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Total Synthesis of Narbonolide and Biotransformation to Pikromycin

Lakshmanan Venkatraman,[†] Christine E. Salomon,[‡] David H. Sherman,[§] and Robert A. Fecik*[,†]

Department of Medicinal Chemistry, 308 Harvard Street Southeast, 8-101 WDH, University of Minnesota, Minneapolis, Minnesota 55455-0353, Department of Microbiology, 420 Delaware Street Southeast, University of Minnesota, Minneapolis, Minnesota 55455, and Departments of Medicinal Chemistry, Microbiology & Immunology, and Chemistry, 210 Washtenaw Avenue, University of Michigan Life Sciences Institute, Ann Arbor, Michigan 48109-2216

fecik001@umn.edu

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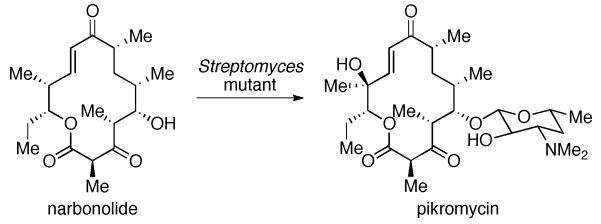

An improved total synthesis of narbonolide and its biotransformation to pikromycin is reported. This total synthesis utilized an intramolecular Nozaki−Hiyama−Kishi coupling that significantly improved macrocyclization yields (90−96%) and allowed for differentiation of the C3- and C5-oxidation states. A *pikAI* deletion mutant of *Streptomyces venezuelae* was used to biotransform synthetic narbonolide to pikromycin by glycosylation and oxidation *in vivo*. This integration of synthetic chemistry and engineered biotransformations holds great promise for the synthesis of novel macrolide analogues of biological interest.

Macrolide antibiotics are a clinically important class of polyketide natural products of current interest for the treatment of drug-resistant bacterial infections, intestinal motility disorders, and inflammatory airway diseases.[1] Recent developments in the metabolic engineering of polyketide biosynthetic pathways have opened new avenues for the production of novel macrolides.[2] Herein, we report an improved total synthesis of narbonolide and its biotransformation to the macrolide pikromycin using a genetically engineered *Streptomyces venezuelae* mutant. This total synthesis significantly improved macrocyclization yields and revealed the effects that protecting groups have on the Nozaki−Hiyama−Kishi (NHK) macrocyclization to 14-membered macrolides.

The modular pikromycin (Pik) polyketide synthase (PKS) system of *S. venezuelae* ATCC 15439 catalyzes the biosynthesis of the 12- and 14-membered macrolactones 10-deoxymethynolide and narbonolide (**1**), respectively (Scheme 1).[3,4] Upon thioesterase (TE)-catalyzed macrolactonization to narbonolide, it undergoes further modifications by the post-PKS tailoring enzymes DesVII and PikC.[3,4] DesVII catalyzes the C5-glycosylation of narbonolide to narbomycin (**2**), which is followed by C12-oxidation to pikromycin (**3**) by PikC. Because the Pik PKS system biosynthesizes both 12- and 14-membered macrolides, the inherent substrate tolerance of this system provides a unique opportunity to study the mechanism and regulation of the size of macrolactone ring formation and the potential of Pik PKS and post-PKS tailoring enzymes as components of the combinatorial biosynthesis toolbox.

To extend the studies by us and others of Pik PKS modules and enzymes with unnatural substrates[5−7] and mimics of the natural chain elongation intermediates,[8−15] we sought to develop a convenient total synthesis of narbonolide that was readily amenable to the synthesis of analogues for the study of DesVII and PikC. We were intrigued by the possibility of utilizing a mutant *S. venezuelae* strain for the biotransformation of narbonolide analogues to novel macrolides. In previous biotransformation studies, narbonolide has been converted to narbomycin[16] and pikromycin[17] by *Streptomyces narbonensis*. Mutant strains of *Streptomyces platensis*[18] and *S. venezuelae*[19] have been used to convert narbonolide into pikromycin analogues with modified sugars. We anticipated that a *S. venezuelae* mutant with a disrupted PKS system would provide a rapid method to glycosylate and oxidize narbonolide analogues with its native enzymes, DesVII and PikC, *in vivo*. Total synthesis

[†] Department of Medicinal Chemistry, University of Minnesota.
[‡] Department of Microbiology, University of Minnesota.
[§] Departments of Medicinal Chemistry, Microbiology & Immunology, and Chemistry, University of Michigan.

(1) Katz, L.; Ashley, G. W. *Chem. Rev.* **2005**, *105*, 499−527.
(2) McDaniel, R.; Welch, M.; Hutchinson, C. R. *Chem. Rev.* **2005**, *105*, 543−558.
(3) Xue, Y.; Zhao, L.; Liu, H.-W.; Sherman, D. H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12111−12116.
(4) Xue, Y.; Sherman, D. H. *Met. Eng.* **2001**, *3*, 15−26.
(5) Beck, B. J.; Aldrich, C. C.; Fecik, R. A.; Reynolds, K. A.; Sherman, D. H. *J. Am. Chem. Soc.* **2003**, *125*, 4682−4683.
(6) Beck, B. J.; Aldrich, C. C.; Fecik, R. A.; Reynolds, K. A.; Sherman, D. H. *J. Am. Chem. Soc.* **2003**, *125*, 12551−12557.
(7) Yin, Y.; Lu, H.; Khosla, C.; Cane, D. E. *J. Am. Chem. Soc.* **2003**, *125*, 5671−5676.
(8) Aldrich, C. C.; Beck, B. J.; Fecik, R. A.; Sherman, D. H. *J. Am. Chem. Soc.* **2005**, *127*, 8441−8452.
(9) Aldrich, C. C.; Venkatraman, L.; Sherman, D. H.; Fecik, R. A. *J. Am. Chem. Soc.* **2005**, *127*, 8910−8911.
(10) Fecik, R. A.; Nguyen, P. L.; Venkatraman, L. *Curr. Opin. Drug Disc. Dev.* **2006**, *8*, 741−747.
(11) He, W.; Wu, J.; Khosla, C.; Cane, D. E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 391−394.
(12) Wu, J.; He, W.; Khosla, C.; Cane, D. E. *Angew. Chem., Int. Ed.* **2005**, *44*, 7557−7560.
(13) Kao, C.-L.; Borisova, S. A.; Kim, H. J.; Liu, H.-w. *J. Am. Chem. Soc.* **2006**, *128*, 5606−5607.
(14) Giraldes, J. W.; Akey, D. L.; Kittendorf, J. D.; Sherman, D. H.; Smith, J. L.; Fecik, R. A. *Nat. Chem. Biol.* **2006**, *2*, 531−536.
(15) Akey, D. L.; Kittendorf, J. D.; Giraldes, J. W.; Fecik, R. A.; Sherman, D. H.; Smith, J. L. *Nat. Chem. Biol*. **2006**, *2*, 537−542.
(16) Suzuki, M.; Hori, T.; Maezawa, I.; Nagahama, N. *J. Chem. Soc., Chem. Commun.* **1971**, 304−305.
(17) Maezawa, I.; Hori, T.; Kinumaki, A.; Suzuki, M. *J. Antibiot.* **1973**, *26*, 771−775.
(18) Maezawa, I.; Kinumaki, A.; Suzuki, M. *J. Antibiot.* **1976**, *29*, 1203−1208.
(19) Hong, J. S. J.; Park, S. H.; Choi, C. Y.; Sohng, J. K.; Yoon, Y. J. *FEMS Microbiol. Lett.* **2004**, *238*, 391−399.

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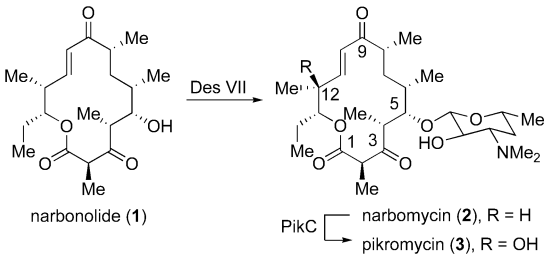
SCHEME 1. Post-PKS Tailoring of Narbonolide in *S. venezuelae* ATCC 15439

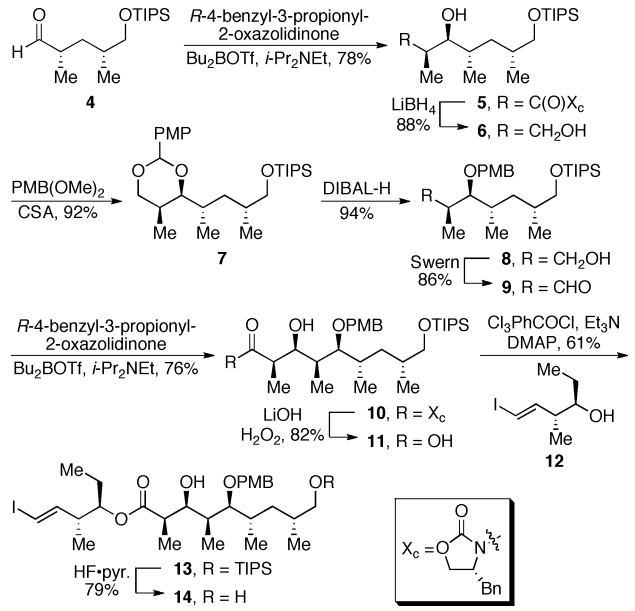


coupled with enzymatic transformations provides a means to gain access to pikromycin analogues that could not be synthesized by natural product degradation.

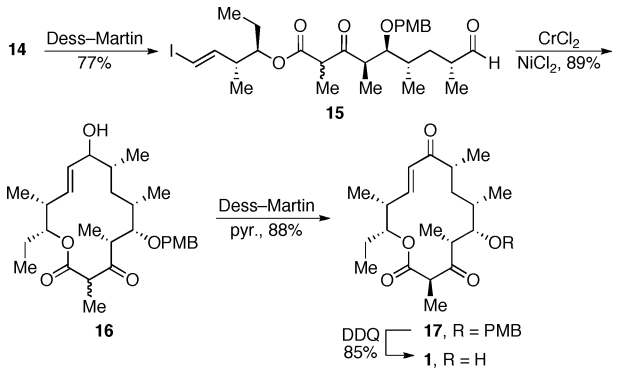
Previous syntheses of narbonolide[20] and pikronolide,[21−23] the pikromycin aglycone, have one or more complicating factors: (1) a low yield of the key macrocyclization reaction, (2) the inability to differentiate the C3- and C5-positions for chemoselective reactions, and (3) highly optimized protecting group strategies that decrease synthetic efficiency. We recently reported a formal total synthesis of narbonolide to evaluate the use of an intramolecular NHK coupling for macrocyclization of 14-membered macrolides.[24] Although we were able to obtain a modest macrocyclization yield (58%), the use of a C3, C5-ketal protecting group prevented us from addressing the problem of selective oxidation of the C3-alcohol. Classic studies on erythromycin by Woodward have demonstrated the effects that protecting groups at the C3- and C5-positions have on the macrolactonization of linear intermediates.[25] On the basis of the protecting group effects observed in the pikronolide syntheses,[21−23] we expected that replacement of the C3, C5-ketal would improve the yield of intramolecular NHK coupling and allow for selective oxidation of the C3-alcohol.

Synthesis of the entire linear C1−C15 chain of narbonolide began with aldehyde **4** (Scheme 2). The Evans asymmetric aldol reaction of aldehyde **4**[15] furnished aldol adduct **5**, which was reduced to 1,3-diol **6**. Protection of the 1,3-diol as its 4-methoxybenzylidene ketal (**7**),[26] followed by DIBAL-H reduction gave the secondary alcohol, corresponding to the C5-position of narbonolide, as PMB ether **8**. Oxidation to aldehyde **9**, an Evans asymmetric aldol reaction to alcohol **10**, and removal of the chiral auxiliary afforded acid **11**. Complete assembly of the entire heptaketide chain of narbonolide was accomplished by

SCHEME 2. Synthesis of the Linear C1−C15 Chain of Narbonolide



SCHEME 3. Completion of the Total Synthesis of Narbonolide



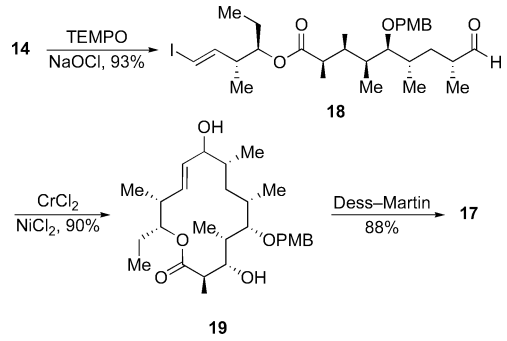
esterification with vinyl iodide **12**[26] to ester **13**, followed by deprotection to primary alcohol **14**.

Treatment of alcohol **14** with the Dess−Martin periodinane and pyridine oxidized both the primary and secondary alcohols to aldehyde **15**; however, the C2-methyl group had epimerized (Scheme 3). We speculated that pyridine might be responsible for the epimerization, but Dess−Martin oxidation without pyridine also furnished aldehyde **15**. We were optimistic that the C2-methyl group could be equilibrated to the desired *R* configuration after macrocyclization, so NHK macrocyclization was performed to give allylic alcohol **16** in high yield. Gratifyingly, Dess−Martin oxidation with pyridine afforded ketone **17** with accompanying equilibration of the C2-methyl group to a single diastereomer. Removal of the C5-protecting group furnished narbonolide (**1**), thus confirming the *R* configuration of the C2-stereocenter.

Although the epimerized aldehyde **15** could be used to complete the total synthesis of narbonolide, we sought to develop an alternate route that avoided epimerization of the C2-methyl group. Treatment of alcohol **14** with TEMPO and NaOCl selectively oxidized the primary alcohol to aldehyde **18** (Scheme 4). Intramolecular NHK coupling afforded allylic alcohol **19**, and Dess−Martin oxidation of the C3- and C9-alcohols to

(20) Kaiho, T.; Masamune, S.; Toyoda, T. *J. Org. Chem.* **1982**, *47*, 1612−1614.
(21) Nakajima, N.; Hamada, T.; Tanaka, T.; Oikawa, Y.; Yonemitsu, O. *J. Am. Chem. Soc.* **1986**, *108*, 4645−4647.
(22) Nakajima, N.; Tanaka, T.; Hamada, T.; Oikawa, Y.; Yonemitsu, O. *Chem. Pharm. Bull.* **1987**, *35*, 2228−2237.
(23) Nakajima, N.; Ubukata, M.; Yonemitsu, O. *Heterocycles* **1997**, *46*, 105−110.
(24) Venkatraman, L.; Aldrich, C. C.; Sherman, D. H.; Fecik, R. A. *J. Org. Chem.* **2005**, *70*, 7267−7272.
(25) Woodward, R. B.; Logusch, E.; Nambiar, K. P.; Sakan, K.; Ward, D. E.; Au-Yeung, B.-W.; Balaram, P.; Browne, L. J.; Card, P. J.; Chen, C. H.; Chênevert, R. B.; Fliri, A.; Frobel, K.; Gais, H.-J.; Garratt, D. G.; Hayakawa, K.; Heggie, W.; Hesson, D. P.; Hoppe, D.; Hoppe, I.; Hyatt, J. A.; Ikeda, D.; Jacobi, P. A.; Kim, K. S.; Kobuke, Y.; Kojima, K.; Krowicki, K.; Lee, V. J.; Leutert, T.; Malchenko, S.; Martens, J.; Matthews, R. S.; Ong, B. S.; Press, J. B.; Rajan Babu, T. V.; Rousseau, G.; Sauter, H. M.; Suzuki, M.; Tatsuta, K.; Tolbert, L. M.; Truesdale, E. A.; Uchida, I.; Ueda, Y.; Uyehara, T.; Vasella, A. T.; Vladuchick, W. C.; Wade, P. A.; Williams, R. M.; Wong, H. N.-C. *J. Am. Chem. Soc.* **1981**, *103*, 3213−3215.
(26) Pilli, R. A.; de Andrade, C. K. Z.; Souto, C. R. O.; de Meijere, A. J. *Org. Chem.* **1998**, *63*, 7811−7819.

SCHEME 4. Alternate Route To Complete the Total Synthesis of Narbonolide



ketone **17** proceeded with no evidence of C2-epimerization. Deprotection of PMB ether **17** (Scheme 3) completed the alternate route to narbonolide (**1**).

To test our hypothesis that a mutant *S. venezuelae* strain lacking a functional PKS can glycosylate and oxidize narbonolide, we elected to use a *pikAI* deletion mutant of *S. venezuelae* (BB138).[27] The BB138 strain does not contain the gene encoding PikAI (modules L, 1, and 2) in the Pik PKS and therefore lacks the ability to biosynthesize narbonolide.[27] *S. venezuelae* BB138 was previously used in genetic complementation studies to produce novel macrolides,[27] but its value in exogenous feeding experiments was unknown. Incubation of synthetic narbonolide with BB138 resulted in exclusive and quantitative bioconversion to pikromycin, as shown by high-performance liquid chromatography (HPLC) (Figure 1). Analysis of the fermentation extract by liquid chromatography−mass spectrometry (LC−MS) confirmed the exclusive metabolite of this bioconversion to be pikromycin (**3**). A control fermentation of *S. venezuelae* BB138 without narbonolide showed no production of narbonolide, narbomycin, or pikromycin by HPLC, confirming that exogenous narbonolide is the sole source for pikromycin production (Figure 1).

One of the challenges in the study of modular PKS systems is access to advanced biosynthetic intermediates for the study of enzymes that catalyze late-stage extension and processing, macrolactonization, and post-PKS modifications. We expect this total synthesis to be valuable in the study of the post-PKS tailoring enzymes DesVII and PikC. Macrocyclization proceeds in high yields (90%) and enables the differentiation of oxygenation states at the C3- and C5-positions. Our results also reveal important structural insights to the NHK macrocyclization of macrolides. Woodward showed that macrolactonization of linear erythronolide intermediates occurs only when the C3- and C5-positions are protected as a cyclic ketal;[25] Martin and co-workers have found one exception to this trend.[28] This work establishes that having the C3- and C5-positions protected as a cyclic ketal is detrimental to the NHK macrocyclization of linear narbonolide intermediates.

Bioconversion of synthetic narbonolide to pikromycin by a *pikAI* deletion mutant of *S. venezuelae* also has important implications for the use of engineered bacteria to transform natural products and their analogues. Despite the lack of a functional Pik PKS system, *S. venezuelae* BB138 retains the fully functional enzymes necessary for the biosynthesis of desosamine and the C5-glycosylation (DesVII) and C12-oxidation (PikC) of narbonolide.

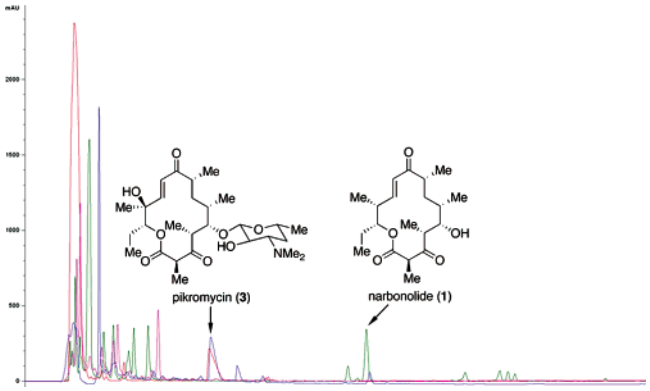


FIGURE 1. HPLC trace of the EtOAc extract from the crude fermentation broth after incubation of narbonolide (**1**) with the *S. venezuelae pikAI* deletion mutant BB138 showing complete conversion to pikromycin (**3**) (blue), overlaid with the HPLC traces of authentic pikromycin (red), control fermentation (pink), and extraction control with narbonolide in media only (green).

In conclusion, we have completed a second-generation total synthesis of narbonolide that markedly improves upon previous syntheses and demonstrated that a *pikAI* deletion mutant of *S. venezuelae* can bioconvert narbonolide to pikromycin. This synthetic route is readily amenable to the synthesis of narbonolide analogues and will enable us to examine the substrate specificity of DesVII and PikC and the utility of *S. venezuelae* BB138 to produce pikromycin analogues. We expect this integration of synthetic chemistry and engineered biotransformations to be of great value in the synthesis of novel macrolides with biological activity.

Experimental Section

(*R*)-3-((2*R*,3*S*,4*S*,5*S*,6*S*,8*R*)-3-Hydroxy-5-(4-methoxybenzyloxy)-2,4,6,8-tetramethyl-9-(triisopropylsilyloxy)nonanoyl)-4-benzyloxazolidin-2-one (10). To a solution of *R*-4-benzyl-3-propionyl-2-oxazolidinone (0.933 g, 4.0 mmol, 1.08 equiv) in CH_2Cl_2 at −78 °C was added freshly distilled Bu_2BOTf (1.22 mL, 4.88 mmol, 1.32 equiv). After 15 min, distilled *i*-Pr_2NEt (0.94 mL, 5.29 mmol, 1.43 equiv) was added, and the temperature was raised to 0 °C; the mixture was stirred for 1 h. The mixture was cooled back to −78 °C, and a solution of aldehyde **9** (1.72 g, 3.7 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) was added. The reaction mixture was stirred at −78 °C for 2 h, followed by stirring at −20 °C for 2 h. The reaction was quenched by the addition of pH 7 phosphate buffer (1 M, 5 mL) and MeOH (12 mL). The mixture was treated with a solution of 30% H_2O_2 (4.5 mL) and MeOH (8 mL) and extracted with Et_2O (5 × 15 mL). Purification by flash chromatography (25% EtOAc/hexanes) afforded the title compound (1.96 g, 76% yield) as a colorless oil: R_f = 0.45 (20% EtOAc/hexanes); $[\alpha]_D^{23}$ = −21.44 (c = 0.49, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.18−7.35 (m, 7H), 6.84 (d, J = 8.4 Hz, 2H), 4.59−4.67 (m, 2H), 4.38 (d, J = 10.8 Hz, 1H), 4.11−4.19 (m, 2H), 3.98 (dd, J = 4.5, 6.6 Hz, 1H), 3.83−3.88 (m, 1H), 3.78 (s, 3H), 3.61 (dd, J = 4.8, 9.6 Hz, 1H), 3.45 (dd, J = 6.6, 9.6 Hz, 1H), 3.31 (dd, J = 2.7, 6.6 Hz, 1H), 3.21 (dd, J = 2.7, 13.5 Hz, 1H), 2.75 (dd, J = 9.6, 13.5 Hz, 1H), 1.59−1.82 (m, 3H), 1.17−1.30 (ovlp, 4H), 1.03−1.15 (ovlp, 22H), 0.99 (d, J = 6.9 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H), 0.96−0.94 (ovlp, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.7, 159.1, 152.8, 135.3, 131.0, 129.6, 129.3, 129.1, 127.6, 113.9, 86.8, 75.3, 73.3, 68.4, 66.3, 55.5, 55.3, 40.9, 38.0, 37.5, 37.4, 33.8, 33.5, 19.1, 18.5, 17.0, 13.9, 12.4, 9.1. HRMS calcd for ($C_{40}H_{63}NO_7Si$ + Na^+): 720.4271, found 720.4288.

(27) Yoon, Y. J.; Beck, B. J.; Kim, B. S.; Kang, H.-Y.; Reynolds, K. A.; Sherman, D. H. *Chem. Biol.* **2002**, *9*, 203−214.

(28) Hergenrother, P. J.; Hodgson, A.; Judd, A. S.; Lee, W.-C.; Martin, S. F. *Angew. Chem., Int. Ed.* **2003**, *42*, 3278−3281.

Narbonolide (1). To a solution of the PMB ether **17** (39.0 mg, 0.0826 mmol) in CH_2Cl_2 (2 mL) and H_2O (0.21 mL, 11.57 mmol, 140 equiv) at 0 °C was added DDQ (37.0 mg, 0.1652 mmol, 2.0 equiv). The reaction was stirred for 1.5 h and quenched by the addition of saturated aqueous $NaHCO_3$ (3 mL). The mixture was diluted with CH_2Cl_2 (10 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic layers were dried (Na_2SO_4), filtered off, and concentrated under reduced pressure. Purification by flash chromatography (40% EtOAc/hexanes) followed by HPLC purification (C18, 40−80% MeCN/0.1% aqueous TFA) afforded the title compound (24.7 mg, 85% yield) as a solid: $R_f = 0.47$ (40% EtOAc/hexanes); $[\alpha]_D^{23} = +66.15$ ($c = 1.24$, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.89 (dd, $J = 5.1$, 16.5 Hz, 1H), 6.08 (dd, $J = 16.5$, 1.8 Hz, 1H), 5.10−5.15 (m, 1H), 3.83−3.86 (m, 1H), 3.70 (q, $J = 6.9$ Hz, 1H), 2.98−3.05 (m, 1H), 2.57−2.72 (m, 1H), 2.21 (br s, 1H), 1.38−1.76 (ovlp, 6H), 1.34 (d, $J = 6.9$ Hz, 3H), 1.13 (d, $J = 6.6$ Hz, 3H), 1.12 (d, $J = 6.9$ Hz, 3H), 1.08 (d, $J = 6.6$ Hz, 3H), 0.92 (d, $J = 6.6$ Hz, 3H), 0.91 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 207.7, 205.1, 171.1, 148.8, 129.0, 76.9, 73.0, 50.6, 40.1, 39.2, 36.7, 35.4, 24.6, 19.0, 18.7, 14.8, 11.2, 10.8. HRMS calcd for ($C_{20}H_{32}O_5 + Na^+$): 375.2147, found 375.2154.

(3*S*,9*R*/*S*)-3,9-Tetrahydro-5-(4-methoxybenzyloxy)narbonolide (19). To a solution of aldehyde **18** (28.0 mg, 0.0466 mmol) in DMSO (19 mL) at room temperature was added $CrCl_2$ (57.2 mg, 0.4660 mmol, 10 equiv) and $NiCl_2$ (0.6 mg, 0.0047 mmol, 0.1 equiv). The reaction was stirred for 12 h, then quenched by the addition of H_2O (10 mL). The mixture was diluted with EtOAc (200 mL), and the layers were separated. The organic layer was washed with H_2O (3 × 20 mL) and saturated aqueous NaCl (20 mL), dried (Na_2SO_4), filtered off, and concentrated under reduced pressure. Purification by flash chromatography (40% EtOAc/hexanes) afforded the title compound (20.0 mg, 90% yield) as a moist solid: $R_f = 0.57$ (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (d, $J = 8.4$ Hz, 2H), 6.87 (d, $J = 8.4$ Hz, 2H), 5.74 (dd, $J = 3.0$, 15.9 Hz, 1H), 5.52 (dd, $J = 6.6$, 15.9 Hz, 1H), 5.06 (dd, $J = 4.5$, 9.6 Hz, 1H), 4.43 (AB system, $J = 10.8$ Hz, ν_{AB} 95.4, 2H), 4.09 (d, $J = 6.6$ Hz, 1H), 3.80 (s, 3H), 3.68−3.72 (m 2H), 3.56−3.60 (m, 1H), 2.59 (pent, $J = 6.6$ Hz, 1H), 2.16−2.46 (m, 3H), 1.64−1.74 (m, 1H), 1.39−1.58 (ovlp, 5H), 1.25−1.32 (m, 3H), 1.22 (d, $J = 6.9$ Hz, 3H), 1.01−1.04 (ovlp, 6H), 0.96 (d, $J = 7.2$ Hz, 3H), 0.88 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.8, 159.5, 135.1, 130.1, 129.9, 129.7, 114.2, 84.3, 78.7, 77.8, 76.7, 70.3, 55.6, 44.0, 38.8, 36.3, 35.9, 32.4, 29.2, 26.7, 17.4, 16.7, 15.8, 11.0, 8.1. HRMS calcd for ($C_{28}H_{44}O_6 + Na^+$): 499.3036, found 499.3039.

Biotransformation of Narbonolide (1) to Pikromycin (3). A concentrated spore stock (10 μL) of the *Streptomyces venezuelae pikAI* deletion mutant BB138[27] was used to inoculate 50 mL of ATCC172 media (10 g of glucose, 20 g of soluble starch, 5 g of yeast extract, 5 g of NZ amine type A, and 1 g of $CaCO_3$ per 1 L of H_2O) in a 250 mL baffled flask and incubated at 30 °C for 2 days with shaking. This seed culture (5 mL) was used to inoculate fresh ATCC172 media (50 mL) containing narbonolide (**1**, 4 mg in 400 μL EtOH) or EtOH (400 μL) and incubated at 30 °C for 3 days with shaking. Sterile media (50 mL) containing narbonolide (**1**, 0.5 mg) was also incubated under the same conditions as an extraction control. Samples were centrifuged and the pH of the decanted supernatant was adjusted to 9.5 with 1 M aqueous NaOH. The supernatant was extracted with EtOAc (2 × 50 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The crude extracts were analyzed by HPLC (C18, 40−80% MeCN/0.1% TFA) and LC−MS (50−100% MeCN/57 mM ammonium acetate, ESI+). MS (ESI+): 524 ($M - H^+$). Pikromycin was purified from the crude fermentation extract by isolation of the corresponding HPLC peak (10.4 min) and was analyzed by MS. MS (ESI−): 548 ($M^+ + Na$), 526 ($M^+ + H^+$).

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Supporting Information Available: General experimental procedures, experimental procedures for compounds **5**−**9**, **11**, **13**−**18**, ^{1}H and ^{13}C NMR spectra for compounds **1**, **5**−**11**, and **13**−**19**, and LC−MS and MS (ESI−) spectra for compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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