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Electronic Supporting Information

A new tool to assess ceramide bioactivity: 6-bromo-7-hydroxycoumarinyl-caged ceramide

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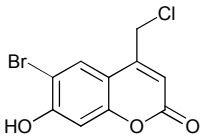
General synthetic methods. Optical rotation was determined on an Autopol III Automatic Polarimeter. High-resolution mass spectra were recorded on an Agilent Technologies G6520A Q-TOF mass spectrometer using electrospray ionization. NMR spectra were recorded on Bruker Avance I and III spectrometers (400 or 500 MHz), and chemical shifts are expressed in parts per million (δ) relative to chloroform (7.26 ppm for ^{1}H and 77.2 ppm for ^{13}C), methanol (3.31 ppm for ^{1}H and 49.0 ppm for ^{13}C) or DMSO (2.50 ppm for ^{1}H and 40.0 ppm for ^{13}C) as an internal reference. TLC was performed on 0.25-mm silica gel 60 F_{254} aluminum-backed plates. Silica gel 60 (230-400 mesh) was used for flash column chromatography. The compounds were visualized by UV light and 10% phosphomolybdic acid in ethanol. THF was distilled from sodium and benzophenone, and hexane and CH_2Cl_2 were dried over calcium hydride. C4-Ceramide (*N*-butyroyl-D-*erythro*-sphingosine, d18:1/4:0) and C16-ceramide (*N*-palmitoyl-D-*erythro*-sphingosine, d18:1/16:0) were purchased from Avanti Polar Lipids. Flash chromatography was performed using a Biotage Isolera One automated system with Flash KP-Sil™ silica cartridges. Preparative TLC was carried out using 1000-μm silica gel GF from Analtech.

Absorption and fluorescence spectroscopy. Absorption spectra were measured with a Cary 5000 UV-vis-NIR spectrophotometer. Emission spectra were recorded using a Horiba Jobin Yvon FL-3 21 2tau spectrofluorometer with slit widths of 1 nm. All spectral measurements were performed at 22 °C using a 1-cm pathlength quartz cuvette. Fluorescence quantum yields were calculated with reference to Coumarin 460 in ethanol (Φ_{fl} = 0.59)[S1] at an excitation wavelength of 374 nm and absorbance ≤ 0.1. Working solutions of the compounds were prepared at 5-10 μM to match the absorbance of 5 μM Coumarin 460 at 374 nm.

Photolysis and photoproduct analysis. Photolysis was performed in a quartz cuvette containing 3 mL of 5 μM caged substrate in 10 mM KMops buffer containing 100 mM KCl (pH 7.4) with 50% ethanol. The sample was irradiated at 350 nm in a Rayonet photochemical reactor (Southern New England Ultraviolet Co., Branford, CT, USA) equipped with four lamps. Aliquots of 20 μL were removed periodically for analysis. Coumarin 480 was added to each aliquot as an internal standard. Loss of starting material and formation of by-product **4** were monitored by reversed-phase HPLC with a SunFire C18 column (Waters Corp., Milford, MA, USA), and eluted with an ethanol/water gradient at a flow rate of 0.8 mL/min. Detection was by absorbance at 325 nm and fluorescence at 480 nm.

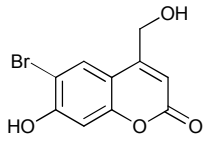
6-Bromo-4-(chloromethyl)-7-hydroxycoumarin (3).



3

A solution of 4-bromoresorcinol **1** (1.00 g, 5.29 mmol) in methanesulfonic acid (8.0 mL) was treated with methyl 4-chloroacetoacetate **2** (0.92 mL, 7.94 mmol) and then was stirred for 2 h at room temperature. The resulting mixture was slowly poured into ice-water (24 mL) and then was stirred for 30 min to give a white precipitate. The precipitate was collected by filtration and washed with cold water (3 × 5 mL). The resulting precipitate was recrystallized with hexane/EtOAc to give compound **3** (1.46 g, 95%): R_f 0.17 (hexane/EtOAc 3:1); ^{1}H NMR (400 MHz; DMSO-d_6) δ_H 5.00 (2H, m), 6.47 (1H, s), 6.92 (1H, s), 7.99 (1H, s), 11.57 (1H, br); ^{13}C NMR (100 MHz; DMSO-d_6) δ_C 41.2, 103.3, 106.2, 110.7, 112.1, 129.1, 150.2, 154.1, 157.6, 159.7.

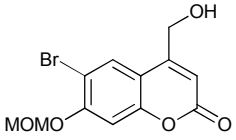
6-Bromo-7-hydroxy-4-hydroxymethylcoumarin (4).



4

A suspension of compound **3** (1.04 g, 3.59 mmol) in water (250 mL) was heated at reflux for 2 days and allowed to cool to room temperature. The resulting mixture was concentrated *in vacuo*, and then the residue was triturated with hexane/Et_2O (2:1, 6 mL) to afford compound **4** (0.96 g, 99%). The resulting compound **4** was used in the next step without further purification: R_f 0.80 (EtOAc); ^{1}H NMR (400 MHz; CD_3OD) δ_H 4.77 (2H, m), 6.38 (1H, s), 6.84 (1H, s), 7.81 (1H, s); ^{13}C NMR (100 MHz; CD_3OD) δ_C 60.8, 104.2, 107.8, 109.1, 112.7, 129.3, 155.5, 157.2, 158.9, 163.3.

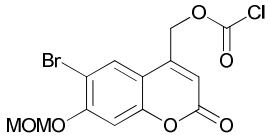
6-Bromo-7-*O*-(methoxymethyl)-4-hydroxymethylcoumarin (5).



5

A mixture of compound **4** (0.80 g, 2.95 mmol) and *N,N*-diisopropylethylamine (DIPEA, 0.57 mL, 2.88 mmol) in dry CH_2Cl_2 (30 mL) was cooled to 0 ^{o}C, and then chloromethyl methyl ether (MOMCl, 0.25 mL, 2.88 mmol) was added dropwise. After being stirred for 2 h at 0 ^{o}C, the reaction mixture was poured into 0.5 M citric acid (20 mL) and extracted with $CHCl_3$ (2 × 20 mL). The combined organic layer was washed with brine (30 mL) and dried over Na_2SO_4. After the solvent was removed *in vacuo*, the residue was triturated with hexane (2 × 5 mL) to afford compound **5** (0.83 g, 89%): R_f 0.34 (hexane/EtOAc 1:1); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 2.05 (1H, t, J = 4.9 Hz), 3.52 (3H, s), 4.86 (2H, m), 5.32 (2H, s), 6.52 (1H, s), 7.16 (1H, s), 7.70 (1H, s); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 56.8, 60.9, 95.3, 104.2, 108.5, 110.6, 112.9, 127.6, 152.9, 154.3, 156.3, 160.8; HRMS $[M+H]^+$ *m/z* calcd for $C_{12}H_{12}BrO_5$ 314.9863, found 314.9863.

6-Bromo-7-*O*-(methoxymethyl)coumarin-4-ylmethoxycarbonyl chloride (6).



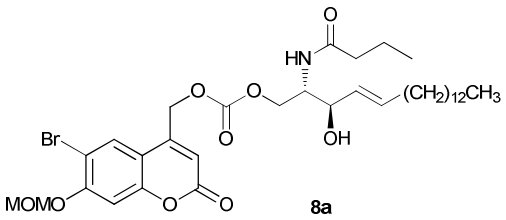
6

A mixture of triphosgene (0.53 g, 1.79 mmol) and Aliquat^{R} 336 (63 µL, 0.14 mmol) in dry hexane (1.5 mL) was stirred at room temperature for 16 h in a pressure tube (bushing type back seal) to generate phosgene. To a suspension of compound **5** (86.7 mg, 0.28 mmol) in dry THF/toluene (3:2, 1.5 mL) was added the phosgene solution at 0 ^{o}C. The resulting mixture was stirred 3 h at 0 ^{o}C and then the solvent was removed *in vacuo*. The residue was triturated with hexane/CH_2Cl_2 (3:1, 2 × 1 mL) to afford the corresponding chloroformate **6** (97.6 mg, 94%). The resulting compound **6** was used in the next step without further purification: R_f 0.87 (hexane/EtOAc 1:1); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 3.53 (3H, s), 5.33 (2H, s), 5.41 (2H, m), 6.44 (1H, s), 7.19 (1H, s), 7.64 (1H, s); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 56.9, 67.2, 95.3, 104.4, 109.0, 111.9, 112.8, 127.4, 145.4, 150.8, 154.4, 156.9, 159.7.

(2*S*,3*R*,4*E*)-2-Butyramido-3-hydroxyoctadec-4-enyl (6-bromo-7-*O*-(methoxymethyl)coumarin-4-yl)methyl carbonate (8a) and (2*S*,3*R*,4*E*)-2-butyramidooctadec-4-ene-1,3-diyl bis(6-bromo-7-*O*-(methoxymethyl)coumarin-4-yl)methyl dicarbonate (8b).

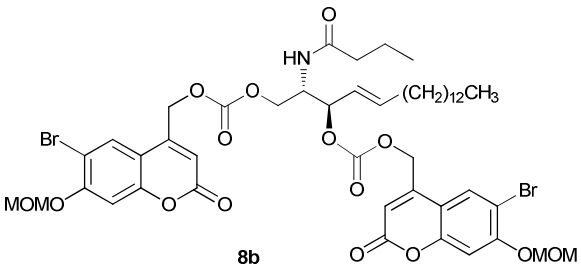
To a mixture of C4-ceramide **7** (*N*-butyroyl-D-*erythro*-sphingosine (d18:1/4:0), 10 mg, 27 µmol) and 6-bromo-7-*O*-(methoxymethyl)coumarin-4-ylmethoxycarbonyl chloride **6** (11 mg, 30 µmol) in dry CH_2Cl_2 (3.0 mL) were added DIPEA (5.2 µL, 30 µmol) and 4-(dimethylamino)pyridine (DMAP, 3.3 mg, 27 µmol) at 0 ^{o}C. The reaction mixture was stirred for 2.5 h at 0 ^{o}C and then was concentrated *in vacuo*. The residue was purified in a Biotage Isolera One flash purification system with hexane/EtOAc gradient

elution to afford mono-Bhc-caged C4-ceramide **8a** (10.4 mg, 54%) and di-Bhc-caged C4-ceramide **8b** (4.0 mg, 14%).



8a

8a: R_f 0.36 (hexane/EtOAc 1:1); $[\alpha]_D^{29}$ -0.6 (*c* 0.58 in $CHCl_3$/MeOH 4:1); ^1H NMR (400 MHz; $CDCl_3$) δ_H 0.88 (3H, t, *J* = 6.8 Hz), 0.95 (3H, t, *J* = 7.4 Hz), 1.25 (20H, m), 1.33 (2H, m), 1.66 (2H, m), 2.05 (2H, m), 2.20 (2H, t, *J* = 7.4 Hz), 2.50 (1H, br), 3.52 (3H, s), 4.26 (2H, m), 4.35 (1H, dd, J_1 = 11.2 Hz, J_2 = 3.4 Hz), 4.47 (1H, dd, J_1 = 11.2 Hz, J_2 = 5.6 Hz), 5.28 (2H, m), 5.32 (2H, s), 5.51 (1H, dd, J_1 = 15.4 Hz, J_2 = 6.5 Hz), 5.78 (1H, dt, J_1 = 15.4 Hz, J_2 = 6.7 Hz), 5.91 (1H, d, *J* = 8.0 Hz), 6.43 (1H, s), 7.17 (1H, s), 7.67 (1H, s); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 13.8, 14.3, 19.2, 22.8, 29.2, 29.4, 29.5, 29.6, 29.8, 32.1, 32.4, 38.8, 52.6, 56.9, 64.4, 67.4, 73.1, 95.3, 104.2, 108.8, 111.8, 112.3, 127.5, 128.4, 135.4, 147.4, 154.3, 154.5, 156.6, 160.1, 173.5; HRMS $[M+H]^+$ *m/z* calcd for $C_{35}H_{53}{}^{79}BrNO_9$ 710.2898, found 710.2901.

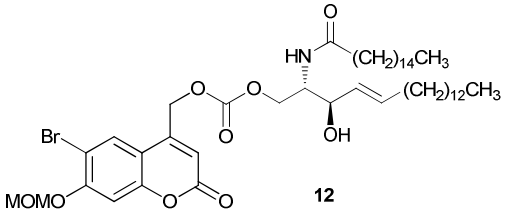


8b

8b: R_f 0.38 (hexane:EtOAc 1:1); $[\alpha]_D^{28}$ -9.2 (*c* 0.59 in $CHCl_3$/MeOH 4:1); ^1H NMR (400 MHz; $CDCl_3$) δ_H 0.87 (3H, t, *J* = 6.8 Hz), 0.94 (3H, t, *J* = 7.4 Hz), 1.24 (20H, m), 1.35 (2H, m), 1.66 (2H, m), 2.06

(2H, m), 2.19 (2H, t, J = 7.4 Hz), 3.52 (6H, s), 4.27 (1H, m), 4.54 (1H, m), 4.56 (1H, m), 5.20 (1H, m), 5.27 (2H, m), 5.28 (2H, m), 5.32 (4H, s), 5.47 (1H, dd, J_1 = 15.4 Hz, J_2 = 8.0 Hz), 5.81 (1H, d, J = 8.6 Hz), 5.94 (1H, dt, J_1 = 15.3 Hz, J_2 = 6.8 Hz), 6.40 (1H, s), 6.42 (1H, s), 7.15 (1H, s), 7.16 (1H, s), 7.67 (1H, s), 7.68 (1H, s); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 13.8, 14.3, 19.2, 22.8, 28.9, 29.4, 29.5, 29.6, 29.8, 32.1, 32.5, 38.7, 50.3, 56.9, 64.5, 64.7, 66.8, 78.8, 95.3, 104.2, 108.8, 111.8, 112.3, 123.2, 127.6, 139.8, 147.3, 147.4, 153.7, 154.3, 154.4, 156.6, 160.0, 172.9; HRMS $[M+H]^+$ m/z calcd for $C_{48}H_{62}{}^{79}Br_2NO_{15}$ 1050.2481, found 1050.2470.

(2*S*,3*R*,4*E*)-2-Palmitoylamido-3-hydroxyoctadec-4-enyl (6-bromo-7-*O*-(methoxymethyl)coumarin-4-yl)methyl carbonate (12).

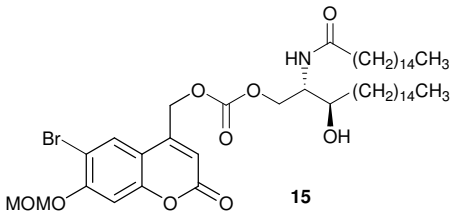


12

To a mixture of C16-ceramide **11** (*N*-palmitoyl-D-*erythro*-sphingosine (d18:1/16:0), 10 mg, 19 μmol) and 6-bromo-7-*O*-(methoxymethyl)coumarin-4-ylmethoxycarbonyl chloride **6** (7.7 mg, 20 μmol) in dry THF/CH_2Cl_2 (2:1, 3.0 mL) were added DIPEA (3.6 μL, 20 μmol) and DMAP (2.3 mg, 19 μmol). The reaction mixture was stirred at room temperature for 36 h and then was concentrated *in vacuo*. Purification of the residue in a Biotage Isolera One flash purification system with hexane/EtOAc gradient elution afforded mono-Bhc-caged C16-ceramide **12** (4.1 mg, 25%): R_f 0.33 (hexane/EtOAc

2:1); $[\alpha]_D^{27}$ +11.0 (*c* 0.31 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 0.88 (6H, t, *J* = 6.8 Hz), 1.25 (46H, m), 1.33 (2H, m), 1.62 (2H, m), 2.04 (2H, m), 2.21 (2H, t, *J* = 7.6 Hz), 2.37 (1H, br), 3.52 (3H, s), 4.25 (2H, m), 4.35 (1H, dd, J_1 = 11.2 Hz, J_2 = 3.4 Hz), 4.48 (1H, dd, J_1 = 11.2 Hz, J_2 = 5.4 Hz), 5.28 (2H, m), 5.32 (2H, s), 5.51 (1H, dd, J_1 = 15.4 Hz, J_2 = 6.5 Hz), 5.78 (1H, dt, J_1 = 15.3 Hz, J_2 = 6.7 Hz), 5.84 (1H, d, *J* = 8.0 Hz), 6.43 (1H, s), 7.18 (1H, s), 7.67 (1H, s); ^{13}C NMR (125 MHz; $CDCl_3$) δ_C 14.3, 22.8, 25.8, 29.4, 29.5, 29.7, 29.8, 32.1, 32.4, 37.0, 52.6, 56.9, 64.5, 67.4, 73.1, 95.3, 104.2, 108.8, 111.8, 112.3, 127.5, 128.4, 135.5, 147.4, 154.3, 154.5, 156.6, 160.1, 173.7; HRMS $[M+H]^+$ *m/z* calcd for $C_{47}H_{77}{}^{79}BrNO_9$ 878.4776, found 878.4772.

(2*S*,3*R*)-2-Palmitoylamido-3-hydroxyoctadecyl (6-bromo-7-*O*-(methoxymethyl)coumarin-4-yl)methyl carbonate (15).



15

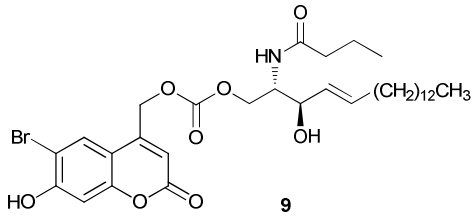
C16-Dihydroceramide **14** (*N*-palmitoyl-D-*erythro*-sphingosine (d18:0/16:0), 10 mg, 19 μmol) was converted to compound **15** (4.4 mg, 27%) using the same procedure as described for compound **12**: R_f 0.34 (hexane/EtOAc 2:1); $[\alpha]_D^{29}$ +11.4 (*c* 0.99 in $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 0.88 (6H, t, *J* = 6.7 Hz), 1.25 (50H, m), 1.52 (2H, m), 1.63 (2H, m), 2.23 (2H, m), 2.47 (1H, br), 3.52 (3H, s), 4.15 (1H, m), 4.26 (1H, m), 4.38 (1H, dd, J_1 = 11.3 Hz, J_2 = 3.3 Hz), 4.51 (1H, m), 5.28 (2H, m), 5.32 (2H, s),

6.01 (1H, d, *J* = 8.5 Hz), 6.43 (1H, s), 7.17 (1H, s), 7.67 (1H, s); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 14.3, 22.8, 25.9, 29.5, 29.6, 29.7, 29.8, 32.1, 34.4, 40.0, 52.6, 56.9, 64.5, 67.6, 68.4, 72.4, 83.3, 95.3, 104.2, 108.8, 111.8, 112.3, 127.5, 147.4, 154.3, 154.6, 156.7, 160.1, 173.6; HRMS $[M+H]^+$ *m/z* calcd for $C_{47}H_{79}{}^{79}BrNO_9$ 880.4933, found 880.4941.

General Procedure for Removal of the MOM Protecting Group to Generate 6-Bromo-7-hydroxycoumarin-4-ylmethyl (Bhc) Caged Ceramides (9, 10, 13, and 16).

To a solution of 4 μmol of MOM-Bhc-caged ceramide (**8a**, **8b**, **12**, or **15**) in 2.0 mL of dry CH_2Cl_2 was added 3 mg of silica-supported sodium hydrogen sulfate ($NaHSO_4{\cdot}SiO_2$) at room temperature. The reaction mixture was stirred at room temperature until the starting material was no longer observed (~2 h, as monitored by TLC). The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC ($CHCl_3$/MeOH 9:1) to afford the corresponding product, which was dissolved in $CHCl_3$ and passed through a 0.45-μm syringe filter to remove the suspended silica gel.

(2*S*,3*R*,4*E*)-2-Butyramido-3-hydroxyoctadec-4-enyl (6-bromo-7-hydroxycoumarin-4-yl)methyl carbonate (9).

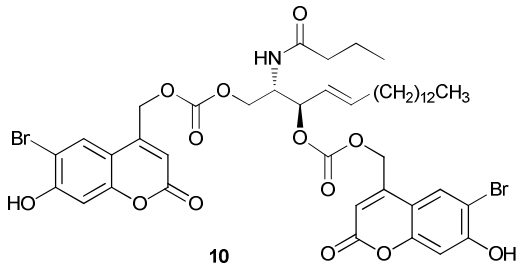


9

Yield 73%; UV ($CHCl_3$/EtOH 4:1) λ_{max} 332 nm; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 0.88 (3H, t, *J* = 6.8

Hz), 0.95 (3H, t, J = 7.4 Hz), 1.25 (20H, m), 1.34 (2H, m), 1.66 (2H, m), 2.05 (2H, m), 2.21 (2H, t, J = 7.4 Hz), 2.31 (1H, br), 4.27 (2H, m), 4.37 (1H, dd, J_1 = 11.3 Hz, J_2 = 3.5 Hz), 4.45 (1H, dd, J_1 = 11.3 Hz, J_2 = 5.9 Hz), 5.26 (2H, m), 5.51 (1H, dd, J_1 = 15.4 Hz, J_2 = 6.6 Hz), 5.79 (1H, dt, J_1 = 15.4 Hz, J_2 = 6.8 Hz), 5.88 (1H, d, J = 8.2 Hz), 6.40 (1H, s), 7.02 (1H, s), 7.61 (1H, s); HRMS $[M+H]^+$ *m/z* calcd for $C_{33}H_{49}{}^{79}BrNO_8$ 666.2636, found 666.2644.

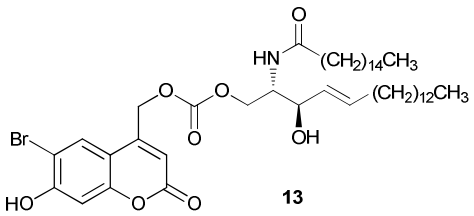
(2*S*,3*R*,4*E*)-2-Butyramidooctadec-4-ene-1,3-diyl bis(6-bromo-7-hydroxycoumarin-4-yl)methyl dicarbonate (10).



10

Yield 68%; UV ($CHCl_3$/EtOH 4:1) λ_{max} 332 nm; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 0.86 (3H, m), 0.93 (3H, m), 1.25 (20H, m), 1.35 (2H, m), 1.66 (2H, m), 2.02 (2H, m), 2.19 (2H, m), 4.15 (1H, m), 4.57 (2H, m), 4.84 (1H, m), 5.26 (4H, m), 5.36 (1H, m), 5.74 (1H, m), 5.90 (1H, m), 6.39 (1H, s), 6.41 (1H, s), 7.00 (1H, s), 7.02 (1H, s), 7.65 (1H, s), 7.67 (1H, s); HRMS $[M-H]^-$ *m/z* calcd for $C_{44}H_{52}{}^{79}Br_2NO_{13}$ 960.1811, found 960.1819.

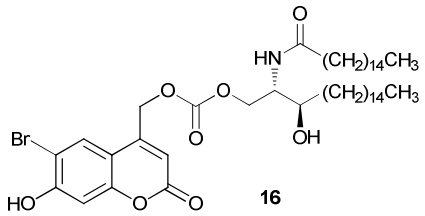
(2*S*,3*R*,4*E*)-2-Palmitoylamido-3-hydroxyoctadec-4-enyl (6-bromo-7-hydroxycoumarin-4-yl)methyl carbonate (13).



13

Yield 70%; UV ($CHCl_3$/EtOH 4:1) λ_{max} 332 nm; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 0.88 (6H, t, J = 6.8 Hz), 1.25 (46H, m), 1.33 (2H, m), 1.62 (2H, m), 2.04 (2H, m), 2.20 (2H, m), 4.24 (2H, m), 4.35 (1H, dd, J_1 = 11.3 Hz, J_2 = 3.4 Hz), 4.47 (1H, dd, J_1 = 11.3 Hz, J_2 = 5.5 Hz), 5.26 (2H, m), 5.50 (1H, dd, J_1 = 15.5 Hz, J_2 = 6.6 Hz), 5.68 (1H, m), 5.85 (1H, d, J = 8.3 Hz), 6.40 (1H, s), 7.02 (1H, s), 7.61 (1H, s); HRMS $[M-H]^-$ *m/z* calcd for $C_{45}H_{71}{}^{79}BrNO_8$ 832.4363, found 832.4362.

(2*S*,3*R*)-2-Palmitoylamido-3-hydroxyoctadecyl(6-bromo-7-hydroxycoumarin-4-yl)methyl carbonate (16).



16

Yield 72%; UV ($CHCl_3$/EtOH 4:1) λ_{max} 332 nm; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 0.88 (6H, t, J = 6.4 Hz), 1.25 (50H, m), 1.52 (2H, m), 1.63 (2H, m), 2.22 (2H, t, J = 7.8 Hz), 4.15 (1H, m), 4.39 (1H, dd, J_1 = 11.4 Hz, J_2 = 3.2 Hz), 4.43 (1H, m), 4.49 (1H, dd, J_1 = 11.5 Hz, J_2 = 5.2 Hz), 5.26 (2H, m), 5.95 (1H,

d, J = 8.2 Hz), 6.40 (1H, s), 7.02 (1H, s), 7.62 (1H, s); HRMS $[M-H]^-$ *m/z* calcd for $C_{45}H_{73}{}^{79}BrNO_8$ 834.4525, found 834.4515.

Preparation of silica-supported sodium hydrogen sulfate ($NaHSO_4 \cdot SiO_2$) catalyst.

A solution of sodium hydrogen sulfate monohydrate ($NaHSO_4 \cdot H_2O$, 1.04 g, 7.53 mmol) in water (5 mL) was treated with 2.50 g of silica gel (SiO_2, 230-400 mesh, grade for column chromatography). The resulting mixture was stirred for 10 min and then was gently heated on a hot plate until the water was removed, providing $NaHSO_4 \cdot SiO_2$ as a free-flowing powder.[S2] The catalyst was further dried and activated in an oven at 120 ^oC for 48 h before use.

Uptake of compound 16 into J774 macrophages.

Mouse macrophages from the J774A.1 cell line were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics at 37 ^oC in a 5% CO_2/95% air incubator. After the macrophages were incubated with 20 μM **16** in KMops containing 5% EtOH for 2 h in the dark with shaking, the cells were gently washed with buffer and UV-irradiated for the time period indicated and imaged using a fluorescence microscope.

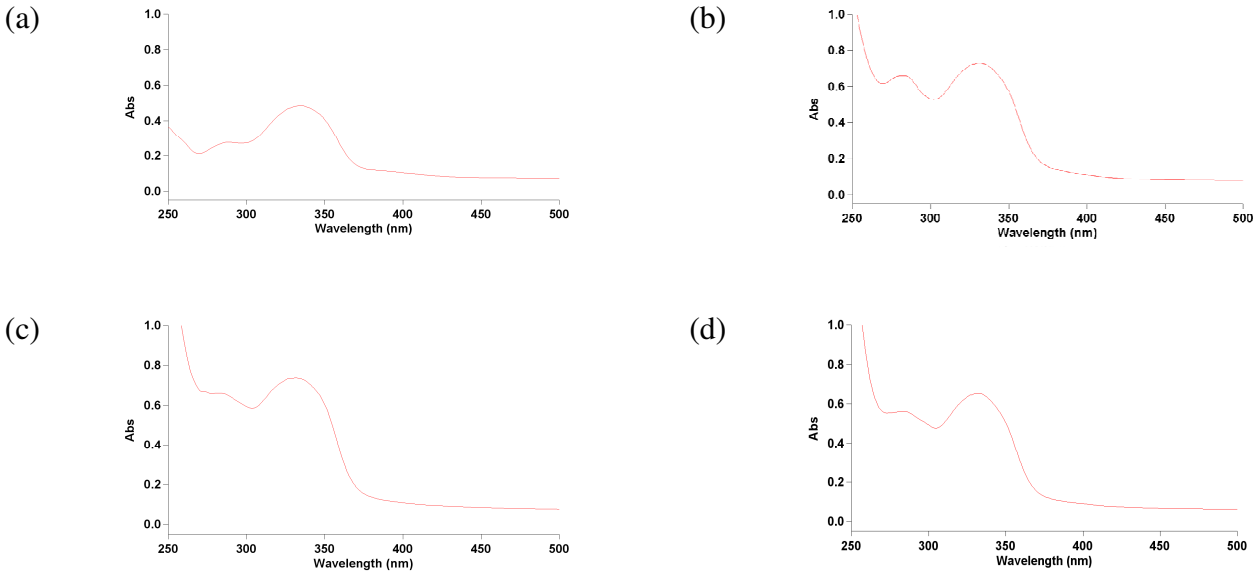
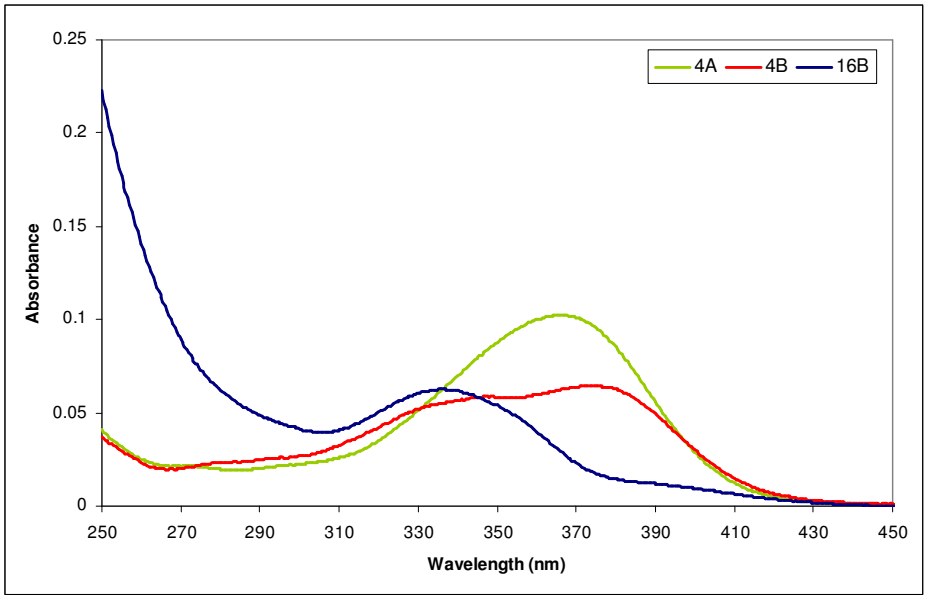


Fig. S1 UV-visible absorption spectra of Bhc-caged ceramides in $CHCl_3$/EtOH (4:1). (a) compound **9**, (b) compound **10**, (c) compound **13**, and (d) compound **16**.



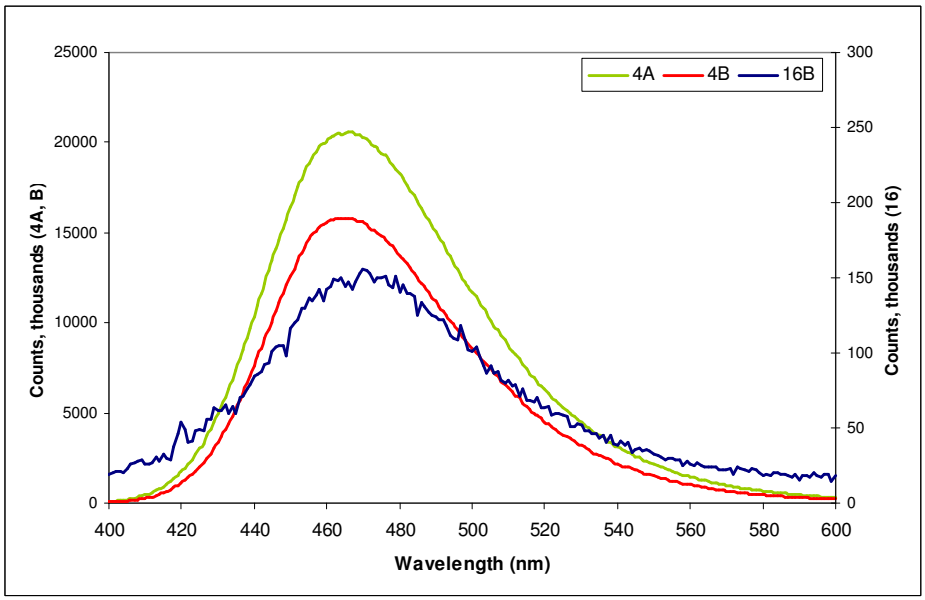


Fig. S2 Absorption (top) and fluorescence emission (bottom) spectra of **4** in KMops buffer, pH 7.4, (4A), and **4** and **16** in KMops containing 50% EtOH (4B and 16B, respectively).

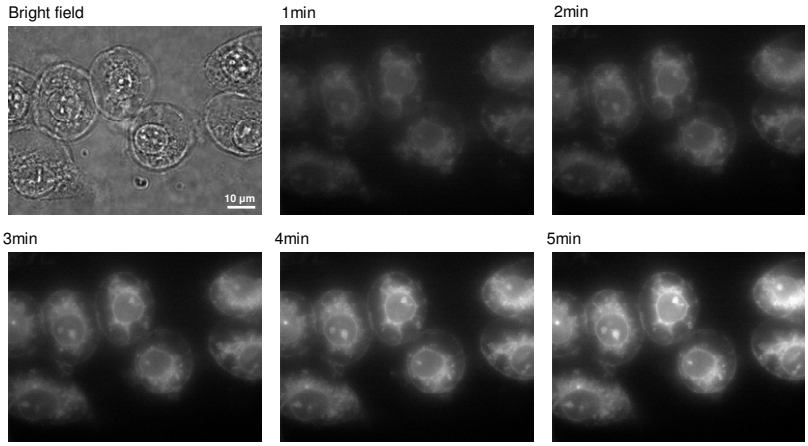


Fig. S3 Uptake of compound **16** by macrophages and time course of photorelease of **4** upon photolysis. Bhc-caged C16-dihydroceramide (20 µM) was incubated with J774 macrophages in KMops buffer (pH 7.4) containing 5% EtOH final concentration (v/v) for 2 h in the dark with continuous shaking. Then the cells were gently washed with buffer and UV-irradiated at 350 nm for the time period indicated and imaged using a fluorescence microscope in the epifluorescence mode. All of the images are background-corrected and displayed on the same intensity scale with respect to the other images in the figure. Similar results were obtained with a lower concentration of **16** (5 µM).

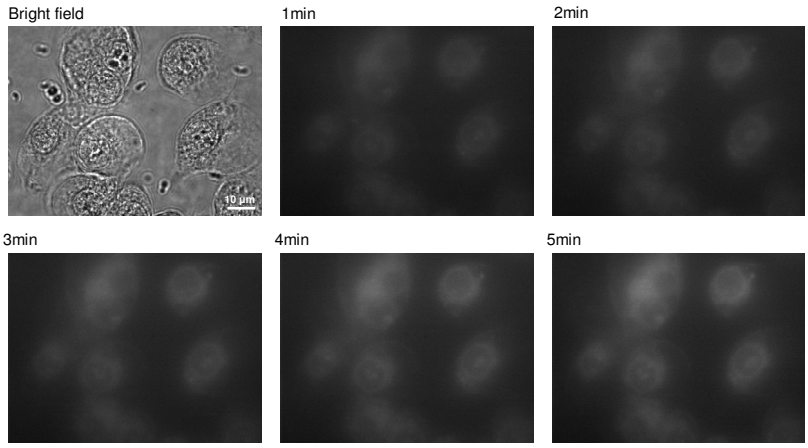


Fig. S4 UV irradiation of J774 macrophages in the absence of **16** (control). Cells were incubated in KMops buffer, pH 7.4 (5% EtOH final concentration, v/v), in the absence of any Bhc-caged compound, and then UV-irradiated at 350 nm for the time periods indicated. All of the images are background-corrected and displayed on the same intensity scale with respect to the other images in the figure.

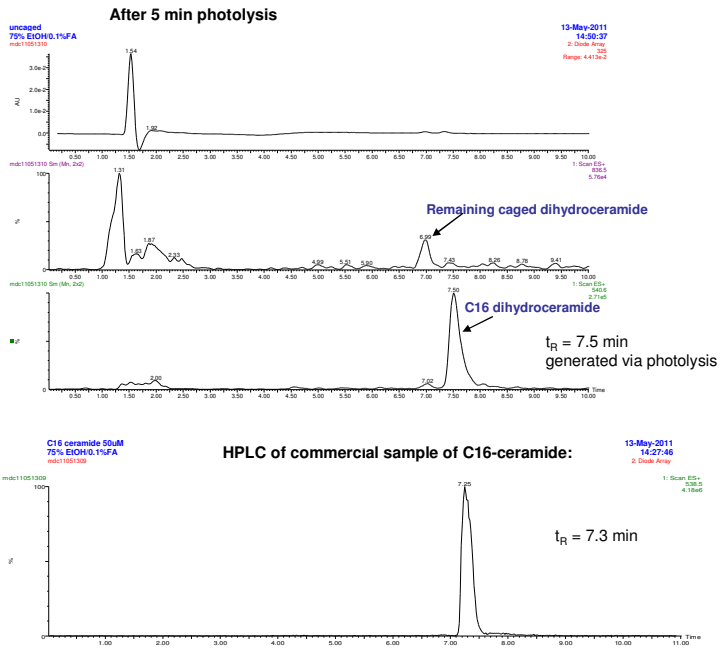
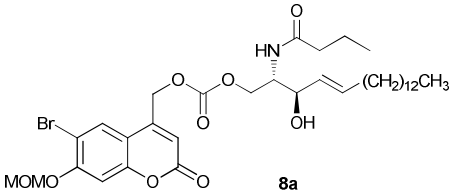


Fig. S5 Release of C16-dihydroceramide from **16** on photolysis at 350 nm. Compound **16** was photolyzed for 5 min in KMops buffer (pH 7.4) containing 50% EtOH. The chromatograms show the HPLC/MS analysis for the uncaging of compound **16** to afford C16-dihydroceramide. Eluent: 75% EtOH, 24.9% H_2O, 0.1% HCO_2H; flow rate, 0.2 mL/min; t_R of C16-dihydroceramide generated by photolysis, 7.5 min; t_R of a commercial sample of C16-ceramide, 7.3 min. MS $[M+H]^+$ *m/z* calcd for $C_{34}H_{70}NO_3^+$ 540.5, found 540.5.

References

S1 G. Jones II, W. R. Jackson and A. M. Halpern, *Chem. Phys. Lett.*, 1980, **72**, 391.

S2 (a) G.W. Breton, *J. Org. Chem.*, 1997, **62**, 8952; (b) T. Nishiguchi and C. Kamio, *J. Chem. Soc., Perkin Trans. 1*, 1989, 707.



8a

Data File	QCBBBB1033A.d	**Sample Name**	YAK-2-125-A
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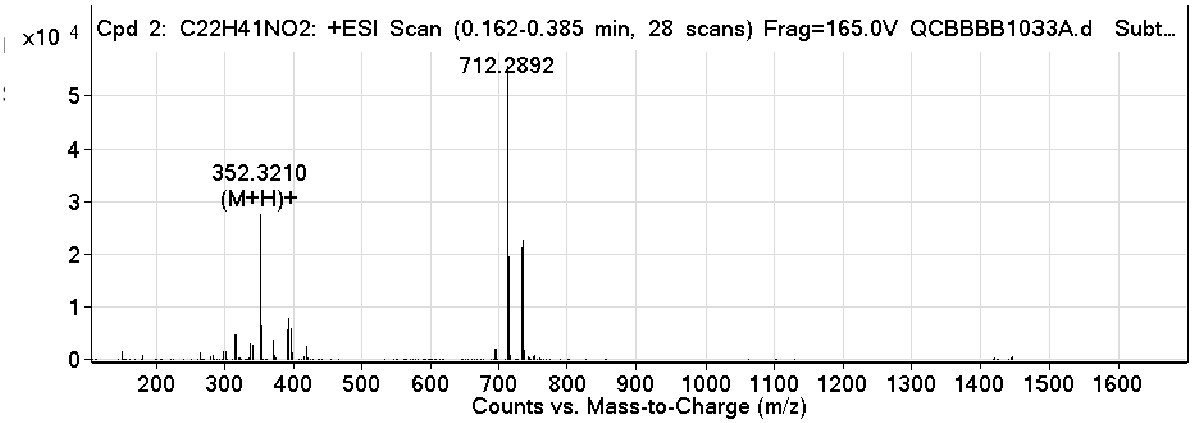
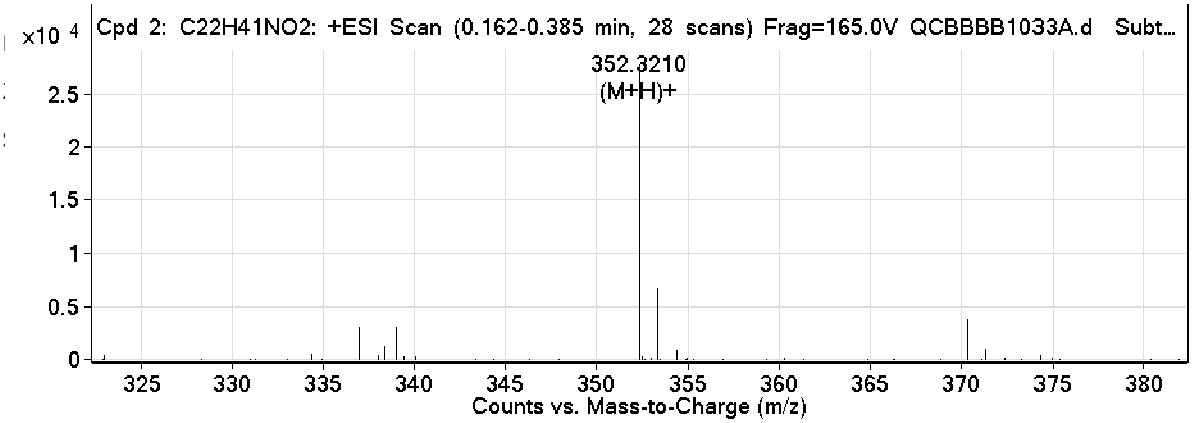
Acq Method		**Acquired Time**	11/4/2010 2:10:26 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=709.2825 EM=HC HC ESI Pos Small Molecule No HPLC.m modified		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 2: C22H41NO2	0.228	351.3137	27816	C22H41NO2	351.3137	-0.07
Cpd 1: C35H52BrNO9	0.228	709.283	70492	C35H52BrNO9	709.2825	0.69

Compound Label	RT	Algorithm	Mass
Cpd 2: C22H41NO2	0.228	Find By Formula	351.3137



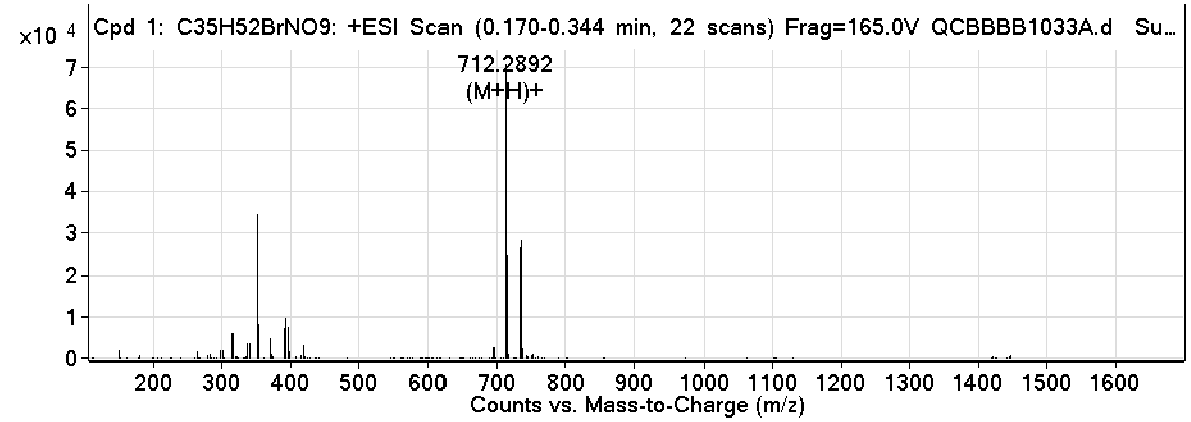


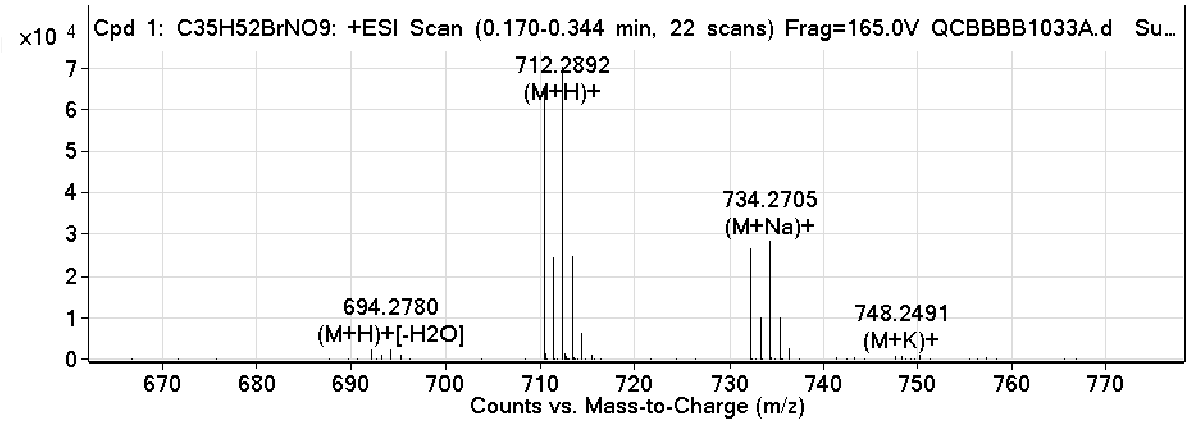
MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
352.321	352.321	-0.07	1	27816	C22 H42 N O2	(M+H)+
353.3242	353.3243	-0.39	1	6768	C22 H42 N O2	(M+H)+
354.3261	354.3274	-3.66	1	1041	C22 H42 N O2	(M+H)+
710.2901				51228		
710.5246				1069		
m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
711.2933				19367		
711.5324				432		
712.2892				55812		
712.5236				1333		
712.6349				487		
713.2917				19709		
713.526				611		
713.6711				372		
714.2944				5155		
715.2997				1052		

Compound Label	RT	Algorithm	Mass
Cpd 1: C35H52BrNO9	0.228	Find By Formula	709.283

MS Spectrum





MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
692.2796	692.2793	0.47	1	2717	C35 H51 Br N O8	(M+H)+[-H2O]
694.278	694.2779	0.07	1	2737	C35 H51 Br N O8	(M+H)+[-H2O]
710.2901	710.2898	0.43		64687	C35 H53 Br N O9	(M+H)+
710.5245				1360		
711.2933	711.2932	0.21		24440	C35 H53 Br N O9	(M+H)+
712.2892	712.2885	1.01		70492	C35 H53 Br N O9	(M+H)+
712.5239				1692		
m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
713.2917	713.2914	0.41		24895	C35 H53 Br N O9	(M+H)+
714.2949	714.2942	1.05		6478	C35 H53 Br N O9	(M+H)+
732.2716	732.2718	-0.26	1	26865	C35 H52 Br N Na O9	(M+Na)+
733.2752	733.2751	0.11	1	10142	C35 H52 Br N Na O9	(M+Na)+
734.2705	734.2704	0.07	1	28693	C35 H52 Br N Na O9	(M+Na)+
735.2737	735.2734	0.41	1	10369	C35 H52 Br N Na O9	(M+Na)+
736.2765	736.2761	0.51	1	2804	C35 H52 Br N Na O9	(M+Na)+
748.2491	748.2457	4.6	1	1067	C35 H52 Br K N O9	(M+K)+

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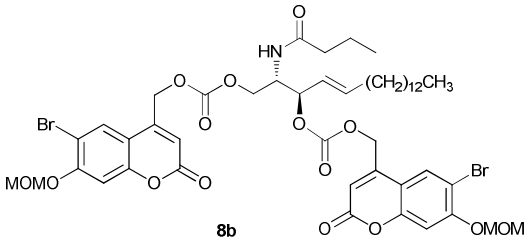


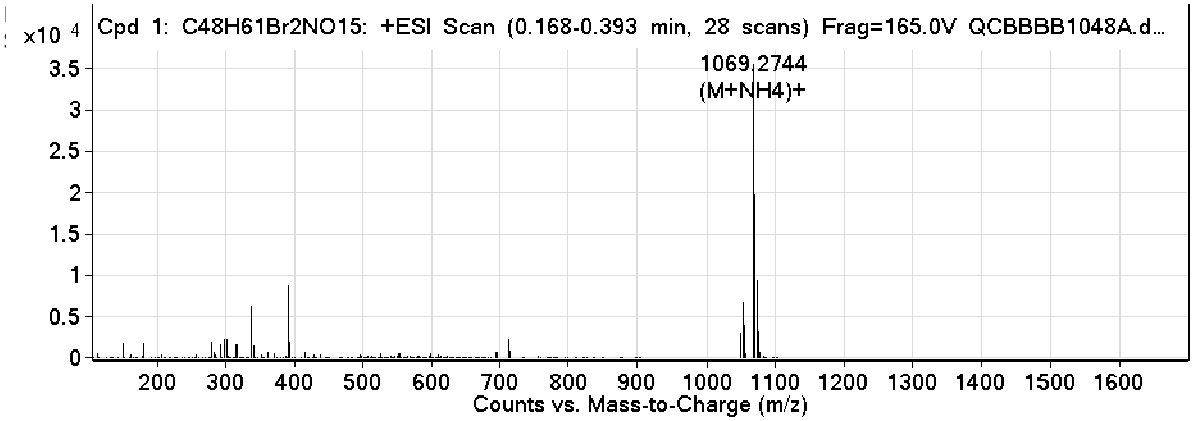
8b

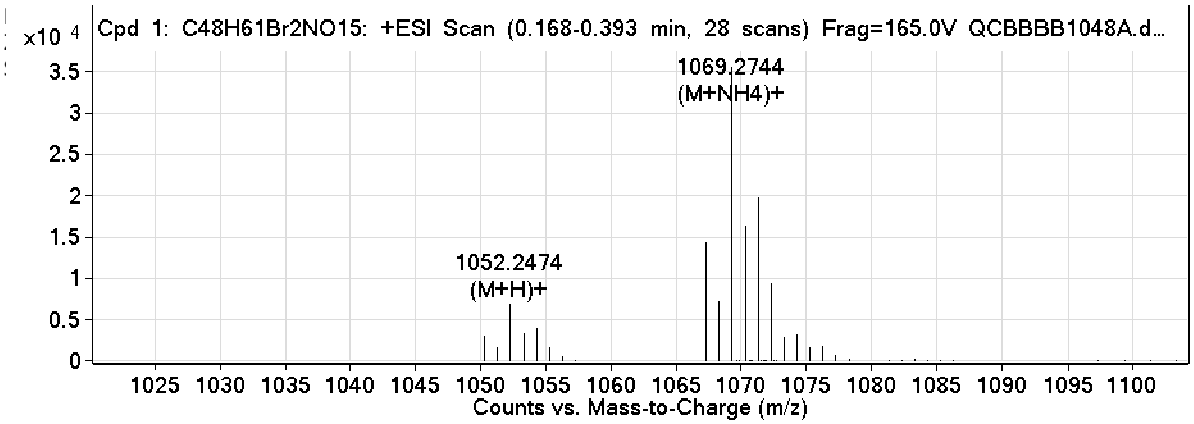
Data File	QCBBBB1048A.d	**Sample Name**	YAK-3-3-B
Sample Type	Sample	**Position**	P1-A2
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	11/24/2010 10:08:47 AM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=1049.2408 EM=HC HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C48H61Br2NO15	0.235	1049.2417	35498	C48H61Br2NO15	1049.2408	0.89

Compound Label	RT	Algorithm	Mass
Cpd 1: C48H61Br2NO15	0.235	Find By Formula	1049.2417

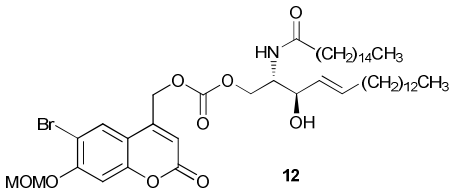




MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
1050.247	1050.2481	-0.99	1	3053	C48 H62 Br2 N O15	(M+H)+
1052.2474	1052.2467	0.65	1	6838	C48 H62 Br2 N O15	(M+H)+
1053.2496	1053.2497	-0.04	1	3387	C48 H62 Br2 N O15	(M+H)+
1054.2465	1054.2462	0.23	1	3952	C48 H62 Br2 N O15	(M+H)+
1055.2479	1055.2484	-0.52	1	1790	C48 H62 Br2 N O15	(M+H)+
m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
1067.2749	1067.2746	0.28		14429	C48 H65 Br2 N2 O15	(M+NH4)+
1068.2782	1068.2779	0.26		7296	C48 H65 Br2 N2 O15	(M+NH4)+
1069.2744	1069.2733	1.12		35498	C48 H65 Br2 N2 O15	(M+NH4)+
1070.2766	1070.2762	0.42		16409	C48 H65 Br2 N2 O15	(M+NH4)+
1071.2734	1071.2728	0.56		19849	C48 H65 Br2 N2 O15	(M+NH4)+
1072.2704	1072.2749	-4.26		9408	C48 H65 Br2 N2 O15	(M+NH4)+
1073.2685	1073.2774	-8.31		2942	C48 H65 Br2 N2 O15	(M+NH4)+
1074.2338	1074.2286	4.79		3319	C48 H61 Br2 N Na O15	(M+Na)+
1075.2343	1075.2316	2.45	1	1679	C48 H61 Br2 N Na O15	(M+Na)+
1076.2278	1076.2282	-0.38	1	1848	C48 H61 Br2 N Na O15	(M+Na)+

--- End Of Report ---



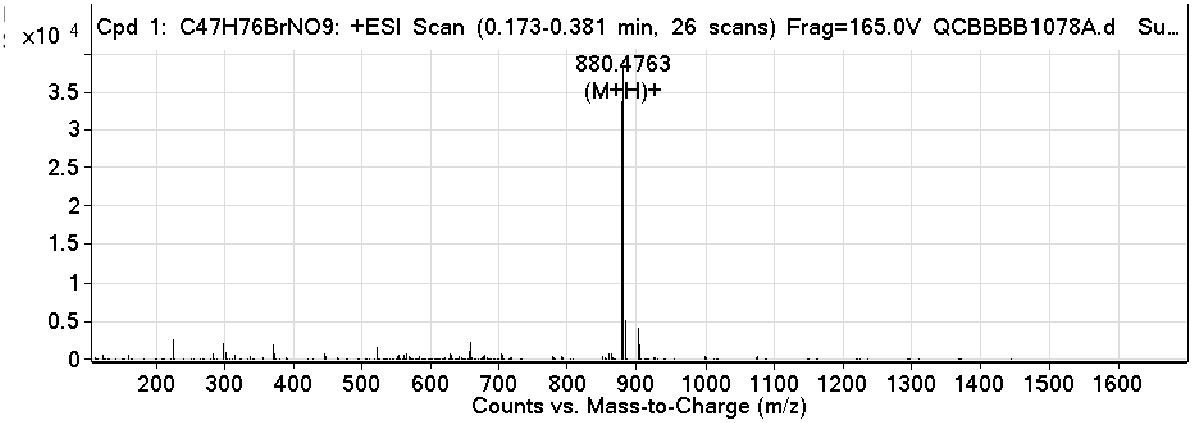
12

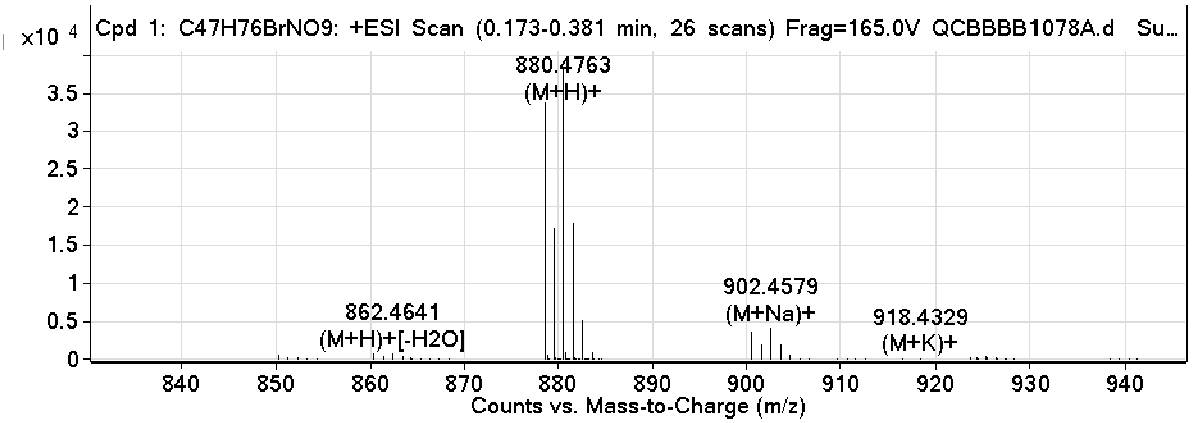
Data File	QCBBBB1078A.d	**Sample Name**	YAK-3-97
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	3/8/2011 4:56:46 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=877.4703 M=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C47H76BrNO9	0.231	877.4699	38608	C47H76BrNO9	877.4703	-0.53

Compound Label	RT	Algorithm	Mass
Cpd 1: C47H76BrNO9	0.231	Find By Formula	877.4699

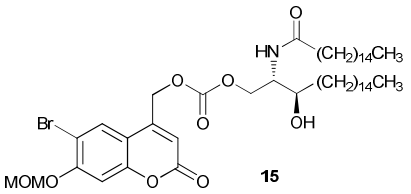




MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
860.4649	860.4671	-2.5	1	768	C47 H75 Br N O8	(M+H)+[-H2O]
862.4641	862.4662	-2.43	1	847	C47 H75 Br N O8	(M+H)+[-H2O]
878.4772	878.4776	-0.52		33882	C47 H77 Br N O9	(M+H)+
879.4803	879.481	-0.77		17432	C47 H77 Br N O9	(M+H)+
880.4763	880.4767	-0.49		38608	C47 H77 Br N O9	(M+H)+
880.7381				967		
m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
881.4788	881.4794	-0.73		17926	C47 H77 Br N O9	(M+H)+
882.4811	882.4823	-1.29		5170	C47 H77 Br N O9	(M+H)+
883.4844	883.4851	-0.81		1144	C47 H77 Br N O9	(M+H)+
900.4588	900.4596	-0.84	1	3842	C47 H76 Br N Na O9	(M+Na)+
901.4622	901.4629	-0.81	1	1991	C47 H76 Br N Na O9	(M+Na)+
902.4579	902.4587	-0.9	1	4205	C47 H76 Br N Na O9	(M+Na)+
903.4603	903.4614	-1.22	1	2057	C47 H76 Br N Na O9	(M+Na)+
904.4634	904.4642	-0.88	1	664	C47 H76 Br N Na O9	(M+Na)+
916.4351	916.4335	1.8	1	133	C47 H76 Br K N O9	(M+K)+

--- End Of Report ---



15

Data File	QCBBBB1069A.d	**Sample Name**	YAK-3-71-A
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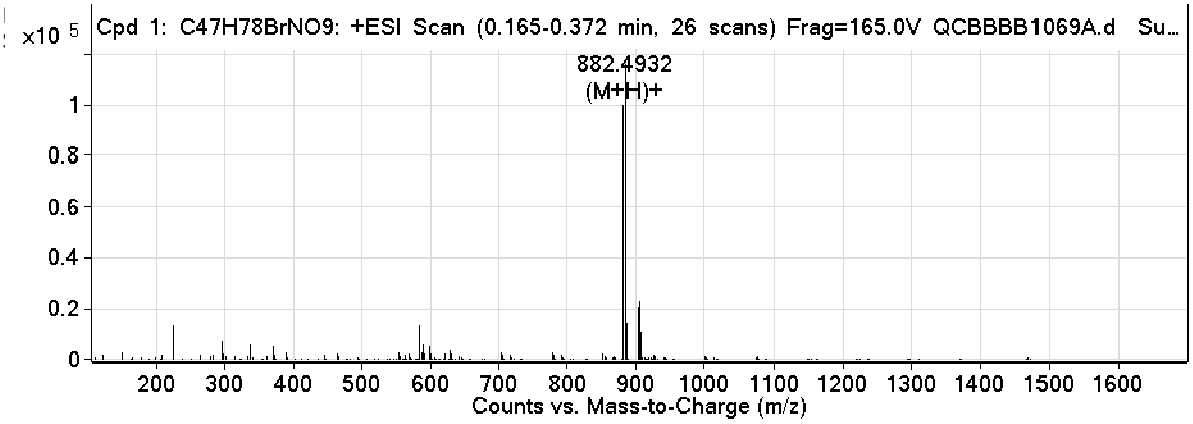
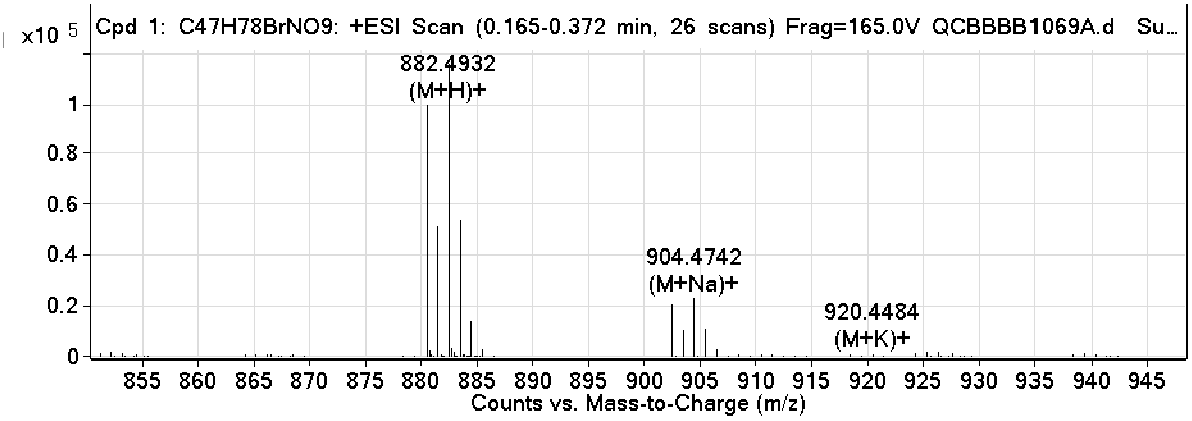
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=879.4860 M=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C47H78BrNO9	0.231	879.4867	115872	C47H78BrNO9	879.486	0.84

Compound Label	RT	Algorithm	Mass
Cpd 1: C47H78BrNO9	0.231	Find By Formula	879.4867

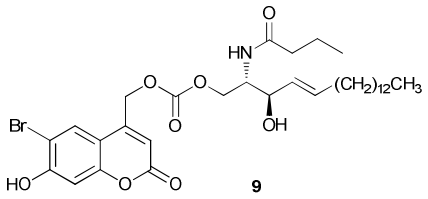




MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
880.4941	880.4933	0.89		100528	C47 H79 Br N O9	(M+H)+
880.7491				2458		
881.4972	881.4966	0.62		51340	C47 H79 Br N O9	(M+H)+
882.4932	882.4924	0.87		115872	C47 H79 Br N O9	(M+H)+
882.7505				3482		
882.8697				1755		
m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
883.4956	883.4951	0.64		53800	C47 H79 Br N O9	(M+H)+
884.498	884.4979	0.06		14673	C47 H79 Br N O9	(M+H)+
885.5005	885.5008	-0.3		3243	C47 H79 Br N O9	(M+H)+
902.4748	902.4752	-0.48	1	20760	C47 H78 Br N Na O9	(M+Na)+
903.4785	903.4786	-0.12	1	10688	C47 H78 Br N Na O9	(M+Na)+
904.4742	904.4743	-0.15	1	23276	C47 H78 Br N Na O9	(M+Na)+
905.4766	905.477	-0.44	1	11335	C47 H78 Br N Na O9	(M+Na)+
906.479	906.4799	-0.97	1	3136	C47 H78 Br N Na O9	(M+Na)+
918.4506	918.4492	1.63	1	1049	C47 H78 Br K N O9	(M+K)+

--- End Of Report ---



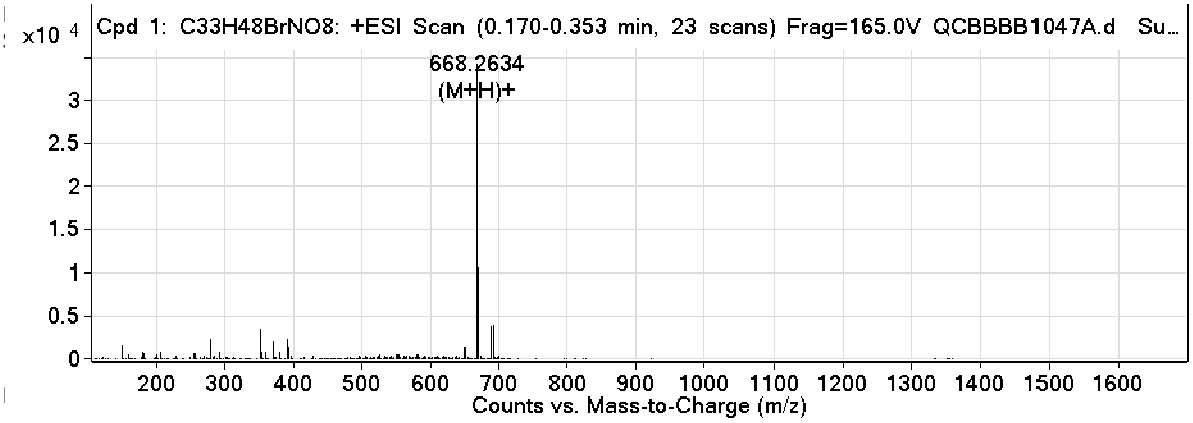
9

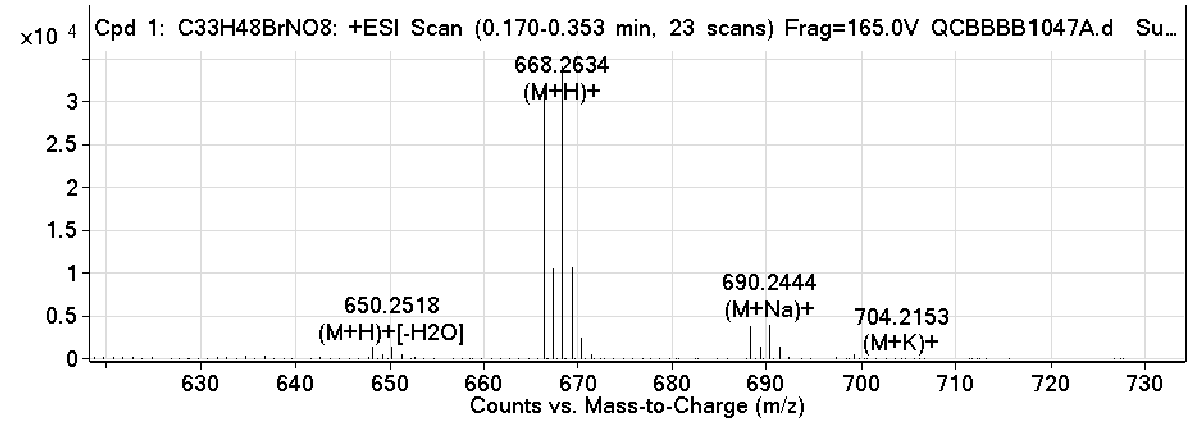
Data File	QCBBBB1047A.d	**Sample Name**	YAK-3-27
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	11/24/2010 9:57:25 AM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=665.2563 EM=HC HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C33H48BrNO8	0.228	665.2573	34225	C33H48BrNO8	665.2563	1.47

Compound Label	RT	Algorithm	Mass
Cpd 1: C33H48BrNO8	0.228	Find By Formula	665.2573

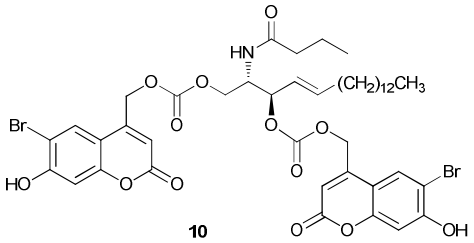




MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
648.2534	648.253	0.6	1	1438	C33 H47 Br N O7	(M+H)+[-H2O]
649.2578	649.2564	2.23	1	564	C33 H47 Br N O7	(M+H)+[-H2O]
650.2518	650.2516	0.24	1	1542	C33 H47 Br N O7	(M+H)+[-H2O]
651.2556	651.2546	1.53	1	542	C33 H47 Br N O7	(M+H)+[-H2O]
666.2644	666.2636	1.16		31583	C33 H49 Br N O8	(M+H)+
667.2673	667.267	0.58		10627	C33 H49 Br N O8	(M+H)+
m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
668.2634	668.2622	1.75		34225	C33 H49 Br N O8	(M+H)+
669.2657	669.2652	0.83		10830	C33 H49 Br N O8	(M+H)+
670.2697	670.2679	2.62		2634	C33 H49 Br N O8	(M+H)+
671.2729	671.2706	3.31		505	C33 H49 Br N O8	(M+H)+
688.246	688.2456	0.6	1	3834	C33 H48 Br N Na O8	(M+Na)+
689.2494	689.2489	0.67	1	1493	C33 H48 Br N Na O8	(M+Na)+
690.2444	690.2442	0.37	1	4122	C33 H48 Br N Na O8	(M+Na)+
691.2474	691.2471	0.33	1	1451	C33 H48 Br N Na O8	(M+Na)+
704.2153	704.2195	-5.95	1	130	C33 H48 Br K N O8	(M+K)+

--- End Of Report ---



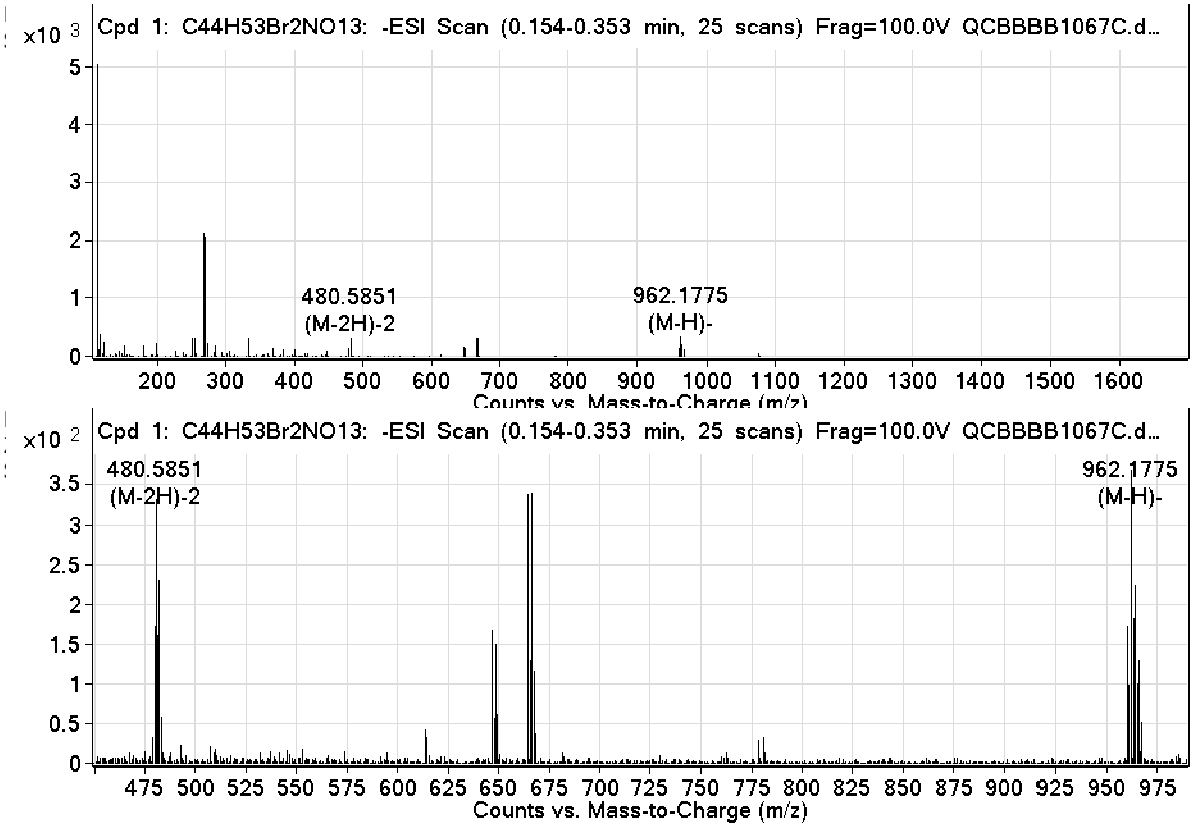
10

Data File	QCBBBB1067C.d	**Sample Name**	YAK-3-61
Sample Type	Sample	**Position**	P1-A6
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	1/4/2011 12:55:40 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=961.1884 EM=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C44H53Br2NO13	0.237	961.1867	334	C44H53Br2NO13	961.1884	-1.71

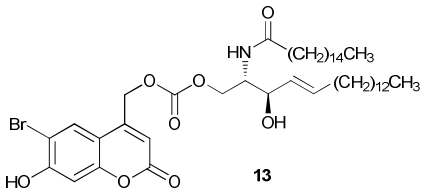
Compound Label	RT	Algorithm	Mass
Cpd 1: C44H53Br2NO13	0.237	Find By Formula	961.1867



MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
112.9857				5060		
113.9891				125		
114.9975				84		
117.0193				417		
479.5849	479.5869	-4.12	-2	173	C44 H51 Br2 N O13	(M-2H)-2
m/z	***Calc m/z***	**Diff(ppm)**	**z**	**Abund**	**Formula**	**Ion**
480.5851	480.5862	-2.24	-2	334	C44 H51 Br2 N O13	(M-2H)-2
481.0865	481.0877	-2.56	-2	161	C44 H51 Br2 N O13	(M-2H)-2
481.5847	481.5858	-2.37	-2	232	C44 H51 Br2 N O13	(M-2H)-2
482.0843	482.087	-5.65	-2	81	C44 H51 Br2 N O13	(M-2H)-2
960.1819	960.1811	0.83	-1	174	C44 H52 Br2 N O13	(M-H)-
961.181	961.1844	-3.57	-1	100	C44 H52 Br2 N O13	(M-H)-
962.1775	962.1796	-2.22	-1	368	C44 H52 Br2 N O13	(M-H)-
963.1793	963.1826	-3.47	-1	184	C44 H52 Br2 N O13	(M-H)-
964.1769	964.179	-2.13	-1	226	C44 H52 Br2 N O13	(M-H)-
965.1807	965.1813	-0.61	-1	102	C44 H52 Br2 N O13	(M-H)-

--- End Of Report ---



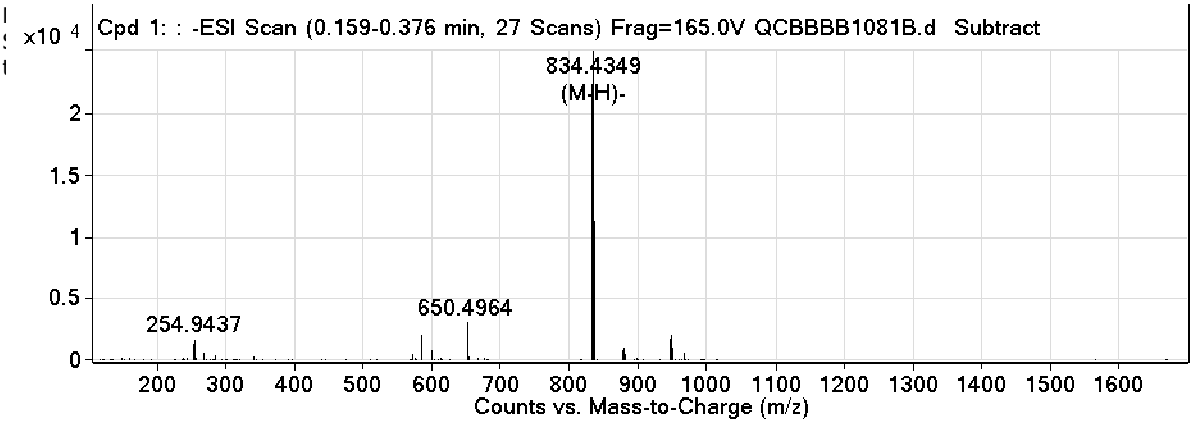
13

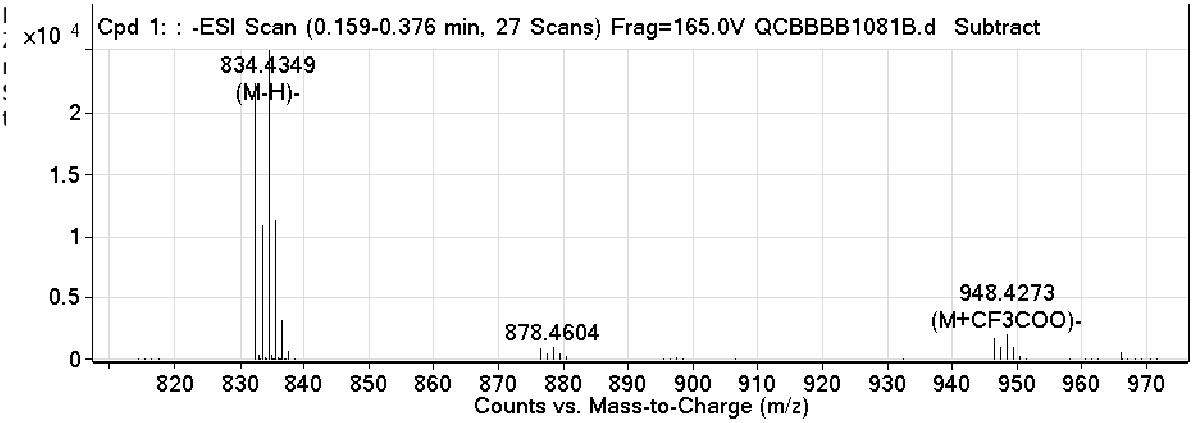
Data File	QCBBBB1081B.d	**Sample Name**	YAK-3-111
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	3/21/2011 1:10:44 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=833.4441 M=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1:	0.234	833.4433	25036	C45H72BrNO8	833.4441	-1.03	C45H72BrNO8	C45H72BrNO8

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1:	951.4224	0.234	Find By Formula	833.4433

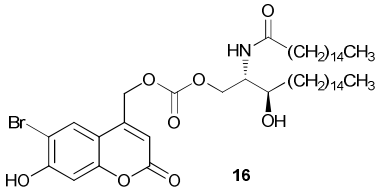




MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
252.9492			1	1455.9		
254.9437			1	1683.2		
582.5093			1	2045.6		
650.4964			1	3049.5		
651.5			1	1193		
832.4362	832.4369	-0.81	1	22411	C45H71BrNO8	(M-H)-
833.439	833.4402	-1.48	1	10976	C45H71BrNO8	(M-H)-
834.4349	834.4359	-1.16	1	25036	C45H71BrNO8	(M-H)-
835.4379	835.4386	-0.9	1	11384	C45H71BrNO8	(M-H)-
836.4406	836.4415	-1.08	1	3187.5	C45H71BrNO8	(M-H)-
878.4604			1	1004.7		
946.4283	946.4297	-1.46	1	1830.3	C47H72BrF3NO10	(M+CF3COO)-
947.4315	947.4331	-1.69	1	968.6	C47H72BrF3NO10	(M+CF3COO)-
948.4273	948.4288	-1.65	1	2094.3	C47H72BrF3NO10	(M+CF3COO)-
949.4293	949.4315	-2.35	1	985.6	C47H72BrF3NO10	(M+CF3COO)-

--- End Of Report ---



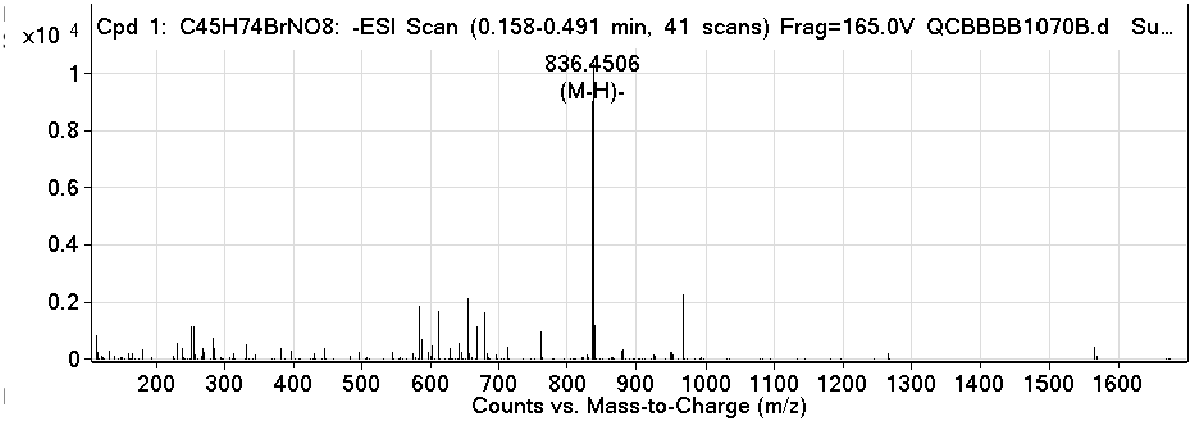
16

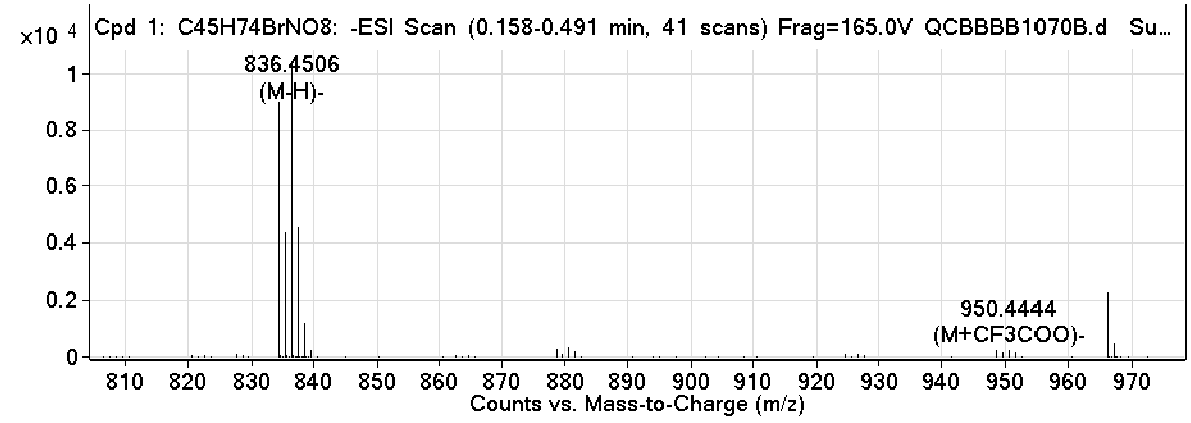
Data File	QCBBBB1070B.d	**Sample Name**	YAK-3-73
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	2/9/2011 5:29:02 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=835.4598 M=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C45H74BrNO8	0.241	835.4588	10310	C45H74BrNO8	835.4598	-1.16

Compound Label	RT	Algorithm	Mass
Cpd 1: C45H74BrNO8	0.241	Find By Formula	835.4588

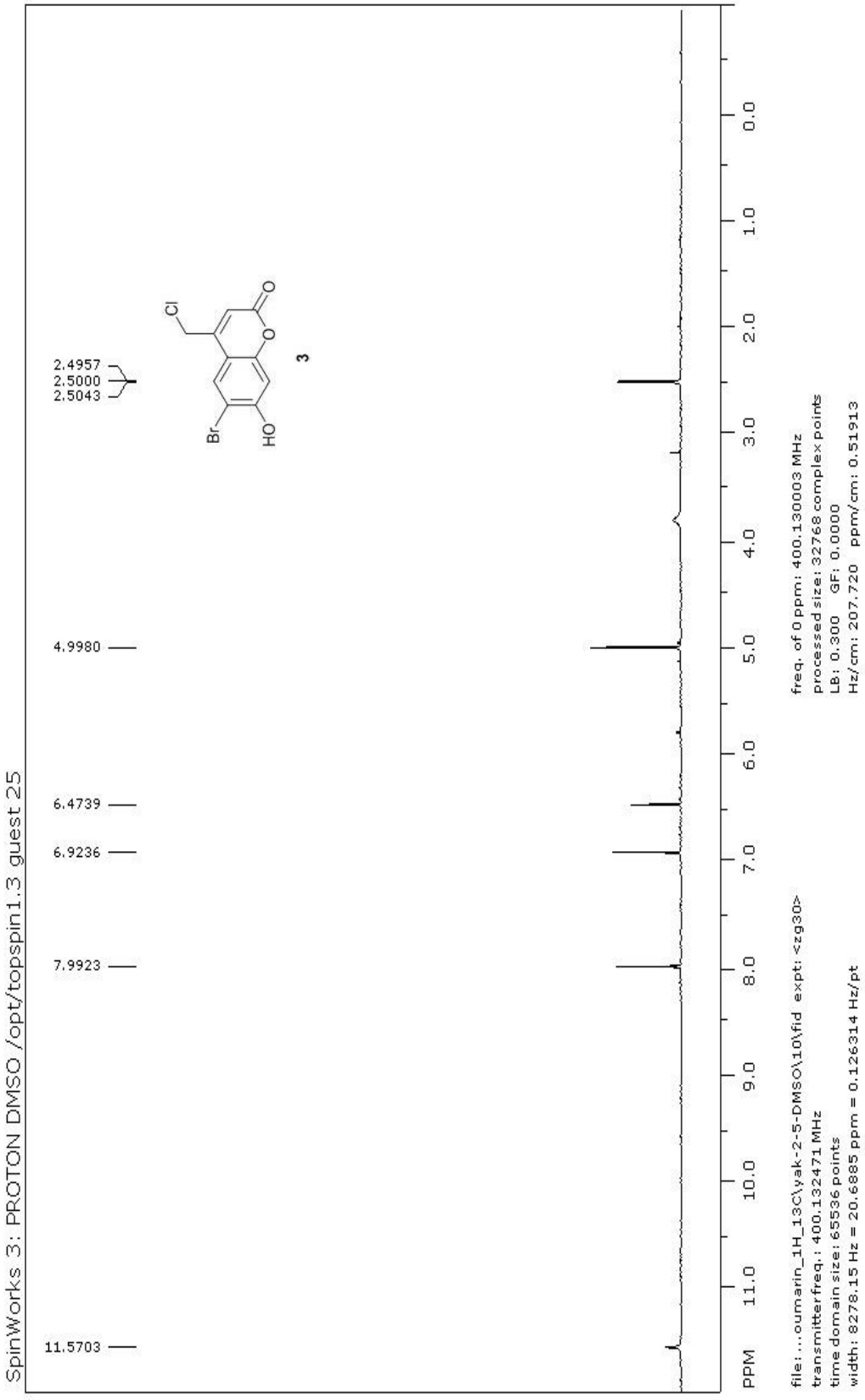




MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
834.4515	834.4525	-1.19		9053	C45 H73 Br N O8	(M-H)-
834.7077				100		
835.4545	835.4559	-1.62		4412	C45 H73 Br N O8	(M-H)-
835.7118				94		
836.4506	836.4515	-1.12		10310	C45 H73 Br N O8	(M-H)-
836.7128				105		
m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
837.4534	837.4543	-1.01		4556	C45 H73 Br N O8	(M-H)-
837.7141				83		
837.7938				80		
838.4555	838.4571	-2.01		1247	C45 H73 Br N O8	(M-H)-
839.4595	839.46	-0.59		263	C45 H73 Br N O8	(M-H)-
948.4424	948.4454	-3.13	-1	280	C47 H74 Br F3 N O10	(M+CF3COO)-
949.4443	949.4487	-4.64	-1	158	C47 H74 Br F3 N O10	(M+CF3COO)-
950.4444	950.4445	-0.1	-1	304	C47 H74 Br F3 N O10	(M+CF3COO)-
951.4445	951.4472	-2.77	-1	166	C47 H74 Br F3 N O10	(M+CF3COO)-

--- End Of Report ---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6.9236
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Cl
O
O
Br
HO
3
PPM
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10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
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width: 8278.15 Hz = 20.6885 ppm = 0.126314 Hz/pt
number of scans: 16
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processed size: 32768 complex points
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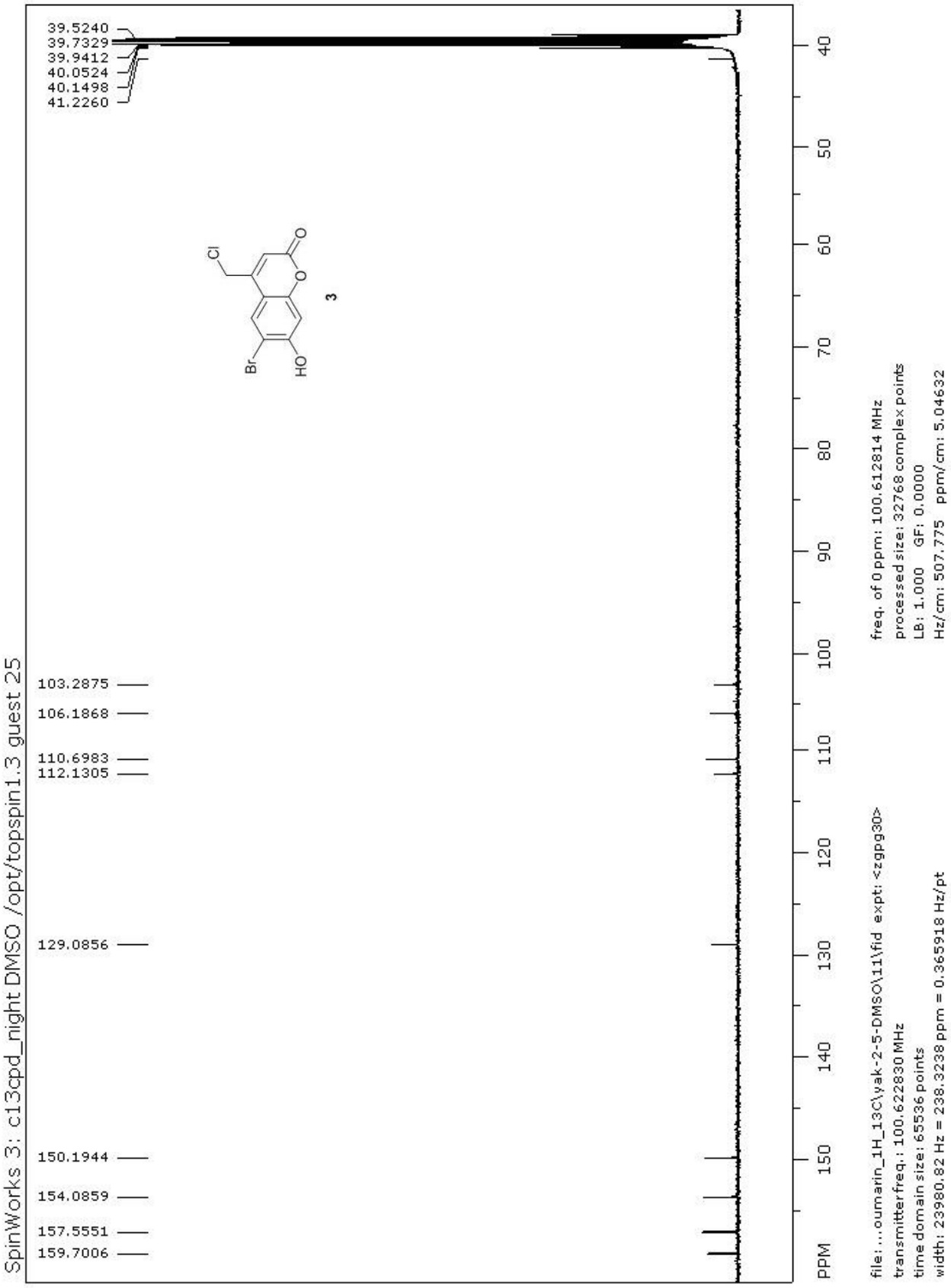
Hz/cm: 207.720 ppm/cm: 0.51913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40.1498
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103.2875
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154.0859
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O
Br
HO
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PPM
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number of scans: 4096
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processed size: 32768 complex points
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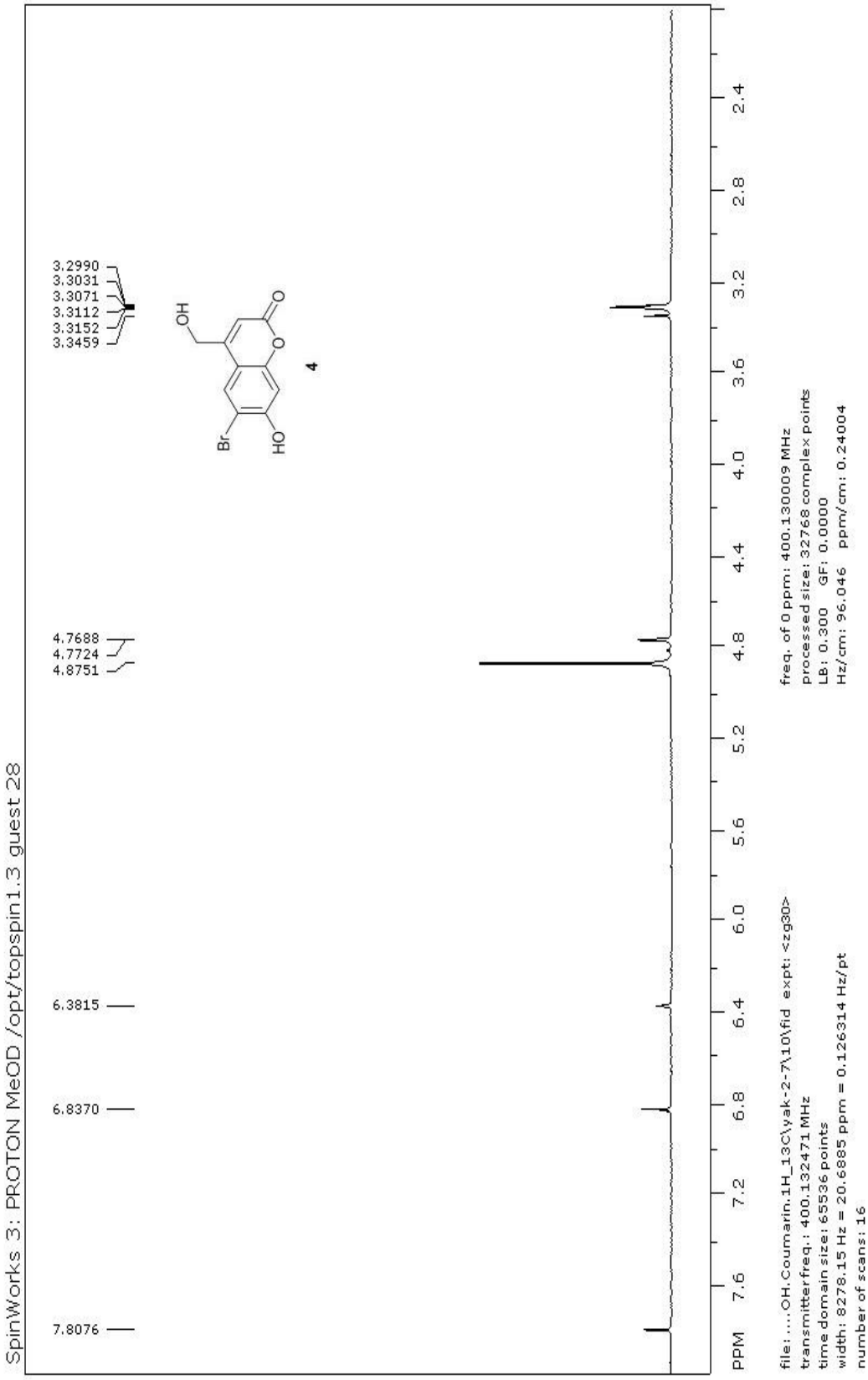
Hz/cm: 507.775 ppm/cm: 5.04632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3.3112
3.3152
3.3459
4.7688
4.7724
4.8751
6.3815
6.8370
7.8076
OH
O
O
Br
HO
4
PPM 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4
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transmitter freq.: 400.132471 MHz
time domain size: 65536 points
width: 8278.15 Hz = 20.6885 ppm = 0.126314 Hz/pt
number of scans: 16
freq. of 0 ppm: 400.130009 MHz
processed size: 32768 complex points
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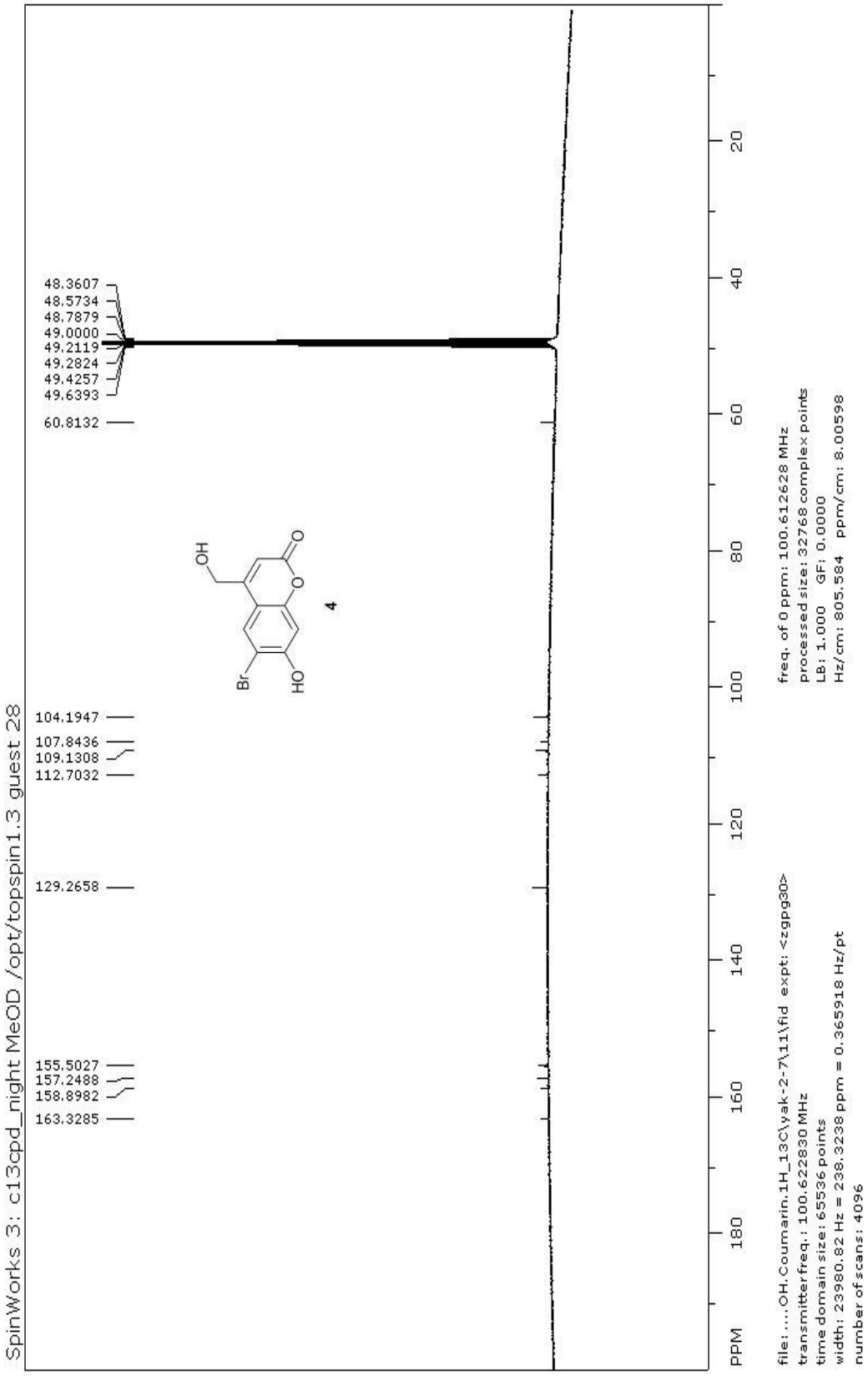
Hz/cm: 96.046 ppm/cm: 0.24004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number of scans: 4096
freq. of 0 ppm: 100.612628 MHz
processed size: 32768 complex points
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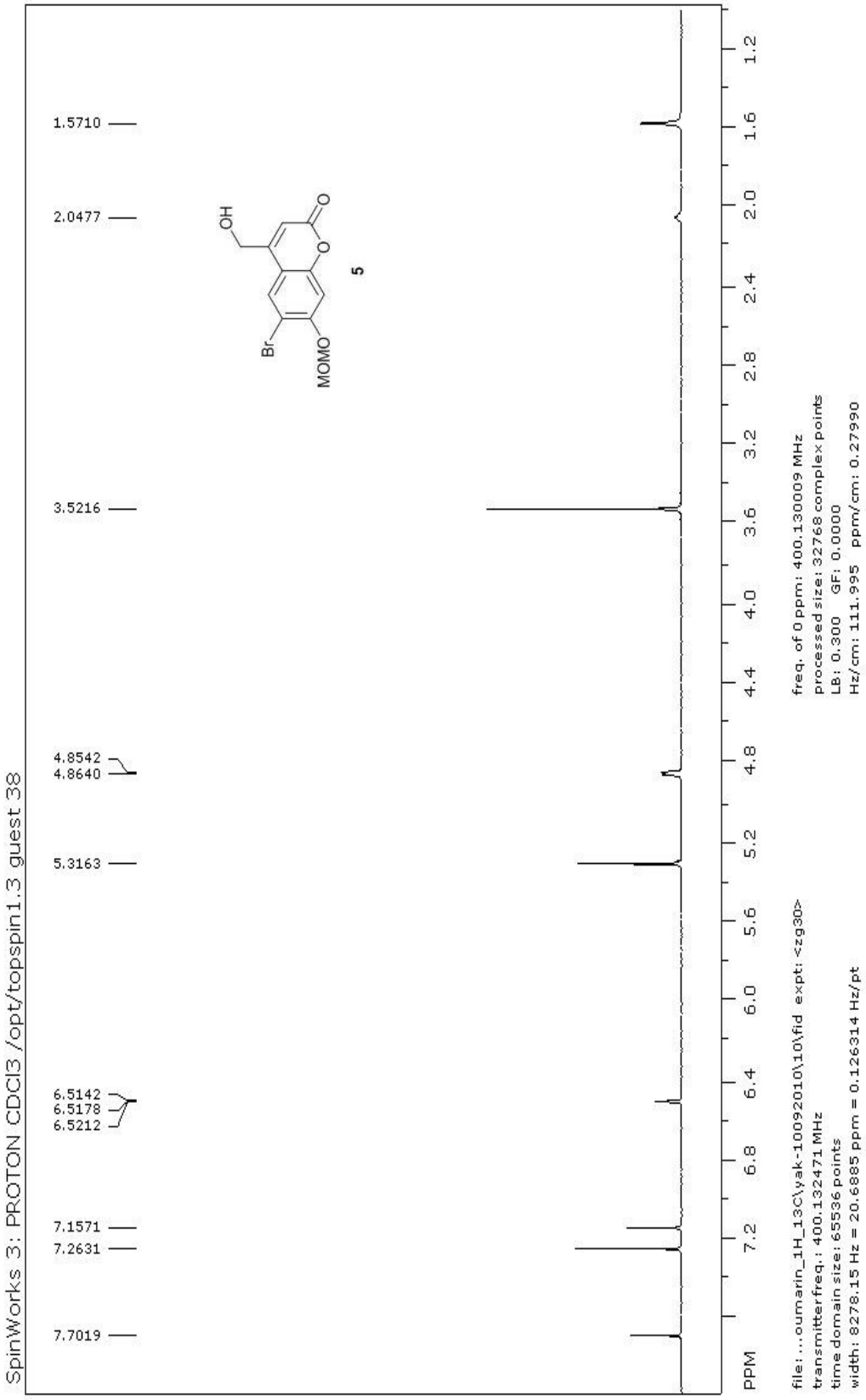
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6.5212
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7.2631
7.7019
OH
O
O
Br
MOMO
5
PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
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3.2
2.8
2.4
2.0
1.6
1.2
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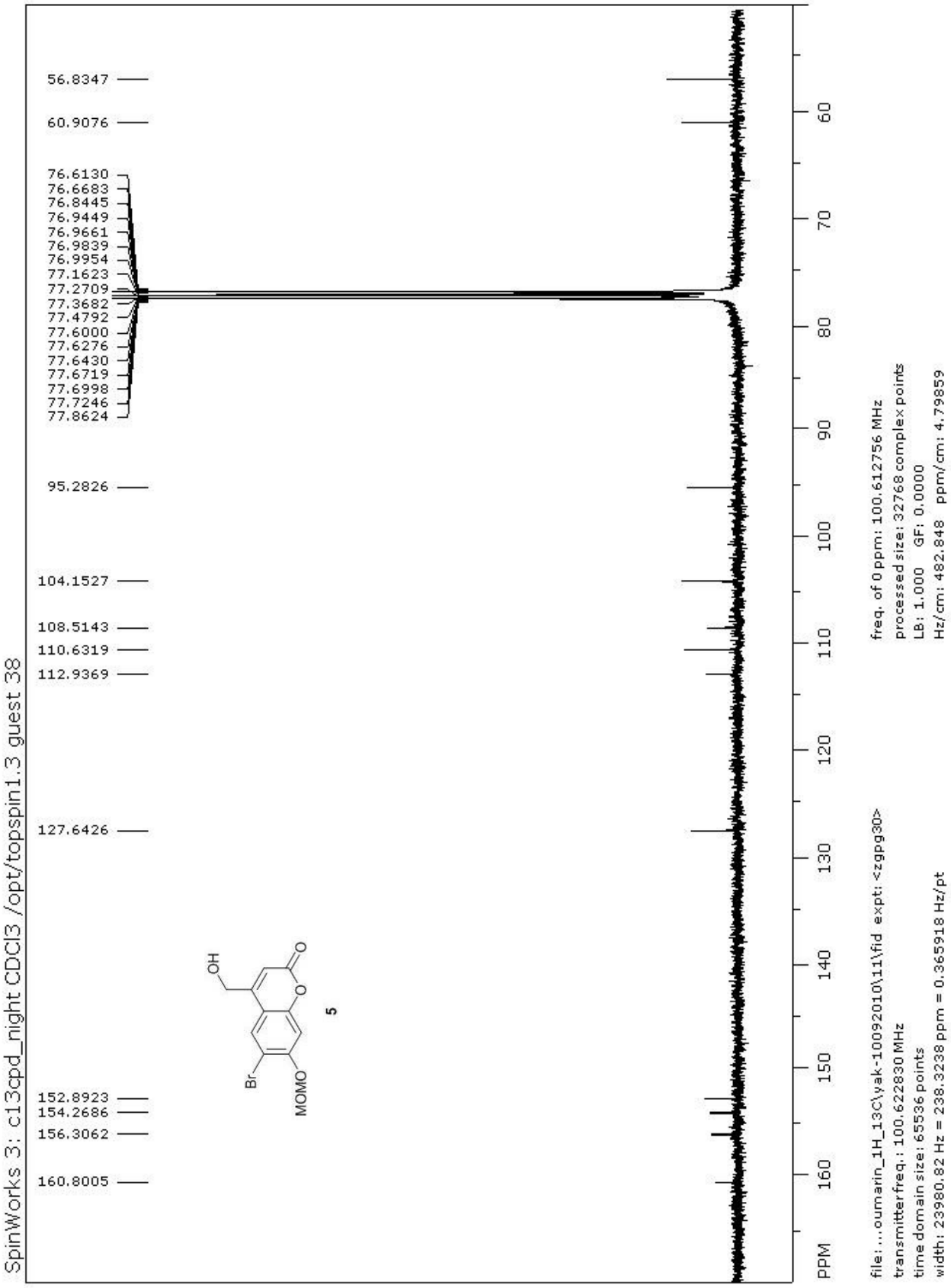
time domain size: 65536 points
width: 8278.15 Hz = 20.6885 ppm = 0.126314 Hz/pt
number of scans: 16
freq. of 0 ppm: 400.130009 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 111.995 ppm/cm: 0.27990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76.6683
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76.9449
76.9661
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77.1623
77.2709
77.3682
77.4792
77.6000
77.6276
77.6430
77.6719
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77.8624
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152.8923
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156.3062
160.8005
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O
Br
MOMO
5
PPM
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150
140
130
120
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80
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processed size: 32768 complex points
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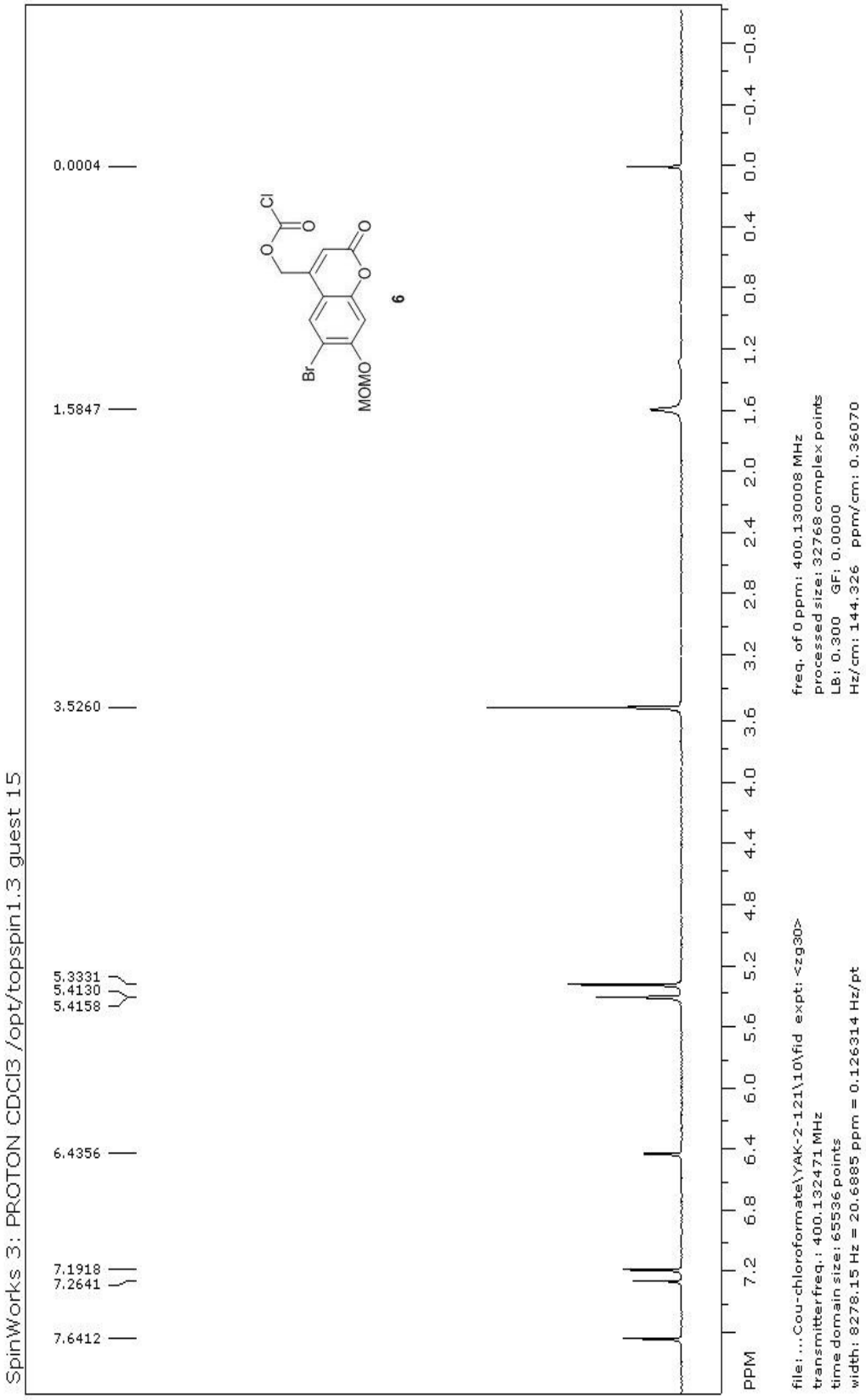
Hz/cm: 482.848 ppm/cm: 4.79859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5.4130
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7.1918
7.2641
7.6412
PPM
7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4 0.0 -0.4 -0.8
Cl
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O
O
O
Br
MOMO
6
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transmitter freq.: 400.132471 MHz
time domain size: 65536 points
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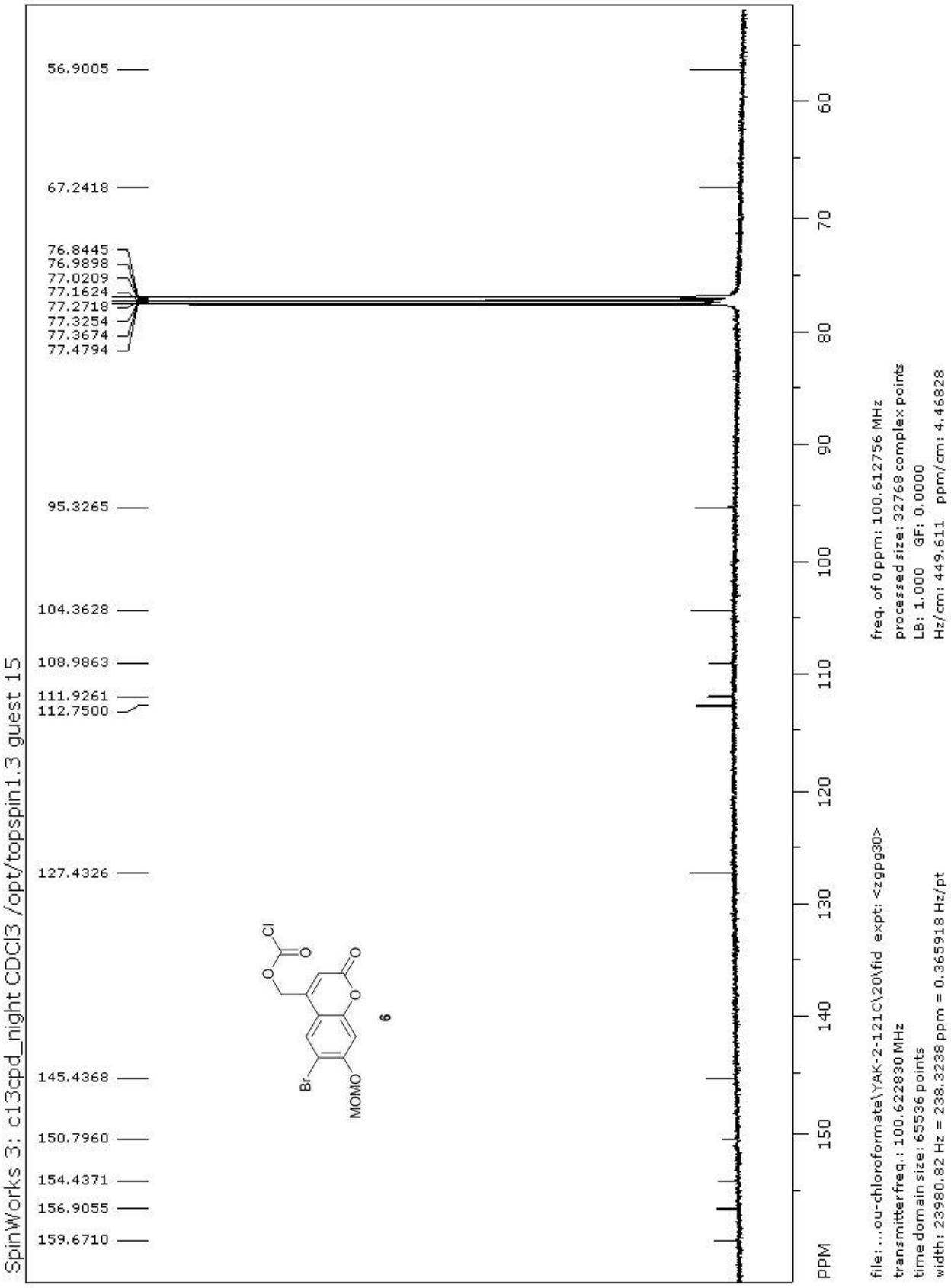
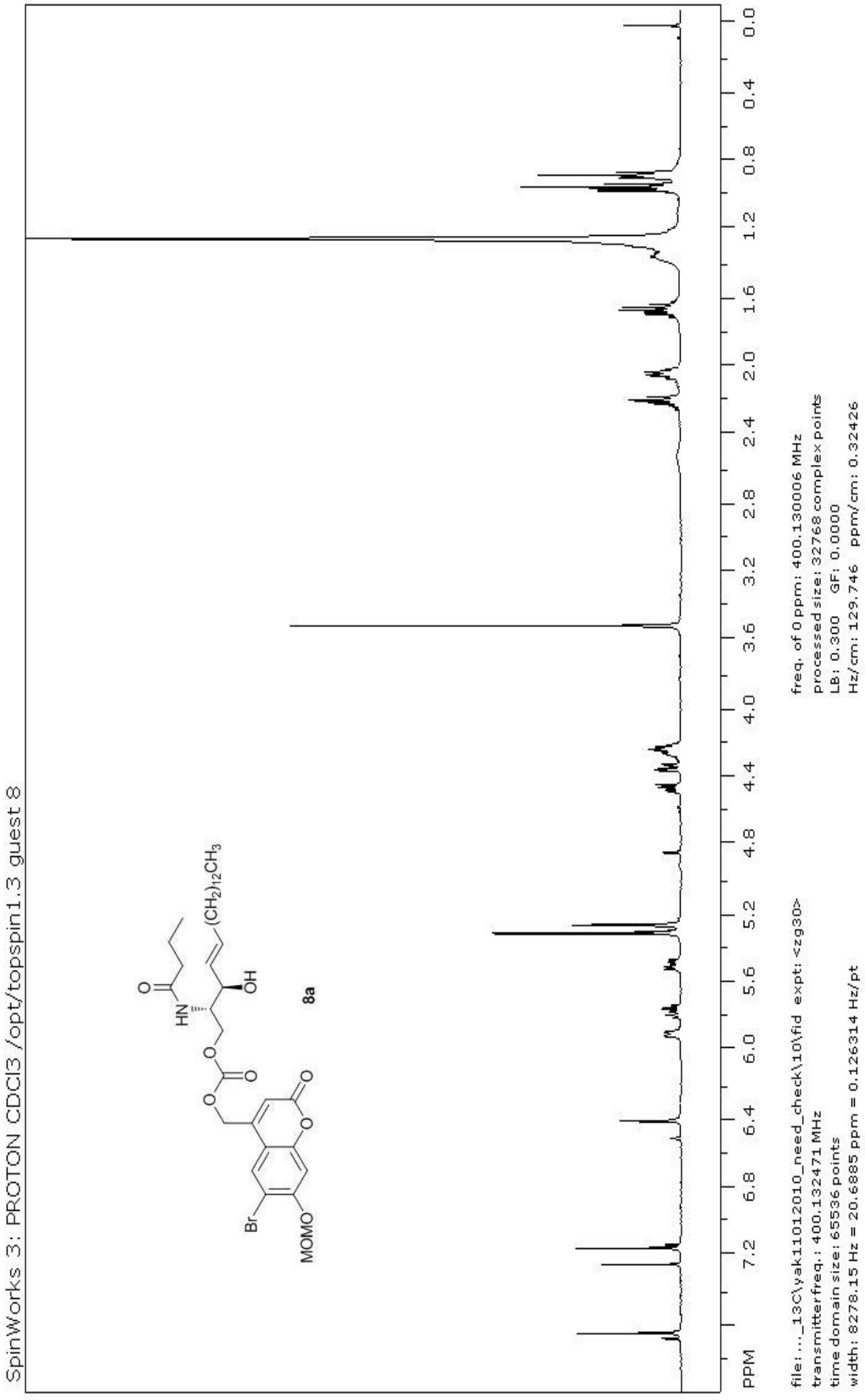
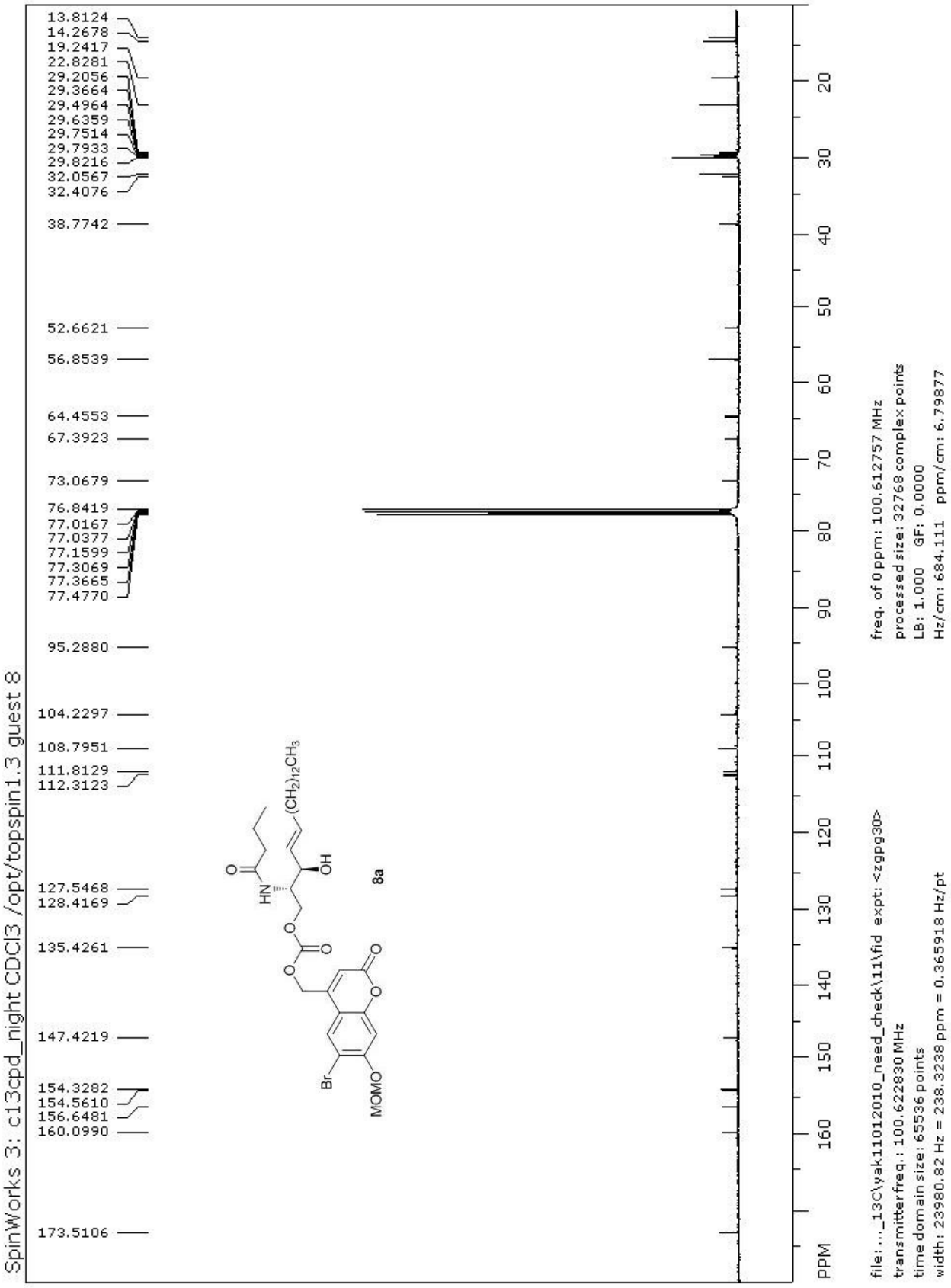
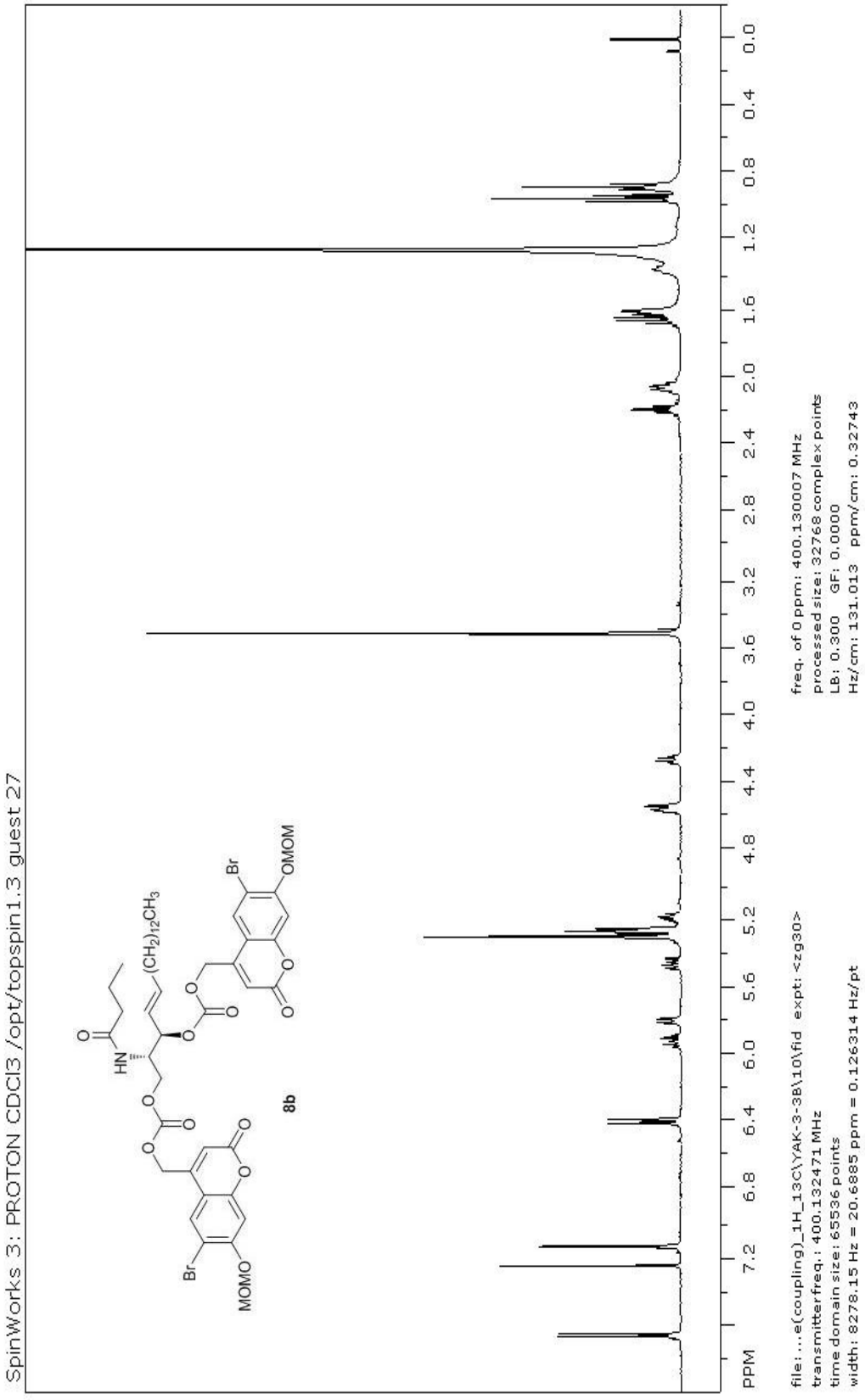
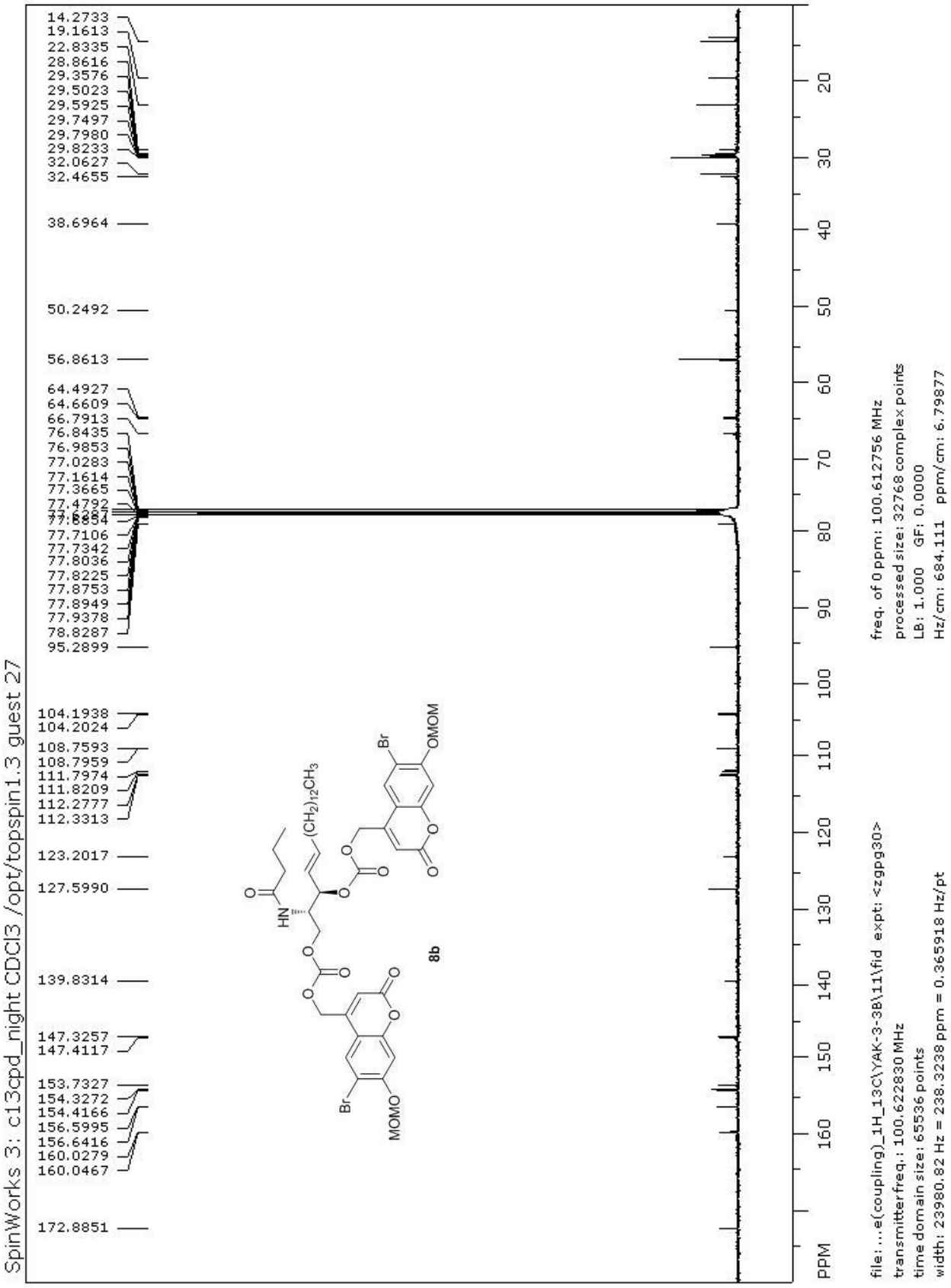
number of scans: 16
freq. of 0 ppm: 400.130008 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 144.326 ppm/cm: 0.36070

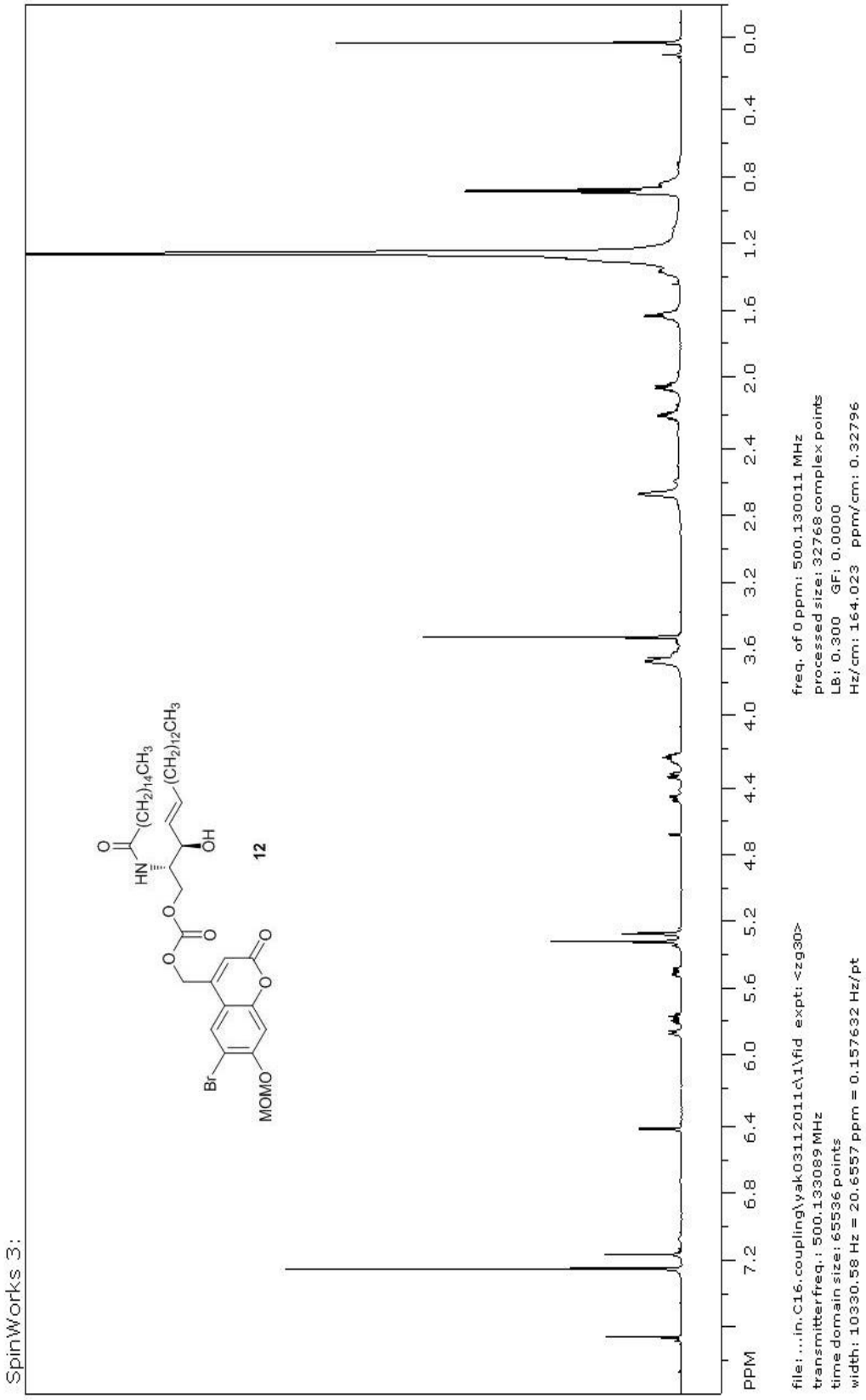

SpinWorks 3: c13cpd_night CDCl3 /opt/topspin1.3 guest 15
56.9005
67.2418
76.8445
76.9898
77.0209
77.1624
77.2718
77.3254
77.3674
77.4794
95.3265
104.3628
108.9863
111.9261
112.7500
127.4326
145.4368
150.7960
154.4371
156.9055
159.6710
PPM
Cl
O
O
O
O
Br
MOMO
6
file: ...ou-chloroformate\YAK-2-121C\20\fid expt: <zgpg30>
transmitter freq.: 100.622830 MHz
time domain size: 65536 points
width: 23980.82 Hz = 238.3238 ppm = 0.365918 Hz/pt
number of scans: 4096
freq. of 0 ppm: 100.612756 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 449.611 ppm/cm: 4.46828

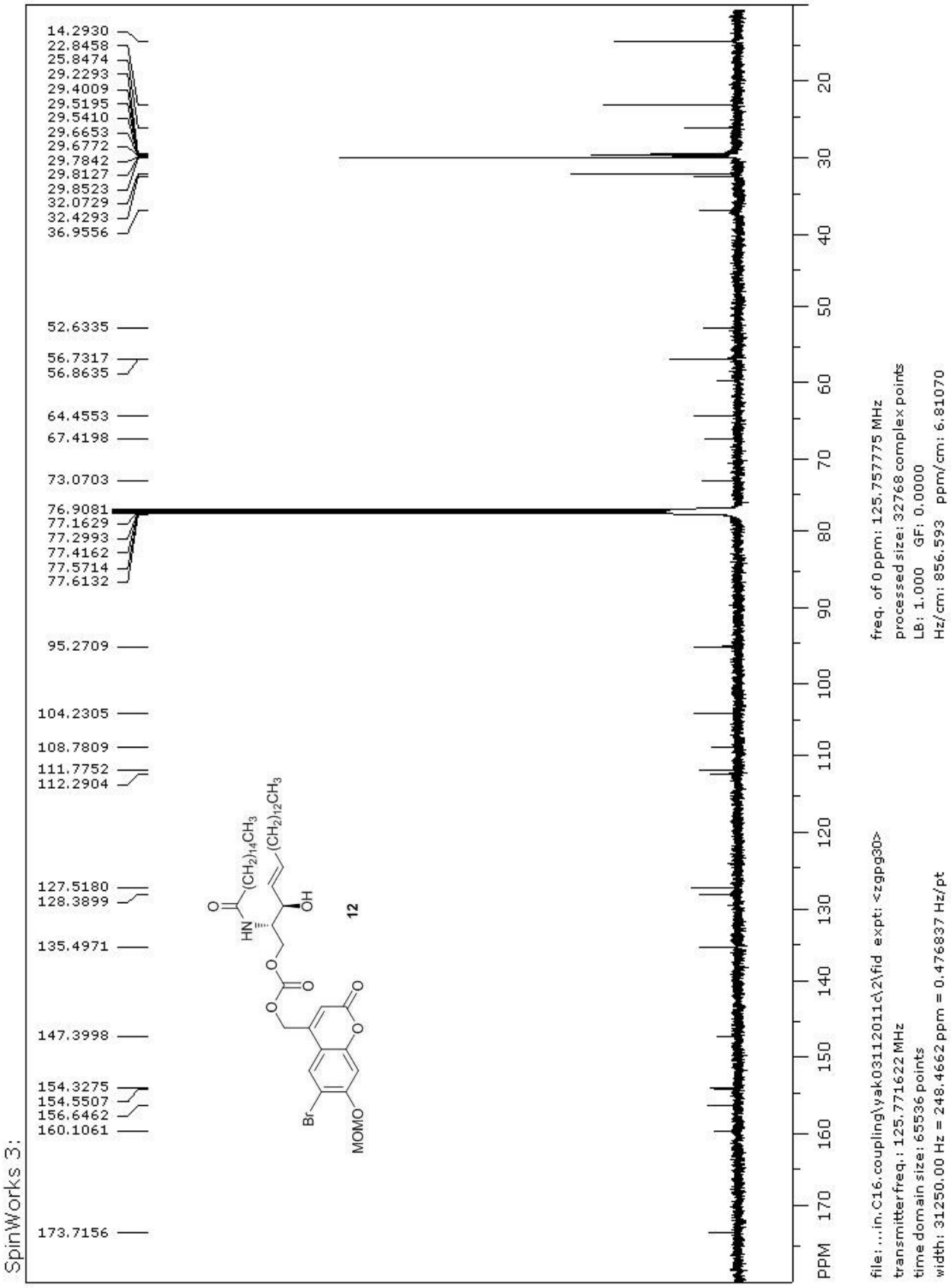

SpinWorks 3: PROTON CDCl3 /opt/topspin1.3 guest 8
8a
PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
file:...13C\yak11012010_need_check\10\fid expt: <zg30>
transmitter freq.: 400.132471 MHz
time domain size: 65536 points
width: 8278.15 Hz = 20.6885 ppm = 0.126314 Hz/pt
number of scans: 16
freq. of 0 ppm: 400.130006 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 129.746 ppm/cm: 0.32426

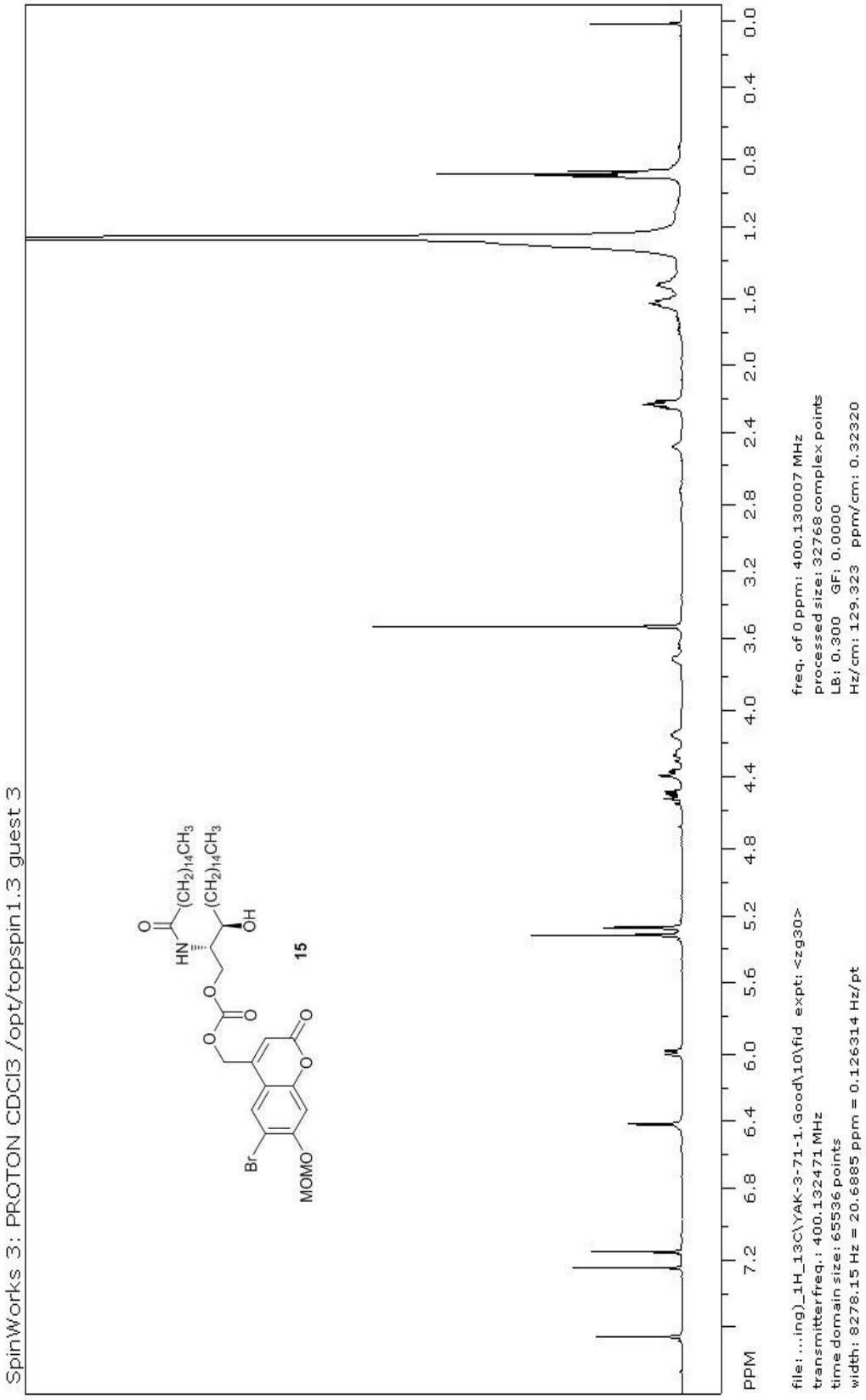

SpinWorks 3: c13cpd_night CDCl3 /opt/topspin1.3 guest 8
13.8124
14.2678
19.2417
22.8281
29.2056
29.3664
29.4964
29.6359
29.7514
29.7933
29.8216
32.0567
32.4076
38.7742
52.6621
56.8539
64.4553
67.3923
73.0679
76.8419
77.0167
77.0377
77.1599
77.3069
77.3665
77.4770
95.2880
104.2297
108.7951
111.8129
112.3123
127.5468
128.4169
135.4261
147.4219
154.3282
154.5610
156.6481
160.0990
173.5106
PPM
8a
file: ...13C\yak11012010_need_check\11\fid expt: <zgpg30>
transmitter freq.: 100.622830 MHz
time domain size: 65536 points
width: 23980.82 Hz = 238.3238 ppm = 0.365918 Hz/pt
number of scans: 6144
freq. of 0 ppm: 100.612757 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 684.111 ppm/cm: 6.79877

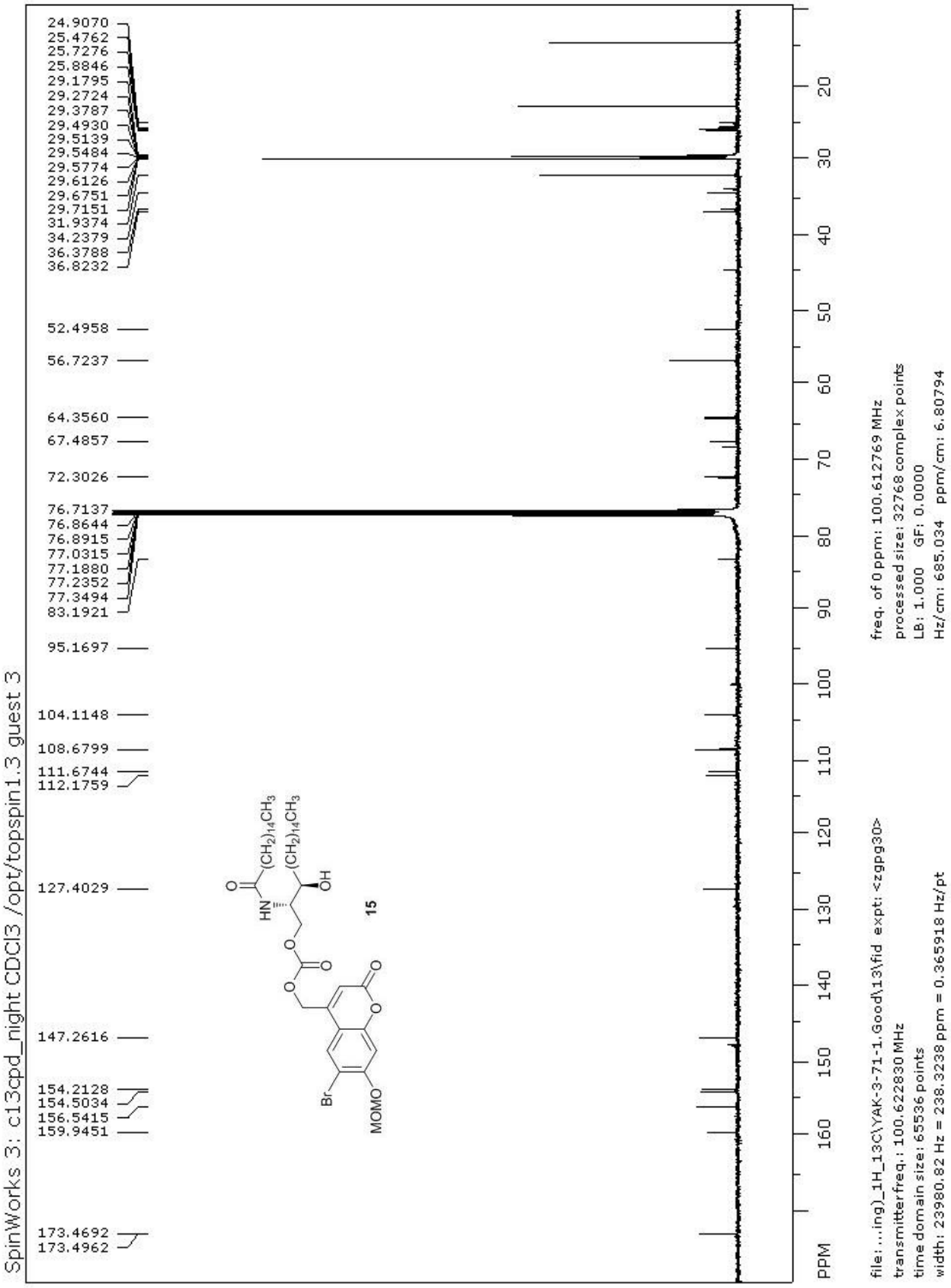

SpinWorks 3: PROTON CDCl3 /opt/topspin1.3 guest 27
8b
PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
file:...e(coupling)_1H_13C\YAK-3-38\10\fid expt:<zg30>
transmitterfreq.: 400.132471 MHz
time domain size: 65536 points
width: 8278.15 Hz = 20.6885 ppm = 0.126314 Hz/pt
number of scans: 16
freq. of 0 ppm: 400.130007 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 131.013 ppm/cm: 0.32743

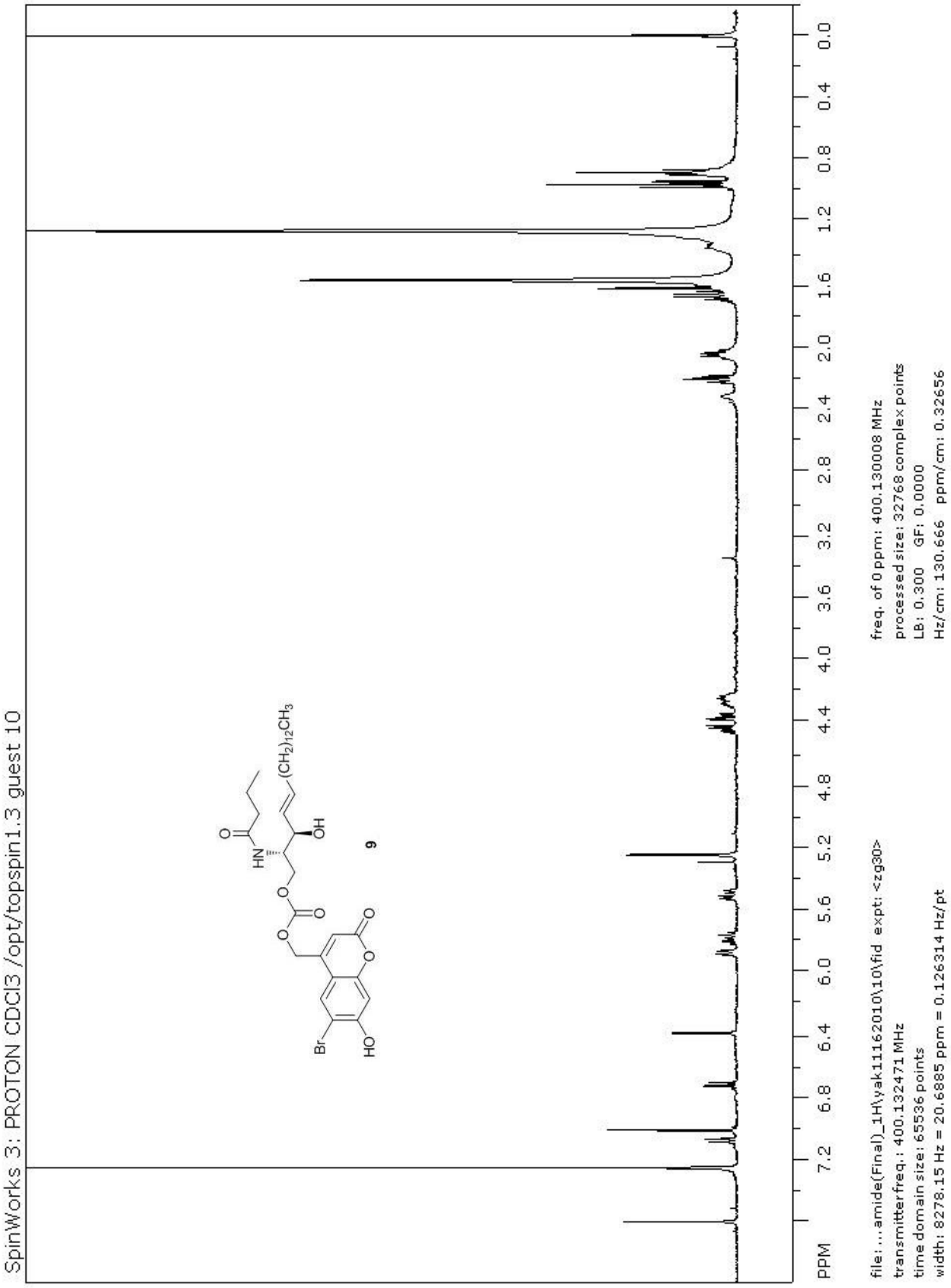

SpinWorks 3: c13cpd_night CDCl3 /opt/topspin1.3 guest 27
14.2733
19.1613
22.8335
28.8616
29.3576
29.5023
29.5925
29.7497
29.7980
29.8233
32.0627
32.4655
38.6964
50.2492
56.8613
64.4927
64.6609
66.7913
76.8435
76.9853
77.0283
77.1614
77.3665
77.4792
77.7106
77.7342
77.8036
77.8225
77.8753
77.8949
77.9378
78.8287
95.2899
104.1938
104.2024
108.7593
108.7959
111.7974
111.8209
112.2777
112.3313
123.2017
127.5990
139.8314
147.3257
147.4117
153.7327
154.3272
154.4166
156.5995
156.6416
160.0279
160.0467
172.8851
PPM
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
HN
(CH2)12CH3
Br
OMOM
MOMO
8b
file: ...e(coupling)_1H_13C\YAK-3-3B\11\fid expt: <zgpg30>
transmitter freq.: 100.622830 MHz
time domain size: 65536 points
width: 23980.82 Hz = 238.3238 ppm = 0.365918 Hz/pt
number of scans: 10240
freq. of 0 ppm: 100.612756 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 684.111 ppm/cm: 6.79877

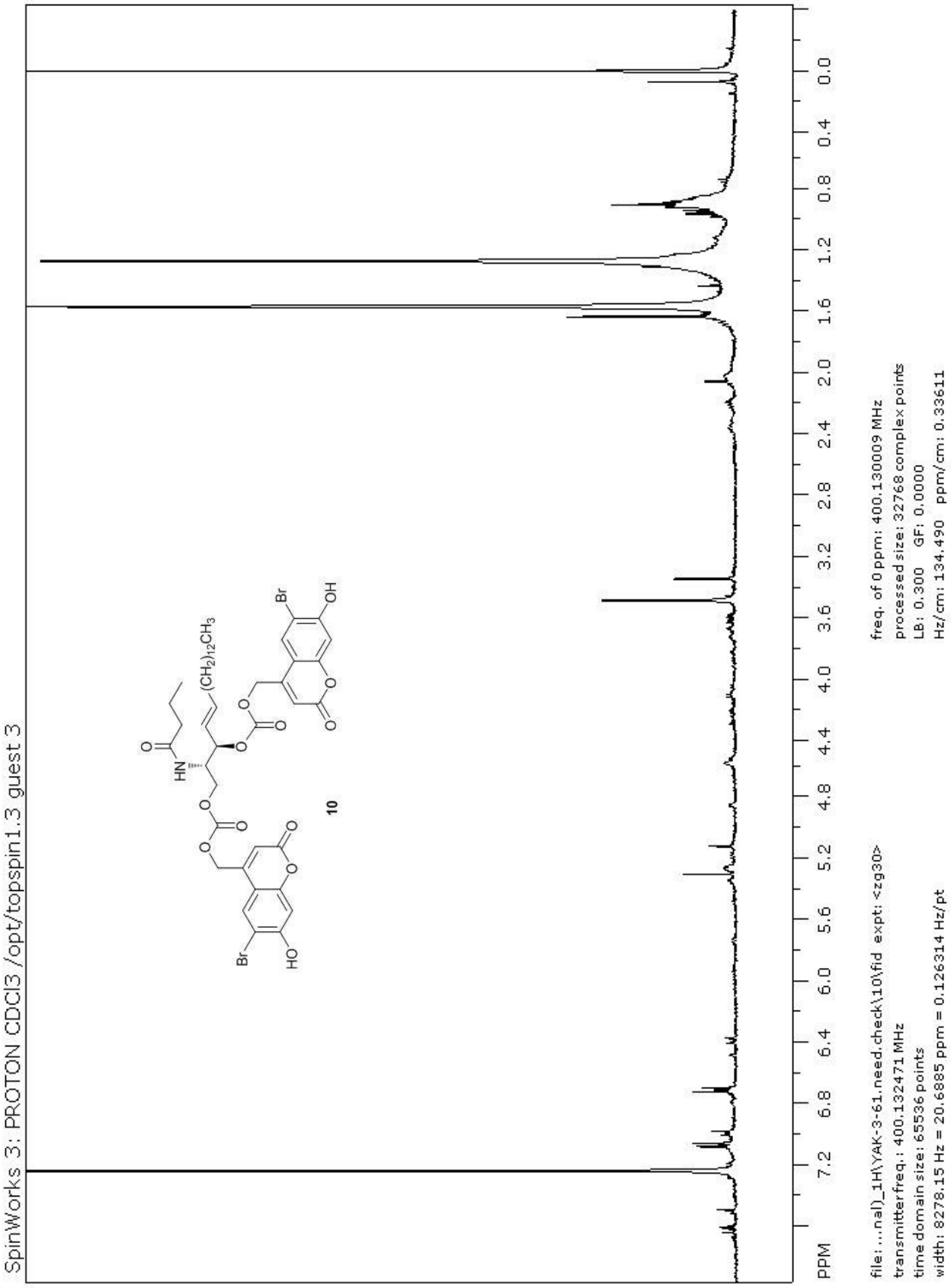

SpinWorks 3:
PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
12
file:...in.C16.coupling\yak03112011c\1\fid expt: <zg30>
transmitterfreq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16
freq. of 0 ppm: 500.130011 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 164.023 ppm/cm: 0.32796

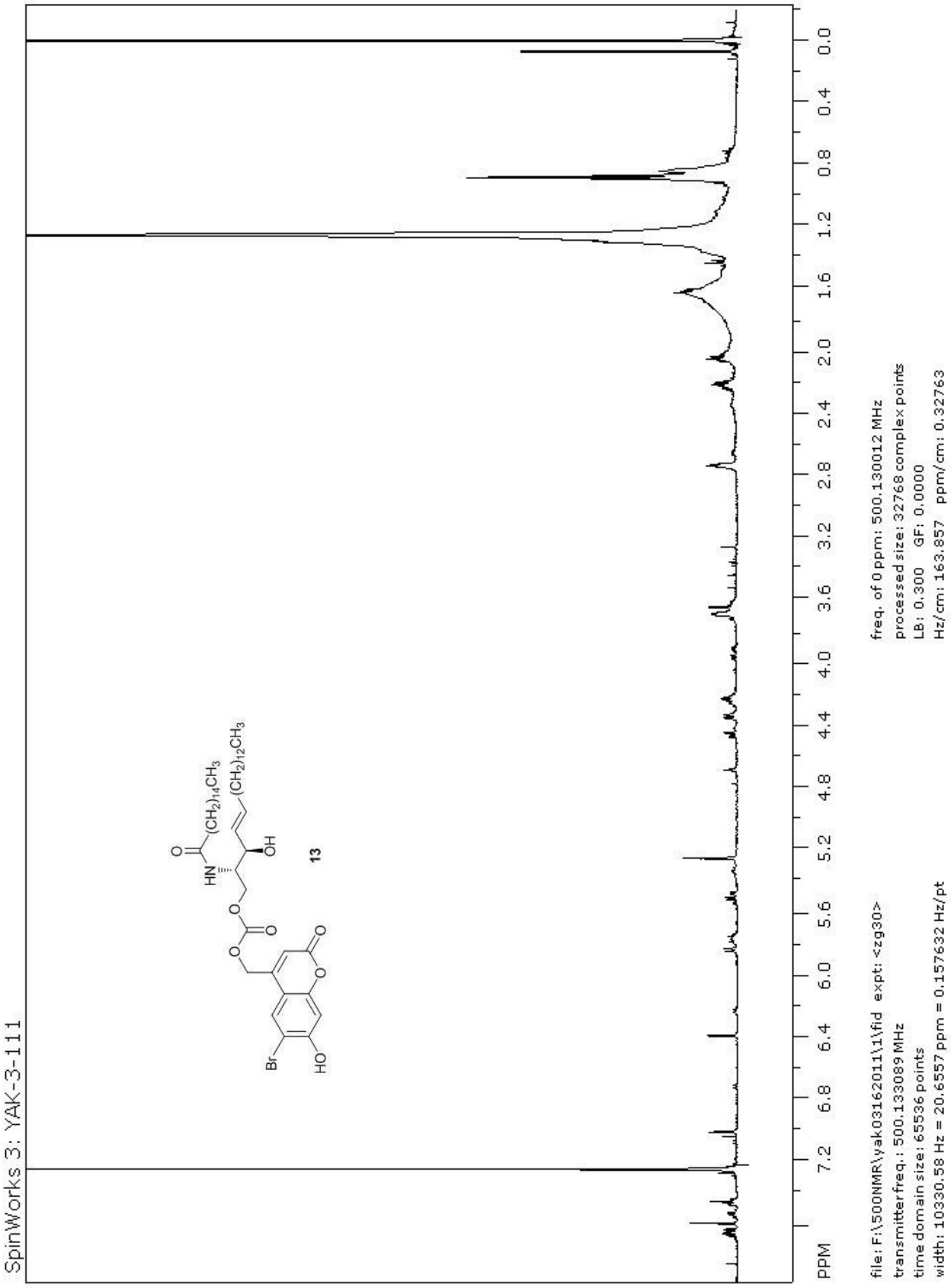

SpinWorks 3:
14.2930
22.8458
25.8474
29.2293
29.4009
29.5195
29.5410
29.6653
29.6772
29.7842
29.8127
29.8523
32.0729
32.4293
36.9556
52.6335
56.7317
56.8635
64.4553
67.4198
73.0703
76.9081
77.1629
77.2993
77.4162
77.5714
77.6132
95.2709
104.2305
108.7809
111.7752
112.2904
127.5180
128.3899
135.4971
147.3998
154.3275
154.5507
156.6462
160.1061
173.7156
12
PPM
file: ...in.C16.coupling\yak03112011c\2\fid expt: <zgpg30>
transmitterfreq.: 125.771622 MHz
time domain size: 65536 points
width: 31250.00 Hz = 248.4662 ppm = 0.476837 Hz/pt
number of scans: 15360
freq. of 0 ppm: 125.757775 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 856.593 ppm/cm: 6.81070

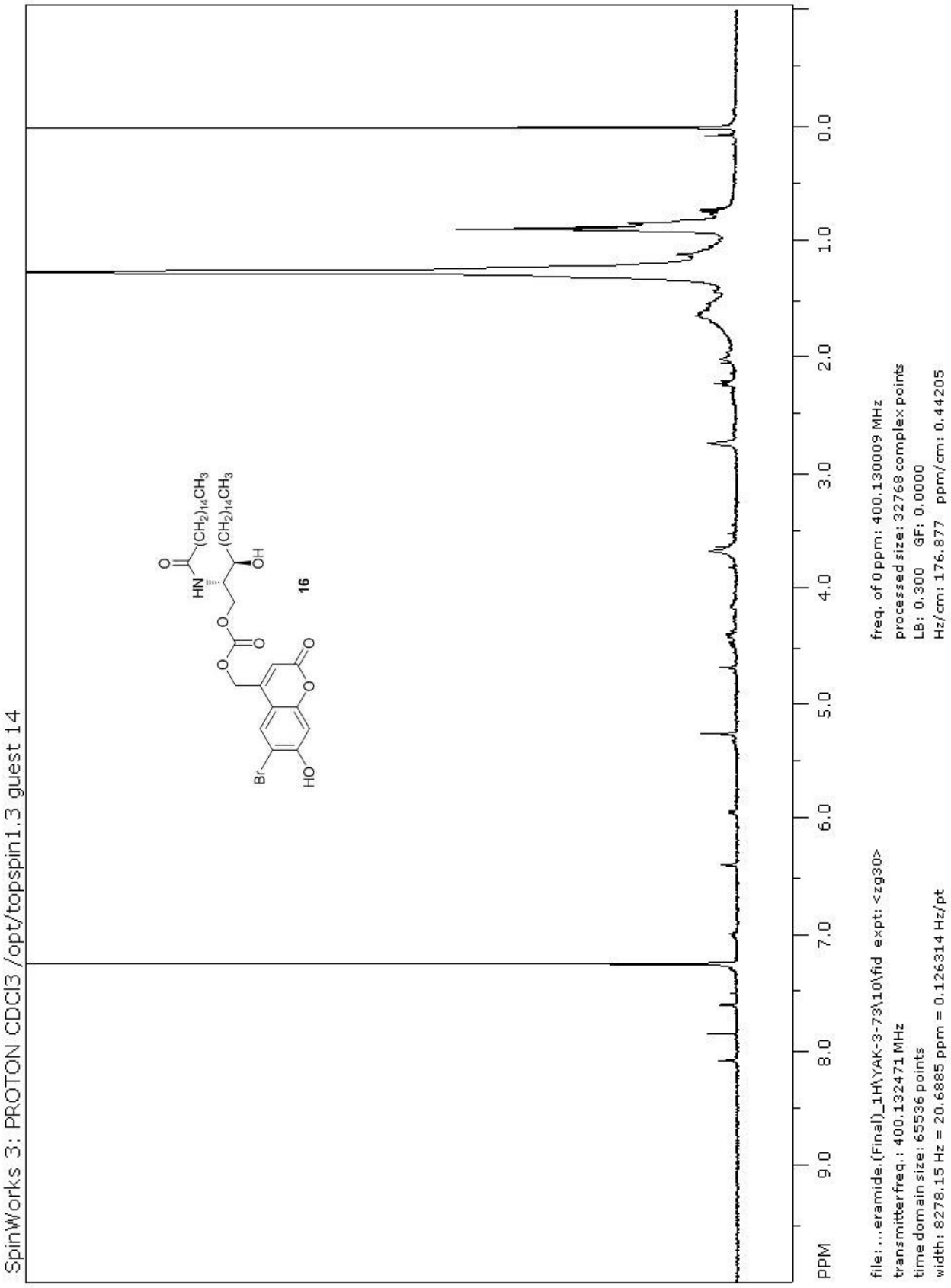

SpinWorks 3: PROTON CDCl3 /opt/topspin1.3 guest 3
15
PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
file:...ing)_1H_13C\YAK-3-71-1.Good\10\fid expt: <zg30>
transmitterfreq.: 400.132471 MHz
time domain size: 65536 points
width: 8278.15 Hz = 20.6885 ppm = 0.126314 Hz/pt
number of scans: 16
freq. of 0 ppm: 400.130007 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 129.323 ppm/cm: 0.32320


SpinWorks 3: c13cpd_night CDCl3 /opt/topspin1.3 guest 3
24.9070
25.4762
25.7276
25.8846
29.1795
29.2724
29.3787
29.4930
29.5139
29.5484
29.5774
29.6126
29.6751
29.7151
31.9374
34.2379
36.3788
36.8232
52.4958
56.7237
64.3560
67.4857
72.3026
76.7137
76.8644
76.8915
77.0315
77.1880
77.2352
77.3494
83.1921
95.1697
104.1148
108.6799
111.6744
112.1759
127.4029
147.2616
154.2128
154.5034
156.5415
159.9451
173.4692
173.4962
PPM
15
MOMO
Br
OH
HN
(CH2)14CH3
(CH2)14CH3
file:...ing)_1H_13C\YAK-3-71-1.Good\13\fid expt: <zgpg30>
transmitterfreq.: 100.622830 MHz
time domain size: 65536 points
width: 23980.82 Hz = 238.3238 ppm = 0.365918 Hz/pt
number of scans: 9216
freq. of 0 ppm: 100.612769 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 685.034 ppm/cm: 6.80794


SpinWorks 3: PROTON CDCl3 /opt/topspin1.3 guest 10
PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
HN
OH
(CH2)12CH3
O
O
O
O
O
Br
HO
9
file: ...amide(Final)_1H\yak11162010\10\fid expt: <zg30>
transmitterfreq.: 400.132471 MHz
time domain size: 65536 points
width: 8278.15 Hz = 20.6885 ppm = 0.126314 Hz/pt
number of scans: 16
freq. of 0 ppm: 400.130008 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 130.666 ppm/cm: 0.32656


SpinWorks 3: PROTON CDCl3 /opt/topspin1.3 guest 3
10
PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
file: ...nal)_1H\YAK-3-61.need.check\10\fid expt: <zg30>
transmitterfreq.: 400.132471 MHz
time domain size: 65536 points
width: 8278.15 Hz = 20.6885 ppm = 0.126314 Hz/pt
number of scans: 80
freq. of 0 ppm: 400.130009 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 134.490 ppm/cm: 0.33611


SpinWorks 3: YAK-3-111
PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
13
file: F:\500NMR\yak03162011\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16
freq. of 0 ppm: 500.130012 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 163.857 ppm/cm: 0.32763


SpinWorks 3: PROTON CDCl3 /opt/topspin1.3 guest 14
16
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
file: ...eramide.(Final)_1H\YAK-3-73\10\fid expt: <zg30>
transmitterfreq.: 400.132471 MHz
time domain size: 65536 points
width: 8278.15 Hz = 20.6885 ppm = 0.126314 Hz/pt
number of scans: 24
freq. of 0 ppm: 400.130009 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 176.877 ppm/cm: 0.44205