Published in final edited form as:

Angew Chem Int Ed Engl. 2015 August 3; 54(32): 9203–9208. doi:10.1002/anie.201504337.

Total Synthesis and Structural Revision of Antibiotic CJ-16,264**

Prof. Dr. K. C. Nicolaou,

Department of Chemistry, BioScience Research Collaborative Rice University, 6100 Main Street, Houston, TX 77005 (USA)

Dr. Akshay A. Shah,

Department of Chemistry, BioScience Research Collaborative Rice University, 6100 Main Street, Houston, TX 77005 (USA)

Dr. Henry Korman,

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093 (USA)

Dr. Tabrez Khan,

Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)

Dr. Lei Shi,

Department of Chemistry, BioScience Research Collaborative Rice University, 6100 Main Street, Houston, TX 77005 (USA)

Dr. Wisuttaya Worawalai, and

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093 (USA)

Prof. Dr. Emmanuel A. Theodorakis

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093 (USA)

Abstract

The total synthesis and structural revision of antibiotic CJ-16,264 is described. Starting with citronellal, the quest for the target molecule featured a novel bis-transannular Diels–Alder reaction that casted stereoselectively the decalin system and included the synthesis of six isomers before demystification of its true structure.

** K.C.N. thanks Drs. D. H. Huang (TSRI), L. Pasternack (TSRI), L. B. Alemany (Rice) and Q. Kleerekoper (Rice) for NMR spectroscopic assistance, Drs. G. Siuzdak (TSRI) and C. Pennington (Rice) for mass spectrometric assistance, and Drs. R. K. Chadha (TSRI) and J. D. Korp (University of Houston) for X-ray crystallographic assistance. This work was supported by the National Institutes of Health (USA) (grant AI055475), the Cancer Prevention & Research Institute of Texas (CPRIT) and The Welch Foundation. H.K. thanks financial support from the National Science Foundation Graduate Research Fellowship Program (Grant No. DGE-1144086). T.K. acknowledges Fulbright India for a Fulbright-Nehru Postdoctoral Fellowship.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.2015xxxxx>.

Keywords

natural products; antibiotic; total synthesis; structural revision; transannular Diels–Alder reaction

Isolated from fungus CL39457, antibiotic CJ-16,264¹ (**1**, Figure 1a) was reported to exhibit impressive activities against drug resistant Gram-positive bacteria such as *Staphylococcus aureus* 01A1120 (MIC = 0.78 µg/mL) and Gram-negative bacteria such as *Moraxella catarrhalis* 87A1055 (MIC = 0.39 µg/mL) and *Escherichia coli* 51A1051 with altered permeability (MIC = 6.25 µg/mL). The assigned structure of this molecule is intriguing in that it is amongst the most complex of its relatives, CJ-16,367 [**2**, Figure 1a, stereochemistry unassigned, isolated from the same fungus (CL39457)]¹, UCS1025A (**3**, Figure 1a)² and UCS1025B (**4**, Figure 1a)², 21-(*S*)- and 21-(*R*)-myceliothermophins C (**5a**)^{3,4} and D (**5b**)^{3,4} (Figure 1b), and the most recently reported antibiotic pyrrolizilactone⁵ (**1a**, Figure 1a). Antibiotic CJ-16,264 (**1**) possesses a challenging tetramethylated decalin system, whose relative and absolute configurations differ from those of its less complex siblings UCS compounds (**3** and **4**), and the myceliothermophins C (**5a**) and D (**5b**) (see Figure 1). Antibiotic CJ-16,264 also appears dissimilar to its close relative antibiotic **1a**. In view of the biological properties of CJ-16,264 (**1**), the doubts over the correctness of its structure raised by the stereochemical differences from its siblings and the incomplete assignment of its absolute configuration^{1,6}, and the challenge presented by its unique polysubstituted decalin system, we embarked on its total synthesis in the hope of solving the puzzle presented by its molecular structure. In this report we describe our synthetic endeavors that led to the total synthesis and structural revision of this target molecule which set the stage for design, synthesis and biological evaluation of its analogs and structural elucidations within this emerging class of antibiotics.

Our retrosynthetic analysis of antibiotic CJ-16,264 (structure **1**, Scheme 1a) pointed to (*S*)-citronellal, the more expensive⁷ of the two enantiomeric forms of this convenient starting material. Mindful of the cost and given our doubts about the structure of our target molecule, we opted to use the less costly (*R*)-citronellal as the starting material for our initial studies, which were directed toward the total synthesis of the antipode of **1** (i.e. *ent*-**1**, Scheme 1a) and the confirmation of the relative stereochemical configuration of the molecule, if not its absolute configuration. At that point we would have the option to apply our developed strategy using (*S*)-citronellal as a starting material in order to obtain the correct enantiomer of CJ-16,264 should our findings dictated the endeavor. Thus, disconnection of *ent*-**1** (Scheme 1a) through the Reformatsky-type reaction employed by Lambert and Danishefsky in their synthesis of UCS1025A (**3**)^{8,9} led to TBS-protected iodide **6** (ideally racemic in order to obtain both diastereoisomers of the final product) and aldehyde **7**, the latter being traced back to (*R*)-citronellal (**12**) through tricyclic lactone **8** (transannular Diels–Alder reaction¹⁰; see *endo* transition state TS-**9**, Scheme 1a) and hydroxy acid **11a** [macrolactonization, (*Z*)- α,β -unsaturated as required for the stereochemical relationship of the C1- and C2-substituents of the initially targeted *ent*-**1**].

Scheme 1b summarizes the construction of the required hydroxy acid **11a** starting from (*R*)-citronellal (**12**). Reaction of **12** with the ylide generated from Ph₃P and CBr₄ gave the

expected 1,1-dibromoolefin (98% yield) whose exposure to *n*-BuLi led to the corresponding terminal acetylene **14** (95% yield)¹¹. Alternatively, acetylene **14** could be prepared from **12** by reaction with the Bestmann–Ohira reagent¹² (85% yield) as shown in Scheme 1b. The latter compound was then converted to vinyl iodide **15** through reductive ozonolysis (O₃; then NaBH₄, 85% yield) followed by a sequence involving carboalumination/iodination¹³ (Cp₂ZrCl₂-AlMe₃; then I₂, overall 84% yield). Subsequent oxidation of **15** (SO₃•py¹⁴, 95% yield) was then followed by selective (*Z*)-olefination of the resulting aldehyde with bis-trifluoroethoxy phosphonate **16**¹⁵ in the presence of KHMDS and 18-crown-6 to furnish (*Z*)-α,β-unsaturated ethyl ester **17** (92% yield, 20:1 *Z*:*E* dr). The vinyl iodide **17** was then coupled with hydroxy vinylstannane **18** using CuTC¹⁶ to afford hydroxy ester **19** (88% yield) which, upon hydrolysis (aq. LiOH), provided the desired (*Z*)-hydroxy acid **11a** (99% yield). We note here that attempted use of palladium-catalyzed¹⁷ coupling reactions to unite vinyl iodide **17** with stannane **18** led to significant amounts of homo-coupling side-products.

With (*Z*)-hydroxy acid **11a** readily available, its macrolactonization was then attempted under various conditions¹⁸, including Yamaguchi¹⁹, Keck²⁰ and Shiina²¹ protocols, all of which failed to produce any of the desired 13-membered macrolide **10** (see Scheme 2). Instead, and under high dilution Shiina conditions (MNBA, Et₃N, DMAP cat.), we observed the formation of the 26-membered macrolide **22** (37% yield plus 8% yield of the corresponding triolide). That the (*2Z*)-olefinic bond of **11a** had isomerized to the (*2E*)-olefinic bond within the diolide **22** was evident from the ¹H NMR spectrum of the latter (*J*_{α,β} = 15.7 Hz). A Michael addition/bond rotation/elimination of DMAP²² or Et₃N²³ under the reaction conditions may account for this observed isomerization of the (*2Z*)-olefinic bond of **11a** to the (*2E*)-olefinic bond within the diolide **22**. The same macrolide (**22**) was prepared from (*2E*)-α,β-unsaturated hydroxy acid **11b** (synthesized from hydroxy vinyl iodide **15** in three steps and 79% overall yield as shown in Scheme 1c) by the same procedure as that used for the macrodimerization of **11a** in 40% yield (plus 8% yield of the corresponding triolide).

Our inability to produce the targeted Diels–Alder precursor **10** coupled with the failure of hydroxy acids **11a** and **11b** to undergo intramolecular [4+2] cycloaddition^{24–27}, led us to attempt the double intramolecular Diels–Alder reaction of the now readily available macrolide **22**. The anticipated incorrect configuration of the product at C1/C1' (see structure **25**, Scheme 2) was expected to be invertible through epimerization. Pleasantly, upon heating **22** in *m*-xylene at 220 °C for 12 h, 10-membered ring macrodiolide **25** was obtained exclusively and in 48% yield, presumably through the *exo* transition state *exo*-TS-**24** (Scheme 2). The racemic form of **25** [i.e. (±)-**25a**, obtained from racemic citronellal via the 6,6'-*anti* diolide **22a** (not shown)] crystallized nicely from hexane (colorless crystals, m.p. 119–123 °C) yielding to X-ray crystallographic analysis²⁸ (see ORTEP representation, Scheme 2), which confirmed the relative configurations of the decalin moiety as depicted in **25**.²⁹

Exposure of diolide **25** to NaOMe in THF: wet MeOH at 65 °C led to the formation of γ-lactone **8** (epimerized at C1, colorless crystals, m.p. 103–105 °C, diethyl ether, see ORTEP representation, Scheme 2)²⁸ as the major product and hydroxy acid **26** (not epimerized at

C1) as the minor product (85% yield, **8:26** ca 3:2, chromatographically separated) (see Scheme 2). The desired diastereoisomer (**8**) for the synthesis of *ent*-**1** was then converted to TBS ether decalin system **27** through a four-step sequence involving (i) LAH reduction (diol, 98% yield); (ii) selective reaction of the less hindered hydroxyl group with the bulky 2,4,6-tri-*iso*-propylbenzenesulfonyl chloride (TPBSCl) in the presence of Ag₂O³⁰ and catalytic amounts of KI [to afford the corresponding sulfonate at the less hindered site (appendage at C2), 63% yield, 79% yield based on 80% conversion]; (iii) silylation (TBSCl, imidazole, 90% yield) of the remaining hydroxyl moiety (on C1 appendage); and (iv) reductive removal of the sulfonate group (LiEt₃BH³¹, 82% yield). The latter compound (i.e. **27**) was then desilylated (TBAF, 91% yield) and the resulting alcohol was oxidized with DMP³² to the targeted aldehyde **7** in 81% yield as shown in Scheme 2.

Coupling of aldehyde **7** with racemic pyrrolizidinone iodide **6** under the influence of BEt₃ (via the corresponding boron enolate of **6**)⁸ proceeded stereoselectively as shown in Scheme 3a furnishing a mixture of alcohols **30** and **31** (87% yield, **30:31** ca 3.5:1 dr, chromatographically separated). The absolute stereochemical configuration of compound **30** (colorless crystals from ethyl acetate-hexane, m.p. 175–178 °C) was confirmed by X-ray crystallographic analysis (see ORTEP representation, Scheme 3a)²⁸. Removal of the TBS group (TASF, 81% yield) from the targeted diastereoisomer **30** (major) followed by DMP oxidation of the secondary alcohol of the resulting diol (**32**) then furnished corrected-*ent*-**1** (epimerized at C7', scheme 3a, 83% yield). While the mass spectrum of synthetic corrected-*ent*-**1** matched that reported for natural CJ-16,264, its ¹H- and ¹³C-NMR data differed significantly from those reported for the natural product, suggesting that the originally assigned structure was in error. The observed epimerization at C7' in corrected-*ent*-**1** upon oxidation at C8' of its precursor (i.e. **32**) confirmed by NMR spectroscopy ($J_{7',7'a} = 0$ Hz for corrected-*ent*-**1**; $J_{7',7'a} = 4.1$ Hz for **32**) is noteworthy. Manual molecular modeling of corrected-*ent*-**1** reveals a dihedral angle closer to 90° in its most sterically favorable conformation (*anti* relationship of H7' and H7'a; see ref. 2b for model study and ref. 5 for structural assignment of pyrrolizilactone **1a**) whereas similar modeling of **32** shows its most sterically favorable conformation exhibiting a dihedral angle of closer to 30° (*syn* relationship of H7' and H7'a; see ref. 2 for model study). The latter conformation is supported by the X-ray analysis of **30** (see ORTEP representation, Scheme 3a, X-ray dihedral angle for H7'-C7'-C7'a-H7'a = 38.6°). It is presumed that thermodynamics spontaneously drive this epimerization to the most stable diastereoisomer (i.e. corrected-*ent*-**1**).

Faced with this predicament, and in order to decipher the true structure of antibiotic CJ-16,264, we then opted to synthesize the diastereoisomeric structure of corrected-*ent*-**1**, isomer **34** (Scheme 3a) derived from enantiomerically pure **7**, and the antipode of the shown enantiomer of **6** via intermediate **31** as shown in Scheme 3a. Thus, chromatographically separated diastereoisomer **31** was converted to **34** through the same two-step sequence used for the conversion of **30** to corrected-*ent*-**1**, and in similar yields, as depicted in Scheme 3a. However, the spectroscopic data of **34** again failed to match those reported for natural CJ-16,264, prompting us to target the remaining two diastereoisomers of this series of compounds, namely those reachable from hydroxy acid **26** (Scheme 2).

To this end, reaction conditions were first optimized to obtain the desired hydroxy acid **26** as the major product of the hydrolysis of **25** (Scheme 2). Thus, treatment of **25** with LiOH in THF: MeOH: H₂O furnished a mixture of **26** and **8** (93% yield) in which the desired hydroxy acid **26** predominated (**26**:**8** ca 2:1, chromatographically separated). The required aldehyde **29** (anti C1/C2) was then synthesized from hydroxy acid **26** through a sequence involving selective methylation of the carboxylic acid moiety (K₂CO₃, MeI, 85% yield), mesylation of the hydroxyl group (MsCl, Et₃N, 90% yield), and reduction of the resulting mesylate methyl ester with Zn dust³³ in the presence of NaI to afford tetramethylated decalin system **28** (83% yield). The methyl ester of the latter was then reduced (DIBAL, 93% yield) and the resulting alcohol was oxidized (DMP, 87% yield) to afford the desired aldehyde **29** as shown in Scheme 2.

Coupling of the now readily available aldehyde **29** with racemic pyrrolizidinone iodide **6** under BEt₃-mediated Reformatsky-type coupling⁸, as shown in Scheme 3b, again proceeded stereoselectively, providing a mixture of crystalline alcohols **35** (colorless crystals from ethyl acetate-hexane, m.p. 142–145 °C) and **36** (colorless crystals from ethyl acetate-hexane, m.p. 130–134 °C) (83% yield, **35**:**36** ca 2.5:1 dr, chromatographically separated). Both **35** and **36** yielded to X-ray crystallographic analysis²⁸ (see ORTEP representations, Scheme 3b) that proved their configurational arrangements as shown in Scheme 3b. The *syn* arrangement of H7' and H7'a in **35** and **36** was consistent with their ¹H-NMR data (*J*_{7',7'a} = 3.9 Hz) as it was for their diastereoisomeric counterpart **30** (*J*_{7',7'a} = 4.0 Hz). Subsequent removal of the TBS group from intermediates **35** and **36** provided diols **37** and **39**, respectively (TASF, 78% yield for **37**, 91% yield for **39**). The targeted diastereoisomeric structures of CJ-16,264 were then obtained from **37** and **39** through DMP oxidation to afford (–)-**38** and **40** in 83% and 82% yield, respectively. Again, we observed epimerization at C7' in both final products (–)-**38** and **40** as evidenced by the *J*_{7',7'a} = 0 Hz coupling constants between H7' and H7'a for both compounds. The same argument and supporting evidence provided above for corrected-*ent*-**1** and **32** apply here for (–)-**38** and **40** and their precursors **37** and **39**, respectively. To our delight, the spectroscopic data (¹H- and ¹³C-NMR) and mass spectrum of structure (–)-**38** were found to be in agreement with the data reported for the natural CJ-16,264,¹ suggesting a structural revision for the antibiotic CJ-16,264. The optical rotation of **38** ([α]_D = –9.8° in MeOH), however, was found to be of the opposite sign to that reported for the natural product ([α]_D = +27.3° in MeOH)¹, pointing to the enantiomer of (–)-**38** as the true structure of antibiotic CJ-16,264. With this information in our possession, we then sought to synthesize the antipode of (–)-**38**, compound (+)-**38** (Scheme 4) in order to fully confirm the absolute configuration and obtain a synthetic sample of the naturally occurring antibiotic CJ-16,264.

The final drive toward the targeted molecule [(+)-**38**] (via intermediates *ent*-**29** plus **6**, *ent*-**35** and *ent*-**37**) proceeded from (*S*)-citronellal (**41**) along the same lines and in similar yields as the synthesis of (–)-**38** (Scheme 3b) as summarized in Scheme 4. The ¹H- and ¹³C-NMR spectroscopic and spectrometric data for synthetic CJ-16,264 matched those reported¹ and provided by Dr. Y. Sugie for the natural product and so did the sign of optical rotation ([α]_D = +7.7° in MeOH). Attempts to recrystallize (+)-**38** from a variety of solvents thus far led to partial formation of the C7'-C8' enol (enol proton, δ = 12.72 ppm, keto:enol ca 4:1)

form and semicrystallization. The enolization of (+)-**38** could be completely reversed by passage of the mixture through a silica gel column (EtOAc: hexanes, hexanes→50% EtOAc in hexanes). The deuterated form (deuteration at C7') of (+)-**38** was also detected through a ¹H-NMR experiment (benzene-*d*₆, D₂O exchange) in which the C7' proton signal decreased significantly. The corresponding diastereoisomer of (+)-**38**, compound *ent*-**40**, was also synthesized from intermediate *ent*-**36**, formed in the coupling of *ent*-**29** and racemic **6**, via *ent*-**39** (see Scheme 4). Interestingly, the proven structure of antibiotic CJ-16,264 [(+)-**38**] resembles closely that proposed for antibiotic **1a** (pyrrolizilactone, Figure 1a), although the latter was not precisely defined with regard to the relative stereochemistry between the decalin and pyrrolizidinone domains and the absolute stereochemistry of the molecule (structure **1a** represented four stereostructures: two enantiomers and two diastereoisomers)⁵.

The reported chemistry led to an enantioselective total synthesis and structural revision of antibiotic CJ-16,264 as structure (+)-**38** and sets the foundation for the full structural elucidation of other members of this growing class of antitumor antibiotics. The devised synthetic strategy features a novel macrolactonization/dimerization and a stereoselective intramolecular bis-[4+2] cycloaddition to forge the highly-substituted decalin system of the molecule in its proper stereochemical configuration. A total of six isomeric structures of the antibiotic have been rendered readily available for biological investigations, underscoring the importance of total synthesis to both biology and structural elucidation. Most significantly, the reported total synthesis of antibiotic CJ-16,264 paves the way for analog design, synthesis, and biological evaluation within this class of natural products.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Sugie Y, Hirai H, Kachi-Tonai H, Kim YJ, Kojima Y, Shiomi Y, Sugiura A, Sugiura A, Suzuki Y, Yoshikawa N, Brennan L, Duignan J, Huang LH, Sutcliffe J, Kojima N. *J Antibiot.* 2001; 54:917–925. [PubMed: 11827034]
2. (a) Nakai R, Ogawa H, Asai A, Ando K, Agatsuma T, Matsumiya S, Akinaga S, Yamashita Y, Mizukami T. *J Antibiot.* 2000; 53:294–296. [PubMed: 10819301] (b) Agatsuma T, Akama T, Nara S, Matsumiya S, Nakai R, Ogawa H, Otaki S, Ikeda S, Saitoh Y, Kanda Y. *Org Lett.* 2002; 4:4387–4390. [PubMed: 12465894]
3. Yang YL, Lu CP, Chen MY, Chen KY, Wu YC, Wu SH. *Chem Eur J.* 2007; 13:6985–6991. [PubMed: 17503417]
4. For the recent total synthesis of myelothermophins C and D, see: Nicolaou KC, Shi L, Lu M, Pattanayak MR, Shah AA, Ioannidou HA, Lamani M. *Angew Chem Int Ed.* 2014; 53:10970–10974. *Angew Chem.* 2014; 126:11150–11154.
5. Nogawa T, Kawatani M, Uramoto M, Okano A, Aono H, Futumura Y, Koshino H, Takahashi S, Osada H. *J Antibiot.* 2013; 66:621–623. [PubMed: 23715039]
6. Sizova, EP. PhD thesis. University of Minnesota; 2009.
7. Pricing information: (*S*)-citronellal (Aldrich Co.) = \$33/1G; (*R*)-citronellal (Takasago International Co.) = \$0.3/1G.
8. Lambert TH, Danishefsky SJ. *J Am Chem Soc.* 2006; 128:426–427. [PubMed: 16402826]

9. For other total synthesis of UCS1025A, see: Hoye TR, Dvornikovs V. *J Am Chem Soc.* 2006; 128:2550–2551. [PubMed: 16492035] De Figueiredo RM, Fröhlich R, Christmann M. *Angew Chem Int Ed.* 2007; 46:2883–2886. *Angew Chem.* 2007; 119:2941–2944. Uchida K, Ogawa T, Yasuda Y, Mimura H, Fujimoto T, Fukuyama T, Wakimoto T, Asakawa T, Hamashima Y, Kan T. *Angew Chem Int Ed.* 2012; 51:12850–12853. *Angew Chem.* 2012; 124:13022–13025.
10. For examples of transannular Diels–Alder reactions incorporating (Z)-dienophiles in total synthesis, see: Marsault E, Toró A, Nowak P, Deslongchamps P. *Tetrahedron.* 2001; 57:4243–4260.
11. Corey EJ, Fuchs PL. *Tetrahedron Lett.* 1972; 13:3769–3772.
12. Ohira S. *Synth Commun.* 1989; 19:561–564.
13. (a) Negishi E, Okukado N, King AO, Van Horn DE, Spiegel BI. *J Am Chem Soc.* 1978; 100:2254–2256. (b) Negishi E. *Pure Appl Chem.* 1981; 53:2333–2356. and references therein.
14. Parikh JR, Doering WVE. *J Am Chem Soc.* 1967; 89:5505–5507.
15. Still WC, Gennari C. *Tetrahedron Lett.* 1983; 24:4405–4408.
16. Allred GD, Liebeskind LS. *J Am Chem Soc.* 1996; 118:2748–2749.
17. Stille JK. *Angew Chem Int Ed.* 1986; 25:502–524.
18. Parenty A, Moreau X, Campagne JM. *Chem Rev.* 2006; 106:911–939. [PubMed: 16522013]
19. Inanaga J, Hirata K, Saeki H, Katsuki T, Yamaguchi M. *Bull Chem Soc Jpn.* 1979; 52:1989–1993.
20. Boden EP, Keck GE. *J Org Chem.* 1985; 50:2394–2395.
21. Shiina I, Kubota M, Ibuka R. *Tetrahedron Lett.* 2002; 43:7535–7539.
22. Rezgui F, Gaied MME. *Tetrahedron Lett.* 1998; 39:5965–5966.
23. Hill JS, Isaacs NS. *Tetrahedron Lett.* 1986; 27:5007–5010.
24. For review of intramolecular Diels–Alder reactions in total synthesis, see: Nicolaou KC, Snyder AA, Montagnon T, Vassilikogiannakis G. *Angew Chem Int Ed.* 2002; 41:1668–1698. *Angew Chem.* 2002; 114:1742–1773.
25. Juhl M, Tanner D. *Chem Soc Rev.* 2009; 38:2983–2992. [PubMed: 19847335]
26. (a) Roush WR, Gillis HR. *J Org Chem.* 1982; 47:4825–4829. (b) Roush WR, Hall SE. *J Am Chem Soc.* 1981; 103:5200–5211.
27. (a) Yoshida K, Grieco PA. *J Org Chem.* 1984; 49:5257–5260. (b) Grieco PA, Nunes JJ, Gaul MD. *J Am Chem Soc.* 1990; 112:4595–4596.
28. Crystallographic data for crystal structures 8, ($\pm$)-25a, *ent*-25, 30, 35 and 36 are available free of charge from the Cambridge Crystallographic Data Centre (www.ccdc.cam.ac.uk/data_request/cif) under deposition number 1059641, 1059642, 1400327, 1059640, 1059639 and 1059638.
29. *ent*-25 (colorless crystals from diethyl ether, m.p. 120–126 °C, obtained as summarized in Scheme 4) also yielded to X-ray crystallographic analysis that confirmed its structure (see supplementary information for ORTEP)²⁸.
30. Bouzide A, Sauvé G. *Org Lett.* 2002; 4:2329–2332. [PubMed: 12098239]
31. Brown HC, Krishnamurthy S. *J Am Chem Soc.* 1973; 95:1669–1671.
32. Dess DB, Martin JC. *J Org Chem.* 1983; 48:4155–4156.
33. Ko ovský P, erný V. *Collect Czech Chem Commun.* 1979; 44:246–250.

Figure 1.

Molecular structures of antibiotic CJ-16,264 and other natural products containing varying substituted decalin systems and pyrrolizidinone (**a**) or pyrrolidinone moieties (**b**).

Scheme 1.

Retrosynthetic analysis for ent-CJ-16,264 (ent-1) and synthesis of (2Z)- and (2E)-hydroxy acids **11a** and **11b**. **b.** Reagents and conditions: a) CBr_4 (2.0 equiv), PPh_3 (4.0 equiv), CH_2Cl_2 , 0 °C, 3 h, 98%; b) $n\text{BuLi}$ (3.0 equiv), THF, -78 °C, 2 h, 95%; c) K_2CO_3 , **13**, MeOH, 25 °C, 10 h, 85%; d) O_3 , CH_2Cl_2 :MeOH (1:1), -78 °C, 2 h; then NaBH_4 (1.1 equiv), -78 → 25 °C, 4 h, 85%; e) Cp_2ZrCl_2 (1.1 equiv), AlMe_3 (4.0 equiv), $\text{ClCH}_2\text{CH}_2\text{Cl}$, 25 °C, 5 h; then I_2 , THF, -20 °C, 2 h, 84%; f) $\text{SO}_3 \cdot \text{py}$ (2.0 equiv), Et_3N (4.0 equiv), CH_2Cl_2 : DMSO (3:1), 0 °C, 1 h, 91%; g) LiHMDS (1.2 equiv), 18-crown-6 (5.0 equiv), **16** (1.2 equiv), THF, 0 °C, 2 h, 92% (95% Z:E dr); h) CuTC (6.0 equiv), **18** (3.0 equiv), DMF, 0 → 25 °C, 2 h, 85%; i) LiOH (35.0 equiv), THF: H_2O (1:1), 60 °C, 18 h, 99%. **c.** Reagents and conditions: j) $\text{SO}_3 \cdot \text{py}$ (2.0 equiv), Et_3N (4.0 equiv), CH_2Cl_2 : DMSO (3:1), 0 °C, 1 h; then **20**, 25 °C, 4 h; 90%; k) CuTC (6.0 equiv), **18** (3.0 equiv), DMF, 0 → 25 °C, 2 h, 88%; l) LiOH (35.0 equiv), THF: H_2O (1:1), 60 °C, 20 h, 99%. Abbreviations: TBS = tert-butyltrimethylsilyl; Cp = cyclopentadienyl; LiHMDS = lithium bis(trimethylsilyl)amide; CuTC = copper(I)-thiophene-2-carboxylate.

Scheme 2.

Transannular Diels–Alder reaction to provide *cis*-fused decalin **25** and syntheses of C1-epimeric aldehydes **7** and **29**. Reagents and conditions: a) conditions included several standard macrolactonization protocols including Yamaguchi, Keck and Shiina conditions; b) MNBA (1.5 equiv), Et₃N (2.0 equiv), DMAP (0.1 equiv), CH₂Cl₂, 25 °C, 10 h, 37–40% for diolide (**22**), 8% for triolide; c) *m*-xylene (0.021 M), 220 °C, 12 h, 48%; d) NaOMe (10.0 equiv), THF: wet MeOH (9:1), 65 °C, 14 h, 85% [ca 3:2 dr at C1 (**8**:**26**)]; e) LAH (1.5 equiv), THF, –78 °C, 1 h, 98%; f) TPBSCl (1.6 equiv), Ag₂O (2.5 equiv), KI (0.2 equiv),

CH₂Cl₂, 25 °C, 20 h, 63% (79% brsm); g) TBSCl (2.0 equiv), imidazole (3.0 equiv), CH₂Cl₂, 0→25 °C, 15 h, 90%; h) LiEt₃BH (4.0 equiv), THF, microwave, 80 °C, 10 min, 82%; i) TBAF (1.5 equiv), THF, 25 °C, 1.5 h, 91%; j) DMP (2.5 equiv), CH₂Cl₂, 0→25 °C, 2 h, 81%; k) LiOH (50.0 equiv), THF:MeOH:H₂O (4:2:1), 80 °C, 24 h, 93% [ca 2:1 dr at C1 (**26:8**)]; l) MeI (1.5 equiv), K₂CO₃ (2.0 equiv), DMF, 25 °C, 2 h, 85%; m) MsCl (2.0 equiv), Et₃N (3.0 equiv), CH₂Cl₂, 0 °C, 2 h, 90%; n) NaI (10.0 equiv), activated Zn dust (20.0 equiv), DME, 95 °C, 2 h, 83%; o) DIBAL-H (1.5 equiv), hexane, −78 °C, 1 h, 93%; p) DMP (2.3 equiv), CH₂Cl₂, 0→25 °C, 1 h, 87%. Abbreviations: MNBA = 2-methyl-6-nitrobenzoic anhydride; DMAP = 4-(dimethylamino)pyridine; LAH = lithium aluminum hydride; DIBAL-H = di-*iso*-butylaluminum hydride; TBSCl = *tert*-butyldimethylsilyl chloride; MsCl = methanesulfonyl chloride; TPBSCl = 2,4,6-tri-*iso*-propylbenzenesulfonyl chloride; DME = 1,2-dimethoxyethane; TBAF = tetra-*n*-butylammonium fluoride; DMP = Dess–Martin periodinane.

Scheme 3.

Syntheses of the enantiomer of the originally assigned structure of CJ-16,264 (corrected-*ent*-1) (**a**) and its diastereoisomers **34** (**a**), **38** (**b**), and **40** (**b**). **a**. Reagents and conditions: a) BEt_3 (3.5 equiv), **6** (3.5 equiv), toluene, -78°C , 2 h, 87% (**30**:**31** ca 3.5:1 dr); b) TASF (2.0 equiv), THF, 0°C , 1 h, 81% for **32**, 73% for **33**; c) DMP (1.5 equiv), CH_2Cl_2 , $0 \rightarrow 25^\circ\text{C}$, 2 h, 80% for corrected-*ent*-1, 83% for **34**. **b**. Reagents and conditions: d) BEt_3 (3.5 equiv), **6** (3.5 equiv), toluene, -78°C , 2 h, 83% (**35**:**36** ca 2.5:1 dr); e) TASF (2.0 equiv), THF, 0°C , 1 h, 78% for **37**, 91% for **39**; f) DMP (1.5 equiv), CH_2Cl_2 , $0 \rightarrow 25^\circ\text{C}$, 2 h, 83% for (-)-**38**, 82% for **40**. Abbreviations: TASF = tris(dimethylamino)sulfoniumdifluoro trimethylsilicate.

Scheme 4.

Synthesis of the revised structure of antibiotic CJ-16,264 [(+)-38] and its diastereoisomer *ent*-40. Reagents and conditions: a) see Schemes 1–3; b) BEt_3 (3.5 equiv), **6** (3.5 equiv), toluene, -78°C , 2 h, 86% (*ent*-35:*ent*-36 ca 2.3:1 dr); c) TASf (2.0 equiv), THF, 0°C , 1 h, 76% for *ent*-37, 88% for *ent*-39; d) DMP (1.5 equiv), CH_2Cl_2 , $0 \rightarrow 25^\circ\text{C}$, 2 h, 83% for (+)-38, 82% for *ent*-40.