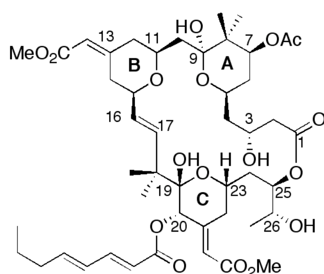
TOTAL SYNTHESIS OF THE BRYOSTATINS AND MEDICINALLY RELEVANT ANALOGUES

Reported by Paul E. Gormisky February 18, 2010

INTRODUCTION

Isolation and Biological Evaluation

The bryostatins are a class of marine macrolides that show extremely promising anti-cancer activity. During a search for anti-cancer agents from marine sources in 1968, the extracts from bryozan invertebrates *Bugula neritina* demonstrated activity against lymphocytic leukemia cell lines *in vitro*. Many years of effort led to the isolation and structural elucidation of bryostatin 1 (**1**) in 1982 by Pettit and coworkers.[1] The structure of bryostatin 1 was determined by X-Ray crystallography. To date a total of 20 bryostatins have been isolated and their structures elucidated by a combination of X-Ray crystallography and NMR spectroscopy. All members of this family share in common a 26-membered macrolactone with three imbedded and highly functionalized pyran rings (A-C). Also conserved throughout the family are an exocylic enoate at C13 and C21, hydroxyl groups at C3 and C26, and a C16-17 *trans*-olefin. The members differ in the functionality of the C-ring and the identity of the C7 and C20 esters. Even after considerable effort, **1** can only be isolated in 0.00014% from tons of wet animal (14 tons yields only 18g of bryostatin 1).[2] Current efforts focus on marine aquaculture; however, the invertebrate can only be cultivated in sea and require a large surface growing area, making this an unsustainable solution. Nonetheless, a combination of isolation and aquaculture has provided sufficient quantities of **1** to be evaluated medicinally.



Bryostatin 1 (**1**)

The bryostatins show remarkable and extremely potent anti-cancer activity. Bryostatin 1 initially showed activity against murine tumors, including P388 lymphocytic leukemia, ovarian sarcoma and B16 melanoma. The efficacy and potency of bryostatin 1 led to an explosion in research on its biological activity and mode of action. Bryostatin 1 has been shown to restore apoptosis in cancer cells,[3] reverse multi-drug resistance,[4] stimulate the immune system[5] and act synergistically with other anticancer agents.[6] All of these effects are observed at remarkably low dose 50 $\mu g/m^2$ in humans, allowing a multi-week clinical trial to be conducted using only 1.2 mg of bryostatin 1 per patient. In fact, it is this potency that has allowed bryostatin 1 to advance as a pharmaceutical agent despite its extreme scarcity. There are currently 38 clinical trials using bryostatin 1 alone or in combination with another agent either active or recently completed in the United States.[7] In addition to this promising anti-cancer activity, **1** and its

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analogues have shown promise in animal models as a lead for treatment of Alzheimer's disease.[8]

Although its exact mode of action is not clear, it is known that bryostatin is a potent binder and inhibitor of various protein kinase C (PKC) isozymes (K_i=1.35 nM).[9] It has been shown to bind to the cysteine-rich domains of PKC in a manner similar to that of the phorbol esters and diacylglycerol (DAG). It is believed that bryostatin acts to protect "tumor suppressing" PKCs, whereas phorbol, a tumor promoter, leads to their degradation.

TOTAL SYNTHESIS

Introduction

The amazing biological activity, molecular complexity and scarcity of the members of the bryostatin family make it an ideal target for total synthesis. The initial syntheses of this family were lengthy (60-70 steps) and showcased some of the most modern methods of the 1990s, particularly aldol methodology and selective reductions for the synthesis of polyacetate chains. As methods and thinking about the role of natural products as medicines evolved, two new approaches emerged. First, Wender and Keck focused on finding simplified and readily synthesized analogues of the bryostatins that retained the original molecule's activity. Later, Trost applied modern transition metal-mediated reactions to complete a much shorter synthesis of bryostatin 16. The history of the bryostatins in this sense reflects the advancement of organic synthesis and the role of the synthetic chemist in producing compounds of biomedical interest.

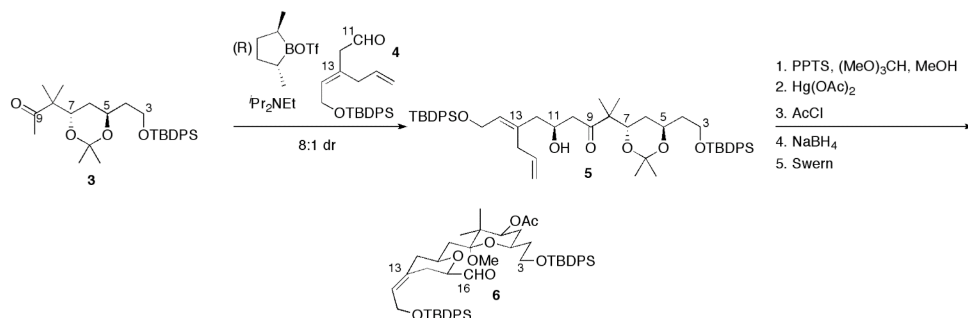
Massamune's Total Synthesis of Bryostatin 7

In 1990, Masamune's group reported the first total synthesis of any member of the bryostatin family.[10] In addition to possessing the previously discussed common structural features of the bryostatins, bryostatin 7 (**2**) differs from **1** only at C20 where an acetate replaces the long chain ester.

The key challenges synthetically are the installation of the polyacetate oxygenation pattern of the A-B rings, two sensitive exocyclic enoates, a sterically congested C16-C17 *trans*-alkene and the 26-membered macrolactone. Retrosynthetically, the top and bottom halves of the molecule can be separated by retro-Julia olefination to break the C16-C17 alkene and by a macrolactonizaton. A series of acetate aldol transforms serve to set the stereochemistry at C7 and C11 on the top half, with the two pyran rings (A and B) being formed by intramolecular ketalization and oxymercuration. The bottom C-ring utilizes substrate controlled nucleophilic addition and ketalization to set the desired stereochemistry and form the ring.

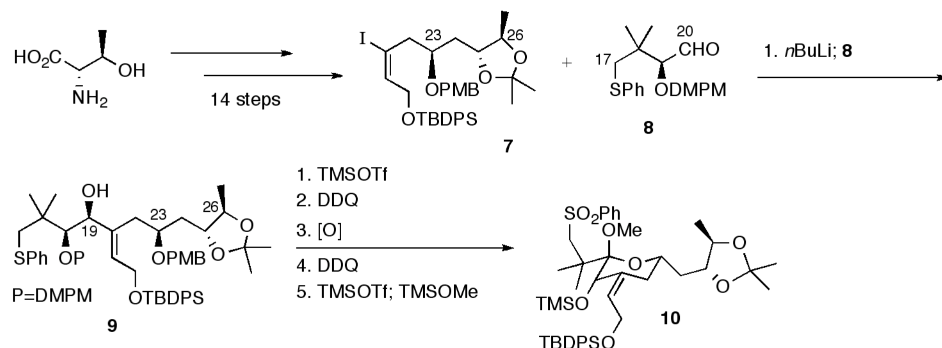
Bryostatin 7 (**2**)

Scheme 1. Masamune Synthesis of the C3-C16 Fragment of Bryostatin 7



The synthesis of the C3-C16 fragment (Scheme 1) begins with an acetate aldol (4:1 selectivity) and an anti-selective reduction to set the C5 and C7 stereochemistry followed by elaboration to ketone **3**. Another aldol reaction between **3** and aldehyde **4** proceeded with 8:1 selectivity to furnish AB-ring precursor **5**. Note that the sensitive C13 enoate is present in latent form as an allyl silyl ether until late in the synthesis. Acid catalyzed acetonide removal followed by ketalization of the C9 ketone closes the A-ring. Oxymercuration closes the B-ring and installs the C16 aldehyde for later Julia olefination.

Scheme 2. Masamune Synthesis of the C17-C27 Fragment of Bryostatin

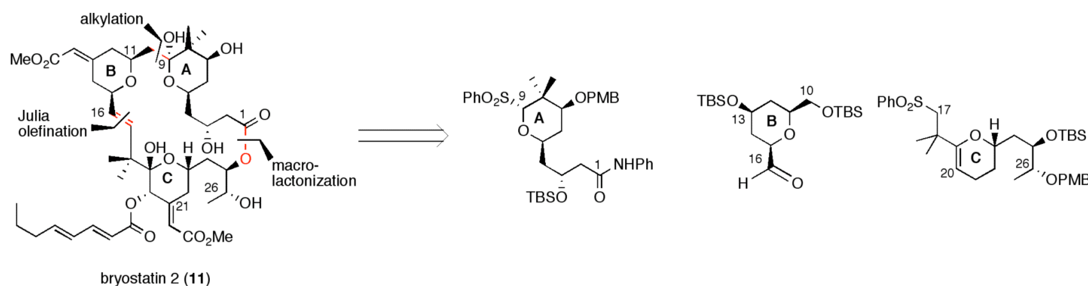


The synthesis of the C17-C27 fragment starts from threonine, which contains the C25 and C26 stereochemistry. It is elaborated to vinyl iodide **7**, whose synthesis features a chelation-controlled zinc addition to set the C23 stereochemistry with 8:1 selectivity and a tin-cuprate conjugate addition to set the olefin geometry. The lithium anion of **7** adds into aldehyde **8** under chelation control to afford keto-thioether **9** in 6:1 selectivity. Protecting group manipulations and ketalization of the C19 ketone forges the C-ring and oxidation of the thioether yields sulfone **10**, the bottom half of bryostatin 7. Julia olefination between aldehyde **6** and sulfone **10** followed by oxidation and acetate aldol to set the C3 stereochemistry sets up macrolactonization and deprotection to complete the synthesis of bryostatin 7.

Other Early Syntheses

The next total synthesis of a bryostatin did not appear for another 9 years until, in 1999, Evans synthesized bryostatin 2 (**11**),[11] which differs from bryostatins 1 and 7 only by the nature or lack of esters at C7 and C20. The Evans retrosynthesis and key fragments are shown in Scheme 3.

Scheme 3. Evans Retrosynthesis of Bryostatin 2



Evans disconnects the top and bottom halves of bryostatins identically to Masamune; however, rather than constructing a linear AB-ring precursor, a C9-C10 disconnect is also made to give three fragments each containing an intact pyran ring. Also similar to the Massamune synthesis, Evans uses aldol methodology to set the 1,3-oxygen stereochemistry of the A-ring in the C1-9 fragment **12**. Of note is the use of a site-selective lactonization to differentiate alcohols at C3 and C5, a strategy which has subsequently been adopted by several groups. Evans also places a sulfonyl group at C9 to allow deprotonation and alkylation with a C10 triflate of the C10-C16 fragment **13**. In the forward direction, Julia olefination unites C16 aldehyde from **13** with sulfone **14**. Alkylation of **12** and macrocyclization provided the cyclized core of **11**. Elaboration of the C-ring and late stage installation of the exocylic enoates at C13 and C21 finishes the synthesis.

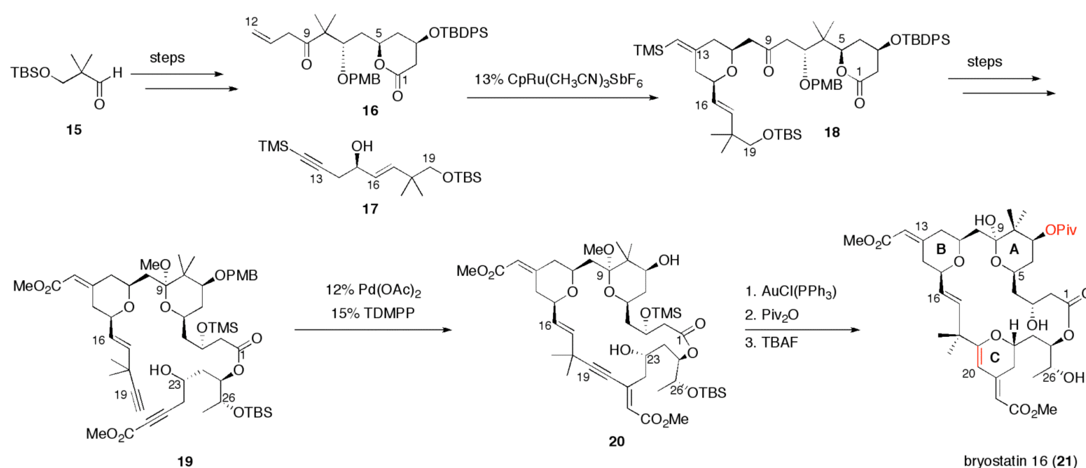
For brevity, Yamamura's and Nishiyama's total synthesis of bryostatins 3[12] will not be discussed here. The authors follow a retrosynthetic plan similar to that of Evans; however, a dithiane coupling is used to form the C9-10 bond and mask the ketone for eventual ketalization. Hale has also reported a formal total synthesis of bryostatin 7.[13] and several other groups[14] are actively pursuing synthesis of various members of the bryostatins family.

Trost's Total Synthesis of Bryostatin 16

In 2008 the Trost group disclosed the shortest synthesis of a bryostatin to date, 39 steps, relying heavily on transition metal mediated reactions.[15] A Pd-mediated alkyne-alkyne coupling was envisioned to forge the C20-21 bond and close the macrocycle, setting up a gold-mediated cyclization to close the C ring. A novel Ru-catalyzed cyclization[16] would simultaneously form the C12-C13 bond and close the B ring.

After elaborating common aldehyde **15** to alkene **16** and alkyne **17**, a Ru-catalyzed cyclization followed by conjugate addition into the resulting enone forms the B-ring and sets the olefin geometry at C13 in pyran **18** (Scheme 4). Elaboration of this intermediate, including alkynylation of the C19 alcohol and esterification of the C1 acid, gives macrocyclization precursor **19**. A palladium-catalyzed alkyne-alkyne coupling closes the macrocycle and sets the C21 enoate geometry. A gold-catalyzed 6-endo-dig cyclization closes the C-ring. Deprotection yields bryostatin 16 in only 39 steps compared to ~70 for previous syntheses. The use of powerful transition-mediated reactions capable of forming multiple bonds in a single step was critical to the efficiency of this synthesis.

Scheme 4. Trost's Synthesis of Bryostatin 16



SYNTHESIS AND BIOLOGICAL EVALUATION OF BRYOSTATIN ANALOGUES

Introduction

Despite the heroic efforts to synthesize members of the bryostatin family, the early syntheses are much too long to provide large-scale quantities of the molecule for clinical trials. Even the Trost synthesis, though much lower in step count, uses somewhat high loadings of toxic metals, making it poorly suited for industrial manufacturing. However, the Trost synthesis illustrates one avenue to make complex natural products accessible for medicinal purposes: develop efficient reactions that are well suited to maximize the yield and convenience of a target-oriented synthesis. The Wender group has pioneered a complementary approach towards bryostatin analogues termed "function-oriented synthesis". This approach posits that natural products are not designed by nature for human therapeutic use, from which it follows that the natural product itself will not necessarily be the one compound most effective for human medicine. Furthermore, rather than using simplifying reactions, the chemist can simplify the structure of the molecule with the end goal of making the molecule easier to synthesize and tuning it to the desired biological activity.

Rational Design of a Bryostatin Analogue

The knowledge that bryostatin competes for the same binding site on PKC as the phorbol esters and DAG provides a starting point for determination of what elements of the bryostatin structure are critical for binding. The Wender group used computer modeling to calculate all possible pharmacophoric triads present in phorbol esters and DAG.[17] The most likely triads were then correlated with the structure of bryostatin 1, revealing C1, C19 and C26 oxygen atoms as likely features key to the binding affinity of bryostatin for PKC. It was therefore hypothesized that the A-B ring segment of bryostatin acts as a spacer domain to place the pharmacophoric elements in the requisite locations. The heteroatom triad-containing C-ring and lower half of the molecule would then act as the recognition domain, responsible for binding.

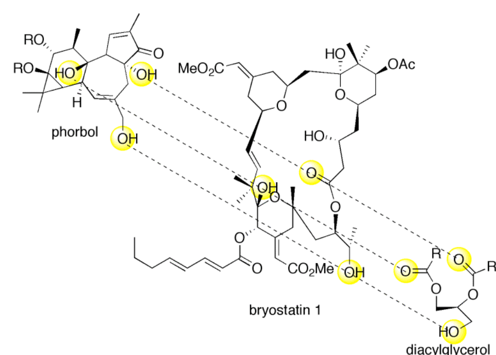
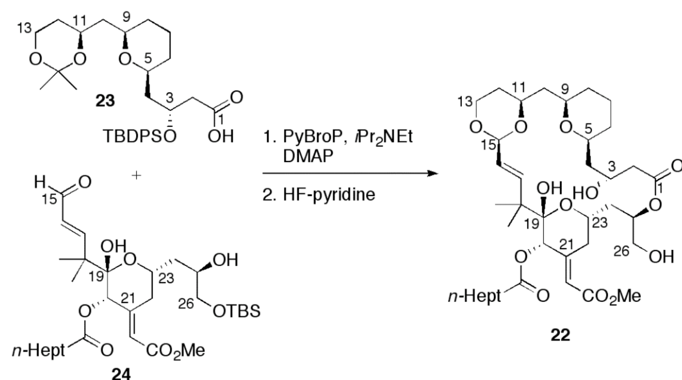


Figure 1. Comparative Identification of a Bryostatin 1 Pharmacophoric Triad

This suggests that a simplified, but conformationally similar spacer domain could retain overall molecular function, if the required hydrogen-bond donors and acceptors and their spatial orientation remained intact. Preparation of a variety of bryostatins analogues indicated that changes on the C-ring had more substantial impact on binding than changes the A and B-rings.[18] Also consistent with the functional hypothesis is the observation that acylation of the C26-hydroxyl nullifies binding. Furthermore, elimination of the spacer domain also prevents binding. In line with this hypothesis, the Wender group synthesized analogue **22** in 30 steps,[19] compared to ~70 for the natural product. This molecule has a K_i of 0.25 nM (compared to the K_i of 1.35 nM for bryostatin 1) and also showed nanomolar GI_{50} s for a wide range of cancer cell lines *in vitro*. Importantly, the spacer domain can be synthesized in only 10 steps in 25% overall yield,[20] because much of the functionality has been removed and the B-ring pyran replaced by an acetal. Key features of the synthesis of **22** include highly enantioselective hydrogenation to set the oxygen stereocenters in the spacer domain **23** and a transacetalization to close the macrolactone with spacer domain/aldehyde **24** (Scheme 5). This analogue is currently undergoing evaluation in animal models.

Scheme 5. Completion of Analogue Synthesis



Evaluation of a Wide Variety of Analogues

The Wender group has subsequently published several papers modifying various portions of the molecule. For example, a variety of esters have been attached at C20. Since the identity of this ester is not critical for binding efficacy, a 3-aminobenzoate ester was installed via acylation and nitro reduction.[21] The nitrogen functionality allows a variety of groups to be installed, most interestingly, fluorophores. The A-ring can also be replaced with various groups, including *tert*-butyl, phenyl and bromophenyl, while still retaining binding affinity.[22] These groups maintain the required structural rigidity. The bromide, for example, can be further elaborated via cross-coupling. Finally, C7 analogues have been prepared. Changes at this position can have a significant effect on binding, depending on size and polarity.[23] Keck has also developed a program focused on the synthesis and structure activity correlation of various bryostatins analogues.[24] By examining the biological profile of these analogues lacking certain functional groups in competition with phorbol esters, the Keck group has conjectured that A-ring functionalization, while not needed for strong binding to PKC, is critical for the biological profile of the bryostatins and analogues compared to that of the phorbol esters.[25] This stands in conflict with the Wender group's analogues, which for the most part lack A-ring functionality. Not enough *in vivo* testing has been reported with the Keck analogues to comment on their efficacy. However, as the Wender analogues have shown promise in animal models and are undergoing further *in vivo* trials, it is clear that the function-oriented synthesis approach to the bryostatins has merit.

CONCLUSION

Three metrics often used to evaluate the importance of a natural product are its structural complexity, its biological activity and its scarcity. The bryostatins embody all three of these qualities, making them high profile natural product targets. Several total syntheses of the bryostatins have been described. These molecules challenged chemists to develop new methods to solve the problem of polyacetate synthesis as well as cleverly employ traditional techniques. The later syntheses of bryostatin

and its analogues also showcase the use of transition metal-catalyzed reactions and function-oriented synthesis. The extraordinary biological profile of the bryostatins will make them interesting subjects of research for years to come.

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