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Isolation, Structure, and Total Synthesis of the Marine Macrolide Mangrolide D

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Abstract

The isolation, characterization and total synthesis of the macrocyclic polyene mangrolide D is reported. A 16-step total synthesis relies on robust Suzuki and ring-closing metathesis reactions, and an iron-catalyzed hydroazidation of an exo-methylene substituted tetrahydropyran as a key step for the synthesis of the appended 4-*epi*-vancosamine sugar. Although mangrolide D did not display antibiotic activity, this work should prove enabling towards the synthesis of the antitubercular tiacumicins which display a virtually identical macrocyclic backbone.

Graphical Abstract

In 2013, Jamison and MacMillan disclosed the isolation, structural characterization, and biological activity of a novel marine-derived macrolide antibiotic termed mangrolide A (**2**, Fig. 1).¹ The original sample of mangrolide A (**2**), isolated from the fermentation broth of the rare marine actinomycete strain *Actinoalloteichus spitensis*, was found to possess Gram-negative antibacterial activity and stimulated us and others to pursue a total synthesis of this material. Gademann and coworkers, based on their very elegant synthesis of the structurally related macrolide fidaxomicin (**3**),² recently reported the total synthesis of mangrolide A (**2**).³ While this study fully corroborated the structure disclosed for the isolated natural product

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ASSOCIATED CONTENT

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Isolation, structural characterization and analytical data for mangrolide D, and experimental procedures and analytical data for new synthetic compounds (PDF)

sample,¹ synthetic mangrolide A was found to be devoid of any antibacterial activity.³ Through our own synthetic work, we had come to the same disconcerting conclusion that our synthetic mangrolide A (**2**),^{4,5} as well as ultra-purified re-isolated natural material, failed to register the Gram-negative activity reported for the original batch of fermented mangrolide A. Therefore, we postulated that the Gram-negative activity of the original sample could be derived from a minor contaminating or interfering metabolite, a phenomenon not uncommon in the field of natural product isolation and bioactivity.⁶ In this context, we reevaluated the founder strain leading to the isolation and structural characterization of a natural analog termed mangrolide D (**1**) as reported herein in conjunction with a total synthesis. Given the structural identity of the mangrolide macrocycle to the macrocyclic core of fidaxomicin (**3**, marketed as Dificid®), an antibiotic used clinically to treat the gastrointestinal infection caused by *C. difficile*,⁷ we were mindful that an efficient synthetic approach towards mangrolides could also be leveraged towards the synthesis of fidaxomicin analogs not available from fermentation.^{8,9}

Large-scale fermentation of *Alloteichus spitensis* strain SNA-018 and subsequent extraction with XAD-7 resin, followed by flash and HPLC C18 purification led to **1** (1.1 mg from 20 L) as a white solid; $[\alpha]_D^{25} +XX$; UV (MeOH) 204, 234, and 260. A molecular formula of $C_{33}H_{53}NO_6$ was derived from the HRMS ESI-TOF with an $[M+H]^+$ of m/z 560.3949 (calcd for $C_{42}H_{70}NO_{12}$, 560.3951). Analysis of the 1H and ^{13}C chemical shifts indicated a polyketide structure with five olefins, four vinyl methyls, and a sugar moiety.¹⁰ Briefly, the aglycon structure shows similarities to the aglycon of tiacumicin B (**3**) and mangrolide A (**2**). However, its macrocyclic core differs from **3** by virtue of the oxidation state at C₂₁ (Me vs CH₂OR) and C₁₈ (Me vs OH).¹¹ The configuration at C₇, C₁₀, C₁₁ and C₁₇ were assigned based on homology to mangrolide A (**2**) and **3**.^{1,2b} The relative configuration of the α -linked 4-*epi*-vancosamine at C₁₁ was assigned based on detailed analysis of the coupling constants as well as NOE measurements.¹⁰ A small coupling of 2.5 Hz from the anomeric proton H1' to H2'-axial establishes the equatorial position for H1'. NOE correlations from the axial methyl protons H7' to H6' and to H2' equatorial, establish the relationship between C1' and C3' as well as the C3'/C4' and C4'/C5' configurations as *anti*, *anti*. This is consistent with previous reports of 4-*epi*-vancosamine.^{12,13}

Our strategy towards mangrolide D (**1**), originally conceived for mangrolide A (**2**) as disclosed in 2013,⁴ is depicted in Scheme 1.¹⁴ With future analog studies in mind, we advanced a flexible and convergent approach that would unite fragments **4-6** via robust and interchangeable bond-forming reactions (cross-coupling, olefin metathesis, esterification). In the event, we settled on the olefin metathesis as the macrocycle-forming step.

The synthesis of the top ester fragment is shown in Scheme 2. Similar to Gademann,³ Lewis acid-assisted opening of (*R*)-isopropylloxirane (**7**)¹⁵ with propynyllithium, followed by Cu(I)-catalyzed boration of homopropargylic alcohol **8**³ yielded multigram quantities of enantiomerically pure vinylborane **5**.³ Esterification of this alcohol **5** with dienolic acid **4** (prepared from olefination of acrolein with phosphorane **10**)¹⁶ provided the desired northern coupling partner **9** in three steps longest linear sequence from commercially available material.

The synthesis of southern fragment **6** commenced with a highly diastereoselective aldol reaction of bornanesultam **12** with (*E*)-3-iodomethacrolein derived from MnO₂-mediated oxidation of known alcohol **11**^{2b,8b} (Scheme 3).¹⁷ The *syn*-aldol product **13** was obtained in 82% yield and protected as silyl ether **14** (91% yield). Reduction of **14** (DIBAL-H, CH₂Cl₂, –78 °C)¹⁸ delivered the corresponding aldehyde (82%) which was homologated to unsaturated ester **15** via treatment with phosphorane **10** in 78% yield. Reduction of the ester, followed by oxidation of the resulting primary alcohol with Dess-Martin periodinane yielded aldehyde **16** (92%, 2 steps). A highly diastereoselective Brown allylation (→ **17**) was followed by silylation of the homoallylic alcohol (→ **18**) and selective deprotection of the triethylsilyl ether to provide southern fragment **6** in 10 steps and 30% overall yield from vinyl iodide **11**.

Next, we focused on the synthesis of the 4-*epi*-vancosamine sugar fragment,^{19,20} which we plan to introduce via the activated 2-deoxy-azido glycosyl donor **27**, the synthesis of which begins with a selective oxidation of the diol derived from commercially available 3,4-di-*O*-acetyl-6-deoxy-*L*-glucal (**19**, Scheme 4).²¹ Oxa-Michael addition of methanol to the resulting enone **20** yielded tetrahydropyranone **21** (75%).²² Extensive experimentation revealed that methylenation of **21** was best achieved using Peterson olefination conditions, providing **22** in 54% yield.²³ Gratifyingly, the key hydroazidation of **22** using conditions first described by Boger and coworkers provided azide **23** in good yield and with desired stereochemistry (10:1).^{24,25} Hydrolysis of the anomeric methoxy substituent (→ **24**) and peracetylation (→ **25**) set the stage for selective hydrazine-mediated removal of the primary acetate (→ **26**) and activation of the anomeric position as the trichloroacetimidate **27**. The latter was not purified and used as such for coupling with fragment **6** as described in Scheme 5.

Treatment of homoallylic alcohol **6** with the above prepared crude imidate **27** in the presence of BF₃-etherate selectively provided the α -anomer of glycosylated product **28** (78%).²⁶ Staudinger reduction of the azide **28** yielded primary amine **29**, a material that underwent uneventful Suzuki cross-coupling with top fragment **9**.^{2,27} Ring-closing metathesis of **30** with second generation Grubbs catalyst provided protected macrocycle **31** as an inseparable mixture of *E/Z*-isomers (1:1).² Fluoride-mediated removal of the silylether was followed by mild acetate removal to yield mangrolide D (**1**) and the corresponding *Z*-isomer (42% combined) that were separated via reversed phase HPLC. The proton and carbon NMR spectra of synthetic mangrolide D fully matched those of the natural product, whereas significant chemical shift differences were observed for the corresponding mangrolide diastereomer containing the enantiomeric 4-*epi*-vancosamine derived from 3,4-di-*O*-acetyl-6-deoxy-*D*-glucal (*ent*-**19**).²¹ The optical rotations for synthetic and natural mangrolide D were of the same sign and similar magnitude (synthetic: [α]_D = –67.5 (*c* = 0.08, CHCl₃); natural: [α]_D = –50.1 (*c* = 0.08, CHCl₃)). Alas, synthetic or natural mangrolide D was poorly active when tested against the Gram-positive strain *B. subtilis* (MIC = 32 μ g/mL) and inactive against the Gram-negative strain *P. aeruginosa* PA01 (MIC > 256 μ g/mL). The control compound streptomycin killed *B. subtilis* with an MIC of 0.125 μ g/mL and gentamicin's antibacterial activity registered with an MIC of 1 μ g/mL against *P.*

aeruginosa PA01. These results imply that mangrolide D was not responsible for the antibacterial activity of the original mangrolide A fermentation sample.

In summary, our search to identify a potential metabolite responsible for the Gram-negative activity of a fermentation sample originally assigned to mangrolide A led to the isolation and structural characterization of mangrolide D. A convergent, efficient total synthesis of mangrolide D (16 total steps longest linear sequence) fully confirmed its structure and assignment of the absolute configuration of the attached 4-*epi*-vancosamine. Other highlights are the efficient approach to both enantiomers of 4-*epi*-vancosamine, a natural aminosugar for which there were virtually no reported synthetic protocols described.^{13,20} In particular, a highly stereoselective iron-catalyzed hydroazidation developed by Boger (**22** → **23**) was key to a successful assembly of the 3-deoxy-4-azido sugar. Although mangrolide D did not register antibacterial activity, our synthetic efforts are nevertheless highly relevant in the context of synthetic macrolide antibiotics.²⁸ The macrocyclic core of mangrolide is virtually identical to the core of the clinically useful fidaxomicin (**3**). Analogs of the latter could find significance in the context of multidrug-resistant tuberculosis if systemic bioavailable analogs can be identified.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Mangrolide A (2) and D (1) and Fidaxomicin (3).

Scheme 1.
Synthetic Strategy

Scheme 2. Synthesis and Coupling of Fragments 4 and 5^a

Scheme 3. Synthesis of Fragment 6^a

Scheme 4. Synthesis of Activated Azidosugar 27^a

Scheme 5. Fragment Coupling and Final Steps^a