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Exploiting the natural metabolic diversity of *Streptomyces venezuelae* to generate unusual reduced macrolides

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Materials. HPLC-grade acetic acid, MeCN, EtOAc, MeOH, and H_2O were from J. T. Baker (Phillipsburg, NJ). The PKS inhibitor cerulenin, Trizma base, phenylmethylsulphonyl fluoride (PMSF), and glass beads (150 to 212 μm in diameter) were obtained from Sigma (St. Louis, MO). Ammonium acetate and 2-mercaptoethanol were from Fluka Chemie GambH (Steinheim, Germany), and Advantec filter paper (6 mm diameter, Toyo Ltd, Tokyo) was purchased for antibacterial bioassay. The propagation and culture conditions of the bacterial strains *Streptomyces venezuelae* ATCC 15439, its engineered derivatives DHS2001, YJ003, and YJ028, as well as five species of streptomycete, *S. avermitilis* NRRL 8165, *S. hygroscopicus* NRRL 5491, *S. fradiae* NRRL 2702, *S. coelicolor* A3(2), and *S. lividans* TK24 have been described previously.[1] *Bacillus subtilis* ATCC 23857, which is susceptible to macrolide antibiotics, was used for the antibacterial bioassay. A reversed-phase preparative HPLC column (ODS-BP, 5 μm, 250 × 10 mm, Watchers, Japan) and a reversed-phase analytical HPLC column (Nova-Pak C_{18}, 4 μm, 150 × 3.9 mm, Waters, Milford, MA) were employed in this study.[2] Pikromycin (**8**), which is routinely used as a precursor for *in vivo* and *in vitro* bioconversion tests, was isolated from extracts obtained from a culture of the wild type strain of *S. venezuelae* ATCC 15439,[2] and its purity was then checked by HPLC-ESI-MS/MS. 10-Deoxymethynolide (**1**) and narbonolide (**2**) were synthesized,[3] after which their purity was checked by HPLC-ESI-MS/MS analysis. Macrolides originating from different actinomycetes including avermectins (**21**), rosamicin (**23**), and rapamycin (**24**) were purchased from Sigma and then used for *in vivo* bioconversion without further purification. However, 5-*O*-desosaminyl tylactone (**22**),[4] heterologously produced by *S. venezuelae* YJ005, was purified by preparative HPLC system followed by solid-phase extraction (SPE) procedure using an OASIS HLB cartridge (Waters), as previously described.[2]

***In vivo* Bioconversion of Macrolides.** The engineered strains of *S. venezuelae* DHS2001 (a *pik* PKS gene-deleted mutant), YJ003 (a *des* gene-deleted mutant), and YJ028 (a both *pik* PKS and *des* genes-deleted mutant) have been described previously.[5] These organisms were initially streaked for isolation on SPA agar plates (0.1% yeast extract, 0.1% beef extract, 0.2% tryptose, 1.0% glucose, 1.5% agar, and trace amount of $FeSO_4$) and then incubated at 30℃ for 2 d. Isolated colonies were then used to inoculate 50 ml of SCM media (1.5% soluble starch, 2.0% soytone, 0.01% CaCl2, 0.15% yeast extract, and 1.0% MOPS) in 500 ml baffled Erlenmeyer flasks. Following 2 d of growth at 30℃, the cultures were fed with 250 μg (final concentration 5 μg/mL, dissolved in <125 μL of MeOH) of one of the macrolactone aglycones, and then further incubated for 3 d. The whole cultures were then extracted and partitioned using an equal volume of EtOAc two times in a 250 mL of separatory funnel, after which the organic extracts were combined and concentrated under a vacuum. The crude residues were then reconstituted in MeOH, after which they were diluted with 9 volumes of H_2O, and then purified by SPE cleanup using an OASIS HLB extraction cartridge (Waters, 60 mg). The eluants (dissolved in MeOH) from the cartridges were then evaporated to dryness at room temperature by vacuum centrifugation. Next, the dried eluants were immediately dissolved in 200 μL of MeOH and a portion of this solvent was subjected to HPLC-ESI-MS analysis. The other *Streptomyces* species were grown for 2 d in different media under the culture conditions recommended by the American Type Culture Collection (ATCC), which were as follows;[1] for *S. avermitilis*, glucose asparagines medium at 26℃; for *S. hygroscopicus*, YEME medium at 26℃; for *S. fradiae*, oatmeal medium at 26℃; for *S. coelicolor*, YEME medium at 28℃, for *S. lividans*, YEME medium at 28℃. The cultures were supplemented with **8** at a final concentration of 5 μg/mL and then incubated for additional 3 d, after which they were subjected to the same extraction procedures described above and analyzed by HPLC-ESI-MS analysis. To determine if the hydrogenation activity of *S. venezuelae* is flexible toward several types of heterologous unsaturated macrolides **21** to **24**, each of these macrolides was supplemented into a *S. venezuelae* YJ028 culture that was then analyzed using the same procedures described above. All of the *in vivo* experiments were independently carried out in duplicate.

***In vitro* Bioconversion of Macrolides Using Cell-free Extracts.** Cell-free extracts (CFEs) of the engineered strains of *S. venezuelae* DHS2001, YJ003, and YJ028 were prepared by glass-bead homogenization.[1,6] To accomplish this, the mycelium (8 g wet weight) of each strain was cultivated in SCM medium for 3 days at 30℃, harvested by centrifugation, washed twice with 0.1 M Tris-HCl (pH 7.6), and then re-suspended in 20 ml of extraction buffer (0.1 M Tris-HCl, 10 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 1 mM PMSF, pH 7.6) at 4℃. Next, 15 g of pre-cooled glass beads (Sigma) were added, after which the mycelium was pulverized by vigorous agitation using a vortex mixer. Specifically, the samples were vortexed ten times for 30 sec each, with intermittent cooling in ice. Next, the glass beads were removed by low-speed centrifugation, and then the cellular debris was removed by centrifugation at 18,000×g for 20 min. The entire process was carried out at 4℃ and generated 10 mL of resulting

supernatant. To each aliquot of CFE 200 μg (finally concentration 20 μg/mL, dissolved in <80 μL of MeOH) of substrate, **8** or 8,9-dihydro-10-deoxymethynolide (**19,** see synthetic procedures described below), were added. The resulting reaction mixtures were then incubated at 30℃ for 12 h before being quenched with 10 mL of EtOAc. Next, the organic residues were subjected to the same extraction and purification steps to prepare them for HPLC-ESI-MS analysis (see conditions described below). The effect of a PKS-inhibitor on the hydrogenation activity of a CFE from *S. venezuelae* DHS2001 was evaluated by adding cerulenin (from 10 to 100 μM) to aliquots of the CFE that had been supplemented with **8**, as described above. Independent experiments were performed in triplicate.

HPLC-ESI-MS/MS Analysis of Macrolides Obtained from Bioconversions. Analyses of the macrolides were performed using a Waters/Micromass Quattro *micro*/MS interface comprised of a Waters 2695 separation module connected directly to a Micromass Quattro *micro* MS. Separation was performed on a 150 × 3.9 mm Nova-Pak C_{18} (4.0 μm, Waters) reversed-phase column. The analytes were eluted at a flow rate of 180 μL/min with a gradient of 5 mM (w/v) ammonium acetate, 0.05% HOAc (v/v) in H_2O (A) and 80% (v/v) MeCN with the same additives concentration (B) at 20% to 70% B for 25 min, to 90% B for 20 min, maintained at 90% B for 10 min, and then to 20% B for another 10 min for column re-equilibration. The column effluent was directed to the ESI-MS, which was operated in the positive ion mode, without splitting. The instrument was calibrated by the direct infusion of a stock solution of authentic **8** (100 μg/ml) into the ion source at a rate of 60 μL/min. The optimization parameters of the ESI-MS/MS system were based on the maximum generation, first of the protonated molecular ions (parents) and then of the corresponding fragment (product) ion (i.e. desosaminyl moiety at *m/z* 158.1). The following calibration parameters were retained to enable the optimum HPLC-ESI-MS/MS detection of the macrolides: the capillary voltage and cone voltage were 4 kV and 35 V, respectively; the source and desolvation temperatures were 130 and 250℃, respectively; the desolvation gas and cone gas flow rates were 500 and 50 liter/h, respectively. The scan mass range was *m/z* 150 to 1,000 Da when run in the scan mode. The collision energy in the MS/MS mode, which concurred with the full argon-induced fragmentation of the parent molecules, was found to be 1.5 V.

Isolation and Identification of the Reduced Macrolides Obtained from *in vivo* Bioconversions. To prepare crude extracts containing 14-membered reduced macrolides, six litres of culture broth from *S. venezuelae* DHS2001, in which 14-membered natural macrolactone **2** was supplemented, were extracted with EtOAc. The extracts were concentrated under vacuum followed by cleanup using OASIS HLB SPE cartridges (see procedures described above). On the other hand, crude extracts containing 12-membered reduced macrolides were prepared from two litres of the culture broth from *S. venezuelae* DHS2001, fed with 12-membered unnatural macrolactones **19** or 8,9-dihydro-neomethynolide (**20**, see synthetic procedures described below), and then treated according to the same procedure described above. The resultant crude extracts were then subjected to chromatographic isolation. To accomplish this, HPLC was performed using a preparative reversed-phase HPLC column (Watchers) and the samples were then eluted with the same mobile phase employed in the analytical HPLC system at a flow rate of 12 mL/min over a period of 100 min. This eluent was fractionated into 5 mL portions that were monitored by analytical HPLC-ESI-MS/MS coupled with a UV detector set to 220 nm to trace and identify the purity of the bio-converted macrolides. Fractions containing the products of interest were then pooled and extracted again using OASIS MCX SPE cleanup, after which they were freeze-dried as described above. NMR samples were prepared by dissolving each compound in 200 μL of $CDCl_3$ (Sigma) and placing the solution in a 5 mm Shigemi advanced NMR microtube (Sigma) matched to the solvent. The ^1H, ^{13}C, and 2D ^1H-^1H COSY NMR spectra were acquired using a Varian INOVA 500 spectrometer at 298 K. Chemical shifts were reported in ppm using TMS as an internal reference. The assignment of each compound was carried out by comparison with previously assigned ^1H NMR spectra and by a combination of 1D and 2D NMR experiments. All NMR data processing was done using the MESTREC (Magnetic Resonance Companion) software.

Antibacterial Assay of the Reduced Macrolides. The bactericidal activity of the reduced macrolides was determined using the method described by the National Committee for Clinical Laboratory Standards (NCCLS) method.[7] Briefly, *B. subtilis* ATCC 23857 was initially grown in Müller-Hilton broth (Difco), and then aliquots of the culture were dispensed into Müller-Hilton agar-based medium. A hundred microgram aliquots of the reduced macrolides and their corresponding macrolides (as positive controls) were reconstituted in an appropriate volume of MeOH, after which they were dispensed into paper bioassay disks. An equal volume of MeOH solvent, which was used as a negative control, was also applied onto a disk. The dried disks were then placed onto agar plates and incubated at 37℃ for 15 h. The diameter of the zone of inhibition was used to evaluate the antibacterial activity. All assays were performed in triplicate.

3-*tert*-Butyldimethylsilanyloxy-8,9-dihydro-10-deoxymethynolide (19b). A solution of lactone (**19a**)[3] (26 mg, 0.063 mmol in 4 mL of MeOH) was stirred under hydrogen (1 atm, balloon) at room temperature in the presence of 5% palladium on charcoal (52 mg). The resulting mixture was stirred at room temperature for 1 h. After filtration through the pad of Celite with ether (3 × 10 mL), the solution was concentrated. Purification by flash chromatography (hexane:EtOAc = 7:1, [*v*/*v*]) afforded the desired reduced lactone (**19b**, 20 mg, 77%) as a colorless oil: $[\alpha]_D^{24.3}$ -0.8° (*c* 1.96, $CHCl_3$); IR(film) ν_{max} 2958, 2881, 2349, 1724, 1604, 1462, 1369, 1253, 1165, 1065 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$,) δ 0.08 (d, J = 3.9 Hz, 6H), 0.92 (m, 18H), 1.16 (m, 6H), 1.39 (m, 2H), 1.50 (m, 2H), 1.59

(m, 2H), 1.87 (m, 3H), 2.12 (m, 1H), 2.51 (m, 1H), 2.74 (m, 1H), 3.12 (dd, *J* = 18.9, 12.6 Hz, 1H), 3.57 (d, *J* = 9.9 Hz, 1H), 4.66 (t, *J* = 6.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.7, 175.2, 80.7, 77.9, 45.8, 44.6, 35.1, 34.1, 33.7, 32.2, 26.2, 24.2, 20.5, 19.3, 18.5, 18.1, 18.0, 16.4, 10.8, -3.2, -3.3; HRMS calc. for $C_{23}H_{44}O_4Si$ 412.3009, found 412.3007.

8,9-Dihydro-10-deoxymethynolide (19). To a solution of reduced lactone (**19b**) (20 mg, 0.048 mmol in 0.5 mL of MeCN) prepared in the previous procedure, two milliliters of solution (HF:H_2O:MeCN = 1:0.5:8.5, [*v*/*v*/*v*]) were added at room temperature. After the resulting mixture was stirred for 18 h, it was neutralized with saturated $NaHCO_3$ (10 mL) and extracted with ether (3 × 10 ml). The combined organic extract was washed with aqueous saturated NaCl (10 mL), dried onto $MgSO_4$, and then concentrated. Purification by flash chromatography (hexane:EtOAc = 3:1, [*v*/*v*]) afforded the desired 8,9-dihydro-10-deoxymethynolide (**19**, 11 mg, 79%) as a colorless oil: T_{ret} (retention time on prep-HPLC) 14.7 min; ESI-MS/MS ([M+H] 299) [M+H–H_2O] 281, [M+H–$2H_2O$] 263, [M+H–$2H_2O$–CO] 235, [M+H–$2H_2O$–2CO] 207, [M+H–$3H_2O$–2CO] 189, [M+H–$4H_2O$–2CO] 171, [M+H–$5H_2O$–3CO] 125; $[\alpha]_D^{26.8}$ -17.5° (*c* 0.42, $CHCl_3$); IR (film) ν_{max} 3491, 2962, 1712, 1458, 1369, 1242, 1173, 1092, 991 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.3 Hz, 3H), 0.94 (d, *J* = 7.1 Hz, 3H), 1.04 (d, *J* = 6.6 Hz, 3H), 1.16 (d, *J* = 6.9 Hz, 3H), 1.27 (d, *J* = 6.6 Hz, 3H), 1.40 (m, 2H), 1.52 (dddd, *J* = 7.4, 7.4, 7.4, 7.4 Hz, 2H), 1.58 (m, 2H), 1.85(m, 3H), 2.13 (ddd, *J* = 18.7, 5.4, 2.5 Hz, 1H), 2.55 (m, 1H), 2.70 (dddd, *J* = 10.3, 6.7, 6.7, 6.7 Hz, 1H), 3.11 (ddd, *J* = 18.8, 12.4, 2.2 Hz, 1H), 3.50 (d, *J* = 10.1 Hz, 1H), 4.69 (m 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 214.4, 174.6, 81.0, 79.0, 45.9, 43.8, 34.0, 33.9, 33.5, 32.2, 24.2, 20.6, 19.4, 18.0, 17.0, 15.4, 10.8; HRMS calc. for $C_{17}H_{30}O_4$ 298.2144, found 298.2140.

3,12-*tert*-Butyldimethylsilanyloxy-8,9-dihydro-neomethynolide (20b). To hydrogenate TBS-protected lactone (**20a**, 28.6 mg, 0.053 mmol), the same synthetic procedures for preparation of **19b** were carried out,[3] except using flash chromatography eluted in different solvent ratio (hexane:EtOAc = 10:1, [*v*/*v*]), yielding the reduced lactone (**20b**, 25 mg, 87%) as a colorless oil: $[\alpha]_D^{18.6}$ -15.5° (*c* 1.64, $CHCl_3$); IR (film) ν_{max} 2947, 2889, 1728, 1462, 1373, 1254, 1080, 937, 837 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 12H), 0.88 (s, 9H), 0.90 (s, 9H), 0.96 (d, *J* = 6.5 Hz, 3H), 1.07 (d, *J* = 7.2 Hz, 3H), 1.16 (m, 9H), 1.33 (m, 2H), 1.78 (m, 3H), 1.90 (m, 1H), 2.13 (dt, *J* = 18.6, 5.4 Hz, 1H), 2.50 (m, 1H), 2.71 (m, 1H), 3.04 (ddd, *J* = 18.5, 8.9, 5.6 Hz, 1H), 3.59 (d, *J* = 9.9 Hz, 1H), 3.99 (dddd, *J* = 7.0, 7.0, 7.0, 7.0 Hz, 1H), 4.71(dd, *J* = 6.6, 1.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.1, 174.7, 81.4, 79.7, 67.7, 46.0, 44.6, 35.1, 34.2, 32.8, 32.0, 26.2, 25.8, 25.1, 21.1, 18.5, 18.1, 18.1, 18.0, 16.3, -3.2, -3.3, -3.9, -4.5; HRMS calc. for $C_{29}H_{58}O_5Si_2$ 542.3823, found 542.3820.

8,9-Dihydro-neomethynolide (20). De-protection procedures of TBS-protected reduced lactone (**20b**, 17 mg, 0.031 mmol) were followed by the same synthetic procedures for preparation of **19**, except employing flash chromatography eluted in different solvent ratio (hexane:EtOAc = 2:1, [*v*/*v*]), affording the resultant 8,9-dihydro-neomethynolide (**20,** 7.9 mg, 81%) as a colorless oil: T_{ret} 13.9 min; ESI-MS/MS ([M+H] 315) [M+H–H_2O] 297, [M+H–$2H_2O$] 279, [M+H–$2H_2O$–CO] 251, [M+H–$2H_2O$–2CO] 223, [M+H–$3H_2O$–2CO] 205, [M+H–$4H_2O$–2CO] 187, [M+H–$5H_2O$–3CO] 141; $[\alpha]_D^{26.8}$ -11.2° (*c* 0.34, $CHCl_3$); IR (film) ν_{max} 3452, 2966, 2935, 1709, 1458, 1373, 1261, 1169, 1068, 995, 906, 798, 733 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (d, *J* = 6.4 Hz, 3H), 1.12 (d, *J* = 7.1 Hz, 3H), 1.16 (d, *J* = 7.0 Hz, 3H), 1.23 (d, *J* = 6.2 Hz, 3H), 1.27 (d, *J* = 6.7 Hz, 3H), 1.37 (m, 2H), 1.60 (ds, 2H), 1.77 (m, 2H), 1.89 (m, 2H), 2.17 (m, 1H), 2.55 (m, 1H), 2.70 (m, 1H), 3.01 (ddd, *J* = 18.6, 10.3, 3.9 Hz, 1H), 3.52 (d, *J* = 10.2 Hz, 1H), 4.02 (dddd, *J* = 6.2, 6.2, 6.2, 6.2 Hz, 1H), 4.68(dd, *J* = 7.7, 1.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.5, 174.2, 81.6, 78.7, 67.1, 45.9, 43.6, 34.1, 33.7, 33.5, 32.1, 24.7, 21.3, 19.0, 17.9, 17.0, 15.4; HRMS calc. for $C_{17}H_{30}O_5$ 314.2093, found 314.2092.

8,9-Dihydro-YC-17 (11) (see **Fig. S1** and **Table S1**)
Formula: $C_{25}H_{45}NO_6$; MW 455; pale solid (1.4 mg);
T_{ret} (retention time on prep-HPLC): 18.5 min;
HRESIMS: [M+H] calc. 456.3247, found 456.6240;
ESI-MS/MS ([M+H] 456): [M+H–desosamine (*des*)] 299, [*des*] 158

8,9-Dihydro-methymycin (12) (see **Fig. S2** and **Table S2**)
Formula: $C_{25}H_{45}NO_7$; MW 471; white solid (2.9 mg);
T_{ret}: 13.4 min;
HRESIMS: [M+H] calc. 472.3196, found 472.4004;
ESI-MS/MS ([M+H] 472): [M+H–H_2O] 454, [M+H–*des*] 315, [*des*] 158

8,9-Dihydro-neomethymycin (13) (see **Fig. S3** and **Table S3**)
Formula: $C_{25}H_{45}NO_7$; MW 471; white solid (2.3 mg);
T_{ret}: 13.0 min;
HRESIMS: [M+H] calc. 472.3196, found 472.3949;
ESI-MS/MS ([M+H] 472): [M+H–H_2O] 454, [M+H–*des*] 315, [*des*] 158

8,9-Dihydro-novamethymycin (14) (see **Fig. S4** and **Table S4**)
Formula: $C_{25}H_{45}NO_8$; MW 487; white solid (1.1 mg);
T_{ret}: 12.4 min; HRESIMS: [M+H] calc. 488.3145, found 488.4010;
ESI-MS/MS ([M+H] 488): [M+H–H_2O] 470, [M+H–$2H_2O$] 452, [M+H–*des*] 331, [*des*] 158

10,11-Dihydro-narbomycin (15) (see **Fig. S5** and **Table S5**)
Formula: $C_{28}H_{49}NO_7$; MW 511; pale solid (1.8 mg);
T_{ret}: 23.3 min;
HRESIMS: [M+H] calc. 512.3509, found 512.4207;
ESI-MS/MS ([M+H] 512): [M+H–*des*] 355, [*des*] 158

10,11-Dihydro-pikromycin (16) (see **Fig. S6** and **Table S6**)
Formula: $C_{28}H_{49}NO_8$; MW 527; white solid (3.0 mg);
T_{ret}: 15.3 min;
HRESIMS: [M+H] calc. 528.3458, found 528.4112;
ESI-MS/MS ([M+H] 528): [M+H–H_2O] 510, [M+H–*des*] 371, [*des*] 158

10,11-Dihydro-neopikromycin (17) (see **Fig. S7** and **Table S7**)
Formula: $C_{28}H_{49}NO_8$; MW 527; white solid (1.9 mg);
T_{ret}: 16.2 min;
HRESIMS: [M+H] calc. 528.3458, found 528.4206;

ESI-MS/MS ([M+H] 528): [M+H–H_2O] 510, [M+H–*des*] 371, [*des*] 158

10,11-Dihydro-novapikromycin (18) (see **Fig. S8** and **Table S8**)
Formula: $C_{28}H_{49}NO_9$; MW 543; white solid (1.5 mg);
T_{ret}: 21.5 min;
HRESIMS: [M+H] calc. 544.3407, found 544.3905;
ESI-MS/MS ([M+H] 544): [M+H–H_2O] 526, [M+H–$2H_2O$] 508, [M+H–*des*] 387, [*des*] 158

10,11-Dihydro-5-*O*-desosaminyl tylactone (26) (see **Fig. S9** and **Table S9**)
Formula: $C_{31}H_{55}NO_7$; MW 553; pale solid (1.2 mg);
T_{ret} 24.8 min;
HRESIMS: [M+H] calc. 554.3407, found 554.3905;
ESI-MS/MS ([M+H] 554): [M+H–*des*] 397, [*des*] 158

10,11-Dihydro-rosamicin (27) (see **Fig. S10** and **Table S10**)
Formula: $C_{31}H_{53}NO_9$; MW 583; white solid (1.9 mg);
T_{ret} 11.5 min;
HRESIMS: [M+H] calc. 584.3407, found 584.3905;
ESI-MS/MS ([M+H] 584): [M+H–*des*] 427, [*des*] 158

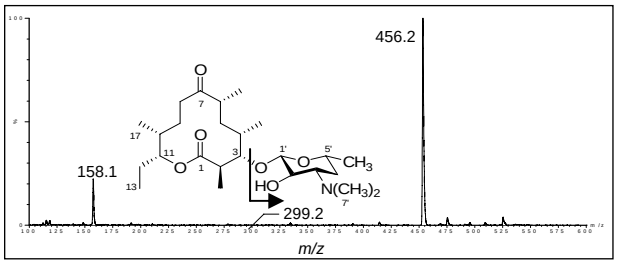

Fig. S1 ESI-MS/MS fragmentation pattern of **11**.

Table S1. NMR data of **11**, compared with those of the corresponding unsaturated macrolide YC-17 (**3**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**11**		**3**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	171.7(s)	—	173.7(s)
2	2.78(dq)	48.0(d)	2.80(dq)	47.8(d)
3	3.41(d)	85.3(d)	3.43(d)	85.3(d)
4	1.96(m)	33.9(d)	1.97(m)	33.1(d)
5	1.63(m)	31.4(t)	1.52(m)	31.1(t)
	1.40(m)		1.31(m)	
6	2.51(m)	43.6(d)	2.53(m)	44.6(d)
7	—	215.4(s)	—	209.7(s)
8	2.49(t)	34.1(t)	6.20(dd)	127.8(d)
	2.41(ddd)			
9	1.60(m)	22.6(t)	6.84(dd)	147.6(d)
	1.35(ddd)			
10	2.22(m)	37.2(d)	2.92(m)	48.5(d)
11	3.94(dd)	80.5(d)	3.91(dd)	83.1(d)
12	1.55(m)	26.2(t)	1.51(m)	26.0(t)
	1.44(m)		1.43(m)	
13	0.96(t)	8.1(q)	0.92(t)	8.6(q)
14	1.23(d)	11.2(q)	1.21(d)	11.5(q)
15	1.08(d)	14.6(q)	1.01(d)	15.0(q)
16	1.19(d)	15.9(q)	1.21(d)	15.3(q)
17	1.06(d)	14.0(q)	1.14(d)	13.2(q)
1’	5.08(d)	101.7(d)	5.01(d)	103.7(d)
2’	3.81(dd)	71.7(d)	3.84(dd)	71.6(d)
3’	2.84(m)	60.8(d)	2.86(m)	60.8(d)
4’	1.66(m)	32.1(t)	1.64(m)	31.7(t)
	1.50(m)		1.51(m)	
5’	3.69(m)	66.1(t)	3.76(m)	65.8(d)
6’	1.20(d)	22.1(q)	1.17(d)	22.0(q)
7’	2.28(s)	42.5(q)	2.29(s)	43.5(q)

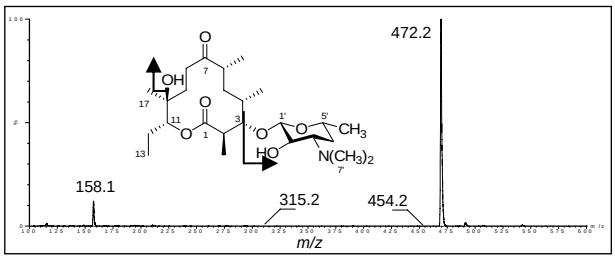

Fig. S2 ESI-MS/MS fragmentation pattern of **12**.

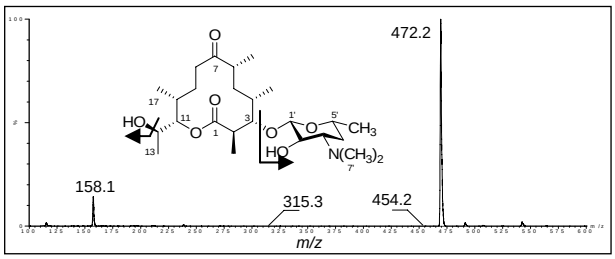

Fig. S3 ESI-MS/MS fragmentation pattern of **13**.

Table S2. NMR data of **12**, compared with those of the corresponding unsaturated macrolide methymycin (**5**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**12**		**5**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	174.0(s)	—	173.5(s)
2	2.80(dq)	48.5(d)	2.81(dq)	48.1(d)
3	3.44(d)	85.3(d)	3.47(d)	85.3(d)
4	1.97(m)	33.5(d)	1.96(m)	33.1(d)
5	1.79(m)	31.6(t)	1.45(m)	31.8(t)
	1.54(m)		1.27(m)	
6	2.53(m)	44.5(d)	2.54(m)	44.3(d)
7	—	223.4(s)	—	200.2(s)
8	2.53(t)	29.4(t)	6.36(d)	130.8(d)
	2.42(ddd)			
9	1.64(t)	32.4(t)	7.02(d)	145.4(d)
	1.36(ddd)			
10	—	71.2(s)	—	75.7(s)
11	4.04(dd)	92.7(d)	4.04(dd)	91.1(d)
12	1.58(m)	18.6(t)	1.56(m)	18.0(t)
	1.45(m)		1.44(m)	
13	0.99(t)	8.8(q)	0.94(t)	8.8(q)
14	1.26(d)	11.5(q)	1.26(d)	11.4(q)
15	1.06(d)	15.3(q)	1.08(d)	15.1(q)
16	1.17(d)	15.8(q)	1.21(d)	15.9(q)
17	1.35(s)	21.1(q)	1.40(s)	22.0(q)
1’	5.05(d)	103.2(d)	5.03(d)	103.6(d)
2’	3.89(dd)	72.7(d)	3.88(dd)	71.6(d)
3’	2.82(m)	65.8(d)	2.81(m)	63.8(d)
4’	1.64(m)	31.3(t)	1.60(m)	31.9(t)
	1.53(m)		1.52(m)	
5’	3.79(m)	66.7(d)	3.77(m)	66.7(d)
6’	1.23(d)	22.7(q)	1.19(d)	23.1(q)
7’	2.27(s)	43.8(q)	2.24(s)	43.7(q)

Table S3. NMR data of **13**, compared with those of the corresponding unsaturated macrolide neomythymycin (**6**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**13**		**6**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	173.8(s)	—	171.9(s)
2	2.86(dq)	48.8(d)	2.81(dq)	47.8(d)
3	3.45(d)	85.1(d)	3.45(d)	85.3(d)
4	1.90(m)	33.6(d)	1.97(m)	33.0(d)
5	1.66(m)	31.8(t)	1.45(m)	31.5(t)
	1.40(m)		1.21(m)	
6	2.52(m)	44.2(d)	2.53(m)	44.3(d)
7	—	224.0(s)	—	201.7(s)
8	2.49(t)	35.4(t)	6.18(dd)	131.0(d)
	2.39(ddd)			
9	1.62(m)	21.4(t)	6.92(dd)	145.3(d)
	1.34(ddd)			
10	—	31.2(d)	—	43.7(d)
11	4.01(dd)	87.7(d)	4.06(dd)	87.3(d)
12	3.58(dq)	67.9(d)	3.92(dq)	67.0(d)
13	1.19(d)	19.8(q)	1.21(d)	19.4(q)
14	1.24(d)	11.0(q)	1.24(d)	12.4(q)
15	1.01(d)	15.1(q)	1.04(d)	15.5(q)
16	1.16(d)	15.7(q)	1.21(d)	16.0(q)
17	1.05(d)	14.8(q)	1.15(d)	15.8(q)
1’	5.09(d)	102.9(d)	5.08(d)	102.4(d)
2’	3.85(dd)	72.6(d)	3.85(dd)	72.6(d)
3’	2.85(m)	65.3(d)	2.83(m)	64.8(d)
4’	1.65(m)	31.2(t)	1.61(m)	31.2(t)
	1.57(m)		1.54(m)	
5’	3.73(m)	66.8(d)	3.71(m)	66.1(d)
6’	1.27(d)	22.4(q)	1.22(d)	22.7(q)
7’	2.23(s)	43.2(q)	2.28(s)	43.3(q)

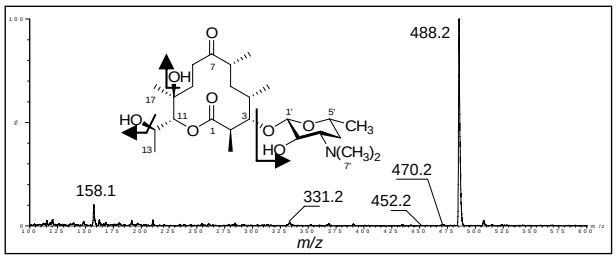

Fig. S4 ESI-MS/MS fragmentation pattern of **14**.

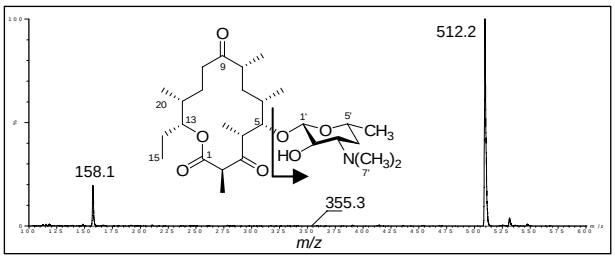

Fig. S5 ESI-MS/MS fragmentation pattern of **15**.

Table S4. NMR data of **14**, compared with those of the corresponding unsaturated macrolide novamethymycin (**7**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**14**		**7**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	171.3(s)	—	172.9(s)
2	2.82(dq)	48.3(d)	2.81(dq)	48.0(d)
3	3.43(d)	85.3(d)	3.41(d)	84.8(d)
4	1.95(m)	33.9(d)	1.97(m)	33.3(d)
5	1.82(m)	32.1(t)	1.41(m)	31.7(t)
	1.56(m)		1.20(m)	
6	2.50(m)	44.2(d)	2.52(m)	44.3(d)
7	—	224.1(s)	—	200.7(s)
8	2.50(t)	29.4(t)	6.33(d)	131.8(d)
	2.40(ddd)			
9	1.60(t)	32.4(t)	7.05(d)	145.4(d)
	1.35(ddd)			
10	—	65.2(s)	—	69.9(s)
11	4.10(dd)	88.7(d)	4.16(dd)	95.3(d)
12	3.98(dq)	61.9(d)	3.93(dq)	62.4(d)
13	1.20(d)	19.6(q)	1.21(d)	19.4(q)
14	1.23(d)	11.2(q)	1.23(d)	11.8(q)
15	1.06(d)	15.5(q)	1.02(d)	15.1(q)
16	1.14(d)	15.9(q)	1.19(d)	15.9(q)
17	1.31(s)	20.8(q)	1.40(s)	22.5(q)
1’	5.09(d)	101.9(d)	5.05(d)	101.7(d)
2’	3.85(dd)	72.3(d)	3.83(dd)	72.3(d)
3’	2.85(m)	65.1(d)	2.85(m)	65.0(d)
4’	1.63(m)	31.0(t)	1.62(m)	31.5(t)
	1.53(m)		1.52(m)	
5’	3.71(m)	66.4(d)	3.70(m)	66.4(d)
6’	1.22(d)	22.1(q)	1.20(d)	22.9(q)
7’	2.25(s)	43.1(q)	2.26(s)	42.7(q)

Table S5. NMR data of **15**, compared with those of the corresponding unsaturated macrolide narbomycin (**4**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**15**		**4**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	172.3(s)	—	172.9(s)
2	3.33(q)	51.3(d)	3.36(q)	51.6(d)
3	—	210.3(s)	—	211.8(s)
4	2.82(dq)	45.9(d)	2.86(dq)	45.3(d)
5	3.02(d)	82.1(d)	3.04(d)	84.7(d)
6	1.96(m)	33.6(d)	1.97(m)	33.7(d)
7	1.65(m)	31.2(t)	1.42(m)	31.8(t)
	1.43(m)		1.18(m)	
8	2.51(m)	52.4(d)	2.55(m)	44.3(d)
9	—	210.4(s)	—	198.2(s)
10	2.54(t)	35.4(t)	6.16(dd)	128.1(d)
	2.44(ddd)			
11	1.61(m)	29.2(t)	6.85(dd)	141.9(d)
	1.38(ddd)			
12	2.27(m)	37.5(d)	2.93(m)	36.4(d)
13	3.90(dd)	82.1(d)	3.91(dd)	82.4(d)
14	1.57(m)	24.4(t)	1.53(m)	24.8(t)
	1.36(m)		1.33(m)	
15	0.97(t)	8.4(q)	0.95(t)	8.5(q)
16	1.32(d)	12.7(q)	1.34(d)	12.6(q)
17	1.18(d)	9.9(q)	1.17(d)	9.8(q)
18	1.06(d)	15.0(q)	1.07(d)	14.7(q)
19	1.16(d)	15.9(q)	1.18(d)	15.6(q)
20	1.03(d)	14.4(q)	1.12(d)	15.0(q)
1’	5.07(d)	101.9(d)	5.05(d)	101.8(d)
2’	3.84(dd)	72.3(d)	3.83(dd)	71.8(d)
3’	2.85(m)	65.1(d)	2.85(m)	65.3(d)
4’	1.62(m)	31.0(t)	1.62(m)	31.2(t)
	1.56(m)		1.52(m)	
5’	3.70(m)	66.4(d)	3.70(m)	66.4(d)
6’	1.24(d)	22.1(q)	1.20(d)	22.3(q)
7’	2.22(s)	43.1(q)	2.26(s)	43.5(q)

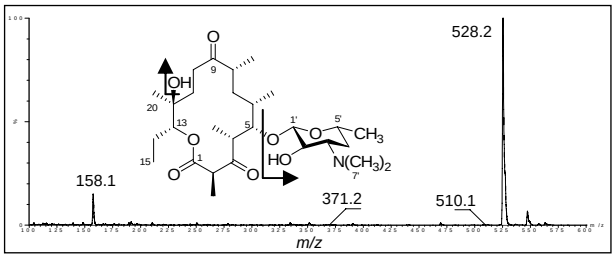

Fig. S6 ESI-MS/MS fragmentation pattern of **16**.

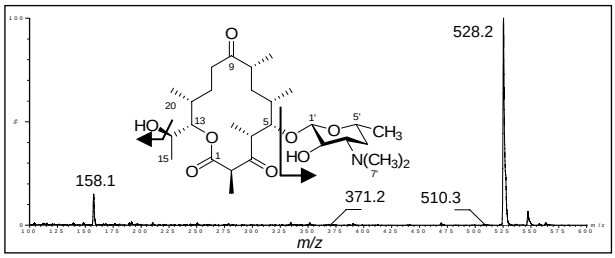

Fig. S7 ESI-MS/MS fragmentation pattern of **17**.

Table S6. NMR data of **16**, compared with those of the corresponding unsaturated macrolide pikromycin (**8**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**16**		**8**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	172.0(s)	—	172.5(s)
2	3.36(q)	51.5(d)	3.31(q)	51.6(d)
3	—	211.9(s)	—	210.8(s)
4	2.83(dq)	45.1(d)	2.84(dq)	45.0(d)
5	3.01(d)	83.8(d)	3.04(d)	84.7(d)
6	1.97(m)	33.7(d)	1.97(m)	33.3(d)
7	1.79(m)	31.4(t)	1.45(m)	31.8(t)
	1.54(m)		1.20(m)	
8	2.52(m)	52.6(d)	2.53(m)	44.3(d)
9	—	209.1(s)	—	198.7(s)
10	2.51(t)	29.4(t)	6.33(d)	129.1(d)
	2.42(ddd)			
11	1.62(t)	31.8(t)	7.05(d)	145.4(d)
	1.36(ddd)			
12	—	76.1(s)	—	76.4(s)
13	4.01(dd)	82.9(d)	4.08(dd)	90.6(d)
14	1.56(m)	18.4(t)	1.57(m)	18.8(t)
	1.35(m)		1.32(m)	
15	0.96(t)	8.7(q)	0.96(t)	8.8(q)
16	1.34(d)	12.7(q)	1.31(d)	12.6(q)
17	1.16(d)	9.5(q)	1.12(d)	9.7(q)
18	1.05(d)	15.3(q)	1.06(d)	15.1(q)
19	1.15(d)	16.0(q)	1.21(d)	15.7(q)
20	1.32(s)	20.6(q)	1.42(s)	22.1(q)
1’	5.10(d)	100.9(d)	5.08(d)	100.8(d)
2’	3.85(dd)	72.3(d)	3.81(dd)	71.5(d)
3’	2.82(m)	65.2(d)	2.82(m)	65.3(d)
4’	1.60(m)	31.0(t)	1.60(m)	31.0(t)
	1.51(m)		1.51(m)	
5’	3.70(m)	66.6(d)	3.67(m)	66.3(d)
6’	1.22(d)	22.6(q)	1.21(d)	22.9(q)
7’	2.23(s)	42.7(q)	2.29(s)	42.1(q)

Table S7. NMR data of **17**, compared with those of the corresponding unsaturated macrolide neopikromycin (**9**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**17**		**9**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	171.2(s)	—	172.0(s)
2	3.32(q)	51.1(d)	3.33(q)	51.9(d)
3	—	211.1(s)	—	211.8(s)
4	2.84(dq)	45.0(d)	2.82(dq)	44.8(d)
5	3.04(d)	84.5(d)	3.05(d)	84.0(d)
6	1.95(m)	33.5(d)	1.97(m)	33.7(d)
7	1.67(m)	31.8(t)	1.43(m)	31.8(t)
	1.41(m)		1.18(m)	
8	2.50(m)	52.5(d)	2.54(m)	44.4(d)
9	—	209.8(s)	—	197.7(s)
10	2.50(t)	34.8(t)	6.15(dd)	127.3(d)
	2.40(ddd)			
11	1.61(m)	29.8(t)	6.85(dd)	139.9(d)
	1.39(ddd)			
12	2.22(m)	31.1(d)	2.91(m)	30.4(d)
13	4.00(dd)	81.0(d)	4.03(dd)	87.6(d)
14	3.96(dq)	67.4(d)	3.97(dq)	66.7(d)
15	1.21(d)	19.7(q)	1.25(d)	19.8(q)
16	1.36(d)	12.6(q)	1.34(d)	12.1(q)
17	1.13(d)	9.8(q)	1.16(d)	9.7(q)
18	1.06(d)	15.0(q)	1.04(d)	15.5(q)
19	1.16(d)	15.9(q)	1.25(d)	15.6(q)
20	1.09(d)	14.7(q)	1.19(d)	16.0(q)
1’	5.01(d)	101.7(d)	5.06(d)	100.2(d)
2’	3.82(dd)	72.0(d)	3.82(dd)	72.4(d)
3’	2.88(m)	64.9(d)	2.85(m)	65.5(d)
4’	1.63(m)	31.4(t)	1.65(m)	30.9(t)
	1.52(m)		1.50(m)	
5’	3.68(m)	66.2(d)	3.67(m)	66.3(d)
6’	1.21(d)	22.8(q)	1.21(d)	22.1(q)
7’	2.24(s)	42.2(q)	2.27(s)	42.4(q)

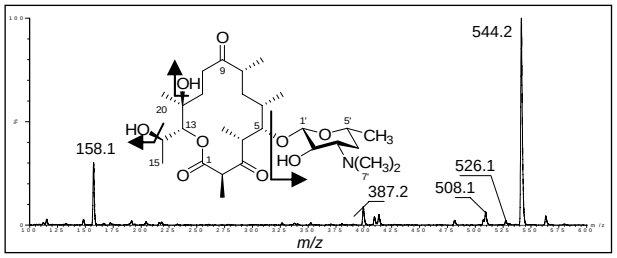

Fig. S8 ESI-MS/MS fragmentation pattern of **18**.

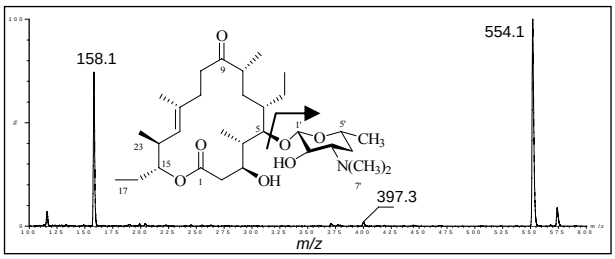

Fig. S9 ESI-MS/MS fragmentation pattern of **26**.

Table S8. NMR data of **18**, compared with those of the corresponding unsaturated macrolide novapikromycin (**10**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**18**		**10**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	172.1(s)	—	172.0(s)
2	3.36(q)	51.5 (d)	3.35(q)	51.8(d)
3	—	211.2(s)	—	211.3(s)
4	2.81(dq)	45.0(d)	2.84(dq)	44.6(d)
5	3.14(d)	84.1(d)	3.04(d)	83.6(d)
6	1.96(m)	33.6(d)	1.97(m)	33.3(d)
7	1.77(m)	31.2(t)	1.45(m)	31.8(t)
	1.54(m)		1.22(m)	
8	2.52(m)	52.6(d)	2.54(m)	44.3(d)
9	—	207.3(s)	—	198.7(s)
10	2.50(t)	29.8(t)	6.33(d)	129.3(d)
	2.41(ddd)			
11	1.64(t)	32.8(t)	6.99(d)	144.7(d)
	1.39(ddd)			
12	—	70.1(s)	—	70.4(s)
13	4.10(dd)	96.3(d)	4.13(dd)	96.1(d)
14	3.93(dq)	61.4(d)	3.98(dq)	61.3(d)
15	1.18(d)	18.7(q)	1.21(d)	19.8(q)
16	1.34(d)	12.2(q)	1.33(d)	12.6(q)
17	1.16(d)	9.8(q)	1.11(d)	9.5(q)
18	1.06(d)	15.1(q)	1.06(d)	15.2(q)
19	1.18(d)	15.7(q)	1.19(d)	15.9(q)
20	1.35(s)	20.9(q)	1.41(s)	22.4(q)
1’	5.03(d)	100.9(d)	5.06(d)	101.3(d)
2’	3.86(dd)	71.6(d)	3.86(dd)	71.4(d)
3’	2.87(m)	64.3(d)	2.87(m)	64.5(d)
4’	1.61(m)	31.3(t)	1.63(m)	31.6(t)
	1.51(m)		1.51(m)	
5’	3.64(m)	66.0(d)	3.64(m)	66.5(d)
6’	1.21(d)	22.6(q)	1.20(d)	22.2(q)
7’	2.21(s)	42.6(q)	2.25(s)	42.9(q)

Table S9. NMR data of **26**, compared with those of the corresponding unsaturated macrolide 5-*O*-desosaminyl tylactone (**22**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**26**		**22**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	171.5(s)	—	172.4(s)
2	2.53(m)	43.2(t)	2.48(m)	43.7(t)
	2.27(m)		2.24(m)	
3	3.82(ddd)	70.1(d)	3.84(ddd)	70.6(d)
4	2.08(dq)	45.3(d)	2.05(dq)	45.6(d)
5	2.80(d)	82.2(d)	2.81(d)	82.0(d)
6	1.79(m)	41.2(d)	1.75(m)	41.8(d)
7	2.26(m)	33.1(t)	1.45(m)	33.4(t)
	2.22(m)		1.22(m)	
8	2.51(m)	44.7(d)	2.51(m)	40.7(d)
9	—	211.6(s)	—	201.1(s)
10	2.53(t)	36.1(t)	6.30(dd)	122.1(d)
	2.44(ddd)			
11	1.60(m)	33.1(t)	7.38(dd)	146.7(d)
	1.35(ddd)			
12	—	132.8(s)	—	134.2(s)
13	5.18(dd)	125.2(d)	5.46(dd)	142.5(d)
14	2.98(m)	42.5(d)	2.91(m)	42.8(d)
15	3.94(dd)	81.4(d)	3.93(dd)	81.5(d)
16	1.57(m)	24.5(t)	1.55(m)	24.9(t)
	1.36(m)		1.35(m)	
17	0.96(t)	8.5(q)	0.94(t)	8.5(q)
18	1.06(d)	8.9(q)	1.06(d)	8.8(q)
19	1.29(m)	23.0(t)	1.27(m)	23.8(t)
	1.18(m)		1.17(m)	
20	0.96(t)	12.4(q)	0.96(t)	12.1(q)
21	1.16(d)	15.9(q)	1.21(d)	15.7(q)
22	1.68(s)	17.3(q)	1.71(s)	16.8(q)
23	1.12(d)	16.0(q)	1.16(d)	16.3(q)
1’	5.09(d)	101.6(d)	5.08(d)	100.8(d)
2’	3.82(dd)	71.3(d)	3.78(dd)	71.9(d)
3’	2.85(m)	64.2(d)	2.81(m)	63.6(d)
4’	1.59(m)	31.0(t)	1.67(m)	31.3(t)
	1.48(m)		1.53(m)	
5’	3.64(m)	66.1(d)	3.66(m)	66.2(d)
6’	1.17(d)	22.9(q)	1.19(d)	22.7(q)
7’	2.21(s)	42.3(q)	2.28(s)	42.5(q)

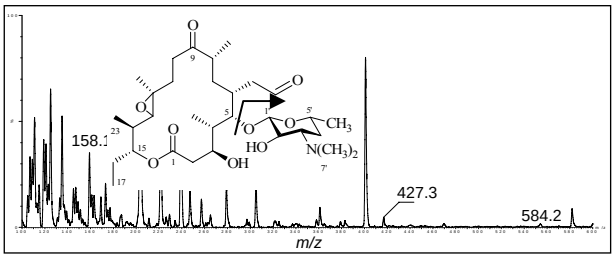

Fig. S10 ESI-MS/MS fragmentation pattern of **27**.

Table S10. NMR data of **27**, compared with those of the corresponding unsaturated macrolide rosamicin (**23**) ($CDCl_3$, ^1H 500 MHz, ^{13}C 100 MHz, 298 K)

Position	**27**		**23**	
	δ_H (m)	δ_C (m)	δ_H (m)	δ_C (m)
1	—	173.3(s)	—	171.9(s)
2	2.54(m)	42.2(t)	2.53(m)	43.2(t)
	2.28(m)		2.26(m)	
3	3.84(ddd)	70.3(d)	3.84(ddd)	70.2(d)
4	2.05(dq)	45.8(d)	2.01(dq)	45.1(d)
5	2.81(d)	81.6(d)	2.84(d)	81.9(d)
6	2.01(m)	32.2(d)	2.02(m)	32.8(d)
7	1.87(m)	32.5(t)	1.44(m)	33.1(t)
	1.62(m)		1.19(m)	
8	2.52(m)	44.5(d)	2.51(m)	40.1(d)
9	—	211.8(s)	—	201.5(s)
10	2.45(t)	31.8(t)	6.33(dd)	124.0(d)
	2.42(ddd)			
11	1.62(t)	31.3(t)	7.06(dd)	143.2(d)
	1.37(ddd)			
12	—	57.8(s)	—	56.9(s)
13	2.51(dd)	73.2(d)	2.55(dd)	67.5(d)
14	2.58(m)	37.5(d)	2.59(m)	37.8(d)
15	3.93(dd)	77.4(d)	3.94(dd)	78.3(d)
16	1.56(m)	23.9(t)	1.57(m)	24.5(t)
	1.38(m)		1.39(m)	
17	0.96(t)	8.3(q)	0.98(t)	8.2(q)
18	1.06(d)	8.8(q)	1.04(d)	8.9(q)
19	2.49(m)	43.1(t)	2.41(m)	43.8(t)
	2.26(m)		2.20(m)	
20	9.72(m)	202.1(d)	9.68(m)	201.8(d)
21	1.16(d)	15.7(q)	1.21(d)	15.9(q)
22	1.30(s)	21.3(q)	1.41(s)	22.3(q)
23	1.02(d)	11.4(q)	1.06(d)	11.7(q)
1’	5.11(d)	100.9(d)	5.08(d)	101.1(d)
2’	3.80(dd)	71.8(d)	3.74(dd)	71.3(d)
3’	2.82(m)	64.4(d)	2.86(m)	63.9(d)
4’	1.51(m)	31.4(t)	1.55(m)	31.0(t)
	1.43(m)		1.48(m)	
5’	3.64(m)	66.1(d)	3.64(m)	66.2(d)
6’	1.13(d)	22.9(q)	1.16(d)	21.7(q)
7’	2.20(s)	42.3(q)	2.21(s)	43.5(q)

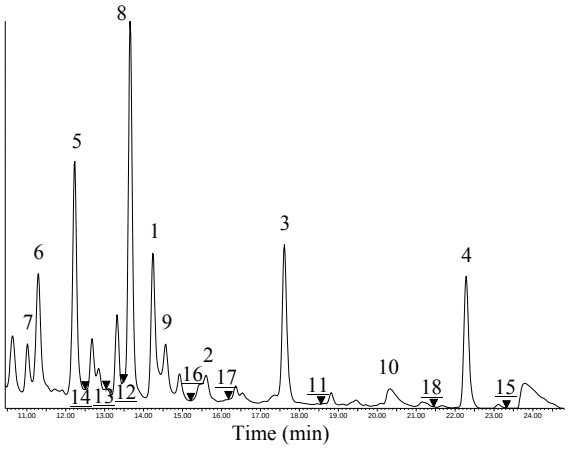

Fig. S11 Preparative UV-profiles in the large-scale fermentation of wild-type *S. venezuelae* ATCC15439. HPLC was monitored at 210 nm. Numbers underlined represent series of reduced macrolides that were detectable by MS analyses.

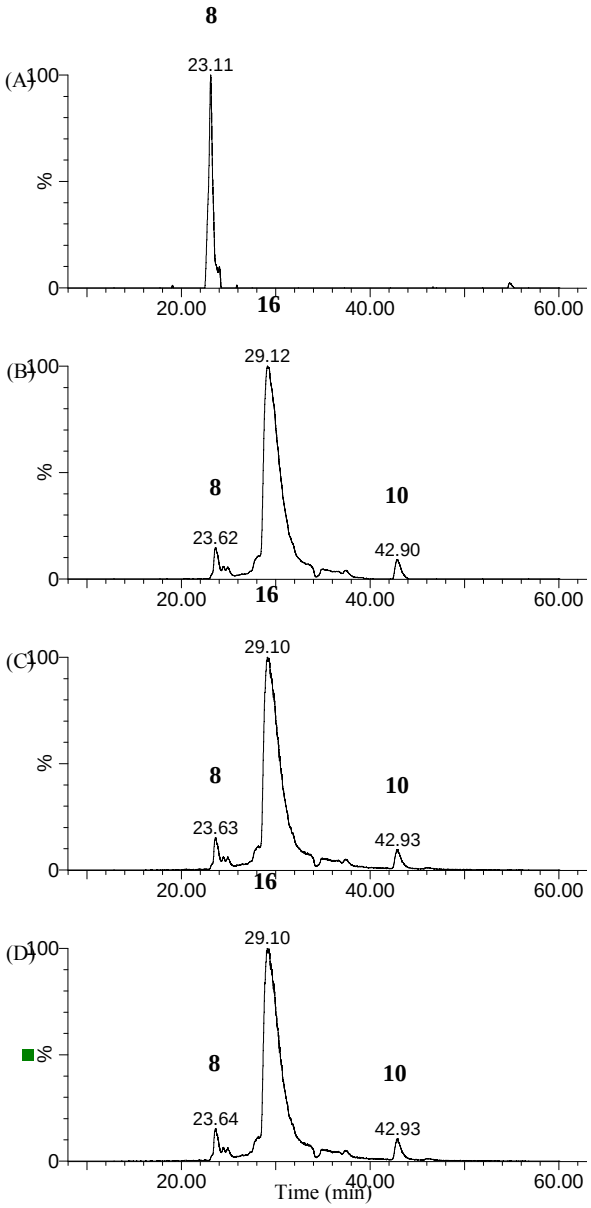

Fig. S12 Selected ion chromatograms of organic extracts obtained from (A) blank SCM medium spiked with 5 µg/mL of pikromycin (**8**), the cultures of recombinant strains of *S. venezuelae* (B) DHS2001, (C) YJ003, and (D) YJ028, that fed with the same level of **8**. Peaks labeled with **8**, novapikromycin (**10**), and 10,11-dihydro-pikromycin (**16**) were selected at molecular $[M+H]^+$ ions 526.0, 542.0, and 528.0, respectively.

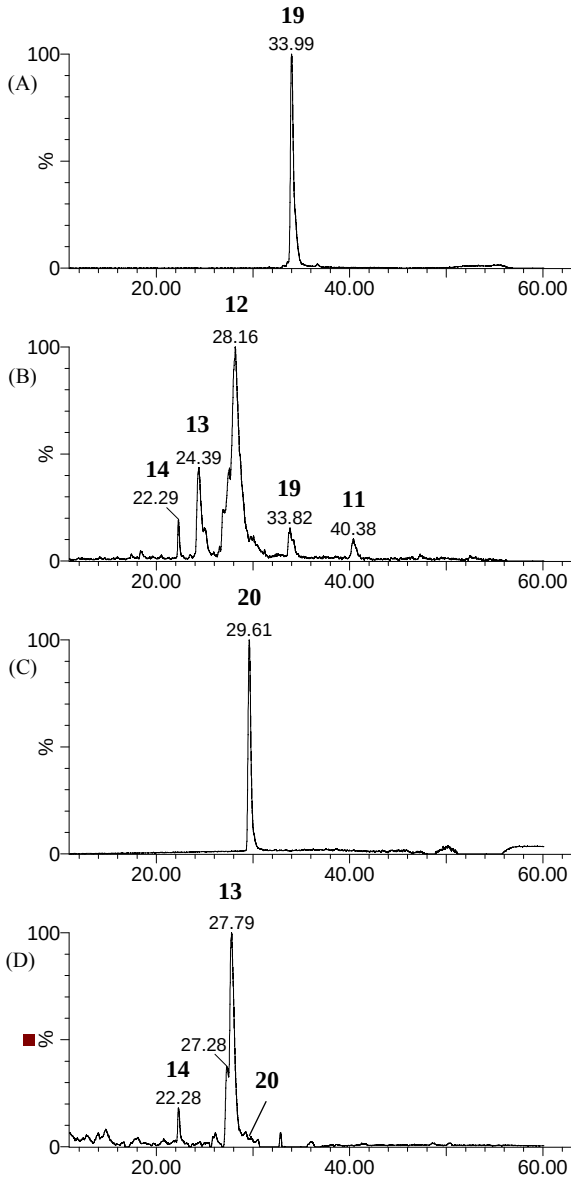

Fig. S13 Selected ion chromatograms of organic extracts obtained from (A) blank SCM medium spiked with 5 µg/mL of 8,9-dihydro-10-deoxymethynolide (**19**), (B) the culture of recombinant strains of *S. venezuelae* DHS2001 fed with the same level of **19**, (C) blank SCM medium spiked with 5 µg/mL of 8,9-dihydro-neomethynolide (**20**), and (D) the culture of recombinant strains of *S. venezuelae* DHS2001 fed with the same level of **20**. Peaks labeled with 8,9-dihydro-YC-17 (**11**), 8,9-dihydro-methymycin (**12**), 8,9-dihydro-neomethymycin (**13**), 8,9-dihydro-novamethymycin (**14**) were selected at molecular $[M+H]^{1+}$ ions 456.0, 472.0, 472.0, 488.0 respectively, whereas peaks labeled with 19 and 20 were selected at sodium adduct $[M+Na]^+$ ions 321.0 and 337.0.

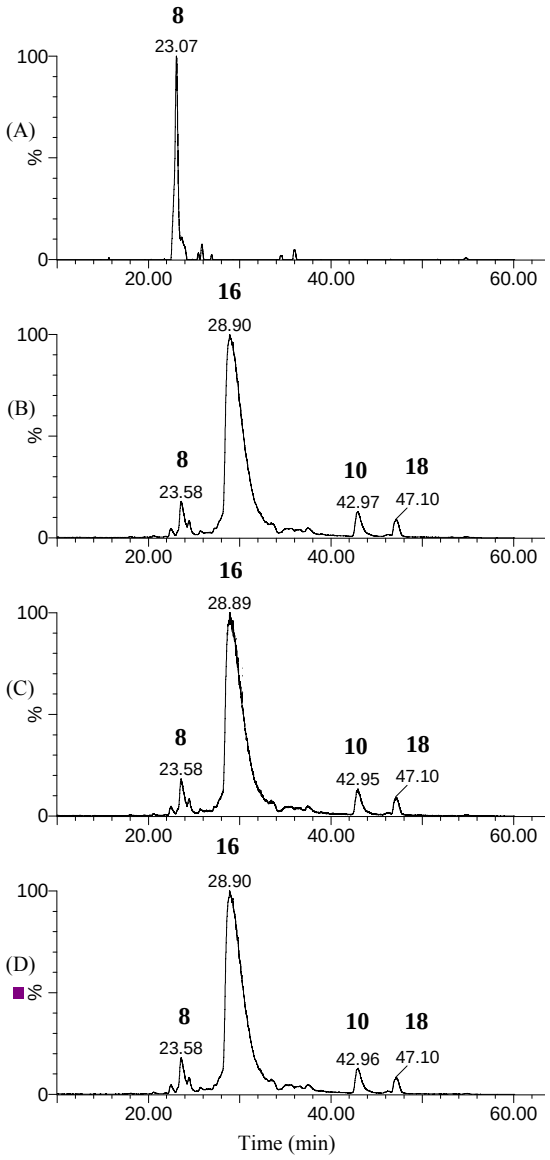

Fig. S14 Selected ion chromatograms obtained from the in vitro reactions using pikromycin (8) as a substrate with (A) boiled cell-free extracts (CFE) prepared from the cultures of recombinant strains of *S. venezuelae* DHS2001, (B) CFE prepared from DHS2001, (C) CFE prepared from YJ003, and (D) CFE prepared from YJ028. Peaks labeled with 8, novapikromycin (**10**), 10,11-dihydro-pikromycin (**16**), and 10,11-dihydro-novapikromycin (**18**) were selected at molecular $[M+H]^+$ ions 526.0, 542.0, 528.0, and 544.0, respectively.

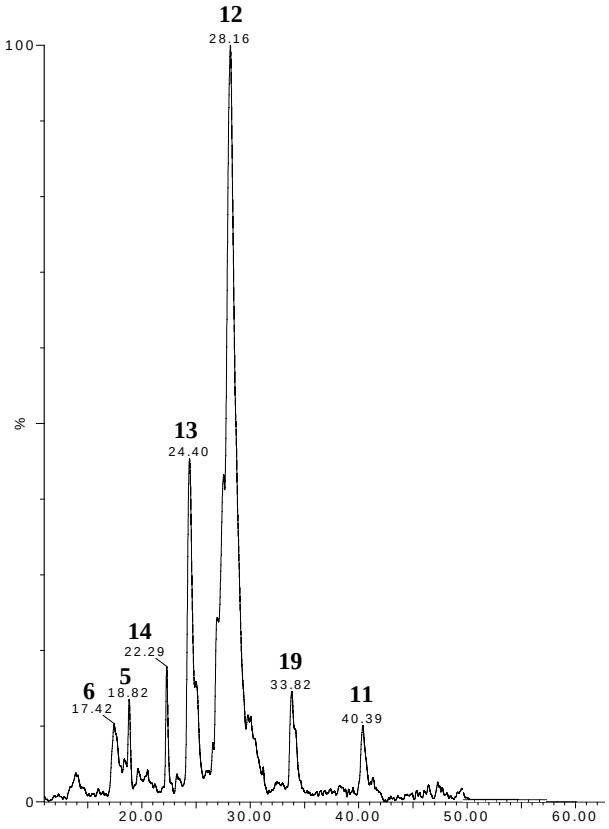

Fig. S15 Selected ion chromatograms obtained from in vitro reaction using 8,9-dihydro-10-deoxymethynolide (**19**) as a substrate with cell-free extracts (CFE) prepared from the cultures of recombinant strains of *S. venezuelae* DHS2001. Peaks labeled with methymycin (**5**), neomethymycin (**6**), 8,9-dihydro-YC-17 (**11**), 8,9-dihydro-methymycin (**12**), 8,9-dihydro-neomethymycin (**13**), 8,9-dihydro-novamethymycin (**14**) were selected at molecular $[M+H]^+$ ions 470.0, 470.0, 456.0, 472.0, 472.0, 488.0 respectively, whereas peaks labeled with 19 was selected at sodium adduct $[M+Na]^+$ ions 321.0.

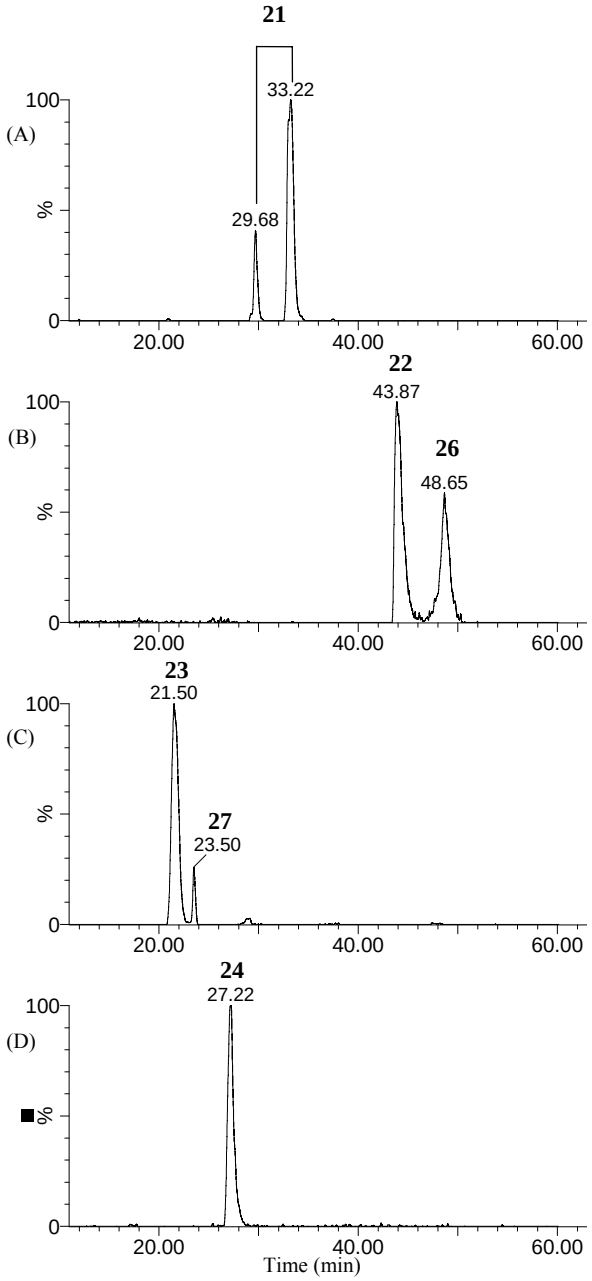

Fig. S16 Selected ion chromatograms of organic extracts obtained from the cultures of recombinant strain of S. venezuelae YJ028 that fed with 5 μg/mL of (A) avermectins (**21**), (B) 5-*O*-desosaminyl tylactone (**22**), (C) rosamicin (**23**) and (D) rapamycin (**24**). Peaks labeled with 21 consist of avermectin A1a and A1b, selected at molecular $[M+NH_4]^+$ ions 904.0 and 890.0. Peaks labeled with 22, 23, 10,11-dihydro-5-*O*-desosaminyl tylactone (**26**), and 10,11-dihydro-rosamicin (**27**) were selected at molecular $[M+H]^+$ ions 552.0, 582.0, 554.0, and 584.0 respectively, whereas 24 was selected at molecular $[M+NH_4]^+$ ion 931.0.

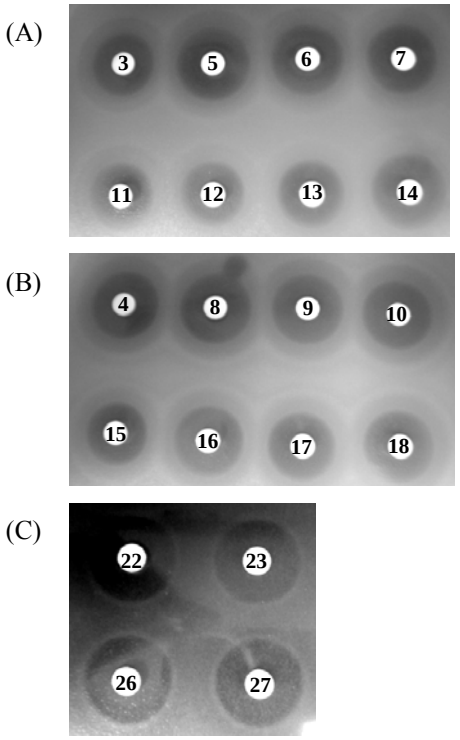

Fig. S17 Bioassay of the unusual saturated macrolides and their corresponding unsaturated macrolides, as described in the main text. (A) 12-membered, (B) 14-membered, and (C) 16-membered macrolides. Upper halos represent the antibacterial activities of previously characterized macrolides as positive control, whereas down halos represent those of macrolides, which were biosynthesized in this study.

Notes and references

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