

Total syntheses and configuration assignments of JBIR-04 and unantimycin A

Yoshinosuke Usuki, Chie Hamada, Tetsuya Satoh

Citation	Organic & Biomolecular Chemistry, 15(35); 7346-7351
Issue Date	2017-08-14
Type	Journal Article
Textversion	Author
Supplementary information	Supplementary information is available at https://doi.org/10.1039/C7OB01732D .
Rights	The following article has been accepted by Organic & Biomolecular Chemistry. The final published version is available at https://doi.org/10.1039/C7OB01732D .
DOI	10.1039/C7OB01732D

Self-Archiving by Author(s)
Placed on: Osaka City University

Total Syntheses and Configuration Assignments of JBIR-04 and Unantimycin A

Yoshinosuke Usuki,* Chie Hamada and Tetsuya Satoh

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x
www.rsc.org/

First total syntheses of JBIR-04 and unantimycin A have been achieved. Comparison of our spectroscopic data with those reported for natural samples verified the structure of the natural products; (2*S*, 4*S*, 6*S*, 7*R*, 9*S*, 28*S*) configuration was thus assigned via total synthesis.

Introduction

Neoantimycin (**1**), first isolated in 1967 from *Streptomyces orinoci*¹, is a ring-extended member of the antimycin class with a 15-membered tetralactone moiety (Figure 1). The recent discovery of prunustatin A (**2**) as a selective GRP78 molecular chaperone

downregulator highlights the potential of the neoantimycin subfamily as research probes^{2,3,4}. This discovery may lead to the development of new approaches for fighting cancer since GRP78 is a molecular chaperone critical to the unfolded protein response. Moreover, it has emerged as a new therapeutic target for drug-resistant cancer cells and cancer stem cells⁵.

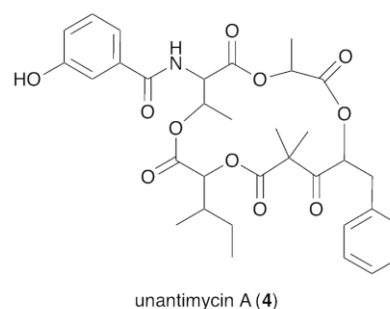
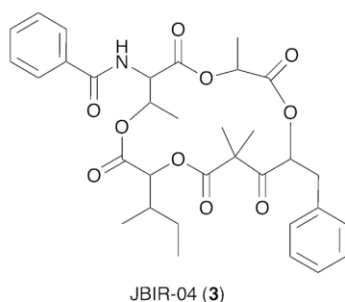
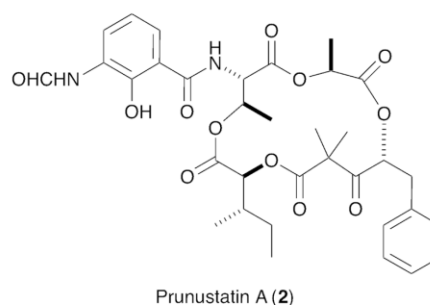
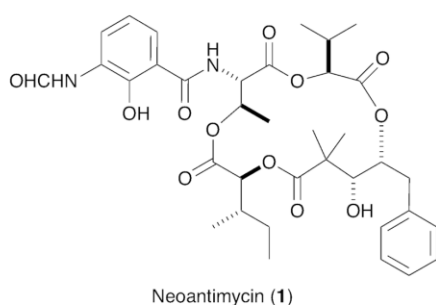
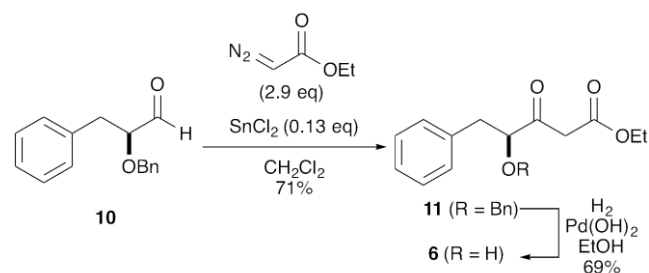
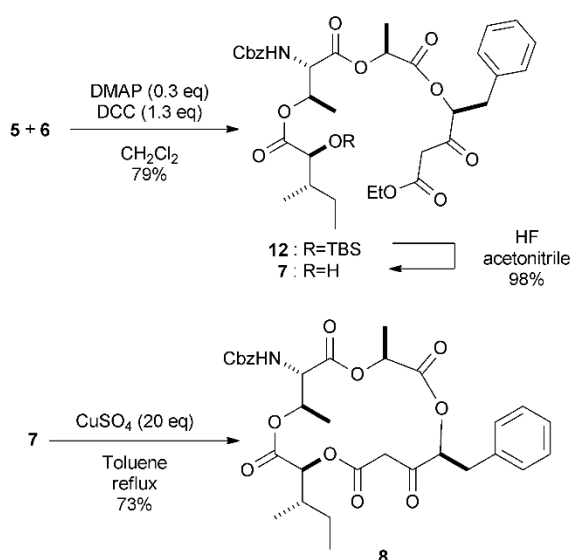


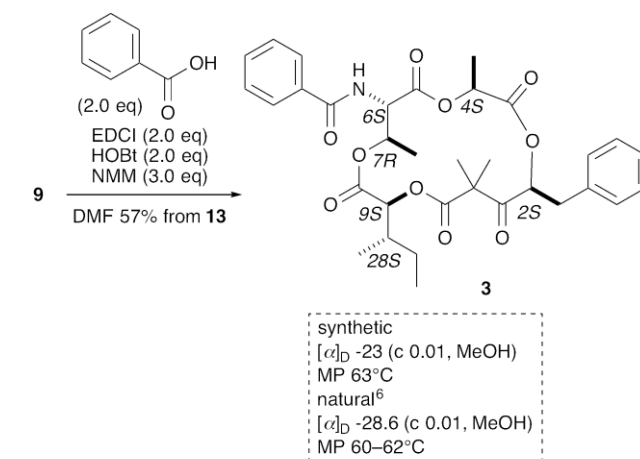
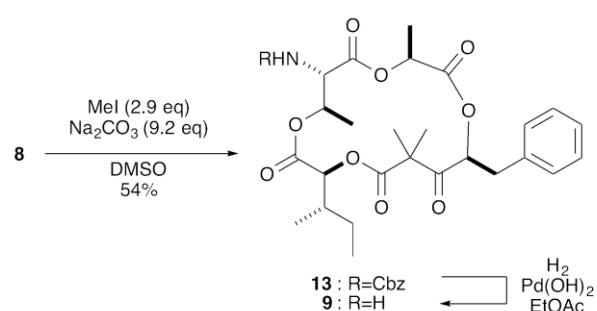
Fig. 1 Structures of neoantimycin (**1**), prunustatin A (**2**), JBIR-04 (**3**), and unantimycin A (**4**).

Scheme 2 Synthesis of β -keto ester 6.

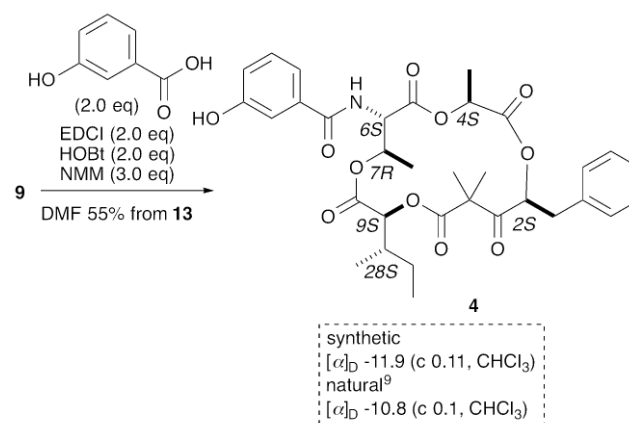
The ring-closure precursor **7** was obtained by condensation of **5**^{10a} and **6** in the presence of DCC and DMAP and subsequent removal of the TBS group with HF in CH₃CN (Scheme 3). A mixture of **7** and anhydrous CuSO₄ (20 equiv.) in toluene was heated under reflux. The desired transesterification proceeded smoothly to provide the 15-membered tetralactone **8** in 73% yield.

Scheme 3 Preparation and transesterification of **7**.

The introduction of *gem*-dimethyl groups at C11 was achieved by treatment of **8** with iodomethane (2.9 equiv.) and Na₂CO₃ (9.2 equiv.) in DMSO at 40°C for 4 h (Scheme 4). The desired product **13** was obtained in 54% yield. To complete the synthesis of **3**, the Cbz group of **13** was removed by hydrogenolysis with Pd(OH)₂ in EtOAc to afford **9**. The subsequent condensation of **9** with benzoic acid was achieved using EDCI, HOBT, and NMM in DMF to provide synthetic JBIR-04 (**3**) in 57% yield. The spectral data of synthetic **3** were identical to those reported for a natural sample⁶. The optical rotation and melting point of synthetic **3** ([α]_D -23.0, c 0.01, MeOH; MP 63°C) were consistent with those of the natural product ([α]_D -28.6, c 0.01, MeOH; MP 60–62°C).

Scheme 4 Endgame towards JBIR-04 (**3**).

We then focused on unantimycin A (**4**). Condensation of **9** and 3-hydroxybenzoic acid was achieved using EDCI, HOBT, and NMM in DMF to provide synthetic unantimycin A (**4**) in 55% yield (Scheme 5). The spectral data of synthetic **4** were identical to those reported for a natural sample⁹. The optical rotation and melting point of synthetic **4** ([α]_D -11.9, c 0.11, CHCl₃) were consistent with those of the natural product ([α]_D -10.8, c 0.1, CHCl₃).

Scheme 5 Endgame towards unantimycin A (**4**).

Conclusions

In summary, the first total syntheses of JBIR-04 and unantimycin A have been achieved. Comparison of our spectroscopic data with those reported for natural samples verified the structure of the natural products; (2*S*, 4*S*, 6*S*, 7*R*, 9*S*, 28*S*) configuration was thus assigned via total synthesis. Further studies on their biological activities are now in progress, and the results will be reported in due course.

Experimental

General information and materials

¹H and ¹³C NMR spectra were recorded on either Bruker Avance 300 (300 and 75 MHz), Bruker Avance 400 (400 and 100 MHz), JEOL ECZ-400S (400 and 100 MHz), or Bruker Avance III 600 (600 and 150 MHz) instruments. Chemical shifts were reported in parts per million (ppm, δ) relative to Me₄Si (0 ppm) and CDCl₃ (7.26 ppm for ¹H NMR and 77.0 ppm for ¹³C NMR) as the internal reference. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad), coupling constant in Hz, integration. Coupling constants were determined directly from ¹H and ¹³C NMR spectra. Mass spectra were obtained on a JEOL JMS-T100LP (DART, ESI) spectrometer. Infrared (IR) spectra were recorded on a JASCO FT/IR-4600 spectrometer. Optical rotations were measured on a JASCO P-2200 with a path length of 0.1 dm at ambient temperature; the concentrations are reported in g/dL.

Most of the reagents and solvents were purchased from Wako Pure Chemical Industries, Ltd., Tokyo Kasei Kogyo Co., and Sigma-Aldrich Co. and were used without further purification unless otherwise noted. All air- and moisture-sensitive reactions were carried out in flame-dried, argon-flushed, two-necked flasks sealed with rubber septa, and dry solvents and reagents were introduced using a syringe. THF was freshly distilled from sodium benzophenone ketyl. Flash column chromatography was carried out on Kanto Chemical silica gel 60 N (spherical, neutral, 40–50 μ m). Analytical and preparative thin-layer chromatography was performed on Merck precoated silica gel (#5715 Kieselgel 60F₂₅₄ 0.25 mm and #5744 Kieselgel 60F₂₅₄ 0.5 mm, respectively).

Ethyl (S)-4-(benzyloxy)-3-oxo-5-phenylpentanoate (11). A solution of aldehyde **10** (1.97 g, 8.24 mmol) in CH₂Cl₂ (14 mL) was added to ethyl diazoacetate (2.68 g, 23.5 mmol) in CH₂Cl₂ (14 mL) and anhydrous SnCl₂ (209 mg, 1.10 mmol) sequentially at 0°C. After being stirred overnight at 0°C, the reaction mixture was washed with aq. sat. NaHCO₃ before being dried over Na₂SO₄ and concentrated. The residue was purified by flash column chromatography (10% EtOAc in *n*-hexane) to give β -keto ester **11** (ca. 2.6 : 1 mixture of tautomers) as a pale yellow oil (1.92 g, 5.88 mmol, 71%); [α]_D = –50.4 (c 1.33, CHCl₃); Major (Keto form): ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.00 (10H, m), 4.51–4.34 (2H, ABq, *J* = 11.7 Hz, ν = 38.7 Hz), 4.18–4.05 (1H, m), 4.12 (2H, q, *J* = 7.2 Hz), 3.58–3.38 (2H, ABq, *J* = 15.9 Hz, ν = 43.2 Hz), 3.08 (1H, dd, *J* = 14.1, 4.2 Hz), 2.95 (1H, dd, *J* = 14.1, 7.8 Hz), 1.22 (3H, t, *J* = 7.2 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 205.17, 167.29, 137.04, 136.72, 129.63, 128.50, 128.46, 127.98, 127.83, 126.83, 85.37, 73.04, 61.35, 45.53, 38.22, 14.11; IR (KBr) ν_{max} 3423, 3029, 2982, 2929, 1750, 1719, 1685, 1654,

1638, 1560, 1542, 1509, 1491, 1457, 1315, 1225, 1095, 1030, 735, 698, 478 cm^{–1}; HRDARTMS Calcd. For C₂₀H₂₃O₄ 327.1596: found 327.1588 [M+H]⁺. Minor (Enol form, diagnostic peaks only): ¹H NMR (400 MHz, CDCl₃) δ 12.10 (1H, s), 5.27 (1H, s), 4.60 (1H, d, *J* = 11.9 Hz), 4.30 (1H, d, *J* = 11.9 Hz), 4.21 (2H, q, *J* = 7.1 Hz), 1.30 (3H, t, *J* = 7.1 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 176.15, 172.23, 89.19, 79.88, 73.01, 60.30, 40.37, 26.91.

Ethyl (S)-4-hydroxy-3-oxo-5-phenylpentanoate (6). To a solution of **11** (509 mg, 1.56 mmol) in ethanol (20 mL), Pd(OH)₂ (224 mg) was added. The resulting suspension was stirred overnight under H₂. Then, the mixture was filtered through a pad of Celite®. The filtrate was concentrated in vacuo. Purification by flash column chromatography (20 % EtOAc in *n*-hexane) afforded **6** as a yellow oil (254 mg, 1.08 mmol, 69%); [α]_D = –8.0 (c 0.87, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.35–7.02 (5H, m), 4.46 (1H, dd, *J* = 8.1, 4.5 Hz), 4.17 (2H, q, *J* = 7.2 Hz), 3.60–3.36 (2H, m), 3.14 (1H, dd, *J* = 14.1, 4.5 Hz), 2.87 (1H, dd, *J* = 14.1, 8.0 Hz), 1.26 (3H, t, *J* = 7.2 Hz). ¹³C NMR (75 MHz, CDCl₃) δ 204.91, 167.11, 136.33, 129.40, 128.69, 127.06, 77.54, 61.69, 45.65, 39.74, 14.08; IR (KBr) ν_{max} 3449, 3032, 2983, 2931, 1735, 1718, 1708, 1686, 1654, 1647, 1627, 1560, 1542, 1509, 1457, 1399, 1368, 1320, 1091, 1029, 942, 730, 701, 477 cm^{–1}; HRDARTMS Calcd. For C₁₃H₁₆NaO₄ 259.0946: found 259.0961 [M+Na]⁺.

Ethyl (S)-4-(((S)-2'-((N-((benzyloxy)carbonyl)-O-((2'S,3'S)-2'-hydroxy-3'-methylpentanoyl)-L-threonyl)oxy)propanoyl)oxy)-3-oxo-5-phenylpentanoate (12). To a stirred mixture of **5** (269 mg, 0.49 mmol), **6** (128 mg, 0.54 mmol), and DMAP (19 mg, 0.16 mmol) in CH₂Cl₂ (2.7 mL), a solution of DCC (188 mg, 0.68 mmol) in CH₂Cl₂ (2.7 mL) was added dropwise at 0°C. The reaction mixture was stirred at r.t. overnight. The reaction mixture was diluted with *n*-hexane and filtered through a pad of Celite®. The filtrate was concentrated in vacuo. The crude residue was purified by flash column chromatography (15% EtOAc in *n*-hexane) to give **12** as a colorless oil (295 mg, 0.38 mmol, 79%); [α]_D = –18.8 (c 0.51, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.38–7.12 (10H, m), 5.47–5.34 (3H, m), 5.13 (2H, s), 5.07 (1H, q, *J* = 7.0 Hz), 4.52 (1H, dd, *J* = 9.7, 3.0 Hz), 4.13 (2H, q, *J* = 7.0 Hz), 4.05–3.97 (1H, m), 3.55–3.40 (2H, ABq, *J* = 16.5 Hz, ν = 43.9 Hz), 3.36–3.23 (1H, m), 3.18 (1H, dd, *J* = 14.5, 5.2 Hz), 3.04 (1H, dd, *J* = 14.5, 7.3 Hz), 1.80–1.67 (1H, m), 1.50–1.11 (8H, m), 0.92–0.78 (6H, m), 0.00 (6H, s); ¹³C NMR (100 MHz, CDCl₃) δ 199.30, 172.24, 169.27, 168.93, 166.26, 156.49, 136.07, 135.03, 129.51, 128.76, 128.65, 128.38, 128.18, 127.36, 79.14, 76.18, 70.64, 69.51, 67.46, 61.67, 57.77, 46.56, 39.47, 36.55, 25.79, 23.89, 18.25, 17.12, 16.77, 15.66, 14.14, 11.72, –4.87, –5.49; IR (KBr) ν_{max} 3448, 3067, 3033, 2960, 2935, 2857, 1752, 1734, 1685, 1654, 1637, 1541, 1509, 1457, 1381, 1253, 1187, 1138, 1090, 1065, 1003, 876, 838, 778, 738, 698, 485 cm^{–1}; HRDARTMS Calcd. For C₄₀H₅₈NO₁₂Si 772.3728: found 772.3728 [M+H]⁺.

Ethyl (S)-4-(((S)-2'-((N-((benzyloxy)carbonyl)-O-((2'S,3'S)-2'-hydroxy-3'-methylpentanoyl)-L-threonyl)oxy)propanoyl)oxy)-3-oxo-5-phenylpentanoate (7). To a solution of **12** (377 mg, 0.49 mmol) in acetonitrile (8.8 mL) at 0°C, 46.0–48.0% HF aq. (1.3 mL) was added. The reaction mixture was stirred at r.t. for 4 h, before being quenched with sat. NaHCO₃ and extracted with EtOAc. The combined organic layers were washed with brine, dried over

Na₂SO₄, and concentrated in vacuo. The product was used in the next step without further purification (315 mg, 0.48 mmol, 98%): [α]_D = -3.28 (c 0.53, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.42–7.07 (10H, m), 5.55 (1H, dq, *J* = 6.4, 2.8 Hz), 5.44–5.34 (2H, m), 5.15 (2H, s), 5.09 (1H, q, *J* = 7.1 Hz), 4.58 (1H, dd, *J* = 9.7, 2.8 Hz), 4.16 (2H, q, *J* = 7.1 Hz), 4.00–3.89 (1H, m), 3.57–3.43 (2H, ABq, *J* = 16.4 Hz, *u* = 38.2 Hz), 3.18 (1H, dd, *J* = 14.4, 5.5 Hz), 3.01 (1H, dd, *J* = 14.4, 7.5 Hz), 2.95–2.74 (1H, m), 1.81–1.68 (1H, m), 1.52–1.16 (8H, m), 0.98–0.84 (6H, m); ¹³C NMR (100 MHz, CDCl₃) δ 198.18, 173.74, 169.15, 169.01, 166.27, 156.54, 135.93, 134.93, 129.39, 128.62, 128.47, 128.39, 128.18, 127.29, 79.09, 74.37, 71.39, 69.38, 67.52, 61.70, 57.42, 46.63, 38.75, 36.31, 23.91, 16.71, 16.53, 15.34, 14.03, 11.80; IR (KBr) ν_{max} 3448, 3066, 3033, 2965, 2936, 2876, 1750, 1734, 1719, 1654, 1637, 1560, 1541, 1509, 1499, 1457, 1384, 1316, 1211, 1132, 1091, 1060, 737, 699, 487 cm⁻¹; HRDARTMS Calcd. For C₃₄H₄₄NO₁₂ 658.2864: found 658.2858 [M+H]⁺.

Benzyl ((3S,6R,7S,10S,15S)-15-benzyl-10-((S)-sec-butyl)-3,7-dimethyl-2,5,9,12,14-pentaoxo-1,4,8,11-tetraoxacyclopentadecan-6-yl)carbamate (8). A 300 mL flask containing a mixture of anhydrous CuSO₄ (748 mg, 4.69 mmol) and **7** (154 mg, 246 μ mol) in toluene (200 mL) was equipped with a small dropping funnel filled with molecular sieves 4A (7.0 g) and a reflux condenser. The mixture was refluxed at 130°C for 5 h. After cooling to r.t., it was filtered to remove CuSO₄. The filtrate was concentrated and purified by flash column chromatography (20% EtOAc in *n*-hexane) to give **8** as a colorless oil (105 mg, 172 μ mol, 73%). [α]_D = 2.4 (c 1.04, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.41–7.10 (10H, m), 5.60 (1H, dq, *J* = 6.4, 2.3 Hz), 5.54 (1H, d, *J* = 9.7 Hz), 5.43 (1H, dd, *J* = 9.5, 5.4 Hz), 5.19–5.11 (2H, m), 5.04 (1H, d, *J* = 9.2 Hz), 4.61 (1H, dd, *J* = 9.8, 2.3 Hz), 3.35–3.18 (2H, ABq, *J* = 14.0 Hz, *u* = 55.0 Hz), 3.25 (1H, dd, *J* = 14.0, 5.4 Hz), 3.12 (1H, dd, *J* = 14.0, 9.6 Hz), 2.05–1.91 (1H, m), 1.55–1.41 (1H, m), 1.38 (3H, d, *J* = 6.4 Hz), 1.25 (3H, d, *J* = 7.1 Hz), 1.21–1.00 (1H, m), 0.94–0.82 (6H, m); ¹³C NMR (75 MHz, CDCl₃) δ 199.37, 169.94, 168.88, 167.86, 165.24, 156.76, 135.96, 135.45, 129.32, 128.75, 128.60, 128.33, 128.14, 127.22, 79.35, 76.35, 71.79, 69.76, 67.51, 57.57, 48.23, 36.95, 35.68, 24.36, 16.57, 16.54, 14.20, 10.53; IR (KBr) ν_{max} 3428, 3370, 3088, 3064, 3031, 2966, 2938, 2879, 1957, 1748, 1722, 1604, 1586, 1515, 1455, 1382, 1346, 1310, 1282, 1192, 1132, 1088, 1061, 1028, 1004, 913, 844, 775, 734, 700, 648, 602, 577, 502, 468 cm⁻¹; HRESIMS Calcd. For C₃₂H₃₆NO₁₁ 610.2288: found 610.2274 [M-H]⁻.

Benzyl ((3S,6S,7R,10S,15S)-15-benzyl-10-((S)-sec-butyl)-3,7,13,13-tetramethyl-2,5,9,12,14-pentaoxo-1,4,8,11-tetraoxacyclopentadecan-6-yl)carbamate (13). To a solution of Na₂CO₃ (92 mg, 1.35 mmol) in DMSO (1.4 mL) at r.t., a solution of β -keto ester **8** (56 mg, 91.6 μ mol) in DMSO (1.3 mL) was added. Then, MeI (18 μ L, 289 μ mol) was added. The reaction mixture was stirred at r.t. for 4 h, diluted with H₂O, and extracted with *n*-hexane/EtOAc (1 : 1, 3x). The combined organic layers were washed with brine, dried over Na₂SO₄, and concentrated in vacuo. Purification by flash column chromatography (20% EtOAc in *n*-hexane) afforded **13** as a colorless oil (31 mg, 54 μ mol, 54%). [α]_D = -23.0 (c 0.94, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.42–7.19 (10H, m), 5.76 (1H, dd, *J* = 10.7, 3.1 Hz), 5.68 (1H, dq, *J* = 6.4, 2.8 Hz), 5.57 (1H, d, *J* = 9.6 Hz), 5.29 (1H, q, *J* = 6.8 Hz), 5.14 (2H, d, *J* = 3.4 Hz), 4.97 (1H, d, *J* = 9.3 Hz), 4.63 (1H, dd, *J* = 9.7, 2.8 Hz), 3.37 (1H, dd, *J* = 14.0, 3.1 Hz), 3.12 (1H, dd, *J* = 14.0, 10.7 Hz), 2.03–1.92 (1H, m),

1.55–1.40 (1H, m), 1.40 (3H, s), 1.38 (3H, d, *J* = 6.4 Hz), 1.25 (3H, d, *J* = 6.8 Hz), 1.24 (3H, s), 1.22–1.06 (1H, m), 0.88 (3H, d, *J* = 6.9 Hz), 0.86 (3H, t, *J* = 7.2 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 203.40, 171.21, 168.87, 168.30, 168.15, 156.78, 136.37, 135.88, 129.41, 128.60, 128.37, 128.33, 128.11, 126.90, 78.77, 75.58, 71.36, 69.58, 67.51, 57.87, 53.80, 37.40, 36.13, 24.36, 22.35, 20.57, 16.42, 16.19, 13.86, 10.23; IR (KBr) ν_{max} 3377, 3065, 3031, 2967, 2938, 2879, 1751, 1718, 1654, 1637, 1604, 1541, 1509, 1499, 1456, 1383, 1342, 1314, 1281, 1242, 1194, 1138, 1102, 1059, 1005, 987, 912, 864, 776, 734, 699, 647, 549, 487 cm⁻¹; HRESIMS Calcd. For C₃₄H₄₀NO₁₁ 638.2601: found 638.2581 [M+H]⁺.

(3S,6S,7R,10S,15S)-6-Amino-15-benzyl-10-((S)-sec-butyl)-3,7,13,13-tetramethyl-1,4,8,11-tetraoxacyclopentadecane-2,5,9,12,14-pentaoxone (9). To a solution of benzyl carbamate **13** (17 mg, 27 μ mol) in EtOAc (2 mL), 20% Pd(OH)₂ (6 mg) was added. The resulting suspension was stirred overnight under H₂. Then, the mixture was filtered through a pad of Celite®. The filtrate was concentrated to give amine **9** (13 mg, 26 μ mol). The product was used in the next step without further purification. [α]_D = -90.3 (c 0.65, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.32–7.17 (5H, m), 5.75 (1H, dd, *J* = 10.7, 3.1 Hz), 5.67 (1H, dq, *J* = 6.5, 2.7 Hz), 5.30 (1H, q, *J* = 6.7 Hz), 4.99 (1H, d, *J* = 9.4 Hz), 3.53 (1H, d, *J* = 2.7 Hz), 3.37 (1H, dd, *J* = 14.4, 3.0 Hz), 3.27 (1H, dd, *J* = 14.4, 10.7 Hz), 2.04–1.90 (1H, m), 1.53–1.42 (1H, m), 1.45 (3H, d, *J* = 6.5 Hz), 1.39 (3H, s), 1.24–1.06 (1H, m), 1.22 (3H, d, *J* = 6.8 Hz), 1.22 (3H, s), 0.88 (3H, d, *J* = 6.7 Hz), 0.86 (3H, t, *J* = 7.5 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 203.76, 172.12, 171.28, 169.24, 168.66, 135.65, 129.59, 128.43, 126.90, 79.00, 75.80, 71.63, 69.23, 58.48, 53.81, 37.53, 36.21, 24.49, 22.53, 20.59, 16.60, 16.51, 13.95, 10.37; IR (KBr) ν_{max} 3221, 3064, 3030, 2967, 2937, 2879, 2363, 1750, 1718, 1654, 1637, 1603, 1560, 1542, 1508, 1497, 1456, 1387, 1343, 1261, 1194, 1153, 1103, 1054, 1014, 986, 647, 548, 488, 468 cm⁻¹; HRESIMS Calcd. For C₂₆H₃₆NO₉ 506.2390: found 506.2437 [M+H]⁺.

JBIR-04 (3). To a solution of **9** (11 mg, 22 μ mol) in DMF (1 mL), benzoic acid (12 mg, 98 μ mol), HOBt (6 mg, 46 μ mol), EDCI•HCl (9 mg, 46 μ mol), and NMM (8 μ L, 73 μ mol) were added successively. The reaction mixture was stirred at r.t. for 14 h, diluted with H₂O, and extracted with EtOAc (3x). The combined organic layers were washed with sat. aq. NaHCO₃ and brine, dried over Na₂SO₄, and concentrated in vacuo. Purification by flash column chromatography (40% EtOAc in *n*-hexane) afforded **3** as a colorless amorphous solid (9.3 mg, 15 μ mol, 57%). MP = 63°C (lit.⁴ MP = 60–62°C). [α]_D = -23 (c 0.01, MeOH) (lit.⁶ [α]_D = -28.6 (c 0.01, MeOH)). ¹H NMR (600 MHz, CDCl₃) δ 7.91 (2H, d, *J* = 7.3 Hz), 7.57 (1H, t, *J* = 7.4 Hz), 7.50 (2H, t, *J* = 7.6 Hz), 7.31–7.25 (5H, m), 6.93 (1H, d, *J* = 9.0 Hz), 5.79 (1H, dd, *J* = 10.7, 3.0 Hz), 5.78 (1H, dq, *J* = 6.5, 3.0 Hz), 5.33 (1H, q, *J* = 6.8 Hz), 5.21 (1H, dd, *J* = 9.0, 3.0 Hz), 5.05 (1H, d, *J* = 9.3 Hz), 3.40 (1H, dd, *J* = 14.5, 3.0 Hz), 3.15 (1H, dd, *J* = 14.5, 10.7 Hz), 2.07–1.98 (1H, m), 1.56–1.47 (1H, m), 1.42 (3H, s), 1.41 (3H, d, *J* = 6.5 Hz), 1.26 (3H, d, *J* = 6.8 Hz), 1.22–1.12 (1H, m), 1.25 (3H, s), 0.90 (3H, d, *J* = 6.8 Hz), 0.89 (3H, t, *J* = 7.7 Hz); ¹³C NMR (150 MHz, CDCl₃) δ 203.39, 171.37, 168.84, 168.38, 168.29, 167.97, 136.34, 133.44, 132.16, 129.42, 128.74, 128.39, 127.27, 126.92, 78.87, 75.65, 71.82, 69.70, 55.92, 53.76, 37.44, 36.13, 30.90, 24.36, 22.48, 20.50, 16.41, 16.36, 13.92, 10.24; IR (KBr) ν_{max} 3423, 3067, 3034, 2967, 2936, 2877, 1752, 1719, 1686, 1655, 1647, 1638, 1579, 1561, 1542, 1526, 1509, 1458, 1381, 1349, 1320, 1240, 1191, 1131, 1103,

1060, 1009, 971, 887, 700, 624, 540, 505, 482, 462, 438 cm^{-1} ; HRESIMS Calcd. For $\text{C}_{33}\text{H}_{39}\text{NNaO}_{10}$ 632.2472; found 632.2472 $[\text{M}+\text{Na}]^+$.

Unantimycin A (4). To a solution of **9** (7.9 mg, 16 μmol) in DMF (0.8 mL), 3-hydroxybenzoic acid (4.3 mg, 31 μmol), HOBt (4.2 mg, 31 μmol), EDCI•HCl (6.0 mg, 31 μmol), and NMM (5.2 μL , 47 μmol) were added successively. The reaction mixture was stirred at r.t. for 17 h, diluted with H_2O , and extracted with EtOAc (3x). The combined organic layers were washed with sat. aq. NaHCO_3 and brine, dried over Na_2SO_4 , and concentrated in vacuo. Purification by flash column chromatography (40% EtOAc in *n*-hexane) afforded **4** as a colorless amorphous solid (7.0 mg, 11 μmol , 55%). MP = 78°C. $[\alpha]_D^{25} = -11.9$ (c 0.11, CHCl_3), (lit.⁹ $[\alpha]_D^{25} = -10.8$ (c 0.1, CHCl_3)). ^1H NMR (600 MHz, CDCl_3) δ 7.49 (1H, dd, $J = 2.5, 1.5$ Hz), 7.43 (1H, dt, $J = 7.8, 1.3$ Hz), 7.36 (1H, t, $J = 7.9$ Hz), 7.31 (4H, m), 7.25 (1H, m), 7.06 (1H, ddd, $J = 7.8, 2.5, 1.0$ Hz), 6.98 (1H, d, $J = 9.1$ Hz), 5.96 (1H, brs), 5.79 (1H, dd, $J = 10.8, 3.2$ Hz), 5.78 (1H, dq, $J = 6.3, 2.7$ Hz), 5.34 (1H, q, $J = 6.8$ Hz), 5.19 (1H, dd, $J = 9.1, 2.8$ Hz), 5.04 (1H, d, $J = 9.2$ Hz), 3.40 (1H, dd, $J = 14.5, 3.1$ Hz), 3.15 (1H, dd, $J = 14.5, 10.8$ Hz), 2.03 (1H, m), 1.51 (1H, m), 1.42 (3H, s), 1.40 (3H, d, $J = 6.4$ Hz), 1.26 (3H, d, $J = 7.1$ Hz), 1.26 (3H, s), 1.17 (1H, m), 0.90 (3H, d, $J = 6.8$ Hz), 0.89 (3H, t, $J = 7.5$ Hz); ^{13}C NMR (150 MHz, CDCl_3) δ 203.39, 171.47, 168.85, 168.32, 168.26, 167.89, 156.31, 136.34, 134.80, 130.10, 129.43, 128.43, 126.96, 119.47, 119.02, 114.70, 78.93, 75.74, 71.74, 69.75, 56.03, 53.80, 37.50, 36.13, 24.36, 22.51, 20.53, 16.46, 16.40, 13.93, 10.26; IR (KBr) ν_{max} 3422, 2965, 2932, 2879, 2751, 1793, 1751, 1735, 1718, 1685, 1671, 1654, 1647, 1560, 1541, 1523, 1509, 1457, 1420, 1387, 1340, 1319, 1194, 1159, 1139, 1103, 1061, 963, 882, 752, 700, 669, 486, 459, 423 cm^{-1} ; HRESIMS Calcd. For $\text{C}_{33}\text{H}_{39}\text{NNaO}_{11}$ 648.2421; found 648.2403 $[\text{M}+\text{Na}]^+$.

Acknowledgements

We are grateful to Professor Kazuo Shin-ya of the National Institute of Advanced Industrial Science and Technology for ^1H and ^{13}C spectra of naturally isolated JBIR-04. We also thank Dr. Matsumi Doe, Analytical Division, Osaka City University, for NMR measurements.

Notes and references

- (1) G. Cassinelli, A. Grein, P. Orezzi, P. Pennella, A. Sanfilippo, *Archiv für Mikrobiologie* 1967, **55**, 358.
- (2) (a) Y. Umeda, S. Chijiwa, K. Furihata, K. Furihata, S. Sakuda, H. Nagasawa, H. Watanabe, K. Shin-ya, *J. Antibiot.* 2005, **58**, 206; (b) Y. Umeda, K. Furihata, S. Sakuda, H. Nagasawa, K. Ishigami, H. Watanabe, M. Izumikawa, M. Takagi, T. Doi, Y. Nakao, K. Shin-ya, *Org. Lett.* 2007, **9**, 4239.
- (3) (a) X. Li, R. Zvanych, S. A. Vanner, W. Wang, N. A. Magarvey, *Bioorg. Med. Chem. Lett.* 2013, **23**, 5123; (b) A. A. Salim, K. J. Cho, L. Tan, M. Quezada, E. Lacey, J. F. Hancock, R. J. Capon, *Org. Lett.* 2014, **16**, 5036; (c) S. Yamakoshi, M. Okamoto, H. Sawamoto, Y. Arai, E. Kawanishi, M. Sasaki, K. Takeda, *Curr. Org. Synth.* 2017, **14**, 299.
- (4) J. Liu, X. Zhu, S. J. Kim, W. Zhang, *Nat. Prod. Rep.* 2016, **33**, 1146.

- (5) (a) B. Luo, A. S. Lee, *Oncogene* 2013, **32**, 805; (b) P. Pyrko, A. H. Schonthal, F. M. Hoffman, T. C. Chen, A. S. Lee, *Cancer. Res.* 2007, **67**, 9809.
- (6) M. Izumikawa, J. Ueda, S. Chijiwa, M. Takagi, K. Shin-ya, *J. Antibiot.* 2007, **60**, 640.
- (7) S. Manaviazar, P. Nockemann, K. J. Hale, *Org. Lett.* 2016, **18**, 2902.
- (8) K. Takahashi, E. Tsuda, K. Kurokawa, *J. Antibiot.* 2001, **54**, 867.
- (9) C. L. Lim, T. Nogawa, A. Okano, Y. Futamura, M. Kawatani, S. Takahashi, D. Ibrahim, H. Osada, *J. Antibiot.* 2016, **69**, 456.
- (10) (a) Y. Usuki, H. Ogawa, K.-i. Yoshida, T. Inaoka, H. Iio, *Asian J. Org. Chem.* 2015, **4**, 737; (b) H. Ogawa, H. Iio, Y. Usuki, *Chem. Lett.* 2015, **44**, 1214.
- (11) C. R. Holmquist, E. J. Roskamp, *J. Org. Chem.* 1989, **54**, 3258.