Articles

Structure Elucidation of Liposidomycins, a Class of Complex Lipid Nucleoside Antibiotics

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Liposidomycins A, B, and C are novel lipid nucleoside antibiotics produced by *Streptomyces griseosporeus*, which strongly inhibit bacterial peptidoglycan synthesis. Detailed structure analysis of liposidomycins A, B, and C and of their chemical degradation products by NMR and mass spectrometry shows that they each possess 5′-substituted uridine, 5-amino-5-deoxyribose-2-sulfate, and perhydro-1,4-diazepine moieties, but differ in the structures of the lipid side chains. Liposidomycins A and C contain 3-(3′-methylglutaryl)-7,10-hexadecadienoic acid and 3-(3′-methylglutaryl)tetradecanoic acid, respectively, and liposidomycin B contains 3-(3′-methylglutaryl)-12-methyltridecanoic acid. Using liposidomycin B as a central structural model, a detailed fragmentation map from collision-induced dissociation was produced using tandem mass spectrometry. The resulting data were used for partial characterization of structural subunits and in establishing sites and order of interconnectivity of subunits in the intact molecule. Structure of the fatty acid component of liposidomycin A as 3-hydroxy-7,10-hexadecadienoic acid, a previously unknown natural product, was established principally by charge-remote fragmentation using a technique involving microscale lipid hydrolysis and lithiation using LiOH. These methods should have general utility in structural studies of complex natural product families whose members share common structural subunits.

Introduction

Nucleoside antibiotics as a class of natural products exhibit an exceptional range of biological activities, similarly matched by great structural diversity.[1-4] The liposidomycins, which strongly inhibit bacterial peptidoglycan synthesis, are among the largest and most complex of the nucleoside antibiotics and constitute a family of structurally novel compounds isolated from *Streptomyces griseosporeus*.[5] The primary site of action of liposidomycin C was determined to be at phospho-*N*-acetylmuramylpentapeptide transferase, the first step in the lipid cycle of peptidoglycan synthesis in the cell wall of *Escherichia coli*.[6] The inhibition is strong, ID_{50} 0.03 μg/mL, and is highly specific. The liposidomycin structure closely resembles the reaction intermediate between uridine diphosphate *N*-acetylmuramylpentapeptide and undecaprenyl phosphate in the lipid cycle. Several other groups of nucleoside antibiotics are known which inhibit microbial cell wall synthesis.[3] These include the polyoxins, which inhibit chitin synthetase, and the nikkomycins and neopolyoxins, all of which act as competitive inhibitors of uridine diphosphate *N*-acetyl-D-glucosamine. Tunicamycins,[7] fatty acyl nucleosides, are reported to inhibit phospho-*N*-acetylmuramylpentapeptide transferase, but the inhibitory activity is more pronounced for inhibition of synthesis of various glycoconjugates including mammal glycoproteins. Mureidomycins[4] and pacidamycins[4] are peptidyl nucleosides active against *Pseudomonas* sp., which were recently shown to inhibit formation of the lipid intermediate.

We presently report the structure elucidation of liposidomycins A, B, and C as **1**, **2**, and **3**, respectively, based principally on ^1H and ^{13}C NMR and tandem mass spectrometry. Using as a principal model liposidomycin B, the structure of which was communicated previously,[8] a method is described in which the structures and points of interconnectivity of subunits are determined in part from collision-induced dissociation (CID) pathways established by tandem mass spectrometry. The overall liposidomycin structure was established by correlation of NMR data and detailed CID maps with structural subunit data determined from the intact molecules and from chemical degradation products. Structure of the fatty acid component of liposidomycin A was established by NMR and by

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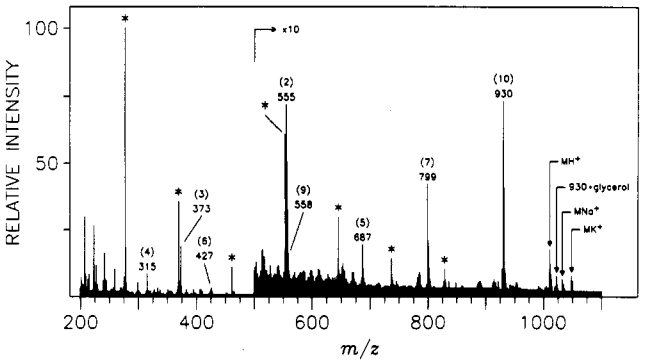
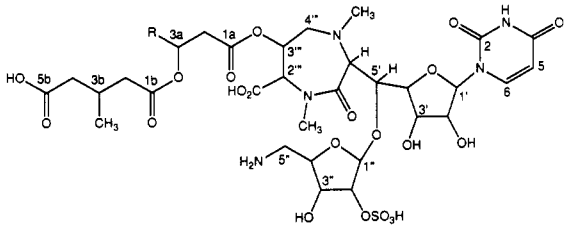


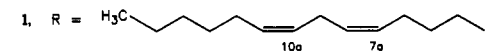
Figure 1. FAB mass spectrum of liposidomycin B (2). Numbered peaks denote ions selected for collision-induced dissociation for construction of the fragmentation map shown in Scheme I. Values in parentheses indicate mass shifts from a separate experiment in which *O*-perdeuterioglycerol was used as FAB matrix. Asterisks indicate matrix-derived ions.

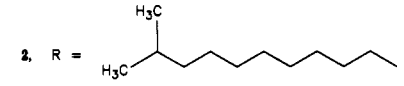
charge-remote fragmentation of the lithium salt of the fatty acid produced by microscale saponification of the nucleoside by LiOH. Taken together, NMR and CID experiments demonstrate the complementarity of the methods described and their applicability to structural studies involving families of compounds which share common structural elements.

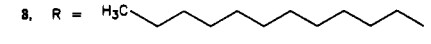
Overall Structural Properties of Liposidomycins A, B, and C

Liposidomycins A, B, and C are members of a family of over 20 complex nucleosides which share common structural features, but differ with respect to fatty acid structure and presence or absence of sulfate, as described in a later section. Molecular weights of 1, 2, and 3 were determined









to be 1033, 1009, and 1009, respectively, by positive and negative ion fast atom bombardment (FAB) mass spectrometry, as shown in Figure 1 for 2. The elemental composition of 2, $C_{42}H_{67}N_5O_{21}S$, supported by measurement of exact mass,[8] was found to be 24.000 u lower than the exact molecular mass of 1, requiring 1 to have two net additional carbon atoms and two unsaturations, a difference shown by NMR and tandem mass spectrometry to reside in the fatty acid moieties as discussed below. Liposidomycin C, an isomer of 2, was found to differ from 2 only in having a normal-chain C_{14} fatty acid rather than the iso structure in 2.

The number of exchangeable (acidic) hydrogen atoms in 2 was unambiguously established as nine from the 10-u shift of the MH^+ ion in the FAB mass spectrum of 2 measured using deuteriated matrix (Figure 1).[9,10] The presence of sulfate was first recognized by the facile and characteristic[11] expulsion of SO_3 as a 79.9517 u neutral from MH^+ as implied in Figure 1 and verified by a constant neutral loss measurement for 80 u (showing m/z 1010 as a precursor of m/z 930), as well as from the IR spectrum.[8]

Collision-Induced Dissociation Pathways Established by Tandem Mass Spectrometry. An extensive map of CID pathways resulting from collisional activation of protonated 2 was determined and is summarized in Scheme I. These data were found to be of considerable use in correlating the structures of structural subunits with those derived from NMR and provided a census of structural elements that matched those found in chemical degradation studies, thus adding a degree of confidence in assembly of the overall structure. Initial experiments, described below, established the mass relationships shown for 2 (e.g., $555^+ \rightarrow 373^+ \rightarrow 245^+$), while later experiments involving NOE (nuclear overhauser effects), HMBC (heteronuclear multiple-bond correlation spectroscopy),[12] and CID measurements on chemical degradation products of 2 were used in parallel to develop the structure assignments shown in Scheme I. The CID map is of considerable value in determining the relative placement of structural subunits in the intact molecule and is similar in some respects to establishing the order of subunits in simpler linear molecules, such as peptides.[13]

The pathways shown in Scheme I were determined by a series of separate experiments in which fragment ions formed by spontaneous dissociation in MS-1 (those peaks labeled in Figure 1) were mass-selected and subjected to collisional activation and the dissociation products measured in MS-2. Although CID of larger ions, such as m/z 930, may be insufficient in a single experiment to generate all possible subunit ions, the entire network of relevant subunit ions (the sum of which is dictated by molecular weight) was observed by sequential analysis of increasingly smaller precursor ions as indicated in each subsequent CID spectrum. There are numerous examples[14] of the high selectivity of tandem mass spectrometry, in which precursor ions are virtually unrecognizable in the primary (MS-1 only) mass spectrum but yield clear and informative spectra in MS-2; however, the favorable results obtained with liposidomycins are undoubtedly aided by high surface activity conferred by the hydrophobic lipid moiety.[15]

An example of the manner in which decomposition sequences in Scheme I were determined is shown in Figure 2. Starting with the $MH^+ - SO_3$ ion (m/z 930), sequential collisional activation of 799^+, 687^+, 555^+, 373^+, and 183^+ leads to the dissociation pathways shown involving losses of uracil and ribose, yielding the 1,4-diazepin-3-one moiety, 3-methylglutaric acid, and the C_{14}-fatty acid as terminal ions. Additional pathways represented in Scheme I were established from collisional dissociation of m/z 558 and 427 ions,[16] corresponding both to species in the collisional dissociation pathway (see Figure 2a) and to products of

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(10) Verma, S.; Pomerantz, S. C.; Sethi, S. K.; McCloskey, J. A. *Anal. Chem.* **1986**, *58*, 2898.
(11) White, K. D.; Sphon, J. A.; Hall, S. *Anal. Chem.* **1986**, *58*, 562.
(12) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
(13) See for example: Biemann, K. *Methods Enzymol.* **1990**, *193*, 455.
(14) Busch, K. L.; Glish, G. L.; McLuckey, S. A. *Mass Spectrometry/Mass Spectrometry*; VCH Publishers: New York, 1988.
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(16) Data are shown in the supplementary material.

Scheme I. Collision-Induced Dissociation Pathways of Protonated Liposidomycins Determined by Tandem Mass Spectrometry

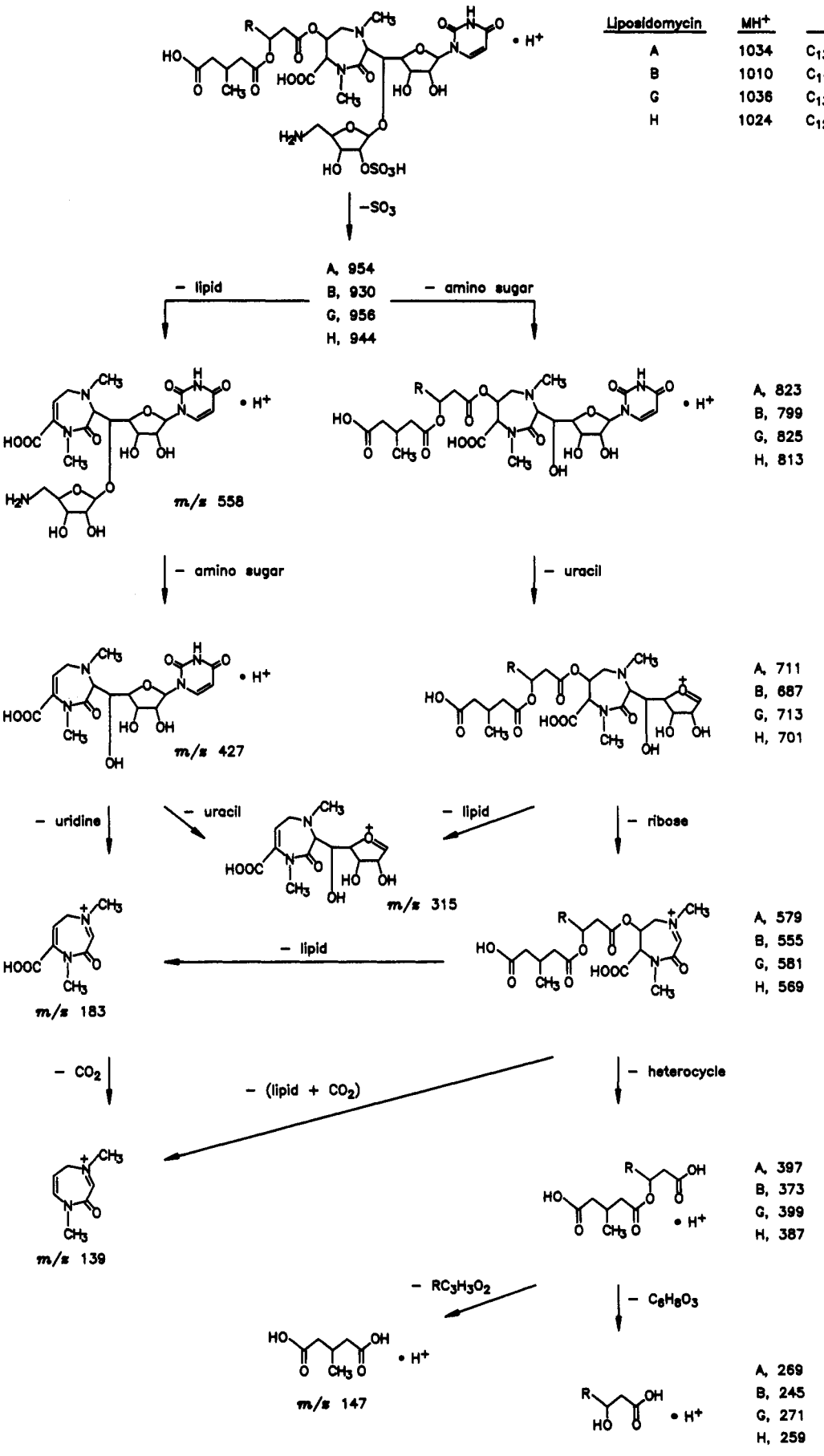


Table I. ^{1}H-NMR Data for Liposidomycins A (1), B(2), and C (3)[a]

position	1 (δ, ppm)[b]	2 (δ, ppm)	3 (δ, ppm)[b]
5		5.76, d, J = 8 Hz	
6		7.82, d, J = 8 Hz	
1′		5.64, d, J = 1.5 Hz	
2′		4.14, overlapping	
3′		4.25, overlapping	
4′		4.29, m	
5′		4.42, bd, J = 10.7 Hz	
6′		3.9, d, J = 10.7 Hz	
1″		5.45, bs	
2″		4.67, d, J = 4.8 Hz	
3″		4.3, dd, J = 4.8, 7.9 Hz	
4″		4.16, m	
5″		3.32, overlapping, 3.22, dd, J = 14, 4.4 Hz	
2‴		4.25, overlapping	
3‴		5.45, m	
4‴		3.1, overlapping, 3.44, bd, J = 14.9 Hz	
>NCH_3		2.46, s	
$CONCH_3$		3.1, s	
2a	2.6, dd, J = 8.9, 16 Hz	2.61, dd, J = 8.5, 15.7 Hz	2.62, dd, J = 8.5, 16 Hz
	2.69, dd, J = 5.2, 16 Hz	2.69, dd, J = 4.9, 15.7 Hz	2.68, dd, J = 5.2, 5.2, 16 Hz
3a	5.23, m	5.2, m	5.23, m
4a	1.67, m	1.61, m	1.63, m
5a	1.40, m	1.3, m, overlapping	1.3, m, overlapping
6a	2.07, m	1.3, m, overlapping	1.3, m, overlapping
7a	5.37, dtt, J = ~1.6, 6.6, 11 Hz	1.3, m, overlapping	1.3, m, overlapping
8a	5.32*, dtt, J = ~1.6, 6.6, 11 Hz	1.3, m, overlapping	1.3, m, overlapping
9a	2.77, t, J = 6.6 Hz	1.3, m, overlapping	1.3, m, overlapping
10a	5.35*, dtt, J = ~1.6, 6.6, 11 Hz	1.3, m, overlapping	1.3, m, overlapping
11a	5.37, dtt, J = ~1.6, 6.6, 11 Hz	1.3, m, overlapping	1.3, m, overlapping
12a	2.07, m	1.51, m, J = 6.8 Hz	1.3, m, overlapping
12a-CH_3		0.88, d, J = 6.8 Hz	
13a	1.35, overlapping	0.88, d, J = 6.8 Hz	1.3, m, overlapping
14a	1.35, overlapping		0.89, t, J = 6.8 Hz
15a	1.32, overlapping		
16a	0.9, t, J = 7 Hz		
2b	2.43†	2.4	2.42
	2.15‡	2.1	2.16
3b	2.37, m	2.37, m	2.42, m
3b-CH_3	1.0, d, J = 7 Hz	1.0, d, J = 6.8 Hz	1.0, d, J = 6.8 Hz
4b	2.25†	2.4	2.42
	2.19‡	2.1	2.16

[a] Assignments were deduced from COSY and decoupling data. CD_3OD, TMS. Assignments with †, ‡ may be interchanged. [b] Data for the nonlipid portions of 1 and 3 are essentially the same as for 2 and are listed in the supplementary material.

acid hydrolysis (molecular weights 557 and 426, respectively) produced as described in a later section. The observed sequence of losses determined from data in Figure 2 places constraints on the interconnectivity of subunits in the intact molecule. For example, based on these data the following conclusions may be reached: (i) the amino sugar must be a side chain rather than a bridging unit between uridine and the remainder of the molecule; (ii) allowable bridging elements are ribose and the diazepine moiety; (iii) the lipid elements in the intact molecule, 3-methylglutaric acid and the fatty acid, are directly bound in a single subunit and are in turn connected to the diazepine ring or its carboxyl group. On the other hand, placement of the sulfate group from these data alone is not possible because of its loss as the first step of dissociation but was established by other means.

Identification of Structural Subunits in Intact Liposidomycins

Evidence for the presence of the major subunit elements as common structural themes in all three liposidomycins is provided by ^{1}H NMR data in Table I and the supplementary material (1–3) and CID maps in Scheme I (1 and 2).

Uracil. Elimination of uracil as a neutral molecule (112 u) was observed in the positive-ion FAB mass spectra of liposidomycins B and C as indicated by ions at m/z 799 and 687 (112.0319 by difference, cf. Figure 1). Evidence for loss of uracil in the FAB mass spectrum is additionally supported by deuterium labeling using *O*-perdeuterioglycerol matrix[9] in which elimination of two exchangeable hydrogens in the 112-u neutral was demonstrated (see Figure 1, deuterium shifts), as required for uracil. The ^{1}H NMR spectra are likewise consistent with the uracil moiety: δ 7.83 (1 H, d, J = 8 Hz), 5.75 (1 H, d, J = 8 Hz) for 1, δ 7.82 (1 H, d, J = 8 Hz), 5.76 (1 H, d, J = 8 Hz) for 2, and δ 7.8 (1 H, d, J = 8 Hz), 5.76 (1 H, d, J = 8 Hz) for 3.

Ribose. A molecule of ribose (132 u) is eliminated from m/z 687 to form m/z 555 as determined from the exact-mass difference of these ions in the primary FAB mass spectrum (Figure 1). The deuterium-exchange information (see Figure 1) demonstrates that loss of 132 u results in elimination of three active hydrogens which supports a C-5′ or other carbon-bound sugar linkage rather than a more common O-5′-type sugar attachment. An O-5′ sugar linkage would otherwise show a net loss of two active hydrogen atoms. Deuterium labeling proved instrumental in establishing the C-5 linkage of the ribose moiety by placing restraints on the hydrogens which were likely to be available for transfer during elimination of the ribosyl moiety.

Amino Sugar and Sulfate. Composition of the amino sugar ($C_5H_9NO_3$) was determined solely from the difference between exact mass values of ions at m/z 930 and 799, corresponding to loss of 131 u in the positive-ion FAB mass

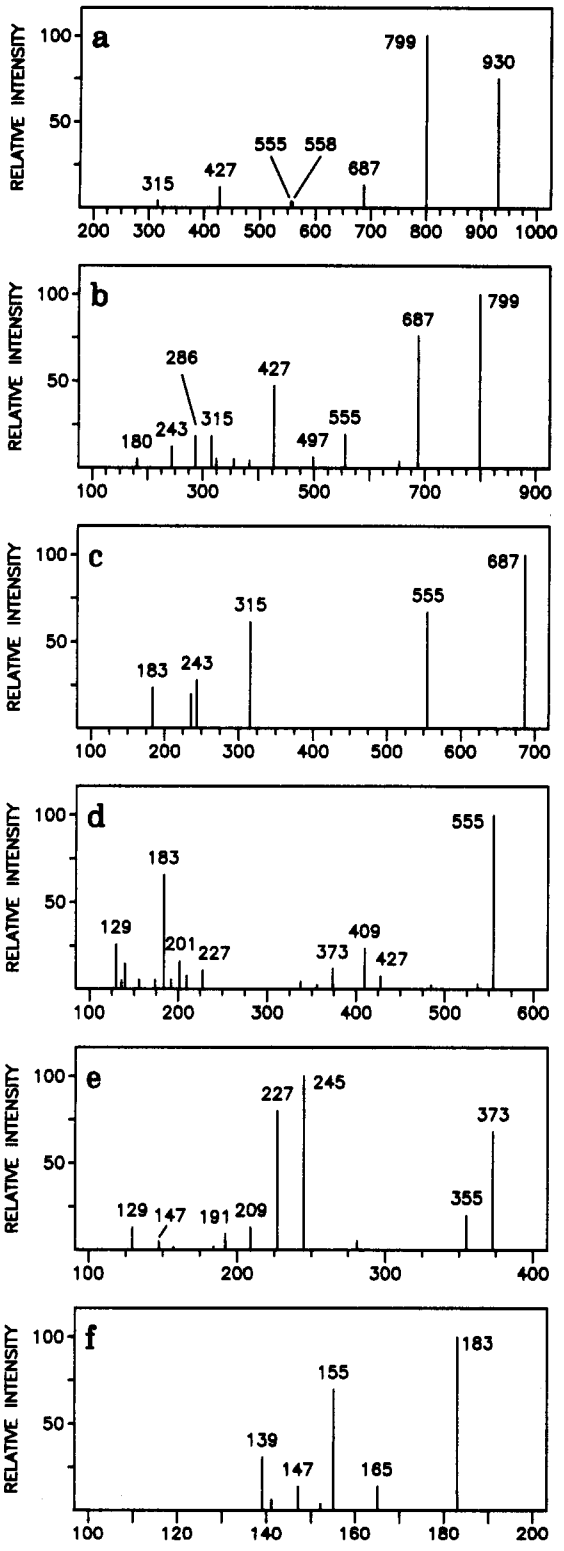


Figure 2. Collision-induced dissociation mass spectra of positive ions formed by fast atom bombardment of liposidomycin B (**2**) shown in Figure 1: (a) m/z 930, (b) m/z 799, (c) m/z 687, (d) m/z 555, (e) m/z 373, (f) m/z 183.

spectrum of **2** (Figure 1). The deuterium-exchange experiment demonstrated that the amino sugar contained four active hydrogens based on mass shifts for ions at m/z 930 and 799 in Figure 1.

The presence of a sulfate group was recognized from the positive-ion FAB mass spectrum (Figure 1) from the highly characteristic[11] elimination of 80 u from the protonated molecule. High-resolution data for $(M - H)^-$ and $MH^+ - 80$ (m/z 930) ions confirmed this difference to be due to SO_3. In addition, the number of active hydrogens remained unchanged following loss of SO_3 from MH^+ as indicated by the deuterium-exchange experiment (Figure 1), as required for sulfated hydroxyl. However, facile loss of SO_3 in the first step of dissociation of the protonated molecule precluded detection of the sulfate group in any of the structural subunits formed by collisional dissociation. On the basis of the predicted localization of charge on sulfate in the liposidomycin anion, CID measurements were carried out on the molecular anion of **2** (m/z 1008) and on the abundant ion source-produced m/z 592 anion,[16] leading to the assignments shown in Scheme II. The composition of the m/z 592 ion is supported by measurement of the exact mass difference (416.237 u) between m/z 1008 and 592 in the primary ionization FAB mass spectrum, which corresponds to loss of the neutral lipid moiety plus CO_2 (calcd for $C_{21}H_{36}O_8$, 416.241). Location of sulfate within the amino sugar moiety is indicated by the assignments shown for $592^- \rightarrow 228^-$, as well as by the absence of appropriate mass differences which would indicate sulfate substitution in any of the subunit losses represented in Scheme II. Other dissociation reactions shown provide direct corroboration of the mass values and interconnectivity patterns derived from positive-ion data in Scheme I, namely that 3-methylglutaric acid and the C_{14} fatty acid constitute one integral structural unit and that the lipid moiety and uracil are not bridging units between other subunits. Additional evidence for the sulfated amino sugar is based on (i) loss of 131.0531 mass units ($C_5H_9NO_3$) from the $MH^+ - SO_3$ ion from **2**; (ii) elemental composition of the structural unit $C_5H_{11}NO_7S$ calculated as the difference between **2** and the sum of isolated hydrolysis products, having a total of three rings or double bonds and four exchangeable hydrogen atoms determined by FAB mass spectrometry using a ^2H exchange method;[9,10] (iii) comparison between data from ^1H- and ^{13}C-NMR of **2**, **3**, and those of isolated hydrolysis products, establishing the presence of 5-amino-5-deoxy-2-*O*-sulfonyl-β-D-ribofuranoside as discussed in a later section.

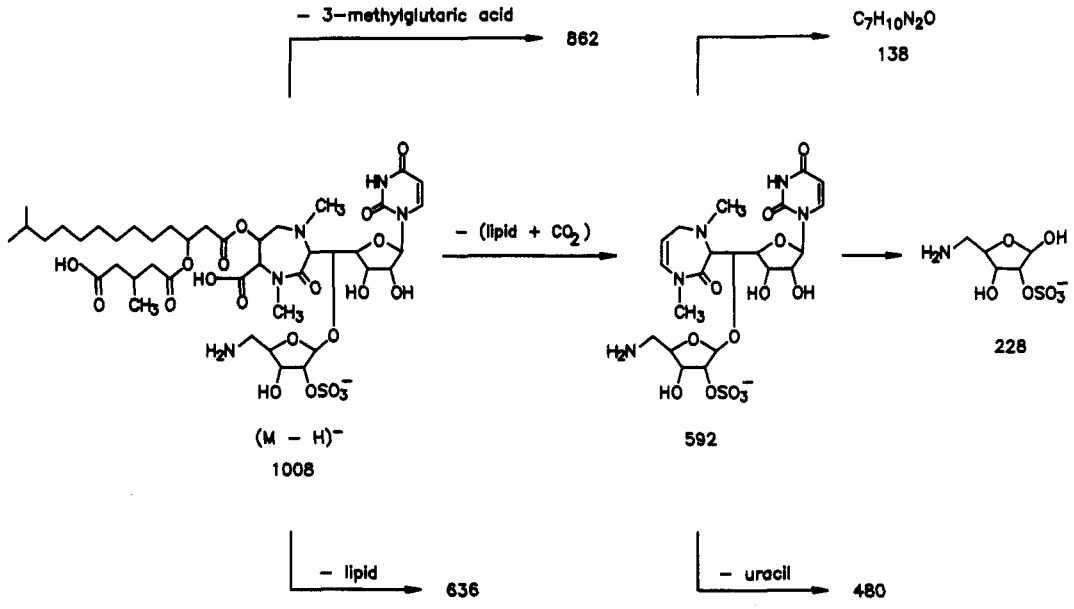
1,4-Diazepin-3-one Heterocycle.[17] Gross structural features of the heterocyclic moiety are evident indirectly from mass spectral data and are supported by NMR data in Table I, but the detailed structure was established by NMR spectra of chemical degradation products as described in later sections. A net difference in total structure composition and elimination of structural components of SO_3, the fatty acid, uracil, amino sugar, and ribose results in a net composition of $C_8H_{11}N_2O_3$ for the remaining part of the structure. High-resolution FAB measurements confirmed this composition from the exact mass difference between m/z 555 and m/z 373 (corresponding to loss of neutral $C_8H_{10}N_2O_3$) in the primary mass spectrum of **2**, 182.0672 (calcd, 182.0691).

Lipid Subunit. As shown by data in Table I and Scheme I, all three liposidomycins contain a C_6 lipid moiety, directly established by high-resolution FAB-MS and GC/MS (discussed below) as 3-methylglutaric acid, which is esterified through a β-hydroxyl group of a long chain fatty acid of variable structure. As indicated in an earlier section, fatty acid modification in all liposidomycins, including **1–3**, can be tracked from molecular weights of the intact antibiotics as well as from selected CID exper-

(17) The numbering system for the seven-membered heterocycle in structure **1** correlates with that of perhydro-1,4-diazepines[18] in which C-2‴ corresponds to C-5 and C-3‴ to C-6.

(18) (a) Intl. Union of Pure and Applied Chemistry. *Nomenclature of Organic Chemistry*; Butterworths: London, 1969. (b) Patterson, A. M.; Capell, L. T. *The Ring Index*; Reinhold: New York, 1940; p 60.

Scheme II. Principal Collision-Induced Dissociation Products of Liposidomycin B (2) Determined by Collisional Activation of Negative Ions m/z 1008 and 592



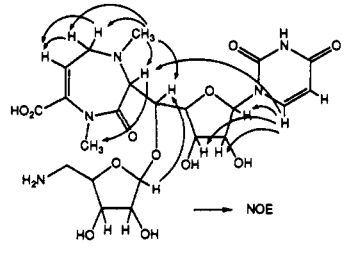


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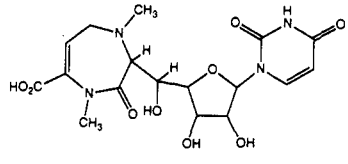
Figure 3. NOE patterns from 10 obtained by DIFNOE.

iments as represented in the dissociation maps (Scheme I).

Acid Hydrolysis Products of Liposidomycins B and C. Hydrolysis of 2 and 3 produced uracil and lipid components whose identification served to corroborate structure assignments obtained from NMR (Table I and supplementary material) and CID mass spectra (Scheme I) from the intact molecules and also provided a sufficient quantity of a common product, nucleoside 4, for detailed



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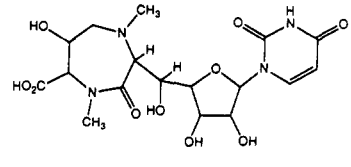

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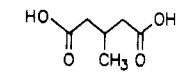
structure analysis of the central core of the molecule by NMR. Hydrolysis of 2 (procedure C) yielded three lipid-related products; 3-methylglutaric acid (5), 3-hydroxy-12-methyltridecanoic acid (6), and a lipid moiety of molecular weight 372 (7). Product 5 was analyzed by GC/MS as its TMS ester (M^+ m/z 290) and compared with the authentic material[19] (data not shown). The elemental composition (MH^+, m/z 245) was also established by high-resolution FAB-MS. The methyl esters of 6 (from 2) and of 3-hydroxytetradecanoic acid (8) from 3 (cf. Ex-



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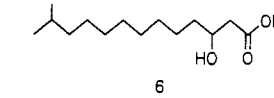

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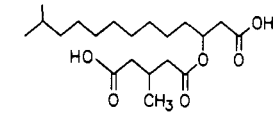

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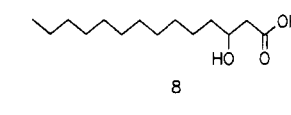

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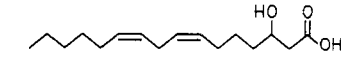

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perimental Section) both produced m/z 185 ($M^+ - C_3H_5O_2$) and m/z 103 ions ($C_4H_7O_3^+$) in their EI mass spectra, characteristic of β-hydroxylation,[20] in support of the assignments shown in Table I. Methanolysis of 3 gave methyl 3-hydroxytetradecanoate instead of methyl 3-hydroxy-12-methyltridecanoate found in 2. The absolute configuration of the fatty acid was inferred to be *R* because the optical rotation of the fatty acid in $CHCl_3$ was levorotatory [$[\alpha]^{20}_D = -12.7°$, *c* 0.14, $CDCl_3$], which is characteristic of 3(*R*)-hydroxy fatty acids.[21-23] Uracil was identified in an acid hydrolysate of 2 (procedure B) by GC/MS following trimethylsilylation [TMS, M^+ m/z 256, and TMS-d_9, M^+ m/z 274] and was confirmed by comparison with mass spectra of authentic silylated uracil.[24]

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Table II. ^{1}H-NMR Data for 4 and 9[a]

position	4	9
5	5.68, d, J = 8.2 Hz	5.82, d, J = 8.3 Hz
6	8.01, d, J = 8.2 Hz	8.2, d, J = 8.3 Hz
1′	5.88, d, J = 5.3 Hz	5.91, d, J = 4.5 Hz
2′	4.38, dd, J = 5, 5.3 Hz	4.33, dd, J = 4.5, 4.5 Hz
3′	4.29, dd, J = 4.4, 5 Hz	4.31, dd, J = 4, 4.5 Hz
4′	4.07, br. d, J = 4.4 Hz	4.13, dd, J = 1.6, 4 Hz
5′	4.18, br. d, J = 9.8 Hz	4.18, dd, J = 1.6, 10 Hz
6′	3.78, d, J = 9.8 Hz	3.68, d, J = 10 Hz
2″		4.22, d, J = 4.8 Hz
3‴	6.49, t, J = 6.8 Hz	4.46, dt, J = 2.7, 4.8 Hz
4‴	3.0, br. dd, J = 6.8, 12.5 Hz	3.16, br. dd, J = 2.7, 4.8 Hz
	3.36, br. dd, J = 6.8, 12.5 Hz	3.21, dd, J = 2.7, 15.3 Hz
>NCH_3	2.45, s	2.41, s
$CONCH_3$	3.02, br. s	3.09, s

[a] Assignments were deduced from decoupling experiments. D_2O, 4.75 ppm.

Hydrolysis by procedures A and B both produced nucleoside 4, shown by HRMS (Experimental Section) to be $C_{17}H_{22}N_4O_9$, mol. wt 426, with five exchangeable hydrogen atoms in the neutral molecule and corresponding to m/z 427 in Scheme I. The related nucleoside 9 was also obtained by methanolysis of a mixture of liposidomycins in a microwave oven[25] (5% HCl–MeOH, 4 min). The structures of 4 and 9 were determined in part by ^{1}H-NMR in comparison with anhydrodeacylliposidomycin (10, structure in Figure 3) as described below. High-voltage paper electrophoresis of 4 showed the presence of both acidic (–COOH) and basic (–N<) groups. The assignments from ^{1}H-NMR of 4 and 9 are shown in Table II. The chemical shifts and coupling constants of 4 (1′: δ_C 90.0, δ_H 5.88, $J_{1',2'}$ = 5.3 Hz, 2′: δ_C 74.3, δ_H 4.38, $J_{2',3'}$ = 5 Hz, 3′: δ_C 71.6, δ_H 4.29, $J_{3',4'}$ = 4.4 Hz) are similar to those of uridine (1′: δ_C 89.5, δ_H 5.9, $J_{1',2'}$ = 5.1 Hz, 2′: δ_C 74.0, δ_H 4.34, $J_{2',3'}$ = 5.1 Hz, δ_C 69.6, δ_H 4.22, $J_{3',4'}$ = 5.5 Hz). Differential NOE measurements between H-6 and H-2′, H-6 and H-3′, and H-1′ and H-4′ of 4 suggest that C-1′, C-2′, C-3′, and C-4′ of 4 possess the same relative configuration as in uridine. The negative Cotton effect at 259 nm in the ORD spectrum of 4 may be explained by the rule for predicting the sign of the Cotton effect.[26] NOEs between H-6 and H-2′/H-3′ of 4 show that the nucleoside 4 has a conformation similar to C-5′-substituted thionucleoside derivatives from albomycin,[27] and the sugar-base torsion angle (ϕ_{CN}) is probably less than −75°.[28]

Alkaline Hydrolysis Products of Liposidomycins B and C. Alkaline hydrolysis of a mixture of liposidomycins produced the anhydrodeacylliposidomycins 10 (mol. wt 557) and 11 (mol. wt 637). The molecular formula $C_{22}H_{31}N_5O_{12}$ was deduced for 10 from high-resolution FAB mass spectra (Experimental Section). The CID mass spectra[16] of the MH^+ ion from 10 (m/z 558) and the principal fragment ion m/z 427 showed that 10 contains the subunits uridine, nonsulfated amino sugar, and the seven-membered heterocycle. Evidence that 11 is a sulfated form of 10 was shown by the characteristic[11] elimination of 80 u from the MH^+ ion in the FAB mass spectrum of 11.[16] HMBC spectroscopy[12] of 10 shows ^{1}H–^{13}C long-range coupling patterns as previously indicated.[5] These data established the seven-membered ring heterocycle as a 1,4-diazepin-3-one, as well as the position of the amino sugar as shown in 10. Phase-sensitive double-quantum-filtered COSY[30] in conjunction with ^{1}H-detected ^{1}H–^{13}C correlation (HMQC)[31] from 10 permitted the complete assignments of ^{1}H- and ^{13}C-NMR signals. NOE data from 10 support the structure as shown in Figure 3. Chemical shifts in ^{13}C-NMR of the aminopentose moiety listed in Table III indicate that the pentose has a ribose-type configuration, for the following reasons. Chemical shifts in ^{13}C-NMR and chemical shifts and coupling constants in ^{1}H-NMR of the aminopentose moiety are comparable to those of methyl 5-amino-5-deoxy-β-D-riboside in D_2O (adjusted to pD 7 with deuteriated acetic acid), which in turn was synthesized by catalytic hydrogenation of methyl 5-azido-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside[32] [10, 1″ (δ 5.2, br. s), 2″ (δ 4.13, br. d, J = 5.5 Hz), 3″ (δ 4.27, dd, J = 5.5, 7.6 Hz), 4″ (δ 4.2, m), 5″ (δ 3.35 and 3.19, each dd); methyl 5-amino-5-deoxy-β-D-ribofuranoside, 1 (δ 4.89, s), 2 (δ 4.03, d, J = 4.6 Hz), 3 (δ 4.16, dd, J = 4.6, 7 Hz), 4 (δ 4.07, m), 5 (δ 3.33 and 3.03, each dd)]. The chemical shifts at C-1″ in the ^{13}C-NMR spectrum of 2 (δ 110), 3 (δ 109.8) and 10 (δ 109.3) characterize the amino sugar anomeric configuration specifically as that of a β-ribofuranoside.[33]

Table III. ^{13}C-NMR Data for 10, 11, and 12[a]

position	10	11	12
2	151.0	151	151.2
4	166.1	166	166.8
5	101.2	101.5	101.3
6	141.6	141.5	141.8
1′	90.6	90.8	90.8
2′	73	73	73.5
3′	68.7	68.5	68.6
4′	81.9	82	81.7
5′	76.2	75.5	76.8
6′	63[b]		62.9
7′	170.5		172
1″	109.3	106.5	108
2″	74.6	80	80.1
3″	69.8	69	69.2
4″	78.3	78	78.5
5″	40.4		39.4
1‴	168.4		173.4
2‴	144	145	59.5
3‴	122.8		68.3
4‴	51.3	51.4	58.4
>NCH_3	40.4	40.2	38.5
$CONCH_3$	33.1	32.8	36.5

[a] Assignments for 10 were deduced from HMQC and HMBC data; assignments for 11 and 12 were performed by comparison with the data from 10. D_2O, CCl_4. [b] Detected by HMQC.

Products of Reductive Cleavage of Liposidomycins. Reductive cleavage of a mixture of liposidomycins with $LiBH_4$ gave 12 (mol. wt 655), concluded to be a hydrated form of compound 11. The position of the fatty acid side chains was deduced by upfield shifts (ca. 1 ppm) of H-3‴ in 12 compared with 2 or 3 as shown in Tables I and IV.[16] An ester linkage between 3-methylglutaric acid and β-

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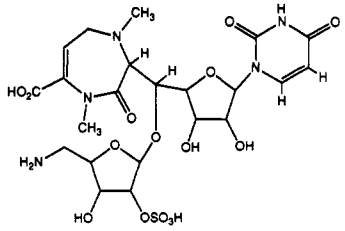

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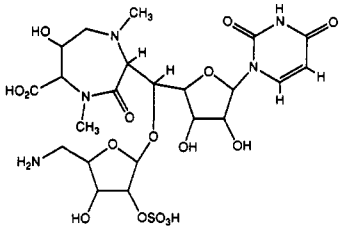

12

hydroxyl of the fatty acid was indicated by downfield shifts (ca. 1 ppm) of the fatty acid β-proton in **2** or **3** as compared with unsubstituted methyl esters of 3-hydroxy fatty acids.[23]

Sulfate Ester Placement in the Amino Sugar. The position of the sulfate ester was concluded to be at C-2″ by comparing ^{1}H- and ^{13}C-NMR of the amino sugar moiety of **10** with those of **11** and **12** as shown in Table III. For example, downfield shifts[34,35] of H-2″ (δ_H 4.13) and C-2″ (δ_C 74.6) were observed in **10** compared with δ_H 4.63 and δ_C 80 in **11**, respectively. The assignments from ^{1}H-NMR spectra of **10–12** are listed in Table IV; Table I and listings in the supplementary material summarize the assignments from ^{1}H-NMR spectra of **2** and **3**.

Structure of Liposidomycin A

Liposidomycin A (**1**) was isolated as a minor component of the liposidomycin family, whose molecular formula (as previously discussed), $C_{44}H_{67}N_5O_{21}S$, shows the molecule to be sulfated and, as indicated by HRMS, contains additional C_2 and two degrees of unsaturation compared with **2**. The CID map of **1** (Scheme I) shows the structural difference with **2** to reside solely in the fatty acid residue, which is therefore isomeric with monohydroxyhexadecadienoic acid. Although an acetylenic function, or one or more cyclopropane rings could not be excluded by these data, the ^{1}H NMR spectrum of **1** (Table I) strongly suggests presence of a methylene-interrupted diene. ^{1}H–^{1}H COSY showed that a triplet proton at δ 2.77 ppm (J = 6.6 Hz, H-9a) is coupled to two protons at δ 5.35 and δ 5.32 (H-8a and H-10a), and two protons at δ 5.37 (H-7a and H-11a) are coupled to two protons at δ 2.07 (H-6a and H-12). The chemical shifts and the coupling pattern were comparable to those of the methylene-interrupted diene moiety of linoleic acid. NOE between the proton at C-9a and the two protons at C-6a and C-12a and the coupling constants ($J_{7a,8a}$ = 11 Hz, $J_{10a,11a}$ = 11 Hz) of the double bonds were confirmed by a spin-decoupling experiment, in which H-9a was irradiated, establishing cis configurations of the double bonds. For characterization of the fatty acid as a 3-hydroxy 7,10-diene, charge remote fragmentation induced by collisional activation was used, and the results were confirmed by variation of a more conventional approach involving chemical derivatization of the double bonds and CID of the resulting products.

Table IV. ^{1}H-NMR Data for **10**, **11**, and **12**[a]

position	10	11	12
5	5.82, d, J = 8.5 Hz	5.83, d, J = 8.2 Hz	5.81, d, J = 8.3 Hz
6	7.7, d, J = 8.5 Hz	7.63, d, J = 8.2 Hz	7.73, d, J = 8.3 Hz
1′	5.62, d, J = 2.8 Hz	5.64, d, J = 3.0 Hz	5.57, d, J = 2.5 Hz
2′	4.28, dd, J = 2.8, 4.6 Hz	4.29, dd, J = 3.0, 5.0 Hz	4.29, dd, J = 2.5, 5.9 Hz
3′	4.12, dd, J = 4.6, 8 Hz[b]	4.25, dd, J = 5.5, 7.5 Hz	4.16, dd, J = 5.9, 8.8 Hz
4′	4.23, m	4.18, overlapping	4.08, dd, J = 1.5, 8.6 Hz
5′	4.35, dd, J = 2.3, 9.6 Hz	4.55, dd, J = 2.7, 8.5 Hz	4.4, overlapping
6′	3.93, d, J = 9.6 Hz	4.18, overlapping	3.84, d, J = 9.7 Hz
1″	5.2, br. s	5.55, br. s	5.42, br. s
2″	4.13, br. d, J = 5.5 Hz[b]	4.63, br. d, J = 5.5 Hz	4.62, br. d, J = 5 Hz
3″	4.27, dd, J = 5.5, 7.6 Hz[b]	4.39, dd, J = 5.5, 7.9 Hz	4.39, overlapping
4″	4.2, m	4.18, m	4.2, m
5″	3.19, dd, J = 5.1, 13.6 Hz	3.22, dd, J = 6.7, 13.4 Hz	3.22, dd, J = 4.6, 14 Hz
	3.35, dd, J = 3.4, 13.6 Hz	3.38, dd, J = 6.7, 13.4 Hz	3.34, dd, J = 4.1, 14 Hz
2‴			4.18, overlapping
3‴	6.5, t, J = 6.8 Hz	6.48, t, J = 7.4 Hz	4.4, overlapping
4‴	2.94, dd, J = 6.8, 12.7 Hz	3.24, br.	2.98, br. dd, J = 3, 15.6 Hz
	3.35, dd, J = 6.8, 12.7 Hz	3.62, br.	3.1, br. dd, J = 2.8, 15.6 Hz
>NCH$_3$	2.4, s	2.68, br. s	2.4, s
CONCH$_3$	2.99, s	3.01, s	3.04, s

[a] Assignments for **10** were deduced from DQF COSY, HMBC, and HMQC; assignments for **11** and **12** were performed by decoupling experiments and comparison with the data from **10**. D_2O, 4.75 ppm. [b] Detected by HMQC.

Characterization of the Fatty Acid Moiety by Charge-Remote Fragmentation. For general application of the charge-remote techniques to fatty acids[36] the sample is dissolved in FAB matrix containing lithium or other suitable salt. The cationized analyte ions resulting from FAB ionization are then collisionally dissociated to produce a fragment ion series indicative of side-chain substitution or modification. The technique is notable because of its experimental simplicity and is also interesting from a mechanistic viewpoint, since the process belongs to a class of gas-phase ion decompositions not involving charge-site initiation of fragmentation. Numerous model compound studies[36,37] have demonstrated the usefulness of charge-remote fragmentation in assigning side-chain functionality, but there have been few reports of its use for structure determination[38] and none involving molecules with the

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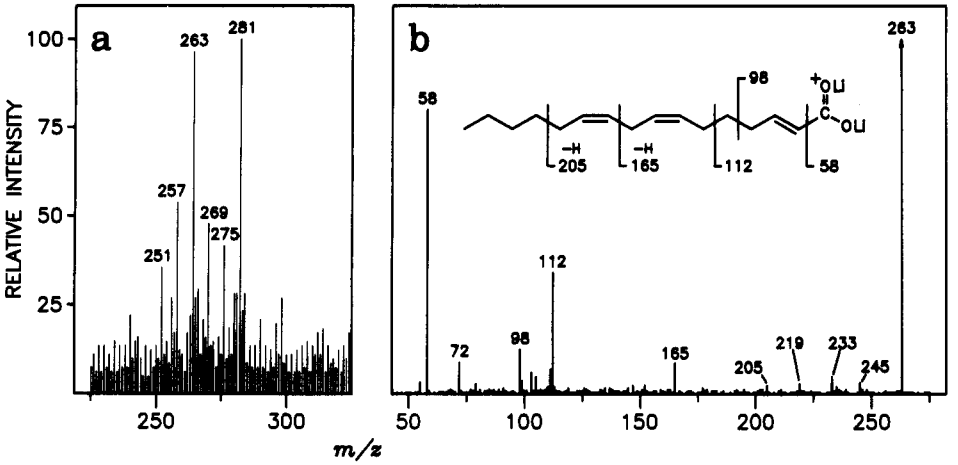

Figure 4. Determination of diene double bond positions in the lipid moiety of 1 by charge-remote fragmentation. (a) FAB mass spectrum of the fatty acid region of a LiOH hydrolysate of 1. See text for ion assignments. (b) CID mass spectrum from m/z 263 in panel a. The 2,3-double bond is shown as *trans* for convenience.

structural complexity of the liposidomycins.

Attempts to generate satisfactory CID mass spectra under a variety of conditions directly from liposidomycin A ions formed in MS-1 were not successful, but useful results were obtained using mild LiOH saponification of 1 prior to FAB analysis. When the resulting hydrolysate is mixed into neat glycerol, the resulting mass spectrum (Figure 4a) exhibits ions from the saponified fatty acid (MH^+ 269, MLi^+ 275, $(M - H + 2Li)^+$ 281) whose mass values are dictated by the molecular weight of the fatty acid moiety (Scheme I). Also observed are ions 18 u lower corresponding to dehydration of the 3-hydroxy function (m/z 251, 257, 263).

Collisional activation of the dilithiated ion m/z 263, the most suitable of the ion species for charge-remote fragmentation of the hydrocarbon chain,[36] produced the mass spectrum in Figure 4b.[39] Ions through m/z 112 in Figure 4b are assigned as charge-proximate fragmentation products and those above m/z 112 as principally representing charge-remote fragments.[37g] The principal diagnostic ions from the liposidomycin A fatty acid are the allylic cleavage product m/z 165 and the aliphatic ion series m/z 205, 219, 233 which represents charge-remote losses of C_nH_{2n+2} distal to the third double bond in the molecule. The mass 233 ion may also have contribution from loss of CO_2 from the molecular ion, a common process in high-energy CID reactions when a double bond is near the lithiated terminus.[37g] It is noted that both even- and odd-electron ions are recorded, an observation of mechanistic interest.[40] Particularly notable are the (odd-electron) distonic radical cations which occur at m/z 98 and m/z 112, which occur by simple homolytic bond cleavage of the two allylic sites in a 1,4-diene system as shown. These important charge-remote odd-electron fragments are vinylogous to m/z 72 and m/z 86, two principal odd-electron ions in the charge-remote fragmentation spectra of the corresponding saturated acids.[37f] The ions m/z 98 and m/z 112 are also prominent in the CID mass spectrum of lithiated *trans*-2-hexadecenoic acid (data not shown), implying that dehydration occurs at C-2,3 as shown. The low mass ions m/z 58 and 72 are assigned[36] as the charge-proximate radical cations $CO_2Li_2^{\bullet+}$ and $CH_2CO_2Li_2^{\bullet+}$, respectively.

A straightforward tally of these ions leads to a structural assignment of 2,7,10-hexadecatrienoic acid, the α,β-unsaturated product of 3-hydroxy-7,10-hexadecadienoic acid, **13**. The position of the hydroxyl group in the native acid has already been established for liposidomycin B (vide supra). The fact that lithiated 3-hydroxy fatty acids can lose water in the FAB matrix even when nonesterified was also established by comparing the primary and product ion scans of a model compound, 3-hydroxypalmitic acid, whose CID product ion spectrum (m/z 267) matches that of *trans*-2-hexadecenoic acid (data not shown). The discovery that liposidomycin A contains a CH_2-interrupted diene moiety was further confirmed by the results of ^{1}H-NMR (Table I), although its position in the chain could not be assigned from these data alone.

Corroboration of Liposidomycin A Fatty Acid Structure by Double-Bond Derivatization and Tandem Mass Spectrometry. The structure of the fatty acid side chain from 1 as 3-hydroxy-7,10-hexadecadienoic acid (**13**) was independently confirmed based on a variation of the dimethylamino alcohol procedure of Puzo et al.[41] In its original form, the technique involves epoxidation of the unsaturated fatty acid with *m*-chloroperbenzoic acid.[42] This is followed in a single step by ring-opening with aqueous dimethylamine to afford, after esterification, dimethylamino alcohol methyl esters which are analyzed by ammonia chemical ionization GC/MS. Each olefinic site gives rise to two ammonium ion fragments which reveal the original site of unsaturation. There have previously been few reported applications of the technique to multiply unsaturated or complex lipid-containing compounds.[43] In the present study, oxidation of the fatty acid was carried out using dimethyldioxirane[44] applied to intact 1, followed in a single step by conversion of the resulting epoxides to dimethylamino alcohols with concommitant saponification of the fatty acid, using aqueous dimethylamine. Dimethyldioxirane as a solution in aqueous acetone is advantageous in being miscible with lipid-containing compounds, and its high volatility permits preparation of clean, uncontaminated products directly amenable to FAB

(39) Collisional activation of the dilithiated hydroxy acid ion m/z 281 led almost entirely to fragmentation by loss of water, with insufficient information for characterization of double bond position.

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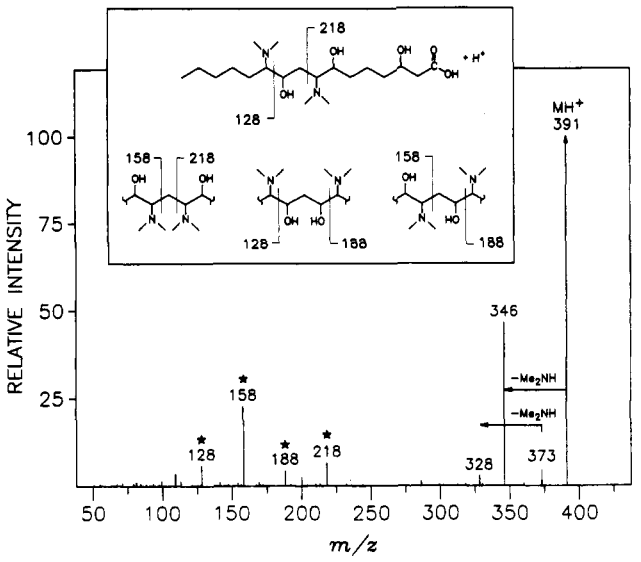

Figure 5. Collision-induced dissociation mass spectrum of the bis(dimethylamino) alcohol isomers of the fatty acid derivative from 1. Asterisks denote double-bond position-determining fragment ions. Ions m/z 373 and 328 arise from losses of H_2O from m/z 391 and 346, respectively. Structures show cleavage assignments for the four possible isomers.

ionization. Mass selection of the MH^+ ion followed by CID generates a set of favored ammonium cleavage ions which clearly mark the sites of the original double bonds. As shown by the substructure drawings in Figure 5, each olefinic site in the fatty acid should produce two possible dimethylamino alcohol isomers, each of which in turn yields two possible dimethylammonium ion fragments when subjected to collisional dissociation, giving a total of four possible ions per original double bond. The mass values of MH^+ selected for CID, as well as mass values of the ammonium fragments, are arithmetically related and are constrained by the mass spectrometrically determined molecular weight of the underivatized fatty acid established as in Scheme I. In addition, for each dimethylamino alcohol produced, one CID fragment occurs with a hydroxyl and one without; thus, the identity of any hydroxyl-bearing fragment is further confirmed by the presence of its dehydrated analog, 18 u lower. Therefore, each derivatized double bond, when subjected to this technique, gave up to six independent measures of its original location. Several of these derivatives were subjected to collisional dissociation, and the structure of liposidomycin A fatty acid was deduced therefrom.

The CID mass spectrum of the bis(dimethylamino)-alcohol (m/z 391) from 1 is shown in Figure 5. Following the expected losses of H_2O and Me_2NH there appears an unambiguous series of ions corresponding to the dimethylammonium fragments of each of the four regioisomers produced. This spectrum exhibits a complete set of dimethylammonium fragment ions, comprising a proof of double bond location and confirming the assignment as **13**, in support of the result obtained by charge-remote fragmentation.

Screening of Liposidomycins by CID Mapping

The construction of a CID map using tandem mass spectrometry, as initially carried out for **2**, Scheme I, provides an effective means for structural screening of biological isolates which may contain related members of the same family. This approach is relatively rapid, requires sample quantities of one microgram or less, and can be applied to isolates that have not been extensively purified.

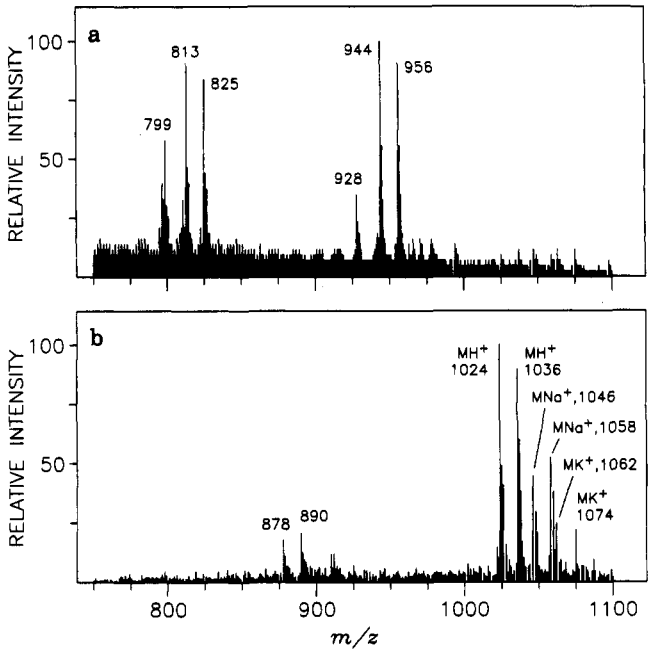

Figure 6. Mass spectra of crude liposidomycin fraction G. (a) FAB mass spectrum (MS-1 only). (b) Tandem mass spectrum for constant neutral loss of 80 u, representing ions in panel a which dissociate by elimination of SO_3.

Specific structural features can be tested for by carrying out appropriate constant neutral-loss experiments,[45] for example, using mass values corresponding to the subunits represented in Scheme I. Alternatively, product (daughter) ion scans can be used by entering the map at any point to test for the presence of a partial structure. Because the overall features of the dissociation map are established, an intermediate ion in the pathway need not necessarily be of sufficient abundance in the primary mass spectrum (from MS-1) to permit an informative CID mass spectrum to be acquired.

An example is given by the analysis of a crude liposidomycin fraction isolated by HPLC,[16] corresponding to the original designation liposidomycin G.[5,46] The primary FAB mass spectrum of the isolate, Figure 6a, exhibits ions representing several components but is without clear indication of molecular ions (MH^+). Constant neutral-loss measurement for 80 u, as a test for sulfate (Figure 6b), provides a conventional pattern of protonated and alkali molecule adducts showing components of mol. wt 1023 (designated liposidomycin H, MH^+ 1024) and 1035 (liposidomycin G, MH^+ 1036). The ions m/z 944 and 956 in Figure 6a are therefore due to loss of SO_3, while m/z 928 is concluded to represent a minor nonsulfated liposidomycin species which is not formed by loss of 80 u and shows no precursor ion in Figure 6b. The fragment ions m/z 813 and 825 in Figure 6a are thus part of the dissociation pathways of liposidomycins H and G, respectively, as listed in the CID map in Scheme I (with the exception that CID measurements on m/z 558 and 427 were not made). Ions of mass 878 and 890 arise from loss of 3-methylglutaric acid from m/z 1024 and 1036, respectively, and appear in Figure 6b because they are precursors to elimination of SO_3. Additional CID mass spectra, acquired as earlier discussed (cf. Figure 2), result in the remainder

(45) Yost, R. A.; Boyd, R. K. *Methods Enzymol.* **1990**, *193*, 154.
(46) The original A through L designations were made solely on the basis of HPLC profiles,[5] without benefit of analysis of each fraction by mass spectrometry. Subsequent molecular weight assignments and state of sulfation data for each of 13 fractions[5] corresponding to the original chromatographic designations are given in the supplementary material.

of assignments for H and G shown in Scheme I. The fatty acid subunits were found in this manner to constitute the sole structural differences compared with the central model 2, with liposidomycin H containing additional CH_2 (14 u) and G with additional C_2H_2 (28 u), presumably representing one olefinic unit. These results suggest that the charge-remote fragmentation technique described above could be directly applied to the crude HPLC fraction for assignment of the fatty acid structures, without the necessity of isolating either antibiotic, or either fatty acid.[47] In these examples the fatty acids have different molecular weights and so would be distinguished (as appropriate derivatives) by mass selection in MS-1. However, the approach described will lead to ambiguous conclusions in the event that more than one component in the mixture gives rise to subunit ions having the same mass, which are then selected for CID. In principle, precursor (parent) ion scan experiments could resolve such uncertainties, if the precursor ions are of different mass.

Experimental Section

General Procedures. The CD spectrum of 4 was obtained at a concentration of 250 μg/mL in H_2O. The scanning conditions were as follows: scanning speed 4 mm/min, temperature 25 °C, cell length 0.1 dm, scale 0.02°/cm. Trimethylsilyl derivatives for mass spectrometry were prepared on a scale of several μg in glass melting point capillaries, using either *N,O*-bis(trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane (Pierce, Rockford, IL) or *N,O*-bis([$^2H_9$]trimethylsilyl)acetamide with 1% [$^2H_9$]trimethylchlorosilane (Merck Isotopes, St. Louis, MO) and pyridine (1:10) by heating 1 h at 100–120 °C.

NMR Spectroscopy. Chemical shifts are reported relative to TMS in MeOH-d_4, residual DHO (^1H, δ 4.75, checked by external TSP), and external CCl_4 (^{13}C, δ 96, checked by dioxane = δ 66.5) in D_2O. HMBC spectra of 10 (3 mg) in D_2O were obtained by a published scheme[12] with 320 scans per t_1. Mixing times Δ_1 and Δ_2 in the sequence were 3.7 and 60 ms, respectively. The HMQC spectrum of 10 in D_2O was obtained by a previously described scheme[31] with 672 scans for each t_1 with a mixing time Δ of 3.75 ms.

Mass Spectrometry. CID mass spectra were acquired using a VG 70-SEQ instrument, consisting of a double-focusing magnetic sector mass analyzer (MS-1), rf-only quadrupole gas collision cell, and quadrupole mass analyzer (MS-2). All samples were dissolved in glycerol, or *O*-perdeuterioglycerol (Merck Isotopes; data in Figure 1), and ionized by fast atom bombardment by Xe, using a saddle-field type FAB gun operated at 7.2–7.6 kV and 1.1 mA discharge current. Precursor ions were mass-selected using MS-1 operated at resolution 1000. All CID spectra recorded by MS-2 were acquired at unit mass or greater resolution, and mass spectra were signal-averaged over five scans. CID mass spectra were produced using 30 eV of translational energy (E_{LAB}) of the incident precursor ion at a collision gas pressure corresponding to transmission of approximately 20% of the precursor ion beam, resulting in predominantly multicollision conditions. GC/MS measurements were made using electron ionization.

Culture Conditions and Isolation of Liposidomycins A, B, and C. *Streptomyces griseosporeus* was cultured at 28 °C for 28 h in tank fermentors as described previously.[6] The 1200-L culture was filtered, and the filtrate was adjusted to pH 7 with HCl. The solution was subjected to 60 L of Diaion HP-10. The eluent with 50% aqueous acetone was concentrated, and the resulting solution was extracted with BuOH. The organic layer was evaporated in vacuo. The resulting residue was purified by silica gel ($CHCl_3$, MeOH, 1:1 → 1:3), and active fractions were combined and concentrated under vacuum. The water-soluble part of the residue was purified by fast flow liquid chromatography (Silica Gel ODS-Q3, 0.1% Et_2NH–HCO_2H (pH 4)/CH_3CN (55:45)) and MCI-GEL chromatography (50% acetone–0.25 N NH_4OH). Active fractions were combined and concentrated in vacuo. The residue was finally purified by HPLC (Nucleosil-5 C_{18}, 0.1% Et_2NH–HCO_2H (pH 4)/CH_3CN (60:40)). After desalting by MCI gel, each fraction gave 2 mg of liposidomycin A (1), 8 mg of B (2), and 12 mg of C (3).

1: colorless powder; mp > 190 °C dec; $[\alpha]^{24}_D$ + 18.7° (*c* 0.3, H_2O); $C_{44}H_{67}N_5O_{21}S$ [$(M + H)^+$ *m/z* 1034, $(M + Na)^+$ *m/z* 1056, $(M + K)^+$ *m/z* 1072]; UV λ_{max} (H_2O) 260 nm ($E^{1\%}_{1cm}$ 60).

2: colorless powder; mp > 190 °C dec; $[\alpha]^{24}_D$ +17.3° (*c* 0.4, H_2O); $C_{42}H_{67}N_5O_{21}S$ [$(M + H)^+$ *m/z* 1010, $(M - H)^-$ *m/z* 1008, $(M + Na)^+$ *m/z* 1032, $(M + K)^+$ *m/z* 1048]; UV λ_{max}(H_2O) 262 nm ($E^{1\%}_{1cm}$ 72); ^{13}C-NMR (δ, ppm in CD_3OD) 167.7, 173.8, 172.6, 171.2, 166.4, 152, 142.5, 110.1, 102.4, 92, 83.5, 81.6, 79.9, 79, 75.8, 73.6, 71.9, 70.5, 70.2, 67.5, 64.6, 58, 43.5, 41.8, 41.2, 40.3, 40.2, 39, 37.2, 35.1, 31, 30.7, 30.6, 30.4, 29.1, 28.7, 28.5, 26.3, 23.1, 20.2, 11.6.

3: colorless powder; mp > 190 °C dec; $[\alpha]^{24}_D$ +18.9° (*c* 0.9, H_2O); $C_{42}H_{67}N_5O_{21}S$ [$(M + H)^+$ *m/z* 1010, $(M + Na)^+$ *m/z* 1032, $(M + K)^+$ *m/z* 1048]; UV λ_{max}(H_2O) 262 nm ($E^{1\%}_{1cm}$ 69); ^{13}C-NMR (δ, ppm in CD_3OD) 173.6, 172.2, 170.8, 166.2, 142.3, 109.8, 102, 91.9, 83.2, 81.4, 79.7, 78.5, 75.6, 73.6, 71.7, 70.6, 70, 66.9, 64.2, 57.8, 43.3, 41.6, 41.5, 41.1, 40.4, 38.7, 37, 34.9, 32.8, 30.5, 30.4, 30.35, 30.3, 30.2, 28.5, 26.1, 23.5, 19.9, 14.2, 11.3.

Acid Hydrolysis of Liposidomycins B and C. A mixture of liposidomycins (26.9 mg) was dissolved in 2 mL of 3 N HCl and 0.2 mL of *n*-BuOH (procedure A). The resulting solution was heated at 100 °C in a sealed tube. After 3 h, the reaction mixture was extracted with EtOAc. The aqueous layer was evaporated under vacuum, and the resulting residue was purified by preparative silica gel TLC (BuOH–MeOH–H_2O (4:1:2)) to give 6.6 mg of white powder 4.

The acid hydrolysis was also carried out by the above procedure by using 0.5 mg of pure 2 or 3, respectively (procedure B), or by hydrolysis of 50 μg of 2 under more gentle conditions by 0.5 N trifluoroacetic acid at 70 °C for 1 h (procedure C). Methyl esters of fatty acids were obtained by methylation of the residue from the organic layer with 5% HCl–MeOH and then analyzed by GC/MS. Fatty acid from 2: methyl 3-hydroxy-12-methyltridecanoate: $(M - H_2O)^+$ *m/z* 240.2091 $C_{15}H_{28}O_2$, $(C_4H_7O_3)^+$ *m/z* 103, from FAB-MS, MH^+ 245.2115. Fatty acid from 3: methyl 3-hydroxytetradecanoate: M^+ *m/z* 258.2189, $(M - H_2O)^+$ *m/z* 240.2054, $(C_4H_7O_3)^+$ *m/z* 103. The aqueous layer was evaporated and subjected to HPLC (RP-ODS, A: 0.25 M NH_4OAc, pH 6, B: H_2O–CH_3CN (60:40), A → B).[48] The major HPLC fraction 4 was trimethylsilylated and analyzed by mass spectrometry. EI: 4-$(TMS)_5$, M^+ *m/z* 786; 4-$(TMS)_4$, M^+ *m/z* 714.786; 4-$(TMS\text{-}d_9)_4$, M^+ *m/z* 750; FAB-MS 4-$(TMS)_4$, MH^+ *m/z* 715.

4: ^{13}C-NMR (δ, ppm in D_2O); 167, 152.6, 143.2, 143.1, 123.7, 103.3, 90.0, 85.2, 74.3, 71.6, 67.6, 51.9, 34.6, CD; $\Delta\epsilon = -9.42$ (H_2O, 260 nm).

Methanolysis of Liposidomycin C (2). Liposidomycin C (18 mg) was dissolved in 10 mL of 5% HCl–MeOH. The resulting mixture was heated at 110 °C in a sealed tube. After 17 h, the reaction mixture was evaporated in vacuo and water added. The resulting solution was extracted with Et_2O. The organic layer was evaporated under vacuum to give 1.4 mg of oily material (methyl 3-hydroxytetradecanoate). The aqueous layer was evaporated in vacuo and purified by preparative silica gel TLC (BuOH–MeOH–H_2O (4:1:2)) to give 0.7 mg of 4 and 1.0 mg of the monomethyl ester of 4.

Methanolysis of Liposidomycins. A mixture of liposidomycins (30 mg) was dissolved in 10 mL of 5% HCl–MeOH in a Teflon-sealed tube. The reaction was carried out in a Toshiba ER-200 microwave oven. After 4 min, the reaction mixture was evaporated under vacuum, and the residue was purified by preparative cellulose TLC (BuOH–MeOH–H_2O (4:1:2)) to yield a mixture of 4 and 9. Further purification by cellulose TLC (PrOH–1 N NH_4OH (7:3)) yielded 2 mg of 4 and 3 mg of 9.

Alkaline Hydrolysis of Liposidomycins. A crude mixture of liposidomycins (400 mg) was dissolved in 100 mL of 0.035 N NaOH and warmed for 4 h at 37 °C. The reaction mixture was subjected to Dowex 50 w (H^+) and eluted with 0.5 N NH_4OH. Cellulose TLC (BuOH–MeOH–H_2O (4:1:2)) gave 5.2 mg of 10 and 3 mg of 11. FAB-MS: 10, MH^+ *m/z* 558.2053, calcd for C_{22}-

(47) Further structural studies on these fatty acids and other liposidomycins[46] are not planned.

(48) Buck, M.; Connick, M.; Ames, B. N. *Anal. Biochem.* **1983**, *129*, 1.

$H_{32}N_5O_{12}$, 558.2048; 11 MH^+ m/z 638.

Reductive Deacylation of Liposidomycins with $LiBH_4$. Under an argon atmosphere, to a solution of a crude mixture of liposidomycins (123 mg) in 20 mL THF was added 10 mg of $LiBH_4$. The reaction mixture was refluxed for 3 h. After the reaction mixture was cooled on an ice bath, the solution was adjusted to pH 4 by adding 5% HCl–MeOH. The resulting solution was extracted with EtOAc, and the aqueous layer was evaporated in vacuo. The residue was purified by preparative cellulose TLC (PrOH–1 N NH_4OH (7:3)). Further purification by cellulose TLC (BuOH–MeOH–H_2O (4:1:2)) gave 10.2 mg of a white powder, which was purified by high-voltage paper electrophoresis (0.1 N Py-HCOOH, pH 2 buffer, 2500 V, 170 mA) to give 1 mg of a pure powder 12: FAB-MS MH^+ m/z 656.

Saponification of Liposidomycin A (1) by LiOH. 1 (5–15 μg) was dissolved in 5 μL of 35 mM LiOH (previously purged with N_2 to remove dissolved oxygen). The glass reaction tube was sealed and incubated ca. 5 h at 37 °C (pH > 9), at which point it was neutralized with 0.25 M NH_4OAc (pH 6) and taken to dryness with a Speed-Vac. The dried residue was then taken up in 2–3 μL of H_2O and mixed directly into neat glycerol for analysis by FAB-tandem mass spectrometry.

Preparation of Amino Alcohol Derivatives of Fatty Acid from 1. Derivatives for charge-initiated fragmentation reactions were prepared by the addition of dimethyldioxirane[44] (ca. 100 mM in aqueous acetone) to a dried sample of 1, contained in 5-mm o.d. glass sample tubes. The reagent was added in moderate excess, mixed, and allowed to stand approximately 45 minutes at room temperature. The highly volatile reagent was then removed via Speed-Vac, and the pure, dry epoxides were treated with excess Me_2NH (40% aqueous solution). Sample tubes were then flame sealed and placed in a 105 °C oven (t > 16 h). The tubes were subsequently scored and opened and the reagent removed by Speed-Vac, yielding a dry mixture of dimethylamino alcohols, which were transferred in MeOH to glycerol for FAB-tandem mass spectrometry.

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Supplementary Material Available: CID mass spectra of m/z 427 and 558 positive ions and m/z 592 negative ion from **2**, ^1H NMR data for 1 and 3, HMBC, DQF COSY, and HMQC spectra of **10**, a HPLC chromatogram of liposidomycin extract, and molecular weights of liposidomycin components (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Crown Ether Derivatives of Tetrathiafulvalene. 1

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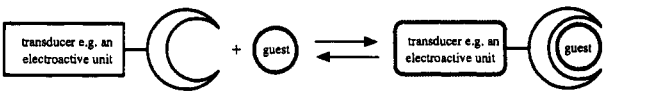
A synthetic procedure leading to derivatives of tetrathiafulvalene (TTF) incorporating crown ether ligands has been developed. The properties of such redox-active ionophores were studied by cyclic voltammetry (CV), plasma desorption mass spectrometry (PDMS), and ^1H-NMR.

Introduction

In recent years substantial progress has been reported in the fields of redox-active macrocyclic ligand systems,[1-4] that is, systems in which the electronic properties of a redox-active center can be influenced by complexation of a guest molecule in another part (the "antenna") of the molecule or vice versa. Such compounds might be used for redox-controlled release or uptake of guest molecules or as sensor molecules where complexation of ions or molecules is detected by a change in redox properties of the host. At the moment, this area is fairly new and applications are rather few, but the progress in host–guest chemistry, supramolecular chemistry, and general synthetic organic chemistry will undoubtedly lead to an increase of activities in this field. It is now possible to carry out creative design of tailor-made molecules by relatively simple combinations of well-studied ligand systems with redox-active moieties.

Scheme I



Sensormolecule — Complex with altered physical properties

In most of the previously studied cases that are relevant to this study the antenna is of the crown ether type and is situated in close proximity to a redox-active center (the "transducer") which in all cases incorporates a transition metal. Some examples are given in Figure 1. The recent system 1 was reported by van Veggel et al.[2] (M_1: Cu, Ni, Zn; M_2: Ba^{2+}, Cs^+; X: O, S) and showed significant shifts (approx. 90 mV) of redox potentials on substituting Ba^{2+} for Cs^+. The systems 2–5 were reported by Green et al.[3] Surprisingly, the redox potential of 2 (cp = cyclopentadienyl) was insensitive to the presence of alkali metal ions. However, significant shifts were observed in the case of 3–5. The largest shifts were observed when 3 was treated with sodium ions, but 400 equiv of sodium ions were necessary before the maximum shift (110 mV) was reached. The 15-crown-5 derivative 5 reached saturation at 70 mV, but after addition of only 5–7 equiv of sodium ions. A very interesting system 6 based on ferrocene has been reported recently by Gokel et al.[4] In that study the

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