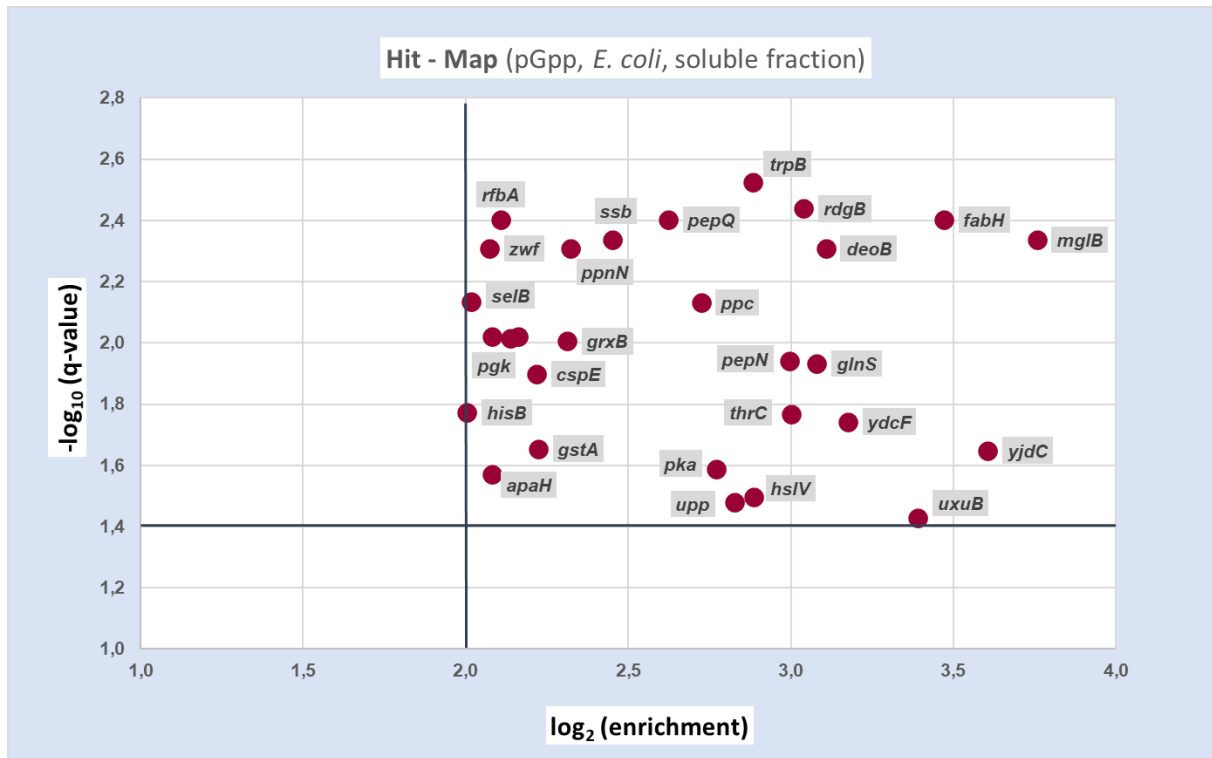


Supporting figure 7: graphic representation of proteins captured by ppGpp-CC4 from the *E. coli* soluble fraction. Only hits complying the threshold criteria are shown in the plot.

4.1.5. pGpp-CC (soluble fraction)

Supporting table 7: putative pGpp receptors captured by pGpp-CC from the soluble fraction of *E. coli* cell lysate.

Nr.	ac	gene	protein	log₂ (enrichment)	q-value
1	P0AEE5	<i>mgIB</i>	D-galactose-binding periplasmic protein	3,8	0,00
2	P0ACU7	<i>yjdC</i>	HTH-type transcriptional regulator	3,6	0,02
3	P0A6R0	<i>fabH</i>	3-oxoacyl-[acyl-carrier-protein] synthase 3	3,5	0,00
4	P39160	<i>uxuB</i>	D-mannonate oxidoreductase	3,4	0,04
5	P34209	<i>ydcF</i>	Uncharacterized protein	3,2	0,02
6	P0A6K6	<i>deoB</i>	Phosphopentomutase	3,1	0,00
7	P00962	<i>glnS</i>	Glutamine--tRNA ligase	3,1	0,01
8	P52061	<i>rdgB</i>	dITP/XTP pyrophosphatase	3,0	0,00
9	P00934	<i>thrC</i>	Threonine synthase	3,0	0,02
10	P04825	<i>pepN</i>	Aminopeptidase N	3,0	0,01
11	P0A7B8	<i>hslV</i>	ATP-dependent protease subunit	2,9	0,03
12	P0A879	<i>trpB</i>	Tryptophan synthase beta chain	2,9	0,00
13	P0A8F0	<i>upp</i>	Uracil phosphoribosyltransferase	2,8	0,03
14	P76594	<i>pka</i>	Protein lysine acetyltransferase	2,8	0,03
15	P00864	<i>ppc</i>	Phosphoenolpyruvate carboxylase	2,7	0,01
16	P21165	<i>pepQ</i>	Xaa-Pro dipeptidase	2,6	0,00
17	P0AGE0	<i>ssb</i>	Single-stranded DNA-binding protein	2,5	0,00
18	P0ADR8	<i>ppnN</i>	Pyrimidine/purine nucleotide 5'-monophosphate nucleosidase	2,3	0,00
19	P0AC59	<i>grxB</i>	Glutaredoxin	2,3	0,01
20	P0A9D2	<i>gstA</i>	Glutathione S-transferase GstA	2,2	0,02
21	P0A972	<i>cspE</i>	Cold shock-like protein CspE	2,2	0,01
22	P06999	<i>pfkB</i>	ATP-dependent 6-phosphofructokinase isozyme 2	2,2	0,01
23	P30749	<i>moaE</i>	Molybdopterin synthase catalytic subunit	2,1	0,01
24	P37744	<i>rfaA</i>	Glucose-1-phosphate thymidyltransferase 1	2,1	0,00
25	P0A799	<i>pgk</i>	Phosphoglycerate kinase	2,1	0,01
26	P05637	<i>apaH</i>	Bis(5'-nucleosyl)-tetraphosphatase	2,1	0,03
27	P0AC53	<i>zwf</i>	Glucose-6-phosphate 1-dehydrogenase	2,1	0,00
28	P14081	<i>selB</i>	Selenocysteine-specific elongation factor	2,0	0,01
29	P06987	<i>hisB</i>	Histidine biosynthesis bifunctional protein	2,0	0,02



Supporting figure 8: graphic representation of proteins captured by pGpp-CC from the *E. coli* soluble fraction. Only hits complying the threshold criteria are shown in the plot.

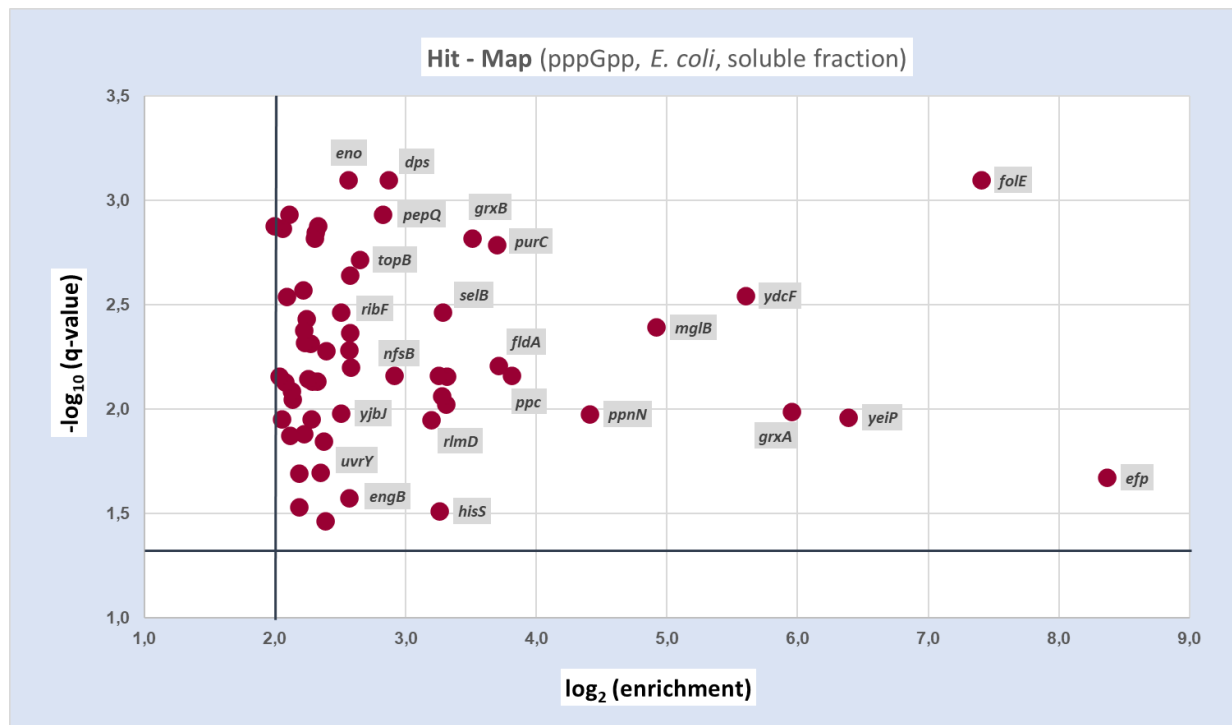
4.1.6. pppGpp-CC (soluble fraction)

Supporting table 8: putative pppGpp receptors captured by pppGpp-CC from the soluble fraction of *E. coli* cell lysate.

Nr.	ac	gene	protein	\log_2 (enrichment)	q-value
1	P0A6N4	<i>efp</i>	Elongation factor P	8,4	0,02
2	P0A6T5	<i>folE</i>	GTP cyclohydrolase 1	7,4	0,00
3	P0A6N8	<i>yeiP</i>	Elongation factor P-like protein	6,4	0,01
4	P68688	<i>grxA</i>	Glutaredoxin 1	6,0	0,01
5	P34209	<i>ydcF</i>	Protein YdcF	5,6	0,00
6	P0AEE5	<i>mgIB</i>	D-galactose-binding periplasmic protein	4,9	0,00
7	P0ADR8	<i>ppnN</i>	Pyrimidine/purine nucleotide 5'-monophosphate nucleosidase	4,4	0,01
8	P00864	<i>ppc</i>	Phosphoenolpyruvate carboxylase	3,8	0,01
9	P61949	<i>fldA</i>	Flavodoxin 1 OS=Escherichia coli	3,7	0,01
10	P0A7D7	<i>purC</i>	Phosphoribosylaminoimidazole-succinocarboxamide synthase	3,7	0,00
11	P0AC59	<i>grxB</i>	Glutaredoxin 2	3,5	0,00
12	P0A7B5	<i>proB</i>	Glutamate 5-kinase	3,3	0,01

13	P0A870	<i>talB</i>	Transaldolase B	3,3	0,01
14	P14081	<i>selB</i>	Selenocysteine-specific elongation factor	3,3	0,00
15	P37330	<i>glcB</i>	Malate synthase G	3,3	0,01
16	P60906	<i>hisS</i>	Histidine--tRNA ligase	3,3	0,03
17	P0A6R0	<i>fabH</i>	3-oxoacyl-[acyl-carrier-protein] synthase 3	3,3	0,01
18	P55135	<i>rlmD</i>	23S rRNA (uracil(1939)-C(5))-methyltransferase RlmD	3,2	0,01
19	P38489	<i>nfsB</i>	Oxygen-insensitive NAD(P)H nitroreductase	2,9	0,01
20	P0ABT2	<i>dps</i>	DNA protection during starvation protein	2,9	0,00
21	P21165	<i>pepQ</i>	Xaa-Pro dipeptidase	2,8	0,00
22	P14294	<i>topB</i>	DNA topoisomerase 3	2,6	0,00
23	P00962	<i>glnS</i>	Glutamine--tRNA ligase	2,6	0,01
24	P42641	<i>obgE</i>	GTPase ObgE/CgtA	2,6	0,00
25	P0A761	<i>nanE</i>	Putative N-acetylmannosamine-6-phosphate 2-epimerase	2,6	0,00
26	P0A6P7	<i>engB</i>	Probable GTP-binding protein	2,6	0,03
27	P07118	<i>valS</i>	Valine--tRNA ligase	2,6	0,01
28	P0A6P9	<i>eno</i>	Enolase	2,6	0,00
29	P68206	<i>yjbJ</i>	UPF0337 protein YjbJ	2,5	0,01
30	P0AG40	<i>ribF</i>	Bifunctional riboflavin kinase/FMN adenylyltransferase	2,5	0,00
31	P00934	<i>thrC</i>	Threonine synthase	2,4	0,01
32	P24203	<i>yjiA</i>	P-loop guanosine triphosphatase	2,4	0,03
33	P0A7B8	<i>hslV</i>	ATP-dependent protease subunit	2,4	0,01
34	P0AED5	<i>uvrY</i>	Response regulator UvrY	2,3	0,02
35	P77735	<i>yajO</i>	1-deoxyxylulose-5-phosphate synthase	2,3	0,00
36	P0AEH3	<i>elaA</i>	Protein ElaA	2,3	0,01
37	P0AFG8	<i>aceE</i>	Pyruvate dehydrogenase E1 component	2,3	0,00
38	P25665	<i>metE</i>	5-methyltetrahydropteroyltriglutamate – homocysteine methyltransferase	2,3	0,00
39	P31120	<i>glmM</i>	Phosphoglucosamine mutase	2,3	0,01
40	P05637	<i>apaH</i>	Bis(5'-nucleosyl)-tetrphosphatase	2,3	0,01
41	P21599	<i>pykA</i>	Pyruvate kinase II	2,3	0,00
42	P52061	<i>rdgB</i>	dITP/XTP pyrophosphatase	2,3	0,01
43	Q46920	<i>queF</i>	NADPH-dependent 7-cyano-7-deazaquinine reductase	2,2	0,00
44	P0A6F9	<i>groS</i>	10 kDa chaperonin	2,2	0,00
45	P76291	<i>cmoB</i>	tRNA U34 carboxymethyltransferase	2,2	0,00
46	P0A796	<i>pfkA</i>	ATP-dependent 6-phosphofructokinase isozyme 1	2,2	0,01
47	P0AE12	<i>amn</i>	AMP nucleosidase	2,2	0,00
48	P08956	<i>hsdR</i>	Type I restriction enzyme EcoKI R	2,2	0,03

49	P06987	<i>hisB</i>	Histidine biosynthesis bifunctional protein	2,2	0,02
50	P00350	<i>gnd</i>	6-phosphogluconate dehydrogenase, decarboxylating	2,1	0,01
51	P0AEZ9	<i>moaB</i>	Molybdenum cofactor biosynthesis protein B	2,1	0,01
52	P30850	<i>rnb</i>	Exoribonuclease 2	2,1	0,01
53	P62768	<i>yaeH</i>	UPF0325 protein YaeH	2,1	0,00
54	P0AB89	<i>purB</i>	Adenylosuccinate lyase	2,1	0,00
55	P0A6E4	<i>argG</i>	Argininosuccinate synthase	2,1	0,01
56	P0A805	<i>frr</i>	Ribosome-recycling factor	2,1	0,00
57	P77570	<i>anmK</i>	Anhydro-N-acetylmuramic acid kinase	2,1	0,01
58	P06612	<i>topA</i>	DNA topoisomerase 1	2,0	0,01
59	P0A7E5	<i>pyrG</i>	CTP synthase	2,0	0,00

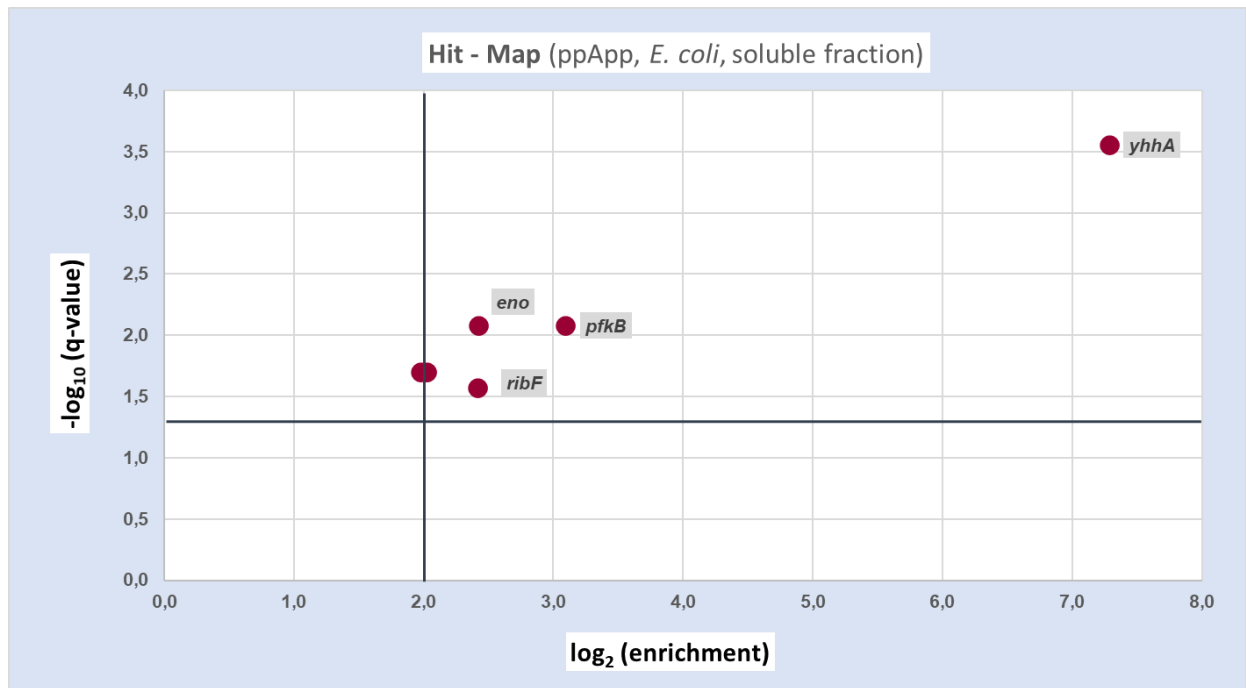


Supporting figure 9: graphic representation of proteins captured by pppGpp-CC from the *E. coli* soluble fraction. Only hits complying the threshold criteria are shown in the plot.

4.1.7. ppApp-CC (soluble fraction)

Supporting table 9: putative ppApp receptors captured by ppApp-CC from the soluble fraction of *E. coli* cell lysate.

Nr.	ac	gene	protein	log ₂ (enrichment)	q-value
1	P0ADX7	<i>yhhA</i>	Uncharacterized protein YhhA	7,3	0,00
2	P06999	<i>pfkB</i>	ATP-dependent 6-phosphofructokinase isozyme 2	3,1	0,01
3	P0A6P9	<i>eno</i>	Enolase	2,4	0,01
4	P0AG40	<i>ribF</i>	Bifunctional riboflavin kinase/FMN adenylyltransferase	2,4	0,03
5	P0ACY3	<i>yeaG</i>	Uncharacterized protein	2,0	0,02
6	P0A717	<i>prs</i>	Ribose-phosphate pyrophosphokinase	2,0	0,02

**Supporting figure 10:** graphic representation of proteins captured by ppApp-CC from the *E. coli* soluble fraction. Only hits complying the threshold criteria are shown in the plot.

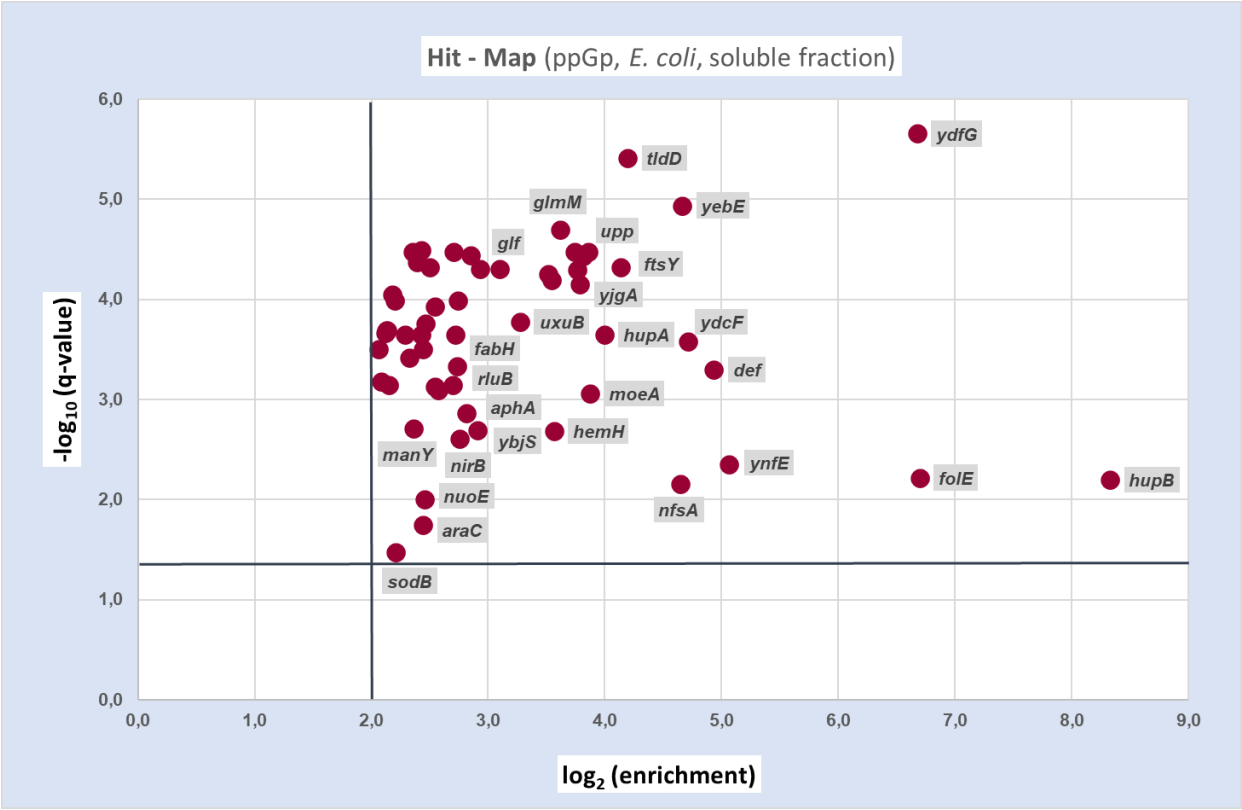
4.1.8. ppGp-CC (soluble fraction)

Supporting table 10: putative ppGp receptors captured by ppGp-CC from the soluble fraction of *E. coli* cell lysate.

Nr.	ac	gene	protein	log₂ (enrichment)	q-value
1	P0ACF4	<i>hupB</i>	DNA-binding protein HU-beta	8,3	0,01
2	P0A6T5	<i>folE</i>	GTP cyclohydrolase 1	6,7	0,01
3	P39831	<i>ydfG</i>	NADP-dependent 3-hydroxy acid dehydrogenase	6,7	0,00
4	P77374	<i>ynfE</i>	Putative dimethyl sulfoxide reductase chain	5,1	0,00
5	P0A6K3	<i>def</i>	Peptide deformylase	4,9	0,00
6	P34209	<i>ydcF</i>	Protein YdcF	4,7	0,00
7	P33218	<i>yebE</i>	Inner membrane protein YebE	4,7	0,00
8	P17117	<i>nfsA</i>	Oxygen-insensitive NADPH nitroreductase	4,6	0,00
9	P0AGG8	<i>tldD</i>	Metalloprotease TldD	4,2	0,00
10	P10121	<i>ftsY</i>	Signal recognition particle receptor	4,1	0,00
11	P0ACF0	<i>hupA</i>	DNA-binding protein	4,0	0,00
12	P12281	<i>moeA</i>	Molybdopterin molybdenumtransferase	3,9	0,00
13	P0A8F0	<i>upp</i>	Uracil phosphoribosyltransferase	3,9	0,00
14	P0AEE5	<i>mgIB</i>	D-galactose-binding periplasmic protein	3,8	0,00
15	P0A8X0	<i>yjgA</i>	UPF0307 protein YjgA	3,8	0,00
16	P0AFI7	<i>pdxH</i>	Pyridoxine/pyridoxamine 5'-phosphate oxidase	3,8	0,00
17	P38489	<i>nfsB</i>	Oxygen-insensitive NAD(P)H nitroreductase	3,7	0,00
18	P31120	<i>glmM</i>	Phosphoglucosamine mutase	3,6	0,00
19	P23871	<i>hemH</i>	Ferrochelatase	3,6	0,00
20	P75960	<i>cobB</i>	NAD-dependent protein deacylase	3,5	0,00
21	P14294	<i>topB</i>	DNA topoisomerase 3	3,5	0,00
22	P39160	<i>uxuB</i>	D-mannonate oxidoreductase	3,3	0,00
23	P37747	<i>glf</i>	UDP-galactopyranose mutase	3,1	0,00
24	P05055	<i>pnp</i>	Polyribonucleotide nucleotidyltransferase	2,9	0,00
25	P75821	<i>ybjS</i>	Uncharacterized protein YbjS	2,9	0,00
26	P26616	<i>maeA</i>	NAD-dependent malic enzyme	2,9	0,00
27	P0AE22	<i>aphA</i>	Class B acid phosphatase	2,8	0,00
28	P08201	<i>nirB</i>	Nitrite reductase (NADH) large subunit	2,8	0,00
29	P0AAG8	<i>mgIA</i>	Galactose/methyl galactoside import ATP-binding protein MglA	2,7	0,00
30	P0A6R0	<i>fabH</i>	3-oxoacyl-[acyl-carrier-protein] synthase 3	2,7	0,00

31	P0A761	<i>nanE</i>	Putative N-acetylmannosamine-6-phosphate 2-epimerase	2,7	0,00
32	P37744	<i>rfbA</i>	Glucose-1-phosphate thymidyltransferase 1	2,7	0,00
33	P37765	<i>rluB</i>	Ribosomal large subunit pseudouridine synthase B	2,7	0,00
34	P21363	<i>yciE</i>	Protein YciE	2,6	0,00
35	P03014	<i>pinE</i>	Serine recombinase PinE	2,5	0,00
36	P0A799	<i>pgk</i>	Phosphoglycerate kinase	2,5	0,00
37	P21177	<i>fadB</i>	Fatty acid oxidation complex subunit alpha	2,5	0,00
38	P0A993	<i>fbp</i>	Fructose-1,6-bisphosphatase class 1	2,5	0,00
39	P0AFD1	<i>nuoE</i>	NADH-quinone oxidoreductase subunit E	2,5	0,01
40	P0A9E0	<i>araC</i>	Arabinose operon regulatory protein	2,4	0,02
41	P08956	<i>hsdR</i>	Type I restriction enzyme EcoKI R protein	2,4	0,00
42	P0A6Z3	<i>htpG</i>	Chaperone protein HtpG	2,4	0,00
43	P0A9L3	<i>fkfB</i>	FKBP-type 22 kDa peptidyl-prolyl cis-trans isomerase	2,4	0,00
44	P0A9G6	<i>aceA</i>	Isocitrate lyase	2,4	0,00
45	P69801	<i>manY</i>	PTS system mannose-specific EIIC component	2,4	0,00
46	P0C0V0	<i>degP</i>	Periplasmic serine endoprotease DegP	2,4	0,00
47	P0A9B2	<i>gapA</i>	Glyceraldehyde-3-phosphate dehydrogenase A	2,3	0,00
48	P0A862	<i>tpx</i>	Thiol peroxidase	2,3	0,00
49	P0AGD3	<i>sodB</i>	Superoxide dismutase [Fe]	2,2	0,03
50	P0A8M6	<i>yeeX</i>	UPF0265 protein YeeX	2,2	0,00
51	P23830	<i>pssA</i>	CDP-diacylglycerol--serine O-phosphatidyltransferase	2,2	0,00
52	P31979	<i>nuoF</i>	NADH-quinone oxidoreductase subunit F	2,2	0,00
53	P64610	<i>yrbL</i>	Uncharacterized protein YrbL	2,1	0,00
54	P75913	<i>ghrA</i>	Glyoxylate/hydroxypyruvate reductase A	2,1	0,00
55	P0A7A5	<i>pcm</i>	Protein-L-isoaspartate O-methyltransferase	2,1	0,00
56	P76010	<i>ycgR</i>	Flagellar brake protein	2,1	0,00
57	P0AEZ9	<i>moaB</i>	Molybdenum cofactor biosynthesis protein B	2,1	0,00
58	P09372	<i>grpE</i>	HSP-70 cofactor	2,1	0,00
59	P10441	<i>lpxB</i>	Lipid-A-disaccharide synthase	2,1	0,00
60	P0A870	<i>talB</i>	Transaldolase B	2,1	0,00
61	P0AF26	<i>narJ</i>	Nitrate reductase molybdenum cofactor assembly chaperone	2,1	0,03
62	P0AC59	<i>grxB</i>	Glutaredoxin 2	2,0	0,00
63	P0AF24	<i>nagD</i>	Ribonucleotide monophosphatase NagD	2,0	0,00
64	P0A6H1	<i>clpX</i>	ATP-dependent Clp protease ATP-binding subunit	2,0	0,00
65	P0AC53	<i>zwf</i>	Glucose-6-phosphate 1-dehydrogenase	2,0	0,00

66	P11868	<i>tdcD</i>	Propionate kinase	2,0	0,00
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Supporting figure 11: graphic representation of proteins captured by ppGp-CC from the *E. coli* soluble fraction. Only hits complying the threshold criteria are shown in the plot.

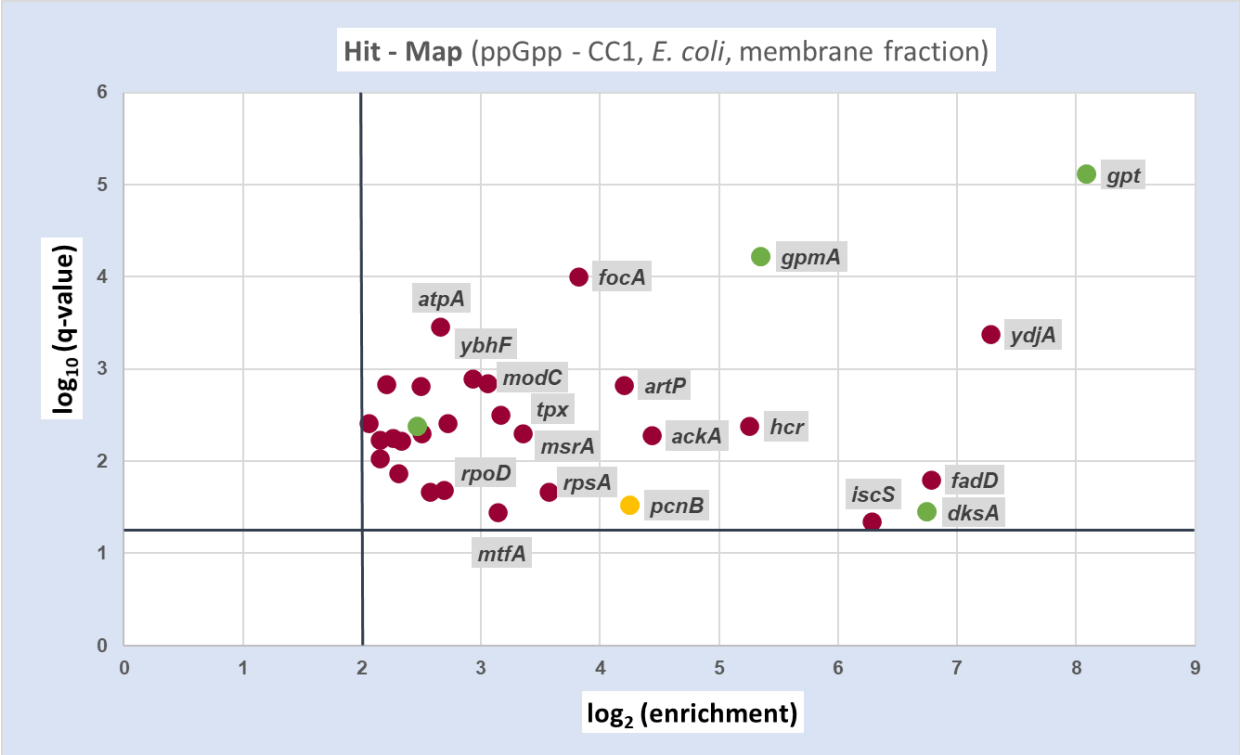
4.2. *E. coli* (membrane fraction)

4.2.1. ppGpp-CC1 (membrane fraction)

Supporting table 11: putative ppGpp receptors captured by ppGpp-CC1 from the membrane fraction of E. coli cell lysate.

Nr.	ac	gene	protein	log ₂ (enrichment)	q-value
1	P0A9M5	<i>gpt</i>	Xanthine phosphoribosyltransferase	8,1	0,00
2	P0ACY1	<i>ydjA</i>	Putative NAD(P)H nitroreductase	7,3	0,00
3	P69451	<i>fadD</i>	Long-chain-fatty-acid--CoA ligase	6,8	0,02
4	P0ABS1	<i>dksA</i>	RNA polymerase-binding transcription factor	6,7	0,04
5	P0A6B7	<i>iscS</i>	Cysteine desulfurase	6,3	0,04
6	P62707	<i>gpmA</i>	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	5,4	0,00
7	P75824	<i>hcr</i>	NADH oxidoreductase	5,3	0,00
8	P0A6A3	<i>ackA</i>	Acetate kinase	4,4	0,01
9	P0ABF1	<i>pcnB</i>	Poly(A) polymerase I	4,3	0,03
10	P0AAF6	<i>artP</i>	Arginine transport ATP-binding protein	4,2	0,00
11	P0AC23	<i>focA</i>	Probable formate transporter 1	3,8	0,00
12	P0AG67	<i>rpsA</i>	30S ribosomal protein S1	3,6	0,02
13	P0A744	<i>msrA</i>	Peptide methionine sulfoxide reductase	3,3	0,01
14	P0A862	<i>tpx</i>	Thiol peroxidase	3,2	0,00
15	P76346	<i>mtfA</i>	uncharacterized protein	3,1	0,04
16	P09833	<i>modC</i>	Molybdenum import ATP-binding protein	3,1	0,00
17	P0A9U1	<i>ybhF</i>	probable multidrug ABC transporter ATP-binding protein	2,9	0,00
18	P23871	<i>hemH</i>	Ferrochelatase	2,7	0,00
19	P00579	<i>rpoD</i>	RNA polymerase sigma factor	2,7	0,02
20	P0ABB0	<i>atpA</i>	ATP synthase subunit alpha	2,7	0,00
21	P0A6K6	<i>deoB</i>	Phosphopentomutase	2,6	0,02
22	P0ABT2	<i>dps</i>	DNA protection during starvation protein	2,5	0,01
23	P64604	<i>mlaD</i>	Intermembrane phospholipid transport system binding protein	2,5	0,00
24	P08200	<i>icd</i>	Isocitrate dehydrogenase	2,5	0,00
25	P0ADU5	<i>ygiW</i>	uncharacterized protein	2,3	0,01
26	P63386	<i>mlaF</i>	Intermembrane phospholipid transport system ATP-binding protein	2,3	0,01
27	P37624	<i>rbbA</i>	Ribosome-associated ATPase	2,3	0,01
28	P0AE52	<i>bcp</i>	Peroxiredoxin	2,2	0,00

29	P0AAG8	<i>mgIA</i>	Galactose/methyl galactoside import ATP-binding protein	2,2	0,01
30	P21645	<i>lpxD</i>	UDP-3-O-(3-hydroxymyristoyl)glucosamine N-acyltransferase	2,1	0,01
31	P37330	<i>glcB</i>	Malate synthase G	2,1	0,00

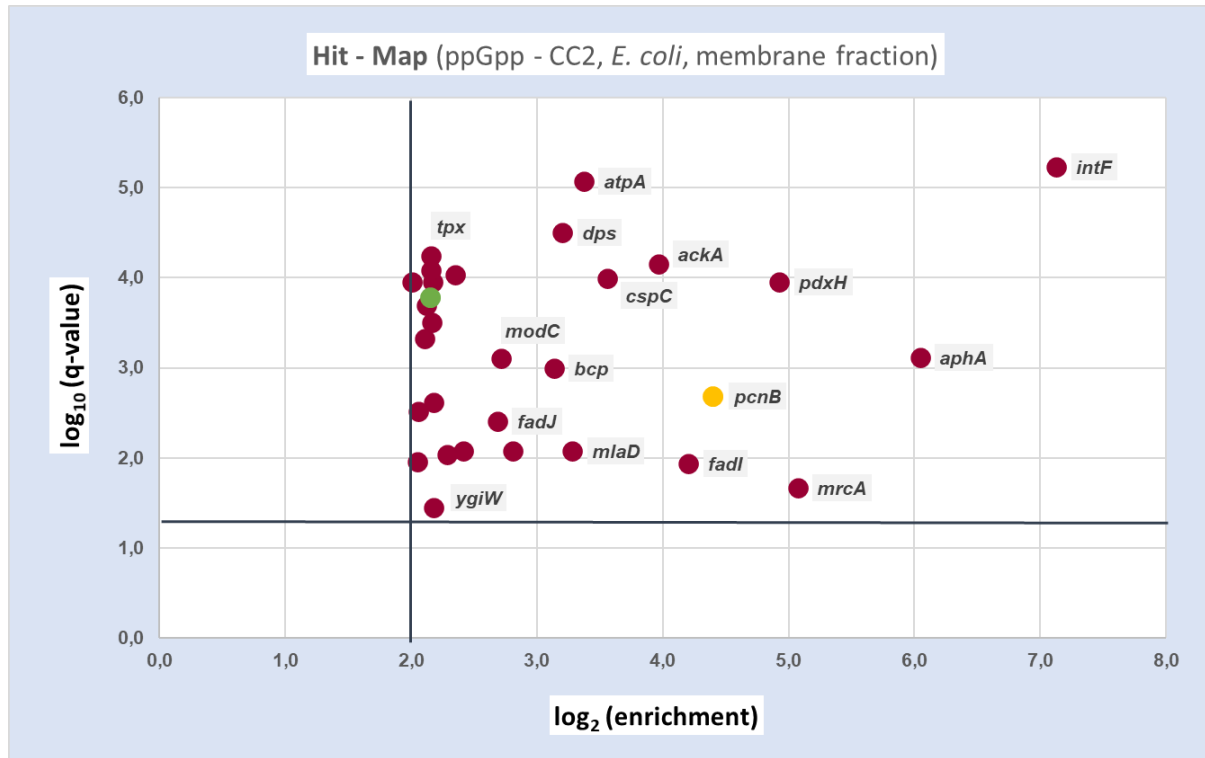


Supporting figure 12: graphic representation of proteins captured by ppGpp-CC1 from the *E. coli* membrane fraction. Only hits complying the threshold criteria are shown in the plot.

4.2.2. ppGpp-CC2 (membrane fraction)

Supporting table 12: putative ppGpp receptors captured by ppGpp-CC2 from the membrane fraction of *E. coli* cell lysate. An extract of this table was already shown in the main part of the manuscript.

Nr.	ac	gene	protein	log ₂ (enrichment)	q-value
1	P71298	<i>intF</i>	Prophage integrase	7,1	0,00
2	P0AE22	<i>aphA</i>	Class B acid phosphatase	6,0	0,00
3	P02918	<i>mrcA</i>	Penicillin-binding protein 1A	5,1	0,02
4	P0AFI7	<i>pdxH</i>	Pyridoxine/pyridoxamine 5'-phosphate oxidase	4,9	0,00
5	P0ABF1	<i>pcnB</i>	Poly(A) polymerase I	4,4	0,00
6	P76503	<i>fadI</i>	3-ketoacyl-CoA thiolase	4,2	0,01
7	P0A6A3	<i>ackA</i>	Acetate kinase	4,0	0,00
8	P0A9Y6	<i>cspC</i>	Cold shock-like protein	3,6	0,00
9	P0ABB0	<i>atpA</i>	ATP synthase subunit alpha	3,4	0,00
10	P64604	<i>mldD</i>	Intermembrane phospholipid transport system binding protein	3,3	0,01
11	P0ABT2	<i>dps</i>	DNA protection during starvation protein	3,2	0,00
12	P0AE52	<i>bcp</i>	Peroxiredoxin	3,1	0,00
13	P37692	<i>rfaF</i>	ADP-heptose--LPS heptosyltransferase 2	2,8	0,01
14	P09833	<i>modC</i>	Molybdenum import ATP-binding protein	2,7	0,00
15	P77399	<i>fadJ</i>	Fatty acid oxidation complex subunit alpha	2,7	0,00
16	P77304	<i>dtpA</i>	Dipeptide and tripeptide permease A	2,4	0,01
17	P0A6M8	<i>fusA</i>	Elongation factor G	2,3	0,00
18	P63386	<i>mldF</i>	Intermembrane phospholipid transport system ATP-binding protein	2,3	0,01
19	P0ADU5	<i>ygiW</i>	uncharacterized protein	2,2	0,04
20	P23173	<i>tnaB</i>	Low affinity tryptophan permease	2,2	0,00
21	P0AFP4	<i>ybbO</i>	Uncharacterized oxidoreductase	2,2	0,00
22	P62517	<i>mdoH</i>	Glucans biosynthesis glucosyltransferase H	2,2	0,00
23	P11557	<i>damX</i>	Cell division protein	2,2	0,00
24	P0A862	<i>tpx</i>	Thiol peroxidase	2,2	0,00
25	P37765	<i>rluB</i>	Ribosomal large subunit pseudouridine synthase B	2,2	0,00
26	P08200	<i>icd</i>	Isocitrate dehydrogenase [NADP]	2,1	0,00
27	P11880	<i>murF</i>	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	2,1	0,00
28	P27836	<i>wecG</i>	UDP-N-acetyl-D-mannosaminuronic acid transferase	2,1	0,00
29	P45537	<i>yhfK</i>	uncharacterized protein YhfK	2,0	0,01
30	P0A972	<i>cspE</i>	cold shock-like protein	2,0	0,00



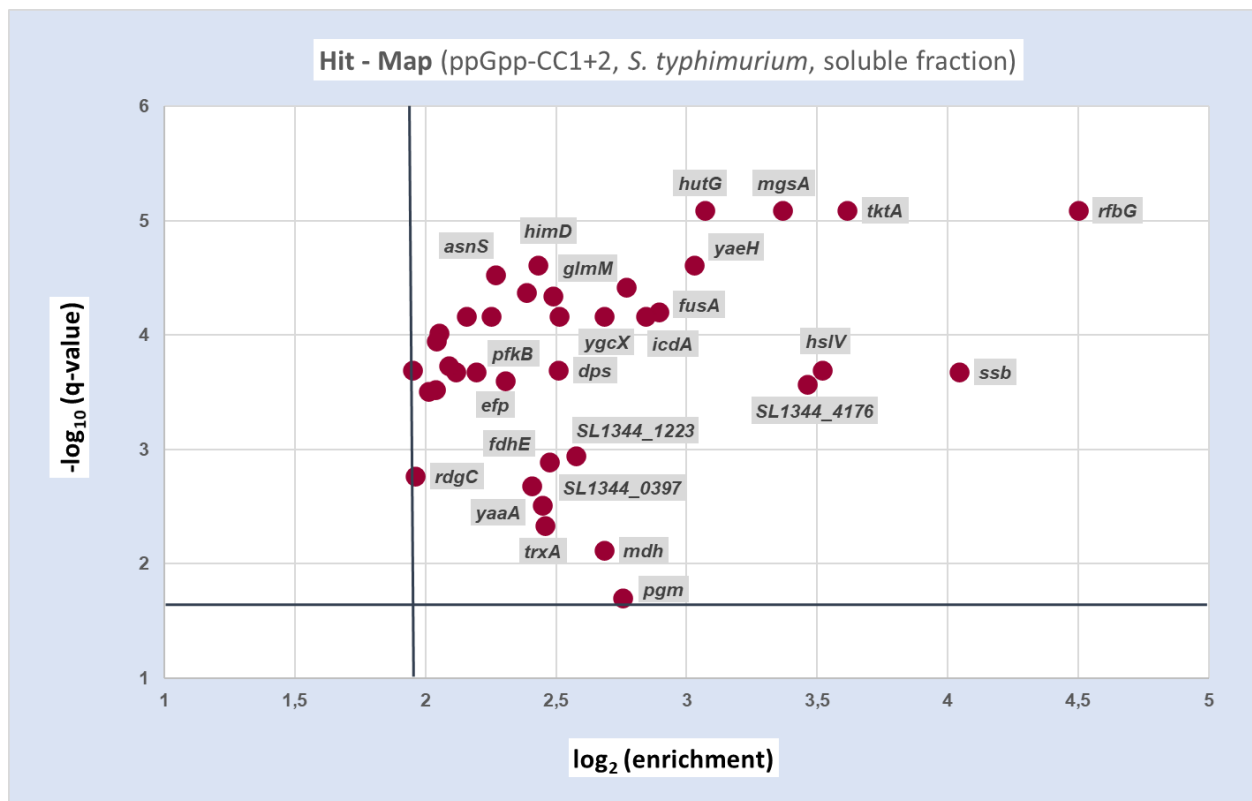
Supporting figure 13: graphic representation of proteins captured by ppGpp-CC2 from the *E. coli* membrane fraction. Only hits complying the threshold criteria are shown in the plot.

4.3. *S. typhimurium* (soluble fraction)

Supporting table 13: putative ppGpp receptors captured by a mixture of ppGpp-CC1 and ppGpp-CC2 from the soluble fraction of *S. typhimurium* cell lysate.

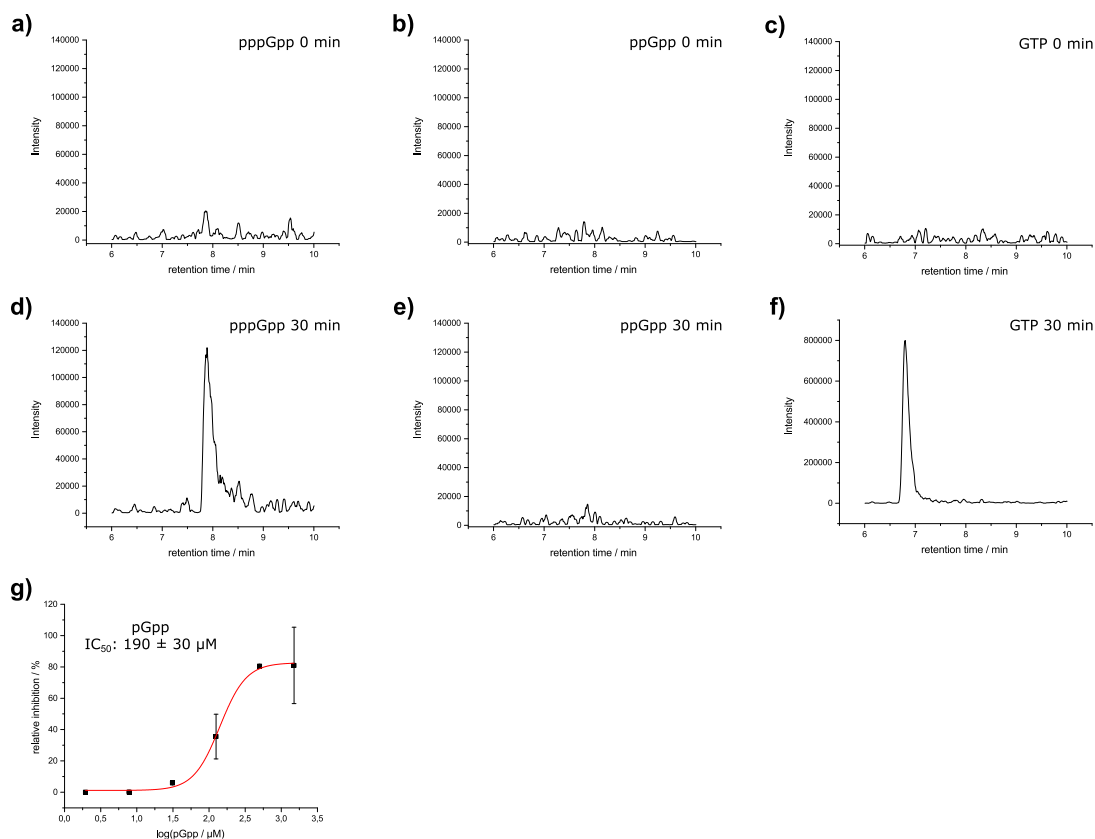
Nr.	ac	gene	protein	log ₂ (enrichment)	q-value
1	E1WH24	<i>rfbG</i>	CDP-glucose 4,6-dehydratase	4,5	0,00
2	E1WEU6	<i>ssb</i>	Single-stranded DNA-binding protein	4,0	0,00
3	E1WHL6	<i>tktA</i>	Transketolase	3,6	0,00
4	E1WEE7	<i>hslV</i>	ATP-dependent protease subunit	3,5	0,00
5	E1WET0	<i>SL1344_4176</i>	Putative uncharacterized protein	3,5	0,00
6	E1W7A8	<i>mgsA</i>	Methylglyoxal synthase	3,4	0,00
7	E1W9S6	<i>hutG</i>	Formimidoylglutamate	3,1	0,00
8	E1W883	<i>yaeH</i>	UPF0325 protein yaeH	3,0	0,00
9	E1WIM3	<i>fusA</i>	Elongation factor G	2,9	0,00
10	E1WFA8	<i>icdA</i>	Isocitrate dehydrogenase [NADP]	2,8	0,00
11	E1WBB1	<i>SL1344_4508</i>	Conserved hypothetical ABC transporter	2,8	0,00
12	E1W9J2	<i>pgm</i>	Phosphoglucomutase	2,8	0,02
13	E1WAK6	<i>ygcX</i>	Probable glucarate dehydratase 1	2,7	0,00
14	E1WIE2	<i>mdh</i>	Malate dehydrogenase OS=Salmonella typhimurium (strain SL1344) GN=mdh	2,7	0,01
15	E1WFF4	<i>SL1344_1223</i>	Putative oxidoreductase	2,6	0,00
16	E1WFF1	<i>SL1344_1220</i>	Putative uncharacterized protein	2,5	0,00
17	E1W9W7	<i>dps</i>	DNA protection during starvation protein	2,5	0,00
18	E1WI76	<i>glmM</i>	Phosphoglucosamine mutase	2,5	0,00
19	E1WE88	<i>fdhE</i>	Protein FdhE	2,5	0,00
20	E1WDY2	<i>trxA</i>	Thioredoxin	2,5	0,00
21	E1W7M8	<i>yaaA</i>	UPF0246 protein yaaA	2,4	0,00
22	E1W713	<i>himD</i>	Integration host factor subunit beta	2,4	0,00
23	E1W8R3	<i>SL1344_0397</i>	Probable peroxidase	2,4	0,00
24	E1WF16	<i>aspA</i>	Aspartate ammonia-lyase	2,4	0,00
25	E1WF24	<i>efp</i>	Elongation factor P	2,3	0,00
26	E1W731	<i>asnS</i>	Asparaginyl-tRNA synthetase	2,3	0,00
27	E1W9M3	<i>gltA</i>	Citrate synthase	2,3	0,00
28	E1WFJ3	<i>pfkB</i>	6-phosphofructokinase isozyme	2,2	0,00
29	E1WHK9	<i>pgk</i>	Phosphoglycerate kinase	2,2	0,00

30	E1WFJ1	SL1344_1259	Putative uncharacterized protein	2,1	0,00
31	E1WGW6	<i>cbiC</i>	Precorrin-8X methylmutase	2,1	0,00
32	E1WHI0	<i>lysS</i>	Lysyl-tRNA synthetase	2,1	0,00
33	E1WHV3	<i>dkgA</i>	2,5-diketo-D-gluconic acid reductase A	2,0	0,00
34	E1WCL8	<i>fabB</i>	3-oxoacyl-[acyl-carrier-protein] synthase I	2,0	0,00
35	E1WAJ7	<i>pyrG</i>	CTP synthase	2,0	0,00
36	E1W8Q3	<i>rdgC</i>	Recombination-associated protein rdgC	2,0	0,00
37	E1W7J4	<i>grxB</i>	Glutaredoxin 2	2,0	0,00



Supporting figure 14: graphic representation of proteins captured by a mixture of ppGpp-CC1 and ppGpp-CC2 from the *S. typhimurium* soluble fraction. Only hits complying the threshold criteria are shown in the plot.

5. Target validation: Bis(5'-nucleosyl)-tetraphosphatase ApaH is regulated by Magic Spot Nucleotides in vitro



Supporting Figure 15: Biochemical characterization of MSN target ApaH: **a) and d)**: extracted ion chromatogram for 522 m/z (triphosphorylated guanosine) of ApaH treated pppGpp for given reaction time. **b) and e)**: extracted ion chromatogram for 442 m/z (diphosphorylated guanosine) of ApaH treated ppGpp for given reaction time. **c) and f)**: extracted ion chromatogram for 362 m/z (monophosphorylated guanosine) of ApaH treated GTP for given reaction time. Representative chromatogram of three experiments. **g)** representative IC_{50} curve of pGpp on diadenosine tetraphosphate hydrolysis by ApaH. $n=2$. Experiments were independently repeated with $n=2$. Error bars indicate standard deviation. Insert: average and standard deviation of independent experiments.

5.1. ApaH in vitro assay

The protocol was derived from Guranowski et al.^[8] In brief, for IC_{50} experiments, 20 μl reaction buffer (62.5 mM Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] pH 7.5, 150 μM $CoCl_2$, 1.25 mM 2-mercaptoethanol), 3 μl 243 μM diadenosine tetraphosphate (Ap4A) and 8 μl H_2O or inhibitor dissolved in H_2O were mixed. 2 μl ApaH solution (4.3 $\mu g/ml$ in 10% glycerol, 5 mM 2-mercaptoethanol, 2 mg/ml bovine serum albumin) were added and the reaction mixture were incubated for 60 s at 37 $^{\circ}C$ and 600 rpm on a thermocycler. For degradation experiments using other nucleotides than Ap4A, 6 μl of 304 μM substrate were used with 125 μM $CoCl_2$ in the reaction buffer and incubated for 30 min. The reaction was quenched by addition of 3 μl 3 mM alkalized ethylenediaminetetraacetic acid solution.

5.2. LC/MS analysis of nucleotides

30 μ l acetonitrile was added to the quenched reaction mixture and after vortexing, the samples were centrifuged for 15 min at 4 °C and 20,000 g. The supernatants were transferred to a vial with glass insert.

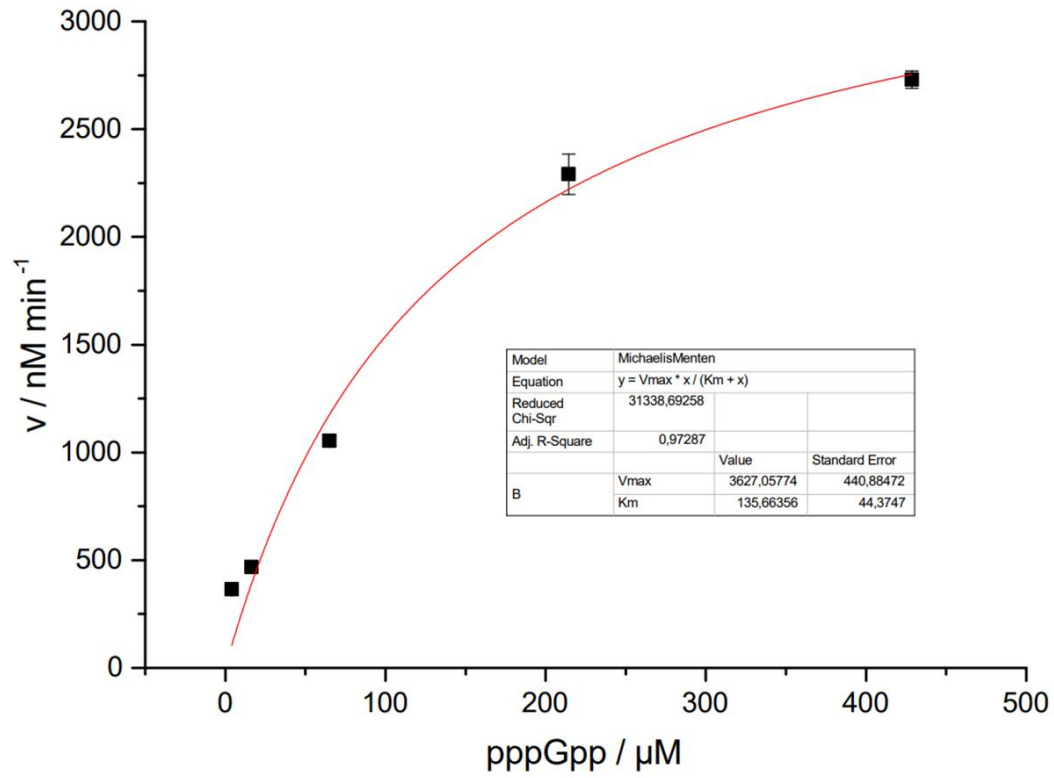
4 μ l of the samples were injected on a Hilic-Z, PEEK-lined column (Agilent technologies, 100 x 2.1 mm) operated at 35 °C. The gradient was: 90 % B (acetonitrile) for 0.5 min, to 40 % B within 11.5 min, hold for 2 min, to 90 % B within 0.5 min, hold for 5.5 min. Solvent A was 20 mM ammonium acetate pH 9. The flow rate was set to 0.4 ml/min. The LC was coupled with a QqQ-MS (Agilent Technologies: G4220A, G4226A, G1316A, G6460A) with the following parameters: Gas temperature 310 °C at 10.7 L/min, sheath gas temperature 310 °C with 10 L/min flow, nebulizer pressure of 30 psi, capillary voltage of 4 kV in negative mode, delta EMV of 400 and 2 kV nozzle voltage. Data were acquired by Agilent MassHunter Data Acquisition (version B.08.02) and analyzed with Agilent MassHunter Qualitative Analysis (version B.07.00, SP1).

5.3. IC₅₀ - determination

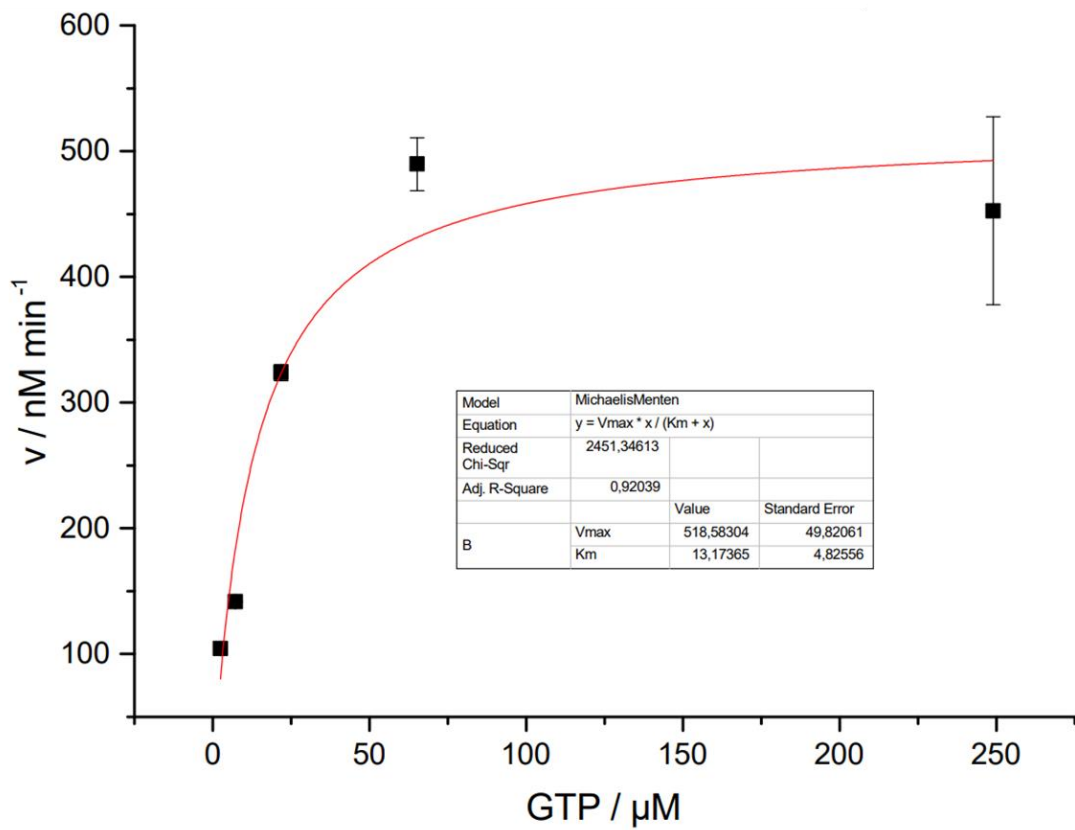
Each IC₅₀ assay was performed with two reactions per condition and reproduced at least once (total n=4). Relative intensity of Ap4A to EDTA treated reactions were determined and the average of each condition was plotted against the log₁₀ concentration of the inhibitor. OriginPro 2017 was used to fit the sigmoidal dose/response curves. The derived functions were used to determine the absolute IC₅₀ values. Averaged IC₅₀ values are given with corresponding standard deviation.

5.4. Determination of kinetic parameters (K_M and K_{cat})

For K_M and k_{cat} determination, 6 μ l of GTP- or pppGpp-solutions were added at different concentrations to 20 μ l reaction buffer (62.5 mM Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] pH 7.5, 125 μ M CoCl₂, 1.25 mM 2-mercaptoethanol) and 2 μ l ApaH. The reaction was incubated at 37 °C and 650 rpm and terminated by addition of EDTA after 30 min. Product concentration was determined by multiple reaction monitoring LC/MS using a standard curve. K_M and v_{max} was determined by Origin 9 using the Michaelis-Menten function. K_{cat} was calculated by dividing v_{max} by the enzyme concentration (9.8 nM).



Supporting Figure 16: Michaelis-Menten model for ApaH-catalyzed hydrolysis of pppGpp towards pGpp.



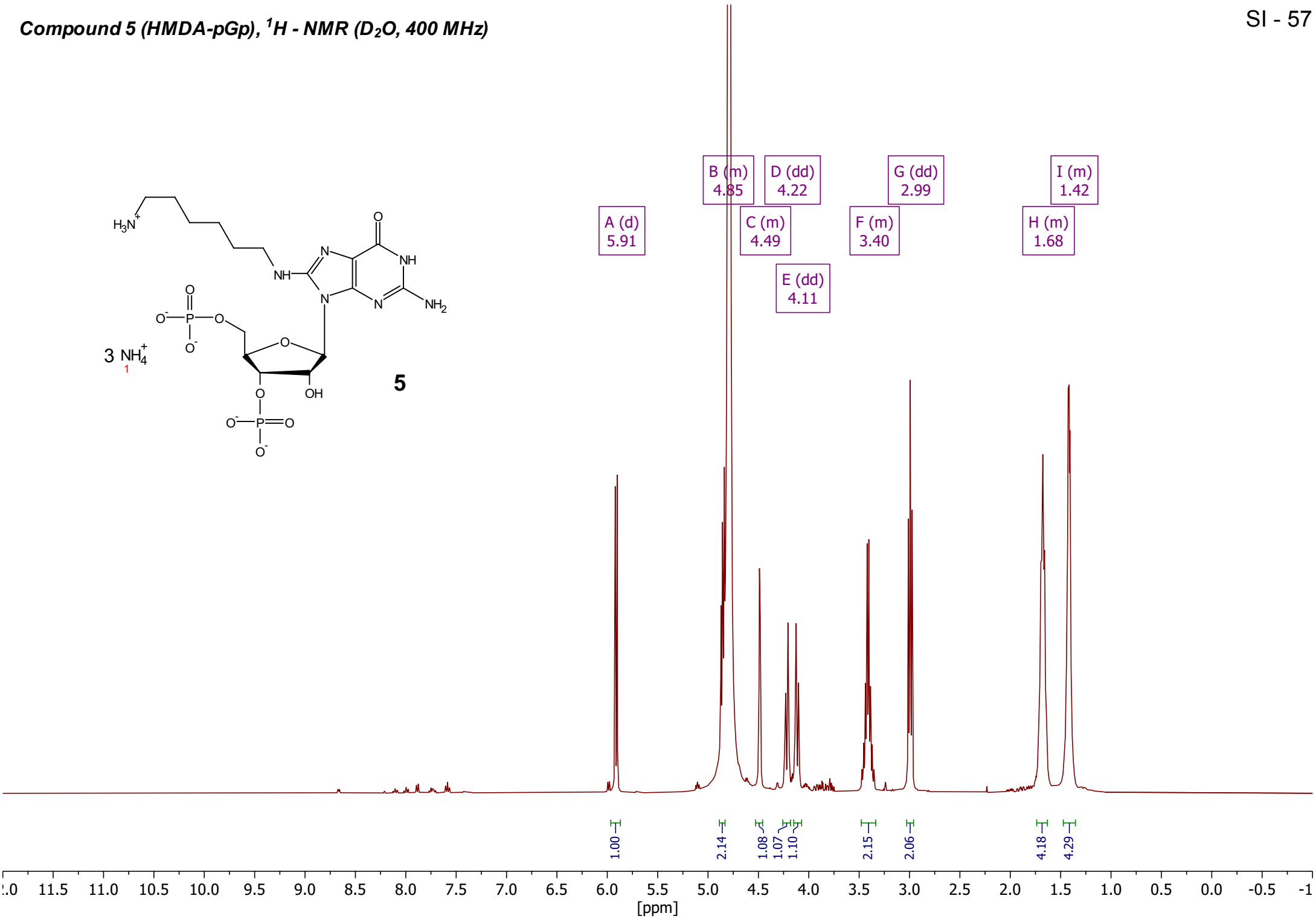
Supporting Figure 17: Michaelis-Menten Fit for ApaH-catalyzed hydrolysis of GTP towards GMP.

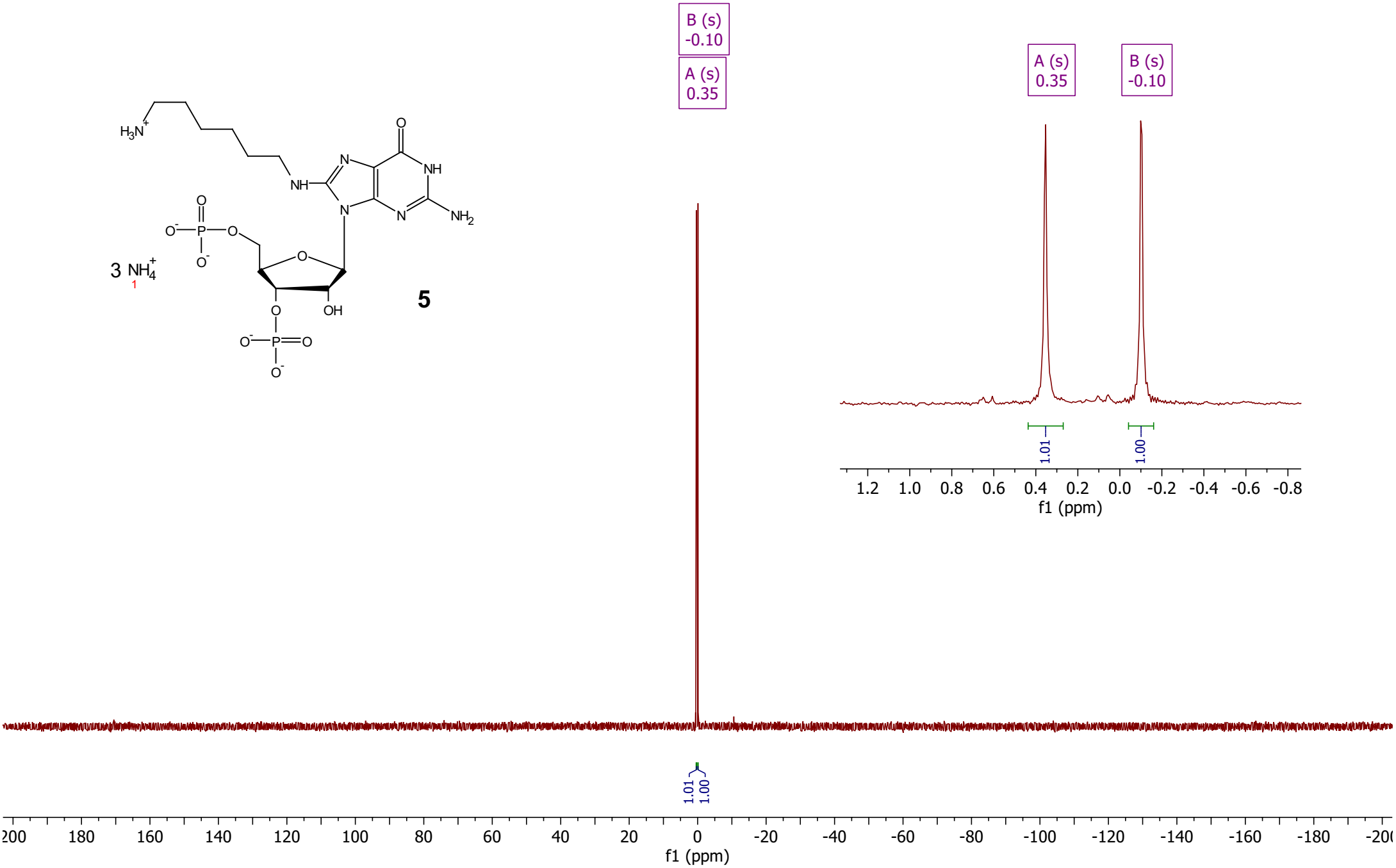
6. Supporting references

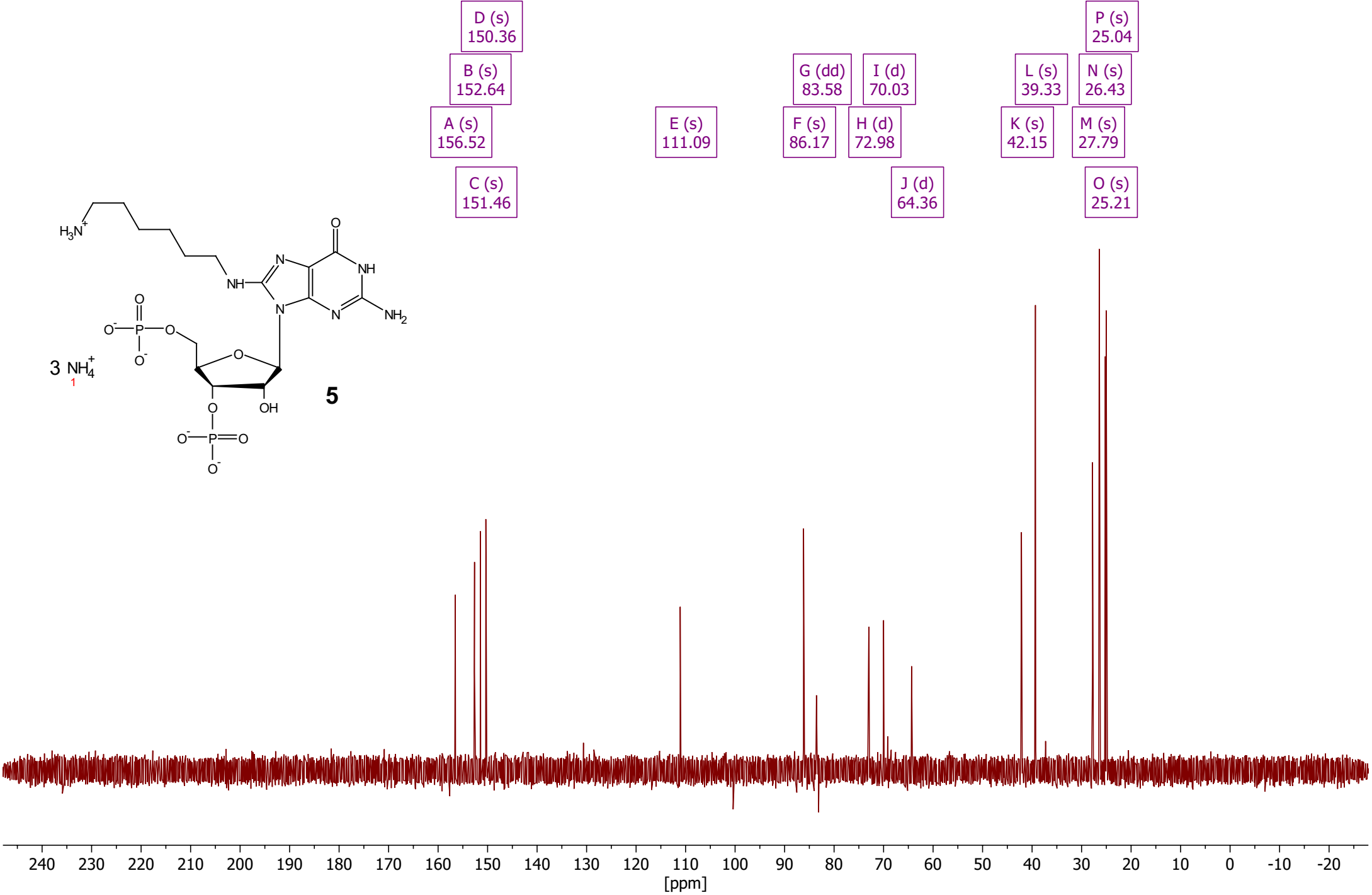
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- [1] L. Bialy, H. Waldmann, *Angew. Chem.* **2002**, *114*, 1819 – 1822; *Angew. Chem. Int. Ed.* **2002**, *41*, 1748 – 1751.
- [2] A. Hofer, G. S. Cremonik, A. C. Müller, R. Giambruno, C. Trefzer, G. Superti-Furga, K. L. Bennett, H. J. Jessen, *Chem. Eur. J.* **2015**, *21*, 10116–10122.
- [3] T. M. Haas, P. Ebensperger, V. B. Eisenbeis, C. Nopper, T. Dürr, N. Jork, N. Steck, C. Jessen-Trefzer, H. J. Jessen, *Chem. Commun.* **2019**, *55*, 5339-5342.
- [4] B.-J. Laventie, T. Glatter, U. Jenal, *Methods Mol. Biol.* **2017**, *1657*, 361.376.
- [5] B.-J. Laventie, J. Nesper, E. Ahrné, T. Glatter, A. Schmidt, U. Jenal, *J. Vis. Exp.* **H. 97**, e51404.
- [6] W. Steinchen, V. Zegarra, G. Bange, *Front. Microbiol.* **2020**, *11*, 2072.
- [7] B. Wang, P. Dai, D. Ding, A. Del Rosario, R. A. Grant, B. L. Pentelute, M. T. Laub, *Nat. Chem. Biol.* **2019**, *15*, 141-150.
- [8] A. Guranowski, H. Jakubowski, E. Holler, *J. Biol. Chem.* **1983**, *258*, 14784–1478.

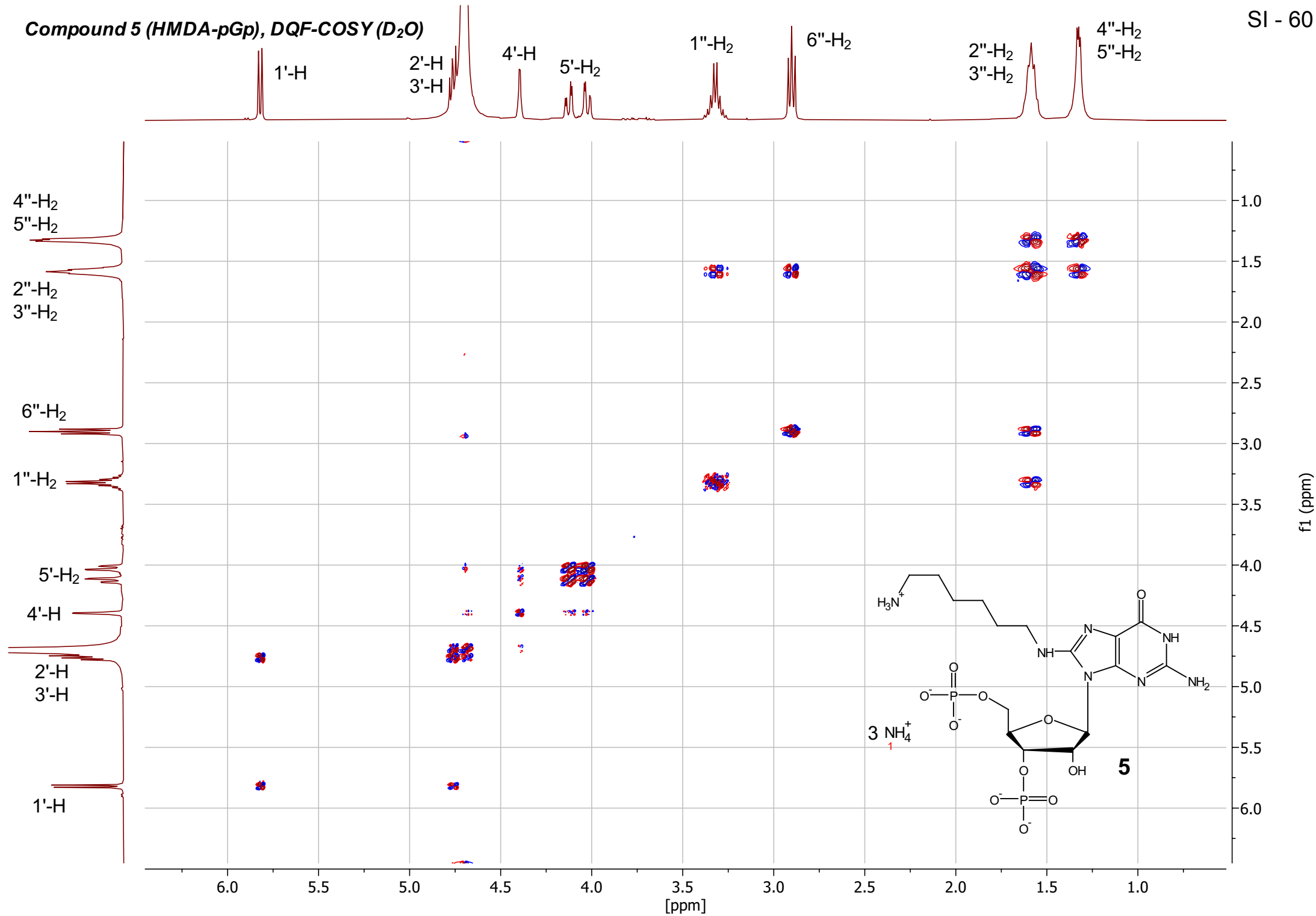
7. NMR - spectra

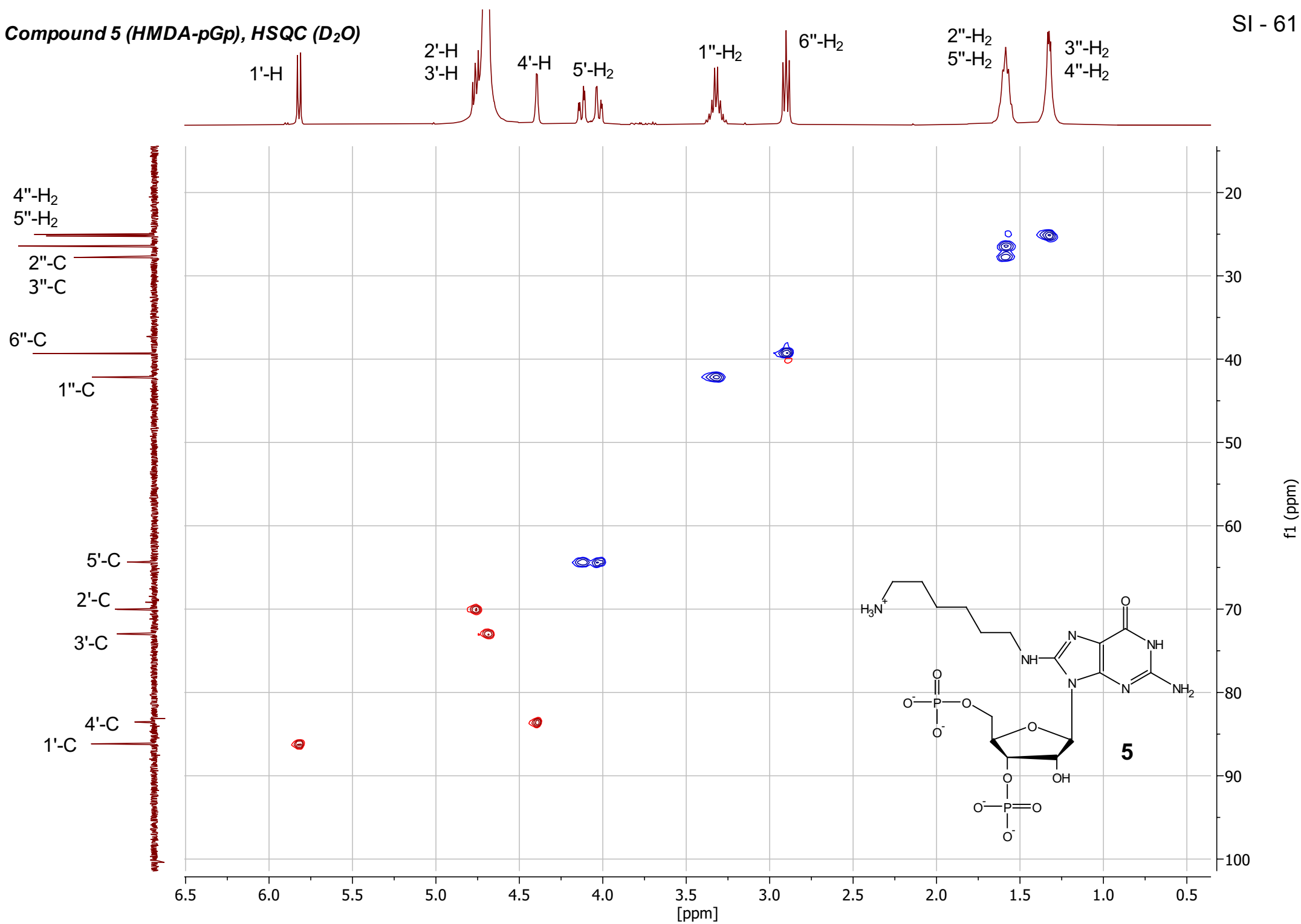
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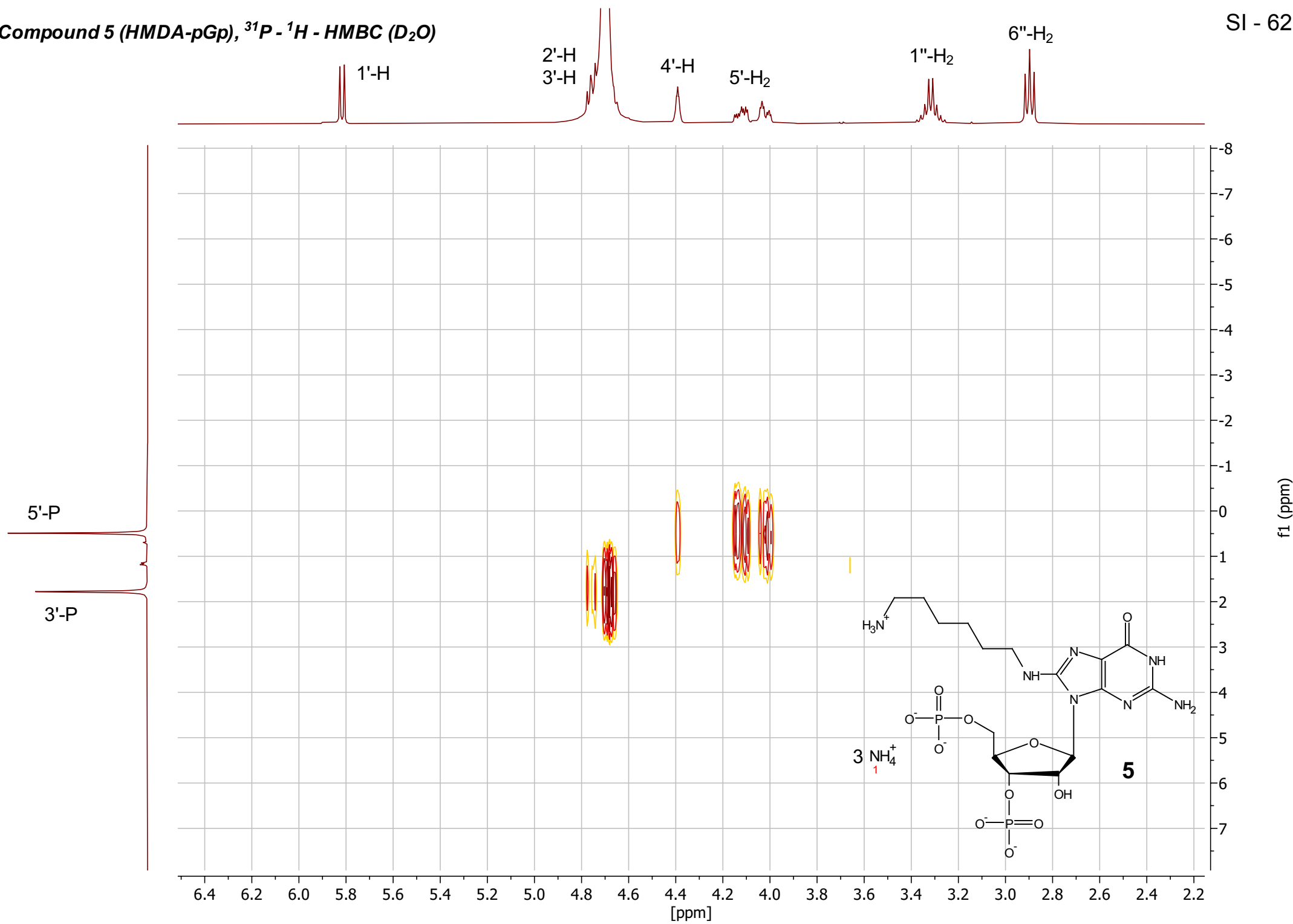


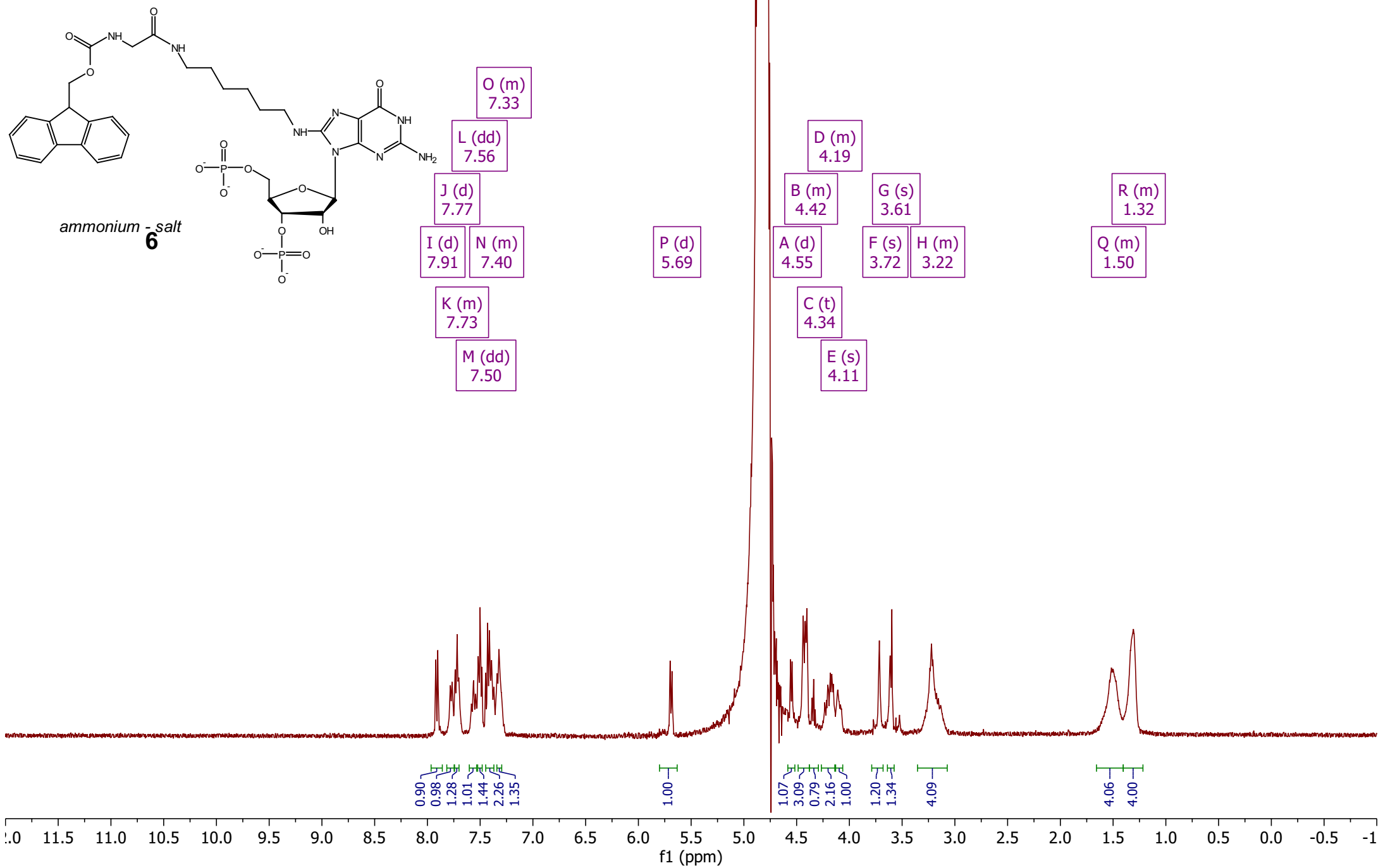


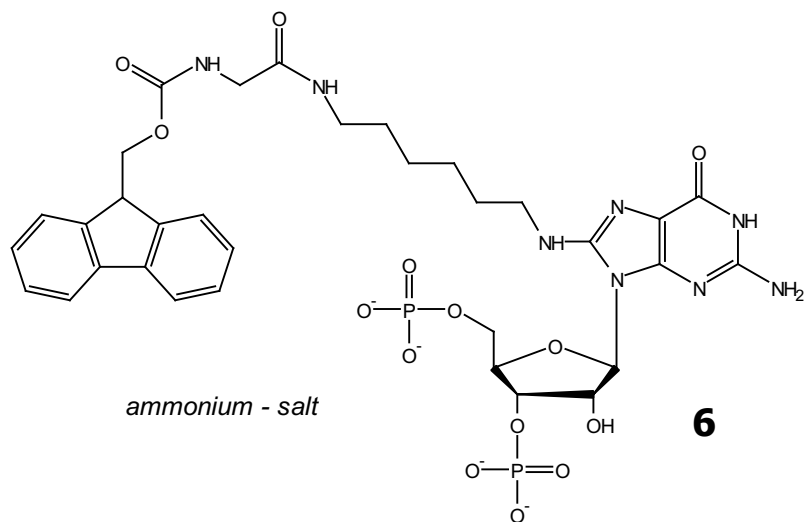












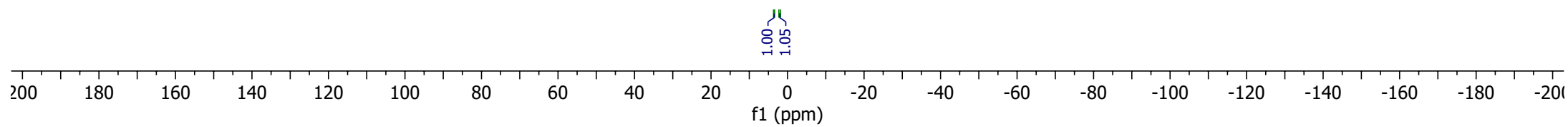
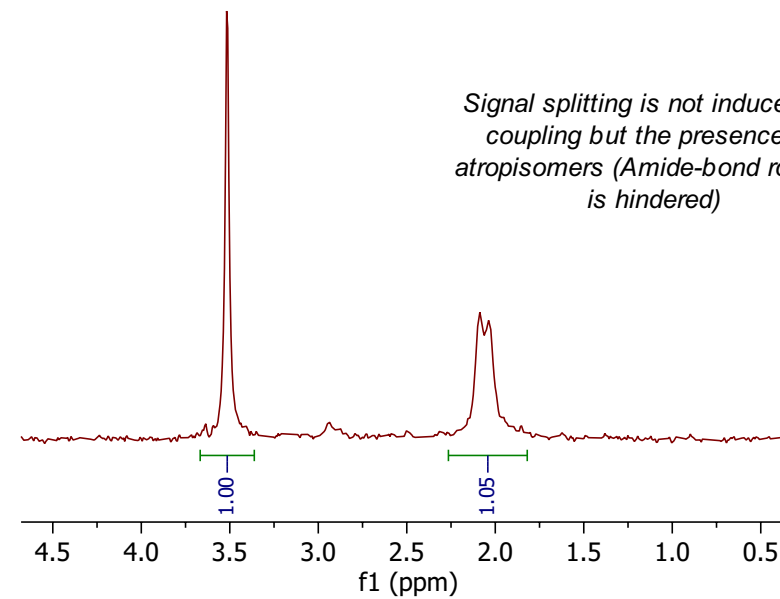
B (s)
3.52

A (d)
2.06

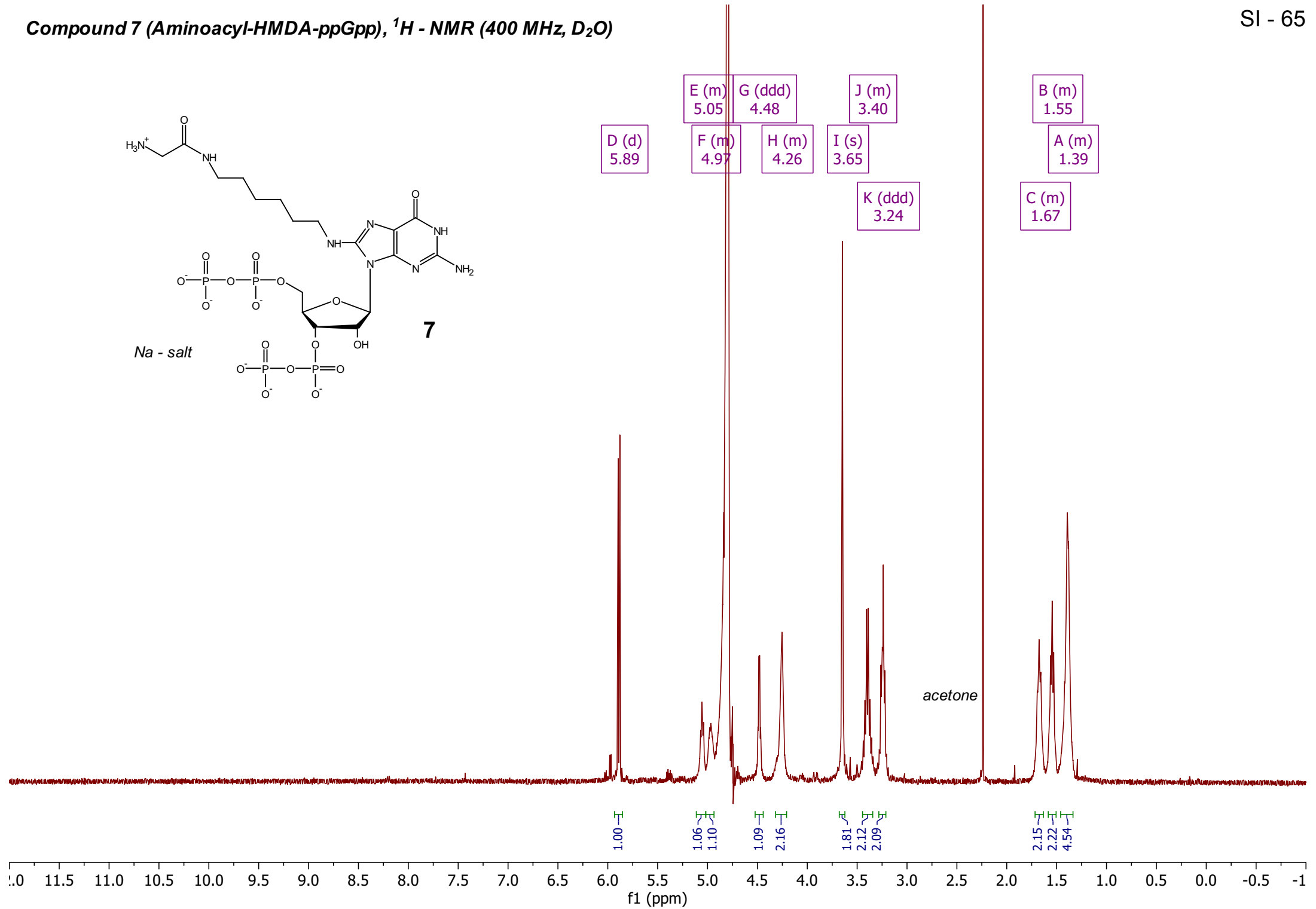
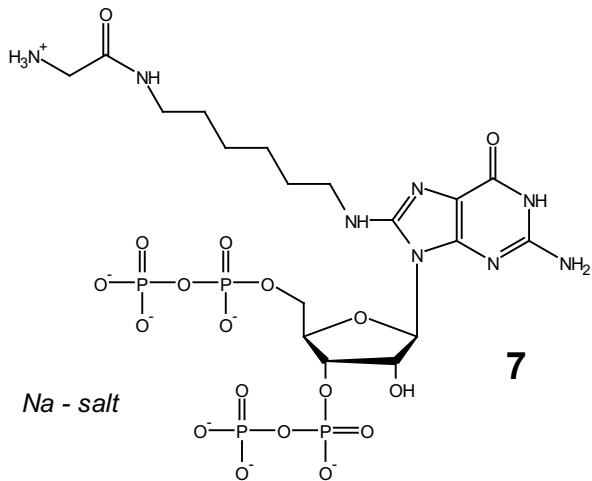
B (s)
3.52

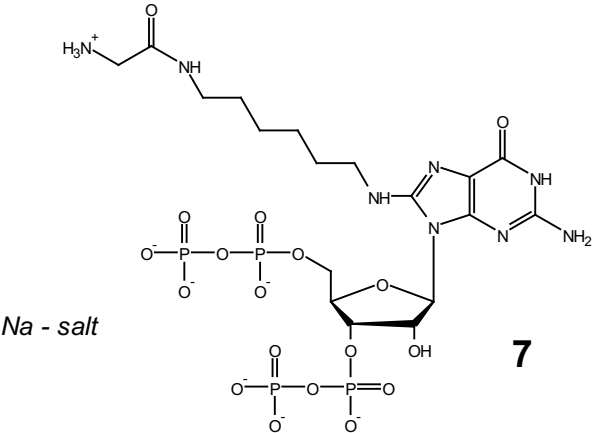
A (d)
2.06

Signal splitting is not induced by coupling but the presence of atropisomers (Amide-bond rotation is hindered)



Compound 7 (Aminoacyl-HMDA-ppGpp), ¹H - NMR (400 MHz, D₂O)





B (d)
-6.26

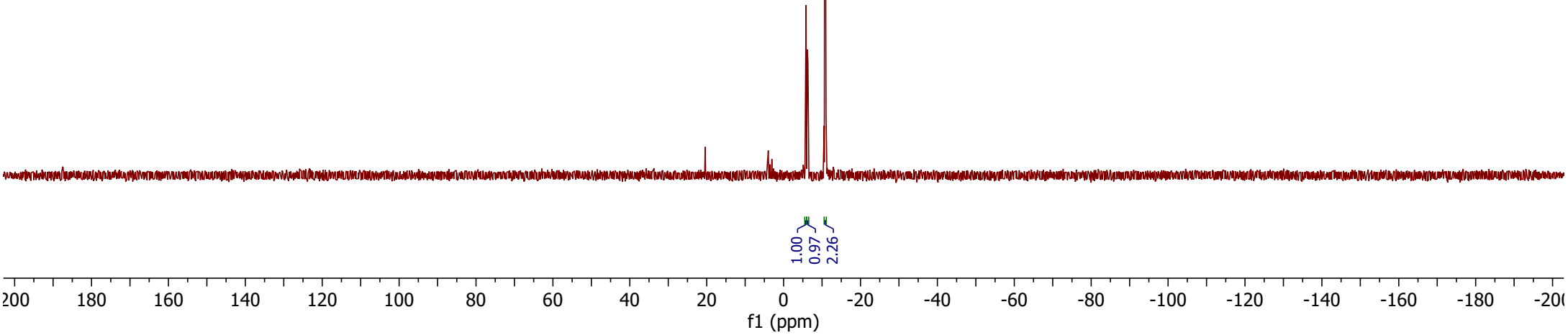
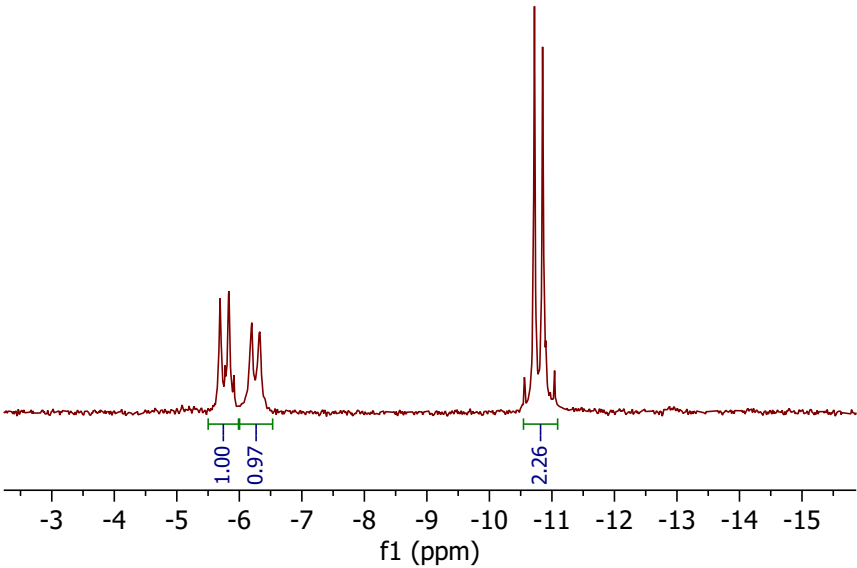
A (d)
-5.76

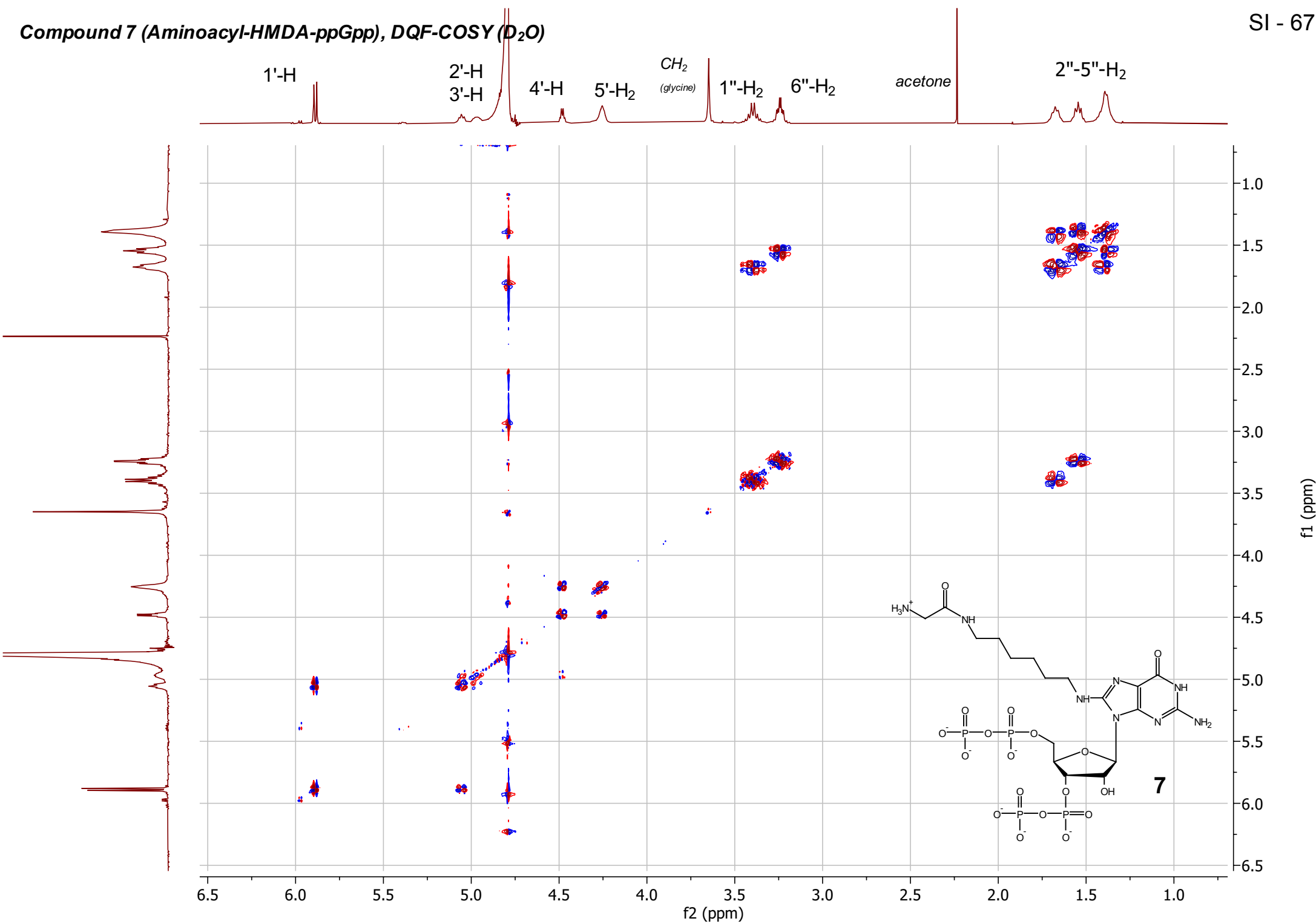
C (d)
-10.79

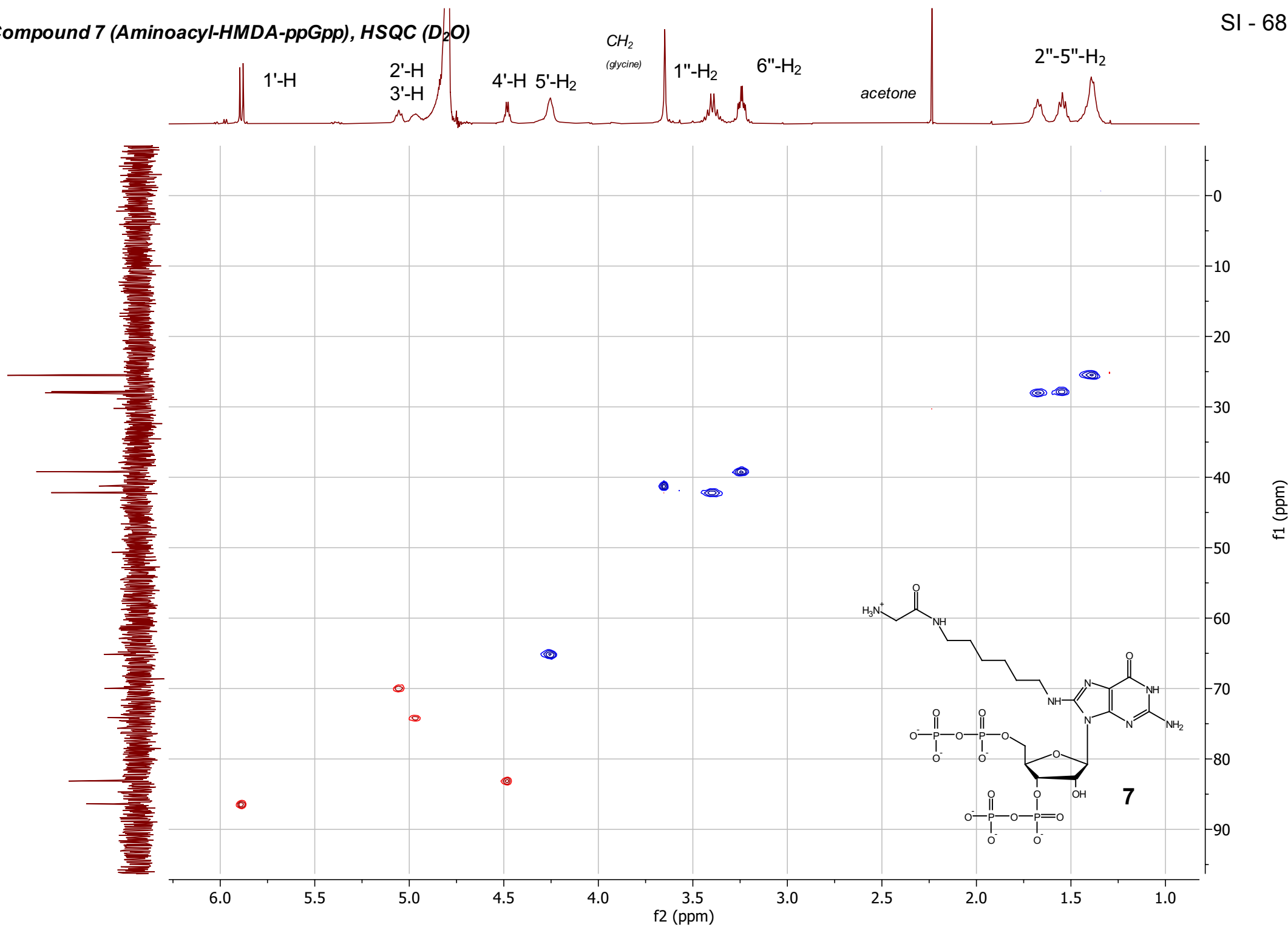
B (d)
-6.26

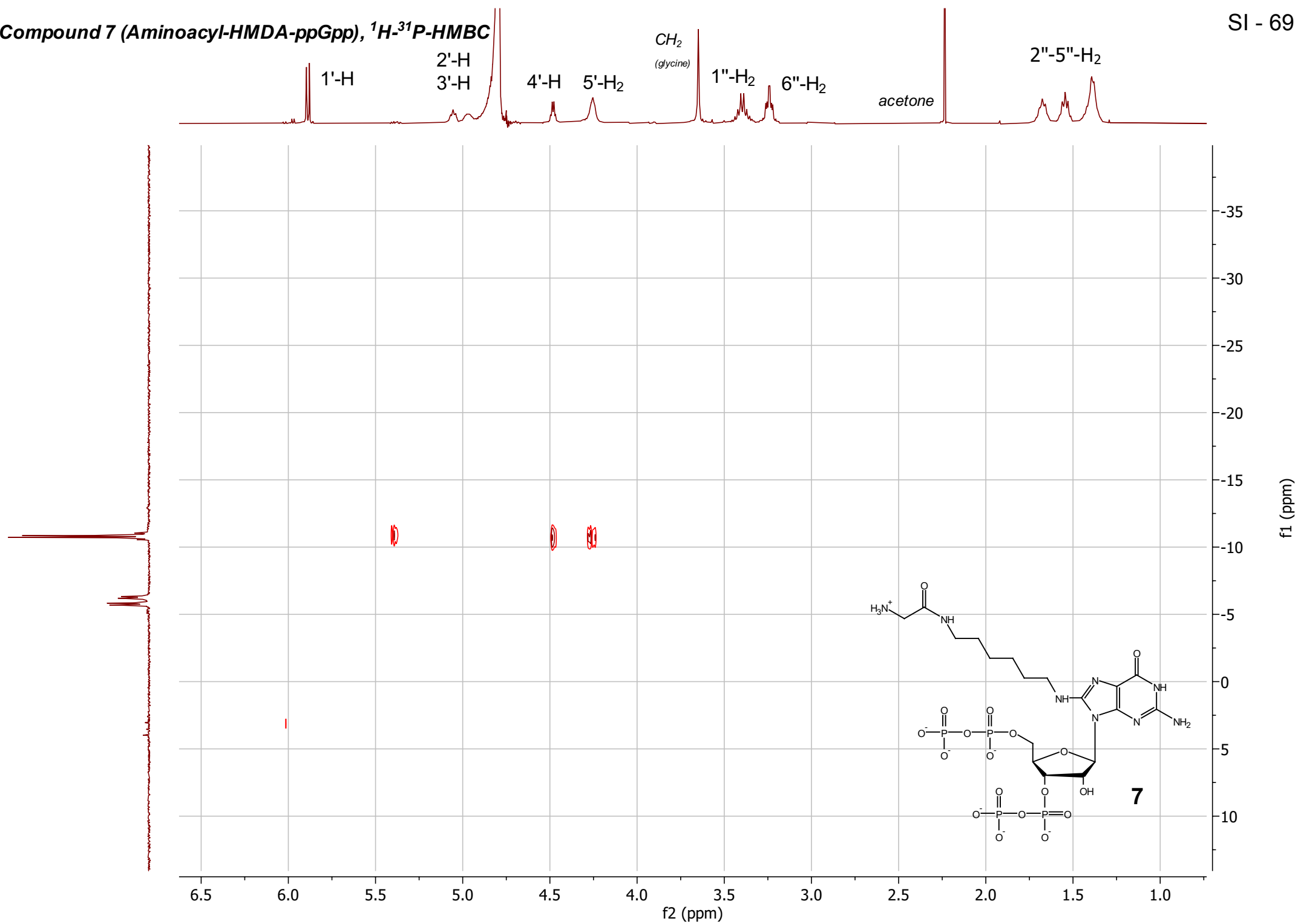
A (d)
-5.76

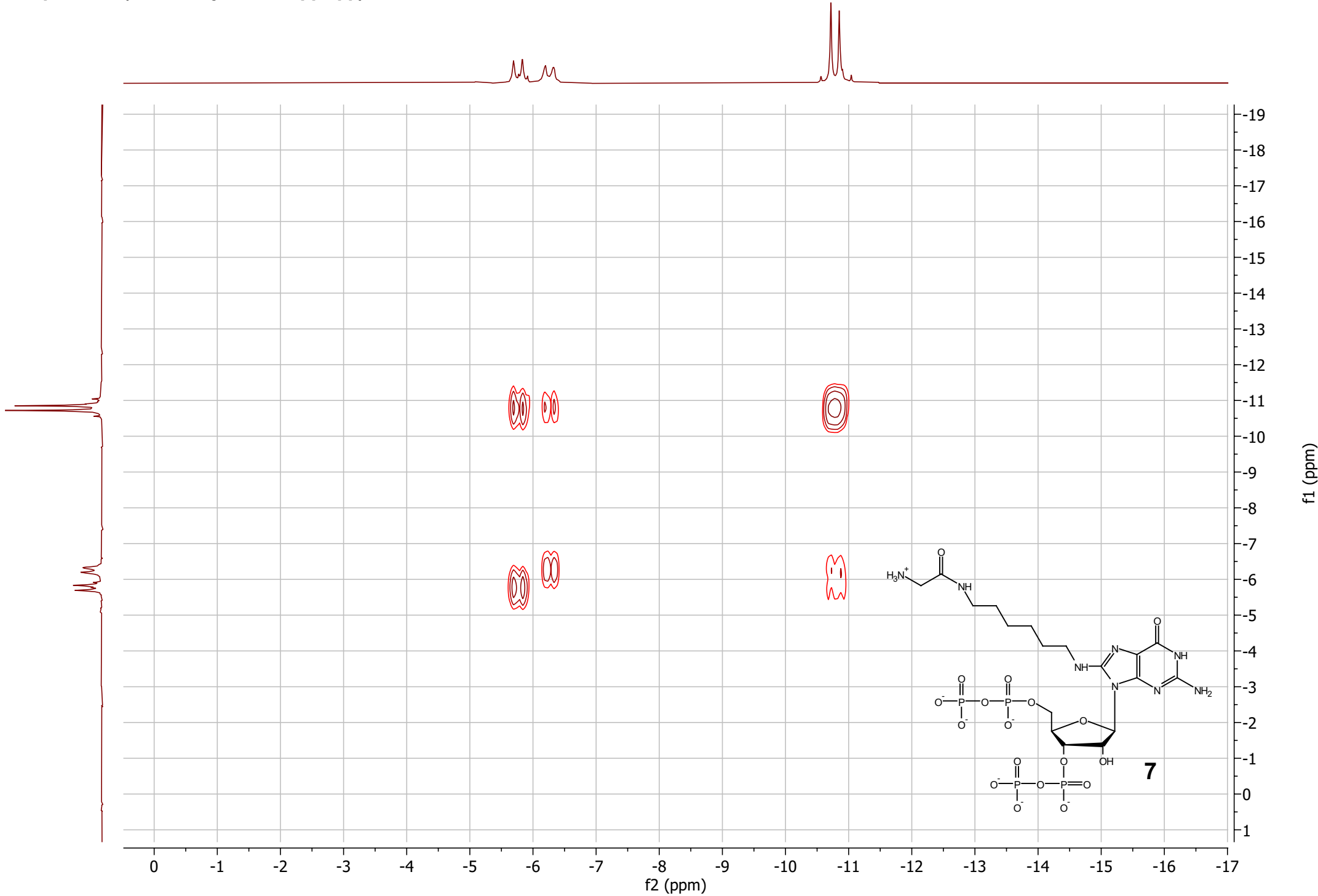
C (d)
-10.79

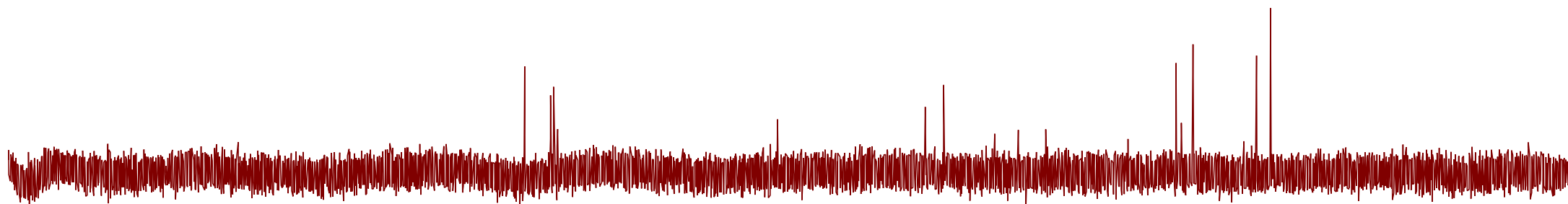
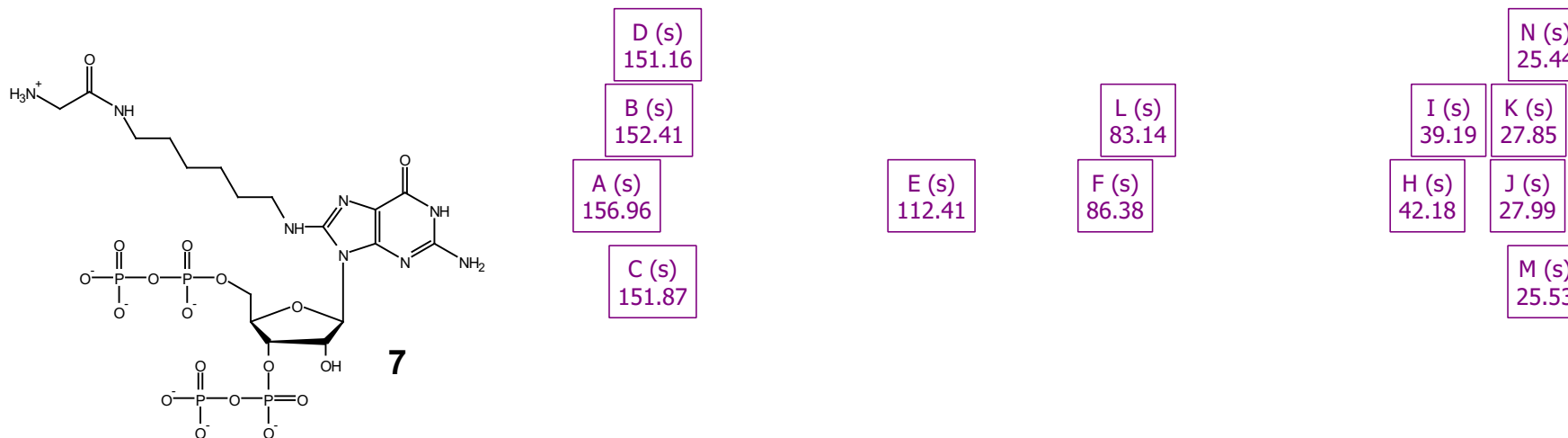




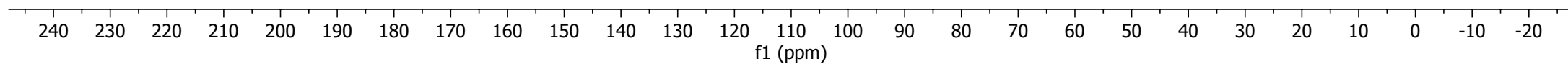


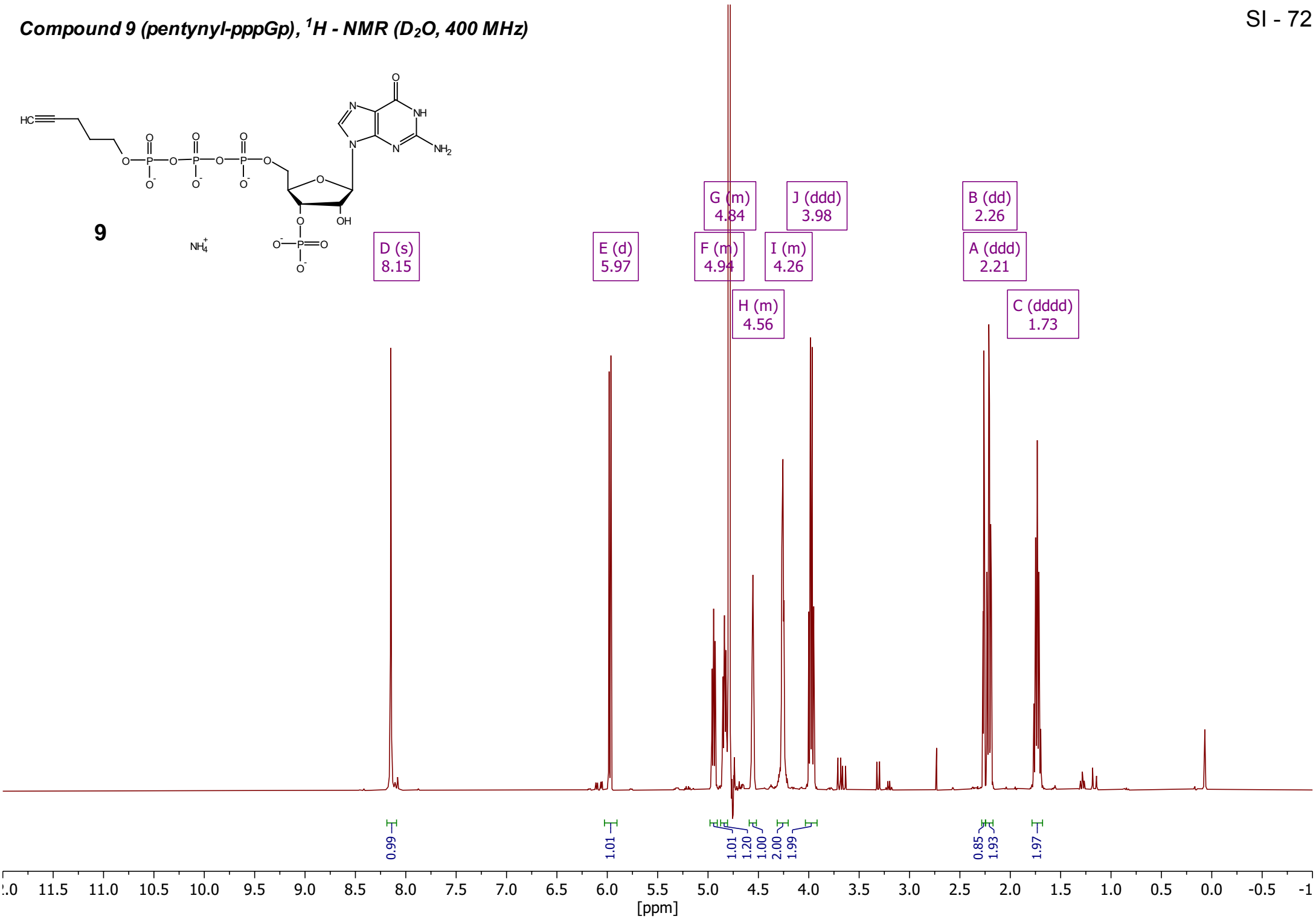


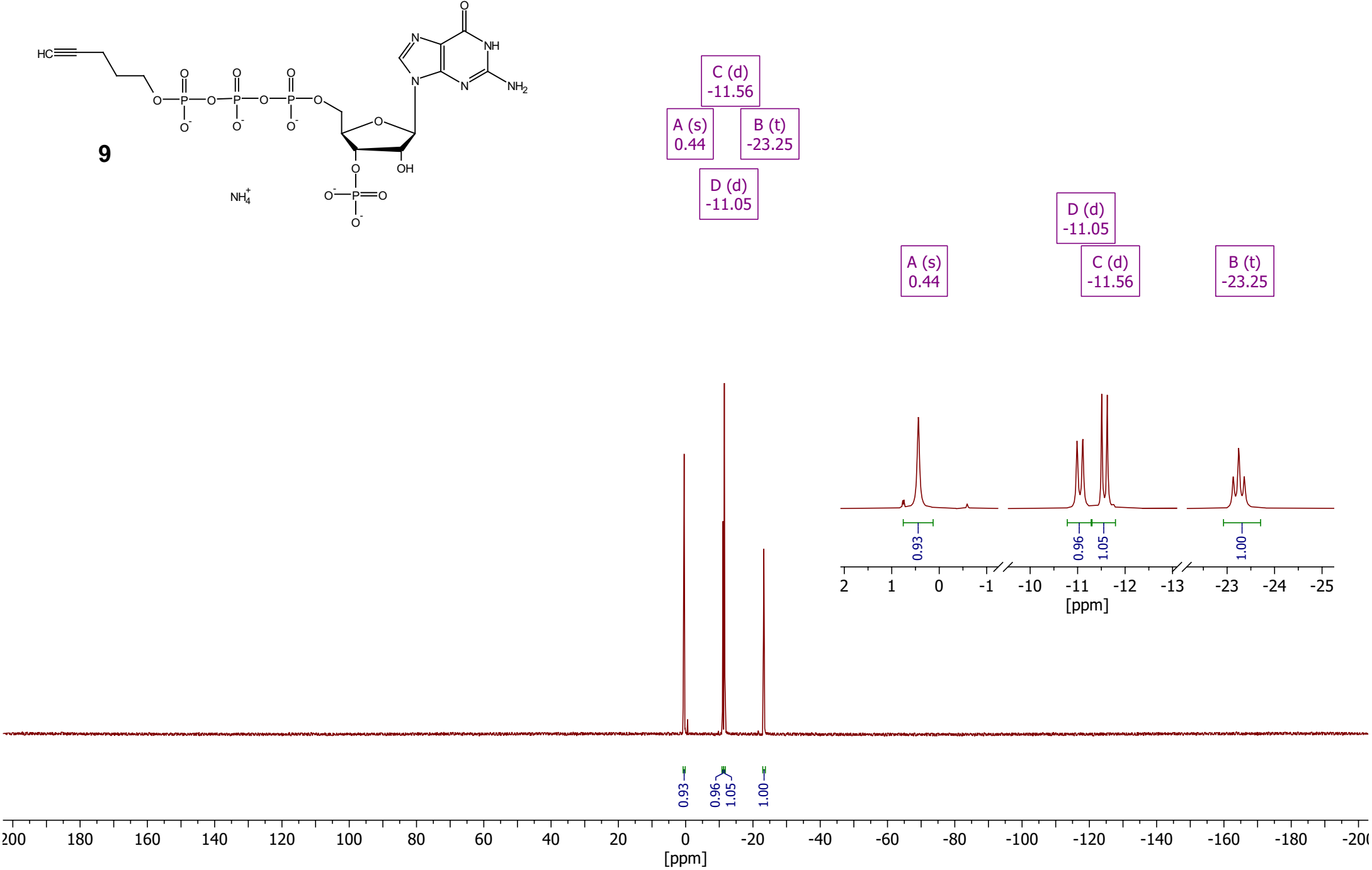


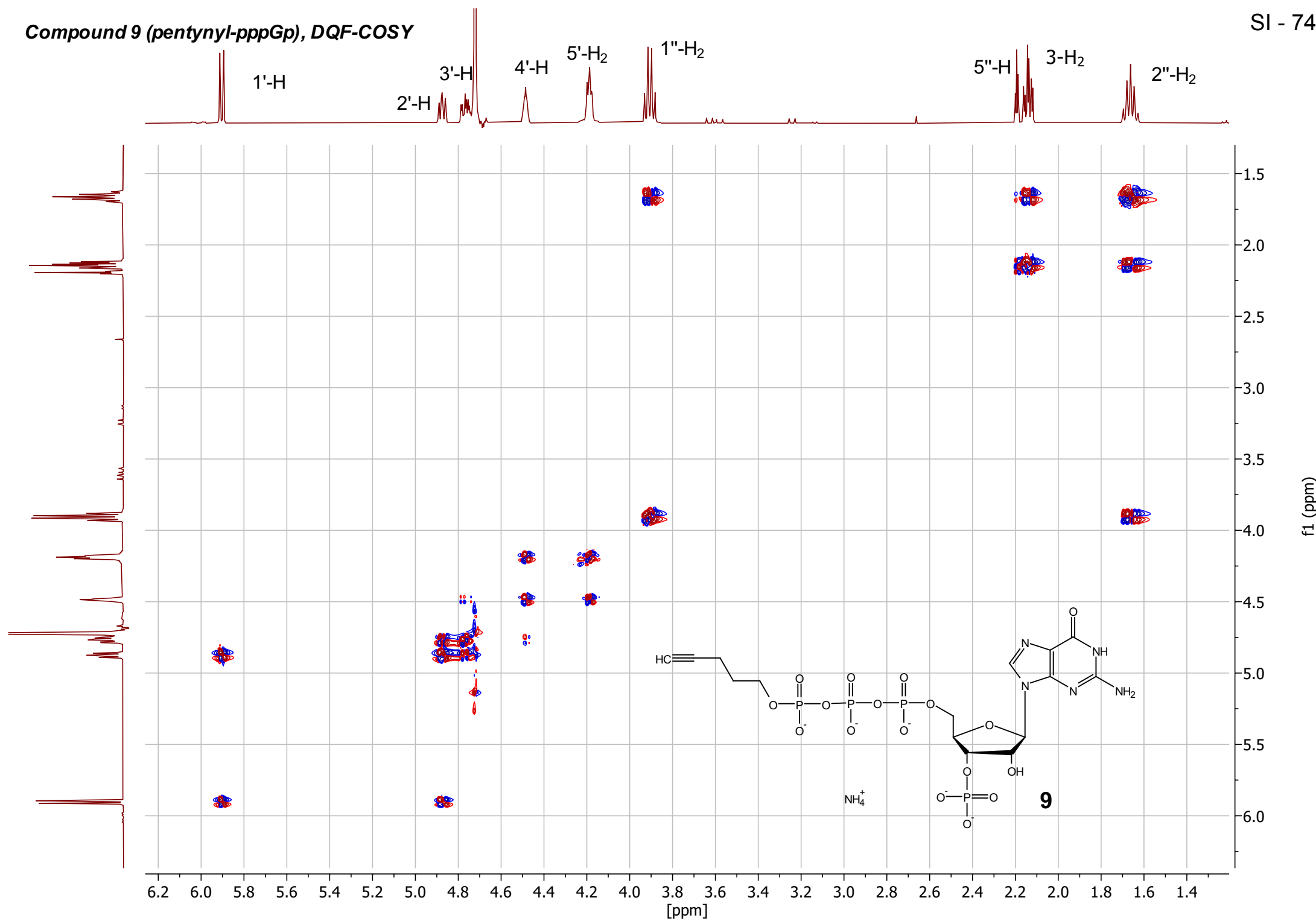


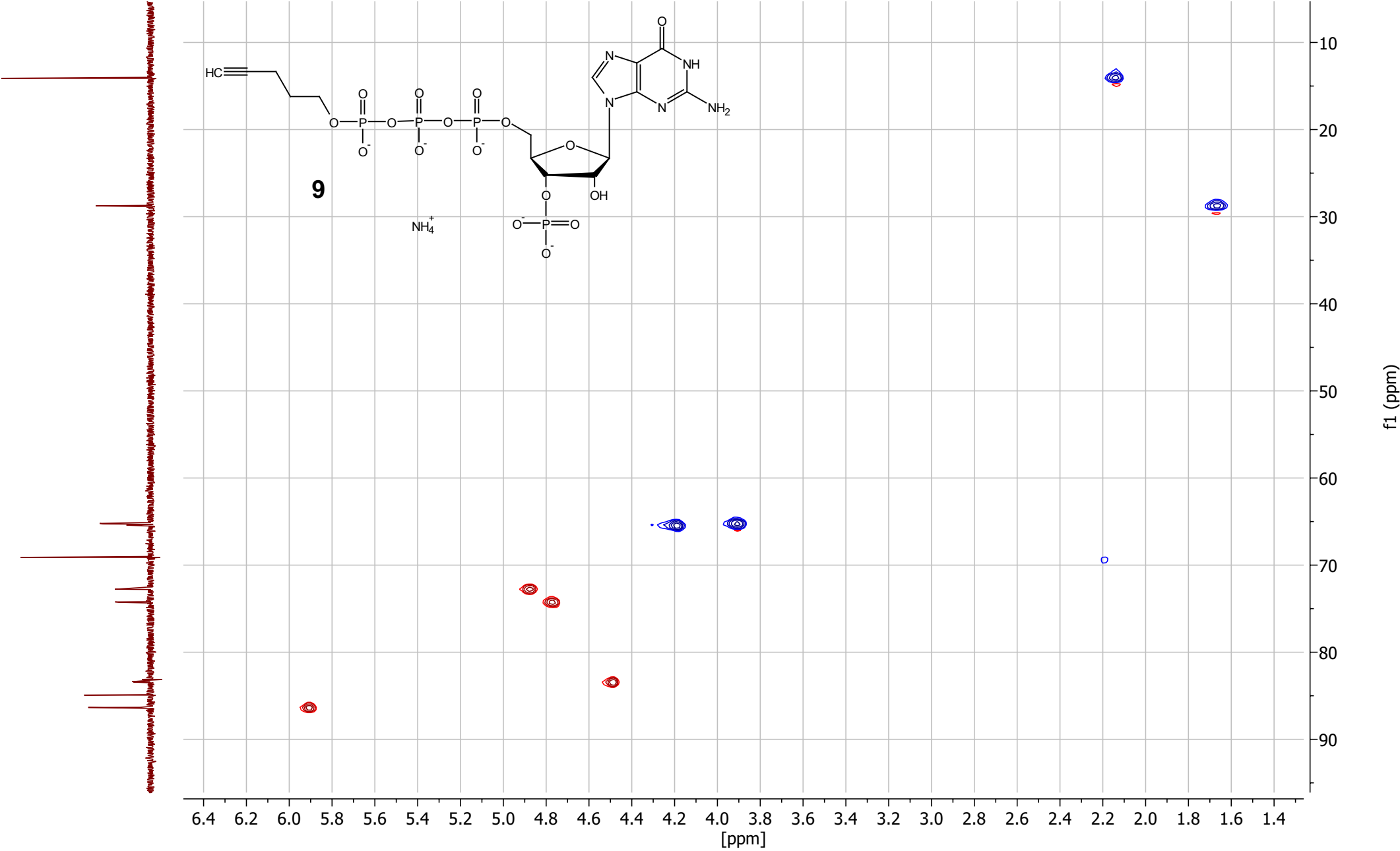
Some peaks couldn't be picked due to the low intensity. But they were manually added into the peak list.

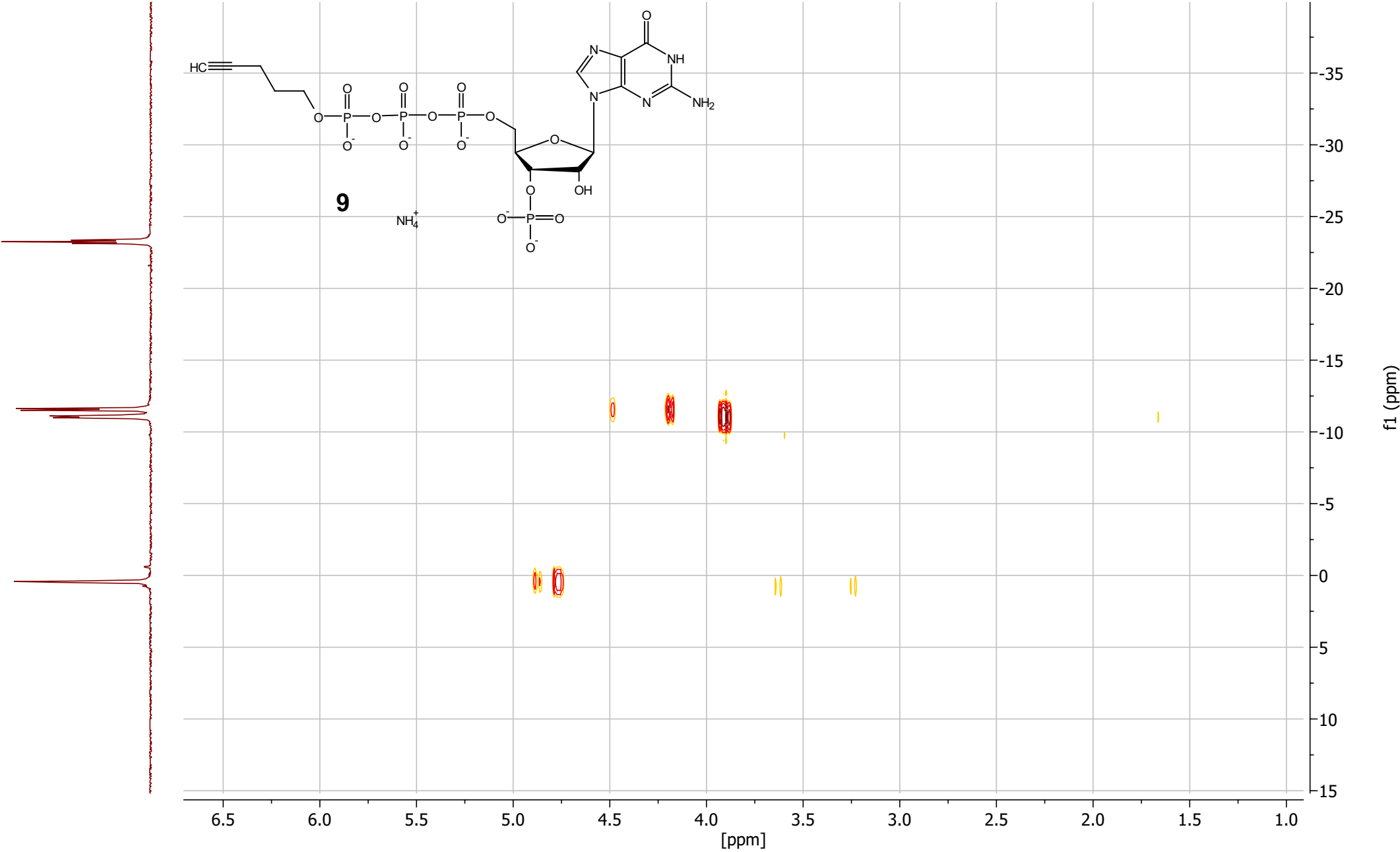


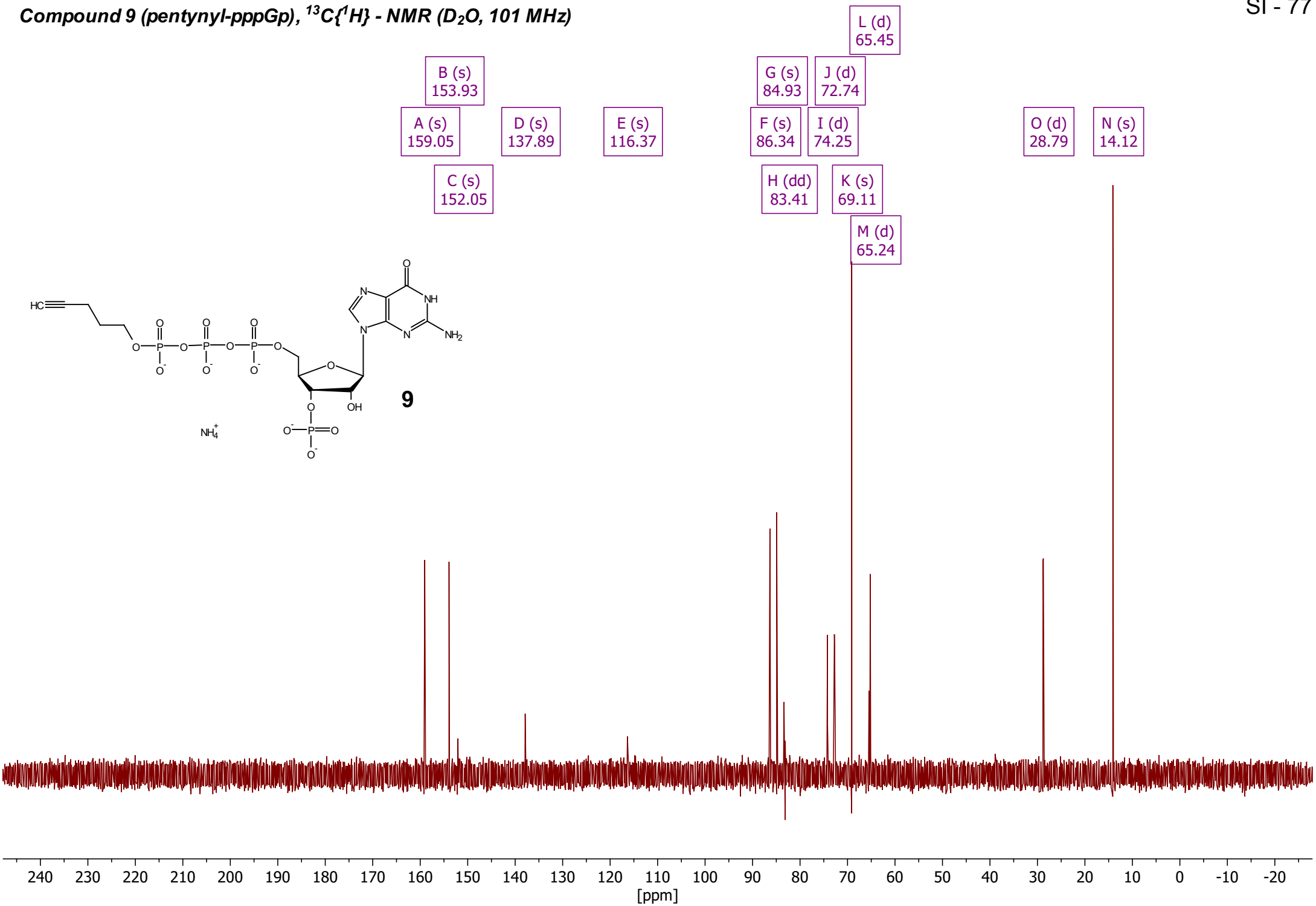
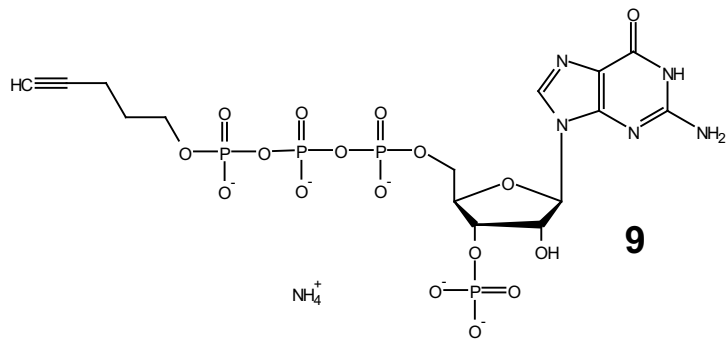


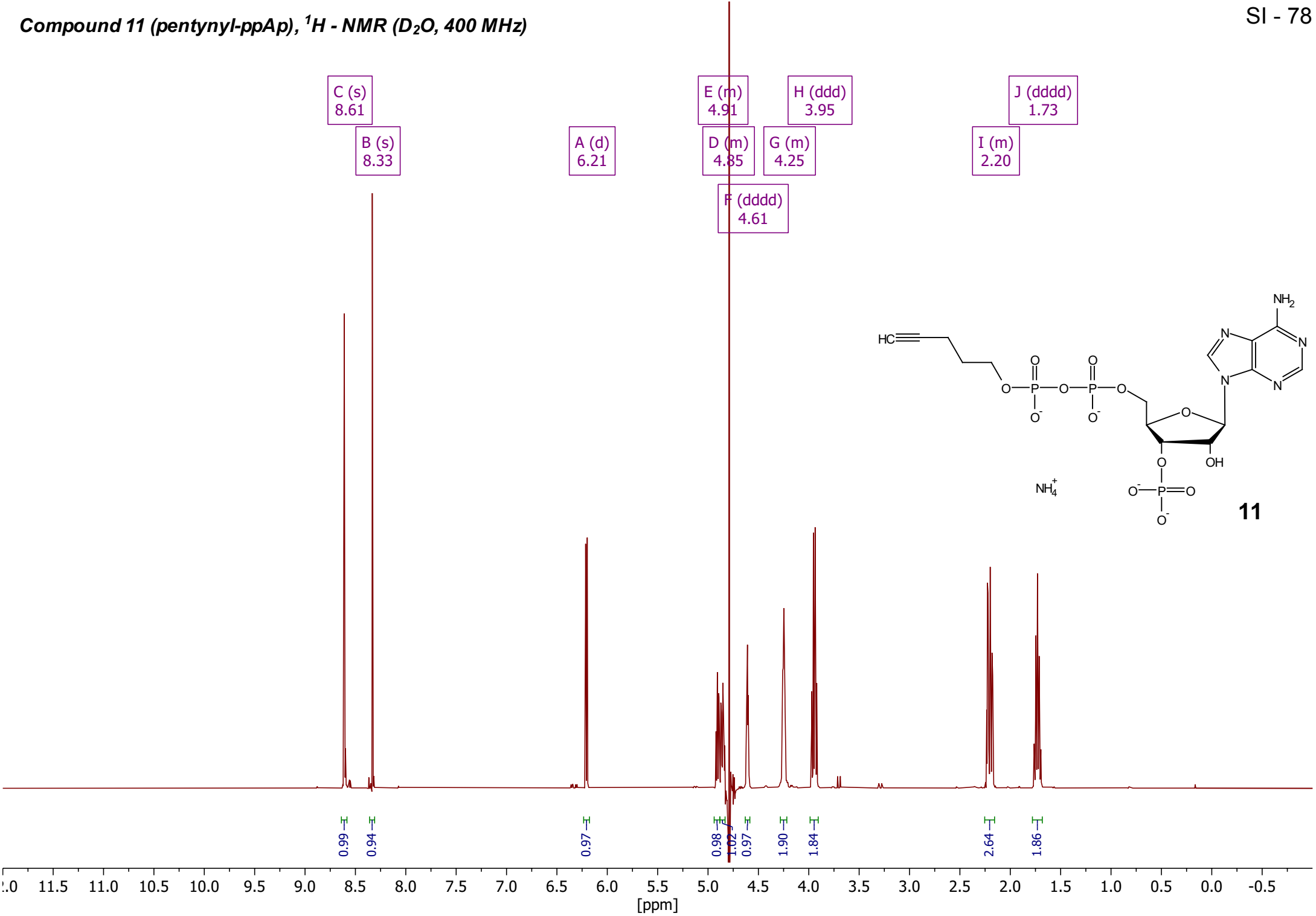


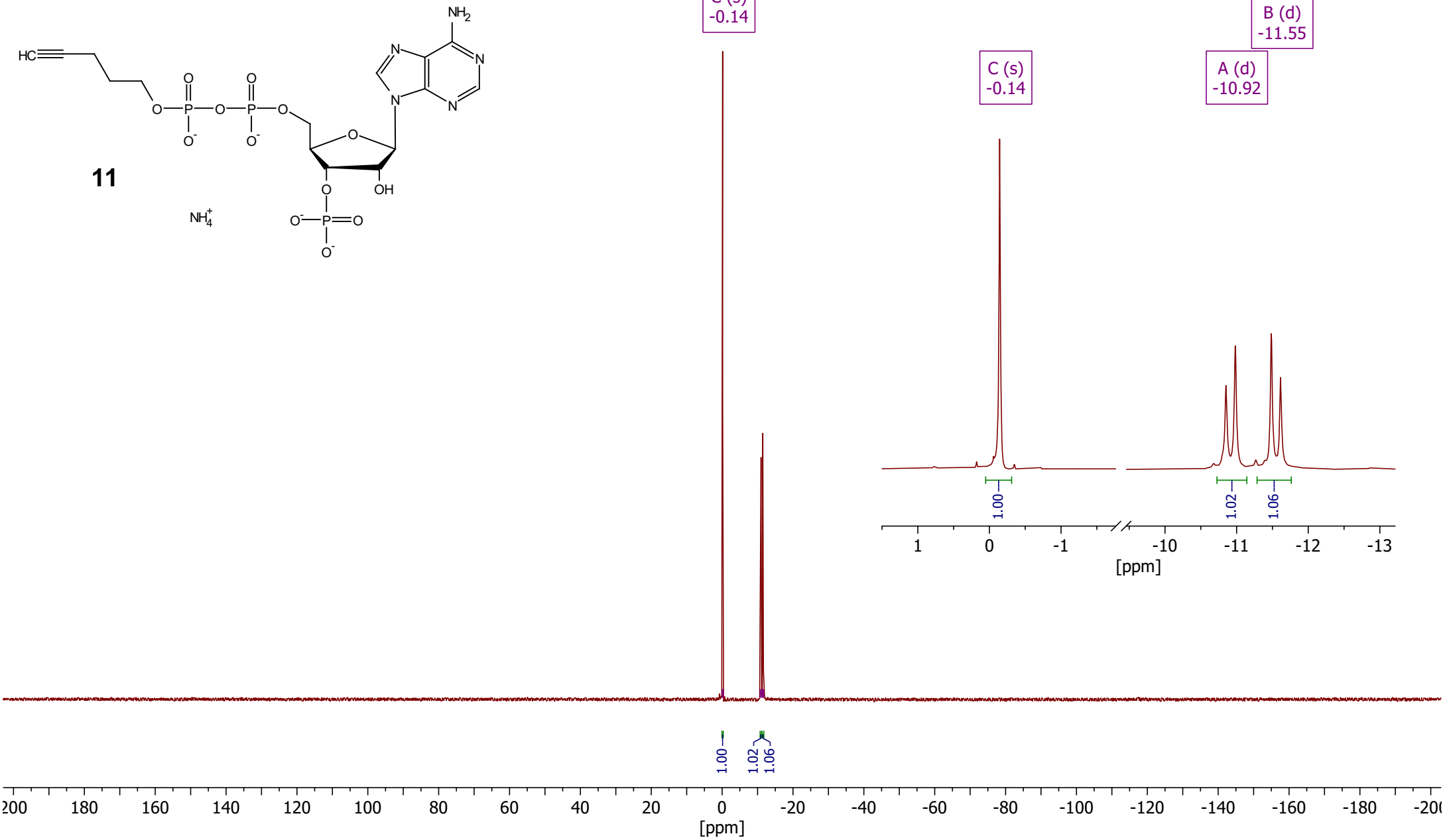


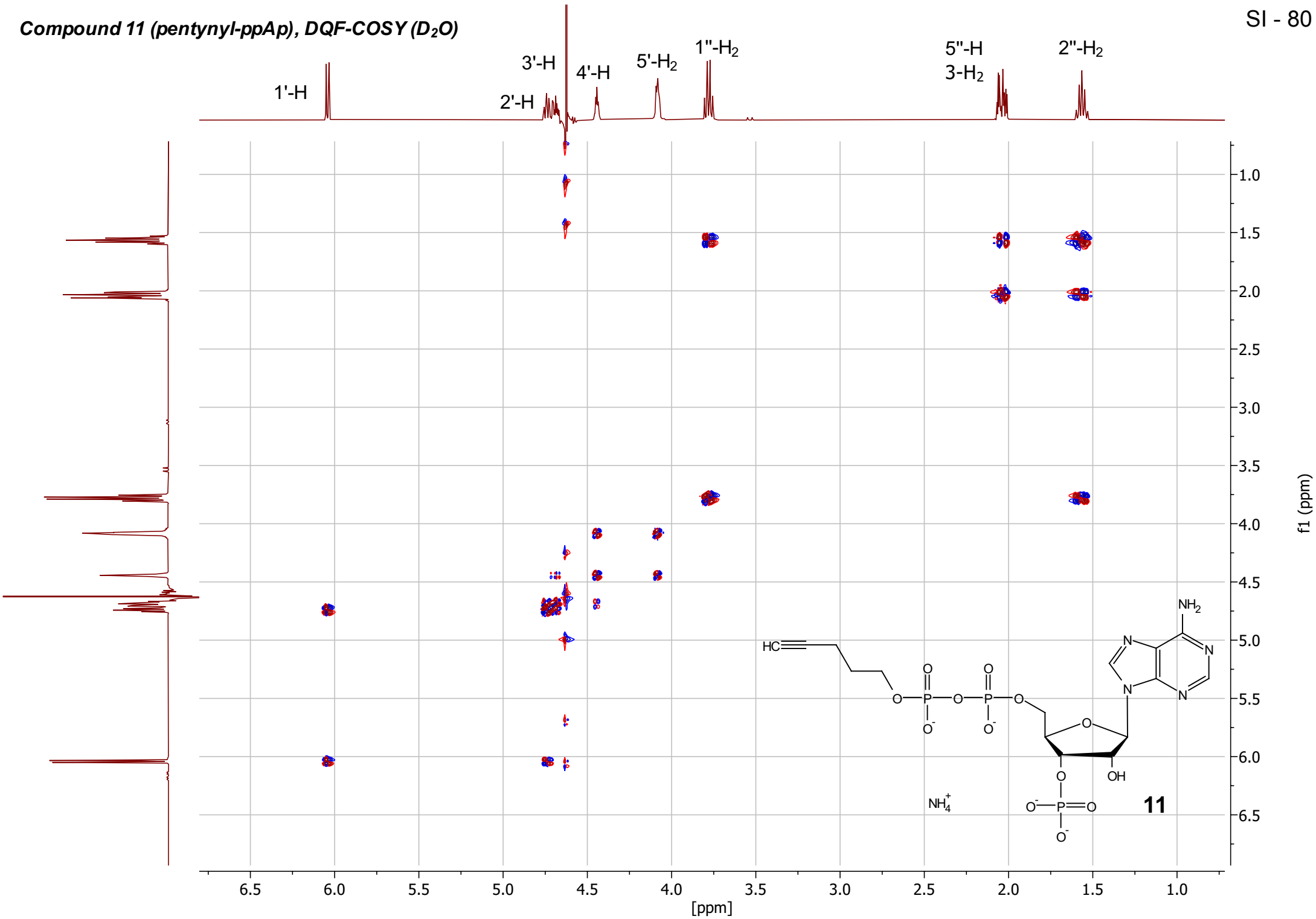


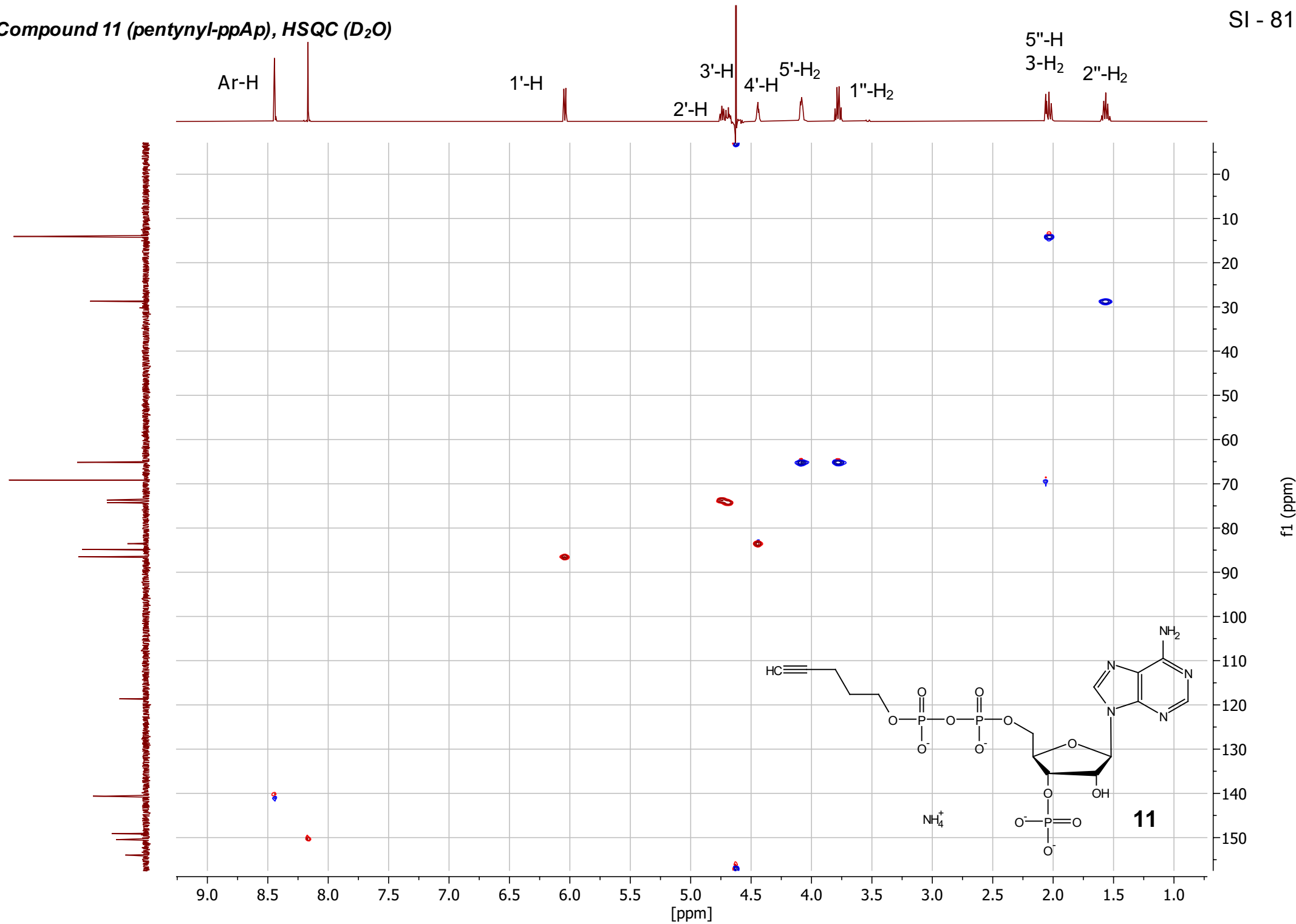


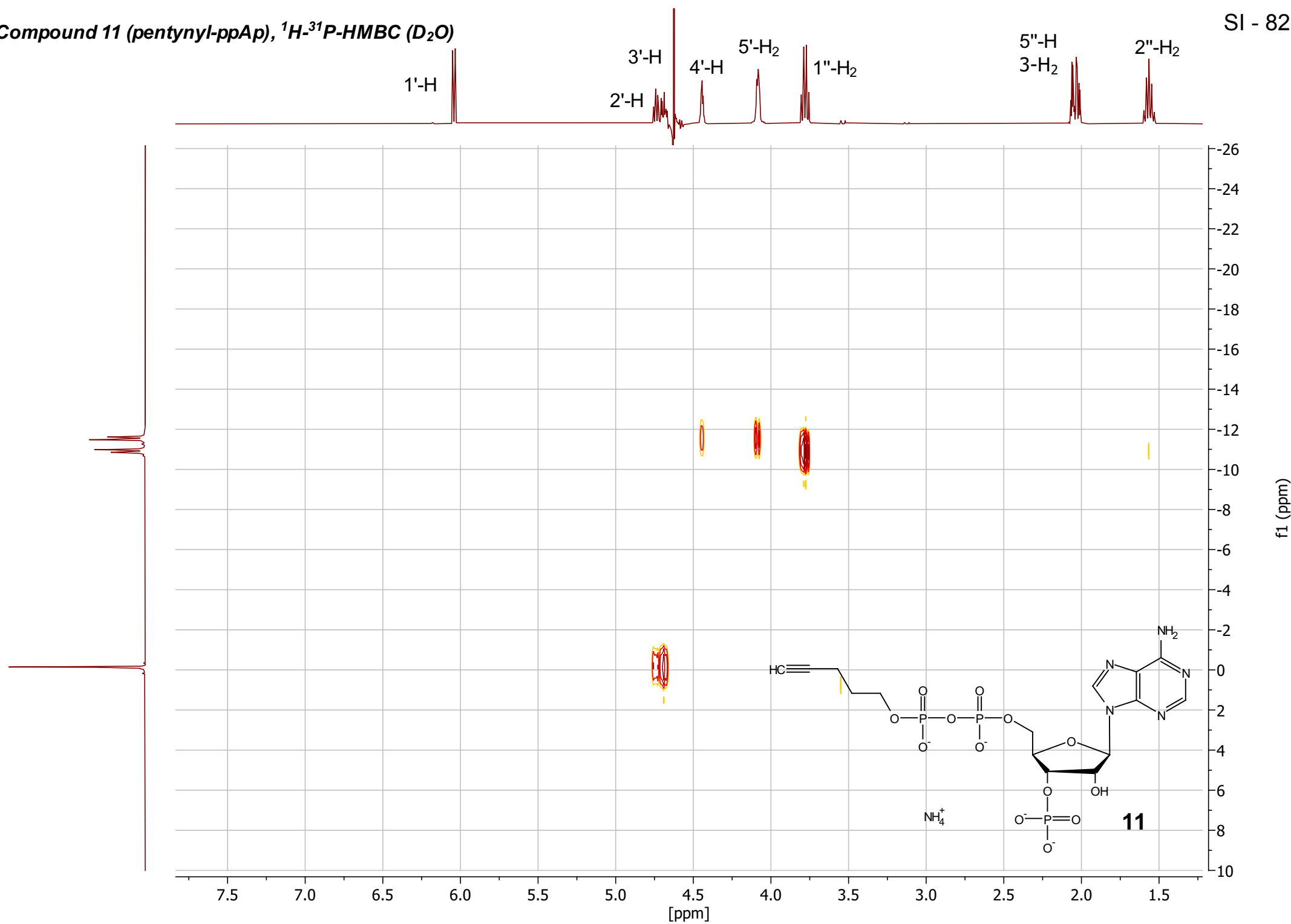


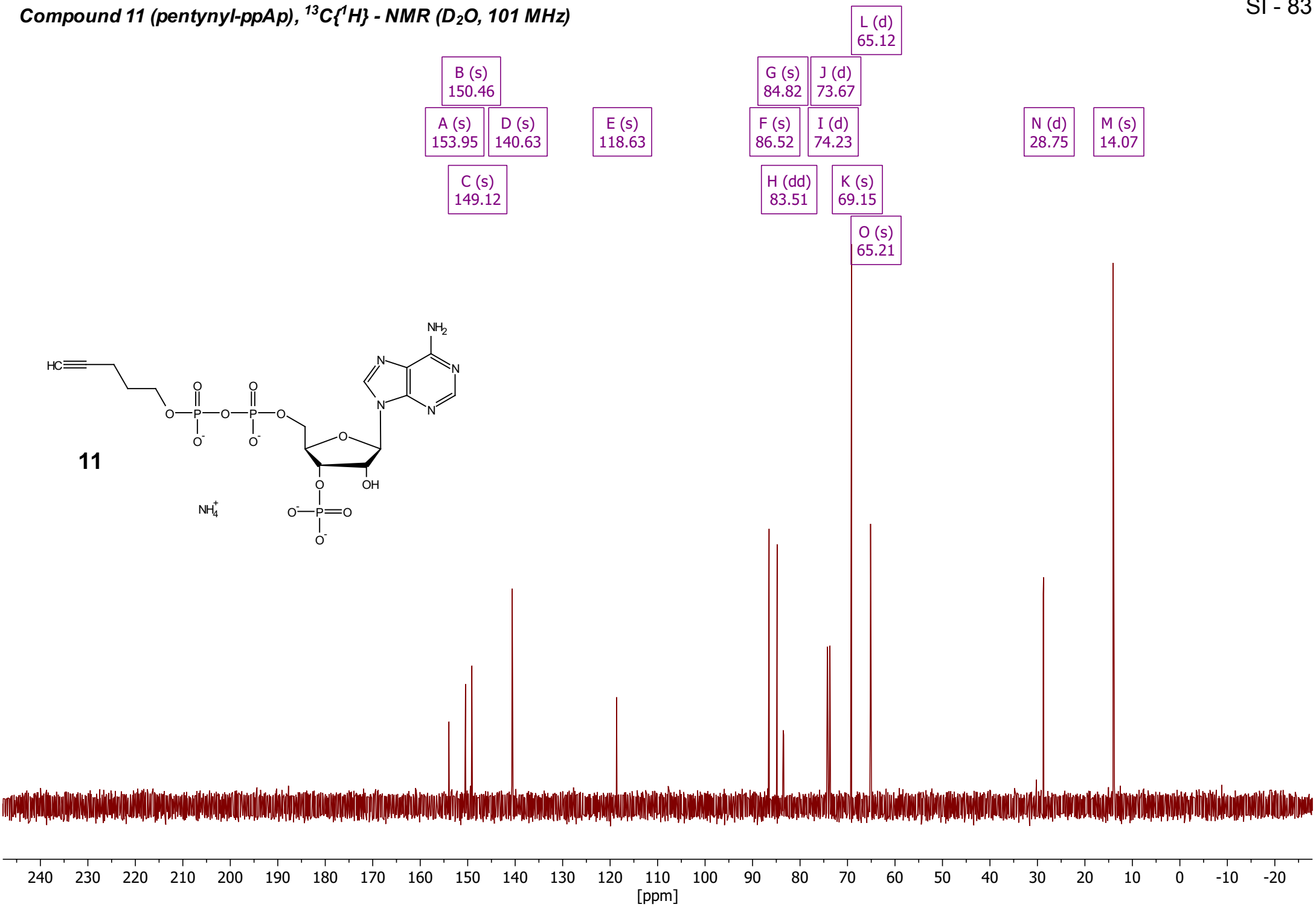


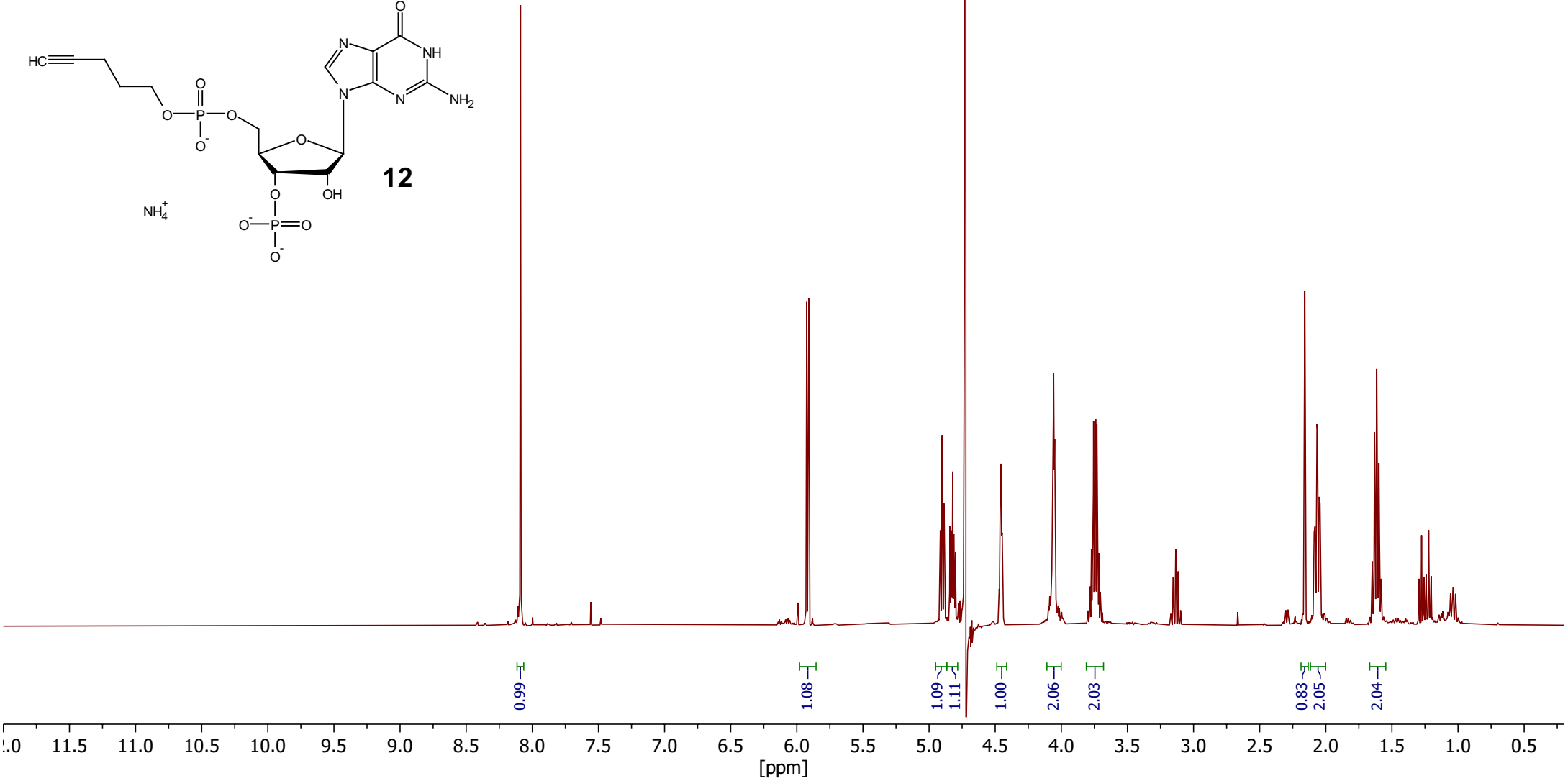


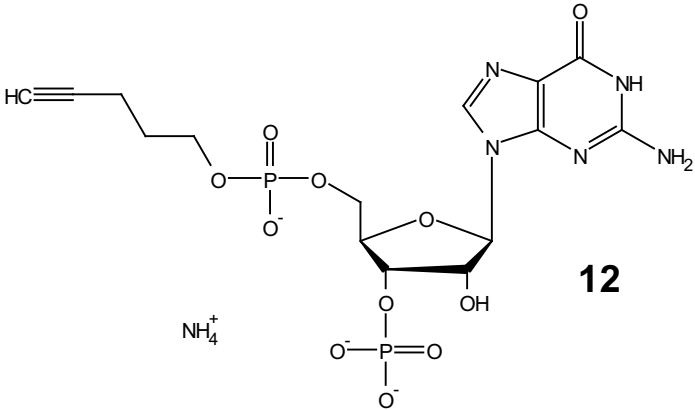












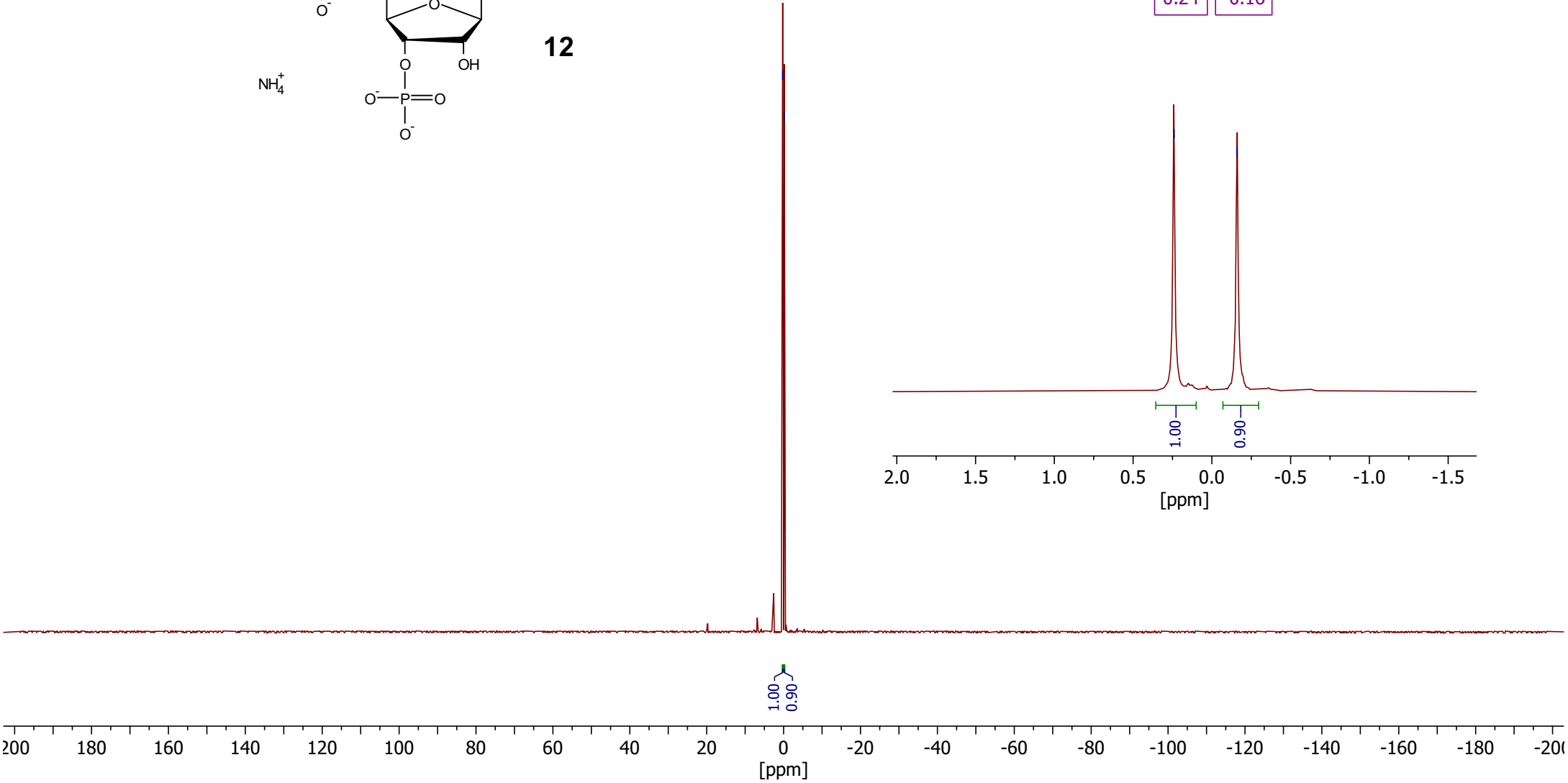
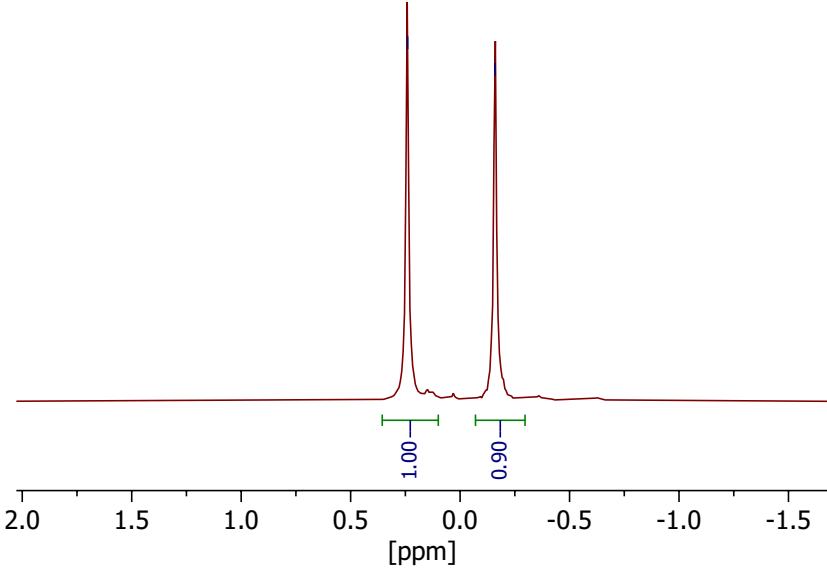
12

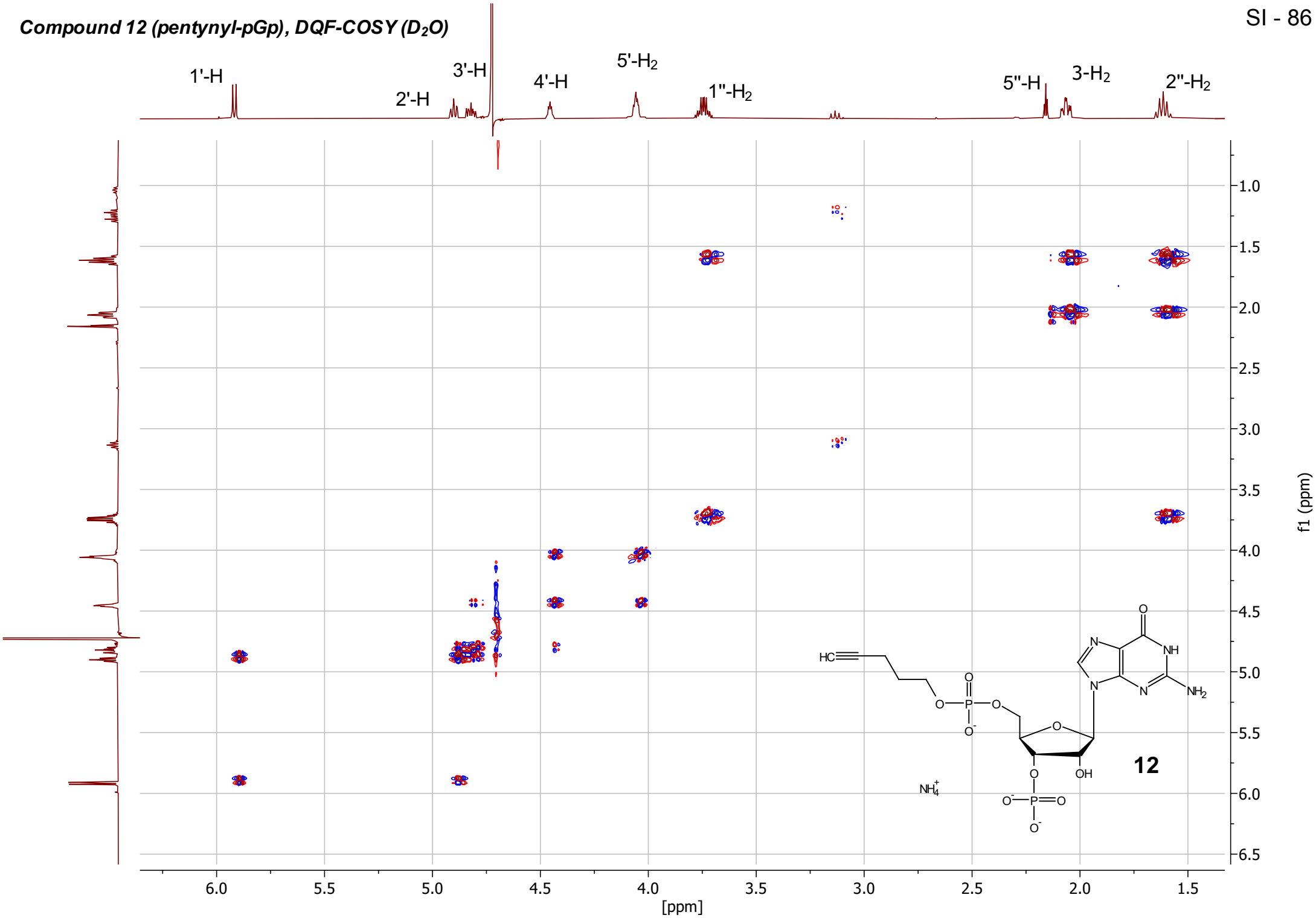
B (s)
-0.16

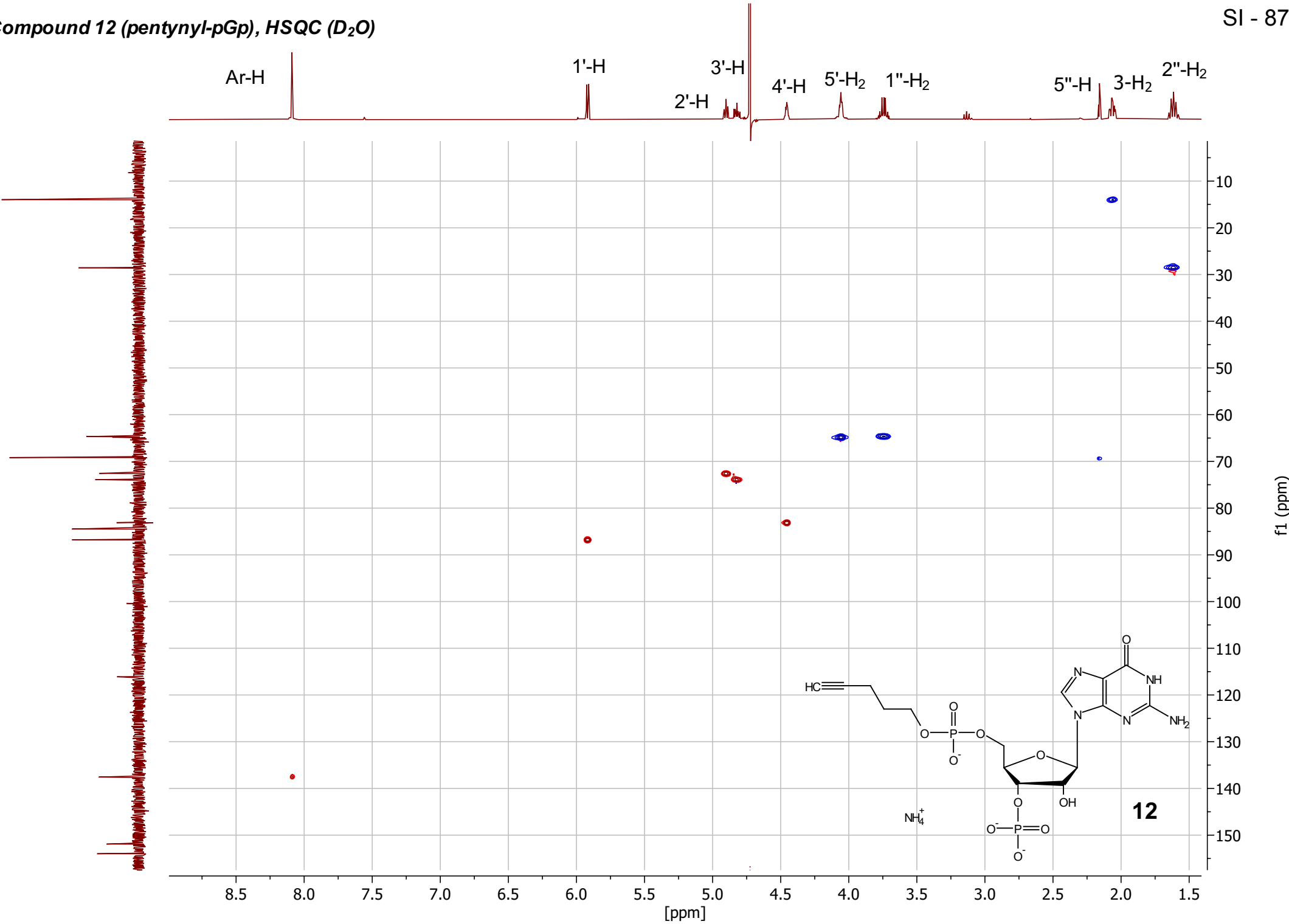
A (s)
0.24

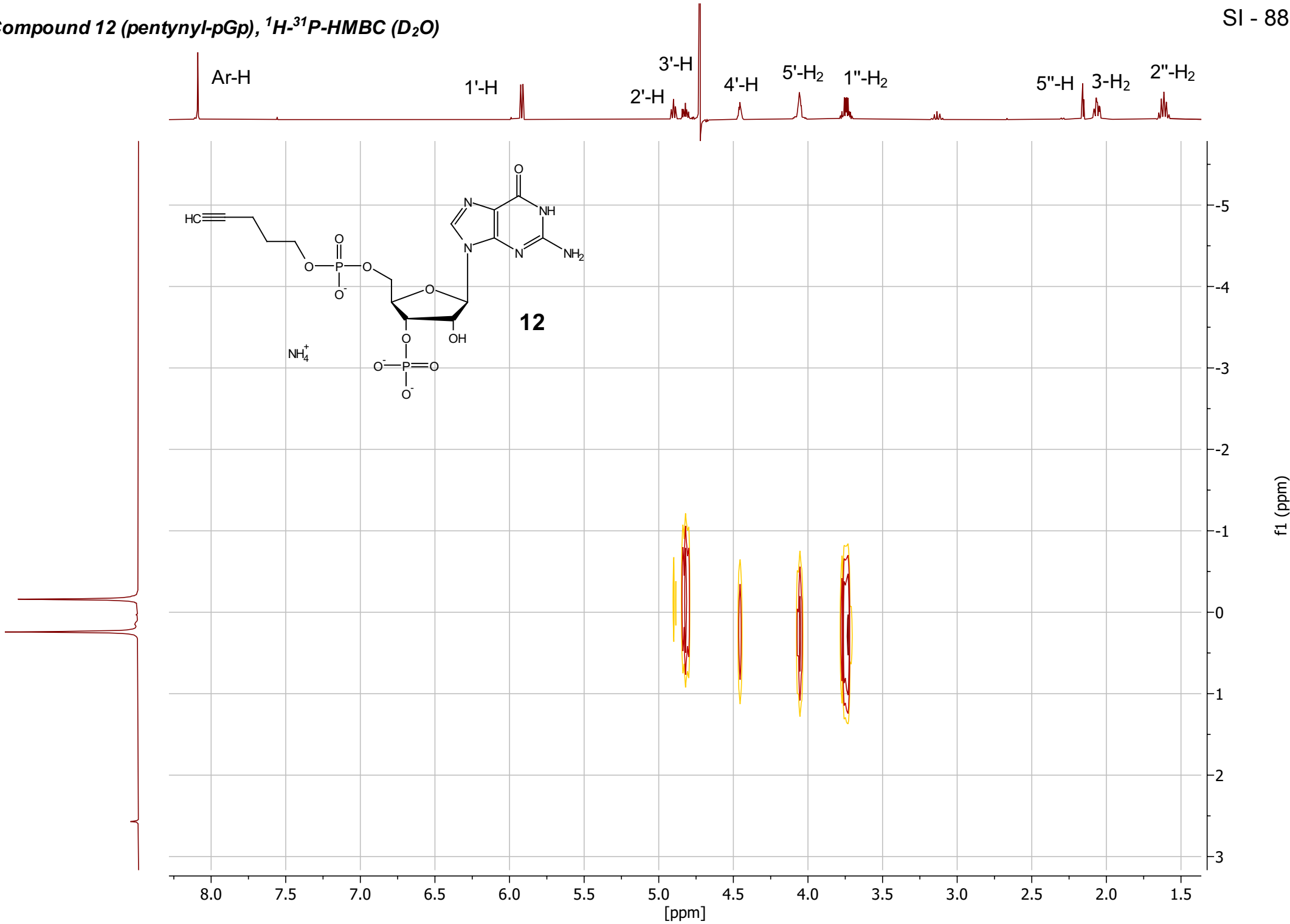
A (s)
0.24

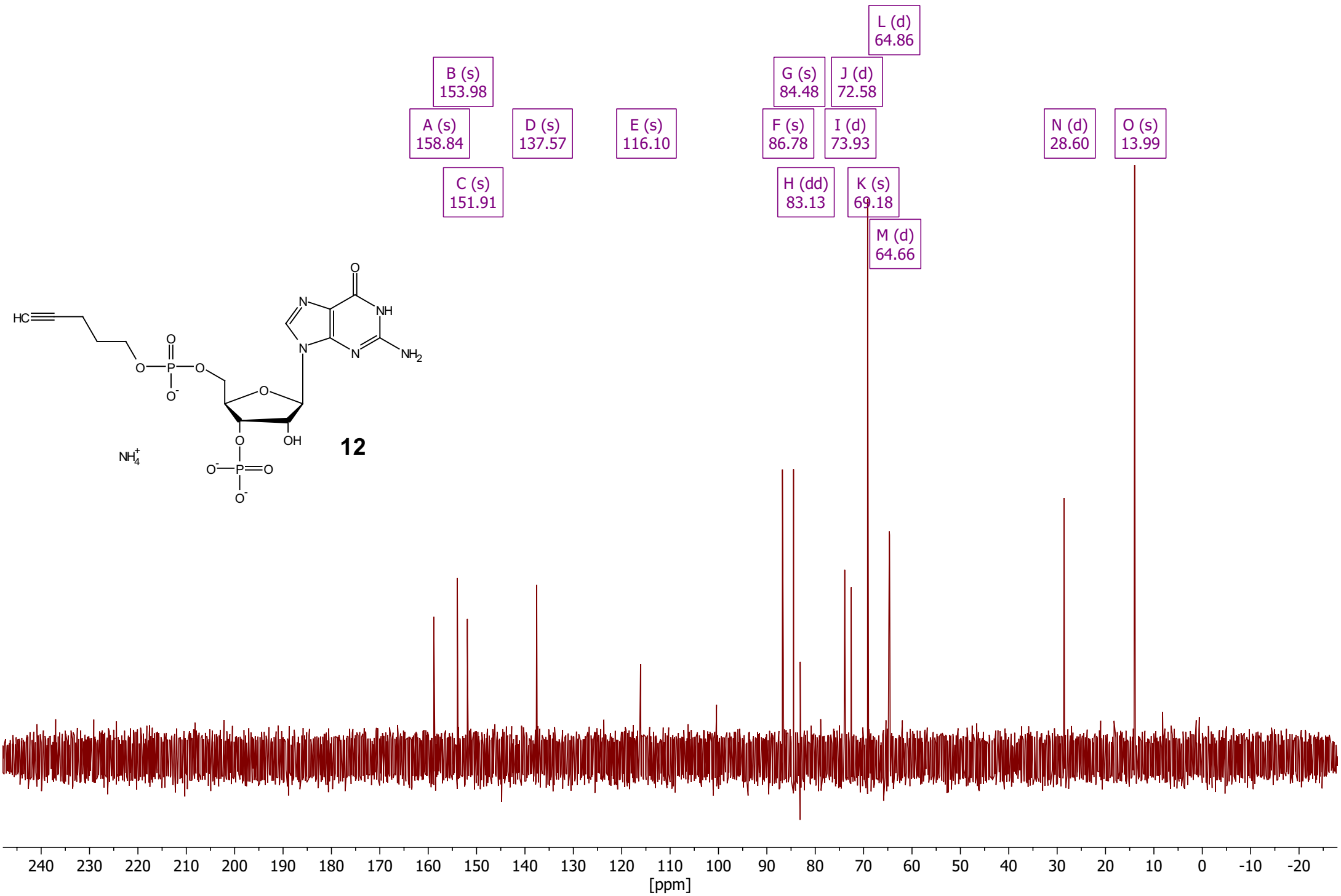
B (s)
-0.16

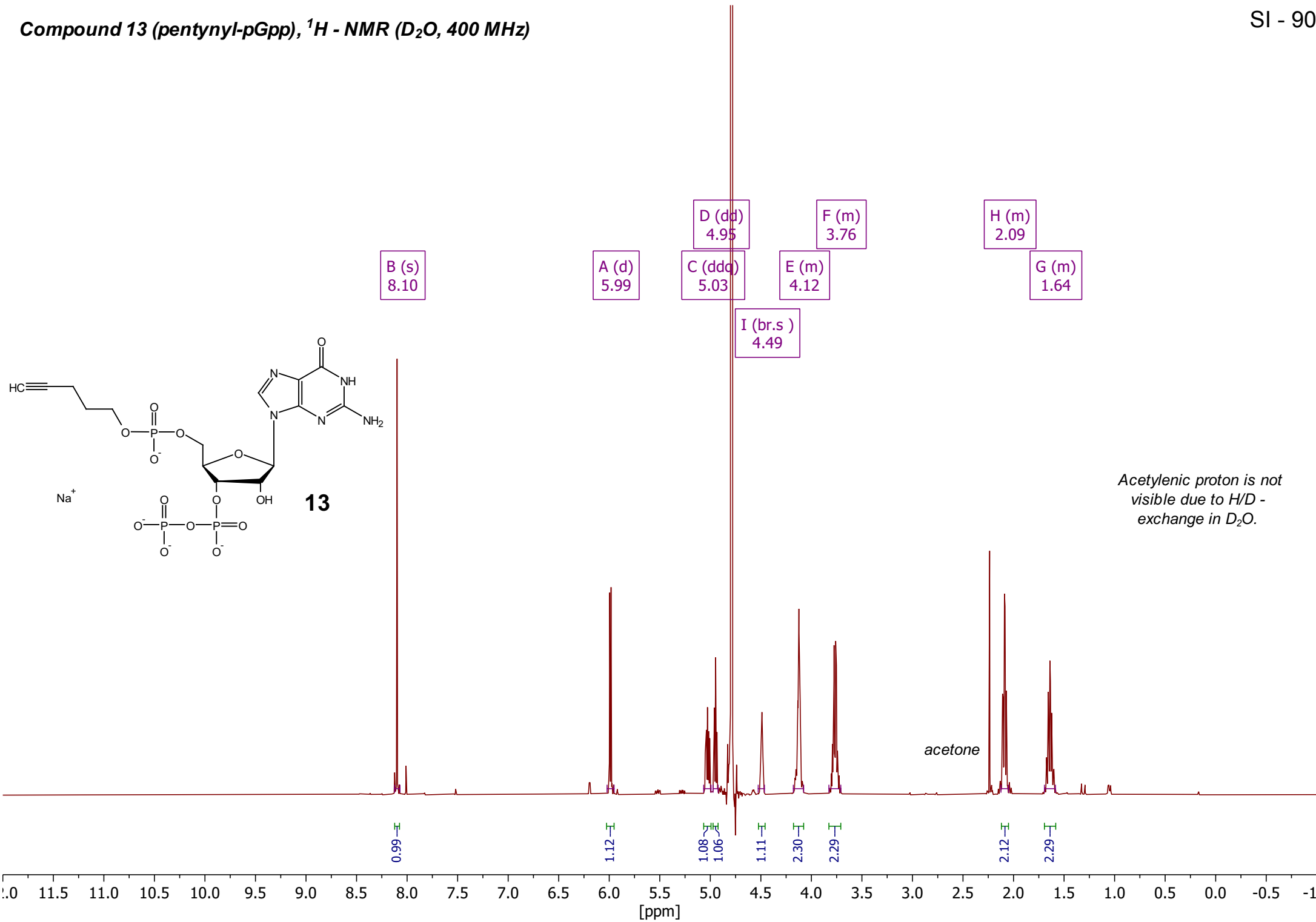


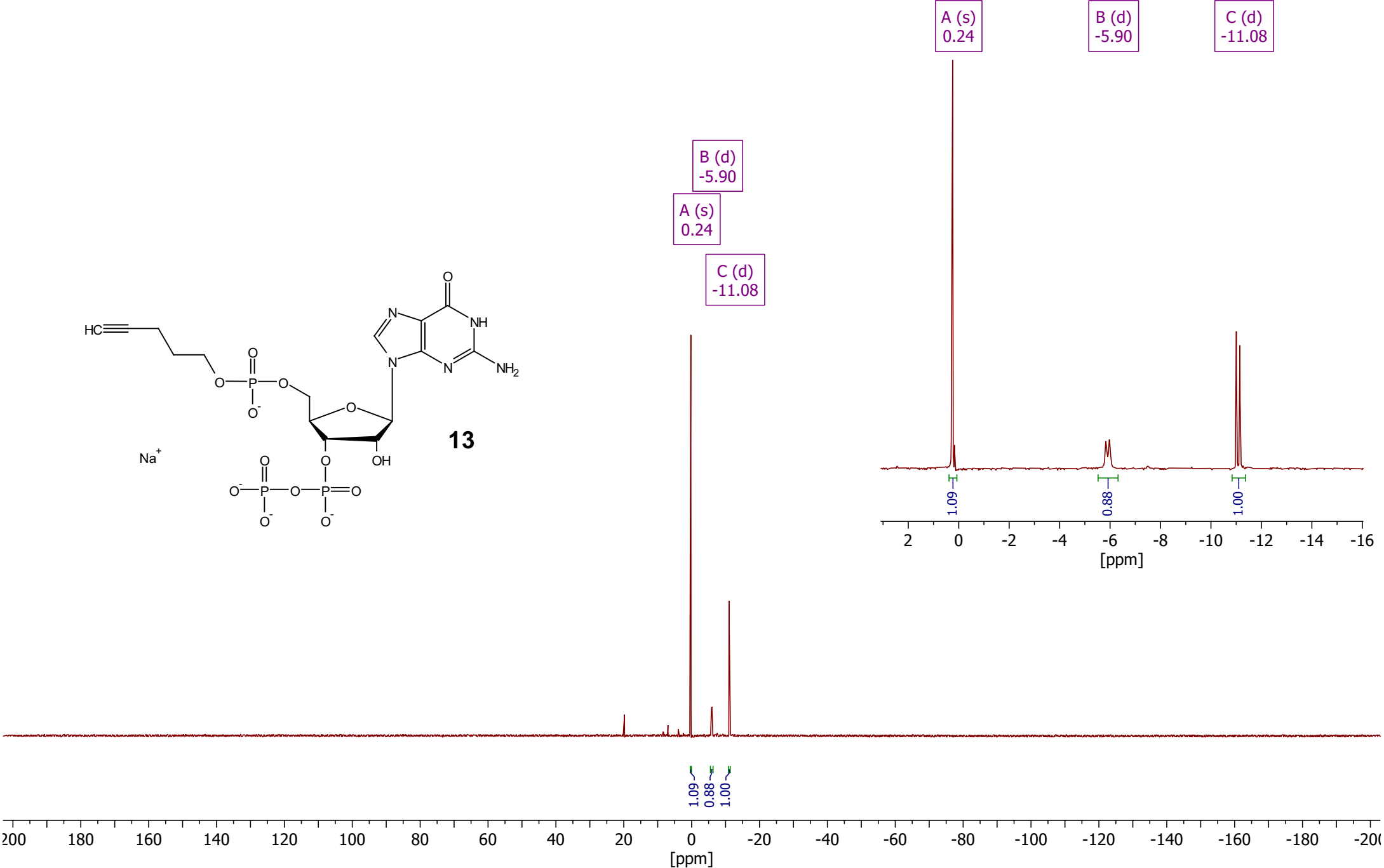


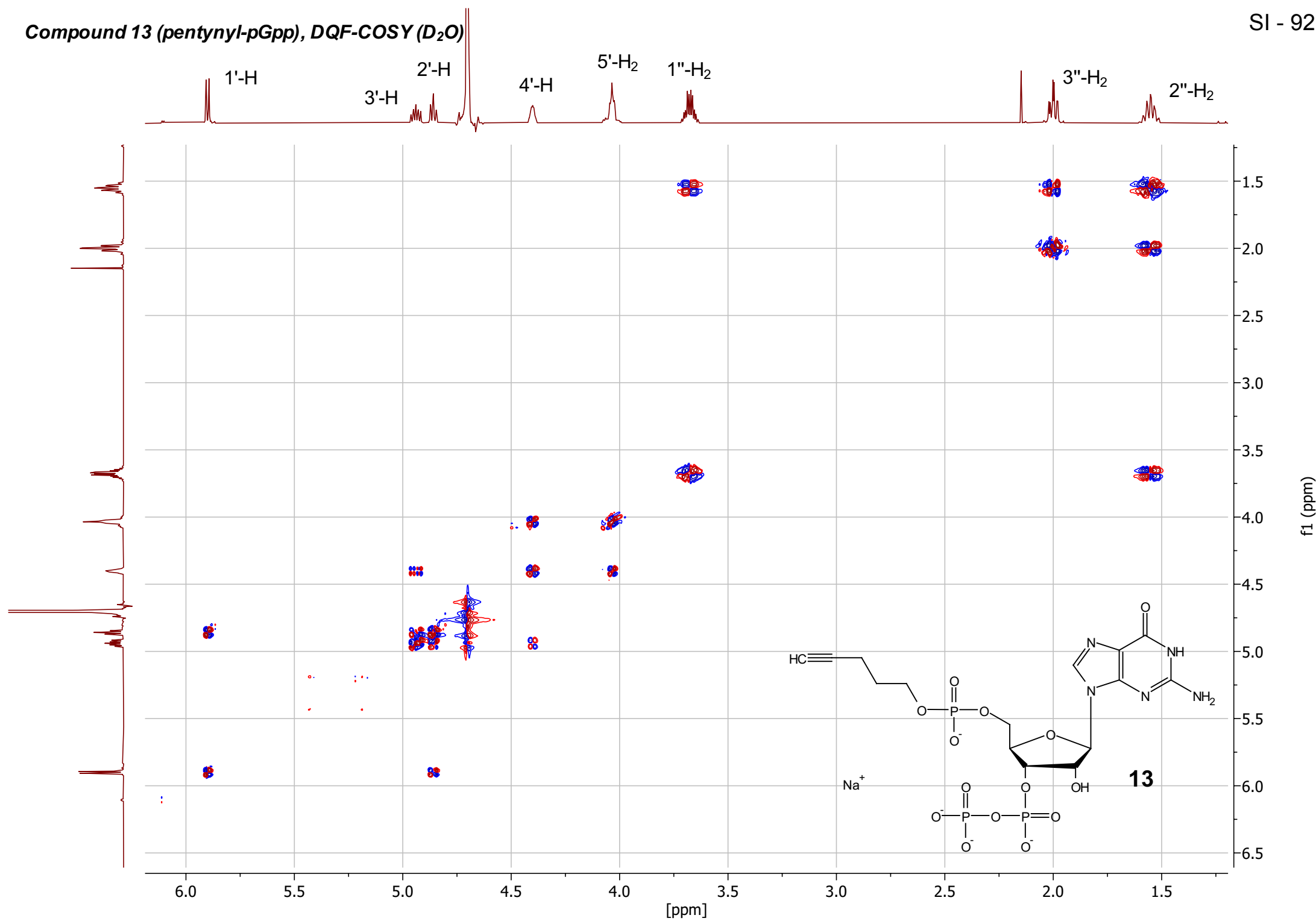


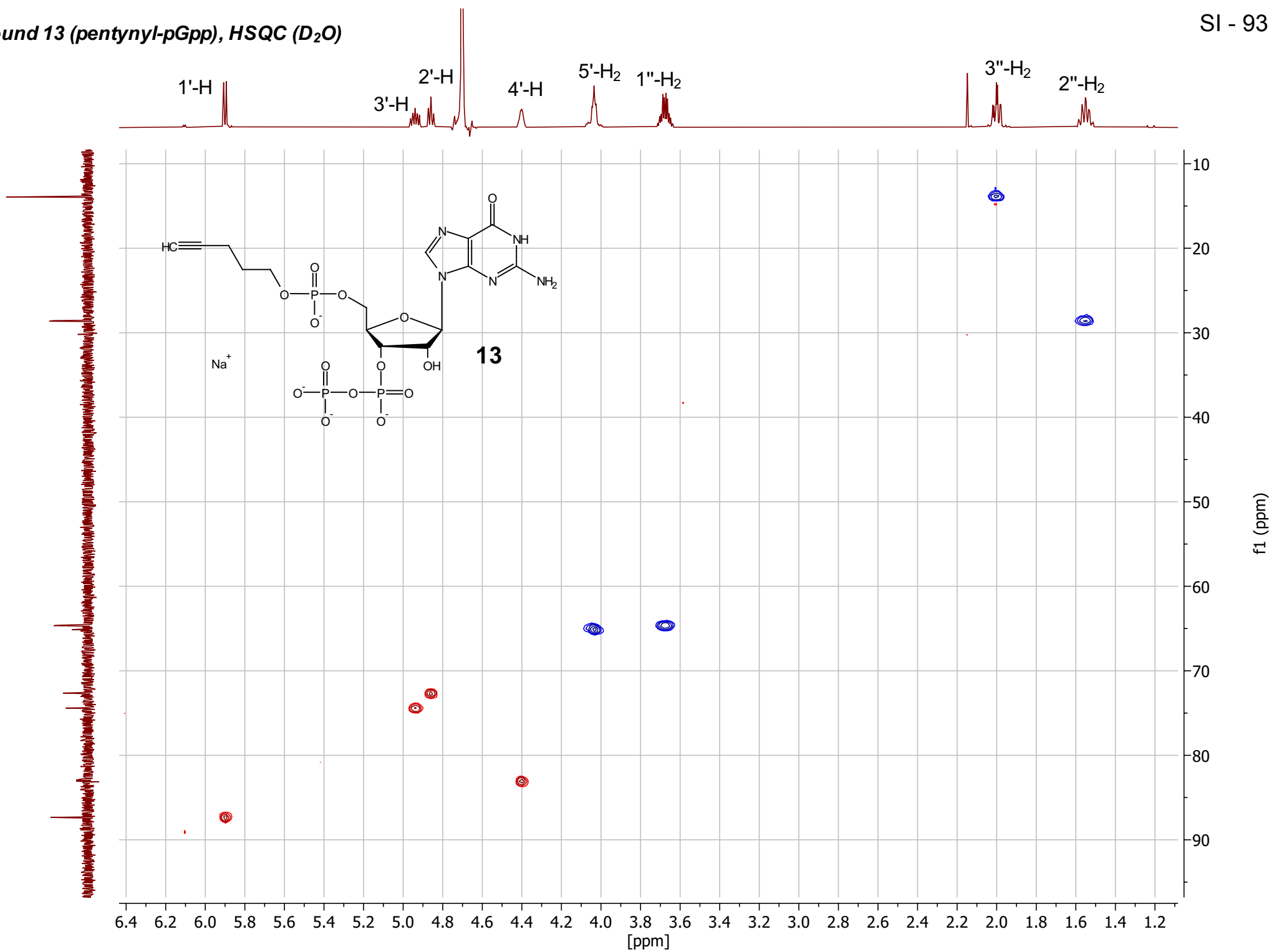


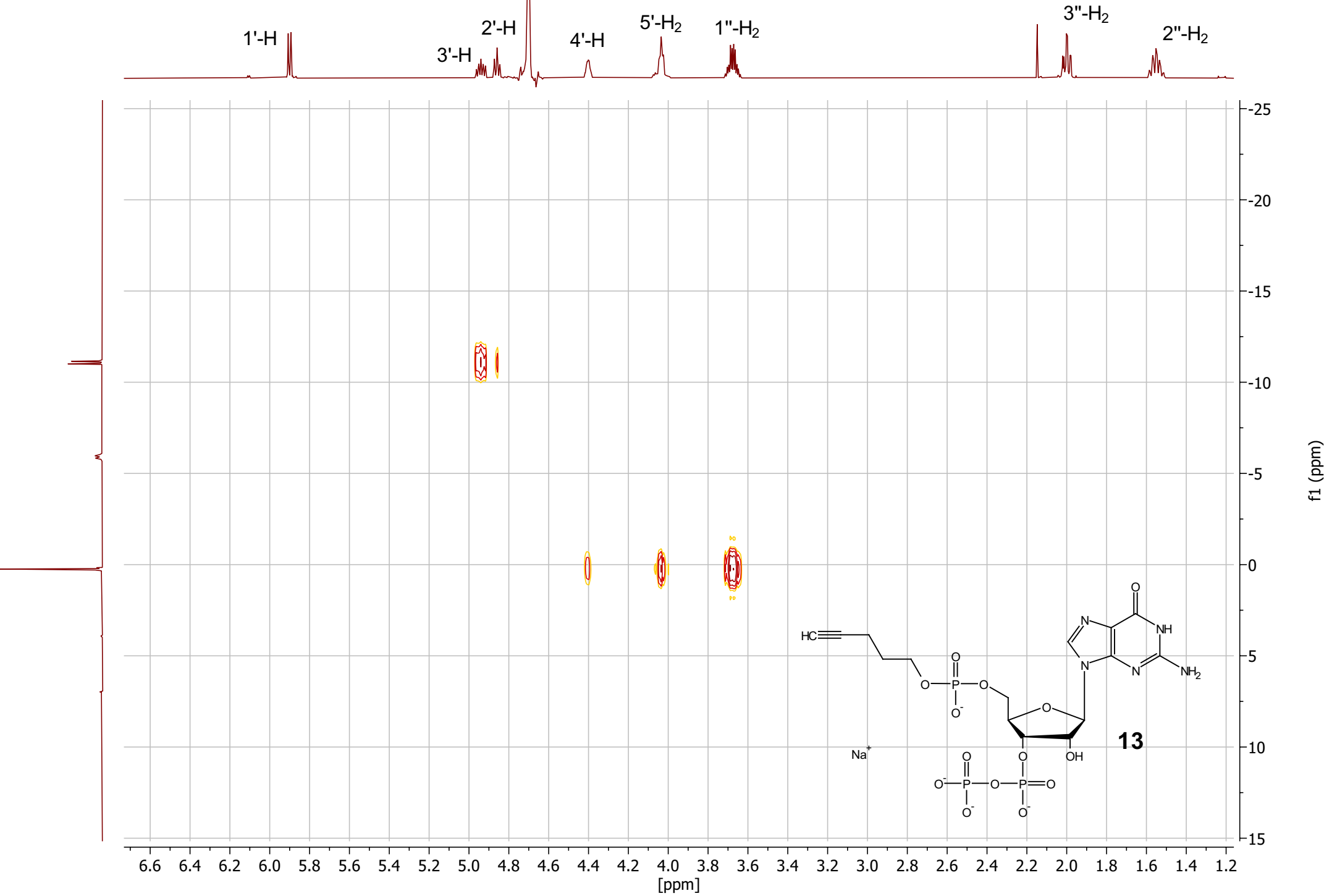


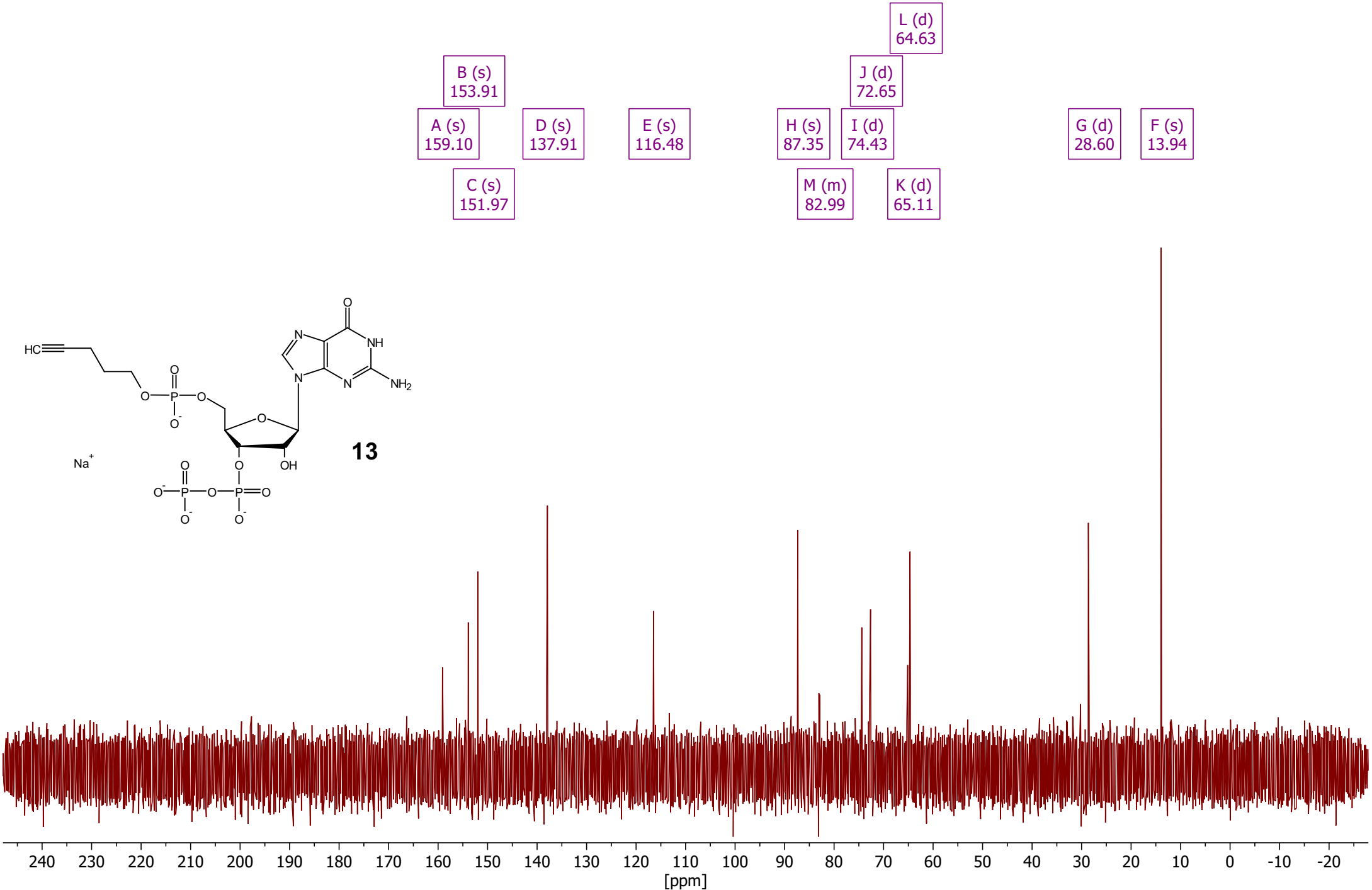


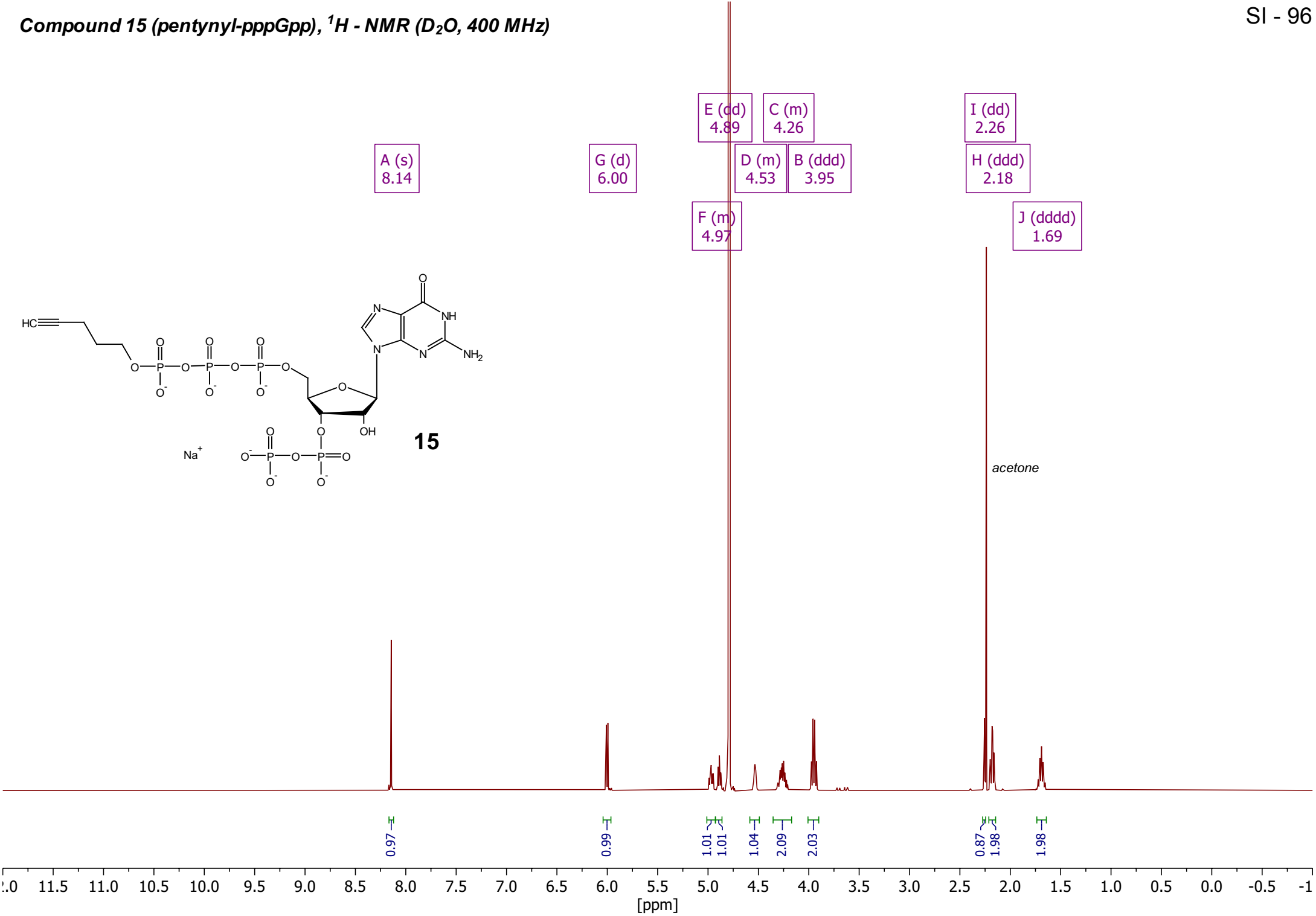


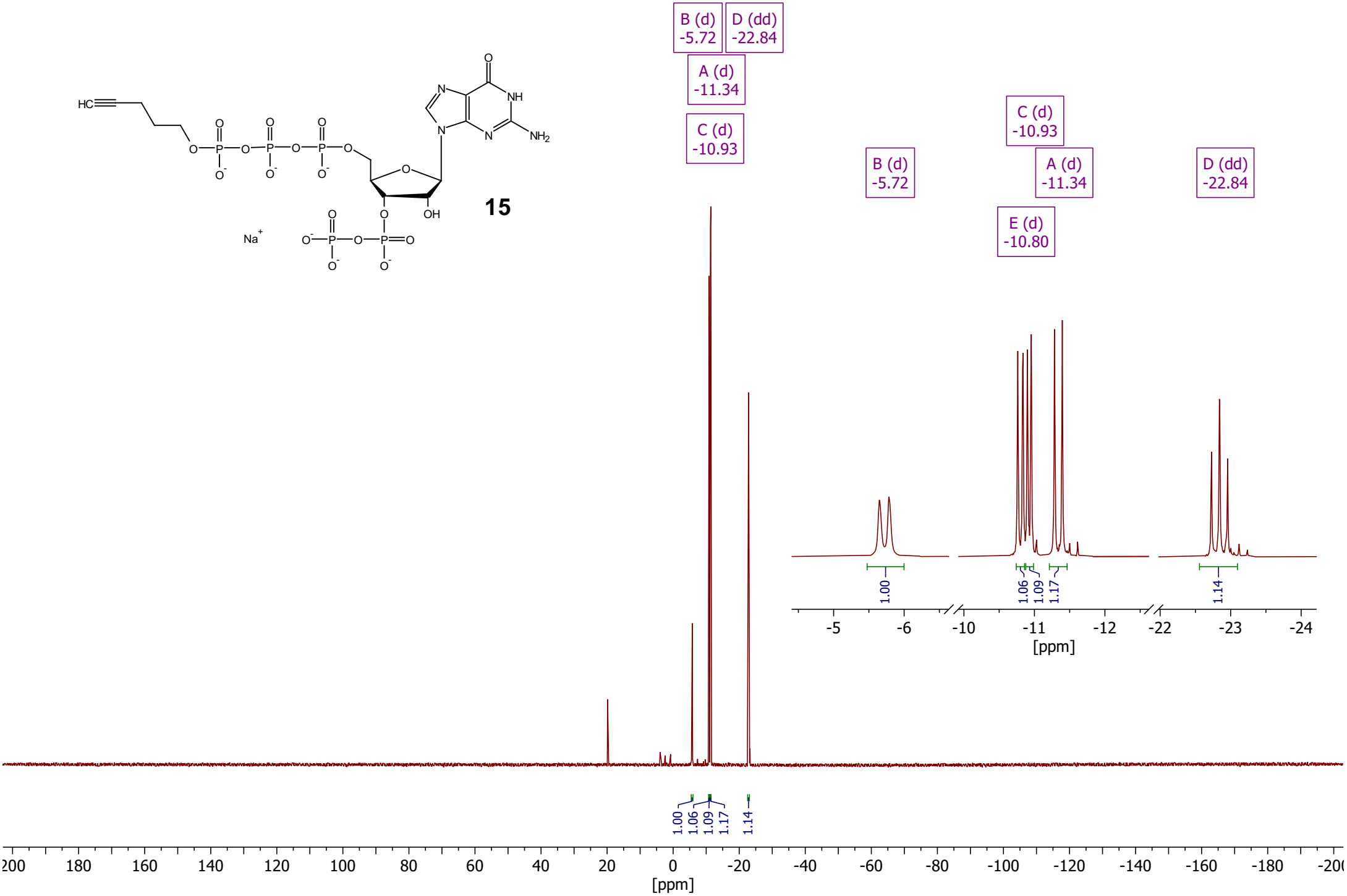


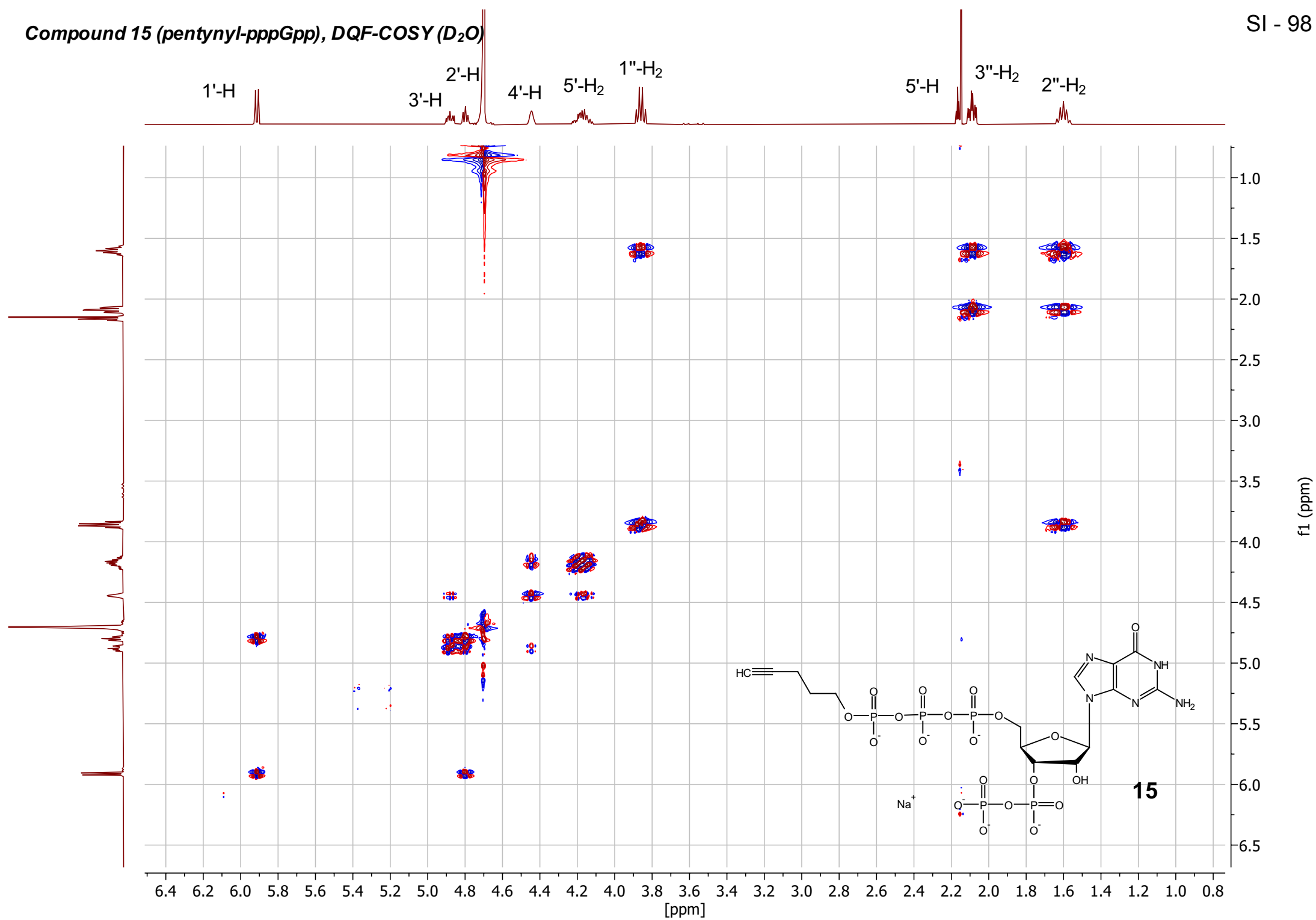


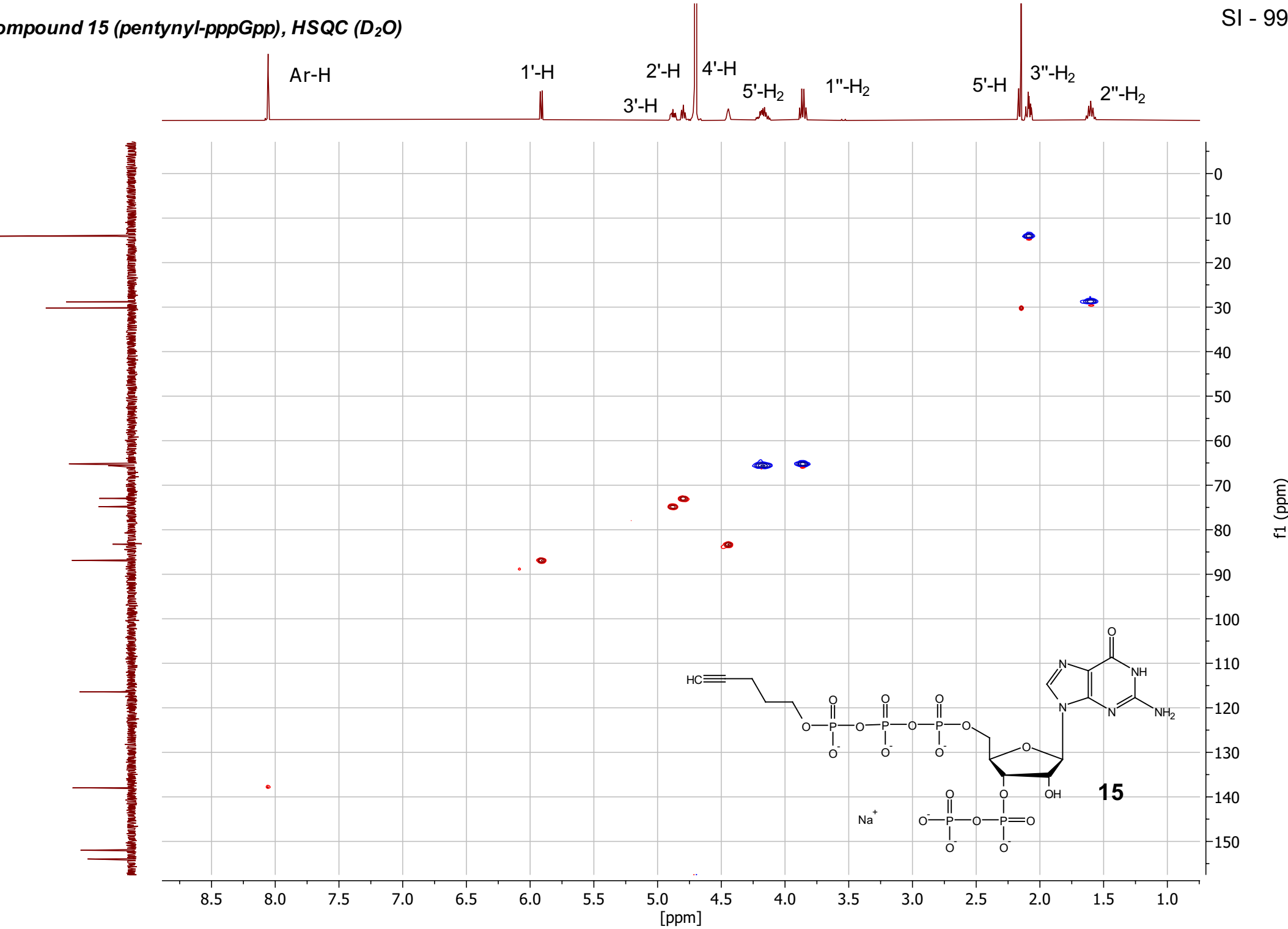


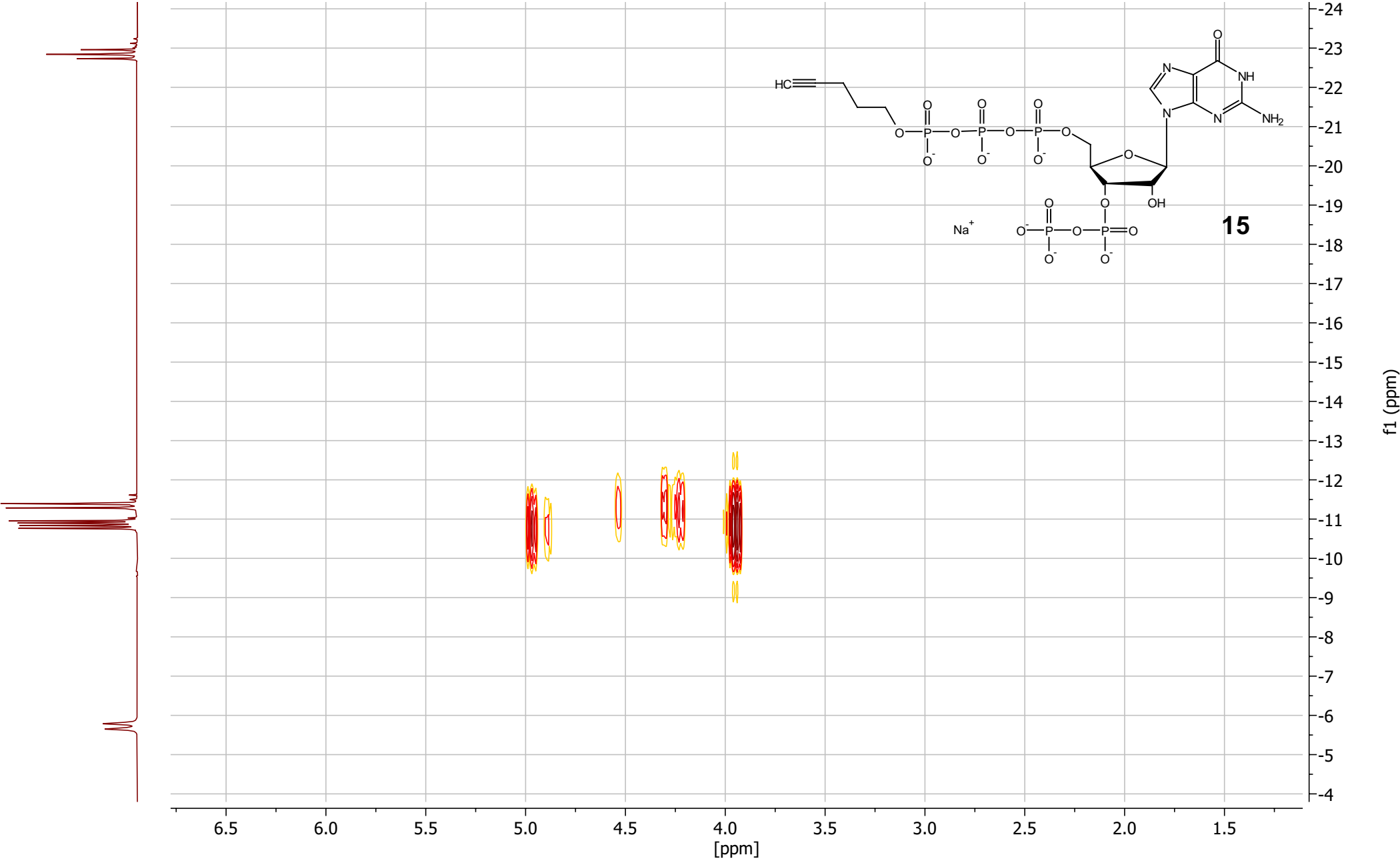


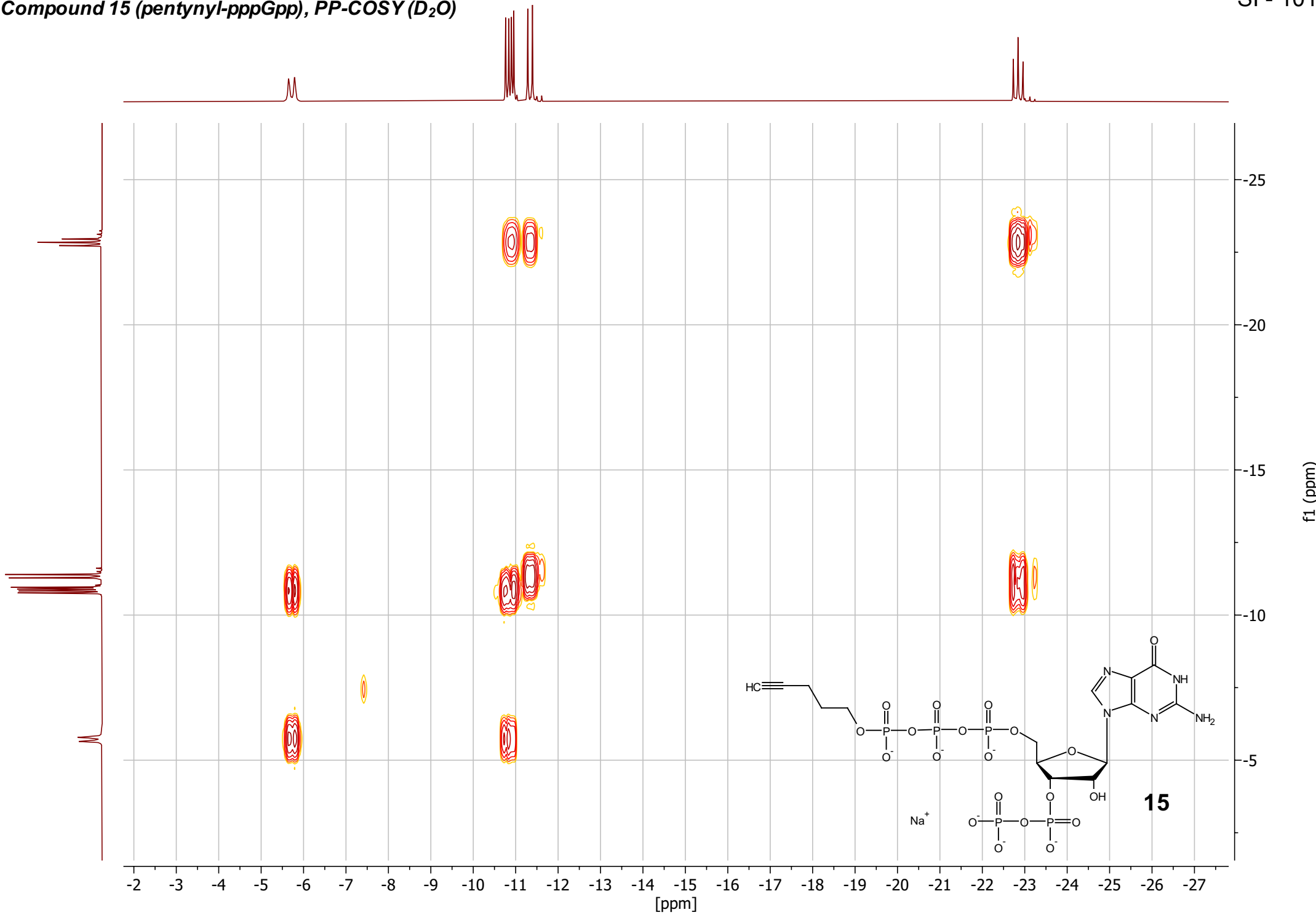


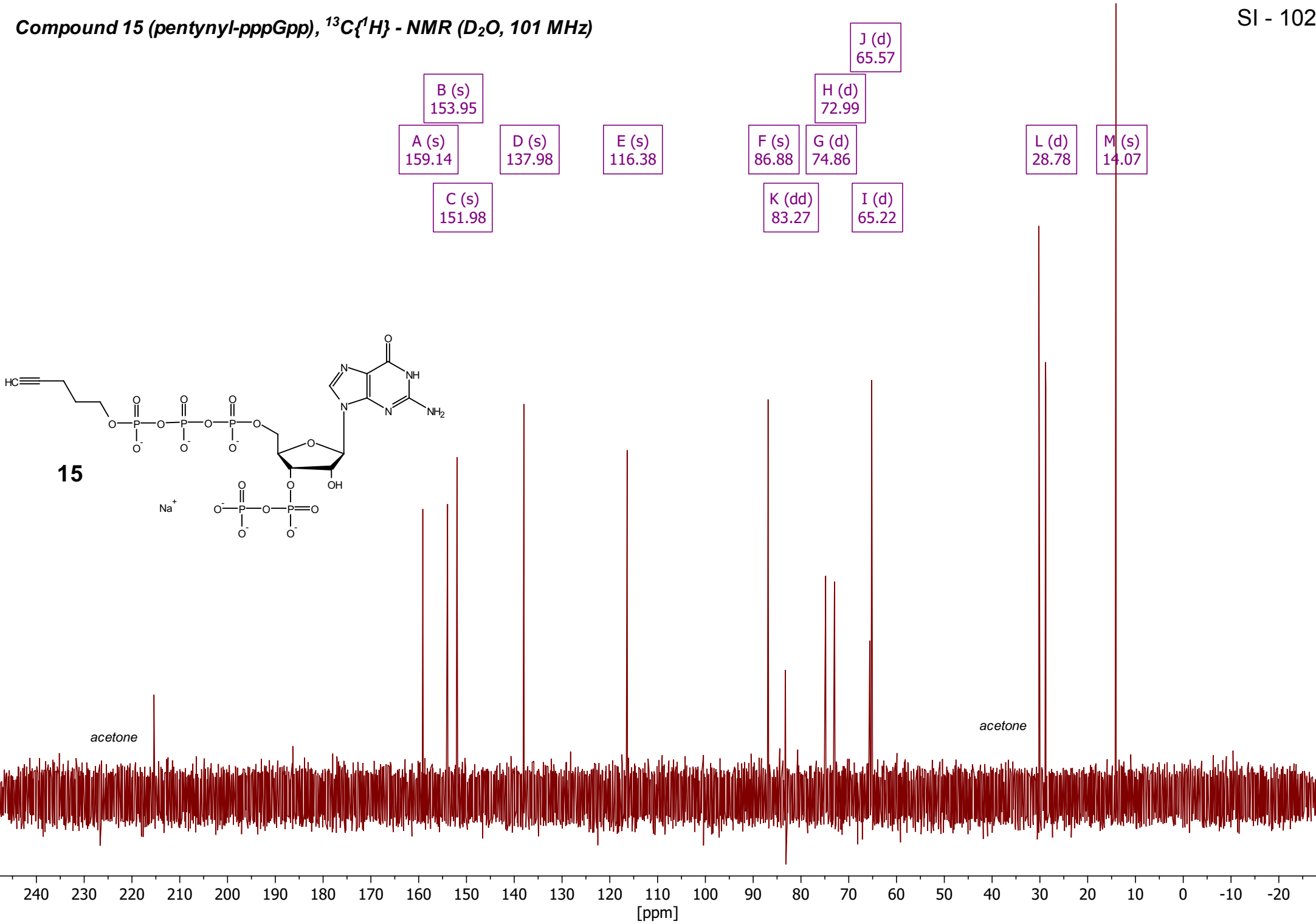
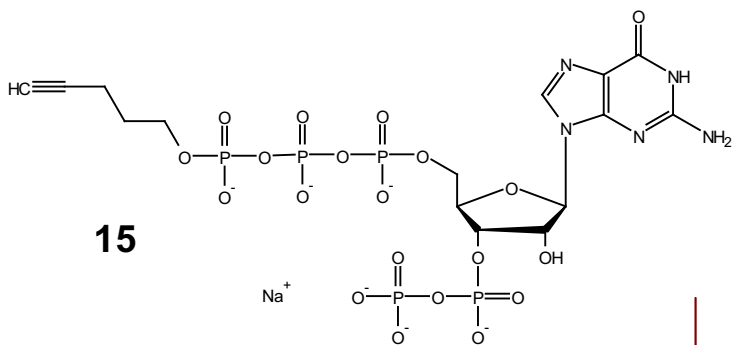


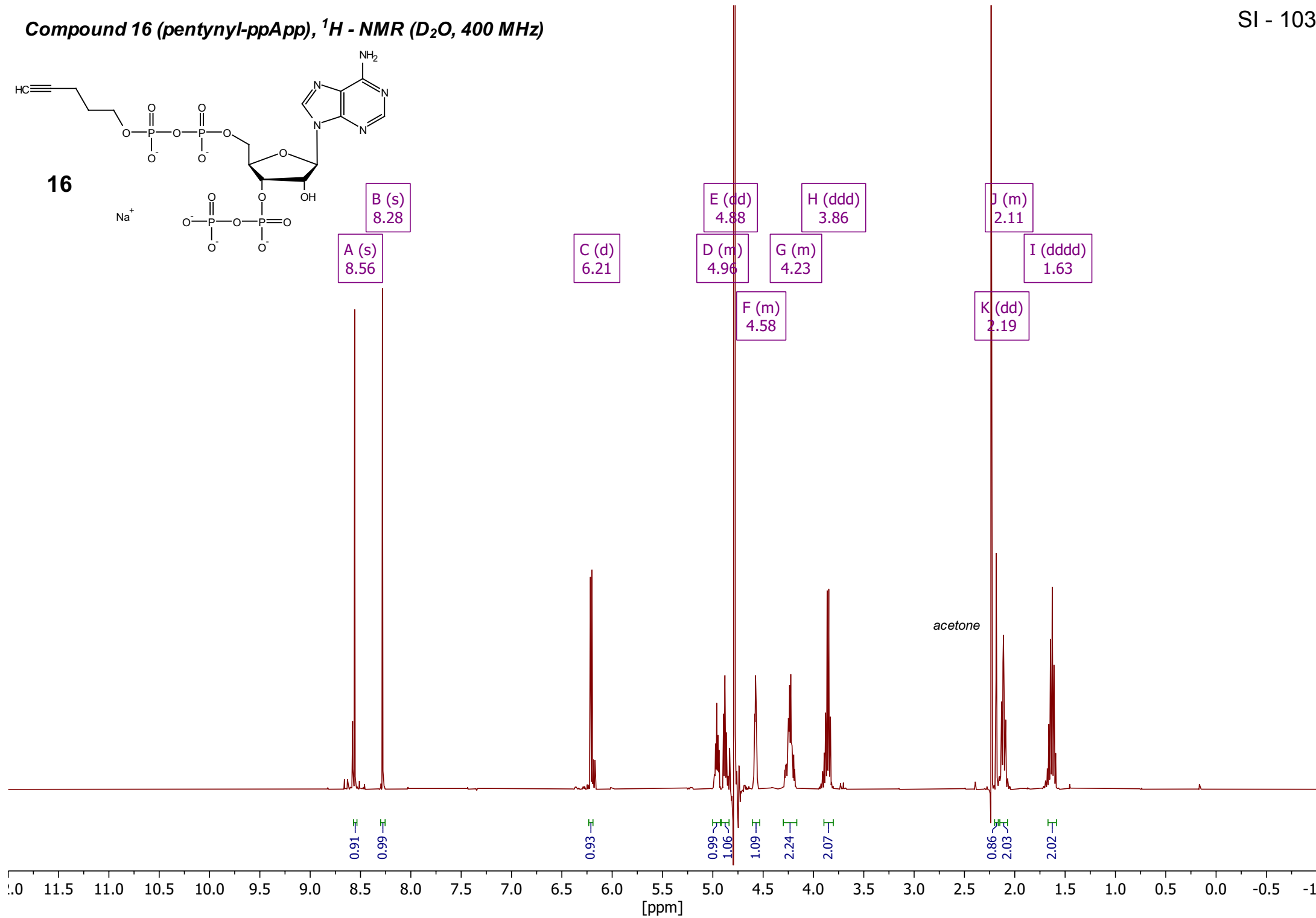
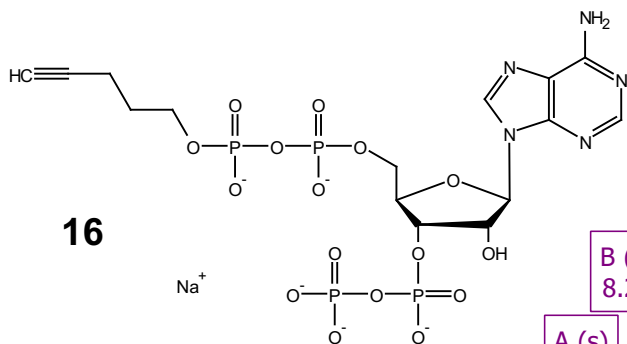


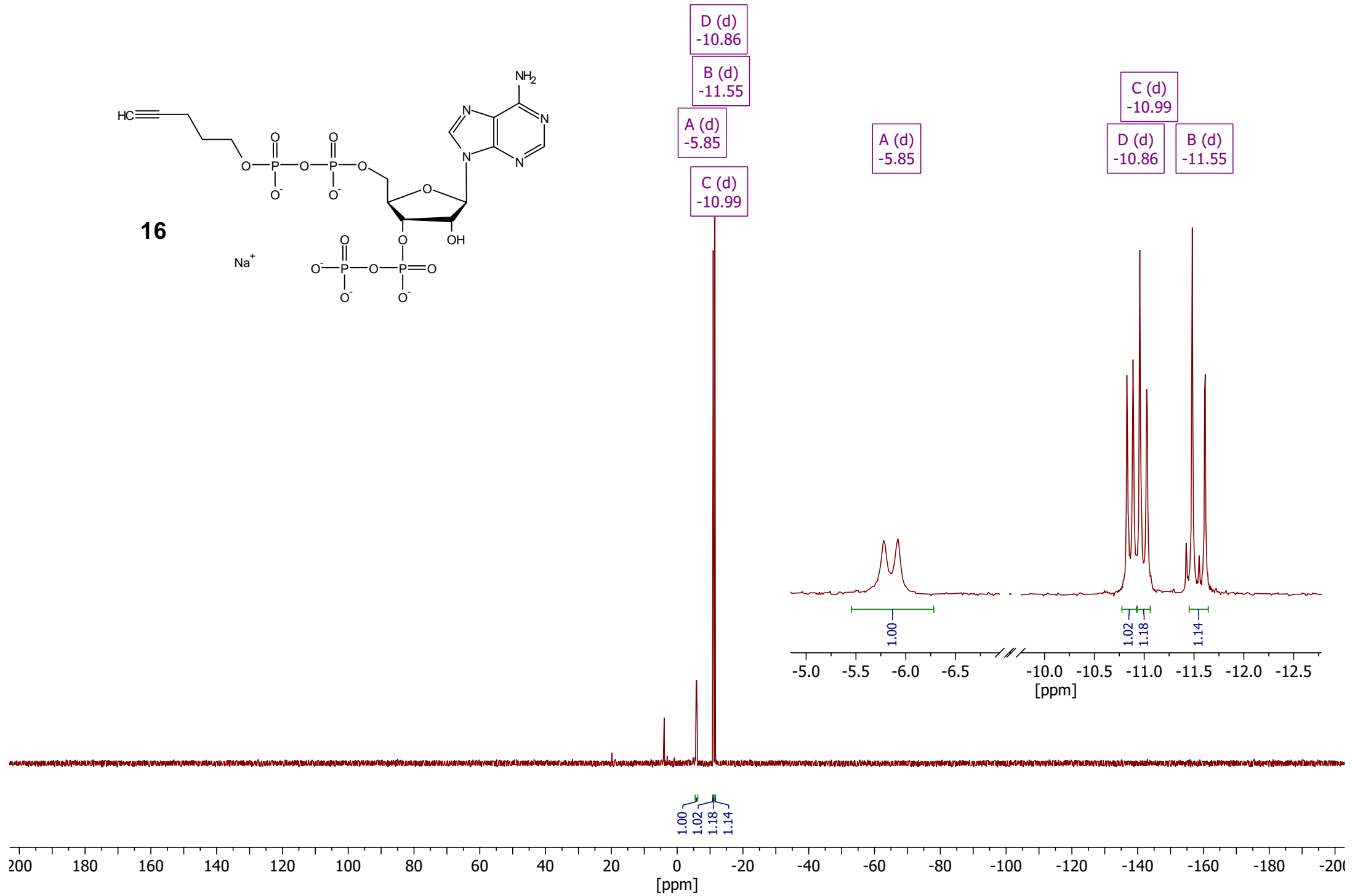


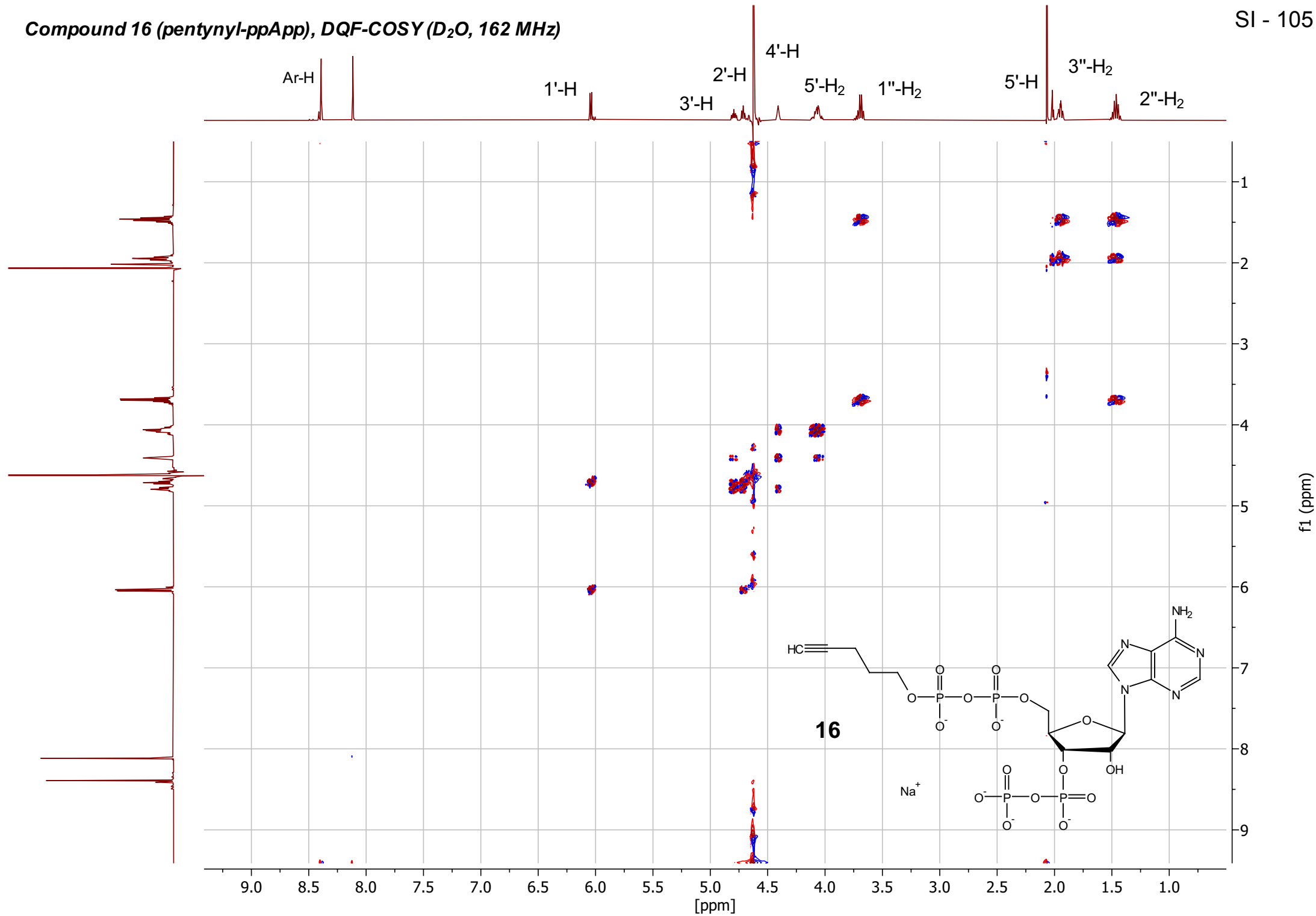


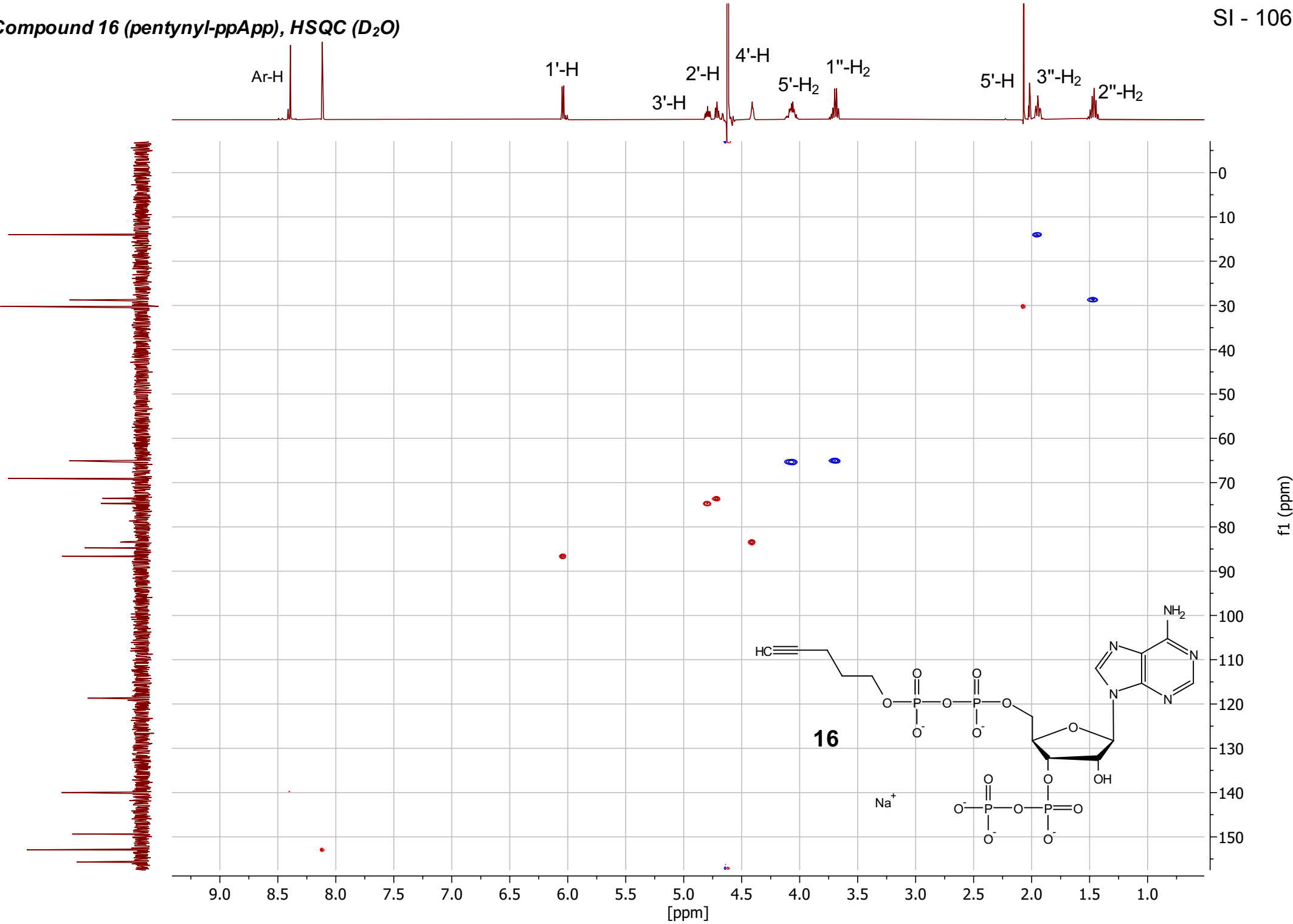


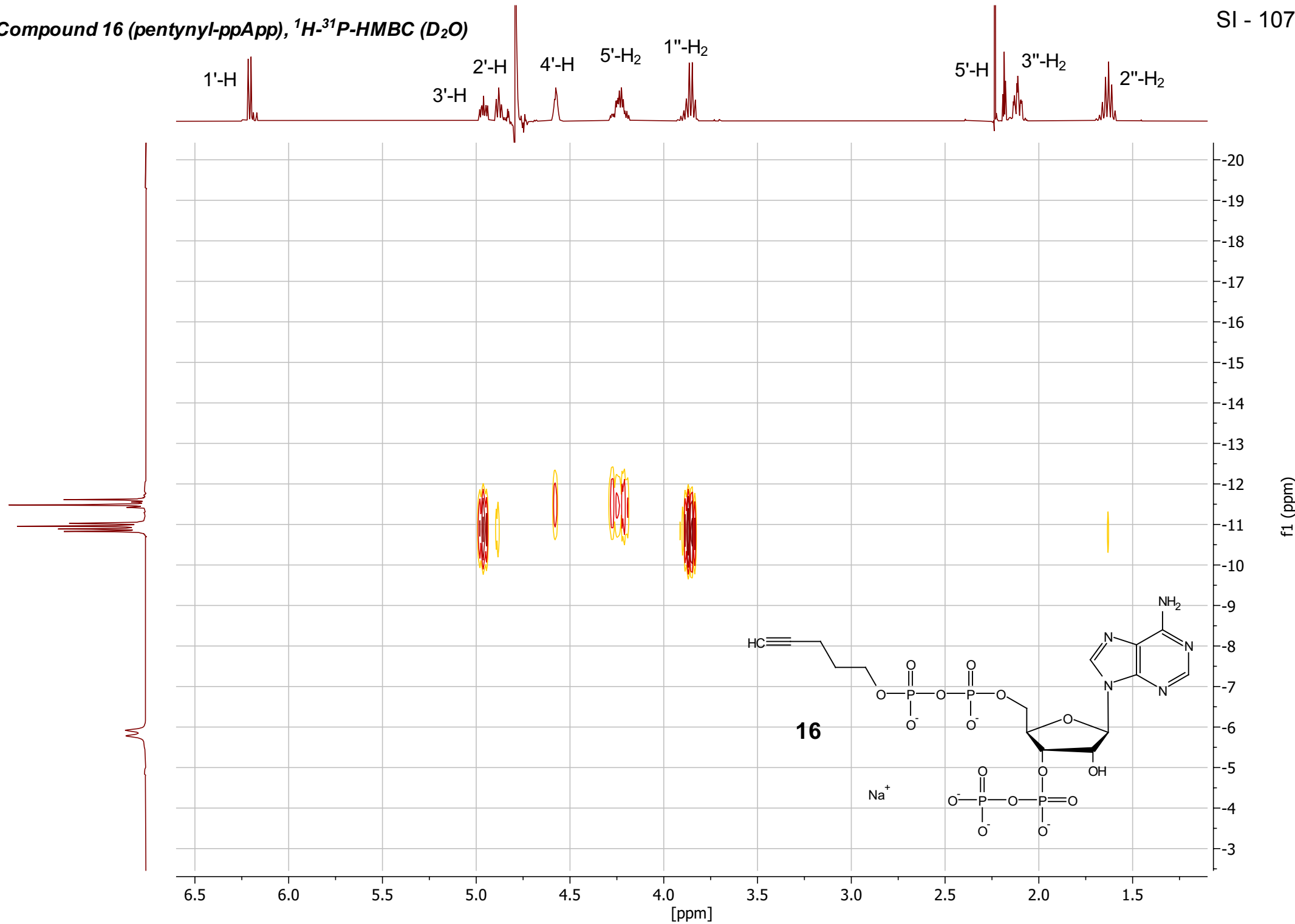


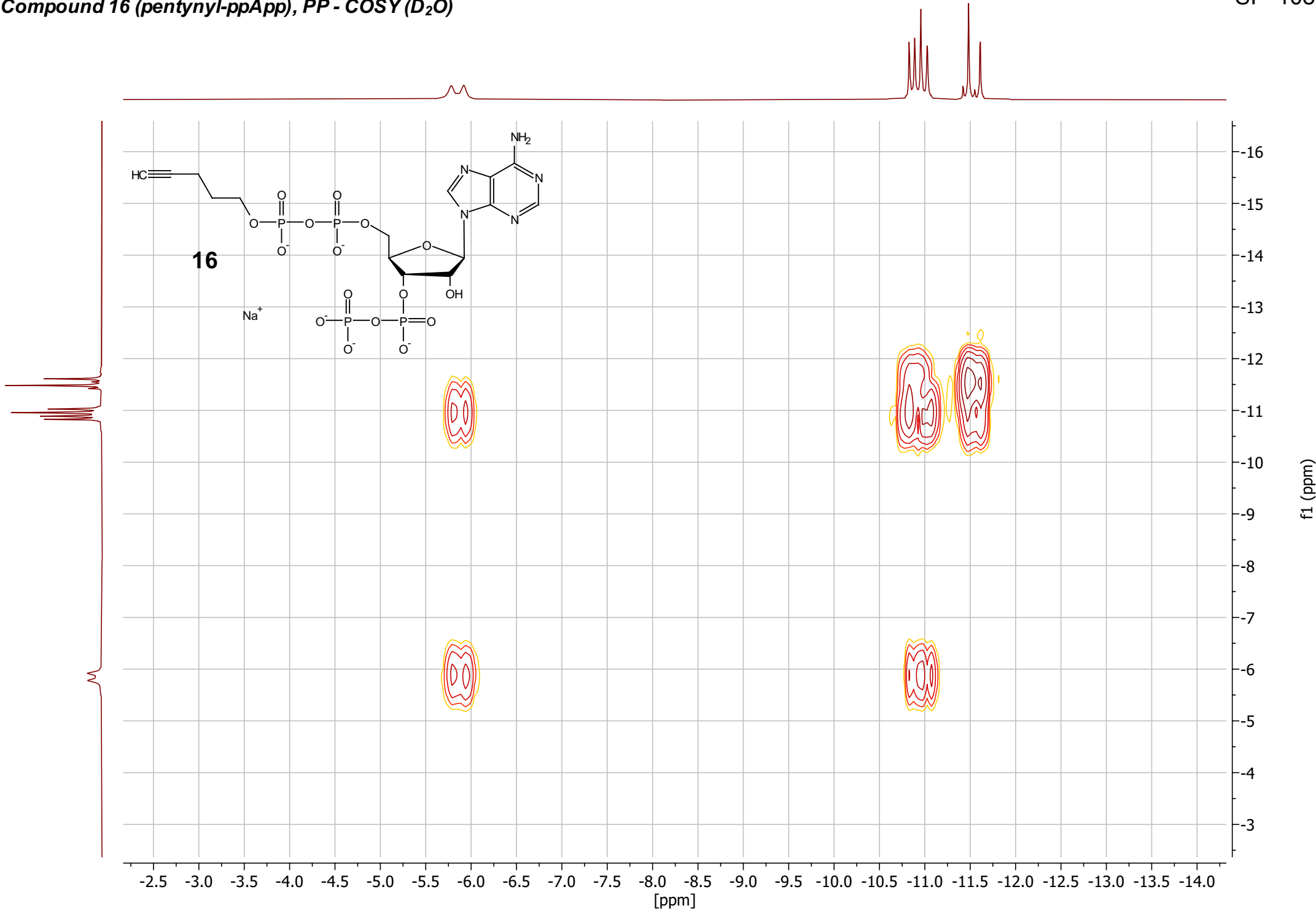


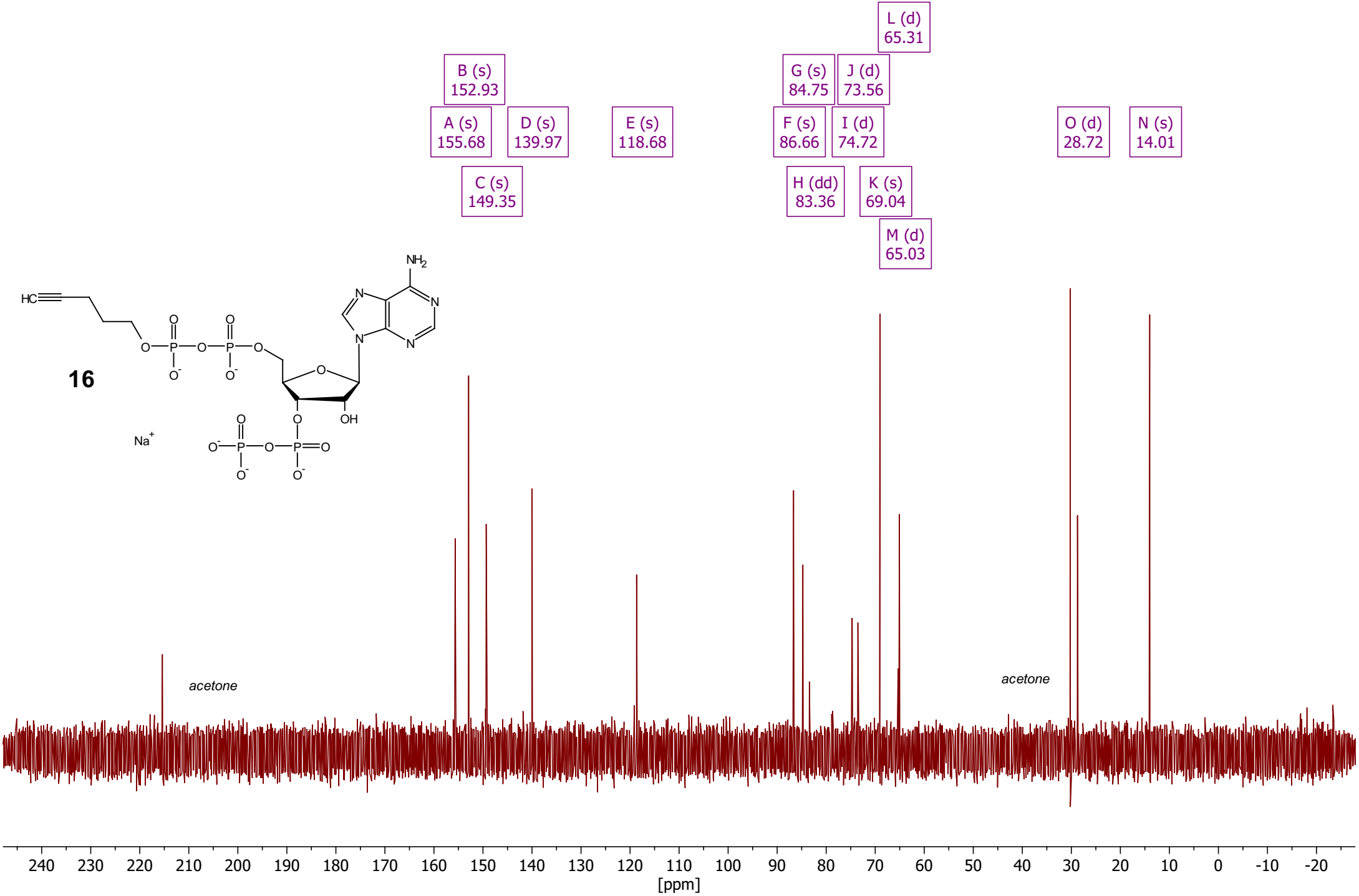


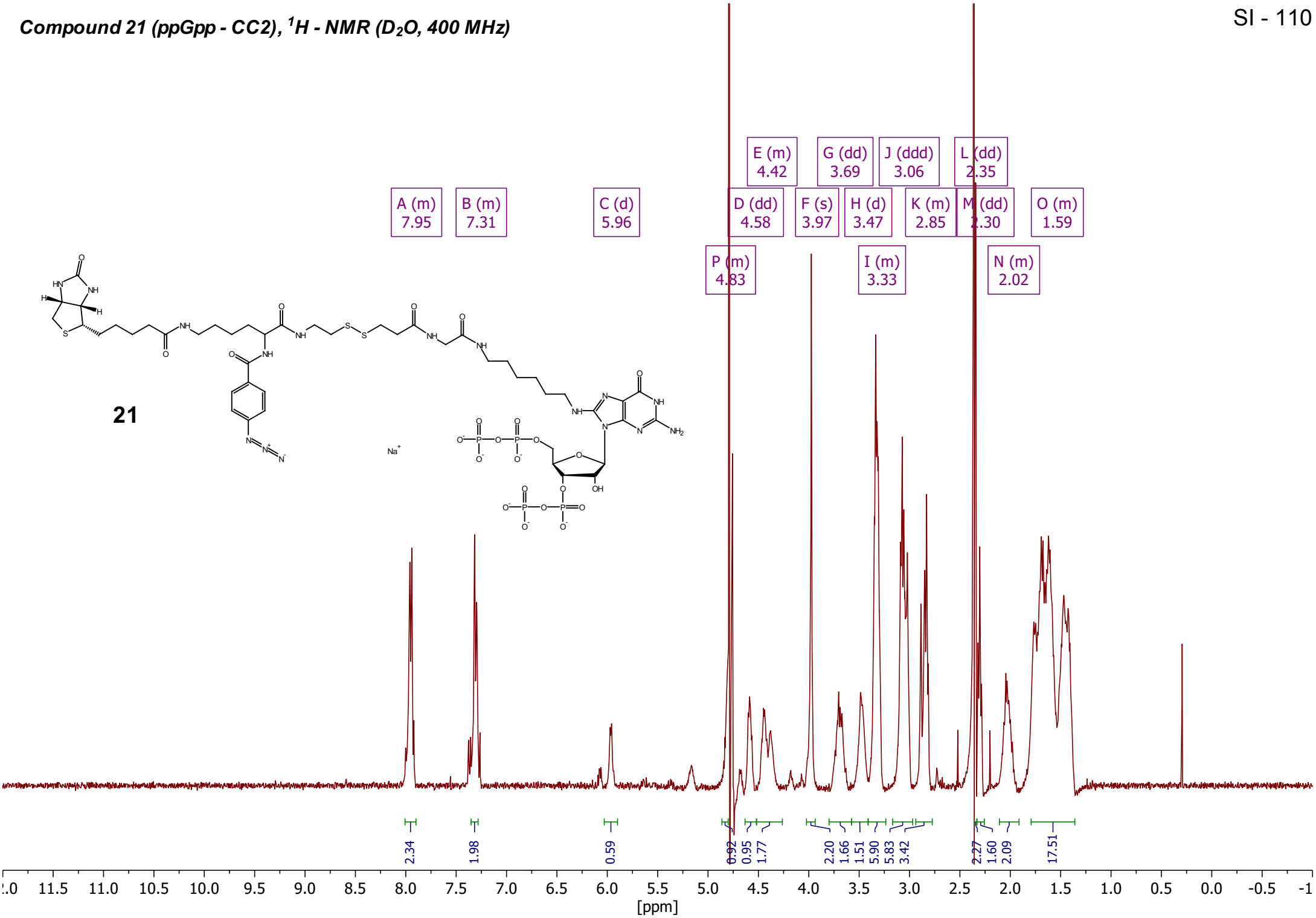


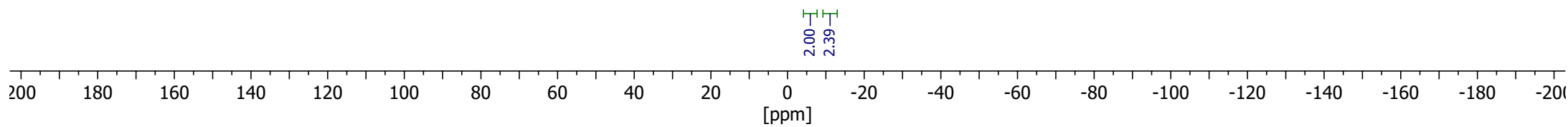
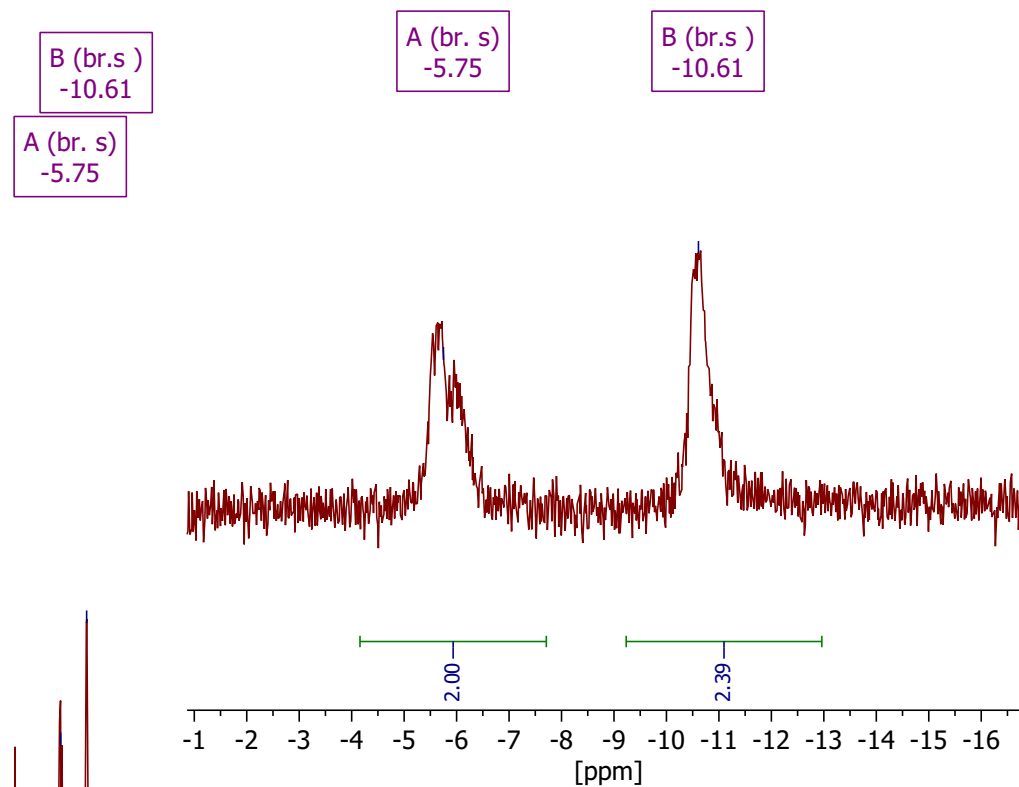


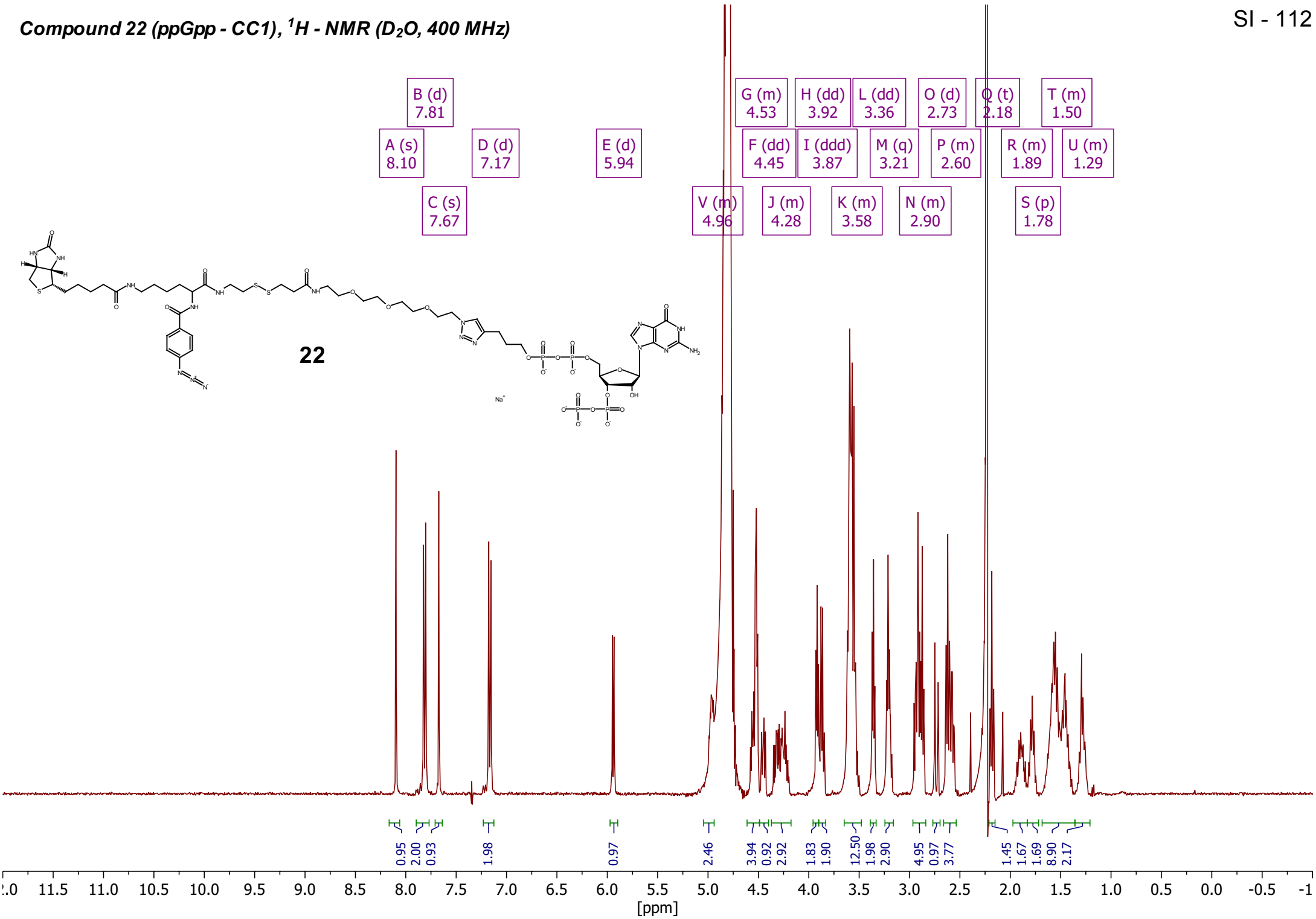


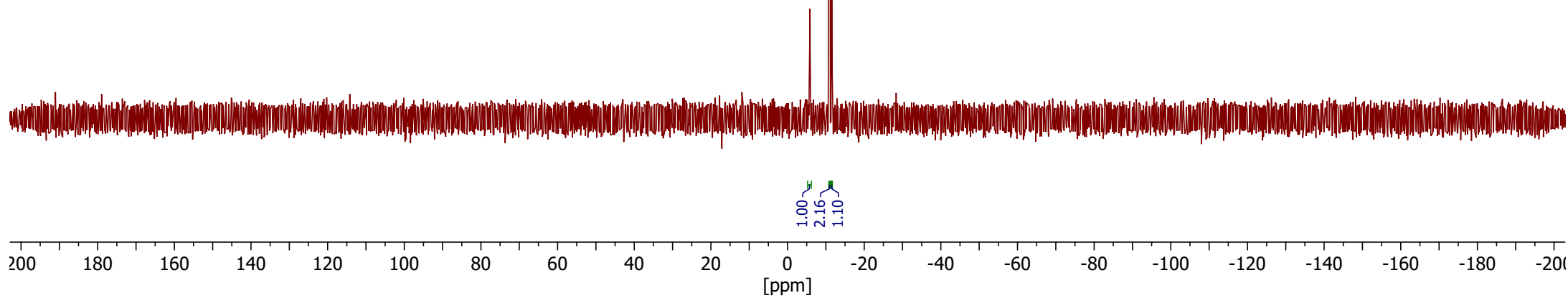
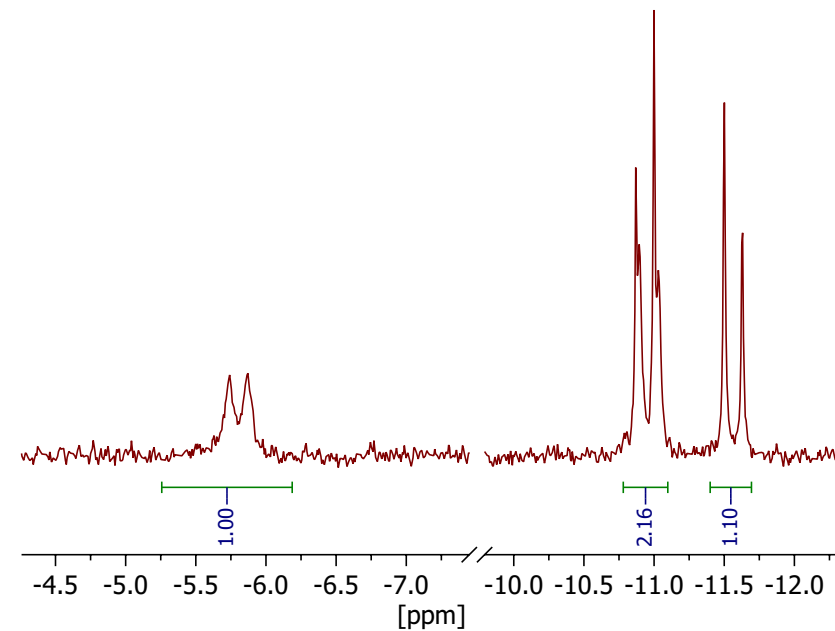
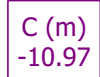


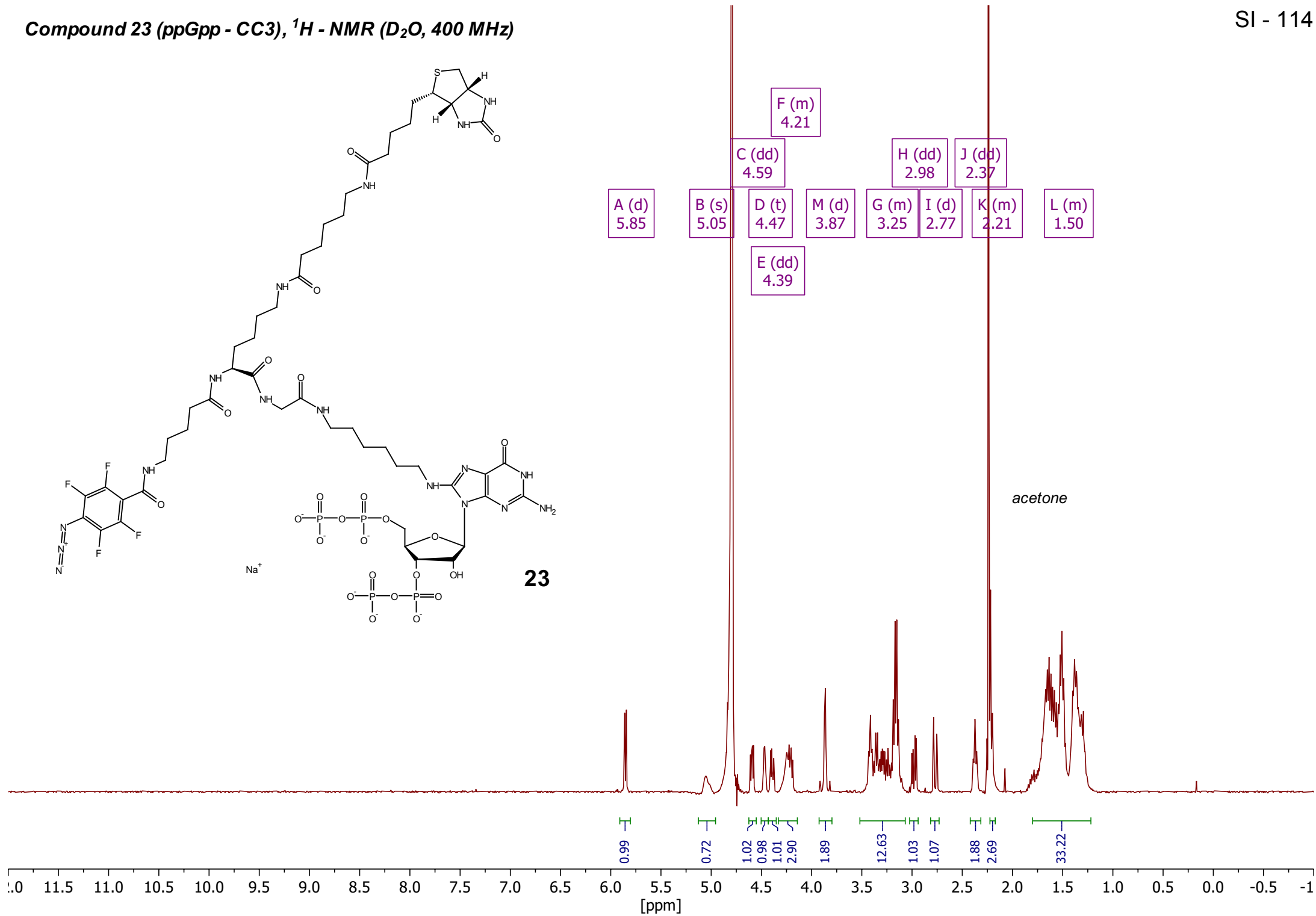


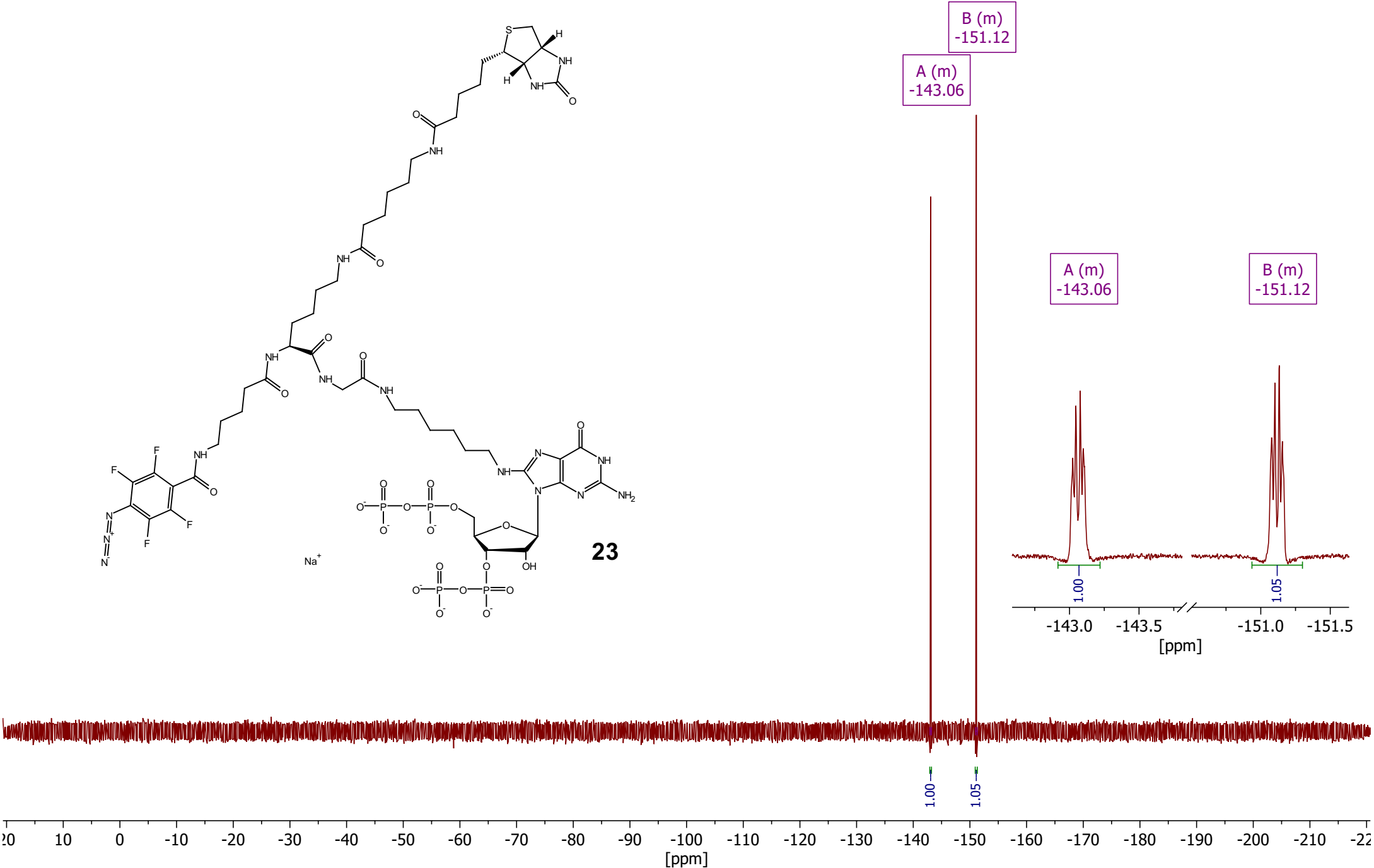


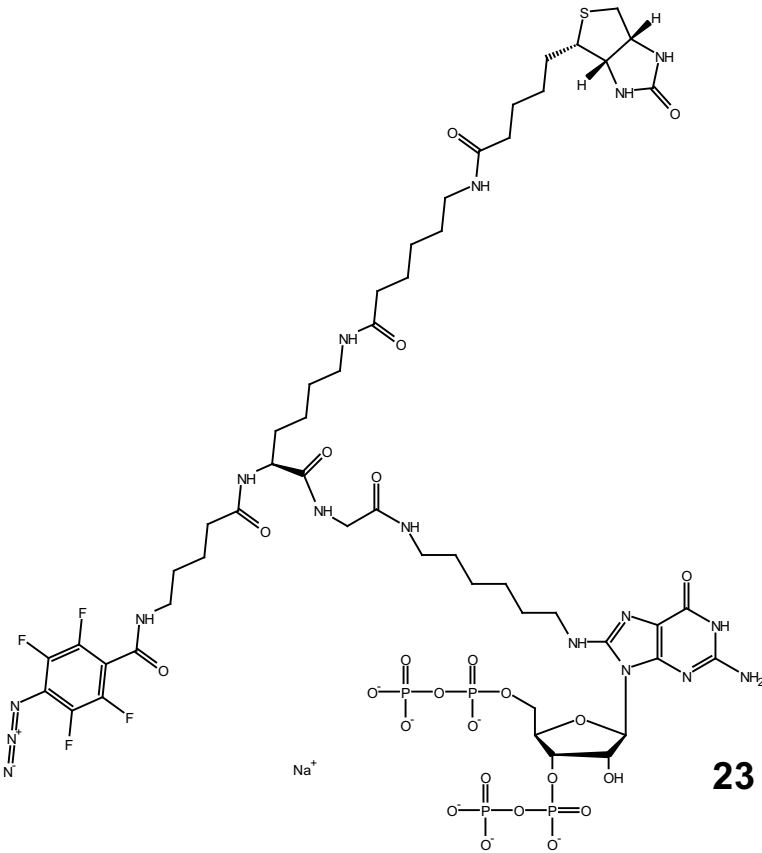












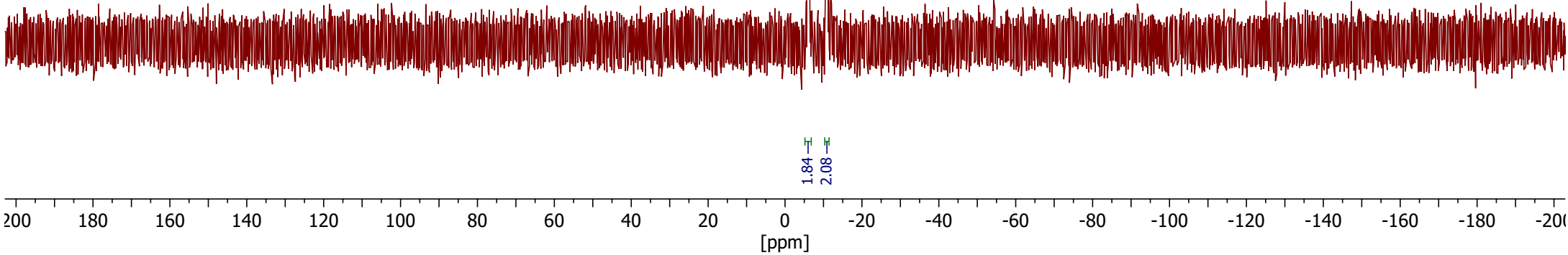
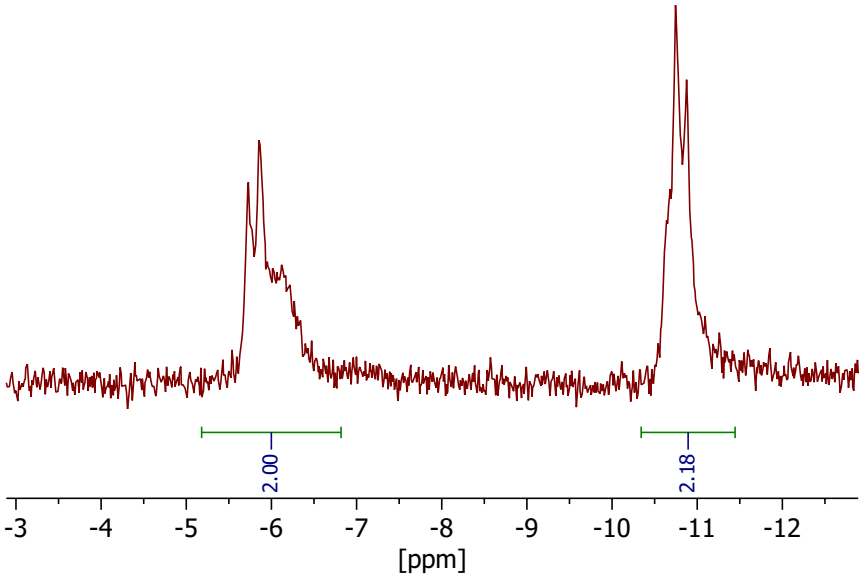
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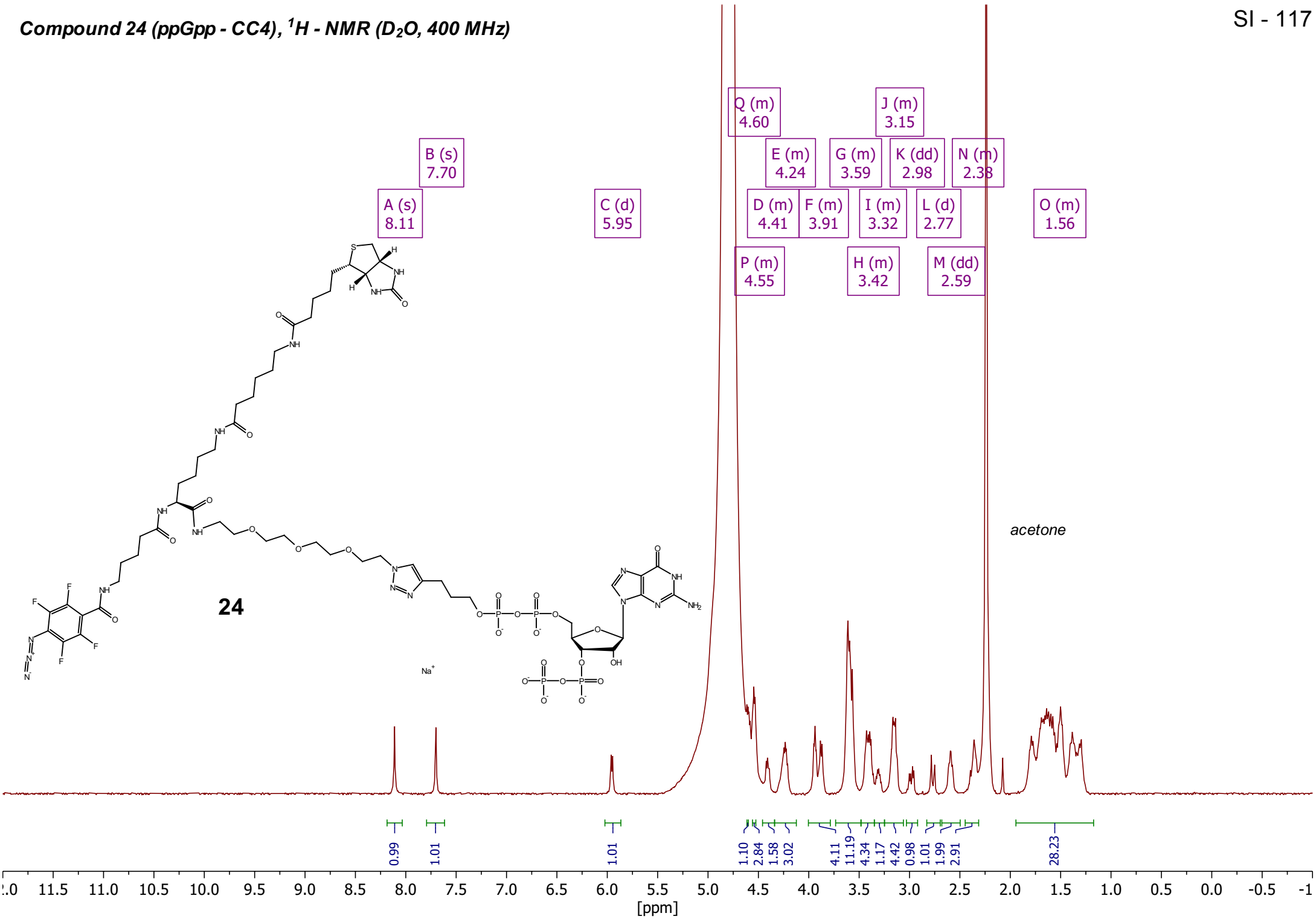
B (d)
-10.81

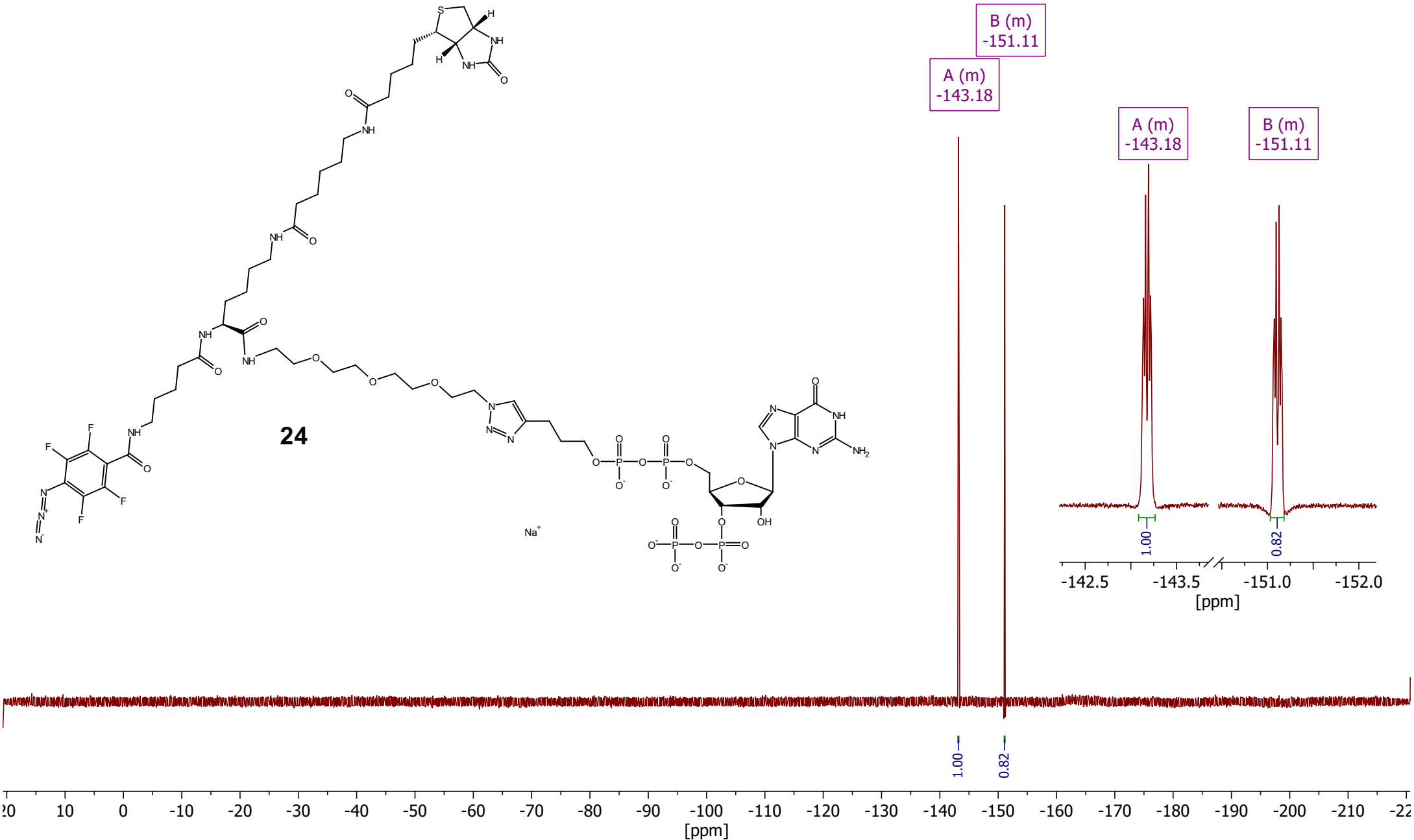
A (d)
-5.79

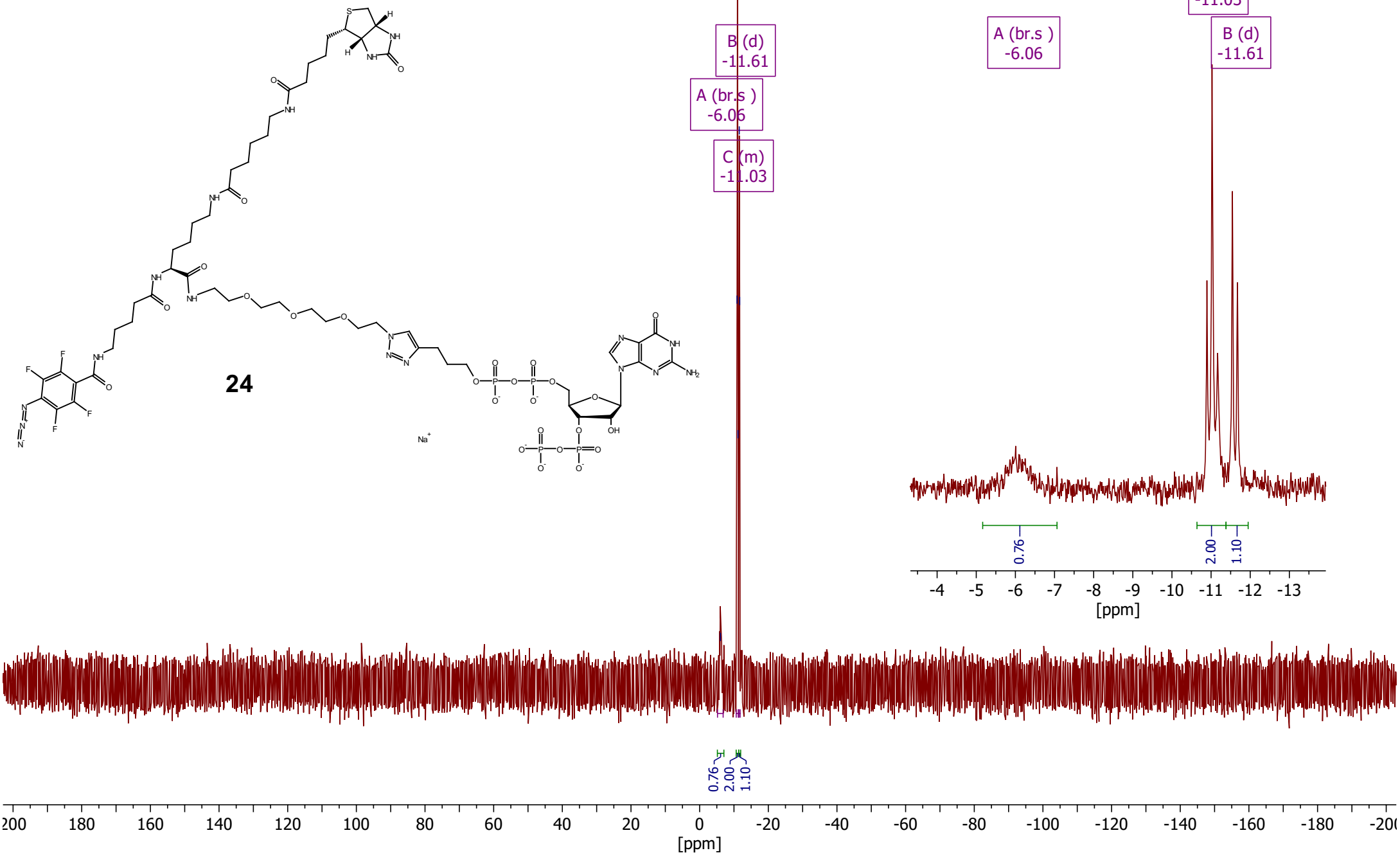
A (d)
-5.79

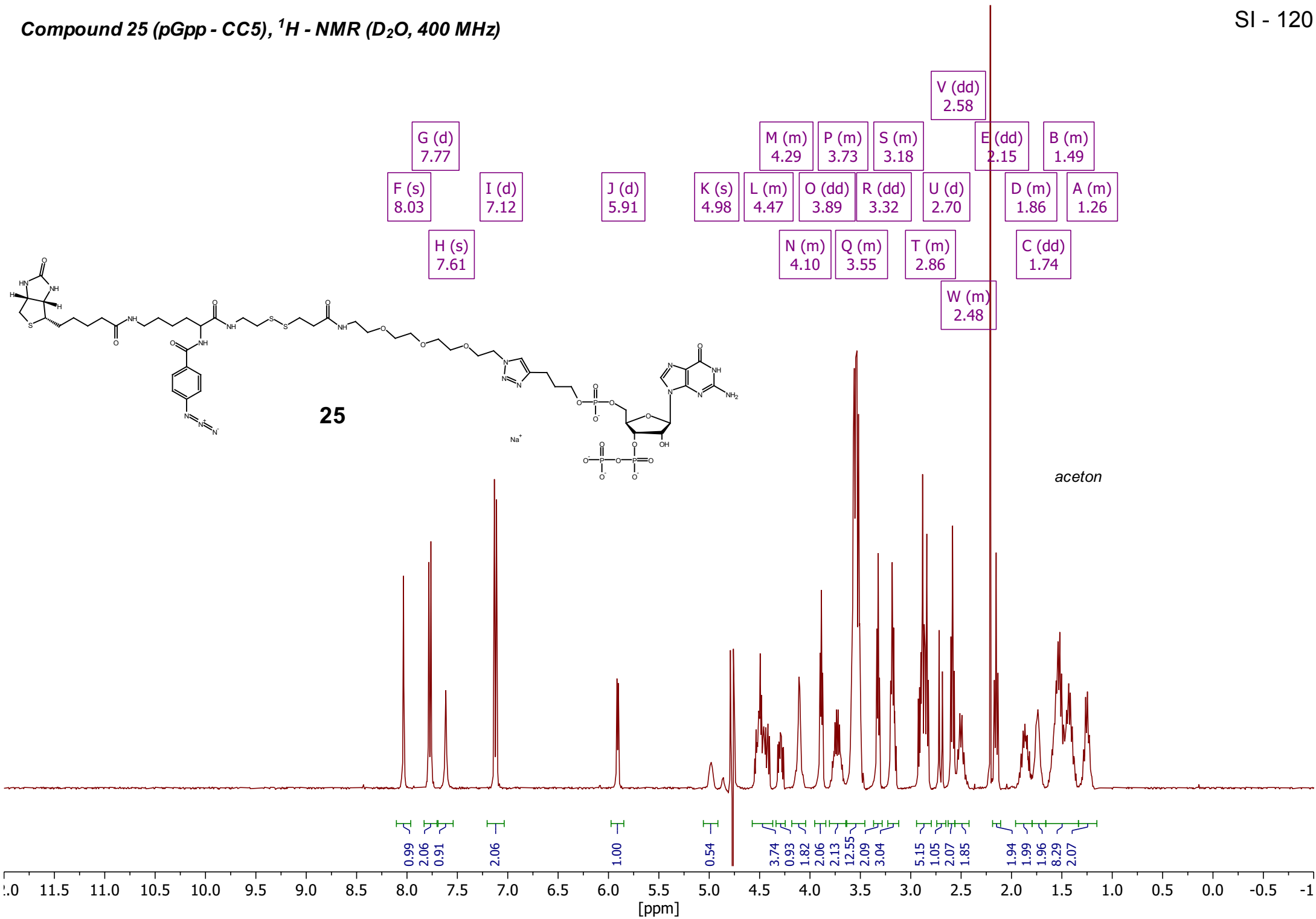
B (d)
-10.81

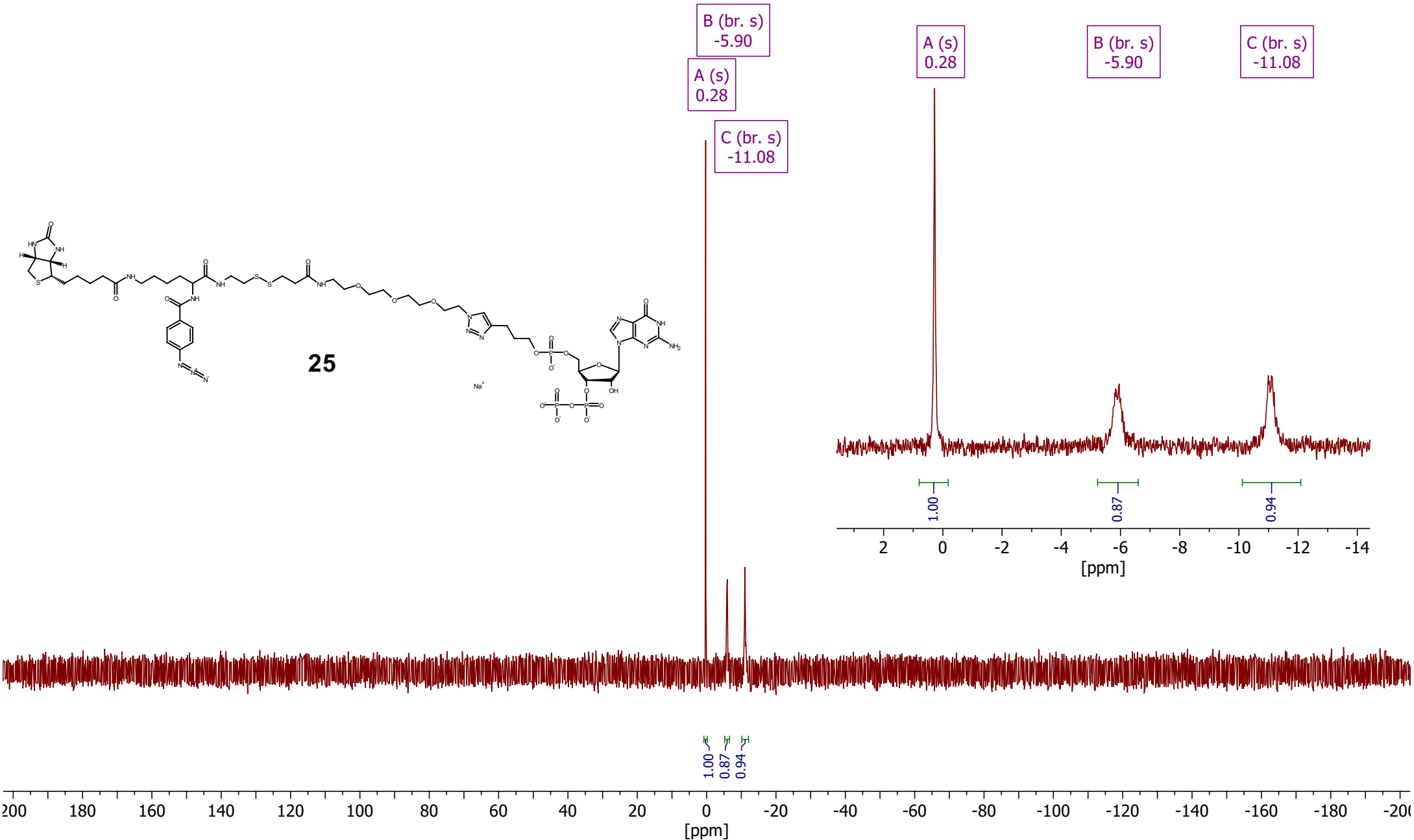


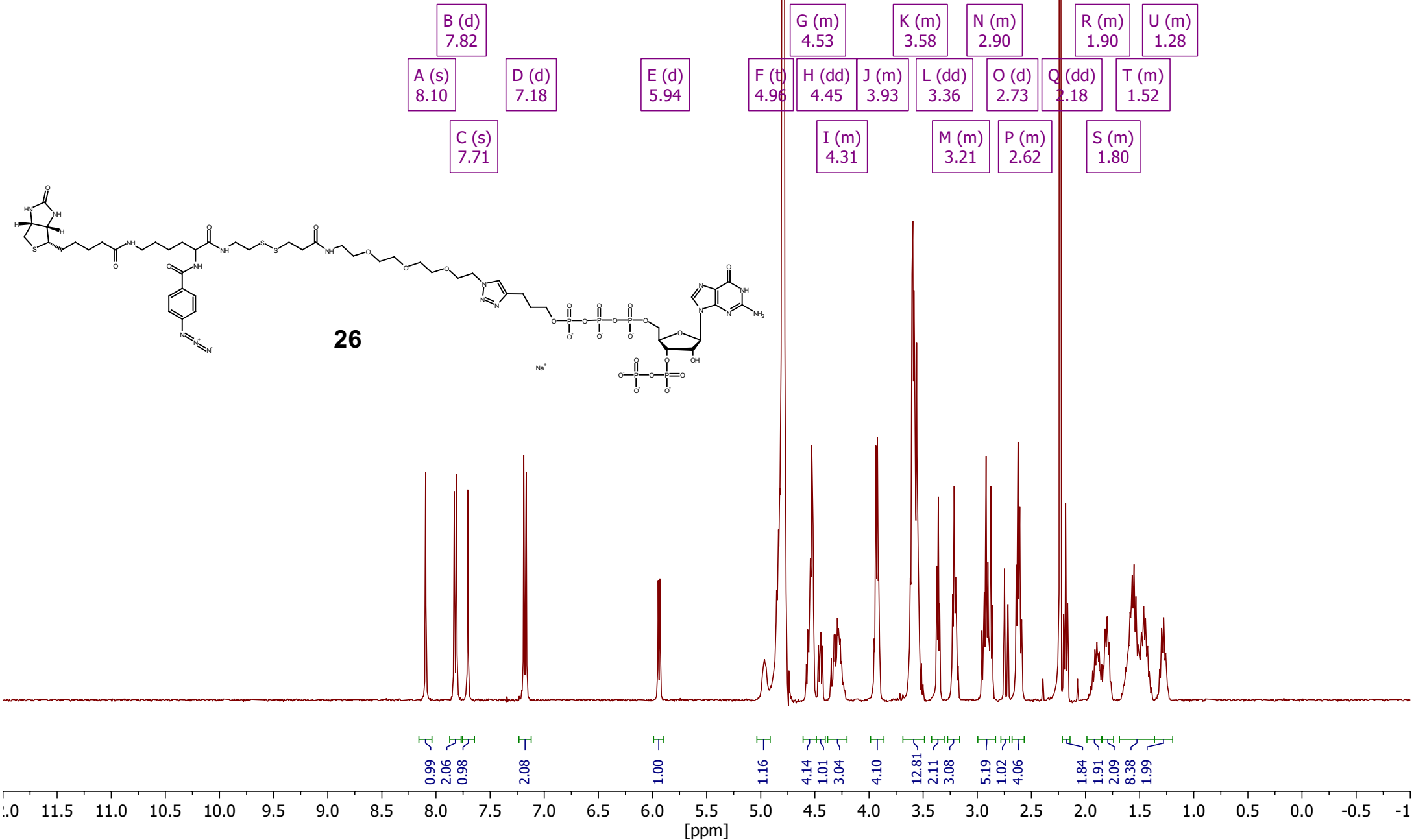


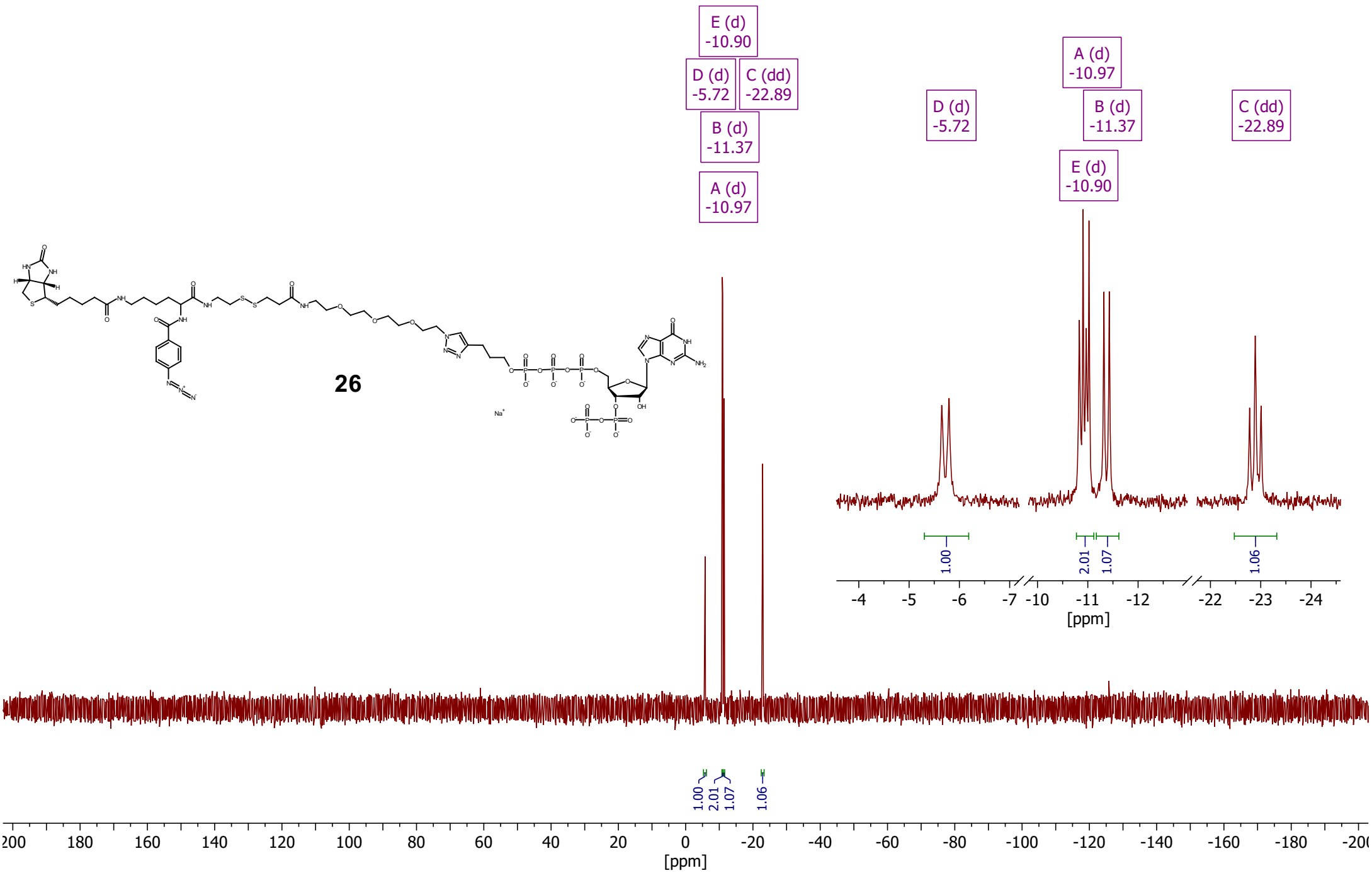


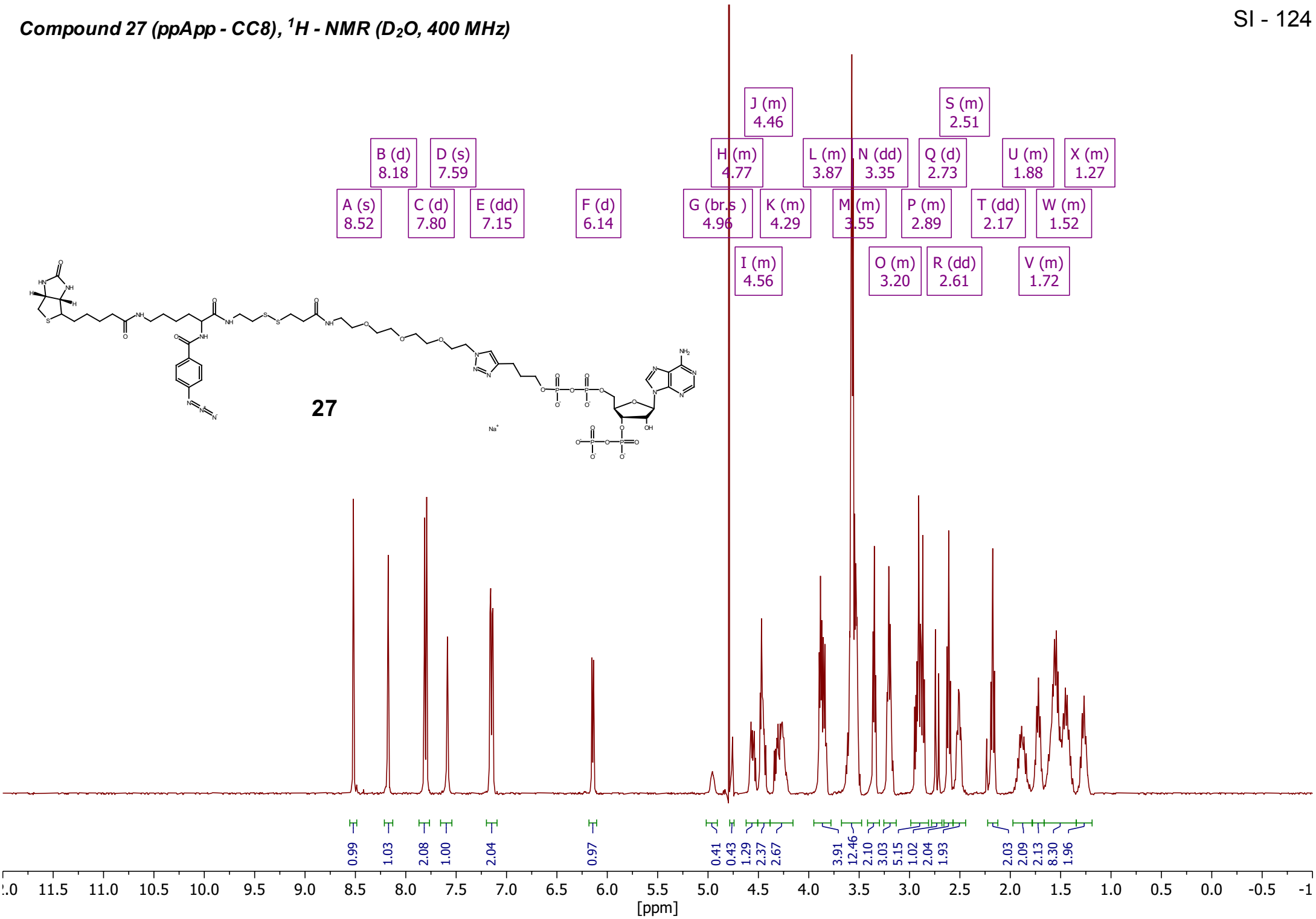


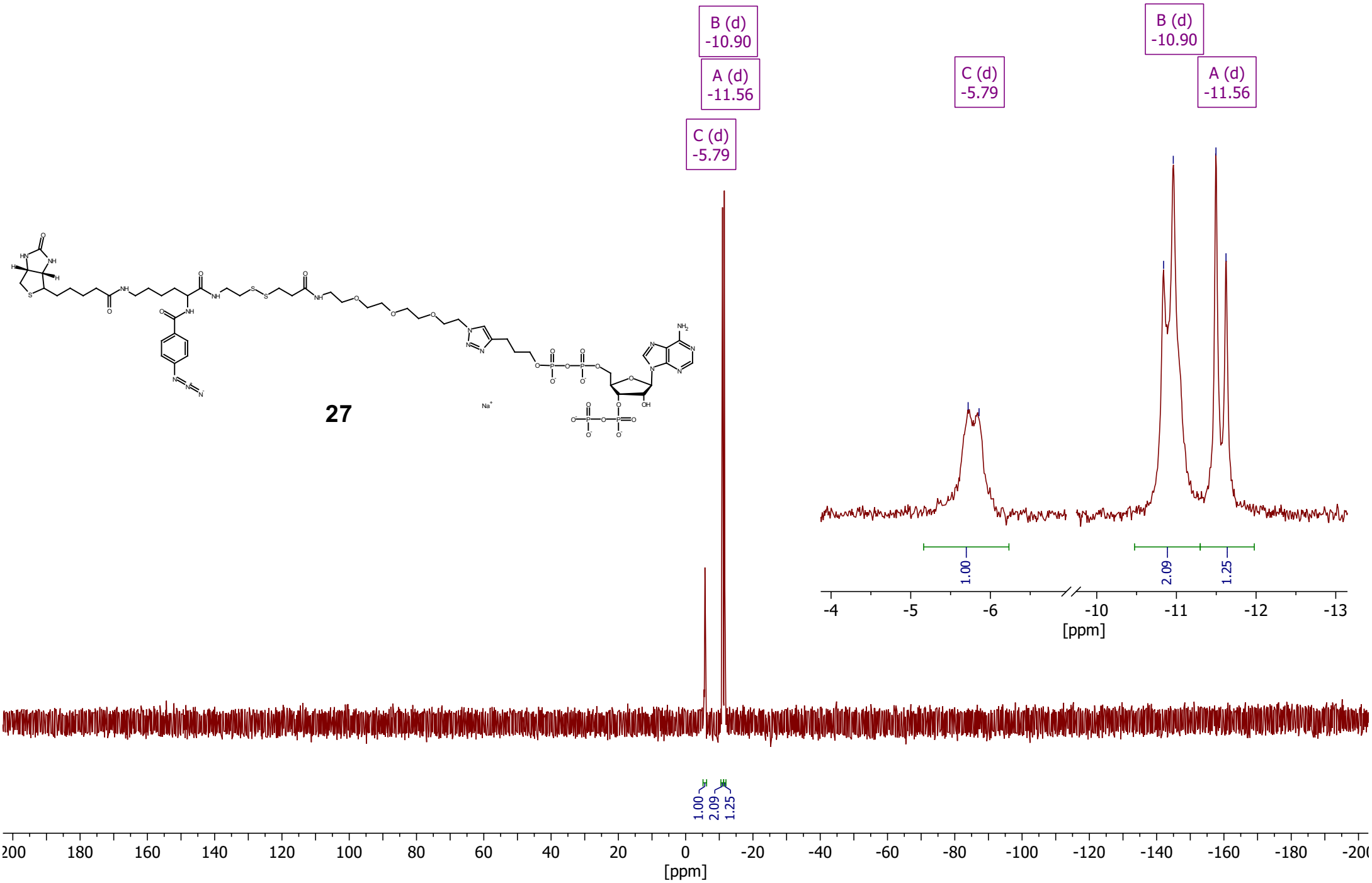


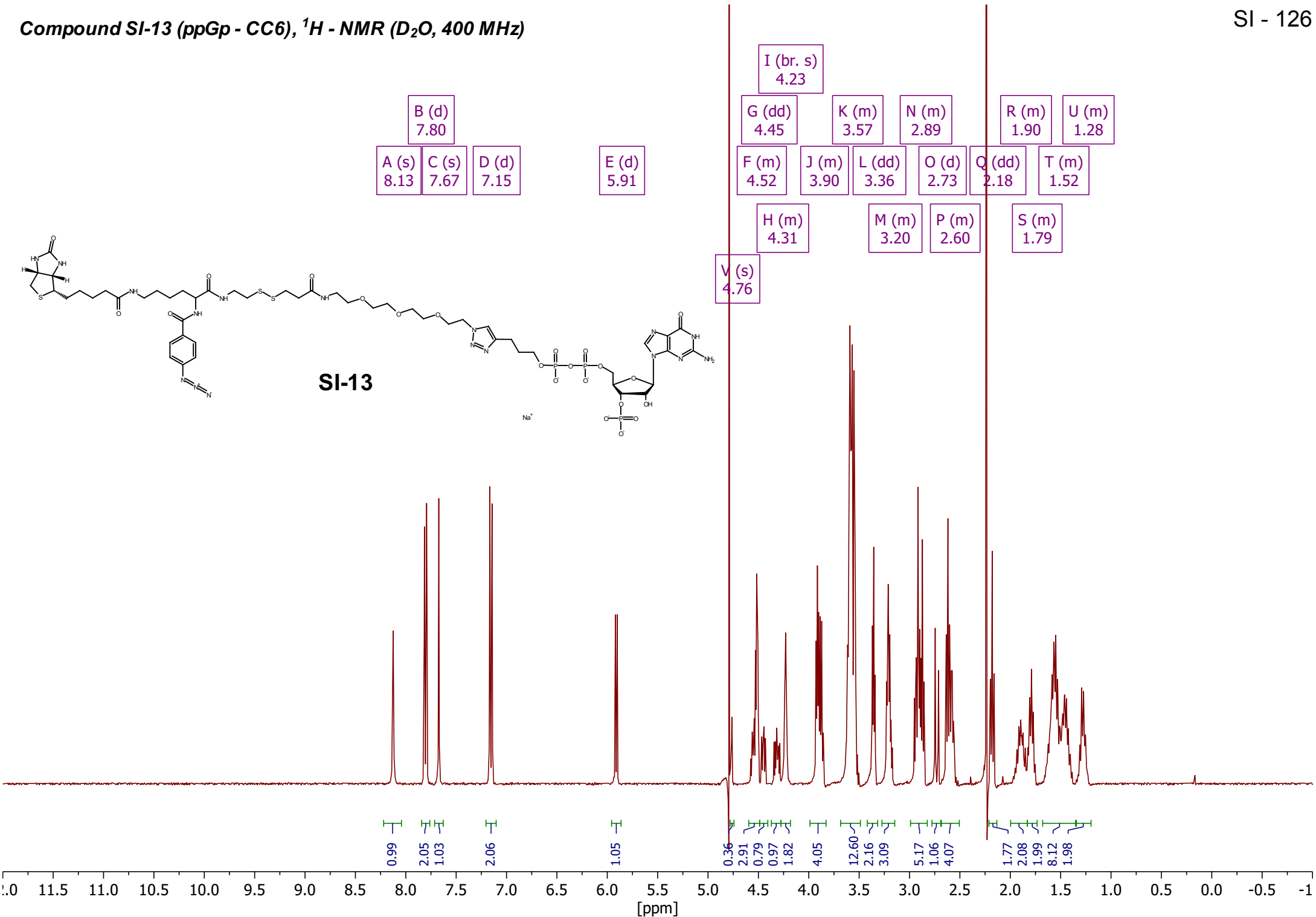


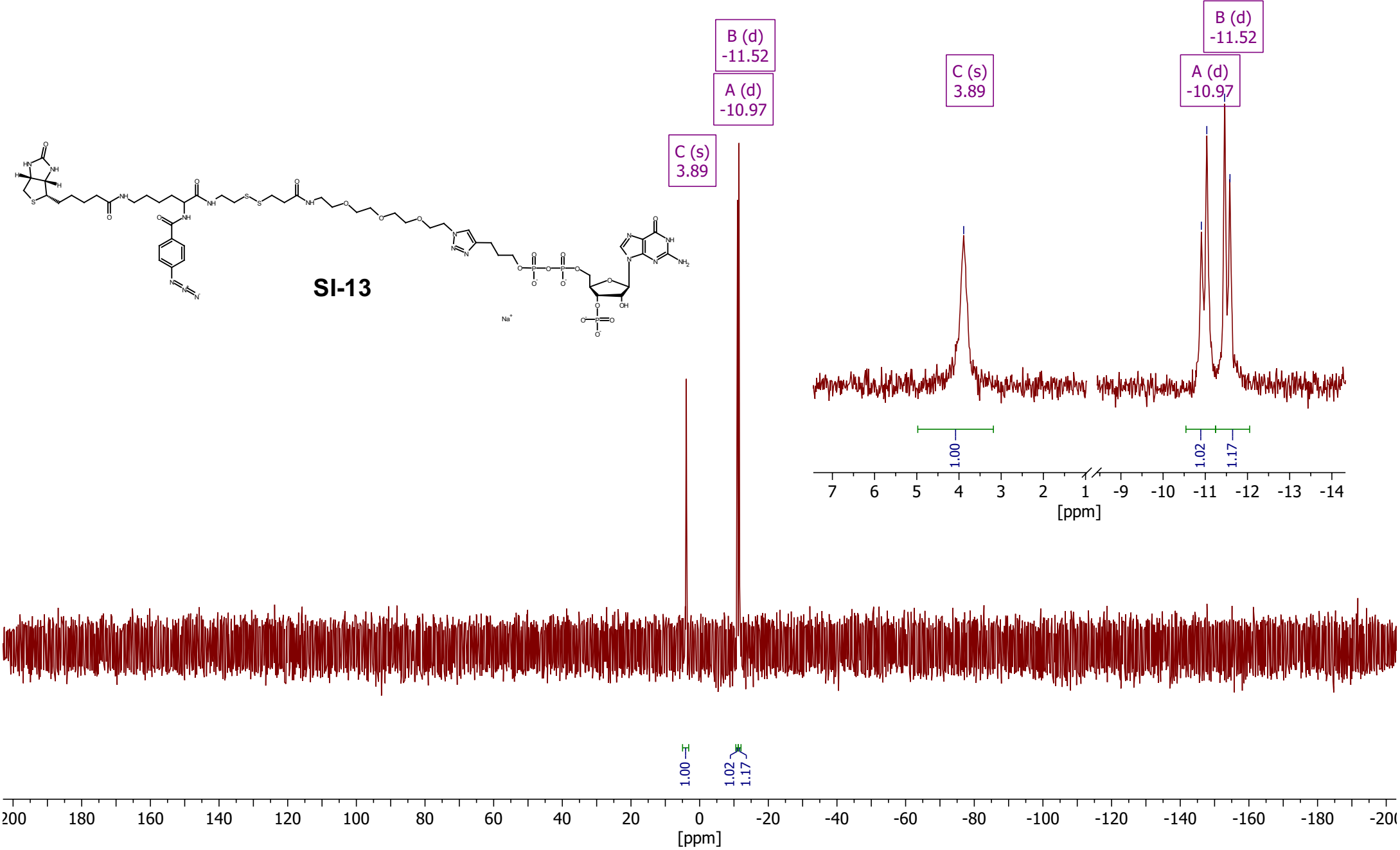


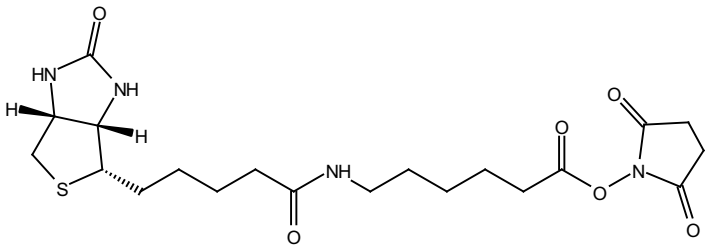




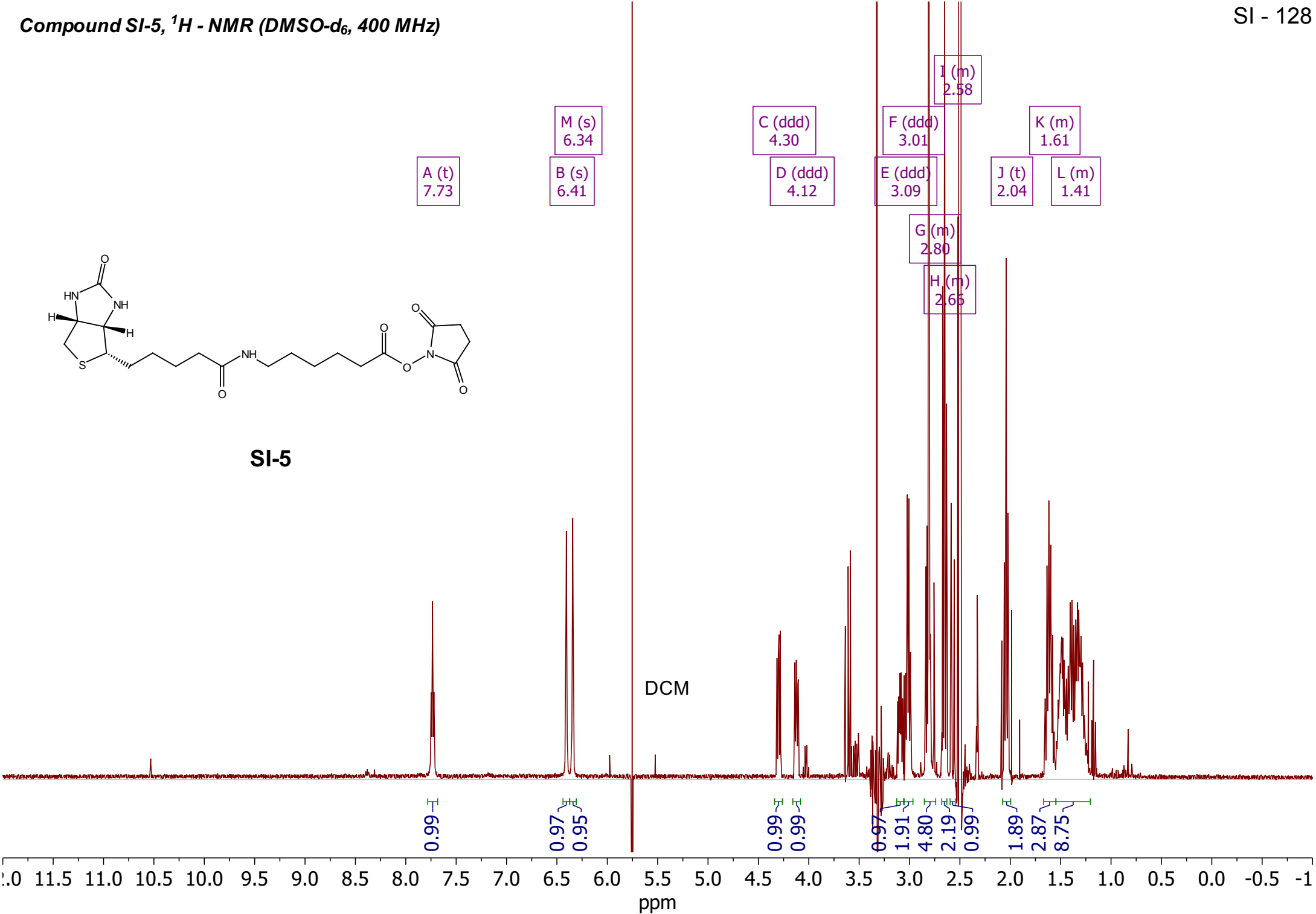


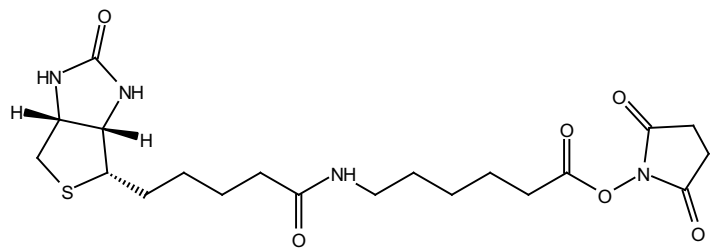




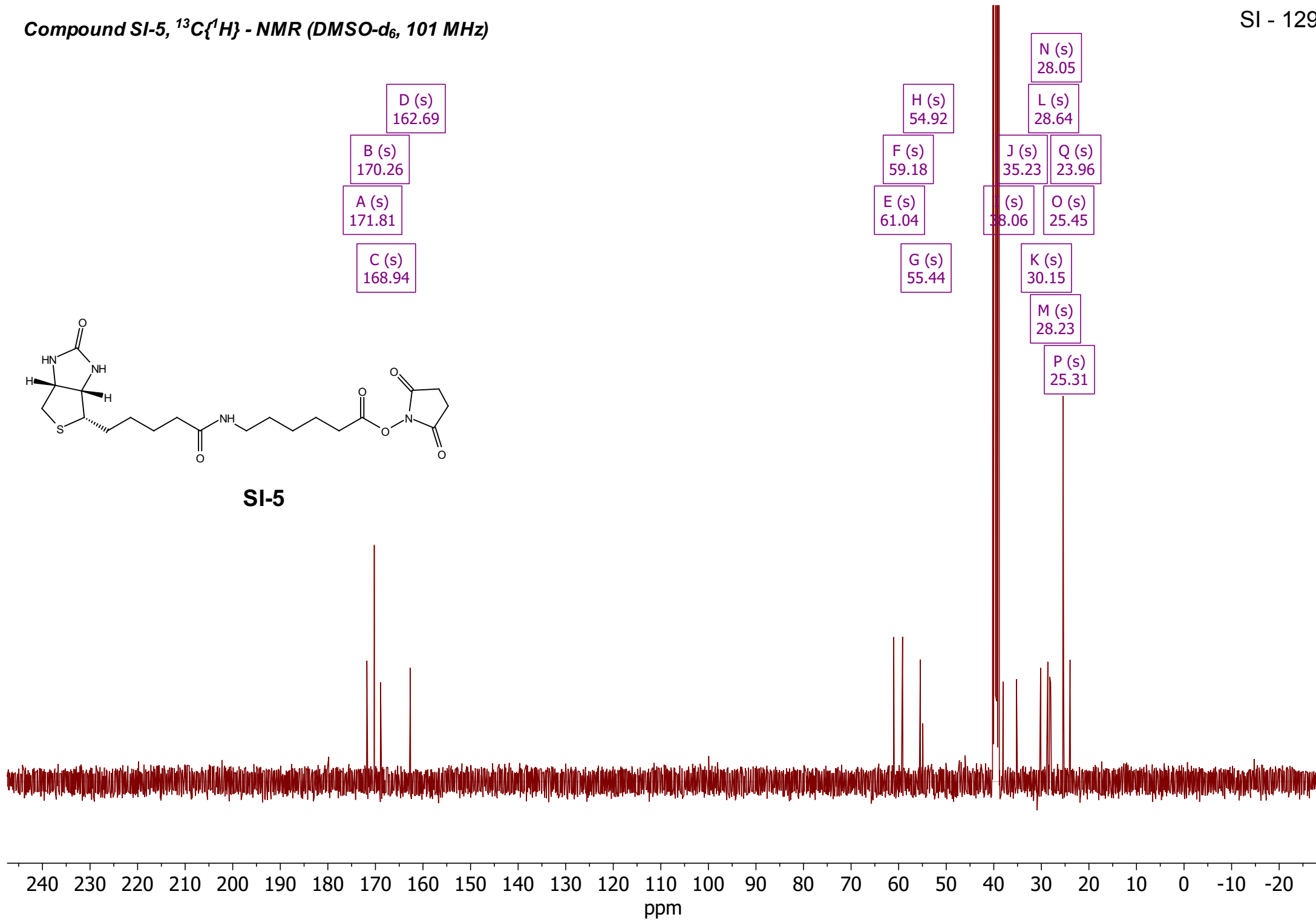


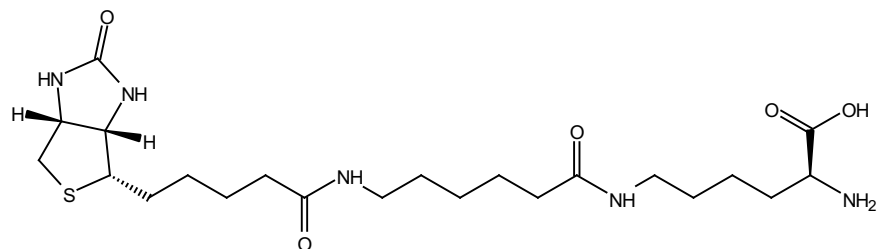
SI-5



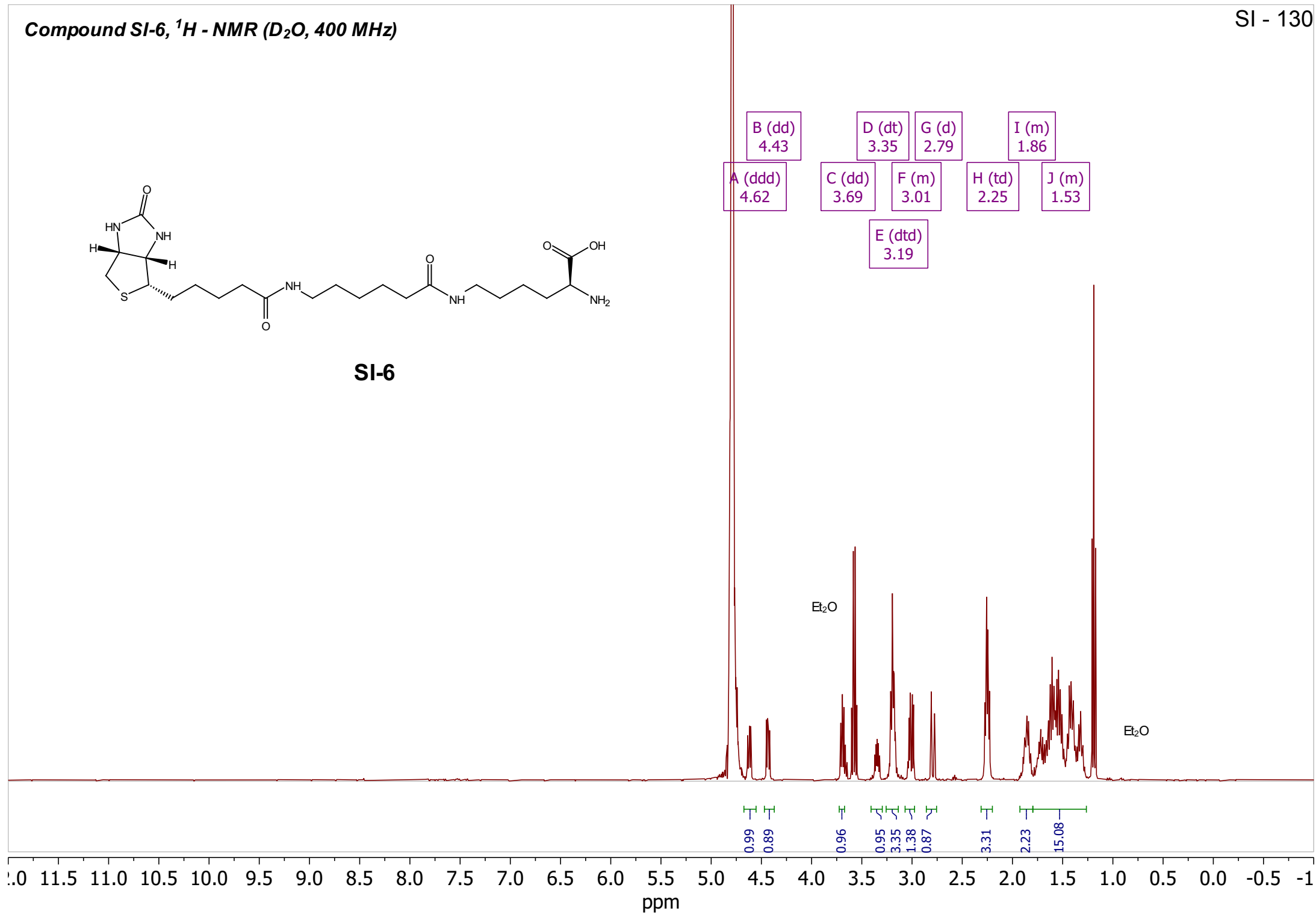


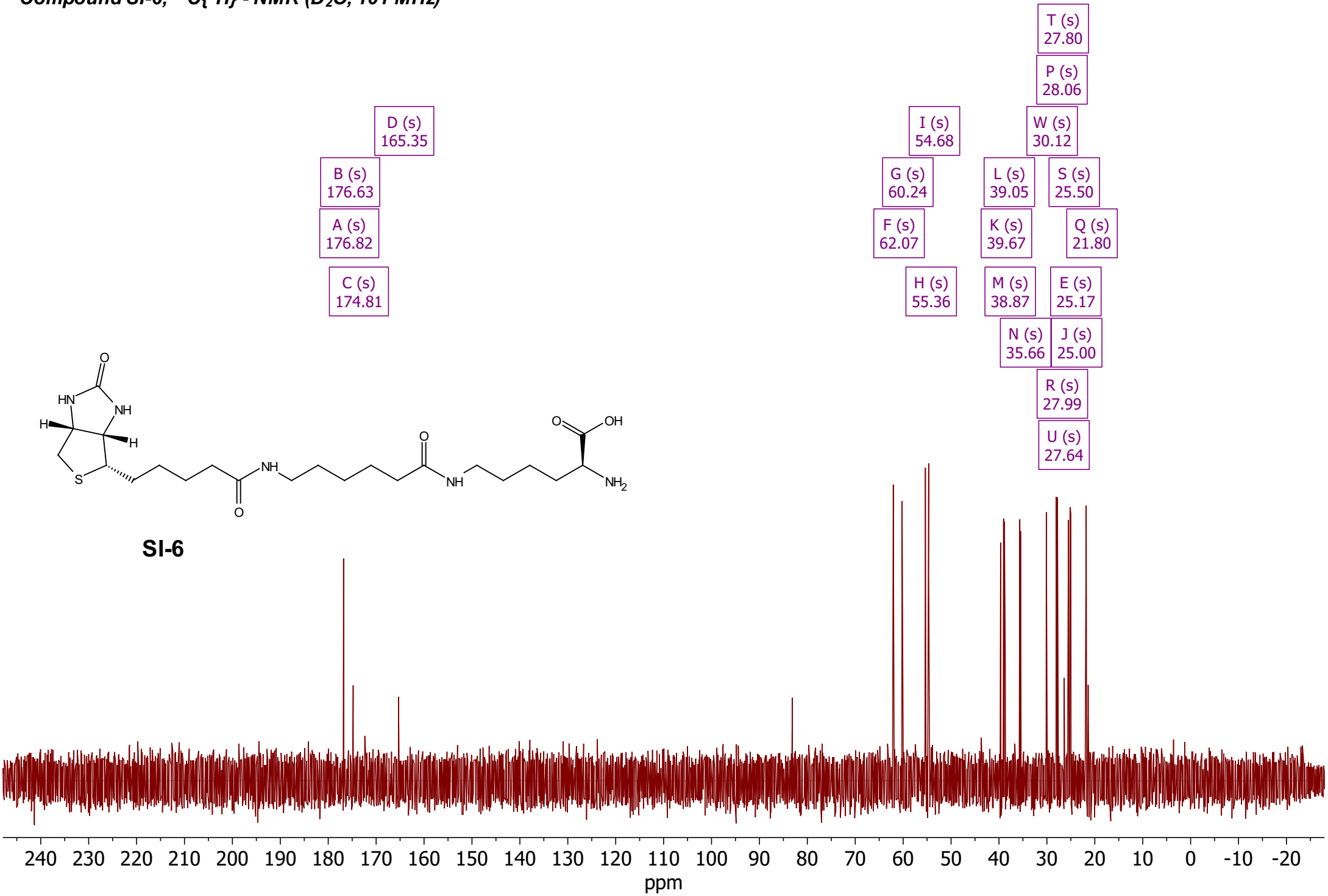
SI-5

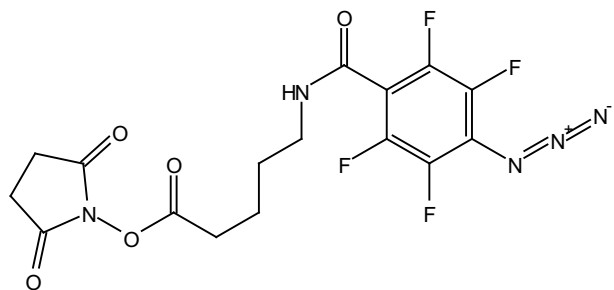
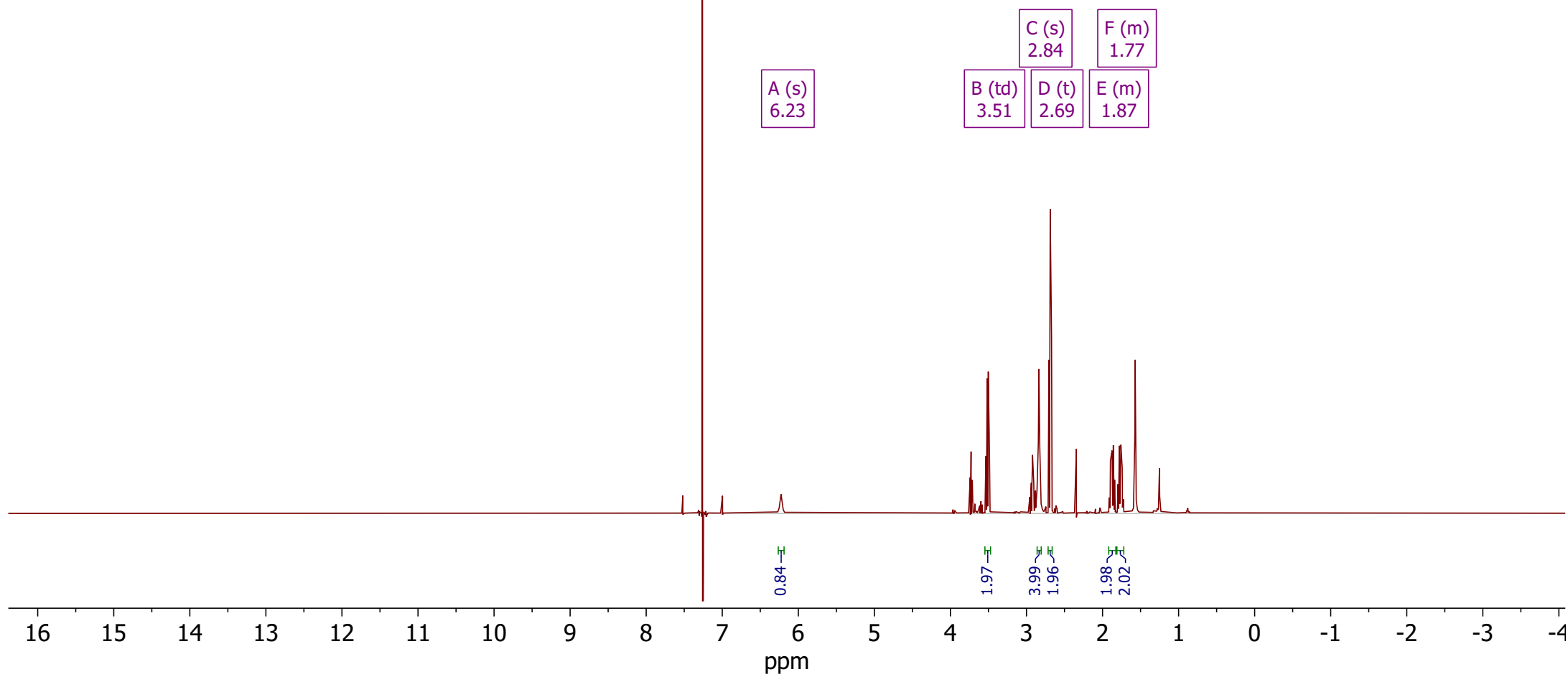


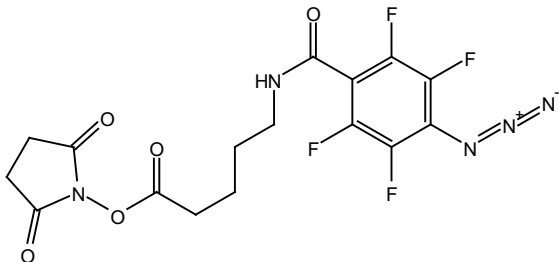


B (dd) 4.43	D (dt) 3.35	G (d) 2.79	I (m) 1.86
A (ddd) 4.62	C (dd) 3.69	F (m) 3.01	H (td) 2.25
	E (dtd) 3.19		J (m) 1.53

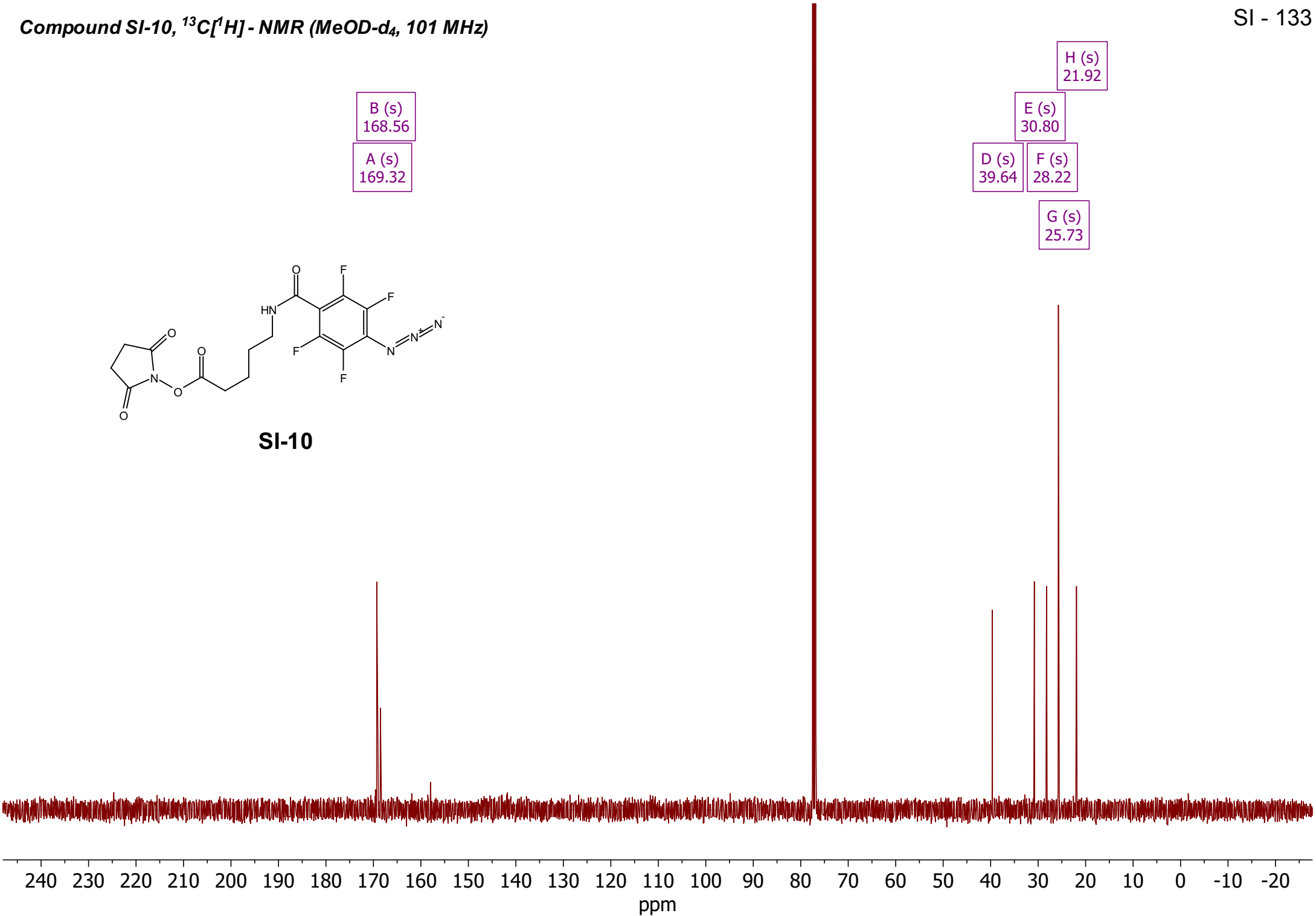




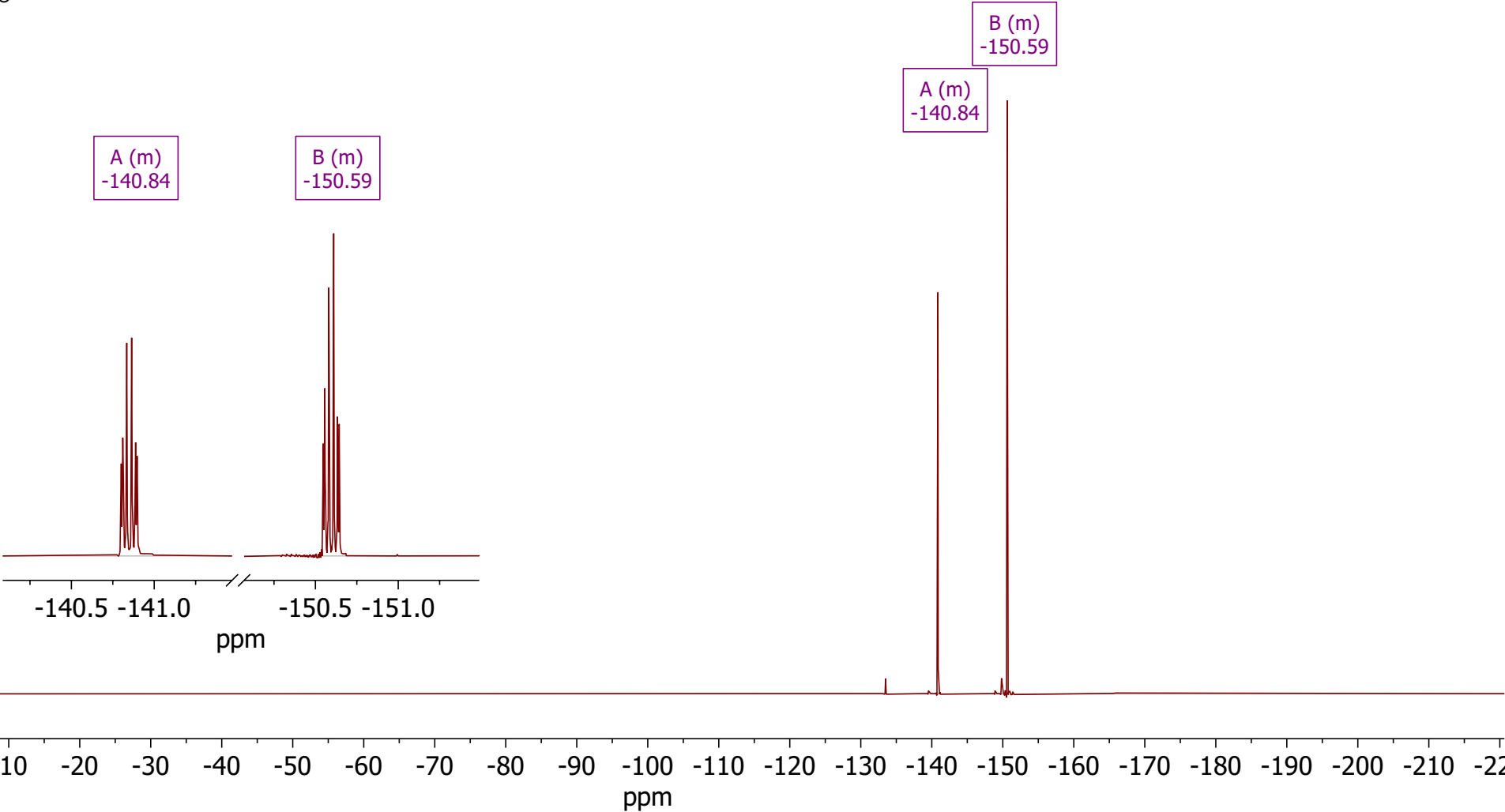
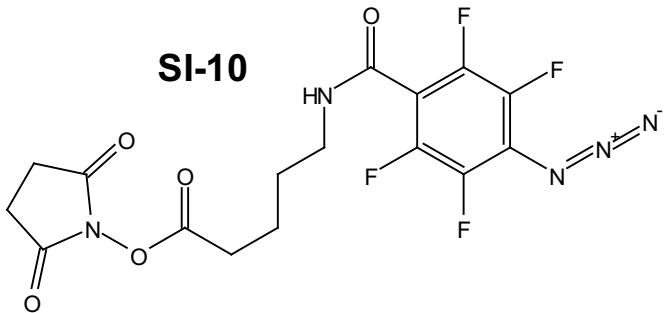
**SI-10**

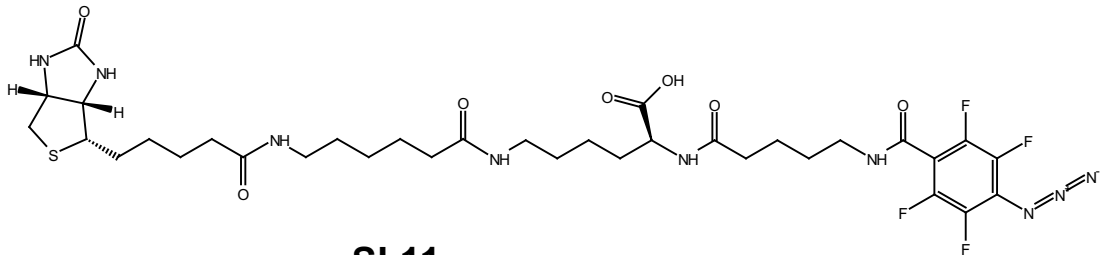


SI-10

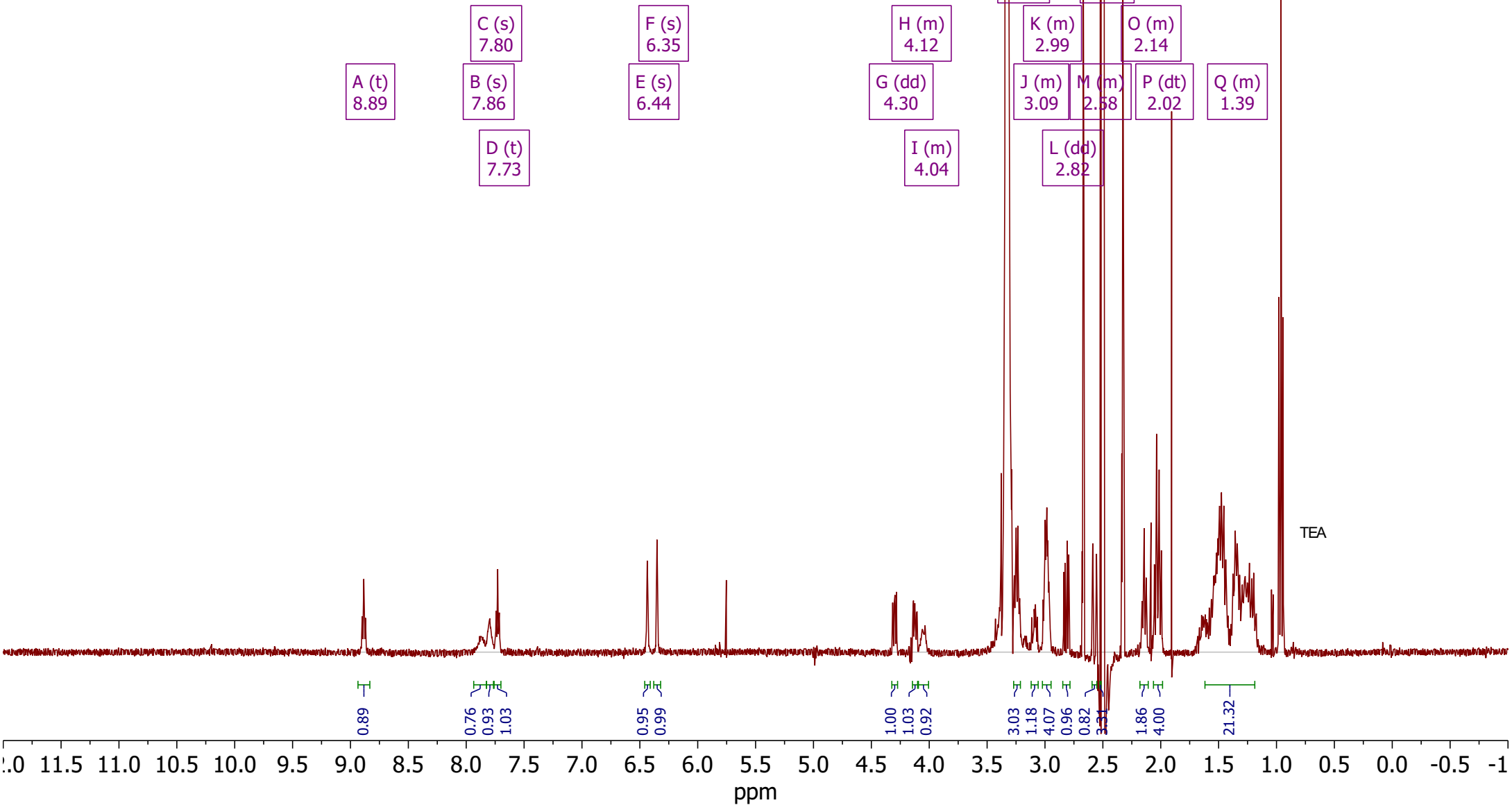


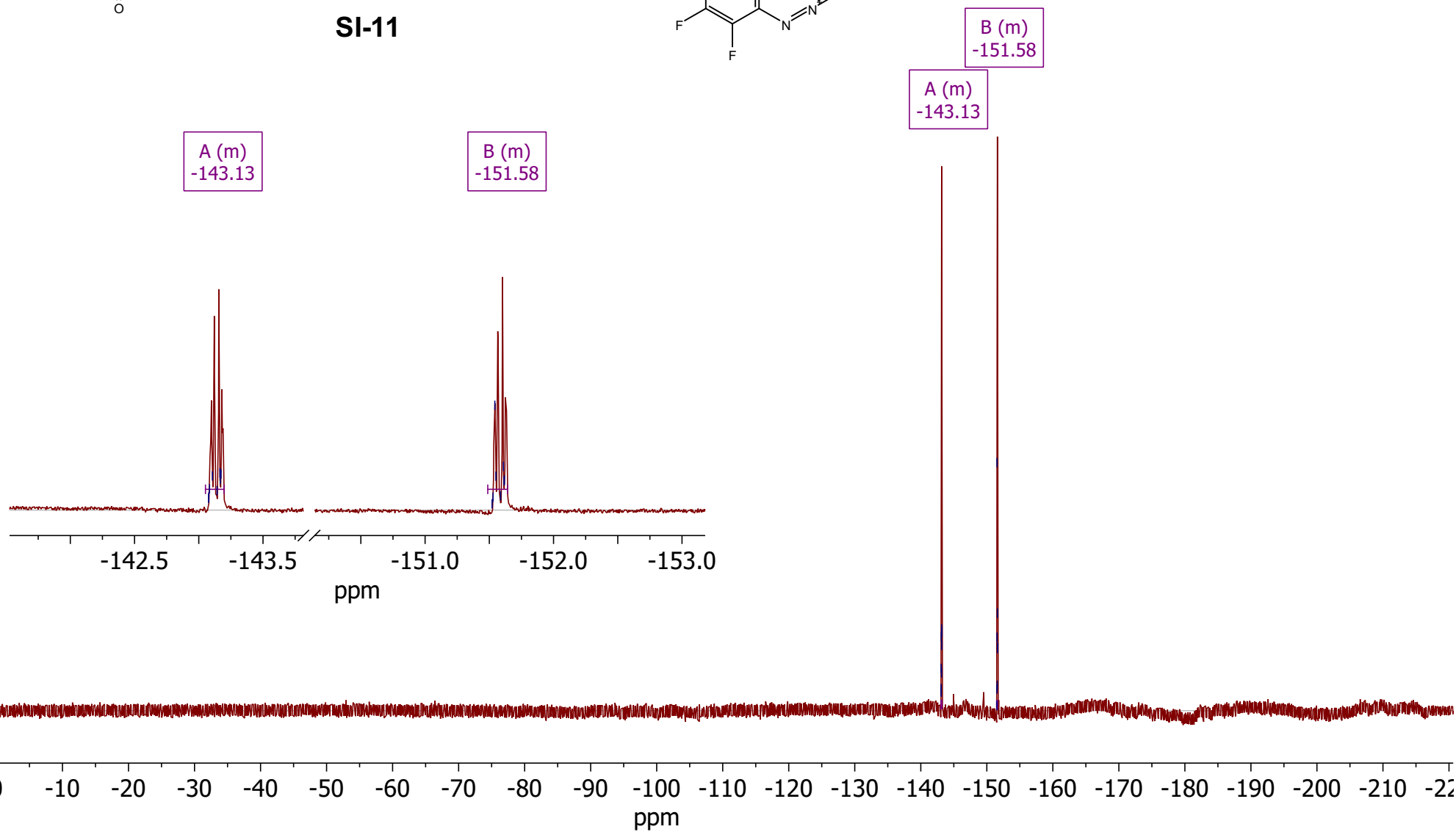
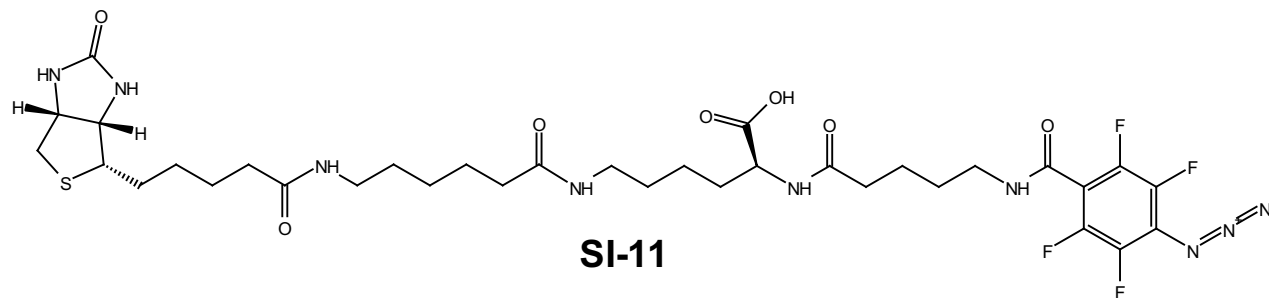
SI-10





SI-11



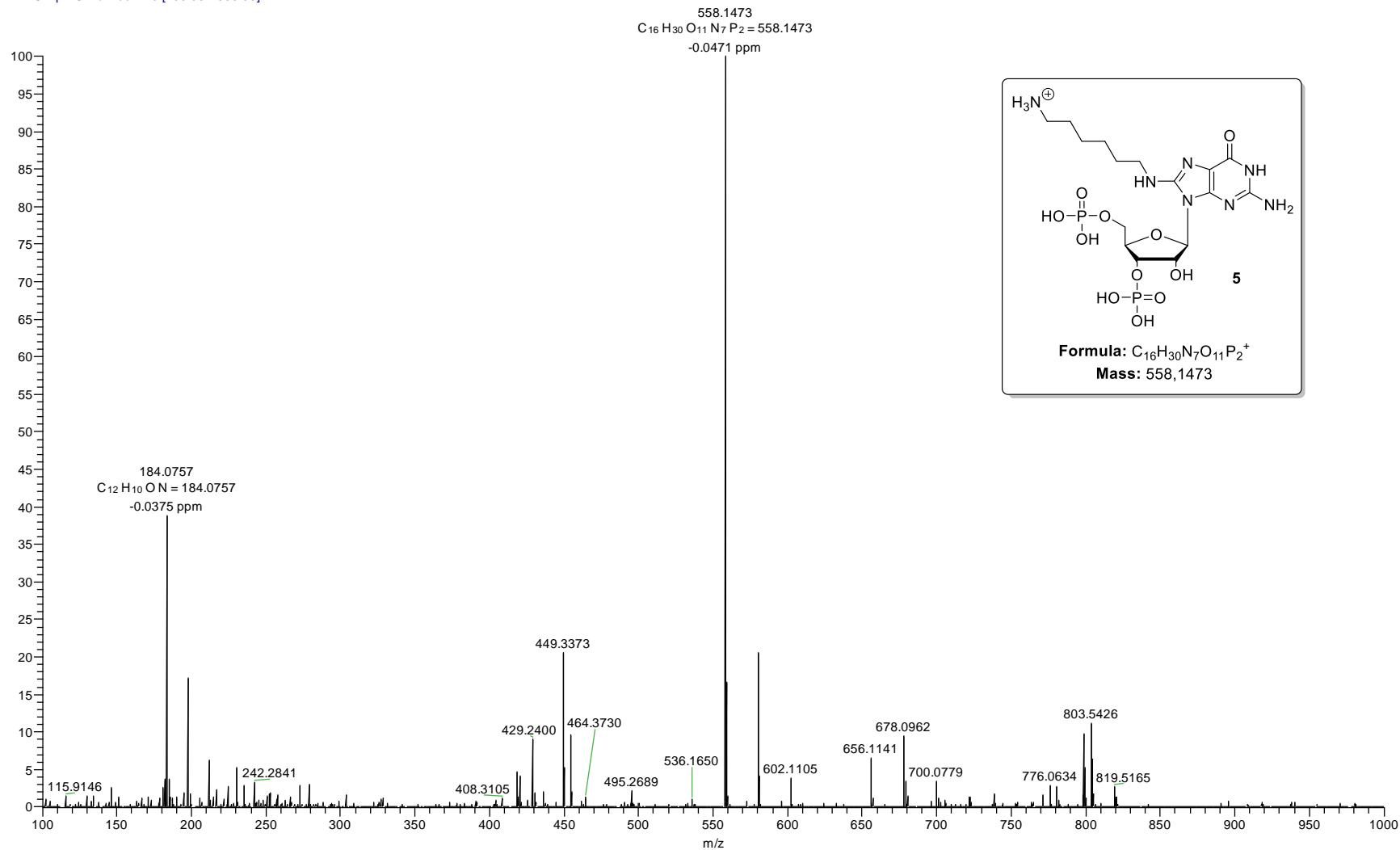


8. MS – spectra

(sorted according to molecule numbering)

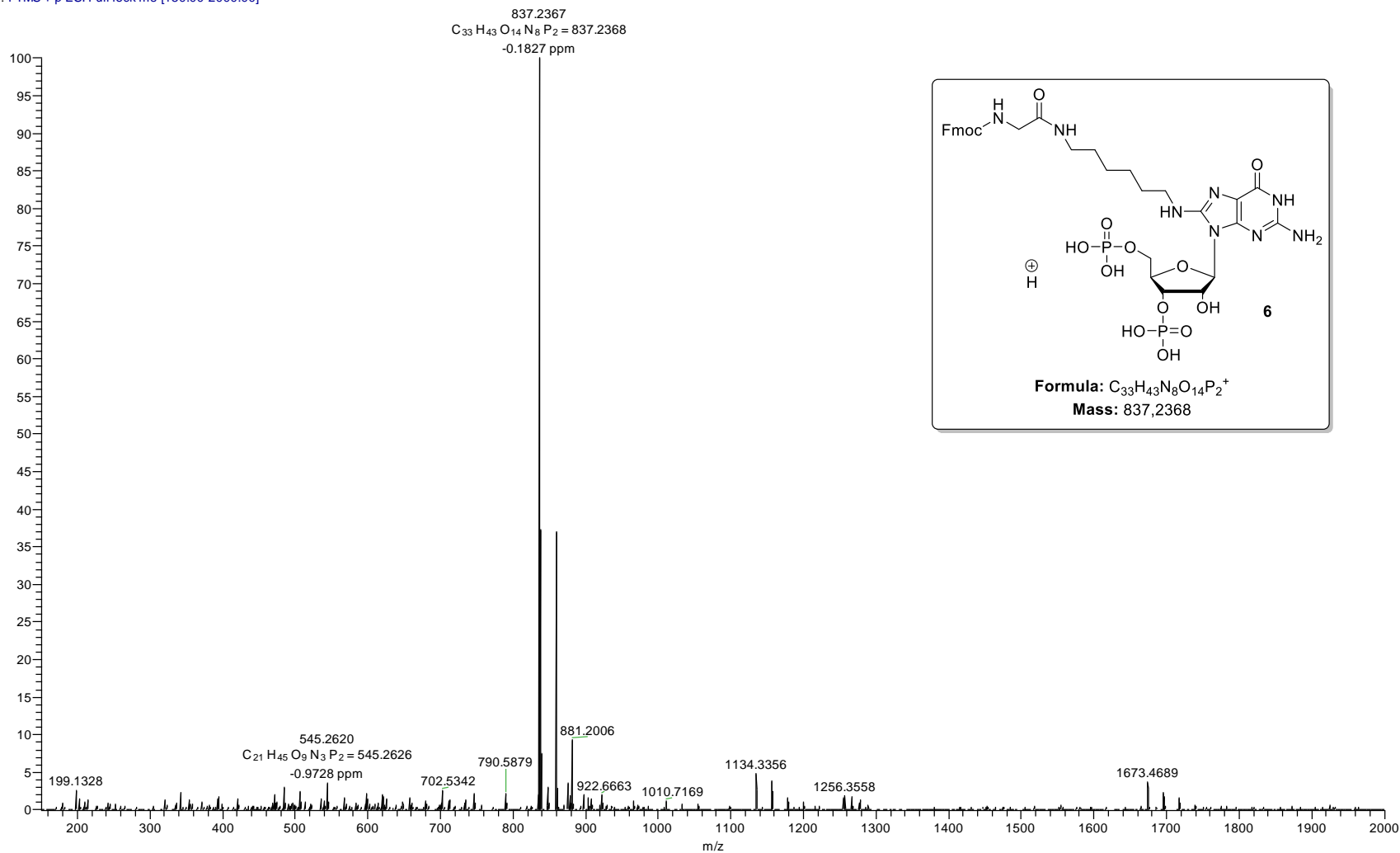
HRMS (ESI) Analysis of compound 5: HMDA – pGp

hsjeb04shr1 #1 RT: 0.02 AV: 1 NL: 2.22E6
T: FTMS + p ESI Full lock ms [100.00-1000.00]



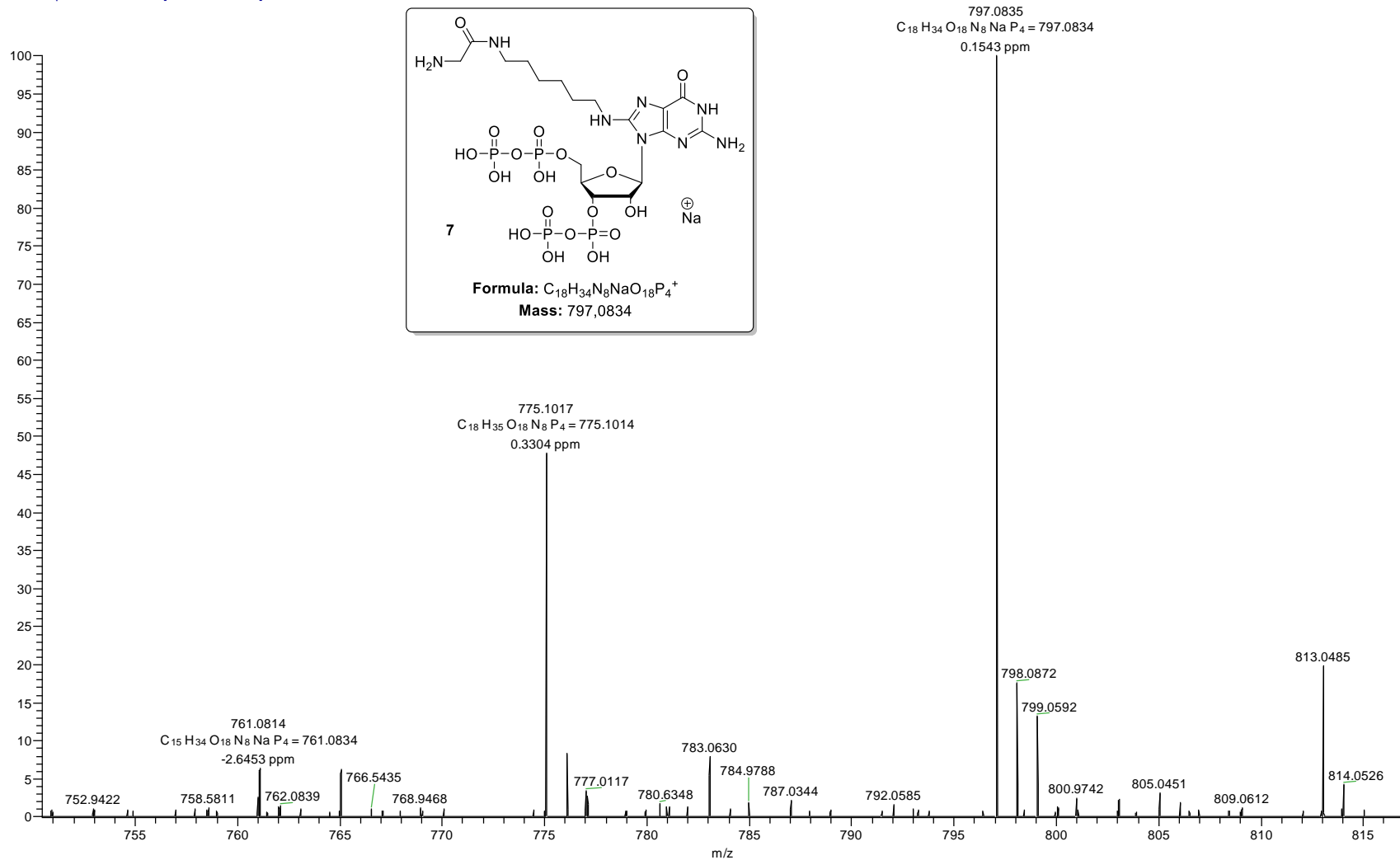
HRMS (ESI) Analysis of compound 6: Fmoc-Glycyl-HMDA – pGp

hsjeb17shr1 #1 RT: 0.03 AV: 1 NL: 1.76E6
T: FTMS + p ESI Full lock ms [150.00-2000.00]



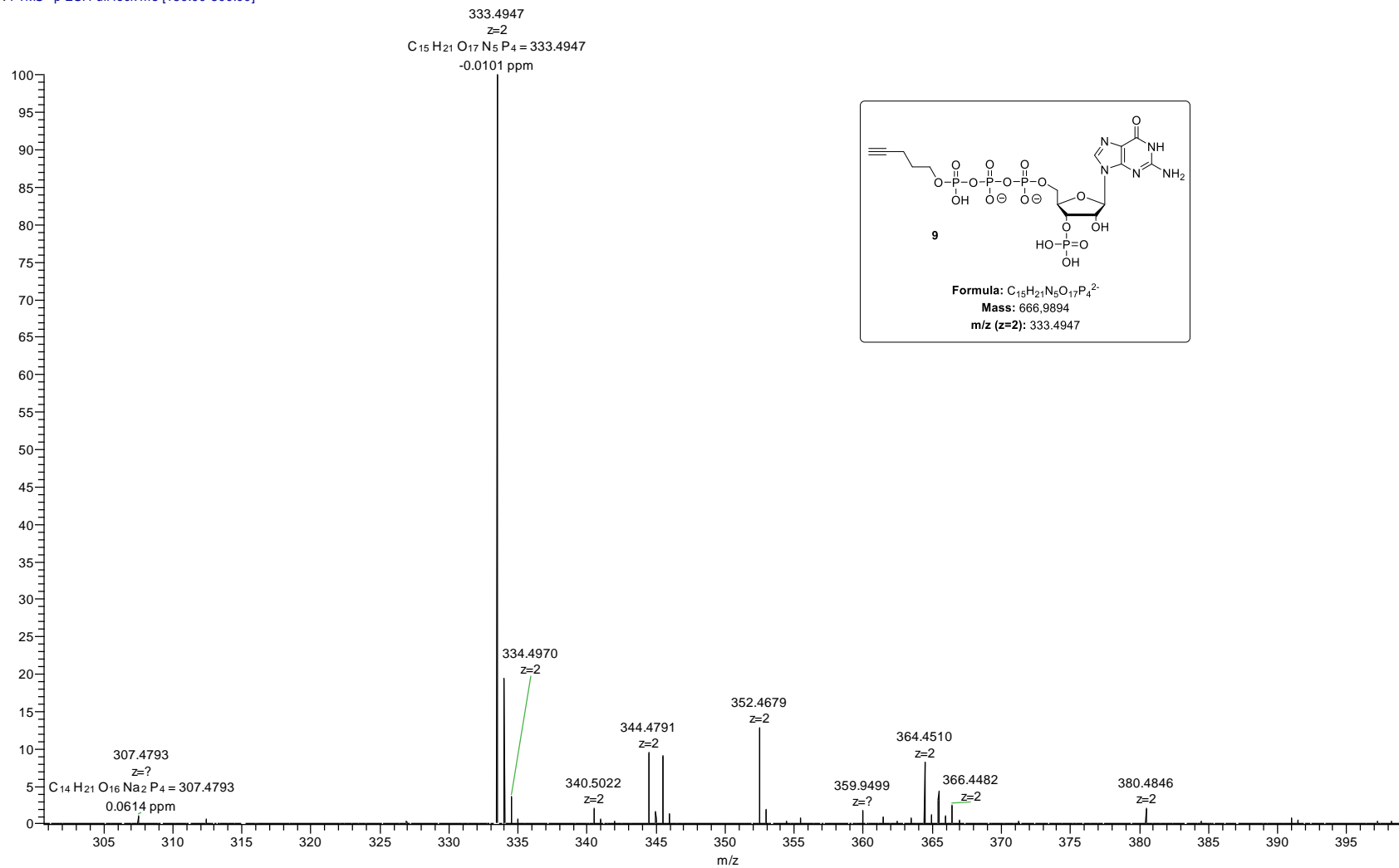
HRMS (ESI) Analysis of compound 7: Glycyl-HMDA – ppGpp

hsjeb23shr01 #1 RT: 0.02 AV: 1 NL: 1.93E5
T: FTMS + p ESI Full lock ms [100.00-1000.00]



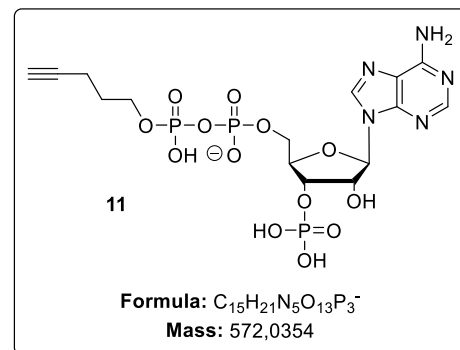
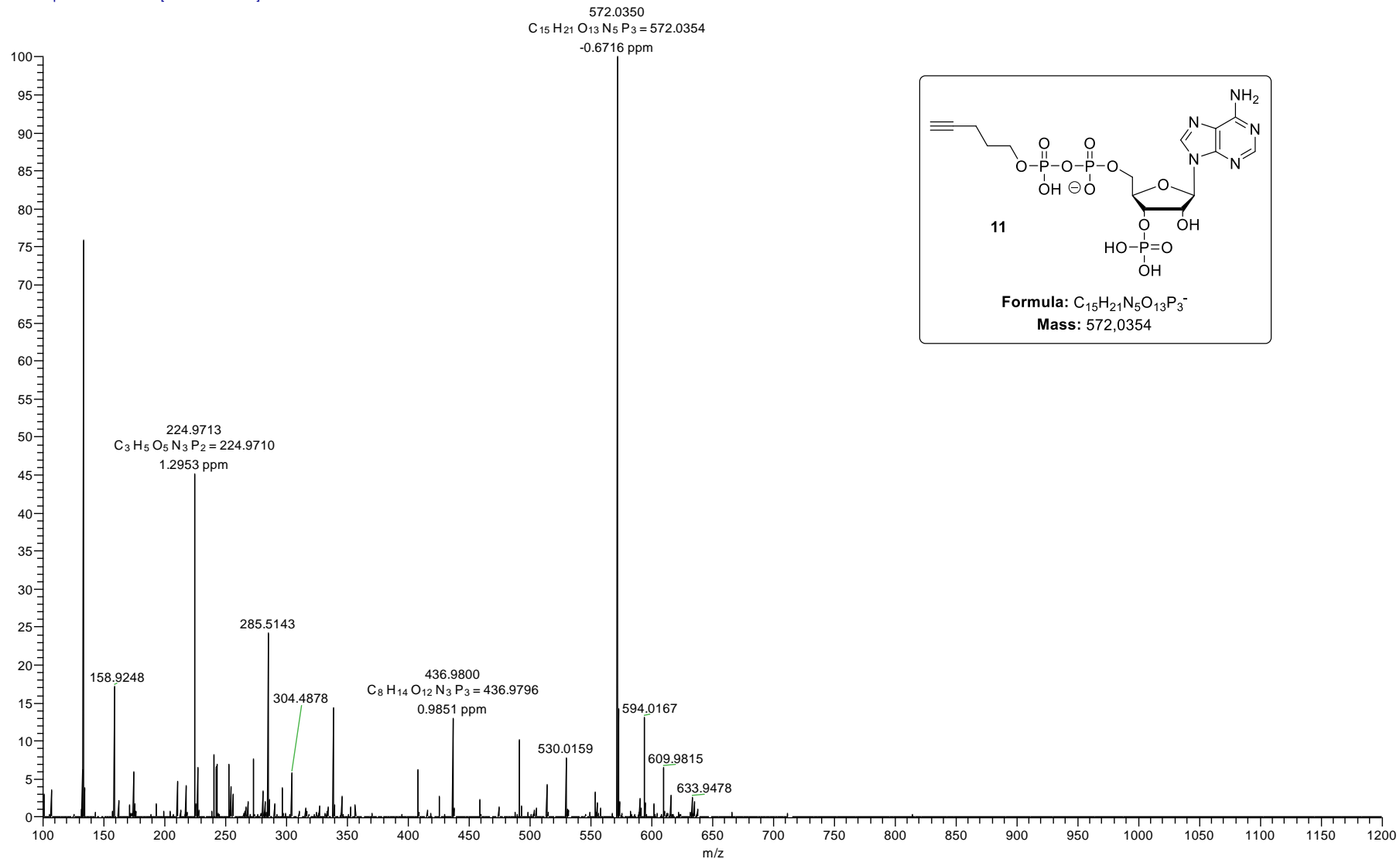
HRMS (ESI) Analysis of compound 9: pentynyl - pppGp

hsjeb34shr1 #1 RT: 0.02 AV: 1 NL: 1.29E7
T: FTMS - p ESI Full lock ms [150.00-800.00]



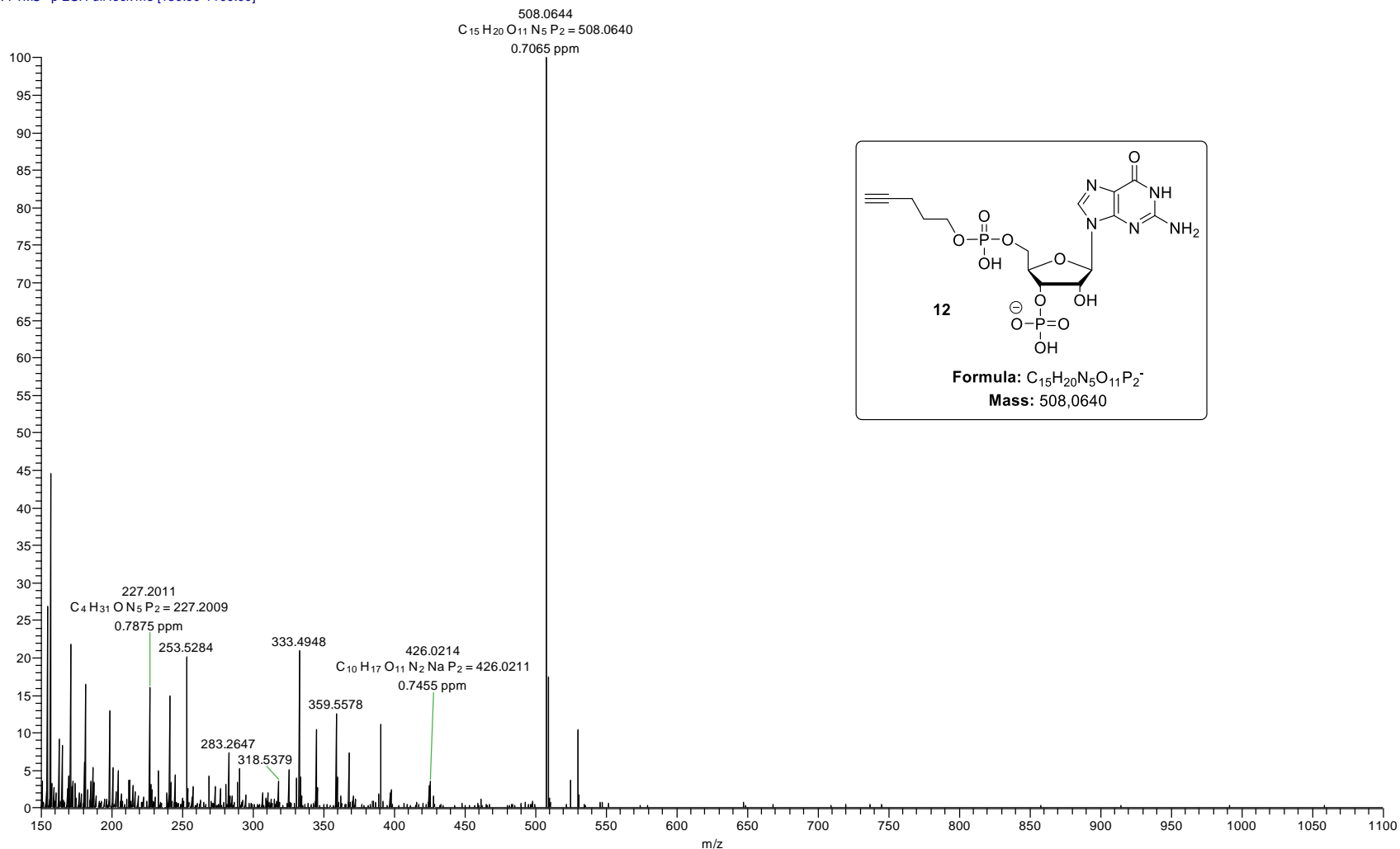
HRMS (ESI) Analysis of compound 11: pentynyl - ppAp

hsjeb49shr2 #1 RT: 0.02 AV: 1 NL: 2.70E7
T: FTMS - p ESI Full lock ms [100.00-1200.00]



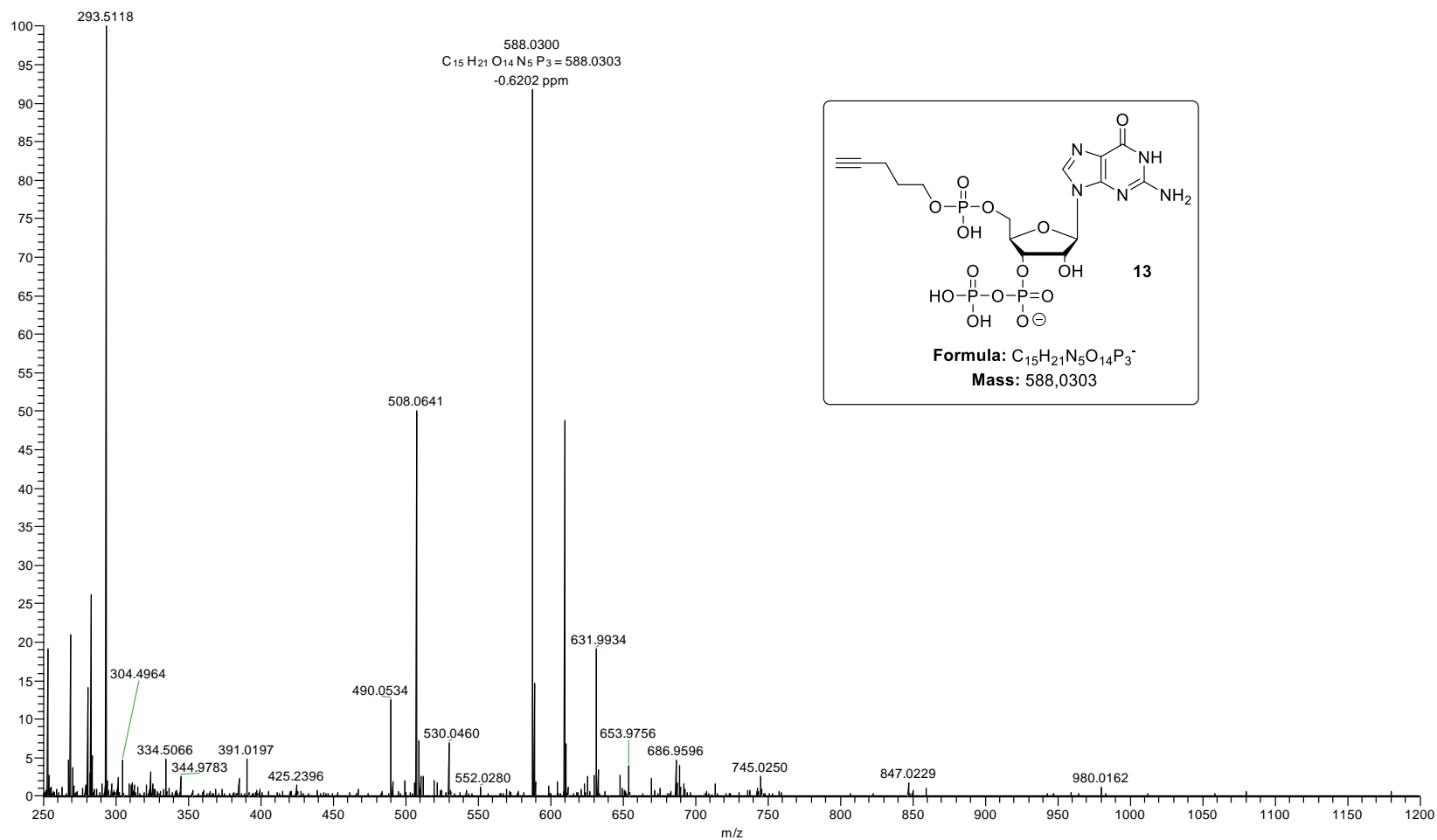
HRMS (ESI) Analysis of compound 12: pentynyl – pGp

hsjeb35shr1 #1 RT: 0.02 AV: 1 NL: 1.37E6
T: FTMS - p ESI Full lock ms [150.00-1100.00]



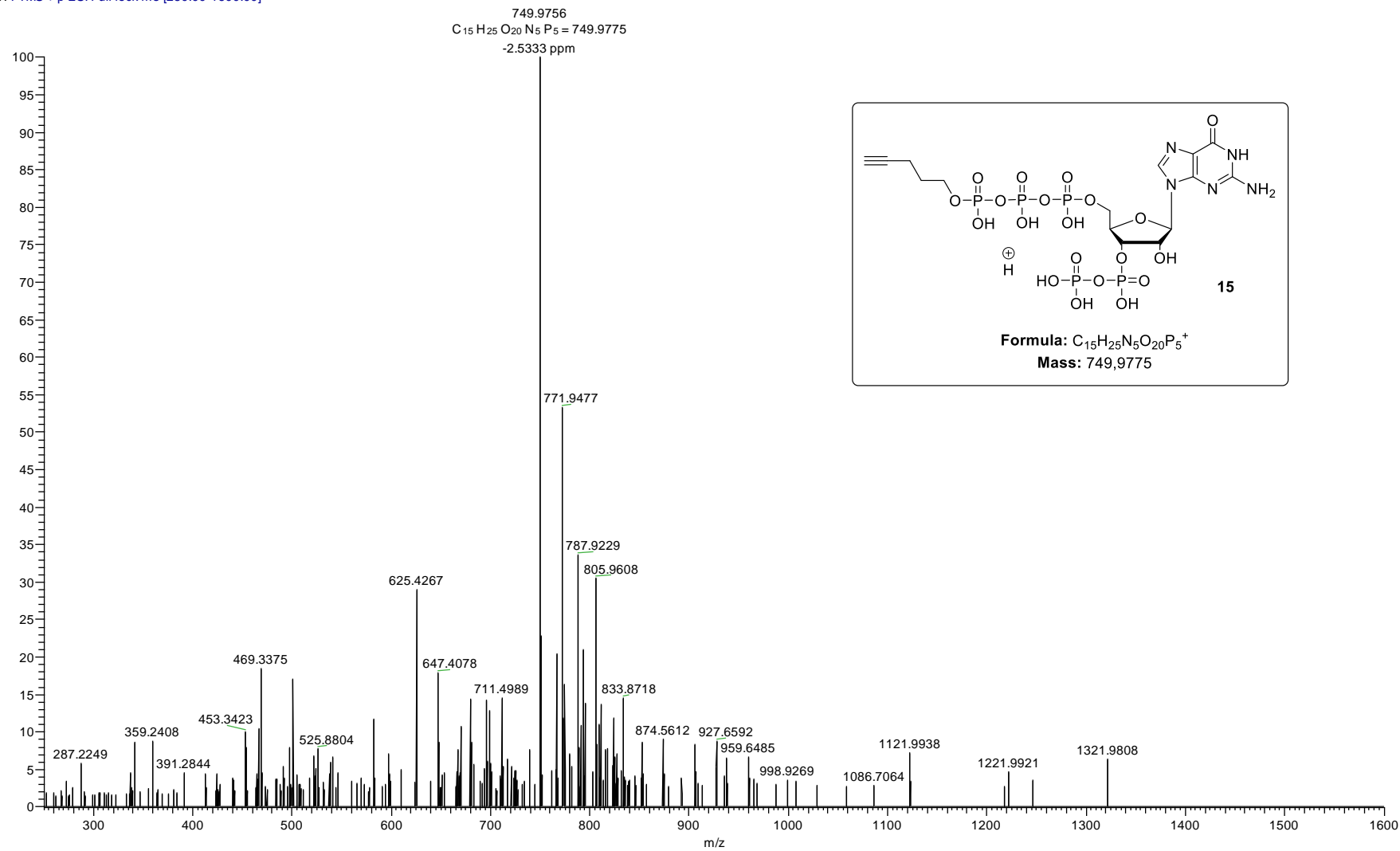
HRMS (ESI) Analysis of compound 13: pentynyl – pGpp

hsjeb38shr2 #1 RT: 0.02 AV: 1 NL: 1.78E6
T: FTMS - p ESI Full lock ms [250.00-1200.00]



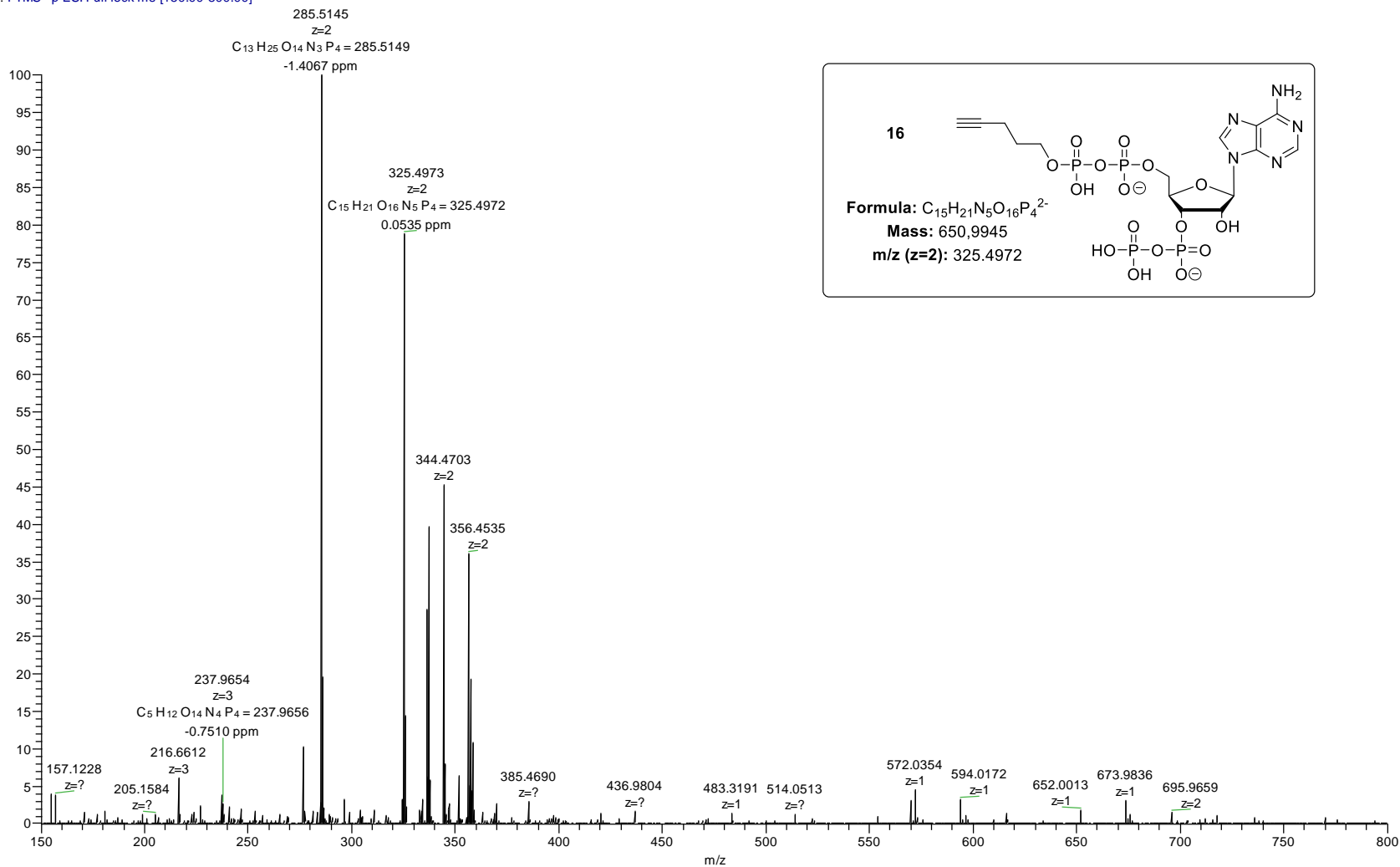
HRMS (ESI) Analysis of compound 15: pentynyl – pppGpp

hsjeb37shr4 #1 RT: 0.02 AV: 1 NL: 1.63E5
T: FTMS + p ESI Full lock ms [250.00-1600.00]



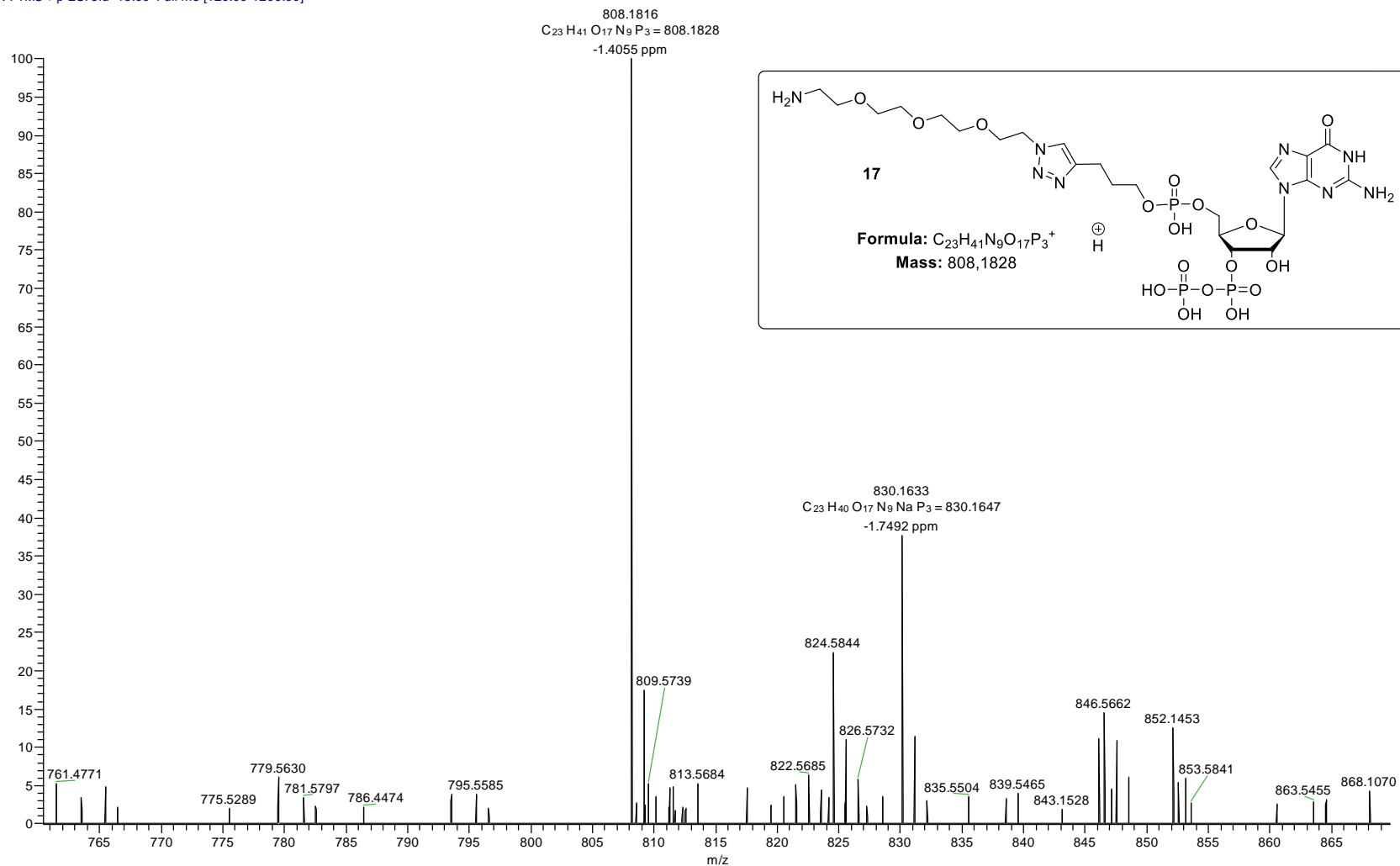
HRMS (ESI) Analysis of compound 16: pentynyl – ppApp

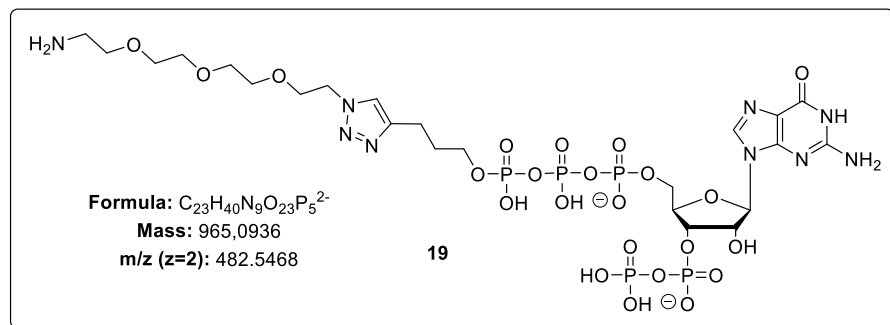
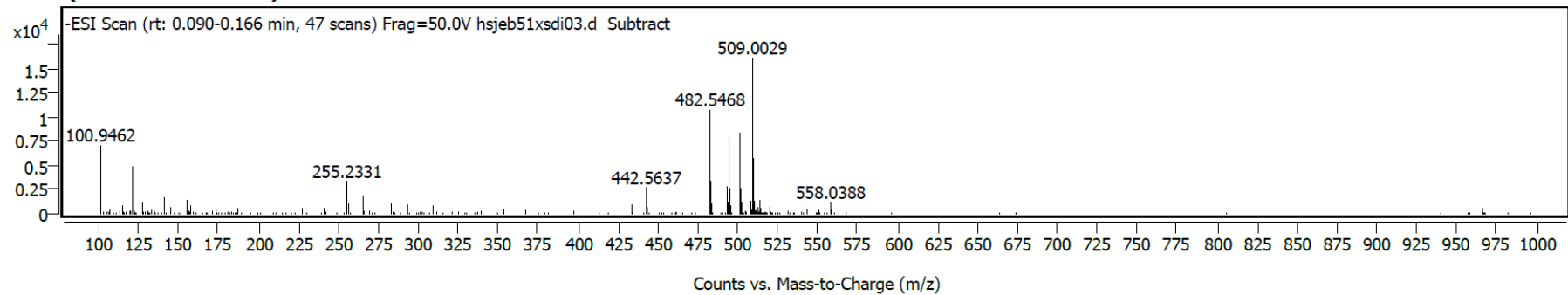
hsjeb50shr1 #1 RT: 0.02 AV: 1 NL: 2.71E6
T: FTMS - p ESI Full lock ms [150.00-800.00]



HRMS (ESI) Analysis of compound 17: amino – pGpp

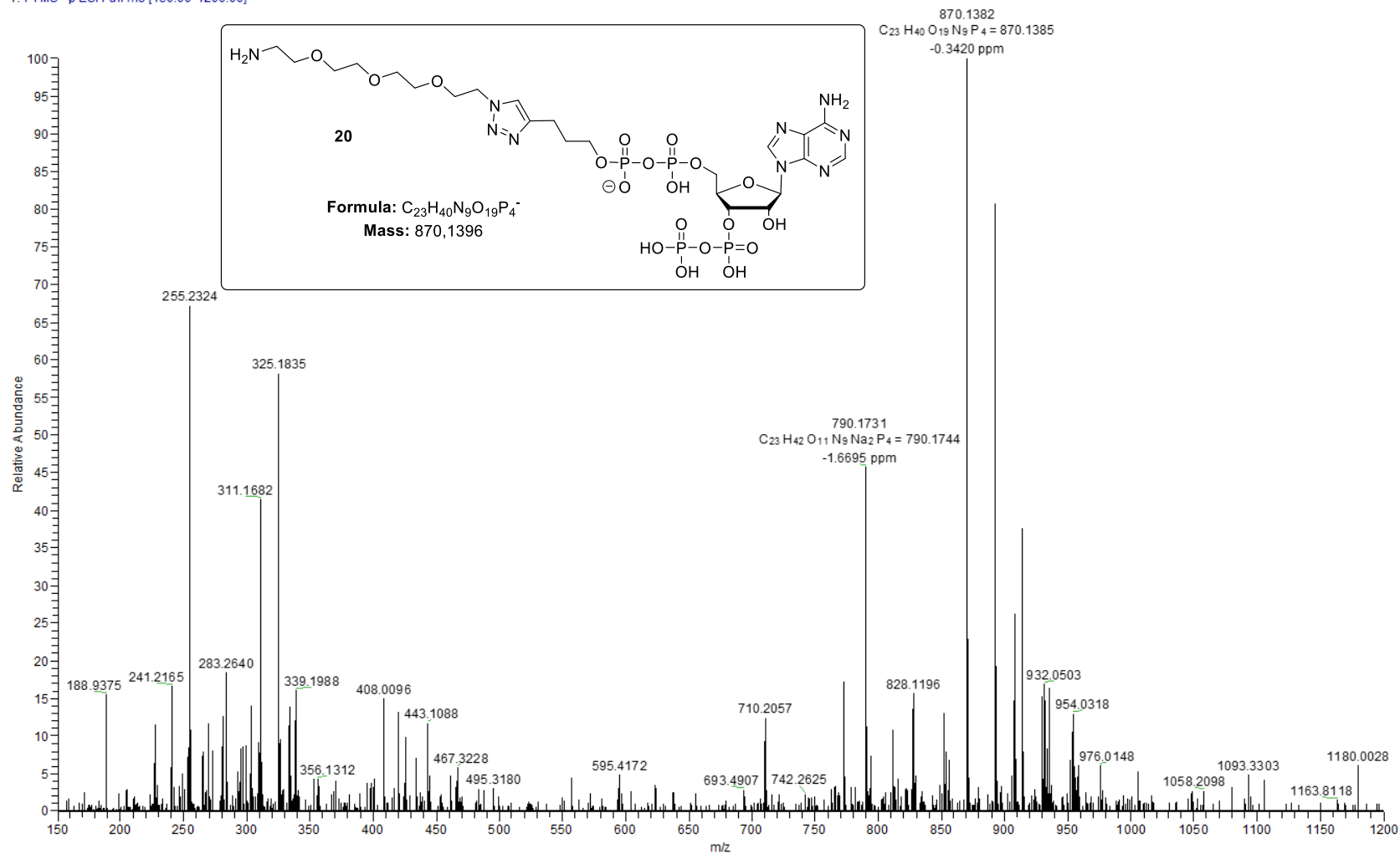
hsjeb52shr4 #1 RT: 0.02 AV: 1 NL: 2.02E5
T: FTMS + p ESI sid=15.00 Full ms [120.00-1200.00]



HRMS (ESI) Analysis of compound 19: amino – pppGpp**Sample Spectra****- Scan (rt: 0.090-0.166 min) Sub**

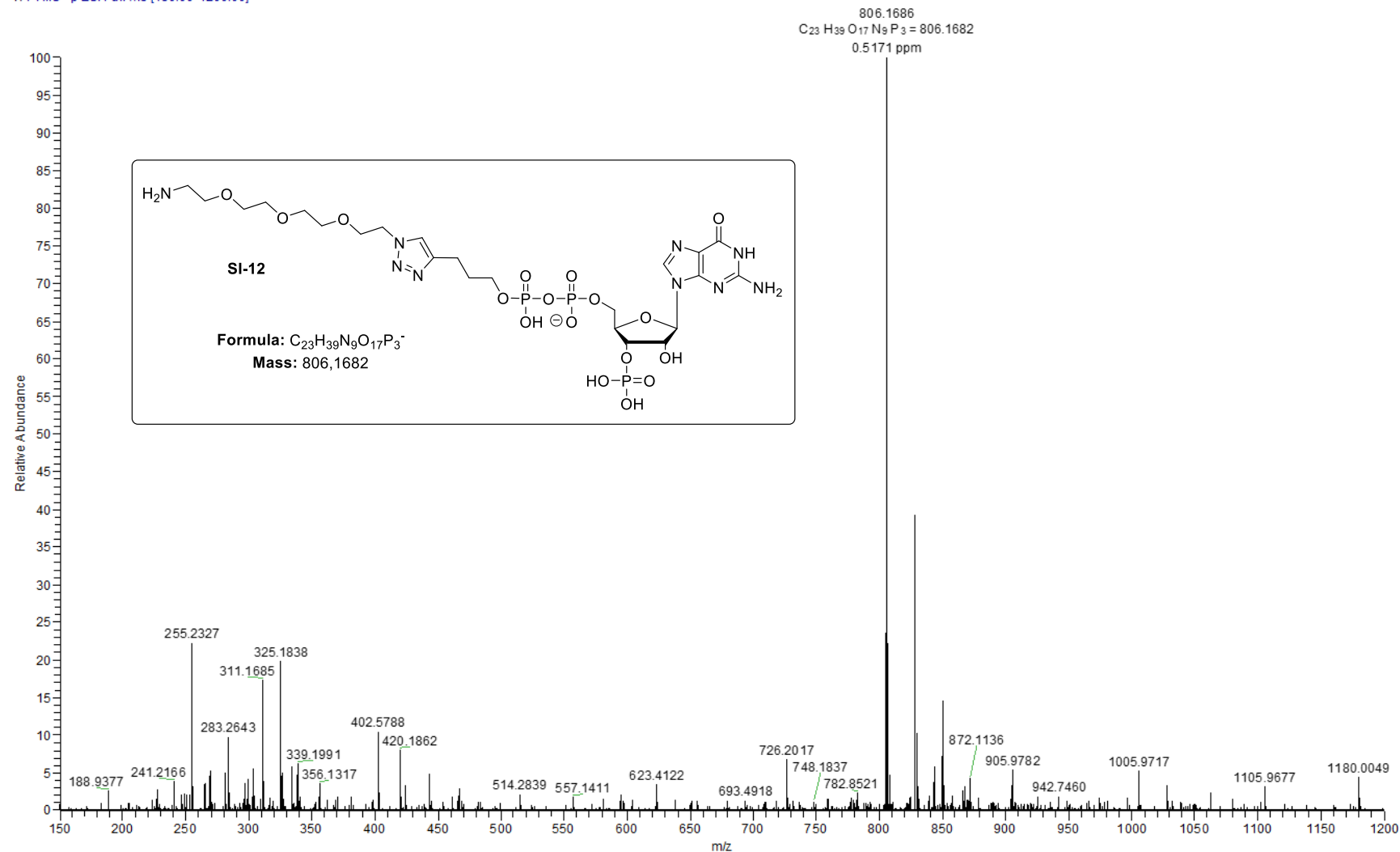
HRMS (ESI) Analysis of compound 20: amino – ppApp

hsjeb54shr5 #1 RT: 0.02 AV: 1 NL: 5.74E5
T: FTMS - p ESI Full ms [150.00-1200.00]



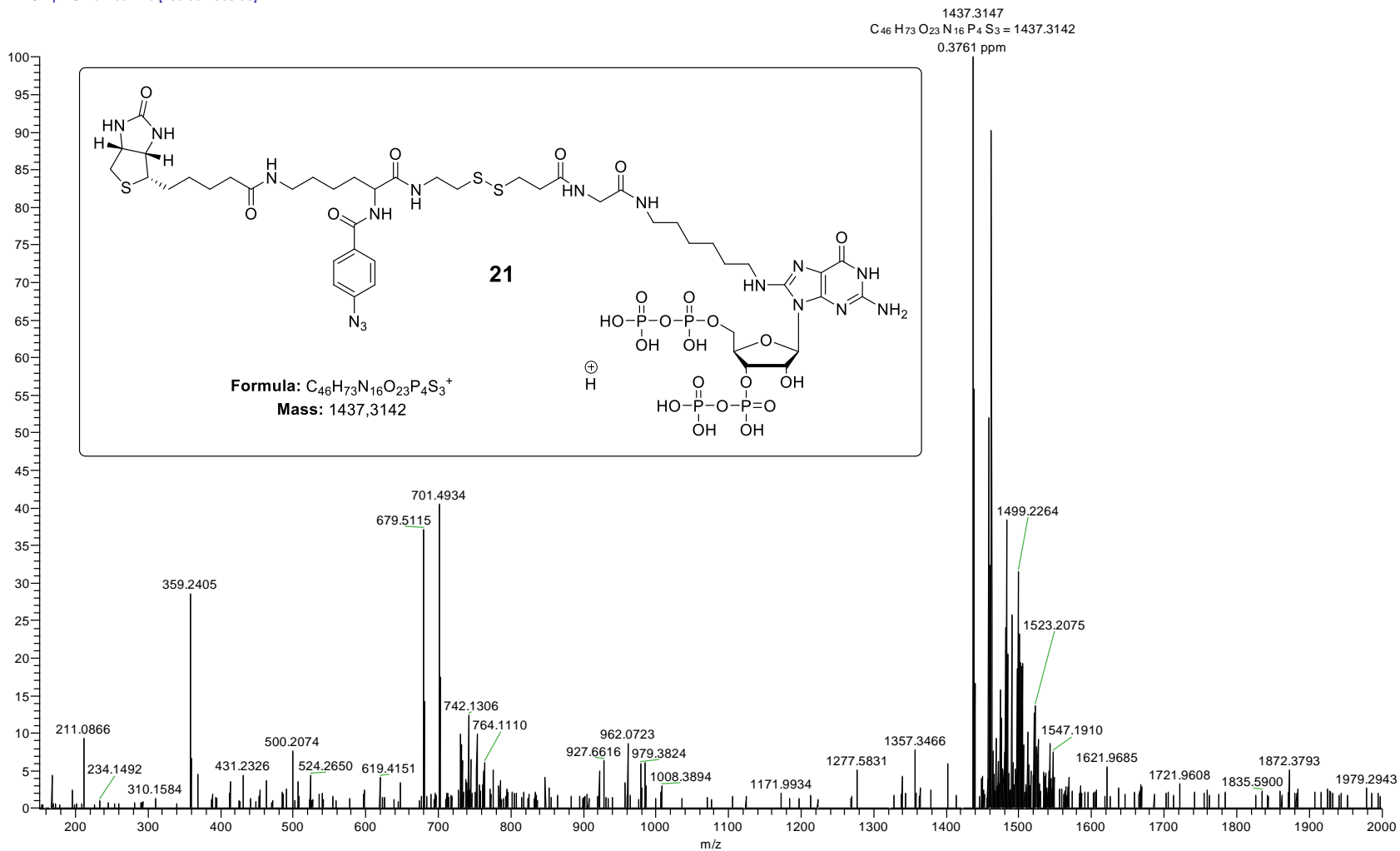
HRMS (ESI) Analysis of compound SI-12: amino – ppGp

hsjeb55shr1 #1 RT: 0.02 AV: 1 NL: 9.71E5
T: FTMS - p ESI Full ms [150.00-1200.00]



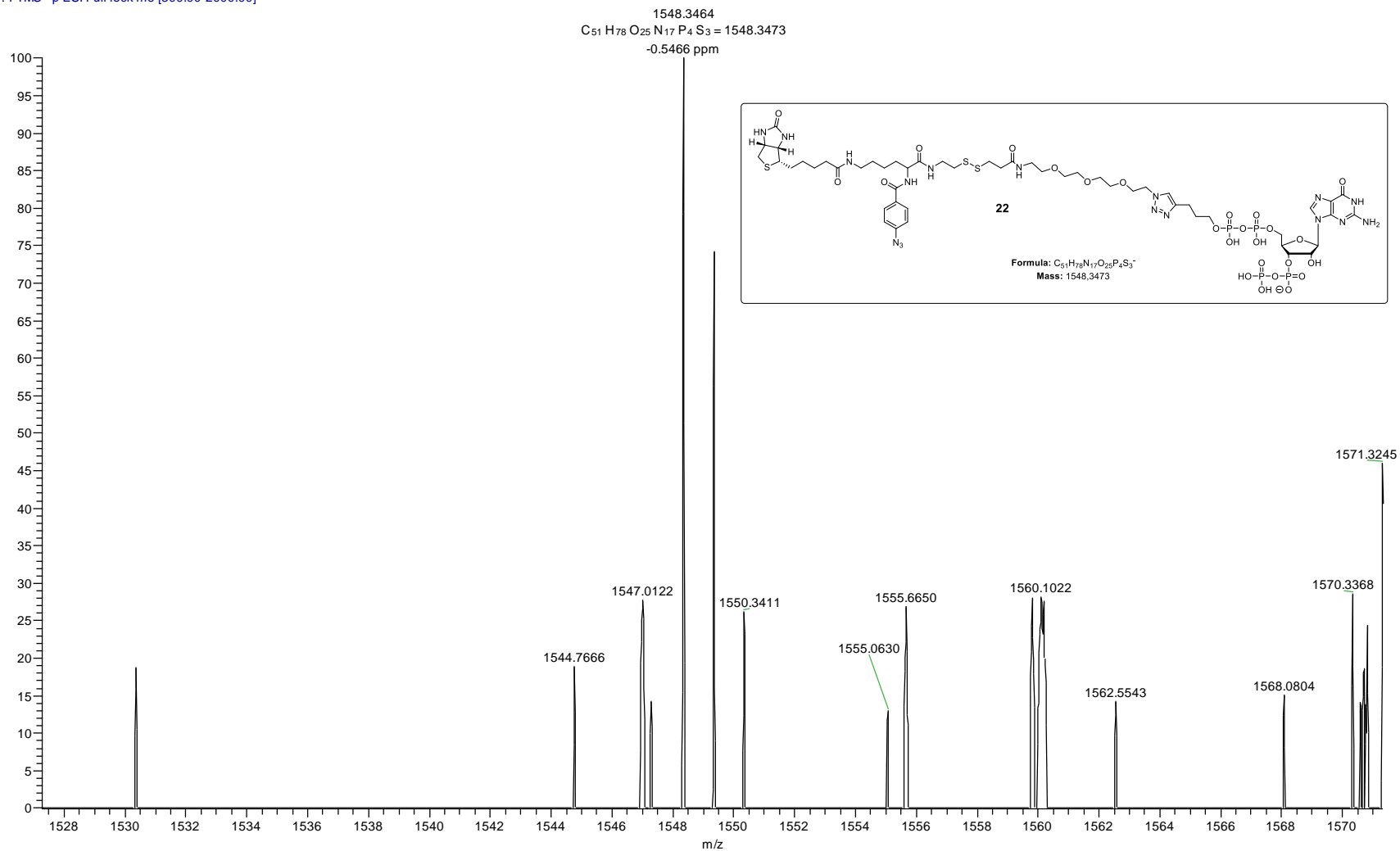
HRMS (ESI) Analysis of compound 21: ppGpp – CC1

hsjeb25shr8 #1 RT: 0.02 AV: 1 NL: 1.91E5
T: FTMS + p ESI Full lock ms [150.00-2000.00]



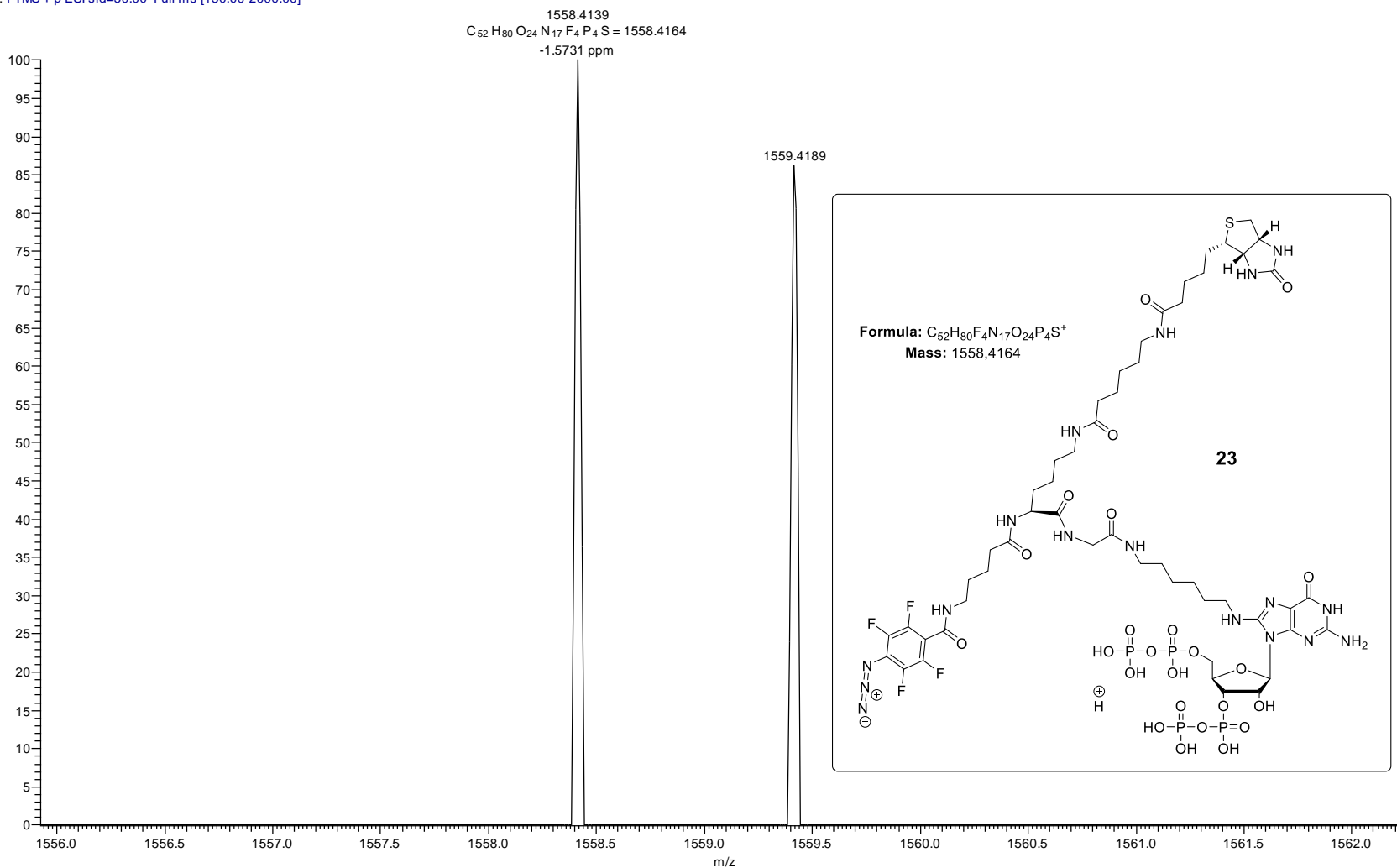
HRMS (ESI) Analysis of compound 22: ppGpp – CC2

hsjeb29shr02 #1 RT: 0.03 AV: 1 NL: 9.10E3
T: FTMS - p ESI Full lock ms [300.00-2000.00]



HRMS (ESI) Analysis of compound 23: ppGpp – CC3

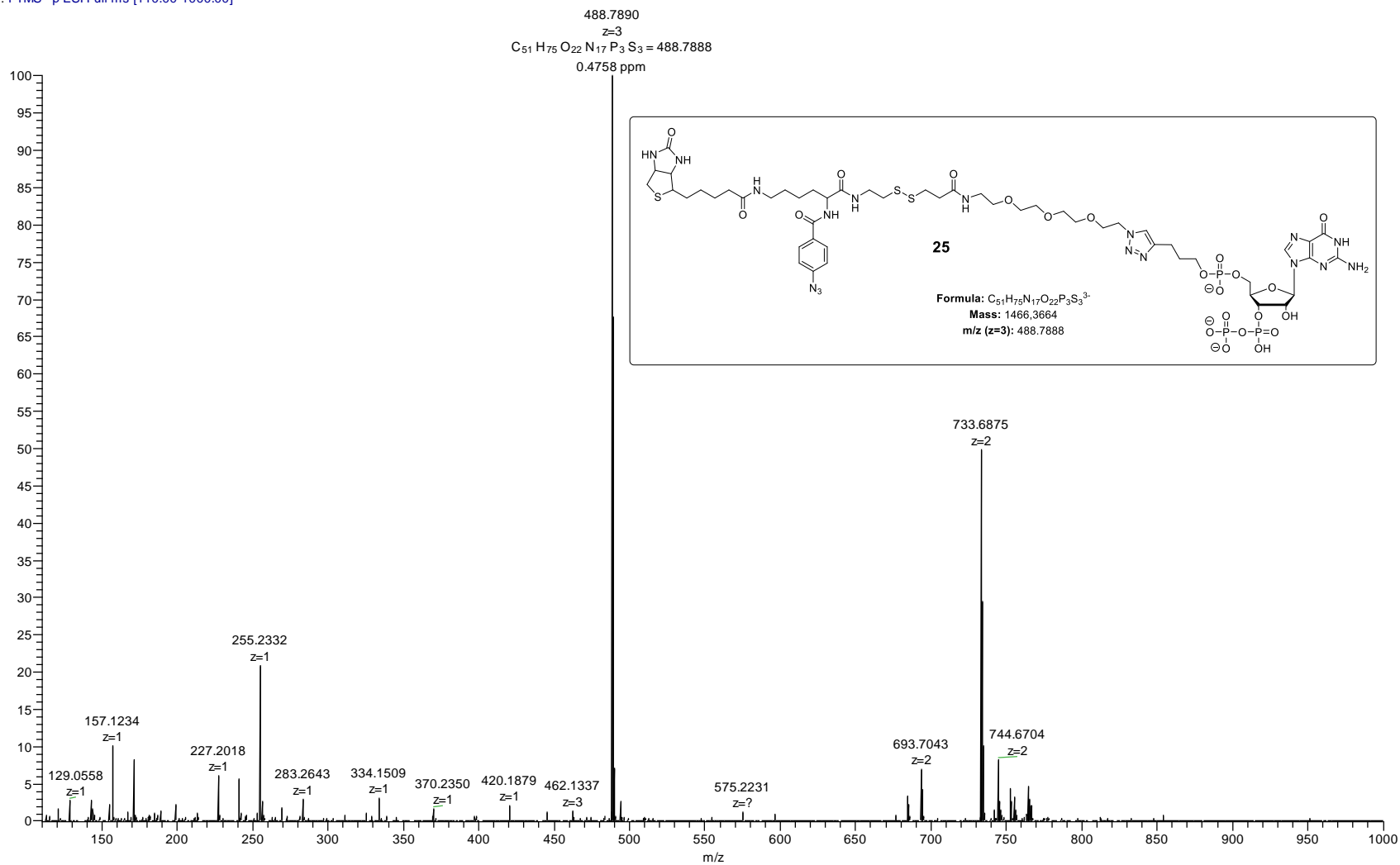
hsjeb39shr3 #1 RT: 0.02 AV: 1 NL: 8.53E3
T: FTMS + p ESI sid=50.00 Full ms [150.00-2000.00]





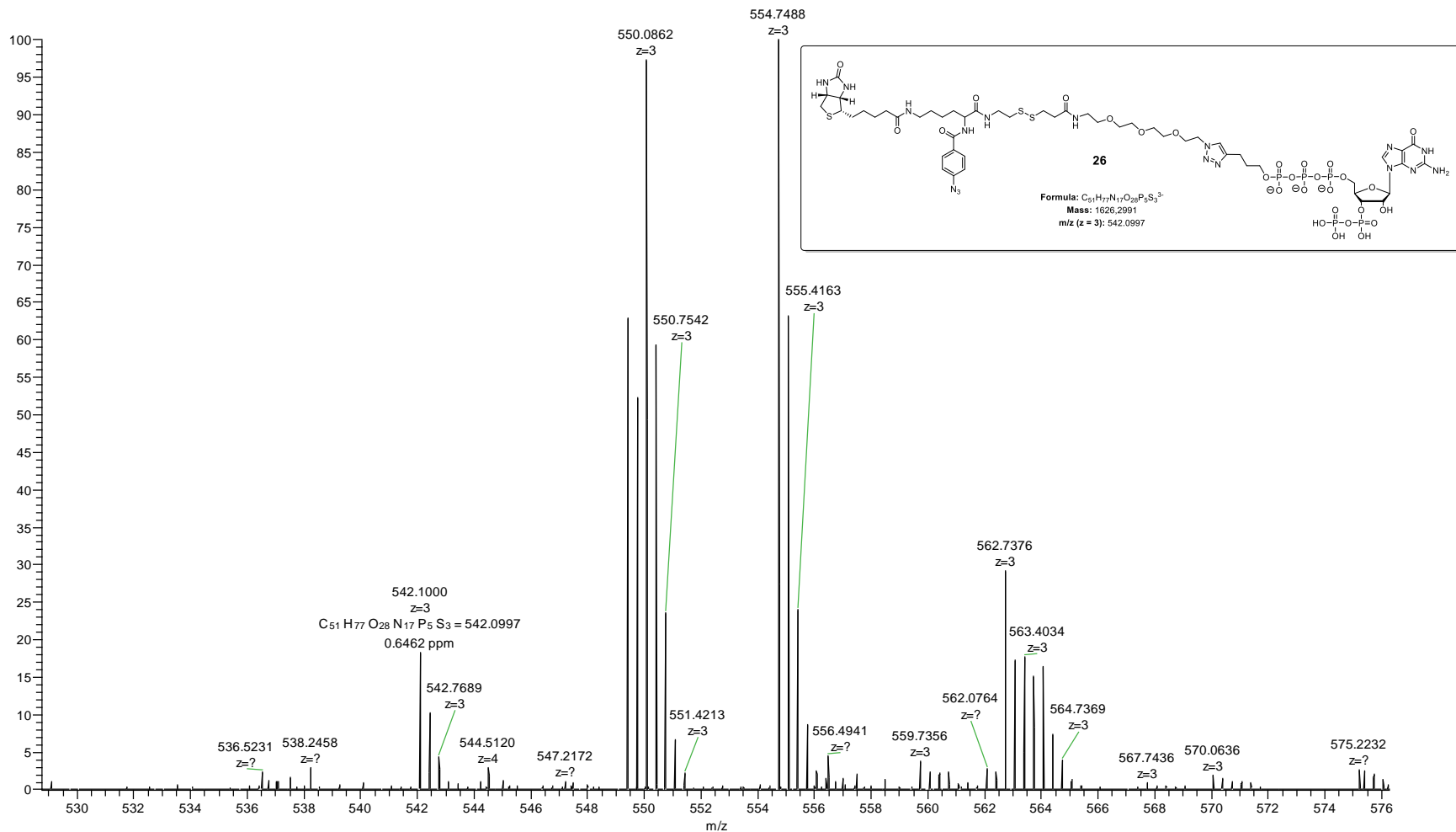
HRMS (ESI) Analysis of compound 25: pGpp - CC

hsjeb57shr3 #1 RT: 0.02 AV: 1 NL: 7.73E6
T: FTMS - p ESI Full ms [110.00-1000.00]



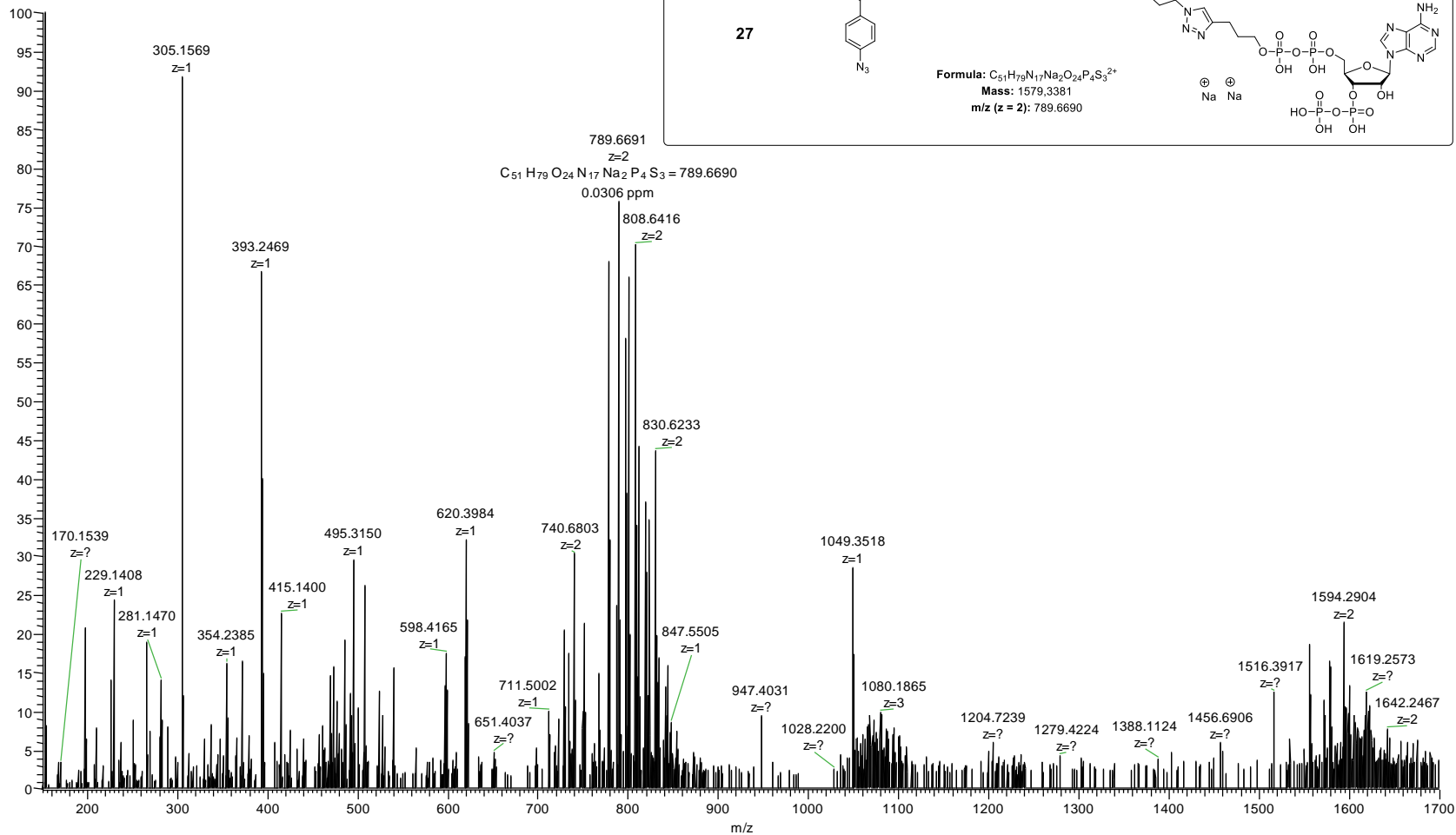
HRMS (ESI) Analysis of compound 26: pppGpp - CC

hsjeb56shr1 #1 RT: 0.02 AV: 1 NL: 2.69E6
T: FTMS - p ESI Full ms [110.00-1000.00]



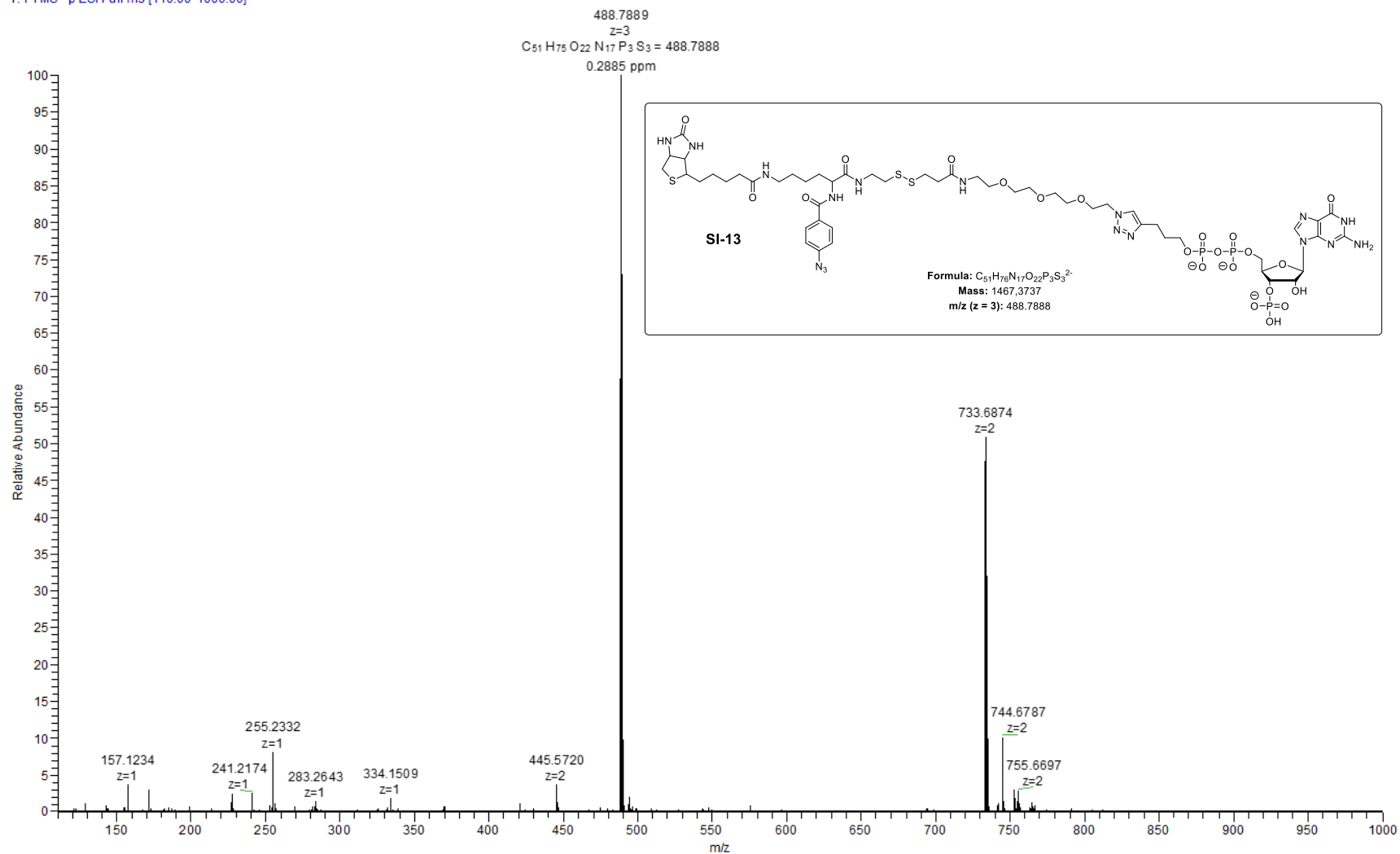
HRMS (ESI) Analysis of compound 27: ppApp - CC

hsjeb59shr7 #1 RT: 0.02 AV: 1 NL: 2.63E5
T: FTMS + p ESI Full lock ms [150.00-1700.00]



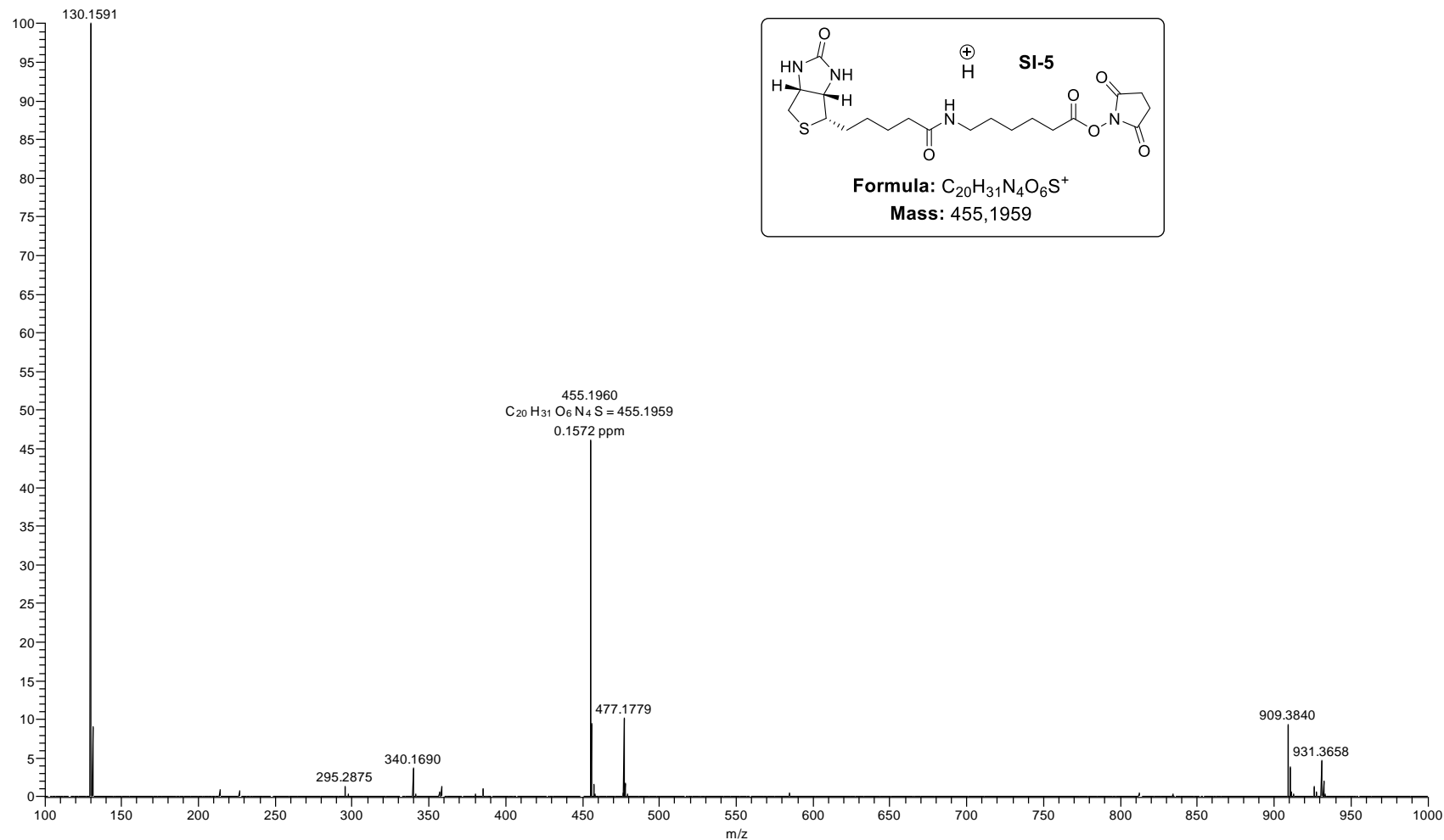
HRMS (ESI) Analysis of compound SI-13: ppGp - CC

hsjeb58shr1#1 RT: 0.02 AV: 1 NL: 1.74E7
T: FTMS - p ESI Full ms [110.00-1000.00]



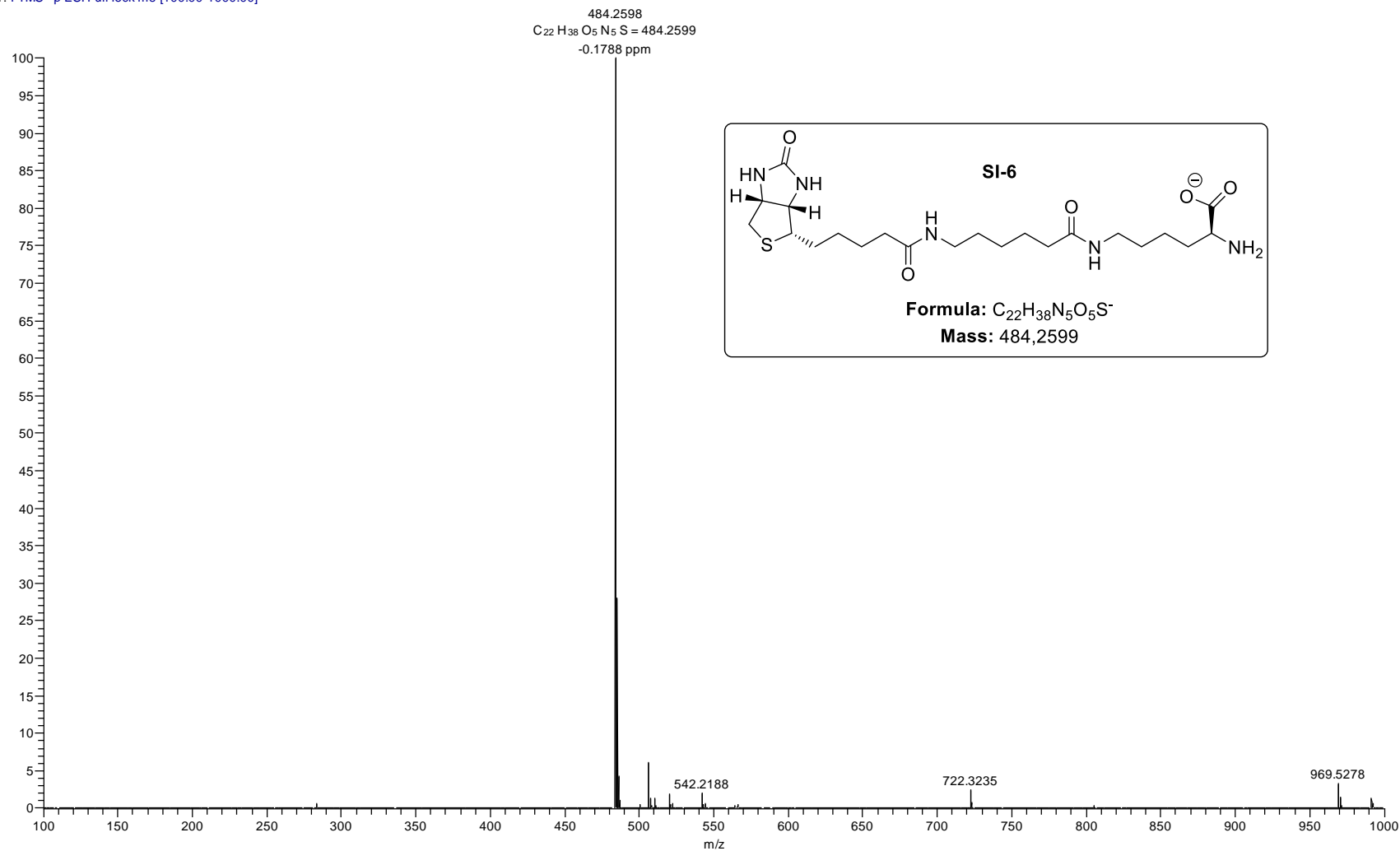
HRMS (ESI) Analysis of compound SI-5: Biotin-NHS derivative

hsjeb20shr08 #1 RT: 0.02 AV: 1 NL: 5.16E7
T: FTMS + p ESI Full ms [100.00-1000.00]



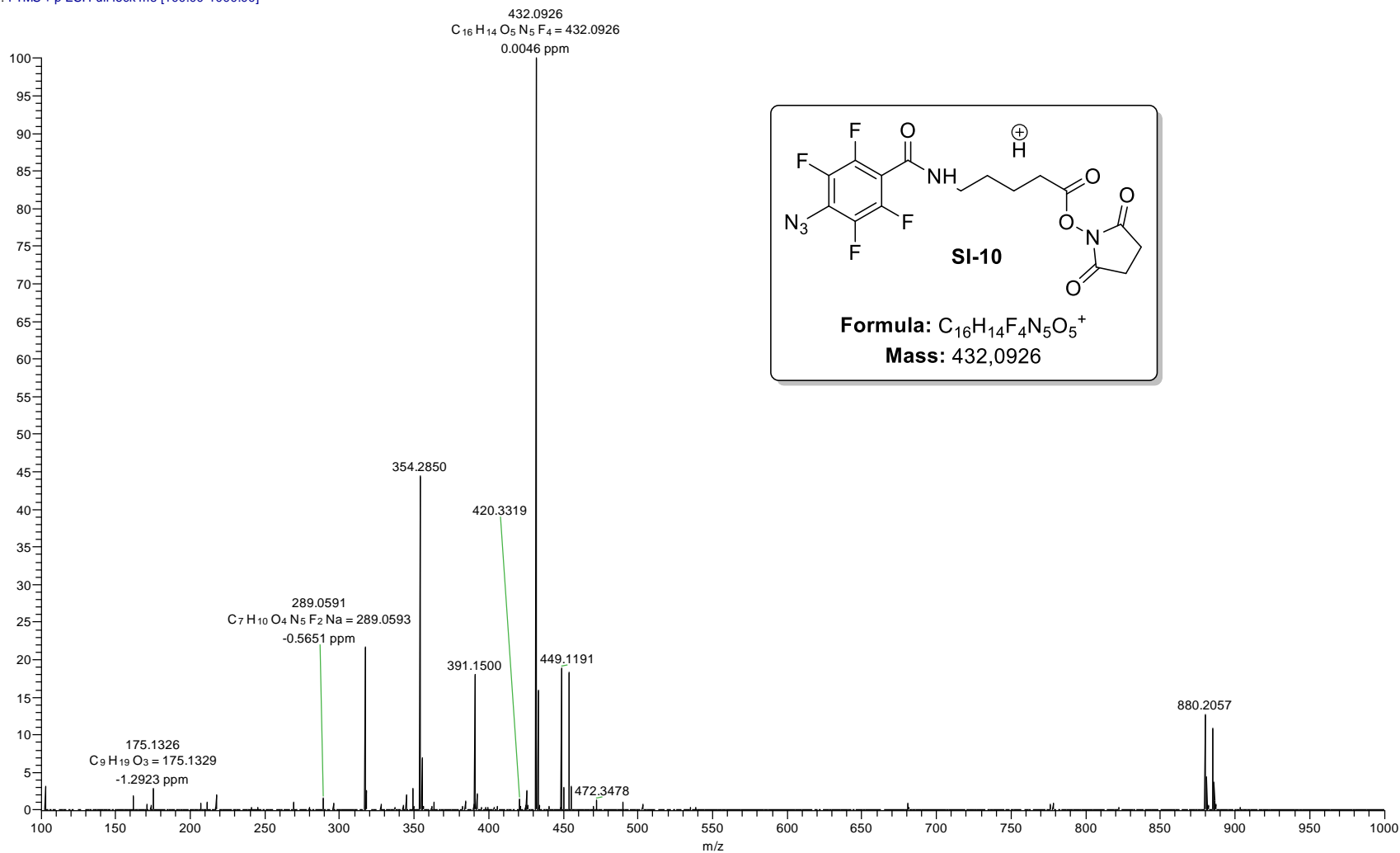
HRMS (ESI) Analysis of compound SI-6: Biotin-lysine moiety

hsjeb28shr02 #1 RT: 0.02 AV: 1 NL: 4.47E7
T: FTMS - p ESI Full lock ms [100.00-1000.00]



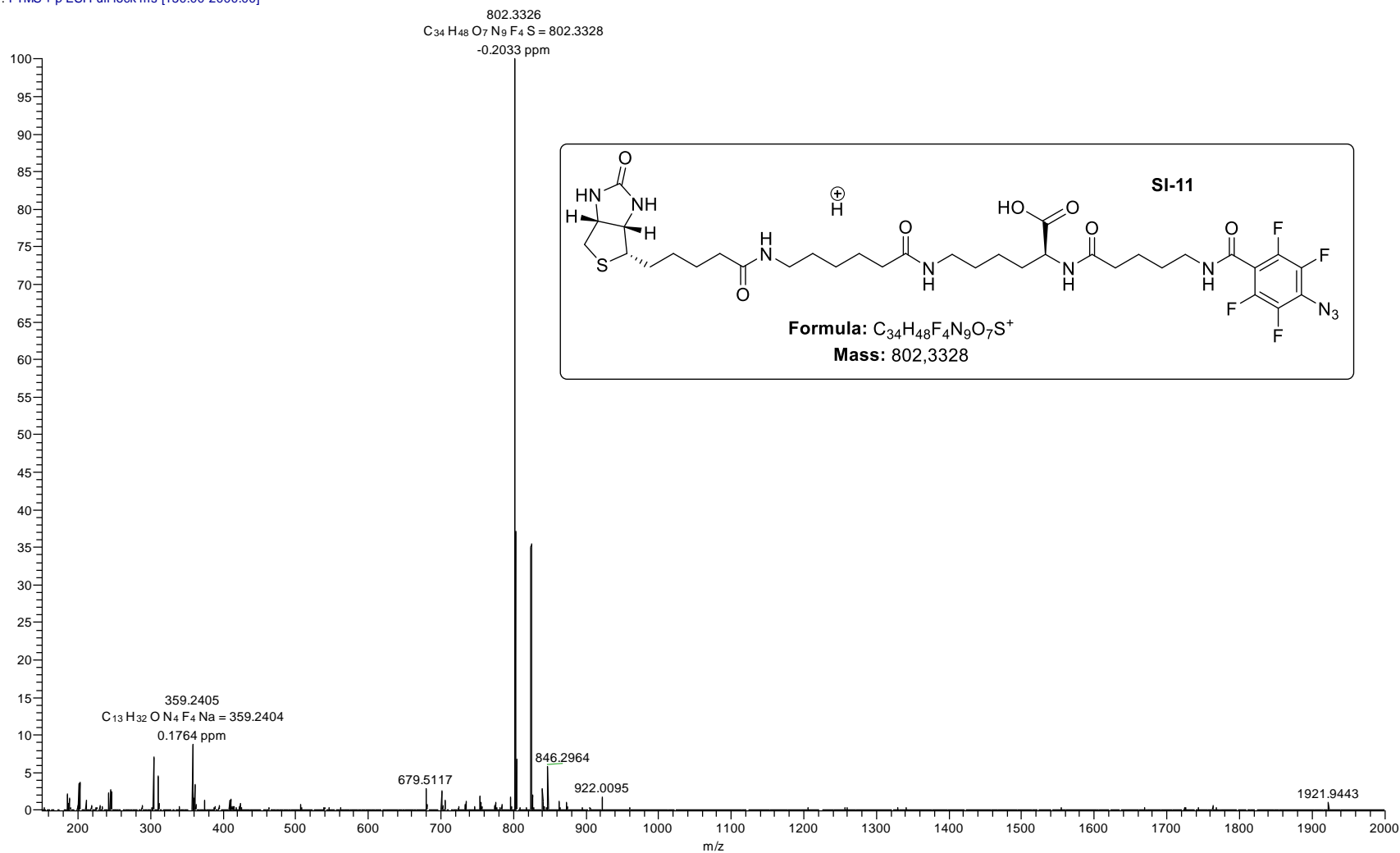
HRMS (ESI) Analysis of compound SI-10: Fluorophenylazide derivative

hsjeb26shr1 #1 RT: 0.02 AV: 1 NL: 2.23E7
T: FTMS + p ESI Full lock ms [100.00-1000.00]



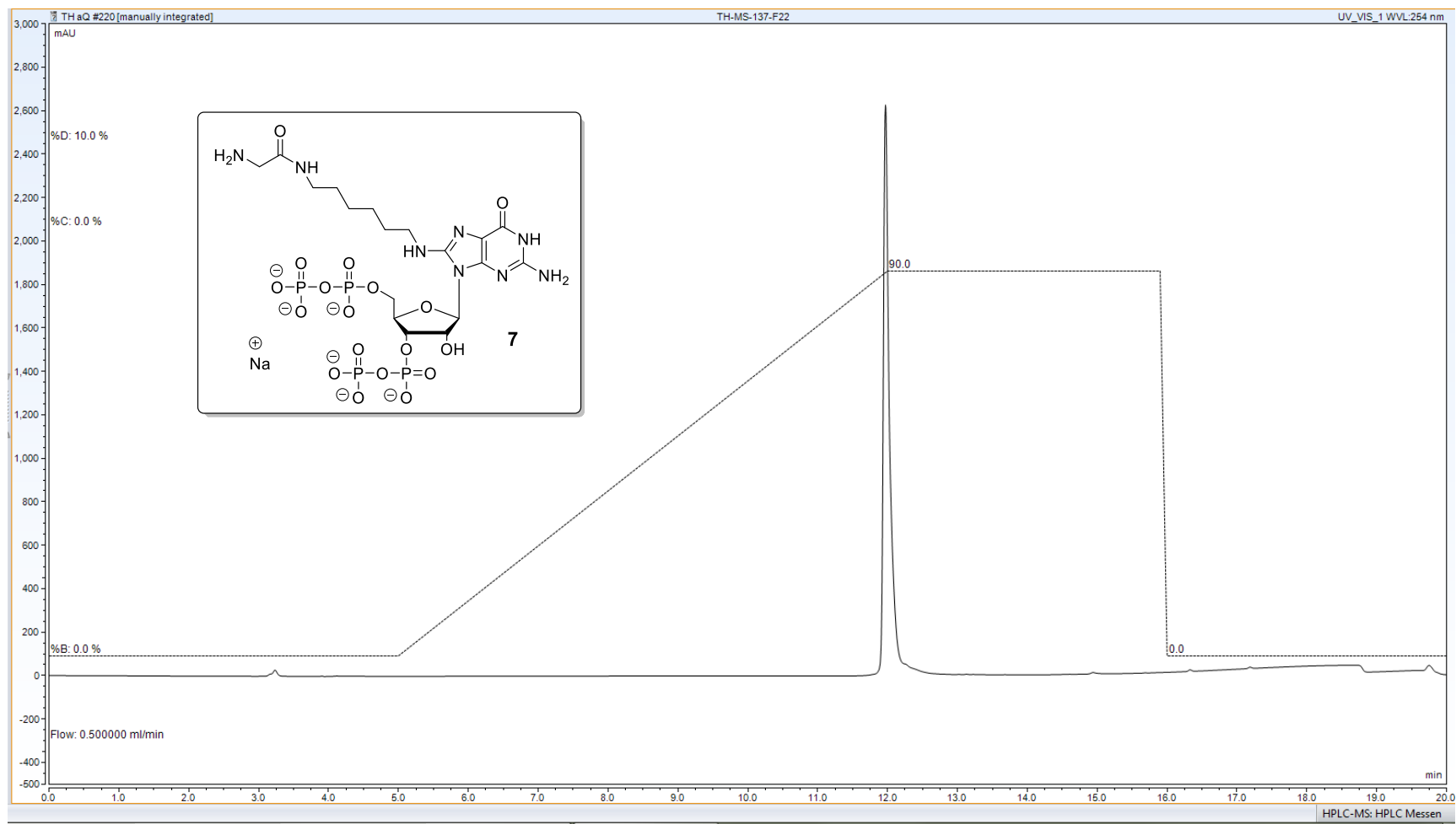
HRMS (ESI) Analysis of compound SI-11: linker structure (carboxylic acid)

hsjeb31shr7 #1 RT: 0.02 AV: 1 NL: 2.24E6
T: FTMS + p ESI Full lock ms [150.00-2000.00]

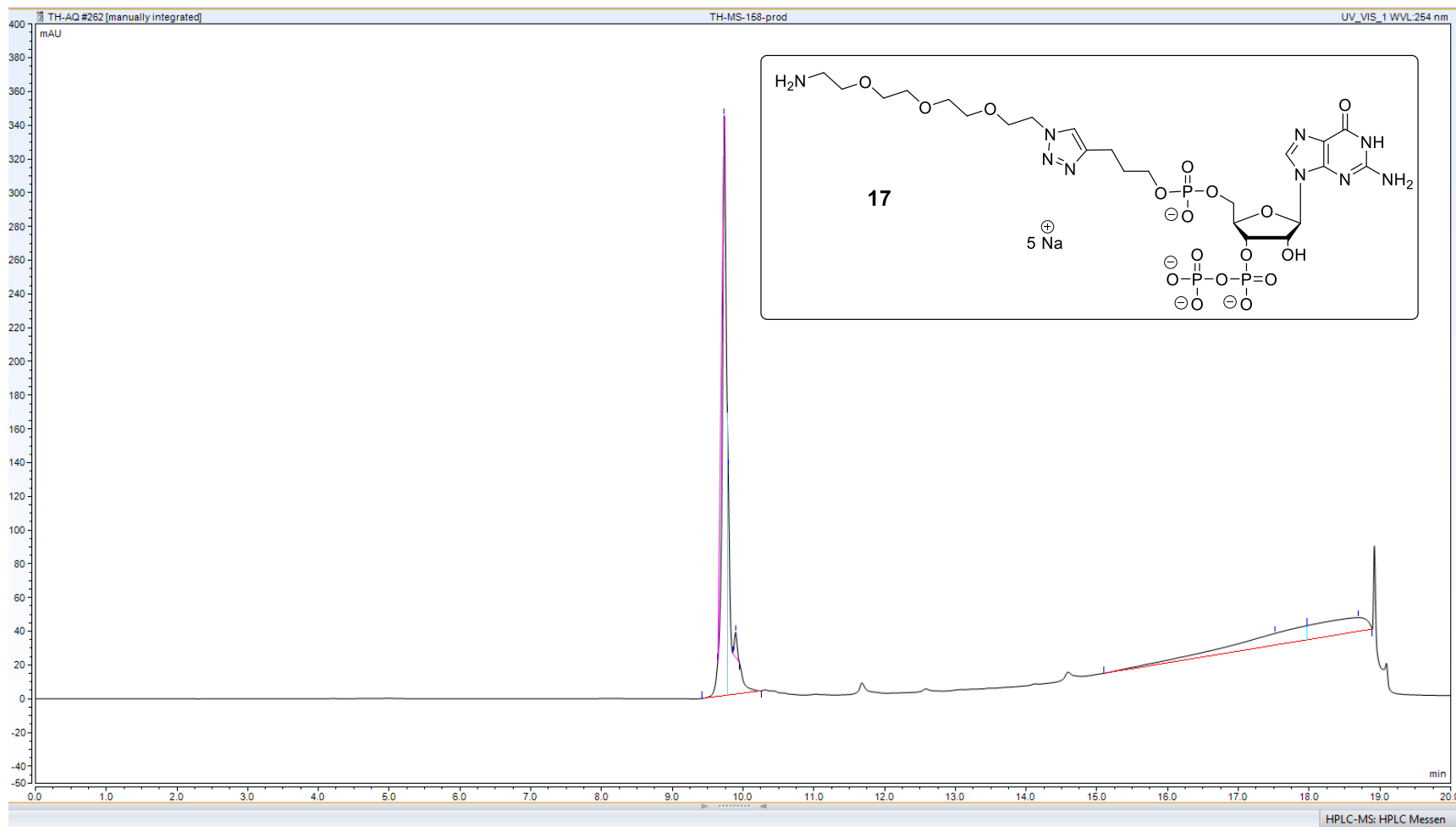


9. HPLC – analysis

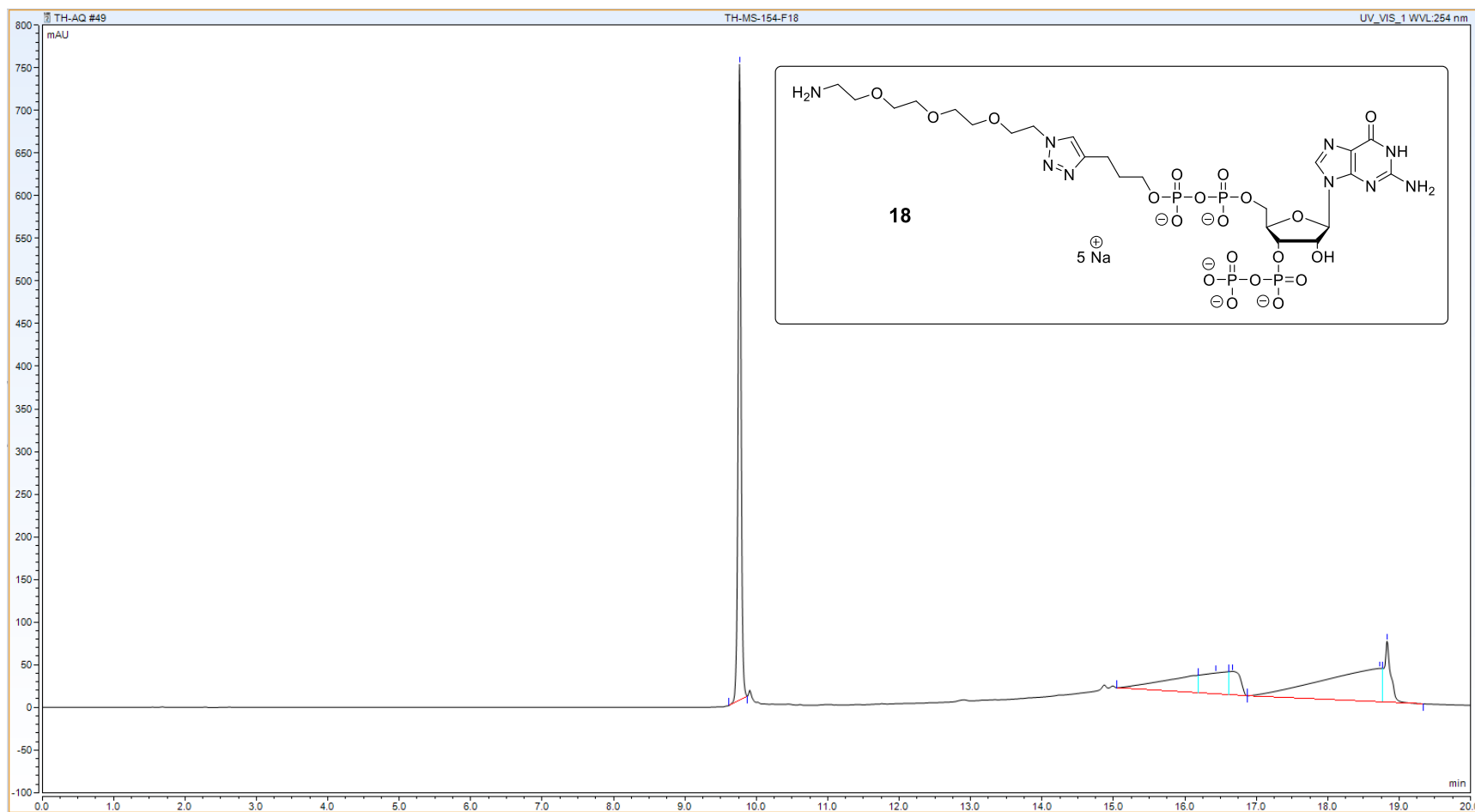
HPLC-UV measurements were performed using a dionex ultimate 3000 system and a C18AQ – column. A gradient of H₂O/MeCN/TEAA(pH 7, 100 mM) was applied.

HPLC – UV – analysis of compound 7: amino – ppGpp 1

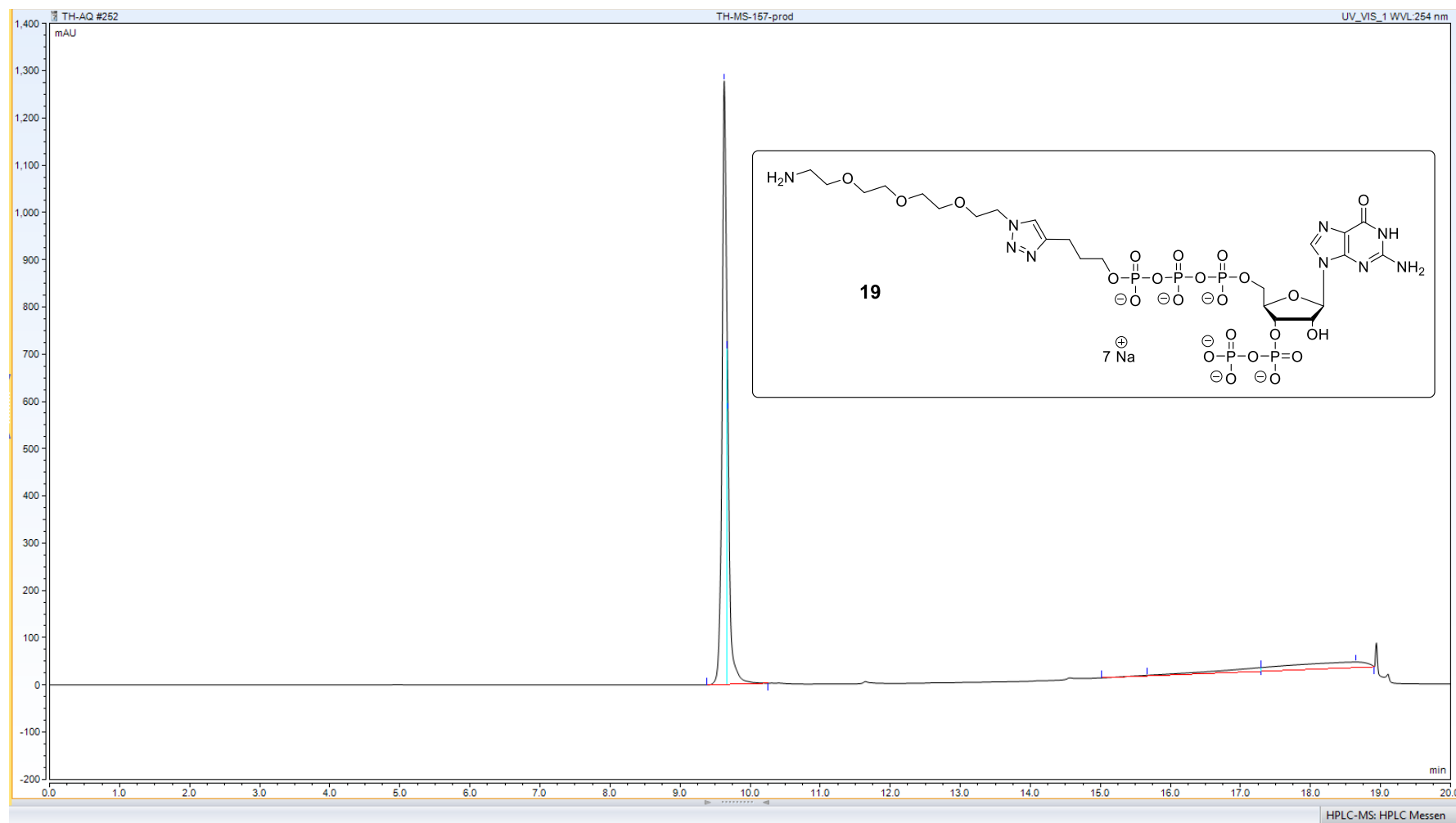
C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound 17: amino – pGpp

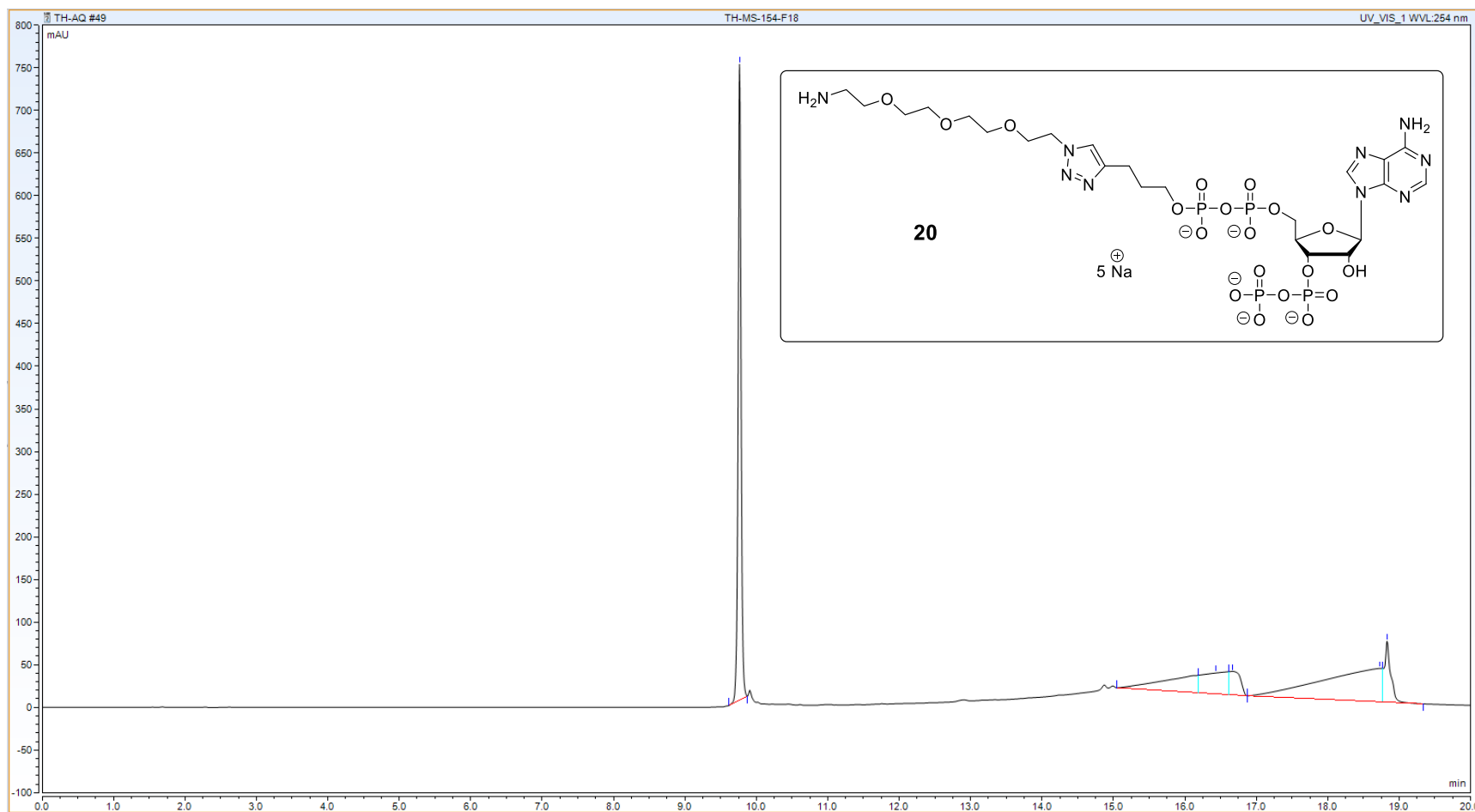
C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound 18: amino – ppGpp 2

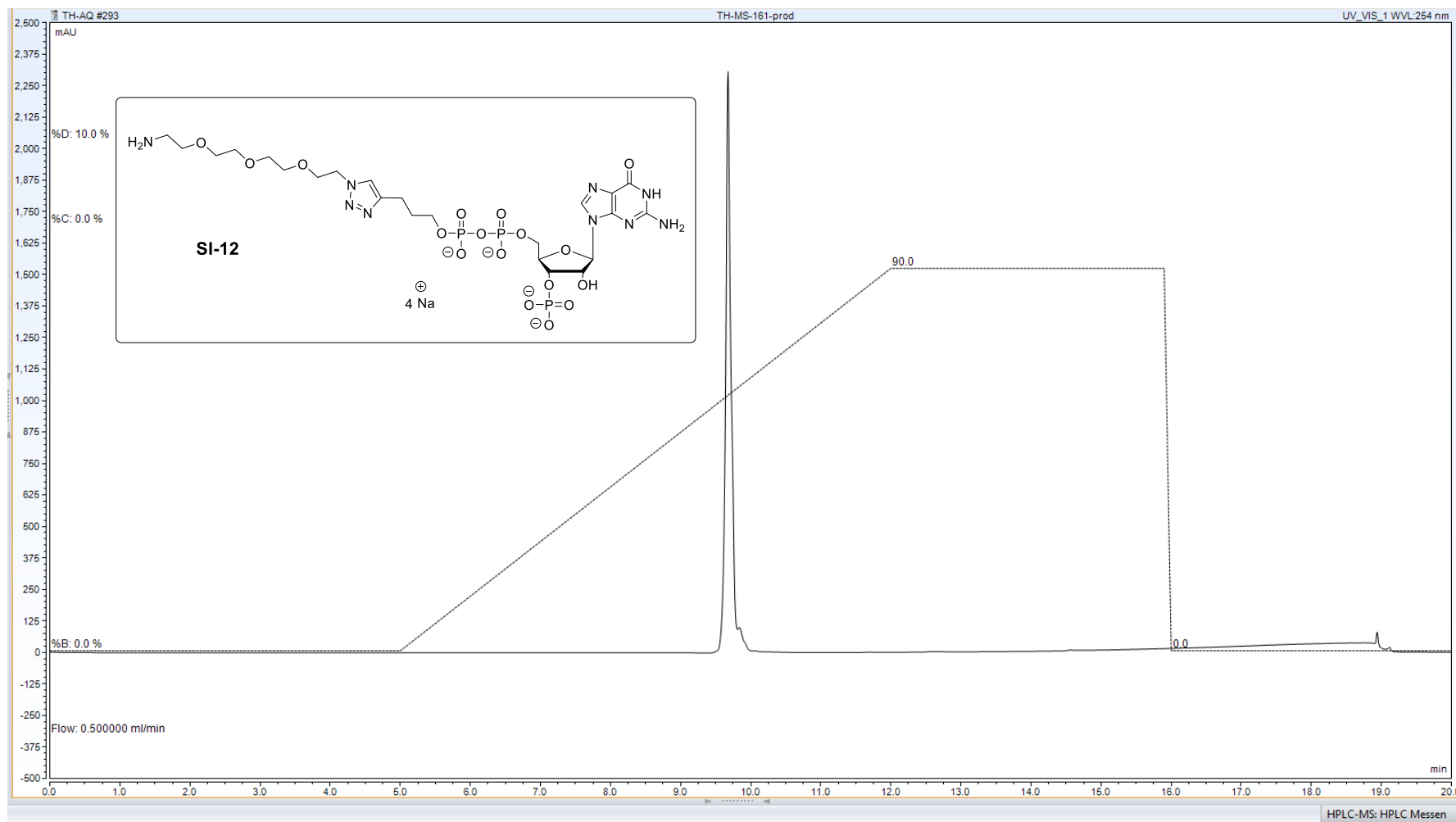
C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound 19: amino – pppGpp

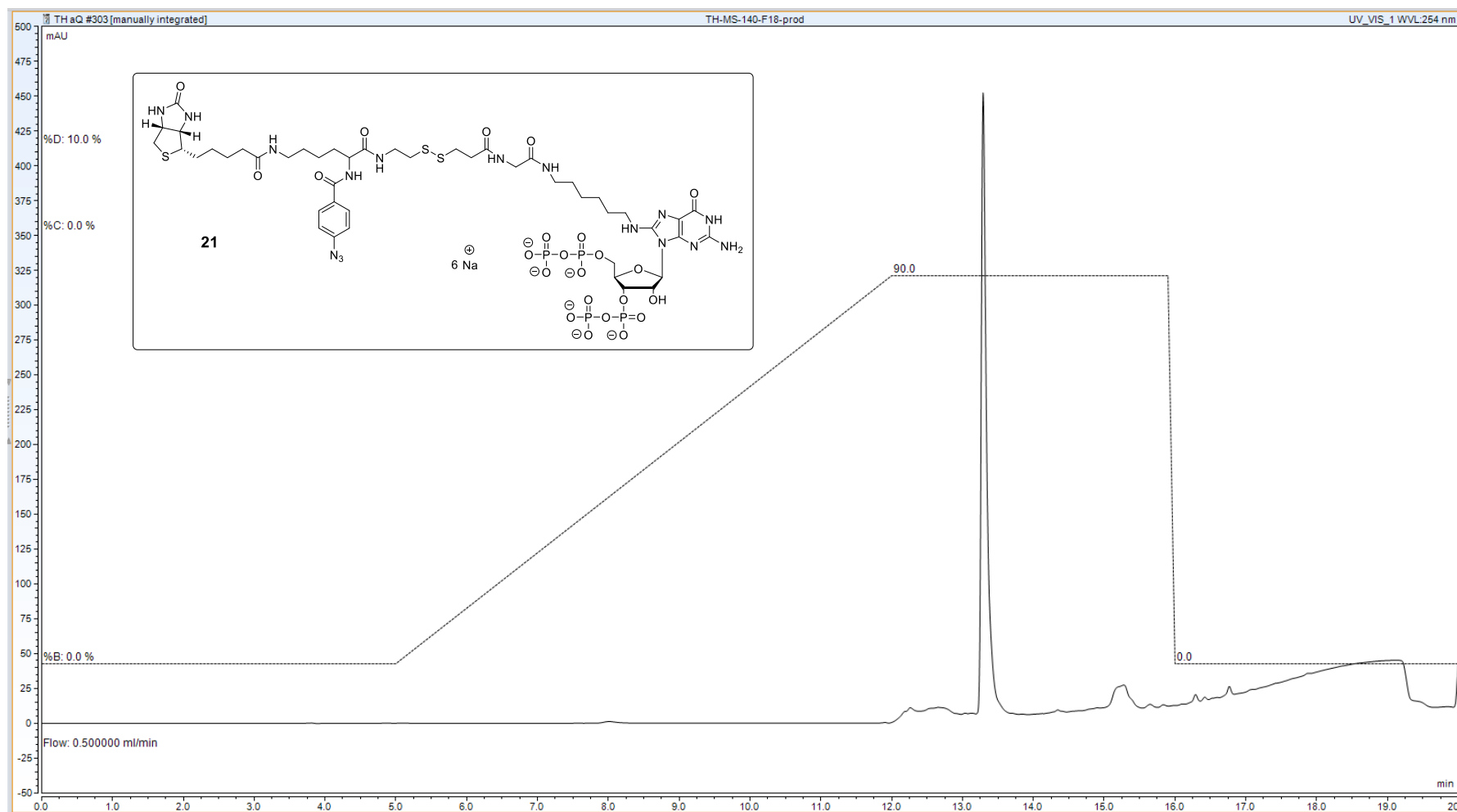
C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound 20: amino – ppApp

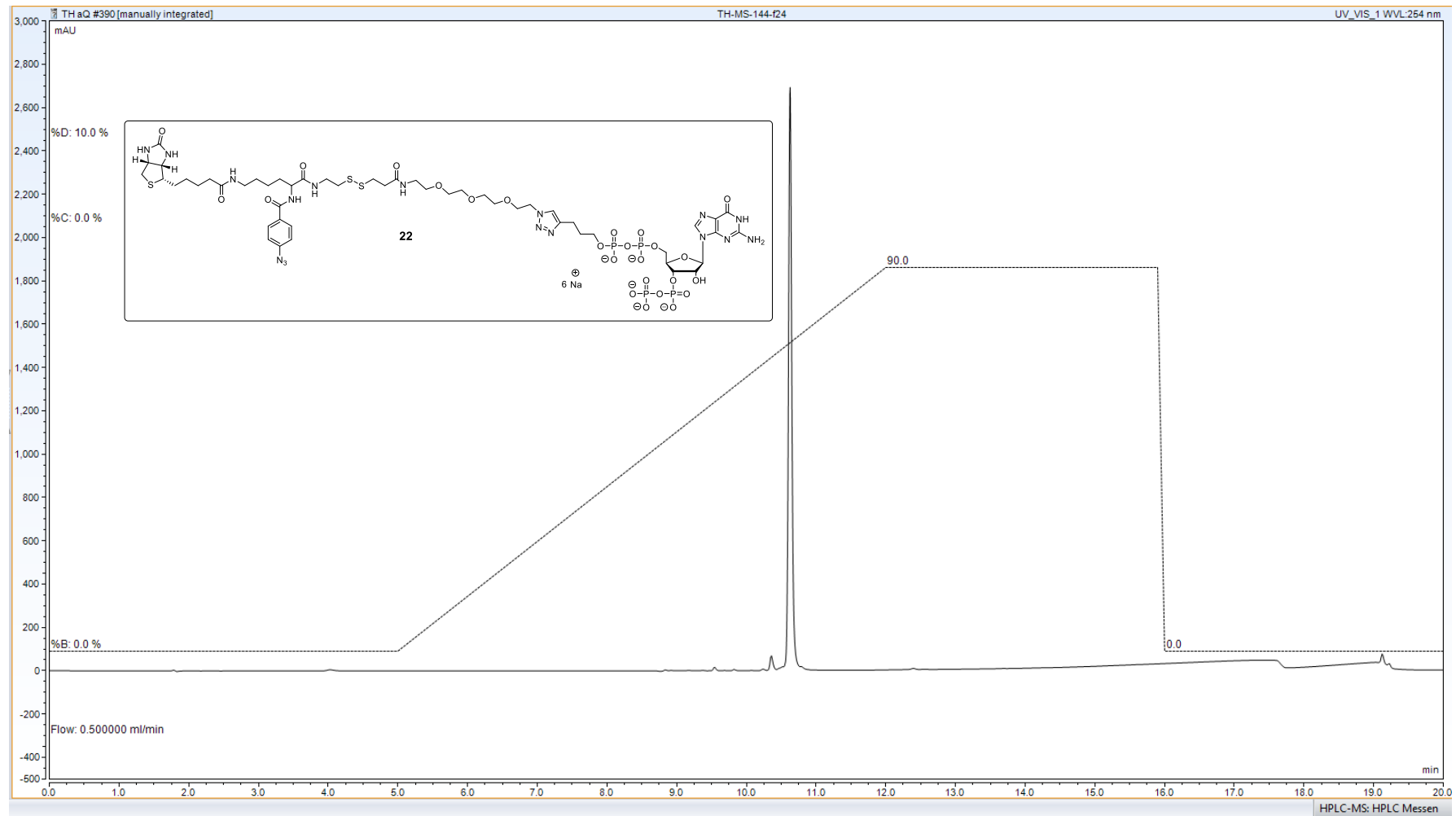
C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound SI-12: amino – ppGp

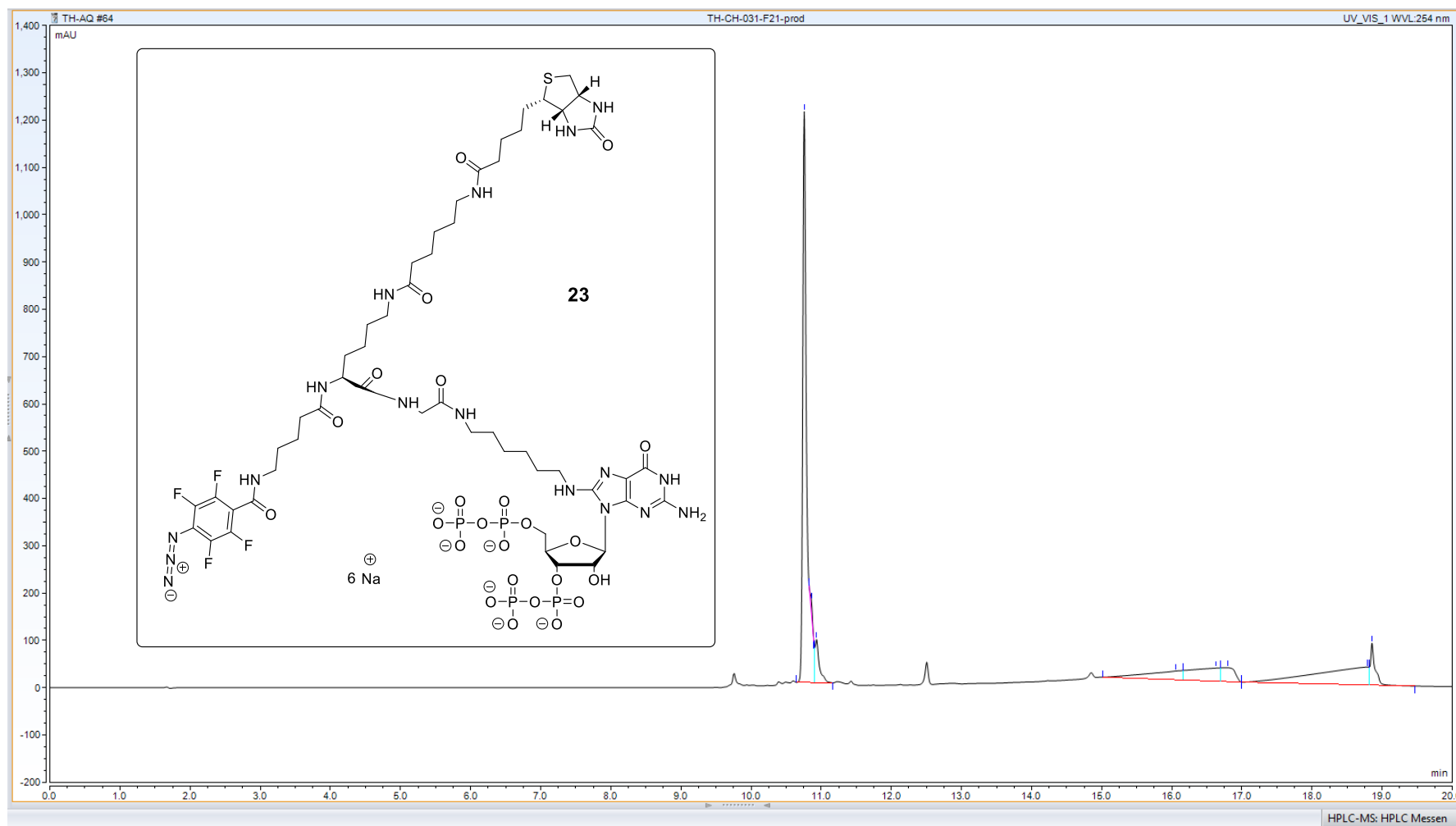
C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound 21: ppGpp – CC1

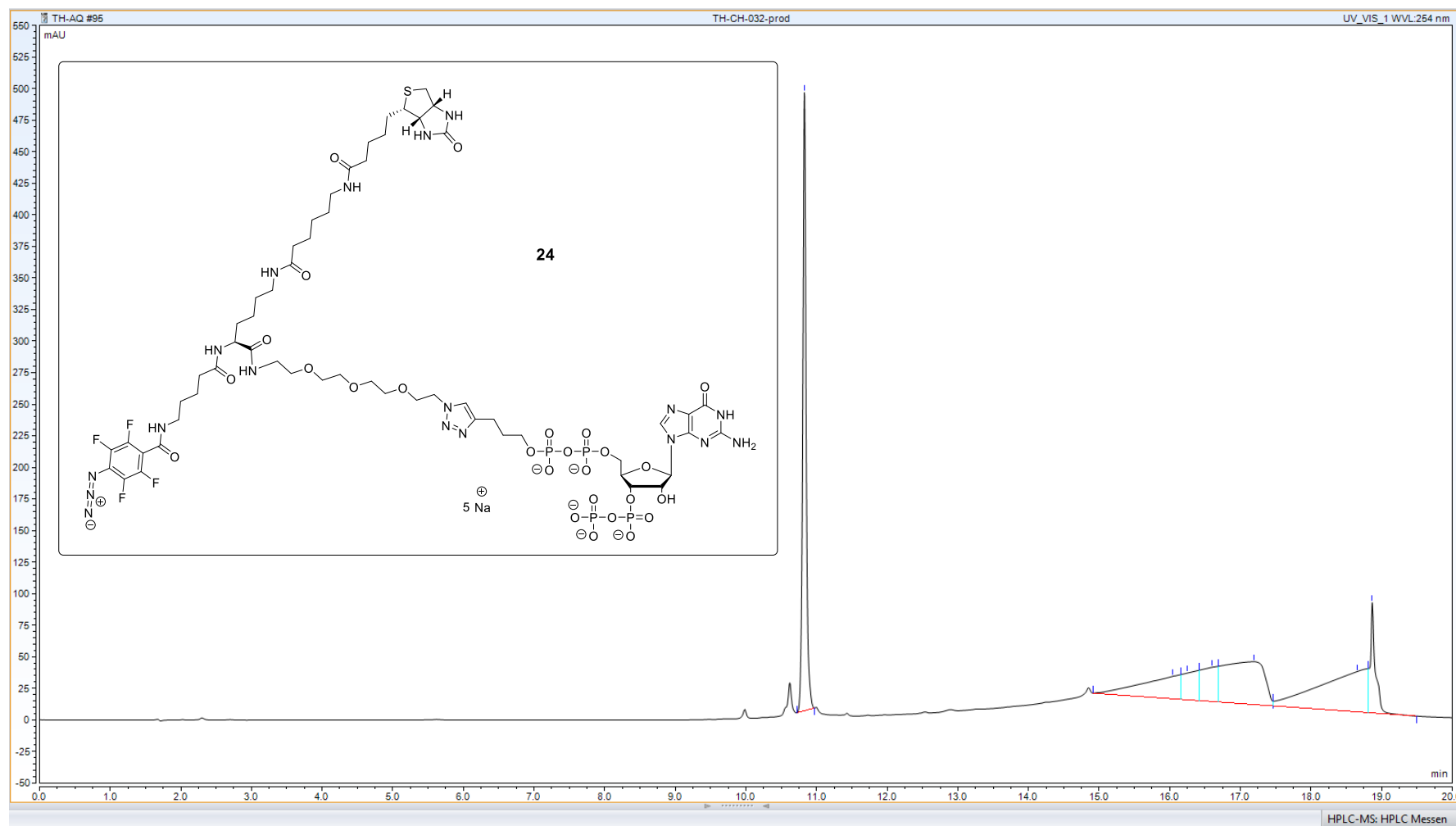
C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound 22: ppGpp – CC2

C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

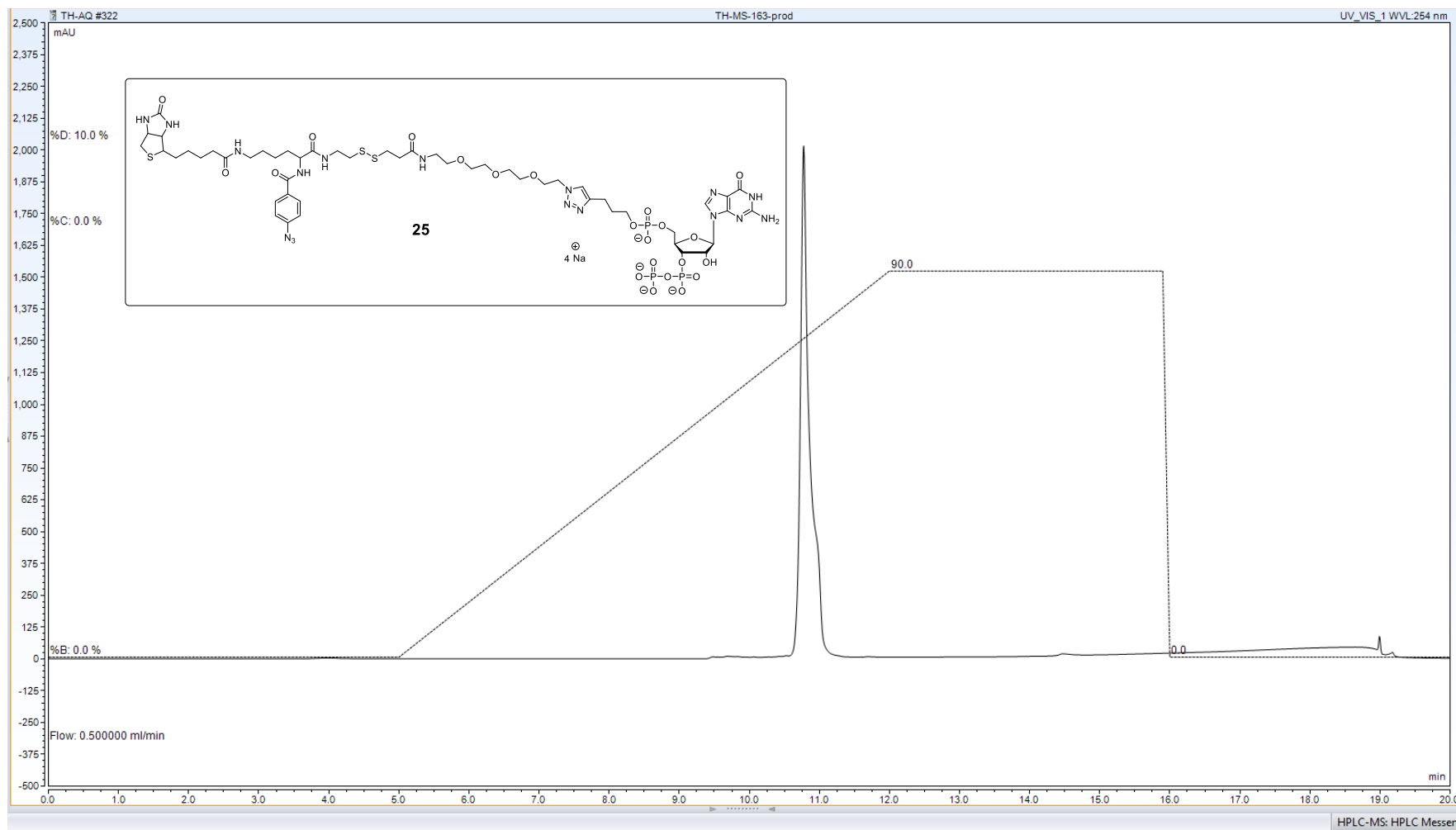
HPLC – UV – analysis of compound 23: ppGpp – CC3

C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

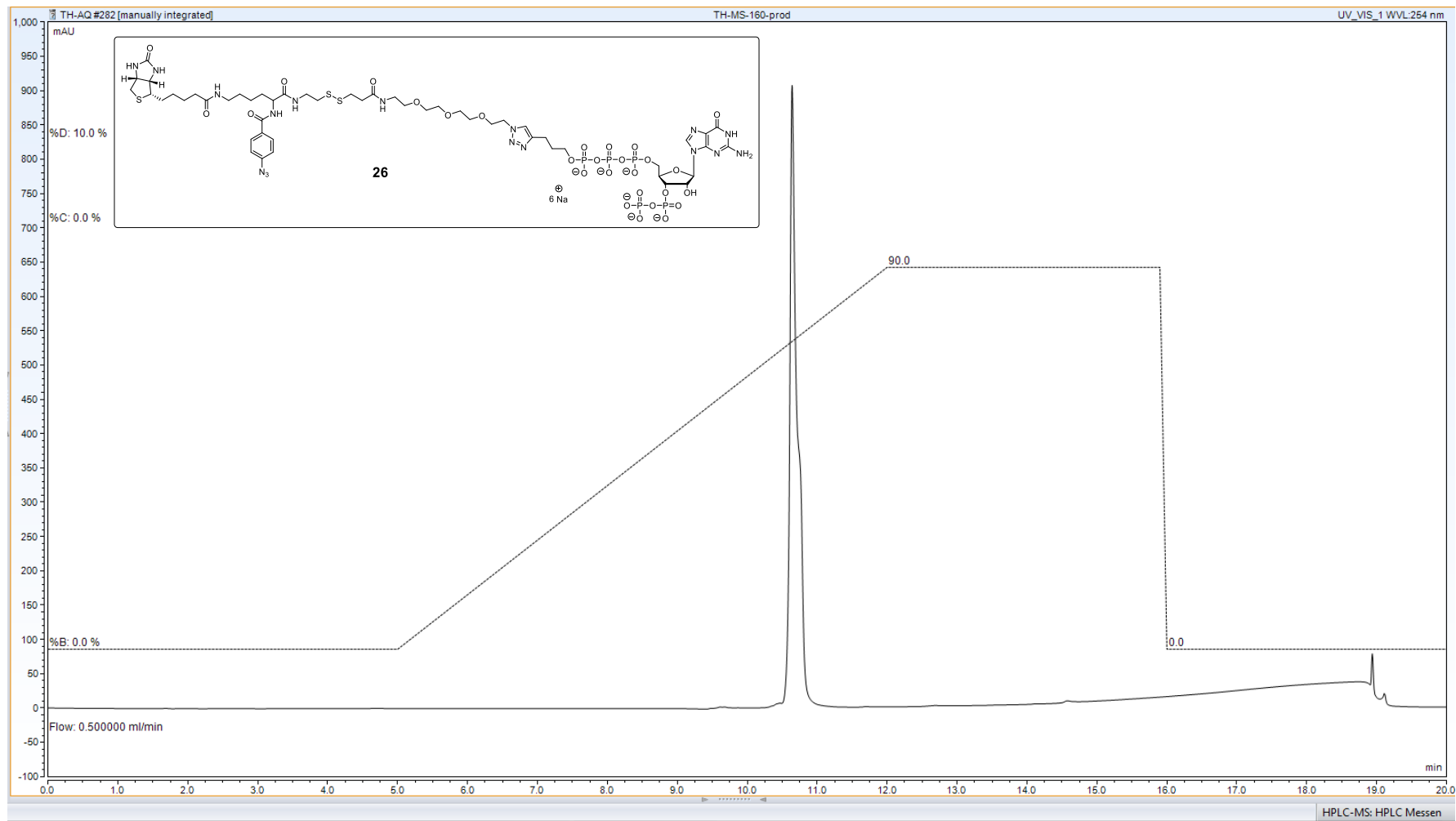
HPLC – UV – analysis of compound 24: ppGpp – CC4

C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

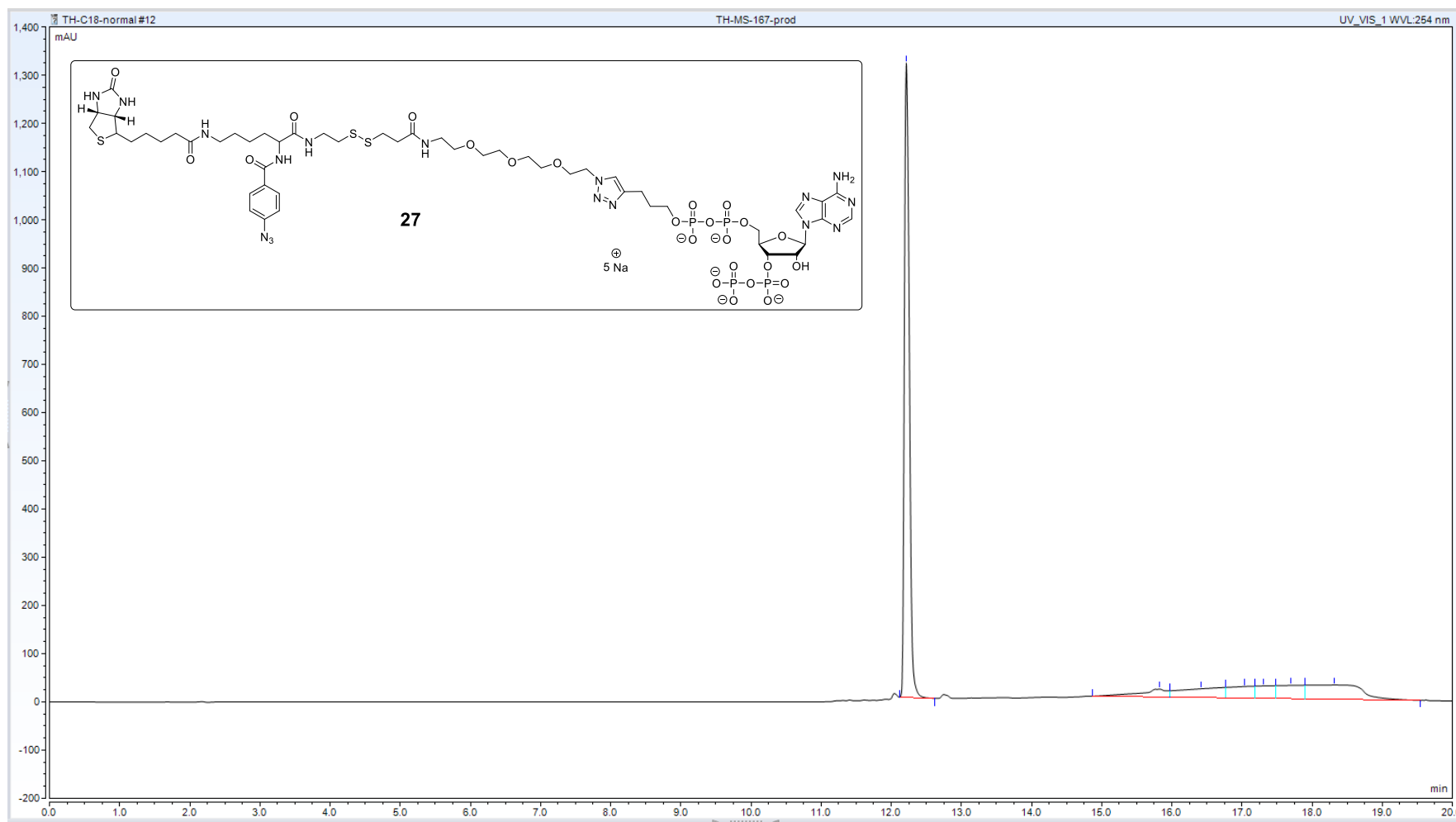
HPLC – UV – analysis of compound 25: pGpp – CC



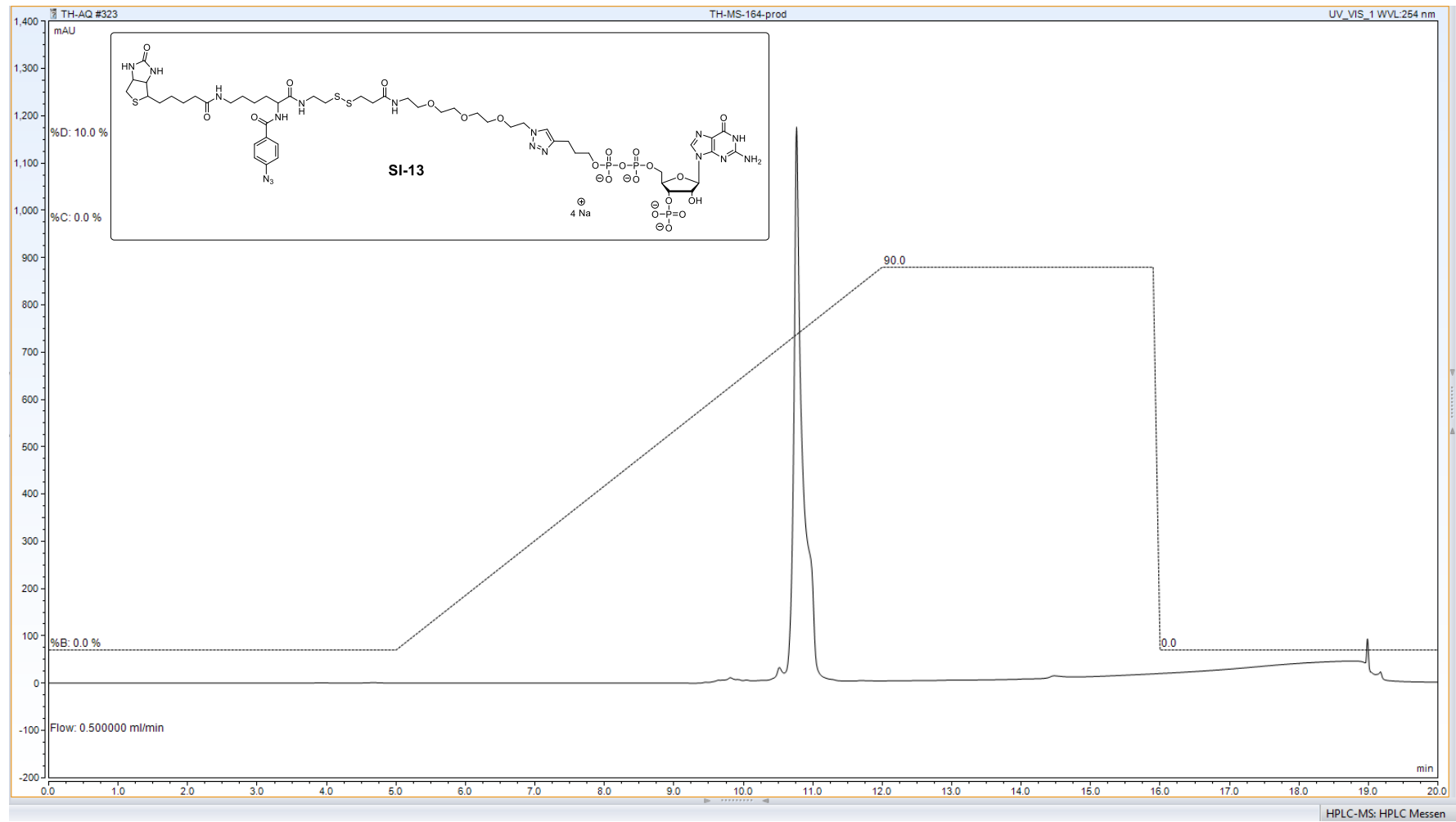
C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound 26: pppGpp – CC

C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound 27: ppApp - CC

C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)

HPLC – UV – analysis of compound SI-13: ppGp - CC

C18 AQ – column (H₂O/MeCN – gradient, 10 mM TEAA)