SUPPORTING INFORMATION

Syntheses of differentially fluorinated triazole-based 1-deoxysphingosine analogues *en route* to SphK inhibitors

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1. General Methods

All reagents were purchased from Sigma Aldrich, Alfa Aesar or Carbosynth chemical companies. Dichloromethane (CH_2Cl_2) was distilled from CaH_2. CH_3CN was pre-died over 4 Å MS, distilled and then stored with activated 4Å MS. Toluene was stored with activated 4Å MS, THF was distilled from sodium and Et_3N was stored with activated 4Å MS. 4Å MS were activated by heating under high vacuum at 260 ºC for 10 h and then were stored at 165 ºC. ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Varian® Mercury VX 400 or on a Bruker® Avance Ultrashield (400 MHz, 100.6 MHz and 376.5 MHz respectively) spectrometer. NMR signals were fully assigned by COSY, HSQC, NOESY and HMBC experiments. All chemical shifts are quoted on the δ scale in ppm using the residual solvent as internal standard (^1H NMR: $CDCl_3$ = 7.26; ^{13}C NMR: $CDCl_3$ = 77.16; ^1H NMR: MeOD = 3.31; ^{13}C NMR: CD_3OD = 49.0). Coupling constants (*J*) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, p = pentuplet, br s = broad singlet, br d = broad doublet, br t = broad triplet and br q = broad quartet. Infrared (IR) spectra were recorded on a JASCO FTIR-600 plus Fourier Transform Infrared Spectrophotometer wavenumbers ($\tilde{\nu}$) in cm^{-1}. ESI MS were run on an Agilent® 1100 Series LC/MSD instrument. Melting points (m.p.) were recorded with Reichert apparatus. Optical rotations were measured on a Perkin–Elmer® 241 polarimeter with a path length of 1.0 dm and are reported with implied units of 10^{-1} deg cm^2 g^{-1}. Concentrations (c) are given in g/100 ml. Thin layer chromatography (TLC) was carried out on 0.25 mm E. Merck® aluminium backed sheets coated with 60 F_{254} silica gel. Visualization of the silica plates was achieved using a UV lamp (λmax = 254 nm) and/or by heating plates that were dipped in a H_2SO_4/ethanol (1:15) or anisaldehyde solution. Flash chromatography was carried out using forced flow of the indicated solvent on Fluka® or Merck® silica gel 60 (230-400 mesh). The biological assays were run on a CLARIOstar® BMG LABTECH's instrument using Corning® 384 low volume well plates.

2. General Procedures

General Procedure for the CuAAC reaction. A solution of $CuSO_4 \cdot 5H_2O$ (12.5mg, 0.05 mol) in 0.6 mL of H_2O was added to a stirring solution of azide **11a-d** (1.05-2.0 mmol) and the terminal alkyne **8** (279 mg, 1 mmol) in 3.5 mL of CH_2Cl_2. Sodium ascorbate (19.8 mg, 0.1 mmol) in 0.7 mL of water was then added dropwise and the mixture was stirred for 48 h at room temperature. The aqueous layer was extracted three times with CH_2Cl_2 and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product **12a-d** was purified by column chromatography (Hexanes:AcOEt 9:1 to 7:3).

General Procedure for the debenzylation or simultaneous reduction of alkenes and debenzylation. 30% Pd/C (percent of catalyst (Pd/C (10 wt%)) loading relative to the substrate by weight)) was added to a flask containing the *N*,*N*-dibenzylamino compound **12a-d** (1 mmol) in 2.6 mL of methanol. The inside air was replaced with H_2 (balloon) by three vacuum/H_2 cycles. The reaction mixture fitted with the hydrogen balloon was then stirred at room temperature until the TLC monitoring indicated the complete consumption of the starting material (24-48 h). The mixture was filtered through Celite, concentrated in vacuo and then purified by flash column chromatography.

General Procedure for *N*,*N*-dimethylation of amines 1a-d to furnish 3a-d. $NaBH_3CN$ (0.090 g, 1.42 mmol) was added to a cold (0 °C) mixture of the free amine (0.129 mmol) and paraformaldehyde (0.039g, 1.29 mmol) in 1 mL of CH_3OH and the resulting reaction mixture was stirred at room temperature for 48 h. The solvent was then evaporated and the crude was redissolved in $CHCl_3$. The resulting organic phase was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give the dimethyl amine derivative.

General Procedure for the monomethylation of amines 1a-d to furnish 2a-d. Boc_2O (0.126 mmol, 1.15 equiv) was added to a solution on free amine derivative **1a-d** (0.110 mmol) in 0.1 mL of CH_3OH. The reaction mixture was stirred for 16 h at room temperature and it was then concentrated under vacuum. The crude product was redissolved in CH_2Cl_2 and washed with brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and

concentrated under reduced pressure. Without further purification, the Boc derivative was dissolved in THF and added dropwise to a suspension of $LiAlH_4$ (3 equiv) and the reaction mixture was refluxed for 24 h and allowed to warm to room temperature. A 1M NaOH solution was then added dropwise and the slurry was filtered through Celite. The resulting filtrate was extracted with AcOEt. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give the monomethyl amine **2a-d**.

General Procedure for the guanidination of amines 1a-d to furnish 15a-d. A mixture the amine **1a-d** (0.200 mmol, 1 equiv.), *N*,*N*'-di-Boc-1*H*-pyrazole-1-carboxiamidine (**14**) (0.070 g, 0.220 mmol, 1.10 equiv) and triethylamine (28 μL, 0.200 mmol, 1 equiv) in 1 mL of a 3:2 v/v mixture of dry CH_2Cl_2:DME was stirred at room temperature under argon atmosphere for 12 h. The solvent was then removed under reduced pressure and the residue was purified by flash chromatography (9:1 to 6:4 hexanes/EtOAc) to give the Boc guanidino derivative **15a-d**.

General Procedure for the Boc deprotection of the protected guanidino derivatives 15a-d to afford 4a-d. A solution of protected guanidino derivatives **15a-d** (0.150 mmol, 1 equiv) in 2 mL of TFA/CH_2Cl_2 (1:1, v/v) was stirred at room temperature for aproximately 4 h (or otherwise shown by TLC). The solution was then concentrated in vacuo and purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1 to 9:3:0.3) to give the free guanidino compound **4a-d**.

3. Synthetic procedures and characterization data

(3*R*,4*S*)-4-(Dibenzylamino)pent-1-yn-3-ol (8):[1] To a solution of oxalyl chloride (0.6 ml, 7.05 mmol, 1.5 equiv.) in CH_2Cl_2 (4.2 ml) a solution of DMSO (0.73 ml, 10.34 mmol, 2.2 equiv.) and CH_2Cl_2 (5.0 ml) was added dropwise at -78 °C. The mixture was stirred for 15 min at the same temperature. Then, a solution of (*S*)-2-dibenzylaminopropan-1-ol 5[2] (1.2 g, 4.70 mmol, 1 equiv.) in 33 mL of CH_2Cl_2 was added dropwise to the mixture. After 30 min, Et_3N (2.6 ml, 18.8 mmol, 4 equiv.) was added and the reaction mixture was allowed to warm to room temperature for 2 h. Then, the reaction was quenched by addition of saturated solution of NH_4Cl and extracted several times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude aldehyde was then dissolved in in 7 mL of THF at -78 °C, and 0.5 M ethynyl magnesium bromide (28.21 mL, 14.1 mmol, 3.0 equiv.) was then added slowly. The mixture was warmed up to -30 °C and allowed to react for 18 hours, after which NH_4Cl (20 mL) was added. The aqueous layer was extracted using dichloromethane (3 x 20 mL portions), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude consisting of a 9:1 *anti*:*syn* diastereomeric mixture was purified using flash column chromatography (hexane : ethyl acetate = 9 : 1) to afford **8** as a dark yellow oil in 86% yield (1.13 g, 4.04 mmol), with spectroscopic data in agreement with those described in the literature: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.34-7.23 (m, 10H), 4.20 (d, 2H, *J* = 13.2 Hz), 4.18 (m, 1H) 3.37 (d, *J* = 13.2 Hz, 2H), 3.04 (m, 1H), 2.44 (d, *J* = 1.2 Hz, 1H), 1.23 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100.6 MHz; $CDCl_3$): δ 139.0, 129.2, 128.5, 127.4, 83.8, 74.3, 62.7, 55.4, 54.6, 9.3.

***N*-((2*S*,3*S*)-3-hydroxypent-4-yn-2-yl)-1*H*-isoindole-1,3(*2H*)-dione and *N*-((2*S*,3*R*)-3-hydroxypent-4-yn-2-yl)-1*H*-isoindole-1,3(*2H*)-dione (9):** A solution of DMSO (0.65 mL, 9.11 mmol, 2.2 equiv.) and CH_2Cl_2 (4.0 ml) was added dropwise at -78 °C to a solution of oxalyl chloride (0.5 mL, 6.21 mmol, 1.5 equiv.) in CH_2Cl_2 (4.0 mL). The mixture was stirred for 15 min at that temperature. A solution of **6**[3] (851 mg, 4.14 mmol, 1 equiv.) in 28 mL of CH_2Cl_2 was then added dropwise to the mixture. After 30 min, Et_3N (2.3 mL, 16.6 mmol, 4 equiv.) was added and the reaction mixture was allowed to warm to room temperature for 2 h. The reaction was then quenched by addition of saturated solution of NH_4Cl and extracted several times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and

concentrated under reduced pressure. The crude aldehyde was then dissolved in 12 mL of THF at -78 °C, and 0.5 M ethynyl magnesium bromide (24.8 mL, 12.42 mmol, 3.0 equiv.) was added slowly. The mixture was warmed up to -30 °C and allowed to react for 14 hours, after which NH_4Cl (20 mL) was added. The aqueous layer was extracted using dichloromethane (3 x 20 mL portions), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified using flash column chromatography (hexane : ethyl acetate = 9 : 1) to afford **9** as a 1:1 mixture of diastereoisomers as a dark yellow oil in a 95% yield (0.9 g, 3.93 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.38 (m, 4H), 4.78 – 4.75 (m, 0.5H), 4.73 (t, *J* = 2.1 Hz, 0.5H), 4.26-4.18 (m, 0.5H), 4.07 (qd, *J* = 7.1 Hz, *J* = 2.9 Hz, 0.5H), 2.46 (d, *J* = 2.1 Hz, 0.5H), 2.42 (d, *J* = 2.1 Hz, 0.5H), 1.56 (d, *J* = 7.1 Hz, 1.5H), 1.56 (d, *J* = 7.1 Hz, 1.5H). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{13}H_{12}NO_3$: 230.0817; found: 230.0820. FT-IR (ATR) ν in cm^{-1}: 3391, 32270 (br), 3093, 3038, 2903, 2280, 1782, 1748, 1349, 1313, 1213, 1127, 1051, 837,672.

(1*S*,2*S*)-2-(dibenzylamino)-1-(1-dodecyl-1*H*-1,2,3-triazol-4-yl)propan-1-ol (12a): A solution of $CuSO_4 \cdot 5H_2O$ (18 mg, 0.07 mmol) in 0.9 mL of H_2O was added to a solution of 1-azidododecane **11a**[4] (605 mg, 2.86 mmol) and alkyne **8** (400 mg, 1.43 mmol) in 5 mL of CH_2Cl_2. Sodium ascorbate (36 mg, 0.143 mmol) in 1.3 mL of H_2O was then added dropwise and the mixture was stirred for 48 h at room temperature. The aqueous layer was extracted three times with CH_2Cl_2 and the combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography (Hexanes:AcOEt 9:1 to 7:3) to afford **12a** (387 mg, 0.788 mmol, 55%) as a pale yellow oil as the single anti diastereoisomer. $[\alpha]_D^{25}$ -21.67 (c 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 – 7.13 (m, 10H), 7.04 (s, 1H), 4.89 (d, *J* = 6.9 Hz, 1H), 4.19 (t, *J* = 7.2 Hz, 2H), 3.91 (br s, 1H), 3.62 (d, *J* = 13.7 Hz, 2H,), 3.41 (d, *J* = 13.7 Hz, 2H), 3.19 (p, *J* = 6.9 Hz, 1H), 1.81 – 1.71 (m, 2H), 1.35 – 1.19 (m, 18H), 0.90 (t, *J* = 6.9 Hz, 3H).^{13}C NMR (100.6 MHz, $CDCl_3$): δ 150.3, 139.6, 128.8, 128.0, 126.8, 120.9, 68.2, 57.3, 54.3, 50.0, 31.8, 30.1, 29.5, 29.4, 29.2, 29.2, 28.9, 26.3, 22.5, 14.0, 9.0. HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{31}H_{47}N_4O$: 491.3750; found: 491.3738. FT-IR (ATR) ν in cm^{-1}: 3329, 2923, 1602, 1494, 1454, 1044, 1028, 1216.

(1*S*,2*S*)-2-amino-1-(1-dodecyl-1*H*-1,2,3-triazol-4-yl)propan-1-ol (1a): 30% Pd/C (Pd/C (10 wt%) to substrate ratio, 114 mg) was added to a flask containing a solution of **12a** (0.380

g, 0.7743 mmol) in 2mL of CH_3OH. The flask was equipped with a hydrogen balloon and the mixture was stirred at r.t. for 24 h. The mixture was filtered through Celite, concentrated *in vacuo* and then purified by flash column chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give 1a as a white wax (0.211 g, 0.681 mmol, 88%). $[\alpha]_D^{25}$ -1.4 (c 0.34, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.89 (s, 1H), 4.67 (dd, *J* = 4.9 Hz, *J* = 0.4 Hz, 1H), 4.40 (t, *J* = 7.1 Hz, 2H), 3.20 (qd, *J*= 6.6 Hz, *J* = 4.9 Hz, 1H), 1.95 – 1.86 (m, 2H), 1.39 – 1.21 (m, 18H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.90 (t, *J* = 6.9 Hz, 3H).^{13}C NMR (100.6 MHz, CD_3OD): δ150.1, 124.0, 72.2, 52.3, 51.4, 33.1, 31.3, 30.8, 30.8, 30.7, 30.6, 30.5, 30.1, 27.5, 23.8, 17.8, 14.5. HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{17}H_{35}N_4O$: 311.2809; found: 311.2814. FT-IR (ATR) ν in cm^{-1}: 3356, 2955, 2922, 1645, 1460, 1149, 1050.

(1*S*,2*S*)-2-(dimethylamino)-1-(1-dodecyl-1*H*-1,2,3-triazol-4-yl)propan-1-ol (3a): Compound **1a** (0.040 g, 0.129 mmol) was treated with paraformaldehyde (0.039 g, 1.29 mmol) and $NaBH_3CN$ (0.090 g, 1.42 mmol) following the general procedure for *N*,*N'*-dimethylation of amines. The crude product was purified by column chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give **3a** as a yellowish wax (33 mg, 0.098 mmol, 76%). $[\alpha]_D^{25}$ +12.3 (c 1.05, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, *J* = 0.6 Hz, 1H), 5.04 (dd, *J* = 4.3 Hz, *J* = 0.6 Hz, 1H), 4.31 (t, *J* = 7.2 Hz, 2H), 2.80 (qd, *J* = 6.8 Hz, *J* = 4.3 Hz, 1H), 2.30 (s, 6H), 1.87 (p, *J* = 7.2 Hz, 2H), 1.33 – 1.19 (m, 18H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.86 (t, *J* = 6.9 Hz, 3H).^{13}C NMR (100.6 MHz, $CDCl_3$): δ 150.0, 121.2, 67.4, 63.5, 50.3, 42.7, 31.8, 30.2, 29.7, 29.6, 29.5, 29.4, 28.9, 26.4, 22.6, 14.1, 9.8. HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{19}H_{39}N_4O$: 339.3124; found: 339.3123. FT-IR (ATR) ν in cm^{-1}: 3327, 2921, 1653, 1457, 1216.

***N*,*N'*-bis(*tert*-butoxycarbonyl)-1-((1*S*,2*S*)-1-(1-dodecyl-1*H*-1,2,3-triazol-4-yl)-1-hydroxypropan-2-yl)guanidine (15a):** Compound **15a** was prepared following the general procedure for the guanidination of amines starting from amine **1a** (94 mg, 0.303 mmol, 1 equiv.). The reaction crude was purified by flash chromatography (9:1 to 6:4 hexanes/EtOAc) to give **15a** (126 mg, 0.228 mmol, 75%) as a white foam. $[\alpha]_D^{25}$ +130.1 (c 1.16, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.41 (s, 1H), 8.37 (d, *J* = 7.0 Hz, 1H), 7.51 (d, *J* = 0.6 Hz, 1H), 6.45 (br s, 1H), 5.04 (dd, *J* = 2.1 Hz, *J* = 0.6 Hz, 1H), 4.49 (pd, *J* = 7.0 Hz, *J* = 2.1 Hz, 1H),

4.30 (t, *J* = 7.3 Hz, 2H), 1.90 – 1.81 (m, 2H), 1.46 (s, 9H), 1.45 (s, 9H), 1.30 – 1.20 (m, 21H), 0.85 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 162.7, 157.0, 152.9, 148.7, 121.7, 83.7, 79.7, 71.9, 53.1, 50.4, 32.0, 30.3, 29.7, 29.6, 29.5, 29.4, 29.1, 28.3, 28.1, 26.5, 22.8, 16.0, 14.2. HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{28}H_{53}N_6O_5$: 553.4077; found: 553.4074. FT-IR (ATR) ν in cm^{-1}: 3284, 2979, 2915, 1719, 1652, 1584, 1130, 1053.

1-((1*S*,2*S*)-1-(1-dodecyl-1*H*-1,2,3-triazol-4-yl)-1-hydroxypropan-2-yl) guanidine (4a): Compound **4a** was prepared following the general procedure for the Boc deprotection of the protected guanidino derivatives **15a-d** starting from **15a** (0.086 g, 0.155 mmol). The residue was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1 to 9:3:0.3). The collected fractions were washed with NaOH 1M to give **4a** (0.054 g, 0.154 mmol, 99%) as a yellowish syrup. $[\alpha]_D^{25}$ -26.13 (c 0.3, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.93 (s, 1H), 4.91 – 4.89 (m, 1H), 4.40 (t, *J* = 7.1 Hz, 2H, H-6), 3.98 (qd, *J* = 6.7 Hz, *J* = 3.9 Hz, 1H), 1.94 – 1.85 (m, 2H), 1.37 – 1.24 (m, 18H), 1.15 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100.6 MHz, CD_3OD): δ 158.7, 149.4, 124.3, 70.4, 53.7, 51.4, 33.1, 31.3, 30.8, 30.7, 30.6, 30.5, 30.1, 27.5, 23.7, 15.7, 14.5. HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{18}H_{37}N_6O$: 353.3029; found: 353.3023. FT-IR (ATR) ν in cm^{-1}: 3328, 3161, 2923, 1669, 1653, 1457, 1202, 1179, 1135.

(1*S*,2*S*)-1-(1-dodecyl-1*H*-1,2,3-triazol-4-yl)-2-(methylamino)propan-1-ol (2a): Compound **2a** was prepared from amine **1a** (36.5 mg, 0.117 mmol), following the general procedure for the monomethylation of amines **1a-d**. The crude product was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give **2a** (23 mg, 0.071 mmol, 61%) as a white wax. $[\alpha]_D^{25}$ +10.4 (c 0.14, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ 7.87 (d, *J* = 0.5 Hz, 1H), 4.93 (dd, *J* = 4.1 Hz, *J* = 0.5 Hz, 1H), 4.39 (t, *J* = 7.1 Hz, 2H), 2.99 (qd, *J* = 6.6 Hz, *J* = 4.1 Hz, 2H), 2.45 (s, 3H), 1.95 – 1.86 (m, 2H), 1.36 – 1.24 (m, 18H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.90 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (100.6 MHz, CD_3OD): δ 150.6, 123.9, 68.9, 60.3, 51.4, 33.5, 33.1, 31.3, 30.8, 30.6, 30.6, 30.5, 30.1, 27.5, 23.7, 14.5, 13.9. HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{18}H_{37}N_4O$: 325.2967; found: 325.2962. FT-IR (ATR) ν in cm^{-1}: 3279, 2953, 2919, 1653, 1466, 1143, 1056.

(1*S*,2*S*)-2-(dibenzylamino)-1-(1-((*Z*)-12,12,12-trifluorododec-9-en-1-yl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (12b): Compound **12b** was prepared following the general

procedure for the CuAAC reaction. A solution of $CuSO_4 \cdot 5H_2O$ (14 mg, 0.056 mmol) in 0.7 mL of H_2O was added to a solution of azide **11b**[4] (0.340 g, 1.29 mmol) and the terminal alkyne **8** (0.328 g, 1.174 mmol) in 4 mL of CH_2Cl_2. Sodium ascorbate (23 mg, 0.117 mmol) in 0.8 mL of H_2O was then added dropwise and the mixture was stirred for 48 h at room temperature. After the work up, the crude product was purified by column chromatography (Hexanes:AcOEt 9:1 to 6:4) to give **12b** (0.310 g, 0.571 mmol, 49 %) as a yellowish oil. $[\alpha]_D^{25}$ –21.74 (*c* 0.15, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 – 7.16 (m, 10H), 7.10 (s, 1H), 5.76 – 5.65 (m, 1H), 5.43 – 5.34 (m, 1H), 4.87 (d, *J* = 6.7 Hz, 1H), 4.22 (t, *J* = 7.2 Hz, 2H), 3.63 (d, *J* = 13.7 Hz, 2H), 3.47 (br s, 1H, OH), 3.42 (d, *J* = 13.7 Hz, 2H), 3.20 (p, *J* = 6.7 Hz, 1H), 2.90 – 2.78 (m, 2H), 2.04 (q, *J* = 6.9 Hz, 2H), 1.81 – 1.72 (m, 2H), 1.41 – 1.18 (m, 13H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 150.3, 139.6, 136.4 (C-14), 128.8, 127.9, 126.7, 126.0 (q, *J* = 276.5 Hz), 120.8, 116.5 (q, *J* = 3.4 Hz), 68.2, 57.3, 54.2, 49.9, 31.9 (q, *J* = 29.5 Hz), 30.0, 29.0, 28.8, 28.8, 28.7, 27.1, 26.2, 8.9. HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{31}H_{42}F_3N_4O$: 543.3311; found: 543.3310. FT-IR (ATR) ν in cm^{-1}: 3381, 3027, 2926, 2854, 1602, 1453, 1347, 1251, 1131, 699, 660.

(1*S*,2*S*)-2-(dibenzylamino)-1-(1-((*Z*)-11,11,12,12,12-pentafluorododec-8-en-1-yl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (12c): Compound **12c** was prepared following the general procedure for the CuAAC reaction. A solution of $CuSO_4 \cdot 5H_2O$ (14 mg, 0.056 mmol) in 0.7 mL of H_2O was added to a mixture of the azide **11c**[4] (0.345 g, 1.15 mmol) and the terminal alkyne **8** (0.307 g, 1.1 mmol) in 3.5 mL of CH_2Cl_2. Sodium ascorbate (22 mg, 0.11 01mmol) in 0.8 mL of H_2O was then added dropwise and the mixture was stirred for 48 h at room temperature. After the work up, the crude product was purified by column chromatography (Hexanes:AcOEt 9:1 to 6:4) to give **12c** (0.425 g, 0.74 mmol, 69%) as a yellowish oil. $[\alpha]_D^{25}$ – 21.7 (c 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 – 7.17 (m, 10H), 7.10 (d, *J* = 0.5 Hz, 1H), 5.77 – 5.67 (m, 1H), 5.44 – 5.34 (m, 1H), 4.90 – 4.83 (m, 1H), 4.21 (t, *J* = 7.0 Hz, 2H), 3.62 (d, *J* = 13.7 Hz, 2H), 3.41 (d, *J* = 13.7 Hz, 3H), 3.20 (p, *J* = 6.8 Hz, 1H), 2.80 (td, *J* = 17.8 Hz, *J* = 7.4 Hz, 2H), 2.06 – 1.98 (m, 2H), 1.81 – 1.71 (m, 2H), 1.38 – 1.17 (m, 11H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 150.5, 139.8, 136.9, 129.0, 128.3, 127.0, 121.1, 115.7 (t, $J_{C,F}$ = 4.3 Hz), 68.5, 57.6, 54.7, 50.2, 30.3, 29.3 (t, $J_{C,F}$ = 22.3 Hz), 29.1, 29.0, 28.9, 27.3, 26.4, 9.2. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -86.35 (s, F-17), -118.47 (t, $J_{F,H}$ = 17.8 Hz, F-

16). HRMS (ESI-TOF): [M+H]+ m/z calcd for $C_{31}H_{40}F_5N_4O$: 579.3122; found: 579.3116. FT-IR (ATR) ν in cm^{-1}: 3309, 3028, 2930, 1646, 1455, 1192, 699, 649.

(1*S*,2*S*)-2-(*N*,*N*'-dibenzylamino)-1-(1-((*Z*)-10,10,11,11,12,12,12-heptafluorododec-7-en-1-yl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (12d) and (1*R*,2*S*)-2-(*N*,*N*'-dibenzylamino)-1-(1-((Z)-10,10,11,11,12,12,12-heptafluorododec-7-en-1-yl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (diast-12d): Compound **12d** was prepared following the general procedure for the CuAAC reaction. A solution of $CuSO_4 \cdot 5H_2O$ (25 mg, 0.098 mmol) in 1.2 mL of H_2O was added to a mixture of the azide **11d**[4] (0.726 g, 2.22 mmol) and the terminal alkyne **8** (0.550 g, 1.97 mmol) in 6.5 mL of CH_2Cl_2. Sodium ascorbate (39 mg, 0.197 mmol) in 1.4 mL of H_2O was then added dropwise and the mixture was stirred for 48 h at room temperature. After the work up, the crude residue was purified by column chromatography (Hexanes:AcOEt 9:1 to 6:4) to give:

12d (major): (0.811 g, 1.32 mmol, 67%) as a yellowish oil. $[\alpha]_D^{25}$ –15.52 (*c* 1.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 – 7.15 (m, 10H), 7.07 (s, 1H), 5.72 (m, 1H), 5.42 (m, 1H), 4.88 (d, *J* = 6.8 Hz, 1H), 4.20 (t, *J* = 7.1 Hz, 2H), 3.78 (br s, 1H), 3.62 (d, *J* = 13.6 Hz, 2H), 3.40 (d, *J* = 13.6 Hz, 2H), 3.20 (p, *J* = 6.8 Hz, 1H), 2.84 (td, *J* = 18.5 Hz, *J* = 7.2 Hz, 2H), 2.02 (q, *J* = 6.5 Hz, 2H), 1.82 – 1.69 (m, 2H), 1.39 – 1.17 (m, 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 150.4, 139.6, 136.7, 128.9, 128.1, 126.9, 120.9, 115.5 (t, *J* = 4.2 Hz), 68.3, 57.4, 54.5, 50.0, 30.0, 29.0 (t, *J* = 22.5 Hz), 28.7, 28.4, 27.1, 26.2, 9.0. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -80.61 (t, *J* = 9.7 Hz,), -114.05 – -114.26 (m), -127.37 – -127.52 (m). HRMS (ESI-TOF): [M+H]+ m/z calcd for $C_{31}H_{38}F_7N_4O$: 615.2934; found: 615.2928. FT-IR (ATR) ν in cm-1: 3327, 3031, 2931, 1494, 1453, 1218, 1171, 1112, 742, 700.

Diast-12d (minor) $[\alpha]_D^{25}$ +62.0 (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 – 7.23 (m, 11H), 5.78 – 5.68 (m, 1H), 5.48 – 5.35 (m, 1H), 4.89 (br s, 1H), 4.73 (d, *J* = 9.9 Hz, 1H), 4.33 – 4.17 (m, 2H, H-6), 3.92 (d, *J* = 13.3 Hz, 2H), 3.40 (d, *J* = 13.3 Hz, 2H), 2.91 – 2.76 (m, 3H), 2.08 – 1.97 (m, 2H), 1.89 – 1.78 (m, 2H), 1.41 – 1.20 (m, 6H), 1.06 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 149.4, 138.6, 136.8, 129.0, 128.5, 127.3, 121.0, 115.5 (t, *J* = 4.3 Hz), 67.3, 59.7, 53.2, 50.1, 30.0, 29.0 (t, *J* = 22.7 Hz), 28.8, 28.4, 27.1, 26.2, 7.9. HRMS (ESI-TOF): $[M+H]^+$ *m/z* calcd for $C_{31}H_{38}F_7N_4O$: 615.2934; found: 615.2931. FT-IR (ATR) ν in cm^{-1}: 3340, 3032, 2930, 1653, 1454, 1219, 1170, 1112, 700, 655.

(1*S*,2*S*)-2-amino-1-(1-(12,12,12-trifluorododecyl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (1b): Compound **1b** was prepared following the general procedure for the reduction of *N*,*N'*-dibenzyl amines, starting from **12b** (0.245 g, 0.451 mmol). The reaction crude was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give **1b** as a yellowish waxy solid (0.122 g, 0.334 mmol, 74%). $[\alpha]_D^{25}$ –6.14 (c 1.2, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ 7.90 (s, 1H), 4.69 (br s, 1H, H-3), 4.40 (t, *J* = 7.1 Hz, 2H), 3.21 (m, 1H), 2.20 – 2.05 (m, 2H), 1.95 – 1.86 (m, 2H), 1.58 – 1.49 (m, 2H), 1.43 – 1.24 (m, 14H), 1.04 (d, *J* = 6.2 Hz, 3H).^{13}C NMR (100.6 MHz, CD_3OD): δ 148.7, 127.5 (q, *J* = 275.4 Hz), 122.6, 70.8, 50.8, 49.9, 33.0 (q, *J* = 28.2 Hz), 29.9, 29.1, 28.9, 28.7, 28.3, 26.1, 21.6 (q, *J* = 2.9 Hz), 16.4. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -69.55 (t, *J* = 10.5 Hz). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{17}H_{32}F_3N_4O$: 365.2528; found: 365.2525. FT-IR (ATR) ν in cm^{-1}: 3312, 3141, 2925, 2854, 1559, 1457, 1254, 1139, 1051.

(1*S*,2*S*)-2-amino-1-(1-(11,11,12,12,12-pentafluorododecyl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (1c): Compound **1c** was prepared following the general procedure for the reduction of *N*,*N'*-dibenzyl amines, starting from **12c** (0.401 g, 0.693 mmol). The reaction crude was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give **1c** as a colourless waxy solid (0.250 g, 0.628 mmol, 90%). $[\alpha]_D^{25}$ +0.899 (c 0.77, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.89 (s, 1H), 4.68 (d, $J_{3,2}$ = 4.9 Hz, 1H), 4.40 (t, $J_{6,7}$ = 7.1 Hz, 2H), 3.24 – 3.16 (m, 1H), 2.18 – 2.01 (m, 2H), 1.97 – 1.85 (m, 2H), 1.62 – 1.52 (m, 2H), 1.45 – 1.25 (m, 12H), 1.03 (d, *J* = 6.6 Hz, 3H).^{13}C NMR (100.6 MHz, CD_3OD): δ 150.1, 124.0, 72.2, 52.3, 51.3, 31.4 (t, *J* = 21.9 Hz), 31.3, 30.5, 30.4, 30.3, 30.1, 30.1, 27.5, 21.4 (t, *J* = 3.4 Hz), 17.8. ^{19}F NMR (376.5 MHz, CD_3OD): δ -87.04 (s, F-17), -119.51 (t, $J_{F,H}$ = 18.9 Hz, F-16). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{17}H_{30}F_5N_4O$: 401.2340; found: 401.2346. FT-IR (ATR) ν in cm^{-1}: 3351, 3292, 2926, 1653, 1472, 1190.

(1*S*,2*S*)-2-amino-1-(1-(10,10,11,11,12,12,12-heptafluorododecyl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (1d): Compound **1d** was prepared following the general procedure for the reduction of *N*,*N'*-dibenzyl amines, starting from **12d** (0.698 g, 1.162 mmol). The reaction crude was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give 1d as a colourless waxy solid (0.411 g, 0.941 mmol, 81%). $[\alpha]_D^{25}$ 0 (c 0.25, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.89 (s, 1H), 4.67 (d, *J* = 4.9 Hz, 1H), 4.40 (t, *J* = 7.1 Hz, 2H),

3.19 (qd, *J* = 6.6 Hz, *J* = 4.9 Hz, 1H), 2.20 – 2.05 (m, 2H), 1.96 – 1.87 (m, 2H), 1.63 – 1.54 (m, 2H), 1.45 – 1.26 (m, 10H), 1.03 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100.6 MHz, CD_3OD): δ 150.1, 124.0, 72.3, 52.3, 51.3, 31.4 (t, *J* = 22.0 Hz), 31.3, 30.3, 30.2, 30.1, 30.0, 27.5, 21.2 (t, *J* = 3.4 Hz), 17.9. ^{19}F NMR (376.5 MHz, CD_3OD): δ -82.28 (t, *J* = 9.9 Hz), -116.38 – -116.61 (m), -129.13 – -129.17 (m). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{17}H_{28}F_7N_4O$: 437.2151; found: 437.2146. FT-IR (ATR) ν in cm^{-1}: 3344, 3284, 2938, 2912, 1607, 1468, 1218, 1117.

(1*S*,2*S*)-2-(dimethylamino)-1-(1-(12,12,12-trifluorododecyl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (3b): Compound **1b** (0.020 g, 0.056 mmol) was treated with paraformaldehyde (0.017 g, 0.560 mmol) and $NaBH_3CN$ (0.039 g, 0.616 mmol) following the general procedure for *N*,*N*-dimethylation of amines. The reaction crude was purified by flash column chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give **3b** as a colourless waxy solid (19.2 mg, 0.049 mmol, 87%). $[\alpha]_D^{25}$ +5.11 (c 0.62, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (s, 1H), 5.12 (d, *J* = 4.0 Hz, 1H), 4.32 (t, *J* = 7.2 Hz, 2H), 3.31 (br s, 1H), 2.91 (qd, *J* = 6.8 Hz, *J* = 4.0 Hz, 1H), 2.37 (s, 6H), 2.11 – 1.97 (m, 2H), 1.93 – 1.83 (m, 2H), 1.58 – 1.48 (m, 2H), 1.39 – 1.16 (m, 14H), 0.96 (d, *J* = 6.8 Hz, 3H).^{13}C NMR (100.6 MHz, $CDCl_3$): δ 149.9, 127.4 (q, *J* = 276.3 Hz), 121.5, 67.4, 64.0, 50.4, 42.8, 33.8 (q, *J* = 28.2 Hz), 30.4, 29.5, 29.4, 29.4, 29.3, 29.1, 28.8, 26.6, 21.95 (q, *J* = 2.8 Hz), 9.8. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -66.43 (t, *J* = 10.9 Hz). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{19}H_{36}F_3N_4O$: 393.2841; found: 393.2843. FT-IR (ATR) ν in cm^{-1}: 3327, 2925, 2855, 1653, 1457, 1254, 1216, 1135, 1042.

(1*S*,2*S*)-2-(dimethylamino)-1-(1-(11,11,12,12,12-pentafluorodode cyl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (3c): Compound **1c** (0.037 g, 0.092 mmol) was treated with paraformaldehyde (0.028 g, 0.92 mmol) and $NaBH_3CN$ (0.0636 g, 1.012 mmol) following the general procedure for *N*,*N*-dimethylation of amines. The reaction crude was purified by flash column chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give **3c** as a colourless waxy solid (27.1 mg, 0.063 mmol, 69%). $[\alpha]_D^{25}$ –2.92 (c 1.11, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, *J* = 0.6 Hz, 1H), 5.06 (dd, *J* = 4.2 Hz, *J* = 0.6 Hz, 1H), 4.32 (t, *J* = 7.2 Hz, 2H), 3.07 (br s, 1H), 2.83 (qd, *J* = 6.8 Hz, *J* = 4.2 Hz, 1H), 2.33 (s, 6H), 2.08 – 1.83 (m, 4H), 1.61 – 1.51 (m, 2H), 1.40 – 1.21 (m, 12H), 0.94 (d, *J* = 6.8 Hz, 3H).^{13}C

NMR (100.6 MHz, $CDCl_3$): δ 150.1, 121.4, 67.5, 63.7, 50.4, 42.9, 30.7 (t, *J* = 22.0 Hz), 30.4, 29.4, 29.3, 29.3, 29.2, 29.1, 26.6, 20.3 (t, *J* = 3.3 Hz), 10.0. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -85.47 (s), -118.31 (t, *J* = 18.2 Hz). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{19}H_{34}F_5N_4O$: 429.2653; found: 429.2660. FT-IR (ATR) ν in cm^{-1}: 3333, 2926, 1653, 1458, 1190, 1043.

(1*S*,2*S*)-2-(dimethylamino)-1-(1-(10,10,11,11,12,12,12-heptafluorododecyl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (3d): Compound **1d** (0.037 g, 0.086 mmol) was treated with paraformaldehyde (0.026 g, 0.86 mmol) and $NaBH_3CN$ (0.059 g, 0.943 mmol) following the general procedure for *N*,*N*-dimethylation of amines. The reaction crude was purified by flash column chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give **3d** as a colourless waxy solid (29.4 mg, 0.063 mmol, 74%). $[\alpha]_D^{25}$ +8.01 (c 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (s, 1H), 5.06 (d, *J* = 4.2 Hz, 1H), 4.33 (t, *J* = 7.2 Hz, 2H), 2.83 (qd, *J* = 6.8 Hz, *J* = 4.2 Hz, 1H), 2.33 (s, 6H), 2.11 – 1.95 (m, 2H), 1.94 – 1.84 (m, 2H), 1.62 – 1.52 (m, 2H), 1.40 – 1.20 (m, 10H), 0.94 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 150.1, 121.4, 67.5, 63.7, 50.4, 42.9, 30.7 (t, *J* = 22.4 Hz), 30.4, 29.3, 29.2, 29.1, 29.0, 26.5, 20.2 (t, *J* = 3.6 Hz), 10.0. HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{19}H_{32}F_7N_4O$: 465.2464; found: 465.2452. FT-IR (ATR) ν in cm^{-1}: 3311, 2926, 1653, 1457, 1219, 1169, 1117.

***N*,*N'*-bis(*tert*-butoxycarbonyl)-1-((1*S*,2*S*)-1-(1-(12,12,12-trifluorododecyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxypropan-2-yl)guanidine (15b):** Compound **15b** was obtained following the general procedure for the guanidination of amines starting from **1b** (30 mg, 0.083 mmol) was treated with *N*-*N*'-di-Boc-1*H*-pyrazole-1-carboxiamidine (**14**) (29 mg, 0.091 mmol) following the general procedure for guanidination of amines. The reaction crude was purified by flash chromatography (silica gel, hexane:ethyl acetate 8:2→1:1) to give **15b** (45 mg, 0.075 mmol, 90%) as a white solid. $[\alpha]_D^{25}$ –43.6 (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.42 (s, 1H), 8.38 (d, *J* = 6.8 Hz, 1H), 7.52 (s, 1H), 6.52 (br s, 1H), 5.06 (d, *J* = 1.9 Hz, 1H), 4.50 (pd, *J* = 6.8 Hz, *J* = 1.9 Hz, 1H), 4.31 (t, *J* = 7.2 Hz, 2H), 2.11 – 1.97 (m, 2H), 1.91 – 1.82 (m, 2H), 1.58 – 1.45 (m, 20H), 1.36 – 1.21 (m, 17H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 162.7, 157.1, 152.9, 148.8, 127.4 (q, *J* = 276.2 Hz), 121.8, 83.7, 79.8, 72.0, 53.2, 50.4, 33.8 (q, *J* = 28.3 Hz), 30.4, 29.5, 29.4, 29.4, 29.3, 29.1, 28.8, 28.3, 28.1, 26.6, 22.0 (q, *J* = 2.9 Hz), 16.0. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -66.71 (t, *J* = 10.5 Hz). HRMS (ESI-

TOF): $[M+H]^+$ *m*/*z* calcd for $C_{28}H_{50}F_3N_6O_5$: 607.3795; found: 607.3794. FT-IR (ATR) ν in cm^{-1}: 3283, 2986, 2934, 1719, 1651, 1469, 1251, 1129.

***N*,*N'*-bis(*tert*-butoxycarbonyl)-1-((1*S*,2*S*)-1-(1-(12,12,12,11,11-pentafluorododecyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxypropan-2-yl)guanidine (15c):** Compound **1c** (0.056 g, 0.139 mmol) was treated with *N*-*N*'-di-Boc-1*H*-pyrazole-1-carboxiamidine (**14**) (0.049 g, 0.153 mmol) following the general procedure for guanidination of amines. The reaction crude was purified by flash chromatography (silica gel, hexane:ethyl acetate 8:2→1:1) to give **15c** (0.074 g, 0.115 mmol, 83%) as a white foam. $[\alpha]_D^{25}$ –48.8 (c 1.03, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 11.41 (s, 1H, NH), 8.36 (d, *J* = 6.8 Hz, 1H), 7.51 (s, 1H), 6.49 (br s, 1H), 5.04 (d, *J* = 1.9 Hz, 1H), 4.49 (pd, *J* = 6.8 Hz, *J* = 1.9 Hz, 1H), 4.30 (t, *J* = 7.2 Hz, 2H), 2.05 – 1.79 (m, 4H), 1.60 – 1.49 (m, 2H), 1.45 (s, 18H), 1.38 – 1.19 (m, 15H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 162.6, 157.0, 152.9, 148.8, 121.7, 83.7, 79.7, 71.9, 53.1, 50.4, 30.7 (t, *J* = 22.1 Hz), 30.3, 29.3, 29.3, 29.2, 29.1, 29.0, 28.3, 28.1, 26.5, 20.3 (t, *J* = 3.4 Hz), 15.9. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -81.88 (s), -114.73 (t, *J* =18.4 Hz). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{28}H_{48}F_5N_6O_5$: 643.3606; found: 643.3601. FT-IR (ATR) ν in cm^{-1}: 3283, 2988, 2924, 1719, 1652, 1611, 1585, 1471, 1194, 1166, 1050**.**

***N*,*N'*-bis(*tert*-butoxycarbonyl)-1-((1S,2S)-1-(1-(12,12,12,11,11,10,10-heptafluorododecyl)-1H-1,2,3-triazol-4-yl)-1-hydroxypropan-2-yl)guanidine (15d):** Compound **1d** (0.036 g, 0.082 mmol) was treated with *N*-*N*'-di-Boc-1*H*-pyrazole-1-carboxiamidine (**14**) (0.029 g, 0.902 mmol) following the general procedure for guanidination of amines. The reaction crude was purified by flash chromatography (silica gel, hexane:ethyl acetate 8:2→1:1) to give 15d (0.051 g, 0.076 mmol, 92%) as a white foam. $[\alpha]_D^{25}$ –33.3 (c 0.73, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 11.42 (s, 1H), 8.38 (d, *J* = 6.7 Hz, 1H), 7.52 (s, 1H), 6.51 (br s, 1H), 5.06 (d, *J* = 1.9 Hz, 1H), 4.50 (pd, *J* = 6.7 Hz, *J* = 1.9 Hz, 1H), 4.32 (t, *J* = 7.3 Hz, 2H), 2.10 – 1.94 (m, 2H), 1.92 – 1.83 (m, 2H), 1.64 – 1.43 (m, 20H), 1.39 – 1.22 (m, 13H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 162.7, 157.1, 152.9, 148.9, 121.8, 83.7, 79.8, 72.0, 53.2, 50.4, 30.7 (t, *J* = 22.3 Hz), 30.3, 29.2, 29.2, 29.1, 29.0, 28.3, 28.1, 26.5, 20.1 (t, *J* = 3.6 Hz), 16.0. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -77.36 (t, *J* = 9.6 Hz), -112.16 (m), -124.62 (br s). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{28}H_{46}F_7N_6O_5$:

679.3418; found: 679.3408. FT-IR (ATR) ν in cm^{-1}: 3283, 2978, 2921, 1718, 1652, 1457, 1129, 1119.

1-((1*S*,2*S*)-1-(1-(12,12,12-trifluorododecyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxypropan-2-yl) guanidinium trifluoroacetate salt (4b): Compound **4b** was prepared following the general procedure for the Boc deprotection of the protected guanidino derivatives starting from **15b** (35 mg, 0.058 mmol). The reaction crude was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1 to $CHCl_3$:MeOH:NH_4OH 7:3:0.3) to give **4b** (27 mg, 0.042 mmol, 72%) as a white wax. $[\alpha]_D^{25}$ –6.7 (c 0.4, CH_3OH). ^1H NMR (400 MHz, CD_3OD): δ 7.93 (s, 1H), 4.91 – 4.89 (m, 1H), 4.41 (t, *J* = 7.1 Hz, 2H), 3.98 (qd, *J* = 6.7 Hz, *J* = 3.9 Hz, 1H), 2.19 – 2.05 (m, 2H), 1.95 – 1.86 (m, 2H), 1.58 – 1.49 (m, 2H,), 1.42 – 1.24 (m, 14H), 1.14 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100.6 MHz, CD_3OD): δ 158.6, 149.3, 128.91 (q, *J* = 275.3 Hz), 124.2, 70.3, 53.7, 51.4, 34.38 (q, *J* = 28.3 Hz), 31.3, 30.5, 30.5, 30.3, 30.1, 29.7, 27.4, 23.02 (q, *J* = 2.9 Hz), 15.7. ^{19}F NMR (376.5 MHz, CD_3OD): δ -68.04 (t, *J* = 10.9 Hz), -76.97 (s). HRMS (ESI-TOF): $[M]^+$ *m*/*z* calcd for $C_{18}H_{34}F_3N_6O^+$: 407.2741; found: 407.2748. FT-IR (ATR) ν in cm^{-1}: 3347, 3178, 2928, 1669, 1463, 1203, 1137, 1054.

1-((1*S*,2*S*)-1-(1-(11,11,12,12,12-pentafluorododecyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxypropan-2-yl) guanidinium trifluoroacetate salt (4c): Compound **4c** was prepared following the general procedure for the Boc deprotection of the protected guanidino derivatives starting from **15c** (63 mg, 0.098 mmol). The reaction crude was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1 to $CHCl_3$:MeOH:NH_4OH 7:3:0.3) to give **4c** (44 mg, 0.066 mmol, 67%) as a colourless syrup. $[\alpha]_D^{25}$ –17.54 (c 0.23, CH_3OH). ^1H NMR (400 MHz, CD_3OD): δ 7.98 (d, *J* = 0.4 Hz, 1H), 4.93 (dd, *J* = 3.9 Hz, *J* = 0.4 Hz, 1H), 4.41 (t, *J* = 7.1 Hz, 2H), 3.98 (qd, *J* = 6.7 Hz, *J* = 3.9 Hz, 1H), 2.17 – 2.01 (m, 2H), 1.95 – 1.86 (m, 2H), 1.62 – 1.52 (m, 2H), 1.44 – 1.25 (m, 12H), 1.13 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100.6 MHz, CD_3OD): δ 158.6, 149.3, 124.4, 70.4, 53.7, 51.4, 31.4 (t, *J* = 21.9 Hz), 31.4, 30.5, 30.4, 30.3, 30.1, 30.1, 27.5, 21.4 (t, *J* = 3.5 Hz), 15.6. ^{19}F NMR (376.5 MHz, CD_3OD): δ -77.01 (s), -87.04 (s), -119.52 (t, *J* = 18.9 Hz). HRMS (ESI-TOF): $[M]^+$ *m*/*z* calcd for $C_{18}H_{32}F_5N_6O^+$: 443.2558; found: 443.2552. FT-IR (ATR) ν in cm^{-1}: 3336, 2944, 2851, 1653, 1457, 1196, 1014.

1-((1*S*,2*S*)-1-(1-(10,10,11,11,12,12,12-heptafluorododecyl)-1*H*-1,2,3-triazol-4-yl)-1-hydroxypropan-2-yl) guanidinium trifluoroacetate salt (4d): Compound **4d** was prepared following the general procedure for the Boc deprotection of the protected guanidino derivatives starting from **15d** (35 mg, 0.052 mmol). The reaction crude was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1 to $CHCl_3$:MeOH:NH_4OH 7:3:0.3) to give **4d** (24 mg, 0.033 mmol, 65%) as a white waxy solid. $[\alpha]_D^{25}$ – 7.2 (c 0.66, CH_3OH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.93 (d, *J* = 0.5 Hz, 1H), 4.90 (dd, *J* = 3.9 Hz, *J* = 0.5 Hz, 1H), 4.41 (t, *J* = 7.0 Hz, 2H), 3.98 (qd, *J* = 6.8 Hz, *J* = 3.9 Hz, 1H), 2.20 – 2.04 (m, 2H), 1.96 – 1.86 (m, 2H), 1.64 – 1.53 (m, 2H), 1.46 – 1.25 (m, 10H), 1.14 (d, *J* = 6.8 Hz, 3H). ^{19}F NMR (376.5 MHz, CD_3OD): δ -76.98 (s), -82.27 (t, $J_{17,16}$ = 10.0 Hz, 3F, F-17), -116.39 – -116.61 (m, 2F, F-16), -129.12 – -129.18 (m, 2F, F-15). HRMS (ESI-TOF): $[M]^+$ *m*/*z* calcd for $C_{18}H_{30}F_7N_6O^+$: 479.2364; found: 479.2378. FT-IR (ATR) ν in cm^{-1}: 3447, 3354, 2929, 1647, 1636, 1472, 1218.

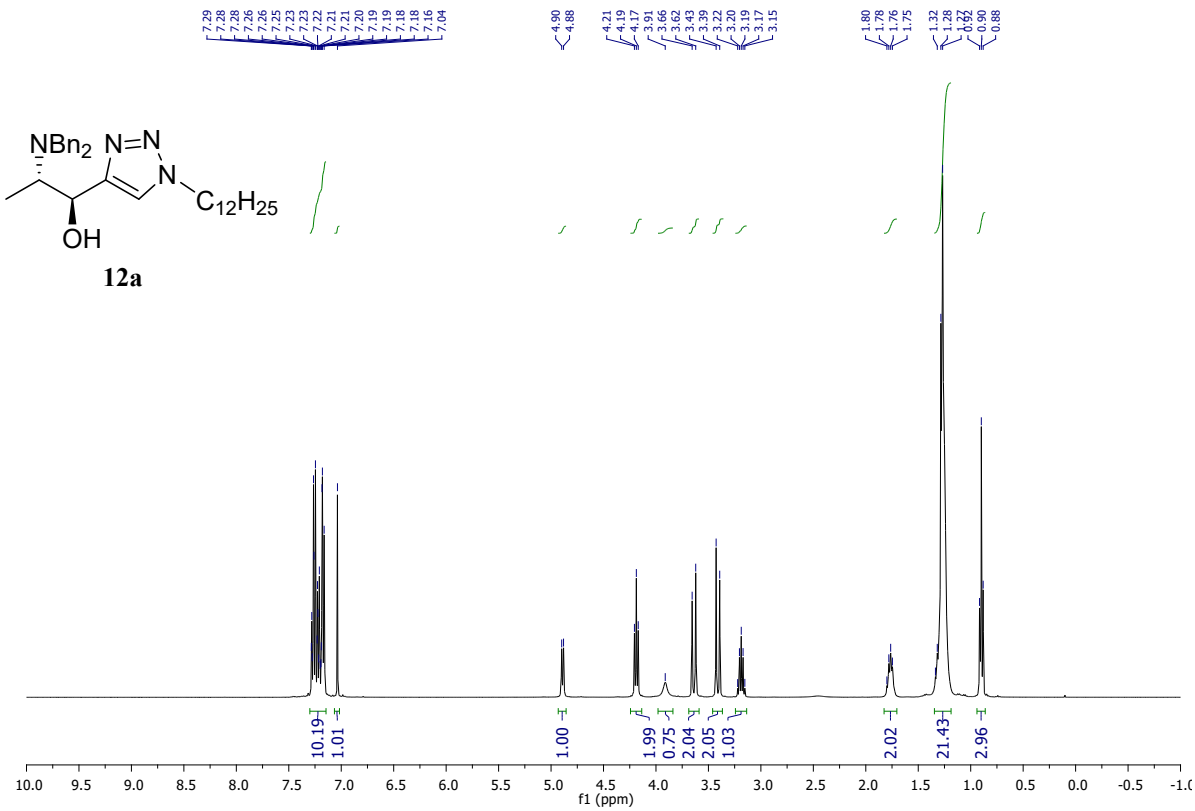
(1*S*,2*S*)-2-(methylamino)-1-(1-(12,12,12-trifluorododecyl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (2b): Compound **2b** was prepared from amine **1b** (40 mg, 0.111 mmol), following the general procedure for the monomethylation of amines. The crude product was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give **2b** (28.3 mg, 0.075 mmol, 68 %) as a colourless waxy solid. $[\alpha]_D^{25}$ –1.89 (c 0.74, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ 7.87 (d, *J* = 0.5 Hz, 1H), 4.92 (dd, *J* = 4.2 Hz, *J* = 0.5 Hz, 1H), 4.39 (t, *J* = 7.1 Hz, 2H), 2.96 (qd, *J* = 6.6 Hz, *J* = 4.2 Hz, 1H), 2.44 (s, 3H), 2.20 – 2.05 (m, 2H), 1.90 (p, *J* = 7.1 Hz, 2H), 1.58 – 1.49 (m, 2H), 1.42 – 1.27 (m, 14H), 1.00 (d, *J* = 6.6 Hz, 3H).^{13}C NMR (100.6 MHz, CD_3OD): δ 150.7, 128.9 (q, *J* = 275.4 Hz), 123.9, 69.0, 60.3, 51.3, 34.4 (q, *J* = 28.2 Hz), 33.6, 31.3, 30.5, 30.5, 30.5, 30.3, 30.1, 29.8, 27.5, 23.0 (q, *J* = 3.0 Hz). ^{19}F NMR (376.5 MHz, CD_3OD) δ -68.02 (t, *J* = 11.3 Hz). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{18}H_{34}F_3N_4O$: 379.2685; found: 379.2691. FT-IR (ATR) ν in cm^{-1}: 3326, 2925, 1653, 1457, 1254, 1138.

(1*S*,2*S*)-2-(methylamino)-1-(1-(11,11,12,12,12-pentafluorodode cyl)-1*H*-1,2,3-triazol-4-yl)propan-1-ol (2c): Compound **2c** was prepared from amine **1c** (44 mg, 0.110 mmol), following the general procedure for the monomethylation of amines. The crude product was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH

9:1:0.1) to give **2c** (38 mg, 0.091 mmol, 83 %) as a colourless waxy solid. $[\alpha]_D^{25}$ –0.6 (c 0.31, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ 7.87 (s, 1H), 4.92 – 4.90 (m, 2H), 4.39 (t, *J* = 7.1 Hz, 2H), 2.94 (qd, *J* = 6.6 Hz, *J* = 4.2 Hz, 1H), 2.42 (s, 3H), 2.17 – 2.01 (m, 2H), 1.95 – 1.86 (m, 2H), 1.62 – 1.52 (m, 2H), 1.44 – 1.26 (m, 12H), 1.00 (d, *J* = 6.6 Hz, 3H).^{13}C NMR (100.6 MHz, CD_3OD): δ 150.8, 123.9, 69.2, 60.3, 51.3, 33.7, 31.4 (t, *J* = 21.9 Hz), 31.3, 30.5, 30.4, 30.3, 30.1, 30.0, 27.5, 21.4 (t, *J* = 3.3 Hz), 14.2. ^{19}F NMR (376.5 MHz, CD_3OD): δ -87.04 (s), -119.49 (t, *J* = 18.9 Hz). HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{18}H_{32}F_5N_4O$: 415.2496; found: 415.2501. FT-IR (ATR) ν in cm^{-1}: 3309, 2924, 2855, 1558, 1457, 1191.

(1*S*,2*S*)-1-(1-(10,10,11,11,12,12,12-heptafluorododecyl)-1*H*-1,2,3-triazol-4-yl)-2-(methylamino)propan-1-ol (2d): Compound **2d** was prepared from amine **1d** (46 mg, 0.105 mmol, 1 equiv.), following the general procedure for the monomethylation of amines. The crude product was purified by flash chromatography (silica gel, $CHCl_3$:MeOH:NH_4OH 9:1:0.1) to give **2d** (28.0 mg, 0.062 mmol, 59%) as a colourless waxy solid. $[\alpha]_D^{25}$ –0.26 (c 0.9, $CHCl_3$). ^{1}H NMR (400 MHz, CD_3OD): δ 7.87 (s, 1H), 4.92 – 4.90 (m, 1H), 4.40 (t, *J* = 7.1 Hz, 2H), 2.95 (qd, *J* = 6.6 Hz, *J* = 4.2 Hz, 1H), 2.43 (s, 3H), 2.21 – 2.05 (m, 2H), 1.96 – 1.87 (m, 2H), 1.64 – 1.54 (m, 2H), 1.45 – 1.26 (m, 10H), 1.00 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (100.6 MHz, CD_3OD): δ 150.8, 123.9, 69.1, 60.3, 51.3, 33.6, 31.4 (t, *J* = 22.0 Hz), 31.3, 30.3, 30.2, 30.1, 30.0, 27.4, 21.2 (t, *J* = 3.7 Hz), 14.1. HRMS (ESI-TOF): $[M+H]^+$ *m*/*z* calcd for $C_{18}H_{30}F_7N_4O$: 451.2308; found: 451.2312. FT-IR (ATR) ν in cm^{-1}:3297, 3147, 2927, 2919, 1653, 1466, 1224, 1132, 1050.

4. ^{1}H, ^{13}C and ^{19}F spectra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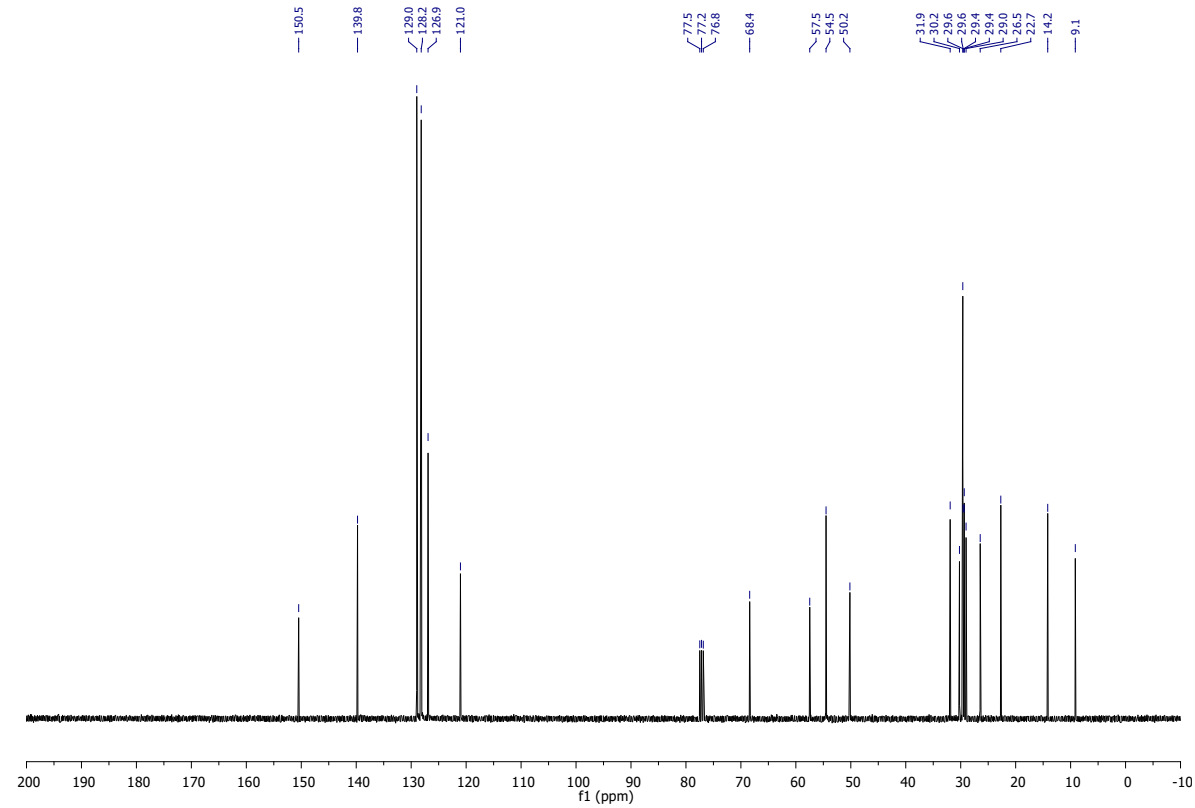
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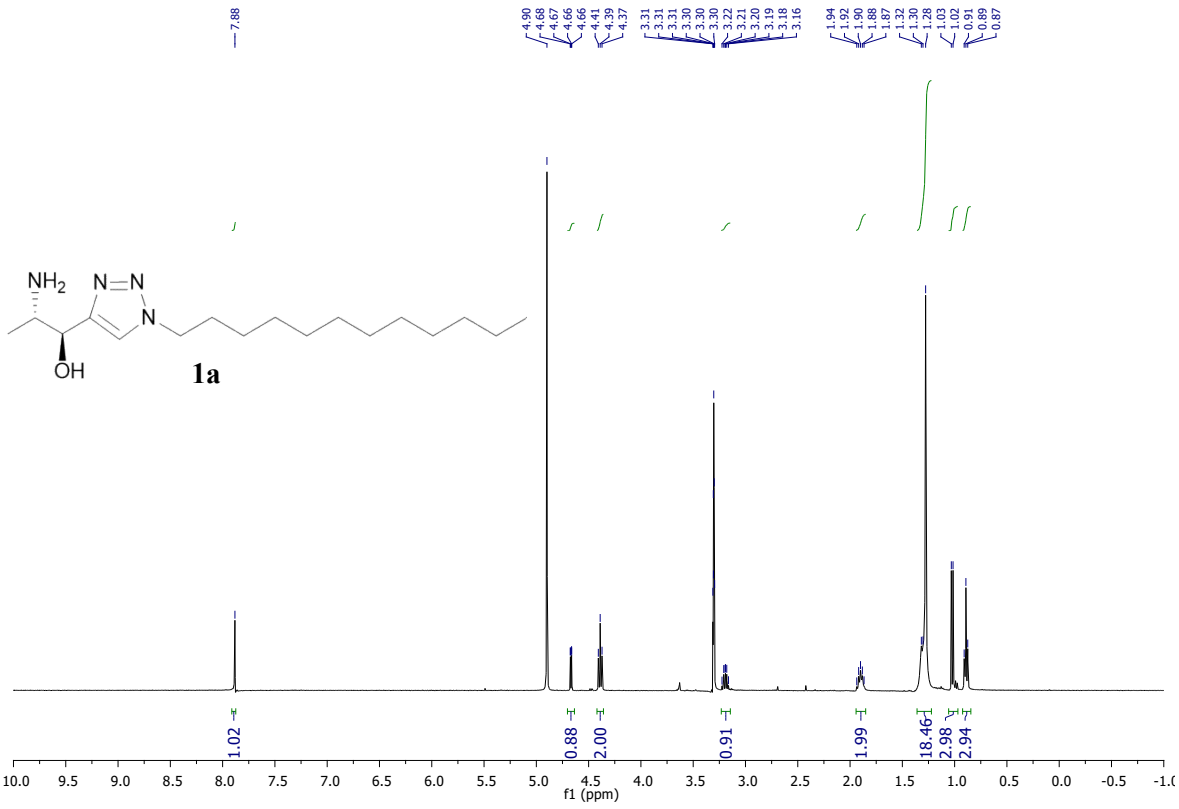
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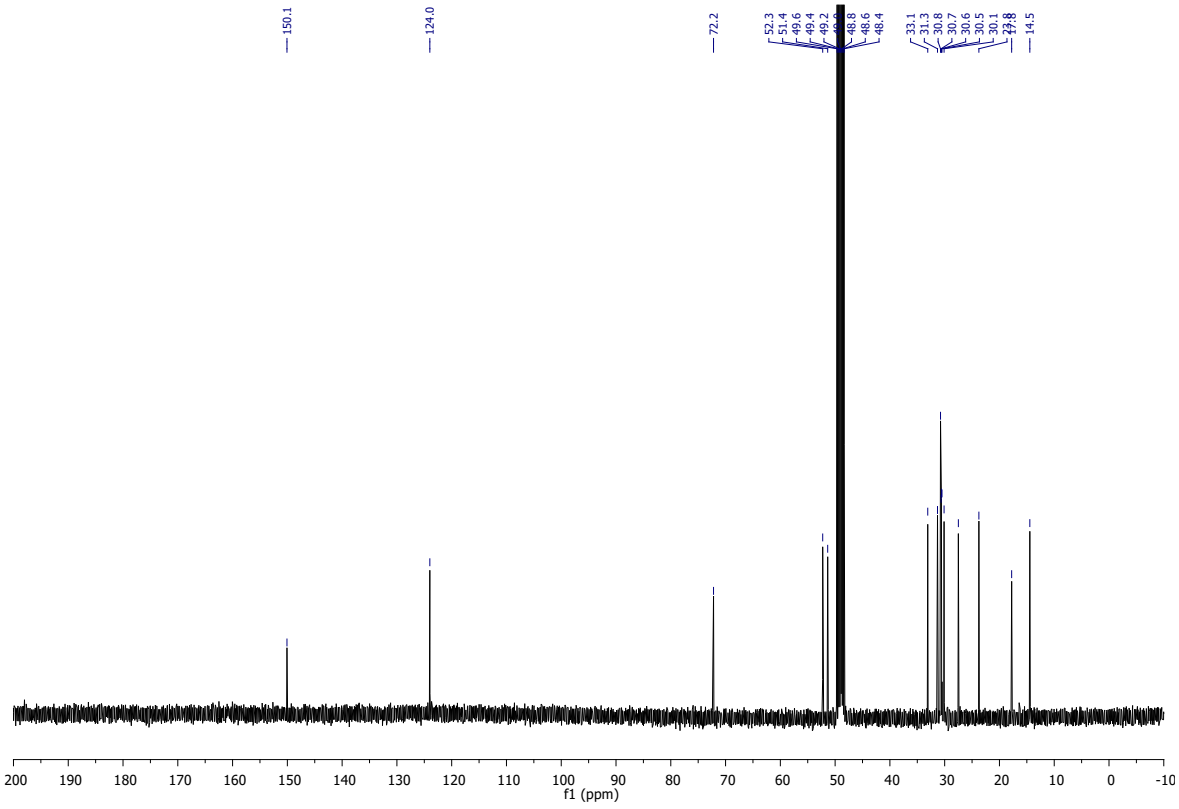
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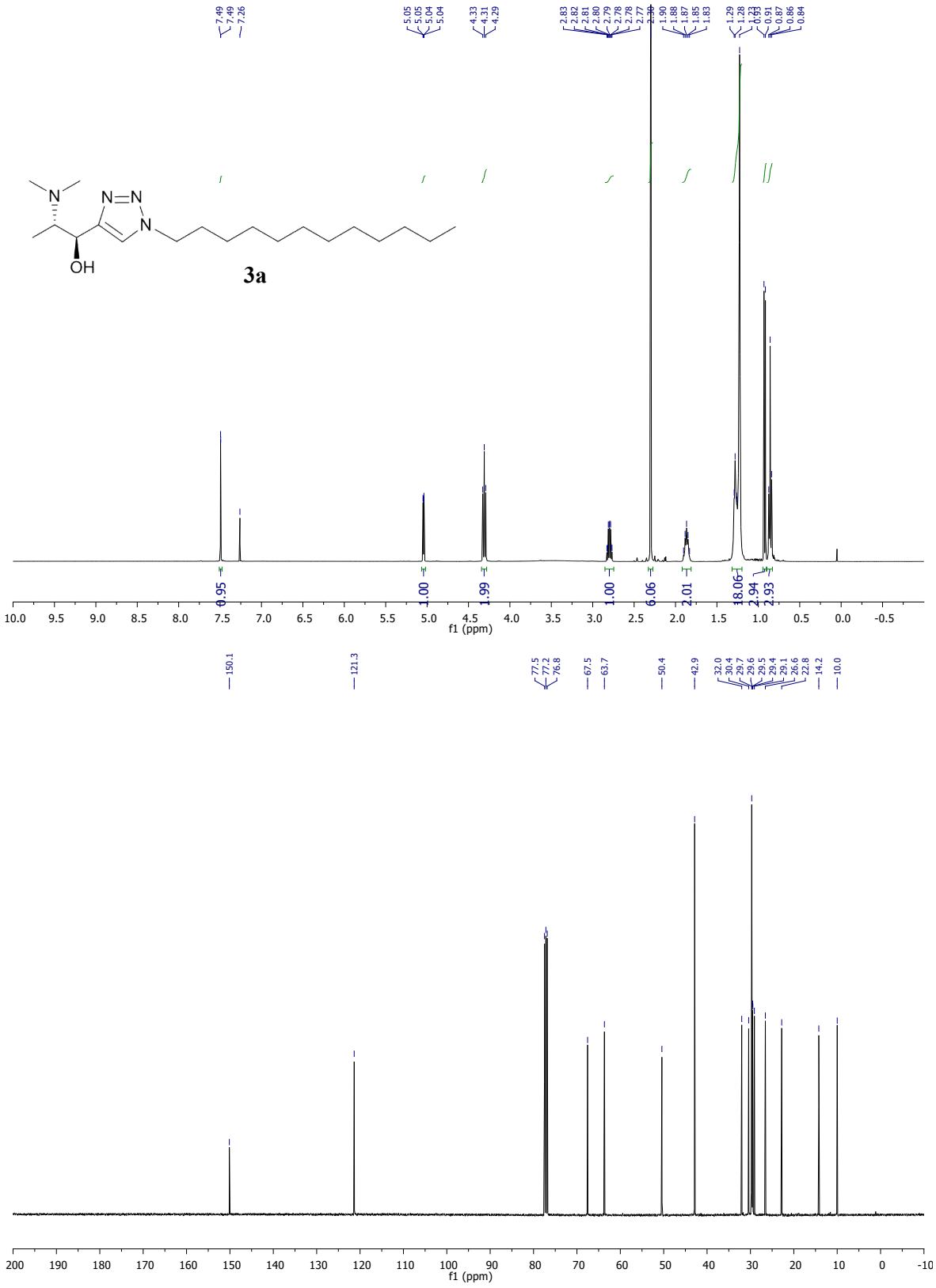
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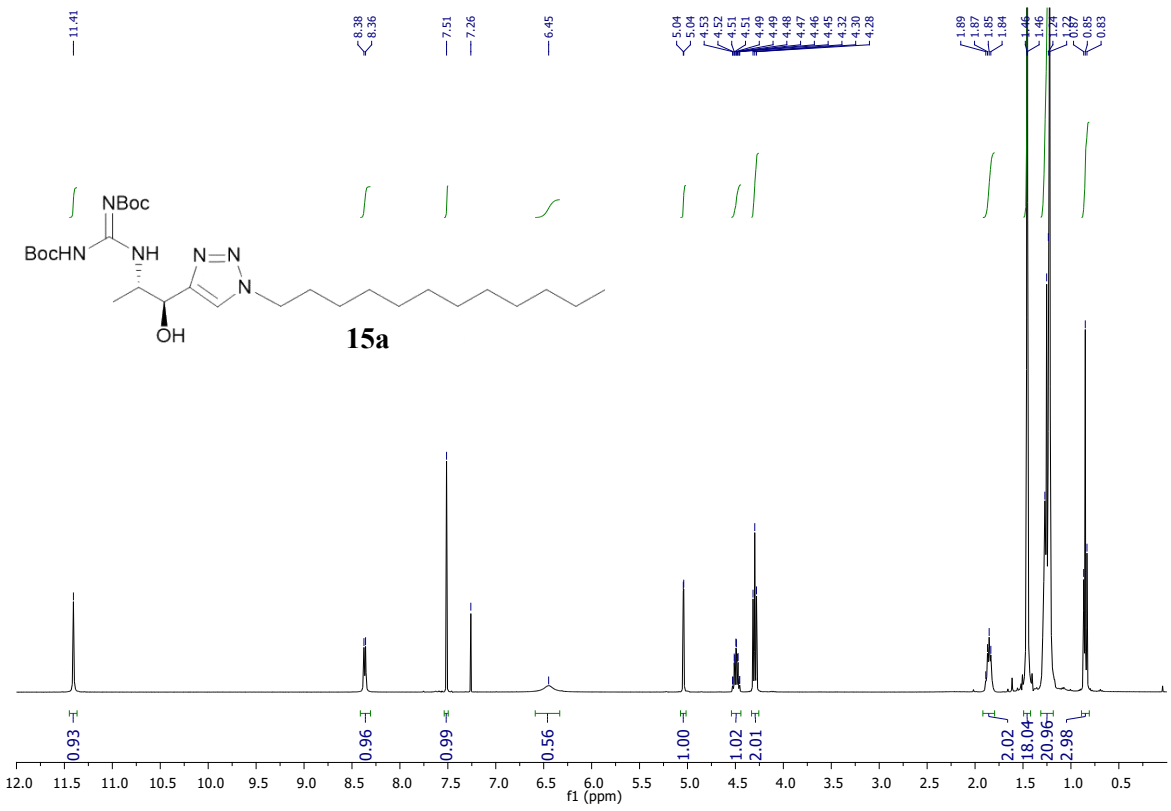
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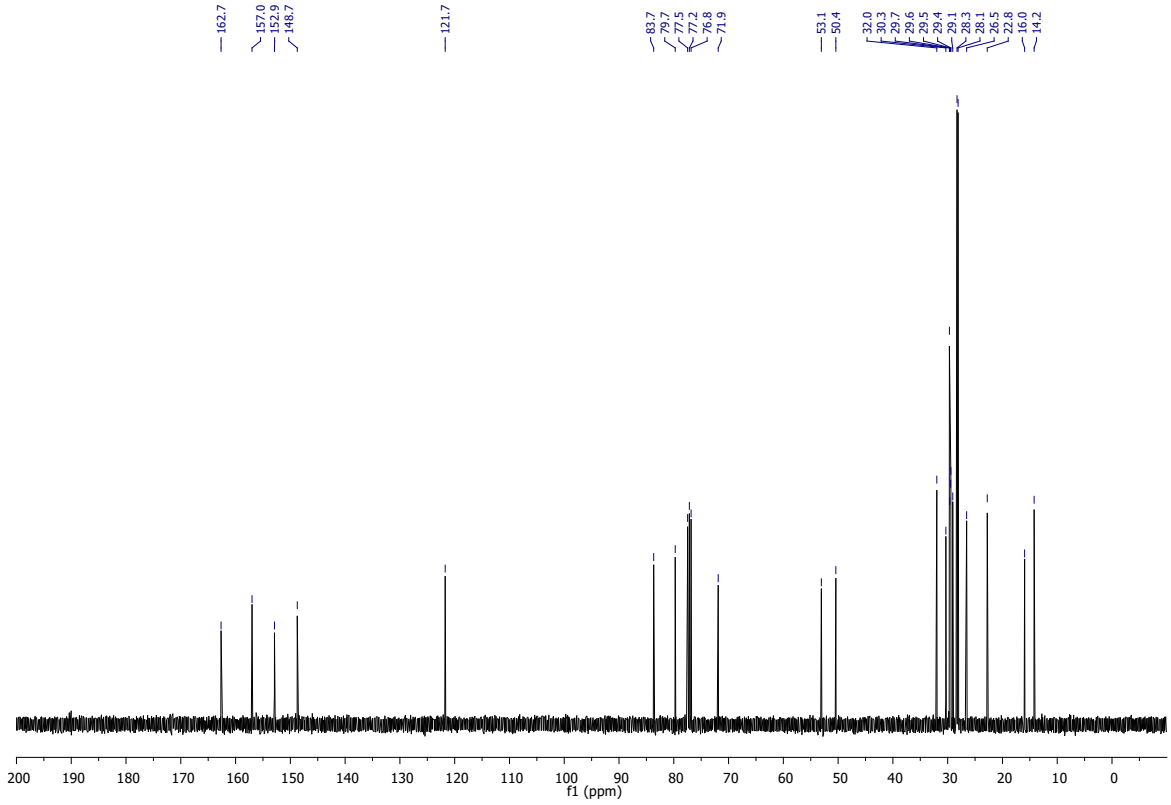
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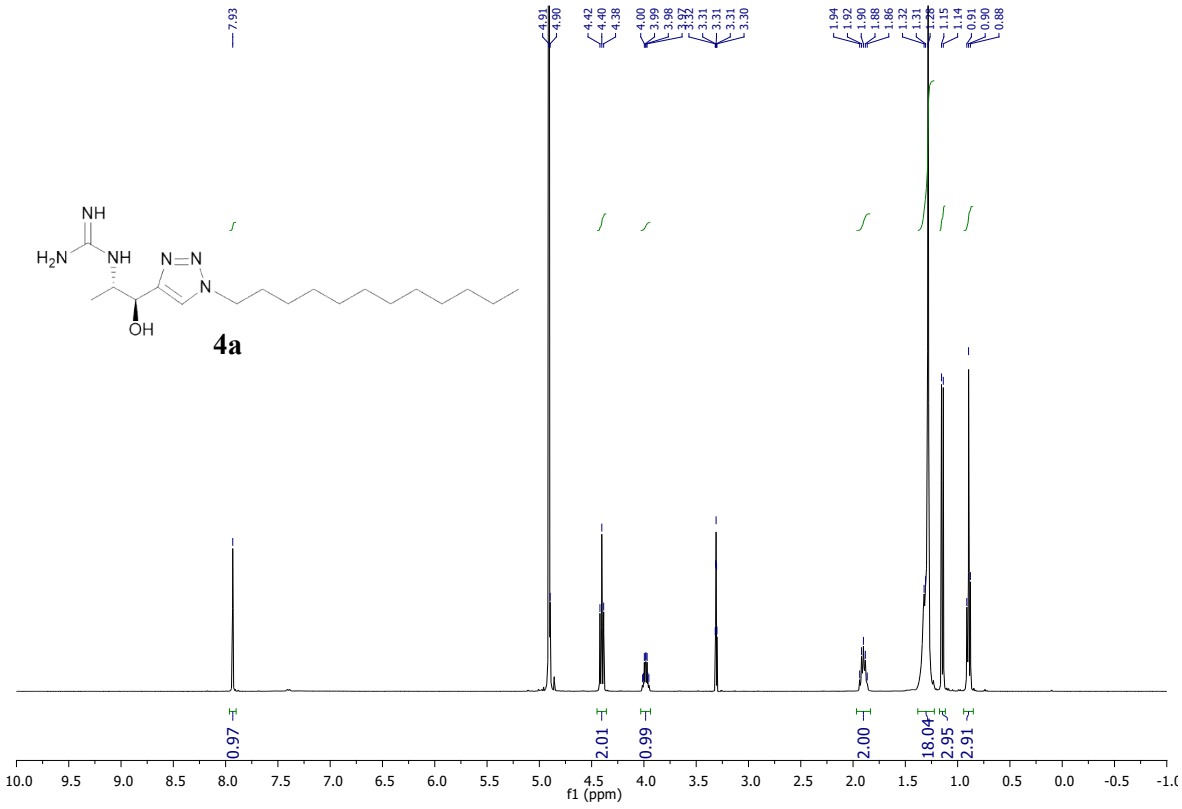
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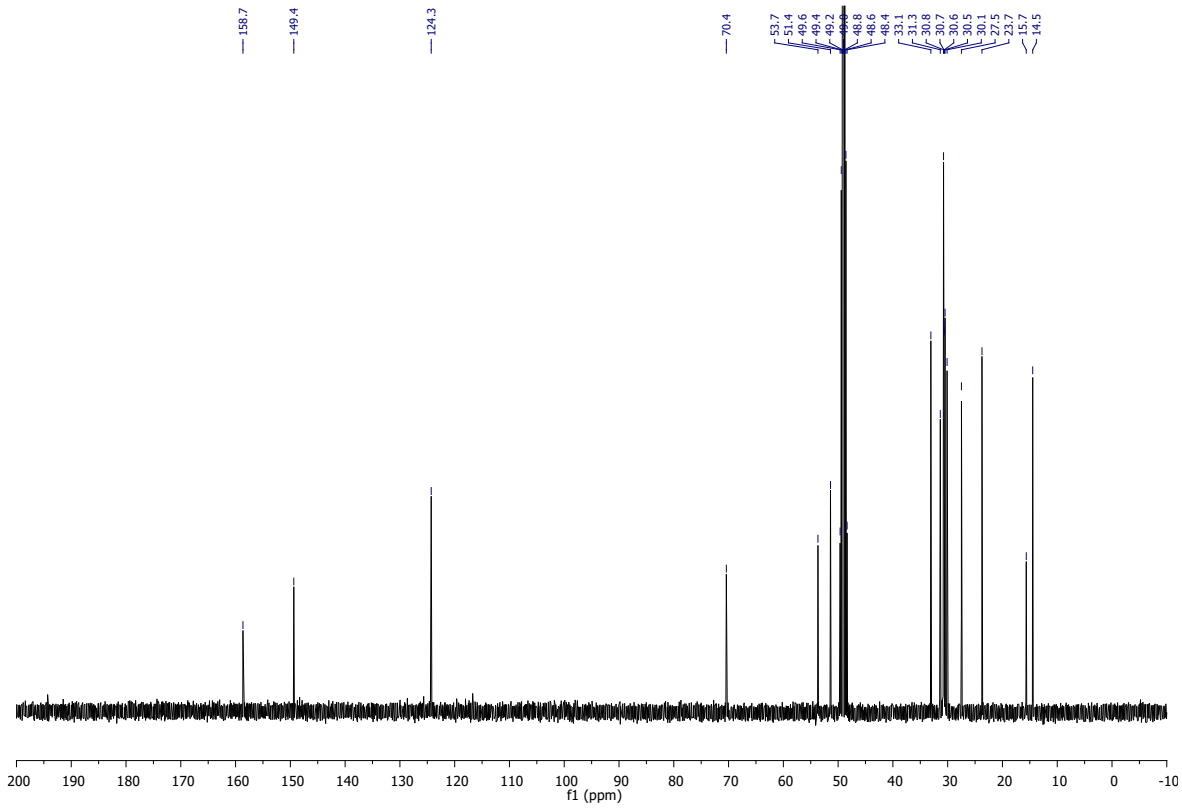
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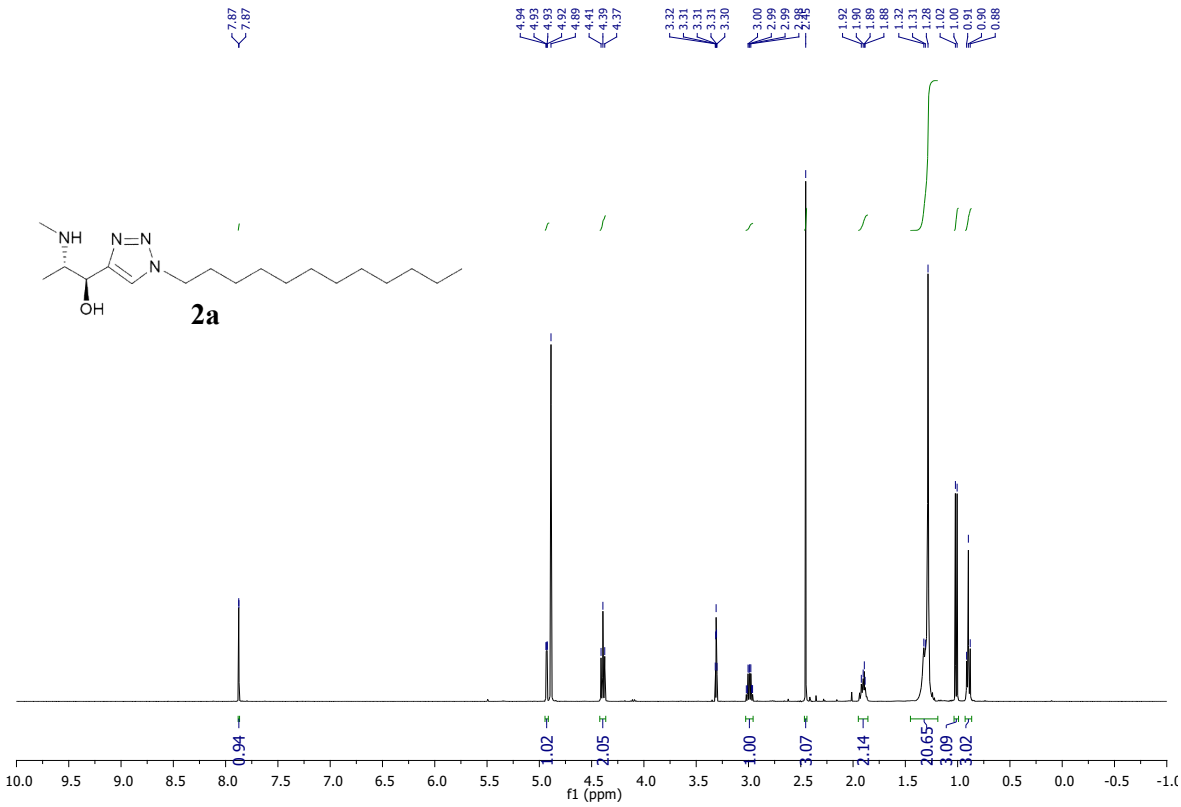
f1 (ppm)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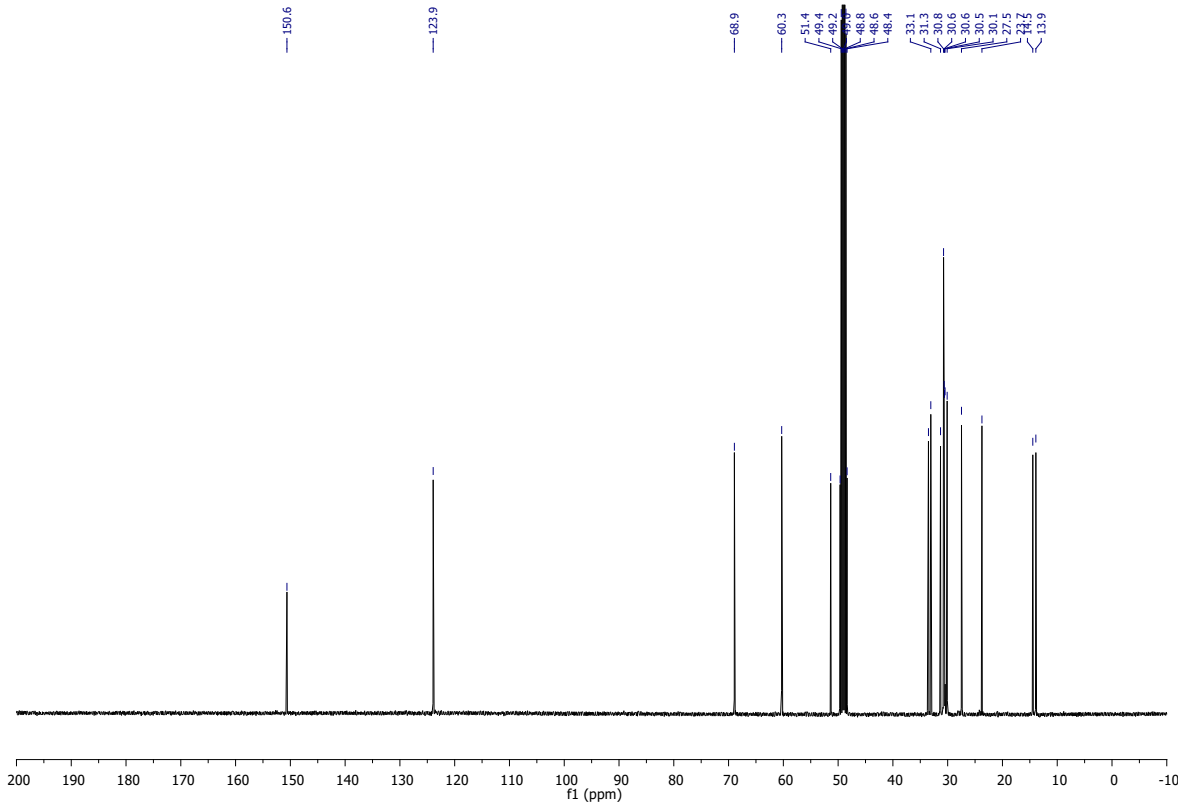
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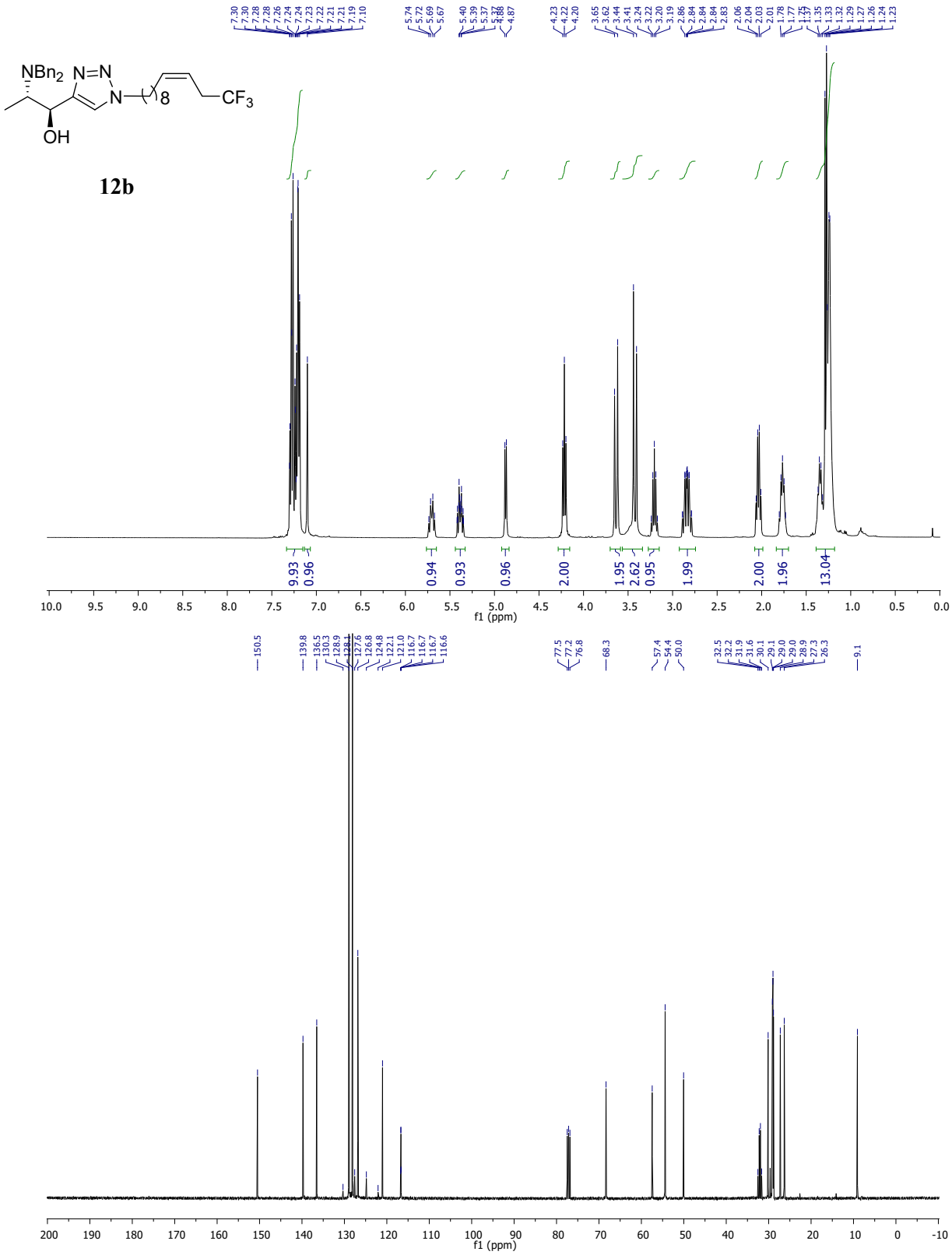
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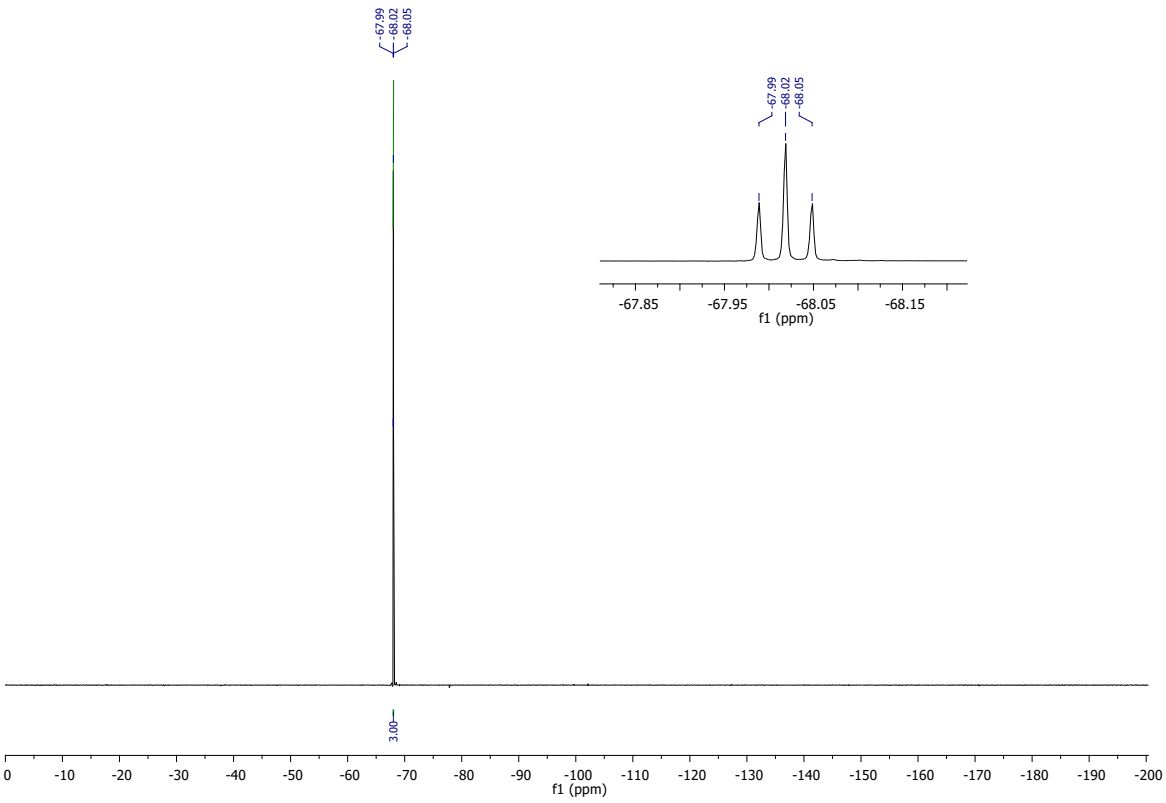
f1 (ppm)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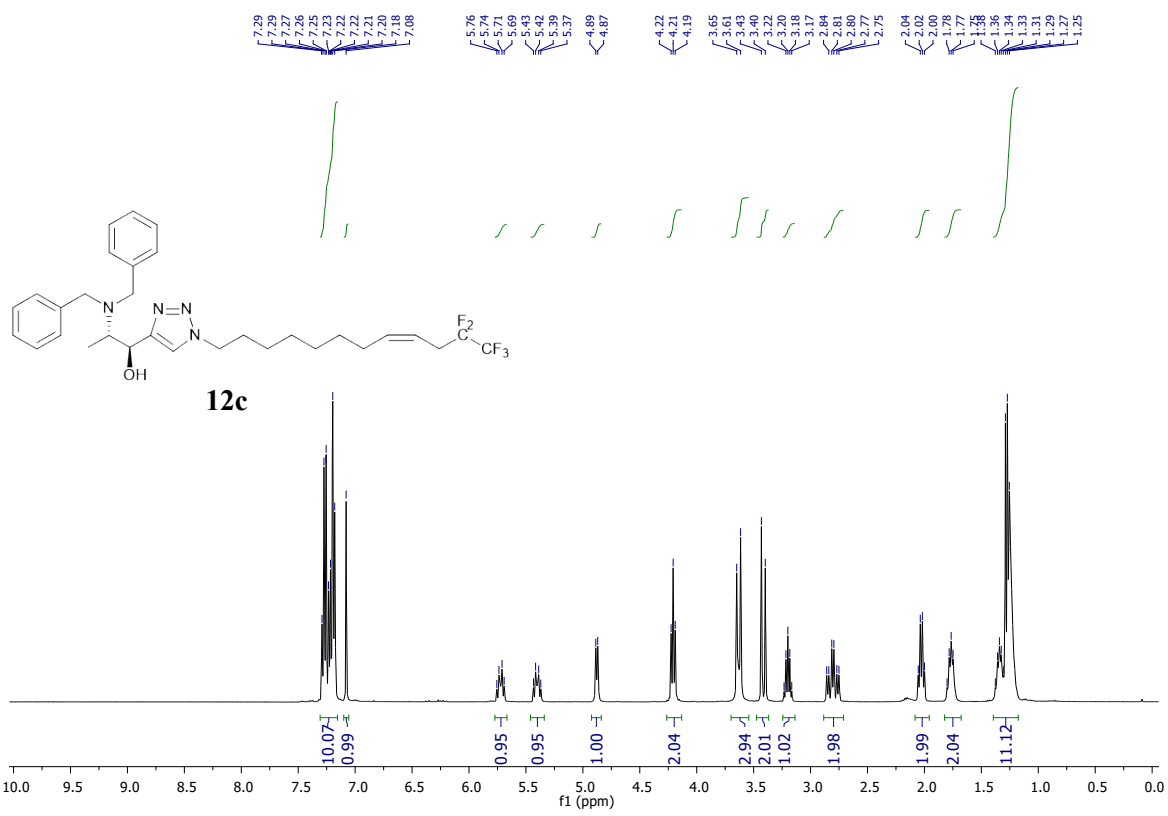
f1 (ppm)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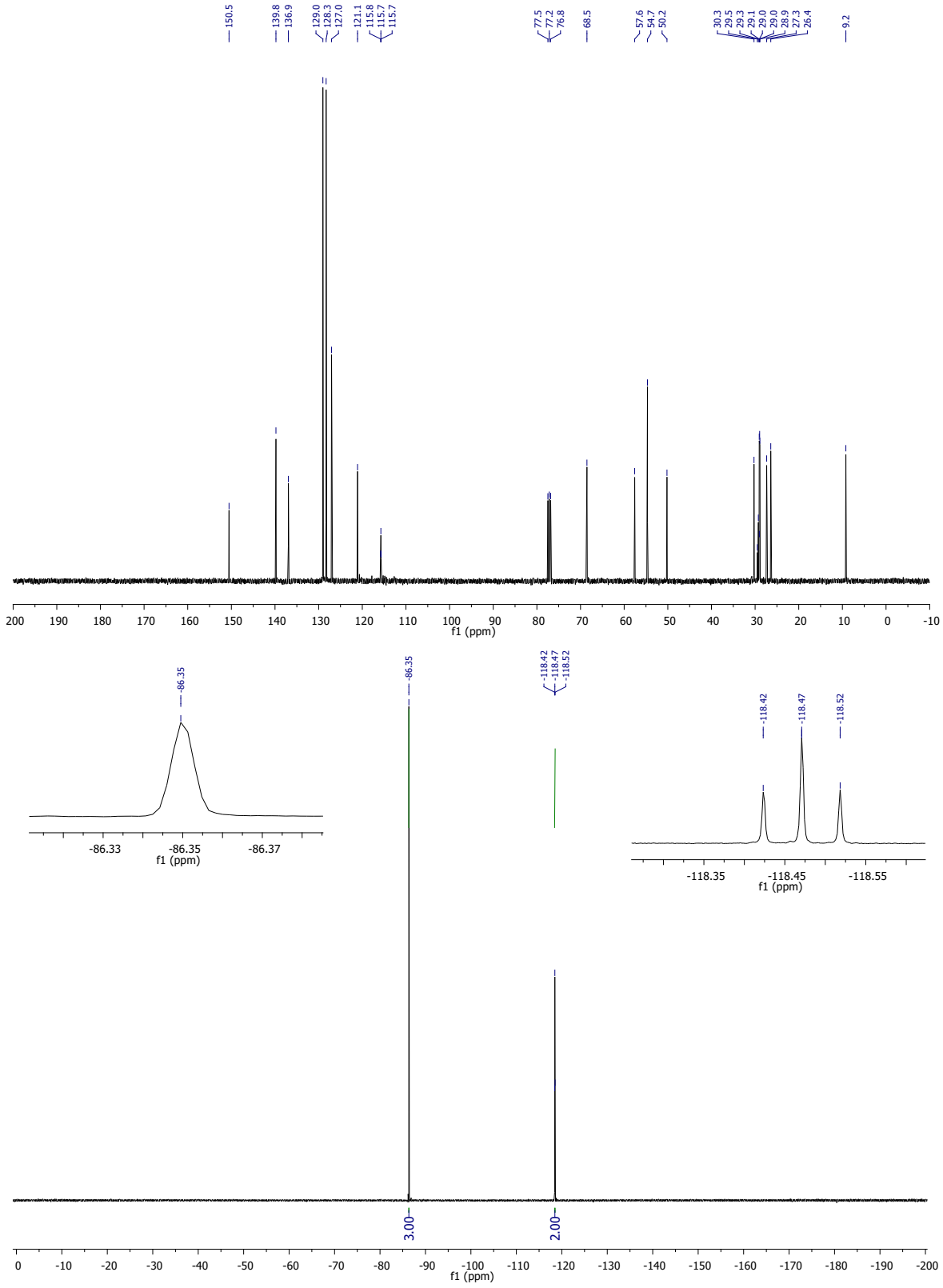
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26.4
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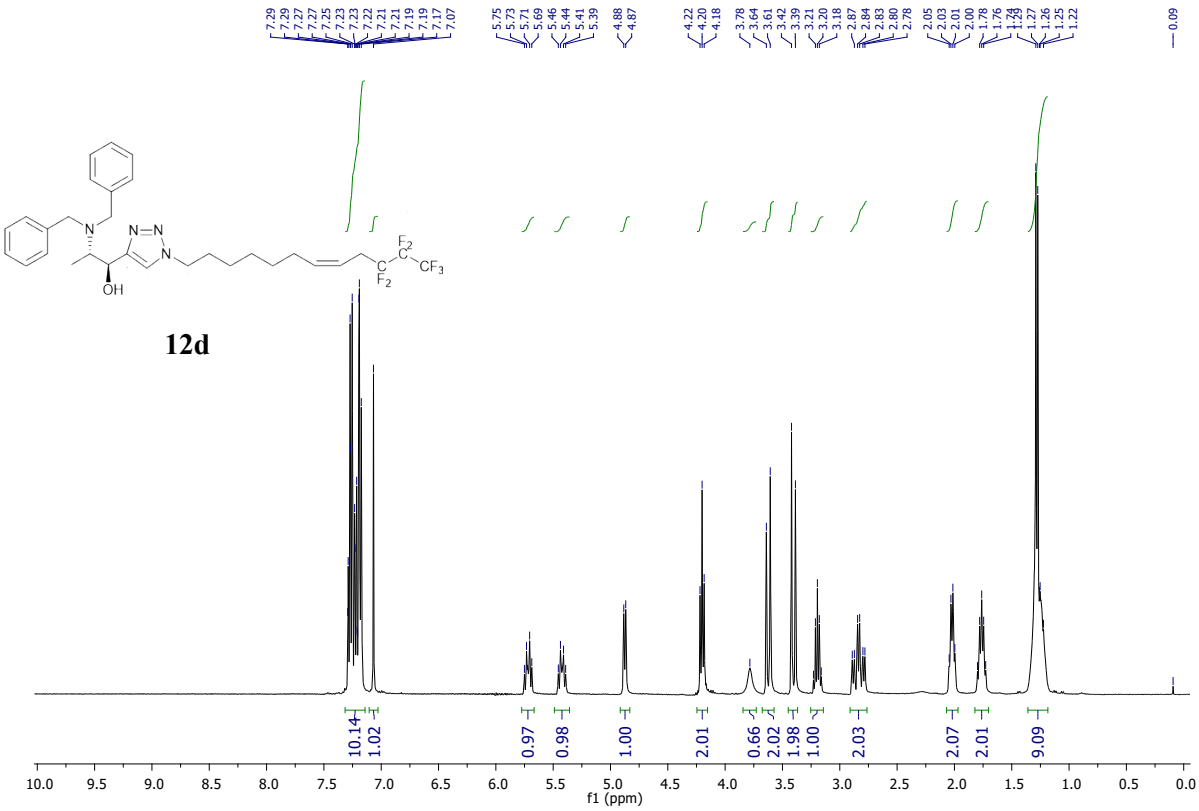
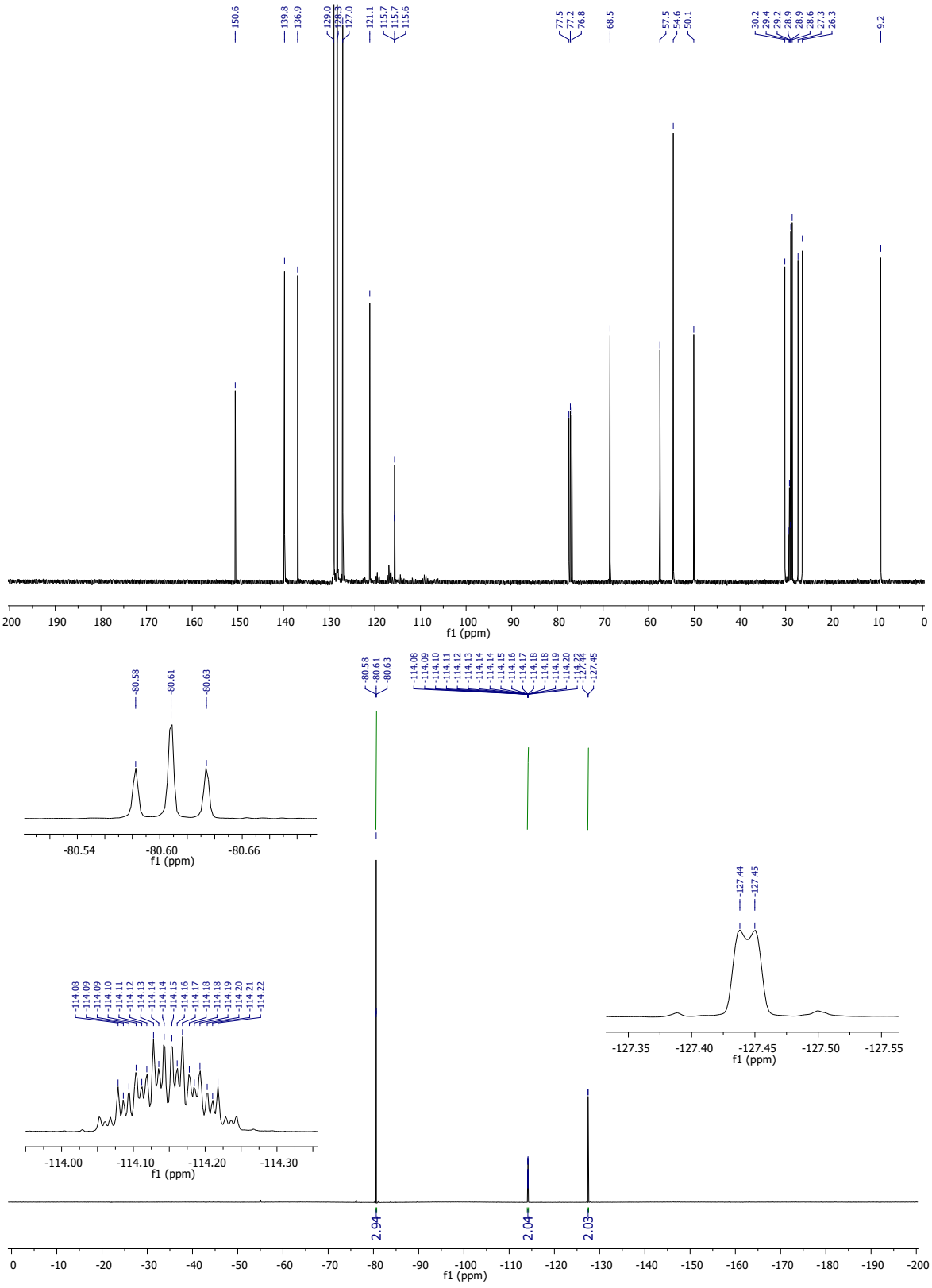
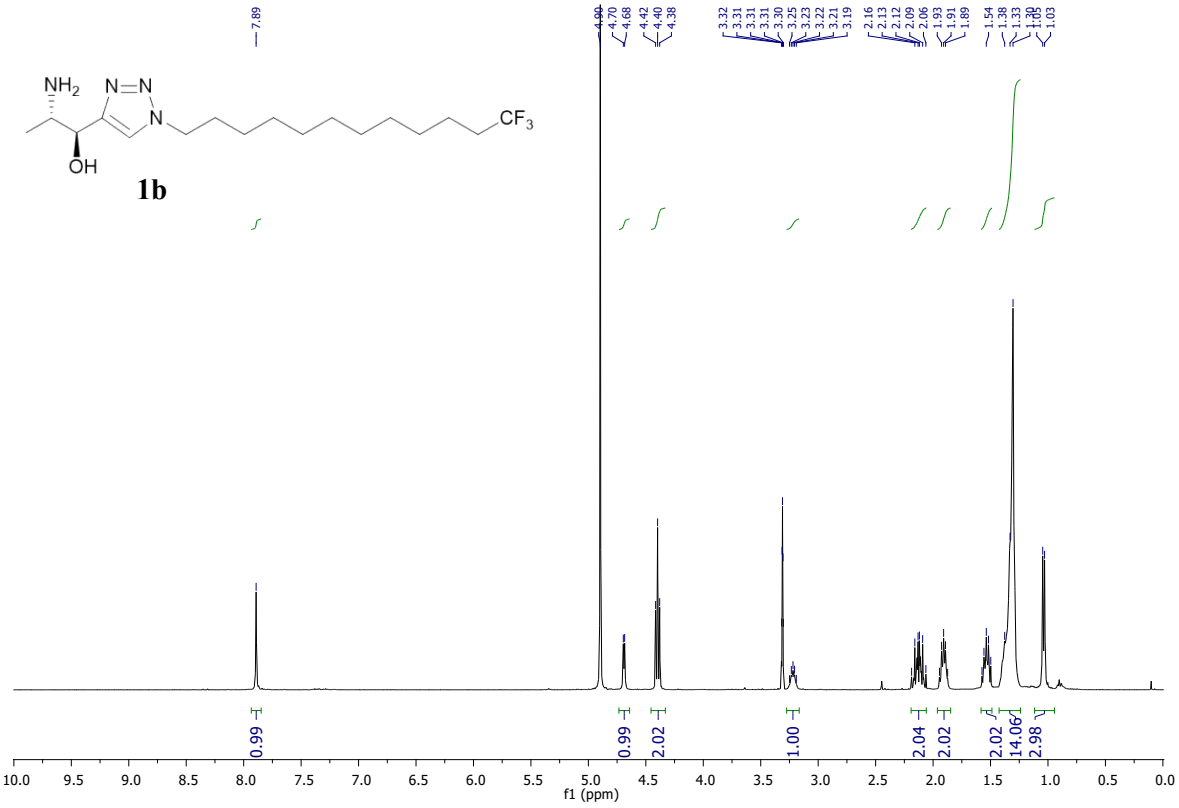
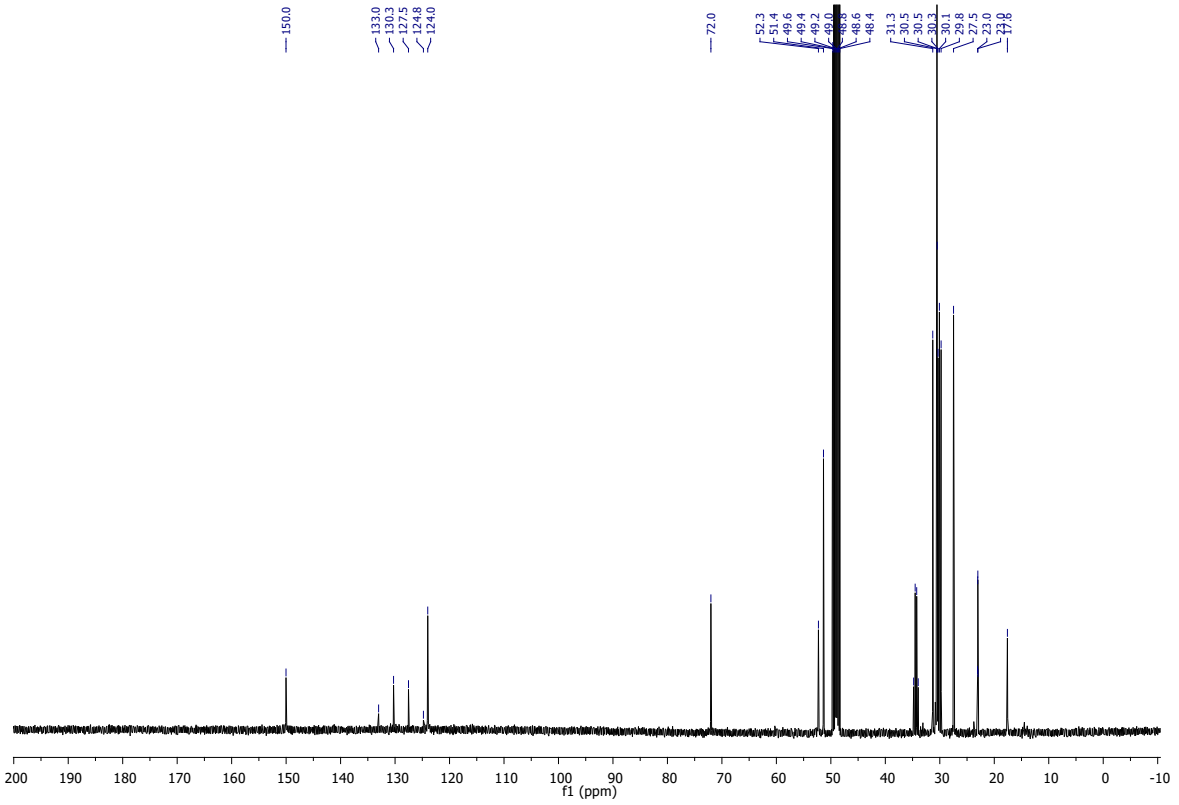
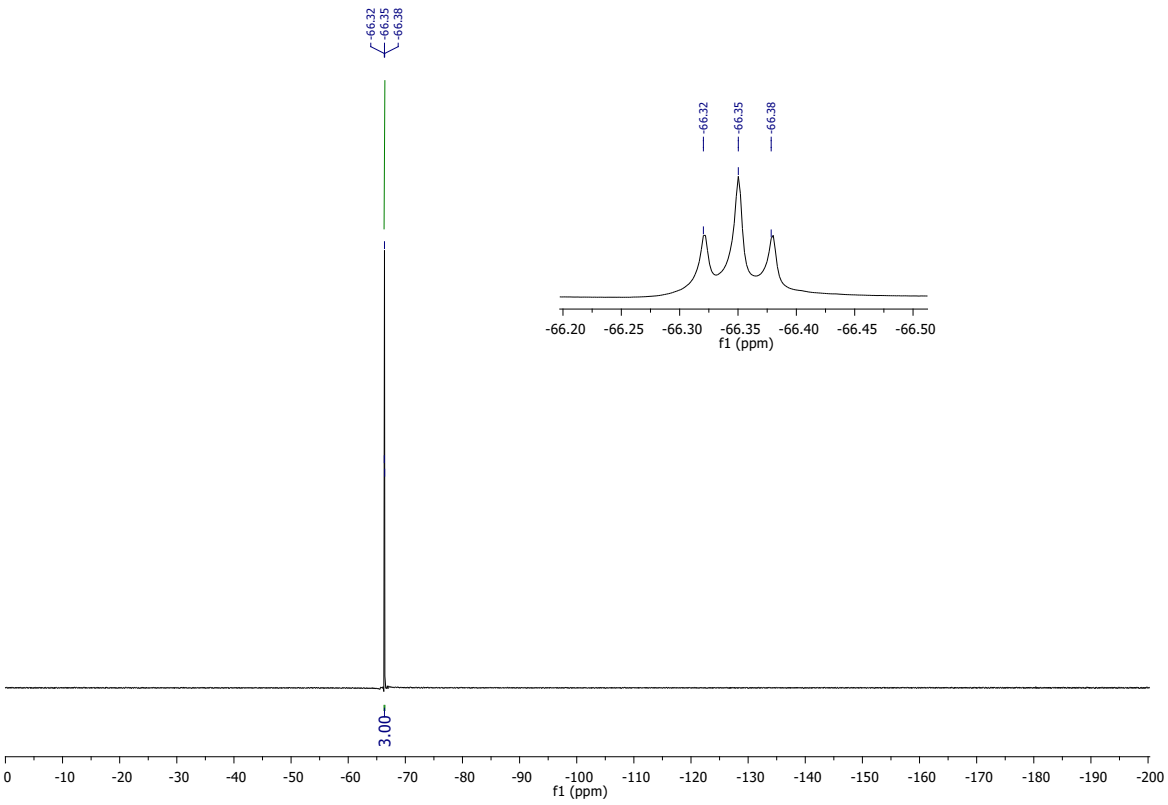
-118.42
-118.47
-118.52
-118.35
-118.45
-118.55
f1 (ppm)
3.00
2.00
f1 (ppm)

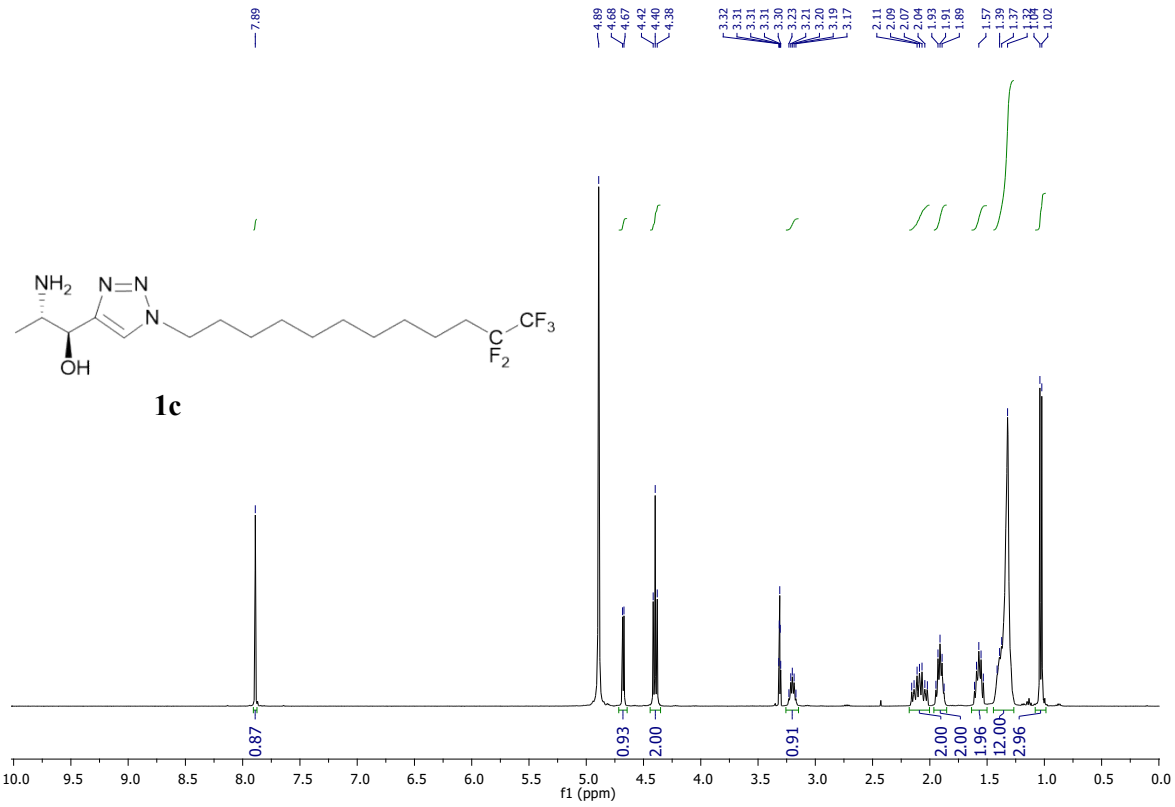

12d
f1 (ppm)


150.6
139.8
136.9
129.0
128.3
127.0
121.1
115.7
115.7
115.6
77.5
77.2
76.8
68.5
57.5
54.6
50.1
30.2
29.4
29.2
28.9
28.9
28.6
27.3
26.3
9.2
f1 (ppm)
-80.58
-80.61
-80.63
-80.54
-80.60
-80.66
f1 (ppm)
-114.08
-114.09
-114.09
-114.10
-114.11
-114.12
-114.13
-114.14
-114.14
-114.15
-114.16
-114.17
-114.18
-114.18
-114.19
-114.20
-114.21
-114.22
-114.00
-114.10
-114.20
-114.30
f1 (ppm)
-127.44
-127.45
-127.35
-127.40
-127.45
-127.50
-127.55
f1 (ppm)
2.94
2.04
2.03
f1 (ppm)

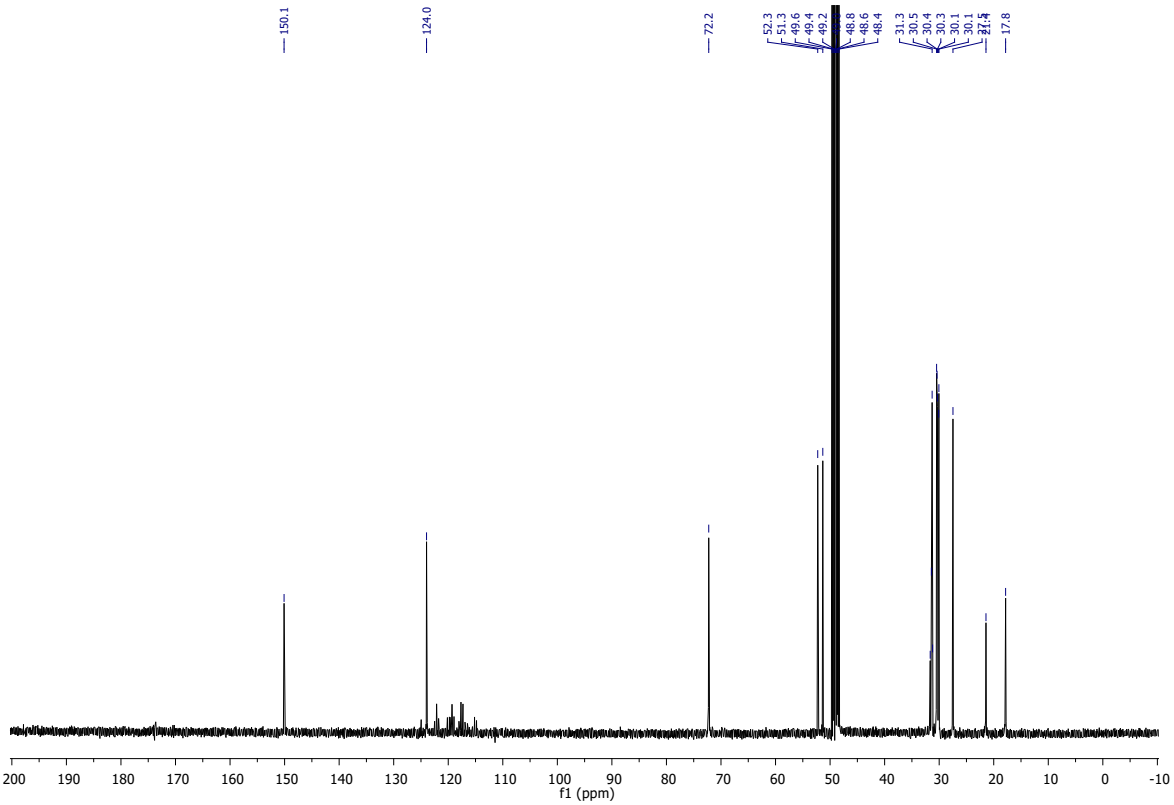

NH2
N=N
N
CF3
OH
1b
f1 (ppm)

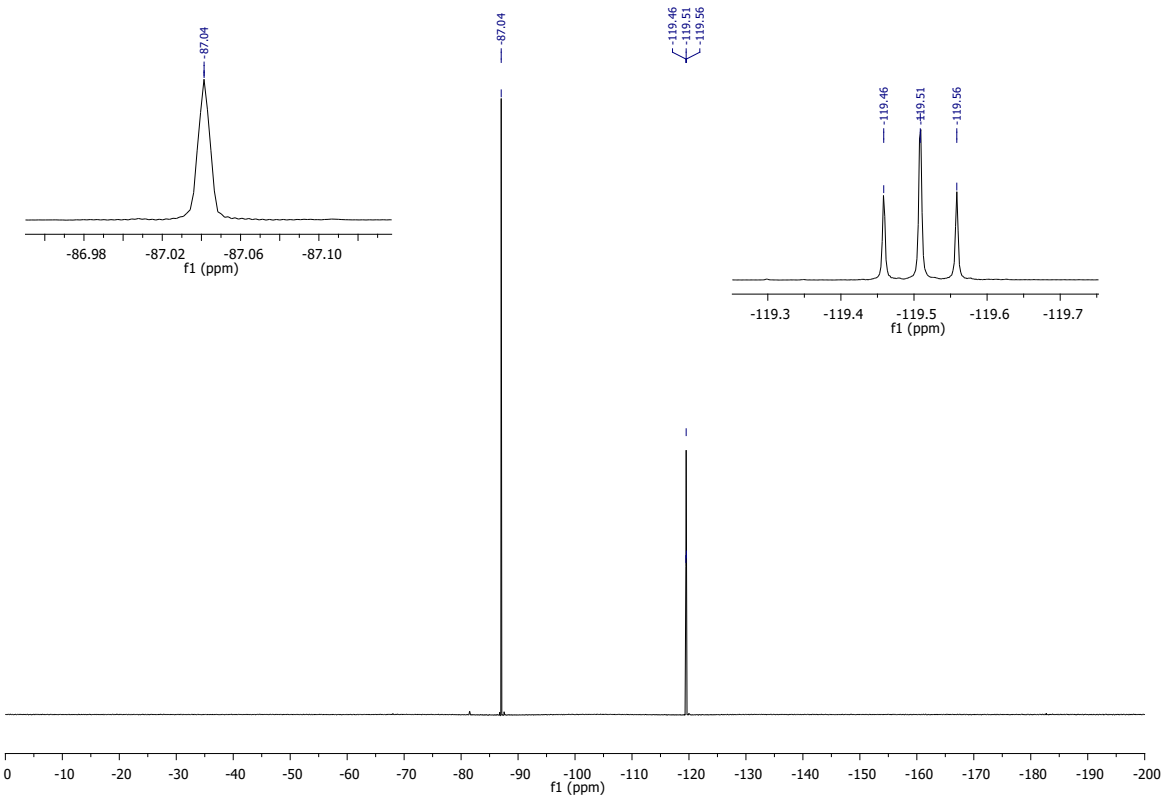

f1 (ppm)

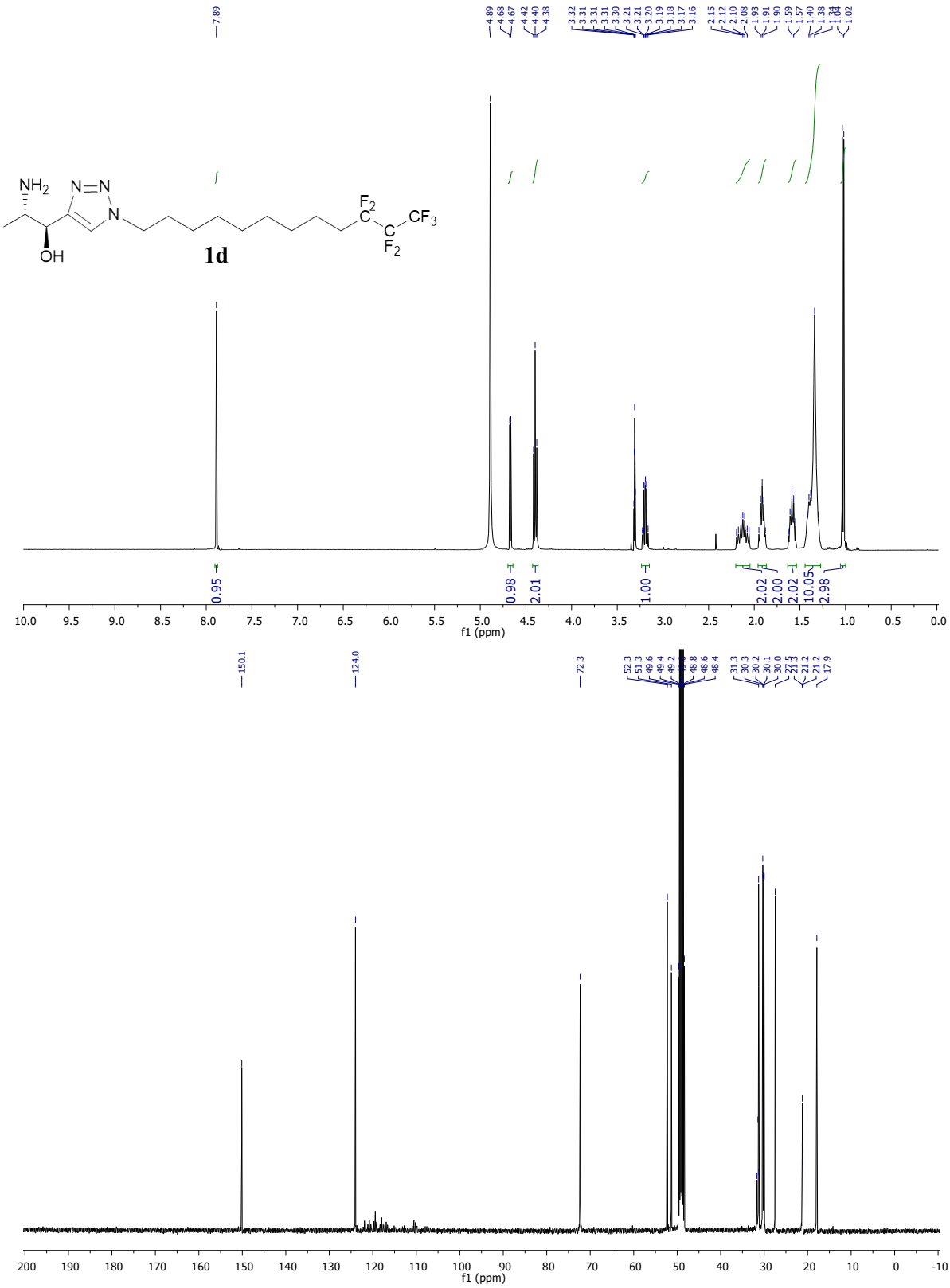

-66.32
-66.35
-66.38
-66.32
-66.35
-66.38
-66.20 -66.25 -66.30 -66.35 -66.40 -66.45 -66.50
f1 (ppm)
3.00
0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

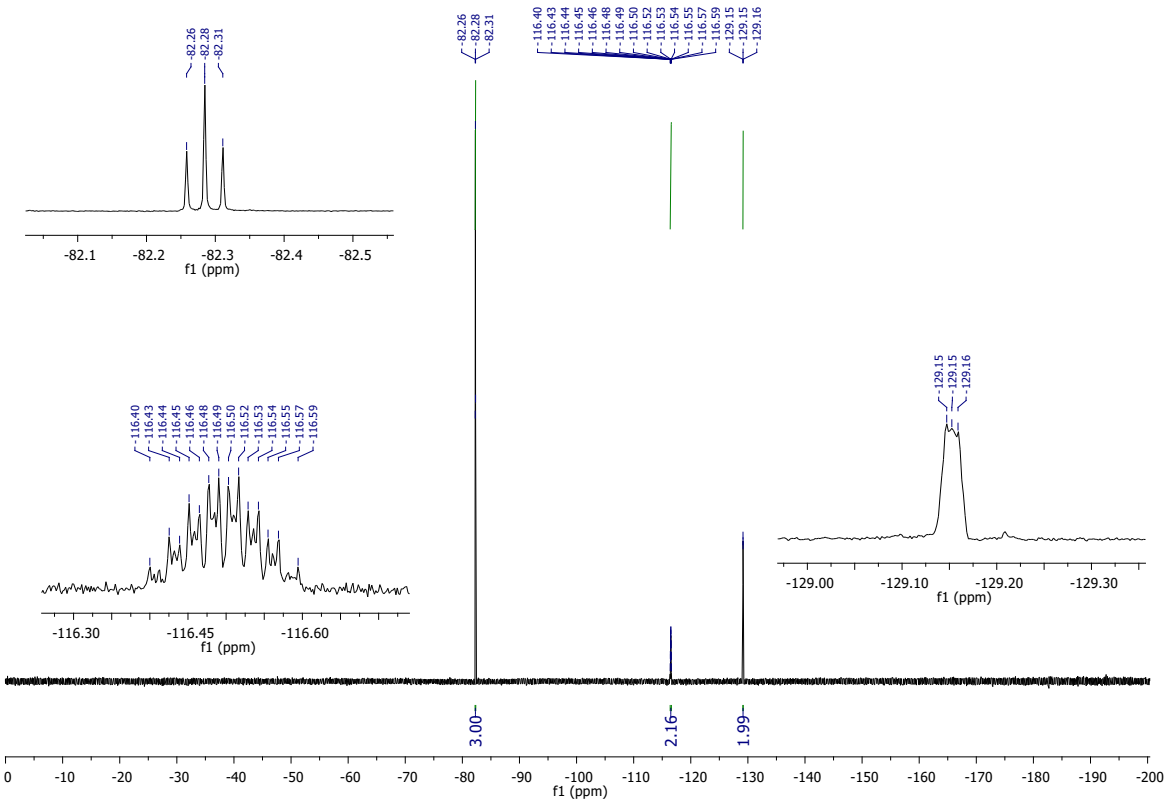

7.89
4.89
4.68
4.67
4.42
4.40
4.38
3.32
3.31
3.31
3.31
3.30
3.23
3.21
3.20
3.19
3.17
2.11
2.09
2.07
2.04
1.93
1.91
1.89
1.57
1.39
1.37
1.04
1.02
NH2
N=N
N
OH
CF3
C
F2
0.87
0.93
2.00
0.91
2.00
2.00
1.96
12.00
2.96
f1 (ppm)

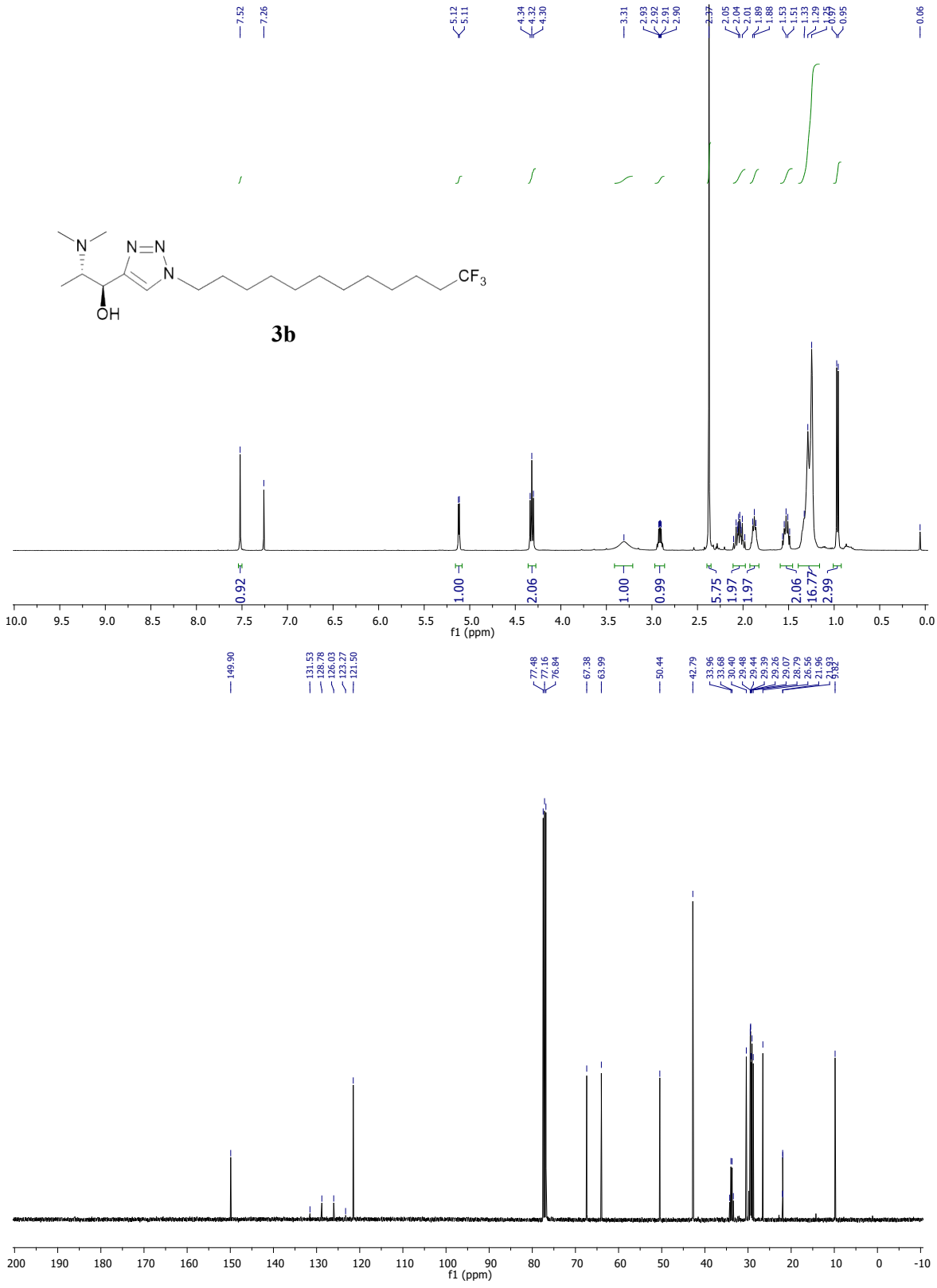
1c

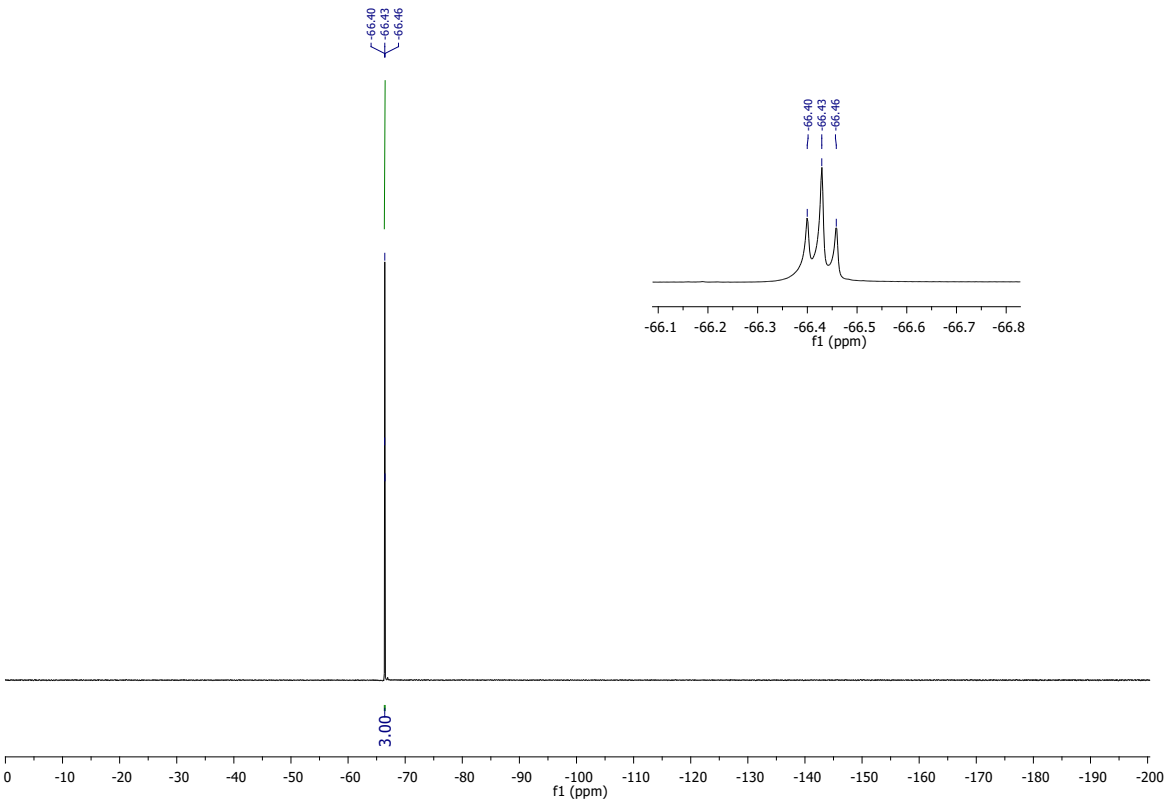

150.1
124.0
72.2
52.3
51.3
49.6
49.4
49.2
48.8
48.6
48.4
31.3
30.5
30.4
30.3
30.1
30.1
27.4
17.8
f1 (ppm)

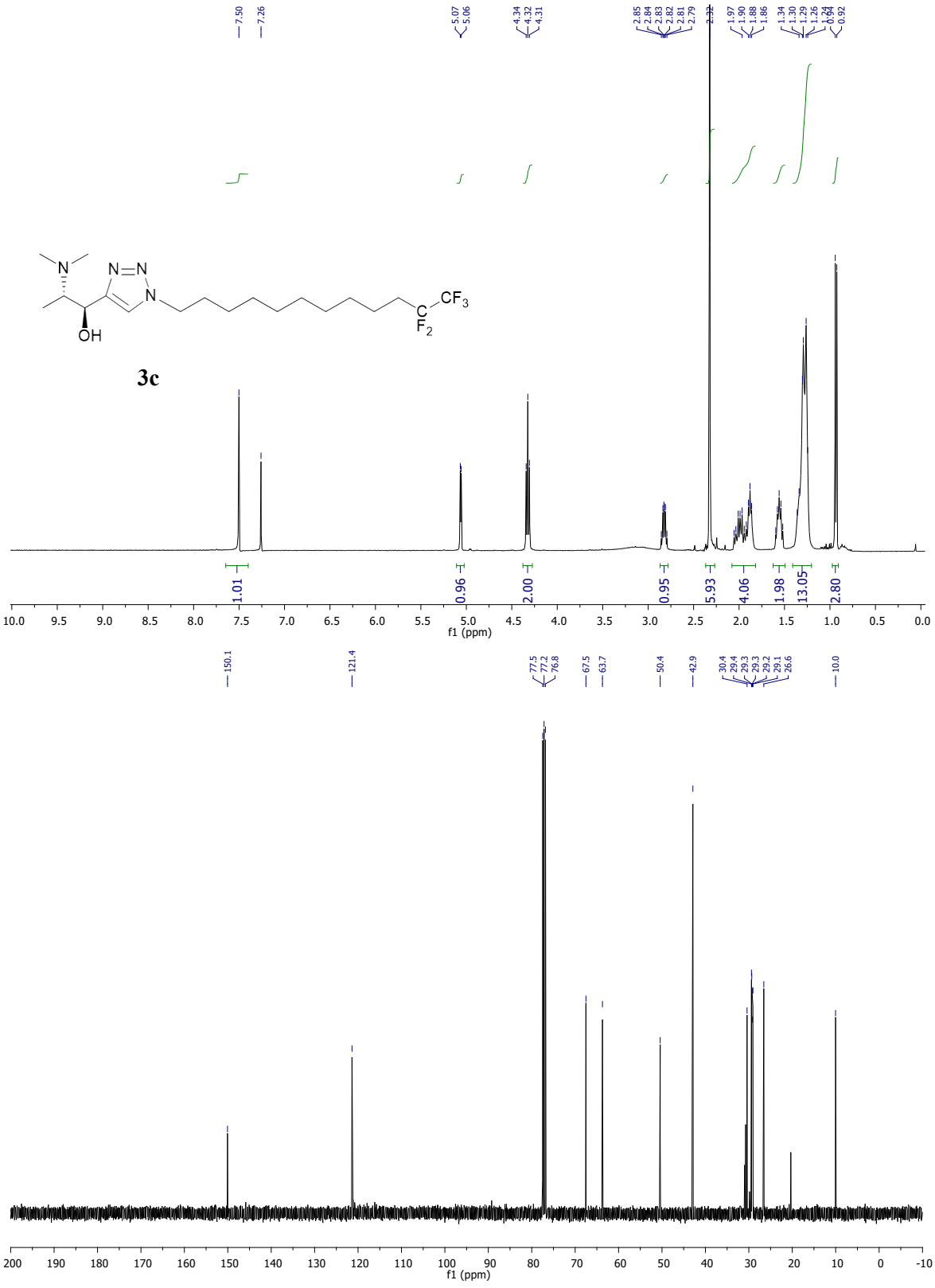

-87.04
-86.98
-87.02
-87.06
-87.10
f1 (ppm)
-87.04
-119.46
-119.51
-119.56
-119.46
-119.51
-119.56
-119.3
-119.4
-119.5
-119.6
-119.7
f1 (ppm)
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)


NH2
N=N
N
OH
F2
C
CF3
C
F2
1d
f1 (ppm)
f1 (ppm)

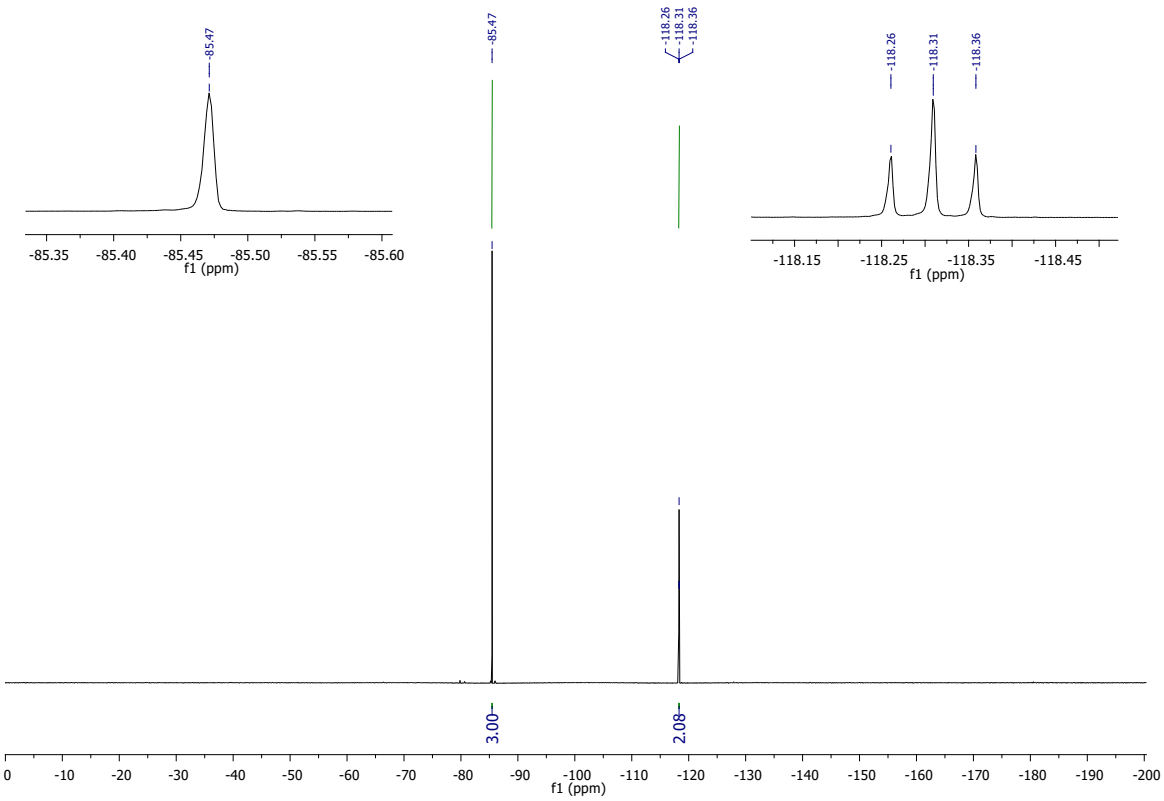

-82.26
-82.28
-82.31
-82.1
-82.2
-82.3
-82.4
-82.5
f1 (ppm)
-116.40
-116.43
-116.44
-116.45
-116.46
-116.48
-116.49
-116.50
-116.52
-116.53
-116.54
-116.55
-116.57
-116.59
-116.30
-116.45
-116.60
f1 (ppm)
-129.15
-129.15
-129.16
-129.00
-129.10
-129.20
-129.30
f1 (ppm)
3.00
2.16
1.99
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)

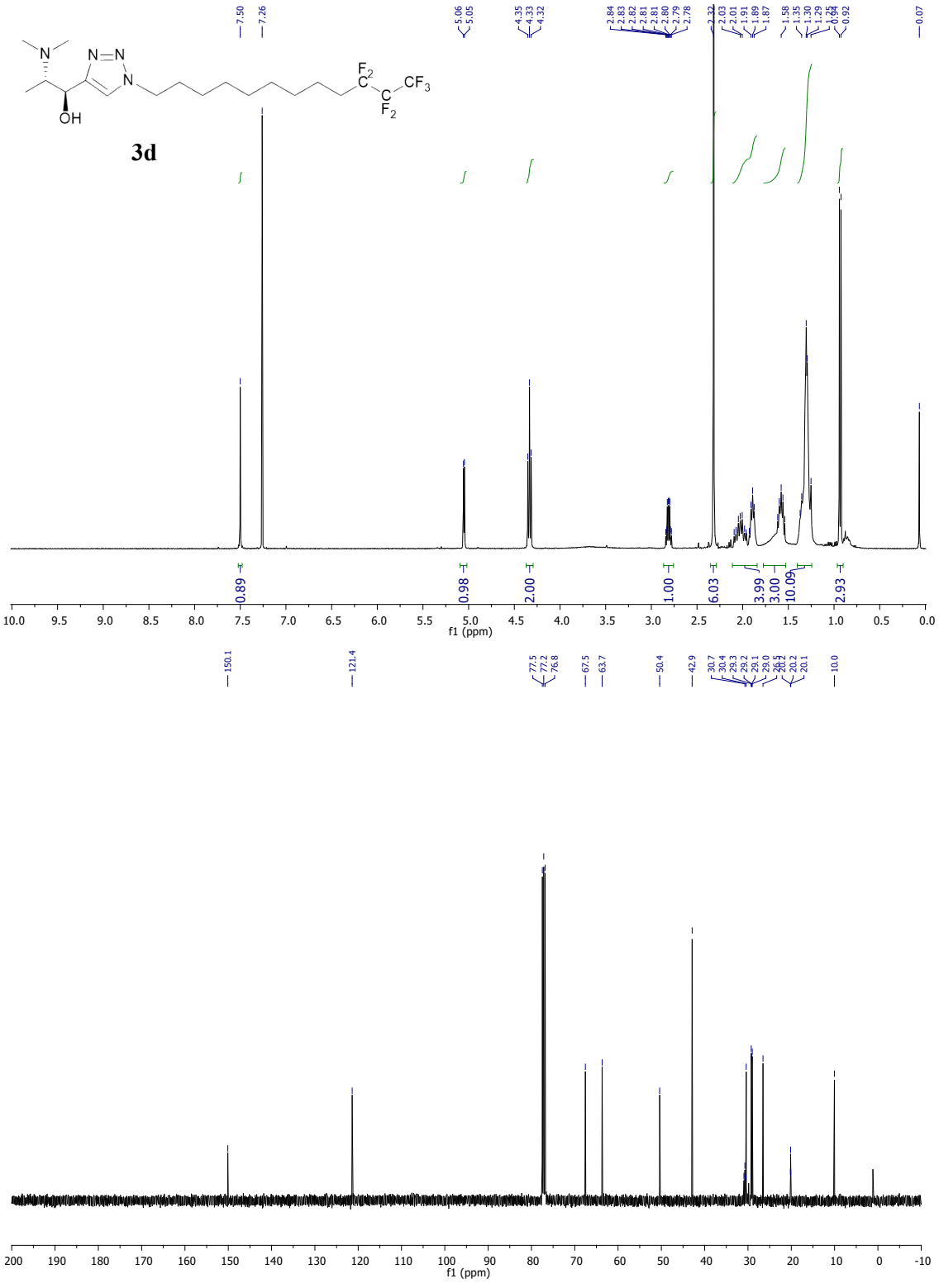

7.52
7.26
5.12
5.11
4.34
4.32
4.30
3.31
2.93
2.92
2.91
2.90
2.37
2.05
2.04
2.01
1.89
1.88
1.53
1.51
1.33
1.29
0.95
0.06
N
N=N
N
CF3
OH
3b
0.92
1.00
2.06
1.00
0.99
5.75
1.97
1.97
2.06
16.77
2.99
f1 (ppm)
149.90
131.53
128.78
128.03
123.27
121.50
77.48
77.16
76.84
67.38
63.99
50.44
42.79
33.96
33.68
30.40
29.48
29.44
29.39
29.26
29.07
28.79
26.56
21.96
21.93
9.82
f1 (ppm)

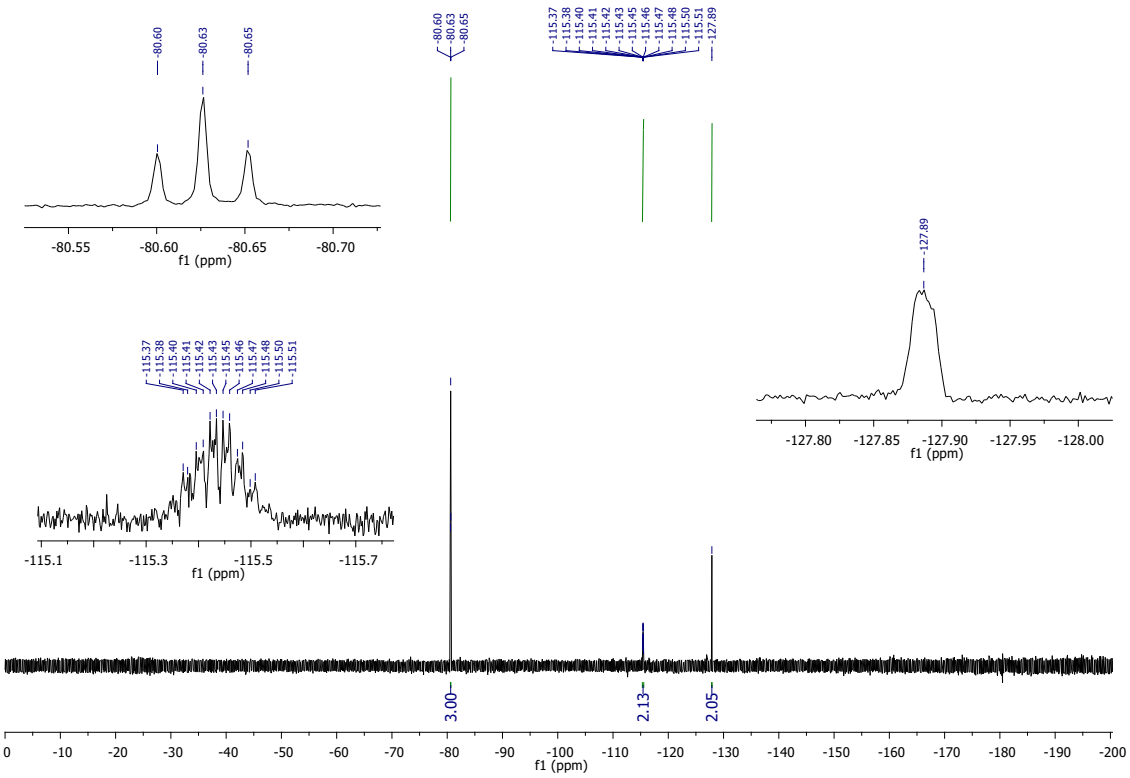

-66.40
-66.43
-66.46
-66.40
-66.43
-66.46
-66.1 -66.2 -66.3 -66.4 -66.5 -66.6 -66.7 -66.8
f1 (ppm)
3.00
0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

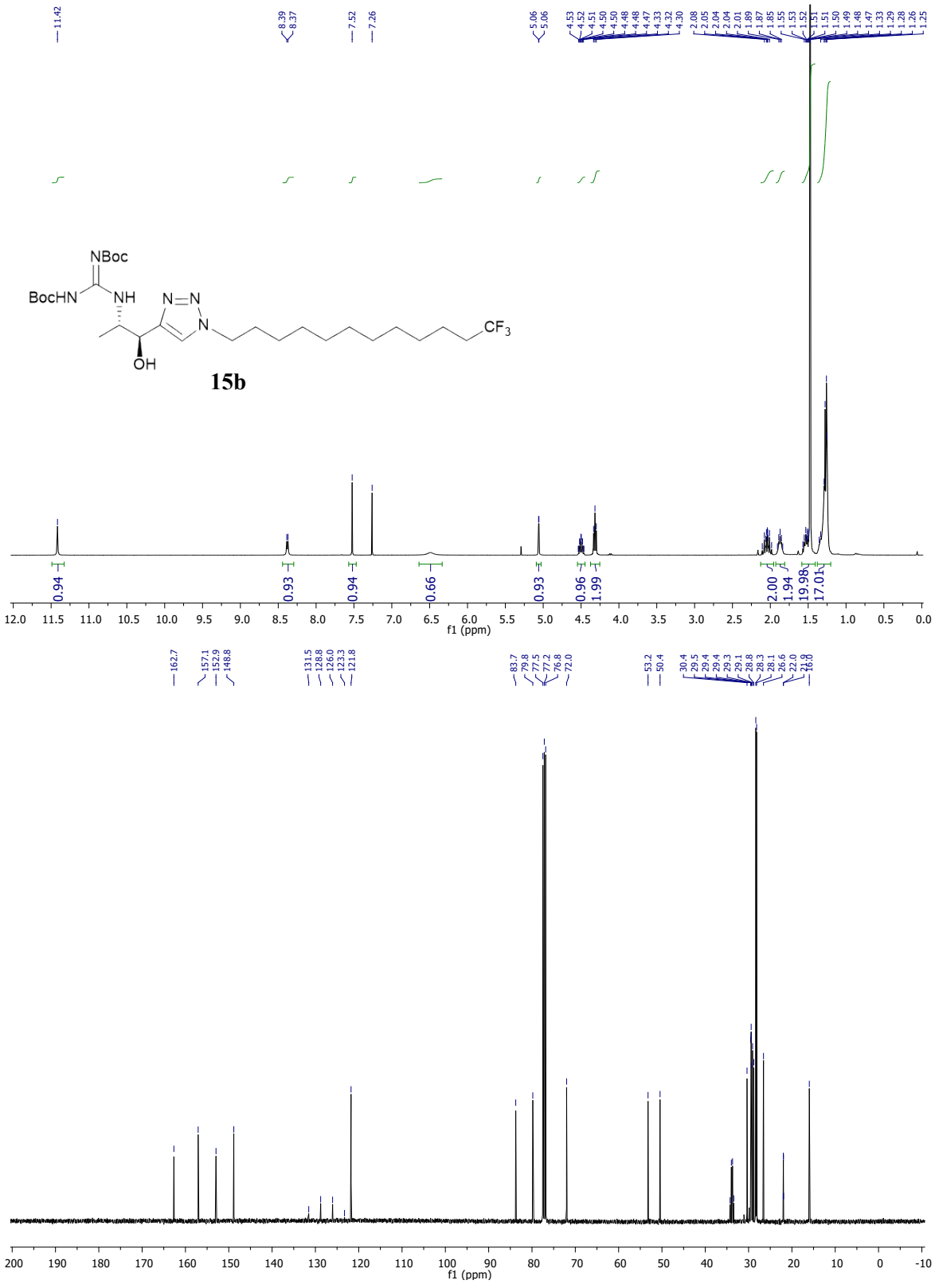

7.50
7.26
5.07
5.06
4.34
4.32
4.31
2.85
2.84
2.83
2.82
2.81
2.79
1.97
1.90
1.88
1.86
1.34
1.30
1.29
1.26
0.92
N
N=N
N
OH
CF3
C
F2
1.01
0.96
2.00
0.95
5.93
4.06
1.98
13.05
2.80
f1 (ppm)
150.1
121.4
77.5
77.2
76.8
67.5
63.7
50.4
42.9
30.4
29.4
29.3
29.3
29.2
29.1
26.6
10.0
f1 (ppm)

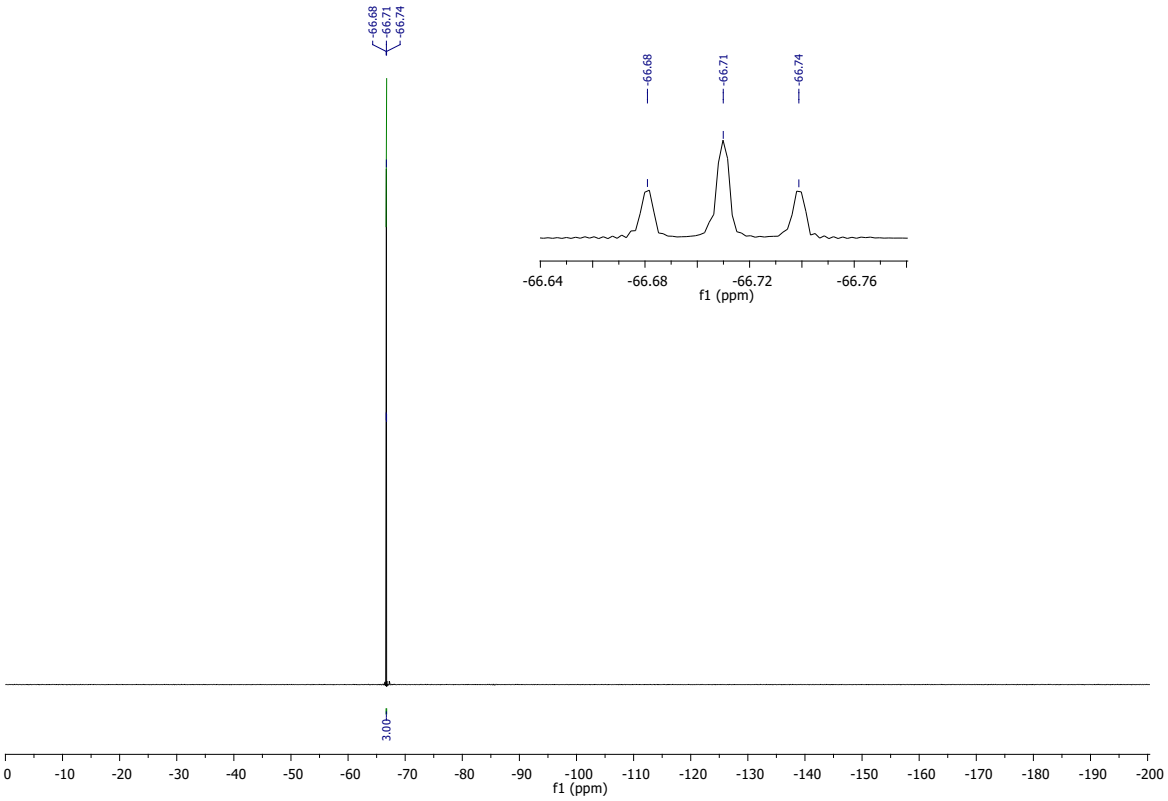
3c

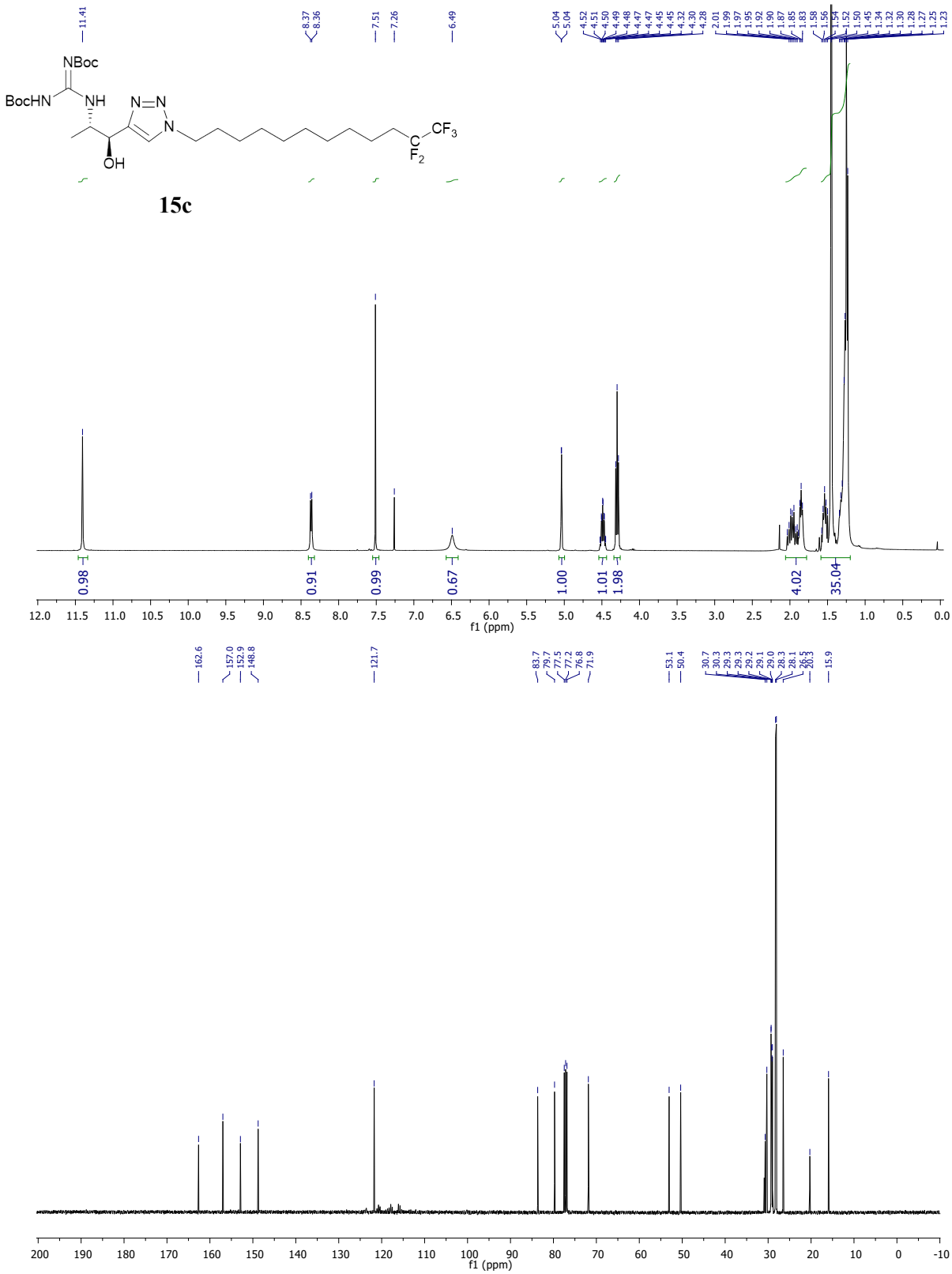

-85.47
-85.35
-85.40
-85.45
-85.50
-85.55
-85.60
f1 (ppm)
-85.47
-118.26
-118.31
-118.36
-118.26
-118.31
-118.36
-118.15
-118.25
-118.35
-118.45
f1 (ppm)
3.00
2.08
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)


3d
f1 (ppm)
f1 (ppm)

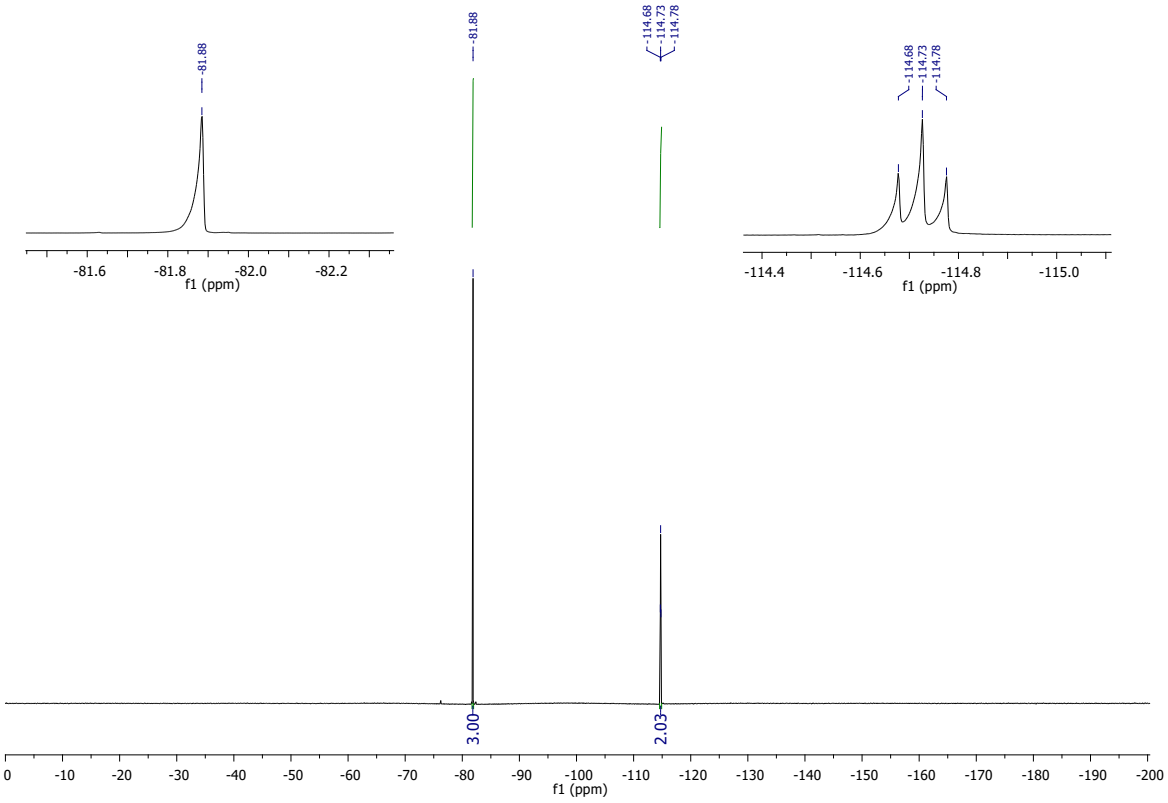

-80.60
-80.63
-80.65
-80.55
-80.60
-80.65
-80.70
f1 (ppm)
-115.37
-115.38
-115.40
-115.41
-115.42
-115.43
-115.45
-115.46
-115.47
-115.48
-115.50
-115.51
-127.89
-115.1
-115.3
-115.5
-115.7
f1 (ppm)
-127.89
-127.80
-127.85
-127.90
-127.95
-128.00
f1 (ppm)
3.00
2.13
2.05
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)

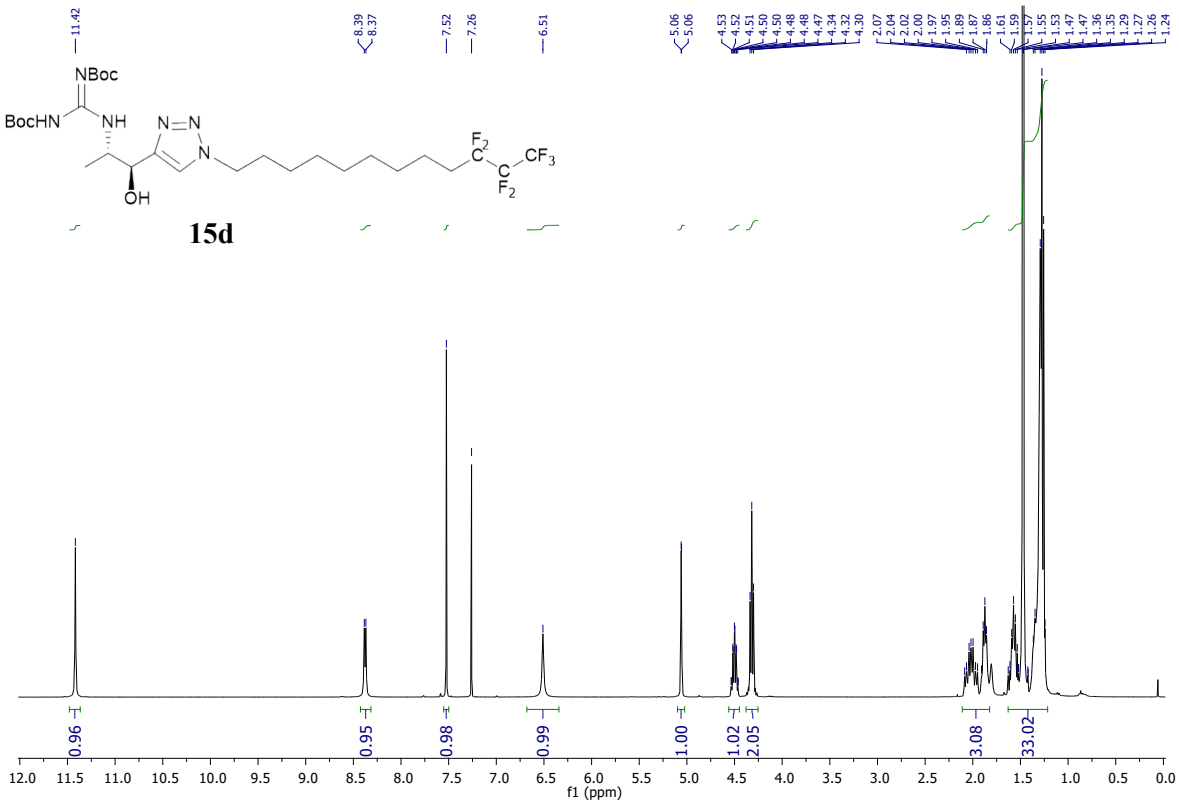

NBoc
BocHN
NH
N=N
N
CF3
OH
15b
f1 (ppm)
f1 (ppm)


-66.68
-66.71
-66.74
-66.64
-66.68
-66.72
-66.76
f1 (ppm)
3.00
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)

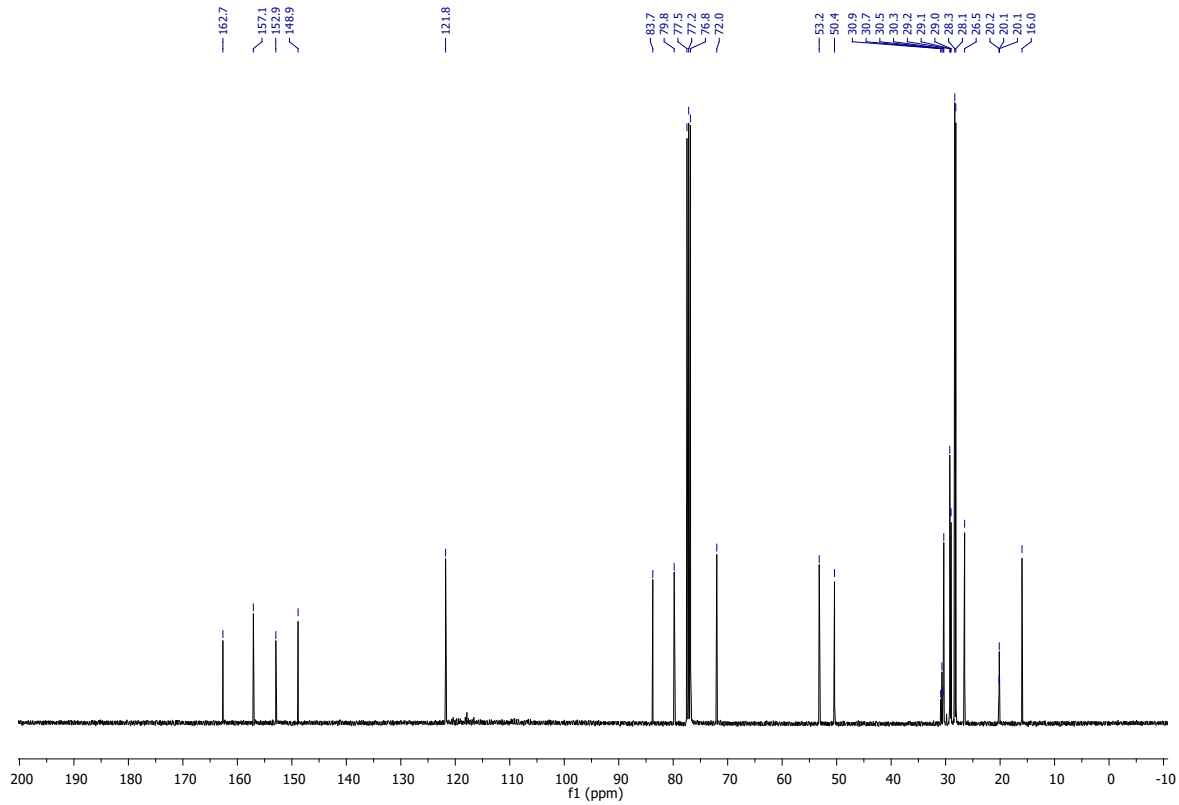

NBoc
BocHN
NH
N=N
N
OH
CF3
F2
11.41
8.37
8.36
7.51
7.26
6.49
5.04
5.04
4.52
4.51
4.50
4.49
4.48
4.47
4.47
4.45
4.45
4.32
4.30
4.28
2.01
1.99
1.97
1.95
1.92
1.90
1.87
1.85
1.83
1.58
1.56
1.54
1.52
1.50
1.45
1.34
1.32
1.30
1.28
1.27
1.25
1.23
0.98
0.91
0.99
0.67
1.00
1.01
1.98
4.02
35.04
f1 (ppm)
162.6
157.0
152.9
148.8
121.7
83.7
79.7
77.5
77.2
76.8
71.9
53.1
50.4
30.7
30.3
29.3
29.3
29.2
29.1
29.0
28.3
28.1
26.5
20.3
15.9
f1 (ppm)

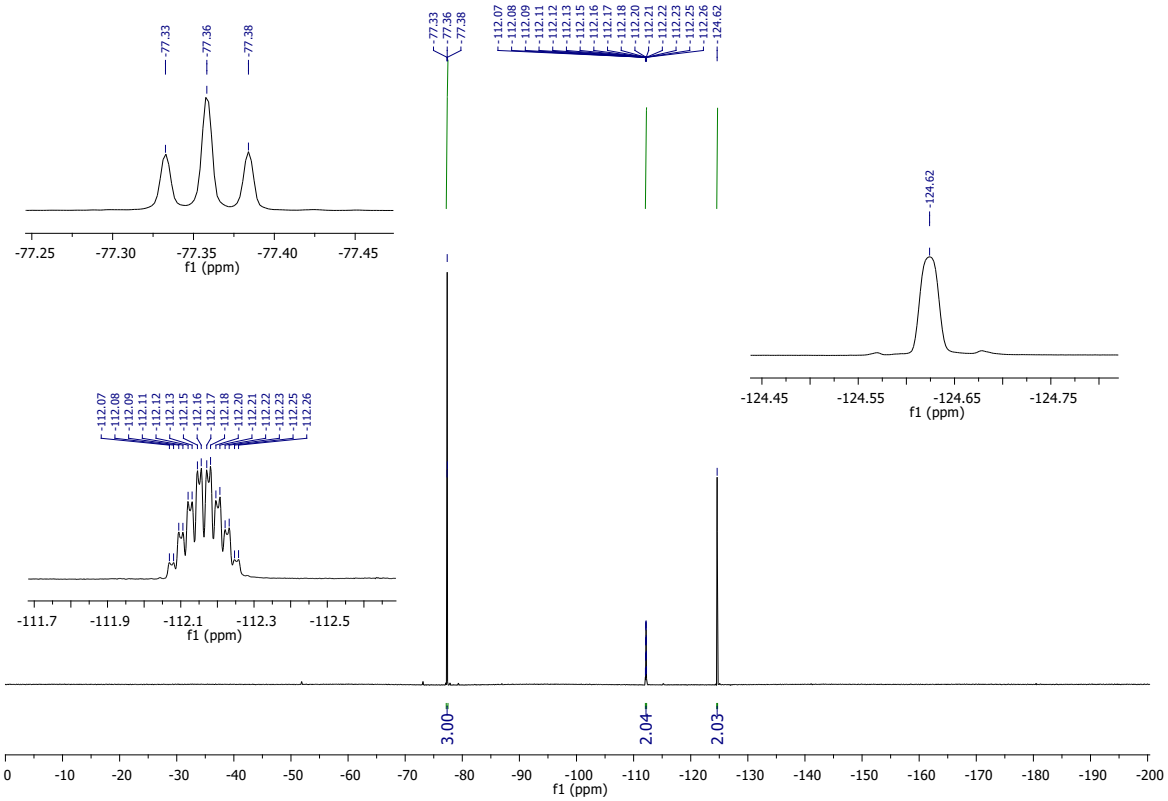
15c

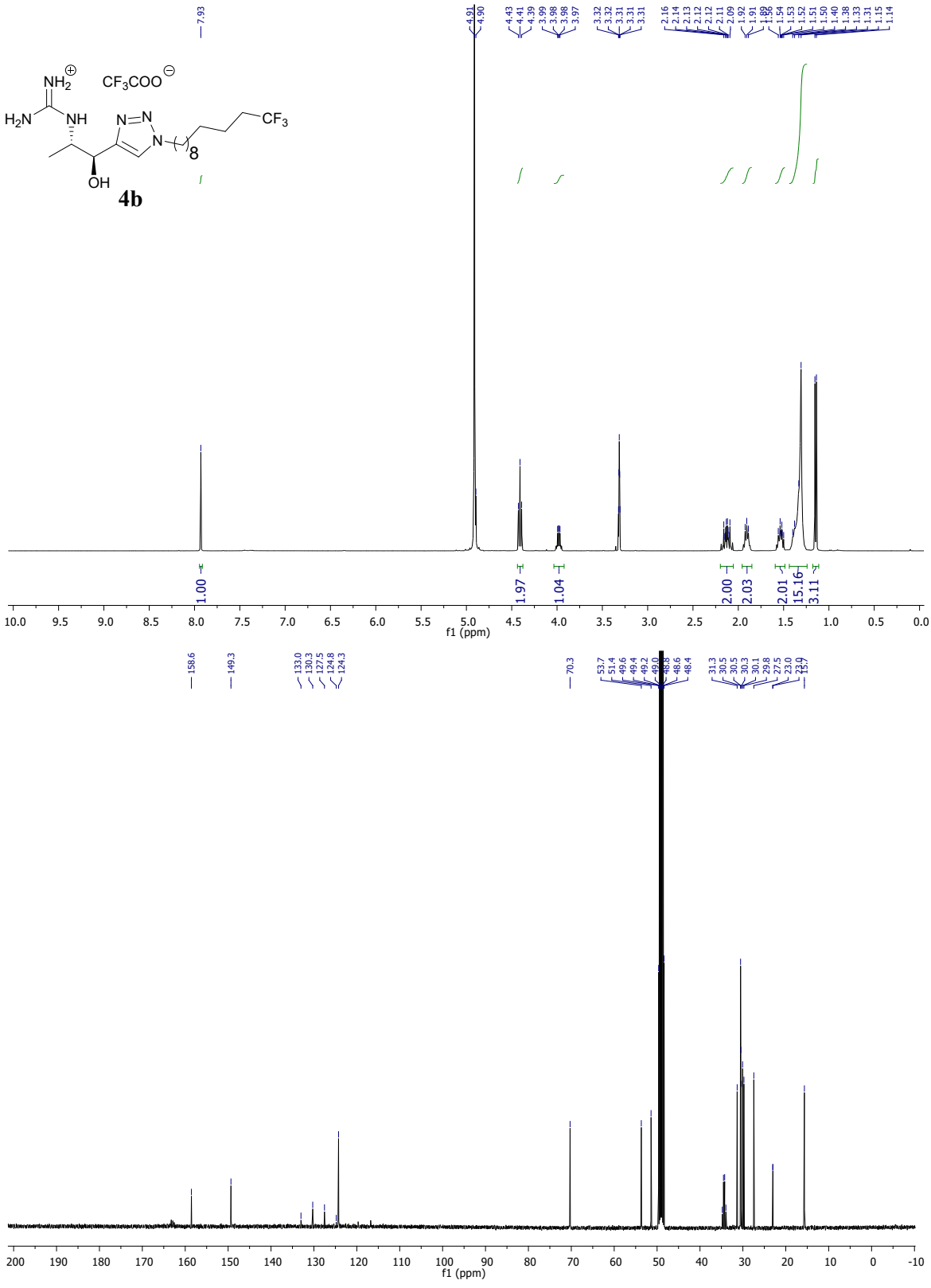

-81.88
-81.6
-81.8
-82.0
-82.2
f1 (ppm)
-81.88
-114.68
-114.73
-114.78
-114.68
-114.73
-114.78
-114.4
-114.6
-114.8
-115.0
f1 (ppm)
3.00
2.03
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)

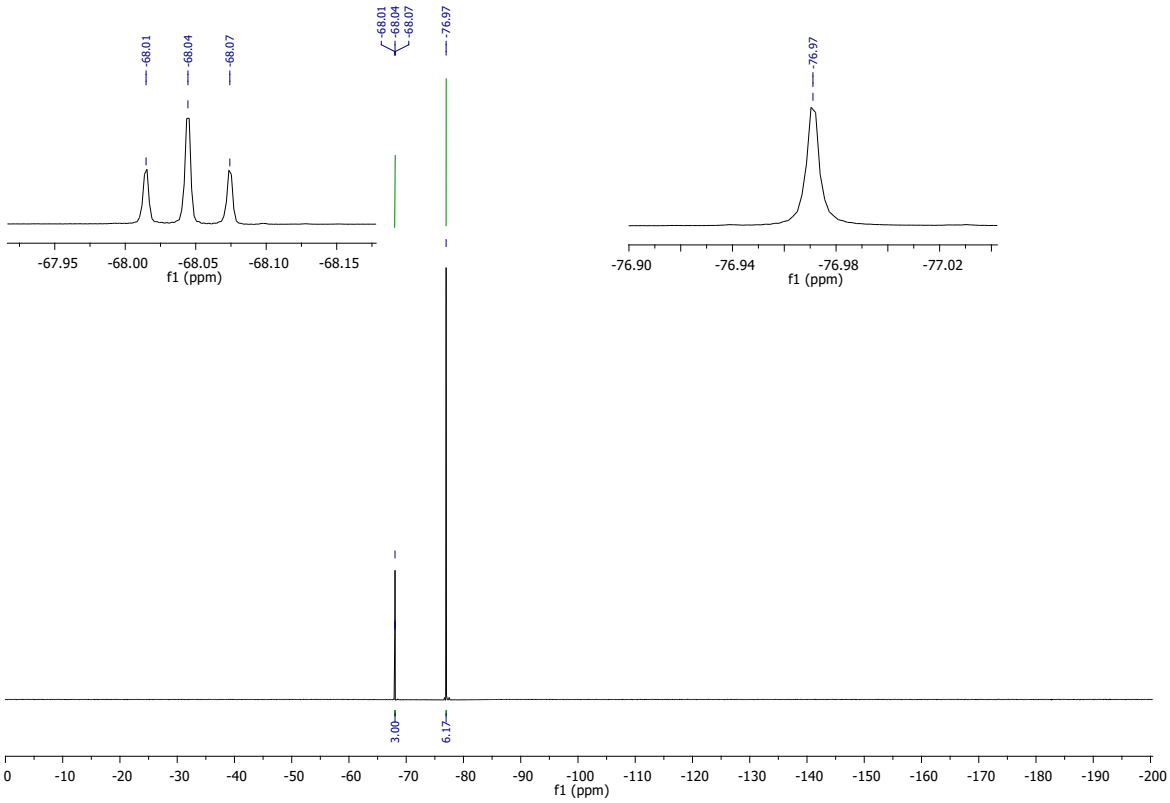

NBoc
BocHN
NH
N=N
N
OH
F2C
CF3
CF2
11.42
8.39
8.37
7.52
7.26
6.51
5.06
5.06
4.53
4.52
4.51
4.50
4.50
4.48
4.48
4.47
4.34
4.32
4.30
2.07
2.04
2.02
2.00
1.97
1.95
1.89
1.87
1.86
1.61
1.59
1.57
1.55
1.53
1.47
1.47
1.36
1.35
1.29
1.27
1.26
1.24
0.96
0.95
0.98
0.99
1.00
1.02
2.05
3.08
33.02
f1 (ppm)

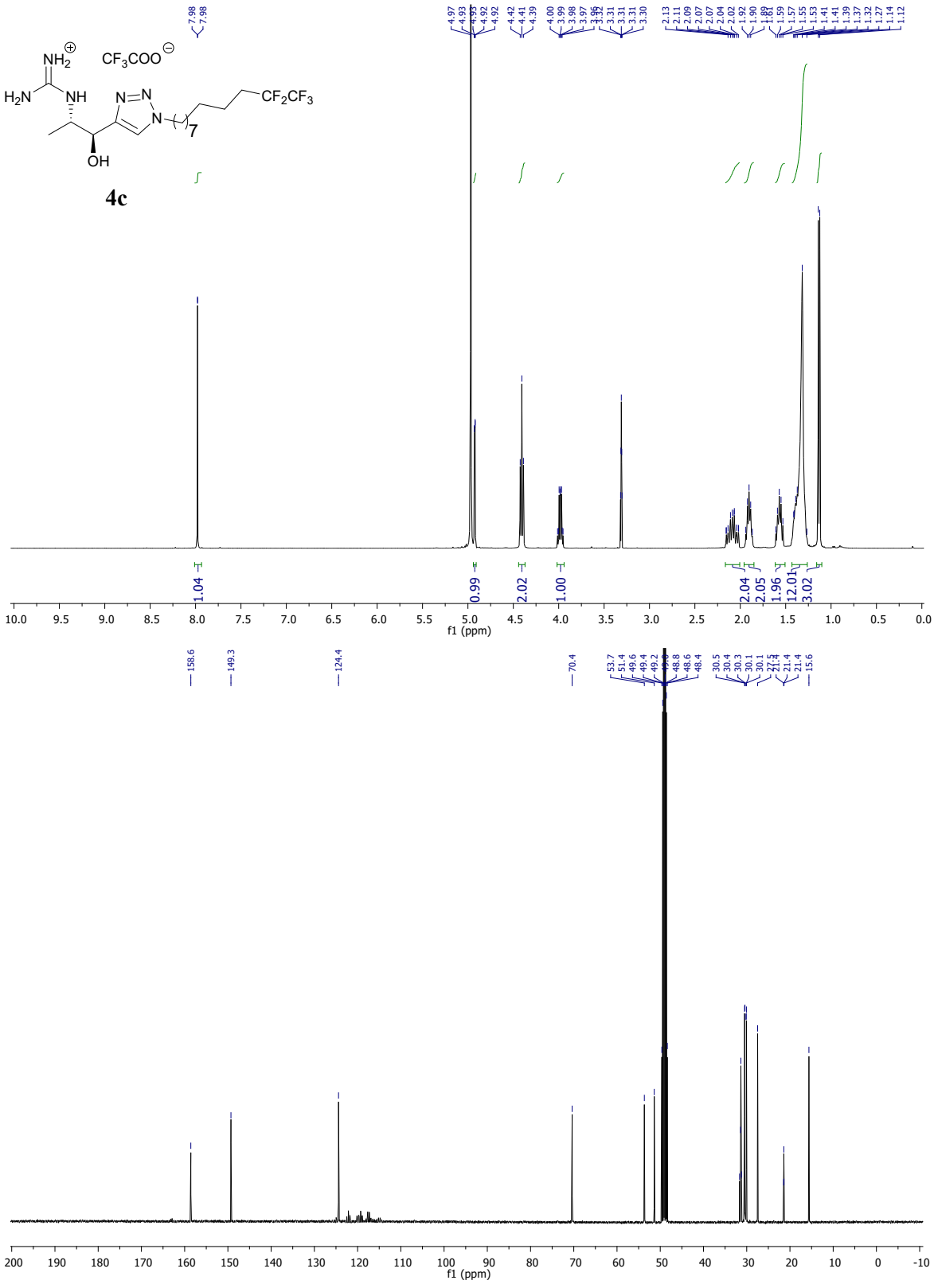
15d

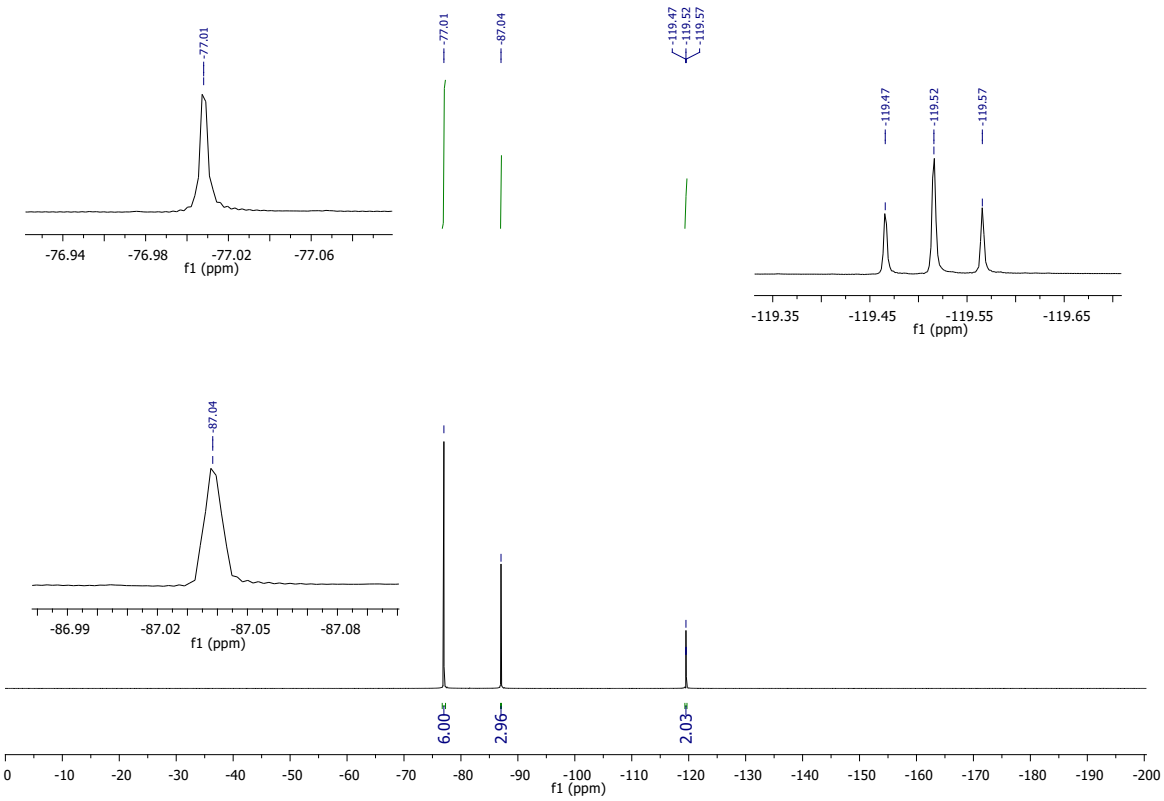

162.7
157.1
152.9
148.9
121.8
83.7
79.8
77.5
77.2
76.8
72.0
53.2
50.4
30.9
30.7
30.5
30.3
29.2
29.1
29.0
28.3
28.1
26.5
20.2
20.1
20.1
16.0
f1 (ppm)


-77.33
-77.36
-77.38
-77.25
-77.30
-77.35
-77.40
-77.45
f1 (ppm)
-112.07
-112.08
-112.09
-112.11
-112.12
-112.13
-112.15
-112.16
-112.17
-112.18
-112.20
-112.21
-112.22
-112.23
-112.25
-112.26
-124.62
-111.7
-111.9
-112.1
-112.3
-112.5
f1 (ppm)
-124.45
-124.55
-124.65
-124.75
f1 (ppm)
3.00
2.04
2.03
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)


NH2
CF3COO
H2N
NH
N=N
N
8
CF3
OH
4b
f1 (ppm)


-68.01
-68.04
-68.07
-76.97
-67.95
-68.00
-68.05
-68.10
-68.15
f1 (ppm)
-76.90
-76.94
-76.98
-77.02
f1 (ppm)
3.00
6.17
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)


NH2
CF3COO
H2N
NH
N
N
7
CF2CF3
OH
4c
f1 (ppm)
f1 (ppm)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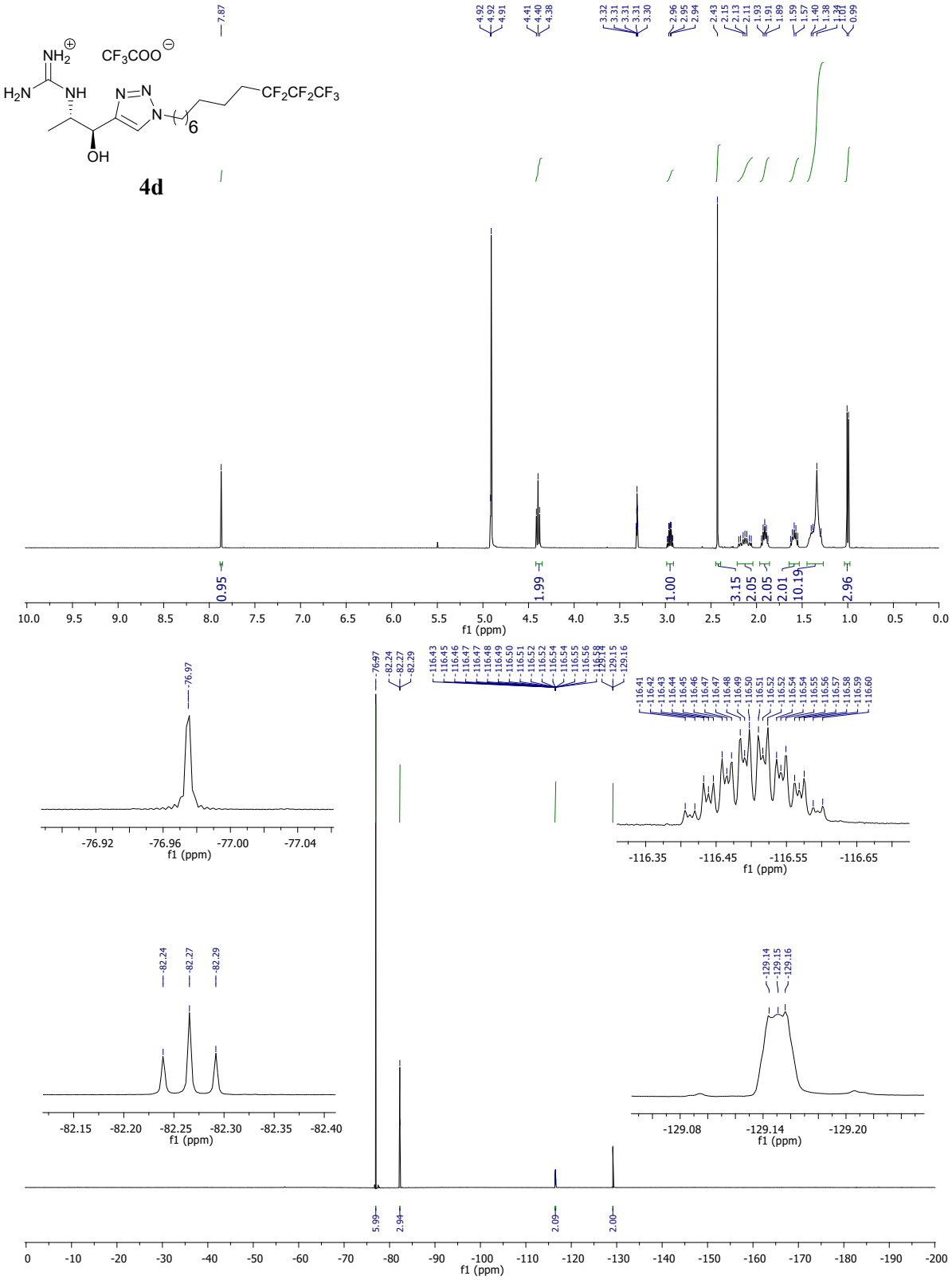
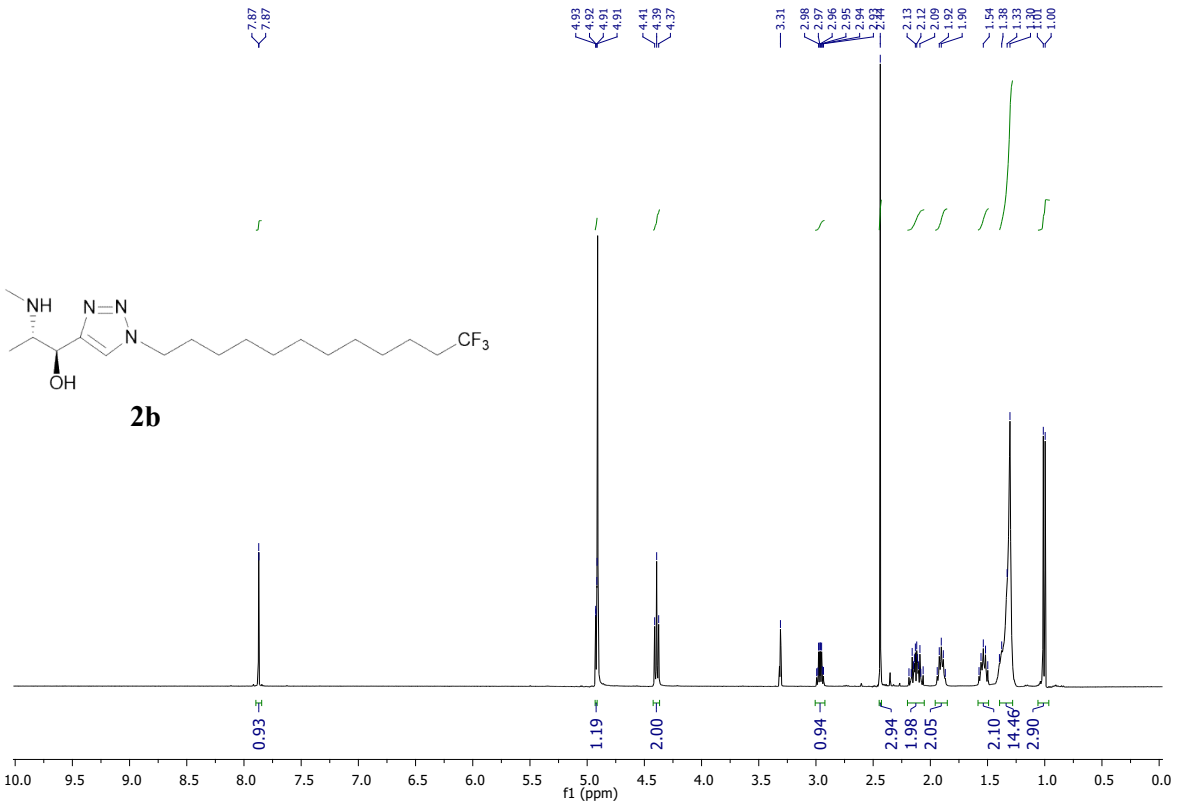
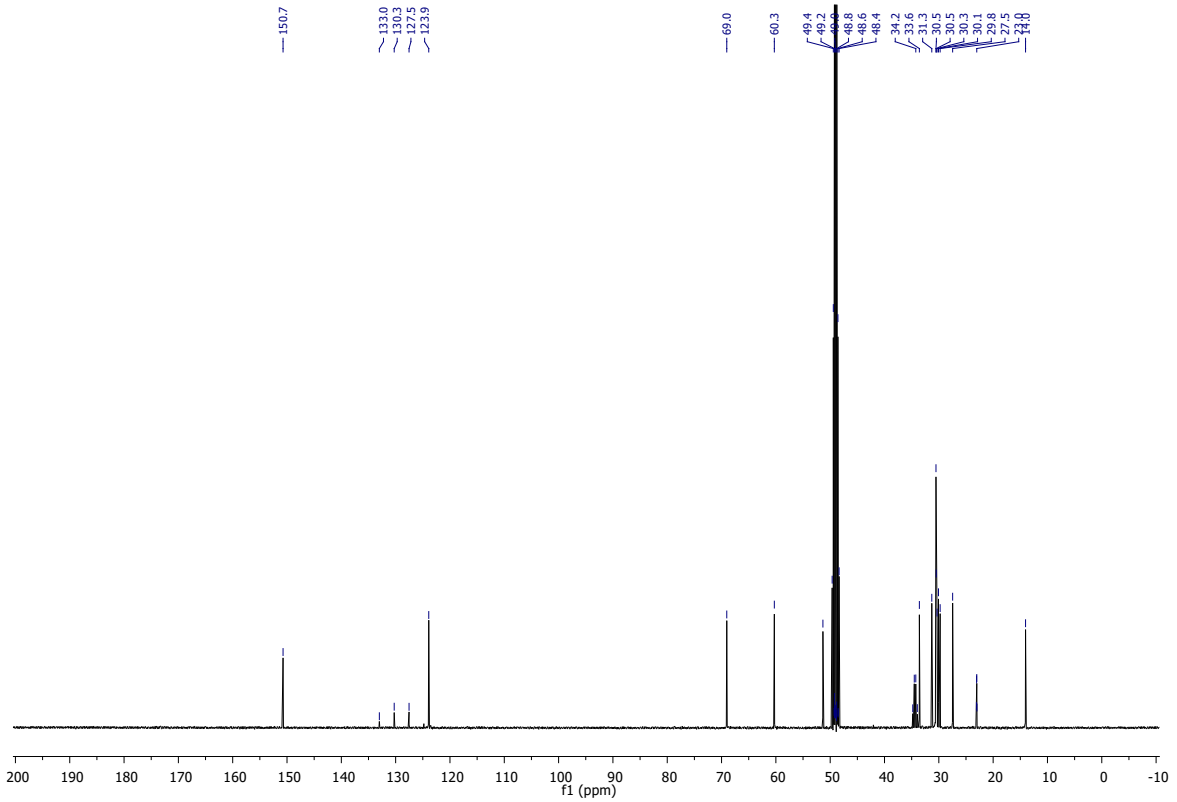
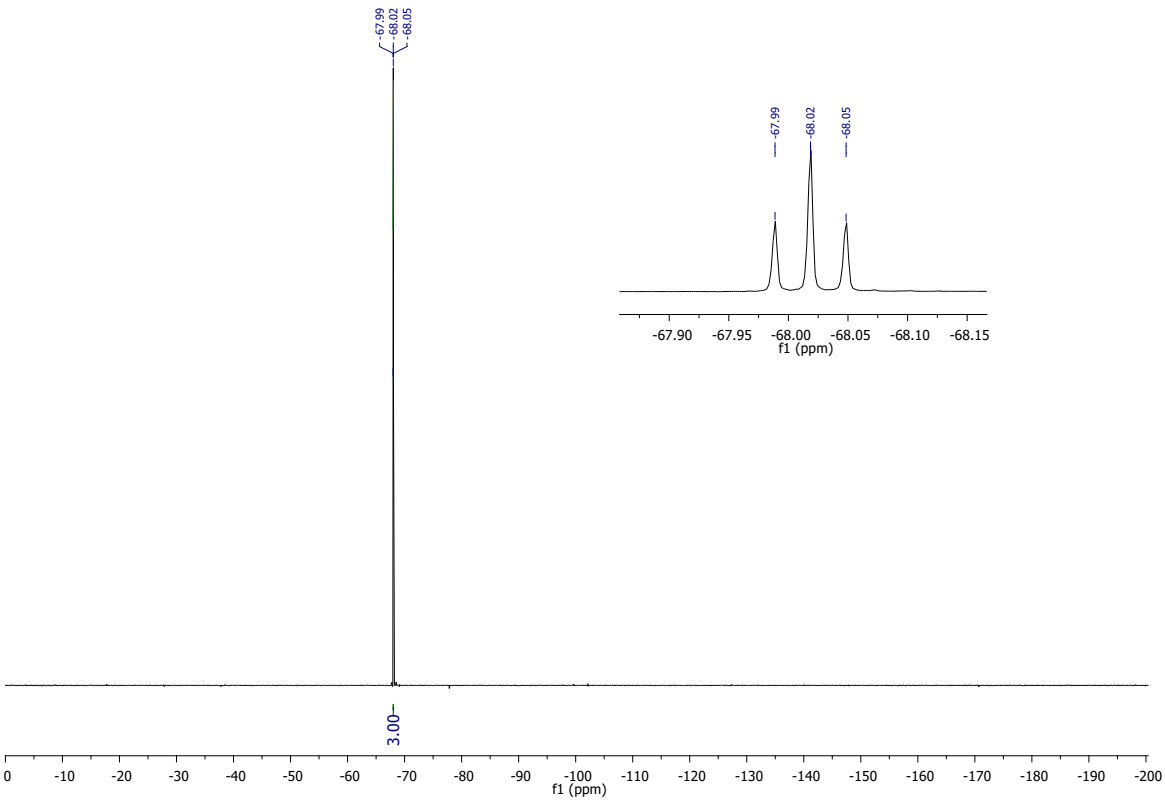
-119.47
-119.52
-119.57
-76.94
-76.98
-77.02
-77.06
f1 (ppm)
-119.35
-119.45
-119.55
-119.65
f1 (ppm)
-86.99
-87.02
-87.05
-87.08
f1 (ppm)
6.00
2.96
2.03
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)


NH2
CF3COO
H2N
NH
N=N
N
6
CF2CF2CF3
OH
4d
f1 (ppm)



2b

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-68.05
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0
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-20
-30
-40
-50
-60
-70
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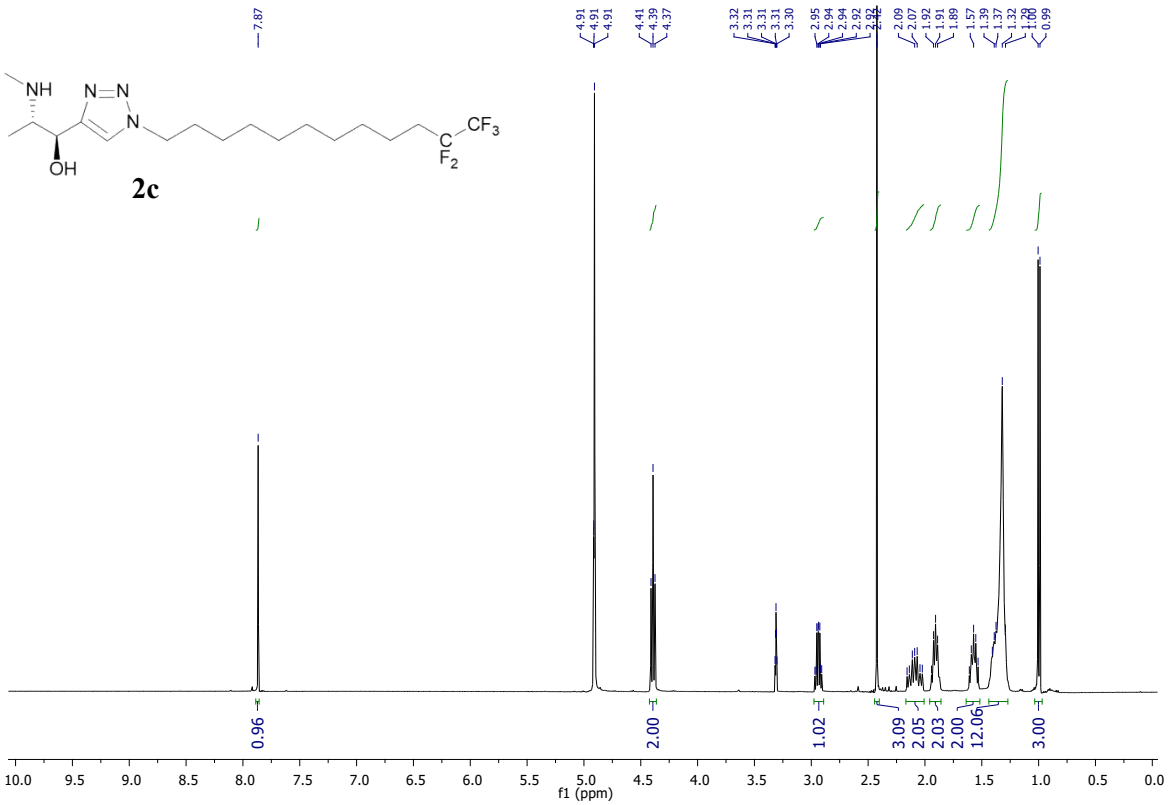
-68.05
-68.10
-68.15
f1 (ppm)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N
OH
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C
F2
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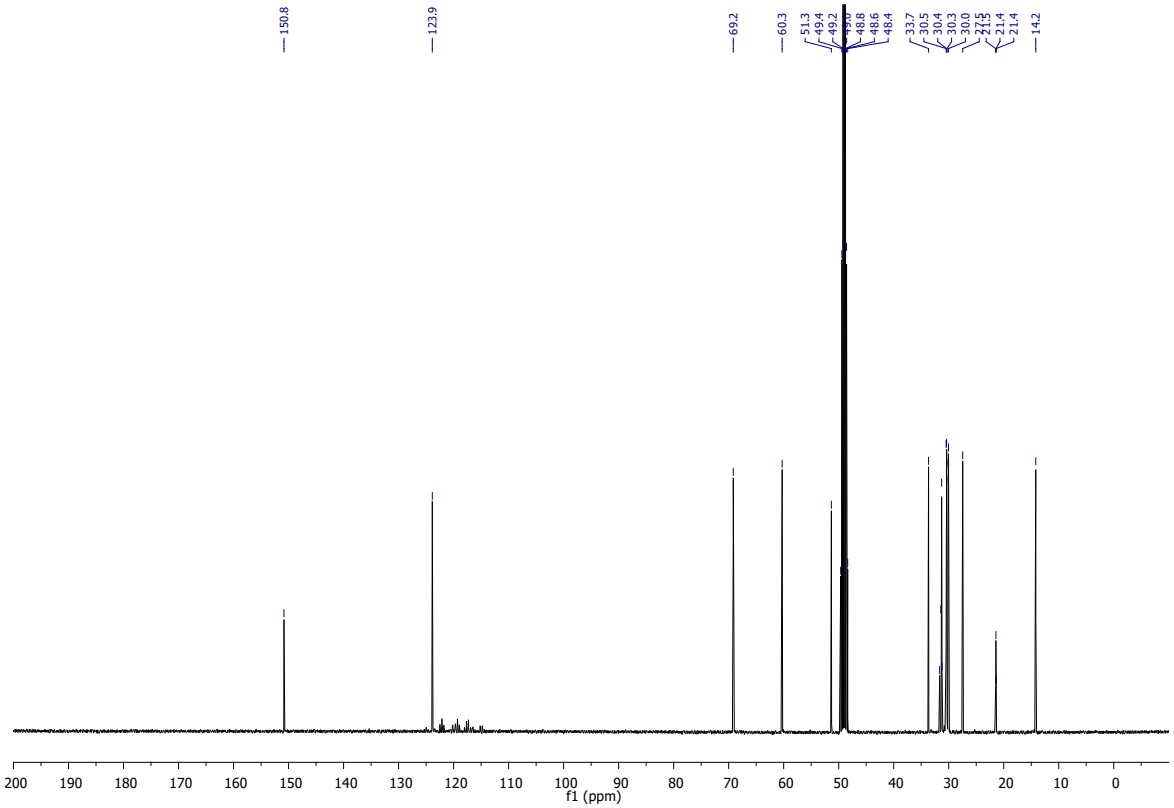
4.91
4.91
4.91
4.41
4.39
4.37
3.32
3.31
3.31
3.31
3.30
2.95
2.94
2.94
2.92
2.92
2.32
2.09
2.07
1.92
1.91
1.89
1.57
1.39
1.37
1.32
1.08
1.00
0.99
0.96
2.00
1.02
3.09
2.05
2.03
2.00
12.06
3.00
f1 (ppm)

2c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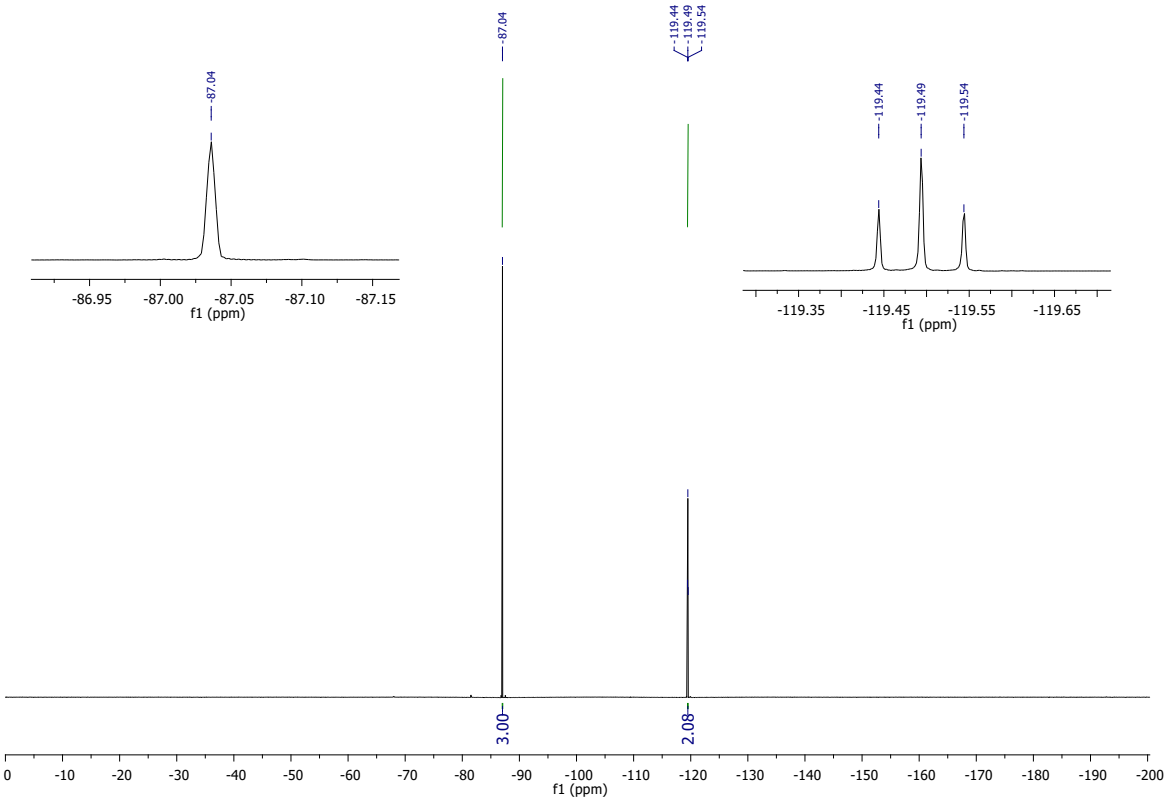
123.9
69.2
60.3
51.3
49.4
49.2
48.8
48.6
48.4
33.7
30.5
30.4
30.3
30.0
27.5
21.4
21.4
14.2
f1 (ppm)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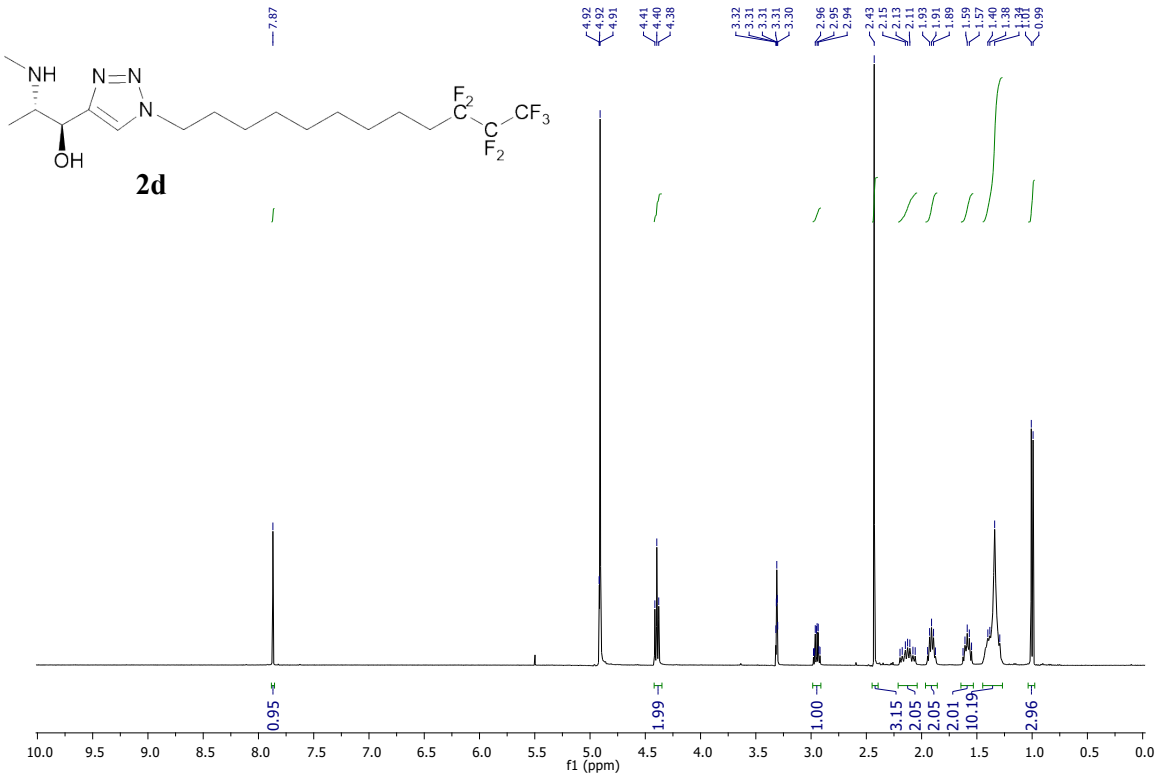
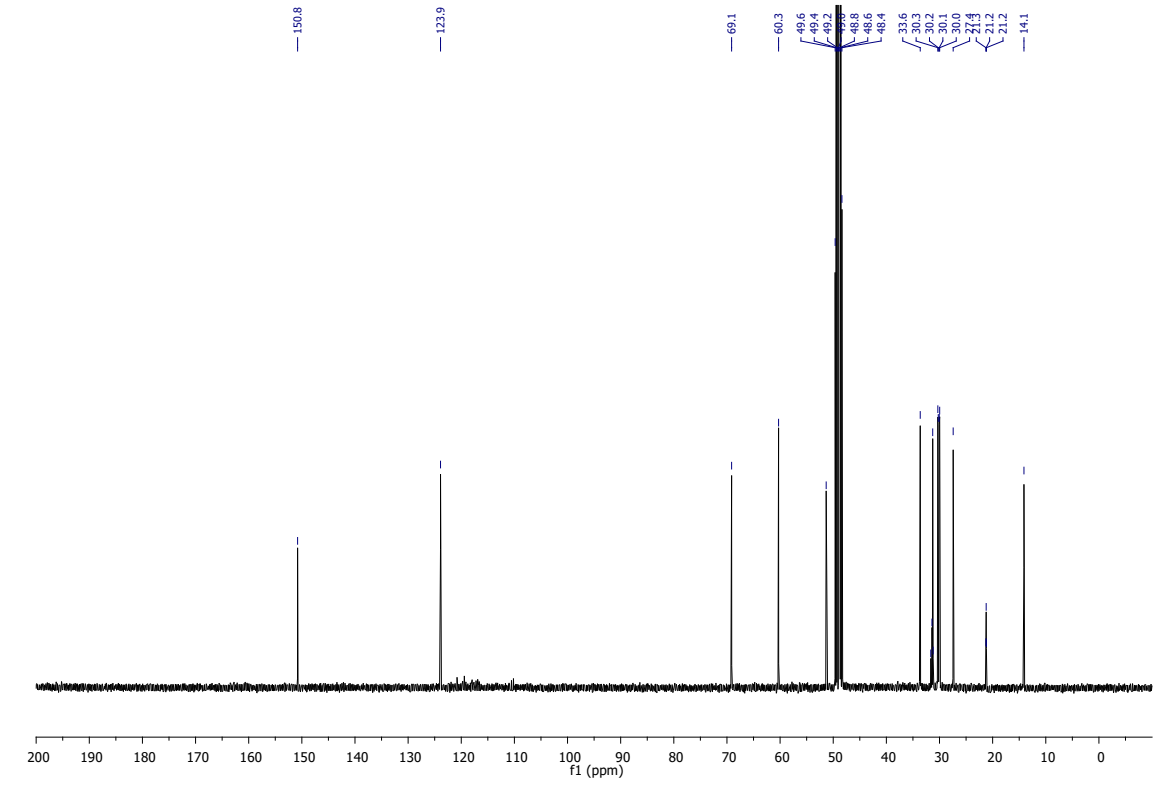
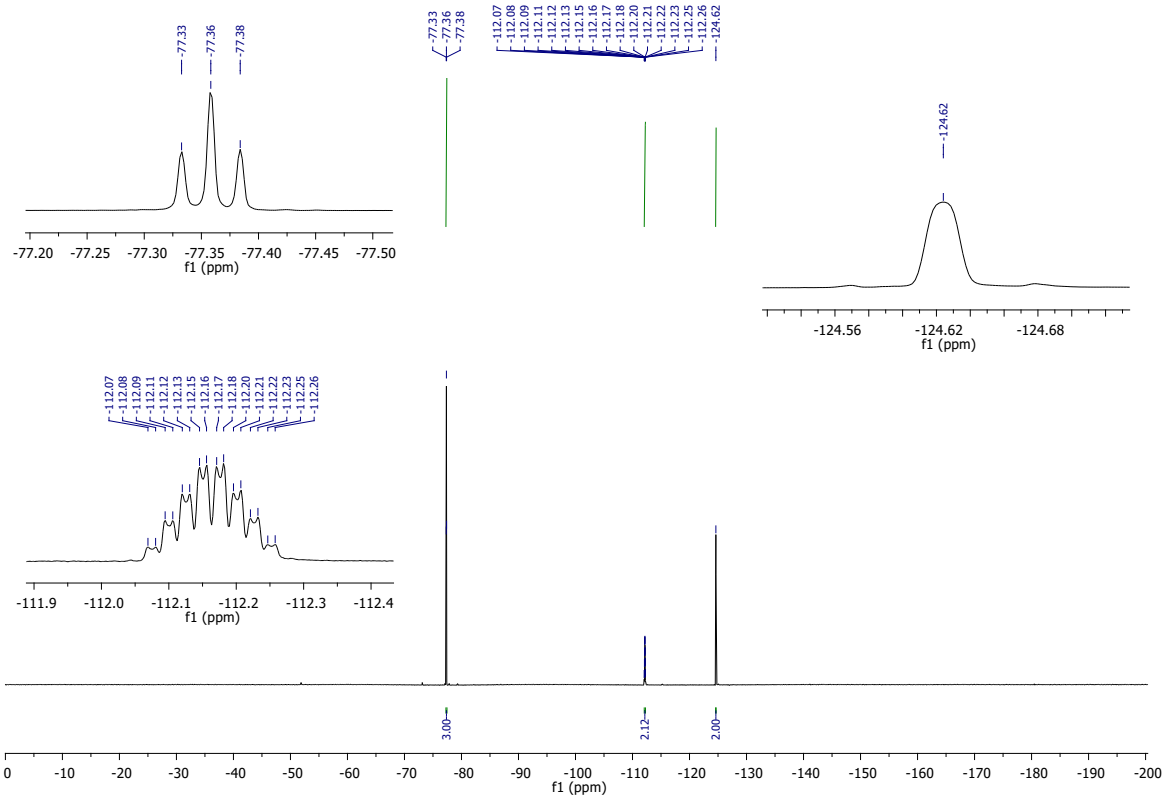
-86.95
-87.00
-87.05
-87.10
-87.15
f1 (ppm)
-119.35
-119.45
-119.55
-119.65
f1 (ppm)
3.00
2.08
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)


NH
N=N
N
OH
F2
C
CF3
C
F2
2d
7.87
4.92
4.92
4.91
4.41
4.40
4.38
3.32
3.31
3.31
3.31
3.30
2.96
2.95
2.94
2.43
2.15
2.13
2.11
1.93
1.91
1.89
1.59
1.57
1.40
1.38
1.01
0.99
0.95
1.99
1.00
3.15
2.05
2.01
10.19
2.96
f1 (ppm)


150.8
123.9
69.1
60.3
49.6
49.4
49.2
48.8
48.6
48.4
33.6
30.3
30.2
30.1
30.0
21.3
21.2
21.2
14.1
f1 (ppm)


-77.33
-77.36
-77.38
-77.20 -77.25 -77.30 -77.35 -77.40 -77.45 -77.50
f1 (ppm)
-112.07
-112.08
-112.09
-112.11
-112.12
-112.13
-112.15
-112.16
-112.17
-112.18
-112.20
-112.21
-112.22
-112.23
-112.25
-112.26
-124.62
-124.62
-124.56 -124.62 -124.68
f1 (ppm)
-111.9 -112.0 -112.1 -112.2 -112.3 -112.4
f1 (ppm)
3.00
2.12
2.00
0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

5. Sphingosine kinase activity quantification.

In order to evaluate the inhibitory capacity of the synthesized compounds, the Sphingosine kinase activity in presence of these inhibitors was determined.

Adapta™ Universal Kinase Assay kit (Invitrogen, Carlsbad, CA, USA) was used following the manufacturer's protocol. Human SphK1 and SphK2 recombinant proteins (Thermo Fisher, Madrid, Spain) were assayed in independent experiments and each condition was analyzed in triplicate at room temperature. SphK1 was used at final concentration of 0.025 ng/µl, SphK2 at 0.8 ng/µl and ATP and sphingosine were used at a final concentration of 1 µM and 5 µM respectively for both kinases.

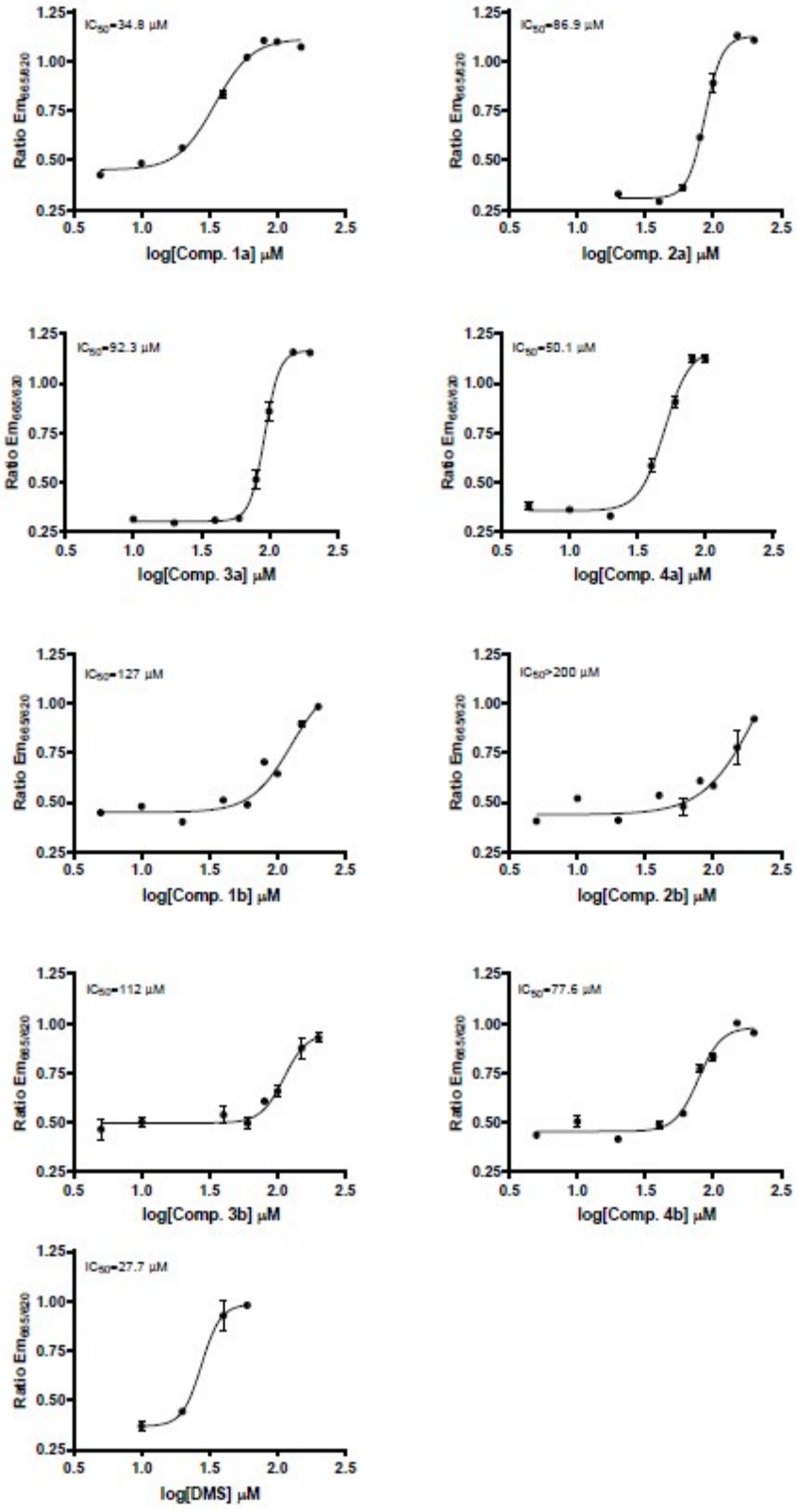
Inhibitors were dissolved in 100% DMSO at an initial concentration of 50 mM. Several dilutions were performed also in DMSO, and a pre-dilution of each sample in 1X Kinase Buffer A was performed. The final concentrations assayed of each compound were 200 µM, 150 µM, 100 µM, 80 µM, 60 µM, 40 µM, 20 µM, 10 µM and 5 µM.

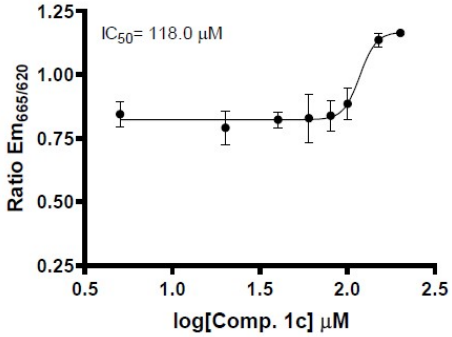
TR-FRET was quantified using a CLARIOstar microplate reader (BMG Labech, Biogen científica SL, Madrid, Spain) using the following parameters: Ex = 340 nm; Em1 = 620/10; Em2 = 665/10; delay = 100 µs; integration time = 200 µs; Focal height = 11 mm. The TR-FRET ratio of each condition was calculated as EM_{665nm}/EM_{620nm} and results expressed as a percentage of inhibition using the following equation:

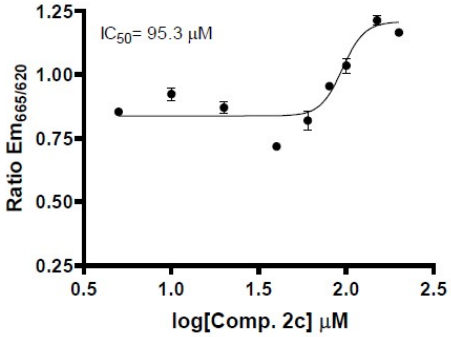
$$\%\ inhibition = \frac{(Ratio_{sample} - Ratio_{0\%\ inhibition})}{(Ratio_{100\%\ inhibition} - Ratio_{0\%\ inhibition})}$$

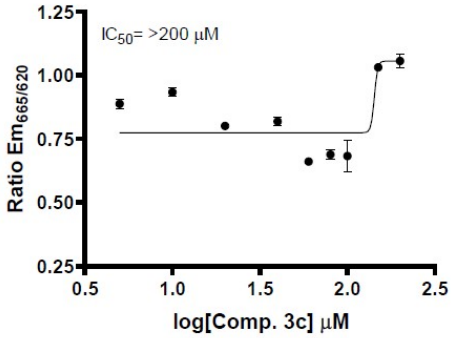
IC_{50} of each compound was calculated fitting the data to a sigmoidal dose-response curve with variable slope.

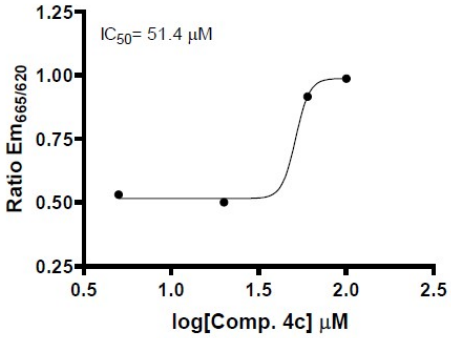
Figure 1. Inhibition curves of each compound for SK1

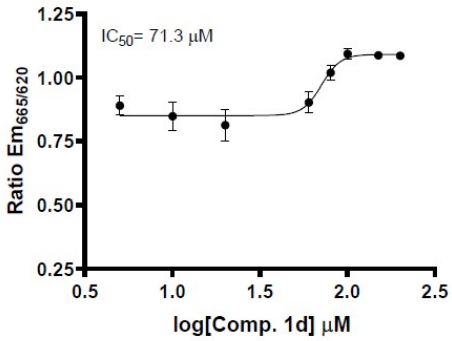


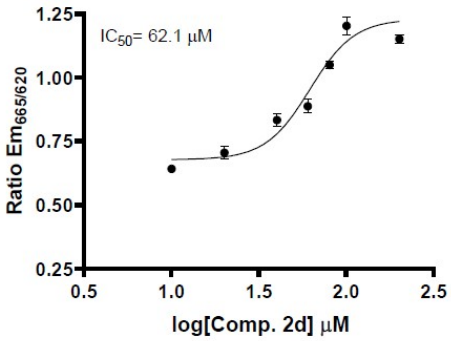

IC50= 118.0 µM
Ratio Em665/620
log[Comp. 1c] µM

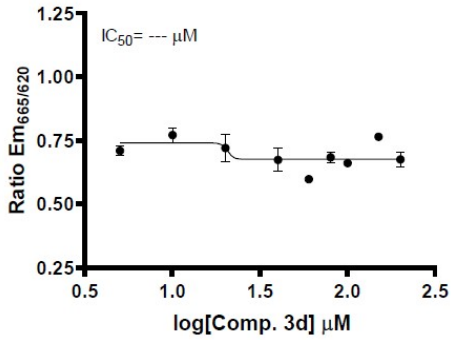

IC50= 95.3 µM
Ratio Em665/620
log[Comp. 2c] µM


IC50= >200 µM
Ratio Em665/620
log[Comp. 3c] µM


IC50= 51.4 µM
Ratio Em665/620
log[Comp. 4c] µM


IC50= 71.3 µM
Ratio Em665/620
log[Comp. 1d] µM


IC50= 62.1 µM
Ratio Em665/620
log[Comp. 2d] µM


IC50= --- µM
Ratio Em665/620
log[Comp. 3d] µM

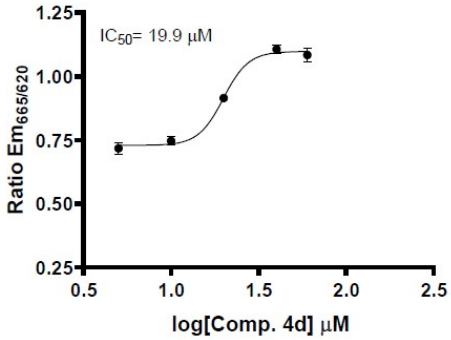
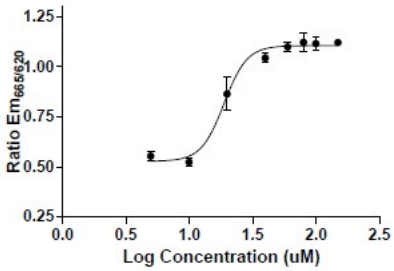
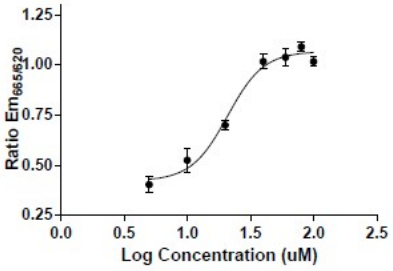

IC50= 19.9 µM
Ratio Em665/620
log[Comp. 4d] µM

Figure 2. Inhibition curves of each compound for SphK2:

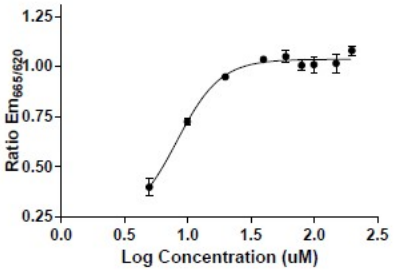
Compound **1a**



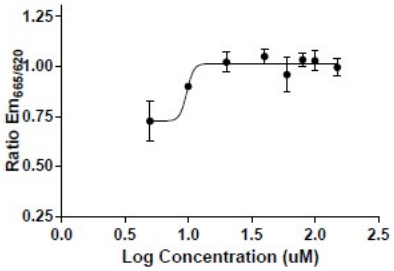
Compound **2a**



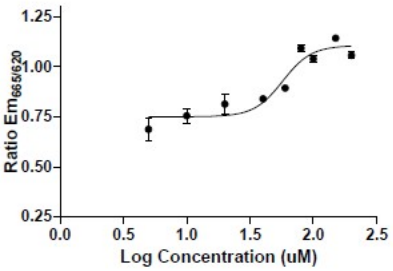
Compound **3a**



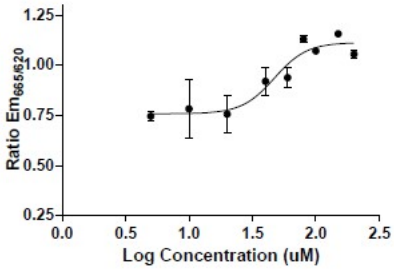
Compound **4a**



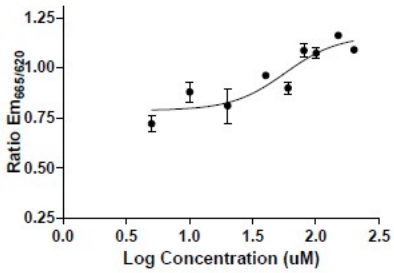
Compound **1b**



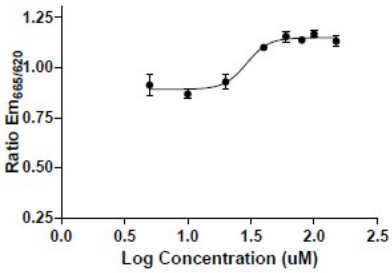
Compound **2b**



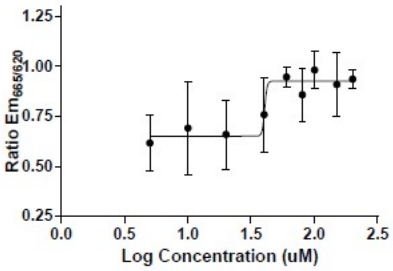
Compound **3b**



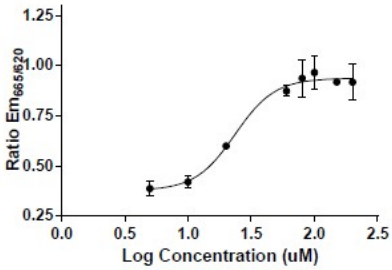
Compound **4b**



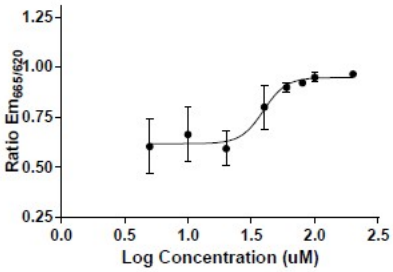
Compound **1c**



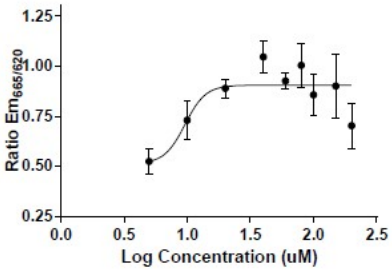
Compound **2c**



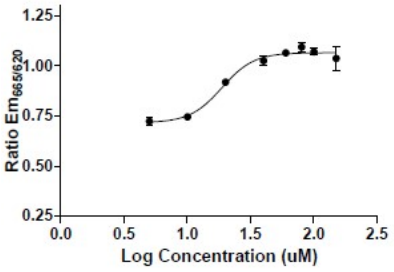
Compound **3c**



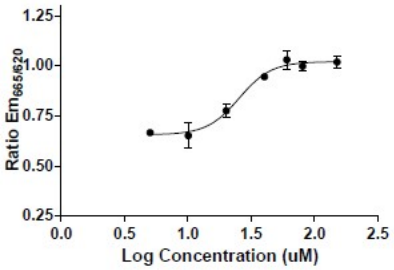
Compound **4c**



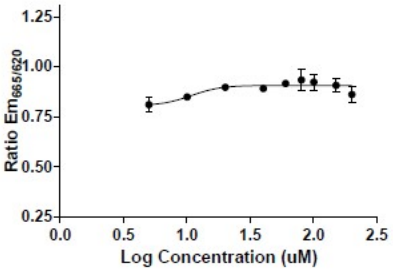
Compound **1d**



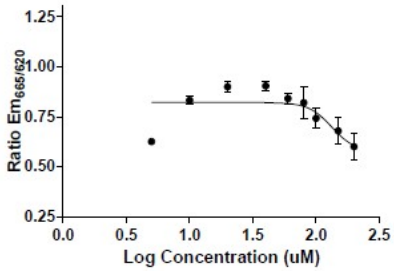
Compound **2d**



Compound **3d**



Compound **4d**



6. Docking studies.

6.1. General procedure

The protein structures of Sphk1 and Sphk2 were set up following the protocol previously described.[4] The crystal structure of Sphk1 (PDB ID: 3VZB, chain A) was prepared using MOE software,[5] retaining a key structural water molecule that interacts with residues Ser298 and Gly456. For Sphk2, which does not have an available crystal structure, a homology model was built in MOE using the same Sphk1 crystal structure, although the water molecule was omitted due to differences in residues. The synthesized molecules under study were prepared using MOE, Openbabel 3,[6] and Corina software.[7] Docking studies were conducted with rDock,[8] which included generating a docking cavity based on the cocrystallized sphingosine ligand, docking the prepared molecules, and scoring the resulting binding poses. The best-scored poses were analysed using PyMol visualization system.

6.2. Additional figures

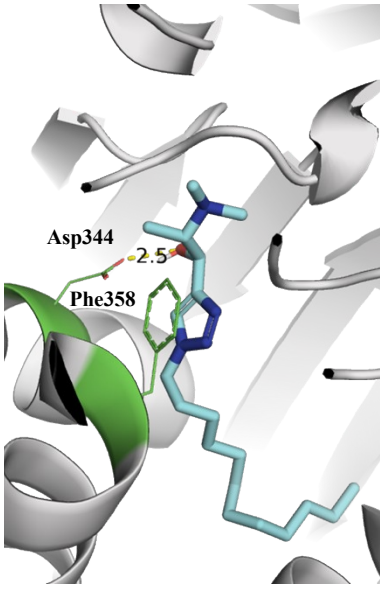


Figure 3. Best binding mode in SphK2 for compound **3a**.

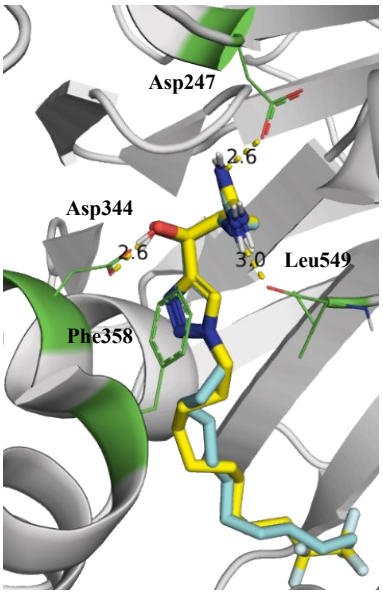


Figure 4. Best binding mode in SphK2 for compound **4c**.

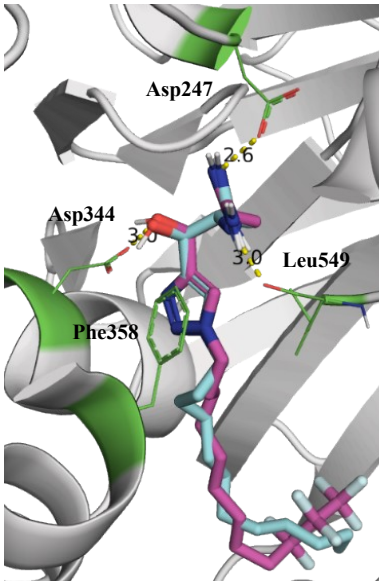


Figure 5. Best binding mode in SphK2 for compound **4d**.

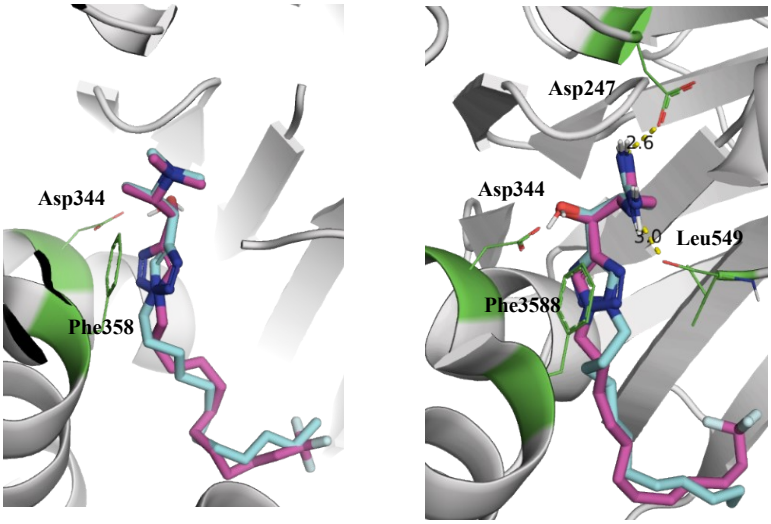


Figure 6. Best binding mode in Sphk2 for the less active compounds **3b** (left, pink in comparison with **3a**, blue) and **4b** (right, pink, in comparison with **4a** (blue).

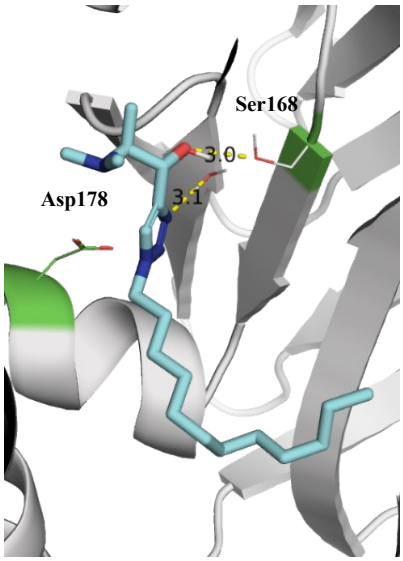


Figure 7. Best binding mode in SphK1 for compound **3a**.

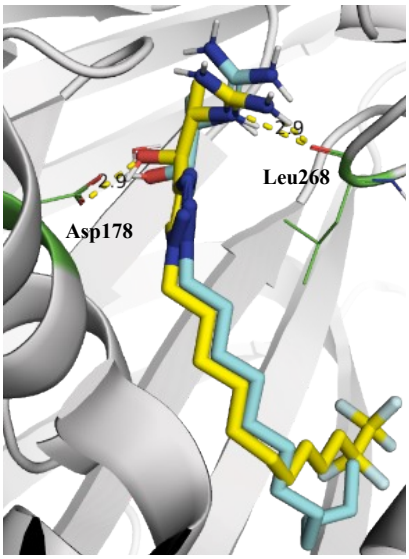


Figure 8. Best binding mode in SphK1 for compound **4c** (yellow) in comparison with **4a** (blue).

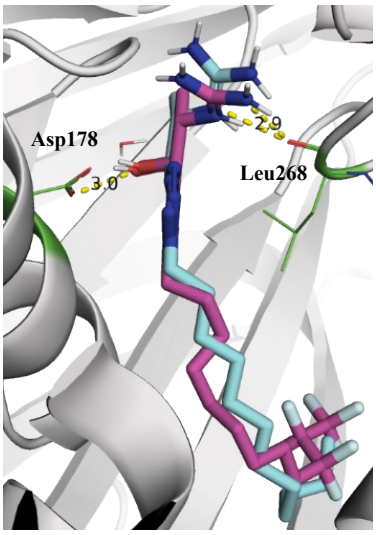


Figure 9. Best binding mode in SphK1 for compound **4d** (pink) in comparison with **4a** (blue).

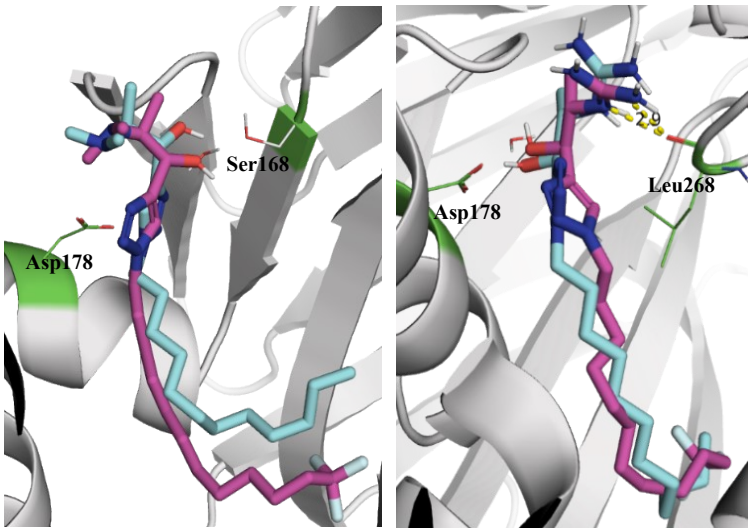


Figure 10. Best binding mode in Sphk1 for the less active compounds **3b** (left, pink in comparison with **3a**, blue) and **4b** (right, pink), in comparison with **4a** (blue).

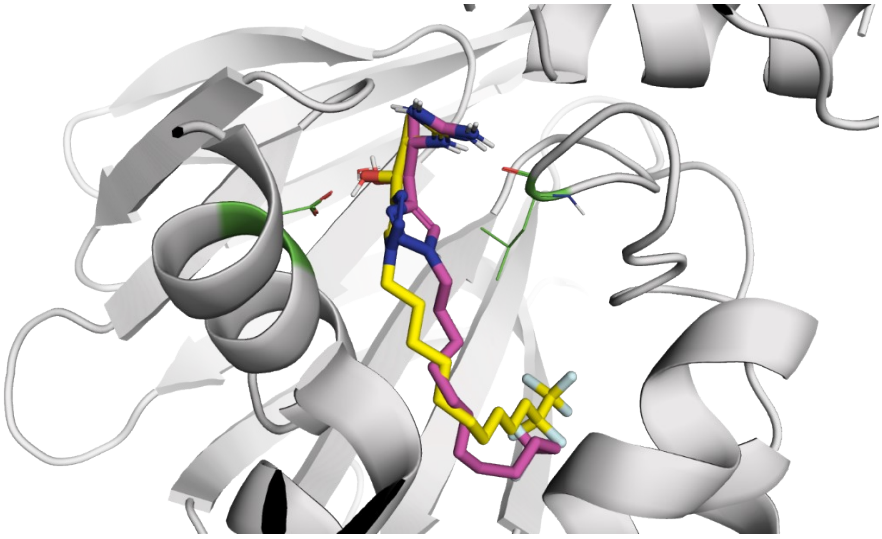


Figure 11. Best binding mode in SphK1 for compound **4c** (yellow) in comparison with **4b** (pink).

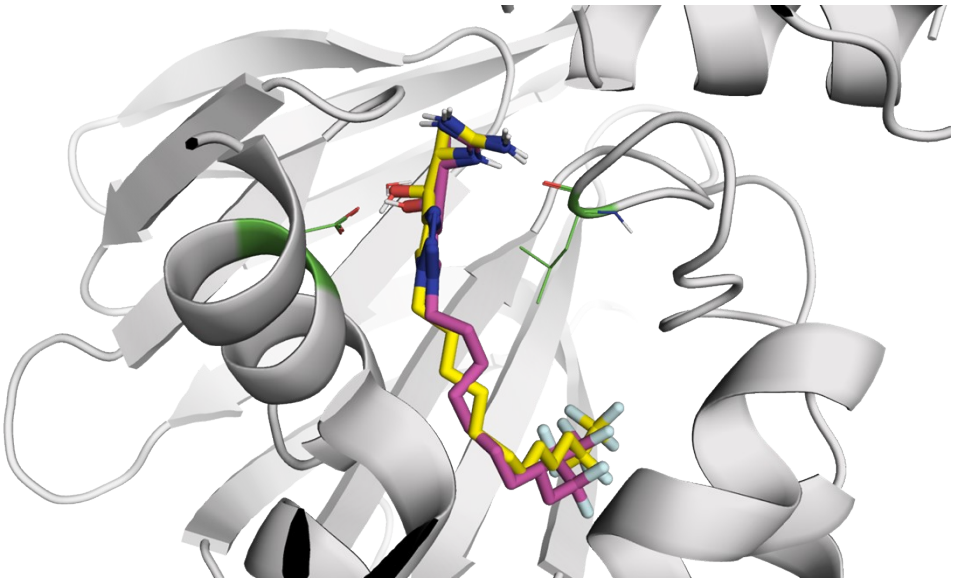


Figure 12. Best binding mode in SphK1 product **4d** (pink) in comparison with **4c** (yellow).

7. References

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