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Efficient Synthetic Access to a New Family of Highly Potent Bryostatin Analogues via a Prins-Driven Macrocyclization Strategy

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Bryostatin 1, the prototypical member of a class of structurally complex macrolides isolated from the marine bryozoan *Bugula neritina*, exhibits remarkable biological activity, including potent antineoplastic activity, restoration of apoptotic function, immune system stimulation, and reversal of multidrug resistance.[1] Recent clinical trials using bryostatin in combination with other agents have shown significant promise for the treatment of certain cancers.[2] The exceptional potency of bryostatin is such that only *~1.5 mg* is needed for a full eight-week clinical treatment cycle.

Recently and quite significantly, this natural product has also been shown to enhance cognition and memory in animals,[3] suggesting its potential use in the treatment of neurological disorders, including Alzheimer's disease, depression, and other cognitive impairments.[4] Bryostatin's activities are thought to arise in part from its modulation of protein kinase C (PKC), a family of C1 domain containing kinases implicated in a variety of biological processes and disease states.[5] Other C1 domain proteins have also been suggested as possible targets.[6]

There are three overarching and interconnected problems that have impeded clinical advancement of the bryostatin lead. It is naturally scarce and difficult to isolate. Second, synthetic routes are not as yet amenable to the step-economical production of the natural product.[7,8] Finally, it is difficult to modify as needed to access analogues for both mechanistic and clinical studies. As a result of these combined problems, the vast majority of preclinical and clinical work has been done only on bryostatin 1; very few analogues have been prepared and even fewer have been advanced preclinically. Facile and scalable access to analogues is critically needed as bryostatin is not optimized for clinical use.

Given that the activity of a molecule can often be attributed to a subset of its functionalities, functionally superior yet structurally simpler molecules amenable to practical synthesis can be designed to address the above-noted bryostatin problems.[1a] The success of this function-oriented synthesis strategy has been demonstrated by simplified analogue **4**,[9] which is more active than bryostatin but is prepared in 40 fewer steps.

Analogue **4** has now been moved into in vitro and in vivo preclinical evaluation. Because of the enormity of this effort, it has become increasingly important to define whether the hydropyranyl B-ring of the natural product offers any advantage in efficacy or off-target selectivity over the dioxane B-ring common thus far to all potent analogues. To address this need, we have targeted analogues **1**−**3**, which incorporate the complete oxycarbocyclic core ring system of the bryostatins. We report herein a highly efficient, step-economical route to this new analogue class based on a novel Prins-driven macrocyclization strategy.

Our approach to dioxane **4** involves an acetalization-driven macrocyclization procedure that proceeds putatively through the formation of an oxocarbenium ion (Figure 1a) and its capture by a suitably situated oxygen nucleophile. We reasoned that the targeted hydropyranyl systems might be formed by positioning a suitable carbon nucleophile in place of the nucleophilic oxygen (Figure 1b), setting the stage for a Prins-driven macrocyclization.[10] The Prins reaction has found much use in the construction of hydropyranyl ring systems, but its adaptation to a process that concomitantly closes a macrocycle is rare.[11] This strategy gains added significance in the case of bryostatin as all total syntheses have relied on an often demanding Julia reaction[12] in combination with a lactonization to produce the macrocycle. Attempts to replace the Julia reaction with milder processes have thus far encountered difficulties.[13]

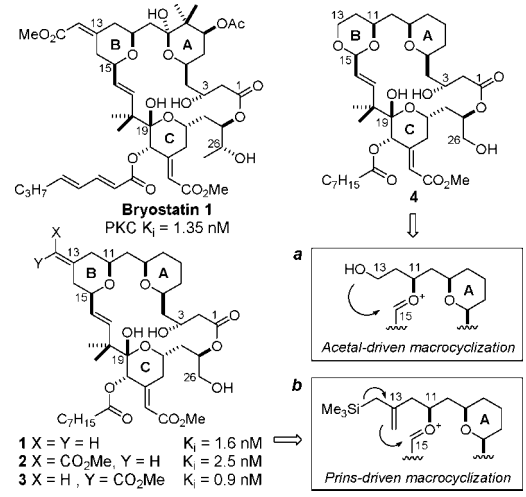

Figure 1. Bryostatin 1, new analogues **1**−**3**, representative B-ring dioxane analogue **4**, and strategies for their synthesis.

The synthesis of analogues **1**−**3** was planned to proceed from a common intermediate **13**, the product of a Prins-driven macrocyclization of intermediate **12**. The latter would, in turn, be convergently assembled from recognition domain **10**[9] and acid **9**. The synthesis of **9** started with the known intermediate **5** (available in seven steps),[14] which was protected as its triethylsilyl ether (Scheme 1) and was converted via a Bunnelle reaction[15] in 69% yield to the corresponding allylsilane **7**. Intermediate **7** was debenzylated using lithium naphthalenide, affording alcohol **8** in 93% yield. Oxidation of **8** cleanly provided the acid **9**, which was subsequently esterified with recognition domain **10** using Yamaguchi's conditions.[16] The triethylsilyl ether was then removed with pyridinium *para*-toluenesulfonate to reveal macrocyclization precursor **12**.

Gratifyingly, upon exposure to TMS-OTf,[17] intermediate **12** was cleanly converted, with complete diastereoselectivity and excellent yield (93%), into bryopyran **13**. Notably, this constitutes a rare and exceptionally high yielding example of a Prins-driven macrocyclization and one of the most complex examples of *any* Prins cyclization. Bryostatin analogue **1** was revealed in 77% yield upon exposure of **13** to HF•pyridine.

The conversion of bryopyran **13** into enoates **2** and **3** was next explored (Scheme 2). The C13 exocyclic olefin in **13** was chemoselectively cleaved with ozone (1 equiv) to afford ketone **14** in 88% yield. Treatment of **14** with the sodium anion of trimethyl phosphonoacetate gave an *E*:*Z* mixture of the corresponding enoates in 87%

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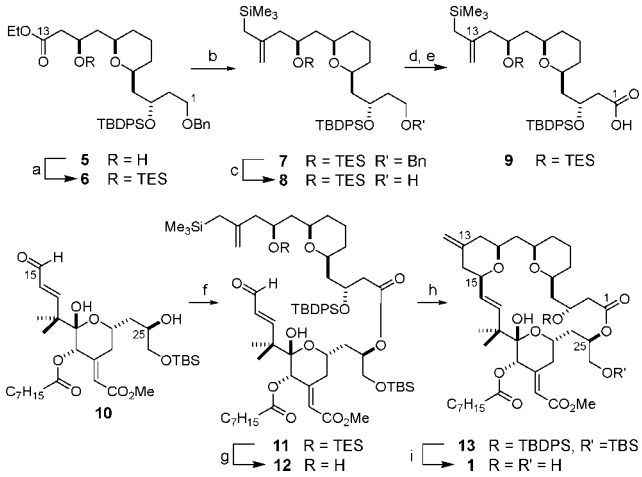
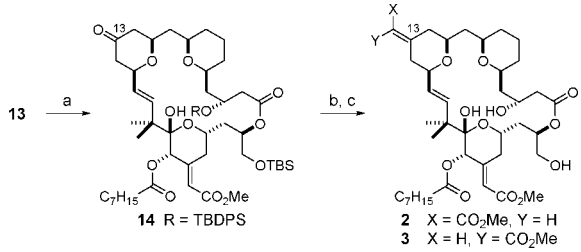
Scheme 1. Synthesis of Bryostatin Analogue **1**[a]



[a] Reagents and conditions: (a) TES-Cl, imidazole, CH_2Cl_2, rt, 97%; (b) (i) $CeCl_3$, $TMSCH_2MgCl$, THF, −78 °C → rt, 12 h, (ii) SiO_2, CH_2Cl_2, rt, 69%; (c) Li^0, naphthalene, THF, −25 °C, 93%; (d) TPAP (10 mol %), NMO (3 equiv), powdered 4Å MS, CH_2Cl_2, rt; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, 2:1 *t*-BuOH:H_2O, 0 °C, 89% from **8**; (f) **9**, 2,4,6-trichlorobenzoyl chloride, Et_3N, toluene, then **10**, DMAP, rt; (g) PPTS (30 mol %), 1:4 H_2O:THF, rt, 71% from **9**; (h) TMS-OTf, Et_2O, −78 → 0 °C, 93%; (i) HF•py, THF, rt, 77%.

Scheme 2. Synthesis of Bryostatin Analogues **2** and **3**[a]



[a] Reagents and conditions: (a) O_3, CH_2Cl_2, −78 °C, then thiourea, CH_2Cl_2: MeOH, rt, 88%; (b) trimethyl phosphonoacetate, NaHMDS, THF, 0 °C, 87% combined yield, 1:1.05 *E*:*Z*; (c) HF•py, THF, rt, 83% combined yield, 32% isolated **2**, 37% isolated **3**.

combined yield.[18] This mixture was desilylated using HF•pyridine to reveal the C13-enoate analogues **2** and **3** in 32 and 37% yield, respectively.

In accord with our pharmacophoric hypothesis,[19] analogues **1**, **2**, and **3** were found to be potent ligands for PKC, with K_i values of 1.6, 2.5, and 0.9 nM, respectively. Given these potent K_i values, we were motivated to test the antileukemic activity of analogues **1**−**3** in vitro. Significantly, these compounds exhibit nanomolar and *subnanomolar* EC_{50} values for growth inhibition of K562 human erythroleukemia cells and MV411 B-myelomonocytic leukemia cells (Table 1).

In summary, the first members of a new class of potent bryostatin analogues containing the complete bryostatin oxycarbocyclic ring system have been synthesized using a novel Prins-driven macrocyclization. Given the remarkable functional group tolerance and efficiency of this process, it is an attractive strategy for the convergent synthesis of tetrahydropyran containing targets. Significantly, initial in vitro assays indicate that these analogues are our most active compounds made to date, exceeding the antiproliferative activity of our most potent dioxane analogue **4** by up to 2 orders of magnitude. Further studies on these promising leads will be reported in due course.

Table 1. Binding and Growth Inhibitory Data

ID	PKC K_i (nM)	K562 (nM)[d]	MV411 (nM)[d]
1	1.63 ± 0.08[a]	2.3 ± 0.6	1.4 ± 0.7
2	2.5 ± 0.1[b]	4 ± 1	0.17 ± 0.05
3	0.9 ± 0.2[a]	0.47 ± 0.09	0.4 ± 0.2
4	3.1 ± 0.3[a,c]	15 ± 2	1.4 ± 0.3

[a] Average of two experiments. [b] Average of four experiments. [c] See Supporting Information for discussion. [d] EC_{50} values are an average of three experiments. All error bars indicate standard error of the mean.

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

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