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Total Synthesis of Bryostatin 16 via Atom Economical and Chemoselective Approaches

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Abstract

Atom economy¹ and chemoselectivity² constitute two of the major challenges for enhancement of synthetic efficiency. The syntheses of complex natural products constitute the most demanding arena for exploration of such principles. The combination of extraordinary promising biological activity and the structural complexity of the bryostatins with the benchmark provided by earlier syntheses of some members make them an excellent target for such studies. Herein, we report a concise total synthesis of bryostatin 16 (1). Bryostatin 16 was chosen as the specific synthetic target, most critically because bryostatin 16 could potentially act as a pivotal parent structure to allow access to almost all other bryostatins. Moreover, its synthesis could provide an essential chemical probe by enabling facile analogue syntheses with variations in most parts of the structure, to allow fine-tuning of the biological functions. Application of atom economical and chemoselective reactions under development in these laboratories provides ready access to polyhydropyrans, common structural features of numerous polyacetate-polypropionate derived natural products. Most notably, our strategy of employing the combination of two transition metal catalysts (palladium and gold) demonstrates a new chemoselective and atom-economical macrocyclization reaction between two different alkynes and subsequent formation of the C-ring dihydropyran in the context of a complex natural product synthesis.

The bryostatins 1–20 (Fig. 1), originally isolated from the marine bryozoan *Bugula neritina*, are a class of structurally complex macrolactone natural products, which exhibit exceptional biological activity, most notable their anticancer activity *in vivo*³. Clinical application of bryostatin in combination with other chemotherapeutic agents has shown significant potential to treat some cancers with high potency^{4,5}. Furthermore, recent studies have revealed that bryostatin significantly affects both cognition and memory enhancement in animals, which suggests potential use of bryostatin for the treatment of Alzheimer's disease, depression and other cognitive impairments⁶. Although bryostatins' activities could be attributed to their strong affinity for protein kinase C (PKC) isozymes⁷, their actual mode of action is still an important research subject. Unfortunately, their clinical advancement is hampered by the limited source of bryostatins from isolation: low yield (ca. $1.6 \times 10^{-4}\%$, 18 g of bryostatin 1, one of the most abundant members, from 14 t of animals on industrial scale⁸) and non-renewable supply. Therefore, efficient total syntheses of these natural products^{9–12} and their analogues^{13–16} remain in high demand.

The structures of bryostatins constitute significant synthetic challenges, which include three heavily substituted polyhydropyran rings, two acid/base-sensitive *exo*-cyclic unsaturated esters, one congested C16–17 *trans*-olefin, as well as numerous oxygen-containing functionalities on a 26-membered lactone. As an example, the challenge posed by the C16–17 double bond led to failure in routes relying on its formation by metathesis reactions¹⁷, even in

the case of a relay metathesis strategy¹⁶. Despite their biological, clinical and structural significance, to date only three of the twenty bryostatins have been accessed by total synthesis (bryostatin 7 by Masamune⁹, bryostatin 2 by Evans¹⁰, and bryostatin 3 by Nishiyama and Yamamura¹¹).

With a goal of streamlining the strategy to these complicated targets to enable more extensive access, we chose bryostatin 16 (**1**)¹⁸ as the specific synthetic target, which was motivated by three circumstances: (i) bryostatin 16 could act as a pivotal parent structure to allow access to all other bryostatins (except bryostatin 3, 19 and 20; see Fig. 1) by elaboration of the electron-rich and relatively reactive C19–20 olefin^{10,16} (for a promising biosynthetic approach, see ref. 19); (ii) the dihydropyran entity of the C-ring in **1** offers an ideal forum for us to explore a Pd-catalyzed alkyne-alkyne coupling as a novel macrocyclization method²⁰, which has not previously existed in a complex natural product synthesis; (iii) new analogues, which are not easily available from other syntheses, might be readily obtained simply by variations in this natural product's synthesis.

From a view of retrosynthetic analysis, the acid and/or base sensitivity of the C-ring of bryostatins (see ref. 10) leads us to conceive a strategy of constructing the C-ring of bryostatin 16 at the very end of the synthesis. The benefits also include flexible late variations for the access to other bryostatins or analogues, as well as minimization of functional group transformation and protecting group usage. While all previous total syntheses have relied on assembling the macrocycle by a demanding Julia olefination followed by a lactonization, we envisioned the use of a Pd-catalyzed chemoselective alkyne-alkyne coupling followed by a metal-catalyzed 6-*endo-dig* cyclization would efficiently construct both the macrocycle and the C-ring of **1** (Fig. 2). Esterification between fragments **4** and **5** would give the requisite diyne precursor. Fragment **5** could be synthesized from vinyl silane **6**. The 4-methylene-2,6-*cis*-tetrahydropyran moiety in intermediate **6** provides a perfect opportunity to examine our Ru-catalyzed alkene-alkyne coupling/Michael addition methodology²¹ between two complex fragments (**7** and **8**) aiming to address some chemoselectivity challenges. Given the difficulty to form the sterically hindered C16–17 olefin in the late stage (either by Julia olefination¹⁰ or ring-closing metathesis^{16,17}), alkyne **8** was specifically designed to install this *trans* alkene in an early stage.

Alkene **7** has been previously synthesized in 16 steps from (*R*)-pantolactone¹⁶. This synthesis can be shortened (Fig. 3) however by starting from aldehyde **2**. Asymmetric Brown allylation²², followed by PMB protection and oxidative olefin-cleavage quickly afforded aldehyde **10** as the same intermediate in our previous synthesis. With this modification, alkene **7** is now available in 11 steps from aldehyde **2**. Enantioselective synthesis of alkyne **8** was achieved in four steps from the same aldehyde **2** (see Fig. 3). Homologation, followed by indium-mediated propargylation²³ efficiently gave racemic **8** in good yield. (*R*)-**8** was then obtained in 90% yield and 90% ee via careful Dess–Martin oxidation²⁴ followed by CBS reduction²⁵ of the corresponding ketone.

With both alkene **7** and alkyne **8** in hand, the Ru-catalyzed tandem alkene-alkyne coupling/Michael addition proceeded beautifully to generate *cis*-tetrahydropyran **6**. The chemoselectivity was demonstrated by the high compatibility of a β,γ -unsaturated ketone, a six-membered lactone, an unprotected allylic alcohol, a PMB ether and two different silyl ethers in this reaction. DCM was found to be the optimal solvent for this reaction, while acetone or a DCM-DMF mixed solvent gave either lower conversion or more decomposition. Notably, only 1.2 equiv of alkene **7** is required in this coupling reaction (see Fig. 4A). Though the yield is moderate presumably due to the fact that additional olefin functionality in the alkyne fragment could lower the turnover number of the Ru catalyst, this result has been proved highly reproducible and both starting materials could be recovered, which guaranteed enough

materials for the rest of the synthesis. Subsequent bromination of the *exo*-cyclic vinyl silane followed by a CSA catalyzed transesterification–methyl ketalization–desilylation all in one event, cleanly gave the desired alcohol **13** containing both the A-ring and B-ring substructures in over 90% yield. The vinyl bromide functionality may serve as a convenient handle for the syntheses of bryostatin analogues via the use of metal-catalyzed coupling reactions. Pd-catalyzed carbonylation was next used to install the *exo*-cyclic conjugated methyl ester. Dess–Martin oxidation of the primary alcohol **14** followed by Ohira–Bestmann alkynylation²⁵ and desilylation provided donor alkyne **15** for the alkyne-alkyne coupling. The challenge of chemoselective hydrolysis of the β -hydroxy methyl ester in the presence of the α,β -unsaturated methyl ester was overcome by the use of trimethyltin hydroxide²⁶ in DCE; we hypothesized that due to the Lewis acidity of trimethyltin hydroxide, the adjacent alcohol could act as a directing group in the saponification reaction. Subsequent TES protection completed the synthesis of acid fragment **5**. Alcohol fragment **4** was synthesized in three steps from the known¹⁶ homo-propargyl alcohol **17** (Fig. 4B).

Esterification between acid **5** and alcohol **4** proceeded in 92% yield using Yamaguchi's conditions²⁸ (Fig. 5). Oxidative removal of the two PMB protecting groups using DDQ gave the macrocyclization precursor **3**. After extensive experimentation, treatment of **3** with 12 mol % Pd(OAc)₂ and 15 mol % TDMPP in toluene at room temperature successfully provided the desired macrocycle **20** with reasonably good yield (56%), while the use of THF or benzene as solvent, or lower ligand/Pd ratio proved less efficient. Like other macrocyclizations, low concentration [0.002M] proved to be critical, otherwise, formation of the dimeric byproducts could be observed. To the best of our knowledge, this is the first example of using Pd-catalyzed alkyne-alkyne coupling as a macrocyclization method in a complex natural product synthesis, which illustrates a new avenue of using C-C bond formation to construct a macrocycle. Mechanistically, the Pd catalyst chemoselectively inserts into the C-H bond of the terminal alkyne that, then, sets the stage for the chemo- and regioselective intramolecular carbametalation of the disubstituted alkyne, which, after reductive elimination of the formed vinylpalladium hydride, creates the macrocycle efficiently in spite of the complexity of the substrate. The remaining challenge was to conduct a 6-*endo-dig* cyclization to form the C-ring of bryostatin. Due to the modest selectivity in the Pd-catalyzed reaction (5-*exo* vs 6-*endo*) and difficulty in separation of these isomers¹⁶, a more regioselective catalyst was sought. After extensive screening of a number of metals, we settled upon a cationic gold complex [Au(PPh₃)]SbF₆ as the catalyst (for a cationic gold-catalyzed 5-*exo* cyclization, see ref. ²⁹). DCM-CH₃CN (10:1) as a mixed solvent in the presence of NaHCO₃ (10 equiv) as a buffer, gave the acid-sensitive 6-*endo* product in a satisfying 73% isolated yield. Subsequent pivalation of the hindered secondary alcohol under rather forcing conditions (Piv₂O 50 equiv, DMAP 80 equiv, 50 °C)³⁰ did afford the pivalate ester **21** in 62% yield. The following global deprotection proved to be nontrivial: HF-pyridine, aq. HF, aq. HOAc, PPTS, etc. gave either decomposition or isomerization. An unreported extreme acid-sensitivity of this natural product was eventually found. In contrast to these acid conditions, treatment of **21** with 5 equiv of TBAF and direct purification by reverse phase HPLC successfully provided bryostatin 16 (**1**), which was spectroscopically identical to the literature (Reported optical rotation [α]_D: + 84, c 0.43, MeOH; Found [α]_D: + 81, c 0.04, MeOH)¹⁸.

In conclusion, we have developed a unique and highly concise strategy (26 longest linear sequence, 39 total steps from aldehyde **2**) for the asymmetric total synthesis of bryostatin 16. The synthetic efficiency can be attributed to a tandem Ru-catalyzed alkene-alkyne coupling/Michael addition to form the B-ring, an acid-catalyzed one-pot cascade to form the A-ring, a directed chemoselective hydrolysis, a Pd-catalyzed alkyne-alkyne coupling as a novel macrocyclization reaction and a new gold-catalyzed 6-*endo-dig* cyclization to form the C-ring of bryostatin 16. We believe these atom-economical and chemoselective approaches could have indications beyond this work. The conciseness of this synthesis readily allows access to

significant quantities of this key bryostatin and implementation of this strategy towards the syntheses of various bryostatins and their analogues, as well as the related biological experiments, is being undertaken.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Structures of bryostatins 1–20

Figure 2. Retrosynthetic Analysis

Figure 3. Synthesis of alkene 7 and alkyne 8

Reaction conditions: *a*) (–)-(Ipc)₂B(allyl), Et₂O, –90 °C, 67%, 94% ee; *b*) PMB-Br, NaH, DMF, 90%; *c*) O₅O₄ (2 mol %), 2,6-lutidine, NaIO₄, dioxane/water (3:1), 87%; *d*) (*Z*)-1-bromo-2-ethoxyethene, *t*-BuLi, Me₂Zn, then **2**, Et₂O, –78 °C; then NaHSO₄, rt, 97%; *e*) (3-bromo-1-propynyl)-trimethylsilane, indium powder, InF₃ (10 mol %), THF, 65 °C, 68%; *f*) (i) Dess–Martin periodinane, NaHCO₃, DCM; (ii) (*S*)-2-methyl-CBS-oxazaborolidine (5 mol %), catechol-borane, DCM, –78 °C, 90%, 90% ee over two steps. Ipc, isopinocampheyl; PMB, *p*-methoxybenzyl; DMF, *N,N*-dimethylformamide. THF, tetrahydrofuran; DCM, dichloromethane. For tabulated spectral data of all depicted compounds, please see the Supplementary Information.

Figure 4. Synthesis of acid 5 and alcohol 4

Reaction conditions: *a*) $\text{CpRu}(\text{CH}_3\text{CN})_3\text{PF}_6$ (13 mol %), DCM, 34% (80% brsm); *b*) NBS, DMF, 98%; *c*) CSA (10 mol %), MeOH, 0 °C, 93–96%; *d*) $\text{PdCl}_2(\text{CH}_3\text{CN})_2$ (10 mol %), dppf (30 mol %), CO (1 atm), MeOH, Et_3N , DMF, 80 °C, 83% (90% brsm); *e*) Dess–Martin periodinane, NaHCO_3 , DCM, 88%; *f*) Ohira–Bestmann reagent, K_2CO_3 , MeOH, 97%; *g*) TBAF, HOAc, THF, 90% (96% brsm); *h*) $(\text{CH}_3)_3\text{SnOH}$, DCE, 80 °C, 84%; *i*) TESOTf, 2,6-lutidine, DCM, –10 °C to 0 °C, 76–79%. *j*) $\text{Cu}(\text{OTf})_2$ (3 mol %), PMBOC(NH) CCl_3 , toluene, –10 °C; *k*) PPTS, MeOH, 93% over two steps; *l*) TBSOTf, 2,6-lutidine, DCM, –78 °C, 71%. Cp, cyclopentadienyl; brsm, based on recovered starting material; NBS, *N*-bromosuccinimide; CSA, camphorsulfonic acid; dppf, 1,1'-bis(diphenylphosphino)ferrocene; TBAF, tetra-*n*-

butylammonium fluoride; DCE, 1,2-dichloroethane; TES, triethylsilyl; OTf, trifluoromethanesulfonate; PPTS, pyridinium *p*-toluenesulfonate; TBS, *t*-butyldimethylsilyl. For tabulated spectral data of all depicted compounds, please see the Supplementary Information.

Figure 5. Synthesis of bryostatin 16

Reaction conditions: *a*) 5, 2,4,6-trichlorobenzoyl chloride, Et₃N, toluene, then 4, DMAP, 92%; *b*) DDQ, pH 7.0 buffer, DCM, 46% 3 and 58% 19; *c*) Pd(OAc)₂ (12 mol %), TDMPP (15 mol %), toluene, 56%; *d*) AuCl(PPh₃) (20 mol %), AgSbF₆ (20 mol %), NaHCO₃, DCM/CH₃CN, 0 °C to rt, 73%; *e*) Piv₂O, DMAP, DCM, 50 °C, 62%; *f*) TBAF, THF, ca 52%. DMAP, *N,N*-4-dimethylaminopyridine; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; TDMPP, tris(2,6-dimethoxyphenyl)phosphine; Piv, pivaloyl. For tabulated spectral data of all depicted compounds, please see the Supplementary Information.