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Precursor Directed Biosynthesis of Novel 6-Deoxyerythronolide B Analogs Containing Non-natural Oxygen Substituents and Reactive Functionalities

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Abstract

Feeding of synthetic precursors to a blocked mutant of 6-deoxyerythronolide B synthase (DEBS) [1] led to production of novel 6-deoxyerythronolide B analogs *in vivo* containing additional non-natural oxygen substituents as well as additional reactive groups. © 1999 Elsevier Science Ltd. All rights reserved.

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Polyketides are a structurally diverse class of natural products that have found extensive use as pharmaceutically active compounds due to their often observed antibiotic, antifungal, anticancer and anti-inflammatory properties [2]. In a process which closely parallels fatty acid biosynthesis, macrolides such as 6-deoxyerythronolide B (6-dEB) [3] are biosynthesized by modular polyketide synthases (PKSs) through repetitive condensations of simple monomers such as acetic or propionic acid derivatives [4]. The vast structural variety of this group of compounds is generated by the use of different starter and elongation units and by variation of the degree of processing of the enzyme bound intermediates. Due to abundant resistance of various pathogens, there is substantial need for generation of novel compounds possessing new or improved pharmacological properties.

We have recently reported the production of novel erythromycin [3] analogs using the promising concept of precursor directed biosynthesis with an engineered 6-deoxyerythronolide B synthase (DEBS) expressed in *Streptomyces coelicolor* [5]. Feeding of synthetic precursor analogs to a DEBS KS1^0 mutant incapable of endogenous polyketide synthesis resulted in formation of the corresponding macrolide aglycone analogs. Depending on their functionality and stereochemistry, precursors could be directed to the KS domain of either module 2 or module 3 of DEBS. Until now, however, only analogs carrying different alkyl and aryl group have been produced by this method. In the present work, we report for the first time the production of novel 6-deoxyerythronolide B analogs containing olefinic and additional heteroatom substituents that could provide reactive handles for further derivatization and modification.

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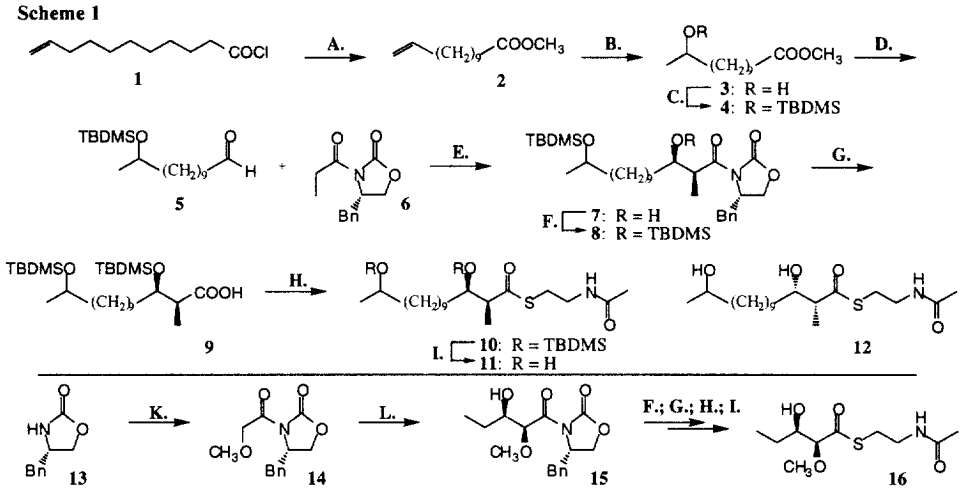
Scheme 1



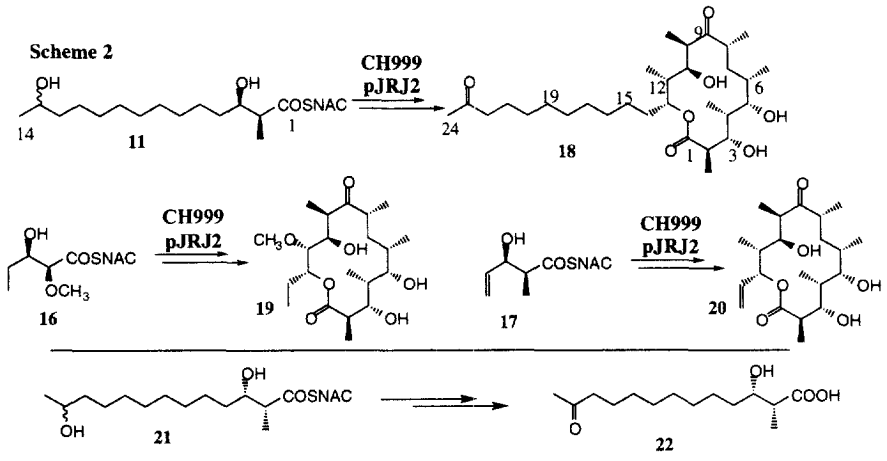
Reagents and conditions: **A.** i) CH_2N_2 ii) cat. Ag(I) benzoate, CH_3OH, 93%; **B.** i) $Hg(OAc)_2$, THF ii) $NaBH_4$, 3N NaOH, 73%; **C.** TBDMSCl, NEt_3, CH_2Cl_2, 0°C→rt , 95%; **D.** DIBAH, CH_2Cl_2, -78 °C, 80%; **E.** i) Bu_2BOTf, NEt_3, CH_2Cl_2, 0 °C ii) then -78 °C, **5**, iii) H_2O_2, 77%; **F.** TBDMSTf, DIEA, CH_2Cl_2, 0 °C; quant; **G.** i) LiOH, H_2O_2, THF/water, ii) Na_2SO_3, 97%; **H.** i) $(PhO)_2PON_3$, NEt_3, DMF, 0 °C, ii) NEt_3, N-acetylcysteamine, 82%; **I.** 30% HF, CH_3CN, 0°C→rt, 88%. **K.** i) BuLi, THF, ii) CH_3OCH_2COCl, 99%; **L.** i) Bu_2BOTf, NEt_3, CH_2Cl_2, 0 °C ii) then -78 °C, propionaldehyde, iii) H_2O_2, 75%.

Precursor synthesis (Scheme 1) started with commercially available undecylenic acid chloride (**1**), which was elongated using Arndt-Eistert synthesis to give methyl dodecylenic acid (**2**). The distal hydroxy group was introduced by treatment with mercuric acetate followed by $NaBH_4$ to give hydroxy compound **3**, which was subsequently protected as a TBDMS ether (compound **4**) [6]. After reduction of the carbonyl group to the aldehyde stage using DIBAH, compound **5** was elongated by means of the Evans aldol methodology [7] in the presence of (*4S*)-4-benzyl-2-propionyl oxazolidinone (**6**), dibutylborontriflate and triethylamine to afford *syn*-aldol product **7** with a (*2S, 3R,13R/S*) configuration as a 1:1 mixture of diastereomers in 70% yield. Subsequent protection of the β-hydroxy group with TBDMS triflate gave oxazolidinone **11**. The chiral auxiliary was then removed by treatment with LiOOH and $NaSO_3$ and the corresponding *bis*-protected acid **12** was coupled with N-acetylcysteamine (NAC) after treatment with diphenylphosphoryl azide and triethylamine to afford thioester **10** in 82% yield. The protecting groups were removed with HF in acetonitrile to afford the desired compound **11** as a 1:1 mixture of diastereomers. Compound **12** with a (*2R, 3S, 13R/S*) configuration was obtained in an analogous way using (*4R*)-4-benzyl-2-oxazolidinone as a chiral auxiliary in the Evans aldol reaction. Synthesis of methoxy diketide **16** was based on the same strategy. (*4S*)-4-Benzyl-2-oxazolidinone (**13**) was coupled with 2-methoxyacetyl chloride after deprotonation with butyllithium to give compound **14** in 99% yield. Evans aldol reaction with propionaldehyde afforded the corresponding *syn*-aldol compound **15** in 75% yield, which was then further elaborated into β-hydroxythioester **16** as described above [8]. The vinyl diketide**17** was prepared in a completely analogous manner starting from acrolein and oxazolidinone **6**.

Synthetic precursors thus obtained were fed to *S. coelicolor* CH999/pJRJ2 (which expresses the $KS1^0$ mutant of DEBS) as described previously [5]. Products were purified from the fermentation media by extraction with ethyl acetate and subsequent silica gel chromatography. Application of precursor **11** to growing cultures of *S. coelicolor* CH999/pJRJ2 resulted in production of aglycone **18** (ca. 4 mg/L), whereas

administration of methoxy derivative **16** led to formation of **19** (ca. 2 mg/L) [9]. Incorporation of **17** was much more efficient, giving ca. 45 mg/L of 14,15-dehydro-6-dEB (**20**). By contrast, no cyclic products were detected when substrate **12** with a (*2R, 3S*) configuration was added to the cultures. In the latter case, non-PKS related oxidation of the distal hydroxyl group appears to occur during the fermentation process (Scheme 2). The timing and efficiency of substrate conversion to the ketone form was tested in a feeding experiment using compound **21** as a substrate. After 7 days of incubation, the main fermentation product was recovered in 85% yield and shown to be ketoacid **22**, arising from hydrolysis of the thioester and oxidation of the distal hydroxy group [10]. This therefore indicates that oxidation of the C13 hydroxyl group of **11** takes place before PKS conversion into **18**. Isolation of free acid **22** also suggests that successful priming of the PKS by exogenously added precursors is crucially dependent on their stability, since other degradative processes can compete with the rather slow polyketide synthesis.

Scheme 2



Compound **20** is the first example a 6-deoxyerythronolide B analog containing non-natural, functionalized side chains which can serve as reactive handles for further modifications. For example, coupling of **17** with a second biologically active compound or to an affinity column as a bioactive ligand may be of particular interest. Furthermore, introduction of methoxy groups as in **18** may be particularly beneficial, since hydroxylation and subsequent methylation at normally non-oxygen carrying positions is a common post-PKS modification of polyketides of related producer strains. All these examples provide further support for the combination of chemical precursor synthesis and subsequent substrate elaboration by engineered organisms as a general strategy for the generation of novel natural products.

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References and Notes:

[1] Abbreviations used: 6-dEB: 6-deoxyerythronolide B; DEBS: 6-dEB Synthase; DIBAH: diisobutylaluminium hydride; DIEA: diisopropylethylamine; KS: keto synthase; PKS: polyketide synthase; NAC: N-acetylcysteamine; TBDMS: *t*-butyldimethylsilyl.

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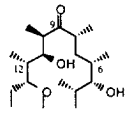
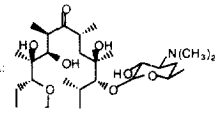
[3] 6-dEB:  Erythromycin A: 

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[8] Spectroscopic data of intermediates were in accordance with the proposed structure. **11**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.84 (t, br, 1H, NH), 3.92 (m, 1H, H3), 3.79 (m, 1H, H13), 3.45 (m, 2H, CH_2-N), 3.03 (m, 2H, CH_2-S), 2.72 (qd, 1H, *J*=7.1, 3.5 Hz), 2.41 (s, br, 1H, OH), 1.97 (s, 3H, $COCH_3$), 1.50-1.24 (m, 18H, 9 CH_2), 1.22 (d, 3H, *J*=7.1 Hz, 2-Me), 1.19 (d, 3H, *J*=6.2 Hz, H14). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 204.2, 170.5, 72.1, 68.1, 53.3, 39.3, 34.1, 29.5, 29.4, 28.5, 25.9, 25.7, 23.3, 23.2, 11.1. **16**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.03 (s, br, NH), 3.77-3.75 (m, 1H, H3), 3.66 (d, 1H, *J*=3.6 Hz, H2), 3.54 (s, 3H, OCH_3), 3.48-3.45 (m, 1H, CHN), 3.43-3.38 (m, 1H, CHN), 3.15-3.09 (m, 1H, CHS), 3.02-2.95 (m, 1H, CHS), 1.96 (s, 3H, CH_3CO), 1.61-1.55 (m, 2H, H4), 0.99 (t, 3H, *J*=7.6 Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 202.9, 170.6, 89.2, 74.2, 60.3, 39.0, 27.9, 26.2, 23.1, 10.0. **17**: ^{1}H-NMR (250 MHz, $CDCl_3$): δ 5.09 (br, 1H, NH), 5.83 (m, 1H, H-4'), 5.32 (dd, 1H, *J*=17.0, 1.6 Hz, H5'a), 5.21 (dd, 1H, *J*=10.1, 1.6 Hz, H5'b), 4.45 (m, 1H, H3'), 3.43 (m, 2H, N-CH_2), 3.04 (m, 2H, S-CH_2), 2.81 (m, 1H, H2'), 1.97 (s, 3H, N-$COCH_3$), 1.21 (d, 3H, *J*=7.2 Hz, 2'-CH_3).

[9] Product structures were confirmed by ^{1}H-NMR, ^{13}C-NMR, ^{1}H-COSY and MS. **17**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.23 (dd, 1H, *J*=6.1, 2.4 Hz, H13), 4.01 (d, 1H, *J*=5.0 Hz, H5), 3.91 (d, 1H, *J*=10.3 Hz, H3), 3.67 (d, br, 1H, *J*=10.2 Hz, H11), 2.81-2.71 (m, 2H, H2, H10), 2.66-2.59 (m, 1H, H8), 2.42 (t, 2H, *J*=7.3 Hz, H22), 2.14 (s, 3H, H24), 2.04-1.98 (m, 1H, H6), 1.86 (q, br, 1H, *J*=6.4 Hz, H4), 1.82-1.75 (m, 1H, H14), 1.75-1.61 (m, 2H, H7, H12), 1.61-1.53 (m, 2H, H21), 1.51-1.42 (m, 1H, H14), 1.39-1.20 (m, 13H, H15-20, H7), 1.29 (d, 3H, *J*=6.8 Hz, 2-Me), 1.07 (d, 3H, *J*=7.2 Hz), 1.05 (d, 3H, *J*=6.6 Hz), 1.04 (d, 3H, *J*=7.0 Hz) 1.02 (d, 3H, *J*=6.6 Hz) (4-Me, 6-Me, 8-Me, 10-Me), 0.89 (d, 3H, *J*=7.0 Hz, 12-Me). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 213.5 (C9), 209.5 (C23), 178.3 (C1), 79.6 (C3), 76.5 (C5), 74.8 (C13), 70.9 (C11), 43.9, 43.8, 43.5 (C2/C10/C22), 40.8 (C12), 39.2 (C8), 37.7 (C4), 37.4 (C7), 35.5 (C6), 32.2, 29.4, 29.3, 29.2, 29.1, 26.2, 23.8 (C14-21/C24), 16.6 (6-Me), 14.7 (2-Me), 13.2 (10-Me), 9.3 (8-Me), 6.9 (4-Me), 6.2 (12-Me). HRMS (FAB$^+$) Calcd for $(C_{30}H_{54}O_7)Cs^+$: 659.2924. Found: 659.2954. **18**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.02 (dd, 1H, *J*=5.8, 4.1 Hz, H13), 4.00 (dd, 1H, *J*=3.3, 2.6 Hz, H5), 3.86 (d, 1H, 10.2 Hz, H3), 3.72-3.69 (m, 1H, H11), 3.51 (s, 3H, OCH_3), 3.11 (d, 1H, *J*=9.7 Hz, H12), 2.89-2.83 (m, 2H, H2, H10), 2.64-2.58 (m, 1H, H8), 2.07-1.96 (m, 2H, H14, H6), 1.95-1.92 (m, 1H, H4), 1.82-1.69 (m, 2H, H7, H14), 1.37-1.29 (m, 1H, H7), 1.33 (d, 3H, *J*=6.6, 2-Me), 1.16 (d, 3H, *J*=6.6 Hz, 10-Me), 1.09-1.05 (3d, 9H, 4-Me, 6-Me, 8-Me), 1.01 (t, 3H, *J*=7.2 Hz, H15). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 214.1 (C9), 178.2 (C1), 83.3 (C12), 80.0 (C3), 76.7 (C5), 76.4 (C13), 69.4 (C11), 61.3 (OMe), 45.3 (C10), 43.6 (C2), 38.6 (C8), 36.8 (C4, C7), 35.4 (C6), 24.8 (C14), 16.5 (6-Me), 14.4 (2-Me), 14.3 (10-Me), 10.7 (15-Me), 9.0 (8-Me), 6.9 (4-Me). HRMS (FAB$^+$) Calcd for $(C_{21}H_{38}O_7)Na^+$: 425.2515. Found: 425.2505. **20**: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.83 (m, 1H, H14), 5.80 (m, 1H, H13), 5.25-5.33 (ddm, 2H, H15), 3.97 (m, 1H, H5), 3.90 (d, 1H, J = 10.6 Hz, H3), 3.69 (dm, 1H, H11), 2.73-2.84 (m, 2H, H2, H10), 2.59 (m, 1H, H8), 1.59-1.97 (m, 4H, H4, H6, H7a, H12), 1.29 (d, 3H, J = 6.8 Hz, 2-CH_3), 1.19-1.35 (m, 1H, H7b), 0.99-1.08 (m, 9H, 4-CH_3, 6-CH_3, 8-CH_3), 0.95 (d, 3H, J = 6.8 Hz, 10-CH_3), 0.88 (d, 3H, J = 6.9 Hz, 12-CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 213.6 (C9), 177.5 (C1), 134.9(C14), 116.6 (C15), 79.4 (C3), 76.6 (C5), 74.2 (C13), 70.9 (C11), 43.8 (C10), 43.2(C2), 41.5 (C12), 39.0 (C8), 37.7 (C4), 37.5 (C7), 35.6 (C6), 16.6 (C6-Me), 14.6 (C2-Me), 13.4 (C10-Me), 9.2 (C12-Me), 6.9 (C4-Me), 6.3 (C8-Me). HRMS (FAB$^+$) Calcd for $(C_{21}H_{36}O_6)H^+$: 385.2590. Found: 385.2580.

[10] **22**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.10 (s, br, 1H, OH), 3.94 (m, 1H, H3), 2.58 (qd, 1H, *J*=7.3, 3.4 Hz, H1), 2.41 (t, 2H, *J*=7.3 Hz, H11), 2.13 (s, 3H, H13), 1.55 (m, 2H), 1.51-1.23 (m, 12H), 1.19 (d, 3H, J=7.3 Hz, 2-Me). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 209.9 (C12), 180.8 (C1), 71.7 (C3), 44.0, 43.8 (2 CH_2), 33.5 (C2), 29.6 (C13), 29.3, 29.3, 29.04, 25.9, 23.8 (5 CH_2), 10.4 (2-Me). MS (CI$^+$): Calcd for $(C_{14}H_{26}O_4)H^+$: 259.0. Found: 259.0.