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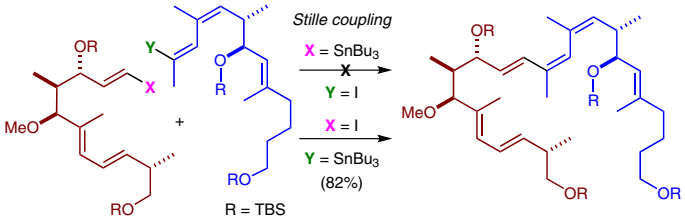
# Synthesis of the $C_1$–$C_{23}$ Fragment of the Archazolids and Evidence for V-ATPase but not COX Inhibitory Activity

**Gregory W. O'Neil**[*a]
**Alexander M. Craig**[a]
**John R. Williams**[a]
**Jeffrey C. Young**[b]
**P. Clint Spiegel**[a]

[a] Department of Chemistry, Western Washington University, Bellingham, WA 98225, USA
oneilg@wwu.edu
[b] Department of Biology, Western Washington University, Bellingham, WA 98225, USA




Received: 23.12.2016
Accepted after revision: 17.01.2017
Published online: 08.02.2017
DOI: 10.1055/s-0036-1588413; Art ID: st-2016-r0865-l

**Abstract** A convergent synthesis of a $C_1$–$C_{23}$ fragment of the archazolids has been completed based on a high-yielding Stille coupling to construct the substituted *Z*,*Z*,*E*-conjugated triene. After removal of the protecting groups, the resulting tetrol exhibited evidence for inhibition of the vacuolar-type ATPase (V-ATPase) but not cyclooxygenase (COX) inhibitory activity.

**Key words** archazolid, natural product, Stille reaction, V-ATPase, cyclooxygenase, total synthesis.

The archazolid natural products[1] (A–F, Figure 1) constitute a family of highly potent (subnanomolar $IC_{50}$) and selective vacuolar-type ATPase (V-ATPase) inhibitors that have shown promising activity against a number of particularly aggressive and lethal cancers including trastuzumab-resistant breast cancer,[2] glioblastoma multiforme (GBM),[3] and T-cell acute lymphoblastic leukemia (T-ALL).[4] More recent studies indicate that the archazolids also block iron metabolism and thereby mediate a therapeutic effect in breast cancers,[5] they modulate release of tumor-promoting cytokines,[6] and when combined with the p53 activator nutlin-3a, synergistically induce tumor cell death.[7]

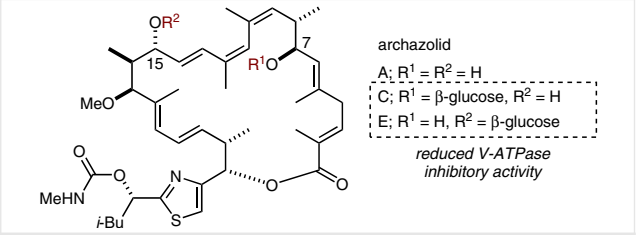



**Figure 1** Structure of the archazolid natural products

All members consist of a structurally similar 24-membered macrolactone and thiazole/carbamate side chain. Glycosylation at either the $C_7$- or $C_{15}$-hydroxyls (archazolids C and E, respectively) significantly reduces their V-ATPase inhibitory activity,[1d] indicating that these two groups form important interactions with the enzyme. Interestingly, these same hydroxyls are connected by a *Z*,*Z*,*E*-conjugated triene unique to the archazolids.

Several synthetic strategies have been reported for the pharmacophorically relevant conjugated triene region of the archazolids (Figure 2). Menche's group ultimately utilized a three-step aldol condensation after attempted Horner–Wadsworth–Emmons reactions failed,[8] which then required a late-stage enantioselective CBS reduction to install the $C_{15}$ hydroxyl. Two palladium-catalyzed cross-couplings have also been described, one a successful, albeit low-yielding, Stille reaction[9] and the other a similar yet simpler Negishi coupling.[10] Recently, our group reported a synthesis of the archazolid triene by cross-metathesis (CM).[11] While

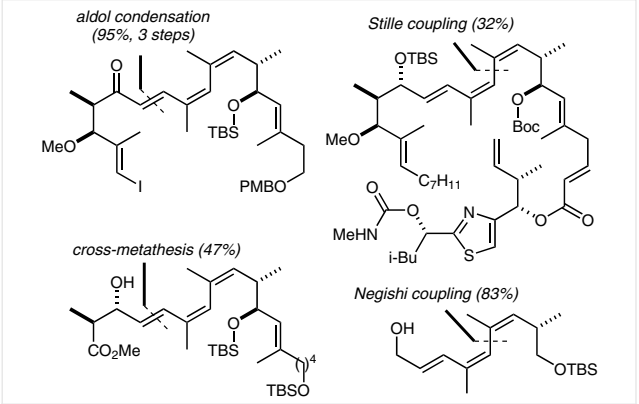



**Figure 2** Reported synthetic approaches to the archazolid conjugated triene



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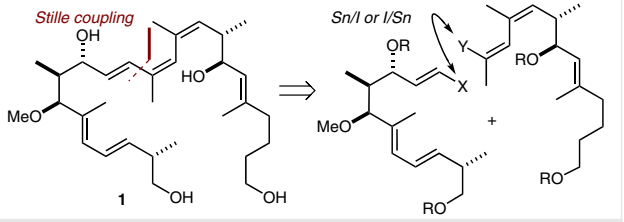
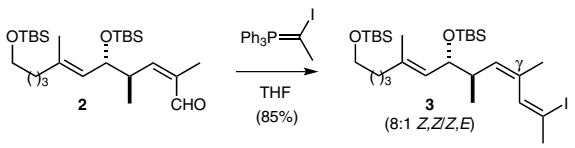
promising, the convergence of this CM strategy is limited, for example due to the requirement of using a *cis* homodimer and issues associated with metathesis back-biting.[12c] Combined with nonideal aspects of the other synthetic approaches (e.g., low yields or requisite late-stage manipulations), we continued to explore alternative disconnections.

Herein we report the synthesis of a $C_1$–$C_{23}$ fragment of the archazolids based on a high-yielding and convergent Stille coupling for construction of the conjugated triene (Scheme 1). The choice of coupling partner identity (i.e., which was the organostannane and halide) proved critical to the success of this reaction. After removal of the protecting groups, the V-ATPase and cyclooxygenase (COX) inhibitory activities of the resulting tetrol **1** were then assayed. The results suggest some level of V-ATPase inhibition by this compound but no significant interaction with COX.



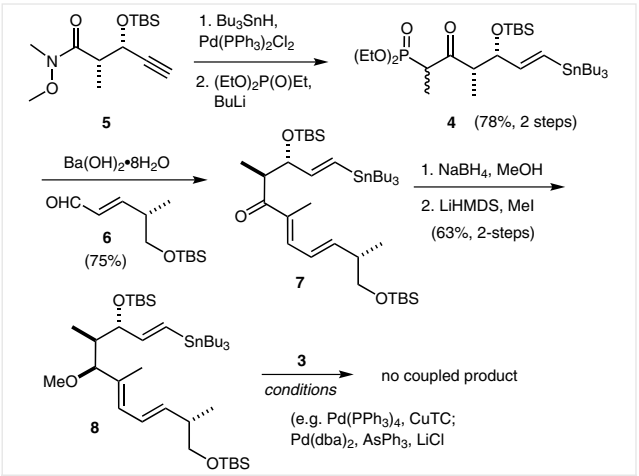

**Scheme 1** Novel Stille coupling-based synthesis of the archazolid triene containing compound **1**

When considering alternative non-metathesis based archazolid syntheses, we nonetheless wanted to make use of the chemistry developed for these prior approaches.[12] To that end, aldehyde **2** was identified as a suitable starting point (Scheme 2). This compound had been previously prepared en route to dihydroarchazolid B.[12c] We argue that saturation of the $C_2$–$C_3$ olefin (Figure 1) would not be expected to negatively impact archazolid biological activity, but may simplify their synthesis and improve stability. In preparation for possible palladium-catalyzed cross-couplings, aldehyde **2** was first converted into vinyl iodide **3** in 85% as an 8:1 mixture of *Z*/*E* isomers by $^1$H NMR analysis at the newly formed alkene.[13]



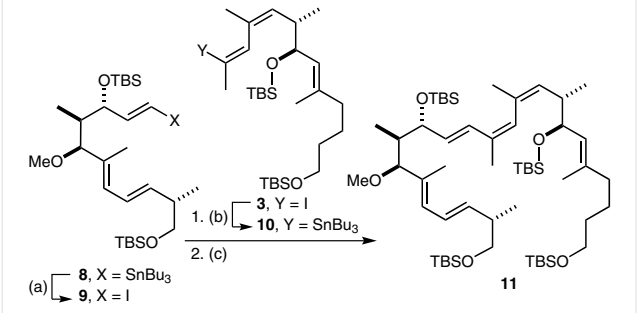

**Scheme 2** Vinyl iodide synthesis for cross-coupling

Our 'western hemisphere' synthesis[12b] was adapted to produce an appropriate vinyl stannane coupling partner, accomplished through the use of phosphonate **4** which is available in two steps from known Weinreb amide **5**[14] (Scheme 3). Horner–Emmons olefination[15] with aldehyde **6**[16] gave ketone **7** which was then reduced and methylated as previously described.[12b] Unfortunately all attempts to couple stannane **8** and iodide **3** failed. In each, unreacted iodide **3** was observed, suggesting difficulties in the oxidative addition step of the catalytic cycle, perhaps sterically hindered by the γ-methyl group.




**Scheme 3** Vinyl stannane synthesis and first attempts at Stille coupling

Gratifyingly, switching the sense of organometallic/halide in these reactions now led to success with the couplings. Specifically, iododestannylation[17] of **8** gave iodide **9**, and iodide **3** was converted into stannane **10** by lithium–halogen exchange and trapping with $Bu_3SnCl$ (Scheme 4).[18] These two compounds underwent very efficient coupling using Fürstner's conditions,[19] providing **11** in 82% yield.




**Scheme 4** *Reagents and conditions*: (a) $I_2$, $CH_2Cl_2$, –10 °C, 70%; (b) *t*-BuLi then $Bu_3SnCl$, 90%; (c) **10** (1.0 equiv), $Pd(PPh_3)_4$ (5 mol%), CuTC (2.0 equiv), $[Ph_2PO_2][NBu_4]$ (3.0 equiv), THF, 15 h, 82%.

We were intrigued about the possibility of a compound of type **11** inhibiting V-ATPase function. Others have commented on the utility of natural product derived fragments for drug discovery,[20] particularly those that maintain essential pharmacophoric features. Previously we had tested both 'western' (**12**) and 'eastern' (**13**) hemispheres of the archazolids using an *Arabidopsis* V-ATPase assay and found



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that neither displayed measurable inhibitory activity (Figure 3).[12b] However, these compounds lacked the important linked $C_7$- and $C_{15}$-hydroxyls present in tetrol **1**.

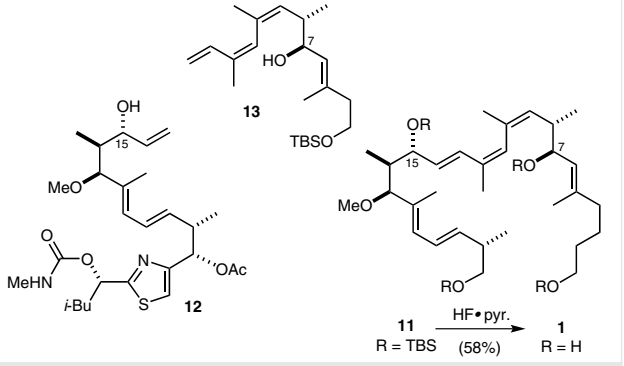
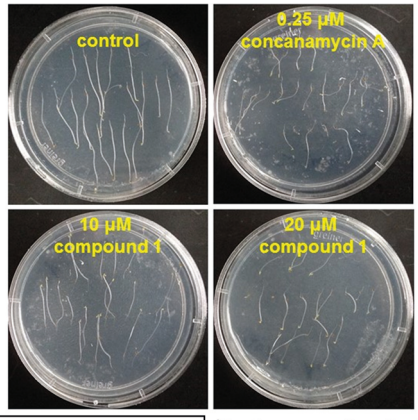



**Figure 3** Synthetic archazolid fragments; only compound **1** bears the linked $C_7$- and $C_{15}$-hydroxyls known to be important for V-ATPase inhibitory activity

As indicated in Figure 4, compound **1**[21] displayed dose-dependent growth inhibition of etiolated *Arabidopsis*.[22] A key component in the etiolated habit is stem elongation driven by V-ATPase-mediated cell expansion,[23] such that monitoring seedling stem length provides a measurement for V-ATPase activity. Previously, we demonstrated that stem elongation in *Arabidopsis* seedlings is inhibited by known V-ATPase inhibitors concanamycin A[24] and bafilomycin,[23] with concanamycin A exhibiting four times the potency of bafilomycin.[12b] The $IC_{50}$ value for compound **1** in this assay was approximately two orders of magnitude greater than that of concanamycin A and thus also the archazolids.[25] Nonetheless, this modest activity is significant given the major structural differences and overall simplification of **1** relative to the natural product.




| Treatment[A] | $IC_{50}$ (µM)[B] |
|---|---|
| concanamycin A | 0.25 |
| compound **12** | >100 |
| compound **13** | >100 |
| compound **1** | 25 |

[A]Seeds were germinated and grown on media containing assayed compounds. [B]Calculated by comparing growth on treated vs. untreated media (control).

**Figure 4** Select *Arabidopsis* V-ATPase assay results[22]

Based on a recent report by Reker et al.,[26] we may also suspect other biochemical targets for compound **1**. In their study, archazolid A (**ArcA**) was dissected into four hypothetical fragments (e.g., **ArcA-1**, Figure 5) from which potential targets were predicted. This computational exercise identified primarily proteins associated with arachidonic acid (e.g., cyclooxygenase, COX). However when tested, the COX-2 inhibitory activity of **ArcA** was weak (24 ± 6% inhibition at 10 µM). Reker et al. suggest this disconnect between predicted hypothetical fragment activities and the actual natural product could be due to the COX active site being buried, allowing for binding of smaller fragments (e.g., **ArcA-1** and arachidonic acid) but not **ArcA**.[26]

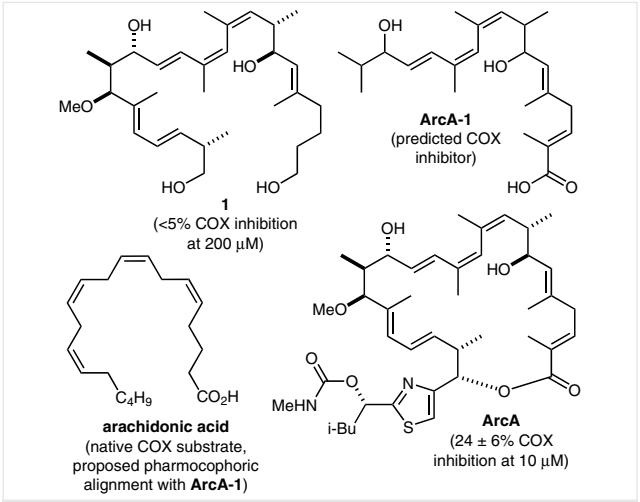



**Figure 5** Structures of synthetic archazolid fragment **1**, a hypothetical archazolid fragment **ArcA-1** used by Reker,[26] archazolid A (**ArcA**), and arachidonic acid

We saw compound **1** as an opportunity to add additional experimental data to this theoretical work, representing a fragment similar to the hypothetical fragment **ArcA-1**. Interestingly, compound **1** did not show a dose response of greater than 5% inhibition of COX-1 or COX-2 at concentrations between 1 and 200 µM.[27] It is possible that compound **1** ($C_{23}$) is similarly too large compared to **ArcA-1** ($C_{17}$) and arachidonic acid ($C_{20}$) to bind COX. Alternatively, the inactivity of **1** could suggest that a carboxylic acid terminus is critical, which is known to be important for other COX inhibitors.[28] However, this would not explain the measureable activity of **ArcA** which also lacks a carboxylic acid. Other factors such as binding entropies[29] might also therefore need to be considered (with **ArcA** being more conformationally restricted than **1**) to understand the differential COX-inhibitory activity of these compounds.

In summary, we have developed an efficient synthesis of the archazolid macrolactone ($C_1$–$C_{23}$) framework. The resulting fragment **1** displayed evidence for inhibition of the V-ATPase, in line with the importance of properly linked $C_7$- and $C_{15}$-hydroxyls for archazolid/V-ATPase binding. Com-

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pound **1** was also assayed for COX inhibition based on previously reported predicted activities for a structurally similar hypothetical fragment. The results showed no significant COX-inhibitory activity for **1** (<5% inhibition at concentrations up to 200 μM) suggesting certain structural requirements for COX binding (i.e., carboxylic acid or macrocycle). Current efforts are aimed at advancing our understanding of archazolid structure–activity-relationships,[30] by utilizing **1** as a starting point to further probe the archazolid/V-ATPase and archazolid/COX interactions.

## Acknowledgment

Financial support from the National Institutes of Health (R15GM101580) is gratefully acknowledged.

## Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1588413.

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(21) **Compound 11**

To a solution of **9** (36 mg, 0.058 mmol, 1.0 equiv) and **10** (45 mg, 0.058 mmol, 1.0 equiv) in degassed THF (1.2 mL) was added $[Ph_2PO_2][NBu_4]$ (75 mg, 0.17 mmol, 3.0 equiv), CuTC (28 mg, 0.07 mmol, 2.5 equiv), and $Pd(PPh_3)_4$ (7 mg, 0.006 mmol, 0.1 equiv), and the mixture was stirred for 15 h. The reaction was quenched with aq $NaHCO_3$ (15 mL) and extracted with MTBE (2 × 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash chromatography on silica (20:1 to 10:1 hexanes–EtOAc) gave **11** (46 mg, 82%) as an oil. $[\alpha]_D^{20}$ –5.2 (*c* 0.5, $CH_2Cl_2$). IR (ATR): 3062, 2983, 1736, 1614, 1415, 1274, 1267, 1129, 1078, 930 $cm^{-1}$. $^1H$ NMR (500 MHz, $C_6D_6$): δ = 6.80 (d, *J* = 16.0 Hz, 1 H), 6.44 (ddd, *J* = 15.3, 10.7, 0.9 Hz, 1 H), 6.17 (d, *J* = 10.4 Hz, 1 H), 6.02 (s, 1 H), 5.90 (dd, *J* = 16.0, 7.2 Hz, 1 H), 5.58 (dd, *J* = 15.4, 8.0 Hz, 1 H), 5.34 (dd, *J* = 8.4, 0.8 Hz, 1 H), 5.29 (ddd, *J* = 9.8, 2.0, 1.0 Hz, 1 H), 5.07 (d, *J* = 7.4 Hz, 1 H), 4.29 (dd, *J* = 9.0, 6.0 Hz, 1 H), 3.63 (d, *J* = 10.0 Hz, 1 H), 3.58–3.53 (m, 2 H), 3.47 (dd, *J* = 9.7, 6.2 Hz, 1 H), 3.38 (dd, *J* = 9.7, 6.8 Hz, 1 H), 3.18 (s, 3 H), 2.71 (m, 1 H), 2.42 (m, 1 H), 1.96 (m, 2 H), 1.91 (s, 3 H), 1.86 (m, 1 H), 1.89 (d, *J* = 1.0 Hz, 3 H), 1.71 (d, *J* = 1.0 Hz, 3 H), 1.59 (s, 3 H), 1.50 (m, 2 H), 1.39 (m, 2 H), 1.08 (s, 6 H), 1.03 (s, 6 H), 1.02 (s, 3 H), 1.01 (s, 6 H), 1.00 (s, 3 H), 0.99 (d, *J* = 7.0 Hz, 3 H), 0.98 (s, 3 H), 0.96 (d, *J* = 7.0 Hz, 3 H), 0.95 (s, 6 H), 0.94 (d, *J* = 7.0 Hz, 3 H), 0.93 (s, 3 H), 0.19 (s, 3 H), 0.17 (s, 3 H), 0.13 (s, 6 H), 0.08 (s, 6 H), 0.05 (s, 6 H). $^{13}C$ NMR (125 MHz, $C_6D_6$): δ = 137.8, 136.0, 134.9, 134.7, 133.4, 133.3, 133.0, 132.5, 130.9, 130.3, 126.3, 125.4, 73.8, 72.9, 68.5, 63.4, 63.4, 56.0, 43.7, 41.5, 40.6, 40.1, 33.1, 31.4, 30.3, 28.5, 26.7, 26.6, 26.5 25.4, 24.8, 24.8, 24.0, 21.0, 19.1, 18.9, 18.8, 17.4, 17.2, 17.1, 16.2, 14.3, 11.2, 9.8, 8.7, –3.0, –3.6, –4.2, –4.3, –4.4, –4.7, –4.8, –4.9. HRMS (ESI$^+$): *m/z* calcd for $C_{55}H_{108}O_5Si_4Na^+$ [M + Na]$^+$: 983.7172; found 983.7179.

**Compound 1**

To a solution of **11** (25 mg, 0.026 mmol) in THF (1.2 mL) at 0 °C was added pyridine (0.3 mL) and HF·py (60% HF, 0.2 mL), and the mixture was allowed to slowly warm to r.t. for 42 h. The reaction was cooled to 0 °C, diluted with EtOAc (15 mL), and quenched with aq $NaHCO_3$ (15 mL). The layers were separated, and the aqueous phase was re-extracted with EtOAc (2 × 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification by flash chromatography on silica (1:1 to 1:2 to 0:1 hexanes– EtOAc) gave **1** (7 mg, 58%) as an oil. $[\alpha]_D^{20}$ –6.0 (*c* 0.5, $CH_2Cl_2$). IR (ATR): 3350, 3955, 2929, 2872, 1716, 1688, 1525, 1471, 1418, 1369, 1244, 1126, 1084, 1008, 963, 919, 828, 730 $cm^{-1}$. $^1H$ NMR (500 MHz, $CD_3OD$): δ = 6.52 (dd, *J* = 16.0, 0.7 Hz, 1 H, H13), 6.29 (ddd, *J* = 15.0, 10.8, 0.7 Hz, 1 H, $H_20$), 5.90 (d, *J* = 11.0 Hz, 1 H, H19), 5.74



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(s, 1 H, H11), 5.70 (dd, *J* = 15.8, 5.9 Hz, 1 H, H14), 5.57 (dd, *J* = 15.2, 7.6 Hz, 1 H, H21), 5.09 (d, *J* = 9.0 Hz, 1 H, H9), 5.07 (d, *J* = 8.0, 1.0 Hz, 1 H, H6), 4.46 (dd, *J* = 6.0, 3.5 Hz, 1 H, H15), 4.05 (dd, *J* = 9.0, 6.1 Hz, 1 H, H7), 3.48 (t, *J* = 6.0 Hz, 2 H, H1a/b), 3.38 (d, *J* = 8.4 Hz, 1 H, H17), 3.37 (dd, *J* = 10.6, 5.5 Hz, 1 H, H23a), 3.33 (dd, *J* = 10.6, 6.5 Hz, 1 H, H23b), 3.09 (s, 3 H, OMe), 2.36–2.28 (m, 2 H, H8/22), 1.97 (t, *J* = 7.3 Hz, 2 H, H4a/b), 1.81 (d, *J* = 1.2 Hz, 3 H, Me10), 1.70 (s, 3 H, Me12), 1.64 (m, 1 H, H16) 1.58 (d, *J* = 1.0 Hz, 3 H, Me5), 1.56 (d, *J* = 1.0 Hz, 3 H, Me18), 1.48–1.40 (m, 4 H, H2/3), 0.96 (d, *J* = 7.0 Hz, 3 H, Me22), 0.81 (d, *J* = 7.0 Hz, 3 H, Me8), 0.59 (d, *J* = 7.0 Hz, 3 H, Me16). $^{13}$C NMR (125 MHz, $CD_3OD$): δ = 139.3, 138.7, 134.7, 134.4, 133.6, 132.7, 131.5, 130.5, 129.9, 127.3, 126.9, 90.4, 73.1, 73.0, 71.7, 68.2, 63.0, 56.3, 42.8, 41.3, 41.2, 40.7, 33.3, 25.3, 25.0, 20.5, 17.2, 17.0, 16.3, 11.1, 10.7. HRMS (ESI$^+$): *m/z* calcd for $C_{31}H_{52}O_5Na^+$ [M + Na]$^+$: 527.3712; found 527.3729.

(22) Assays were conducted side-by-side for compound **1** and concanamycin at concentrations of 0.125, 0.25, 0.5, 1.0, 2.0, 10.0, and 20.0 µM. See Supporting Information for details.

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