Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting information

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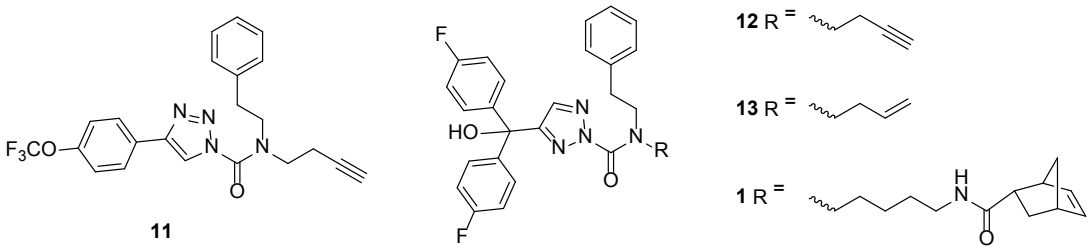
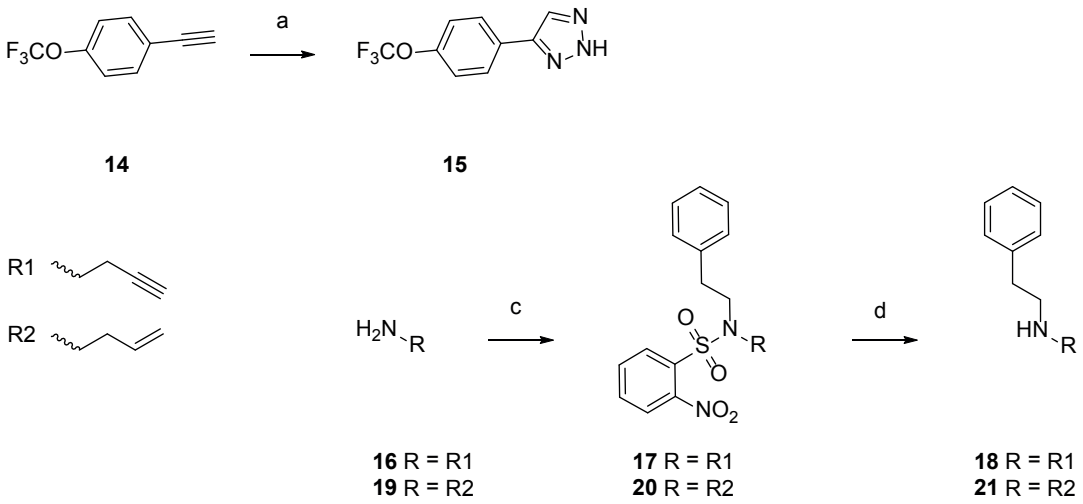
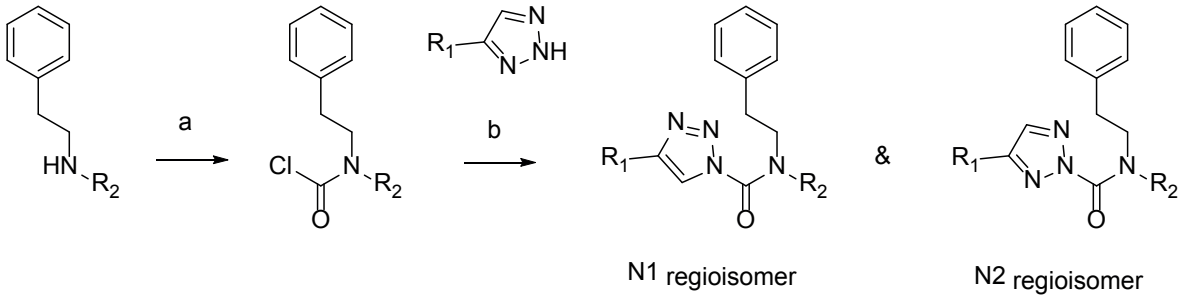


SI Figure 1. Hybrid probes of DH376 and HT-01 **11** - **13**, which were used for the structural assignment of probe **1.**



SI Scheme 1. Synthesis of triazole and amines for the hybrid probes **11 - 13**. Reagents and conditions: (a) TMS-N_3, CuI, DMF/MeOH, 100 °C, 18 h, 27%; (c) i. NsCl, Et_3N, THF; ii. $Ph(CH_2)_2Br$, Cs_2CO_3, CH_3CN, 80 °C, **17**: 93%; **20**: 70%; (d) PhSH, Cs_2CO_3, CH_3CN, **18**: 74%; **21**: 81%.



SI Scheme 2. Triphosgene coupling for synthesis of triazole ureas. Reagents and conditions: (a) triphosgene, DIPEA, THF, 0 °C; (b) DIPEA, DMAP, THF, 60 °C. Isolated yields: **11**: 37%; **22**: 41%; **23**: 33%; **12**: 40%; **24**: 29%; **13**: 11% (Table 2).

Assignment regioisomers

Table 1. Computed values of the ^{13}C shift of the triazole carbon (indicated with the red circle). Simplified structures of N1 and N2 regioisomers for DFT calculations are shown.

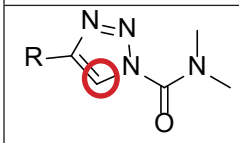
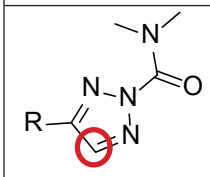
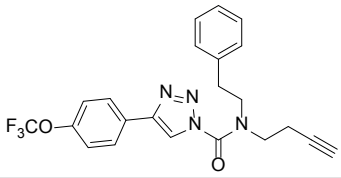
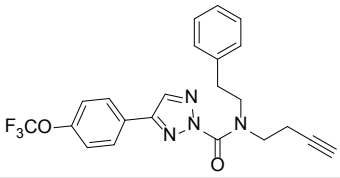
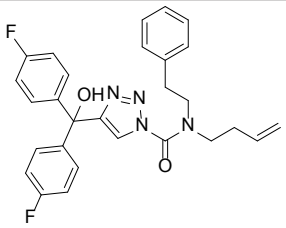
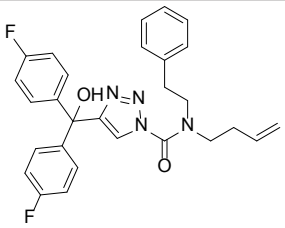
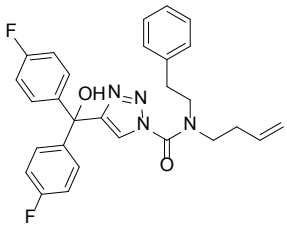
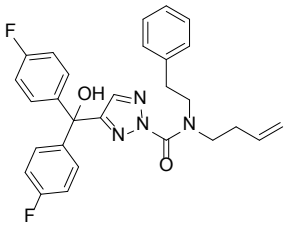
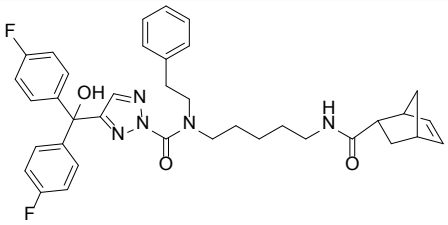
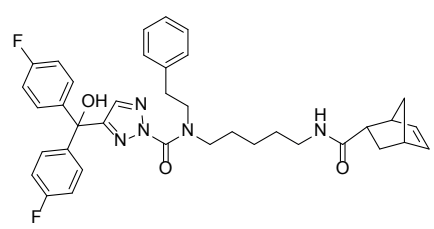
Structure	Theoretical δ (ppm)
	122 (R = Ph) 124 (R = Me)
	135 (R = Ph) 138 (R = Me)

Table 2. Final compounds obtained from triphosgene couplings (Scheme 1, SI Scheme 2) and the ^{13}C NMR chemical shift of the triazole carbon of 1,2,3-triazole urea regioisomers (measured in DMSO).

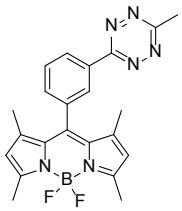
Entry	Structure	^{13}C ppm	Entry	Structure	^{13}C ppm
11		126	**22**		134
23		123	**12**		135
24		123	**13**		135
			1		135

SI Figure 2



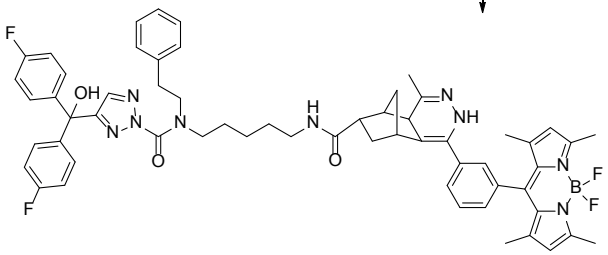
1

Chemical Formula: $C_{37}H_{39}F_2N_5O_3$
Exact Mass: 639.30

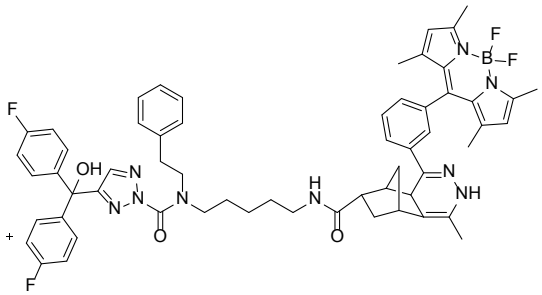


10

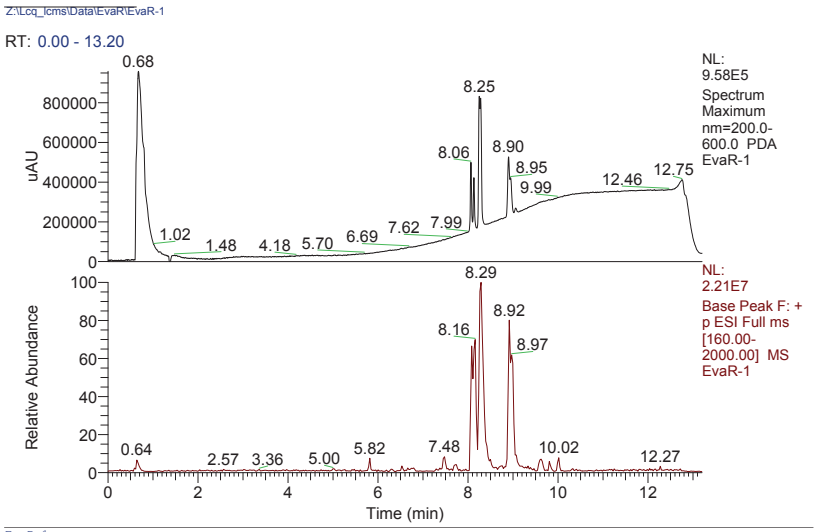
Chemical Formula: $C_{22}H_{21}BF_2N_6$
Exact Mass: 418.19

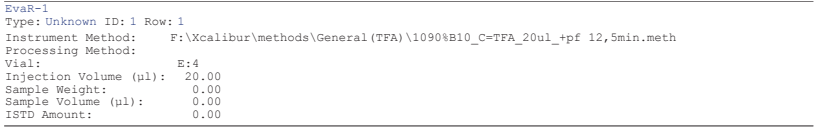


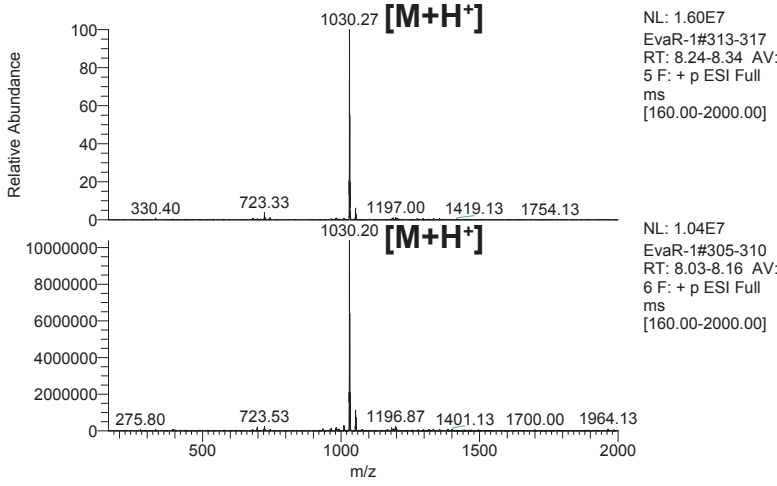
Chemical Formula: $C_{59}H_{60}BF_4N_9O_3$
Exact Mass: 1029.48



Chemical Formula: $C_{59}H_{60}BF_4N_9O_3$
Exact Mass: 1029.48







Experimental

Synthesis

General methods. Reagents were purchased from Sigma Aldrich, Acros or Merck and used without further purification unless noted otherwise. Reactions under dry conditions were performed using oven or flame-dried glassware and dry solvents (dried for a minimum of 24 h over activated molecular sieves of appropriate (3 - 4Å pore size). Traces of water were removed from starting compounds by co-evaporation with toluene. All moisture sensitive reactions were performed under an argon or nitrogen atmosphere. Flash chromatography was performed using SiliCycle silica gel type SilicaFlash P60 (230 – 400 mesh). HPLC purification was performed on a preparative LC-MS system (Agilent 1200 series) with an Agilent 6130 Quadruple MS detector. TLC analysis was performed on Merck silica gel 60/ Kieselguhr F254, 0.25 mm. Compounds were visualized using UV-irradiation and/or a $KMnO_4$ stain (K_2CO_3 (40 g), $KMnO_4$ (6 g), H_2O (600 mL) and 10% NaOH (5 mL)). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AV-400 MHz spectrometer at 400 (^{1}H) and 100 (^{13}C) MHz using $CDCl_3$, CD_3OD or $(CD_3)_2SO$ as solvent, unless stated otherwise. Spectra were analyzed using MestReNova 11.0.3. Chemical shift values are reported in ppm with tetramethylsilane or solvent resonance as the internal standard ($CDCl_3$, δ 7.26 for ^{1}H, δ 77.16 for ^{13}C; CD_3OD, δ 3.31 for ^{1}H, δ 49.00 for ^{13}C; $(CD_3)_2SO$, δ 2.50 for ^{1}H, δ 39.52 for ^{13}C). Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, td = triple doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constants *J* (Hz), and integration. LC-MS analysis was performed on a Finnigan Surveyor HPLC system with a Gemmi C18 50x4.60 mm column (detection at 200-600 nm), coupled to a Finnigan LCQ Adantage Max mass spectrometer with ESI. The applied buffers were H_2O, MeCN and 1.0% TFA in H_2O (0.1% TFA end concentration). High resolution mass spectra (HRMS) were recorded by direct injection on a q-TOF mass spectrometer (Synapt G2-Si) equipped with an electrospray ion source in positive mode with leu-enkephalin H^+ (*m/z* = 556.2771) as an internal lock mass. The instrument was calibrated prior to measurement using the MS/MS spectrum of glu-1-fibrinopeptide B. Molecules shown are drawn using ChemDraw v16.0.

***tert*-Butyl (5-((2-nitro-*N*-phenethylphenyl)sulfonamido)pentyl)carbamate (3).** To a solution of *N*-Boc-cadaverine (**2**, 0.70 g, 3.5 mmol) in THF (14 mL) were added 2-nitrobenzenesulfonyl chloride (0.77 g, 3.5 mmol) and Et_3N (0.73 mL, 5.2 mmol). The reaction mixture was stirred for 1.5 h, poured into H_2O (60 mL) and extracted with EtOAc (3x 30 mL). The combined organic layers were washed with H_2O, brine, dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in CH_3CN (28 mL) and Cs_2CO_3 (3.4 g, 10 mmol) and phenethyl bromide (2.2 mL, 16 mmol) were added. The reaction mixture was stirred at 80 °C for 18 h, poured into H_2O (70 mL) and extracted with EtOAc (3 x 35 mL). The combined organic layers were washed with H_2O, brine, dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica gel column chromatography (20 > 30% EtOAc in pentane) yielded the title compound (1.6 g, 3.2 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (dd, *J* = 7.4, 1.9 Hz, 1H), 7.71 – 7.57 (m, 3H), 7.29 – 7.23 (m, 2H), 7.22 – 7.13 (m, 3H), 4.53 (br s, 1H), 3.54 – 3.45 (t, *J* = 8.0 Hz, 2H), 3.33 (t, *J* = 7.6 Hz, 2H), 3.07 (d, *J* = 6.4 Hz, 2H), 2.93 – 2.79 (t, *J* = 8.0 Hz, 2H), 1.57 (p, *J* = 7.6 Hz, 2H), 1.44 (m, 11H), 1.33 – 1.20 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.08, 148.12, 138.13, 133.69, 133.50, 131.72, 130.75, 128.87, 126.77, 124.26, 79.07, 48.90, 47.71, 40.39, 35.22, 29.72, 27.85, 23.78. LC-MS *m/z*: 391.9 (M-Boc), 513.9 $[M+Na]^+$.

***N*-(5-Aminopentyl)-2-nitro-*N*-phenethylbenzenesulfonamide (4).** To a solution of **3** (0.72 g, 1.5 mmol) in DCM (13.5 mL) was added TFA (1.4 mL). The reaction mixture was stirred for 1 h, concentrated and co-evaporated with toluene (3 x) to yield the title compound as TFA adduct (0.74 mg, 1.5 mmol, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, *J* = 7.4, 1.8 Hz, 1H), 7.69 – 7.54 (m, 3H), 7.29 – 7.07 (m, 5H), 6.4 (br s, 2H), 3.46 (t, *J* = 7.9 Hz, 2H), 3.32 (t, *J* = 7.2 Hz, 2H), 2.97 (s, 2H), 2.79 (t, *J* = 7.9 Hz, 2H), 1.66 (t, *J* = 7.7 Hz, 2H), 1.57 (q, *J* = 7.4 Hz, 2H), 1.34 (q, *J* = 7.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.06, 137.93, 133.81, 133.19, 132.07, 130.29, 128.85, 128.74, 126.84, 124.37, 49.14, 47.56, 40.11, 35.07, 27.49, 26.75, 23.01. LC-MS *m/z*: 391.9 $[M+H]^+$, 782.8 $[2M + H]^+$.

2,5-Dioxopyrrolidin-1-yl (1*S*,4*S*)-bicyclo[2.2.1]hept-5-ene-2-carboxylate (5). To a solution of 5-norbornene-2-carboxylic acid (Sigma Aldrich, mixture of *endo* and *exo*, predominantly *endo,* 0.60 mL, 4.9 mmol) in DCE (50 mL) were added EDC (3.8 g, 20 mmol) and *N*-hydroxysuccinimide (2.3 g, 20 mmol). The reaction mixture was stirred for 18 h, washed with 1M HCl (3 x 40 mL), dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica

gel column chromatography yielded the title compound as a 1 : 0.3 mixture of endo and exo isomers (1.1 g, 4.5 mmol, 92%). TLC: R_f = 0.45 (1:2 EtOAc:pentane). NMR assignment for major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.24 (dd, *J* = 5.7, 3.1 Hz, 1H), 6.12 (dd, *J* = 5.7, 2.9 Hz, 1H), 3.40 (m, 1H), 3.25 (dt, *J* = 9.0, 3.8 Hz, 1H), 2.99 (d, *J* = 1.6 Hz, 1H), 2.80 (s, 4H), 2.04 – 1.98 (m, 1H), 1.53 – 1.48 (m, 2H), 1.39 – 1.32 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.99, 138.15, 132.17, 49.66, 46.46, 42.53, 40.61, 29.57, 25.61.

(1*S*,2*S*,4*S*)-*N*-(5-((2-Nitro-*N*-phenethylphenyl)sulfonamido)pentyl)bicyclo[2.2.1]hept-5-ene-2-carboxamide (6). To a solution of **5** (0.34 g, 1.5 mmol) in DMF (10 mL) were added **4** (0.74 g, 1.5 mmol) and DIPEA (0.76 mL, 4.4 mmol). The reaction mixture was stirred for 2 h, poured into a 1:1 mixture of 1 M HCl and brine (100 mL) and extracted with Et_2O (2 x 50 mL). The combined organic layers were washed with brine (3 x 50 mL), dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica gel column chromatography (1:1 pentane:EtOAc) yielded two stereoisomers: endo and exo. Title compound **6** (endo isomer): 0.23 g, 0.46 mmol, 33%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.72 – 7.56 (m, 3H), 7.31 – 7.10 (m, 5H), 6.23 (dd, *J* = 5.7, 3.1 Hz, 1H), 5.96 (dd, *J* = 5.8, 2.8 Hz, 1H), 5.51 (s, 1H), 3.55 – 3.45 (m, 2H), 3.34 (t, *J* = 7.4 Hz, 2H), 3.16 (dt, *J* = 13.2, 6.6 Hz, 3H), 2.94 – 2.79 (m, 4H), 1.98 – 1.86 (m, 1H), 1.58 (p, *J* = 7.5 Hz, 2H), 1.52 – 1.41 (m, 3H), 1.35 – 1.26 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.57, 148.13, 138.08, 137.87, 133.63, 133.54, 132.39, 131.76, 130.69, 128.84, 128.72, 126.79, 124.29, 50.14, 48.92, 47.62, 46.31, 44.91, 42.84, 39.27, 35.18, 30.05, 29.10, 27.70, 23.71.
Exo isomer: 0.14 g, 0.26 mmol, 18%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 – 7.91 (m, 1H), 7.72 – 7.56 (m, 3H), 7.29 – 7.14 (m, 5H), 6.18 – 6.05 (m, 2H), 5.61 (s, 1H), 3.55 – 3.44 (m, 2H), 3.40 – 3.32 (m, 2H), 3.28 – 3.19 (m, 2H), 2.91 (dd, *J* = 3.0, 1.4 Hz, 2H), 2.88 – 2.80 (m, 2H), 2.01 – 1.85 (m, 4H), 1.71 (d, *J* = 8.3 Hz, 1H), 1.60 (t, *J* = 7.4 Hz, 2H), 1.55 – 1.48 (m, 2H), 1.33 (dd, *J* = 5.9, 2.9 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.87, 138.29, 138.09, 136.20, 133.66, 133.54, 131.77, 130.76, 128.86, 128.74, 126.81, 124.31, 48.90, 47.59, 47.37, 46.46, 44.83, 41.71, 39.41, 35.17, 30.64, 29.83, 27.69, 23.69.

(1*S*,2*S*,4*S*)-*N*-(5-(Phenethylamino)pentyl)bicyclo[2.2.1]hept-5-ene-2-carboxamide (7). To a solution of **6** (0.23 g, 0.46 mmol) in CH_3CN (6 mL) were added Cs_2CO_3 (0.45 g, 1.4 mmol) and thiophenol (70 µL, 0.70 mmol). The reaction mixture was stirred for 18h, quenched with $NaHCO_3$ (sat. aq., 10 mL) and extracted with DCM (3 x 30 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica gel column chromatography (silica neutralized with Et_3N, 1:19 MeOH:DCM) yielded the title compound (62 mg, 0.19 mmol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.27 (m, 2H), 7.26 – 7.18 (m, 3H), 6.21 (dd, *J* = 5.7, 3.1 Hz, 1H), 5.94 (dd, *J* = 5.7, 2.8 Hz, 1H), 5.90 (t, *J* = 6.3 Hz, 1H), 4.30 (s, 2H), 3.24 – 3.11 (m, 3H), 3.05 (m, 2H), 2.96 – 2.76 (m, 4H), 1.91 (ddd, *J* = 11.7, 9.3, 3.7 Hz, 1H), 1.72 (p, *J* = 7.5 Hz, 2H), 1.55 – 1.23 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.24, 139.99, 137.75, 132.33, 128.75, 128.53, 126.23, 51.21, 50.09, 49.68, 46.29, 44.87, 42.79, 39.36, 36.30, 29.98, 29.67, 29.61, 24.68.

Methyl 1*H*-1,2,3-triazole-5-carboxylate (8). This protocol is based on literature procedure.[18] A mixture of azidotrimethylsilane (2.6 mL, 20 mmol) and methyl propiolate (1.8 mL, 20 mmol) was heated for 4 h at 90 °C, concentrated and coevaporated with MeOH to yield the title compound (1.74 g, 14 mmol, 68%). ^{1}H NMR (400 MHz, MeOD) δ 8.36 (s, 1H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, MeOD) δ 162.61, 139.63, 131.92, 52.53.

***bis*(4-Fluorophenyl)(2*H*-1,2,3-triazol-4-yl)methanol (9).** To a cooled (0 °C) solution of **8** (0.69 g, 5.4 mmol) in THF (70 mL) was added 4-fluorophenylmagnesium bromide (2.0 M in THF, 9.5 mL, 19 mmol). The reaction mixture was stirred for 2 h at rt, quenched with NH_4Cl (sat. aq.) and extracted with DCM. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica gel column chromatography (20 > 40% EtOAc in pentane) yielded the title compound (1.2 g, 4.0 mmol, 74%). ^{1}H NMR (300 MHz, MeOD) δ 7.58 (s, 1H), 7.40 – 7.29 (m, 4H), 7.09 – 6.97 (m, 4H). ^{13}C NMR (75 MHz, MeOD) δ 174.84, 165.07, 130.38, 130.27, 115.64, 115.35, 103.14, 103.12, 100.62.

***N*-(5-((1*S*,2*S*,4*S*)-bicyclo[2.2.1]hept-5-ene-2-carboxamido)pentyl)-4-(bis(4-fluorophenyl)(hydroxy)methyl)-*N*-**

phenethyl-2*H*-1,2,3-triazole-2-carboxamide (1). To a cooled (0 °C) solution of **7** (36 mg, 0.11 mmol) in THF (1 mL) were added DIPEA (58 µL, 0.33 mmol) and triphosgene (15 mg, 0.05 mmol). The reaction mixture was stirred for 1.5 h, quenched with H_2O (10 mL) and extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (2 x 10 mL), dried (Na_2SO_4), filtered and concentrated. The residue was dissolved in THF (2 mL) and DIPEA (58 µL, 0.33 mmol), DMAP (15 mg, 0.11 mmol) and **9** (33 mg, 0.11 mmol) were added. The reaction mixture was stirred for 3.5 h at 60 °C, quenched with NH_4Cl (sat. aq., 10 mL) and extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with H_2O (10 mL), brine (10 mL), dried (Na_2SO_4), filtered and concentrated. Purification of the residue by silica gel column chromatography (3:2 PE:EtOAc) yielded the title compound as the lower TLC spot (26 mg, 0.04 mmol, 37%). ^{1}H NMR (400 MHz, DMSO, 80 °C) δ 7.95 (s, 1H), 7.37 (m, 4H), 7.29 – 7.07 (m, 9H), 6.07 (dd, *J* = 5.7, 3.0 Hz, 1H), 5.81 (dd, *J* = 6.0, 2.7 Hz, 1H), 3.70 (t, *J* = 7.6 Hz, 1H), 3.59 (s, 1H), 3.45 (s, 1H), 3.30 (s, 1H), 3.07 – 2.71 (m, 6H), 1.73 (ddd, *J* = 12.6, 9.4, 3.8 Hz, 1H), 1.66 – 1.15 (m, 13H). ^{13}C NMR (100 MHz, DMSO, 80 °C) δ 162.19, 159.76, 155.28, 149.18, 148.63, 142.22, 141.79, 136.26, 134.59, 131.87, 128.71, 128.61, 128.53, 128.30, 128.22, 128.02, 127.96, 126.02, 125.95, 114.21, 114.11, 113.99, 113.90, 49.01, 45.26, 43.19, 41.79, 38.02, 28.55, 28.50, 28.47, 28.29, 23.21. HRMS *m/z* calculated for $C_{37}H_{39}F_2N_5O_3$ $[M+Na]^+$: 662.2913, found: 662.2923.

4-(4-(Trifluoromethoxy)phenyl)-2*H*-1,2,3-triazole (15). To a solution of 4-trifluoromethoxyphenylacetylene (**14**, 0.61 mL, 4.0 mmol) in a mixture of DMF (27 mL) and MeOH (5.3 mL) were added CuI (75 mg, 0.4 mmol) and azidotrimethylsilane (0.8 mL, 6 mmol). The reaction mixture was stirred at 100 °C for 18 h, quenched with brine (20 mL), extracted with DCM (3 x 50 mL), dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica gel column chromatography (1:4 EtOAc:pentane) yielded the title compound (0.25 g, 1.1 mmol, 27%). ^{1}H NMR (400 MHz, MeOD) δ 8.25 – 8.11 (br s, 1H), 7.93 (d, *J* = 8.8 Hz, 2H), 7.34 (d, *J* = 7.9 Hz, 2H).^{13}C NMR (100 MHz, MeOD) δ 150.30, 130.44, 128.56, 123.18, 122.56, 120.64. LC-MS *m/z*: 230.1 $[M+H]^+$.

***N*-(But-3-yn-1-yl)-2-nitro-*N*-phenethylbenzenesulfonamide (17).** This protocol is based on literature procedure.[5] To a solution of 1-amino-3-butyne (**9**, 0.52 g, 7.5 mmol) in DCM (30 mL) were added *O*-nitrophenylsulfonyl chloride (1.7 g, 7.5 mmol) and Et_3N (1.6 mL, 11 mmol). The reaction mixture was stirred for 4 h, poured into H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O, brine, dried (Na_2SO_4), filtered and concentrated. The residue was dissolved in CH_3CN (60 mL) and Cs_2CO_3 (7.3 g, 23 mmol) and phenethyl bromide (3.0 mL, 22 mmol) were added. The reaction mixture was stirred at 80 °C for 2 h, poured into H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O, brine, dried (Na_2SO_4), filtered and concentrated. Purification of the residue by silica gel column chromatography (0 > 25% EtOAc in pentane) yielded the title compound (2.5 g, 7.0 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.71 – 7.57 (m, 3H), 7.27 – 7.14 (m, 5H), 3.64 – 3.50 (m, 4H), 2.93 – 2.83 (m, 2H), 2.46 (td, *J* = 7.2, 2.7 Hz, 2H), 2.00 (t, *J* = 2.7 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.99, 137.81, 133.73, 133.25, 131.88, 130.62, 128.79, 128.66, 126.75, 124.29, 80.66, 70.74, 49.58, 46.46, 35.07, 19.09.

***N*-Phenethylbut-3-yn-1-amine (18).** To a solution of **17** (2.5 g, 7.0 mmol) in CH_3CN (70 mL) were added Cs_2CO_3 (6.8 g, 21 mmol) and benzenethiol (1.2 mL, 12 mmol). The reaction mixture was stirred for 18 h, quenched with $NaHCO_3$ (sat. aq., 200 mL) and extracted with DCM (2 x 150 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated. Purification of the residue by silica gel column chromatography (neutralized column with Et_3N, 0 > 10% MeOH in DCM) yielded the title compound (0.90 g, 5.2 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.25 (m, 2H), 7.21 (m, 3H), 2.89 (dd, *J* = 8.4, 6.5 Hz, 2H), 2.80 (m, 4H), 2.36 (td, *J* = 6.7, 2.6 Hz, 2H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.47 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.93, 128.75, 128.50, 126.21, 82.41, 69.56, 50.68, 47.87, 36.42, 19.58.

***N*-(But-3-en-1-yl)-2-nitro-*N*-phenethylbenzenesulfonamide (20).** To a solution of 3-butenylamine hydrochloride (**19**, 0.23 g, 2.1 mmol) in THF (8.4 mL) were added *O*-nitrophenylsulfonyl chloride (0.46 g, 2.1 mmol) and DIPEA (0.5 mL, 3 mmol). The reaction mixture was stirred for 18 h, poured into H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O, brine, dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in CH_3CN (17 mL) and Cs_2CO_3 (2.1 g, 6.3 mmol) and phenethyl bromide (1.4 mL, 11 mmol) were added. The reaction mixture was stirred at 80 °C for 2 h, poured into H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O, brine, dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica gel column chromatography (5 > 30% EtOAc in pentane) yielded the title compound (0.53 g, 1.5 mmol, 70%). ^{1}H

NMR (400 MHz, $CDCl_3$) δ 7.96 (dd, *J* = 7.5, 1.8 Hz, 1H), 7.71 – 7.57 (m, 3H), 7.30 – 7.14 (m, 5H), 5.70 (ddt, *J* = 17.1, 10.2, 6.8 Hz, 1H), 5.12 – 4.99 (m, 2H), 3.58 – 3.50 (m, 2H), 3.46 – 3.38 (m, 2H), 2.90 – 2.82 (m, 2H), 2.32 (q, *J* = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.08, 134.26, 133.72, 133.54, 131.74, 130.79, 128.86, 128.73, 126.79, 124.28, 117.62, 49.01, 47.17, 35.15, 32.80.

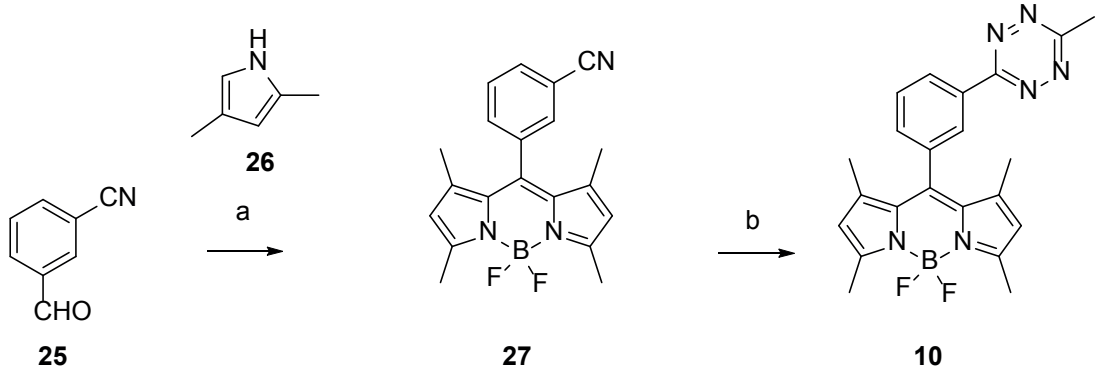
***N*-Phenethylbut-3-en-1-amine (21).** To a solution of **20** (0.54 g, 1.5 mmol) in CH_3CN (15 mL) were added Cs_2CO_3 (1.5 g, 4.5 mmol) and benzenethiol (0.23 mL, 2.3 mmol). The reaction mixture was stirred for 18 h, quenched with $NaHCO_3$ (sat. aq.) and extracted with DCM. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. Purification of the residue by silica gel column chromatography (1 > 20% MeOH in DCM) yielded the title compound (0.21 g, 1.2 mmol, 81%). ^{1}H NMR (300 MHz, MeOD) δ 7.34 – 7.12 (m, 5H), 5.75 (ddt, *J* = 17.1, 10.2, 6.8 Hz, 1H), 5.12 – 4.83 (m, 3H), 2.81 (m, 4H), 2.65 (t, *J* = 7.2 Hz, 2H), 2.24 (q, *J* = 7.1 Hz, 2H). ^{13}C NMR (75 MHz, MeOD) δ 140.73, 136.92, 129.65, 129.55, 127.31, 117.04, 51.81, 49.36, 36.48, 34.50.

***N*-(But-3-yn-1-yl)-*N*-phenethyl-4-(4-(trifluoromethoxy)phenyl)-1*H*-1,2,3-triazole-1-carboxamide (11) and *N*-(but-3-yn-1-yl)-*N*-phenethyl-4-(4-(trifluoromethoxy)phenyl)-2*H*-1,2,3-triazole-2-carboxamide (22).** To a cooled (0 °C) solution of **18** (85 mg, 0.49 mmol) in THF (5 mL) were added DIPEA (0.26 mL, 1.5 mmol) and triphosgene (77 mg, 0.26 mmol). The reaction mixture was stirred at 0 °C for 1 h, quenched with H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in THF (5 mL) and DIPEA (0.26 mL, 1.5 mmol), DMAP (61 mg, 0.5 mmol) and **15** (0.12 g, 0.5 mmol) were added. The reaction mixture was stirred for 4 h at 60 °C, quenched with NH_4Cl (sat. aq.) and extracted with EtOAc. The combined organic layers were washed with brine (2 x), dried (Na_2SO_4), filtered and concentrated. Purification of the residue by silica gel column chromatography (5 > 10% EtOAc in pentane) yielded the title compounds. LC-MS *m/z*: 428.9 $[M+H]^+$. **11** (apolar): 79 mg, 0.18 mmol, 37%. ^{1}H NMR (400 MHz, DMSO-d_6, 90 °C) δ 8.85 (s, 1H), 8.10 – 8.02 (m, 2H), 7.49 – 7.39 (m, 2H), 7.35 – 7.15 (m, 5H), 3.85 (dd, *J* = 8.6, 6.6 Hz, 2H), 3.70 (t, *J* = 7.0 Hz, 2H), 3.00 (t, *J* = 7.6 Hz, 2H), 2.75 (d, *J* = 2.2 Hz, 1H), 2.65 – 2.56 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6, 20 °C) δ 148.89, 148.25, 144.66, 144.54, 138.51, 138.09, 128.92, 128.89, 128.74, 128.50, 127.48, 126.46, 126.40, 123.00, 122.65, 122.56, 121.68, 120.95, 119.25, 117.55, 81.43, 81.23, 73.30, 72.95, 51.19, 50.00, 48.15, 47.11, 34.24, 32.61, 18.19, 16.51. HRMS *m/z* calculated for $C_{22}H_{19}F_3N_4O_2$ $[M+Na]^+$: 451. 1352, found: 451.1360. **22** (polar): 84 mg, 0.20 mmol, 41%. ^{1}H NMR (400 MHz, DMSO-d_6, 90 °C) δ 8.57 (s, 1H), 8.11 – 8.01 (m, 2H), 7.53 – 7.43 (m, 2H), 7.38 – 7.12 (m, 5H), 3.77 (dd, *J* = 8.9, 6.5 Hz, 2H), 3.63 (t, *J* = 7.1 Hz, 2H), 2.99 (t, *J* = 7.7 Hz, 2H), 2.73 (s, 1H), 2.59 (td, *J* = 7.0, 2.7 Hz, 2H). ^{13}C NMR (150 MHz, DMSO, 20 °C) δ 149.37, 149.35, 148.94, 147.14, 138.53, 138.04, 134.06, 133.99, 128.85, 128.59, 128.42, 128.26, 128.09, 126.39, 122.58, 121.72, 120.88, 119.18, 117.48, 81.42, 81.09, 73.05, 72.83, 51.08, 50.09, 48.15, 47.21, 34.32, 32.56, 18.22, 16.44. HRMS *m/z* calculated for $C_{22}H_{19}F_3N_4O_2$ $[M+Na]^+$: 451.1352, found: 451.1355.

4-(*bis*(4-Fluorophenyl)(hydroxy)methyl)-*N*-(but-3-yn-1-yl)-*N*-phenethyl-1*H*-1,2,3-triazole-1-carboxamide (23) and 4-(*bis*(4-fluorophenyl)(hydroxy)methyl)-*N*-(but-3-yn-1-yl)-*N*-phenethyl-2*H*-1,2,3-triazole-2-carboxamide (12). To a cooled (0 °C) solution of **18** (78 mg, 0.45 mmol) in THF (5 mL) were added DIPEA (0.26 mL, 1.5 mmol) and triphosgene (77 mg, 0.26 mmol). The reaction mixture was stirred at 0 °C for 1.5 h, quenched with H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in THF (5 mL) and DIPEA (0.26 mL, 1.5 mmol), DMAP (61 mg, 0.5 mmol) and **9** (0.13 g, 0.45 mmol) were added. The reaction mixture was stirred for 4 h at 60 °C, quenched with NH_4Cl (sat. aq.) and extracted with EtOAc. The combined organic layers were washed with brine (2 x), dried (Na_2SO_4), filtered and concentrated. Purification of the residue by silica gel column chromatography (12 > 20% EtOAc in pentane) yielded the title compounds. LC-MS *m/z*: 487.0 $[M+H]^+$. **23** (apolar): 72 mg, 0.15 mmol, 33%. ^{1}H NMR (400 MHz, DMSO, 90 °C) δ 8.01 (s, 1H), 7.48 – 7.36 (m, 4H), 7.31 – 7.07 (m, 8H), 6.59 (d, *J* = 2.2 Hz, 1H), 3.79 (dd, *J* = 8.7, 6.6 Hz, 2H), 3.67 (t, *J* = 7.0 Hz, 2H), 2.95 (t, *J* = 7.6 Hz, 2H), 2.72 (s, 1H), 2.58 (td, *J* = 7.0, 2.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO, 90 °C) δ 162.08, 159.66, 152.82, 148.60, 142.09, 137.78, 128.63, 128.54, 128.20, 127.92, 125.91, 123.23, 113.99, 113.77, 80.84, 74.67, 72.04, 50.17, 47.24, 33, 17. HRMS *m/z* calculated for $C_{28}H_{24}F_2N_4O_2$ $[M+Na]^+$: 509.1760, found:

509.1760. **12** (polar): 88 mg, 0.18 mmol, 40%. ^{1}H NMR (400 MHz, DMSO, 90 °C) δ 7.97 (s, 1H), 7.43 – 7.34 (m, 4H), 7.30 – 7.06 (m, 8H), 6.77 (s, 1H), 3.69 (dd, *J* = 8.8, 6.7 Hz, 2H), 3.53 (t, *J* = 7.2 Hz, 2H), 2.89 (d, *J* = 7.9 Hz, 2H), 2.69 (d, *J* = 1.8 Hz, 1H), 2.46 (s, 2H). ^{13}C NMR (100 MHz, DMSO, 90 °C) δ 162.13, 159.71, 155.41, 149.02, 141.64, 137.83, 134.78, 128.53, 128.45, 128.13, 127.87, 125.84, 114.09, 113.87, 80.79, 74.98, 71.92, 65.99, 50.10, 47.48. HRMS *m/z* calculated for $C_{28}H_{24}F_2N_4O_2$ [M+Na]$^+$: 509.1760, found: 509.1766.

4-(*bis*(4-Fluorophenyl)(hydroxy)methyl)-*N*-(but-3-en-1-yl)-*N*-phenethyl-1*H*-1,2,3-triazole-1-carboxamide (24) and 4-(*bis*(4-fluorophenyl)(hydroxy)methyl)-*N*-(but-3-en-1-yl)-*N*-phenethyl-2*H*-1,2,3-triazole-2-carboxamide (13). To a cooled (0 °C) solution of **21** (0.11 g, 0.62 mmol) in THF (6.2 mL) were added DIPEA (0.3 mL, 1.7 mmol) and triphosgene (96 mg, 0.32 mmol). The reaction mixture was stirred at 0 °C for 1 h, quenched with H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in THF (6.2 mL) and DIPEA (0.3 mL, 1.7 mmol), DMAP (76 mg, 0.62 mmol) and **8** (0.18 g, 0.62 mmol) were added. The reaction mixture was stirred for 18 h at 60 °C, quenched with NH_4Cl (sat. aq.) and extracted with EtOAc. The combined organic layers were washed with brine (2x), dried (Na_2SO_4), filtered and concentrated. Purification of the residue by silica gel column chromatography (10 > 25% EtOAc in pentane) yielded the title compounds. **24** (apolar): 89 mg, 0.18 mmol, 29%. NMR assignment major rotamer: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.8 (s, 1H), 7.49 – 6.83 (m, 13H), 5.94 – 5.52 (m, 1H), 5.25 – 4.91 (m, 2H), 3.81 – 3.45 (m, 4H), 3.07 – 2.79 (m, 2H), 2.38 (dd, *J* = 50.6, 8.3 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 162.89, 160.47, 153.62, 149.41, 142.99, 138.98, 138.45, 135.55, 135.15, 129.53, 129.45, 129.16, 128.88, 126.88, 124.26, 123.84, 117.75, 117.63, 115.05, 114.84, 75.36, 51.23, 50.45, 49.25, 47.86, 34.71, 33.06, 31.59, 21.20. HRMS *m/z* calculated for $C_{28}H_{26}F_2N_4O_2$ [M+H]$^+$: 489.2097, found: 489.2098. **13** (polar): 32 mg, 65 µmol, 11%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.40 – 6.87 (m, 13H), 5.62 (m, 1H), 5.25 – 4.76 (m, 2H), 3.68 – 3.48 (m, 3H), 3.30 (s, 1H), 2.84 (d, *J* = 78.8 Hz, 2H), 2.26 (dd, *J* = 109.5, 7.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 162.91, 160.48, 156.10, 149.90, 142.60, 138.98, 138.38, 135.60, 135.04, 129.42, 129.34, 128.98, 128.82, 126.82, 117.59, 117.45, 115.14, 114.93, 75.68, 50.93, 50.42, 49.08, 48.16, 34.65, 33.09, 32.73, 31.52, 29.47. HRMS *m/z* calculated for $C_{28}H_{26}F_2N_4O_2$ [M+Na]$^+$: 511.1916, found: 511.1924.



Scheme 3. Synthesis of BODIPY-tetrazine **10**. Reagents and conditions: (a) *i*. TFA (catalytic), DCM; *ii*. DDQ; *iii*. DIPEA, $BF_3{\cdot}OEt_2$; 42%; (b) *i*. $Zn(OTf)_2$, CH_3CN, NH_2NH_2, 60 °C; *ii*. $NaNO_2$; 0.3%.

3-(5,5-Difluoro-1,3,7,9-tetramethyl-5*H*-4λ4,5λ4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-10-yl)benzonitrile (27). This protocol is based on literature procedure.[11] To a solution of 3-formylbenzonitrile (**25**, 0.81 g, 6.1 mmol) and 2,4-dimethylpyrrole (**26,** 1.4 mL, 13 mmol) in DCM (160 mL) were added four drops of TFA. After stirring at rt for 30 min, a solution of DDQ (1.4 g, 6.1 mmol) in DCM (160 mL) was added, followed by DIPEA (12.5 mL, 73 mmol) and $BF_3 \cdot OEt_2$ (12.5 mL, 100 mmol). The reaction was stirred for 18 h, diluted with water and extracted with DCM. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (75 > 100% toluene in pentane) to yield the title compound (0.89 g, 2.6 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.51 (m, 4H), 6.01 (s, 2H), 2.56 (s, 6H), 1.36 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.70, 142.63, 138.09, 136.65, 132.92, 132.83, 131.91, 131.13, 130.27, 121.94, 117.99, 113.65, 14.82, 14.77.

5,5-Difluoro-1,3,7,9-tetramethyl-10-(3-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-5*H*-4λ4,5λ4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinine (10). Adapted from literature procedure, omitted DMF as a co-solvent.[11] To a suspension of **27** (175 mg, 0.5 mmol) and $Zn(OTf)_2$ (91 mg, 0.25 mmol) in CH_3CN (0.26 mL) in a sealed microwave tube under argon was added hydrazine (0.8 mL, 25 mmol). The reaction mixture was stirred at 60 °C for 18 h and allowed to cool down to rt before addition of $NaNO_2$ (0.7 g in 5 mL H_2O). The mixture was acidified with 1M HCl and extracted with DCM. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified with column chromatography (0 > 0.5% CH_3CN in toluene, followed by 4:1 pentane:EtOAc) 0.3% isolated yield after preperative HPLC. NMR data agrees with literature values.[11] ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 – 8.57 (m, 1H), 8.41 – 8.32 (m, 1H), 7.88 (t, *J* = 7.8 Hz, 1H), 7.75 (dt, *J* = 7.7, 1.4 Hz, 1H), 6.22 (s, 2H), 3.01 (s, 3H), 2.48 (s, 6H), 1.41 (s, 6H). HRMS *m/z* calculated for $C_{22}H_{21}BF_2N_6$ $[M+H]^+$: 419.1962, found: 419.1962.

LC-MS validation of chemical ligation.

In an LC-vial containing 160 µL MilliQ water 20 µL **1** (1 mM in DMSO) and 20 µL **10** (1 mM in DMSO) were added. The contents were mixed thoroughly by pipetting and reacted for 1 h at 37 °C. 20 µL of this mixture was then directly injected and resolved on LC-MS (ThermoFisher LCQ as in Synthetic Methods). Gradient elution 10% -> 90% acetonitrile in H_2O, 1% trifluoroacetic acid. Final concentrations: 0.1 mM **1**, 0.1 mM **10**, 20% DMSO.

Computational methods

The triazole urea structures were initially optimized by a conformer distribution search included in the Spartan 10 program.[19] The conformer distribution was calculated in the gas phase at the DFT level of theory using B3LYP as hybrid functional and 6-31G(d) as basis set. The resulting structure library was further refined using the Gaussian 09 program revision A.02,[20] with the use of the ωB97XD long-range corrected hybrid functional and 6-311+G(d,p) as basis set. Optimization was done in gas-phase and subsequently corrections for solvent effects were done by use of a polarizable continuum model (PCM), using DMSO as solvent parameter. Gas-phase free energies were computed using the quasi-harmonic approximation in the gas phase according to the work of Truhlar - the quasi-harmonic approximation is the same as the harmonic oscillator approximation except that vibrational frequencies lower than 100 cm^{-1} were raised to 100 cm^{-1} as a way to correct for the breakdown of the harmonic oscillator model for the free energies of low-frequency vibrational modes. The denoted free Gibbs energy was calculated using Equation (1) in which $^{\Delta}E_{gas}$ is the gas-phase energy (electronic energy), $^{\Delta}G^{T}_{gas}$ (T = 298.15 K and pressure = 1 atm.) is the sum of corrections from the electronic energy to free Gibbs energy in the harmonic oscillator approximation also including zero-point-vibrational energy, and $^{\Delta}G^{T}_{solv}$ is their corresponding free solvation Gibbs energy.

$$\Delta G^{T}_{in\ solution} = \Delta E_{gas} + \Delta G^{T}_{gas} + \Delta G_{solv} \quad (1)$$

$$= \Delta G^{T}_{gas} + \Delta G_{solv}$$

All found minima were checked for negative frequencies. Based on the lowest energy structures according to the optimisation described above, the chemical shifts were calculated with the use of the Gauge-Independent Atomic Orbital (GIAO) method using WC04/6-311+G(2d,p) and a PCM model with as solvent DMSO. No additional scaling was used for the denoted chemical shifts.

Biochemical methods

Mouse brain membrane proteome. Mouse brains (C57Bl/6) were isolated according to guidelines approved by the ethical committee of Leiden University (DEC#13191), frozen in N_2 (l), and stored at -80 °C until use. Tissues were

thawed on ice, dounce homogenized in appropriate volumes (2-4 mL) of cold lysis buffer (20 mM HEPES, pH 7.2, 2 mM DTT, 250 mM sucrose, 1 mM $MgCl_2$, 2.5 U/mL benzonase) and incubated on ice (15 min), followed by two low-speed spins (3 min, 1,400−2,500 *g*, 4°C) to remove debris. The supernatant fraction was collected for further use. The membrane and cytosolic fractions of cell or tissue lysates were separated by ultracentrifugation (93,000 *g*, 45 min, 4°C). The supernatant was collected (cytosolic fraction) and the membrane pellet was resuspended in cold storage buffer (20 mM Hepes, pH 7.2, 2 mM DTT) by thorough pipetting and passage through an insulin needle. Protein concentrations were determined by a Quick Start™ Bradford Protein Assay and samples were diluted to 2.0 mg/mL with cold storage buffer, aliquoted, flash frozen in N_2 (l) and stored at -80 °C until further use.

SDS-PAGE. Mouse brain proteome or cell lysate (15 µL, 2.0 or 1.0 mg/mL, membrane fraction or whole lysate) was pre-incubated with vehicle or inhibitor (0.375 µL 40 x inhibitor stock, 30 min, rt) followed by an incubation with the activity based probe (0.375 µL 40 x probe stock, 20 min, rt). Final concentrations for the inhibitors are indicated in the main text and figure legends. Reactions were quenched with 4x Laemmli buffer (5 µL, 240 mM Tris (pH 6.8), 8% (w/v) SDS, 40% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, 0.04% (v/v) bromophenol blue). 10 or 20 µg per reaction was resolved on a 10% acrylamide SDS-PAGE gel (180 V, 75 min). Gels were scanned using Cy2, Cy3 and Cy5 multichannel settings on a ChemiDoc MP (Bio-Rad) and stained with Coomassie after scanning. Fluorescence was normalized to Coomassie staining and quantified with Image Lab v5.2.1 (Bio-Rad). IC_{50} curves were fitted with Graphpad Prism® v7 (Graphpad Software Inc.).

Cell culture. U2OS cells were cultured at 37 °C under 7% CO2 in DMEM containing phenol red, stable glutamine, 10% (v/v) New Born Calf Serum (Thermo Fisher), and penicillin and streptomycin (200 µg/mL each; Duchefa). Medium was refreshed every 2-3 days and cells were passaged twice a week at 80-90% confluence by trypsinization, followed by resuspension in fresh medium. Cells lines were purchased from ATCC and were regularly tested for mycoplasma contamination. Cultures were discarded after 2-3 months of use.

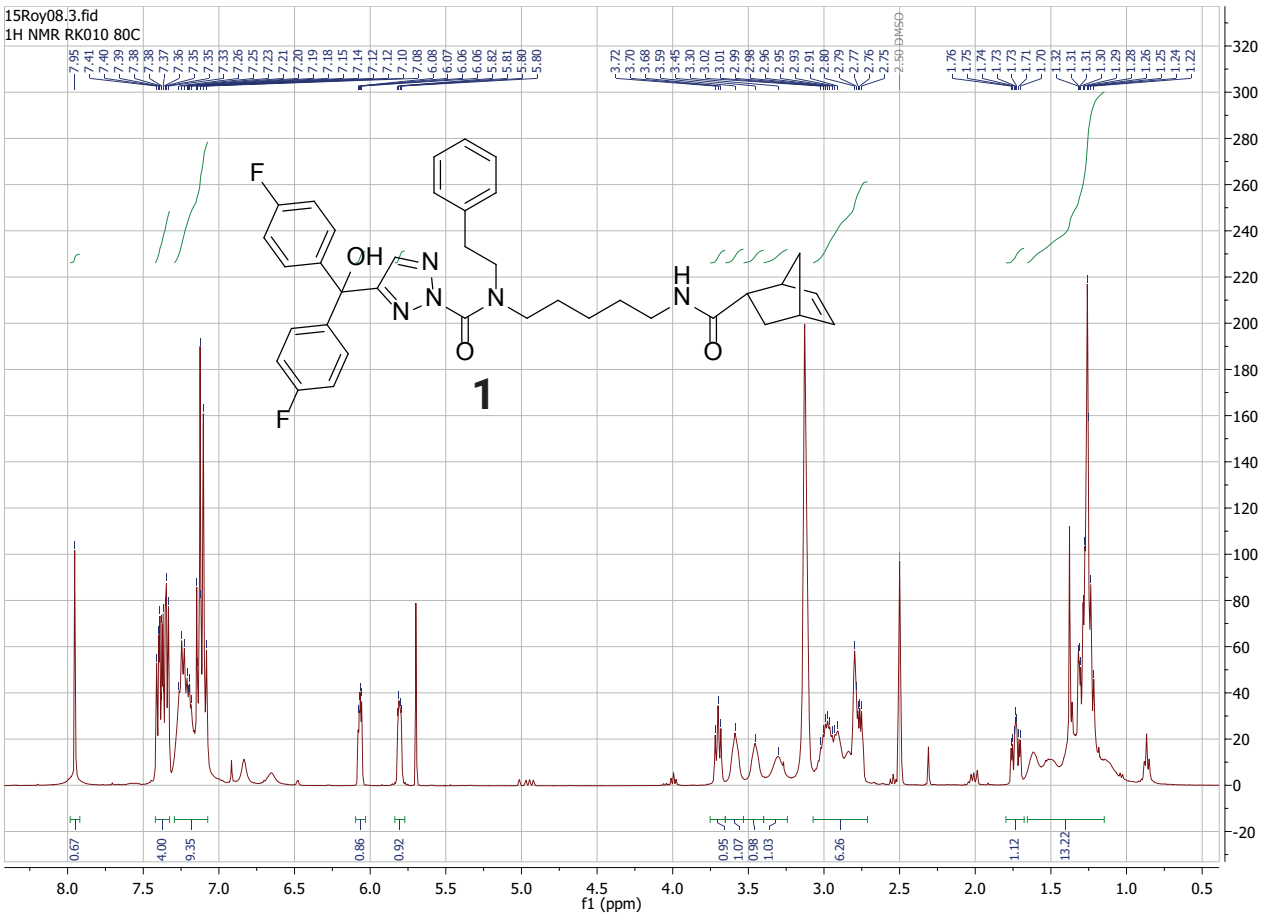
Plasmids. The DAGLα and DAGLα-S472A plasmids (both containing a FLAG tag) were constructed as described before.[21] Briefly, full length human cDNA of hDAGL-α was purchased from Biosource and cloned into mammalian expression vector pcDNA3.1, containing genes for ampicillin and neomycin resistance. A FLAG-linker was made from primers and cloned into the vector at the C-terminus of hDAGL-α. Two step PCR mutagenesis was performed to substitute the active site serine for an alanine in the hDAGL-α-FLAG, to obtain hDAGL-α-S472A-FLAG. All plasmids were grown in XL-10 Z-competent cells and prepped (Maxi Prep, Qiagen). The sequences were confirmed by sequence analysis at the Leiden Genome Technology Centre.

Transfection. U2OS cells were seeded at 500.000 cells/well in 6-well plates 24 h prior to transfection in 2 mL DMEM containing 10% serum. Prior to transfection, culture medium was aspirated and 1 mL medium was added per well. A 3:1 (m:m) mixture of polyethyleneimine (PEI, 4.5 µg/well) and plasmid DNA (1.5 µg/well) was prepared in serum free culture medium and incubated for 15 min at rt. Transfection was performed by dropwise addition of the PEI/DNA mixture (200 µL/well) to the cells. 24 h post-transfection, the medium was refreshed and after 48 h cells were harvested or used for *in situ* treatments.

***In situ* tetrazine labeling & Western blot.** 48 h post-transfection, the cells were washed with serum-free medium (3 x 1 mL). The cells were treated (1 mL serum-free medium / well) with either DMSO as vehicle or probe **1** (5 µM final concentration, 1% DMSO) for 1 h at 37°C. After incubation, the cells were washed with serum-free medium (3 x, 1 mL) and treated with either DMSO as vehicle, HT-01 (1 µM final concentration), or compound **10** (5 µM final concentration) for 1 h at 37 °C. The cells were subsequently washed with PBS (3 x 1 mL) and harvested by scraping into 1 mL PBS. The cells were pelleted by centrifugation (5 min, 1000 *g*). The supernatant was discarded and cell pellets were frozen in N_2 (l) and stored at -80 °C until sample preparation for SDS-PAGE. Cell pellets were thawed on ice, resuspended in cold lysis buffer (20 mM HEPES, pH 7.2, 2 mM DTT, 250 mM sucrose, 1 mM MgCl2, 2.5 U/mL benzonase) and incubated on ice (15 min). Protein concentrations were determined by Bradford Protein Assay. The

cell lysate was diluted to 1.5 mg/mL in cold lysis buffer. Samples (15 µL) were denatured in Laemlli buffer (5 µL) (240 mM Tris (pH 6.8), 8% (w/v) SDS, 40% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, 0.04% (v/v) bromophenol blue) for 30 min at rt. Samples were resolved on a 10% acrylamide SDS-PAGE gel (15 µg / sample, 200 V, 65 min). Gels were scanned using Cy2, Cy3 and Cy5 multichannel settings on a ChemiDoc MP (Bio-Rad) and were used for Coomassie staining or Western Blot analysis. Experiment was performed in duplo.

Cell survival assay. U2OS cells were seeded at 100.000 cells/well in 6-well plates 24 h prior to transfection in 2 mL DMEM containing 10% serum. Prior to treatment cells were washed with serum-free medium (3 x, 0.5 mL). Per well 150 µL serum-free medium was added, containing either DMSO as vehicle (1%), DH376 (1 µM final concentration, 1% DMSO), or probe **1 (**5 µM final concentration, 1% DMSO) and incubated for 1 h at 37 °C. Cells were washed with serum-free medium (1x 1 mL) and subsequently treated with 150 uL serum-free medium per well, containing either DMSO as vehicle (1 %), compound **10** (10 µM final concentration, 1% DMSO), HT-01 (1 µM final concentration, 1% DMSO), or Cy5 Click mix (50 mM copper sulphate, 3 mM sodium ascorbate, 1 mM THPTA, 1 uM Cy5-Azide, 1% DMSO) for 1 h at 37 °C. After incubation, cells were washed with serum-free medium (1 x, 0.5 mL) and incubated with 100 uL of Trypsin in PBS/EDTA. Cells were suspended in 500 uL medium containing 10% serum. A Live/Dead cell count was performed using Trypan Blue on a TC20 Cell counter (Bio-Rad). The experiment was performed in triplo.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O
F
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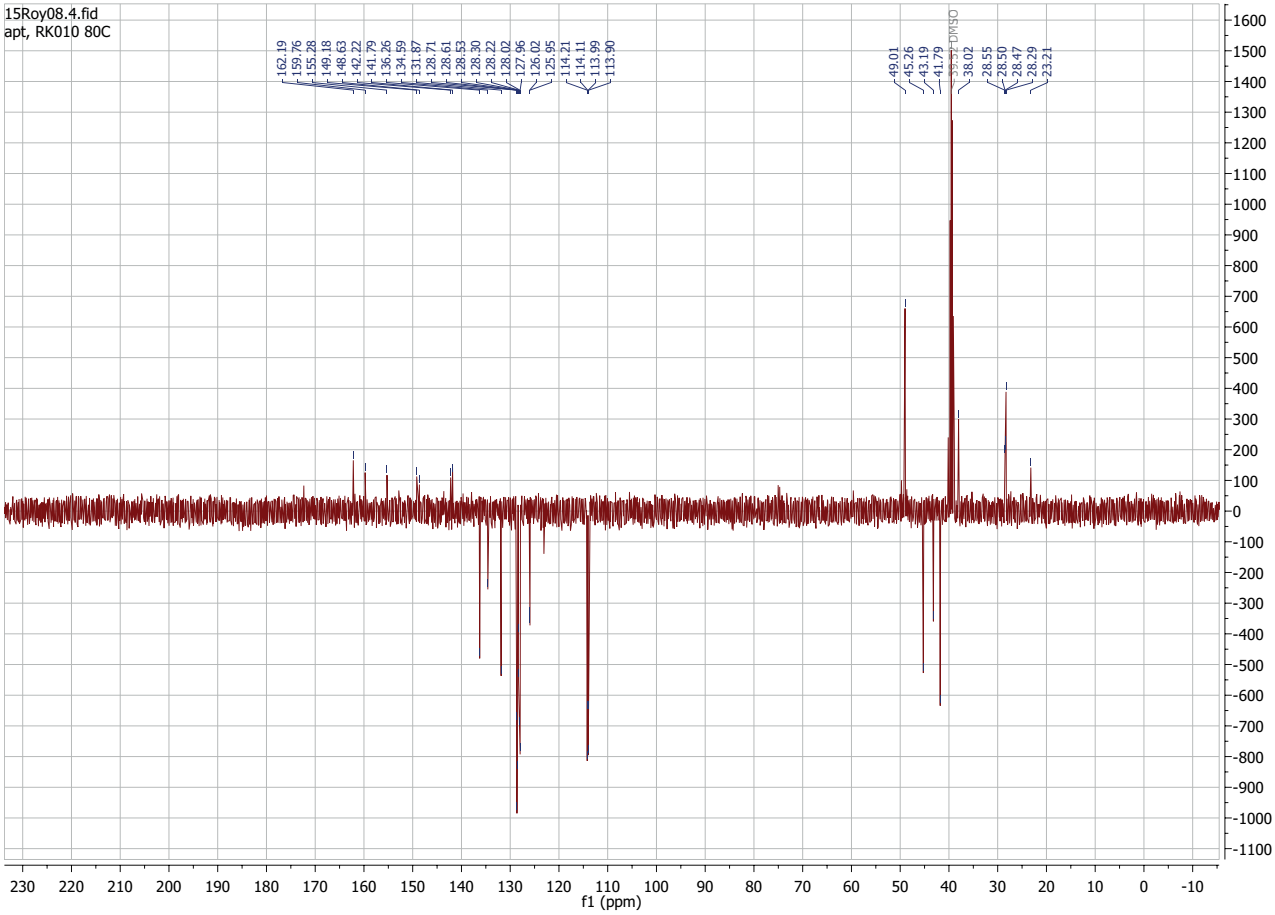
f1 (ppm)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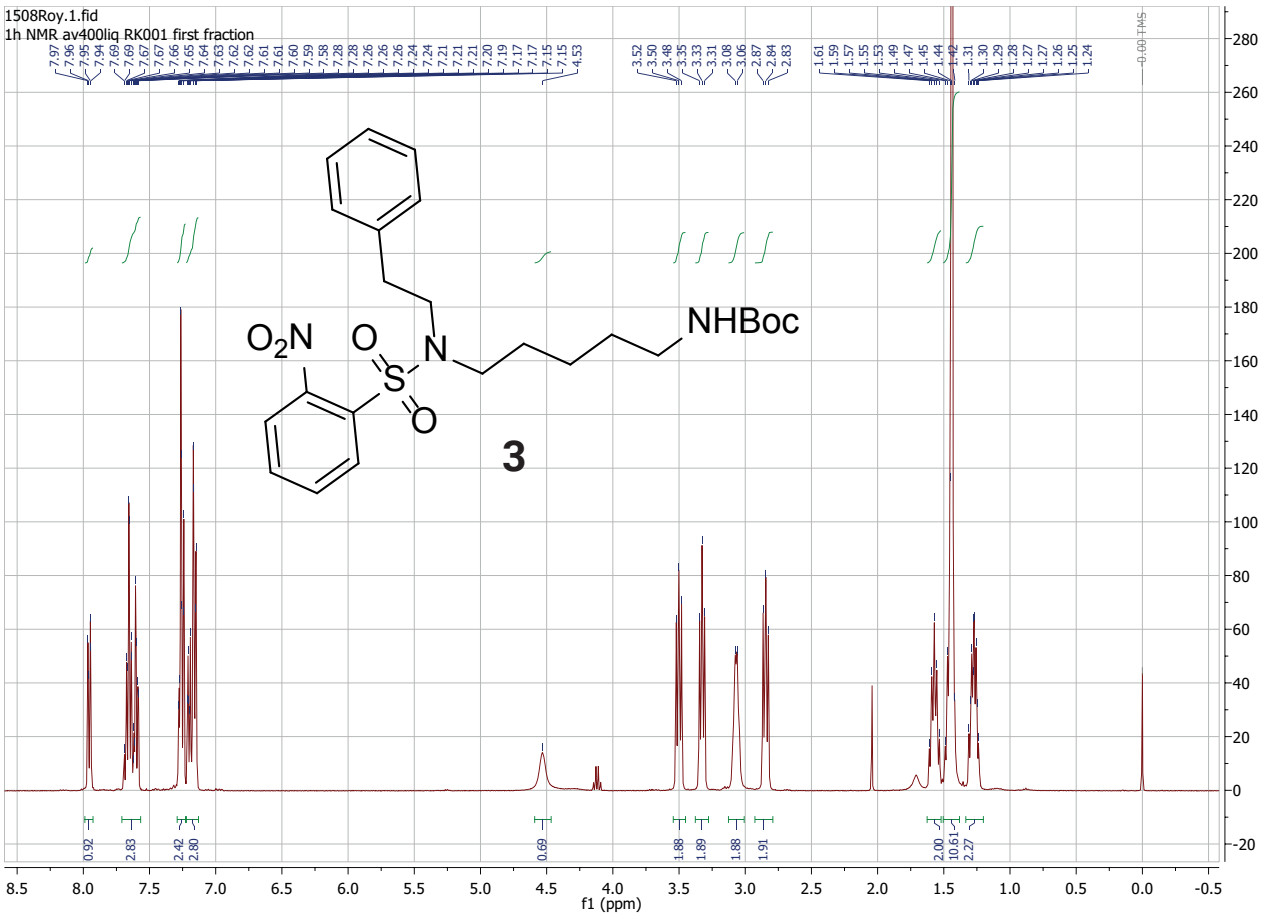
f1 (ppm)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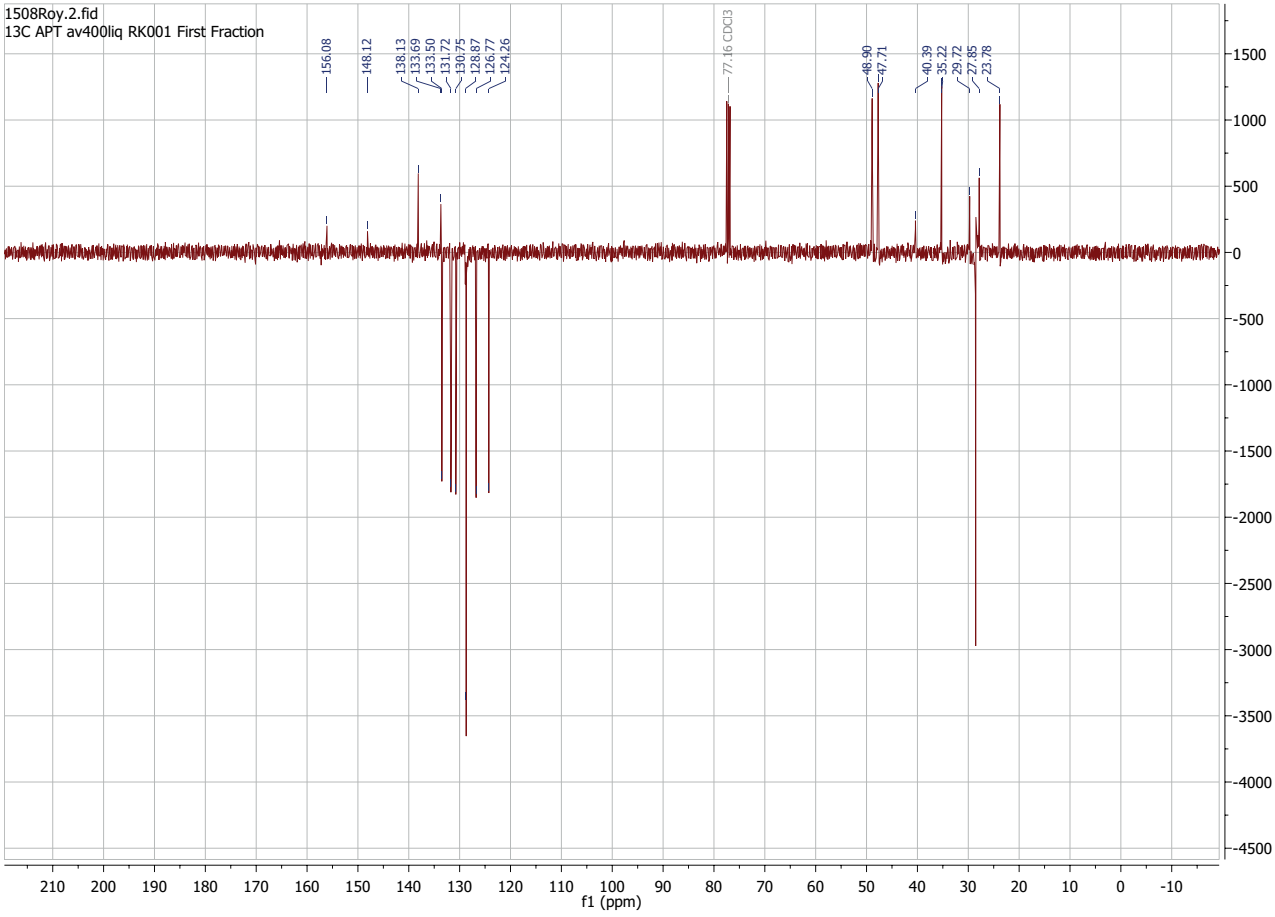
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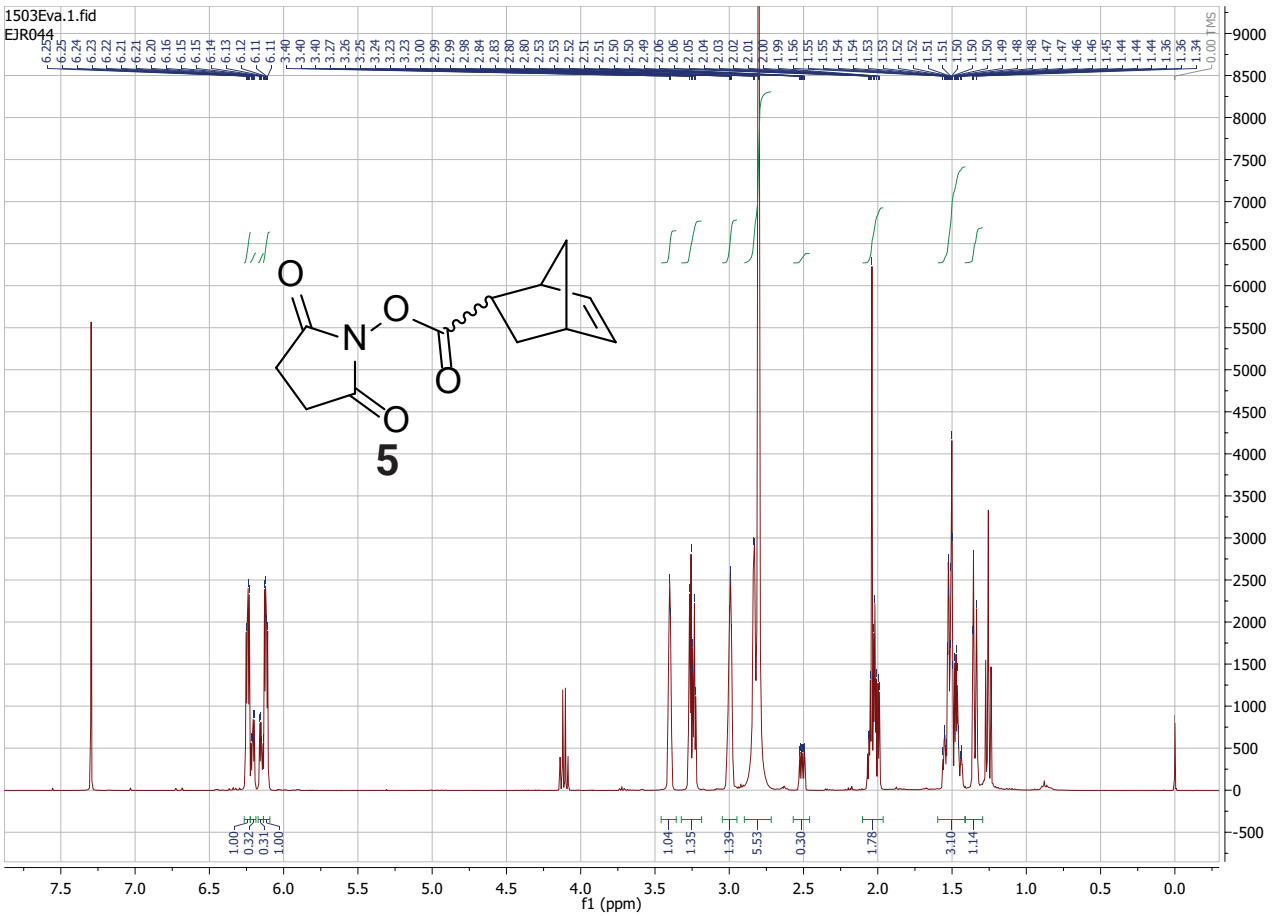
f1 (ppm)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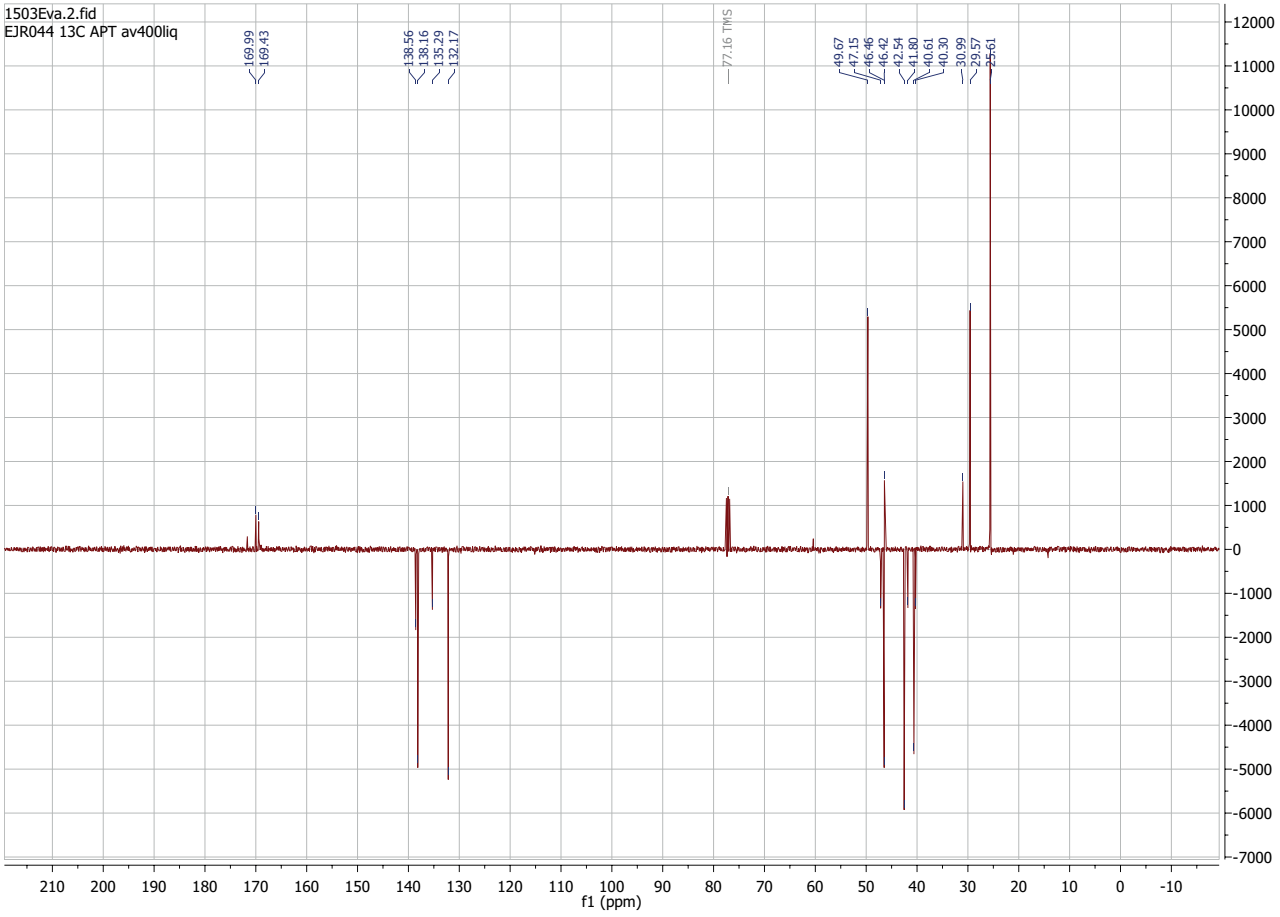
5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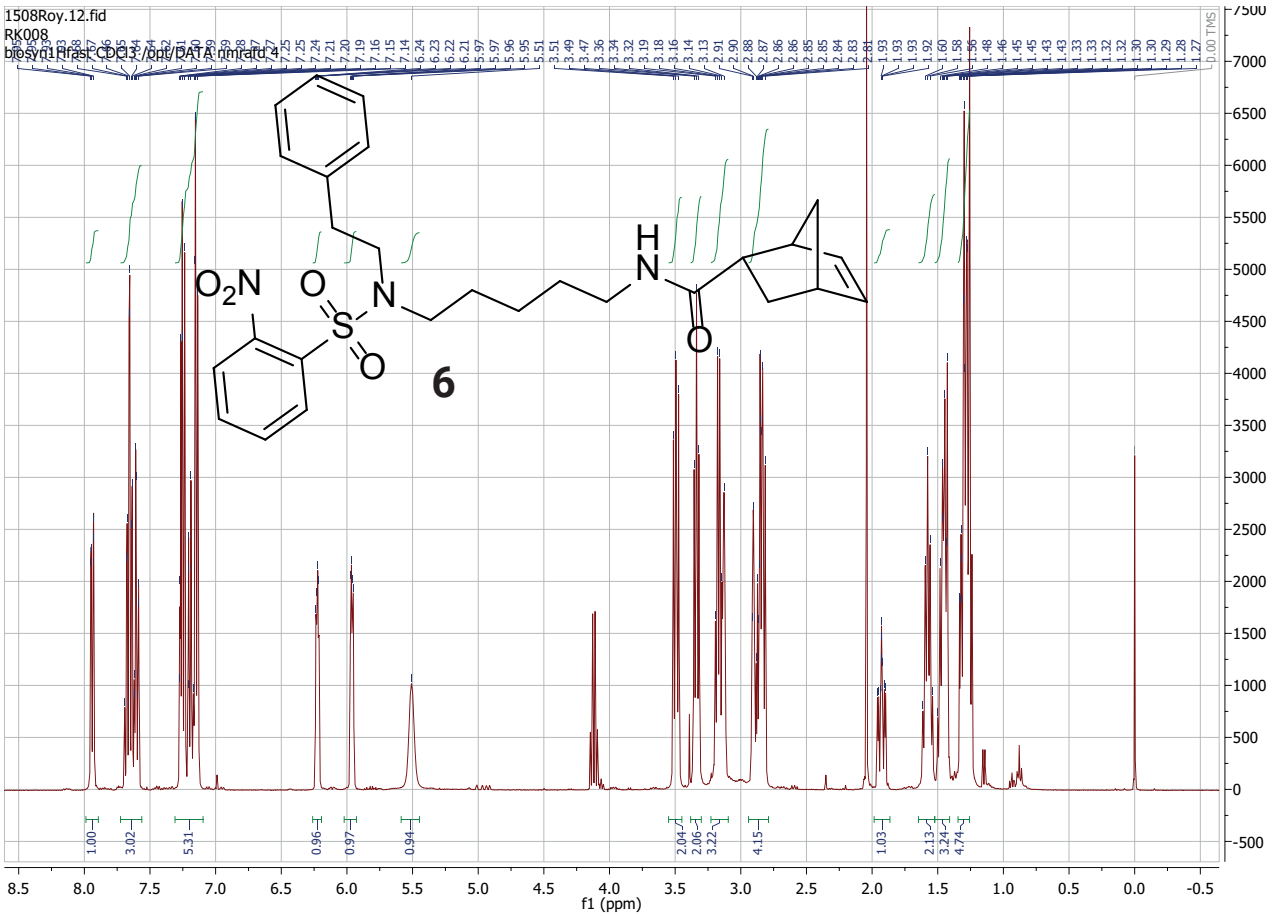
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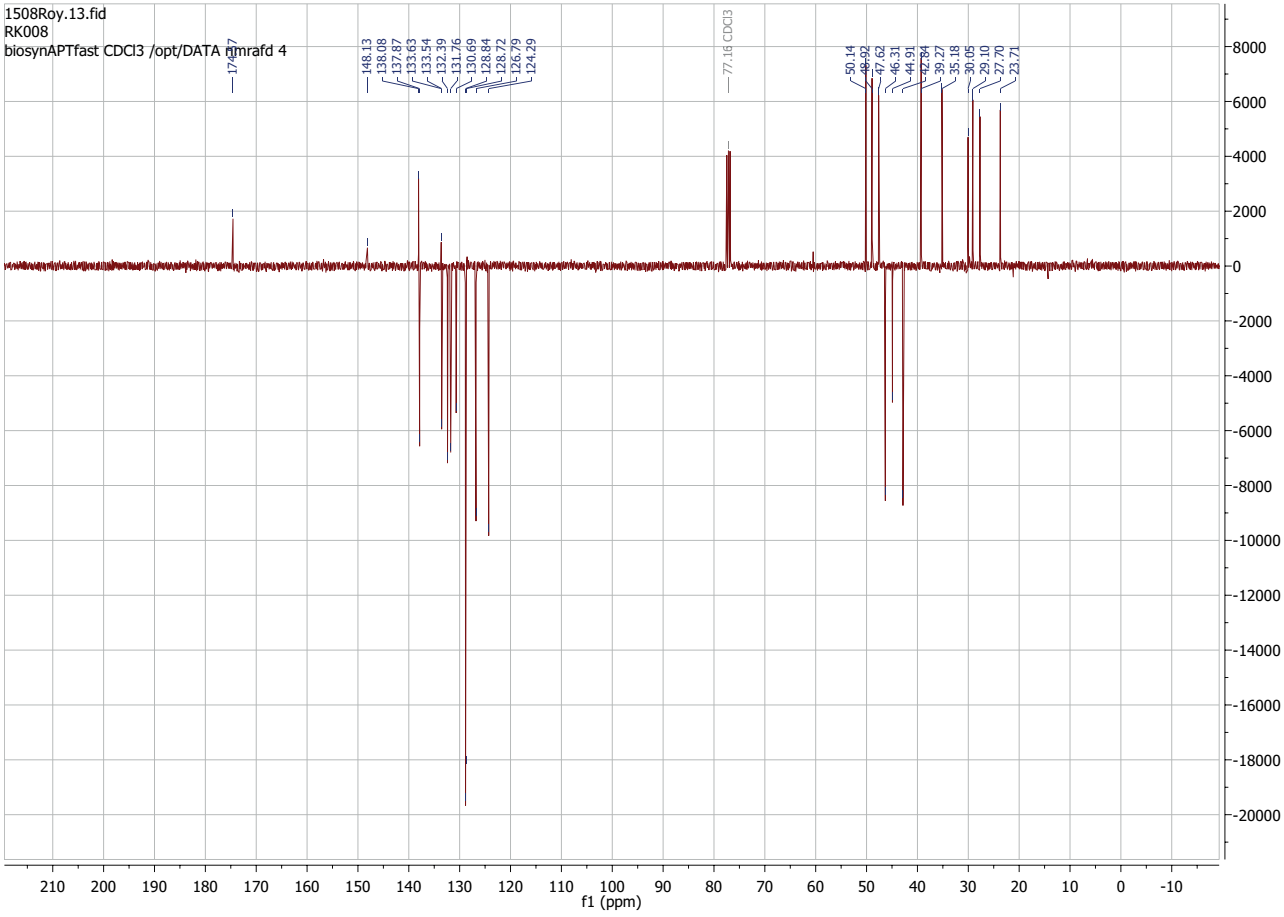
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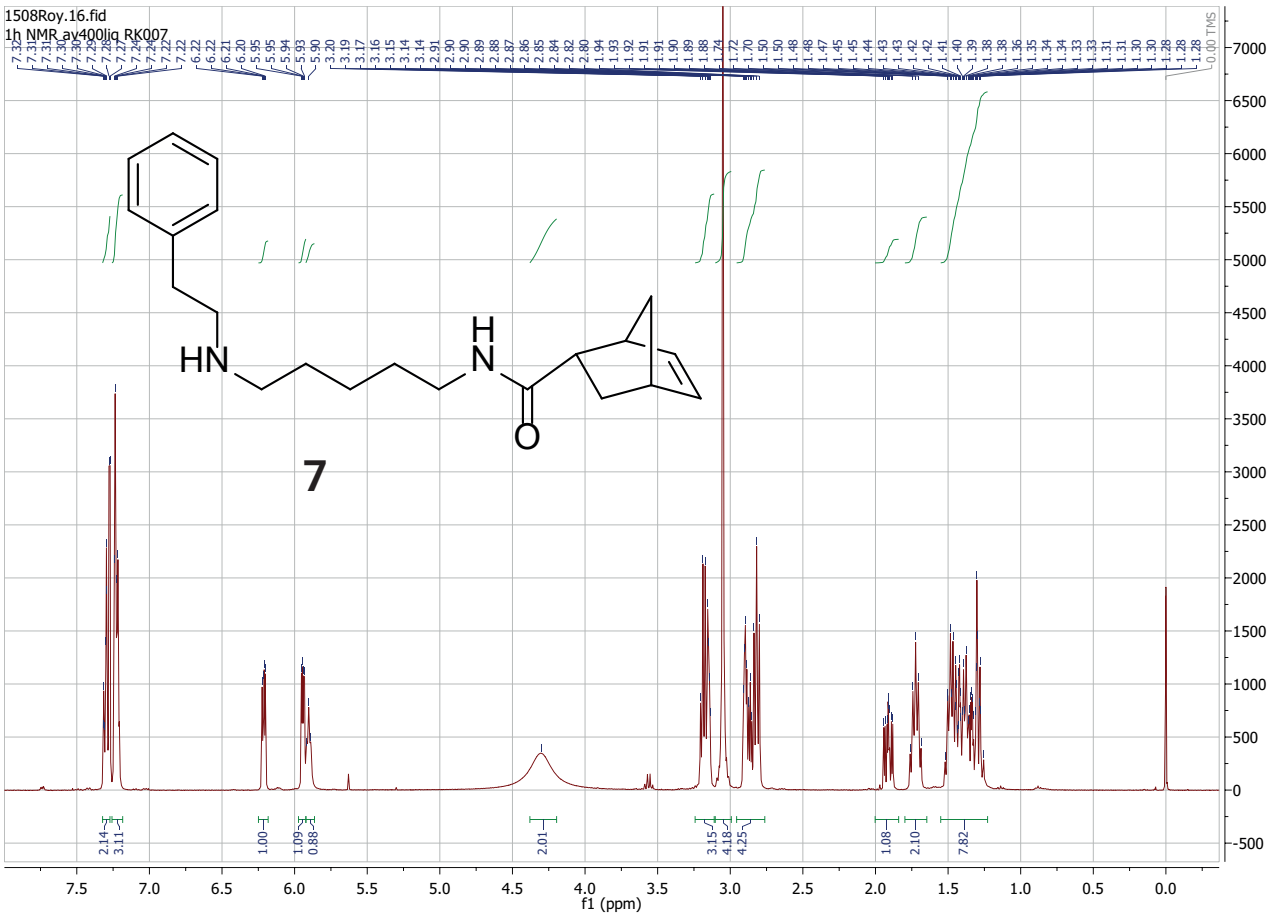
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O
7
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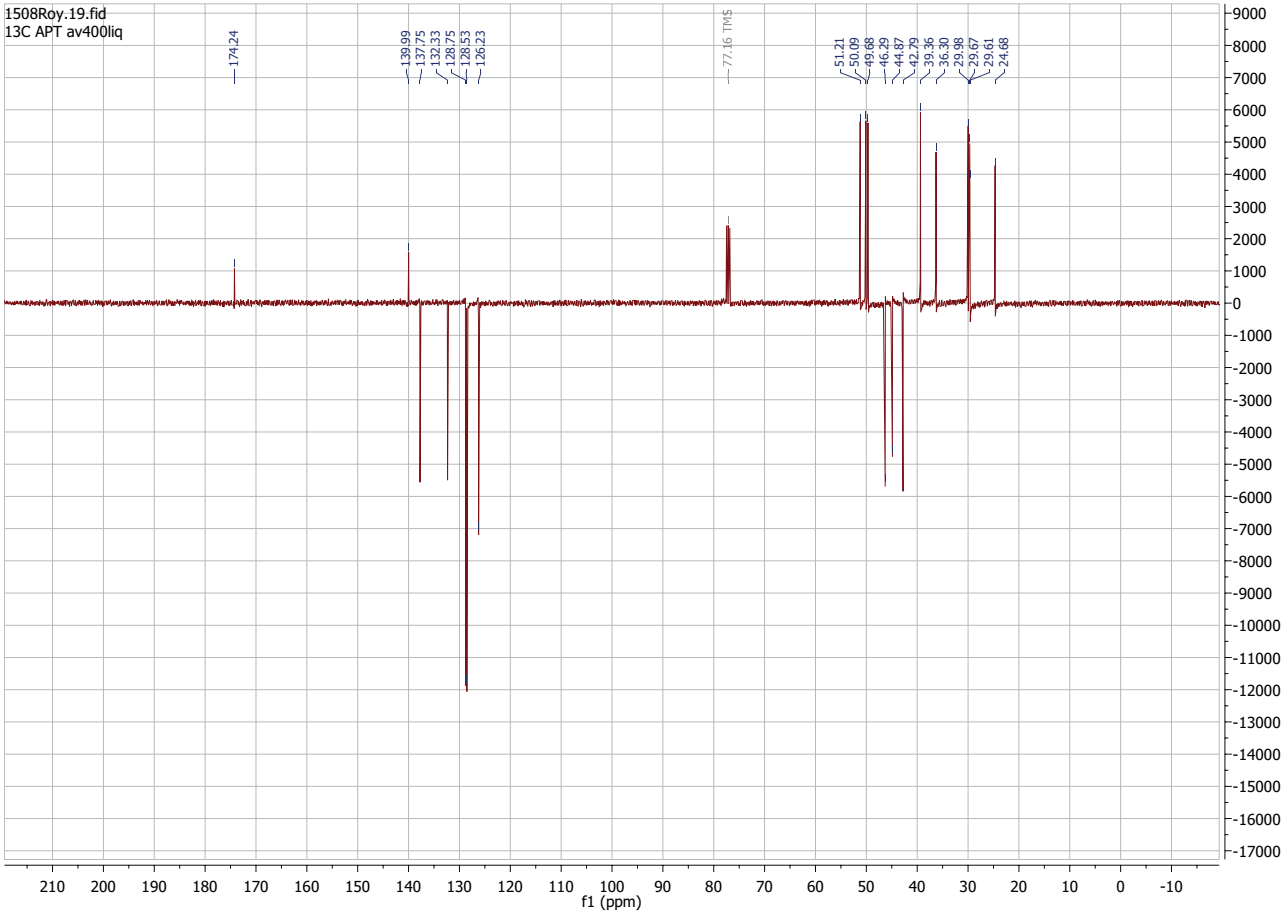
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f1 (ppm)


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O
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B (s)
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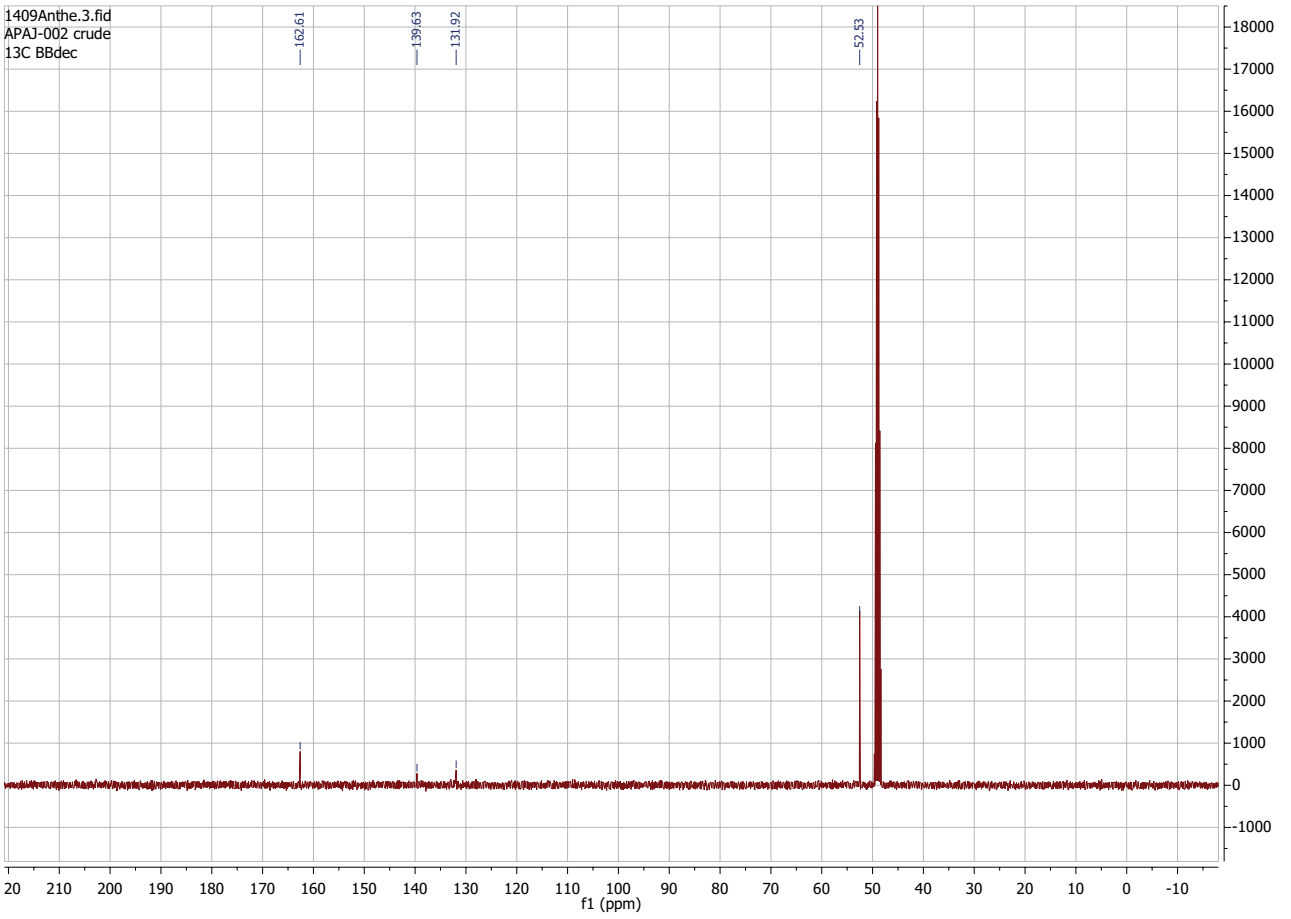
3.26
f1 (ppm)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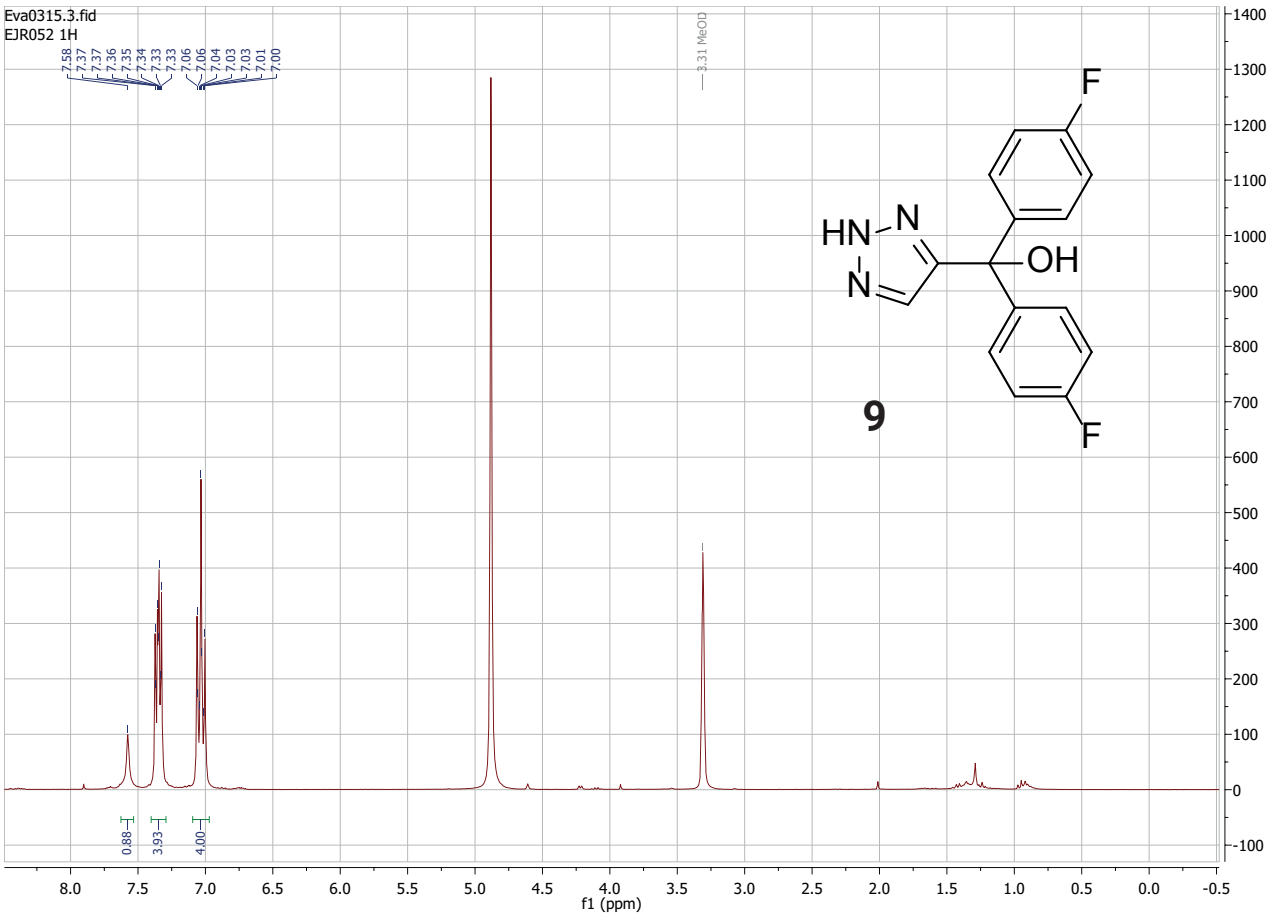
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7.06
7.04
7.03
7.03
7.01
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F
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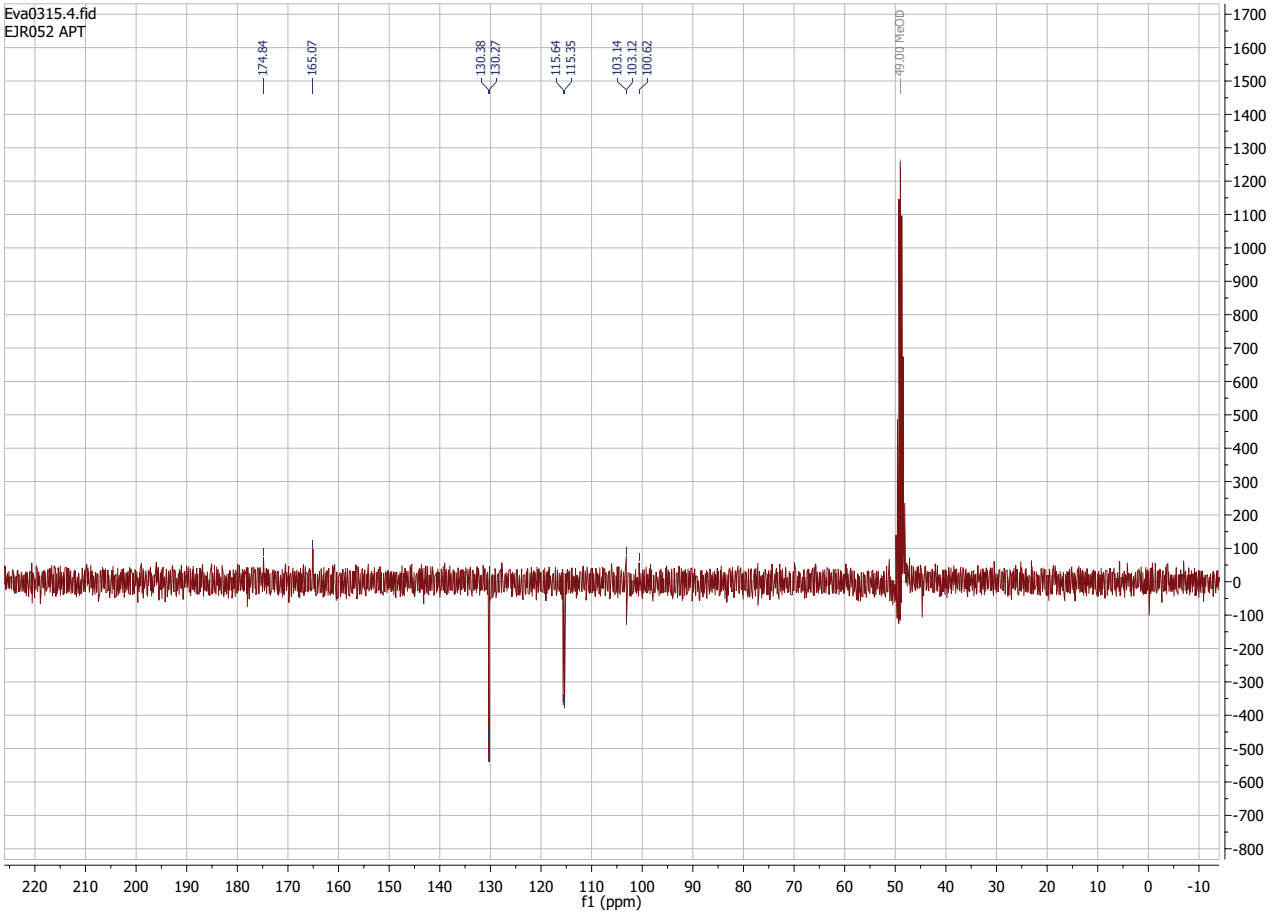
f1 (ppm)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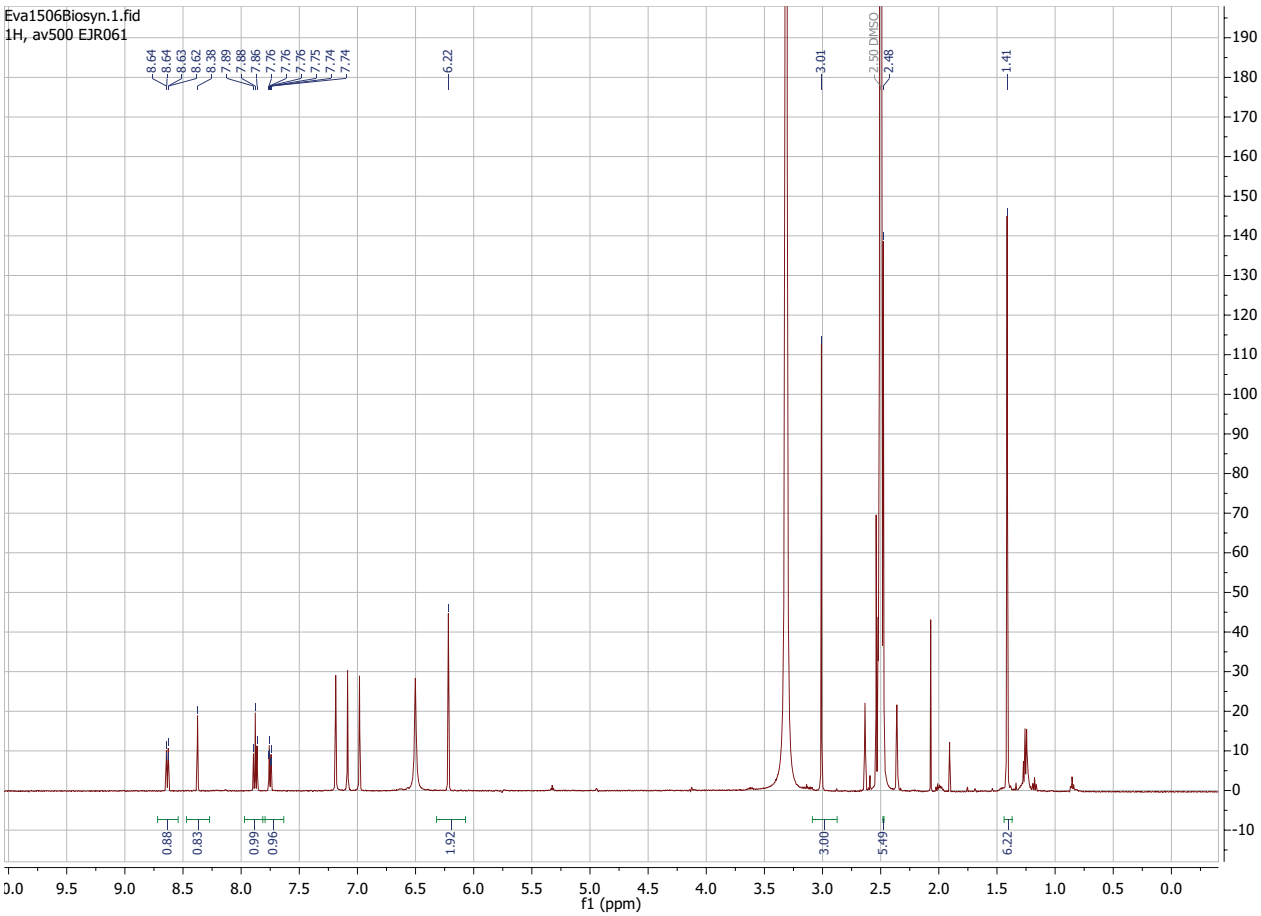
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7.76
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6.22
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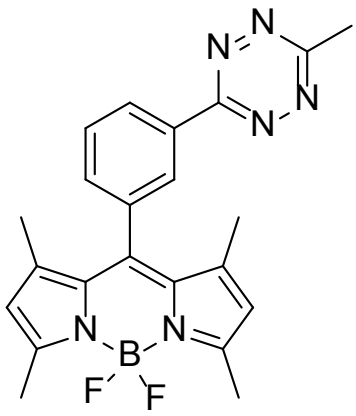
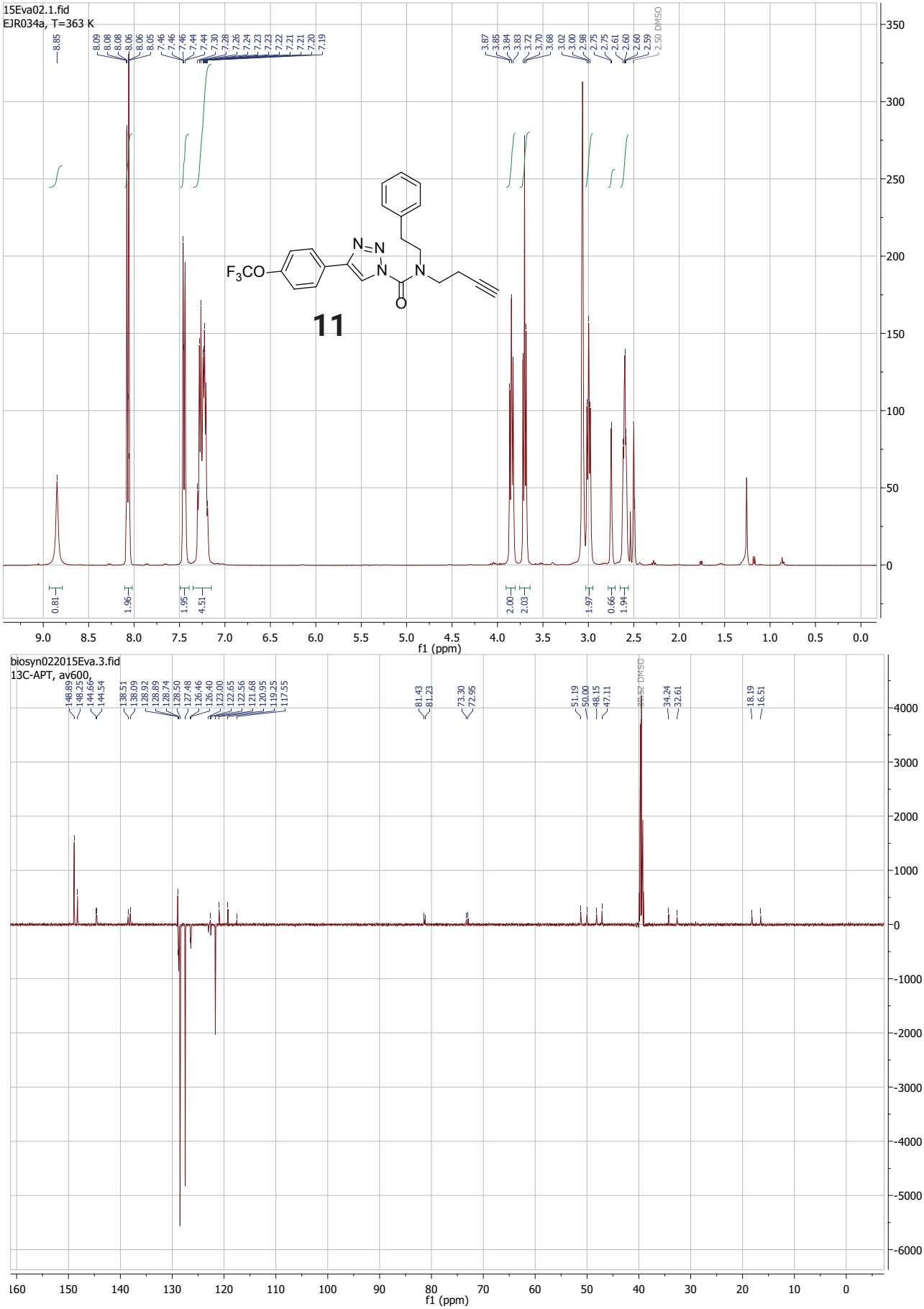
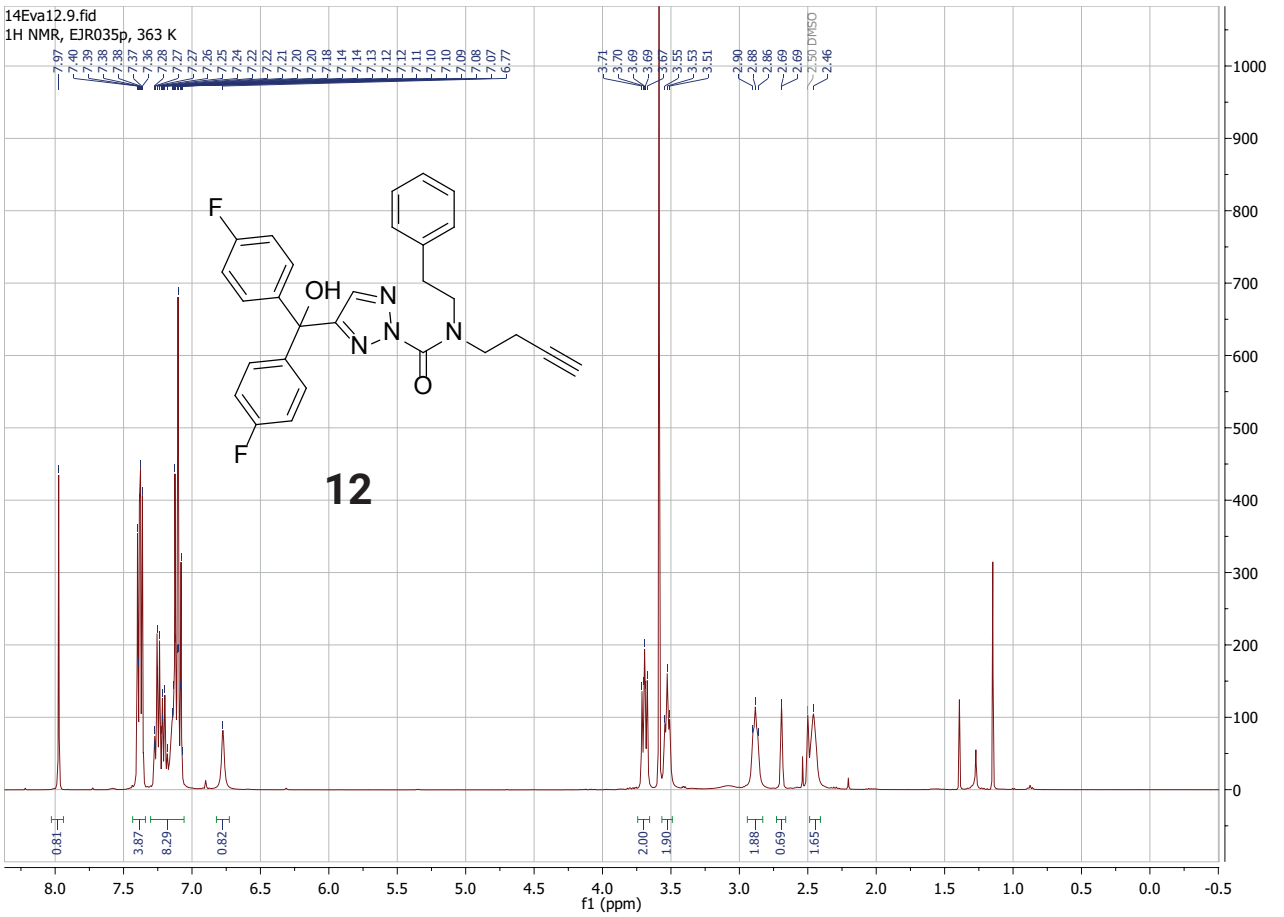


10

^{1}H and ^{13}C spectra

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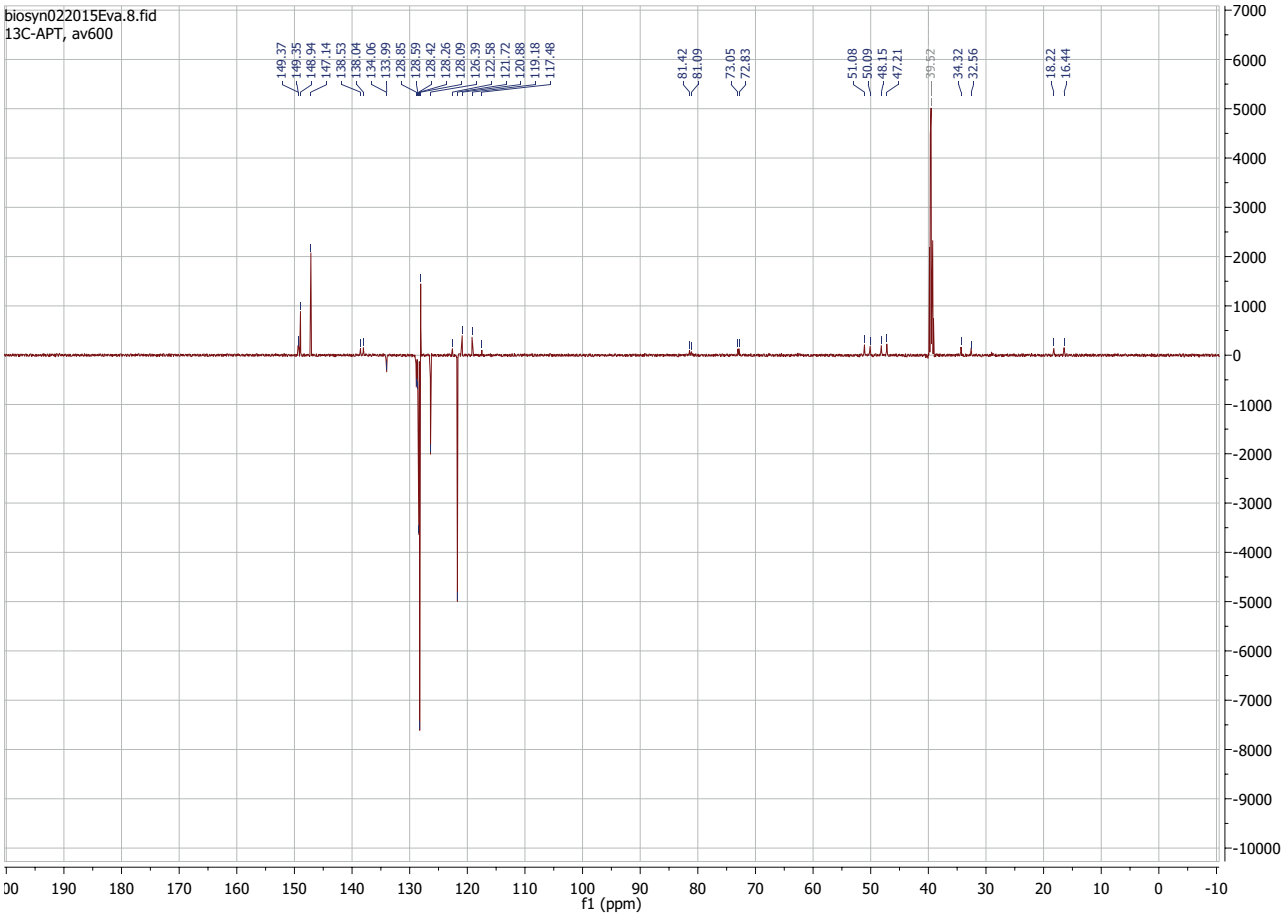
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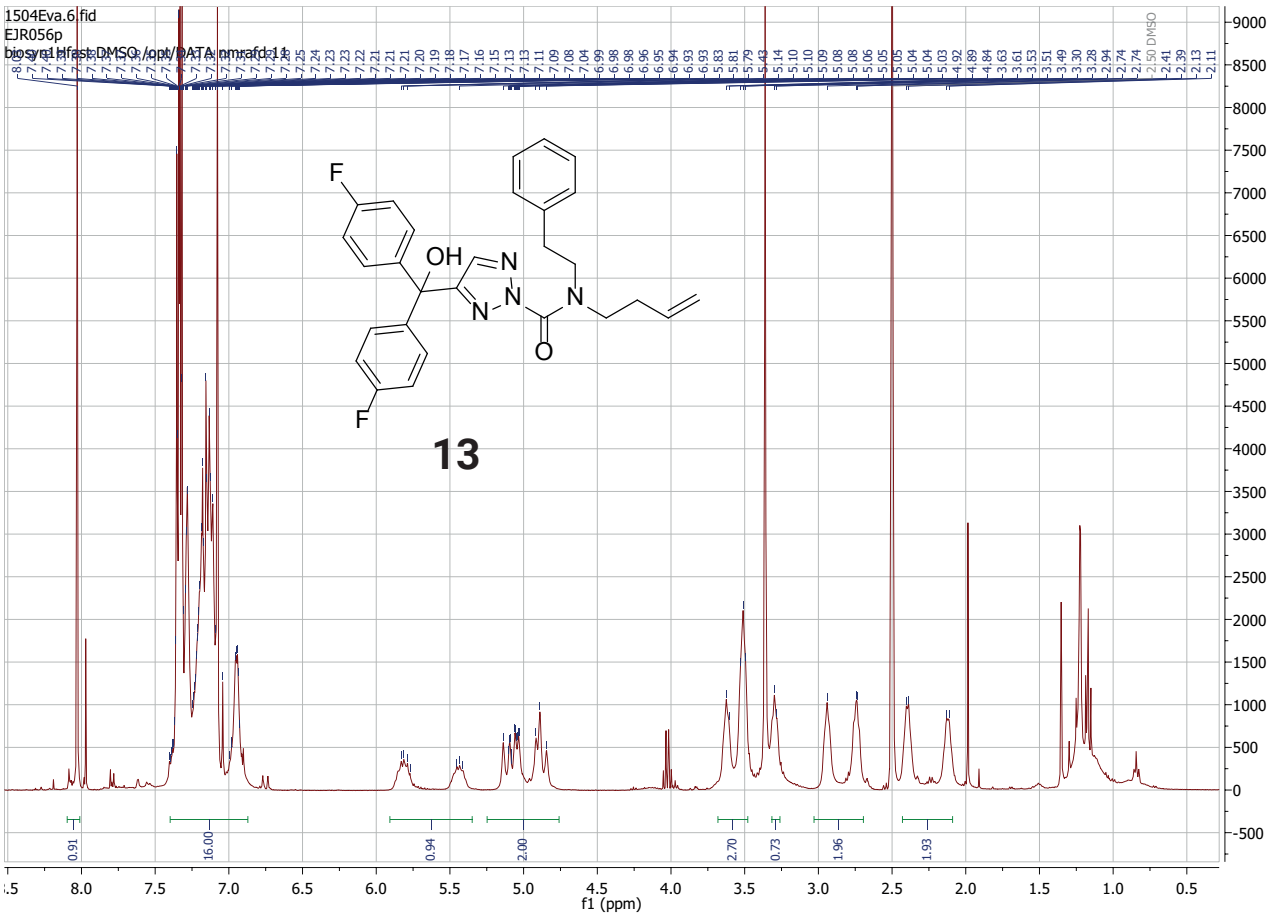
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13
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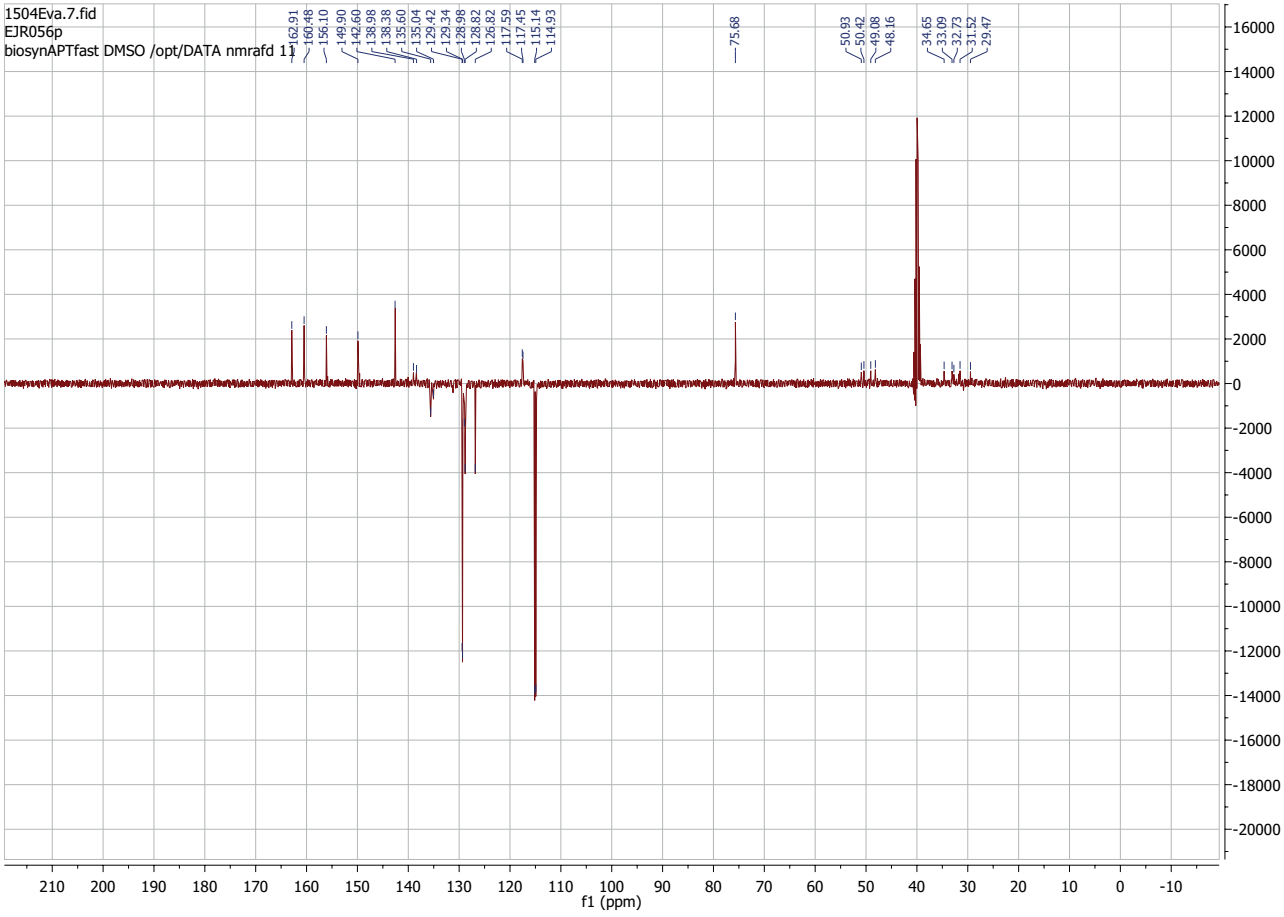
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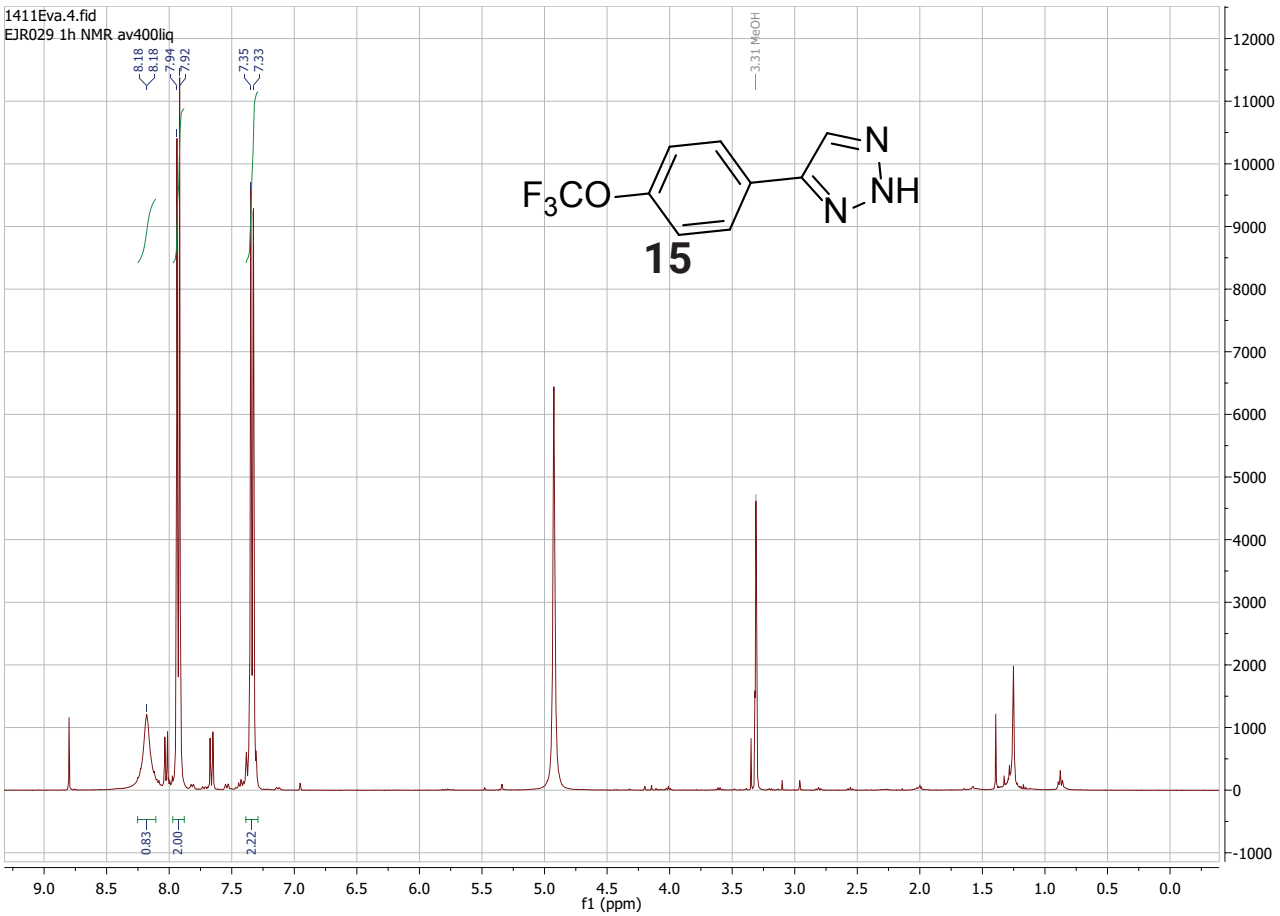
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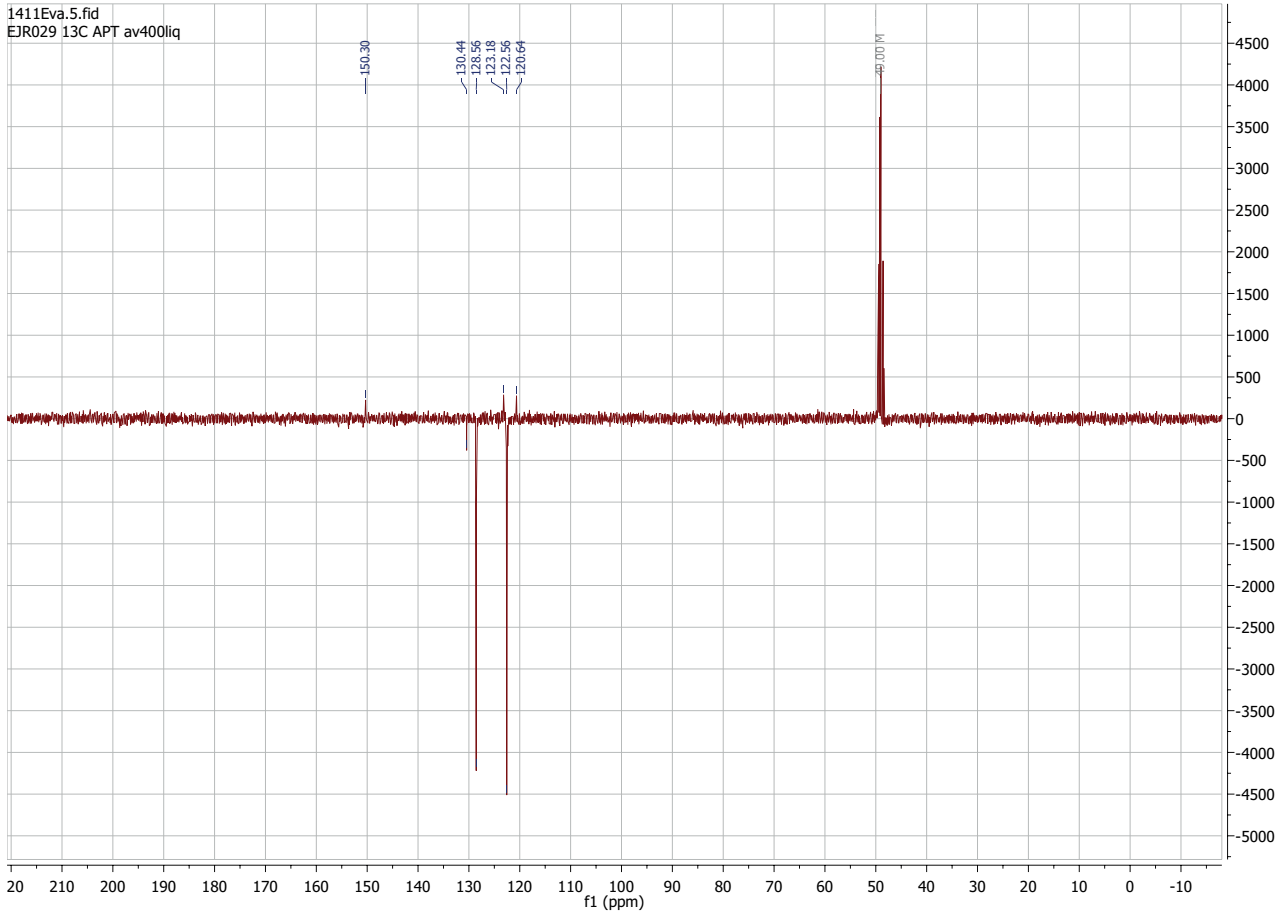
f1 (ppm)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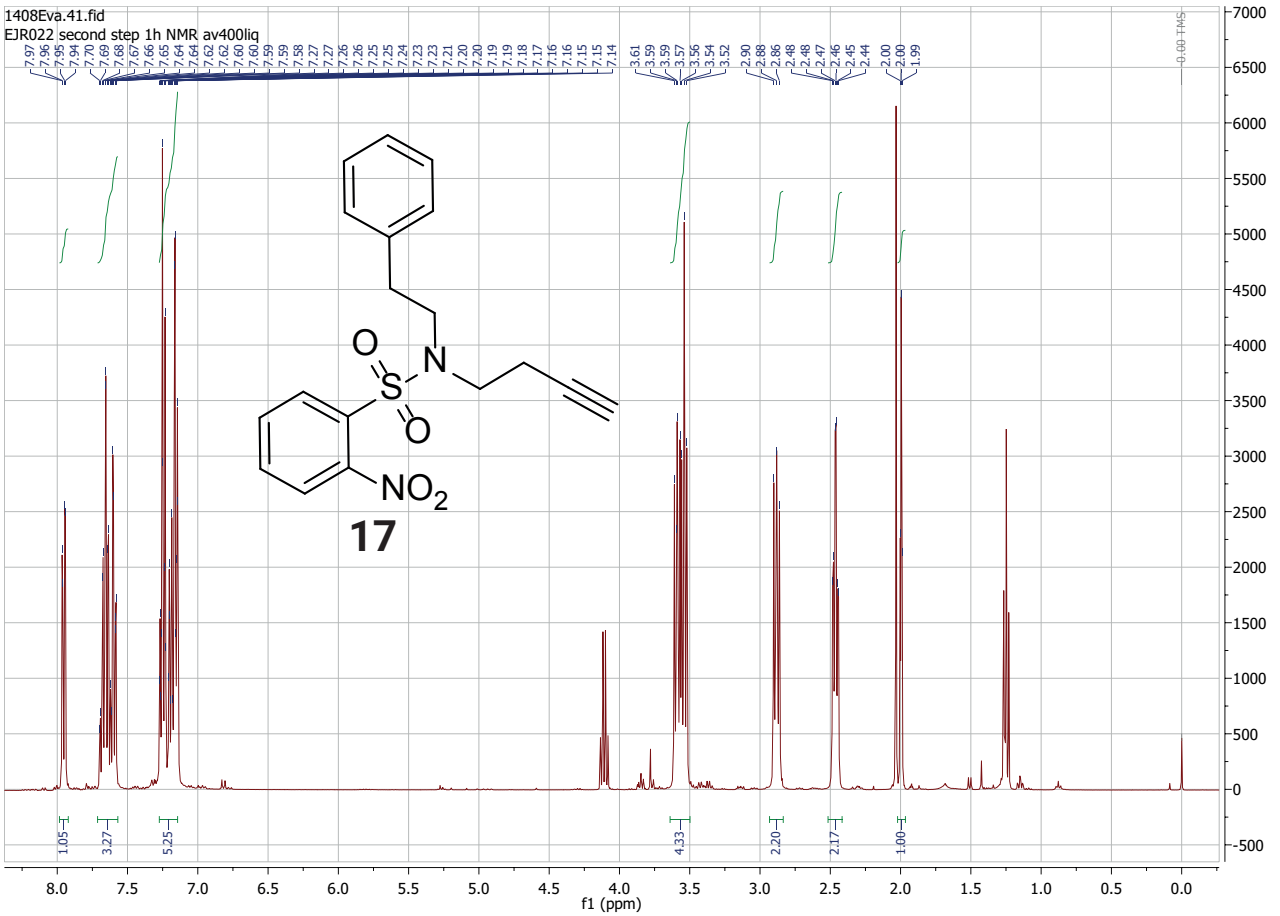
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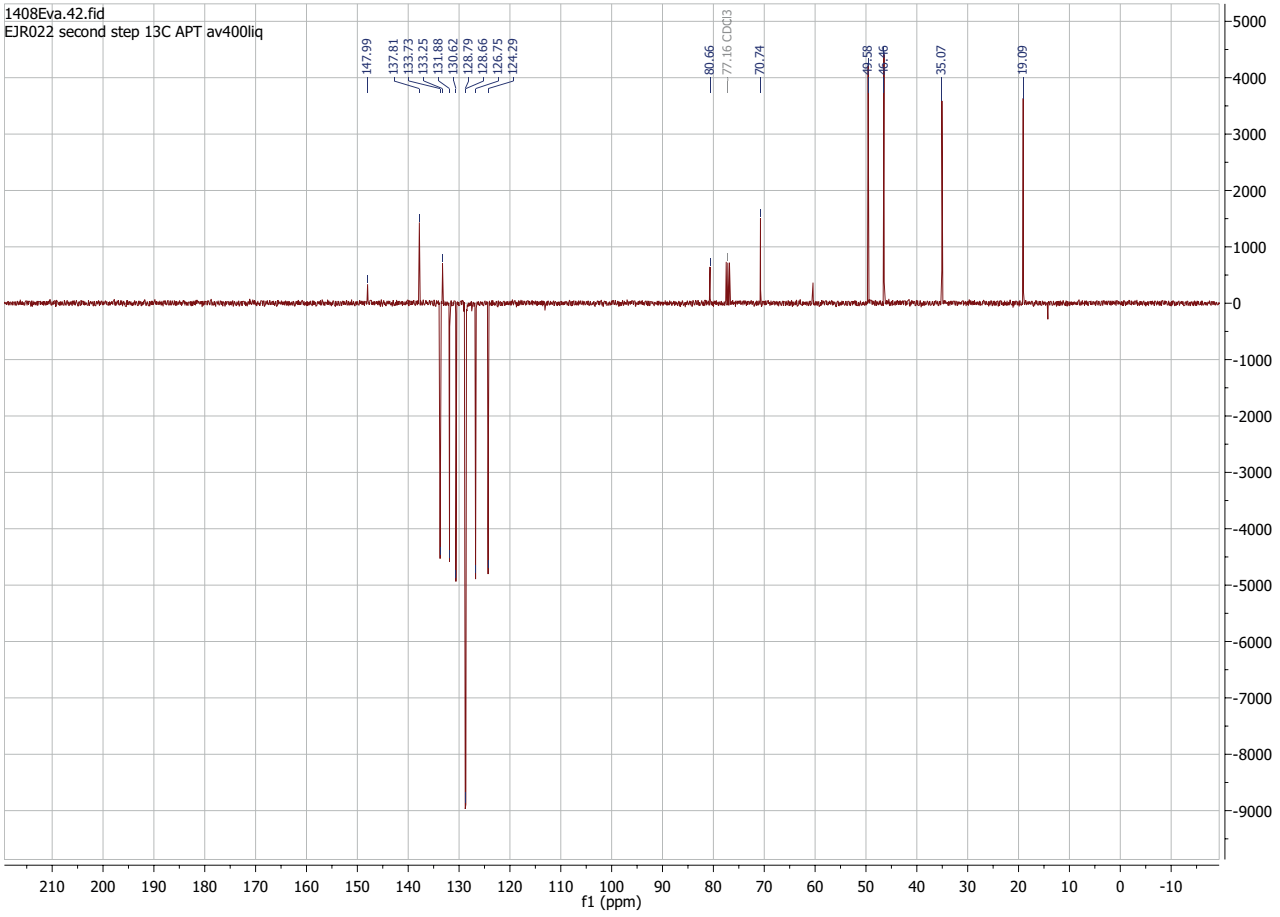
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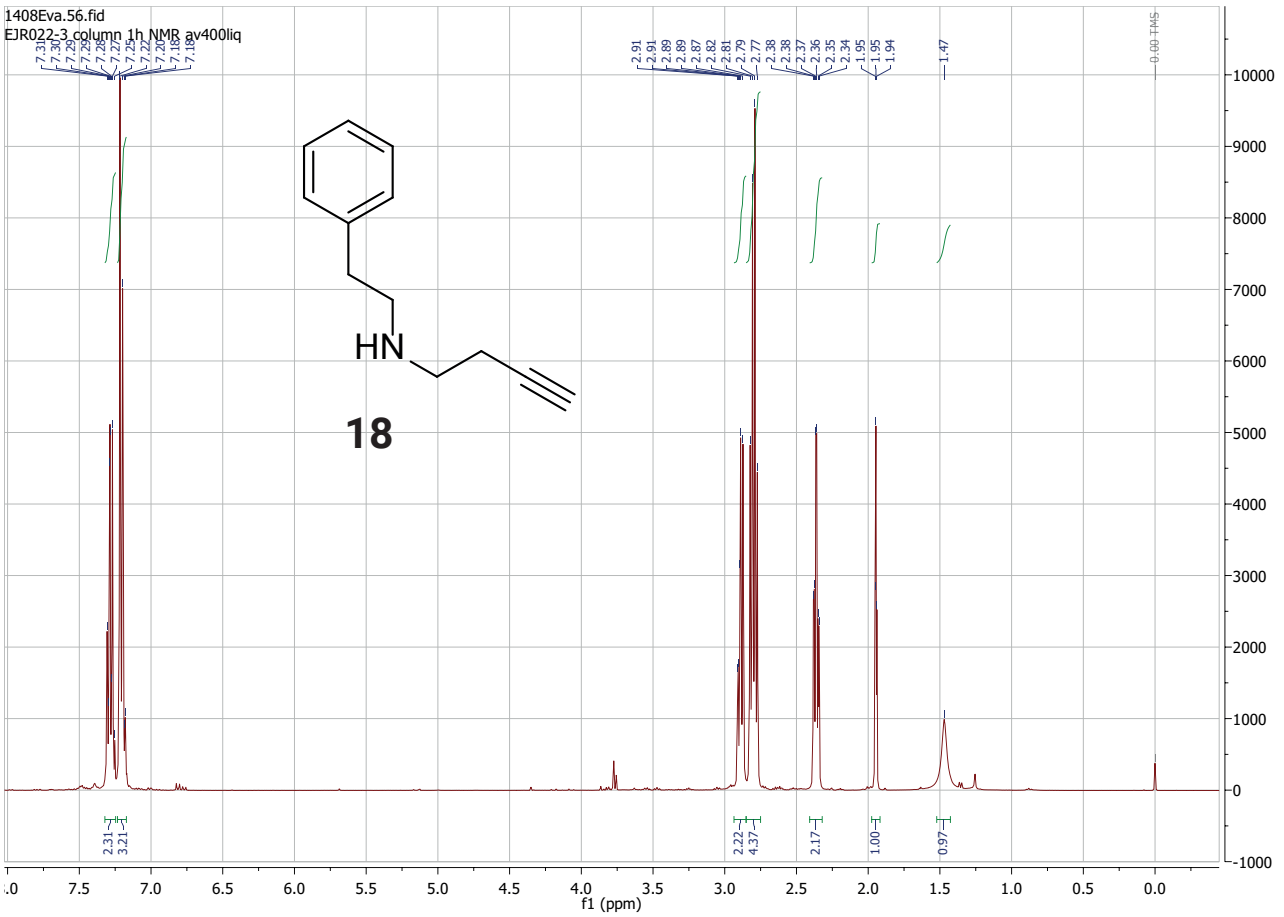
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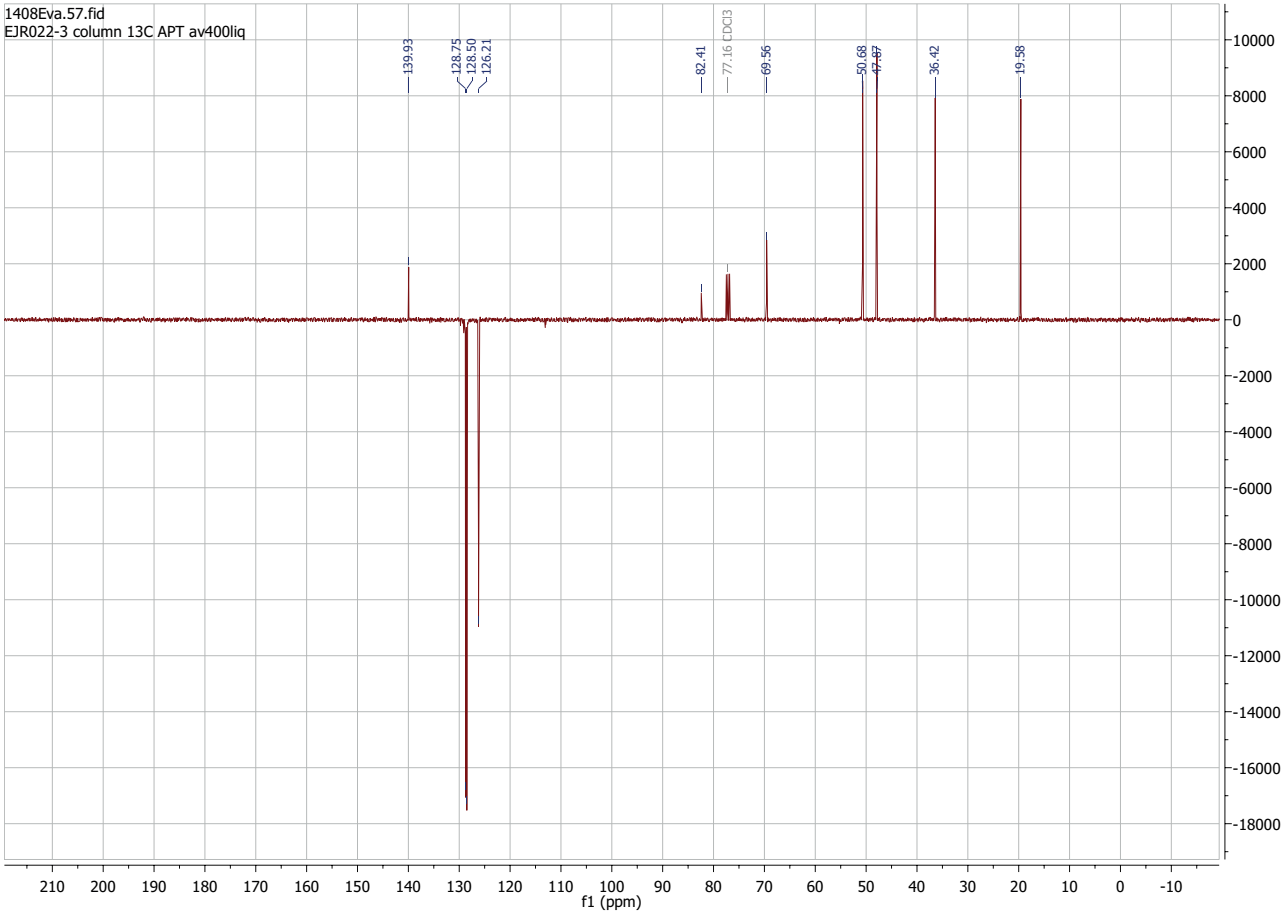
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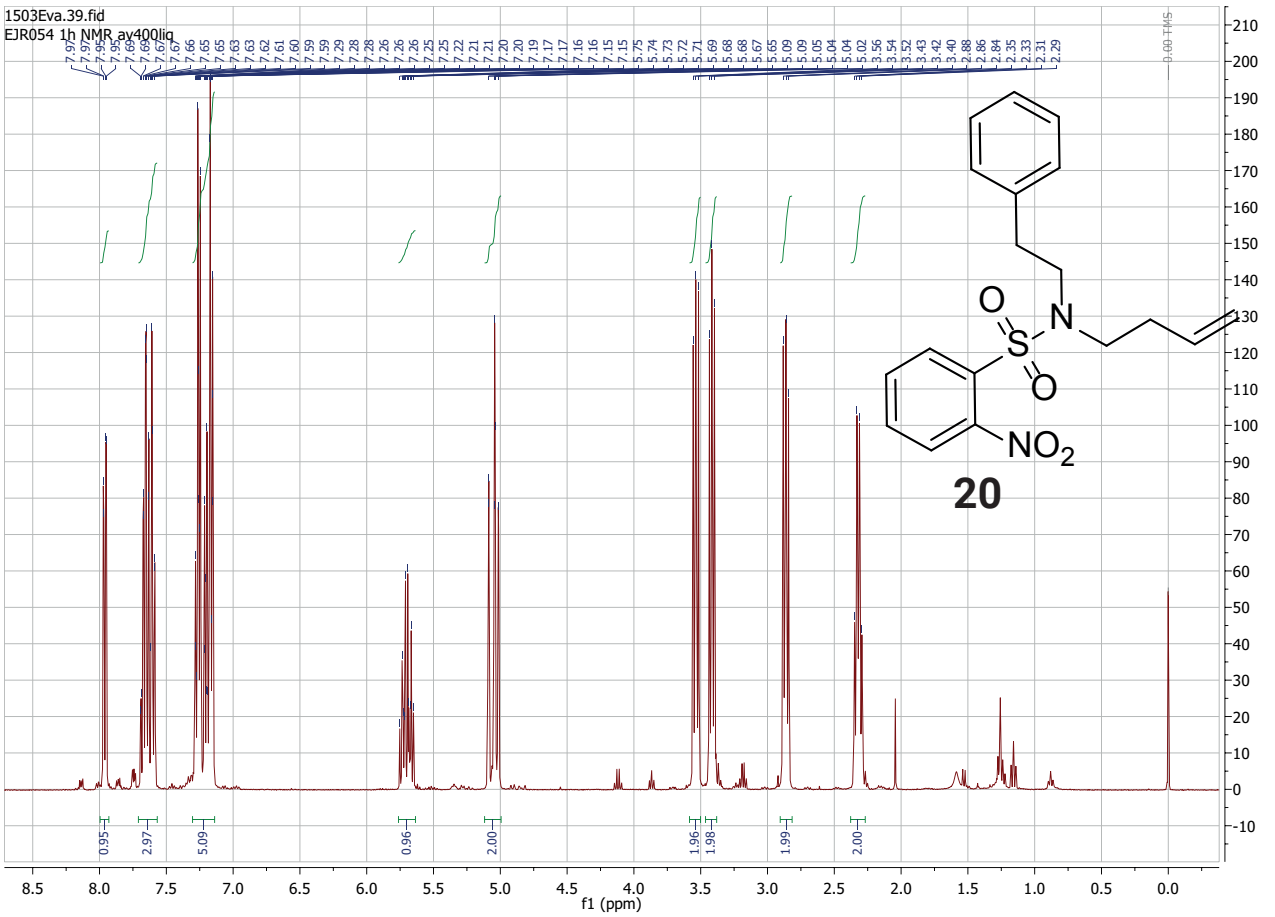
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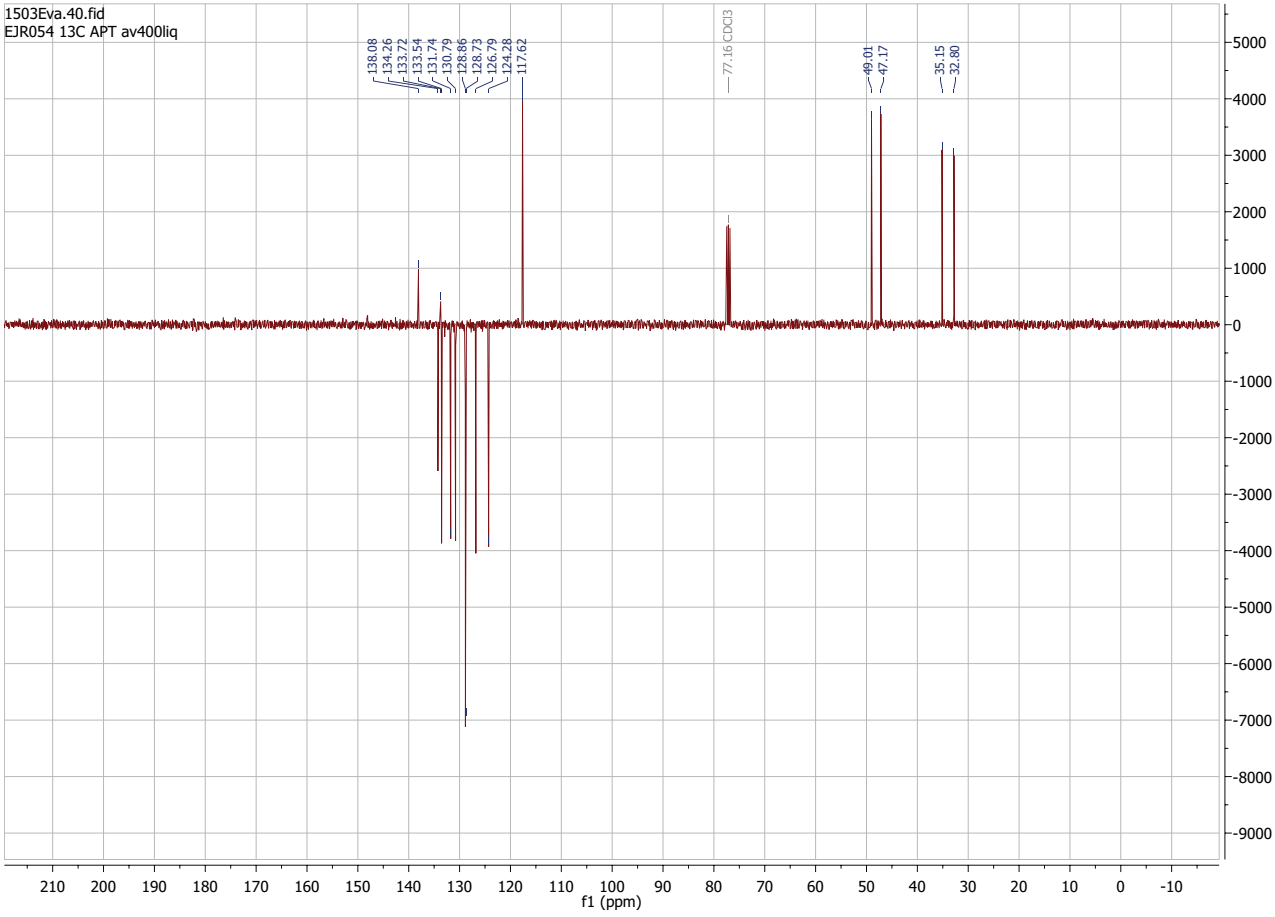
f1 (ppm)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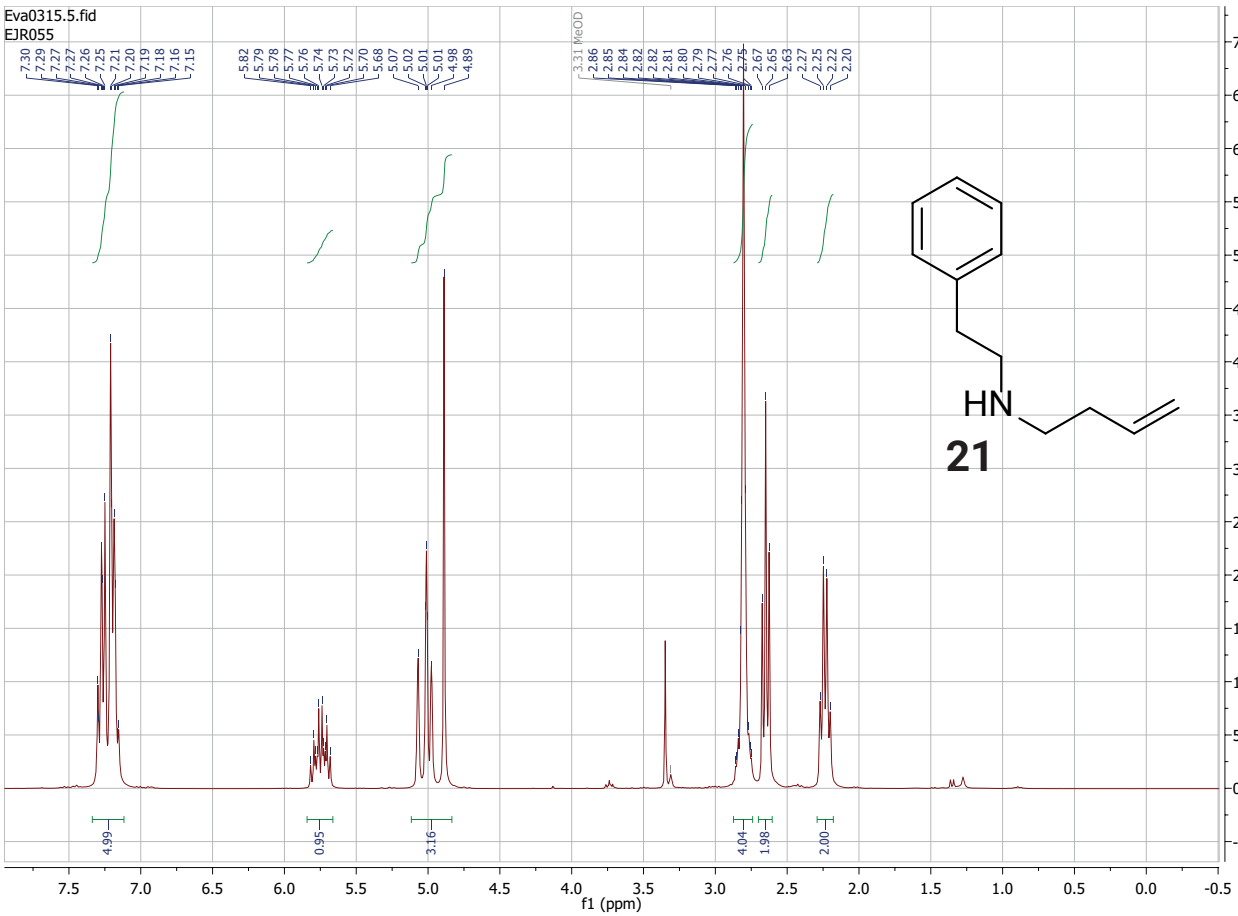
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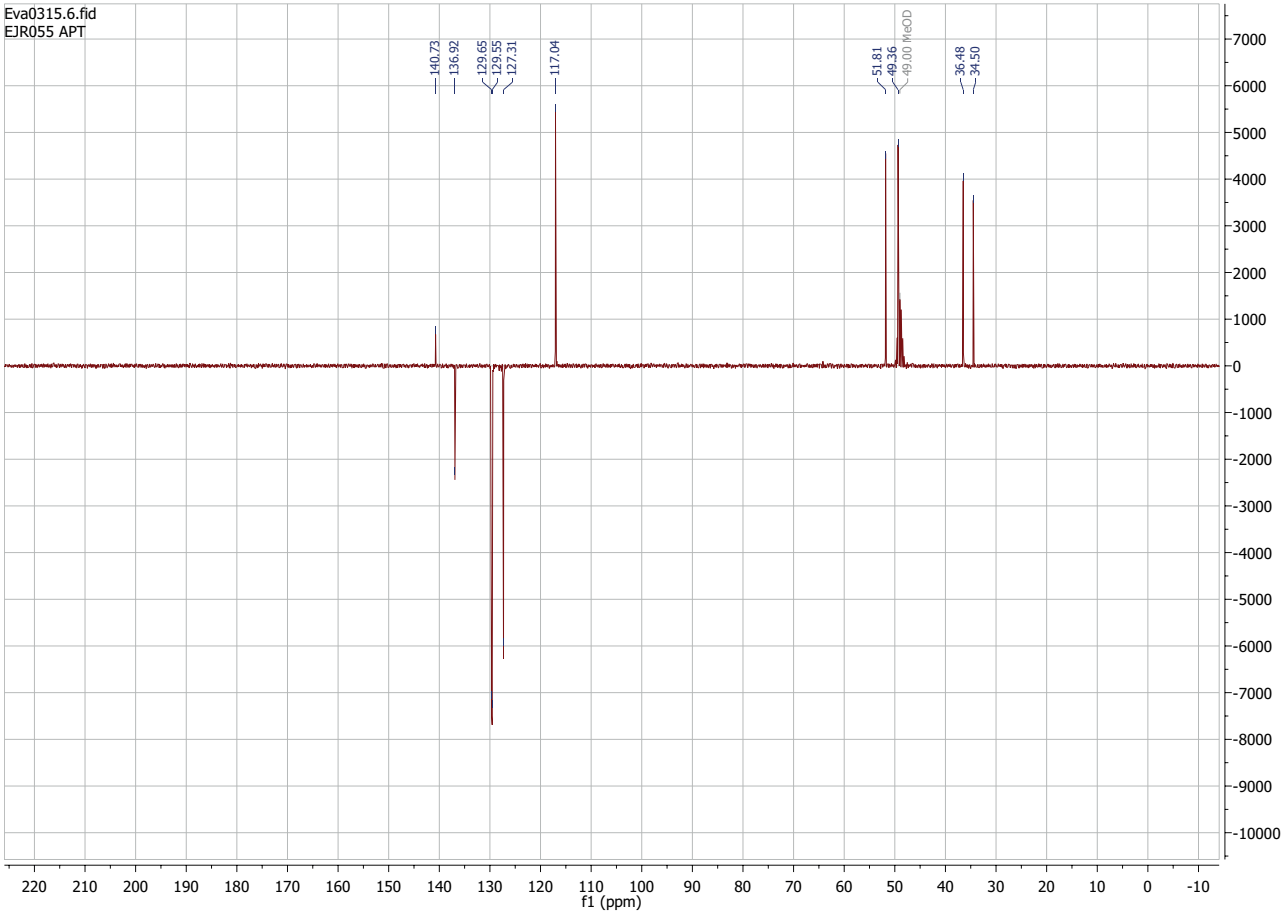
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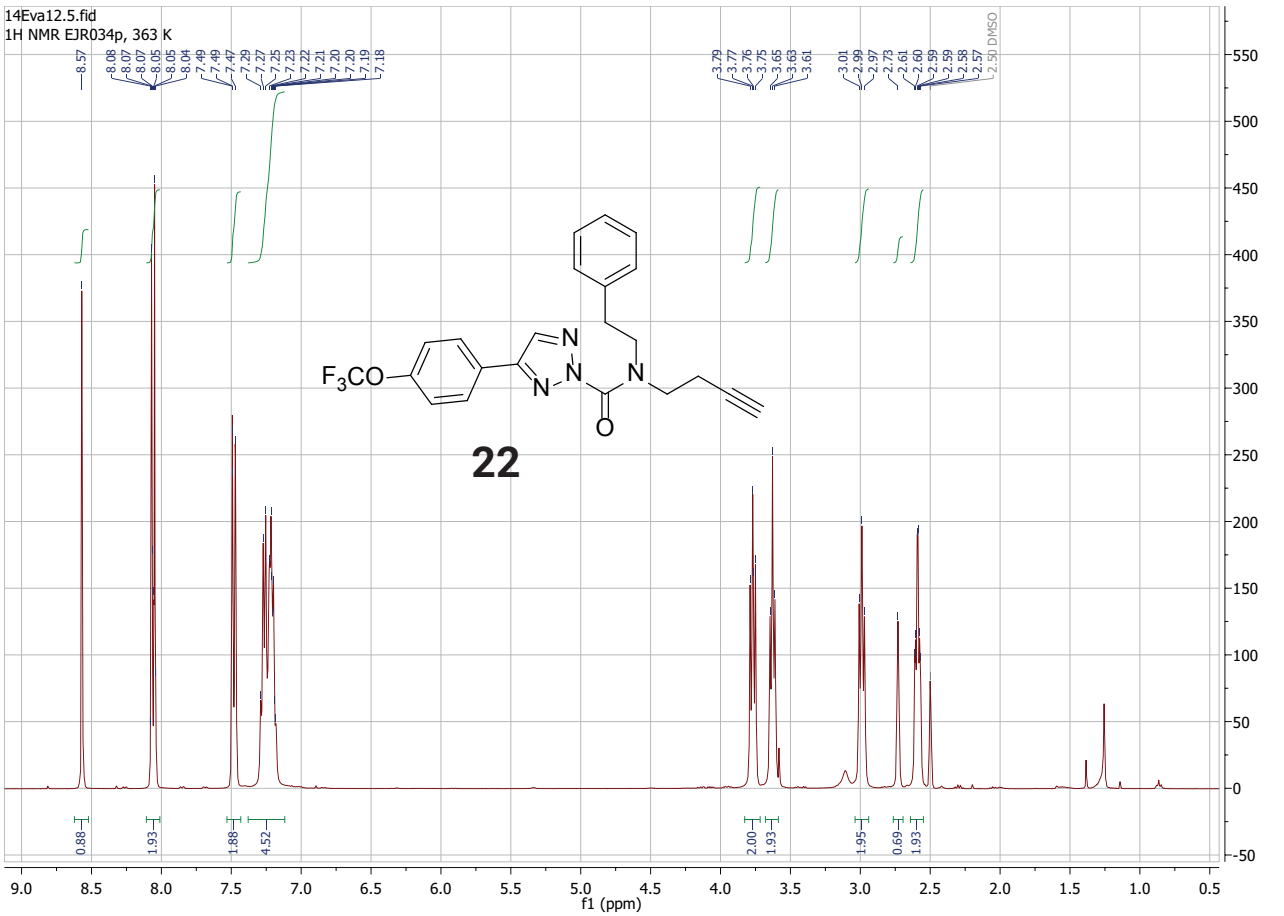
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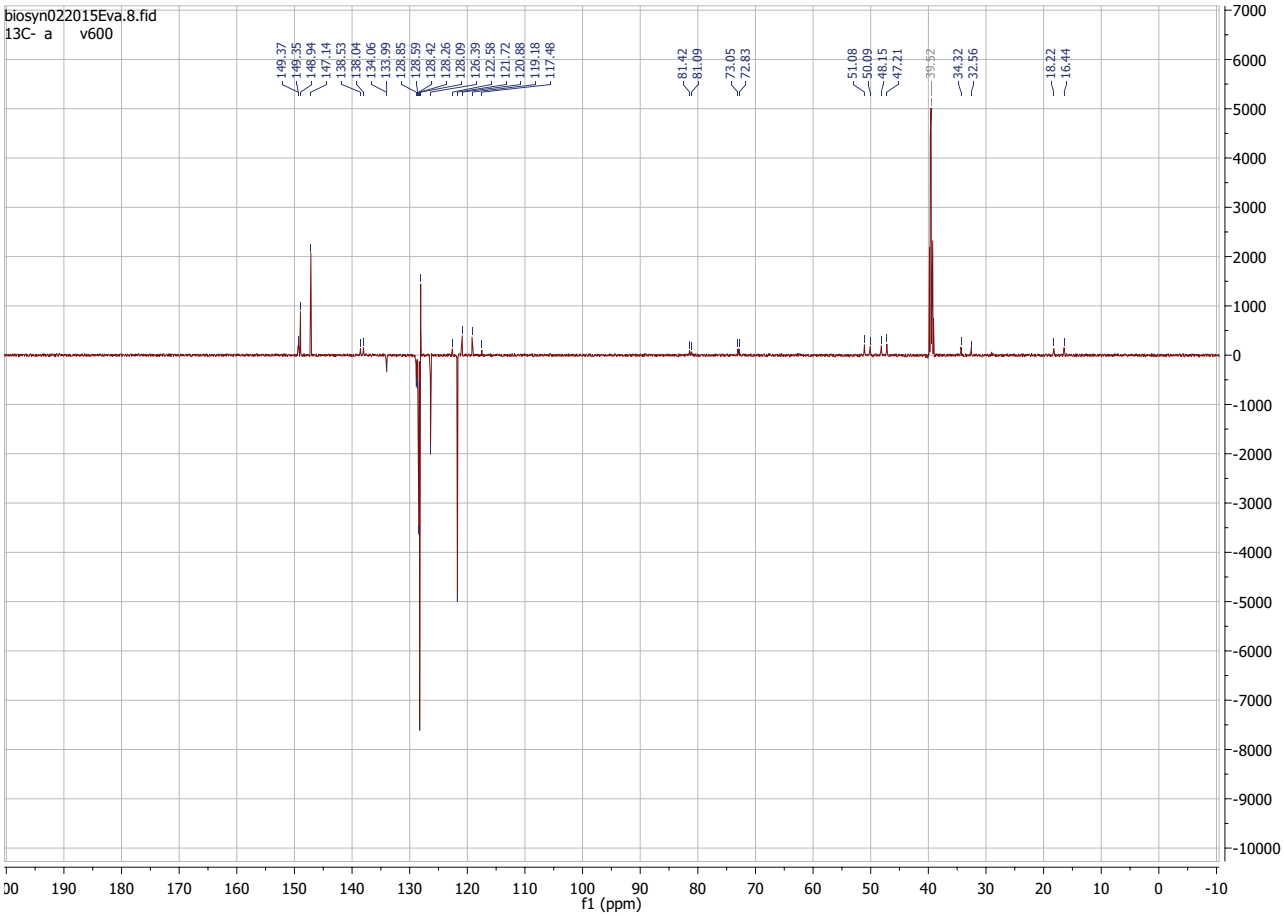
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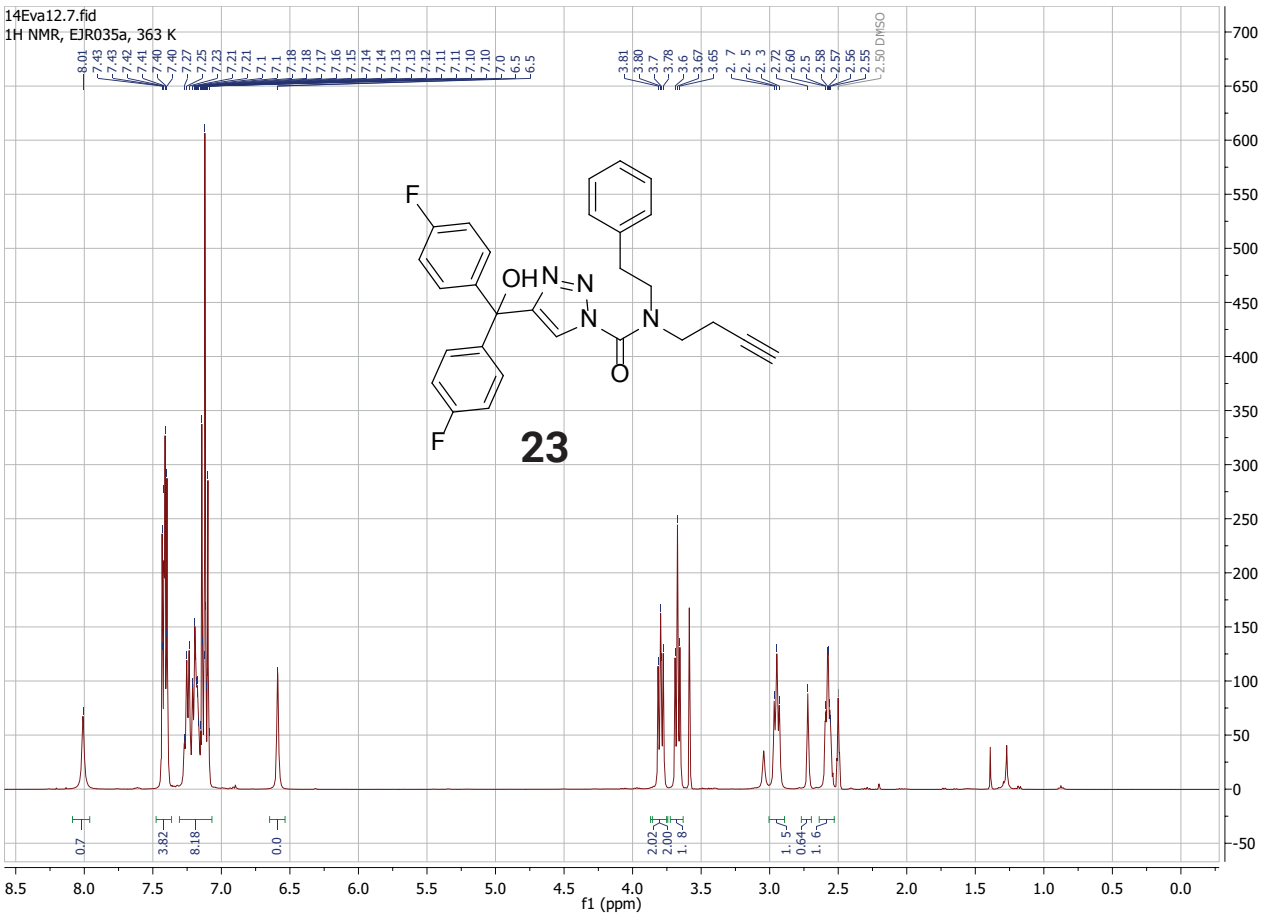
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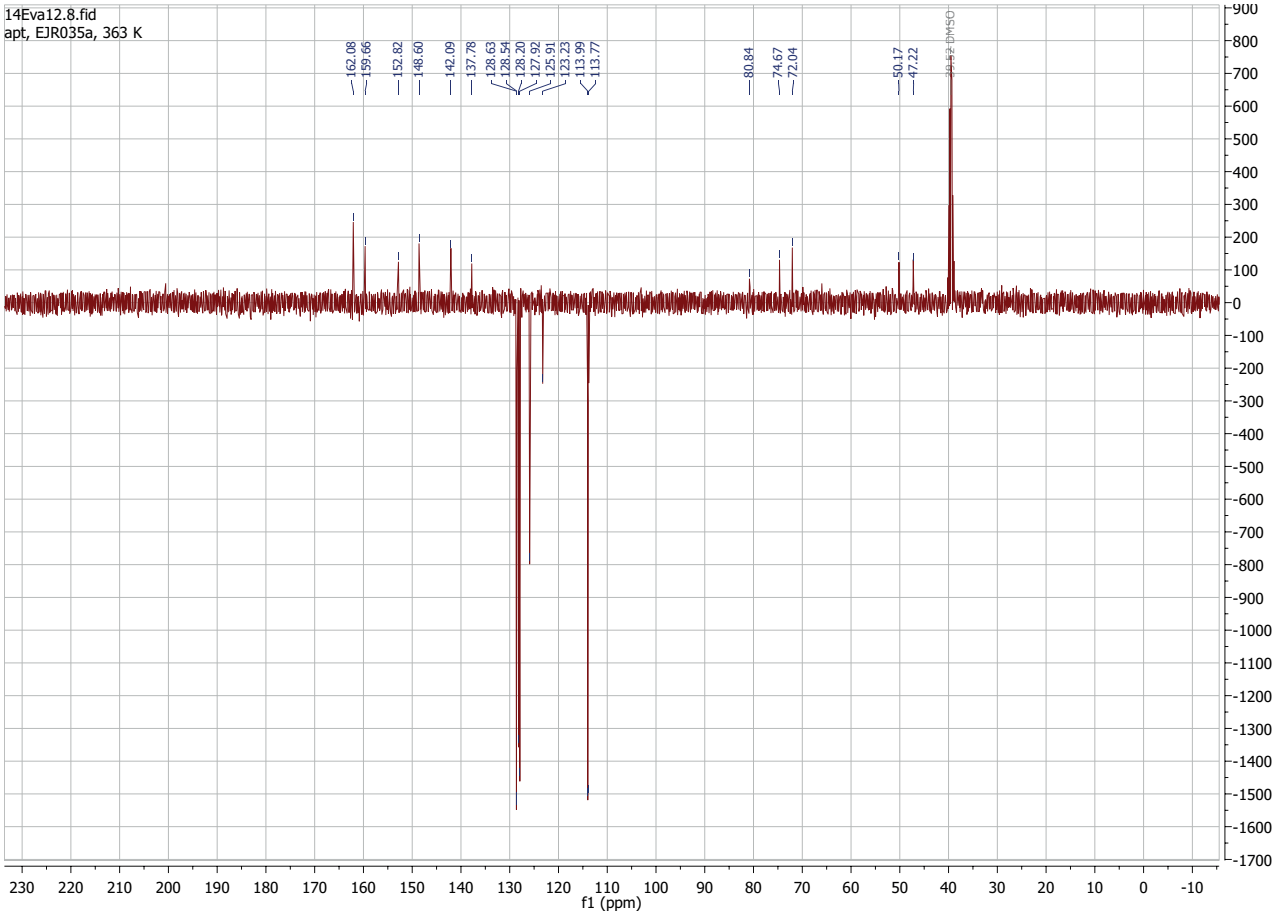
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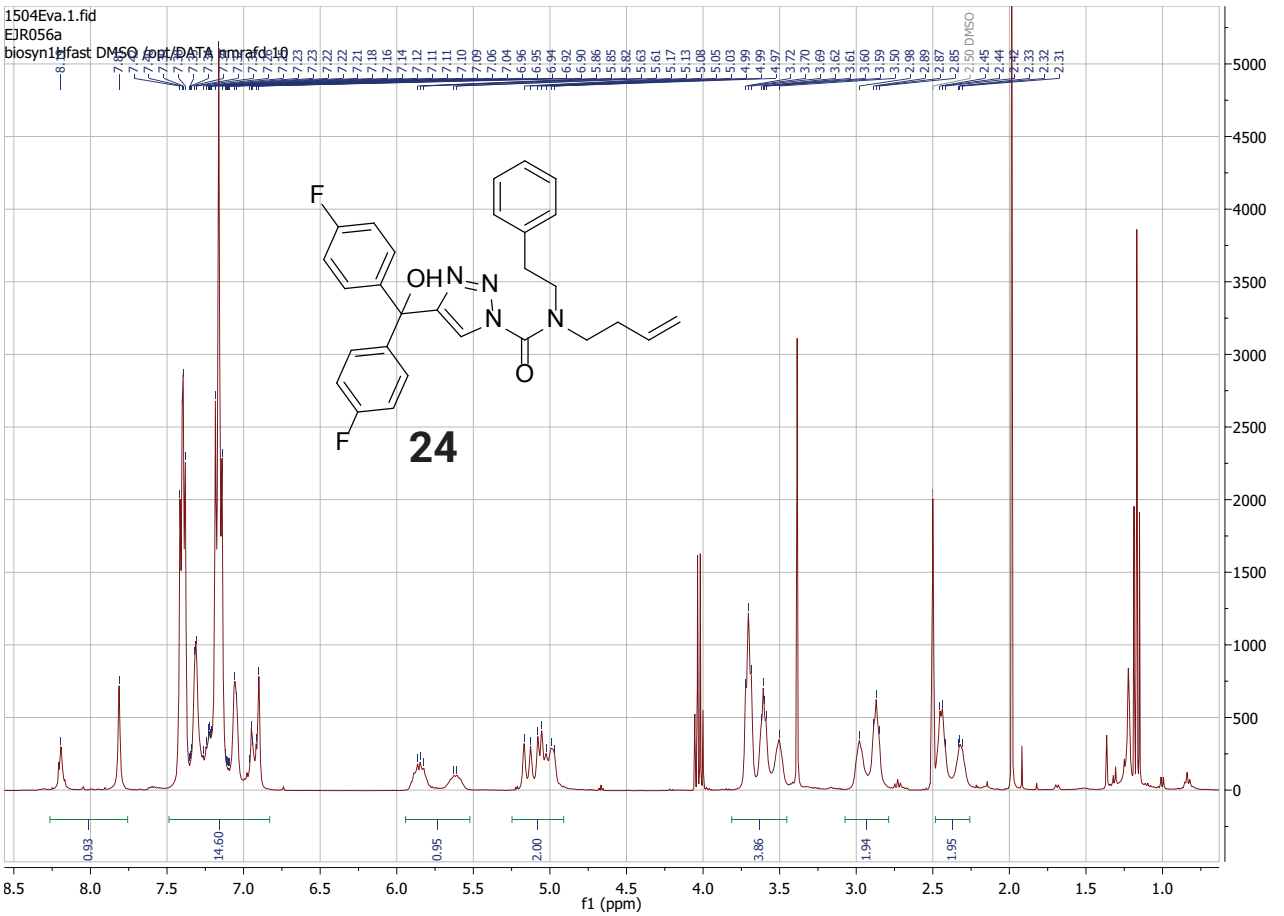
162.08
159.66
152.82
148.60
142.09
137.78
128.63
128.54
128.20
127.92
125.91
123.23
113.99
113.77
80.84
74.67
72.04
50.17
47.22
39.52 DMSO
f1 (ppm)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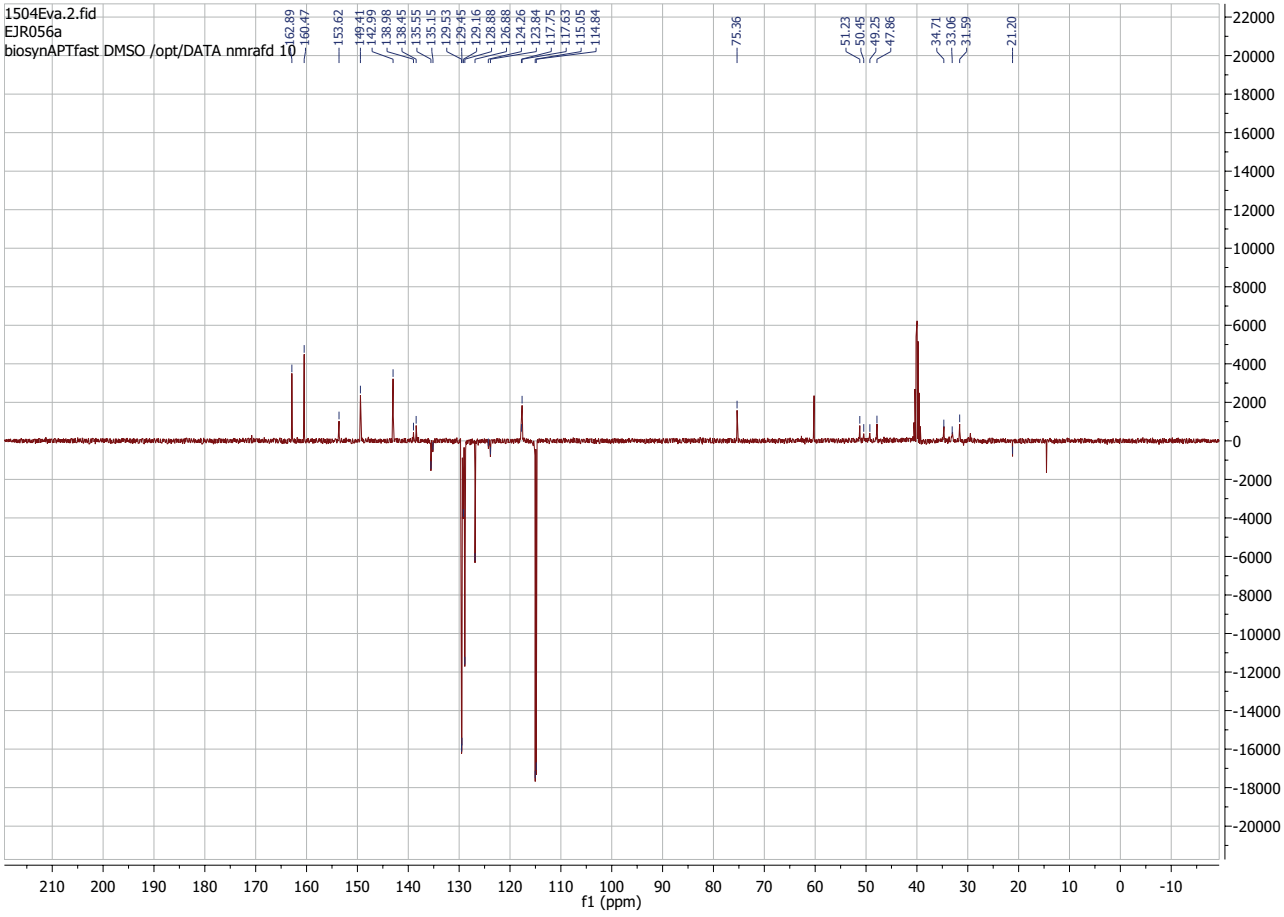
EJR056a
biosyn1Hfast DMSO /opt/DATA nmrafd 10
F
OH
N
N
N
N
O
F
24
f1 (ppm)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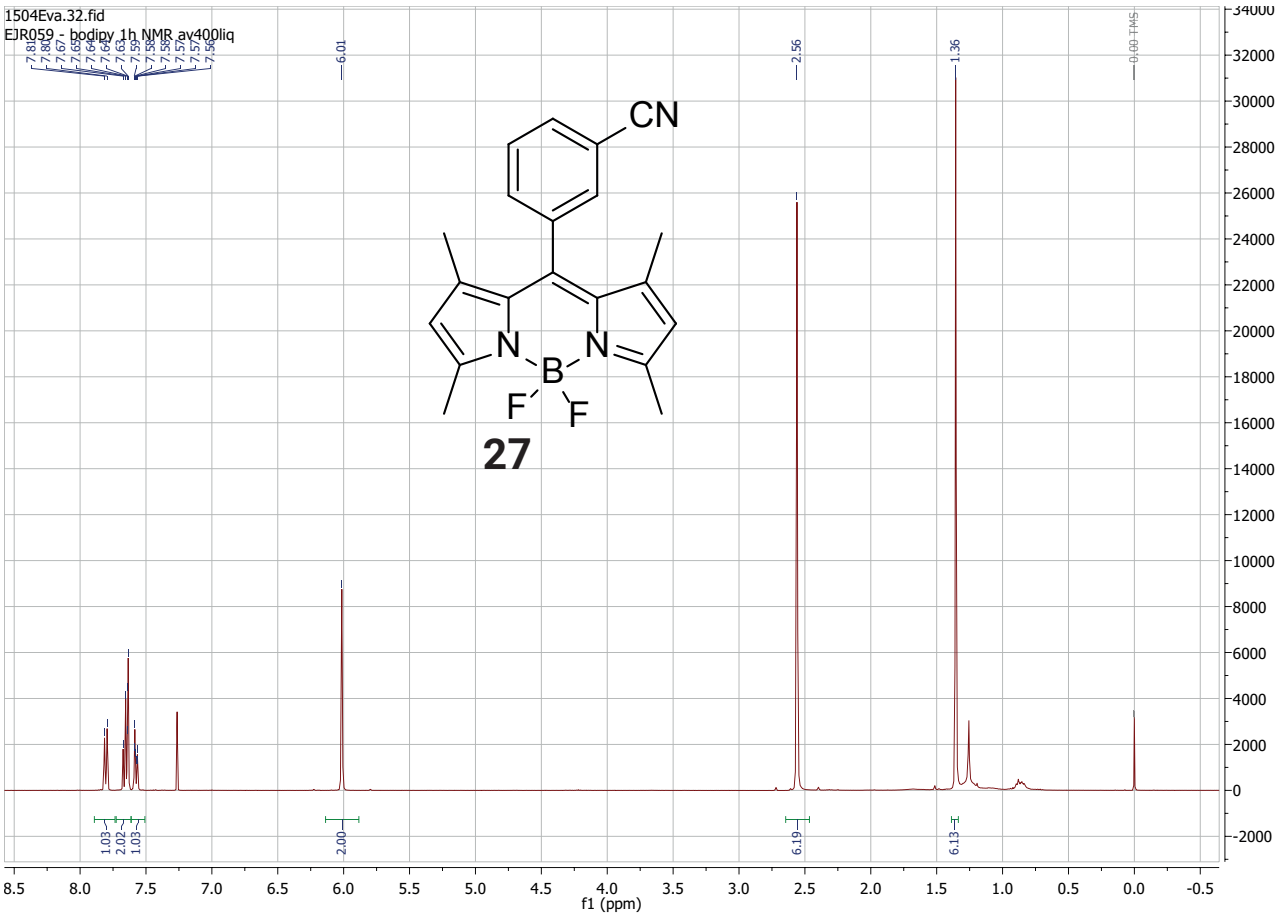
biosynAPTfast DMSO /opt/DATA nmrafd 10
f1 (ppm)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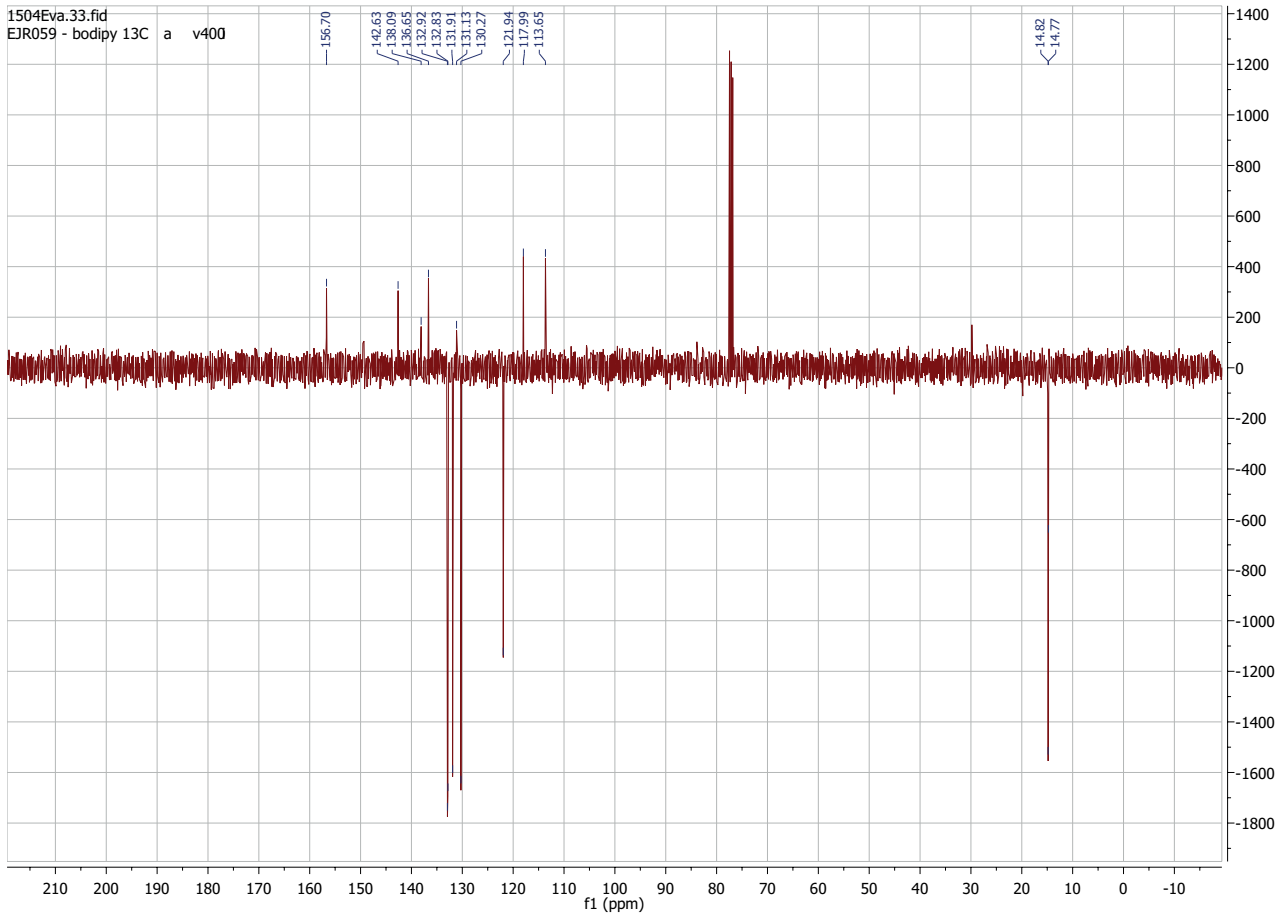
EJR059 - bodipy 1h NMR av400liq
CN
N
B
N
F
F
27
f1 (ppm)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EJR059 - bodipy 13C a v400
f1 (ppm)