Scalable synthesis of bryostatin 1 and analogs, adjuvant leads against latent HIV

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Bryostatin 1 is an exceedingly scarce marine-derived natural product that is in clinical development directed at HIV/AIDS eradication, cancer immunotherapy, and the treatment of Alzheimer’s disease. Despite this unique portfolio of indications, its availability has been limited and variable, thus impeding research and clinical studies. Here, we report a total synthesis of bryostatin 1 that proceeds in 29 total steps (19 in the longest linear sequence, >80% average yield per step), collectively produces grams of material, and can be scaled to meet clinical needs (~20 grams per year). This practical solution to the bryostatin supply problem also opens broad, facile, and efficient access to derivatives and potentially superior analogs.

Several approaches to solving bryostatin’s supply problem have been pursued since its first isolation in 1968 (12, 13). The NCI’s original “hand collection” of 14 tons of the marine organism Bugula neritina provided only 18 g of bryostatin 1 (0.00014% yield) (14). Improvements in the extraction of bryostatin 1 from its marine source using supercritical CO2 have been reported (15) but are ultimately limited by its low and variable natural production and the challenges of sustainable, large-scale harvesting in the delicate and changing marine ecosystem (16). Related efforts to boost production of bryostatin 1 by aquaculture of B. neritina were intensely pursued but later abandoned because of high capitalization costs for “in-sea” culture and low natural product yields for “in-tank” culture (17).

As an alternative to marine harvesting, synthetic biological approaches have been reported but remain in early stages because cultivation of the symbiotic bacterium associated with bryostatin production is difficult (18, 19). Representing a third supply strategy, chemical syntheses of various members of the bryostatin family have improved over the years, from as many as 90 steps to as few as 36 steps (20–26). The only reported synthesis of bryostatin 1 required 57 steps (24).

Here, we report a solution to the bryostatin 1 supply problem in the form of a step-economical, multigram-producing synthesis that addresses both clinical and research needs and serves additionally as a practical platform for accessing new and potentially superior analogs. Our convergent synthesis proceeds in 29 steps, with a longest linear sequence (LLS) of 19 steps and 4.8% overall yield (>80% average yield per step), and has collectively produced >2 g of bryostatin 1. All steps, except the final step for safety reasons, were conducted on a gram to multigram scale. This synthesis can thus readily supply the amount of material needed to further advance clinical evaluation, as a single gram of bryostatin 1 can treat ~1000 cancer patients (9, 11) or ~2000 Alzheimer’s patients (4) according to currently used clinical dosing.

The synthetic challenge posed by bryostatin 1 is defined in part by its macrocyclic lactone structure, which incorporates three embedded hydrocarbon rings, 11 stereocenters, and a formidable array of multiple alkene, alcohol, ether, hemiketal, and ester functionalities. Our approach to this challenge (Fig. 1) was inspired by our earlier syntheses of bryostatin 9 (25) and analogs (27–29) and is designed to initially produce in parallel the less complex A- and C-ring subunits of the target. These precursors are then conjoined through a Yamaguchi esterification and Prins macrocyclization to produce the B-ring and, after four subsequent steps, bryostatin 1. An additional advantage of this convergent strategy is that either subunit or final-stage intermediates can be modified to access derivatives and analogs.
The synthesis of bryostatin's C-ring subunit (Fig. 2, aldehyde 2), which incorporates C15 to C26 of the macrocycle and important structural elements that influence its PKC affinity (30), started with the hydrolysis and in situ prenylation of inexpensive dihydropyran 3 ($3/mol) to form known diol 4 (>20 g scale) (31). This and all subsequent reactions were conducted on multiple scales by multiple investigators to ensure reproducibility and information transfer. Further, although all isolable products were purified and characterized, many steps were designed to be conducted without product purification to reduce time, cost, and waste-generating chromatographies. As such, crude diol 4 was doubly oxidized to afford 1,5-ketoaldehyde 5 (>25 g scale, 81% yield), setting the stage for C-ring formation. Although previously unexplored on dicarbonyl compounds, Nokami's crotylation procedure involving ketoaldehyde 5 and crotyl transfer reagent 6 (32) was found to proceed with high chemo- and enantioselectivity to set the C23 stereochemistry in alcohol 7 (>98% enantiomeric excess (ee)). This process can be conducted in one flask along with an in situ cyclodehydration and enol ether oxidation to yield pyran 9 as a 2:1 inconsequential mixture of C20 epimers. Pyran 9 proved to be unstable and was thus directly oxidized to provide the isolable C20 ketone 10 (69% yield from ketoaldehyde 5). Compound 10 is the first intermediate in the sequence that required chromatographic purification.

The C-ring enolate of bryostatin 1 was introduced using a one-step aldol condensation protocol, which afforded exclusively the E-isomer 12 in 84% yield (27). The C20 ketone was then

**Fig. 2. Reaction sequence for the C-ring subunit, 2.** Reagents and conditions: a. NH$_4$Cl, H$_2$O; then zinc powder (3.4 equiv), prenyl bromide (1.7 equiv), tetrahydrofuran (THF). b. Oxalyl chloride (3 equiv), dimethyl sulfoxide (DMSO) (4 equiv), CH$_2$Cl$_2$, –78°C; then 4; then Et$_3$N (8 equiv), –78° to –30°C. c. Reagent 6 (2 equiv), p-toluenesulfonic acid (p-TsOH-H$_2$O) (10 mol %), CHCl$_3$; then 4 Å molecular sieves, 70°C; then magnesium monoperoxyphthalate (MMPP) hexahydrate (0.45 equiv), NaHCO$_3$ (2 equiv), MeOH, 0°C. d. Dess–Martin periodinane (1.5 equiv), pyridine (10 equiv), CH$_2$Cl$_2$, 0°C. e. Glyoxylate 11 (5 equiv), K$_2$CO$_3$ (5.5 equiv), 4.4:1 THF/MeOH. f. CeCl$_3$-7H$_2$O (0.5 equiv), N,N-dimethylformamide (DMF). g. N,N-dimethylformamide (DMF), Me, methyl; Et, ethyl; Bu, butyl.
selectively reduced and esterified to afford octynoate 13. The introduction of the octynoate at C20 as a less reactive, masked equivalent of the target octadecanoate was designed to enable the projected chemoselective oxidation of three different alkenes (see below). This approach was predicated on the ambitious expectation that an yne-to-diene isomerization could be effected on an advanced intermediate at the end of the synthesis.

The first test of our serial alkene oxidation plan was encountered with the dihydroxylated C25/C26 acetonide and C19 ketal groups were removed and the C26 alcohol protected to afford aldehyde 21. Overall, this route to bryostatin 1 has been a standard Evans-Saksena reaction conditions (88% yield) after in situ hydrolysis. Subsequently, the C25/C26 acetonide and C19 ketal groups were reduced and the C26 alcohol protected to afford aldehyde 21 (1 equiv.).

The homologation of aldehyde 21 has been a longstanding chemoselectivity challenge because of its steric encumbrance (including unanticipated long-range effects arising from the C25/C26 protecting group) (33–35) as well as the potential for competing deprotonation and Michael additions involving the ynoate and unsaturated ester moieties. Of the many nonbasic nucleophiles we surveyed (including vinyl zinc (36) and cerium (37) reagents), only vinyl zinic 16 cleanly engaged the C17 aldehyde to afford aldehyde 18 (78% yield) after in situ hydrolysis. Subsequently, the C25/C26 acetonide and C19 ketal groups were removed and the C26 alcohol protected to afford aldehyde 21. Overall, this route to bryostatin’s C-ring subunit, 2, proceeded in 13 steps (LLS) and a highly efficient 16% overall yield.

The synthesis of the A-ring subunit of bryostatin 1 (Fig. 3, acid 1) began with the condensation of t-butyl acetate and propionate 19, a rare example of a Claisen reaction between two enolizable coupling partners. We found that sterically large substituents on both the electrophile (i.e., 3,3-dioxy groups) and nucleophile (t-butyl group) were necessary to suppress enolate exchange events leading to undesired products. The resultant β-ketoester 20 was reduced with Noyori’s catalyst (96% ee) and straightforwardly converted to aldehyde 21. This three-step sequence from 19 to 21 was routinely carried out on 30-g batches with one chromatographic purification in a single week (~80% yield per step).

Aldehyde 21 was combined with β-diketone 22 in a substrate-controlled boron aldol reaction to afford hydroxyketone 23 in equilibrium with its hemiketal isomer (86% combined yield, 2:1 dr). Alternative aldol methods failed to selectively deliver the 1,3-aryl adduct. For example, Paterson’s disopinocamphyl (1p) protocol (38) led only to reduction (39) of the C9 carboxyl group of β-diketone 22, whereas Mukaiyama (Lewis acids: BF₄, AlMe₃, Cl, TiCl₄, SnCl₄, Tf₂NH) and metal-enolate reactions (Li, Zn, Sn) generally gave complex mixtures that were at best ~1:1 dr. Notwithstanding the moderate 2:1 selectivity for this boron aldol reaction, this process was selected for use because of its favorable throughput on multigram scales.

The chemo- and diastereoselective reduction of ketone 23 was accomplished by modifying standard Evans-Saksena reaction conditions (40). We found that application of either Me₃N- or
NaBH(OAc)₃ in AcOH/MeCN resulted in low diastereoselectivity (2:1 dr); however, after the introduction of acetone as a co-solvent, which likely suppressed intermolecular reduction events, the dr improved to >15:1. Ketalization at C9 (using trimethyl orthoformate as a dehydrating agent), followed by a one-flask acylation at C7 and chemo-selective acetal hydrolysis (41) at C11, then afforded aldehyde 25.

The diastereoselective allylation of aldehyde 25 proceeded in 84% yield and 10:1 dr (decagram scale) using Leighton’s chiral diamine controller with silane 26 (42). The high efficiency of this Leighton allylation belies the many difficulties we experienced in surveying allylation platforms, most of which could not be extended to incorporate the sensitive trimethylsilyl moiety [e.g., 1pc method (43)] or did not react with the highly oxygenated and β-disubstituted aldehyde 25 [e.g., chiral allylstannane platforms (44, 45)]. Silylation of the C11 alcohol and hydrolysis of the C1 ester (46) then completed the A-ring subunit, 1, in 13% overall yield (10 steps).

A Yamaguchi esterification united the A- and C-ring fragments, 1 and 2, in 82% yield (Fig. 4A) and set the stage for another key step, a Prins macrocyclization (28, 47). Catalyzed by pyridinium
p-toluenesulfonate (PPTS) in methanol, this process formed bryostatin’s B-ring while closing the macrocycle (70% yield of 29 and 11% yield of C26 desilylated product). The resultant macroalicone incorporating four different p-systems served as a third test of our selective alkene oxidation pattern. Gratifyingly, a stoichiometric ozonolysis of 29 occurred chemoselectively to yield ketone 30 [80% yield, 90% based on recovered starting material (brsm)]. We found that by performing this reaction in the presence of methanol (versus dichloromethane alone), the yield increased from ~50% to 80%, likely by mitigating the negative effects arising from ionization of the C9 ketal.

At this point, the long-deferred alkynoate-to-diene isomerization was ready to be tested. Using a modification of a procedure reported by Rychnovsky (49), the C20 ynoate of 30 was isomerized with triphenylphosphine and 2,4,6-trimethylphenol to afford dienoate 31 in 90% yield. This practical and efficient conversion ranks among the most complex examples of an ynoate isomerization process reported to date (49).

The B-ring enoate was installed by a Horner-Wadsworth-Emmons reaction using Fuji’s phosphonate 32 (21, 50). We found that this 3,3’-dimethyl-BINOL phosphonate gave a significantly higher Z:E ratio than the corresponding unsubstituted reagent (11:1 vs. 3:1 Z:E). Finally, the silyl ethers and C9 ketal of 33 were cleaved with buffered HF-pyridine followed by in situ hydrolis to yield bryostatin 1 in 80% yield (30% overall yield from buffered HF-pyridine followed by in situ hydrolysis). Gratifyingly, a stoichiometric ozonolysis of 33 occurred chemoselectively to yield ketone 34 and 35 (Fig. 4C). However, these compounds displayed different isomeric selectivities relative to bryostatin 1, which binds all conventional and novel PKC isoforms with single-digit nanomolar affinity. Because different isoforms are associated with different therapeutic indications, access to isoform-selective PKC modulators enables the development of more therapeutically relevant, disease-specific leads (5, 6, 52, 53). This work opens sustainable research access to bryostatin 1 as well as more synthetically accessible analogs that are proving to be more effective and better tolerated in comparative studies with cells, disease models in animals, and ex vivo samples taken from HIV-positive patients (27, 54).

REFERENCES AND NOTES

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/358/6360/218/suppl/DC1 Materials and Methods
Figs. S1 to S3
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References (S5–S8)
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A gram-scale route to bryostatin

Scientists once accumulated 14 tons of the red, bushy, tufted sea creature Bugula neritina to extract 18 grams of bryostatin 1. The macrocyclic organic compound is under study for treatment of HIV, cancer, and Alzheimer’s disease but has proven frustratingly scarce. Wender et al. report a 29-step chemical synthesis of bryostatin 1 that proceeds in 4.8% overall yield and provides gram quantities of the compound (see the Perspective by Lanman). Intermediates along the pathway can be straightforwardly modified to produce analogs, two of which were prepared en route and studied in vitro. Science, this issue p. 218; see also p. 166