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Total synthesis of isoneoantimycin†

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Antimycins are one of the well-known antifungal metabolites produced by *Streptomyces* bacteria. Neoantimycin and its analogues, the ring-expanded antimycins featuring a 15-membered tetraester ring, have been shown to be effective regulators of the oncogenic proteins GRP78/BiP and K-Ras. Isoneoantimycin was isolated from *Streptomyces fradiae* IFO12773 (ISP 5063) as a minor metabolite during the fermentation of neoantimycin and is the first reported antibiotic of the antimycin family without the macrolide core. In this study, we explored the total synthesis and stereochemical assignment of isoneoantimycin as an approach to perform structure–activity studies on neoantimycins. Taking the neoantimycin biosynthesis pathway into account, we presumed that the stereochemistry of isoneoantimycin is the same as that of neoantimycin. The synthesis of our target molecule with the (1*S*,2*R*,5*S*,6*S*,14*R*,15*R*,17*S*) configuration has been achieved by using chiral-pool building blocks. A comparison of the spectroscopic data between the synthetic and natural samples verified our presumption of the stereochemistry of natural isoneoantimycin.

Introduction

Isoneoantimycin (**1**) was isolated from *Streptomyces fradiae* IFO12773 (ISP 5063) as a minor metabolite produced during the fermentation of neoantimycin (**2**) by Takeda and co-workers in 1998.[1] 2D NMR analyses and EIMS fragmentation revealed that the planar structure of compound **1** comprises units identical to those of neoantimycin; isoneoantimycin is the first reported antibiotic of the antimycin family without the macrolide core and consists of five components, namely γ-butyrolactone, α-hydroxy-isovaleric acid, L-threonine, α-hydroxy-β-methyl-valeric acid, and 3-formylaminosalicylic acid, as shown in Fig. 1. However, the absolute configuration of **1** could not be elucidated at that time.

After the discovery of antimycin A in 1949,[2] many antimycin-class depsipeptides have been reported.[3] However, very few papers on compounds without a macrolactone structure have been published.[1,4] The diverse biological activities of these antibiotics, including anti-cancer, antifungal, and immunosuppressant properties, have intrigued researchers and attracted significant attention from them.[5] Neoantimycin and its analogues, the ring-expanded antimycins, have been shown

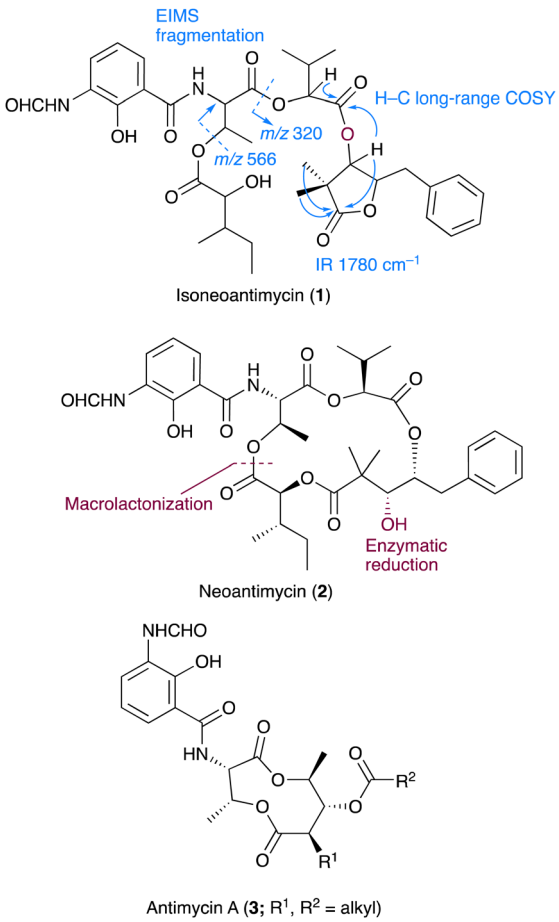

Fig. 1 Structures of isoneoantimycin (**1**), neoantimycin (**2**), and antimycin A (**3**).

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†Electronic supplementary information (ESI) available: Comparisons of NMR data of natural and synthetic compounds (Table S1), HREIMS fragmentation (Fig. S1), experimental details for known compounds and NMR spectra for new compounds. See DOI: https://doi.org/10.1039/d3ob00099k

to be effective regulators of the oncogenic proteins GRP78/BiP and K-Ras.[6]

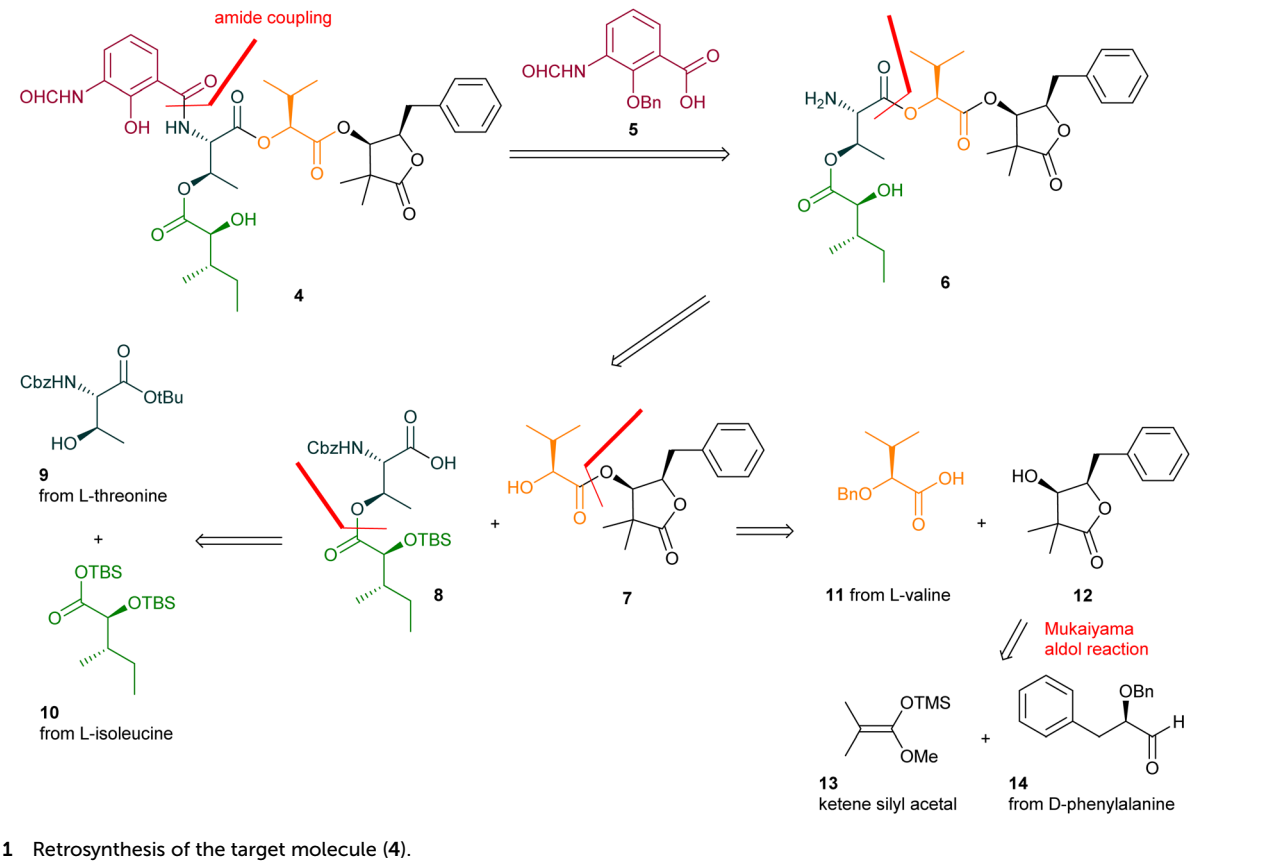
During our exploration of antimycin-type antibiotics,[7] we were engaged in studies directed toward the total synthesis and stereochemical assignment of **1** as an approach to perform structure–activity studies on neoantimycins. Herein, we report our success in these endeavours.

Results and discussion

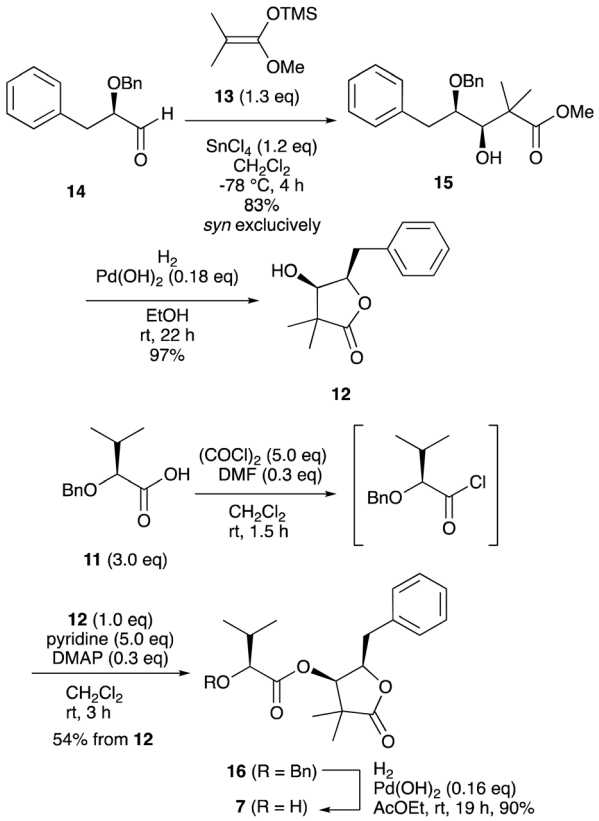
The absolute configuration of **2** has been confirmed, as shown in Fig. 1, by total synthesis.[7b] Recently, the biosynthetic pathway for neoantimycin production was proposed, wherein 3-formylaminosalicylic acid is employed as the starting substrate in the hybrid NRPS (nonribosomal peptide synthetase)/PKS (polyketide synthase) machinery for the growth of the depsipeptidal chain.[8] The subsequent macrolactonization and ketoreductase reduction provide neoantimycin. Taking the neoantimycin biosynthesis pathway into account, we presumed that the stereochemistry of **1** is the same as that of neoantimycin. Compound **4** was set as the target molecule for our synthesis (Scheme 1). Our synthetic strategy involves amide formation between amine **6** (four components linearly connected) and the protected 3-formylaminosalicylic acid **5**[9] to afford the desired compound (1*S*,2*R*,5*S*,6*S*,14*R*,15*R*,17*S*)-**4**. Conceptually, **6** could be divided into left and right units; the left part **8** would be obtained by the condensation of *N*-Cbz L-threonine *tert*-butyl ester **9**[10] and α-hydroxy carboxylic acid derivative **10**.[11] The right part **7** would be obtained by the condensation of α-hydroxy carboxylic acid **11**[12] with 3-hydoxy-γ-butyrolactone **12**, which is derived from D-phenylalanine.

The Mukaiyama aldol reaction of aldehyde **14**,[13] derived from D-phenylalanine, with the commercially available silyl ketene acetal **13** in the presence of $SnCl_4$ proceeded exclusively to afford the corresponding *syn*-**15** in 83% yield.[14] Hydrogenolysis of **15** with $Pd(OH)_2$ in EtOH resulted in the removal of the benzyl ether protecting group and this was followed by a spontaneous intramolecular trans-esterification to provide **12**[7b] in 97% yield. The *O*-Bn protected acid **11**,[12] prepared from L-valine, was treated with oxalyl chloride and a catalytic amount of DMF to produce the corresponding acid chloride, and the subsequent condensation with **12** provided the corresponding **16** in 54% yield. Reductive deprotection of the benzyl group with $Pd(OH)_2$ in AcOEt under a hydrogen atmosphere afforded the desired right fragment **7** in 90% yield (Scheme 2).

The commercially available *N*-Cbz L-threonine was converted into *tert*-Bu ester **9**[10] in 97% yield. Bis-TBS protected **10**, prepared from L-isoleucine, was treated with oxalyl chloride and a catalytic amount of DMF to produce the corresponding acid chloride intermediate and the subsequent condensation with **9** provided **17** in 93% yield. The *tert*-Bu group of **17** was selectively removed with TESOTf-2,6-lutidine in 1,2-dichlor-



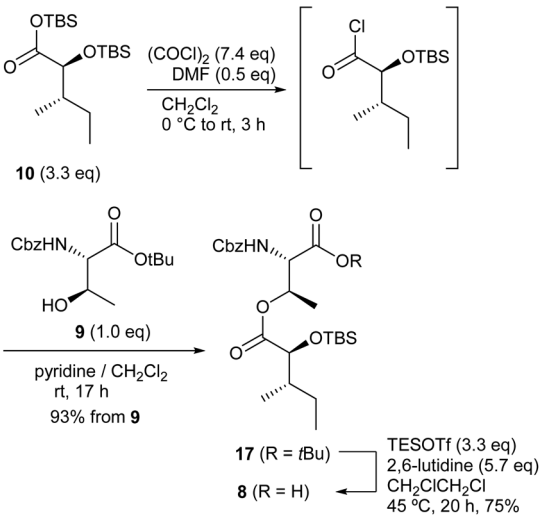
Scheme 1 Retrosynthesis of the target molecule (**4**).



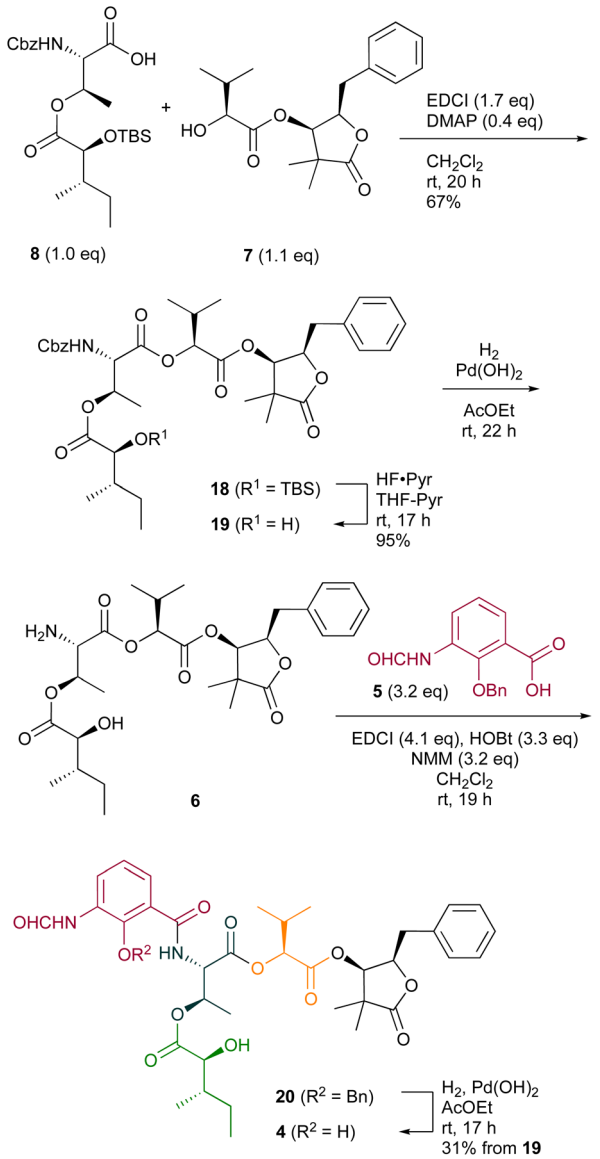
Scheme 2 Synthesis of the left fragment **7**.

oethane to afford the left fragment **8** in 75% yield (Scheme 3).[15]

Condensation of **7** and **8** with EDCI and DMAP provided the corresponding **18** in 67% yield (Scheme 4). In the endgame of the total synthesis of **4**, the TBS group of **18** was removed with HF·Pyr in THF-Pyr to afford **19** in 95% yield. The subsequent hydrogenolysis with $Pd(OH)_2$ in EtOAc provided



Scheme 3 Synthesis of the right fragment **6**.



Scheme 4 Endgame in the synthesis of **4**.

amine **6**. The subsequent condensation of **6** with benzyl ether **5**[9] using EDCI, HOBt, and NMM in DCM provided the corresponding amide **20**. Finally, the reductive removal of the benzyl ether protecting group was accomplished in the presence of a catalytic amount of $Pd(OH)_2$ in EtOAc under a hydrogen atmosphere to provide synthetic isoneoantimycin (**4**) in 31% yield from **19**.[16]

The optical rotation of synthetic **4** ($[\alpha]_D$ +40.9, *c* 0.51, EtOH) was consistent with that of the natural product ($[\alpha]_D$ +53.8, *c* 0.26, EtOH).[1] A comparison of the ^{1}H NMR chemical shifts of synthetic **4** with those reported for the natural sample **1** showed that the chemical shifts of the two methine CH protons at C6 and C18 were misperceived in ref. 1 (Fig. 2(a) red bar). Swapping the $\Delta\delta_H$ of C6 and the $\Delta\delta_H$ of C18 resulted in a good agreement (Fig. 2(a) blue bar). The ^{13}C NMR spectra of synthetic **4** was almost identical to those of the natural

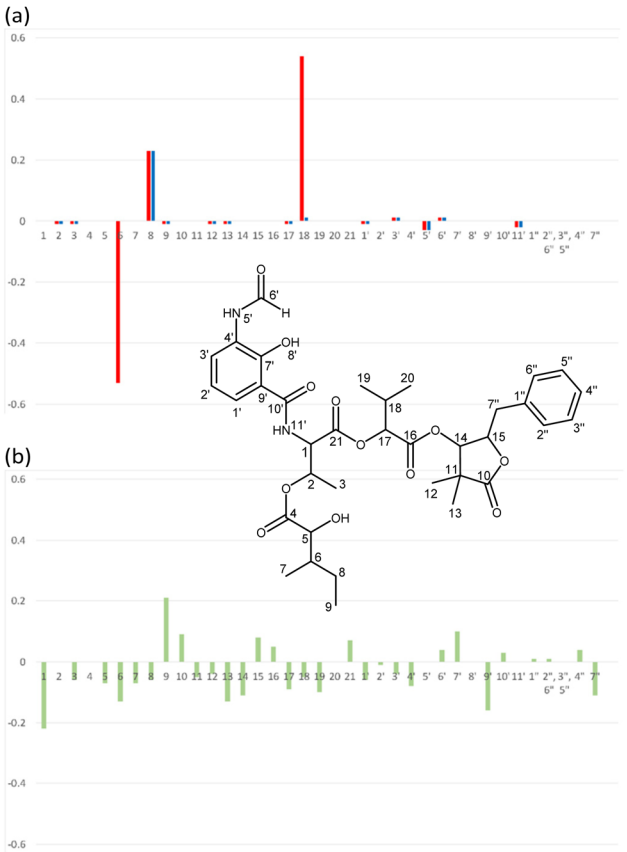


Fig. 2 Differences between the NMR chemical shifts of natural isoneoantimycin (**1**) and synthetic **4**. (a) $\Delta\delta_H$ (in ppm) = δ_H of **4** − originally reported δ_H of **1** (red bar) or corrected δ_H of **1** (blue bar). (b) $\Delta\delta_C$ (in ppm) = δ_C of **4** − originally reported δ_C of **1**.

product (Fig. 2(b)). Two fragment ions were observed at *m*/*z* 320.1625 and 566.2268 in the HREIMS spectrum of **4**, as before (Fig. S1†). However, chromatographic profiles have not been reported for the natural sample of isoneoantimycin as they not available at this moment. We could not ensure the absolute configuration of natural **1** any further.

Conclusions

In summary, the total synthesis of isoneoantimycin with the (1*S*,2*R*,5*S*,6*S*,14*R*,15*R*,17*S*) configuration has been achieved. A comparison of the spectroscopic data between synthetic **4** and natural **1** verified our presumption. Further studies on the biological activities of synthetic **4** are now in progress, and the results will be reported in due course.

Experimental

General information and materials

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Biospin AVANCE III HD 400 (400 MHz) or JEOL JNM-ECZ400S (400 and 100 MHz) or Bruker Avance III 600 (600 and 150 MHz) instrument. 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 NMR and ^{13}C NMR spectra. The chemical shifts are reported in δ (ppm) values relative to $CHCl_3$ (δ 7.26 ppm for ^{1}H NMR and δ 77.0 ppm for ^{13}C NMR) and to Me_4Si (δ 0.00 ppm for ^{1}H NMR). Mass spectra were obtained on a JEOL AccuTOF LC-plus JMS-T100LP (DART, ESI) spectrometer. HREIMS data were collected using a JEOL JMS-700 mass spectrometer. Infrared absorption spectra (IR) were measured using a JASCO FT/IR-4600 Fourier transform infrared spectrometer. UV spectra were recorded on a Shimadzu UV-1900 spectrophotometer. 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^{-1}.

All air and moisture-sensitive reactions were carried out in an argon-flushed 2-necked flask sealed with a rubber septum, and dried solvents and reagents were introduced using a syringe. Tetrahydrofuran (THF) was freshly distilled under an argon atmosphere from sodium benzophenone ketyl. Flash column chromatography was carried out using Kanto Chemical silica gel 60N (spherical, neutral, 40–50 μm), and pre-coated Merck silica gel plates (Art5715 Kieselgel 60F_{254}, 0.25 mm) were used for thin-layer chromatography (TLC). TLC visualization was performed using UV (254 nm) or a charring solution (ethanoic *p*-anisaldehyde, ethanoic phosphomolybdic acid).

Methyl (4*R*,3*R*)-4-benzyloxy-3-hydroxy-2,2-dimethyl-5-phenylpentanoate (15). To a solution of **14** [13] (1.47 g, 6.1 mmol) in CH_2Cl_2 (26 mL) was added $SnCl_4$ (1.0 M CH_2Cl_2 solution, 7.4 mL, 7.4 mmol) dropwise at −78 °C. After being stirred for 40 min at −78 °C, commercially available ketene silyl acetal **13** (1.61 g, 9.2 mmol) was added and then the mixture was stirred for 4 h at −78 °C. The reaction mixture was allowed to warm up to room temperature and quenched with sat. aq. $NaHCO_3$ (40 mL). The aqueous layer was extracted with Et_2O. The combined organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography (5% AcOEt in *n*-hexane) to give ester **15** (1.743 g, 5.1 mmol, 83%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.10 (m, 10H), 4.55 (d, J = 10.7 Hz, 1H), 4.36 (d, J = 10.7 Hz, 1H), 3.70 (ddd, J = 9.0, 5.2, 1.3 Hz, 1H), 3.39 (d, J = 11.0 Hz, 1H), 3.32 (s, 3H), 3.26 (dd, J = 10.9, 1.3 Hz, 1H), 3.08 (dd, J = 13.1, 5.2 Hz, 1H), 3.00 (dd, J = 13.1, 9.0 Hz, 1H), 1.21 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.34, 138.10, 137.70, 129.51, 128.53, 128.27, 128.16, 127.75, 126.30, 79.21, 77.36, 71.75, 51.52, 44.79, 36.95, 23.79, 22.21; HRMS (ESI$^+$) *m*/*z* calcd for $C_{21}H_{26}NaO_4$ $[M + Na]^+$ 365.17288, found 365.17324. IR (KBr): 3448, 3029, 2948, 2926, 1735, 1142, 1065, 743, 699 cm^{-1}; $[\alpha]_D$ = +14.0 (*c* 1.20, $CHCl_3$).

(4*R*,5*R*)-5-Benzyl-4-hydroxy-3,3-dimethylhydrofran-2-one (12). To a solution of **15** (731 mg, 2.13 mmol) in 99.5% EtOH (37 mL) was added 20% $Pd(OH)_2$ (273 mg). The inner atmosphere was purged with H_2. After being stirred overnight at

room temperature, the resulting mixture was filtered through a pad of Celite® and the filtrate was concentrated. The residue was purified by flash column chromatography (30% AcOEt in *n*-hexane) to give **12** (455 mg, 2.07 mmol, 97%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.21 (m, 5H), 4.70 (td, J = 7.2, 3.4 Hz, 1H), 3.97–3.85 (m, 1H), 3.20 (dd, J = 13.9, 7.1 Hz, 1H), 3.10 (dd, J = 13.9, 7.4 Hz, 1H), 1.88 (br s, 1H), 1.28 (s, 3H), 1.23 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.76, 136.65, 129.28, 128.67, 126.84, 81.58, 76.48, 45.86, 34.66, 22.57, 17.78; HRMS (ESI$^+$) m/z calcd for $C_{13}H_{17}O_3$ $[M + H]^+$ 221.11777, found 221.11857; IR (KBr): 3369, 3027, 2977, 2942, 1752, 1092, 1007, 717, 697 cm^{-1}; $[\alpha]_D$ = +63.8 (c 0.91, $CHCl_3$). Lit[7b] $[\alpha]_D$ = +70.5 (c 0.12, $CHCl_3$).

(2*R*,3*R*)-2-Benzyl-4,4-dimethyl-5-oxotetrahydrofuran-3-yl (*S*)-2-benzyloxy-3-methylbutanoate (16). To a mixture of **11**[12] (565 mg, 2.7 mmol) and DMF (2 drops) in CH_2Cl_2 (5 mL) was added oxalyl chloride (390 μL, 4.5 mmol) dropwise at 0 °C. The mixture was stirred at room temperature for 1.5 h. Then, the volatiles were removed *in vacuo*. To the crude carboxylic acid chloride thus obtained were added CH_2Cl_2 (1.0 mL), pyridine (370 μL, 4.5 mmol) and DMAP (30 mg, 0.25 mmol) at room temperature. After being stirred for 10 min, a solution of **12** (198 mg, 0.90 mmol) in CH_2Cl_2 (1.0 mL) was added and then stirred at room temperature for 3 h. The reaction mixture was quenched by adding brine (30 mL). The aqueous layer was extracted with AcOEt (60 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography (30% AcOEt in *n*-hexane) to give **16** (0.200 g, 0.488 mmol, 54%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.17 (m, 10H), 5.40 (d, J = 3.8 Hz, 1H), 4.80 (dt, J = 9.1, 4.0 Hz, 1H), 4.71 (d, J = 11.6 Hz, 1H), 4.40 (d, J = 11.6 Hz, 1H), 3.85 (d, J = 4.4 Hz, 1H), 2.99 (dd, J = 14.5, 9.1 Hz, 1H), 2.83 (dd, J = 14.5, 4.2 Hz, 1H), 2.23–2.11 (m, 1H), 1.34 (s, 3H), 1.21 (s, 3H), 1.04 (d, J = 6.9 Hz, 3H), 0.98 (d, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.24, 171.63, 137.38, 136.52, 129.22, 128.89, 128.59, 128.15, 128.10, 127.25, 82.70, 80.08, 78.26, 73.12, 44.84, 35.58, 31.62, 22.98, 19.57, 18.51, 17.01; HRMS (ESI$^+$) m/z calcd for $C_{25}H_{30}NaO_5$ $[M + Na]^+$ 433.19909, found 433.19734; IR (KBr): 2965, 1750, 1497, 1457, 1387, 1347, 1190, 1133, 1109, 1073, 1003, 733, 697, 620 cm^{-1}; $[\alpha]_D$ = +32.9 (c 0.54, CH_2Cl_2).

(2*R*,3*R*)-2-Benzyl-4,4-dimethyl-5-oxotetrahydrofuran-3-yl (*S*)-2-hydroxy-3-methylbutanoate (7). To a solution of **16** (312 mg, 0.759 mmol) in AcOEt (13 mL) was added 20% $Pd(OH)_2$ (83 mg). The inner atmosphere was purged with H_2. After being stirred overnight at room temperature, the resulting mixture was filtered through a pad of Celite® and the filtrate was concentrated. The residue was purified by flash column chromatography (30% AcOEt in *n*-hexane) to give **7** (218 mg, 0.68 mmol, 90%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.04 (m, 5H), 5.31 (d, J = 3.7 Hz, 1H), 4.76 (dt, J = 8.6, 4.2 Hz, 1H), 4.12 (dd, J = 6.4, 3.0 Hz, 1H), 2.99 (dd, J = 14.4, 8.8 Hz, 1H), 2.79 (dd, J = 14.4, 4.8 Hz, 1H), 2.49 (d, J = 6.9 Hz, 1H), 2.08 (pd, J = 6.8, 3.0 Hz, 1H), 1.35 (s, 3H), 1.21 (s, 3H), 1.11 (d, J = 6.9 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.04, 174.30, 136.09, 129.16, 128.94, 127.31, 79.98, 79.04, 75.24, 44.77, 35.37, 32.01, 22.96, 19.52, 18.41, 15.54. HRMS (ESI$^+$) m/z calcd for $C_{18}H_{24}NaO_5$ $[M + Na]^+$ 343.15214, found 343.15006.

IR (KBr): 3490, 2973, 2936, 2876, 1765, 1458, 1393, 1207, 1148, 2048, 1025, 1005, 979, 711, 624 cm^{-1}; $[\alpha]_D$ = +108.8 (c 1.78, CH_2Cl_2).

(2*R*,3*S*)-3-Benzyloxycarbonylamino-4-*tert*-butoxy-4-oxobutan-2-yl (2*S*,3*S*)-2-*tert*-butyldimethylsilyloxy-3-methylpentanoate (17). To a mixture of freshly prepared **10**[11] (1.10 g) and DMF (35 μL, 0.45 mmol) in CH_2Cl_2 (9.0 mL) was added oxalyl chloride (0.55 mL, 6.41 mmol) dropwise at 0 °C. The mixture was stirred at 0 °C for 2 h and then warmed to room temperature and stirred for an additional 1 h. Then, the volatiles were removed *in vacuo*. To the crude carboxylic acid chloride thus obtained were added a solution of alcohol **9**[10] (269 mg, 0.869 mmol) in CH_2Cl_2 (0.5 mL) and pyridine (3.7 mL) dropwise at room temperature. After being stirred for 14 h, the reaction mixture was diluted with THF (120 mL) and filtered through a pad of Celite® and the filtrate was concentrated *in vacuo*. The crude residue was dissolved in AcOEt (50 mL) and then washed with H_2O, sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash column chromatography (10% AcOEt in *n*-hexane) afforded ester **17** as a colorless oil (436 mg, 0.81 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.28 (m, 5H), 5.43 (td, J = 6.4, 2.3 Hz, 1H), 5.39 (d, J = 9.8 Hz, 1H), 5.21–5.07 (m, 2H), 4.37 (dd, J = 9.7, 2.3 Hz, 1H), 4.18 (d, J = 3.7 Hz, 1H), 3.99 (d, J = 4.2 Hz, 1H), 1.83–1.69 (m, 1H), 1.57–1.46 (m, 1H), 1.44 (s, 9H), 1.29 (d, J = 6.4 Hz, 3H), 1.23–1.11 (m, 1H), 0.94 (s, 9H), 0.91 (s, 3H), 0.89 (s, 9H), 0.85 (t, J = 7.4 Hz, 3H), 0.13 (d, J = 7.3 Hz, 3H), 0.10 (s, 3H), 0.02 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.29, 168.82, 156.75, 136.36, 128.68, 128.35, 128.21, 82.91, 77.36, 76.41, 71.08, 67.36, 58.28, 39.65, 28.11, 25.85, 24.00, 18.33, 17.41, 15.78, 11.76, −4.67, −5.20; HRMS (ESI$^+$) m/z calcd for $C_{28}H_{47}NaO_7Si$ $[M + Na]^+$ 560.30197, found 560.29795. IR (KBr): 2960, 2933, 2858, 1734, 1517, 1461, 1255, 1143, 1065, 838, 778 cm^{-1}; $[\alpha]_D$ = −2.6 (c 1.06, CH_2Cl_2).

***N*-Benzyloxycarbonyl-*O*-(2*S*,3*S*)-2-*tert*-butyldimethylsilyloxy-3-methylpentanoyl-L-threonine (8).** To a stirred solution of *tert*-butyl ester **17** (491 mg, 0.913 mmol) in CH_2ClCH_2Cl (9.8 mL) were added 2,6-lutidine (0.60 mL, 5.18 mmol) and TESOTf (0.65 mL, 3.02 mmol) successively at 0 °C. The reaction mixture was stirred at 45 °C for 20 h at room temperature, diluted with phosphate buffer (pH 7), acidified with 1 M aq. $KHSO_4$ and then extracted with AcOEt (23 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash column chromatography (30% to 100% AcOEt in *n*-hexane) afforded carboxylic acid **8** as a colorless oil (328 mg, 0.68 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (br s, 1H), 7.41–7.29 (m, 5H), 5.50 (dq, J = 6.3, 2.5 Hz, 1H), 5.42 (d, J = 9.6 Hz, 1H), 5.23–5.08 (m, 2H), 4.56 (dd, J = 9.7, 2.6 Hz, 1H), 3.97 (d, J = 4.9 Hz, 1H), 1.80–1.63 (m, 1H), 1.47–1.36 (m, 1H), 1.33 (d, J = 6.4 Hz, 3H), 1.15 (m, 1H), 0.89 (s, 9H), 1.29 (d, J = 6.3 Hz, 3H), 1.25–1.10 (m, 1H), 0.94 (s, 6H), 0.89 (s, 9H), 0.87–0.82

(m, 3H), 0.00 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.04, 174.30, 136.09, 129.16, 128.94, 127.31, 79.98, 79.04, 77.36, 75.24, 44.77, 35.37, 32.01, 22.96, 19.52, 18.41, 15.54; HRMS (ESI^+) m/z calcd for $C_{24}H_{39}NNaO_7Si$ $[M + Na]^+$ 504.23935, found 504.23448; IR (KBr): 2961, 2933, 1751, 1735, 1710, 1509, 1253, 1155, 1144, 1089, 1065, 839, 777 cm^{-1}; $[\alpha]_D$ = +4.14 (c 1.85, CH_2Cl_2).

(2*R*,3*S*)-4-(((*S*)-1-(((2*R*,3*R*)-2-Benzyl-4,4-dimethyl-5-oxotetrahydrofuran-3-yl)oxy)-3-methyl-1-oxobutan-2-yl)oxy)-3-benzyloxycarbonylamino-4-oxobutan-2-yl-(2*S*,3*S*)-2-*tert*-butyldimethylsilyloxy-3-methylpentanoate (18). To a stirred solution of **8** (228 mg, 0.47 mmol), **7** (167 mg, 0.52 mmol) and DMAP (25 mg, 0.21 mmol) in CH_2Cl_2 (2.8 mL) was added a solution of EDCI (125 mg, 0.81 mmol) in CH_2Cl_2 (2.9 mL) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 20 h. The crude residue was diluted with water (40 mL) and then extracted with CH_2Cl_2 (12 mL × 3); the combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (30% AcOEt in *n*-hexane) to give compound **18** as a colorless oil (249 mg, 0.32 mmol, 67%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.21 (m, 10H), 5.51–5.43 (m, 1H), 5.38 (d, J = 9.6 Hz, 1H), 5.32–5.27 (m, 1H), 5.14 (s, 2H), 4.96 (d, J = 2.9 Hz, 1H), 4.75 (dt, J = 9.1, 3.8 Hz, 1H), 4.61 (dd, J = 9.6, 3.2 Hz, 1H), 4.06 (d, J = 3.9 Hz, 1H), 3.07 (dd, J = 14.7, 9.1 Hz, 1H), 2.88 (dd, J = 14.7, 3.9 Hz, 1H), 2.35–2.29 (m, 1H), 1.82–1.71 (m, 1H), 1.38 (d, J = 6.4 Hz, 3H), 1.30 (s, 3H), 1.18 (s, 3H), 1.10 (d, J = 6.9 Hz, 3H), 0.97 (d, J = 6.9 Hz, 3H), 0.90 (s, 9H), 0.84 (t, J = 7.4 Hz, 3H), 0.02 (s, 3H), −0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.29, 168.82, 156.75, 136.36, 128.68, 128.35, 128.21, 82.91, 77.36, 76.41, 71.08, 67.36, 58.28, 39.65, 28.11, 25.85, 24.00, 18.33, 17.41, 15.78, 11.76, −4.67, −5.20; HRMS (ESI^+) m/z calcd for $C_{42}H_{61}NO_{11}Si$ $[M + Na]^+$ 806.3912, found 806.3883; IR (KBr): 3435, 1655, 1560, 1459, 1088, 820 cm^{-1}; $[\alpha]_D$ = +26.9 (c 0.88, CH_2Cl_2).

(2*R*,3*S*)-4-(((*S*)-1-(((2*R*,3*R*)-2-Benzyl-4,4-dimethyl-5-oxotetrahydrofuran-3-yl)oxy)-3-methyl-1-oxobutan-2-yl)oxy)-3-benzyloxycarbonylamino-4-oxobutan-2-yl-(2*S*,3*S*)-2-hydroxy-3-methylpentanoate (19). Silyl ether **18** (246 mg, 0.314 mmol) was weighed into a Teflon test tube. THF (3.6 mL) and pyridine (0.62 mL) were added, and then HF-Py (0.60 mL) was added to the solution. The mixture was stirred for 17 h. The reaction was quenched with sat. aq. $NaHCO_3$ and the mixture was extracted with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash column chromatography (30% AcOEt in *n*-hexane) afforded **19** as a colorless oil (201 mg, 0.30 mmol, 95%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.21 (m, 10H), 5.62 (qd, J = 6.3, 2.8 Hz, 1H), 5.39 (d, J = 9.6 Hz, 1H), 5.28 (d, J = 3.6 Hz, 1H), 5.14 (s, 2H), 4.97 (d, J = 2.9 Hz, 1H), 4.75 (td, J = 9.4, 3.7 Hz, 1H), 4.68 (dd, J = 9.7, 2.8 Hz, 1H), 4.03–3.97 (m, 1H), 3.04 (dd, J = 14.6, 9.3 Hz, 1H), 2.87–2.77 (m, 1H), 2.30 (pd, J = 6.7, 2.8 Hz, 1H), 1.77 (m, 1H), 1.68 (q, J = 1.5 Hz, 1H), 1.38 (d, J = 6.4 Hz, 3H), 1.30 (s, 3H), 1.17 (s, 3H), 1.10 (d, J = 6.9 Hz, 3H), 0.96 (t, J = 6.5 Hz, 6H), 0.89 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.00, 173.88, 169.43, 168.34, 156.66, 136.65, 135.98, 129.37, 128.81, 128.72, 128.51, 128.30, 127.11, 80.39, 79.20, 77.54, 74.61, 71.64, 67.69, 57.50, 44.67, 38.83, 35.35, 29.96, 23.98, 22.88, 19.53, 18.41, 16.81, 16.34, 15.43, 11.87; HRMS (ESI^+) m/z calcd for $C_{36}H_{47}NNaO_{11}$ $[M + Na]^+$ 692.30468, found 692.30167; IR (KBr): 3449, 3351, 2968, 2936, 1751, 1514, 1392, 1203, 1131, 1063, 735, 698 cm^{-1}; $[\alpha]_D$ = +43.2 (c 1.27, CH_2Cl_2).

(2*R*,3*S*)-3-Amino-4-(((*S*)-1-(((2*R*,3*R*)-2-benzyl-4,4-dimethyl-5-oxotetrahydrofuran-3-yl)oxy)-3-methyl-1-oxobutan-2-yl)oxy)-4-oxobutan-2-yl-(2*S*,3*S*)-2-hydroxy-3-methylpentanoate (6). To a solution of **19** (178 mg, 0.27 mmol) in AcOEt (17 mL) was added 20% $Pd(OH)_2$ (112 mg). The inner atmosphere was purged with H_2. After being stirred overnight at room temperature, the resulting mixture was filtered through a pad of Celite® and the filtrate was concentrated to give amine **6**. This was used for the next reaction without further purification.

Isoneoantimycin (4, synthetic). To a solution of **6** (132 mg, 0.25 mmol) in CH_2Cl_2 (10 mL) were added 2-benzyloxy-3-formylaminobenzoic acid **5** [9](216 mg, 0.80 mmol), HOBt (109 mg, 0.81 mmol), EDCI·HCl (155 mg, 1.00 mmol), and NMM (87 μL, 0.79 mmol) successively. The reaction mixture was stirred at room temperature overnight, diluted with water, and extracted 3 times with AcOEt. The combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The resulting amide **20** was used for the next step without further purification.

To a solution of this material (401 mg) in AcOEt (15 mL) was added 20% $Pd(OH)_2$ (90 mg). The inner atmosphere was purged with H_2. After being stirred overnight at room temperature, the resulting mixture was filtered through a pad of Celite® and the filtrate was concentrated. The residue was purified by flash column chromatography (30% AcOEt in *n*-hexane) to give the white solid **4** (57.8 mg, 83 μmol, 31% for 3 steps) as a 6 : 1 mixture of rotamers; ^{1}H NMR (600 MHz, $CDCl_3$) Major isomer δ 12.54 (s, 1H), 8.54 (dd, J = 8.1, 1.3 Hz, 1H), 8.49 (d, J = 1.7 Hz, 1H), 7.91 (s, 1H), 7.34–7.24 (m, 3H), 7.22 (dd, J = 8.1, 1.3 Hz, 1H), 7.22 (dd, J = 8.1, 1.3 Hz, 1H), 7.01 (d, J = 8.8 Hz, 1H), 6.92 (t, J = 8.1 Hz, 1H), 5.68 (qd, J = 6.5, 3.0 Hz, 1H), 5.32 (d, J = 3.7 Hz, 1H), 5.14 (dd, J = 8.8, 3.1 Hz, 1H), 5.02 (d, J = 2.9 Hz, 1H), 4.77 (dt, J = 9.5, 3.5 Hz, 1H), 4.10 (d, J = 4.0 Hz, 1H), 3.02 (dd, J = 14.7, 9.5 Hz, 1H), 2.83 (dd, J = 14.6, 3.5 Hz, 1H), 2.37–2.31 (m, 1H), 1.84–1.77 (m, 1H), 1.42 (d, J = 6.4 Hz, 3H), 1.43–1.37 (m, 1H), 1.32 (s, 3H), 1.31–1.24 (m, 1H), 1.19 (s, 3H), 1.13 (d, J = 6.9 Hz, 3H), 1.00 (d, J = 6.9 Hz, 3H), 0.97 (d, J = 6.9 Hz, 3H), 0.91 (d, J = 7.4 Hz, 1H); minor isomer (diagnostic peaks only) δ 12.40 (s, 1H), 8.77 (d, J = 11.5 Hz, 1H), 7.78 (d, J = 11.4 Hz, 1H), 7.37 (d, J = 7.9 Hz, 1H), 1.51 (d, J = 6.4 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) Major isomer δ 178.95, 173.96, 170.29, 169.03, 168.31, 159.10, 150.71, 136.67, 129.36, 128.86, 127.58, 127.20, 125.02, 120.20, 119.26, 112.90, 80.34, 79.45, 77.87, 74.59, 71.86, 55.44, 44.71, 39.13, 35.45, 30.01, 24.40, 22.92, 19.56, 18.43, 17.10, 16.36, 15.29, 11.97; minor isomer (diagnostic peaks only) δ 161.07, 151.43, 129.32, 127.30, 120.97, 120.54; HRMS (ESI^+) m/z calcd for $C_{36}H_{46}N_2NaO_{12}$ $[M + Na]^+$ 721.29484, found 721.29106; UV λ_{max}^{EtOH} nm (ε) 346 (3842), 182 (16 384); IR (KBr): 3369, 2968, 2935, 2877, 1751, 1686, 1534, 1364, 1251, 1200, 1132, 1067 cm^{-1}; $[\alpha]_D^{25}$ = +42.0 (c 0.19, EtOH).

Conflicts of interest

There are no conflicts to declare.

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16 The ^{1}H and ^{13}C NMR spectra indicated that synthetic **4** existed as an *ca.* 6 : 1 mixture of two rotamers. This phenomenon was also observed in the total synthesis of 15-membered ring neoantimycin antibiotics.7*a*,*b*.

