Measuring Binding of Protein to Gel-Bound Ligands with Magnetic Levitation

Supporting Information

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General Methods. All chemicals and reagents were purchased from Sigma-Aldrich, Oakwood Products, or MP Biomedicals and used without further purification. A Varian Inova spectrometer operating at 500 MHz (^{1}H) was used for NMR experiments. PL-PEGA resin (0.2 mmol·g^{-1}, 300-500 μm diameter wet bead size) was produced by Varian Inc. and purchased from Agilent Technologies. Filter mesh was purchased from McMaster-Carr. A Varian Inova spectrometer operating at 500 or 600 MHz (^{1}H) was used for NMR experiments. The osmolality of the standard levitation solution (300 mM Gd(DTPA), and 0.05% polysorbate 20 dissolved in PBS buffer, pH 7.4) was determined by freezing point depression using an Advanced Instruments Model 3300 Osmometer. The magnets were purchased from Applied Magnets (www.magnet4less.com). The strength of the magnetic field at the surface of the magnets was measured using a handheld DC magnetometer (AlphaLab Inc, www.trifield.com). Calibrated density standards (± 0.0002 g·cm^{-3} at 23°C) were purchased from American Density Materials (Stauton, VA; www.densitymaterials.com).

Functionalization of PEGA Beads.

PEGA beads (0.25 g, 10% in methanol, containing ~5 μmol primary amine) were rinsed three times with DMSO (5 mL) for 5 minutes. The beads were then suspended in a solution of DMSO (2 mL) containing an appropriate dye (e.g. Rhodamine isothiocyanate, 0.54 mg, 1 μmol, 0.2 equiv.). After gently rocking the beads for two hours, the reaction media was removed and the beads were resuspended in a solution of DMSO (5 mL) to which the desired carboxylic acid (10 equiv., 50 μmol) was added, followed by *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC, 10 equiv.), *N*,*N*-Diisopropylethylamine (30 equiv., 150 μmol), and *N*-hydroxysuccinimide (NHS, 10 equiv.). This reaction mixture was gently rocked overnight, after which time the beads

were thoroughly rinsed with DCM (2 x 10 mL), DMSO (2 x 10 mL), MeOH (2 x 10 mL), and 10 mM phosphate buffered saline, pH 7.4 (5 x 10 mL). The beads were stored in 10 mM phosphate buffered saline, pH 7.4, containing 0.1% w/v NaN_3.

Determining the Magnetic Susceptibility and Density of the Standard Levitation Buffer. For our experiments, the strength of the magnetic field at the surface of the magnets (B_0) was 0.38 T (measured using a handheld DC magnetometer). The magnets were separated by 45 mm in an anti-Helmholtz configuration. We performed all experiments at room temperature (20 ± 2 ºC). We measured the levitation height of seven density-standard beads (± 0.0002 g·cm^{-3} at 23°C) at 20 ºC in the standard levitation buffer (300 mM Gd(DTPA), and 0.05% polysorbate 20 dissolved in PBS buffer, pH 7.4) (Figure S1).

We fitted a plot of levitation height (*h,* in mm) versus density of the density-standard beads (ρ_s, in g·mL^{-1}) to Eqn. S1. In this equation, ρ_{bead} and ρ_m, (both g·mL^{-1}) and χ_{bead} and χ_m (both unitless) are the densities and the magnetic susceptibilities of the bead and the paramagnetic medium, respectively; g is the acceleration due to gravity (m·s^{-2}) , μ_0 is the magnetic permeability of free space (N·A^{-2}) , d is the distance between the magnets (m), and B_0 is the magnitude of the magnetic field at the surface of the magnets (0.38 T).

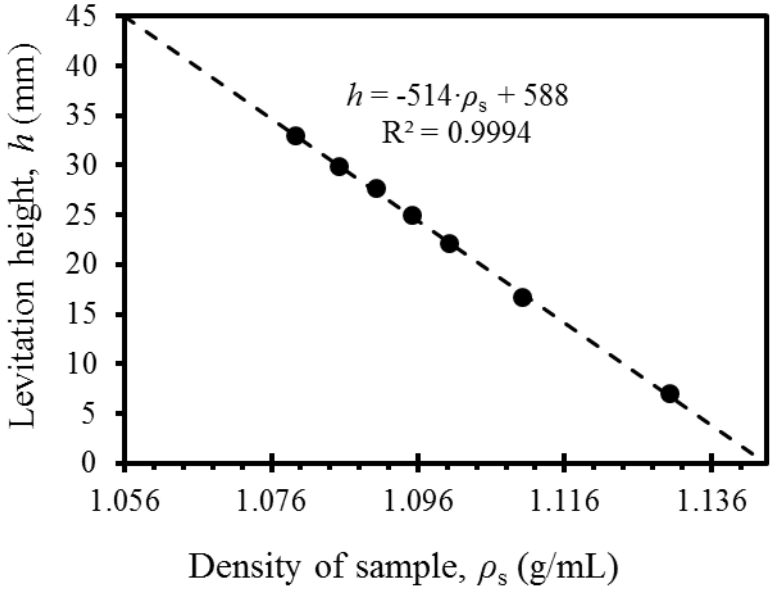
$$h = -A\cdot\rho_s + B \tag{S1a}$$

$$A = \frac{\mu_0 g}{\chi_m - \chi_{bead}}\left(\frac{d}{2B_0}\right)^2 \tag{S1b}$$

$$B = \frac{\mu_0 g \rho_m}{\chi_m - \chi_{bead}}\left(\frac{d}{2B_0}\right)^2 + \frac{d}{2} \tag{S1c}$$

Solving for $\chi_m - \chi_{bead}$, and ρ_m gave values of 8.400×10^{-5} and 1.099 g·mL^{-1}, respectively.

Figure S1. Determining the magnetic susceptibility and density of the standard levitation buffer. The levitation height of seven density standard beads is plotted against the density of the beads. The linear fit can be used to estimate the magnetic susceptibility and density of the levitation buffer.



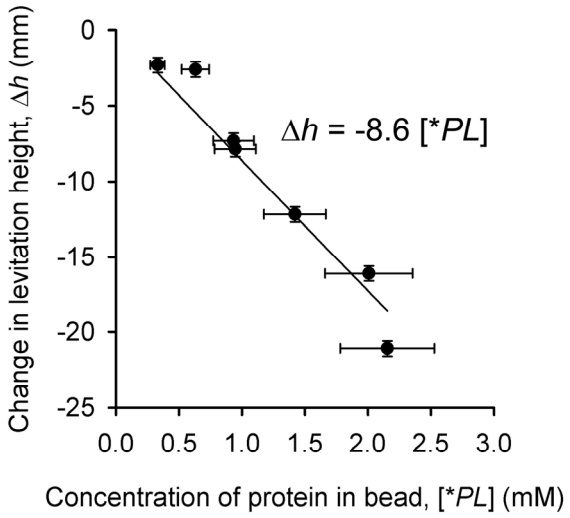
Quantifying the Amount of Protein Bound Per Bead Using MagLev.

A useful feature of MagLev is its ability to correlate the amount of substance covalently attached to bead with the levitation height of the bead.[1] We aimed to extend this strategy to non-covalent interactions by building a calibration curve to correlate empirically the amount of protein bound per bead with the levitation height of the bead. We also examine this correlation analytically in the main text using Eqns. 2-4.

We performed the following experiment to generate the plot in Figure S2: We suspended a batch of beads (100 beads) in a solution of the standard levitation buffer containing 50 μM BCA. Periodically, we removed a set of these beads (~15-20 beads), rinsed them, and levitated them in a fresh solution of the standard levitation buffer. We measured the levitation heights and diameters of the beads, which we used to calculate the total volume of the beads used for each experiment. We then transferred the beads to a 1 mL solution of PBS (10 mM phosphate) containing 1 mM $CF_3SO_2NH_2$, a ligand with high affinity for BCA ($K_d \sim 2 – 13$ nM).[2] After incubating the beads in this solution for one hour, we quantified the amount of protein displaced from the beads my measuring the absorbance of the solution at 280 nm ($\varepsilon = 55300\ M^{-1}\cdot cm^{-1}$)[2]. Control experiments showed that no additional protein was displaced with longer incubation times. We then calculated the concentration of protein present in the beads from the amount of protein displaced and the volume of the beads. Figure S2 correlates changes in levitation height with the concentration of protein present in the beads.

We analyzed these results using Eqn. 4. From this experiment, the change in levitation height, Δh, is related to the concentration of protein bound in the bead, [*PL], by a linear relationship: $\Delta h = -8.6[*PL]$ (note that the slope is expressed in units of $mm\cdot mM^{-1}$).

Figure S2. Quantitative measurement of carbonic anhydrase binding to resin-bound *p*-carboxy benzenesulfonamide. The plot shows the dependence of the change in levitation height (Δh) on the concentration of protein bound in the beads. Vertical error bars are equal to the error in measurements of levitation height (± 0.1 mm)



Determining the Diffusion Coefficient of BCA in PEGA beads and the Partition Coefficient of BCA Between the Beads and Solution.

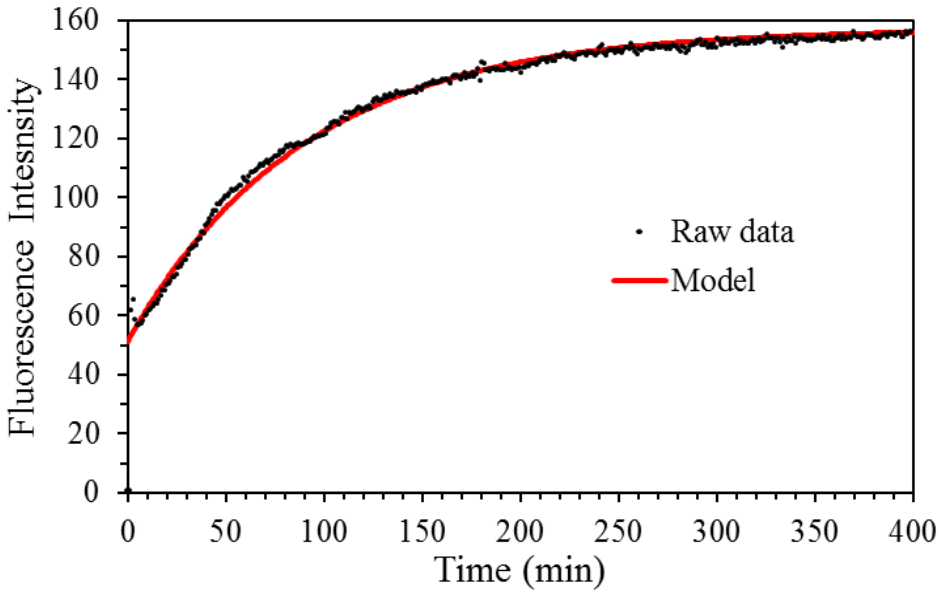
Briefly, we functionalized PEGA beads with *N,N*-dimethyl benzene sulfonamide **2**, which has a very low affinity for BCA (K_d > 10 mM).[2] These beads were soaked in a solution of FITC-BCA (110 μM) and allowed to reach equilibrium over five days. The beads were transferred to a solution of 1 x PBS buffer (137 mM NaCl, 2.7 mM KCl, 11.8 mM phosphate, pH 7.4). Diffusion of FITC-BCA from the beads to the solution resulted in an increase in the fluorescence of the solution over time (Figure S3). From this plot, we determined the diffusion coefficient by measuring the average size of the beads and fitting the kinetic data to Eqn. S1.[3]

$$\frac{M_{sol}}{M_{\infty}} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}e^{-\frac{D_{bead}n^2\pi^2 t}{R^2}} \qquad \text{(S1)}$$

In this equation, M_{sol} and M_{∞} are the amounts of protein in the solution at time t and at steady-state respectively. Using this approach, we estimate the diffusion coefficient (D_{bead}) of BCA in PEGA gel to be ~ 5 x 10^{-13} $m^2 \cdot s^{-1}$, which is approximately two orders of magnitude slower than the diffusion constant in water (~ 9 x 10^{-11} $m^2 \cdot s^{-1}$).[4] Similarly, the partition coefficient of the protein between the beads and solution ($K_{bead/sol}$) was determined by comparing the concentration of BCA in the beads, $[BCA]_{bead}$, with the concentration of BCA in the protein stock solution, $[BCA]_{stock}$ (Eqn. S2). The concentration of BCA in the beads was calculated from the concentration of BCA in the PBS buffer after the beads were added and the protein was fully desorbed, $[BCA]_{sol}$, and the volume of the beads that were added, V_{beads}. We estimate the partition coefficient ($K_{bead/sol}$) to be ~ 0.4.

$$K_{\frac{bead}{sol}} = \frac{[BCA]_{bead}}{[BCA]_{stock}} = \frac{[BCA]_{sol}}{V_{beads}[BCA]_{stock}} \qquad \text{(S2)}$$

Figure S3. Diffusion of protein from PEGA beads to solution. We suspended PEGA beads (functionalized with sulfonamide **2**) in a solution of FITC-labeled BCA (110 μM) and allowed the beads and solution to reach equilibrium (5 days). We then transferred the beads to a solution of 1 x PBS buffer. This figure shows the resulting increase in the fluorescence of the solution over time as FITC-BCA diffuses from the beads to the solution.



Separating PEGA Beads Based on Their Diameters.

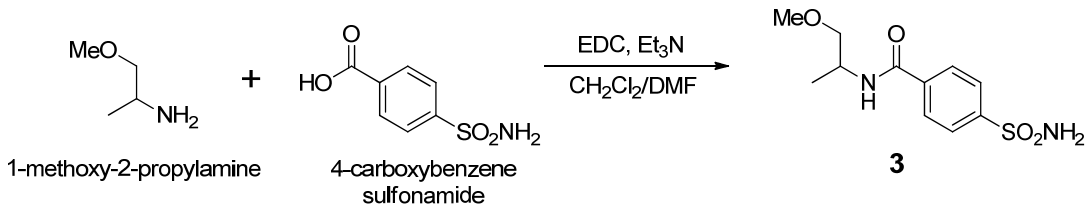
We filtered a batch of carboxybenzene sulfonamide **1** functionalized rhodamine-dyed PEGA beads through several sizes of polyester mesh. The beads were first vacuum filtered through 45.7 x 45.7 mesh (openings per sq. inch, 375 micron rating). The largest beads remained on the top of the mesh. The remaining smaller beads were subsequently vacuum filtered through 86 x 86 mesh (143 micron rating). Only the smallest beads passed through this filter and were collected as the batch of small beads.

Quantification of Gadolinium Partitioning.

We suspended a batch of beads in a solution of the standard levitation buffer and allowed the system to equilibrate for 3 days. We subsequently measured the magnetic susceptibility (SI units) of both the beads ($\chi = 4.9 \times 10^{-5}$) and the solution ($\chi = 6.9 \times 10^{-5}$) using a Magnetic Susceptibility Balance Mark 1 (Johnson Matthey). While the value for the magnetic susceptibility of the solution is slightly higher, this is to be expected considering that the beads contain some portion of polymer. The partition coefficient of gadolinium between the beads and the solution is, therefore, assumed to be unity.

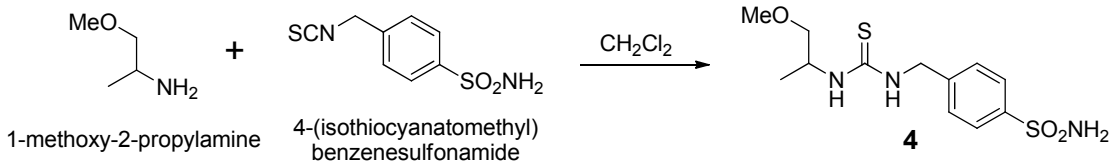
Synthesis of Solution-Phase Analogues of the Immobilized Ligands.

In order to provide a basis for comparing the observed dissociation constants of BCA with the on-bead ligands, we synthesized benzenesulfonamides **3 – 6** (as racemates) as analogues of the on-bead ligands.



1-methoxy-2-propylamine 4-carboxybenzene sulfonamide **3**

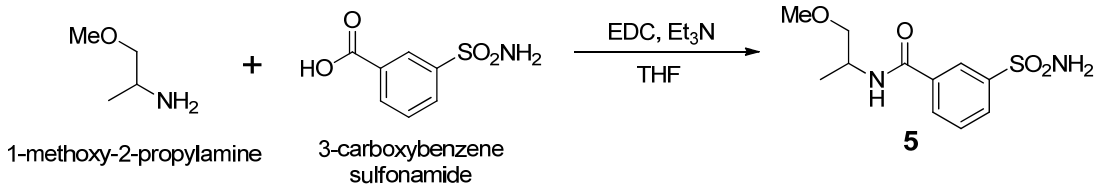
To a stirred solution of 4-carboxybenzene sulfonamide (760 mg, 3.8 mmol, 1 equiv) dissolved in 15 mL CH_2Cl_2:DMF (1:2) was added sequentially triethylamine (0.75 mL, 5.4 mmol, 1.4 equiv), 1-methoxy-2-propylamine (0.80 mL, 7.6 mmol, 2 equiv.), and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC, 880 mg, 5.7 mmol, 1.5 equiv.). The reaction mixture was stirred at room temperature for 18 hours, diluted with ethyl acetate (10 mL) and saturated aqueous ammonium chloride (10 mL). The organic and aqueous layers were separated, and the aqueous layer was extracted with ethyl acetate (2 x 15 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude mixture was purified by flash column chromatography on silica gel (CH_2Cl_2 → 5% MeOH in CH_2Cl_2) to yield the desired sulfonamide as a white solid (550 mg, 53% yield). ^{1}H NMR (DMSO-d_6, 500 Mhz): δ 8.41 (d, 1H, J = 7.8 Hz), 7.99 (app d, 2H, J = 8.7 Hz), 7.89 (app d, 2H, J = 8.7 Hz), 7.47 (bs, 2H), 4.20 (septet, 1H, J = 6.8 Hz), 3.41 (dd, 1H, J = 9.6, 6.4 Hz), 3.30 (dd, 1H, J = 9.6, 6.4 Hz), 3.27 (s, 3H), 1.15 (d, 3H, J = 6.8 Hz). ^{13}C NMR (DMSO-d_6, 125 Mhz): δ 164.7, 146.1, 137.5, 127.9, 125.5, 74.9, 58.1, 44.7, 17.2. HRMS (EI) calc. for $[C_{11}H_{16}N_2O_4S + H]^+$ 273.0904, found 237.0903.



1-methoxy-2-propylamine 4-(isothiocyanatomethyl) benzenesulfonamide **4**

To a stirred solution of 1-methoxy-2-propylamine (52 μL, 0.5 mmol, 1.3 equiv.) in CH_2Cl_2 (5 mL) at room temperature was added 4-(isothiocyanatomethyl)benzenesulfonamide (87 mg, 0.38 mmol, 1 equiv.). The reaction mixture was stirred overnight, concentrated, and purified by flash

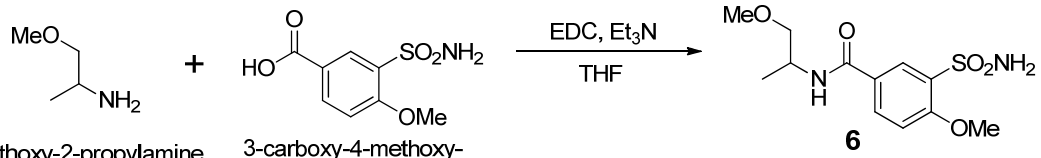
column chromatography on silica gel (CH_2Cl_2 → 60% EtOAc in CH_2Cl_2) to yield the desired sulfonamide as a white solid (109 mg, 90% yield). ^{1}H NMR (CD_3OD, 500 Mhz): δ 7.85 (app d, 2H, J = 8.2 Hz), 7.47 (app d, 2H, J = 8.2 Hz) 4.82 (s, 2H), 4.47 (bs, 1H), 3.43 (dd, 1H, J = 9.6, 5.0 Hz), 3.39 (dd, 1H, J = 9.6, 5.0 Hz), 3.34 (s, 3H), 1.19 (d, 3H, J = 6.4 Hz). ^{13}C NMR (CD_3OD, 125 Mhz): δ 145.2, 143.7, 131.2, 128.9, 127.4, 76.8, 59.4, 51.0, 48.3, 17.7. HRMS (EI) calc. for $[C_{12}H_{19}N_3O_3S_2 + H]^+$ 318.0941, found 318.0947.



1-methoxy-2-propylamine

3-carboxybenzene sulfonamide

To a stirred solution of 3-carboxybenzene sulfonamide (65 mg, 0.32 mmol, 1 equiv) dissolved in 1.5 mL tetrahydrofuran was added sequentially triethylamine (0.20 mL, 1.4 mmol, 4.5 equiv), 1-methoxy-2-propylamine (170 μL, 1.6 mmol, 5 equiv.), and EDC (124 mg, 0.65 mmol, 2 equiv.). The reaction mixture was stirred at room temperature for 18 hours, diluted with ethyl acetate (5 mL) and saturated aqueous ammonium chloride (4 mL). The organic and aqueous layers were separated, and the aqueous layer was extracted with ethyl acetate (2 x 5 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude mixture was purified by flash column chromatography on silica gel (30% EtOAc in CH_2Cl_2 → 100% EtOAc) to yield the desired sulfonamide as a white solid (50 mg, 57% yield). ^{1}H NMR (DMSO-d_6, 500 Mhz): δ 8.49 (d, 1H, J =7.8 Hz), 8.31 (s, 1H), 8.05 (d, 1H, J = 7.0 Hz), 7.96 (d, 1H, J = 7.8 Hz), 7.67 (t, 1H, J = 7.8 Hz), 7.43 (bs, 2H), 4.23 (septet, 1H, J = 7.0 Hz), 3.43 (dd, 1H, J = 9.6, 6.4 Hz), 3.31 (dd, 1H, J = 9.6, 6.4 Hz), 3.27 (s, 3H), 1.15 (d, 3H, J = 7.0 Hz). ^{13}C NMR (DMSO-d_6, 125 Mhz): δ 164.6, 144.3, 135.3, 130.3, 129.0, 128.0, 124.7, 74.9, 58.1, 44.6, 17.2. HRMS (EI) calc. for $[C_{11}H_{16}N_2O_4S + H]^+$ 273.0904, found 273.0905.



To a stirred solution of 3-carboxy-4-methoxybenzene sulfonamide (123 mg, 0.53 mmol, 1 equiv) dissolved in 1.5 mL tetrahydrofuran was added sequentially triethylamine (0.30 mL, 2.2 mmol, 4.1 equiv), 1-methoxy-2-propylamine (280 μL, 2.7 mmol, 5 equiv.), and EDC (204 mg, 1.1 mmol, 2 equiv.). The reaction mixture was stirred at room temperature for 18 hours, diluted with ethyl acetate (5 mL) and saturated aqueous ammonium chloride (4 mL). The organic and aqueous layers were separated, and the aqueous layer was extracted with ethyl acetate (2 x 5 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude mixture was purified by flash column chromatography on silica gel (30% EtOAc in CH_2Cl_2 → 100% EtOAc) to yield the desired sulfonamide as a white solid (115 mg, 72% yield). ^{1}H NMR (DMSO-d_6, 500 Mhz): δ 8.15-8.10 (m, 2H), 7.89-7.85 (m, 1H), 7.33-7.28 (m, 3H), 4.16 (septet, 1H, J = 6.8 Hz), 3.94 (s, 3H), 3.41 (dd, 1H, J = 9.6, 5.5 Hz), 3.31 (dd, 1H, J = 9.6, 5.5 Hz), 3.30(s, 3H), 1.15 (d, 3H, J = 6.8 Hz). ^{13}C NMR (DMSO-d_6, 125 Mhz): δ 164.0, 159.7, 136.9, 130.3, 128.9, 124.3, 113.1, 75.6, 59.0, 57.3, 45.3, 18.1. HRMS (EI) calc. for $[C_{12}H_{18}N_2O_5S + H]^+$ 303.1009, found 303.0998.

Determination of the Binding Affinity of Solution-Phase Analogues of the Immobilized Ligands for BCA.

Using fluorescence titration,[5] we measured the dissociation constants of sulfonamides **3-6** from BCA in the standard levitation buffer (Table S1).

Table S1. Dissociation constants of sulfonamides **3** – **6** from BCA in the standard levitation buffer, as measured using fluorescence titration.

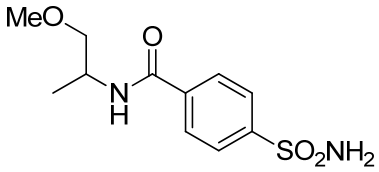
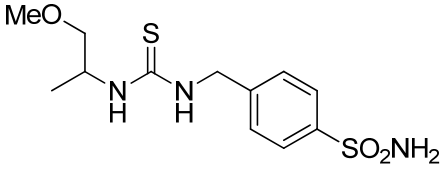
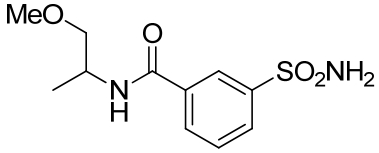
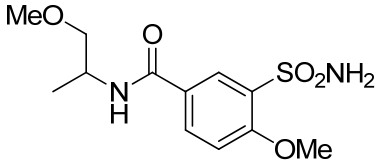
Compound		K_d (μM)
	**3**	0.70
	**4**	0.10
	**5**	12
	**6**	56

Figure S4. Detecting BCA using MagLev. Shown here is a reprint of Figure 7A, with a logarithmic concentration axis. For thesef experiments, beads were levitated in the high sensitivity levitation buffer. We incubated individual beads, functionalized with sulfonamide **1**, in solutions (20 μL) of BCA (40 nM – 40 μM) dissolved in 1 x PBS at room temperature (20 °C), for either 3 or 24 hours. After incubation, we transferred the beads into microcuvettes containing the levitation buffer described above, and measured their levitation heights (in comparison to a bead that had been incubated in a solution free of BCA). We then repeated this experiment, changing the incubation media to whole blood. The error bars show one standard deviation (n = 7).

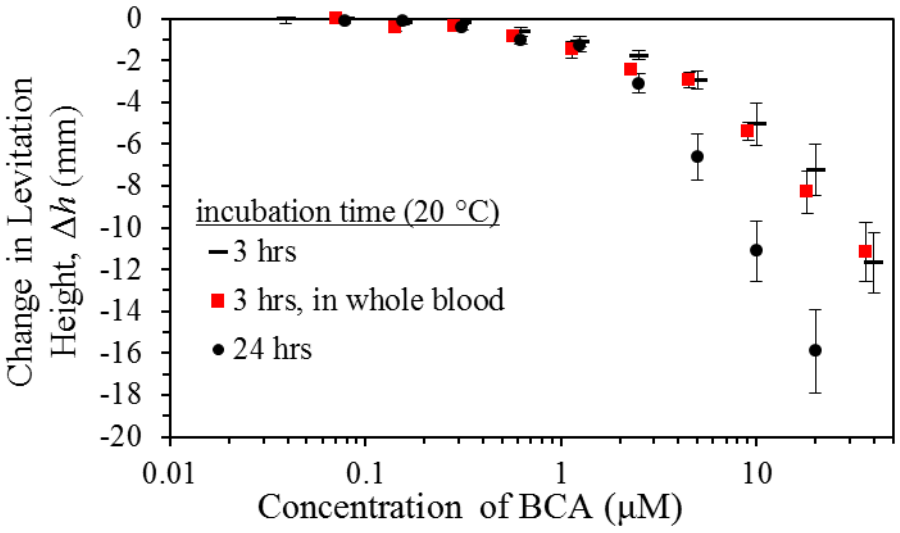


Figure S5. Preliminary screen of dyes for a MagLev-based multiplexed protein-ligand binding assay. Ten percent of the available amines on 300-500 μm PEGA-NH_2 beads were covalently labeled with (from left to right) Reactive Black 5, Reactive Blue 4, DAC isothiocyanate (ITC), Malachite Green ITC, Fluorescein ITC, Reactive yellow 2, 4-chloro-7-nitrobenzofurazan, Eosin ITC, Rhodamine B ITC, Cibacron Brilliant Red 3B-A, Procion Red MX-5B. Photographs of the beads under visible light (**A**) and UV light irradiation (**B**).

A

B

References

(1) Mirica, K. A.; Phillips, S. T.; Shevkoplyas, S. S.; Whitesides, G. M. *J. Am. Chem. Soc.* **2008**, *130*, 17678–17680.
(2) Krishnamurthy, V. M.; Kaufman, G. K.; Urbach, A. R.; Gitlin, I.; Gudiksen, K. L.; Weibel, D. B.; Whitesides, G. M. *Chem. Rev*. **2008**, *108*, 946–1051.
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(4) Tyn, M. T.; Gusek, T. W. *Biotech. Bioeng*. **1990**, *35*, 327.
(5) (a) Wang, Z.-X. *FEBS Lett.* **1995,** *360*, 111-114. (b) V. M. Krishnamurthy, V. Semetey, P. J. Bracher, N. Shen, G. M. Whitesides, *J. Am. Chem. Soc.* **2007**, *129*, 1312-1320.

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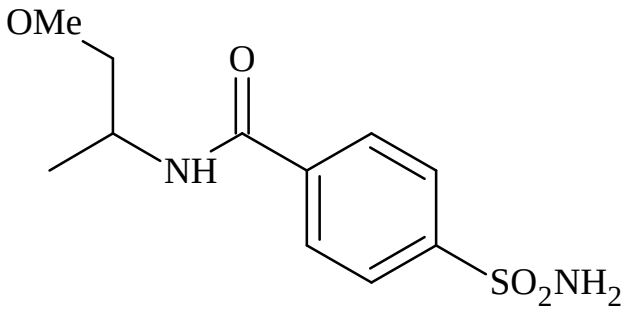

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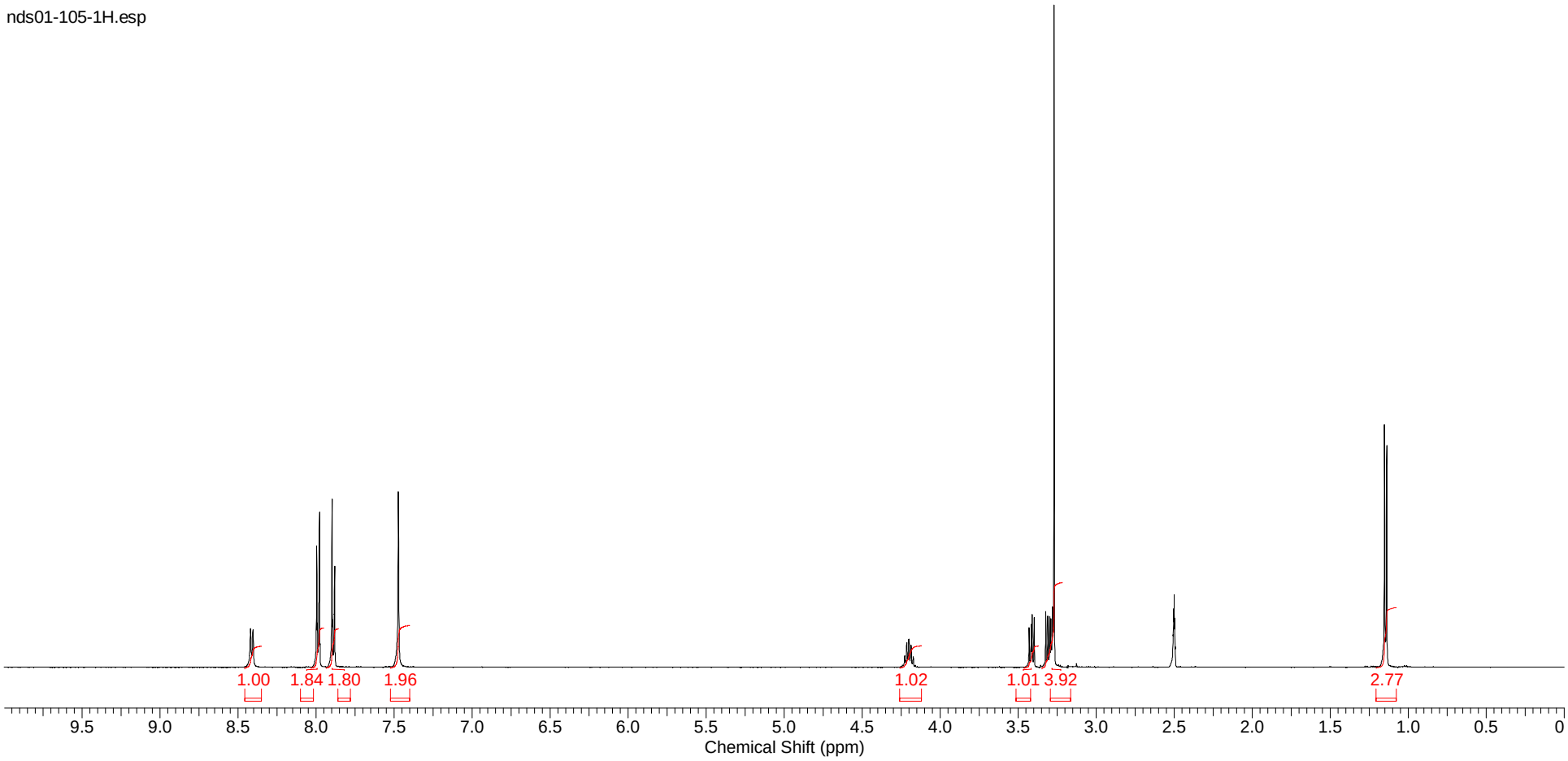

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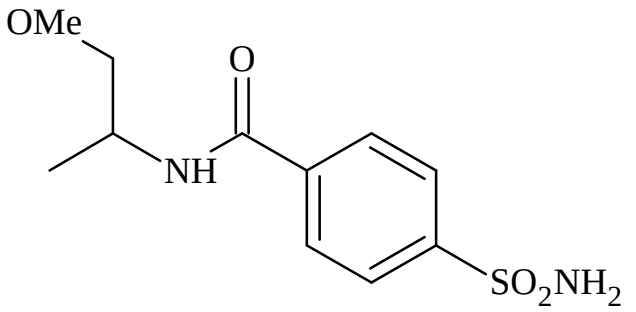

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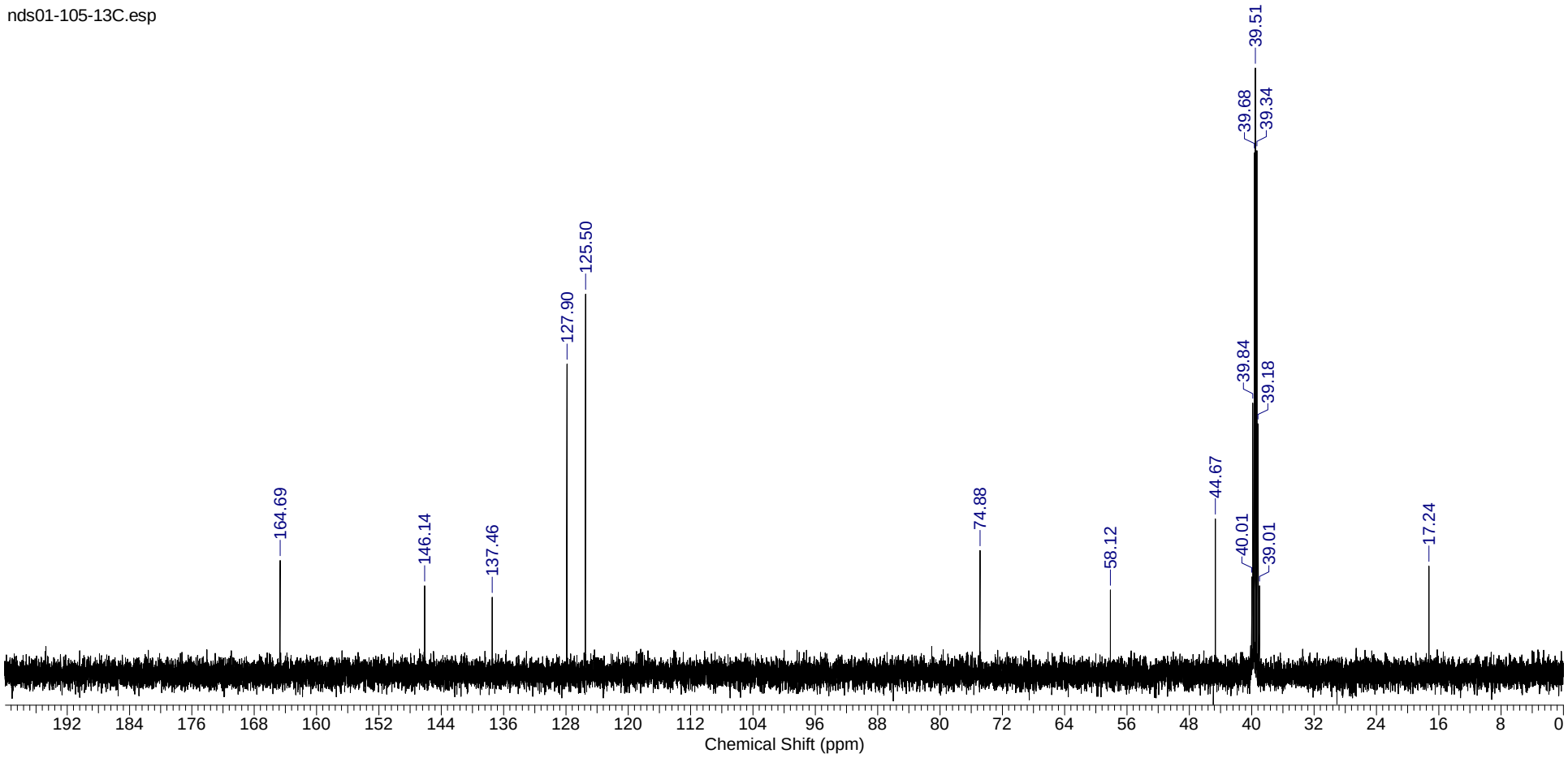

Acquisition Time (sec)	2.1842	***Comment***	S/N = 351	***Date***	Nov 18 2011	***Date Stamp***	Nov 18 2011
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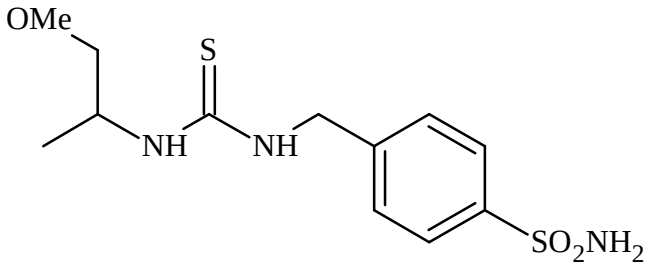
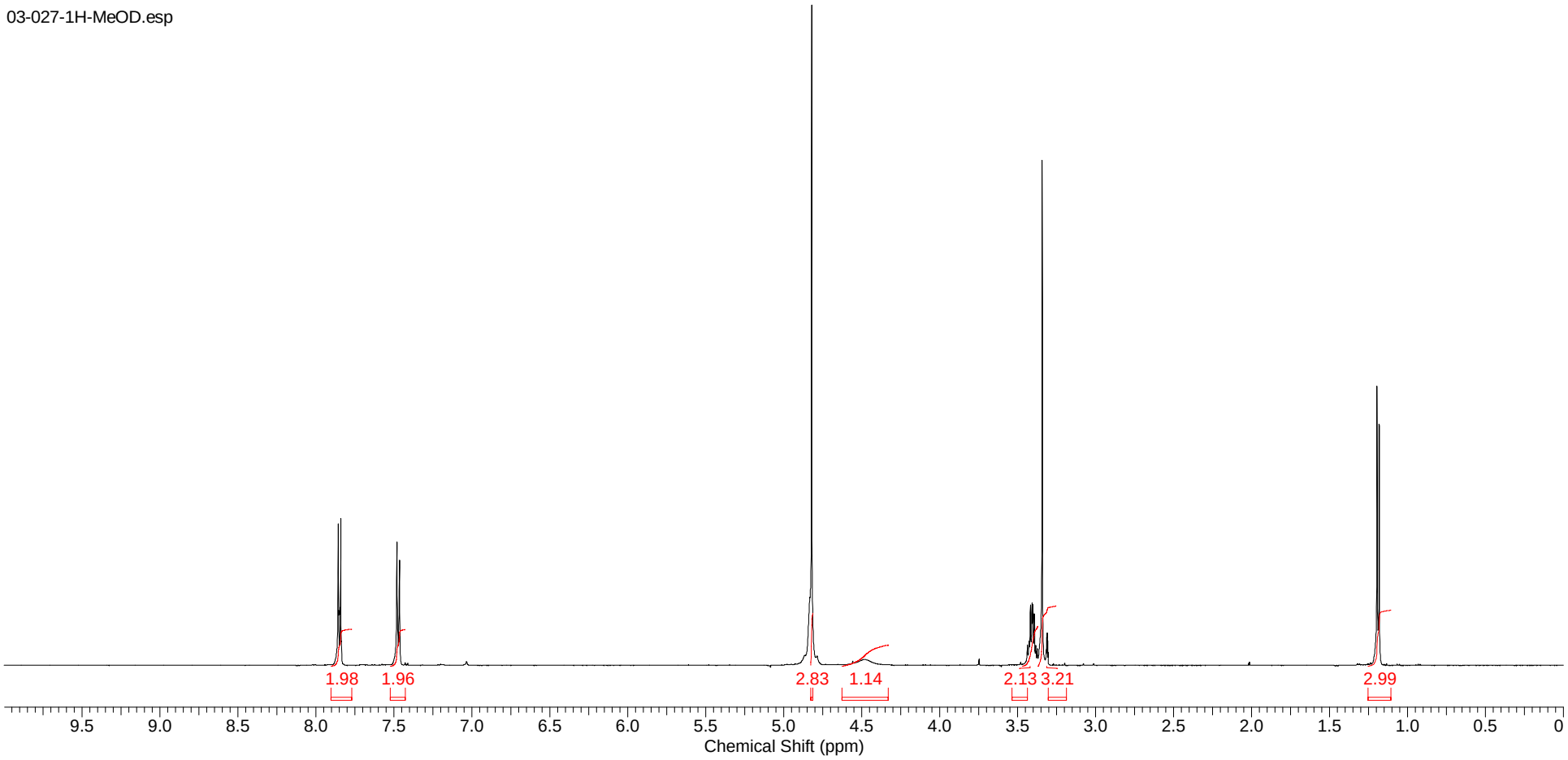
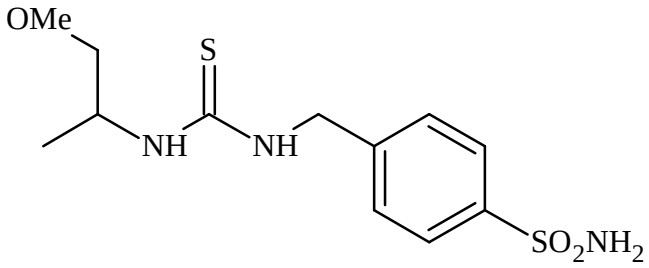
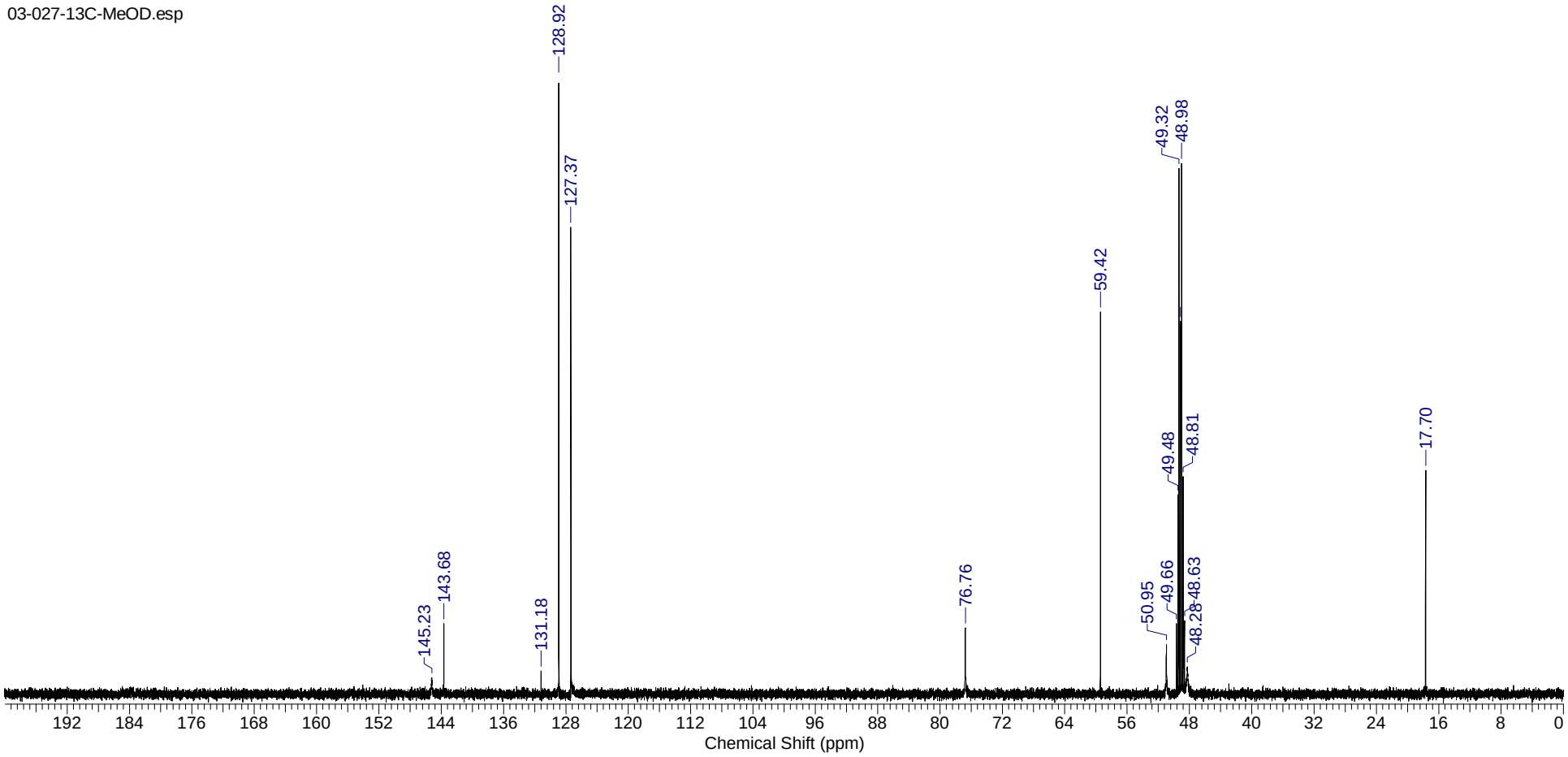
Nucleus	1H	***Number of Transients***	4	***Original Points Count***	16384	***Points Count***	16384
Pulse Sequence	s2pul	***Receiver Gain***	28.00	***Solvent***	METHANOL-d4		
Spectrum Offset (Hz)	3292.5981	***Spectrum Type***	STANDARD	***Sweep Width (Hz)***	7501.17	***Temperature (degree C)***	25.000



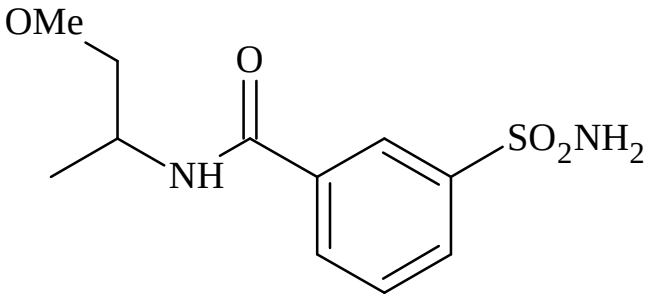


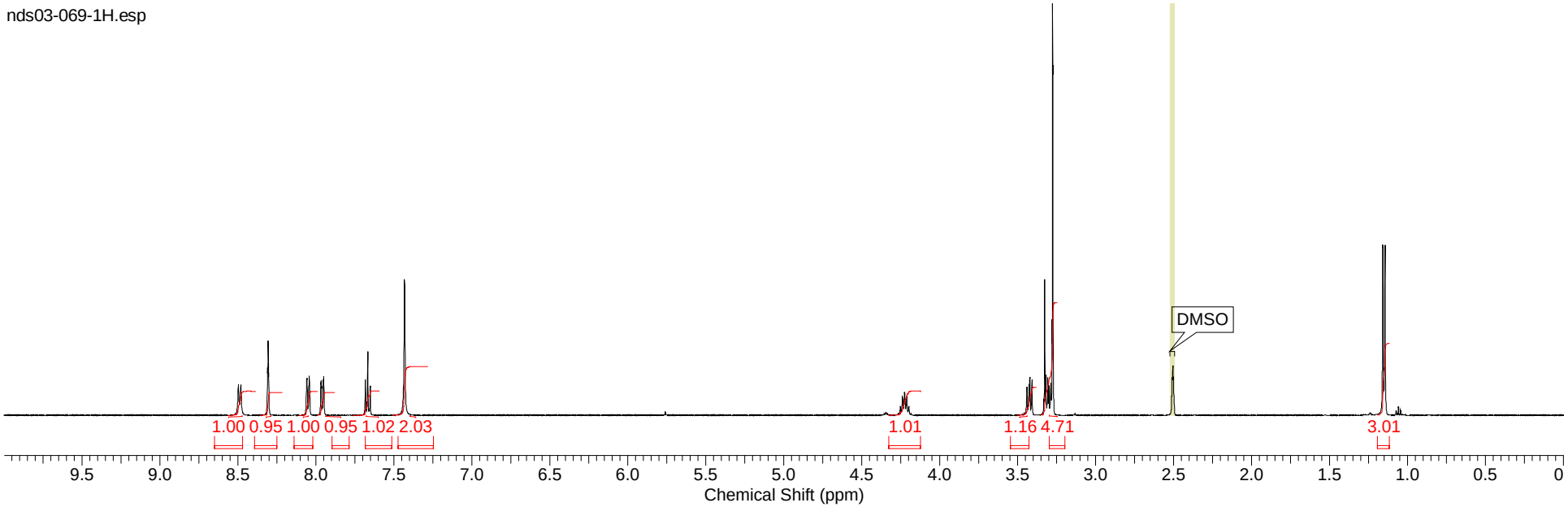
Acquisition Time (sec)	1.0924	***Comment***	STANDARD CARBON PARAMETERS			***Date***	Nov 18 2011
Date Stamp	Nov 18 2011	***File Name***	C:\Users\Nathan\Documents\Dropbox\NMR\nds\03-027-13C-MeOD.fid\fid				
Frequency (MHz)	125.71	***Nucleus***	13C	***Number of Transients***	717	***Original Points Count***	32768
Points Count	32768	***Pulse Sequence***	s2pul	***Receiver Gain***	60.00	***Solvent***	METHANOL-d4
Spectrum Offset (Hz)	14100.2783	***Spectrum Type***	STANDARD	***Sweep Width (Hz)***	29996.25	***Temperature (degree C)***	25.000





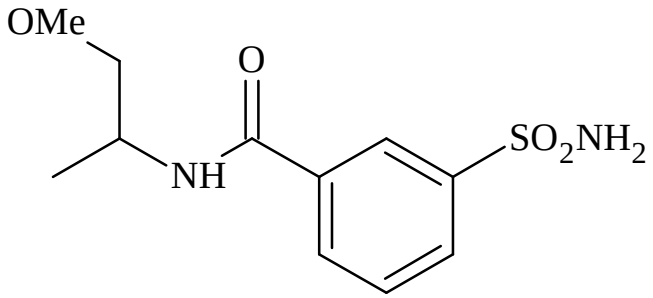
Acquisition Time (sec)	2.1842	***Comment***	S/N = 351	***Date***	Oct 25 2011	***Date Stamp***	Oct 25 2011
File Name	C:\Users\Nathan\Documents\Dropbox\NMR\nds\nds03-069-1H.fid\fid					***Frequency (MHz)***	499.88
Nucleus	1H	***Number of Transients***	8	***Original Points Count***	16384	***Points Count***	16384
Pulse Sequence	s2pul	***Receiver Gain***	40.00	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	3299.4624
Spectrum Type	STANDARD	***Sweep Width (Hz)***	7501.17	***Temperature (degree C)***	25.000		



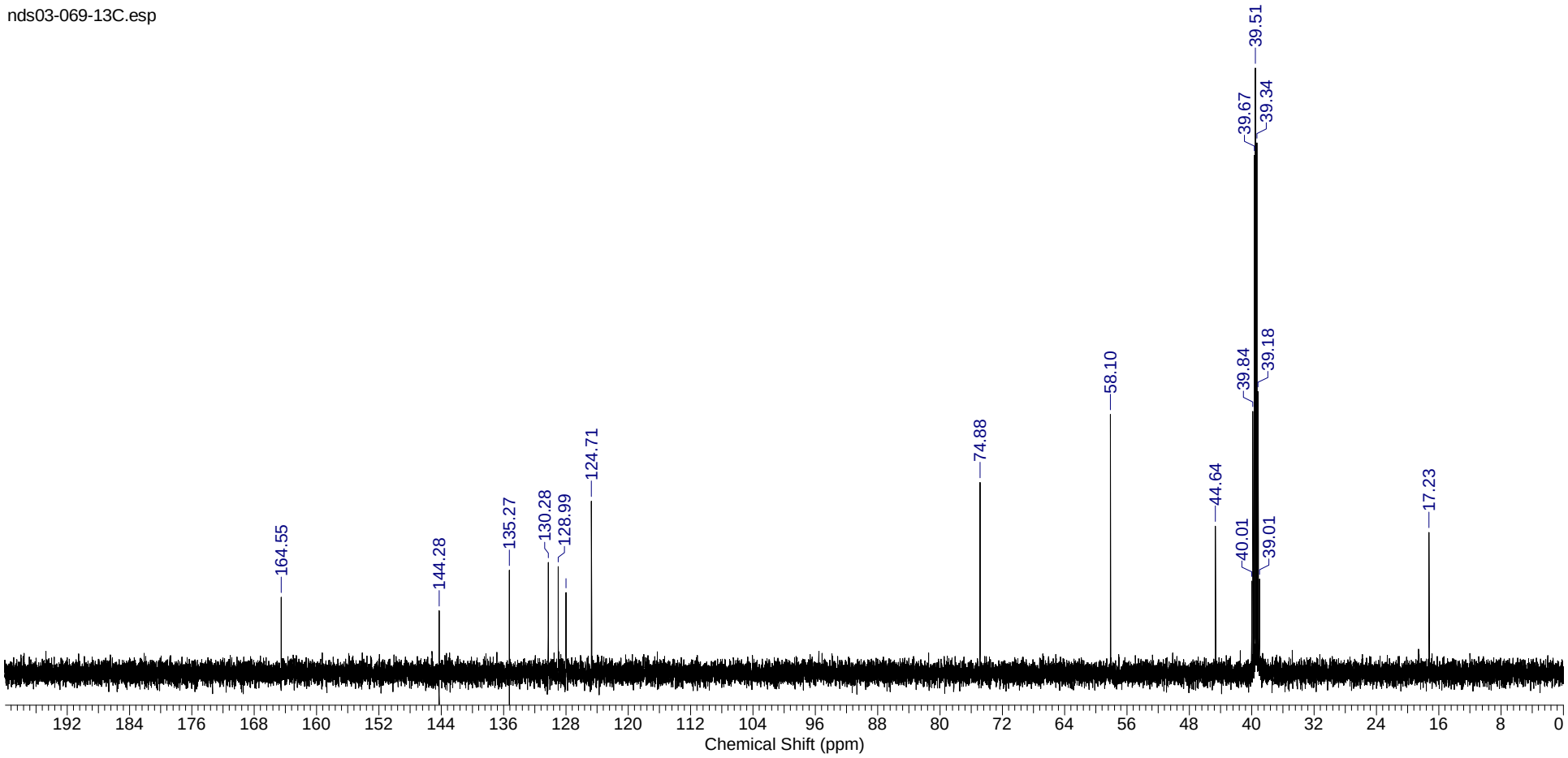


No.	(ppm)	Annotation	Layer No.	Created By	Created At	Modified By	Modified At
1	[2.49 .. 2.52]	DMSO	1	Nathan	Fri 11/18/2011 12:47:27 PM		

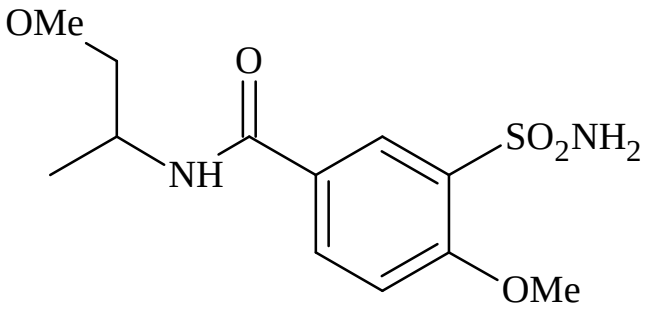
Acquisition Time (sec)	1.0924	***Comment***	STANDARD CARBON PARAMETERS			***Date***	Oct 25 2011
Date Stamp	Oct 25 2011	***File Name***	C:\Users\Nathan\Documents\Dropbox\NMR\nds\nds03-069-13C.fid\fid				
Frequency (MHz)	125.71	***Nucleus***	13C	***Number of Transients***	197	***Original Points Count***	32768
Points Count	32768	***Pulse Sequence***	s2pul	***Receiver Gain***	60.00	***Solvent***	DMSO-d6
Spectrum Offset (Hz)	13851.5078	***Spectrum Type***	STANDARD	***Sweep Width (Hz)***	29996.25	***Temperature (degree C)***	25.000



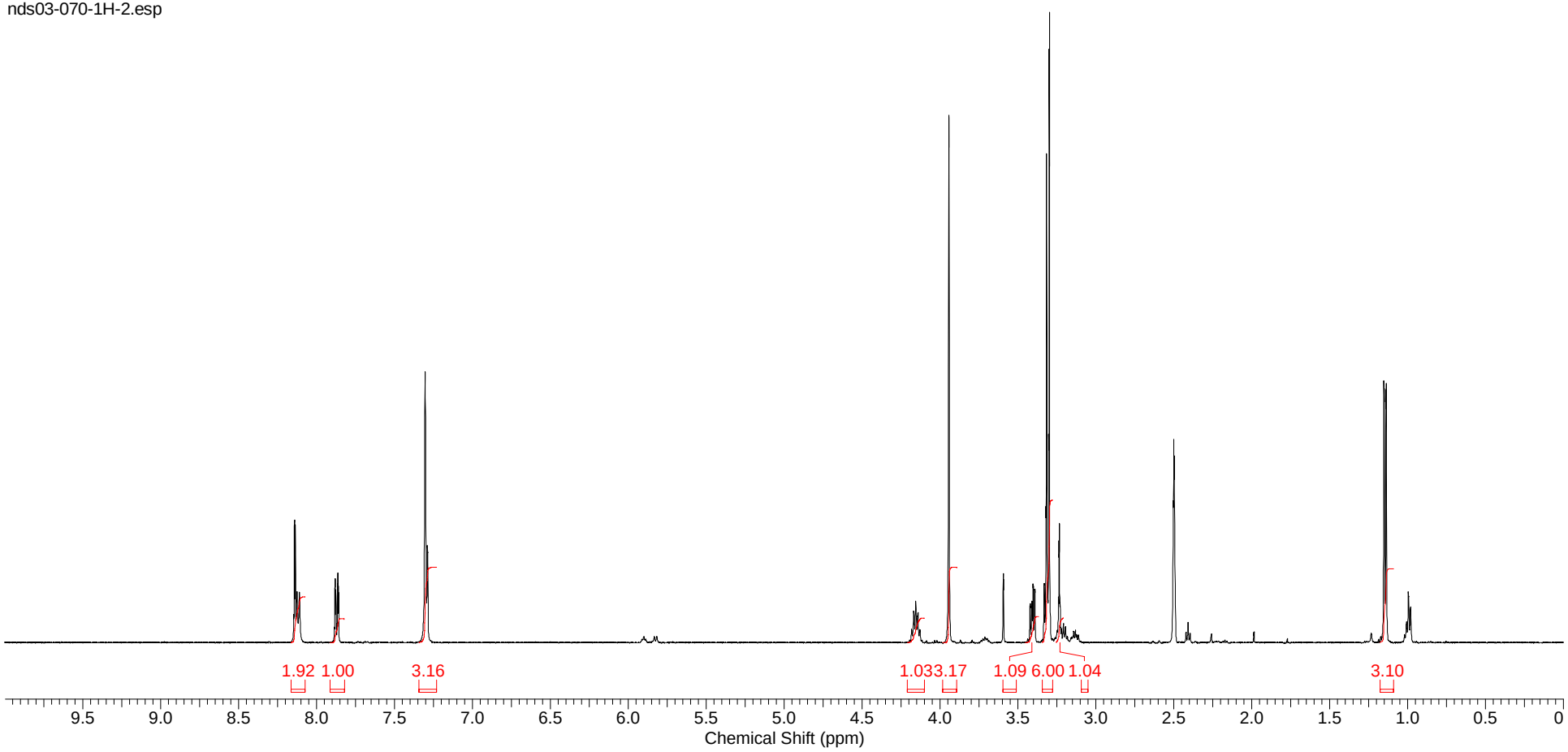
nds03-069-13C.esp



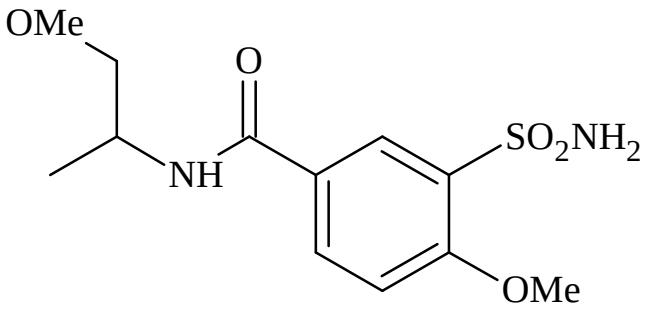
Acquisition Time (sec)	2.1842	***Comment***	S/N = 351	***Date***	Oct 25 2011	***Date Stamp***	Oct 25 2011
File Name	C:\Users\Nathan\Documents\Dropbox\NMR\nds\nds03-070-1H-2.fid\fid					***Frequency (MHz)***	499.88
Nucleus	1H	***Number of Transients***	8	***Original Points Count***	16384	***Points Count***	16384
Pulse Sequence	s2pul	***Receiver Gain***	40.00	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	3296.5706
Spectrum Type	STANDARD	***Sweep Width (Hz)***	7501.17	***Temperature (degree C)***	25.000		



nds03-070-1H-2.esp



Acquisition Time (sec)	1.0924	***Comment***	STANDARD CARBON PARAMETERS			***Date***	Oct 25 2011
Date Stamp	Oct 25 2011	***File Name***	C:\Users\Nathan\Documents\Dropbox\NMR\nds\nds03-070-13C.fid\fid				
Frequency (MHz)	125.71	***Nucleus***	13C	***Number of Transients***	351	***Original Points Count***	32768
Points Count	32768	***Pulse Sequence***	s2pul	***Receiver Gain***	60.00	***Solvent***	DMSO-d6
Spectrum Offset (Hz)	13942.3623	***Spectrum Type***	STANDARD	***Sweep Width (Hz)***	29996.25	***Temperature (degree C)***	25.000



nds03-070-13C.esp