Total synthesis of bryostatin 3

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Bryostatins are a family of 21 complex marine natural products with a wide range of potent biological activities. Among all the 21 bryostatins, bryostatin 3 is structurally the most complex. Whereas nine total syntheses of bryostatins have been achieved to date, bryostatin 3 has only been targeted once and required the highest number of steps to synthesize (43 steps in the longest linear sequence and 88 total steps). Here, we report a concise total synthesis of bryostatin 3 using 22 steps in the longest linear sequence and 31 total steps through a highly convergent synthetic plan by the use of highly atom-economical and chemoselective transformations in which alkynes played a major role in reducing step count.

The bryostatins, first isolated by Pettit et al. (1, 2) from the marine bryozoan Bugula neritina, are a family of 21 macrolides (3–6) with potent antineoplastic (7, 8), immunopotentiating (9), synaptogeneration-inducing (10), and latent HIV-modulating (11) activity. Beneficial effects as a post-stroke treatment (12) and for restoring the blood-brain barrier after traumatic blast injuries (13) have also been demonstrated. Although the exact mechanism of action remains an ongoing area of research (14), it has become clear that bryostatins act as agonists of protein kinase C (PKC), with low nanomolar affinities for their target. Their pharmacological potential together with their intriguingly complex structures have attracted the attention of numerous synthetic organic chemists over the past 30 years. To date, nine completed total syntheses have been reported (Fig. 1: bryostatin 1 (Keck, 2011; Wender, 2017) (15, 16), bryostatin 2 (Evans, 1999) (17), bryostatin 3 (Yamamura, 2000) (18), bryostatin 7 (Masamune, 1990; Kriese, 2011) (19, 20), bryostatin 8 (Song, 2018) (21), bryostatin 9 (Wender, 2011) (22), and bryostatin 16 (Trost, 2008) (23). In addition, Hale has developed a formal synthesis of bryostatin 7 (24–27), and other groups also have made important contributions to this area (28–32). All of these synthetic studies serve as guidelines toward the ultimate goal of a concise route to bryostatins that is practical and flexible, addressing the supply problem and allowing for ready access to analogs for structure-activity-relationship (SAR) studies.

Topologically, all bryostatins share a 26-membered lactone and three highly functionalized tetrahydropyran rings integrated in the macrocycle. Bryostatin 3, 19, and 20 are rather exceptional because of an additional butenolide unit directly fused to the macrocycle (Fig. 1, bryostatin 3, for example). This distinguishing butenolide unit is found in the region of the molecule crucial for binding to its biological target, PKC (33). Although changes in this recognition domain substantially attenuated binding affinities in SAR studies, bryostatin 3 retained low nanomolar affinity for PKC [inhibition constant (Ki) = 2.75 nM] (33), comparable with that of bryostatin 1 (Ki = 1.35 nM). From a synthetic perspective, the butenolide unit renders such bryostatins, especially bryostatin 3, structurally more complex and thus more challenging to synthesize. Among all the completed syntheses (15–23), only one targeted the butenolide-containing bryostatin: the Yamamura group’s synthesis of bryostatin 3 (18). Not surprisingly, both the longest linear step count (43) and the total step count (88) are highest of all the bryostatin syntheses. With the goal of developing practical and flexible approaches to the bryostatin family by using atom-economical and selective reactions, we report a concise total synthesis of bryostatin 3 by using only 22 steps in the longest linear sequence and 31 total steps.

Retrosynthetically, we proposed that bryostatin 3 could be synthesized from the macrocyclic intermediate 1 through a late-stage oxidative functionalization of the dihydro- pyran ring C and a palladium-catalyzed carbo- nylative esterification of the steroidal vinyl bromide (Fig. 2). Intermediate 1 could then be disassembled into intermediate 2 and fragment 3 on the basis of three key transformations: the palladium-catalyzed alkyne-alkyne coupling reaction to construct the C20–C21 bond, the gold-catalyzed 6-endo-dig cyclization to generate the dihydropryan ring C, and the Yamaguchi macrolactonization to form the macrocycle from the C1 carboxylic acid and the C25 alcohol. Furthermore, intermediate 2 could be disconnected into fragments 4 and 5 based on another two key

Fig. 1. Prior total syntheses of bryostatins.
Butyne developed a three-step synthesis from 3-methylpentenenitrile to furnish diol with the Sharpless asymmetric dihydroxylation yielding our attention toward diyne fragment yield and moderate diastereoselectivity. Turn-12 with methyl propiolate to generate alkynoate geometric isomer, which was then cross-coupled to %11-bromo-2-ethoxyethene (Fig. 3A) with comparable complexity (35). Another asymmetric dihydroxylation of enyne furnished fragment in excellent yield and moderate diastereoselectivity. Turning our attention toward diyne fragment, we developed a three-step synthesis from 3-methylbutyne. Double lithiation of enyne and successive quench with N,N'-dimethylformamide (DMF) and triethylchlorosilane (TESCl) delivered the alkyne aldehyde in exquisite chemo- and regioselectivity (Fig. 3B). This intermediate was exposed to a vinyl zinc reagent derived from (Z)-1-bromo-2-ethoxyethene, followed by elimination under acidic conditions with aqueous sodium bisulfate to give enal. A catalytic enantioselective propargylation of afforded the diyne fragment in 86% yield and 98% enantiomeric excess (36). The last key fragment was prepared in eight steps according to Krische’s procedure in their synthesis of Bryostatin 7 (20).

With fragments, and in hand, we proceeded with the fragment couplings and downstream elaborations. In the first key coupling, fragments and were assembled into tetrahydropyran through a ruthenium-catalyzed alkene-alkyne coupling—Michael addition cascade (Fig. 3C) (37). The diyne fragment underwent the desired coupling reaction with perfect chemoselectivity for the sterically more accessible alkyne. The cascade Michael addition reaction to construct the tetrahydropyran ring was highly diastereoselective, with a >20/1 syn/anti ratio. The coupling product was isolated in 48% yield (87% yield based on recovered starting material). Attempts to optimize the coupling reaction—including changing solvents [such as ethyl acetate, 3-pentanone, cyclopentanone, DMF, tetrahydrofuran (THF), and CH2Cl2], using additives [such as acetic acid, camphorsulfonic acid (CSA), acetonitrile, trimethylacetonitrile, DMF, and 2,6-di-tert-butyl-4-methylpyridine], and varying temperature and time—all led to detrimental effects. Fortunately, although the catalyst turnover was not ideal, the reaction was highly reproducible and easily performed at room temperature in air without precautions, and both unreacted coupling partners could be recovered in good yield and reused for multiple runs. The resulting vinyl silane was ipso-brominated with complete retention of olefin geometry to furnish the vinyl bromide.

Fig. 2. Retrosynthetic analysis of bryostatin 3.
the desired cyclization. After full conversion was indicated by means of thin-layer chromatography (TLC), a stock solution of ZrCl₄ in methanol was added to the above crude reaction mixture, again with no need of solvent removal or exchange, to hydrolyze the C25-C26 cyclopentylidene acetal in situ. After the above three orthogonal operations in one pot, the triol 27 could be isolated in 48% overall yield. Although the C3-OTBS group was desilylated during the acetonide deprotection process, it was reinstalled by subjecting the triol to silylation conditions that resulted in bis-silylated product 28 in 60% yield. Moving forward with intermediate 28, trimethyltin...
hydroxide selectively hydrolyzed the methyl ester to give seco-acid 29 in 80% yield (40), which was then cyclized under the modified Yamaguchi conditions to afford the macrocyclic intermediate 1 in near quantitative yield (41).

With an established route to the macrocyclic intermediate 1, we proceeded to complete the total synthesis by means of an oxidative functionalization of the dihydropyran ring C, an esterification of the B-ring vinyl-bromide, and a global deprotection (Fig. 5). The dihydropyran ring C of intermediate 1 was chemoselectively epoxidized with methylrhenium trioxide/urea hydroperoxide (MTO/UHP) and 1-methylimidazole under the Yamazaki conditions (42). 1-Methylimidazole proved to be the superior additive for this process, presumably by buffering the Lewis acidity of MTO, accelerating the reaction and increasing the metal complex’s lifetime. Although the reaction was relatively clean with high conversion according to nuclear magnetic resonance (NMR) monitoring of the crude product, the epoxide 30 was rather acid sensitive, and attempts to purify it only led to low and variable yields. Thus, after workup, the crude epoxide was directly subjected to the following acid-promoted ring-opening methanolysis step to furnish the α-hydroxyl ketal intermediate 31, which was again unstable for purification and storage. Consequently, the crude α-hydroxyl ketal 31 was esterified immediately through acylation by using 2,4-octadienoic anhydride to give a stable intermediate 33, which could be purified and stored regularly. The three-step, one-purification process with purification only at the end was highly reproducible and scalable. With the C-ring fully functionalized, we then turned to the carboxylation of the B-ring vinyl-bromide to install the requisite exocyclic vinyl ester. Treatment of the vinyl-bromide 33 with Pd2(dba)3(CHCl3) (20 mol %) and Xantphos (60 mol %) under one atmosphere of CO and with MeOH as a cosolvent furnished the desired vinyl ester 34 in 50% yield. At this stage, a protected form of bryostatin 3, 34, was obtained. All that remained was deprotection by means of hydrolysis of the two methyl ketals and removal of the two silyl groups. Previously, Song’s synthesis of bryostatin 8 (21) required the same type of final global deprotection. However, distinct from their synthesis in which aqueous HF in acetonitrile enabled the global deprotection in one pot, the treatment of our intermediate 34 under similar conditions only led to disappointingly complicated mixtures, as indicated by the messy NMR spectra and streaky TLC plates. Inspired by Yamamura’s synthesis of bryostatin 3 (18), we performed the final global deprotection in two separate steps: The protected bryostatin 3 34 was first treated with aqueous hydrofluoride (HF) in acetonitrile to remove the two silyl groups and one methyl ketal. After workup, the crude residue was stirred in trifluoroacetic acid (TFA)–H2O–CH2Cl2 for 1 hour to hydrolyze the remaining methyl ketal. Through the two-step deprotection procedure, 0.9 mg of bryostatin 3 was obtained in 60% overall yield from 34.

The identification of our synthetic bryostatin 3 was initially problematic because its 1H-NMR was observed to be concentration dependent. Such a property has also been documented for other bryostatins (15, 16, 43). We were able to closely compare our synthetic bryostatin 3 with 1 mg of bryostatin 3 from K. Ohmori and found that the data of TLC, 1H-NMR, 13C-NMR, optical rotation, and high-performance liquid chromatography matched excellently (supplementary materials).
This concise total synthesis showcases the value of the alkyne functionality that emanates from its chemoselectivity and its flexibility in elaborating the required structural details. The synthetic strategy and synthetic technologies in this work are expected to have implications toward the ultimate goal of practical, flexible, and concise synthesis of bryostatins and their bioactive analogs.

REFERENCES AND NOTES

Fig. 5. Completion of bryostatin 3 synthesis. UHP, urea hydroperoxide; DMAP, 4-dimethylaminopyridine; DIPEA, N,N-diisopropylethylamine; Xanthos, 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthene; DMF, N,N'-dimethylformamide; TFA, trifluoroacetic acid.

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Stitching alkynes into bryostatin 3
The bryostatin family of marine natural products has been explored for a wide variety of pharmaceutical applications but remains challenging to source. The general structure comprises a macrocycle that contains three smaller, six-membered rings. Bryostatin 3 is distinguished by the added complexity of a fourth, fused lactone ring. Trost et al. report a convergent synthesis of this complex molecule, taking advantage of alkyne coupling reactions to stitch together three main fragments and asymmetric dihydroxylation and propargylation reactions to set stereochemistry. Science, this issue p. 1007