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COMMUNICATION

Synthesis and activity of the archazolid western hemisphere†

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A convergent and scalable synthesis of the archazolid western hemisphere has been completed. The V-ATPase inhibitory activity of this compound along with a previously prepared eastern domain was then tested using a convenient *Arabidopsis*-based V-ATPase assay.

The vacuolar-type H^+-ATPase (V-ATPase) is among the most widely distributed ATP-driven ion pumps in nature. It is expressed in all eukaryotic cells, where it participates in the acidification of intracellular organelles that are essential for many cellular processes.[1] The elucidation of the physiological role of the V-ATPase has revealed the important function these proteins play in a wide array of pathological processes including osteoporosis,[2] renal acidosis,[3] and cancer.[4] Thus, the V-ATPase is regarded as a promising pharmacological target and selective inhibitors of this enzyme represent important leads toward a better understanding of these diseases and the development of effective drugs for their therapy.

In 2003, as part of a program directed toward the identification of new anti-cancer agents, Höfle and coworkers reported the isolation of two novel cytotoxic compounds from the culture broth of strains of the myxobacteria *Archangium gephyra*.[5] Archazolids A (**1**) and B (**2**) showed high activity against a number of human and mammalian cancer cell lines with IC_{50} values ranging from 0.1 to 1 ng ml^{-1} (Fig. 1). An incubation of PtK_2 potoroo cells with archazolid A (5 ng ml^{-1}) led to the formation of vacuoles in the endoplasmic reticulum, a phenomenon that is typical for inhibitors of V-ATPases. Later studies of PtK_2 kidney cells incubated with archazolid A showed that these cells lost intact acidic lysosomes, also suggesting that the archazolids interfere with vacuolar-type ATPase.[6] This was confirmed by measuring the inhibitory activity of archazolids A and B on purified V-ATPase from *M. sexta*. Both archazolids A and B were shown to inhibit the activity of the purified V-ATPase holoenzyme half-maximally at a concentration of 20 nM (IC_{50} *ca.* 0.8 nmol per mg enzyme), in the same range as the well-established plecomacrolidic V-ATPase inhibitors bafilomycin[7] and concanamycin.[8] Previous studies had demonstrated that the archazolids share, at least partially, the same binding site within subunit *c* of the V_0 domain as bafilomycin and concanamycin.[6] Very recent point mutation experiments combined with the use of a labeled semi-synthetic archazolid derivative, however, indicate that archazolid V-ATPase binding is strikingly different than the plecomacrolides.[9]

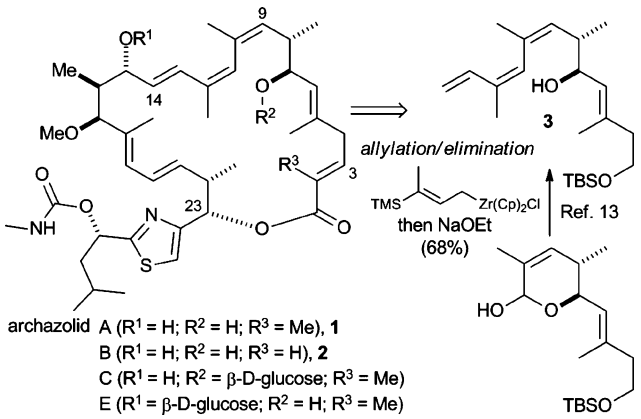

Fig. 1 Archazolid natural products.

Stereochemical assignment of archazolids A and B was first reported in 2006,[10] and was later confirmed by total synthesis.[12] Since their initial discovery, the family has been extended to include archazolids C–F.[11] Each of the archazolids share a 30-carbon linear polyketide backbone that has been incorporated into a highly functionalized 24-membered macrolactone. Embedded within the core is a C_9–C_{14} *Z*,*Z*,*E*-conjugated triene unique to the archazolids. Recently our group reported a direct approach to this subunit utilizing a tandem lactol TMS-allylation/Peterson elimination and a completion of the C_3–C_{13} fragment (**3**).[13]

Elaboration of **3** into archazolids A and B was envisioned to occur by first olefination at C_2–C_3 followed by macrocycle formation by ring-closing metathesis (RCM) (Fig. 2). Herein we report a concise synthesis of the remaining C_{14}–C_{23} and side-chain fragment (**4**) common to each of the archazolids required to complete our synthesis. Key disconnections include Horner–Emmons (HWE) olefination at $\Delta^{18,19}$ and side-chain installation by addition of a suitable organometallic reagent derived from thiazole **5**. Additionally, a tractable *Arabidopsis*-based V-ATPase assay has been developed that was then used to test the V-ATPase

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† Electronic supplementary information (ESI) available: Complete experimental details including spectroscopic and analytical data for compounds **4**, **11**, **12**, **16**, **17**, **18**, **19**, and **20**, Mosher ester data for compound **4**, and V-ATPase assay dose response curves and methods for compounds **3**, **20**, and **21**. See DOI: 10.1039/c1ob06446k

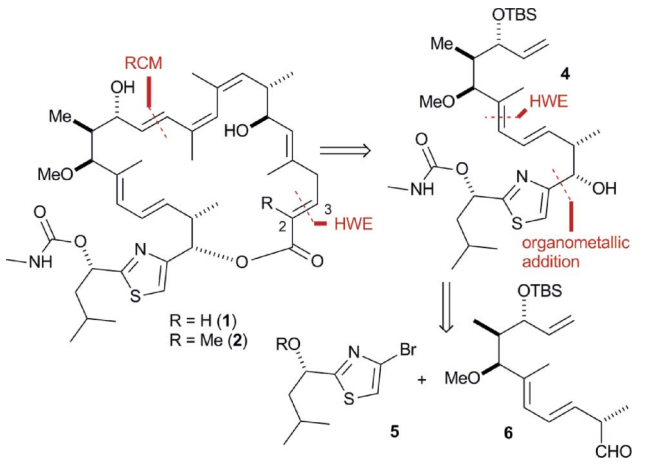
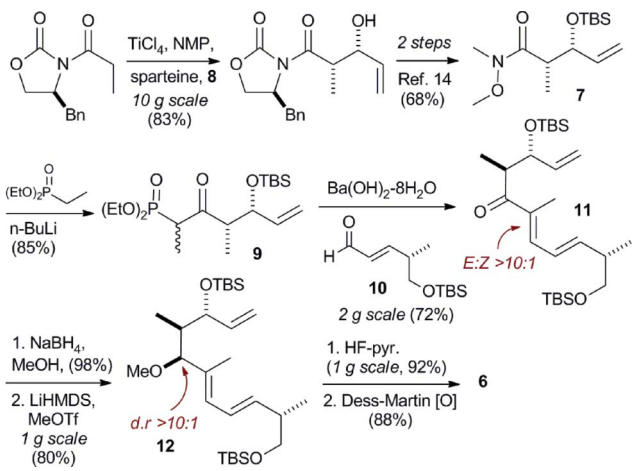


Fig. 2 Archazolid retrosynthesis.

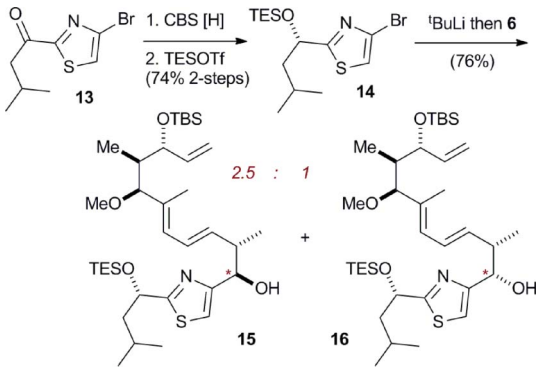
inhibitory activity of both the "eastern" and "western" archazolid hemispheres.

Our synthesis began from known Weinreb amide **7**,[14] prepared on a ten-gram scale by a titanium-mediated Evans' aldol reaction with acrolein (**8**) (Scheme 1).[15] Conversion to the ketophosphonate **9** then allowed for a Horner–Emmons coupling[16] with (*R*)-Roche ester derived aldehyde **10**.[17] A comparison of deprotonation methods revealed that $Ba(OH)_2{\cdot}8H_2O$[18] proceeded with the highest *trans*-selectivity and has delivered multigram quantities of **11**. Reduction of the ketone with $NaBH_4$ gave the corresponding alcohol with >10 : 1 diastereoselectivity as determined by NMR analysis.[19] After some experimentation, it was found that methylation of the newly generated hydroxyl-group could be performed on gram scale by deprotonation with a freshly prepared precooled solution of LiHMDS followed by alkylation with methyl triflate (MeOTf) at −78 °C. Allowing the reaction mixture to warm resulted in a significant amount of 1,3-silyl migration[20] and a mixture of products. Selective removal of the primary TBS-group[21] in **12** and oxidation with Dess–Martin periodinane[22] then completed a synthesis of aldehyde **6**.



Scheme 1 Gram-scale synthesis of aldehyde **6**.

The final fragment was prepared from known ketothiazole **13**,[23] available in one step from commercially available 2,4-dibromothiazole (Scheme 2). Enantioselective reduction[24] and



Scheme 2 Thiazole fragment coupling.

TES-protection gave bromide **14**. Treatment of **14** with *tert*-butyllithium resulted in a rapid lithium–halogen exchange[25] giving the corresponding highly-colored organolithium that added smoothly to **6** producing an inseparable mixture of **15** and **16** with modest Felkin-control (*vide infra*).

Oxidation of this mixture with Dess–Martin periodinane gave **17** which then allowed for installation of the desired C_{23}-*O* stereocenter by selective hydride addition (Scheme 3).[26] It was found that L-Selectride gave the best balance of yield to selectivity, affording **16** in 73% yield as a 10 : 1 (NMR) mixture of diastereomers (Table 1).[27]

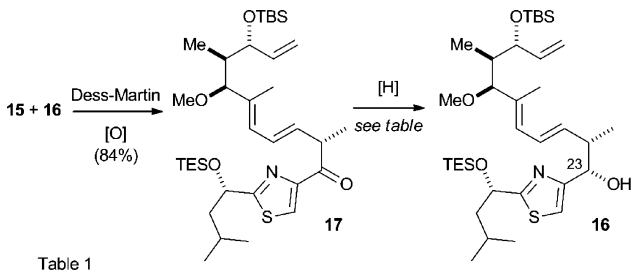


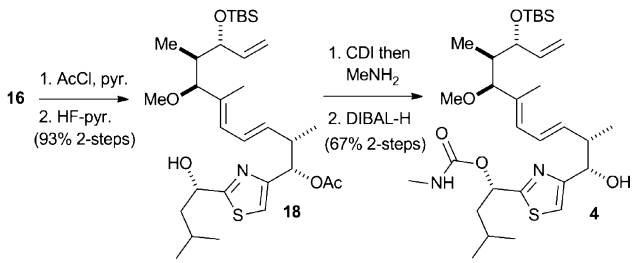
Table 1

conditions	ratio (**16**):(**15**)[a]	yield[b]
$NaBH_4$	3.1:1	78%
DIBAL-H	3.3:1	72%
$LiAlH_4$	3.1:1	64%
$LiB(Et)_3H$	20:1	46%
L-selectride	10:1	73%

[a]Ratios determined by NMR.
[b]Isolated yield.

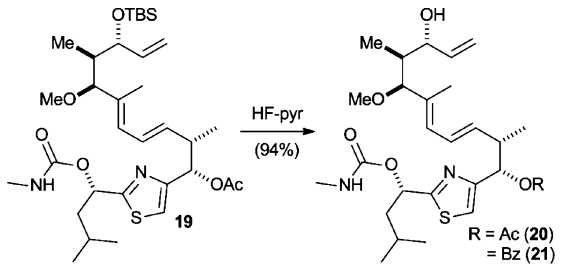
Scheme 3 Installation of the C_{23}-*O* stereocenter.

Completion of the fragment was then achieved in four steps from **16** by acylation of the free hydroxyl followed by selective TES-deprotection affording alcohol **18** (Scheme 4). Installation of the carbamate and removal of the acetate with DIBAL-H delivered **4** in 62% overall yield from **16**.



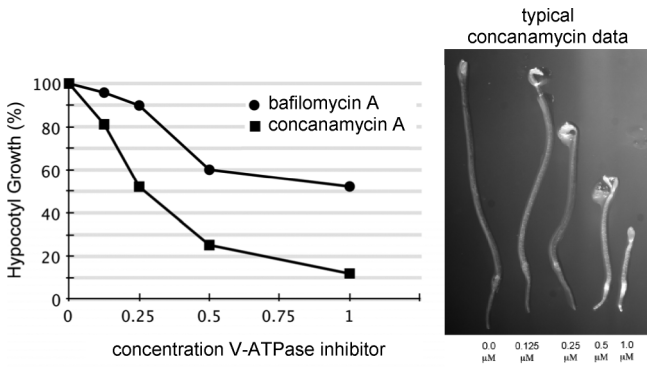
Scheme 4 Completion of the archazolid western hemisphere.

It was thought that the dense functionalization and various rigidifying elements within **4** might render compounds of this type V-ATPase inhibitors themselves.[28] To test this, the V-ATPase inhibitory activity of compounds **20**, **21**, and the previously prepared C_3–C_{13} fragment (**3**) was measured using a convenient *Arabidopsis*-based V-ATPase assay (Scheme 5).



Scheme 5 Synthetic western hemisphere derivatives.

Acidification of the plant vacuole by the V-ATPase facilitates cell expansion by generating turgor pressure through solute accumulation.[29] Selective inhibition by bafilomycin A, in fact, was a key component in identifying the first plant V-ATPase[29] and it is known that the selective inhibition of concanamycin A inhibits *Arabidopsis* cell elongation in a dose dependent manner.[30] The first V-ATPase mutant identified in plants, *det3*, exhibits a reduction in subunit *c* and in V-ATPase activity, resulting in the loss of the etiolated (dark) growth habit in seedlings.[31] A key component in the etiolated habit is stem elongation driven by V-ATPase mediated cell expansion. Cell growth in *Arabidopsis* seedlings was assayed by measuring etiolated seedling stem length in the presence of varying amounts of inhibitors (Fig. 3).[32]



Treatment[A]	IC_{50} (μM)
concanamycin A	0.25
bafilomycin A	1.0
compound **20**	>100
compound **21**	>100
compound **3**	>100

[A]Seeds were germinated and grown on media containing assayed compounds.

Fig. 3 *Arabidopsis* hypocotyl-growth V-ATPase assay.

While the plants proved highly sensitive to the known V-ATPase inhibitors bafilomycin A and concanamycin A, none of the synthetic archazolid derivatives displayed any significant V-ATPase inhibitory activity in this assay.[33] This data would suggest that, like the plecomacrolides,[34] macrolactone formation is essential for archazolid V-ATPase binding and inhibition.

Conclusions

A convergent synthesis of the archazolid "western hemisphere" has been completed that is particularly well-suited for the large-scale preparation of advanced intermediates and access to a series of side-chain analogues. The V-ATPase inhibitory activity of this material was then evaluated using a tractable *Arabidopsis*-based V-ATPase assay. Assay results indicate that the macrocyclic structure of the archazolids is critical to their V-ATPase inhibitory activity. Efforts are ongoing to complete the synthesis of the natural product and analogues for future SAR studies using our plant-based assay method.

Acknowledgements

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