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Journal Name

ARTICLE

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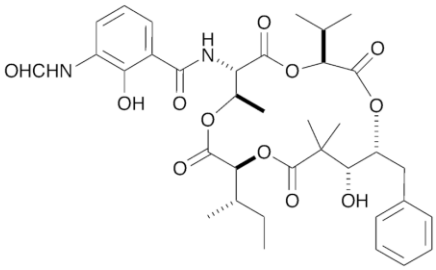
Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x
www.rsc.org/

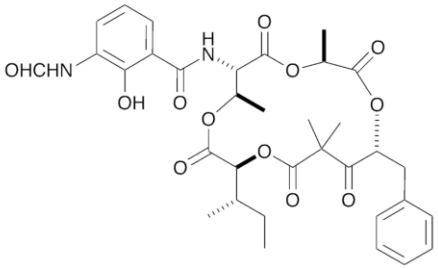
First total syntheses of JBIR-04 and unantimycin A have been achieved. Comparison of our spectroscopic data with those reported for natural samples verified the structure of the natural products; (*2S*, *4S*, *6S*, *7R*, *9S*, *28S*) configuration was thus assigned via total synthesis.

Introduction

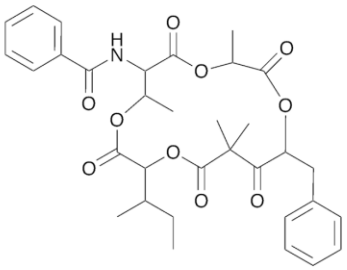
Neoantimycin (**1**), first isolated in 1967 from *Streptomyces orinoci*[1], is a ring-extended member of the antimycin class with a 15-membered tetralactone moiety (Figure 1). The recent discovery of prunustatin A (**2**) as a selective GRP78 molecular chaperone downregulator highlights the potential 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 cells[5].



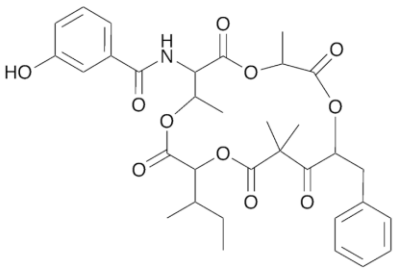
Neoantimycin (**1**)



Prunustatin A (**2**)



JBIR-04 (**3**)



unantimycin A (**4**)

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

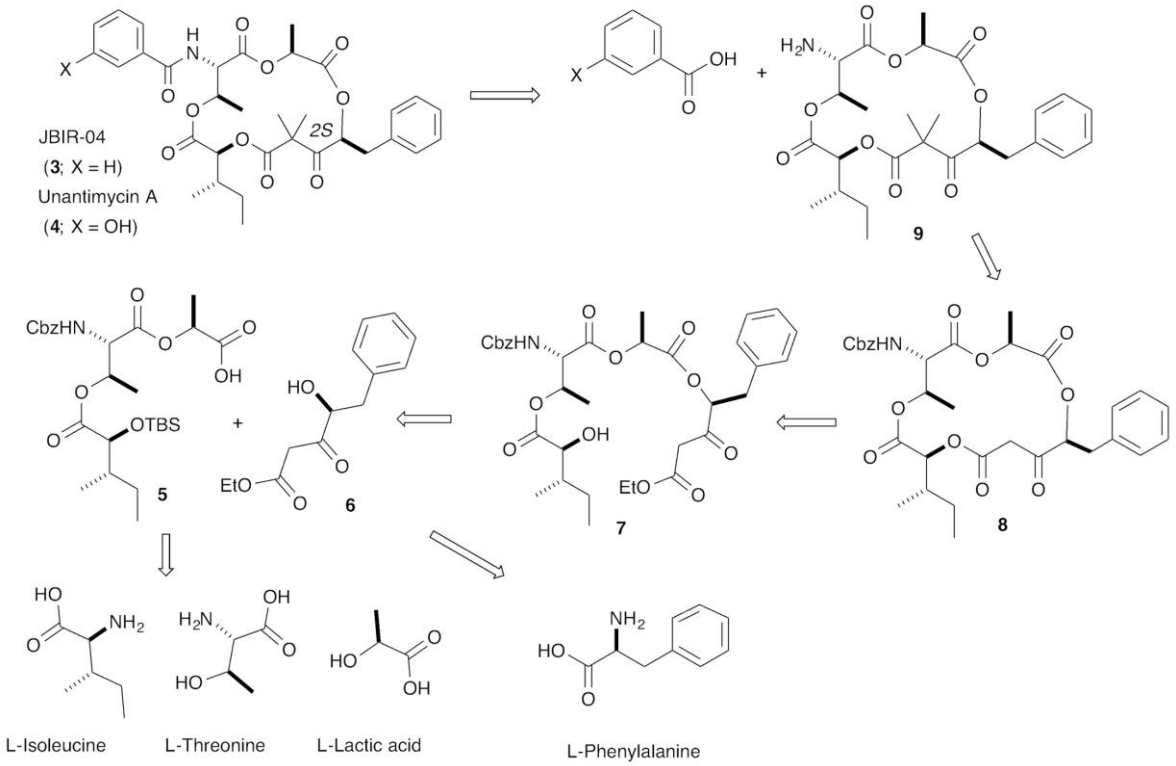
JBIR-04 (**3**) was isolated from *Streptomyces violaceusniger* 4541-SVS3 by Shin-ya and coworkers in 2007[6]. It showed an inhibitory activity against the GRP78 expression induced by the stimulation of 2-deoxyglucose in HT1080 cells. Analyses of spectroscopic data revealed that the planar structure of **3** is a 15-membered tetralactone of neoantimycin-class antibiotics. Nevertheless, the absolute stereostructure of **3** has not yet been determined. In 2016, Hale reported spectroscopic comparison between natural JBIR-04 and its synthetic compound with a *2R* configuration[7], which is the same as that observed for prunustatin A and SW-163A[8], other members of the neoantimycin family. Significant differences were observed between the chemical shifts and coupling constants of H2 and H12: H2 natural δ 5.78 (dd, J = 10.9, 2.9 Hz), synthetic δ 5.67 (t, J = 5.8 Hz); H12 natural δ 3.40 (dd, J = 14.5, 2.9 Hz), δ 3.15 (dd, J = 14.5, 10.9 Hz), synthetic δ 3.35 (dd, J = 14.0, 6.0 Hz), δ 3.26 (dd, J = 14.0, 5.6 Hz). This result confirms that the absolute stereochemistry of JBIR-04 differs from that of prunustatin A; that is, it has a *2S* configuration.

Unantimycin A (**4**), isolated in 2016 by Osada and coworkers from a culture broth of *Streptomyces* sp. RK88-1355, is a unique member of the neoantimycin family of natural products[9]. The absolute stereostructure of **4** has also not yet been determined. The NMR data, including the H12 chemical shifts and coupling constants, of **4** were identical to those of **3**. The negative values of specific rotation were reported for **3** and **4** (−28.6° (c 0.01, MeOH) and −10.8° (c 0.1, $CHCl_3$), respectively), indicating that **4** would have the same 15-membered ring as that of **3**.

As an extension of our synthetic studies on neoantimycin-class antibiotics[10], we have been engaged in studies directed toward the total synthesis and stereochemical assignment of **3** and **4**. Herein, we report our success in these endeavors.

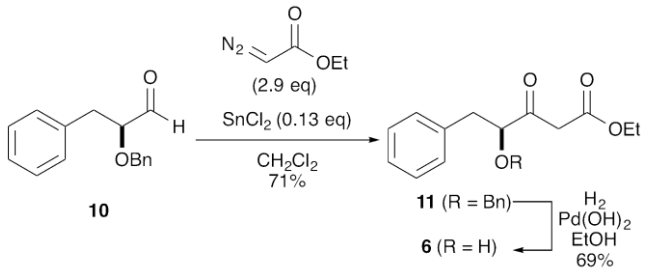
Results and discussion

We adopted a synthetic strategy for **3** and **4**, involving cyclization via transesterification of β-keto ester **7** followed by the late-stage introduction of *gem*-dimethyl groups at C11 (Scheme 1). Metal salt-mediated transesterification of β-keto ester would proceed under mild conditions, which minimizes the epimerization at other stereogenic centers



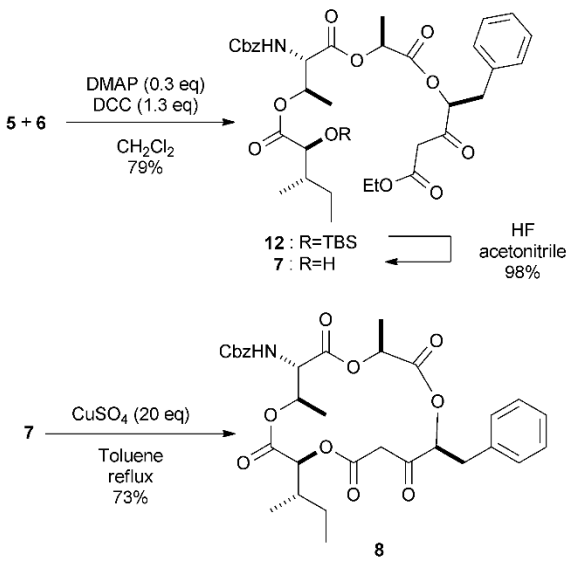
Scheme 1 Retrosynthetic analysis of JBIR-04 (**3**) and unantimycin A (**4**).

Our total synthesis of **3** and **4** commenced with the reaction of aldehyde **10**, derived from L-phenylalanine, with ethyl diazoacetate in the presence of $SnCl_2$ (0.13 equiv.) [11], which provided the corresponding β-keto ester **11** in 71% yield as a ca. 2.6 : 1 mixture of tautomers. Removal of the benzyl ether protecting group using $Pd(OH)_2$ in EtOH resulted in the formation of **6** in 69% yield (Scheme 2).



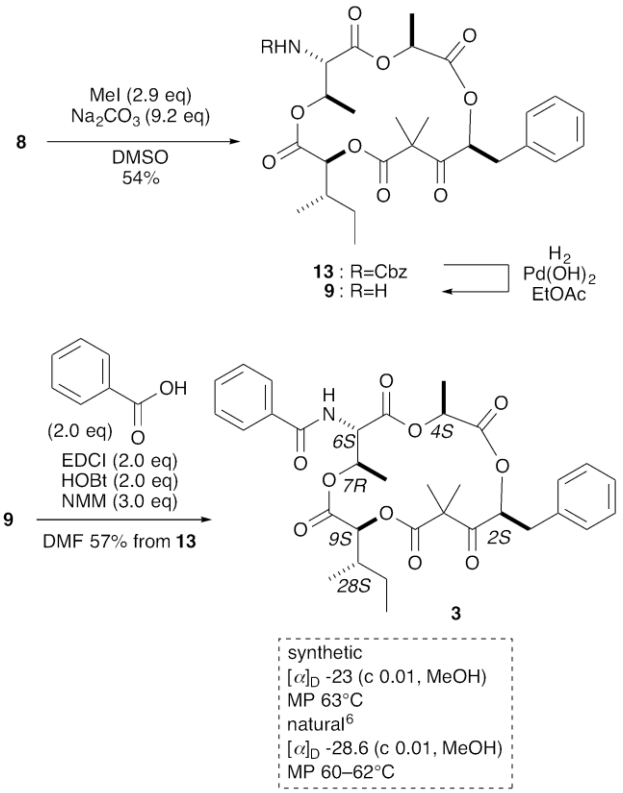
Scheme 2 Synthesis of β-keto ester **6**.

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 CH_3CN (Scheme 3). A mixture of **7** and anhydrous $CuSO_4$ (20 equiv.) in toluene was heated under reflux. The desired transesterification proceeded smoothly to provide the 15-membered tetralactone **8** in 73% yield.



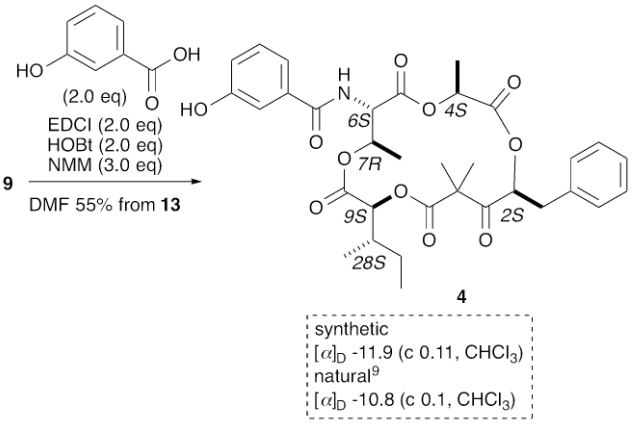
Scheme 3 Preparation and transesterification of **7**.

The introduction of *gem*-dimethyl groups at C11 was achieved by treatment of **8** with iodomethane (2.9 equiv.) and Na_2CO_3 (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 sample[6]. The optical rotation and melting point of synthetic **3** ($[\alpha]_D$ −23.0, c 0.01, MeOH; MP 63°C) were consistent with those of the natural product ($[\alpha]_D$ −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 sample[9]. The optical rotation and melting point of synthetic **4** ($[\alpha]_D$ −11.9, c 0.11, $CHCl_3$) were consistent with those of the natural product ($[\alpha]_D$ −10.8, c 0.1, $CHCl_3$).



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; (*2S*, *4S*, *6S*, *7R*, *9S*, *28S*) 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

^{1}H and ^{13}C 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 Me_4Si (0 ppm) and $CDCl_3$ (7.26 ppm for ^{1}H NMR and 77.0 ppm for ^{13}C 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 ^{1}H and ^{13}C 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 μm). Analytical and preparative thin-layer chromatography was performed on Merck precoated silica gel (#5715 Kieselgel 60F_{254} 0.25 mm and #5744 Kieselgel 60F_{254} 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 CH_2Cl_2 (14 mL) was added to ethyl diazoacetate (2.68 g, 23.5 mmol) in CH_2Cl_2 (14 mL) and anhydrous $SnCl_2$ (209 mg, 1.10 mmol) sequentially at 0°C. After being stirred overnight at 0°C, the reaction mixture was washed with aq. sat. $NaHCO_3$ before being dried over Na_2SO_4 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%): $[\alpha]_D$ = −50.4 (c 1.33, $CHCl_3$); Major (Keto form): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.00 (10H, m), 4.51–4.34 (2H, ABq, J = 11.7 Hz, υ = 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, υ = 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{20}H_{23}O_4$ 327.1596: found 327.1588 $[M+H]^+$. Minor (Enol form, diagnostic peaks only): ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 H_2. 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%): $[\alpha]_D$ = −8.0 (c 0.87, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 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). ^{13}C NMR (75 MHz, $CDCl_3$) 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 $C_{13}H_{16}NaO_4$ 259.0946: found 259.0961 $[M+Na]^+$.

Ethyl (*S*)-4-(((*5'S,8'R,9'S,12'S*)-9'-(((benzyloxy)carbonyl)amino)-5'-((*S*)-sec-butyl)-2',2',3',3',8',12'-hexamethyl-6',10'-dioxo-4',7',11'-trioxa-3'-silatridecan-13'-oyl)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 CH_2Cl_2 (2.7 mL), a solution of DCC (188 mg, 0.68 mmol) in CH_2Cl_2 (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%): $[\alpha]_D$ = −18.8 (c 0.51, CHCl3); ^{1}H NMR (400 MHz, $CDCl_3$) 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, υ = 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{40}H_{58}NO_{12}Si$ 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

Na_2SO_4, and concentrated in vacuo. The product was used in the next step without further purification (315 mg, 0.48 mmol, 98%): $[\alpha]_D$ = −3.28 (c 0.53, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 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, υ = 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{34}H_{44}NO_{12}$ 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 $CuSO_4$ (748 mg, 4.69 mmol) and **7** (154 mg, 246 µmol) 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 $CuSO_4$. 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 µmol, 73%). $[\alpha]_D$ = 2.4 (c 1.04, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) 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, *υ* = 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); ^{13}C NMR (75 MHz, $CDCl_3$) 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 $C_{32}H_{36}NO_{11}$ 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 Na_2CO_3 (92 mg, 1.35 mmol) in DMSO (1.4 mL) at r.t., a solution of β-keto ester **8** (56 mg, 91.6 µmol) in DMSO (1.3 mL) was added. Then, MeI (18 µL, 289 µmol) was added. The reaction mixture was stirred at r.t. for 4 h, diluted with H_2O, and extracted with *n*-hexane/EtOAc (1 : 1, 3x). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash column chromatography (20% EtOAc in *n*-hexane) afforded **13** as a colorless oil (31 mg, 54 µmol, 54%). $[\alpha]_D$ = −23.0 (c 0.94, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{34}H_{40}NO_{11}$ 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-pentaone (9). To a solution of benzyl carbamate **13** (17 mg, 27 µmol) in EtOAc (2 mL), 20% $Pd(OH)_2$ (6 mg) was added. The resulting suspension was stirred overnight under H_2. Then, the mixture was filtered through a pad of Celite®. The filtrate was concentrated to give amine **9** (13 mg, 26 µmol). The product was used in the next step without further purification. $[\alpha]_D$ = −90.3 (c 0.65, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{26}H_{36}NO_9$ 506.2390: found 506.2437 $[M+H]^+$.

JBIR-04 (3). To a solution of **9** (11 mg, 22 µmol) in DMF (1 mL), benzoic acid (12 mg, 98 µmol), HOBt (6 mg, 46 µmol), EDCI•HCl (9 mg, 46 µmol), and NMM (8 µL, 73 µmol) were added successively. The reaction mixture was stirred at r.t. for 14 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 3 as a colorless amorphous solid (9.3 mg, 15 µmol, 57%). MP = 63°C (lit.[4] MP = 60–62°C). $[\alpha]_D$ = −23 (c 0.01, MeOH) (lit.[6] $[\alpha]_D$ = −28.6 (c 0.01, MeOH)). ^{1}H NMR (600 MHz, $CDCl_3$) 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); ^{13}C NMR (150 MHz, $CDCl_3$) 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 $C_{33}H_{39}NNaO_{10}$ 632.2472: found 632.2472 $[M+Na]^+$.

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. $[\alpha]_D$ = −11.9 (c 0.11, $CHCl_3$), (lit.[9] $[\alpha]_D$ = −10.8 (c 0.1, $CHCl_3$)). ^{1}H 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 $C_{33}H_{39}NNaO_{11}$ 648.2421: found 648.2403 $[M+Na]^+$.

Acknowledgements

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

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