solution was then cooled to 0 °C, under an argon atmosphere, and the crude chloroformate solution was added. The resulting mixture was stirred at 0 °C for 30 min and then at room temperature for 3 h. The reaction was then diluted with chloroform and washed with pH 4.0 buffer, and the aqueous phase was back-extracted with chloroform. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated to afford 156 mg (87%) of **35**: IR (KBr) 1795, 1712, 1620 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 8.56 (s, 1 H, C-2H), 8.09 (d, J = 12, 1 H, C-5H), 7.29 (s, 1 H, C-8H), 6.11–5.92 (m, 2 H, vinyl CH's), 5.69 (m, 1 H, β-lactam CH), 5.61–5.27 (m, 4 H, vinyl CH_2's), 5.53, 5.21 (AB q, J = 7.9, 2 H, CH_2OCON), 4.85–4.67 (m, 4 H, allyl CH_2O's), 3.81, 3.53 (ABX, J_{AB} = 16.4, J_{AX} = 3.8, J_{BX} = 1.4, 2 H, β-lactam CH_2), 3.73 (bs, 4 H, CH_2N), 3.43 (m, 1 H, cyclopropyl CH), 3.25 (bs, 4 H, CH_2N), 1.33 (m, 2 H cyclopropyl CH_2), 1.14 (m, 2 H cyclopropyl CH_2); MS m/z 639 (M + H). Anal. Calcd for $C_{31}H_{31}N_4O_8FS$: C, 58.30; H, 4.89; N, 8.77. Found: C, 58.15; H, 4.99; N, 8.77.

***rac*-3-[[[[4-(3-Carboxy-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-quinolinyl)-1-piperazinyl]carbonyl]oxy]methyl]-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Monosodium Salt (36).** A solution of **35** (60 mg, 0.10 mmol) in 1.5 mL of 1:1 dichloromethane/ethyl acetate, containing triphenylphosphine (10 mg, 0.04 mmol) and sodium 2-ethylhexanoate (53 mg, 0.32 mmol), was cooled to 0 °C under an argon atmosphere. Tetrakis(triphenylphosphine)palladium(0) (11 mg, 0.01 mmol) was added and the mixture was stirred at 0 °C for 40 min. The mixture was then precipitated from ether and the solid portion was purified by chromatography on C_{18} silica gel using a gradient from 1:9 to 1:1 acetonitrile/water as eluant to afford 7.4 mg (13%) of **36**: IR (KBr) 3425, 1773, 1708, 1628 cm^{-1}; ^1H-NMR (D_2O + trace CD_3CN) δ 8.70 (s, 1 H, enone CH), 7.8 (m, 1 H, ArH), 7.55 (m, 1 H, ArH), 5.74 (bs, 1 H, C-5 H), 5.52, 5.17 (AB, J = 14.8, 2 H, $CH_2O(CO)$), 3.81, 3.53 (AB of ABX, J_{AB} = 17.2, J_{AX} = 3.0, J_{BX} = 0, 2 H, C-6 CH_2), 3.70 (m, 4 H), 3.35 (m, 5 H, NCH and piperazine CH_2's), 1.4 (m, 2 H, cyclopropyl CH_2), 1.1 (bs, 2 H, cyclopropyl CH_2); MS m/z 581 (M + H).

[5*R*]-[5α-6-α(*R)]-6-(1-Hydroxyethyl)-3-(hydroxymethyl)-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Monosodium Salt (37).** A solution of **24** (1.1 g, 2.14 mmol) in dry tetrahydrofuran (200 mL) was stirred under an atmosphere of argon in an icewater bath. Glacial acetic acid (1.15 mL, 10.1 mmol) was added dropwise over 1 min, followed by the dropwise addition of 1 M tetrabutylammonium fluoride (8.6 mL, 8.6 mmol) over 10 min. After the last addition, stirring was continued and the bath was allowed to slowly come to room temperature. Stirring was continued at room temperature for 24 h. The reaction mixture was allowed to stand at room temperature for an additional 20 h and concentrated to dryness under reduced pressure on a rotary evaporator (bath temperature < 25 °C). Ethyl acetate (300 mL) was added to the residue and washed by extraction with icewater, cold saturated sodium bicarbonate, and again with water. Following drying over magnesium sulfate, the desiccant was filtered off and the ethyl acetate evaporated. The residue was chromatographed on silica gel, eluting with 1:1 ethyl acetate/hexane to afford 192 mg (31%) of the diol.

A solution of the diol obtained above (190 mg, 0.67 mmol), ethyl acetate (10 mL), methylene chloride (10 mL), sodium 2-ethylhexanoate (165 mg, 1.0 mmol), triphenylphosphine (35 mg, 0.134 mmol), and tetrakis(triphenylphosphine)palladium(0) (38 mg, 0.033 mmol) was stirred under an atmosphere of dry argon for 1 h in an icewater bath. Several volumes of ether were added, and the precipitate was centrifuged. The insoluble portion was triturated with ether and centrifuged three times. The crude product was dissolved in water and chromatographed on C_{18} silica gel, eluting with water to afford 115 mg (64%) of the known diol **37**.[11,20]

Acknowledgment. We thank Dr. George Beskid and Ms. Anne M. Bertasso for their help during the course of these studies. We also thank Ms. Betty Ann Hedemus for the preparation of this manuscript.

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Synthetic Modification of a Novel Microbial Ionophore: Exploration of Anticoccidial Structure–Activity Relationships

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While fermentation-derived polyether ionophores such as salinomycin are the dominant class of anticoccidial feed additives, there is little information concerning the structural features which confer optimal potency/efficacy in this important series. The recently discovered microbial polyether **1a**, featuring potent, broad-spectrum anticoccidial activity, was employed as a template to explore structure–activity relationships. A number of single-step synthetic modifications targeted structural changes in both the lipophilic carbon backbone and the ion-binding cavity of **1a**. Although previous semisynthetic transformations among the polyether ionophores almost always resulted in a substantial loss of anticoccidial activity, we obtained several analogues, altered on the periphery of the ionophore–ion complex, which retain good potency and efficacy. Monoglycone **7** (semduramicin sodium) has the most impressive anticoccidial profile of this series, and is undergoing further biological testing under field conditions.

Avian coccidiosis, an enteric protozoal infection caused by pathogenic species of *Eimeria*, is a ubiquitous problem in the high-intensity rearing systems characteristic of the poultry industry.[1] This has resulted in a universal dependence on anticoccidial feed additives affording prophylactic disease control. Fermentation-derived polyether carboxylic acid ionophores, such as salinomycin and monensin, have been the dominant class of anticoccidial feed additives for nearly two decades. They have achieved this position because they provide excellent disease control and are relatively refractory to resistance development.[2] The anticoccidial utility of the ionophore antibiotics is well established, but there is little information concerning the structural features which confer optimal potency/efficacy in this important series.[3] While semisynthetic modifica-

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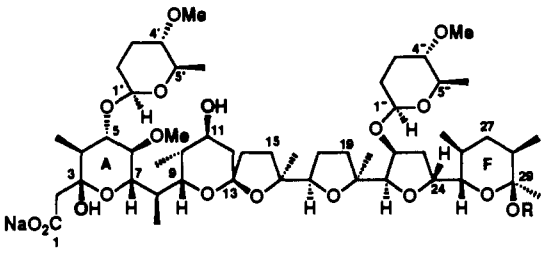
tion has proved to be a useful tool for understanding structure–activity relationships and enhancing biological activity for other natural products, its application to the carboxylic acid ionophores has been relatively limited.[3] Cullen et al. recently discovered a novel microbial polyether **1a** (UK-58,852) with potent, broad-spectrum anticoccidial activity.[4] This paper describes the use of **1a** as a template for the systematic exploration of anticoccidial structure–activity relationships through a series of single-step synthetic transformations.[5]



1a; R = H
1b; R = Me

Although the anticoccidial mode of action of the ionophores is not unambiguously established, it is thought to be related to the ability of these molecules to solubilize physiologically important cations in, and transport them across, lipophilic cell membranes.[2c,6] The diffusion of cations through lipid-rich membranes is facilitated by the characteristic molecular configuration of the ionophores, in which a cation such as sodium or potassium is reversibly bound by an array of polar oxygen ligands in a cavity surrounded by a lipophilic exterior carbon backbone.[7a] The lipophilic nature of the resulting ionophore–cation complex is an important factor in the catalysis of ion

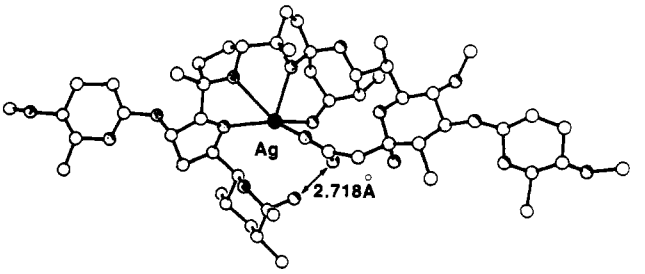


Figure 1. Crystal structure of **1a** (silver salt).

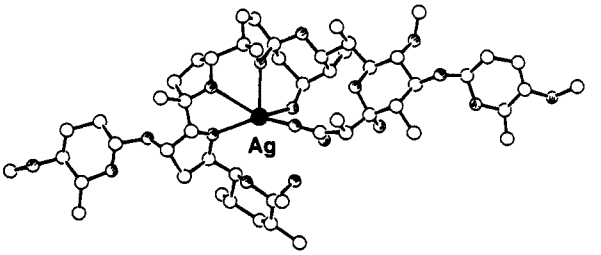
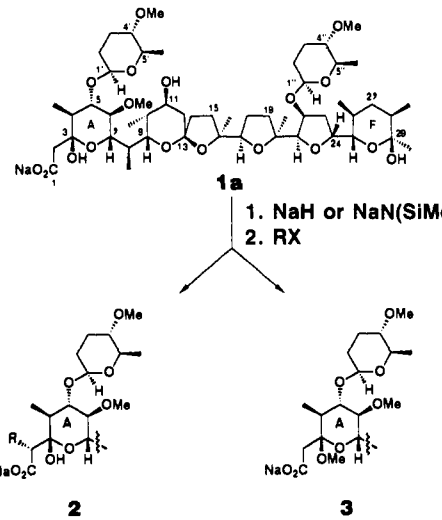


Figure 2. Crystal structure of **2a** (silver salt).

Scheme I



R[a]	Compound 2
CH_3	a
CH_2CH_3	b
$CH_2CH_2CH_3$	c
$CH_2CH=CH_2$	d
$CH_2C\equiv CH$	e,f

[a] stereochemistry not assigned at C-2 for 2b-f

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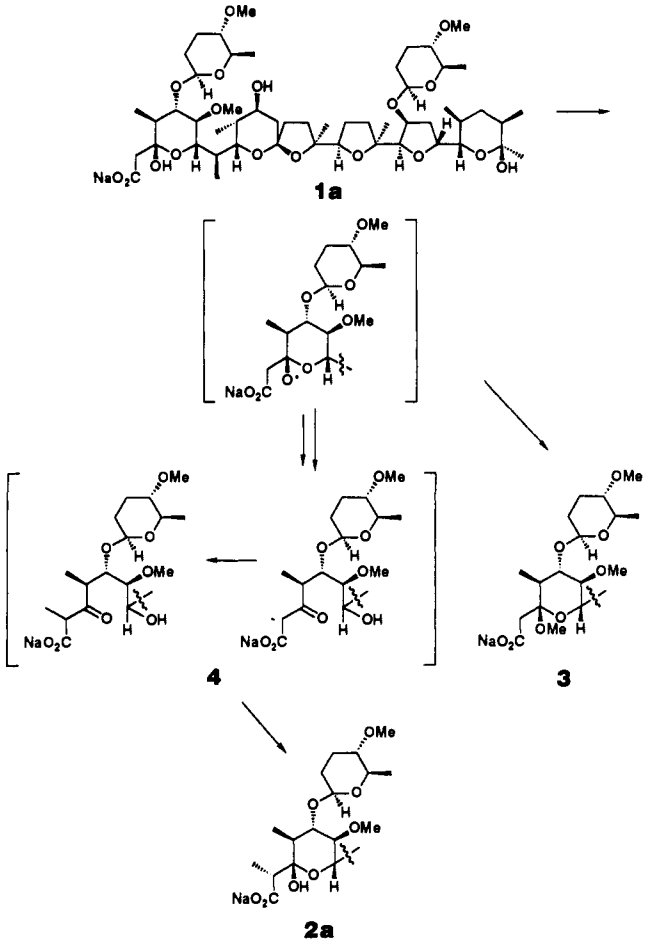
Scheme II



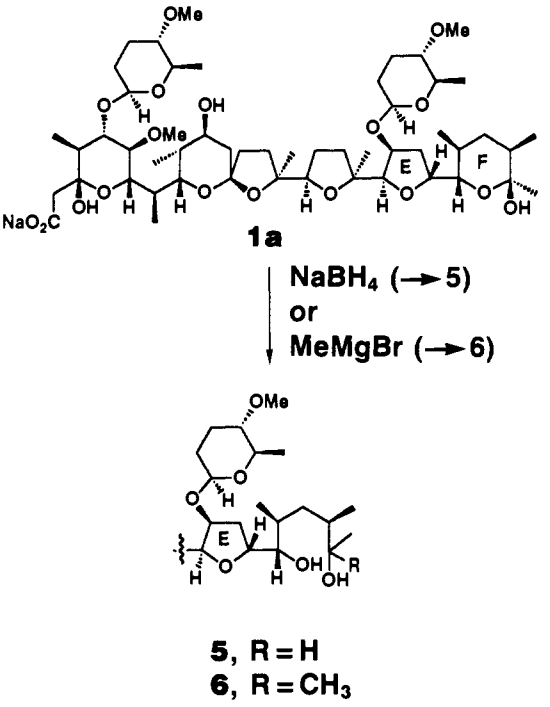
transport across lipid bilayers.[7b] We have explored the relationship between anticoccidial activity and structural changes in both the lipophilic carbon backbone and the ion-binding cavity of **1a**.

Chemistry

The X-ray structure of **1a** (Figure 1) reveals that the C-11 hydroxyl is directly involved in ion complexation, while the C-29 hydroxyl is within 2.718 Å of the carboxyl carbon and can participate in the formation of a strong hydrogen bond. This hydrogen bond is a key factor in the maintenance of the cage-like ionophore structure.[7a] Our strategy for modifying the ion-binding cavity of **1a** was based in part on etherification of these hydroxyl moieties. Etherification at the F-ring hemiketal is well precedented for ionophores related to **1a**, and is readily accomplished by acid-catalyzed reaction with low molecular weight alcohols.[3a] Accordingly, methyl ketal **1b** can be prepared from **1a** by treatment of the free acid with methanol.[4a] Ketal **1b** may also be obtained as a coproduct during the preparation of **1a** by fermentation.[4a]

Ether formation at the C-11 or C-29 position was not observed under basic alkylation conditions. Rather, this methodology resulted in an unexpected series of analogues substituted at C-2 of the carbon backbone (Scheme I), along with a small amount of ether formation at the C-3 hydroxyl of the A-ring hemiketal. Specifically, treatment of **1a** with 1 equiv of sodium hydride [or sodium bis(trimethylsilyl)amide] and excess iodomethane (1.5 equiv) in THF at ambient temperature afforded a mixture of products. Following silica gel chromatography, we isolated significant quantities (31%) of unchanged **1a**, along with a novel 2-methyl derivative (**2a**, 20%) and an isomeric A-ring methyl ketal (**3**, 4%); F-ring ketal **1b** was not detected in the crude product mixture. The absolute stereochemistry of compound **2a** was established by X-ray crystallography (Figure 2).[8] These reaction conditions were also employed to prepare several congeneric 2-alkyl derivatives (**2b-f**), albeit in low yield. The formation of ketal **3** is thought to proceed by alkylation of the corresponding alkoxide. Alternatively, alkylation of the unsubstituted methylene group of **1a** might involve the intermediacy of keto acids **4** (Scheme II).

Scheme III



Other approaches to altering the structure of the ion-binding cavity involved cleavage of the F-ring ketal. Nucleophilic ring opening of hemiketals with either hydride[9] or Grignard reagents[10] has been described for other ionophores, and this chemistry was readily applied to **1a**. Reaction of **1a** with sodium borohydride (Scheme III) gave the dihydro derivative **5** as a mixture of diastereomers (95% yield). Opening of the F-ring ketal was also accomplished by reaction of **1a** with excess methylmagnesium bromide, providing the *gem*-dimethyl analogue **6** in modest yield (30%).

The X-ray structure of **1a** (Figure 1) also indicates that the A- and E-ring deoxy sugars are part of the lipophilic scafolding surrounding the ion-binding region of **1a**. We viewed the hydrolytic cleavage of these groups as a straightforward approach to altering the properties of the **1a** backbone (Scheme IV). Thus, treatment of **1a** with *p*-toluenesulfonic acid (1.3 equiv) in acetonitrile/water (5/3) afforded a mixture of monoglycone[5] **7** and aglycone **8**. The composition of the reaction mixture varied with time. After 4 h, monoglycone **7** was the predominant

(8) Corresponding α-diastereomer was not isolated, but its presence in the crude reaction product cannot be ruled out; alkylation of the related ionophore maduramicin with methyl iodide afforded both α- and β-diastereomers (data not shown).

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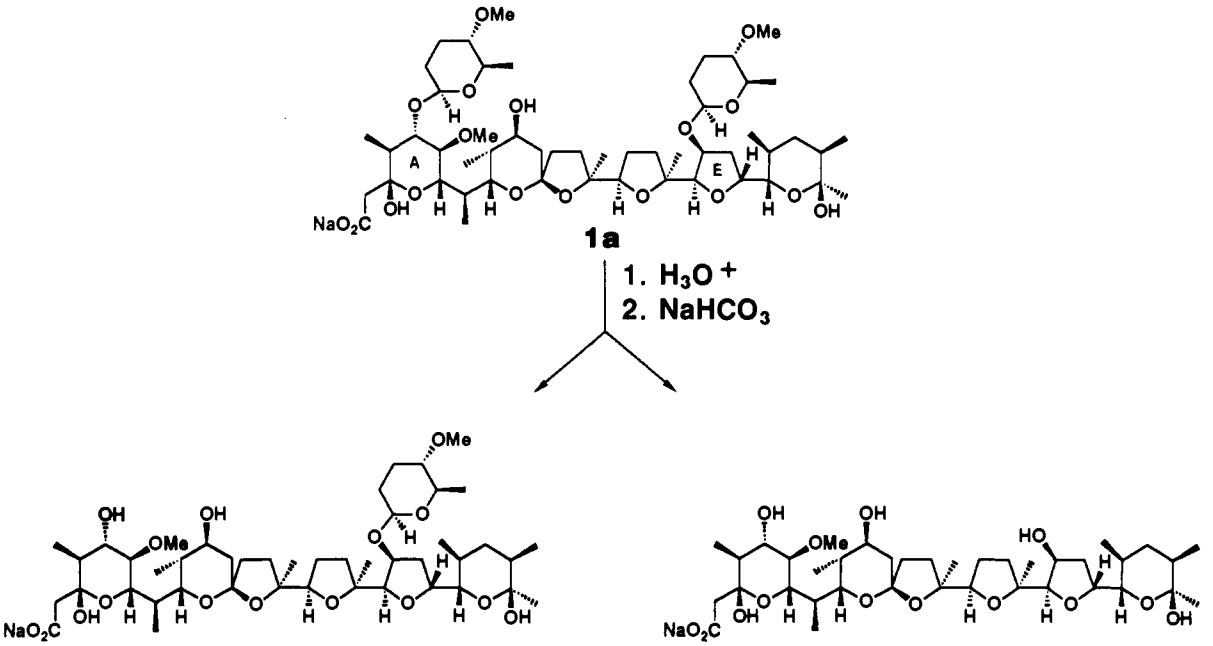
Scheme IV



hydrolysis product, while 7 and 8 were present in almost equal amounts at 24 h. Interestingly, the monoglycone resulting from cleavage of the E-ring sugar alone was not observed.

Results and Discussion

The fermentation derived ionophore **1a** and its derivatives were evaluated in chickens against *Eimeria tenella* (cecal coccidiosis). Test compounds were administered continuously in feed from 1 day prior to infection until the birds were sacrificed to measure lesions at 6 days after infection.[11] Anticoccidial activity is reported in Table I as the minimum concentration (MEC) required to provide control of lesions equivalent to noninfected, nonmedicated birds. The ionophores were also tested against *Eimeria tenella* in vitro[12] in order to assess potential differences in intrinsic potency in this series.

Among analogues modified on the periphery of the ionophore–cation binding complex (**2**, **7**, and **8**), only aglycone **8** lacked significant anticoccidial activity either in vivo (MEC > 50 ppm) or in vitro. The remaining ionophores in this group were not substantially different in the in vitro *Eimeria* assay, but were readily distinguished on the basis of anticoccidial potencies observed in the chick screen. In contrast to aglycone **8**, monoglycone **7** was fully efficacious in the chick model down to 25 ppm. With an MEC of 7.5 ppm, the 2-methyl analogue **2a** is the most potent **1a** derivative in this series, and is among the most potent anticoccidial ionophores described in the literature.[2a] Nevertheless, small changes in chain length at the C-2 position beyond methyl (**2b–f**) resulted in a substantial drop in potency. In addition, stereochemical configuration at this position also seems important. The propargyl analogue **2f** provided excellent control of lesions at 60 ppm, but diastereomer **2e** was completely inactive at this dose level. Although analogues featuring alterations at or near ion-binding ligands (**1b**, **3**, **5**, and **6**) had intrinsic anticoccidial activities similar to those modified at the perimeter of the molecule, none of the compounds in this structural category exhibited in vivo potency comparable to that of the parent ionophore. The F-ring methyl ketal **1b** had the best activity in this group, but was only one-third as potent as **1a**.

Table I

compd	IC_{50}[a]	MEC[b]
1a	0.03	15
1b	0.02	50
2a	0.03	7.5
2b	c	30
2c	0.01	60
2d	0.01	60
2e	0.01	>60
2f	0.01	60
3	c	120
5	0.05	80
6	0.03	>120
7 (semduramicin sodium)	0.03	25
8	>1.0	>50

[a] Drug concentration (ppm) which inhibits growth of *E. tenella* by 50% in vitro relative to nonmedicated controls. [b] Minimum concentration (ppm) of drug in feed which provides control of *E. tenella* lesions equivalent to noninfected, nonmedicated controls. [c] Not determined.

Conclusions

With the exception of aglycone **8**, we have found a wide range of synthetic transformations around **1a** to be consistent with good intrinsic anticoccidial activity. Conversely, in vivo anticoccidial potency varied considerably among these semisynthetically derived ionophores, with the most potent analogues featuring alterations in the carbon backbone of **1a**. Whether these results reflect differences in drug metabolism and distribution is unknown, but it is expected that the series of closely related ionophores generated in this work will function as tools

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to more fully understand the complex structural and physicochemical requirements for in vivo activity in this important anticoccidial class.

While previous semisynthetic transformations among the polyether ionophores have almost always resulted in a substantial loss of anticoccidial activity,[3] our work with **1a** has provided several new analogues which retain good potency and efficacy. Monoglycone **7** (now designated semduramicin sodium[13]) has proven to have the most impressive anticoccidial profile in more extended testing,[14] and biological characterization of this novel ionophore under field conditions is now underway.

Experimental Section

Melting points (uncorrected) were determined on a Thomas-Hoover capillary apparatus. ^{13}C-NMR spectra were recorded on a Bruker AM250 or a Varian XL300 instrument using deuteriochloroform ($CDCl_3$) with tetramethylsilane as internal standard. FAB-MS were obtained on a VG Analytical 70/250-S mass spectrometer using a dithiothreitol–dithioerythritol (3:1) matrix. Optical rotations were recorded using a Perkin-Elmer 141 polarimeter.

Alkylation of 1a: 2a–f and 3. To a 500-mL round-bottom flask containing 80 mL of dry tetrahydrofuran was added 1.12 g (0.028 mol) of sodium hydride (60% dispersion in mineral oil). The solvent was drawn off to remove the mineral oil, and 180 mL of additional THF was introduced. This was followed by the addition of 2.6 mL (5.96 g, 0.042 mol) of iodomethane. The reaction was cooled to 5 °C with an ice bath, and 28.6 g (0.028 moles) of antibiotic **1a** was added in portions as a solid. The reaction mixture was stirred under nitrogen and allowed to warm to ambient temperature. The progress of the alkylation was followed by TLC, and after 2 h, a small volume of water was added, and the reaction was concentrated to a hard foam. This material was dissolved in dichloromethane, washed with water and saturated aqueous sodium chloride and dried over anhydrous sodium sulfate. Resolution of the complex product mixture was accomplished by silica gel chromatography (Waters Prep 500; 2–4% ethanol/dichloromethane). The following materials were isolated and characterized:

(a) 7.3 g of an amorphous solid, less polar than **1a** by HPLC analysis; this material was further purified by recrystallization from heptane to give 5.59 g (20%) of **2a** as a crystalline compound: mp 194–195 °C; $[\alpha]^{25}_D$ = 12.4° (c = 0.5, MeOH); positive FAB-MS had diagnostic peaks at m/z 1038 $(M + Na)^+$ and 976 $(M - Na - CO_2 - H_2O)^+$; NMR ($CDCl_3$) δ_c 180.96 (0), 107.48 (0), 103.27 (1), 102.49 (1), 99.77 (0), 96.88 (0), 86.98 (1), 84.51 (0), 84.21 (0), 82.63 (1), 82.41 (1), 82.15 (1), 80.92 (1), 80.57 (1), 80.29 (1), 79.95 (1), 74.45 (1), 74.68 (1), 72.97 (1), 70.13 (1), 67.70 (1), 67.31 (1), 59.82 (3), 56.90 (3), 56.87 (3), 46.69 (1), 40.90 (1), 39.99 (1), 38.96 (2), 36.51 (2), 33.85 (2), 33.66 (1), 33.60 (1), 33.58 (2), 33.25 (1), 32.62 (2), 32.31 (2), 31.90 (2), 31.15 (2), 30.65 (2), 27.42 (2), 27.35 (3), 27.02 (2), 26.05 (3), 23.34 (3), 18.34 (3), 18.45 (3), 17.55 (3), 17.07 (3), 11.56 (3), 11.67 (3), 11.02 (3), 10.38 (3); absolute stereochemistry assigned by X-ray (Figure 2). Anal. ($C_{53}H_{89}O_{18}Na$) C, H.

(b) 9.1 g (31%) of unchanged **1a**, characterized by comparison with an authentic sample.

(c) 1.3 g (4%) of **3** as an amorphous solid, mp 182–193 °C; positive FAB-MS had diagnostic peaks at m/z 1059.6 $(M + 2Na - H)^+$, 1037.6 $(M + Na)^+$, 961.6 $(M + Na - CO_2 - CH_3OH)^+$; NMR ($CDCl_3$) δ_c 176.31 (0), 107.28 (0), 103.09 (1), 102.02 (1), 101.86 (0), 97.11 (0), 86.91 (1), 84.41 (0), 83.99 (0), 82.33 (1), 81.50 (1), 80.75 (1), 80.38 (1), 79.86 (1), 79.57 (1), 74.54 (1), 74.34 (1), 73.15 (1), 71.60 (1), 70.00 (1), 65.06 (1), 59.91 (3), 56.72 (3), 47.67 (3), 42.92 (2), 39.97 (1), 39.83 (1), 38.80 (2), 36.42 (2), 34.69 (1), 33.66 (2), 33.43 (2), 33.29 (1), 33.10 (1), 32.52 (2), 32.29 (2), 30.87 (2), 30.53 (2), 27.62 (3), 27.22 (2), 27.03 (2), 26.89 (2), 26.31 (3), 23.45 (3), 18.32 (3), 18.18 (3), 17.21 (3), 16.87 (3), 11.23 (3), 10.40 (3), 8.16 (3). Anal. ($C_{53}H_{89}O_{18}Na \cdot H_2O$) C, H.

According to a procedure similar to that described above for compound **2a**, additional C-2 alkyl analogues were prepared:

2b. To a 75-mL round-bottom flask containing a solution of 10.22 g (0.01 mol) of antibiotic **1a** in 30 mL (0.375 mol) of ethyl iodide was added 10.0 mL of a 1.0 M solution (0.01 mol) of sodium bis(trimethylsilyl)amide in THF. The reaction mixture was stirred 16 h at ambient temperature and subsequently poured into 500 mL of dichloromethane. The resulting solution was washed with water and saturated aqueous sodium chloride and dried over anhydrous sodium sulfate. Removal of the solvent afforded the crude product mixture, which was purified by silica gel chromatography to give 0.275 g (2.6%) of **2b**: mp 155–162 °C; positive FAB-MS had diagnostic peaks at m/z 1052 $(M + Na)^+$ and 990 $(M + Na - CO_2 - H_2O)^+$; NMR ($CDCl_3$) δ_c 180.07 (0), 107.44 (0), 103.12 (1), 102.3 (1), 99.98 (0), 96.94 (0), 86.75 (1), 84.51 (0), 84.26 (0), 82.41 (1), 82.25 (1), 80.79 (1), 80.42 (1), 80.11 (1), 79.99 (1), 79.81 (1), 74.52 (1), 74.57 (1), 72.86 (1), 69.94 (1), 67.77 (1), 67.44 (1), 59.67 (3), 56.76 (3), 56.72 (3), 53.26 (1), 40.97 (1), 39.89 (1), 38.87 (2), 36.35 (2), 33.76 (2), 33.45 (1), 33.43 (2), 33.38 (1), 33.15 (1), 32.45 (2), 32.15 (2), 31.00 (2), 29.35 (2), 30.52 (2), 27.42 (3), 27.26 (2), 26.87 (2), 26.79 (2), 25.88 (3), 23.18 (3), 18.33 (3), 18.19 (3), 17.41 (3), 16.93 (3), 13.06 (3), 11.90 (3), 11.02 (3), 10.26 (3). Anal. ($C_{54}H_{91}O_{18}Na \cdot H_2O$) C, H.

2c. This analogue was prepared by a procedure similar to that described for compound **2b** except that 0.5 molar equiv of silver oxide was added as a catalyst, and the reaction time was 2 h. Workup involved removal of excess propyl iodide by vacuum distillation, dissolution of the residue in ether, and successive washes of the ether layer with solutions of sodium bicarbonate and sodium chloride. Following removal of the solvent, the crude product was purified by chromatography on silica gel, affording 0.282 g (2.7%) of **2c**, a broad melting semisolid; positive FAB-MS had diagnostic peaks at m/z 1066 $(M + Na)^+$ and 1004 $(M + Na - CO_2 - H_2O)^+$; NMR ($CDCl_3$) δ_c 180.13 (0), 107.55 (0), 103.26 (1), 102.50 (1), 100.10 (0), 97.03 (0), 86.93 (1), 84.59 (0), 84.34 (0), 82.54 (1), 82.45 (1), 80.90 (1), 80.96 (1), 80.25 (1), 80.56 (1), 79.95 (1), 74.65 (1), 74.44 (1), 72.95 (1), 70.06 (1), 67.85 (1), 67.46 (1), 59.79 (3), 56.87 (3), 56.37 (3), 51.93 (1), 41.08 (1), 40.07 (1), 38.97 (2), 36.46 (2), 33.87 (2), 33.59 (1), 33.57 (1), 33.28 (1), 32.57 (2), 32.30 (2), 31.14 (2), 30.66 (2), 29.42 (2), 27.39 (2), 27.00 (2), 26.85 (2), 25.97 (3), 23.37 (3), 18.47 (3), 18.34 (3), 17.53 (3), 17.05 (3), 14.68 (3), 12.01 (3), 11.14 (3), 10.39 (3).

2d. To a 300-mL flask containing a solution of 10.22 g (0.01 mol) of antibiotic **1a** and 1.3 mL (0.015 mol) of allyl iodide in 120 mL of dry THF was added 10.0 mL (0.01 mol) of a 1.0 M solution of sodium bis(trimethylsilyl)amide. The reaction was monitored by TLC, and maximum product formation was noted at 1 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in dichloromethane. The organic layer was washed with aqueous sodium chloride and dried over anhydrous sodium sulfate. Following evaporation of the solvent, the crude reaction product was purified by silica gel chromatography to give 0.385 g (3.6%) of **2d**: mp 103–111 °C; positive FAB-MS had diagnostic peaks at m/z 1217 $(M - H + C_4H_{10}O_2S_2$ (matrix)), 1155 $(M - H + C_4H_{10}O_2S_2$ (matrix) $- CO_2 - H_2O)^+$, 1064 $(M + Na)^+$ and 1002 $(M + Na - CO_2 - H_2O)^+$; NMR ($CDCl_3$) δ_c 179.58 (0), 140.09 (1), 113.00 (2), 107.42 (0), 103.11 (1), 102.42 (1), 99.79 (0), 96.88 (0), 86.82 (1), 84.45 (0), 84.20 (0), 82.24 (1), 80.77 (1), 80.40 (1), 80.12 (1), 79.93 (1), 79.78 (1), 74.52 (1), 74.30 (1), 72.84 (1), 69.95 (1), 67.62 (1), 67.57 (1), 59.71 (3), 56.76 (3), 56.73 (3), 51.57 (1), 40.73 (1), 39.88 (1), 38.84 (2), 36.33 (2), 33.71 (2), 33.47 (1), 33.40 (2), 33.12 (1), 32.45 (2), 32.17 (2), 31.04 (2), 30.99 (2), 30.52 (2), 27.29 (3), 27.26 (2), 26.94 (2), 26.85 (2), 25.90 (3), 23.21 (3), 18.33 (3), 18.21 (3), 17.41 (3), 16.93 (3), 11.75 (3), 11.00 (3), 10.25 (3). Anal. ($C_{55}H_{91}O_{18}Na$) C, H.

2e,f. The experimental procedure was identical to that described for compound **2d**, except that propargyl bromide was used as the alkylating agent. The reaction stirred at room temperature under nitrogen for 16 h. Following the usual workup, the crude

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product mixture was resolved using silica gel chromatography. Two isomeric propargyl analogues were isolated. The more lipophilic isomer was recrystallized from heptane to give 0.390 g (3.7%) of **2e**: mp 140–143 °C; positive FAB-MS had diagnostic peaks at m/z 1062 $(M + Na)^+$ and 1000 $(M + Na - CO_2 - H_2O)^+$; as with compound **6d**, several higher molecular weight peaks incorporating matrix were present in the FAB-MS of **2e** (not shown); NMR ($CDCl_3$) δ_c 178.31 (0), 107.40 (0), 103.15 (1), 102.43 (1), 99.51 (0), 96.98 (0), 86.82 (1), 84.51 (0), 84.26 (0), 82.27 (1), 82.20 (1), 80.79 (1), 80.43 (1), 80.13 (1), 79.81 (1), 74.56 (1), 74.33 (1), 72.92 (1), 70.02 (1), 67.60 (1), 67.51 (1), 67.37 (1), 59.78 (3), 56.77 (3), 51.37 (1), 41.17 (1), 39.87 (1), 38.86 (2), 36.37 (2), 33.66 (2), 33.59 (1), 33.44 (1), 33.43 (2), 33.15 (1), 32.48 (2), 32.17 (2), 31.02 (2), 30.56 (2), 27.31 (3), 27.30 (2), 26.90 (2), 26.85 (2), 25.96 (3), 23.22 (3), 18.36 (3), 18.24 (3), 17.44 (3), 16.97 (3), 16.06 (2), 12.60 (3), 11.07 (3), 10.30 (3). Anal. ($C_{55}H_{89}O_{18}Na$) C, H.

The more polar isomer (**2f**), 0.358 g (3.4%), mp 150–155 °C, could be further purified by recrystallization from heptane: mp 158–168 °C; positive FAB-MS had diagnostic peaks at m/z 1062 $(M + Na)^+$ and 1000 $(M + Na - CO_2 - H_2O)^+$; as with compound **2e**, several higher molecular weight peaks incorporating matrix were present in the FAB-MS of **2f** (not shown); NMR ($CDCl_3$) δ_c 179.84 (0), 107.29 (0), 103.16 (1), 103.10 (1), 98.94 (0), 96.74 (0), 86.80 (1), 84.53 (0), 84.20 (0), 82.26 (1), 81.28 (1), 80.75 (1), 80.11 (1), 79.72 (1), 76.82 (1), 74.47 (1), 74.26 (1), 72.85 (1), 69.91 (1), 68.26 (1), 67.51 (1), 59.93 (3), 56.70 (3), 56.63 (0), 51.18 (1), 40.28 (1), 39.71 (1), 38.83 (2), 36.33 (2), 33.62 (1), 33.61 (2), 33.34 (2), 33.16 (1), 33.04 (1), 32.41 (2), 32.31 (2), 30.78 (2), 30.44 (2), 27.61 (3), 27.05 (2), 26.81 (2), 26.80 (3), 23.24 (3), 18.27 (3), 18.23 (3), 17.44 (3), 16.9 (2), 16.82 (3), 10.67 (3), 10.12 (3), 8.96 (3). Anal. ($C_{55}H_{89}O_{18}Na \cdot H_2O$) C, H.

Sodium Borohydride Reduction: 5. To a solution of 7.00 g (0.007 mol) of antibiotic **1a** in 350 mL of *p*-dioxane was added (portionwise) 2.10 g (0.055 mol) of sodium borohydride. The resulting suspension was treated with sufficient water (30 mL) to cause the solids to dissolve. After 3.5 h, the solvent was removed and the residue slurried with water. The excess borohydride was destroyed with dilute (1 N) hydrochloric acid. The reaction was extracted with dichloromethane, and the organic layer was washed successively with water, aqueous solutions of sodium bicarbonate, and sodium chloride. Removal of the solvent gave **5** (6.65 g, 95%) as a diastereomeric mixture (ratio ~1:1 based on HPLC analysis): mp 143–185 °C; positive FAB-MS had diagnostic peaks at m/z 1026 $(M + Na)^+$ and 964 $(M + Na - CO_2 - H_2O)^+$; NMR ($CDCl_3$) δ_c (given the complexity of the spectrum of this diastereomeric mixture, no attempt was made to assign peak multiplicities) 178.54, 136.22, 114.34, 107.51, 103.11, 102.25, 99.42, 97.99, 85.53, 85.50, 84.15, 82.41, 81.63, 81.53, 80.92, 80.88, 80.22, 79.51, 79.65, 79.61, 79.51, 79.46, 77.53, 77.10, 77.02, 76.92, 76.68, 74.44, 74.18, 69.90, 68.79, 67.37, 60.51, 59.54, 56.61, 44.66, 44.53, 39.94, 39.31, 36.39, 36.36, 34.27, 33.21, 33.04, 32.98, 32.87, 32.78, 32.65, 31.98, 31.94, 31.89, 30.89, 30.86, 30.24, 27.15, 27.10, 27.06, 26.96, 26.91, 26.76, 26.57, 26.52, 22.86, 22.28, 18.25, 18.14, 18.02, 17.47, 16.32, 12.25, 11.16, 10.30. Anal. ($C_{52}H_{89}O_{18}Na \cdot H_2O$) C, H.

Grignard Addition Product: 6. A solution of 0.35 mL (0.001 mol) of methylmagnesium bromide in ether was diluted with an additional 5 mL of dry ether. To this was added 1.00 g (0.001 mol) of **1a** in 15 mL of dry ether. The reaction was heated to reflux for 1 h. On the basis of TLC analysis, the reaction was only partly complete at this point. An additonal 0.014 mol of methylmagnesium bromide was then added portionwise over 1 h at room temperature. The reaction was then poured slowly into aqueous ammonium chloride, and the resulting solution was extracted with ethyl acetate. The organic layer was washed successively with water, aqueous sodium bicarbonate, and sodium chloride and dried over sodium sulfate. The crude product was purified by chromatography and trituration with heptane to give 0.31 g (30%) of **6**: mp 185–187 °C; FAB-MS had diagnostic peaks at m/z 1040 $(M + Na)^+$ and 978 $(M + Na - CO_2 - H_2O)^+$; NMR ($CDCl_3$) δ_c 180.09 (0), 107.87 (0), 102.92 (0), 102.42 (1), 98.17 (0), 86.69 (1), 85.45 (0), 84.18 (0), 82.54 (1), 82.22 (1), 81.73 (1), 81.27 (1), 80.31 (1), 80.50 (1), 79.95 (1), 75.75 (1), 74.69 (1), 74.48 (1), 70.07 (1), 69.16 (1), 67.84 (1), 59.58 (3), 56.88 (3), 45.60 (2), 44.83 (1), 43.53 (1), 39.31 (2), 39.08 (2), 35.39 (1), 34.23 (2), 33.40 (1), 33.22 (2), 33.23 (1), 31.91 (2), 31.68 (2), 31.12 (2), 30.71 (2), 30.64 (2), 29.16 (3), 28.09 (3), 27.38 (2), 27.02 (2), 26.74 (2), 23.33 (3), 22.58 (3), 18.59 (3), 18.45 (3), 18.32 (3), 17.04 (3), 12.40 (3), 11.44 (3), 10.28 (3). Anal. ($C_{53}H_{91}O_{18}Na \cdot 2H_2O$) C, H.

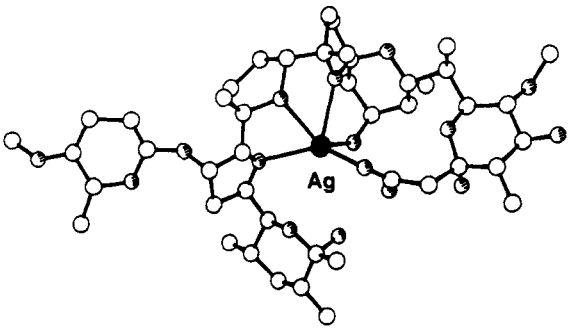

Figure 3. Crystal structure of **7** (semduramicin silver salt).

Acid Hydrolysis of UK-58,852: 7 and 8. To a solution of 0.60 g (0.587 mmol) of antibiotic **1a** in acetonitrile/water (5/3) was added 0.150 g (0.785 mmol) of *p*-toluenesulfonic acid. After stirring at room temperature for 4 h, a product more polar than **1a** (compound **7**) predominated in the reaction mixture. Following more extended reaction times (≥24 h), an equivalent amount of an additional polar component (compound **8**) was detected. At this point, the mixture was treated with 5.0 g of solid sodium bicarbonate and concentrated to dryness under vacuum. The resulting solid was dissolved in ether and washed with a saturated aqueous solution of sodium bicarbonate. The bicarbonate washings were extracted with ether, and all the ether layers were combined and washed successively with water and saturated sodium chloride. The ether solution was dried over anhydrous sodium sulfate and evaporated to dryness under vacuum. Preparative resolution of the crude reaction mixture was accomplished by silica gel chromatography (ethyl acetate) to give the following reaction products:

(a) A white solid, further purified by recrystallization from isopropyl ether to give 0.2 g (38%) of **7**: mp 175–176 °C; $[\alpha]^{25}_D$ = 20.3° (c = 0.5, MeOH); positive FAB-MS had diagnostic peaks at m/z 896 $(M + Na)^+$ and 834 $(M + Na - CO_2 - H_2O)^+$; NMR[15] ($CDCl_3$) δ_c 179.09 (0), 107.45 (0), 103.22 (1), 97.70 (0), 96.89 (0), 86.96 (1), 84.51 (0), 84.15 (0), 82.28 (1), 81.95 (1), 80.92 (1), 80.22 (1), 79.83 (1), 74.74 (1), 74.57 (1), 73.01 (1), 70.03 (1), 67.61 (1), 66.80 (1), 59.03 (3), 56.86 (3), 45.38 (3), 45.28 (1), 39.81 (1), 38.89 (2), 36.40 (2), 33.77 (2), 33.74 (1), 33.54 (1), 33.39 (2), 33.11 (1), 32.47 (2), 32.25 (2), 30.55 (2), 27.56 (3), 26.92 (2), 26.83 (2), 26.05 (3), 23.2 (3), 18.38 (3), 17.51 (3), 16.99 (3), 12.10 (3), 11.05 (3), 10.43 (3); structure unambiguously established by X-ray (Figure 3). Anal. ($C_{45}H_{75}O_{16}Na \cdot H_2O$) C, H.

(b) A white solid which was recrystallized as above to give 0.240 g (53%) of **8**: mp 154 °C; positive FAB-MS had diagnostic peaks at m/z 768 $(M + Na)^+$ and 706 $(M + Na^+ - CO_2 - H_2O)^+$; NMR ($CDCl_3$) δ_c 179.26 (0), 107.61 (0), 97.79 (0), 97.03 (0), 87.44 (1), 84.63 (0), 84.30 (0), 82.28 (1), 82.07 (1), 79.97 (1), 74.73 (1), 73.04 (1), 71.73 (1), 70.23 (1), 67.68 (1), 66.93 (1), 59.10 (3), 45.53 (2), 45.40 (1), 39.98 (1), 38.99 (2), 36.50 (2), 34.85 (2), 33.96 (2), 33.87 (1), 33.71 (1), 33.50 (2), 33.31 (1), 31.96 (2), 27.70 (3), 27.00 (2), 26.11 (3), 23.24 (3), 17.59 (3), 17.05 (3), 12.18 (3), 11.11 (3), 10.55 (3). Anal. ($C_{38}H_{63}O_{14}Na \cdot 1.5H_2O$) C, H.

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Supplementary Material Available: X-ray data for compounds **1a**, **2a**, and **7** (55 pages); structure factors for **1a** (21 pages). Ordering information is given on any current masthead page.