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The synthesis and colloidal characterization of several triscationic amphiphilic antibacterial agents

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The Synthesis and Colloidal Characterization of Several Triscationic Amphiphilic Antibacterial Agents

An Honors Program Project Presented to
the Faculty of the Undergraduate
Department of Chemistry and Biochemistry
James Madison University

by Kristin R. McKenna
May 2016

Accepted by the faculty of the Department of Chemistry and Biochemistry, James Madison University, in partial fulfillment of the requirements for the Honors Program.

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PUBLIC PRESENTATION

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Acknowledgements

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Abstract

M-P,12,12, a triscationic amphiphile, was characterized for its antibacterial and colloidal properties as well as its ability to associate with sodium dodecyl sulfate (SDS). This amphiphile has a mesitylene core, one pyridinium headgroup, and two dimethyldodecylammonium tails. The critical aggregation concentration (CAC) for M-P,12,12 was determined by three different methods. According to isothermal titration calorimetry (ITC), the CAC is 2.54 mM, conductivity studies determined the CAC to be 2.07 and the degree of ionization to be 0.188, and nuclear magnetic resonance (NMR) spectroscopy yielded a CAC value of 1.73 mM. The NMR study of the water-insoluble solid formed by M-P,12,12 and SDS found that the two associate in an approximately 1 to 3 ratio of M-P,12,12 to SDS. In addition to this study, several reactions were undertaken to install either a caffeine or acridine headgroup onto the mesitylene core as well as to produce long-chain amines to be added to M-1,1. These reactions were variably efficient and may or may not warrant future work to optimize conditions.
Introduction

In recent years, antibiotic-resistant bacteria have become a major global problem, especially in hospitals and assisted-living facilities. Novel antimicrobial compounds could be useful for treating these infections and preventing the proliferation of these antibiotic-resistant bacteria. Amphiphiles, molecules with both hydrophilic and hydrophobic portions, have been used as antibacterials since 1935. The research in the Caran lab is dedicated to the synthesis and characterization of novel amphiphilic antibacterials. The most recent series of compounds synthesized in the Caran lab contain a mesitylene core, three ammonium cations, and either one or two saturated alkyl chains, varying in length from eight to twenty-two carbons. The amphiphiles are each named with M (for the mesitylene core) followed by a label for the non-tail bearing headgroup(s) (1 for trimethylammonium and P for pyridinium) then the length of each hydrocarbon chain. For example, the compound in figure 1 would be termed M-1,8,8 for its mesitylene core, 1 trimethylammonium head group, and two chains that are each eight carbons long. The solution of each compound also includes three bromide counterions. In the solid state, these counterions are ionically bound to the amphiphiles.

Figure 1. Molecular structure of M-1,8,8.

The antibacterial activities of these compounds have been measured by the Seifert laboratory in the Department of Biology. This is done by determining the minimum inhibitory concentration (MIC). The MIC is the lowest concentration of a molecule that prevents the growth of bacteria. Several of these compounds have comparable MIC values to existing
antibacterials. The compounds with the strongest antibacterial activity across six strains of bacteria in previous studies performed by students in the Seifert laboratory were found to be M-P,12,12, M-1,12,12, and M-1,1,18 (Figure 2).

**Figure 2.** The molecular structure of M-P,12,12 (left), M-1,12,12 (center), and M-1,1,18 (right).

The MIC values for these compounds against *Staphylococcus aureus* were 4 μM, 4 μM, and 16 μM, respectively.\(^9,10\) Comparatively, ciprofloxacin and fluconazole, commonly used broad-spectrum antibiotics, each have minimum inhibitory concentrations of 6.25 μM for *S. aureus*.\(^11\) Therefore, M-P,12,12 and M-1,1,12 are more effective against *S. aureus* than these current antibacterial agents, but M-1,1,18 is not. Since M-1,1,18 had a higher MIC against *S. aureus*, increasing its antibacterial activity is desirable.\(^10\) In order to do this, a modified version of M-1,1,18 could be synthesized.

Modifications in the tail of the molecules impact both the CAC and the antibacterial properties of the amphiphiles.\(^9,10\) One such modification is an unsaturated hydrocarbon tail. Previous studies by Zawrah et al. and Prasad et al. have demonstrated that antibacterial activity for compounds containing multiple bonds have greater antibacterial activity than their saturated analogs.\(^11,15\) There have also been experiments in which anionic amphiphiles with unsaturated hydrophobic portions have been characterized, including 2-alkynoic fatty acids and chalcone derivatives.\(^13,14\) The 2-alkynoic fatty acids displayed MIC values for *S. aureus* between 2.5 μM and >1000 mM.\(^13\) The lower end of this range is comparable to existing antibacterials.\(^11\)
Given the effectiveness of amphiphiles with unsaturated hydrophobic regions and M-1,1,18, an analog of M-1,1,18 with an unsaturated hydrocarbon chain was among the compounds that this study aimed to synthesize. M-1,1,18 contains a mesitylene core, two trimethylammonium headgroups, and one $N,N,N$-dimethyloctadecylammonium group (Figure 2). The compound M-1,1,oleyl was the M-1,1,18 analog that is proposed to be synthesized and characterized in this study. The only structural difference between M-1,1,oleyl and M-1,1,18 is that M-1,1,oleyl contains a cis double bond between carbons 9 and 10 on the octadecyl chain (Figure 3). Sodium oleate, an amphiphile with an unsaturated hydrocarbon tail, displays a CAC that is three times higher than its saturated analog. This may be because unsaturated hydrocarbon chains are more rigid. This makes them less apt to forming aggregates, since the tails cannot freely conform to the conformation that is most favorable for aggregation. Therefore, M-1,1,oleyl was predicted to have a higher CAC than M-1,1,18.

Figure 3. The molecular structure of M-1,1,oleyl.

In addition to the efforts to synthesize M-1,1,oleyl, this study involved further characterization of M-P,12,12 the most effective antibacterial compound in the M-P, M-1, or M-1,1 series. The colloidal properties measure how a compound aggregates in solution. The critical aggregation concentration (CAC) is the lowest concentration at which aggregates form in solution. The CAC can be determined by several methods, including isothermal titration
calorimetry (ITC), conductivity, and NMR differential concentration studies. ITC uses the heat exchange that accompanies aggregation or deaggregation to determine the CAC. Conductivity uses the differences in the conductive properties between aggregates and free molecules to determine CAC. NMR spectra show the chemical environment of certain atoms in solution. The differences in the chemical environment of the protons in aggregates versus those in free molecules also gives a measure of the CAC. The CAC value for M-P,12,12 was previously determined to be 2.54 mM by ITC. In this study, the CAC for M-P,12,12 is determined by NMR and conductivity methods in order to compare those values to the result found by ITC.

Previous studies had found that similar molecules that differed only in the non-tail-bearing head group had very similar CAC and MIC values. This study also aimed to synthesize other intermediates to produce another series of amphiphiles to determine if steric hindrance would have an impact on CAC and/or MIC.

The combination of two oppositely charged small, organic molecules in solution can form new compounds. Formation of new solids or gels that have different properties than the original solutions can be used for industrial and medical applications. In this study, M-P,12,12 was combined with sodium dodecyl sulfate (SDS), a commonly used, negatively charged surfactant.
Experimental

Materials

1,3,5-tris(bromomethyl)benzene was used as obtained from Synthonix. Trimethylamine (33% wt. in ethanol), dimethylamine (40% wt. in water), oleylamine, 1,6-dibromohexane, paraformaldehyde, and 2,2,2-trifluoroethanol were used as obtained from ACROS. Sodium borohydride, pyridine, caffeine, and acridine were used as obtained from Aldrich. Sodium dodecylsulfate (SDS), N,N-dimethyldodecylamine, and N,N-dimethyltetradecylamine were used as received from MP Biomedicals, Inc. 1-bromoeicosane and 1-bromodocosane were used as received from Tokyo Chemical Industry Co., Ltd.

Synthesis of M-1,1

This synthesis follows the previously reported methods.9,10 1,3,5-tris(bromomethyl)benzene is combined with two equivalents of trimethylamine in acetone starting at 0 °C then gradually warming up to room temperature (Scheme 1). The products are then purified by suspension in a 3:100 ethanol/acetone solution with approximately 27 mL solvent per 100 g of solid. Yields of M-1,1 were approximately 65%.

Scheme 1. Synthesis of M-1,1 from 1,3,5-tris(bromomethyl)benzene and trimethylamine.

Synthesis of N,N-dimethyloleylamine

A modified version of the procedure for the demethylation of primary amines developed by Tajbakhsh et al. was used to dimethylate oleylamine.16 Oleylamine was suspended in five mL
of 2,2,2-trifluoroethanol per mmol of oleylamine. After the solution was allowed to stir for five minutes, five equivalents of paraformaldehyde were added. The solution was then stirred for five more minutes followed by the addition of two equivalents of sodium borohydride. The resulting solution was stirred under reflux (80-85 °C) overnight (Scheme 2).

![Scheme 2. Synthesis of N,N-dimethyloleylamine from oleylamine and paraformaldehyde.](image)

**Synthesis of M-1,1,oleyl**

This synthesis followed the previously reported methods for the installation of the tail-bearing headgroup.\(^9,10\) 1.2 equivalents of N,N-dimethyloleylamine was combined with one equivalent of M-1,1 in ethanol at reflux (~80 °C) (Scheme 3). This reaction yielded a mixture of products that were not separated to date.

![Scheme 3. Synthesis of M-1,1, oleyl from M-1,1 and N,N-dimethyloleylamine.](image)
**Synthesis of N,N-dimethyleicosylamine, N,N-dimethyldocosylamine, and bis(N,N-dimethyl)hexylamine**

A 40% aqueous solution of dimethylamine (75 equiv.) was added to one equivalent of 1-bromoeicosane or 1-bromodocosane in approximately ten mL per gram of bromoalkane at -78 °C. The products of this reaction were dried with nitrogen gas. The dimethylalkylamine products were extracted from 2 M NaOH with diethylether. The resulting solution was dried with sodium sulfate. The sodium sulfate was filtered out, and the solvent was removed by rotary evaporation. When the product was dried without heating, the percent yield was approximately 60-75%. It is notable that the yield was significantly diminished by drying at 80 °C, as compared to room temperature. The synthesis of bis(N,N-dimethyl)hexylamine followed a similar procedure, with the sole major difference being that 150 equivalents of 1,6-dibromohexane were combined with 1 equivalent of dimethylamine.

**Synthesis of M-6-M (Bolaamphiphile with 6-carbon linker)**

1,6-bis(N,N-dimethylamino)hexane in ethanol was added to 2 equivalents of M-1,1 in ethanol at room temperature. The solution was then filtered and the solid product was dried with a 97.6% yield.

**Synthesis of M-P,Br,Br**

A solution of 1,3,5-tris(bromomethyl)benzene in ethanol was stirred at room temperature. Pyridine (2 equiv.) was then added dropwise to this solution. The resulting solution was stirred overnight with an air condenser used to prevent excessive evaporation of the solvent. The solution was then filtered to give a 95% yield.
Synthesis of M-P,12,12

A solution of M-P,Br,Br in ethanol was brought 80 °C. Once the solution was at reflux, 2.4 equivalents of N,N-dimethyldodecylamine were added, and this reaction was allowed to proceed overnight. The solvent was filtered off, and the solid product was recrystallized in ethanol and acetone. The percent yields for the dried, recrystallized products were generally approximately 40%.

Syntheses of M-1,1,14, M-1,1,20, and M-1,1,22

N,N-dimethyloctadecylamine or N,N-dimethyldocosylamine (1.2 equiv.) were added to one equivalent of M-1,1 in ethanol at 80 °C for the synthesis of M-1,1,14 or M-1,1,22, respectively. The solvent was filtered off, and the solid product was recrystallized in ethanol and acetone. The percent yield of the dried, recrystallized product was 14.4% for M-1,1,14 and 40.8% for M-1,1,22.

Synthesis of M-caffeine and M-acridine

In each reaction, either caffeine or acridine was dissolved in one of four solvents. These solvents included acetone, ethanol, dichloromethane, and dimethylformamide. The acridine or caffeine solution was added dropwise to one equivalent of 1,3,5-tris(bromomethyl)benzene. These reactions were performed in acetone, ethanol, dichloromethane, and dimethylformamide. The reaction did not proceed considerably after 48 hours in any of the solvents, as determined by NMR spectroscopy.

Synthesis of the M-P,12,12/SDS Combination Solid

M-P,12,12 and SDS were combined in water at variable ratios with a combined osmolarity of approximately 10 mM. The chemical environment was studied using 1H NMR, as indicated by changes in chemical shift from the pure compounds.
**Determination of CAC**

CAC was determined with isothermal titration calorimetry (ITC), NMR spectroscopy, and conductivity studies. These studies were all performed at 37 °C. ITC and conductivity studies involved M-P,12,12 in nanopure water, whereas NMR studies involved M-P,12,12 in D$_2$O.

**SEM**

Scanning electron microscopy (SEM) was utilized to study the compound formed by combining M-P,12,12 and SDS. The solid produced from combining three equivalents of SDS and one equivalent of M-P,12,12 in water was filtered from aqueous solution, dried at ambient pressure, and mounted on an SEM stub with conductive carbon tape. A Leo 1430VP scanning electron microscope was used.

**MIC Measurements**

The MIC values for each amphiphile were measured by students in the Seifert laboratory, as previously reported. Solutions of each amphiphile were serially diluted. Each dilution was dispensed into 6 wells (100 µL per well) on a 96 well plate. Each well was then inoculated with one of the six strains of bacteria utilized in this study. The plates were then incubated at 37 °C for 72 hours. The MIC was defined as the minimum concentration of amphiphile that prevented visible growth of the organism.
Results and Discussion

CAC Studies of M-P,12,12

The CAC of M-P,12,12 was determined using several different methods. The ratio of the slopes of the lines in the conductivity graph gives the α value, which is 0.188 for M-P,12,12. This value represents the degree of ionization of the aggregate.\(^{12}\) The CAC is then found by solving for the x-coordinate of the intersection of the two lines. Points in the region of the slope change were not included in the linear regressions because around the CAC, conductivity does not change linearly with concentration. Using the equations found on the graph, the CAC determined by the conductivity experiment is 2.07 mM. This is approximately 19% less than 2.54 mM, the value found by ITC.\(^{9,10}\) This is consistent with data from other compounds in the M-1 and M-1,1 series, in which the CAC found by conductivity is around 20% less than that found by ITC.\(^{17}\)

![Graph](Figure_4.png)

**Figure 4.** Conductivity of a solution of M-P,12,12 (mA) versus its concentration. The equation for the linear regions above and below the CAC were determined. The x-coordinate of the intercept of these lines is the CAC.
Various concentrations of M-P,12,12 both above and below the CAC values found by ITC and conductivity measurements were analyzed by NMR spectroscopy. The average difference in chemical shift between each sample and the lowest concentration sample was calculated and is reported in Table 1. Figure 6 shows the dependence of the shift in one of the NMR peaks on concentration.

Table 1. The average change in chemical shift between all $^1$H NMR peaks in various concentrations of M-P,12,12.

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Average Change in Chemical Shift (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.676</td>
<td>0.0451</td>
</tr>
<tr>
<td>5.842</td>
<td>0.0426</td>
</tr>
<tr>
<td>5.007</td>
<td>0.0415</td>
</tr>
<tr>
<td>4.173</td>
<td>0.0363</td>
</tr>
<tr>
<td>3.338</td>
<td>0.0334</td>
</tr>
<tr>
<td>2.921</td>
<td>0.0265</td>
</tr>
<tr>
<td>2.504</td>
<td>0.0227</td>
</tr>
<tr>
<td>2.086</td>
<td>0.0146</td>
</tr>
<tr>
<td>1.669</td>
<td>0.0074</td>
</tr>
<tr>
<td>1.252</td>
<td>0.0053</td>
</tr>
<tr>
<td>0.835</td>
<td>0.0065</td>
</tr>
<tr>
<td>0.626</td>
<td>0.0030</td>
</tr>
<tr>
<td>0.501</td>
<td>0.0004</td>
</tr>
<tr>
<td>0.250</td>
<td>0.0004</td>
</tr>
</tbody>
</table>
Figure 6. An example of one of the peaks in the M-P,12,12 samples that shifted with concentration. The highest concentration sample is at the top and the other samples follow in order of descending concentration. This peak represents the protons in the -CH$_2$ group on the hydrocarbon tail that is adjacent to the ammonium.

The data from Table 1 was plotted as average change in chemical shift vs. the inverse of concentration. The CAC is the inverse of the x-coordinate of the intercept of the two lines, which is 1.73 mM (Figure 7). This is 16% less than the CAC found by conductivity and 32% less than the value found by ITC.$^{9,10}$ This difference is partially due to the difference in the solvents used in NMR. Solutions for the NMR experiments were dissolved in D$_2$O, whereas solutions for ITC and conductivity studies were prepared in nanopure water (H$_2$O).
Figure 7. The average change in chemical shift versus inverse concentration for M-P,12,12. Lines of best fit were determined for the linear regions above and below the CAC.

M-P,12,12/SDS Combination Solid

The solid formed by the combination of M-P,12,12 and SDS was studied using $^1$H NMR. An example of the comparison of one of the samples with pure M-P,12,12 and pure SDS is shown in Figure 8.
Figure 8. $^1$H NMR data for pure M-P,12,12 (top), pure SDS (middle), and an 89.8:10.2 M-P,12,12/SDS mixture (bottom). Peak M1 represents the protons adjacent to the nitrogen in the pyridinium group. Peak M2 represents the protons on the phenyl group in the $\gamma$ position relative to the pyridinium group. Peak S1 represents the two protons on the $-\text{CH}_2$ group adjacent to the sulfate group. Peak S3 represents the protons on the $-\text{CH}_2$ groups on the dodecyl chain excluding the two closest to the sulfate group and the terminal methyl group.

Table 2 and Figure 9 represent how the chemical shift of four peaks (two from M-P,12,12 and two from SDS) vary as a function of the ratio of M-P,12,12 to SDS. The general trend seems to be that the largest magnitude change is seen in the 24.7% M-P,12,12/75.3% SDS or 27.7% M-P,12,12/72.3% SDS samples, which corresponds with an approximately 3:1 ratio of SDS to M-P,12,12 for the association. This is in line with predictions because M-P,12,12 has a +3 overall charge without the bromine counterion, whereas SDS has a -1 overall charge without the sodium counterion. These experiments help give insight into the molecular structure of the solid, which is made up of M-P,12,12 and SDS in an approximately one to three ratio.
Table 2. Changes in chemical shift relative to pure M-P,12,12 and SDS in solutions of M-P,12,12 and SDS. The dashes are put in for peaks that did not produce a clear enough signal to be recognized by NMR. In the case of the 100% M-P,12,12 and 100% SDS samples, this is because only one of the compounds is present. In the other samples, the peaks may be resolved if more scans are performed.

<table>
<thead>
<tr>
<th>% M-P,12,12</th>
<th>Peak M1</th>
<th>Peak M2</th>
<th>Peak S1</th>
<th>Peak S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>89.8</td>
<td>-0.0018</td>
<td>-0.0026</td>
<td>0.017</td>
<td>-0.1271</td>
</tr>
<tr>
<td>74.7</td>
<td>-0.0056</td>
<td>-0.0078</td>
<td>0.0216</td>
<td>-0.1285</td>
</tr>
<tr>
<td>66.3</td>
<td>-0.0093</td>
<td>-0.0128</td>
<td>0.0248</td>
<td>-0.1299</td>
</tr>
<tr>
<td>49.6</td>
<td>-0.0251</td>
<td>-0.0345</td>
<td>0.0333</td>
<td>-0.1326</td>
</tr>
<tr>
<td>32.9</td>
<td>-0.0558</td>
<td>-0.0727</td>
<td>0.026</td>
<td>-</td>
</tr>
<tr>
<td>29.7</td>
<td>-0.0561</td>
<td>-0.0788</td>
<td>0.0317</td>
<td>-0.134</td>
</tr>
<tr>
<td>27.7</td>
<td>-0.0696</td>
<td>-0.0603</td>
<td>0.0325</td>
<td>-0.1872</td>
</tr>
<tr>
<td>24.7</td>
<td>-0.0601</td>
<td>-0.0656</td>
<td>0.0293</td>
<td>-</td>
</tr>
<tr>
<td>22.7</td>
<td>-0.0534</td>
<td>-</td>
<td>-</td>
<td>0.0063</td>
</tr>
<tr>
<td>19.7</td>
<td>-0.0358</td>
<td>-0.0383</td>
<td>0.0092</td>
<td>-0.0107</td>
</tr>
<tr>
<td>9.85</td>
<td>-0.0229</td>
<td>-0.0412</td>
<td>0.0027</td>
<td>-0.0111</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 9. Changes in chemical shift (in ppm) in solutions ranging from 89.8% M-P,12,12/10.2% SDS to 9.85% M-P,12,12/90.15% SDS. The orange and blue circles represent the changes in chemical shift of peaks that originate from M-P,12,12 (peaks M1 and M2), whereas the grey and black triangles represent changes in peaks that originate from SDS (peaks S1 and S3).

SEM images showed that the M-P,12,12/SDS material formed lamellar aggregates with each layer being approximately 200 nm thick (Figure 10).

Figure 10. SEM images of the solid formed by the association of M-P,12,12 and SDS.
**M-1,1,20 and M-1,1,22**

The MIC values for M-1,1,20 and M-1,1,22 are reported in Table 4 as well as the MIC values for M-1,1,18.\textsuperscript{17} M-1,1,20 has similar MIC values to M-1,1,18; however, when they do differ, M-1,1,20 has the higher value. Therefore, M-1,1,18 is the more potent antibacterial overall. Both M-1,1,18 and M-1,1,20 had MIC values below M-1,1,22 for all of the strains tested. Therefore, out of these three compounds, M-1,1,22 is the least potent antibacterial agent.

**Table 4.** MIC values for M-1,1,18, M-1,1,20, and M-1,1,22 for six strains of bacteria, as reported in Gallagher et al.\textsuperscript{17} G\textsuperscript{+} indicates a gram positive organism, whereas G\textsuperscript{-} indicates a gram negative organism.

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>Bacillus cereus</em> (G\textsuperscript{+})</th>
<th><em>Enterococcus faecalis</em> (G\textsuperscript{+})</th>
<th><em>Streptococcus agalactiae</em> (G\textsuperscript{+})</th>
<th><em>Staphylococcus aureus</em> (G\textsuperscript{+})</th>
<th><em>Escherichia coli</em> (G\textsuperscript{-})</th>
<th><em>Pseudomonas aeruginosa</em> (G\textsuperscript{-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1,1,18</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>16</td>
<td>16</td>
<td>125</td>
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<tr>
<td>M-1,1,20</td>
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<td>4</td>
<td>16</td>
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<td>250</td>
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<td>M-1,1,22</td>
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<td>&gt;250</td>
<td>&gt;250</td>
<td>&gt;250</td>
<td>63</td>
<td>&gt;250</td>
</tr>
</tbody>
</table>

**M-1,1,oleyl**

Oleylamine was synthesized and combined with M-1,1 in an effort to produce M-1,1,oleyl. However, this produced a mixture of products that was not separated. M-1,1,oleyl did not stand out as a major product (Figure 11). However, it is possible that the conditions for the reaction could be optimized in order to produce M-1,1,oleyl.
Figure 11. Products of the attempted reaction of M-1,1 with oleylamine in dimethylsulfoxide-d6 on a 400 MHz NMR instrument.

**M-caffeine**

M-caffeine was not produced under any of the conditions used to combine 1,3,5-tris(bromomethyl)benzene and caffeine. Each attempted reaction yielded only starting materials after 48 hours, as confirmed by $^1$H NMR (Figure 12).
Figure 12. $^1$H NMR spectra of (from top to bottom) 1,3,5-tris(bromomethyl)benzene, caffeine, and an attempted reaction between the two compounds. Each of these spectra was obtained on a 400 MHz instrument in dimethylsulfoxide-d6.
Conclusion

In conclusion, several amphiphiles were synthesized and purified in this study, including M-1,1,20, M-1,1,22, and M-6-M. N,N-dimethyloleyleamine was also synthesized; however, M-1,1,oleyl was not synthesized in good yield or purified. Although the synthetic procedure for M-P,12,12 was previously optimized, the colloidal properties were further characterized beyond simply one method. The differences found between the different methods used may give insight into the aggregation of the compound. It is also important to note that several reactions that did not proceed as planned have been identified, which eliminates certain headgroups from being suitable candidates for further study. A novel ionic mixture was produced and characterized as well from the combination of M-P,12,12 and SDS. Its potential uses are currently unknown; however, it may prove to have utile or scientifically interesting properties.
Future Studies

Future studies could involve optimizing conditions to produce pure M-1,1,oleyl. Further CAC studies can be performed on M-1,1,20 and M-1,1,22. Powder x-ray diffraction can additionally characterize the M-P,12,12/SDS combination solid. Also, other non-tail-bearing headgroups can be tested to determine whether a specific headgroup can ameliorate antibacterial activity or otherwise provide useful information.
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