LETTERS

Total synthesis of bryostatin 16 using atom-economical and chemoselective approaches

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Of the concepts used to improve the efficiency of organic syntheses, two have been especially effective: atom economy[1] (the use of routes in which most of the atoms present in the reactants also end up in the product) and chemoselectivity[2] (the use of reactions that take place only at desired positions in a molecule). Synthesis of complex natural products is the most demanding arena in which to explore such principles. The bryostatin family of compounds are especially interesting targets, because they combine structural complexity with promising biological activity[3–7]. Furthermore, synthetic routes to some bryostatins have already been reported[9–12], providing a benchmark against which new syntheses can be measured. Here we report a concise total synthesis of bryostatin 16 (1), a parent structure from which almost all other bryostatins could in principle be accessed. Application of atom-economical and chemoselective reactions currently under development provides ready access to polyhydropyran motifs in the molecule, which are common structural features of many other natural products. The most notable transformations are two transition-metal-catalysed reactions. The first is a palladium-catalysed reaction of two different alkynes to form a large ring. The product of this step is then converted into a dihydropyran (the 'C ring' of bryostatins) in the second key reaction, which is catalysed by a gold compound. Analogues of bryostatin that do not exist in nature could be readily made by following this route, which might allow the biological activity of bryostatins to be fine-tuned.

The bryostatins 1–20 (Fig. 1), which were originally isolated from the marine bryozoan *Bugula neritina*, are a class of structurally complex macrolactone natural products that exhibit exceptional biological activity, most notable their anticancer activity *in vivo*[3]. Clinical application of bryostatin in combinatizon with other chemotherapeutic agents has shown significant potential in treating 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 its potential use in the treatment of Alzheimer's disease, depression and other cognitive impairments[6]. Although bryostatins' activities could be attributed to their strong affinity for protein kinase C isozymes[7], their actual mode of action is still an important research subject. However, their clinical advancement is hampered by the limited availability of bryostatins from isolation, due to low yield ($\sim 1.6 \times 10^{-4}$%; 18 g of bryostatin 1, one of the most abundant members, from 14 t of animals on an industrial scale[8]) and there being a 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 pose significant challenges to their synthesis; the structures include three heavily substituted tetrahydropyran rings, two acid/base-sensitive *exo*-cyclic unsaturated esters and one congested C16–C17 *trans*-alkene, as well as numerous oxygen-containing functionalities on a 26-membered lactone. As

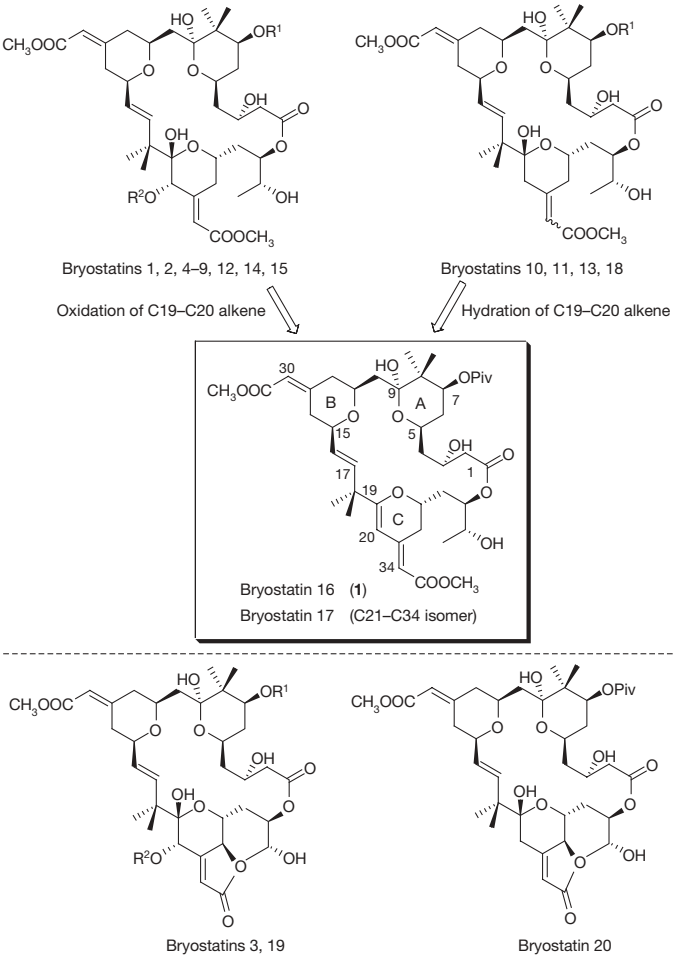

Figure 1 | Structures of bryostatins 1–20. Piv, pivaloyl.

an example, the challenge posed by the C16–C17 double bond led to failure in routes relying on its formation by metathesis reactions[17], even in the case of a relay metathesis strategy[16]. Despite their biological, clinical and structural significance, until now only three of the 20 bryostatins have been accessed by total synthesis (bryostatin 7 (ref. 9), bryostatin 2 (ref. 10) and bryostatin 3 (ref. 11)).

With a goal of streamlining the strategy to these complicated targets to enable better access, we choose bryostatin 16 (**1**)[18] as the specific synthetic target, for three reasons. First, bryostatin 16 could act as a pivotal parent structure allowing access to all other bryostatins (except bryostatins 3, 19 and 20; see Fig. 1) by elaboration of the electron-rich and relatively reactive C19–C20 alkene[10,16] (for a biosynthetic approach, see ref. 19). Second, the dihydropyran entity of

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the C ring in **1** offers an ideal forum for us to explore a palladium-catalysed alkyne–alkyne coupling as a macrocyclization method[20] for complex natural product synthesis. Third, new analogues, which are not easily available from other syntheses, might be readily obtained simply by variations in this natural product's synthesis.

From the viewpoint of retrosynthetic analysis, the acid and/or base sensitivity of the C ring of bryostatins[10] leads us to a strategy of constructing the C ring of bryostatin 16 at the very end of the synthesis. The benefits of this also include flexible late variations for access to other bryostatins or analogues, as well as minimization of functional group transformation and protecting group usage. Whereas all previous total syntheses have relied on assembling the macrocycle by performing a difficult Julia olefination followed by a lactonization, we predict that the use of a palladium-catalysed chemoselective alkyne–alkyne coupling followed by a metal-catalysed 6-*endo*-*dig* cyclization will efficiently produce both the macrocycle and the C ring of **1** (Fig. 2). Esterification between fragments **4** and **5** will give the requisite diyne precursor. Fragment **5** can be synthesized from vinyl silane **6**. The 4-methylene-2,6-*cis*-tetrahydropyran moiety in intermediate **6** provides us with an opportunity to examine our ruthenium-catalysed alkene–alkyne coupling/Michael addition methodology[21] between two complex fragments (**7** and **8**), with the aim of addressing some questions in chemoselectivity. Given the difficulty of forming the sterically hindered C16–C17 alkene in the late stage (either by Julia olefination[10] or ring-closing metathesis[16,17]), alkyne **8** is specifically designed to install this *trans*-alkene in an early stage.

Alkene **7** has previously been synthesized in 16 steps from (*R*)-pantolactone[16]. This synthesis can be shortened (Fig. 3), however, by starting from aldehyde **2**. Asymmetric Brown allylation[22], followed by protection of the resulting alcohol with *p*-methoxybenzyl (PMB) group and oxidative alkene-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** is achieved in four steps from aldehyde **2** (Fig. 3). Homologation, followed by indium-mediated propargylation[23] efficiently gives racemic **8** in good yield. (*R*)-**8** is then obtained in 90% yield and 90% enantiomeric excess through careful Dess–Martin oxidation[24] followed by Corey–Bakshi–Shibata reduction[25] of the corresponding ketone.

With both alkene **7** and alkyne **8** in hand, we proceed with the ruthenium-catalysed tandem alkene–alkyne coupling/Michael addition to generate *cis*-tetrahydropyran **6**. The chemoselectivity is 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. We find dichloromethane (DCM) to be the optimal solvent for this reaction, whereas acetone or a dichloromethane–*N*,*N*-dimethylformamide mixed solvent gives either lower conversion or more decomposition. Notably, only 1.2 equiv. of alkene **7** is required in this coupling reaction (Fig. 4a). Although the yield is moderate, presumably because additional alkene functionality in the alkyne fragment could lower the turnover number of the ruthenium catalyst, this result has been proved to be highly reproducible and both starting materials could be recovered, guaranteeing enough materials for the rest of the synthesis. Subsequent bromination of the *exo*-cyclic vinyl silane followed by a camphorsulfonic-acid-catalysed transesterification/methyl ketalization/desilylation all in one event cleanly gives 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 through the use of metal-catalysed coupling reactions. We next use palladium-catalysed carbonylation to install the *exo*-cyclic conjugated methyl ester. Dess–Martin oxidation of the primary alcohol **14** followed by Ohira–Bestmann alkynylation[26] and desilylation provides donor alkyne **15** for the alkyne–alkyne coupling. We overcome the challenge of chemoselective hydrolysis of the β-hydroxy methyl ester in the presence of the α,β-unsaturated methyl ester by using of trimethyltin hydroxide[27] in 1,2-dichloroethane; we hypothesize that, owing to the Lewis acidity of trimethyltin hydroxide, the adjacent alcohol could act as a directing group in the saponification reaction. Subsequent protection of the alcohol with triethylsilyl group completes the synthesis of acid fragment **5**. Alcohol fragment **4** is synthesized in three steps from the known[16] homo-propargyl alcohol **17** (Fig. 4b).

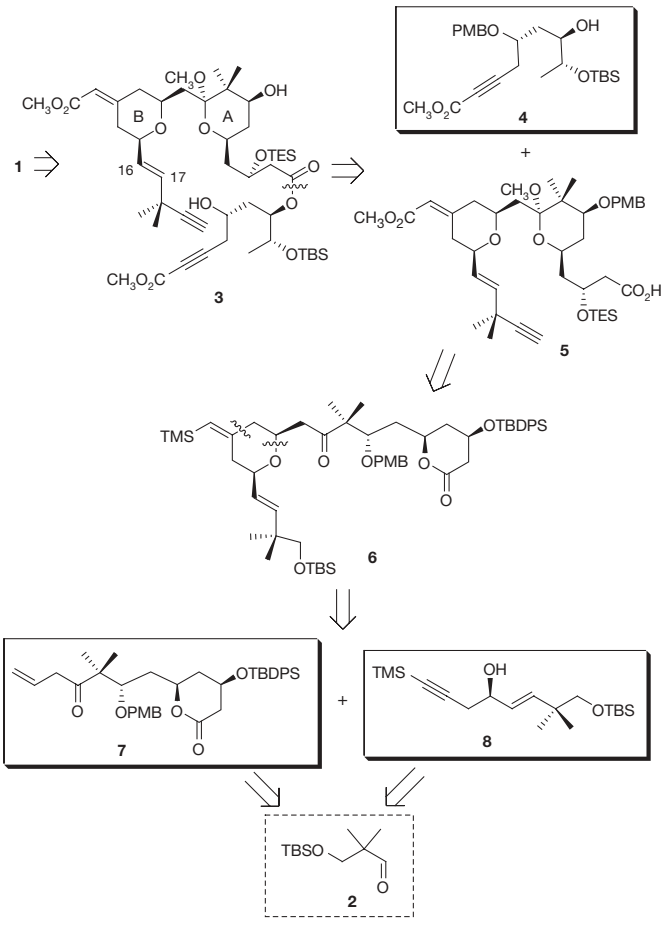

Figure 2 | Retrosynthetic analysis. TES, triethylsilyl; TBS, *t*-butyldimethylsilyl; PMB, *p*-methoxybenzyl; TMS, trimethylsilyl; TBDPS, *tert*-butyldiphenylsilyl.

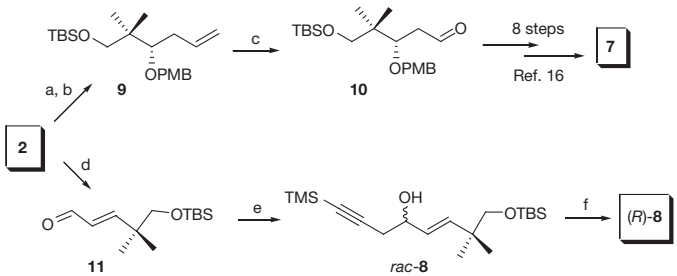

Figure 3 | Synthesis of alkene 7 and alkyne 8. Reaction conditions: (a) (–)-$(Ipc)_2B(allyl)$, Et_2O, –90 °C, 67%, 94% enantiomeric excess (e.e.); (b) PMB-Br, NaH, DMF, 90%; (c) OsO_4 (2 mol%), 2,6-lutidine, $NaIO_4$, dioxane/water (3:1), 87%; (d) (*Z*)-1-bromo-2-ethoxyethene, *t*-butyllithium, $(CH_3)_2Zn$, then **2**, $(C_2H_5)_2O$, –78 °C; then $NaHSO_4$, room temperature (20–25 °C), 97%; (e) (3-bromo-1-propynyl)-trimethylsilane, indium powder, InF_3 (10 mol%), THF, 65 °C, 68%; (f)(i) Dess–Martin periodinane, $NaHCO_3$, DCM; (f)(ii) (*S*)-2-methyl-CBS-oxazaborolidine (5 mol%), catecholborane, DCM, –78 °C, 90%, 90% e.e. over two steps. Ipc, isopinocamphenyl; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; DCM, dichloromethane; CBS, Corey–Bakshi–Shibata. For tabulated spectral data of all depicted compounds, please see the Supplementary Information.

Esterification between acid **5** and alcohol **4** proceeds in 92% yield using Yamaguchi's conditions[28] (Fig. 5). Oxidative removal of the two PMB protecting groups using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone gives the macrocyclization precursor **3**. After extensive experimentation, we find that treatment of **3** with 12 mol% $Pd(OAc)_2$ and 15 mol% tris(2,6-dimethoxyphenyl)phosphine in toluene at room temperature (20–25 °C) successfully provides the desired macrocycle **20** with reasonably good yield (56%), whereas the use of tetrahydrofuran or benzene as solvent, or a lower ligand/palladium ratio proves less efficient. As for other macrocyclizations, low concentration (0.002 M) proves to be critical; otherwise, formation of the dimeric by-products could be observed. This example of using palladium-catalysed alkyne–alkyne coupling as a macrocyclization method in a complex natural product synthesis illustrates a new way of constructing a macrocycle using carbon–carbon bond formation. Mechanistically, the palladium catalyst chemoselectively inserts into the carbon–hydrogen bond of the terminal alkyne; this sets the stage for the chemo- and regioselective intramolecular carbometalation of the disubstituted alkyne, which, after reductive elimination of the formed vinyl palladium hydride, creates the macrocycle efficiently in spite of the complexity of the substrate.

The remaining challenge is to conduct a 6-*endo*-*dig* cyclization to form the C ring of bryostatin. Owing to the modest selectivity in the palladium-catalysed reaction (5-*exo* versus 6-*endo*) and the difficulty in separating these isomers[16], we seek a more regioselective catalyst. After extensive screening of a number of metals, we settle upon a cationic gold complex, $[Au(PPh_3)]SbF_6$, as the catalyst (for a cationic gold-catalysed 5-*exo* cyclization, see ref. 29). DCM-CH_3CN (10:1), as a mixed solvent in the presence of $NaHCO_3$ (10 equiv.) as a buffer, gives the acid-sensitive 6-*endo* product in 73% isolated yield. Subsequent pivalation of the hindered secondary alcohol under forcing conditions (Piv_2O, 50 equiv.; *N*,*N*-4-dimethylaminopyridine, 80 equiv.; 50 °C)[30] does afford the pivalate ester **21** in 62% yield. The following global deprotection proves to be nontrivial: hydrogen fluoride-pyridine, aqueous hydrofluoric acid, aqueous acetic acid, pyridinium *p*-toluenesulfonate and so on give either decomposition or isomerization. We eventually find an extreme acid sensitivity of this natural product. By contrast with these acid conditions, treatment of **21** with 5 equiv. of tetra-*n*-butylammonium fluoride and direct purification by reverse-phase high-performance liquid chromatography successfully provides bryostatin 16 (**1**) that is spectroscopically identical to previously reported samples (reported optical

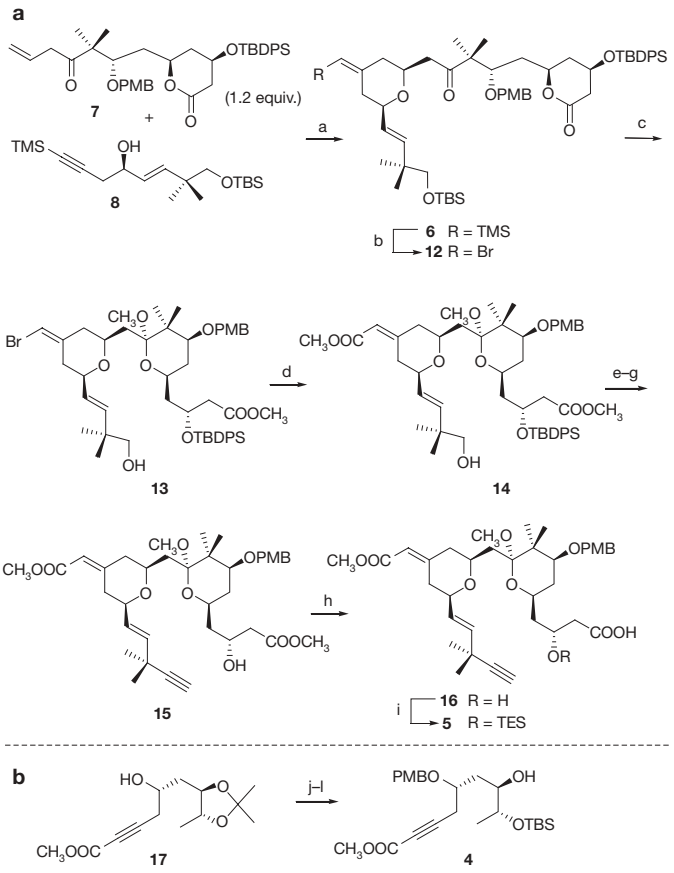

Figure 4 | Synthesis of acid 5 and alcohol 4. a, Synthesis of **5**. Reaction conditions: (a) $CpRu(CH_3CN)_3PF_6$ (13 mol%), DCM, 34% (80% b.r.s.m.); (b) NBS, DMF, 98%; (c) CSA (10 mol%), CH_3OH, 0 °C, 93–96%; (d) $PdCl_2(CH_3CN)_2$ (10 mol%), dppf (30 mol%), CO (1 atm), CH_3OH, $(C_2H_5)_3N$, DMF, 80 °C, 83% (90% b.r.s.m.); (e) Dess–Martin periodinane, $NaHCO_3$, DCM, 88%; (f) Ohira–Bestmann reagent, K_2CO_3, CH_3OH, 97%; (g) TBAF, HOAc, THF, 90% (96% b.r.s.m.); (h) $(CH_3)_3SnOH$, DCE, 80 °C, 84%; (i) TESOTf, 2,6-lutidine, DCM, −10 °C to 0 °C, 76–79%. **b**, Synthesis of **4**. Reaction conditions: (j) $Cu(OTf)_2$ (3 mol%), $PMBOC(NH)CCl_3$, toluene, –10 °C; (k) PPTS, CH_3OH, 93% over two steps; (l) TBSOTf, 2,6-lutidine, DCM, –78 °C, 71%. Cp, cyclopentadienyl; b.r.s.m., based on recovered starting material; NBS, *N*-bromosuccinimide; CSA, camphorsulfonic acid; dppf, 1,1′-bis(diphenylphosphino)ferrocene; TBAF, tetra-*n*-butylammonium fluoride; HOAc, acetic acid; DCE, 1,2-dichloroethane; OTf, trifluoromethanesulfonate; PPTS, pyridinium *p*-toluenesulfonate. For tabulated spectral data of all depicted compounds, please see the Supplementary Information.

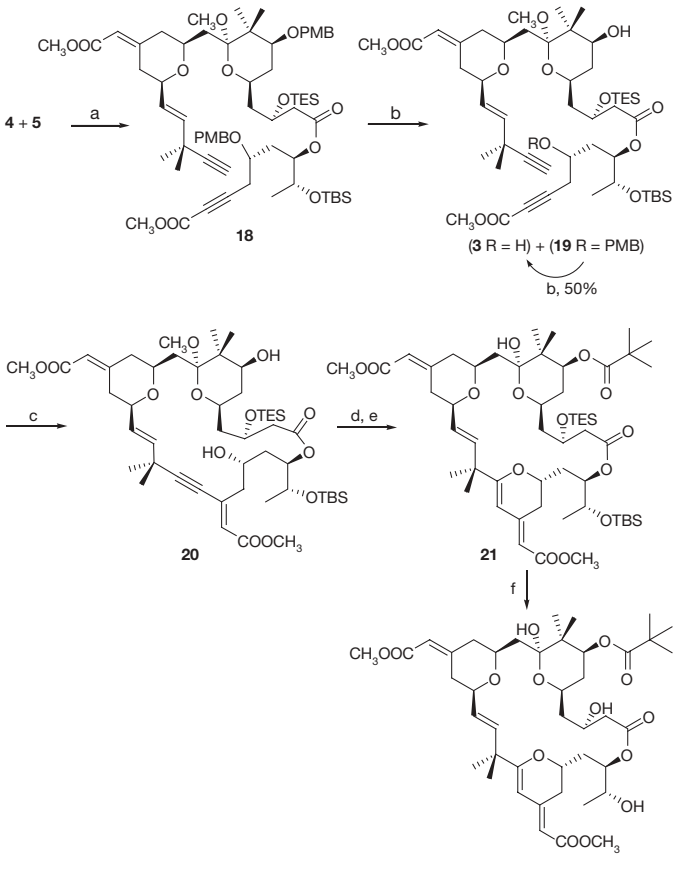

Figure 5 | Synthesis of bryostatin 16. Reaction conditions: (a) **5**, 2,4,6-trichlorobenzoyl chloride, $(C_2H_5)_3N$, toluene, then **4**, DMAP, 92%; (b) DDQ, pH 7.0 buffer, DCM, 46% **3** and 58% **19**; (c) $Pd(OAc)_2$ (12 mol%), TDMPP (15 mol%), toluene, 56%; (d) $AuCl(PPh_3)$ (20 mol%), $AgSbF_6$ (20 mol%), $NaHCO_3$, DCM/CH_3CN, 0 °C to room temperature, 73%; (e) Piv_2O, DMAP, DCM, 50 °C, 62%; (f) TBAF, THF, ~52%. DMAP, *N*,*N*-4-dimethylaminopyridine; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; TDMPP, tris(2,6-dimethoxyphenyl)phosphine. For tabulated spectral data of all depicted compounds, please see the Supplementary Information.

rotation: $[\alpha]_D^{20}$, $+84^\circ\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.43\,g\,cm^{-3}$, in CH_3OH); found: $[\alpha]_D^{20}$, $+81^\circ\,cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.04\,g\,cm^{-3}$, in CH_3OH)[18].

In conclusion, we have developed a highly concise strategy (26-step 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 ruthenium-catalysed alkene–alkyne coupling/Michael addition to form the B ring, an acid-catalysed one-pot cascade to form the A ring, a directed chemoselective hydrolysis, a palladium-catalysed alkyne–alkyne coupling as a macrocyclization reaction and a gold-catalysed 6-*endo*-*dig* cyclization to form the C ring of bryostatin 16. We believe that all these atom-economical and chemoselective approaches could have implications 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 synthesis of various bryostatins and their analogues, and performance of the related biological experiments, is being undertaken.

Received 5 September; accepted 10 October 2008.

1. Trost, B. M. The atom economy–a search for synthetic efficiency. *Science* **254,** 1471–1477 (1991).
2. Trost, B. M. Selectivity: a key to synthetic efficiency. *Science* **219,** 245–250 (1983).
3. Hale, K. J., Hummersone, M. G., Manaviazar, S. & Frigerio, M. The chemistry and biology of the bryostatin antitumour macrolides. *Nat. Prod. Rep.* **19,** 413–453 (2002).
4. Newman, D. J. & Cragg, G. M. Marine natural products and related compounds in clinical and advanced preclinical trials. *J. Nat. Prod.* **67,** 1216–1238 (2004).
5. Dowlati, A. *et al.* Phase I and correlative study of combination bryostatin 1 and vincristine in relapsed B-cell malignancies. *Clin. Cancer Res.* **9,** 5929–5935 (2003).
6. Hongpaisan, J. & Alkon, D. L. A structural basis for enhancement of long-term associative memory in single dendritic spines regulated by PKC. *Proc. Natl Acad. Sci. USA* **104,** 19571–19576 (2007).
7. Dell'Aquilla, M. L., Harold, C. L., Kamano, Y., Pettit, G. R. & Blumberg, P. M. Differential effects of bryostatins and phorbol esters on arachidonic acid metabolite release and epidermal growth factor binding in C3H 10T1/2 cells. *Cancer Res.* **48,** 3702–3708 (1988).
8. Schaufelberger, D. E. *et al.* The large-scale isolation of bryostatin 1 from Bugula neritina following current good manufacturing practices. *J. Nat. Prod.* **54,** 1265–1270 (1991).
9. Kageyama, M. *et al.* Synthesis of bryostatin 7. *J. Am. Chem. Soc.* **112,** 7407–7408 (1990).
10. Evans, D. A. *et al.* Total synthesis of bryostatin 2. *J. Am. Chem. Soc.* **121,** 7540–7552 (1999).
11. Ohmori, K. *et al.* Total synthesis of bryostatin 3. *Angew. Chem. Int. Ed.* **39,** 2290–2294 (2000).
12. Manaviazar, S. *et al.* Enantioselective formal total synthesis of the antitumor macrolide bryostatin 7. *Org. Lett.* **8,** 4477–4480 (2006).
13. Wender, P. A. & Verma, V. A. The design, synthesis and evaluation of C7 diversified bryostatin analogs reveals a hot spot for PKC affinity. *Org. Lett.* **10,** 3331–3334 (2008).
14. Wender, P. A., DeChristopher, B. A. & Schrier, A. J. Efficient synthetic access to a new family of highly potent bryostatin analogues via a Prins-driven macrocyclization strategy. *J. Am. Chem. Soc.* **130,** 6658–6659 (2008).
15. Keck, G. E. *et al.* Convergent assembly of highly potent analogues of bryostatin 1 via pyran annulation: bryostatin look-alikes that mimic phorbol ester function. *J. Am. Chem. Soc.* **130,** 6660–6661 (2008).
16. Trost, B. M., Yang, H., Thiel, O. R., Frontier, A. J. & Brindle, C. S. Synthesis of a ring-expanded bryostatin analogue. *J. Am. Chem. Soc.* **129,** 2206–2207 (2007).
17. Ball, M. *et al.* A preliminary evaluation of a metathesis approach to bryostatins. *Tetrahedr. Lett.* **47,** 2223–2227 (2006).
18. Pettit, G. R. *et al.* Antineoplastic agents. 340. Isolation and structural elucidation of bryostatins 16–18. *J. Nat. Prod.* **59,** 286–289 (1996).
19. Sudek, S. *et al.* Identification of the putative bryostatin polyketide synthase gene cluster from ''Candidatus Endobugula sertula'', the uncultivated microbial symbiont of the marine ryozoan Bugula neritina. *J. Nat. Prod.* **70,** 67–74 (2007).
20. Trost, B. M., Matsubara, S. & Caringi, J. J. Cycloisomerization of α,ω-diynes to macrocycles. *J. Am. Chem. Soc.* **111,** 8745–8746 (1989).
21. Trost, B. M., Yang, H. & Wuitschik, G. A. Ru-catalyzed tandem alkyne-enone coupling/Michael addition: synthesis of 4-methylene-2,6-*cis*-tetrahydropyrans. *Org. Lett.* **7,** 4761–4764 (2005).
22. Brown, H. C. & Jadhav, P. K. Asymmetric carbon-carbon bond formation via β-allyldiisopinocampheylborane. Simple synthesis of secondary homoallylic alcohols with excellent enantiomeric purities. *J. Am. Chem. Soc.* **105,** 2092–2093 (1983).
23. Lin, M. & Loh, T. Indium-mediated reaction of trialkylsilyl propargyl bromide with aldehydes: highly regioselective synthesis of allenic and homopropargylic alcohols. *J. Am. Chem. Soc.* **125,** 13042–13043 (2003).
24. Dess, D. B. & Martin, J. C. A useful 12-I-5 triacetoxyperiodinane (the Dess-Martin periodinane) for the selective oxidation of primary or secondary alcohols and a variety of related 12-I-5 species. *J. Am. Chem. Soc.* **113,** 7277–7287 (1991).
25. Corey, E. J. & Helal, J. C. Reduction of carbonyl compounds with chiral oxazaborolidine catalysts: A new paradigm for enantioselective catalysis and a powerful new synthetic method. *Angew. Chem. Int. Ed.* **37,** 1986–2012 (1998).
26. Roth, G. J., Liepold, B., Müller, S. G. & Bestmann, H. J. Further improvements of the synthesis of alkynes from aldehydes. *Synthesis* 59–62 (2004).
27. Nicolaou, K. C., Estrada, A. A., Zak, M., Lee, S. H. & Safina, B. S. A mild and selective method for the hydrolysis of esters with trimethyltin hydroxide. *Angew. Chem. Int. Ed.* **44,** 1378–1382 (2005).
28. Inanaga, J., Hirata, K., Saeki, H., Katsuki, T. & Yamaguchi, M. A rapid esterification by mixed anhydride and its application to large-ring lactonization. *Bull. Chem. Soc. Jpn* **52,** 1989–1993 (1979).
29. Liu, Y., Song, F., Song, Z., Liu, M. & Yan, B. Gold-catalyzed cyclization of (Z)-2-en-4-yn-1-ols: highly efficient synthesis of fully substituted dihydrofurans and furans. *Org. Lett.* **7,** 5409–5412 (2005).
30. Pettit, G. R., Sengupta, D., Herald, C. L., Sharkey, N. A. & Blumberg, P. M. Antineoplastic agents. 192. Synthetic conversion of bryostatin 2 to bryostatin 1 and related bryopyrans. *Can. J. Chem.* **69,** 856–860 (1991).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We acknowledge the National Institutes of Health (GM 13598) for their support of our programs. G.D. is a Stanford Graduate Fellow. We thank H. Yang for discussions and for help characterizing compound **21**, and we additionally thank him and C. S. Brindle for providing synthetic intermediates. Palladium and ruthenium salts were supplied by Johnson Matthey. We acknowledge S. R. Lynch for his help with two-dimensional nuclear magnetic resonance analysis. We also thank the Wender group's assistance with reverse-phase high-performance liquid chromatography.

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