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TMEDA-derived biscationic amphiphiles: An economical preparation of potent antibacterial agents

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Dedicated to Professor Amos B. Smith, III, in celebration of his 40 years of mentoring scientists

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ABSTRACT

Bis-alkylated derivatives of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) represent a well-known class of versatile biscationic amphiphiles, owing to their low cost and ease of preparation. Asymmetric TMEDA derivatives, however, have been studied significantly less, particularly in regards to their antimicrobial properties. We have thus prepared a series of 36 mono- and bis-alkylated TMEDA derivatives to evaluate their inhibition of bacterial growth. This series of compounds showed low micromolar activity against a panel of four bacteria. Optimal inhibition was observed when the biscationic amphiphiles possessed modest asymmetry and were composed of between 20 and 24 total carbon atoms in the side chains. These amphiphiles were prepared in a simple two-step procedure, utilizing inexpensive materials and atom-economical reactions, making them practical for further development.

The preparation of chemical agents to counter the spread of human pathogens has been a challenge long before the term medicinal chemistry was coined.[1] From the fermentation of beverages to the preparation of bleach, the facile production of compounds to minimize the pathogenic effects of microbes has been a key concern.[2] Development of bacterial resistance to even the most potent of antibiotics has ensured that continued research into antimicrobial compounds will remain crucial.[3]

Cationic amphiphiles have a unique and longstanding history in addressing this problem.[4] Highlights include the introduction of benzalkonium chloride (*N*-alkyl-*N*-benzyl-*N*,*N*-dimethylammonium chloride) in the 1930s[5] and formulation of this series of structures into commercially important agents such as Lysol® brand products.

Cationic amphiphiles have been traditionally thought of as membrane disruptors,[6,7] capitalizing on electrostatic interactions with the predominantly anionic bacterial cell membrane. The nonpolar chain then intercalates, leading to membrane disruption and ultimately bacterial cell lysis.[8] This mechanism may be minimally susceptible to bacterial resistance.[9] In addition, other mechanisms of action have been suggested, including internalization of amphiphiles into bacterial cells.[10]

Of obvious concern for the development of antimicrobial agents is economy of preparation,[11] and many cationic amphiphiles benefit from facile assembly.[12,13] Other structural classes such as antimicrobial peptides and synthetic mimics thereof (SMAMPs), a promising group of structures that often serve as cationic amphiphiles, are oftentimes challenging to obtain or prepare, though improvements in this area are being sought.[14] We have thus chosen to pursue the synthesis of potent amphiphilic antimicrobials with high levels of atom economy,[15] utilizing short and user-friendly preparations.

Our research program has aimed to develop polycephalic (multi-headed) amphiphiles to optimize antibacterial action.[16,17] A focus on asymmetric disposition of alkyl chains around an easily accessible bis-ammonium core has led to simple and efficient preparation of a series of amphiphiles with low micromolar activity. Starting with 4,4′-bipyridine, we prepared 35 symmetric and asymmetric amphiphiles in short order[17] and observed trends that parallel literature precedent.[18] First, bioactivity peaked at an optimal range of alkyl carbons on the nonpolar tails (roughly 22–24 side chain carbons). Additionally, modest amounts of asymmetry seemed to ensure good solubility of amphiphiles with longer alkyl

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chains.[17] We also noted that the nature of the counterion was less influential.

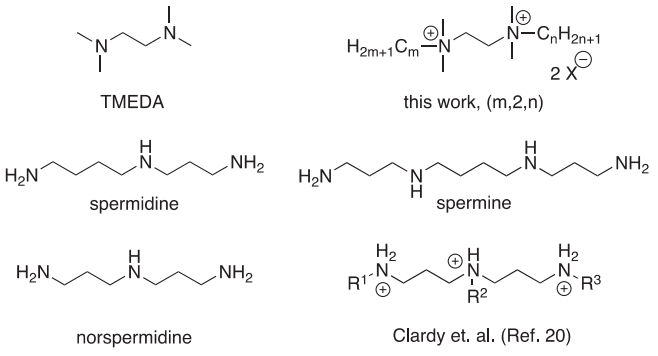
Presently, we have chosen to focus on a commonplace series of bis-amine structures as our synthetic core, starting with *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA), which is available at a cost of approximately $20 per mol.[19] Analogous structures with increased linker distance between the amines, as well as those with an increased number of amines such as spermidine and spermine (Scheme 1), are also available at reasonable cost. The Clardy group has been investigating protonated polyamine compounds for biological activity, reporting the biofilm disrupting capability of norspermidine derivatives.[20] In this work we will detail the preparation of the compounds designated (m,2,n), where 2 is the linker between two amines, and m and n are the number of carbons of each *n*-alkyl hydrophobic side chain.

While the preparation of symmetric (gemini)[21] bis-alkylated derivatives of TMEDA is well-precedented,[22–24] and select antimicrobial activities have been reported,[25,26] we found that the preparation of asymmetric bis-alkylated TMEDA derivatives has scarcely been published.[27] Optimization of antimicrobial activity for such asymmetric derivatives has remained uninvestigated. Since we envisioned expedient syntheses of such compounds and anticipated powerful antimicrobial activities thereof,[16] we set out to prepare a series of such asymmetric bis-alkylated TMEDA derivatives.

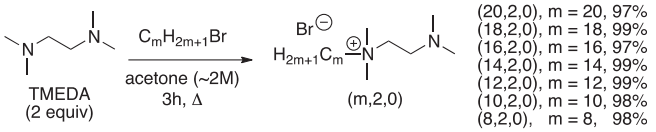
Monoalkylation of TMEDA can be accomplished in a straightforward and atom-economical manner, with exposure of an excess (2 molar equivalents) of the bisamine to a variety of alkyl bromides in minimal-solvent conditions (Scheme 2; see Supporting information for full synthetic detail of all compounds). Simple removal of excess TMEDA in vacuo led to pure (>98%) monoalkylated crystalline products in nearly quantitative yields, without workup or chromatography.

Subsequent exposure to a different alkyl bromide, again in high concentration (~2 M in acetonitrile), followed by filtration, led to good yields (43–92%) of the desired asymmetric biscationic amphiphiles, as shown in Scheme 3. Recrystallization was performed as necessary to ensure compound purity >98%, as determined by NMR. It was found to be operationally advantageous to start with the longer-chained monocationic compounds for installation of the second chain, i.e., preparing (20,2,10) from (20,2,0) and not from (10,2,0). This perhaps reflects the hygroscopic nature of the smaller-chained compounds. It was noted that the largest compound prepared, (20,2,18), suffered from poor water solubility; it was thus not evaluated for bioactivity.

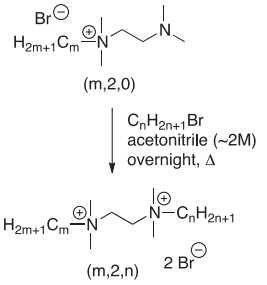
Additionally, two compounds with odd numbers of carbons in one chain [(13,2,10) and (11,2,10)] were prepared from (10,2,0); yields were comparable to the other preparations (Scheme 4). These preparations were prompted by observations from our



Scheme 1. Commercially available polyamines, and some ammonium derivatives thereof.



Scheme 2. Preparation of monoalkylated TMEDA derivatives (m,2,0).



Starting material	n of Alkyl bromide ($C_nH_{2n+1}Br$)					
	18	16	14	12	10	8
(20,2,0)	54%*	77%	81%	73%	82%	88%
(18,2,0)		78%	65%	47%	54%	72%
(16,2,0)			92%	78%	91%	87%
(14,2,0)				43%	66%	71%
(12,2,0)					63%	89%
(10,2,0)						49%

Scheme 3. Yields of synthetic preparation of asymmetric bis-alkylated TMEDA derivatives. Asterisk indicates a water-insoluble compound. See Supporting information for full experimental detail of all compounds.

4,4′-bipyridinium series, wherein we saw that we could optimize efficiency by judicious choice of chain length.

Finally, for comparative purposes, symmetrical TMEDA amphiphiles were prepared according to literature precedent[28] (Scheme 5). Thus, exposure of TMEDA to excess alkyl bromide (3 equiv) in acetonitrile, followed by filtration, led to (n,2,n) compounds, which were recrystallized as necessary.

With a series of 36 amphiphiles in hand, MIC values against the Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis* and Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* were determined by standard methods (Table 1). Comparison was made to a commercially-available benzalkonium chloride solution. The broth microdilution for determining the MIC of the compounds was performed as previously reported;[17] details are reported in the Supporting information.

Examination of the antibacterial activity of the prepared amphiphiles revealed strong antimicrobial activity in many cases, reaching MIC levels as low as 1 μM, with 14 compounds showing activity superior to that of benzalkonium chloride. Some clear trends were also uncovered. First, monocationic compounds were generally less effective at inhibiting the Gram negative bacteria tested (*E. coli* and *P. aeruginosa*) as compared to the bis-alkylated counterparts. For example, (18,2,0) highlighted this trend, displaying MIC values of 2–4 μM versus the Gram positive bacteria and 63 μM versus both Gram negative species.

In accordance with literature precedent,[17] compounds with an aggregate of 20–24 side chain carbons displayed optimal activity. Six of these presented MIC values strictly in the single digit micromolar range. Accordingly, (16,2,8), (14,2,10), and (12,2,12) were highly potent '24-carbon' compounds; (14,2,8) and (12,2,10) were bioactive '22-carbon' compounds; (12,2,8) was the most active of the compounds with 20 carbons in the side chains. We were surprised, however, to see a relative uniformity of bioactivity, as many of these strongly inhibitory compounds showed nearly identical MIC values. Furthermore, while we generally saw preferential activity of many compounds against the Gram positive bacteria tested (*S. aureus* and *E. faecalis*), there was little differentiation in activity for the strongest compounds between Gram positive and Gram negative bacteria.

Two compounds with an odd number of side chain carbons were prepared: (13,2,10) and (11,2,10), which allowed for examination of compounds with 23 and 21 carbons in the side chains. Gratifyingly, this led to our most potent compound: (13,2,10),