Isolation, Structural Assignment, and Total Synthesis of Barmumycin

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Barmumycin was isolated from an extract of the marine actinomycete Streptomyces sp. BOSC-022A and found to be cytotoxic against various human tumor cell lines. On the basis of preliminary one- and two-dimensional ¹H and ¹³C NMR spectra, the natural compound was initially assigned the structure of macrolactone-type compound 1, which was later prepared by two different routes. However, major spectroscopic differences between isolated barmumycin and 1 led to revision of the proposed structure as E-16. On the basis of the synthesis of this new compound, and subsequent spectroscopic comparison of it to an authentic sample of barmumycin, the structure of the natural compound was indeed confirmed as that of E-16.

Introduction

Natural products from terrestrial plants and microorganisms have long been a traditional source of drugs; however, over the past few years, marine organisms have garnered ever-increasing attention as a rich bank of new bioactive compounds.¹ Marine actinomycetes have also proven to be an important source of biologically active compounds.²

Among the marine actinomycetes that our group has studied, those of the genus Streptomyces have clearly shown the most pharmacological potential; however, in many bioactive cultures they have yielded only compounds that are already known. During ongoing research efforts to explore the biosynthetic potential of rare marine microorganisms, we isolated two known compounds, pretomaymycin 3 and oxotomaymycin 4 (Figure 1), plus the previously unknown compound barmumycin from the culture broth of the marine actinomycete Streptomyces sp. BOSC-022A, isolated from a tunicate collected off the Scottish coast. Barmumycin and its diacetate show antitumor activity at micromolar concentrations in all 12 cancer cell lines tested (see Table 1 in the Supporting Information). Herein we report the isolation, total synthesis, and structure elucidation of barmumycin.

Results and Discussion

The molecular formula of barmumycin was determined to be C₁₅H₁₉NO₄ by HRMS MALDI-TOF; it gave an (M + H)⁺ ion at m/z 278.13840 (calcd m/z 278.13869 for C₁₅H₂₀NO₄). Reaction of barmumycin with acetic anhydride and pyridine gave a diacetyl derivative, confirmed by MS, pointing the presence of two OH and/or NH protons (see Figure 2).

¹H NMR shows three groups of protons (see Table 2 in the Supporting Information). The first group is in the aromatic region and contains three protons at 6.90 (d), 7.03 (d), and 7.08 (s) ppm; the chemical shifts indicated a 1,2,4-substituted electron-rich benzene ring. The second group corresponds to a single vinylic proton at 5.34 ppm (m). The third region comprises an upfield CH shift at 4.67 ppm (m); two methyl...
groups, seen at 3.90 ppm (s) and 1.62 ppm (d); and the signals of three CH₂ at 4.07 (bt), 4.18 (bd), 3.75 (m), 2.29 (m) and 2.71 (m) ppm. On the basis of these data, the MS findings, and further data from one-dimensional and two-dimensional (COSY, HMBC, and NOESY) ¹H and ¹³C NMR experiments, we initially proposed that the structure of the isolated natural product was that of benzomacrolactone 1, derived from 5-methoxy-2-aminobenzoic acid with an exocyclic (E)-double bond via Wittig reaction; dihydroxylation of a 2,2-dimethoxyacetaldehyde with allyl bromide and indium powder in water (95% yield). ⁷

Decanolides are chemical entities abundant in terrestrial organisms, though only a few (e.g., modiolides A and B) have been reported; see: Crestia, D.; Gu  et al.  J. Org. Chem. 2003, 68, 412–415. ⁸

We sought to synthesize 1 to compare it against an authentic sample of barmumycin in order to assess its structural assignment. Our retrosynthetic analysis of 1 entailed formation of the exocyclic double bond via Wittig reaction; dihydroxylation of a double bond to give the alcohol required for lactonization; and finally, introduction of a functionalized five-carbon chain onto the nitrogen of methyl 2-amino-5-methoxybenzoate (Scheme 1).

The functionalized five-carbon chain on the aniline nitrogen was introduced by two different ways: via reductive amination (Scheme 2) and via N-alkylation (Scheme 3).

Hom ally l alcohol 2 was obtained by Barbier reaction of 2,2-dimethoxyacetaldehyde with allyl bromide and indium powder in water (95% yield).⁷ Attempts at direct deprotection of the dimethyl acetal under acidic conditions led to polymerization of 2; therefore, the alcohol had to be protected. Acetylation of the alcohol to give compound 3, followed by dimethyl acetal deprotection using LiBF₄ in MeCN–H₂O ⁹ gave the aldehyde 4 in excellent yield.

Reductive amination of 4 with aniline ⁵ required special conditions due to the poor nucleophilicity of the aniline (which is deactivated by the methyl ester group in the ortho position); thus, reaction of 4, 5, phenylsiline, and dibutyltin dichloride under microwave irradiation for short reaction times gave the aminal ⁶a in 67% yield. ¹¹,¹² Attempts at protecting the aniline NH in ⁶a as a ‘Bu carbamate failed due to its poor reactivity; therefore, ⁶a was treated with K₂CO₃ in MeOH to give the deacylated derivative ⁶b. However, all attempts at oxidizing ⁶b to its keto derivative resulted in decomposition of the starting material. ¹³ Thus, the aniline had to be protected, but this was not possible in the presence of the unprotected alcohol. Exploiting the lack of reactivity of the aromatic amine toward Boc protection, and using standard conditions, ⁶b was converted into its ‘Bu carbonate derivative ⁶c in 43% yield. ¹⁴ The aniline group of ⁶c was then orthogonally protected using (CF₃CO)₂O in pyridine to afford the trfluoroacetamide derivative ⁶d in quantitative yield. Treatment of ⁶d with 10% TFA in CH₂Cl₂ to remove the carbonate gave the free alcohol ⁶e in quantitative yield. Compound ⁶e was then oxidized with Dess–Martin periodinane (DMP) ¹⁵ to yield the ketone ⁶f in 93% yield. Slow addition of ⁶f to N-methylmorpholine oxide (NMO) and a catalytic amount of OsO₄ in acetone–H₂O to generate the corresponding diol ⁶g while preventing double-bond isomerization gave ⁶g in good yield. Diol ⁶g was further protected by conversion into its 2,2-dimethyl-1,3-dioxolane derivative ⁶h using 2,2-dimethoxypropane plus pyridinium p-toluensulfonate (PPTS) as catalyst (quantitative yield). Deprotection of the amine in ⁶h via mild basic hydrolysis gave the free amine ⁶i in 97% yield.

A faster and better yielding synthesis of ⁶i (Scheme 3) was done in parallel to the route described above. The first step was dihydroxylation of isobutyl but-3-enoate. The introduction of the bromomethyl residue was planned for a later step. The oxidation conditions described above afforded isobutyl 3,4-dihydroxybutanote (11), which was then further protected as the 2,2-dimethyl-1,3-dioxolane derivative ¹², in excellent overall yield for both steps. The key step, transformation of ¹² into the bromoketone ¹³ using bromomethyl lithium, gave ¹³ in 49% yield. N-Alkylation of ¹³ with ¹⁴ under microwave irradiation gave ¹⁴. Wittig chemistry was employed to introduce the ethyldiene chain. Reaction of ¹⁴ with the Wittig ylide derived from ethyltriphenylphosphonium bromide yielded ¹⁵ (43%) as a mixture of Z/E diastereomers. ¹⁶

Z/E-¹⁴ was transformed into ¹⁴ in three successive reactions: hydrolysis of the methyl ester, acetonide protection under acidic conditions, and macrocyclization. The acid ¹⁵ was obtained by purification of the Z/E mixture of acids by semipreparative HPLC. ¹⁷ Racemic Z-1 was obtained in 35% yield by acetal deprotection followed by macrocyclization using EDC·HCl and HNCl. ¹⁸

(11) Kangasmetsa, J. J.; Johnson, T. Org. Lett. 2005, 7, 5653–5655.
(12) Other reduction conditions proved unsuccessful. These included NaBH₄ (OAc)₂ in THF at room temperature for 16 h, NaBH(OAc)₂ in CH₂Cl₂/MeOH at room temperature for 5 h, and NaBH(OAc)₂ in toluene at 110 °C for 2 h.
(13) The aniline ⁵ was isolated from the oxidation degradation mixture. Its formation could be rationalized through hydrolysis of the enamine resulting from enolization of the keto compound.
(14) Under these conditions, methyl 2-(5-allyl-2-oxooxazolidin-3-yl)-5-methoxybenzoate was isolated as a byproduct in 27% yield.
(15) Dess, D. B.; Martin, J. C. J. Org. Chem. 1983, 48, 4155–4156.
(16) The Z/E stereoisomers were in a ratio of 73:27 (based on NMR signal areas).
(17) The NOESY correlations between ⁶H₂O (2.27 and 2.41 ppm) and the vinyl proton (5.58 ppm) confirmed the stereochemistry of (Z)-¹⁵ (see NOESY interactions in the Supporting Information).

J. Org. Chem. Vol. 75, No. 24, 2010 8509

FIGURE 1. Two known compounds isolated from Streptomyces sp. BOSC-022A.

FIGURE 2. Structure of ¹ showing ¹H NMR (blue) and ¹³C NMR (red) chemical shifts (left) and its HMBC and NOE correlations (right).
solid-supported DMAP in a 5 mM CH$_2$Cl$_2$ solution. The $^1$H NMR spectrum of Z-1 showed two doublets for the CH$_3$ linked to the double bond (1.42 ppm and 1.45 ppm) and two quadruplets for the vinylic proton (5.51 and 5.53 ppm). These data could be explained by the presence of two highly populated conformations of Z-1 at room temperature. Therefore, we studied peak coalescence by $^1$H NMR run at different temperatures. Spectra from the initial experiments, run up to 55°C in CDCl$_3$ as solvent, exhibited this trend, but coalescence was not reached at this temperature limit. Finally, coalescence was almost reached in DMSO-$d_6$ as solvent at 145°C (see Table 4 in the Supporting Information). Moreover, comparison of spectroscopic data for baromycin with those for Z-1 revealed dramatic differences in the chemical shifts (see Tables 2 and 3 in the Supporting Information). This discrepancy, despite the conflicting stereochemistry of the two compounds (Z-1 and E-baromycin), led us to pursue a new structural assignment.

Re-evaluation of all possible alternative structures led us to systematic elucidation of E-16 as a novel structure for baromycin (Figure 3). Interestingly, the very close structural resemblance of 16 to the pretomaymycin and oxotomaymycin isolated from the extract (Figure 1) suggests that all three molecules could derive from the same biogenetic pathway.

In order to confirm that the structure of baromycin is actually that of E-16, we synthesized the latter and subsequently compared it to an authentic sample of the former. This began with selective silyl protection of the primary alcohol in the commercially available N-Boc-trans-4-hydroxy-L-prolinol followed by oxidation of the secondary alcohol in the derivative 17, which afforded ketone 18 in 62% yield over two steps (Scheme 4). Wittig chemistry was again employed to introduce the ethylidene chain: reaction of 18 with the Wittig ylide derived from ethyltriphenylphosphonium bromide yielded 19 as a 9:1 mixture of Z/E-diastereomers. Z/E-19 was used directly without separation, as a single purification was planned for the final step of the synthesis. The TMS ether and the tert-butyl carbamate of Z/E-19 were deprotected with 10% TFA in CH$_2$Cl$_2$ to give the pyrrolidine derivative Z/E-20. Condensation of Z/E-20 to vanillic acid using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and N,N-diisopropylethylamine (DIEA) gave a 9:1 mixture of Z/E-diastereomers. The configuration of the double bond was established by the NOESY correlations in Z/E-16 between 5CH$_2$ (4.00–4.20 ppm) and the vinyl proton (5.51 and 5.53 ppm), confirmed the stereochemistry of (Z)-1 (see NOESY interactions in the Supporting Information).

(18) The NOESY correlations between $^1$CH$_3$ (4.35 and 4.82 ppm) and CH$_3$ (1.42 and 1.45 ppm), and between $^3$CH$_2$ (2.09–2.20 ppm) and the vinyl proton (5.51 and 5.53 ppm), confirmed the stereochemistry of (Z)-1 (see NOESY interactions in the Supporting Information).

(19) The reaction was performed with (EtO)$_2$P(O)CH$_2$CH$_3$ and either LDA, K’BuO, or NaHMDS as base.
the desired 19. However, the Kocienski variant of the Julia–Lythgoe olefination20 afforded a 2:1 mixture of E/Z-19. This process entails nucleophilic addition of 5-(ethylsulfonyl)-1-phenyl-1H-tetrazole anion to the ketone followed by transposition and elimination to give the double bond. Again, deprotection of the hydroxyl group and the amine group was obtained using 10% TFA in CH2Cl2, and condensation of E/Z-20 to vanillic acid using PyBOP and DIEA gave a 2:1 mixture of E/Z-16. This mixture was purified by semipreparative HPLC to obtain E-16 as a single diastereomer. The configuration of the double bond was established by the NOESY correlations in E-16 between CH2 (4.00–4.22 ppm) and the vinyl proton (5.30–5.38 ppm) (see the NOESY correlations in the Supporting Information).

Comparison of spectroscopic data obtained for E-16 and barmumycin confirmed that the revised structure is indeed the structure of the natural product.

In summary, the previously unreported marine compound barmumycin was isolated, and its chemical formula was determined via mass spectrometry. On the basis of preliminary NMR data, barmumycin was initially assigned the structure of compound 1. To confirm this assignment, compound 1 was synthesized following two different strategies starting from an o-aminobenzoic ester: one based on reductive amination, and one based on N-alkylation, which was shorter and higher yielding. However, comparison of the NMR spectra for 1 with those for isolated barmumycin showed dramatic differences. The structure of barmumycin was reassessed, and most probable option conceived was compound E-16, which was subsequently prepared (in five steps and 18% overall yield) for comparison with the natural compound. The spectroscopic data for E-16 fully coincided with that for barmumycin, thereby confirming that the two structures are equivalent. This work is a new example of the importance of total synthesis for structural characterization and confirmation of natural products.21

**Experimental Section**

See the Supporting Information for general procedures.

**Extraction and Isolation of Barmumycin.** The culture broth (10 L) was separated by filtration into a mycelial cake and cultured filtrate (9 L). A 500 mL aliquot of the absorber resin XAD-1180 was added to the filtrate. Compound barmumycin was eluted from the resin by double extraction with a 3:1:1 mixture of EtOAc-MeOH-H2O (1.8 L). The active fractions were concentrated in the organic phase, which was concentrated to dryness in vacuo to yield 950 mg of crude extract. This extract was purified by vacuum flash chromatography using a mixture of n-hexane-EtOAc and EtOAc-MeOH, whereby the fractions containing barmumycin (220 mg) were eluted with 9:1 EtOAc-MeOH. The active fractions were purified by silica gel chromatography using CHCl3-MeOH mixtures. Cytotoxicity was detected in the fractions eluted with 96:4 CHCl3-MeOH (20 mg). Further purification with a C18 column by HPLC afforded 6 mg of pure barmumycin (elution with 54:46 H2O-MeOH). This quantity of barmumycin was treated with 0.5 mL of pyridine and 0.5 mL of Ac2O to afford 7 mg of the corresponding diacetate. The molecular formula of barmumycin was determined to be C15H19NO4 by HPLC-APESI MS, in which it gave an (M+Na)+ peak at 300 and (M−H)− 276. Barmumycin gave an (M+H)+ peak at 278 and (M−H)− 276 in HPLC-APCI MS and it gave an (M+H)+ ion at m/z 278.13840 (calcd m/z 278.13869 for C15H19NO4) in HRMALDI-TOF MS.

The diacetyl derivative of barmumycin gave an (M+Na)+ peak at 362 and an (M+Na)+ peak at 384 by HPLC−APCI MS and HPLC−ESI MS.

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2-Acetoxypent-4-enal (4). LiBF₄ (6.05 g, 64.5 mmol) was added to a solution of 3 (4.05 g, 21.5 mmol) in 98:2 MeCN/H₂O (110 mL) and the mixture was stirred for 72 h at room temperature. The solvents were removed in vacuo. The crude was dissolved in CH₂Cl₂, washed with water and brine, and then concentrated in vacuo to yield 4 (2.82 g, 92%) as a yellowish oil. IR (KBr film): ν 3397, 3308, 2923, 1744, 1373, 1237, 1043 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 2.18 (s, 3H); 2.32–2.65 (m, 2H); 5.06–5.30 (m, 3H); 5.65–5.87 (m, 1H); 9.54 (s, 1H). ¹³C NMR (100.6 MHz, CDCl₃): δ 20.4 (q); 33.1 (t); 77.2 (d); 119.0 (t); 131.4 (d); 170.4 (s). MS (ESI-TOF): 143 (M⁺) 170.6 (s). MS (ESI-TOF): 308 (M⁺). 13C NMR (100.6 MHz, CDCl₃): δ 82.3 (s); 116.4 and 116.8 (d); 118.5 and 118.7 (d); 118.6 (t); 132.2 and 37.1 (t); 52.7 (t); 54.0 and 55.8 (t); 55.7 (q); 73.3 and 73.7 (d); 82.3 (s); 116.4 and 116.8 (d); 118.5 and 118.7 (d); 118.6 (t); 132.2 and 132.3 (d); 132.7 (d); 154.8 (s); 149.6 (s); 153.2 (s); 168.5 (s). MS (ESI-TOF): 306 (M⁺ + 1, 100). HRMS: m/z calcd for C₁₉H₂₈NO₆ 366.1917, found 366.1917.

Methyl 2-(2-Acetoxypent-4-enamino)-5-methoxybenzoate (6a), PhSiH₃ (2.01 g, 18.6 mmol) was added to a THF solution of 5 (2.02 g, 9.3 mmol) and then 2-Acetoxypent-4-enylamino (4) (1.32 g, 9.3 mmol). The mixture was heated to 100 °C under MW irradiation for 15 min. The solvents were removed in vacuo. Purification by silica gel column chromatography (100:0 to 95:5 hexane/EtOAc) yielded 6a (543 mg, 43%) as a yellowish oil. IR (KBr film): δ 3369, 3078, 1730, 1709, 1500, 1368 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 1.47 (s, 9H); 2.41–2.48 (m, 2H); 3.39 (d, J = 6.1 Hz, 2H); 3.76 (3H, OMe); 3.85 (3H, OMe); 4.91 (p, J = 6.1 Hz, 1H); 5.12 (m, 1H); 5.17 (m, 1H); 5.82 (m, 1H); 6.74 (d, J = 9.2 Hz, 1H); 7.04 (dd, J = 9.2, 3.1 Hz, 1H); 7.42 (d, J = 3.1 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 27.7 (q); 36.5 (t); 45.9 (t); 51.6 (q); 56.0 (t); 74.4 (d); 82.2 (s); 110.4 (s); 112.9 (d); 114.4 (d); 118.4 (t); 123.3 (d); 123.9 (d); 145.8 (s); 149.6 (s); 153.2 (s); 168.5 (s). MS (ESI-TOF): 306 (M⁺ + 1, 100). HRMS: m/z calcd for C₁₉H₂₈NO₆ 366.1917, found 366.1917. 19 F NMR (376 MHz, CDCl₃): δ −59.2 (m) 18.48 (m) 27.6 and 27.7 (3q); 36.7 and 37.1 (t); 52.7 (q); 54.0 and 55.8 (t); 55.7 (q); 73.3 and 73.7 (d); 82.3 (s); 116.4 and 116.8 (d); 118.5 and 118.7 (d); 118.6 (t); 132.2 and 132.3 (d); 132.7 (d); 154.8 (s); 149.6 (s); 153.2 (s); 168.5 (s). MS (ESI-TOF): 306 (M⁺ + 1, 100). HRMS: m/z calcd for C₁₉H₂₈NO₆ 366.1917, found 366.1917.

Methyl 2-(2-(tert-Butyrocarboxyloxy)pent-4-enylamino)-5-methoxybenzoate (6c), Boc₂O (823 mg, 3.77 mmol) was added to a solution of 6b (909.4 mg, 3.43 mmol) and DMAP (125.6 mg, 1.03 mmol) in dry CH₂Cl₂ (50 mL). The reaction mixture was stirred for 40 h at room temperature and then concentrated in vacuo. Purification by silica gel column chromatography (100:0 to 95:5 hexane–EtOAc) yielded 6c (543 mg, 43%) as a yellowish oil. IR (KBr film): δ 3369, 3078, 1730, 1709, 1500, 1368 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 1.47 (s, 9H); 2.41–2.48 (m, 2H); 3.39 (d, J = 6.1 Hz, 2H); 3.76 (3H, OMe); 3.85 (3H, OMe); 4.91 (p, J = 6.1 Hz, 1H); 5.12 (m, 1H); 5.17 (m, 1H); 5.82 (m, 1H); 6.74 (d, J = 9.2 Hz, 1H); 7.04 (dd, J = 9.2, 3.1 Hz, 1H); 7.42 (d, J = 3.1 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃): δ 27.7 (q); 36.5 (t); 45.9 (t); 51.6 (q); 56.0 (t); 74.4 (d); 82.2 (s); 110.4 (s); 112.9 (d); 114.4 (d); 118.4 (t); 123.3 (d); 123.9 (d); 145.8 (s); 149.6 (s); 153.2 (s); 168.5 (s). MS (ESI-TOF): 306 (M⁺ + 1, 100). HRMS: m/z calcd for C₁₉H₂₈NO₆ 366.1917, found 366.1917. 19 F NMR (376 MHz, CDCl₃): δ −59.2 (m) 18.48 (m) 27.6 and 27.7 (3q); 36.7 and 37.1 (t); 52.7 (q); 54.0 and 55.8 (t); 55.7 (q); 73.3 and 73.7 (d); 82.3 (s); 116.4 and 116.8 (d); 118.5 and 118.7 (d); 118.6 (t); 132.2 and 132.3 (d); 132.7 (d); 154.8 (s); 149.6 (s); 153.2 (s); 168.5 (s). MS (ESI-TOF): 306 (M⁺ + 1, 100). HRMS: m/z calcd for C₁₉H₂₈NO₆ 366.1917, found 366.1917.
Methyl 5-Methoxy-2-[N-(2-hydroxypent-4-etyl)]trifluoroacetamido]benzoate (6a). A 10% solution of TFA in CH₂Cl₂ (50 mL) was added to 6d (494.9 mg, 1.07 mmol), and the mixture was stirred at room temperature for 25 min. Elimination of the solvent gave 6e (387 mg, quant) as a yellow oil. IR (KBr film): ν 3770, 2950, 1688, 1519, 1457, 1222, 1074 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 2.20 – 2.30 (2H, CH₂), 3.46 – 3.49 and 3.54 – 3.61 (1H, 3H, OMe); 3.90 (s, 3H, OMe); 4.02 – 4.15 (1H, 2H); 4.89 (bs, 1H, OH); 5.10 – 5.14 (2H, 2H); 5.72 – 5.81 (1H, 1H), 7.09 – 7.14 (1H, 1H); 7.31 and 7.39 (2d, J = 8.8 Hz, 1H); 7.55 (d, J = 2.5 Hz, 1H). ¹³C NMR (100.6 MHz, CDCl₃): δ 39.3 and 39.4 (t), 52.9 (q), 55.8 (q); 58.4 and 59.0 (t); 62.8 and 68.8 (d); 114.9 (s); 116.2 and 116.7 (d); 118.8 (t); 118.9 and 119.0 (d); 120.1 (s); 120.9 (s); 122.8 (s); 131.1 (s); 131.9 and 132.1 (d); 131.3 and 134.3 (d); 159.8 (s). ¹⁹F NMR (376 MHz, CDCl₃): δ −68.7 (s). MS (ESI-TOF): 434 (M + 1, 100).

Isobutyl 3,4-Dihydroxybutanoate (11). A solution of isobutyl but-3-enoate (10.5 g, 73.6 mmol) in acetonitrile (50 mL) was added dropwise over 20 h to a solution of N-methylmorpholine oxide (10.9 g, 80.9 mmol) and a catalytic amount of O₂ in 60:40 acetone/H₂O (250 mL). The reaction was quenched with NaHSO₄, 40% aq solution (3 mL) and concentrated in vacuo. The crude was dissolved in EtOAc and filtered through silica gel, and the eluent was concentrated in vacuo to yield 11 (12.6 g, 97%) as a yellow oil. IR (KBr film): ν 3402, 2962, 1729, 1470, 1381, 1170, 1043 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 0.94 (d, J = 6.8 Hz, 6H); 2.00 – 1.89 (m, 1H); 2.51 (dd, J = 16.4 and 4.0 Hz, 1H, CH₂); 2.58 (dd, J = 16.4 and 8.4 Hz, 1H, CH₂); 5.38 (dd, J = 11.2 and 3.6 Hz, 1H, CH₂); 3.91 (d, J = 6.8 Hz, 2H); 4.10 – 4.18 (m, 1H). ¹³C NMR (100.6 MHz, CDCl₃): δ 19.2 (2q); 27.8 (d); 37.9 (t); 65.9 (t); 68.8 (d); 71.2 (t); 172.8 (s). MS (ESI-TOF): 177 (M + 1, 45); 199 (M + Na, 100). HRMS: (+ESI): m/z calculated for C₇H₁₃O₄Na as 247.0845, found 247.0846.

Isobutyl 2,2-Dimethyl-1,3-dioxolane-4-ylacetate (12). Pyridinium p-toluenesulfonate (120 mg, 0.48 mmol) was added to a solution of 11 (15.4 g, 87.26 mmol) in 50:50 2,2-dimethoxypropane/CH₂Cl₂ (300 mL). The reaction mixture was stirred at room temperature for 10 h, and then the solvent was removed in vacuo. Purification by silica gel column chromatography (50:50 hexane/EtOAc) yielded 12 (17.4 g, 92%) as a yellowish oil. IR (KBr film): ν 1736, 1380, 1370 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 0.93 (d, J = 6.8 Hz, 6H); 1.35 (3H, 3H); 1.41 (3H, 3H); 1.98 – 1.87 (m, 1H); 2.52 (dd, J = 15.7 and 7.6 Hz, 1H, CH₂); 2.72 (dd, J = 15.7 and 6.4 Hz, 1H, CH₂); 3.65 (dd, J = 8.4 and 6.4 Hz, 1H, CH₂); 3.88 (d, J = 6.4 Hz, 2H); 4.16 (dd, J = 8.4 and 6.0 Hz, 1H, CH₂); 4.40 – 4.51 (m, 1H). ¹³C NMR (100.6 MHz, CDCl₃): δ 19.0 (2q); 25.5 (q); 26.9 (q); 27.6 (d); 39.0 (t); 69.2 (t); 70.8 (s); 71.2 (d); 109.1 (s); 170.6 (s).

1-Bromo-3-(2,2-dimethyl-1,3-dioxolane-4-yl)prop-2-ene (13). A 1.6 M solution of MeLi in Et₂O (5 mL, 8 mmol) was added to a solution of 12 (865 mg, 4 mmol) and dibromomethane (557 μL, 5 mmol) in THF (20 mL) at −116 °C. The solution was stirred for 3 h and then quenched with saturated NH₄Cl (60 mL). The residue was immediately extracted with CH₂Cl₂. The organic layers were dried over MgSO₄, filtered, and then concentrated in vacuo. Purification by silica gel column chromatography (50:50 hexane/EtOAc) yielded 13 (27.6 g, 49%) as a yellow oil. IR (KBr film): ν 1719, 1370, 840 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 1.35 (3H, 3H); 1.42 (3H, 3H); 2.82 (dd, J = 16.6 and 6.0 Hz, 1H, CH₂); 3.07 (dd, J = 16.6 and 6.8 Hz, 1H, CH₂); 3.60 (dd, J = 8.4 and 6.4 Hz, 1H, CH₂); 3.94 (2s, 2H); 4.18 (dd, J = 8.4 and 6.0 Hz, 1H, CH₂); 4.44 – 4.51 (1H, 1H). ¹³C NMR (100.6 MHz, CDCl₃): δ 25.4 (q); 26.8 (q); 34.6 (t); 44.16 (t); 69.2 (t); 71.8 (s); 109.3 (s); 199.7 (s). MS (ESI-TOF): 259 (M⁺Br⁻ + Na, 100); 261 (M⁺Br⁻ + Na, 98). HRMS: (+ESI): m/z calculated for C₁₃H₁₉O₂BrNa (M + Na) 293.0944, found 293.0943.
(12 mL). The reaction mixture was stirred at 40°C for 15 min under microwave irradiation. Purification by silica gel column chromatography (95:5 to 80:20 hexane–EtOAc) yielded 10 (1.44 g, 74%) as a yellow oil. IR (KBr film): ν 3350, 1705, 1692, 1521, 1286, 1225, 1045 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 1.34 (s, 3H); 1.40 (s, 3H); 2.64 (dd, J = 16.2, 6.5 Hz, 1H, CH₂); 2.94 (dd, J = 16.2, 6.5 Hz, 1H, CH₂); 3.58 (dd, J = 8.4, 6.7 Hz, 1H); 3.76 (s, 3H, OMe); 3.89 (s, 3H, OMe); 4.08 (dd, 2H, CH₂); 4.18 (dd, J = 8.4 and 6.0 Hz, 1H); 4.46–4.52 (m, 1H); 6.64 (d, J = 9.1 Hz, 1H); 7.02 (dd, J = 9.1 and 3.1 Hz, 1H); 7.45 (d, J = 3.1 Hz, 1H); 7.99 (bt, 1H, NH). ¹³C NMR (100.6 MHz, CDCl₃): δ 25.4 (q); 26.8 (q); 44.2 (t); 51.7 (q); 54.2 (t); 59.9 (q); 71.8 (d); 109.1 (s); 110.8 (s) (d); 114.8 (d); 123.1 (d); 144.7 (s); 149.9 (s); 168.4 (s); 204.7 (s). MS (ESI-TOF): 338 (M + 1, 47); 675 (2M + 1, 100). HRMS: m/z calculated for C₁₇H₂₁N₂O₇Na 338.1598, found 338.1603.

(Z/E)-3-Methyl-2-[2-(2,2-Dimethyl-1,3-dioxolan-4-ylmethy1)-but-2-enylamino]-5-methoxybenzoic acid (Z/E-14). A 2.5 M solution of BuLi in hexane (1.27 mL, 3.17 mmol) was added to a mixture of ethyltriphenylphosphonium bromide (1.18 g, 3.17 mmol) in anhydrous THF (13 mL). The reaction mixture was stirred at room temperature for 24 h. The crude reaction mixture was then concentrated under reduced pressure, filtered, and the solvent was removed under reduced pressure. The crude product was purified by cartridge chromatography with hexane–EtOAc (95:5 to 80:20) to yield Z-14 (337 mg, 1.58 mmol) in anhydrous THF (3 mL) was added dropwise to the reaction mixture. The reaction mixture was stirred at −78°C for 30 min and subsequently allowed to warm to room temperature for an additional 30 min. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography with hexane–EtOAc (100:95 to 80:20) to yield Z-14 (337 mg, 1.58 mmol) and E-14 (250 mg, quant; 73.27 Z/E, as determined by ¹H NMR). IR (KBr film): ν 3373, 1639, 1517, 1225, 1065 cm⁻¹. Z-14. ¹H NMR (400 MHz, CDCl₃): δ 1.33 (s, 3H); 1.40 (s, 3H); 1.74 (d, J = 6.9 Hz, 3H); 2.23–2.44 (m, 2H, CH₂); 3.53 (dd, J = 7.8 and 7.4 Hz, 1H); 3.76 (s, 3H, OMe); 3.82 (bs, 2H, CH₂); 3.85 (s, 3H, OMe); 4.00 (dd, J = 7.8 and 6.0 Hz, 1H); 4.19–4.26 (m, 1H); 5.56 (q, J = 6.9 Hz, 1H); 6.63 (d, J = 9.2 Hz, 1H); 7.03 (dd, J = 9.2 and 3.2 Hz, 1H); 7.42 (d, J = 3.2 Hz, 1H). E-14. ¹H NMR (100.6 MHz, CDCl₃): δ 1.35 (s, 3H); 1.43 (s, 3H); 1.65 (d, J = 6.9 Hz, 3H); 2.23–2.44 (m, 2H, CH₂); 3.56 (dd, J = 7.6 and 7.6 Hz, 1H); 3.75 (s, 3H, OMe); 3.82 (bs, 2H, CH₂); 3.86 (s, 3H, OMe); 4.02–4.05 (m, 1H); 4.19–4.26 (m, 1H); 5.62 (q, J = 6.9 Hz, 1H); 6.63 (d, J = 9.2 Hz, 1H); 7.00 (dd, J = 9.2 and 3.1 Hz, 1H); 7.41 (d, J = 3.1 Hz, 1H). ¹³C NMR (400 MHz, CDCl₃): δ 13.4 (q); 25.7 (q); 27.0 (q); 32.6 and 42.8 (t); 40.0 (t); 51.5 (q); 56.0 (q); 69.3 (t); 75.0 (d); 109.8 (s); 112.8 and 114.1 (d); 114.4 (d); 123.3 (d); 125.4 (d); 133.0 (s); 137.7 (s); 144.6 (s); 149.4 (s); 168.6 (s). MS (ESI-TOF): 350 (M + 1, 35); 372 (M + Na, 45); 721 (2M + Na, 100). HRMS: m/z calculated for C₁₉H₂₈NO₇Na 350.1962, found 350.1966.
Lorente et al.

(Z)-1-tert-Butoxy carbonyl-4-ethyliden-2-(trimethylsilyloxy)-methyl pyrrolidine (Z-19).BuOK (213 mg, 1.9 mmol) was added to a solution of ethyltriphenylphosphonium bromide (705 mg, 1.9 mmol) in THF (5 mL), and the mixture was stirred for 1 h. After this time, 18 (180 mg, 0.62 mmol) was added, and the mixture was stirred for additional 30 min. Water was added, the residue was extracted with EtOAc dried over MgSO4, and filtered, and the solvent was removed under reduced pressure. Purification by silica gel column chromatography with hexane-EtOAc (95:5) yielded Z- E-19 (136 mg, 73%) in a 9:1 ratio as a colorless oil. IR (KBr film): ν 1702, 1397, 1251, 1109 cm-1. 1H NMR (400 MHz, CDCl3): δ 0.09 (s, 9H); 1.48 (s, 9H); 1.58 (d, J = 6.8 Hz, CH3); 2.45–2.54 (m, 1H, CH2); 2.56–2.67 (m, 1H, CH2); 3.20–3.45 (m, 1H, CH2); 3.55–3.67 (m, 1H, CH2); 3.78–4.05 (m, 3H, CH + CH3); 5.32–5.40 (m, 1H, CH). 13C NMR (100.6 MHz, CDCl3): δ 29.1 (t); 50.1 (t); 61.2 (t); 62.7 (d); 121.0 (d); 133.1 (s). HRMS: m/z calcd for C15H18NO1 Si 300.1989, found 300.1990.

(Z,E)-2-(hydroxymethyl)pyrrolidine (Z-20). A solution of Z/E-19 in a 9:1 ratio (136 mg, 0.45 mmol) in 10% TFA in CH2Cl2 (5 mL) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography with CH2Cl2–MeOH (98:2 to 90:10) to obtain Z/E-20 (105 mg) in a 9:1 ratio in quantitative yield. IR (KBr film): ν 3380, 1677, 1435, 1135 cm-1. 1H NMR (400 MHz, CDCl3): δ 1.63 (d, J = 6.7 Hz, 3H, CH3); 2.21–2.30 (m, 1H, CH2); 2.58–2.68 (m, 1H, CH3); 3.65–3.95 (m, 5H); 5.50–5.58 (m, 1H, CH). 13C NMR (100.6 MHz, CDCl3) (Ediastereomer): δ 14.7 (q); 28.5 (q); 34.0 and 34.5 (t); 47.5 (t) 57.5 and 57.7 (d); 62.7 and 63.1 (t); 79.4 (s); 115.9 and 116.2 (d); 136.7 (s); 154.2 (s). HRMS: m/z calcd for C15H16NO.Si 300.1989, found 300.1990.

(Z)-4-Ethyliden-2-(hydroxymethyl)pyrrolidine (Z-21). A solution of Z/E-20 in a 9:1 ratio (136 mg, 0.45 mmol) in 10% TFA in CH2Cl2 (5 mL) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography with CH2Cl2–MeOH (98:2 to 90:10) to obtain Z/E-21 (95 mg, 0.31 mmol) in 1:2 ratio in quantitative yield. IR (KBr film): ν 3380, 1677, 1435, 1135 cm-1. 1H NMR (400 MHz, CDCl3) (Ediastereomer): δ 1.63 (d, J = 6.7 Hz, 3H, CH3); 2.21–2.30 (m, 1H, CH2); 2.58–2.68 (m, 1H, CH3); 3.65–3.95 (m, 5H); 5.50–5.58 (m, 1H, CH). 13C NMR (100.6 MHz, CDCl3) (Ediastereomer): δ 14.7 (q); 28.5 (q); 34.0 and 34.5 (t); 47.5 (t) 57.5 and 57.7 (d); 62.7 and 63.1 (t); 79.4 (s); 115.9 and 116.2 (d); 136.7 (s); 154.2 (s). HRMS: m/z calcd for C15H16NO.Si 300.1989, found 300.1990.

(Z)-4-Ethyldien-2- (hydroxymethyl) pyrroldine (Z-22). A solution of Z/E-21 in a 1:2 ratio (95 mg, 0.31 mmol) in 10% TFA in CH2Cl2 (5 mL) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography with CH2Cl2–MeOH (98:2 to 90:10) to obtain Z/E-22 (95 mg, 0.31 mmol) in 1:2 ratio in quantitative yield. IR (KBr film): ν 3380, 1677, 1435, 1135 cm-1. 1H NMR (400 MHz, CDCl3) (Ediastereomer): δ 1.63 (d, J = 6.7 Hz, 3H, CH3); 2.21–2.30 (m, 1H, CH2); 2.58–2.68 (m, 1H, CH3); 3.65–3.95 (m, 5H); 5.50–5.58 (m, 1H, CH). 13C NMR (100.6 MHz, CDCl3) (Ediastereomer): δ 14.7 (q); 28.5 (q); 34.0 and 34.5 (t); 47.5 (t) 57.5 and 57.7 (d); 62.7 and 63.1 (t); 79.4 (s); 115.9 and 116.2 (d); 136.7 (s); 154.2 (s). HRMS: m/z calcd for C15H16NO.Si 300.1989, found 300.1990.

(Z)-4-Ethyliden-2-hydroxyethyl)pyrrolidine (Z-23). A solution of Z/E-22 in a 1:2 ratio (95 mg, 0.31 mmol) in 10% TFA in CH2Cl2 (5 mL) was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography with CH2Cl2–MeOH (98:2 to 90:10) to obtain Z/E-23 (95 mg, 0.31 mmol) in 1:2 ratio in quantitative yield. IR (KBr film): ν 3380, 1677, 1435, 1135 cm-1. 1H NMR (400 MHz, CDCl3) (Ediastereomer): δ 1.63 (d, J = 6.7 Hz, 3H, CH3); 2.21–2.30 (m, 1H, CH2); 2.58–2.68 (m, 1H, CH3); 3.65–3.95 (m, 5H); 5.50–5.58 (m, 1H, CH). 13C NMR (100.6 MHz, CDCl3) (Ediastereomer): δ 14.7 (q); 28.5 (q); 34.0 and 34.5 (t); 47.5 (t) 57.5 and 57.7 (d); 62.7 and 63.1 (t); 79.4 (s); 115.9 and 116.2 (d); 136.7 (s); 154.2 (s). HRMS: m/z calcd for C15H16NO.Si 300.1989, found 300.1990.

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Supporting Information Available: General procedures; tables of the bioactivity of isolated barmycin and of its diacetyl derivative; NMR data Tables 2 and 3; 1H and 13C NMR spectra of compounds 6a–c, 7–14, 17, 18, (Z/E)-19, and (Z/E)-20; and the 1H and 13C NMR spectra with two-dimensional NMR experiments for compounds (Z)-15, (Z)-1, (Z)-16, and (E)-16. This information is available free of charge via the Internet at http://pubs.acs.org.

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