SUPPLEMENTARY INFORMATION

# Biasing Reaction Pathways with Mechanical Force

**Charles R. Hickenboth, Jeffrey S. Moore, Scott R. White, Nancy R. Sottos, Jerome Baudry, and Scott R. Wilson**

## Table of Contents

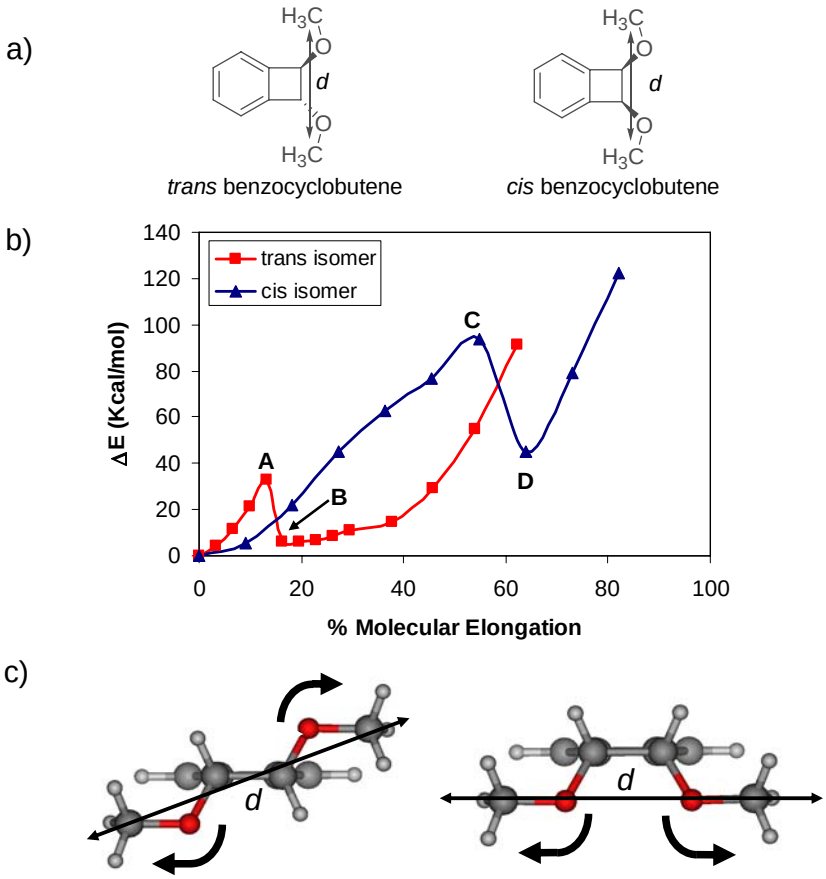



**Figure 1**. Results of the COGEF study of *trans* and *cis* benzocyclobutenes. a) To simulate the effect of mechanical force on benzocyclobutenes, the molecules were systematically elongated along the vector represented by the distance *d*. b) At each step, all other degrees of freedom were allowed to relax and the energy was calculated at the DFT B3LYP/6-31G* level. The energy versus percent molecular elongation was then plotted. Each isomer is predicted to undergo a force-induced ring opening to the E,E-*o*QDM intermediate. Percent molecular elongation is defined as $((d\text{-}d_0)/d_0)*100$. All *ab initio* calculation were performed using the program Gaussian 03 (Gaussian 03, Revision B.04, Gaussian, Inc., Pittsburgh PA, 2003.). Default criteria for convergence were used (i.e. threshold values for maximum force and RMS force of $4.5 * 10^{-4}$ and $3 * 10^{-4}$ au, respectively, and threshold values for maximum displacement and RMS displacement of $1.8 * 10^{-3}$ and $1.2 * 10^{-3}$ au, respectively). c) It was found that the simulated elongational tensile force distorts the two isomers in a different rotational sense. In the *trans* isomer (left), elongation along the vector represented by *d* induces a conrotatory torquing motion of the substituents, leading to the E,E-*o*QDM intermediate. In the *cis* isomer (right), elongation along the vector represented by *d* induces a disrotatory torquing motion of the substituents, again leading to the E,E-*o*QDM intermediate.

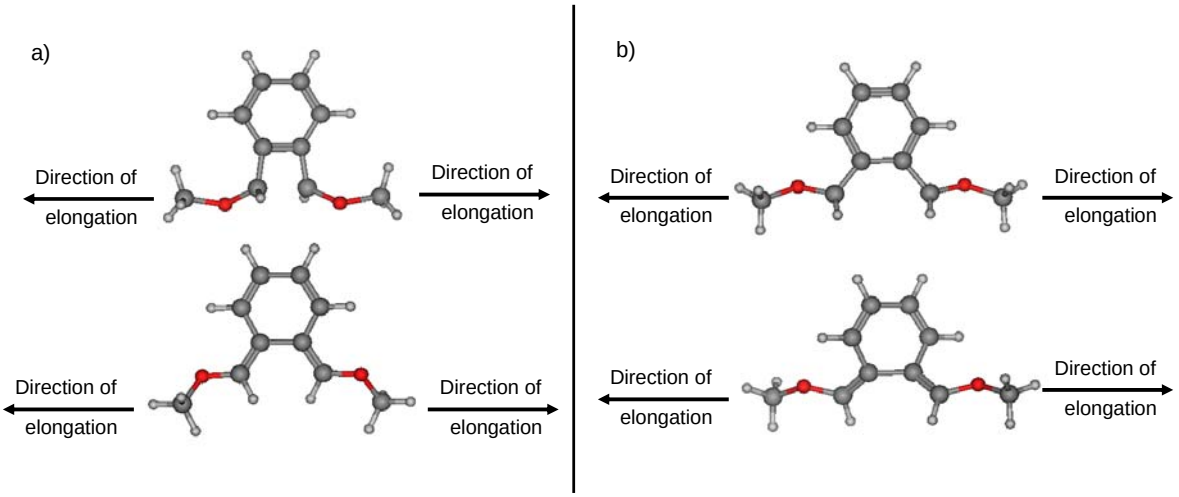



**Figure 2**. Molecular models displaying the force-induced transformation predicted by DFT B2LYP/6-31G* calculations. a) *Trans* benzocyclobutene at points A (top) and B (bottom) in Figure 1. b) *Cis* benzocyclobutene at point C (top) and D (bottom) in Figure 1. Coordinates were extracted from the Gaussian output in xyz format and converted to PDB format using the program VMD[1]; and then read using the program MOE (Chemical Computing Group Inc., Montreal, Canada) which assigns bond orders based on interatomic distances.

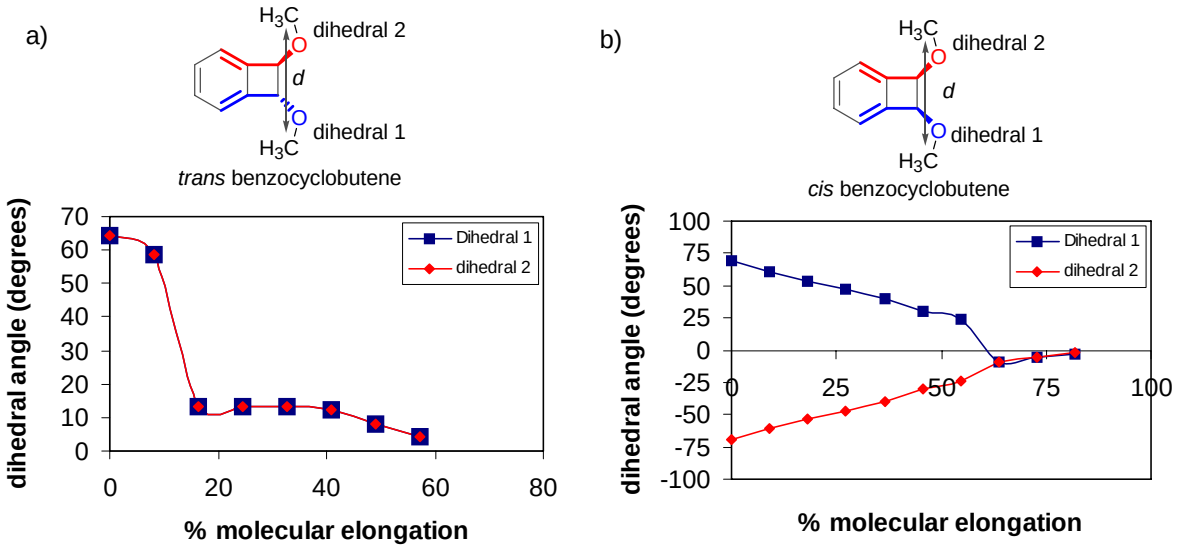



**Figure 3.** Plots of dihedral angle versus percent molecular elongation for a) *trans* and b) *cis* 1,2-dimethoxybenzocyclobutene calculated at the DFT B3LYP/6-31G* level. Percent molecular elongation is defined as $((d\text{-}d_0)/d_0)*100$. For the *trans* isomer, it is predicted that the simulated force induces a conrotatory torquing motion leading to the E,E-oQDM intermediate For the *cis* isomer, the simulated force induces a disrotatory torquing motion, leading to the same E,E-*o*QDM intermediate.

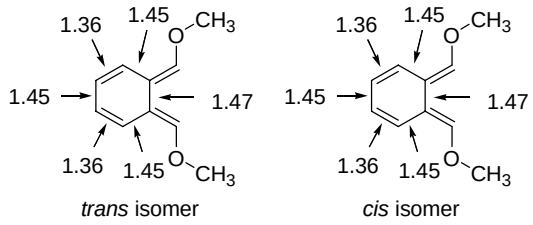



**Figure 4**. Computational results confirming that activation of both isomers with tensile force is predicted to yield the same E,E-*o*QDM intermediate for both isomers. The structure at points B and D in Figure 1 were minimized at the DFT B3LYP/6-31G* level without constraints. It was found that structures obtained differed only in their chirality (enantiomers due to an out-of-plane twist). This result supports the idea that the same intermediate is predicted to be formed under mechanochemical conditions from both *trans* and *cis* benzocyclobutenes.

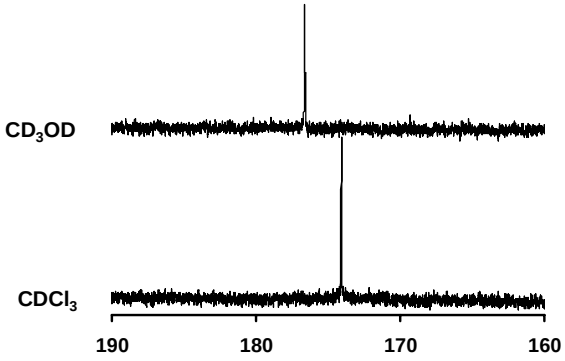



**Figure 5.** A portion of the $^{13}C$ NMR spectra showing a 1:1 mixture of polymers **3** and **4** after sonication in the presence of **5**$^{13}$**C**. In both $CDCl_3$ and $CD_3OD$ the mixture shows only a single peak. This is strong evidence that LFPs **3** and **4** yield the same Diels-Alder adduct, since it is highly improbable that the adduct formed from the E,E-*o*QDM and the E,Z-*o*QDM would have identical shifts in two different solvents. Both spectra were recorded on a spectrometer operating at a $^{13}C$ frequency of 125 MHz and at a concentration of about 12 mg/mL.

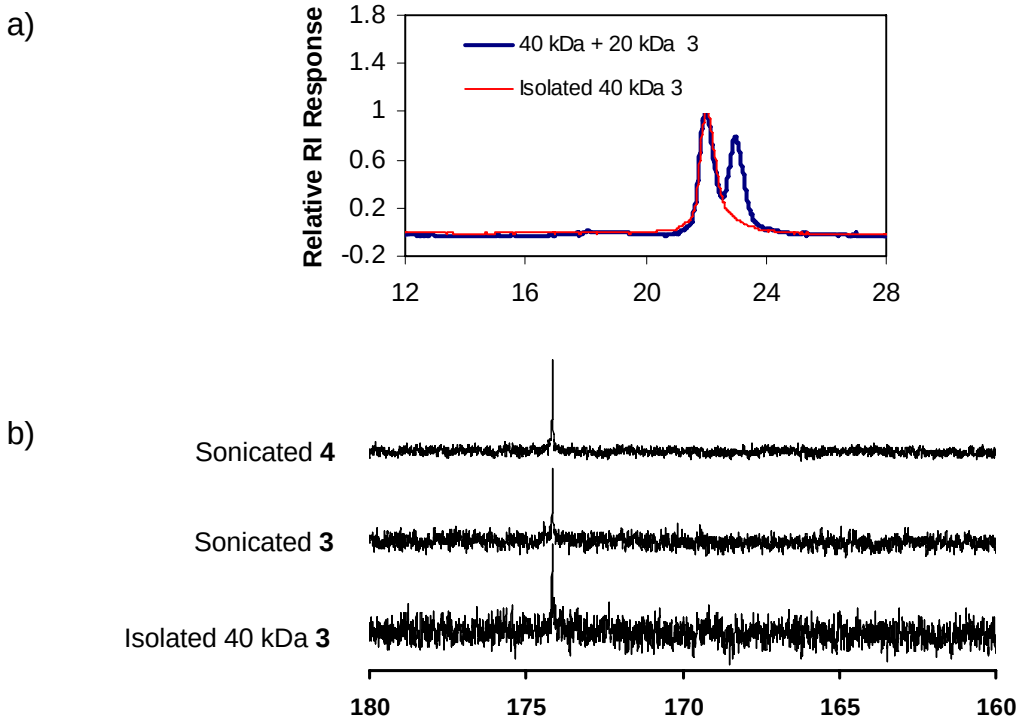



**Figure 6**. Results of experiment designed to detect benzocyclobutene activation without chain scission. **a)** Overlay of the GPC chromatograms (RI signal only) of 40 kDa LFP **3** after sonication (blue) and after isolation of the MW corresponding to 40 kDa chains (red). The crude mixture, immediately after sonication, consists of a bimodal mixture of 40 kDa and 20 kDa polymers, presumably corresponding to unbroken and broken chains, respectively. The 40 kDa material was then isolated by preparatory scale GPC and analyzed by $^{13}C$ NMR. **b)** A portion of the $^{13}C$ NMR spectra of the isolated 40 kDa chains after sonication of LFP **3** with that of polymers **3** and **4** prior to separation. The presence of the Diels-Alder adduct in the 40 kDa chains after sonication suggests that the benzocyclobutene unit can be activated without chain scission. All $^{13}C$ spectra were recorded on a spectrometer operating at a $^{13}C$ frequency of 125 MHz instrument in $CDCl_3$ at a concentration of approximately 12 mg/mL.

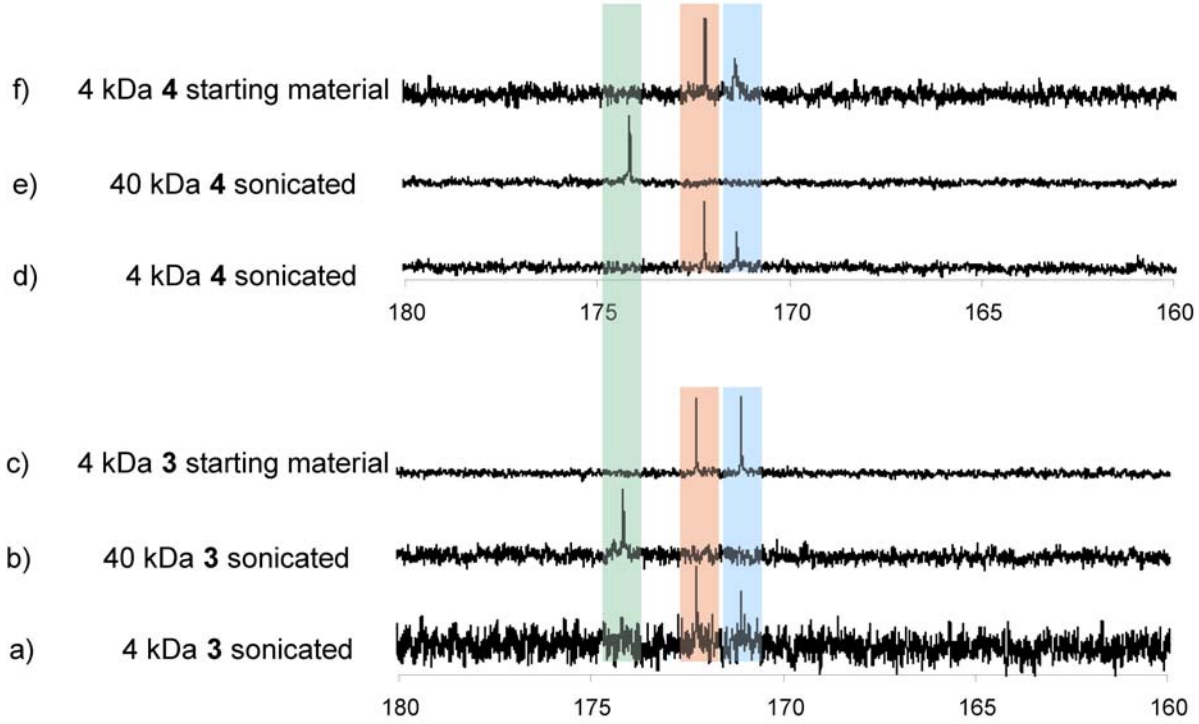



**Figure 7**. Portions of the $^{13}C$ NMR spectra of polymers **3** and **4** showing the MW dependence of formation of the *o*QDM intermediate, followed by cycloaddition to $\mathbf{5}^{\mathbf{13}}\mathbf{C}$. **a**) 4 kDa polymer **3** after sonication. **b**) 40 kDa polymer **3** after sonication. **c**) 4 kDa polymer **3** before sonication. **d**) 4 kDa polymer **4** after sonication. **e**) 40 kDa polymer **4** after sonication. **f**) 4 kDa polymer **4** before sonication. After sonication of both 4 kDa **3** and 4 kDa **4**, the only $^{13}C$ resonances observed are due to the amide carbonyl (colored red) and the ester carbonyl (colored blue) in the starting material. At a MW of 4 kDa, the concentration of the benzocyclobutene units is high enough to allow for detection at natural abundance. The resonance due to the Diels-Alder adduct is not observed (colored green in the spectra of 40 kDa **3** and **4**). In the case of 40 kDa **3** and **4**, the concentration of the benzocyclobutene units is too low to allow detection of the amide and ester carbonyls at natural abundance. All $^{13}C$ spectra were recorded on a spectrometer operating at a $^{13}C$ frequency of 125 MHz instrument in $CDCl_3$ at a concentration of approximately 12 mg/mL.

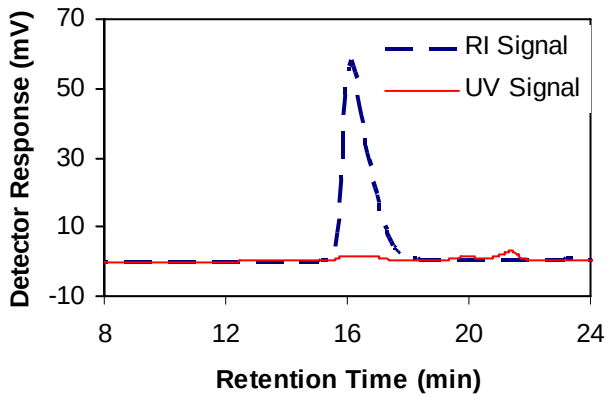



**Figure 8**. GPC chromatogram acquired after subjecting 40 kDa LFP **3** to all experimental conditions except sonication. No significant UV absorbance increase was observed, suggesting that ultrasound is necessary for the observed UV absorbance increase.

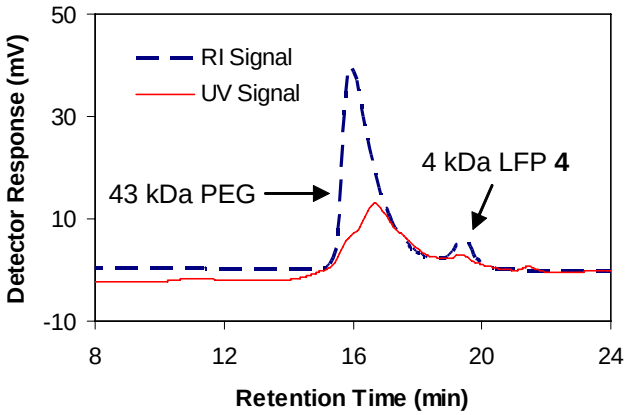



**Figure 9**. GPC chromatogram after a 1:1 mol ratio of 43 kDa PEG and 4 kDa LFP **4** was subjected to ultrasound. The final UV/RI ratio was consistent with the background reaction for both polymers. This result suggests that the linker must be embedded within a polymer and the polymer must be above the critical value to observe reactivity; mixing alone is not enough.

## Experimental

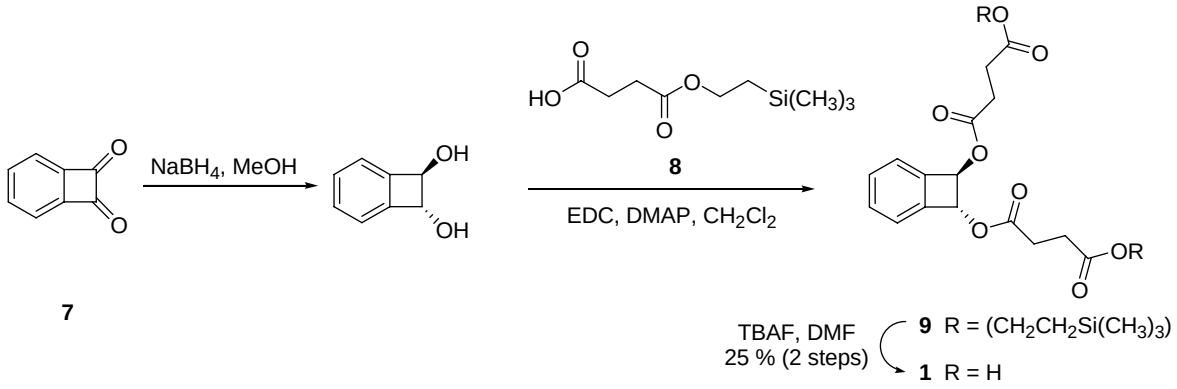
General Procedures: Unless otherwise stated, all starting materials and reagents were obtained from commercial suppliers and used without further purification. α-methoxy-ω-amino poly(ethylene glycol) was purchased from Nektar Transforming Therapeutics (2, 5, 10, 20 kDa). PEG standards were purchased from Polymer Labs. 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 98%) was purchased from TSI. 1,4-$^{13}$C-maleic anhydride (99% isotope purity) was purchased from Cambridge Isotope Labs. 4-(dimethylamino)pyridine (DMAP, 99%), 1-aminopyrene (95%), and TBAF (1.0 M soln in THF) were purchased from Aldrich. $ZnBr_2$ was purchased from Strem, and 1,1,1,3,3,3-hexamethyldisilazide was purchased from Acros. *N*,*N*'-dicyclohexylcarbodiimide (DCC) was obtained from Avocado. Reagent grade ether was purchased from Malinckrodt. Anhydrous methylene chloride was purchased from Acros and stored over 3Å molecular sieves. ACS grade acetonitrile was purchased from Aldrich and stored over 3Å molecular sieves.

Preparatory gel permeation chromatography (prep GPC) analyses were performed with a Waters 515 HPLC pump, a Waters 2487 UV detector, a 410 Differential Diffractometer, and a series of three Waters columns (19 X 300 mm, Ultrastyragel $10^4$ Å THF, $10^2$ Å THF, 500 Å THF) in a solution of THF (99.9%+, HPLC grade), inhibitor free. Analytical gel permeation chromatography (GPC) analyses were performed with a Waters 515 HPLC pump, a Viscotek TDA Model 300 triple detector array, a Thermoseparations Trace Series AS100 autosampler, and series of three Viscotek Viscogel columns (7.8 X 300 mm, 2 GMHXL16141 and one G3000HXL16136) in a solution of 89% tetrahydrofuran and 10% methanol, with 1% triethylamine, at 30 $^{o}$C. The GPC was calibrated with monodisperse polystyrene standards.

Ultrasound experiments were performed on a Vibra Cell 505 liquid processor with a ½" diameter solid probe from Sonics and Materials. The distance between the titanium tip and bottom of the Suslick cell[2] was approximately 1 cm. The Suslick cells were made by the School of Chemical Sciences' (SCS) Glass Shop at the University of Illinois.

Flash chromatrography was conducted with silica gel 60 (230-400 mesh) from EM science. The $^{1}$H and $^{13}$C NMR specra were recorded on Varian Unity 500 MHz spectrometers in the NMR laboratory, SCS, University of Illinois. The residual solvent protons were used as an internal standard. Coupling constants (*J*) are reported in Hertz (Hz), and splitting patterns are

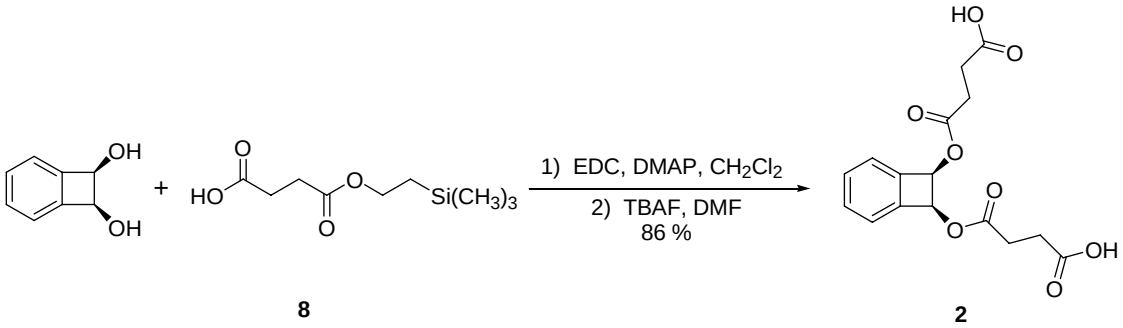
designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Mass spec were obtained through the Mass Spectrometry Facility, SCS, University of Illinois.




***Trans*-1,2-bis[(3-carboxypropanoyl)oxy]-1,2-dihydrobenzocyclobutene 1**: To a 50 mL round bottom flask equipped with a magnetic stirrer and rubber stopper and purged with $N_2$ was added benzocyclobutene diketone **7**[3] (0.148 g, 1.12 mmol) and methanol (33.7 mL). The resulting solution was cooled in an ice water bath, and $NaBH_4$ (85 mg, 2.25 mmol) was added in several portions. The mixture was stirred for 30 min with cooling then quenched with acetone (2 mL) and the solvent was removed under vacuum at 0 ºC. The residue was purified by flash chromatography at 4 ºC in a cold box, using 3:2 hexane:EtOAc as the eluent. The later eluting spot, which consisted of the *trans* diol, was concentrated under vacuum at 0 ºC, placed in a precooled flask equipped with a magnetic stirrer and rubber stopper and suspended in $CH_2Cl_2$ (2 mL). EDC (0.494 g, 2.58 mmol) and DMAP (27 mg, 0.225 mmol) was then added, followed by 4-(2-(trimethylsilyl)ethyl) ester butanoic acid **8**[4] (0.49 g, 2.25 mmol). The solution was stirred with ice bath cooling, and slowly warmed to room temperature. After 1.5 h the solvent was removed under vacuum. The residue was placed on a silica plug and the plug was eluted with 1:1 hexane:EtOAc. The filtrate was concentrated under vacuum, yielding an orange oil.

The residue obtained above was taken up in DMF (2-3 mL). TBAF in THF (1 M) was added in portions (0.5 mL) every 20 min until the reaction was complete by TLC, then partitioned between 1 M HCl and EtOAc (25 mL each). The layers were separated, and the aqueous layer was extracted with EtOAc (4 X 25 mL). The combined organics were dried over $Na_2SO_4$ and the solvent was removed under vacuum. The residue was placed on a silica plug and eluted with 200 mL of 2:1 hexane:EtOAc with 10% AcOH. The eluent was concentrated

under vacuum, and purified by column chromatography (3:1 hexane:EtOAc with 10% AcOH/ silica) yielding **1** (53 mg, 28%) as a colorless oil: $^{1}$H NMR (500 MHz, $CD_3OD$) δ 7.41 (dd, $J$ = 2.9, 5.4 Hz, 2H), 7.30 (dd, $J$ = 3.1, 5.6 Hz, 2H), 5.79 (s, 2H,), 2.66 (m, 4H), 2.62 (m, 4H); $^{13}$C NMR ($CD_3OD$, 125 MHz) δ 178.7, 176.4, 145.2, 134.6, 127.9, 80.1, 32.6, 32.5; HRMS (ESI) calcd for $C_{16}H_{17}O_8$ $(M+H)^+$ 337.0923, found 337.0920.

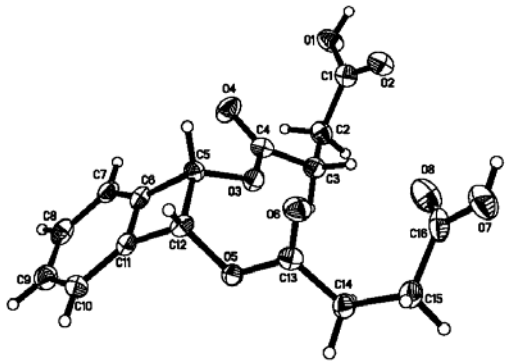



**8** **2**

***Cis*-1,2-bis[(3-carboxypropanoyl)oxy]-1,2-dihydrobenzocyclobutene 2**: *Cis*-benzocyclobuten-1,2-diol[5] (0.1774 g, 1.3 mmol) was suspended in $CH_2Cl_2$ (2 mL) in a 20 mL scintillation vial equipped with a magnetic stirrer and rubber stopper and purged with $N_2$. The suspension was cooled in an ice bath, then added was a solution of acid **8**[4] (0.567 g, 2.6 mmol) in $CH_2Cl_2$ (0.6 mL), followed by EDC (0.569 g, 2.98 mmol) and 4-dimethylaminopyridine (31 mg, 0.25 mmol). The rubber stopper was replaced with a plastic screw cap and the solution was stirred and slowly warmed to room temperature. After stirring for 10 h, the reaction was diluted with $CH_2Cl_2$ (5 mL) and washed with water (5 mL), 1 M HCl (5 mL), and satd $NaHCO_3$ (5 mL), and dried over $Na_2SO_4$. The solvent was removed under vacuum and the residue was used in the subsequent reaction without further purification.

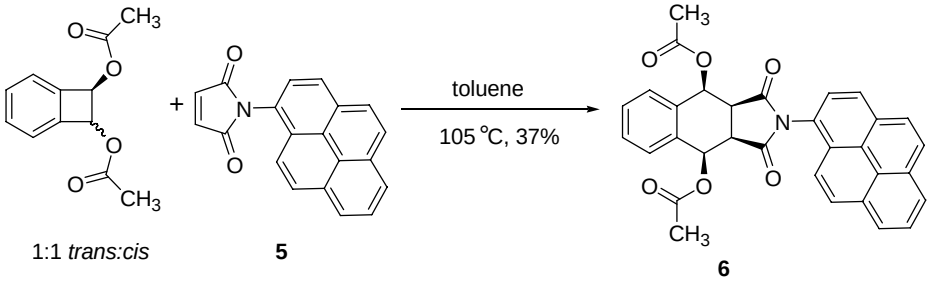
The residue obtained above was placed in a 20 mL scintillation vial equipped with a magnetic stirrer and plastic screw cap and dissolved in DMF (6.5 mL) A solution of TBAF in THF (3.9 mL of 1.0 M soln, 3.9 mmol) was then added, and the solution was stirred overnight. It was then partitioned between 1.0 M HCl (75 mL) and EtOAc (75 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 X 50 mL). The combined organics were washed with 1.0 M HCl and dried over $Na_2SO_4$. The solvent was removed under vacuum, and the yellow residue obtained was filtered through a silica plug, eluting with 6:3:1 hexane:EtOAC:AcOH. The filtrate was concentrated under vacuum, yielding **2** (0.381 g, 86 %)

as a white solid: $^{1}$H NMR (500 MHz, $CD_3OD$) δ 7.42 (dd, *J* = 3.2, 5.5 Hz, 2H), 7.31 (dd, *J* = 3.2, 5.5 Hz, 2H), 6.22 (s, 2H), 4.90 (s, 2H), 2.68 (m, 4H), 2.61 (m, 4H); $^{13}$C NMR (125 MHz, $CD_3OD$) δ 175.9, 173.7, 145.0, 131.2, 125.2, 75.8, 29.9, 29.6; HRMS (ESI) calcd for $C_{16}H_{16}O_8Na$ $(M+Na)^+$ 359.0743, found 359.0730.




ORTEP thermal ellipsoid diagram at the 50% probability for diacid **2**.

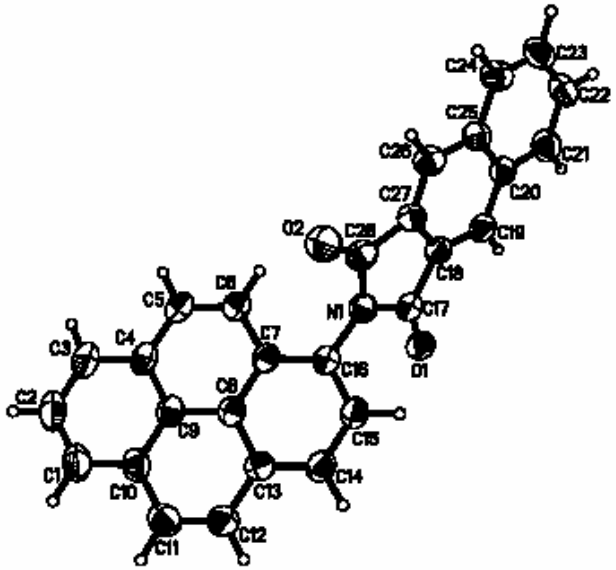
**Preparation of $^{13}$C labeled maleimide 5$^{13}$C**: This compound was prepared according to the general procedure reported by Reddy and coworkers,[6] except 1,4-$^{13}$C-enriched maleic anhydride was substituted for unenriched maleic anhydride. Both **5** and **5$^{13}$C** were used without further purification other than that described by Reddy and coworkers.



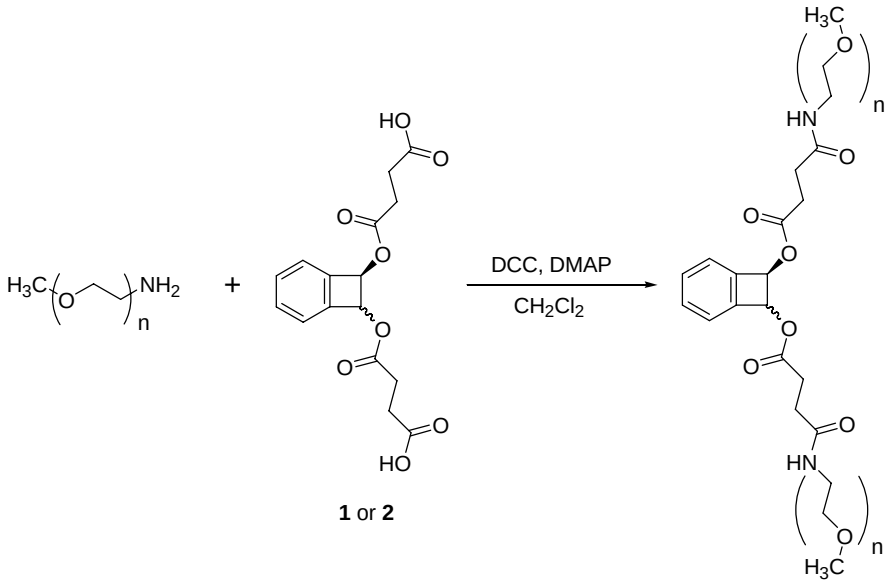

1:1 *trans:cis* **5** **6**

**Diels-Alder cycloaddition between *trans*-benzocyclobutene-1,2-diacetate and pyrene-labeled maleimide 5:** A 1:1 mixture of *trans* and *cis* benzocyclobutene-1,2-diacetate[5] (0.11 g, 0.249 mmol of *trans* isomer) was placed into 15 mL round bottom flask equipped with a magnetic stirrer and reflux condenser. Maleimide **5** (70.4 mg, 0.237 mmol) was added, followed by toluene (7.8 mL). The resulting solution was warmed to 105 ºC and stirred for 48h. The

solvent was removed under vacuum and the residue was purified by column chromatography (3:1 chloroform:diethyl ether), yielding **6** (45.6 mg, 37 %) as a red solid: $^{1}$H NMR ($CDCl_3$, 500 MHz) δ 8.26 (t, *J* = 8.4 Hz, 2H), 8.19 (d, *J* = 7.5 Hz, 1H), 8.15 (d, *J* = 9.0 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 8.05 (t, *J* = 7.9 Hz, 1H), 7.95 (d, *J* = 9 Hz, 1H), 7.82 (d, *J* = 7.7 Hz, 1H), 7.74 (dd, *J* = 3.7, 5.3 Hz, 2H), 7.60 (br s, 1H), 7.54 (dd, *J* = 3.4, 5.4 Hz, 2H), 6.52 (app q, *J* = 1.8 Hz, 2H), 3.92 (app q, *J* = 2.2 Hz, 2H), 2.20 (s, 6H); $^{13}$C NMR ($CDCl_3$, 125 MHz) δ 174.2, 169.7, 133.8, 132.5, 131.2, 130.9, 130.0, 129.9, 129.8, 129.0, 128.9, 127.8, 127.3, 126.7, 126.5, 126.0, 125.8, 125.5, 124.9, 124.6, 121.3, 67.5, 43.4, 21.2; HRMS (EI) calc'd for $C_{32}H_{23}NO_6$ 517.1525, found 517.1523. Also isolated from the reaction mixture was *N*-pyrene-2,3-naphthimide: mp = > 260 ºC; $^{1}$H NMR ($CDCl_3$, 500 MHz) δ 8.58 (s, 2H), 8.33 (d, *J* = 9.3 Hz, 1H), 8.27 (d, *J* = 8.0 Hz, 1H), 8.23 (d, *J* = 8.0 Hz, 1H), 8.16 (m, 4H), 8.13 (d, *J* = 8 Hz, 1H), 8.06 (t, *J* = 6.8 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 9.4 Hz, 1H), 7.79 (d, *J* = 3.2 Hz, 1H), 7.78 (d, *J* = 3.2 Hz, 1H); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 168.0, 136.0, 132.3, 131.3, 131.0, 130.7, 129.7, 129.1, 128.7, 128.4, 128.0, 127.4, 126.6, 126.5, 126.2, 126.1, 125.8, 125.7, 125.6, 125.3, 124.7, 121.8; LRMS (EI, 70 eV) *m/z* 397 ($M^+$), 369 $(M\text{-}CO)^+$, 322, 297, 269, 248, 236, 210, 183, 168, 136, 118, 101, 75.




ORTEP thermal ellipsoid diagram at the 50% probability for *N*-pyrene-2,3-naphthimide.




**1** or **2**

**3** or **4**

MW 2,5,10,20,30 kDa

MW 4,10,20,40,60 kDa

**Synthesis of Center Link-Functionalized Polymers. General Procedure:** α-methoxy-ω-aminopoly(ethylene glycol) (mPEG-$NH_2$, 2 equiv), diacid linker **1** or **2** (1 equiv), *N*,*N*'-dicyclohexylcarbodiimide (DCC, 5 equiv) and DMAP, (1 equiv) were dissolved in a small amount of $CH_2Cl_2$ (approximately 1.5 mL per 1 g of polymer) and stirred at room temp for 24 h in the dark. The dicyclohexylisourea byproduct (DCU) was removed by filtration through a cotton plug. The filtrate was then slowly precipitated into approximately 200 times its volume of ether. The polymer was collected by filtration through a fritted funnel and washed with ether, then dried under vacuum. Typical conversions from the starting polymer to the link-functionalized polymer with a MW double the starting value ranged from 60-90% by analytical GPC analysis.

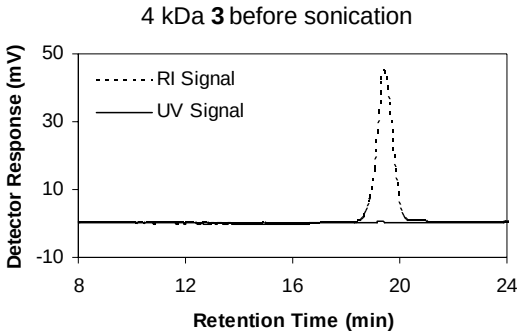
**Set-up of the ultrasound apparatus:** For each ultrasound experiment, an acetonitrile solution of the polymer to be sonicated was placed in a Suslick cell[1] that was inserted into a collar, and screwed onto the transducer. A thermocouple and an argon line were threaded through septa on two of the Suslick cell side arms and placed in contact with the solution, ensuring that they did not touch the probe. The third side arm of the cell was sealed with a plastic screw cap. Argon was bubbled through the solution for 30 min prior to each experiment as well as during the

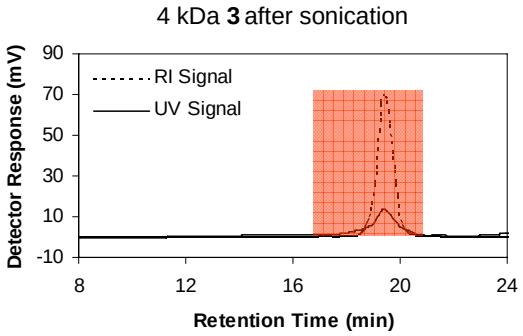
experiment. The entire system was cooled in an ice bath to maintain a temperature of 0-9 $^{o}$C throughout sonication.

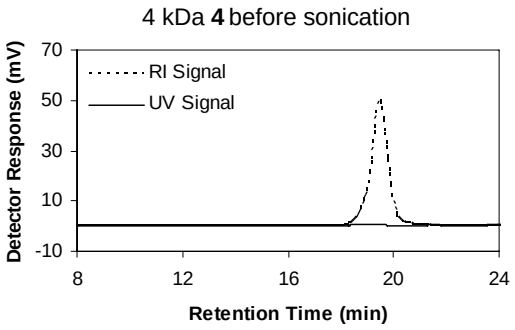
**Calibration of the sonication equipment:** Calorimetry was used to calibrate the ultrasonic intensity produced by the probe. A small dewar was filled with exactly 250 mL of Millipore water and the probe was submerged approximately ½" into the water. The exact height was marked for reproducibility. A thermocouple was then introduced into the water and the temperature was allowed to stabilize. The amplitude on the processor was set to 21%, the initial temperature was recorded, and sonication was started. The temperature of the water was recorded every 15 s for 3 min, and these values were plotted against time to determine Δ temperature/Δ time by the slope of the line. This procedure was repeated for amplitudes of 30, 40, 50, 60, 70, and 80%.

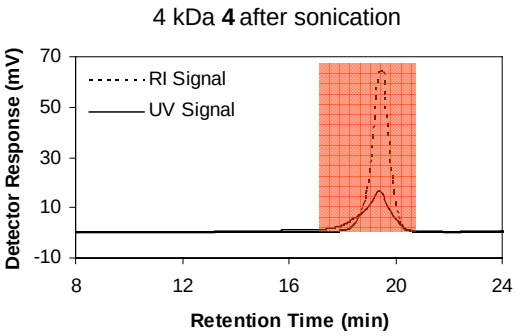
The heat due to cavitation in J/s, or W, was determined from the following equation: q = specific heat ($Jg^{-1}$ $^{o}C^{-1}$) X mass (g) X Δ temperature/Δ time ($^{o}$C/s) where the specific heat of water = 4.179 $Jg^{-1}$ $^{o}C^{-1}$, the mass of water = 250 g, and Δ temperature/Δ time = the slope of the line. The resulting value, q, was divided by the surface area of the horn tip (1.27 $cm^2$ for a 5" tip) to find $W/cm^2$. Finally, the power produced was plotted against the percentage of amplitude to generate the calibration curves.

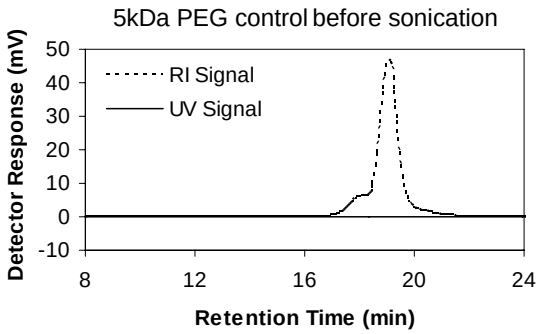
**Figure 10**. Representative GPC chromatograms before (left) and after (right) sonication for 4 kDa, 10 kDa, 20 kDa, and 60 kDa LFPs **3** and **4**, and for 5 kDa, 10 kDa, 20 kDa, 26 kDa and 60 kDa PEG. The regions shaded red indicate the area of the GPC curve considered for the determination of the UV and RI signal areas. Typically, only regions corresponding to elution times of unbroken chains were analyzed.[7]

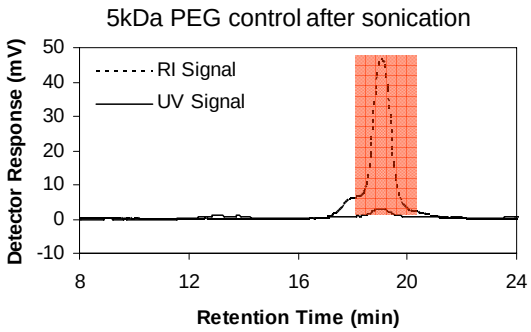


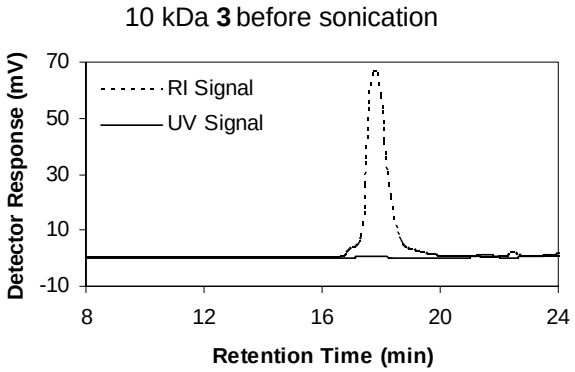



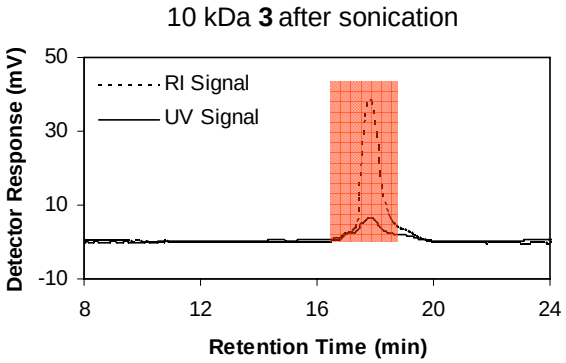



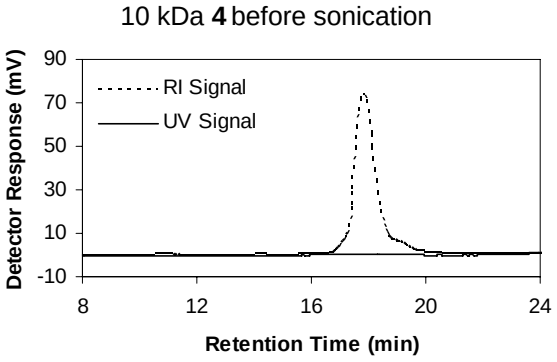



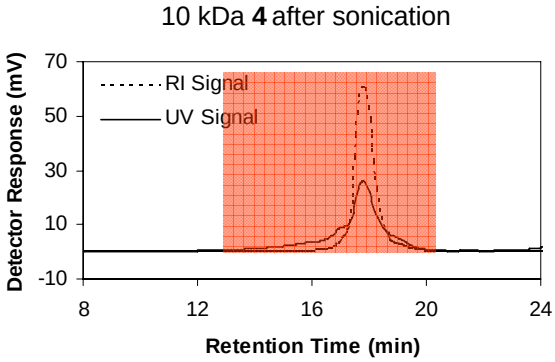



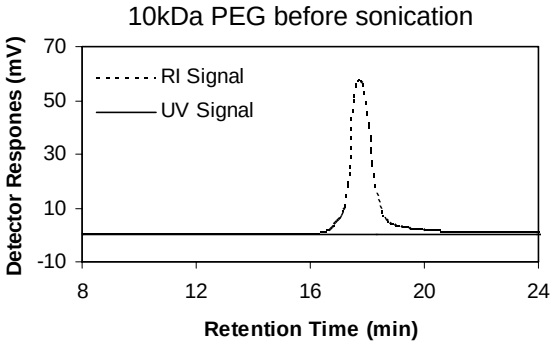



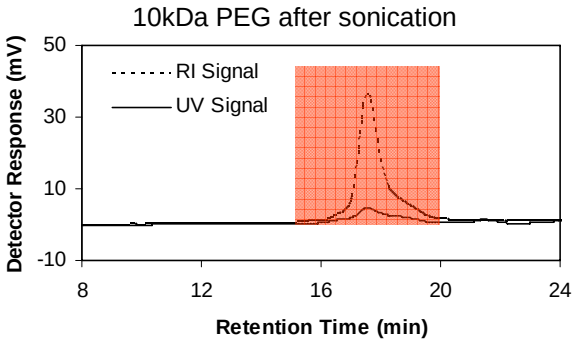

10 kDa **3** before sonication
RI Signal
UV Signal
Detector Response (mV)
70
50
30
10
-10
8
12
16
20
24
Retention Time (min)

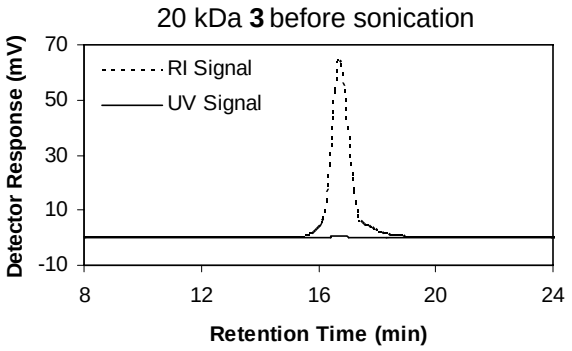

10 kDa **3** after sonication
RI Signal
UV Signal
Detector Response (mV)
50
30
10
-10
8
12
16
20
24
Retention Time (min)

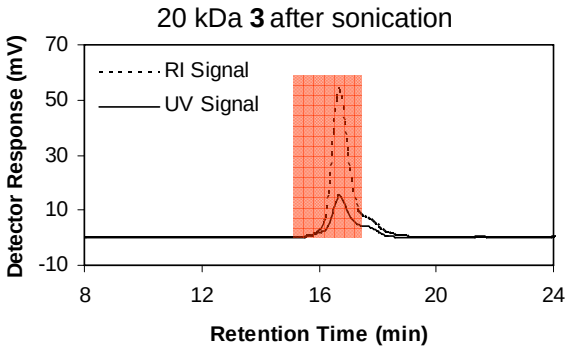

10 kDa **4** before sonication
RI Signal
UV Signal
Detector Response (mV)
90
70
50
30
10
-10
8
12
16
20
24
Retention Time (min)

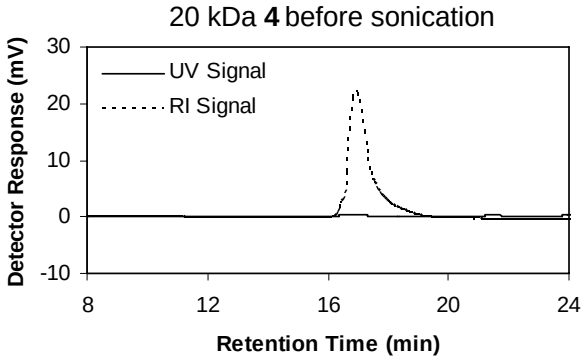

10 kDa **4** after sonication
RI Signal
UV Signal
Detector Response (mV)
70
50
30
10
-10
8
12
16
20
24
Retention Time (min)

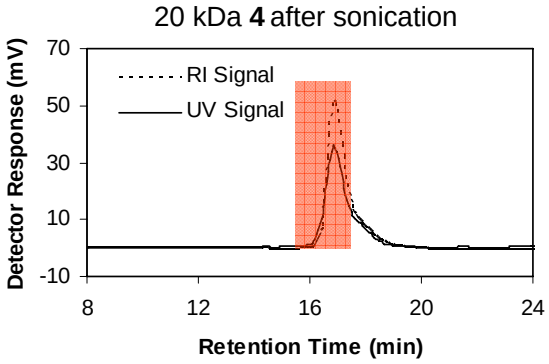

10kDa PEG before sonication
RI Signal
UV Signal
Detector Respones (mV)
70
50
30
10
-10
8
12
16
20
24
Retention Time (min)

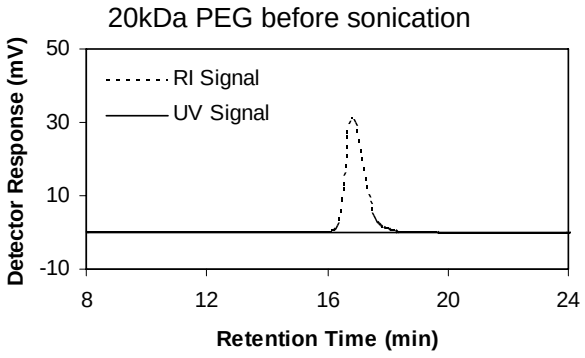

10kDa PEG after sonication
RI Signal
UV Signal
Detector Response (mV)
50
30
10
-10
8
12
16
20
24
Retention Time (min)

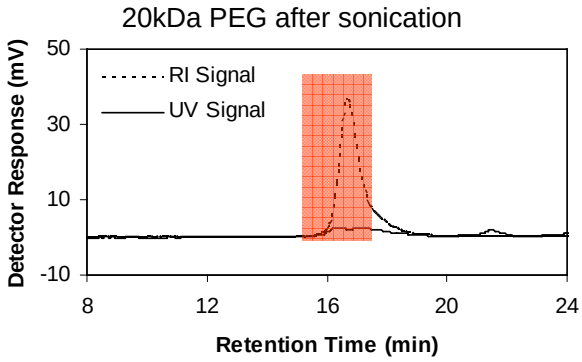

20 kDa **3** before sonication
Detector Response (mV)
70
50
30
10
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)

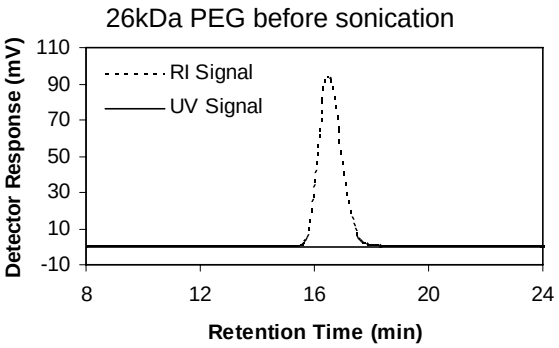

20 kDa **3** after sonication
Detector Response (mV)
70
50
30
10
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)

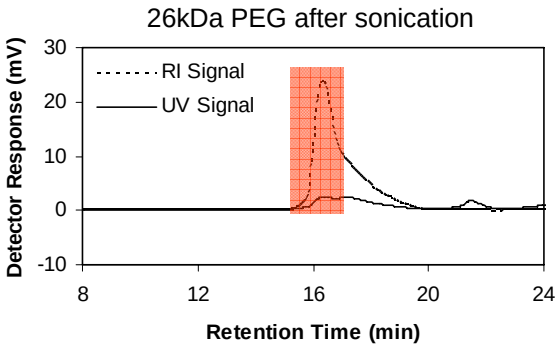

20 kDa **4** before sonication
Detector Response (mV)
30
20
10
0
-10
UV Signal
RI Signal
8
12
16
20
24
Retention Time (min)

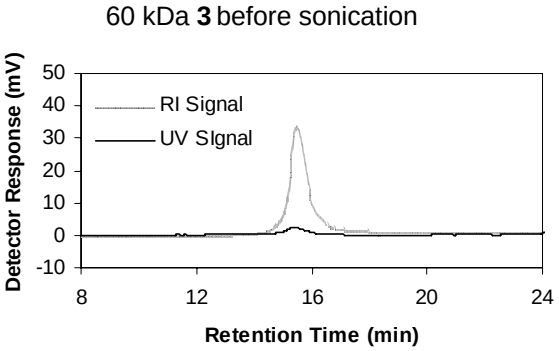

20 kDa **4** after sonication
Detector Response (mV)
70
50
30
10
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)

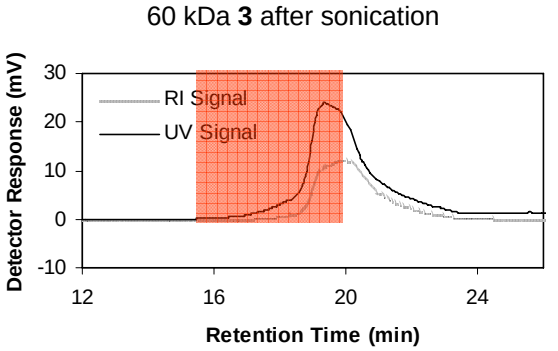

20kDa PEG before sonication
Detector Response (mV)
50
30
10
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)

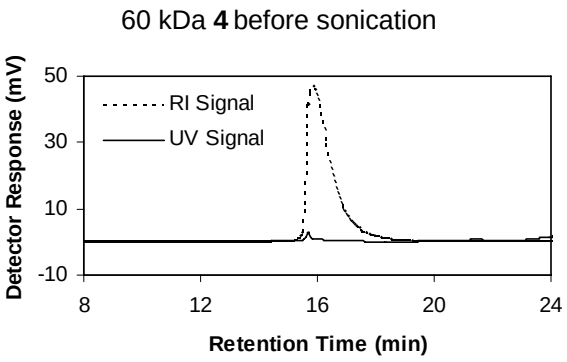

20kDa PEG after sonication
Detector Response (mV)
50
30
10
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)

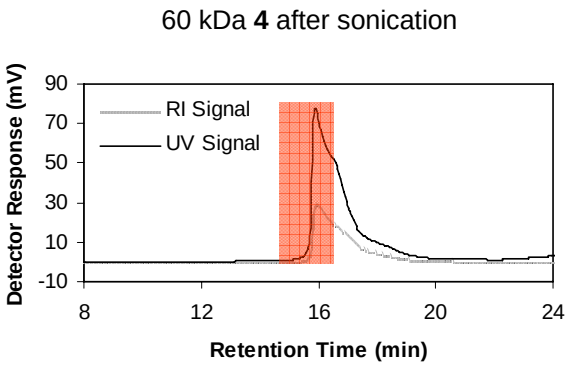

26kDa PEG before sonication
Detector Response (mV)
110
90
70
50
30
10
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)

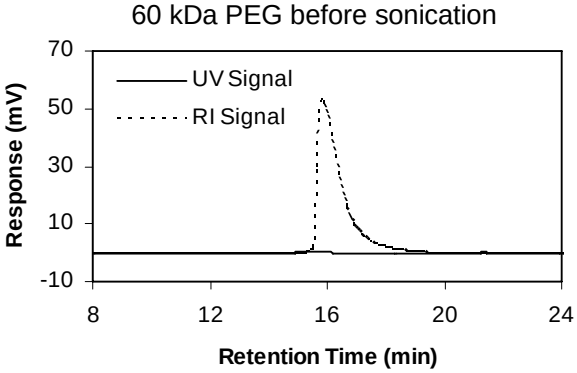

26kDa PEG after sonication
Detector Response (mV)
30
20
10
0
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)

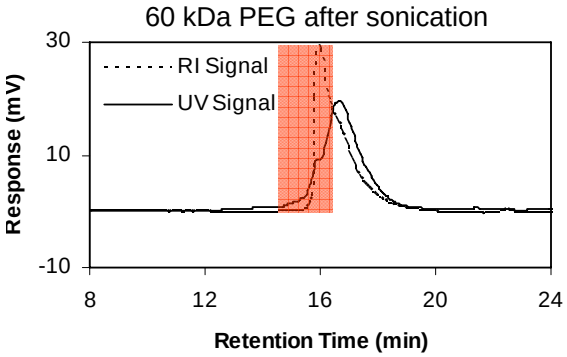

60 kDa 3 before sonication
Detector Response (mV)
50
40
30
20
10
0
-10
RI Signal
UV SIgnal
8
12
16
20
24
Retention Time (min)

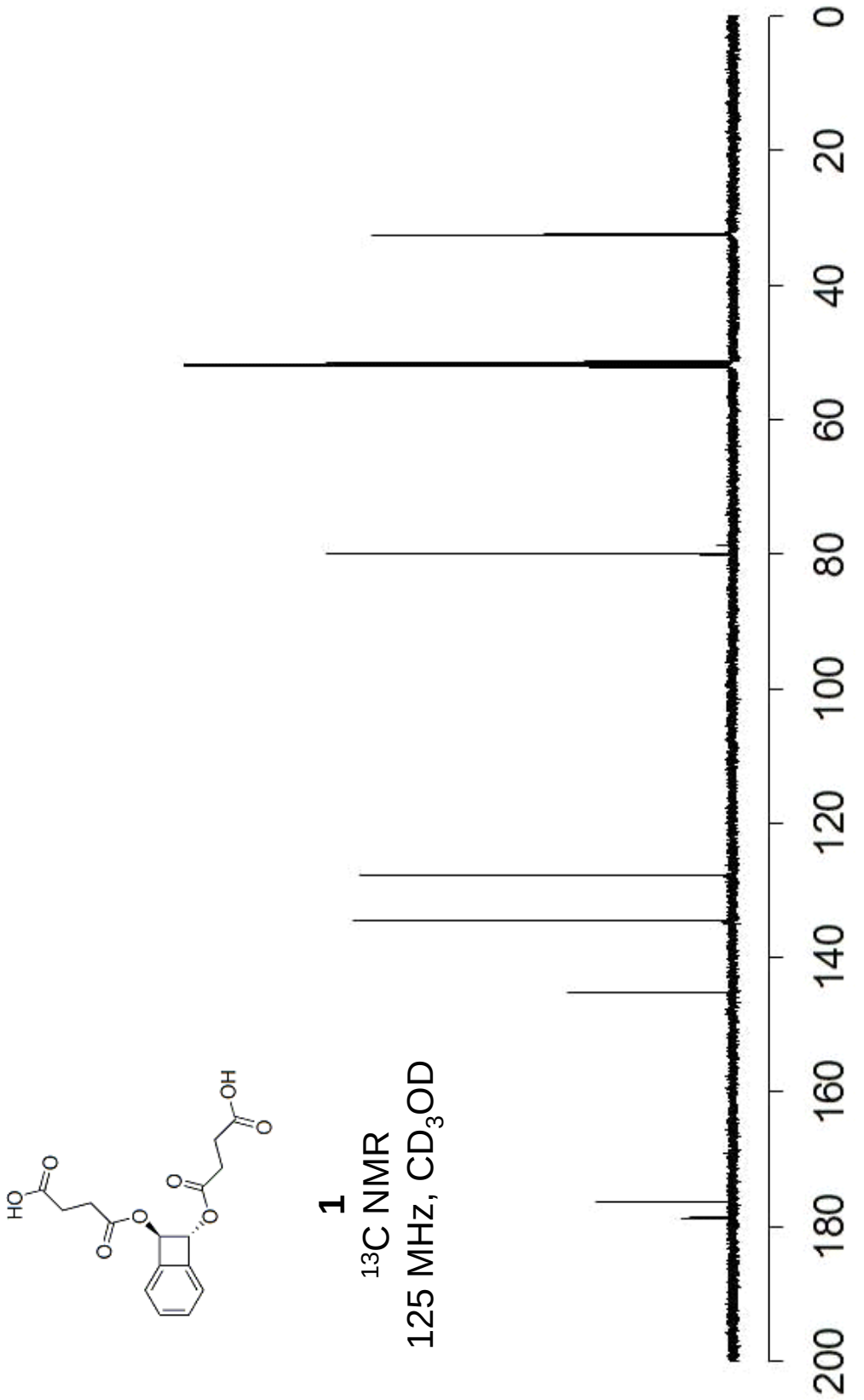

60 kDa 3 after sonication
Detector Response (mV)
30
20
10
0
-10
RI Signal
UV Signal
12
16
20
24
Retention Time (min)

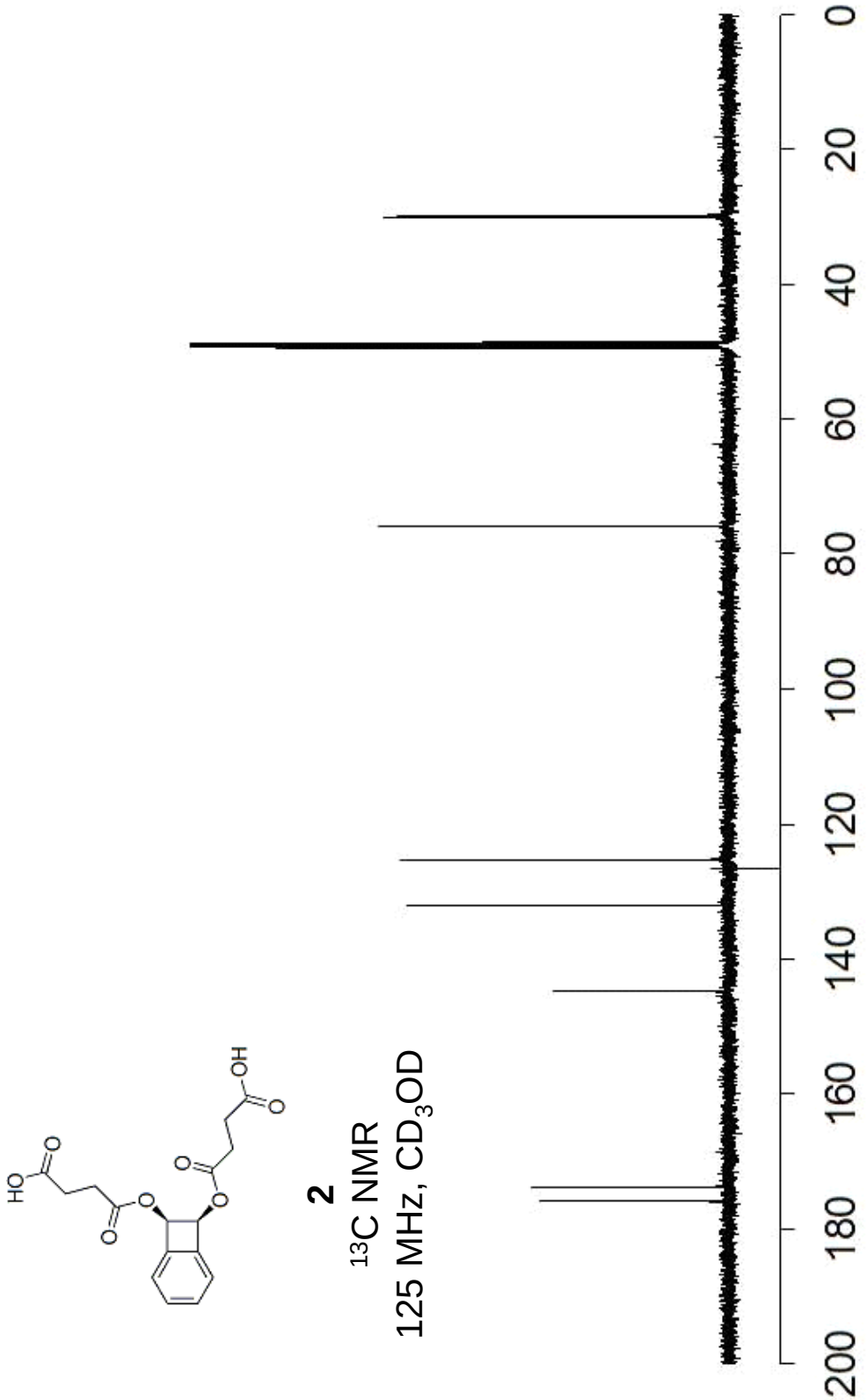

60 kDa 4 before sonication
Detector Response (mV)
50
30
10
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)

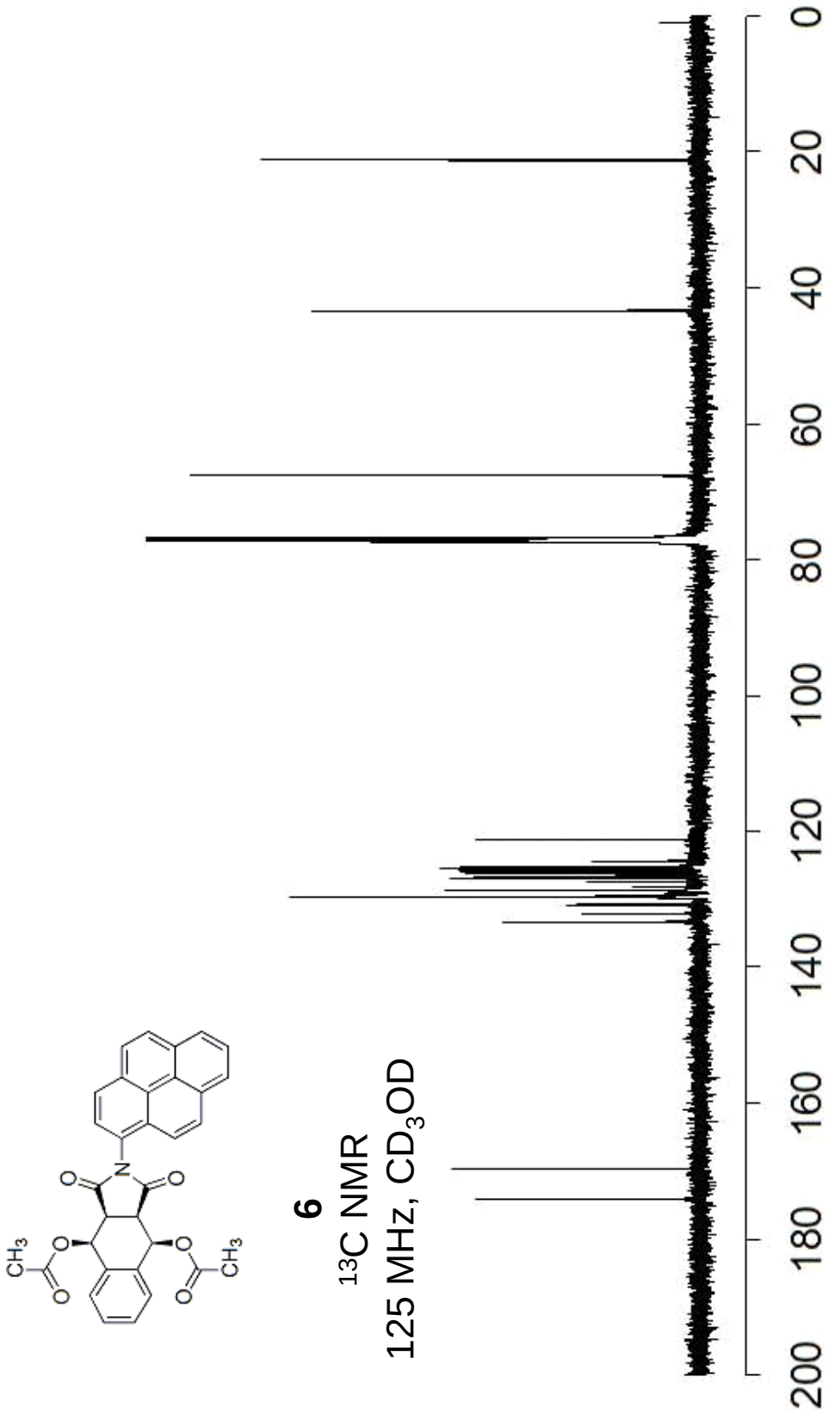

60 kDa 4 after sonication
Detector Response (mV)
90
70
50
30
10
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)


60 kDa PEG before sonication
Response (mV)
70
50
30
10
-10
UV Signal
RI Signal
8
12
16
20
24
Retention Time (min)


60 kDa PEG after sonication
Response (mV)
30
10
-10
RI Signal
UV Signal
8
12
16
20
24
Retention Time (min)


HO
O
O
O
O
O
OH
O
1
13C NMR
125 MHz, CD3OD
0
20
40
60
80
100
120
140
160
180
200


HO
O
O
O
O
O
OH
O
2
13C NMR
125 MHz, CD3OD
0
20
40
60
80
100
120
140
160
180
200


6
$^{13}$C NMR
125 MHz, $CD_3OD$
0
20
40
60
80
100
120
140
160
180
200
N
O
O
O
O
$CH_3$
$CH_3$

References:

(1) Humphrey, W; Dalke, A; Schulten, K. VMD - Visual Molecular Dynamics *J. Molec. Graphics* **14**, 33-38 (1996).

(2) Suslick, K. S.; Goodale, J. W.; Schubert, P. F.; and Wang, H. H. Sonochemistry and sonocatalysis of metal carbonyls. *J. Am. Chem. Soc.* **105**, 5781-5785 (1983).

(3) South, M.S., Liebeskind, L.S. Practical multigram synthesis of benzocyclobutenediones. *J. Org. Chem*. **47**, 3815-3821 (1982).

(4) Pouzair, V.; Drasar, P.; Cerny, I.; Havel, M. A novel, indirect preparation of hemisuccinates. **14**, 501 (1984).

(5) Nozaki, H.; Noyori, R.; Kozaki, N. Acetolysis of *trans*-1,2-dibromobenzocyclobutene. *Tetrahedron* **20**, 641 (1964).

(6) Reddy, P.Y., Kondo, S., Fujita, S., Toru, T. Efficient synthesis of fluorophore-linked maleimide derivatives. *Synthesis*, 999-1002 (1998).

(7) In the 60 kDa PEG control polymer, the broken 30 kDa chains were observed to incorporate label more than PEG of lower MW. We speculate that this is due to a radical polymerization of the maleimide trap from the macroradicals resulting from chain scission. Radical polymerizations of unsaturated monomers initiated from the macroradicals formed upon chain scission have been previously reported.

(8) On occasion, the use of maleimide **5 or 5$^{13}$C** after recrystallization from toluene resulted in significant baseline drift in the UV signal after sonication. This baseline drift did not show up in the RI curves. We speculate that the drift is due to small amounts of a radical polymerization of maleimide initiated by the ultrasound conditions. The polymerization of unsaturated monomers initiated by ultrasound has been previously described. See, for example, Price, G.J. The use of ultrasound for the controlled degradation of polymer solutions. *Advances in Sonochemistry* **1**, 231-287 (1990). This idea is consistent with the fact that addition of a radical inhibitor, such as BHT, significantly reduces the UV baseline drift. Control experiments with crude **5** and with recrystallized **5** and added BHT show that the presence of a radical inhibitor has no effect on the final UV/RI ratio for both 40 kDa **3**, 40 kDa **4**, and 43 kDa PEG. It is currently not clear why this occurs only with recrystallized **5** or **5$^{13}$C**.