Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information for: (*E,E*)-1,5-cyclooctadiene: a small and fast click-chemistry multitalent

Henning Stöckmann,[†,‡] André A. Neves,[‡] Henry A. Day,[†] Shaun R. Stairs,[†] Kevin M. Brindle,[‡] and Finian J. Leeper[*,†]

University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW, UK, and Cancer Research UK Cambridge Research Institute, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, UK

E-mail: fjl1@cam.ac.uk

Phone: ++44 (0) 1223 336 403. Fax: ++44 (0) 1223 336 362

Cell culture

Murine T-cell lymphoma (EL4, from ATCC, Teddington, UK) cells were grown in RPMI-1640 medium (Invitrogen, Paisley, UK); 2.0 g L^{-1} glucose, 300 mg L^{-1} L-glutamine) supplemented with 10% FBS (fetal bovine serum, PAA laboratories, Yeovil, UK) and maintained in a 5% CO_2, water-saturated atmosphere at 37 °C.

[*]To whom correspondence should be addressed
[†]Department of Chemistry
[‡]Cancer Research UK Cambridge Research Institute

Cell surface azidoglycan labeling and detection by flow cytometry

EL4 cells were incubated for 24 h in media containing 50 μM Ac_4GalNAz. Control cells were grown in the absence of Ac_4GalNAz. The following procedure was followed for both azidosugar-pulsed and non-pulsed sets of cells. The medium was removed from the flasks and cells were washed with warm PBS (phosphate buffered saline; water, NaCl, KCl, Na_2HPO_4, KH_2PO_4; Fisher Scientific, Loughborough, UK). The contents of each flask were transferred to a centrifuge tube, centrifuged (600 g, 4 °C, 4 min), resuspended in cold FACS buffer (1% FBS in 20 mM HEPES, 150 mM NaCl, pH 7.4) and transferred to 1.5 mL Eppendorf tubes. Cells were centrifuged and resuspended in 100 μL labeling buffer (100 μM **11** in FACS buffer) or as a control 100 μL FACS buffer. The Eppendorf tubes were incubated in a hot block with orbital shaking (500 rpm, 37 °C, 30 min). The cells were washed three times with 700 μL ice cold FACS buffer, centrifuged and resuspended in 100 μL labeling buffer (10 μM **12** in FACS buffer containing 50 nM SYTOX Green, Invitrogen) or as a control 100 μL 50 nM SYTOX Green in FACS buffer, followed by incubation as above for 20 min. Cells were then filtered through a 50 μm cut-off membrane into flow cytometry tubes and kept on ice. Each sample was analysed by a flow cytometer (model LSRII, BD Oxford, UK) using 20,000 events.

Data analysis was performed using FlowJo flow cytometry analysis software (Tree Star, Ashland, OR). The viable cell population (population of interest) was determined by gating cells to exclude those with a low NADH auto-fluorescence and those with high levels of SYTOX Green (cell death marker). The far-red median fluorescence intensity (MFI, Alexa Fluor 647 fluorophore) of the viable cell population was then assessed (Figure S1).[†] Data points were collected in triplicate.

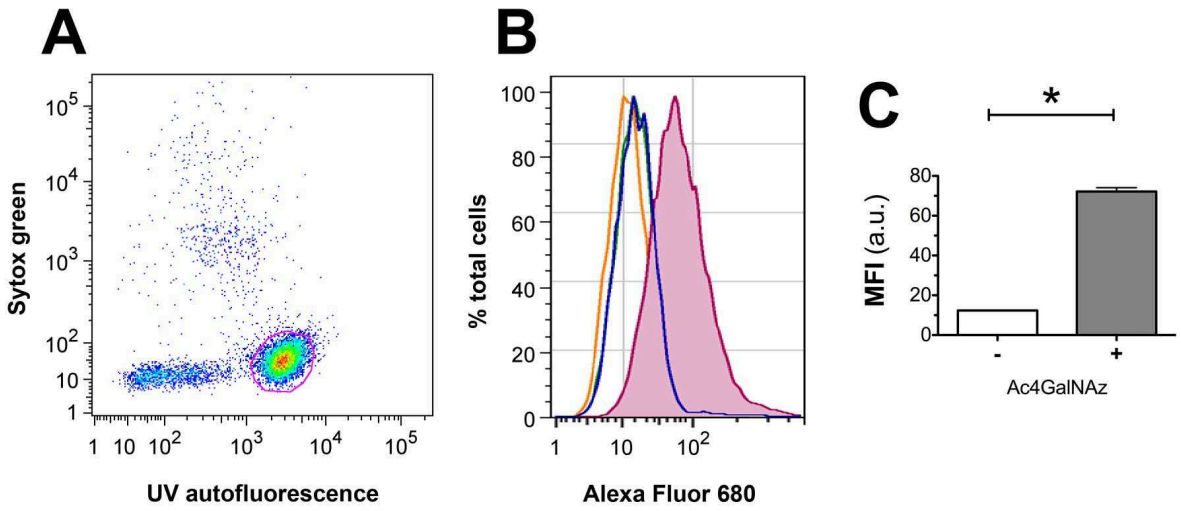
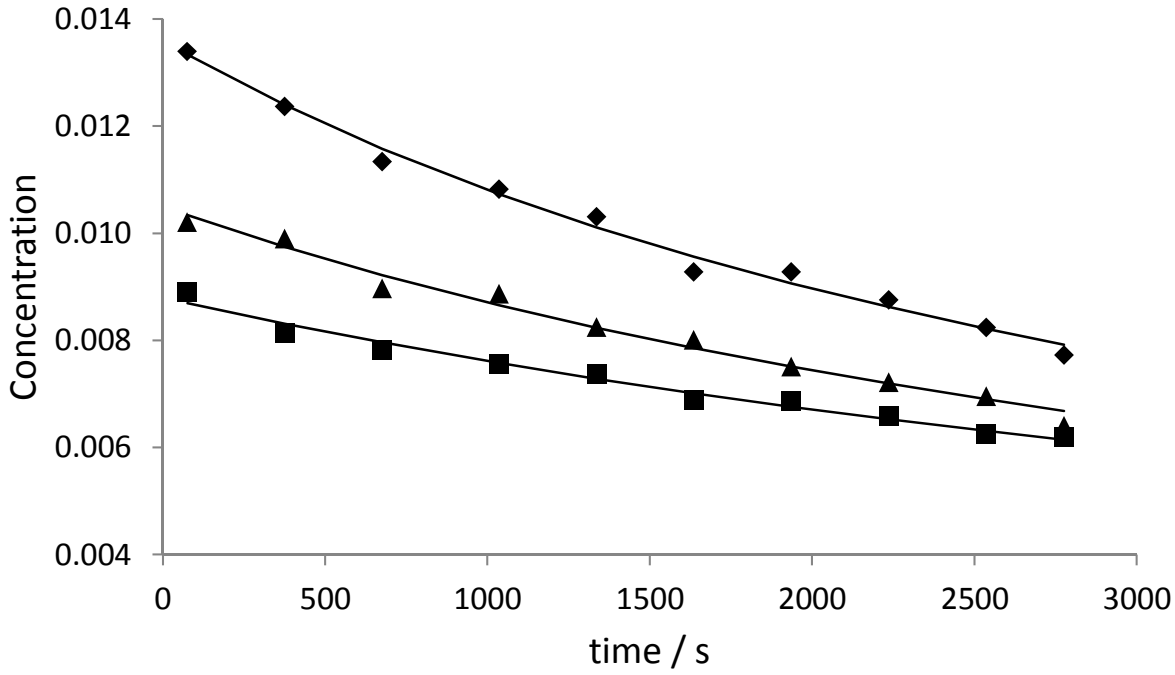


Figure S1: Flow cytometry analysis of EL4 cell labelling using **11** and tetrazine **12**. Cells were cultured in the presence or absence of Ac_4GalNAz (50 mM) for 24h, then incubated with a 100 μM solution of **11** for 30 min, 37 °C, 500 r.p.m. After washing, cells were reacted with **12**, 10 μM, for 20 min, 37 °C, 500 r.p.m., re-washed and analyzed by flow cytometry against controls. The population of viable cells (population of interest; pink circle in (A) was identified from the high levels of UV autofluorescence, caused by intracellular NADH, and low levels of Sytox green (cell death marker); Histogram analysis (B) of EL4 cells revealed a ca. 6-fold difference (C) between Ac_4GalNAz-treated (+) and non-treated (-) cells. MFI, median fluorescence intensity. Histogram color scale: orange: cells with no dyes, blue: cells + **12**, green: cells+**11**+**12**, red: cells+Ac_4GalNAz+**11**+**12**. Data points (C) were collected in triplicate and are shown as mean ± SD (error bars lie within the chart bars when not visible). * $p < 0.05$, one-tailed t-test with Mann Whitney correction.

Kinetic evaluation of the [3+2] cycloaddition of (*E,E*)-1,5-cyclooctadiene with benzyl azide

Kinetic experiments for the first [3+2] cycloaddition were performed under second-order conditions. (*E,E*)-1,5-cyclooctadiene was mixed in a 1:1 molar ratio with benzyl azide in $CDCl_3$ and the reaction was monitored by ^{1}H-NMR (500 MHz) at 25 °C probe temperature. The second-order rate constants were determined by fitting the experimental data to the second-order rate equation.

time / s	integral	concentration / M	fit	error
0	0.264	0.0136	0.0136	0.0E+00
75	0.260	0.0134	0.0133	3.7E-09
375	0.240	0.0124	0.0124	7.9E-10
675	0.220	0.0113	0.0116	5.8E-08
1035	0.210	0.0108	0.0107	8.6E-09
1335	0.200	0.0103	0.0101	3.8E-08
1635	0.180	0.0093	0.0096	8.1E-08
1935	0.180	0.0093	0.0091	4.4E-08
2235	0.170	0.0088	0.0086	1.9E-08
2535	0.160	0.0082	0.0082	7.4E-10
2775	0.150	0.0077	0.0079	3.7E-08
			Sum	**2.9E-07**



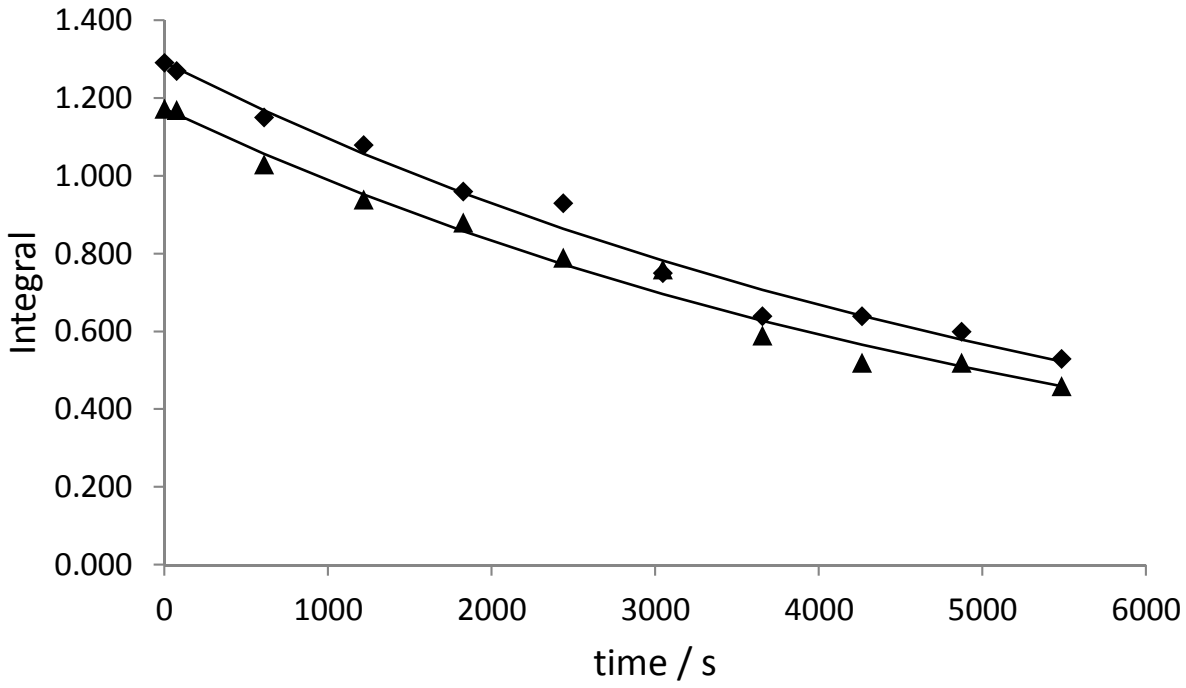
Entry	c_0 / M	k / s^{-1} x M^{-1}
1	0.0136	0.0190
2	0.0105	0.0196
3	0.0177	0.0177
	average	**0.0187**
	error	**0.0010**

Figure S2: Kinetic evaluation of [3+2] cycloaddition of (*E*,*E*)-1,5-cyclooctadiene with benzyl azide. Top: Example experimental data and fitted data from one experiment. Center: Plots of benzyl azide concentration *vs.* time from three different experiments. Bottom: Summary of data (starting concentrations and rate constants) from three different experiments.

Kinetic evaluation of the second [3+2] cycloaddition of (*E*,*E*)-1,5-cyclooctadiene with benzyl azide

Kinetic experiments for the second [3+2] cycloaddition were performed under pseudo first order conditions. Triazoline **8** was mixed in a ca. 1:20 molar ratio with benzyl azide in $CDCl_3$ and the reaction was monitored by ^{1}H-NMR (500 MHz) at 25 °C probe temperature. The pseudo first order rate constants were determined by fitting the experimental data to the pseudo first order rate equation.

time / s	integral	fit	error
0	1.292	1.2919	0.0E+00
75	1.270	1.2761	3.7E-05
609	1.150	1.1686	3.4E-04
1218	1.080	1.0570	5.3E-04
1827	0.960	0.9560	1.6E-05
2436	0.930	0.8648	4.3E-03
3045	0.750	0.7822	1.0E-03
3654	0.640	0.7075	4.6E-03
4263	0.640	0.6399	5.1E-09
4872	0.600	0.5788	4.5E-04
5481	0.530	0.5235	4.2E-05
		Sum	**1.1E-02**



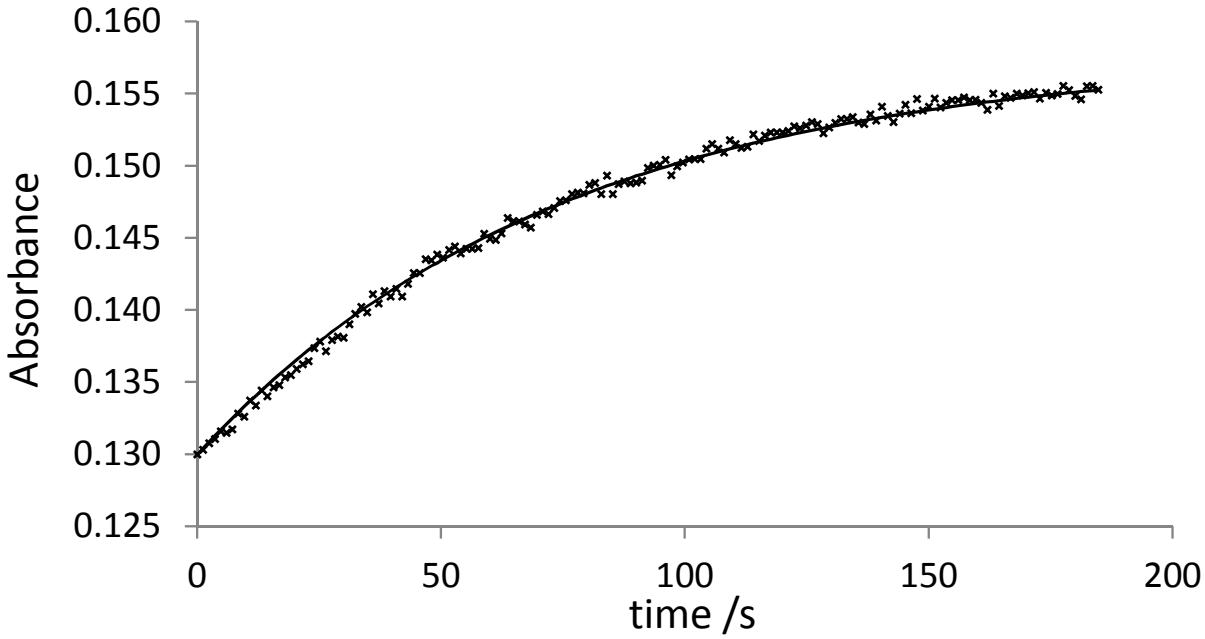
Entry	c_0 / M	k' / s^{-1}	k / s^{-1} x M^{-1}
1	0.5380	1.65E-04	3.06E-04
2	0.5800	1.71E-04	2.95E-04
		average	**3.0E-04**
		error	**8.1E-06**

Figure S3: Kinetic evaluation of the second [3+2] cycloaddition of (*E*,*E*)-1,5-cyclooctadiene with benzyl azide. Top: Example experimental data and fitted data from one experiment. Center: Plots of benzyl azide concentration *vs.* time from three different experiments. Bottom: Summary of data (starting concentrations and rate constants) from two different experiments.

Kinetic evaluation of inverse-electron-demand Diels-Alder reactions with tetrazine 5

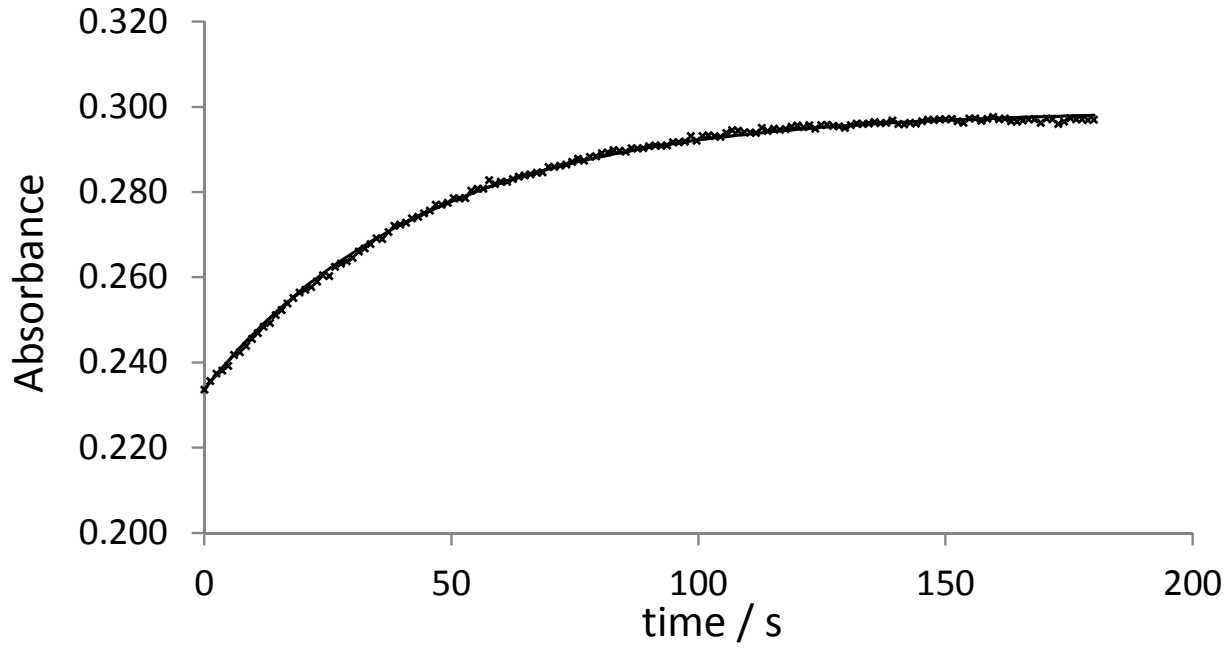
Kinetic experiments for inverse-electron-demand Diels-Alder reactions were performed under pseudo first order conditions by UV-Vis spectroscopy. The pseudo first order rate constants were determined by fitting the experimental data to the corresponding rate equation.

The reactions between trans-cyclooctenes **6** and **8** and 3,6-di-(2-pyridyl)-*s*-tetrazine (**5**) were monitored by UV-Vis spectroscopy at 340 nm using the reagents in a 1:10 molar ratio in MeOH at 25 °C. The concentrations of excess reagent are given in the tables.



Entry	k'	A_{oo}	c_0 / M	k / s^{-1} x M^{-1}
1	0.0134	0.1576	5.00E-05	267.04
2	0.0159	0.1494	5.00E-05	318.02
3	0.0153	0.1598	5.00E-05	305.49
			average	**296.85**
			error	**26.56**

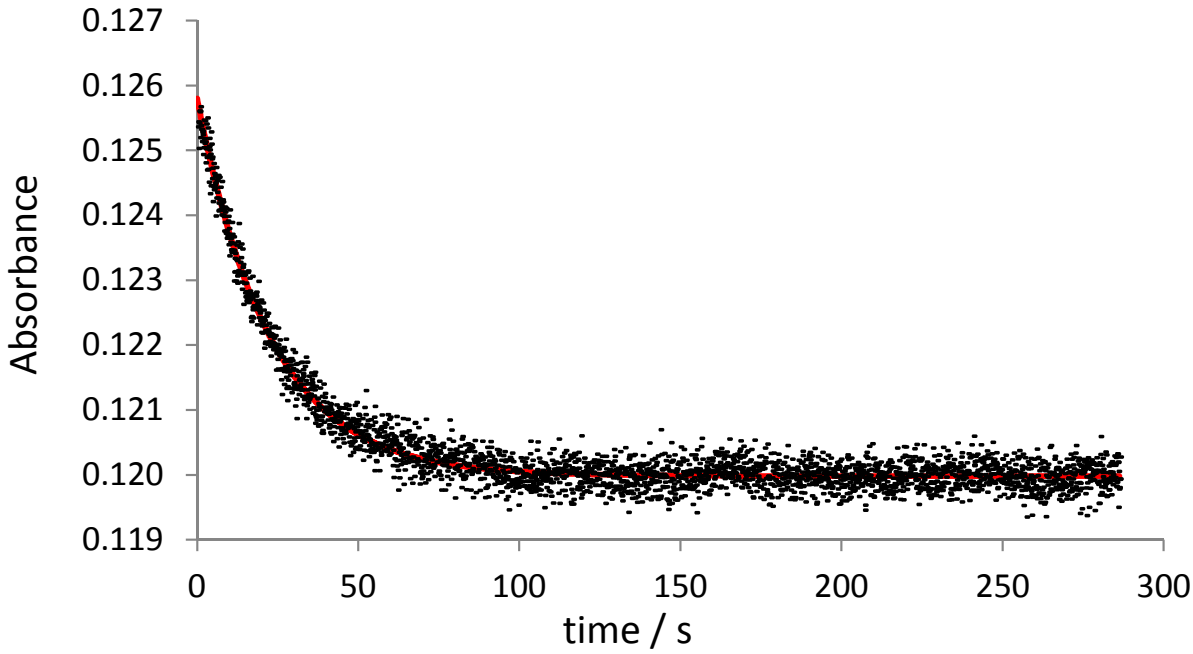
Figure S4: Kinetic evaluation of inverse-electron-demand Diels-Alder reaction of triazoline **8** with 3,6-di-(2-pyridyl)-*s*-tetrazine (**5**). Top: Plot of absorbance *vs.* time. Bottom: Summary of data (pseudo first order rate, final absorbance, concentration of excess substrate and second order rate constants) from three different experiments.



Entry	k'	A_{oo}	A_0	c_0 / M	k / s^{-1} x M^{-1}
1	0.0224	0.2992	0.2336	5.00E-05	448.52
2	0.0221	0.2982	0.2363	5.00E-05	442.28
3	0.0235	0.2816	0.2219	5.00E-05	470.41
				average	**453.74**
				error	**14.77**

Figure S5: Kinetic evaluation of inverse-electron-demand Diels-Alder reaction of trans-cyclooctene **6** with 3,6-di-(2-pyridyl)-*s*-tetrazine (**5**). Top: Plot of absorbance *vs.* time. Bottom: Summary of data (pseudo first order rate, final absorbance, concentration of excess substrate and second order rate constants) from three different experiments.

The reaction between (*E*,*E*)-1,5-cyclooctadiene **4** and 3,6-di-(2-pyridyl)-*s*-tetrazine (**5**) was monitored by UV-Vis spectroscopy at 295 nm using (*E*,*E*)-1,5-cyclooctadiene and tetrazine in a 1:10 molar ratio in THF at 25 °C. The concentrations of excess reagent are given in the table.



Entry	k' / s^{-1}	A_{oo}	c_0 / M	k / s^{-1} x M^{-1}
1	0.0441	0.1200	5.00E-06	8819.22
2	0.0506	0.1234	5.00E-06	10126.70
3	0.0385	0.1185	5.00E-06	7709.28
			average	**8885.07**
			error	**1210.05**

Figure S6: Kinetic evaluation of inverse-electron-demand Diels-Alder reaction of (*E*,*E*)-1,5-cyclooctadiene **4** with 3,6-di-(2-pyridyl)-*s*-tetrazine (**5**). Top: Plot of absorbance *vs.* time. Bottom: Summary of data (pseudo first order rate, final absorbance, concentration of excess substrate and second order rate constants) from three different experiments.

General methods and materials

NMR spectra were recorded on the following instruments: Bruker DRX500, Bruker Avance BB, Bruker Avance TCI, Bruker AM400 and Bruker DRX400. All chemical shifts are quoted in ppm, relative to tetramethylsilane, using the residual solvent peak as a reference standard. All coupling constants are quoted in Hz. Infrared spectra were recorded on a Perkin Elmer Spectrum One (FT-IR) spectrophotometer. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier TOF mass spectrometer with electrospray and modular Lockspray interface. Analytical thin layer chromatography (TLC) was carried out on Merck Kieselgel 60 F254 plates with visualisation by ultra violet light (254 nm), potassium permanganate or ninhydrin or phosphomolybdic acid /$Ce(SO_4)_2$ dip. Flash column chromatography was carried out using Merck Kieselgel 60 (230-400 mesh). HPLC was carried out on a Varian ProStar system, UV detection at 254 nm, Phenomenex Jupiter C18 column, particle size 5 μm. LC-MS analysis was performed on a Waters 2795 system, UV detection at 254 nm, Supelco ABZ+plus column, 3.3cm x 4.6mm, particle size 3 μm. All solvent mixtures are reported as % vol/vol unless otherwise stated. Reagents and solvents were purified using standard means. All other chemicals were used as received unless noted otherwise. Extractive procedures were performed using distilled solvents and evaporation of solvents was performed under reduced pressure. All aqueous solutions used were saturated. Unless otherwise stated, all non-aqueous reactions were carried out under an argon atmosphere using anhydrous conditions and oven-dried glassware. Standard techniques were employed for handling air-sensitive materials.

Synthetic procedures

cis,cis-5,10-Dioxatricyclo[7.1.0.0^{4,6}]decane (2)[1]

Sodium carbonate (53.9 g, 508 mmol, 1.1 eq) was added to a stirred solution of (Z,Z)-1,5-cyclooctadiene (50 g, 462 mmol, 1.0 eq) in DCM (200 mL). The suspension was cooled to 0 °C and peracetic acid (36%, 179 mL, 971 mmol, 2.1 eq) was added. The mixture was warmed to r.t. and

stirred until the reaction had gone to completion (typically 2 d). The pH was adjusted to ca. 11 with 40% NaOH at 0 °C, followed by extraction with Et_2O (4 x 150 mL). The combined organic layers were dried over $MgSO_4$, concentrated and distilled to give 5,10-dioxatricyclo[7.1.0.0^{4,6}]decane **2** (45.8 g, 327 mmol, 70.7% yield), b.p. 69-70 °C at 0.3 mm Hg, which crystallized on standing. The spectroscopic data were in accordance with the literature.

β-hydroxy-diphenylphosphine oxides

***rel*-((1S,2S,5S,6S)-2,6-dihydroxycyclooctane-1,5-diyl)bis(diphenylphosphine oxide) (3a)** and ***rel*-((1R,4S,5S,8R)-5,8-dihydroxycyclooctane-1,4-diyl)bis(diphenylphosphine oxide) (3b)**

To a solution of diphenylphosphine (8.4 g, 44.9 mmol, 2.1 eq.) and cis,cis-5,10-dioxatricyclo-[7.1.0.0^{4,6}]decane (**2**) (3.0 g, 21.4 mmol, 1.0 eq.) in THF (86 mL), BuLi (1.5 M solution in hexanes, 30.0 mL, 44.9 mmol, 2.1 eq.) was added dropwise at -78 °C. The mixture was stirred for 1 h, allowed to warm to room temperature and stirred for 12 h. The brown solution was cooled to 0 °C, diluted with THF (86 mL) and quenched by the addition of AcOH (3.7 mL, 3.86 g, 64.2 mmol, 3 eq) and and H_2O_2 (30%, 6.6 mL, 64.2 mmol, 3 eq). The mixture became clear, followed by the formation of a white precipitate. The mixture was removed from the ice bath and stirred vigorously at 25 °C for 2 h whereupon the white precipitate dissolved. The clear solution was then transferred to a separatory funnel, Et_2O (86 mL) added, the aqueous layer removed, and the organic layer washed with brine (2 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to afford a white solid. The remaining AcOH was azeotropically removed with toluene to yield an isomeric mixture of phosphine oxides (11.07 g, 20.3 mmol, 95%) as a white solid, mp 112-120 °C.

R_f (10% MeOH in DCM) 0.61 and 0.71;

δ_H(500 MHz, MeOD) 7.93-7.40 (20H, m), 4.16 (1H, app q, J = 8.0), 4.06 (1H, app q, J = 7.1), 3.19 (1H, app q, J = 8.3), 3.05 (1H, app q, J = 9.2), 2.12-1.94 (3H, m), 1.94-1.83 (1H, m), 1.83-1.71 (1H, m), 1.71-1.48 (3H, m);

δ_C(125 MHz, MeOD) 135.01 (d, J = 58.2), 134.22 (d, J = 54.6), 133.96 (d, J = 54.6), 133.19 (d, J

= 54.5), 133.03 (d, *J* = 2.4), 132.90 (d, *J* = 2.4), 132.76 (d, *J* = 2.6), 132.62 (d, *J* = 2.7), 132.34 (d, *J* = 8.8), 132.07 (d, *J* = 9.4), 131.96 (d, *J* = 8.8), 131.83 (d, *J* = 9.2), 129.95 (d, *J* = 11.4), 129.86 (d, *J* = 11.4), 129.60 (d, *J* = 11.8), 129.58 (d, *J* = 12.0), 71.17 (d, *J* = 3.2), 70.62 (d, *J* = 2.4), 44.01 (d, *J* = 69.8), 42.20 (d, *J* = 71.2), 34.74 (dd, *J* = 9.6, 9.6), 32.42 (d, *J* = 9.2), 24.29 (d, *J* = 13.9), 21.59;

ν_{max}(solid)/cm^{-1} 3319.37, 3057.03, 2923.32, 1590.98, 1483.93, 1437.23, 1313.20;

m/z (EI) 545.2021 (M+H$^+$. $C_{32}H_{35}O_4P_2$ requires 545.2011).

(*E*,*E*)-1,5-cyclooctadiene (4)

The mixture of phosphine oxides **3a** and **3b** (3.00 g, 5.5 mmol, 1.0 eq) was dissolved in DMF (30 mL) with stirring under an Ar atmosphere. The clear solution became cloudy and NaH (60% in mineral oil, 0.72 g, 17.1 mmol, 3.1 eq) was added in portions over 5 min at r.t. under a positive Ar stream. The reaction mixture was stirred for 12 h at room temperature, cooled to 0 °C, diluted with pentane (25 mL) and quenched with half-saturated NH_4Cl (11 mL). The aqueous layer was extracted with pentane (25 mL) and the combined organic layers were washed with water (5 x 22 mL) and brine (1 x 22 mL) to give (*E*,*E*)-1,5-cyclooctadiene (50 mL of a 25 mM solution in pentane, 1.3 mmol, 23% yield).

The concentration of (*E*,*E*)-1,5-cyclooctadiene was determined by titration with 3,6-Di-2-pyridyl-1,2,4,5-tetrazine, color change from pink to yellow.

Transfer of the product into other organic solvents: The 10 mL of the pentane solution was extracted with an equal volume of aqueous 0.1 M $AgNO_3$. The pentane layer was discarded, the desired volume of organic solvent was added, followed by the addition of 10 mL of 35 % aqueous NH_3 and vigorous shaking. The organic layer was washed with water (10 mL) and brine (10 mL).

R_f (pentane) 0.43;

δ_H(500 MHz, D_2O) 5.40-5.27 (4H, m), 2.83-2.70 (4H, m), 2.58-2.41 (4H, m) ;

δ_C(125 MHz, D_2O) 126.63, 31.85.

Tetrazine monoadduct 6

***rel*-(4aS,10aS,p*R*)-1,4-di(pyridin-2-yl)-4a,5,6,9,10,10a-hexahydrocycloocta[*d*]pyridazine**

To (*E*,*E*)-1,5-cyclooctadiene (2 mL, 0.02 molar in pentane, 40 μmol, 1.0 eq) was added 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (4.7 mg, 20 μmol, 0.5 eq) with stirring at room temperature. A small amount of solid that formed during the reaction (bis-adduct) was removed by filtration and the filtrate concentrated to give the desired product (10.8 mg, 34 μmol, 85% yield) as a yellow solid, mp 198-202 °C.

Rf=0.27 (5% MeOH in /DCM);

δ_H(500 MHz, $CDCl_3$) 8.64 (2H, qd, *J* = 0.88, 4.78), 8.28 (2H, td, *J* = 0.99, 7.98), 7.74 (2H, dt, *J* = 1.78, 7.73), 7.31 (2H, ddd, *J* = 1.16, 4.79, 7.49), 5.92-5.82 (2H, m), 3.56 (2H, d, *J* = 7.65), 2.64 (2H, d, *J* = 11.80), 2.58-2.42 (2H, m), 1.84 (2H, dd, *J* = 5.18, 13.03), 1.72-1.60 (2H, m);

δ_C(125 MHz, $CDCl_3$) 163.26, 154.53, 148.87, 136.33, 135.22, 124.64, 122.83, 36.81, 36.54, 36.28;

ν_{max}(solid)/cm^{-1} 3045.75, 2999.87, 2950.88, 2862.98, 1596.41, 1560.02, 1546.85, 1463.16, 1426.46, 1364.35;

m/z (EI) 317.1757 ($M+H^+$. $C_{20}H_{21}N_4$ requires 317.1761).

Tetrazine bisadduct 7

***rel*-(4a*S*,6a*S*,10a*S*,12a*S*)-1,4,7,10-tetra(pyridin-2-yl)-4a,5,6,6a,10a,11,12,12a-octahydrocycloocta-[1,2-d:5,6-*d*']dipyridazine**

To (*E*,*E*)-1,5-cyclooctadiene (2 mL, 0.02 molar in pentane, 40 μmol, 1.0 eq) was added 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (19.0 mg, 80 μmol, 2.0 eq) with stirring at room temperature. The solid that formed during the reaction was isolated by filtration, washed with hexane and dried to give the desired product (19.0 mg, 36 μmol, 89% yield) as a yellow solid, mp 250-252 °C. Impurities are presumably due to tautomerization of the product.

δ_H(500 MHz, $CDCl_3$) 8.63 (2H, qd, *J* = 0.85, 4.75), 8.36 (2H, td, *J* = 0.96, 8.03), 7.75 (2H, dt, *J* = 1.77, 7.76), 7.30 (2H, ddd, *J* = 1.13, 4.80, 7.43), 4.51 (4H, s), 2.18-1.99 (8H, m);

δ_C(125 MHz, $CDCl_3$) 162.16, 154.55, 148.95, 136.26, 124.59, 123.05, 36.43, 35.66;

ν_{max}(solid)/cm^{-1} 2941.10, 1595.37, 1561.35, 1545.55, 1461.80, 1429.72, 1388.14, 1355.43.

m/z (EI) 525.2512 ($M+H^+$. $C_{32}H_{29}N_8$ requires 525.2510).

Benzyl azide monoadduct 8

***rel*-(3a*S*,9a*S*, p*R*)-1-benzyl-3a,4,5,8,9,9a-hexahydro-1*H*-cycloocta[*d*][1,2,3]triazole**

To (*E*,*E*)-1,5-cyclooctadiene (470 μl, 0.2 molar in DCM, 94 μmol, 1.1 eq) was added benzyl azide (11.4 mg, 86 μmol, 1.0 eq) with stirring at r.t. No precautions were taken to exclude moisture or air from the reaction mixture. When the reaction had finished (typically 2 h, TLC), the solvent and excess (*E*,*E*)-1,5-cyclooctadiene were removed by rotary evaporation giving giving the desired product (20.5 mg, 85 μmol, 99% yield) as a white crystalline solid, mp 113-115 °C.

R_f (50% Et_2O in hexane) 0.34;

δ_H(500 MHz, $CDCl_3$) 7.36-7.25 (3H, m), 7.21-7.16 (2H, m), 5.51-5.38 (1H, ddd, *J* = 3.4, 11.1, 15.5), 5.34-5.24 (1H, ddd, *J* = 3.4, 11.1, 15.5), 4.92 (1H, d, *J* = 15.3), 4.41 (1H, d, *J* = 15.3), 3.87 (1H, td, *J* = 1.5, 11.3), 2.68-2.63 (1H, m), 2.61 (1H, td, *J* = 1.5, 11.3), 2.42-2.34 (2H, m), 2.34-2.25 (1H, m), 2.13-2.00 (2H, m), 1.64-1.52 (1H, m), 1.45-1.34 (1H, m);

δ_C(125 MHz, $CDCl_3$) 136.36, 135.89, 134.28, 128.86,128.55,127.97, 87.22, 65.55, 52.76, 37.85, 35.74, 33.52, 32.60;

ν_{max}(solid)/cm^{-1} 2934.65, 2919.09, 2854.46, 1497.02, 1454.83, 1438.84;

m/z (EI) 242.1662 ($M+H^+$. $C_{15}H_{20}N_3$ requires 242.1652).

PEG adduct 9a

***rel*-2-(2-(2-(2-((3aR,9aS,p*R*)-3a,4,5,8,9,9a-hexahydro-1*H*-cycloocta[*d*][1,2,3]triazol-1-yl)ethoxy)-ethoxy)ethoxy)ethanamine**

To (*E*,*E*)-1,5-cyclooctadiene (345 μL, 0.2 molar in DCM, 69 μmol, 1.1 eq) was added 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanamine (13.7 mg, 63 μmol, 1.0 eq) with stirring at r.t. When the reaction had finished (typically 2 h, TLC), the solvent and excess (*E*,*E*)-1,5-cyclooctadiene were

removed by rotary evaporation giving the desired product (20.5 mg, 63 μmol, 100% yield) as a clear oil.

Rf=0.20 (10% MeOH/1%TEA/DCM);

δ_H(500 MHz, $CDCl_3$) 5.55-5.40 (2H, m), 3.84 (1H, t, *J* = 11.1), 3.77-3.55 (11H, m), 3.55-3.44 (1H, m),3.49 (2H, t, *J* = 5.3), 2.97 (1H, app t, *J* = 11.1), 2.85 (2H, t, *J* = 5.3), 2.70 (1H, dd, *J* = 4.6, 13.8), 2.50-2.39 (2H, m), 2.39-2.29 (1H, m), 2.26-2.13 (2H, m), 1.72-1.54 (4H, m);

δ_C(125 MHz, $CDCl_3$) 135.69, 134.45, 86.77, 73.45, 70.74, 70.72, 70.51, 70.39, 69.56, 67.39, 48.09, 41.84, 37.83, 36.07, 33.51, 32.65;

ν_{max}(film)/cm^{-1} 3428.61, 2940.31, 1725.74;

m/z (EI) 327.2401($M+H^+$. $C_{16}H_{31}N_4O_3$ requires 327.2391).

PEG adduct 9b

***rel*-32-((3aS,9aS,p*R*)-3a,4,5,8,9,9a-hexahydro-1*H*-cycloocta[*d*][1,2,3]triazol-1-yl)-3,6,9,12,15,-18,21,24,27,30-decaoxadotriacontan-1-amine**

To (*E*,*E*)-1,5-cyclooctadiene (300 μL, 0.2 M in DCM, 60 μmol, 1.2 eq) was added 32-azido-3,6,9,12,15,18,21,24,27,30-decaoxadotriacontan-1-amine (26.3 mg, 50 μmol, 1 eq) with stirring at room temperature. When the reaction had finished (typically 2 h, TLC), the solvent and excess (*E*,*E*)-1,5-cyclooctadiene were removed by rotary evaporation giving the desired product (31.5 mg, 50 μmol, 99% yield) as a clear oil.

Rf=0.26 (10% MeOH/1%TEA/DCM);

δ_H(500 MHz, $CDCl_3$) 5.53-5.38 (2H, m), 3.83 (1H, td, *J* = 1.5, 11.3), 3.66-3.57 (40H, m), 3.55 (2H, t, *J* = 5.2), 3.53-3.44 (1H, m), 2.97 (1H, td, *J* = 1.3, 11.0), 2.88 (2H, t, *J* = 1.5, 11.3), 2.76-2.62 (4H, m),2.49-2.38 (2H, m), 2.38-2.28 (1H, m), 2.24-2.12 (2H, m), 1.70-1.50 (2H, m);

δ_C(125 MHz, $CDCl_3$) 135.69, 134.45, 86.78, 72.40, 70.71, 70.66, 70.65, 70.62, 70.61, 70.57, 70.55, 70.50, 70.32, 69.59, 67.39, 48.09, 41.66, 37.82, 36.06, 33.51, 32.65;

ν_{max}(film)/cm^{-1} 3428.61, 2940.31, 1725.74;

m/z (EI) 635.4244 ($M+Na^+$. $C_{30}H_{59}N_4O_{10}$ requires 635.4226).

TMDIBO-PEG adduct 11

To Carbonic acid-2',3',2",3"-tetramethoxy-7,8-didehydro-1,2:5,6-dibenzocyclocta-1,5,7-triene-3-yl 4-nitrophenyl ester[2] (20.7 mg, 41 μmol, 1.0 eq) and PEG adduct **9b** (26.0 mg, 41 μmol, 1.0 eq) in CH_2Cl_2 (5 mL) was added NEt_3 (29 μL, 205 μmol, 5.0 eq) with stirring at room temperature. When the reaction had finished (typically 4 h, TLC), the solvent was removed by rotary evaporation followed by column chromatography (1% MeOH/DCM) giving the desired product (35.0 mg, 35 μmol, 85% yield) as a clear oil.

Rf=0.15 (10% MeOH/DCM);

δ_H(500 MHz, $CDCl_3$) 7.03 (1H, s), 6.87 (1H, s), 6.80 (1H, s), 6.79 (1H, s), 5.62-5.38 (3H, m), 3.90 (6H, s), 3.86 (3H, s), 3.85 (3H, s), 3.84 (1H, td, *J* = 1.5, 11.3), 3.73-3.55 (42H, m), 3.52-3.47 (1H, m), 3.41-3.37 (1H, m), 3.06 (1H, dd, *J* = 2.0, 15.0), 2.97 (1H, td, *J* = 1.3, 11.0), 2.80 (1H, dd, *J* = 3.6, 15.0), 2.73-2.65 (1H, m), 2.49-2.38 (2H, m), 2.38-2.28 (1H, m), 2.24-2.12 (2H, m), 2.01-1.90 (1H, br s), 1.70-1.50 (2H, m);

δ_C(125 MHz, $CDCl_3$) 155.56, 149.06, 148.82, 147.86, 147.82, 145.78, 144.57, 135.69, 134.45, 115.67, 113.99, 113.26, 112.52, 109.41, 109.39, 109.16, 108.81, 108.20, 86.78, 70.71, 70.65, 70.50, 70.36, 70.21, 69.59, 67.39, 56.20, 56.15, 48.09, 46.53, 41.05, 37.83, 36.06, 33.50, 32.65;

ν_{max}(film)/cm^{-1} 2862.81, 2335.41, 1721.16, 1601.90, 1561.42, 1504.90;

m/z (EI) 1023.5143 ($M+Na^+$. $C_{51}H_{76}O_{16}N_4$ requires 1023.5149).

Benzyl azide-tetrazine bisadduct 10

***rel*-(3aS,5aS,9aS,11aS)-1-benzyl-6,9-di(pyridin-2-yl)-3a,4,5,5a,9a,10,11,11a-octahydro-1*H*-[1,2,3]-triazolo[4',5':5,6]cycloocta[1,2-*d*]pyridazine**

Benzyl azide monoadduct (**8**) (20.0 mg, 83 μmol, 1.0 eq) was dissolved in DCM (800 μl) and 3,6-di(pyridin-2-yl)-1,2,4,5-tetrazine (19.6 mg, 83 μmol, 1.0 eq) was added with stirring. The solvent was removed by rotary evaporation to give the desired product (37.0 mg, 82 μmol, 99% yield) as a yellow solid, mp 185-187 °C.

R_f (10% MeOH in DCM) 0.54;

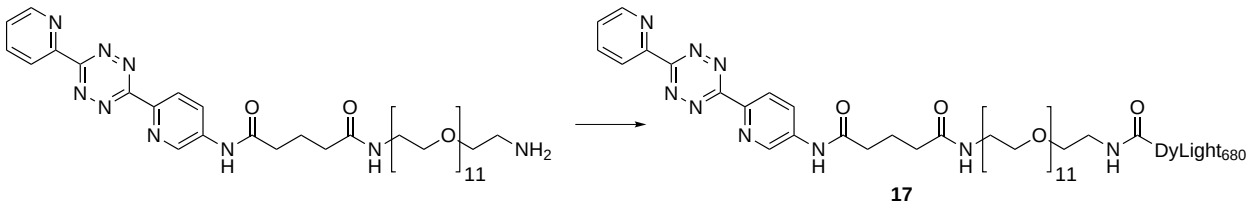
δ_H(500 MHz, $CDCl_3$) 8.56 (1H, app d, *J* = 4.7), 8.49 (1H, app d, *J* = 4.7), 8.27 (1H, app d, *J* = 8.0), 8.25 (1H, app d, *J* = 8.0), 7.69 (2H, app tt, *J* = 2.1, 7.7), 7.32-7.22 (7H, m), 4.73 (1H, d, *J* = 15.4), 4.61 (1H, d, *J* = 15.4), 4.61 (1H, app t, *J* = 11.5), 3.65 (1H, dd, *J* = 3.4, 8.5), 3.47 (1H, dd, *J* = 3.4, 8.5), 3.35 (1H, app td, *J* = 2.1, 11.5), 2.74-2.66 (1H, app dd, *J* = 4.1, 15.2), 2.11-1.95 (2H, m), 1.91-1.80 (3H, m), 1.64-1.52 (1H, m), 1.44-1.32 (1H, m);

δ_C(125 MHz, $CDCl_3$) 161.72, 161.71, 154.13, 154.12, 149.08, 149.01, 136.66, 136.57, 128.77, 128.48, 127.77, 125.02, 124.97, 123.03, 123.01, 83.76, 65.16, 53.39, 35.69, 35.27, 34.63,33.97, 33.78;

ν_{max}(solid)/cm^{-1} 3062.08, 2956.11, 2929.45, 2850.02, 1599.69, 1562.51, 1549.56, 1489.16, 1464.54, 1455.94, 1440.16, 1429.25;

m/z (EI) 450.2417 ($M+H^+$. $C_{27}H_{28}N_7$ requires 450.2401).

Tetrazine 12



17

The precursor to tetrazine **12** was prepared as previously described.[3]

*N*1-(35-amino-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyl)-*N*5-(6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)glutaramide (1.9 mg, 2 μmol, 2.0 eq) was dissolved in DMF (200 μL) followed by the addition of DyLight680 NHS ester (1 mg, 1 μmol, 1.0 eq) and Hunig's Base (1.0 μL, 5 μmol, 5.0 eq). The solution was stirred overnight at room temperature, concentrated and purified by HPLC to give the desired product (analysis by LC-MS).

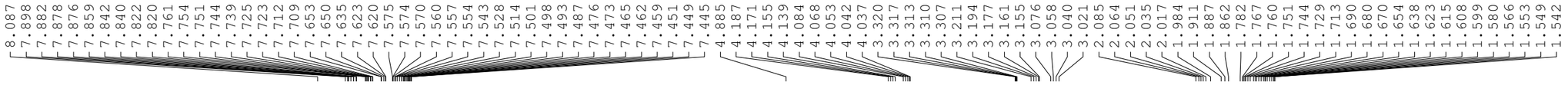
m/z (ES) 1683.6 ($M+H^+$).

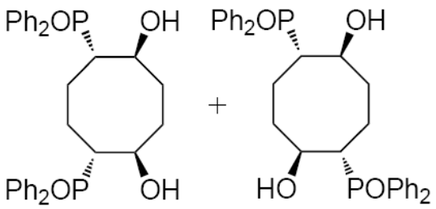
References

(1) Newton, P. F.; Whitham, G. H. *J.C.S. Perkin I* **1979**, 3067–3071.

(2) Stöckmann, H.; Neves, A. A.; Stairs, S.; Ireland-Zecchini, H.; Brindle, K. M.; Leeper, F. J. *Chem. Sci*. **2011**, DOI: 10.1039/C0SC00631A.

(3) Rossin, R.; Verkerk, P. R.; van den Bosch, S. M.; Vulders, R. C. M.; Verel, I.; Lub, J.; Robillard, M. S. *Angew Chem Int Ed Engl* **2010**, *49*, 3375–3378.



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PROCNO                 1

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INSTRUM           drx500
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PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     0
SWH              8503.401 Hz
FIDRES           0.129752 Hz
AQ               3.8535669 sec
RG                  11.3
DW                 58.800 usec
DE                  6.00 usec
TE                 300.0 K
D1              2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                  14.75 usec
PL1                 -6.00 dB
SFO1          500.0532723 MHz

F2 - Processing parameters
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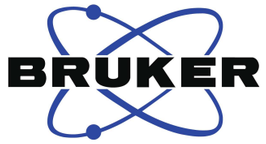

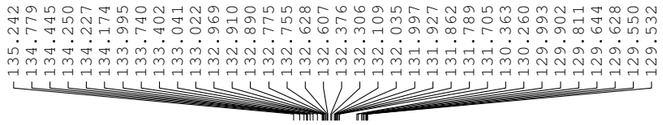

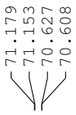

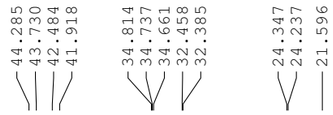

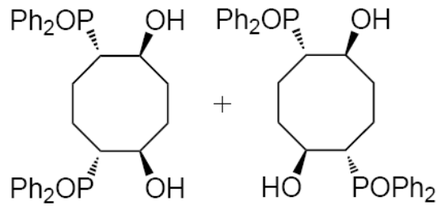


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PROCNO                 1

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PULPROG           zgpg30
TD                 65536
SOLVENT             MeOD
NS                   256
DS                     8
SWH            34013.605 Hz
FIDRES          0.519006 Hz
AQ             0.9634292 sec
RG                  4096
DW                14.700 usec
DE                 6.00 usec
TE                300.0 K
D1            3.00000000 sec
d11           0.03000000 sec
DELTA         2.90000010 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                  9.80 usec
PL1                -6.00 dB
SFO1         125.7520828 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2                -6.00 dB
PL12               10.62 dB
PL13               15.00 dB
SFO2         500.0517480 MHz

F2 - Processing parameters
SI                 65536
SF           125.7374972 MHz
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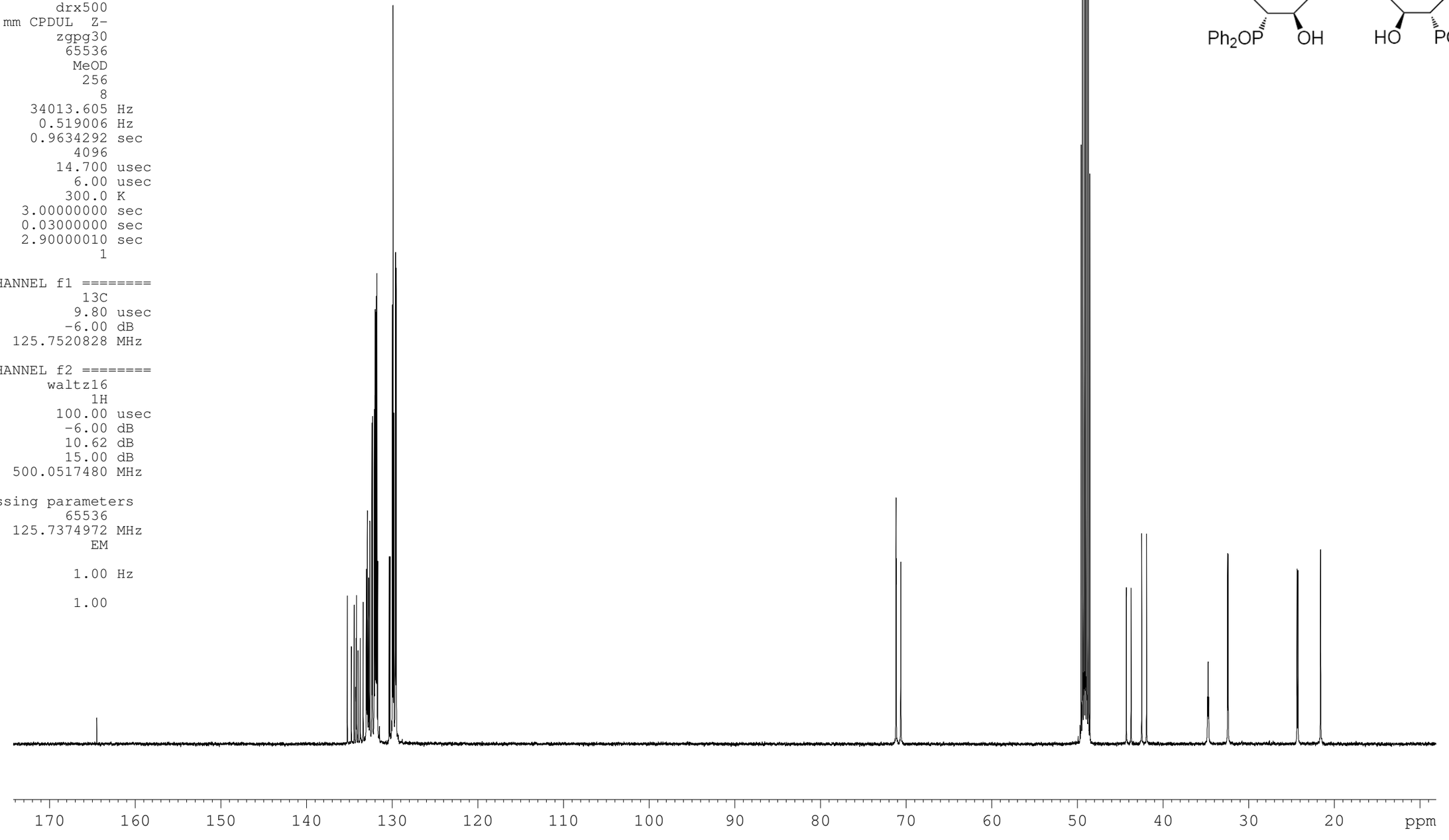

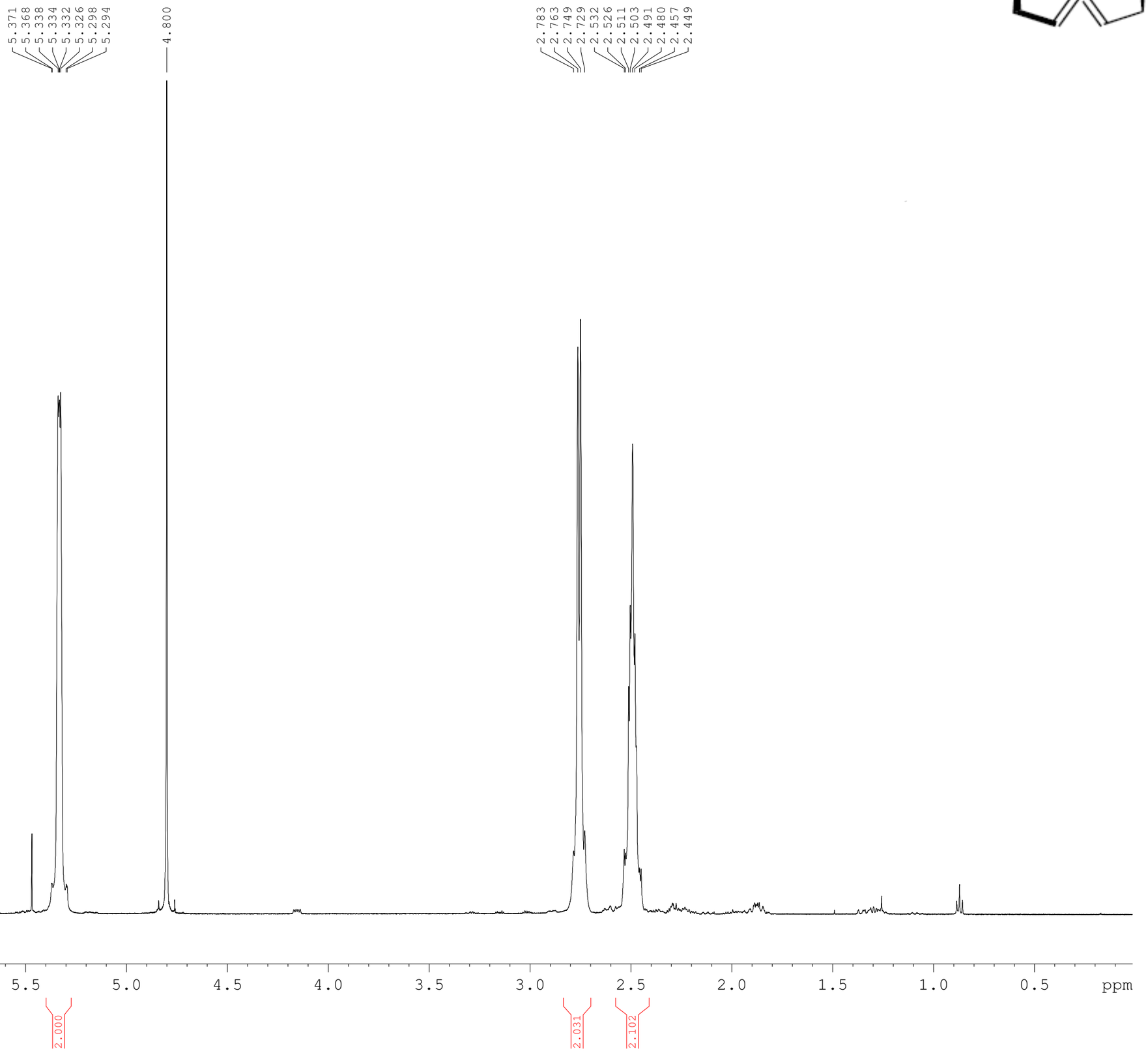


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PROCNO                 1

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PULPROG             zg30
TD                 65536
SOLVENT              D2O
NS                    16
DS                     0
SWH              8503.401 Hz
FIDRES           0.129752 Hz
AQ              3.8535669 sec
RG                   22.6
DW                 58.800 usec
DE                  6.00 usec
TE                 300.0 K
D1              2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 14.75 usec
PL1                -6.00 dB
SFO1         500.0532723 MHz

F2 - Processing parameters
SI                 65536
SF           500.0499527 MHz
WDW                   no
SSB       0
LB        0 Hz
GB        0
PC                  1.00
```

```
Current Data Parameters
NAME         fjl-hs503b
EXPNO                11
PROCNO                1

F2 - Acquisition Parameters
Date_          20110203
Time              11.06
INSTRUM           drx500
PROBHD   5 mm CPDUL  Z-
PULPROG           zgpg30
TD                65536
SOLVENT             D2O
NS                    256
DS                      8
SWH           34013.605 Hz
FIDRES         0.519006 Hz
AQ            0.9634292 sec
RG                 4096
DW                14.700 usec
DE                 6.00 usec
TE                300.0 K
D1           3.00000000 sec
d11          0.03000000 sec
DELTA        2.90000010 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                 9.80 usec
PL1               -6.00 dB
SFO1        125.7520828 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2                -6.00 dB
PL12               10.62 dB
PL13               15.00 dB
SFO2        500.0517480 MHz

F2 - Processing parameters
SI                 65536
SF          125.7376786 MHz
WDW                   no
SSB      0
LB       0 Hz
GB       0
PC                 1.00
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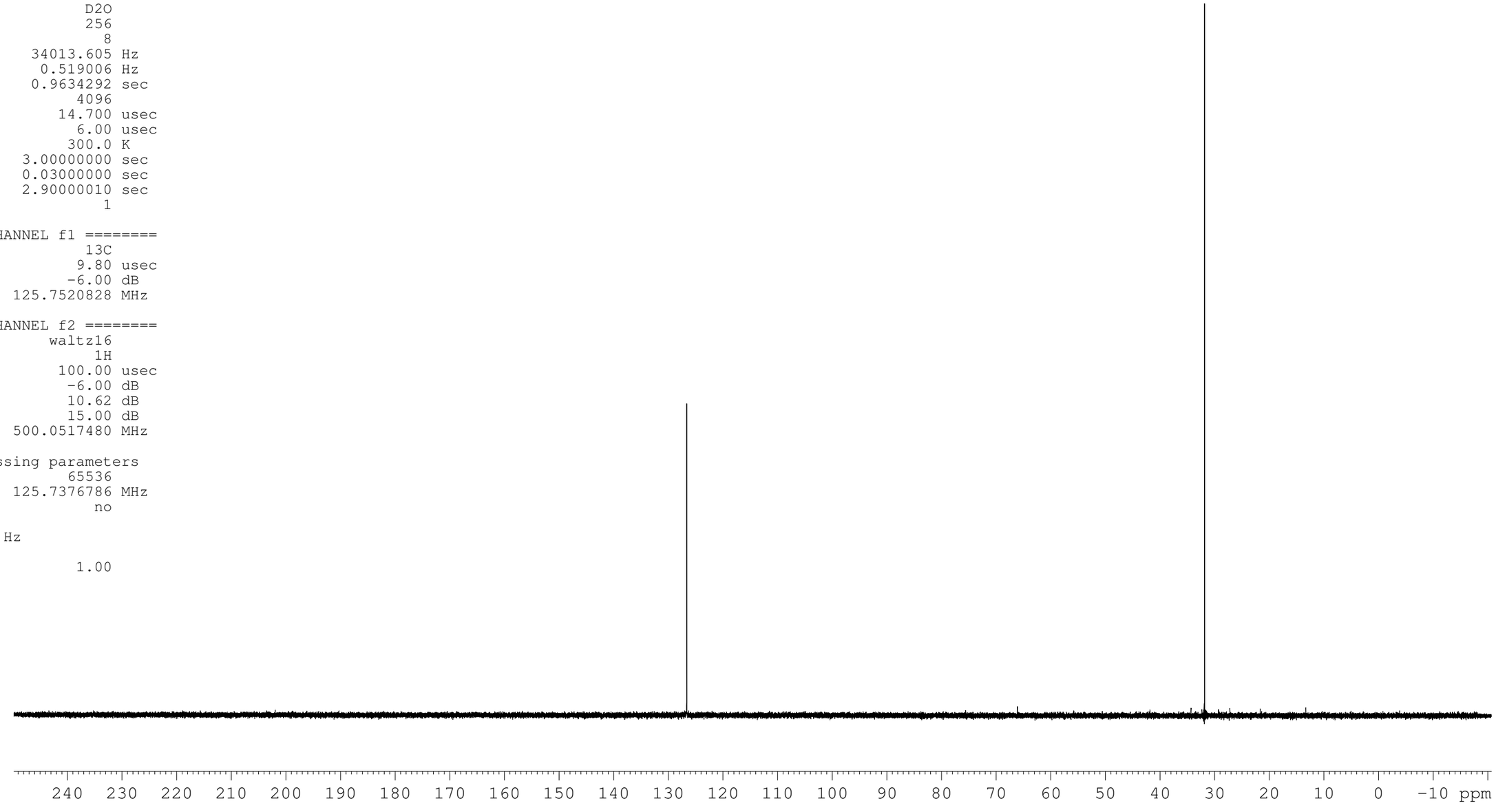

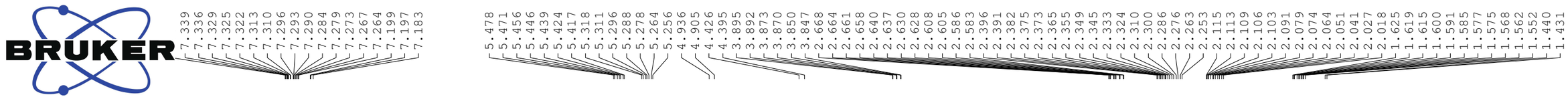

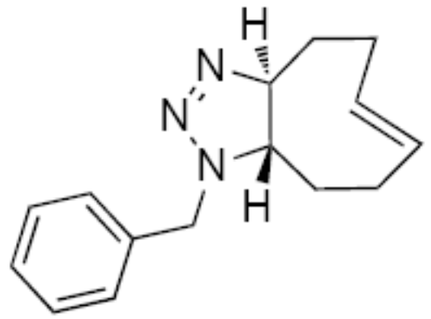


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PROCNO                1

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Time              10.59
INSTRUM          drx500
PROBHD   5 mm CPDUL  Z-
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  16
DS                   0
SWH           8503.401 Hz
FIDRES        0.129752 Hz
AQ            3.8535669 sec
RG                11.3
DW               58.800 usec
DE                6.00 usec
TE               300.0 K
D1           2.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                 1H
P1               14.75 usec
PL1              -6.00 dB
SFO1        500.0532723 MHz

F2 - Processing parameters
SI                65536
SF          500.0500257 MHz
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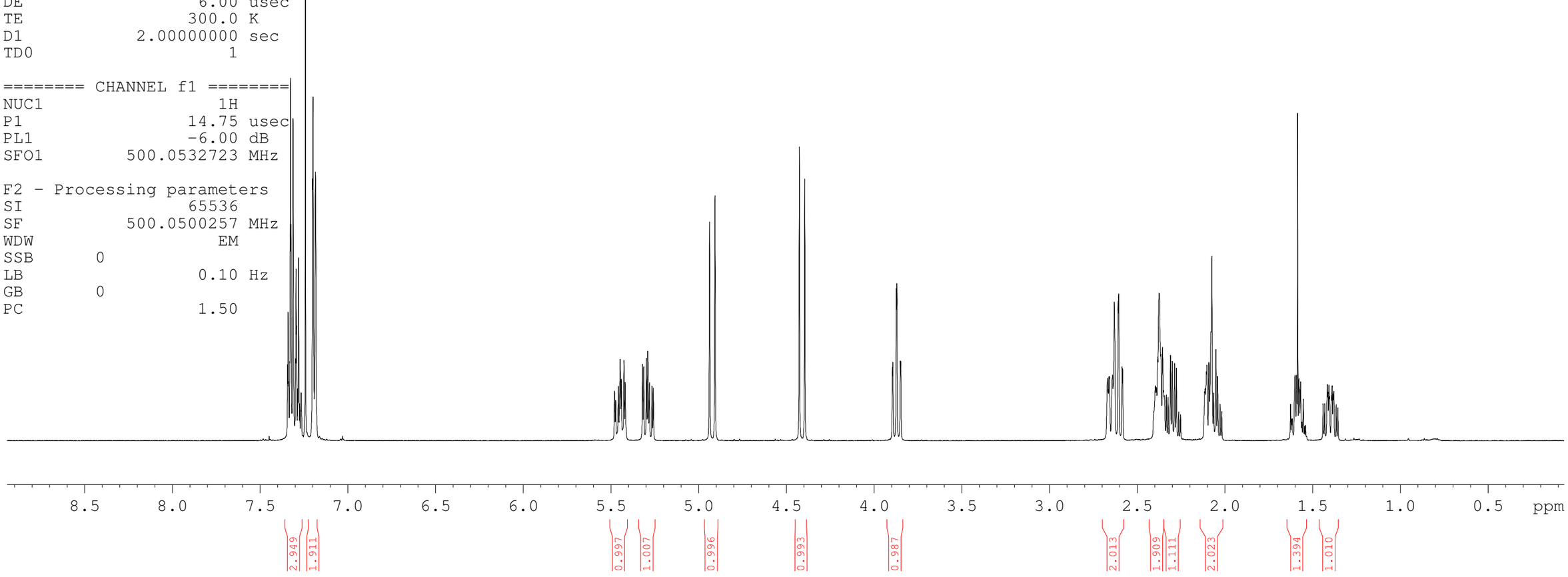

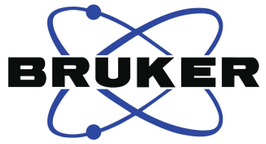

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EXPNO                21
PROCNO                1

F2 - Acquisition Parameters
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Time              11.17
INSTRUM          drx500
PROBHD   5 mm CPDUL  Z-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    8
SWH           34013.605 Hz
FIDRES         0.519006 Hz
AQ            0.9634292 sec
RG                 4096
DW                14.700 usec
DE                 6.00 usec
TE                300.0 K
D1           3.00000000 sec
d11          0.03000000 sec
DELTA        2.90000010 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                13C
P1                 9.80 usec
PL1               -6.00 dB
SFO1        125.7520828 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                1H
PCPD2           100.00 usec
PL2              -6.00 dB
PL12             10.62 dB
PL13             15.00 dB
SFO2        500.0517480 MHz

F2 - Processing parameters
SI                65536
SF          125.7376590 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
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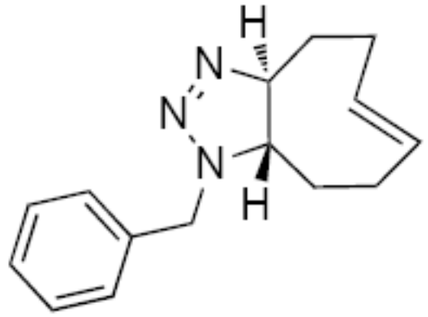

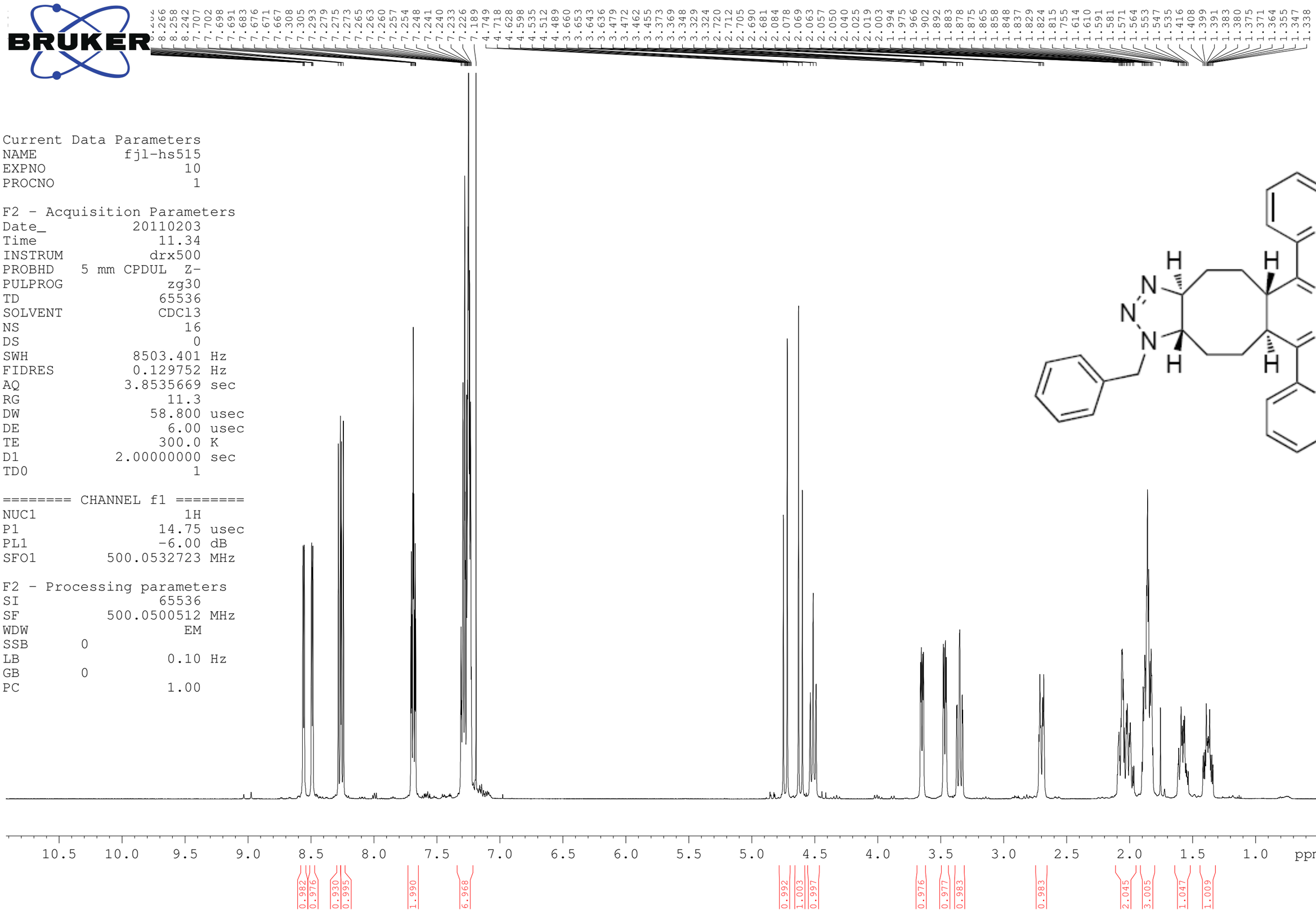

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PROCNO                1

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Time              11.52
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PULPROG          zgpg30
TD                65536
SOLVENT            CDCl3
NS                   256
DS                     8
SWH            34013.605 Hz
FIDRES          0.519006 Hz
AQ             0.9634292 sec
RG                  4096
DW                14.700 usec
DE                 6.00 usec
TE                300.0 K
D1            3.00000000 sec
d11           0.03000000 sec
DELTA         2.90000010 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  13C
P1                   9.80 usec
PL1                 -6.00 dB
SFO1         125.7520828 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2                -6.00 dB
PL12               10.62 dB
PL13               15.00 dB
SFO2         500.0517480 MHz

F2 - Processing parameters
SI                 65536
SF           125.7376619 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.00
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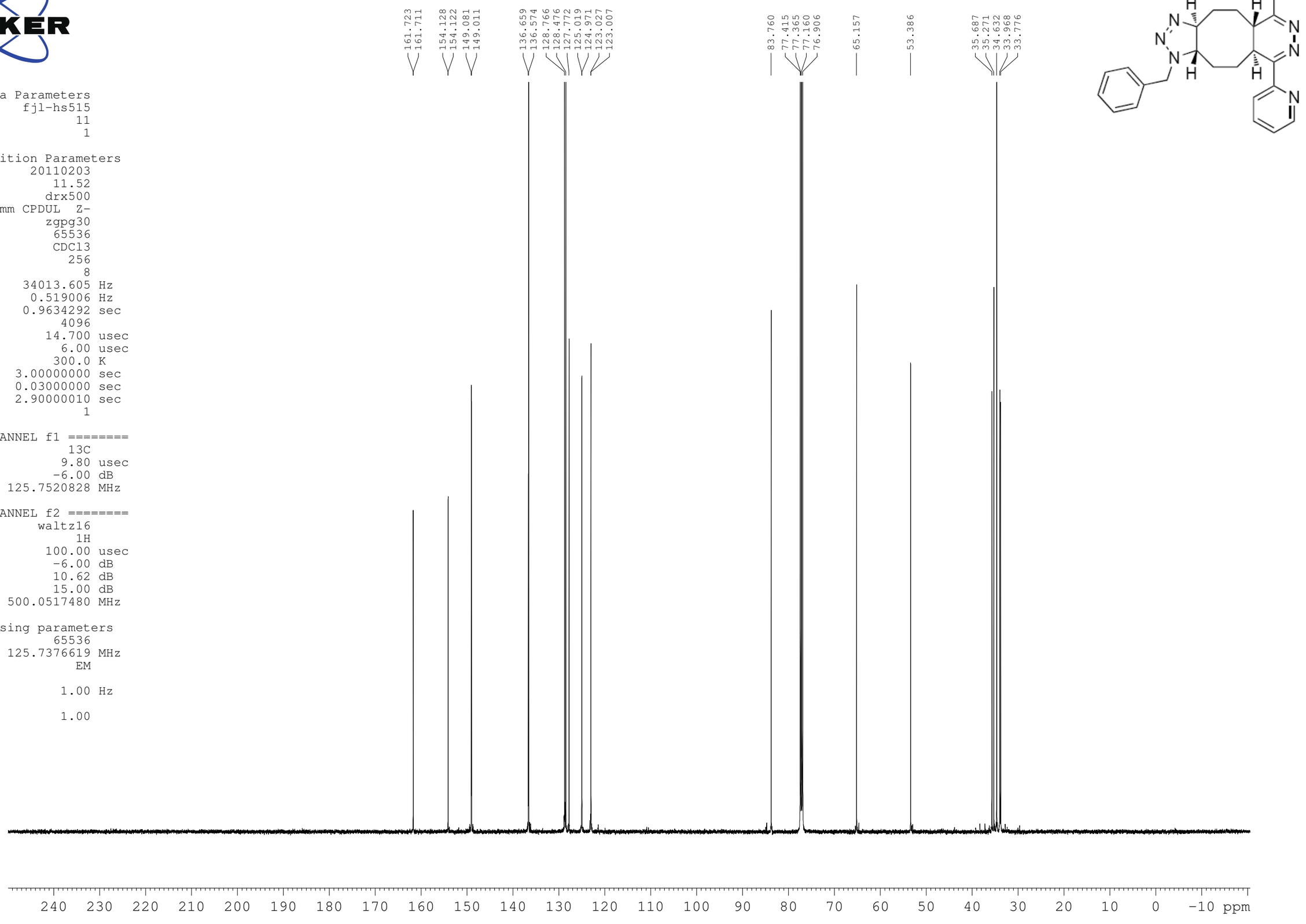
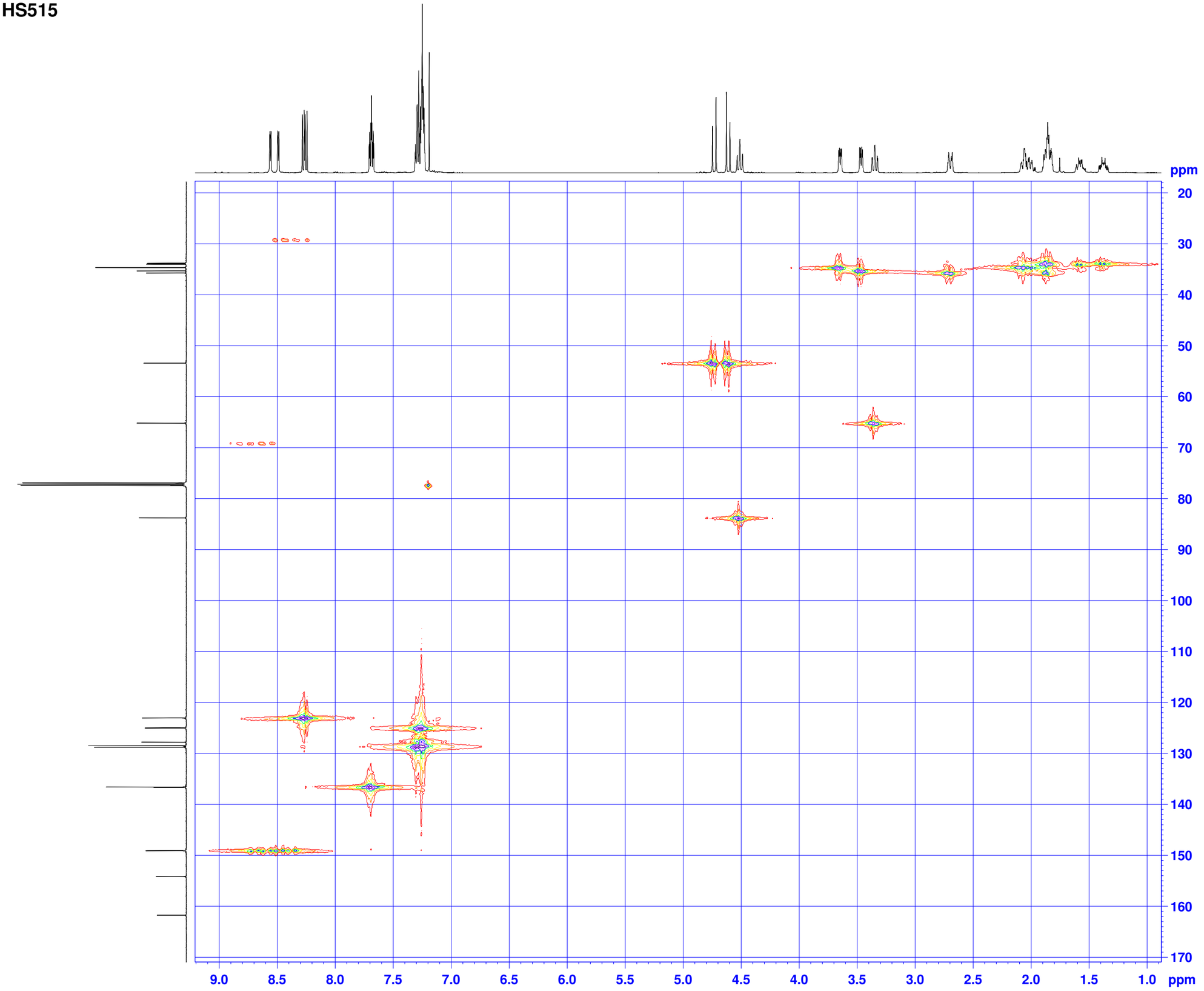
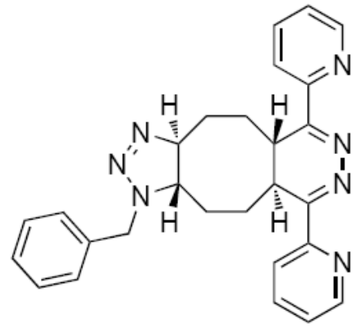


HS515



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TD                  2048
SOLVENT             CDCl3
NS                     2
DS                    16
SWH              6009.615 Hz
FIDRES           2.934382 Hz
AQ               0.1704436 sec
RG                  2048
DW                 83.200 usec
DE                  6.00 usec
TE                 300.0 K
CNST2         145.0000000
d0             0.00000300 sec
D1             2.00000000 sec
d2             0.00344828 sec
d12            0.00002000 sec
d13            0.00000400 sec
D16            0.00010000 sec
DELTA1         0.00232428 sec
IN0            0.00001990 sec

======== CHANNEL f1 ========
NUC1                1H
P1                14.75 usec
p2                29.50 usec
PL1               -6.00 dB
SFO1         500.0525002 MHz

======== CHANNEL f2 ========
CPDPRG2             garp
NUC2                13C
P3                 9.25 usec
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.74 dB
SFO2         125.7476820 MHz

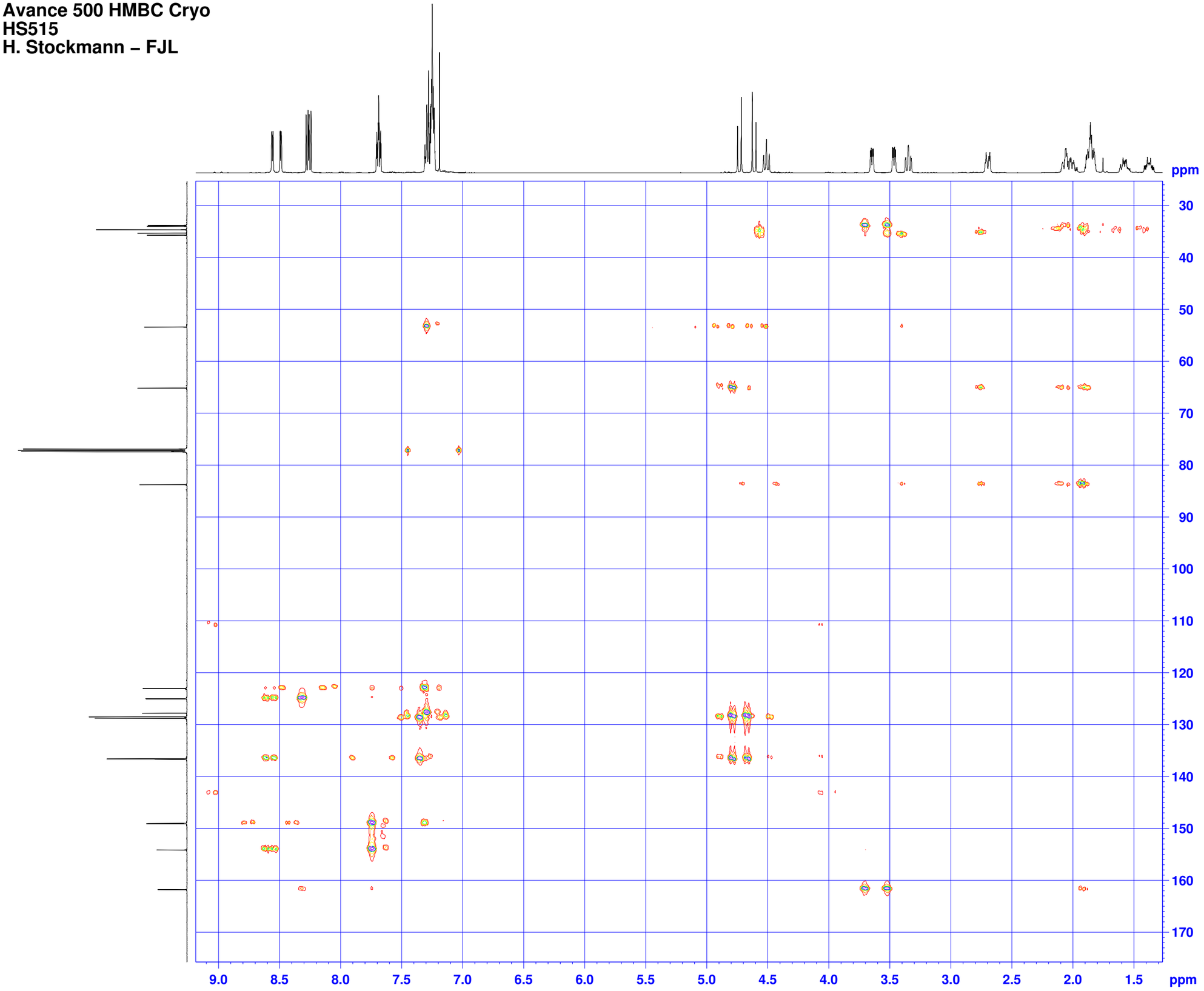
====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPNAM2          SINE.100
GPNAM3          SINE.100
GPZ1               50.00 %
GPZ2               30.00 %
GPZ3               40.10 %
P16              1000.00 usec

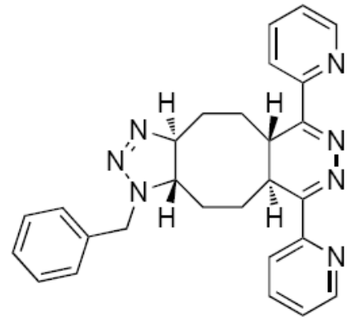
F1 - Acquisition parameters
TD                  256
SFO1          125.7477 MHz
FIDRES         98.146988 Hz
SW              199.810 ppm
FnMODE               QF

F2 - Processing parameters
SI                 1024
SF          500.0500440 MHz
WDW                SINE
SSB                   4
LB        0 Hz
GB        0
PC                 1.00

F1 - Processing parameters
SI                 1024
MC2                  QF
SF          125.7376451 MHz
WDW              States
SSB                   4
LB        0 Hz
GB        0
```

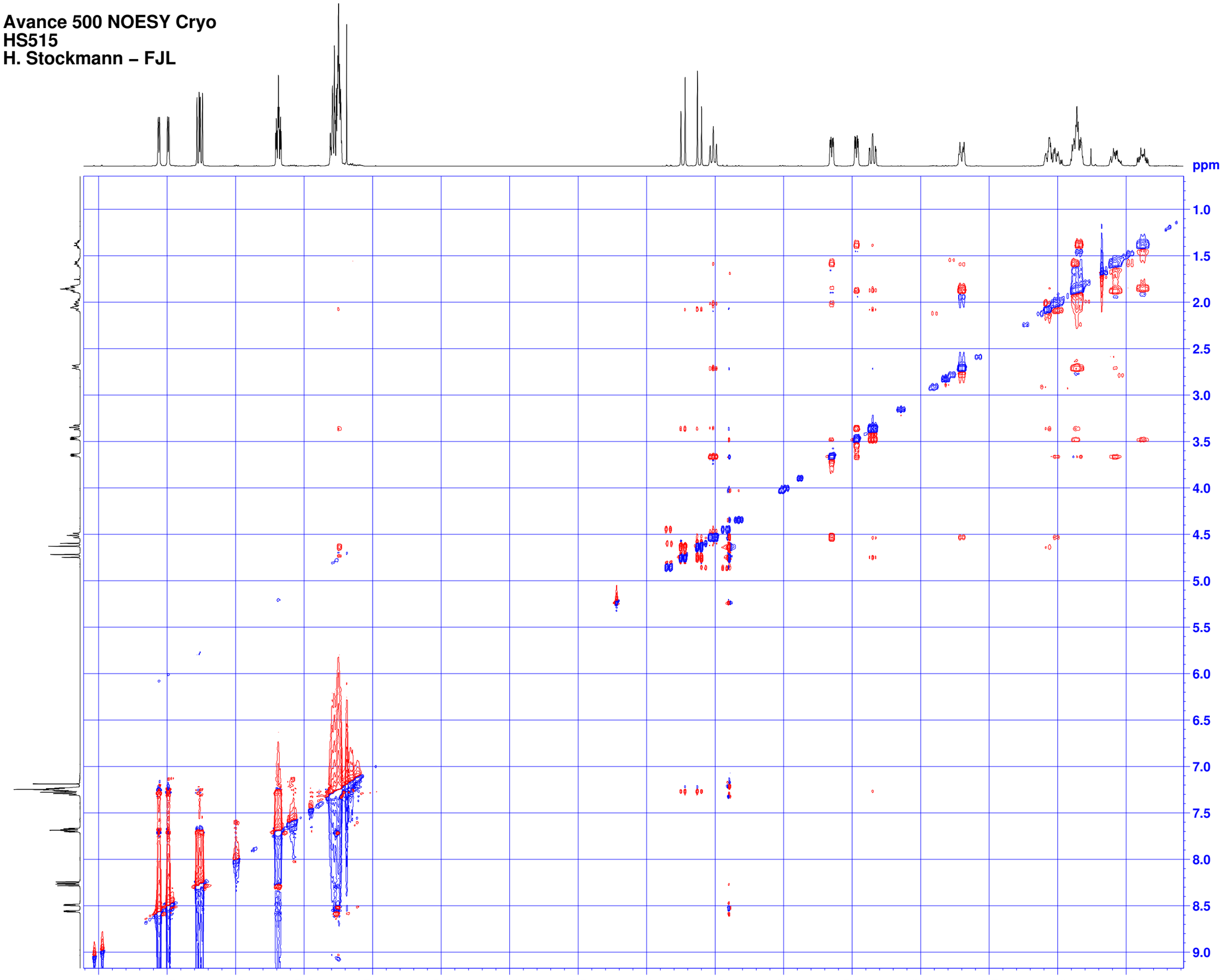
Avance 500 HMBC Cryo
HS515
H. Stockmann – FJL

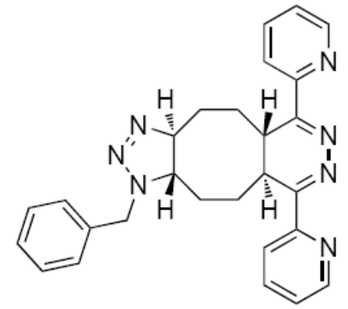




Time	21.42
INSTRUM	drx500
PROBHD	5 mm CPDUL Z-
PULPROG	hmbcgplpndqf
TD	2048
SOLVENT	CDCl3
NS	2
DS	8
SWH	5482.456 Hz
FIDRES	2.676980 Hz
AQ	0.1868276 sec
RG	4096
DW	91.200 usec
DE	6.00 usec
TE	300.0 K
CNST2	145.0000000
CNST13	10.0000000
d0	0.00000300 sec
D1	2.00000000 sec
d2	0.00344828 sec
d6	0.05000000 sec
D16	0.00010000 sec
IN0	0.00001730 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	14.75 usec
p2	29.50 usec
PL1	-6.00 dB
SFO1	500.0525002 MHz
======== CHANNEL f2 ========	
NUC2	13C
P3	9.25 usec
PL2	-6.00 dB
SFO2	125.7508255 MHz
====== GRADIENT CHANNEL =====	
GPNAM1	SINE.100
GPNAM2	SINE.100
GPNAM3	SINE.100
GPZ1	50.00 %
GPZ2	30.00 %
GPZ3	40.10 %
P16	1000.00 usec
F1 - Acquisition parameters	
TD	256
SFO1	125.7508 MHz
FIDRES	112.897400 Hz
SW	229.833 ppm
FnMODE	QF
F2 - Processing parameters	
SI	1024
SF	500.0500219 MHz
WDW	SINE
SSB	4
LB	0 Hz
GB	0
PC	1.00
F1 - Processing parameters	
SI	1024
MC2	QF
SF	125.7376786 MHz
WDW	States
SSB	4
LB	0 Hz
GB	0

Avance 500 NOESY Cryo
HS515
H. Stockmann – FJL






```
Cu
NA
EX
PR

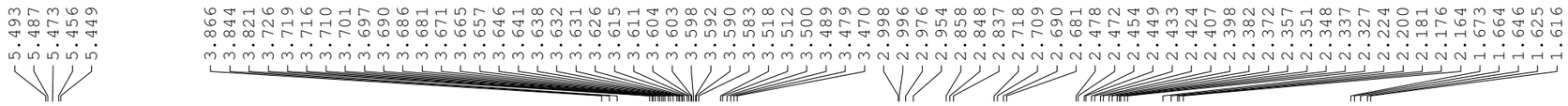
F2
Date_              20110208
Time                 22.03
INSTRUM              drx500
PROBHD    5 mm CPDUL  Z-
PULPROG             noesyph
TD                     2048
SOLVENT               CDCl3
NS                        8
DS                        4
SWH               5000.000 Hz
FIDRES               2.441406 Hz
AQ                0.2048500 sec
RG                     10.1
DW                 100.000 usec
DE                   6.00 usec
TE                   300.0 K
d0                0.00008122 sec
D1                2.00000000 sec
D8                1.20000005 sec
IN0               0.00020000 sec

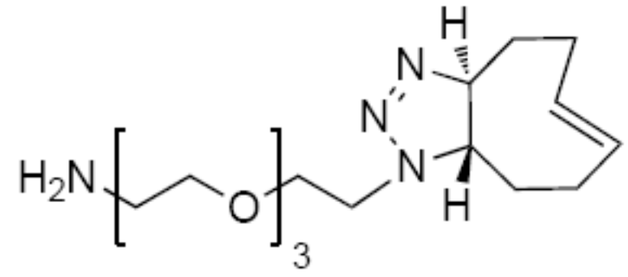
======== CHANNEL f1 ========
NUC1                    1H
P1                   14.75 usec
PL1                  -6.00 dB
SFO1          500.0522502 MHz

F1 - Acquisition parameters
TD                      320
SFO1               500.0523 MHz
FIDRES            15.625000 Hz
SW                    9.999 ppm
FnMODE                 TPPI

F2 - Processing parameters
SI                     2048
SF            500.0500512 MHz
WDW                   QSINE
SSB                     2.5
LB          0 Hz
GB          0
PC                     1.00

F1 - Processing parameters
SI                     2048
MC2                    TPPI
SF            500.0500418 MHz
WDW           States-TPPI
SSB                     2.5
LB          0 Hz
GB          0
```



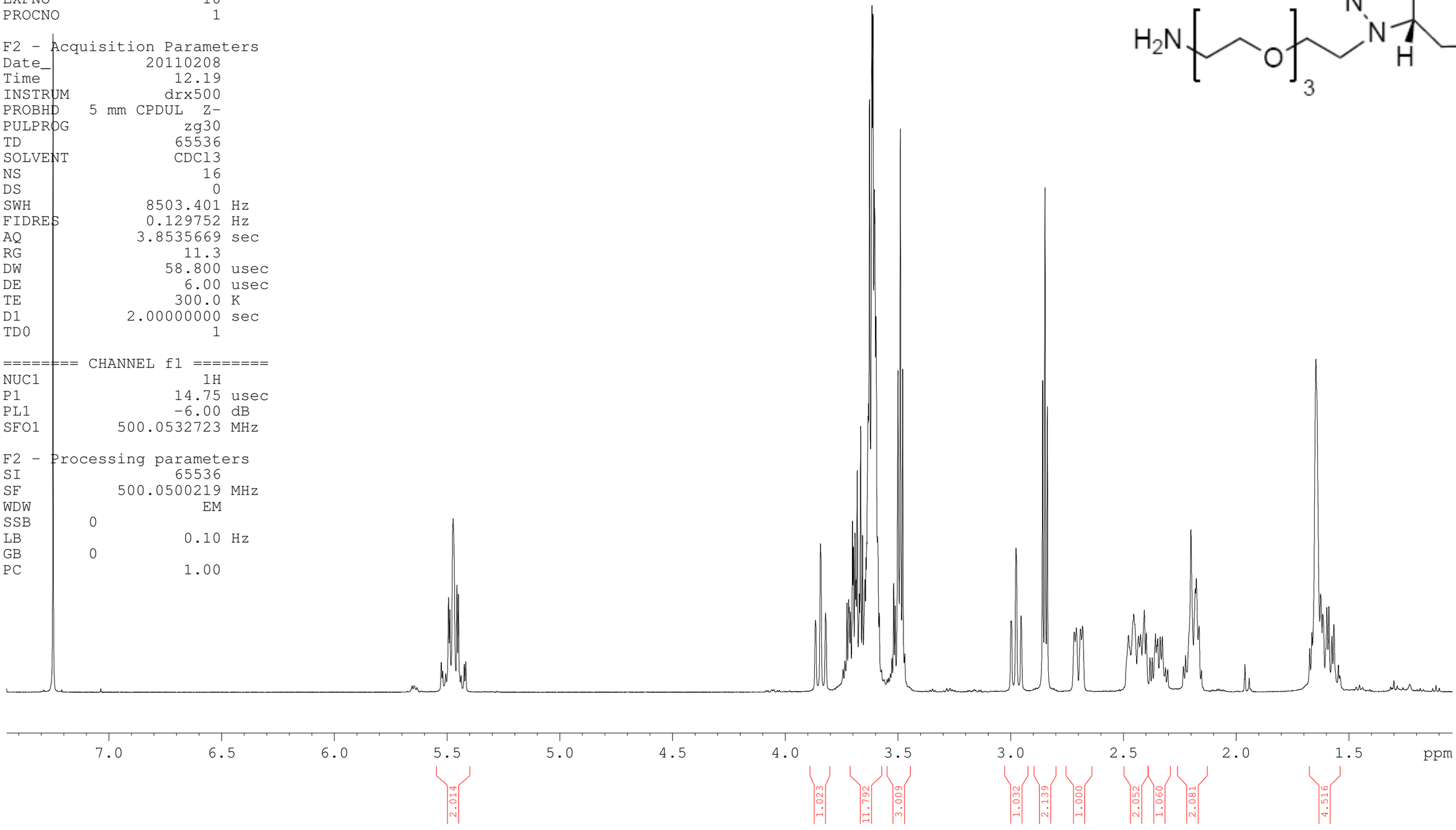



```
Current Data Parameters
NAME         fjl-hs516-2
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110208
Time               12.19
INSTRUM           drx500
PROBHD   5 mm CPDUL  Z-
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    0
SWH            8503.401 Hz
FIDRES         0.129752 Hz
AQ             3.8535669 sec
RG                  11.3
DW                58.800 usec
DE                 6.00 usec
TE                300.0 K
D1           2.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                14.75 usec
PL1               -6.00 dB
SFO1        500.0532723 MHz

F2 - Processing parameters
SI                65536
SF          500.0500219 MHz
WDW                  EM
SSB       0
LB                 0.10 Hz
GB        0
PC                 1.00
```



rance 500 13C Cryo
HS516-2
. Stockmann - FJL

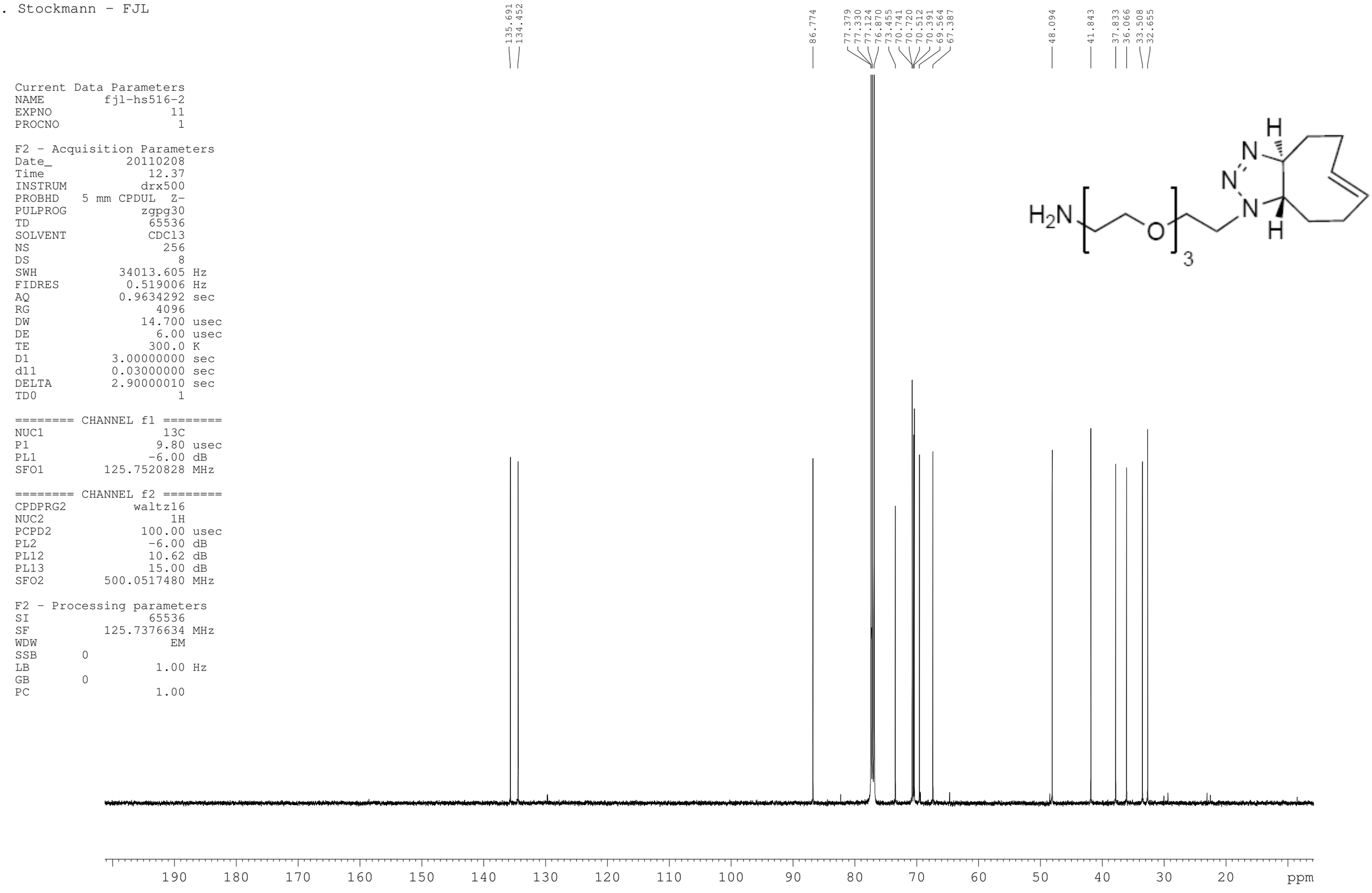
```
Current Data Parameters
NAME         fjl-hs516-2
EXPNO                 11
PROCNO                 1

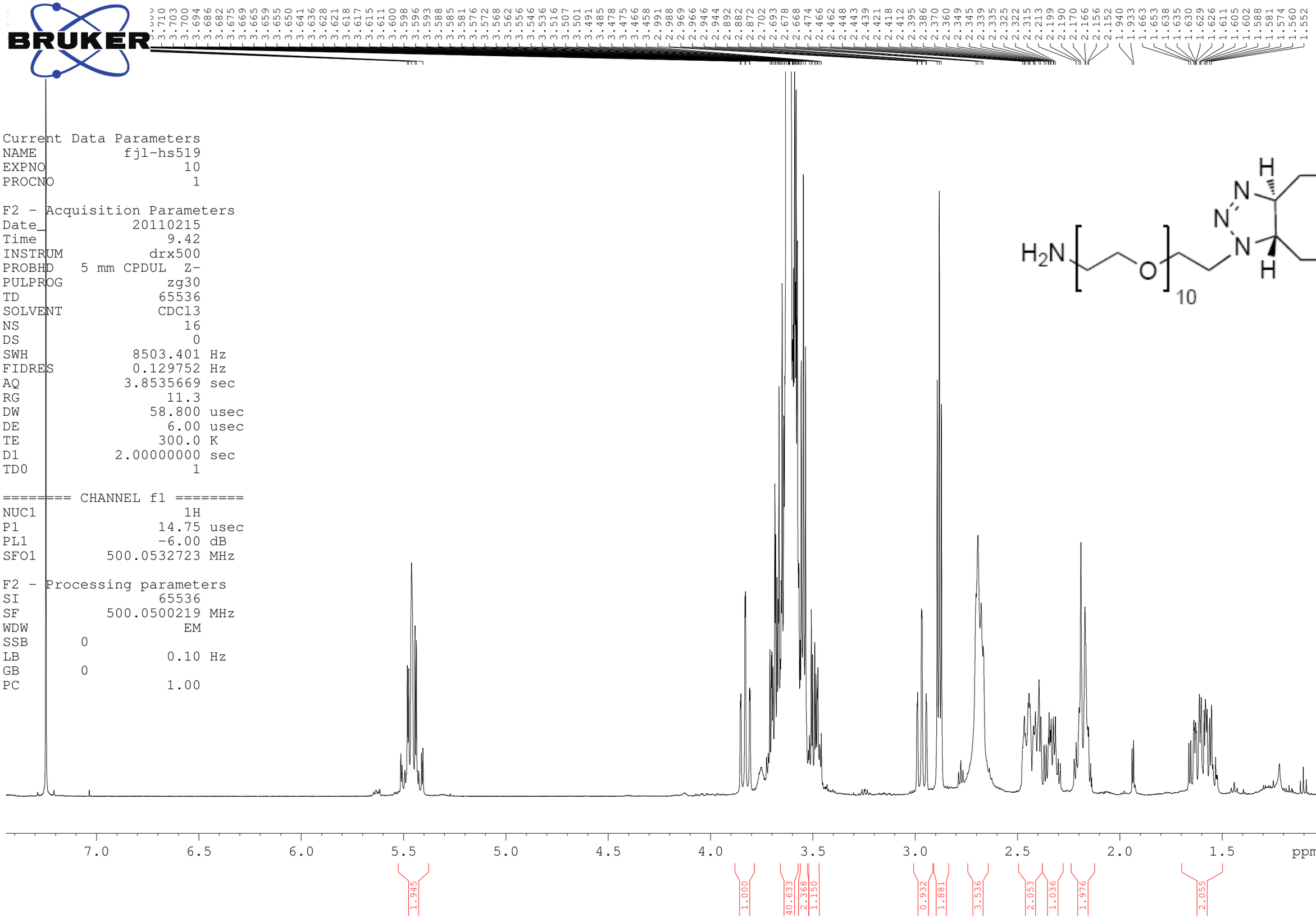
F2 - Acquisition Parameters
Date_           20110208
Time               12.37
INSTRUM           drx500
PROBHD   5 mm CPDUL  Z-
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   256
DS                     8
SWH            34013.605 Hz
FIDRES          0.519006 Hz
AQ             0.9634292 sec
RG                  4096
DW                14.700 usec
DE                 6.00 usec
TE                300.0 K
D1            3.00000000 sec
d11           0.03000000 sec
DELTA         2.90000010 sec
TD0                    1

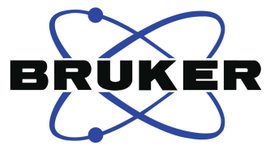
======== CHANNEL f1 ========
NUC1                 13C
P1                  9.80 usec
PL1                -6.00 dB
SFO1         125.7520828 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2                -6.00 dB
PL12               10.62 dB
PL13               15.00 dB
SFO2         500.0517480 MHz

F2 - Processing parameters
SI                 65536
SF           125.7376634 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.00
```







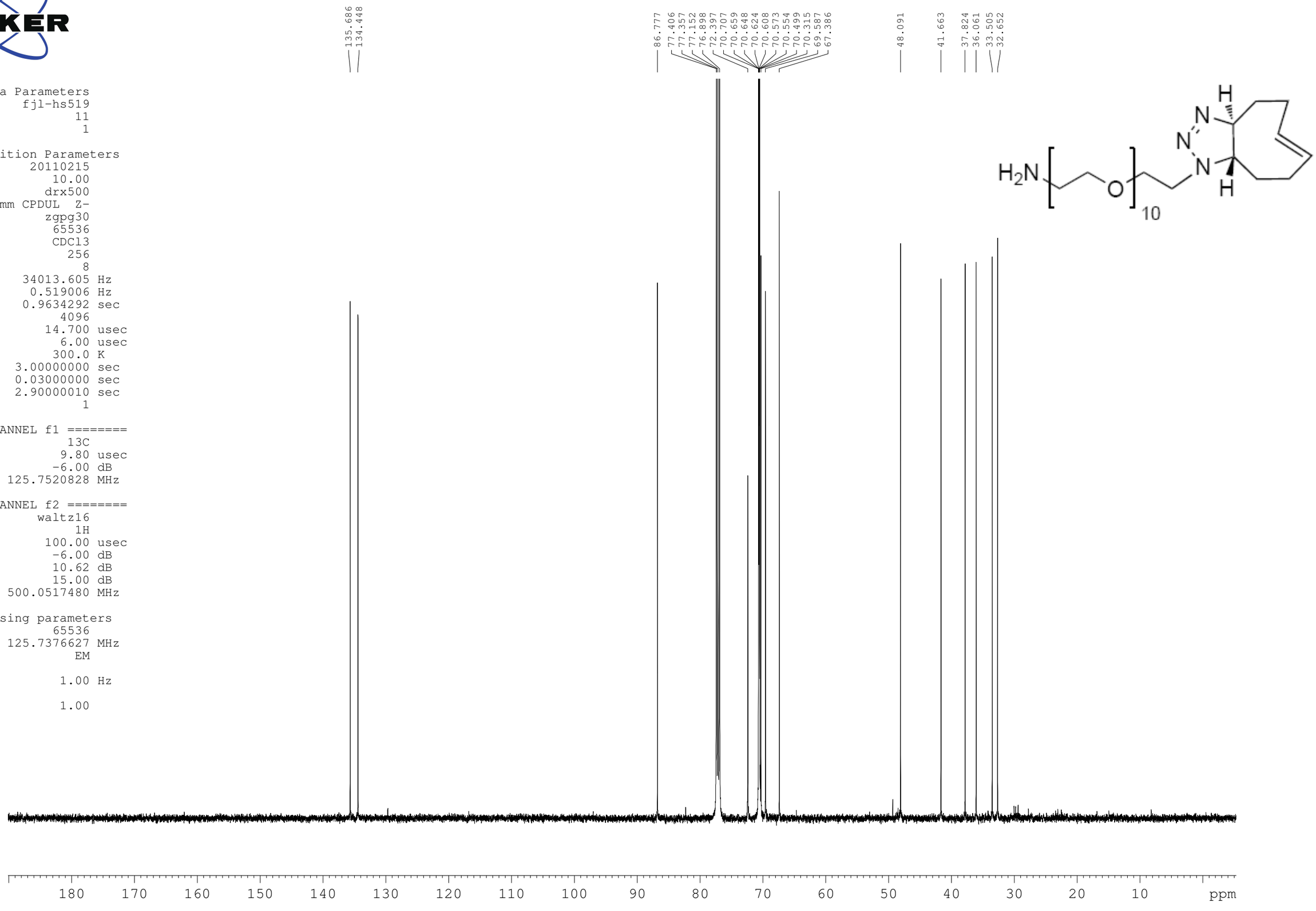
```
Current Data Parameters
NAME          fjl-hs519
EXPNO                11
PROCNO                1

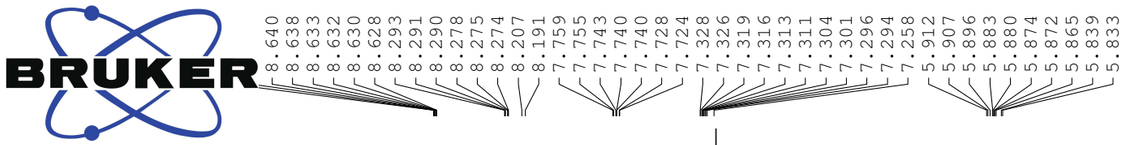
F2 - Acquisition Parameters
Date_          20110215
Time              10.00
INSTRUM           drx500
PROBHD   5 mm CPDUL  Z-
PULPROG           zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    8
SWH           34013.605 Hz
FIDRES         0.519006 Hz
AQ            0.9634292 sec
RG                 4096
DW                14.700 usec
DE                 6.00 usec
TE                300.0 K
D1           3.00000000 sec
d11          0.03000000 sec
DELTA        2.90000010 sec
TD0                   1

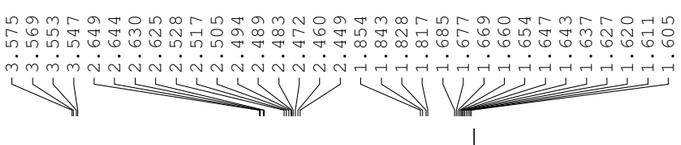
======== CHANNEL f1 ========
NUC1                 13C
P1                 9.80 usec
PL1               -6.00 dB
SFO1        125.7520828 MHz

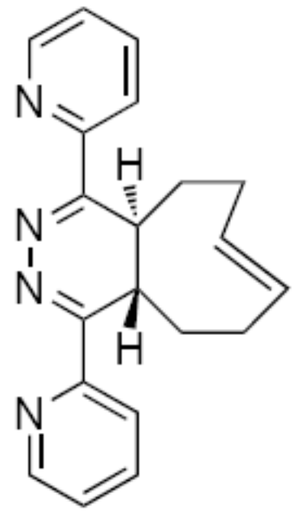
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2            100.00 usec
PL2               -6.00 dB
PL12              10.62 dB
PL13              15.00 dB
SFO2        500.0517480 MHz

F2 - Processing parameters
SI                65536
SF          125.7376627 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.00
```







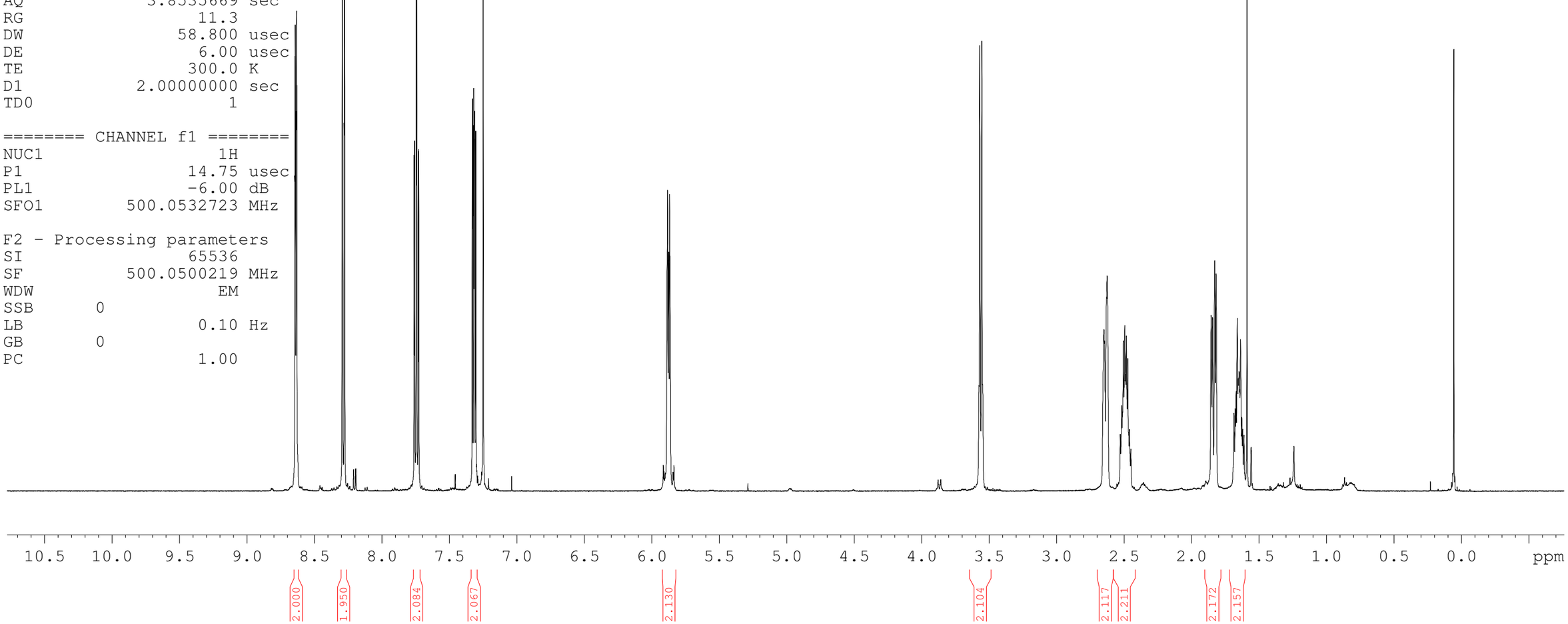


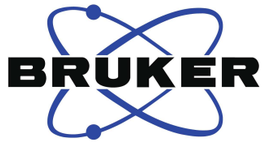

```
Current Data Parameters
NAME          fjl-hs529
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_          20110224
Time              15.00
INSTRUM          drx500
PROBHD   5 mm CPDUL  Z-
PULPROG           zg30
TD               65536
SOLVENT          CDCl3
NS                  24
DS                   0
SWH           8503.401 Hz
FIDRES        0.129752 Hz
AQ            3.8535669 sec
RG                11.3
DW              58.800 usec
DE               6.00 usec
TE              300.0 K
D1           2.00000000 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                1H
P1              14.75 usec
PL1             -6.00 dB
SFO1        500.0532723 MHz

F2 - Processing parameters
SI                65536
SF          500.0500219 MHz
WDW                 EM
SSB       0
LB                0.10 Hz
GB        0
PC                1.00
```





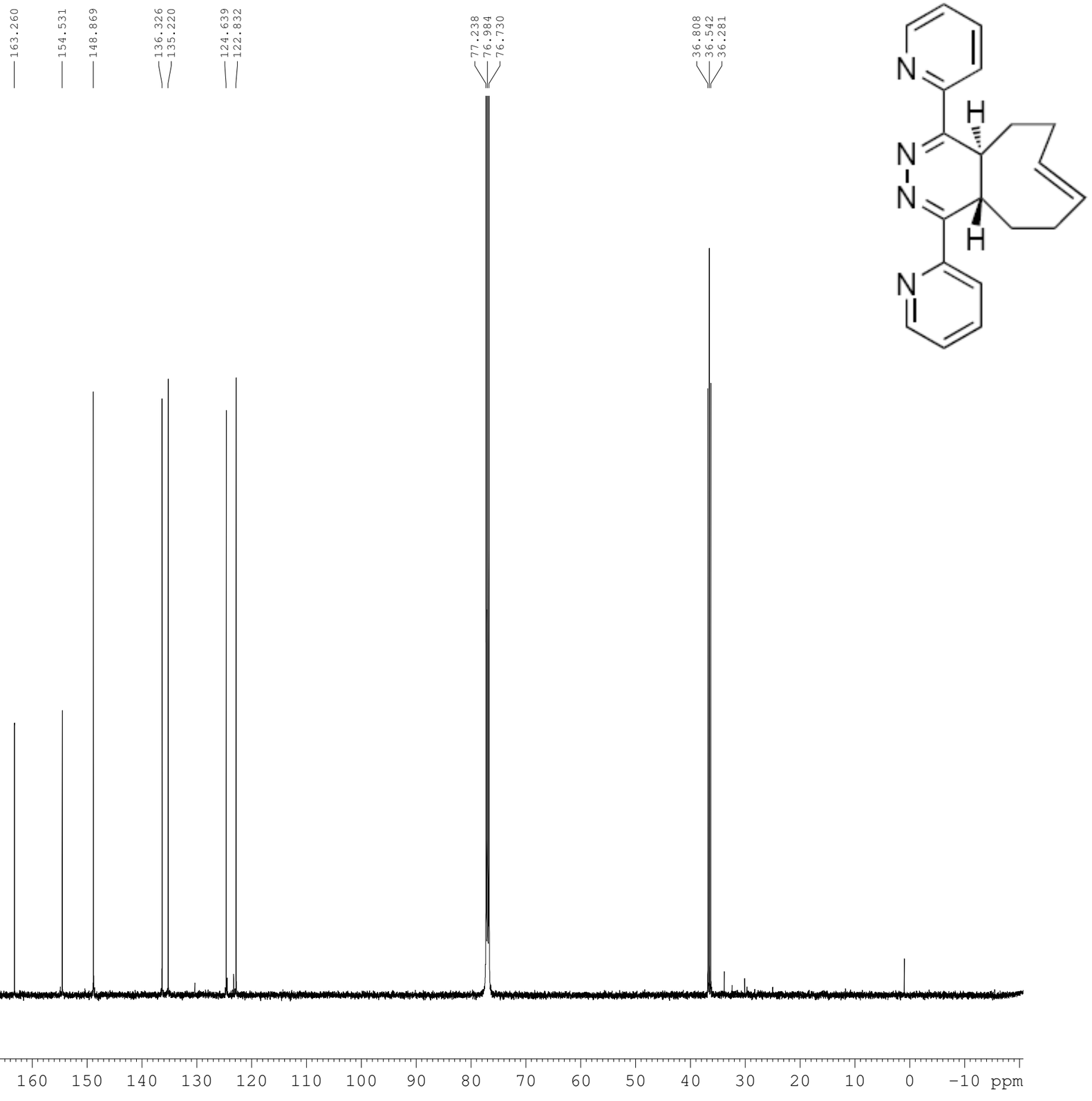
```
Current Data Parameters
NAME          fjl-hs529
EXPNO                11
PROCNO                1

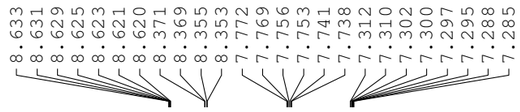
F2 - Acquisition Parameters
Date_          20110224
Time              15.36
INSTRUM          drx500
PROBHD   5 mm CPDUL  Z-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  512
DS                    8
SWH           34013.605 Hz
FIDRES         0.519006 Hz
AQ            0.9634292 sec
RG                 4096
DW                14.700 usec
DE                 6.00 usec
TE                300.0 K
D1           3.00000000 sec
d11          0.03000000 sec
DELTA        2.90000010 sec
TD0                   1

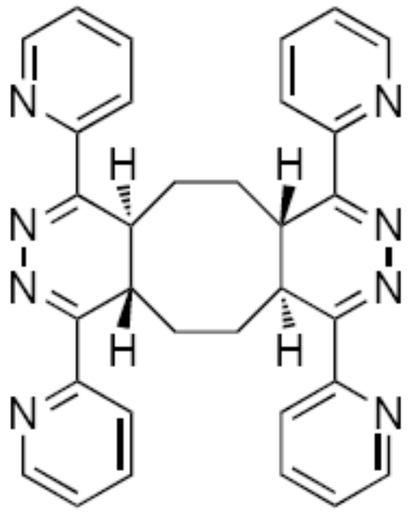
======== CHANNEL f1 ========
NUC1                 13C
P1                  9.80 usec
PL1                -6.00 dB
SFO1        125.7520828 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2                -6.00 dB
PL12               10.62 dB
PL13               15.00 dB
SFO2        500.0517480 MHz

F2 - Processing parameters
SI                 65536
SF          125.7376786 MHz
WDW                   EM
SSB      0
LB                 1.00 Hz
GB       0
PC                 1.00
```





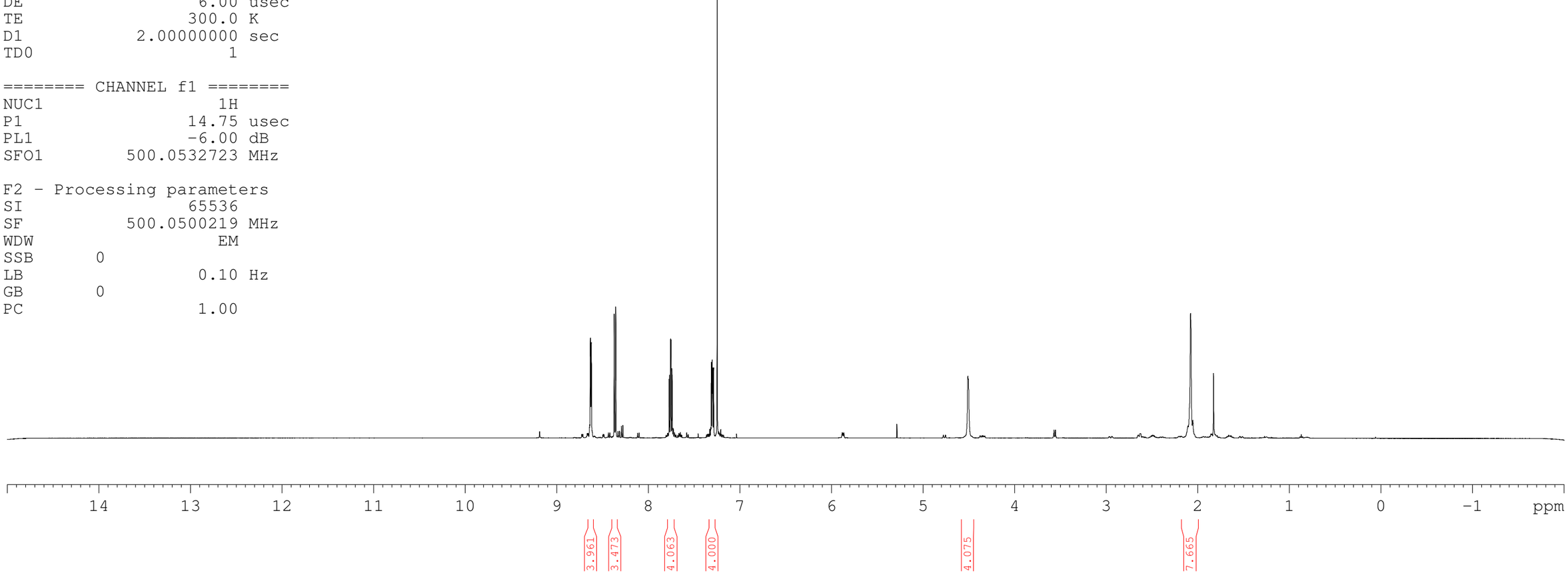


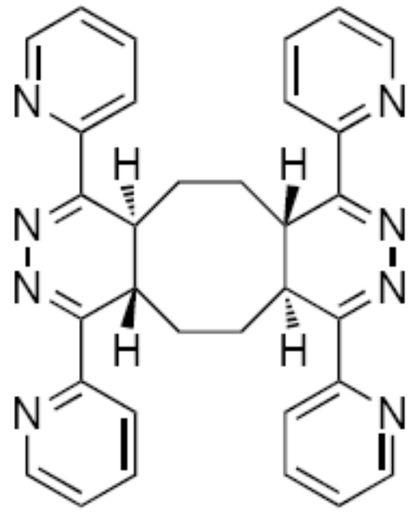
```
Current Data Parameters
NAME          fjl-hs530
EXPNO                10
PROCNO                1

F2 - Acquisition Parameters
Date_          20110301
Time              10.32
INSTRUM          drx500
PROBHD   5 mm CPDUL  Z-
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    0
SWH            8503.401 Hz
FIDRES         0.129752 Hz
AQ             3.8535669 sec
RG                 11.3
DW                58.800 usec
DE                 6.00 usec
TE                300.0 K
D1           2.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                14.75 usec
PL1               -6.00 dB
SFO1        500.0532723 MHz

F2 - Processing parameters
SI                65536
SF          500.0500219 MHz
WDW                  EM
SSB       0
LB                0.10 Hz
GB        0
PC                1.00
```





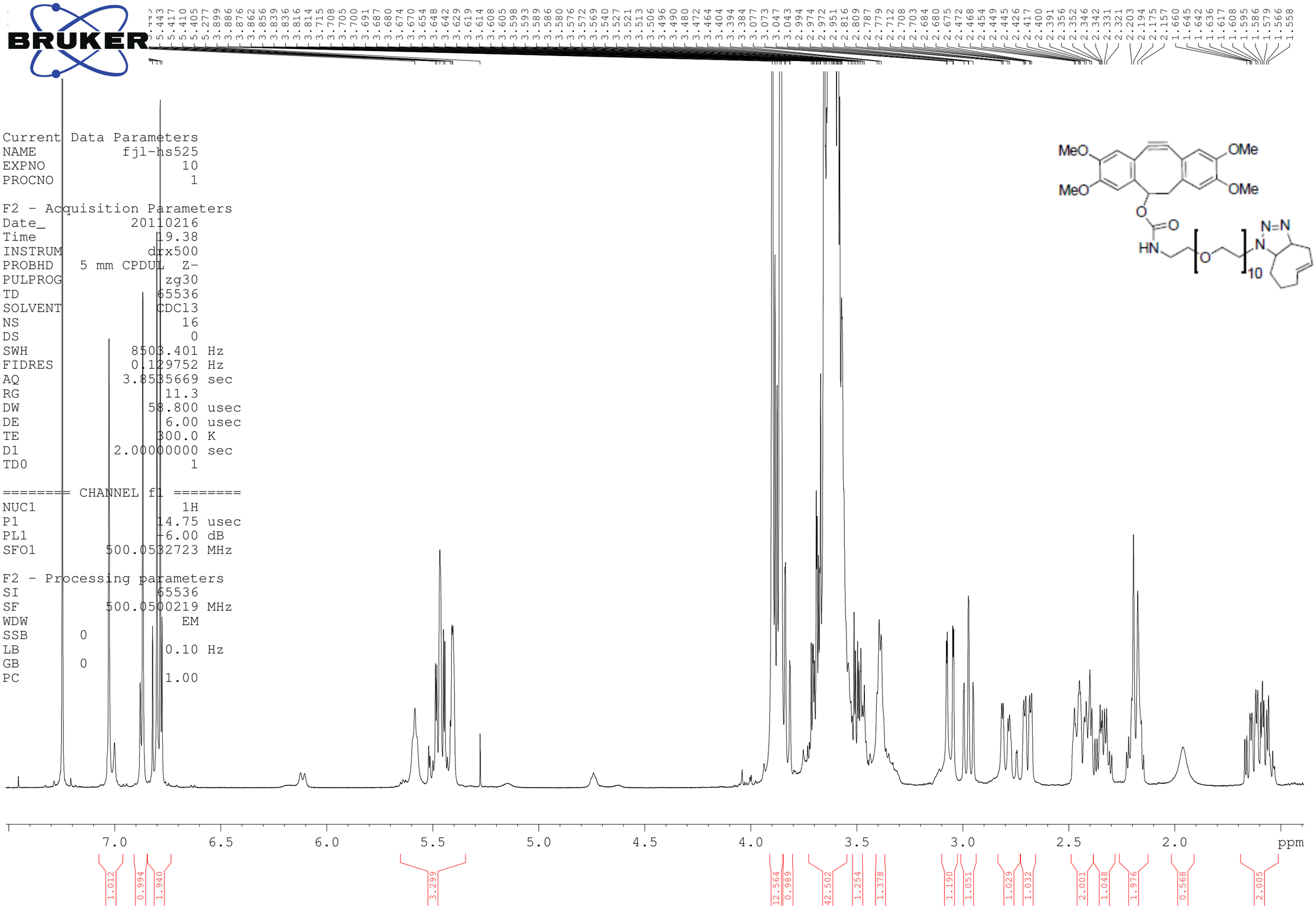
```
Current Data Parameters
NAME          fjl-hs530
EXPNO                11
PROCNO                1

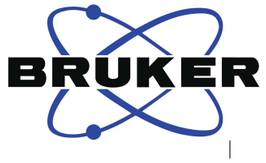
F2 - Acquisition Parameters
Date_          20110301
Time              10.50
INSTRUM          drx500
PROBHD   5 mm CPDUL  Z-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    8
SWH           34013.605 Hz
FIDRES         0.519006 Hz
AQ            0.9634292 sec
RG                 4096
DW                14.700 usec
DE                 6.00 usec
TE                300.0 K
D1           3.00000000 sec
d11          0.03000000 sec
DELTA        2.90000010 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  9.80 usec
PL1                -6.00 dB
SFO1        125.7520828 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2                -6.00 dB
PL12               10.62 dB
PL13               15.00 dB
SFO2        500.0517480 MHz

F2 - Processing parameters
SI                 65536
SF          125.7376786 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.00
```





149.065
148.822
147.857
147.818
145.780
144.565
135.687
134.446
115.666
113.992
113.259
112.523
109.411
109.387
109.162
108.811
108.197
86.781
70.710
70.647
70.498
70.363
70.208
69.594
67.389
56.197
56.152
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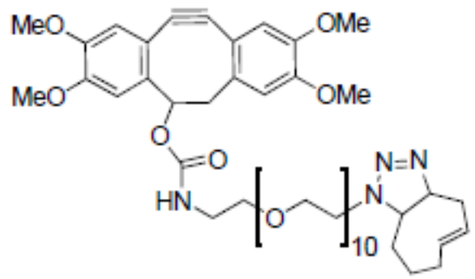
46.529
41.047
37.825
36.059
33.504
32.652




```
Current Data Parameters
NAME          fjl-hs525
EXPNO                11
PROCNO                1

F2 - Acquisition Parameters
Date_          20110216
Time              19.56
INSTRUM          drx500
PROBHD   5 mm CPDUL  Z-
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  256
DS                    8
SWH           34013.605 Hz
FIDRES         0.519006 Hz
AQ            0.9634292 sec
RG                 4096
DW                14.700 usec
DE                 6.00 usec
TE                300.0 K
D1           3.00000000 sec
d11          0.03000000 sec
DELTA        2.90000010 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  9.80 usec
PL1                -6.00 dB
SFO1        125.7520828 MHz

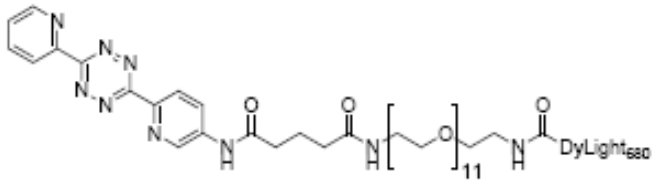
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2                -6.00 dB
PL12               10.62 dB
PL13               15.00 dB
SFO2        500.0517480 MHz

F2 - Processing parameters
SI                 65536
SF          125.7376638 MHz
WDW                   EM
SSB      0
LB                 1.00 Hz
GB       0
PC                 1.00
```

hs-509

F_Leeper2804-1

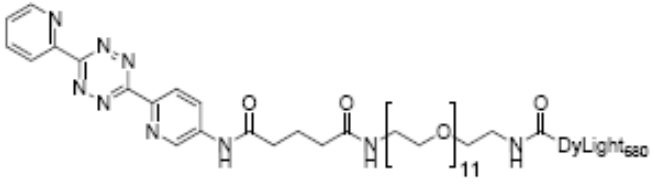
3: Diode Array
Range: 4.782e+1



hs-509

F_Leeper2804-1 129 (3.023) Cm (127:132-(118:120+140:142))

2: Scan ES+
3.69e4



100
%
0

1683.57
1684.74
1682.49
1690.49
1705.62
1699.91
1694.55
1711.34
1714.91
1722.08
1733.13
1756.64
1670.56
1674.43
1718.30
1731.19
1666.84
1738.91
1742.85
1754.91
1759.21
1746.00
1764.53

1670 1680 1690 1700 1710 1720 1730 1740 1750 1760 m/z