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The Butenolide Signaling Molecules SRB1 and SRB2 Induce Lankacidin and Lankamycin Production in *Streptomyces rochei*

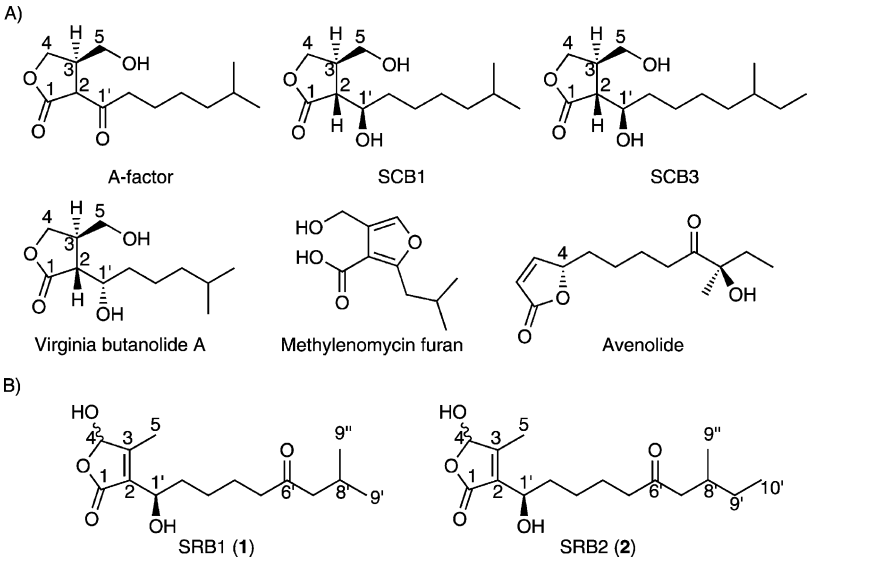
Kenji Arakawa,* Naoto Tsuda, Akihiro Taniguchi, and Haruyasu Kinashi[a]

New signaling molecules that induce lankacidin and lankamycin production in *Streptomyces rochei* were extracted from the culture filtrate and purified by Sephadex LH20 and silica gel chromatography with the help of bioassay. Chiral HPLC and ESI-MS analyses indicated the presence of two active components—SRB1 and SRB2—and their molecular formulas were established to be $C_{15}H_{24}O_5$ and $C_{16}H_{26}O_5$, respectively. By extensive NMR analysis, SRB1 and SRB2 were determined to be 2-(1′-hydroxy-6′-oxo-8′-methylnonyl)-3-methyl-4-hydroxybut-2-en-1,4-olide and 2-(1′-hydroxy-6′-oxo-8′-methyldecyl)-3-methyl-4-hydroxybut-2-en-1,4-olide, respectively. These structures were finally confirmed by chemical synthesis and the absolute configuration at C-1′ was determined to be *R* in each case. The synthetic 1′*R* isomers induced production of lankacidin and lankamycin at around 40 nM concentrations. SRB1 and SRB2 are therefore distinct from the well-known 2,3-disubstituted γ-butyrolactone molecules such as A-factor, virginia butanolide, and SCB1 and and belong, like avenolide, recently isolated from *Streptomyces avermitilis*, to the γ-butenolide family.

Introduction

The filamentous soil bacteria *Streptomyces* are characterized by the ability to produce a wide variety of secondary metabolites, including antibiotics. In many *Streptomyces* species, antibiotic production and morphological differentiation are controlled by small diffusible signaling molecules.[1,2] The well-studied family of signaling molecules identified up to date have 2,3-disubstituted γ-butyrolactone skeletons and are active at nanomolar concentrations.[3] The most extensively characterized molecule is A-factor (2-isocapryloyl-3*R*-hydroxymethyl-γ-butyrolactone; Scheme 1 A), which is responsible for streptomycin production and morphological differentiation in *Streptomyces griseus*.[4] When A-factor produced in a growth-dependent manner reaches a critical concentration, it binds to a specific receptor protein (ArpA), and the A-factor/ArpA complex then dissociates from the promoter region of *adpA*, a global transcriptional activator gene. The de-repressed gene product AdpA binds to its target genes to activate streptomycin production and morphological differentiation.[5] At the top of this regulatory cascade, the *afsA* gene encodes a key enzyme in A-factor biosynthesis.[6,7]

Additional γ-butyrolactone-type signaling molecules and their specific receptors have been identified in several *Streptomyces* species, including *Streptomyces coelicolor* A3(2),[8] *Streptomyces virginiae*,[9] and *Streptomyces lavendulae* FRI-5.[10] The γ-butyrolactone-type signaling molecules have been classified



Scheme 1. Structures of *Streptomyces* signaling molecules. A) Known signaling molecules: the 2,3-disubstituted γ-butyrolactone type (A-factor, SCB1, SCB3, virginia butanolide A), the furan type (methylenomycin furan), and the 4-monosubstituted butenolide type (avenolide). B) The new 2,3-disubstituted butenolides SRB1 (**1**) and SRB2 (**2**) isolated from *S. rochei* 7434AN4.

[a] *Prof. Dr. K. Arakawa, N. Tsuda, A. Taniguchi, Prof. Dr. H. Kinashi*
Department of Molecular Biotechnology
Graduate School of Advanced Sciences of Matter
Hiroshima University
1-3-1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8530 (Japan)
E-mail: karakawa@hiroshima-u.ac.jp

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into three major groups based on the reduction state and the stereochemistry at C-1′ (Scheme 1 A): 1) the 1′-keto type (e.g., A-factor in *S. griseus*), 2) the (1′*R*)-hydroxy type [SCB1 in *S. coelicolor* A3(2)], and 3) the (1′*S*)-hydroxy type (virginia butanolides in *S. virginiae*).

New signaling molecules have recently been identified in *S. coelicolor* and *Streptomyces avermitilis*. A furan-type signaling molecule— methylenomycin furan (Scheme 1 A)—was shown to induce methylenomycin production in *S. coelicolor*.[11] A 4-monosubstituted butenolide named avenolide (Scheme 1 A) induces avermectin production in *S. avermitilis* with a minimum inducing concentration of 4 nM.[12] Signaling molecules of various types are thus involved in controlling antibiotic biosynthesis in *Streptomyces* species.

Streptomyces rochei strain 7434AN4 contains three linear plasmids (pSLA2-L, -M, and -S) and produces two structurally unrelated polyketide antibiotics: lankacidin and lankamycin (Figure S1 in the Supporting Information).[13] Lankacidin and lankamycin inhibit peptide synthesis synergistically by targeting neighboring sites in the ribosome.[14] The biosynthetic gene clusters of these antibiotics are located on the giant linear plasmid pSLA2-L.[15–17] In addition, many regulatory genes have been identified on pSLA2-L, including a synthesis gene of the signaling molecule (*srrX*), six *tetR*-type repressor genes (*srrA-F*), and three SARP (*Streptomyces* antibiotic regulatory protein) genes (*srrY*, *srrZ*, and *srrW*). We revealed that *srrX* and *srrA* constitute the signaling molecule/receptor system in *S. rochei*.[18] Extensive mutational and transcriptional analyses revealed that the SARP gene *srrY* is a target of SrrA and that the signaling pathway goes from *srrX* through *srrA* to *srrY*, leading to lankacidin and lankamycin production.[19] Furthermore, it was shown that SrrY directly activates transcription of the second SARP gene *srrZ* for LM production.[20] In spite of these accumulated data on the regulatory cascade of antibiotic production in *S. rochei* 7434AN4, the signaling molecules themselves had not been isolated. Here we report the isolation, structural elucidation, and biological activities of the signaling molecules termed "SRBs" (*Streptomyces rochei* butenolides), which induce lankacidin and lankamycin production at nanomolar concentrations.

Results

Isolation of SRBs

S. rochei KA61, a disruptant of the SARP-family activator gene *srrY*, was used to isolate the SRB molecules. This strain does not produce lankacidin or lankamycin, due to a shutdown of the regulatory cascade downstream of *srrY*, which improves the isolation efficiency of the SRB molecules without the disturbance of antibiotics. A 160 L culture of strain KA61 was grown in a 200 L jar fermenter at 28 °C for 43 h. The culture filtrate was passed through an Amberlite XAD16 column to absorb hydrophobic metabolites, including SRBs. The column was extracted with EtOAc, and the resulting oil (18 g) was purified by silica gel chromatography with hexane/EtOAc (2:1 to 1:2 *v/v*). Each fraction was subjected to bioassay to check its antibiotic-inducing activity. The active fractions were combined and purified on Sephadex LH20 with methanol as eluent. The active fractions were then collected and further purified by series of silica gel chromatographic separations with the following three solvent systems to give a single spot on TLC (R_f = 0.20 in hexane/EtOAc 1:1 *v/v*): $CHCl_3$/methanol (50:1–30:1 *v/v*), toluene/EtOAc (2:1–1:1 *v/v*), and hexane/EtOAc (2:1–1:1 *v/v*). No other fractions showed any inducing activity, indicating that the purified component is an inducing factor of lankacidin and lankamycin in *S. rochei*.

The active component was further analyzed by chiral HPLC (TCI Chiral MB-S column; macroporous silica gel coated with optically active N-substituted polymaleimides) with aqueous acetonitrile (10%) as mobile phase (flow rate 1.0 mL min^{-1}) with detection at 210 nm. In contrast with the results obtained with HPLC and TLC, two peaks were detected: at 9.1 min (SRB1) and 17.3 min (SRB2; Figure 1 A). Because of the limited amounts of the two compounds available (total ≈250 μg; estimated later by UV absorbance), however, structural analysis was carried out as a mixture. ESI-MS analysis also indicated the presence of SRB1 and SRB2 in 1:1 ratio (Figure 1 B). Their molecular formulas were established to be $C_{15}H_{24}O_5$ for SRB1 and $C_{16}H_{26}O_5$ for SRB2, suggesting that SRB1 and SRB2 are homologues with a difference consisting of one methylene unit. With regard to the HPLC chromatogram of the SRBs (Figure 1 A), it is noteworthy that their retention times differed by 8.2 min. A similar relationship was observed in the *S. coelicolor* signaling molecules SCB1 and SCB3 (Scheme 1 A): SCB1 (*iso*-type C_8 side chain) eluted at 22–23 min, whereas SCB3 (*antei-so*-type C_9 side chain) eluted at 40–48 min.[21]

Structural elucidation of SRBs

The ^{13}C NMR and HMBC spectra of the mixture of SRB1 and SRB2 (Figure 1 D) revealed two carbonyl groups (C-1 and C-6′; δ_C = 170.7 and 213.1 ppm) and one C=C double bond (C-2 and C-3; δ_C = 129.4 and 157.1 ppm) for each compound. No olefinic protons were observed in the ^1H NMR spectrum (Figure 1 C), suggesting that the C=C double bond was fully substituted. The existence of cyclic skeletons in the SRBs was therefore inferred from the degree of unsaturation. A strong absorbance at 1750 cm^{-1} in the IR spectrum indicated the presence of an α,β-unsaturated γ-lactone ring. The singlet H-5 protons at δ_H = 2.08 ppm were assigned to a methyl group at C-3 (δ_C = 157.1) by HMBC of the H-5 protons to C-3 (Figure 1 E-i). The H-5 protons showed long-range HMBC correlations with the C-4 methine carbon (δ_C = 98.7 ppm) and the C-2 quaternary carbon (δ_C = 129.4 ppm). The C-4 carbon is bound to a highly deshielded broad-singlet proton (δ_H = 5.88 ppm), indicating that C-4 is hydroxylated. Furthermore, the broad-singlet H-4 proton showed a HMBC correlation with the C-1 carbonyl carbon (δ_C = 170.7 ppm). The H-5 protons showed moderate NOEs with the deshielded H-1′ proton (δ_H = 4.50 ppm) of the alkyl side chain as well as with H-4. From these data, a 2,3-disubstituted γ-hydroxybutenolide ring, in which C-3 was substituted with a methyl group and C-2 with an alkyl side chain, was deduced as a partial structure (Figure 1 E-i). The broad-singlet signal of

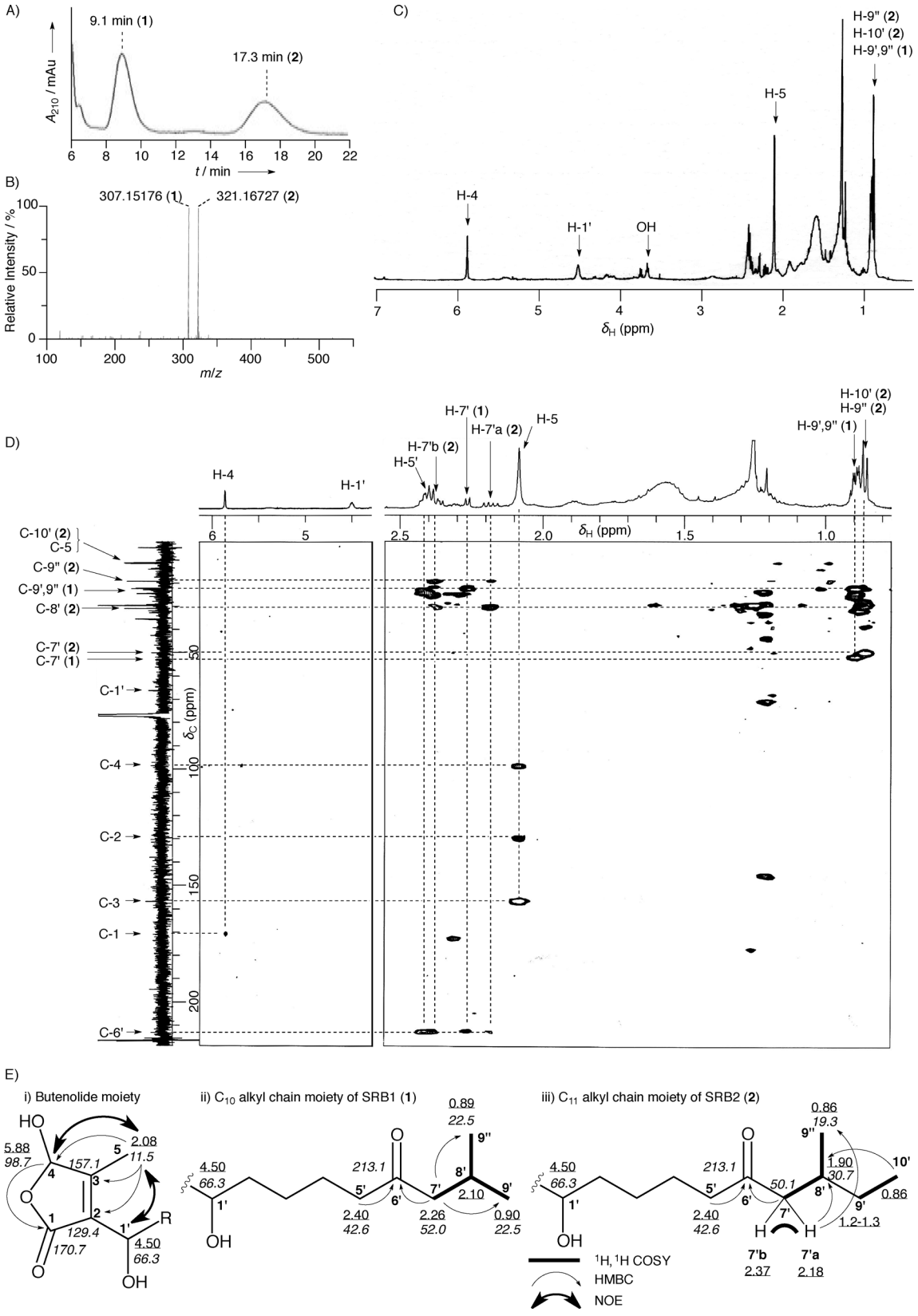


Figure 1. Spectral analysis of natural SRBs. A) Chiral HPLC analysis. Elution profiles were monitored by UV absorbance at 210 nm. B) ESI-MS spectrum. C) ^{1}H NMR spectrum. D) HMBC spectrum and the key cross-peaks. E) ^{1}H,^{1}H COSY and HMBC correlations: i) in the butenolide moieties in both SRBs, ii) in the C_{10} alkyl chain moiety of SRB1, and iii) in the C_{11} alkyl chain moiety of SRB2. The δ_H and δ_C values are shown underlined and in italics, respectively.

H-4 implied that the two compounds exist as mixtures of interconverting epimers at C-4. These assignments showed a good agreement with those of known 2,3-disubstituted γ-hydroxybutenolides (R = isopropyl or *sec*-butyl; Figure 1 E-i) previously isolated from *Streptomyces antibioticus* Tü99.[22]

The common partial structure of the two SRBs indicated that the alkyl side chains branched at C-2 have the formulas $C_{10}H_{19}O_2$ for SRB1 and $C_{11}H_{21}O_2$ for SRB2. The C-6′ carbonyl carbon at $\delta_C = 213.1$ ppm was assigned to a ketone and showed HMBC correlations with proton signals at $\delta_H = 2.26$ (d), 2.18 (dd), and 2.37–2.40 ppm (m) (Figure 1 D). These signals correspond to the methylene protons: $\delta_H = 2.26$ ppm at $\delta_C =$ 52.0 ppm (C-7′ of SRB1; Figure 1 E-ii), $\delta_H = 2.18$ and 2.37 ppm at $\delta_C = 50.1$ ppm (C-7′ of SRB2; Figure 1 E-iii), and $\delta_H = 2.40$ ppm at $\delta_C = 42.6$ ppm (C-5′ of SRB1 and SRB2; Figure 1 E-ii and -iii). These spectral data indicate that the methylene groups (C-5′ and C-7′) on the two sides of C-6′ have different chemical environments.

With regard to SRB1, the doublet H-7′ proton ($\delta_H =$ 2.26 ppm) showed a ^{1}H,^{1}H COSY correlation with the proton at $\delta_H = 2.10$ ppm, which in turn connected to the two methyl groups H-9′ and H-9″ at $\delta_H = 0.89$–0.90 ppm. A partial structure of the alkyl chain of SRB1 was thus elucidated as shown in Figure 1 E-ii.

With regard to SRB2, the double–doublet H-7′a proton ($\delta_H =$ 2.18 ppm) showed ^{1}H,^{1}H COSY correlations with signals at $\delta_H =$ 2.37 ppm and $\delta_H = 1.90$ ppm, the former being assigned to a geminal H-7′b proton by HMQC experiment. The latter proton ($\delta_H = 1.90$ ppm) connected to C-8′ showed a further ^{1}H,^{1}H COSY correlation with the H-9′ methyl protons ($\delta_H =$ 0.86 ppm; Figure 1 E-iii). In addition, the C-8′ carbon ($\delta_C =$ 30.7 ppm) showed HMBC correlations with the double-doublet H-7′a proton and the H-10′ methyl protons ($\delta_H = 0.86$ ppm), the latter of which showed a ^{1}H,^{1}H COSY correlation with the H-9″ methylene protons ($\delta_H = 1.2$–1.3 ppm). These assignments led to a partial structure of the alkyl chain of SRB2 as illustrated in Figure 1 E-iii. Although further assignments of the three additional methylene groups corresponding to C2′–C4′ of SRB1 and SRB2 were difficult due to overlapping signals, SRB1 and SRB2 were assigned as 2-(1′-hydroxyl-6′-oxo-8′-methylnonyl)-3-methyl-4-hydroxybut-2-en-1,4-olide (**1**) and 2-(1′-hydroxyl-6′-oxo-8′-methyldecyl)-3-methyl-4-hydroxybut-2-en-1,4-olide (**2**). Each compound contains a 2,3-disubstituted γ-hydroxybutenolide skeleton, which is distinct from those of the known γ-butyrolactone molecules such as A-factor, virginia butanolide, and SCB1 (Scheme 1).

Synthesis of SRB molecules

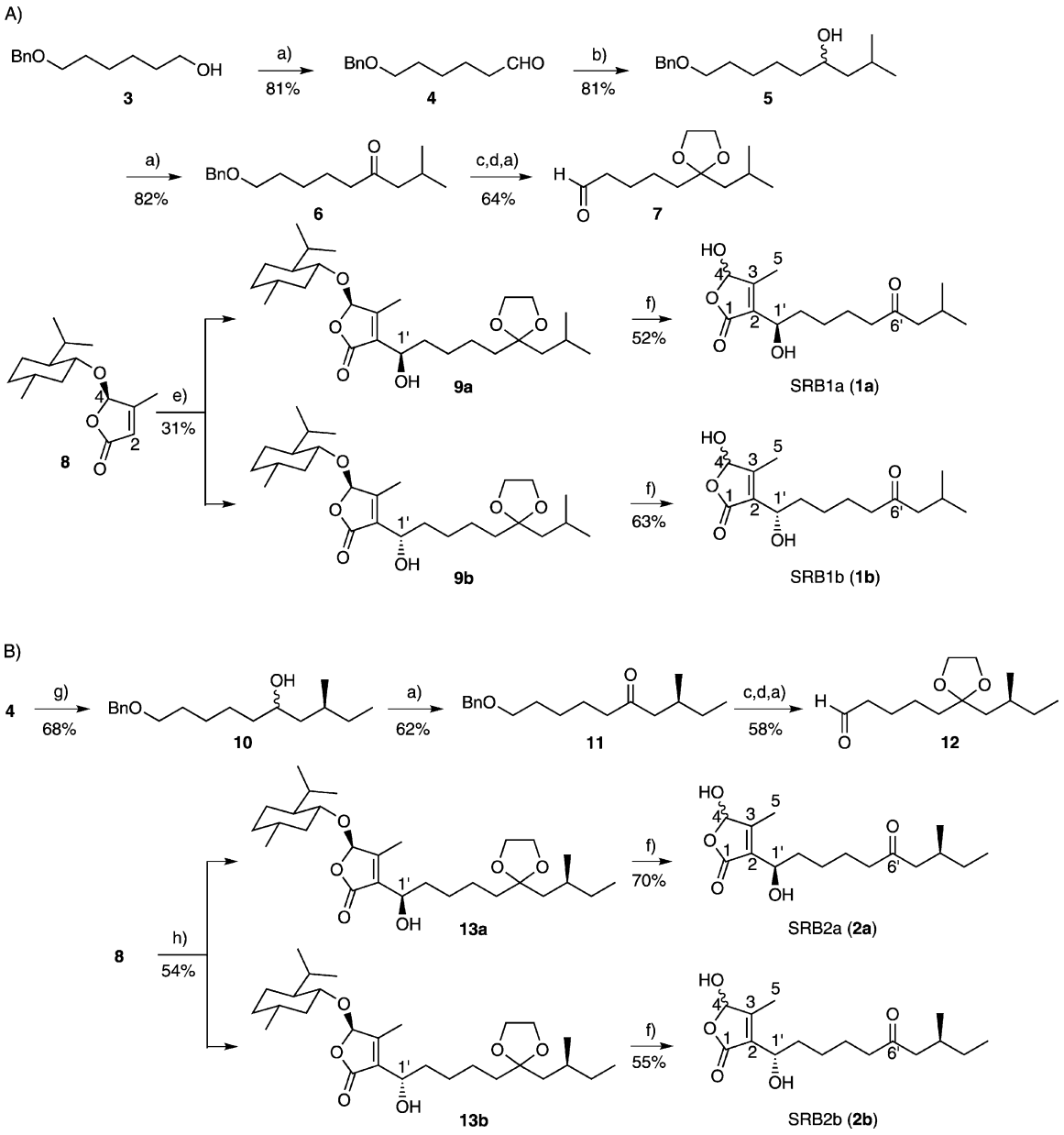
To confirm the proposed structures of **1** and **2** and to determine their C-1′ configurations, the 1′*R* isomers (**1a** and **2a**) and the 1′*S* isomers (**1b** and **2b**) were synthesized as shown in Scheme 2.

For the synthesis of the alkyl chains of **1a** and **1b** (Scheme 2 A), the readily available 1-(benzyloxy)hexan-6-ol (**3**)[23] was oxidized with pyridinium chlorochromate (PCC) to give aldehyde **4** in 81% yield, **4** was then coupled with the Grignard reagent isobutylmagnesium bromide to give the C_{10} unit **5** in 81% yield, and the newly generated hydroxy group was then oxidized with PCC to afford ketone **6** in 82% yield. Protection of the ketone group in **6** with ethylenedioxybis(trimethylsilane), removal of the benzyl group, and subsequent oxidation with PCC afforded aldehyde **7** in 64% yield (three steps).

Enantiomerically pure 3-methyl-4-(L-menthyloxy)but-2-en-1,4-olide (**8**) was prepared by condensation of propanal with glyoxylic acid and L-menthol, followed by two-stage recrystallization from petroleum ether.[24,25] The key coupling reaction between the anion derived from **8** [with lithium diisopropylamide (LDA)] and the aldehyde **7** provided a diastereomeric mixture of **9a** and **9b** in 2:1 ratio and in 31% yield. The large protecting group (L-menthyl) at the C-4 hydroxy function of **8** prevented removal of the more acidic H-5 proton,[26] leading to the production of the desired 2,3-disubstituted γ-(L-menthyloxy)butenolide. The diastereomeric mixture of **9a** and **9b** was separated by repeated flash chromatography runs with hexane/EtOAc. Each of the purified compounds had 94% diastereomeric purity as judged from the two well-separated hemiacetal H-4 proton signals in the ^{1}H NMR spectra ($\delta_H =$ 5.69 ppm for **9a** and $\delta_H = 5.71$ ppm for **9b**). The C-1′ configurations of these compounds were determined from the $\Delta\delta$ ($\delta_S - \delta_R$) values of their (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)-α-phenylacetate (MTPA) ester derivatives.[27] Characteristic H-4 and H-5 proton signals in the γ-butenolide moiety of **9a** showed distinct negative $\Delta\delta$ values (−25 and −98 Hz, respectively), whereas the H-2′ proton showed a slightly positive value (+5 Hz). The C′-1 configuration of **9a** was thus determined to be *R*, whereas that of **9b** is *S*. Finally, removal of the L-menthyl and 1,3-dioxolane groups in **9a** and **9b** was performed simultaneously with boron tribromide to afford SRB1a (**1a**) and SRB1b (**1b**) in 52 and 63% yields, respectively.

SRB2a (**2a**) and SRB2b (**2b**, Scheme 2 B) were synthesized in a similar way as for **1a** and **1b** but a different Grignard reagent, (*S*)-(2-methylbutyl)magnesium chloride, was used for construction of the C_{11} unit **10**.

The retention times of SRB1 and SRB2 (9.1 and 17.3 min) on a chiral HPLC column (mobile phase; 10% aqueous acetonitrile containing 0.1% trifluoroacetic acid) at a flow rate of 1.0 mL min^{-1} were identical to those of the synthetic 1′*R* isomers **1a** and **2a**, whereas the synthetic 1′*S* isomers **1b** and **2b** eluted slightly earlier, at 8.7 and 16.6 min (Figure 2 A). Furthermore, the natural SRB mixture showed a positive optical rotation value ($[\alpha]_D^{22} = +17.5$), which was in good agreement with those of the synthetic 1′*R* isomers (+18.4 for **1a**, +22.4 for **2a**). The synthetic 1′*S* isomers exhibited negative values of optical rotation (−9.32 for **1b**, −5.71 for **2b**). Consequently, the absolute C-1′ configurations in SRB1 and SRB2 were both determined to be *R* (Scheme 1B). As shown in Figure 2 B, spectra of the natural SRBs showed good agreement with those of the synthetic compounds **1a** and **2a**. In the ^{1}H and ^{13}C NMR spectra of the synthetic SRBs (**1a**, **1b**, **2a**, **2b**), small separate resonances ($\Delta\delta < 0.03$ ppm in ^{1}H NMR and $\Delta\delta < 0.3$ ppm in ^{13}C NMR) due to the presence of interconverting epimers at C-



Scheme 2. Synthesis of *S. rochei* butenolides: A) SRB1, and B) SRB2. a) Pyridinium chlorochromate, CH_2Cl_2; b) isobutylmagnesium bromide, THF; c) TMSO-$(CH_2)_2$OTMS, TMSOTf, CH_2Cl_2; d) H_2, Pd-C (10%), EtOAc; e) LDA, THF/HMPA, and then **7**; f) BBr_3, CH_2Cl_2; g) (*S*)-(2-methylbutyl)magnesium chloride, THF; h) LDA, THF/HMPA, and then **12**.

4 are observed (Table 1). These hemiacetalic epimers interconvert rapidly[28] and were inseparable even by chiral HPLC.

Biological activities of SRB molecules

The minimum concentrations of the synthetic SRBs necessary for antibiotic production were determined by bioassay. The 1′*R* isomers **1a** and **2a** showed inducing activities at 42 and 40 nM, whereas the 1′*S* isomers **1b** and **2b** were active at 660 and 630 nM, respectively. The values for **1a** and **2a** corresponded to that for the natural SRBs (ca. 50 nM). The 1′*S* isomers (**1b** and **2b**) showed much lower activities (around 6%) than the 1′*R* isomers, suggesting that the 1′*R* configuration is important for antibiotic-inducing activity. In this respect, we cannot exclude the possibility that the low inducing activities of **1b** and **2b** might be due to contamination by **1a** and **2a**. The average yield of SRBs in strain KA61 was around 6 μg per liter of the culture. The metabolic profiles of strain KA20, a *srrX–srrB* double mutant, separately supplemented with SRB1 and SRB2 were comparable (Figure 3), suggesting that SRB1 and SRB2 play equal roles in inducing lankacidin and lankamycin production in *S. rochei*.

Discussion

Two signaling molecules, SRB1 and SRB2, which induce production of lankacidin and lankamycin in *S. rochei* 7434AN4, were isolated and their structures were determined. The SRB

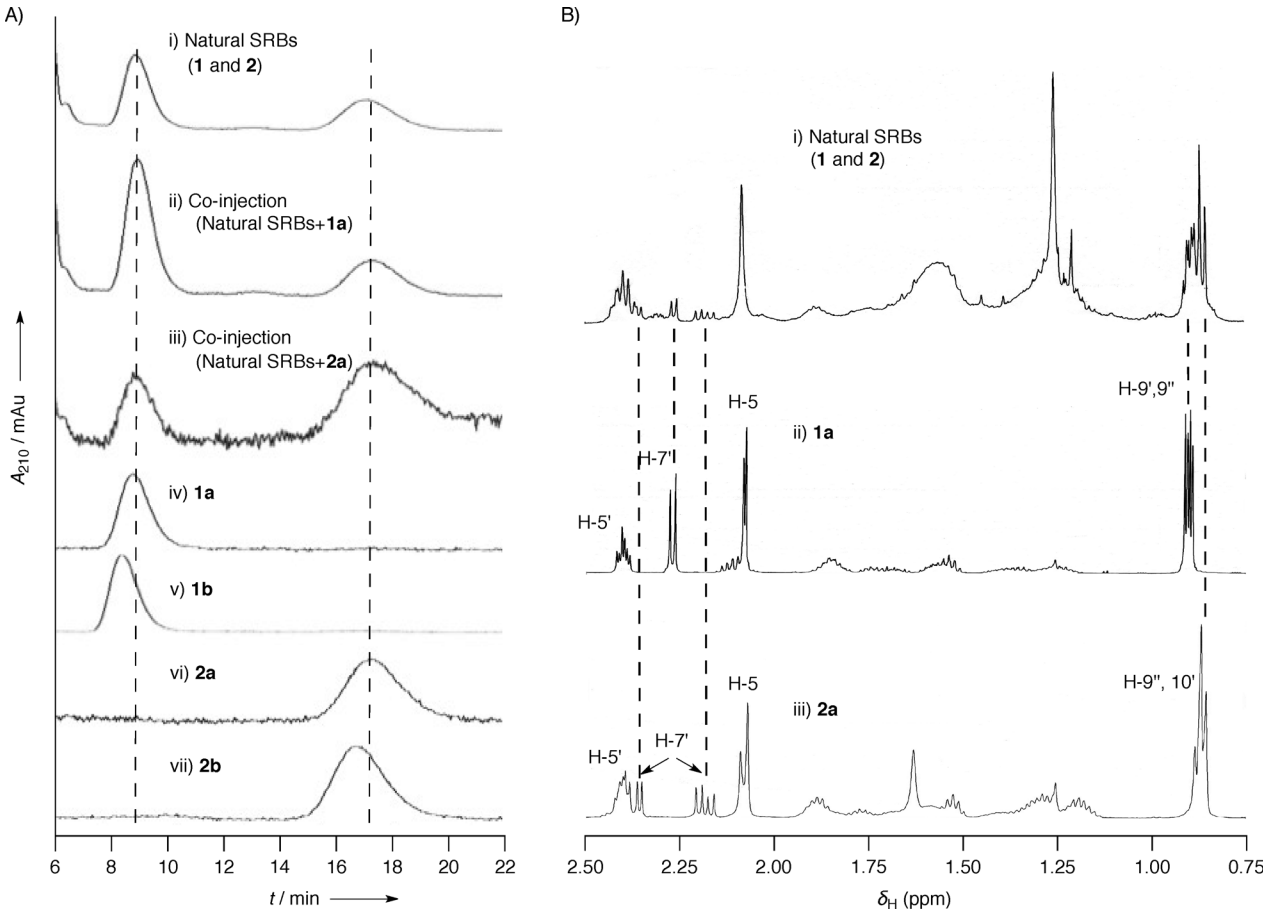


Figure 2. Determination of the C-1′ configurations in natural SRBs. A) Chiral HPLC analysis of: i) natural SRBs (**1** and **2**), ii) co-injection of natural SRBs and synthetic **1a**, iii) co-injection of natural SRBs and synthetic **2a**, iv) synthetic **1a**, v) **1b**, vi) **2a**, and vii) **2b**. Elution profiles were monitored by UV absorbance at 210 nm. B) ^{1}H NMR spectra of: i) natural SRBs, ii) synthetic **1a**, and iii) **2a** (δ_H = 0.75–2.50 ppm).

Table 1. NMR data for **1a** and **2a** in $CDCl_3$.[a]

Pos.	SRB1a (**1a**)		SRB2a (**2a**)	
	δ_C (mult.)[b]	δ_H (mult., *J* [Hz])[b]	δ_C (mult.)[b]	δ_H (mult., *J* [Hz])[b]
1	171.4/171.5 (s)	–	171.4/171.5 (s)	–
2	129.9/130.0 (s)	–	129.9/130.0 (s)	–
3	157.6/157.6 (d)	–	157.6/157.7 (d)	–
4	98.7/98.9 (d)	5.85 (brs)	98.7/98.9 (d)	5.85 (brs)
5	11.5/11.6 (d)	2.07/2.09 (s)	11.5/11.6 (q)	2.08/2.09 (s)
1′	66.4/66.5 (d)	4.49 (m)	66.4/66.5 (d)	4.48 (m)
2′	35.6/35.7 (t)	1.70–1.85 (m)	35.5/35.7 (t)	1.70–1.85 (m)
3′	24.7/24.8 (t)	1.25, 1.36 (m)	24.7/24.8 (t)	1.22, 1.35 (m)
4′	23.0/23.1 (t)	1.53 (m)	23.0/23.2 (t)	1.53 (m)
5′	42.7/42.9 (t)	2.40 (dt, 3.4, 7.1)	42.7/42.9 (t)	2.40 (m)
6′	211.6/212.5 (s)	–	211.8/212.8 (s)	–
7′	51.9/52.0 (t)	2.26 (d, 7.1)	50.0/50.1 (t)	2.18 (dd, 7.9, 8.3), 2.37 (m)
8′	24.7 (d)	2.09 (m)	30.9 (d)	1.90 (m)
9′	22.5/22.6 (d)	0.89 (d, 6.8)[c]	29.5 (t)	1.18, 1.28 (m)
10′	–	–	11.3 (q)	0.87 (d, 6.7)
9′′	22.5/22.6 (d)	0.90 (d, 6.7)[c]	19.3/19.4 (q)	0.86 (d, 7.0)

[a] Spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C. [b] The residential solvent signal (δ_C = 77 ppm) and internal standard tetramethylsilane (δ_H = 0 ppm) were used as references. [c] Assignments are exchangeable.

molecules each contain a 2,3-disubstituted γ-hydroxybutenolide skeleton. Structurally related butenolides have been isolated from *S. antibioticus* Tü99 as antibiotics, which exhibited moderate antimicrobial activity against *Pseudomonas aeruginosa*.[22] About 60% of *Streptomyces* species use γ-butyrolactone-type signaling molecules to induce antibiotic production,[29] and fourteen molecules of this type have previously been identified.[3,21] New types of signaling molecules have recently been isolated from several *Streptomyces* species. A furan-type signaling molecule, methylenomycin furan, was identified as an autoregulator for methylenomycin production in *S. coelicolor* A3(2).[11] In addition, avenolide, a new 4-monosubstituted γ-butenolide-type molecule, was identified as an inducing factor for avermectin production in *S. avermitilis*.[12] It is noteworthy that the *avaR* gene (*sav2269*) in *S. avermitilis*, which encodes a homologue of the AfsA-family proteins, is not involved in avenolide production. We have not determined the stereochemistry at C-8′ in SRB2. Nevertheless the *S* configuration of C-8′ in **2** seems more plausible in view of the following evidence. 8-Methyldecanoyl-ACP, a possible biosynthetic intermediate of **2**, is synthesized by condensation of four malonyl-CoA units with a starter (*S*)-2-methylbutyryl-CoA, derived from L-isoleucine, in primary fatty acid biosynthesis. Hafner et al.

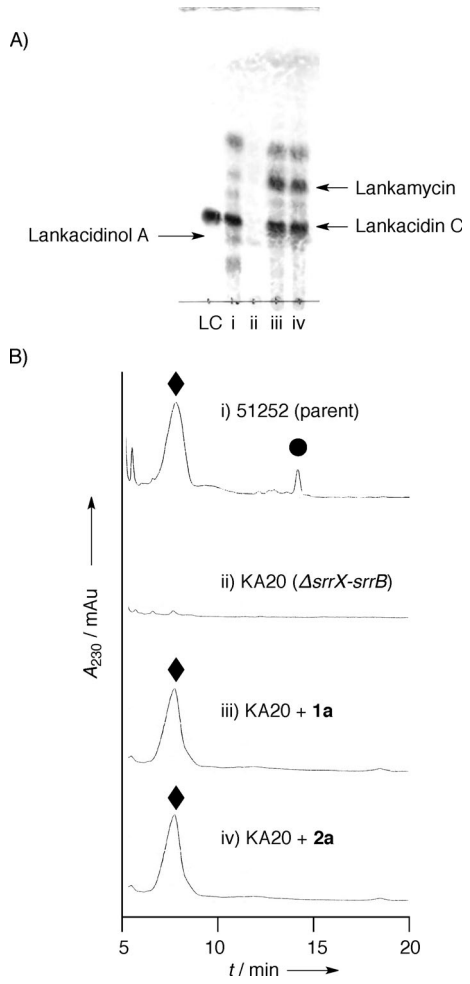


Figure 3. Effects of SRB1 and SRB2 on lankacidin and lankamycin production. A) TLC analysis of metabolites. i) Parent 51252; ii) strain KA20; iii) strain KA20 supplemented with **1a** (840 nM); iv) strain KA20 supplemented with **2a** (840 nM). Lane LC is a standard sample of lankacidin C. B) HPLC analysis of metabolites. Elution profiles were monitored by UV absorbance at 230 nm. Closed diamonds and circle indicate the peaks of lankacidin C and lankacidinol A, respectively.

reported that the branched-chain 2-oxo acid decarboxylase is specific to the *S* isomer of 3-methyl-2-oxopentanoic acid for the synthesis of *anteiso*-type fatty acids and avermectins in *S. avermitilis*.[30]

The minimum concentrations of SRB1 (**1** = **1a**) and SRB2 (**2** = **2a**) for induction of antibiotic production in *S. rochei* were 42 nM and 40 nM, respectively. These values were lower than those of SCB1 (128–256 nM) for actinorhodin and undecylprodigiosin production in *S. coelicolor*[31] and higher than those of A-factor (around 1 nM) for streptomycin in *S. griseus*[6] and of virginia butanolides (around 3 nM) for virginiamycin in *S. virginiae*.[32] The synthetic 1′*S* isomers **1b** and **2b** exhibited significantly weaker activities than the 1′*R* isomers **1a** and **2a**, indicating that the stereochemistry at C-1′ is crucial for induction of activity. Our preliminary experiments demonstrated that 2,3-dihydro-SRB1, prepared from **1a** by catalytic hydrogenation, showed no ability to induce antibiotic production in *S. rochei* at a concentration of 33.5 μM, indicating the importance of the C2=C3 double bond for activity. The function(s) of the C-4 hemiacetal hydroxy and the C-6′ ketone groups together with the chain length should also be studied in due course. It was reported that higher concentrations of SCB1 (>1000 nM) inhibited antibiotic production in *S. coelicolor*, but did not affect growth and morphological development.[31] A similar effect on antibiotic production was also observed for virginiae butanolides in *S. virginiae*.[33] In contrast, no inhibitory effect on antibiotic production and morphological differentiation was observed for SRBs in *S. rochei* even at a concentration of 6700 nM. We have previously observed a negative effect of *srrX* on spore formation on the basis of gene inactivation experiments.[18] However, the present work clearly indicates that SRBs themselves have no inhibitory effect on spore formation. It might therefore be possible that the SrrX protein has a regulatory function in morphological differentiation.

With regard to the biosynthesis of SRB1 and SRB2, we speculated that *srrX* encodes a key enzyme like *afsA* in *S. griseus*, based on the following evidence. 1) SRB1 and SRB2 resemble possible intermediates of the γ-butyrolactone signaling molecules. 2) The culture extract of the *srrX* mutant has no antibiotic-production-inducing activity in *S. rochei*. 3) No other positive signals were detected in *S. rochei* DNA when *srrX* was used as a probe for hybridization. Consistently with these speculation, several possible genes for SRB biosynthesis were found around *srrX* (*orf85*) on pSLA2-L: an NAD-dependent dehydrogenase gene *srrG* (*orf81*), a phosphatase gene *srrP* (*orf83*), a P450 hydroxylase gene *srrO* (*orf84*), and a thioesterase gene *srrH* (*orf86*). Analogously to the biosyntheses of A-factor[7] and virginia butanolides,[34] SrrX could be responsible for coupling between a C_3 unit and a C_{12} or C_{13} β-keto acid derived from fatty acid biosynthesis, followed by spontaneous intramolecular aldol condensation to form a butenolide skeleton. The butenolide intermediate might then be further modified by biosynthetic enzymes including SrrG to synthesize SRBs. The SrrG protein is homologous to BarS1, which is involved in the reduction of the C-1′ ketone group to produce virginia butanolides in *S. virginiae*.[35] Our preliminary data showed that a mutation of *srrG* abolished lankacidin and lankamycin production in *S. rochei* (unpublished result). Comprehensive analyses of the SRB biosynthetic pathway by gene inactivation and feeding experiments are in progress in our laboratory.

Experimental Section

Strains and culture conditions: *S. rochei* wild-type strain 7434AN4 and strain 51252, which carries only pSLA2-L, were described previously.[13,15] Strain KA61, a disruptant of the SARP-family transcriptional activator gene *srrY*, was used as an SRB producer.[20] Strain KA20, a double mutant of *srrX* and the transcriptional repressor gene *srrB*, was used as an SRB indicator strain.[18] YM medium [yeast extract (0.4%), malt extract (1.0%), D-glucose (0.4%), pH 7.3] was used for SRB synthesis and bioassay.

Spectroscopic instruments: NMR spectra were recorded with a JEOL LA-500 spectrometer fitted with a field gradient accessory. $CDCl_3$ (99.8 atom% enriched, Acros) was used as a solvent. Chemical shifts were recorded as δ values based on a resident solvent

signal ($\delta_C = 77.0$ ppm) or on the internal standard signal of tetramethylsilane ($\delta_H = 0$ ppm). ESI and atmospheric pressure chemical ionization (APCI) mass spectra were measured with a LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, USA). Optical rotations were measured with a JASCO DIP-370 polarimeter. IR spectra were recorded with a JASCO FT/IR-5300 spectrometer. UV spectra were obtained with an Ultrospec 3300pro UV/visible spectrophotometer (Amersham Biosciences).

Isolation of SRB molecules: An inoculum of strain KA61 was grown by use of a two-stage seed culture system. The first-stage seed culture (50 mL) was used as an inoculum for 4 L medium and was grown at 28 °C and 90 rpm for 28 h. The resulting second-stage seed culture was inoculated into YM medium (160 L) and cultivated at 28 °C and 110 rpm with an airflow rate of 0.5 v v^{-1} min^{-1} (air volume/liquid volume per minute) for 43 h. The culture supernatant was passed through a column of Amberlite XAD16 resin (5 kg, Sigma) at a flow rate of 10 L h^{-1} to absorb hydrophobic compounds, including SRBs. The resin was extracted three times with equal volumes of EtOAc. The combined organic phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The resulting residue (18 g) was purified by silica gel chromatography with hexane/EtOAc (1:1 to 1:2, *v/v*). Each fraction was subjected to bioassay with use of strain KA20 as a test organism (see below). Active fractions were collected and purified on Sephadex LH-20 (GE Healthcare) with methanol and further purified by a series of silica gel column chromatographic separations with three different solvent systems [$CHCl_3$/MeOH (50:1–30:1 *v/v*), toluene/EtOAc (2:1–1:1 *v/v*), and hexane/EtOAc (2:1–1:1 *v/v*)]. The purified sample was analyzed by ESI-MS and NMR (Figure 1). The ^{1}H and ^{13}C NMR assignments were supported by ^{1}H,^{1}H COSY, HMQC, and HMBC experiments (see the Supporting Information).

Compound ***1*** *(SRB1)*: HRMS (ESI): *m/z* calcd for $C_{16}H_{26}O_5Na$: 321.1673 $[M+Na]^+$; found: 321.1671.

Compound ***2*** *(SRB2)*: HRMS (ESI): *m/z* calcd for $C_{15}H_{24}O_5Na$: 307.1516 $[M+Na]^+$; found: 307.1513.

Optical rotation of natural SRBs (mixture): $[\alpha]_D^{22} = +17.5$ ($c = 0.012$, $CHCl_3$).

SRB assay: The *srrX–srrB* double mutant KA20 was used as a highly sensitive SRB indicator, because this strain, like the *srrB* mutant, produces two antibiotics when SRBs are added. A seed culture of strain KA20 (100 μL) supplemented with an aliquot of chromatographic fraction (10 μL) was grown in YM liquid medium (5 mL) at 28 °C for 24 h. Antibiotic production was analyzed by HPLC and TLC as follows. The crude extract was placed on a COSMOSIL 5C18-MS-II column (4.6×250 mm, Nakarai Tesque, Japan) and eluted with acetonitrile/sodium phosphate (10 mM) buffer (3:7 *v/v*, pH 8.2) at a flow rate of 1.0 mL min^{-1}. The eluate was monitored at 230 nm with a JASCO MD-2010 multi-wavelength photodiode array detector. Lankacidin C and lankacidinol A were detected at 8.5 and 16.9 min, respectively. TLC was developed with a mixture of $CHCl_3$/methanol (15:1 *v/v*) and baked after spraying with anisaldehyde/H_2SO_4.

1-(Benzyloxy)hexan-6-al (4): A mixture of 1-(benzyloxy)hexan-6-ol (**3**,[23] 8.00 g, 38.4 mmol), pyridinium chlorochromate (PCC, 12.8 g, 59.5 mmol), and sodium acetate (1.40 g, 17.1 mmol) in CH_2Cl_2 (200 mL) was stirred at room temperature for 1.5 h. The mixture was diluted with ether (300 mL) and filtered through a pad of Celite. The filtrate and washings were concentrated in vacuo. The residue was purified over silica gel with hexane/EtOAc (7:1 *v/v*) to give **4** (6.45 g, 81%) as a colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 1.40$–1.45 (m, 2H; H-3), 1.61–1.68 (m, 4H; H-2 and H-4), 2.35 (t, $J = 7.4$ Hz, 1H; H-5a), 2.43 (dt, $J = 1.8$, 7.4 Hz, 1H; H-5b), 3.47 (t, $J = 6.4$ Hz, 2H; H-1), 4.50 (s, 2H; PhC*H*$_2$), 7.25–7.37 (m, 5H; *Ph*CH$_2$), 9.75 ppm (t, $J = 1.8$ Hz, 1H; H-6); ^{13}C NMR ($CDCl_3$): $\delta = 21.9$ (C-3), 24.5 (C-4), 29.4 (C-2), 43.7 (C-5), 70.0 (C-1), 202.7 ppm (C-6); ^{13}C NMR ($CDCl_3$, benzyl resonances): $\delta = 72.9$ (PhCH$_2$), 127.5, 127.6, 128.4, 138.6 ppm; IR (neat): $\tilde{\nu} = 2938$, 2861, 1725, 1454, 1364, 1101, 739, 698 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{13}H_{18}O_2Na$: 229.1199 $[M+Na]^+$; found: 229.1199.

1-(Benzyloxy)-8-methylnonan-6-ol (5): 1-Bromo-2-methylpropane (9.00 mL, 83.4 mmol) was added at 0 °C to a suspension of magnesium (2.00 g, 82.3 mmol) and iodine (160 mg) in THF (40 mL), and the mixture was stirred at 0 °C for 2 h. A solution of aldehyde **4** (2.82 g, 13.7 mmol) in THF (10 mL) was added dropwise to the mixture at 0 °C, and the mixture was stirred at room temperature for 2.5 h. Saturated aqueous NH_4Cl (50 mL) was added, and the mixture was extracted twice with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified over silica gel with hexane/EtOAc (7:1–5:1 *v/v*) to give **5** (2.93 g, 81%) as a colorless oil.

Diastereomer mixture: ^{1}H NMR ($CDCl_3$): $\delta = 0.91$ (t, $J = 7.0$ Hz, 6H; H-9 and H-9′), 1.22 (m, 1H; H-7a), 1.32–1.46 (m, 7H; H-3,-4,-5,-7b), 1.63 (t, $J = 7.0$ Hz, 2H; H-2), 1.76 (m, 1H; H-8), 3.47 (t, $J = 6.7$ Hz, 2H; H-1), 3.66 (br s, 1H; H-6), 4.50 (s, 2H; PhC*H*$_2$), 7.26–7.34 ppm (m, 5H; *Ph*CH$_2$); ^{13}C NMR ($CDCl_3$): $\delta = 22.0$ (C-9), 23.5 (C-9′), 24.6 (C-8), 25.4 (C-4), 26.3 (C-3), 29.7 (C-2), 38.0 (C-5), 46.8 (C-7), 69.9 (C-6), 70.3 ppm (C-1); ^{13}C NMR ($CDCl_3$, benzyl resonances): $\delta = 72.8$ (PhCH$_2$), 127.5, 127.6, 128.3, 138.6 ppm; IR (neat): $\tilde{\nu} = 3397$, 2932, 2863, 1454, 1366, 1101, 735, 698 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{17}H_{28}O_2Na$: 287.1982 $[M+Na]^+$; found: 287.1981.

1-(Benzyloxy)-8-methylnonan-6-one (6): A mixture of alcohol **5** (2.90 g, 11.0 mmol), PCC (3.75 g, 17.4 mmol), and sodium acetate (290 mg, 3.54 mmol) in CH_2Cl_2 (60 mL) was stirred at room temperature for 2 h. The mixture was diluted with ether (100 mL), and filtered through a pad of Celite. The filtrate and washings were concentrated in vacuo. The residue was purified over silica gel with hexane/EtOAc (10:1 *v/v*) to give **6** (2.37 g, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$): $\delta = 0.90$ (d, $J = 6.4$ Hz, 6H; H-9 and H-9′), 1.34–1.40 (m, 2H; H-3), 1.55–1.66 (m, 4H; H-2 and H-4), 2.13 (m, 1H; H-8), 2.26 (d, $J = 7.0$ Hz, 2H; H-7), 2.37 (t, $J = 7.4$ Hz, 2H; H-5), 3.46 (t, $J = 6.4$ Hz, 2H; H-1), 4.49 (s, 2H; PhC*H*$_2$), 7.25–7.35 ppm (m, 5H; *Ph*CH$_2$); ^{13}C NMR ($CDCl_3$): $\delta = 22.6$ (C-9 and C-9′), 23.5 (C-4), 24.6 (C-8), 25.8 (C-3), 29.5 (C-2), 43.2 (C-5), 51.8 (C-7), 70.1 (C-1), 211.0 ppm (C-6); ^{13}C NMR ($CDCl_3$,benzyl resonances): $\delta = 72.9$ (PhCH$_2$), 127.5, 127.6, 128.3, 138.6 ppm; IR (neat): $\tilde{\nu} = 2936$, 2866, 1711, 1454, 1366, 1103, 737, 698 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{17}H_{26}O_2Na$, 285.1825 $[M+Na]^+$; found: 285.1822.

5-(2′-Isobutyl-1′,3′-dioxolan-2′-yl)pentanal (7): Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 300 μL, 1.66 mmol) was added at −78 °C to a solution of ketone **6** (2.30 g, 8.77 mmol) and ethylenedioxybis(trimethylsilane) (4.20 mL, 17.1 mmol) in CH_2Cl_2 (80 mL) and the mixture was stirred at −78 °C for 1 h and at room temperature for 7 h. Pyridine (1 mL) and water (10 mL) were added at 0 °C, and the mixture was extracted twice with CH_2Cl_2. The combined organic phases were washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified over silica gel with hexane/EtOAc (20:1 *v/v*) to give the corresponding dioxolane derivative (2.43 g, 90%) as a colorless oil. A mixture of the dioxolane derivative (2.40 g, 7.83 mmol) and Pd-C (10%, 750 mg) in EtOAc (60 mL) was stirred under hydrogen at room

temperature for 24 h. The mixture was passed through a pad of Celite. The filtrate and washings were concentrated in vacuo. The residue was purified over silica gel with hexane/EtOAc (4:1–2:1 *v/v*) to give the deprotected alcohol (1.61 g, 95%) as a colorless oil. A mixture of this alcohol (1.61 g, 7.44 mmol), PCC (3.40 g, 15.8 mmol), and sodium acetate (280 mg, 3.41 mmol) in CH_2Cl_2 (60 mL) was stirred at room temperature for 4 h. The mixture was diluted with ether (50 mL) and filtered through a pad of Celite. The filtrate and washings were concentrated in vacuo. The residue was purified over silica gel with hexane/EtOAc (5:1 *v/v*) to give **7** (1.20 g, 75%) as a colorless oil. Overall yield 64% (three steps). ^{1}H NMR ($CDCl_3$): $\delta = 0.94$ (d, $J = 6.7$ Hz, 6H; $2\times CH_3$), 1.37–1.44 (m, 2H; H-4), 1.51 (m, 2H; *i*PrCH_2), 1.60–1.67 (m, 4H; H-3 and H-5), 1.75 (m, 1H, Me_2CH), 2.43 (m, 2H; H-2), 3.91 [m, 4H; $(CH_2O–)_2$], 9.76 ppm (t, $J = 1.9$ Hz, 1H; H-1); ^{13}C NMR ($CDCl_3$): $\delta = 22.3$ (C-3), 23.4 (C-4), 24.0 (Me_2CH), 24.1 (Me_2CH), 37.1 (C-5), 43.9 (C-2), 45.2 (*i*PrCH_2), 64.6 [$(CH_2O–)_2$], 111.7 (C-2′), 202.6 ppm (C-1); IR (neat): $\tilde{\nu} =$ 2953, 2872, 1711, 1466, 1366, 1090, 949 cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{12}H_{23}O_3$: 215.1642 $[M+H]^+$; found: 215.1642.

(1′*R*)-2-(1′-hydroxyl-5′-(2″-isobutyl-1″,3″-dioxolan-2″-yl)pentyl)-3-methyl-4-(L-menthyloxy)but-2-en-1,4-olide (9a) and (1′*S*)-2-(1′-hydroxyl-5′-(2″-isobutyl-1″,3″-dioxolan-2″-yl)pentyl)-3-methyl-4-(L-menthyloxy)but-2-en-1,4-olide (9b): A solution of *n*-butyllithium (1.65 M in hexane, 2.80 mL, 4.62 mmol) was added dropwise at 0 °C to a solution of diisopropylamine (640 μL, 4.55 mmol) in THF (10 mL). After the system had been stirred for 30 min, hexamethylphosphoric triamide (HMPA, 4 mL) was added dropwise at 0 °C. A solution of readily available 3-methyl-4-(L-menthyloxy)but-2-en-1,4-olide (**8**, 1.15 g, 4.55 mmol)[24,25] in THF (12 mL) was added dropwise at −78 °C, and the mixture was further stirred at the same temperature for 30 min. A solution of aldehyde **7** (1.05 g, 4.90 mmol) in THF (10 mL) was then added dropwise at −78 °C over 10 min, and the mixture was further stirred at the same temperature for 1.5 h. Saturated aqueous NH_4Cl (10 mL) was added, and the mixture was extracted twice with CH_2Cl_2. The combined organic phases were washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by silica gel chromatography with hexane/EtOAc (4:1 *v/v*) to give a mixture of **9a** and **9b** (2:1, 660 mg, 31%) as a colorless oil; these were further separated by repeated flash chromatography runs. The absolute configurations at C-1′ were established by the modified Mosher method.[27] The differences in chemical shifts ($\Delta\delta$) were obtained by subtracting the δ values for the (*R*)-MTPA ester from their counterparts for the (*S*)-MTPA ester ($\delta_S - \delta_R$).

Compound 9a: $[\alpha]_D^{27} = -58.8$ ($c = 1.10$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta =$ 0.91 (d, $J = 6.7$ Hz, 3H; Me_2CH), 0.93 (d, $J = 6.8$ Hz, 3H; Me_2CH), 1.28–1.42 (m, 2H; C-3′), 1.50 (d, $J = 6.1$ Hz, 2H; *i*PrCH_2), 1.59–1.63 (m, 5H; H-2′a,-4′,-5′), 1.75 (m, 1H, Me_2CH), 1.83 (m, 1H; H-2′b), 1.97 (s, 3H; H-5), 2.86 (br s, 1H; C1′–O*H*), 3.91 [m, 4H; $(CH_2O–)_2$], 4.46 (t, $J = 6.7$ Hz, 1H; H-1′), 5.69 ppm (s, 1H; H-4); ^{1}H NMR ($CDCl_3$, menthyl resonances): $\delta = 0.81$ (d, $J = 6.8$ Hz, 3H), 0.86 (m, 1H), 0.88 (d, $J =$ 7.1 Hz, 3H), 0.96 (d, $J = 6.4$ Hz, 3H), 1.02 (m, 2H), 1.22–1.27 (m, 1H), 1.28–1.42 (m, 1H), 1.64–1.70 (m, 2H), 2.08–2.14 (m, 2H), 3.62 ppm (dt, $J = 4.3$, 11 Hz, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 11.5$ (C-5), 23.5 (C-4′), 24.0 (Me_2CH), 24.1 (Me_2CH), 25.7 (C-3′), 36.6 (C-2′), 37.2 (C-5′), 45.2 (*i*Pr-CH_2), 64.6 ($(CH_2O-)_2$), 66.9 (C-1′), 100.9 (C-4), 111.9 (C-2″), 130.6 (C-2), 155.4 (C-3), 171.4 ppm (C-1); ^{13}C NMR ($CDCl_3$, menthyl resonances): $\delta = 15.9$, 20.8, 22.2, 23.2, 25.3, 31.4, 34.2, 40.5, 47.7, 79.6 ppm; IR (neat): $\tilde{\nu} = 2953$, 2870, 1759, 1458, 1370, 1331, 1094, 947, 756 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{27}H_{46}O_6Na$: 489.3187 $[M+Na]^+$; found: 489.3182.

Compound 9b: $[\alpha]_D^{28} = -99.5$ ($c = 0.760$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.91$ (d, $J = 6.4$ Hz, 3H; Me_2CH), 0.93 (d, $J = 6.8$ Hz, 3H; Me_2CH), 1.28–1.42 (m, 2H; C-3′), 1.50 (d, $J = 6.1$ Hz, 2H; *i*PrCH_2), 1.59–1.72 (m, 5H; H-2′a,4′,5′), 1.75 (m, 1H; Me_2CH), 1.83 (m, 1H; H-2′b), 1.99 (s, 3H; H-5), 3.91 [m, 4H; $(CH_2O–)_2$], 4.48 (t, $J = 7.3$ Hz, 1H; H-1′), 5.71 ppm (s, 1H; H-4); ^{1}H NMR ($CDCl_3$, menthyl resonances): $\delta =$ 0.80 (d, $J = 7.0$ Hz, 3H), 0.86 (m, 1H), 0.87 (d, $J = 7.1$ Hz, 3H), 0.96 (d, $J = 6.7$ Hz, 3H), 1.05 (m, 2H), 1.22–1.27 (m, 1H), 1.28–1.42 (m, 1H), 1.59–1.72 (m, 2H), 2.10–2.15 (m, 2H), 3.62 ppm (dt, $J = 4.3$, 11 Hz, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 11.5$ (C-5), 23.5 (C-4′), 24.0 (Me_2CH), 24.1 (Me_2CH), 25.7 (C-3′), 36.4 (C-2′), 37.2 (C-5′), 45.1 (*i*Pr-CH_2), 64.5 ((CH_2O-)$_2$), 66.7 (C-1′), 100.7 (C-4), 111.8 (C-2″), 130.7 (C-2), 155.4 (C-3), 171.4 ppm (C-1); ^{13}C NMR ($CDCl_3$, menthyl resonances): $\delta = 15.7$, 20.8, 22.2, 23.1, 25.1, 31.4, 34.2, 40.4, 47.7, 79.5 ppm; IR (neat): $\tilde{\nu} =$ 2953, 2870, 1759, 1456, 1370, 1333, 1096, 947 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{27}H_{46}O_6Na$: 489.3187 $[M+Na]^+$; found: 489.3184.

SRB1a (1a): BBr_3 solution in CH_2Cl_2 (10%, 400 μL, 420 μmol) was added at −78 °C to a solution of **9a** (82 mg, 0.18 μmol) in CH_2Cl_2 (3.0 mL), and the mixture was stirred at −78 °C for 1.5 h. Saturated aqueous $NaHCO_3$ (2 mL) was carefully added, and the mixture was extracted twice with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by silica gel chromatography with hexane/EtOAc (1:1 *v/v*) to give SRB1a (**1a**, 26 mg, 52%) as a colorless oil.

Mixture of C-4 epimers: $[\alpha]_D^{24} = +18.4$ ($c = 0.860$, $CHCl_3$); ^{1}H NMR and ^{13}C NMR assignments are listed in Table 1; IR (neat): $\tilde{\nu} = 3389$, 2957, 2872, 1752, 1705, 1464, 1370, 1335, 1088, 955, 758 cm^{-1}; UV/Vis (MeOH): λ_{max} ($\log\varepsilon$) = 210 nm (3.98 mol^{-1} dm^3 cm^{-1}); HRMS (ESI): *m/z* calcd for $C_{15}H_{24}O_5Na$: 307.1516 $[M+Na]^+$; found: 307.1519.

SRB1b (1b): Compound **9b** (23 mg, 50 μmol) was treated in the same manner as described for the preparation of **1a** to give SRB1b (**1b**, 8.9 mg, 63%) as a colorless oil.

Mixture of C-4 epimers: $[\alpha]_D^{25} = -9.32$ ($c = 0.400$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.89$ (d, $J = 6.4$ Hz, 3H; H-9′ or H-9″), 0.90 (d, $J = 6.4$ Hz, 3H; H-9″ or H-9′), 1.25 (m, 1H; H-3′a), 1.36 (m, 1H; H-3′b), 1.53 (m, 2H; H-4′), 1.69–1.81 (m, 2H; H-2′), 2.08/2.09 (s, 3H; H-5), 2.09 (m, 1H; H-8′), 2.26 (d, $J = 7.1$ Hz, 2H; H-7′), 2.40 (dt, $J = 2.2$, 7.1 Hz, 2H; H-5′), 4.46 (m, 1H; H-1′), 5.85 ppm (br s, 1H; H-4); ^{13}C NMR ($CDCl_3$): $\delta = 11.5/11.6$ (C-5), 22.5/22.6 (C-9′ and C-9″), 22.9/23.2 (C-4′), 24.5/24.7 (C-8′), 24.7/24.8 (C-3′), 35.6/35.7 (C-2′), 42.6/42.8 (C-5′), 51.9/52.0 (C-7′), 66.4/66.6 (C-1′), 98.5/98.8 (C-4), 129.9/130.3 (C-2), 157.1/157.2 (C-3), 171.1/171.2 (C-1), 211.4/212.6 ppm (C-6′); IR (neat): $\tilde{\nu} =$ 3389, 2957, 2872, 1752, 1705, 1464, 1370, 1335, 1088, 955, 758 cm^{-1}; UV/Vis (MeOH): λ_{max} ($\log\varepsilon$) = 210 nm (4.02 mol^{-1} dm^3 cm^{-1}); HRMS (ESI): *m/z* calcd for $C_{15}H_{24}O_5Na$: 307.1516 $[M+Na]^+$; found: 307.1515.

(8*S*)-1-(Benzyloxy)-8-methyldecan-6-ol (10): (*S*)-(+)-1-Chloro-2-methylbutane (9.00 mL, 75.1 mmol) was added at room temperature to a suspension of magnesium (2.05 g, 84.3 mmol) and iodine (135 mg) in THF (35 mL), and the mixture was stirred at 70 °C for 2 h. A solution of aldehyde **4** (2.78 g, 13.5 mmol) in THF (10 mL) was added dropwise at 0 °C to the mixture, which was stirred at room temperature for 1 h. Saturated aqueous NH_4Cl (30 mL) was added, and the mixture was extracted twice with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified over silica gel with hexane/EtOAc (7:1–5:1 *v/v*) to give **10** (2.55 g, 68%) as a colorless oil.

Diastereomer mixture: ^{1}H NMR ($CDCl_3$): $\delta = 0.85$–0.92 (m, 6H; H-9′ and H-10), 1.11 (m, 1H; H-9a), 1.25–1.50 (m, 10H; H-3,-4,-5,-7,-8,-9b), 1.63 (t, $J = 6.7$ Hz, 2H; H-2), 3.47 (t, $J = 6.7$ Hz, 2H; H-1), 3.68 (m, 1H; H-6), 4.50 (s, 2H; PhCH_2), 7.25–7.34 ppm (m, 5H; *Ph*CH_2); ^{13}C NMR ($CDCl_3$): $\delta = 11.1/11.3$ (C-10), 18.8/19.8 (C-9′), 25.3/25.5 (C-4), 26.3 (C-3), 29.0/30.4 (C-9), 29.7 (C-2), 30.8/31.1 (C-8), 37.7/38.3 (C-5), 44.6/44.8 (C-7), 69.6/69.9 (C-6), 70.3 ppm (C-1); ^{13}C NMR ($CDCl_3$, benzyl resonances): $\delta = 72.8$ (PhCH_2), 127.5, 127.6, 128.3, 138.7 ppm; IR (neat): $\tilde{\nu} = 3397$, 2930, 2857, 1454, 1101, 735, 698 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{18}H_{30}O_2Na$: 301.2138 $[M+Na]^+$; found: 301.2142.

(8*S*)-1-(Benzyloxy)-8-methyldecan-6-one (11): The alcohol **10** (2.50 g, 8.98 mmol) was treated in the same manner as described for the preparation of **6** to give **11** (1.53 g, 62%) as a colorless oil. $[\alpha]_D^{26} = +2.40$ ($c = 0.500$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.86$ (d, $J = 6.7$ Hz, 3H; H-9′), 0.87 (t, $J = 7.4$ Hz, 3H; H-10), 1.18 (m, 1H; H-9a), 1.30 (m, 1H; H-9b), 1.37 (m, 2H; H-3), 1.55–1.65 (m, 4H; H-2 and H-4), 1.91 (m, 1H; H-8), 2.18 (dd, $J = 8.0$, 16 Hz, 1H; H-7a), 2.35–2.42 (m, 3H; H-5 and H-7b), 3.46 (t, $J = 6.7$ Hz, 2H; H-1), 4.49 (s, 2H; PhCH_2), 7.25–7.35 ppm (m, 5H; *Ph*CH_2); ^{13}C NMR ($CDCl_3$): $\delta = 11.3$ (C-10), 19.4 (C-9′), 23.5 (C-4), 25.8 (C-3), 29.5 (C-9), 29.6 (C-2), 30.8 (C-8), 43.2 (C-5), 49.9 (C-7), 70.2 (C-1), 211.2 ppm (C-6); ^{13}C NMR ($CDCl_3$, benzyl resonances): $\delta = 72.9$ (PhCH_2), 127.5, 127.6, 128.3, 138.6 ppm; IR (neat): $\tilde{\nu} = 2936$, 2861, 1713, 1456, 1366, 1103, 737, 698 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{18}H_{28}O_2Na$: 299.1982 $[M+Na]^+$; found: 299.1983.

5-(2′-((2″*S*)-2″-methylbutyl)-1′,3′-dioxolan-2′-yl)pentanal (12): Compound **11** (1.50 g, 5.43 mmol) was treated in the same manner as described for the preparation of **7** to give **12** (720 mg, 58% in three steps) as a colorless oil. $[\alpha]_D^{27} = +5.92$ ($c = 1.09$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.86$ (t, $J = 7.4$ Hz, 3H; *Me*CH_2), 0.93 (d, $J = 6.4$ Hz, 3H; *Me*CH), 1.17 (m, 1H; MeCH_2a), 1.36–1.44 (m, 4H; H-4 and MeCHCH_2), 1.51 (m, 1H; MeC*H*), 1.60–1.67 (m, 5H; H-3, -5, and MeCH_2b), 2.44 (dt, $J = 1.9$, 7.4 Hz, 2H; H-2), 3.91 [m, 4H; (CH_2O–)$_2$], 9.76 ppm (t, $J = 1.9$ Hz, 1H; H-1); ^{13}C NMR ($CDCl_3$): $\delta = 11.3$ (*Me*CH_2), 20.5 (*Me*CH), 22.3 (C-3), 23.4 (C-4), 30.4 (MeCH), 30.6 (MeCH_2), 37.1 (C-5), 43.1 (MeCHCH_2), 43.9 (C-2), 64.6/64.7 [(CH_2O–)$_2$], 111.9 (C-2′), 202.6 ppm (C-1); IR (neat): $\tilde{\nu} = 2957$, 2876, 1725, 1462, 1377, 1138, 1082, 949 cm^{-1}; HRMS (APCI): *m/z* calcd for $C_{13}H_{25}O_3$: 229.1798 $[M+H]^+$; found: 229.1796.

(1′*R*)-2-(1′-hydroxyl-5′-(2″-((2‴*S*)-2′-methylbutyl-1″,3″-dioxolan-2″-yl)pentyl)-3-methyl-4-(L-menthyloxy)but-2-en-1,4-olide (13a) and (1′*S*)-2-(1′-hydroxyl-5′-(2″-((2‴*S*)-2′-methylbutyl-1″,3″-dioxolan-2″-yl)pentyl)-3-methyl-4-(L-menthyloxy)but-2-en-1,4-olide (13b): Compound **12** (670 mg, 2.93 mmol) was treated in the same manner as described for the preparation of **9** to give a mixture (2:1) of **13a** and **13b** (656 mg, 54%) as a colorless oil, which was also further separated by flash chromatography.

Compound 13a: $[\alpha]_D^{23} = -72.4$ ($c = 1.27$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.86$ (t, $J = 7.3$ Hz, 3H; *Me*CH_2), 0.93 (d, $J = 6.8$ Hz, 3H; *Me*CH), 1.14–1.20 (m, 1H; MeCH_2a), 1.31–1.42 (m, 6H; H-3′, -4′, MeCH_2b, and MeCHCH_2a), 1.50 (m, 1H; MeC*H*), 1.59–1.63 (m, 4H; H-2′a, -5′, and MeCHCH_2b), 1.84 (m, 1H; H-2′b), 1.97 (s, 3H; H-5), 2.85 (d, $J = 9.2$ Hz, 1H; C1′O*H*), 3.91 [m, 4H; (CH_2O–)$_2$], 4.46 (q, $J = 8.2$ Hz, 1H; H-1′), 5.69 ppm (s, 1H; H-4); ^{1}H NMR ($CDCl_3$, menthyl resonances): $\delta = 0.81$ (d, $J = 7.1$ Hz, 3H), 0.86 (m, 1H), 0.87 (d, $J = 7.3$ Hz, 3H), 0.96 (d, $J = 6.7$ Hz, 3H), 1.10 (m, 2H), 1.25 (m, 1H), 1.31–1.42 (m, 1H), 1.64–1.71 (m, 2H), 2.10 (m, 2H), 3.62 ppm (dt, $J = 4.3$, 11 Hz, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 11.3$ (*Me*CH_2), 11.5 (C-5), 20.4 (*Me*CH), 23.5 (C-4′), 25.7 (C-3′), 30.3 (MeCH), 30.6 (MeCH_2), 36.6 (C-2′), 37.2 (C-5′), 43.1 (MeCHCH_2), 64.5/64.7 [(CH_2O–)$_2$], 66.9 (C-1′), 100.9 (C-4), 112.0 (C-2″), 130.6 (C-2), 155.4 (C-3), 171.4 ppm (C-1); ^{13}C NMR ($CDCl_3$, menthyl resonances): $\delta = 15.9$, 20.8, 22.2, 23.2, 25.4, 31.5, 34.2, 40.5, 47.7, 79.6 ppm; IR (neat): $\tilde{\nu} = 2955$, 2926, 1755, 1456, 1372, 1331, 1096, 949, 758 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{28}H_{48}O_6Na$: 503.3343 $[M+Na]^+$; found: 503.3339.

Compound 13b: $[\alpha]_D^{22} = -74.4$ ($c = 0.860$, $CHCl_3$). ^{1}H NMR ($CDCl_3$): $\delta = 0.86$ (t, $J = 7.3$ Hz, 3H; *Me*CH_2), 0.93 (d, $J = 6.4$ Hz, 3H; *Me*CH), 1.14–1.20 (m, 1H; MeCH_2a), 1.31–1.42 (m, 6H; H-3′, -4′, MeCH_2b, and MeCHCH_2a), 1.50 (m, 1H; MeC*H*), 1.59–1.71 (m, 4H; H-2′a, -5′, and MeCHCH_2b), 1.82 (m, 1H; H-2′b), 1.98 (s, 3H; H-5), 2.39 (m, 1H; C1′O*H*), 3.91 [m, 4H; (CH_2O–)$_2$], 4.47 (t, $J = 7.3$ Hz, 1H; H-1′), 5.71 ppm (s, 1H; H-4); ^{1}H NMR ($CDCl_3$, menthyl resonances): $\delta =$ 0.80 (d, $J = 6.7$ Hz, 3H), 0.86 (m, 1H), 0.87 (d, $J = 7.1$ Hz, 3H), 0.96 (d, $J = 6.7$ Hz, 3H), 1.10 (m, 2H), 1.26 (m, 1H), 1.31–1.42 (m, 1H), 1.59–1.71 (m, 2H), 2.12 (m, 2H), 3.62 ppm (dt, $J = 4.3$, 11 Hz, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 11.3$ (*Me*CH_2), 11.5 (C-5), 20.4 (*Me*CH), 23.5 (C-4′), 25.7 (C-3′), 30.3 (MeCH), 30.6 (MeCH_2), 36.4 (C-2′), 37.2 (C-5′), 43.1 (MeCHCH_2), 64.5/64.6 [(CH_2O–)$_2$], 66.8 (C-1′), 100.7 (C-4), 112.0 (C-2″), 130.7 (C-2), 155.4 (C-3), 171.4 ppm (C-1); ^{13}C NMR ($CDCl_3$, menthyl resonances): $\delta = 15.7$, 20.8, 22.2, 23.1, 25.2, 31.4, 34.2, 40.4, 47.7, 79.5 ppm; IR (neat): $\tilde{\nu} = 2955$, 2872, 1761, 1456, 1372, 1331, 1096, 949 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{28}H_{48}O_6Na$: 503.3343 $[M+Na]^+$; found: 503.3341.

SRB2a (2a): Compound **13a** (20 mg, 42 μmol) was treated in the same manner as described for the preparation of **1a** to give SRB2a (**2a**, 8.4 mg, 70%) as a colorless oil.

Mixture of C-4 epimers: $[\alpha]_D^{22} = +22.4$ ($c = 0.800$, $CHCl_3$); ^{1}H NMR and ^{13}C NMR assignments are listed in Table 1; IR (neat): $\tilde{\nu} = 3397$, 2959, 2932, 2876, 1750, 1705, 1460, 1381, 1337, 1090, 957, 756 cm^{-1}; UV/Vis (MeOH): λ_{max} ($\log \varepsilon$) = 210 nm (4.06 mol^{-1} dm^3 cm^{-1}); HRMS (ESI): *m/z* calcd for $C_{16}H_{26}O_5Na$: 321.1673 $[M+Na]^+$; found: 321.1676.

SRB2b (2b): Compound **13b** (20 mg, 42 μmol) was treated in the same manner as described for the preparation of **1a** to give SRB2b (**2b**, 6.8 mg, 55%) as a colorless oil.

Mixture of C-4 epimers: $[\alpha]_D^{24} = -5.71$ ($c = 0.700$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 0.86$ (d, $J = 7.0$ Hz, 3H; H-9″), 0.87 (t, $J = 6.7$ Hz, 3H; H-10′), 1.15–1.20 (m, 1H; H-9′a), 1.21–1.32 (m, 2H; H-3′), 1.30–1.40 (m, 1H; H-9′b), 1.51–1.59 (m, 2H; H-4′), 1.65–1.80 (m, 1H; H-2′a), 1.81–1.93 (m, 2H; H-2′b and H-8′), 2.08/2.09 (s, 3H; H-5), 2.17 (d, $J = 7.9$, 8.0 Hz, 1H; H-7′a), 2.35–2.42 (m, 3H; H-5′ and H-7′b), 3.03 (br s, 1H; C1′O*H*), 4.49 (m, 1H; H-1′), 5.85 ppm (br s, 1H; H-4); ^{13}C NMR ($CDCl_3$): $\delta = 11.3/11.3$ (C-10′), 11.5/11.6 (C-5), 19.3/19.4 (C-9″), 23.0/23.2 (C-4′), 24.6/24.8 (C-3′), 29.5/29.5 (C-9′), 30.9 (C-8′), 35.6 (C-2′), 42.7/42.9 (C-5′), 50.0/50.1 (C-7′), 66.4/66.5 (C-1′), 98.7/98.9 (C-4), 129.9/130.1 (C-2), 157.4/157.5 (C-3), 171.3/171.4 (C-1), 211.7/212.8 ppm (C-6′); IR (neat): $\tilde{\nu} = 3397$, 2959, 2932, 2876, 1750, 1705, 1460, 1381, 1337, 1090, 957, 756 cm^{-1}; UV/Vis (MeOH): λ_{max} ($\log \varepsilon$) = 210 nm (4.03 mol^{-1} dm^3 cm^{-1}); HRMS (ESI): *m/z* calcd for $C_{16}H_{26}O_5Na$: 321.1673 $[M+Na]^+$; found: 321.1676.

Chiral HPLC analysis: Natural SRB1 and SRB2 (**1** and **2**) and synthetic **1a**, **1b**, **2a**, and **2b** were dissolved in acetonitrile (30 μg mL^{-1}) and an aliquot of each sample (10 μL) was analyzed by HPLC with a Chiral MB-S column (macroporous silica gel coated with optically active *N*-substituted polymaleimides, 4.6×250 mm, Tokyo Chemical Industry, Co., Ltd., Japan) with aqueous acetonitrile (10%) containing trifluoroacetic acid (0.1%) as solvent at a flow rate of 1.0 mL min^{-1}, and with detection at 210 nm. Natural SRB1 (**1**) and SRB2 (**2**) were eluted at 9.1 and 17.3 min. Synthetic **1a**, **1b**, **2a**, and **2b** were eluted at 9.1, 8.7, 17.3, and 16.6 min, respectively.

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K. Arakawa, N. Tsuda, A. Taniguchi, H. Kinashi*

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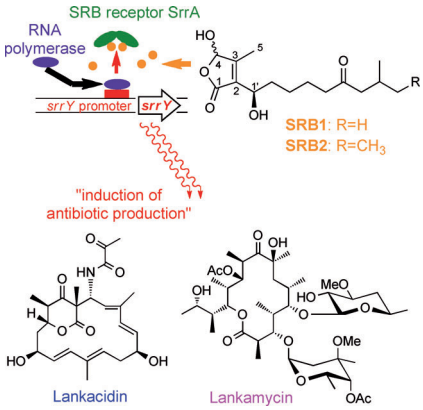
The Butenolide Signaling Molecules SRB1 and SRB2 Induce Lankacidin and Lankamycin Production in *Streptomyces rochei*



Control of antibiotic production: Two signaling molecules—SRB1 and SRB2—that induce production of lankacidin and lankamycin in *Streptomyces rochei* 7434AN4 were isolated and their structures were determined. Each contains a 2,3-disubstituted γ-hydroxybutenolide skeleton, and the stereochemistry at C-1′ is crucial for inducing activity.

