Semisynthetic Approaches to Laspartomycin Analogues[⊥]

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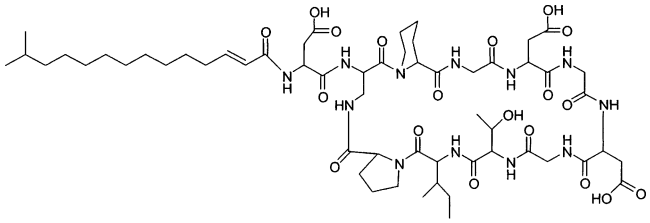
Laspartomycin C (**1**), a lipopeptide antibiotic related to amphomycin, consists of a cyclic peptide core and an aspartic acid unit external to the core and linking this to a C_{15}-2,3-unsaturated fatty acid. This was reported initially to be active against *Staphylococcus aureus*, and more recent studies have shown that it is active against VRE, VISA, and MRSA isolates. The enzymatic cleavage of the fatty acid tail was accomplished with a deacylase produced by *Actinoplanes utahensis* and resulted in two peptides, designated Peptide 1 and Peptide 2. Semisynthetic derivatives of both peptides have been made, and the principal requirement for biological activity appears to be the presence of an acylaspartic acid.

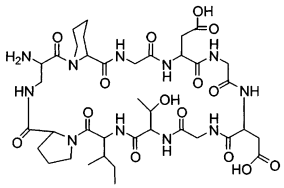
Laspartomycin was originally reported by Umezawa et al. in 1968 as a lipopeptide antibiotic related to amphomycin with a 2,3-unsaturated C_{15}-fatty acid side chain.[1] At that time, full chemical and spectroscopic characterization was very difficult and the structure has remained unknown until relatively recently.[2] Subsequently glycinocin A was reported from another *Streptomyces* sp. and is apparently identical with or a stereoisomer of laspartomycin C (**1**).[3] Laspartomycin C consists of a cyclic peptide core and an aspartic acid external to the core linking the core to a C_{15}-2,3-unsaturated fatty acid. It was initially reported to be active against *Staphylococcus aureus*, and current studies have shown that it is active against vancomycin-resistant enterococci (VRE), methicillin-resistant *S. aureus* (MRSA), and vancomycin-intermediate *S. aureus* (VISA) isolates. The enzymatic cleavage of laspartomycin components with a deacylase produced by *Actinoplanes utahensis* results in two peptides[4] shown below (Peptide 1 and Peptide 2), and both of these peptides have been converted by synthetic modifications to derivatives active against *S. aureus*. The purpose of this study was to examine the influence of the side chain on the structure−activity of this antibiotic. To this end, a variety of derivatives have been prepared including the conversion of Peptide 1 to Peptide 2.

A number of new antibiotics were prepared by derivatizing the laspartomycin cyclic peptide.[5,6] The MIC values of these compounds against *S. aureus* in Mueller Hinton broth were determined in the presence and absence of 4 mM calcium chloride. Without the added calcium the MIC values were significantly higher. In the case of tsushimycin, which is closely related to amphomycin, this calcium ion dependence of biological activity was explained by a confirmation change stabilized by Ca^{2+} bound within the peptide ring and the formation of a dimer that interacted with the bacterial cell membrane. In this study the structure of tsushimycin was determined in the presence of calcium ions by X-ray crystallography.[8] The side chains of the laspartomycin cyclic peptide contained acyl amino acids, acyl dipeptides, and acyl tripeptides. The stereochemistry of the amino acids and peptides was explored in the present investigation. The acyl groups were both aliphatic and aromatic. Some of the structure−activity relationships observed are summarized in Table 1. The length of the side chain was very important since the octanoyl derivative was not active compared to the pentadecanoyl compound (**4** and **5**). Derivatives containing *p*-substituted long-chain alkyl aromatic acyl side chains showed good activity (**6** and **7**), but when the aromatic portion was at the end of chain, the compound was not active (**8**).



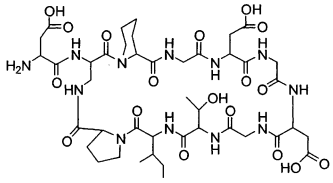
laspartomycin C ($C_{57}H_{90}N_{12}O_{19}$)

1



Peptide 1 ($C_{38}H_{59}N_{11}O_{15}$)

2



Peptide 2 ($C_{42}H_{64}N_{12}O_{18}$)

3

Pentadecanoyl dipeptides showed that the L-aspartic was essential for activity since the D-isomer was inactive (**10**). In addition, aromatic amino acids had greater potencies (**9**, **11**, and **12**) and the stereochemistry of this amino acid did not affect the results since the D-phenylalanine was also active. The pentadecanoyl glycine and alanine derivatives were not active (**13** and **14**), but the corresponding L-lysine compound (**15**) did show moderate activity.

The importance of the L-aspartic side chain is also evident since the pentadecanoyl derivative of Peptide 1 was inactive (**16**), and surprisingly the corresponding L-glutamic acid (**17**) also had poor activity. However one compound containing L-asparagine (**18**) did show moderate activity. Appending the side chain of the lipodepsipeptide antibiotic daptomycin to Peptide 2 produced an inactive derivative (**27**), whereas extending the lipid tail containing the

[⊥] Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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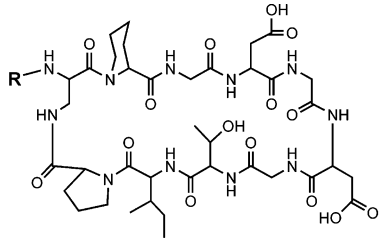
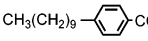
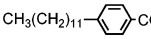
Table 1. Structure−Activity Relationships of Laspartomycin Analogues from Side-Chain Modifications



	compound number	side chain (R)	MIC vs. MSSA[a]
position and size of alkyl groups	**1**	laspartomycin	2
	4	$CH_3(CH_2)_8$ CO-L-Asp-	>64
	5	$CH_3(CH_2)_{13}$ CO-L-Asp-	2
	6	$CH_3(CH_2)_9$  CO-L-Asp-	1-2
	7	$CH_3(CH_2)_{11}$  CO-L-Asp-	2
	8	 O-$(CH_2)_{10}$CO-L-Asp-	>64
stereochemistry of side-chain amino acids	**9**	$CH_3(CH_2)_{13}$CO-L-Trp-L-Asp-	8
	10	$CH_3(CH_2)_{13}$CO-L-Trp-D-Asp-	>64
	11	$CH_3(CH_2)_{13}$CO-L-Phe-L-Asp-	4
	12	$CH_3(CH_2)_{13}$CO-D-Phe-L-Asp-	4
aromatic vs. aliphatic amino acids	**13**	$CH_3(CH_2)_{13}$CO-Gly-L-Asp-	>64
	14	$CH_3(CH_2)_{13}$CO-L-Ala-L-Asp-	>64
	15	$CH_3(CH_2)_{13}$CO-L-Lys-L-Asp-	16

	compound number	side chain (R)	MIC vs. MSSA[a]
importance of aspartic acid in side chain	**16**	$CH_3(CH_2)_{13}CO$	>64
	5	$CH_3(CH_2)_{13}$CO-L-Asp-	2
	17	$CH_3(CH_2)_{13}$CO-L-Glu-	64
	9	$CH_3(CH_2)_{13}$CO-L-Trp-L-Asp-	8
	18	$CH_3(CH_2)_{13}$CO-L-Trp-L-Asn-	8
linker effects	**19**	$CH_3(CH_2)_{11}$NHCO-L-Asp-	4
	20	$CH_3(CH_2)_{15}$NHCO-L-Asp-	2
	21	$CH_3(CH_2)_{13}$NHCO-L-Phe-L-Asp-	4
	22	$CH_3(CH_2)_{11}$OCO-L-Asp-	8
	23	$CH_3(CH_2)_{11}SO_2$-L-Asp-	16
	24	$CH_3(CH_2)_{15}SO_2$-L-Phe-L-Asp-	4
	25	$CH_3(CH_2)_{15}SO_2$-L-Trp-L-Asp-	4
	26	$CH_3(CH_2)_{15}SO_2$-L-Trp-	>64
	27	$CH_3(CH_2)_8$CO-L-Trp-L-Asn-L-Asp-	>64
	28	$CH_3(CH_2)_{13}$CO-L-Trp-L-Asn-L-Asp-	16
	29	$CH_3(CH_2)_{13}$CO-L-Asp-Gly-	>128
	30	$CH_3(CH_2)_{13}$CO-L-Asp-Gly-L-Asp-	>128

[a] MIC values are minimum inhibitor concentrations expressed as μg/mL. Assay organism was a methicillin-sensitive *Staphylococcus aureus* assayed by the tube dilution method.

pentadecanoyl rather than the decanoyl moiety (**28**) resulted in moderate activity. Replacement of the aspartic acids in the core peptide by asparagine or various amide functions resulted in complete loss of activity.

In order to assess the effect of different types of linkers, the corresponding long-chain ureas, sulfonamides, and a carbamate were prepared (**19**, **20**, **21**, **22**, **23**, and **24**) All of these compounds showed reasonably potent biological activity (Table 1).

Catalytic hydrogenation of **1** afforded the dihydro-laspartomycin, which had approximately the same in vitro potency as **1**. Conversion of laspartomycin C to the dihydroxy compound with dilute potassium permanganate proceeded readily, but the product lacked biological activity.

Table 2 shows representative laspartomycin analogues with activity against staphylococci and entercococci that are resistant to clinically used antibiotics. Thus it is clear from this work that changing the side chain of laspartomycin to various sulfonyl or carbamoyl derivatives produces compounds with interesting biological activity, but none were more potent than the parent antibiotic.

Experimental Section

General Experimental Procedures. FABMS were obtained on a VG model ZAB-2SE mass spectrometer. All semisynthetic compounds were characterized also by HPLC/UV using a Hewlett-Packard Model 1100 instrument.

All the condensation reactions on Peptide 1 or Peptide 2 were carried out in anhydrous dimethylformamide using either dicyclohexylcarbodiimide and 1-hydroxybenzotriazole or *O*-[(ethoxycarbonyl)cyanomethyleneamino]-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TOTU) as the coupling agents (Figure 1).

All of the intermediate acids either were available commercially or were synthesized. The amino acid and peptide derivatives were prepared mainly by the activated ester method using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole except for the tripeptide derivatives, which were prepared using standard solid-phase techniques. The protecting groups were removed by standard procedures using 95% trifluoroacetic acid for the *tert*-butyl esters, *N*-*tert*-butyloxycarbonyl, and *O*-trityl groups. Catalytic hydrogenation with 5% palladium on carbon was used for removing benzyl and carbobenzoxy moieties. Examples are provided in the Experimental Section.

Table 2. Activity of Laspartomycin Analogues against Resistant Strains of Bacteria

		MIC (μg/mL)[a]					
compound	side chain (R)	MSSA	MRSA	MRSE	VISA	VSE	VRE, MDR
5	$CH_3(CH_2)_{13}$CO-L-Asp−	4	4	8	16	4	4
21	$CH_3(CH_2)_{13}$NH-CO-L-Phe-L-Asp−	4	4	4	8	2−4	8
24	$CH_3(CH_2)_{13}SO_2$-L-Trp-L-Asp−	>64	8	4	>64	2	4
11	$CH_3(CH_2)_{13}$CO-L-Phe-L-Asp−	4	4	4	32	4	8

[a] MIC values are minimum inhibitory concentrations for MSSA (methicillin-sensitive *Staphylococcus aureus*, Smith), MRSA (methicillin-resistant *S. aureus*), MRSE (methicillin-resistant *S. epidermidis*), VISA (vancomycin-intermediate *S. aureus*), VRE (vancomycin-resistant enterococci), and MDR (multidrug-resistant enterococci).

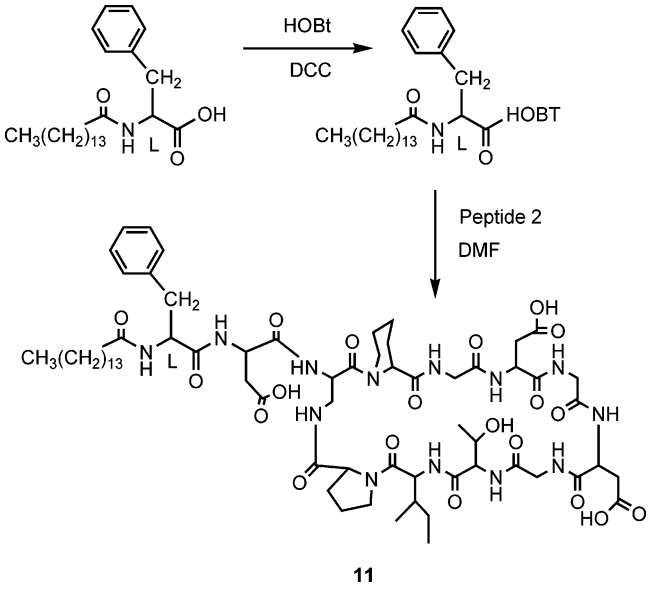


Figure 1. Peptide coupling with DCC and HOBT. The procedure was repeated with D-phenylalanine to produce compound **12**.

The products from these reactions were purified by the procedures outlined below, and identifications of the compounds were confirmed by FABMS. Product purity from these procedures general ranged from 80 to 93% based on HPLC area percent when the chromatograms were monitored at 215 nm.

Pentadecanoyl-L-aspartic Acid 4-*O*-Benzyl Ester. L-Aspartic acid 4-*O*-benzyl ester (0.2578 g. 1.156 mmol) was added to 2 mL of water and 2 mL of tetrahydrofuran followed by 1 mL of saturated sodium bicarbonate solution, and the mixture was stirred until solution was complete. A slurry of pentadecanoyl hydroxybenzotriazole (0.2798 g, 0.758 mmol) in 5 mL of water and 5 mL of tetrahydrofuran was added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into 20 mL of water and acidified to pH 1.0 with 6 N hydrochloric acid. The resulting precipitate was chilled, filtered, and dried to afford 0.2792 g of product, $C_{22}H_{41}NO_5$: FABMS *m*/*z* 448 $(M + H)^+$, 470 $(M + Na)^+$, 492 $(M + 2Na - H)^+$ (calcd for $C_{22}H_{41}NO_5$, 448.3).

Pentadecanoyl-L-aspartic Acid 4-*O*-Benzylhydroxybenzotriazole Ester. A mixture of pentadecanoyl-L-aspartic acid 4-*O*-benzyl ester (0.2619 g 0.5851 mmol) 1-hydroxybenzotriazole (0.0895 g, 0.5851 mmol) and dicyclohexylcarbodiimide (0.1205 g, 0.5851 mmol) in 5.0 mL of tetrahydrofuran was stirred at room temperature overnight. The reaction mixture was filtered and evaporated to dryness at reduced pressure. The resulting oil was slurred in hexane to give a crystalline product. Yield: 0.2933 g.

Pentadecanoyl-L-Aspartic Acid 4-*O*-Benzyl Derivative of Peptide 1. A mixture of Peptide 1 (14.8 mg, 0.0162 mmol) and diisopropylethylamine (0.023 mL, 0.1319 mmol) was added to 0.5 mL of dimethylformamide and stirred at room temperature. To this was added two 0.20 mL aliquots of a solution of the above hydroxybenzotriazole ester (44.9 mg, 0.0794 mmol) at the beginning and after 2.5 h. After 5 h, water was added and the product adsorbed onto a 2.5 × 5.0 cm styrene−divinylbenzene resin column (ENVI-Chrom P) and eluted with 0.05 M phosphate buffer (pH 7.2) in 10% and 25% acetonitrile. Fractions containing the desired product were desalted as described for compound **25** and freeze-dried to obtain 6.0 mg of white powder: FABMS *m*/*z* 1339 $(M + H)^+$ 1361 $(M + Na)^+$, and 1377 $(M + K)^+$ (calcd for $C_{64}H_{99}N_{12}O_{19}$, 1339.7).

Compound 5. A mixture of 3.0 mg of the benzyl derivative, 11 mg of 5% palladium on carbon, and 1.0 mL of methanol was hydrogenated at atmospheric pressure overnight (balloon technique). The mixture was filtered through Celite, evaporated to dryness, slurried in water, and lyophilized to give 2.0 mg of compound **5**: FABMS *m*/*z* 1287 $(M + K)^+$ (calcd for $C_{57}H_{92}N_{12}O_{19}K$, 1287.6).

Dihydrolaspartomycin. A mixture of 21.3 mg of laspartomycin (**1**), 35 mg of 5% palladium on carbon, and 2.5 mL of methanol was hydrogenated at atmospheric pressure overnight (balloon technique). The mixture was filtered through Celite, evaporated to dryness, slurried in water, and lyophilized to give 19.4 mg of the desired product: FABMS *m*/*z* 1248 $(M + H)^+$, 1270 $(M + Na)^+$ (calcd for $C_{57}H_{93}N_{12}O_{19}$, 1249.7).

Dodecylcarbamoyl−Peptide 2 (19). A mixture of dodecylisocyanate (0.0104 g, 0.043 mmol) and Peptide 2 (0.044 g, 0.043 mmol) was stirred in DMF (0.60 mL). After 60 min at room temperature, a second 0.0104 mL aliquot of the isocyanate was added and stirred for 60 min. The reaction was quenched, and the product was isolated on Sephadex LH-20 as described for compound **25**. Product-containing fractions were pooled, methanol was removed under vacuum, and the product was freeze-dried from aqueous solution. Yield: 32 mg of a white solid, 77% by HPLC (215 nm area %); FABMS *m*/*z* 1259 $(M + Na)^+$ (calcd for $C_{55}H_{89}N_{13}O_{19}Na$,1258.6).

Dodecyloxycarbonyl−Peptide 2 (22). A mixture of dodecylchloroformate (0.010 mL) and Peptide 2 (0.035 g, 0.034 mmol) in DMF (0.80 mL) was stirred at room temperature. The reaction mixture was diluted with 4 mL of methanol, and the product was isolated on a Sephadex LH-20 column as in the preparation of compound **25**. Methanol was removed under vacuum from the product-containing fractions; the residue was dissolved in 10 mL of 14% acetonitrile 0.10 M in ammonium phosphate (aqueous pH 7.2). This solution was desalted by application to a styrene−divinylbenzene resin cartridge (0.5 g, Supelco EnviChrom-P, conditioned with 10 mL of acetonitrile and 6 mL of 14% acetonitrile. The sample-loaded cartridge was rinsed with 6 mL of salt-free 14% acetonitrile. The product was stripped off using 6 mL of salt-free 67% acetonitrile, which was removed under vacuum, and the product was freeze-dried from aqueous solution. Yield: 11 mg of a white solid, 81% by HPLC (215 nm area %); FABMS *m*/*z* 1238 $(M + H)^+$, 1260 $(M + Na)^+$, 1276 $(M + K)^+$ (calcd for $C_{55}H_{89}N_{12}O_{20}$, 1237.6).

Hexadecylcarbamoyl−Peptide 2 (20). A mixture of Peptide 2 (0.0438 g) and hexadecylisocyanate (0.013 mL) in 0.50 mL of DMF was stirred at room temperature. After 70 min, a second aliquot of the isocyanate was added. The reaction mixture was quenched and the product isolated on a Sephadex LH-20 column as described for compound **25**. Methanol was removed under vacuum from the product-containing fractions, yielding 29 mg of a yellow solid. The product was further purified by low-resolution reversed-phase chromatography. The product was eluted with 40% acetonitrile 0.10 M in ammonium phosphate (aqueous pH7.2). This fraction was diluted with an equal volume of distilled water and desalted as described for compound **25**. Eighteen milliliters of 67% acetonitrile were necessary to elute the product. Acetonitrile was removed under vacuum, and the product was freeze-dried from aqueous solution. Yield: 24 mg of an off-white solid, 83% by HPLC (215 nm area %); FABMS *m*/*z* 1292 $(M + H)^+$, 1314 $(M + Na)^+$, 1330 $(M + K)^+$ (calcd for $C_{59}H_{98}N_{13}O_{19}$, 1292.7).

Hexadecylsulfonyl-L-Phenylalanyl−Peptide 2 (24). Hexadecylsulfonyl-L-phenyl alanine (60 mg., 0.132 mmol), hydroxybenzotriazole (19 mg, 0.132 mmol), and dicyclohexylcarbodiimide (32 mg, 0.151

mmol) was stirred for 40 min in 0.67 mL of DMF. A 0.30 mL aliquot of this solution was added to a solution of Peptide 2 (50 mg, 0.0488 mmol) in 0.40 mL of DMF. The progress of the reaction was monitored by HPLC, and the product was isolated by chromatography in a similar fashion to that described for compound **25**. The product was a white powder, 13 mg; FABMS *m*/*z* 1460.5 $(M + H)^+$, 1482.4 $(M + Na)^+$, 1498.4 $(M + K)^+$ (calcd for $C_{67}H_{106}N_{13}O_{21}S$, 1460.7).

Hexadecylsulfonyl-L-Tryptophan Methyl Ester. A solution of hexadecylsulfonyl chloride (260 mg, 0.80 mmol), tryptophan methyl ester hydrochloride (254 mg, 1.0 mmol), and 0.34 mL of triethylamine (2.45 mmol) in 2.0 mL of DMF was stirred at room temperature for 4.0 h. The mixture was diluted with 10 mL of 1.0 N HCl and extracted with 20 mL of ethyl acetate. The ethyl acetate solution was washed with water and saturated salt solution and dried over magnesium sulfate, then evaporated to give beige crystals: yield 261 mg; FABMS *m*/*z* 507 $(M + H)^+$ (calcd for $C_{28}H_{47}N_2O_4S$, 507.3).

Hexadecylsulfonyl-L-tryptophan. A mixture of hexadecylsulfonyl-L-tryptophan methyl ester (260 mg, 0.514 mmol) and 0.50 mL of 1.0 N NaOH in 2.0 mL of methanol and 2.0 mL of tetrahydrofuran was stirred at room temperature for several h. Thin-layer chromatography indicated the reaction was incomplete. An additional 0.50 mL of 1.0 N NaOH was added and the mixture stirred until the reaction was complete (18 h). The reaction was worked up as described in the above example to afford 196 mg of product: FABMS *m*/*z* 493 $(M + H)^+$ (calcd for $C_{27}H_{45}N_2O_4S$, 493.3).

Hexadecylsulfonyl-L-Tryptophanyl−Peptide 2 (25). Hexadecylsulfonyl-L-tryptophan (90 mg, 0183 mmol), hydroxybenzotriazole (28 mg, 0.183 mmol), and dicyclohexylcarbodiimide (38 mg, 0.183 mmol) was stirred for 40 min in 1.0 mL of DMF. A 0.30 mL aliquot of this solution was added to a solution of Peptide 2 (94.5 mg, 0.0439 mmol) in 0.20 mL of DMF. The progress of the reaction was monitored by HPLC. At the completion of the reaction, the reaction mixture was diluted with 5 mL of methanol and 1.5 M NH_4OH was added to an apparent pH of about 7. The filtered sample solution was applied to a 2.5 × 44 cm size exclusion column (Sephadex LH-20 fine, swelled in methanol), which was eluted with methanol at about 0.8 mL/min. The product eluted in about 25 mL of eluate starting at about 105 mL. The methanol was removed from the product pool by evaporation under vacuum at or below 30 °C. The solid residue was dissolved in about 12 mL of 10% acetonitrile buffered with 0.08 M ammonium phosphate (aqueous pH 7.2). This solution was applied to a 2.5 × 5 cm styrene−divinylbenzene resin column (Supelco ENVI-Chrom P resin) and eluted with increasing concentrations of acetonitrile buffered with pH 7.2 ammonium phosphate. The product eluted in about 36 mL using 48% acetonitrile as eluent. Acetonitrile was removed from this fraction by evaporation under vacuum (prior to applying the sample to this resin, the resin column was washed with 100 mL aliquots of 70% acetonitrile, then 100% acetonitrile, and finally 20% acetonitrile.). The aqueous solution of the sample was then applied to the column for desalting. The column was rinsed with 28 mL of 21% acetonitrile (unbuffered), and the desalted product was stripped from the column using 67% acetonitrile. Acetonitrile was removed by evaporation under vacuum, and the product was freeze-dried. Yield: 14 mg, white solid; FABMS *m*/*z* 1499 $(M + H)^+$, 1521 $(M + Na)^+$, 1537 $(M + K)^+$(calcd for $C_{69}H_{107}N_{14}O_{21}S$, 1499.7).

MIC Determinations. MIC values against organisms were determined using the broth microdilution method in accordance with the U.S. Clinical and Laboratory Standard Institute Guideline M7-A6 (**7**) using cation-adjusted Mueller-Hinton broth with 50 μg/mL of calcium. MSSA (methicillin-sensitive *Staphylococcus aureus* Smith, ATCC 19636), MRSA (methicillin-resistant *S. aureus*, ATCC 43300), MRSE (methicillin-resistant *S. epidermidis*, ATCC 51625), VISA (vancomycin-intermediate *S. aureus*, CDC HIP 5836), VRE/MDR (vancomycin-resistant *Enterococcus faecium*, ATCC 51559), and VSE (vancomycin-sensitive *E. faecalis*) ATCC 29212 were used. MIC values for compounds in Table 1 were obtained by the tube dilution method against a MSSA in Mueller-Hinton broth determined in the presence and absence of 4 mM calcium chloride.

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