Synthesis, Critical Micelle Concentrations, and Antimycobacterial Properties of Homologous, Dendritic Amphiphiles. Probing Intrinsic Activity and the "Cutoff" Effect

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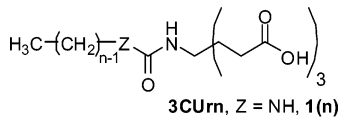
Newkome-type, 1→3 *C*-branched dendrons make an excellent headgroup for amphiphiles with ultralong, saturated, linear alkyl chains. Synthesis of a homologous series of five such amphiphiles from 14 to 22 carbons—$RNHCONHC(CH_2CH_2CO_2H)_3$, R = *n*-$C_nH_{2n+1}$, n = 14, 16, 18, 20, 22—proceeds readily. These amphiphiles are soluble in aqueous solutions of triethanolamine. Surface-tension measurements on this homologous series reveal an unusually gradual decrease in log critical micelle concentration (CMC) as the chain length increases. In fact, the tetradecyl homologue does not appear to form micelles. Further, measurements of minimal inhibitory concentration (MIC) by broth microdilution against *Mycobacterium smegmatis* as a function of the initial cell density provide a direct measure of the intrinsic activity (MIC_0) of each homologue. The hexadecyl homologue is the most active at inhibiting growth with an MIC_0 equal to 3.5×10^{-5} M, which is 100-fold below the CMC.

Introduction

Natural, saturated fatty acids have a long history as microbicides against many pathogens.[1−6] However, the very low solubility of natural, saturated fatty acids in aqueous solutions[7] creates uncertainties in measurements of inhibitory activities because the precise concentration of fatty acid available to the microorganism is unknown. Furthermore, as the chain length of natural, saturated fatty acids increases to octadecyl and beyond, the aqueous solubility cannot be measured.[7] Yet, water-soluble amphiphiles with chain lengths longer than octadecyl might be active against mycobacteria, microorganisms with especially hydrophobic surfaces.[8]

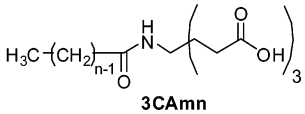
Knowing the solubilities and the critical micelle concentrations (CMCs[a]) of a homologous series of water-soluble amphiphiles enables speculation about how chain length affects intrinsic, antimicrobial activity. In a homologous series, antimicrobial activity can show a "cutoff" effect,[9] earlier termed the parabolic case,[10] where antimicrobial activity increases up to an optimal point as chain length increases; after that, the activity decreases. To conclude that the "cutoff" effect is due to the intrinsic activities[11,12] (i.e., the maximal response that a compound induces) of the homologous amphiphiles, one must ascertain that the amphiphiles fully dissolve in aqueous media. However, the more soluble an amphiphile is, the less likely it will partition into a hydrophobic membrane or cell wall. As Gruber[11] cautioned earlier, one must vary the drug−cell ratios to provide an indication of how membrane partitioning affects activity. Finally, if intrinsic activity occurs near or at the CMC, one must consider detergency[13,14] as a contributing mechanism of action.

The reactions of Newkome-type, 1→3 *C*-branched dendrons[15,16] can be adapted to construct homologous series of water-soluble amphiphiles that have very hydrophobic groups. As shown by Kaifer and collaborators, Newkome-type dendrons (first, second, and third generation) that terminate with carboxylates impart water solubility to hydrophobic groups—those containing a ferrocene,[17] dansyl,[18] pyrene,[19] and viologen.[20] In the case of the dansyl group, the first generation dendron provides the most polar microenvironment.[18] Examples of novel multi-headed, multi-tailed amphiphiles from Hirsch's laboratory contain two second-generation Newkome-type dendritic heads and lipophilic tails attached to calixarene[21] and fullerene.[22] A recent report[23] from Weber's laboratory describes froth-flotation studies with oligofunctional surfactants that have first- and second-generation Newkome-type dendrons. Using the Newkome-type first-generation dendron, we[24] have made a homologous series of water-soluble amphiphiles that have linear alkyl chains with 20 or more carbons—ultralong chains,[25] which have been rarely studied in surfactant chemistry.[26−31]



3CUrn, Z = NH, **1(n)**

3CCbn, Z = O



3CAmn

To determine how various microorganisms respond to water-soluble amphiphiles with long chain lengths, we have measured the minimal inhibitory concentrations (MICs) of three homologous series—4-(3-alkylureido)- (**3CUrn**, **1(n)**), 4-alkoxycarbonylamino- (**3CCbn**), and, 4-alkanoylamino-4-(2-carboxyethyl)-heptanedioic acids (**3CAmn**), where **3C** = three carboxyl dendritic headgroup, **Ur** = ureido linker, **Cb** = carbamato linker, **Am** = amido linker, and **n** = the number of carbons in the fatty chain—of these dendritic amphiphiles as inhibitors of growth against a broad array of microorganisms.[32] These amphiphiles have good activity against Gram-positive bacteria, yeast, fungi, and mycobacteria. Considerably lower concentra-

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[a] Abbreviations: ATR, attenuated total reflection; BHIB, brain heart infusion broth; CFU, colony forming units; CMC, critical micelle concentration; *I*, inoculum; I_{tr}, inoculum at the threshold; logD, calculated log of the distribution coefficient; MIC, minimal inhibitory concentration; MIC_0, intrinsic activity; S, sucrose.

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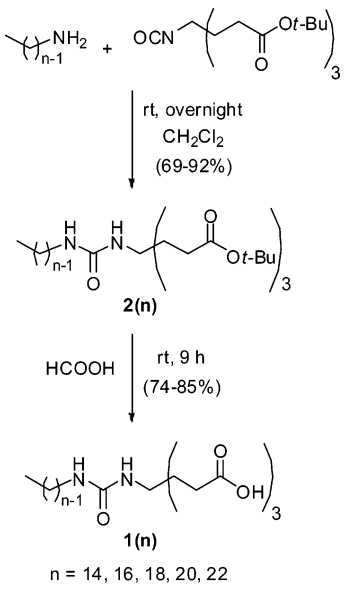
Scheme 1. Synthesis of **1(*n*)**



tions of these amphiphiles compared to natural, saturated fatty acids inhibit growth of *Mycobacterium smegmatis*.[32] Furthermore, the chain length for optimal activity is longer for these amphiphiles than those for natural, saturated fatty acids.

One of our goals is to design amphiphiles that have excellent activity independent of any detergency, which occurs at or near the CMC.[13,14] Toward this goal, we measure CMCs and explore a simple, in vitro method[11] to determine the intrinsic antimycobacterial activity. When two amphiphiles have identical tails, one with a tricarboxylato headgroup should have a higher CMC than one with a monocarboxylato headgroup.[33,34] A homologous series with relatively high CMCs and low MICs enables exploring how chain length affects activity independent of detergency.

Herein, we report the details of the synthesis of the **1(n)** series of amphiphiles synthesized from a dendritic isocyanate and even-numbered alkan-1-amines from 14 to 22 carbons. We also report the CMCs of the corresponding tris(triethanolammonium) salts and the antimycobacterial activity of this homologous series against *M. smegmatis* as a function of initial cell density to probe intrinsic activity.

Results and Discussion

Synthesis. The addition[35] of alkan-1-amines to the dendritic isocyanate gave triesters **2(n)** in good to high yields of purified products (Scheme 1). Icosan-1-amine and docosan-1-amine were prepared from the corresponding alcohols in three steps—mesylate formation, azide substitution, and catalytic reduction—in good overall yields of recrystallized products (see Supporting Information). Formolysis of **2(n)** produced the triacids, **1(n)**, in good yields of recrystallized products. All compounds were fully characterized, including a single-crystal X-ray structural analysis of **2(16)**, which crystallizes with four molecules of amphiphile and two molecules of water in the unit cell (see Supporting Information).

Critical Micelle Concentrations in Aqueous Triethanolamine. The choice of the counterion followed from a study[36] of *N*-lauroyl-L-glutamate in water; the triethanolammonium salt dissolved to a much greater concentration than did the potassium salt. Because chain length can affect the pK_a of fatty acids due to aggregation,[37] we explored different amounts of base to achieve the maximum solubility in aqueous solutions of triethanolamine (pK_a 7.76).[38] The tricarboxylic acids dissolved readily in a solution of triethanolamine/water [~5% (wt/vol)]; the final solution contained ≥9:1 mol equiv of triethanolamine: **1(n)**.

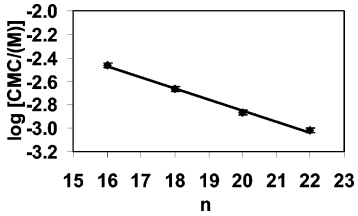


Figure 1. Effect of alkyl chain length (n) on log CMC for **1(n)**. Error limits are estimated at 5% for each measurement. The solid line is a linear-regression analysis, where log CMC = −0.094 ± 0.005 × (n) − 0.97 ± 0.09.

By using a pendent-drop analyzer to measure surface tensions, the CMCs of **1(16)**−**1(22)** were estimated in triethanolamine/water [~6% (wt/vol)] (Figure 1). These solutions contained more triethanolamine than in the antimycobacterial assays because higher concentrations of amphiphile were required to measure the CMCs than those required to inhibit growth of *M. smegmatis*. The pH of the solutions ranged from 9.3 to 10.0 depending on the concentration of the amphiphiles. Unlike **1(18)**−**1(22)**, the surface tension data for **1(14)** did not show a leveling off with increasing concentrations up to 10 mM (see Supporting Information), suggesting that it failed to form micelles in the expected concentration range. The surface tension data for **1(16)** showed a leveling off then continued to decrease with increasing concentration. We used this leveling off point as the CMC for **1(16)**.

Two noteworthy results emerge from the data in Figure 1. First, there is a very modest dependence of log CMC on alkyl chain length. The slope of the line in Figure 1 is −0.094 ± 0.005; this value contrasts with a value of −0.3 for a similar plot of the CMCs of potassium salts of natural, saturated fatty acids.[33] Slopes of similar plots for potassium salts of di- and tricarboxylato amphiphiles have values of −0.22.[33,34] The CMCs of triethanolammonium salts of natural, saturated fatty acids show a biphasic dependence on chain length.[39] The slopes have values of −0.06 for octanoate to tetradecanoate and −0.6 for tetradecanoate to octadecanoate. Second, the CMC for the longest homologue, **1(22)**, is 1 mM, which is quite large for an ultralong chain. As such, this value is 8-fold higher than the MIC required for completely inhibiting the growth of *M. smegmatis* (see below). Studies are planned to probe the structure of the micelles and how (1) elongating the chain and (2) different counterions affect the CMC.

Antimycobacterial Assays. To probe the intrinsic activities of these amphiphiles, we measured the MICs of **1(14)**−**1(22)** at several different initial cell densities, expressed as colony-forming units (CFU)/mL, of *M. smegmatis*. Experiments were performed at two overlapping ranges of initial inocula—32 to 3.2×10^7 CFU/mL and 500 to 5.0×10^8 CFU/mL. The MIC was measured, by successive 2-fold dilution in 96-well microtiter plates, as the lowest concentration at which an amphiphile completely inhibited the growth after 4 days at 37 °C.

The "Inoculum Effect". In studies of antimicrobial activity, the MIC is often observed to increase as initial cell density (i.e., inoculum) increases. This phenomenon is referred to as the "inoculum effect".[40−42] The "inoculum effect" presumably reflects the increased demand for a given drug as the number of cells and targets increases.[42] A mathematical model[43] (eq 1) of the "inoculum effect"

$$\log \mathrm{MIC} = \log \mathrm{MIC}_0 + (e^{k(\log I - \log I_{tr})} - 1) \qquad (1)$$

describes the independence of log MIC at low inoculum by defining log MIC_0 as the intrinsic activity, log I as the inoculum, and log I_{tr} as the inoculum at the threshold immediately before the rise in MIC, and k is a constant that describes the rate of increase in MIC. Equation 1 is flat at low inoculum and rises exponentially after I_{tr}, which varies by drug (Figure 2). From a bioorganic mechanistic perspective, the "inoculum effect" may provide information about a change in the mechanism of action.

MICs of 1(n). All five homologues show an "inoculum effect" and follow eq 1. Figure 2 illustrates that the antimycobacterial activity of these amphiphiles is constant as the initial cell density increases up to I_{tr}. These plateaus equal log MIC_0, the intrinsic activity. Note the constancy of the value of MIC_0 for each homologue in these plateaus. On the basis of seven (or more) separate measurements of MIC_0 for each amphiphile, we conclude that the differences in the values of MIC_0 for each homologue are reliable even though individual measurements overlap within error. Among the amphiphiles, **1(16)** has the lowest MIC_0. The log I_{tr} varies among the amphiphiles. The lowest log I_{tr} (4.7) is for **1(16)**; the highest log I_{tr} (6.7) occurs for both **1(14)** and **1(20)**. If partitioning into a hydrophobic part of the cell were a dominant part of the mechanism, the log I_{tr} should be lowest for **1(14)**. After the threshold, the increases in log MICs with log initial cell density are approximately the same for all the amphiphiles.

Figure 3 shows plots of log MIC vs alkyl chain length at four different initial cell densities to better illustrate the effect of alkyl chain length on activity. Figure 3 also illustrates Gruber's thesis[11] that at initial cell densities above the lowest threshold, one measures apparent values and can be misled as to which chain length yields the optimal intrinsic activity. At initial cell densities of $\leq 3.2 \times 10^4$ CFU/mL, MIC is constant and MIC_0 is revealed. The most active compound is **1(16)**; though MIC_0 for **1(16)** is only one 2-fold dilution lower than that of **1(18)**. Homologue **1(16)** is also the most active at 5×10^8 CFU/mL. At this inoculum, the data show almost no variation among the amphiphiles, except that the MIC for **1-(16)** is only one 2-fold dilution lower than the rest. In contrast, at intermediate inocula of 5×10^6 and 3.2×10^5, both **1(16)** and **1(18)** are the most active compounds.

Comparison with Previous Work. The discovery that the homologue with the hexadecyl chain gives the best activity contrasts with the results for natural, saturated fatty acids. In an earlier study[5] of several mycobacteria, tetradecanoic acid is slightly more active than dodecanoic acid at inhibiting the growth of *M. smegmatis*. In a very recent study,[44] dodecanoic acid is the most active of the natural, saturated fatty acids up to 26 carbons for several mycobacteria, including *M. smegmatis*.

If partitioning of these amphiphiles into both outer and cytoplasmic mycobacterial membranes[8] is a significant component of the mechanism of action, then calculating partitioning coefficients could be used to compare intrinsic activities. For a homologous series, partition coefficients increase as the alkyl chain length increases. For compounds with ionizable groups, the distribution coefficient (D), which comprises the partition coefficient and the pK_a's, provides a better estimate of the partitioning between octan-1-ol and water at given pH. At pH 7.4, calculated[45] logD ranges from −5.1 for **1(14)** to −1.9 for **1(22)** in increments of 0.8 for each additional $-CH_2-CH_2-$ unit. At pH 7.4, calculated[45] logD for natural, saturated fatty acids ranges from 2.4 for tetradecanoic acid to 5.6 for docosanoic acid. These calculations suggest that the **1(n)** series substantially favors the hydrophilic phase and that natural, saturated fatty acids favor the hydrophobic phase. Therefore, the activities of **1(n)** and natural, saturated fatty acids are not likely explained by the same mechanism.

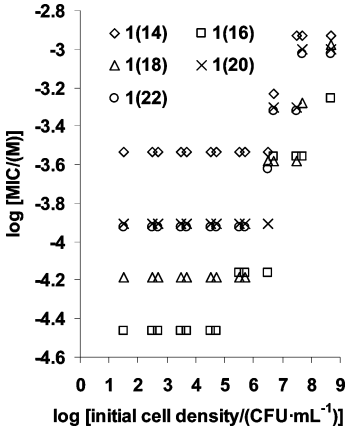


Figure 2. Effect of initial cell density on inhibiting the growth of *M. smegmatis* for **1(n)**. Combined data of two separate assays—32 to 3.2 $\times 10^7$ CFU/mL and 500 to 5.0×10^8 CFU/mL. Error bars (not shown for clarity) are ±0.3.

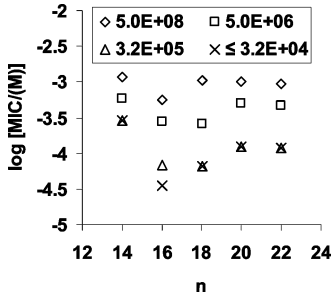


Figure 3. Effect of alkyl chain length **(n)** on inhibiting the growth of *M. smegmatis* for **1(n)** as a function of the initial cell density. Error bars (not shown for clarity) are ±0.3.

Comparison of MIC and CMC. As MIC_0 of each homologue (Figure 2) is lower than the corresponding CMC (Figure 1), detergency is not the likely mechanism of action for the intrinsic activity. In this comparison, the MIC_0 of **1(16)** is 100-fold smaller than the CMC and the MIC_0 of **1(22)** is 8-fold smaller than the CMC. The data at the highest inoculum (Figure 3) illustrate conditions where the amphiphiles may not be saturating the cells. In this comparison, the MIC of **1(16)** is 5-fold smaller than the CMC, and the MIC of **1(22)** equals the CMC. We tentatively conclude that detergency is the likely mechanism of action at the highest initial cell density for **1(22)** and **1(20)**. For the other members of the series, detergency likely contributes moderately to the mechanism of action.

We initiated this project to design long-chain amphiphiles with high CMCs. The underlying thesis is that the higher the CMC, the less likely detergency will contribute to the mechanism of action. Although these amphiphiles do not have MICs low enough to be considered as leads for drug development, keep in mind that the therapeutic index (ratio of cytotoxicity to potency) is the ultimate criterion for potential applications. In this regard, these amphiphiles have potential application as affordable topical anti-infectives. If cytotoxicity is only related to detergency (hence CMC), then a therapeutic index of 100 looks more promising. Although cytotoxicity is unlikely to be that simple, designing amphiphiles with high CMCs is essential for developing nondetergent amphiphilic microbicides.

Conclusions

Water-soluble amphiphiles that have ultralong chains can be made readily from first-generation, Newkome-type dendrons. As tris(triethanolammonium) salts, these amphiphiles show excellent solubility in water. This homologous series of alkyl chains that extends to docosyl reveals that the "cutoff" for antimycobacterial activity is the hexadecyl chain. The intrinsic activity is well below the CMC, suggesting a mechanism of action that does not involve detergency.

Experimental Section

General Methods. Unless specified, solvents and reagents were used as received from commercial suppliers. THF was distilled from sodium/benzophenone ketyl. Weisocyanate was prepared as described.[46] Analytical thin layer chromatography was performed on polyester-coated silica gels (60 Å) and detected by treating with 10% ethanolic phosphomolybdic acid reagent (20 wt % solution in ethanol). Flash column chromatography was carried out on 60 Å ultrapure silica gels. Solutions were concentrated by rotary evaporation. Uncorrected melting ranges were determined in open capillary tubes. NMR spectra were recorded at 400 and 100 MHz for ^{1}H and ^{13}C, respectively, and reported in ppm. IR spectra of neat samples were obtained with an FTIR equipped with a diamond ATR system and reported in cm^{-1}. HRMS data were obtained on a dual-sector mass spectrometer in FAB mode with 2-nitrobenzyl alcohol as the proton donor. Elemental analyses were obtained from a commercial laboratory.

General Procedure for the Preparation of Long-Chain Ureido Tri-*tert*-butyl Esters, 2(n), n = 14, 16, 18, 20, 22. An amine (5.43 mmol) was added slowly to a solution of Weisocyanate (5.43 mmol) in CH_2Cl_2 (45 mL). The resulting transparent solution was stirred at room temperature. After the solution was stirred overnight, the solvent was removed to leave a crude yellow oil (**n** = 14) and a crude white solid (**n** = 16, 18, 20, 22). The crude yellow oil was purified by flash column chromatography (hexane:EtOAc = 5:1), while the crude white solid was purified by recrystalization with EtOH−H_2O. Both purification methods gave a pure white solid (69−92%). Details for **2(16)**−**2(22)** can be found in Supporting Information.

Di-*tert*-butyl 4-(2-*tert*-butoxycarbonylethyl)-4-(3-tetradecylureido)heptanedioate, 2(14). The general procedure described above afforded a white solid; mp 55.0−55.6 °C; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H), 1.20−1.35 (bm, 22H), 1.35−1.50 (bm, 29H), 1.94 (m, 6H), 2.24 (m, 6H), 3.14 (q, 2H), 4.08 (m, 1H), 4.57 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 26.9, 28.1, 29.3, 29.57, 29.58, 29.62 29.65, 29.9, 30.2, 30.6, 31.9, 40.6, 56.4, 80.5, 156.8, 173.1; IR 3327, 2919, 2850, 1721, 1673, 1646, 1559, 1148; HRMS calcd for $C_{37}H_{71}N_2O_7$ [M + H]$^+$ 655.5261, found 655.5246. Anal. ($C_{37}H_{70}N_2O_7$) C, H, N.

General Procedure for the Preparation of Long-Chain Ureido Triacids, 1(n) n = 14, 16, 18, 20, 22. Compound **2(n)** (5.02 mmol) was added to formic acid (20 mL). The resulting mixture was stirred to give a transparent solution. For some compounds, it was necessary to warm the solution to dissolve **2(n)**. The transparent solution was stirred at room temperature for 9 h; the complete reaction was identified by the formation of milky solution. The solution was concentrated to yield a white solid, which was recrystallized with acetic acid and hexane to give a white solid (74−85%). Details for **1(16)**−**1(22)** can be found in Supporting Information.

4-(2-Carboxyethyl)-4-(3-tetradecylureido)heptanedioic Acid, 1(14). The general procedure described above afforded a white solid; mp 159.0−159.5 °C; ^{1}H NMR (CD_3OD) δ 0.90 (t, 3H), 1.25−1.35 (bm, 22H), 1.44 (bm, 2H), 1.95 (m, 6H), 2.27 (m, 6H), 3.05 (t, 2H); ^{13}C NMR (DMSO-d_6) δ 14.0, 22.1, 26.4, 28.2, 28.7, 28.8, 29.0, 29.1, 30.0, 31.3, 38.8, 55.0, 157.0, 174.5; IR 3395, 3352, 2916, 2849, 1709, 1693, 1610, 1559; HRMS calcd for $C_{25}H_{47}N_2O_7$ [M + H]$^+$ 487.3383, found 487.3384. Anal. ($C_{25}H_{46}N_2O_7$) C, H, N.

Determination of the CMCs. A video system (mounted on a vibration isolation table) that captures images of a pendent drop from an 18-gauge stainless-steel needle (1.27 mm) was used to determine surface tension. To help maintain humidity levels and ensure that the drop size did not vary significantly during the measurements, the pendent drop was enclosed in a standard glass cuvette that contained 0.5 mL of aqueous triethanolamine (∼9 mg/mL) dissolved in ultrapure (Type I) water. A hole (2.54 mm) was drilled in the Teflon lid to accommodate the needle. Calibration of the instrument entailed measuring the tip width of the needle with a micrometer and using that measurement to perform an initial calibration of the video camera's magnification.

Surface tension values for an individual static drop were determined for each solution via drop-shape analysis, from 20 images of each drop (one image was taken by the software every 0.5 s) to produce an average surface tension. Reproducibility of the measurements was determined by performing the drop-shape analysis on five different drops of the same solution to obtain values with standard deviations of approximately ±0.2 mN/m. Plots of surface tension versus log [amphiphile] were made (see Supporting Information); linear least-squares analyses of both the points before and the points after the break were used to determine the CMC.

Antimicrobial Assays. Microbial Strains, Culture Conditions, and Preparations of Inocula for Susceptibility Testing. *M. smegmatis* was obtained from the Virginia Tech Microbiology culture collection. Two separate assays with initial cell densities ranging from 32 to 3.2 × 10^7 CFU/mL and 500 to 5.0 × 10^8 CFU/mL were performed. The different initial cell densities were prepared in the following manner. A single isolated colony of *M. smegmatis* on M7H10 agar was used to inoculate sterile M7H9 broth (2 mL) in a screw-capped tube (16 × 125 mm). After incubation at 37 °C for 5 d without shaking, 1 mL was used to inoculate M7H9 broth (18 mL) in a 125 mL flask and then incubated at 30 °C for 5 d with aeration (120 rpm). The culture was transferred to a sterile 50 mL screw-capped centrifuge tube; centrifugation (5000*g*) for 20 min was performed. The supernatant-spent medium was discarded, and the cells were suspended in 1/10-strength BHIB medium (2 mL). A 10-fold dilution series was prepared in 1/10-strength BHIB by transferring the concentrated cell suspension (0.5 mL) into 4.5 mL of 1/10-strength BHIB to yield a 10-fold to 10^7-fold dilution series. The CFU/mL of the concentrated cell suspension was measured by spreading different dilutions (0.1 mL each) on M7H10 agar. The agar plates were incubated at 37 °C and colonies were counted after 4 d. The average of the colony counts was calculated. MIC measurements were performed within 24 h after the dilutions were performed.

Quality Assurance. For the work reported here, all cultures and suspensions that were used as inocula were uncontaminated; colonies had the expected morphologies. To check for viability and contamination, broth cultures were streaked on plate count agar; the plates were incubated at 37 °C for 4 d. All viable, uncontaminated inocula were stored up to 14 d at 4 °C until used.

Measurement of MICs. Microtiter plates (96 wells: rows A−H, columns 1−12) were filled by the following protocol to give 2-fold serial dilutions of **1(n)**. Aliquots (50 μL) of 1/10-strength BHIB+S (pH 7.4) were placed in all the wells except for those in column 1. Solutions of ≥9:1 triethanolamine:**1(n)** (100 μL) were placed in column 1. An aliquot (50 μL) was removed from a well in column 1 and mixed with the BHIB+S in the well of column 2. Then, an aliquot (50 μL) of this mixture was removed and mixed with BHIB+S in column 3. This process was repeated through column 11, at which point an aliquot (50 μL) was removed and discarded. Column 12 was the blank (positive growth) control, BHIB+S only, for each row. An aliquot (50 μL) of the microbial inoculum at a given cell density was added to all wells in a row. Aqueous triethanolamine without **1(n)** was also tested for antimicrobial activity by using the same protocol; no antimicrobial activity was found. The concentration of **1(n)** ranged from 0.57 mg/mL to 0.55 μg/mL. After the plates were incubated at 37 °C for 4 d, MIC results were read by comparing the turbidity (due to growth of microbes) of each test well to the positive control wells. MIC was defined as

the lowest concentration completely inhibiting the growth. The experiments were run in duplicate for the initial cell densities of 32 to 3.2×10^7 CFU/mL and in single for the initial cell densities of 500 to 5.0×10^8 CFU/mL.

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Supporting Information Available: Experimental procedures for the synthesis of icosan-1-amine and docosan-1-amine. Characterization data for **2(16)**−**2(22)** and **1(16)**−**1(22)**. Description of a single-crystal X-ray analysis of **2(16)**. Data from surface-tension measurements for **1(14)**−**1(22)** in aqueous triethanolamine. This material is available free of charge via the Internet at http://pubs.acs.org.

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