Precursor-Directed Biosynthesis of 16-Membered Macrolides by the Erythromycin Polyketide Synthase

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Abstract: *Streptomyces coelicolor* CH999/pJRJ2 harbors a plasmid encoding DEBS(KS1°), a mutant form of 6-deoxyerythronolide B synthase that is blocked in the formation of 6-deoxyerythronolide B (**1**, 6-dEB) due to a mutation in the active site of the ketosynthase (KS1) domain that normally catalyzes the first polyketide chain elongation step of 6-dEB biosynthesis. Administration of (2*E*,4*S*,5*R*)-2,4-dimethyl-5-hydroxy-2-heptenoic acid, *N*-acetylcysteamine thioester (**6**) an unsaturated triketide analogue of the natural triketide chain elongation intermediate to cultures of *S. coelicolor* CH999/pJRJ2 results in formation of a 16-membered macrolactone, which is isolated in the hemiketal form **33**. The formation of the octaketide **33** indicates that the triketide substrate has been processed by DEBS module 2 as if it were a diketide analogue. The substrate specificity of this novel reaction has been explored by the incubation of three additional analogues of the unsaturated triketide **6**, compounds **18**, **31**, and **32**, with *S. coelicolor* CH999/pJRJ2, resulting in the formation of the corresponding macrolactones **34**, **35**, and **36**. By contrast, the unsaturated triketide **10**, lacking a methyl group at C-2, did not give rise to any detectable macrolactone product when incubated with *S. coelicolor* CH999/pJRJ2.

Modular polyketide synthases (PKSs) are large (M_r 100−1000 kDa) multifunctional enzyme systems that mediate the biosynthesis of extraordinarily complex natural products from simple 2-, 3-, and 4-carbon building blocks such as acetyl-CoA, propionyl-CoA, and butyryl-CoA and their activated derivatives malonyl-, methylmalonyl-, and ethylmalonyl-CoA.[1] The resulting polyketide natural products display an enormous range of pharmacologically important activities, including antibiotic, antifungal, antiparasitic, antitumor, and immunosuppressive properties.[2] Modular polyketide synthases are organized into groups of active sites, known as modules, in which each module is responsible for one complete cycle of polyketide chain extension and functional group modification. Each module consists of a set of catalytic domains of 100−400 amino acids each that are analogous in both function and sequence to the individual enzymes of fatty acid biosynthesis. The key chain-building step of polyketide biosynthesis, catalyzed by a β-keto acylthioester synthase (ketosynthase, KS) domain, is a decarboxylative condensation reaction analogous to the chain elongation step of classical fatty acid biosynthesis. Indeed polyketide synthases and fatty acid synthases show remarkable genetic, protein structural, and mechanistic similarities.[3] All PKS modules possess at a minimum ketosynthase (KS), acyl transferase (AT), and acyl carrier protein (ACP) domains. The degree of functional group modification taking place during each chain elongation cycle is determined by the specific combination of keto reductase (KR), dehydrase (DH), and enoyl reductase (ER) domains found in each module. Additional degrees of complexity arise from the use of different starter and chain elongation units as well as the generation of new stereocenters. Modular PKSs are responsible for the biosynthesis of the polyketide precursors of a wide variety of metabolites including erythromycin,[4] methymycin,[5] tylosin,[6] epothilone,[7] rapamycin,[8] and rifamycin.[9]

The most thoroughly studied modular PKS is the 6-deoxyerythronolide B synthase (DEBS) of *Saccharopolyspora erythraea*, which catalyzes the formation of 6-deoxyerythronolide B (6-dEB, **1a**), the parent macrolide aglycon of the widely used, broad-spectrum antibiotic erythromycin A.[4] (Scheme 1) The formation of 6-dEB requires six condensation steps beginning with a propionyl-CoA primer and six methylmalonyl-CoA extender units. The six modules of DEBS are organized into

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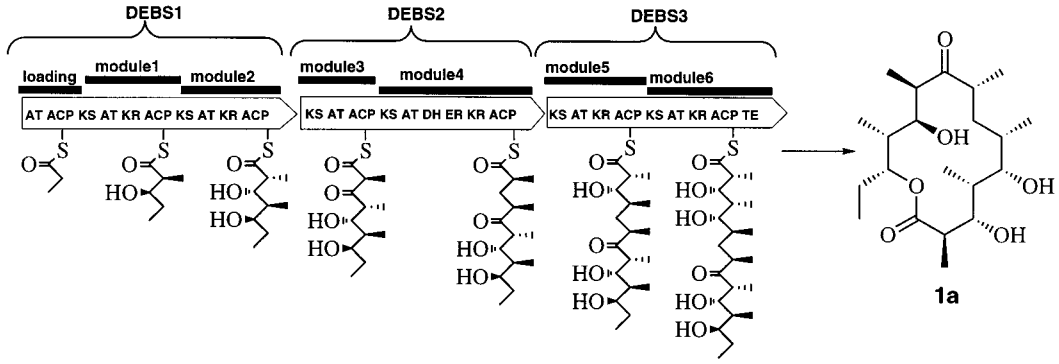
Scheme 1



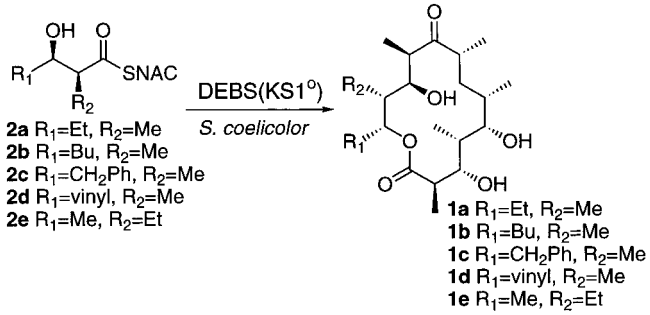
three large polypeptides, DEBS1, DEBS2, and DEBS3, each M_r > 330 kDa and each consisting of two modules.[10] Upstream of the N-terminus of module 1 in DEBS1 are specialized AT and ACP domains that control the loading of the propionyl-CoA primer unit, while the C-terminus of DEBS3 carries a TE domain implicated in release of the finished heptaketide product from the PKS with concomitant formation of the 14-membered macrolactone.

One of the most powerful tools for the study of PKS function and mechanism has been the ability to express entire PKSs or combinations of individual modules and domains in heterologous organisms using specially engineered host−vector systems.[11] The resultant engineered organisms have served as vehicles for examination of in vivo function of the encoded PKSs, or as sources of active enzymes for detailed in vitro mechanistic experimentation. Thus, not only has the entire set of DEBS structural genes been expressed in the Actinomycete host *Streptomyces coelicolor* CH999, leading to the formation of more than 40 mg/L of 6-dEB, but the derived cellular extracts have been shown to harbor the expected polyfunctional proteins, DEBS1, DEBS2, and DEBS3, which after purification were together able to mediate the formation of 6-dEB from propionyl-CoA, methylmalonyl-CoA, and NADPH.[12,13]

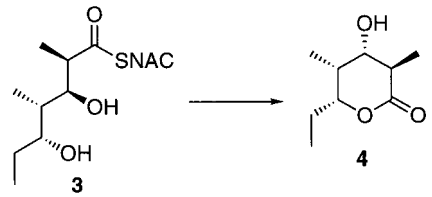
Inactivation by site-directed mutagenesis of the KS1 (β-ketoacyl-ACP synthase) domain that normally catalyzes the first condensation step of 6-dEB biosynthesis, gives a mutant construct, DEBS(KS1°), that no longer produces 6-dEB. We have reported that formation of 6-dEB (**1a**) is restored, however, when **2a**, an *N*-acetyl cysteamine thioester (SNAC) derivative of the normal diketide chain elongation intermediate, is administered to cultures of *S. coelicolor* CH999 harboring plasmid pJRJ2 encoding DEBS(KS1°)[14a] (Scheme 2). Moreover, by feeding synthetic analogues of **2a** to these mutant cultures, unnatural polyketide analogues of 6-dEB were generated.[14b]

In principle, this precursor-directed biosynthetic approach can be extended to the administration of analogues of higher-order intermediates such as triketides, tetraketides, etc., provided that

Scheme 2



Scheme 3



(a) the analogues themselves be sufficiently stable chemically under the feeding conditions used and (b) the analogues in question be recognized and processed by the appropriate downstream module. Direct feeding or incubation of **3**, the SNAC analogue of the natural erythromycin triketide intermediate is precluded by the rapid lactonization of the 3,5-hydroxy acylthioester to the corresponding triketide lactone **4** (Scheme 3). This problem can be avoided by the use of the corresponding 2,3-dehydro-analogue **5** cyclization of which is prevented by the presence of the trans double bond. Administration of **5** and its C-4 diastereomer **6** to *S. coelicolor* CH999/pJRJ2 encoding DEBS(KS1°) gave surprising results. Although feeding of the synthetic triketide **5**, having the natural erythromycin diketide stereochemistry at C-4 and C-5, yielded the predicted unsaturated 14-membered lactone, 10,11-anhydro-6-dEB (**7**),[15] administration of the diastereomeric triketide **6** gave none of the expected C-12 epimer **8**, but instead was found to lead to production of the 16-membered macrolactone product **9**[14a] (Scheme 4). The latter result indicated that unsaturated triketide **6** was apparently being selectively recognized by DEBS module 2 rather than DEBS module 3 and thereby processed as if it were a *diketide* analogue. To explore the generality of these surprising observations and to elucidate the structural basis of this unexpected stereochemical discrimination, we have now incubated a series of structural and stereochemical analogues of **5** and **6** with *S. coelicolor* CH999/pJRJ2. The results of these studies are detailed below.

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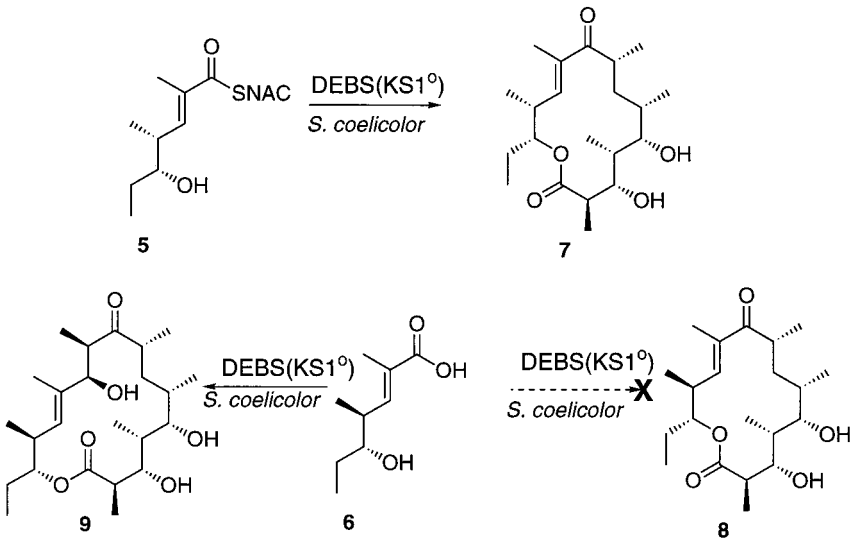
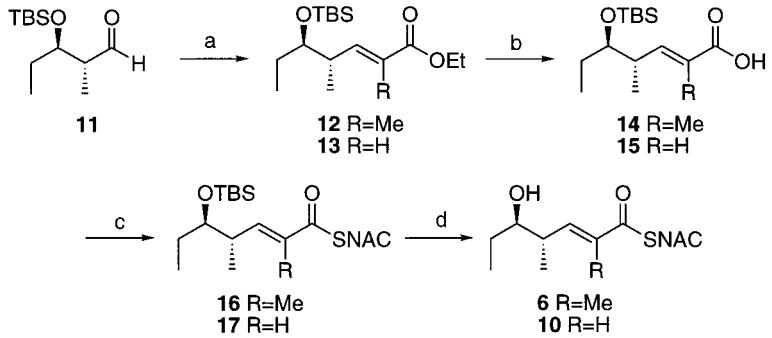
Scheme 4

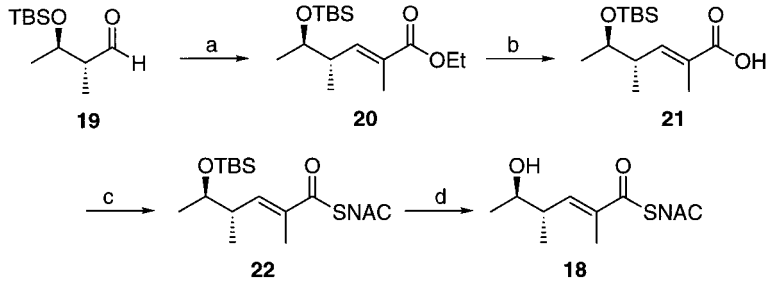


Scheme 5[a]



[a] (a) **11** → **12**, $Ph_3P = C(CH_3)CO_2Et$, THF; **11** → **13**, $Ph_3P = CHCO_2Et$, THF; (b) K_2CO_3, $MeOH/H_2O$; (c) i. $Ph_2P(O)N_3$, Et_3N, DMF; ii. HSNAC; (d) HF, CH_3CN/H_2O.

Scheme 6[a]



[a] (a) $Ph_3P = C(CH_3)CO_2Et$, THF; (b) K_2CO_3, $MeOH/H_2O$; (c) i. $Ph_2P(O)N_3$, Et_3N, DMF; ii. HSNAC; (d) HF, CH_3CN/H_2O.

Results

Preparation of Triketide Analogues. The unsaturated (4*S*,5*R*)-4-methyl-5-hydroxy triketides **6** and **10** were readily prepared from the known protected (2*S*,3*S*)-2-methyl-3-hydroxy aldehyde **11**[16] using the appropriate Emmons reagent to generate the corresponding unsaturated esters **12** and **13** which were then converted to the desired -SNAC thioesters in a conventional manner (Scheme 5). In a similar fashion, the lower homologue **18** was conveniently synthesized from the protected β-hydroxy-aldehyde **19**[17] (Scheme 6). Finally, the TBS-protected (3*R*)-3-hydroxypentanal **23**[18] and (3*R*)-3-*tert*-butyldimethylsilyloxy-butyraldehyde **24**[19] were converted in like manner to the corresponding (5*R*)-5-hydroxy-2-methylheptenoic and -2-methylhexenoic NAC thioesters **31** and **32** by straightforward variation of the same sequence of reactions (Scheme 7).

Feeding of Unsaturated Triketides to *S. coelicolor* CH999/pJRJ2. To facilitate comparison among various substrates that were to be used in precursor-directed biosynthesis experiments, a set of standardized feeding and isolation protocols was developed. After considerable experimentation, it was found that satisfactory yields of macrolide products could be reproducibly obtained by supplementing 3-day-old agar cultures of *S.*

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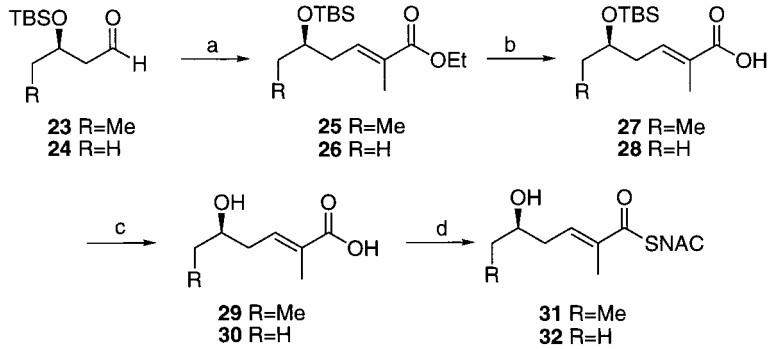
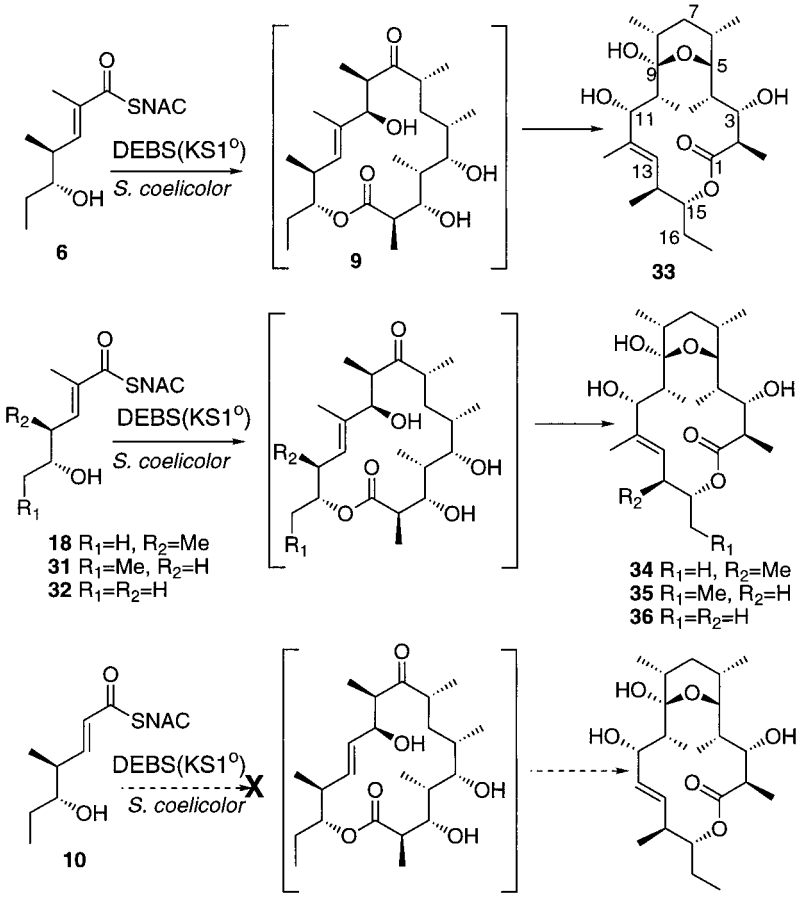
Scheme 7[a]



[a] (a) $Ph_3P = C(CH_3)CO_2Et$, THF; (b) K_2CO_3, $MeOH/H_2O$; (c) HF, CH_3CN/H_2O; (d) i. $Ph_2P(O)N_3$, Et_3N, DMF; ii. HSNAC.

Scheme 8



coelicolor CH999/pJRJ2 encoding DEBS(KS1°) with 1 mL each of a 5 mM solution of unsaturated triketide-SNAC substrate in 5% DMSO−water. After an additional 4 days of incubation, the entire mycelia and media from 20 25-mL plates were homogenized and extracted with ethyl acetate, and any derived macrolide products were purified by preparative TLC from the concentrated extract. The products obtained were then analyzed by 1D and 2D NMR and by mass spectrometry to assign their individual structures.

Feeding of the (2*E*,4S,5*R*)-2,4-dimethyl-5-hydroxy-2-heptenoic acid, *N*-acetylcysteamine thioester **6**, to a total of 500 mL of surface culture of *S. coelicolor* CH999/pJRJ2 gave the 16-membered ring macrolide, previously assigned[14a] the structure **9** (10 mg, 23% yield) (Scheme 8). Unexpectedly, the ^{13}C NMR spectrum revealed that the macrolide was actually present as the corresponding 5,9-hemiketal **33** and not as the free ketone **9**, as evidenced by the characteristic resonance at δ 99.8 for the C-9 hemiketal carbon, and the absence of the corresponding carbonyl carbon signal at ∼200 ppm. In fact, comparison with the ^{13}C NMR spectrum of the previously reported sample of macrolactone obtained from earlier feedings of **6** confirmed that the two macrolactone samples were indeed identical. Analogous incorporation experiments with the unsaturated triketides **18**, **31**, and **32** gave the corresponding 16-membered macrolactones **34** (20 mg, 48% yield), **35** (10 mg, 24% yield), and **36** (8 mg, 20% yield) (Scheme 8). The ^1H NMR spectrum of **34** was assigned using 2D $^1H-^1H$ COSY spectroscopy, with both DEPT and $^1H-^{13}C$ HETCOSY used to assign the ^{13}C NMR signals. In addition, the structure of **34**, including the stereochemistry of the 5,9-hemiketal, was fully confirmed by single-crystal X-ray diffraction analysis (Figure 1). The structure and stereochemistry of **35** and **36** were readily assigned by detailed comparison of the ^1H- and ^{13}C NMR spectra of each with those of the reference macrolactones **33** and **34**. No other 14- or 16-membered

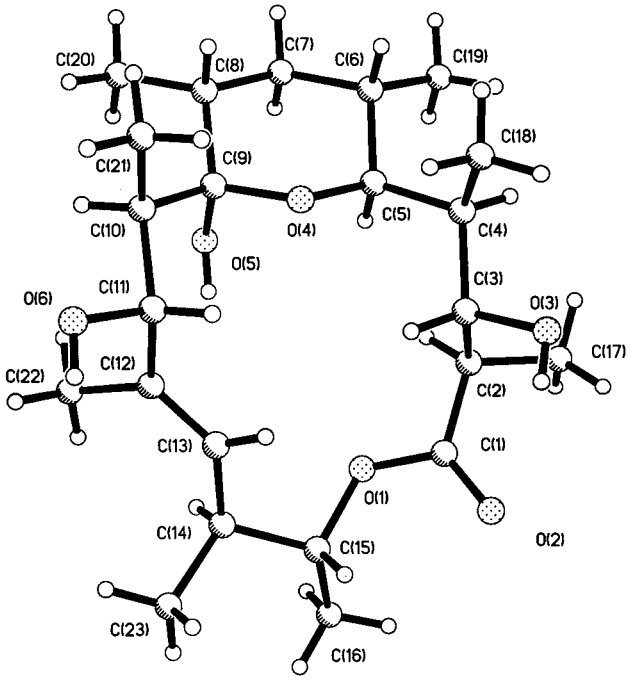


Figure 1. X-ray structure of **34**.

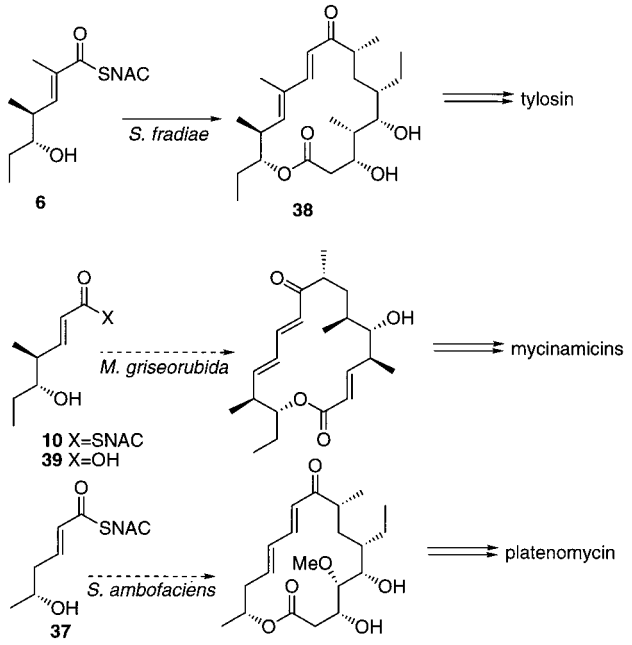
macrolactones were detected in the fermentation extract. Interestingly, attempted incorporation of the unsaturated triketide substrate **10**, lacking a methyl group at C-2, did not give rise to detectable quantities of any macrolactone products.

Discussion

Feeding to *S. coelicolor* CH999/pJRJ2 of the unsaturated triketide **5**, with the (4*R*,5*R*)-4-methyl-5-hydroxy substitution pattern and stereochemistry of the natural 6-dEB triketide precursor, results in formation of the corresponding 10,11-anhydro-6-dEB analogue **7**.[15] **5** is thus apparently recognized and processed as a triketide by DEBS module 3, despite the absence of the normal (3*S*)-hydroxyl moiety. By contrast, administration of the diastereomeric (4*S*,5*R*)-4-methyl-5-hydroxy unsaturated triketide **6** leads predominantly to the unexpected formation of the 16-membered macrolactone **33**, the 5,9-hemiketal of **9**,[14a] whose formation must be the consequence of the processing of triketide **6** by module 2. The resultant polyketide intermediates are apparently processed by the downstream DEBS modules, leading, after a total of five rounds of polyketide chain elongation and functional group modification, to the observed macrolactone **33**.

To explore the structural and stereochemical features influencing recognition and processing of the unsaturated triketide surrogates, we examined the incorporation of a series of analogues of **6** in which the methyl groups at C-2, C-4, and C-6 were systematically replaced by a proton. Triketides **18**, **31**, and **32**, lacking methyl groups at C-6, at C-4, or both C-6 and C-4, respectively, were each converted with comparable efficiency to the corresponding 16-membered lactone analogues **34**, **35**, and **36**. Feeding of the unsaturated triketide **10**, lacking a methyl group at C-2, did not give rise to any detectable macrolactone product, however, under the conditions of the feeding experiments that were used. Of particular interest was the observation that, while the absence of the (4*S*)-methyl group in the unsaturated triketide substrates **31** and **32** did not impede the formation of a 16-membered lactone, the presence of an epimeric (4*R*)-methyl in **5** is known to result exclusively in the formation of 14-membered lactone **7**.

Scheme 9



Although none of the four unsaturated triketide analogues that are accepted and processed by DEBS module 2 is itself a natural substrate for DEBS, two triketides, **6** and **10**, as well as the closely related compound **37**, are in fact demonstrated or presumptive precursors of the 16-membered ring macrolide antibiotics tylosin, mycinamicin, and platenomycin, respectively (Scheme 9). For example, **6** has previously been incorporated intact into tylactone (**38**), the parent aglycon of tylosin,[21] while **39**, the free acid corresponding to **10**, has been isolated from the culture filtrate of a mutant of *Micromonospora griseorubida* blocked in the formation of mycinamicins.[22] Interestingly, all known 16-membered macrolides with a methyl substituent at C-14 have the (14*S*)-stereochemistry characteristic of tylosin and mycinamicin, while all known 12- and 14-membered ring macrolides that carry a methyl group at the corresponding C-10 or C-12 positions, respectively, have the epimeric methyl configuration found in 6-dEB (**1a**).

The results reported here demonstrate that DEBS module 2 can process a number of unsaturated triketide analogues of varied structures and stereochemistries, while strictly discriminating against other stereoisomers. The biochemical basis for this striking discrimination is currently being investigated using purified preparations of individual DEBS modules, and the results will be reported in due course.

Experimental Section

Ethyl (2*E*,4*S*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoate (12). The aldehyde (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-methylpentanal (**11**),[16] (1.55 g, 6.73 mmol, 1.0 equiv) was dissolved in 100 mL of THF and treated with 7.97 g (20.7 mmol, 3.0 equiv) of (carbethoxyethylidene)triphenylphosphorane. The mixture was stirred at reflux for 24 h. After cooling to room temperature, the reaction mixture was subjected to SiO_2 gel (100 g) chromatography and eluted with 5% EtOAc in hexanes to afford 2.12 g (100%) of ethyl (2E,4*S*,5*R*)-

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5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoate (**12**) as a colorless oil: R_f 0.56 (5% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.03−0.09 (m, 6H, dimethyl in TBS), 0.85−0.93 (m, 12H, *t*-Bu in TBS and H7), 0.98 (d, 3H, C4-Me), 1.32 (t, 3H, $COOCH_2CH_3$), 1.35−1.52 (m, 2H, H6), 1.87 (s, 3H,C2-Me), 2.58−2.71 (m, 1H, H4), 3.48−3.56 (m, 1H, H5), 4.20 (m, 2H, $COOCH_2CH_3$), 6.76 (d, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.3, 144.7, 127.2, 76.5, 60.3, 37.8, 27.3, 25.7, 18.1, 17.2, 16.0, 14.2, 12.5, 9.5, −4.6, −4.9; $[\alpha]_D$ −13.6° (*c* = 2.0, $CHCl_3$); HRFAB-MS ($[M + Na]^+$, NBA/NaI): Calculated for $(C_{17}H_{34}O_3Si)Na^+$: 337.2175, Found: 337.2168.

(2*E*,4*S*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoic Acid (14). To a 100 mL flask under nitrogen charged with 30 mL of MeOH and 10 mL of distilled H_2O was added 1.75 g (12.7 mmol, 5.0 equiv) of potassium carbonate (K_2CO_3) and 800 mg (2.54 mmol, 1.0 equiv) of ethyl (2*E*,4*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoate (**12**). The solution was heated under reflux for 3 h. Methanol was removed by rotary evaporation, and the remaining aqueous layer was acidified to pH 2 with concentrated HCl, followed by saturation with NaCl and extraction with ethyl ether (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flash SiO_2 gel chromatography (18 g, elution 5% EtOAc in hexanes) to afford (2*E*,4*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoic acid (**14**, 545 mg, 75%) as a colorless oil. R_f 0.12 (5% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.02−0.12 (m, 6H, dimethyl in TBS), 0.83−0.93 (m, 12H, *t*-Bu in TBS and H7), 1.03 (d, 3H, C4-Me), 1.35−1.63 (m, 2H, H6), 1.88 (s, 3H, C2-Me), 2.58−2.72 (m, 1H, H4), 3.53−3.58 (m, 1H, H5), 6.87 (d, 1H, H3).

(2*E*,4*S*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoic Acid, *N*-Acetylcysteamine Thioester (16). (2*E*,4*S*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoic acid (**14**) (545 mg, 1.90 mmol, 1.0 equiv) was dissolved in 10 mL DMF under nitrogen atmosphere. After cooling to 0 °C, the solution was treated with diphenylphosphoryl azide (0.62 mL, 2.85 mmol, 1.5 equiv) and triethylamine (0.53 mL, 3.80 mmol, 2.0 equiv). The mixture was stirred at 0 °C for 2 h, and *N*-acetylcysteamine (HSNAC: 271 mg 2.28 mmol, 1.2 equiv) was added. The reaction was allowed to stir at room temperature for 2 h followed by addition of water (10 mL) and extraction with EtOAc (3 × 30 mL). The organic layers were dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by flash SiO_2 gel column chromatography with 40% EtOAc in hexanes afforded (2*E*,4*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoic acid, *N*-acetylcysteamine thioester (**16**) (474 mg, 64%) as a colorless oil: R_f 0.33 (40% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.02−0.09 (m, 6H, dimethyl in TBS), 0.83−0.93 (m, 12H, *t*-Bu in TBS and H7), 1.03 (d, 3H, C4-Me), 1.33−1.48 (m, 1H, H6a), 1.45−1.53 (m, 1H, H6b), 1.88 (s, 3H, C2-Me), 2.00 (s, 3H, N-$COCH_3$), 2.65−2.76 (m, 1H, H4), 3.06 (m, 2H, S−CH_2), 3.45 (m, 2H, N-CH_2), 3.50−3.60 (m, 1H, H5), 6.11 (b, 1H, N*H*), 6.80 (d, 1H, H3).

(2*E*,4*S*,5*R*)-2,4-Dimethyl-5-hydroxy-2-heptenoic Acid, *N*-Acetylcysteamine Thioester (6). (2*E*,4*S*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoic acid, *N*-acetylcysteamine thioester (**16**) (474 mg, 1.22 mmol) was dissolved in 10 mL of acetonitrile and 2 mL of water. To the mixture, HF (1 mL of a 48% aqueous solution, 31 mmol) was added dropwise. The mixture was stirred at room temperature for 2 h before addition of saturated aqueous $NaHCO_3$ at 0 °C until the pH of the solution reached 7.5. Acetonitrile was removed in vacuo, and the resulting mixture was extracted with EtOAc (3 × 30 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by flash SiO_2 gel chromatography (5% methanol in $CHCl_3$) to afford (2*E*,4*S*,5*R*)-2,4-dimethyl-5-hydroxy-2-heptenoic acid, *N*-acetylcysteamine thioester (**6**)[22] (334 mg, 100%) as a colorless oil: R_f 0.28 (5% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, 3H, H7), 1.03 (d, 3H, C4-Me), 1.32−1.40 (m, 1H, H6a), 1.35−1.52 (m, 1H, H6b), 1.82 (s, 3H, C2-Me), 1.92 (s, 3H, N-$COCH_3$), 2.52−2.62 (m, 1H, H4), 3.04 (m, 2H, S−CH_2), 3.44 (m, 3H, N-CH_2 and H5), 6.11 (b, 1H, N*H*), 6.67 (d, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 194.0, 170.9, 143.3, 136.0, 76.4, 39.7, 38.9, 28.3, 27.8, 22.9, 16.4, 12.7, 10.0; $[\alpha]_D$ −13.4° (*c* = 2.0, $CHCl_3$); HRFAB-MS ($[M + Na]^+$, NBA/NaI): Calculated for $(C_{13}H_{23}NO_3S)Na^+$: 296.1297, Found: 296.1296.

Ethyl (2*E*,4*S*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-4-methyl-2-heptenoate (13). The aldehyde (2*R*,3*R*)-3-(*tert*-butyldimethylsilyloxy)-2-methylpentanal (**11**, 1.50 g, 6.51 mmol, 1.0 equiv) was dissolved in 100 mL of THF and treated with 7.33 g (20.0 mmol, 3.1 equiv) of (carbethoxymethylene)triphenylphosphorane. The mixture was stirred at reflux for 24 h and the product isolated and purified as described for **2** to afford 1.43 g (73%) of ethyl (2*E*,4*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoate (**13**) as a colorless oil: R_f 0.57 (10% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.03−0.09 (m, 6H, dimethyl in TBS), 0.85−0.93 (m, 12H, *t*-Bu in TBS and H7), 1.08 (d, 3H, C4-Me), 1.32 (t, 3H, $COOCH_2CH_3$), 1.35−1.55 (m, 2H, H6), 2.42−2.55 (m, 1H, H4), 3.51−3.57 (m, 1H, H5), 4.20 (q, 2H, $COOCH_2CH_3$), 5.79 (d, 1H, H2), 6.98 (dd, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 166.7, 151.5, 121.0, 76.5, 60.0, 41.3, 26.8, 25.8, 18.1, 15.1, 14.2, 9.5, −4.4, −4.6; $[\alpha]_D$ −12.7° (*c* = 2.0, $CHCl_3$); HRFAB-MS ($[M + Na]^+$, NBA/NaI): Calculated for $(C_{16}H_{32}O_3Si)Na^+$: 323.2019, Found: 323.2027.

(2*E*,4*S*,5*R*)-4-Methyl-5-hydroxy-2-heptenoic Acid, *N*-Acetylcysteamine Thioester (10). Ethyl (2*E*,4*R*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-4-methyl-2-heptenoate (**13**, 600 mg, 2.0 mmol) was hydrolyzed by treatment with 5.0 equiv of K_2CO_3 in refluxing in 3:1 MeOH−water for 3 h and then purified as described above to give, after flash SiO_2 gel chromatography (15 g, elution with 5% EtOAc in hexanes), (2*E*,4*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoic acid (**15**) (530 mg, 97%) as a colorless oil (R_f 0.61 (20% EtOAc/hexanes)). Treatment of **15** with $Ph_2P(O)N_3$ (0.64 mL, 2.93 mmol, 1.5 equiv) and Et_3N (0.55 mL, 3.90 mmol, 2.0 equiv) in 10 mL of DMF and then HSNAC (463 mg 3.90 mmol, 2.0 equiv) gave after flash SiO_2 gel column chromatography (20% EtOAc in hexanes) (2*E*,4*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-4-methyl-2-heptenoic acid, *N*-acetylcysteamine thioester (**17**) (506 mg, 69%) as a colorless oil: R_f 0.33 (40% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.02−0.09 (m, 6H, dimethyl in TBS), 0.83−0.95 (m, 12H, *t*-Bu in TBS and H7), 1.08 (d, 3H, C4-Me), 1.35−1.56 (m, 2H, H6), 2.02 (s, 3H, N-$COCH_3$), 2.42−2.55 (m, 1H, H4), 3.12 (m, 2H, S−CH_2), 3.42−3.59 (m, 3H, N-CH_2 and H5), 6.15 (d, 1H, H2), 3.50−3.60 (m, 1H, H5), 6.10 (d, 1H, H2), 6.14 (b, 1H, N*H*), 6.98 (dd, 1H, H3). The TBS group was removed by treatment with aqueous HF (50 equiv) in 5:1 CH_3CN−water, as described for **6**, to give (2*E*,4*S*,5*R*)-4-methyl-5-hydroxy-2-heptenoic acid, *N*-acetylcysteamine thioester (**10**) (321 mg, 92%) as a colorless oil: R_f 0.24 (5% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, 3H, H7), 1.16 (d, 3H, C4-Me), 1.36−1.63 (m, 2H, H6), 2.01 (s, 3H, N-$COCH_3$), 2.37−2.51 (m, 1H, H4), 3.10 (m, 2H, S−CH_2), 3.48 (m, 3H, N-CH_2 and H5), 6.15 (d, 1H, H2), 6.20 (b, 1H, N*H*), 6.98 (dd, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 190.2, 170.9, 147.9, 128.6, 76.8, 42.1, 39.6, 28.1, 27.6, 22.9, 15.9, 10.0; $[\alpha]_D$ −9.6° (*c* = 2.0, $CHCl_3$); HRFAB-MS ($[M + Na]^+$, NBA/NaI): Calculated for $(C_{12}H_{21}NO_3S)Na^+$: 282.1140, Found: 282.1146.

Ethyl (2*E*,4*S*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-2,4-dimethyl-2-hexenoate (20). (2*R*,3*R*)-3-(*tert*-Butyldimethylsilyloxy)-2-methylbutanal (**19**)[17] (1.0 g, 4.62 mmol, 1.0 equiv) was reacted with 5.07 g (14.0 mmol, 3.0 equiv) of (carbethoxyethylidene)triphenylphosphorane as described above to afford 727 mg (52%) of ethyl (2*E*,4*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-2-hexenoate (**20**) as a colorless oil: R_f 0.52 (5% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.03−0.09 (m, 6H, dimethyl in TBS), 0.85−0.93 (m, 9H, *t*-Bu in TBS), 0.98 (d, 3H, C4-Me), 1.09 (d, 3H, H6), 1.28 (t, 3H, $COOCH_2CH_3$), 1.86 (s, 3H, C2-Me), 2.54−2.64 (m, 1H, H4), 3.68−3.78 (m, 1H, H5), 4.20 (m, 2H, $COOCH_2CH_3$), 6.69 (d, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.3, 144.9, 127.3, 71.6, 60.3, 41.0, 25.8, 21.4, 16.0, 14.2, 12.5, −4.4, −4.9; $[\alpha]_D$ −16.2° (*c* = 2.0, $CHCl_3$); HRFAB-MS ($[M + Na]^+$, NBA/NaI): Calculated for $(C_{16}H_{32}O_3Si)Na^+$: 323.2019, Found: 323.2028.

(2*E*,4*R*,5*R*)-2,4-Dimethyl-5-hydroxy-2-hexenoic Acid, *N*-Acetylcysteamine Thioester (18). The ester **20** (327 mg, 1.09 mmol) was hydrolyzed in methanolic K_2CO_3 to give 260 mg of crude (2*E*,4*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-2-heptenoic acid (**21**) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.03−0.09 (m, 6H, dimethyl in TBS), 0.87−0.94 (m, 9H, *t*-Bu in TBS), 1.00 (d, 3H, C4-Me), 1.11 (d, 3H, H6), 1.89 (s, 3H, C2-Me), 2.48−2.59 (m, 1H, H4), 3.69−3.76 (m, 1H, H5), 6.85 (d, 1H, H3). Treatment of **21** with Ph_2P-

(O)N_3 (308 μL, 1.43 mmol, 1.5 equiv) and Et_3N (266 μL, 1.90 mmol, 2.0 equiv) followed by HSNAC (115 mg 1.0 mmol, 1.0 equiv) gave, after flash SiO_2 gel column chromatography (40% EtOAc in hexanes), (2*E*,4*S*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-2-hexenoic acid, *N*-acetylcysteamine thioester (**22**) (258 mg, 73%) as a colorless oil: R_f 0.28 (40% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.03−0.10 (m, 6H, dimethyl in TBS), 0.86−0.93 (m, 12H, *t*-Bu in TBS), 1.03 (d, 3H, C4-Me), 1.12 (d, 3H, H6), 1.88 (s, 3H, C2-Me), 1.99 (s, 3H, N-COCH_3), 2.48−2.60 (m, 1H, H4), 3.08 (m, 2H, S−CH_2), 3.46 (m, 2H, N-CH_2), 3.68−3.80 (m, 1H, H5), 5.97 (b, 1H, N*H*), 6.75 (d, 1H, H3). **22** was deprotected by treatment with HF (45 equiv) in CH_3-CN−water to afford after purification (2*E*,4*S*,5*R*)-2,4-dimethyl-5-hydroxy-2-hexenoic acid, *N*-acetylcysteamine thioester (**18**) (180 mg, 100%) as a colorless oil: R_f 0.30 (4% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.07 (d, 3H, C4-Me), 1.22 (d, 3H, H6), 1.92 (s, 3H, C2-Me), 1.98 (s, 3H, N-COCH_3), 2.50−2.62 (m, 1H, H4), 3.10 (m, 2H, S−CH_2), 3.46 (m, 3H, N-CH_2), 3.71−3.81 (m, 1H, H5), 6.07 (b, 1H, N*H*), 6.68 (d, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 194.1, 171.0, 143.6, 136.4, 71.2, 41.0, 39.8, 28.4, 22.9, 21.0, 16.2, 12.8; $[\alpha]_D$ −25.1° (c = 2.0, $CHCl_3$); HRFAB-MS ([M + Na]$^+$, NBA/NaI): Calculated for ($C_{12}H_{21}NO_3S$)Na^+: 282.1140, Found: 282.1152.

Ethyl (2*E*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-2-methyl-2-heptenoate (25). (3*R*)-3-(*tert*-Butyldimethylsilyloxy)pentanal (**23**)[18] (1.53 g, 7.08 mmol, 1.0 equiv) was reacted with 10.3 g (28.32 mmol, 4.0 equiv) of (carbethoxyethylidene)triphenylphosphorane in the usual manner to give, after purification, 2.14 g (100%) of ethyl (2*E*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2-methyl-2-heptenoate (**25**) as a colorless oil: R_f 0.71 (5% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04−0.09 (m, 6H, dimethyl in TBS), 0.85−0.93 (m, 12H, *t*-Bu in TBS and H7), 1.32 (t, 3H, $COOCH_2CH_3$), 1.42−1.55 (m, 2H, H6), 1.86 (s, 3H, C2-Me), 2.29−2.37 (m, 2H, H4), 3.67−3.77 (m, 1H, H5), 4.20 (q, 2H, $COOCH_2CH_3$), 6.83 (t, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.1, 139.0, 133.3, 72.6, 60.3, 36.1, 30.2, 25.8, 18.0, 17.3, 14.2, 12.5, 9.6, −4.6, −4.9; $[\alpha]_D$ −1.2° (c = 2.0, $CHCl_3$); HRFAB-MS ([M + Na]$^+$, NBA/NaI): Calculated for ($C_{16}H_{32}O_3Si$)Na^+: 323.2019, Found: 323.2007.

(2*E*,5*R*)-5-Hydroxy-2-methyl-2-heptenoic Acid, *N*-Acetylcysteamine Thioester (31). 25 (740 mg, 2.46 mmol) was treated with aqueous methanolic K_2CO_3 to give (2*E*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2-methyl-2-heptenoic acid (**27**) which was deprotected with HF in aqueous acetonitrile to afford (2*E*,5*R*)-5-hydroxy-2-methyl-2-heptenoic acid (**29**) (430 mg). The crude **29** was reacted with $Ph_2P(O)N_3$ (611 μL, 2.84 mmol, 1.5 equiv) and Et_3N (527 μL, 3.78 mmol, 2.0 equiv) followed by HSNAC (203 mg 1.70 mmol, 0.9 equiv) and the product isolated and purified as described above to give (2*E*,5*R*)-5-hydroxy-2-methyl-2-heptenoic acid, *N*-acetylcysteamine thioester (**31**) (380 mg, 74%) as a colorless oil: R_f 0.26 (4% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, 3H, H7), 1.43−1.62 (m, 2H, H6), 1.89 (s, 3H, C2-Me), 1.97 (s, 3H, N-COCH_3), 2.30−2.50 (m, 2H, H4), 3.07 (m, 2H, S−CH_2), 3.43 (m, 2H, N-CH_2), 3.67−3.78 (m, 1H, H5), 6.11 (b, 1H, N*H*), 6.86 (t, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 193.8, 171.0, 138.3, 137.3, 72.1, 39.7, 36.2, 30.6, 28.3, 22.9, 12.7, 9.9; $[\alpha]_D$ −8.4° (c = 2.0, $CHCl_3$); HRFAB-MS ([M + Na]$^+$, NBA/NaI): Calculated for ($C_{12}H_{21}NO_3S$)Na^+: 282.1140, Found: 282.1139.

Ethyl (2*E*,5*R*)-5-(*tert*-Butyldimethylsilyloxy)-2-methyl-2-hexenoate (26). The aldehyde (3*R*)-3-(*tert*-butyldimethylsilyloxy)butanal (**24**)[19] (1.20 g, 5.93 mmol, 1.0 equiv) was treated with 5.93 g (16.4 mmol, 2.8 equiv) of (carbethoxyethylidene)triphenylphosphorane to give, after purification, 1.50 g (88%) of ethyl (2*E*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2-methyl-2-hexenoate (**26**) as a colorless oil: R_f 0.70 (5% EtOAc/hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04−0.09 (m, 6H, dimethyl in TBS), 0.85−0.93 (m, 9H, *t*-Bu in TBS), 1.19 (d, 3H, H6), 1.30 (t, 3H, $COOCH_2CH_3$), 1.86 (s, 3H, C2-Me), 2.25−2.35 (m, 2H, H4), 3.87−3.97 (m, 1H, H5), 4.20 (q, 2H, $COOCH_2CH_3$), 6.83 (t, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.1, 138.9, 128.8, 67.9, 60.3, 38.9, 25.8, 23.9, 14.2, 12.5, −4.6, −4.9; $[\alpha]_D$ −12.6° (c = 2.0, $CHCl_3$); HRFAB-MS ([M + Na]$^+$, NBA/NaI): Calculated for ($C_{15}H_{30}O_3Si$)Na^+: 309.1862, Found: 309.1868.

(2*E*,5*R*)-5-Hydroxy-2-methyl-2-hexenoic Acid, *N*-Acetylcysteamine Thioester (32). Ethyl (2*E*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2-methyl-2-hexenoate (**26**, 600 mg, 2.09 mmol) was hydrolyzed with aqueous methanolic K_2CO_3 to afford 520 mg of crude (2*E*,5*R*)-5-(*tert*-butyldimethylsilyloxy)-2-methyl-2-hexenoic acid (**28**) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.05−0.09 (m, 6H, dimethyl in TBS), 0.87−0.94 (m, 9H, *t*-Bu in TBS), 1.19 (d, 3H, H6), 1.87 (s, 3H, C2-Me), 2.27−2.43 (m, 2H, H4), 3.89−3.98 (m, 1H, H5), 6.98 (d, 1H, H3). **28** was deprotected with HF in aqueous CH_3CN give 300 mg of (2*E*,5*R*)-5-hydroxy-2-methyl-2-hexenoic acid (**30**). Treatment of crude **30** with $Ph_2P(O)N_3$ (672 μL, 3.12 mmol, 1.5 equiv) and Et_3N (580 μL, 4.16 mmol, 2.0 equiv) followed by HSNAC (248 mg 2.08 mmol, 1.0 equiv) gave, after purification by flash SiO_2 gel column chromatography with 2% MeOH in $CHCl_3$, (2*E*,5*R*)-5-hydroxy-2-methyl-2-hexenoic acid, *N*-acetylcysteamine thioester (**32**) (280 mg, 55%) as a colorless oil: R_f 0.24 (4% MeOH/$CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (d, 3H, H6), 1.89 (s, 3H, C2-Me), 1.98 (s, 3H, N-COCH_3), 2.38−2.46 (m, 1H, H4), 3.01 (m, 2H, S−CH_2), 3.46 (m, 3H, N-CH_2), 3.95−4.05 (m, 1H, H5), 6.05 (b, 1H, N*H*), 6.88 (d, 1H, H3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 193.8, 171.0, 137.9, 137.3, 66.8, 39.6, 38.2, 28.3, 23.3, 22.9, 12.7; $[\alpha]_D$ −11.1° (c = 2.0, $CHCl_3$); HRFAB-MS ([M + Na]$^+$, NBA/NaI): Calculated for ($C_{11}H_{19}NO_3S$)Na^+: 268.0984, Found: 268.0989.

General Feeding and Extraction Conditions. *S. coelicolor* CH999/pJRJ2 encoding DEBS(KS1°)[14a] was cultivated at 30 °C on 25-mL R2YE agar[23] plates for 3 days. Each plate was then overlaid with 1 mL of 5 mM substrate solution in 5% DMSO/water. After an additional 4 days, the combined mycelia and agar (20 plates, 500 mL total) were homogenized and extracted with EtOAc (3 × 500 mL) at ∼40 °C. The combined organic extract was dried over $MgSO_4$, concentrated to ∼5 mL in vacuo, and then washed with saturated $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4, and concentrated in vacuo.

Purification and Structure Determination. The crude mixture obtained from each precursor feeding was subjected to initial purification by SiO_2 column chromatography (∼1 g of SiO_2 in a Pasteur pipet) with 20% EtOAc/hexanes. The relevant fraction thus obtained was further purified by preparative TLC plate (6 cm × 10 cm) using 40% EtOAc/hexanes as eluent. The product band was eluted with EtOAc, and the resultant product was characterized by ^{1}H (300 MHz)- and ^{13}C NMR (75 MHz) spectroscopy (Bruker Avance 300), mass spectrometry (Kratos MS80RFA), and FT IR (Perkin-Elmer model 1600). The ^{1}H NMR spectra of products were assigned using two-dimensional ^{1}H−^{1}H COSY spectroscopy. The ^{13}C NMR spectrum of **34** was fully assigned by a combination of DEPT and ^{1}H−^{13}C HETCOSY spectroscopy. NMR assignments of the other three 16-membered lactones **33**, **35**, and **36** were based on comparison to the spectra of **34**.

Conversion of (2*E*,4*S*,5*R*)-2,4-Dimethyl-5-hydroxy-2-heptenoic Acid, *N*-Acetylcysteamine Thioester (6) to 33. Feeding of 20 mL of 5 mM (2*E*,4*S*,5*R*)-2,4-dimethyl-5-hydroxy-2-heptenoic acid, *N*-acetylcysteamine thioester (**6**) to *S. coelicolor* CH999/pJRJ2 gave 10 mg of **33**[14a] (23% yield) as a white powder: R_f 0.47 (20% EtOAc/hexanes); IR (film) ν 3505, 3382, 2985, 2926, 1712, 1455, 1380, 1190, 1001, 982 cm^{-1}; ^{1}H NMR (400 MHz, CD_2Cl_2): δ 5.34 (dd, 1H, J = 8.0, 1.2 Hz, H13), 4.65−4.75 (m, 1H, H15), 3.97 (d, 1H, J = 10.0 Hz, H11), 3.80 (d, 1H, J = 9.7 Hz, H3), 3.40 (dd, 1H, J = 10.0, 1.5 Hz, H5), 2.75 (q, 1H, J = 7.1 Hz, H2), 2.52−2.63 (m, 1H, H14), 2.52 (d, 1H, J = 2.1 Hz, C3-OH), 2.15 (dq, 1H, J = 10.0, 6.7 Hz, H10), 1.90−2.00 (m, 1H, H8), 1.75−1.88 (m, 1H, H16a), 1.72 (d, 3H, J = 1.2 Hz, C12-Me), 1.67−1.74 (m, 1H, H4), 1.55−1.65 (m, 2H, H6 and H16b), 1.38−1.46 (m, 1H, H7a), 1.15−1.25 (m, 1H, H7b), 1.10 (d, 3H, J = 6.6 Hz, C10-Me), 1.07 (d, 3H, J = 7.0 Hz, C2-Me), 0.98 (d, 3H, J = 6.9 Hz, C4-Me), 0.97 (d, 3H, J = 6.8 Hz, C14-Me), 0.90 (t, 3H, J = 7.1 Hz, H17), 0.85 (d, 3H, J = 6.8 Hz, C8-Me), 0.74 (d, 3H, J = 6.6 Hz, C6-Me); ^{13}C NMR (100 MHz, CD_2Cl_2): δ 178.4 (C1), 139.1 (C12), 133.3 (C13), 99.8 (C9), 78.5 (C11), 78.4 (C15), 73.6 (C5), 72.1 (C3), 41.8 (C10), 39.4 (C2), 37.0 (C14), 36.3 (C7), 34.6 (C4), 34.4 (C8), 31.5 (C6), 24.9 (C16), 16.2 (C6-Me, C14-Me), 15.1 (C8-Me), 12.1 (C10-Me), 10.1 (C12-Me), 8.7 (C4-Me), 8.0 (C17), 7.8 (C2-Me); HRFAB-MS ([M + Na]$^+$, NBA/NaI): Calculated for ($C_{24}H_{42}O_6$)Na^+: 449.2879, Found: 449.2883.

(23) Hopwood, D. A.; Bibb, M. J.; Chater, K. F.; Kieser, T.; Bruton, C. J.; Kieser, H. M.; Lydiate, D. J.; Smith, C. P.; Ward, J. M.; Schrempf, H. *Genetic Manipulation of Streptomyces. A Laboratory Manual*; John Innes Foundation: Norwich, UK, 1985; pp 235−236.

Conversion of (2*E*,4*S*,5*R*)-2,4-Dimethyl-5-hydroxy-2-hexenoic Acid, *N*-Acetylcysteamine Thioester (18) to 34. Feeding of 20 mL of 5 mM **18** to *S. coelicolor* CH999/pJRJ2 gave 20 mg of **34** (48% yield) as colorless crystals, mp 158−160 °C. X-ray-quality single crystals of **34** were grown as colorless prisms from a mixture of EtOAc−hexanes. R_f 0.27 (20% EtOAc/hexanes); IR (neat) ν 3503, 3371, 2972, 2930, 1710, 1454, 1378, 1333, 1195, 1131, 1003, 985, 857 cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 5.39 (dd, 1H, J = 8.0, 0.9 Hz, H13), 4.59−4.71 (m, 1H, H15), 3.98 (d, 1H, J = 10.1 Hz, H11), 3.81 (d, 1H, J = 10.4 Hz, H3), 3.41 (dd, 1H, J = 10.0, 1.3 Hz, H5), 2.73 (q, 1H, J = 7.0 Hz, H2), 2.56 (d, 1H, J = 2.1 Hz, C3−OH), 2.48−2.56 (m, 1H, H14), 2.15 (dq, 1H, J = 10.0, 6.7 Hz, H10), 1.89−2.01 (m, 1H, H8), 1.66−1.80 (m, 1H, H4), 1.72 (d, 3H, J = 1.1 Hz, C12-Me), 1.48−1.65 (m, 1H, H6), 1.38−1.46 (m, 1H, H7a), 1.29 (d, 3H, J = 6.1, H16), 1.21−1.30 (m, 1H, H7b), 1.10 (d, 3H, J = 6.7 Hz, C10-Me), 1.03 (d, 3H, J = 7.1 Hz, C2-Me), 0.99 (d, 3H, J = 6.8 Hz, C14-Me), 0.96 (d, 3H, J = 6.7 Hz, C4-Me), 0.85 (d, 3H, J = 6.8 Hz, C8-Me), 0.74 (d, 3H, J = 6.6 Hz, C6-Me); ^{13}C NMR (75.5 MHz, CD_2Cl_2): δ 177.1 (C1), 138.9 (C12), 133.4 (C13), 99.9 (C9), 78.6 (C11), 74.6 (C15), 73.7 (C5), 72.3 (C3), 42.0 (C10), 39.9 (C2), 38.5 (C14), 37.0 (C7), 34.9 (C4), 34.5 (C8), 31.6 (C6), 18.1 (C16), 16.3 (C6-Me), 16.1 (C14-Me), 15.1 (C8-Me), 12.2 (C10-Me), 10.2 (C12-Me), 8.0 (C4-Me), 7.2 (C2-Me); HRFAB-MS ([M + Na]$^+$, NBA/NaI): Calculated for $(C_{23}H_{40}O_6)Na^+$: 435.2723, Found: 435.2720.

Conversion of (2*E*,5*R*)-5-Hydroxy-2-methyl-2-heptenoic Acid, *N*-Acetylcysteamine Thioester (31) to 35. Feeding of 20 mL of 5 mM (2*E*,5*R*)-5-hydroxy-2-methyl-2-heptenoic acid, *N*-acetylcysteamine thioester (**31**) to *S. coelicolor* CH999/pJRJ2 gave 10 mg of **35** (24% yield) as a white powder: R_f 0.29 (20% EtOAc/hexanes); IR (film) ν 3474, 2974, 2935, 1723, 1456, 1380, 1332, 1266, 1183, 987 cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 5.34 (t, 1H, J = 8.0, Hz, H13), 4.96−5.07 (m, 1H, H15), 4.04 (d, 1H, J = 10.0 Hz, H11), 3.84 (d, 1H, J = 10.2 Hz, H3), 3.41 (dd, 1H, J = 10.0, 1.7 Hz, H5), 2.74 (q, 1H, J = 7.1 Hz, H2), 2.62 (d, J = 2.1 Hz, C3−OH), 2.31−2.39 (m, 2H, H14), 2.13 (dq, 1H, J = 10.0, 6.7 Hz, H10), 1.87−2.02 (m, 1H, H8), 1.65−1.80 (m, 1H, H4), 1.70 (d, 3H, J = 1.2 Hz, C12-Me), 1.57−1.69 (m, 2H, H16), 1.49−1.60 (m, 1H, H6), 1.38−1.47 (m, 1H, H7a), 1.22−1.34 (m, 1H, H7b), 1.11 (d, 3H, J = 6.7 Hz, C10-Me), 1.07 (d, 3H, J = 7.1 Hz, C2-Me), 0.97 (d, 3H, J = 6.8 Hz, C4-Me), 0.93 (t, 3H, J = 7.5 Hz, C17), 0.85 (d, 3H, J = 6.8 Hz, C8-Me), 0.75 (d, 3H, J = 6.6 Hz, C6-Me); ^{13}C NMR (75.5 MHz, CD_2Cl_2): δ 178.3 (C1), 140.7 (C12), 126.9 (C13), 99.9 (C9), 78.3 (C11), 74.4 (C15), 73.8 (C5), 72.3 (C3), 42.3 (C10), 40.1 (C2), 36.9 (C7), 34.9 (C4), 34.6 (C8), 31.6 (C6), 29.3 (C14), 27.5 (C16), 16.3 (C6-Me), 15.1 (C8-Me), 12.1 (C10-Me), 10.8 (C12-Me), 9.1 (C17), 8.0 (C4-Me), 7.7 (C2-Me); HRFAB-MS ([M + Na]$^+$, NBA/NaI): Calculated for $(C_{23}H_{40}O_6)Na^+$: 435.2723, Found: 435.2724.

Conversion of (2*E*,5*R*)-5-Hydroxy-2-methyl-2-hexenoic Acid, *N*-Acetylcysteamine Thioester (32) to 36. Feeding of 20 mL of 5 mM (2*E*,5*R*)-5-hydroxy-2-methyl-2-hexenoic acid, *N*-acetylcysteamine thioester (**32**) to *S. coelicolor* CH999/pJRJ2 gave 8 mg of **36** (20% yield) as a white powder: R_f 0.16 (20% EtOAc/hexanes); IR (neat) ν 3444, 2973, 2933, 1726, 1459, 1380, 1330, 1199, 1101, 1042, 982, 736 cm^{-1}; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 5.34 (td, 1H, J = 7.2, 1.3 Hz, H13), 4.99−5.10 (m, 1H, H15), 4.03 (d, 1H, J = 10.0 Hz, H11), 3.83 (d, 1H, J = 10.3 Hz, H3), 3.41 (dd, 1H, J = 10.0, 1.5 Hz, H5), 2.71 (q, 1H, J = 7.0 Hz, H2), 2.64 (d, 1H, J = 2.1 Hz, C3−OH), 2.44−2.58 (m, 1H, H14a), 2.30−2.35 (m, 1H, H14b), 2.11 (dq, 1H, J = 10.0, 6.7 Hz, H10), 1.88−2.01 (m, 1H, H8), 1.65−1.80 (m, 1H, H4), 1.69 (d, 3H, J = 0.9 Hz, C12-Me), 1.51−1.66 (m, 1H, H6), 1.37−1.43 (m, 1H, H7a), 1.35 (d, 3H, J = 7.2, H16), 1.21−1.31 (m, 1H, H7b), 1.11 (d, 3H, J = 6.7 Hz, C10-Me), 1.04 (d, 3H, J = 7.1 Hz, C2-Me), 0.96 (d, 3H, J = 6.7 Hz, C4-Me), 0.85 (d, 3H, J = 6.8 Hz, C8-Me), 0.74 (d, 3H, J = 6.6 Hz, C6-Me). ^{13}C NMR (75.5 MHz, CD_2Cl_2): δ 177.8 (C1), 140.2 (C12), 126.7 (C13), 99.8 (C9), 78.6 (C11), 78.1 (C15), 73.6 (C5), 72.3 (C3), 42.2 (C10), 40.2 (C2), 36.7 (C7), 35.0 (C4), 34.2 (C8), 31.4 (C6), 29.1 (C14), 19.7 (C16), 16.3 (C6-Me), 14.9 (C8-Me), 11.9 (C10-Me), 10.6 (C12-Me), 7.8 (C4-Me), 7.0 (C2-Me); HRFAB-MS ([M + Na]$^+$, NBA/NaI): Calculated for $(C_{22}H_{38}O_6)Na^+$: 421.2566, Found: 421.2552.

X-ray Crystallography. X-ray diffraction data were collected in 0.3° steps on a four-circle diffractometer in the ϕ-scan mode equipped with a Bruker SMART CCD 1K detector ($Mo_{K\_}$ radiation, λ = 71.073 pm). The structure of **34** was solved by direct methods and refined with full matrix least-squares on all reflections based on F^2 using the SHELXTL programs commercially available from Bruker Analytical Instruments. Crystallographic data for lactone **34**: crystallographic asymmetric unit $(C_{23}H_{40}O_6)_2$ M_r = 825.10; clear, colorless crystal of dimension 0.03 × 0.025 × 0.015 mm mounted on a quartz fiber; monoclinic space group $P2_1$; a = 10.52(6) Å, b = 18.02(4) Å, c = 13.08 (8) Å; V = 2462 (3) Å^3; Z = 2; ρ_{calcd} = 1.113 g cm^{-3}; μ = 0.079 mm^{-1} (no correction applied); 11 366 reflections collected, 6656 independent (R_{int} = 0.0991); θ range 1.94 to 23.43°, 99.2% completeness; 523 parameters (1 restraint); R_1 = 0.0829, wR^2 = 0.1929 [$I > 2\sigma(I)$] for 6656 data; GooF on F^2 1.009; max/min +0.267 & −0.250 eÅ^{-3}; H atoms located in density maps and refined in fixed idealized positions. The coordinates have been deposited with the Cambridge Crystallographic database and can be obtained by reference to CCDC 157840.

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Supporting Information Available: Tables of crystal data and structure refinement, atomic coordinates, bond lengths and bond angles, anisotropic displacement parameters, and hydrogen coordinates and isotropic displacement factors for **34**, figure illustrating nonsymmetry-related components of the unit cell (PDF). X-ray crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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