Electronic supplementary information for:

"Synthesis of Bioinspired Carbohydrate Amphiphiles that Promote and Inhibit Biofilms"

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Contents

GENERAL METHODS AND INSTRUMENTATION

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian 500 MHz spectrometer. FTIR spectra were obtained by pressing a small amount of dry sample onto a horizontal attenuated total reflectance (ATR) adapter on a Nicolet FT-IR. WAXS data was obtained using a Rigaku MiniFlex Powder Diffractometer with a CuK_{α} X-ray source. The powder sample, approximately 20-30 mgs, was packed into a 16mm x 20mm x 0.4mm depression in a glass slide. Polymer molecular weights were determined by gel permeation chromatography (GPC) versus polystyrene standards using THF as the eluent at a flow rate of 1.0 mL/min with a Styragel column (HR4E, 7.8 x 300 mm) in series with a refractive index detector. Dynamic light scattering (DLS) measurements were performed on a Brookhaven 90plus Nano-particle Sizer at a scattering angle of 90°. Surface tension measurements were performed on a Kruss DSA100 contact angle goniometer by the hanging drop method using Young-Laplace drop shape analysis. MALDI-tof analysis was performed at HT Labs, San Diego, CA using 2,5-dihydroxybenzoic acid as the matrix. Unless otherwise mentioned, all chemicals were purchased from Sigma-Aldrich, Alfa-Aesar, or Acros and used as received. Monomers **3** and **4** were synthesized using the previously reported method.[1]

Critical Micelle Concentration. Stock dispersions of the amphiphiles with concentrations ranging from 0.4-0.2 mM based on Mn_{NMR} (reported in Table S2) were prepared in PBS buffer. In a 48-well plate, dispersions were serially diluted (2-fold) for a total of 16 concentrations ranging from 0.4-0.2 mM to less than 0.5 μM. In a separate 48-well plate, 5.0 μL of a 0.02 mM pyrene stock solution in methanol was placed in each well and allowed to evaporate. The diluted solutions were transferred to the plate containing pyrene and incubated for 30 minutes at 37°C. For the assay, the pyrene concentration was 0.5 μM for all samples. In a 100 μL quartz cuvette, pyrene fluorescence was recorded with excitation at 340 nm. Pyrene fluorescence intensity at 370 nm was plotted against concentration on a logarithmic scale. The CMC was determined by the intersection of fitted-line segments of the concentrations below the CMC (nearly horizontal) and the concentrations showing a rapid change in fluorescence as described and illustrated in Astafieva *et al*.[2] CMC determinations were repeated twice and the average of both determinations is reported in Figure 2. Standard deviations were less than 2 μM for each pair of determinations.

Biofilm and swarming motility assays. All strains were routinely cultured on lysogeny broth (LB) medium, which was solidified with 1.5% agar when necessary. For biofilm assays, M63 minimal salts medium was supplemented with $MgSO_4$ (1 mM) and arginine (0.4%), as reported.[3] Swarming motility plates were comprised of M8 medium supplemented with $MgSO_4$, glucose, and CAA and solidified with 0.5% agar. For each strain tested, 2 μl of LB grown overnight cultures was inoculated onto the surface of the swarm plates and incubated for 16 h at 37°C.[4] Stock solutions of amphiphiles were prepared at 4 mg/mL in PBS buffer and filtered through 0.2 μm syringe filters prior to use. After filtering, amphiphile $\mathbf{1b_{20}}$ was incubated at 37 °C for 16 hours before use.

Electron microscopy. Compounds were negatively stained with 1% ammonium molybdate pH 7.0. TEM images were captured at 100kV on a FEI Tecnai F20ST FEG, equipped with a digital camera (XR-41B; Advanced Microscopy Techniques). Compounds were analyzed at the concentrations indicated in the text.

Quantifying c-di-GMP levels. The bulk levels of c-di-GMP were quantified as reported, with one modification.[5] Bacteria were harvested from the surface of swarming agar after growth in 6-well plates, rather than full-sized petri plates. All strains were analyzed in triplicate.

SYNTHETIC PROCEDURES

General Procedure for Synthesis of Amphiphile 1' Series.
In an oven-dried flask, lactam **3** (0.250 g, 0.54 mmol) and initiator **5** (a-group) or **6** (b-group) (10 mol% for DP_{th} = 10, 5 mol% for DP_{th} = 20) were dissolved in 4 mL of distilled tetrahydrofuran (without BHT) which had been dried over molecular sieves. The reaction flask was cooled to 0 °C in an ice bath. Next, the appropriate volume of a 0.5 M solution of LiHMDS in THF (25 mol% for DP_{th} = 10 and 12.5 mol% for DP_{th} = 20) was added and the

solution was stirred for 0.5 hr, at which time the reaction was allowed to warm to room temperature. After 0.5 hr at room temperature, complete consumption of **3** was confirmed by thin-layer chromatography with 1:1 ethyl acetate and hexane. To quench the reaction, a drop of saturated NH_4Cl aqueous solution was added. The THF was removed and the resulting solid was redissolved in diethyl ether (25 mL) and washed with 1 M HCl, sat'd $NaHCO_3$, and brine. After drying over sodium sulfate, the product was isolated by evaporation of solvent. The product was an amorphous solid that adhered to the walls of the flask. Residual hydrophobic impurities were removed by washing with pentane that was then decanted. After drying under high vacuum, a white powder was isolated with quantitative or close to quantitative yield.

Example Procedure

Amphiphile 1'a$_{10}$

In an oven-dried flask, lactam **3** (0.250 g, 0.54 mmol) and initiator **5** (29.1 mg, 0.054 mmol, 10 mol%) were dissolved in 4 mL of distilled tetrahydrofuran dried over molecular sieves. The reaction flask was cooled to 0 °C in an ice bath. Next, 0.28 mL of a 0.5 M solution of LiHMDS in THF (0.140 mmol, 26 mol%) was added and the solution was stirred for 0.5 hr, at which time the reaction was allowed to warm to room temperature. Complete consumption of **3** was confirmed by thin-layer chromatography with 1:1 ethyl acetate and hexane. To quench the reaction, a drop of saturated NH_4Cl aqueous solution was added. The THF was removed and the resulting solid was redissolved in diethyl ether (25 mL) and washed with 1 M HCl, sat'd $NaHCO_3$, and brine. After drying over sodium sulfate, the product was isolated by evaporation of solvent. The product was washed with pentane and the pentane was decanted from the flask. This process was repeated twice. After drying under high vacuum, 0.253 g (96%) of a white powder was isolated.

General Procedure for Synthesis of Amphiphile 2' Series.

In an oven-dried flask, lactam **3** (0.125 g, 0.27 mmol), lactam **4** (0.125 g, 0.27 mmol), and initiator **5** (a-group) or **6** (b-group) (10 mol% for DP_{th} = 10, 5 mol% for DP_{th} = 20) were dissolved in 4 mL of distilled tetrahydrofuran (without BHT) which had been dried over molecular sieves. The reaction flask was cooled to 0 °C in an ice bath. Next, the appropriate volume of a 0.5 M solution of LiHMDS in THF (25 mol% for DP_{th} = 10 and 12.5 mol% for DP_{th} = 20) was added and the solution was stirred for 0.5 hr, at which time the reaction was allowed to warm to room temperature. After 0.5 hr at room temperature, complete consumption of **3** and **4** was confirmed by thin-layer chromatography with 1:1 ethyl acetate and hexane. To quench the reaction, a drop of saturated NH_4Cl aqueous solution was added. The THF was removed and the resulting solid was redissolved in diethyl ether (25 mL) and washed with 1 M HCl, sat'd $NaHCO_3$, and brine. After drying over sodium sulfate, the product was isolated by evaporation of solvent. The product was washed with pentane and the pentane was decanted from the flask. This process was repeated twice. After drying under high vacuum, a white powder was isolated with quantitative or close to quantitative yield.

General Procedure for Debenzylation of Amphiphiles

Amphiphile **1'** or **2'** (0.200 - 0.250 g depending on sample) was dissolved in 5.0 mL of tetrahydrofuran. Next, 1.5 equiv. of LiHMDS (from 1.0 M stock solution in tetrahydrofuran) was added to this solution. The solution was stirred for 5 minutes at room temperature. The solution was added dropwise into a rapidly stirred solution of sodium in anhydrous liquid ammonia (50 mL) at −78 °C under nitrogen. Sodium was washed in toluene and hexane and cut into small pieces before addition. The solution's deep blue color was maintained by adding additional sodium. After 1 hr at −78 °C, sat'd ammonium chloride was added until the blue color disappeared. After evaporation of the ammonia at room temperature, the resulting aqueous layer was washed with diethyl ether twice. The aqueous solution was dialyzed with 100-500 dalton MWCO tubing for 2 days with 3 water changes. After lyophilization, the resulting white solid was washed with methanol (10 mL), in which none of the samples were soluble, and collected by filtration as white powders. Yields are reported over both steps, polymerization and deprotection, and ranged from 71 – 96%.

Initiator Syntheses

6-palmitamidohexanoic acid

In a flask, 5.00 g (0.038 mol, 1.0 equiv) of 6-aminohexanoic acid and 3.8 g of sodium hydroxide (0.95 mol, 2.5 equiv) were dissolved in approximately 25 mL of water. In a separate flask, 11.5 g of palmitoyl chloride (0.042 mol, 1.1 equiv) was dissolved in approximately 50 mL of diethyl ether. With cooling in an ice bath, the ether solution was added dropwise to the aqueous solution with vigorous stirring and a precipitate formed. After 2 hours, the reaction mixture was poured into 100 mL of 1.0 M HCl and stirred to acidify. The precipitate was collected by filtration and then recrystallized from refluxing methanol with 5% glacial acetic acid. Following filtration and drying under vacuum, 12.2 g (87% yield) of a white powder was isolated. ^{1}H NMR (500 MHz, d_6-DMSO): δ 7.72 (br, 1H, N*H*), 2.99 (td, *J* = 6.9, 5.5 Hz, 2H), 2.17 (t, *J* = 7.4 Hz, 2H), 2.01 (t, *J* = 7.4 Hz, 2H), 1.52 – 1.41 (m, 4H), 1.36 (p, *J* = 7.2 Hz, 2H), 1.22 (m, 24H), 0.85 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, d_6-DMSO) δ 174.5, 172.0, 38.3, 35.5, 33.7, 31.3, 29.1 (3), 29.0 (2), 28.9, 28.8 (2), 28.7, 26.0, 25.4, 24.3, 22.1, 14.0. IR (ATR): 3310, 3284, 2915, 2849, 1714, 1695, 1637 (amide I), 1568 and 1547 (amide II) cm^{-1}. HRMS (m/z): [M+H]+ calcd. for $C_{22}H_{44}NO_3$, 370.3321; found, 370.3335.

pentafluorophenyl 6-palmitamidohexanoate (5)

In a flask, 5.00 g (0.014 mol, 1.0 equiv) of 6-palmitamidohexanoic acid, 2.74 g (0.015 mol, 1.1 equiv) of pentafluorophenol, and 3.07 g (0.015 mol, 1.1 equiv) of dicyclohexylcarbodiimide were dissolved in approximately 50 mL of dichloromethane and stirred for 2 hours. The reaction was filtered to remove dicyclohexyl urea and the solvent was removed. The crude solid was washed with methanol to remove any excess dicyclohexyl urea and then recrystallized from a mixture of diethyl ether and dichloromethane cooled to – 20 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.48 (br, 1H, N*H*), 3.26 (m, 2H), 2.67 (t, *J* = 7.3 Hz, 2H), 2.15 (m, 2H), 1.79 (p, *J* = 7.4 Hz, 2H), 1.61 (m, 2H), 1.55 (m, 2H), 1.46 (m, 2H), 1.31-1.24 (m, 24H), 0.87 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.3, 169.5, 39.2, 37.1, 33.3, 32.1, 29.8 (5), 29.7, 29.5 (4), 26.2, 26.0, 24.5, 22.8, 14.3, pentafluorophenol signals were not observed. IR (ATR): 3345, 2916, 2849, 1783, 1637 (amide I), 1520 (amide II) cm^{-1}. [M+Na]+ calcd. for $C_{28}H_{42}F_5NO_3Na$, 558.2983; found, 558.2995.

N,N'-dipalmitoyl L-lysine

In a flask, 5.00 g (0.0274 mol, 1.0 equiv) of L-lysine HCl and 5.00 g of sodium hydroxide (0.125 mol, 4.5 equiv) were dissolved in approximately 25 mL of water. In a separate flask, 16.6 g of palmitoyl chloride (0.061 mol, 2.2 equiv) was dissolved in approximately 50 mL of diethyl ether. With cooling in an ice bath, the ether solution was added dropwise to the aqueous solution with vigorous stirring and a precipitate formed. After 2 hours, the reaction mixture was poured into 100 mL of 1.0 M HCl and stirred to acidify. The precipitate was collected by filtration and then recrystallized from refluxing methanol with 5% glacial acetic acid. Following filtration and drying under vacuum, 13.5 g (79% yield) of a white powder was isolated. The ^{1}H, ^{13}C, and IR matched those previously reported.[6]

N,N'-dipalmitoyl L-lysine pentafluorophenyl ester (6)

In a flask, 0.500 g (0.803 mmol, 1.0 equiv) of N,N'-dipalmitoyl L-lysine, 0.162 g (0.883 mmol, 1.1 equiv) of pentafluorophenol, and 0.140 mL (0.883 mol, 1.1 equiv) of diisopropylcarbodiimide were dissolved in approximately 50 mL of chloroform and stirred for 16 hours at room temperature. The reaction was concentrated and the solid was suspended in methanol and filtered. Subsequently, it was washed with ethyl ether on the filter pad and dried. A white solid (0.527 g, 83% yield) was isolated and was found to be pure by NMR. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.56 (d, *J* = 7.0 Hz, 1H), 5.64 (t, *J* = 6.2 Hz, 1H), 4.81 (ddd, *J* = 8.4, 6.9, 4.4 Hz, 1H), 3.38 – 3.21 (m, 2H), 2.28 (td, *J* = 7.4, 2.6 Hz, 2H), 2.18 (t, *J* = 7.6 Hz, 2H), 1.98 (m, 2H), 1.69-1.54 (m, 6H), 1.52 – 1.41 (m, 2H),1.40 – 1.15 (m, 50H), 0.87 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.1, 173.9, 168.9, 52.0, 38.2, 37.0, 36.4, 32.1, 30.9, 29.9, 29.8 (3), 29.7, 29.6, 29.5 (3), 29.4, 25.9, 25.7, 22.8, 22.0, 14.3. IR (ATR): 3416 br, 3309, 2915, 2849, 1780, 1652 and 1638 (amide I), 1552 and 1550 (amide II) cm^{-1}. [M+Na]+ calcd. for $C_{44}H_{74}F_5N_2O_4$, 789.5569; found, 789.5595.

References Cited:

1. Kałuża, Z.; Abramski, W.; Bełżecki, C.; Grodner, J.; Mostowicz, D.; Urbański, R.; Chmielewski, M., Cycloaddition of chlorosulfonyl isocyanate to sugar vinyl ethers. *Synlett* **1994,** (07), 539-541.

2. Astafieva, I.; Zhong, X. F.; Eisenberg, A., Critical micellization phenomena in block polyelectrolyte solutions. *Macromolecules* **1993,** *26* (26), 7339-7352.

3. O'Toole, G. A.; Pratt, L. A.; Watnick, P. I.; Newman, D. K.; Weaver, V. B.; Kolter, R., Genetic approaches to study of biofilms. In *Methods Enzymol.*, Ron, J. D., Ed. Academic Press: 1999; Vol. Volume 310, pp 91-109.

4. Kuchma, S. L.; Brothers, K. M.; Merritt, J. H.; Liberati, N. T.; Ausubel, F. M.; O'Toole, G. A., BifA, a Cyclic-Di-GMP Phosphodiesterase, Inversely Regulates Biofilm Formation and Swarming Motility by *Pseudomonas aeruginosa* PA14. *J. of Bacteriol.* **2007,** *189* (22), 8165-8178.

5. Kuchma, S. L.; Griffin, E. F.; O'Toole, G. A., Minor Pilins of the Type IV Pilus System Participate in the Negative Regulation of Swarming Motility. *J. of Bacteriol.* **2012,** *194* (19), 5388-5403.

6. Takeoka, S.; Mori, K.; Ohkawa, H.; Sou, K.; Tsuchida, E., Synthesis and Assembly of Poly(ethylene glycol)−Lipids with Mono-, Di-, and Tetraacyl Chains and a Poly(ethylene glycol) Chain of Various Molecular Weights. *J. Am. Chem. Soc.* **2000,** *122* (33), 7927-7935.

Table S1. Molecular Weight Characterization of Protected Amphiphiles

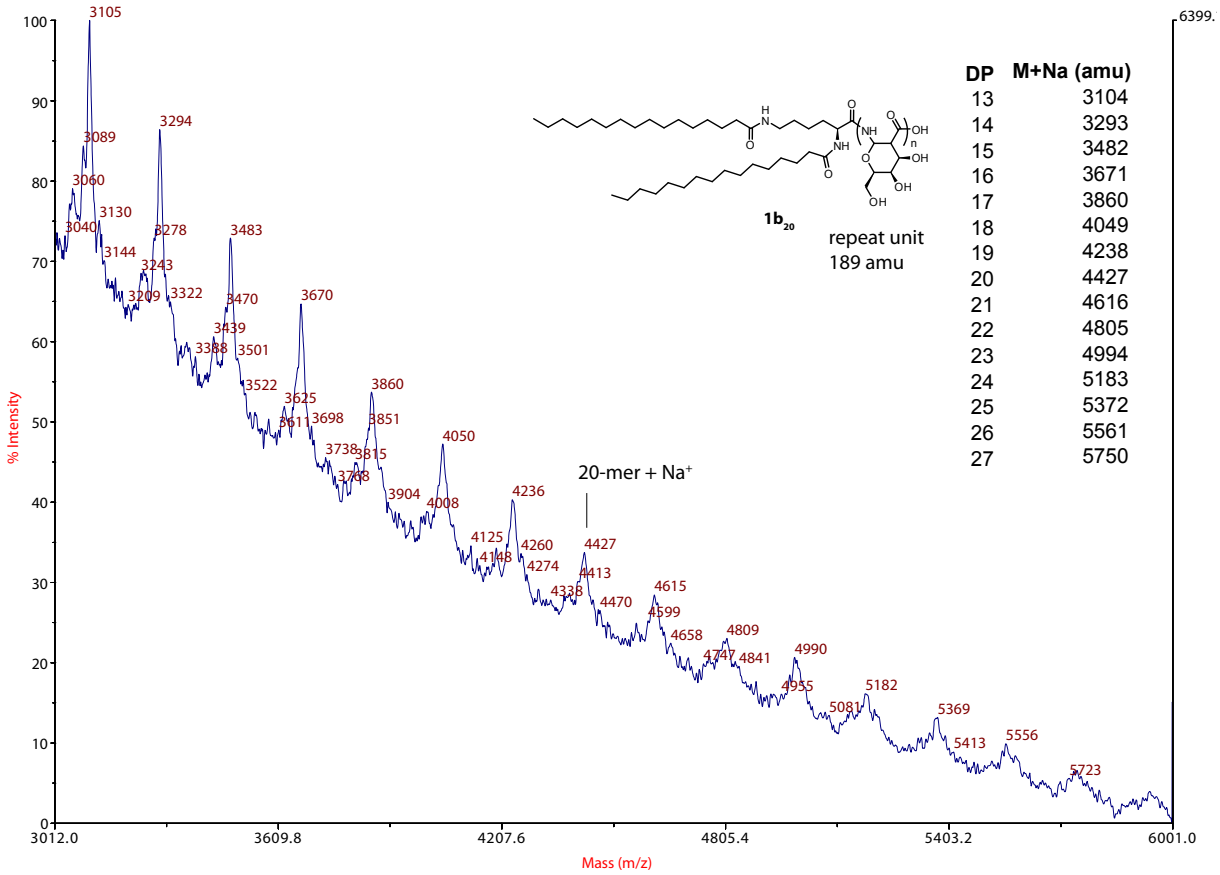
	$M_{n(th)}$ (kDa)	$M_{n(GPC)}$[a] (kDa)	$M_{w(GPC)}$[a] (kDa)	Đ[b]	DP_{th}	DP_{GPC}	DP_{NMR}[c]
1'a$_{10}$	4.9	4.0	4.5	1.1	10	8	10
1'a$_{20}$	9.5	8.9	9.8	1.1	20	19	21
1'b$_{10}$	5.2	4.9	5.9	1.2	10	9	10
1'b$_{20}$	9.8	10.1	11.2	1.1	20	21	20
2'a$_{10}$	4.9	4.1	4.7	1.1	10	8	9
2'a$_{20}$	9.5	8.5	9.4	1.1	20	18	19
2'b$_{10}$	5.2	4.6	5.3	1.2	10	10	11
2'b$_{20}$	9.8	10.0	10.8	1.1	20	21	20

[a] Determined using GPC with THF as eluent against polystyrene standards. [b] M_w/M_n [c] Determined by comparison of the integration of the benzyl aromatic signal (representing 15 protons per repeat unit) to the aliphatic signal (representing 26 or 50 protons per endgroup for the a- or b-groups, respectively) centered at 1.26 ppm.

Table S2. Molecular Weight Characterization of Amphiphiles

	$M_{n(th)}$ (kDa)	DP_{th}	$M_{n(NMR)}$ (kDa)	DP_{NMR}[a]
1a$_{10}$	2.2	10	1.9	8
1a$_{20}$	4.1	20	3.6	17
1b$_{10}$	2.5	10	2.5	10
1b$_{20}$	4.4	20	4.4	20
2a$_{10}$	2.2	10	2.2	10
2a$_{20}$	4.1	20	4.3	21
2b$_{10}$	2.5	10	2.5	10
2b$_{20}$	4.4	20	4.6	21

[a]Determined by comparison of the integration of the aliphatic signal (representing 26 or 50 protons per end group for the a- or b-groups, respectively) centered near 1.2 ppm with the integration of PAS repeat unit signals (see spectra for greater detail).



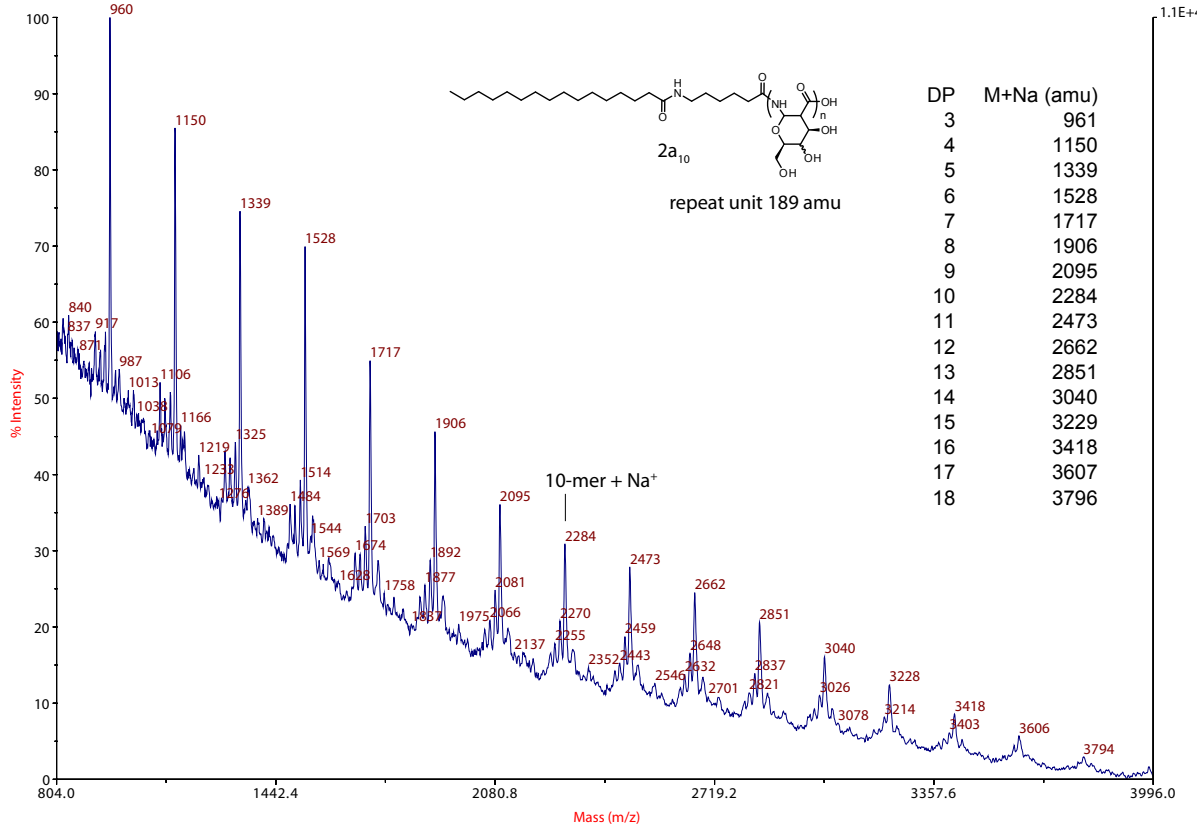
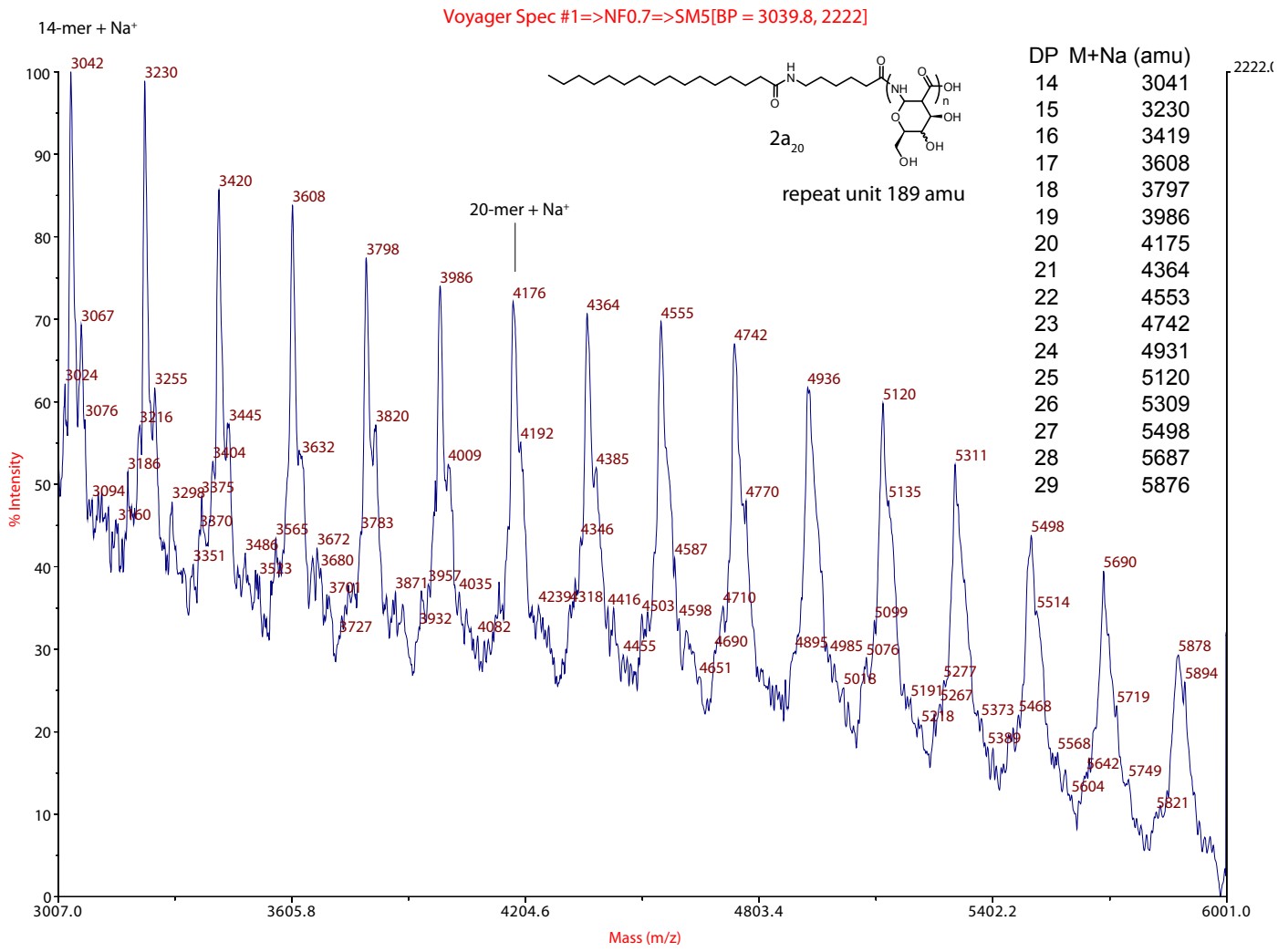


Figure S1. MALDI-tof analysis of **1b**$_{20}$, **2a**$_{10}$, and **2a**$_{20}$. Samples were prepared with a 2,5-dihydroxybenzoic acid matrix at a concentration of approximately 0.1 mg/mL and evaluated using positive ion mode. The intensities of peaks may not be representative of the actual distribution of molecules due to fragmentation and/or preferential desorption of smaller molecules relative to larger molecules. (continued on next page)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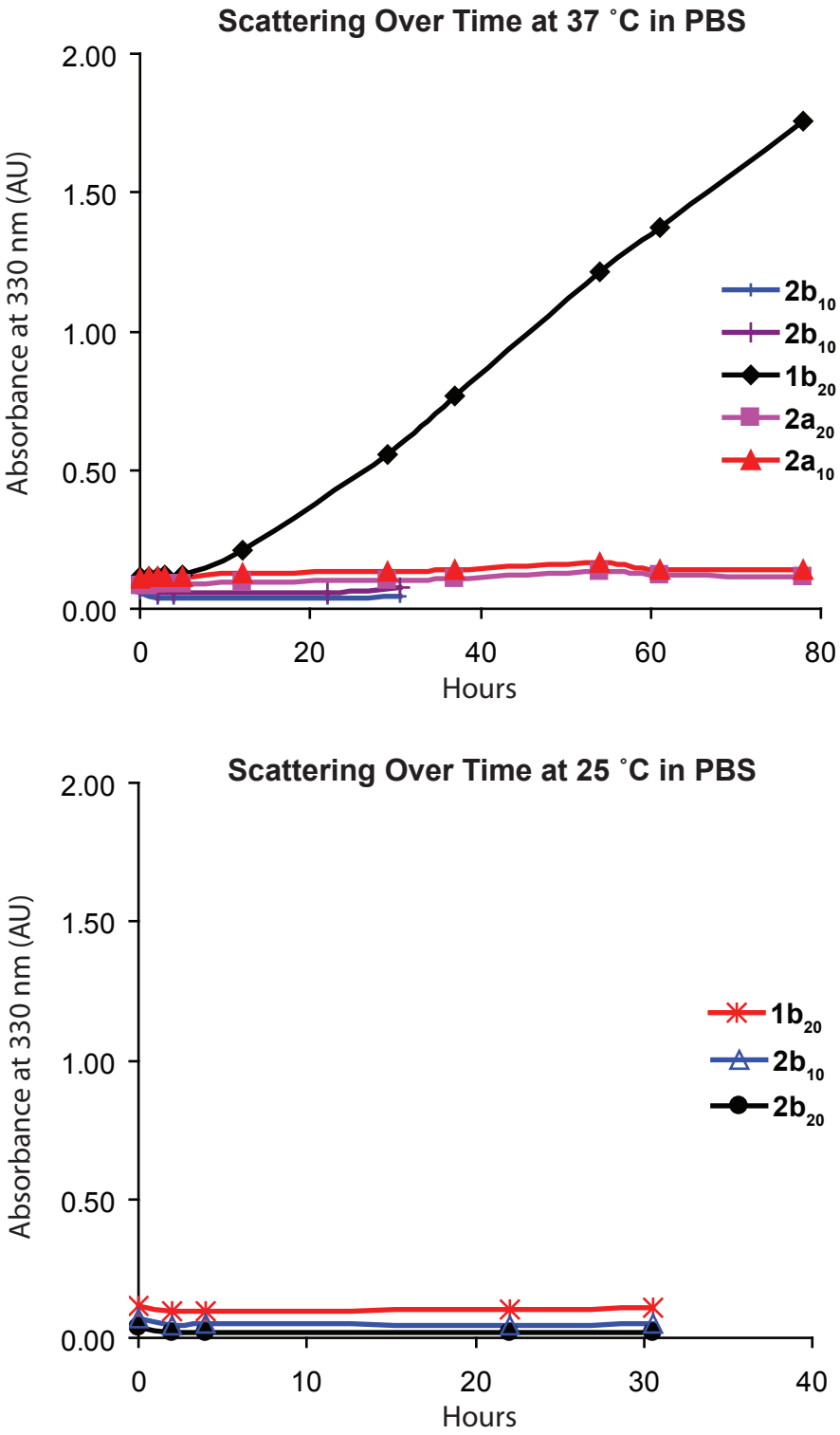


Figure S2. The graphs show the change in the amount of light scattering measured by the absorbance at 330 nm over time when samples were incubated at 37 °C (top) or 25 °C (bottom). The concentration of amphiphile was 1.0 mg/mL in PBS buffer and the spectra were recorded in a 1 cm pathlength cuvette. Sample **1b**$_{20}$ at 37 °C was the only sample that showed an increase in scattering over the time periods investigated.

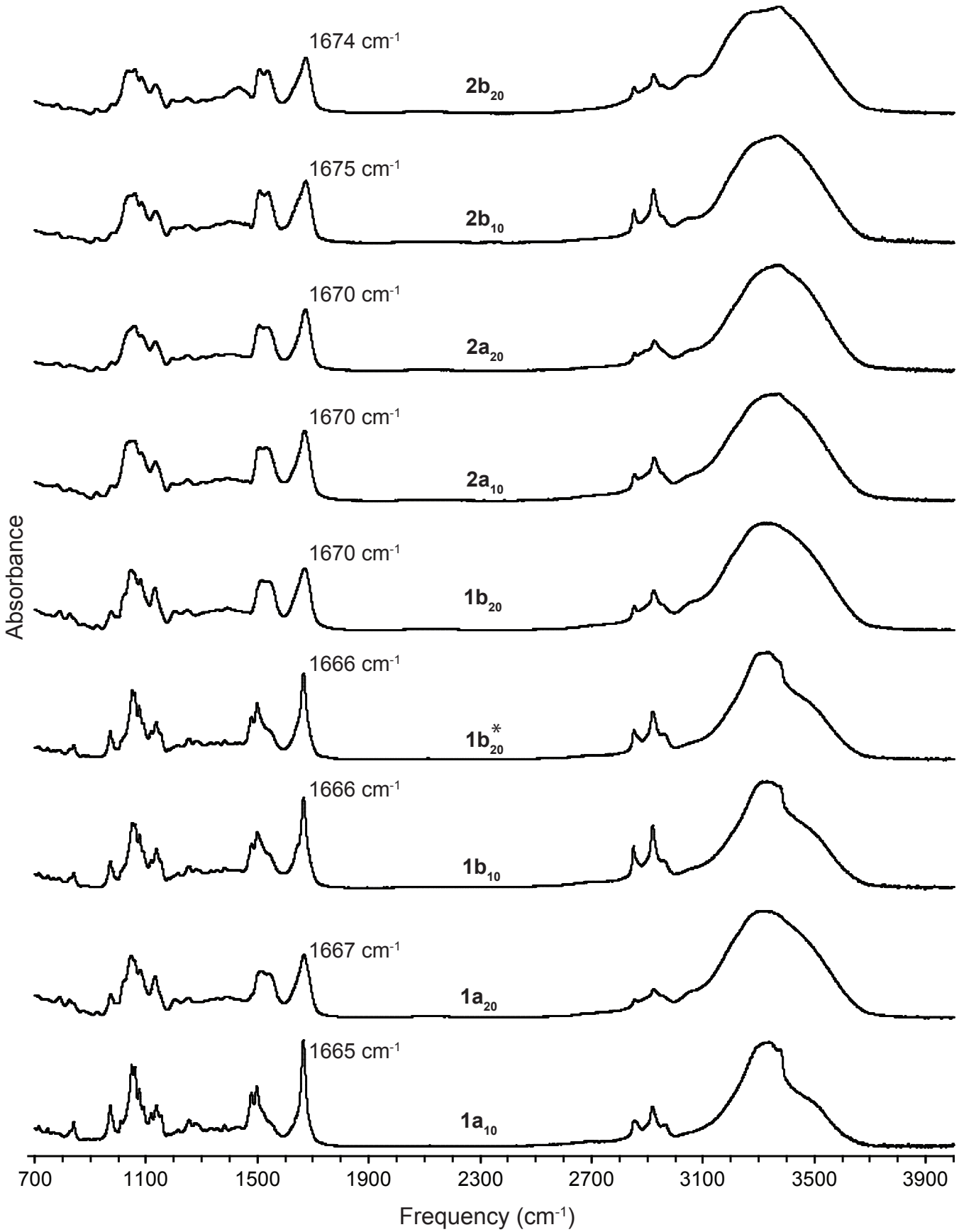


Figure S3. IR spectra for the PAS amphiphiles. Sample **1b$_{20}$*** was incubated overnight at 37 °C in PBS, dialyzed, and then lyophilized.

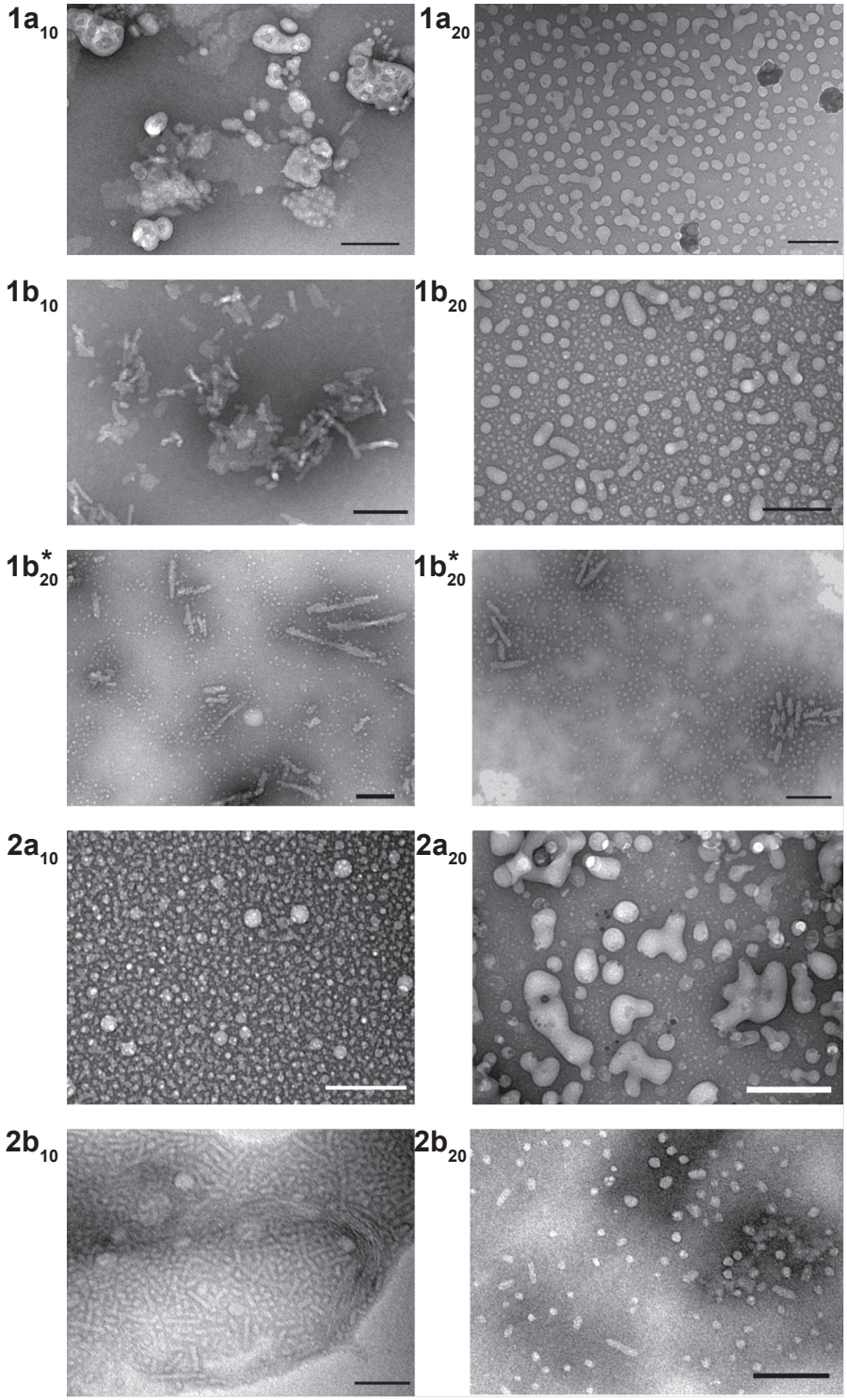


Figure S4. TEM micrographs of PAS amphiphiles. All samples were negatively stained with 1% ammonium molybdate, pH 7.0. Concentrations for solutions: **1a**$_{10}$ (1.0 mg/mL), **1a**$_{20}$ (0.25 mg/mL),**1b**$_{10}$ (4.0 mg/mL),**1b**$_{20}$ (1.0 mg/mL), **1b**$_{20}$***** (4.0 mg/mL), **2a**$_{10}$ (2.0 mg/mL), **2a**$_{20}$ (0.8 mg/mL), **2b**$_{10}$ (2.0 mg/mL), **2b**$_{20}$ (2.0 mg/mL). The two micrographs for **1b**$_{20}$***** were prepared from solutions that were incubated at a concentration of 4.0 mg/mL in PBS at 37 °C for 16 hours before preparation. Amphiphiles **1a**$_{20}$, **1b**$_{20}$ (before incubation), **2a**$_{10}$, **2a**$_{20}$, and **2b**$_{20}$ show structures consistent with a globular micellar assembly. Amphiphiles **1b**$_{10}$, **1b**$_{20}$*****, and **2b**$_{10}$ display structures suggesting worm-like or rod-like micellar assemblies. The structures formed by **1a**$_{10}$ are not easily classified. All scale bars are 100 nm.

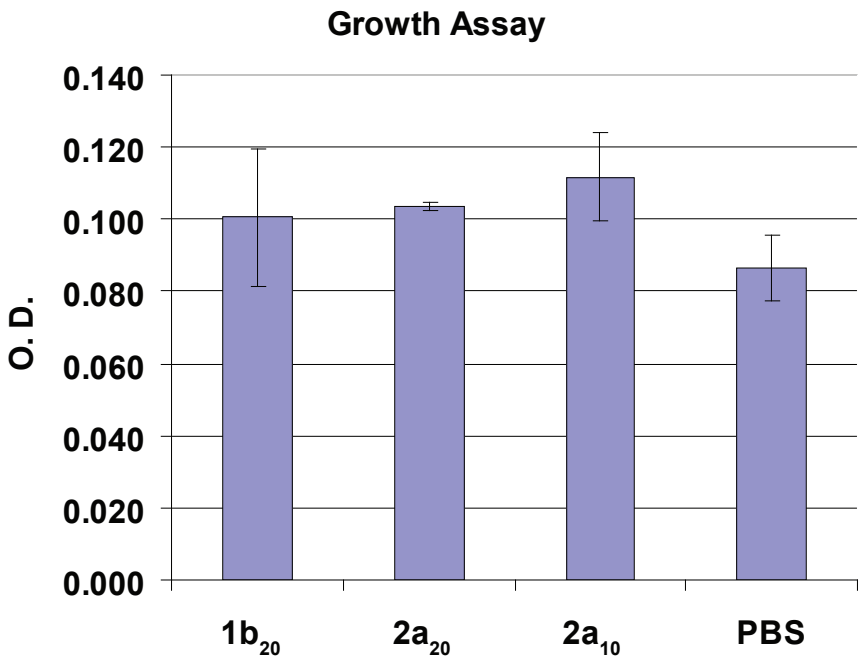


Figure S5. The bar graph shows the effect of each amphiphile on the planktonic growth of *P. aeruginosa* PA14, as measured by the optical density (O.D.) at 600 nm when the samples were incubated at 37 °C overnight. The concentration of amphiphile was 0.5 mg/mL for all samples, n = 3, and error bars show the standard deviation.

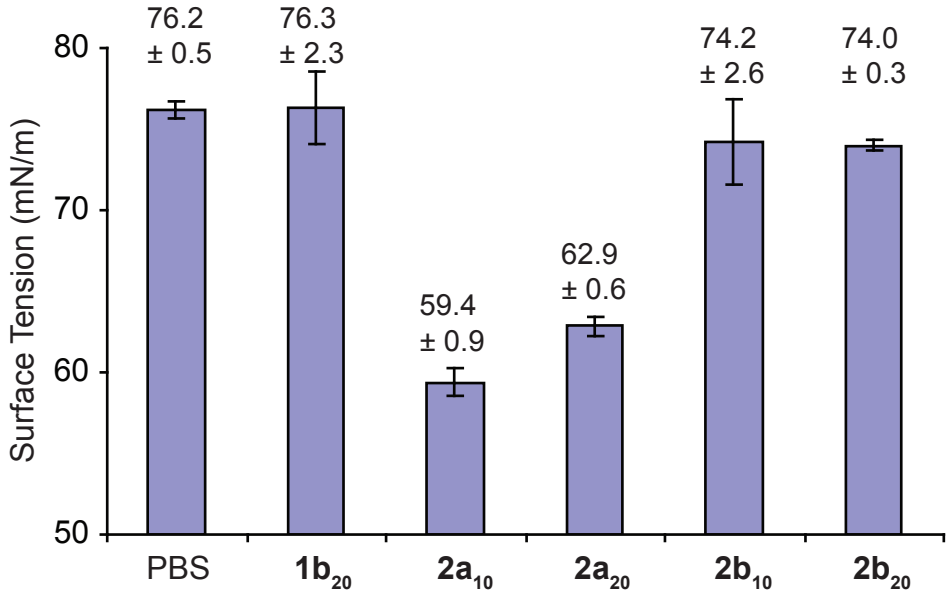
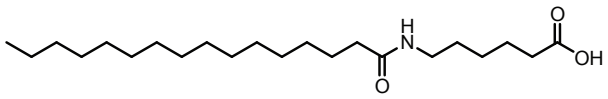
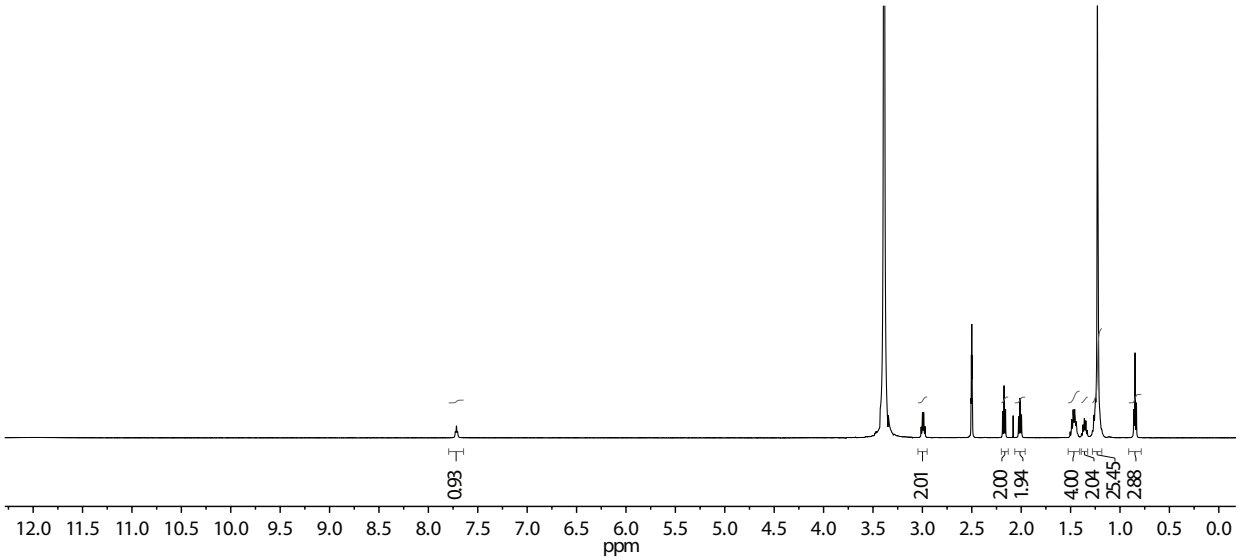


Figure S6. Comparison of the effect of PAS amphiphiles on surface tension measured by the hanging drop method. All amphiphile solutions were 1.0 mg/mL in PBS buffer and measurements (n=4) were taken at 24˚C and errors are reported as standard deviation of the mean. Amphiphiles **2a**$_{10}$ and **2a**$_{20}$ lower the surface tension of an aqueous solution, whereas amphiphiles **1b**$_{20}$, **2b**$_{10}$, and **2b**$_{20}$ display little or no ability to lower the surface tension at the concentration tested.

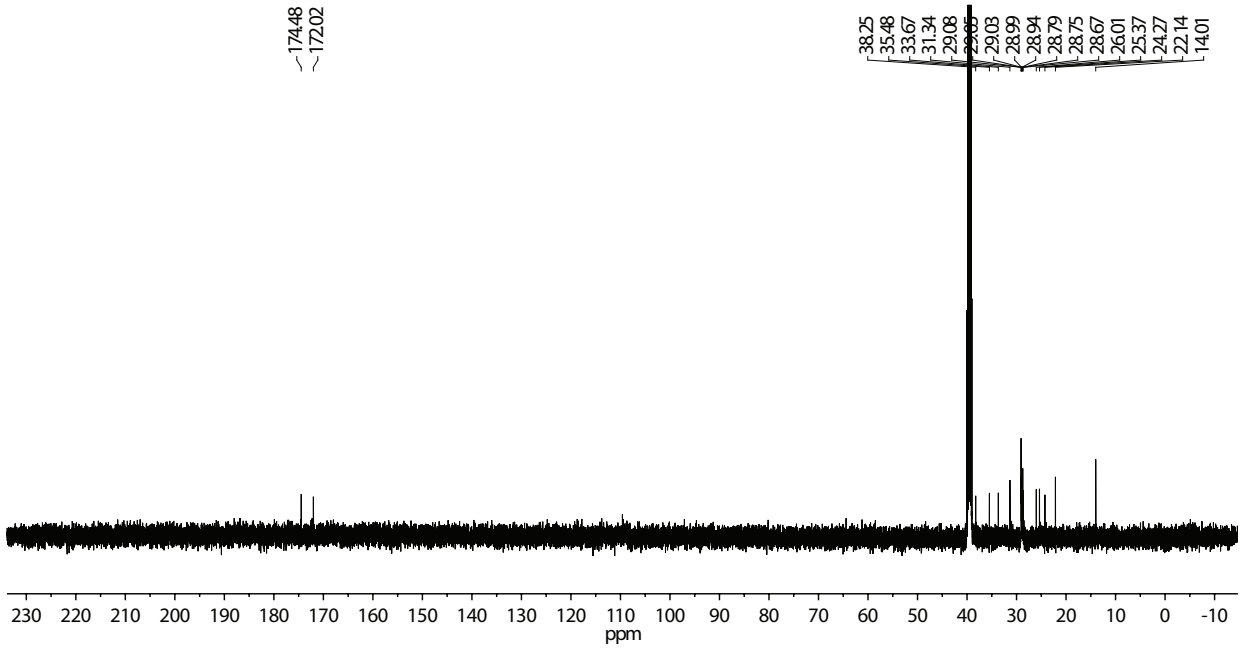
^{1}H NMR (500 MHz, d_6-DMSO)



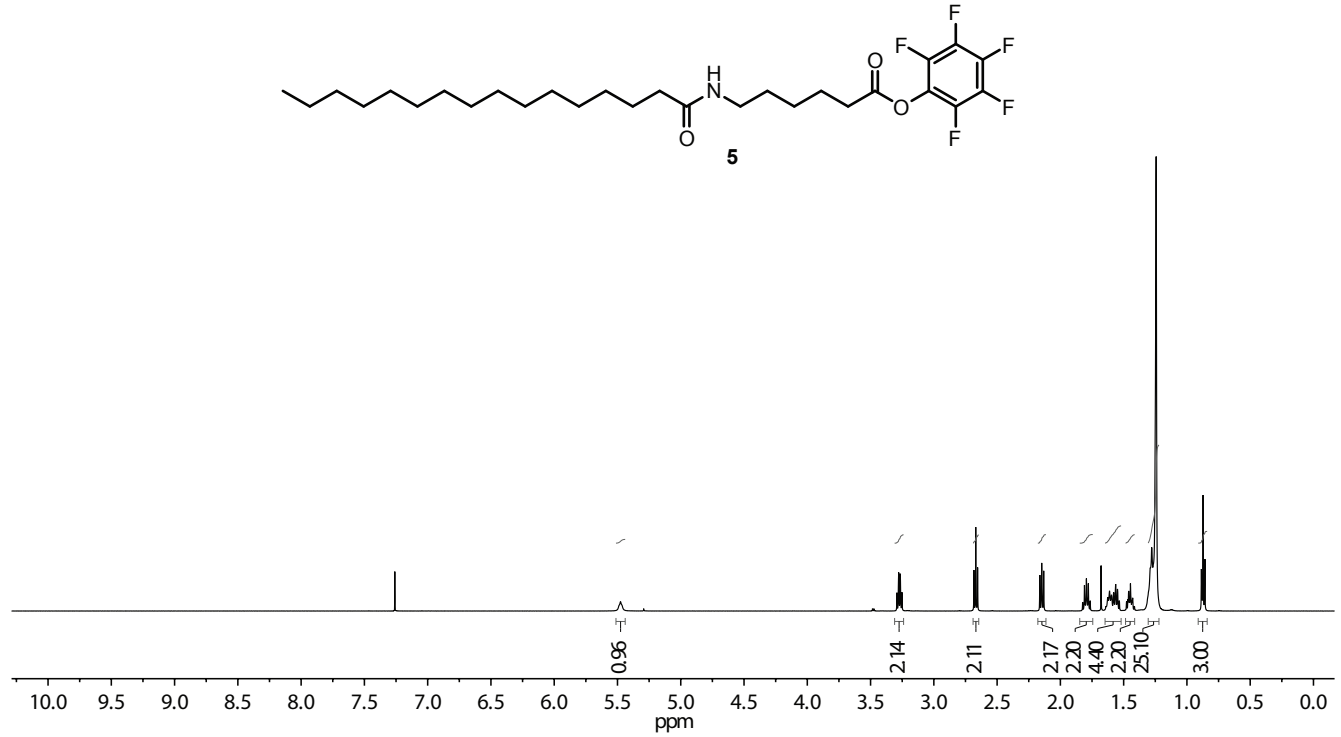
6-palmitamidohexanoic acid



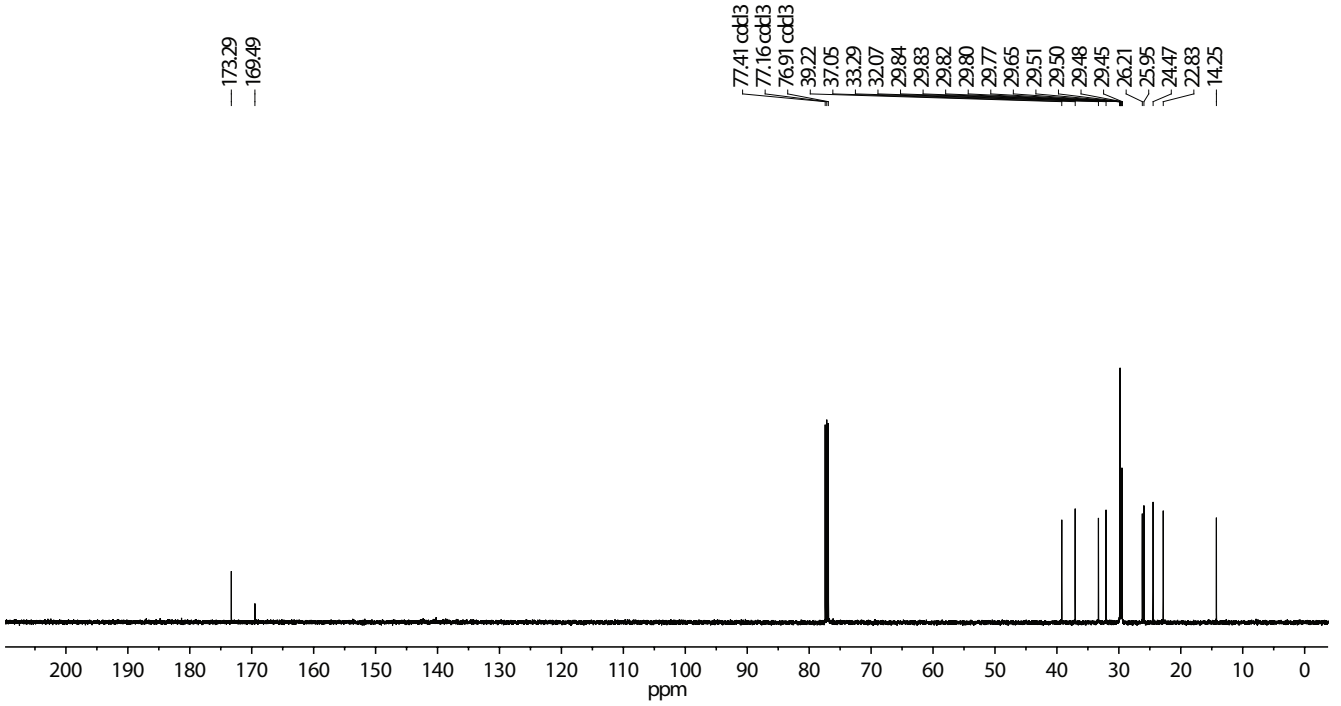
^{13}C NMR (126 MHz, d_6-DMSO)



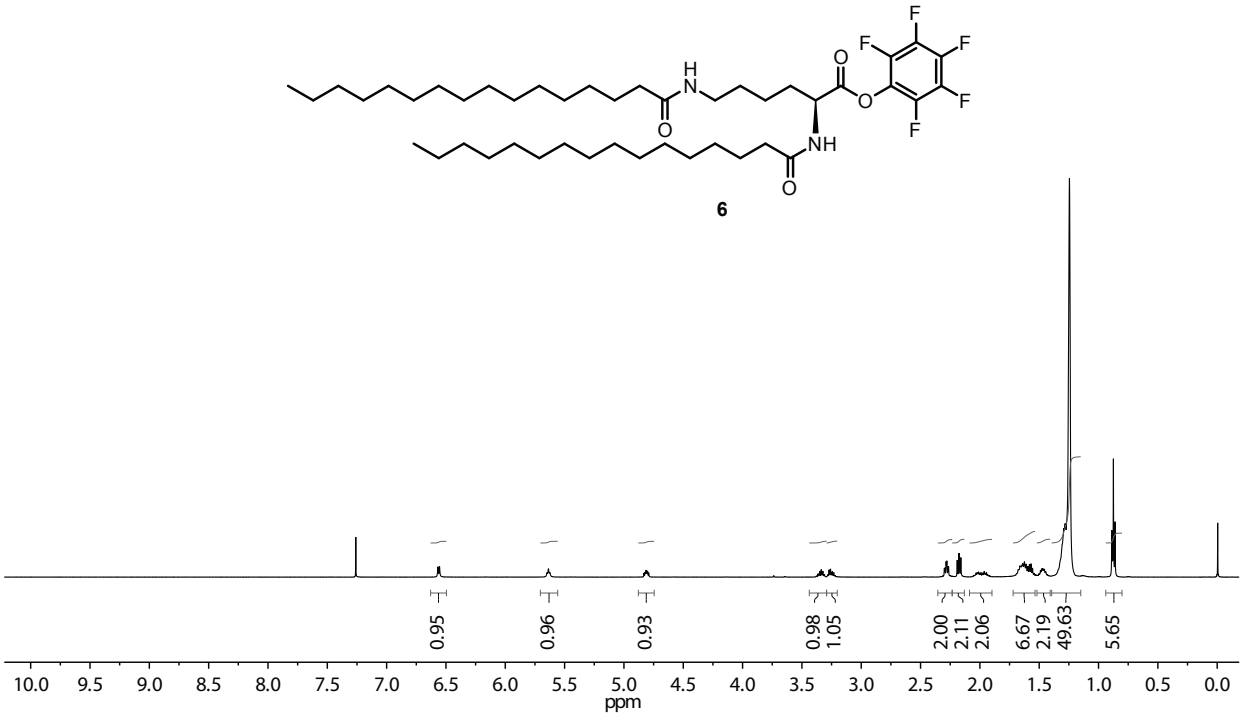
^{1}H NMR (500 MHz, $CDCl_3$)



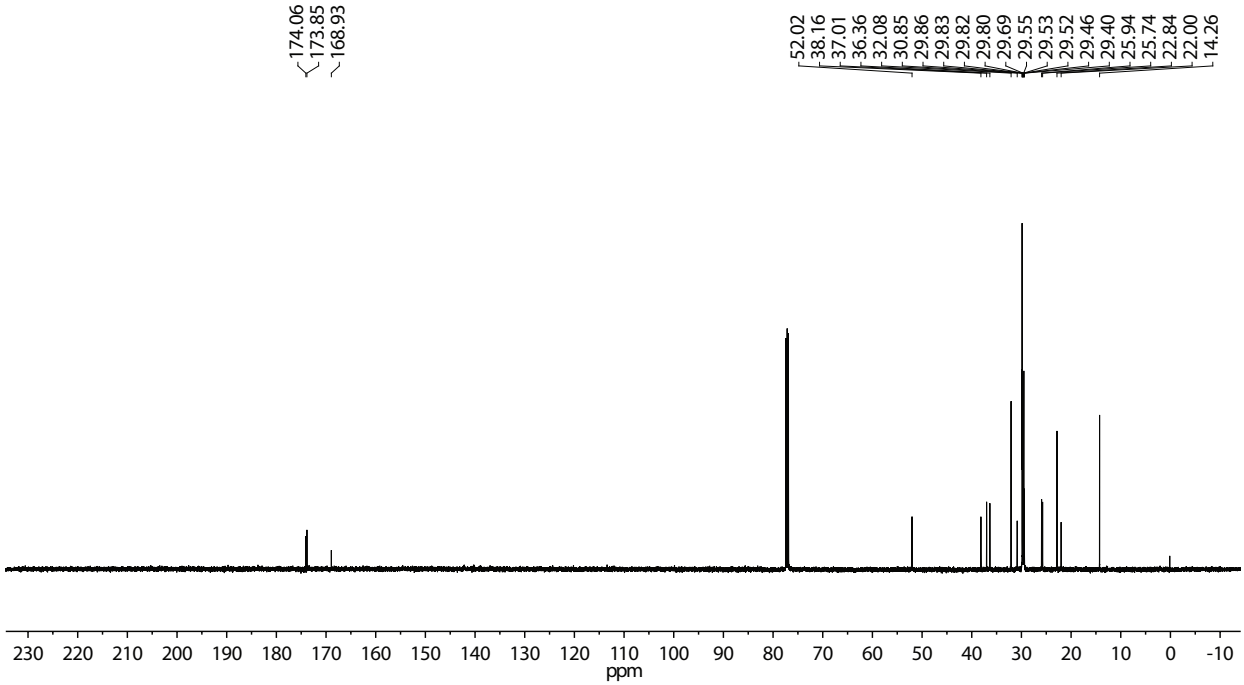
^{13}C NMR (126 MHz, $CDCl_3$)

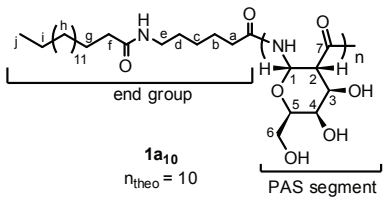


^{1}H NMR (500 MHz, $CDCl_3$)



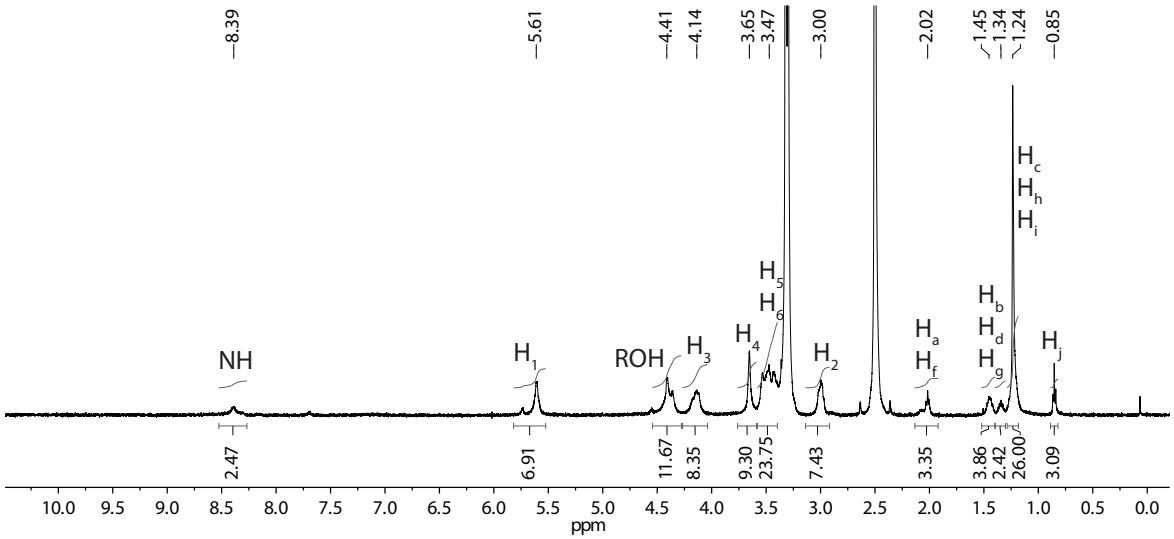
^{13}C NMR (126 MHz, $CDCl_3$)



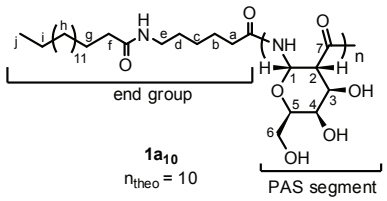


The signal centered at 1.24 is assumed to contain 26 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 26. The degree of polymerization based on NMR (DP_{NMR}) is estimated by taking the average of the integrations of H_1, H_2, H_3, and H_4. Hence, the DP_{NMR} = 8.0.

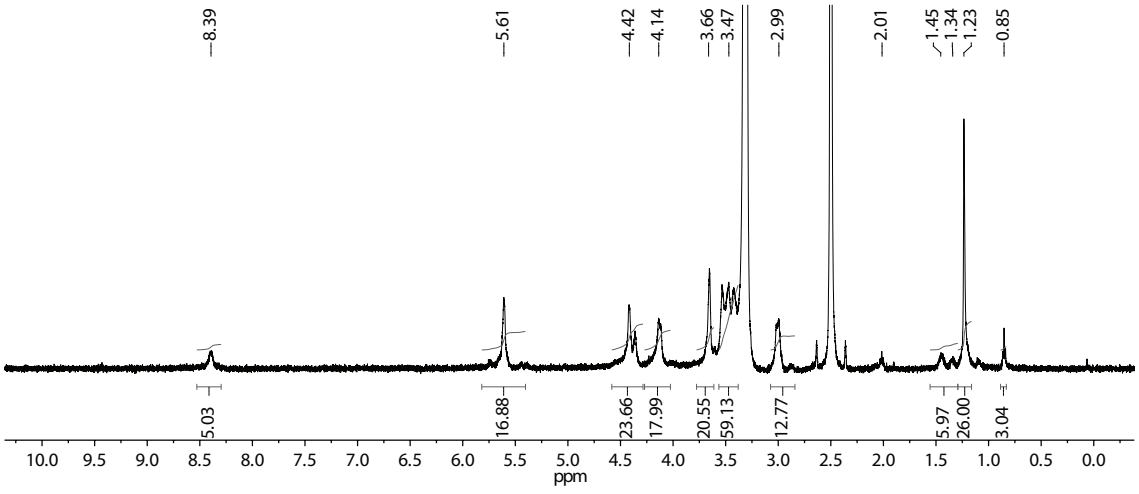
^{1}H NMR (500 MHz, d_6-DMSO)



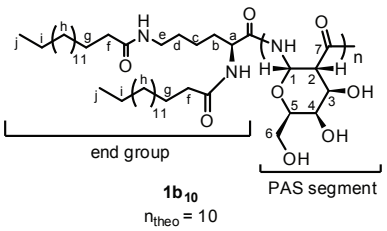
^{1}H NMR (500 MHz, d_6-DMSO)



DP_{NMR} = 17.

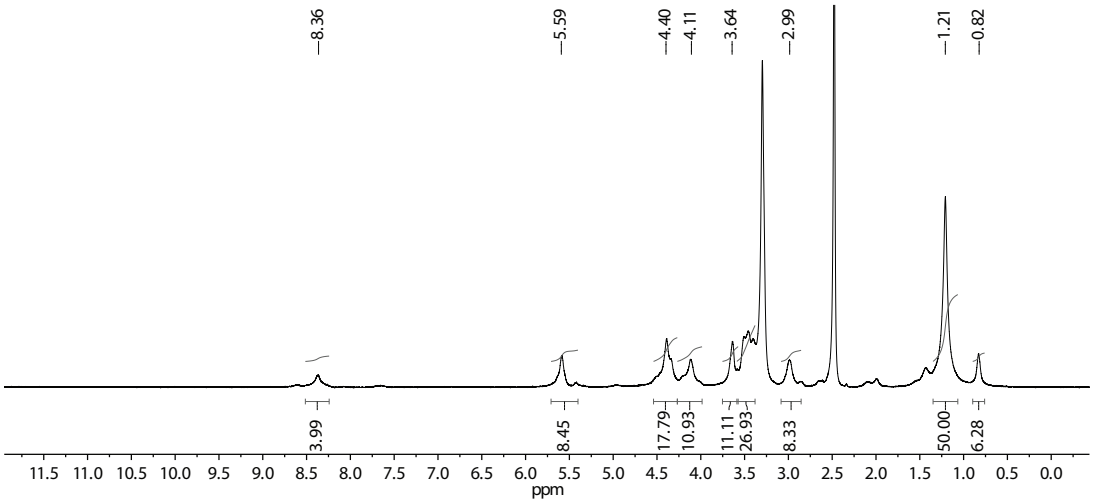


^{1}H NMR (500 MHz, d_6-DMSO)

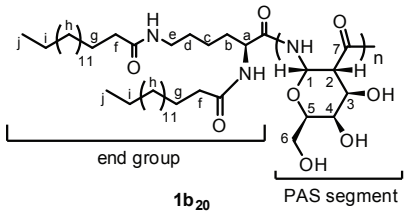


1b$_{10}$
n_{theo} = 10

The signal centered at 1.21 is assumed to contain 50 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 50. The degree of polymerization based on NMR (DP_{NMR}) is estimated by taking the average of the integrations of H_1, H_2, H_3, and H_4. Hence, the DP_{NMR} = 9.7.

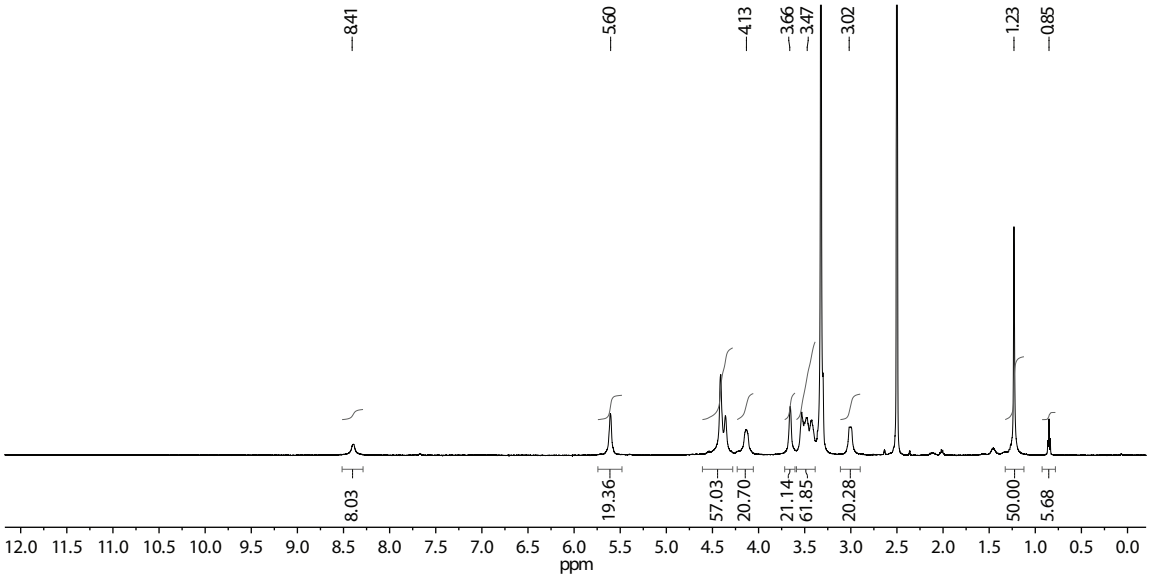


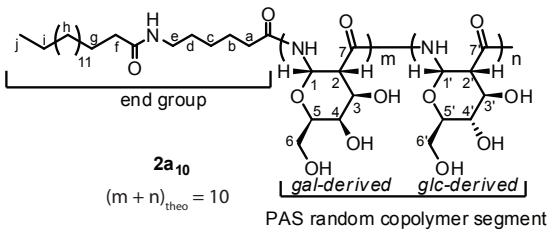
^{1}H NMR (500 MHz, d_6-DMSO)



1b$_{20}$
n_{theo} = 20

DP_{NMR} = 20.4



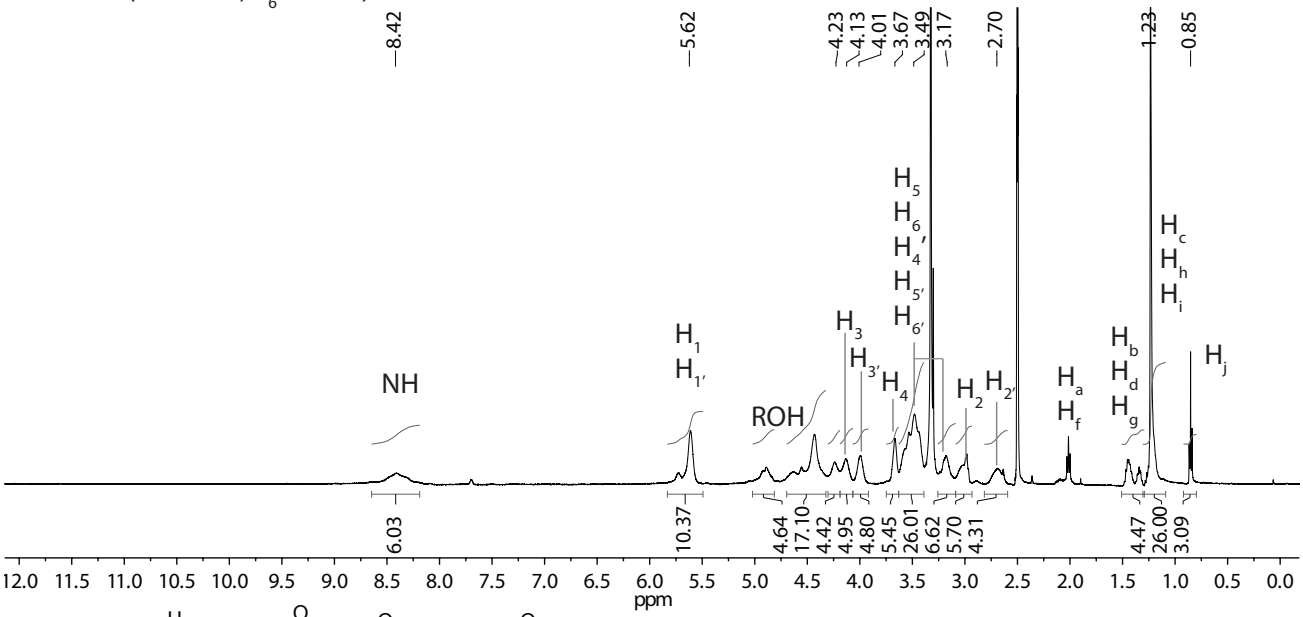


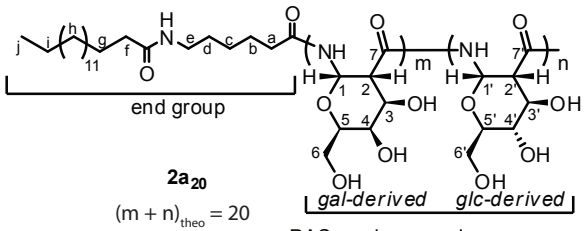
2a$_{10}$

$(m + n)_{theo} = 10$

The signal centered at 1.24 is assumed to contain 26 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 26. The degree of polymerization based on NMR (DP_{NMR}) is estimated by the integration of H_1, which overlaps in both of the comonomers. Hence, the DP_{NMR} = 10.4.

^{1}H NMR (500 MHz, d_6-DMSO)



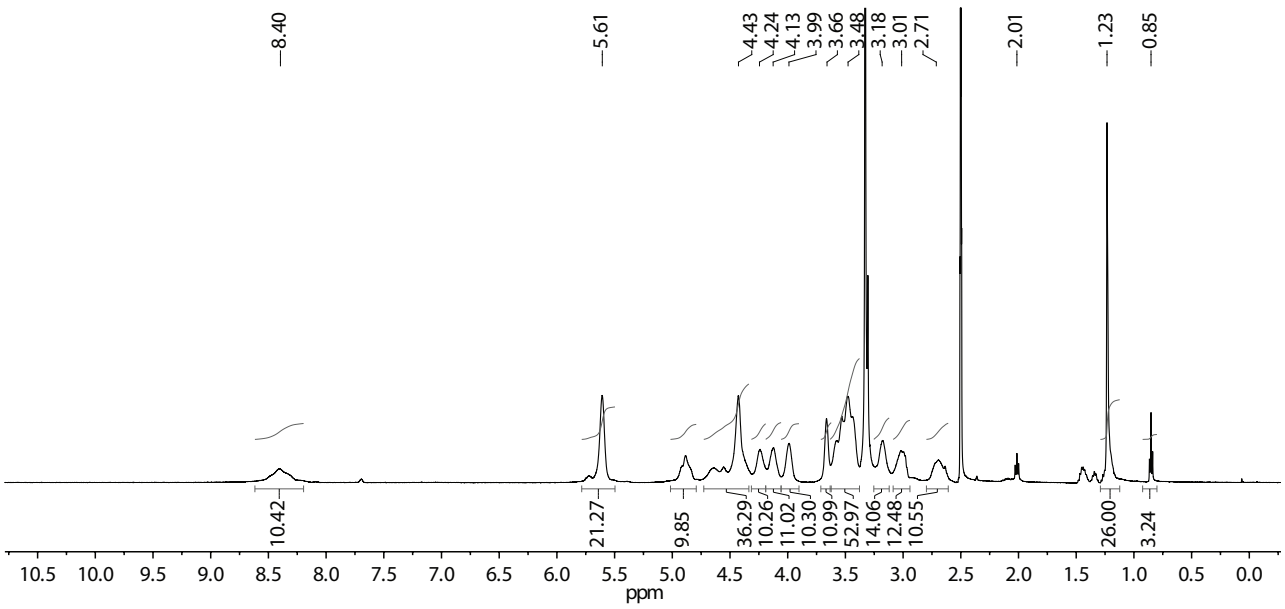


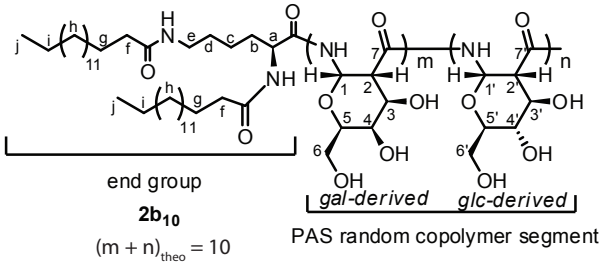
2a$_{20}$

$(m + n)_{theo} = 20$

DP_{NMR} = 21.3

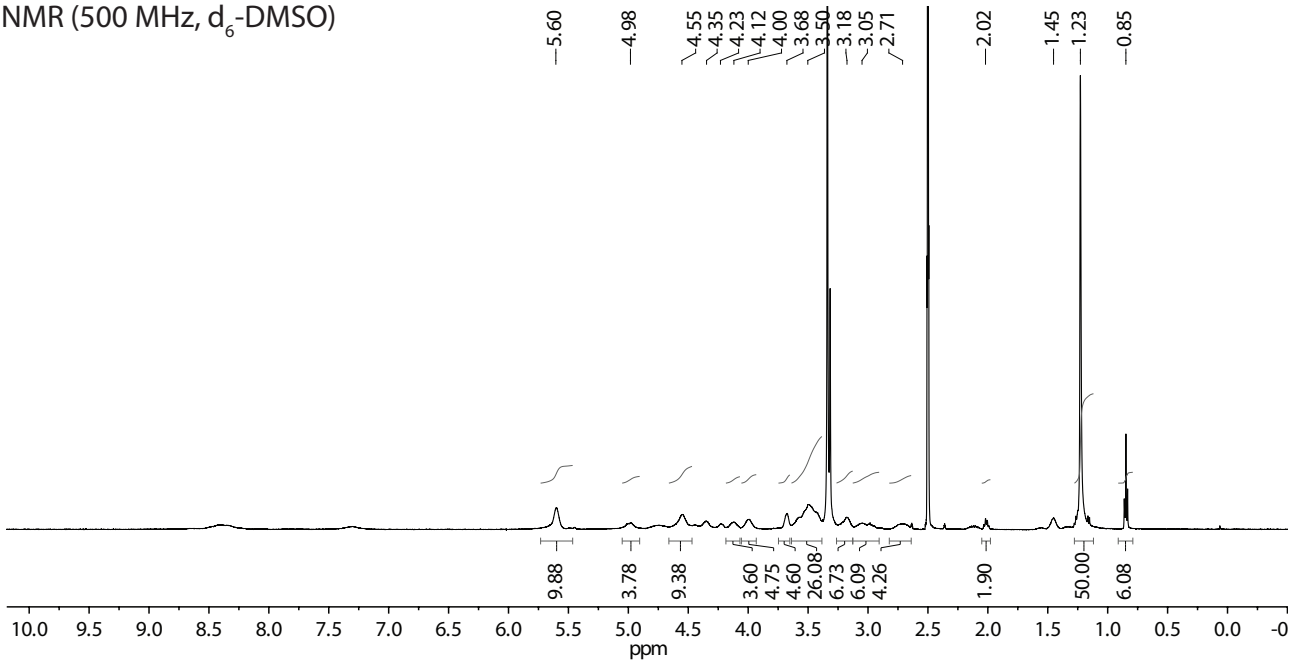
^{1}H NMR (500 MHz, d_6-DMSO)

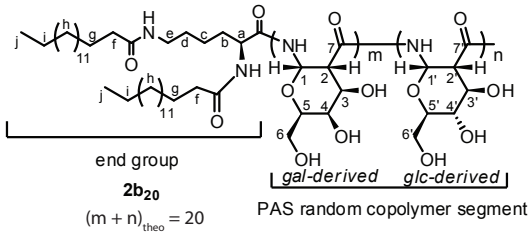




The signal centered at 1.23 is assumed to contain 50 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 50. The degree of polymerization based on NMR (DP_{NMR}) is estimated by the integrations of H_1 and $H_{1'}$ signals that overlap. Hence, the DP_{NMR} = 9.9.

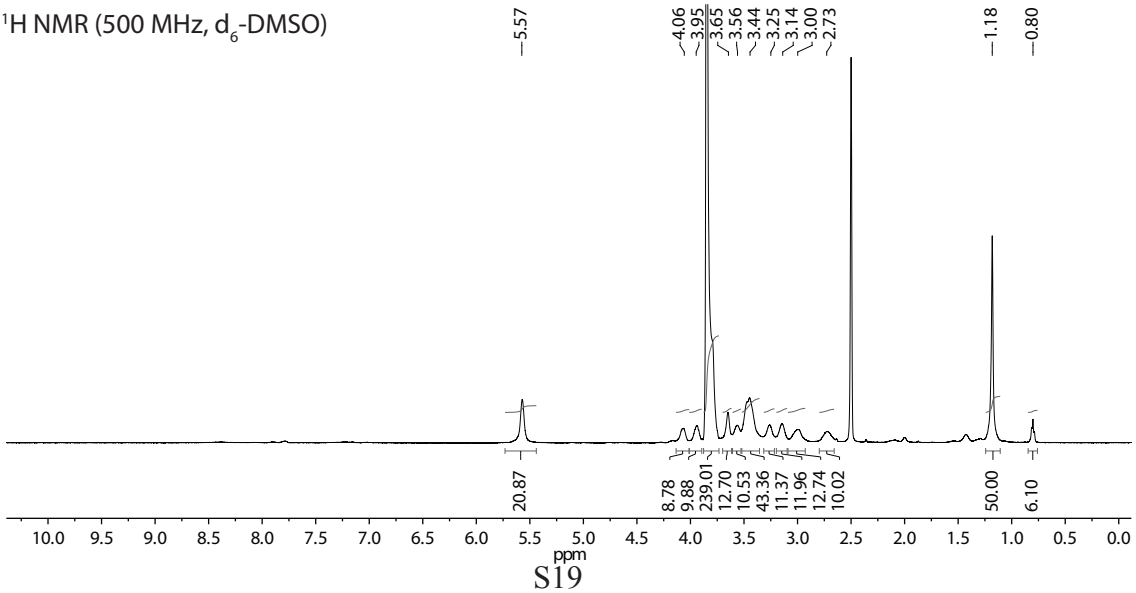
^{1}H NMR (500 MHz, d_6-DMSO)

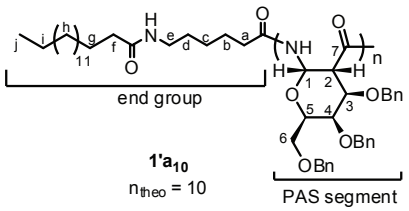




DP_{NMR} = 20.9

^{1}H NMR (500 MHz, d_6-DMSO)



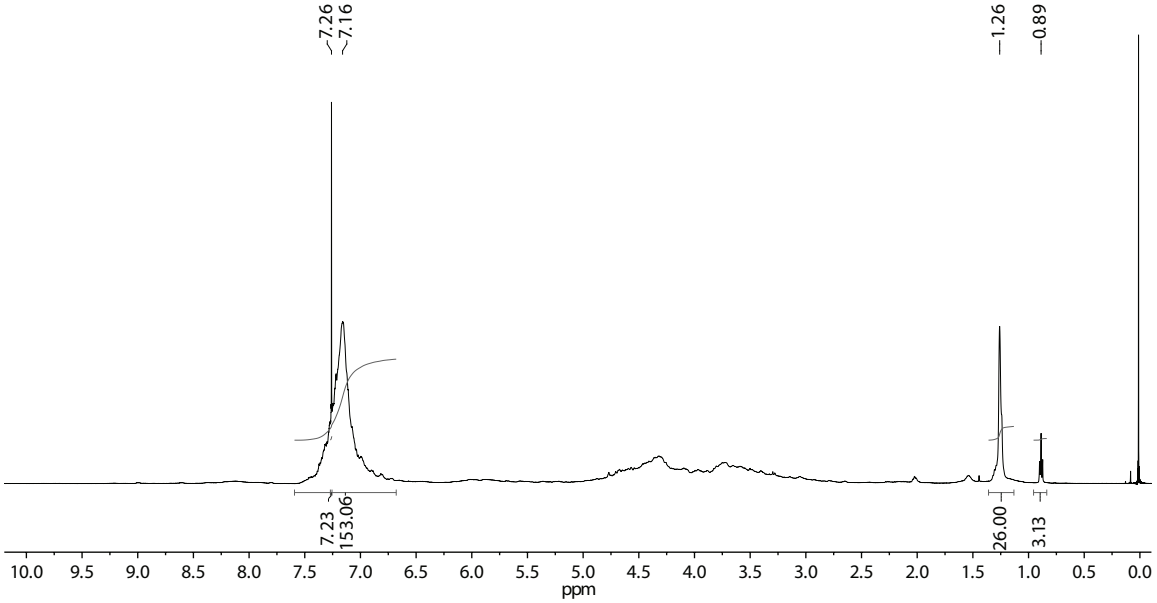


The signal centered at 1.26 is assumed to contain 26 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 26. The aromatic region is integrated and signal due to chloroform is subtracted, and the following equation is used to estimate DP.

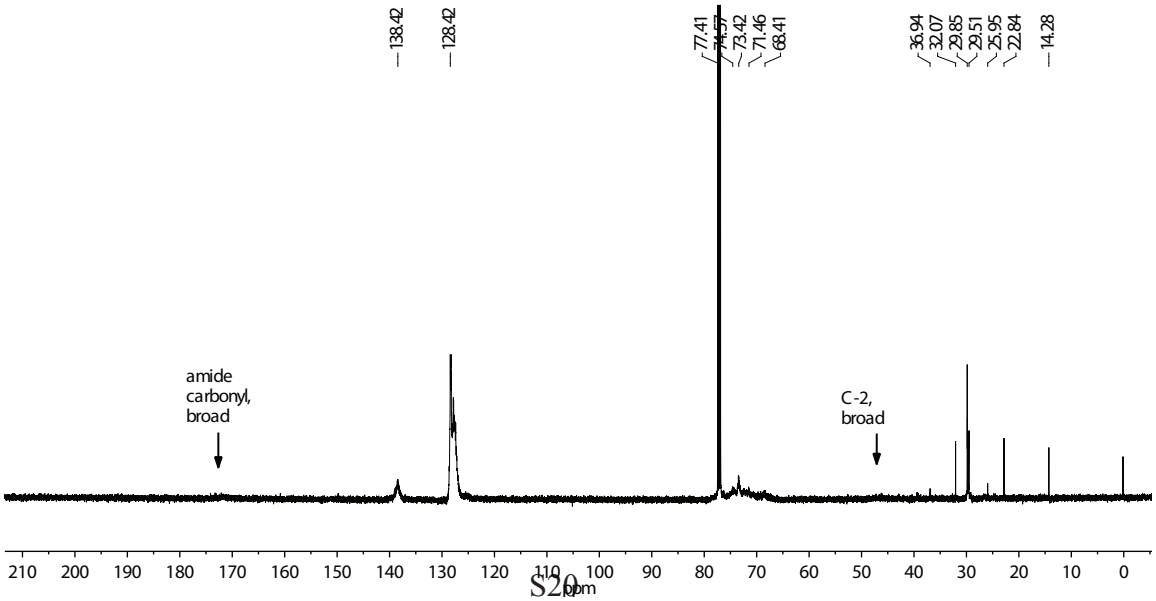
$$DP_{NMR} = \frac{\text{integration of aromatic region}}{\text{number of aromatic protons in repeat unit}}$$

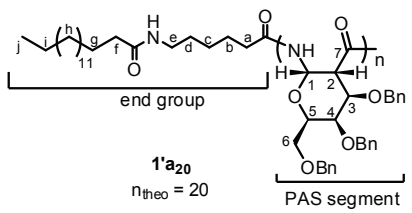
$$DP_{NMR} = \frac{145.8}{15} = 9.7$$

^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)



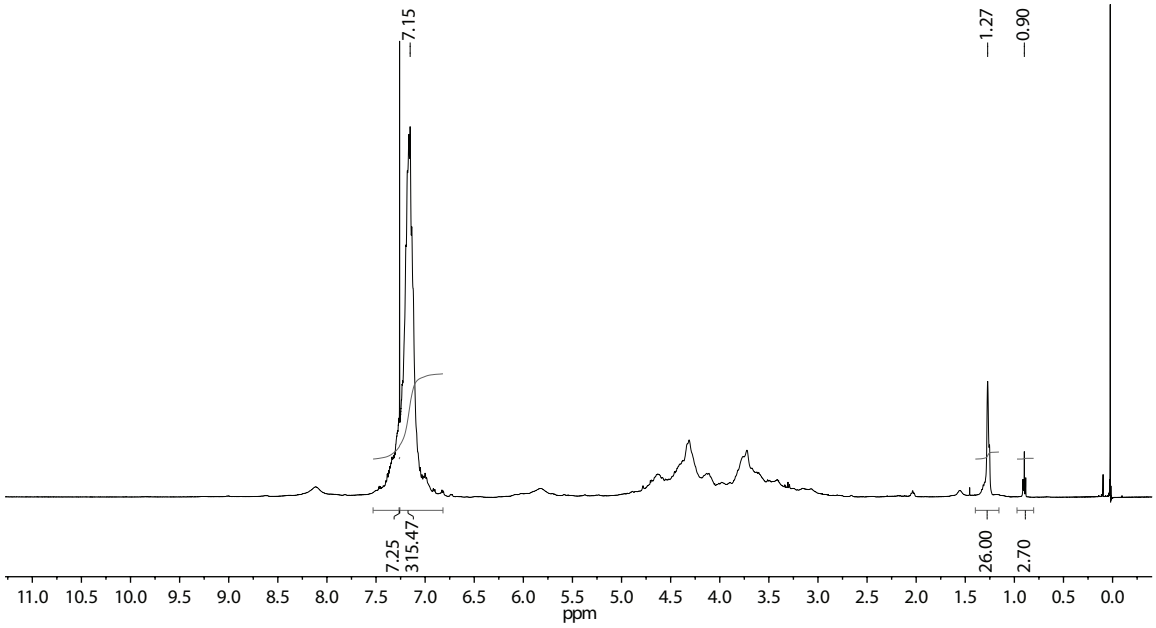


The signal centered at 1.26 is assumed to contain 26 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 26. The aromatic region is integrated and signal due to chloroform is subtracted, and the following equation is used to estimate DP.

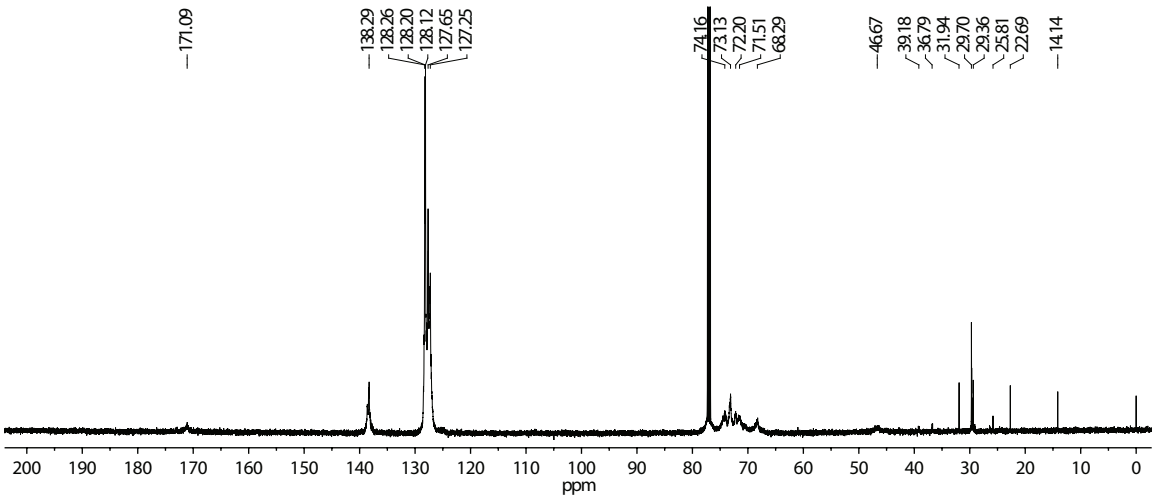
$$DP_{NMR} = \frac{\text{integration of aromatic region}}{\text{number of aromatic protons in repeat unit}}$$

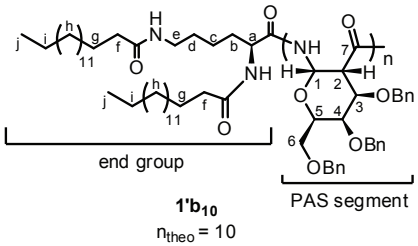
$$DP_{NMR} = \frac{308.2}{15} = 20.5$$

^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)





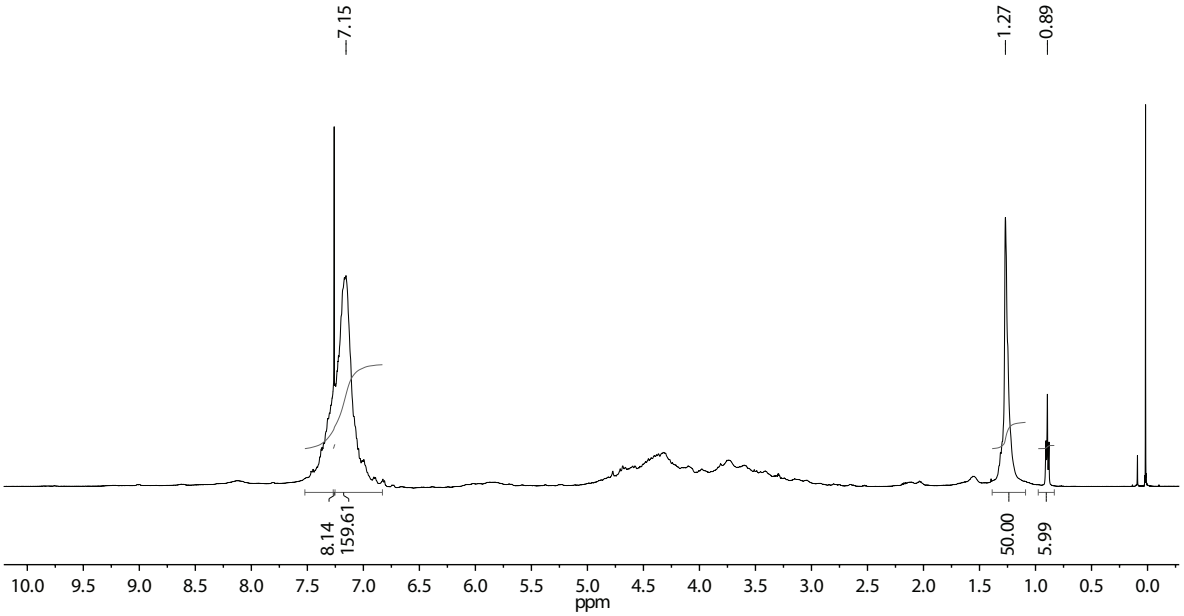
1'b$_{10}$
n_{theo} = 10

The signal centered at 1.26 is assumed to contain 50 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 50. The aromatic region is integrated and signal due to chloroform is subtracted, and the following equation is used to estimate DP.

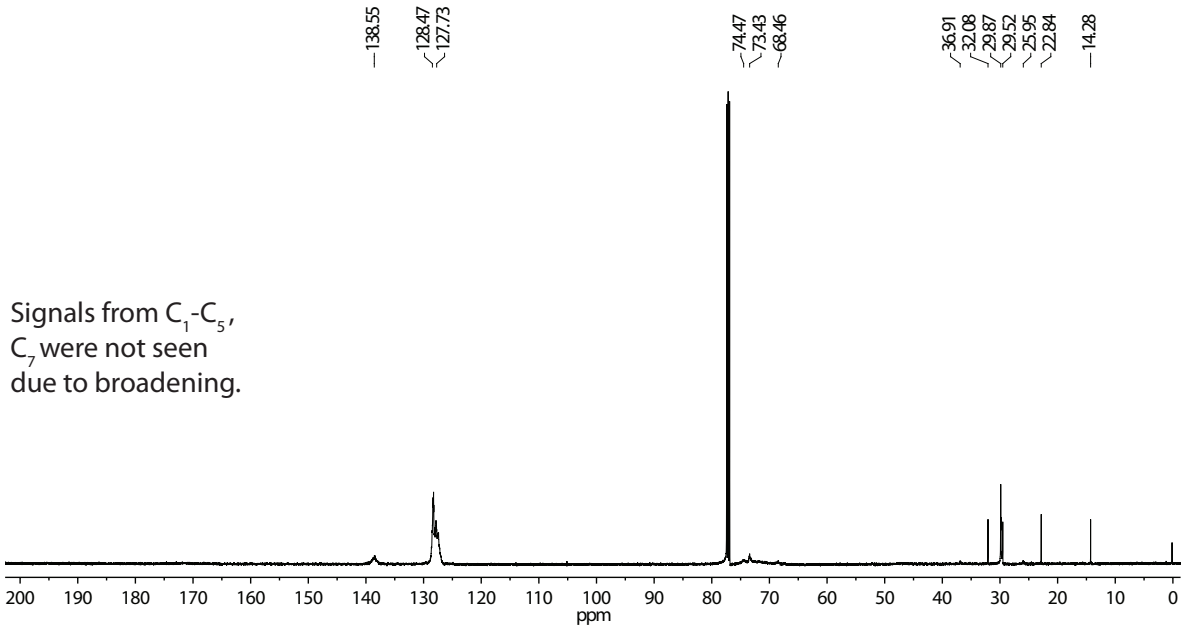
$$DP_{NMR} = \frac{\text{integration of aromatic region}}{\text{number of aromatic protons in repeat unit}}$$

$$DP_{NMR} = \frac{151.5}{15} = 10.1$$

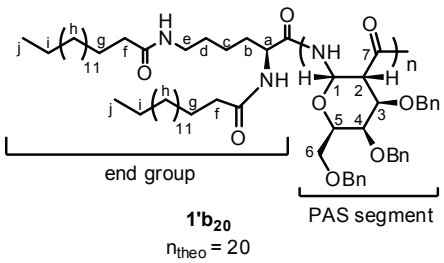
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)



Signals from C_1-C_5, C_7 were not seen due to broadening.

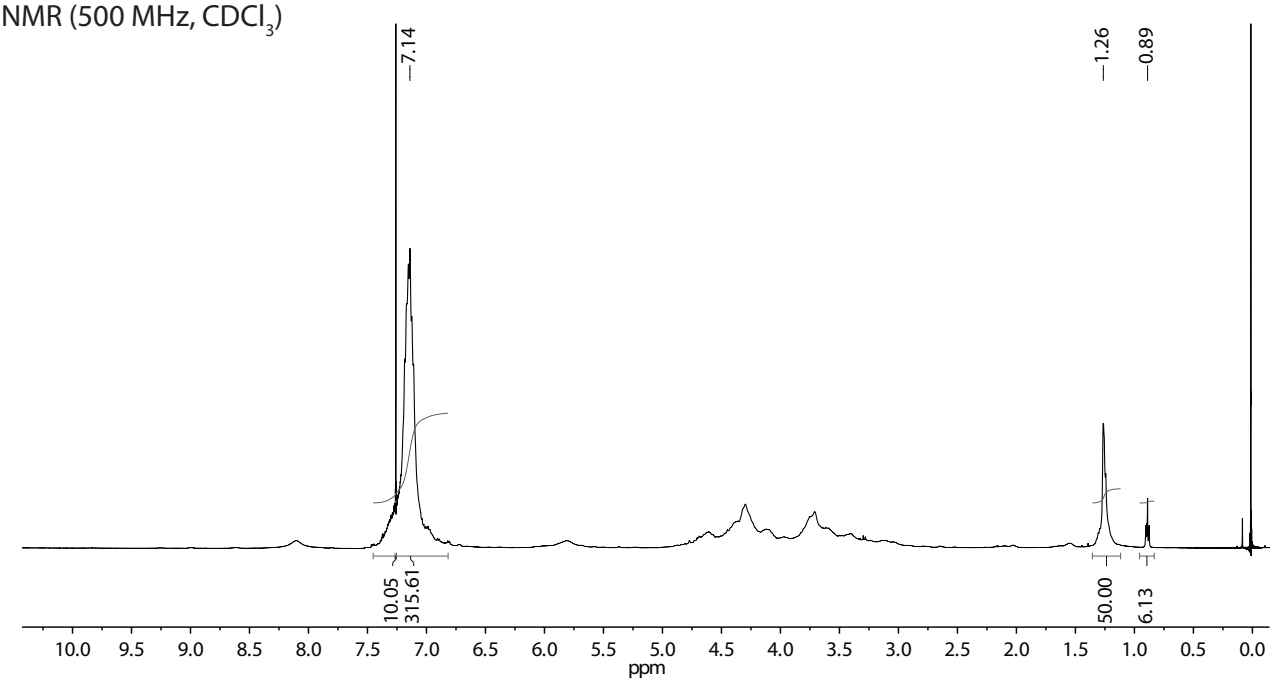


The signal centered at 1.26 is assumed to contain 50 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 50. The aromatic region is integrated and signal due to chloroform is subtracted, and the following equation is used to estimate DP.

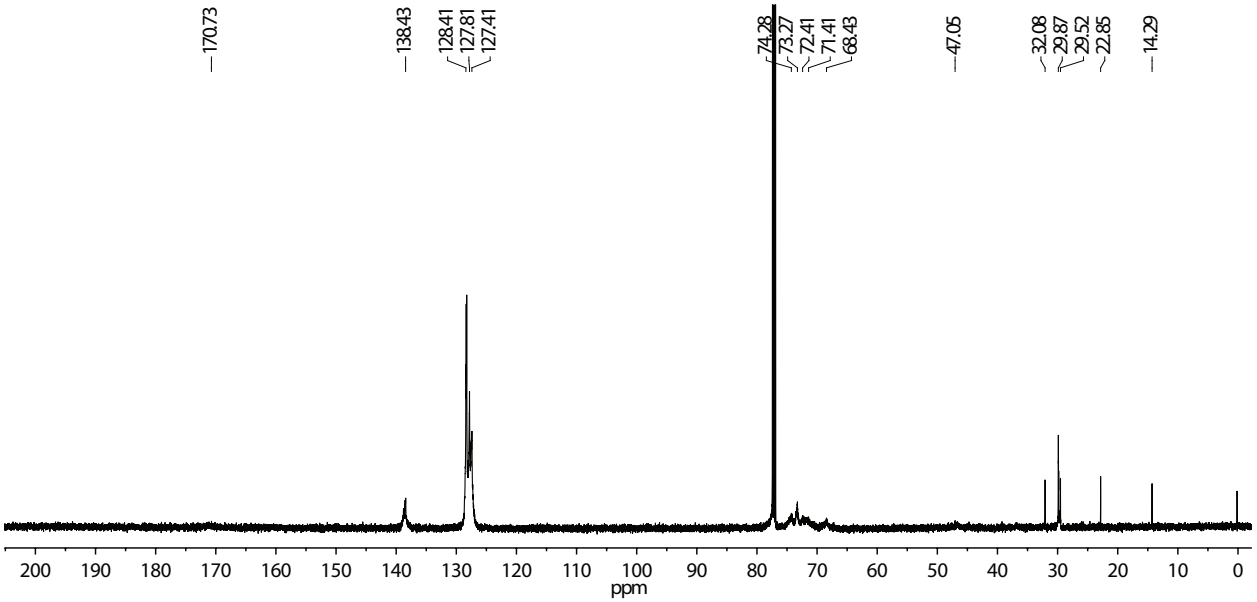
$$DP_{NMR} = \frac{\text{integration of aromatic region}}{\text{number of aromatic protons in repeat unit}}$$

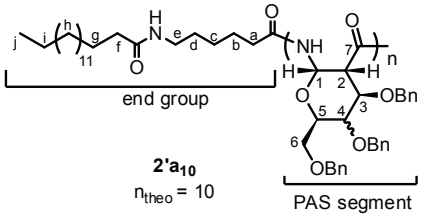
$$DP_{NMR} = \frac{305.6}{15} = 20.4$$

^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)



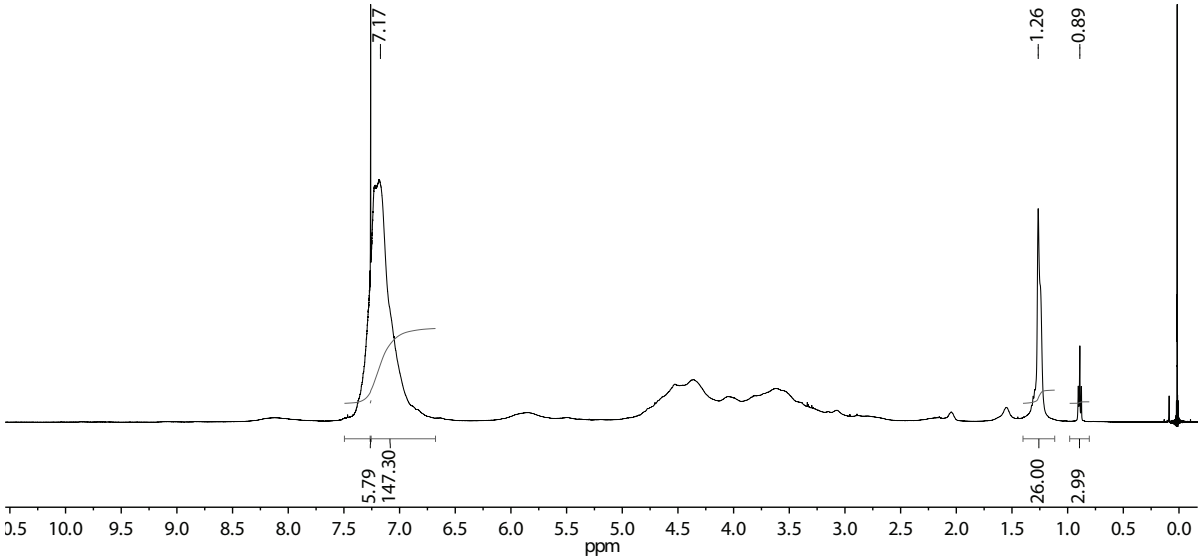


The signal centered at 1.26 is assumed to contain 26 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 26. The aromatic region is integrated and signal due to chloroform is subtracted, and the following equation is used to estimate DP.

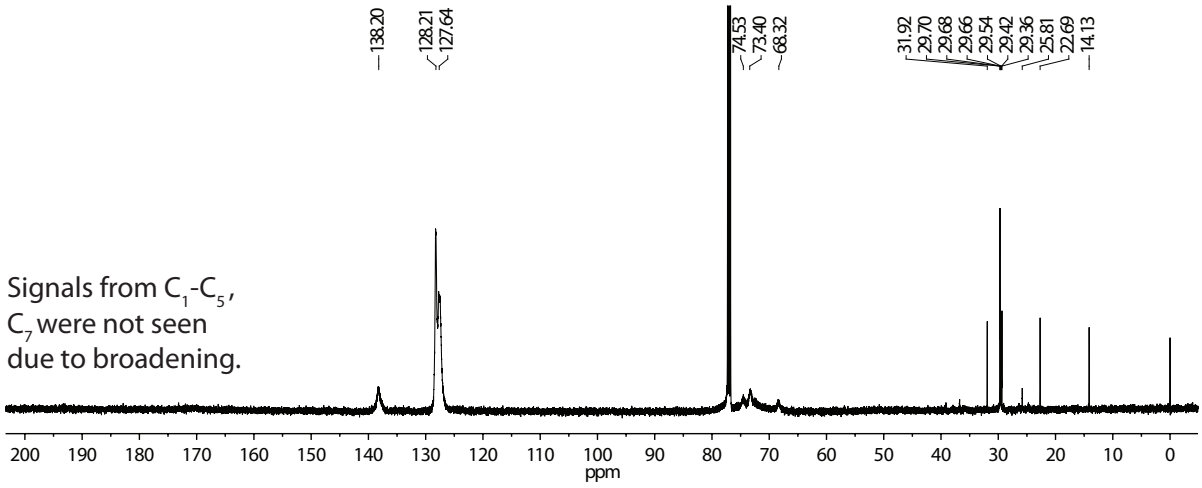
$$DP_{NMR} = \frac{\text{integration of aromatic region}}{\text{number of aromatic protons in repeat unit}}$$

$$DP_{NMR} = \frac{141.5}{15} = 9.4$$

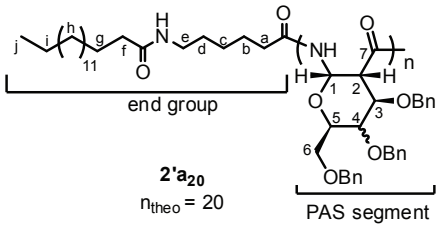
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)



Signals from C_1-C_5, C_7 were not seen due to broadening.

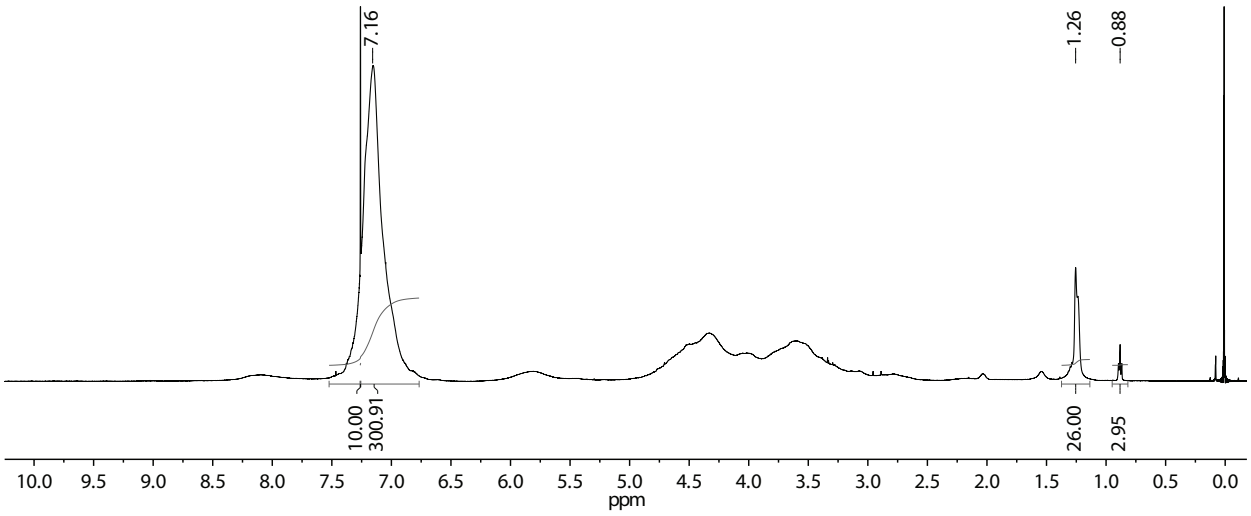


The signal centered at 1.26 is assumed to contain 26 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 26. The aromatic region is integrated and signal due to chloroform is subtracted, and the following equation is used to estimate DP.

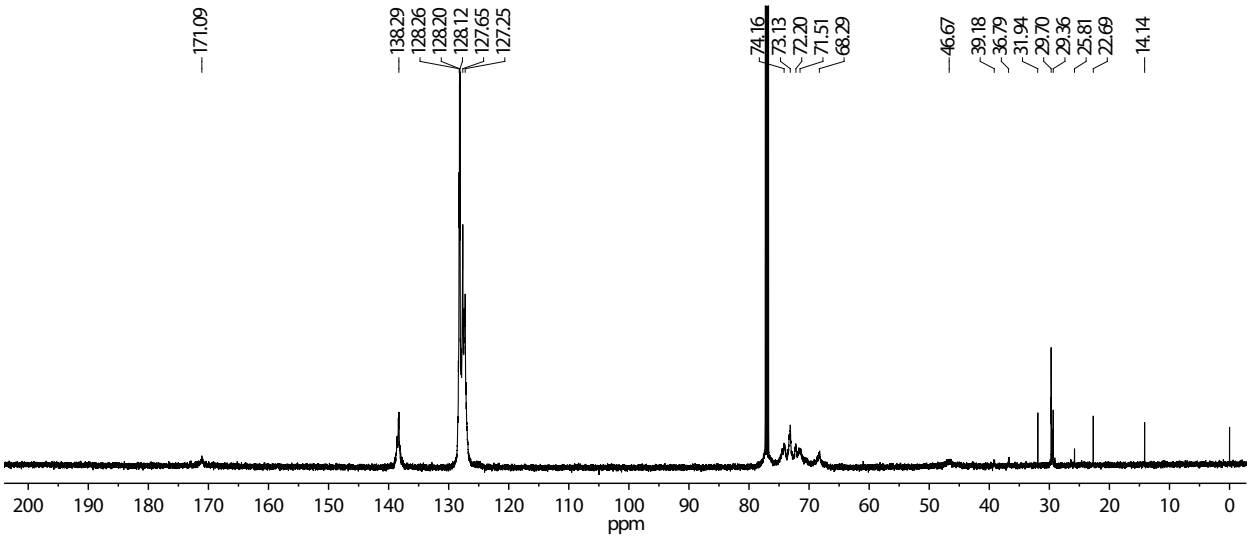
$$DP_{NMR} = \frac{\text{integration of aromatic region}}{\text{number of aromatic protons in repeat unit}}$$

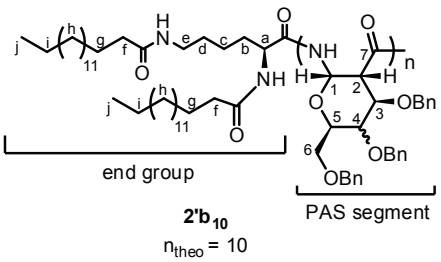
$$DP_{NMR} = \frac{290.9}{15} = 19.4$$

^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)





2'b$_{10}$

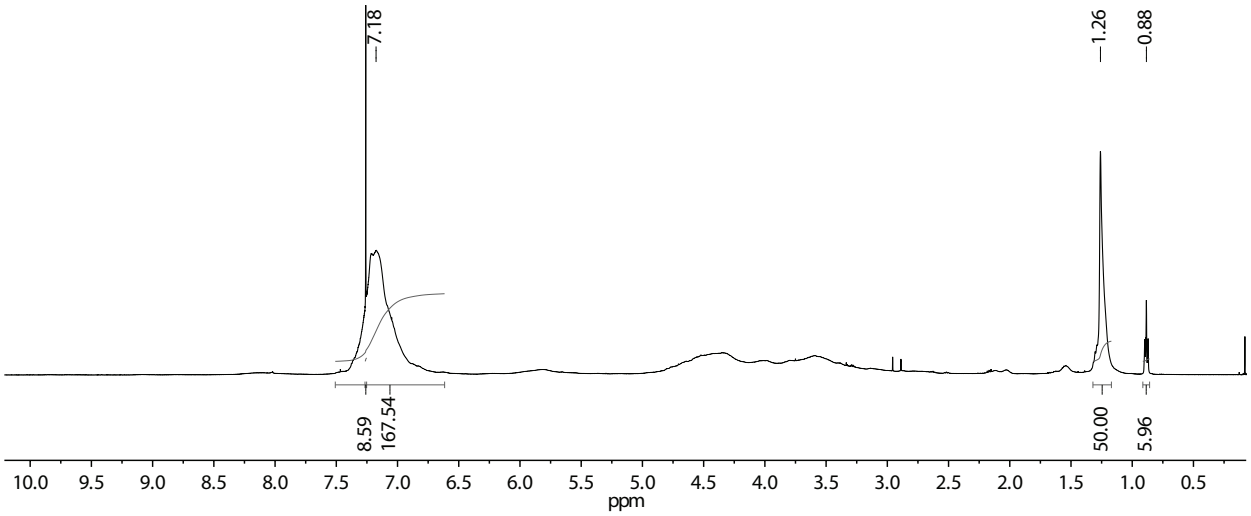
n_{theo} = 10

The signal centered at 1.26 is assumed to contain 50 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 50. The aromatic region is integrated and signal due to chloroform is subtracted, and the following equation is used to estimate DP.

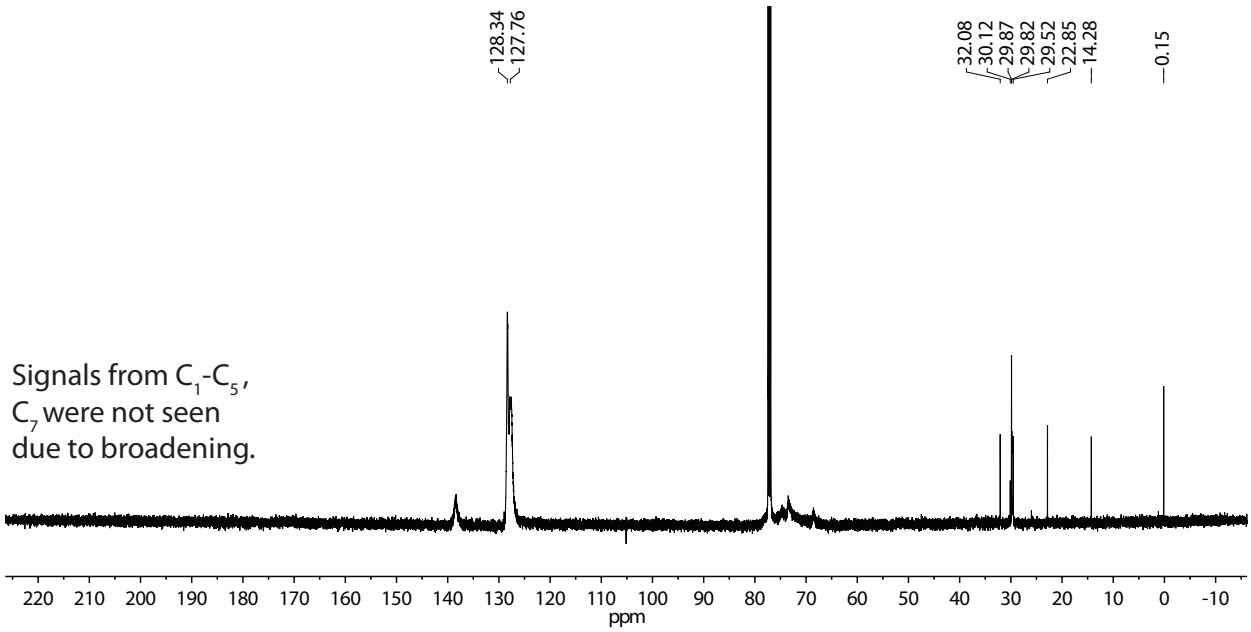
$$DP_{NMR} = \frac{\text{integration of aromatic region}}{\text{number of aromatic protons in repeat unit}}$$

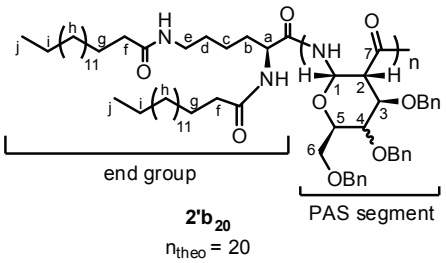
$$DP_{NMR} = \frac{158.9}{15} = 10.6$$

^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)





2'b$_{20}$

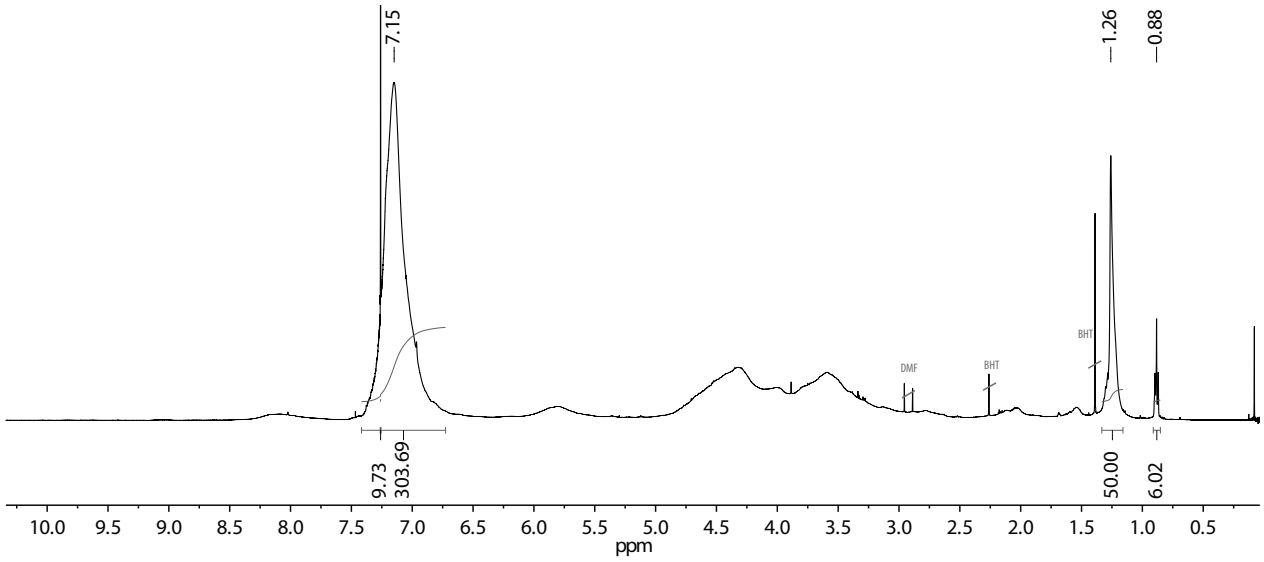
n_{theo} = 20

The signal centered at 1.26 is assumed to contain 50 proton signals (H_i, H_h, and H_c), and is therefore set to an integration value of 50. The aromatic region is integrated and signal due to chloroform is subtracted, and the following equation is used to estimate DP.

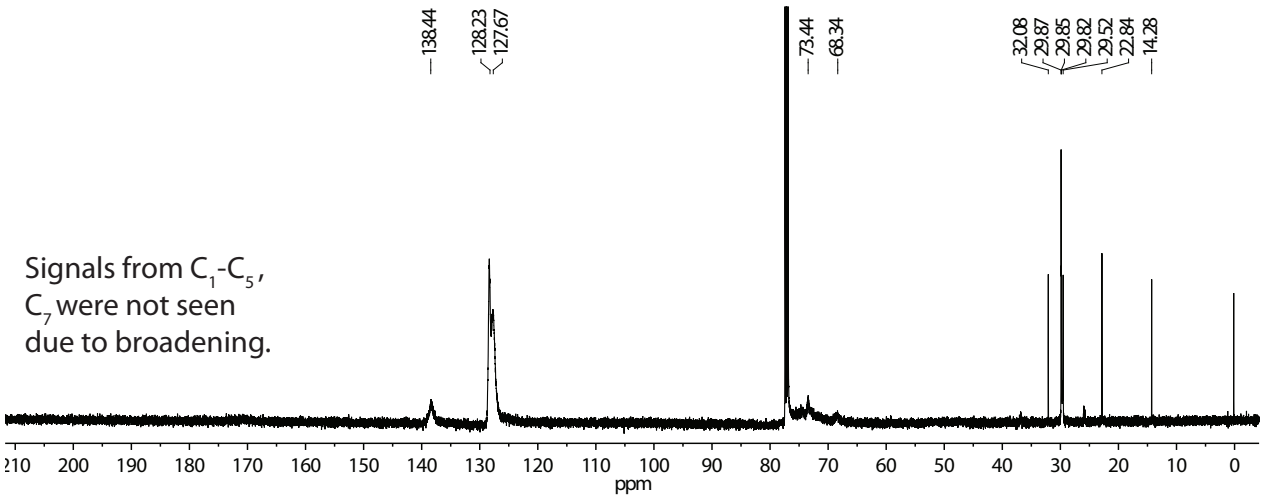
$$DP_{NMR} = \frac{\text{integration of aromatic region}}{\text{number of aromatic protons in repeat unit}}$$

$$DP_{NMR} = \frac{294}{15} = 19.6$$

^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (126 MHz, $CDCl_3$)



Signals from C_1-C_5, C_7 were not seen due to broadening.