

Supplementary data

Investigation of porphyrin-forming reactions. Part 1. Pyrrole + aldehyde oligomerization in two-step, one-flask syntheses of meso-substituted porphyrins

G. Richard Geier III and Jonathan S. Lindsey*

Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695-8204

USA

LD-MS Control and Validation Experiments. Control experiments were performed to determine conditions for LD-MS analysis of crude, oxidized reaction mixtures (from pyrrole + benzaldehyde reactions), and to assess the reliability and repeatability of the LD-MS analysis method. In general, LD-MS analysis was found to be robust. Spectra with good peak resolution and signal-to-noise ratio were repeatedly obtained with minimal difficulty under a wide range of reaction conditions. The LD-MS analysis method was insensitive to variations in samples other than the oligomer composition (e.g., different acid concentrations). These findings support the application of LD-MS as a qualitative tool for probing oligomer molecular weight range and oligomer diversity in crude, oxidized reaction mixtures formed under diverse reaction conditions. A comprehensive summary is provided below of the control experiments we performed to investigate the utility of the LD-MS analysis method.

- (1) *Matrix requirement.* Recording mass spectra in the presence of a matrix (α -cyano-4-hydroxycinnamic acid) did not improve the quality of the mass spectra. In fact, the presence of the matrix complicated the mass spectra in the region of $m/z < 400$ due to the presence of matrix peaks. Thus, all spectra were recorded in the absence of added matrix as described in the experimental section. The experiment probing matrix effects was performed as follows. A saturated solution of α -cyano-4-hydroxycinnamic acid in CH_2Cl_2 was mixed in a variety of proportions with a crude reaction mixture (8 mM benzaldehyde, 24 mM pyrrole, 3 mM BF_3 -etherate in CH_2Cl_2 for 1 h at room temperature followed by addition of 35 mM DDQ). Mass spectra were recorded and compared.
- (2) *Pre-analysis stability of crude reaction mixtures.* The LD-MS spectra were found to not change during the time elapsed from completion of the reaction to LD-MS analysis, if the crude sample from the reaction of pyrrole + benzaldehyde was spotted onto the LD-MS target within a few min of the addition of DDQ. Four different reactions were performed

(10 mM reactants with 20 mM TFA or 1.0 mM BF₃-etherate, and 100 mM reactants with 64 mM TFA or 10 mM BF₃-etherate) and a target was spotted with each sample shortly after addition of DDQ. LD-MS spectra were recorded immediately, and again after 7 h and 27 h. The target was stored at room temperature in the dark between analyses. Additionally, at 7 h and 27 h fresh spots were made on the target from the original crude, oxidized reaction mixtures that had been stored tightly capped at room temperature in the dark. The samples spotted on the target shortly after DDQ addition provided identical mass spectra over the 27 h. Samples freshly spotted on the target at 7 h and 27 h were minimally different in the case of BF₃-etherate catalysis and significantly different in the case of TFA catalysis. Thus, all crude reaction mixtures prepared in this study were spotted on the LD-MS target within 5 min of DDQ addition, and analysis was performed within 12 h.

- (3) *Day-to-day repeatability.* The day-to-day repeatability of the LD-MS analysis method was found to be good. A number of experimental conditions were performed multiple times (up to ~30) over the course of 2 years. Spectra recorded 2 years apart from samples prepared under “identical” conditions showed only minor differences. Typically, the peak resolution and signal-to-noise ratio varied slightly, but the detection of peaks and their relative intensities were largely unchanged. The only significant day-to-day variation was the intensity of the peak at m/z 220, particularly with BF₃-etherate catalysis.
- (4) *Repeatability over multiple target locations.* To examine the degree of heterogeneity of the sample spot deposited on the LD-MS target, sample spots were sampled from multiple locations. Generally, spectra recorded from different locations on the same spot were in excellent agreement both in terms of the particular peaks present and the peak

intensities. Regardless, to avoid anomalous spectra, all samples were examined at at least two locations. If the spectra were in poor agreement, additional locations were sampled.

- (5) *Effect of sample concentration.* The concentration of the sample (and thereby the amount of sample spotted on the target) was found to have little effect on the mass spectra over a broad range. Five control reactions were performed with concentrations of reactants (pyrrole + benzaldehyde) from 10 to 320 mM. Three reactions were catalyzed by TFA and two by BF_3 -etherate. Each crude reaction mixture was serially diluted in CH_2Cl_2 prior to spotting on the target. The mass spectra of the diluted and undiluted samples were compared. No significant differences were observed.
- (6) *Effect of DDQ concentration.* Addition of DDQ beyond 0.75 molar equiv relative to pyrrole was found to have no effect on the mass spectra. Reactions were performed at 10 mM reactants and 20 mM TFA or 1.0 mM BF_3 -etherate and oxidation was performed with variable amounts of DDQ (3/16 to 4 molar equiv relative to pyrrole). Mass spectra were compared and no changes were observed with concentrations of DDQ greater than the theoretically required amount (3/4 molar equiv of DDQ relative to pyrrole).
- (7) *Effect of low molecular weight oligomers on detection of higher molecular weight oligomers.* Low molecular weight oligomers were found to dominate the mass spectra only if present in moderate amounts in the crude reaction mixture. Small amounts of low molecular weight oligomers did not affect the detection of higher molecular weight oligomers. A reaction was performed that provided peaks of almost exclusively $m/z > 700$ (320 mM reactants, 20 mM TFA). After addition of DDQ, the crude reaction mixture was blended in varying proportions with one of three different samples containing low molecular weight oligomers (10 mM reactants, 20 mM TFA; 10 mM reactants, 1.0 mM BF_3 -etherate; and a TPP stock solution). Mass spectral analysis

showed that about 20-40% of the low molecular weight sample was required to cause loss of detection of the high molecular weight peaks. The shift in the molecular weight range observed by LD-MS smoothly followed the increase in the percentage of low molecular weight material in the sample blend.

- (8) *Effect of acid (identity)*. Reactions were performed using 10 mM reactants under catalysis by 20 mM TFA or 1.0 mM BF₃-etherate. After DDQ oxidation, the opposite acid was added to each crude reaction mixture (e.g., TFA to the BF₃-etherate reaction). Mass spectra were compared and the addition of the second acid had no effect on the appearance of the mass spectra.
- (9) *Effect of acid (concentration)*. Four control reactions were performed (10 mM reactants with 20 mM TFA or 1.0 mM BF₃-etherate, and 100 mM reactants with 64 mM TFA or 10 mM BF₃-etherate). After DDQ addition, each reaction was doped with increasing amounts of the acid catalyst. Mass spectra were compared and doping with additional acid was observed to have no effect on the mass spectra.
- (10) *Effect of sample neutralization*. Neutralization of the acid catalysts by addition of triethylamine (TEA) had a modest effect on the mass spectra. After DDQ oxidation, crude reaction mixtures were neutralized with 2- or 4-fold excess TEA (relative to the acid). The comparison of mass spectra showed no change in terms of the peaks detected. However, the signal-to-noise ratio in the TEA-containing samples was much poorer than in the absence of TEA, in part due to an intense signal for TEA. Given this finding, TEA was not added to the samples prior to LD-MS analysis. The crude, oxidized samples were spotted on the target for LD-MS analysis; TEA was then added to the reaction samples prior to analysis by UV-vis and HPLC.

SEC Control and Validation Experiments. Control experiments were performed to determine conditions for SEC analysis of crude, oxidized reaction mixtures from the reaction of pyrrole + benzaldehyde and to assess the reliability and repeatability of SEC analysis. In general, SEC analysis was not particularly informative. Peak resolution was poor and detection by a UV-vis detector complicated interpretation (different reaction products provide different absorption spectra). The appearance of the chromatograms was sensitive to variations in samples other than that of the oligomer composition. Chromatograms obtained from authentic materials provided anomalous retention times. Taken together, these findings illustrate the difficulties of using SEC as a qualitative tool for probing oligomer molecular weight range and oligomer diversity in crude, oxidized reaction mixtures formed under a range of conditions. A comprehensive list of experiments is provided below.

- (1) *Solvent selection.* THF was chosen as the mobile phase due to good solubility of the crude, oxidized reaction mixtures in THF and the favorable UV cut-off of THF. Toluene was also considered, but crude, oxidized reaction mixtures were poorly soluble in toluene.
- (2) *Column selection.* A crosslinked polystyrene column with a nominal pore size of 50 Å was used. A comparison of 100-Å and 50-Å columns individually, and columns in series (50 Å and 100 Å; 100 Å and 500 Å) led to that selection. The 50-Å column offered the widest retention-time range per analysis time for crude reaction mixtures from the reaction of pyrrole + benzaldehyde.
- (3) *Detection wavelength selection.* The eluant was monitored at five wavelength ranges (250-300, 300-350, 350-400, 400-450, and 450-500 nm). The absorbance from TPP dominated the chromatograms recorded below 450 nm.

- (4) *Pre-analysis sample stability.* Chromatograms obtained from identical samples after pre-analysis delays of varying length were found to show differences. The observed magnitude and type of change varied. In about half of the samples the changes were significant. In general, the peaks due to higher molecular weight material declined in intensity, perhaps due to gradual precipitation. The greatest change occurred between samples analyzed immediately and after 1 h. At times longer than 1 h, the chromatograms were fairly constant. Given this finding, all crude reaction mixtures were analyzed by SEC within 5 min of DDQ oxidation.
- (5) *Day-to-day repeatability.* Repeatability was generally good. Identical sets of conditions were performed in triplicate over multiple days. Very similar chromatograms were obtained.
- (6) *Effect of sample neutralization.* SEC analysis was affected by pre-analysis neutralization of the acid catalyst by TEA. Two BF_3 -etherate- and two TFA-catalyzed reactions were neutralized with varying amounts of TEA (from 1 to 10 equiv). Major differences were observed with > 2 equiv of TEA, with TFA catalyzed reactions affected to a larger extent than BF_3 -etherate catalyzed reactions. Given this finding, samples were not treated with TEA prior to analysis.
- (7) *Effect of DDQ.* Control reactions were oxidized with varying amounts of DDQ. BF_3 -etherate catalyzed reactions were largely unaffected by excess DDQ, whereas TFA catalyzed reactions were affected (the peak with retention time of 6 min was enhanced). To avoid the intense signal from DDQ and to neutralize residual acid, the crude, oxidized reaction mixtures prior to analysis were washed with an aqueous solution containing 5% NaOH and 5% $\text{Na}_2\text{S}_2\text{O}_4$.

(8) *Analysis of control samples.* Samples of DDQ, 5-phenyldipyrromethane mixed with DDQ, 5,10-diphenyltripyrane mixed with DDQ, and TPP were analyzed independently. The comparison of the retention times was not consistent with changes in molecular weight and size. In particular, DDQ and 5,10-diphenyltripyrane mixed with DDQ provided peaks with anomalously short retention times (shorter than that of TPP).

SEC Analyses of Crude, Oxidized Reaction Mixtures as a Function of Time. Four reaction conditions were monitored by SEC as a function of time in the reactions of pyrrole + benzaldehyde. The optimal conditions for 10 mM reactants of 20 mM TFA (Figure S1) or 1.0 mM BF₃-etherate (Figure S2) were examined as were two additional conditions (100 mM TFA, Figure S3; 10 mM BF₃-etherate, Figure S4) which showed a dramatic turnover in yield and truncation of oligomers by LD-MS analysis. Figure S1 shows the increase of a low molecular weight peak at long reaction time and no increase in high molecular weight material; this result is consistent with the LD-MS data although not as dramatic. Figure S2 shows little change in the SEC chromatograms at long reaction times, consistent with LD-MS data. Figure S3 shows some loss of high molecular weight material accompanied by a modest increase in low molecular weight material, though again the changes were not as dramatic as shown in LD-MS spectra from the identical reactions. Figure S4 also shows some loss of high molecular weight material accompanied by a modest increase in low molecular weight material, though again the changes were not as dramatic as shown in LD-MS spectra from the identical reactions. In general, the SEC data were in agreement with the LD-MS data though the changes were not as large. However, under no condition examined did the amount of high molecular weight material increase at long reaction times.

Figure S1. SEC data as a function of time for 10 mM reactants, 20 mM TFA. Detection wavelength was 325 nm.

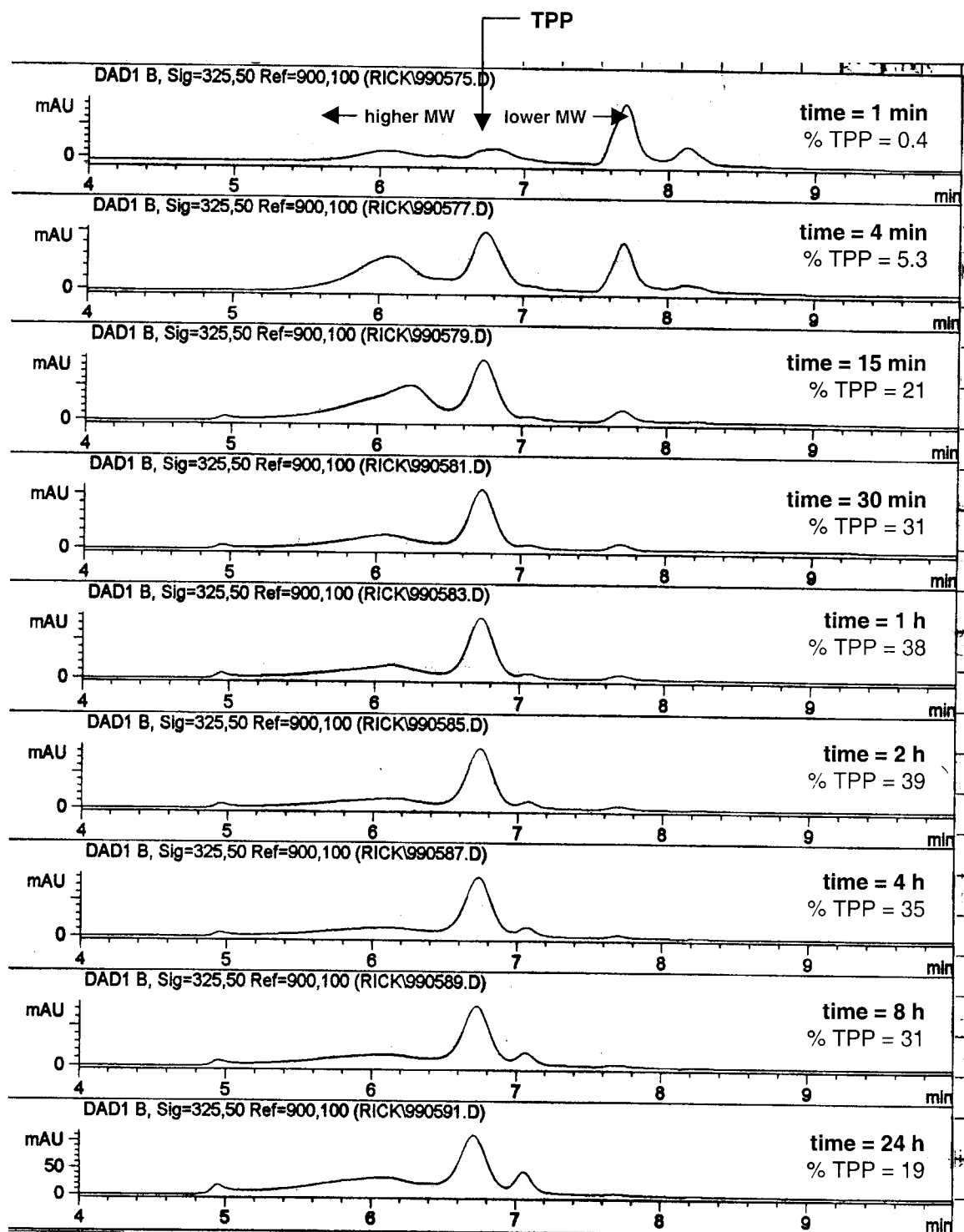


Figure S2. SEC data as a function of time for 10 mM reactants, 1.0 mM BF₃-etherate. Detection wavelength was 325 nm.

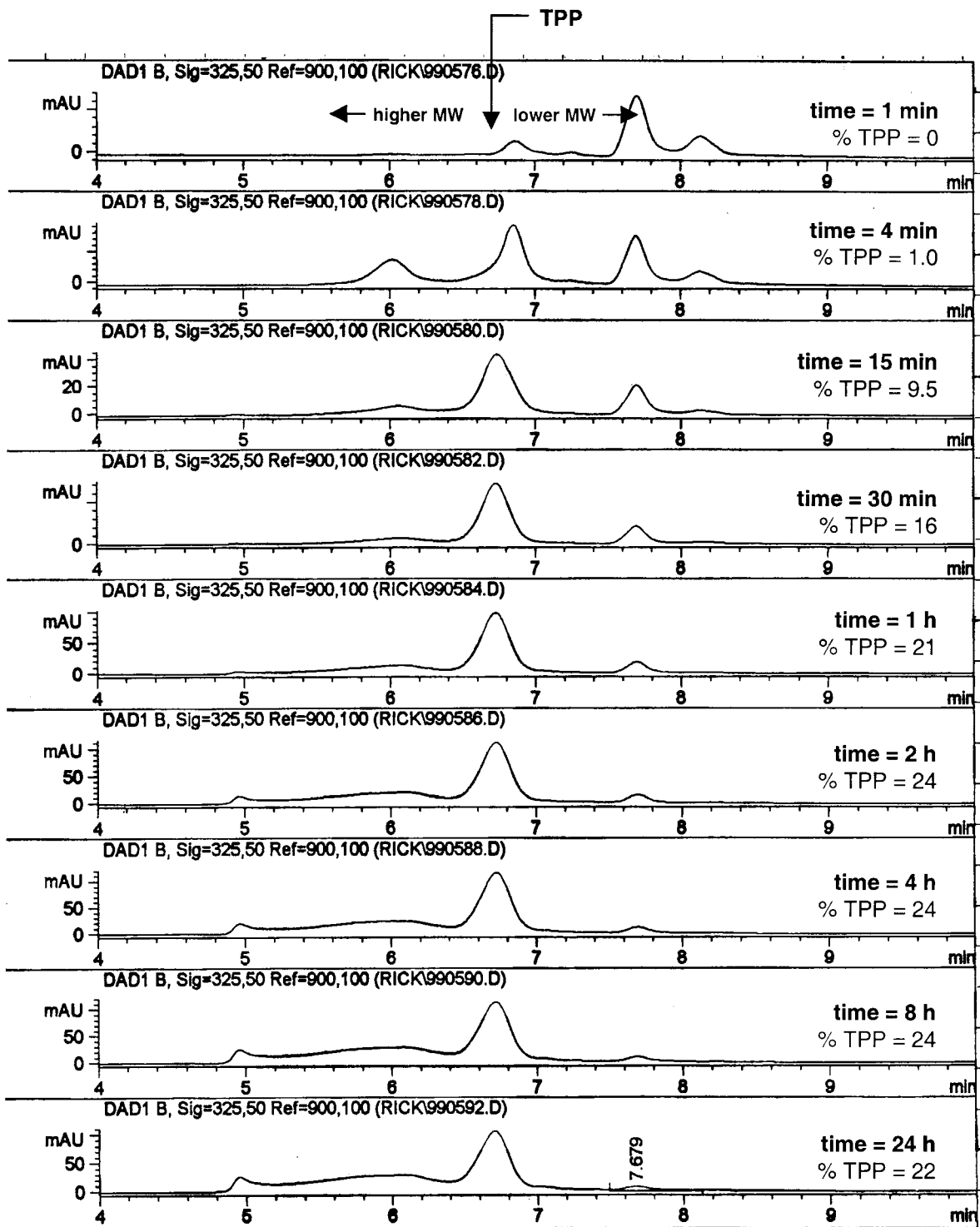


Figure S3. SEC data as a function of time for 10 mM reactants, 100 mM TFA. Detection wavelength was 325 nm.

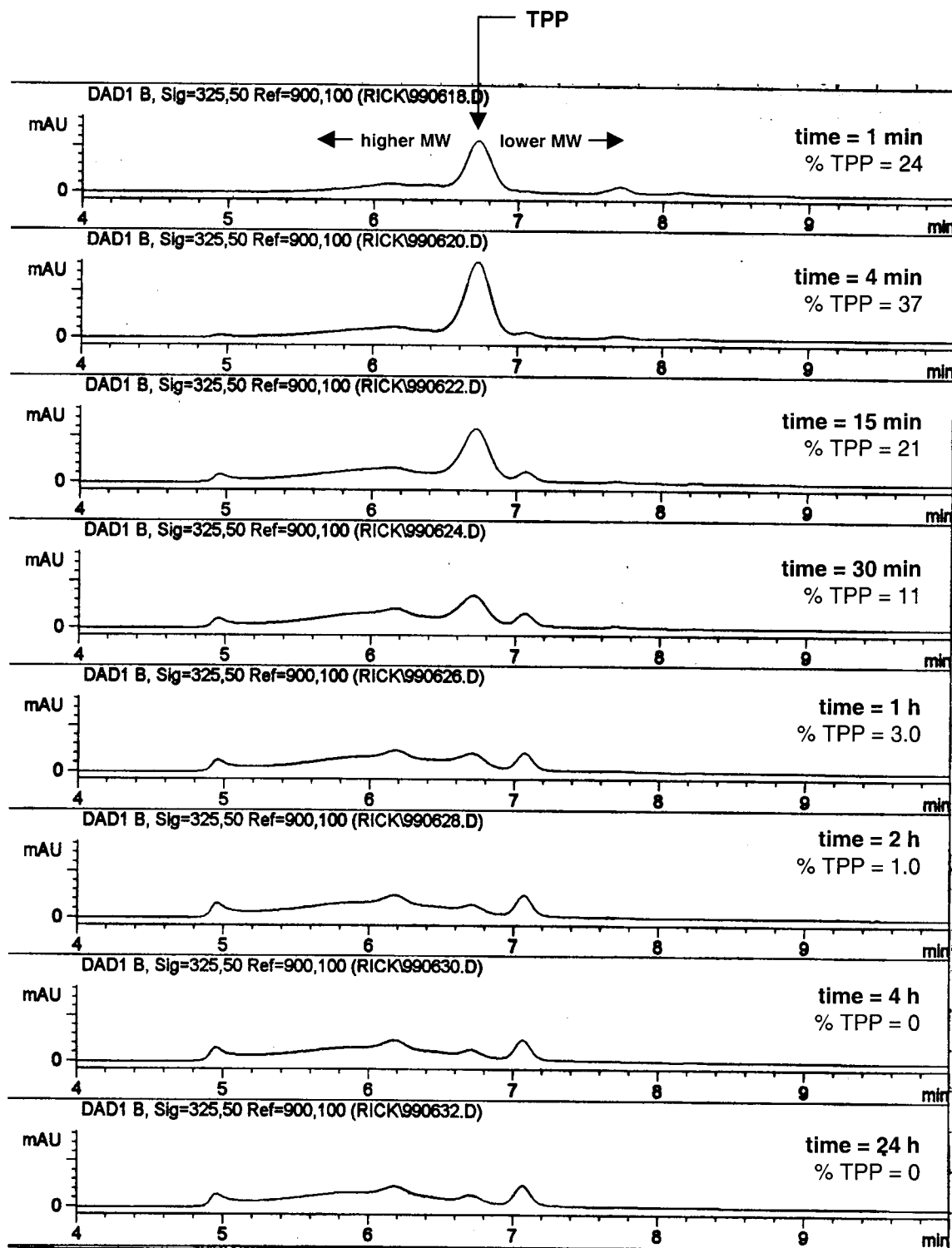


Figure S4. SEC data as a function of time for 10 mM reactants, 10 mM BF₃-etherate. Detection wavelength was 325 nm.

