Incremental Conversion of Outer-Membrane Permeabilizers into Potent Antibiotics for Gram-Negative Bacteria

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Abstract: Cholic acid derivatives appended with amine groups have been prepared for use as permeabilizers of the outer membranes of Gram-negative bacteria. These compounds interact synergistically with antibiotics such as erythromycin and novobiocin to inhibit growth of Gram-negative bacteria. When a hydrophobic chain is included on the permeabilizers, they can be converted into potent antibiotics. The role of the hydrophobic chain is to facilitate self-promoted transport of the cholic acid derivatives across the outer membrane of Gram-negative bacteria. These compounds share activities found with polymyxin B and derivatives.

Introduction

The outer membrane common to Gram-negative bacteria is unique among lipid bilayers in its structure and function. The outer leaflet of the outer membrane of most species of Gram-negative bacteria is comprised primarily of lipid A (**1**;[1] Figure 1). Lipopolysaccharide (LPS) or Gram-negative endotoxin consists of lipid A linked to a core oligosaccharide, which is typically bonded to a polysaccharide.[2] Lipid A molecules are believed to form intermolecular noncovalent bridging interactions via divalent cations (Mg^{2+} and Ca^{2+}), providing a substantial permeability barrier to hydrophobic molecules, lysozymes, and proteases.[3] Disruption of the lipid A cross bridging significantly increases the permeability of the outer membrane. Cross bridging can be disrupted by metal ion chelators such as EDTA,[4] by amines including tris(hydroxymethyl)aminomethane (TRIS) at high concentrations (>30 mM),[5] or at much lower concentrations by compounds that selectively bind LPS.[6−8]

Compounds known to bind LPS and increase the permeability of the outer membrane of Gram-negative bacteria include bactericidal/permeability increasing protein,[6] a number of small cationic peptides,[7] the polymyxin family of antibiotics, and polymyxin derivatives.[8] Many of these compounds have been shown to bind the lipid A portion of LPS.[9] Polymyxin B_1 and B_2 (used as a mixture and collectively termed PMB) (**2**; Figure 2) are the most commonly used members of the polymyxin

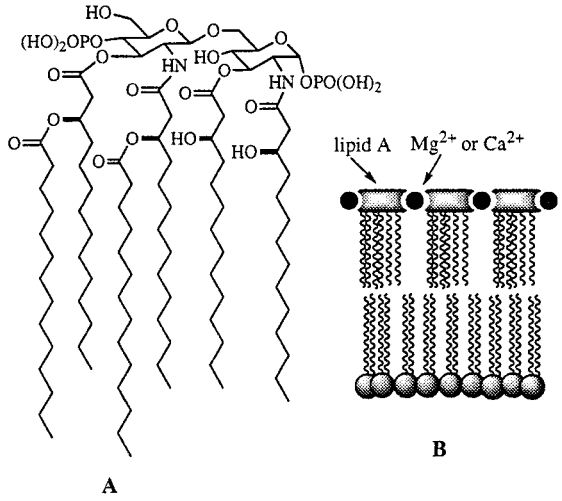

Figure 1. (A) Structure of lipid A from *E. coli*. (B) Schematic representation of the permeability barrier provided by lipid A−divalent cation interactions.

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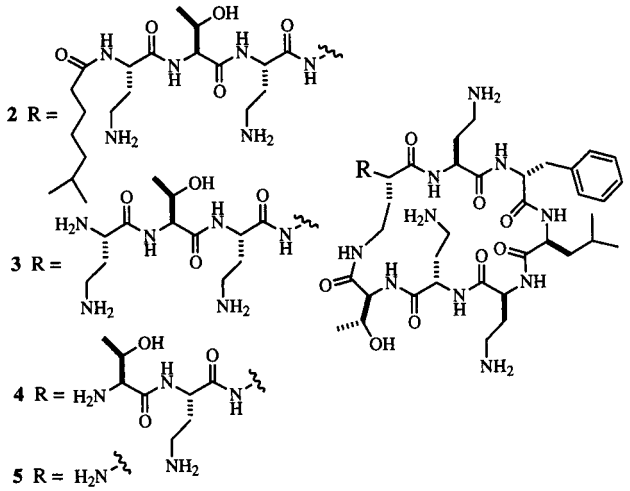


Figure 2. Polymyxin B_2 (**2**), deacyl polymyxin B (**3**), polymyxin B nonapeptide (**4**), and polymyxin B heptapeptide (**5**).

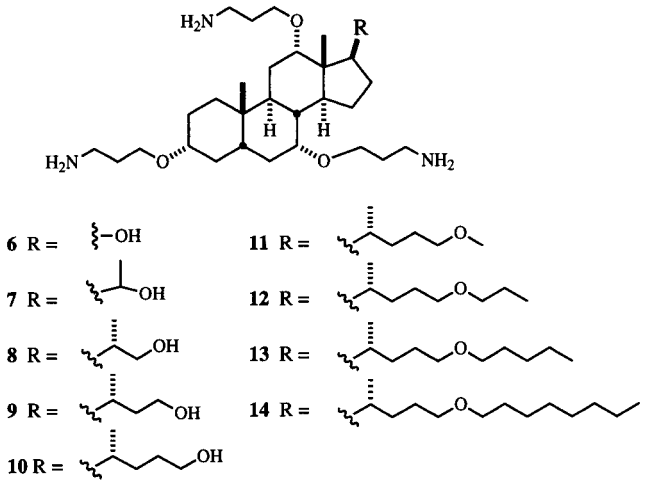
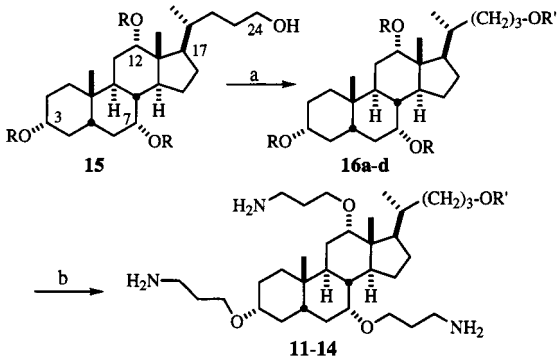


Figure 3. Compounds **6**−**14** with varied side chain lengths.

Scheme 1



for **15, 16a-d**, R = -$(CH_2)_3N_3$

for **16a, 11**, R' = -CH_3 for **16c, 13**, R' = -$(CH_2)_4CH_3$

for **16b, 12**, R' = -$(CH_2)_2CH_3$ for **16d, 14**, R' = -$(CH_2)_7CH_3$

Reagents (reaction yields in parentheses): a) NaH, DMF, CH_3I, $CH_3(CH_2)_2Br$, $CH_3(CH_2)_4Br$, or $CH_3(CH_2)_7Br$ (85-90%). b) $LiAlH_4$, THF (55-70%).

family of antibiotics which includes polymyxins A, B_1, B_2, D_1, E_1, and E_2, circulin A, and octapeptins A_1, A_2, A_3, B_1, B_2, B_3, and C_1. PMB is bactericidal to Gram-negative strains of bacteria, and its proposed mechanism of action involves association with lipid A[9c,d] on the surface of bacteria, self-promoted transport through the outer membrane, and disruption of the cytoplasmic membrane, resulting in cell death.[3a,10] In contrast, deacylpolymyxin B (DAPB) (**3**; Figure 2),[8e] polymyxin B nonapeptide (PMBN) (**4**; Figure 2),[8j] and polymyxin B heptapeptide (PMBH) (**5**; Figure 2)[8d] are much less bactericidal than PMB or not bactericidal at all. Nevertheless, DAPB, PMBN, and PMBH retain the ability to bind LPS and increase the permeability of the outer membranes of Gram-negative bacteria to hydrophobic antibiotics.[8] On the basis of the behaviors of PMB and its derivatives, two distinct activities of these compounds have been categorized as (1) sublethal permeabilization of the outer membrane (a result of LPS binding) and (2) lethal disruption of the cytoplasmic membrane (requiring self-promoted transport[3c] through the outer membrane).[3a] PMB is capable of both types of activity, while DAPB, PMBN, and PMBH are primarily limited to permeabilization of the outer membrane.

We recently reported the development of cholic acid-derived compounds that effectively increase the permeability of the outer membrane of Gram-negative bacteria.[11] These compounds were designed to provide nonpeptide mimics of the structure of PMBH, the LPS binding domain of PMB. Importance was placed on reproducing in our cholic acid derivatives the relative spatial arrangement of the primary amines of the diaminobutyric acid residues in PMBH which are conserved among the polymyxin family of antibiotics. Our continued efforts have yielded compounds that permeabilize not only the outer membrane of Gram-negative bacteria, but also specific derivatives that rival PMB in bactericidal activity. These compounds exhibit the two activities demonstrated by PMB and derivatives, i.e., sublethal permeabilization of the outer membrane and/or a second lethal activity which appears to be disruption of the cytoplasmic membrane. Through our design process we have incrementally converted cholic acid-derived permeabilizers into potent antibiotics by varying the hydrophobic nature of the side chain extending from C-17 of the steroid nucleus as in series **6**−**14** (Figure 3). The length of the side chain appears to influence the ability of the compounds to pass through the outer membrane, and variations in the chain yield compounds with a range of combinations of sublethal and lethal activity. These variations contrast the abrupt structural changes and activities observed upon conversion of PMB to DAPB, PMBN, or PMBH.

Results and Discussion

Our initial design included primary amine groups attached to a steroid nucleus derived from cholic acid as a mimic of the structure of PMBH. However, no function was anticipated for the carbon chain extending from C-17. Nevertheless, we observed dramatic effects in the antibacterial activity of the cholic acid derivatives upon altering the nature of the steroid side chain. Consequently, to fully explore the role of the steroid side chain in sublethal and lethal activities, we prepared a series of compounds in which the side chain was incrementally varied in length.

Preparation of compounds in which the steroid side chain was lengthened from C-24 (**11**−**14**) was straightforward (Scheme 1). Reaction of alcohol **15**[11] with methyl iodide, propyl bromide, pentyl bromide, or octyl bromide followed by reduction gave **11**−**14**. Synthesis of compounds with truncated side chains (**6**−**10**) required oxidative cleavage of carbons from the steroid side chain, and published methods were used to prepare starting materials **17**[12] and **25**[13] (Schemes 2 and 3). For the synthesis of **7** (Scheme 2), the ketone of **17** was protected followed by

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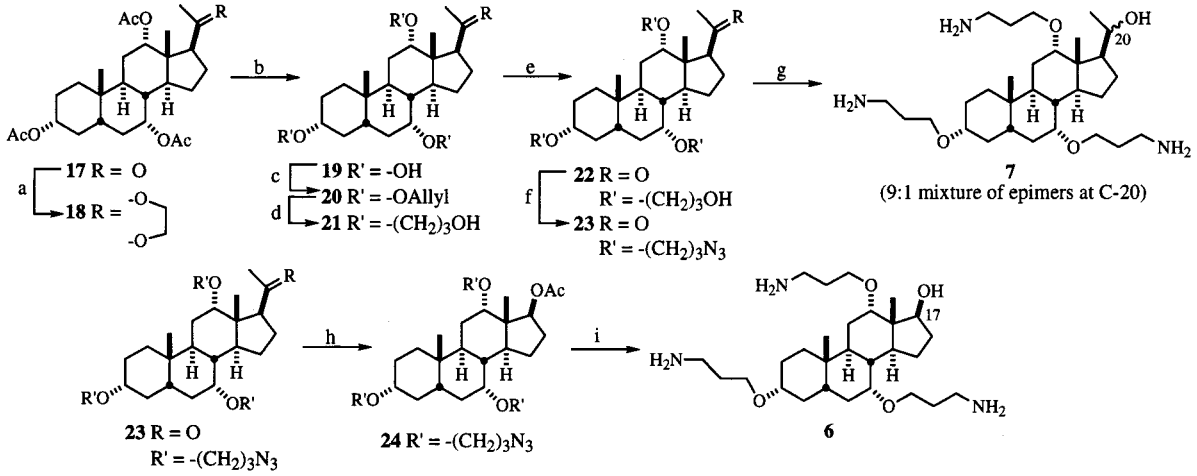
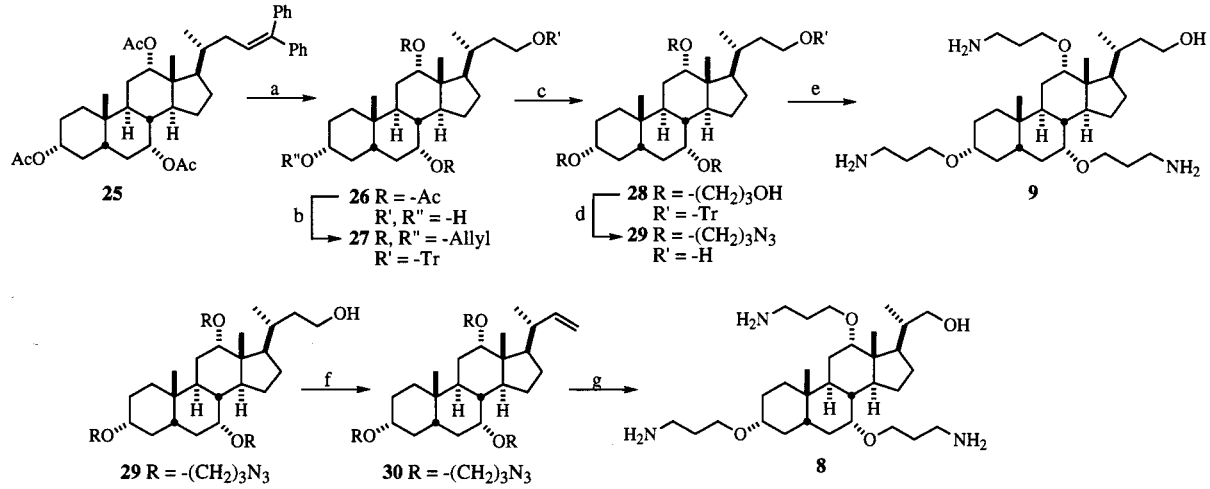
Scheme 2



Reagents (reaction yields in parentheses): a) ethylene glycol, TsOH, benzene (~100%). b) NaOH, MeOH (96%). c) allyl bromide, NaH, THF (90%). d) 9-BBN, THF; H_2O_2, NaOH (55%). e) PPTS, acetone, H_2O (98%). f) MsCl, Et_3N, CH_2Cl_2; NaN_3, DMSO (88%). g) $LiAlH_4$, THF (69%). h) urea-hydrogen peroxide complex, trifluoroacetic anhydride, CH_2Cl_2 (55%). i) NaOH, MeOH; $LiAlH_4$, THF (43%).

Scheme 3



Reagents (reaction yields in parentheses): a) O_3, CH_2Cl_2, MeOH; Me_2S; $NaBH_4$ (76%). b) NaOH, MeOH; TrCl, Et_3N, DMAP, DMF; allylbromide, NaH, THF (64%). c) 9-BBN, THF; H_2O_2, NaOH (93%). d) MsCl, Et_3N, CH_2Cl_2; NaN_3, DMSO; TsOH, MeOH, CH_2Cl_2 (94%), e) $LiAlH_4$, THF (71%). f) *o*-$NO_2C_6H_4SeCN$, Bu_3P, THF; H_2O_2. (36%). g) O_3, CH_2Cl_2, MeOH; Me_2S; $LiAlH_4$, THF (68%).

installation of the propylene-linked azides, giving **23**. Reduction of **23** provided **7** as a 9:1 mixture of epimers at C-20. Compound **7** was tested as the mixture. Truncation to C-17 yielding **6** was achieved using a Baeyer−Villiger oxidation of ketone **23**, followed by deprotection and reduction. Preparation of **9** (Scheme 3) employed ozonolysis of **25** and reduction to give alcohol **26**. After hydrolysis of the esters in **26**, the pathway used to prepare **10**[11] was followed to give **9**. The side chain of **29** was also shortened by a carbon, via ozonolysis of alkene **30**, to give **8** after reduction.

The compounds in the series **6**−**14** were tested for the ability both to act as antibiotics alone and to permeabilize the outer membrane of Gram-negative bacteria, causing sensitization to hydrophobic antibiotics that ineffectively cross the outer membrane. We anticipated that variations in the length of the steroid side chain would have a direct effect on antibiotic behavior and that this effect would differ from that observed on the permeabilization behavior.

We focused our studies on *Escherichia coli* K-12 strain ATCC 10798; however, to demonstrate that activity of the cholic acid derivatives was not species dependent, we also measured the activity of selected compounds with *Pseudomonas aeruginosa* (ATCC 27853). To characterize the activity of the cholic acid derivatives, we measured minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values. To measure permeabilization of the outer membrane of the bacteria, we used erythromycin and novobiocin. These antibiotics are very active against Gram-positive bacteria,[14] but, due to the permeability barrier of the outer membrane, are much less active against Gram-negative organisms. We measured the MICs of erythromycin and novobiocin against *E. coli* (ATCC 10798) as 70 and >500 μg/mL, respectively. Our threshold measure of permeabilization was the concentration of the cholic acid derivatives required to lower the MIC of either erythromycin or novobiocin to 1 μg/mL.[15]

Results of the MIC, MBC, and permeabilization (with erythromycin) measurements are plotted in Figure 4. Note that the MIC and MBC values of the compounds dropped dramatically as the steroid side chain length increased. In contrast, the

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(14) Conte, J. E., Jr. *Manual of Antibiotics and Infectious Diseases*; Williams & Wilkins: Philadelphia, 1995.

(15) Many clinically useful antibiotics against Gram-negative bacteria have MIC values of ca.1 μg/mL (see ref 14).

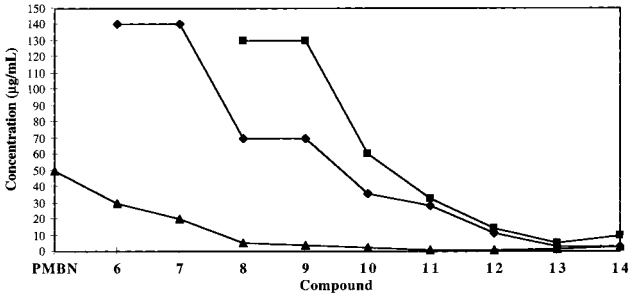

Figure 4. Compound number in order of increasing steroid side chain length vs (▲) concentration required to lower the MIC of erythromycin from 70 to 1 μg/mL (note that PMBN (**4**) is included), (◆) MIC, (■) MBC with *E. coli* (10798).

concentrations required to permeabilize the outer membrane reached a near minimum with shorter side chain lengths than are required for minimum MIC or MBC values. The data describing permeability include a measurement using PMBN.[16]

Permeabilization of the outer membrane of Gram-negative bacteria alone does not cause cell death,[17] suggesting that the cholic acid derivatives must pass through the outer membrane to kill the bacteria. Because longer side chains result in lower MIC and MBC values, the apparent role of the hydrophobic steroid side chain is to facilitate membrane insertion and self-promoted transport after initial association with the outer membrane (Figure 5). This mechanism of action is similar to a model proposed for the activity of PMB and derivatives:[3a,18] binding to the outer membrane increases permeability while self-promoted transport, requiring a hydrophobic side chain, is required for antibacterial action.

In our results, we found a limited correlation between side chain length and permeabilization; i.e., some side chain is required for maximal permeabilization as seen in the difference in activity of compounds **6**−**8**. We propose that the side chains in compounds such as **8**−**11** initiate limited membrane insertion, and that membrane insertion yields greater membrane permeability than association with LPS on the cell surface alone (see Figure 5). A similar effect is observed with PMB: in studies with reconstituted outer membranes of Gram-negative bacteria, Seydel and co-workers[19] found that PMB, which is capable of self-promoted transport, transiently increased the permeability of the membrane to ions, whereas PMBN caused no increase in ion permeability.

To quantify the synergistic behavior of our compounds with erythromycin and novobiocin, fractional inhibition concentration (FIC) values were calculated.[20] An FIC value is a standard measure of the ability of two antibiotics to inhibit bacterial growth synergistically and is defined as FIC = [A]/MIC_A + [B]/MIC_B where [A] and [B] are the concentrations of compounds A and B that in combination inhibit bacterial growth and MIC_A and MIC_B are the MICs of compounds A and B, respectively. Synergism between antibiotics is indicated by FIC values of less than 0.5. With the exception of **14**, the compounds display FIC values of less than 0.5 with erythromycin, with some values near 0.05 (Table 1).

Table 1. MIC, MBC, Permeabilization, and FIC Data for **6**−**14** with *E. Coli* (ATCC 10798)

compd	MIC (μg/mL)	MBC (μg/mL)	*a* (μg/mL)	*b* (μg/mL)	FIC[c]	*d* (μg/mL)	FIC[e]
6	140	>200	30	160	0.23	60	0.43
7	140	>160	20	180	0.16	40	0.29
8	70	130	5.0	140	0.086	14	0.20
9	70	130	4.0	80	0.071	12	0.17
10	36	60	2.0	50	0.070	4.0	0.11
11	28	33	1.0	25	0.050	3.0	0.11
12	11	14	0.6	4.0	0.069	1.0	0.093
13	3.0	5.0	1.5	2.5	0.51	0.8	0.27
14	3.0	10	3.0	3.0	1.0	nd[f]	nd[f]

[a] Concentration required to lower the MIC of erythromycin from 70 to 1 μg/mL. [b]MBC with 1 μg/mL erythromycin. [c]FIC values with erythromycin. [d]Concentration required to lower the MIC of novobiocin from >500 to 1 μg/mL. [e]FIC values with novobiocin. [f]nd = not determined.

Table 2. MIC, Permeabilization, and FIC Data for **6**, **8**, **11**, and **13** with *P. Aeruginosa* (ATCC 27853)

compd	MIC (μg/mL)	a	FIC[b]
6	150	125	0.83
8	70	13	0.19
11	10	2.5	0.25
13	2.0	2.0	1.0

[a] Concentration required to lower the MIC of novobiocin from 70 to 1 μg/mL. [b]FIC values with novobiocin.

Details from studies with novobiocin are also shown in Table 1. The fact that results with erythromycin and novobiocin were comparable demonstrates that the activity of the cholic acid derivatives is not antibiotic dependent. Suggesting that the activity is not species dependent, similar trends were observed with *E. coli* (ATCC 10798) and *P. aeruginosa* (ATCC 27853), although, as expected,[21] *P. aeruginosa* proved to be more resistant than *E. coli* (Table 2).

Though the cholic acid derivatives were designed to mimic the behavior of PMB and derivatives, they share some structural features with squalamine (**31**; Figure 6), an antibiotic isolated from sharks.[22] However, the ionic topology of squalamine differs markedly from the cholic acid derivatives we prepared; under physiological conditions squalamine offers termini of opposite charges with a hydrophobic core, while the cholic acid derivatives are facially amphiphilic. Nevertheless, a proposed mechanism of action for squalamine involves membrane disruption, and a proposed active conformation of squalamine and mimics has an intramolecular salt bridge between the terminal amine of the spermidine group and the sulfate at C-24.[23] This salt bridge results in positioning of amines above a face of the steroid. This conformation minimally resembles the arrangement of amines found in the cholic acid derivatives; however, the number and positioning of the amines are different in the two types of steroid derivatives. To observe whether including a sulfate at C-24 in our cholic acid derivatives would increase the activity of the compounds, we prepared **32**. Treatment of **15** with SO_3−pyridine complex and reduction of the azides with triphenylphosphine gave **32** in 66% yield. The MIC of **32** with *E. coli* (ATCC 10798) was measured as 60 μg/mL. The

(16) Obtained from Boehringer Mannheim as 95% pure PMBN and used as received.

(17) Permeabilization is classified as a "sublethal action"; see ref 3a. DAPB (**3**), PMBN (**4**), PMBH (**5**), and **6**−**11** can cause increases in permeability at concentrations much lower than their corresponding MBC values.

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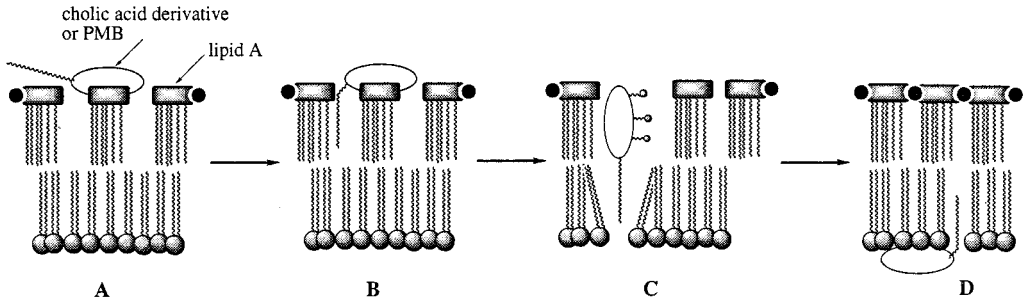

Figure 5. Proposed mechanism for action of cholic acid derivatives and PMB (see refs 2a and 16). (A) Association of cholic acid derivatives or PMB with LPS disrupts the lipid A cross bridging and increases the permeability of the membrane. (B) A hydrophobic chain (if present) inserts into the membrane, facilitating incorporation of the remainder of the molecule into the membrane. (C) Insertion of the molecule into the membrane further increases the permeability of the membrane and allows self-promoted transport. **(**D) As the compound passes through the outer membrane, it gains access to the cytoplasmic membrane.

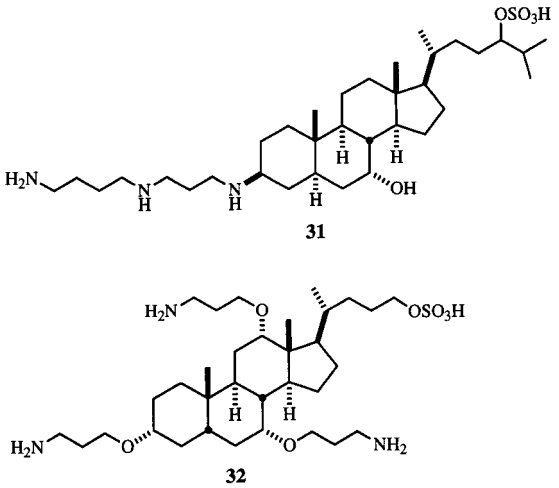

Figure 6. Squalamine (**31**) and **32**.

concentration required to lower the MIC of erythromycin to 1 μg/mL was 4.0 μg/mL with the same strain. As compared to the parent alcohol (**10**), the antibiotic and permeabilization activities of **32** are decreased by addition of a sulfate at C-24.

To develop more potent antibiotics, we noted that PMB contains amines in the exocyclic chain, and therefore, we included an amine in the side chain of two cholic acid derivatives: one with primary amines (**33**) and the other with guanidine groups (**34**) (Figure 7). As observed earlier,[11] incorporation of guanidine groups increases the activity of the cholic acid derivatives as compared to compounds containing primary amines. As a control, we prepared **35**, lacking a hydrophobic side chain. The three compounds were prepared from **15**, via mesylation of the alcohol followed by reaction with octylamine and reduction to give **33** in 65% yield from the mesylate. Compound **33** was reacted with aminoiminomethanesulfonic acid to give **34** in 91% yield. Reaction of the mesylate of **15** with sodium azide and reduction gave **35** in 61% yield.

The MIC of the control (**35**) was relatively high, as expected, as was the MBC (Figure 8). In contrast, the MICs of **33** and **34** were very low; in fact they rival PMB in activity. Notably, the MBCs of **33**, **34**, and PMB were very similar to the MICs; that is, at a threshold concentration these compounds kill all of the bacteria in solution. Through a range of concentrations (1−100 μg/mL), we found no evidence of aggregation with **33** or **34**. These results are consistent with the model proposed for activity in Figure 5: a hydrophobic chain is necessary for self-promoted transport, and the compounds must traverse the outer membrane to effect cell death.

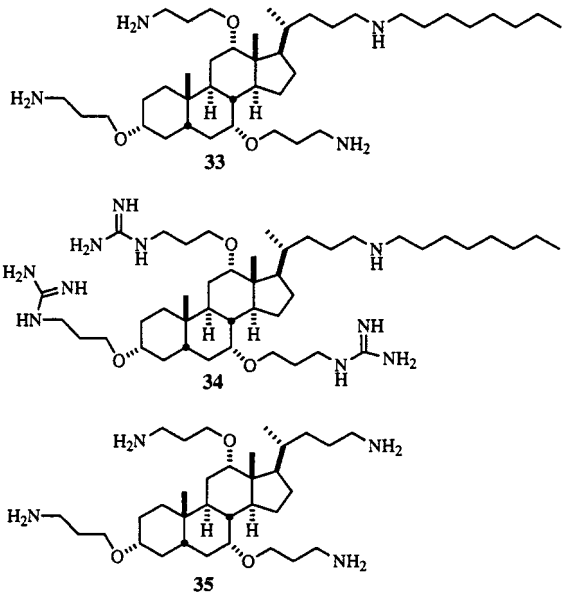

Figure 7. Compounds **33**−**35**.

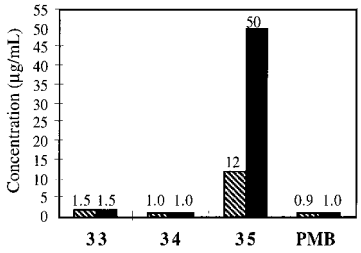

Figure 8. MIC (hatched bars) and MBC (solid bars) values for **33**−**35** and PMB (**2**) measured with *E. coli* (ATCC 10798).

As an additional means of demonstrating the membrane-disrupting capabilities of the cholic acid derivatives, we used a luciferin/luciferase-based cell lysis assay.[24] In this assay *E. coli*, containing an inducible luciferase coding plasmid, was incubated with the inducing agent (toluene) and then treated with a lysis buffer containing either PMB or one of the cholic acid derivatives and Triton X-100, followed by addition of luciferin and ATP. Cell lysis resulted in luminescence. The concentrations of the membrane-disrupting agents (PMB and the cholic acid derivatives) were varied, and the resulting luminescence was measured. In the absence of the membrane-disrupting agents

(24) (a) Willardson, B. M.; Wilkins, J. F.; Rand, T. A.; Schupp, J. M.; Hill, K. K.; Keim, P.; Jackson, P. J. *Appl. Environ. Microbiol.* **1998**, *64*, 1006. (b) Schupp, J. M.; Travis, S. E.; Price, L. B.; Shand, R. F.; Keim, P. *Biotechniques* **1995**, 19, *18.*

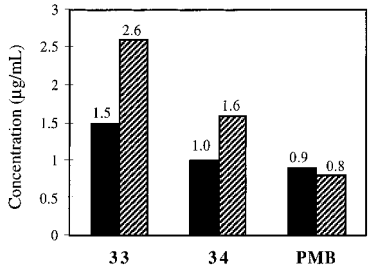

Figure 9. MIC values (solid bars) with *E. coli* (ATCC 10798) and concentrations required for half-maximal luminescence (hatched bars) (see the text) for **33**, **34**, and PMB (**2**).

no luminescence was observed. In Figure 9, comparison is made of the MICs of **33**, **34**, and PMB as compared to the concentrations required for half-maximal luminescence (luminescence maxima were similar for all compounds tested). The luminescence data correlate better with MIC values than with permeabilization of the outer membrane as measured with erythromycin and novobiocin. For example, the concentration required for half-maximal luminescence of **7** (115 μg/mL) is comparable to its MIC with *E. coli* (10798) (140 μg/mL), while the concentration at which **7** lowered the MIC of erythromycin to 1 μg/mL (20 μg/mL) is much lower. Notably, the concentration at which PMBN caused half-maximal luminescence was >100 μg/mL. In the luminescence assay as in MIC values, compounds **33** and **34** rival PMB in activity.

Conclusions

We have demonstrated that by appropriately arranging amine or guanidine groups on a steroid nucleus, it is possible to prepare compounds that demonstrate activities similar to those of PMB derivatives. Furthermore, by including elements found in the exocyclic chain of PMB in the cholic acid derivatives, we have incrementally converted membrane permeabilizers into potent antibiotics. This process has demonstrated the role of a hydrophobic chain in initiating self-promoted transport through the outer membrane of Gram-negative bacteria. Compounds lacking the chain permeabilize the outer membrane, but are ineffective in killing the cell. A sufficient hydrophobic chain facilitates traversal of the outer membrane, leading to effective bactericidal activity.

Effects of the cholic acid derivatives on Gram-positive bacteria and on mammalian cells are being determined and will be communicated in due course. Additionally, the ability of the cholic acid derivatives to inhibit the effects of LPS on human monocytes is being actively investigated.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR 500 (500 MHz) or Varian Unity 300 (300 MHz) spectrometer and are referenced to TMS, residual C*H*Cl$_3$ (^{1}H), residual C*H*D$_2$OD (^{1}H), *C*DCl$_3$ (^{13}C), or *C*D$_3$OD (^{13}C). IR spectra were recorded on a Perkin-Elmer 1600 FTIR instrument. Mass spectrometric data were obtained on a JEOL SX 102A spectrometer. THF was dried over Na°/benzophenone, and CH_2Cl_2 was dried over CaH_2 prior to use. Other reagents and solvents were obtained commercially and used as received unless otherwise noted.

16a−d. Representative Procedure. **Preparation of 16b**. (Compound **16d** was reported in ref 9.) NaH (0.06 g, 60% in mineral oil, 1.49 mmol) and propyl bromide (0.136 mL, 1.49 mmol) were added to a DMF solution of compound **15**[11] (0.096 g, 0.149 mmol). The suspension was stirred under N_2 for 24 h. H_2O (20 mL) was added, and the mixture was extracted with hexanes (3 × 10 mL). The combined extracts were dried over Na_2SO_4 and concentrated in vacuo. Silica gel chromatography (10% EtOAc in hexanes) afforded the desired product (92 mg, 90% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.68−3.64 (m, 1 H), 3.61−3.57 (m, 1 H), 3.52 (t, J = 6.1 Hz, 2 H), 3.49 (br s, 1 H), 3.46−3.35 (m, 10 H), 3.25 (d, J = 2.4 Hz, 1 H), 3.23−3.19 (m, 1 H), 3.16−3.11 (m, 1 H), 3.09−3.03 (m, 1 H), 2.17−2.03 (m, 3 H), 1.95−1.55 (m, 17 H), 1.51−1.40 (m, 4 H), 1.38−1.17 (m, 5 H), 1.11−0.96 (m, 3 H), 0.93−0.89 (m, 9 H), 0.65 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 80.64, 79.79, 76.08, 72.67, 71.59, 65.01, 64.44, 64.33, 49.04, 48.94, 48.75, 46.61, 46.40, 42.68, 42.00, 39.83, 35.72, 35.45, 35.30, 35.10, 32.38, 29.81, 29.77, 29.72, 29.09, 27.88, 27.76, 27.65, 26.52, 23.55, 23.12, 23.04, 22.87, 18.06, 12.60, 10.79; HRFAB-MS (thioglycerol + Na^+ matrix) m/e ([M + Na]$^+$) 708.4910 (23.5%), calcd 708.4920.

11−13. Representative Procedure. Preparation of 12. (Compound **14** was reported in ref 11.) Compound **16b** (0.092 g, 0.134 mmol) was dissolved in THF (10 mL) followed by the addition of $LiAlH_4$ (0.031 g, 0.81 mmol). The suspension was stirred under N_2 for 12 h. $Na_2SO_4 \cdot 10H_2O$ (~1 g) was then carefully added. After the gray color in the suspension dissipated, anhydrous Na_2SO_4 was added, and the precipitate was removed by filtration. Concentration and silica gel chromatography (CH_2Cl_2/MeOH/28% $NH_3 \cdot H_2O$, 12:6:1 and then 10:5:1) yielded a glass which was dissolved in 1 M HCl (2 mL). The resulting clear solution was washed with Et_2O (2 × 10 mL). A 20% NaOH solution was added to the aqueous phase until the solution became strongly basic. CH_2Cl_2 (3 × 10 mL) was used to extract the basic solution. The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the desired product (0.045 g, 55% yield) as a white glass. Data for **12:** ^{1}H NMR (~20% $CDCl_3$ in CD_3OD, 500 MHz) δ 4.73 (br s, 6 H), 3.74−3.70 (m, 1 H), 3.65−3.61 (m, 1 H), 3.55 (t, J = 6.3 Hz, 2 H), 3.42−3.38 (m, 4 H), 3.33−3.30 (m, 2 H), 3.16−3.10 (m, 2 H), 2.83−2.73 (m, 6 H), 2.18−2.06 (m, 3 H), 1.96−1.20 (series of multiplets, 26 H), 1.12−0.98 (m, 3 H), 0.95−0.92 (m, 9 H), 0.70 (s, 3 H); ^{13}C NMR (~20% $CDCl_3$ in CD_3OD, 75 MHz) δ 81.67, 80.49, 77.04, 73.44, 72.28, 67.77, 67.71, 67.06, 47.74, 47.08, 43.75, 42.82, 41.21, 40.60, 40.56, 40.12, 36.47, 36.19, 36.04, 35.74, 34.09, 33.82, 33.78, 33.16, 29.49, 28.87, 28.43, 27.18, 24.22, 23.66, 23.49, 23.40, 18.64, 13.04, 11.03; HRFAB-MS (thioglycerol + Na^+ matrix) m/e ([M + H]$^+$) 608.5348 (100%), calcd 608.5330. Data for **11:** ^{1}H NMR (~20% $CDCl_3$ in CD_3OD, 500 MHz) δ 4.79 (br s, 6H), 3.74−3.71 (m, 1 H), 3.66−3.62 (m, 1 H), 3.55 (t, J = 6.1 Hz, 2 H), 3.52 (br s, 1 H), 3.38−3.28 (series of multiplets, 4 H), 3.33 (s, 3 H), 3.16−3.10 (m, 2H), 2.83−2.72 (m, 6 H), 2.19−2.07 (m, 3 H), 1.97−1.62 (series of multiplets, 15 H), 1.58−1.20 (series of multiplets, 9 H), 1.13−0.98 (m, 3 H), 0.95 (d, J = 6.3 Hz, 3 H), 0.93 (s, 3 H), 0.70 (s, 3 H); ^{13}C NMR (~20% $CDCl_3$ in CD_3OD, 75 MHz) δ 81.82, 80.65, 77.20, 74.43, 67.85, 67.18, 58.90, 47.80, 47.22, 43.91, 43.01, 41.31, 40.78, 40.69, 40.22, 36.63, 36.35, 36.18, 35.86, 34.27, 33.97, 33.26, 29.60, 29.03, 28.58, 28.53, 27.14, 24.33, 23.61, 23.45, 18.68, 13.06; HRFAB-MS (thioglycerol + Na^+ matrix) m/e ([M + Na]$^+$) 602.4855 (100%), calcd 602.4873. Data for **13:** ^{1}H NMR (~50% $CDCl_3$ in CD_3OD, 500 MHz) δ 4.08 (br s, 6 H), 3.71−3.67 (m, 1 H), 3.62−3.58 (m, 1 H), 3.53 (t, J = 6.3 Hz, 2 H), 3.49 (br s, 1 H), 3.43−3.38 (m, 4 H), 3.31−3.27 (m, 2 H), 3.14−3.07 (m, 2 H), 2.83−2.73 (m, 6 H), 2.16−2.03 (m, 3 H), 1.93−1.17 (series of multiplets, 30 H), 1.10−0.96 (m, 3 H), 0.93−0.89 (m, 9 H), 0.67 (s, 3 H); ^{13}C NMR (~50% $CDCl_3$ in CD_3OD, 75 MHz) δ 80.51, 79.35, 75.85, 71.29, 70.83, 66.73, 66.62, 65.96, 46.68, 45.98, 42.59, 41.63, 40.20, 39.53, 39.43, 39.21, 35.34, 35.04, 35.00, 34.71, 33.11, 32.90, 32.82, 32.00, 29.15, 28.49, 28.15, 27.75, 27.35, 26.22, 23.18, 22.60, 22.45, 22.34, 17.77, 13.75, 12.22; HRFAB-MS (thioglycerol + Na^+ matrix) m/e ([M + H]$^+$) 636.5679 (100%), calcd 636.5669.

18. A mixture of **17**[12] (1.00 g, 2.10 mmol), ethylene glycol (3.52 mL, 63 mmol), and *p*-TsOH (20 mg, 0.105 mmol) was refluxed in benzene under N_2 for 16 h. Water formed during the reaction was removed by a Dean−Stark moisture trap. The cooled mixture was washed with saturated aqueous $NaHCO_3$ (50 mL), and the aqueous phase was extracted with Et_2O (50 mL, 2 × 30 mL). The combined extracts were washed with brine (75 mL) and dried over anhydrous Na_2SO_4. Removal of the solvent in vacuo gave the desired product (1.09 g, 100%) as a glass: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.10 (t, J = 2.7 Hz, 1 H), 4.92 (d, J = 2.7 Hz, 1 H), 4.63−4.52 (m, 1 H), 3.98−

3.80 (m, 4 H), 2.32 (t, $J = 9.5$ Hz, 1 H), 2.13 (s, 3 H), 2.08 (s, 3 H), 2.05 (s, 3 H), 2.00−1.40 (series of multiplets, 15 H), 1.34−0.98 (m, 3 H), 1.20 (s, 3 H), 0.92 (s, 3 H), 0.82 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.69, 170.63, 170.47, 111.38, 75.07, 74.23, 70.85, 64.95, 63.43, 49.85, 44.73, 43.39, 41.11, 37.37, 34.84, 34.80, 34.52, 31.42, 29.18, 27.02, 25.41, 24.16, 22.72, 22.57, 22.44, 21.73, 21.63, 13.40; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ($[M + H]^+$) 521.3106 (38.6%), calcd 521.3114.

19. Compound **18** (1.09 g, 2.10 mmol) was dissolved in MeOH (50 mL). NaOH (0.84 g, 21 mmol) was added to the solution. The suspension was refluxed under N_2 for 24 h. MeOH was removed in vacuo, and the residue was dissolved in Et_2O (100 mL), washed with H_2O (100 mL) and brine (100 mL), and dried over anhydrous Na_2SO_4. The desired product (0.80 g, 96% yield) was obtained as white solid after removal of solvent in vacuo: mp 199−200 °C; ^{1}H NMR (10% CD_3OD in $CDCl_3$, 300 MHz) δ 4.08−3.83 (series of multiplets, 9 H), 3.44−3.34 (m, 1 H), 2.41 (t, $J = 9.3$ Hz, 1 H), 2.22−2.10 (m, 2 H), 1.96−1.50 (series of multiplets, 12 H), 1.45−0.96 (series of multiplets, 4 H), 1.32 (s, 3 H), 0.89 (s, 3 H), 0.78 (s, 3 H); ^{13}C NMR (10% CD_3OD in $CDCl_3$, 75 MHz) δ 112.11, 72.35, 71.57, 68.09, 64.54, 63.24, 49.36, 45.90, 41.48, 41.45, 39.18, 38.79, 35.29, 34.71, 34.45, 29.90, 27.26, 26.60, 23.65, 22.54, 22.44, 22.35, 13.46; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ($[M + Na]^+$) 417.2622 (87.3%), calcd 417.2617.

20. To a round-bottom flask were added **19** (0.80 g, 2.03 mmol) and dry THF (100 mL) followed by NaH (60% in mineral oil, 0.81 g, 20.3 mmol). The suspension was refluxed under N_2 for 30 min, and allyl bromide (1.75 mL, 20.3 mmol) was added. After 48 h at reflux, another 10 equiv of NaH and allyl bromide were added. After an additional 48 h, cold water (50 mL) was added to the cooled suspension. The resulting mixture was extracted with Et_2O (60 mL, 2 × 30 mL). The combined extracts were washed with brine (100 mL) and dried over anhydrous Na_2SO_4. Silica gel chromatography (6% EtOAc in hexanes) gave the desired product (0.94 g, 90% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.02−5.84 (m, 3 H), 5.31−5.04 (m, 6 H), 4.12−4.05 (m, 2 H), 4.01−3.81 (m, 7 H), 3.70 (dd, $J =$ 12.9, 5.6 Hz, 1 H), 3.55 (t, $J = 2.6$ Hz, 1 H), 3.33 (d, $J = 2.9$ Hz, 1 H), 3.18−3.08 (m, 1 H), 2.65 (t, $J = 10.0$ Hz, 1 H), 2.32−2.14 (m, 3 H), 1.84−1.45 (series of multiplets, 10 H), 1.41−1.22 (m, 3 H), 1.27 (s, 3 H), 1.14−0.92 (m, 2 H), 0.89 (s, 3 H), 0.75 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.38, 136.07, 136.00, 116.31, 115.54, 115.38, 112.34, 80.07, 79.22, 75.05, 69.83, 69.34, 68.82, 65.14, 63.24, 48.80, 45.96, 42.47, 42.15, 39.40, 35.55, 35.16, 35.15, 29.04, 28.22, 27.52, 24.21, 23.38, 23.11, 22.95, 22.58, 13.79; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ($[M + Na]^+$) 537.3549 (100%), calcd 537.3556.

21. To a solution of **20** (0.94 g, 1.83 mmol) in THF (50 mL) was added 9-BBN (0.5 M solution in THF, 14.7 mL, 7.34 mmol). The mixture was stirred under N_2 for 12 h followed by the addition of 20% NaOH solution (4 mL) and 30% H_2O_2 solution (4 mL). The resulting mixture was refluxed for 1 h followed by the addition of brine (100 mL). The mixture was extracted with EtOAc (4 × 30 mL), and the combined extracts were dried over anhydrous Na_2SO_4. After the removal of solvent in vacuo, the residue was purified by SiO_2 column chromatography (EtOAc followed by 10% MeOH in CH_2Cl_2) to give the product (0.559 g, 54% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.02−3.52 (series of multiplets, 17 H), 3.41−3.35 (m, 1 H), 3.29 (d, $J = 2.4$ Hz, 1 H), 3.22−3.15 (m, 3 H), 2.58 (t, $J = 10.0$ Hz, 1 H), 2.27−1.95 (m, 3 H), 1.83−1.48 (series of multiplets, 16 H), 1.40−0.93 (series of multiplets, 5 H), 1.27 (s, 3 H), 0.90 (s, 3 H), 0.75 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 112.41, 80.09, 79.09, 76.31, 66.70, 66.02, 65.93, 64.80, 63.26, 61.53, 61.25, 60.86, 48.59, 45.80, 42.51, 41.72, 39.10, 35.36, 35.02, 34.98, 32.87, 32.52, 32.40, 28.88, 27.94, 27.21, 24.33, 23.02, 22.84 (2 C's), 22.44, 13.69; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ($[M + Na]^+$) 591.3881 (100%), calcd 591.3873.

22. To a solution of **21** (0.559 g, 0.98 mmol) in acetone (40 mL) and water (4 mL) was added PPTS (0.124 g, 0.49 mmol), and the solution was refluxed under N_2 for 16 h. The solvent was removed in vacuo. Water (40 mL) was added to the residue, and the mixture was extracted with EtOAc (40 mL, 2 × 20 mL). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. SiO_2 column chromatography (8% MeOH in CH_2Cl_2) afforded the desired product (0.509 g, 98% yield) as a clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.83−3.72 (m, 8 H), 3.66 (t, $J = 5.6$ Hz, 2 H), 3.54 (br s, 2 H), 3.43−3.28 (m, 4 H), 3.24−3.12 (m, 2 H), 2.26−2.00 (m, 4 H), 2.08 (s, 3 H), 1.98−1.50 (series of multiplets, 15 H), 1.42−0.96 (series of multiplets, 6 H), 0.90 (s, 3 H), 0.62 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 210.49, 78.87, 76.30, 66.86, 66.18, 65.69, 61.74, 61.43, 60.71, 55.31, 48.05, 43.02, 41.58, 39.53, 35.28, 35.09, 34.96, 32.77, 32.70, 32.31, 31.12, 28.72, 27.88, 27.14, 23.47, 22.75, 22.47, 22.34, 13.86; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ($[M + Na]^+$) 547.3624 (100%), calcd 547.3611.

23. Et_3N (0.168 mL, 1.20 mmol) was added to the solution of **22** (0.18 g, 0.344 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C followed by the addition of mesyl chloride (0.088 mL, 1.13 mmol). After 10 min, H_2O (3 mL) and brine (30 mL) were added. The mixture was extracted with EtOAc (30 mL, 2 × 10 mL), and the extracts were washed with brine (50 mL) and dried over anhydrous Na_2SO_4. After removal of solvent, the residue was dissolved in DMSO (5 mL), and NaN_3 (0.233 g, 3.44 mmol) was added. The suspension was heated to 50 °C under N_2 for 12 h. H_2O (50 mL) was added to the cooled suspension. The mixture was extracted with EtOAc (30 mL, 2 × 10 mL), and the extracts were washed with brine (50 mL) and dried over anhydrous Na_2SO_4. SiO_2 column chromatography (EtOAc/hexanes, 1:5) afforded the product (0.191 g, 88% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.72−3.64 (m, 2 H), 3.55−3.24 (series of multiplets, 11 H), 3.18−3.02 (m, 2 H), 2.22−2.02 (m, 4 H), 2.08 (s, 3 H), 1.95−1.46 (series of multiplets, 15 H), 1.38−0.96 (series of multiplets, 6 H), 0.89 (s, 3 H), 0.62 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 210.36, 79.69, 79.22, 75.98, 65.08, 64.80, 64.53, 55.31, 48.93, 48.86, 48.76, 48.06, 43.03, 41.91, 39.66, 35.44, 35.31, 35.12, 31.04, 29.77, 29.69, 29.67, 28.99, 28.10, 27.65, 23.60, 22.99, 22.95, 22.50, 14.00; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ($[M + Na]^+$) 622.3820 (100%), calcd 622.3805.

7. Compound **23** (0.191 g, 0.319 mmol) was dissolved in THF (20 mL) followed by the addition of $LiAlH_4$ (60.4 mg, 1.59 mmol). The gray suspension was stirred under N_2 for 12 h. $Na_2SO_4 \cdot 10\ H_2O$ (~1 g) powder was carefully added. After the gray color in the suspension disappeared, anhydrous Na_2SO_4 was added, and the precipitate was removed by filtration. After the removal of solvent in vacuo, the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2/28% $NH_3 \cdot H_2O$, 3:3:1). The relevant fractions were collected, and 1 M HCl (2 mL) was added to dissolve the residue. The resulting clear solution was washed with Et_2O (2 × 10 mL). To the aqueous phase was added 20% NaOH solution until the solution became strongly basic. The solution was extracted with CH_2Cl_2 (20 mL, 2 × 10 mL), and the combined extracts were dried over anhydrous Na_2SO_4. Removal of solvent in vacuo gave the desired product (0.115 g, 69% yield) as a colorless oil. From ^{1}H NMR, it is apparent that the product is a mixture of two stereoisomers at C_{20} in a ratio of 9:1. No attempt was made to separate the stereoisomers; they were tested as a mixture: ^{1}H NMR (20% $CDCl_3$ in CD_3OD, 300 MHz) δ 4.69 (br s, 7 H), 3.76−3.69 (m, 1 H), 3.63−3.53 (m, 5 H), 3.50−3.40 (m, 1 H), 3.29 (br s, 1 H), 3.18−3.07 (m, 2 H), 2.94−2.83 (m, 1 H), 2.81−2.66 (m, 5 H), 2.23−2.06 (m, 4 H), 1.87−1.50 (series of multiplets, 15 H), 1.39−0.96 (series of multiplets, 6 H), 1.11 (d, $J = 6.1$ Hz, 3 H), 0.93 (s, 3 H), 0.75 (s, 3 H); ^{13}C NMR (20% $CDCl_3$ in CD_3OD, 75 MHz) δ 81.46, 80.67, 77.32, 70.68, 67.90, 67.66, 67.18, 50.32, 47.17, 43.30, 43.06, 40.74, 40.64, 40.38, 40.26, 36.31, 36.28, 35.93, 34.30, 34.02, 33.29, 29.63, 29.31, 28.43, 26.10, 24.67, 24.09, 23.96, 23.50, 13.30 for the major isomer; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ($[M + H]^+$) 524.4431 (64.2%), calcd 524.4427.

24. Compound **23** (0.256 g, 0.489 mmol) was dissolved in CH_2Cl_2 (10 mL), and cooled to 0 °C followed by the addition of Na_2HPO_4 (0.69 g, 4.89 mmol) and urea−hydrogen peroxide complex (UHP) (0.069 g, 0.733 mmol). Trifluoroacetic anhydride (TFAA) (0.138 mL, 0.977 mmol) was then added dropwise. The suspension was stirred for 12 h, and additional UHP (23 mg, 0.25 mmol) and TFAA (0.069 mL, 0.49 mmol) were added. After another 12 h, H_2O (30 mL) was added, and the resulting mixture was extracted with EtOAc (3 × 20 mL). The combined extracts were washed with brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. SiO_2 chromatography (EtOAc/hexanes, 1:5) afforded the desired product (0.145 g, 55% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.21 (dd, $J = 9.3$

and 7.3 Hz, 1 H), 3.70−3.57 (m, 2 H), 3.55 (t, J = 6.0 Hz, 2 H), 3.43−3.37 (m, 6 H), 3.32−3.25 (m, 3 H), 3.17−3.02 (m, 2 H), 2.28−2.05 (m, 4 H), 2.03 (s, 3 H), 1.86−1.19 (series of multiplets, 19 H), 0.97 (dd, J = 14.5 and 3.3 Hz, 1 H), 0.90 (s, 3 H), 0.78 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.08, 79.71, 78.03, 75.72, 75.53, 65.41, 65.04, 64.53, 48.79, 48.70, 46.49, 41.92, 39.44, 37.81, 35.45, 35.22, 35.10, 29.73, 29.63, 28.89, 28.33, 27.50, 27.34, 23.39, 22.97, 22.92, 21.28, 12.72; HRFAB-MS (thioglycerol + Na^+ matrix) m/e ([M − H]$^+$) 614.3798 (24.5%), calcd 614.3778.

6. Compound **24** (0.145 g, 0.236 mmol) was dissolved in CH_2Cl_2 (2 mL) and MeOH (1 mL). A 20% NaOH solution (0.2 mL) was added. The mixture was stirred for 12 h, and anhydrous Na_2SO_4 was used to remove water. After concentration in vacuo, the residue was purified by silica gel chromatography (EtOAc/hexanes, 1:3) to afford the desired product (0.124 g, 92% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.29 (br s, 1 H), 3.69−3.60 (m, 2 H), 3.52 (t, J = 6.0 Hz, 2 H), 3.45−3.32 (m, 8 H), 3.26 (d, J = 2.7 Hz, 1 H), 3.17−3.02 (m, 2 H), 2.19−1.94 (m, 4 H), 1.90−1.62 (series of multiplets, 13 H), 1.57−1.20 (series of multiplets, 7 H), 0.97 (dd, J = 14.3 and 3.1 Hz, 1 H), 0.90 (s, 3 H), 0.73 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 79.69, 78.03, 75.47, 73.38, 65.46, 65.00, 64.47, 48.87, 48.68, 46.83, 41.93, 39.71, 37.87, 35.43, 35.20, 35.09, 29.96, 29.69, 29.59, 29.53, 28.89, 28.44, 27.48, 23.72, 22.91, 22.71, 11.77. The alcohol (0.124 g, 0.216 mmol) was dissolved in dry THF (20 mL) followed by the addition of $LiAlH_4$ (33 mg, 0.866 mmol). The gray suspension was stirred under N_2 for 12 h. $Na_2SO_4 \cdot 10H_2O$ (∼2 g) was carefully added. After the gray color in the suspension dissipated, anhydrous Na_2SO_4 was added, and the precipitate was removed by filtration. After the removal of solvent, the residue was purified by column chromatography (SiO_2, MeOH/CH_2Cl_2/28% $NH_3 \cdot H_2O$, 2.5:2.5:1). After concentration of the relevant fractions, 1 M HCl (2 mL) was added to dissolve the milky residue. The resulting clear solution was washed with Et_2O (2 × 10 mL). To the aqueous phase was added 20% NaOH solution until the solution became strongly basic. CH_2Cl_2 (20 mL, 2 × 10 mL) was used to extract the basic solution. The combined extracts were dried over anhydrous Na_2SO_4, and removal of solvent gave the desired product (0.050 g, 47% yield) as a colorless oil: ^{1}H NMR (20% $CDCl_3$ in CD_3OD, 300 MHz) δ 4.77 (s, 7 H), 4.25 (t, J = 8.5 Hz, 1 H), 3.75−3.68 (m, 1 H), 3.66−3.58 (m, 1 H), 3.55 (t, J = 6.1 Hz, 2 H), 3.48−3.41 (m, 1 H), 3.34 (br s, 1 H), 3.30 (d, J = 3.6 Hz, 1 H), 3.17−3.08 (m, 2 H), 2.86−2.70 (m, 6 H), 2.20−1.91 (m, 4 H), 1.88−1.16 (series of multiplets, 19 H), 1.00 (dd, J = 14.2 and 3.0 Hz, 1 H), 0.93 (s, 3 H), 0.73 (s, 3 H); ^{13}C NMR (20% $CDCl_3$ in CD_3OD, 75 MHz) δ 80.62, 79.12, 76.74, 73.77, 68.50, 67.79, 67.17, 47.69, 43.04, 40.76, 40.64, 40.62, 40.22, 39.01, 36.32, 36.25, 35.94, 34.27, 33.97, 33.72, 30.13, 29.53, 28.43, 24.48, 23.58, 23.40, 12.38; HRFAB-MS (thioglycerol + Na^+ matrix) m/e ([M + H]$^+$) 496.4108 (100%), calcd 496.4114.

26. Compound **25**[13] (2.30 g, 3.52 mmol) was dissolved in MeOH (50 mL) and CH_2Cl_2 (100 mL). A small amount of Et_3N was added, and the solution was cooled to −78 °C. Ozone was bubbled through the solution until a blue color persisted. Me_2S (4 mL) was introduced followed by the addition of $NaBH_4$ (0.266 g, 0.703 mmol) in MeOH (10 mL). The resulting solution was allowed to warm and stir overnight. The solution was concentrated in vacuo, and brine (60 mL) was added. The mixture was extracted with EtOAc (40 mL, 2 × 30 mL), and the combined extracts were washed with brine and dried over anhydrous Na_2SO_4. Silica gel chromatography (EtOAc) afforded the product (1.24 g, 76% yield) as a white solid: mp 219−220 ° C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.10 (t, J = 2.8 Hz, 1 H), 4.90 (d, J = 2.7 Hz, 1 H), 3.73−3.59 (m, 2 H), 3.56−3.44 (m, 1 H), 2.13 (s, 3 H), 2.09 (s, 3 H), 2.07−0.95 (series of multiplets, 23 H), 0.91 (s, 3 H), 0.83 (d, J = 6.3 Hz, 3 H), 0.74 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.84, 170.82, 75.63, 71.77, 71.03, 60.73, 48.10, 45.26, 43.54, 41.16, 38.78, 37.89, 35.00, 34.43, 32.26, 31.50, 30.60, 29.07, 27.50, 25.70, 22.96, 22.71, 21.81, 21.63, 18.18, 12.35; HRFAB-MS (thioglycerol + Na^+ matrix) m/e ([M + H]$^+$) 465.3197 (20%), calcd 465.3216.

27. Compound **26** (1.24 g, 2.67 mmol) was dissolved in MeOH (30 mL), and NaOH (0.54 g, 13.4 mmol) was added. The suspension was refluxed under N_2 for 24 h. The MeOH was removed in vacuo followed by the addition of H_2O (50 mL). The precipitate was filtered, washed with H_2O, and then dried in vacuo to give a white solid (1.02 g). This solid was dissolved in DMF (40 mL) followed by the sequential addition of NEt_3 (1.12 mL, 8.02 mmol), DMAP (16.3 mg, 0.13 mmol), and trityl chloride (1.49 g, 5.34 mmol). The suspension was stirred under N_2 for 12 h and then heated to 50 °C for 24 h. H_2O (100 mL) was added to the cooled suspension, and the mixture was extracted with EtOAc (3 × 50 mL). The combined extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Silica gel chromatography (EtOAc) afforded the product (1.20 g, 72% yield) as a pale yellow glass. To this glass were added dry THF (80 mL) and NaH (60% in mineral oil, 0.77 g, 19.3 mmol). The suspension was refluxed under N_2 for 1/2 h before the introduction of allyl bromide (1.67 mL, 19.3 mmol). After 48 h at reflux, another 10 equiv of NaH and allyl bromide were introduced. After another 48 h, the reaction mixture was cooled, and H_2O (100 mL) was slowly added. The resulting mixture was extracted with hexanes (3 × 50 mL), and the combined extracts were washed with brine (100 mL) and dried over anhydrous Na_2SO_4. Silica gel chromatography (5% EtOAc in hexanes) afforded the product (1.27 g, 64% yield for all three steps) as a clear glass: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46−7.43 (m, 6 H), 7.29−7.16 (m, 9 H), 5.98−5.81 (m, 3 H), 5.29−5.18 (m, 3 H), 5.14−5.03 (m, 3 H), 4.11−3.97 (m, 4 H), 3.75−3.67 (m, 2 H), 3.49 (br s, 1 H), 3.32−3.13 (d, J = 2.4 Hz, 1 H), 3.20−3.13 (m, 2 H), 3.00 (m, 1 H), 2.33−2.12 (m, 3 H), 2.03−0.92 (series of multiplets, 19 H), 0.88 (s, 3 H), 0.78 (d, J = 6.6 Hz, 3 H), 0.65 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 144.71, 136.08, 136.04, 135.94, 128.80, 127.76, 126.86, 116.30, 115.57, 86.53, 80.77, 79.20, 74.96, 69.42, 69.34, 68.81, 62.00, 46.87, 46.48, 42.67, 42.11, 39.90, 36.15, 35.50, 35.14, 35.10, 33.23, 28.99, 28.09, 27.75, 27.56, 23.36, 23.32, 23.12, 18.24, 12.66; HRFAB-MS (thioglycerol + Na^+ matrix) m/e ([M + Na]$^+$) 765.4875 (100%), calcd 765.4859.

28. To a THF (40 mL) solution of **27** (1.27 g, 1.71 mmol) was added 9-BBN (0.5 M solution in THF, 17.1 mL). The mixture was stirred for 12 h before the addition of NaOH (20% solution, 10 mL) and H_2O_2 (30% solution, 10 mL). The resulting mixture was refluxed for 1 h followed by the addition of brine (100 mL) and extraction with EtOAc (4 × 30 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Silica gel chromatography (5% MeOH in CH_2Cl_2) afforded the product (1.26 g, 93% yield) as a clear glass: ^{1}H NMR (5% CD_3OD in $CDCl_3$, 300 MHz) δ 7.46−7.43 (m, 6 H), 7.32−7.20 (m, 9 H), 3.94 (s, 3 H), 3.78−3.56 (m, 10 H), 3.48 (br s, 1 H), 3.32−3.26 (m, 2 H), 3.24−3.12 (m, 3 H), 3.00 (dd, J = 8.2 and 6.1 Hz, 1 H), 2.23−1.96 (m, 3 H), 1.90−0.95 (series of multiplets, 25 H), 0.90 (s, 3 H), 0.77 (d, J = 6.6 Hz, 3 H), 0.66 (s, 3 H); ^{13}C NMR (5% CD_3OD in $CDCl_3$, 75 MHz) δ 144.52, 128.64, 127.64, 126.76, 86.43, 80.55, 79.31, 77.65, 77.23, 76.80, 76.06, 66.17, 66.01, 65.41, 61.93, 61.20, 60.73, 60.39, 47.29, 46.08, 42.65, 41.62, 39.49, 36.02, 35.10, 34.89, 34.77, 32.89, 32.71, 32.41, 32.26, 28.68, 27.70, 27.51, 27.19, 23.26, 22.66, 22.50, 18.23, 12.34; HRFAB-MS (thioglycerol + Na^+ matrix) m/e ([M + Na]$^+$) 819.5169 (100%), calcd 819.5099.

29. To a CH_2Cl_2 (50 mL) solution of compound **28** (1.26 g, 1.58 mmol) at 0 °C was added Et_3N (0.92 mL, 6.60 mmol) followed by mesyl chloride (0.47 mL, 6.05 mmol). After 15 min, H_2O (10 mL) was added followed by brine (80 mL). The mixture was extracted with EtOAc (60 mL, 2 × 30 mL), and the combined extracts were dried over anhydrous Na_2SO_4. After removal of solvent in vacuo, the residue was dissolved in DMSO (10 mL), and NaN_3 (1.192 g, 18.3 mmol) was added. The suspension was heated to 60 °C under N_2 overnight. H_2O (100 mL) was added, and the mixture was extracted with EtOAc (3 × 40 mL). The combined extracts were washed with brine and dried over anhydrous Na_2SO_4. Removal of the solvent in vacuo afforded a pale yellow oil. The oil was dissolved in MeOH (10 mL) and CH_2Cl_2 (20 mL), and TsOH (17.4 mg, 0.092 mmol) was added. After 12 h, saturated aqueous $NaHCO_3$ (20 mL) and brine (50 mL) were added, and the mixture was extracted with EtOAc (3 × 40 mL).The combined extracts were washed with brine (50 mL) and dried over anhydrous Na_2SO_4. Silica gel chromatography (EtOAc/hexanes, 1:3) afforded the desired product (0.934, 94%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.75−3.70 (m, 1 H), 3.68−3.63 (m, 2 H), 3.62−3.57 (m, 1 H), 3.53 (t, J = 6.1 Hz, 2 H), 3.50 (br s, 1 H), 3.46−3.38 (m, 6 H), 3.26 (d, J = 2.4 Hz, 1 H), 3.24−3.20 (m, 1 H), 3.16−3.12 (m, 1 H), 3.10−3.04 (m, 1 H), 2.17−2.04 (m, 3 H), 1.96−1.63 (m, 14 H), 1.53−1.45 (m, 3 H), 1.35−1.20 (m, 7 H), 1.08−1.00 (m, 1 H), 0.97−0.88

(m, 1 H), 0.94 (d, J = 6.8 Hz, 3 H), 0.89 (s, 3 H), 0.67 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 80.64, 79.81, 76.06, 65.05, 64.49, 64.34, 61.03, 49.02, 48.98, 48.78, 46.93, 46.53, 42.76, 42.01, 39.83, 39.14, 35.46, 35.33, 35.12, 32.97, 29.79, 29.73, 29.10, 27.90, 27.68, 23.56, 23.06, 22.88, 18.24, 12.60; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ([M + Na]$^+$) 652.4285 (100%), calcd 652.4295.

9. Compound **29** (0.245 g, 0.391 mmol) was dissolved in THF (30 mL) followed by the addition of $LiAlH_4$ (59 mg, 1.56 mmol). The gray suspension was stirred under N_2 for 12 h. Na_2SO_4·10H_2O powder (~1 g) was carefully added. After the gray color in the suspension dissipated, anhydrous Na_2SO_4 was added, and the precipitate was removed by filtration. After the removal of solvent, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/28% NH_3·H_2O, 10:5:1 and then 10:5:1.5). The solvent was removed from relevant fractions, and 1 M HCl (4 mL) was added to dissolve the residue. The resulting clear solution was extracted with Et_2O (3 × 10 mL). A 20% NaOH solution was added until the solution became strongly basic. CH_2Cl_2 (4 × 10 mL) was used to extract the basic solution. The combined extracts were dried over anhydrous Na_2SO_4, and removal of solvent in vacuo gave the desired product (0.15 g, 71% yield) as a colorless oil: ^{1}H NMR (~20% CD_3OD in $CDCl_3$, 500 MHz) δ 4.73 (br s, 7 H), 3.74−3.70 (m, 1 H), 3.65−3.60 (m, 2 H), 3.56−3.52 (m, 4 H), 3.31−3.28 (m, 2 H), 3.16−3.09 (m, 2 H), 2.82−2.71 (m, 6 H), 2.19−2.06 (m, 3 H), 1.97−1.66 (series of multiplets, 15 H), 1.58−1.48 (m, 3 H), 1.38−0.98 (m, 7 H), 0.96 (d, J = 6.8 Hz, 3 H), 0.93 (s, 3 H), 0.71 (s, 3 H); ^{13}C NMR (~20% CD_3OD in $CDCl_3$, 75 MHz) δ 81.80, 80.60, 77.17, 67.88, 67.86, 67.18, 60.73, 48.11, 47.28, 43.93, 42.99, 41.34, 40.76, 40.72, 40.24, 39.70, 36.33, 36.18, 35.86, 34.29, 33.99, 33.96, 33.83, 29.60, 29.00, 28.57, 28.54, 24.33, 23.59, 23.48, 18.86, 13.04; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ([M + H]$^+$) 552.4756 (100%), calcd 552.4772.

30. *o*-$NO_2C_6H_4SeCN$ (0.094 g, 0.21 mmol) and Bu_3P (0.095 mL, 0.38 mmol) were stirred in dry THF (5 mL) at 0 °C for 1/2 h followed by the addition of compound **29** (0.10 g, 0.159 mmol) in THF (2 mL). The suspension was stirred for 1 h followed by the addition of H_2O_2 (30% aqueous solution, 2 mL). The mixture was stirred for 12 h followed by extraction with hexanes (4 × 10 mL). The combined extracts were dried over anhydrous Na_2SO_4. The desired product (0.035 g, 36% yield) was obtained as a pale yellow oil after silical gel chromatography (10% EtOAc/hexanes): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.73−5.66 (ddd, J = 17.1, 10.2, 8.3 Hz, 1 H), 4.90 (dd, J = 17.1, 2.0 Hz, 1 H), 4.82 (dd, J = 10.2 Hz, 1.96 Hz, 1 H), 3.68−3.64 (m, 1 H), 3.62−3.58 (m, 1 H), 3.54−3.26 (m, 9 H), 3.25−3.22 (m, 2 H), 3.15−3.11 (m, 1 H), 3.10−3.04 (m, 1 H), 2.17−1.62 (series of multiplets, 18 H), 1.51−1.43 (m, 2 H), 1.35−1.18 (m, 4 H), 1.06−0.91 (m, 2 H), 1.02 (d, J = 6.3 Hz, 3 H), 0.90 (s, 3 H), 0.68 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 145.50, 111.72, 80.60, 79.82, 76.09, 65.06, 64.50, 64.45, 49.05, 48.97, 48.79, 46.43, 46.13, 42.76, 42.03, 41.30, 39.84, 35.49, 35.34, 35.15, 29.82, 29.80, 29.75, 29.11, 28.00, 27.84, 27.68, 23.56, 23.08, 22.95, 19.79, 12.87; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ([M + Na]$^+$) 634.4167 (90.6%), calcd 634.4169.

8. Compound **30** (0.105 g, 0.172 mmol) was dissolved in CH_2Cl_2 (5 mL) and MeOH (5 mL) at −78 °C. O_3 was bubbled into the solution for ca. 20 min. Me_2S (1 mL) was added , and the solvent was removed in vacuo. The residue was dissolved in THF (15 mL), and $LiAlH_4$ (0.033 g, 0.86 mmol) was added. The suspension was stirred for 12 h. Na_2SO_4·10H_2O (~2 g) was carefully added. After the gray color of the suspension dissipated, anhydrous Na_2SO_4 was added, and the precipitate was removed by filtration. Concentration and silica gel chromatography (CH_2Cl_2/MeOH/28% NH_3·H_2O, 10:5:1.5 and then 9:6:1.8) yielded a white glass. To this material was added 1 M HCl (4 mL). The resulting clear solution was washed with Et_2O (3 × 10 mL). A 20% NaOH solution was added to the aqueous phase until the solution became strongly basic. CH_2Cl_2 (4 × 10 mL) was used to extract the basic solution. The combined extracts were dried over anhydrous Na_2SO_4, and removal of solvent gave the desired product (0.063 g, 68% yield) as a colorless oil: ^{1}H NMR (~10% CD_3OD in $CDCl_3$, 500 MHz) δ 4.76 (br s, 7 H), 3.75−3.71 (m, 1 H), 3.66−3.62 (m, 1 H), 3.58−3.52 (m, 4 H), 3.33−3.29 (m, 2 H), 3.22 (dd, J = 10.5 and 7.6 Hz, 1 H), 3.15−3.09 (m, 2 H), 2.81 (t, J = 6.8 Hz, 2 H), 2.76−2.71 (m, 4 H), 2.19−2.08 (m, 3 H), 2.00−1.66 (series of multiplets, 14 H), 1.58−1.45 (m, 3 H), 1.40−1.08 (m, 5 H), 1.03 (d, J = 6.8 Hz, 3 H), 1.02−0.96 (m, 1 H), 0.93 (s, 3 H), 0.72 (s, 3 H); ^{13}C NMR (~10% CD_3OD in $CDCl_3$, 75 MHz) δ 81.74, 80.64, 77.23, 67.95, 67.87, 67.18, 47.32, 44.59, 43.72, 43.01, 41.26, 40.80, 40.71, 40.23, 40.02, 36.36, 36.20, 35.87, 34.27, 33.99, 33.90, 29.60, 29.05, 28.58, 28.08, 24.49, 23.62, 23.46, 16.84, 13.12; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ([M + H]$^+$) 538.4578 (4.7%), calcd 538.4584.

32. Compound **15** (0.118 g, 0.183 mmol) was dissolved in dry CH_2Cl_2 (10 mL), and SO_3·pyridine complex (0.035 g, 0.22 mmol) was added. The suspension was stirred for 12 h. The solvent was removed in vacuo to give a white powder. To the white powder was added 1 M HCl (10 mL), and the resulting mixture was extracted with CH_2Cl_2 (4 × 10 mL). The combined extracts were dried over anhydrous Na_2SO_4. The desired product (0.11 g, 84%) was obtained as a pale yellow oil after silica gel chromatography (10% MeOH in CH_2Cl_2): ^{1}H NMR (~10% CD_3OD in $CDCl_3$, 500 MHz) δ 4.03 (t, J = 6.8 Hz, 2 H), 3.69−3.65 (m, 1 H), 3.62−3.58 (m, 1 H), 3.55 (t, J = 6.1 Hz, 2 H), 3.51 (br s, 1 H), 3.46−3.38 (m, 6 H), 3.27 (d, J = 2.4 Hz, 1 H), 3.26−3.21 (m, 1 H), 3.18−3.07 (m, 2 H), 2.18−2.03 (m, 3 H), 1.95−1.47 (series of multiplets, 19 H), 1.40−0.96 (series of multiplets, 9 H), 0.92 (d, J = 6.8 Hz, 3 H), 0.91 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR (~10% CD_3OD in $CDCl_3$, 75 MHz) δ 80.43, 79.68, 75.87, 69.30, 64.82, 64.32, 64.14, 48.78, 48.73, 48.50, 46.44, 46.21, 42.49, 41.76, 39.61, 35.36, 35.17, 35.06, 34.85, 31.73, 29.53, 29.46, 29.44, 28.84, 27.68, 27.48, 27.38, 25.91, 23.30, 22.75, 22.66, 17.70, 12.32; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ([M − H + 2Na]$^+$) 768.3831 (100%), calcd 768.3843. The azides were reduced by treating the triazide (0.11 g, 0.15 mmol) with Ph_3P (0.20 g, 0.77 mmol) in THF (10 mL) and H_2O (1 mL). The mixture was stirred for 3 days. The solvent was removed in vacuo, and the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/28% NH_3·H_2O, 12:6:1 and then 10:5:1.5) to afford the desired product (0.077 g, 78% yield) as a glass. HCl in Et_2O (1 M, 0.5 mL) was added to the glass to give the corresponding HCl salt: ^{1}H NMR (~10% $CDCl_3$ in CD_3OD, 500 MHz) δ 4.81 (s, 10 H), 4.07−3.97 (m, 2 H), 3.82 (br s, 1 H), 3.71 (br s, 1 H), 3.65 (t, J = 5.2 Hz, 2 H), 3.57 (br s, 1 H), 3.37−3.30 (m, 2 H), 3.22−3.02 (m, 8 H), 2.12−1.71 (series of multiplets, 17 H), 1.65−1.01 (series of multiplets, 13 H), 0.97 (d, J = 6.8 Hz, 3 H), 0.94 (s, 3 H), 0.73 (s, 3 H); ^{13}C NMR (~10% $CDCl_3$ in CD_3OD, 75 MHz) δ 81.89, 80.58, 77.50, 70.04, 66.71, 66.56, 66.02, 47.11, 46.76, 44.20, 42.66, 40.50, 39.60, 39.40, 36.24, 36.11, 35.89, 35.67, 32.28, 29.38, 29.23, 29.10, 28.94, 28.49, 26.06, 24.21, 23.46, 23.30, 18.50, 12.86; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ([M + Na]$^+$) 668.4271 (100%), calcd 668.4258.

33. The mesylate derived from **15**[11] (0.19 g, 0.264 mmol) was stirred with excess octylamine (2 mL) at 80 °C for 12 h. After removal of octylamine in vacuo, the residue was chromatographed (silica gel, EtOAc/hexanes, 1:4, with 2% Et_3N) to afford the desired product (0.19 g, 95% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.69−3.37 (series of multiplets, 11 H), 3.26−3.00 (m, 4 H), 2.61−2.53 (m, 4 H), 2.20−2.02 (m, 3 H), 1.98−0.99 (series of multiplets, 40 H), 0.92−0.85 (m, 9 H), 0.65 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 80.60, 79.74, 76.05, 64.97, 64.40, 64.28, 50.79, 50.25, 49.00, 48.90, 48.71, 46.47, 46.34, 42.65, 41.96, 39.80, 35.77, 35.41, 35.27, 35.05, 33.73, 31.96, 30.25, 29.76, 29.74, 29.67, 29.39, 29.05, 27.84, 27.61, 27.55, 26.70, 23.50, 23.00, 22.82, 22.79, 18.06, 14.23, 12.54; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e* ([M + H]$^+$) 755.6012 (100%), calcd 755.6024. The triazide (0.18 g, 0.239 mmol) was dissolved in THF (10 mL) and EtOH (10 mL). Lindlar catalyst (44 mg) was added, and the suspension was shaken under H_2 (50 psi) for 12 h. After removal of the solvent in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/28% NH_3·H_2O, 10:5:1 and then 10:5:1.5). To the product was added 1 M HCl (2 mL), and the resulting clear solution was extracted with Et_2O (2 × 10 mL). A 20% NaOH solution was added until the solution became strongly basic. CH_2Cl_2 (20 mL, 2 × 10 mL) was used to extract the basic solution. The combined extracts were dried over anhydrous Na_2SO_4, and removal of solvent in vacuo gave the desired product (0.114 g, 68% yield) as a clear oil: ^{1}H NMR (~20% $CDCl_3$ in CD_3OD, 500 MHz) δ 4.79 (br s, 7 H), 3.74−3.70 (m, 1 H), 3.66−3.61 (m, 1 H), 3.56−3.51 (m, 3 H), 3.31−3.29 (m, 2 H), 3.16−3.09 (m, 2 H), 2.88−2.72 (m, 6 H), 2.59−2.51 (m, 4 H), 2.18−2.07 (m, 3 H), 1.97−1.66 (series of multiplets,

14 H), 1.62−0.97 (series of multiplets, 25 H), 0.95 (d, $J = 6.3$ Hz, 3 H), 0.93 (s, 3 H), 0.89 (t, $J = 6.8$ Hz, 3 H), 0.70 (s, 3 H); ^{13}C NMR (∼20% $CDCl_3$ in CD_3OD, 75 MHz) δ 81.82, 80.63, 77.23, 67.85, 67.19, 51.20, 50.69, 47.82, 47.24, 43.92, 43.01, 41.30, 40.80, 40.68, 40.22, 36.74, 36.38, 36.20, 35.87, 34.66, 34.15, 33.87, 32.90, 30.54, 30.39, 30.30, 29.64, 29.03, 28.59, 28.41, 26.96, 24.37, 23.65, 23.48, 18.75, 14.63, 13.09; HRFAB-MS (thioglycerol + Na^+ matrix) *m*/*e* ($[M + H]^+$) 677.6309 (46.6%), calcd 677.6309.

34. Compound **33** (0.08 g, 0.12 mmol) was dissolved in $CHCl_3$ (5 mL) and MeOH (5 mL), aminoiminosulfonic acid (0.045 g, 0.36 mmol) was added, and the suspension was stirred for 12 h. The solvent was removed in vacuo, and the residue was dissolved in 1 M HCl (6 mL) and H_2O (10 mL). The solution was washed with Et_2O (3 × 5 mL), and a 20% NaOH solution was then added dropwise until the solution became strongly basic. The basic mixture was extracted with CH_2Cl_2 (4 × 5 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the desired product (0.087 g, 91% yield) as a white glass: ^{1}H NMR (∼20% $CDCl_3$ in CD_3OD, 500 MHz) δ 4.96 (br s, 13 H), 3.74−3.68 (m, 1 H), 3.65−3.50 (m, 4 H), 3.38−3.18 (series of multiplets, 10 H), 2.60−2.50 (m, 4 H), 2.15−1.99 (m, 3 H), 1.88−1.72 (m, 14 H), 1.60−0.99 (series of multiplets, 25 H), 0.94 (br s, 6 H), 0.89 (t, $J = 6.6$ Hz, 3 H), 0.71 (s, 3 H); ^{13}C NMR (∼20% $CDCl_3$ in CD_3OD, 75 MHz) δ 159.00, 158.87, 158.72, 81.68, 79.93, 76.95, 66.59, 65.93, 65.45, 50.82, 50.40, 47.64, 46.94, 43.67, 42.27, 40.18, 39.25, 36.19, 35.66, 35.40, 34.21, 32.45, 30.51, 30.26, 30.18, 30.10, 29.86, 29.35, 28.71, 28.15, 28.00, 26.87, 23.94, 23.44, 23.23, 23.12, 18.61, 14.42, 12.98; HRFAB-MS (thioglycerol + Na^+ matrix) *m*/*e* ($[M + H]^+$) 803.6958 (18.4%), calcd 803.6953.

35. The mesylate derived from **15**[11] (0.092 g, 0.128 mmol) was dissolved in DMSO (2 mL) followed by the addition of NaN_3 (0.0167 g, 0.256 mmol). The suspension was heated to 70 °C for 12 h. H_2O (20 mL) was added to the cooled suspension, and the mixture was extracted with EtOAc/hexanes (1:1) (20 mL, 3 × 10 mL). The combined extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the product (0.081 g, 95% yield) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.69−3.36 (m, 11 H), 3.25−3.02 (m, 6 H), 2.20−2.02 (m, 3 H), 1.97−1.60 (m, 15 H), 1.55−0.98 (m, 13 H), 0.92 (d, $J = 6.3$ Hz, 3 H), 0.89 (s, 3 H), 0.66 (s, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 80.59, 79.77, 76.03, 65.01, 64.46, 64.30, 52.12, 48.99, 48.95, 48.76, 46.44, 46.42, 42.70, 41.99, 39.82, 35.56, 35.44, 35.31, 35.09, 33.09, 29.79, 29.77, 29.71, 29.08, 27.88, 27.78, 27.66, 25.65, 23.53, 23.03, 22.85, 18.00, 12.58; HRFAB-MS (thioglycerol + Na^+ matrix) *m*/*e* ($[M + Na]^+$) 691.4512 (100%), calcd 691.4496. The tetraazide (0.081 g, 0.12 mmol) was dissolved in THF (5 mL) and EtOH (10 mL). Lindlar catalyst (30 mg) was added, and the suspension was shaken under H_2 (50 psi) for 12 h. After removal of the solvent in vacuo, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH/28% $NH_3 \cdot H_2O$, 5:3:1 and then 2:2:1). To the product was added 1 M HCl (2 mL), and the resulting solution was washed with Et_2O (2 × 10 mL). A 20% NaOH solution was added to the aqueous phase until the solution became strongly basic. CH_2Cl_2 (10 mL, 2 × 5 mL) was used to extract the basic solution. The combined extracts were dried over anhydrous Na_2SO_4, and concentration in vacuo gave the desired product (0.044 g, 64% yield) as a colorless oil: ^{1}H NMR (∼20% $CDCl_3$ in CD_3OD, 500 MHz) δ 4.79 (br s, 8 H), 3.74−3.70 (m, 1 H), 3.66−3.62 (m, 1 H), 3.56−3.52 (m, 3 H), 3.31−3.27 (m, 2 H), 3.16−3.10 (m, 2 H), 2.82−2.70 (m, 6 H), 2.64−2.54 (m, 2 H), 2.19−2.07 (m, 3 H), 1.99−1.66 (series of multiplets, 14 H), 1.58−0.96 (series of multiplets, 13 H), 0.96 (d, $J = 6.6$ Hz, 3 H), 0.93 (s, 3 H), 0.70 (s, 3 H); ^{13}C NMR (∼20% $CDCl_3$ in CD_3OD, 75 MHz) δ 81.96, 90.76, 77.33, 67.92, 67.26, 47.84, 47.33, 44.04, 43.24, 43.15, 41.40, 40.91, 40.78, 40.29, 36.82, 36.48, 36.28, 35.96, 34.39, 34.11, 30.59, 29.69, 29.13, 28.68, 28.64, 24.43, 23.69, 23.48, 18.77, 13.06; HRFAB-MS (thioglycerol + Na^+ matrix) *m*/*e* ($[M + H]^+$) 565.5041 (100%), calcd 565.5057.

MIC and MBC Measurements. A known concentration of bacteria (∼10^6 cells/mL) was incubated with varied concentrations of a compound of interest for 24 h in a nutrient broth (tryptic soy, Difco). Bacterial growth was monitored via cell counting and turbidity measurements. The MIC value was the concentration of the studied compound at which the number of bacteria remained constant or decreased during incubation. The MBC value was determined as the concentration at which fewer than 0.1% of the bacteria survived incubation with the compound of interest for 24 h. For an example see ref 25.

Luciferin/Luciferase-Based Cell Lysis Assay. The assay was performed as published,[24] with the following exceptions: *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA) was not used, 0.1% Triton X 100 was used instead of 4%, and cells were incubated in the lysis buffer for 5 min before being exposed to luciferin and ATP.

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Supporting Information Available: IR spectral data and ^{1}H and ^{13}C spectra for compounds **6**−**14** and **32**−**35**. This material is available free of charge via the Internet at http://pubs.acs.org.

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