



Precursor-Directed Biosynthesis of 12-Ethyl Erythromycin

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Received 5 February 1998; accepted 13 April 1998

Abstract—A precursor-directed method for the biosynthesis of novel 6-deoxyerythronolide B derivatives has been extended to allow alteration of the functionality at C-12. We recently described a simple and practical method for harnessing the biosynthetic potential of the erythromycin pathway to generate novel molecules of natural product-like complexity by feeding designed synthetic molecules to an engineered mutant strain having an altered 6-deoxyerythronolide B synthase (DEBS). Our initial applications of this technique focused on alteration of the ethyl side chain of 6-dEB (C14-C15). We now report the extension of this approach to modification of the C-12 substituent. An appropriately designed substrate is shown to incorporate into 6-dEB biosynthesis, yielding a 6-dEB analogue bearing a 12-ethyl group. This 6-dEB analogue is a substrate for post-polyketide tailoring enzymes, and is converted into the corresponding analogue of erythromycin C. These results show that many of the downstream active sites are tolerant of this unnatural functionality and suggest that a wide variety of erythromycin derivatives should be accessible by this approach or by total biosynthesis via genetic engineering. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

Polyketides comprise a large and diverse class of natural products, many of which possess important biological and medicinal properties.¹ A tremendous diversity of structures, many of which are highly complex, are produced via a common biosynthetic pathway which closely parallels the biosynthesis of fatty acids.² Like fatty acid biosynthesis, polyketide biosynthesis proceeds by repetitive condensation of simple carboxylic acid monomers. Structural complexity is introduced by variation in the degree of reduction after each condensation, by the generation of stereocenters, and by variation of monomers. Additional complexity is introduced by 'post-polyketide' enzymes which carry out oxidations, alkylations, glycosylations and other transformations. The abundance of effective, polyketide-derived pharmaceuticals makes this class of compounds an attractive target for drug discovery.¹

Modular polyketide synthases such as 6-deoxyerythronolide B synthase (DEBS) utilize a separate set of active sites for each condensation step. The biosynthesis of 6-deoxyerythronolide B (6dEB) involves six condensation steps, and consequently DEBS is organized into six groups of active sites, called 'modules', each of which is responsible for one cycle of extension and processing (Fig. 1(A)).³ Each module possesses domains analogous to the ketosynthase (KS), acyl transferase (AT), and acyl carrier protein (ACP) enzymes of fatty acid biosynthesis. Other domains analogous to keto-reductase (KR), dehydratase (DH), enoyl reductase (ER), and thioesterase (TE) enzymes are present in various combinations, giving rise to the complex final structure of 6-dEB (1). The six modules of DEBS are organized into three large polypeptides (each > 300 kDa) with two modules contained in each of the DEBS1, DEBS2, and DEBS3 proteins.

The modular structure of these enzymes has made them attractive targets for genetic engineering despite a lack of protein structural information. The feasibility of

Key words: Polyketide synthase; erythromycin; diketide; mutational biosynthesis.

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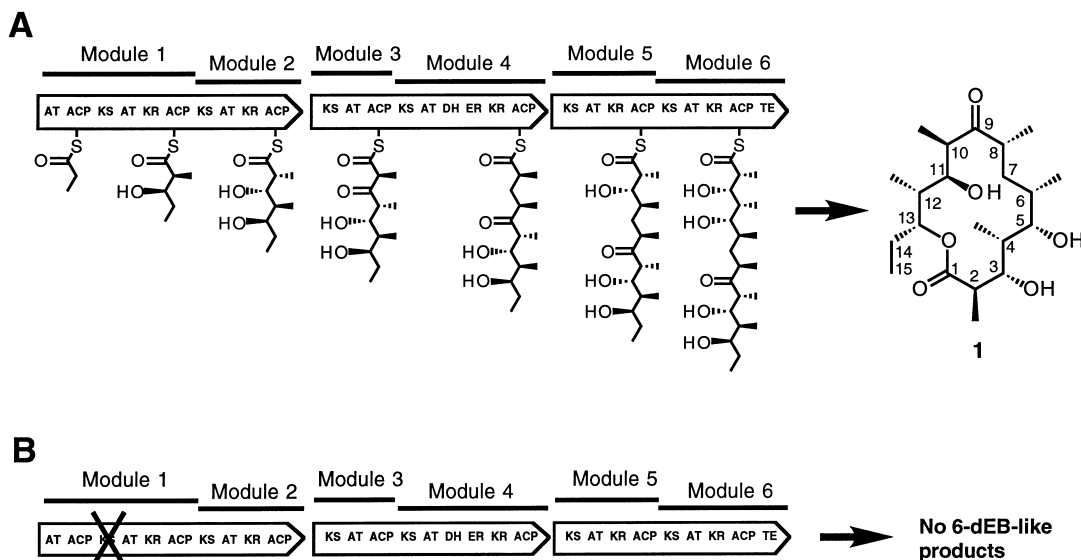


Figure 1. (A) Genetic model of DEBS. DEBS consists of three large polypeptides, each of which contains two ‘modules’. Each module catalyzes the addition of a single methylmalonyl-COA extender unit, along with any reductive steps. Each module contains a variety of domains including a ketosynase (KS), acyltransferase (AT), and acyl carrier protein (ACP). All modules except Module 3 have active ketoreductase (KR) domains, and Module 4 contains dehydratase (DH) and enoyl reductase (ER) domains. Module 6 bears a thioesterase (TE) domain which catalyzes the cyclization of the heptaketide intermediate to form 6-dEB (**1**). Proposed enzyme-bound intermediates are shown for each module. (B) KS1⁰ mutant of DEBS (pJRJ2). Mutation of an active site cysteine residue in the Module 1 ketosynthase domain (KS1) inactivates this domain. No 6-dEB-like products are observed in fermentations of strains expressing this DEBS mutant.

generating new polyketides has been demonstrated via loss-of-function mutagenesis within reductive domains,^{3–5} replacement of acyltransferase domains to alter starter or extender unit specificity,^{6–9} replacement of a reductase domain to alter product stereochemistry,¹⁰ and gain-of-function mutagenesis to introduce novel catalytic activities within modules.^{11,12} However, these approaches are time consuming and limited to metabolically available precursors.

We recently reported the development of a generally applicable, fermentation-based strategy in which chemically synthesized, cell-permeable, non-natural precursors are transformed into novel, natural product-like molecules by a genetically engineered PKS.¹³ DEBS KS1⁰ (Fig. 1(B)) carries an inactivating mutation in the KS domain of module 1. Because it is unable to synthesize the first (diketide) intermediate, no 6-dEB (**1**) is produced. However, when provided with a synthetic analogue of this intermediate (**2**), DEBS KS1⁰ efficiently converts the diketide substrate to 6-dEB.

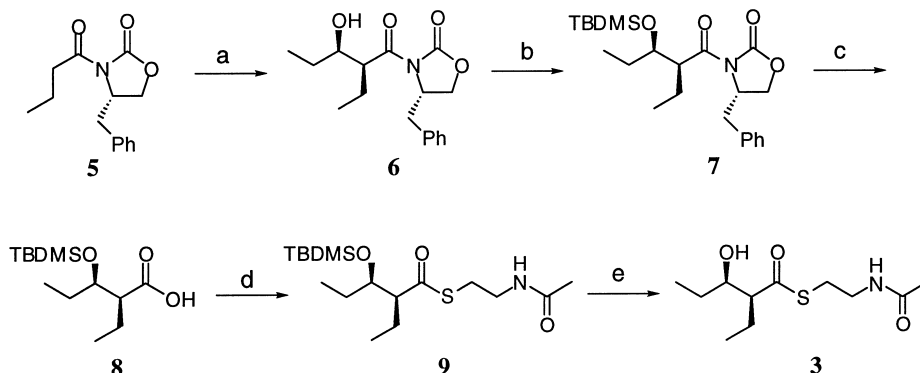
In addition to converting diketide **2** to 6-dEB, DEBS KS1⁰ was shown to utilize ‘unnatural’ substrates and convert these to corresponding analogues of 6-dEB. Initial studies focused primarily on introduction of novel functionality at C15 of 6-dEB. We now report the

precursor-directed biosynthesis of a C12 derivative of 6-dEB in which the methyl side chain found on C12 of 6-dEB has been substituted with an ethyl side chain to give 12-desmethyl-12-ethyl-6-deoxyerythronolide B (**4**).

Results

Construction of the strain CH999/pJRJ2, which expresses the DEBS KS1⁰ mutant, has been described previously.¹³ When grown on R2YE medium, CH999/pJRJ2 produces no detectable 6-dEB-like products. However, when substrate **2** was added to growing cultures of this strain, 6-dEB was efficiently produced (Fig. 2). Our previous studies with unnatural substrates have focused primarily on alterations to the ethyl side chain of 6-dEB (C14–C15). In order to test the generality of this method we prepared substrate **3** which contains a C2-ethyl group. We expected that this substrate would be taken up by module 2 of the DEBS KS1⁰ mutant and converted into 12-desmethyl-12-ethyl-6-dEB.

Substrate **3** (Scheme 1) was prepared by methods analogous to those described previously for the synthesis of substrate **2**.^{13–15} The Evans chiral oxazolidinone was



Scheme 1. Synthesis of **3**. Reagents for transformations: (a) Bu_2BOTf , $\text{EtN}(\text{iPr})_2$, EtCHO ; (b) TBDMSOTf , $\text{EtN}(\text{iPr})_2$; (c) LiOH , H_2O_2 ; (d) $(\text{PhO})_2\text{P}(\text{O})\text{N}_3$, Et_3N , *N*-acetylcysteamine; (e) HF .

acylated with butyryl chloride to provide compound **5**. The asymmetric aldol condensation of **5** with propionaldehyde afforded the desired diketide as the oxazolidinone imide **6**. Imide **6** was protected with *tert*-butyldimethylsilyl triflate to give imide **7**, and the chiral auxiliary was removed with lithium hydroperoxide to give carboxylic acid **8**. The free acid was coupled to *N*-acetylcysteamine (NAC) to afford thioester **9**. NAC thioesters have been used as acyl donors in a variety of studies of fatty acid and polyketide synthases. Finally, the silyl protecting group was removed with hydrofluoric acid to give substrate **3**. Substrate **3** was administered to growing cultures of CH999/pJRJ2 on R2YE agar plates. Polyketide products were obtained by extraction of the agar medium with ethyl acetate. Silica

gel chromatography gave 5 mg of 12-desmethyl-12-ethyl-6-dEB (**4**) from 2 L of agar plates. The product structure was identified by ^1H NMR, ^{13}C NMR and ^1H - ^1H COSY NMR. All spectra are very similar to those of 6-dEB (**1**). Notable differences include the appearance of a new methyl triplet (0.88 ppm) and a shift of the signal for the proton attached to C11. The high-resolution mass spectrum confirms a molecular formula of $\text{C}_{22}\text{H}_{40}\text{O}_6$, which differs from 6-dEB by a single methylene unit.

The successful processing of these unnatural intermediates by the ‘downstream’ modules of DEBS led us to investigate whether the post-PKS enzymes in the erythromycin biosynthetic pathway might also process

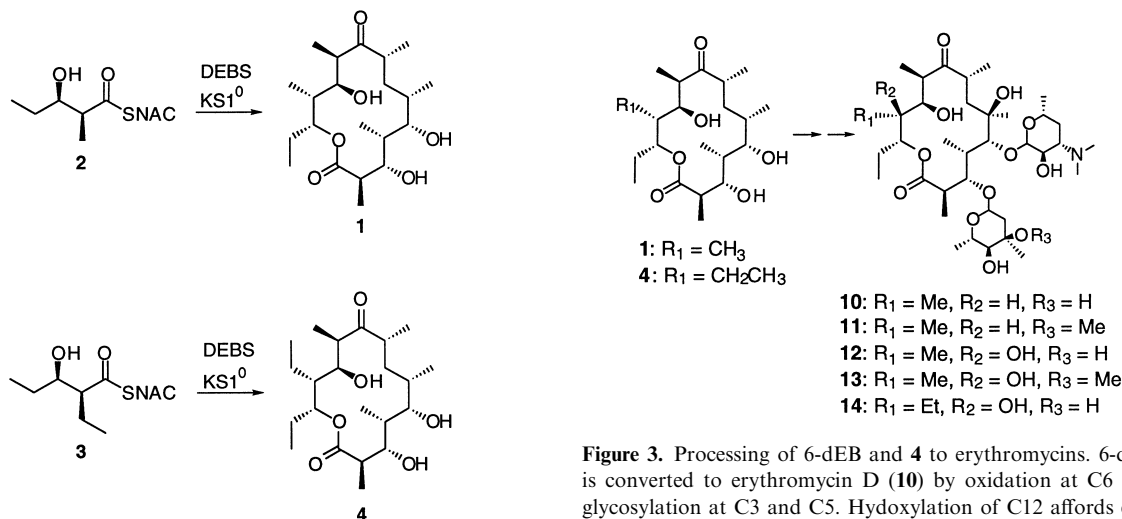


Figure 2. Conversion of diketide substrates by DEBS KS1⁰. Substrate **2** is utilized by DEBS KS1⁰ and efficiently converted to 6-dEB. Substrate **3** is converted to **4**, an analog of 6-dEB in which the methyl group attached to C12 has been replaced by an ethyl group.

Figure 3. Processing of 6-dEB and **4** to erythromycins. 6-dEB is converted to erythromycin D (**10**) by oxidation at C6 and glycosylation at C3 and C5. Hydroxylation of C12 affords erythromycin C (**12**), and subsequent methylation of the mycarose, sugar affords erythromycin A (**13**). Methylation of mycarose without C12 oxidation gives erythromycin B (**11**). *S. erythraea* A34 (which expresses all postpolyketide processing enzymes) converts the 12-ethyl derivative of 6-dEB (**4**) to its erythromycin C analog (**14**).

6-dEB analogue **4**. In the natural producer organism, *Saccharopolyspora erythraea*, 6-dEB undergoes several enzyme-catalyzed transformations. Oxidation at C6 and glycosylations at C3 and C5 afford erythromycin D (**10**) and subsequent transformations afford erythromycins B (**11**), C (**12**), and A (**13**) (Fig. 3). In order to test the ability of the modification enzymes to act on **4**, we utilized an *S. erythraea* mutant (*A34*)¹⁶ which is unable to synthesize 6-dEB. This strain produces no erythromycin when grown on R2YE plates (as judged by the ability of extracts to inhibit growth of the erythromycin-susceptible bacterium *Bacillus cereus*). However, when 6-dEB (which has no antibacterial activity) is added to the culture medium, extracts exhibited potent antibacterial activity. A sample of 6-dEB derivative **4** was assayed for conversion by this strain. A crude extract demonstrated inhibition of *B. cereus* growth, and mass spectrometry was used to identify the major component of the extract as **14** (Fig. 3), an analogue of erythromycin C.

Discussion

The importance of erythromycin as an antibiotic makes it an attractive target for medicinal chemistry, however the challenges involved in total synthesis of macrolides¹⁷ make this approach impractical for the preparation of derivatives. Recent advances have demonstrated that new derivatives of erythromycin and other macrolides are likely to yield valuable new pharmaceuticals.^{18–20} While the synthetic modification of natural products is a proven method for generating derivatives, engineered biosynthesis offers a complementary approach which allows the exploration of different types of modifications which might be difficult or impossible to achieve by semisynthesis.

Precursor-directed biosynthesis combines the power of synthetic chemistry to generate a wide variety of substructures with the power of biosynthesis to selectively and rapidly incorporate these substructures into a complex scaffold of proven pharmacological importance. In addition to our approach which utilizes a KS1 knockout mutation and synthetic diketide substrates, another method of precursor-directed biosynthesis was recently reported which utilizes an engineered PKS with relaxed starter-unit specificity.²¹ Although this engineered PKS can efficiently incorporate simple carboxylic acids into erythromycin derivatives, this approach is limited to alteration of the ethyl side chain of 6-dEB (C14–C15).

The ability to generate new derivatives at C12 of erythromycin may provide a useful route to the investigation of new pharmaceuticals since this region of the molecule of particular medicinal interest.^{19,22} Substrates which bear reactive functional groups might be

used to introduce 'handles' for the synthetic modification of products. A similar approach was recently reported in which a biosynthetic pathway was used to incorporate a reactive ketone group into a cell surface glycoside, allowing specific modifications to the surfaces of intact cells.²³

It is somewhat surprising that the post-polyketide enzymes of *S. erythraea* convert **4** to an erythromycin C analogue. Earlier studies with unnatural 6-dEB derivatives showed that the derivatives examined were converted to erythromycin D analogues.¹³ Despite the fact that it differs from 6-dEB at C12, the 12-ethyl derivative is apparently a substrate for the hydroxylase which acts on C12 (*ery K* gene product). The erythromycin C analogue (**14**) is, however, apparently not a substrate for the methylase (*ery G* gene product) which converts erythromycin C to erythromycin A.

In addition to being a source of potentially useful molecules, chemo-biosynthesis can also provide valuable information to guide genetic engineering. Ethyl side chains, derived from incorporation of ethylmalonyl-CoA extender units, are observed in some macrolides such as tylosin. Although DEBS normally utilizes only methylmalonyl-CoA extender units, the successful incorporation of substrate **3** demonstrates tolerance of intermediates bearing ethyl side chains by DEBS (modules 2–6) as well as other enzymes involved in erythromycin biosynthesis. This suggests that substitution of the module 1 AT domain for a heterologous ethylmalonyl transferase domain should allow for production of **4** by total biosynthesis. Importantly, the use of substrate **3** as a chemical probe allowed this determination to be made without additional genetic manipulations and without addressing the availability of ethylmalonyl-CoA. Ethylmalonyl-CoA is an unusual metabolite which requires a dedicated pathway for its biosynthesis and is absent from many bacteria including *Saccharopolyspora coelicolor*. The facile, precursor-directed synthesis of derivatives such as **4** and **14** should be useful in assessing which engineered mutants of DEBS or other polyketide synthases would be most likely to yield interesting products.

Experimental

General methods

Reagents and chemicals were obtained from commercial suppliers and used without further purification.

(4S)-4-benzyl-3-butyryl-2-oxazolidinone (5). A solution of (4S)-4-benzyl-2-oxazolidinone (1.3 g, 7.1 mmol) in 25 mL freshly distilled THF was cooled to -78°C . *n*-Butyllithium (4.5 mL of a 1.6 M solution in hexanes) was added dropwise by syringe. After 5 min, butyryl

chloride (0.83 mL, 8.0 mmol) was added dropwise by syringe. The solution was stirred for 30 min at -78°C , then warmed slowly to room temperature. After an additional 30 min, the reaction was quenched by addition of 4.5 mL saturated aqueous ammonium chloride. The mixture was concentrated in vacuo, and the residue was extracted (2×50 mL CH_2Cl_2). The combined extracts were washed (50 mL saturated aqueous NaHCO_3 followed by 50 mL brine), dried (MgSO_4) and concentrated in vacuo to afford the product (1.6 g, 89%) as a colorless oil. R_f 0.37 (20% ethyl acetate in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 1.01 (t, 3H, $J=7.4$ Hz, $\text{C}(4')\text{-H}_3$), 1.67–1.78 (m, 2H, $\text{C}(3')\text{-H}_2$), 2.77 (dd, 1H, $J=9.5$ Hz, 13.4 Hz, one of Ph-CH_2), 2.83–3.01 (m, 2H, $\text{C}(2')\text{-H}_2$), 3.30 (dd, 1H, $J=3.2$ Hz, 13.3 Hz, one of Ph-CH_2), 4.14–4.22 (m, 2H, O-CH_2), 4.61–4.71 (m, 1H, N-CH), 7.20 ± 7.36 (m, 5H, Ph-H_5).

(4*S*,2'*S*,3'*R*)-3-(2'-Ethyl-3'-hydroxypentanoyl)-4-benzyl-2-oxazolidinone (6). To 120 mL of dry CH_2Cl_2 was added 8.2 g (33 mmol) of (4*S*)-3-butyryl-4-benzyl-2-oxazolidinone, and the solution was cooled to 0°C . Bu_2BOTf (39 mL of a 1.0 M solution in CH_2Cl_2) was added by syringe, followed by dropwise addition of diisopropylethylamine (7.7 mL, 44 mmol).²⁴ The reaction was cooled to -78°C and freshly distilled propionaldehyde (2.7 mL, 37 mmol) was added. The reaction was stirred at -78°C for 30 min then warmed to 0°C and stirred for an additional 2 h. A mixture of 40 mL phosphate buffer (1.0 M sodium phosphate, pH 7.4) and 100 mL methanol was added to the reaction followed by dropwise addition of 100 mL 2:1 methanol:30% H_2O_2 . After 1.5 h, organic solvents were evaporated in vacuo and the residual material resuspended in 100 mL of 5% aqueous NaHCO_3 and extracted (3×100 mL CH_2Cl_2). The combined extracts were washed (100 mL 5% aqueous NaHCO_3 followed by 100 mL brine), dried (MgSO_4) and concentrated in vacuo. Flash chromatography (6 $\times$ 15 cm silica gel, gradient of 15–30% ethyl acetate in hexanes) afforded 6.0 g (59%) of the desired product as a colorless oil. R_f 0.49 (50% ethyl acetate in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 0.99 (t, 6H, $J=7.4$ Hz, $\text{C}(5')\text{-H}_3$, $\text{C}(2')\text{CH}_2\text{-CH}_3$), 1.51–1.58 (m, 2H, $\text{C}(2')\text{-CH}_2$), 1.70–1.77 (m, 2H, one of $\text{C}(4')\text{-CH}_2$), 1.86–1.92 (m, 2H, one of $\text{C}(4')\text{-CH}_2$), 2.48 (br d, 1H, OH), 2.71 (dd, 1H, $J=10.3$ Hz, 13.2 Hz, one of Ph-CH_2), 3.37 (dd, 1H, $J=3.3$ Hz, 13.2 Hz, one of Ph-CH_2), 3.78–3.83 (m, 1H, $\text{C}(2')\text{-H}$), 3.99–4.04 (m, 1H, $\text{C}(3')\text{-H}$), 4.14–4.22 (m, 2H, O-CH_2), 4.71–4.76 (m, 1H, N-CH), $7.21\text{--}7.36$ (m, 5H, Ph-H_5).

(4*S*,2'*S*,3'*R*)-(2'-Ethyl-3'-*tert*-butyldimethylsiloxy)pentanoyl)-4-benzyl-2-oxazolidinone (7). To 6.0 g (20 mmol) of oxazolidinone **6** in 100 mL CH_2Cl_2 at 0°C , diisopropylethylamine (5.8 mL, 33 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (6.8 mL, 30 mmol) were

added.²⁵ After 2 h, 150 mL of water was added, and the aqueous layer was extracted (3×100 mL CH_2Cl_2). The organic layers were combined, dried (MgSO_4), and concentrated in vacuo. Flash chromatography (6 $\times$ 10 cm silica gel, 40% ethyl acetate in hexanes) afforded product **7** as a colorless oil (7.9 g, 96%). R_f 0.86 (50% ethyl acetate in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 0.02 (s, 3H, Si- CH_3), 0.03 (s, 3H, Si- CH_3), 0.84–0.96 (m, 15H, Si- $\text{C}(\text{CH}_3)_3$, $\text{C}(2')\text{CH}_2\text{-CH}_3$, $\text{C}(5')\text{-H}_3$), 1.50–1.90 (m, 4H, $\text{C}(4')\text{-H}_2$, $\text{C}(2')\text{-CH}_2$), 2.72 (dd, 1H, $J=10.1$ Hz, 13.2 Hz, one of Ph-CH_2), 3.36 (dd, 1H, $J=3.1$ Hz, 13.2 Hz, one of Ph-CH_2), 3.94–3.99 (m, 2H, $\text{C}(2')\text{-H}$, $\text{C}(3')\text{-H}$), 4.09–4.18 (m, 2H, O-CH_2), 4.62–4.68 (m, 1H, N-CH), $7.21\text{--}7.36$ (m, 5H, Ph-H_5).

(2*S*,3*R*)-2-Ethyl-3-(*tert*-butyldimethylsiloxy)-pentanoic acid (8). Oxazolidinone **7** (7.9 g, 19 mmol) was dissolved in 145 mL THF. Water (50 mL) was added and the solution was cooled to 0°C with vigorous stirring. Hydrogen peroxide (18 mL of a 30% aqueous solution) was added followed by lithium hydroxide (1.6 g, 38 mmol).²⁶ The reaction was allowed to warm slowly to room temperature. After 24 h, the reaction was quenched by addition of sodium sulfite (115 mL of a 20% aqueous solution). After stirring for 10 min, the reaction was concentrated in vacuo to remove THF and then acidified to pH 2 by slow addition of 1 N HCl. The resulting cloudy mixture was extracted (3×30 mL CH_2Cl_2). The organic layers were combined, dried (MgSO_4) and concentrated in vacuo. The residue was purified by chromatography (6 $\times$ 15 cm silica gel, 15% ethyl acetate in hexanes) to afford the desired product (2.25 g, 46%). R_f 0.73 (50% ethyl acetate in hexanes). ^1H NMR (400 MHz, CDCl_3) δ 0.10 (s, 6H, Si- $(\text{CH}_3)_2$), 0.84–0.96 (m, 15H, Si- $\text{C}(\text{CH}_3)_3$, $\text{C}(2)\text{CH}_2\text{-CH}_3$, $\text{C}(5)\text{-H}_3$), 1.45–1.81 (m, 4H, $\text{C}(2)\text{-CH}_2$, $\text{C}(4)\text{-H}_2$), 2.40–2.45 (m, 1H, $\text{C}(2)\text{-H}$), 3.84–3.88 (m, 1H, $\text{C}(3)\text{-H}$).

(2*S*,3*R*)-2-Ethyl-3-hydroxypentanoyl-*N*-acetylcysteamine thioester (3). In 100 mL of dry dimethylformamide was dissolved 2.1 g (8.1 mmol) of carboxylic acid **8**. The resulting solution was cooled to 0°C , and diphenylphosphoryl azide (5.2 mL, 24 mmol) and triethylamine (4.5 mL, 32 mmol) were added.¹⁴ After 2 h, *N*-acetylcysteamine (2.6 mL, 24 mmol) was added dropwise. The reaction was allowed to warm slowly to room temperature. After 24 h, 200 mL of water was added, and the resulting mixture was extracted with ether (3×100 mL). The combined organics were dried (MgSO_4) and concentrated in vacuo. The residue was partially purified by chromatography (6 $\times$ 15 cm silica gel, 50% ethyl acetate in hexanes) to give intermediate **9**. The protected thioester **9** (1.8 g, 5 mmol) was dissolved in acetonitrile (96 mL) and water (19 mL), and hydrofluoric acid (14 mL, 280 mmol, 48% aqueous) was added. After 2 h, saturated aqueous NaHCO_3

(approximately 300 mL) was added until the pH reached 7.5. The acetonitrile was removed in vacuo, and the aqueous residue extracted (5×75 mL CH₂Cl₂). The organic layers were combined and dried (MgSO₄). The residue was chromatographed (3×15 cm silica gel, 80% ethyl acetate in hexanes) to give the desired product (540 mg, 29%, two steps). *R_f* 0.16 (70% ethyl acetate in hexanes). ¹H NMR (400 MHz, CDCl₃) δ 0.95 (t, 3H, *J*=7.4 Hz, C(2)CH₂-CH₃), 0.98 (t, 3H, *J*=7.3 Hz, C(5)-H₃), 1.46–1.54 (m, 2H, C(4)-H₂), 1.65–1.84 (m, 2H, C(2)-CH₂), 1.97 (s, 3H, NCO-CH₃), 2.28 (br s, 1H, OH), 2.58–2.64 (m, 1H, C(2)-H), 3.00–3.12 (m, 2H, S-CH₂), 3.39–3.52 (m, 2H, N-CH₂), 3.69–3.75 (m, 1H, C(3)-H), 5.88 (br s, 1H, NH). ¹³C NMR (100 MHz, CDCl₃) δ 10.4, 12.1, 20.5, 23.2, 27.4, 28.6, 39.6, 61.4, 73.8, 170.5, 203.5.

Production and purification of polyketides

The construction of CH999/pJRJ2, which expresses a KS1^o mutant of DEBS has been described previously.¹³ Lawns of *S. coelicolor* CH999/pJRJ2 were grown on R2YE agar plates containing 0.3 mg/mL sodium propionate. After three days, each agar plate was overlaid with 1.5 mL of a 20 mM substrate solution in 9:1 water:DMSO. After an additional 4 days, the agar media (2.0 L) were homogenized and extracted three times with ethyl acetate. The combined extracts were dried (MgSO₄) and filtered through silica gel (1.2×4 cm, eluted with 25 mL of 50% ethyl acetate in hexanes). The extract was purified by silica gel chromatography (1.2×14 cm silica gel, gradient of 15–25% ethyl acetate in hexanes) to afford the product (**4**). *R_f* 0.62 (60% ethyl acetate in hexanes). ¹H NMR (500 MHz, CDCl₃) δ 0.88 (t, 3H, *J*=7.4 Hz, C12CH₂-Me), 0.95 (t, 3H, *J*=7.3 Hz, H15), 1.03–1.09 (m, 12H, C4-Me, C6-Me, C8-Me, C10-Me), 1.30 (d, 3H, *J*=6.8 Hz, C2-Me), 1.35–1.45 (m, 2H, H7b), 1.50–1.92 (m, 7H, H4, H7a, H12, H12-CH₂, H14a, H14b), 2.02–2.10 (m, 1H, H6), 2.63–2.83 (m, 2H, H8, H10), 2.78 (dq, 1H, *J*=10.5 Hz, 6.7 Hz, H2), 3.85 (ddd, 1H, *J*=10.2 Hz, 4.6 Hz, 2.3 Hz, H11), 3.94 (d, 1H, *J*=10.4 Hz, H3), 4.02 (d, 1H, *J*=4.4 Hz, H5), 5.16 (ddd, 1H, *J*=9.7 Hz, 4.0 Hz, 1.6 Hz, H13); ¹³C NMR (100 MHz, CDCl₃) δ 7.1 (C4-Me), 10.8 (C15), 11.6 (C8-Me), 12.5 (C12-Me), 13.3 (C10-Me), 14.7 (C2-Me), 16.4 (C6-Me), 27.3 (C12-CH₂), 25.6 (C14), 35.5 (C6), 37.4 (C7), 37.6 (C4), 39.4 (C8), 43.8 (C2), 44.0 (C10), 46.2 (C12), 69.3 (C11), 76.5 (C13), 77.2 (C5), 79.7 (C3), 178.6 (C1), 213.8 (C9); HRMS (FAB⁺) Calcd for (C₂₂H₄₀O₆)Cs⁺: 533.1879. Found 533.1898.

Post-polyketide processing of 12-desmethyl-12-ethyl-6-dEB

Purified **4** (2 mg dissolved in 1 mL ethanol) was layered onto R2YE plates (80 mL) and allowed to dry. *S. ery-*

threa A34 was then applied so as to give lawns. After 8 days of growth, the media were homogenized and extracted three times with 98.5:1.5 ethyl acetate:triethylamine. Extracts were dried (MgSO₄) and concentrated. The crude extracts were examined by TLC and mass spectrometry. Mass analysis suggests conversion of **4** to the corresponding erythromycin C analogue (**14**): *R_f* 0.41 (streak) (80:20:0.1, CHCl₃:MeOH:NH₄OH). MS (APCI⁺): 733 (M⁺).

Qualitative assay for antibacterial activity. Crude extracts from 80 mL cultures of *S. erythrea* A34 fed with either 2 mg 6-dEB, 2 mg 12-desmethyl-12-ethyl-6-dEB, or no polyketide were each dissolved in 1.4 mL ethanol. Filter discs were soaked in these ethanolic solutions, dried and laid over freshly-plated lawns of *Bacillus cereus*.²⁷ After incubation for 12 h at 37 °C, inhibition of bacterial growth was evident for both erythromycin derivatives but not for the control extract.

Acknowledgements

The authors thank H. Fu for mass spectroscopy of erythromycin derivatives. Research was supported by grants from the National Institutes of Health (CA66736 to C.K. and GM22172 to D.E.C.), an NSF Young Investigator Award (to C.K.), and a David and Lucille Packard Fellowship for Science and Engineering (to C.K.). J.R.J. is a recipient of a National Institute of General Medical Sciences postdoctoral fellowship (1 F32 GM18590-01).

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