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Total Synthesis of Bryostatin 1

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Supporting Information

ABSTRACT: Bryostatin 1 is a marine natural product that is a very promising lead compound because of the potent biological activity it displays against a variety of human disease states. We describe herein the first total synthesis of this agent. The synthetic route adopted is a highly convergent one in which the preformed, heavily functionalized pyran rings A and C are united by "pyran annulation", the TMSOTf-promoted reaction between a hydroxyallylsilane appended to the A-ring fragment and an aldehyde contained in the C-ring fragment, with concomitant formation of the B ring. Further elaborations of the resulting very highly functionalized intermediate include macrolactonization and selective cleavage of just one of five ester linkages present.

Bryostatin 1 is a now well-known natural product originally isolated by Pettit and co-workers from the marine organism *Bugula neritina*.[1] Since that time, other members of this family have been isolated, and some 20 members are now known (Figure 1).[2] It has also been established that the true source of the bryostatins is not actually *B. neritina* but rather a bacterial symbiont.[3]

Interest in the bryostatins, and bryostatin 1 in particular, has been intense because of the wide range of potent bioactivity associated with bryostatin 1. Bryostatin 1 has shown activity against a range of cancers and has also shown synergism with established oncolytic agents such as Taxol.[4] This has led to the use of bryostatin 1 in numerous clinical trials for cancer, despite the absence of any renewable supply for this compound at present. In addition, bryostatin 1 has shown promising activity relevant to a number of other diseases and conditions, including diabetes,[5] stroke,[6] and Alzheimer's disease.[7] A clinical trial for Alzheimer's disease is commencing.[8] This wide range of promising potential indications for bryostatin 1 becomes more understandable when it is recognized that at least one mechanism for function of this agent involves activity on protein kinase C (PKC) isozymes and other proteins containing C1 domains.[9] These signaling proteins are known to regulate some of the most critical cellular processes and properties, including proliferation, differentiation, motility and adhesion, inflammation, and apoptosis.[10]

In view of this backdrop, it is not surprising that synthetic activity directed toward the bryostatins has been intense. What is surprising, perhaps, is that bryostatin 1 itself has not been previously synthesized, while other members of the family have been prepared. Previous total syntheses include those of bryostatin 7 (**2**, by Masamune), bryostatin 2 (**3**, by Evans), bryostatin 3 (**4**, by Yamamura), and bryostatin 16 (**5**, by Trost).[11] In addition, Hale has described a formal synthesis of bryostatin 7, and Trost has recently reported a synthesis of C20-*epi*-bryostatin 7.[11]

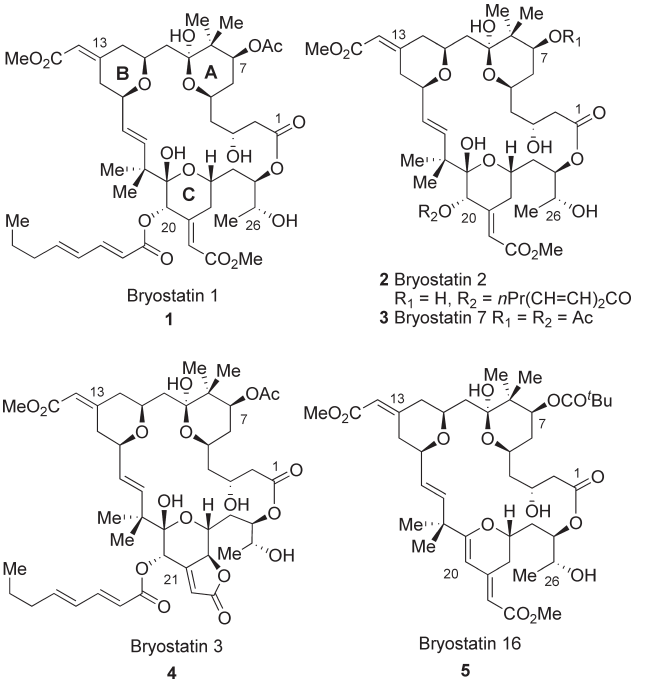

Figure 1. Bryostatin structures.

Another very important aspect of bryostatin synthesis has been the area of analogue synthesis, originally introduced by Wender and co-workers.[12] This group targeted analogues in which the B-ring pyran was replaced by an acetal linkage for ease of synthesis. Recent work in our laboratory directed at establishing structure−activity relationships for bryostatin 1 has led to the synthesis of a number of bryopyrans that are close structural analogues of the bryostatin natural products using methodology developed explicitly for this purpose.[13] The pyran annulation process has been used to prepare the analogues Merle 23 (**6**) and Merle 27 (**7**)[14], which display phorbol ester-like biology in U937 leukemia cells, as well as analogues Merle 28 (**8**) and Merle 30 (**9**), which are predominantly bryo-like in the same differential assay (Figure 2).[15] In addition, Wender has prepared the bryopyran analogues **10** and **11** using the intramolecular variant of the pyran annulation reaction as well as numerous other analogues incorporating the previously mentioned scaffold variation in which the B-ring pyran is replaced by an acetal.[16]

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The work directed at Merle 23 and 27 in comparison with that directed toward Merle 28 and 30 afforded an opportunity to compare two convergent strategies for the union of A-ring and C-ring fragments with concomitant formation of the B ring. On the basis of this previous experience, a strategy of combining an A-ring hydroxyallylsilane and a C-ring aldehyde was selected for an attempted synthesis of bryostatin 1.

The synthesis of the fully functionalized C-ring fragment used a new approach that was more convergent than the one utilized previously for the Merle analogues (Scheme 1). Carboxylic acid **12** (available in four steps) was esterified with the previously reported homoallylic alcohol **13** [prepared from commercial (*R*)-isobutyl lactate in six steps].[13b] Selective oxidative functionalization at the terminus of the less hindered alkene followed by Wittig chain extension afforded **16**, which was then utilized in a Rainier metathesis reaction to construct glycal **17** in 80% yield.[17] Oxidative functionalization of the glycal afforded methoxyketone **18** in 66% yield over two steps. Aldol condensation with methyl glyoxylate then afforded enoate **19** in 82% isolated yield.[18] Luche reduction gave an intermediate C20 alcohol that was found to be unstable with respect to storage or purification by column chromatography. Immediate acetylation of the alcohol after isolation gave the C20 acetate derivative **20** in 84% yield for the two steps of reduction and acylation. This was converted to aldehyde **21** in 91% yield by TBS removal and Ley oxidation using TPAP and NMO.

The A-ring hydroxyallylsilane **22** was prepared in three steps via allylation of the corresponding previously reported aldehyde.[19] The crucial pyran annulation reaction between the A-ring hydroxyallylsilane **22** and the C-ring aldehyde **21** then provided the tricyclic intermediate **23** in 61% isolated yield (Scheme 2). This represents one of the most complex examples of such a pyran annulation to date. The major byproduct was a spirocylic structure formed via intramolecular cyclization of the silane at the C9 position of **22**. However, attempts to suppress this side reaction by increasing the amount of aldehyde or the concentration of the reaction were not successful.

Hydrolysis of the thiolester to reveal the carboxylic acid was attempted next. Selective hydrolysis using various reagents such as $LiOH/H_2O_2$, $AgNO_3/H_2O$, or $Hg(OCOCF_3)_2$ was complicated because of the competitive hydrolysis of other esters, hydrolysis of the methyl ketals, or oxymercuration of the olefins. After considerable experimentation, it was discovered that this hydrolysis could be carried out selectively using $LiOH/H_2O_2$ when the C_3 hydroxyl was free. The exact origin of the selectivity is unclear, but a decrease in the steric hindrance around the thiol ester and activation of the C1 carbonyl due to hydrogen bonding with the C3 alcohol seems to be the most reasonable explanation. To obtain the free alcohol, the BPS group was removed using HF·py under buffered conditions. The presence of methanol during the silyl deprotection was necessary to avoid any hydrolysis

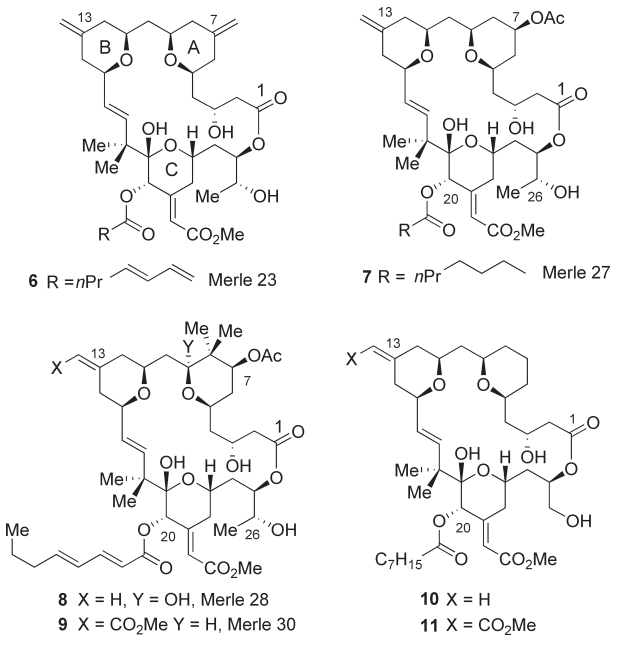
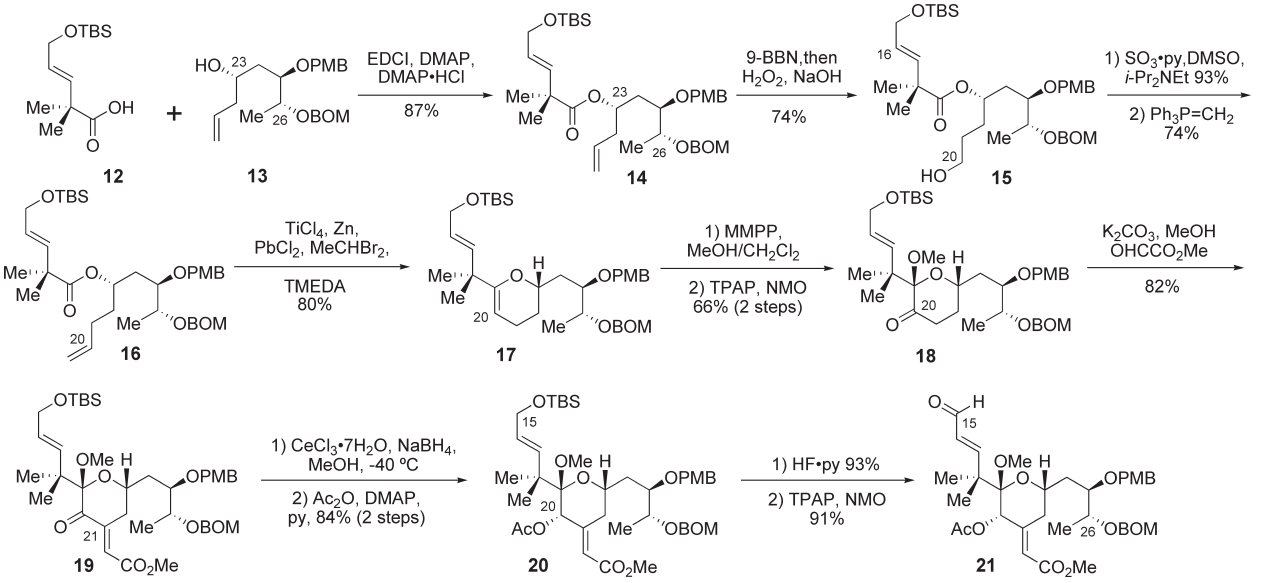


Figure 2. Representative bryopyran analogues.

Scheme 1. Synthesis of C-Ring Aldehyde 21 Using the Rainier Metathesis Reaction



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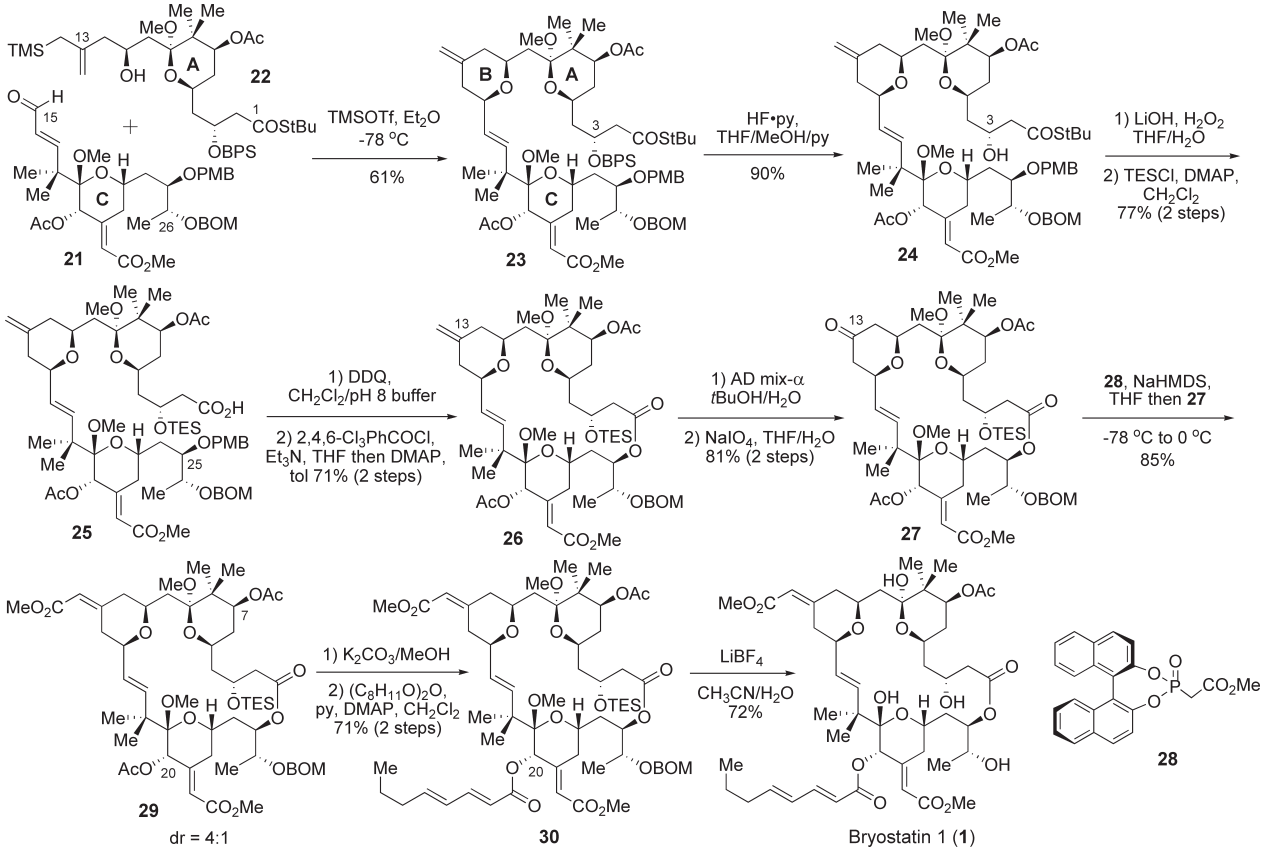
Scheme 2. Synthesis of Bryostatin 1 via Pyran Annulation



of the methyl ketals at C19 and particularly C9. Treatment of the β-hydroxy thiol ester with $LiOH/H_2O_2$ in aqueous THF then cleanly afforded the β-hydroxycarboxylic acid. Since the free hydroxyl group at the C3 position was found to interfere with the subsequent macrolactonization, it was protected as the TES ether. Following removal of the PMB group using DDQ, the resulting seco acid was subjected to Yamaguchi macrolactonization conditions,[20] which afforded macrolactone **26** in 71% yield over two steps.

At this point, the regioselective oxidative cleavage of the C13−C30 olefin of **26** could be achieved by either ozonolysis or reaction with $OsO_4/NaIO_4$. However, a protocol involving Sharpless asymmetric dihydroxylation followed by periodate oxidation was found to be superior to the other conditions and provided ketone **27** in 81% yield.[21] An asymmetric Horner−Wadsworth−Emmons reaction on the ketone using Fuji's chiral BINOL phosphonate **28** provided a 4:1 *Z*/*E* mixture α,β-unsaturated methyl esters in favor of the desired isomer.[22] The geometric isomers were easily separated using preparative thin-layer silica gel chromatography.

We were pleased to find that when bisacetate **29** was exposed to K_2CO_3/MeOH, selective methanolysis of the C20 acetate occurred in just 45 min, providing the desired C20 alcohol. The regioselectivity observed in this reaction could best be explained by activation of the C20 acetate due to the inductive effects associated with the neighboring groups. Since the C20 alcohol was again found to be unstable, it was immediately esterified by reaction with (2*E*,4*E*)-octa-2,4-dienoic anhydride to give **30**, a protected version of bryostatin 1, in 71% yield from bisacetate **29**.

When protected bryostatin 1 derivative **30** was subjected to global deprotection using $LiBF_4$ in acetonitrile/water at 80 °C,[23] two methyl ketals, the TES ether, and the BOM group were all removed without cleavage of any of the five esters present, providing bryostatin 1 in 72% yield. It should be noted that similar deprotection of the bisacetate **29** would provide bryostatin 7. In addition, it is apparent that introduction of other side chains at the C20 position as described above would lead to the synthesis of other naturally occurring bryostatins, such as bryostatins 9 and 15, as well as new bryostatin analogues.

The analytical data for synthetic bryostatin 1 were found to be in excellent agreement with those of a sample of natural bryostatin 1 on the basis of several criteria, such as thin-layer chromatography, ^{1}H NMR, ^{13}C NMR, and ^{13}C distortionless enhancement by polarization transfer (DEPT) spectroscopy, high-resolution mass spectrometry, optical rotation, and LC−MS. As previously reported in the literature and observed by us, the ^{1}H NMR spectra of the bryostatins are highly concentration dependent and very sensitive to trace water or D_2O.[24] A change of just 2-fold in concentration gave observable changes in the proton NMR spectra. Thus, the richly detailed ^{13}C NMR spectra (all carbons observed) of synthetic and natural samples serve as a more reliable fingerprint.

In summary, the total synthesis of bryostatin 1 has been achieved by the convergent union of the A-ring hydroxyallylsilane **22** with the C-ring aldehyde **21**. An almost perfect balance in convergence was realized, as **21** was prepared in 18 steps while **22** was made in 17 steps. After union of these two fragments, an additional 11 steps afforded bryostatin 1. This first total synthesis

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of bryostatin 1 was thus accomplished in 30 steps for the longest linear sequence from commercially available (*R*)-isobutyl lactate.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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