

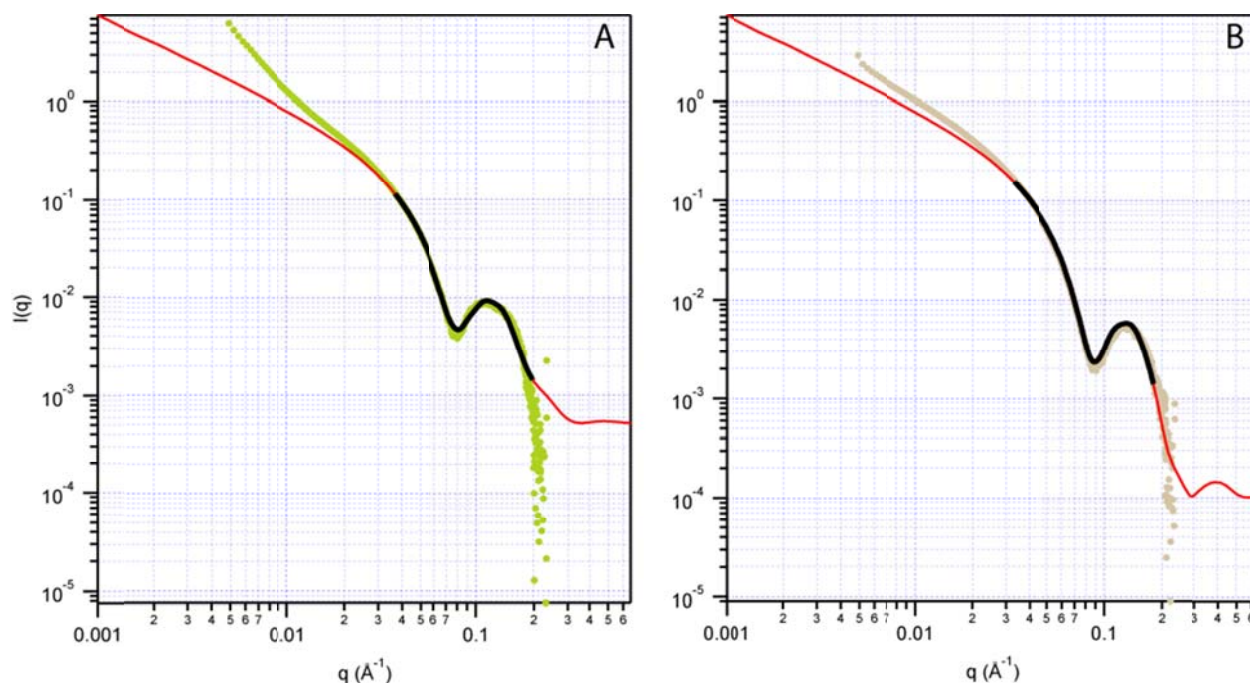
## Supporting Information

### Drug Release from Hydrazone-containing Peptide Amphiphiles

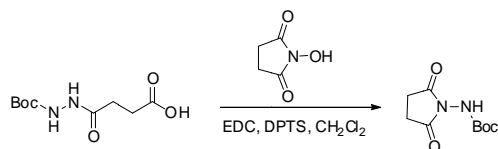
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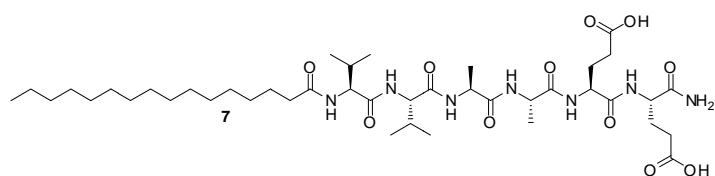
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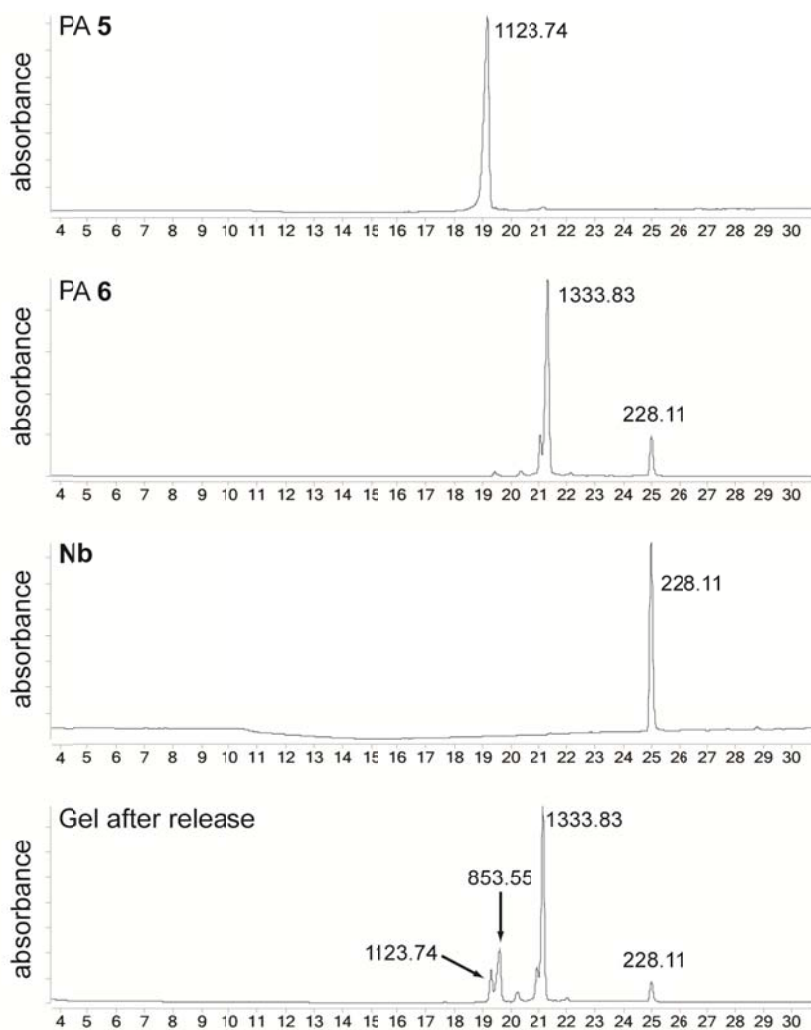
**Figure S1.** SAXS patterns of 0.5 wt% PA **5** (A); and 0.5 wt% PA **6** (B) in aqueous solution. The green (A) and grey (B) dots represent the raw data. The solid red line represents the best fit to a core-shell cylinder form factor given by the equations below, where the core was allowed to be polydisperse according to a log-normal distribution. The solid black line represents the portion of the curves where fits were performed. The best parameters obtained from this fit are a core radius of 17.9 Å, a shell thickness of 17.8 Å, and a  $\sigma$  of 0.23 for PA **5**, and a core radius of 13.1 Å, a shell radius of 22.0 Å and a  $\sigma$  of 0.24 for PA **6**.



**Scheme S1.** Putative intramolecular cyclization reaction of Boc-NHNHC(O)CH<sub>2</sub>CH<sub>2</sub>COOH. <sup>1</sup>H and <sup>13</sup>C NMR spectra are consistent with the proposed product.



**Figure S2.** Chemical structure of  $C_{16}V_2A_2E_2$  (PA 7).



**Figure S3.** LCMS characterization of PA 5, PA 6, Nb and the PA gel after release. The MW value of 853.55 corresponds to PA 7. PA 6 is detected as two peaks due to the *cis* and *trans* isomers of the hydrazone. A small amount of Nb is observed here due to hydrazone hydrolysis during sample processing. LCMS of the gel after release shows the expected species (PA 5, PA 6, PA 7 and Nb), confirming minimal peptide degradation and that Nb is indeed released from the gel.

## Materials and Methods

Rink Amide MBHA resin and Fmoc-protected amino acids purchased from Novabiochem Corporation. Boc<sub>2</sub>O was purchased from Oakwood Products and used as received. EDC was purchased from Novabiochem Corporation and used as received (EDC = *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride). DPTS was synthesized as reported (DPTS = dimethylaminopyridinium *p*-toluene sulfonate).<sup>1</sup> Nabumetone was purchased from LKT Laboratories, Inc. and used as received. All other reagents were purchased from Aldrich and used as received. CH<sub>2</sub>Cl<sub>2</sub> for organic synthesis was dried over activated alumina on a solvent purification system before use. All other solvents were ACS reagent grade and purchased from Mallinckrodt and reagents were purchased from Aldrich and used as received. NMR spectra were recorded on a Varian Unity Plus 500 spectrometer, with working frequencies of 499.4 MHz for <sup>1</sup>H and 125.6 MHz for <sup>13</sup>C. Chemical shifts are reported in ppm and referenced to the residual non-deuterated solvent frequencies. High resolution mass spectra were taken by the Integrated Molecular Structure Education and Research Center (IMSERC) at Northwestern University.

**PA purification.** Purification by preparative-scale HPLC was carried out on a Varian Prostar 210 HPLC system, eluting with of 2% ACN to 100% ACN in water on a Phenomenex C18 Gemini NX column (150 x 30 mm) with 5 μm pore size and 110 Å particle size. 0.1% NH<sub>4</sub>OH was added to both mobile phases to aid PA solubility. Product-containing fractions were confirmed by ESI mass spectrometry (Agilent 6510 Q-TOF LC/MS), combined, and lyophilized after removing ACN by rotary evaporation.

**LCMS.** Analytical LCMS was performed on an Agilent 1200 system with an Agilent 6250 quadrupole-time-of-flight mass spectrometer. using a Phenomenex Gemini C18 column (5 μm particle size, 150 x 1.0 mm) eluting with a gradient of 5% ACN to 95% ACN in water, with each solvent containing 0.1% NH<sub>4</sub>OH. UV absorbance was monitored at 220 nm.

**SEM.** SEM samples were prepared by dissolving powders of PA (1:1 w/w of PA **6**:PA **7**, 300 μg total), which had been lyophilized from HFIP, in 27 μL NaOH (10 mM), followed by gelation with 3 μL CaCl<sub>2</sub> (100 mM). After standing over night, gels were fixed with 4% glutaraldehyde and 3% sucrose in PBS by layering fixative on top of the gel for 90 min. Gel samples were then dehydrated using a graded ethanol series, followed by drying at the critical point using a Tousimis Samdri-795 critical point dryer. Dry samples were mounted on SEM stubs with carbon conductive cement (Electron Microscopy Sciences) and coated with 8 nm OsO<sub>4</sub> using an osmium plasma coater (Structure Probe, Inc.). Images were taken using a Hitachi S-4800-II SEM.

**SAXS collection.** SAXS measurements were performed using beam line 5ID-D, in the DuPont-Northwestern-Dow Collaborative Access team (DND-CAT) Synchrotron Research Center at the Advanced Photon Source, Argonne National Laboratory. An energy of 15 keV corresponding to a wavelength  $\lambda=0.83 \text{ \AA}^{-1}$  was selected using a double-crystal monochromator. The data were collected using a CCD detector (MAR) positioned 245 cm behind the sample. The scattering intensity was recorded in the interval  $0.005 < q < 0.23 \text{ \AA}^{-1}$ . The wave vector defined as  $q = (4\pi/\lambda) \sin(\theta/2)$ , where  $\theta$  is the scattering angle. Samples were analyzed in 1.5 mm quartz capillaries at 0.5% by weight in 0.1% NH<sub>4</sub>OH. The 2-dimensional SAXS images were azimuthally averaged

to produce one-dimensional profiles of intensity ( $I$ ) vs  $q$ , using the two-dimensional data reduction program FIT2D. Scattering of a capillary containing only solvent was also collected and subtracted from the corresponding data. No attempt was made to convert the data to an absolute scale.

**SAXS modeling.** Data analysis was based on fitting the scattering curve to an appropriate model by a least-squares method using software provided by NIST (NIST SANS analysis version 7.0 on IGOR).<sup>2</sup> The scattering intensity of a monodisperse system of particles of identical shape can be described as:

$$I(q) = NP(q)S(q)$$

where  $N$  is the number of particles per unit volume,  $P(q)$  is the form factor revealing the specific size and shape of the scatterers and  $S(q)$  is the structure factor that accounts for the interparticle interactions.<sup>3</sup> In dilute solutions, where the interactions between the objects can be neglected,  $S(q)$  equals one. In a polydisperse system of particles having identical shape, the total intensity scattered from a can be described by:

$$I(q) = N \int_0^{\infty} D_n(R) P(q, R) dR$$

where  $D_n(R)$  is a distribution function and  $D_n(R)dR$  is the number of particles, the size of which is between  $R$  and  $R + dR$ , per unit volume of sample.

A form factor for a simple polydisperse core-shell cylinder, where the core and the shell have a uniform electron density, is given by:

$$P(q) = \int_0^{\pi/2} \sin \theta \cdot d\theta \cdot \left[ V_l(\rho_l - \rho_{solv}) \frac{\sin\left(\frac{qH_l \cos \theta}{2}\right)}{\frac{qH_l \cos \theta}{2}} \frac{2J_1(qR_l \sin \theta)}{qR_l \sin \theta} + V_p(\rho_p - \rho_l) \frac{\sin\left(\frac{qH_p \cos \theta}{2}\right)}{\frac{qH_p \cos \theta}{2}} \frac{2J_1(qR_p \sin \theta)}{qR_p \sin \theta} \right]^2$$

$$V_x = \pi R_x^2 H_x$$

where  $J_1(x)$  is the first order Bessel function. Theta is defined as the angle between the cylinder axis and the scattering vector,  $q$ .  $R_p$  and  $R_l$  are the core and shell radii respectively.  $H_p$  and  $H_l$  are the core and shell lengths and  $\rho$  is electron density.

The polydispersity of the core radius is modeled using a log-normal distribution

$$D_n(R_p) = \frac{\exp\left(-\frac{1}{2} \left[ \frac{\ln(R_p/R_0)}{\sigma_p} \right]^2\right)}{\sqrt{(2\pi)} \sigma_p R_p}$$

where  $R_0$  is the mean core radius and  $\sigma$  is equivalent to the standard deviation of the log-normal distribution.

**TEM.** TEM samples were cast from 0.1 wt. % solutions in 0.1%  $\text{NH}_4\text{OH}$  onto carbon film on copper 300 mesh TEM grids (Electron Microscopy Sciences). Samples were then stained with a 2% solution of uranyl acetate in water. Images were taken on a JEOL 1230 TEM with a Hamamatsu ORCA camera.

**Adipic acid monobenzyl ester (1).** A suspension of adipic acid (9.90 g, 67.7 mmol) in Ac<sub>2</sub>O (25 mL) was heated at reflux for 2.5 h. The Ac<sub>2</sub>O was then removed in vacuo, chasing with toluene (4 x 20 mL) to remove most of the solvent. The viscous oil became a white solid over a few hours. This solid was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and pyridine (8.2 mL, 101 mmol). The solution was cooled to 0 °C, at which point DMAP (826 mg, 6.7 mmol) was added. Once homogenous, benzyl alcohol (8.4 mL, 81.1 mmol) was added dropwise via an addition funnel over 3 min. The reaction mixture was allowed to warm to room temperature as the ice melted. After 1.5 h the reaction mixture was washed with 0.5 N HCl (2 x 30 mL), and the solvent was removed in vacuo. The residue was taken up in sat. aq. NaHCO<sub>3</sub> (60 mL) and washed with Et<sub>2</sub>O (30 mL). The NaHCO<sub>3</sub> solution was then acidified to pH=1 with conc. HCl and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 60 mL). The combined CH<sub>2</sub>Cl<sub>2</sub> extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent was removed in vacuo to yield a pale yellow oil in 29% yield (4.68 g). <sup>1</sup>H NMR(CDCl<sub>3</sub>): 7.27-7.39 (m, 5H), 5.13 (s, 2H), 2.37-2.42 (m, 4H), 1.68-1.75 (m, 4H). <sup>13</sup>C NMR(CDCl<sub>3</sub>): 179.46, 173.31, 136.01, 128.62, 128.29, 66.34, 33.91, 33.68, 24.31, 24.10. HRMS: (M+Na): calc'd: 259.0941; found: 259.0944.

**1-[N-(*tert*-Butyloxycarbonyl)hydrazide]-6-benzyl ester adipate (2).** A solution of *tert*-butyl carbazate (9.87 g, 74.7 mmol) in DMF (20 mL) was cooled to 0 °C. A second solution was prepared containing carboxylic acid **1** (6.0 g, 25.4 mmol) in DMF (70 mL), to which was added EDC (6.28 g, 32.7 mmol) and DPTS (700 mg, 2.4 mmol). After 15 min, the activated acid solution was added to the cooled *tert*-butyl carbazate solution via addition funnel over 15 min. The reaction mixture was allowed to warm to room temperature as the ice melted. After 2 h, the DMF was removed in vacuo until the reaction mixture was concentrated to approximately 20 mL. A solution of glyoxylic acid (13.5 g) in 0.4M Na<sub>2</sub>HPO<sub>4</sub> (30 mL) at pH=5 (adjusted using 2N NaOH) was then added to the DMF solution. After overnight stirring, the reaction mixture was diluted with sat. aq. NaHCO<sub>3</sub> (100 mL). This aqueous solution was extracted with Et<sub>2</sub>O (3 x 100 mL). The combined Et<sub>2</sub>O extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent was removed in vacuo to yield a pale yellow oil in 83% yield (7.40 g). <sup>1</sup>H NMR(CDCl<sub>3</sub>): 8.08 (br s, 1H), 7.31-7.7.37 (m, 5H), 6.82 (s, 1H), 5.10 (s, 2H), 2.38 (t, J=7 Hz, 2H), 2.21-2.30 (m, 2H), 1.67-1.70 (m, 4H), 1.45 (s, 9H). <sup>13</sup>C NMR(CDCl<sub>3</sub>): 173.50, 172.52, 155.92, 136.07, 128.70, 128.37, 128.35, 81.85, 66.40, 33.97, 33.62, 28.29, 24.76, 24.40. HRMS(M+Na): calc'd: 373.1734; found: 373.1731.

**1-[N, N, N'-Tris((*tert*-butyloxycarbonyl))hydrazide]-6-benzyl ester adipate (3).** To an oven- and flame-dried, 3-necked, roundbottom flask equipped with an addition funnel under N<sub>2</sub> flow was added Boc<sub>2</sub>O (8.40 g, 63.6 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (15 mL). The solution was cooled to 0 °C, then DMAP (762 mg, 6.2 mmol) was added. A solution of mono-Boc protected hydrazide **2** (7.40 g, 21.1 mmol) in NEt<sub>3</sub> (8.8 mL, 63.1 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was added dropwise over 15 min. via the addition funnel to the Boc<sub>2</sub>O/DMAP solution, which was kept at 0 °C during addition. After addition, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature. After an additional 2 h, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL), and the organic solution was washed with sat. aq. KH<sub>2</sub>PO<sub>4</sub> (3 x 30 mL). The CH<sub>2</sub>Cl<sub>2</sub> layer was then dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent was removed in vacuo to yield a pale yellow oil in 94% yield (10.89 g). <sup>1</sup>H NMR(CDCl<sub>3</sub>): 7.31-7.35 (m, 5H), 5.10 (s, 1H), 2.90 (m, 2H), 2.39 (m, 2H), 1.71 (m, 4H), 1.45-1.50 (m, 27H). <sup>13</sup>C NMR(CDCl<sub>3</sub>): 173.28, 171.75, 150.81,



149.39, 136.18, 128.68, 128.32, 128.30, 84.33, 83.96, 66.29, 36.66, 34.16, 28.02, 27.99, 24.51, 24.23. HRMS: (M+Na): calc'd: 573.2780; found: 573.2783.

**1-[N, N, N'-Tris((*tert*-butyloxycarbonyl))hydrazide]-adipic acid (4).** A solution of benzyl ester **3** (10.88 g, 19.75 mmol) was dissolved in MeOH (100 mL). To this solution was added Pd/C (10 wt% loading, 1.08 g), and a balloon of H<sub>2</sub> gas was added. After 4 h the reaction mixture was filtered through Celite, eluting with CH<sub>2</sub>Cl<sub>2</sub>. Removal of the solvent in vacuo yielded a clear oil in 86% yield (7.87 g). <sup>1</sup>H NMR(CDCl<sub>3</sub>): 9.21 (br s, 1H), 2.88 (t, J= 7 Hz, 2H) 2.34 (t, J= 7 Hz, 2H), 1.64-1.76 (m, 4H), 1.42-1.50 (m, 27H). <sup>13</sup>C NMR(CDCl<sub>3</sub>): 178.05, 171.84, 150.78, 149.42, 84.40, 84.06, 36.65, 34.08, 28.02, 27.99, 24.37, 24.21. HRMS (M+H): calc'd: 483.2313; found: 483.2322.

**Hydrazide-containing PA (5).** Synthesis of hydrazide-containing PA **5** was performed on a 1 mM scale from Rink Amide MBHA resin using standard solid phase synthesis conditions. For each coupling, 4 equiv of Fmoc-protected amino acid was added using 4 equiv HBTU and 6 equiv DIEA in 20 ml DMF (HBTU = O-benzotriazole-N,N,N',N'-tetramethyluronium-hexafluorophosphate; DIEA = N,N-diisopropylethylamine). Fmoc removal was accomplished with 30% piperidine in DMF. For addition of the hydrazido acid building block (**4**), Fmoc-Lys(Mtt)-OH was added to the resin, and the Mtt group was removed on-resin using 4% TFA and 4% TIPS in CH<sub>2</sub>Cl<sub>2</sub>, washing until yellow color was no longer visible (TFA = trifluoroacetic acid; TIPS = triisopropylsilane). The resin was briefly washed with 5% DIEA in DMF, then carboxylic acid **4** (2.5 equiv) was added with 2.5 equiv HBTU and 4 equiv DIEA in DMF. A Kaiser (ninhydrin) test showed a negative result after coupling. The palmitic acid tail was added using the same conditions. The PA was cleaved from the resin using a peptide cleavage solution of 95% TFA, 2.5% TIPS and 2.5% H<sub>2</sub>O. Concentration of the cleavage solution in vacuo and precipitation of the residue into cold Et<sub>2</sub>O afforded the crude product, which was purified by preparative HPLC. MS (M+H): calc'd: 1123.72; found: 1123.72.

**Nabumetone-containing PA (6).** To a suspension of PA **5** in DMSO (100 mg/mL) was added **Nb** (1.2 equiv). The suspension was sonicated at 40 °C until complete dissolution and gelation was observed (about 30 min). After standing overnight at rt, 0.1% aqueous NH<sub>4</sub>OH was added, and the gel was broken up and dissolved with sonication and vortexing. This mixture was filtered and purified by HPLC. A small amount of free **Nb** was observed after lyophilization due to hydrazone hydrolysis during workup. MS (M+H): calc'd: 1333.83; found: 1333.83.

**Release experiments.** PA mixtures were prepared for release experiments by dissolving stock solutions of PA **6** and PA **7** (C<sub>16</sub>V<sub>2</sub>A<sub>2</sub>E<sub>2</sub>) in HFIP at 10 mg/mL (HFIP = 1,1,1,3,3,3-hexafluoro-2-propanol), mixing solutions 1:1 (v/v) and lyophilizing the HFIP mixture to afford a powder of 1312.5 mg PA **6** and 1312.5 mg PA **7**. The powder was taken up in 10 mM NaOH (236.2 μL) and divided into 5 aliquots of 45 μL each in Eppendorf tubes (total Nb content per tube = 42.7 μg). Each solution was individually gelled with 5 μL CaCl<sub>2</sub> (100 mM) and allowed to stand overnight. 1 mL of release buffer (5% DMSO in 100 mM phosphate buffer with pH = 7.4) was added on top of each gel. At each timepoint, the entire release solution was removed and replaced with fresh buffer. No degradation of the gel was apparent over the course of study. **Nb** content was quantified by measuring absorbance at 330 nm using an M5 Spectramax Plate Reader (Molecular Devices). The average of two measurements was taken for sample, and the

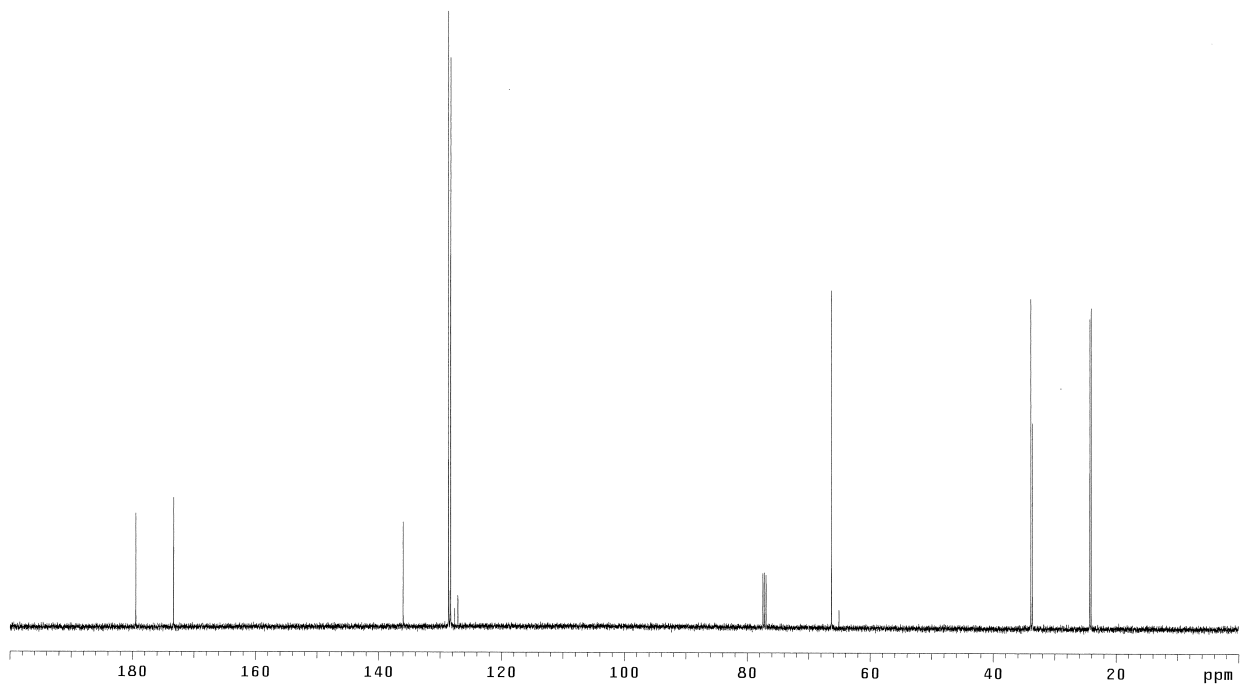
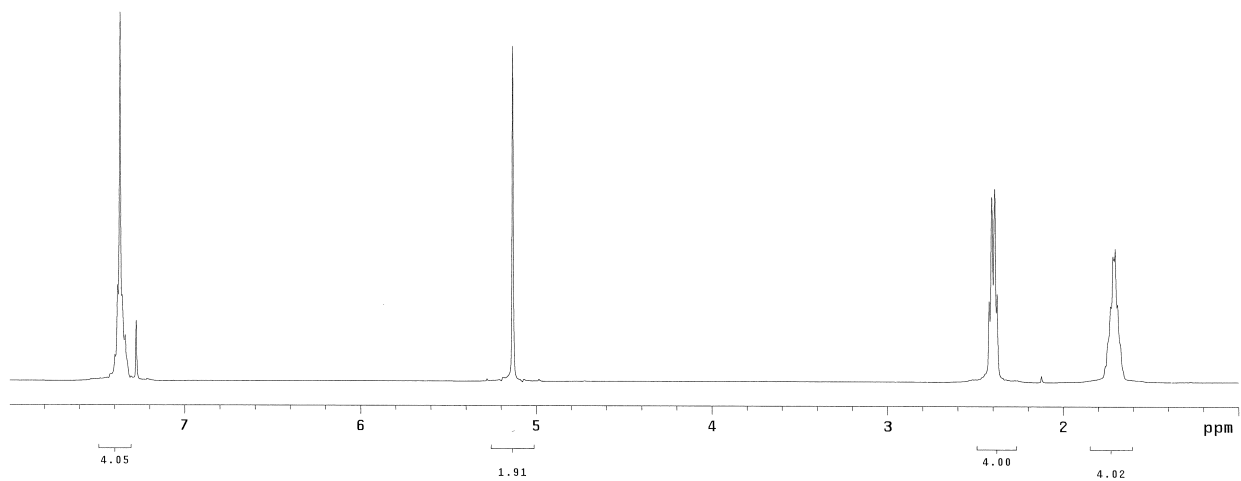
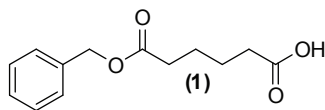
average of the 5 samples are shown in Figure 2, with error bars corresponding to the standard deviation of the cumulative release.

## References

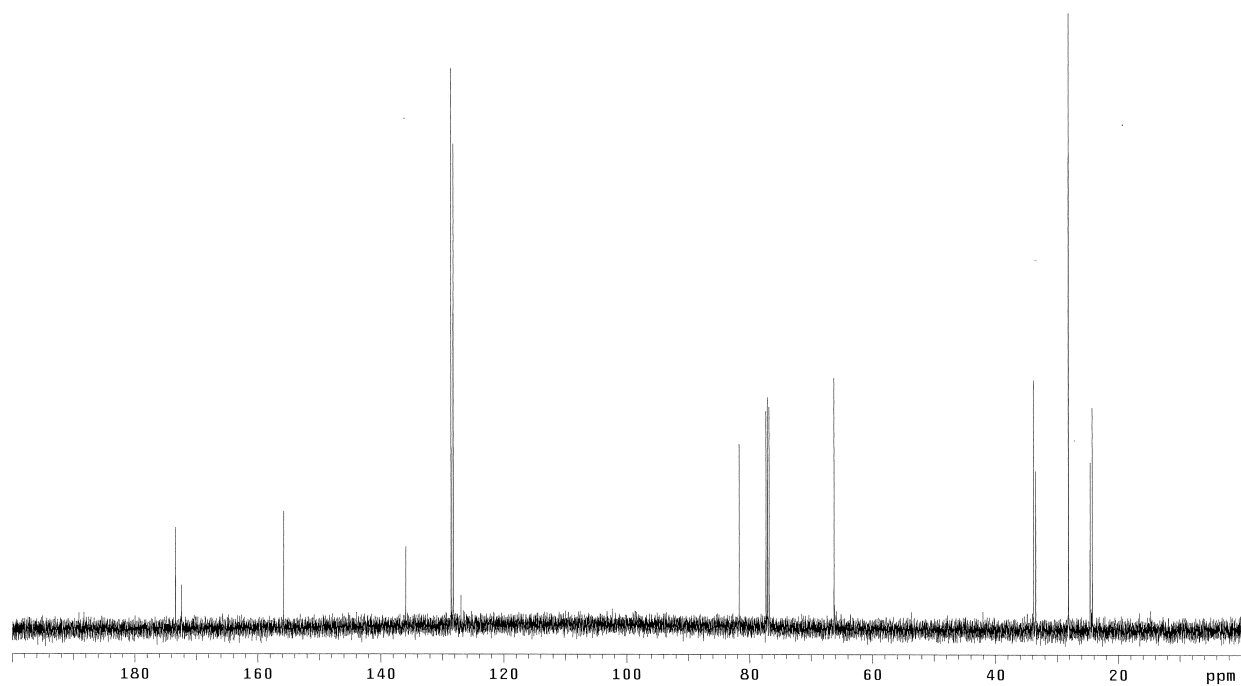
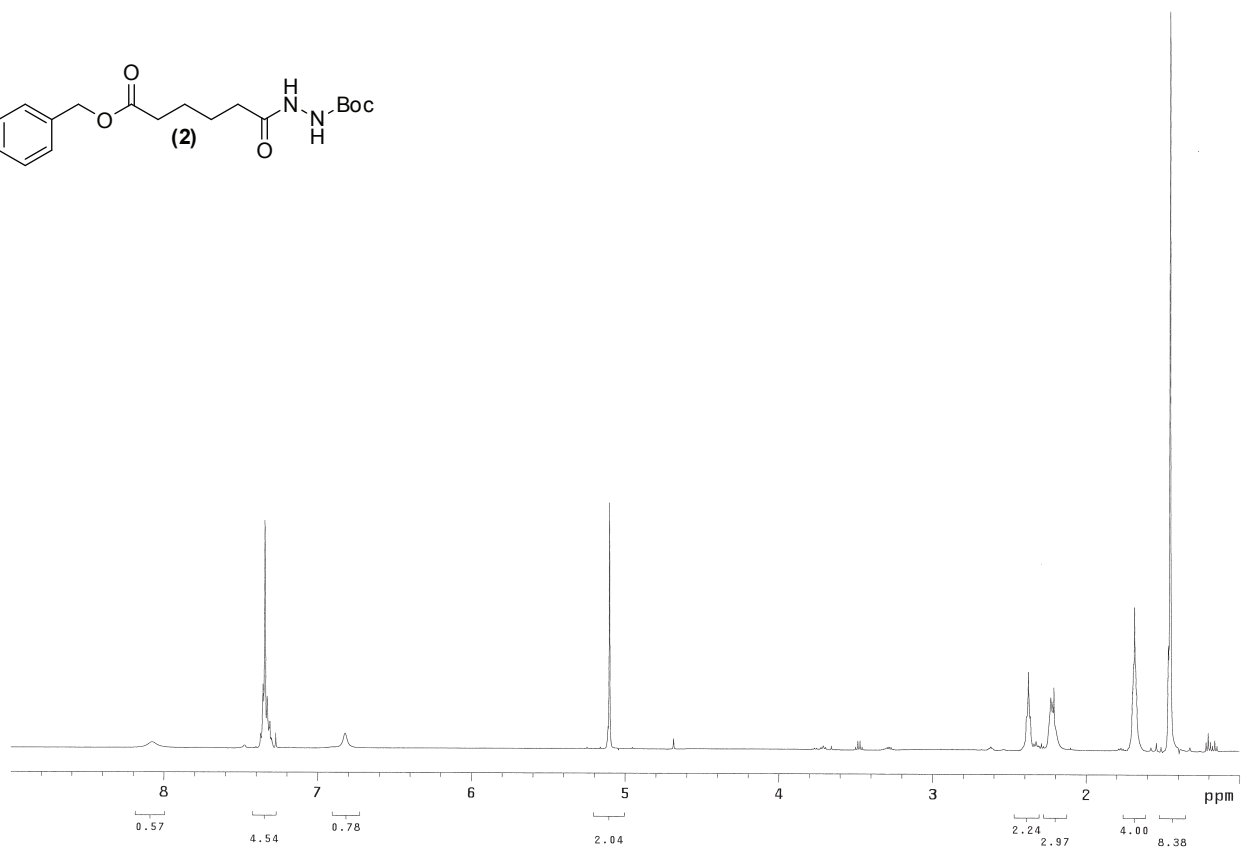
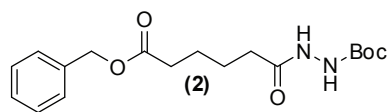
<sup>1</sup> J. S. Moore and S. I. Stupp, *Macromolecules*, 1990, **23**, 65-70.

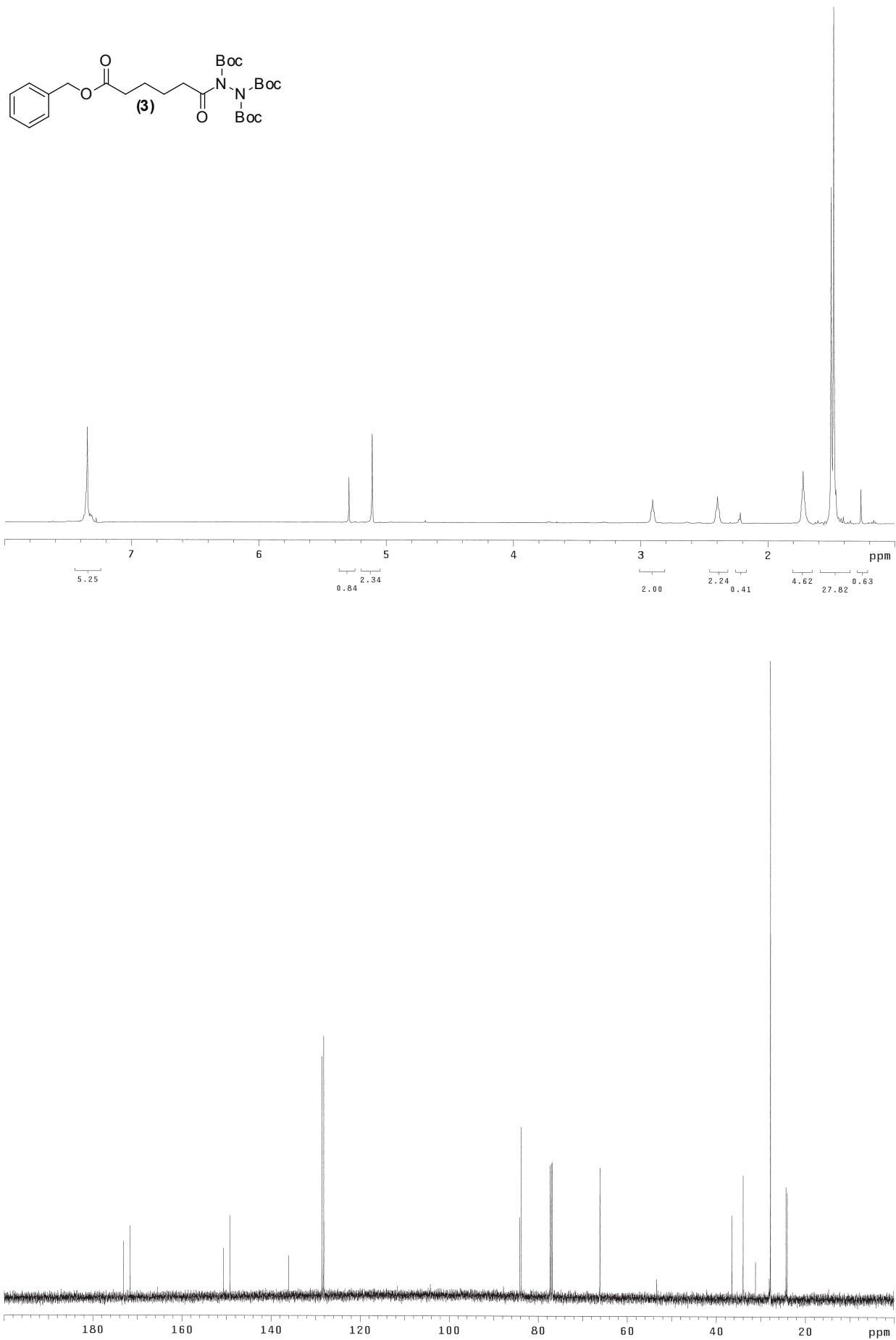
<sup>2</sup> S. R. Kline, *Journal of Applied Crystallography*, 2006, **39**, 895-900.

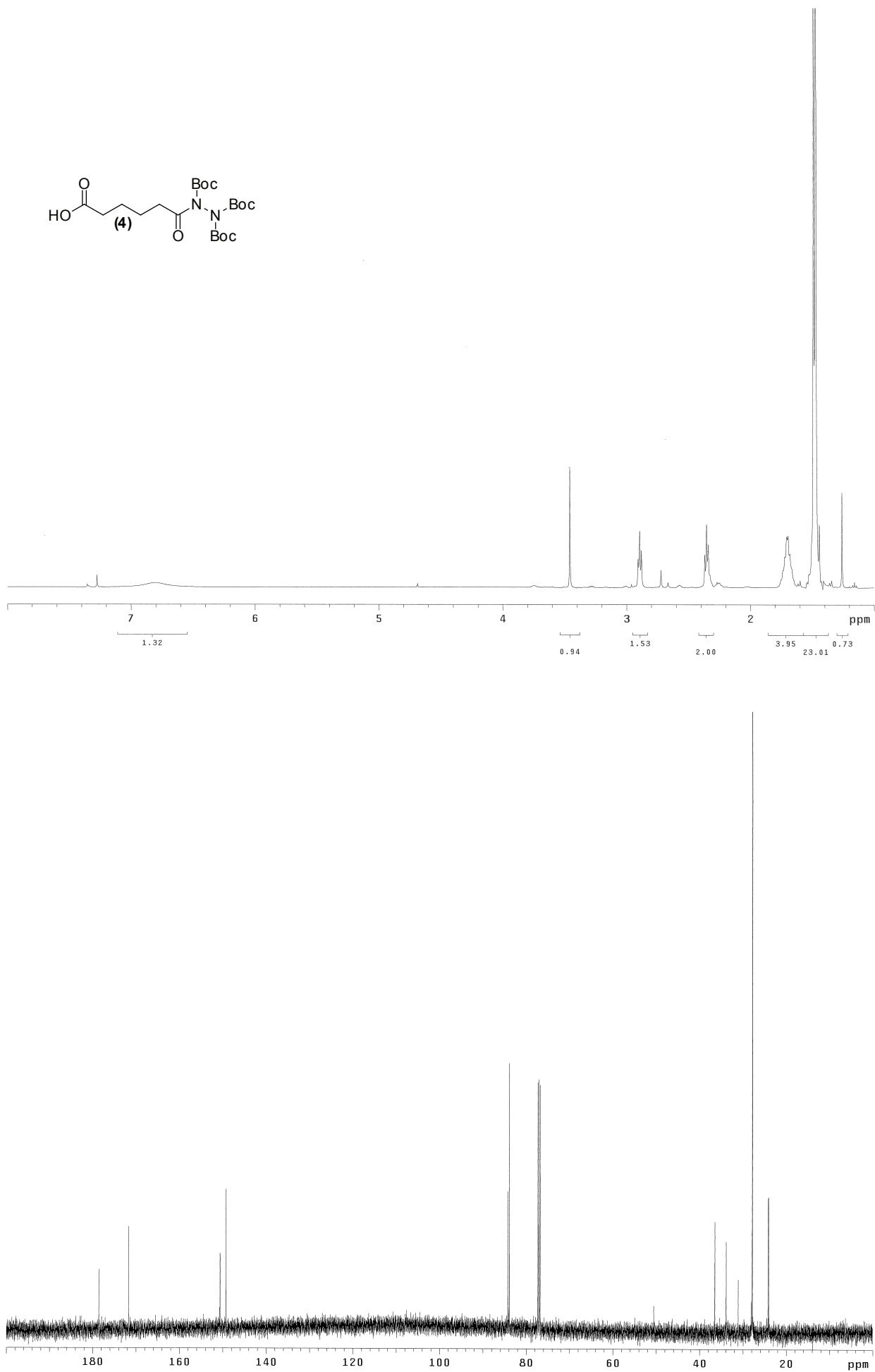
<sup>3</sup> O. Glatter and O. Kratky, *Small angle x-ray scattering*, Academic Press, London ; New York, 1982.

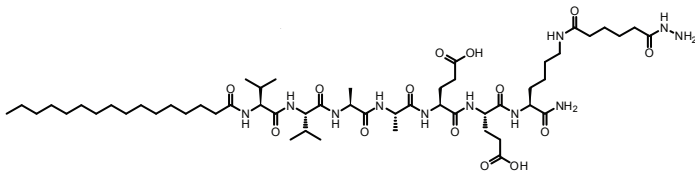




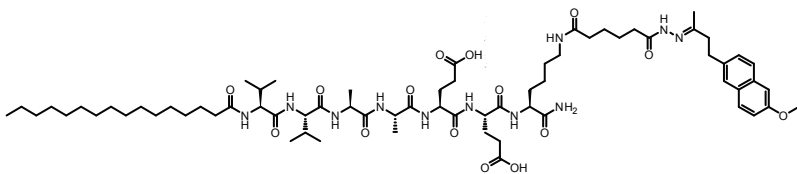
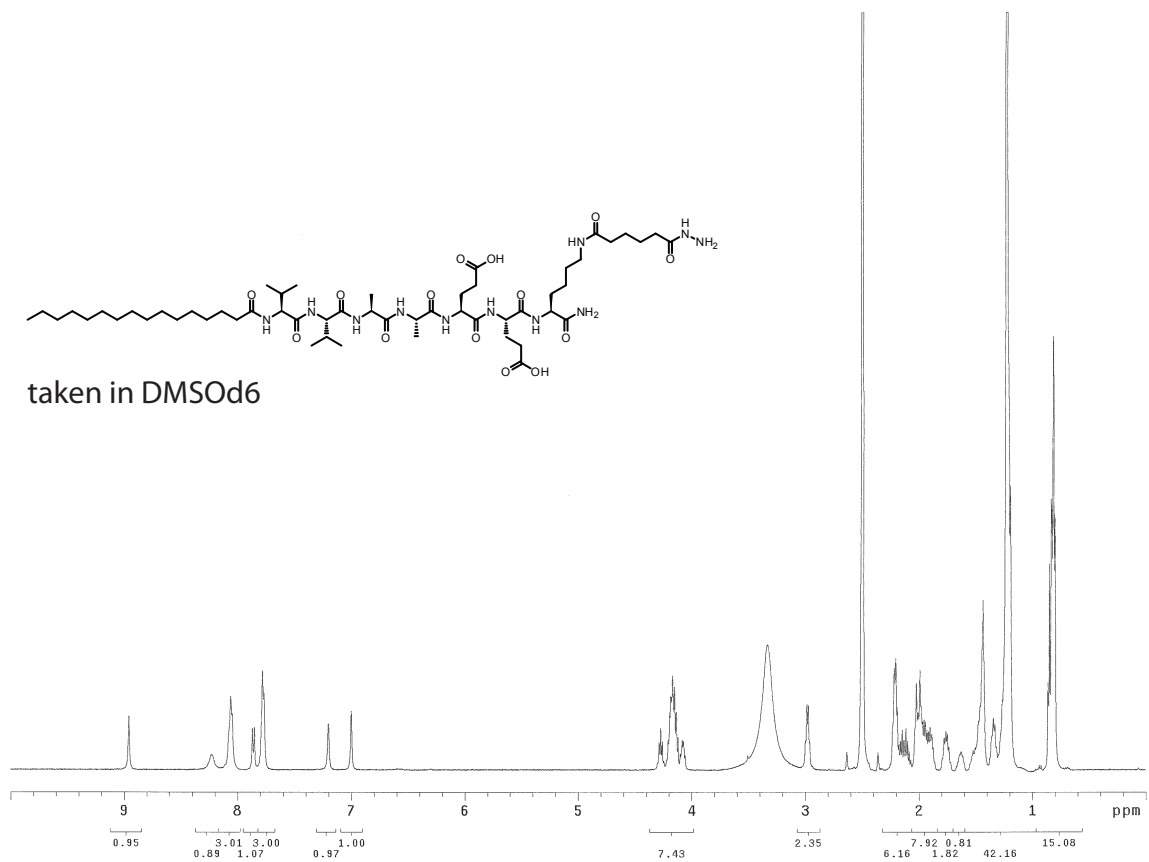








taken in DMSOd6



taken in DMSOd6

