Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 3 October 2007, Revised 22 January 2008, Accepted 2 February 2008 Published online 27 March 2008 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1507

Efficient synthesis of ^{13}C-labelled erythromycin biosynthetic intermediate. 1: *S*-2-acetylaminoethyl (2*R*,3*R*,4*R*,5*R*)-3,5-diacetoxy-2,4-dimethyl-4-([^{13}C]methoxy)-heptanethioate

Katsumi Iida,[a]* Masahiro Kajiwara,[a] Tomoko Inoue-Tanihata,[b] Mineo Fukui✠,[b] Tadashi Nakata†,[b] and Takeshi Oishi‡[b]

An efficient ^{13}C-labelling synthesis of the putative erythromycin biosynthetic intermediate, *S*-2-acetylaminoethyl (2*R*,3*R*,4*R*,5*R*)-3,5-diacetoxy-2,4-dimethyl-4-([^{13}C]methoxy)heptanethioate, which would be useful for the investigation of the chain elongation mechanism in erythromycin biosynthesis, was achieved by utilizing iodo[^{13}C]methane and (2*S*,3*R*,4*R*,5*R*)-4-hydroxy-3,5-*O*-isopropylidene-2,4-dimethylheptanol, obtained in our previous studies on erythromycin A synthesis.

Keywords: ^{13}C-labelling synthesis; erythromycin biosynthetic intermediate; iodo[^{13}C]methane

Introduction

Erythromycin A, a 14-membered macrolide antibiotic produced by *Saccharopolyspora erythraea*, is widely used in clinical medicine. Corcoran *et al.* investigated the origin of the carbon atoms of 6-deoxyerythronolide B, the first biosynthetic macrolide intermediate of erythromycin A, by means of feeding experiments with ^{14}C-labelled compounds in *S. erythraea*.[1,2] Subsequently, Cane *et al.* evaluated the chain elongation mechanism leading to 6-deoxyerythronolide B by means of feeding experiments with ^{2}H-, ^{13}C-, and/or ^{18}O-labelled compounds, especially ^{2}H- and/or ^{13}C-labelled *S*-2-acetylaminoethyl (2*S*,3*R*)-3-hydroxy-2-methylpentanethioate.[3–7] Groups led by Leadlay and Katz employed a genetic approach to examine the biosynthetic pathways to erythromycin.[8–11] Based on the domain organization of the 6-deoxyerythronolide B synthase proteins encoded in the *ery A* region of the *S. erythraea* genome, they predicted the biosynthetic pathways to 6-deoxyerythronolide B.

We were interested in the chain elongation mechanism in erythromycin biosynthesis, and aimed at approaching this issue by using ^{13}C-labelled erythromycin biosynthetic intermediates. The intact incorporation of a ^{13}C-labelled triketide into tylosine, a 16-membered macrolide antibiotic, was reported by Hutchinson and Coworkers,[12] but the incorporation of other labelled putative polyketide intermediates into erythromycin has not been described. Here, we describe the efficient ^{13}C-labelling synthesis of a putative erythromycin biosynthetic intermediate.

Results and discussion

Methods previously reported for the synthesis of erythromycin[13] are unsuitable for efficient ^{13}C-labelling, and we required a more efficient synthetic strategy. Here, we focused on the introduction of a ^{13}C-label into the optically active synthetic intermediates obtained in our previous work.[14,15]

Initially, we looked at the ^{13}C-labelled putative erythromycin biosynthetic intermediate (**3**), which is the equivalent of the C-9–C-21 segment of erythromycin A (**1**) shown inside the dotted square in Scheme 1, though the incorporation of **3** in *S. erythraea* might lead to 12-([^{13}C]methoxy)erythromycin A (**2**). As shown in Scheme 1, **3** should be obtainable from the primary alcohol **5** via oxidation of the hydroxyl group, thioesterification with 2-acetylaminoethanethiol, and deprotection of the

✠*Deceased.*

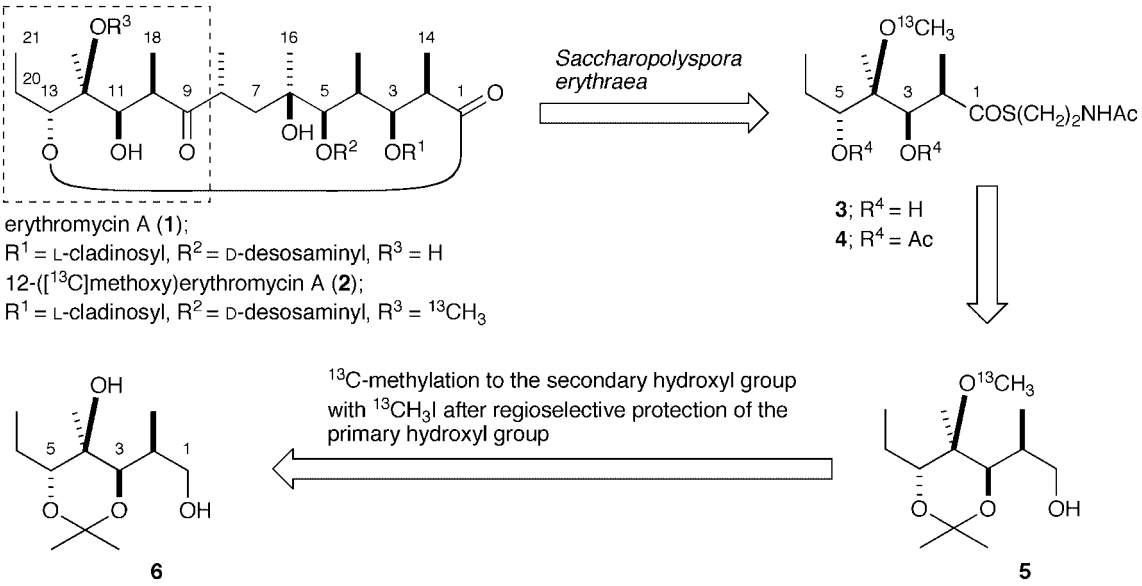
†*Present address: Department of Chemistry, Faculty of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601, Japan.*

‡*Retired.*

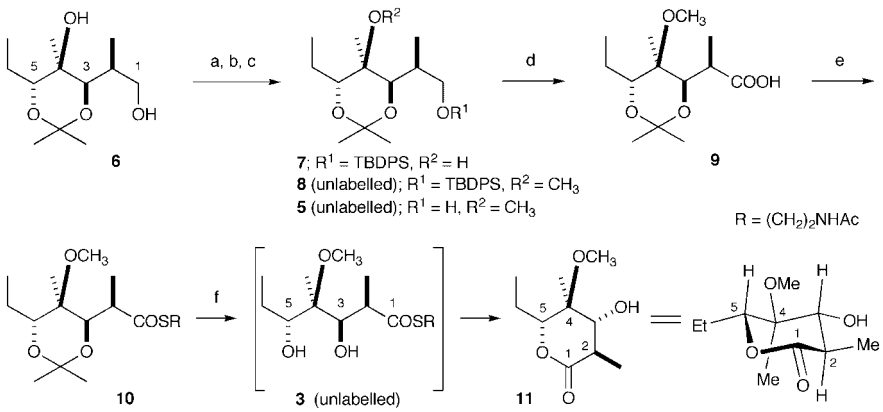
[a]*Department of Medicinal Chemistry, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose-shi, Tokyo 204-8588, Japan*

[b]*RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan*

**Correspondence to: Katsumi Iida, Department of Medicinal Chemistry, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose-shi, Tokyo 204-8588, Japan. E-mail: iida@my-pharm.ac.jp*



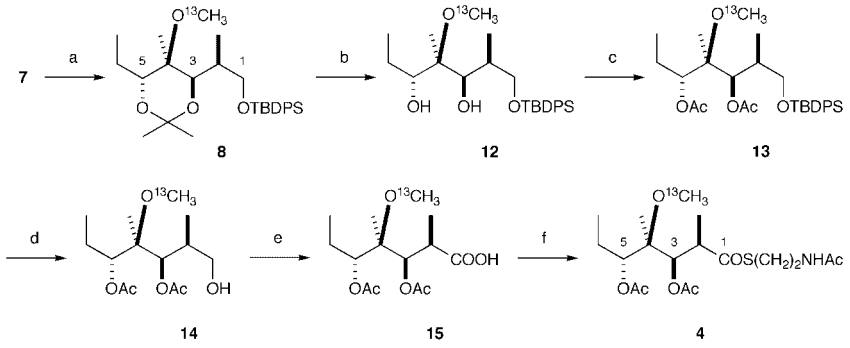
Scheme 1. Strategy for syntheses of ^{13}C-labelled putative erythromycin intermediates **3** or **4** from $^{13}CH_3I$ and **6**.



Reaction conditions for synthesis of **3** (unlabelled) from CH_3I and **6**: (a) TBDPSCl, imid., DMF, rt, 1 h, quant.; (b) CH_3I, KH, THF, 0 °C to rt, each 30 min, 82%; (c) *n*-Bu_4NF, THF, rt, 96 h, 99%; (d) RuO_4, $NaIO_4$, CCl_4, CH_3CN, H_2O, rt, 2 h, 68%; (e) $HS(CH_2)_2NHAc$, $(PhO)_2P(O)N_3$, Et_3N, DMF, rt, 16 h, 95%; (f) 48% HF/CH_3CN (5:95), rt, 1h, 67%.

Scheme 2

acetonide. The primary alcohol **5** should be obtainable from diol **6** synthesized in our previous work[14] via ^{13}C-methylation of the secondary hydroxyl group with iodo[^{13}C]methane after regioselective protection of the primary hydroxyl group with *tert*-butyldiphenylsilyl chloride (TBDPSCl), followed by desilylation. As shown in Scheme 2, we attempted to synthesize **3** (unlabelled) using **6** and iodomethane. Regioselective silylation of the primary hydroxyl group of **6** with TBDPSCl in the presence of imidazole under an argon atmosphere at room temperature for 1 h quantitatively afforded the secondary alcohol **7**. Methylation of the secondary hydroxyl group of **7** with KH and iodomethane under an argon atmosphere at 0°C for 30 min and then at room temperature for 30 min gave ether **8** (unlabelled) in an 82% yield. The desilylation of **8** (unlabelled) with *n*-tetrabutylammonium fluoride (*n*-Bu_4NF) in THF at room temperature for 96 h afforded the primary alcohol **5** (unlabelled) in a 99% yield. Oxidation of **5** (unlabelled) with ruthenium (IV) oxide in the presence of sodium metaperiodate ($NaIO_4$) in a two-phase solution of CCl_4, CH_3CN, and 0.1 M Na phosphate buffer at room temperature for 2 h gave acid **9** in a 68% yield. The thioesterification of **9** with 2-acetylaminoethanethiol in DMF in the presence of diphenylphosphoryl azide and Et_3N under an argon atmosphere at room temperature for 16 h gave thioester **10** in a 95% yield.[6,16] However, deprotection of the acetonide of **10** did not afford diol **3** (unlabelled), but δ-lactone **11** was obtained in a 67% yield. The chair form of **11** shown in Scheme 2 might be the lowest-energy chair conformation, as C-2–Me, C-3–OH, and C-5–Et of **11** can take equatorial positions. Therefore, **11** might be generated from **10** through the attack of the 5-hydroxyl oxygen on the carbonyl carbon with the elimination of 2-acetylaminoethanethioxyl after desilylation. Consequently, the synthesis of **3** appeared to be difficult.



Reaction conditions for synthesis of **4** from $^{13}CH_3I$ and **7**: (a) $^{13}CH_3I$, KH, THF, 0 °C to rt, 30 min, 82%; (b) PPTS, CH_3OH, rt, 27 h, **8** recovered was subjected to the same reaction, overall 88%; (c) Ac_2O, DMAP, pyridine, rt, 23 h, 94%; (d) *n*-Bu_4NF, AcOH, THF, rt, 89 h, 78%; (e) RuO_4, $NaIO_4$, CCl_4, CH_3CN, H_2O, rt, 1 h, 81%; (f) $HS(CH_2)_2NHAc$, $(PhO)_2P(O)N_3$, Et_3N, DMF, rt, 14 h, 75%.

Scheme 3

As shown in Scheme 1, we next examined the synthesis of the ^{13}C-labelled putative erythromycin biosynthetic intermediate **4**, in which the 3,5-dihydroxyl groups of **3** are protected with acetyl groups to exclude formation of δ-lactone **11**. As shown in Scheme 3, ether **8** was obtained via ^{13}C-methylation of **7** with iodo[^{13}C]methane as before (Scheme 2). Treatment of **8** with pyridinium *p*-toluenesulfonate (PPTS) in MeOH at room temperature for 27 h gave diol **12**. This reaction was repeated twice more with recovered **8**, and **12** was obtained in an overall yield of 88%. Diacetylation of the hydroxyl groups of **12** in pyridine and acetic anhydride in the presence of 4-dimethylaminopyridine (DMAP) at room temperature for 23 h afforded ester **13** in a 94% yield. The desilylation of **13** with *n*-Bu_4NF in the presence of acetic acid, which was added to decrease the loss of the acetyl group from C-3 or C-5, in THF at room temperature for 89 h gave the primary alcohol **14** in a 78% yield. Oxidation of **14** with ruthenium (IV) oxide in the presence of $NaIO_4$ in a two-phase solution of CCl_4, CH_3CN, and 0.1 M Na phosphate buffer at room temperature for 1 h provided acid **15** in an 81% yield. The thioesterification of **15** with 2-acetylaminoethanethiol in DMF in the presence of diphenylphosphoryl azide and Et_3N under an argon atmosphere at room temperature for 14 h gave the desired thioester **4** in a 75% yield.

Experimental

Materials and instruments

Iodo[^{13}C]methane (99 atom% ^{13}C) was purchased from Cambridge Isotope Laboratories. (2*S*,3*R*,4*R*,5*R*)-4-Hydroxy-3,5-*O*-isopropylidene-2,4-dimethylheptanol (**6**) (>95% e.e.) synthesized in our previous work[14] was used. All other chemicals were of analytical grade and commercially available. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL GX-400, GX-500, or GSX-400 (^1H: 400 or 500 MHz and ^{13}C: 100 MHz) spectrometer. IR spectra were recorded on a Jasco VALORA-III FT-IR spectrometer. MS spectra were obtained on a JEOL JMS-DX302 spectrometer.

(2*S*,3*R*,4*R*,5*R*)-1-*tert*-Butyldiphenylsilyloxy-4-hydroxy-3,5-*O*-isopropylidene-2,4-dimethylheptanol (7)

To a solution of **6** (4.97 g, 21.0 mmol) and imidazole (3.57 g, 52.4 mmol) in dry DMF (20 ml) was added dropwise TBDPSCl (6.0 ml, 23.7 mmol) at 0°C under an argon atmosphere. The mixture was stirred for 1 h at room temperature. The reaction was quenched with water (5 ml) at 0°C, and the whole was extracted with Et_2O. The combined extract was washed with water and brine, dried over anhydrous $MgSO_4$, and evaporated to give **7** (10.38 g, quant.); ^1H NMR ($CDCl_3$) δ: 0.86 (d, 3H, *J* = 6.7 Hz, 2-C<u>H</u>$_3$), 1.00 (t, 3H, *J* = 7.0 Hz, 7-<u>H</u>$_3$), 1.04 (s, 9H, Si-C(C<u>H</u>$_3$)$_3$), 1.20 (s, 3H, 4-C<u>H</u>$_3$), 1.29 (s, 3H, isopropylidene-C<u>H</u>$_3$), 1.36 (s, 3H, isopropylidene-C<u>H</u>$_3$), 1.38 (m, 2H, 6-<u>H</u>$_2$), 2.16 (m, 1H, 2-<u>H</u>), 3.22 (s, 1H, 4-O<u>H</u>), 3.45 (dd, 1H, *J* = 2.8, 6.7 Hz, 5-<u>H</u>), 3.54 (d, 1H, *J* = 6.3 Hz, 3-<u>H</u>), 3.59 (dd, 1H, *J* = 4.9, 9.8 Hz, 1-<u>H</u>), 3.67 (dd, 1H, *J* = 7.9, 9.8 Hz, 1-<u>H</u>), 7.65–7.68 (m, 10H, phenyl-<u>H</u>$_{10}$).

(2*S*,3*R*,4*R*,5*R*)-1-*tert*-Butyldiphenylsilyloxy-3,5-*O*-isopropylidene-2,4-dimethyl-4-([^{13}C]methoxy)heptanol (8)

To a suspension of KH (50% in oil, 574 mg, 7.2 mmol; previously washed with dry hexane) in dry THF (10 ml) was added dropwise a solution of **7** (3.04 g, 6.5 mmol) in dry THF (6 ml) at 0°C under an argon atmosphere. The suspension was stirred for 30 min, then iodo[^{13}C]methane (0.45 ml, 7.2 mmol) at 0°C was added dropwise under an argon atmosphere. The whole was stirred for 30 min at 0°C, and then for 30 min at room temperature. The reaction was quenched with sat. NH_4Cl aq., and the mixture was extracted with Et_2O. The combined extract was washed with brine, dried over anhydrous $MgSO_4$, and evaporated. Chromatography of the crude product on silica gel, eluting with Et_2O:hexane (1:50–5:95) and then AcOEt/hexane (5:95–1:9–1:4), gave **8** (1.90 g, 82%); ^1H NMR ($CDCl_3$) δ: 0.93 (d, 3H, *J* = 7.3 Hz, 2-C<u>H</u>$_3$), 0.98 (t, 3H, *J* = 7.3 Hz, 7-<u>H</u>$_3$), 1.05 (s, 9H, SiC(C<u>H</u>$_3$)$_3$), 1.08 (s, 3H, 4-C<u>H</u>$_3$), 1.28 (s, 3H, isopropylidene-C<u>H</u>$_3$), 1.35 (s, 3H, isopropylidene-C<u>H</u>$_3$), 1.38 (m, 1H, 6-<u>H</u>), 1.54 (m, 1H, 6-<u>H</u>), 2.01 (m, 1H, 2-<u>H</u>), 3.26 (d, 3H, *J* = 141 Hz, 4-O^{13}C<u>H</u>$_3$), 3.49 (dd, 1H, *J* = 4.9, 9.8 Hz, 1-<u>H</u>), 3.56 (dd, 1H, *J* = 7.9, 9.8 Hz, 1-<u>H</u>), 3.57 (dd, 1H, *J* = 2.4, 11.0 Hz, 5-<u>H</u>), 3.76 (d, 1H, *J* = 3.7 Hz, 3-<u>H</u>), 7.65–7.68 (m, 10H, phenyl-<u>H</u>$_{10}$), data of **8** (unlabelled) synthesized by using iodomethane; ^1H NMR ($CDCl_3$) δ: 0.93 (d, 3H, *J* = 7.3 Hz, 2-C<u>H</u>$_3$), 0.98 (t, 3H, *J* = 7.3 Hz, 7-<u>H</u>$_3$), 1.05 (s, 9H, SiC(C<u>H</u>$_3$)$_3$), 1.07 (s, 3H, 4-C<u>H</u>$_3$), 1.27 (s, 3H, isopropylidene-C<u>H</u>$_3$), 1.36 (s, 3H, isopropylidene-C<u>H</u>$_3$), 1.38 (m, 1H, 6-<u>H</u>), 1.54 (m, 1H, 6-<u>H</u>), 2.01 (m, 1H, 2-<u>H</u>), 3.26 (s, 3H, 4-OC<u>H</u>$_3$), 3.49 (dd, 1H, *J* = 4.9, 9.8 Hz, 1-<u>H</u>), 3.56 (dd, 1H, *J* = 7.9, 9.8 Hz, 1-<u>H</u>), 3.57 (dd, 1H, *J* = 2.2, 10.1 Hz, 5-<u>H</u>), 3.60 (d, 1H, *J* = 3.7 Hz, 3-<u>H</u>), 7.65–7.68 (m, 10H, phenyl-<u>H</u>$_{10}$).

(2*S*,3*R*,4*R*,5*R*)-3,5-*O*-Isopropylidene-2,4-dimethyl-4-methoxyheptanol (5) (unlabelled)

To a solution of **8** (unlabelled) (891 mg, 2.5 mmol) in THF (3 ml) was added *n*-$Bu_4NF \cdot 3H_2O$ (945 mg, 3.6 mmol) at room temperature, and the mixture was stirred for 96 h. The reaction was quenched with sat. NH_4Cl aq., and the mixture was extracted with Et_2O. The combined extract was washed with brine, dried over anhydrous $MgSO_4$, and evaporated. Chromatography of the crude product on silica gel with AcOEt/hexane (1:4–1:2–1:1) gave **5** (unlabelled) (608 mg, 99%); ^{1}H NMR ($CDCl_3$) δ: 0.99 (t, 3H, *J* = 7.3 Hz, 7-H_3), 1.03 (d, 3H, *J* = 6.8 Hz, 2-CH_3), 1.15 (s, 3H, 4-CH_3), 1.29 (s, 3H, isopropylidene-CH_3), 1.37 (s, 3H, isopropylidene-CH_3), 1.38 (m, 1H, 6-H), 1.54 (m, 1H, 6-H), 2.03 (m, 1H, 2-H), 3.08 (m, 1H, 1-OH), 3.38 (d, 1H, *J* = 4.9 Hz, 3-H), 3.39 (s, 3H, 4-OCH_3), 3.58 (t, 2H, *J* = 4.9 Hz, 1-H_2), 3.72 (dd, 1H, *J* = 2.0, 10.5 Hz, 5-H).

(2*R*,3*R*,4*R*,5*R*)-3,5-*O*-Isopropylidene-2,4-dimethyl-4-methoxyheptanoic acid (9)

To a two-phase solution of **5** (unlabelled) (598 mg, 2.4 mmol) in CCl_4 (5 ml), CH_3CN (5 ml) and 0.1 M Na phosphate buffer (7.5 ml) were added $NaIO_4$ (1.56 g, 7.3 mmol) and ruthenium (IV) oxide (55%, 19 mg, 63.4 μmol) at room temperature, and the whole was stirred vigorously for 2 h. The reaction mixture was diluted with sat. NaCl aq. and extracted with Et_2O. The combined extract was dried over anhydrous $MgSO_4$ and evaporated. Chromatography of the crude product on silica gel with AcOEt/hexane (1:3–1:1–2:1–4:1) gave **9** (430 mg, 68%); ^{1}H NMR ($CDCl_3$) δ: 0.98 (t, 3H, *J* = 6.8 Hz, 7-H_3), 1.16 (s, 3H, 4-CH_3), 1.29 (d, 3H, *J* = 7.3 Hz, 2-CH_3), 1.33 (s, 3H, isopropylidene-CH_3), 1.39 (s, 3H, isopropylidene-CH_3), 1.39 (m, 1H, 6-H), 1.53 (m, 1H, 6-H), 2.80 (quintet, 1H, *J* = 7.3 Hz, 2-H), 3.31 (s, 3H, 4-OCH_3), 3.60 (dd, 1H, *J* = 2.9, 8.8 Hz, 5-H), 3.76 (d, 1H, *J* = 7.3 Hz, 3-H).

***S*-2-Acetylaminoethyl (2*R*,3*R*,4*R*,5*R*)-3,5-*O*-Isopropylidene-2,4-dimethyl-4-methoxyheptanethioate (10)**

To a solution of **9** (171 mg, 0.65 mmol) and 2-acetylaminoethanethiol (386 mg, 3.2 mmol) in DMF (0.4 ml) was added diphenylphosphoryl azide (0.28 ml, 1.3 mmol) at room temperature under an argon atmosphere. To this solution was added dropwise Et_3N (0.36 ml, 2.6 mmol) at 0°C, and the whole was stirred for 16 h at room temperature. The reaction was quenched with sat. NH_4Cl aq. and extracted with Et_2O. The combined extract was washed with 10% NaOH aq. and brine, dried over anhydrous $MgSO_4$, and evaporated. Chromatography of the crude product on silica gel with AcOEt/hexane (2:1–4:1)–$CHCl_3$/MeOH (20:1) gave **10** (224 mg, 95%); ^{1}H NMR ($CDCl_3$) δ: 0.98 (t, 3H, *J* = 7.1 Hz, 7-H_3), 1.06 (s, 3H, 4-CH_3), 1.26 (d, 3H, *J* = 7.1 Hz, 2-CH_3), 1.31 (s, 3H, isopropylidene-CH_3), 1.36 (s, 3H, isopropylidene-CH_3), 1.38 (m, 1H, 6-H), 1.49 (m, 1H, 6-H), 1.95 (s, 3H, $NHCOCH_3$), 3.02 (dt, 2H, *J* = 2.9, 6.6 Hz, SCH_2), 3.06 (dq, 1H, *J* = 1.2, 8.1 Hz, 2-H), 3.32 (s, 3H, 4-OCH_3), 3.45 (m, 2H, SCH_2CH_2), 3.65 (dd, 1H, *J* = 2.0, 8.8 Hz, 5-H), 3.80 (d, 1H, *J* = 8.3 Hz, 3-H), 5.93 (brs, 1H, NH).

(2*R*,3*R*,4*S*,5*R*)-2,4-Dimethyl-3-hydroxy-4-methoxyheptan-5-olide (11)

A solution of **10** (50 mg, 0.14 mmol) in 48% HF/CH_3CN (5:95, 1 ml) was stirred for 1 h at room temperature, then added dropwise to a suspension of $NaHCO_3$ in CH_2Cl_2, and the whole was stirred for 30 min at room temperature. To this suspension was added anhydrous $MgSO_4$, and the whole was stirred for 30 min at room temperature. The suspension was filtered and the filtrate was evaporated. Preparative TLC (0.5 mm × 20 cm^2) of the crude product on silica gel and development twice with $CHCl_3$/MeOH (20:1) gave **11** (19 mg, 67%); ^{1}H NMR ($CDCl_3$) δ: 1.09 (t, 3H, *J* = 7.3 Hz, 7-H_3), 1.18 (s, 3H, 4-CH_3), 1.42 (d, 3H, *J* = 7.0 Hz, 2-CH_3), 1.61 (m, 1H, 6-H), 1.75 (m, 1H, 6-H), 2.26 (m, 1H, 3-OH), 2.47 (dq, 1H, *J* = 3.1, 7.0 Hz, 2-H), 3.37 (s, 3H, 4-OCH_3), 3.72 (d, 1H, *J* = 10.1 Hz, 3-H), 3.97 (dd, 1H, *J* = 2.1, 10.4 Hz, 5-H).

(2*S*,3*R*,4*R*,5*R*)-1-*tert*-Butyldiphenylsilyloxy-3,5-dihydroxy-2,4-dimethyl-4-([^{13}C]methoxy)heptanol (12)

To a solution of **8** (1.02 g, 2.1 mmol) in MeOH (5 ml) was added PPTS (400 mg, 1.6 mmol) at room temperature, and the mixture was stirred for 27 h. The reaction mixture was diluted with Et_2O, washed with sat. $NaHCO_3$ aq. and brine, dried over anhydrous $MgSO_4$, and evaporated. Chromatography of the crude product on silica gel with AcOEt/hexane (5:95–1:9–1:3) gave **12** and recovered **8**, and the recovered **8** was subjected to the same reaction twice more (overall 823 mg, overall 88%); ^{1}H NMR ($CDCl_3$) δ: 0.02 (d, 3H, *J* = 7.0 Hz, 2-CH_3), 0.11 (s, 9H, $SiC(CH_3)_3$), 0.14 (t, 3H, *J* = 7.3 Hz, 7-H_3), 0.53 (m, 2H, 6-H_2), 0.61 (s, 3H, 4-CH_3), 1.00 (m, 1H, 2-H), 2.01 (d, 1H, *J* = 6.1 Hz, 5-OH), 2.31 (d, 1H, *J* = 6.4 Hz, 3-OH), 2.34 (d, 3H, *J* = 141 Hz, 4-O$^{13}CH_3$), 2.61 (dd, 1H, *J* = 4.9, 9.8 Hz, 1-H), 2.68 (dd, 1H, *J* = 7.3, 9.8 Hz, 1-H), 2.68 (m, 1H, 5-H), 3.17 (m, 1H, 3-H), 7.65–7.68 (m, 10H, phenyl-H_{10}).

(2*S*,3*R*,4*R*,5*R*)-3,5-Diacetoxy-1-*tert*-butyldiphenylsilyloxy-2,4-dimethyl-4-([^{13}C]methoxy)heptanol (13)

Pyridine (2 ml, 24.7 mmol) and acetic anhydride (2 ml, 21.2 mmol) were added to **12** (401 mg, 0.9 mmol) at room temperature, and the mixture was stirred for 3 h. To this solution was added DMAP (30 mg, 0.25 mmol) at room temperature, and the whole was stirred for 23 h. The reaction mixture was evaporated. Chromatography of the residue on silica gel with AcOEt/hexane (5:95–1:9) gave **13** (446 mg, 94%); ^{1}H NMR ($CDCl_3$) δ: 0.89 (d, 3H, *J* = 7.0 Hz, 2-CH_3), 0.90 (t, 3H, *J* = 7.6 Hz, 7-H_3), 1.05 (s, 9H, $SiC(CH_3)_3$), 1.15 (s, 3H, 4-CH_3), 1.56 (m, 1H, 6-H), 1.72 (m, 1H, 6-H), 2.02 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.27 (m, 1H, 2-H), 3.36 (d, 3H, *J* = 142 Hz, 4-O$^{13}CH_3$), 3.40 (dd, 1H, *J* = 6.4, 9.8 Hz, 1-H), 3.48 (dd, 1H, *J* = 8.5, 9.8 Hz, 1-H), 5.17 (d, 1H, *J* = 2.1 Hz, 3-H), 5.23 (dd, 1H, *J* = 2.1, 10.4 Hz, 5-H), 7.65–7.68 (m, 10H, phenyl-H_{10}).

(2*S*,3*R*,4*R*,5*R*)-3,5-Diacetoxy-2,4-dimethyl-4-([^{13}C]methoxy)-heptanol (14)

To a solution of **13** (104 mg, 0.20 mmol) in THF (0.5 ml) were added dropwise acetic acid (30 μl, 0.52 mmol) and *n*-Bu_4NF (1.0 M in THF, 0.4 ml, 0.4 mmol) at room temperature, and the mixture was stirred for 89 h. The reaction was quenched with sat. NH_4Cl aq., and the whole was extracted with Et_2O. The combined extract was washed with sat. $NaHCO_3$ aq. and brine, dried over anhydrous $MgSO_4$, and evaporated. Chromatography of the crude product on silica gel with AcOEt/hexane (1:4–1:2) gave **14** (54 mg, 78%); ^{1}H NMR ($CDCl_3$) δ: 0.91 (t, 3H, *J* = 7.3 Hz, 7-H_3), 0.93 (d, 3H, *J* = 7.0 Hz, 2-CH_3), 1.20 (s, 3H, 4-CH_3), 1.58 (m, 1H, 6-H), 1.72 (m, 1H, 6-H), 2.03 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$), 2.21 (m, 1H, 2-H), 2.94 (dd, 1H, *J* = 3.1, 5.5 Hz, 1-OH), 3.18 (ddd, 1H, *J* = 3.1, 5.8, 9.5 Hz, 1-H), 3.38 (d, 3H, *J* = 142 Hz, 4-O$^{13}CH_3$), 3.44 (ddd, 1H, *J* = 5.5, 8.6, 9.5 Hz, 1-H), 5.01 (d, 1H, *J* = 2.1 Hz, 3-H), 5.26 (dd, 1H, *J* = 1.8, 10.4 Hz, 5-H).

(2*R*,3*R*,4*R*,5*R*)-3,5-Diacetoxy-2,4-dimethyl-4-([^{13}C]methoxy)-heptanoic acid (15)

To a two-phase solution of **14** (711 mg, 2.1 mmol) in CCl_4 (5 ml), CH_3CN (5 ml), and 0.1 M Na phosphate buffer (7.5 ml) were added $NaIO_4$ (1.33 g, 6.2 mmol) and ruthenium (IV) oxide (55%, 8.0 mg, 26.7 μmol) at room temperature, and the whole was stirred vigorously for 1 h. The reaction mixture was diluted with sat. NaCl aq. and extracted with Et_2O. The combined extract was dried over anhydrous $MgSO_4$ and evaporated. Chromatography of the crude product on silica gel with AcOEt/hexane (1:2–2:1–4:1) gave **15** (615 mg, 81%); ^{1}H NMR ($CDCl_3$) δ: 0.90 (t, 3H, J = 7.3 Hz, 7-H$_3$), 1.14 (d, 3H, J = 7.3 Hz, 2-CH$_3$), 1.18 (s, 3H, 4-CH$_3$), 1.52 (m, 1H, 6-H), 1.74 (m, 1H, 6-H), 2.05 (s, 3H, COCH$_3$), 2.08 (s, 3H, COCH$_3$), 3.06 (quintet, 1H, J = 7.3 Hz, 2-H), 3.28 (d, 3H, J = 142 Hz, 4-O^{13}CH$_3$), 5.23 (dd, 1H, J = 2.7, 10.4 Hz, 5-H), 5.34 (d, 1H, J = 7.3 Hz, 3-H).

***S*-2-Acetylaminoethyl (2*R*,3*R*,4*R*,5*R*)-3,5-diacetoxy-2,4-dimethyl-4-([^{13}C]methoxy)heptanethioate (4)**

To a solution of **15** (278 mg, 0.77 mmol) and 2-acetylaminoethanethiol (826 mg, 6.9 mmol) in DMF (0.4 ml) was added diphenylphosphoryl azide (0.5 ml, 2.3 mmol) at room temperature under an argon atmosphere. To this solution was added dropwise Et_3N (0.65 ml, 4.7 mmol) at 0°C, and the whole was stirred for 14 h at room temperature. The reaction was quenched with sat. NH_4Cl aq. and the mixture was extracted with Et_2O. The combined extract was washed with 10% NaOH aq. and brine, dried over anhydrous $MgSO_4$, and evaporated. Chromatography of the crude product on silica gel with AcOEt/hexane (2:1–4:1) gave **4** (238 mg, 75%); ^{1}H NMR ($CDCl_3$) δ: 0.91 (t, 3H, J = 7.6 Hz, 7-H$_3$), 1.19 (d, 3H, J = 7.3 Hz, 2-CH$_3$), 1.21 (s, 3H, 4-CH$_3$),1.52 (m, 1H, 6-H), 1.74 (m, 1H, 6-H), 1.96 (s, 3H, NHCOCH$_3$), 2.05 (s, 3H, COCH$_3$), 2.09 (s, 3H, COCH$_3$), 3.05 (m, 2H, SCH$_2$), 3.17 (m, 1H, 2-H), 3.30 (d, 3H, J = 142 Hz, 4-O^{13}CH$_3$), 3.47 (m, 2H, SCH$_2$CH$_2$), 5.23 (dd, 1H, J = 2.1, 10.4 Hz, 5-H), 5.34 (d, 1H, J = 5.5 Hz, 3-H), 6.14 (brs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ: 51.78 (4-O^{13}CH$_3$); IR ($CHCl_3$) cm^{-1}: 3450, 3401, 3008, 2940, 1733, 1668, 1526, 1460, 1373, 1248, 1085, 1056, 965; FAB-MS (glycerol) *m/z*: 407 (MH$^+$).

Conclusion

Reaction of the optically active erythromycin A synthetic intermediate **6** obtained in our previous work with iodo[^{13}C]-methane did not afford the desired **3**, as δ-lactone **11** was generated during deprotection of the 3,5-dihydroxyl groups in the final step. Therefore, the 3,5-dihydroxyl groups of **3** were protected by diacetylation, and **4** was successfully obtained for biosynthetic studies on erythromycin.

References

[1] T. Kaneda, J. C. Butte, S. B. Taubman, J. W. Corcoran, *J. Bio. Chem.* **1962**, *237*, 322–328.
[2] S. M. Friedman, T. Kaneda, J. W. Corcoran, *J. Bio. Chem.* **1964**, *239*, 2386–2391.
[3] D. E. Cane, H. Hasler, T.-C. Liang, *J. Am. Chem. Soc.* **1981**, *103*, 5960–5962.
[4] D. E. Cane, H. Hasler, P. B. Taylor, T.-C. Liang, *Tetrahedron* **1983**, *39*, 3449–3455.
[5] D. E. Cane, T.-C. Liang, P. B. Taylor, C. Chang, C.-C. Yang, *J. Am. Chem. Soc.* **1986**, *108*, 4957–4964.
[6] D. E. Cane, C.-C. Yang, *J. Am. Chem. Soc.* **1987**, *109*, 1255–1257.
[7] D. E. Cane, P. C. Prabhakaran, W. Tan, W. R. Ott, *Tetrahedron Lett.* **1991**, *32*, 5457–5460.
[8] J. Cortes, S. F. Haydock, G. A. Roberts, D. J. Bevitt, P. F. Leadlay, *Nature* **1990**, *348*, 176–178. DOI: 10.1038/348176a0.
[9] J. S. Tuan, J. M. Weber, M. J. Staver, J. O. Leung, S. Donadio, L. Katz, *Gene* **1990**, *90*, 21–29.
[10] D. J. Bevitt, J. Cortes, S. F. Haydock, P. F. Leadlay, *Eur. J. Biochem.* **1992**, *204*, 39–49.
[11] S. Donadio, M. J. Staver, J. B. McAlpine, S. J. Swanson, L. Katz, *Science* **1991**, *252*, 675–679.
[12] S. Yue, J. S. Duncan, Y. Yamamoto, C. R. Hutchinson, *J. Am. Chem. Soc.* **1987**, *109*, 1253–1255.
[13] S. Masamune, P. A. McCarthy, Total synthesis of macrolides. In *Macrolide Antibiotics-Chemistry, Biology, and Practice, Chapter 4.* (Ed.: S. Omura), Academic Press, New York, **1984**, pp. 127–198.
[14] T. Nakata, M. Fukui, T. Oishi, *Tetrahedron Lett.* **1988**, *29*, 2219–2222.
[15] T. Nakata, M. Fukui, T. Oishi, *Tetrahedron Lett.* **1988**, *29*, 2223–2226.
[16] S. Yamada, Y. Yokoyama, T. Shioiri, *J. Org. Chem.* **1974**, *39*, 3302–3303.