$ROCH_2Ph$), 4.51 (d, 1 H, J = 11.7 Hz, $ROCH_2Ph$), 4.47 (d, 1 H, J = 12.0 Hz, $ROCH_2Ph$), 4.46 (d, 1 H, J = 11.7 Hz, $ROCH_2Ph$), 4.25 (m, 1 H, H2), 4.24 (dd, 1 H, J = 3.6, 6.5 Hz, H4), 4.09 (dd, 1 H, J = 2.9, 6.5 Hz, H3), 3.70 (m, 1 H, H5), 3.58-3.60 (overlapping m, 2 H, H6, H6'), 2.75 (t, 2 H, J = 8.0 Hz, $ArCH_2R$), 2.37 (t, 2 H, J = 7.2 Hz, CH_2), 1.00-1.57 (m, 16 H), 0.82-0.86 (overlapping t, 6 H, CH_3).

3'-(Benzyloxy)-6'-[[4''-[(benzyloxy)carbonyl]-5''-(1-heptyl)-3''-hydroxyphenoxy]carbonyl]-5'-(1-heptyn-1-yl)phenyl 2,3-Di-*O*-benzyl-L-galactofuranoside (*ent*-23). This compound was prepared on a 0.04-mmol scale in 69% yield from acid ***ent*-21** as described above for the D-antipode **23**: $[\alpha]^{22}_D$ = +58.0° (*c* 1.07, $CHCl_3$); FABLRMS (NOBA + NaI) *m/e* (relative intensity) 1027 (2.6), 663 (6.3), 411 (6.24), 321 (100); FABHRMS calcd for $C_{62}H_{68}O_2Na$ 1027.4608, found 1027.4668. Anal. Calcd for $C_{62}H_{68}O_{12}$: C, 74.08; H, 6.82. Found: C, 73.79; H, 6.56.

KS-501 (1). Compound **22** (14 mg, 0.016 mmol) was hydrogenated over 10% Pd/C (15 mg) under 1 atm of H_2 in EtOH (3 mL) for 36 h. The mixture was filtered, evaporated, and passed through a short pad of silica gel (eluted with 15% MeOH in $CHCl_3$) to leave 10.4 mg (100%) of pure **1** as a clear oil that solidified on standing: $[\alpha]^{23}_D$ = -54.3° (*c* 0.67, MeOH) (lit.[1] $[\alpha]^{23}_D$ = -53° (*c* 0.3, MeOH)); identical by TLC mobility, UV, and ^1H NMR spectra to the natural material.

***ent*-KS-501 (*ent*-1).** Compound ***ent*-22** (17 mg, 0.02 mmol) was hydrogenated over 10% Pd/C (20 mg) under 1 atm of H_2 in EtOH (4 mL) for 36 h. The mixture was filtered, evaporated, and passed through a short pad of silica gel (eluted with 15% MeOH in $CHCl_3$) to leave 10.4 mg (88%) of pure ***ent*-1** as a clear oil that solidified on standing: $[\alpha]^{23}_D$ = +53.5° (*c* 0.68, MeOH); identical by TLC mobility, UV, and ^1H NMR spectra to the natural isomer.

KS-502 (2). Compound **23** (120 mg, 0.12 mmol) was hydrogenated over 10% Pd/C (100 mg) under 1 atm of H_2 in EtOH (10 mL) for 14 h. The mixture was filtered, evaporated, and passed through a short pad of LiChroprep RP-18 (eluted with MeOH) to leave 77 mg (100%) to pure **2** as a clear oil that solidified on standing: $[\alpha]^{22}_D$ = -42.8° (*c* 0.54, MeOH) (lit.[1] $[\alpha]^{23}_D$ = -45° (*c* 0.3, MeOH)); identical by TLC mobility, UV, and ^1H NMR spectra to the natural material.

***ent*-KS-502 (*ent*-2).** Compound ***ent*-23** (19 mg, 0.019 mmol) was hydrogenated over 10% Pd/C (15 mg) under 1 atm of H_2 in EtOH (3 mL) for 24 h. The mixture was filtered, evaporated, and passed through a short pad of LiChroprep RP-18 (eluted with MeOH) to leave 13 mg (100%) of pure ***ent*-2** as a clear oil that solidified on standing: $[\alpha]^{22}_D$ = +42.0° (*c* 0.40, MeOH); identical by TLC mobility, UV, and ^1H NMR spectra to the natural isomer.

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Synthesis of a Cyanobacterial Sulfolipid: Confirmation of Its Structure, Stereochemistry, and Anti-HIV-1 Activity

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Abstract: The total synthesis of a cyanobacterial sulfolipid is described. The key steps involve the epoxidation of a glycal followed by conversion to a 1β-fluoro-2α-hydroxy moiety. After protection of the alcohol, the anomeric β-fluoro substituent is used to fashion an α-glycoside of a glycerol. A sulfonic acid is introduced at the 6-position by oxidation of a thioacetate with Oxone in the presence of a triene subunit.

By taking advantage of a newly developed soluble formazan assay to screen for the cytopathic effects of HIV-1 and for inhibitors of these effects,[1] scientists at the National Cancer Institute (NCI) were able to identify active principles from various cyanobacterial (blue-green algae) media.[2] From these cultures were isolated a series of related sulfolipids. Painstaking chromatographic separation provided four homogeneous compounds, which were tentatively assigned as structures **1-4**. The formulation of the sites of the nonidentical side chains in the diacylglycerol moiety followed from the analysis of mass spectrometric fragmentation data.[2] The *S* configuration at C_2 of the glycerol was presumed from the similarity of the optical rotation of the bis-deacylated product with that of material of known configuration.[3,4]

In light of their activity (EC_{50} 0.1-1 μg/mL depending upon the target cell line) and their undetermined mechanism of action, the sulfolipids were selected by the NCI for further preclinical investigation and for evaluation as to their possible clinical usefulness against AIDS.[2] In particular, questions regarding both the administration of the sulfolipids and their stability as well as efficacy in vivo require immediate attention. Unfortunately, progress toward these goals has been impeded by difficulties in the fermentation process and by the complexities of separating the closely related components of the mixture.[5]

In addition to the relevance of the compounds to issues of current medical concern, the development of a synthetic route to the cyanobacterial sulfolipid series was seen as providing an opportunity to implement strategies which our laboratory has been devising in the field of carbohydrate synthesis. Since we had developed concise methodology for the total synthesis of glycals using the LACDAC reaction,[6] it was our intention to reach compound **1** via a glycal. The use of a glycal starting material was clearly not the only way to reach any of the compounds of

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(6) (a) Danishefsky, S. J. *Chemtracts: Org. Chem.* **1989**, *2*, 273. (b) Danishefsky, S. J.; DeNinno, M. P. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 15. (c) Danishefsky, S. J. *Aldrichimica Acta* **1986**, *19*, 59.

0002-7863/92/1514-659$03.00/0

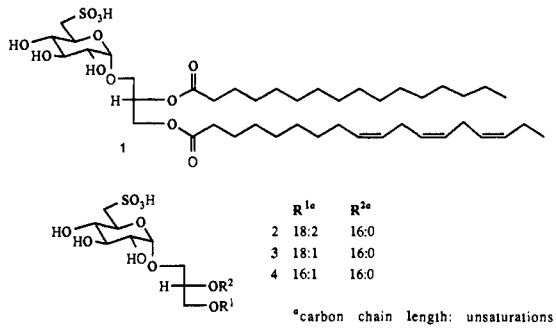
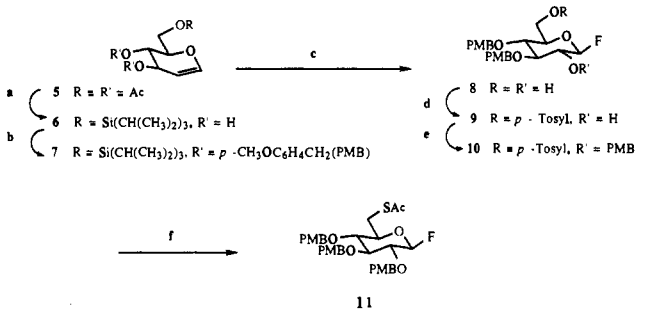


Figure 1.

Scheme I[a]



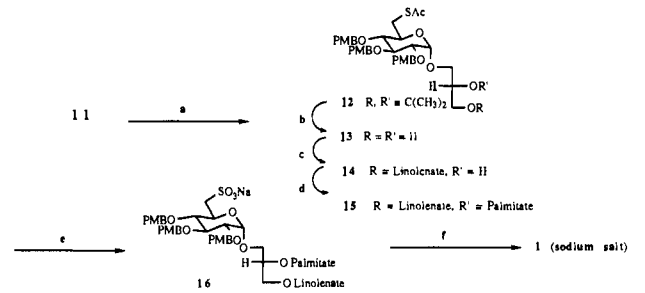
[a](a) MeONa, MeOH, room temperature; (*i*-Prp)$_3$SiCl, Im, DMF, room temperature, 84%. (b) PMBBr, NaH, DMF, room temperature, 99%. (c) Dimethyldioxirane, CH_2Cl_2, acetone, 0 °C; TBAF, THF, room temperature, 56%. (d) *p*-Tosyl chloride, DMAP, pyr, 35 °C, 76%. (e) PMBBr, NaH, DMF, room temperature, 99%. (f) KSAc, EtOH, 78 °C, 85%.

the natural series. However, a recently demonstrated capability to synthesize virtually any glycal, as either the D or L antipode (using enzymatic methods)[7] suggested that these systems could be particularly promising intermediates en route to structurally varied analogues of the sulfolipids. The principal challenges from the standpoint of synthesis lay in the need to install a sulfonic acid at C_6 and the α-glycosidic linkage at the anomeric carbon. As the glycosyl acceptor we hoped to use the readily available, enantiomerically pure (*S*)-isopropylideneglycerol. However, previous use of this acceptor was complicated by its facile racemization under the conditions necessary to effect glycosylation.[8] We hoped to devise conditions to obviate this problem.

The required deprotection of alcohol functions at carbon 2, 3, and 4 while maintaining the positional integrity of the two acyl side chains was to emerge as an additional complexity. To address these questions we first focused on a synthesis of **1**, which was seen to be the most challenging member of the group due to its unsaturation pattern (Figure 1). This unsaturation pattern would impose restraints on the possible deprotection protocols to liberate the hydroxyl functions at carbons 2, 3, and 4. Below we describe the synthesis of sulfolipid **1**.

Methanolysis of tri-*O*-acetyl-D-glucal **5** generated D-glucal, which was converted to its mono-TIPS derivative **6** (84% for two steps).[9] The hydroxyl functions at carbons 3 and 4 were protected

Scheme II[a]



[a](a) $SnCl_2$, $AgClO_4$, 2,6-di-*t*-Bupyr, 4-Å molecular sieves, 1,2-isopropylidene-*sn*-glycerol, Et_2O, room temperature, 86%. (b) MeOH, C_6H_6, 1 N HCl, room temperature, 84%. (c) Linolenic acid, DMAP, EDCI, CH_2Cl_2, room temperature, 61%. (d) Palmitic acid, DMAP, EDCI, CH_2Cl_2, room temperature, 91%. (e) AcOK, AcOH, Oxone, room temperature, 19%. (f) TMSI, $CHCl_3$, room temperature, 70%.

to give the bis(*p*-methoxybenzyl) derivative **7** (99%). Reaction of this glycal with 2,2-dimethyldioxirane[10,11] occurred under the usual conditions, generating apparently a single epoxide, which reacted with tetra-*n*-butylammonium fluoride.[12] As a result of this treatment, the TIPS protecting group was cleaved and the homogeneous glycosyl fluoride **8** was obtained in 56% overall yield. At this stage, the primary and secondary alcohols were readily distinguished by tosylation of the former. Compound **9**, thus available in 76% yield, was converted to its 2-*O*-PMB derivative (99% yield). The ability to carry out the benzylation of the C_2 alcohol in the presence of the C_6 tosylate saved several steps. The tosyloxy function suffered displacement upon treatment of **10** with potassium thioacetate. Compound **11** was thus obtained in 85% yield (Scheme I).

Fortunately, the thioacetate function at C_6 survived the glycosylation step with (*S*)-isopropylideneglycerol.[13] Thus, activation of the anomeric fluoride **11** (stannous chloride, silver(I) perchlorate, ether)[14] and coupling with the glycerol derivative, in the presence of 2,6-di-*tert*-butylpyridine, afforded an 86% yield of **12** *as a single diastereomer*. In the absence of the pyridine base, the reaction product, as shown by 500-MHz ^{1}H NMR analysis, was a mixture of α-glycosides. We assume that this arises from the partial racemization of the glycosyl acceptor prior to glycosylation. Since the 500-MHz ^{1}H NMR spectrum arising from the reaction containing the base shows no corresponding multiplicity of signals, we are confident that the product is a single entity. With the homogeneous β-fluoro anomer **11** as the glycosyl donor, the formation of the α-glycoside product was also apparently stereospecific.[15] Since the consequences of using the α-fluoro isomer were not examined, the connectivity between homogeneity of glycosyl donor and stereospecificity remains to be established.

The acetonide linkage of **12** was cleaved with methanolic HCl to give diol **13** (84%). Reaction of **13** with linolenic acid (DMAP, EDCI, methylene chloride, room temperature) afforded **14** in 61% yield along with 10–15% each of **13** and the bis-linolenoyl product. Palmitoylation of the secondary alcohol of **14** under similar conditions, using palmitic acid, led to **15** (91% yield) (Scheme II).

(7) Berkowitz, D. D. Yale University, New Haven, CT, Personal communication, 1991.

(8) (a) For the synthesis of a sulfolipid bearing identical saturated sixteen-carbon fatty acid residues, see: Gigg, R.; Penglis, A. A. E.; Conant, R. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2490. (b) Preliminary results of a program directed toward the preparation of these sulfolipids have been communicated: Jahangir; Hebbler, A. K.; Morris, P. E.; Gentile, J. N.; Baker, D. C. *Abstracts of Papers*, 199th National Meeting of the American Chemical Society, Boston, MA, April 1990; American Chemical Society: Washington, DC, 1990; CARB-44. (c) The use of isopropylideneglycerol is precluded in a number of glycosylations due to its facile racemization in the presence of Lewis or Bronsted acids, see: Jahangir; Lin, T. H.; Baker, D. C. *Abstracts of Papers*, 201st National Meeting of the American Chemical Society, Atlanta, GA, April 1991; American Chemical Society: Washington, DC, 1991, CARB-70.

(9) All new compounds displayed satisfactory ^{1}H NMR, IR, $[\alpha]_D$, combustion analysis, and HRMS characteristics.

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(13) Eibl, H. *Chemistry and Physics of Lipids* **1981**, *28*, 1.

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(15) The use of a homogeneous β-fluoro glycoside simplifies the technical aspects of the synthesis and has been found to expedite the glycosylation reaction in other work currently under way in these laboratories.

We next faced the question of oxidation of the C_6 thioacetate function in the presence of the triene. In the event, oxidation of **15** with Oxone (potassium acetate–acetic acid)[16,17] afforded the sulfonic acid, isolated as its sodium salt **16**. Though the isolated yield of homogeneous product was only 19%, the success of the process in the presence of the triene is noteworthy. In the final step of the synthesis, the three PMB groups were cleaved upon reaction of **16** with iodotrimethylsilane.[18,19] Compound **1** was isolated as its sodium salt. The ^1H and ^{13}C NMR, infrared, and mass spectra of the synthetic and naturally derived material were identical. We do note a small disparity in the optical rotation of the synthetic material relative to that reported ($[\alpha]^{22}_D$ +42.8° (*c* 1.00, CH_3OH), lit.[2] $[\alpha]_D$ +55.5° (*c* 0.98, CH_3OH). We have every reason to have full confidence in the purity of our synthetic material and regard measurements from synthetically obtained samples as more reliable than measurements conducted on the products derived from fermentation. Most importantly, the anti-HIV-1 activity of synthetic **1** was confirmed by independent measurements by Cardellina and associates at the National Cancer Institute. The potency and formulability of the synthetic material were the same as were exhibited by material previously produced by fermentation.[5]

In summary, a stereospecific route from D-glucal to the α-glyceryl glycoside has been established. The stereochemical assignment for **1** has been confirmed. A route which is presumably applicable to any of the naturally occurring sulfolipid diglycerides or congeners has been demonstrated. The synthesis described could facilitate studies to evaluate the mechanism[20] and clinical usefulness of this class of carbohydrates. The survival of the acyl linkages in the context of in vivo administration is open to questions. It may well be that an analogue program will be necessary to improve potency and drug availability. The work described above could be applied to such an effort.

Experimental Section

6-*O*-(Triisopropylsilyl)-D-glucal (6). To a solution of 3,4,6-tri-*O*-acetyl-D-glucal (**5**) (5.0 g, 18.4 mmol) in anhydrous methanol (50 mL) was added sodium methoxide (49.7 mg, 920 μmol). After the mixture was stirred at ambient temperature for 4 h, the solvent was removed in vacuo. The residue was dissolved in anhydrous dimethylformamide (50 mL, DMF) and the solution blanketed with N_2. Imidazole (3.74 g, 55 mmol) was added followed by chlorotriisopropylsilane (4.28 mL, 20 mmol), and the mixture was stirred at ambient temperature for 10 h. The reaction was diluted with H_2O (100 mL) and washed with ethyl acetate (5 × 60 mL). The combined organic layers were washed with H_2O (5 × 50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (33% ethyl acetate in hexane) to give **6** (4.68 g, 15.5 mmol, 84%): $[\alpha]^{22}_D$ −0.83° (*c* 1.45, $CHCl_3$); IR ($CHCl_3$) 3350, 2950, 2880, 1650, 1465, 1240, 1110, 1060, 890 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 6.28 (d, 1 H, *J* = 6.02 Hz, H-1), 4.71 (dd, 1 H, *J* = 6.03 and 1.87 Hz, H-2), 4.30–4.23 (m, 1 H, H-3), 4.14–3.93 (m, 2 H, H-4 and H-5), 3.89–3.76 (m, 2 H, H-6 and H-6′), 3.32 (bs, 1 H, C-3-OH), 2.41 (bs, 1 H, C-4-OH), 1.16–0.97 (m, 21 H, $Si(CH(CH_3)_2)_3$). Anal. Calcd for $C_{15}H_{30}O_4Si$: C, 59.56; H, 10.00. Found: C, 59.53; H, 9.86.

3,4-Di-*O*-(4-methoxybenzyl)-6-*O*-(triisopropylsilyl)-D-glucal (7). To a solution of **6** (9.1 g, 30.1 mmol) and 4-methoxybenzyl bromide (14.1 g, 70 mmol) in anhydrous DMF (70 mL) was added NaH (2.8 g, 60% dispersion, 70 mmol), and the mixture was stirred at ambient temperature for 4 h. The reaction was diluted with cold H_2O (4 °C, 120 mL) and washed with diethyl ether (5 × 40 mL). The combined organic layers were washed with H_2O (5 × 20 mL) and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (6% ethyl acetate in hexane) to give **7** (16.29 g, 30 mmol, 99%): $[\alpha]^{22}_D$ −3.02° (*c* 2.85, $CHCl_3$); IR ($CHCl_3$) 3060, 2940, 2870, 1645, 1610, 1570, 1520, 1465, 1250, 1100, 1040 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.30–7.26 (m, 4 H, ArH), 6.91–6.86 (m, 4 H, ArH), 6.38 (dd, 1 H, *J* = 6.07 and 1.35 Hz, H-1), 4.82 (dd, 1 H, *J* = 6.06 and 2.67 Hz, H-2), 4.74 (AB q, 2 H, *J* = 27.08 and 10.91 Hz, $ArCH_2$), 4.57–4.55 (m, 2 H, $ArCH_2$), 4.18 (m, 1 H, H-3), 4.03–3.99 (m, 2 H, H-4 and H-5), 3.91–3.89 (m, 2 H, H-6 and H-6′), 3.82 (s, 6 H, OCH_3), 1.12–1.04 (m, 21 H, $Si(CH(CH_3)_2)_3$). Anal. Calcd for $C_{31}H_{46}O_6Si$: C, 68.60; H, 8.54. Found: C, 68.33; H, 8.26.

3,4-Di-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl Fluoride (8). A solution of glucal **7** (1.48 g, 2.7 mmol) in CH_2Cl_2 (50 mL) was cooled to 0 °C, and dimethyldioxirane (75 mM in acetone, 40 mL, 3.0 mmol) was added in one portion. After the mixture was stirred for 1 h at 0 °C, TLC showed complete consumption of starting material, and the solvent was removed in vacuo. The residue was dissolved in anhydrous tetrahydrofuran (30 mL, THF), and a solution of tetra-*n*-butylammonium fluoride (30 mL, 1 M in THF) was added. After being stirred at ambient temperature overnight, the reaction was diluted with H_2O (50 mL) and washed with diethyl ether (4 × 40 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (20% ethyl acetate in CH_2Cl_2) to give **8** (640 mg, 1.51 mmol, 56%): $[\alpha]^{22}_D$ +12.4° (*c* 2.26, $CHCl_3$); mp 95–6 °C; IR ($CHCl_3$) 3580, 3390, 3020, 3000, 2900, 2825, 1610, 1580, 1510, 1460, 1355, 1300, 1250, 1100, 1040, 820 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.29–7.20 (m, 4 H, ArH), 6.89–6.84 (m, 4 H, ArH), 5.14 (dd, 1 H, *J* = 53.29 and 6.35 Hz, H-1), 4.83–4.70 (m, 3 H, $ArCH_2$), 4.59 ($^1/_2$ AB q, 1 H, *J* = 10.73 Hz, $ArCH_2$), 3.79 (s, 6 H, OCH_3), 3.84–3.50 (m, 6 H, H-2, H-3, H-4, H-5, H-6, and H-6′), 2.32 (d, 1 H, *J* = 2.97 Hz, C-2-OH), 1.82 (dd, 1 *H*, *J* = 7.32 and 6.40 Hz, C-6-OH). Anal. Calcd for $C_{22}H_{27}FO_7$: C, 62.55; H, 6.44. Found: C, 62.40; H, 6.48.

3,4-Di-*O*-(4-methoxybenzyl)-6-*O*-(4-tolylsulfonyl)-β-D-glucopyranosyl Fluoride (9). To a stirred solution of diol **8** (423 mg, 1.0 mmol) in pyridine (10 mL) was added 4-(dimethylamino)pyridine (13.5 mg, 110 μmol, DMAP) followed by 4-toluenesulfonyl chloride (300 mg, 1.58 mmol). The solution was maintained at 35 °C under N_2 for 12 h, diluted with cold H_2O (4 °C, 30 mL), and washed with ethyl acetate (3 × 40 mL). The combined organic layers were washed with 0.1 N HCl (30 mL) and brine (30 mL), diluted with *n*-heptane (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (6% ethyl acetate in CH_2Cl_2) to give **9** (437 mg, 758 μmol, 76%): $[\alpha]^{22}_D$ +20.4° (*c* 3.12, $CHCl_3$); mp 101–2 °C; IR ($CHCl_3$) 3580, 3020, 2990, 2940, 2900, 2820, 1725, 1610, 1510, 1460, 1360, 1295, 1250, 1175, 1090, 1035 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.76 (d, 2 H, *J* = 8.29 Hz, ArH), 7.33–7.15 (m, 6 H, ArH), 6.91–6.85 (m, 4 H, ArH), 5.10 (dd, 1 H, *J* = 52.39 and 5.35 Hz, H-1), 4.72–4.65 (m, 3 H, $ArCH_2$), 4.51 ($^1/_2$ AB q, 1 H, *J* = 10.66 Hz, $ArCH_2$), 4.26–4.15 (m, 2 H, H-6 and H-6′), 3.82 (s, 3 H, OCH_3), 3.81 (s, 3 H, OCH_3), 3.80–3.56 (m, 4 H, H-2, H-3, H-4, and H-5), 2.60 (bs, 1 H, C-2-OH), 2.43 (s, 3 H, $ArCH_3$). Anal. Calcd for $C_{29}H_{33}FO_9S$: C, 60.40; H, 5.77. Found: C, 60.13; H, 5.42.

2,3,4-Tri-*O*-(4-methoxybenzyl)-6-*O*-(4-tolylsulfonyl)-β-D-glucopyranosyl Fluoride (10). To a solution of fluoride **9** (156 mg, 270 μmol) and 4-methoxybenzyl bromide (60 mg, 300 μmol) in anhydrous DMF (3 mL) was added NaH (16.2 mg, 406 μmol, 60% dispersion), and the mixture was stirred at ambient temperature for 3 h. The reaction was diluted with cold H_2O (4 °C, 10 mL) and washed with ethyl acetate (3 × 15 mL). The combined organic layers were washed with H_2O (3 × 10 mL) and brine (15 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (33% ethyl acetate in hexane) to give **10** (187 mg, 268 μmol, 99%): $[\alpha]^{22}_D$ +25.4° (*c* 4.05, $CHCl_3$); IR ($CHCl_3$) 3020, 3000, 2960, 2925, 2900, 2830, 1730, 1610, 1580, 1515, 1465, 1370, 1300, 1270, 1250, 1180, 1100, 830 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.76 (d, 2 H, *J* = 8.30 Hz, ArH), 7.31–7.09 (m, 8 H, ArH), 6.90–6.81 (m, 6 H, ArH), 5.13 (dd, 1 H, *J* = 52.75 and 6.40 Hz, H-1), 4.79–4.42 (m, 6 H, $ArCH_2$), 4.21–4.10 (m, 2 H, H-6 and H-6′), 3.80 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.57–3.42 (m, 4 H, H-2, H-3, H-4, and H-5), 2.41 (s, 3 H, $ArCH_3$); MS *m/z* 575 ($M^+ - C_7H_6OCH_3$); HRMS calcd for $C_{37}H_{41}FNaO_{10}S$ $(M + Na)^+$ 719.2303, found 719.2309. Anal. Calcd for $C_{37}H_{41}FO_{10}S$: C, 63.78; H, 5.93. Found: C, 63.73; H, 6.10.

6-Deoxy-2,3,4-tri-*O*-(4-methoxybenzyl)-6-(thioacetyl)-β-D-glucopyranosyl Fluoride (11). A vigorously stirred suspension of tosylate **10** (1.77 g, 2.54 mmol) and potassium thioacetate (1.45 g, 12.7 mmol) in anhydrous ethanol (30 mL) was heated at reflux for 3 h. After cooling to ambient temperature, the mixture was diluted with H_2O (50 mL) and washed with ethyl acetate (5 × 30 mL). The combined organic layers

(16) For leading references on the use of Oxone to oxidize sulfides to sulfoxides and sulfones in the presence of olefins, see: Trost, B. M.; Curran, D. P. *Tetrahedron Lett.* **1981**, *22*, 1287. (b) Evans, T. L.; Grade, M. M. *Synth. Commun.* **1986**, *16*, 1207.

(17) For the oxidation of saturated thioacetyl compounds to sulfonic acids using Oxone, see: Reddie, R. N. *Synth. Commun.* **1987**, *17*, 1129.

(18) Treatment of **16** with DDQ or CAN gave mixtures of products in which the olefins had been transformed and/or the linolenoyl ester had been hydrolyzed.

(19) For the use of TMSI to remove benzyl protecting groups, see: Jung, M. E.; Lyster, M. A. *J. Org. Chem.* **1977**, *42*, 3761.

(20) In light of the similarity of these sulfolipids to cell membrane components, we speculate that their incorporation into the cellular membrane interferes with viral recognition of cell surface receptors, see: Spargo, B. J.; Crowe, L. M.; Ioneda, T.; Beaman, B. L.; Crowe, J. H. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 737.

were washed with brine (40 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (20% ethyl acetate in hexane) to give **11** (1.30 g, 2.16 mmol, 85%): $[\alpha]^{22}_D$ +24.1° (*c* 2.66, $CHCl_3$); mp 58–9 °C; IR ($CHCl_3$) 3010, 2960, 2940, 2905, 2830, 1690, 1610, 1520, 1460, 1350, 1300, 1255, 1100, 830 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.30–7.22 (m, 6 H, ArH), 6.89–6.84 (m, 6 H, ArH), 5.19 (dd, 1 H, *J* = 52.82 and 6.64 Hz, H-1), 4.84–4.52 (m, 6 H, $ArCH_2$), 3.81 (s, 3 H, OCH_3), 3.80 (s, 6 H, OCH_3), 3.65–3.41 (m, 5 H, H-2, H-3, H-4, H-5, and H-6), 3.05 (dd, 1 H, *J* = 13.72 and 6.76 Hz, H-6′), 2.35 (s, 3 H, $C(O)CH_3$); MS *m/z* 479 (M^+ – $C_7H_6OCH_3$); HRMS calcd for $C_{32}H_{38}FO_8S$ ($M + H)^+$ 601.2272, found 601.2292. Anal. Calcd for $C_{32}H_{37}FO_8S$: C, 63.98; H, 6.21. Found: C, 63.80; H, 6.45.

3-*O*-[6-Deoxy-2,3,4-tri-*O*-(4-methoxybenzyl)-6-(thioacetyl)-α-D-glucopyranosyl]-1,2-*O*-isopropylidene-*sn*-glycerol (12). A 25-mL round-bottom flask (flask I) was charged with powdered 4-Å molecular sieves (500 mg), flame-dried under high vacuum for 5 min, and allowed to cool to ambient temperature under N_2. In an Ar-filled glove bag a 5-mL round-bottom flask (previously flame-dried as above) was charged with $SnCl_2$ (103 mg, 546 μmol) and $AgClO_4$ (113 mg, 546 μmol). A solution of fluoride **11** (164 mg, 273 μmol) in anhydrous diethyl ether (10 mL) was added by syringe to flask I followed by 1,2-*O*-isopropylidene-*sn*-glycerol (337 μL, 2.73 mmol) and 2,6-di-*tert*-butylpyridine (184 μL, 819 μmol). The metals were added to flask I under a cone of N_2, and the flask was sealed with a polystyrene cap. After being stirred at ambient temperature for 48 h, the mixture was diluted with diethyl ether (50 mL) and filtered through a plug of Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ (20 mL), H_2O (20 mL), and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (33% ethyl acetate in hexane) to give **12** (167 mg, 234 μmol, 86%): $[\alpha]^{22}_D$ +23.8° (*c* 3.55, $CHCl_3$); IR ($CHCl_3$) 3010, 2995, 2920, 2825, 1685, 1610, 1580, 1510, 1460, 1370, 1350, 1300, 1250, 1070, 1040, 830 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.28–7.24 (m, 6 H, ArH), 6.88–6.81 (m, 6 H, ArH), 4.86 ($^1/_2$ AB q, 1 H, *J* = 10.38 Hz, $ArCH_2$), 4.80 ($^1/_2$ AB q, 1 H, *J* = 10.42 Hz, $ArCH_2$), 4.72–4.66 (m, 3 H, $ArCH_2$ and H′-1), 4.55 ($^1/_2$ AB q, 1 H, *J* = 9.73 Hz, $ArCH_2$), 4.51 ($^1/_2$ AB q, 1 H, *J* = 8.53 Hz, $ArCH_2$), 4.32 (app quintet, 1 H, *J* = 6.06 Hz, H-2), 4.04 (dd, 1 H, *J* = 8.38 and 6.29 Hz, H-1), 3.86–3.67 (m, 3 H, H-1′, H-3, H′-6), 3.79 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.61–3.36 (m, 4 H, H-3′, H′-2, H′-3, and H′-5), 3.23 (dd, 1 H, *J* = 9.60 and 9.08 Hz, H′-4), 2.95 (dd, 1 H, *J* = 13.64 and 7.69 Hz, H′-6′), 2.31 (s, 3 H, $C(O)CH_3$), 1.40 (s, 3 H, $C(CH_3)_2$), 1.34 (s, 3 H, $C(CH_3)_2$); MS *m/z* 591 (M^+ – $C_7H_6OCH_3$); HRMS calcd for $C_{38}H_{49}O_{11}S$ ($M + H)^+$ 713.2997, found 713.2980. Anal. Calcd for $C_{38}H_{48}O_{11}S$: C, 64.03; H, 6.79. Found: C, 63.81; H, 6.77.

3-*O*-[6-Deoxy-2,3,4-tri-*O*-(4-methoxybenzyl)-6-(thioacetyl)-α-D-glucopyranosyl]-*sn*-glycerol (13). Hydrochloric acid (2 mL, 1 N) was added to a stirred solution of pyranose **12** (915 mg, 1.28 mmol) in methanol (18 mL) and benzene (4 mL). After 8 h at ambient temperature, the reaction was diluted with saturated aqueous $NaHCO_3$ (15 mL) and brine (15 mL) and washed with CH_2Cl_2 (6 × 50 mL). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (33% ethyl acetate in methylene chloride to 100% ethyl acetate) to give **13** (725 mg, 1.08 mmol, 84%): $[\alpha]^{22}_D$ +18.0° (*c* 1.08, $CHCl_3$); mp 73–4 °C; IR ($CHCl_3$) 3450, 3015, 2995, 2930, 2830, 1680, 1610, 1580, 1510, 1460, 1350, 1300, 1250, 1060 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.28–7.22 (m, 6 H, ArH), 6.88–6.83 (m, 6 H, ArH), 4.86–4.68 (m, 4 H, $ArCH_2$ and H′-1), 4.63–4.50 (m, 3 H, $ArCH_2$), 3.89–3.25 (m, 10 H, H-1, H-1′, H-2, H-3, H-3′, H′-2, H′-3, H′-4, H′-5, and H′-6), 3.80 (s, 3 H, OCH_3), 3.79 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 2.98 (dd, 1 H, *J* = 13.70 and 7.58 Hz, H′-6′), 2.90 (bs, 1 H, OH), 2.31 (s, 3 H, $C(O)CH_3$), 1.56 (bs, 1 H, OH); MS *m/z* 551 (M^+ – $C_7H_6OCH_3$); HRMS calcd for $C_{35}H_{44}NaO_{11}S$ ($M + Na)^+$ 695.2503, found 695.2529. Anal. Calcd for $C_{35}H_{44}O_{11}S$: C, 62.48; H, 6.59. Found: C, 62.30; H, 6.46.

3-*O*-[6-Deoxy-2,3,4-tri-*O*-(4-methoxybenzyl)-6-(thioacetyl)-α-D-glucopyranosyl]-1-*O*-linolenoyl-*sn*-glycerol (14). Linolenic acid (29 μL, 95 μmol) was added to a solution of diol **13** (67 mg, 100 μmol), DMAP (12.2 mg, 100 μmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (38.3 mg, 200 μmol, EDCI) in CH_2Cl_2 (5 mL). After being stirred at ambient temperature for 6 h, the mixture was diluted with CH_2Cl_2 (30 mL) and washed with H_2O (15 mL). The aqueous layer was washed with CH_2Cl_2 (2 × 15 mL), and the combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (33% ethyl acetate in hexane) to give **14** (57 mg, 61 μmol, 61%): $[\alpha]^{22}_D$ +19.0° (*c* 0.92, $CHCl_3$); IR ($CHCl_3$) 3440, 3015, 2990, 2930, 1730, 1680, 1610, 1580, 1510, 1460, 1350, 1300, 1250, 1070, 1030 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.29–7.21 (m, 6 H, ArH), 6.88–6.82 (m, 6 H, ArH), 5.40–5.27 (m, 6 H, vinyl H), 4.87–4.66 (m, 4 H, $ArCH_2$), 4.61 (d, 1 H, *J* = 3.62 Hz, H′-1), 4.56–4.49 (m, 2 H, $ArCH_2$), 4.14–3.70 (m, 7 H), 3.79 (s, 3 H, OCH_3), 3.78 (s, 6 H, OCH_3), 3.45 (dd, 1 H, *J* = 9.55 and 3.65 Hz, H′-2), 3.40–3.21 (m, 3 H), 3.00 (dd, 1 H, *J* = 13.69 and 7.42 Hz, H′-6), 2.78 (app t, 4 H, *J* = 5.63 Hz, $CHCH_2CH$), 2.36–2.28 (m, 5 H, $C(O)CH_2$ and $C(O)CH_3$), 2.08–2.00 (m, 4 H, CH_2CH), 1.64–1.58 (m, 2 H, $C(O)CH_2CH_2$), 1.29–1.23 (bs, 8 H, CH_2), 0.95 (t, 3 H, *J* = 7.58 Hz, $CHCH_2CH_3$); HRMS calcd for $C_{53}H_{72}NaO_{12}S$ ($M + Na)^+$ 955.4644, found 955.4678. Anal. Calcd for $C_{53}H_{72}O_{12}S$: C, 68.21; H, 7.78. Found: C, 68.48; H, 7.67.

3-*O*-[6-Deoxy-2,3,4-tri-*O*-(4-methoxybenzyl)-6-(thioacetyl)-α-D-glucopyranosyl]-1-*O*-linolenoyl-2-*O*-palmitoyl-*sn*-glycerol (15). To a solution of alcohol **14** (46 mg, 49 μmol), DMAP (6.1 mg, 50 μmol), and palmitic acid (25.6 mg, 100 μmol) in CH_2Cl_2 (2 mL) was added EDCI (19.2 mg, 100 μmol), and the solution was stirred at ambient temperature for 10 h. The reaction was diluted with CH_2Cl_2 (10 mL) and washed with H_2O (10 mL). The aqueous layer was washed with CH_2Cl_2 (2 × 10 mL), and the combined organic layers were washed with brine (10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (16% ethyl acetate in hexane) to give **15** (52.4 mg, 44.7 μmol, 91%): $[\alpha]^{22}_D$ +21.9° (*c* 0.94, $CHCl_3$); IR ($CHCl_3$) 3015, 3000, 2935, 2850, 1730, 1685, 1610, 1510, 1460, 1355, 1300, 1250, 1070 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.27–7.22 (m, 6 H, ArH), 6.88–6.82 (m, 6 H, ArH), 5.40–5.19 (m, 6 H, vinyl H), 4.87–4.61 (m, 4 H, $ArCH_2$), 4.58 (d, 1 H, *J* = 3.59 Hz, H′-1), 4.53–4.49 (m, 2 H, $ArCH_2$), 4.37 (dd, 1 H, *J* = 12.01 and 3.73 Hz), 4.17 (dd, 1 H, *J* = 11.96 and 6.31 Hz), 3.89–3.67 (m, 3 H), 3.79 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.48–3.20 (m, 5 H), 3.00 (dd, 1 H, *J* = 13.55 and 7.37 Hz, H′-6), 2.78 (app t, 4 H, *J* = 5.60 Hz, $CHCH_2CH$), 2.31 (s, 3 H, $C(O)CH_3$), 2.30–2.24 (m, 4 H, $C(O)CH_2$), 2.08–2.00 (m, 4 H, $CHCH_2$), 1.58–1.55 (m, 4 H, $C(O)CH_2CH_2$), 1.28–1.23 (m, 32 H, CH_2), 0.95 (t, 3 H, *J* = 7.58 Hz, $CHCH_2CH_3$), 0.86 (bt, 3 H, $(CH_2)_{14}CH_3$); HRMS calcd for $C_{69}H_{102}NaO_{13}S$ ($M + Na)^+$ 1193.6942, found 1193.7014. Anal. Calcd for $C_{69}H_{102}O_{13}S$: C, 70.74; H, 8.78. Found: C, 70.53; H, 8.48.

3-*O*-[6-Deoxy-2,3,4-tri-*O*-(4-methoxybenzyl)-6-sulfo-α-D-glucopyranosyl]-1-*O*-linolenoyl-2-*O*-palmitoyl-*sn*-glycerol Sodium Salt (16). To a solution of thioacetate **15** (364 mg, 311 μmol) in glacial acetic acid (7 mL) was added anhydrous potassium acetate (1.00 g, 10.2 mmol) followed by Oxone (400 mg, 651 μmol, $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$), and the resulting mixture was stirred vigorously for 8 h at ambient temperature. The reaction was diluted with H_2O (10 mL) and 6 N NaOH (19 mL) and washed with ethyl acetate (5 × 40 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (8% methanol in chloroform) and reversed-phase chromatography (RP-18, methanol) to give **16** (71 mg, 58.3 μmol, 19%): $[\alpha]^{22}_D$ +20.4° (*c* 0.71, $CHCl_3$); IR ($CHCl_3$) 3400, 3010, 2930, 2850, 1730, 1610, 1510, 1460, 1250, 1175, 1040 cm^{-1}; ^1H NMR (250 MHz, $(CD_3)_2SO$) δ 7.27–7.15 (m, 6 H, ArH), 6.88–6.82 (m, 6 H, ArH), 5.36–5.16 (m, 6 H, vinyl H), 4.75–4.41 (m, 7 H, $ArCH_2$ and H′-1), 4.29 (dd, 1 H, *J* = 2.80 and 1.08 Hz), 4.15–4.04 (m, 2 H), 3.96–3.87 (m, 1 H), 3.73 (s, 3 H, OCH_3), 3.72 (s, 6 H, OCH_3), 3.66 (app t, 1 H, *J* = 8.88 Hz), 3.43–3.36 (m, 2 H), 3.32–3.28 (m, 2 H), 3.07 (app t, 1 H, *J* = 9.50 Hz), 2.85–2.73 (m, 5 H, $CHCH_2CH$ and 1 H), 2.29–2.20 (m, 4 H, $C(O)CH_2$), 2.04–1.96 (m, 4 H, CH_2CH), 1.54–1.43 (m, 4 H, $C(O)CH_2CH_2$), 1.33–1.09 (m, 32 H, CH_2), 0.90 (t, 3 H, *J* = 7.56 Hz, $CHCH_2CH_3$), 0.83 (bt, 3 H, $(CH_2)_{14}CH_3$); HRMS calcd for $C_{67}H_{100}NaO_{15}S$ ($M + H)^+$ 1199.6684, found 1199.6627. Anal. Calcd for $C_{67}H_{99}NaO_{15}S{\cdot}H_2O$: C, 66.09; H, 8.36. Found: C, 65.96; H, 8.45.

3-*O*-[6-Deoxy-6-sulfo-α-D-glucopyranosyl]-1-*O*-linolenoyl-2-*O*-palmitoyl-*sn*-glycerol Sodium Salt (1). To a solution of **16** (66 mg, 54.2 μmol) in $CHCl_3$ (2 mL) was added iodotrimethylsilane (30 μL, 211 μmol). After being stirred at ambient temperature for 15 min, the mixture was diluted with anhydrous methanol (5 mL), and after an additional 15 min the solvent was removed in vacuo. The residue was purified by SiO_2 chromatography (11% methanol in chloroform to 25% methanol in chloroform) and reversed-phase chromatography (RP-18, 8% H_2O in methanol) to give **1** (32.4 mg, 37.8 μmol, 70%): $[\alpha]^{22}_D$ +42.8° (*c* 1.00, CH_3OH); IR (CCl_4) 3400, 2920, 2860, 1735, 1460, 1175, 1030 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 5.39–5.31 (m, 6 H, vinyl H), 4.76 (d, 1 H, *J* = 3.71 Hz, H′-1), 4.49 (dd, 1 H, *J* = 11.82 and 3.24 Hz), 4.22–4.05 (m, 3 H), 3.65–3.56 (m, 2 H), 3.43–3.30 (m, 3 H), 3.09 (dd, 1 H, *J* = 9.82 and 8.90 Hz), 2.92 (dd, 1 H, *J* = 14.36 and 9.12 Hz), 2.81 (app t, 4 H, *J* = 5.64 Hz, $CHCH_2CH$), 2.37–2.29 (m, 4 H, $C(O)CH_2$), 2.11–2.06 (m, 4 H, $CHCH_2$), 1.63–1.54 (m, 4 H, $C(O)CH_2CH_2$), 1.44–1.24 (m, 32 H, CH_2), 0.97 (t, 3 H, *J* = 7.57 Hz, $CHCH_2CH_3$), 0.90 (bt, 3 H, $(CH_2)_{14}CH_3$); ^{13}C NMR (250 MHz,

CD_3OD) δ 175.09, 174.91, 132.75, 131.07, 129.22, 128.87, 128.25, 100.10, 74.90, 73.44, 71.70, 69.78, 67.17, 64.32, 54.32, 35.21, 34.99, 33.09, 30.82, 30.51, 30.36, 30.26, 28.21, 26.56, 26.44, 26.03, 23.74, 21.51, 14.68, 14.45; MS m/z 839 $(M + H)^+$; HRMS calcd for $C_{43}H_{76}NaO_{12}S$ $(M + H)^+$ 839.4958, found 839.4928.

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Biosynthesis of Virginiae Butanolide A, a Butyrolactone Autoregulator from *Streptomyces*

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Abstract: Virginiae butanolide A (VB A) (**3**) is one of the virginiamycin-inducing factors from *Streptomyces virginiae* and has a unique 2,3-disubstituted butanolide skeleton which is common to other signal molecules in *Streptomyces*. The biosynthesis of **3** in *Streptomyces antibioticus*, a high producer of **3**, was studied by experiments with labeled precursors. ^{13}C and ^{2}H NMR results as well as CI-MS analyses of dibenzoate samples indicated that the probable biosynthetic pathway to **3** involved coupling between a β-keto acid derivative and a C_3 unit from glycerol, such as dihydroxyacetone or a derivative.

Signal molecules which regulate secondary metabolite production or cytodifferentiation of *Streptomyces* have been known for nearly 20 years. The first one was A factor (**1**) found by Khokhlov et al., which induces formation of streptomycin, aerial mycelium, and spores in *Streptomyces griseus*.[1] After the discovery of A factor, Gräfe et al. isolated factor 1 (**2**) from the culture broth of *Streptomyces viridochromogenes* as an inducer of the formation of aerial mycelia and leukaemomycin in *S. griseus*.[2] Then anthracycline-inducing factors in *S. griseus* were found in the culture broth of *Streptomyces bikiniensis* and *Streptomyces cyaneofuscatus* by Gräfe et al.[3] They assigned these structures to what we will refer to as **3**, **4**, and **5**. Recently, we have reported the isolation and structural elucidation of virginiae butanolides (VB) A-E **3**, **6**, **7**, **8**, and **9**, which induce the production of virginiamycin in *Streptomyces virginiae*,[4] and more recently IM-2 (**10**), which induces the production of a blue pigment in *Streptomyces* sp. FRI-5.[5] All of these molecules have common structural features. They possess a 2,3-disubstituted butanolide skeleton but differ in the C-2 side chain containing functional groups, such as 6-hydroxy or 6-keto groups, and in the length or branching of the alkyl chain. A 2,3-cis or -trans configuration was once proposed for autoregulators which have a C-6 hydroxyl group, but recently the stereochemistry was revised as shown in Figure 1.[6] The absolute configurations of A factor and VB A, B, and C have been assigned to **1**, **3**, **6**, and **7** with their chiral synthesis being carried out by Mori et al.[7]

Table I. ^{13}C Abundances in **3a** Obtained from Feeding Experiments with ^{13}C-Labeled Precursors

carbon no.	δ_C	relative ^{13}C abundances[a]			
		[1-^{13}C]-acetate	[2-^{13}C]-acetate	[1-^{13}C]-isovalerate	[1,3-$^{13}C_2$]-glycerol
1	175.6	6.6	1.6	nd[b]	nd
2	46.4	0.8	10.7	0.8	4.5
3	36.4	0.8	1.0	0.8	nd
4	69.2	0.9	1.1	0.9	6.2
5	65.2	0.9	1.3	1.1	6.1
6	73.1	5.8	1.5	0.9	nd
7	33.0	0.9	9.3	0.9	3.7
8	23.2	0.9	0.9	7.3	nd
9	38.4	0.8	1.2	1.1	nd
10	27.7	1.0	1.0	1.0	nd
11	22.4	1.1	1.3	0.9	nd
12	22.4	0.9	1.2	0.7	nd

[a] Peak height ratio of ^{13}C enriched to natural abundance. [b] nd = Not detected.

This unique butanolide skeleton is hitherto known only in metabolites of *Streptomyces*. These molecules are efficient at extremely low concentrations, and specific receptor proteins are involved in the expression of their activity.[8] Biosynthetic studies on these signal molecules are very important as a new approach to understanding the mechanism of secondary metabolite production in *Streptomyces*. However, it has been very difficult to study the biosynthesis of these molecules because they are mainly produced in trace amounts in culture broths; e.g., only a few micrograms of VB A were obtained from 1 L of *S. virginiae* broth. Recently we found a strain of *Streptomyces antibioticus* which produces several milligrams of VB A per liter of culture broth,[9] and this finding has made it possible to elucidate the biosynthesis of **3** by feeding experiments with ^{13}C-labeled precursors. We have

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