Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
This journal is © The Royal Society of Chemistry 2019

Supplemental Information

Photoaffinity probes for nematode pheromone receptor identification

Ying K. Zhang‡[a], Douglas K. Reilly‡[b], Jingfang Yu[a], Jagan Srinivasan*[b], and Frank C. Schroeder*[a]

[a] *Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY. E-mail: fs31@cornell.edu*

[b] *Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA. E-mail: jsrinivasan@wpi.edu*

‡ *These authors contributed equally*

Contents

1. Supplemental Figure

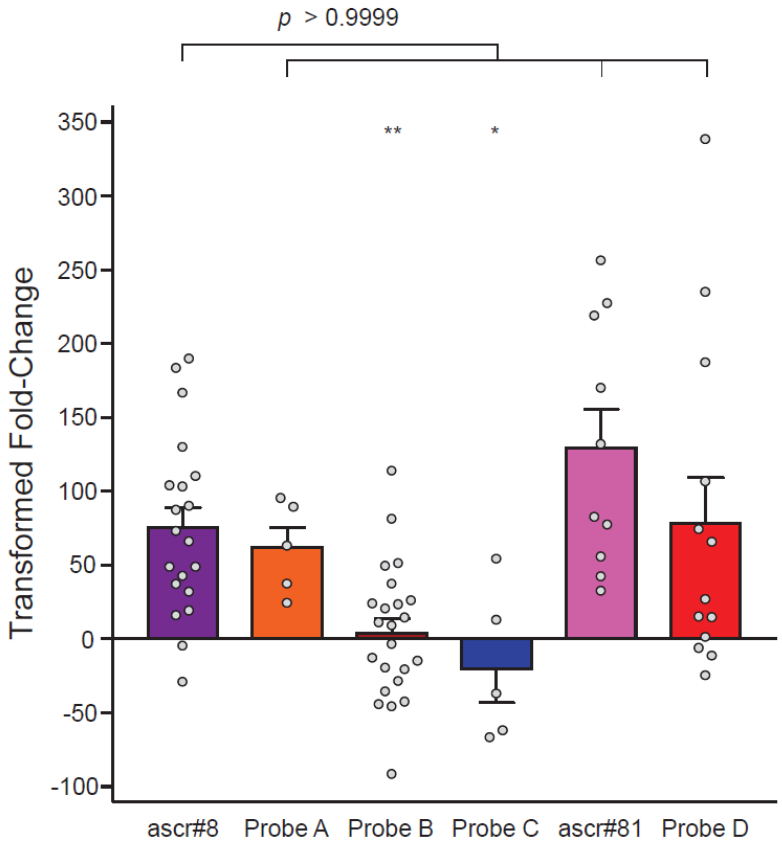


Figure S1. Transformed fold-change of *C. elegans* attraction to ascr#8 and derivatives. Male dwell times in ascr#8 (**1**) and its derivatives, normalized to vehicle control. Data presented as mean ± SEM, $n \geqslant 5$. Kruskal-Wallis test ($p < 0.0001$), * $p = 0.0226$, ** $p = 0.0019$.

2. Experimental section

2.1. Spot retention assay

Bioassays were performed as previously described[1-3]. In short, 50-60 larval stage 4 (L4) male *him-5*(e1490) *C. elegans* were isolated from hermaphrodites and transferred to an NGM plate seeded with OP50 *E. coli*, and left at 20 °C for 5 to 16 h, as needed to complete development to young adults. Immediately before the assay, 0.6 µL of a vehicle control and 1 µM ascr#8 (**1**) (or its derivatives, **9, 10, 11, 2, 12**) were placed in the two scoring regions of a 6 cm-NGM plate coated with a lawn of OP50 *E. coli*. The ascarosides tested were prepared in Milli-Q-purified water from millimolar stock solutions in ethanol. Equivalent dilutions of ethanol into purified water were used as vehicle controls. For each assay, five animals were placed on the two pre-marked spots (**Figure 3A**), equidistant from each cue, before the plate was transferred to a microscope with an attached camera. The plate was then monitored for 20 minutes. The duration of each visit to either cue was scored (if greater than 10 seconds). For each plate, the average dwell time in each scoring region was calculated. This was performed for at least five plates for each compound tested. Plates in which the mean dwell time of either condition was two standard deviations greater than that condition's average were deemed outliers and removed from the data. The CB4088 [*him-5(e1490)*] strain of *C. elegans* used was provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

2.2. Statistical Analyses

Each data set was analyzed for a normal Gaussian distribution using a Shapiro-Wilk normality test. The mean dwell time of each ascaroside (**1, 9, 10, 11, 2, 12**) was compared to the mean dwell time in respective vehicle controls via either a two-tailed paired *t*-test or two-tailed Wilcoxon matched-pairs signed rank test, depending on the results of the Shapiro-Wilk test. Comparison of attraction to ascaroside derivative vs. control was calculated by transforming the measured dwell times using a base-2 exponentiation. This transformed all data points into non-zero values, allowing for the calculation of the fold-change as the log(base 2) of the ratio of the transformed ascaroside dwell time divided by the transformed vehicle dwell time for each plate (see Equation 1). This transformation enabled normalization of the data across biological repeats, as varying baseline dwell times made it difficult to directly compare across conditions (Figure S1).

$$Transformed\ Fold\ Change = \log_2\left(\frac{2^{ascaroside\ dwell\ time}}{2^{vehicle\ dwel\ time}}\right) \quad (1)$$

Since not all data sets were normally distributed, according to a Shapiro-Wilk normality test, a Kruskal-Wallis test was performed, followed by a Dunn's multiple comparisons test. Each probe or ascr#8 derivative was compared to ascr#8 (**1**). An α-value of 0.05 was used in all statistical tests.

2.3. General synthetic procedures

Unless otherwise stated, all oxygen- or moisture-sensitive reactions were carried out under argon atmosphere in flame-dried glassware. Solutions and solvents sensitive to moisture and oxygen were transferred via standard syringe and cannula techniques. All commercial reagents were purchased as reagent grade and, unless otherwise stated, used without further purification. Tetrahydrofuran, dichloromethane, and dimethylformamide were dried over 4Å molecular sieves prior to use; *N*,*N*-diisopropylethylamine was distilled from calcium hydride under argon. Thin-layer chromatography (TLC) was performed using J.T.Baker Silica Gel IB2-F. Flash chromatography was performed using Teledyne Isco CombiFlash systems and Teledyne Isco RediSep Rf silica columns. Nuclear Magnetic Resonance (NMR) spectra were recorded on Varian INOVA 600 (600 MHz) or Varian INOVA 400 (400 MHz) spectrometers. ^{1}H NMR chemical shifts are reported in ppm (δ) relative to residual solvent peaks (7.26 ppm for $CDCl_3$, 3.31 ppm for CD_3OD). NMR-spectroscopic data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants (Hz), and integration. ^{13}C NMR chemical shifts are reported in ppm (δ) relative to $CHCl_3$ (δ 77.2) in $CDCl_3$, CH_3OH (δ 49.0) in CD_3OD. Yields were calculated based on amount of starting material used in the reaction, unless indicated as based on recovered starting material (BRSM).

2.4. Synthesis of Probe A

(*E*)-6-ethoxy-6-oxohex-4-enoic acid (14). To a solution of 4-pentenoic acid (500 mg, 4.99 mmol) dissolved in dry dichloromethane (20 mL), Grubbs catalyst 2nd generation (94 mg, 0.15 mmol) and ethyl acrylate (1.6 ml, 15 mmol) were added at room temperature. The reaction mixture was allowed to stir 20 h and was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-30 % ethyl acetate in hexanes afforded **14** (744 mg, 75%) as a clear oil. Spectroscopic data were identical to those reported previously[4].

Ethyl (*E*)-6-oxo-8-(trimethylsilyl)oct-2-en-7-ynoate (15). Oxalyl chloride (0.32 ml, 3.75 mmol) was added to a solution of **14** (372 mg, 1.88 mmol) in dry dichloromethane (10 mL) at 0 °C. One drop of dimethylformamide (0.05 ml) was added, and the solution was stirred for 20 min at 0 °C. The solution was then concentrated *in vacuo* and redissolved in dry dichloromethane (10 ml). After cooling to 0 °C, bis(trimethylsilyl)ethyne (0.47 ml, 2.07 mmol) followed by aluminum chloride (300 mg, 2.25 mmol) were added. The solution was stirred at 0 °C for 30 min and another 2 hours at room temperature. The reaction was then poured into cold 1 M HCl, extracted with dichloromethane, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-25% ethyl acetate in hexanes afforded **15** (123 mg, 26%) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 6.91 (dt, *J* = 15.6, 6.9 Hz, 1H), 5.84 (d, *J* = 15.6 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.74 (t, *J* = 7.1 Hz, 2H), 2.54 (q, *J* = 7.0 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H), 0.24 (s, 9H).

Ethyl (*S*,*E*)-6-hydroxy-8-(trimethylsilyl)oct-2-en-7-ynoate (SI-1). To a solution of Lithium aluminum hydride (36 mg, 0.95 mmol) in diethyl ether (2 ml), (1*R*,2*S*)-(−)-*N*-

methylephedrine (170.5 mg, 0.95 mmol) in diethyl ether (4 ml) was added slowly. After stirring at reflux for an hour, *N*-ethylaniline (0.24 ml, 1.9 mmol) was added dropwise. After another hour of stirring, the resulting Terashima reagent was cooled to room temperature, and was added dropwise to a solution of **15** (120 mg, 0.48 mmol) in diethyl ether (5 ml) at -78 °C. After 30 min, the reaction was quenched with saturated $NaHCO_3$, extracted with dichloromethane, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using isocratic dichloromethane afforded **SI-1** (117.8 mg, 98%) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 6.97 (dt, *J* = 15.6, 6.9 Hz, 1H), 5.85 (d, *J* = 15.6 Hz, 1H), 4.42-4.35 (m, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 2.42-2.34 (m, 2H), 1.88-1.81 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 0.17 (s, 9H).

Ethyl (*S*,*E*)-6-((*tert*-butyldimethylsilyl)oxy)-8-(trimethylsilyl)oct-2-en-7-ynoate (SI-2). To a solution of **SI-1** (52.6 mg, 0.21 mmol) dissolved in dry dimethylformamide (3 mL), imidazole (42.2 g, 0.62 mmol) and *tert*-butyldimethylsilyl chloride (93.5 mg, 0.62 mmol) were added at room temperature. The reaction mixture was allowed to stir 3 h and was concentrated *in vacuo*. Flash column chromatography on silica using isocratic dichloromethane afforded **SI-2** (72 mg, 94%) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 6.98 (dt, *J* = 15.6, 6.9 Hz, 1H), 5.83 (d, *J* = 15.6 Hz, 1H), 4.36 (t, *J* = 6.2 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 2.39-2.30 (m, 2H), 1.84-1.76 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 0.89 (s, 9H), 0.15 (s, 9H), 0.13 (s, 3H), 0.10 (s, 3H).

(*S*,*E*)-6-((*tert*-butyldimethylsilyl)oxy)oct-2-en-7-ynoic acid (16). To a solution of **SI-2** (72 mg, 0.20 mmol) in 1,4-dioxane (3 mL), lithium hydroxide monohydrate (24.6 mg, 0.59 mmol) in H_2O (0.5 mL) was added, and the resulting mixture was stirred at 60 °C overnight. Glacial acetic acid (1 mL) was added, and the reaction was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-10% methanol in dichloromethane afforded **16** (26 mg, 50%) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 7.10 (dt, *J* = 15.6, 6.9 Hz, 1H), 5.85 (d, *J* = 15.6 Hz, 1H), 4.39 (dt, *J* = 6.1, 2.0 Hz, 1H), 2.45-2.37 (m, 2H), 1.87-1.80 (m, 2H), 0.90 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H).

Ethyl (*S*,*E*)-4-(6-((*tert*-butyldimethylsilyl)oxy)oct-2-en-7-ynamido)benzoate (SI-3). Oxalyl chloride (16.4 µl, 0.19 mmol) was added to a solution of **16** (25.7 mg, 96 µmol) in dry dichloromethane (1 mL) at 0 °C. One drop of dimethylformamide (0.05 ml) was added, and the solution was stirred for 20 min at 0 °C. The solution was then concentrated *in vacuo* and redissolved in dry dichloromethane (1 ml). After cooling to 0 °C, benzocaine (47.4 mg, 0.29 mmol) followed by *N*,*N*-diisopropylethylamine (50 µl, 0.29 mmol) were added. The solution was stirred at 0 °C for 30 min and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-30% ethyl acetate in hexanes afforded **SI-3** (34.5 mg, 87%) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 8.01 (d, *J* = 8.4 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.51-7.42 (m, 1H), 7.04 (dt, *J* = 15.2, 6.9 Hz, 1H), 5.85 (d, *J* = 15.2 Hz, 1H), 4.41 (br t, *J* = 6.1 Hz, 1H), 4.35 (q, *J* = 7.2 Hz, 2H), 2.46-2.37 (m, 3H), 1.88-1.81 (m, 2H), 1.38 (t, *J* = 7.2 Hz, 3H), 0.90 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H).

Ethyl (*S*,*E*)-4-(6-hydroxyoct-2-en-7-ynamido)benzoate (17). To a stirred solution of **SI-3** (34 mg, 0.08 mmol) in acetonitrile (2 ml), 2 drops of 40% HF/H_2O was added at room temperature. After stirring for 1.5 hours, the solution was neutralized with sat. $NaHCO_3$, extracted with dichloromethane, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 20-70% ethyl acetate in hexanes afforded **17** (20.9 mg, 85%) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 8.01 (d, *J* = 8.6 Hz, 2H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.02 (dt, *J* = 15.3, 6.9 Hz, 1H), 6.00 (d, *J* = 15.3 Hz, 1H), 4.42 (dt, *J* = 6.4, 1.8 Hz, 1H), 4.36 (q, *J* = 7.1 Hz, 2H), 2.50 (d, *J* = 1.8 Hz, 1H), 2.44 (q, *J* = 7.4 Hz, 2H), 1.92-1.86 (m, 2H), 1.38 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR (151 MHz, methanol-*d*$_4$):** δ (ppm) 166.4, 164.1, 146.2, 142.3, 131.0, 126.1, 124.5, 119.2, 84.3, 73.8, 61.6, 61.1, 35.9, 27.8, 14.5.

Ethyl 4-((*S*,*E*)-6-(((2*R*,3*R*,5*R*,6*S*)-3-(benzoyloxy)-5-((*tert*-butyldiphenylsilyl)oxy)-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)oct-2-en-7-ynamido)benzoate (19). To a stirred solution of **18** (6.3 mg, 12.8 μmol) in dry dichloromethane (1 mL), trichloroacetonitrile (2.6 μL, 25.7 μmol) and 1,8-diazabicycloundec-7-ene (0.19 μL, 1.3 μmol) were added at room temperature. After 90 min, the reaction was concentrated *in vacuo* to evaporate most solvent. Flash column chromatography on silica using a gradient of 0-20% ethyl acetate in hexanes afforded **SI-4** as a clear oil. A stirred solution of **SI-4** and **17** (4.8 mg, 16 μmol) was cooled to 0 °C in an ice bath, trimethylsilyl trifluoromethanesulfonate (0.7 μL, 3.6 μmol) was added, and the solution was allowed to warm to room temperature. After an hour, the reaction was quenched with sat. $NaHCO_3$, extracted with dichloromethane, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-40% ethyl acetate in hexanes afforded **19** (5.0 mg, 50% from **23,** as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 8.02 (d, *J* = 8.5 Hz, 2H), 7.75 (d, *J* = 7.8 Hz, 2H), 7.67-7.62 (m, 6H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.41-7.29 (m, 8H), 7.10 (dt, *J* = 15.3, 6.9 Hz, 1H), 6.04 (d, *J* = 15.3 Hz, 1H), 5.02 (br s, 1H), 5.00-4.97 (m, 1H), 4.46 (dt, *J* = 6.3, 1.8 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 3.74 (dq, *J* = 9.4, 6.3 Hz, 1H), 3.67 (ddd, *J* = 10.9, 9.4, 4.3 Hz, 1H), 2.55-2.50 (m, 2H), 2.45 (d, *J* = 1.8 Hz, 1H), 2.03-1.97 (m, 3H), 1.92 (dt, *J* = 13.9, 3.9 Hz, 1H), 1.39 (t, *J* = 7.2 Hz, 3H), 1.27 (d, *J* = 6.3 Hz, 3H), 1.05 (s, 9H). **^{13}C NMR (151 MHz, methanol-*d*$_4$):** δ (ppm) 166.3, 165.4, 164.0, 146.3, 142.3, 136.04, 135.96, 134.1, 133.3, 133.2, 131.0, 130.0, 129.94, 129.92, 129.89, 128.4, 127.8, 127.7, 126.1, 124.5, 119.1, 93.7, 81.0, 75.4, 71.1, 70.5, 70.2, 64.5, 61.5, 33.8, 33.5, 27.9, 27.2, 19.5, 18.6, 14.5.

Ethyl 4-((*S*,*E*)-6-(((2*R*,3*R*,5*R*,6*S*)-3-(benzoyloxy)-5-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)oct-2-en-7-ynamido)benzoate (SI-5). To a stirred solution of **19** (5 mg, 6.5 μmol) in tetrahydrofuran (0.5 mL), tetrabutylammonium fluoride solution (1 M in THF, 32 μL, 32 μmol) was added. After stirring overnight, the reaction was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 10-50% ethyl acetate in hexanes afforded ethyl 4-((*S*,*E*)-6-(((2*R*,3*R*,5*R*,6*S*)-3-(benzoyloxy)-5-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)oct-2-en-7-ynamido)benzoate (**SI-5**, 3.2 mg, 93%) as a clear oil. For hydrolysis, lithium hydroxide monohydrate (1.5 mg, 36 μmol) in H_2O (0.1 mL) was added to a solution of **SI-5** (3.2 mg, 6 μmol) in 1,4-dioxane (0.5 mL), and the resulting mixture was stirred at 60 °C overnight. Glacial acetic acid (0.2 mL) was added, and the reaction was

concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-10% methanol in dichloromethane afforded **9** (1.5 mg, 62%) as a clear oil. See **Table S1** for spectroscopic data of probe A. HRMS ESI+: 404.17038 $[M+H]^+$, 426.15192 $[M+Na]^+$. ESI-: 402.15506 $[M-H]^-$.

2.5. Synthesis of Probe B

(2*S*,5*R*,6*R*)-5-(benzyloxy)-6-methoxy-2-methyldihydro-2*H*-pyran-3(4*H*)-one (21). To a stirred solution of **20** (2 g, 4.08 mmol) in tetrahydrofuran (30 mL), tetrabutylammonium fluoride solution (1M in THF, 16.3 mL, 16.3 mmol) was added. After stirring overnight, the reaction was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 10-50% ethyl acetate in hexanes afforded (2*S*,3*R*,5*R*,6*R*)-5-(benzyloxy)-6-methoxy-2-methyltetrahydro-2*H*-pyran-3-ol **(SI-6**, 980 mg, 95%) as a clear oil. Pyridinium chlorochromate (4.9 g, 22.8 mmol) was suspended in methylene chloride (50 ml) and to the reaction mixture 4Å molecular sieves powder was added. After stirring for 30 min, **SI-6** (1.6 g, 6.5 mmol in 20 ml of DCM) was added at room temperature. After 4 hours the reaction was filtered over silica gel and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-30% ethyl acetate in hexanes afforded **21** (1.2 g, 74%) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 7.37-7.27 (m, 5H), 4.81 (d, *J* = 2.4 Hz, 1H), 4.59 (s, 2H), 4.14 (q, *J* = 6.8 Hz, 1H), 3.85 (ddd, *J* = 5.8, 4.6, 2.7 Hz, 1H), 3.48 (s, 3H), 2.75 (dd, *J* = 15.6, 4.3 Hz, 1H), 2.67 (dd, *J* = 15.6, 5.8 Hz, 1H), 1.34 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR (151 MHz, chloroform-*d*):** δ (ppm) 208.0, 137.7, 128.6, 128.1, 127.9, 100.0, 76.5, 71.5, 71.3, 55.6, 40.5, 15.2.

(4*S*,6*R*,7*R*)-7-(benzyloxy)-6-methoxy-4-methyl-5-oxa-1,2-diazaspiro[2.5]oct-1-ene (22). To a stirred solution of **21** (1.2 g, 4.79 mmol) in methanol (2 mL), 7N NH_3 (5.6 ml) and *p*-TsOH (154.6 mg, 0.81 mmol) were added under Ar. After stirring for 3 hours, a solution of hydroxylamine-*O*-sulfonic acid (830 mg, 7.33 mmol) in methanol (1 ml) was added, and the mixture was stirred for 16 hours. Excess ammonia was removed by blowing Ar through the reaction for an hour, and the suspension was filtered, redissolved in methanol, and then cooled to 0 °C. Triethylamine (1 ml, 7.19 mmol) was added to the reaction and after 5 min stirring, a solution of iodine in methanol was added dropwise until color maintained. The reaction was washed with 20 ml 1 M aq. HCl, 20 ml 10% aq. $Na_2S_2SO_3$, 20 ml sat. aq. NaCl, extracted with ethyl acetate, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-20% ethyl acetate in hexanes afforded **22** (492 mg, 39% over three steps) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 7.39-7.27 (m, 5H), 4.76 (br s, 1H), 4.59 (s, 2H), 4.61 (d, *J* = 12.4 Hz, 1H), 4.55 (d, *J* = 12.4 Hz, 1H), 4.23 (q, *J* = 6.7 Hz, 1H), 3.60-3.57 (m, 1H), 3.42 (s, 3H), 2.19 (dd, *J* = 14.8, 3.4 Hz, 1H), 0.86 (dd, *J* = 14.8, 3.7 Hz, 1H), 0.56 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR (151 MHz, chloroform-*d*):** δ (ppm) 138.1, 128.6, 127.91, 127.89, 99.6, 74.2, 71.1, 64.6, 55.2, 29.5, 27.6, 16.5.

(4*S*,6*R*,7*R*)-6-methoxy-4-methyl-5-oxa-1,2-diazaspiro[2.5]oct-1-en-7-yl benzoate (23). To a vigorously stirred solution of **22** (200 mg, 0.76 mmol) in dichloromethane (15 mL), acetonitrile (15 mL) and ruthenium (III) chloride hydrate (23.72 mg, 0.11 mmol) in 15 mL

water was added, followed by the addition of sodium periodate (2.77 g, 12.95 mmol). After stirring for 5 hours, the reaction was diluted with water and extracted with three 4-mL portions of dichloromethane. The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-15% ethyl acetate in hexane afforded **23** (151 mg, 72%) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 8.24-8.20 (m, 2H), 7.62-7.59 (m, 1H), 7.52-7.48 (m, 2H), 5.15-5.13 (m, 1H), 4.87 (br s, 1H), 4.42 (q, *J* = 6.7 Hz, 1H), 3.49 (s, 3H), 2.64 (dd, *J* = 15.3, 3.3 Hz, 1H), 0.81 (dd, *J* = 15.3, 2.9 Hz, 1H), 0.56 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR (151 MHz, chloroform-*d*):** δ (ppm) 165.9, 133.5, 130.2, 128.7, 98.1, 70.0, 64.6, 55.5, 30.0, 27.3, 16.8.

(4*S*,6*R*,7*R*)-6-hydroxy-4-methyl-5-oxa-1,2-diazaspiro[2.5]oct-1-en-7-yl benzoate (24). To a solution of **23** (136 mg, 0.49 mmol) in dichloromethane (3 mL) cooled to -78 °C in a dry ice/acetone bath, boron tribromide solution (1 M in dichloromethane, 0.6 mL, 0.6 mmol) was added dropwise under argon. After 5 min, the reaction was quenched with sat. $NaHCO_3$, extracted with ethyl acetate, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-20% ethyl acetate in hexanes afforded **24**, containing small amounts of two by-products (61.3 mg, 48%, 71% BRSM) as a white powder. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 8.22-8.19 (m, 2H), 7.61-7.57 (m, 1H), 7.50-7.46 (m, 2H), 5.39 (br s, 1H), 5.18-5.15 (m, 1H), 4.64 (q, *J* = 6.7 Hz, 1H), 2.69 (dd, *J* = 15.3, 3.3 Hz, 1H), 0.84 (dd, *J* = 15.3, 2.9 Hz, 1H), 0.54 (d, *J* = 6.7 Hz, 3H). **^{13}C NMR (151 MHz, chloroform-*d*):** δ (ppm) 166.0, 133.6, 130.2, 128.7, 91.5, 70.4, 64.8, 29.4, 27.2, 16.8.

4-((*R*,*E*)-6-(((4*S*,6*R*,7*R*)-7-hydroxy-4-methyl-5-oxa-1,2-diazaspiro[2.5]oct-1-en-6-yl)oxy)hept-2-enamido)benzoic acid (10, probe B). To a stirred solution of **24** (33 mg, 0.13 mmol) in dry dichloromethane (3 mL), trichloroacetonitrile (25.2 μL, 0.25 mmol) and 1,8-diazabicycloundec-7-ene (0.5 μL, 3 μmol) were added at room temperature. After 90 min, the reaction was concentrated *in vacuo* to evaporate most solvent. Flash column chromatography on silica using a gradient of 15-25% ethyl acetate in hexanes afforded **SI-7** (43.5 mg, 85%) as a clear oil. A stirred solution of **SI-7** and *N*-(6'*R*-hydroxy-2'*E*-heptenoyl)-4-aminobenzoic acid ethyl ester (55 mg, 0.19 mmol, prepared following a previously reported method[2]) was cooled to 0 °C in an ice bath, trimethylsilyl trifluoromethanesulfonate (4.6 μL, 25 μmol) was added, and the solution was allowed to warm to room temperature. After an hour, the reaction was quenched with sat. $NaHCO_3$, extracted with dichloromethane, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-15% ethyl acetate in hexanes afforded ethyl 4-((*R*,*E*)-6-(((4*S*,6*R*,7*R*)-7-(benzoyloxy)-4-methyl-5-oxa-1,2-diazaspiro[2.5]oct-1-en-6-yl)oxy)hept-2-enamido)benzoate **(25**, 38.1 mg, 57%) as a clear oil. To a solution of **25** (35 mg, 0.07 mmol) in 1,4-dioxane (2 mL), lithium hydroxide monohydrate (16.4 mg, 0.39 mmol) in H_2O (0.3 mL) was added and the resulting mixture was stirred at 60 °C for 4 hours. Glacial acetic acid (1 mL) was added, and the reaction was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 10-60% ethyl acetate in hexanes afforded **10** (20.6 mg, 79%) as a clear oil. See **Table S2** for spectroscopic data of probe B. HRMS ESI+: 404.18213 $[M+H]^+$. ESI-: 402.16693 $[M-H]^-$.

2.6. Synthesis of Probe C

4-((*R*,*E*)-6-(((2*R*,3*R*,5*R*,6*S*)-5-((*tert*-butyldiphenylsilyl)oxy)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)hept-2-enamido)benzoic acid (SI-8). To a stirred solution of **18** (375 mg, 0.76 mmol) in dry dichloromethane (10 mL), trichloroacetonitrile (0.15 mL, 1.53 mmol) and 1,8-diazabicycloundec-7-ene (11 μL, 0.08 mmol) were added at room temperature. After 90 min, the reaction was concentrated *in vacuo* to evaporate most solvent. Flash column chromatography on silica using a gradient of 0-20% ethyl acetate in hexanes afforded **SI-4** as a clear oil. A stirred solution of **SI-4** and *N*-(6'*R*-hydroxy-2'*E*-heptenoyl)-4-aminobenzoic acid ethyl ester (267 mg, 0.92 mmol, prepared following previous reported method[2]) was cooled to 0 °C in an ice bath, trimethylsilyl trifluoromethanesulfonate (28 μL, 0.15 mmol) was added, and the solution was allowed to warm to room temperature. After an hour, the reaction was quenched with sat. $NaHCO_3$, extracted with dichloromethane, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was used for next step without further purification. To a solution of crude **26** in 1,4-dioxane (5 mL), lithium hydroxide monohydrate (58 mg, 1.39 mmol) in H_2O (1 mL) was added and the resulting mixture was stirred at 60 °C for 4 hours. Glacial acetic acid (1 mL) was added, and the reaction was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 20-80% ethyl acetate in hexanes afforded **SI-8** (61.8 mg, 13% over 2 steps) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 8.08-8.00 (m, 2H), 7.71-7.61 (m, 6H), 7.43-7.32 (m, 6H), 7.08 (dt, *J* = 15.2, 6.8 Hz, 1H), 6.01 (d, *J* = 15.2 Hz, 1H), 4.61 (br s, 1H), 3.88-3.61 (m, 4H), 2.48-2.29 (m, 2H), 1.90-1.58 (m, 4H), 1.16 (d, *J* = 6.2 Hz, 3H), 1.13 (d, *J* = 6.2 Hz, 3H), 1.06 (s, 9H).

4-((*R*,*E*)-6-(((2*R*,5*R*,6*S*)-5-((*tert*-butyldiphenylsilyl)oxy)-6-methyl-3-oxotetrahydro-2*H*-pyran-2-yl)oxy)hept-2-enamido)benzoic acid (27). To a stirred solution of **SI-8** (59 mg, 0.09 mmol) in dry dichloromethane (1 mL), Dess–Martin periodinane (60 mg, 0.14 mmol) was added at room temperature. After stirring overnight, the reaction was quenched with sat. $Na_2S_2SO_3$, extracted with dichloromethane, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-60% ethyl acetate in hexanes afforded **27** (30 mg, 51%) as a clear oil. **^{1}H NMR (600 MHz, chloroform-*d*):** δ (ppm) 8.08-8.05 (m, 2H), 7.70-7.61 (m, 6H), 7.45-7.36 (m, 6H), 7.08 (dt, *J* = 15.2, 6.8 Hz, 1H), 6.02 (d, *J* = 15.2 Hz, 1H), 4.57 (br s, 1H), 4.10 (dq, *J* = 9.0, 6.2 Hz, 1H), 3.90-3.81 (m, 1H), 3.62 (ddd, *J* = 10.6, 9.0, 5.0 Hz, 1H), 2.73 (dd, *J* = 14.2, 10.6 Hz, 1H), 2.52 (dd, *J* = 14.2, 5.0 Hz, 1H), 2.47-2.28 (m, 2H), 1.84-1.62 (m, 2H), 1.21 (d, *J* = 6.3 Hz, 3H), 1.15 (d, *J* = 6.2 Hz, 3H), 1.06 (s, 9H).

4-((*R*,*E*)-6-(((4*R*,6*S*,7*R*)-7-hydroxy-6-methyl-5-oxa-1,2-diazaspiro[2.5]oct-1-en-4-yl)oxy)hept-2-enamido)benzoic acid (11, probe C). To a stirred solution of **27** (30 mg, 0.05 mmol) in methanol (1 mL), 7 N NH_3 (56 μl) was added under Ar. After stirring for 4 hours, a solution of hydroxylamine-*O*-sulfonic acid (8.1 mg, 0.07 mmol) in methanol (0.1 ml) was added and the mixture was stirred for 16 hours. Excess ammonia was removed by blowing Ar through the reaction for an hour and the suspension was filtered and redissolved in methanol, cooling to 0 °C. Triethylamine (10 μl, 0.07 mmol) was added to the reaction and after 5 min

stirring, a solution of iodine in methanol was added dropwise until color maintained. The reaction was washed with 20 ml 1 M HCl, 20 ml 10% $Na_2S_2SO_3$, 20 ml sat. NaCl, extracted with ethyl acetate, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was used for next step without further purification. To a stirred solution of crude **28** in tetrahydrofuran (0.5 mL), tetrabutylammonium fluoride solution (1M in THF, 39 μL, 0.04 mmol) was added. After stirring for 12 hours, the reaction was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 10-50% ethyl acetate in hexanes afforded **11** (2.6 mg, 13% over 4 steps) as a clear oil. See **Table S3** for spectroscopic data of probe C. HRMS ESI+: 404.18198 $[M+H]^+$. ESI-: 402.16693 $[M-H]^-$.

2.7. Synthesis of Probe D

(*R*,*E*)-4-(6-((*tert*-butyldimethylsilyl)oxy)hept-2-enamido)benzoic acid (SI-9). To a solution of **29** (37.2 mg, 0.09 mmol) in 1,4-dioxane (1 mL), lithium hydroxide monohydrate (11.5 mg, 0.28 mmol) in H_2O (0.5 mL) was added, and the resulting mixture was stirred at 60 °C overnight. Glacial acetic acid (0.2 mL) was added, and the reaction was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-20% ethyl acetate in hexane afforded **SI-9** (24 mg, 69%) as a clear oil. **^{1}H NMR (600 MHz, methanol-d_4):** δ (ppm) 7.97 (d, *J* = 8.7 Hz, 2H), 7.73 (d, *J* = 8.7 Hz, 2H), 6.98 (dt, *J* = 15.3, 6.9 Hz, 1H), 6.13 (d, *J* = 15.3 Hz, 1H), 3.94-3.86 (m, 1H), 2.40-2.23 (m, 2H), 1.62-1.57 (m, 2H), 1.16 (d, *J* = 6.1 Hz, 3H), 0.08 (s, 6H). **^{13}C NMR (151 MHz, methanol-d_4):** δ (ppm) 169.6, 166.8, 147.9, 144.4, 131.7, 127.1, 124.9, 120.2, 69.2, 39.3, 29.5, 26.4, 24.2, 18.9, -4.2, -4.6.

(*R*,*E*)-*N*-(2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethyl)-4-(6-((*tert*-butyldimethylsilyl)oxy)hept-2-enamido)benzamide (SI-10). To a stirred solution of **SI-9** (24 mg, 0.06 mmol) in dry dichloromethane (1 mL), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC•HCl) (36.6 mg, 0.19 mmol) and 4-dimethylaminopyridine (23.3 mg, 0.19 mmol) were added at room temperature. The resulting mixture was stirred for 30 min, and 2-(3-(but-3-yn-1-yl)-3H-diazirin-3-yl)ethan-1-amine (17.4 mg, 0.13 mmol) in dry dichloromethane (0.5 mL) was added. After 2 hours, the reaction was washed with sat. $NaHCO_3$, extracted with ethyl acetate, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-50% ethyl acetate in hexanes afforded **SI-10** (16.7 mg, 53%) as a clear oil.
^{1}H NMR (600 MHz, methanol-d_4): δ (ppm) 7.80 (d, *J* = 8.7 Hz, 2H), 7.73 (d, *J* = 8.7 Hz, 2H), 6.98 (dt, *J* = 15.3, 6.9 Hz, 1H), 6.13 (d, *J* = 15.3 Hz, 1H), 3.94-3.86 (m, 1H), 3.28 (t, *J* = 7.2 Hz, 2H), 2.40-2.24 (m, 3H), 2.04 (dt, *J* = 7.5, 2.5 Hz, 2H), 1.73 (t, *J* = 7.2 Hz, 2H), 1.67 (t, *J* = 7.5 Hz, 2H), 1.63-1.58 (m, 2H), 1.17 (d, *J* = 6.1 Hz, 3H), 0.91 (s, 9H), 0.08 (s, 6H). **^{13}C NMR (151 MHz, methanol-d_4):** δ (ppm) 169.6, 166.8, 147.8, 143.3, 130.7, 129.2, 124.9, 120.4, 83.6, 70.4, 69.2, 39.3, 36.0, 33.5, 33.3, 29.5, 27.9, 26.4, 24.2, 18.9, 13.9, -4.1, -4.5.

(*R*,*E*)-*N*-(2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethyl)-4-(6-hydroxyhept-2-enamido)benzamide (30). To a stirred solution of **SI-10** (16.7 mg, 34 μmol) in acetonitrile (0.5 ml), 2 drops of 40% HF/H_2O was added at room temperature. After stirring for 1.5 hours, the solution was neutralized with sat. $NaHCO_3$, extracted with dichloromethane, dried over

Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-10% methanol in dichloromethane afforded **30** (11.8 mg, 92%) as a clear oil. **^{1}H NMR (600 MHz, methanol-*d*$_4$):** δ (ppm) 7.80 (d, *J* = 8.8 Hz, 2H), 7.72 (d, *J* = 8.7 Hz, 2H), 6.98 (dt, *J* = 15.3, 6.9 Hz, 1H), 6.13 (d, *J* = 15.3 Hz, 1H), 3.80-3.73 (m, 1H), 3.28 (t, *J* = 7.2 Hz, 2H), 2.44-2.28 (m, 2H), 2.27 (t, *J* = 2.7 Hz, 1H), 2.04 (dt, *J* = 7.6, 2.7 Hz, 2H), 1.73 (t, *J* = 7.2 Hz, 2H), 1.67 (t, *J* = 7.5 Hz, 2H), 1.64-1.58 (m, 2H), 1.19 (d, *J* = 6.2 Hz, 3H). **^{13}C NMR (151 MHz, methanol-*d*$_4$):** δ (ppm) 169.6, 166.8, 147.6, 143.2, 130.7, 129.2, 125.0, 120.4, 83.6, 70.4, 67.8, 38.6, 36.0, 33.5, 33.3, 29.5, 28.0, 23.5, 13.9.

(2*R*,3*R*,5*R*,6*S*)-2-(((*R*,*E*)-7-((4-((2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethyl)carbamoyl)phenyl)amino)-7-oxohept-5-en-2-yl)oxy)-5-hydroxy-6-methyltetrahydro-2*H*-pyran-3-yl benzoate (SI-12). To a stirred solution of **18** (13.8 mg, 28 μmol) in dry dichloromethane (0.5 mL) and *N*,*N*-dimethylformamide (0.5 mL), trichloroacetonitrile (5.6 μL, 56 μmol) and 1,8-diazabicycloundec-7-ene (0.4 μL, 2.8 μmol) were added at room temperature. After 90 min, the reaction was concentrated *in vacuo* to evaporate most solvent. Flash column chromatography on silica using a gradient of 0-20% ethyl acetate in hexanes afforded **SI-4** as a clear oil. A stirred solution of **SI-4** and **30** (11.8 mg, 31 μmol) was cooled to 0 °C in an ice bath, trimethylsilyl trifluoromethanesulfonate (1 μL, 5.6 μmol) was added, and the solution was allowed to warm to room temperature. After an hour, the reaction was quenched with sat. $NaHCO_3$, extracted with dichloromethane, dried over Na_2SO_4, and concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-40% ethyl acetate in hexanes afforded **SI-11** (2 mg, 8% over 2 steps) containing some residual *N*,*N*-dimethylformamide. To a stirred solution of impure **SI-11** in tetrahydrofuran (0.2 mL), tetrabutylammonium fluoride solution (1 M in THF, 8.4 μL, 8.4 μmol) was added. After stirring for 12 hours, the reaction was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 30-70% ethyl acetate in hexanes afforded **SI-12** (1 mg, 77%) as a clear oil. The crude product was used in the next step without further purification.

***N*-(2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethyl)-4-((*R*,*E*)-6-(((2*R*,3*R*,5*R*,6*S*)-3,5-dihydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)hept-2-enamido)benzamide (12, probe D)**. To a solution of **SI-12** (1 mg, 1.6 μmol) in 1,4-dioxane (0.2 mL), lithium hydroxide monohydrate (0.2 mg, 4.9 μmol) in H_2O (50 μL) was added, and the resulting mixture was stirred at 60 °C overnight. Glacial acetic acid (50 μL) was added, and the reaction was concentrated *in vacuo*. Purification of part of the reaction mixture *via* HPLC provided a pure sample of **12** (0.5 mg, 4% over 3 steps from **30**) as a clear oil. See **Table S4** for spectroscopic data of probe D. HRMS ESI+: 513.27130 $[M+H]^+$, 535.25311 $[M+Na]^+$. ESI-: 511.25616 $[M-H]^-$.

2.7. Click reaction with ascr#18-alkyne (SI-13)

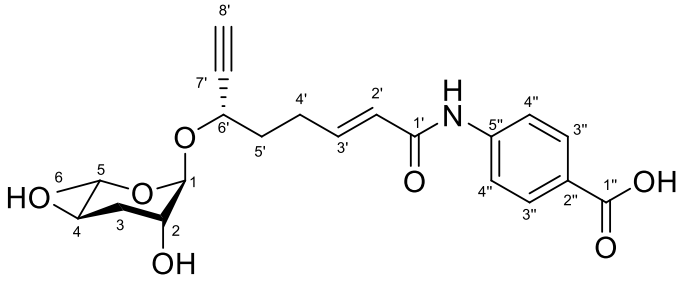
10-(((2*R*,3*R*,5*R*,6*S*)-3,5-dihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)dodec-11-ynoic acid (ascr#18-alkyne, SI-13). To a suspension of Pd/C (1 g) in 20 mL MeOH, (*E*)-10-ethoxy-10-oxodec-8-enoic acid (1.05 g, 4.59 mmol), prepared as described for **14**, was added under argon at 20 °C. After stirring under hydrogen for 1 hour, the solution was filtered and

concentrated *in vacuo*, providing 10-ethoxy-10-oxodecanoic acid (895.9 mg, 85%). Of this material, (325 mg, 1.047 mmol) were reacted, following the procedure described for **SI-1**, to prepare ethyl 10-hydroxy-12-(trimethylsilyl)dodec-11-ynoate (245mg, 75%). Subsequently, a stirred solution of (2*S*,3*R*,5*R*,6*S*)-2-methyl-6-(2,2,2-trichloro-1-iminoethoxy)tetrahydro-2H-pyran-3,5-diyl dibenzoate, prepared as described previously[5] (81.2mg, 0.163 mmol) and ethyl 10-hydroxy-12-(trimethylsilyl)dodec-11-ynoate (72.8 mg, 0.233 mmol) was cooled to 0 °C in an ice bath, trimethylsilyl trifluoromethanesulfonate (5.6 μL, 31 μmol) was added, and the solution was allowed to warm to room temperature. After an hour, the reaction was quenched with sat. $NaHCO_3$, extracted with dichloromethane, dried over Na_2SO_4, and concentrated *in vacuo*. Following flash column chromatography on silica using a gradient of ethyl acetate in hexanes, crude (2*R*,*3R*,*5R*,*6S*)-2-((12-ethoxy-12-oxo-1-(trimethylsilyl)dodec-1-yn-3-yl)oxy)-6-methyltetrahydro-2H-pyran-3,5-diyl dibenzoate (91.9 mg, 67%) was obtained, which was dissolved in a mixture of H_2O (200 μL) and dioxane (1 mL). After adding lithium hydroxide monohydrate (20.9 mg, 0.5 mmol) the resulting mixture was stirred at 60 °C overnight. Glacial acetic acid was added, and the reaction was concentrated *in vacuo*. Flash column chromatography on silica using a gradient of 0-10% methanol in dichloromethane afforded **SI-13** (21.8 mg, 77%) as a clear oil. **^{1}H NMR (600 MHz, methanol-*d*$_4$):** δ (ppm) 5.49 (s, 1H), 4.36 (dt, *J* = 6.9, 2.4 Hz, 1H), 3.76 (s, 1H), 3.58-3.45 (m, 2H), 2.85 (d, *J* = 2.1 Hz, 1H), 2.28 (t, *J* = 7.68 Hz, 2H), 1.97 (d, *J* = 12.5, 3.2 Hz, 1H), 1.80-1.68 (m, 3H), 1.60 (t, *J* = 7.6 Hz, 2H), 1.49 (s, 1H), 1.34 (s, 8H), 1.23 (d, *J* = 6.0 Hz, 3H). **^{13}C NMR (151 MHz, methanol-*d*$_4$):** δ (ppm) 177.7, 97.4, 83.1, 75.1, 71.5, 69.5, 68.1, 65.8, 36.7, 35.9, 35.0, 30.4, 30.6, 30.25, 30.21, 26.4, 26.1, 18.1. HRMS ESI+: 343.21094 $[M+H]^+$, 365.19244 $[M+Na]^+$. ESI-: 341.19678 $[M-H]^-$.

10-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-10-(((2*R*,3*R*,5*R*,6*S*)-3,5-dihydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)decanoic acid (SI-14). To a stirred solution of **SI-13** (2mg, 5.6 μmol) and benzyl azide (1.05 μL, 8.4 μmol) in a mixture of water and acetonitrile (360 μL; v/v, 2:1), $CuSO_4 \cdot 5H_2O$ (10 mol%) and hydrazine hydrate (0.36 μL, 5.6 μmol) were added, and the solution was stirred at 50 °C. After 5 hours, the reaction was concentrated *in vacuo* and chromatographed on silica using a mixture of Hexane/Ethyl acetate/Methanol (v/v/v, 2:6:1), providing **SI-14** (0.2mg, 7.5%) as a clear oil. See **Table S5** for spectroscopic data of **SI-14**. HRMS ESI+: 476.27472 $[M+H]^+$, 498.25696 $[M+Na]^+$. ESI-: 341.19678 $[M-H]^-$.

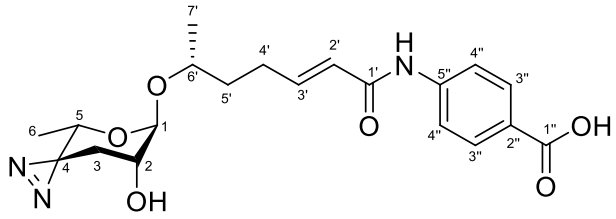
3. Spectroscopic data for synthesized probes

3.1. Table S1. Spectroscopic data for Probe A (9)



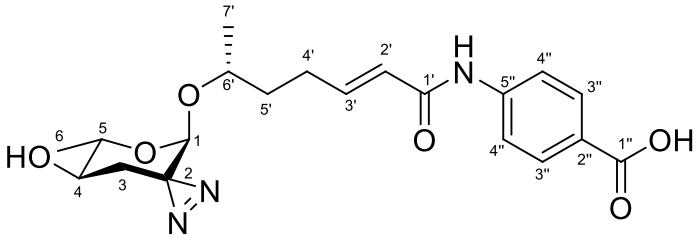
Position	δ ^{1}H [ppm]	δ ^{13}C [ppm]	^{1}H-^{1}H-coupling constants [Hz]	HMBC correlations
1	4.93	97.1		C-2, C-3, C-5, C-6’
2	3.79	69.0	$J_{2,3a}$ =3.0	C-4
3a	1.77	35.7	$J_{3a,3b}$ = 13.5, $J_{3a,4}$ =11.1	C-4, C-5
3b	1.99	35.7	$J_{3b,4}$ =4.3	C-1, C-2, C-4, C-5
4	3.54	67.8	$J_{4,5}$ = 9.5	C-3, C-5, C-6
5	3.51	71.2	$J_{5,6}$ = 6.0	C-1, C-3, C-4, C-6
6	1.22	17.8		C-1, C-4, C-5
1′		166.2		
2′	6.18	125.3	$J_{2',3'}$ = 15.3	C-1’, C-4’
3′	6.98	146.2	$J_{3',4'}$ = 6.9	C-1', C-2’, C-4’, C-5’
4′	2.49	28.7		C-2’, C-3', C-5’, C-6’
5′	1.96	35.0	$J_{5',6'}$ = 6.3	C-3’, C-4’, C-6’, C-7’
6′	4.45	64.7	$J_{6',8'}$ = 1.8	C-1, C-4’, C-5’, C-7’, C-8’
7′		82.3		
8′	2.93	75.7		C-6’, C-7’
1″		170.9		
2″		143.2		
3″	7.96	131.3	$J_{3'',4''}$ = 8.4	C-1’’, C-2’’, C-4’’,
4″	7.69	119.9		C-2’’, C-5’’
5″		129.3		

3.2. Table S2. Spectroscopic data for Probe B (10)



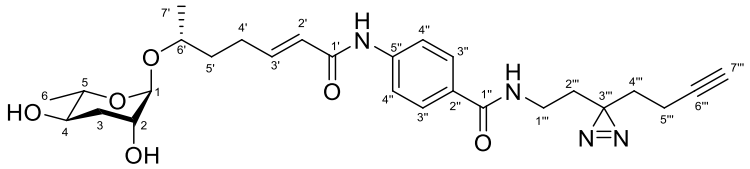
Position	δ ^{1}H [ppm]	δ ^{13}C [ppm]	^{1}H-^{1}H-coupling constants [Hz]	HMBC correlations
1	4.88	98.2	$J_{1,2} = 2.0$	C-2, C-3, C-5, C-6’
2	3.75	69.2	$J_{2,3a} = 3.3$ $J_{2,3b} = 3.5$	C-4
3a	2.31	33.2	$J_{3a,3b} = 14.7$	C-1, C-2, C-4
3b	0.68			C-1, C-2, C-4, C-5
4		28.3		
5	4.31	66.1	$J_{5,6} = 6.8$	C-1, C-6
6	0.48	16.6		C-4, C-5
1′		166.7		
2′	6.17	125.0	$J_{2',3'} = 15.2$	C-1', C-4’
3′	7.02	147.6	$J_{3',4'} = 6.8$	C-1', C-2’ (weak), C-4’, C-5’
4′	2.43	29.5		C-2’, C-3', C-5’, C-6’
5′	1.76	36.9		C-3’, C-4’, C-6’, C-7’
6′	3.91	72.4	$J_{6',7'} = 6.1$	C-1, C-4’, C-5’
7′	1.22	19.2		C-5’, C-6’
1″		170.1		
2″		144.0		
3″	7.97	131.7	$J_{3'',4''} = 8.6$	C-1’’, C-2’’, C-4’’,
4″	7.72	120.2		C-2’’, C-5’’
5″		127.9		

3.3. Table S3. Spectroscopic data for Probe C (11)



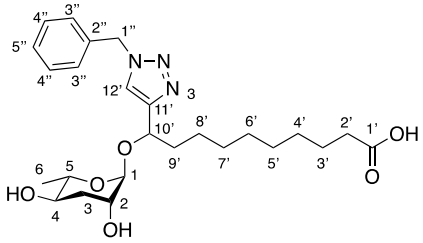
Position	δ ^{1}H [ppm]	δ ^{13}C [ppm]	^{1}H-^{1}H-coupling constants [Hz]	HMBC correlations
1	3.57	97.5		C-2, C-3, C-5, C-6’
2		28.9		
3a	2.10	35.1	$J_{3a,3b}$ = 13.8, $J_{3a,4}$ =11.1	C-2, C-4
3b	0.81	35.1	$J_{3b,4}$ =5.3	C-1, C-2, C-4
4	3.51	70.7	$J_{4,5}$ = 9.3	C-5, C-6
5	3.80	70.4	$J_{5,6}$ = 6.3	C-1, C-3, C-4
6	1.29	17.6		C-5
1′		166.5		
2′	6.18	125.0	$J_{2',3'}$ = 15.3	C-1', C-4’
3′	7.02	147.2	$J_{3',4'}$ = 6.9	C-1', C-2’ (weak), C-4’, C-5’
4′	2.44	29.1		C-2’, C-3', C-5’, C-6’
5′	1.74	36.5		C-3’, C-4’, C-6’, C-7’
6′	3.76	71.7	$J_{6',7'}$ = 6.1	C-1, C-4’, C-5’
7′	1.05	18.9		C-5’, C-6’
1″		169.8		
2″		143.9		
3″	7.97	131.4	$J_{3'',4''}$ = 8.8	C-1”, C-2”, C-4”,
4″	7.72	120.0		C-2” (weak), C-5”
5″		127.6		

3.4. Table S4. Spectroscopic data for Probe D (12)



Position	δ ^{1}H [ppm]	δ ^{13}C [ppm]	^{1}H-^{1}H-coupling const. [Hz]	HMBC correlations
1	4.68	97.1		C-2, C-3, C-5, C-6’
2	3.74	69.6	*$J_{2,3a}$ =3.0, $J_{2,3b}$ =3.8*	C-4
3a	1.79	35.7	*$J_{3a,3b}$ = 13.1, $J_{3a,4}$ =11.3*	C-4, C-5
3b	1.97		*$J_{3b,4}$ =4.5*	C-1, C-2, C-4, C-5
4	3.54	68.0	*$J_{4,5}$ = 9.5*	C-5, C-6
5	3.63	71.0	*$J_{5,6}$ = 6.3*	C-1, C-3, C-4, C-6
6	1.22	17.8		C-4, C-5
1′		166.6		
2′	6.16	124.9	*$J_{2',3'}$ = 15.4*	C-1', C-4’
3′	7.00	147.3	*$J_{3',4'}$ = 6.9*	C-1', C-2’, C-4’, C-5’
4′	2.41	29.1		C-2’, C-3', C-5’, C-6’
5′	1.72	36.7		C-3’, C-4’, C-6’, C-7’
6′	3.85	71.4	*$J_{6',7'}$ = 6.1*	C-1, C-4’, C-5’
7′	1.18	18.9		C-5’, C-6’
1″		169.4		
2″		143.0		
3″	7.80	128.9	*$J_{3'',4''}$ = 8.7*	C-1”, C-2”, C-4”
4″	7.72	120.2		C-2”, C-3”, C-5”
5″		130.5		
1‴	3.29	35.7	*$J_{1''',2'''}$ = 7.2*	C-1”, C-2”’, C-3”’
2‴	1.74	33.3		C-1”’, C-3”’, C-4”’
3‴		27.7		
4‴	1.67	33.0	*$J_{4''',5'''}$ = 7.4*	C-2”’, C-3”’, C-5”’, C-6”’
5‴	2.05	13.5		C-3”’, C-4”’, C-6”’, C-7”’
6‴		83.2		
7‴	*2.26*	*69.9*	*$J_{5''',7'''}$ = 2.7*	

3.5. Table S5. Spectroscopic data for SI-14

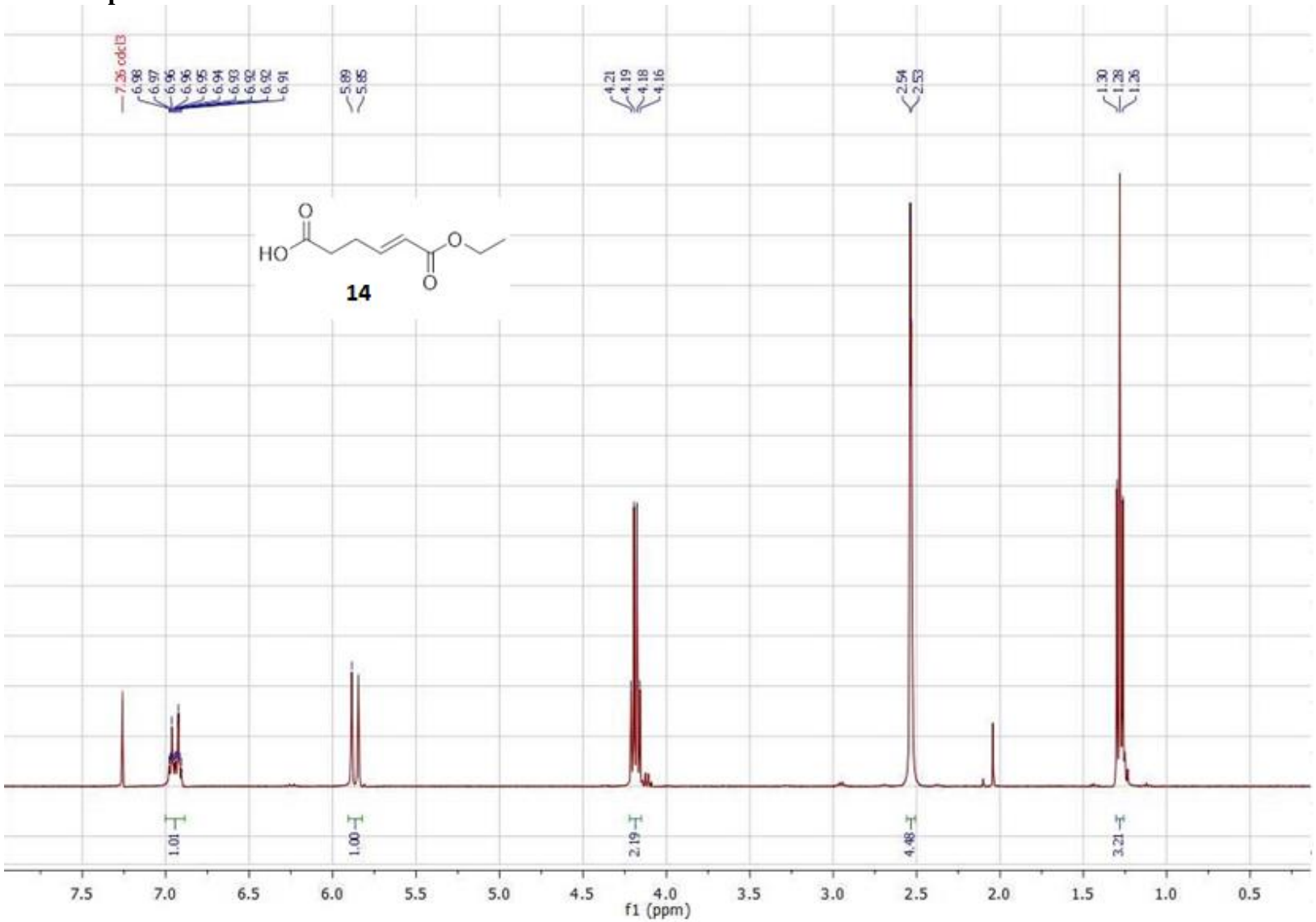


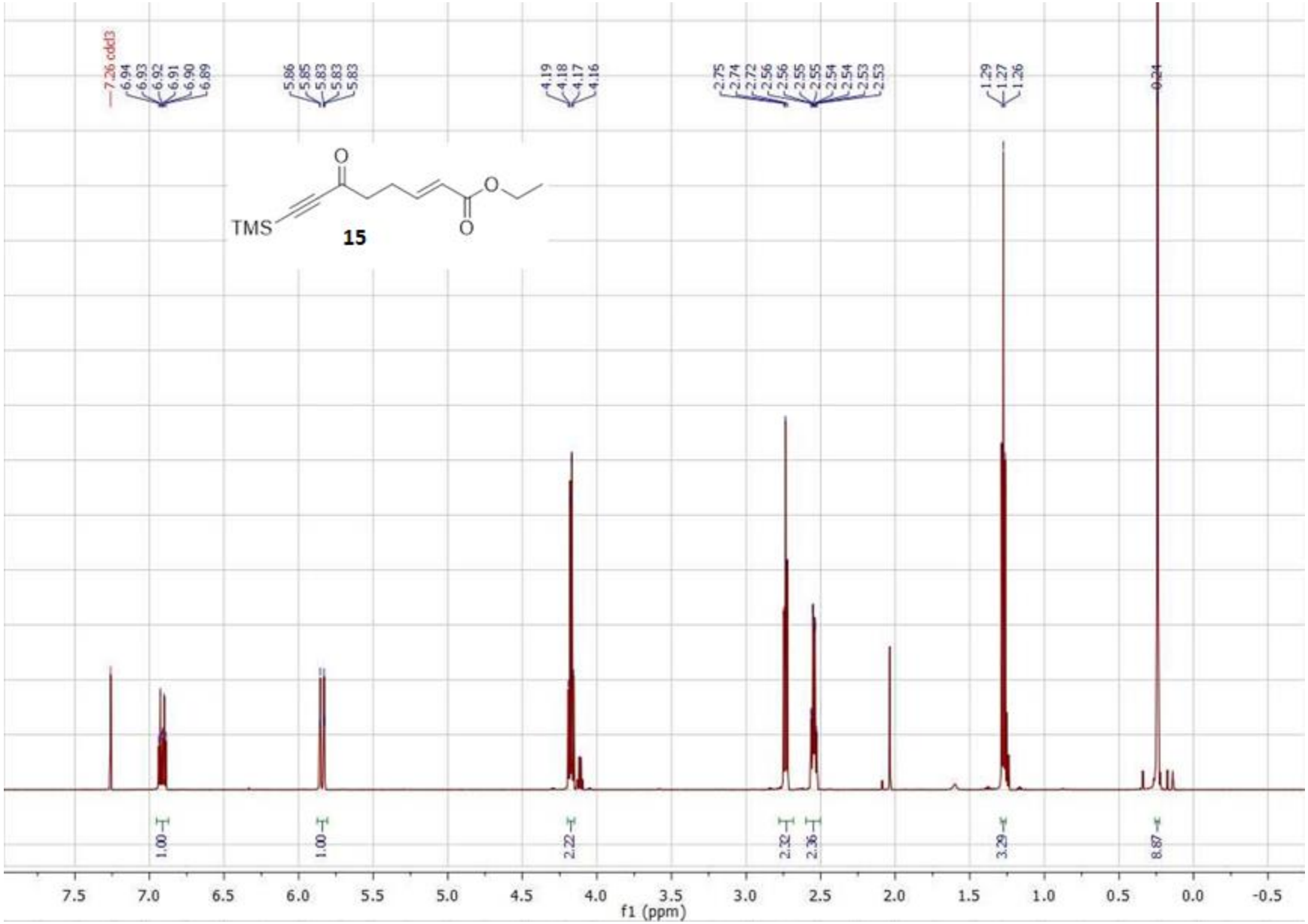
Position	δ ^{1}H [ppm]	δ ^{13}C [ppm]	^{1}H-^{1}H-coupling constants [Hz]
1	4.37	97.2	$J_{1,2}$ =2.9
2	3.68	69.1	$J_{2,3a}$ =3.4, $J_{2,3b}$ =3.1
3a	1.93	36.8	$J_{3a,3b}$ =12.8, $J_{3a,4}$ =11.3
3b	1.80	36.8	$J_{3b,4}$ =5.3
4	3.51	67.9	$J_{4,5}$ = 9.5
5	3.65	71.7	$J_{5,6}$ = 6.2
6	1.25	17.8	
1′		179.8	
2′	2.20	38.5	
3′	1.60	26.6	
4′, 5′, 6′, 7’	1.35-1.27	29.6-30.6	
8′a	1.34	29.6-30.6	
8′b	1.46	29.6-30.6	
9’a	1.79	35.7	$J_{9'a,10}$ =6.1
9′b	1.96	35.7	$J_{9'b,10'}$ = 7.8
10′	4.86	70.1	
11’		149.5	
12’	7.92	123.8	
1″	5.58	54.7	
2″		136.5	
3″	7.33	128.8	$J_{3'',4''}$ = 8.6, $J_{3'',5''}$ = 12.5
4″	7.39	129.9	
5″	7.34	129.5	

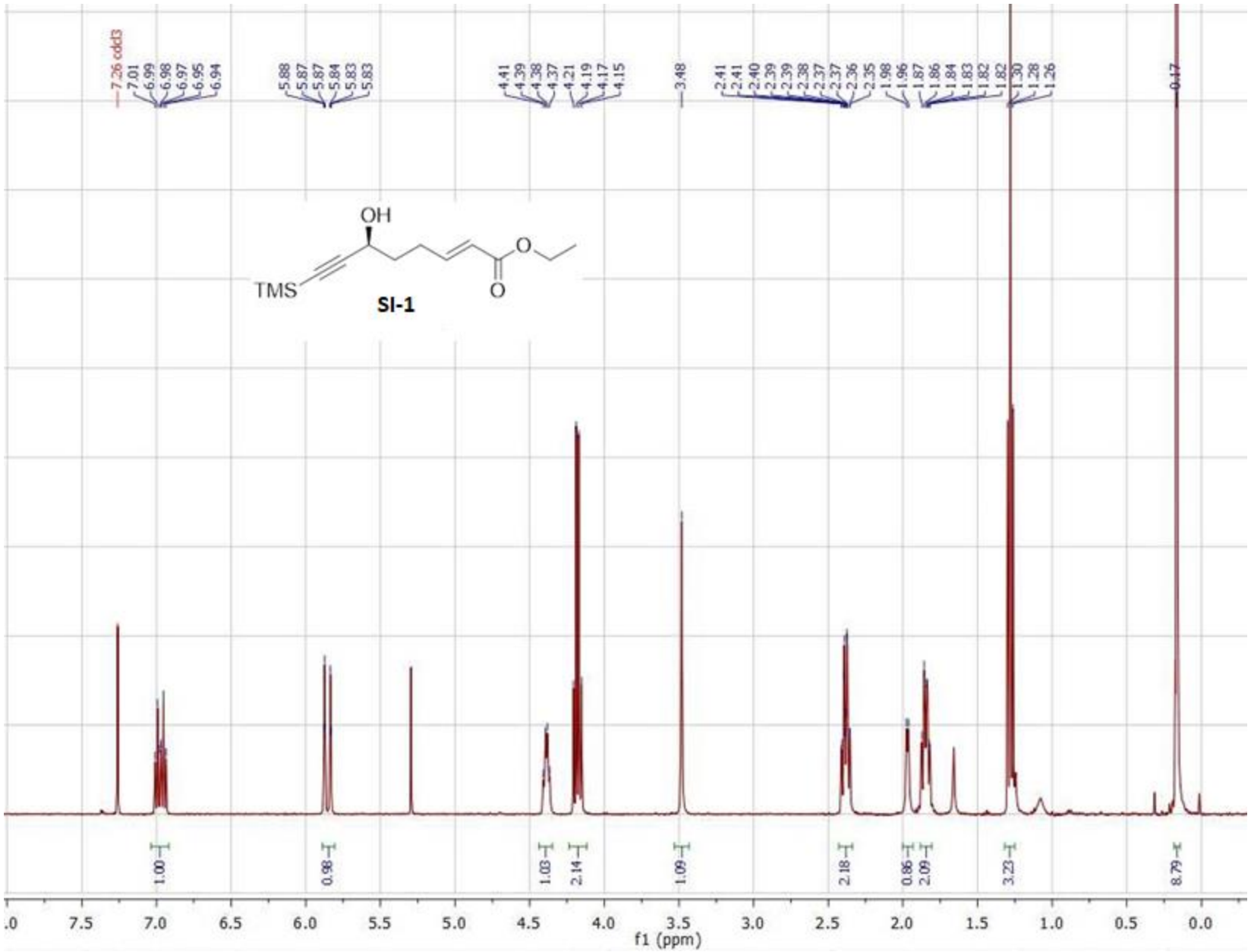
4. References

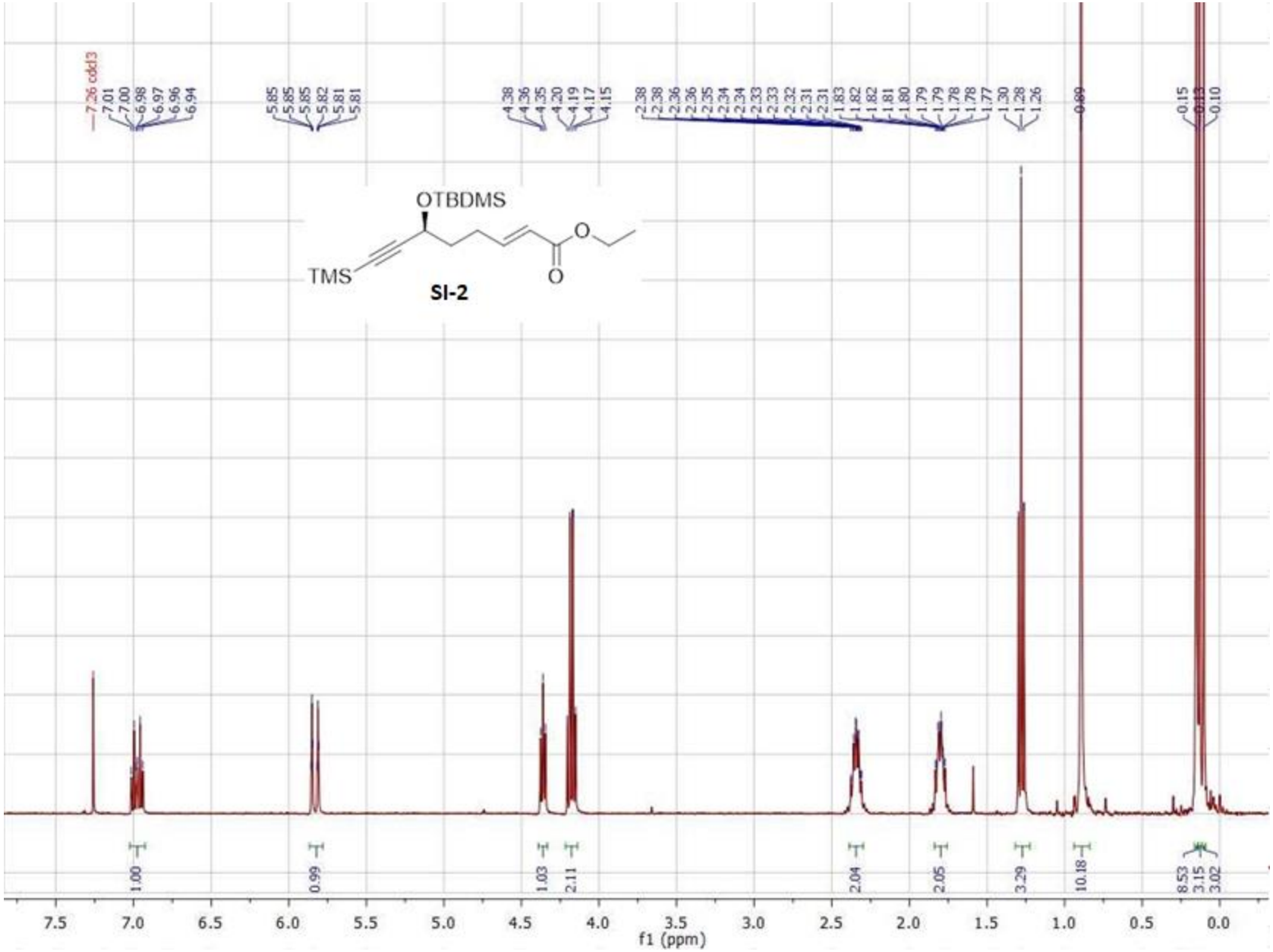
1. A. Narayan, V. Venkatachalam, O. Durak, D. K. Reilly, N. Bose, F. C. Schroeder, A. D. Samuel, J. Srinivasan and P. W. Sternberg, *Proc Natl Acad Sci U S A*, 2016, **113**, E1392-1401.
2. C. Pungaliya, J. Srinivasan, B. W. Fox, R. U. Malik, A. H. Ludewig, P. W. Sternberg and F. C. Schroeder, *Proc Natl Acad Sci U S A*, 2009, **106**, 7708-7713.
3. J. Srinivasan, F. Kaplan, R. Ajredini, C. Zachariah, H. T. Alborn, P. E. Teal, R. U. Malik, A. S. Edison, P. W. Sternberg and F. C. Schroeder, *Nature*, 2008, **454**, 1115-1118.
4. R. A. Bunce and D. M. Herrod, *Organic Preparations and Procedures International*, 2000, **32**, 577-580.
5. Y. K. Zhang, M. A. Sanchez-Ayala, P. W. Sternberg, J. Srinivasan and F. C. Schroeder, *Org Lett*, 2017, **19**, 2837-2840.

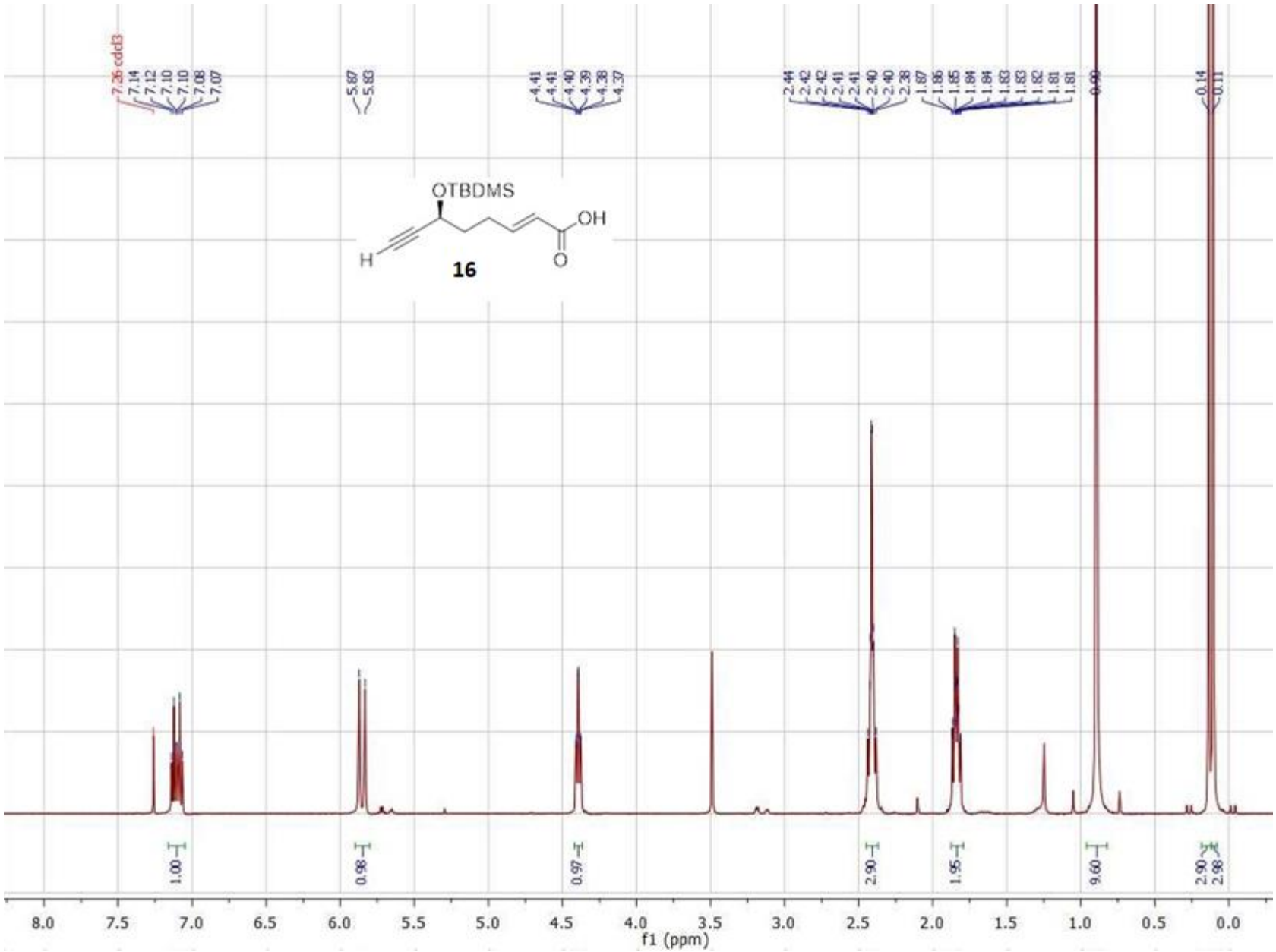
5. NMR spectra

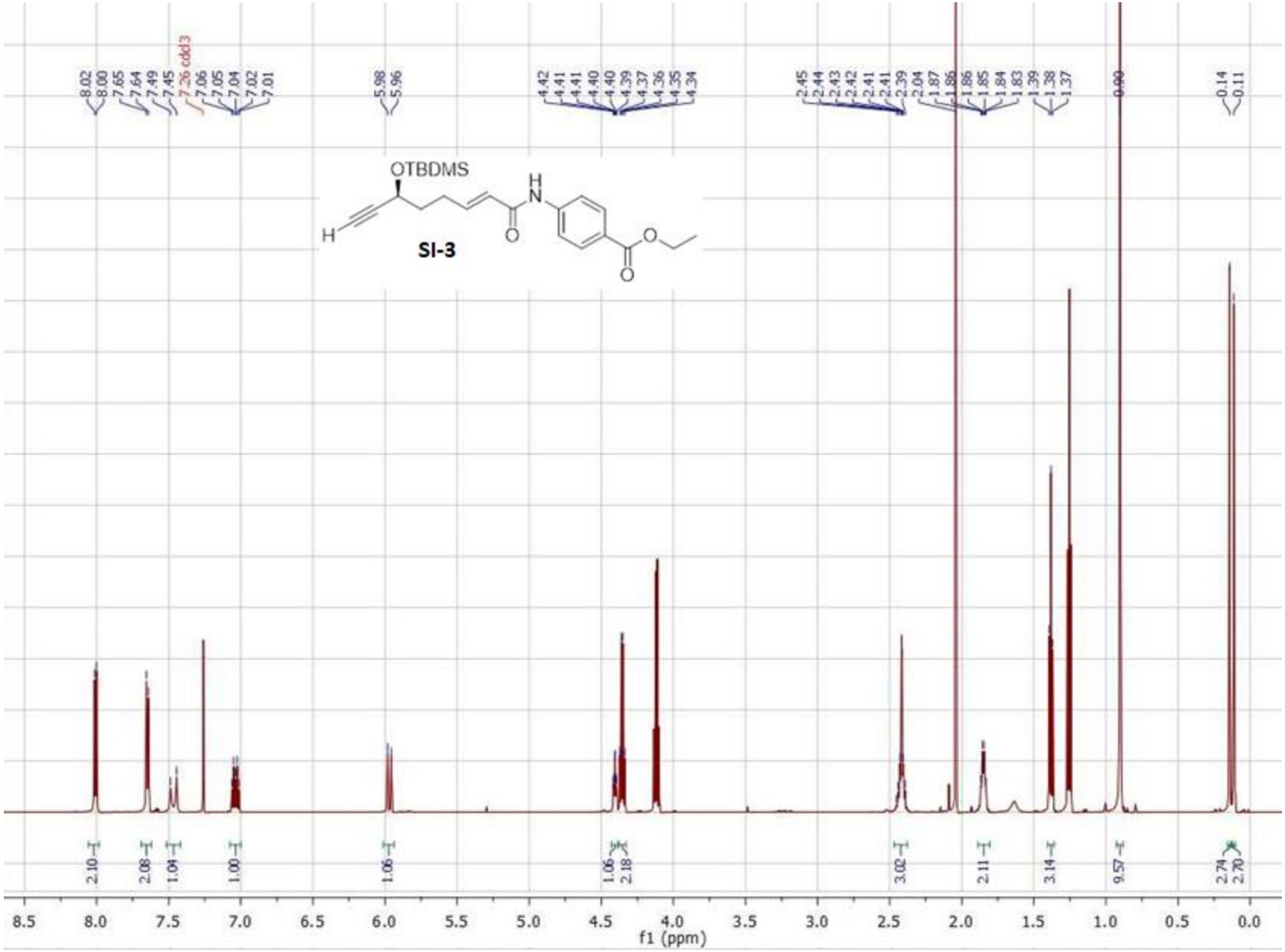


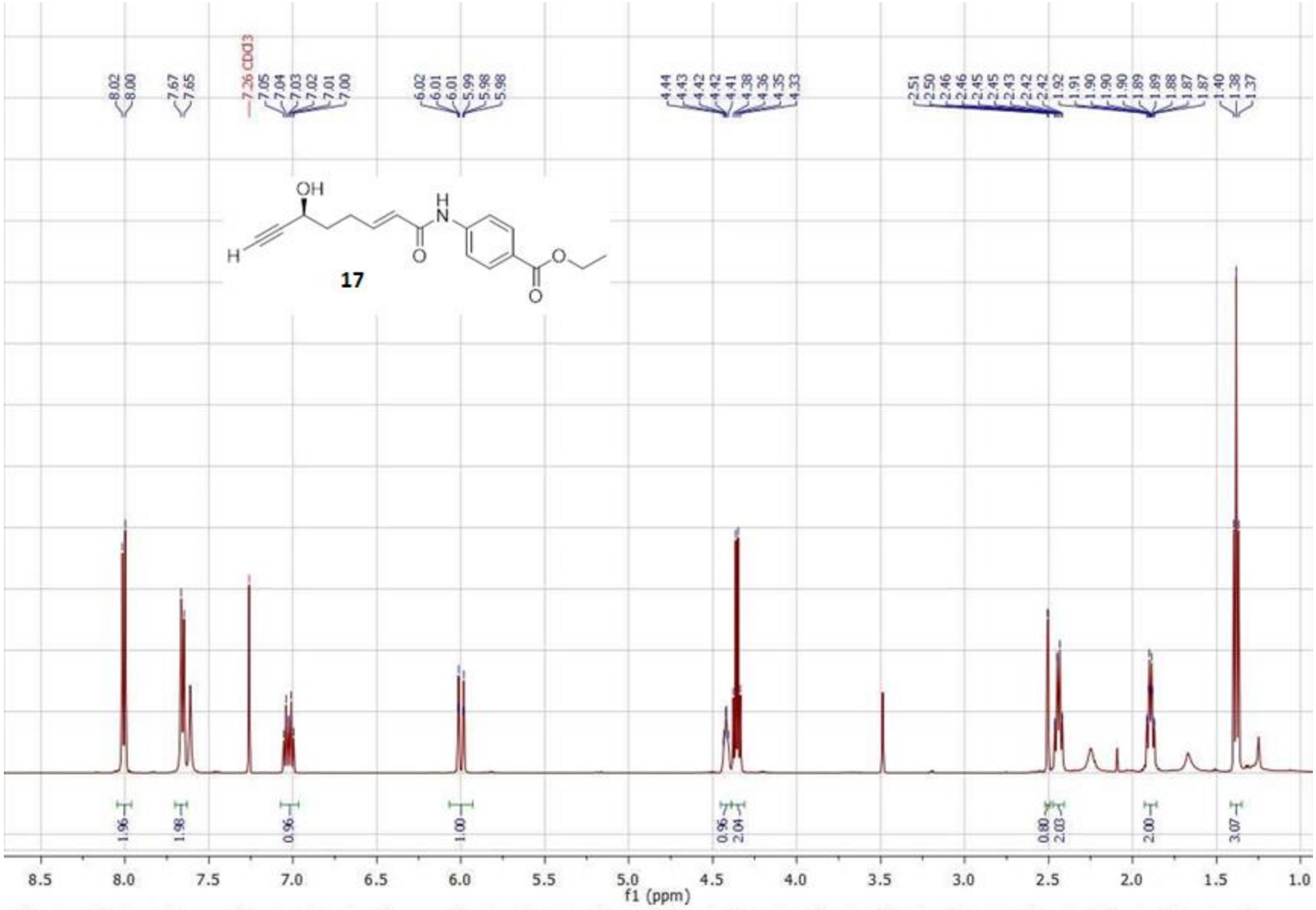

7.26 cdcl3
6.94
6.93
6.92
6.91
6.90
6.89
5.86
5.85
5.83
5.83
5.83
4.19
4.18
4.17
4.16
2.75
2.74
2.72
2.56
2.56
2.55
2.55
2.54
2.54
2.53
2.53
1.29
1.27
1.26
0.24
TMS
15
1.00
1.00
2.22
2.32
2.36
3.29
8.87
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

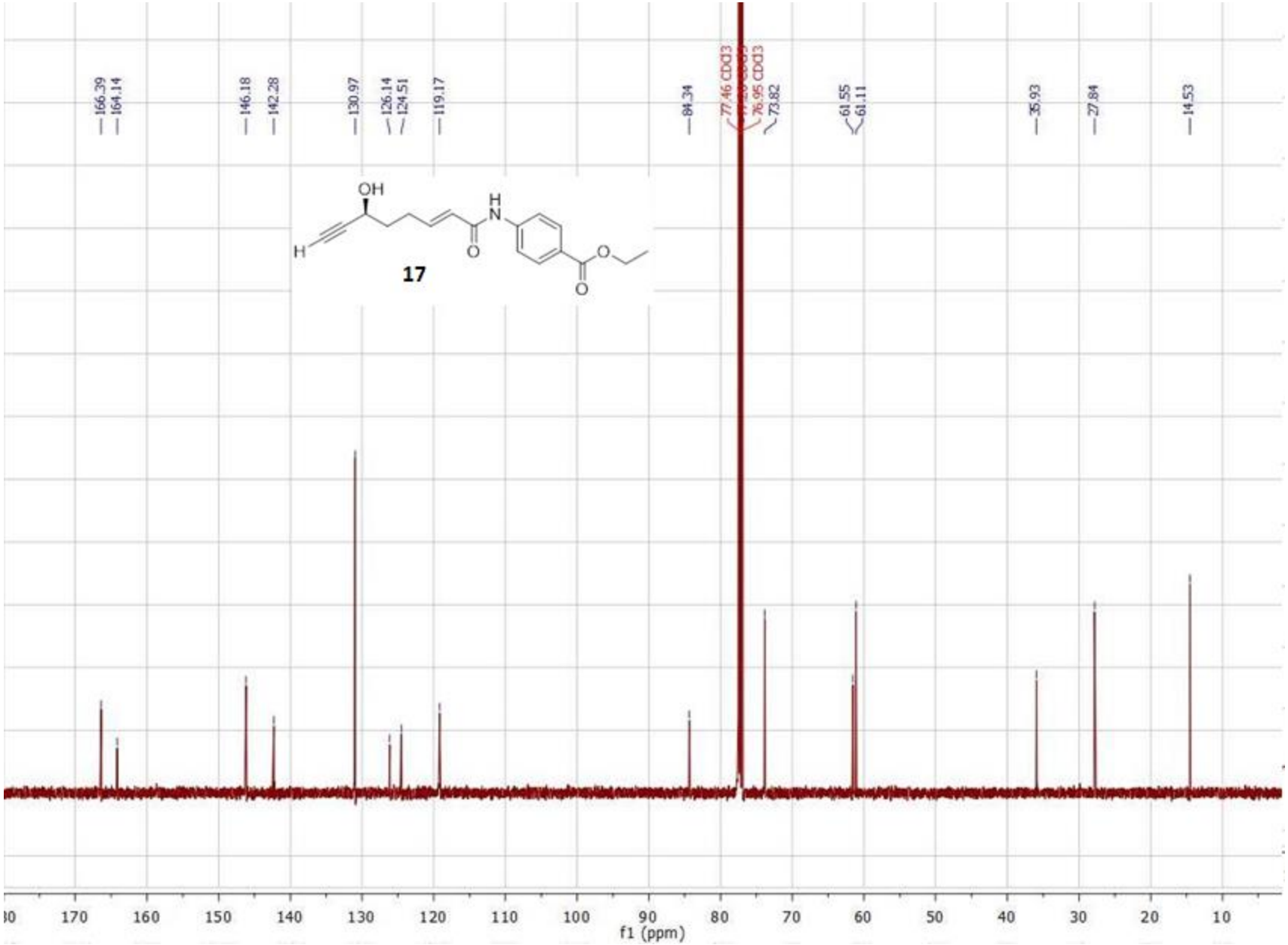

7.26 cdcl3
7.01
6.99
6.98
6.97
6.95
6.94
5.88
5.87
5.87
5.84
5.83
5.83
4.41
4.39
4.38
4.37
4.21
4.19
4.17
4.15
3.48
2.41
2.41
2.40
2.39
2.39
2.38
2.37
2.37
2.36
2.35
1.98
1.96
1.87
1.86
1.84
1.83
1.82
1.82
1.30
1.28
1.26
0.17
OH
TMS
O
O
SI-1
1.00
0.98
1.03
2.14
1.09
2.18
0.86
2.09
3.23
8.79
f1 (ppm)

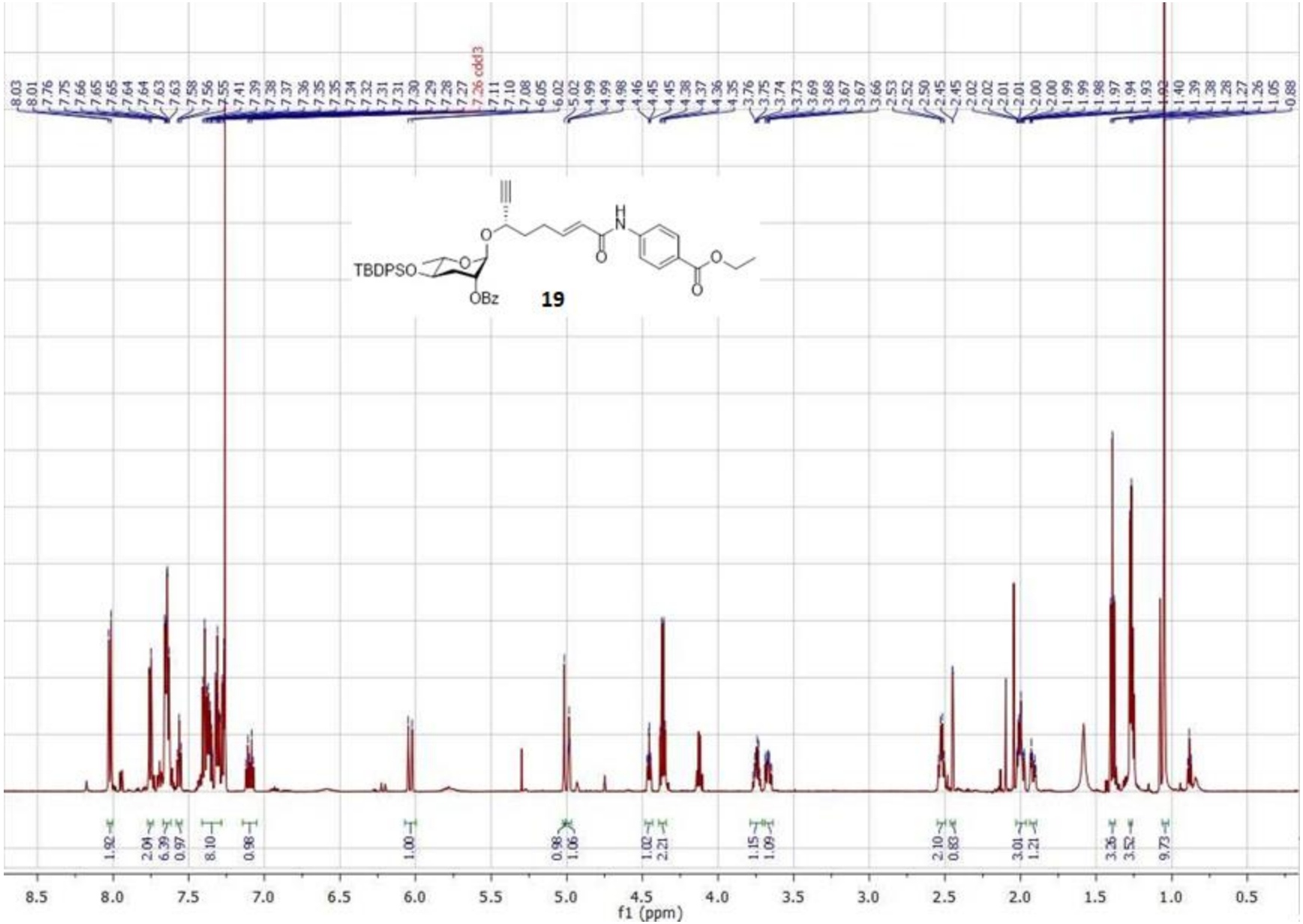

OTBDMS
TMS
O
O
SI-2
f1 (ppm)

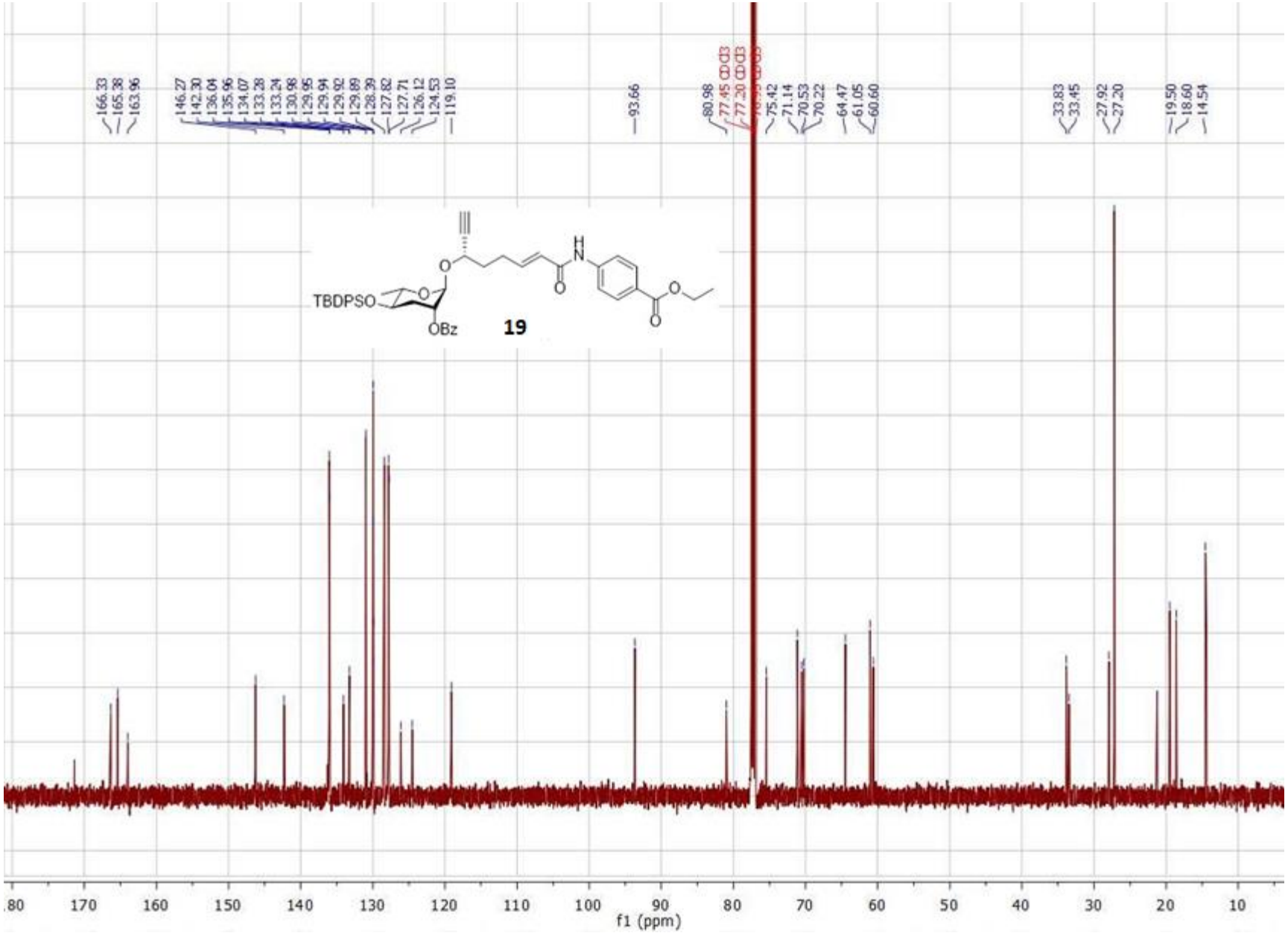

OTBDMS
OH
H
O
16
f1 (ppm)

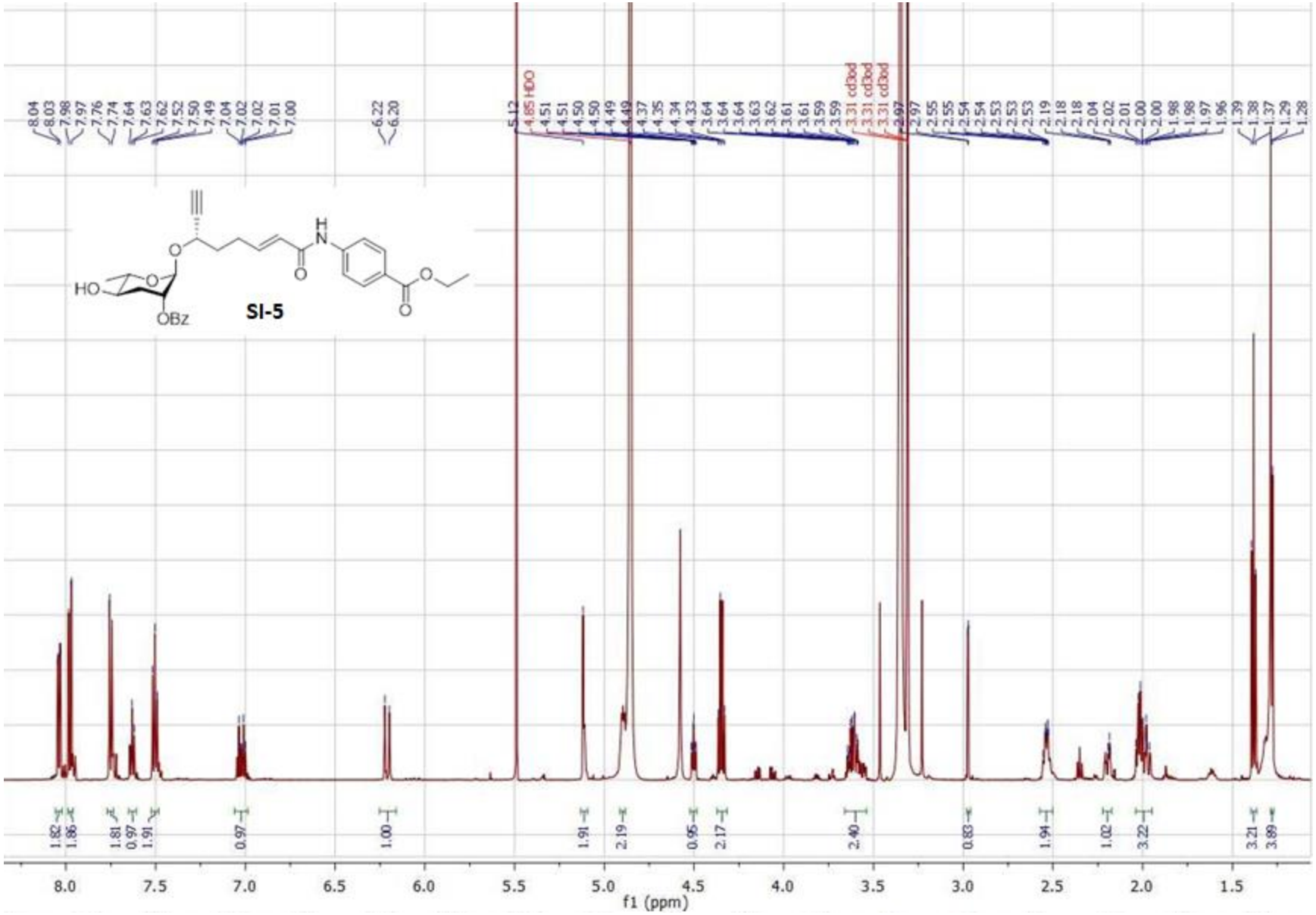

OTBDMS
H
N
O
O
O
H
SI-3
8.02
8.00
7.65
7.64
7.49
7.45
7.26 cdcl3
7.06
7.05
7.04
7.02
7.01
5.98
5.96
4.42
4.41
4.41
4.40
4.40
4.39
4.37
4.36
4.35
4.34
2.45
2.44
2.43
2.42
2.41
2.41
2.39
2.04
1.87
1.86
1.86
1.85
1.84
1.83
1.39
1.38
1.37
0.00
0.14
0.11
2.10
2.08
1.04
1.00
1.06
1.06
2.18
3.02
2.11
3.14
9.57
2.74
2.70
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

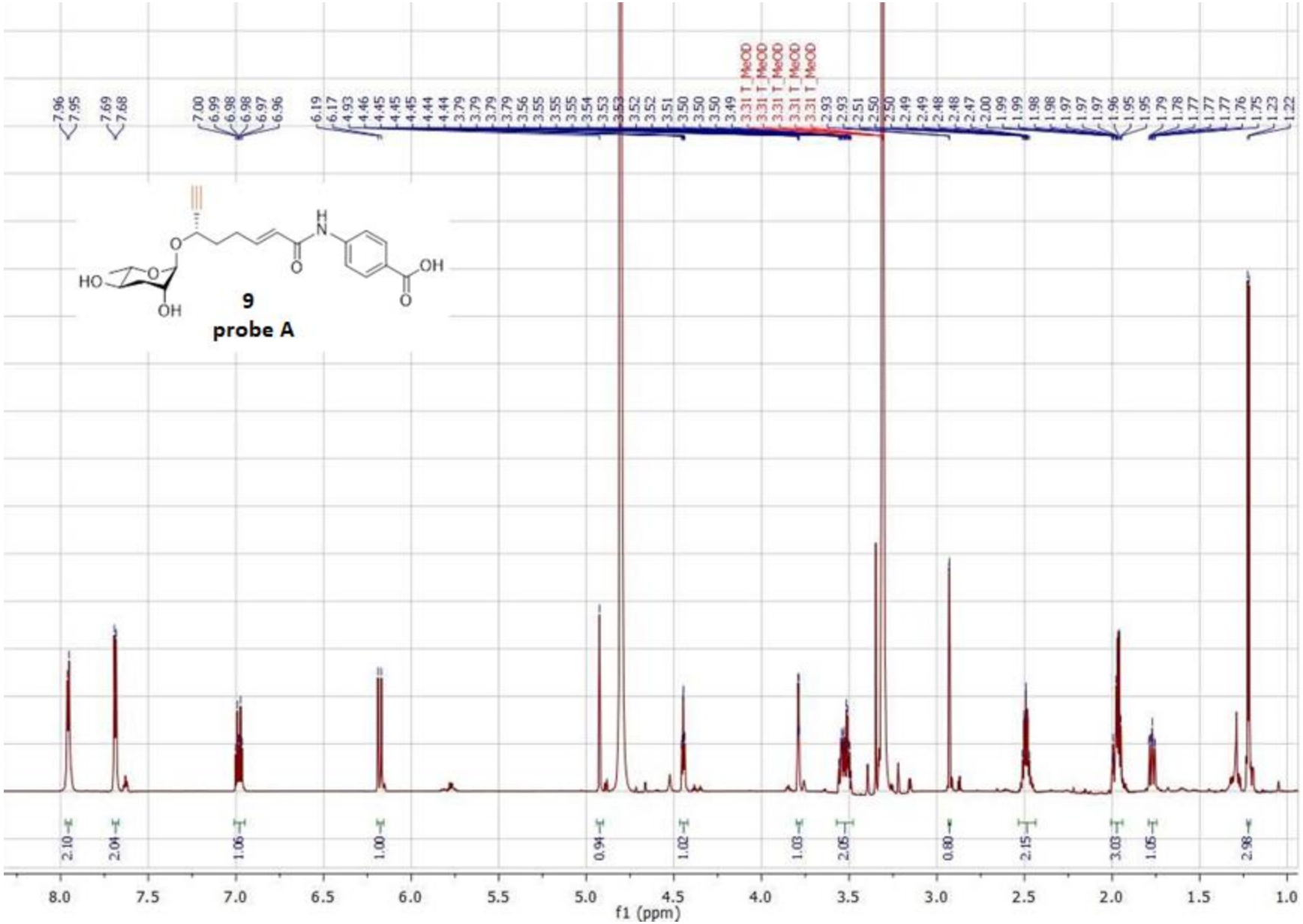

8.02
8.00
7.67
7.65
7.26 CDCl3
7.05
7.04
7.03
7.02
7.01
7.00
6.02
6.01
6.01
5.99
5.98
5.98
4.44
4.43
4.42
4.42
4.41
4.38
4.36
4.35
4.33
2.51
2.50
2.46
2.46
2.45
2.45
2.43
2.42
2.42
1.92
1.91
1.90
1.90
1.90
1.89
1.89
1.88
1.87
1.87
1.40
1.38
1.37
OH
H
N
O
O
O
H
17
1.96
1.98
0.96
1.00
0.96
2.04
0.80
2.03
2.00
3.07
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

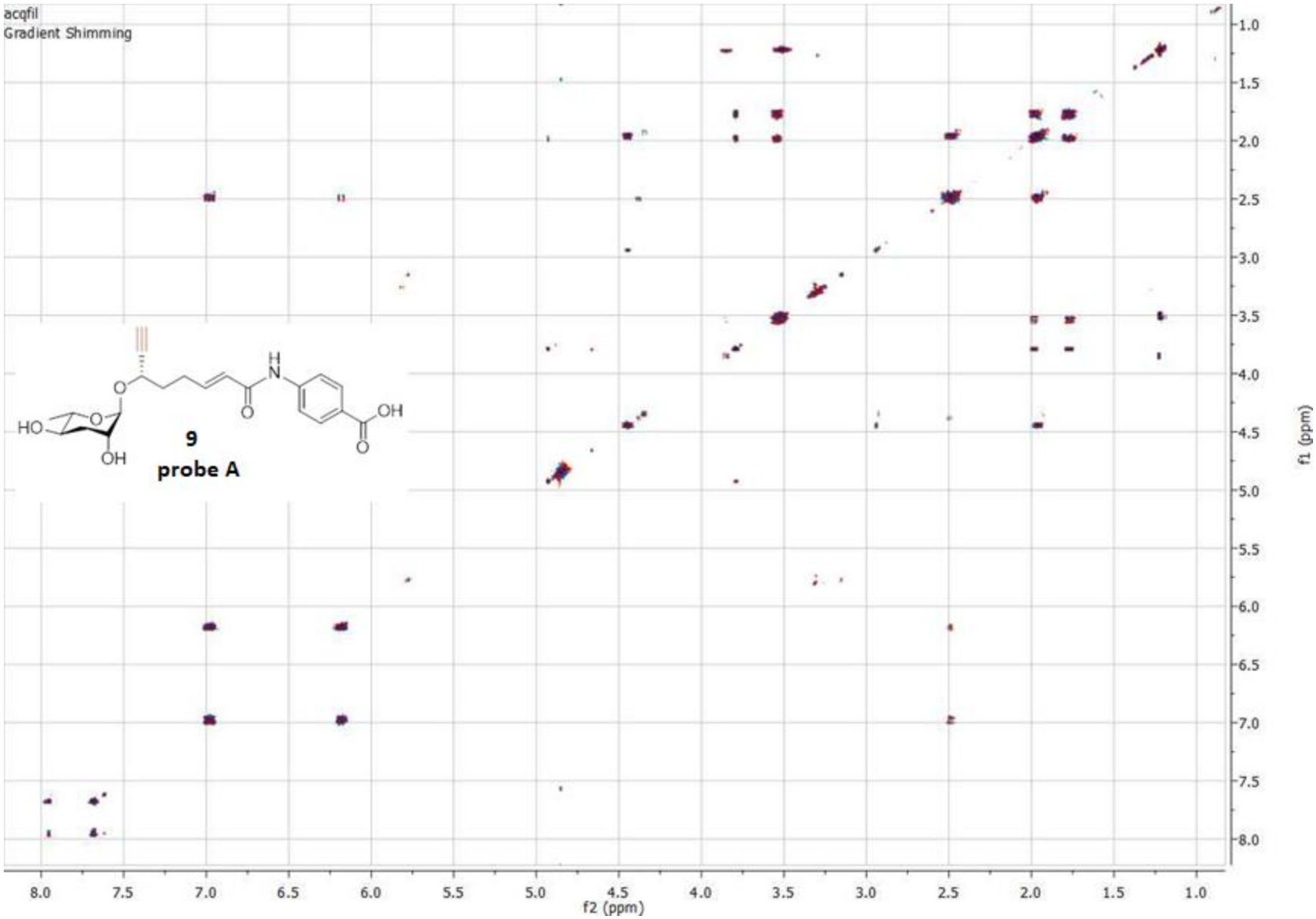

166.39
164.14
146.18
142.28
130.97
126.14
124.51
119.17
84.34
77.46 CDCl3
76.95 CDCl3
73.82
61.55
61.11
35.93
27.84
14.53
OH
H
N
O
H
O
O
17
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

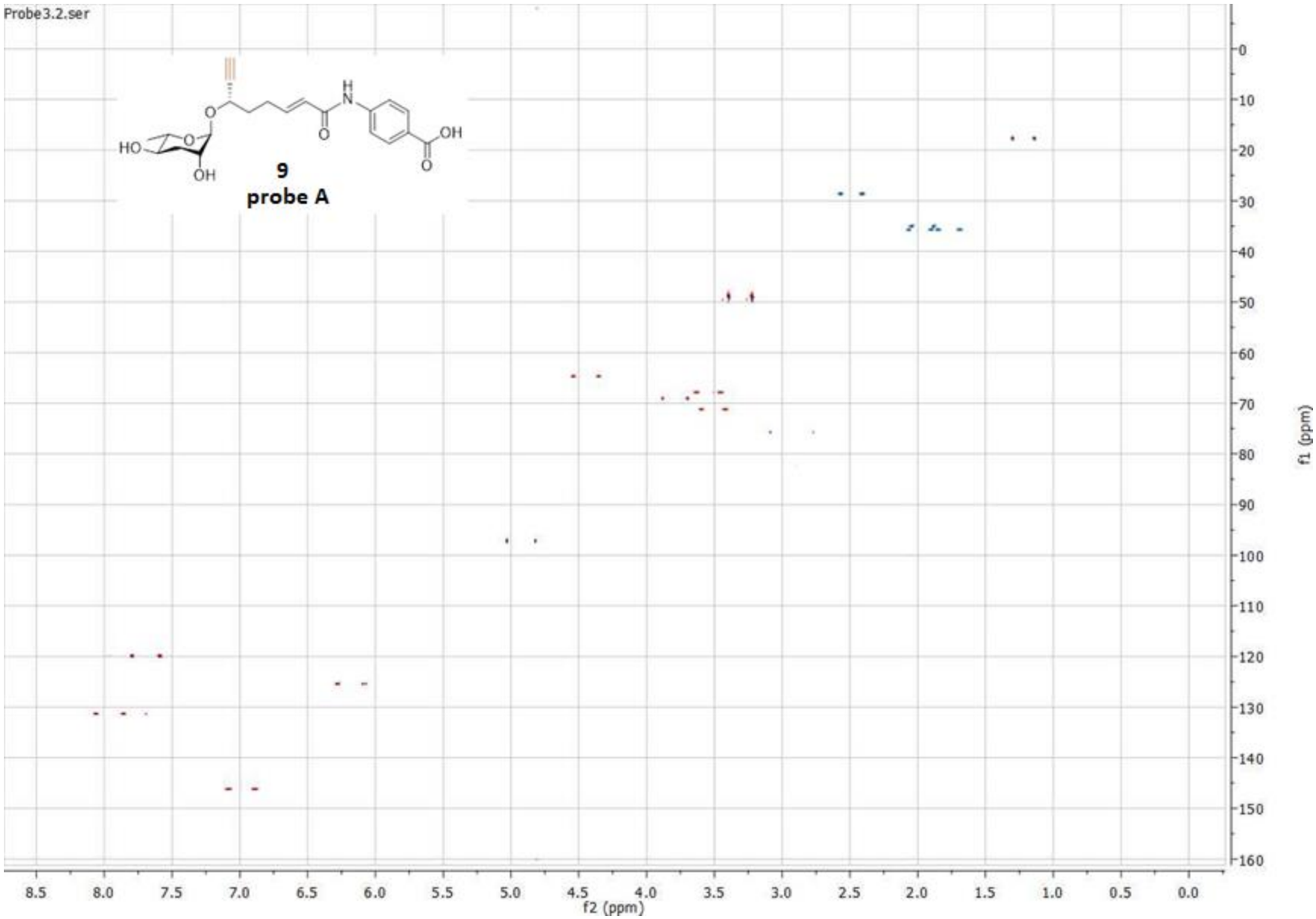

TBDPSO
OBz
19
f1 (ppm)

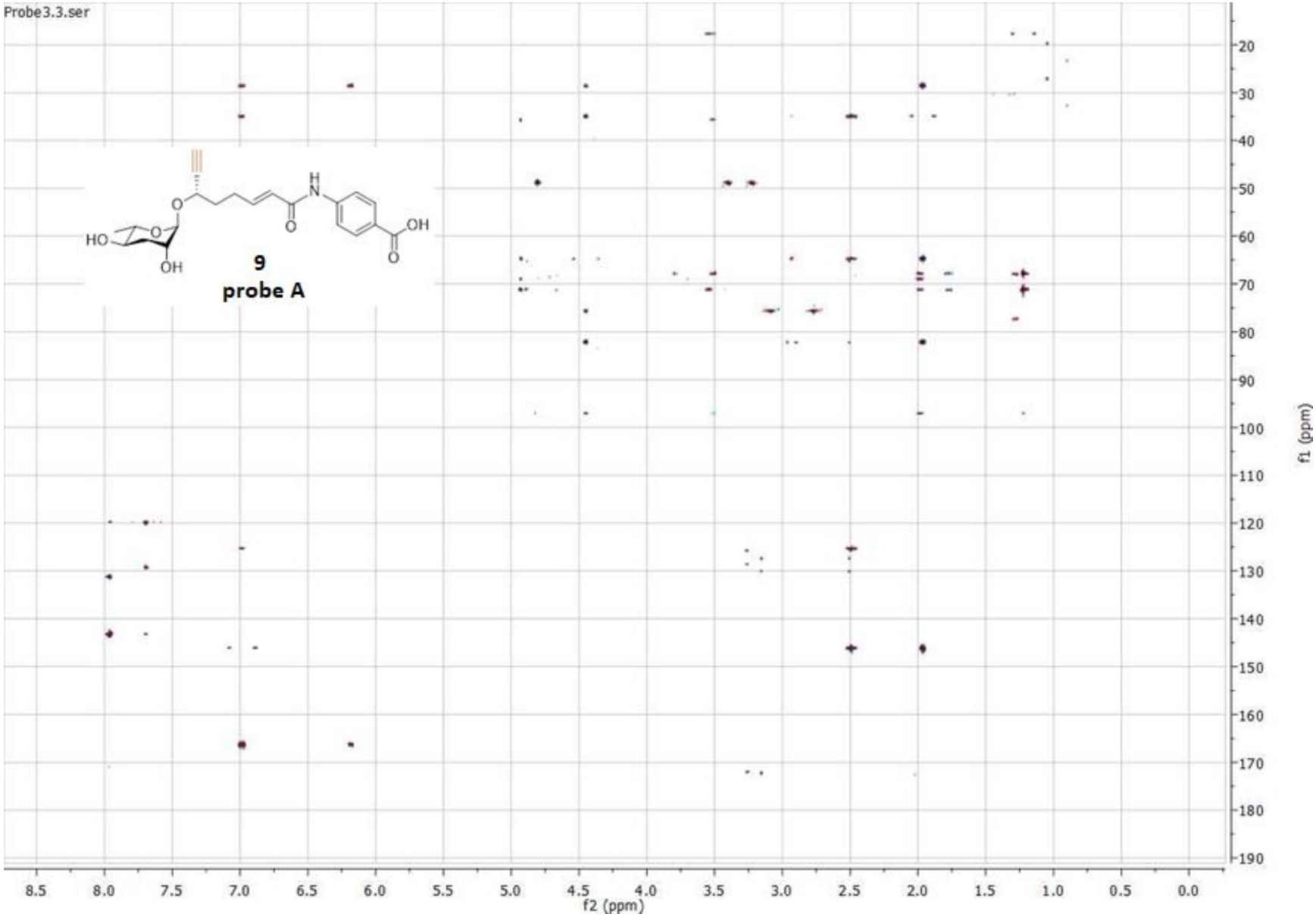

166.33
165.38
163.96
146.27
142.30
136.04
135.96
134.07
133.28
133.24
130.98
129.95
129.94
129.92
129.89
128.39
127.82
127.71
126.12
124.53
119.10
93.66
80.98
77.45 CDCl3
77.20 CDCl3
75.42
71.14
70.53
70.22
64.47
61.05
60.60
33.83
33.45
27.92
27.20
19.50
18.60
14.54
TBDPSO
OBz
19
f1 (ppm)

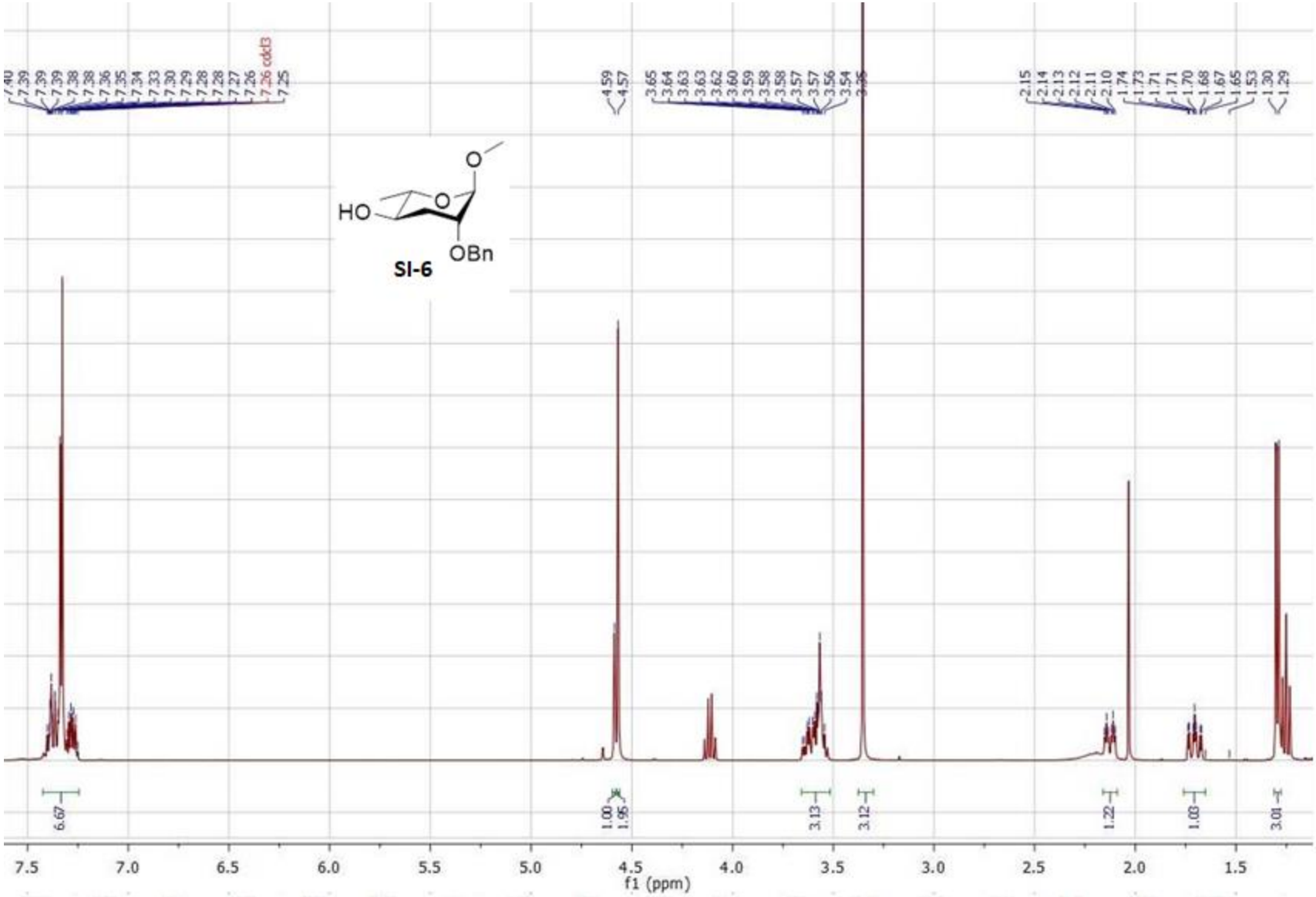

8.04
8.03
7.98
7.97
7.76
7.74
7.64
7.63
7.62
7.52
7.50
7.49
7.04
7.02
7.02
7.01
7.00
6.22
6.20
5.12
4.85 HDO
4.51
4.51
4.50
4.50
4.49
4.49
4.37
4.35
4.34
4.33
3.64
3.64
3.64
3.63
3.62
3.61
3.61
3.59
3.59
3.31 cd3od
3.31 cd3od
3.31 cd3od
2.97
2.97
2.55
2.55
2.54
2.54
2.53
2.53
2.53
2.19
2.18
2.18
2.04
2.02
2.01
2.00
2.00
1.98
1.98
1.97
1.96
1.39
1.38
1.37
1.29
1.28
HO
OBz
SI-5
1.82
1.86
1.81
0.97
1.91
0.97
1.00
1.91
2.19
0.95
2.17
2.40
0.83
1.94
1.02
3.22
3.21
3.89
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)

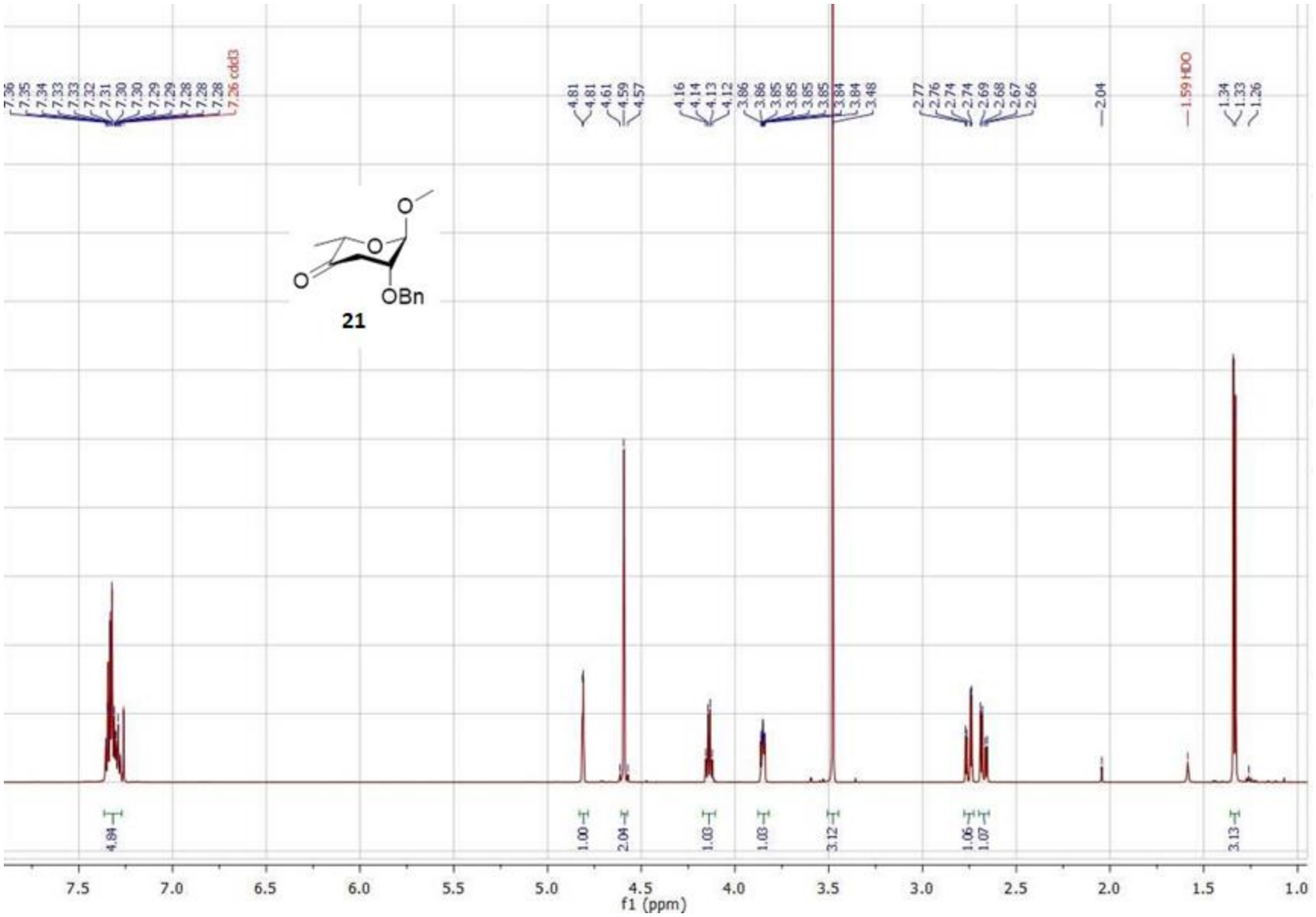

9
probe A
f1 (ppm)

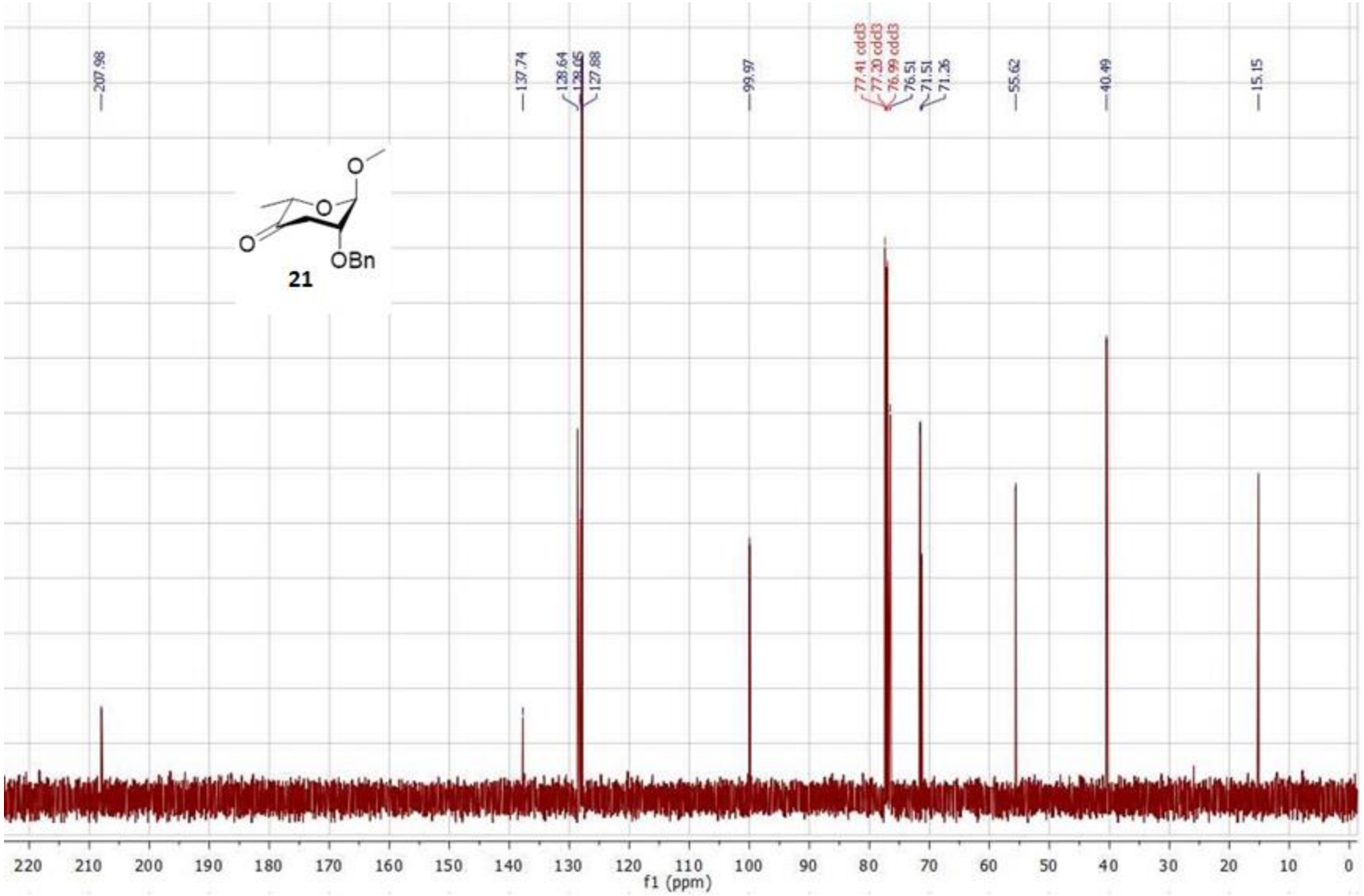

acqfil
Gradient Shimming
HO
O
O
OH
H
N
O
OH
O
9
probe A
f2 (ppm)
f1 (ppm)

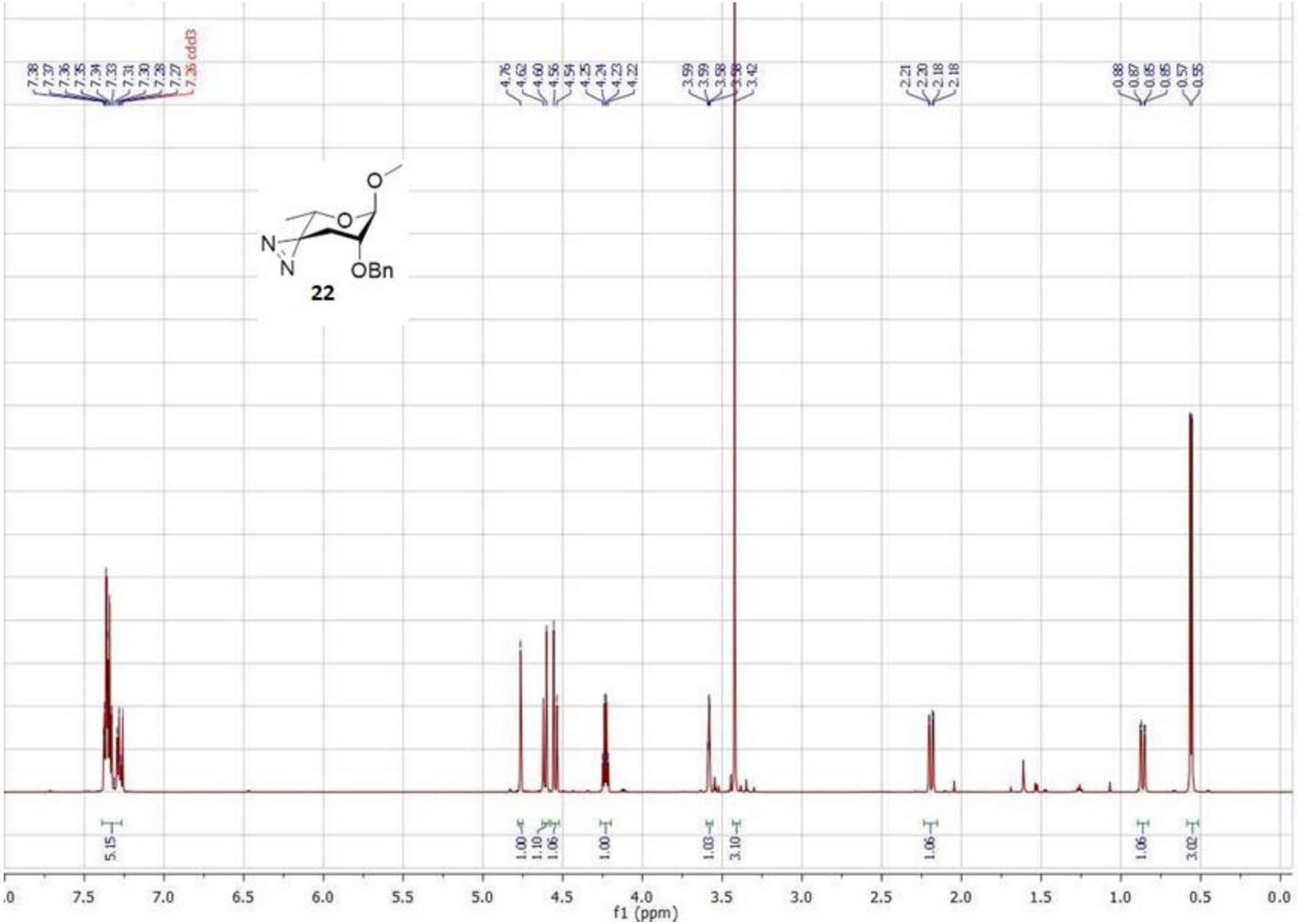

Probe3.2.ser
HO
O
OH
O
H
N
O
OH
O
9
probe A
f1 (ppm)
f2 (ppm)

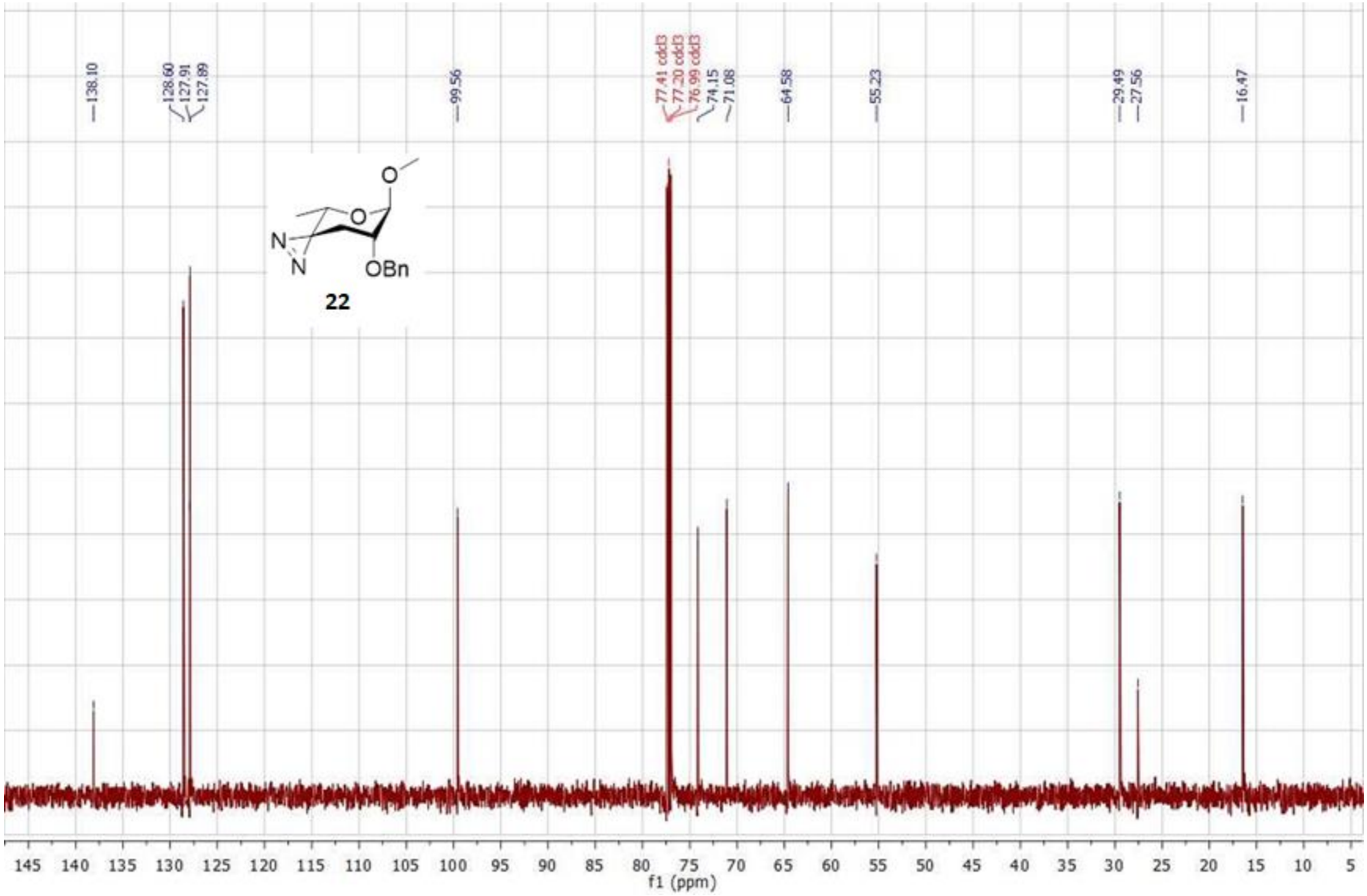

Probe3.3.ser
HO
O
OH
O
H
N
O
OH
O
9
probe A
f2 (ppm)
f1 (ppm)

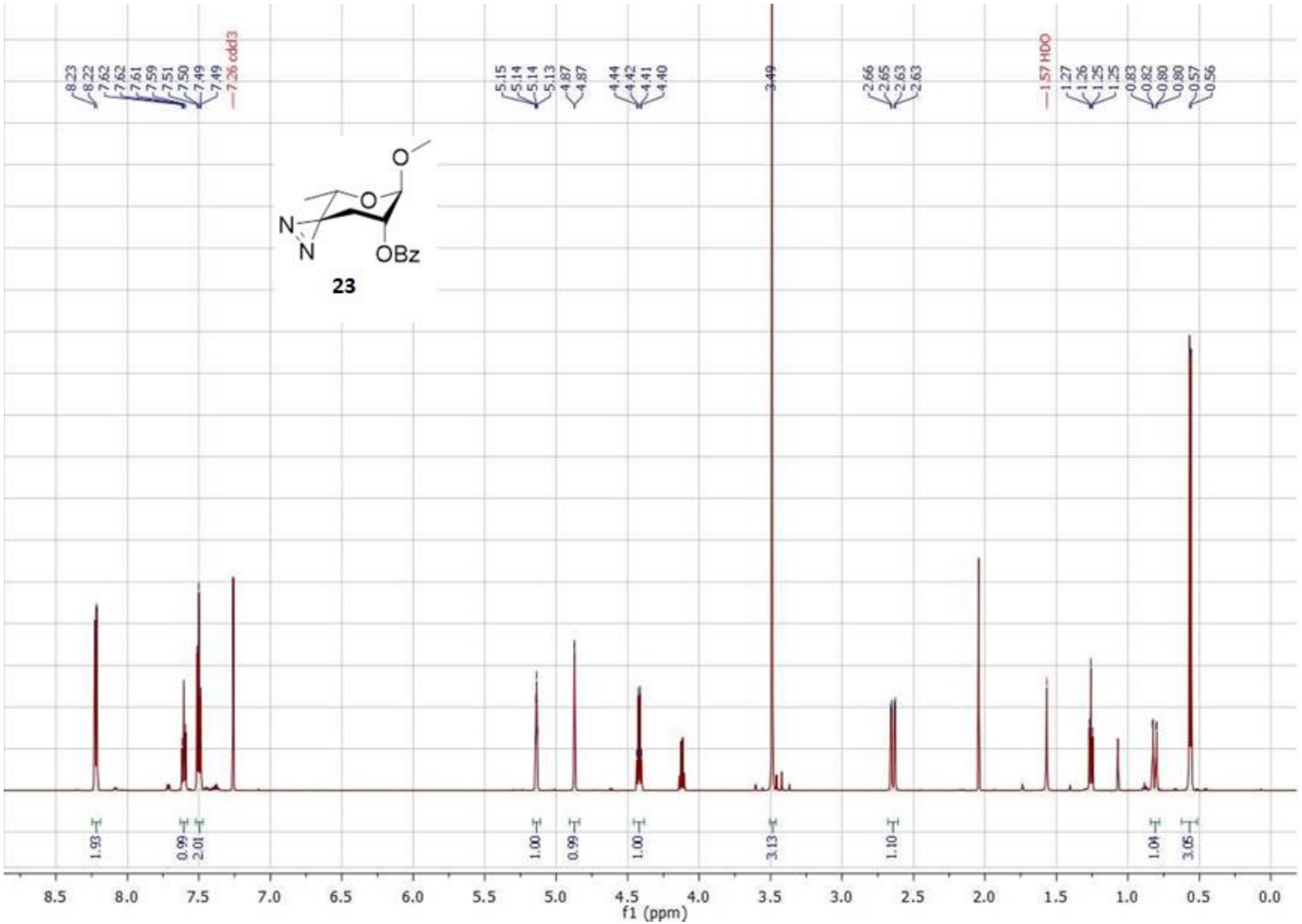

HO
O
O
OBn
SI-6
f1 (ppm)

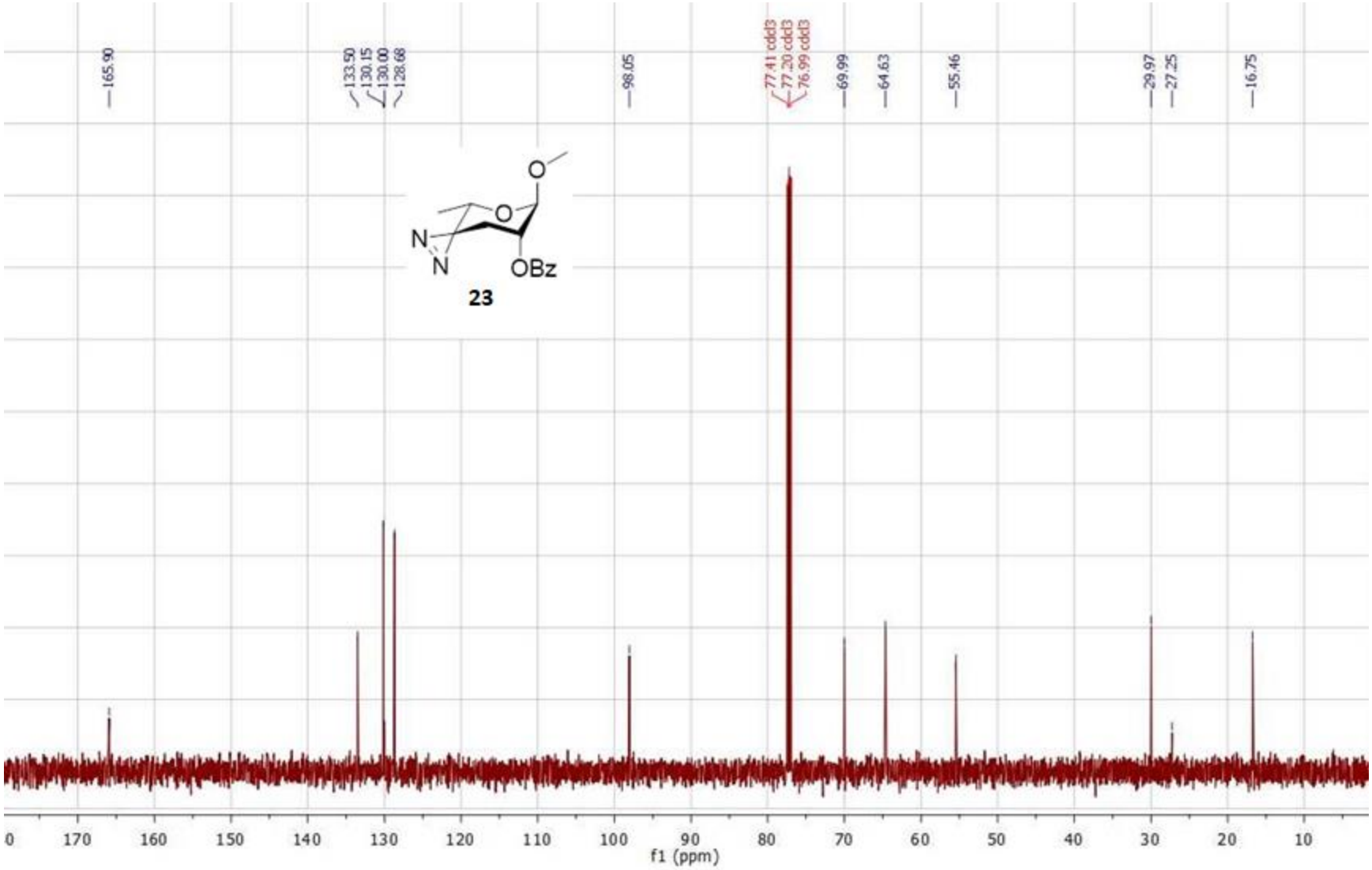

7.36
7.35
7.34
7.33
7.33
7.32
7.31
7.30
7.30
7.29
7.29
7.28
7.28
7.28
7.26 cdcl3
4.81
4.81
4.61
4.59
4.57
4.16
4.14
4.13
4.12
3.86
3.86
3.85
3.85
3.85
3.85
3.84
3.84
3.48
2.77
2.76
2.74
2.74
2.69
2.68
2.67
2.66
2.04
1.59 HDO
1.34
1.33
1.26
OBn
21
4.84
1.00
2.04
1.03
1.03
3.12
1.06
1.07
3.13
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

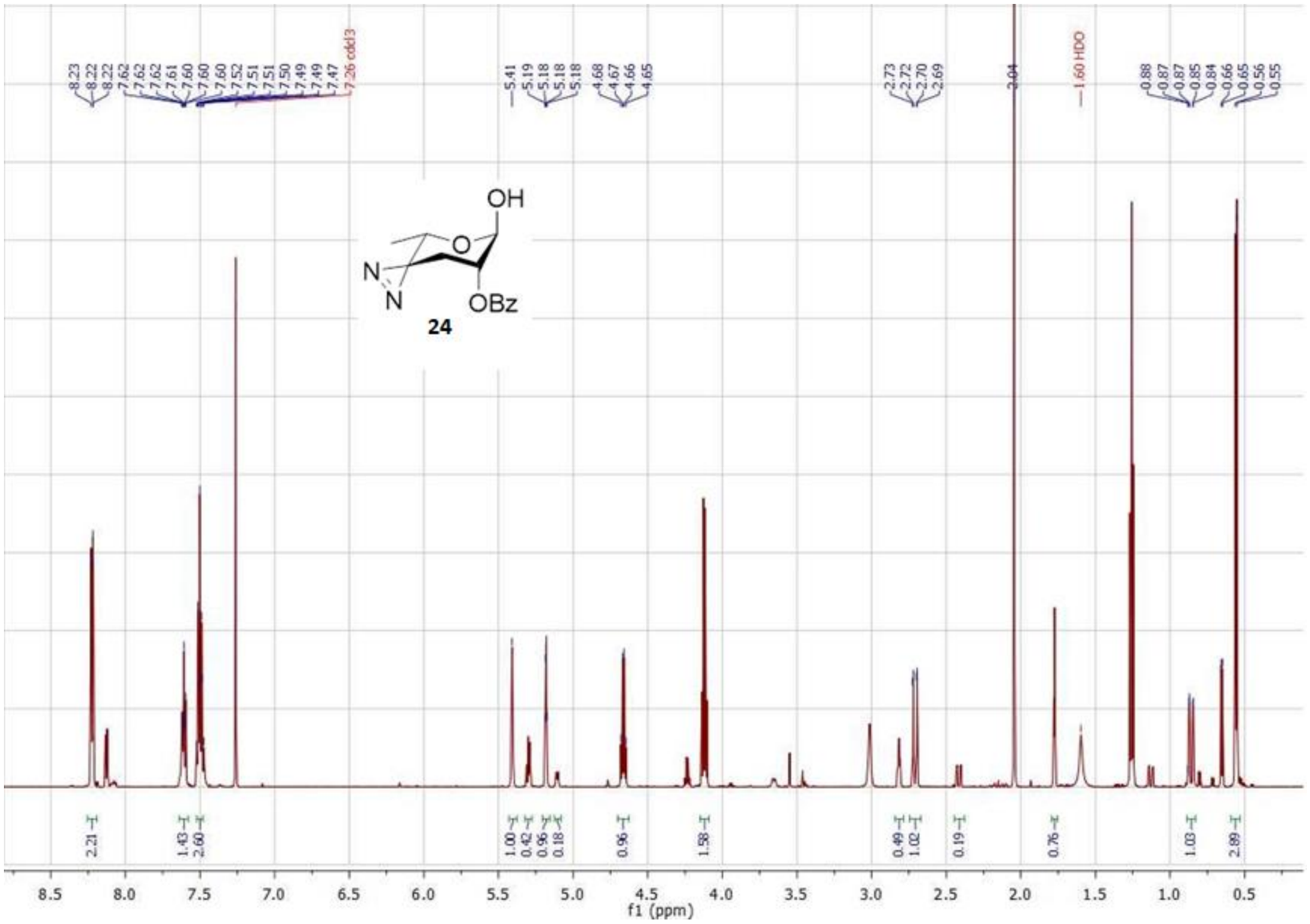

207.98
137.74
128.64
128.05
127.88
99.97
77.41 cdcl3
77.20 cdcl3
76.99 cdcl3
76.51
71.51
71.26
55.62
40.49
15.15
O
O
O
OBn
21
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

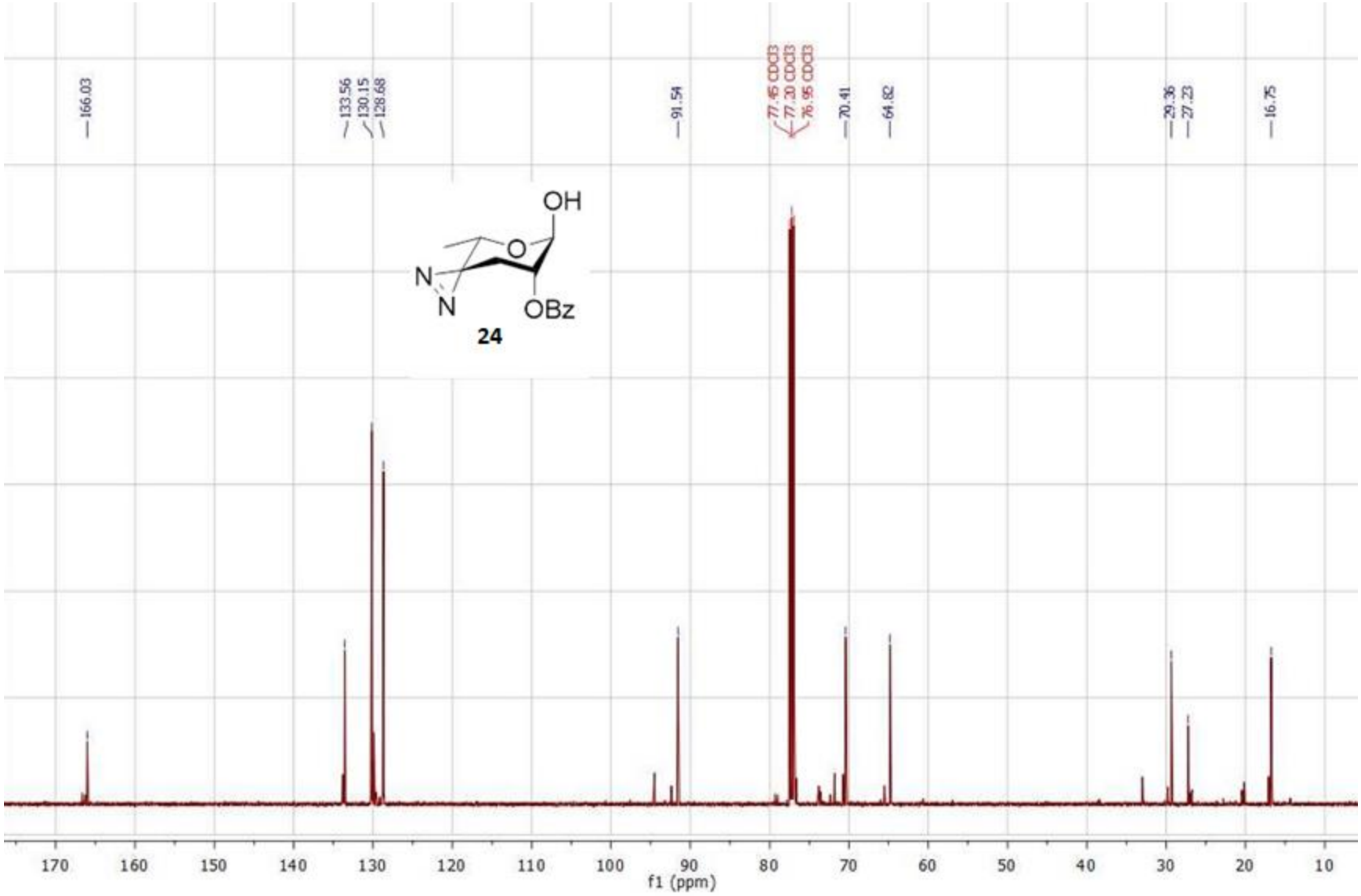

7.38
7.37
7.36
7.35
7.34
7.33
7.31
7.30
7.28
7.27
7.26 cdd3
4.76
4.62
4.60
4.56
4.54
4.25
4.24
4.23
4.22
3.59
3.59
3.58
3.42
2.21
2.20
2.18
2.18
0.88
0.87
0.85
0.85
0.57
0.55
O
O
N
N
OBn
22
5.15
1.00
1.10
1.06
1.00
1.03
3.10
1.06
1.06
3.02
f1 (ppm)

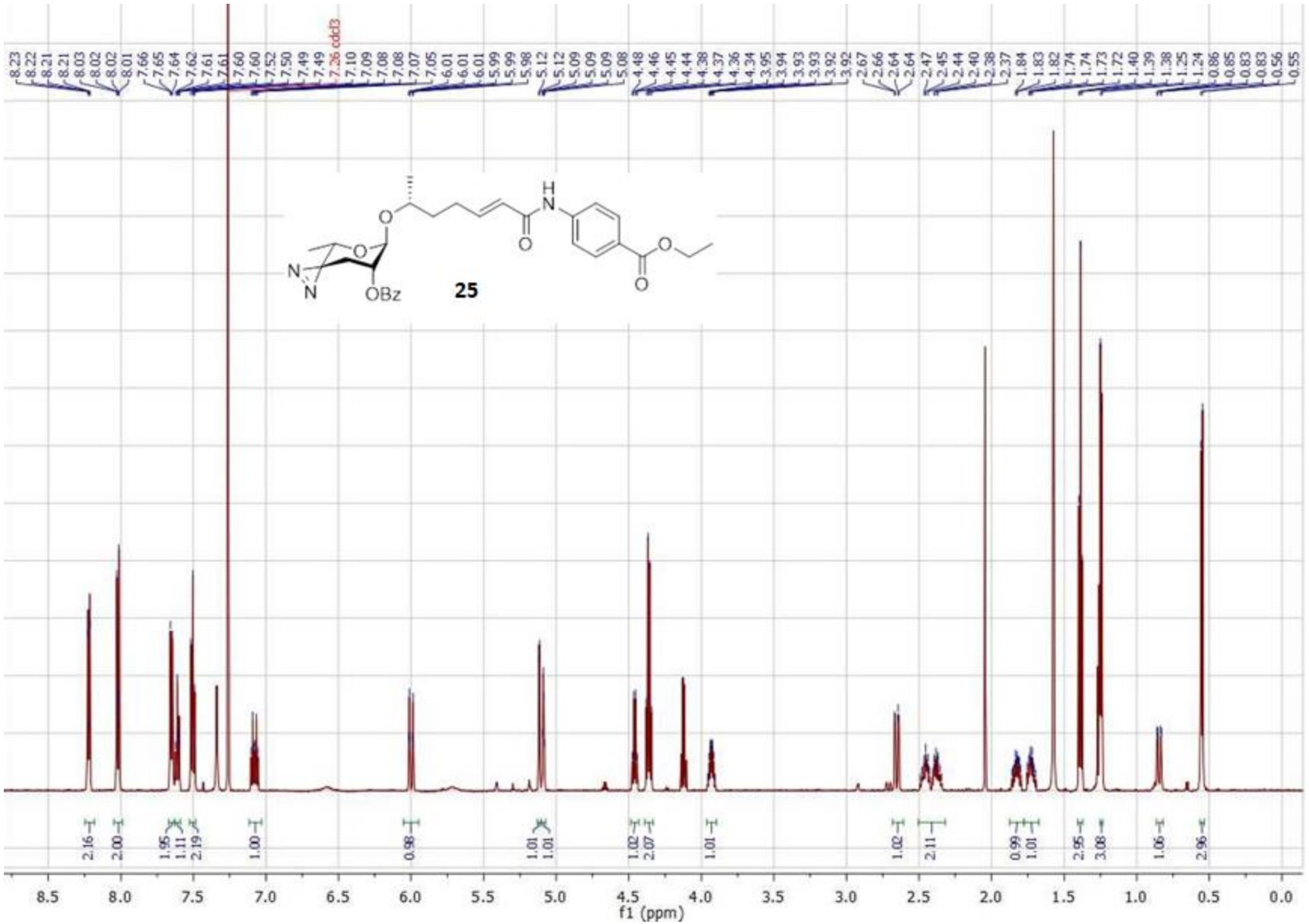

138.10
128.60
127.91
127.89
99.56
77.41 cdcl3
77.20 cdcl3
76.99 cdcl3
74.15
71.08
64.58
55.23
29.49
27.56
16.47
O
O
N
N
OBn
22
145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
f1 (ppm)

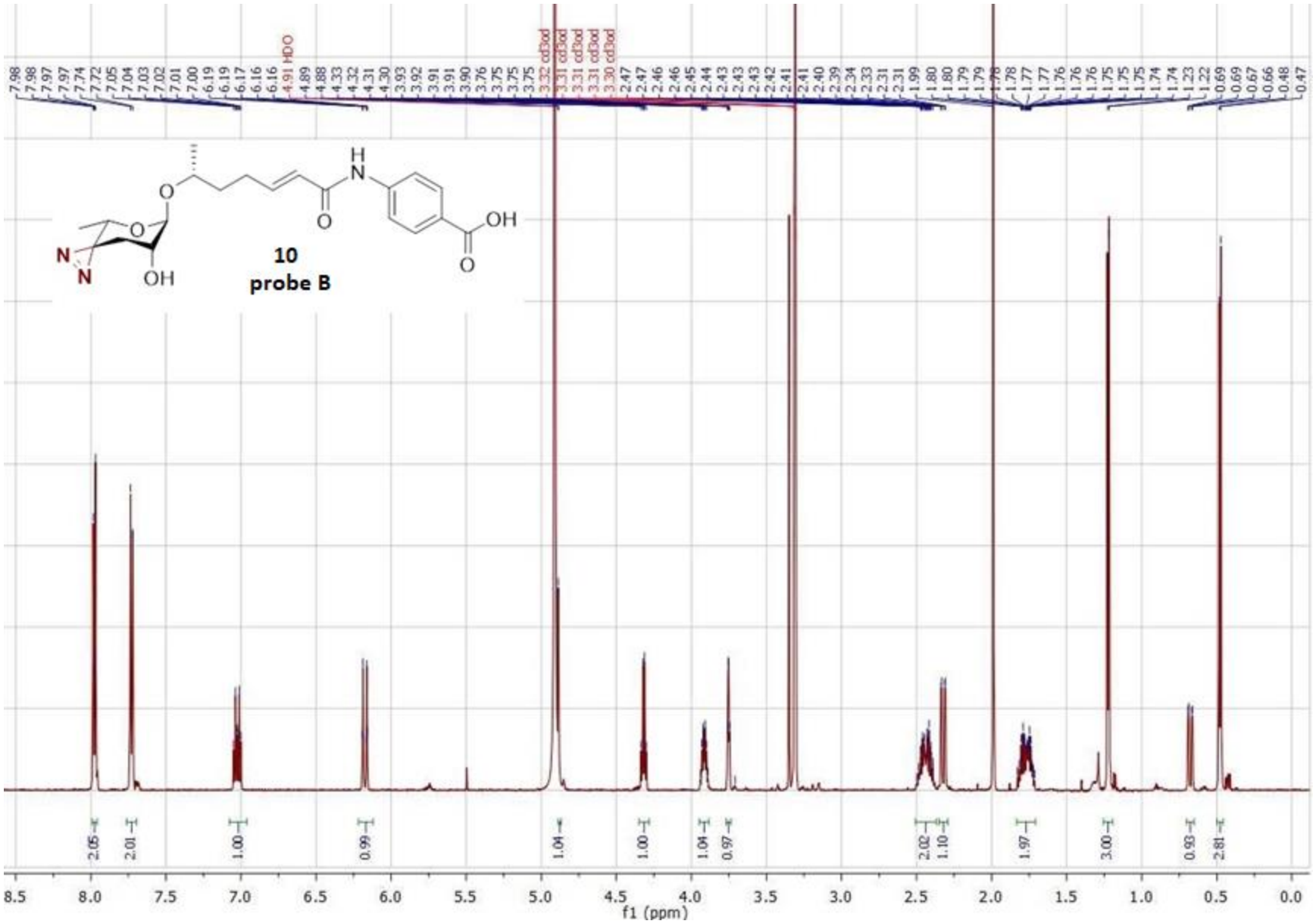

8.23
8.22
7.62
7.62
7.61
7.59
7.51
7.50
7.49
7.49
7.26 cdd3
5.15
5.14
5.14
5.13
4.87
4.87
4.44
4.42
4.41
4.40
3.49
2.66
2.65
2.63
2.63
1.57 H2O
1.27
1.26
1.25
1.25
0.83
0.82
0.80
0.80
0.57
0.56
O
O
N
N
OBz
23
1.93
0.99
2.01
1.00
0.99
1.00
3.13
1.10
1.04
3.05
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

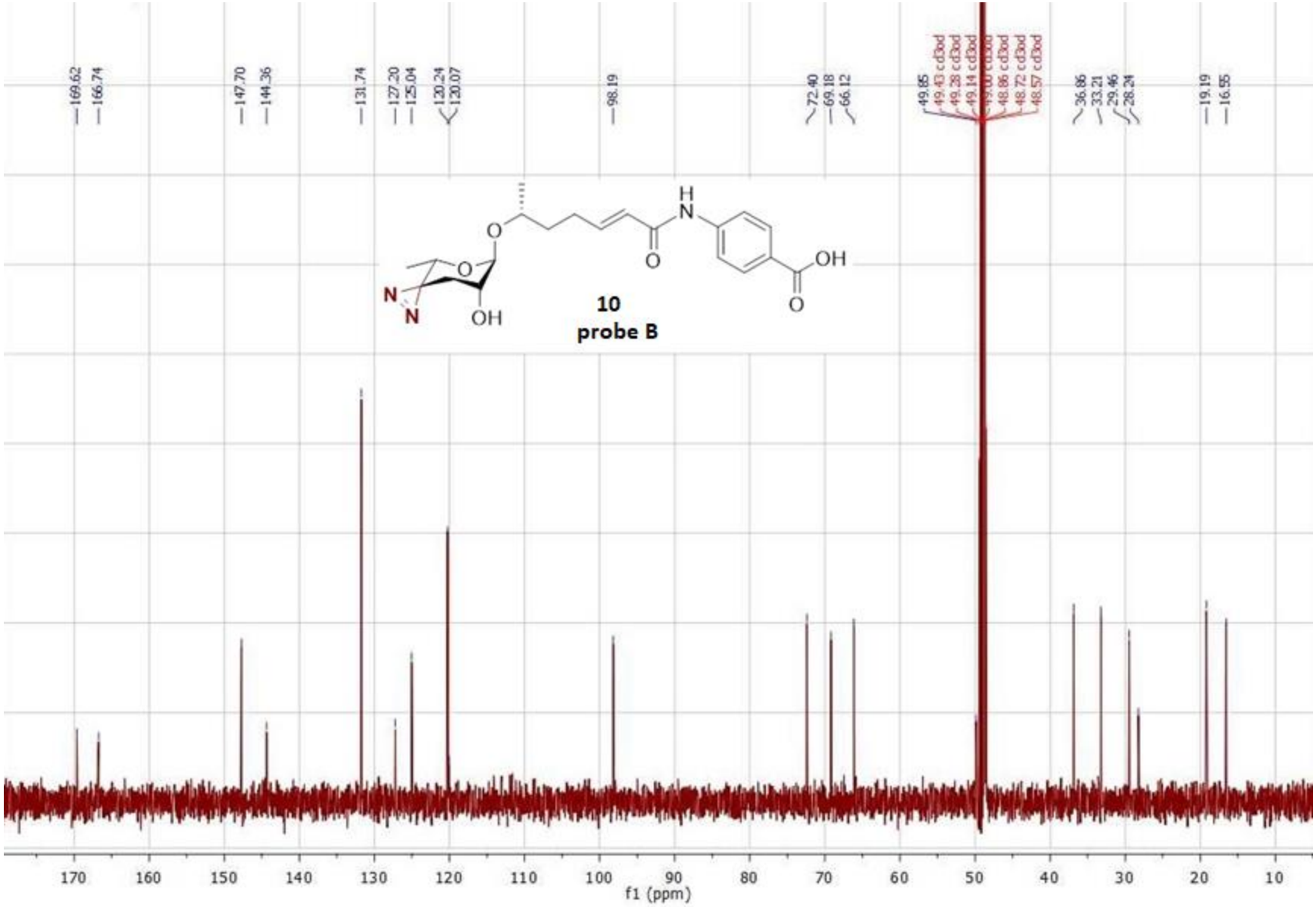

165.90
133.50
130.15
130.00
128.68
98.05
77.41 cdcl3
77.20 cdcl3
76.99 cdcl3
69.99
64.63
55.46
29.97
27.25
16.75
O
O
N
N
OBz
23
0
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

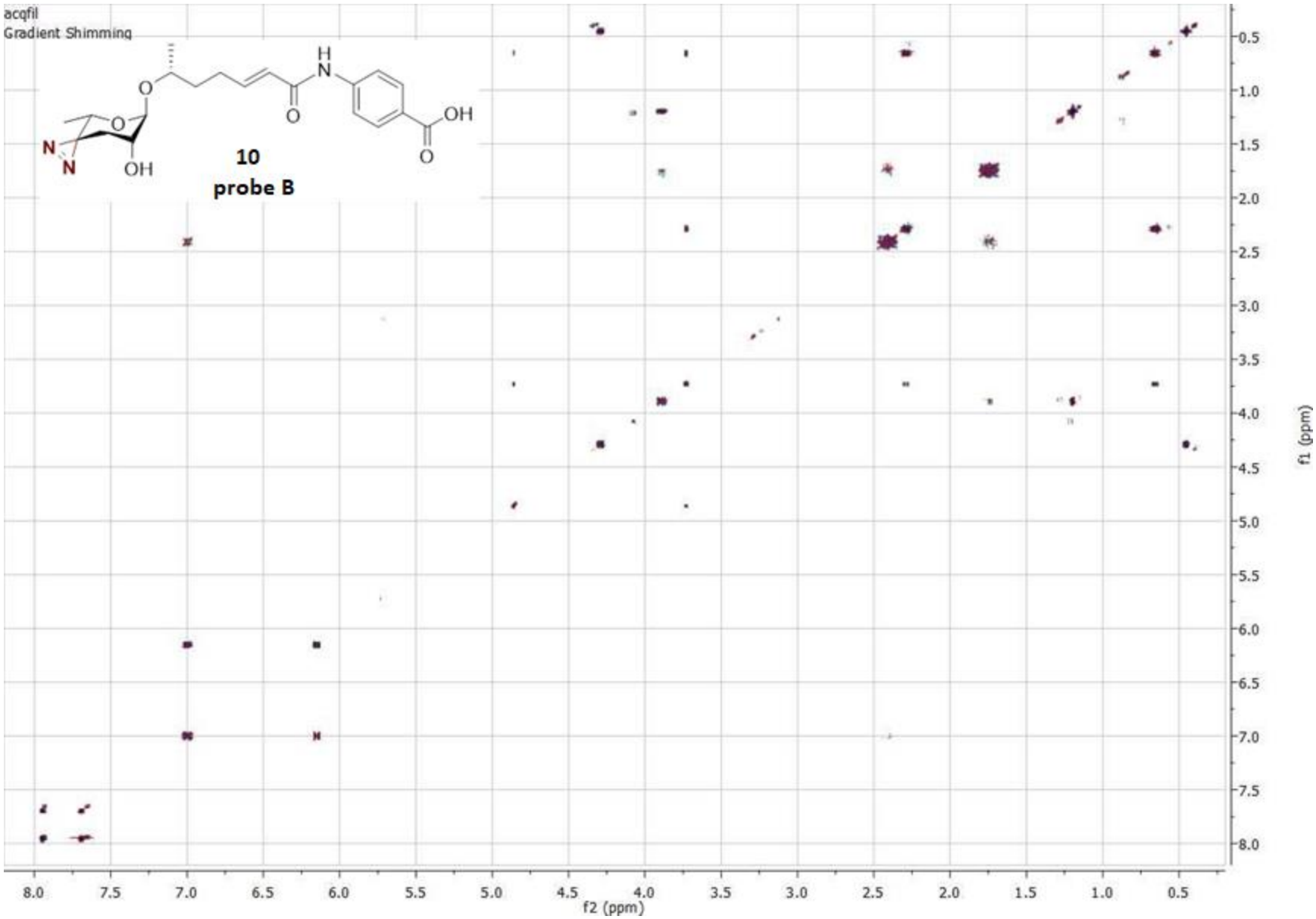

8.23
8.22
8.22
7.62
7.62
7.62
7.61
7.60
7.60
7.60
7.52
7.51
7.51
7.50
7.49
7.49
7.47
7.26 cdcl3
5.41
5.19
5.18
5.18
5.18
4.68
4.67
4.66
4.65
2.73
2.72
2.70
2.69
2.04
1.60 HDO
0.88
0.87
0.87
0.85
0.84
0.66
0.65
0.56
0.55
OH
O
N
N
OBz
24
2.21
1.43
2.60
1.00
0.42
0.96
0.18
0.96
1.58
0.49
1.02
0.19
0.76
1.03
2.89
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

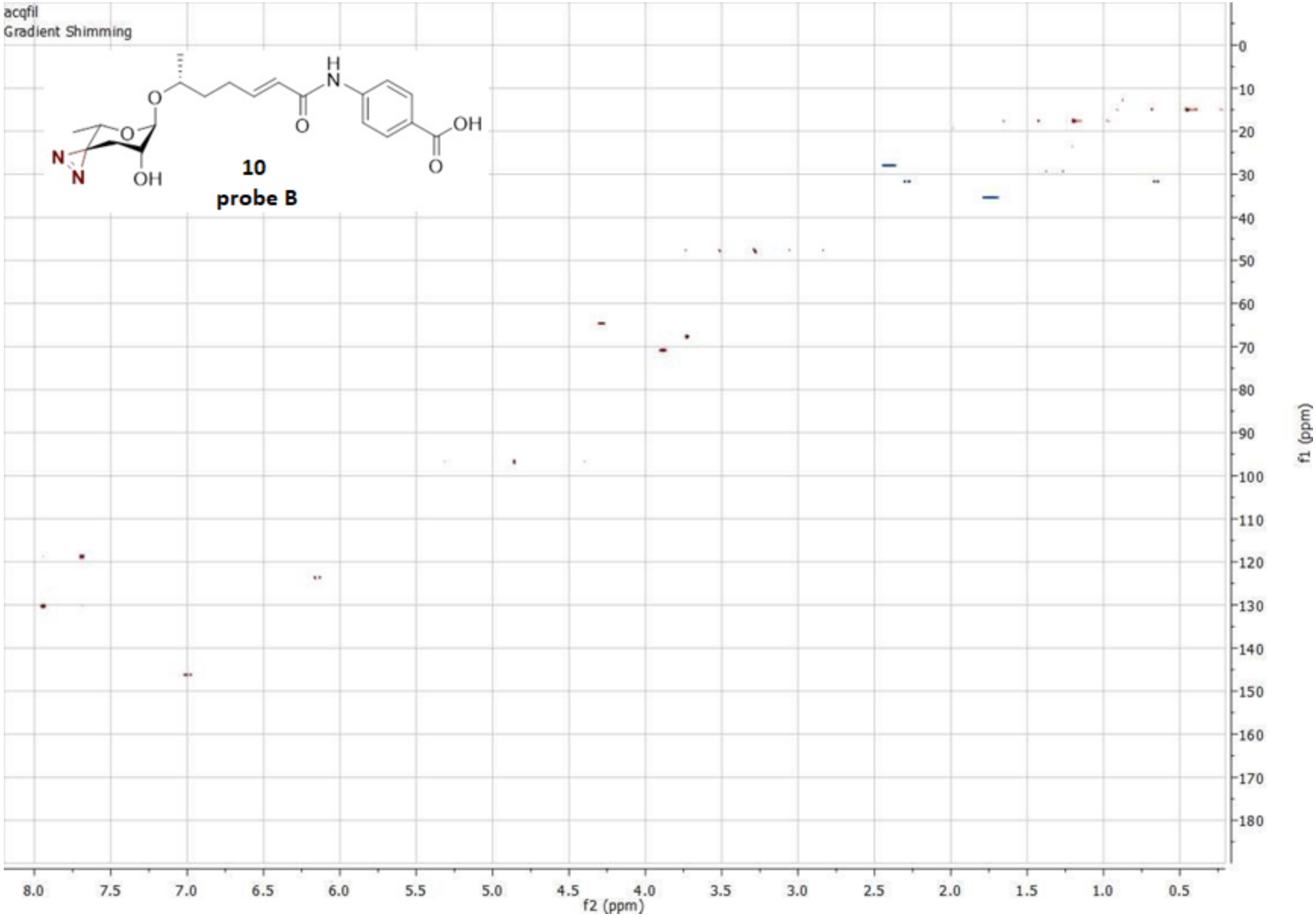

166.03
133.56
130.15
128.68
91.54
77.45 CDCl3
77.20 CDCl3
76.95 CDCl3
70.41
64.82
29.36
27.23
16.75
OH
O
N
N
OBz
24
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

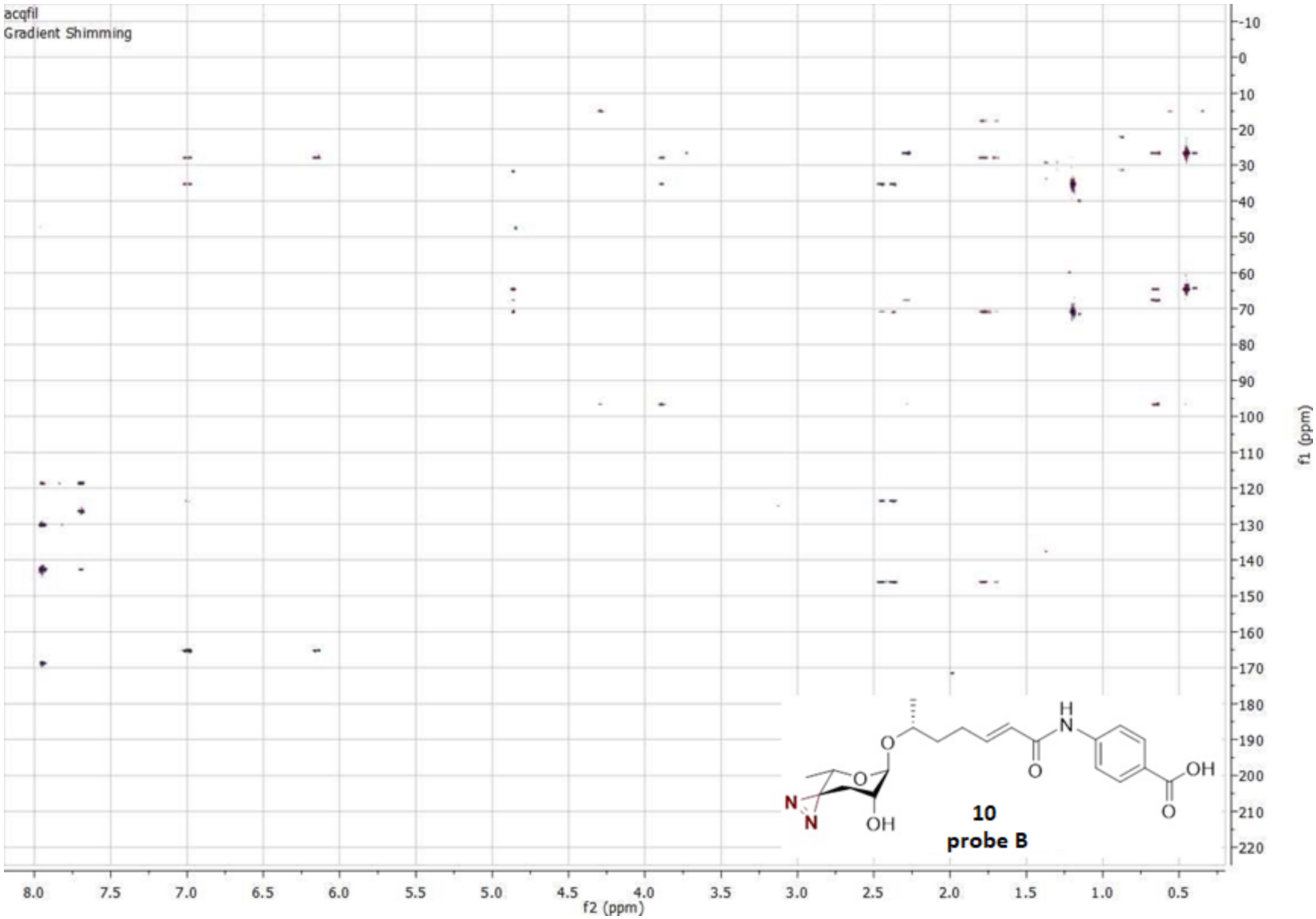

25
OBz
f1 (ppm)

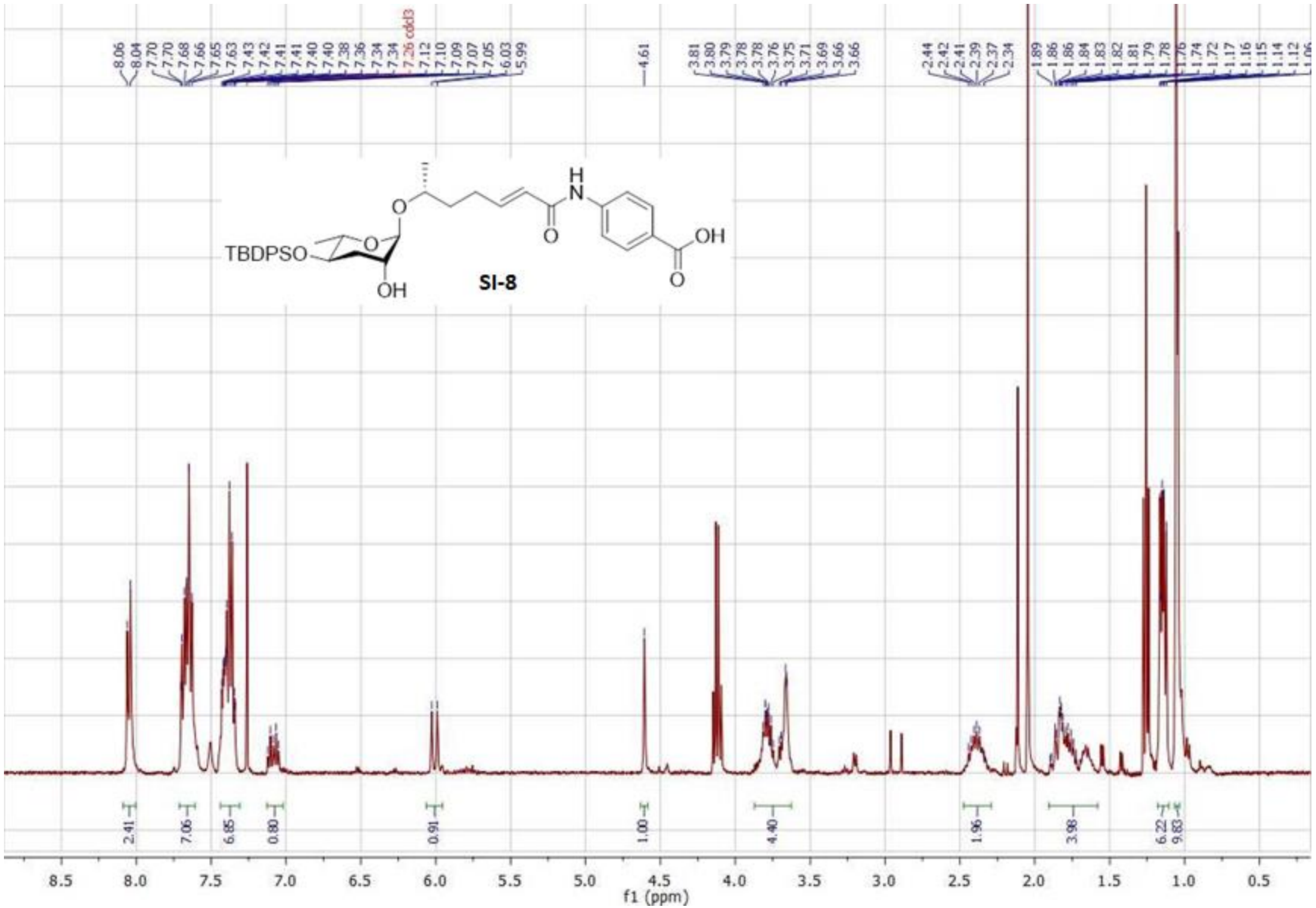

10
probe B
f1 (ppm)

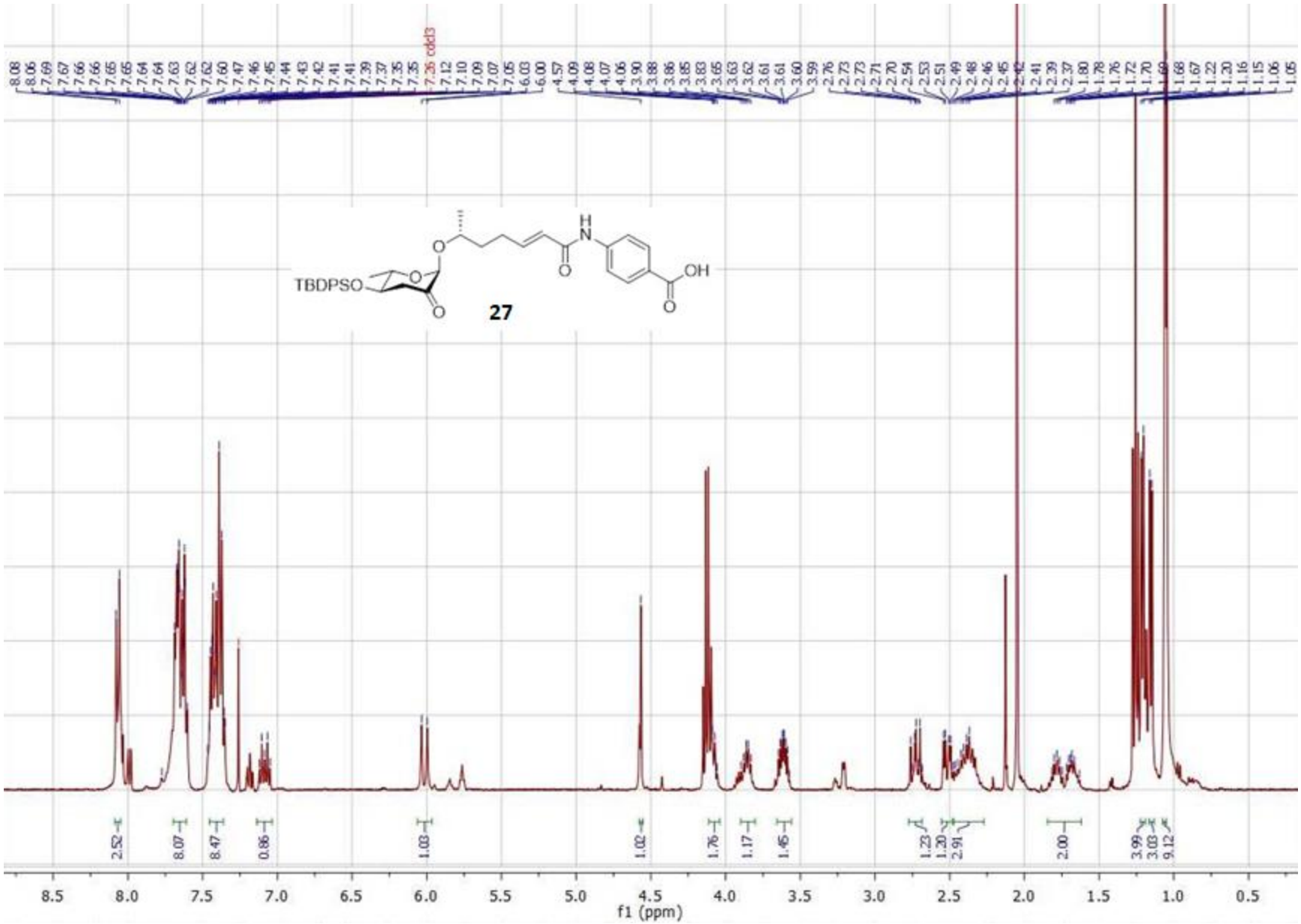

169.62
166.74
147.70
144.36
131.74
127.20
125.04
120.24
120.07
98.19
72.40
69.18
66.12
49.85
49.43 cd3od
49.28 cd3od
49.14 cd3od
48.86 cd3od
48.72 cd3od
48.57 cd3od
36.86
33.21
29.46
28.24
19.19
16.55
O
O
N
N
OH
H
N
O
OH
O
10
probe B
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

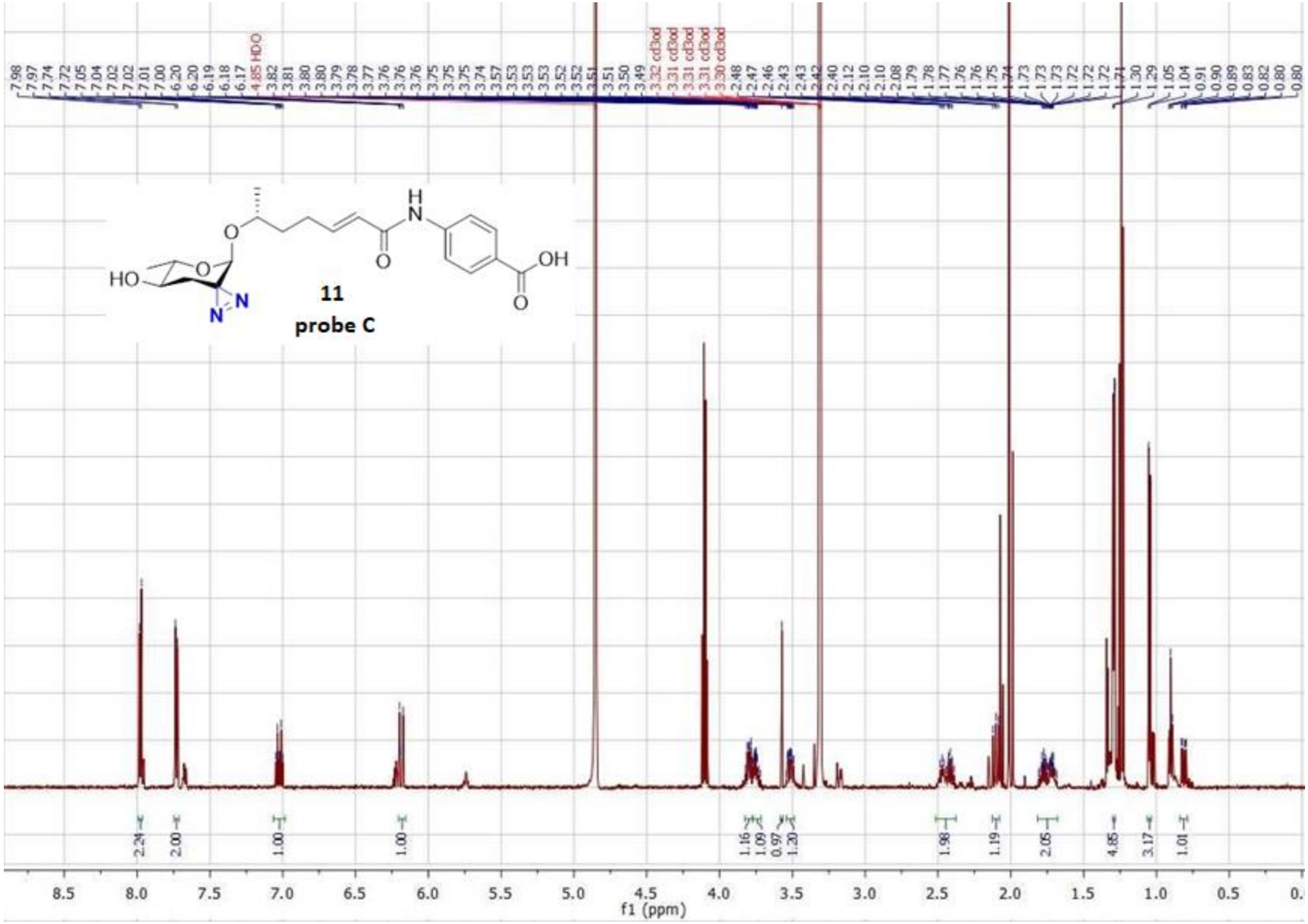

acqfil
Gradient Shimming
10
probe B
f2 (ppm)
f1 (ppm)

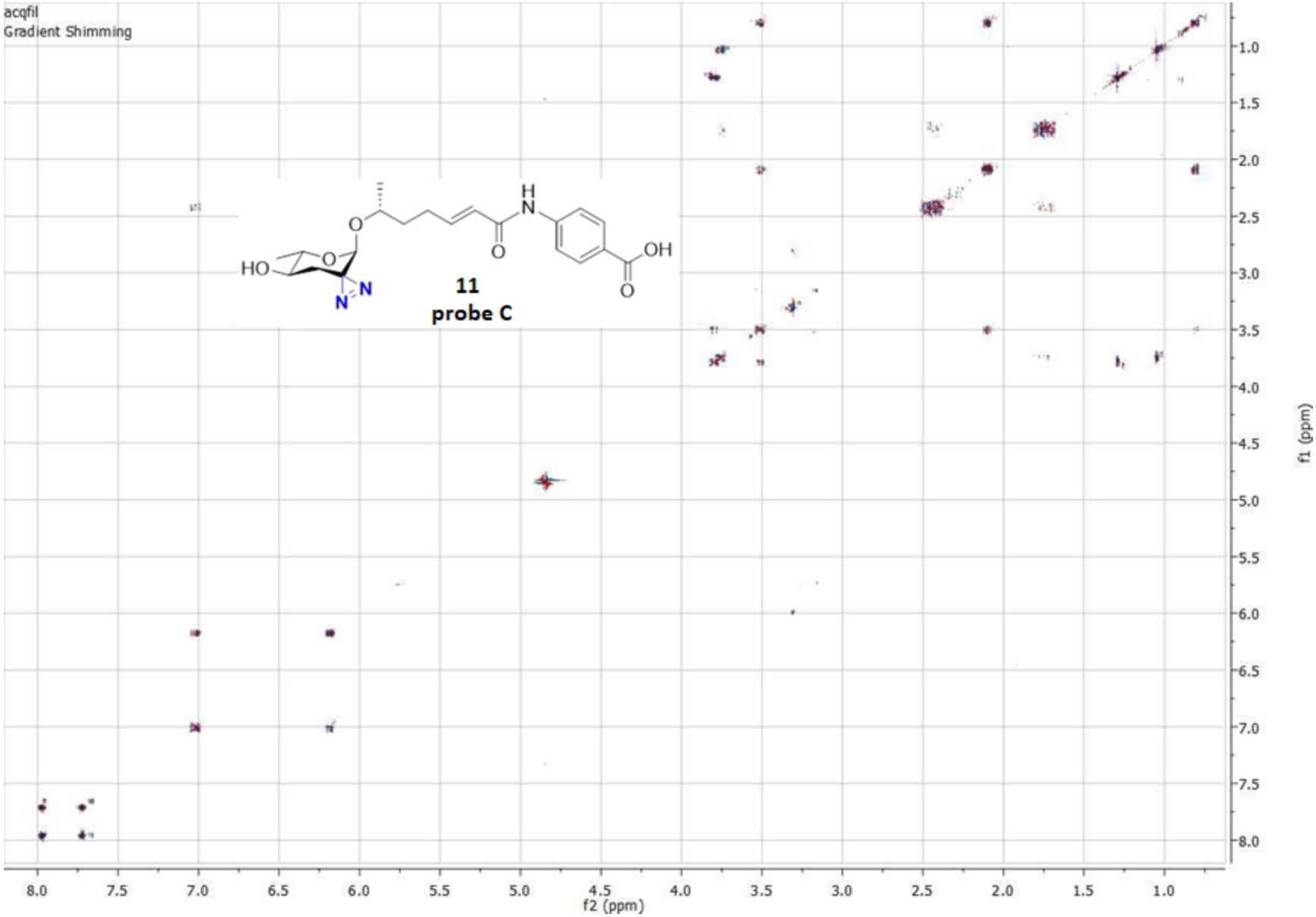

acqfil
Gradient Shimming
10
probe B
f2 (ppm)
f1 (ppm)

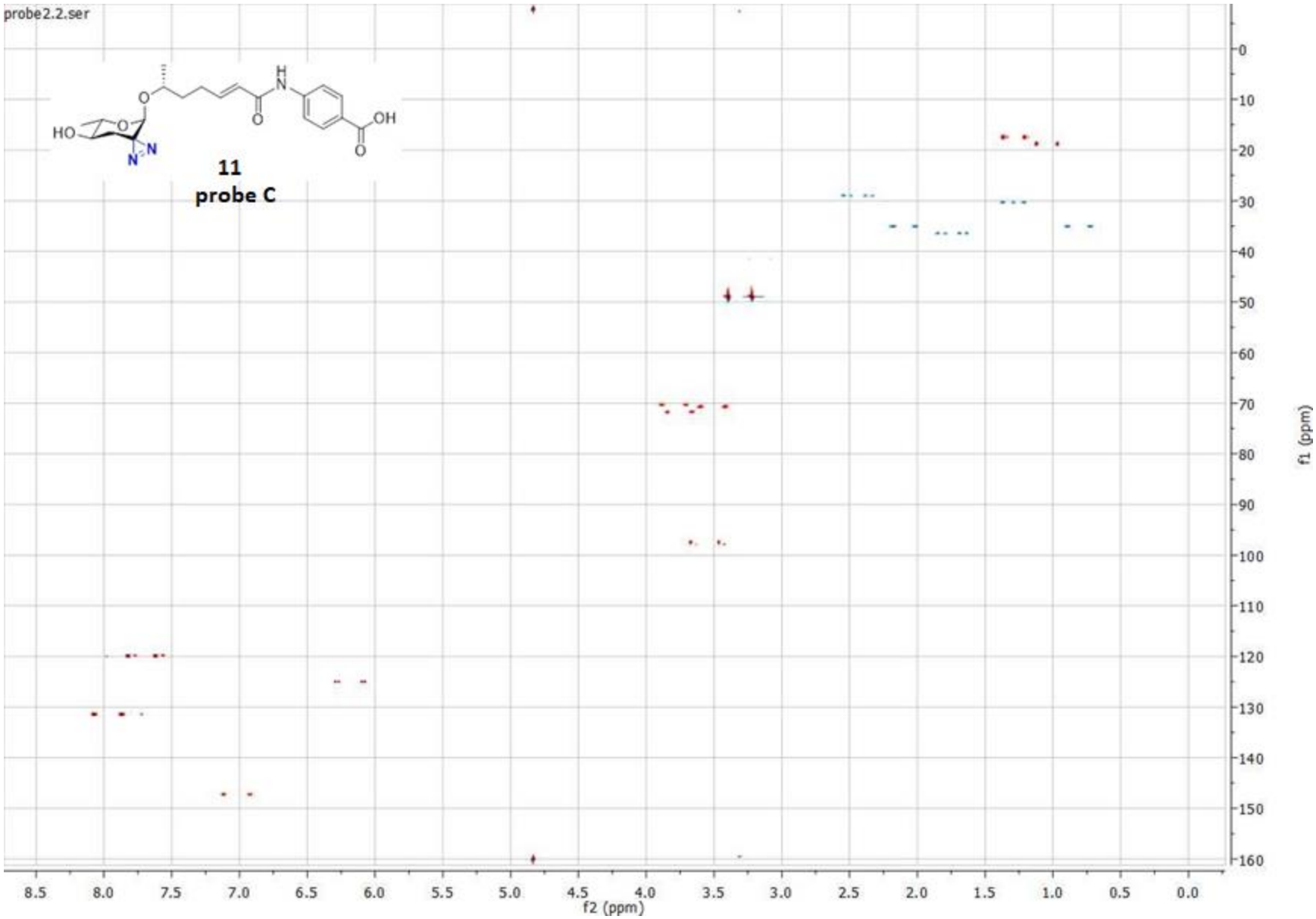

acqfil
Gradient Shimming
f2 (ppm)
f1 (ppm)
10
probe B

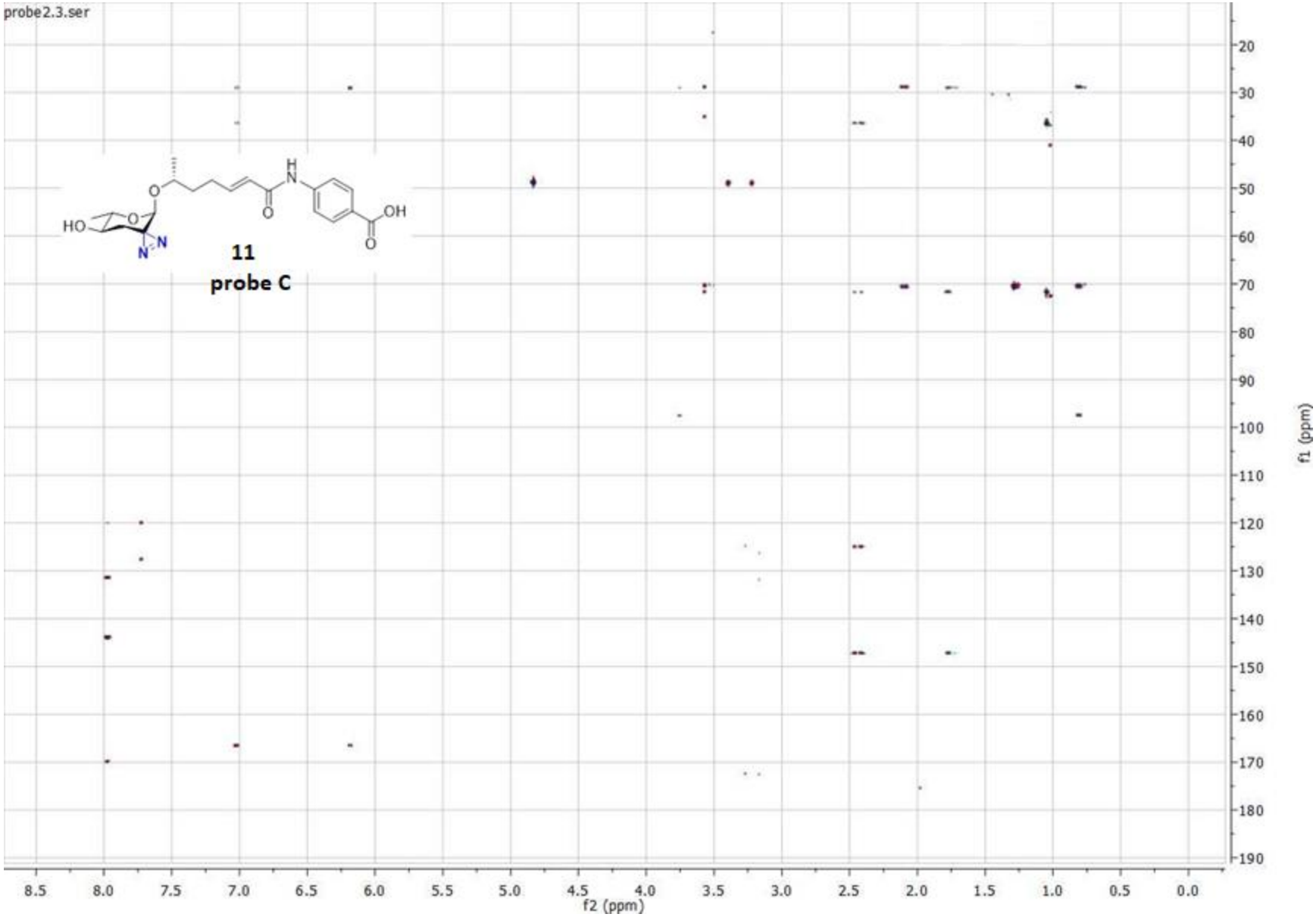

TBDPSO
OH
O
O
H
N
O
OH
O
SI-8
f1 (ppm)

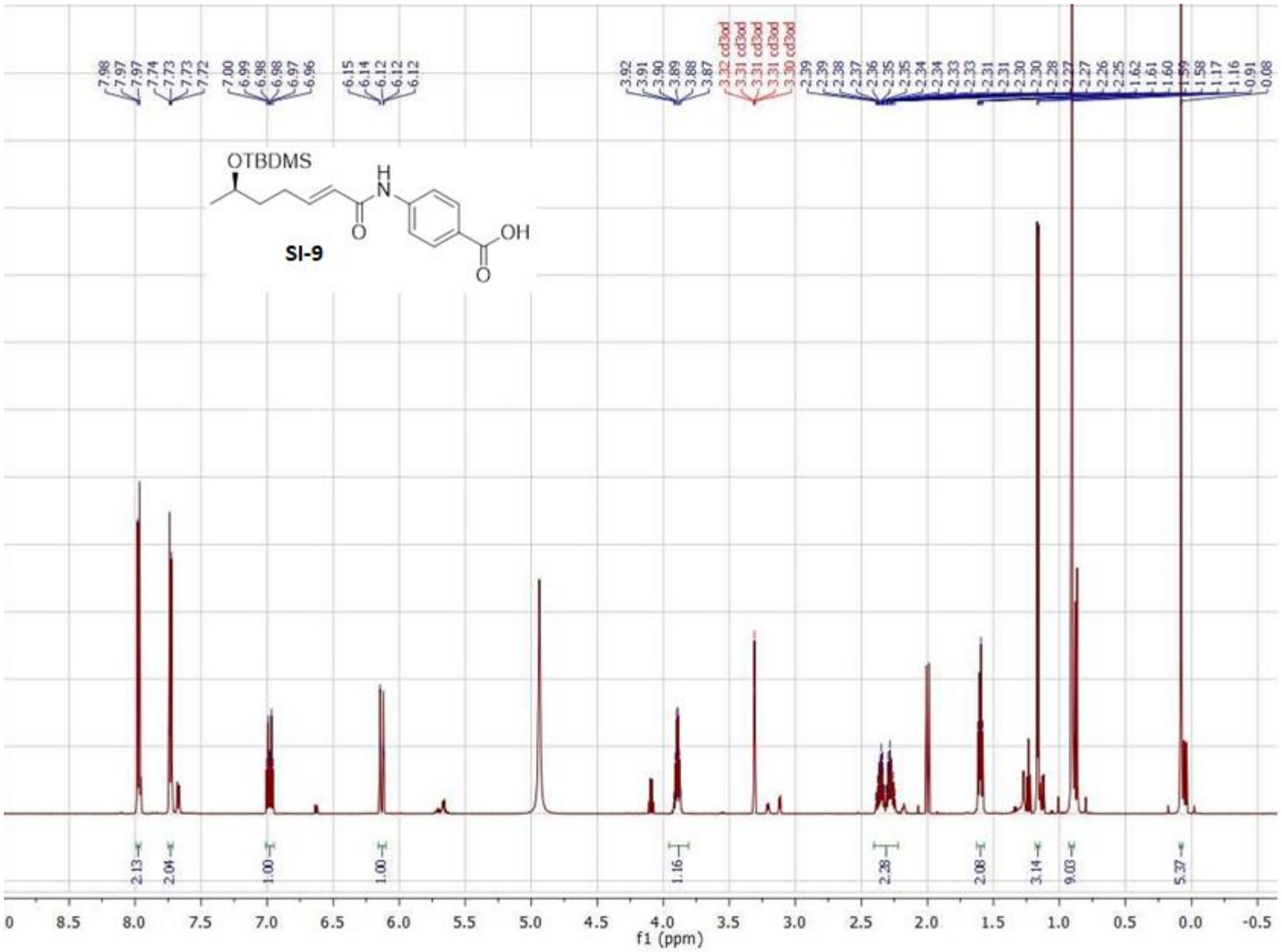

TBDPSO
O
O
O
H
N
O
OH
O
27
f1 (ppm)

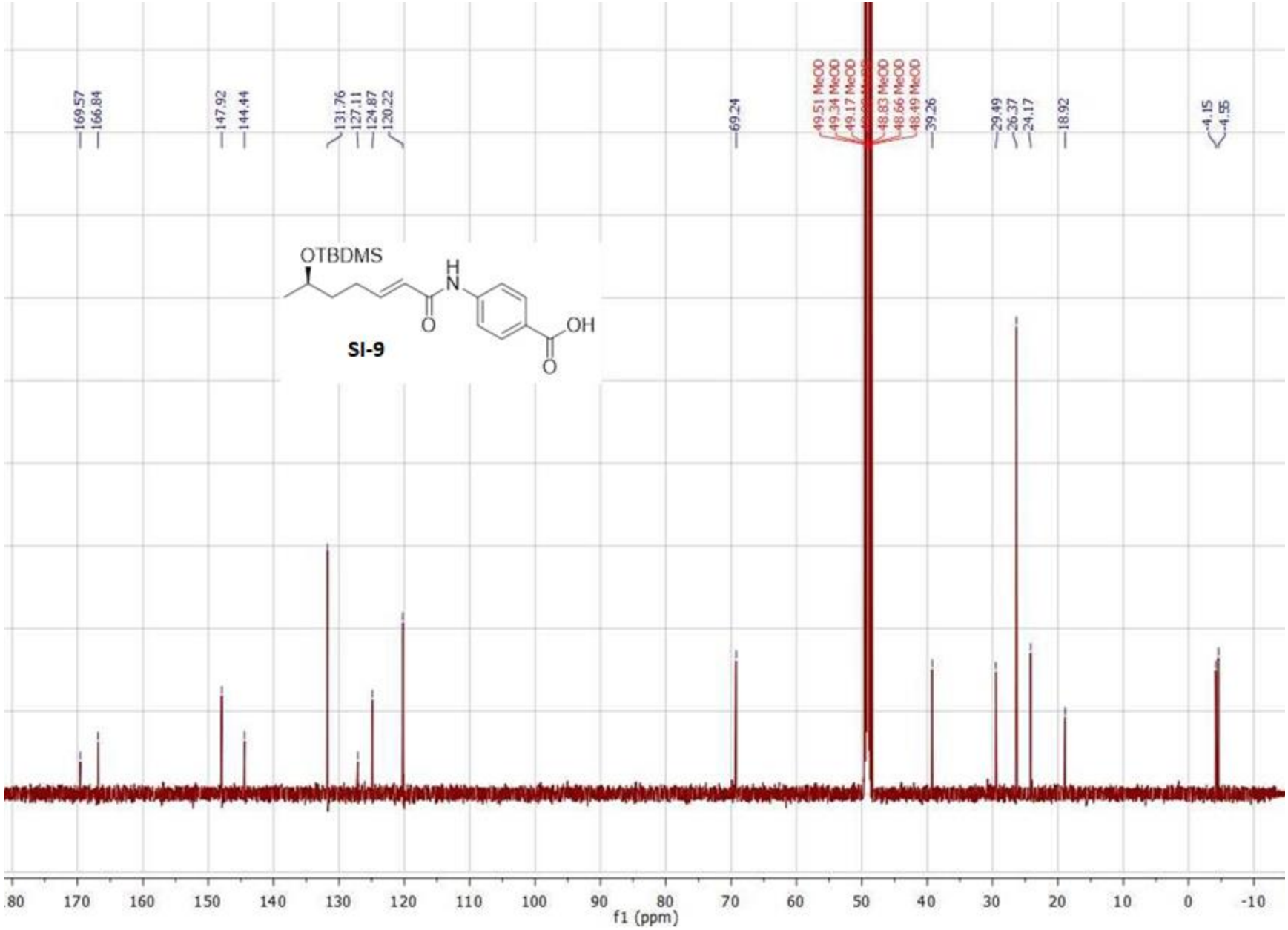

11
probe C
f1 (ppm)

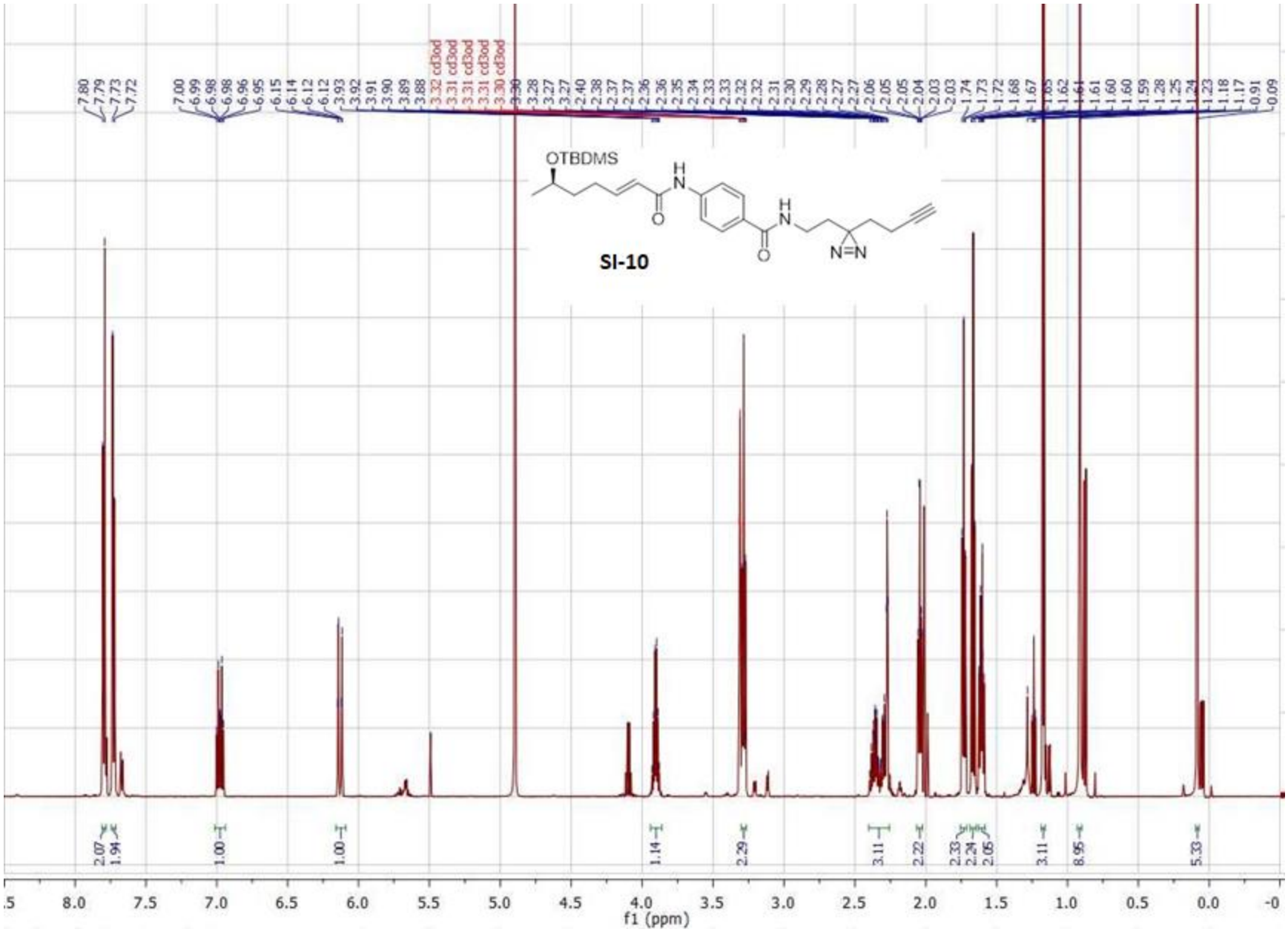

acqfil
Gradient Shimming
HO
O
O
N
N
H
N
O
OH
O
11
probe C
f2 (ppm)
f1 (ppm)

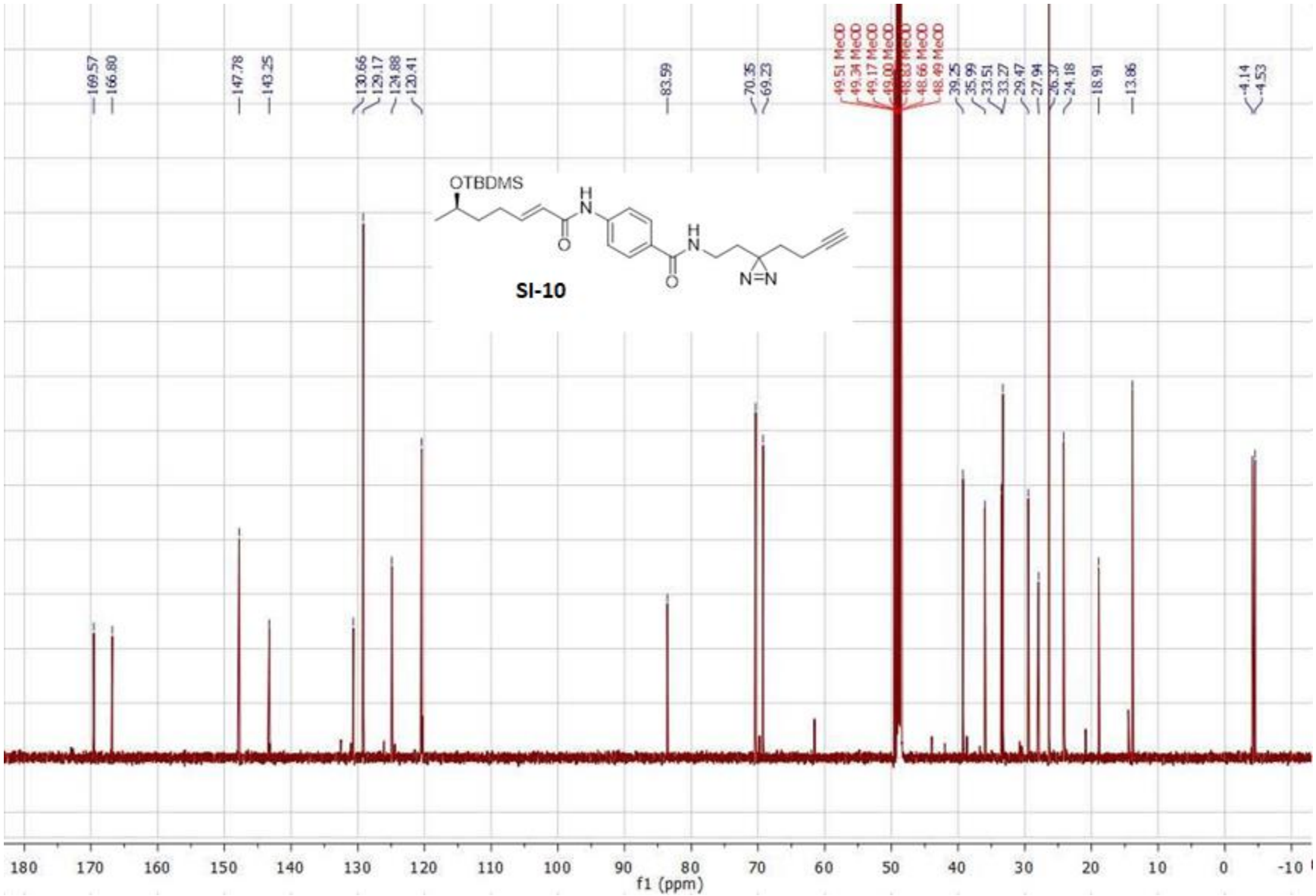

probe2.2.ser
HO
O
O
N
N
H
N
O
OH
O
11
probe C
f2 (ppm)
f1 (ppm)

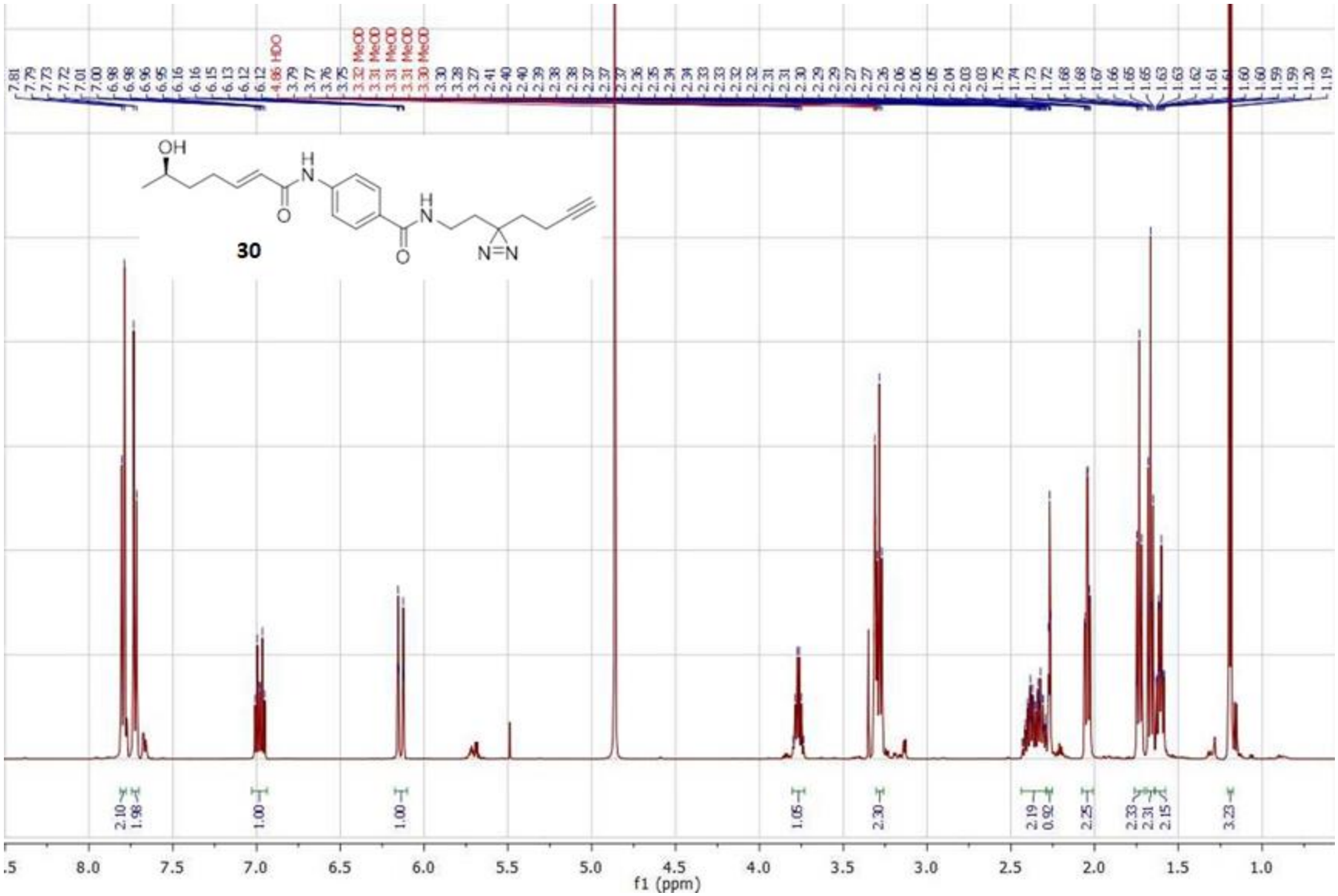

probe2.3.ser
HO
O
O
N
N
H
N
O
OH
O
11
probe C
f2 (ppm)
f1 (ppm)

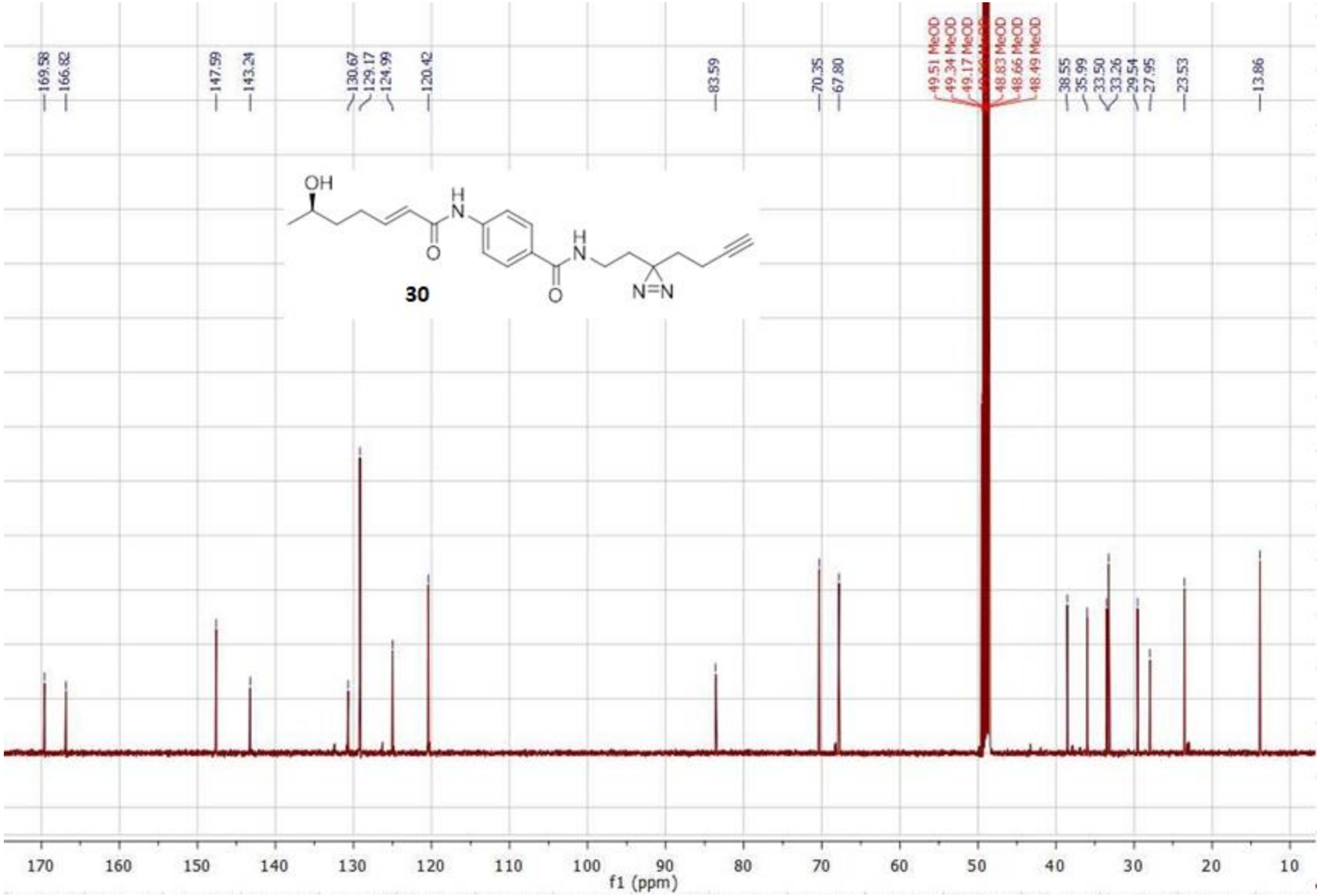

OTBDMS
H
N
O
OH
O
SI-9
f1 (ppm)

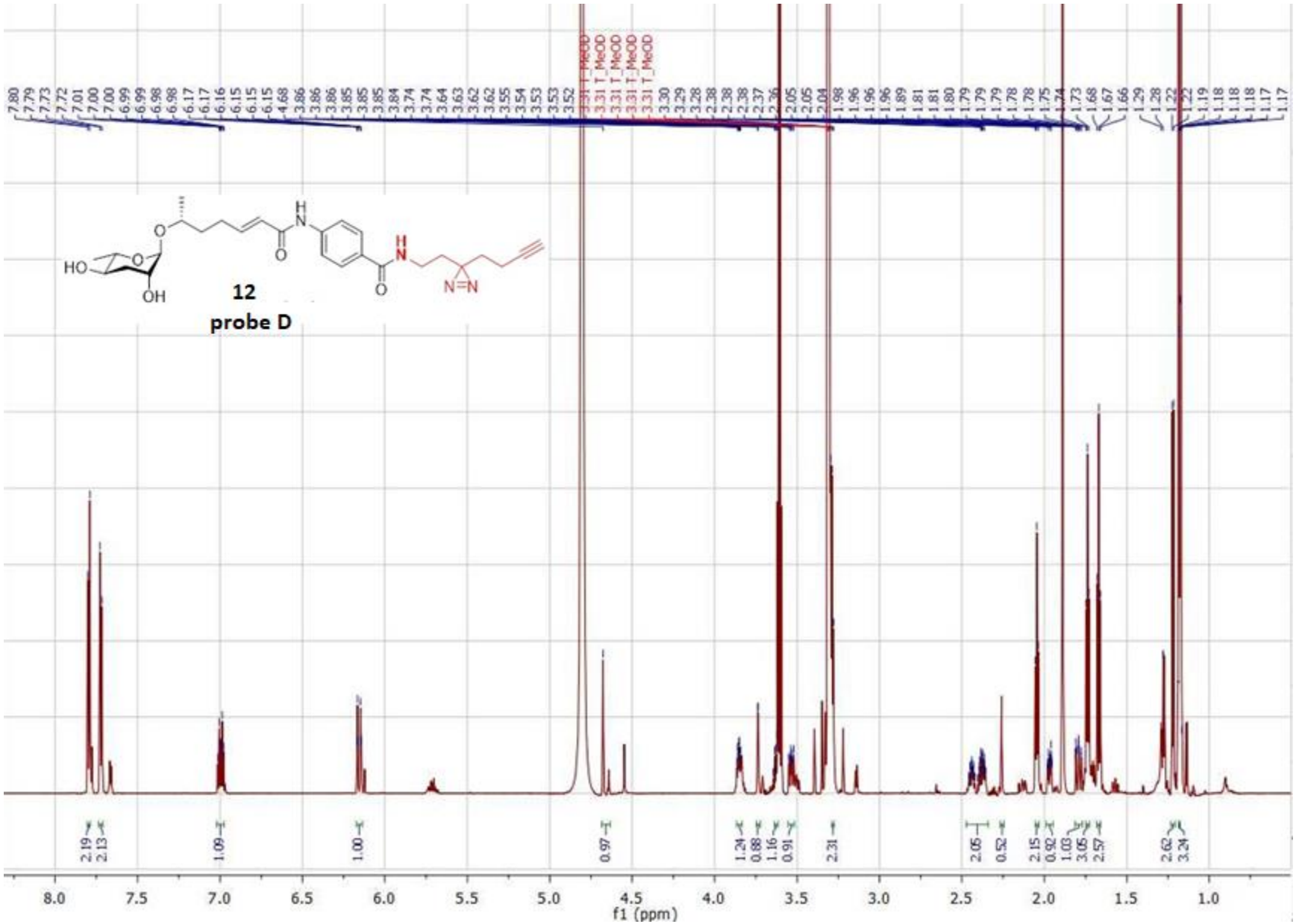

169.57
166.84
147.92
144.44
131.76
127.11
124.87
120.22
69.24
49.51 MeOD
49.34 MeOD
49.17 MeOD
48.83 MeOD
48.66 MeOD
48.49 MeOD
39.26
29.49
26.37
24.17
18.92
-4.15
-4.55
OTBDMS
H
N
O
OH
O
SI-9
f1 (ppm)

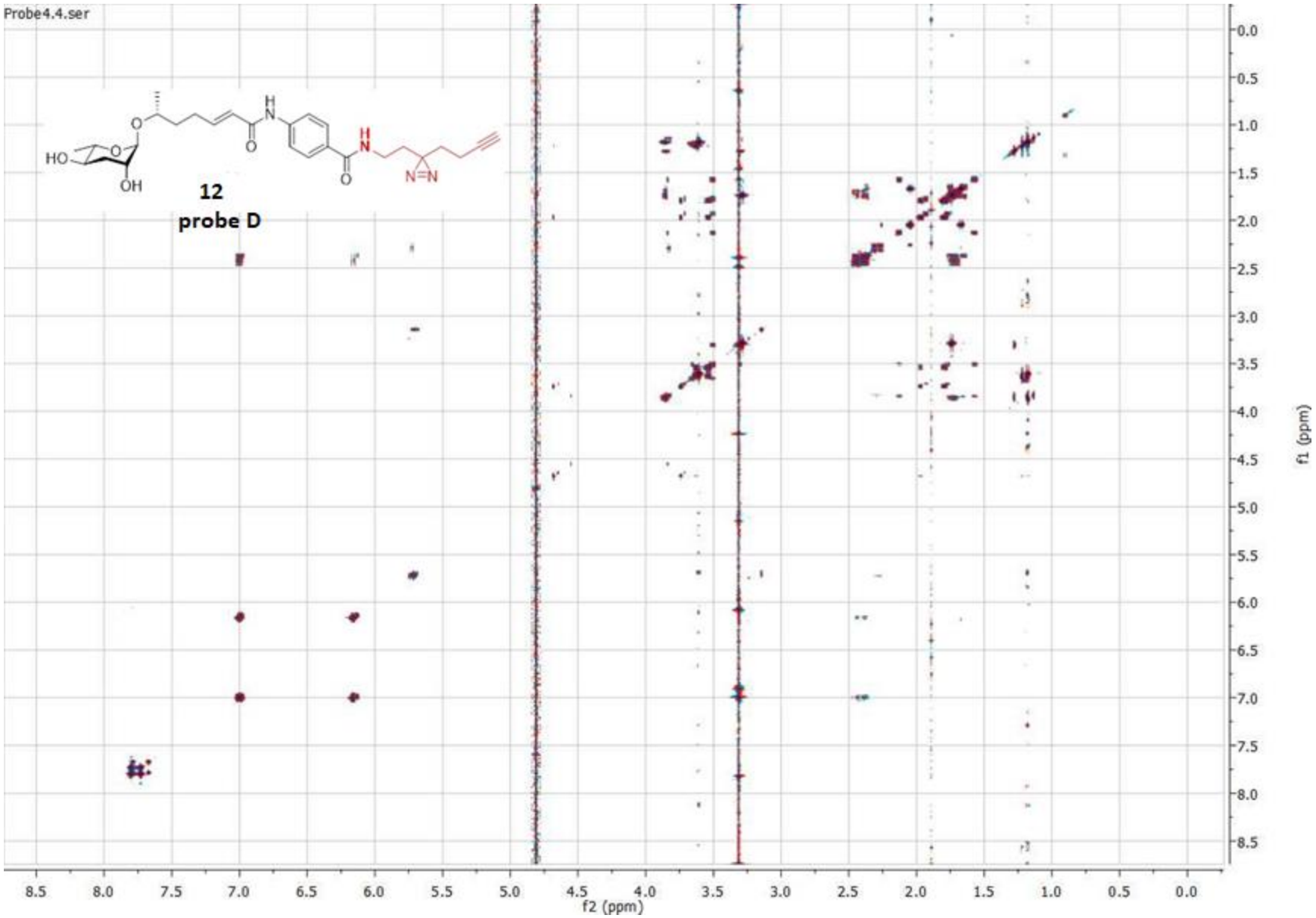

OTBDMS
SI-10
f1 (ppm)

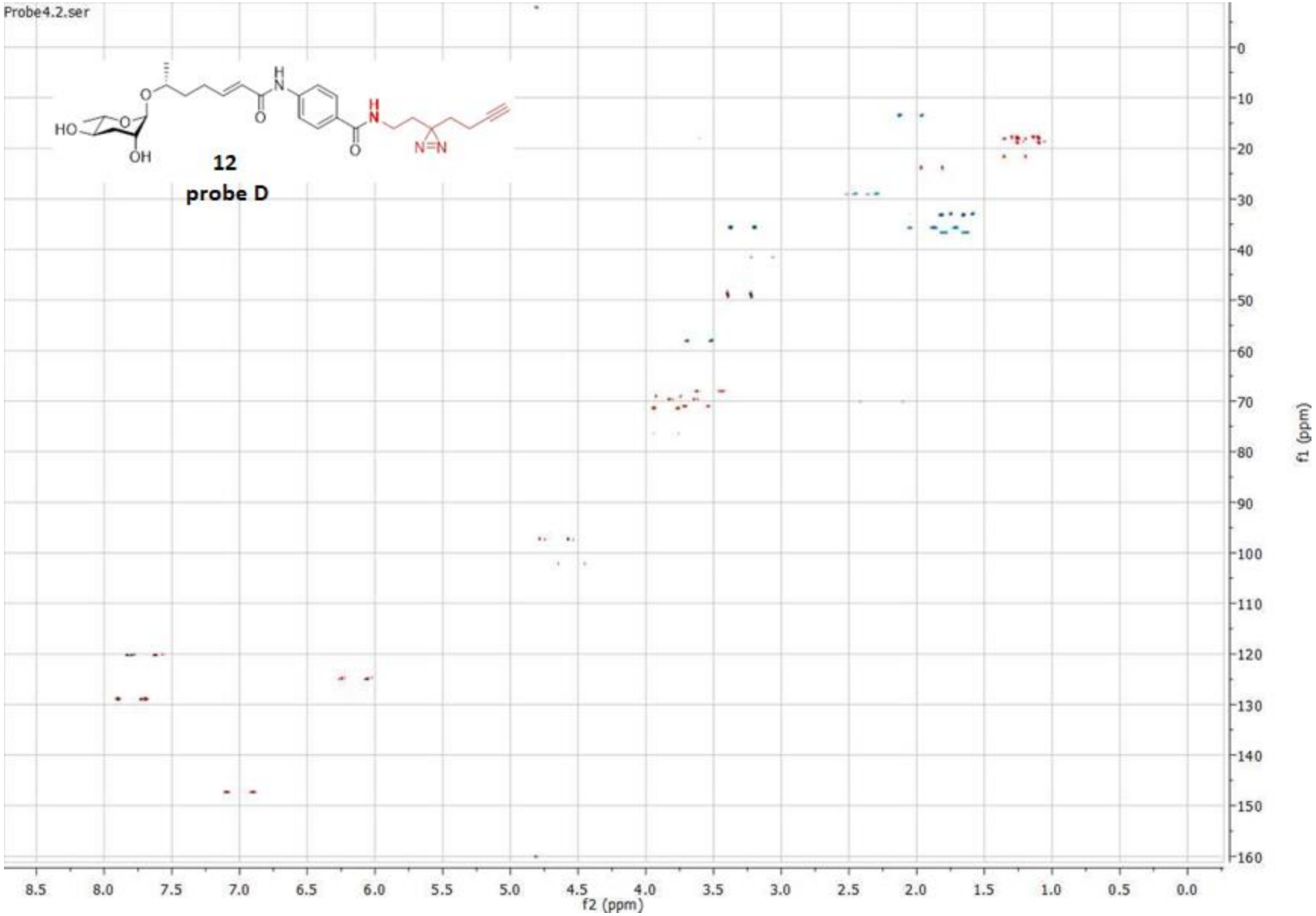

169.57
166.80
147.78
143.25
130.66
129.17
124.88
120.41
83.59
70.35
69.23
49.51 MeOD
49.34 MeOD
49.17 MeOD
49.00 MeOD
48.83 MeOD
48.66 MeOD
48.49 MeOD
39.25
35.99
33.51
33.27
29.47
27.94
26.37
24.18
18.91
13.86
-4.14
-4.53
OTBDMS
H
N
O
H
N
O
N=N
SI-10
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

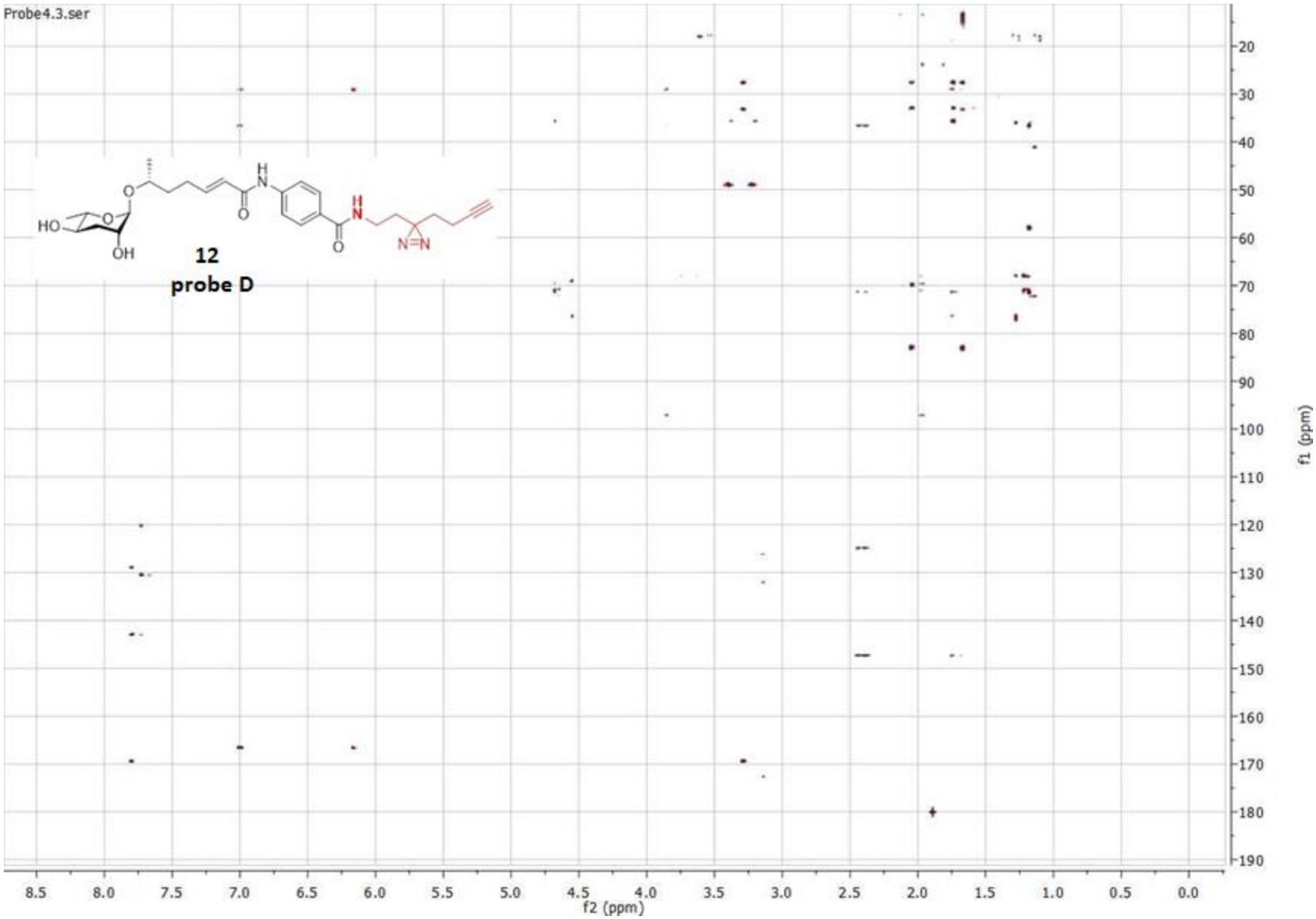

OH
H
N
O
H
N
O
N=N
30
f1 (ppm)

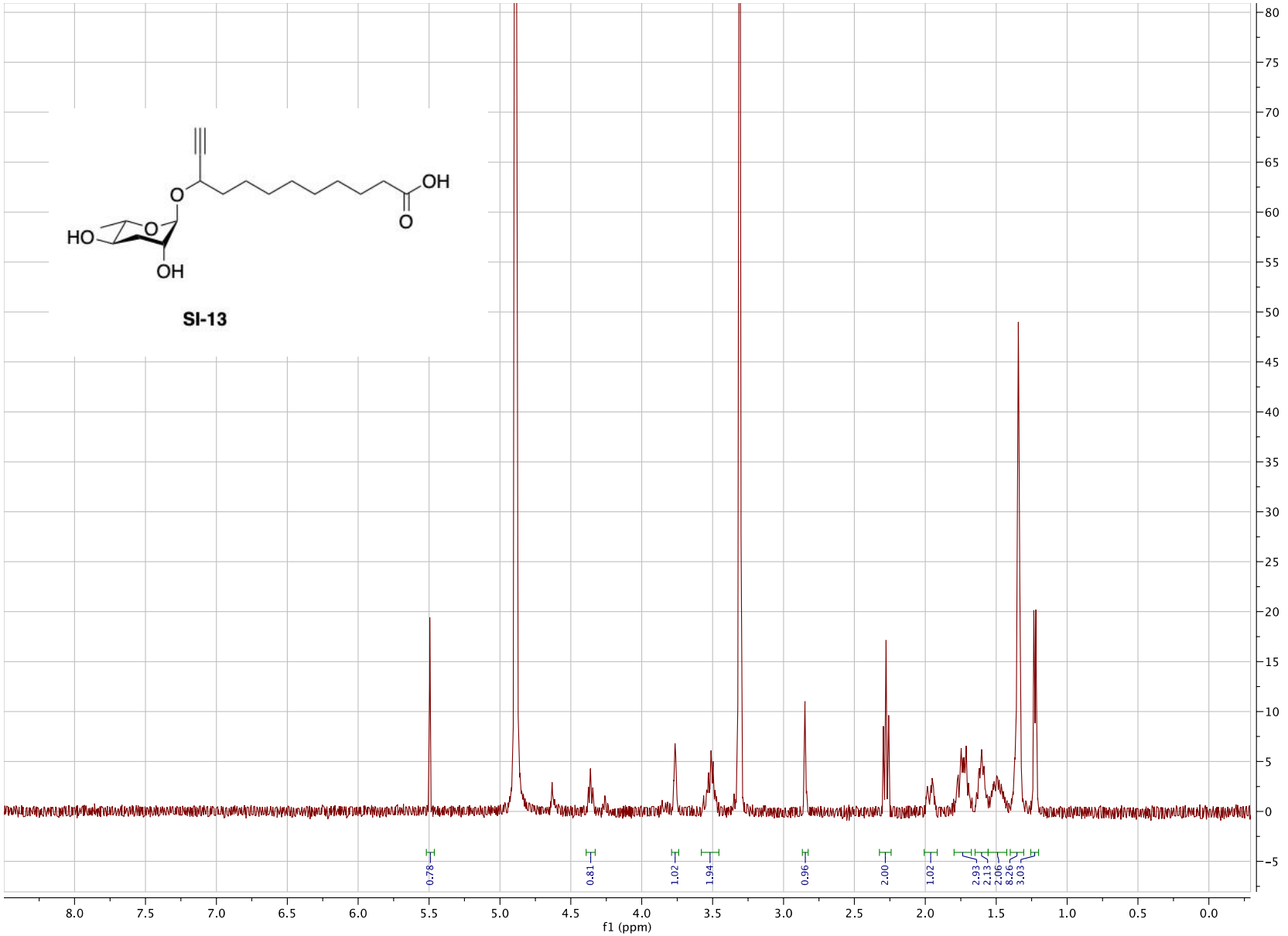

169.58
166.82
147.59
143.24
130.67
129.17
124.99
120.42
83.59
70.35
67.80
49.51 MeOD
49.34 MeOD
49.17 MeOD
48.83 MeOD
48.66 MeOD
48.49 MeOD
38.55
35.99
33.50
33.26
29.54
27.95
23.53
13.86
OH
H
N
O
H
N
O
N=N
30
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

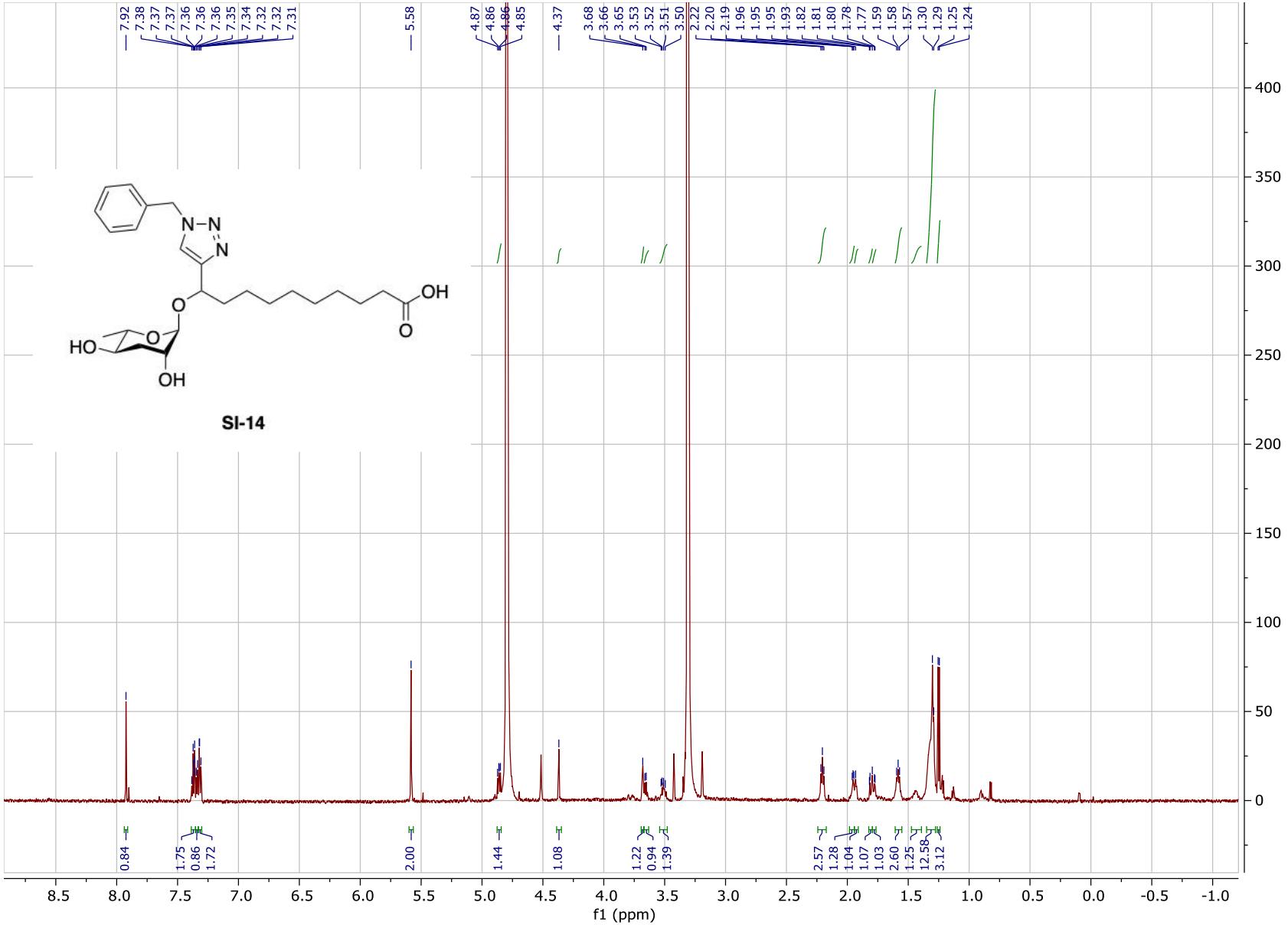

12
probe D
f1 (ppm)


Probe4.4.ser
HO
OH
O
O
H
N
O
H
N
O
N=N
12
probe D
f2 (ppm)
f1 (ppm)


Probe4.2.ser
HO
O
OH
O
H
N
O
H
N
O
N=N
12
probe D
f2 (ppm)
f1 (ppm)


Probe4.3.ser
HO
O
OH
H
N
O
H
N
O
N=N
12
probe D
f2 (ppm)
f1 (ppm)


SI-13
HO
O
O
OH
OH
O
0.78
0.81
1.02
1.94
0.96
2.00
1.02
2.93
2.13
2.06
8.26
3.03
f1 (ppm)


7.92
7.38
7.37
7.37
7.36
7.36
7.36
7.35
7.34
7.32
7.32
7.31
5.58
4.87
4.86
4.86
4.85
4.37
3.68
3.66
3.65
3.53
3.52
3.51
3.50
2.22
2.20
2.19
1.96
1.95
1.95
1.93
1.82
1.81
1.80
1.78
1.77
1.59
1.58
1.57
1.30
1.29
1.25
1.24
N–N
N
O
OH
O
HO
O
OH
SI-14
400
350
300
250
200
150
100
50
0
0.84
1.75
0.86
1.72
2.00
1.44
1.08
1.22
0.94
1.39
2.57
1.28
1.04
1.07
1.03
2.60
1.25
12.58
3.12
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)