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

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V-ATPase is an important anti-cancer target and archazolid natural products represent a promising starting point for the development of V-ATPase-based cancer therapies. Over the last few years, the intensity of cancer research involving archazolid natural products has increased, as evidenced by several recent studies demonstrating promising activity for these compounds against particularly lethal and aggressive types of cancer. This follows an increasing appreciation for the role of the vacuolar-type ATPase (V-ATPase) in cancer.

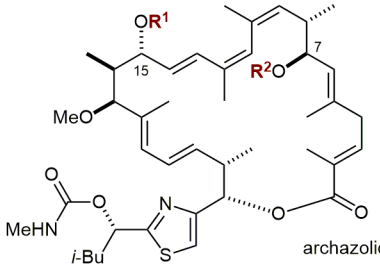
However, archazolids remain scarcely available, which has hindered their development as V-ATPase inhibitor therapeutics. "Only small quantities of archazolids are available from the natural source, and yet the vast majority of biological studies have utilized extracted and laboriously purified material. This argues for continuing efforts toward a more 'ideal' total synthesis," said Professor Gregory O'Neil from Western Washington University (USA). In a recent *Synlett* article, Professor O'Neil's group described what promises to be a highly efficient synthesis of the archazolids based on a convergent Stille coupling to construct the substituted *Z*,*Z*,*E*-conjugated triene unique to this class of compounds.

Professor O'Neil noted: "It is interesting that this reaction was only successful when using the 'eastern' fragment as the stannane and the 'western' fragment as the iodide (which we rationalize as being due to steric interactions during the oxidative addition step). Since tin and iodide groups are interchangeable, the lesson here seems to be that it is prudent to investigate both coupling scenarios when attempting and/or encountering difficulties with complex Stille couplings."

The group's advanced archazolid intermediate displayed dose-dependent inhibition of the V-ATPase. "We were quite excited by the V-ATPase assay results obtained for our advanced fragment **1**, despite its modest activity (~100 times less potent than the natural product), given the overall structural simplifications relative to the natural product," remarked Professor O'Neil. He continued: "The results make a further case for the C_7–C_{15} region of the natural products being pharmacophorically relevant, and gives us a starting point for future SAR studies. It is also planned to scale up our synthesis of **1** to make this compound more widely available as a new tool for V-ATPase/cancer research."

Despite a computational suggestion that the compound would inhibit the cyclooxygenase (COX) enzyme, no COX inhibitory activity was measured.

"There are a number of interesting questions that have been raised by our COX inhibition results in light of the previous theoretical study," concluded Professor O'Neil. "For instance, does the inactivity of our compound arise from it being too large and/or because it lacks a carboxylic acid terminus? Are other factors such as binding entropies at play? These are all questions that we hope to address through synthesis."



	enzyme inhibition V_1/V_0 holoenzyme: IC_{50} [nmol/mg enzyme]	growth inhibition L-929: IC_{50} [nM]
archazolid A (**1**)	0.6	0.81
archazolid C (**2**)	210	1600
archazolid E (**3**)	n.d	510

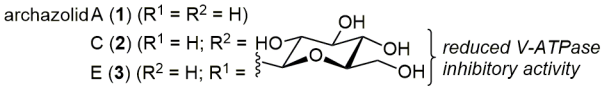
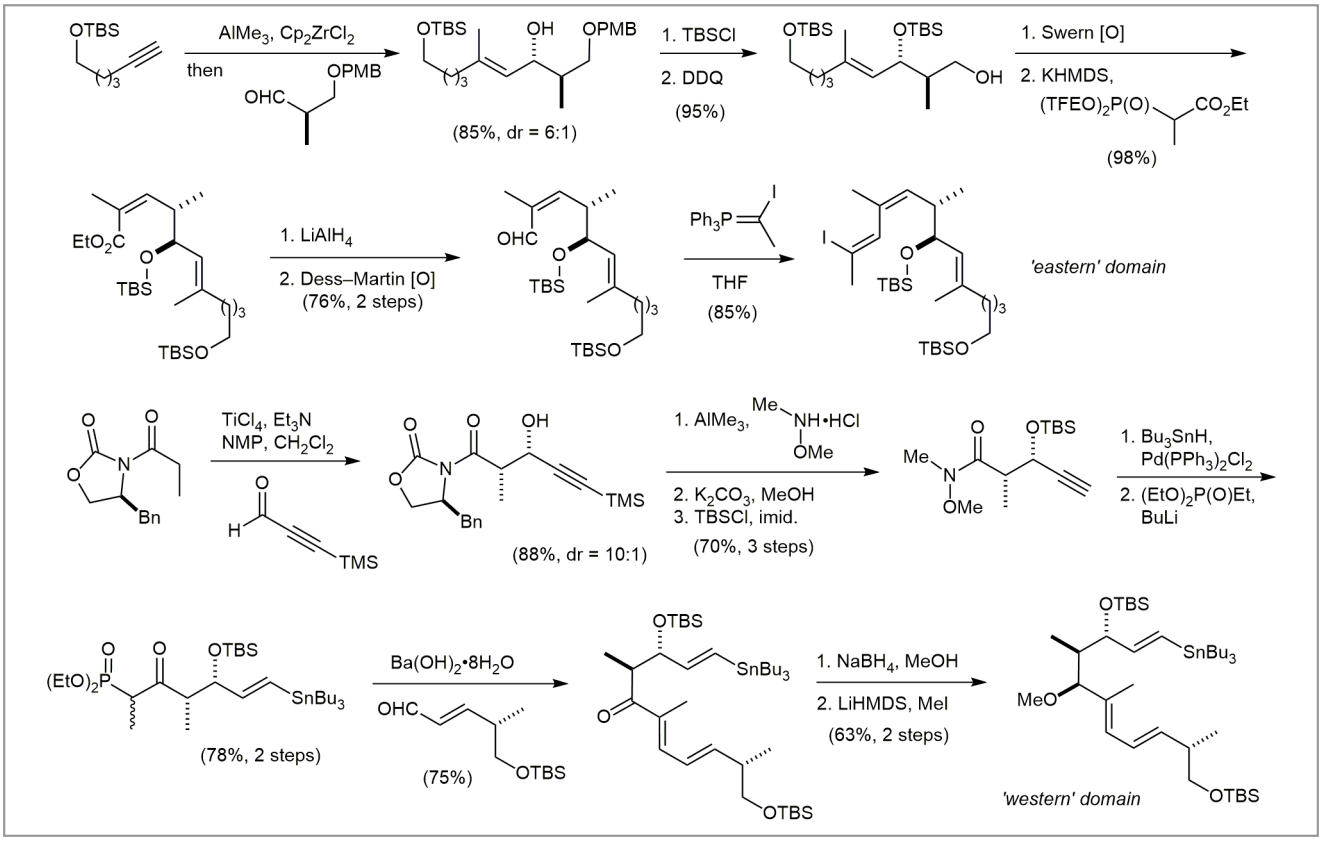
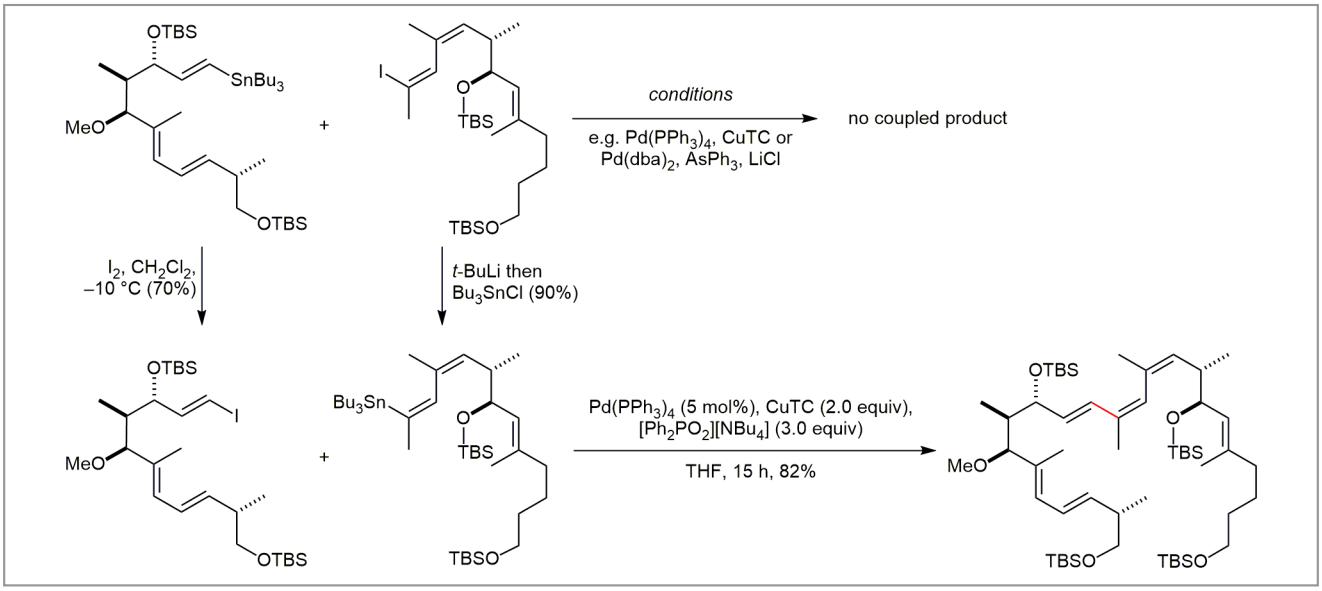


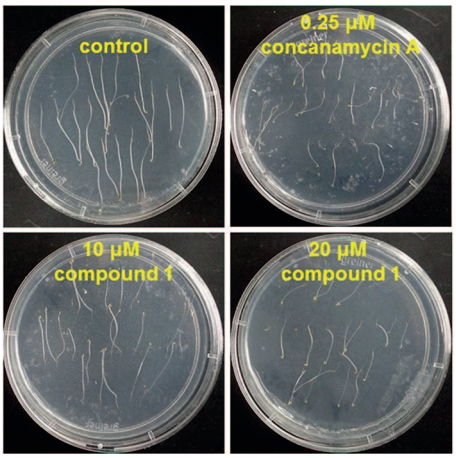
Figure 1 Structure of the archazolid natural products. Glycosylation at either the C_7 or C_{15} hydroxyl significantly reduces their V-ATPase inhibitory activity, indicating that these two groups form important interactions with the enzyme. Interestingly, these same hydroxyls are connected by a *Z*,*Z*,*E*-conjugated triene unique to the archazolids.

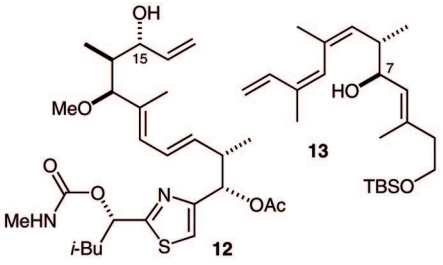


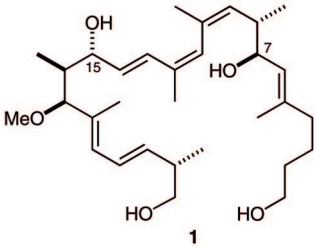
Scheme 1 Synthesis of the 'eastern' and 'western' domains of the archazolids



Scheme 2 Completion of the C_1–C_{23} fragment (**1**) of the archazolids by a complex Stille coupling. First attempts using the 'western' stannane and 'eastern' iodide all failed. Switching the sense of organometallic/halide in these reactions led to success with the reaction.



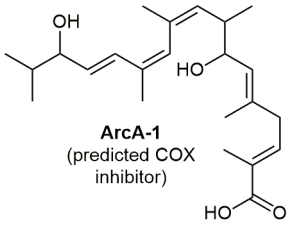




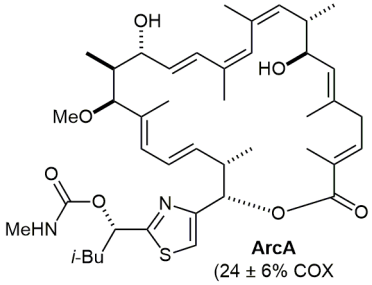
Treatment[a]	IC_{50} (µM)[b]
concanamycin A	0.25
compound **12**	>100
compound **13**	>100
compound **1**	25

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

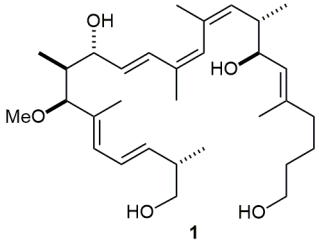
Figure 2 *Arabidopsis* V-ATPase assay results for synthetic archazolid fragments **1**, **12**, and **13** along with a known V-ATPase inhibitor concanamycin A. Whereas compounds **12** and **13** were inactive, tetrol **1** displayed dose-dependent growth inhibition of etiolated *Arabidopsis* as an indicator for inhibition of V-ATPase. The results provide further evidence for the importance of properly linked C_7- and C_{15}-hydroxyls for archazolid/V-ATPase binding.



ArcA-1
(predicted COX inhibitor)



ArcA
(24 ± 6% COX inhibition at 10 µM)



1
(<5% COX inhibition at 200 µM)

Figure 3 Predicted and measured COX inhibitory activity of hypothetical archazolid fragment **ArcA-1**, archazolid A (**ArcA**), and synthesized archazolid fragment **1**. The inactivity of 1 suggests certain structural requirements for COX binding (i.e. carboxylic acid or macrocycle).

About the authors

Dr. G. O'Neil

Gregory O'Neil grew up in New Jersey (USA). He received his B.S. degree in 2002 from Boston College (USA) where he conducted undergraduate research under the guidance of Professor Larry Scott. He then earned a Ph.D. in 2006 from the University of Colorado, Boulder (USA) under the supervision of Professor Andrew Phillips. Before starting his independent career at Western Washington University (USA) in 2008, he received an Alexander-von-Humboldt Postdoctoral Fellowship to work with Professor Alois Fürstner at the Max-Planck Institut für Kohlenforschung (Germany). His research is focused primarily on synthesizing biologically and environmentally compelling natural products.

A. Graig

Alexander Craig was born in Olympia (USA) in 1994. He studied biochemistry at Western Washington University where he completed his B.S. degree in 2016, performing research with Professor O'Neil.

J. Williams II

John Williams II received his B.S. degree from Western Washington University (USA) in 2014. He went on to complete his M.Sc. from Western Washington University in 2016 under the direction of Professor O'Neil. That same year he joined the Washington State Department of Agriculture as an analytical chemist. His current research interests concern analysis of organic residues in complex mixtures utilizing mass spectrometry.

Prof. J. Young

Jeffery Young was born in Berkeley (USA) and received his Ph.D. in 1994 from The Ohio State University (USA) under the guidance of Professor R. Hangarter. In 1994, he joined the lab of Professor M. Sussman at the University of Wisconsin (USA) and studied the role of p-type proton ATPases in plants. His postdoctoral work included the development of T-DNA mutagenesis as a reverse genetic tool in *Arabidopsis thaliana*. He moved to the Biology Department at Western Washington University in 1999 where he currently holds a Full Professor position.

Prof. P. C. Spiegel

P. Clint Spiegel was born in Oregon (USA), and received his B.S. in Biochemistry and Biophysics from Oregon State University. He completed his Ph.D. studies from the University of Washington (USA) in the lab of Barry Stoddard at the Fred Hutchinson Cancer Research Center in 2004. Following graduate studies, he joined Harry Noller's lab as a Jane Coffin Childs Memorial Foundation Postdoctoral Fellow at the University of California, Santa Cruz (USA) to study ribosome structure and function. In 2007, he joined the Chemistry Department at Western Washington University as an Assistant Professor of Chemistry, and in 2015 was promoted to Full Professor. In his independent lab, he employs structural biology approaches to understand the immune response to hemophilia A treatment and takes a biochemical approach to understand the inhibition and activation of ribosome-dependent GTPases.

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