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Supporting Information

Cu(I)-assisted Click Chemistry Strategy for Conjugation of Non-protected Cross-bridged Macrocyclic Chelators to Tumour-targeting Peptides

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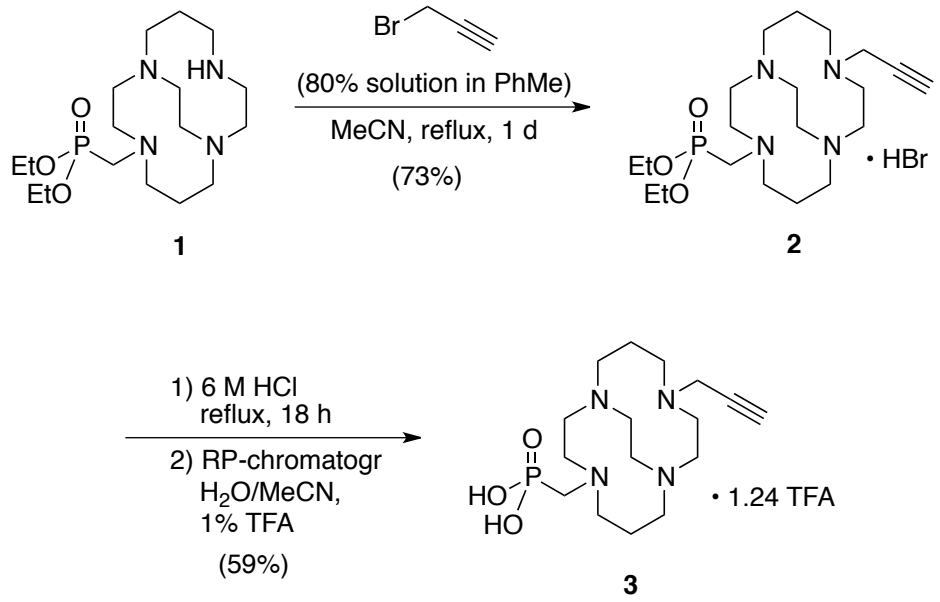
1. Synthesis

1.1 General Information: Unless otherwise specified, all reagents and solvents employed at Pittsburgh were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Matrigel was purchased from BD Biosciences, Bedford, MA. Reactions were monitored by TLC on 0.25 mm silica gel glass plates containing F-254 indicator. Visualization by TLC was monitored by UV light, $KMnO_4$, or radioactivity. Flash chromatography was performed using 200 mesh silica gel. ^{1}H NMR spectra were recorded on a Bruker DRX 400 MHz NMR spectrometer (Pittsburgh), a Varian Mercury 400 MHz NMR spectrometer (NH), or a Varian Unity INOVA 500 MHz NMR spectrometer (NH). Chemical shifts are reported in ppm relative to tetramethylsilane. Coupling constants are reported in Hertz. $^{13}C\{^{1}H\}$ NMR spectra were obtained on a Bruker DRX 400 MHz NMR spectrometer (Pittsburgh), a Varian Mercury 400 MHz NMR spectrometer at 100 MHz (NH), or a Varian Unity INOVA 500 MHz NMR spectrometer at 125 MHz (NH). $^{31}P\{^{1}H\}$ NMR spectra were obtained on a Varian Unity INOVA 500 MHz NMR spectrometer at 202 MHz (NH). ESI-MS at Pittsburgh were obtained on a Waters LCT-Premier XE LC-MS station (Milford, MA). ESI+ HRMS of **2** and **3** were obtained at the Mass Spectrometry and Proteomics Facility at the University of Notre Dame (Notre Dame, IN). $^{64}CuCl_2$ was purchased from Washington University School of Medicine (St. Louis, MO) and University of Wisconsin-Madison (Madison, WI). Aqueous solutions were prepared using ultrapure water (resistivity, 18 MΩ). The Wang resin (loading, 0.61 mmol/g) and all Fmoc-protected amino acids were purchased from Chem-Impex International, Inc. (Wood Dale, IL). Reversed-phase high-performance liquid chromatography (HPLC) were performed either on a Waters 600E (Milford, MA) chromatography system with a Waters 991 photodiode array detector and an Ortec model 661 (EG&G Instruments, Oak Ridge, TN) radioactivity detector, or a Waters 1525 Binary HPLC pump (Milford, MA) with a Waters 2489 UV/visible detector and a model 105-S-1 (Carroll& Ramsey Associates; Berkeley, CA) radioactivity detector. HPLC samples were analyzed on an analytical C18

column (Phenomenex, Torrance, CA) and purified on a semi-preparative C18 column (Phenomenex, Torrance, CA). H_2O (0.1% TFA; solvent A) and acetonitrile (0.1% TFA; solvent B) were used as mobile phase. Radioactive samples were counted using an automated well-type gamma-counter (8000; Beckman, Irvine, CA). PET/CT data were acquired using an Inveon preclinical PET scanner (Siemens Medical Solutions, Erlangen, Germany).

1.2 Discussion-Synthesis of 3: Propargyl cross-bridged cyclam phosphonic acid BFC **3** was synthesized as shown in Scheme S1. Mono-diethyl phosphonate-armed cross-bridged cyclam **1**[1] was *N*-alkylated in MeCN (85 °C, 17 h) with propargyl bromide to give propargyl diethyl phosphonate intermediate **2**, which was characterized as the free base. It was found most convenient in practice to take **2** to the next step directly as its initially formed hydrobromide. Thus, **2**•HBr was hydrolyzed in refluxing 6 N aq HCl (18 h) and purified by reversed-phase flash chromatography (C_{18} column; gradient elution, 100% H_2O + 1% CF_3CO_2H (TFA) to 5% MeCN in H_2O + 1% TFA) to give **3** as a TFA salt. The stoichiometry of the product salt, **3**•$(CF_3CO_2H)_{1.24}$, was established by ^{19}F NMR integration after quantitative addition of an aliquot of $PhCF_3$ to a weighed sample of lyophilized product. This material was used for bioconjugation. The identities of **2** and **3** were established by ^{1}H and ^{13}C NMR analysis as well as HRMS. Phosphorus-carbon and phosphorus-hydrogen coupling was especially helpful, the former $J_{P,C}$ values being established by running $^{13}C\{^{1}H\}$ spectra at two different Larmor frequencies (100 and 125 MHz).



Scheme S1. Synthesis of CB-TE1P1P' (**3**).

1.3 Diethyl (11-(prop-2-ynyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecan-4-yl)methylphosphonate hydrobromide (2•HBr): To a solution of diethyl (1,4,8,11-tetraazabicyclo[6.6.2]hexadecan-4-yl)methyl-phosphonate (**1**)[1] (137 mg, 0.364 mmol) in dry MeCN (2.4 mL) was added a toluene solution of propargyl bromide (80% in tol, 120 µL, 1.11 mmol). The solution was heated at reflux (85 °C) for 17 h. The crude mixture was filtered through a pad of Celite, which was washed with copious volumes of CH_2Cl_2. The combined organic filtrates were concentrated under reduced pressure to give a yellow oil, which was purified by column chromatography (SiO_2; 20% (vol/vol) MeOH/CH_2Cl_2) to give the product as a white solid hydrobromide salt (**2**•HBr) (120 mg, 0.242 mmol, 66.5%). For full characterization of the compound in free base form (**2**), 42 mg of the chromatographed material was made basic with cold 20% (w/v) aq NaOH solution to pH 14. The basic solution was extracted with cold toluene (4 × 15 mL), dried over anhyd Na_2SO_4, and concentrated under reduced pressure to give the product **2** as a colorless gum (31 mg; calc total yield 88.6 mg, 0.214 mmol, 59%); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.32 (3H, t, *J* = 7.1 Hz, P(O)CH_2CH_3), 1.33 (3H, t, *J* = 7.1 Hz, P(O)CH_2CH_3), 1.30–1.49 (4H, m), 2.10 (1H, t, X of ABX, $^4J_{AX} = {}^4J_{BX}$ = 2.3

Hz, propargyl $CH_AH_BCCH_X$), 2.26–2.51 (8H, m), 2.59–2.885 (8H, m), 2.89 (1H, dd (t_{app}), $^2J_{H,H}$ = -15.9 Hz, $^2J_{P,H}$ = -15.9 Hz, NCH*H*P) 2.97–3.16 (2H, m), 3.21 (1H, B of ABX with additional d long-range w-coupling, $^2J_{AB}$ = -17.1 Hz, $^4J_{BX}$ = 2.3 Hz, ^4J_w = 0.8 Hz, propargyl $CH_AH_BCCH_X$), 3.285 (1H, A of ABX with additional d long-range w-coupling, $^2J_{AB}$ = -17.1 Hz, $^4J_{BX}$ = 2.3 Hz, ^4J_w = 1.08 Hz, propargyl $CH_AH_BCCH_X$), 3.55 (1H, td, *J* = 11.5, 4.8 Hz), 3.85 (1H, tt, *J* = 11.8, 4.3 Hz), 4.07–4.17 (4H, m, $P(O)(OCH_2CH_3)_2$); $^{13}C\{^1H\}$ NMR ($CDCl_3$, 125.7 MHz, central peak of $CDCl_3$ resonance set to 77.16 ppm) δ 16.67 (d having downfield line coincident with upfield line of 16.71 resonance, $^3J_{PC}$ = 5.6 Hz, one $P(O)OCH_2CH_3$ of diastereotopic pair), 16.71 (d having upfield line coincident with downfield line of 16.67 resonance, $^3J_{P,C}$ = 5.6 Hz, one $P(O)OCH_2CH_3$ of diastereotopic pair), 27.4, 28.3, 42.1, 50.5 (d, $^1J_{P,C}$ = 162.7 Hz, NCH_2P), 51.0, 51.4, 51.6, 56.0 (d, $^3J_{P,C}$ = 15.9 Hz), 56.3, 56.84, 56.86, 57.0, 58.0, 60.2, 61.4 (d, $^2J_{P,C}$ = -6.9 Hz, $P(O)OCH_2CH_3$), 61.7 (d, $^2J_{P,C}$ = -7.0 Hz, $P(O)OCH_2CH_3$), 71.1 (propargyl CH_2CCH), 81.1 (propargyl CH_2CCH) [Note: ^{31}P–^{13}C couplings were confirmed by obtaining $^{13}C\{^1H\}$ spectra at two different frequencies, 125 and 100 MHz.]; $^{31}P\{^1H\}$ NMR (D_2O, 202.3 MHz, external reference 85% phosphoric acid set to δ 0.00) 27.11 (s; a minor impurity singlet also appeared at 25.21, 3% of major peak); IR (neat, thin film) 3245 (br), 2853, 1670, 1457, 1419, 1175, 1124, 1047 cm^{-1}; HRMS, ESI+ (*m/z)* $[M+H]^+$ exact mass for $C_{20}H_{40}N_4O_3P$: 415.2833; Found: 415.2830 (error 1.1 ppm). On the basis of small impurity peaks in the ^1H and $^{13}C\{^1H\}$ NMR spectra, the purity of the free-base material is estimated to be ~95%.

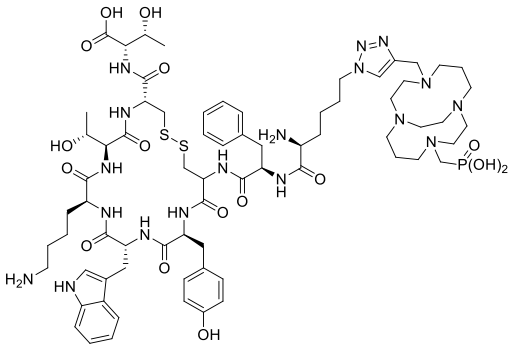
In a subsequent run of the alkylation, diethyl (1,4,8,11-tetraazabicyclo[6.6.2]hexadecan-4-yl)-methylphosphonate (**1**) (149 mg, 0.396 mmol) in dry MeCN (2.4 mL) was treated with a toluene solution of propargyl bromide (80% in tol, 120 µL, 1.11 mmol). The solution was heated at reflux (85 °C) for 23 h. The crude mixture was filtered through a pad of Celite, washed with CH_2Cl_2 as above, and the combined organic filtrates were concentrated under reduced pressure, and purified by column chromatography (SiO_2; 20% (vol/vol) $MeOH/CH_2Cl_2$) to give the product as a white solid hydrobromide salt hydrate (**2**•HBr•H_2O) (148 mg, 0.288 mmol, 73%); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.35 (6H, t, *J* = 7.1 Hz,

P(O)CH_2CH_3), 1.60–1.85 (4H, m), 1.60–1.78 (2H, m), 1.73 (2H, br s, H_2O), 2.28 (1H, t, X of ABX, $^4J_{AX} = {}^4J_{BX}$ = 2.3 Hz, propargyl $CH_AH_BCCH_X$), 2.86–3.54 (21H, m), 3.35 & 3.53 (2H, AB of ABX, $^2J_{AB}$ = -16.9 Hz, $^4J_{AX} = {}^4J_{BX}$ = 2.4 Hz, propargyl $CH_AH_BCCH_X$); 3.70-3.81 (1H, m), 4.09–4.20 (4H, m, P(O)$(OCH_2CH_3)_2$). This material was taken on directly to the phosphonate hydrolysis reaction.

1.4 (11-(Prop-2-ynyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecan-4-yl)methylphosphonic acid trifluoroacetate salt (3•$(CF_3CO_2H)_{1.24}$): Alkynyl phosphonate ester hydrobromide **2**•HBr•H_2O (146 mg, 0.284 mmol) was dissolved in 6 M hydrochloric acid (6 mL) and heated to reflux under nitrogen for 18 h. The resulting solution was concentrated under reduced pressure. The crude product was then dissolved in deionised water (20 mL), water was removed under reduced pressure, and the process was repeated three more times. Purification by reversed–phase flash chromatography (C_{18} column; gradient elution, 100% H_2O + 1% CF_3CO_2H (TFA) to 5% MeCN in H_2O + 1% TFA) gave 119 mg of a glass after solvent removal under reduced pressure. To this was added a total of 20 mL of H_2O (in two separate vials) followed by lyophilization to give the desired product as a white solid trifluoroacetate salt, **3**•$(CF_3CO_2H)_{1.24}$ (84.0 mg, 0.168 mmol, 59%). [Note: The stoichiometry of the product salt was established by ^{19}F NMR integration after quantitative addition of an aliquot of $PhCF_3$ to a weighed sample of lyophilized product in CD_3OD.] ^1H NMR (D_2O, 500 MHz, internal CH_3CN set to δ 2.06) δ 1.76–1.81 (2H, m), 2.31–2.44 (2H, m), 2.67–2.88 (4H, m), 2.98–3.14 (5H, m), 3.13 (1H, t, X of ABX, $^4J_{AX} = {}^4J_{BX}$ = 2.5 Hz, propargyl $CH_AH_BCCH_X$), 3.18 (1H, dd, $^2J_{H,H}$ = -15.4 Hz, $^2J_{PH}$ = -10.9 Hz, NCH*H*P), 3.22–3.45 (6H, m), 3.53 (1H, dd, $^2J_{H,H}$ = -15.4 Hz, $^2J_{P,H}$ = -14.0 Hz, NCH*H*P), 3.51–3.58 (2H, m), 3.60-3.75 (3H, m), 4.224 & 4.322 (2H, AB of ABX, $^2J_{AB}$ = -17.0 Hz, $^4J_{AX} = {}^4J_{BX}$ = 2.5 Hz, propargyl $CH_AH_BCCH_X$); $^{13}C\{^1H\}$ NMR (D_2O, 125.7 MHz, internal CH_3CN set to δ 1.47) δ 20.1, 20.3, 44.9, 47.5, 48.8, 49.1, 51.4 (d, $^3J_{P,C}$ = 7.7 Hz), 52.3 (d, $^1J_{P,C}$ = 140.8 Hz), 53.8, 54.4, 56.8, 58.9, 59.1, 73.5 (propargyl CH_2CCH), 80. 7 (propargyl CH_2CCH), 116.8 (q, $^1J_{C,F}$ = 291.1 Hz, CF_3COO^-), 163.2 (q, $^2J_{C,F}$ = 35.1 Hz, CF_3COO^-) [Note: $^{31}P–^{13}C$ couplings were confirmed by

obtaining $^{13}C\{^1H\}$ spectra at two different frequencies, 125 and 100 MHz.]; $^{31}P\{^1H\}$ (202.33 MHz; D_2O; external reference 85% phosphoric acid set to δ 0.00) δ 9.25 (s); IR (neat, thin film) 3245 (br), 2853, 2254, 2128, 1670, 1457, 1419, 1175, 1124, 1047, 964, 916, 829, 800, 718 cm^{-1}; HRMS, ESI+ (*m/z*) $[M+H]^+$ exact mass for $C_{16}H_{32}N_4O_3P$: 359.2207; Found: 359.2208 (error -0.4 ppm).

1. Young, M. J., Wong, L. S. M., Bist, S., Dugan, K. E., Wilk, D. G., Li, B. T. Y., Stigers, D. J., Widger, P. C. B., Kispert, B. J., Wong, E. H., Weisman, G. R. Synthesis of orthogonally protected cross-bridged tetraazamacrocycles. In preparation.



1.5 CB-TE1P1T-Y3-TATE (**7**): (N_3)-K-Y3-TATE (**5**) on resin (50 mg, 0.274 mmol/g, 13.7 μmol) was swelled with $CHCl_3$ (1 mL). To the mixture was added CB-TE1P1P' (**3**) (4.9 mg, 13.7 μmol), CuI (5.2 mg, 27.4 μmol), DIEA (1 mL). The mixture was vortexed at rt for overnight. The reaction was monitored by microcleaving a small amount of resin till completion of the reaction. EDTA aqueous solution (50 mM) was used to wash the excess amount of Cu ion. The resin was cleaved was TFA/PhOH/TIPS/H_2O (85/5/5/5, 5 mL) for 3 h at rt then transferred to ice-cooled Et_2O, and centrifuged to obtain the peptide. To the peptide, aqueous Na_2S solution (213 equiv) was added, sonicated, centrifuged, and filtered. DMSO (0.2 mL) and water (2 mL) were then added, with adjustment of pH with NH_4OAc to 7. The mixture was stirred at rt for 1 day till LC-MS showed complete conversion to the disulfide. HPLC

purification of the crude mixture yielded the clicked copper-free product **7** (0.9 mg, 25%). ESI-MS: m/z 781.7 $[M + 2H]^{2+}/2$ (calculated for 781.4).

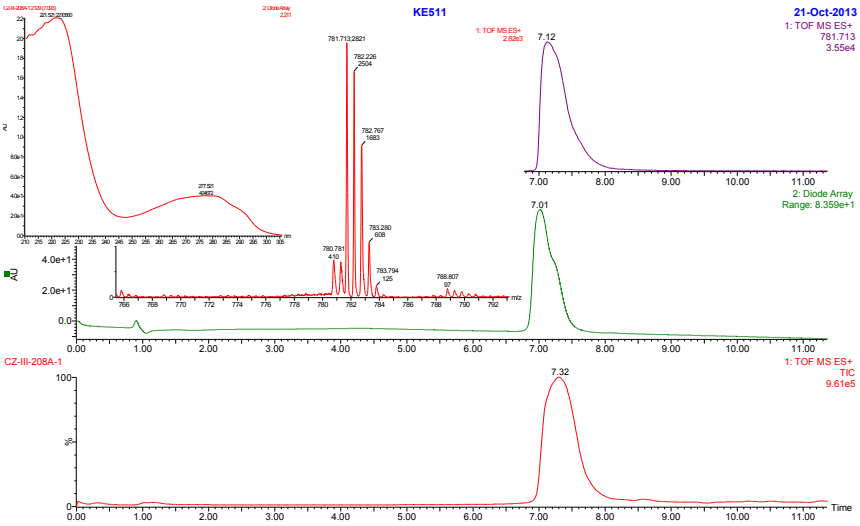


Figure S1. LC-MS profile of purified **7**.

1.6 ^{64}Cu-CB-TE1P1T-Y3-TATE (**6**): To a solution of CB-TE1P1T-Y3-TATE (**7**) (1 nmol) in aqueous NH_4OAc buffer (pH = 8.1, 0.1 M, 100 µL) was added $^{64}Cu(OAc)_2$ (37 MBq). The mixture was vortexed at 70 °C for 10 min. Radio-TLC showed that the reaction was finished. Radio-HPLC confirmed the identity of the radiolabeled product. Radiochemical yield based on radio-TLC and radio-HPLC was more than 95%. The reaction solution was diluted with appropriate buffers for in vitro and in vivo studies without any further purification.

Post-labelling strategy for synthesis of ^{64}Cu-CB-TE1P1T-Y3-TATE (**6**): To a solution of CB-TE1P1P' (**3**) (1 nmol) in aqueous NH_4OAc buffer (pH = 8.1, 0.1 M, 100 µL) was added $^{64}Cu(OAc)_2$ (37 MBq). The mixture was vortexed at 40 °C for 15 min. Radio-TLC showed that the reaction was finished. Radio-HPLC confirmed the identity of the radiolabeled product. Radiochemical yield based on radio-TLC and radio-HPLC was more than 95%. Then (N_3)-K-Y3-TATE (**5**) (1 nmol) was added, followed by $CuSO_4$, TBTA, and

sodium ascorbate. The reaction mixture was heated to 90 °C for 15 min. Radio-HPLC showed 51% conversion of the radiolabelled ^{64}Cu-CB-TE1P1P' (**4**), to ^{64}Cu-CB-TE1P1T-Y3-TATE (**6**).

Table 1. Reaction conditions of click reaction: concentration of reagents and catalysts.

Reagents	Concentration
CB-TE1P1P' (**3**)	3.2 µM
(N_3)-K-Y3-TATE (**5**)	16 µM
$CuSO_4$	1 mM
TBTA	1 mM
Na-Asc	3 mM

Table 2. Reaction conditions of click reaction: reaction times and corresponding yields.

Reaction Time (min) at 90 °C	$^{64}Cu(OAc)_2$	^{64}Cu-CB-TE1P1P' (**4**)	^{64}Cu-CB-TE1P1T-Y3-TATE (**6**)
5	none	60%	40%
10	none	55%	45%
15	none	49%	51%

2. Biodistribution of 6 and 1A1P

All animal studies were conducted according to the procedures outlined by the University of Pittsburgh and Washington University Institutional Animal Care and Use Committees (IACUC). Human colorectal HCT116 cells (kindly provided by Dr. Bert Vogelstein, Johns Hopkins University) were transfected with sstr2 as previously described.[1] Cell media (Iscove's) was obtained from Gibco (Carlsbad, California) and supplemented with 10% fetal bovine serum (FBS, Gibco) and 1 mM Zeocin (Gibco). Female, mice 4-6 weeks old athymic nude from Taconic (Hudson, New York) were injected with 0.8-1.2 million of sstr2-positive HCT116 tumor cells mixed with Matrigel, and were allowed to grow for 9-12 days.

Table 3. Biodistribution of Clicked **6**

	1h	4h	4h block	24 h
Blood	0.34 ± 0.13	0.20 ± 0.03	0.22 ± 0.04	0.15 ± 0.06
Liver	1.13 ± 0.16	1.30 ± 0.10	1.32 ± 0.30	1.05 ± 0.10
Kidney	15.87 ± 3.03	21.03 ± 2.84	16.13 ± 5.03	14.68 ± 2.53
Muscle	0.22 ± 0.15	0.07 ± 0.02	0.09 ± 0.03	0.06 ± 0.00
Bone	0.46 ± 0.16	0.50 ± 0.24	0.20 ± 0.04	0.35 ± 0.10
Adrenal	1.14 ± 0.46	1.24 ± 0.40	0.37 ± 0.05	1.82 ± 1.83
Pancreas	6.71 ± 0.22	4.41 ± 0.95	0.30 ± 0.07	1.84 ± 0.64
Tumour	4.73 ± 1.17	7.54 ± 1.43	0.69 ± 0.22	4.39 ± 1.71

Table 4. Biodistribution of ^{64}Cu-CB-TE1A1P-Y3-TATE (1A1P)

	1h	4h	4h block	24 h
Blood	0.24 ± 0.06	0.18 ± 0.01	0.19 ± 0.05	0.12 ± 0.03
Liver	1.24 ± 0.24	1.89 ± 0.29	1.38 ± 0.22	1.30 ± 0.28
Kidney	8.96 ± 1.75	8.93 ± 1.65	6.73 ± 1.52	5.14 ± 1.01
Muscle	0.07 ± 0.00	0.10 ± 0.03	0.07 ± 0.01	0.06 ± 0.01
Bone	0.44 ± 0.18	0.47 ± 0.26	0.20 ± 0.04	0.17 ± 0.08
Adrenal	0.93 ± 0.45	1.35 ± 0.21	0.43 ± 0.07	0.67 ± 0.16
Pancreas	7.18 ± 2.14	5.64 ± 1.34	0.27 ± 0.03	0.86 ± 0.12
Tumour	7.94 ± 3.10	12.52 ± 2.65	0.64 ± 0.06	5.53 ± 0.94

1. K. Nguyen, J. J. Parry, B. E. Rogers and C. J. Anderson, *Nucl. Med. Biol.*, 2012, **39**, 187-197.

3. NMR Spectra of 2 and 3 — Pages

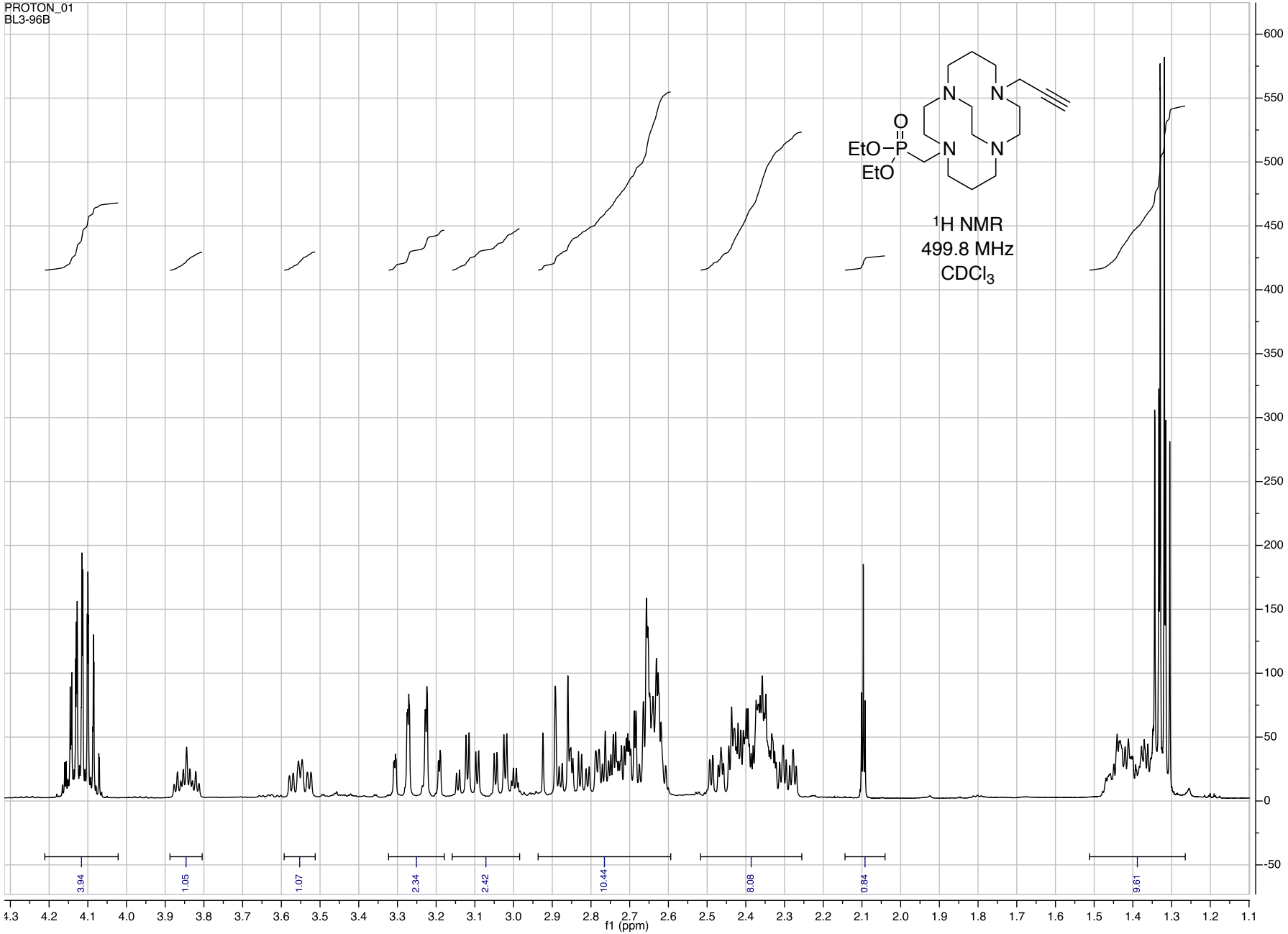
^{1}H NMR spectra of **2** S10-S23

^{13}C{^{1}H} NMR spectra of **2** S24-S28

^{1}H NMR spectra of **2**•HBr•H_2O S29-S36

^{1}H NMR spectra of **3**•1.24 TFA S37-S58

^{13}C{^{1}H} NMR spectra of **3**•1.24 TFA S59-S65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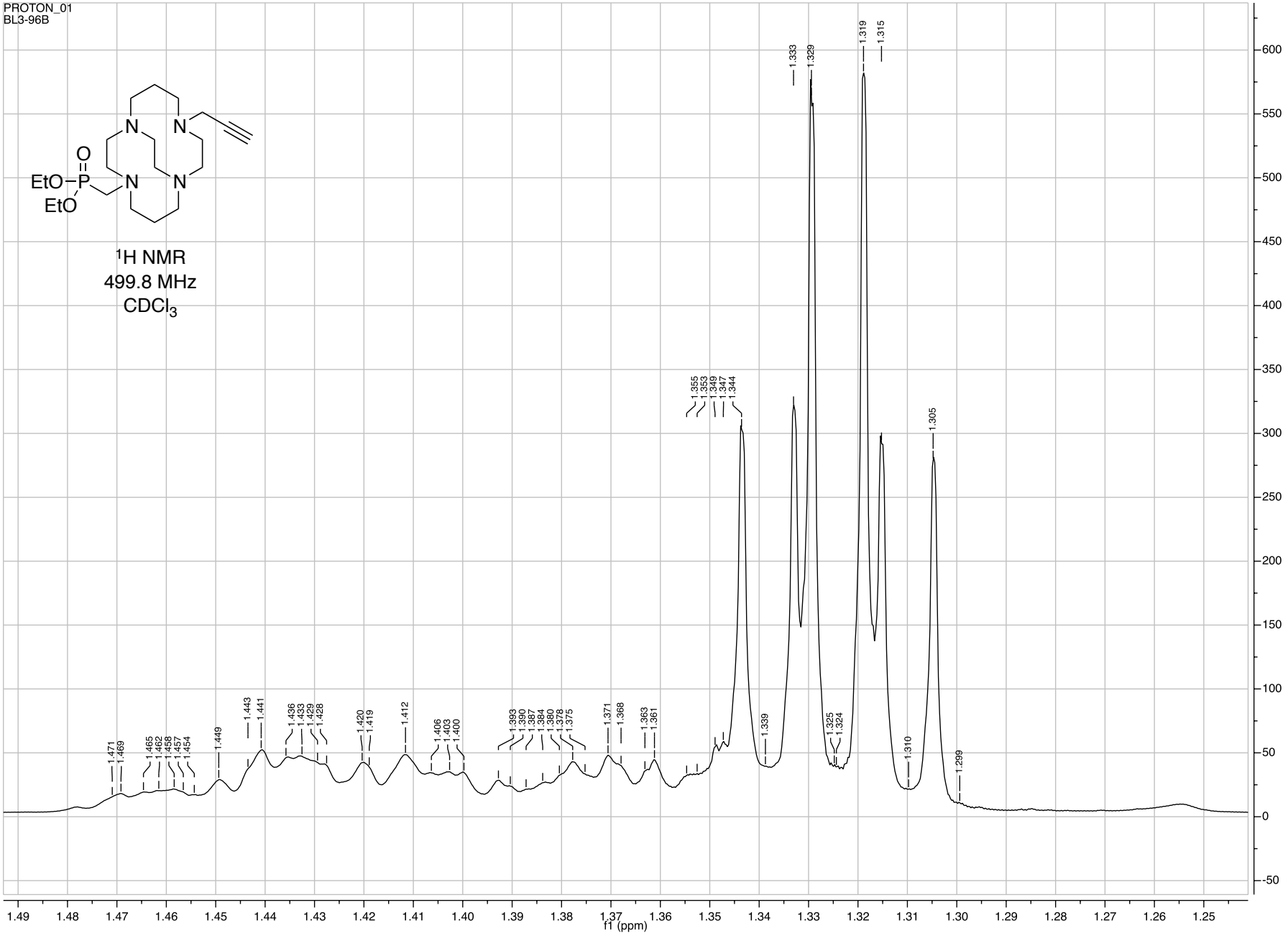
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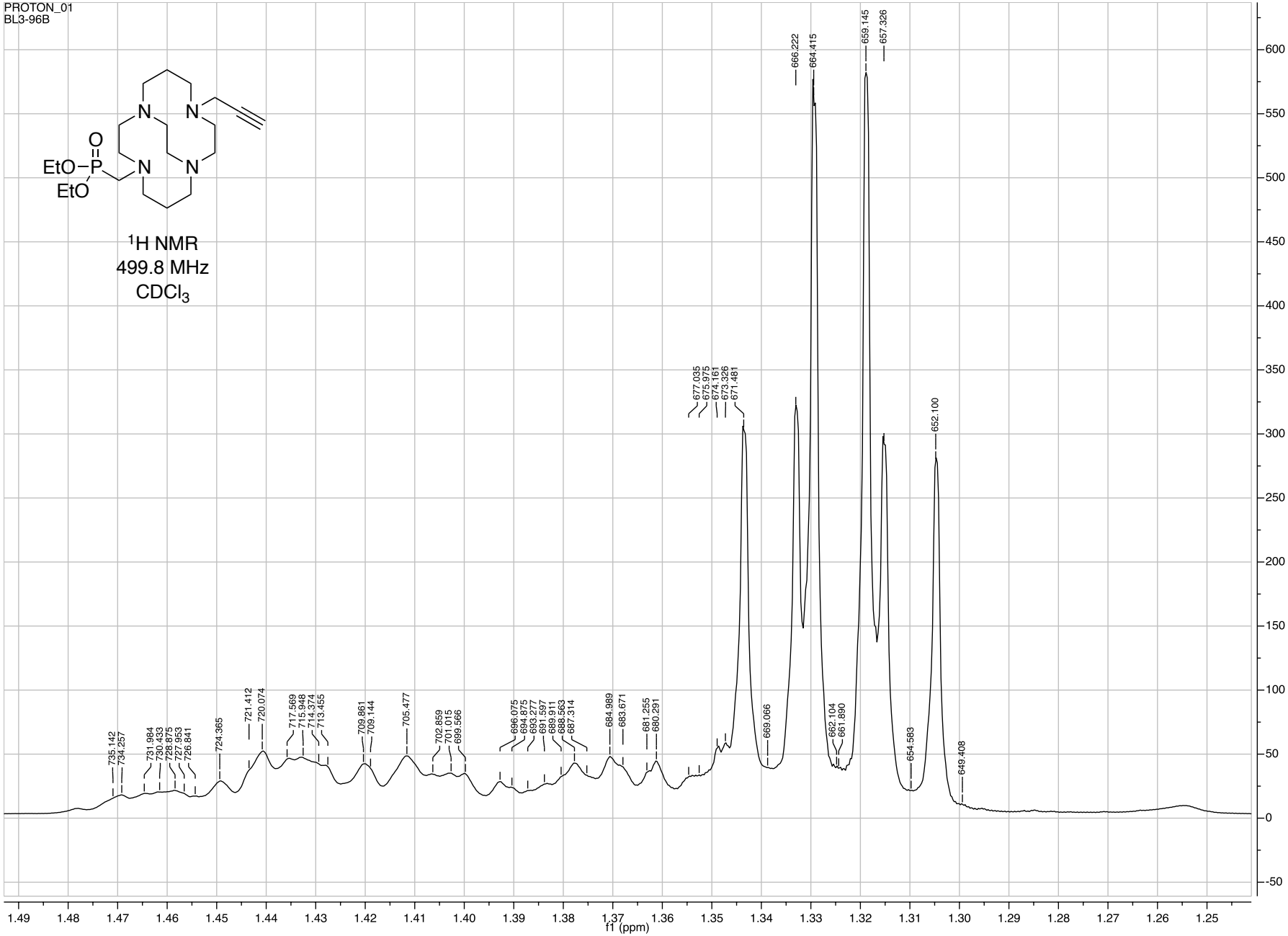
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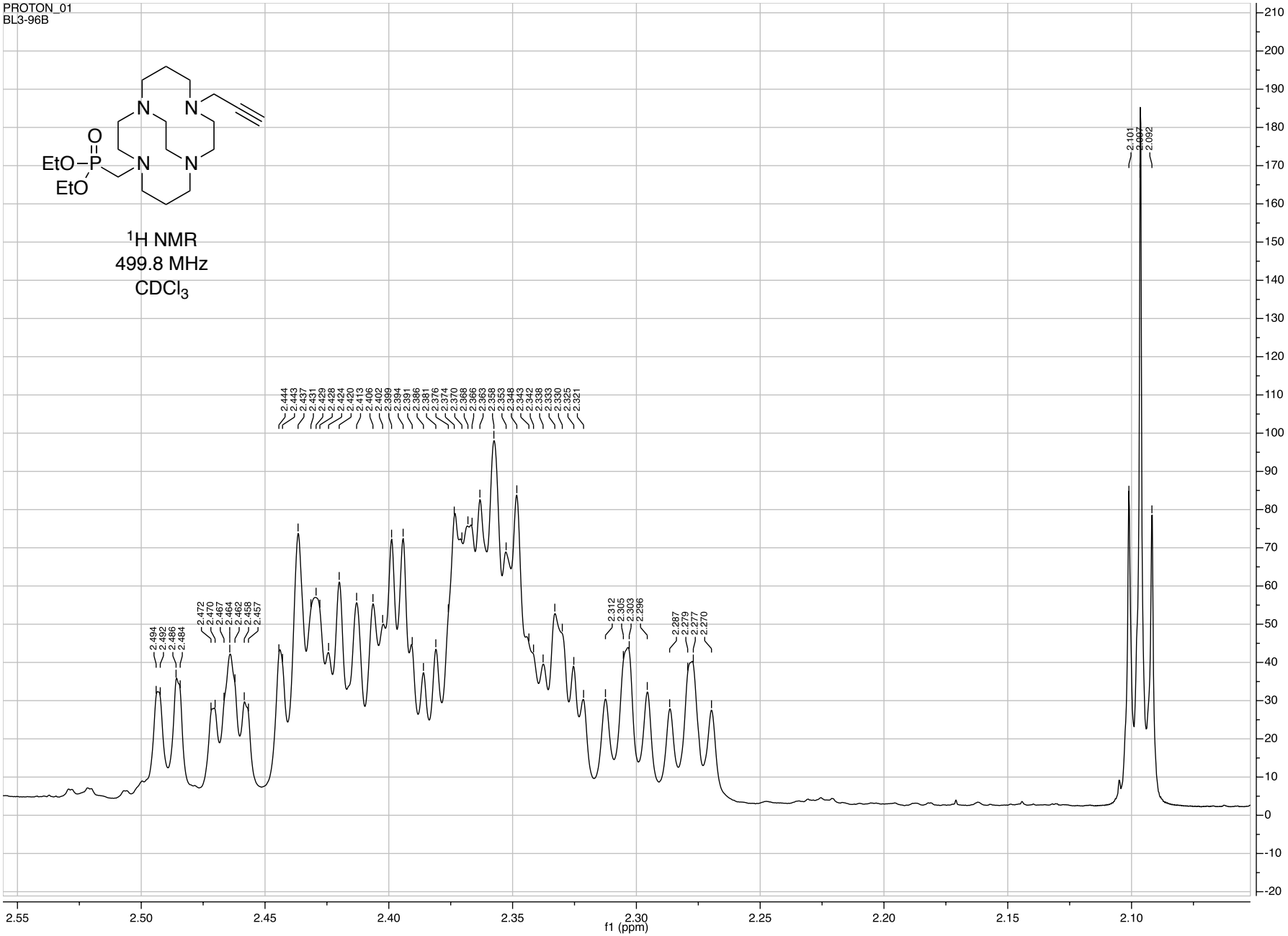
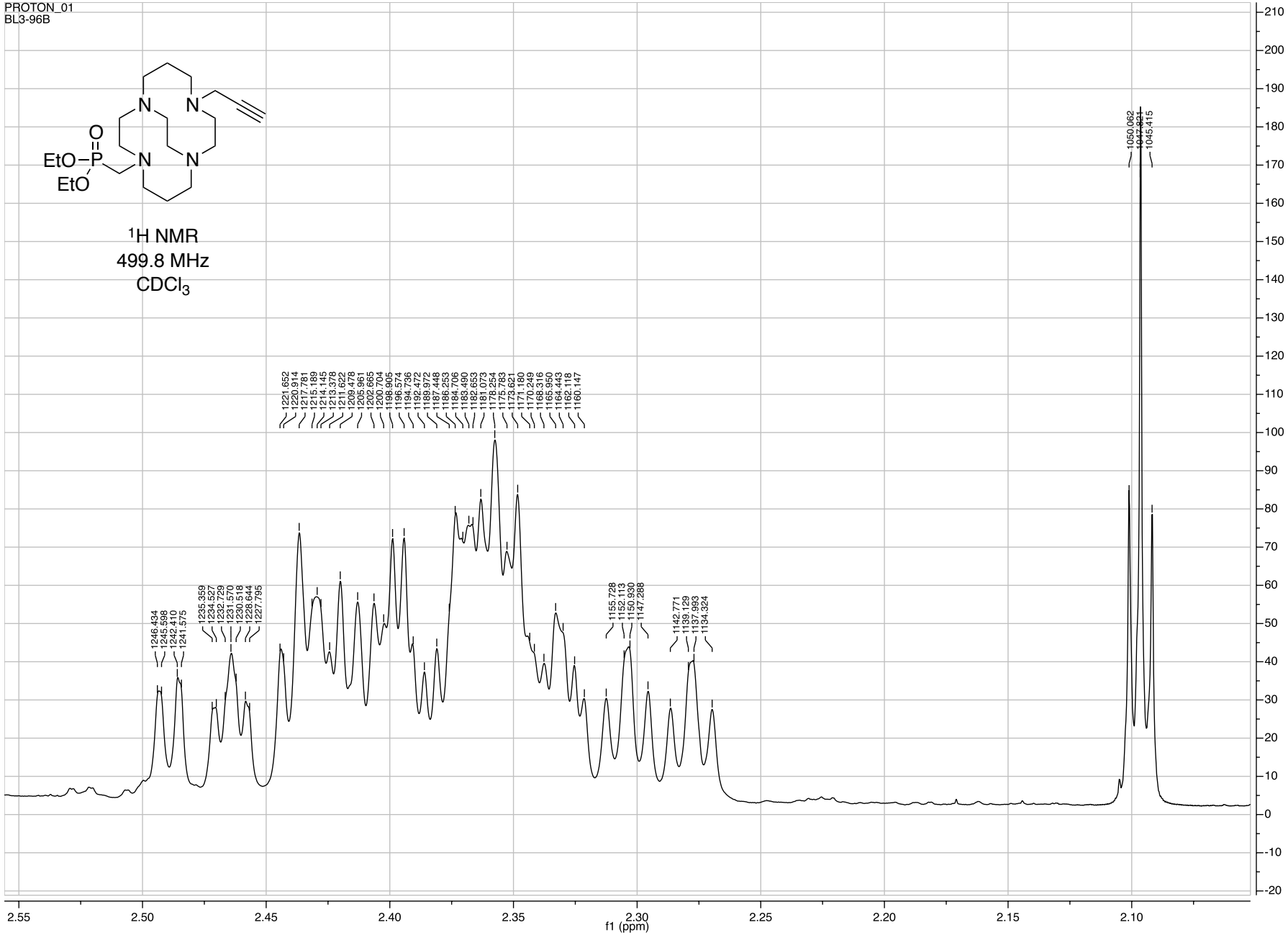
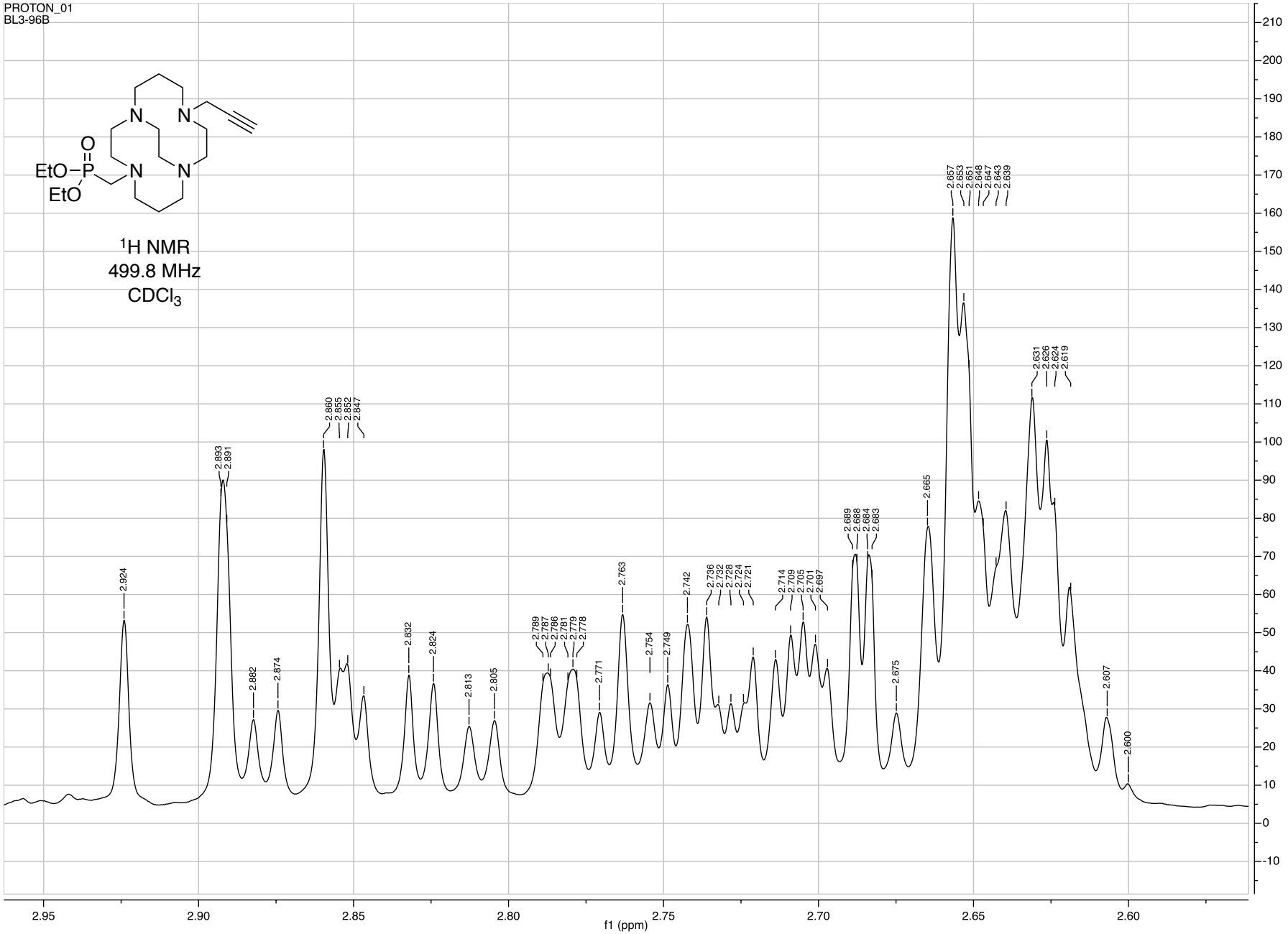
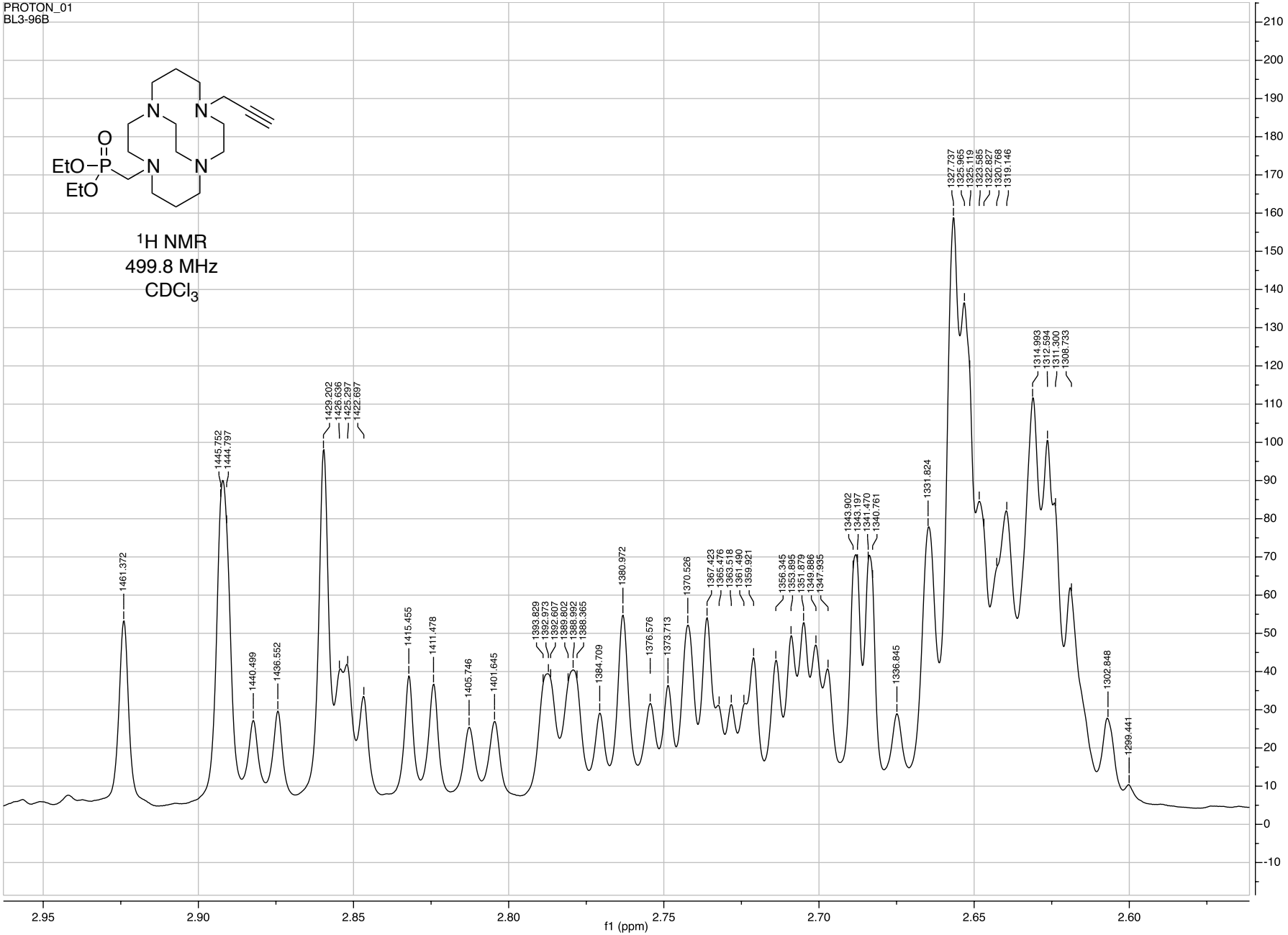
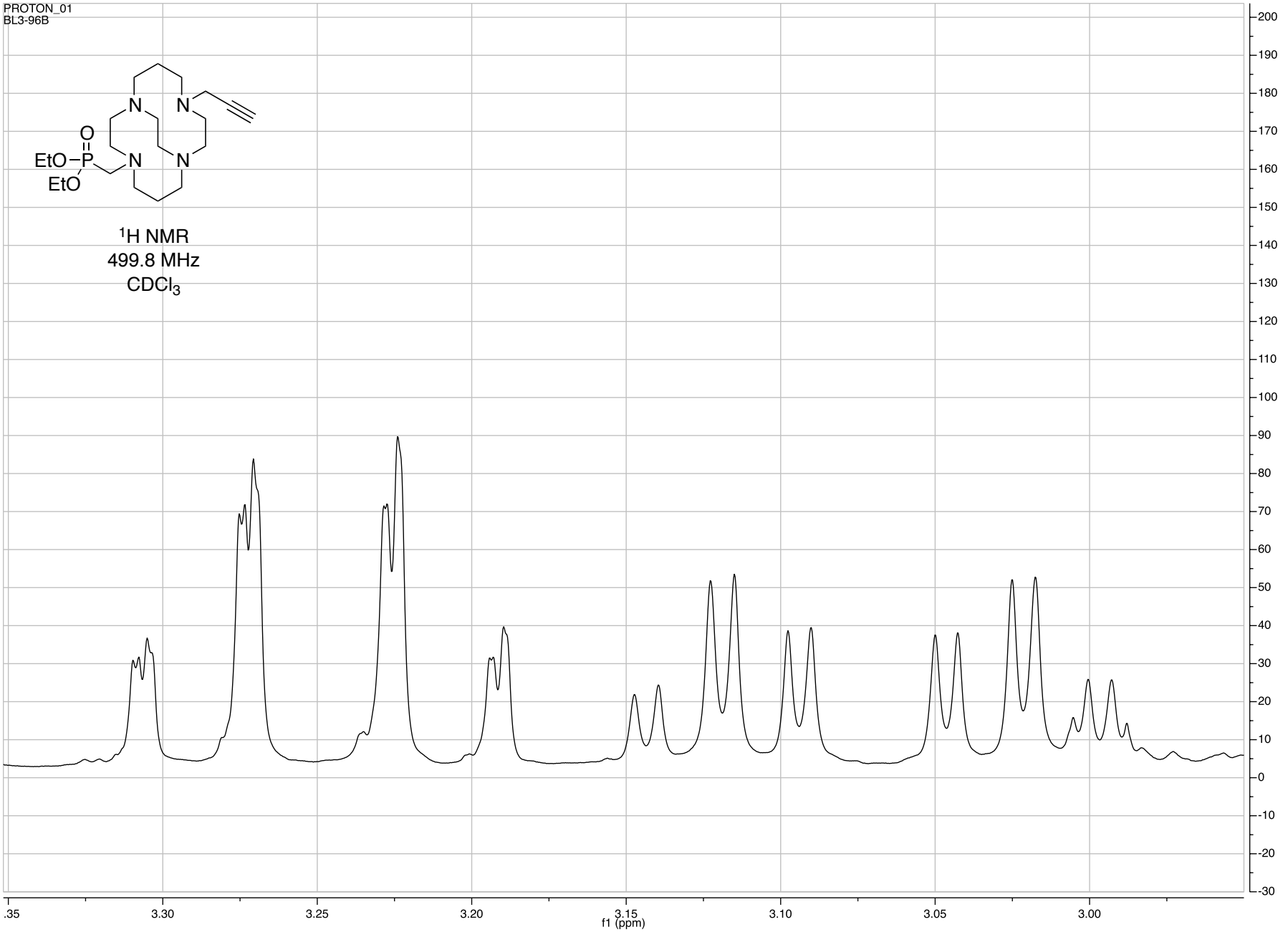
f1 (ppm)

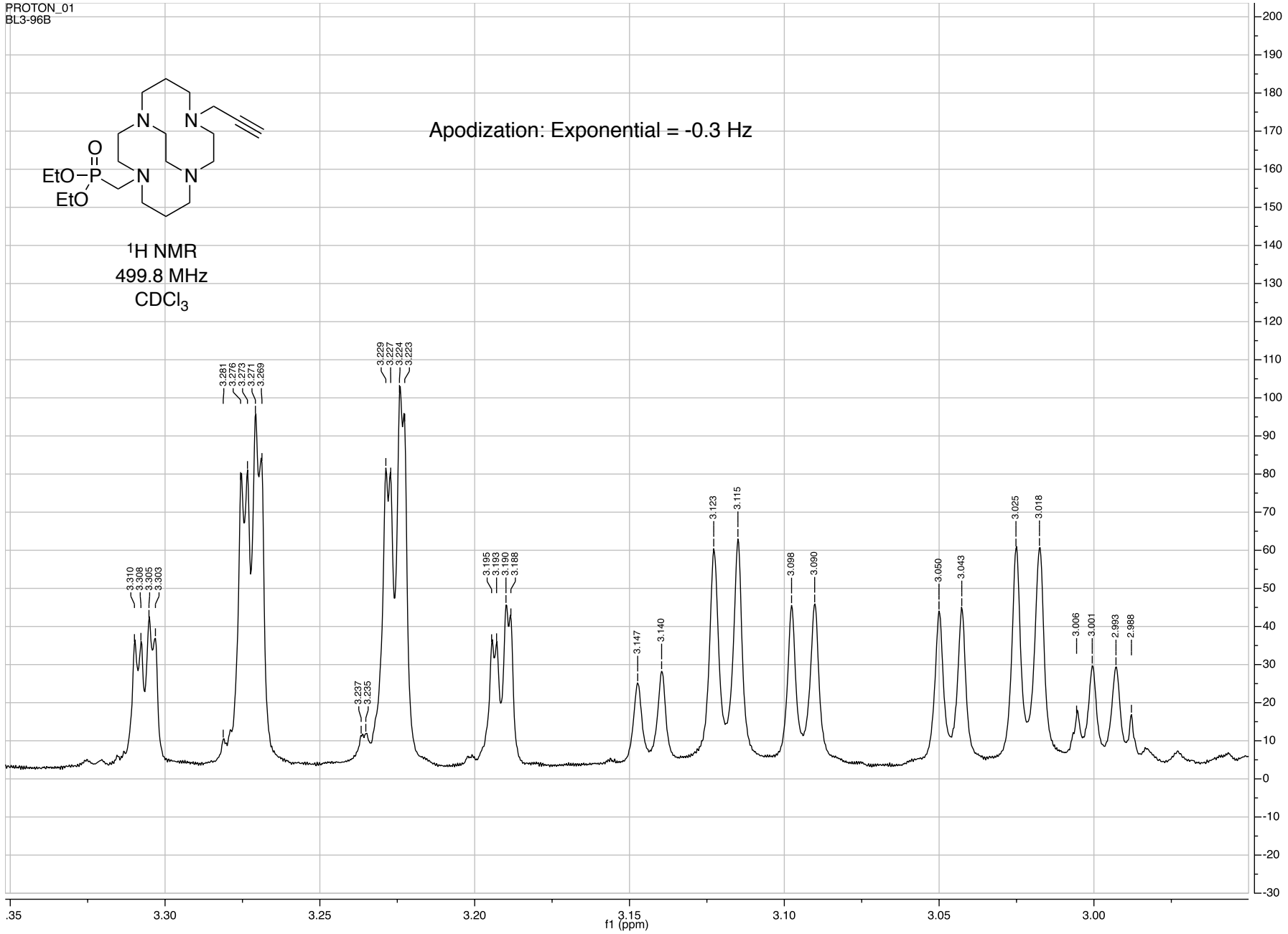

PROTON_01
BL3-96B
EtO
EtO
O
P
N
N
N
N
^{1}H NMR
499.8 MHz
$CDCl_3$
2.494
2.492
2.486
2.484
2.472
2.470
2.467
2.464
2.462
2.458
2.457
2.444
2.443
2.437
2.431
2.429
2.428
2.424
2.420
2.413
2.406
2.402
2.399
2.394
2.391
2.386
2.381
2.376
2.374
2.370
2.368
2.366
2.363
2.358
2.353
2.348
2.343
2.342
2.338
2.333
2.330
2.325
2.321
2.312
2.305
2.303
2.296
2.287
2.279
2.277
2.270
2.101
2.097
2.092
f1 (ppm)

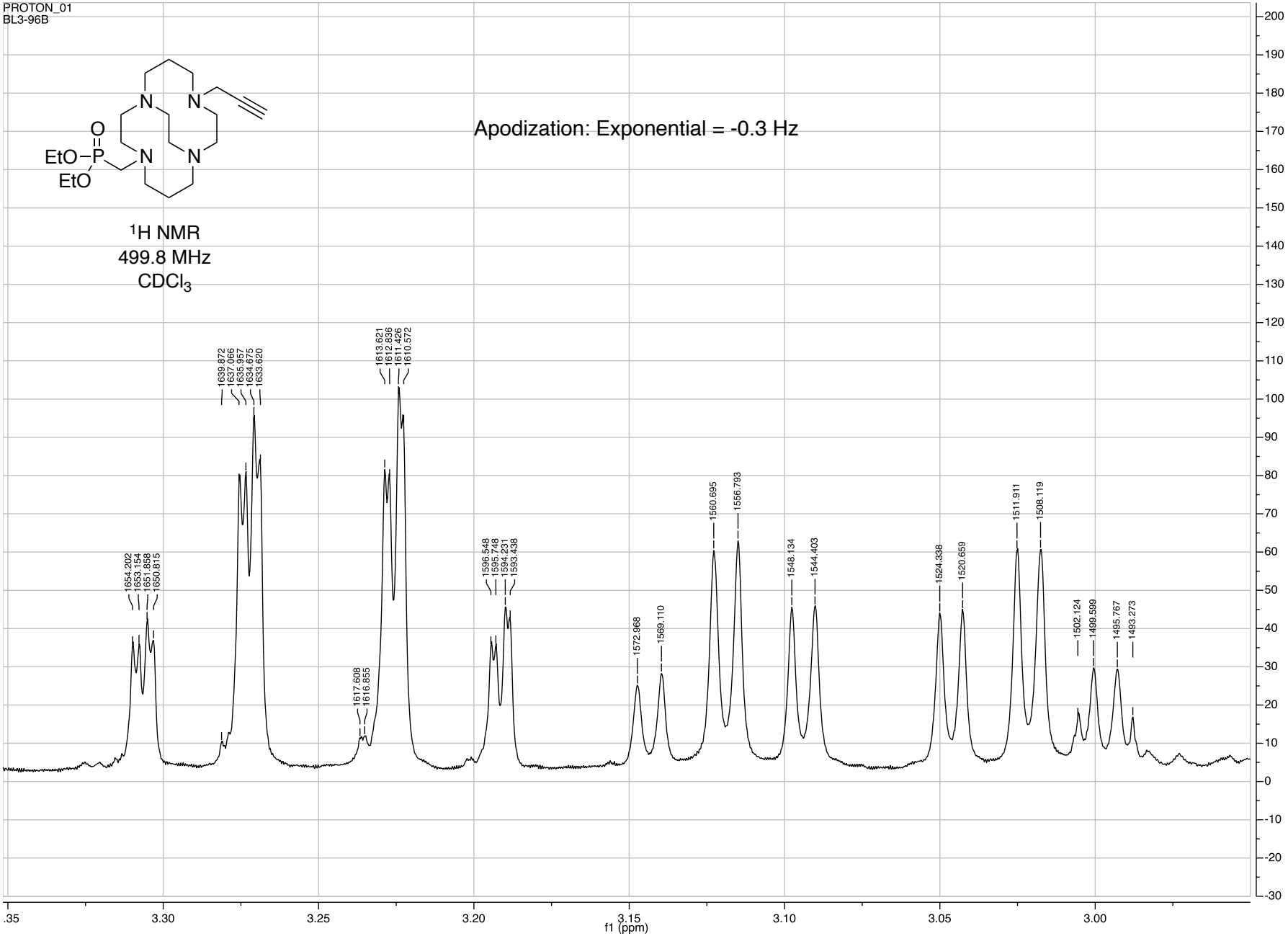

PROTON_01
BL3-96B
EtO
EtO
O
P
N
N
N
N
1H NMR
499.8 MHz
CDCl3
1246.434
1245.598
1242.410
1241.575
1235.359
1234.527
1232.729
1231.570
1230.518
1228.644
1227.795
1221.652
1220.914
1217.781
1215.189
1214.145
1213.378
1211.622
1209.478
1205.961
1202.665
1200.704
1198.905
1196.574
1194.736
1192.472
1189.972
1187.448
1186.253
1184.706
1183.490
1182.653
1181.073
1178.254
1175.783
1173.621
1171.180
1170.249
1168.316
1165.950
1164.443
1162.118
1160.147
1155.728
1152.113
1150.930
1147.288
1142.771
1139.129
1137.993
1134.324
1050.062
1047.821
1045.415
f1 (ppm)

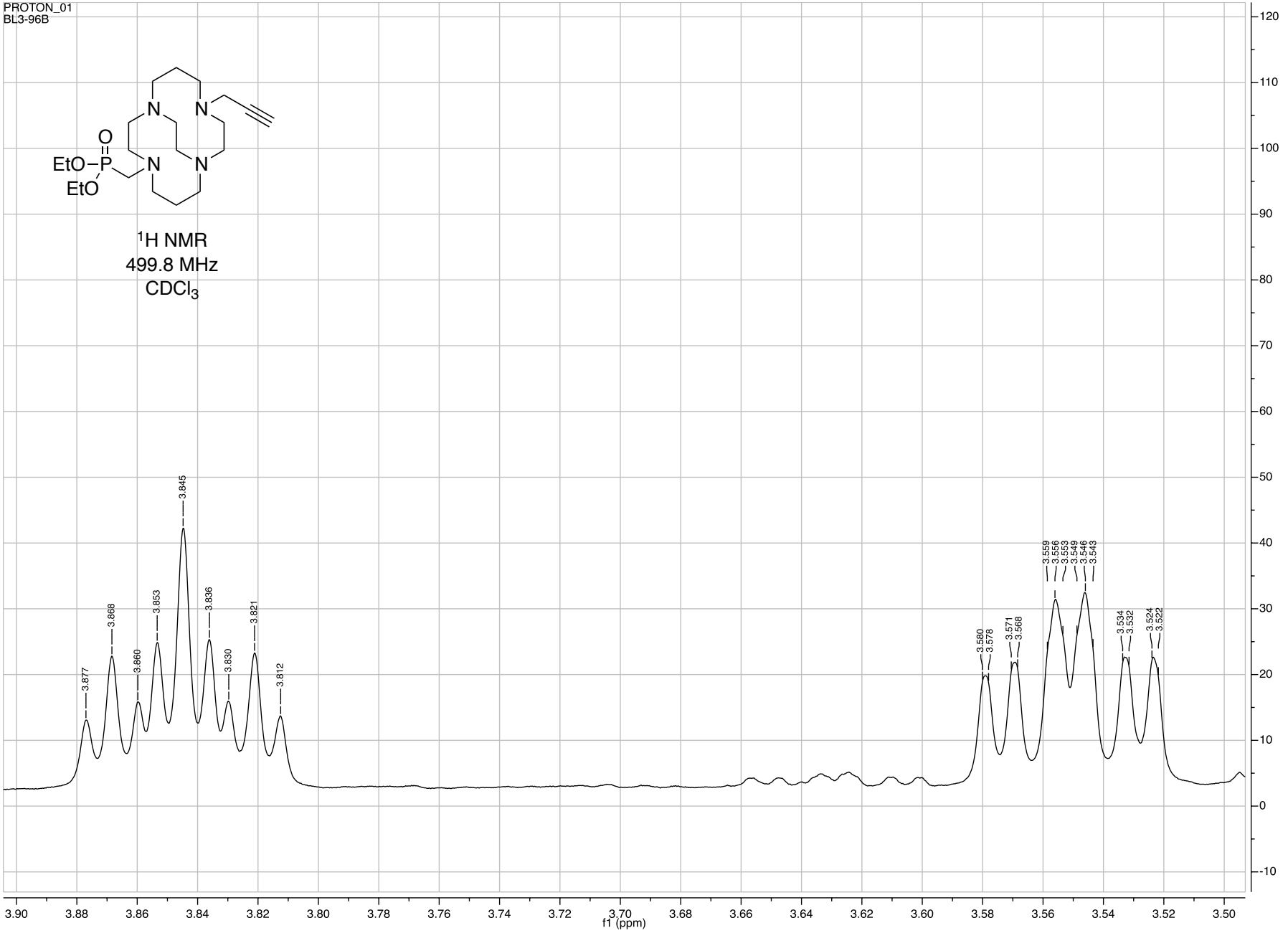

PROTON_01
BL3-96B
EtO–P
EtO
O
N
N
N
N
^{1}H NMR
499.8 MHz
$CDCl_3$
2.924
2.893
2.891
2.882
2.874
2.860
2.855
2.852
2.847
2.832
2.824
2.813
2.805
2.789
2.787
2.786
2.781
2.779
2.778
2.771
2.763
2.754
2.749
2.742
2.736
2.732
2.728
2.724
2.721
2.714
2.709
2.705
2.701
2.697
2.689
2.688
2.684
2.683
2.675
2.665
2.657
2.653
2.651
2.648
2.647
2.643
2.639
2.631
2.626
2.624
2.619
2.607
2.600
f1 (ppm)

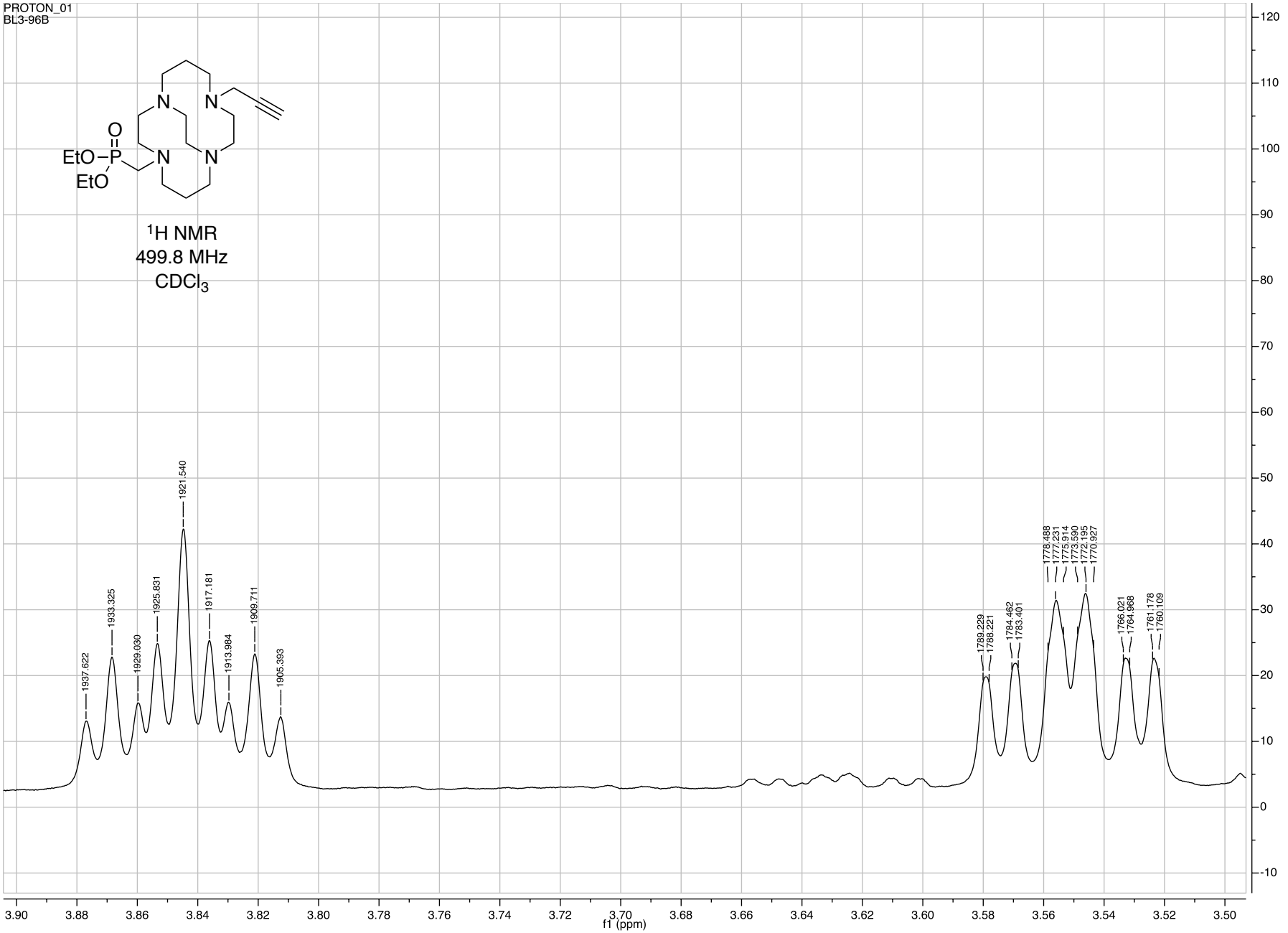

PROTON_01
BL3-96B
EtO
EtO
O
P
N
N
N
N
^{1}H NMR
499.8 MHz
$CDCl_3$
1461.372
1445.752
1444.797
1440.499
1436.552
1429.202
1426.636
1425.297
1422.697
1415.455
1411.478
1405.746
1401.645
1393.829
1392.973
1392.607
1389.802
1388.992
1388.365
1384.709
1380.972
1376.576
1373.713
1370.526
1367.423
1365.476
1363.518
1361.490
1359.921
1356.345
1353.895
1351.879
1349.886
1347.935
1343.902
1343.197
1341.470
1340.761
1336.845
1331.824
1327.737
1325.965
1325.119
1323.585
1322.827
1320.768
1319.146
1314.993
1312.594
1311.300
1308.733
1302.848
1299.441
2.95
2.90
2.85
2.80
2.75
2.70
2.65
2.60
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

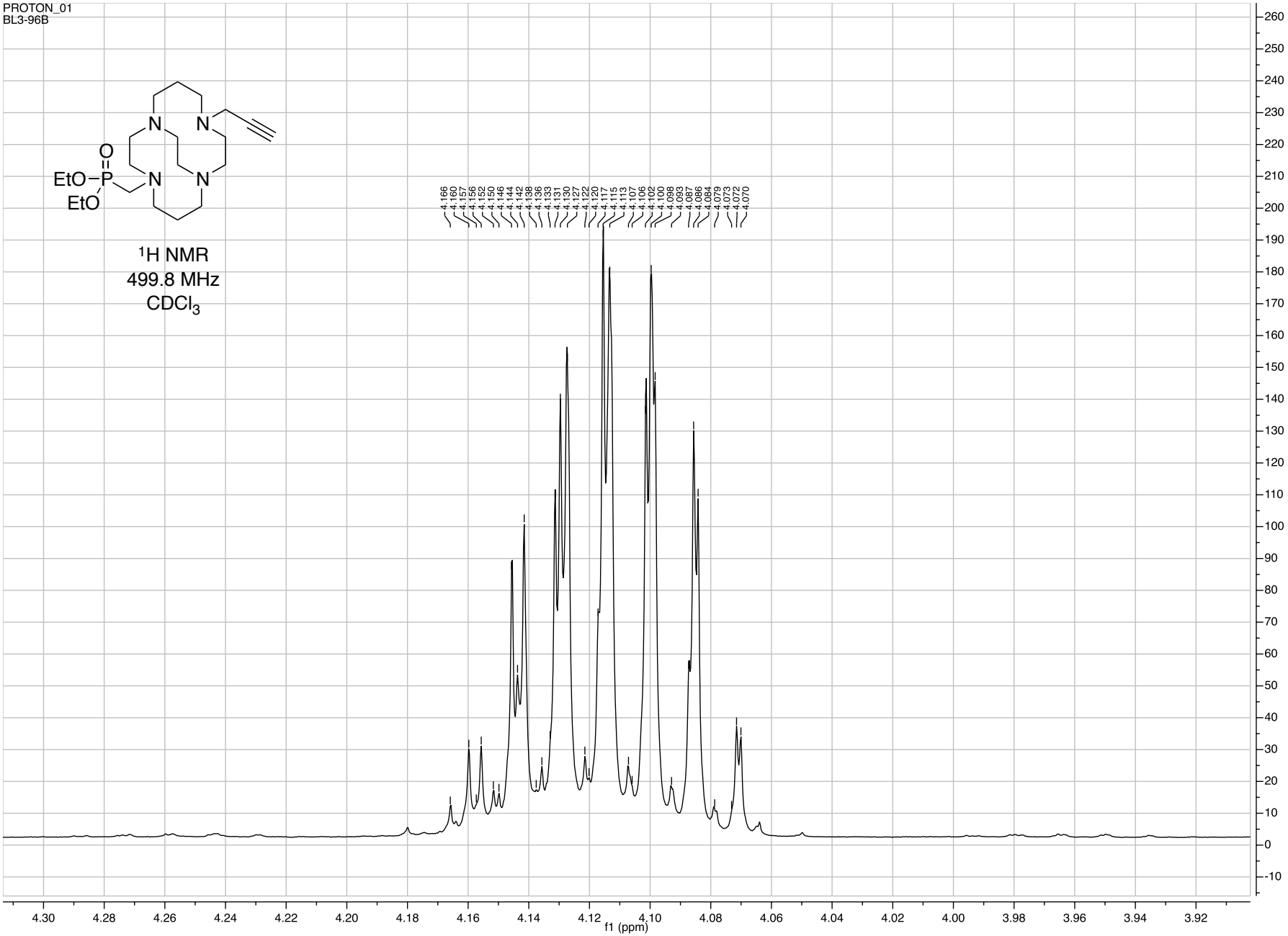

PROTON_01
BL3-96B
O
EtO
P
EtO
N
N
N
N
1H NMR
499.8 MHz
CDCl3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
.35
3.30
3.25
3.20
3.15
3.10
3.05
3.00
f1 (ppm)

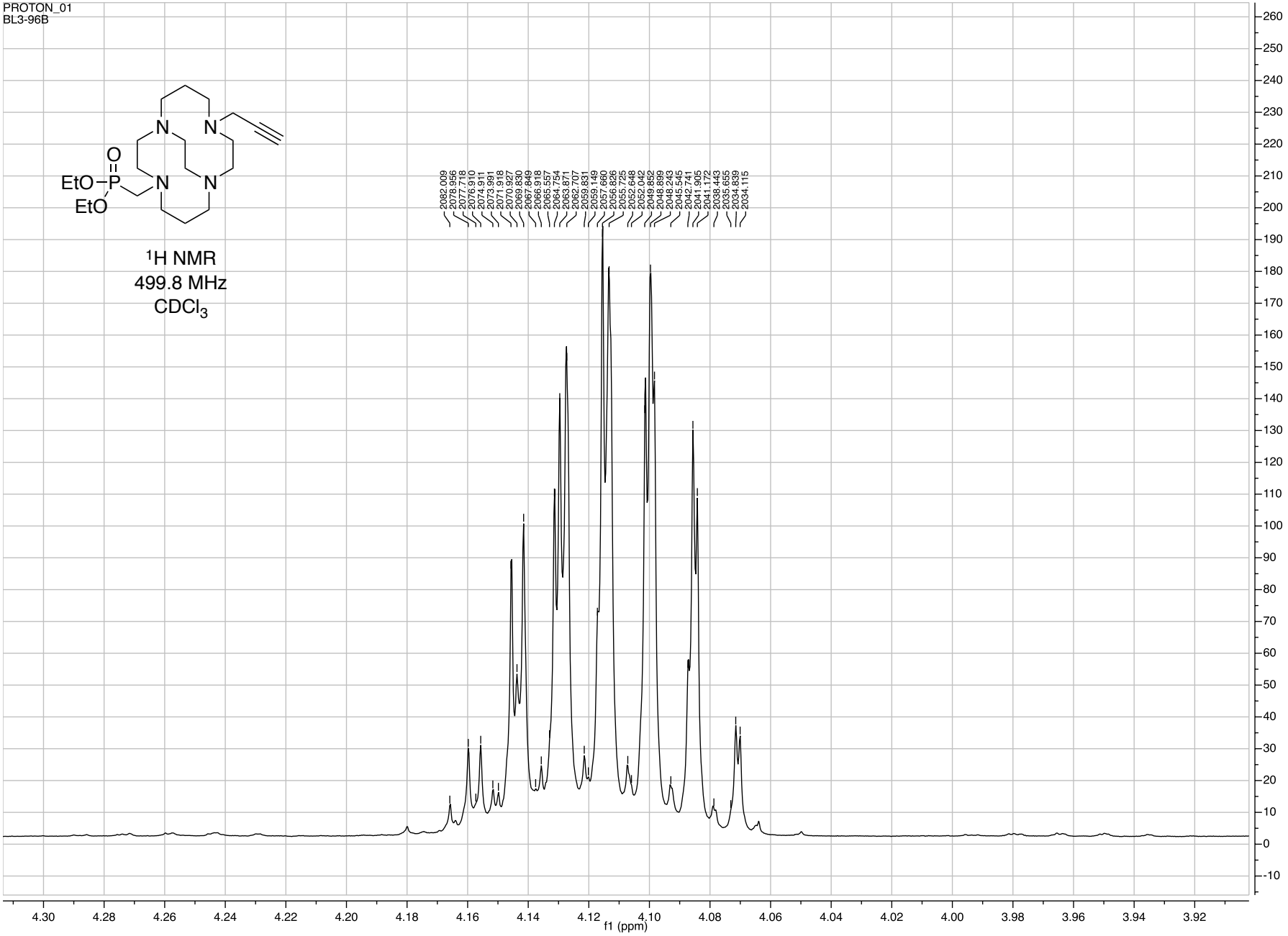

PROTON_01
BL3-96B
Apodization: Exponential = -0.3 Hz
^{1}H NMR
499.8 MHz
$CDCl_3$
3.310
3.308
3.305
3.303
3.281
3.276
3.273
3.271
3.269
3.237
3.235
3.229
3.227
3.224
3.223
3.195
3.193
3.190
3.188
3.147
3.140
3.123
3.115
3.098
3.090
3.050
3.043
3.025
3.018
3.006
3.001
2.993
2.988
f1 (ppm)

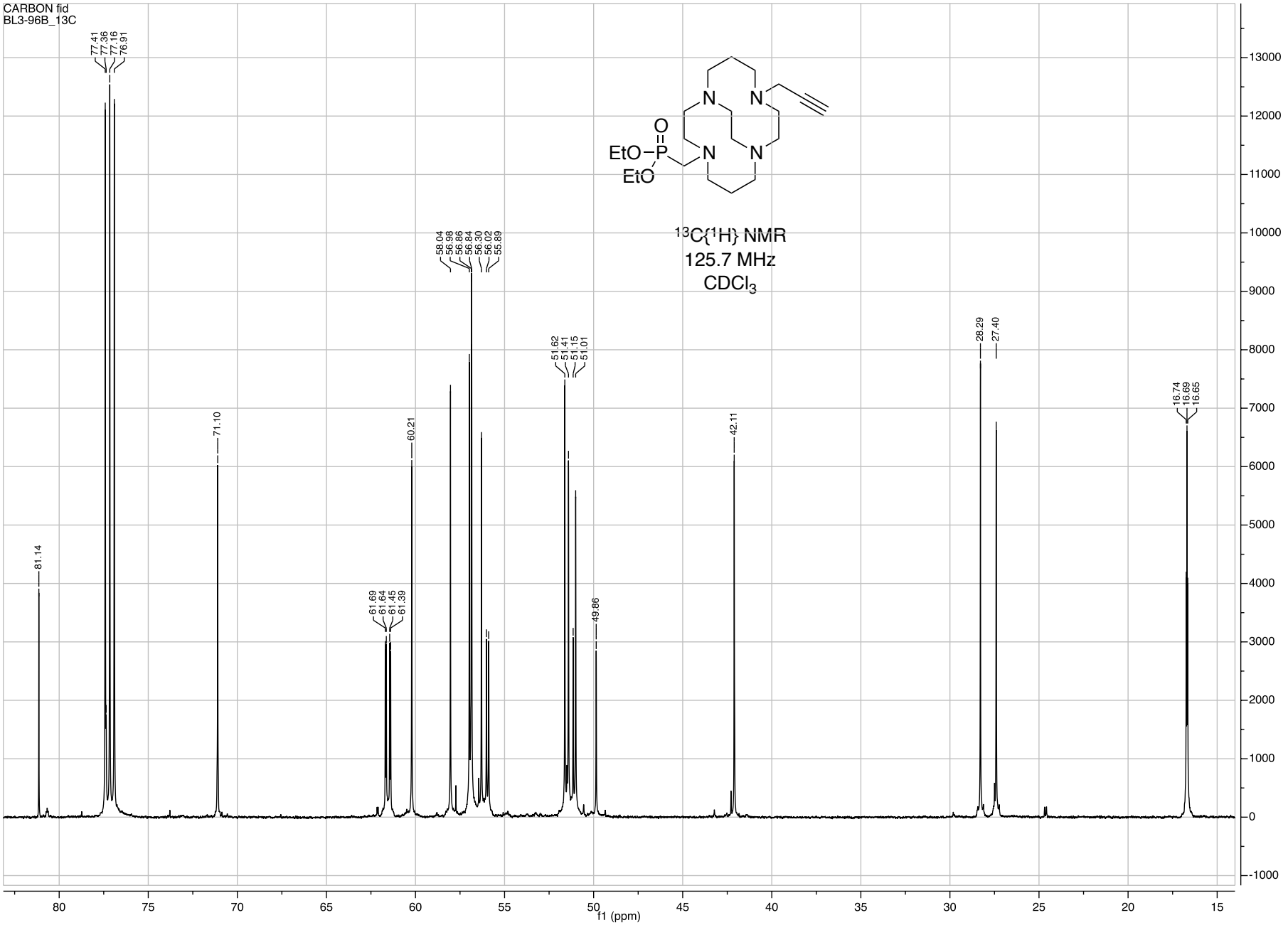

PROTON_01
BL3-96B
Apodization: Exponential = -0.3 Hz
1H NMR
499.8 MHz
CDCl3
1654.202
1653.154
1651.858
1650.815
1639.872
1637.066
1635.957
1634.675
1633.620
1617.608
1616.855
1613.621
1612.836
1611.426
1610.572
1596.548
1595.748
1594.231
1593.438
1572.968
1569.110
1560.695
1556.793
1548.134
1544.403
1524.338
1520.659
1511.911
1508.119
1502.124
1499.599
1495.767
1493.273
f1 (ppm)

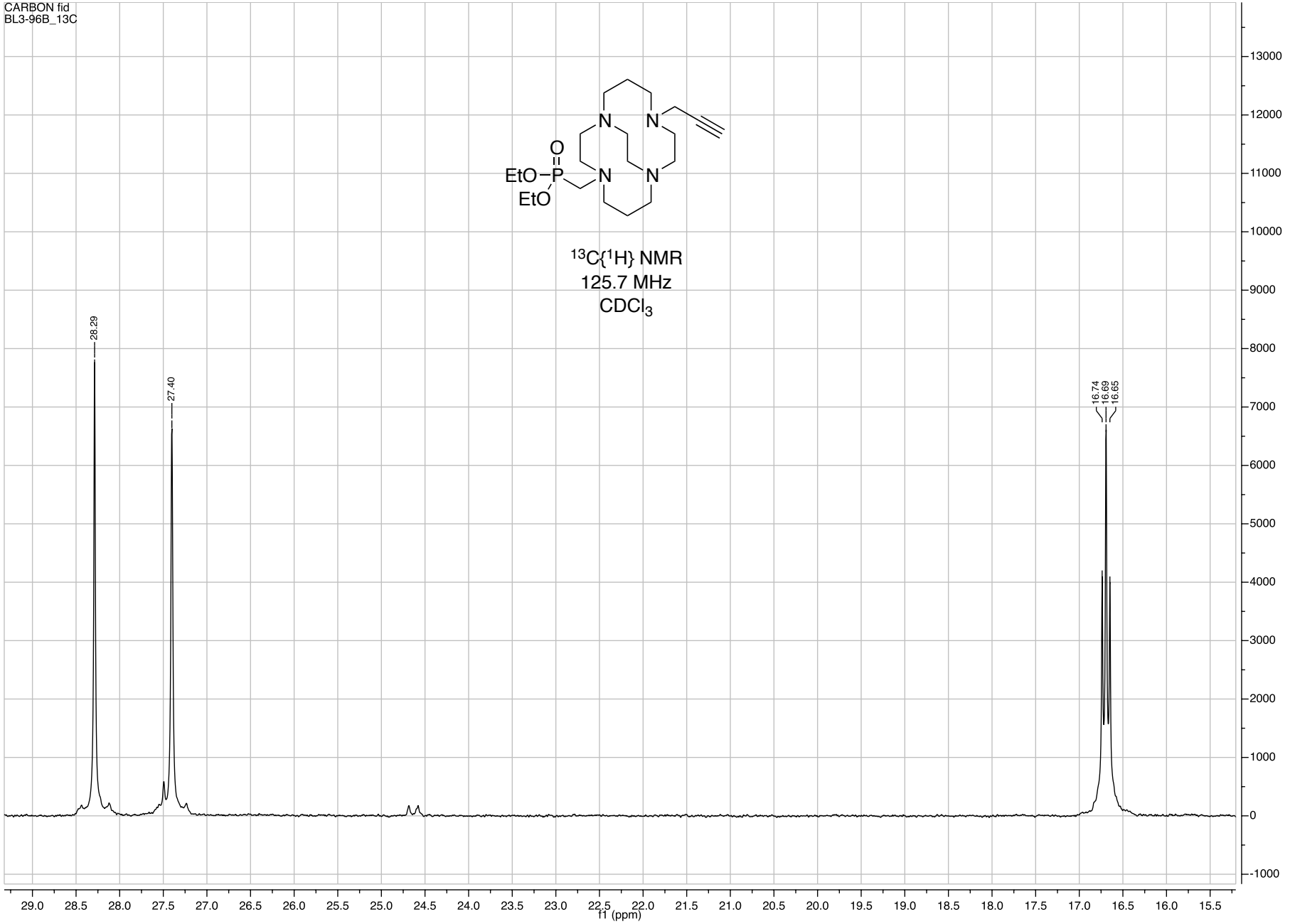

PROTON_01
BL3-96B
^{1}H NMR
499.8 MHz
$CDCl_3$
3.877
3.868
3.860
3.853
3.845
3.836
3.830
3.821
3.812
3.580
3.578
3.571
3.568
3.559
3.556
3.553
3.549
3.546
3.543
3.534
3.532
3.524
3.522
f1 (ppm)


PROTON_01
BL3-96B
EtO
EtO
O
P
N
N
N
N
^{1}H NMR
499.8 MHz
$CDCl_3$
1937.622
1933.325
1929.030
1925.831
1921.540
1917.181
1913.984
1909.711
1905.393
1789.229
1788.221
1784.462
1783.401
1778.488
1777.231
1774.614
1773.590
1772.195
1770.927
1766.021
1764.968
1761.178
1760.109
f1 (ppm)

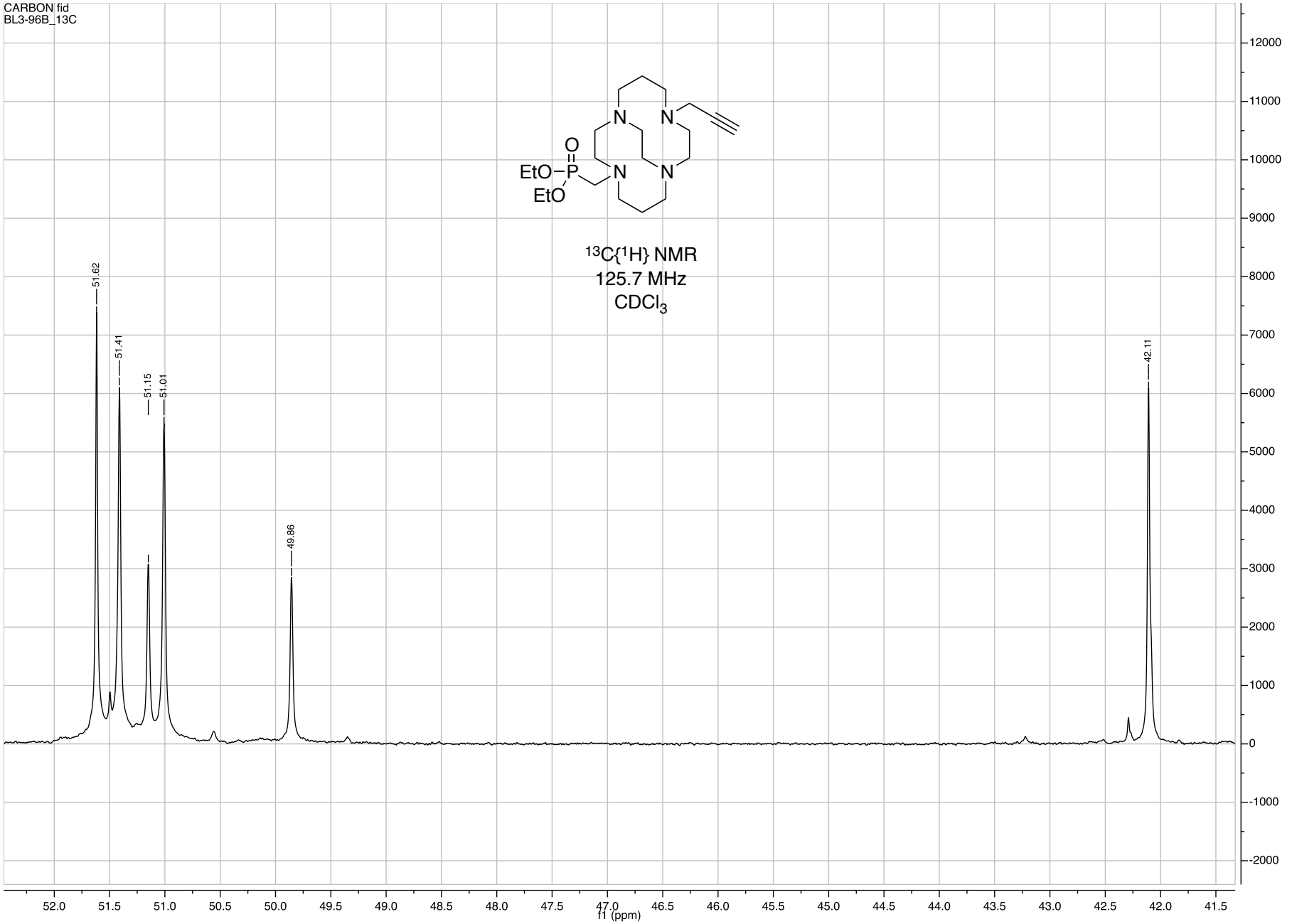

PROTON_01
BL3-96B
EtO
EtO
O
P
N
N
N
N
1H NMR
499.8 MHz
CDCl3
4.166
4.160
4.157
4.156
4.152
4.150
4.146
4.144
4.142
4.138
4.136
4.133
4.131
4.130
4.127
4.122
4.120
4.117
4.115
4.113
4.107
4.106
4.102
4.100
4.098
4.093
4.087
4.086
4.084
4.079
4.073
4.072
4.070
f1 (ppm)

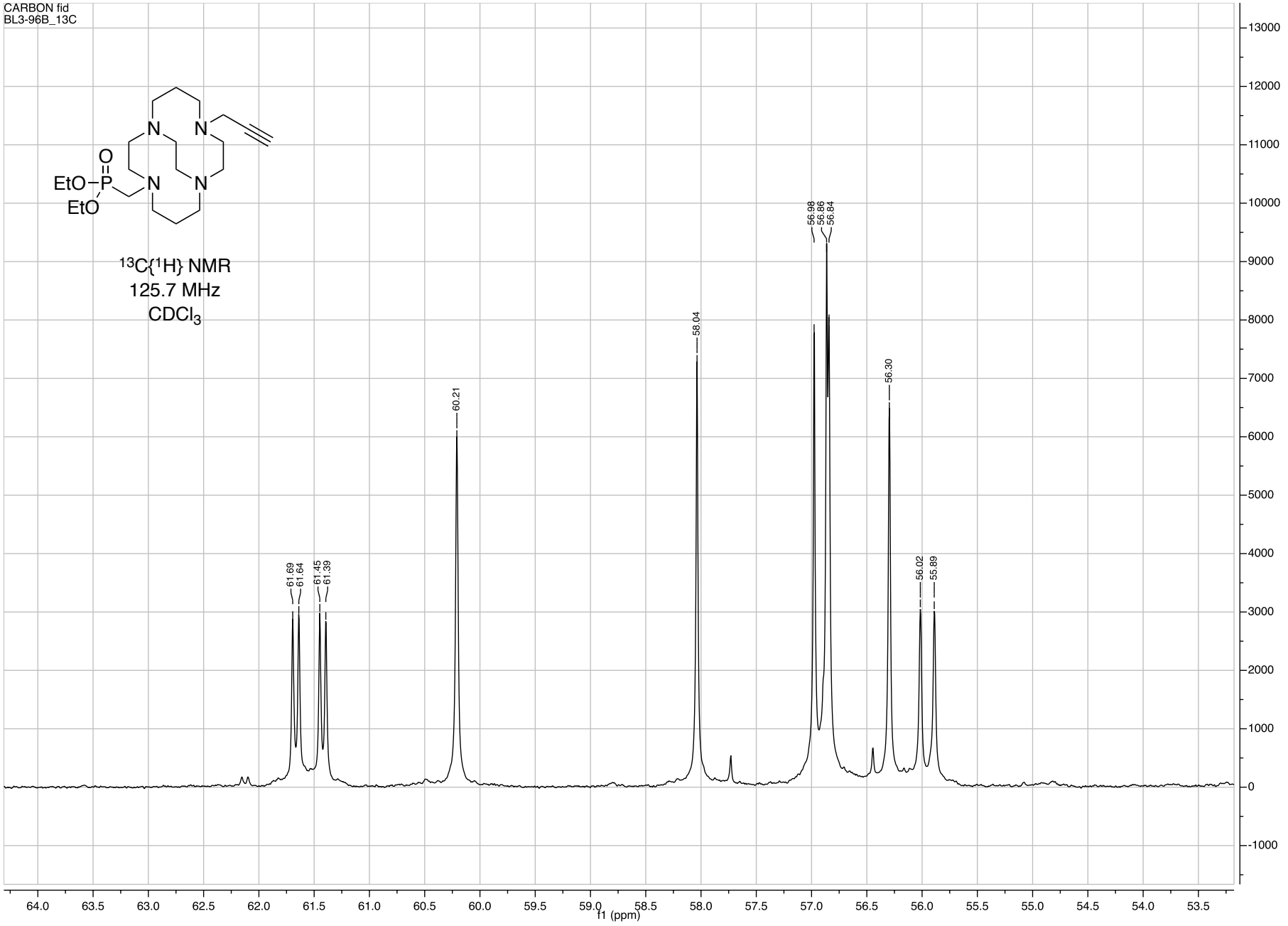

PROTON_01
BL3-96B
^{1}H NMR
499.8 MHz
$CDCl_3$
2082.009
2078.956
2077.718
2076.910
2074.911
2073.991
2071.918
2070.927
2069.830
2067.849
2066.918
2065.557
2064.754
2063.871
2062.707
2059.831
2059.149
2057.660
2056.826
2055.725
2052.648
2052.042
2049.852
2048.899
2048.243
2045.545
2042.741
2041.905
2041.172
2038.443
2035.655
2034.839
2034.115
f1 (ppm)

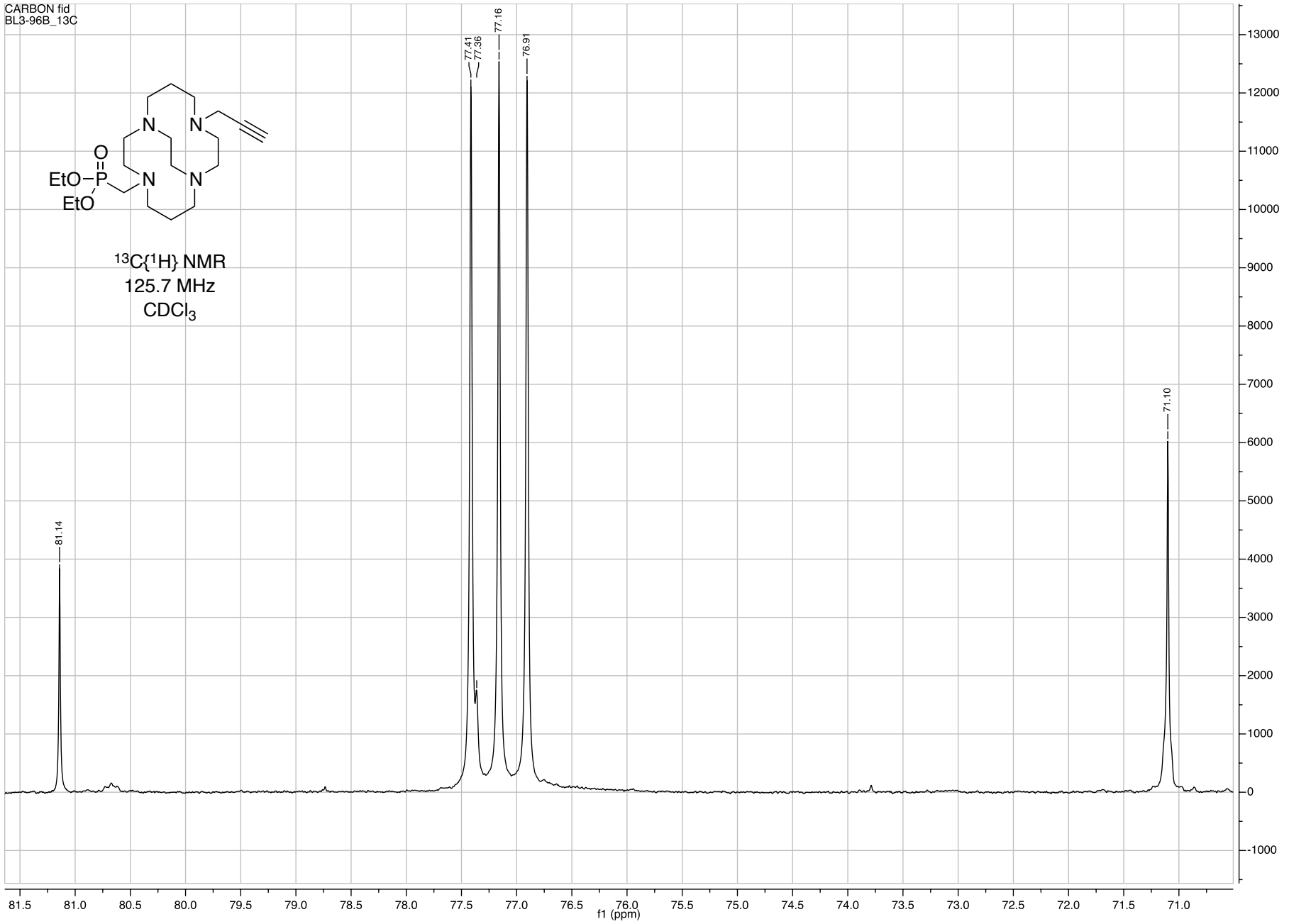

CARBON fid
BL3-96B_13C
77.41
77.36
77.16
76.91
81.14
71.10
61.69
61.64
61.45
61.39
60.21
58.04
56.98
56.86
56.84
56.30
56.02
55.89
51.62
51.41
51.15
51.01
49.86
42.11
28.29
27.40
16.74
16.69
16.65
O
EtO–P
EtO
N
N
N
N
13C{1H} NMR
125.7 MHz
CDCl3
f1 (ppm)

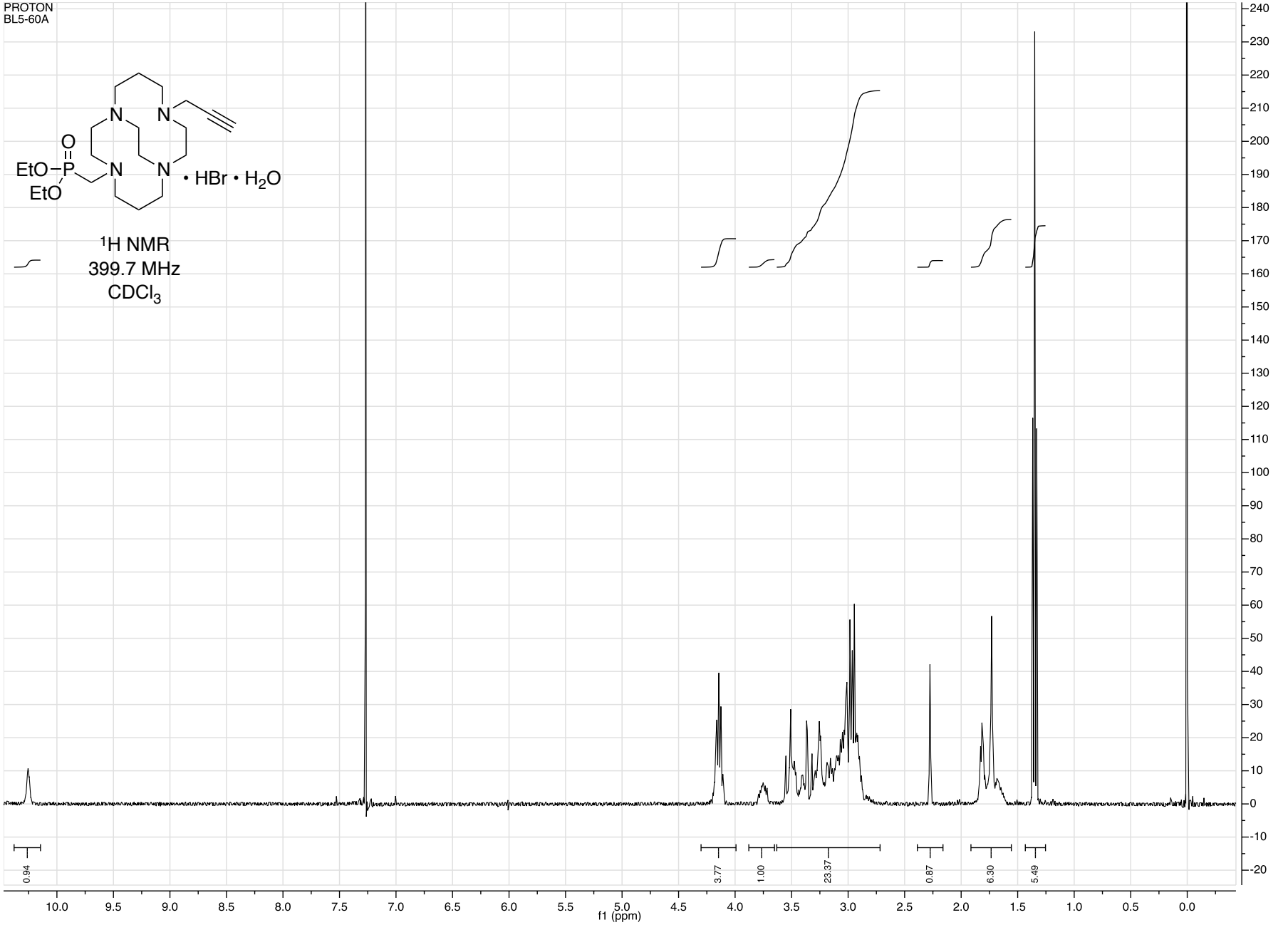


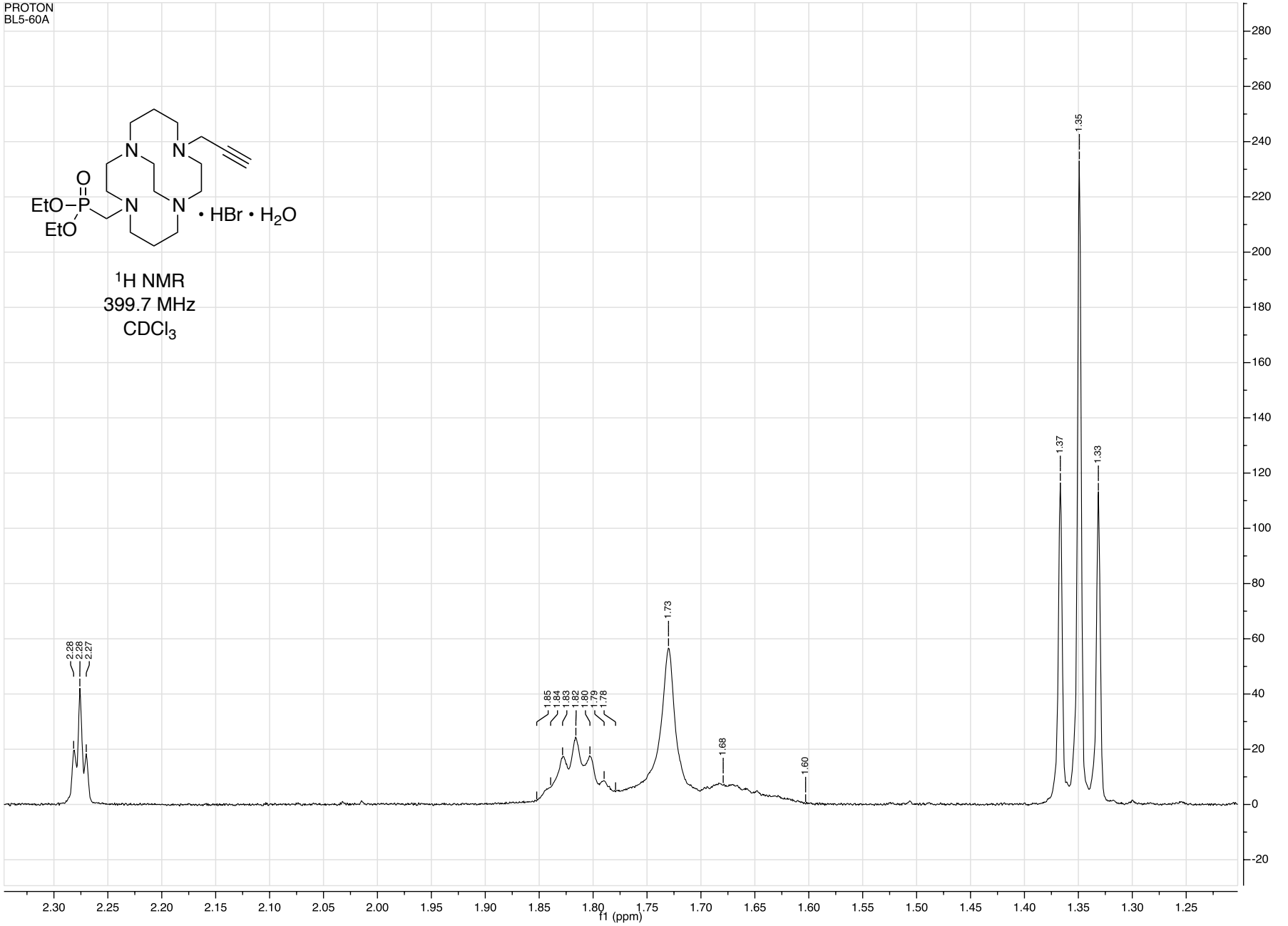
$^{13}C\{^{1}H\}$ NMR
125.7 MHz
$CDCl_3$

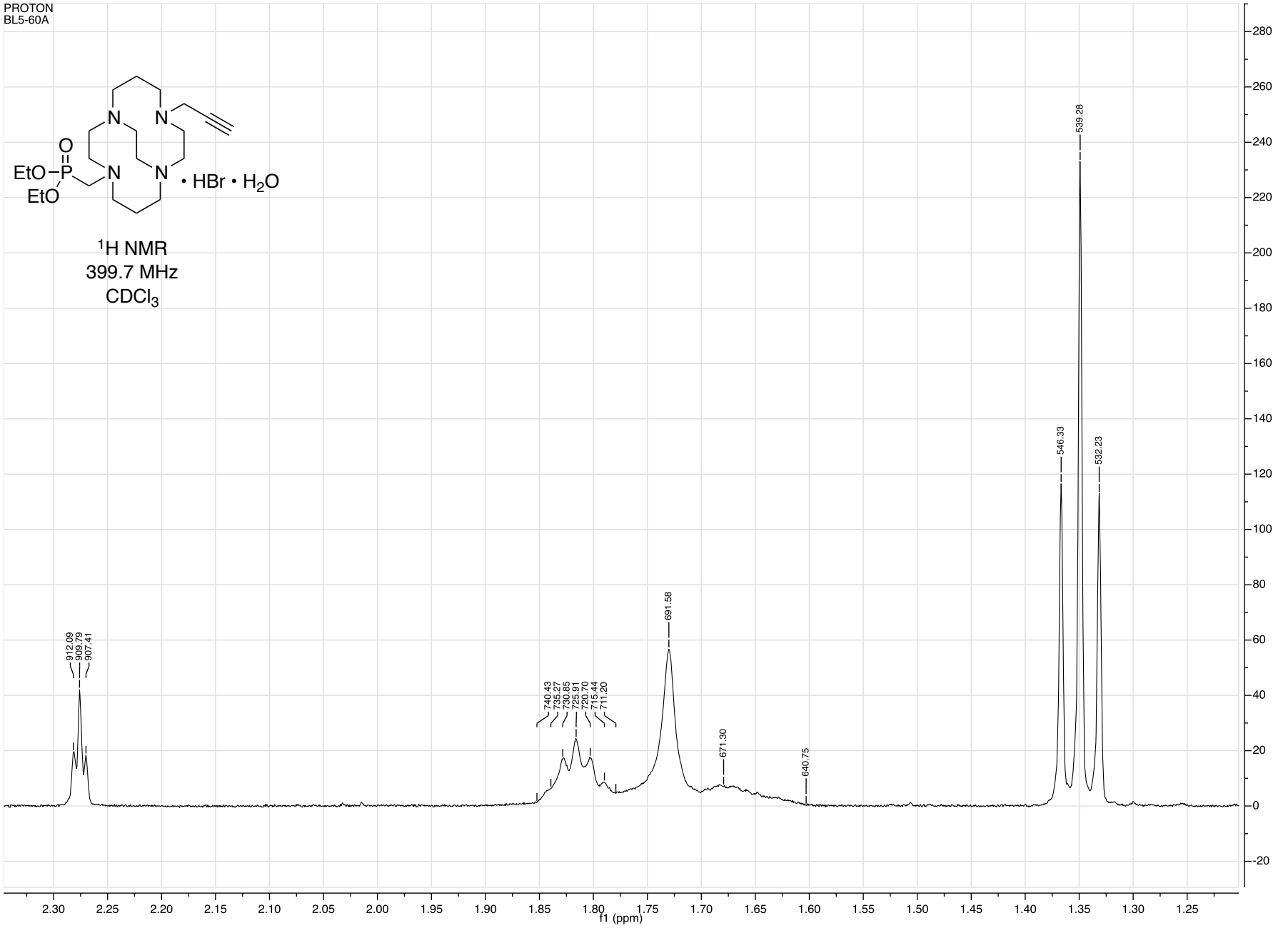

CARBON fid
BL3-96B_13C
51.62
51.41
51.15
51.01
49.86
42.11
$^{13}C\{^{1}H\}$ NMR
125.7 MHz
$CDCl_3$
f1 (ppm)

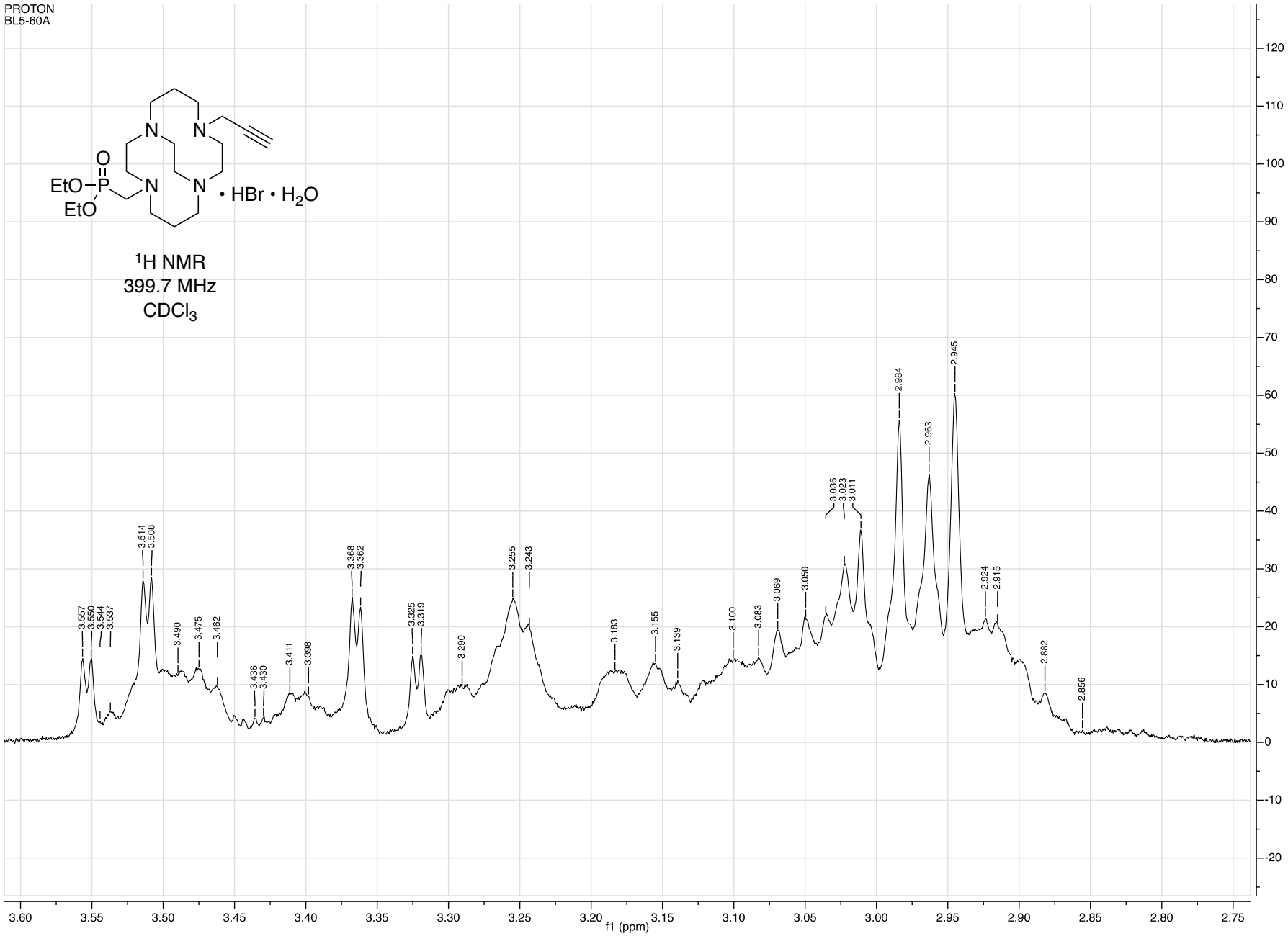

CARBON fid
BL3-96B_13C
EtO
EtO
O
P
N
N
N
N
$^{13}C\{^{1}H\}$ NMR
125.7 MHz
$CDCl_3$
61.69
61.64
61.45
61.39
60.21
58.04
56.98
56.86
56.84
56.30
56.02
55.89
f1 (ppm)

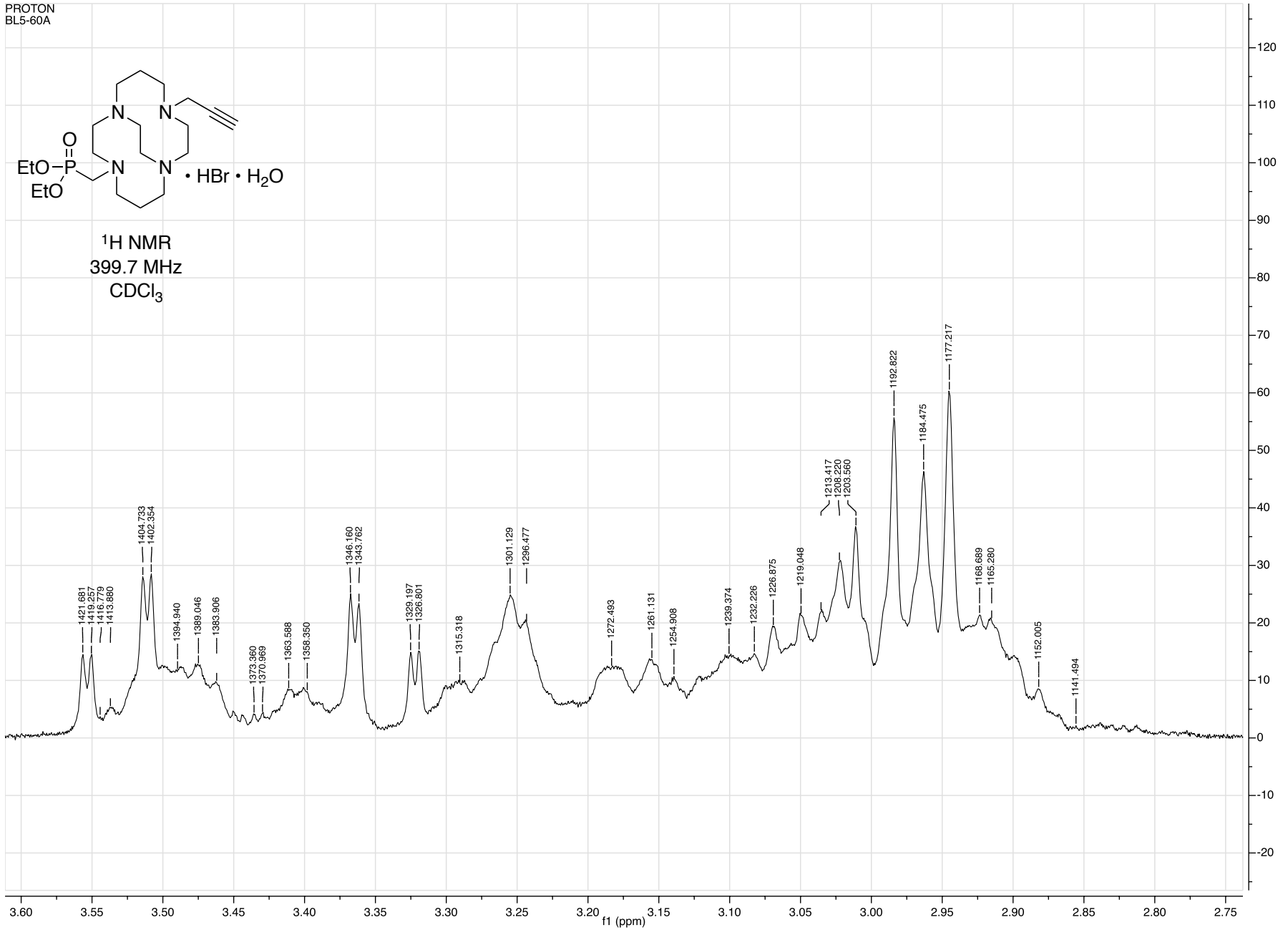

CARBON fid
BL3-96B_13C
77.41
77.36
77.16
76.91
71.10
81.14
$^{13}C\{^{1}H\}$ NMR
125.7 MHz
$CDCl_3$
f1 (ppm)

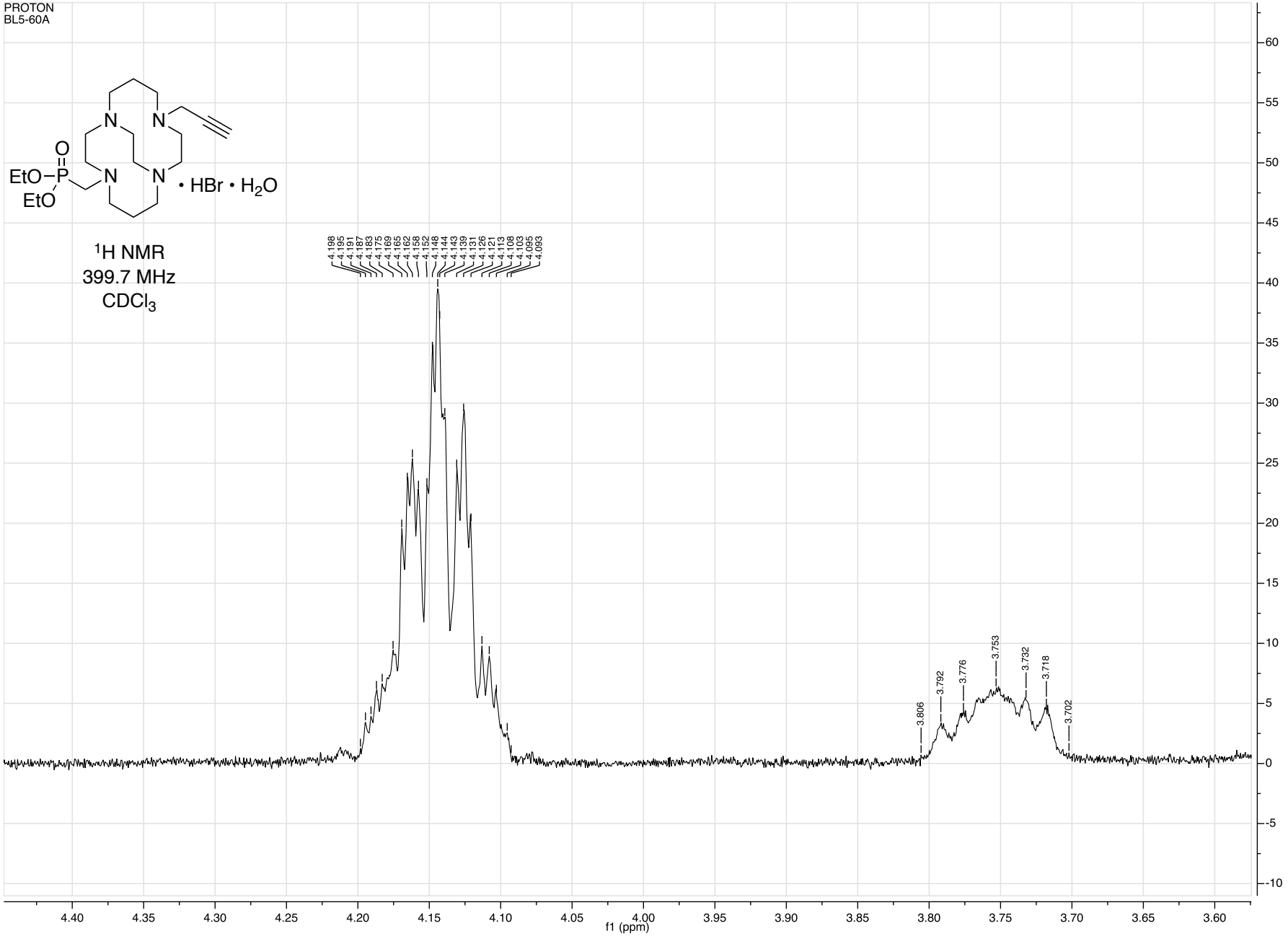

PROTON
BL5-60A
EtO
EtO
O
P
N
N
N
N
• HBr • H2O
1H NMR
399.7 MHz
CDCl3
0.94
3.77
1.00
23.37
0.87
6.30
5.49
f1 (ppm)

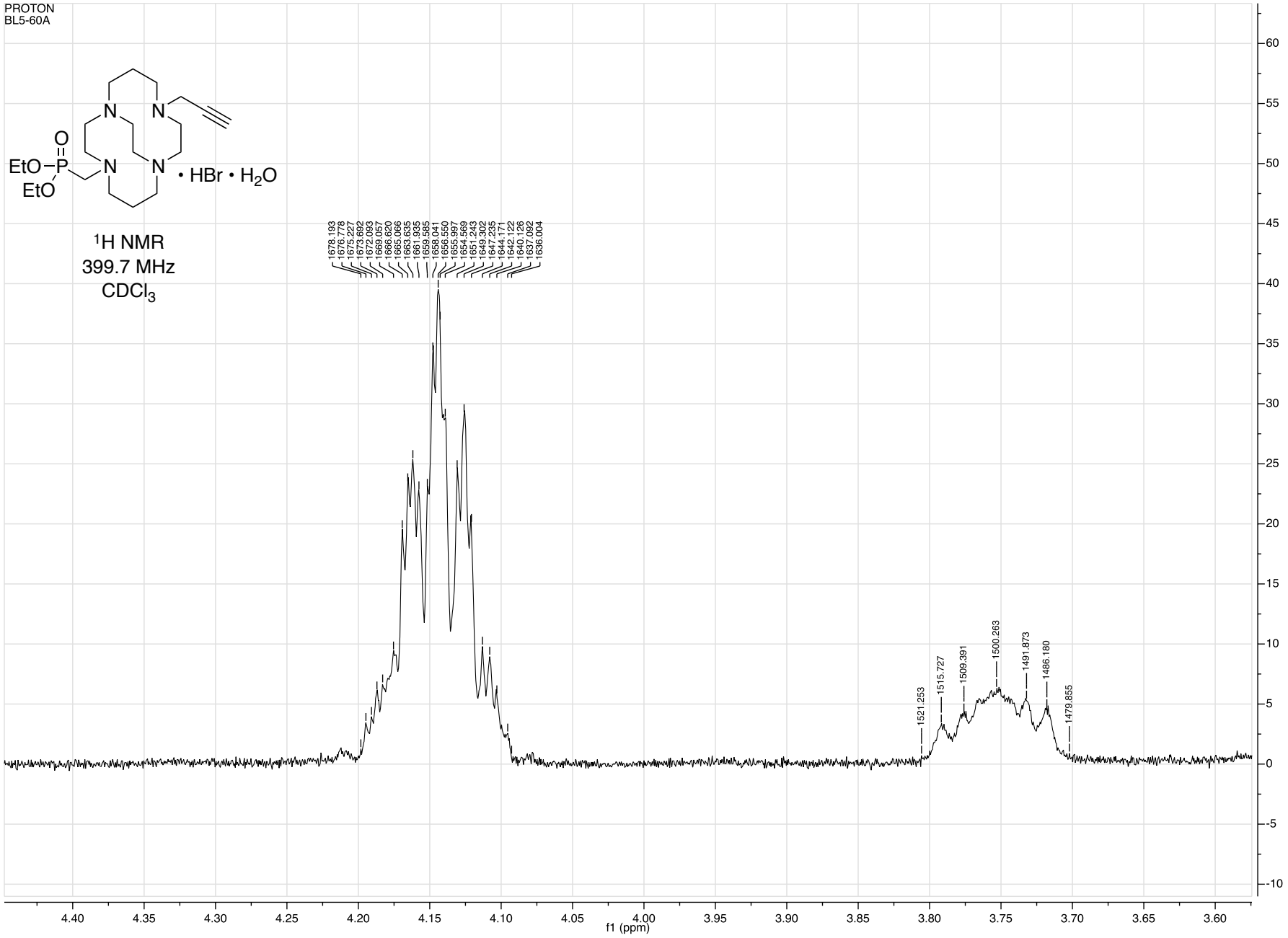

PROTON
BL5-60A
EtO–P
O
EtO
N
N
N
N
• HBr • H2O
1H NMR
399.7 MHz
CDCl3
2.28
2.28
2.27
1.85
1.84
1.83
1.82
1.80
1.79
1.78
1.73
1.68
1.60
1.37
1.35
1.33
f1 (ppm)


PROTON
BL5-60A
EtO
EtO
O
P
N
N
N
N
• HBr • H2O
1H NMR
399.7 MHz
CDCl3
912.09
909.79
907.41
740.43
735.27
730.85
725.91
720.70
715.44
711.20
691.58
671.30
640.75
546.33
539.28
532.23
f1 (ppm)

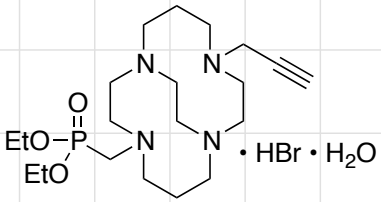

PROTON
BL5-60A
EtO
EtO
O
P
N
N
N
N
• HBr • H2O
1H NMR
399.7 MHz
CDCl3
3.557
3.550
3.544
3.537
3.514
3.508
3.490
3.475
3.462
3.436
3.430
3.411
3.398
3.368
3.362
3.325
3.319
3.290
3.255
3.243
3.183
3.155
3.139
3.100
3.083
3.069
3.050
3.036
3.023
3.011
2.984
2.963
2.945
2.924
2.915
2.882
2.856
f1 (ppm)


PROTON
BL5-60A
EtO
EtO
O
P
N
N
N
N
• HBr • H2O
1H NMR
399.7 MHz
CDCl3
1421.681
1419.257
1416.779
1413.880
1404.733
1402.354
1394.940
1389.046
1383.906
1373.360
1370.969
1363.588
1358.350
1346.160
1343.762
1329.197
1326.801
1315.318
1301.129
1296.477
1272.493
1261.131
1254.908
1239.374
1232.226
1226.875
1219.048
1213.417
1208.220
1203.560
1192.822
1184.475
1177.217
1168.689
1165.280
1152.005
1141.494
f1 (ppm)

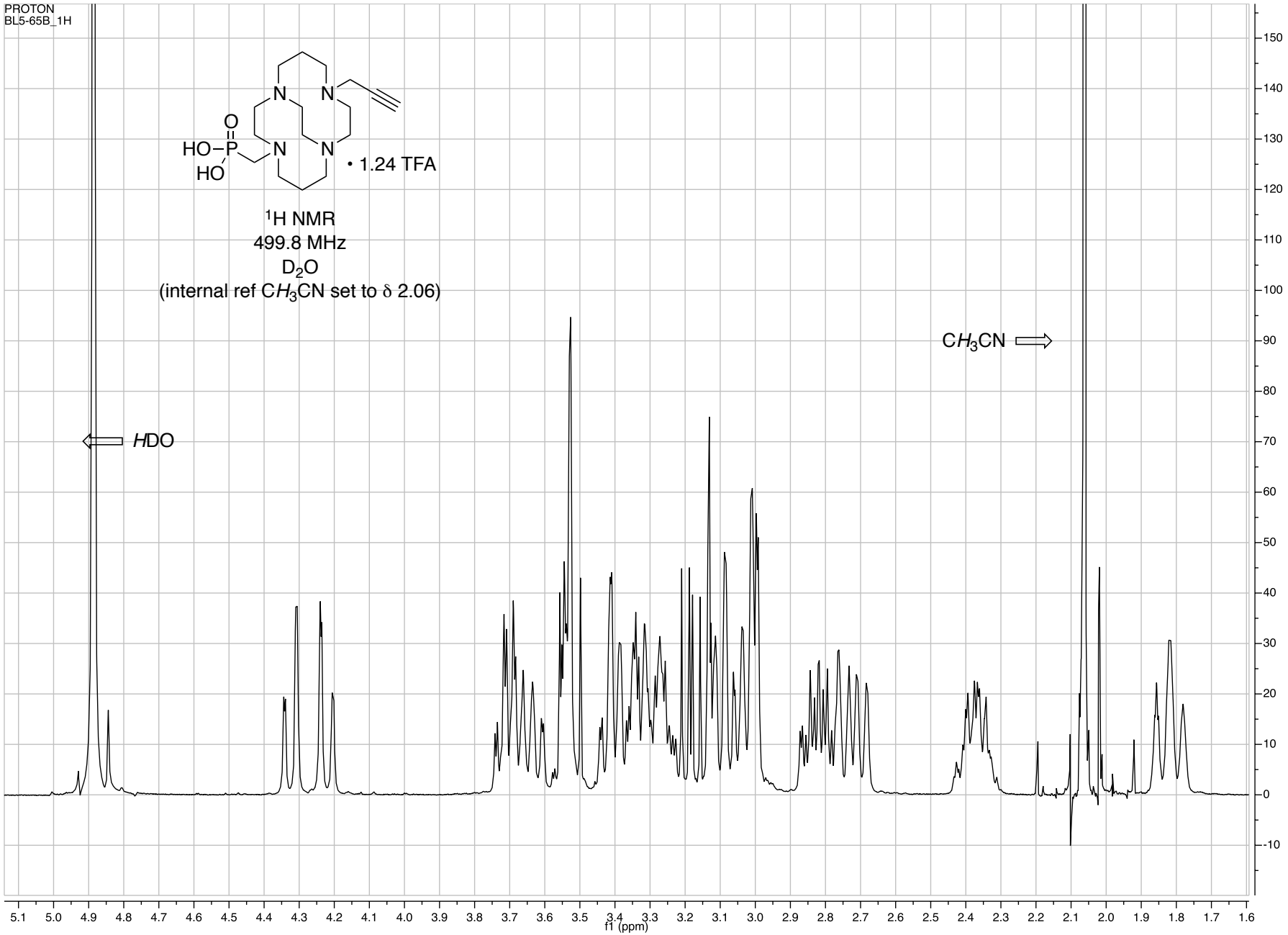

PROTON
BL5-60A
EtO–P
O
EtO
N
N
N
N
• HBr • H2O
1H NMR
399.7 MHz
CDCl3
4.198
4.195
4.191
4.187
4.183
4.175
4.169
4.165
4.162
4.158
4.152
4.148
4.144
4.143
4.139
4.131
4.126
4.121
4.113
4.108
4.103
4.095
4.093
3.806
3.792
3.776
3.753
3.732
3.718
3.702
f1 (ppm)

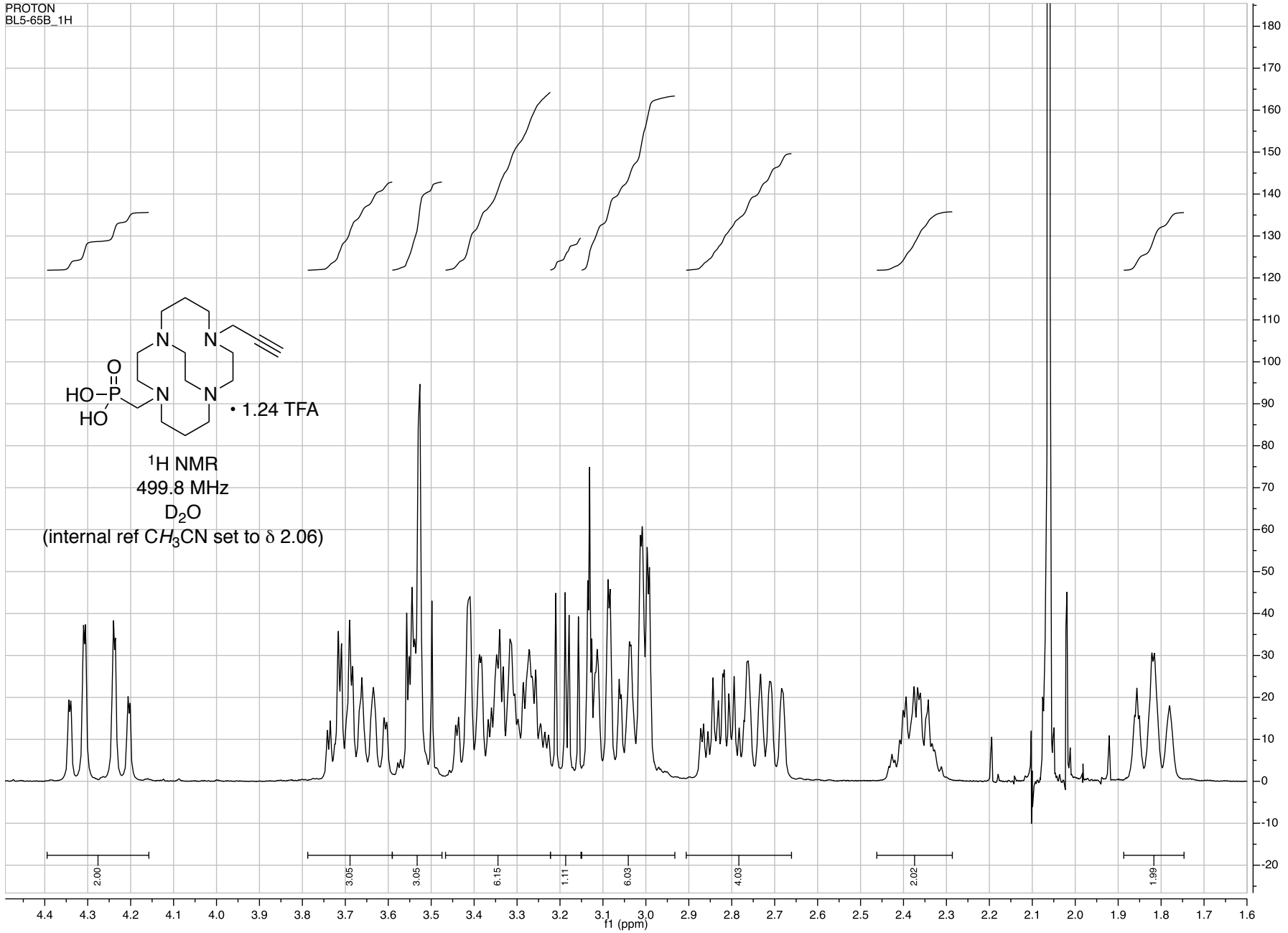

PROTON
BL5-60A
EtO
EtO
• HBr • H2O
1H NMR
399.7 MHz
CDCl3
1678.193
1676.778
1675.227
1673.692
1672.093
1669.057
1666.620
1665.066
1663.635
1661.935
1659.585
1658.041
1656.550
1655.997
1654.569
1651.243
1649.302
1647.235
1644.171
1642.122
1640.126
1637.092
1636.004
1521.253
1515.727
1509.391
1500.263
1491.873
1486.180
1479.855
f1 (ppm)

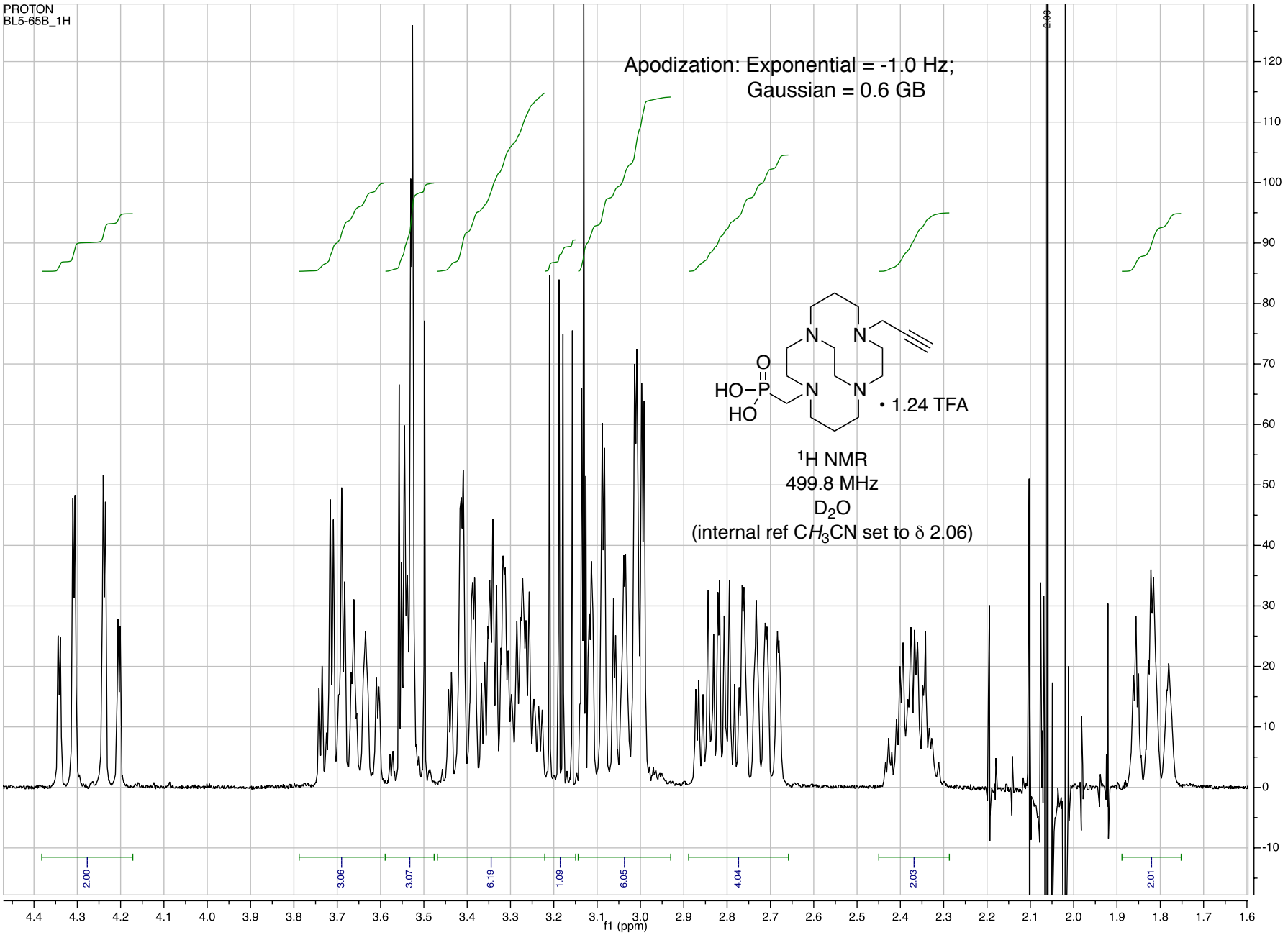
PROTON
BL5-60A

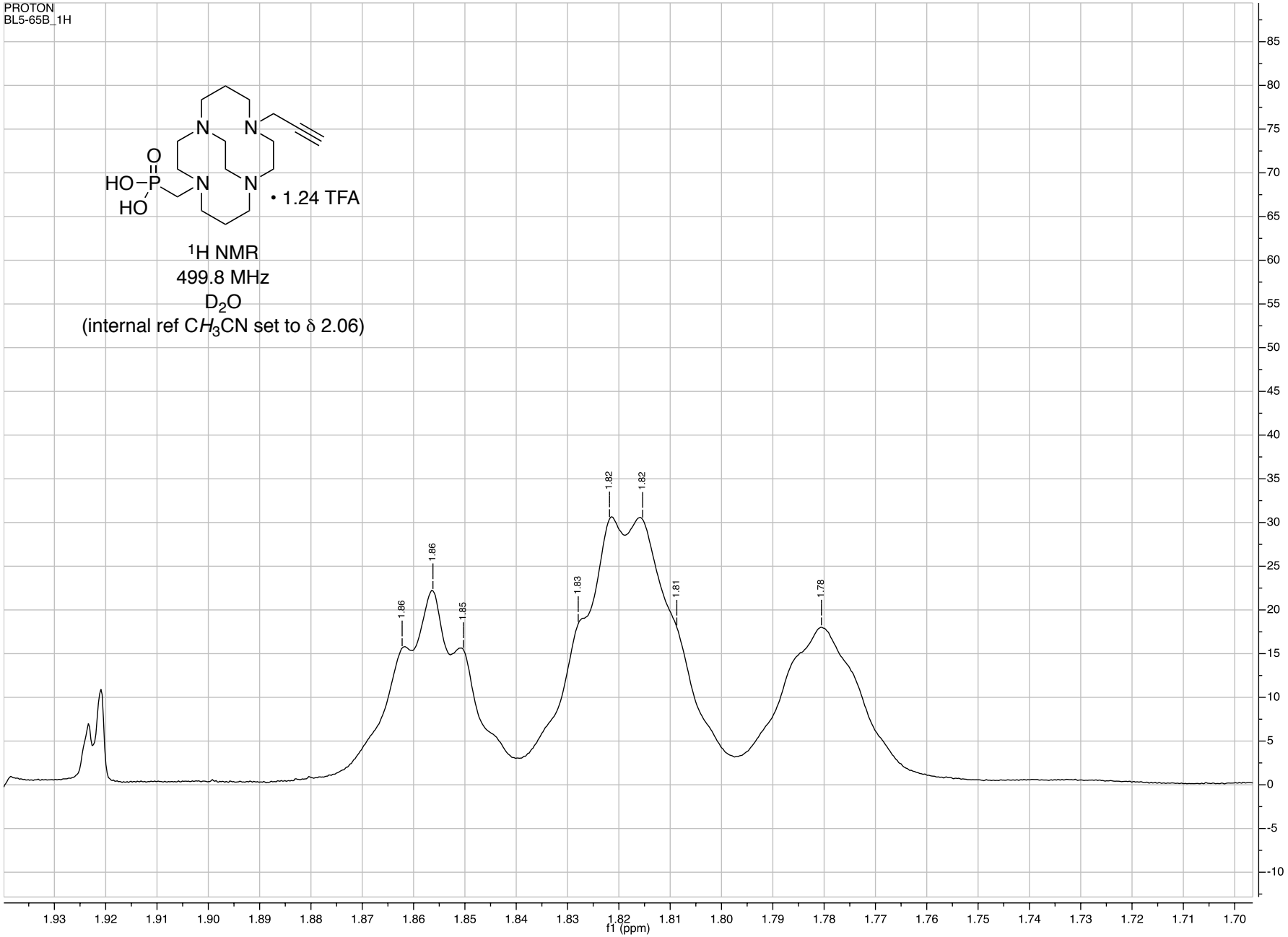


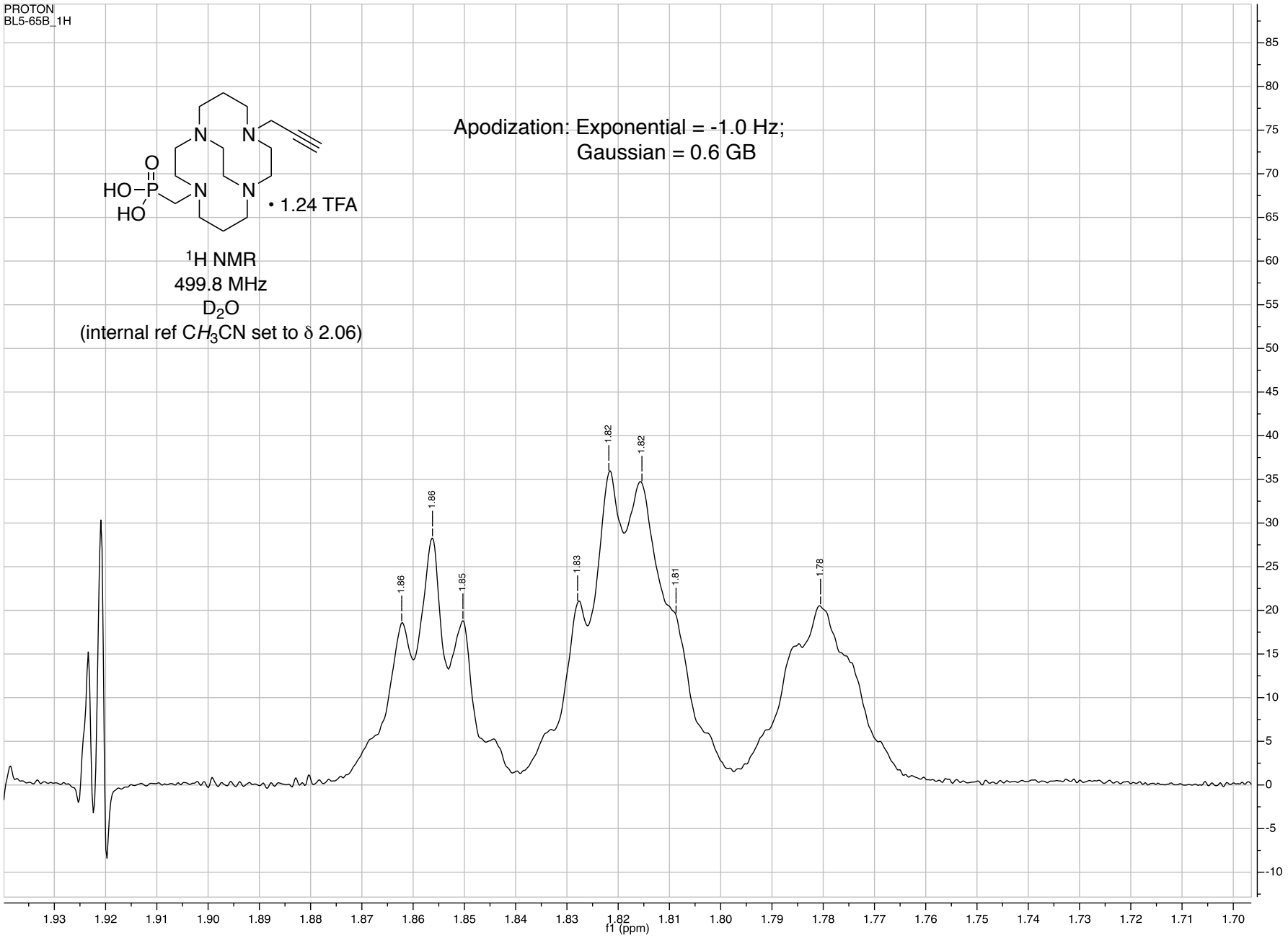
^{1}H NMR
399.7 MHz
$CDCl_3$


PROTON
BL5-65B_1H
HO–P
HO
O
N
N
N
N
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref C*H*$_3$CN set to δ 2.06)
*H*DO
C*H*$_3$CN
f1 (ppm)

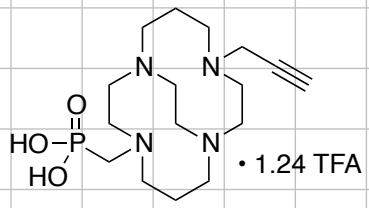

PROTON
BL5-65B_1H
HO–P
O
HO
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
2.00
3.05
3.05
6.15
1.11
6.03
4.03
2.02
1.99
f1 (ppm)


PROTON
BL5-65B_1H
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
HO–P
O
HO
N
N
N
N
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
2.00
3.06
3.07
6.19
1.09
6.05
4.04
2.03
2.01
f1 (ppm)


PROTON
BL5-65B_1H
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
1.86
1.86
1.85
1.83
1.82
1.82
1.81
1.78
f1 (ppm)

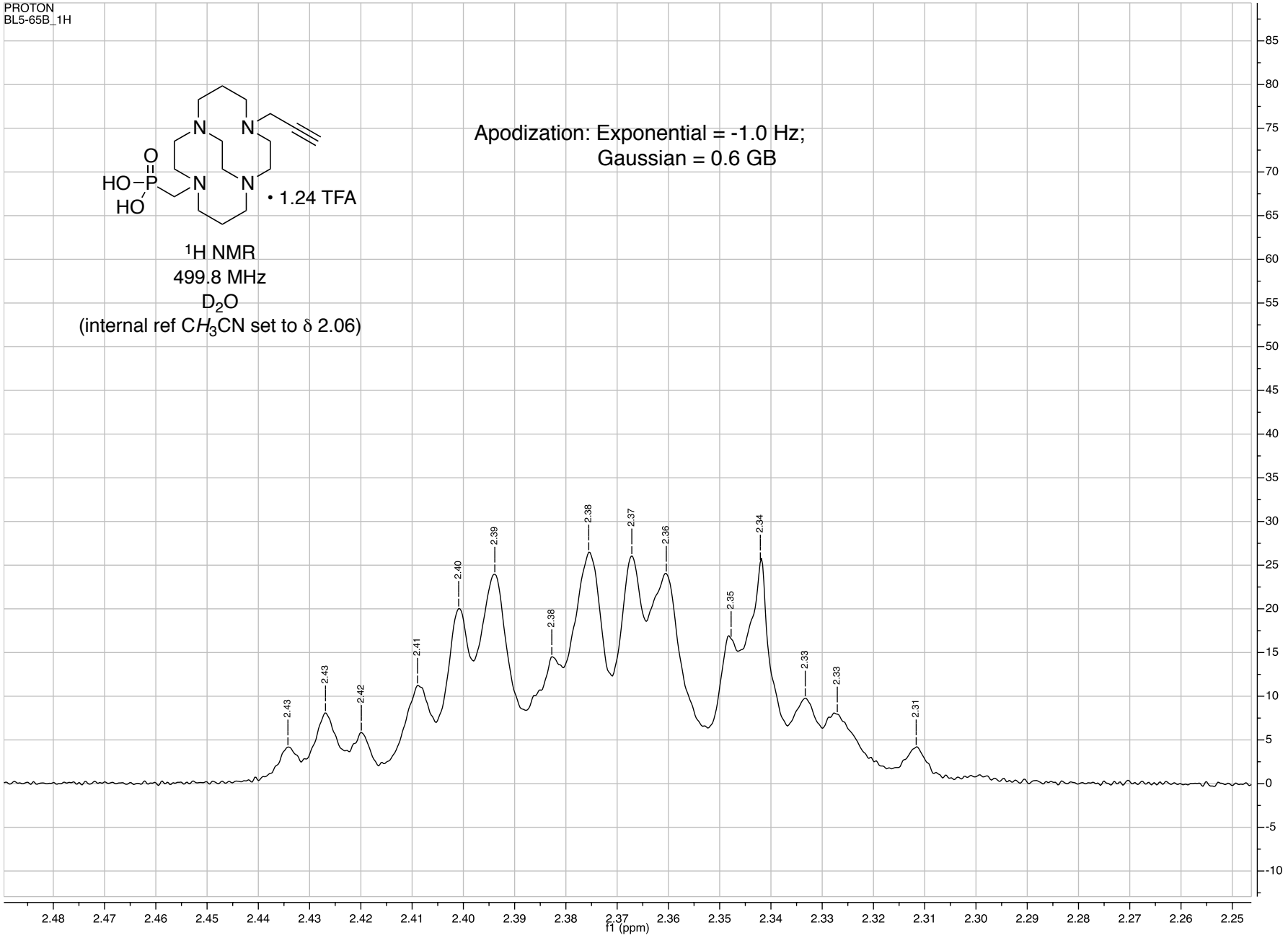

PROTON
BL5-65B_1H
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
1.86
1.86
1.85
1.83
1.82
1.82
1.81
1.78
f1 (ppm)

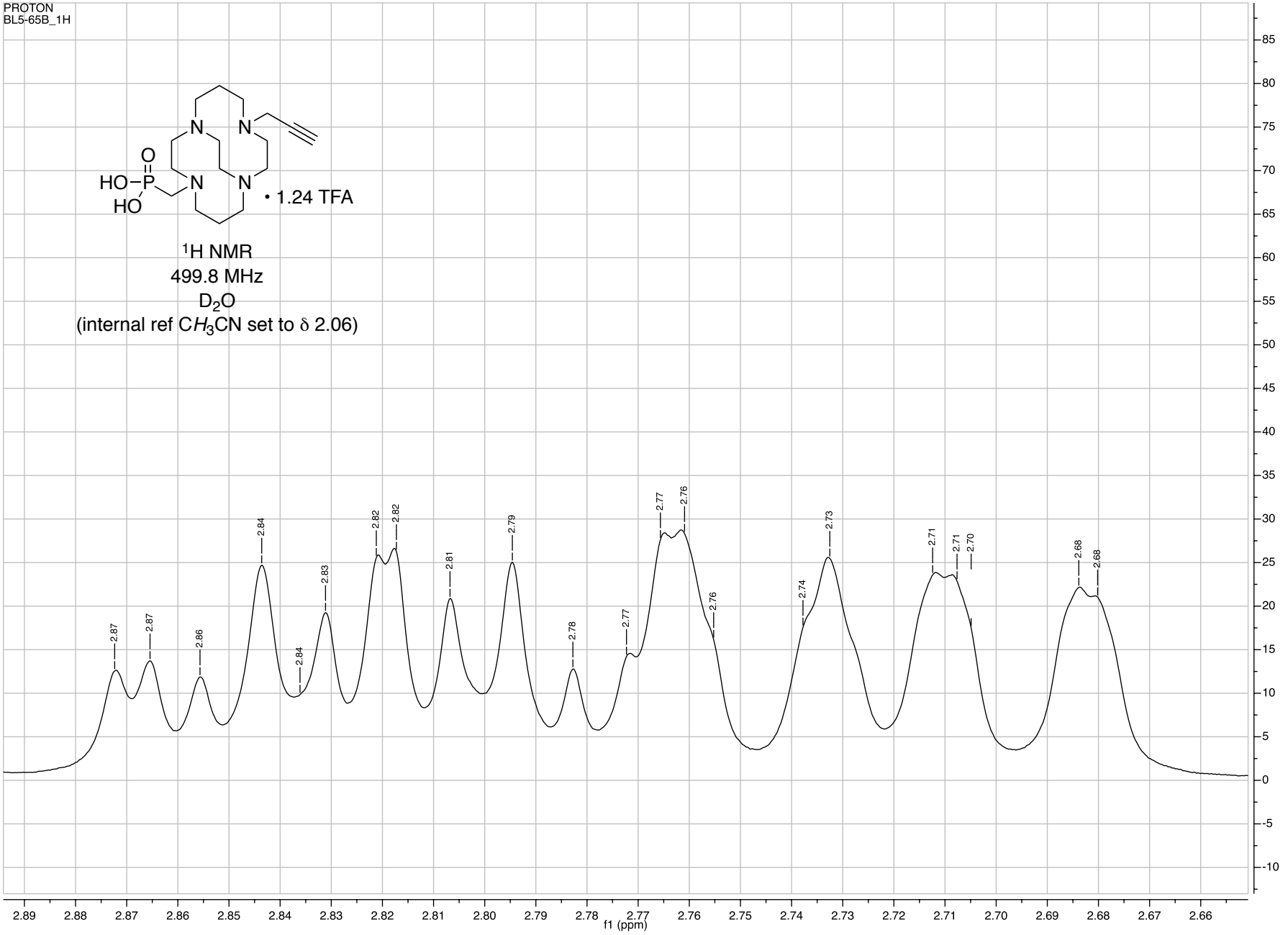
PROTON
BL5-65B_1H

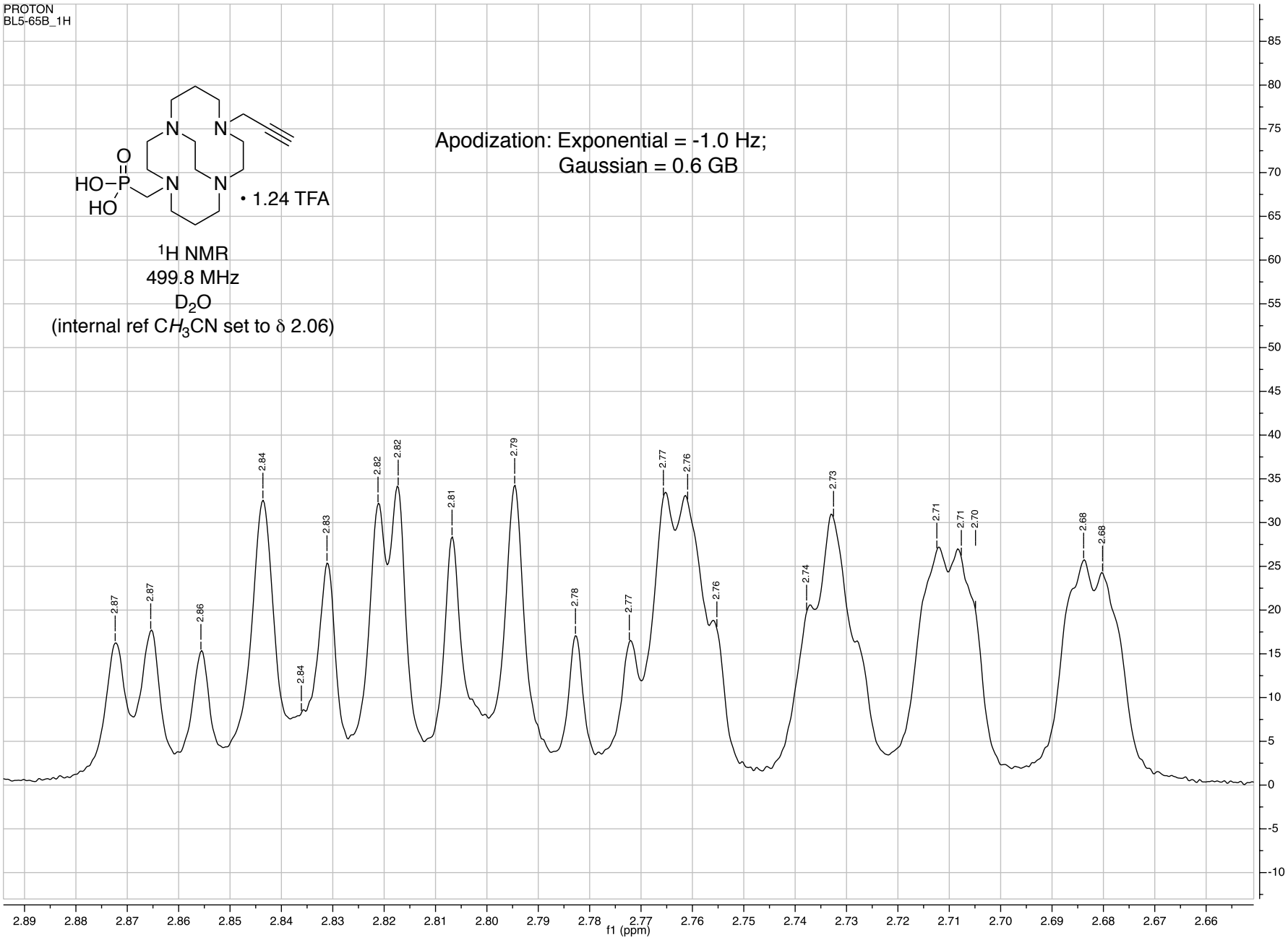


• 1.24 TFA

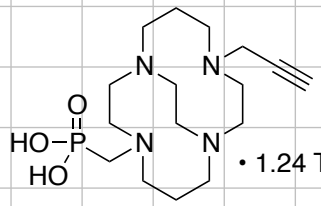
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)


PROTON
BL5-65B_1H
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
2.43
2.43
2.42
2.41
2.40
2.39
2.38
2.38
2.37
2.36
2.35
2.34
2.33
2.33
2.31
f1 (ppm)


PROTON
BL5-65B_1H
HO
HO
O
P
N
N
N
N
• 1.24 TFA
1H NMR
499.8 MHz
D2O
(internal ref CH3CN set to δ 2.06)
2.87
2.87
2.86
2.84
2.84
2.83
2.82
2.82
2.81
2.79
2.78
2.77
2.77
2.76
2.76
2.74
2.73
2.71
2.71
2.70
2.68
2.68
f1 (ppm)


PROTON
BL5-65B_1H
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
f1 (ppm)

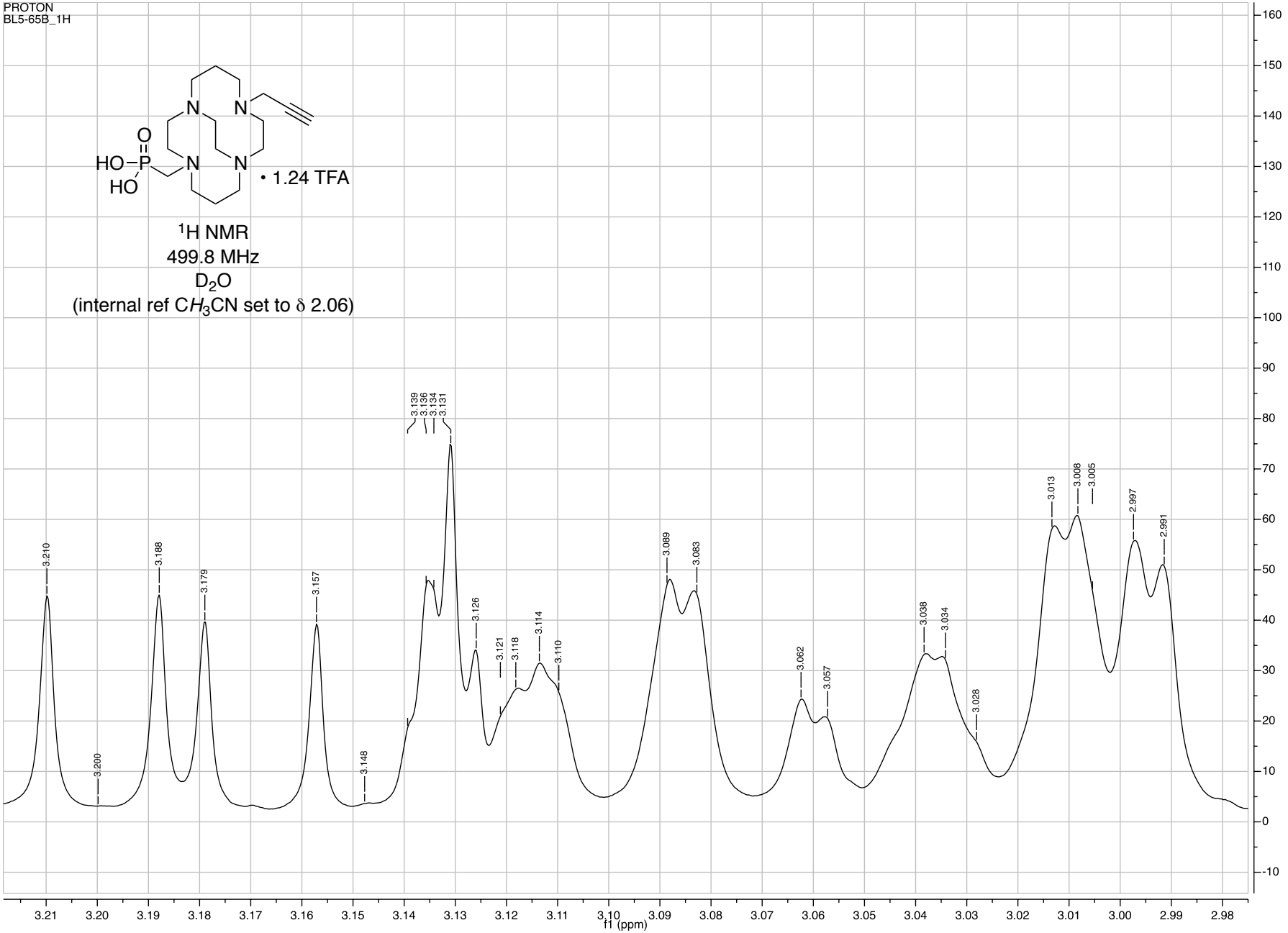
PROTON
BL5-65B_1H

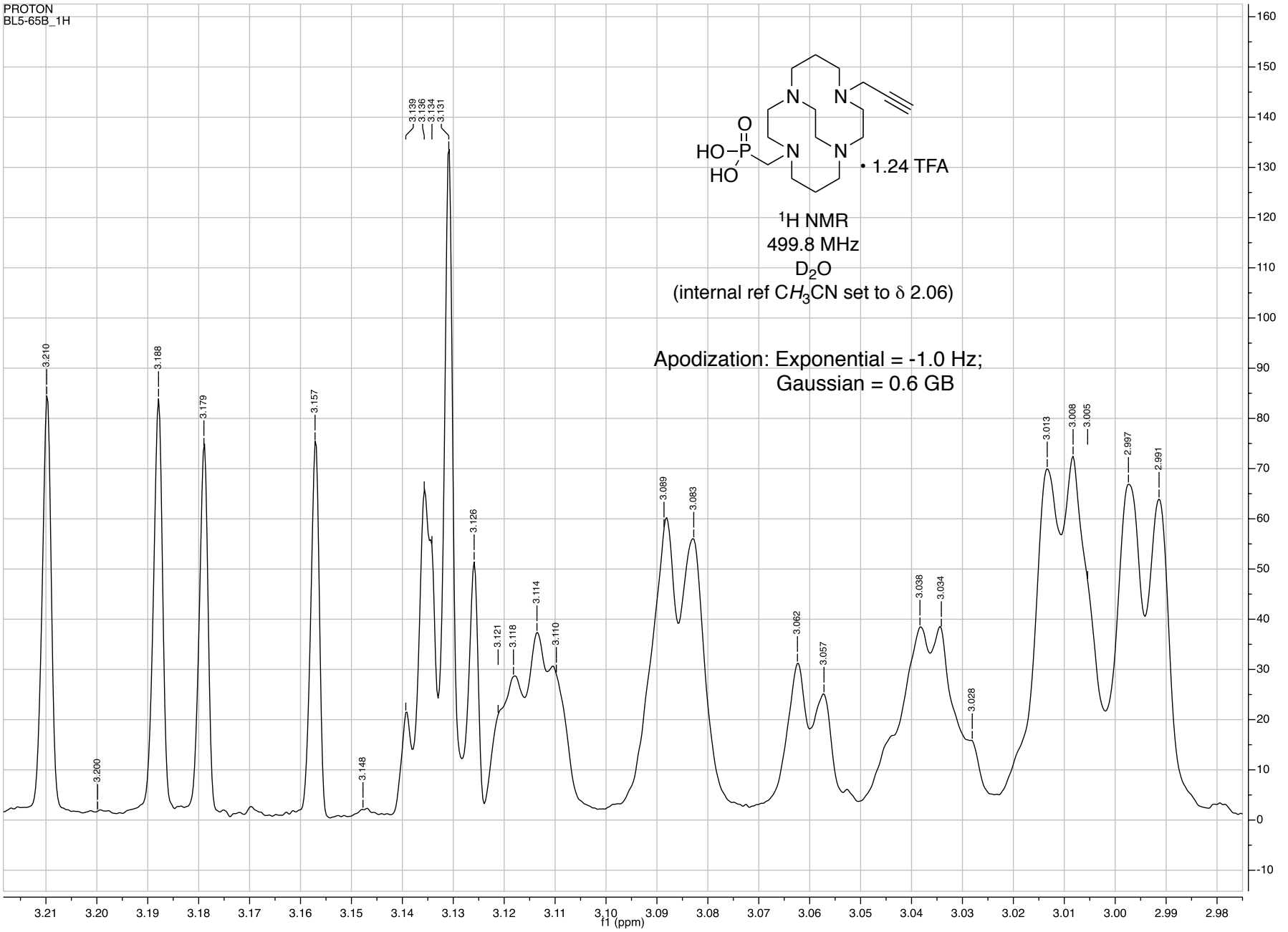


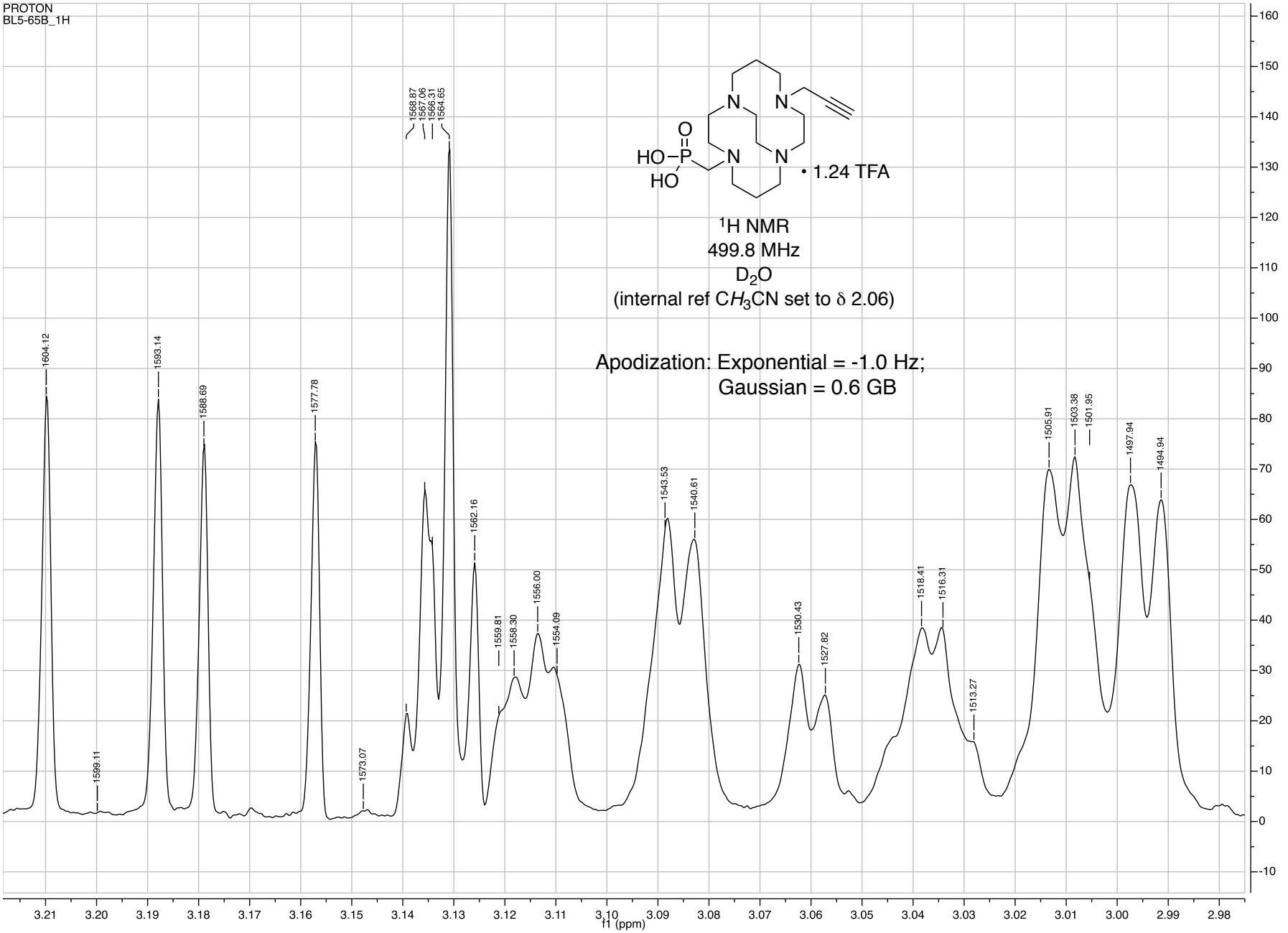
• 1.24 TFA

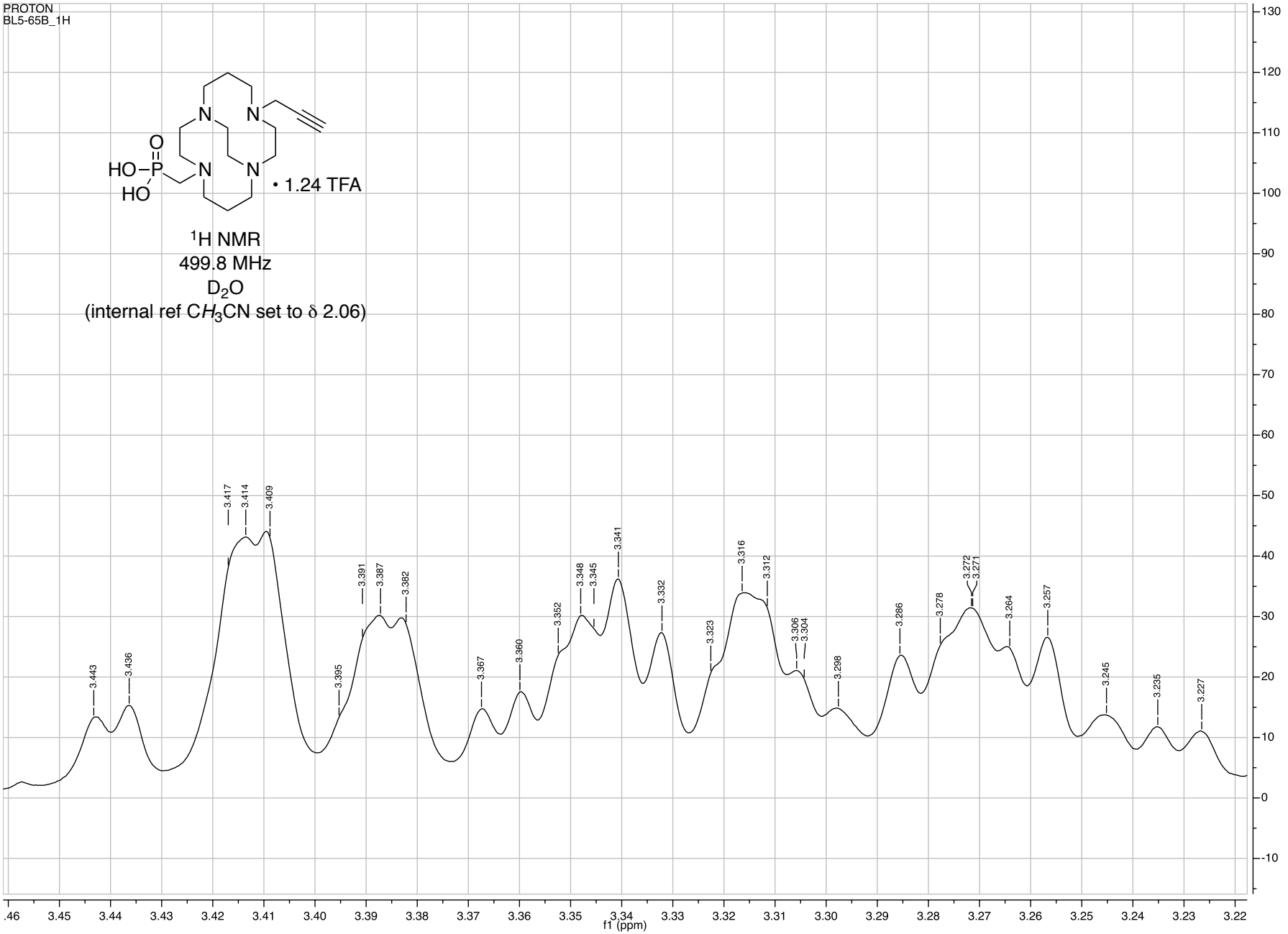
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB

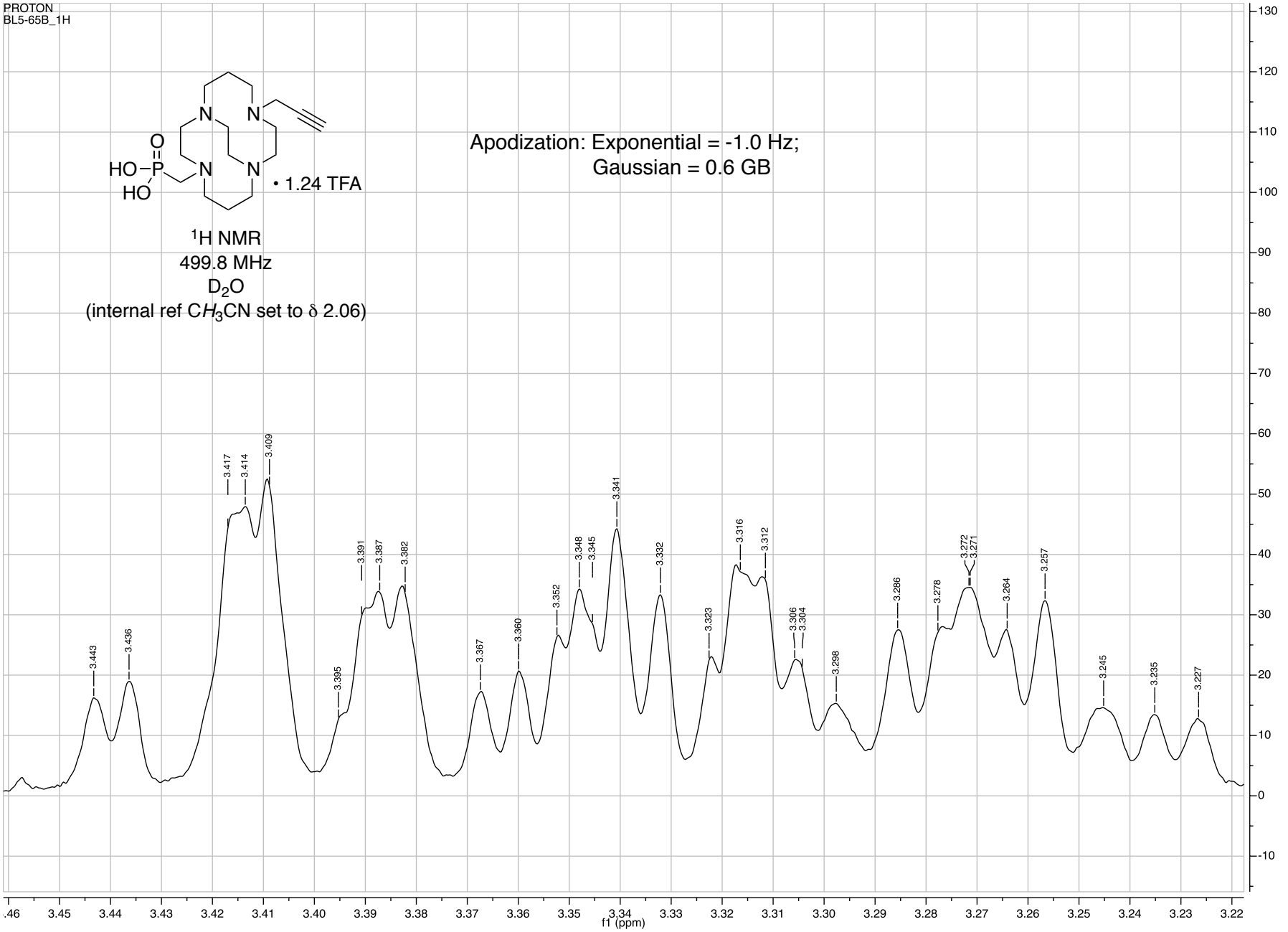
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)

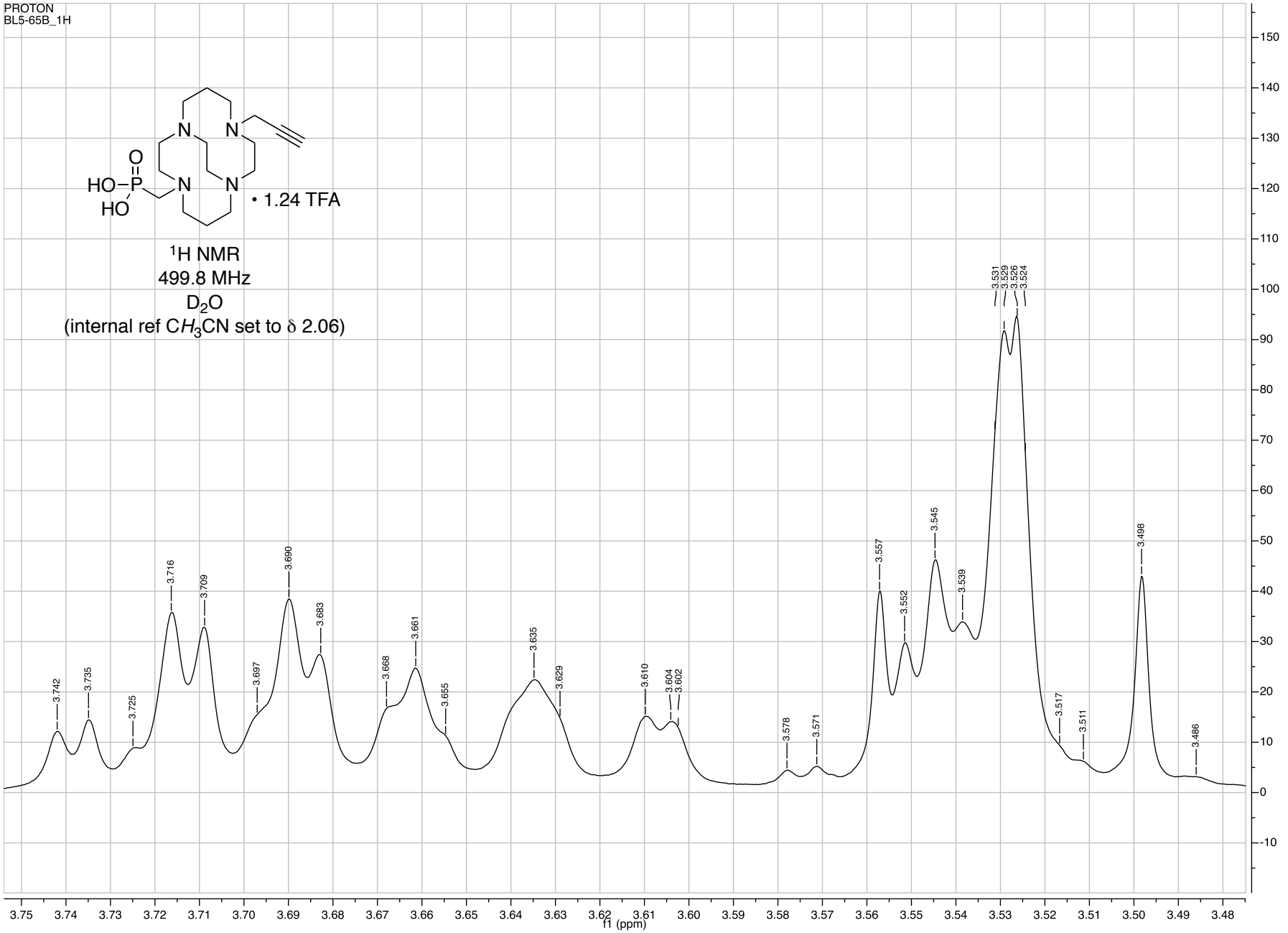

PROTON
BL5-65B_1H
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
3.210
3.200
3.188
3.179
3.157
3.148
3.139
3.136
3.134
3.131
3.126
3.121
3.118
3.114
3.110
3.089
3.083
3.062
3.057
3.038
3.034
3.028
3.013
3.008
3.005
2.997
2.991
f1 (ppm)

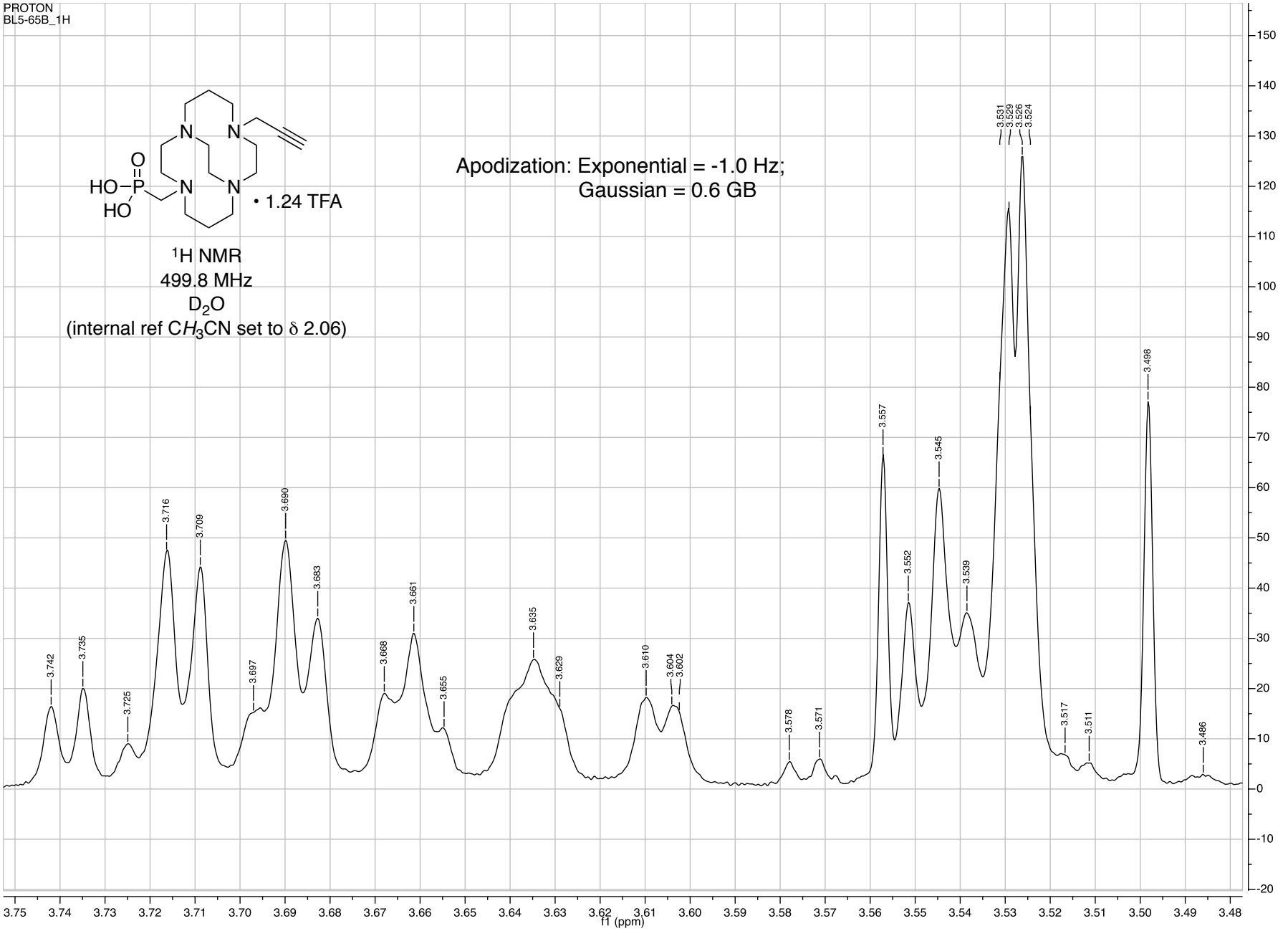

PROTON
BL5-65B_1H
HO–P
HO
O
N
N
N
N
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
3.210
3.200
3.188
3.179
3.157
3.148
3.139
3.136
3.134
3.131
3.126
3.121
3.118
3.114
3.110
3.089
3.083
3.062
3.057
3.038
3.034
3.028
3.013
3.008
3.005
2.997
2.991
f1 (ppm)

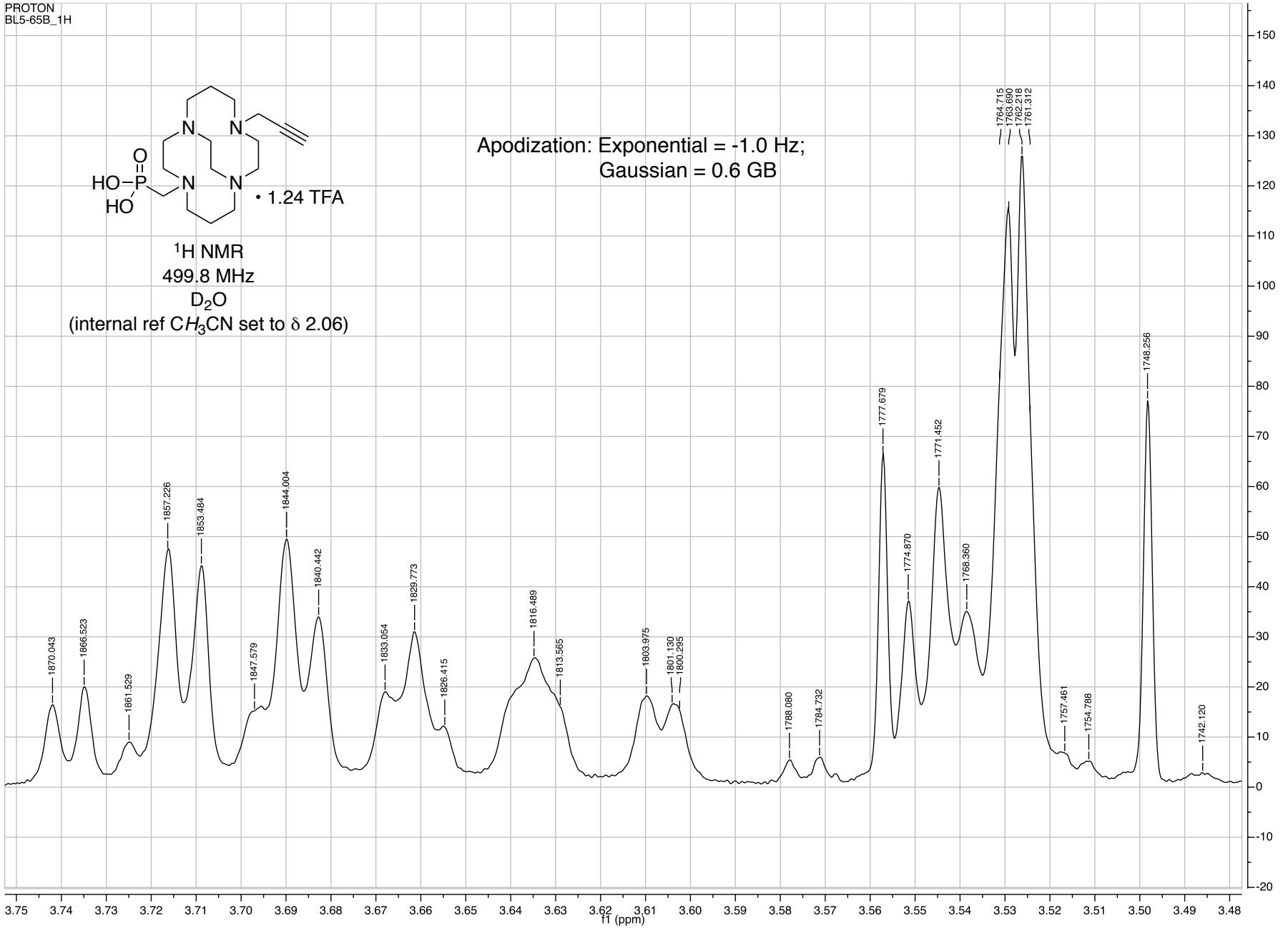

PROTON
BL5-65B_1H
1604.12
1599.11
1593.14
1588.69
1577.78
1573.07
1568.87
1567.06
1566.31
1564.65
1562.16
1559.81
1558.30
1556.00
1554.09
1543.53
1540.61
1530.43
1527.82
1518.41
1516.31
1513.27
1505.91
1503.38
1501.95
1497.94
1494.94
HO–P
O
HO
N
N
N
N
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
f1 (ppm)

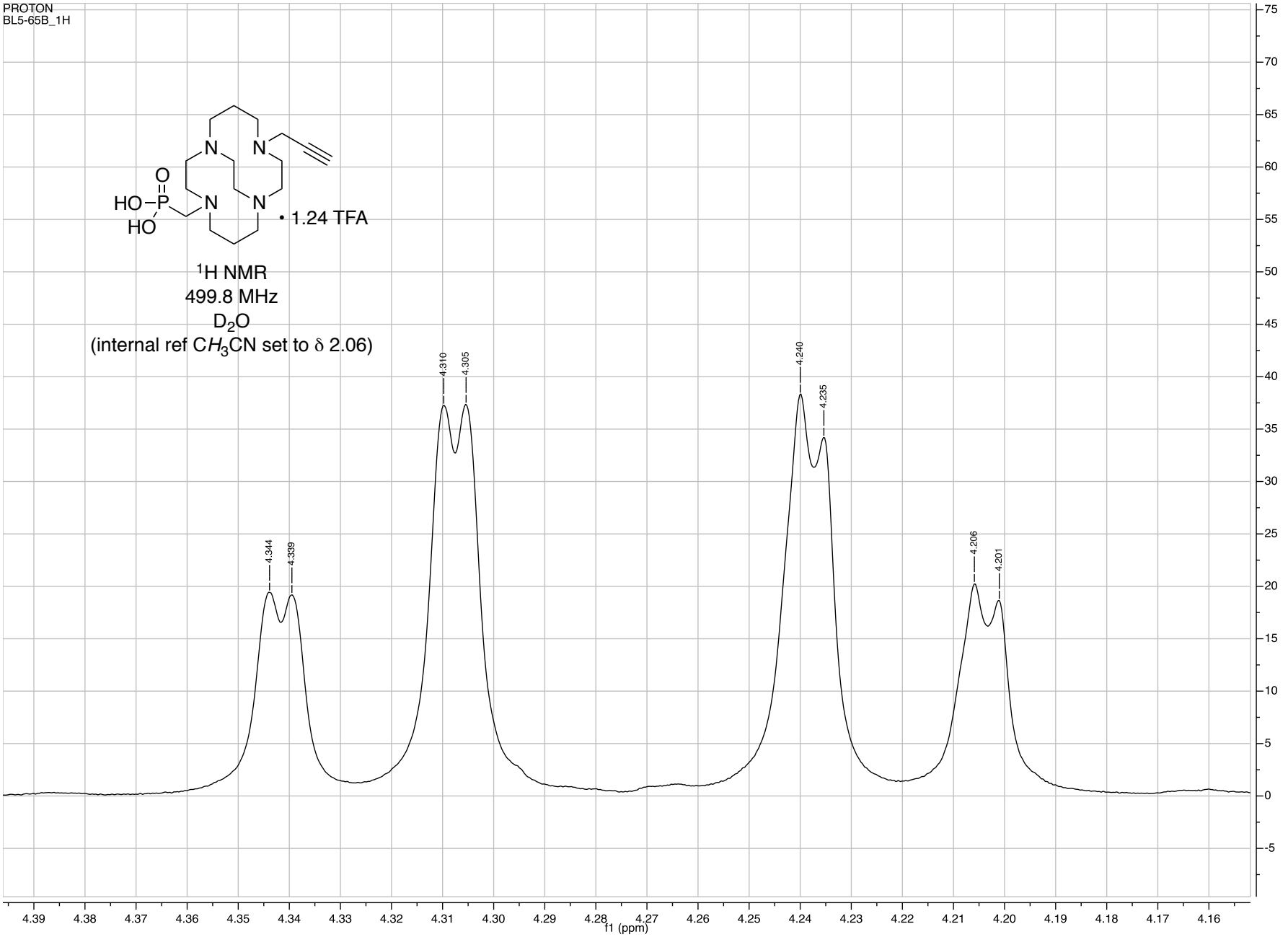

PROTON
BL5-65B_1H
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
3.443
3.436
3.417
3.414
3.409
3.395
3.391
3.387
3.382
3.367
3.360
3.352
3.348
3.345
3.341
3.332
3.323
3.316
3.312
3.306
3.304
3.298
3.286
3.278
3.272
3.271
3.264
3.257
3.245
3.235
3.227
f1 (ppm)

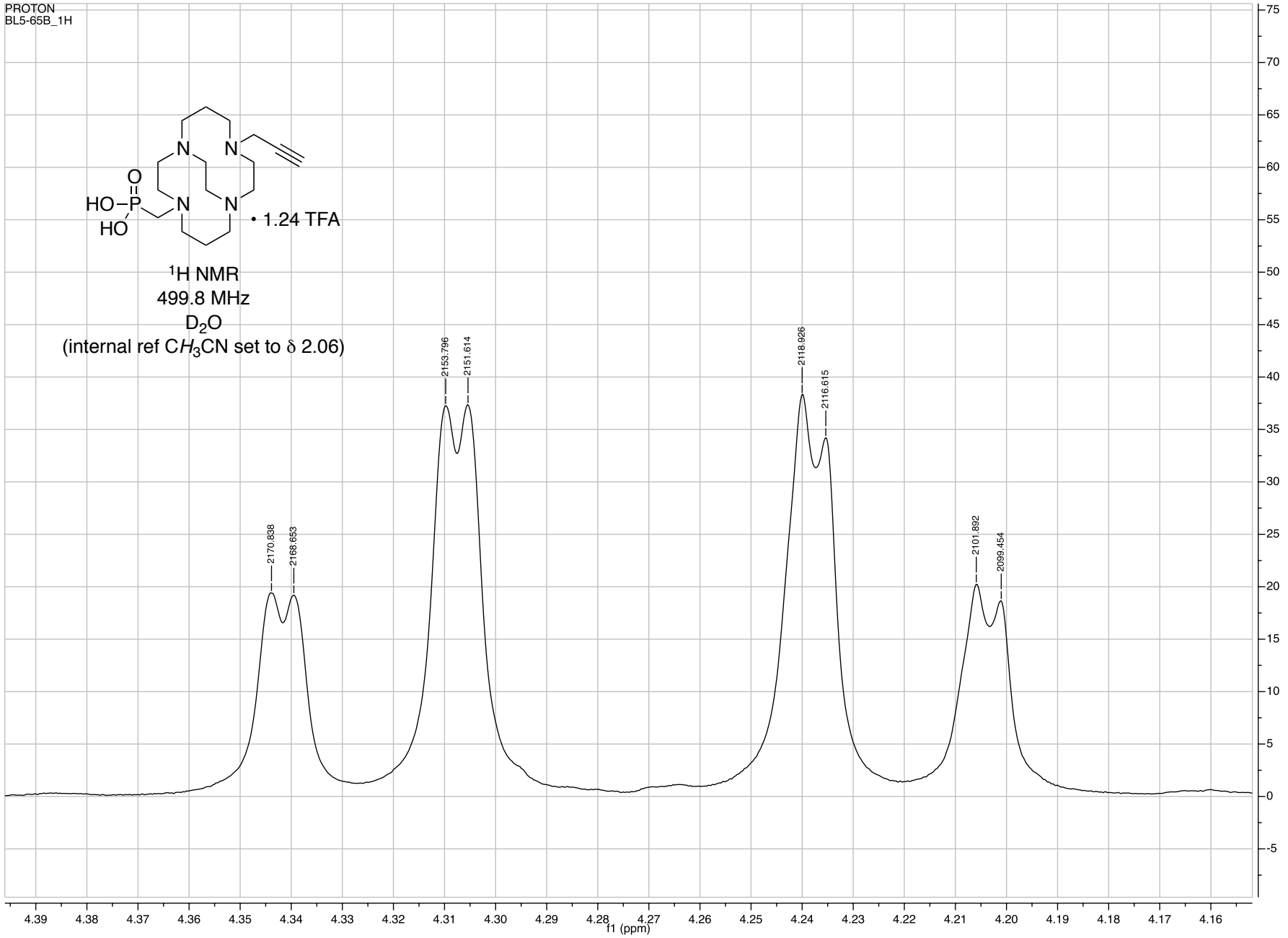

PROTON
BL5-65B_1H
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
3.443
3.436
3.417
3.414
3.409
3.395
3.391
3.387
3.382
3.367
3.360
3.352
3.348
3.345
3.341
3.332
3.323
3.316
3.312
3.306
3.304
3.298
3.286
3.278
3.272
3.271
3.264
3.257
3.245
3.235
3.227
f1 (ppm)

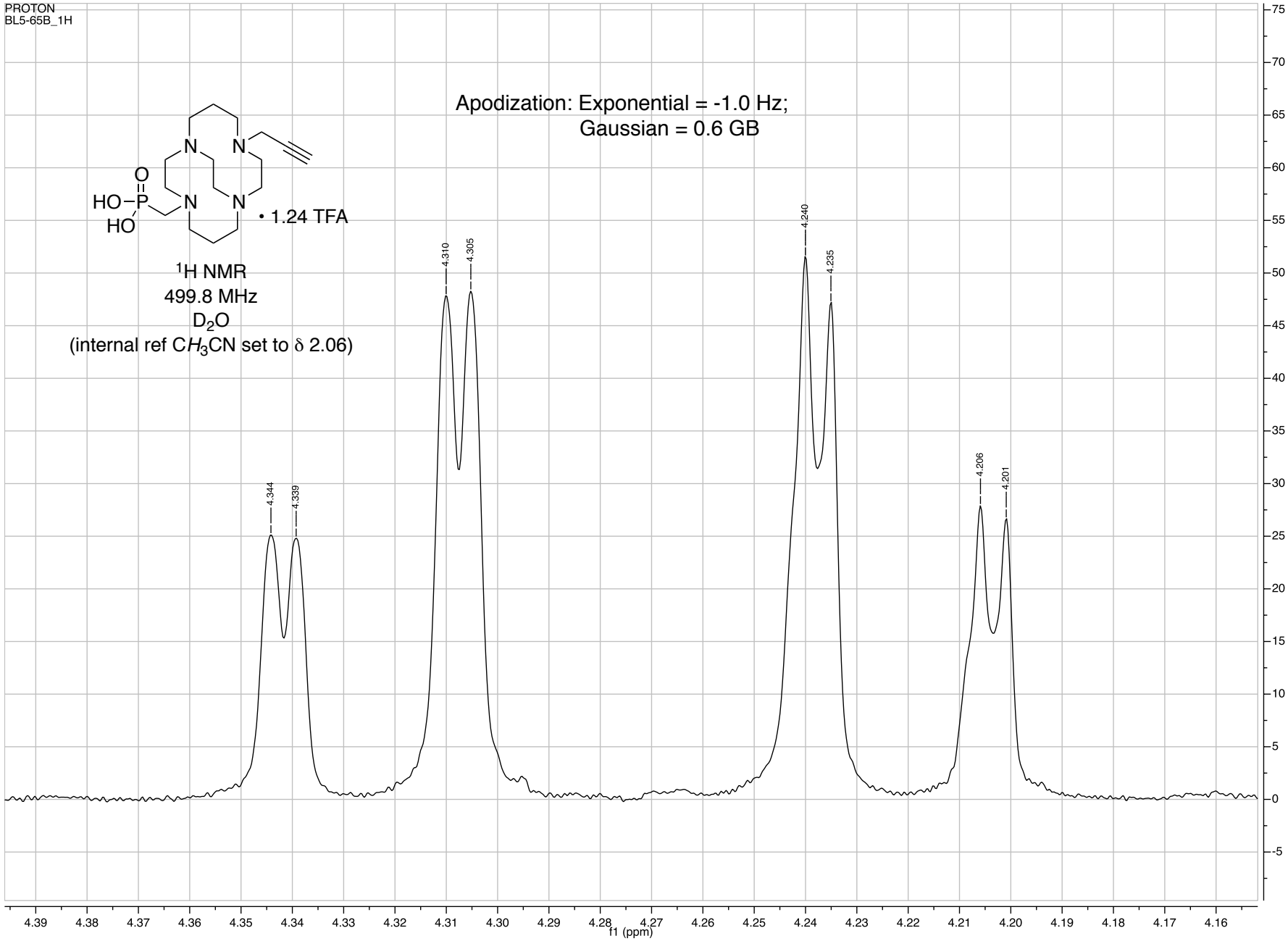

PROTON
BL5-65B_1H
HO–P
O
HO
N
N
N
N
• 1.24 TFA
1H NMR
499.8 MHz
D2O
(internal ref CH3CN set to δ 2.06)
3.742
3.735
3.725
3.716
3.709
3.697
3.690
3.683
3.668
3.661
3.655
3.635
3.629
3.610
3.604
3.602
3.578
3.571
3.557
3.552
3.545
3.539
3.531
3.529
3.526
3.524
3.517
3.511
3.498
3.486
f1 (ppm)

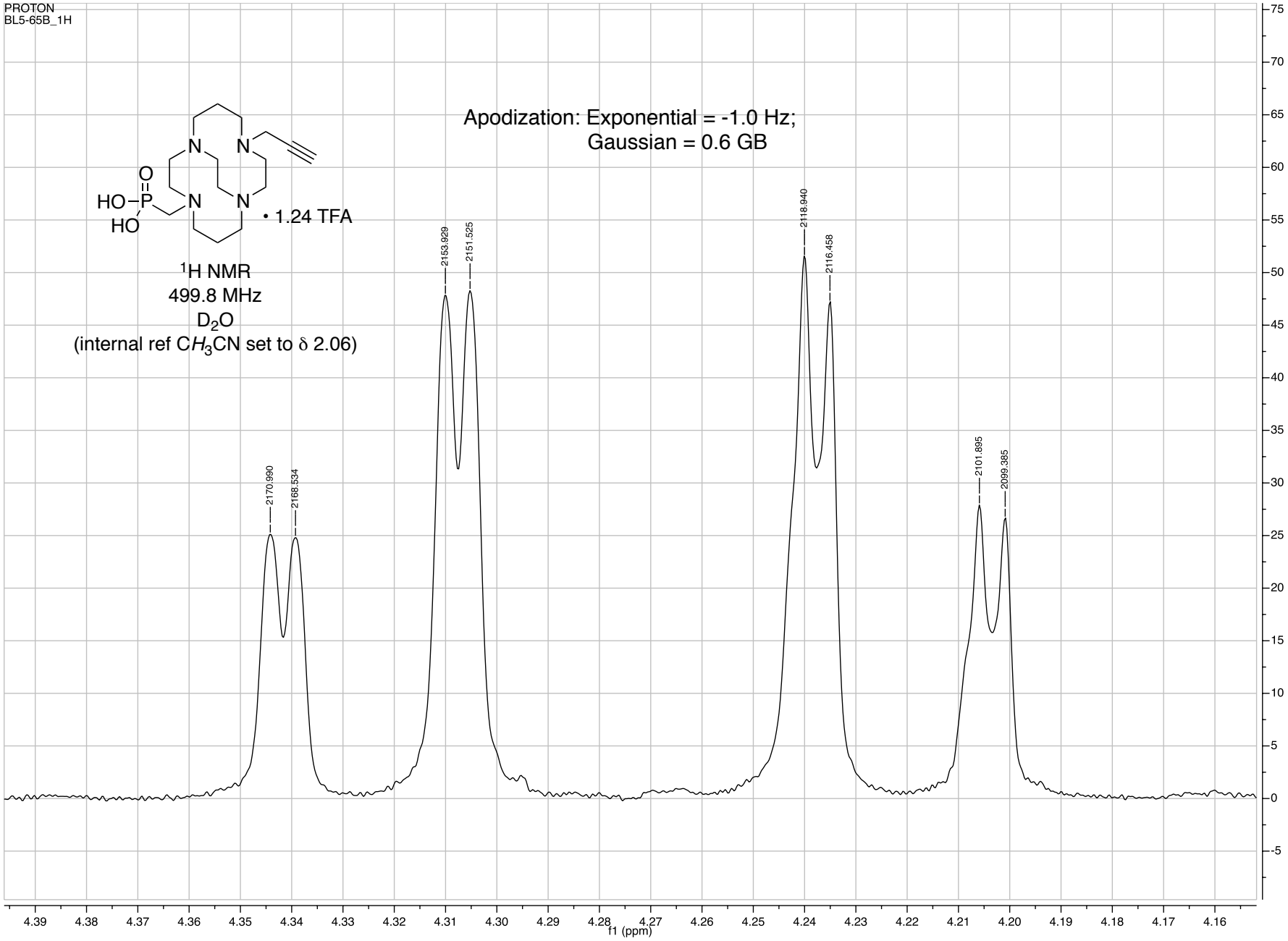

PROTON
BL5-65B_1H
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
3.742
3.735
3.725
3.716
3.709
3.697
3.690
3.683
3.668
3.661
3.655
3.635
3.629
3.610
3.604
3.602
3.578
3.571
3.557
3.552
3.545
3.539
3.531
3.529
3.526
3.524
3.517
3.511
3.498
3.486
f1 (ppm)

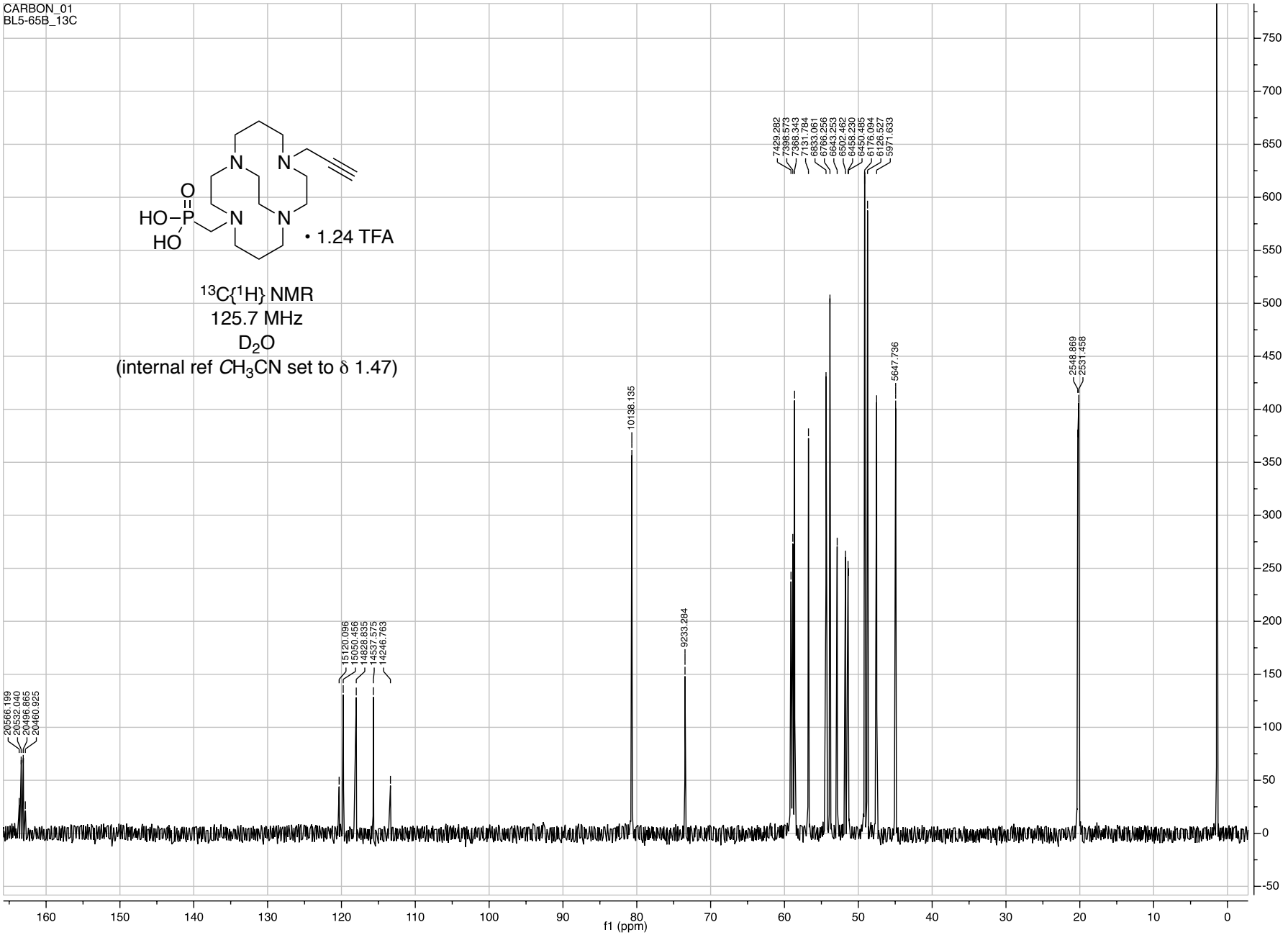

PROTON
BL5-65B_1H
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
1870.043
1866.523
1861.529
1857.226
1853.484
1847.579
1844.004
1840.442
1833.054
1829.773
1826.415
1816.489
1813.565
1803.975
1801.130
1800.295
1788.080
1784.732
1777.679
1774.870
1771.452
1768.360
1764.715
1763.690
1762.218
1761.312
1757.461
1754.788
1748.256
1742.120
f1 (ppm)


PROTON
BL5-65B_1H
HO–P
HO
O
N
N
N
N
N
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
4.344
4.339
4.310
4.305
4.240
4.235
4.206
4.201
f1 (ppm)

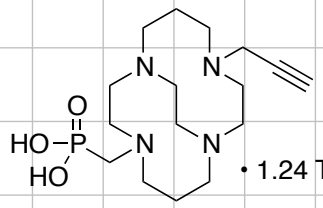

PROTON
BL5-65B_1H
HO–P
HO
O
N
N
N
N
• 1.24 TFA
1H NMR
499.8 MHz
D2O
(internal ref CH3CN set to δ 2.06)
2170.838
2168.653
2153.796
2151.614
2118.926
2116.615
2101.892
2099.454
f1 (ppm)


PROTON
BL5-65B_1H
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
HO
HO
O
P
N
N
N
N
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
4.344
4.339
4.310
4.305
4.240
4.235
4.206
4.201
f1 (ppm)


PROTON
BL5-65B_1H
Apodization: Exponential = -1.0 Hz;
Gaussian = 0.6 GB
• 1.24 TFA
^{1}H NMR
499.8 MHz
D_2O
(internal ref CH_3CN set to δ 2.06)
2170.990
2168.534
2153.929
2151.525
2118.940
2116.458
2101.895
2099.385
f1 (ppm)


CARBON_01
BL5-65B_13C
• 1.24 TFA
$^{13}C\{^{1}H\}$ NMR
125.7 MHz
D_2O
(internal ref CH_3CN set to δ 1.47)
20566.199
20532.040
20496.865
20460.925
15120.096
15050.456
14828.835
14537.575
14246.763
10138.135
9233.284
7429.282
7398.573
7368.343
7131.784
6833.061
6766.256
6643.253
6502.462
6458.230
6450.485
6176.094
6126.527
5971.633
5647.736
2548.869
2531.458
f1 (ppm)

CARBON_01
BL5-65B_13C

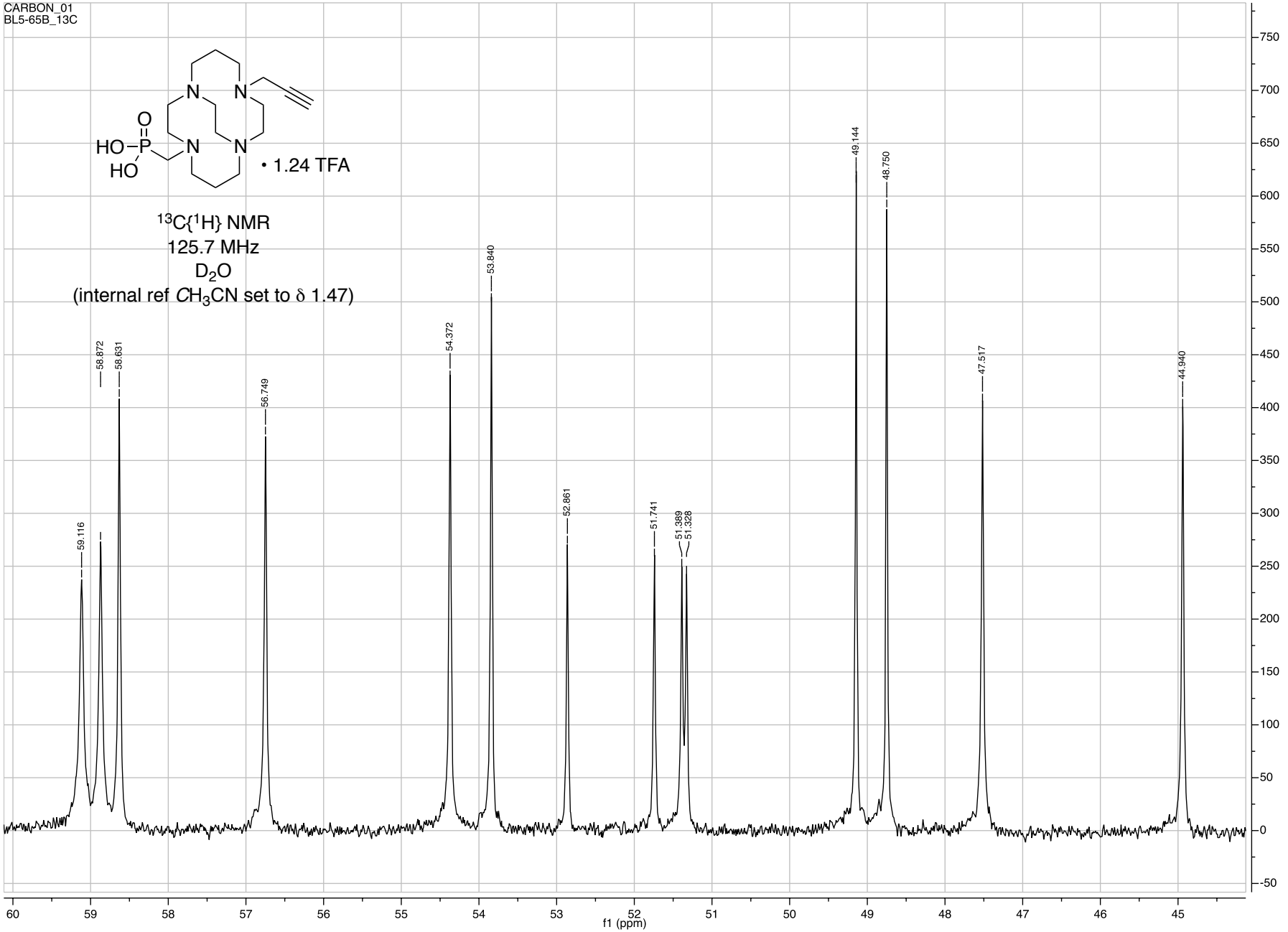


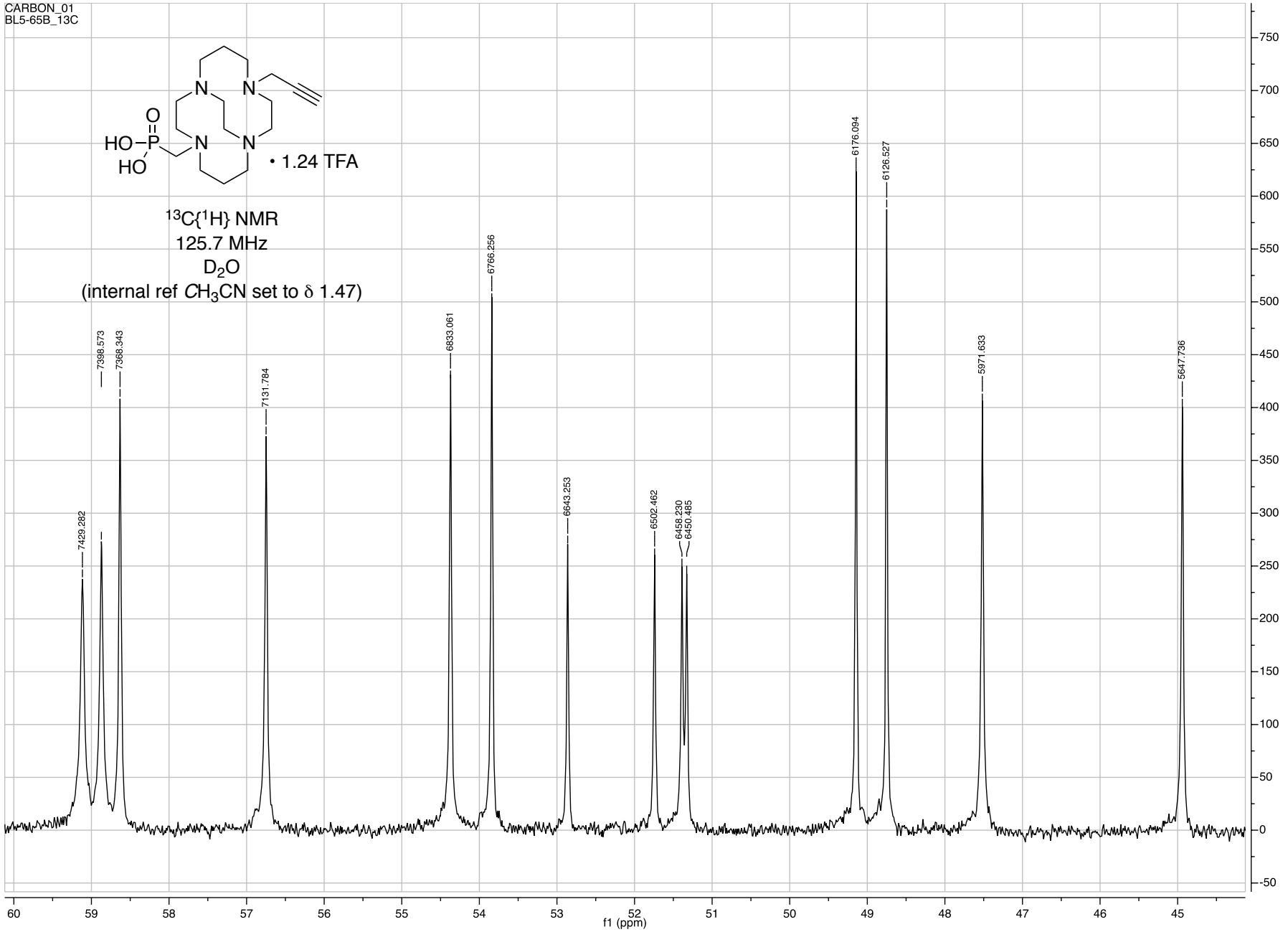
• 1.24 TFA

$^{13}C\{^{1}H\}$ NMR
125.7 MHz
D_2O
(internal ref CH_3CN set to δ 1.47)

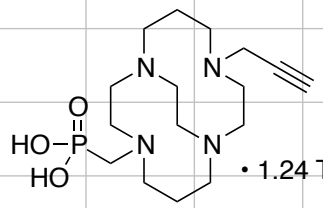
20.28
20.14

f1 (ppm)


CARBON_01
BL5-65B_13C
• 1.24 TFA
$^{13}C\{^{1}H\}$ NMR
125.7 MHz
D_2O
(internal ref CH_3CN set to δ 1.47)
59.116
58.872
58.631
56.749
54.372
53.840
52.861
51.741
51.389
51.328
49.144
48.750
47.517
44.940
f1 (ppm)


CARBON_01
BL5-65B_13C
• 1.24 TFA
$^{13}C\{^{1}H\}$ NMR
125.7 MHz
D_2O
(internal ref CH_3CN set to δ 1.47)
7429.282
7398.573
7368.343
7131.784
6833.061
6766.256
6643.253
6502.462
6458.230
6450.485
6176.094
6126.527
5971.633
5647.736
f1 (ppm)

CARBON_01
BL5-65B_13C



• 1.24 TFA

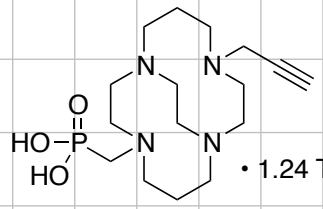
$^{13}C\{^{1}H\}$ NMR
125.7 MHz
D_2O
(internal ref CH_3CN set to δ 1.47)

80.67

73.47

f1 (ppm)

CARBON_01
BL5-65B_13C



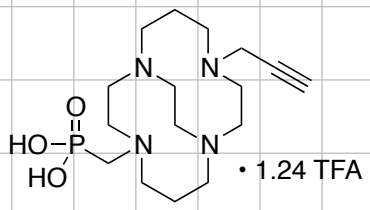
• 1.24 TFA

$^{13}C\{^{1}H\}$ NMR
125.7 MHz
D_2O
(internal ref CH_3CN set to δ 1.47)

163.65
163.38
163.10
162.81
120.31
119.76
118.00
115.68
113.36

f1 (ppm)

CARBON_01
BL5-65B_13C



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