Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Synthesis and Fluorescence Characteristics of ATP-based FRET Probes

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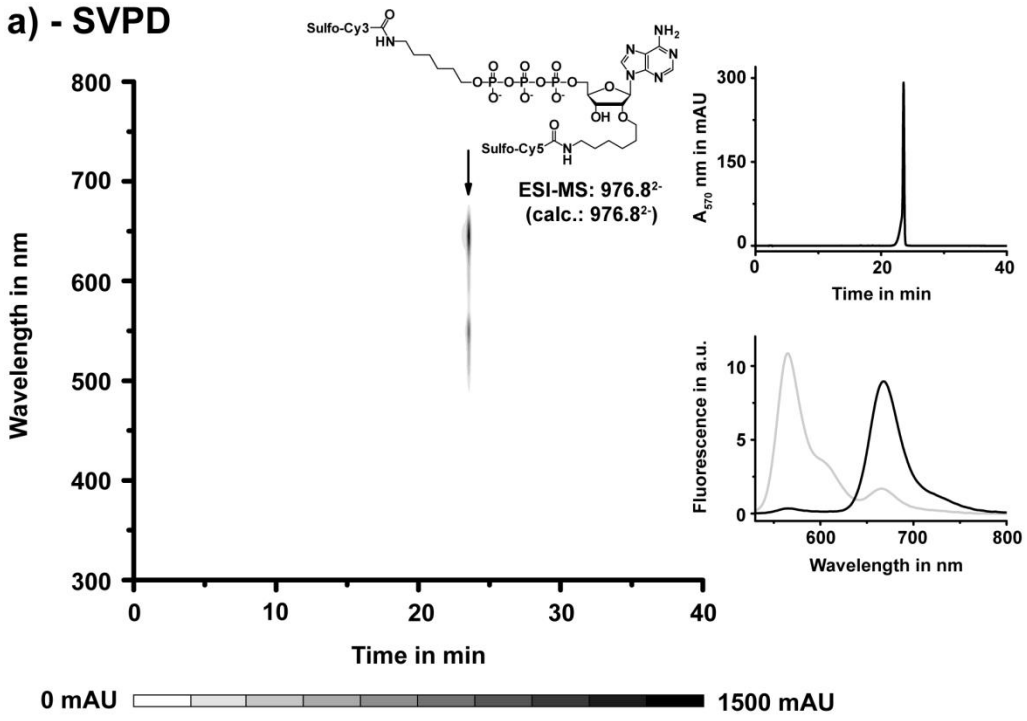
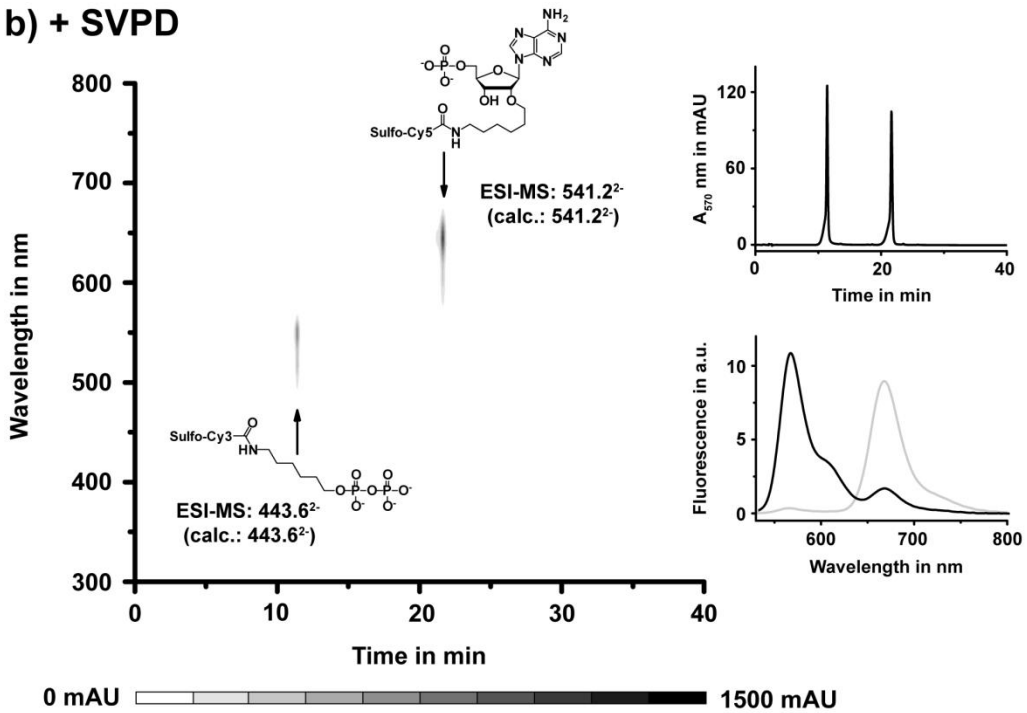
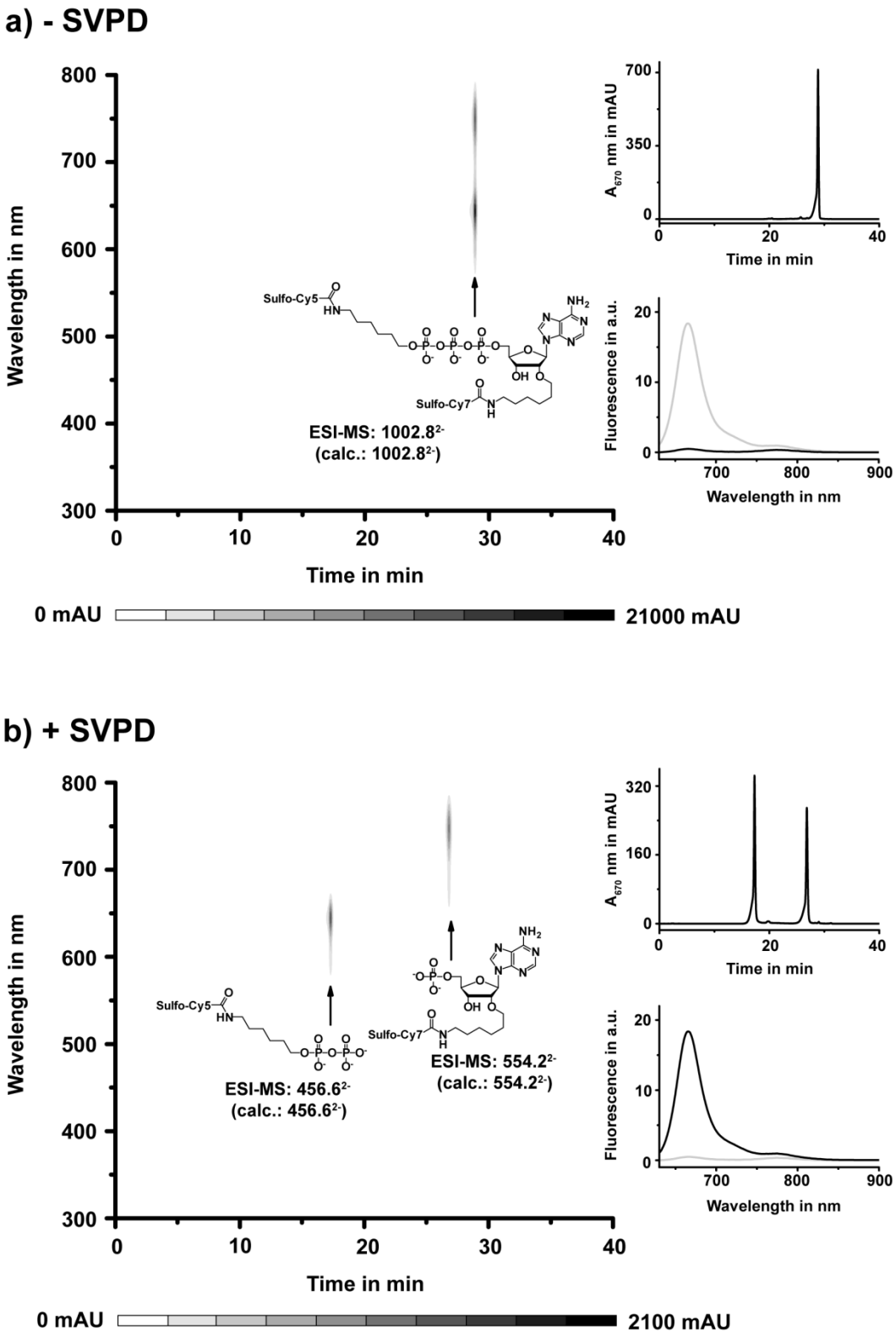
Content

Supplementary Results

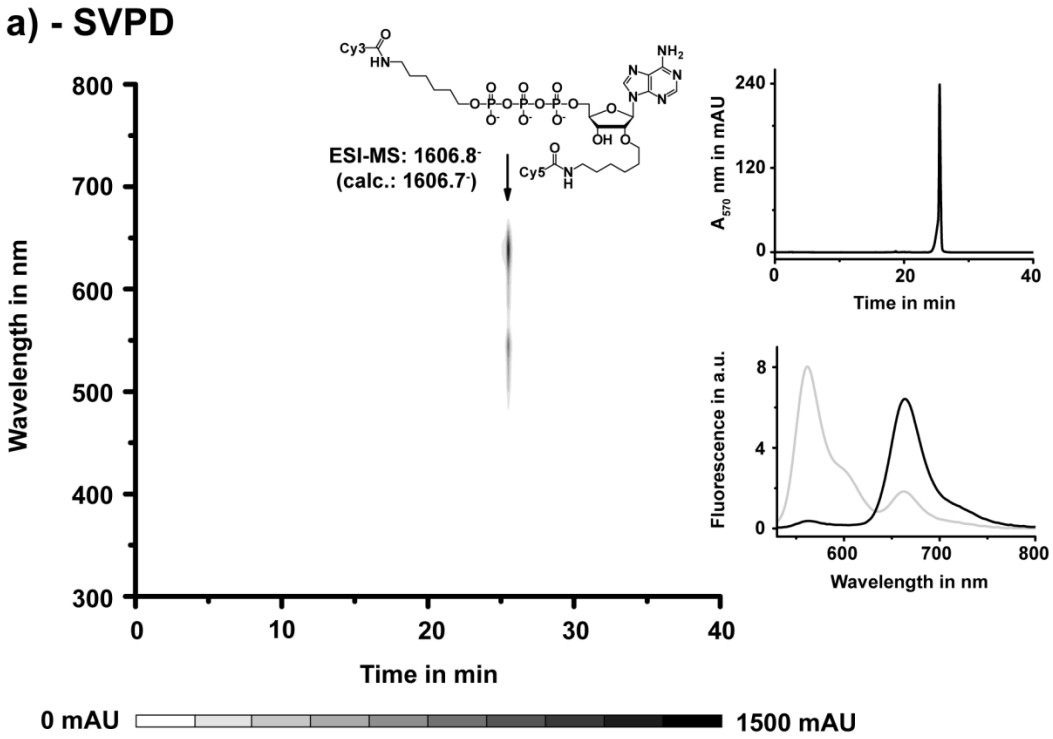


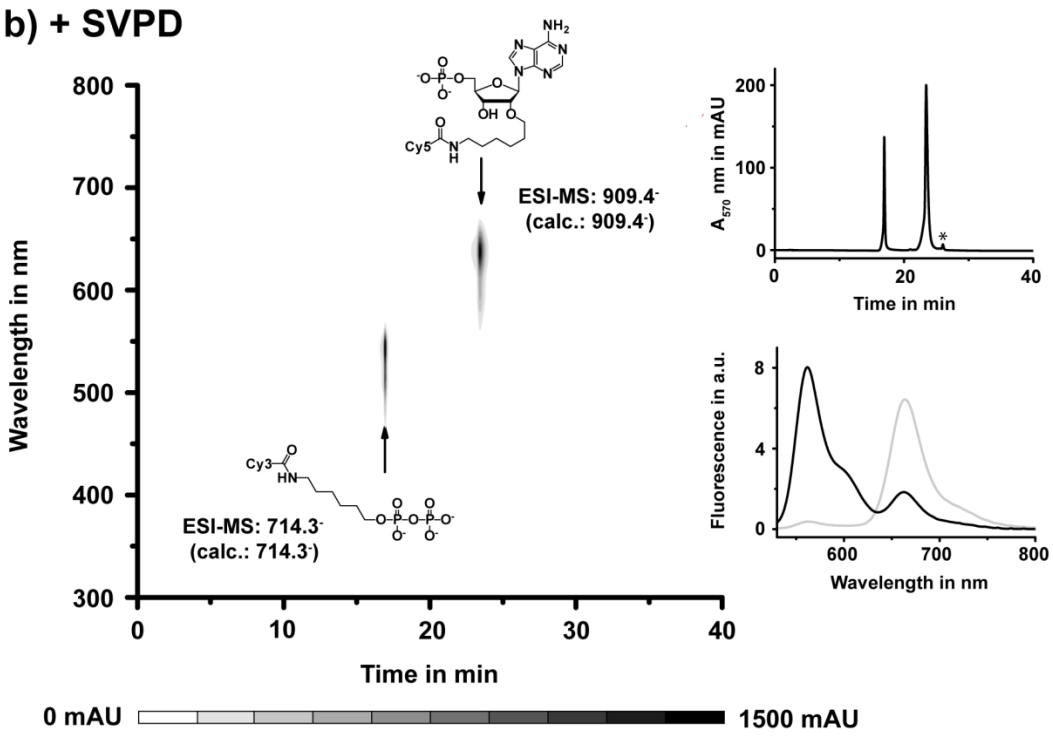


Supplementary Fig. S1 HPLC analysis of incubation of **7a** without (a) or with (b) phosphodiesterase I of *C. adamanteus* (SVPD). Left panels: 2D-RP-HPLC analysis of the reaction (inlets: HR-ESI-MS analysis of the peaks of the RP-HPLC analysis). Right upper panels: Extract of the RP-HPLC analysis at 570 nm. Right lower panels: Fluorescence spectra measured with excitation at 510 nm (black line: fluorescence spectrum of the indicated reaction, grey line: fluorescence spectrum of the other reaction for comparison).

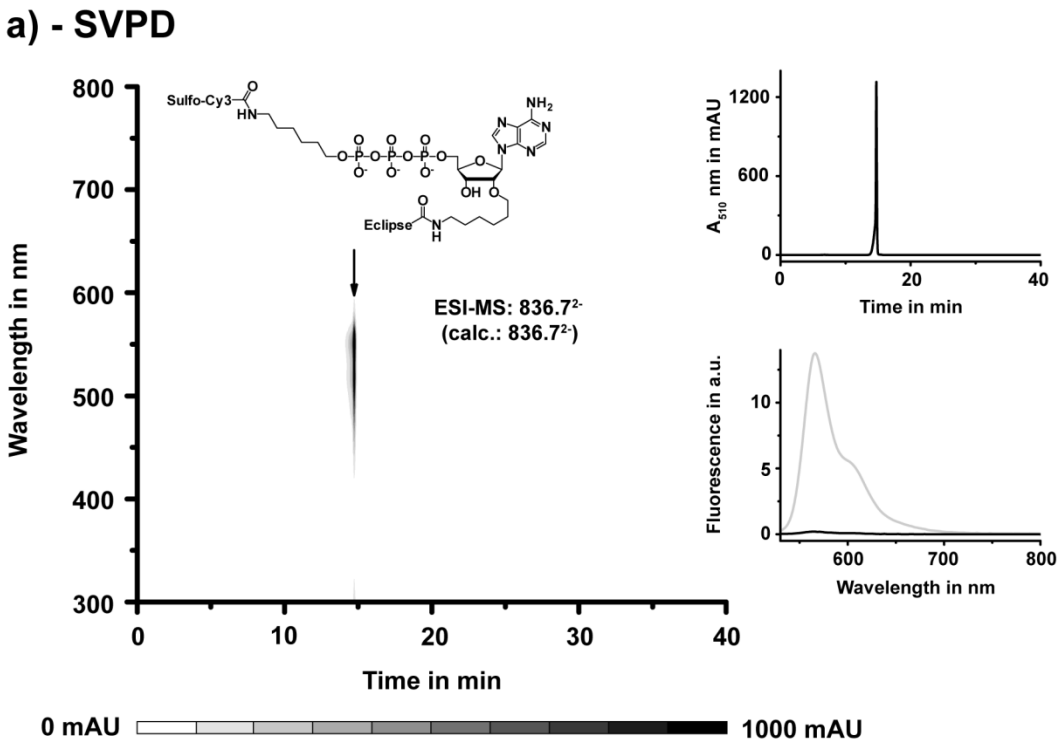


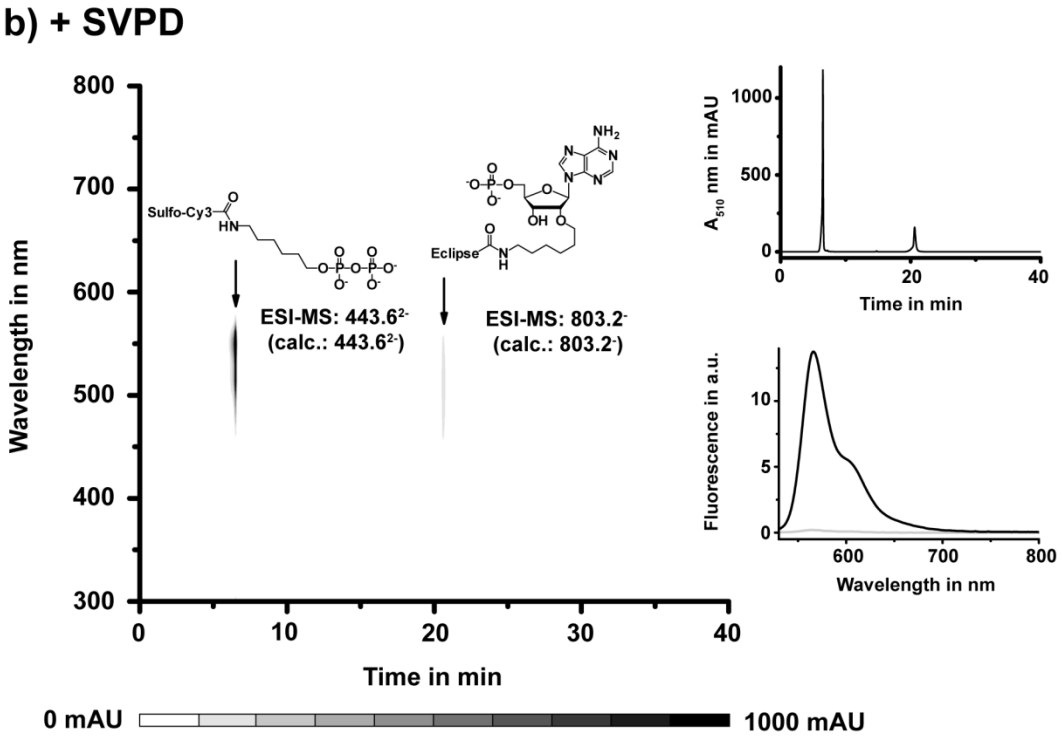
Supplementary Fig. S2 HPLC analysis of incubation of **7b** without (a) or with (b) phosphodiesterase I of *C. adamanteus* (SVPD). Left panels: 2D-RP-HPLC analysis of the reaction (inlets: HR-ESI-MS analysis of the peaks of the RP-HPLC analysis). Right upper panels: Extract of the RP-HPLC analysis at 670 nm. Right lower panels: Fluorescence spectra measured with excitation at 610 nm (black line: fluorescence spectrum of the indicated reaction, grey line: fluorescence spectrum of the other reaction for comparison).



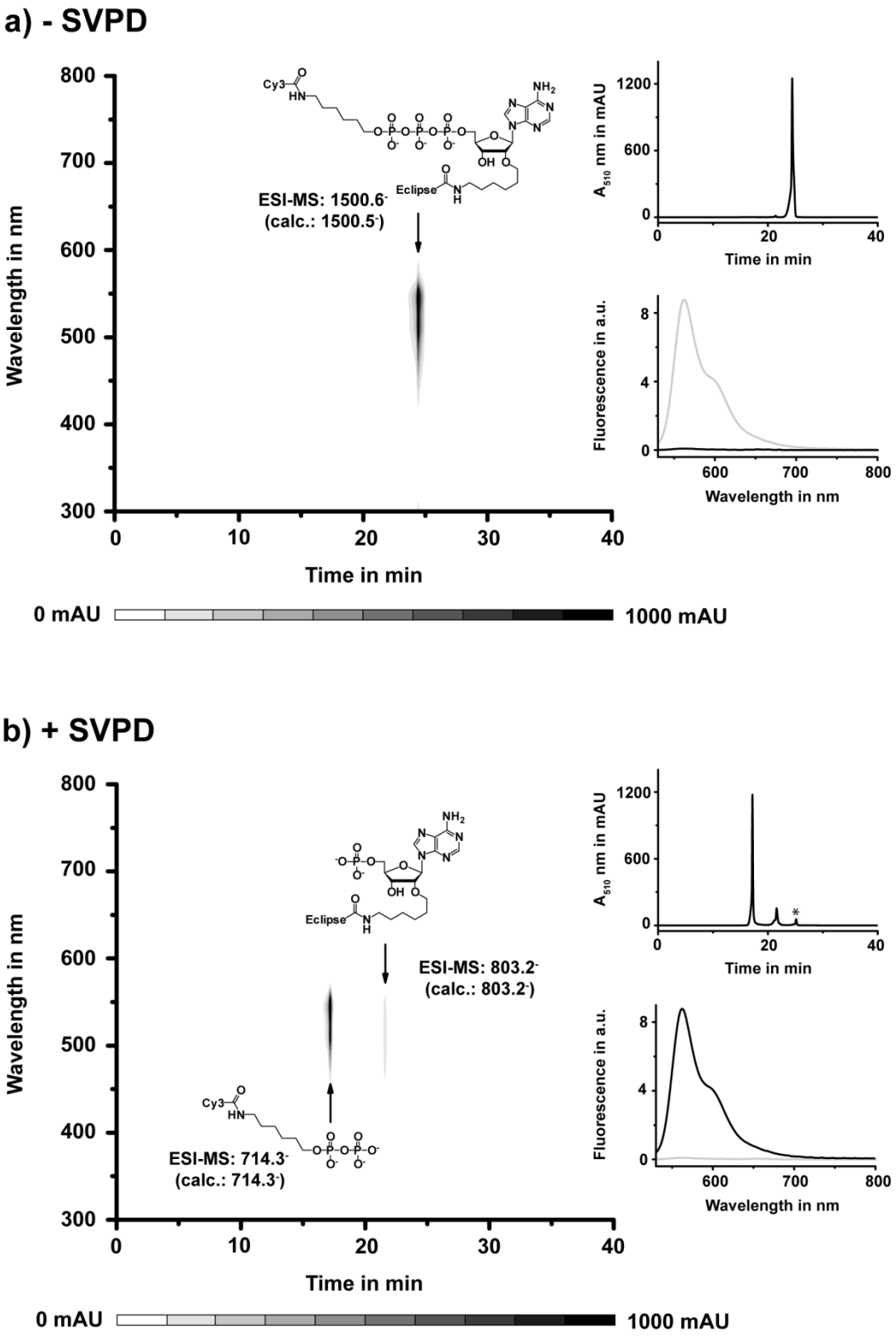


Supplementary Fig. S3 HPLC analysis of incubation of **7c** without (a) or with (b) phosphodiesterase I of *C. adamanteus* (SVPD). Left panels: 2D-RP-HPLC analysis of the reaction (inlets: HR-ESI-MS analysis of the peaks of the RP-HPLC analysis). Right upper panels: Extract of the RP-HPLC analysis at 570 nm. Right lower panels: Fluorescence spectra measured with excitation at 510 nm (black line: fluorescence spectrum of the indicated reaction, grey line: fluorescence spectrum of the other reaction for comparison).

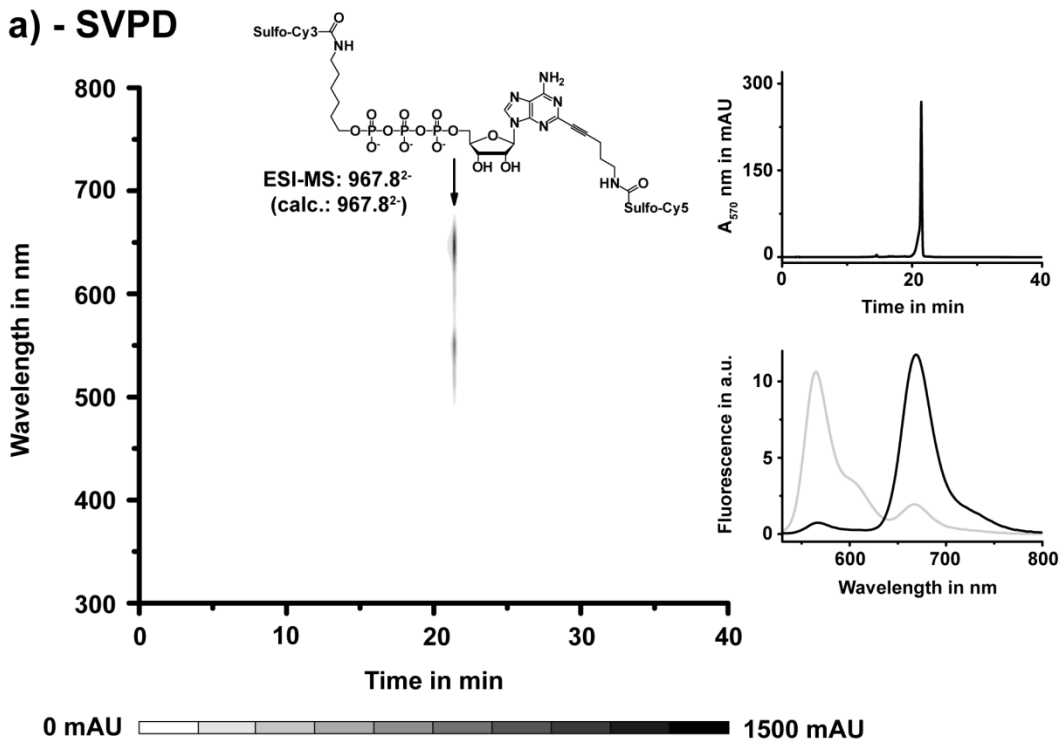


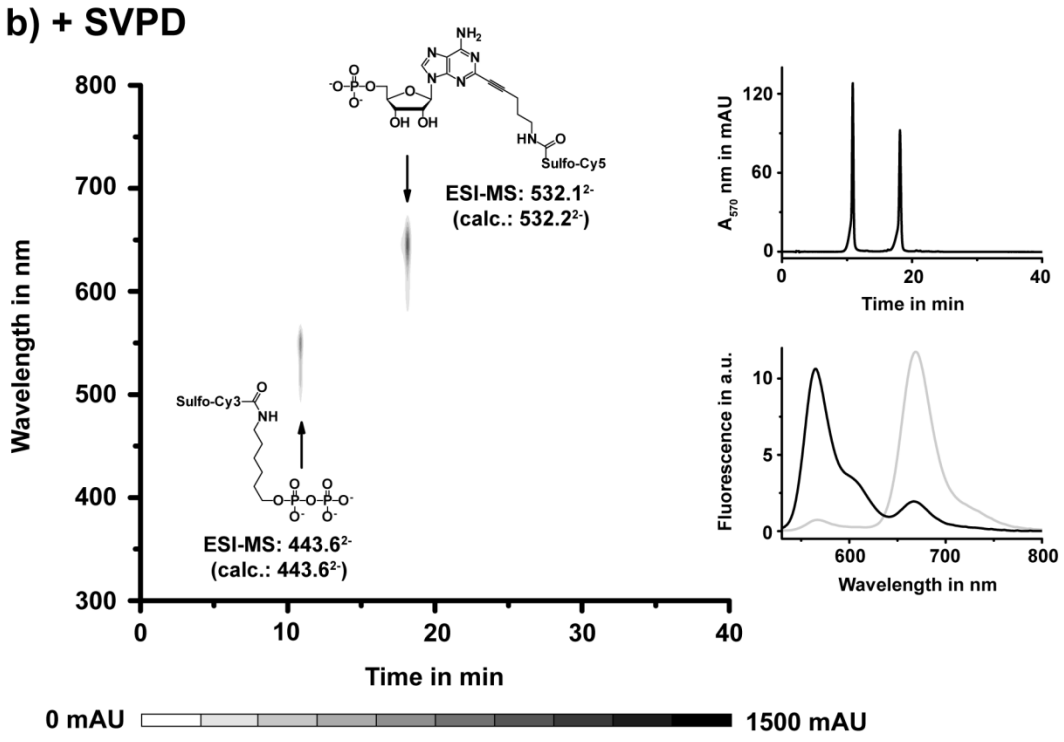


Supplementary Fig. S4 HPLC analysis of incubation of **7d** without (a) or with (b) phosphodiesterase I of *C. adamanteus* (SVPD). Left panels: 2D-RP-HPLC analysis of the reaction (inlets: HR-ESI-MS analysis of the peaks of the RP-HPLC analysis). Right upper panels: Extract of the RP-HPLC analysis at 510 nm. Right lower panels: Fluorescence spectra measured with excitation at 510 nm (black line: fluorescence spectrum of the indicated reaction, grey line: fluorescence spectrum of the other reaction for comparison).

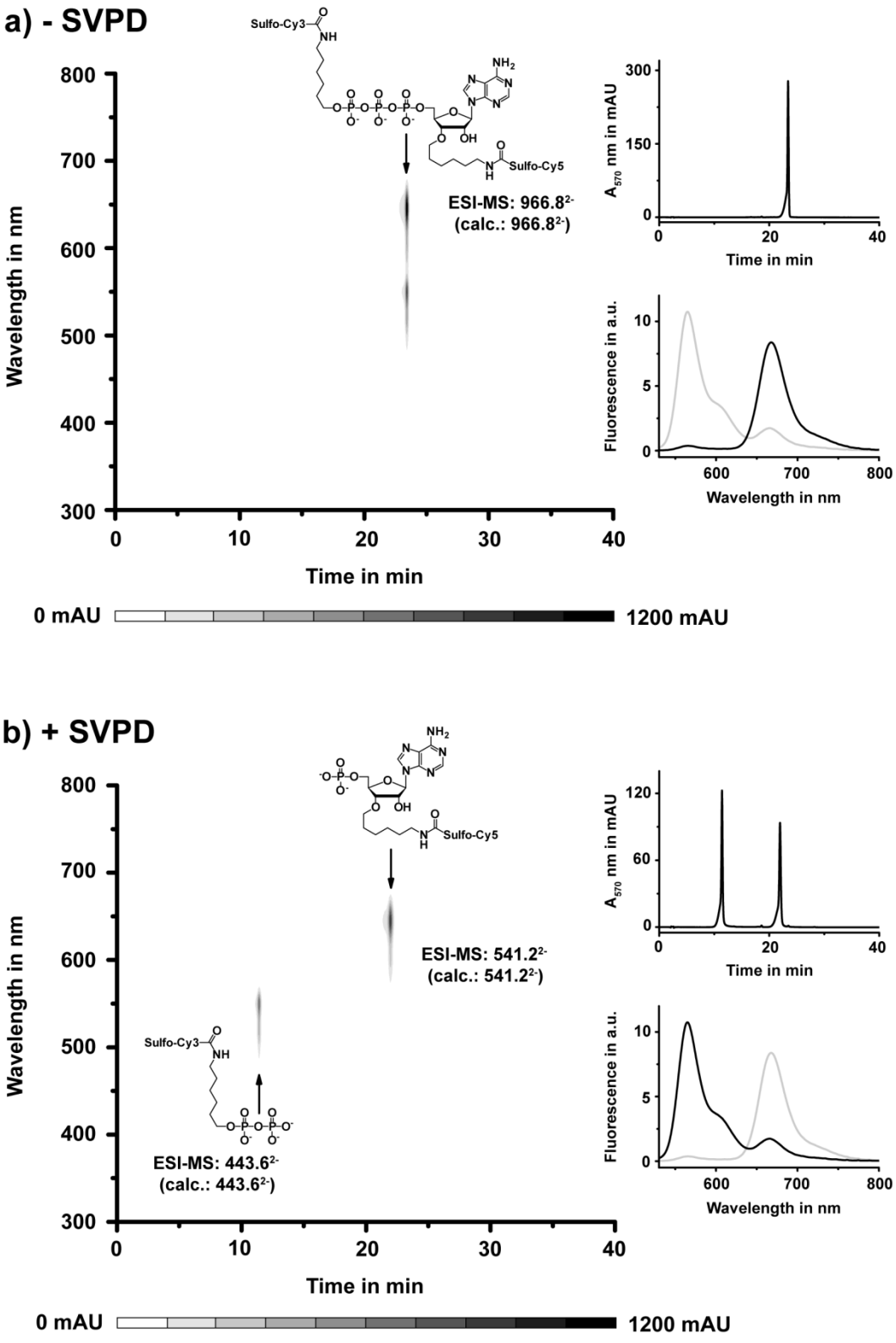


Supplementary Fig. S5 HPLC analysis of incubation of **7e** without (a) or with (b) phosphodiesterase I of *C. adamanteus* (SVPD). Left panels: 2D-RP-HPLC analysis of the reaction (inlets: HR-ESI-MS analysis of the peaks of the RP-HPLC analysis). Right upper panels: Extract of the RP-HPLC analysis at 510 nm. Right lower panels: Fluorescence spectra measured with excitation at 510 nm (black line: fluorescence spectrum of the indicated reaction, grey line: fluorescence spectrum of the other reaction for comparison).





Supplementary Fig. S6 HPLC analysis of incubation of **13** without (a) or with (b) phosphodiesterase I of *C. adamanteus* (SVPD). Left panels: 2D-RP-HPLC analysis of the reaction (inlets: HR-ESI-MS analysis of the peaks of the RP-HPLC analysis). Right upper panels: Extract of the RP-HPLC analysis at 570 nm. Right lower panels: Fluorescence spectra measured with excitation at 510 nm (black line: fluorescence spectrum of the indicated reaction, grey line: fluorescence spectrum of the other reaction for comparison).



Supplementary Fig. S7 HPLC analysis of incubation of **17** without (a) or with (b) phosphodiesterase I of *C. adamanteus* (SVPD). Left panels: 2D-RP-HPLC analysis of the reaction (inlets: HR-ESI-MS analysis of the peaks of the RP-HPLC analysis). Right upper panels: Extract of the RP-HPLC analysis at 570 nm. Right lower panels: Fluorescence spectra measured with excitation at 510 nm (black line: fluorescence spectrum of the indicated reaction, grey line: fluorescence spectrum of the other reaction for comparison).

Supplementary Methods

General experimental details

All temperatures quoted are uncorrected. All reagents are commercially available and used without further purification. All solvents are dried over molecular sieves and used directly without further purification. All reactions were conducted under exclusion of air and moisture. Anion-exchange chromatography of triphosphates was performed on a BioLogic DuoFlow System (Bio-Rad Laboratories) with DEAE Sephadex™ A-25 (GEHealthcare Bio-SciencesAB) column using a linear gradient of triethylammonium bicarbonate buffer (TEAB, pH 7.5) (0.1–1.0 M, flow 2 mL/min, pH 7.5). For medium pressure liquid chromatography (MPLC), a Büchi unit with a Büchi controller C-620, two pumps C-605, a UV monitor C-630 (λ = 254 nm) and fraction collector C-660 was used. For the purification of nucleosides and nucleotides, a 310-25 LiChroprep® RP-18 ready-to-use column (Merck, 40–63 mm) with a linear gradient (5 to 100%) of acetonitrile in 50 mM aqueous triethylammonium acetate (TEAA buffer, pH 7.0) was used. Reversed phase high pressure liquid chromatography (RP-HPLC) was performed using a Shimadzu unit. For the purification of nucleotides a EC 250/4 NUCLEODUR 100-5 C18 ec (Macherey-Nagel), VP 250/10 NUCLEODUR 100-5 C18 ec (Macherey-Nagel) or VP 250/21 NUCLEODUR C18 HTec, 5µm (Macherey-Nagel) column and a linear gradient (5 to 100%) of acetonitrile in 50 mM TEAA buffer (pH 7.0) was used. NMR spectra: Bruker Avance III 400 MHz spectrometer and Bruker AVIII 600 MHz spectrometer. ^{1}H and ^{13}C chemical shifts are reported relative to the residual solvent peak and are given in ppm (δ). A BBFOplus probe with actively shielded z-gradient was used with its inner (BB-) coil tuned to ^{19}F and ^{31}P, respectively. Flash chromatography: Merck silica gel G60. TLC: Merck precoated plates (silica gel 60 F_{254}). ESI-IT: Bruker Esquire 3000 plus. HRMS: Bruker Daltronics micrOTOF-Q II ESI-Qq-TOF. The reported yield refers to the analytically pure substance and is not optimized. Molecular sieve with 4 Å pore size was used for alkylation of triphosphates. Snake venom phosphor diesterase (SVPD) was purchased from Worthington Biochemical Corporation. For fluorescent measurements a Perkin Elmer Luminescence Spectrometer LS50 was used.

Synthesis of Compounds

General procedure 1: synthesis of triphosphates

The respective nucleoside (1 eq., appr. 50 mM) and proton sponge (1.5 eq.) were dissolved in trimethylphosphate and cooled to 0°C. To this, phosphorous oxychloride (1.2 eq.) was added dropwise. The solution was kept at 0°C for 1 hour. Tributylamine (10 eq.) and bis-(tributylammonium)-pyrophosphate (5 eq., 0.5 M in DMF) were added and the solution was stirred at room temperature for 1 hour. The reaction was quenched by addition of 0.1 M TEAB buffer and stirring for 30 minutes at room temperature. The reaction mixture was extracted three times with ethyl acetate and the aqueous phase was evaporated. The product was purified by anion-exchange chromatography and MPLC or RP-HPLC. Fractions containing the product were evaporated and the product repeatedly freeze dried from water to give the triphosphate.

General procedure 2: γ-phosphate alkylation

The respective triphosphate was converted into its tetrabutylammonium salt by passing through a column containing Chelex100 preequilibrated with Bu_4NBr. The tetrabutylammonium triphosphate (1eq., appr. 20 mM) and the alkylation reagent (3 eq., appr. 60 mM) were separately dissolved in DMF and dried over molecular sieves overnight. The two solutions were combined and slightly stirred at room temperature overnight. The solvents were evaporated under reduced pressure and the crude product was purified by anion-exchange chromatography and MPLC or RP-HPLC or directly subjected to deprotection of the trifluoroacetamide group. Fractions containing the product were evaporated and the product repeatedly freeze dried from water to give the alkylated triphosphate.

General procedure 3: trifluoroacetamide deprotection

The trifluoroacetamide protected triphosphate (1 eq., appr. 10 mM) was dissolved in 10% $NH_{3\ aq}$. The reaction was stirred at room temperature for 2 - 4 hours until the reaction was complete. The solvents were evaporated under reduced pressure and the crude product was purified by anion-exchange chromatography (if crude starting material was used) and MPLC or RP-HPLC. Fractions containing the product were evaporated and the product repeatedly freeze dried from water to give the free amine.

General procedure 4: azide reduction

The azide modified triphosphate (1 eq., appr. 3 mM) was dissolved in water/methanol/triethylamine (2:2:1) and tris-(2-carboxyethyl)-phosphine hydrochloride (7 eq.) was added rapidly and stirred at room temperature for 3 – 12 hours until complete conversion. The solvents were evaporated under reduced pressure. The compound was purified by MPLC or RP-HPLC. Fractions containing the product were evaporated and the product repeatedly freeze dried from water to give the free amine.

General procedure 5: NHS ester coupling

The triphosphate containing a free amine (1 eq., appr. 5 mM) was dissolved in 0.1M $NaHCO_3$ (pH 8.7) and the appropriate NHS ester (1.5 eq.), dissolved in DMF (appr. 30 mM), was added. The solution was stirred at room temperature for 2 – 12 hours until complete conversion was achieved. For the less polar NHS esters of Cy3, Cy5 and Eclipse higher amounts of DMF (up to DMF/0.1 M $NaHCO_3$ 1:1) had to be used to maintain solubility. The solvents were evaporated under reduced pressure. The compound was purified by MPLC or RP-HPLC. The solvent was evaporated and the product repeatedly freeze dried from water to give the labeled triphosphate.

γ-(6-Azidohexyl)-*O*2'-(6-aminohexyl)-adenosine triphosphate 2

This compound was synthesized using general procedure 2 and 3 starting from O2'-(6-trifluoroacetamidohexyl)-adenosine triphosphate **1** (103 µmol) and 6-azido-1-bromohexane (512 µmol) in 25% yield.

^{1}H NMR (D_2O, 400 MHz): δ 8.64 (s, 1H, H-8), 8.28 (s, 1H, H-2), 6.18 (d, J = 7.0 Hz, 1H, H-1'), 4.68 (dd, J = 2.1 Hz, J = 5.0 Hz, 1H, H-3'), 4.56 (dd, J = 5.1 Hz, J = 6.9 Hz, 1H, H-2'), 4.46 – 4.42 (m, 1H, H-4'), 4.34 – 4.20 (m, 2H, H5'a, H5'b), 3.91 (q, J = 6.8 Hz, 2H, γP-O-C**H**$_2$), 3.71 (dt, J = 6.3 Hz, J = 10.4 Hz, 1H, 2'-O-

C**H**$_2$a), 3.54 (dt, J = 6.2 Hz, J = 10.4 Hz, 1H, 2'-O-C**H**$_2$b), 3.21 (t, J = 6.8 Hz, 2H, C**H**$_2$-N$_3$), 2.87 (t, 7.4 Hz, 2H, C**H**$_2$-NH$_2$), 1.57 – 1.36 (m, 8H, 4x C**H**$_2$-linker), 1.24 – 0.93 (m, 8H, 4x C**H**$_2$-linker).

^{31}P NMR (D$_2$O, 162 MHz): -11.0 (d, J = 18.2 Hz, 1P), -11.6 (d, J = 18.2 Hz, 1P), -23.3 (t, J = 18.2 Hz, 1P).

HR-ESI-MS: found: 730.1858; calculated: 730.1875 (M-H$^+$, $C_{22}H_{39}N_9O_{13}P_3^-$); deviation: 2.3 ppm.

_O_2'-(6-azidohexyl)-adenosine triphosphate 3

This compound was synthesized using general procedure 1 starting from O2'-(6-azidohexyl)-adenosine (170 µmol) in 39% yield.

^{1}H NMR (D$_2$O, 400 MHz): δ 8.65 (s, 1H, H-8), 8.35 (s, 1H, H-2), 6.23 (d, J = 7.2 Hz, 1H, H-1'), 4.73 (dd, J = 2.1 Hz, J = 5.2 Hz, 1H, H-3'), 4.63 (dd, J = 5.2 Hz, J 0 7.2 Hz, 1H, H-2'), 4.52 – 4.47 (m, 1H, H-4'), 4.39 – 4.32 (m, 1H, H-5'a), 4.32 – 4.25 (m, 1H, H-5'b), 3.84 – 3.75 (m, 1H, 2'-O-C**H**$_2$a), 3.62 – 5.53 (m, 1H, 2'-O-C**H**$_2$b), 3.15 (t, J = 6.9 Hz, 2H, C**H**$_2$-N$_3$), 1.53 – 1.21 (m, 4H, 2x C**H**$_2$-linker), 1.17 – 0.92 (m, 4H, 2x C**H**$_2$-linker).

^{31}P NMR (D$_2$O, 162 MHz): d -10.7 (d, J = 17.1 Hz, 1P), -11.4 (d, J = 19.6 Hz, 1P), -21.1 (t, J = 19.3 Hz, 1P).

HR-ESI-MS: found: 631.0819; calculated: 631.0827 (M-H$^+$, $C_{16}H_{26}N_8O_{13}P_3^-$); deviation: 1.3 ppm.

γ-(6-Aminohexyl)-_O_2'-(6-azidohexyl)-adenosine triphosphate 4

This compound was synthesized using general procedure 2 and 3 starting from O2'-(6-azidohexyl)-adenosine triphosphate **3** (390 µmol) and 1-iodo-6-trifluoroacetamidohexane (1170 µmol) in 25% yield.

^{1}H NMR (MeOD-d$_4$, 400 MHz): δ 8.65 (s, 1H, H-8), 8.21 (s, 1H, H-2), 6.16 (d, J = 5.0 Hz, 1H, H-1'), 4.64 (t, J = 4.6 Hz, 1H, H-3'), 4.41 (t, J = 5.0 Hz, 1H, H-2'), 4.37 – 4.22 (m, 3H, H-4', H-5'a, H-5'b), 4.03 (q, J = 6.2 Hz, 2H, γP-O-C**H**$_2$), 3.72 (dt, J 9.6 Hz, J = 6.5 Hz, 1H, 2'-O-C**H**$_2$a), 3.57 (dt, J = 9.6 Hz, J = 6.5 Hz, 1H, 2'-O-C**H**$_2$b), 3.22 – 3.16 (m, 2H, C**H**$_2$-N$_3$), 2.98 (t, J = 6.7 Hz, 2H, C**H**$_2$-NH$_2$), 1.75 – 1.59 (m, 4H, 2x CH$_2$-linker), 1.59 – 1.39 (m, 8H, 4x CH$_2$-linker), 1.36 – 1.19 (m, 4H, 2x CH$_2$-linker).

^{31}P NMR (MeOD-d$_4$, 162 MHz): δ -10.6 (d, J = 17.1 Hz, 1P), -11.3 (d, J = 19.5 Hz, 1P), -22.2 - -22.8 (m, 1P).

HR-ESI-MS: found: 730.1862; calculated: 730.1875 (M-H$^+$, $C_{22}H_{39}N_9O_{13}P_3^-$); deviation: 1.8 ppm.

γ-(6-Azidohexyl)-_O_2'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 5

This compound was synthesized using general procedure 5 starting from γ-(6-Azidohexyl)-O2'-(6-aminohexyl)-adenosine triphosphate **2** (11.7 µmol) and Sulfo-Cy5 NHS ester (19.8 µmol) in 70% yield.

^{1}H NMR (D_2O, 400 MHz): δ 8.58 (s, 1H, H-8), 8.15 (s, 1H, H-2), 7.91 (t, J = 13.1 Hz, 2H, H-β-Sulfo-Cy5, H-β'-Sulfo-Cy5), 7.87 – 7.77 (m, 4H, H-Ar-Sulfo-Cy5), 7.32 (d, J = 8.3 Hz, 1H, H-Ar-Sulfo-Cy5), 7.29 (d, J = 8.5 Hz, 1H, H-Ar-Sulfo-Cy5), 6.46 (t, J = 12.5 Hz, 1H, H-γ-Sulfo-Cy5), 6.17 (d, J = 13.5 Hz, 1H, H-α-Sulfo-Cy5), 6.14 (d, J = 13.5 Hz, 1H, H-α'-Sulfo-Cy5), 6.10 (d, J = 6.5 Hz, 1H, H-1'), 4.63 (dd, J = 3.0 Hz, J = 4.9 Hz, 1H, H-3'), 4.48 (t, J = 5.6 Hz, 1H, H-2'), 4.38 – 4.33 (m, 1H, H-4'), 4.29 – 4.15 (m, 2H, H-5'a, H-5'b), 4.12 – 3.96 (m, 4H, 2x Sulfo-Cy5-N-C**H**$_2$), 3.87 (q, J = 6.6 Hz, 2H, γP-O-C**H**$_2$), 3.66 (dt, J = 10.0 Hz, J = 6.3 Hz, 1H, 2'-O-C**H**$_2$a), 3.48 (dt, J = 10.0 Hz, J = 6.2 Hz, 1H, 2'-O-C**H**$_2$b), 3.14 (t, J = 7.0 Hz, 2H, C**H**$_2$-N_3), 2.93 (t, J = 6.9 Hz, 2H, C**H**$_2$-NH-CO), 2.18 (t, J = 6.8 Hz, 2H, NH-CO-C**H**$_2$), 1.83 – 0.89 (m, 37H, 11x CH_2-linker, 5x CH_3).

^{31}P NMR (D_2O, 162 MHz): δ -11.1 (d, J = 18.3 Hz, 1P), -11.7 (d, J = 20.1 Hz, 1P), -23.4 (t, J = 18.3 Hz, 1P).

HR-ESI-MS: found: 683.6940; calculated: 683.6956 (M-2H$^+$, $C_{55}H_{76}N_{11}O_{20}P_3S_2^{2-}$); deviation: 2.3 ppm.

γ-(6-Aminohexyl)-*O*2'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 6

This compound was synthesized using general procedure 4 starting from γ-(6-Azidohexyl)-O2'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate **5** (8.2 µmol) in 68% yield.

^{1}H NMR (D_2O, 400 MHz): δ 8.56 (s, 1H, H-8), 8.12 (s, 1H, H-2), 7.95 (t, J = 13.1 Hz, 2H, H-β-Sulfo-Cy5, H-β'-Sulfo-Cy5), 7.86 – 7.75 (m, 4H, H-Ar-Sulfo-Cy5), 7.29 (d, J = 8.4 Hz, 1H, H-Ar-Sulfo-Cy5), 7.27 (d, J = 8.4 Hz, 1H, H-Ar-Sulfo-Cy5), 6.43 (t, J = 12.5 Hz, 1H, H-γ-Sulfo-Cy5), 6.16 (d, 13.5 Hz, 1H, H-α-Sulfo-Cy5), 6.12 (d, J = 13.5 Hz, 1H, H-α'-Sulfo-Cy5), 6.08 (d, J = 6.7 Hz, 1H, H-1'), 4.66 – 4.60 (m, 1H, H-3'), 4.53 (t, J = 5.8 Hz, 1H, H-2'), 4.40 – 4.32 (m, 1H, H-4'), 4.30 – 4.14 (m, 2H, H-5'a, H-5'b), 4.11 – 3.96 (m, 4H, 2x Sulfo-Cy5-N-C**H**$_2$), 3.90 (q, J = 6.4 Hz, 2H, γP-O-C**H**$_2$), 3.71 – 3.61 (m, 1H, 2'-O-C**H**$_2$a), 3.53 – 3.44 (m, 1H, 2'-O-C**H**$_2$b), 2.97 – 2.88 (m, 4H, C**H**$_2$NH$_2$, C**H**$_2$-NH-CO), 2.18 (t, J = 6.7 Hz, 2H, C**H**$_2$-CO-NH), 1.82 – 0.89 (m, 37H, 11x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (D_2O, 162 MHz): δ -11.0 (d, J = 17.5 Hz, 1P), -11.7 (d, J = 18.4 Hz), -23.0 - -23.8(m, 1P).

HR-ESI-MS: found: 670.6995; calculated: 670.7003 (M-2H$^+$, $C_{55}H_{78}N_9O_{20}P_3S_2^{2-}$); deviation: 1.2 ppm.

γ-(6-Sulfo-Cy3-amidohexyl)-*O*2'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 7a (from 2)

This compound was synthesized using general procedure 5 starting from γ-(6-Aminohexyl)-O2'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate **6** (5.6 µmol) and Sulfo-Cy3 NHS ester (11.2 µmol) in 64% yield.

^{1}H NMR (MeOD-d_4, 600 MHz): δ 8.64 (s, 1H, H-8), 8.56 (t, J = 13.5 Hz, 1H, H-β-Sulfo-Cy3), 8.31 (t, J = 13.0 Hz, 1H, H-β-Sulfo-Cy5), 8.30 (t, J = 13.0 Hz, 1H, H-β'-Sulfo-Cy5), 8.18 (s, 1H, H-2), 7.98 – 7.86 (m, 8H, H-Ar-Sulfo-Cy), 7.43 (d, J = 8.3 Hz, 1H, H-Ar-Sulfo-Cy), 7.42 (d, J = 8.3 Hz, 1H, H-Ar-Sulfo-Cy), 7.35 (t, J = 7.6 Hz, 2H, H-Ar-Sulfo-Cy), 6.70 (t, J = 12.5 Hz, 1H, H-γ-Sulfo-Cy5), 6.59 (d, J = 13.5 Hz, 1H, H-α-Sulfo-Cy3), 6.55 (d, J = 13.4 Hz, 1H, H-α'-Sulfo-Cy3), 6.36 (d, J = 13.7 Hz, 1H, H-α-Sulfo-Cy5), 6.35 (d, J = 13.5 Hz, 1H, H-α'-Sulfo-Cy5), 6.15 (d, J = 5.2 Hz, 1H, H-1'), 4.60 (t, J = 4.0 Hz, 1H, H-3'), 4.41 (t, J =

4.9 Hz, 1H, H-2'), 4.33 – 4.22 (m, 5H, H-4', 2x Sulfo-Cy-N-C**H**$_2$), 4.21 – 4.15 (m, 4H, H-5'a, H-5'b, Sulfo-Cy-N-C**H**$_2$), 4.12 (t, J = 7.3 Hz, 2H, Sulfo-Cy-N-C**H**$_2$), 3.99 – 3.91 (m, 2H, γP-O-C**H**$_2$), 3.67 – 3.58 (m, 1H, 2'-O-C**H**$_2$a), 3.51 – 3.44 (m, 1H, 2'-O-C**H**$_2$b), 3.12 (t, J = 7.0 Hz, 2H, C**H**$_2$-NH-CO), 3.07 (t, J = 7.1 Hz, 2H, C**H**$_2$-NH-CO), 2.21 (t, J = 7.4 Hz, 2H, C**H**$_2$-CO-NH), 2.18 (t, J = 2.18, 2H, C**H**$_2$-CO-NH), 1.88 – 1.14 (m, 58H, 14x C**H**$_2$-linker, 10x C**H**$_3$).

^{31}P NMR (MeOD-d$_4$, 162 MHz): δ -9.8 - -10.5 (m, 1P), -10.6 - -11.1 (m, 1P), -20.6 - -21.4 (m, 1P).

HR-ESI-MS: found: 976.7944; calculated: 976.7985 (M-2H$^+$, $C_{86}H_{114}N_{11}O_{27}P_3S_4^{2-}$); deviation: 4.2 ppm.

γ-(6-Sulfo-Cy3-amidohexyl)-*O*2'-(6-azidohexyl)-adenosine triphosphate 8a

This compound was synthesized using general procedure 5 starting from γ-(6-Aminohexyl)-O2'-(6-azidohexyl)-adenosine triphosphate **4** (35.0 µmol) and Sulfo-Cy3 NHS ester (60.4 µmol) in 71% yield.

^{1}H NMR (MeOD-d$_4$, 400 MHz): 8.59 (s, 1H, H-8), 8.57 (t, J = 13.4 Hz, 1H, H-β-Sulfo-Cy3), 8.20 (s, 1H, H-2), 7.97 – 7.89 (m, 4H, H-Ar-Sulfo-Cy3), 7.44 (d, J = 8.4 Hz, 1H, H-Ar-Sulfo-Cy3), 7.42 (d, J = 8.4 Hz, 1H, H-Ar-Sulfo-Cy3), 6.57 (d, J = 13.7 Hz, 1H, H-α-Sulfo-Cy3), 6.53 (d, J = 13.6 Hz, 1H, H-α'-Sulfo-Cy3), 6.15 (d, J = 5.4 Hz, 1H, H-1'), 4.62 (t, J = 4.4 Hz, 1H, H-3'), 4.47 (t, J = 5.2 Hz, 1H, H-2'), 4.38 – 4.21 (m, 5H, H-4', H-5'a, H-5'b, Sulfo-Cy3-N-C**H**$_2$), 4.18 (t, J = 7.5 Hz, 2H, Sulfo-Cy3-N-C**H**$_2$), 4.00 (q, J = 6.6 Hz, 2H, γP-O-C**H**$_2$), 3.71 (dt, J = 9.5 Hz, J = 6.4 Hz, 1H, 2'-O-C**H**$_2$a), 3.54 (dt, J = 9.5 Hz, J = 6.5 Hz, 1H, 2'-O-C**H**$_2$b), 3.22 – 3.14 (m, 2H, C**H**$_2$-N$_3$), 3.13 (t, J = 7.0 Hz, 2H, C**H**$_2$-NH-CO), 2.22 (t, J = 7.3 Hz, 2H, C**H**$_2$-CO-NH), 1.89 – 1.19 (m, 37H, 11x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD-d$_4$, 162 MHz): -11.2 (d, J = 18.3 Hz, 1P), -11.7 (d, J = 18.3 Hz, 1P), -23.1 (t, J = 16.5 Hz, 1P).

HR-ESI-MS: found: 670.6863; calculated: 670.6877 (M-2H$^+$, $C_{53}H_{74}N_{11}O_{20}P_3S_2^{2-}$); deviation: 2.1 ppm.

γ-(6-Sulfo-Cy5-amidohexyl)-*O*2'-(6-azidohexyl)-adenosine triphosphate 8b

This compound was synthesized using general procedure 5 starting from γ-(6-Aminohexyl)-O2'-(6-azidohexyl)-adenosine triphosphate **4** (20 µmol) and Sulfo-Cy5 NHS ester (36.2 µmol) in 81% yield.

^{1}H NMR (MeOD-d$_4$, 400 MHz): δ 8.60 (s, 1H, H-8), 8.31 (t, J = 13.1 Hz, 1H, H-β-Sulfo-Cy5), 8.30 (t, J = 13.1 Hz, 1H, H-β'-Sulfo-Cy5), 8.19 (s, 1H, H-2), 7.92 – 7.32 (m, 4H, H-Ar-Sulfo-Cy5), 7.38 – 7.32 (m, 2H, H-Ar-Sulfo-Cy5), 6.68 (t, J = 12.5 Hz, 1H, H-γ-Sulfo-Cy5), 6.37 (d, J = 13.7 Hz, 1H, H-α-Sulfo-Cy5), 6.33 (d, J = 13.6 Hz, 1H, H-α'-Sulfo-Cy5), 6.16 (d, J = 5.6 Hz, 1H, H-1'), 4.62 (t, J = 4.2 Hz, 1H, H-3'), 4.47 (t, J = 5.3 Hz, 1H, H-2'), 4.38 – 4.23 (m, 3H, H-4', H-5'a, H-5'b), 4.23 – 4.15 (m, 2H, Sulfo-Cy5-N-C**H**$_2$), 4.15 – 4.08 (m, 2H, Sulfo-Cy5-N-C**H**$_2$), 4.00 (q, J = 6.5 Hz, 2H, γP-O-C**H**$_2$), 3.70 (dt, J = 9.5 Hz, J = 6.4 Hz, 1H, 2'-O-C**H**$_2$a), 3.54 (dt, J = 9.5 Hz, J = 6.6 Hz, 1H, 2'-O-C**H**$_2$b), 3.18 (t, J = 6.9 Hz, 2H, C**H**$_2$-N$_3$), 3.16 – 3.09 (m, 2H, C**H**$_2$-NH-CO), 2.21 (t, J = 7.4 Hz, 2H, C**H**$_2$-CO-NH), 1.88 – 1.19 (m, 37H, 11x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD-d4, 162 MHz): δ -11.0 (d, J = 18.2 Hz, 1P), -11.5 (d, J = 18.4 Hz, 1P), -22.3 - -23.0 (m, 1P).

HR-ESI-MS: found: 683.6934; calculated: 683.6956 ($M-2H^+$, $C_{55}H_{76}N_{11}O_{20}P_3S_2^{2-}$); deviation: 3.2 ppm.

γ-(6-Cy3-amidohexyl)-*O*2'-(6-azidohexyl)-adenosine triphosphate 8c

This compound was synthesized using general procedure 5 starting from γ-(6-Aminohexyl)-O2'-(6-azidohexyl)-adenosine triphosphate **4** (35 µmol) and Cy3 NHS ester (52.5 µmol) in 36% yield.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.61 (s, 1H, H-8), 8.52 (t, J = 13.5 Hz, 1H, H-β-Cy3), 8.17 (s, 1H, H-2), 7.53 (d, J = 7.4 Hz, 2H, H-Ar-Cy3), 7.44 (d, J = 7.8 Hz, 2H, H-Ar-Cy3), 7.39 -. 7.27 (m, 4H, H-Ar-Cy3), 6.44 (d, J = 13.5 Hz, 1H, H-α-Cy3), 6.42 (d, J = 13.4 Hz, d, 1H, H-α'-Cy3), 6.15 (d, J = 5.4 Hz, 1H, H-1'), 4.65 (t, J = 4.4 Hz, 1H, H-3'), 4.44 (t, J = 5.2 Hz, 1H, H-2'), 4.41 – 4.33 (m, 1H, H-5'a), 4.32 – 4.22 (m, 2H, H-4', H-5'b), 4.14 (t, J = 7.4 Hz, 2H, Cy3-N-C**H**$_2$), 4.02 (q, J = 6.5 Hz, 2H, γP-O-C**H**$_2$), 3.74 – 3.66 (m, 4H, 2'-O-C**H**$_2$a, Cy3-N-C**H**$_3$), 3.54 (dt, J = 9.5 Hz, J = 6.6 Hz, 1H, 2'-O-C**H**$_2$b), 3.20 – 3.15 (m, 2H, C**H**$_2$-N_3), 3.12 (t, J = 6.9 Hz, 2H, C**H**$_2$-NH-CO), 2.22 (t, J = 7.3 Hz, 2H, C**H**$_2$-CO-NH), 1.90 – 1.19 (m, 34H, 11x C**H**$_2$-linker, 4x C**H**$_3$).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -11.1 (d, J = 18.3 Hz, 1P), -11.6 (d, J = 18.3 Hz, 1P), -23.0 (t, J = 18.4 Hz, 1P).

HR-ESI-MS: found: 583.7212; calculated: 583.7231 ($M-2H^+$, $C_{52}H_{72}N_{11}O_{14}P_3^{2-}$); deviation: 3.3 ppm.

γ-(6-Sulfo-Cy3-amidohexyl)-*O*2'-(6-aminohexyl)-adenosine triphosphate 9a

This compound was synthesized using general procedure 4 starting from γ-(6-Sulfo-Cy3-amidohexyl)-O2'-(6-azidohexyl)-adenosine triphosphate **8a** (24.9 µmol) in 90% yield.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.67 (s, 1H, H-8), 8.56 (t, J = 13.4 Hz, 1H, H-β-Sulfo-Cy3), 8.19 (s, 1H, H-2), 7.99 – 7.87 (m, 4H, H-Ar-Sulfo-Cy3), 7.44 (d, J = 8.3 Hz, 1H, H-Ar-Sulfo-Cy3), 7.42 (d, J = 8.4 Hz, 1H, H-Ar-Sulfo-Cy3), 6.57 (d, J = 13.6 Hz, 1H, H-α-Sulfo-Cy3), 6.53 (d, J = 13.6 Hz, 1H, H-α'-Sulfo-Cy3), 6.17 (d, J = 6.3 Hz, 1H, H-1'), 4.64 (dd, J = 4.6 Hz, J = 2.4 Hz, 1H, H-3'), 4.54 (t, J = 5.4 Hz, 1H, H-2'), 4.36 – 4.12 (m, 7H, H-4', H-5'a, H-5'b, 2x Sulfo-Cy3-N-C**H**$_2$), 3.99 (q, J = 6.6 Hz, 2H, γP-O-C**H**$_2$), 3.66 – 3.52 (m, 2H, 2'-O-C**H**$_2$a, 2'-O-C**H**$_2$b), 3.13 (t, J = 7.0 Hz, C**H**$_2$-NH-CO), 2.95 – 2.80 (m, 2H, C**H**$_2$-NH_2), 2.21 (t, J = 7.3 Hz, 2H, C**H**$_2$-CO-NH), 1.89 – 1.11 (m, 37H, 11x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -11.0 (d, J = 18.1 Hz, 1P), -11.7 (d, J = 18.3 Hz, 1P), -22.2 - -22.9 (m, 1P).

HR-ESI-MS: found: 657.6917; calculated: 657.6925 ($M-2H^+$, $C_{53}H_{76}N_9O_{20}P_3S_2^{2-}$); deviation: 1.2 ppm.

γ-(6-Sulfo-Cy5-amidohexyl)-*O*2'-(6-aminohexyl)-adenosine triphosphate 9b

This compound was synthesized using general procedure 4 starting from γ-(6-Sulfo-Cy5-amidohexyl)-O2'-(6-azidohexyl)-adenosine triphosphate **8b** (16.2 µmol) in 79% yield.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.69 (s, 1H, H-8), 8.30 (t, J = 13.0 Hz, 1H, H-β-Sulfo-Cy5), 8.29 (t, J = 13.0 Hz, 1H, H-β'-Sulfo-Cy5), 8.19 `(s, 1H, H-2), 7.91 – 7.86 (m, 4H, H-Ar-Sulfo-Cy5), 7.38 – 7.32 (m, 2H, H-Ar-Sulfo-Cy5), 6.68 (t, J = 12.4 Hz, 1H, H-γ-Sulfo-Cy5), 6.36 (d, J = 13.7 Hz, 1H, H-α-Sulfo-Cy5), 6.34 (d, J = 13.7 Hz, 1H, H-α'-Sulfo-Cy5), 6.18 (d, J = 6.5 Hz, 1H, H-1'), 4.66 (dd, J = 4.6 Hz, J = 2.2 Hz, 1H, H-3'), 4.56 (dd, J = 6.3 Hz, J = 5.1 Hz, 1H, H-2'), 4.36 – 4.07 (m, 7H, H-4', H-5'a, H-5'b, 2x Sulfo-Cy5-N-C**H**$_2$), 4.00 (q, J = 6.6 Hz, 2H, γP-O-C**H**$_2$), 3.66 – 3.54 (m, 2H, 2'-O-C**H**$_2$a, 2'-O-C**H**$_2$b), 3.13 (t, J = 7.1 Hz, 2H, C**H**$_2$-NH-CO), 2.95 – 2.79 (m, 2H, C**H**$_2$-NH$_2$), 2.21 (t, J = 7.3 Hz, 2H, C**H**$_2$-CO-NH), 1.89 – 1.10 (m, 37H, 11x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -10.9 (d, J = 18.3 Hz, 1P), -11.6 (d, J = 18.0 Hz, 1P), -22.0 - -22.8 (m, 1P).

HR-ESI-MS: found: 670.6988; calculated: 670.7003 (M-2H$^+$, $C_{55}H_{78}N_9O_{20}P_3S_2^{2-}$); deviation: 2.2 ppm.

γ-(6-Cy3-amidohexyl)-*O*2'-(6-aminohexyl)-adenosine triphosphate 9c

This compound was synthesized using general procedure 4 starting from γ-(6-Cy3-amidohexyl)-O2'-(6-azidohexyl)-adenosine triphosphate **8c** (12.6 µmol) in 91% yield.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.72 (s, 1H, H-8), 8.50 (t, J = 13.4 Hz, 1H, H-β-Cy3), 8.16 (s, 1H, H-2), 7.52 (d, J = 7.5 Hz, 2H, H-Ar-Cy3), 7.46 – 7.39 (m, 2H, H-Ar-Cy3), 7.37 – 7.25 (m, 4H, H-Ar-Cy3), 6.42 (d, J = 13.4 Hz, 1H, H-α-Cy3), 6.40 (d, J = 13.3 Hz, 1H, H-α'-Cy3), 6.17 (d, J = 6.7 Hz, 1H, H-1'), 4.67 (dd, J = 4.5 Hz, J = 1.7 Hz, 1H, H-3'), 4.55 (dd, J = 6.5 Hz, J = 4.8 Hz, 1H, H-2'), 4.37 – 4.29 (m, 1H, H-5'a), 4.27 – 4.17 (m, 2H, H-4', H-5'b), 4.13 (t, J = 7.4 Hz, 2H, Cy3-N-C**H**$_2$), 4.01 (q, J = 6.6 Hz, 2H, γP-O-C**H**$_2$), 3.69 (s, 3H, Cy3-N-C**H**$_3$), 3.63 – 3.50 (m, 2H, 2'-O-C**H**$_2$a, 2'-O-C**H**$_2$b), 3.12 (t, J = 6.8 Hz, 2H, C**H**$_2$-NH-CO), 2.95 – 2.78 (m, 2H, C**H**$_2$-NH$_2$), 2.22 (t, J = 7.3 Hz, 2H, C**H**$_2$-CO-NH), 1.89 – 0.97 (m, 34H, 11x C**H**$_2$-linker, 4x C**H**$_3$).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -10.8 (d, J = 18.8 Hz, 1P), -11.7 (d, J = 19.6 Hz, 1P), -22.5 (t, J = 18.8 Hz, 1P).

HR-ESI-MS: found: 572.7412; calculated: 572.7435 (M-2H$^+$, $C_{52}H_{74}N_9O_{14}P_3^{2-}$); deviation: 4.0 ppm.

γ-(6-Sulfo-Cy3-amidohexyl)-*O*2'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 7a (from 4)

This compound was synthesized using general procedure 5 starting from γ-(6-Sulfo-Cy3-amidohexyl)-O2'-(6-aminohexyl)-adenosine triphosphate **9a** (11.2 µmol) and Sulfo-Cy5 NHS ester (14.8 µmol) in 44% yield.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.62 (s, 1H, H-8), 8.56 (t, J = 13.4 Hz, 1H, H-β-Sulfo-Cy3), 8.29 (t, J = 13.2 Hz, 2H, H-β-Sulfo-Cy5, H-β'-Sulfo-Cy5), 8.17 (s, 1H, H-2), 7.97 – 7.85 (m, 8H, H-Ar-Sulfo-Cy), 7.42 (d, J = 8.4 Hz, 1H, H-Ar-Sulfo-Cy), 7.41 (d, J = 8.3 Hz, 1H, H-Ar-Sulfo-Cy), 7.38 – 7.31 (m, 2H, H-Ar-Sulfo-Cy), 6.69 (t, J = 12.4 Hz, 1H, H-γ-Sulfo-Cy5), 6.58 (d, J = 13.6 Hz, 1H, H-α-Sulfo-Cy3), 6.54 (d, J = 13.7 Hz, 1H, H-α'-Sulfo-Cy3), 6.35 (d, J = 13.6 Hz, 1H, H-α-Sulfo-Cy5), 6.34 (d, J = 13.6 Hz, 1H, H-α'-Sulfo-Cy5), 6.15 (s, 1H, H-1'), 4.61 (t, J = 4.2 Hz, 1H, H-3'), 4.41 (t, J = 5.1 Hz, 1H, H-2'), 4.35 – 4.08 (m,

11H, H-4', H-5'a, H-5'b, 4x Sulfo-Cy-N-C**H**$_2$), 3.97 (q, J = 6.4 Hz, 2H, γP-O-C**H**$_2$), 3.69 – 3.60 (m, 1H, 2'-O-C**H**$_2$a), 3.55 – 3.44 (m, 1H, 2'-O-C**H**$_2$b), 3.12 (t, J = 6.9 Hz, 2H, C**H**$_2$-NH-CO), 3.07 (t, J = 6.9 Hz, 2H, C**H**$_2$-NH-CO), 2.21 (t, J = 7.4 Hz, 2H, C**H**$_2$-CO-NH), 2.18 (t, J = 7.3 Hz, 2H, C**H**$_2$-CO-NH), 1.87 – 1.16 (m, 58H, 14x C**H**$_2$-linker, 10x C**H**$_3$).

^{31}P NMR (MeOD-d$_4$, 162 MHz): δ -10.3 (d, J = 17.7 Hz, 1P), -10.9 (d, J = 17.7 Hz, 1P), -20.8 - -21.4 (m, 1P).

HR-ESI-MS: found: 650.8615; calculated: 650.8629 (M-3H$^+$, $C_{86}H_{113}N_{11}O_{27}P_3S_4^{3-}$); deviation: 2.2 ppm.

γ-(6-Sulfo-Cy5-amidohexyl)-*O*2'-(6-Sulfo-Cy7-amidohexyl)-adenosine triphosphate 7b

This compound was synthesized using general procedure 5 starting from γ-(6-Sulfo-Cy5-amidohexyl)-O2'-(6-aminohexyl)-adenosine triphosphate **9b** (12.8 µmol) and Sulfo-Cy7 NHS ester (36 µmol) in 48% yield.

^{1}H NMR (MeOD-d$_4$, 400 MHz): δ 8.69 (s, 1H, H-8), 8.29 (t, J = 13.2 Hz, 2H, H-β-Sulfo-Cy5, H-β'-Sulfo-Cy5), 8.27 (s, 1H, H-2), 7.96 (t, J = 13.1 Hz, 1H, H-β-Sulfo-Cy7), 7.95 (t, J = 13.0 Hz, 1H, H-β'-Sulfo-Cy7), 7.91 – 7.84 (m, 8H, H-Ar-Sulfo-Cy), 7.64 (t, J = 12.7 Hz, 1H, H-δ-Sulfo-Cy7), 7.35 (d, J = 8.9 Hz, 1H, H-Ar-Cy), 7.34 (d, J = 8.7 Hz, 1H, H-Ar-Sulfo-Cy), 7.31 (d, J = 9.0 Hz, 1H, H-Ar-Sulfo-Cy), 7.30 (d, J = 8.8 Hz, 1H, H-Ar-Sulfo-Cy), 6.70 (t, J = 12.5 Hz, 1H, H-γ-Sulfo-Cy5), 6.61 (t, J = 12.6 Hz, 1H, H-γ-Sulfo-Cy7), 6.60 (t, J = 12.5 Hz, 1H, H-γ'-Sulfo-Cy7), 6.41 – 6.29 (m, 4H, H-α-Sulfo-Cy5, H-α'-Sulfo-Cy5, H-α-Sulfo-Cy7, H-α'-Sulfo-Cy7), 6.14 (d, J = 4.4 Hz, 1H, H-1'), 4.57 (t, J = 4.8 Hz, 1H, H-3'), 4.38 – 4.06 (m, 12H, H-2', H-4', H-5'a, H-5'b, 4x Sulfo-Cy-N-C**H**$_2$), 4.01 (q, J = 6.6 Hz, 2H, γP-O-C**H**$_2$), 3.72 – 3.65 (m, 1H, 2'-O-C**H**$_2$a), 3.62 – 3.53 (m, 1H, 2'-O-C**H**$_2$b), 3.12 (t, J = 7.0 Hz, 2H, C**H**$_2$-NH-CO), 3.08 (t, J = 7.1 Hz, 2H, C**H**$_2$-NH-CO), 2.20 (t, J = 7.4 Hz, 2H, C**H**$_2$-CO-NH), 2.19 (t, J = 7.2 Hz, 2H, C**H**$_2$-CO-NH), 1.86 – 1.18 (m, 58H, 14x C**H**$_2$-linker, 10x C**H**$_3$).

^{31}P NMR (MeOD-d$_4$, 162 MHz): δ -10.9 (d, J = 18.3 Hz, 1P), -11.4 (d, J = 17.6 Hz, 1P), -22.0 - -22.6 (m, 1P).

HR-ESI-MS: found: 1002.8105; calculated: 1002.8142 (M-2H$^+$, $C_{90}H_{118}N_{11}O_{27}P_3S_4^{2-}$); deviation: 3.7 ppm.

γ-(6-Cy3-amidohexyl)-*O*2'-(6-Cy5-amidohexyl)-adenosine triphosphate 7c

This compound was synthesized using general procedure 5 starting from γ-(6-Cy3-amidohexyl)-O2'-(6-aminohexyl)-adenosine triphosphate **9c** (6.8 µmol) and Cy5 NHS ester (10.2 µmol) in 12% yield.

^{1}H NMR (MeOD-d$_4$, 400 MHz): δ 8.64 (s, 1H, H-8), 8.49 (t, J = 13.5 Hz, 1H, H-β-Cy3), 8.20 (t, J = 13.1 Hz, 2H, H-β-Cy5, H-β'-Cy5), 8.11 (s, 1H, H-2), 7.55 – 7.21 (m, 16H, H-Ar-Cy), 6.59 (t, J = 12.5 Hz, 1H, H-γ-Cy5), 6.45 (d, J = 13.5 Hz, 1H, H-α-Cy3), 6.43 (d, J = 13.4 Hz, 1H, H-α'-Cy3), 6.27 (d, J = 13.6 Hz, 1H, H-α-Cy5), 6.24 (d, J = 13.6 Hz, 1H, H-α'-Cy5), 6.14 (d, J = 5.5 Hz, 1H, H-1'), 4.68 (t, J = 4.0 Hz, 1H, H-3'), 4.44 – 4.33 (m, 2H, H-2', H-5'a), 4.31 – 4.21 (m, 2H, H-4', H-5'b), 4.17 – 4.06 (m, 4H, 2x Cy-N-C**H**$_2$), 4.02 (q, J = 6.3 Hz, 2H, γP-O-C**H**$_2$), 3.70 (s, 3H, Cy-N-C**H**$_3$), 3.68 – 3.64 (m, 1H, 2'-O-C**H**$_2$a), 3.62 (s, 3H,

Cy-N-C**H**$_3$), 3.55 – 3.46 (m, 1H, 2'-O-C**H**$_2$b), 3.12 (t, J = 6.4 Hz, 2H, C**H**$_2$-NH-CO), 3.05 (t, J = 7.0 Hz, 2H, C**H**$_2$-NH-CO), 2.23 (t, J = 7.4 Hz, 2H, C**H**$_2$-CO-NH), 2.19 (t, J = 7.2 Hz, 2H, C**H**$_2$-CO-NH), 1.89 – 1.11 (m, 52H, 14x C**H**$_2$-linker, 8x C**H**$_3$).

^{31}P NMR (MeOD-d$_4$, 162 MHz): δ -10.3 (d, J = 18.1 Hz, 1P), -10.9 (d, J = 18.3 Hz, 1P), -20.8 - -21.3 (m, 1P).

HR-ESI-MS: found: 804.8811; calculated: 804.8849 (M-2H$^+$, $C_{84}H_{110}N_{11}O_{15}P_3^{2-}$); deviation: 4.7 ppm.

γ-(6-Sulfo-Cy3-amidohexyl)-*O*2'-(6-Eclipse-amidohexyl)-adenosine triphosphate 7d

This compound was synthesized using general procedure 5 starting from γ-(6-Sulfo-Cy3-amidohexyl)-O2'-(6-aminohexyl)-adenosine triphosphate **9a** (11.2 µmol) and Eclipse NHS ester (14.2 µmol) in 27% yield.

^{1}H NMR (MeOD-d$_4$, 400 MHz): δ 8.61 (s, 1H, H-8), 8.53 (t, J = 13.3 Hz, 1H, H-β-Sulfo-Cy3), 8.37 (d, J = 2.3 Hz, 1H, H-Ar-Eclipse), 8.18 (dd, J = 9.0 Hz, J = 2.3 Hz, 1H, H-Ar-Eclipse), 8.16 (s, 1H, H-2), 7.98 – 7.83 (m, 6H, 4x H-Ar-Sulfo-Cy3, 2x H-Ar-Eclipse), 7.78 (d, J = 9.0 Hz, 1H, H-Ar-Eclipse), 7.40 (d, J = 8.2 Hz, 1H, H-Ar-Sulfo-Cy3), 7.38 (d, J = 8.2 Hz, 1H, H-Ar-Sulfo-Cy3), 6.85 (d, J = 9.3 Hz, 2H, H-Ar-Eclipse), 6.52 (d, J = 13.5 Hz, 1H, H-α-Sulfo-Cy3), 6.48 (d, J =13.4 Hz, 1H, H-α'-Sulfo-Cy3), 6.14 (d, J = 5.1 Hz, 1H, H-1'), 4.61 (t, J = 4.4 Hz, 1H, H-3'), 4.39 (t, J = 5.0 Hz, 1H, H-2'), 4.35 – 4.08 (m, 7H, H-4', H-5'a, H-5'b, 2x Sulfo-Cy3-N-C**H**$_2$), 3.98 (q, J = 6.4 Hz, 2H, γP-O-C**H**$_2$), 3.69 – 3.59 (m, 1H, 2'-O-C**H**$_2$a), 3.55 – 3.47 (m, 3H, 2'-O-C**H**$_2$b, Eclipse-N-C**H**$_2$), 3.15 – 3.07 (m, 7H, Eclipse-N-C**H**$_3$, 2x C**H**$_2$-NH-CO), 2.26 (t, J = 7.2 Hz, 2H, C**H**$_2$-CO-NH), 2.22 (t, J = 7.3 Hz, 2H, C**H**$_2$-CO-NH), 2.00 -1.10 (m, 39H, 12x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD-d$_4$, 162 MHz): δ -10.4 (d, J = 18.2 Hz, 1P), -10.9 (d, J = 18.1 Hz, 1P), -21.0 - -21.7 (m, 1P).

HR-ESI-MS: found: 836.7305; calculated: 836.7341 (M-2H$^+$, $C_{70}H_{91}ClN_{13}O_{23}P_3S_2^{2-}$); deviation: 4.3 ppm.

γ-(6-Cy3-amidohexyl)-*O*2'-(6-Eclipse-amidohexyl)-adenosine triphosphate 7e

This compound was synthesized using general procedure 5 starting from γ-(6-Cy3-amidohexyl)-O2'-(6-aminohexyl)-adenosine triphosphate **9c** (6.8 µmol) and Eclipse NHS ester (8.7 µmol) in 13% yield.

^{1}H NMR (MeOD-d$_4$, 400 MHz): δ 8.60 (s, 1H, H-8), 8.48 (t, J = 13.5 Hz, 1H, H-β-Cy3), 8.36 (d, J = 2.3 Hz, 1H, H-Ar-Eclipse), 8.17 (dd, J = 9.1 Hz, J = 2.3 Hz, 1H, H-Ar-Eclipse), 8.14 (s, 1H, H-2), 7.87 (d, J = 9.0 Hz, 2H, H-Ar-Eclipse), 7.78 (d, J = 8.9 Hz, 1H, H-Ar-Eclipse), 7.53 – 7.48 (m, 2H, H-Ar-Cy3), 7.45 – 7.38 (m, 2H, H-Ar-Cy3), 7.35 – 7.25 (m, 4H, H-Ar-Cy3), 6.84 (d, J = 9.4 Hz, 2H, H-Ar-Eclipse), 6.40 (d, J = 13.5 Hz, 1H, H-α-Cy3), 6.38 (d, J = 13.4 Hz, 1H, H-α'-Cy3), 6.13 (d, J = 5.3 Hz, 1H, H-1'), 4.61 (t, J = 4.4 Hz, 1H, H-3'), 4.39 (t, J = 5.1 Hz, 1H, H-2'), 4.36 – 4.20 (m, 3H, H-4', H-5'a, H-5'b), 4.11 (t, J = 7.5 Hz, Cy3-N-C**H**$_2$), 3.98 (q, J = 6.4 Hz, 2H, γP-O-C**H**$_2$), 3.70 – 3.59 (m, 4H, 2'-O-C**H**$_2$a, Cy3-N-C**H**$_3$), 3.55 – 3.46 (m, 3H, 2'-O-C**H**$_2$b, Eclipse-N-C**H**$_2$), 3.13 – 3.07 (m, 7H, Eclipse-N-C**H**$_3$, 2x C**H**$_2$-NH-CO), 2.25 (t, J = 7.1 Hz, 2H, C**H**$_2$-CO-NH), 2.21 (t, J = 7.2 Hz, 2H, C**H**$_2$-CO-NH), 2.00 – 1.16 (m, 36H, 12x C**H**$_2$-linker, 4x C**H**$_3$).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -10.4 (d, J = 18.3 Hz, 1P), -11.0 (d, J = 18.4 Hz, 1P), -21.0 - -21.6 (m, 1P).

HR-ESI-MS: found: 1500.5435; calculated: 1500.5474 (M-H$^+$, $C_{69}H_{90}ClN_{13}O_{17}P_3^-$); deviation: 2.6 ppm.

γ-(6-Azidohexyl)-*C*2-(5-trifluoroacetamidopent-1-yn-1-yl)-adenosine triphosphate

This compound was synthesized using general procedure 2 starting from 2-(5-trifluoroacetamidopent-1-yn-1-yl)-adenosine triphosphate (89.4 µmol) and 6-azido-1-bromo-hexane (267 µmol) in 70% yield.

^{1}H NMR (D_2O, 400 MHz): δ 8.60 (s, 1H, H-8), 6.11 (d, J = 5.7 Hz, 1H, H-1'), 4.77 – 4.72 (m, 1H, H-2'), 4.57 (dd, J = 4.9 Hz, J = 3.7 Hz, 1H, H-3'), 4.43 – 4.38 (m, 1H, H-4'), 4.30 – 4.25 (m, 2H, H-5'a, H-5'b), 3.90 – 3.80 (m, 2H, γP-O-CH_2), 3.54 (t, J = 6.8 Hz, 2H, NHTFA-C**H**$_2$), 3.20 (t, J = 7.0 Hz, 2H, N_3-C**H**$_2$), 2.58 (t, J = 6.9 Hz, 2H, CC-C**H**$_2$), 2.01 – 1.94 (m, 2H, CC-CH_2-C**H**$_2$), 1.46 – 1.34 (m, 4H, 2x C**H**$_2$-linker), 1.16 – 1.09 (m, 4H, 2x C**H**$_2$-linker).

^{19}F NMR (D_2O, 376 MHz): δ -75.8 (s, 3F).

^{31}P NMR (D_2O, 162 MHz): δ -11.0 (d, J = 18.4 Hz, 1P), -11.6 (d, J = 18.4 Hz, 1P), -23.4 (t, J = 19.4 Hz, 1P).

HR-ESI-MS: found: 808.1255; calculated: 808.1228 (M-H$^+$, $C_{23}H_{32}F_3N_9O_{14}P_3^-$); deviation: 3.3 ppm.

γ-(6-Azidohexyl)-*C*2-(5-aminopent-1-yn-1-yl)-adenosine triphosphate 10

This compound was synthesized using general procedure 3 starting from γ-(6-Azidohexyl)-2-(5-trifluoroacetamidopent-1-yn-1-yl)-adenosine triphosphate (62.4 µmol) in 53% yield.

^{1}H NMR (D_2O, 400 MHz): δ 8.57 (s, 1H, H-8), 6.09 (d, J = 5.9 Hz, 1H, H-1'), 4.90 – 4.85 (m, 1H, H-2'), 4.63 – 4.57 (m, 1H, H-3'), 4.44 – 4.39 (m, 1H, H-4'), 4.33 – 4.25 (m, 2H, H-5'a, H-5'b), 3.91 – 3.83 (m, 2H, γP-O-C**H**$_2$), 3.23 – 3.13 (m, 4H, N_3-C**H**$_2$, C**H**$_2$-NH_2), 2.67 (t, J = 6.8 Hz, 2H, CC-C**H**$_2$), 2.11 – 2.01 (m, 2H, CC-CH_2-C**H**$_2$), 1.49 – 1.36 (m, 4H, 2x C**H**$_2$-linker), 1.17 – 1.09 (m, 4H, 2x C**H**$_2$-linker).

^{31}P NMR (D_2O, 162 MHz): δ -11.0 (d, J = 21.2 Hz, 1P), -11.6 (d, J = 20.3 Hz, 1P), -23.4 (t, J = 19.5 Hz, 1P).

HR-ESI-MS: found: 713.1423; calculated: 713.1405 (M-H$^+$, $C_{21}H_{33}N_9O_{13}P_3^-$); deviation: 2.5 ppm.

γ-(6-Azidohexyl)-*C*2-(5-Sulfo-Cy5-amidopent-1-yn-1-yl)-adenosine triphosphate 11

This compound was synthesized using general procedure 5 starting from γ-(6-Azidohexyl)-2-(5-aminopent-1-yn-1-yl)-adenosine triphosphate **10** (33.1 µmol) and Sulfo-Cy5 NHS ester (66 µmol) in 58% yield.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.62 (s, 1H, H-8), 8.33 (t, J = 13.0 Hz, 2H, H-β-Sulfo-Cy5, H-β'-Sulfo-Cy5), 7.95 – 7.82 (m, 4H, H-Ar-Sulfo-Cy5), 7.37 (d, J = 8.8 Hz, 1H, H-Ar-Sulfo-Cy5), 7.35 (d, J = 8.6 Hz, 1H, H-Ar-Sulfo-Cy5), 6.71 (t, J = 12.4 Hz, 1H, H-γ-Sulfo-Cy5), 6.37 (d, J = 13.6 Hz, 2H, H-α-Sulfo-Cy5, H-α'-Sulfo-Cy5), 6.08 (d, J = 5.4 Hz, 1H, H-1'), 4.67 (t, J = 5.1 Hz, 1H, H-2'), 4.58 – 4.51 (m, 1H, H-3'), 4.38 – 4.26 (m, 1H, H-4'), 4.25 – 4.07 (m, 6H, H-5'a, H-5'b, 2x Sulfo-Cy5-N-C**H**$_2$), 3.99 (q, J = 6.5 Hz, 2H, γP-O-C**H**$_2$), 3.29 – 3.24 (m, 4H, C**H**$_2$-N$_3$, C**H**$_2$-NH-CO), 2.45 (t, J = 7.1 Hz, 2H, CC-C**H**$_2$), 2.22 (t, J = 7.0 Hz, 2H, C**H**$_2$-CO-NH), 1.81 – 1.34 (m, 31H, 8x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -11.2 (d, J = 18.5 Hz, 1P), -11.8 (d, J = 18.6 Hz, 1P), -23.1 (t, J = 14.9 Hz, 1P).

HR-ESI-MS: found: 1350.3569; calculated: 1350.3526 (M-H$^+$, $C_{54}H_{71}N_{11}O_{20}P_3S_2^-$); deviation: 3.2 ppm.

γ-(6-Aminohexyl)-*C*2-(5-Sulfo-Cy5-amidopent-1-yn-1-yl)-adenosine triphosphate 12

This compound was synthesized using general procedure 4 starting from γ-(6-Azidohexyl)-2-(5-Sulfo-Cy5-amidopent-1-yn-1-yl)-adenosine triphosphate **11** (19.1 µmol) in 66% yield.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.66 (s, 1H, H-8), 8.31 (t, J = 12.9 Hz, 2H, H-β-Sulfo-Cy5, H-β'-Sulfo-Cy5), 7.92 – 7.84 (m, 4H, H-Ar-Sulfo-Cy5), 7.36 (d, J = 8.5 Hz, 1H, H-Ar-Sulfo-Cy5), 7.34 (d, J = 8.6 Hz, 1H, H-Ar-Sulfo-Cy5), 6.70 (t, J = 12.3 Hz, 1H, H-γ-Sulfo-Cy5), 6.36 (d, J = 13.6 Hz, 2H, H-α-Sulfo-Cy5, H-α'-Sulfo-Cy5), 6.08 (d, J = 4.8 Hz, 1H, H-1'), 4.62 (t, J = 4.8 Hz, 1H, H-2'), 4.59 – 4.51 (m, 1H, H-3'), 4.34 – 4.26 (m, 1H, H-4'), 4.26 – 4.08 (m, 6H, H-5'a, H-5'b, 2x Sulfo-Cy5-N-C**H**$_2$), 4.02 – 3.91 (m, 2H, γP-O-C**H**$_2$), 3.28 (t, J = 6.8 Hz, 2H, C**H**$_2$-NH-CO), 2.95 (t, J = 6.8 Hz, 2H, C**H**$_2$-NH$_2$), 2.44 (t, J = 6.9 Hz, 2H, CC-C**H**$_2$), 2.22 (t, J = 6.9 Hz, 2H, C**H**$_2$-CO-NH), 1.83– 1.34 (m, 31H, 8x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -11.1 (d, J = 18.5 Hz, 1P), -11.8 (d, J = 19.4 Hz, 1P), -22.9 (t, J = 18.4 Hz, 1P).

HR-ESI-MS: found: 1324.3663; calculated: 1324.3621 (M-H$^+$, $C_{54}H_{73}N_9O_{20}P_3S_2^-$); deviation: 3.2 ppm.

γ-(6-Sulfo-Cy3-amidohexyl)-*C*2-(5-Sulfo-Cy5-amidopent-1-yn-1-yl)-adenosine triphosphate 13

This compound was synthesized using general procedure 5 starting from γ-(6-Aminohexyl)-2-(5-Sulfo-Cy5-amidopent-1-yn-1-yl)-adenosine triphosphate **12** (12.6 µmol) and Sulfo-Cy3 NHS ester (29 µmol) in 87% yield.

^{1}H NMR (MeOD-d_4, 600 MHz): δ 8.64 (s, 1H, H-8), 8.52 (t, J = 13.4 Hz, 1H, H-β-Sulfo-Cy3), 8.33 (t, J = 12.9 Hz, 2H, H-β- Sulfo-Cy5, H-β'-Sulfo-Cy5), 7.98 – 7.84 (m, 8H, H-Ar-Sulfo-Cy), 7.45 (d, J = 8.3 Hz, 1H, H-Ar-Sulfo-Cy), 7.44 (d, J = 8.3 Hz, 1H, H-Ar- Sulfo-Cy), 7.37 (d, J = 8.8 Hz, 1H, H-Ar-Sulfo-Cy), 7.35 (d, J = 8.9 Hz, 1H, H-Ar-Sulfo-Cy), 6.75 (t, J = 12.3 Hz, 1H, H-γ-Sulfo-Cy5), 6.58 (d, J = 13.0 Hz, 2H, H-α-Sulfo-Cy3), 6.55 (d, J = 13.0 Hz, 2H, H-α-Sulfo-Cy3), 6.39 (d, J = 13.6 Hz, 2H, H-α- Sulfo-Cy5, H-α'-Sulfo-Cy5), 6.10 (d, J = 5.7 Hz, 1H, H-1'), 4.78 – 4.66 (m, 1H, H-2'), 4.58 – 4.53 (m, 1H, H-3'), 4.36 – 4.11 (m, 11H, H-4', H-5'a, H-5'b, 4x Sulfo-Cy-N-C**H**$_2$-linker), 3.99 (q, J = 6.5 Hz, 2H, γP-O-C**H**$_2$), 3.33 – 3.28 (m, 2H,

C**H**$_2$-NH-CO), 3.12 (t, J = 6.9 Hz, 2H, C**H**$_2$-NH-CO), 2.39 (t, J = 6.9 Hz, 2H, CC-C**H**$_2$), 2.25 – 2.17 (m, 4H, 2x C**H**$_2$-CONH), 1.85 - 1.28 (m, 52H, 11x C**H**$_2$-linker, 10x C**H**$_3$).

^{31}P NMR (MeOD-d$_4$, 162 MHz): δ -11.2 (d, J = 18.3 Hz, 1P), -11.7 (d, J = 18.1 Hz, 1P), -22.1 – -23.8 (m, 1P).

HR-ESI-MS: found: 967.7786; calculated: 967.7750 (M-2H$^+$, $C_{85}H_{108}N_{11}O_{27}P_3S_4^{2-}$); deviation: 3.7 ppm.

γ-(6-Azidohexyl)-*O*3'-(6-aminohexyl)-adenosine triphosphate 14

This compound was synthesized using general procedure 2 and 3 starting from O3'-(6-trifluoroacetamidohexyl)-adenosine triphosphate (191 µmol) and 6-azido-1-bromo-hexane (1024 µmol) in 18% yield.

^{1}H NMR (MeOD-d$_4$, 400 MHz): δ 8.51 (s, 1H, H-8), 8.20 (s, 1H, H-2), 6.06 (d, J = 5.8 Hz, 1H, H-1'), 4.83 (t, J = 5.2 Hz, 1H, H-2'), 4.33 – 4.19 (m, 4H, H-3', H-4', H-5'a, H-5'b), 4.00 (q, J = 6.6 Hz, 2H, γP-O-C**H**$_2$), 3.86 – 3.77 (m, 1H, 3'-O-C**H**$_2$a), 3.74 – 3.65 (m, 1H, 3'-O-C**H**$_2$b), 3.25 (t, J = 6.9 Hz, 2H, C**H**$_2$-N$_3$), 3.92 – 2.88 (m, 2H, C**H**$_2$-NH$_2$), 1.79 – 1.35 (m, 16H, 8x C**H**$_2$-linker).

^{31}P NMR (MeOD-d$_4$, 162 MHz): δ -10.8 (d, J = 17.9 Hz, 1P), -11.5 (d, J = 19.4Hz, 1P), -22.7 (t, J = 18.8 Hz, 1P).

HR-ESI-MS: found: 730.1859; calculated: 730.1875 (M-H$^+$, $C_{22}H_{39}N_9O_{13}P_3^-$); deviation: 2.2 ppm.

γ-(6-Azidohexyl)-*O*3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 15

This compound was synthesized using general procedure 5 starting from γ-(6-Azidohexyl)-O3'-(6-aminohexyl)-adenosine triphosphate **14** (30.5 µmol) and Sulfo-Cy5 NHS ester (51.8 µmol) in 72% yield.

^{1}H NMR (MeOD-d$_4$, 400 MHz): δ 8.63 (s, 1H, H-8), 8.30 (t, J =13.1 Hz, 2H, H-β-Sulfo-Cy5, H-β'-Sulfo-Cy5), 8.18 (s, 1H, H-2), 7.91 – 7.86 (m, 4H, H-Ar-Sulfo-Cy5), 7.38 – 7.32 (m, 2H, H-Ar-Sulfo-Cy5), 6.69 (t, J = 12.4 Hz, 1H, H-γ-Sulfo-Cy5), 6.36 (d, J = 13.8 Hz, 1H, H-α-Sulfo-Cy5), 6.35 (d, J = 13.7 Hz, 1H, H-α'-Sulfo-Cy5), 6.10 (d, J = 7.4 Hz, 1H, H-1'), 4.98 – 4.90 (m, 1H, H-2'), 4.32 – 4.27 (m, 1H, H-4'), 4.26 – 4.08 (m, 7H, H-3', H-5'a, H-5'b, 2x Sulfo-Cy5-N-C**H**$_2$), 4.00 (q, J = 6.6 Hz, 2H, γP-O-C**H**$_2$), 3.78 – 3.70 (m, 1H, 3'-O-C**H**$_2$a), 3.70 – 3.62 (m, 1H, 3'-O-C**H**$_2$b), 3.24 (t, J = 6.9 Hz, 2H, C**H**$_2$N$_3$), 3.19 – 3.14 (m, 2H, C**H**$_2$-NH-CO), 2.20 (t, J = 7.3 Hz, 2H, C**H**$_2$-CO-NH), 1.87 – 1.21 (m, 37H, 11x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD-d$_4$, 162 MHz): δ -11.1 (d, J = 18.3 Hz, 1P), -11.7 (d, J = 18.3 Hz, 1P), -22.6 - - 23.3 (m, 1P).

HR-ESI-MS: found: 683.6948; calculated: 683.6956 (M-2H$^+$, $C_{55}H_{76}N_{11}O_{20}P_3S_2^{2-}$); deviation: 1.2 ppm.

γ-(6-Aminohexyl)-*O*3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 16

This compound was synthesized using general procedure 4 starting from γ-(6-Azidohexyl)-O3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate **15** (22.1 µmol) in 80% yield.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.64 (s, 1H, H-8), 8.30 (t, J = 13.2 Hz, 2H, H-β-Sulfo-Cy5, H-β'-Sulfo-Cy5), 8.18 (s, 1H, H-2), 7.91 – 7.85 (m, 4H, H-Ar-Sulfo-Cy5), 7.38 – 7.32 (m, 2H, H-Ar-Sulfo-Cy5), 6.69 (t, J = 12.4 Hz, 1H, H-γ-Sulfo-Cy5), 6.35 (d, J = 13.6 Hz, 1H, H-α-Sulfo-Cy5), 6.34 (d, J = 13.6 Hz, 1H, H-α'-Sulfo-Cy5), 6.10 (d, J = 7.0 Hz, 1H, H-1'), 4.93 – 4.88 (m, 1H, H-2'), 4.33 – 4.27 (m, 1H, H-4'), 4.26 – 4.08 (m, 7H, H-3', H-5'a, H-5'b, 2x Sulfo-Cy5-N-C**H**$_2$), 4.01 (q, J = 6.2 Hz, 2H, γP-O-C**H**$_2$), 3.76 – 3.69 (m, 1H, 3'-O-C**H**$_2$a), 3.69 – 3.61 (m, 1H, 3'-O-C**H**$_2$b), 3.19 – 3.12 (m, 2H, C**H**$_2$-NH-CO), 2.98 – 2.90 (m, 2H, C**H**$_2$-NH$_2$), 2.20 (t, J = 7.3 Hz, 2H, C**H**$_2$-CO-NH), 1.88 – 1.19 (m, 37H, 11x C**H**$_2$-linker, 5x C**H**$_3$).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -10.8 (d, J = 18.5 Hz, 1P), -11.5 (d, J = 18.0 Hz, 1P), -22.4 - -23.0 (m, 1P).

HR-ESI-MS: found: 670.6997; calculated: 670.7003 (M-2H$^+$, $C_{55}H_{78}N_9O_{20}P_3S_2^{2-}$); deviation: 0.9 ppm.

γ-(6-Sulfo-Cy3-amidohexyl)-*O*3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 17

This compound was synthesized using general procedure 5 starting from γ-(6-Aminohexyl)-O3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate **16** (17.6 µmol) and Sulfo-Cy3 NHS ester (29.9 µmol) in 61% yield.

^{1}H NMR (MeOD-d_4, 400 MHz): δ 8.63 (s, 1H, H-8), 8.56 (t, J = 13.4 Hz, 1H, H-β-Sulfo-Cy3), 8.30 (t, J = 12.9 Hz, 2H, H-β-Sulfo-Cy5, H-β'-Sulfo-Cy5), 8.18 (s, 1H, H-2), 7.97 – 7.86 (m, 8H, H-Ar-Sulfo-Cy), 7.44 (d, J = 8.4 Hz, 1H, H-Ar-Sulfo-Cy), 7.43 (d, J = 8.4 Hz, 1H, H-Ar-Sulfo-Cy), 7.39 – 7.33 (m, 2H, H-Ar-Sulfo-Cy), 6.71 (t, J = 12.4 Hz, 1H, H-γ-Sulfo-Cy5), 6.59 (d, J = 13.4 Hz, 1H, H-α-Sulfo-Cy3), 6.55 (d, J = 13.4 Hz, 1H, H-α'-Sulfo-Cy3), 6.37 (d, J =13.7 Hz, 1H, H-α-Sulfo-Cy5), 6.36 (d, J = 13.7 Hz, 1H, H-α'-Sulfo-Cy5), 6.10 (d, J = 7.1 Hz, 1H, H-1'), 4.96 – 4.90 (m, 1H, H-2'), 4.32 – 4.09 (m, 12H, H-3', H-4', H-5'a, H-5'b, 4x Sulfo-Cy-N-C**H**$_2$), 3.98 (q, J = 6.5 Hz, 2H, γP-O-C**H**$_2$), 3.77 – 3.69 (m, 1H, 3'-O-C**H**$_2$a), 3.68 – 3.60 (m, 1H, 3'-O-C**H**$_2$b), 3.17 – 3.09 (m, 4H, 2x C**H**$_2$-NH-CO), 2.20 (t, J = 7.2 Hz, 4H, 2x C**H**$_2$-CO-NH), 1.92 – 1.19 (m, 58H, 14x C**H**$_2$-linker, 10x C**H**$_3$).

^{31}P NMR (MeOD-d_4, 162 MHz): δ -10.9 (d, J = 18.5 Hz, 1P), -11.5 (d, J = 18.3 Hz, 1P), -21.9 - -23.0 (m, 1P).

HR-ESI-MS: found: 650.8620; calculated: 650.8629 (M-3H$^+$, $C_{86}H_{113}N_{11}O_{27}P_3S_4^{3-}$); deviation: 1.4 ppm.

Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Synthesis and Fluorescence Characteristics of ATP-based FRET Probes

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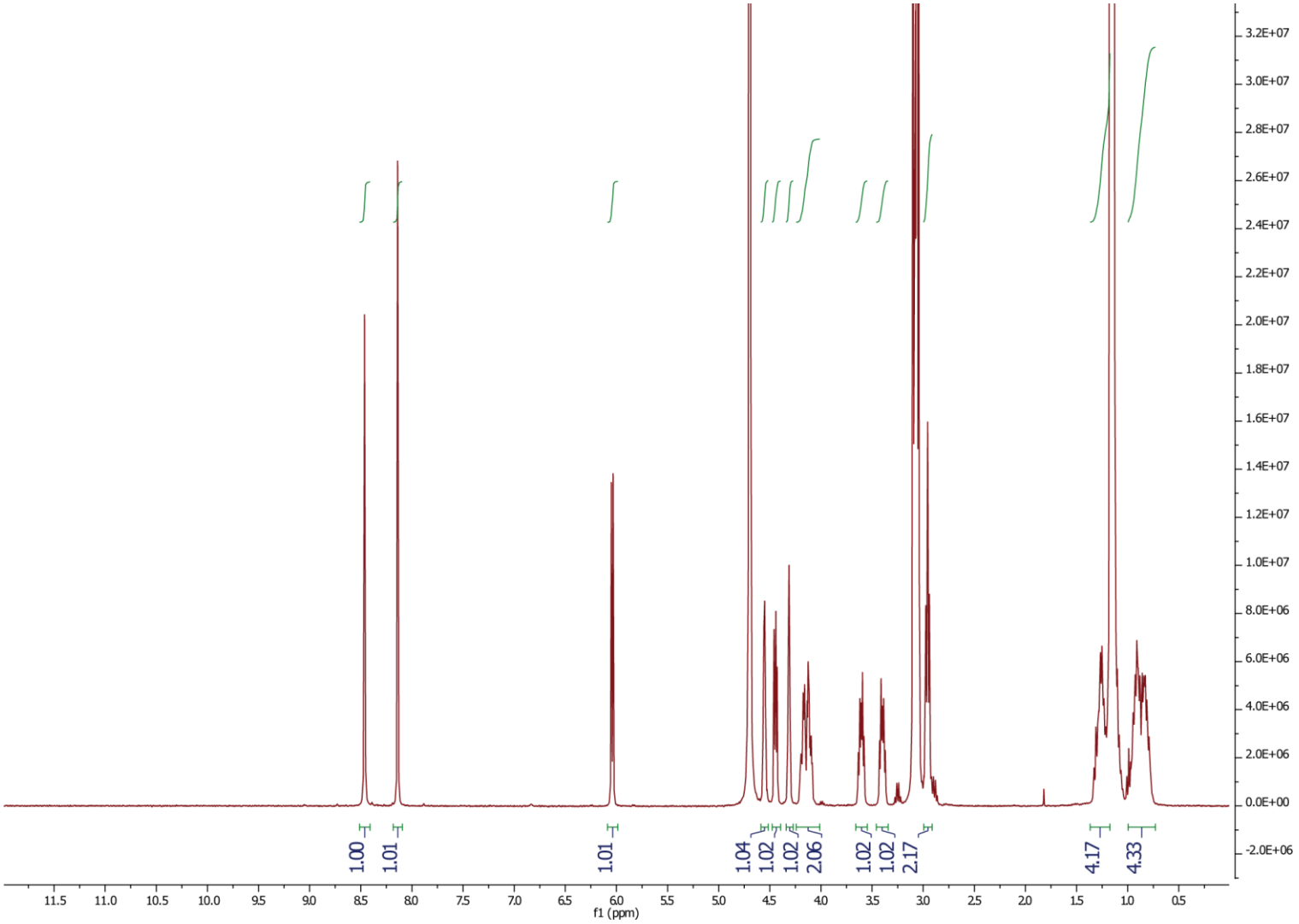
Content

_O_2'-(6-trifluoroacetamidohexyl)-adenosine triphosphate 1 (^{1}H NMR)



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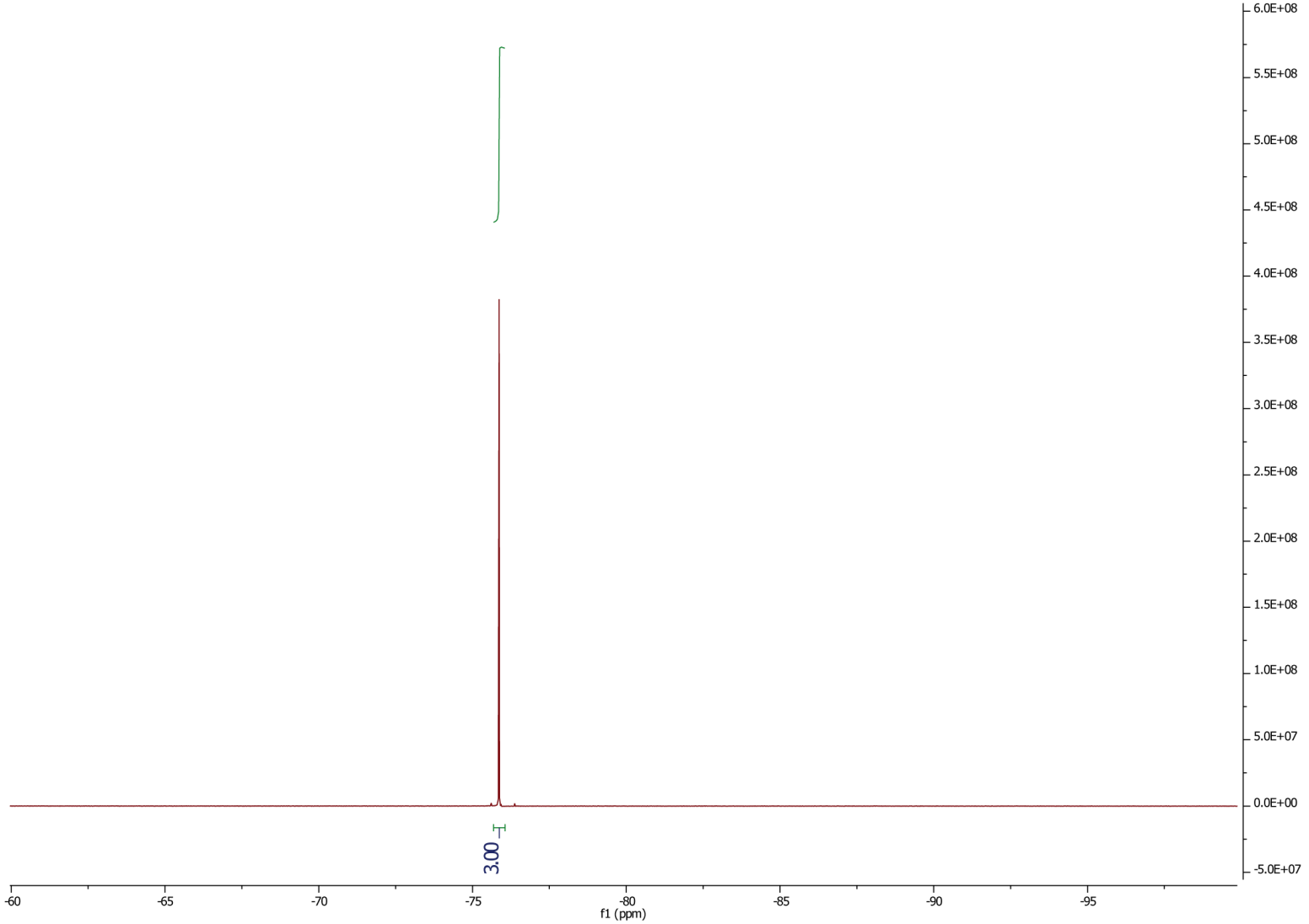

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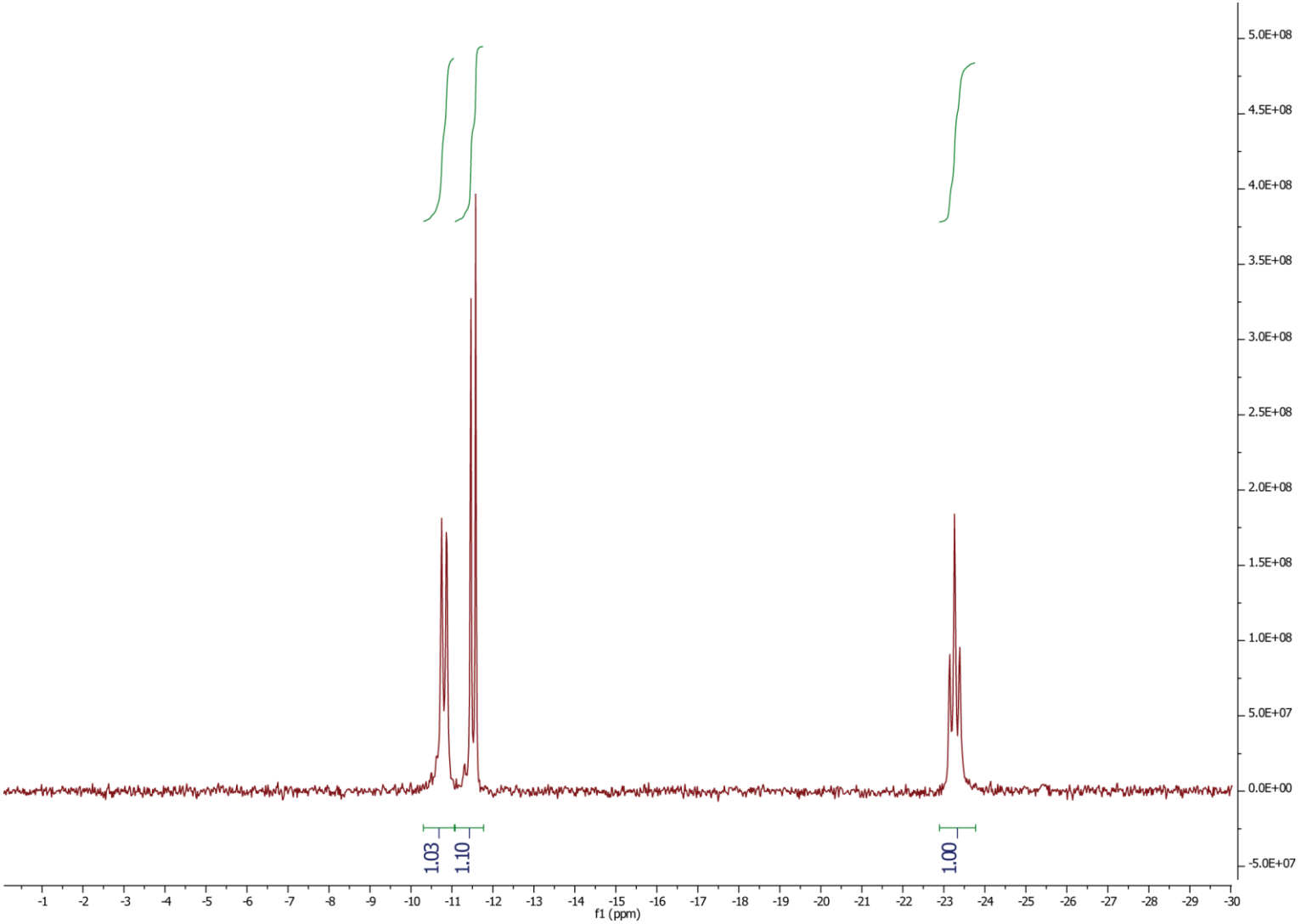

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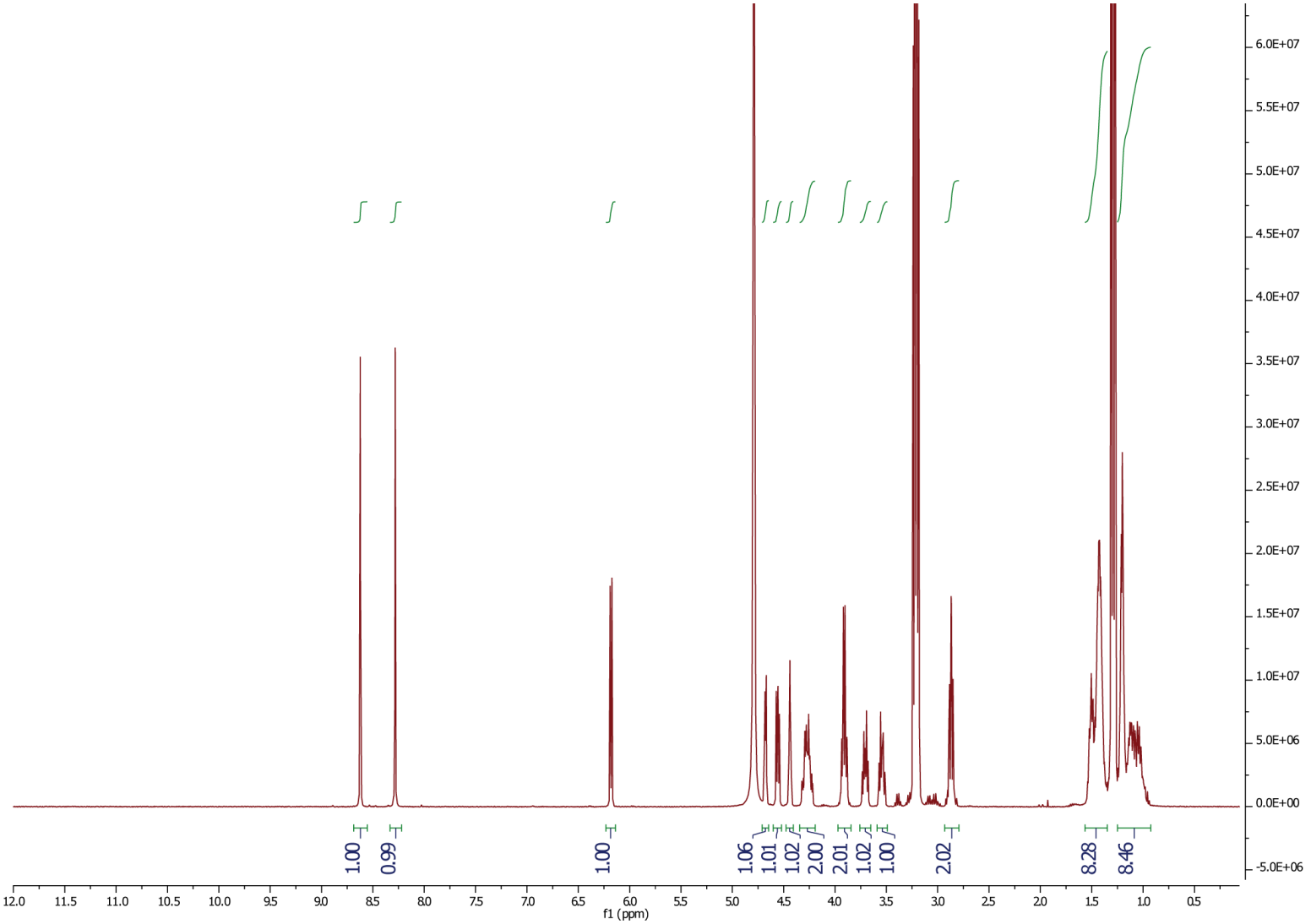

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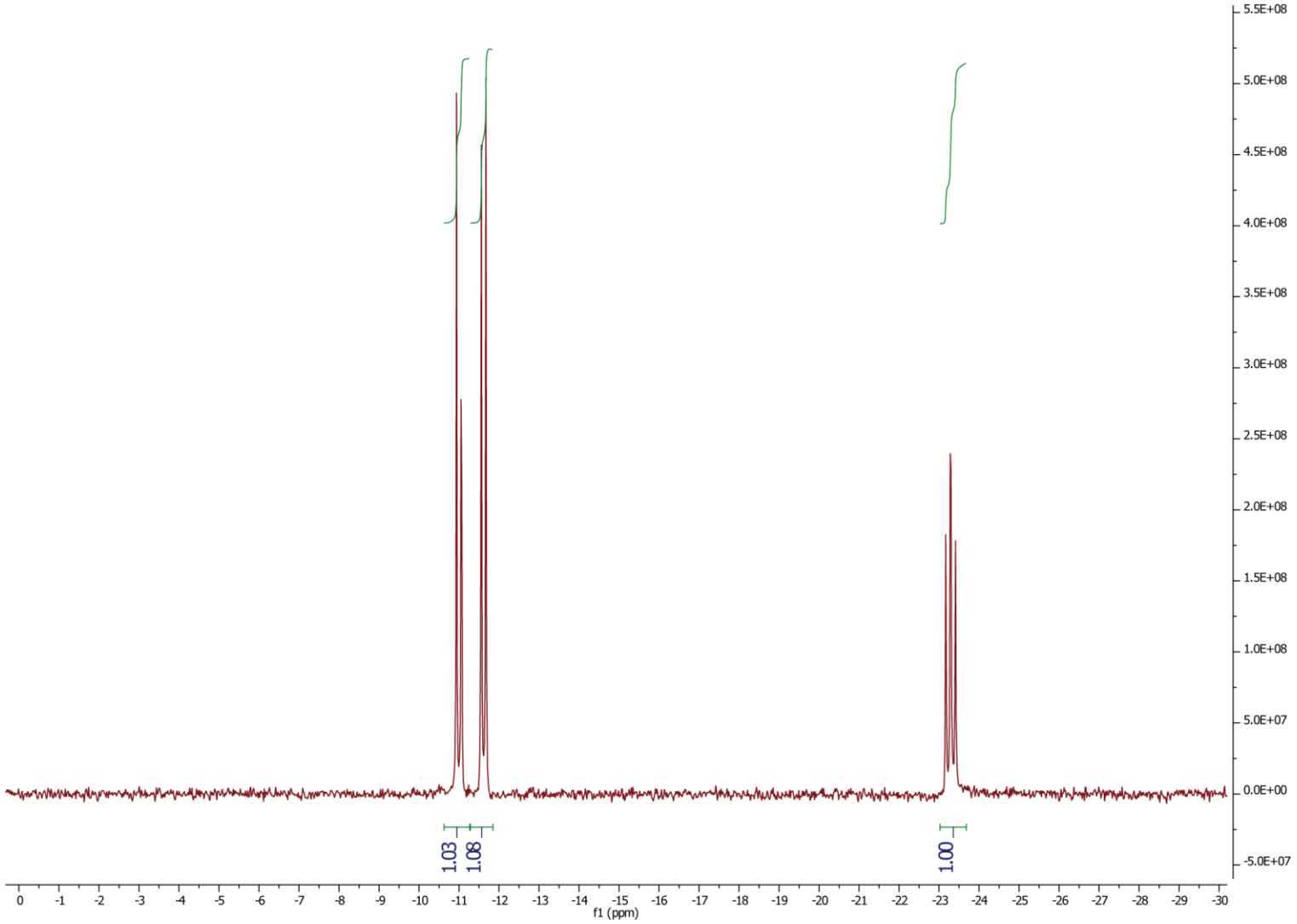

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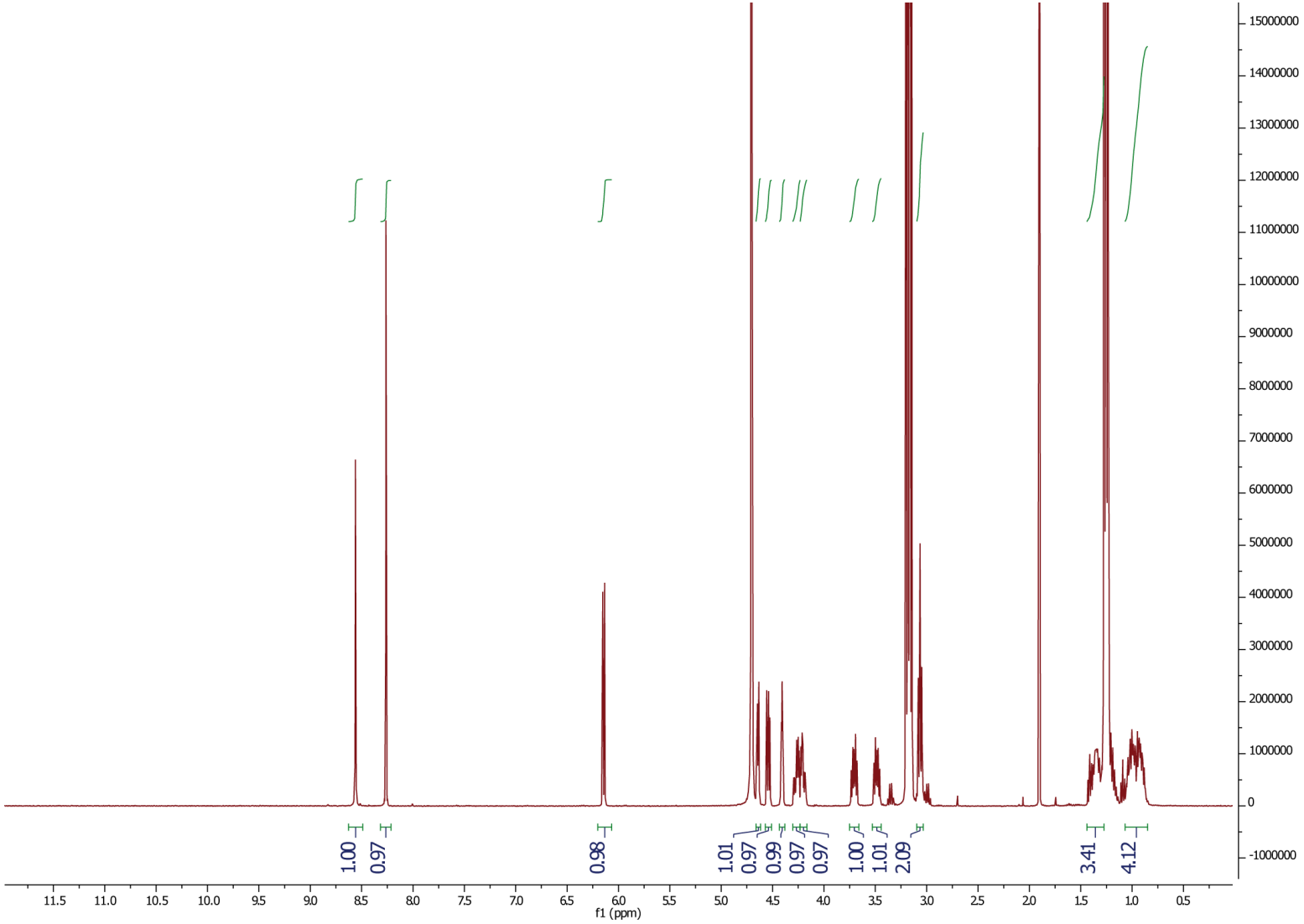

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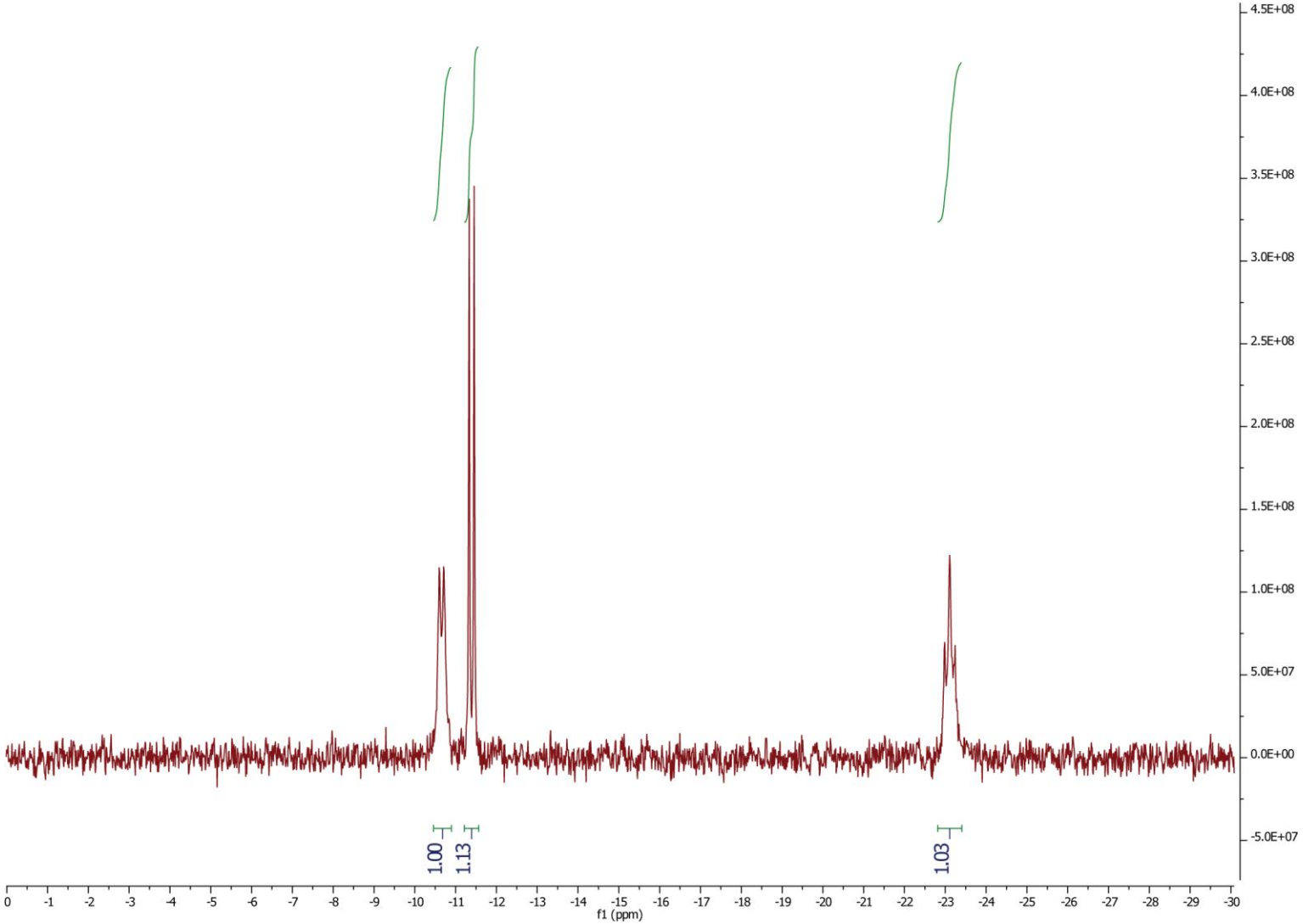

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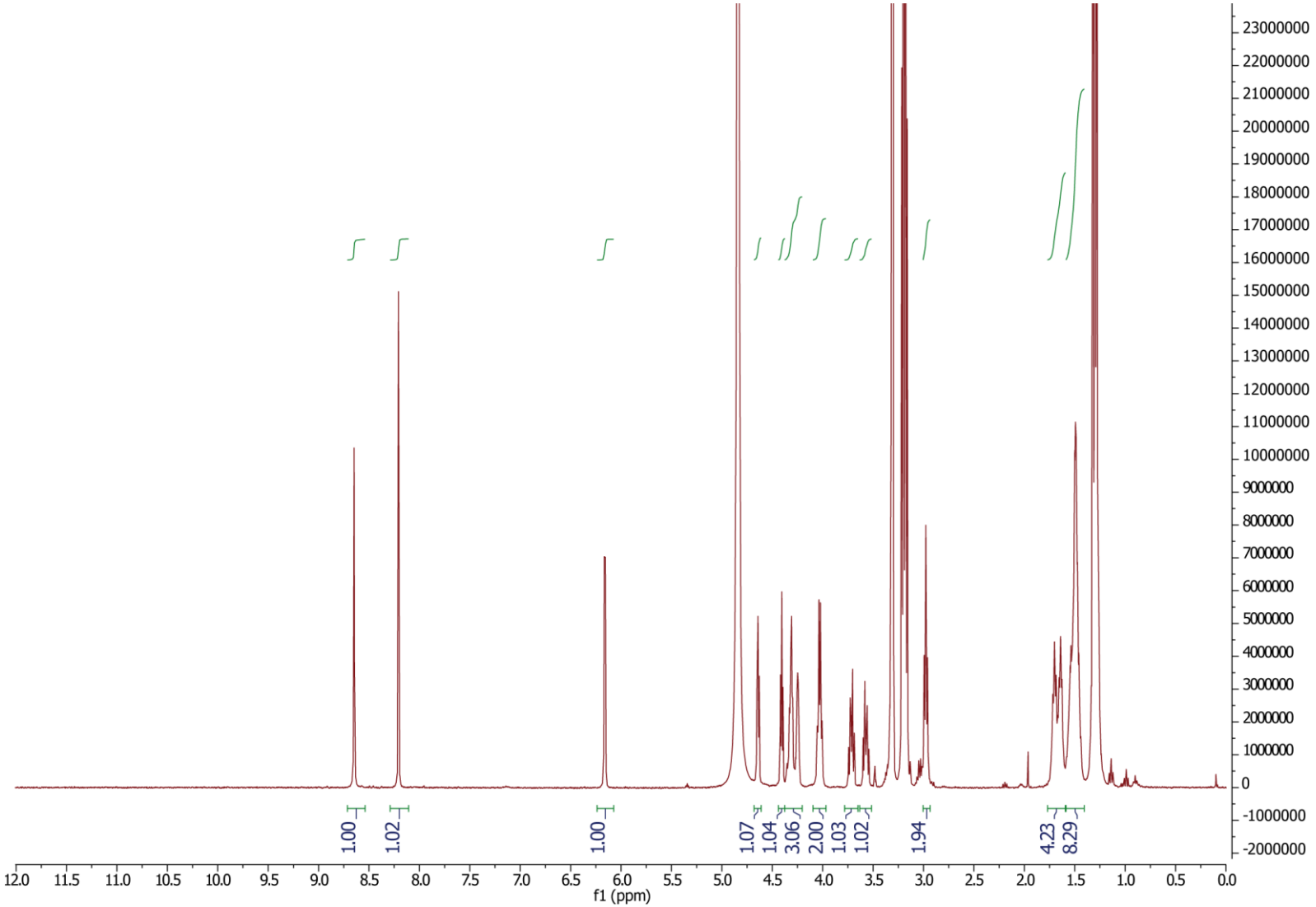

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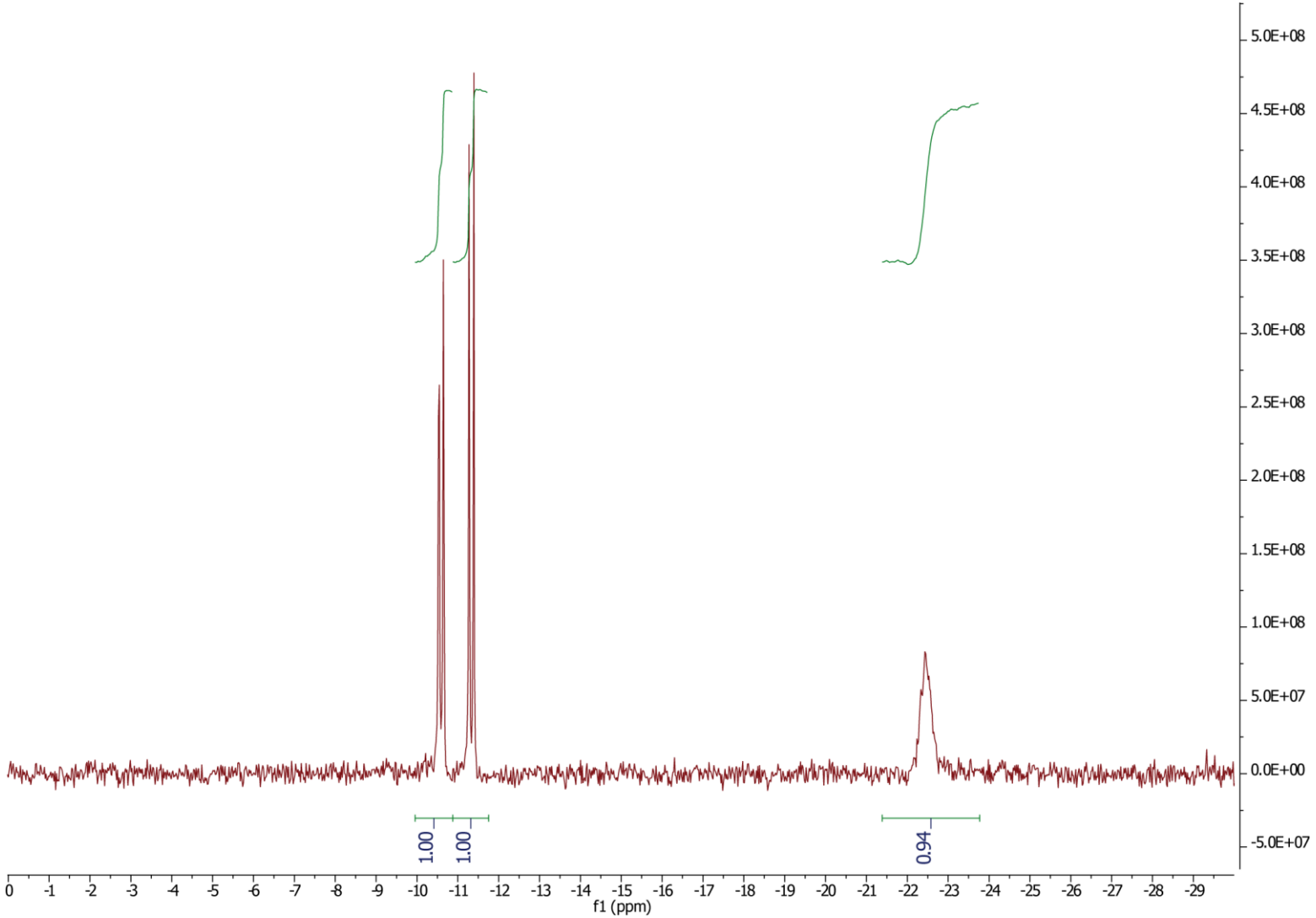

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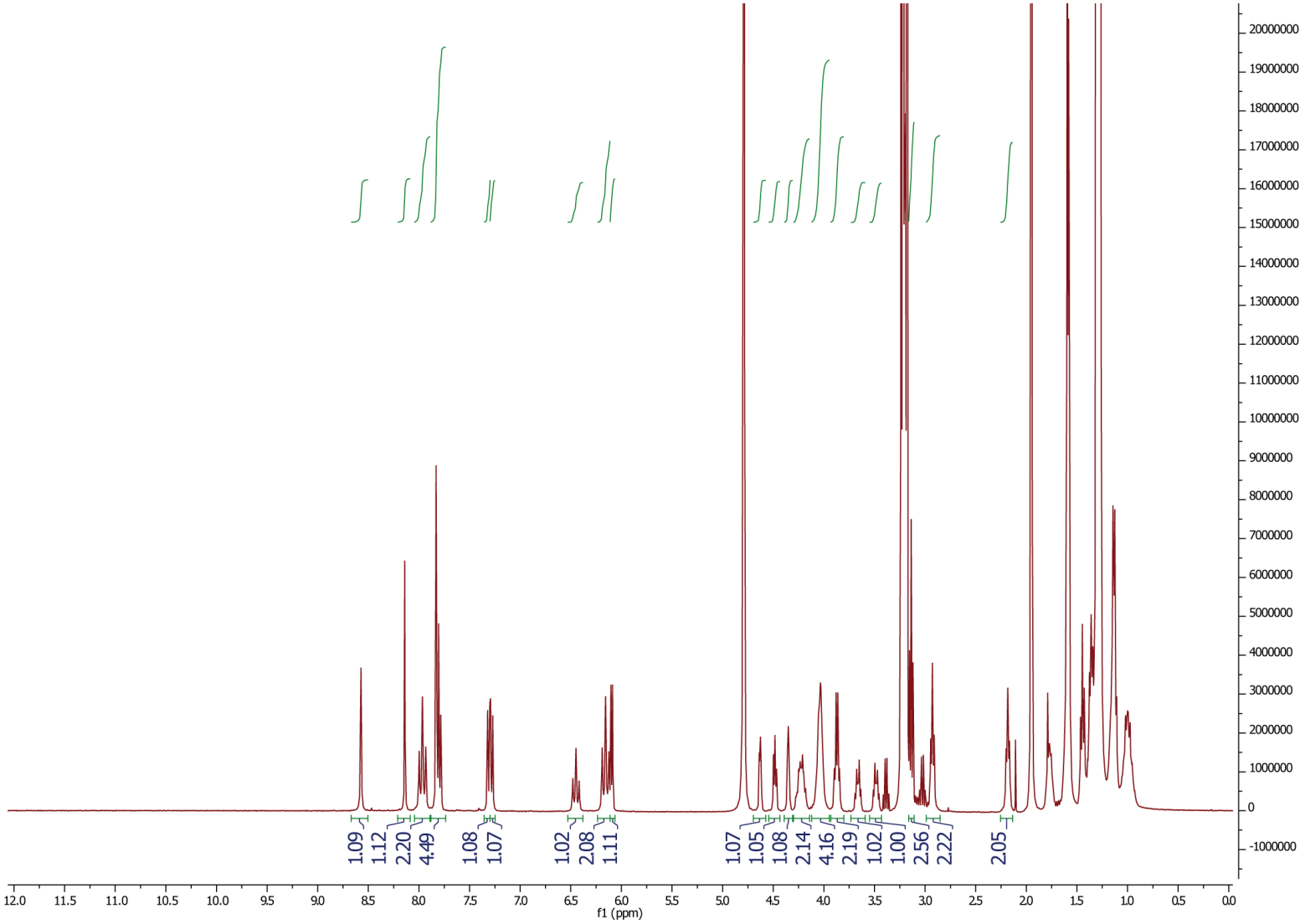

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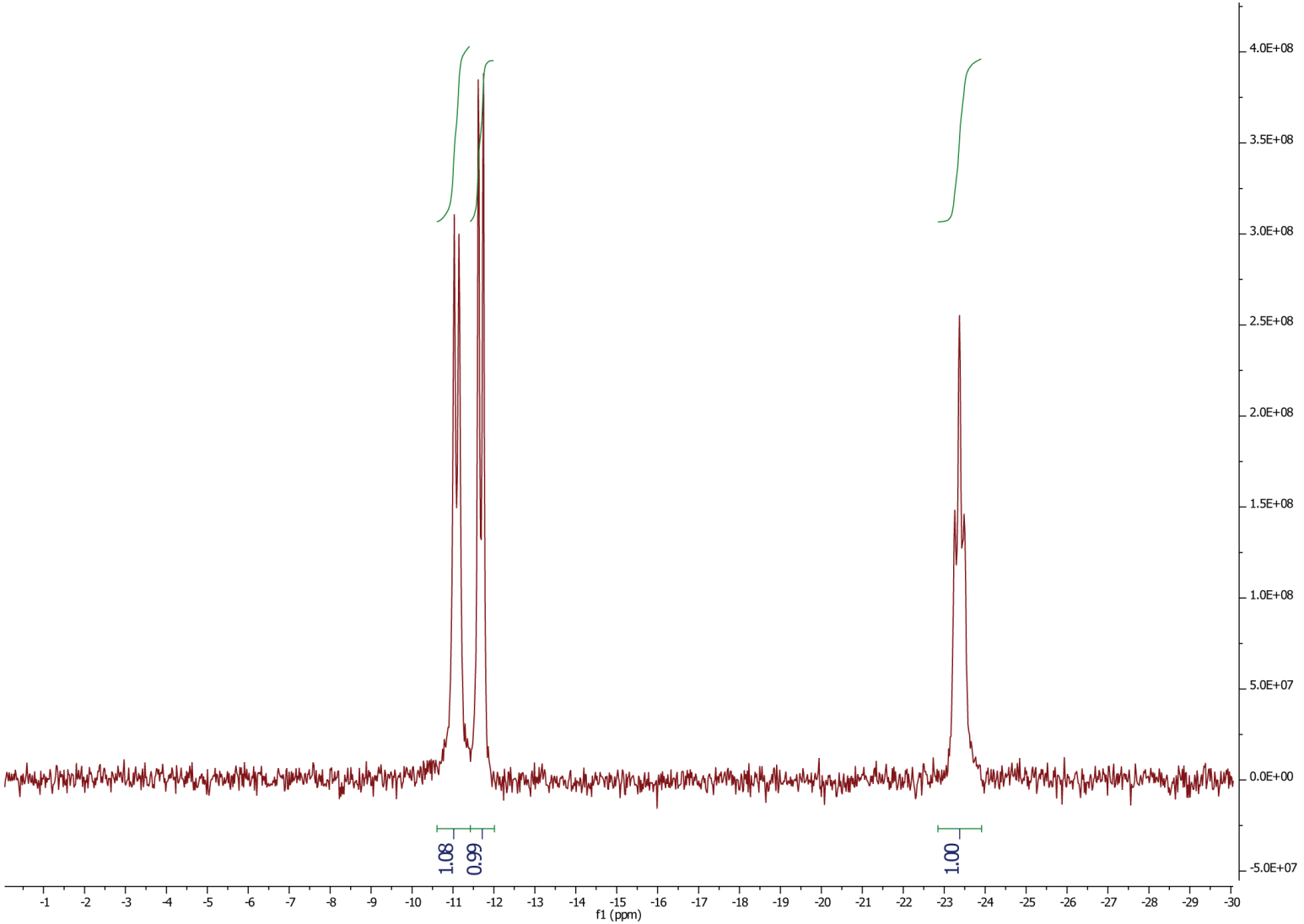

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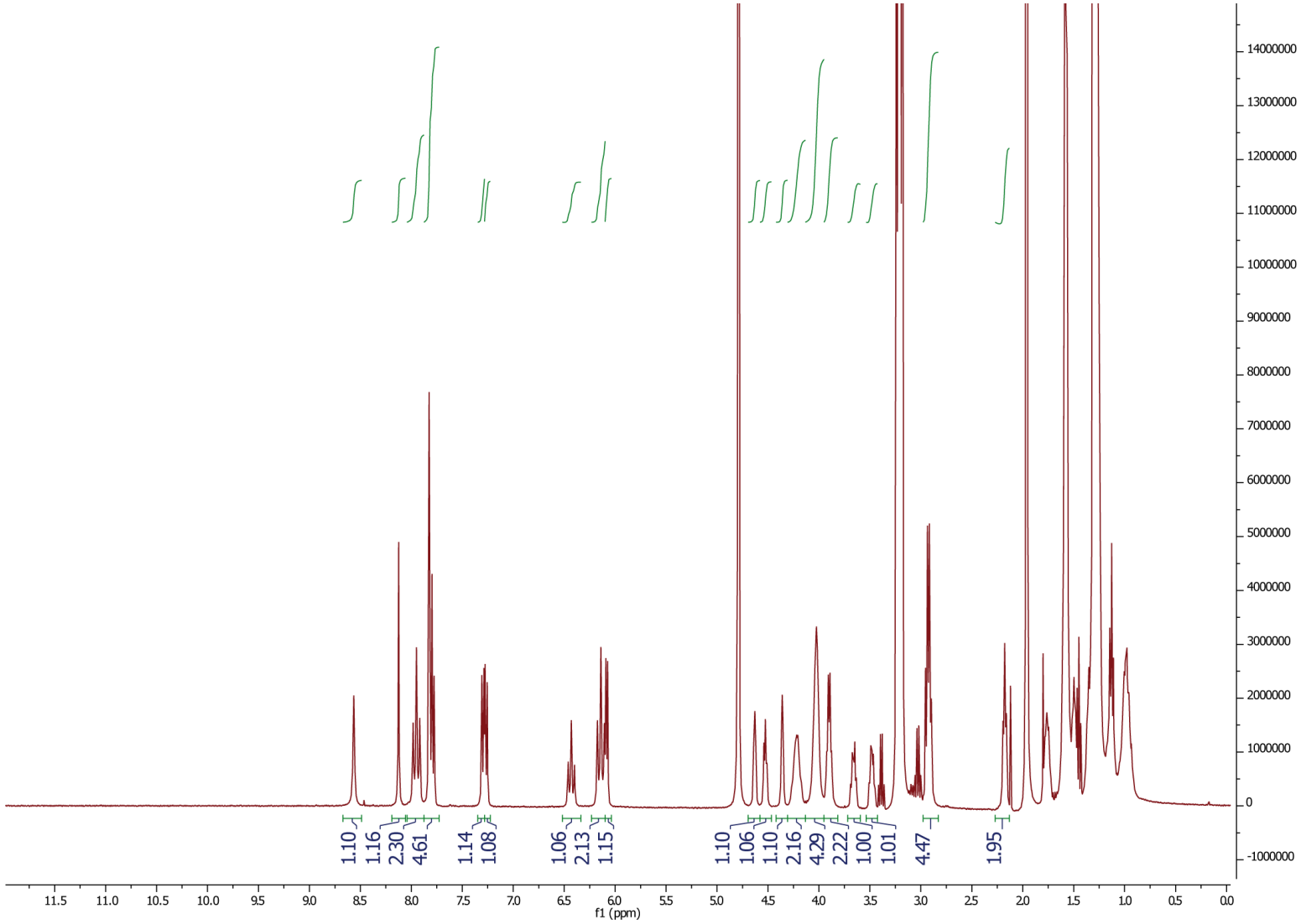

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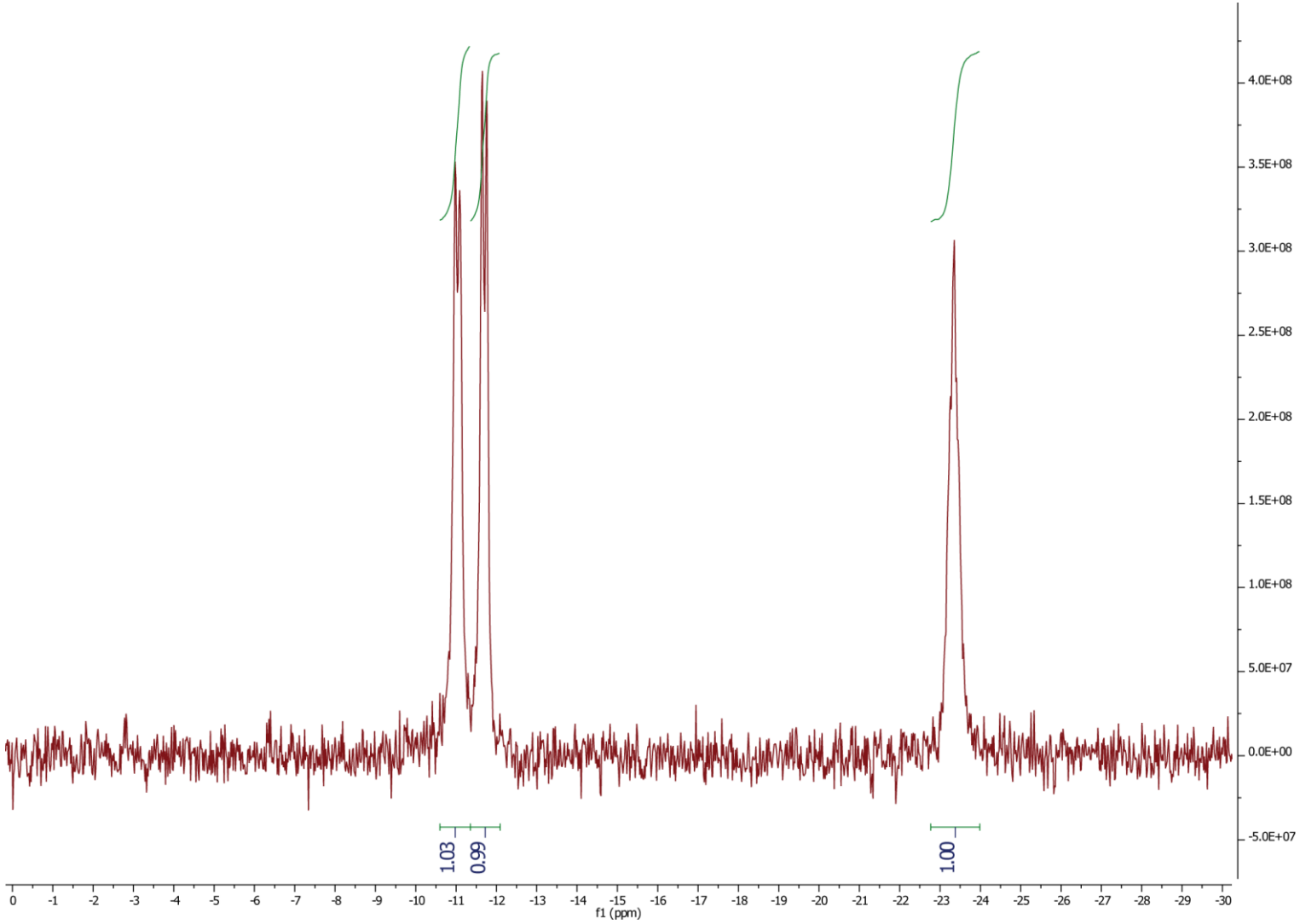

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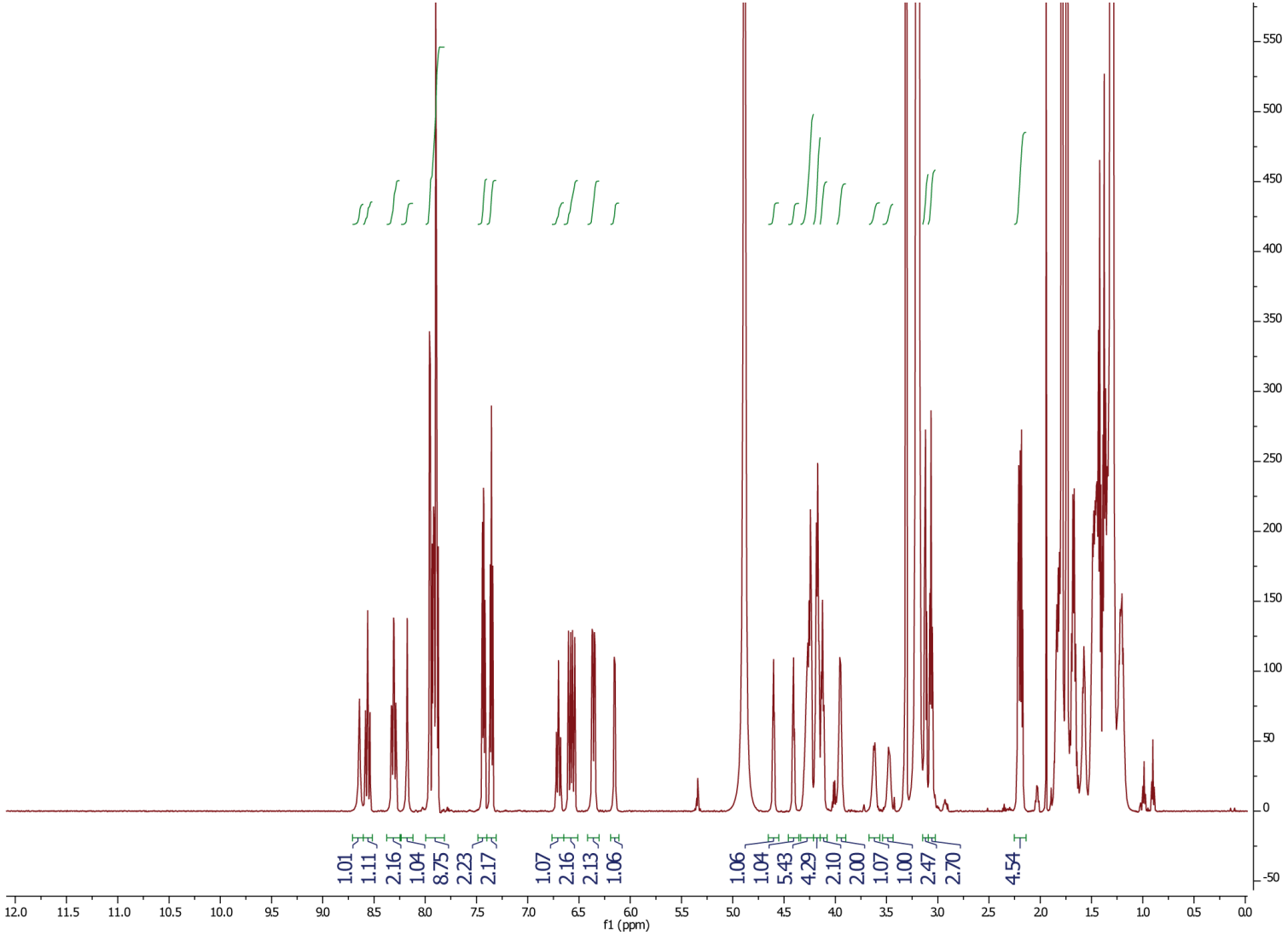

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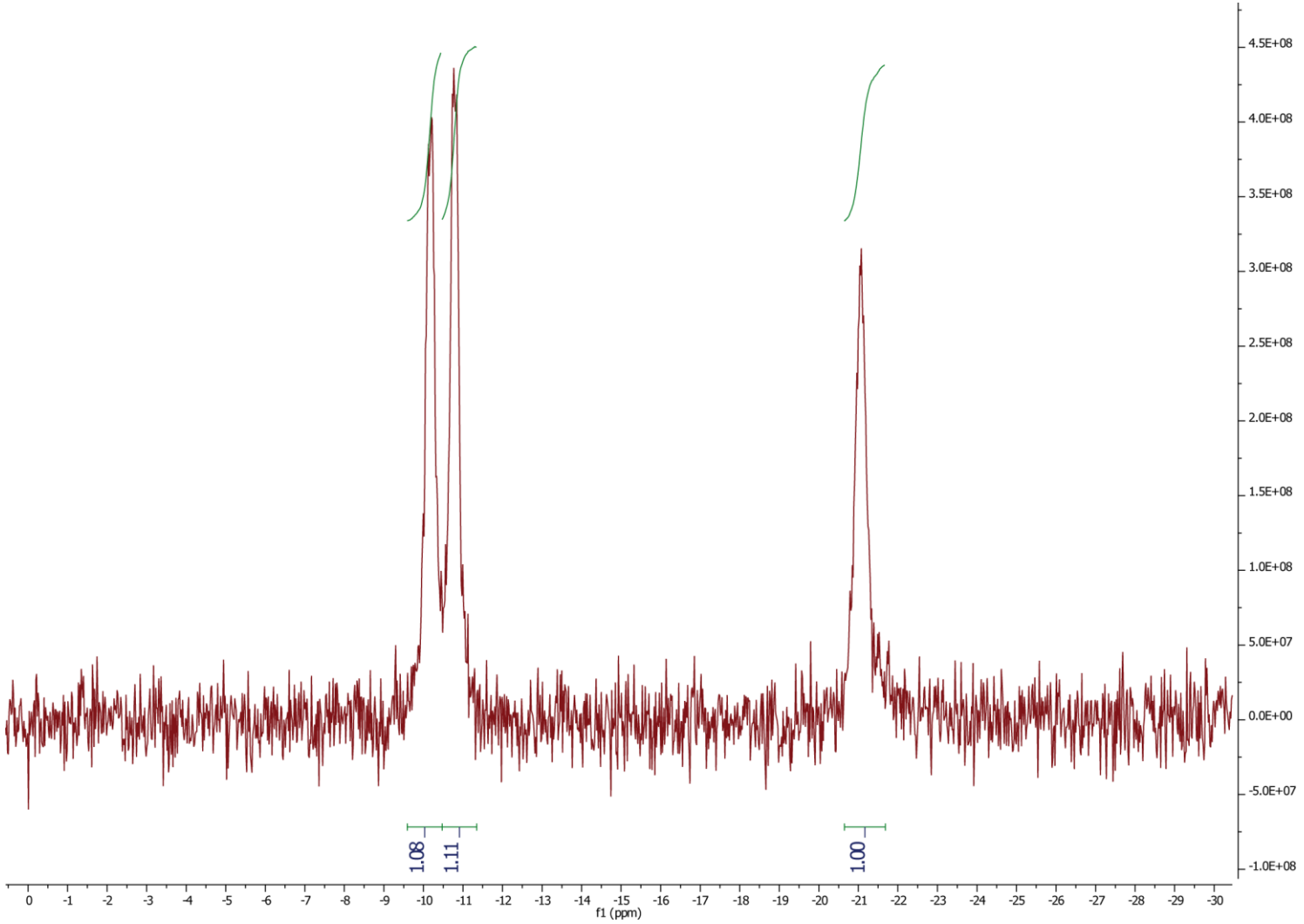

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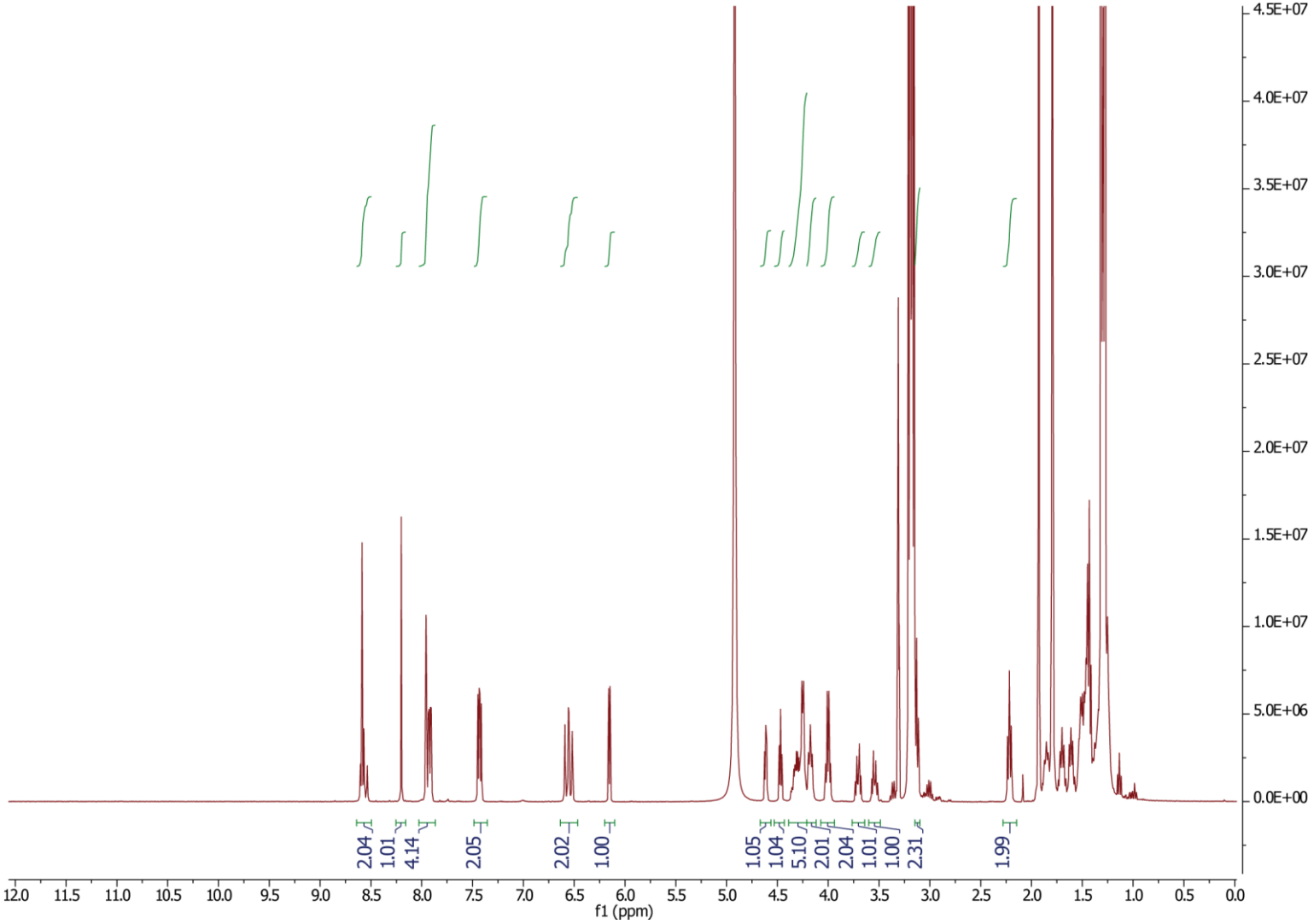

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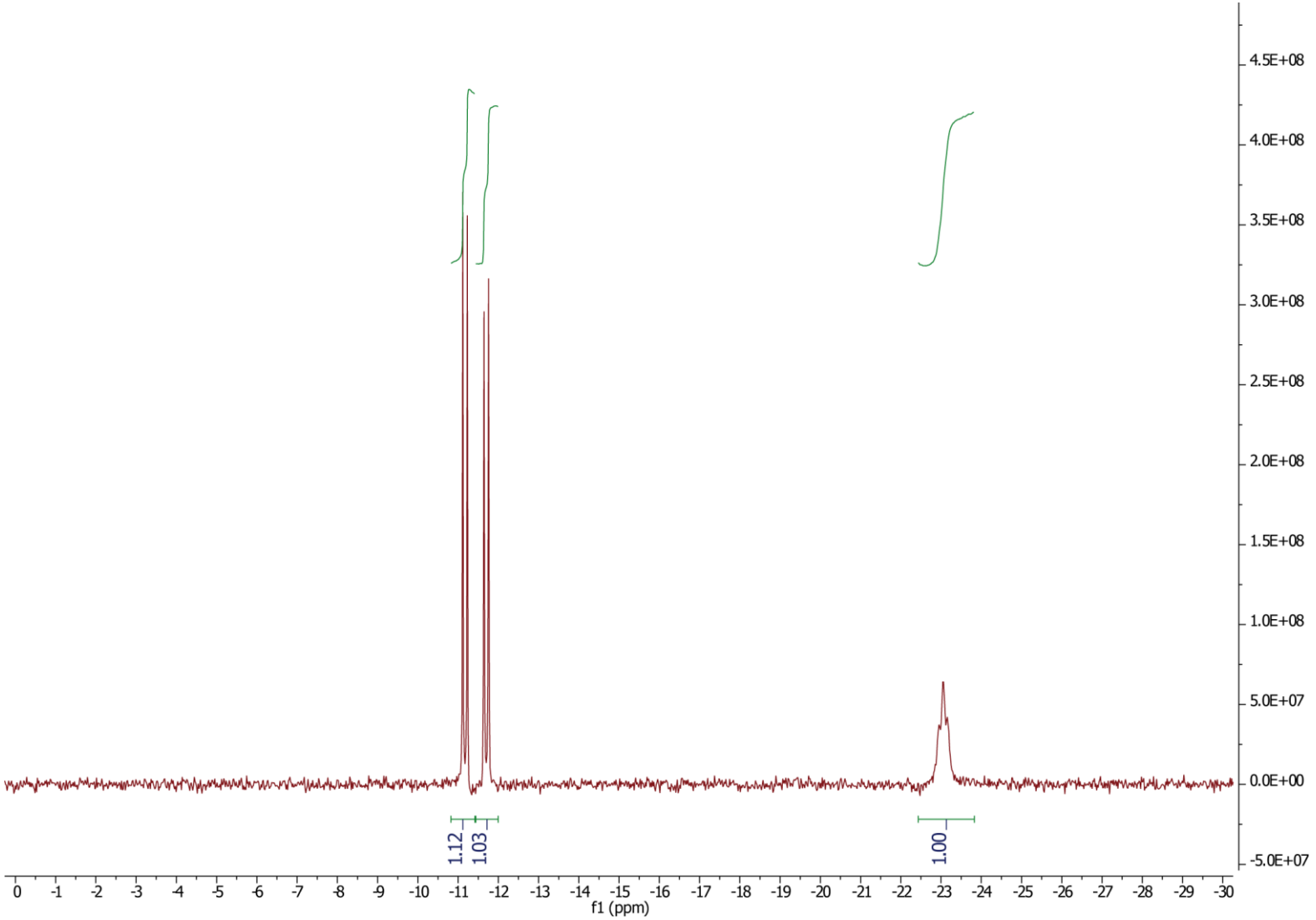

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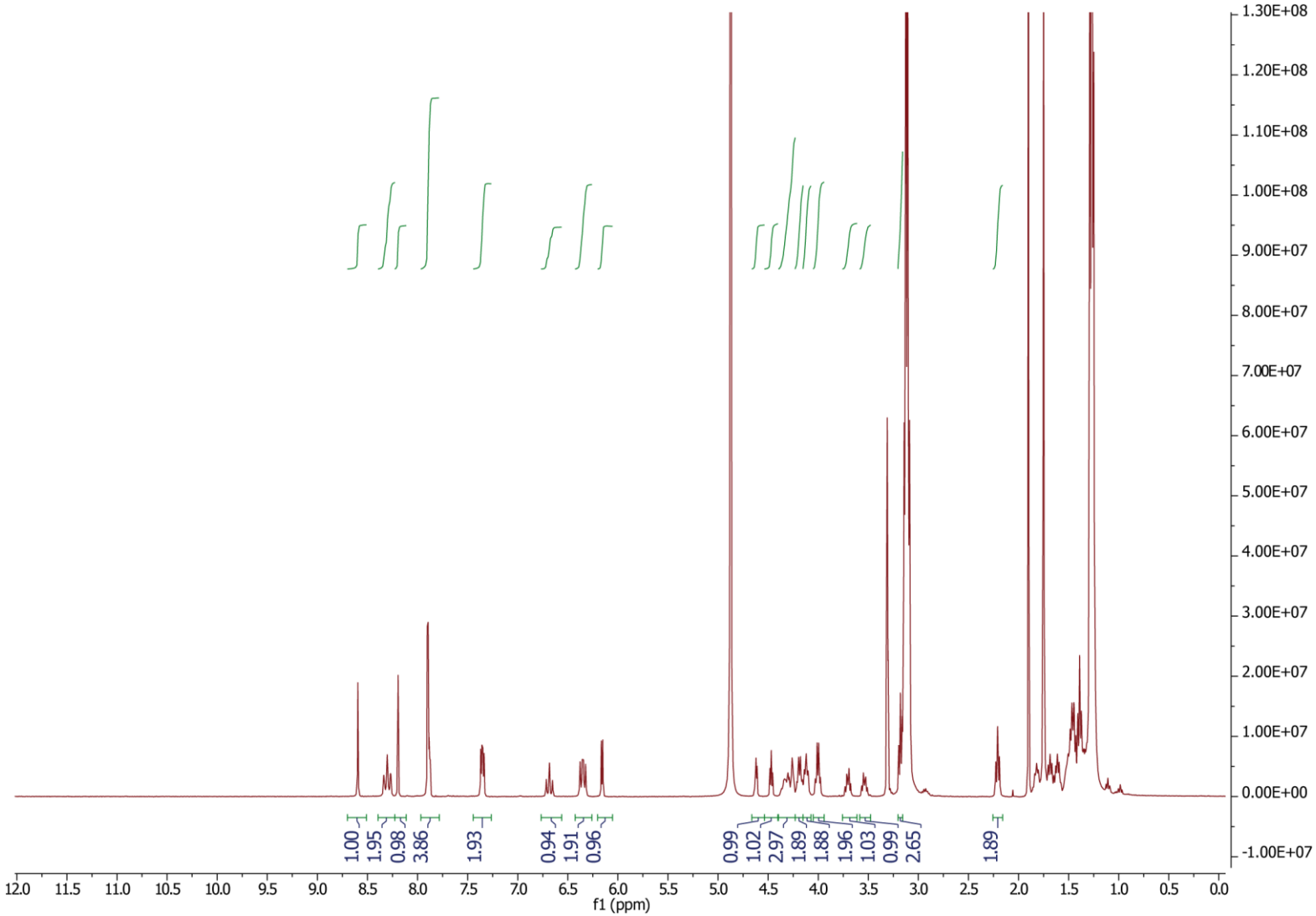

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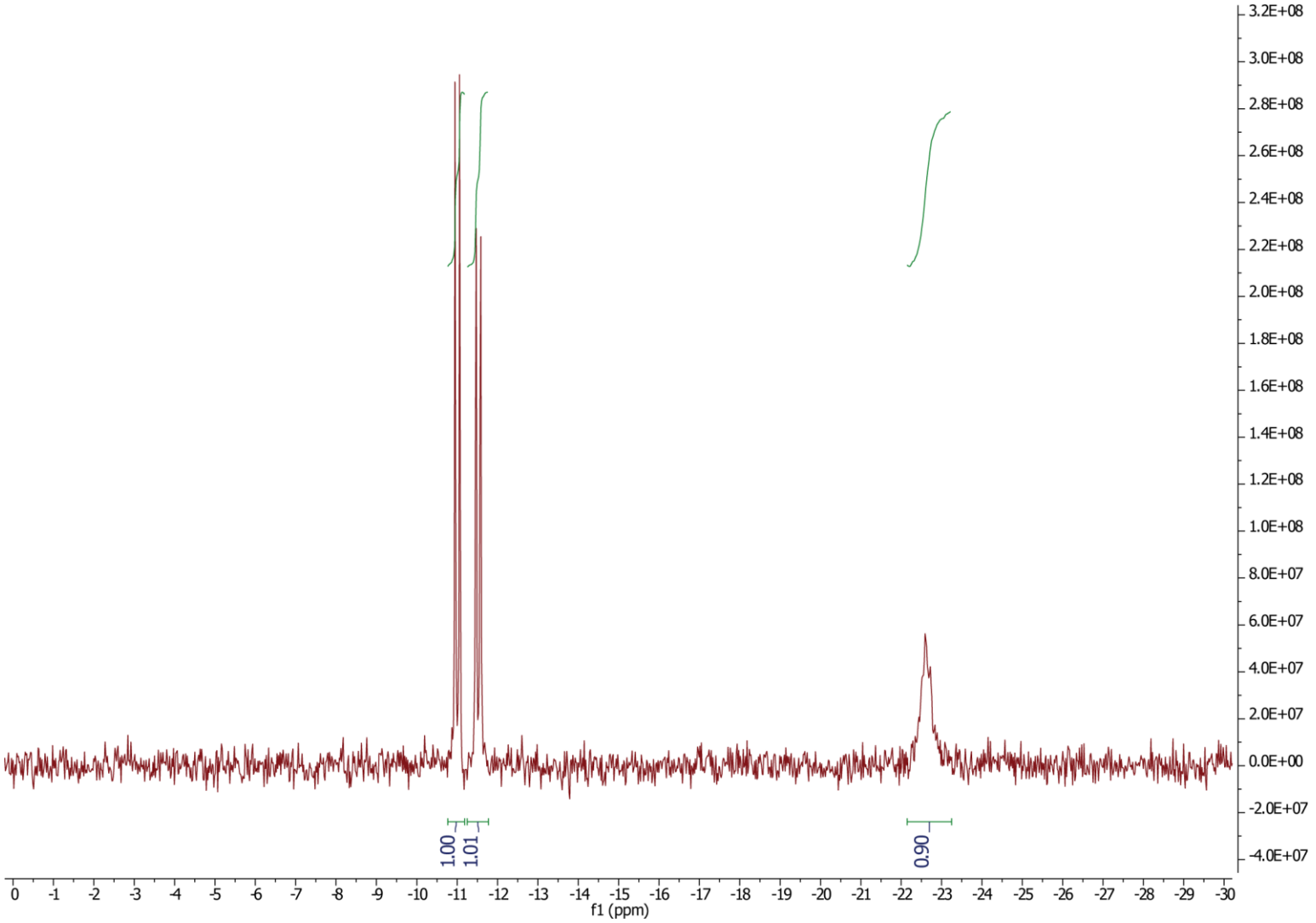

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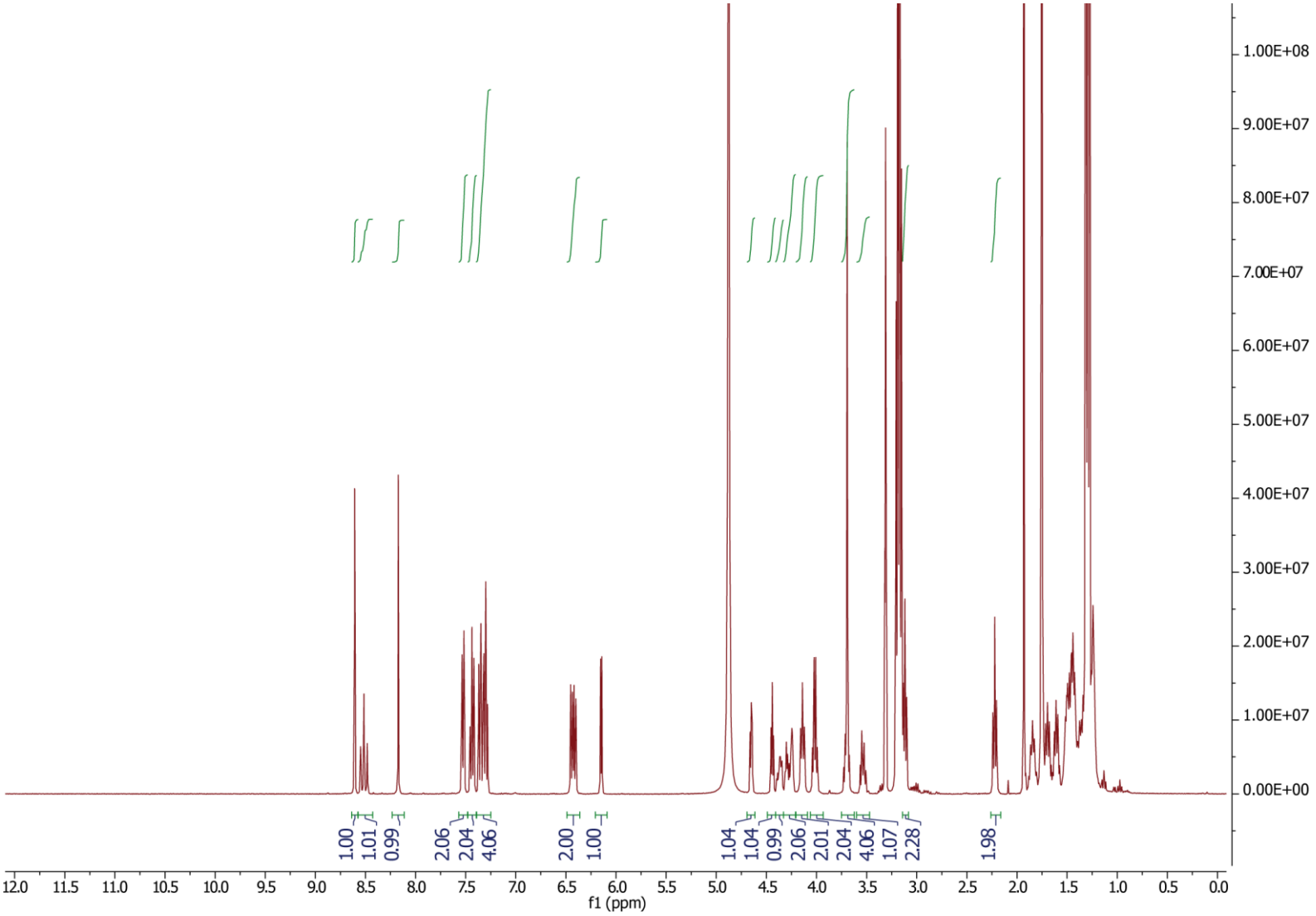

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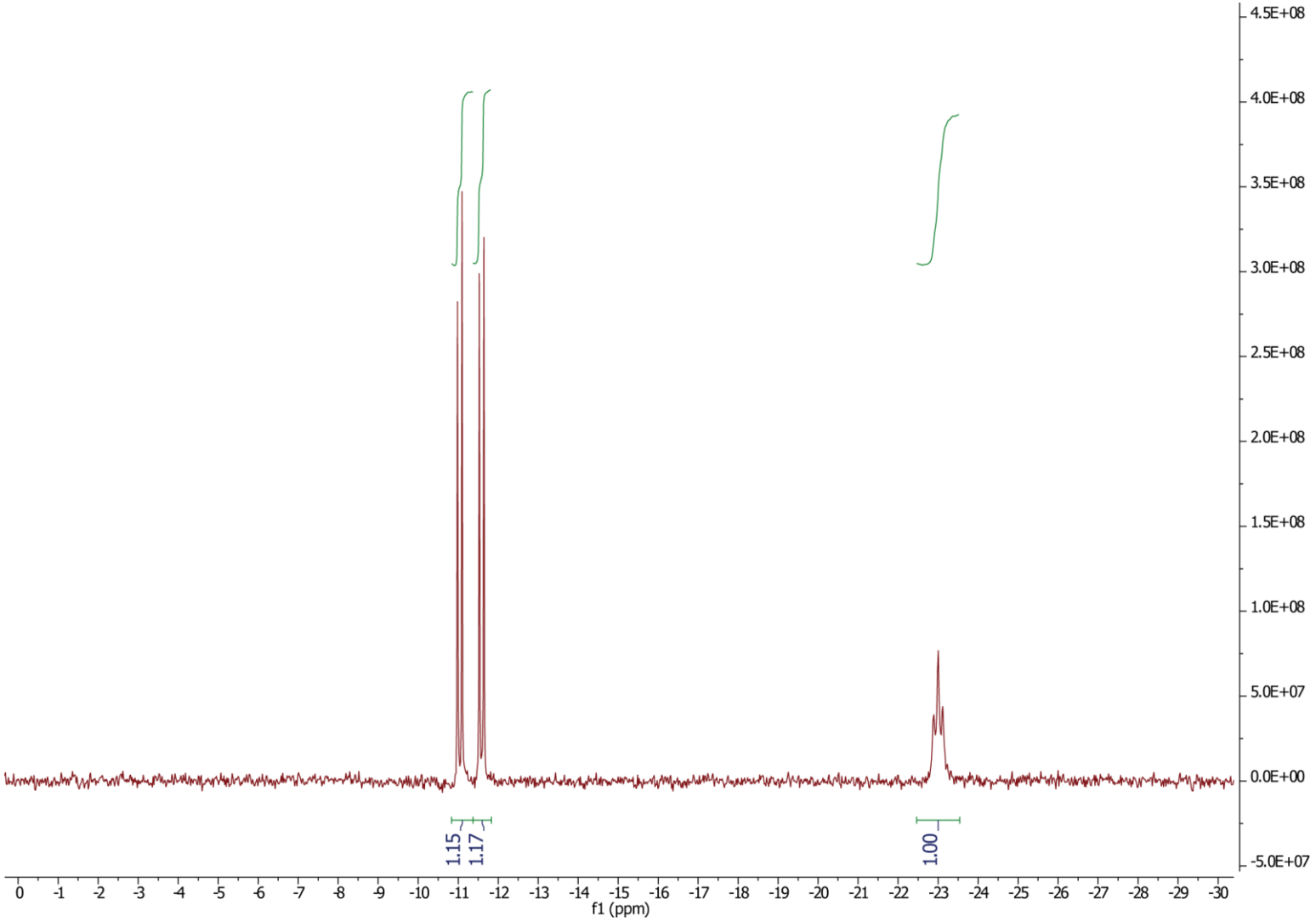

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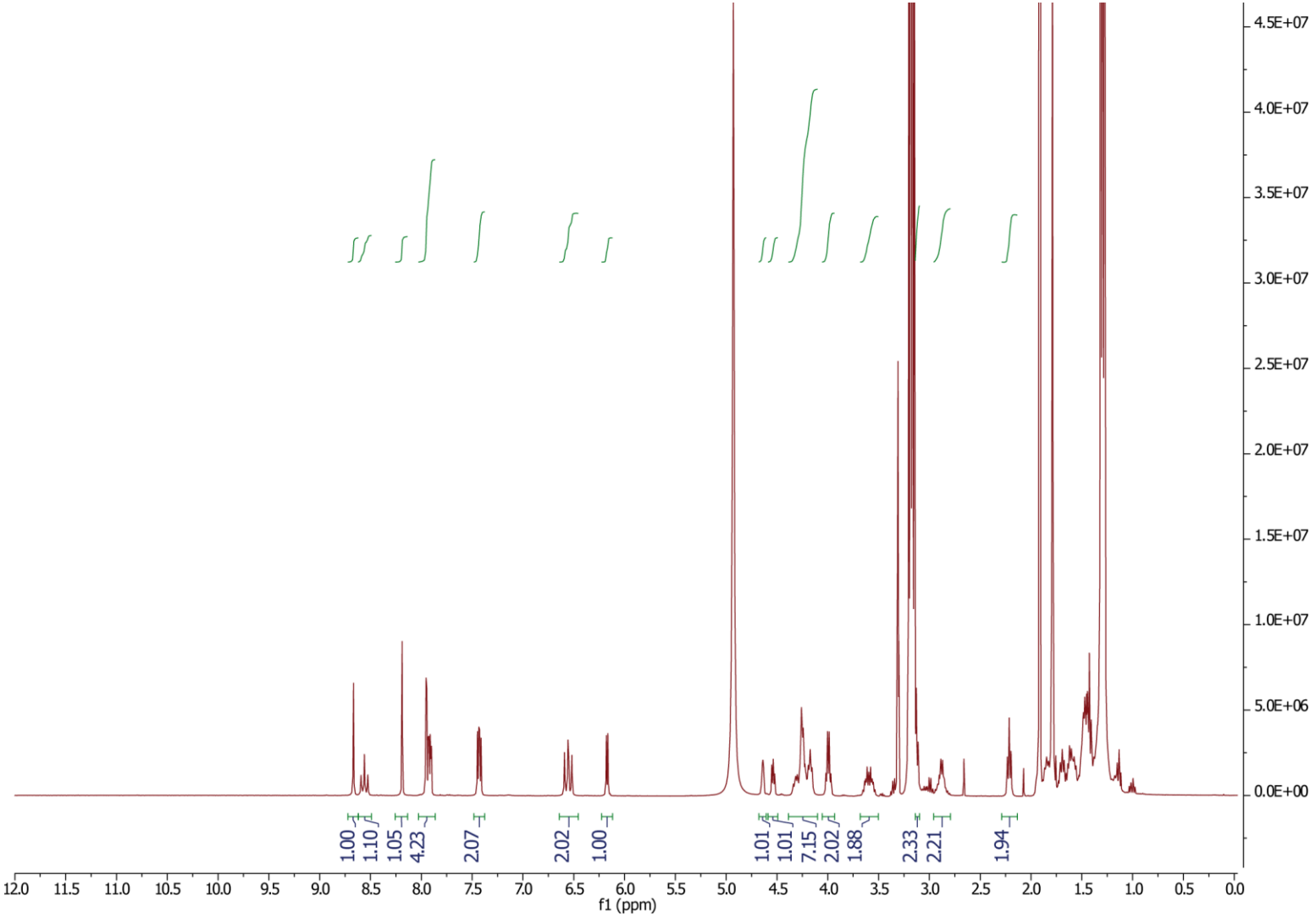

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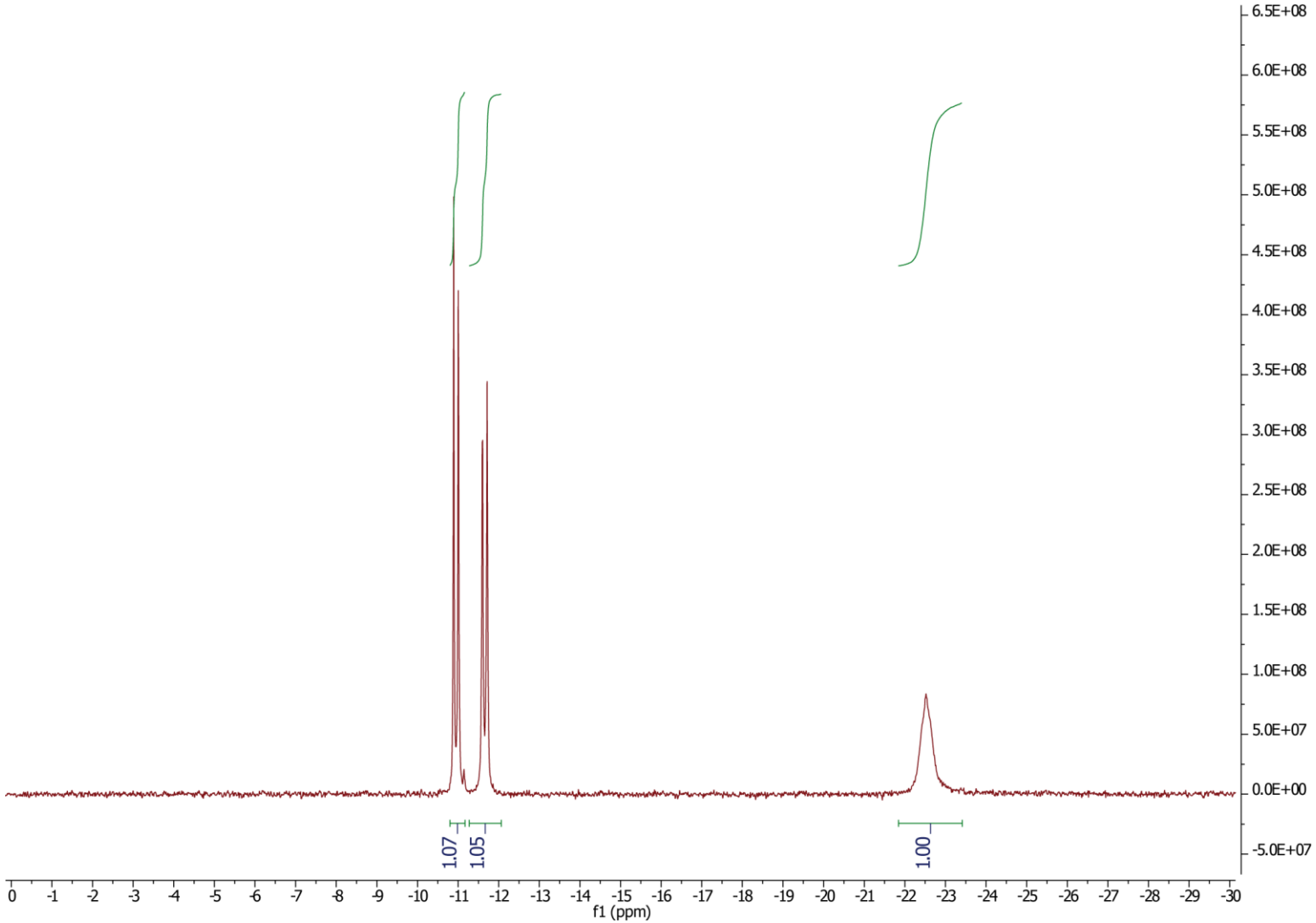

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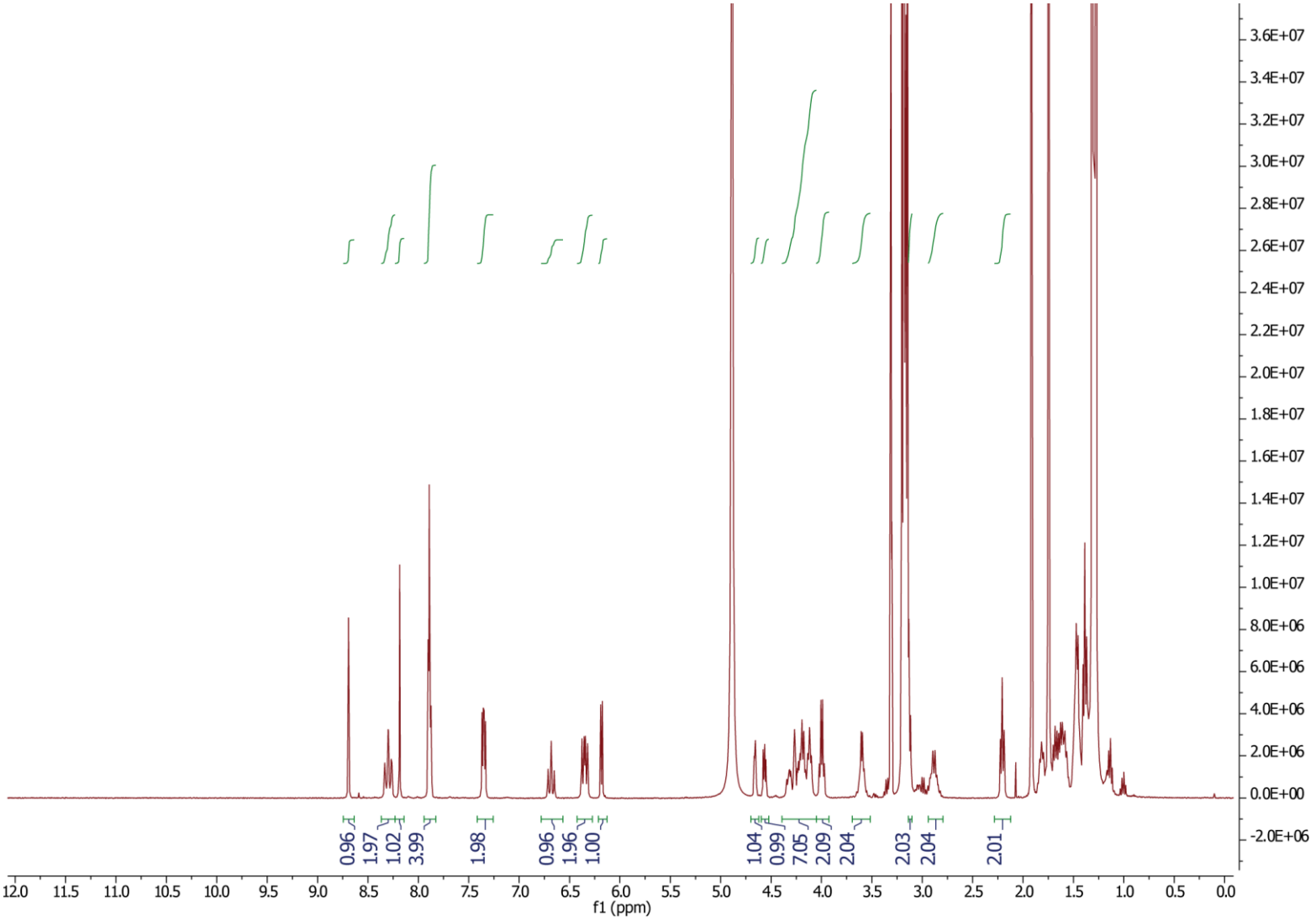

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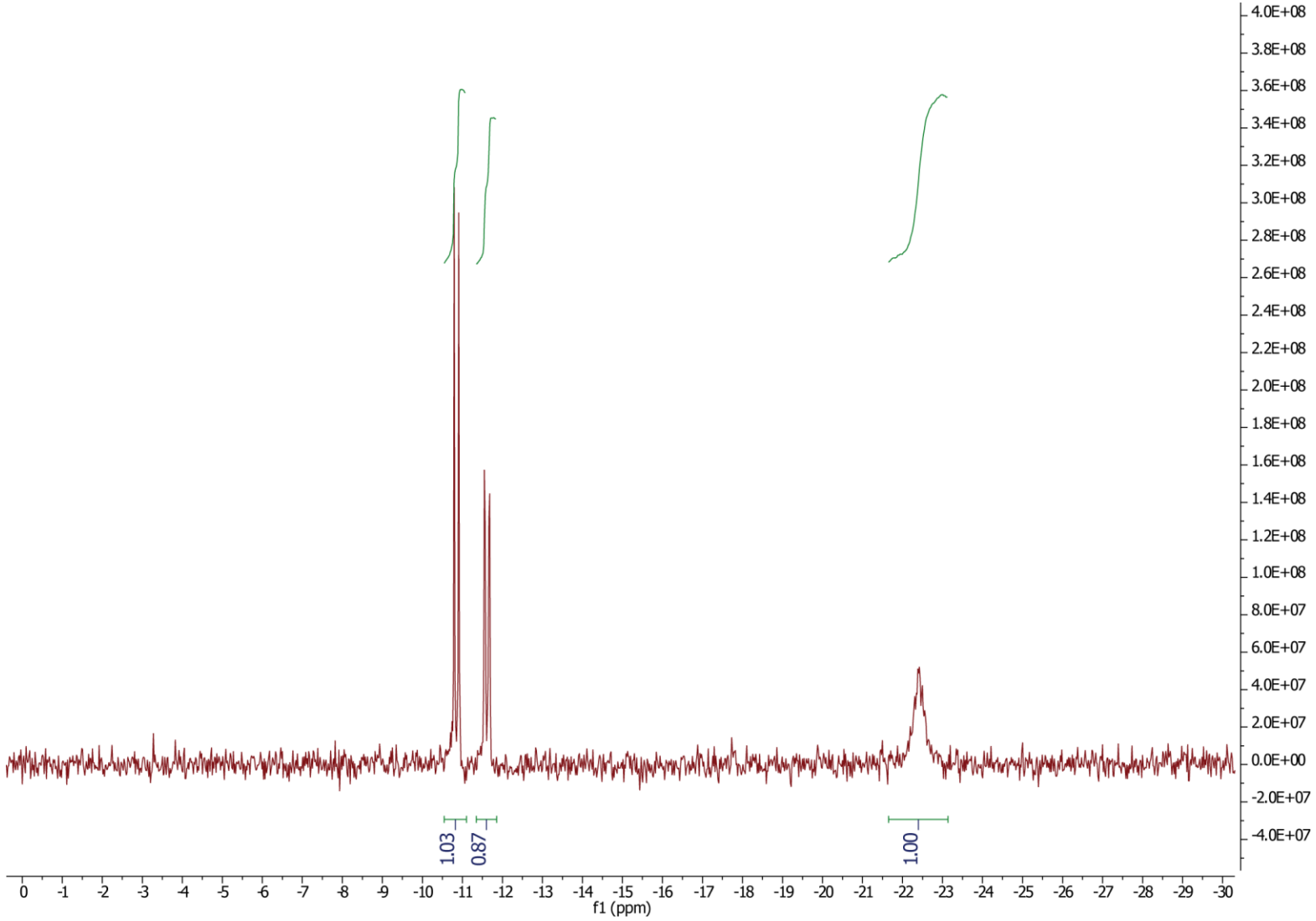

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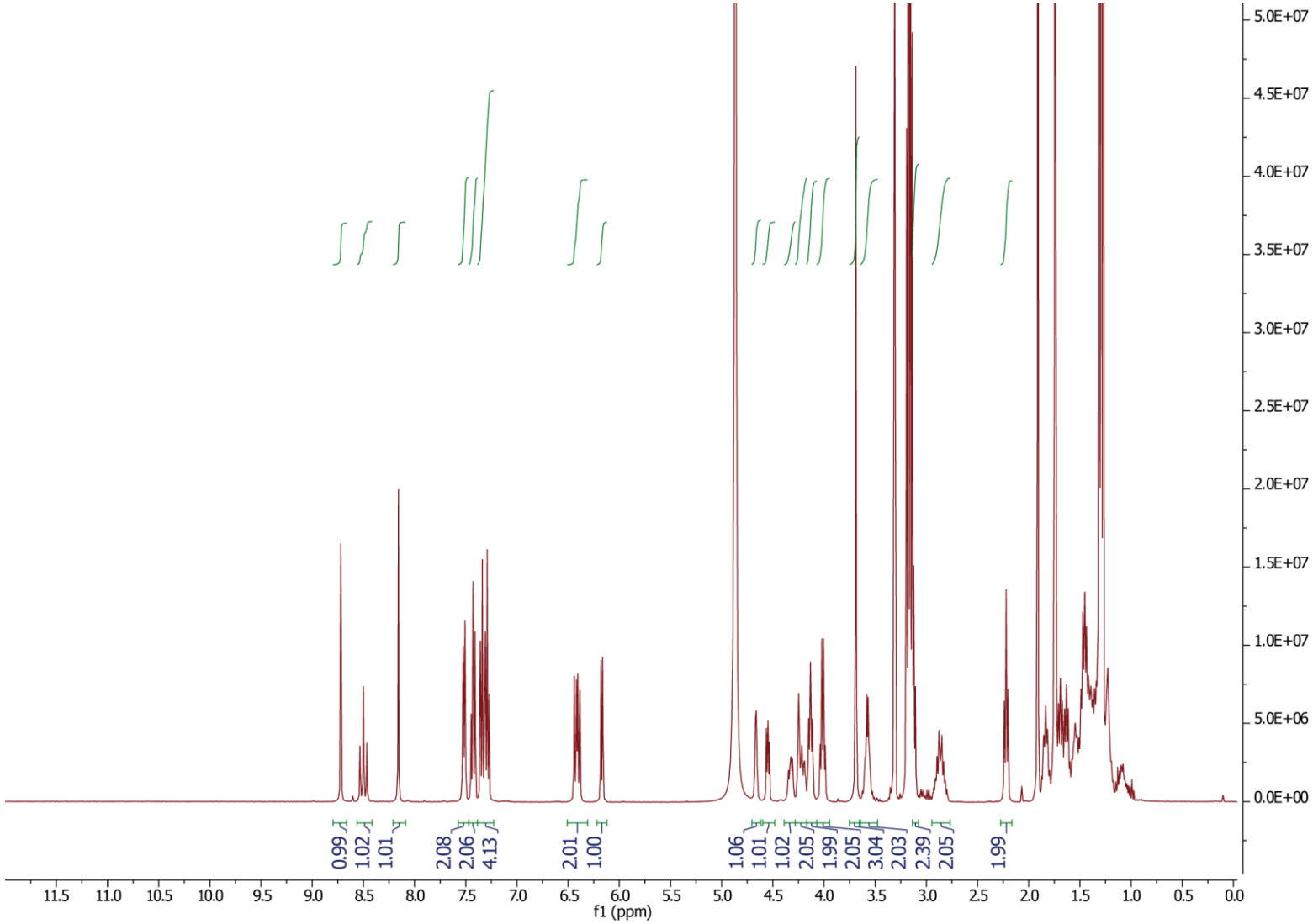

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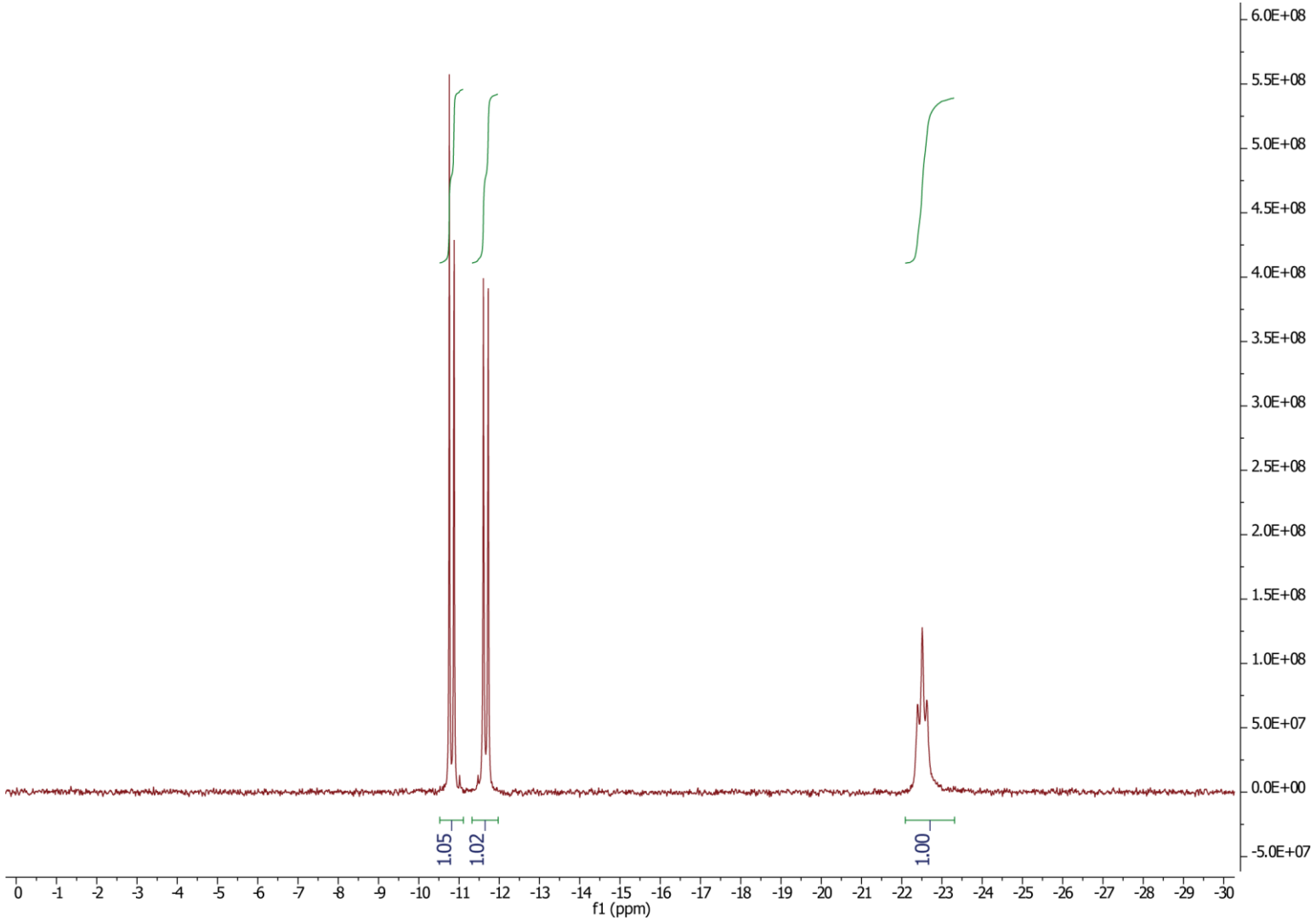

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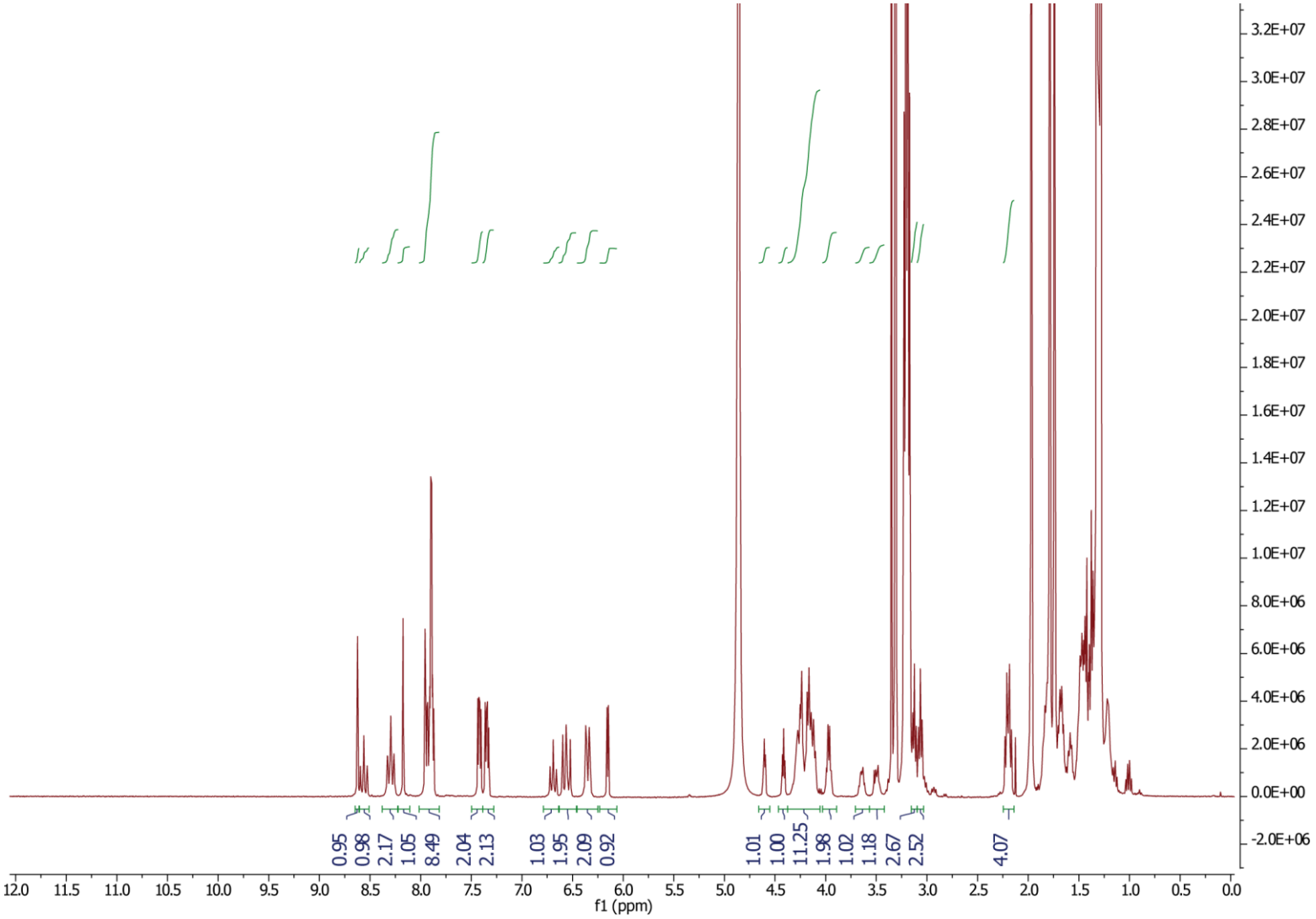

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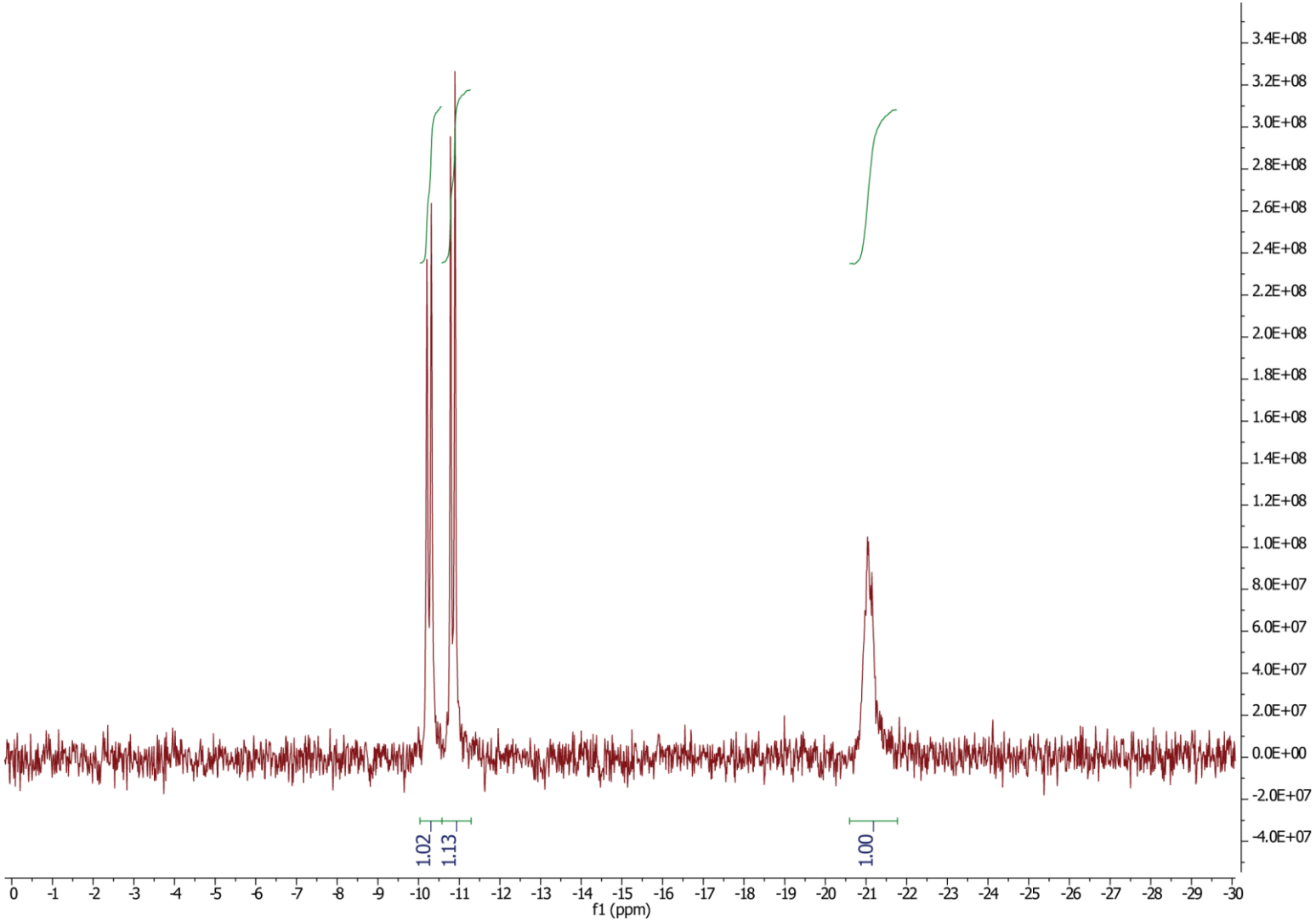

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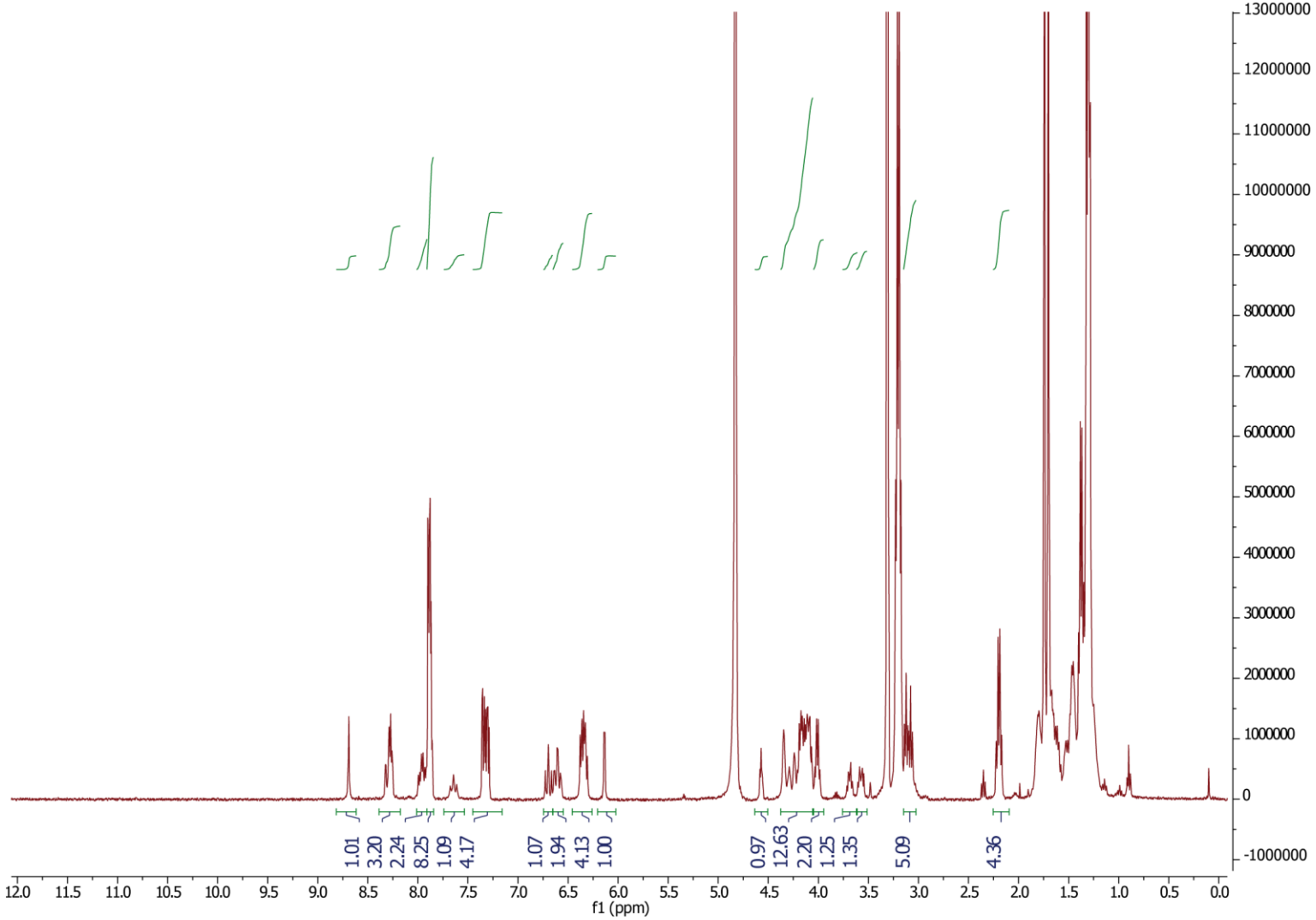

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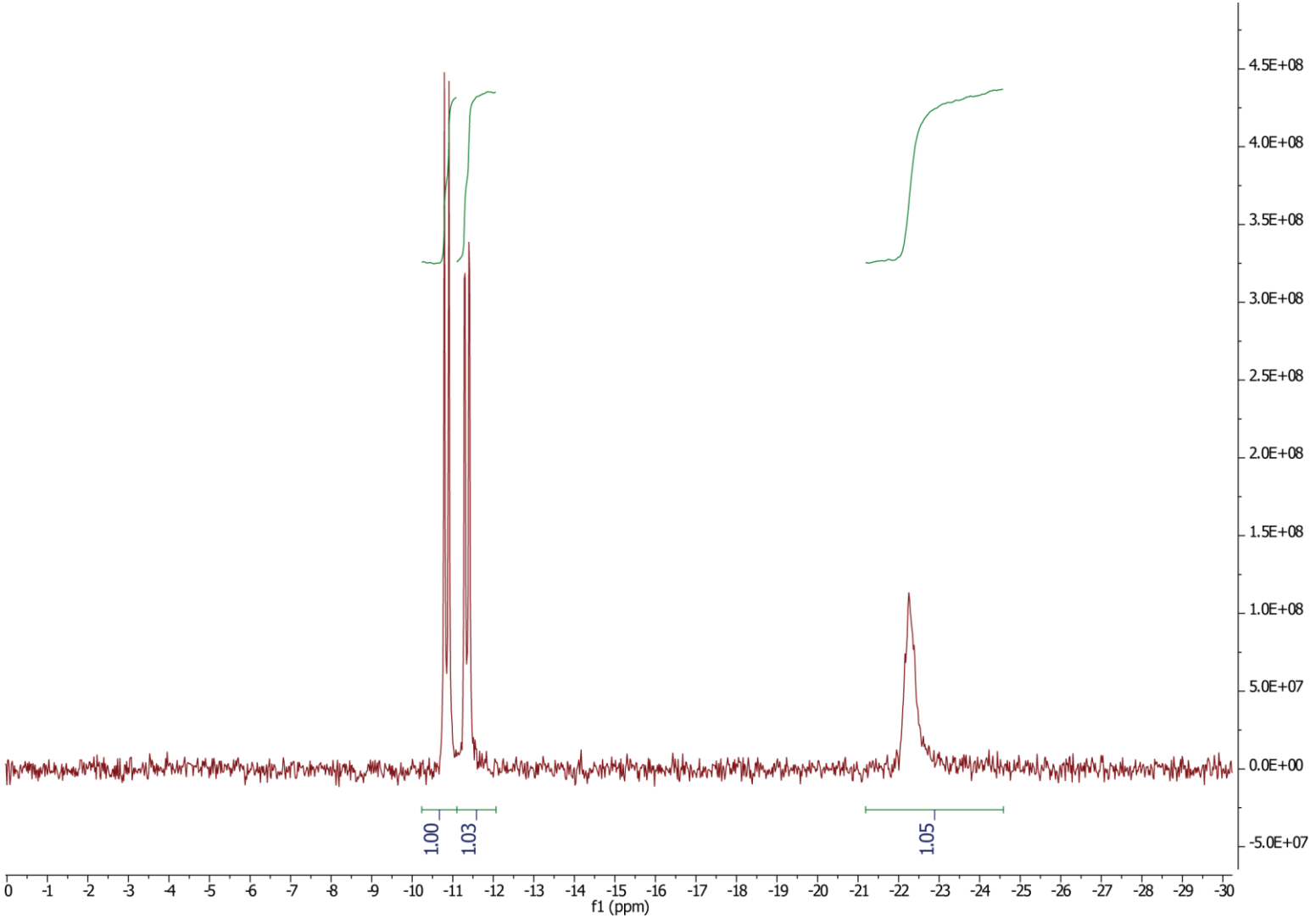

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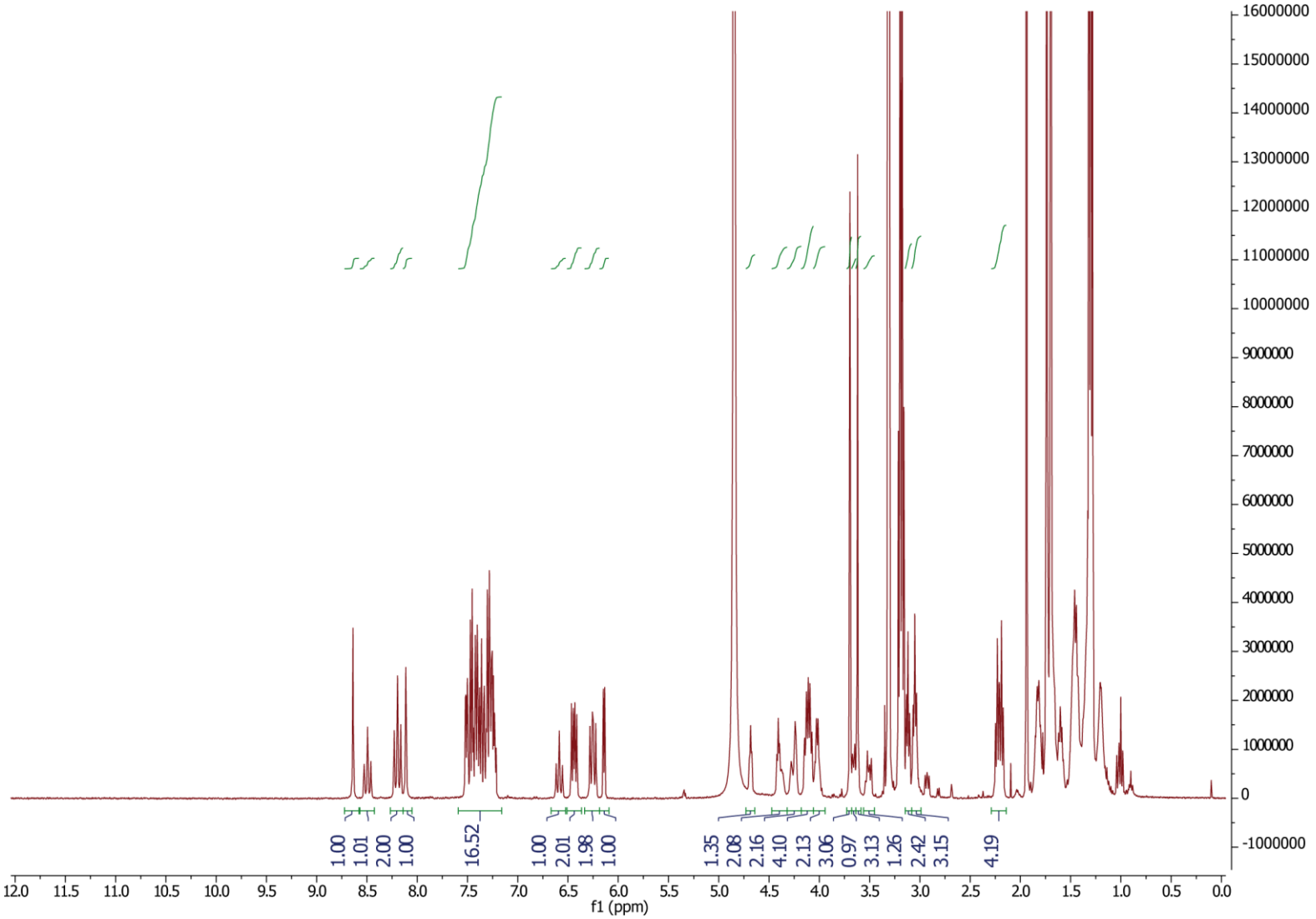

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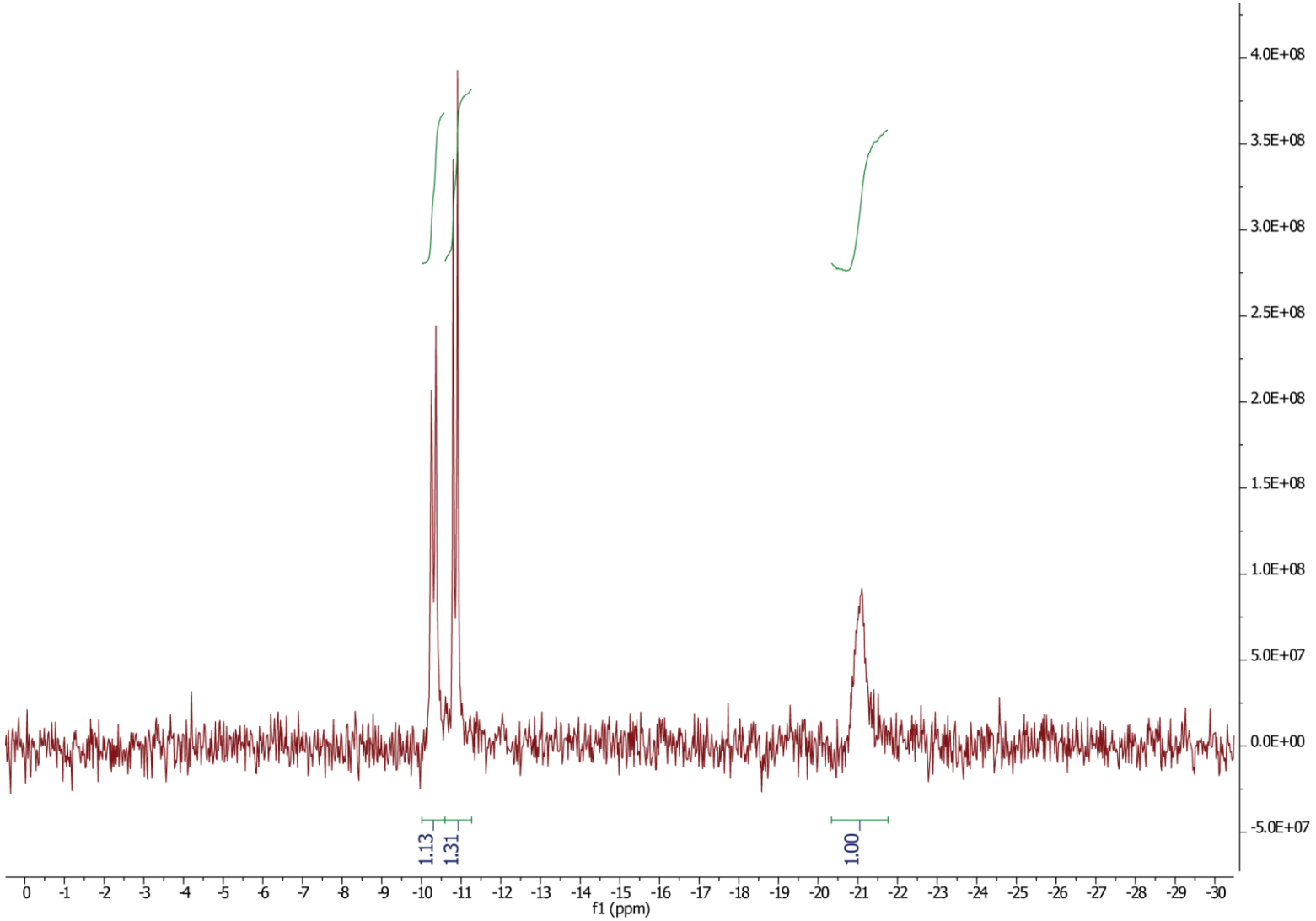

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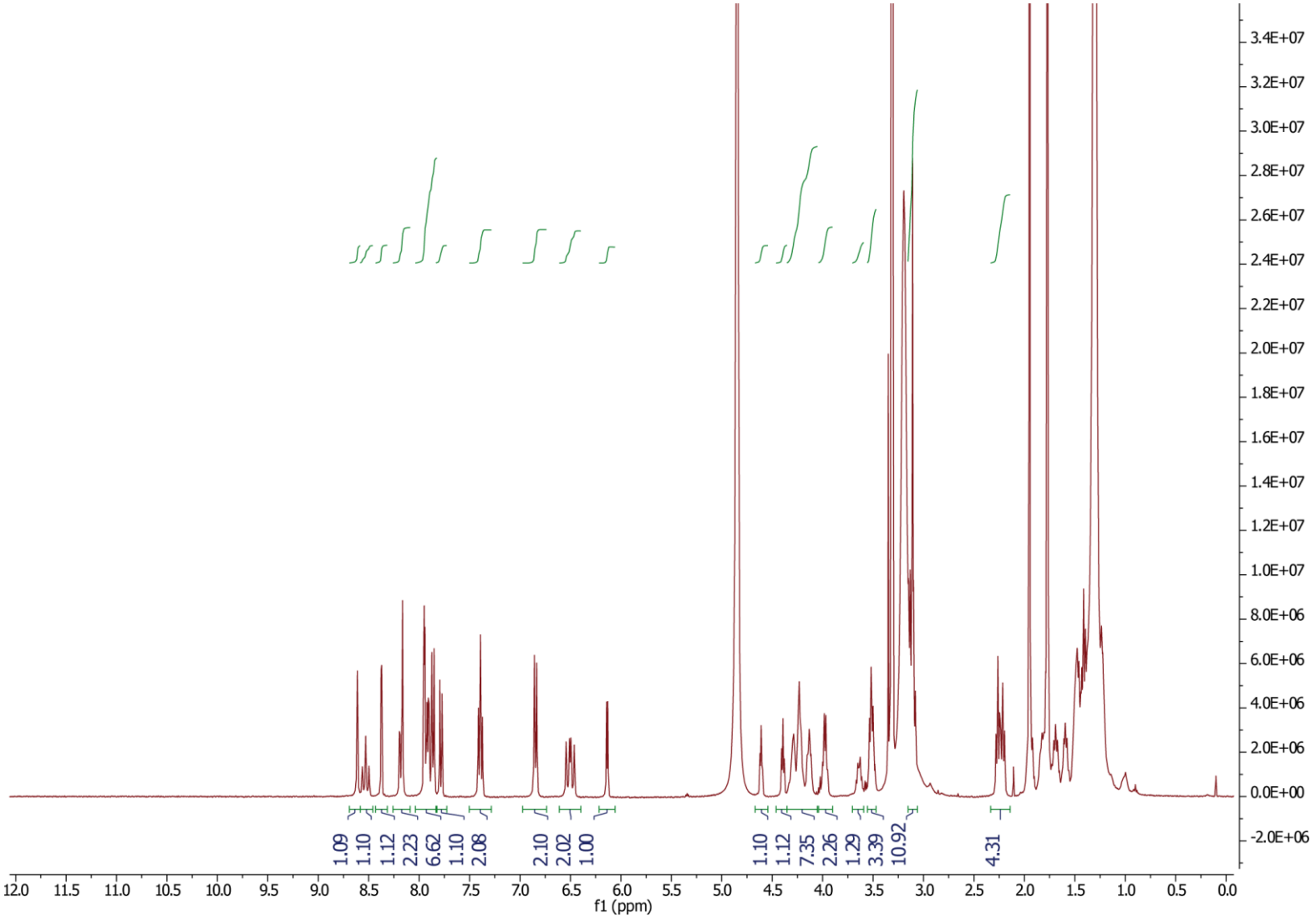

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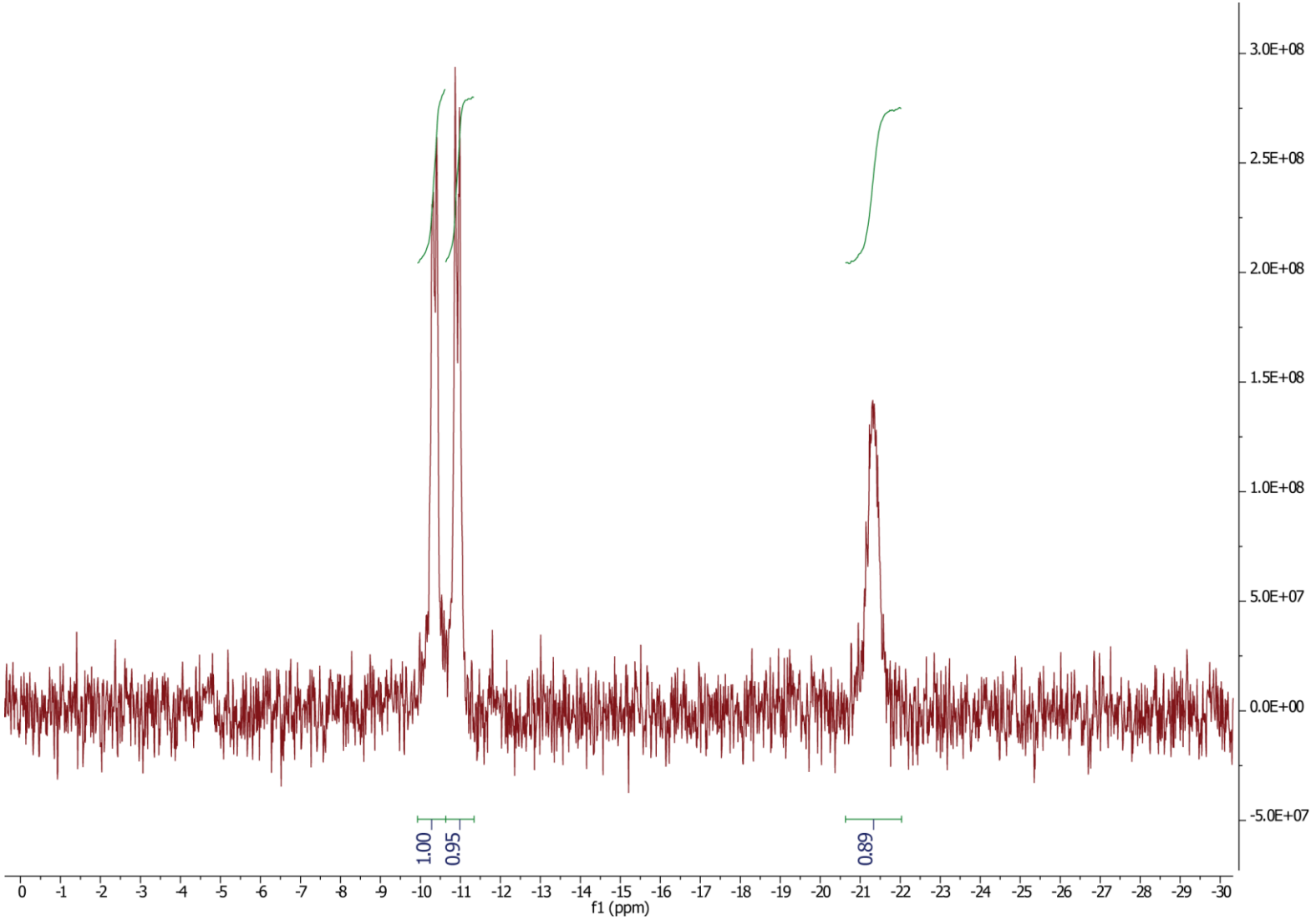

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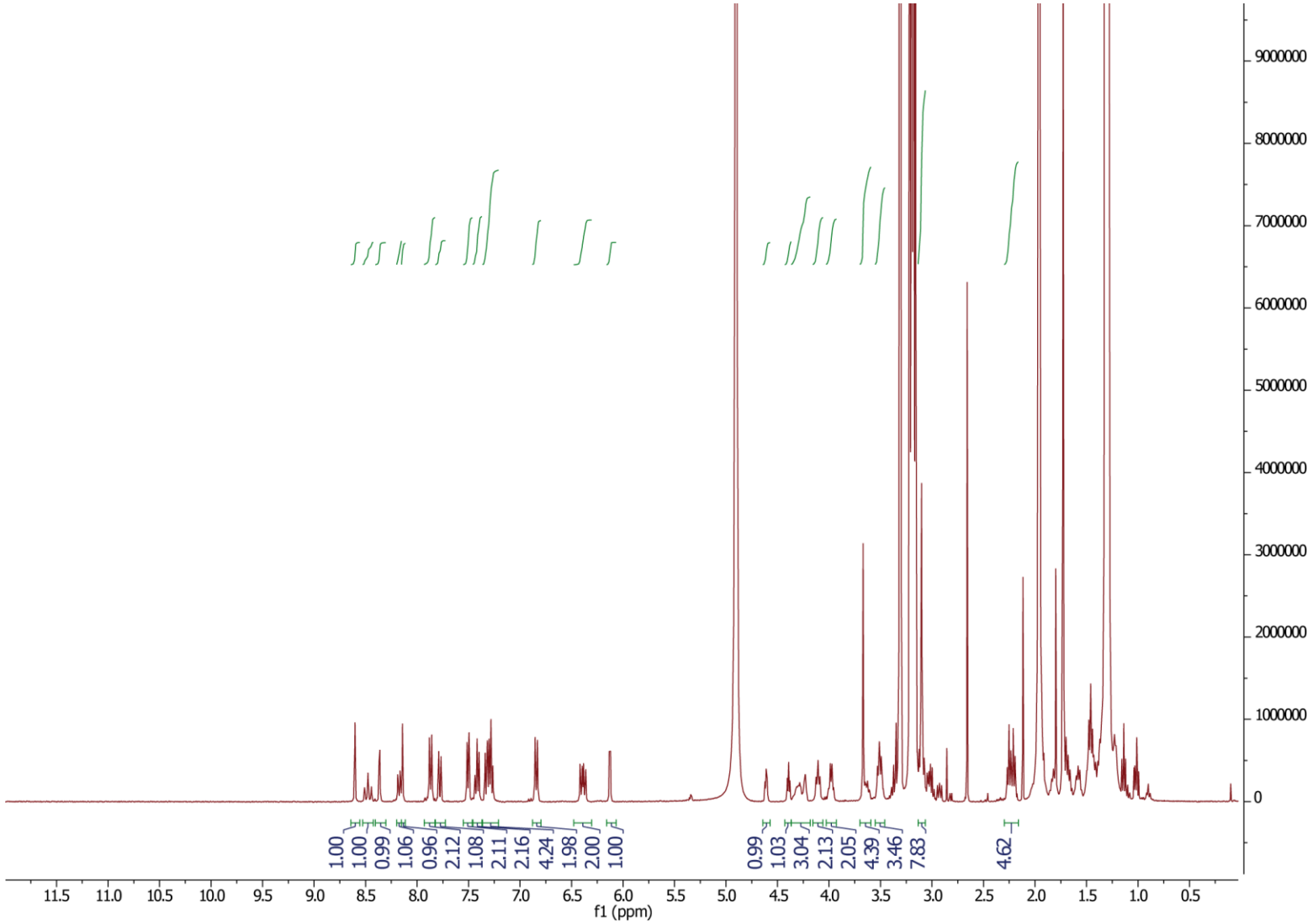

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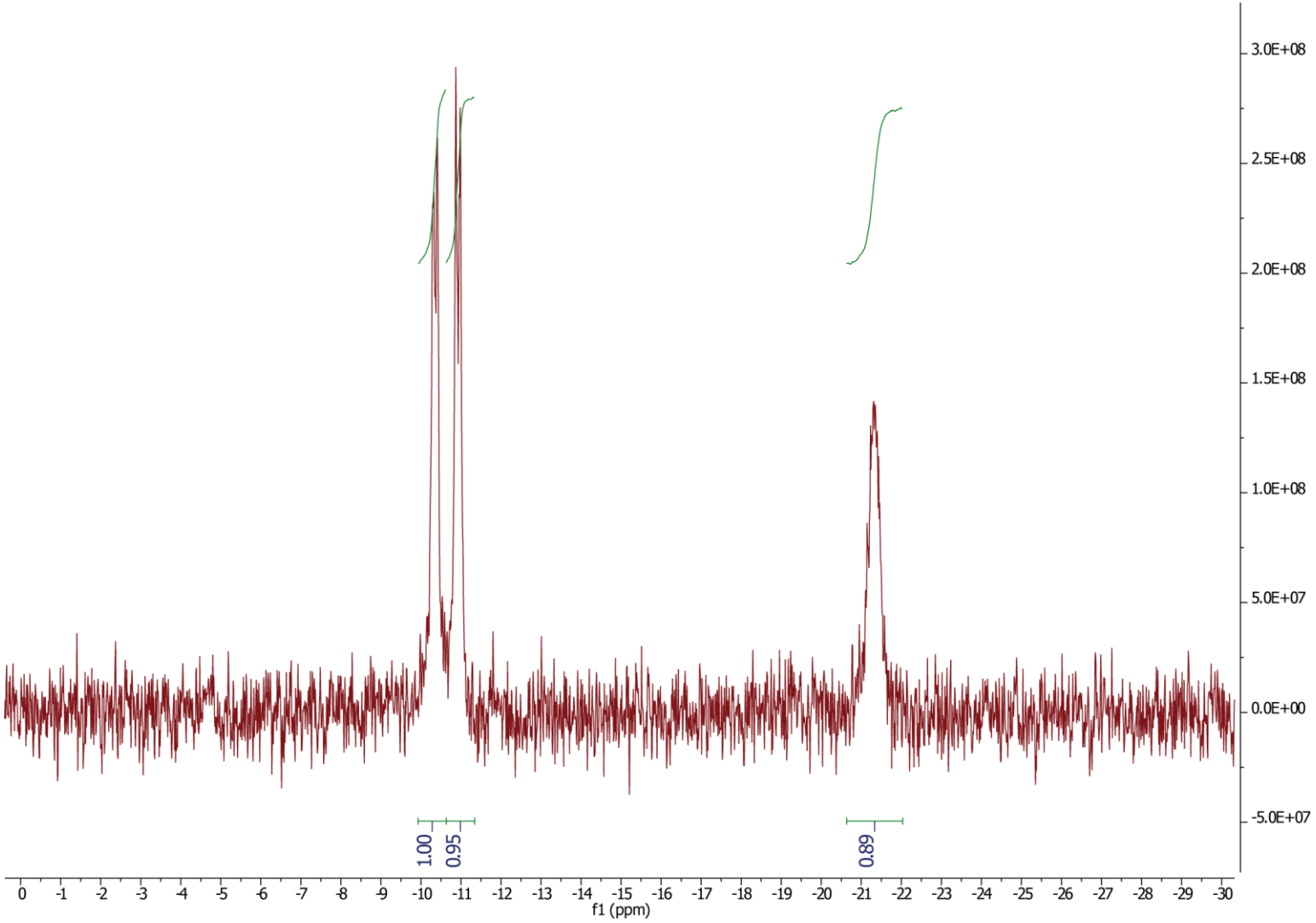

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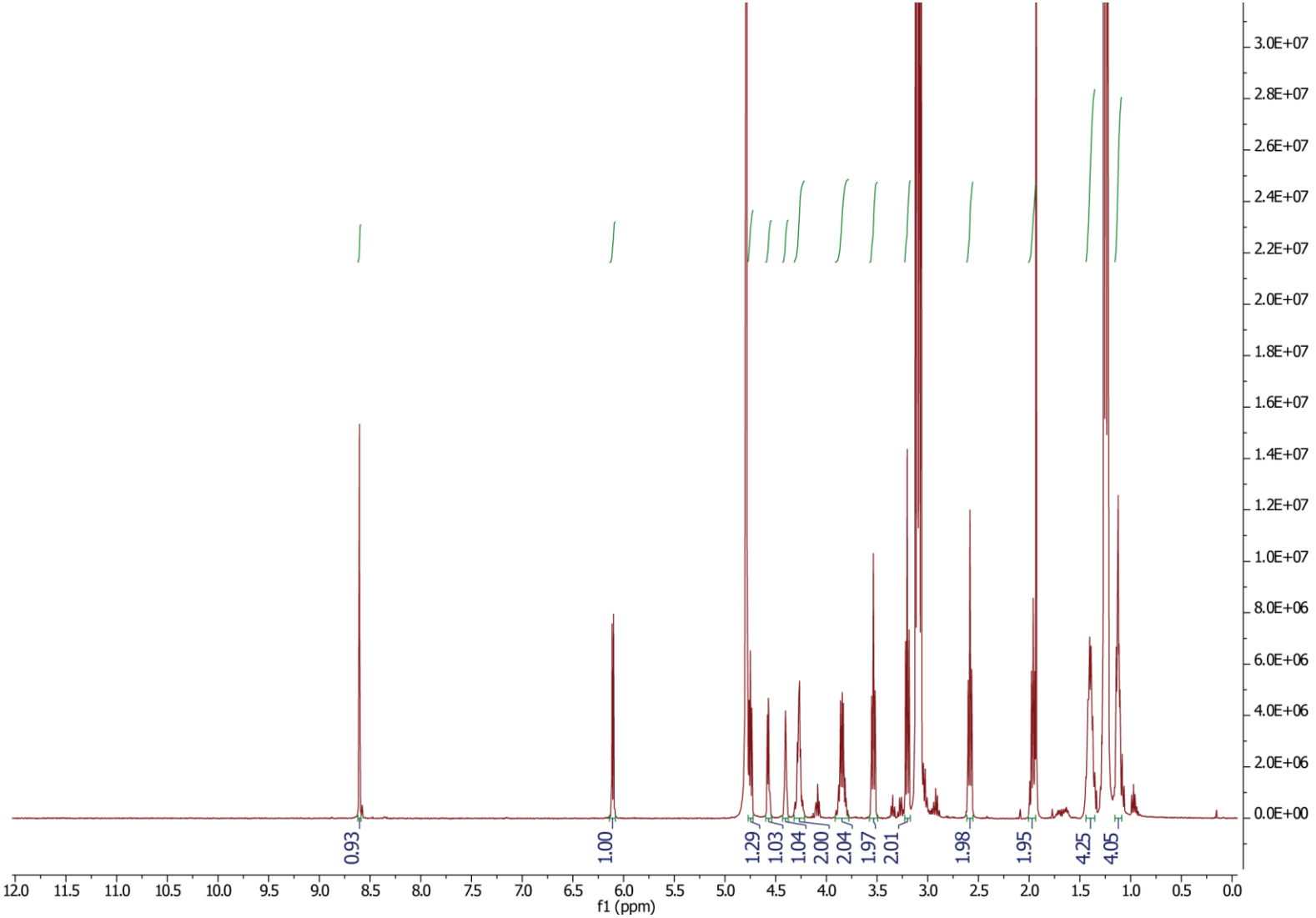

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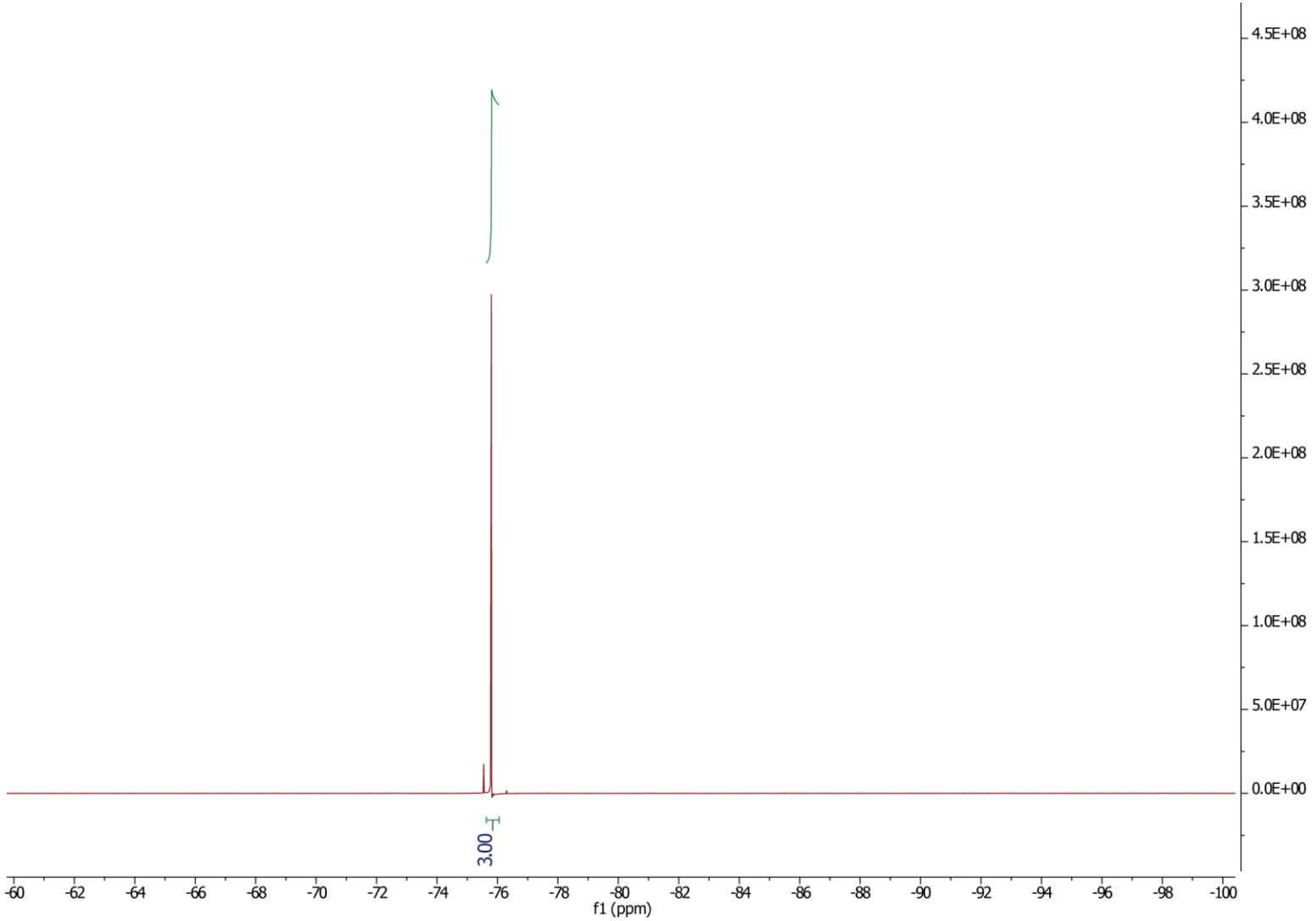

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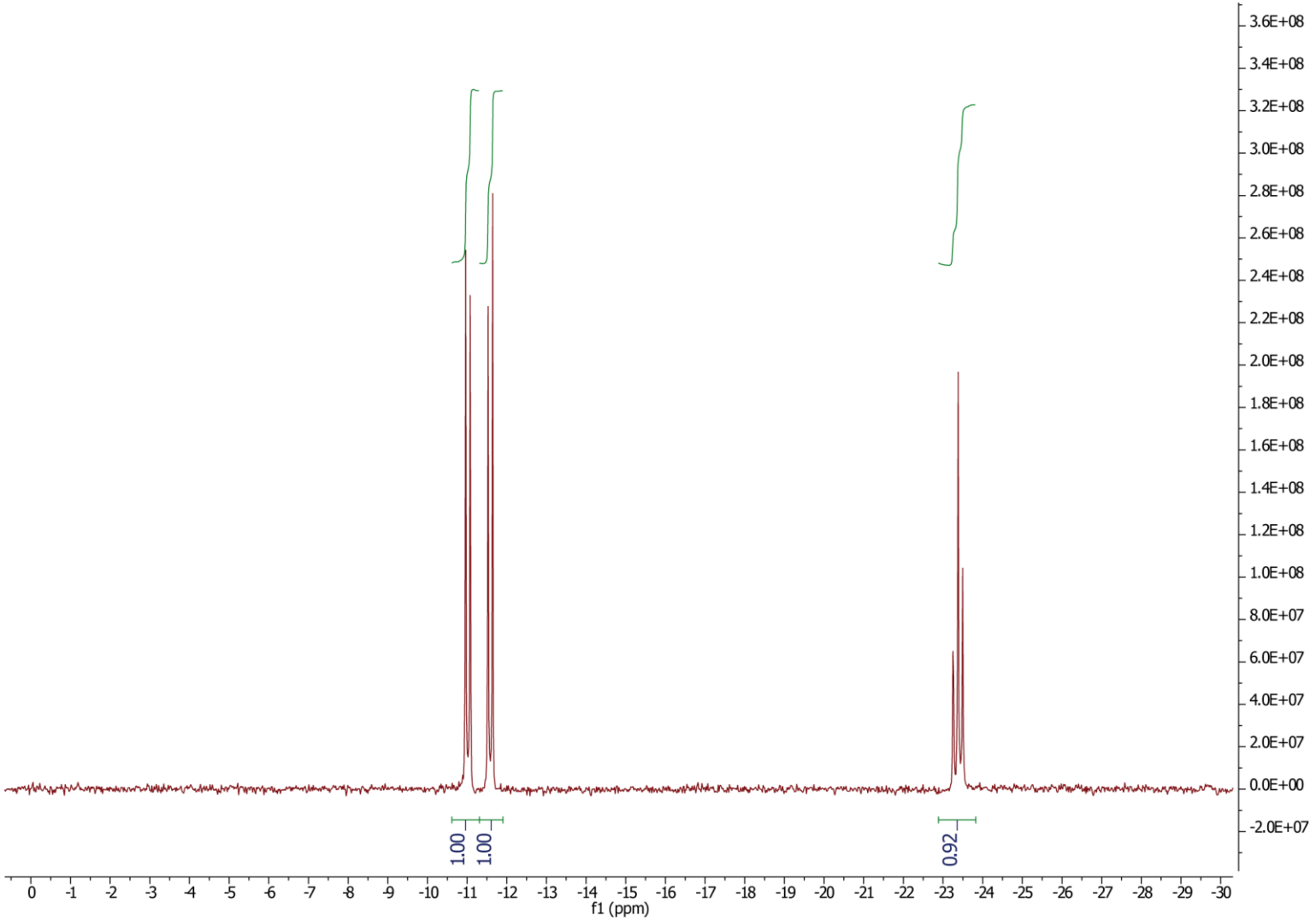

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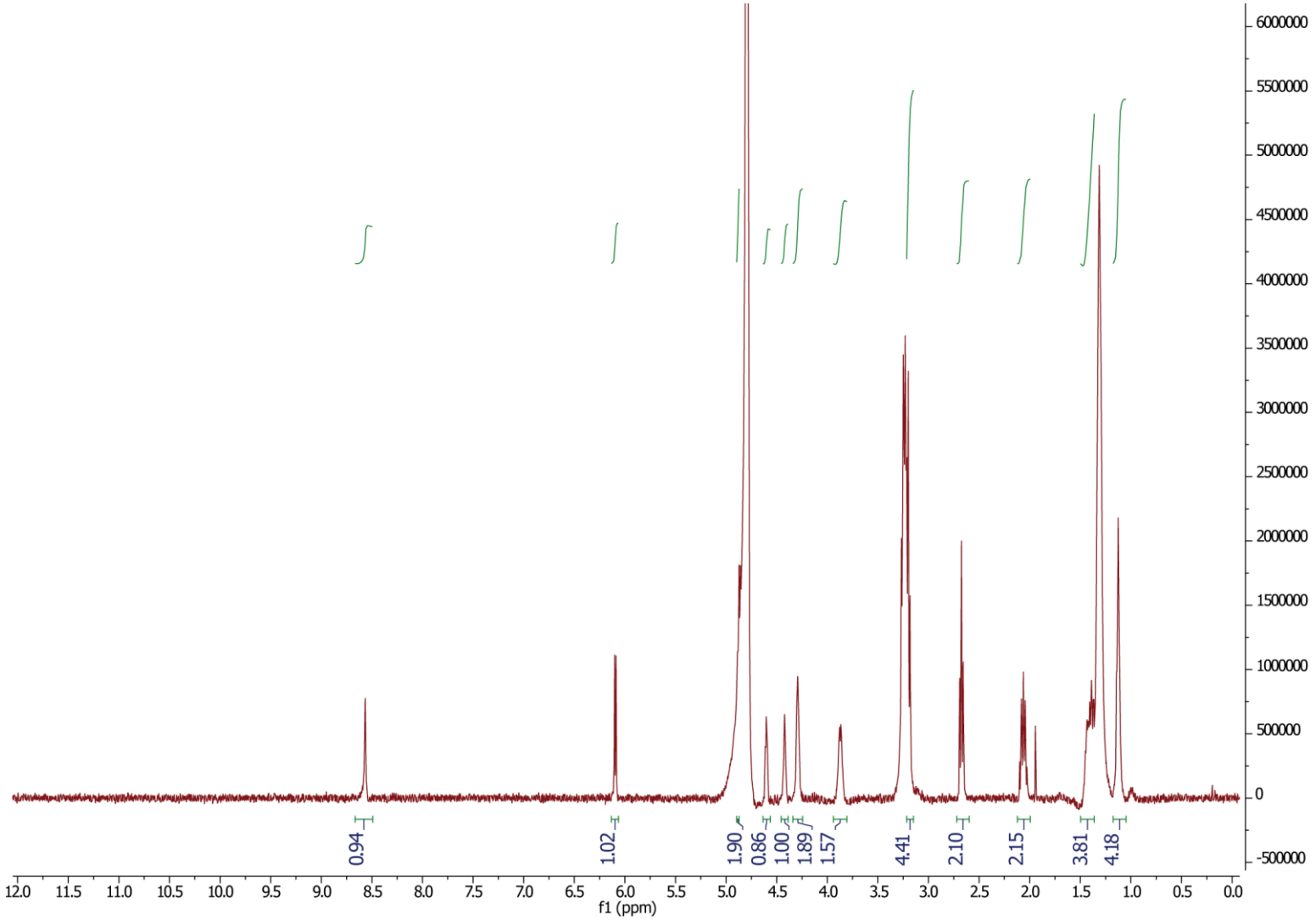

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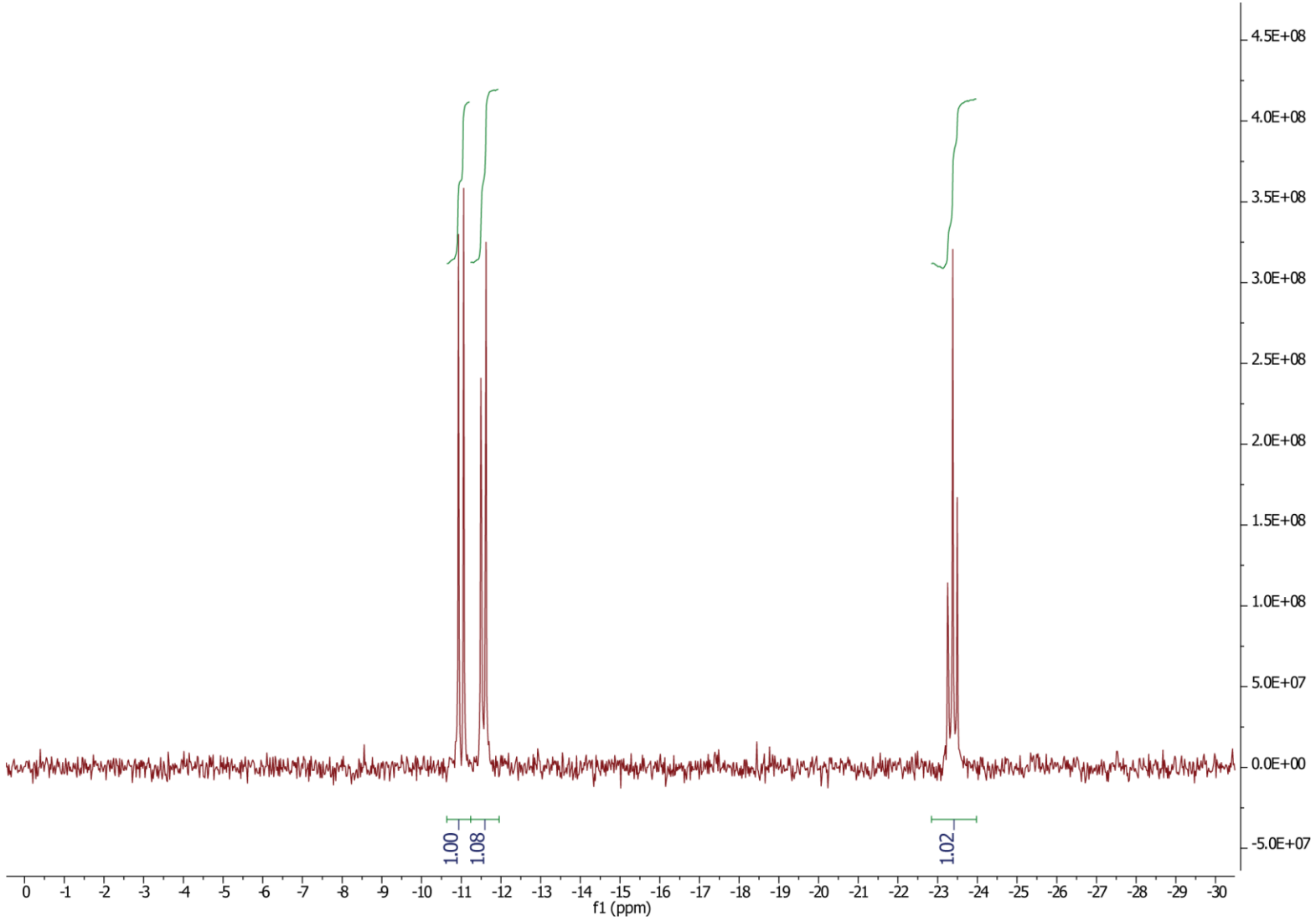

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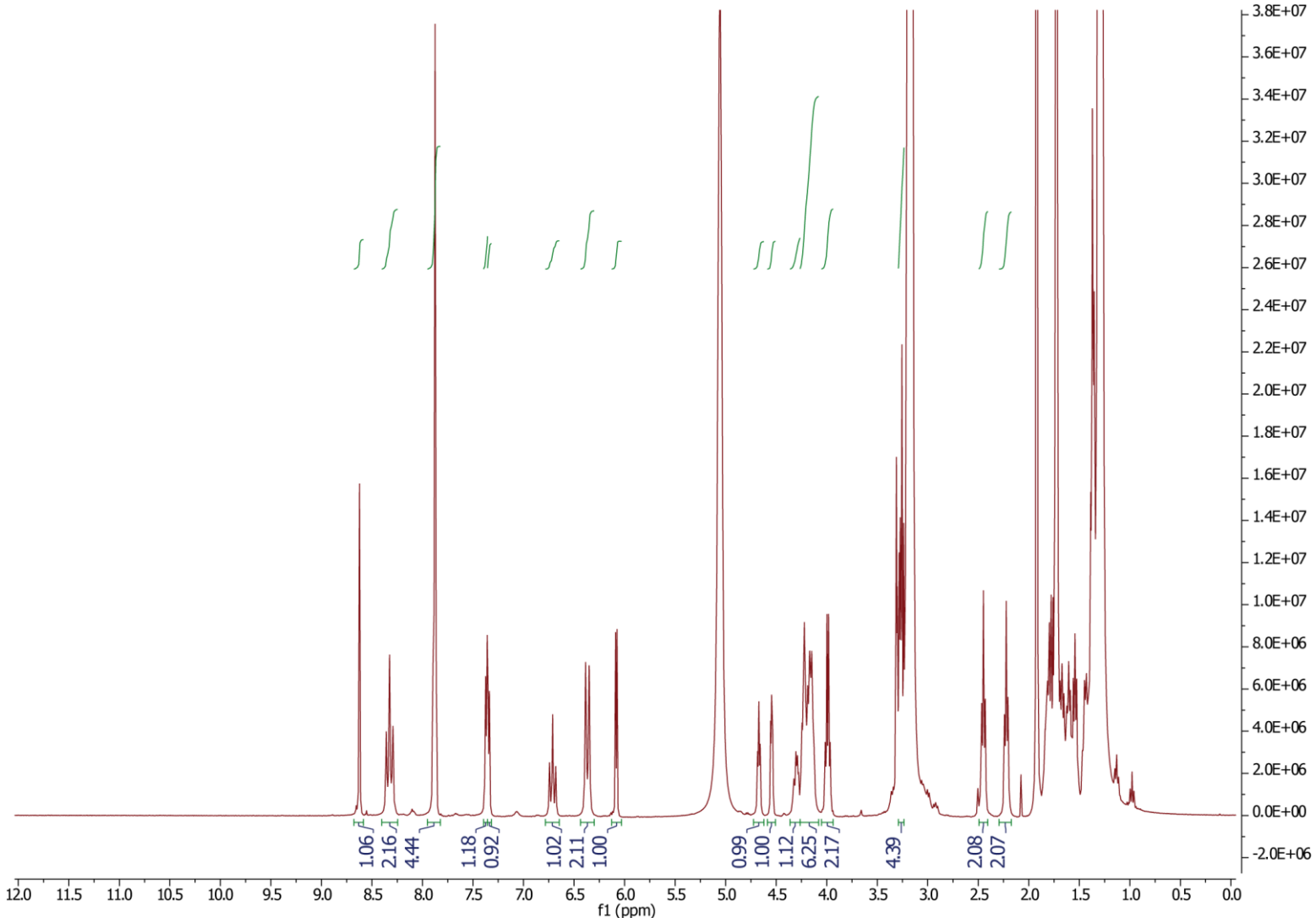

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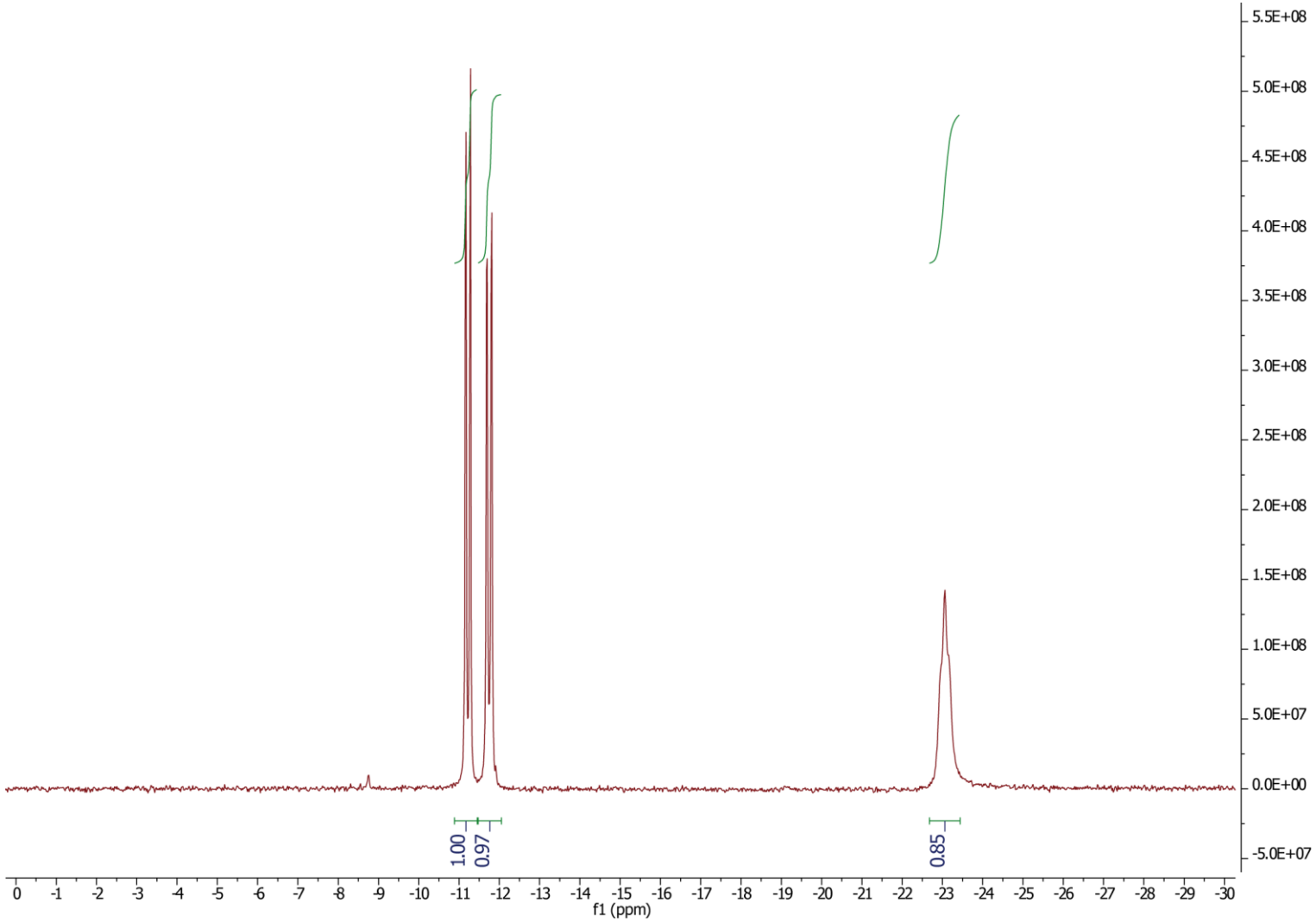

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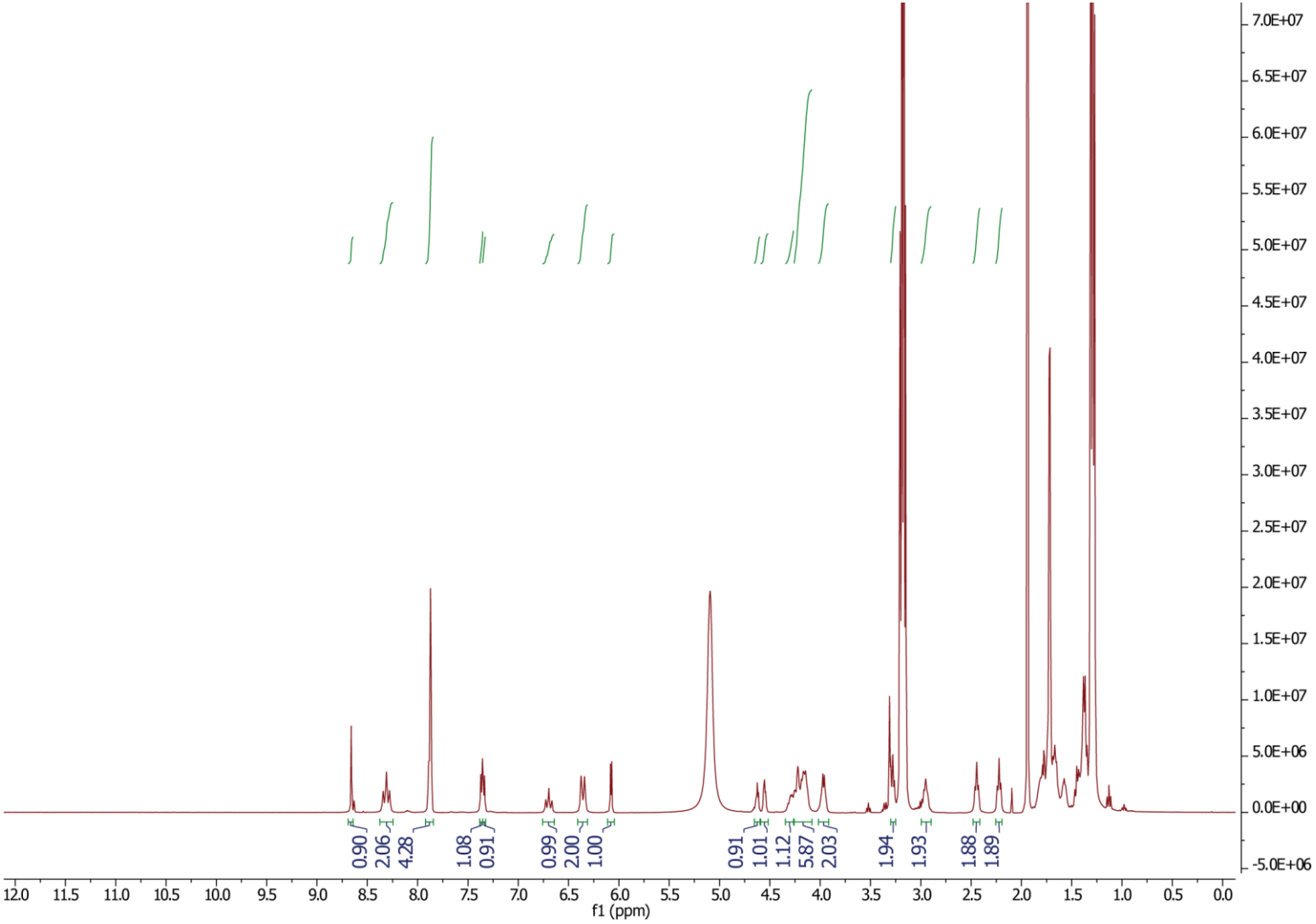

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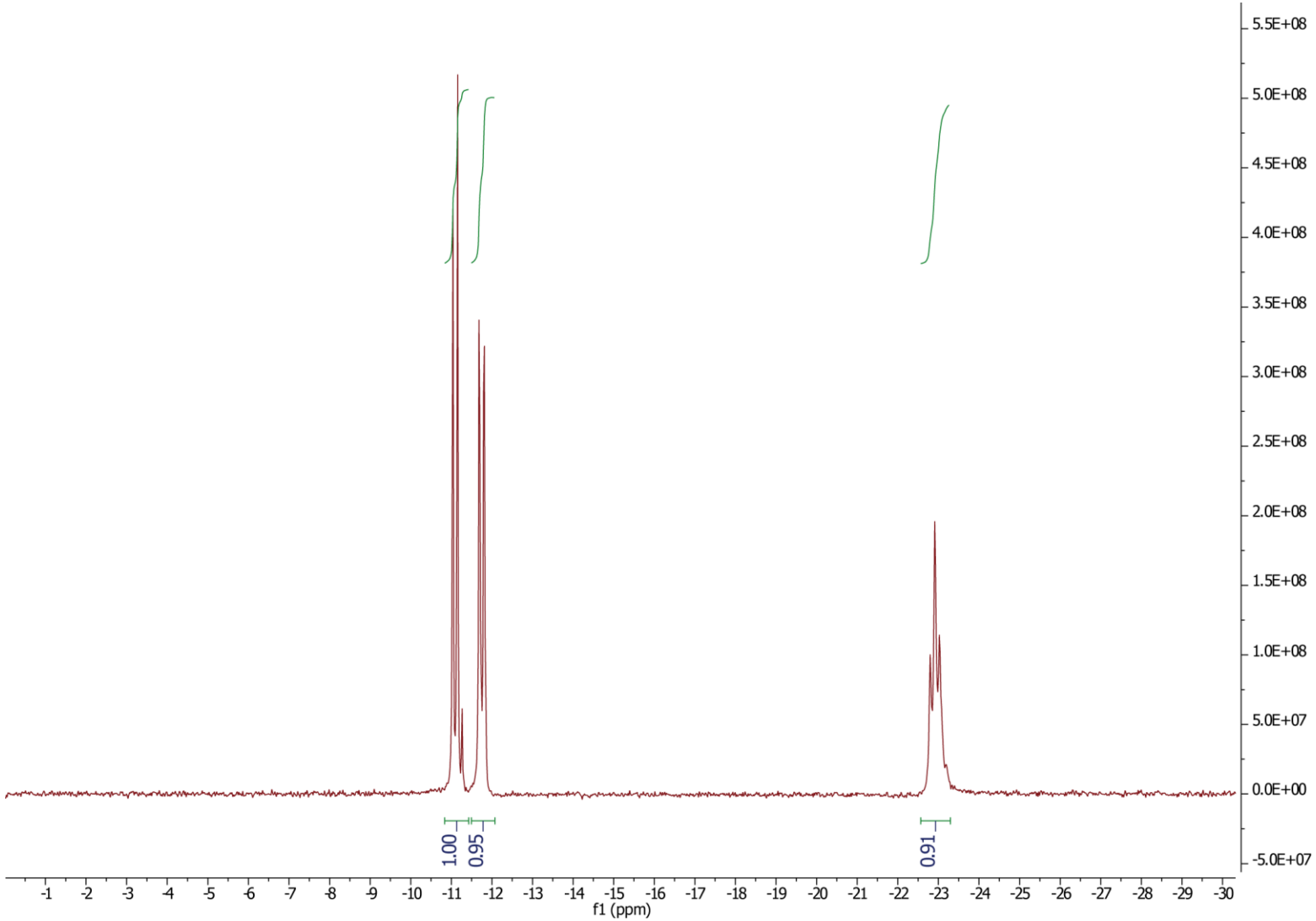
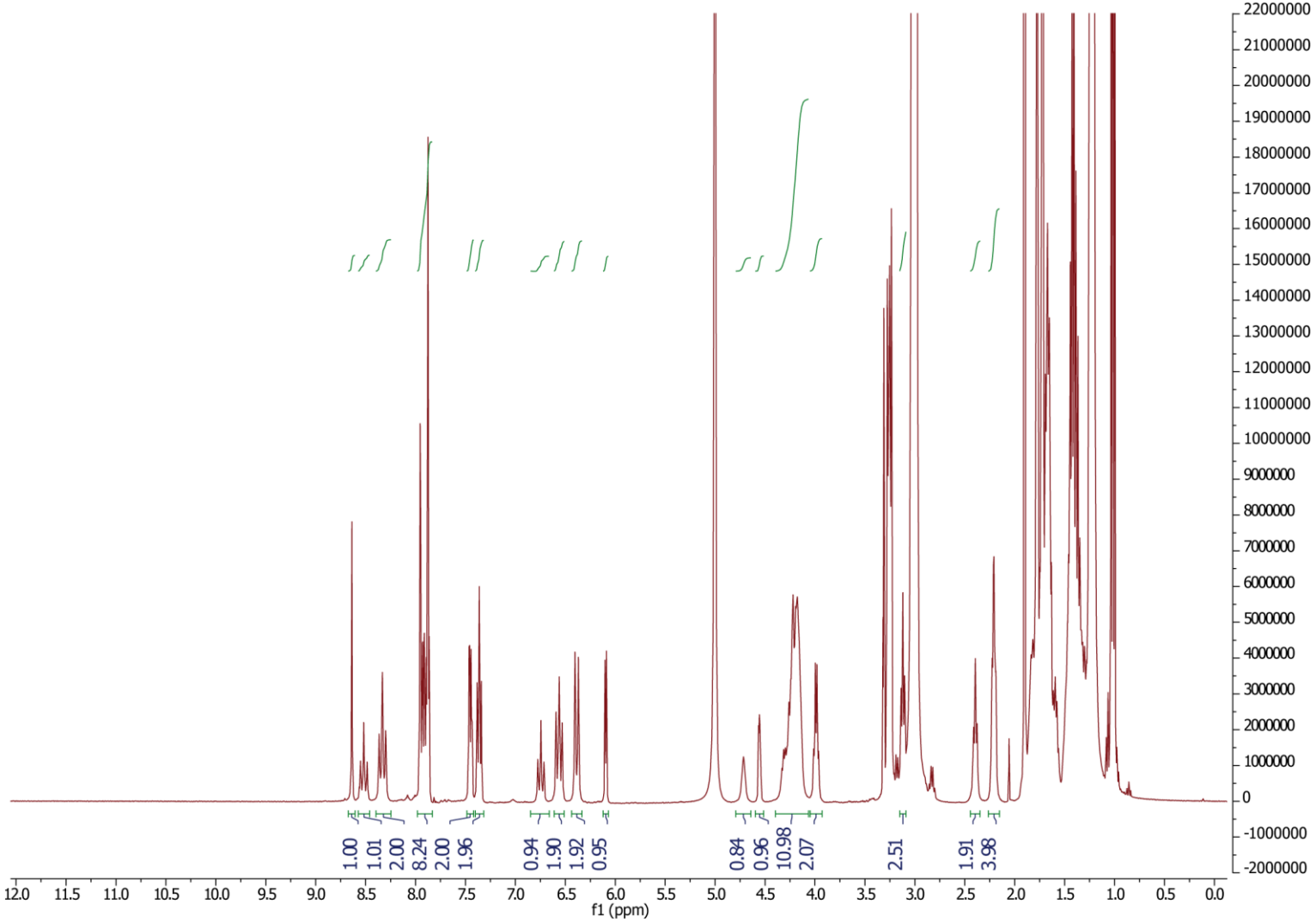
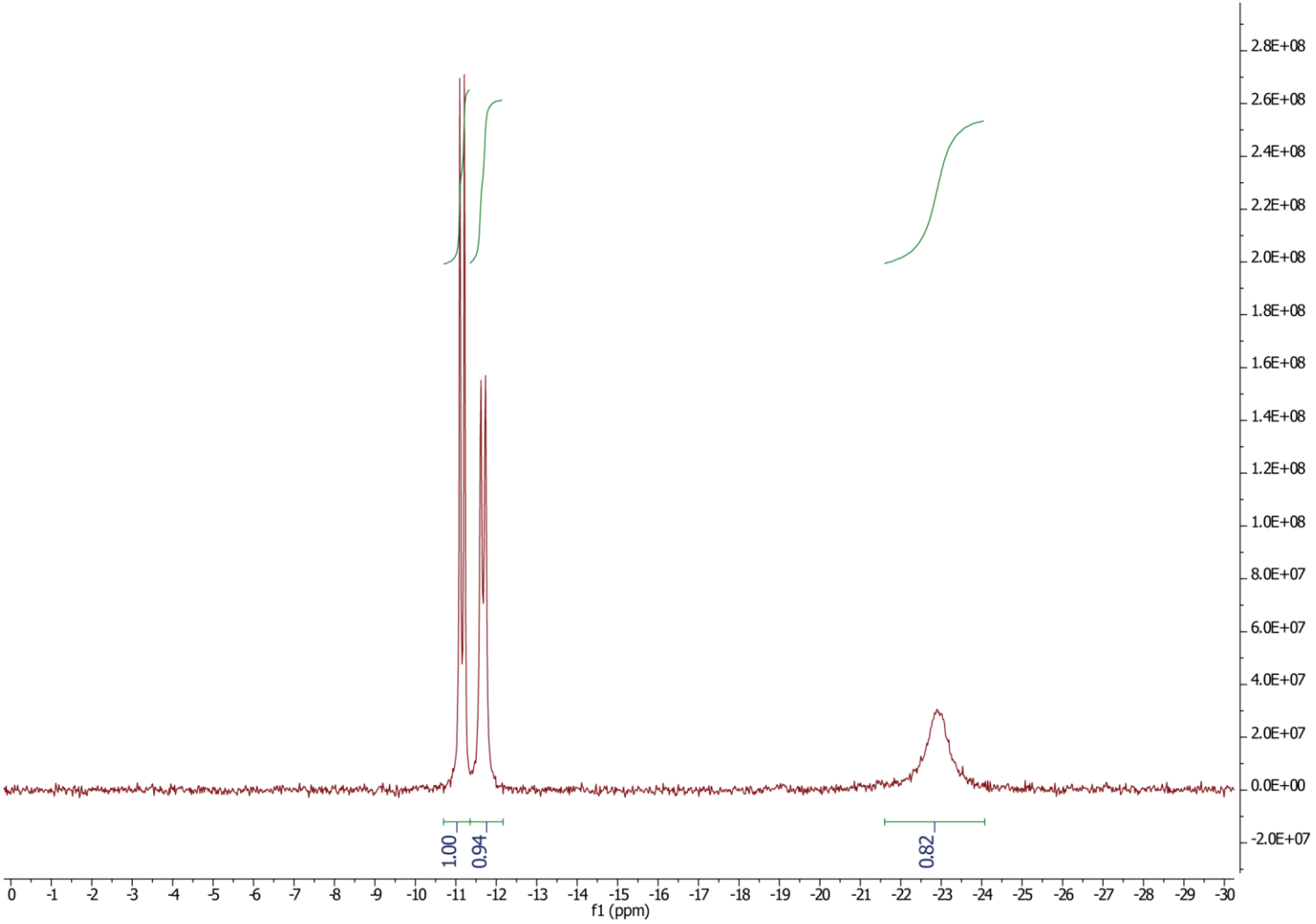


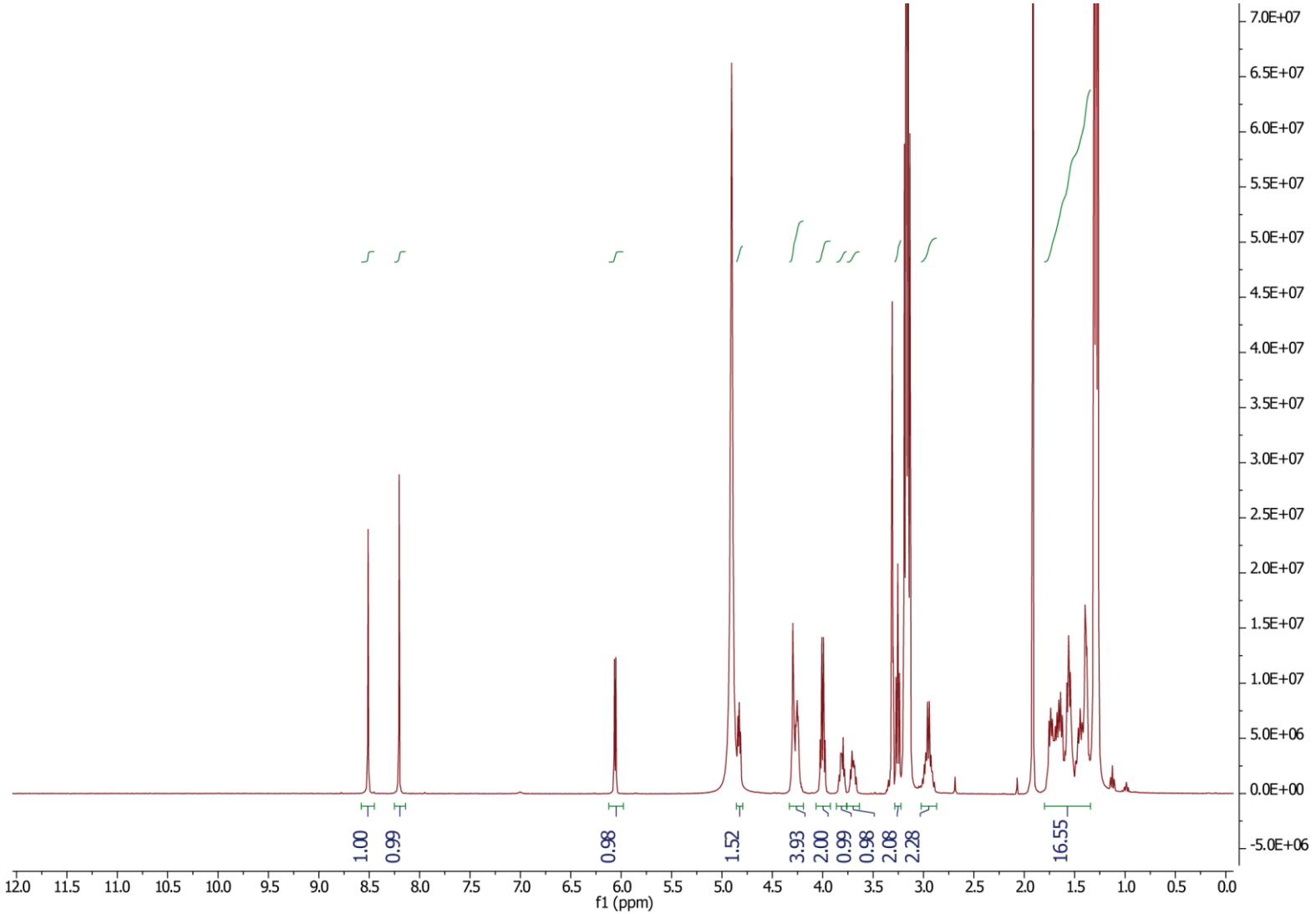
γ-(6-Sulfo-Cy3-amidohexyl)-*C*2-(5-Sulfo-Cy5-amidopent-1-yn-1-yl)-adenosine triphosphate 13 (^{1}H NMR)



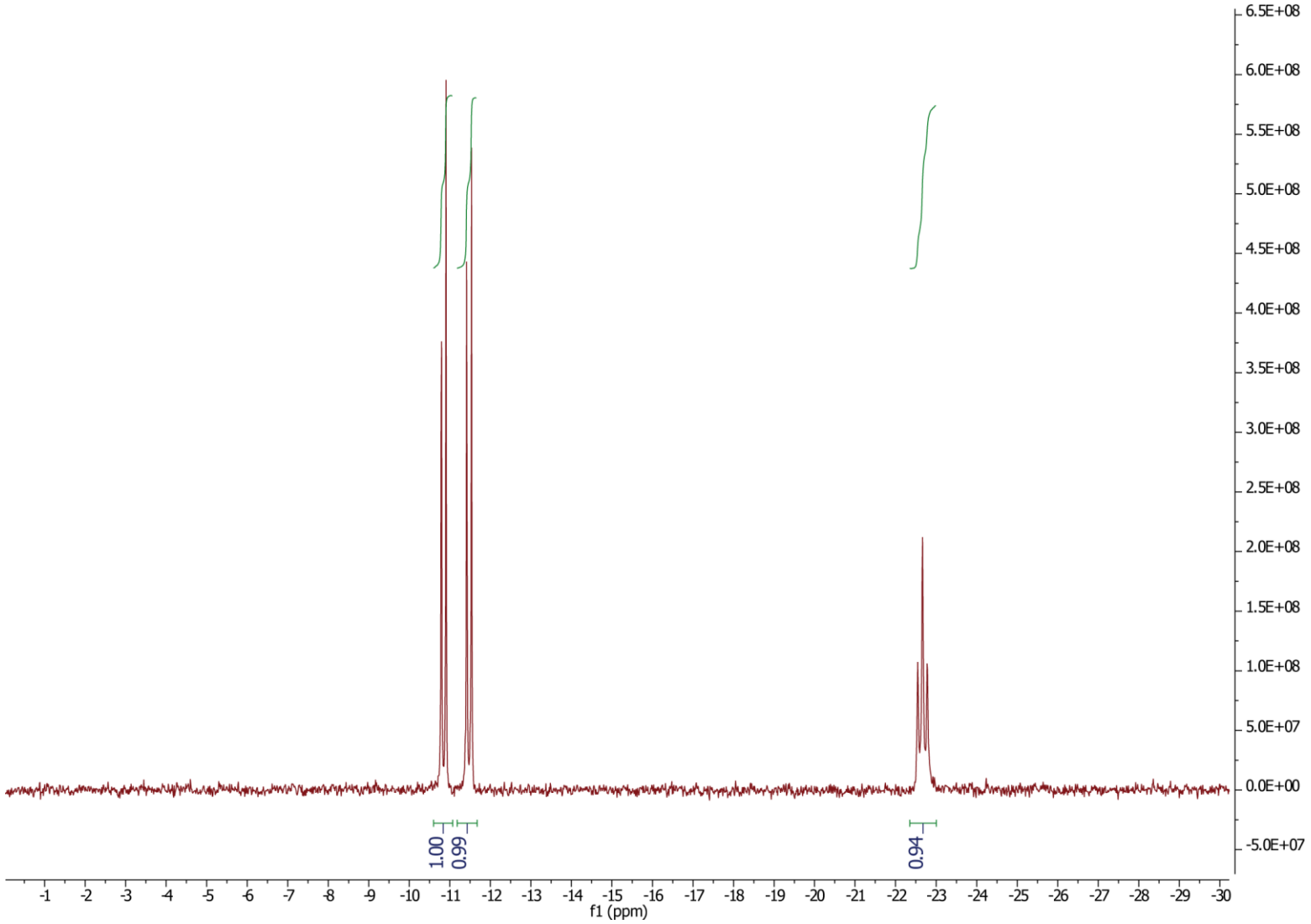
γ-(6-Sulfo-Cy3-amidohexyl)-*C*2-(5-Sulfo-Cy5-amidopent-1-yn-1-yl)-adenosine triphosphate 13 (^{31}P NMR)



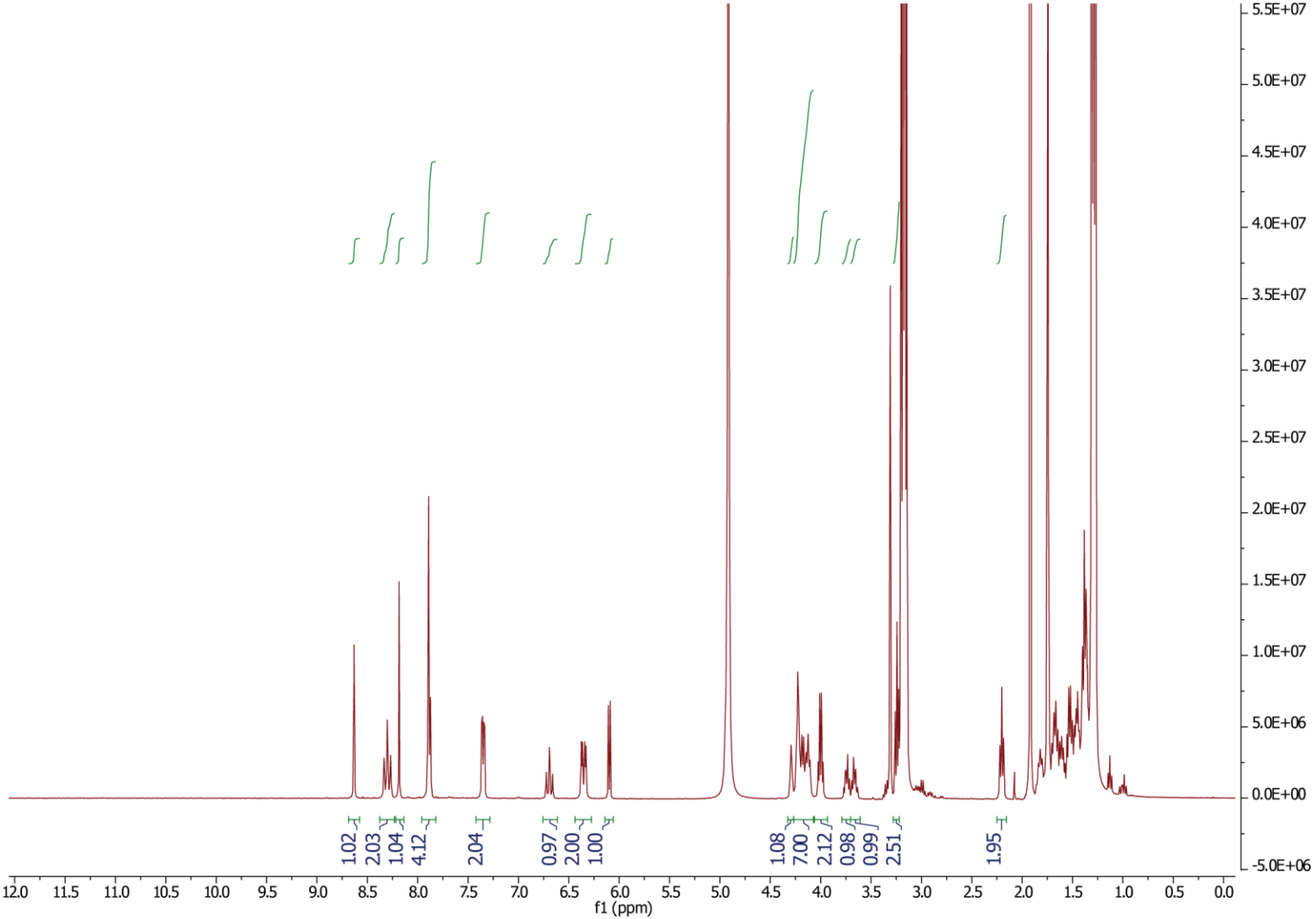
γ-(6-Azidohexyl)-*O*3'-(6-aminohexyl)-adenosine triphosphate 14 (^{1}H NMR)



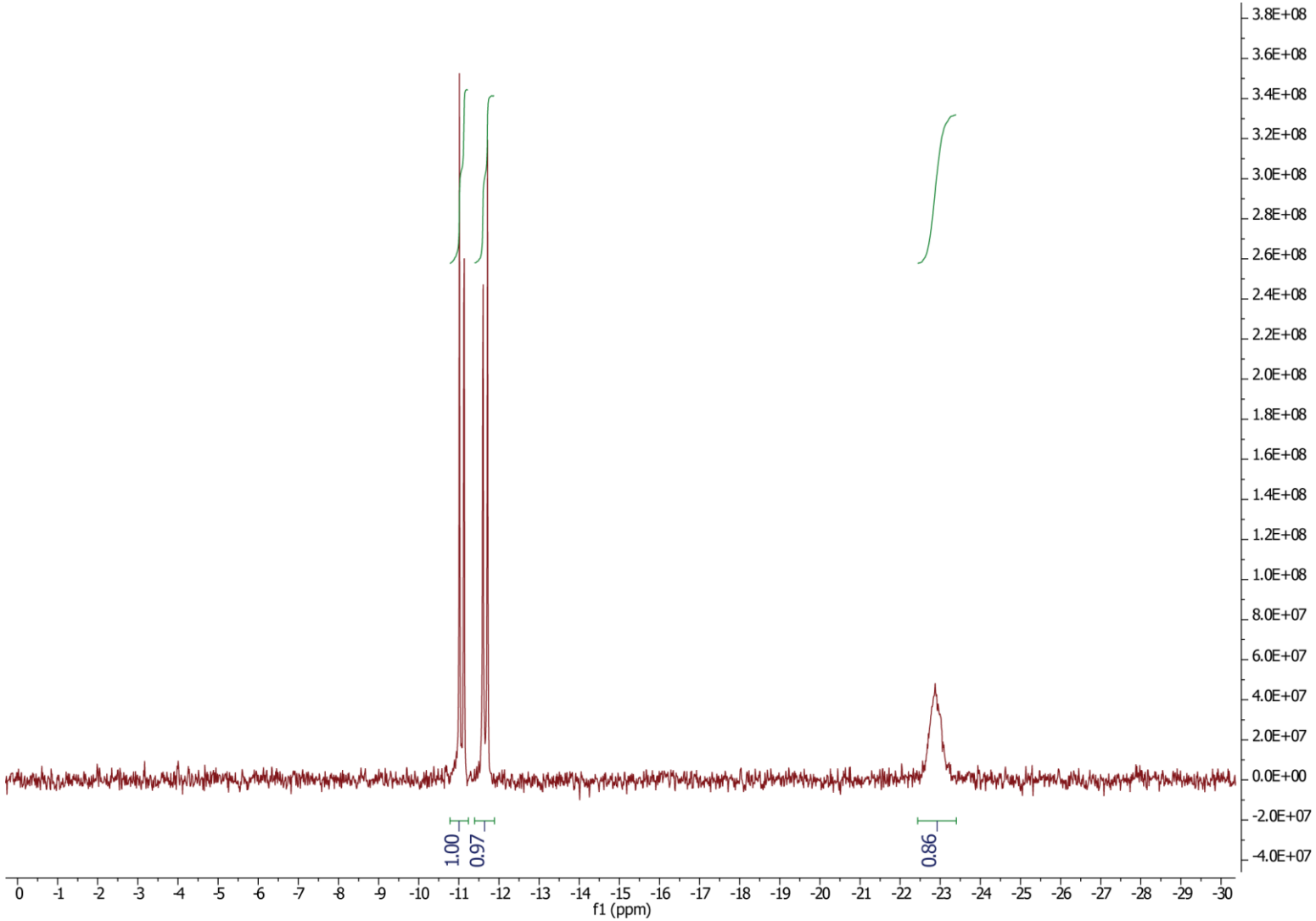
γ-(6-Azidohexyl)-*O*3'-(6-aminohexyl)-adenosine triphosphate 14 (^{31}P NMR)



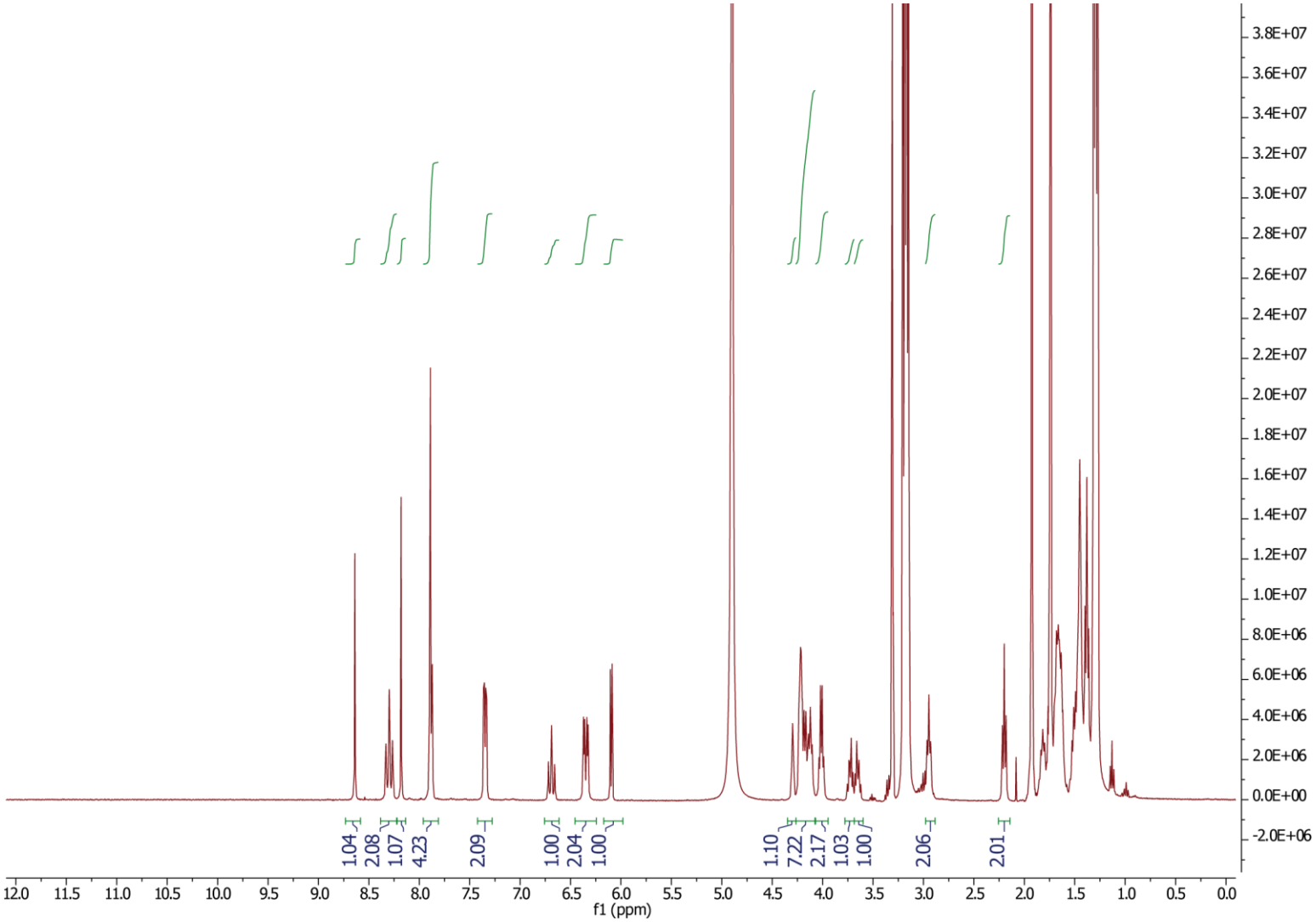
γ-(6-Azidohexyl)-*O*3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 15 (^{1}H NMR)



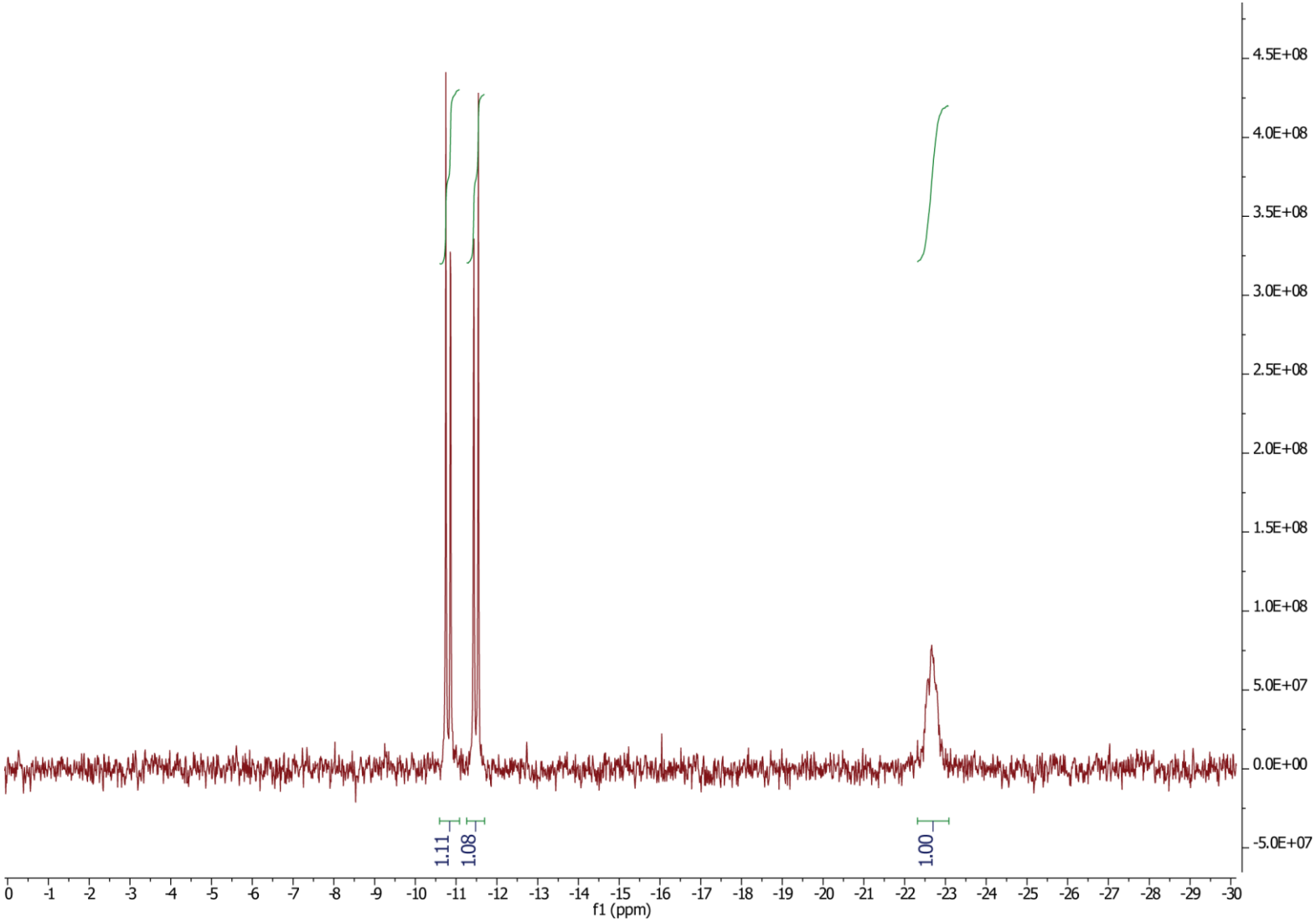
γ-(6-Azidohexyl)-*O*3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 15 (^{31}P NMR)



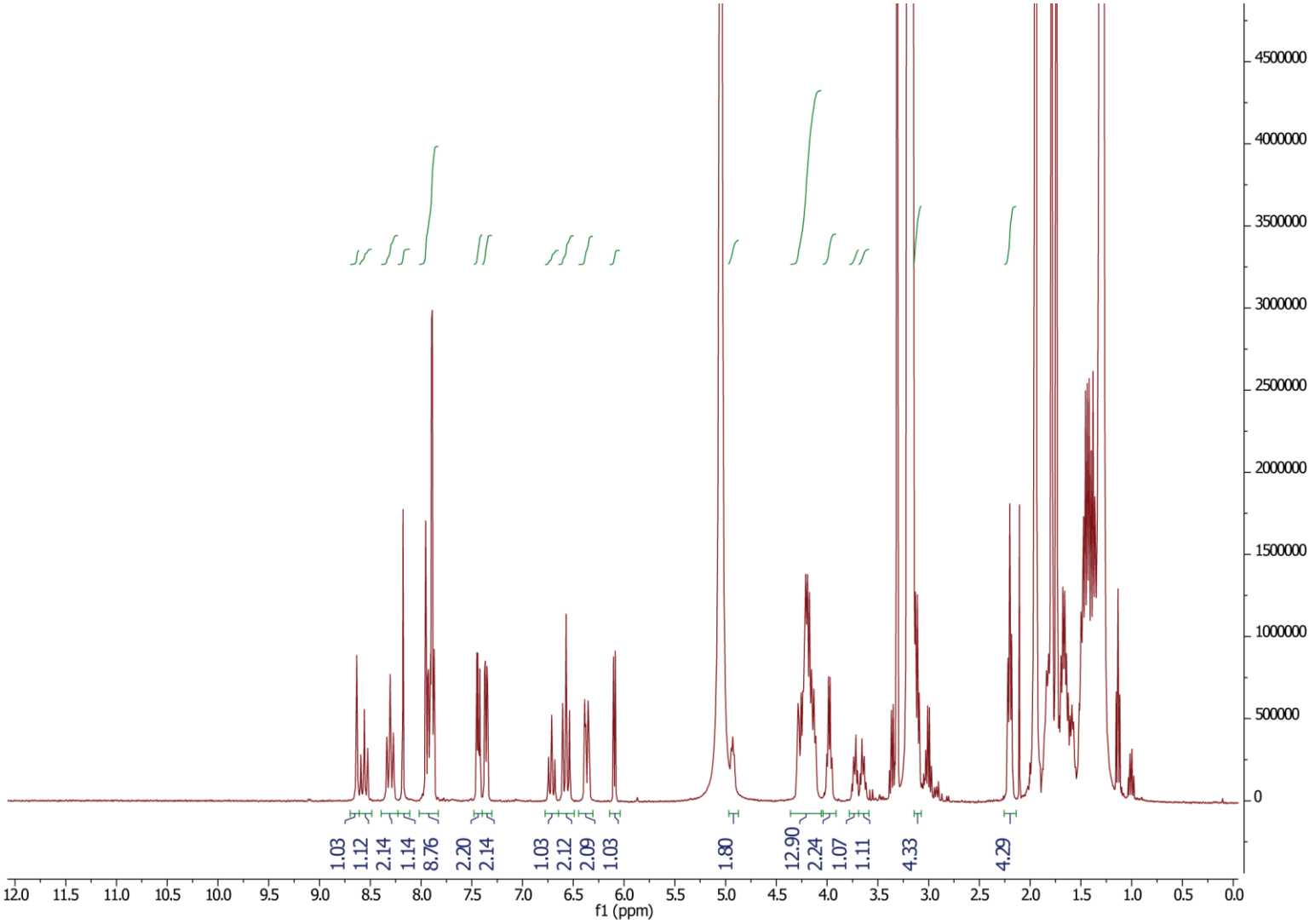
γ-(6-Aminohexyl)-*O*3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 16 (^{1}H NMR)



γ-(6-Aminohexyl)-*O*3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 16 (^{31}P NMR)



γ-(6-Sulfo-Cy3-amidohexyl)-*O*3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 17 (^{1}H NMR)



γ-(6-Sulfo-Cy3-amidohexyl)-*O*3'-(6-Sulfo-Cy5-amidohexyl)-adenosine triphosphate 17 (^{31}P NMR)