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Total Synthesis and Initial Biological Evaluation of New B-Ring-Modified Bryostatin Analogs

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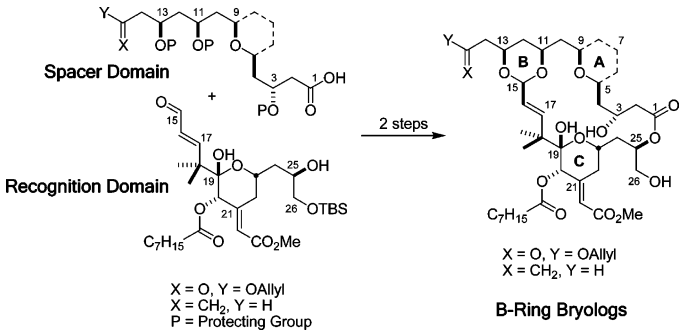
ABSTRACT



The total synthesis and preliminary biological evaluation of the first bryostatin analogs (bryologs) to incorporate B-ring substitution are reported. Asymmetric syntheses of two new polyketide "spacer" domains are described, one exploiting the pseudosymmetry of the C1–C13 region. These fragments are convergently joined to the "recognition" domain through a remarkably versatile macrotransacetalization process. The resulting new analogs exhibit potent nanomolar or picomolar affinity to protein kinase C (PKC), comparable to or better than that found for bryostatin.

Bryostatin 1 (Figure 1) is a complex, marine-derived macrolactone[1] that is currently in phase I and II clinical trials for the treatment of cancer.[2] This compound exhibits a unique range of biological activities including induction of apoptosis, reversal of multidrug resistance, and immune system modulation.[3] Of special therapeutic importance, bryostatin 1 has been shown to enhance the overall efficacy of other oncolytic agents, suggesting its potential use in combination therapy.[4] A study reported earlier this year, for example, showed that bryostatin in combination with paclitaxel produced a superior response rate in patients with untreated, advanced gastric or gastroesophageal junction adenocarcinoma compared to paclitaxel alone.[5] Bryostatin also exhibits activity that could have therapeutic implications for Alzheimer's disease, including an ability to improve memory and learning in animal models.[6]

Unfortunately, bryostatin is not readily obtained from its source organism.[7] This scarcity has hampered clinical studies,

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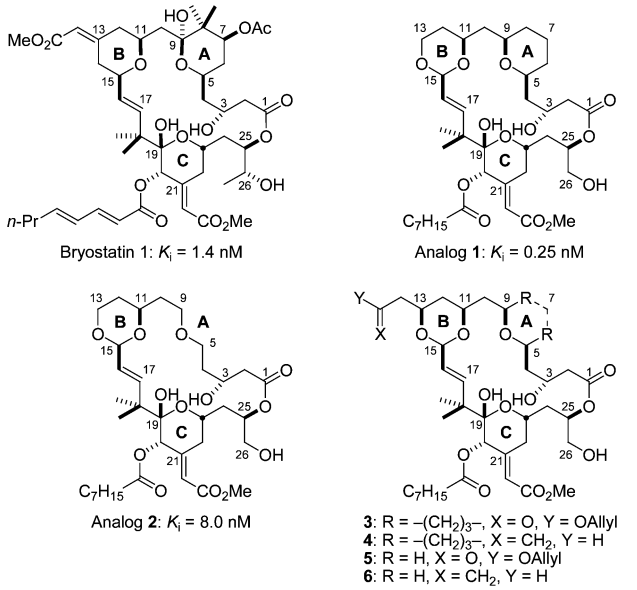

Figure 1. Bryostatin 1 and synthetic analogs.

research on its mode of action, and access to clinically superior structural or functional analogs. While three impressive total syntheses of the bryostatins have been completed, each is over 70 steps and thus not able as yet to provide sufficient material to impact the clinical supply.[8] More importantly, bryostatin 1 is produced in nature for uses other than human therapy and is therefore not an optimized therapeutic agent. Prompted by these considerations and the unique activity profile of bryostatin, our group initiated a program directed at the design and synthesis of simplified bryostatin analogs that can be produced in a practical, step-economical fashion and tuned for optimal performance in the clinic.[9] This function-oriented design and synthesis approach[10] has produced several promising leads of which analogs **1** and **2** (Figure 1) are representative. Analog **1** exhibits in vitro and in vivo biological activities comparable to or better than bryostatin 1 in various assays.[11] Analog **2**, which lacks the A-ring found in bryostatin 1, represents the simplest analog reported to date that maintains high binding affinity.[10] The availability of these analogs in quantity and the ability to tune them for performance has now enabled several mode of action and preclinical studies to move forward.

Bryostatin's activity is thought to arise from its binding to the regulatory domain of certain proteins, including kinases such as protein kinase C (PKC). Since this domain is found in only a small subset of the human kinome, targeting this domain could lead to selective kinase regulation.[12] In addition, unlike many ligands that serve as kinase inhibitors at the ATP binding site, binding to the C1 domain can result in inhibition or activation. This "gain of function" activity has many basic and therapeutic ramifications. A purpose of our ongoing studies in this area is to design agents that would bind to the C1 domain and offer, as needed, selective regulation of kinase isoforms. Bryostatin binds to two subclasses of PKC, the conventional and novel isoforms.[13] It has been hypothesized that the "recognition" domain is responsible for direct interaction with the binding pocket,[10] and as such, functionality on the A- and B-rings of bryostatin could be responsible for selectivity between these two classes of PKC. Previous work has shown that the B-ring could be replaced with a 5- or 6-membered dioxolane ring while maintaining high potency and affecting selectivity.[14] Recent efforts have been directed at a new family of analogs incorporating B-ring functionalities at C13 that could be diversified to probe for potency and selective binding to PKC isoforms. An ester was chosen in order to mimic the B-ring ester of bryostatin, and terminal olefins were chosen in order to diversify analogs through late-stage cross-metathesis. We describe herein the synthesis of four new spacer domains and their incorporation into the synthesis of the first bryostatin analogs (**3**−**6**) derivatized at C13.

The spacer domains of analogs **5** and **6** are pseudo-C_2-symmetric with respect to the axis bisecting the A-ring oxygen.[15] This pseudosymmetry was exploited to efficiently and step economically synthesize B-ring analogs lacking the A-ring (Scheme 1). Toward this end, the Blaise reaction[16] proceeded in high yield to join 2 equiv of acetate **7** to symmetric ether **8** to produce the symmetric bis-β-keto ester **9**. This diketone smoothly underwent a double Noyori asymmetric reduction,[17] selectively producing only one

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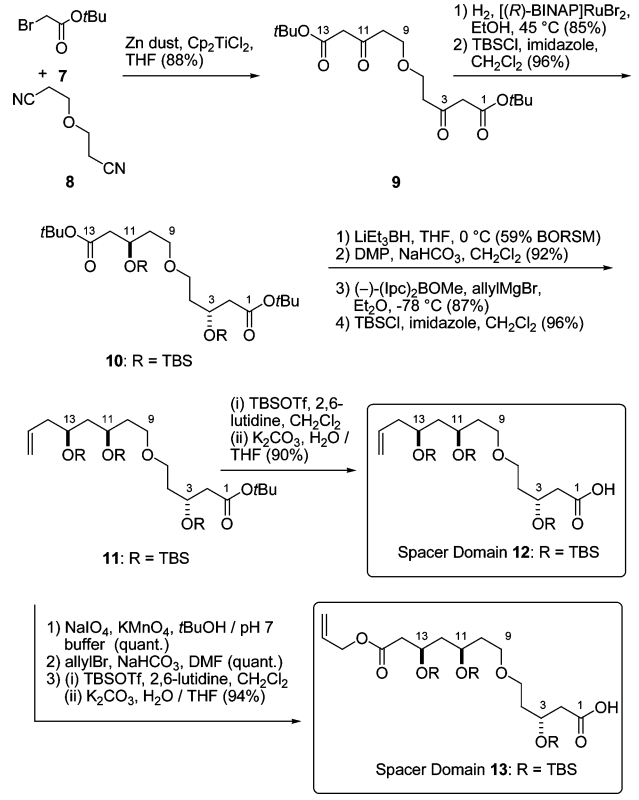
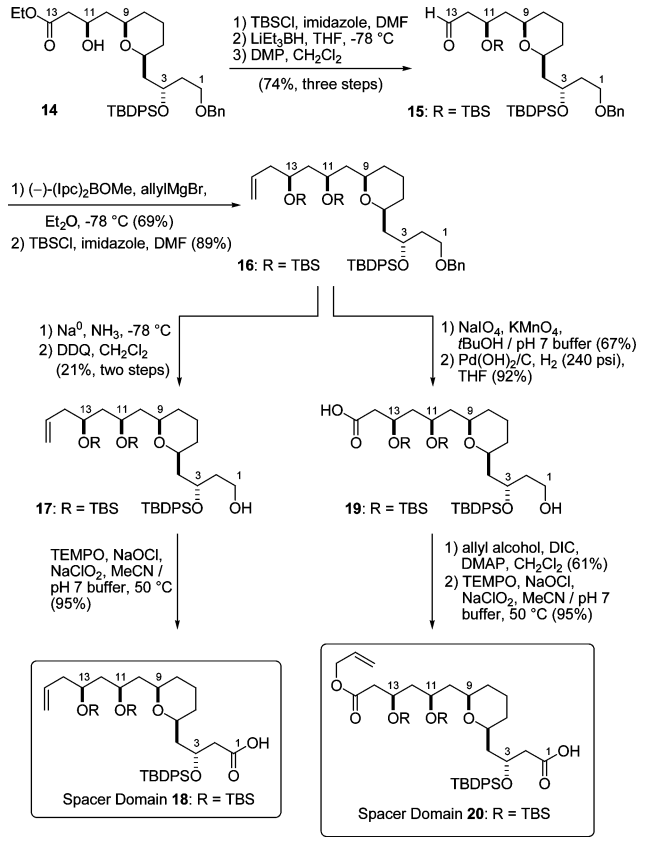
Scheme 1. Synthesis of Spacer Domains **12** and **13**



Scheme 2. Synthesis of Spacer Domains **18** and **20**



detectable isomer, which was subsequently silyl protected. Desymmetrization via monoreduction of one *tert*-butyl ester to the alcohol was followed by oxidation to the aldehyde. Brown's allylation[18] and subsequent protection furnished **11**. This intermediate was then taken on to spacer domain **12** by cleavage of the *tert*-butyl ester. This synthesis provided the completed spacer domain in 29% overall yield over 8 steps.

Separately, intermediate **11** was also converted to a second spacer domain through a three-step sequence. Oxidative cleavage of the terminal olefin and conversion to the allyl ester was followed by selective deprotection of the *tert*-butyl ester to give completed spacer domain **13** in 31% overall yield over 10 steps.

The synthesis of the spacer domains for analogs **3** and **4** began with silyl protection of known hydroxy ester **14**[19] (seven steps from commercially available methyl glutaryl chloride) followed by reduction and reoxidation to provide aldehyde **15** (Scheme 2). Asymmetric allylation was then used to set the C13 stereocenter giving a homoallylic alcohol that was silylated to provide **16**.[18] The configuration of the newly set secondary alcohol was confirmed by analysis of the corresponding C11/C13 acetonide using Rychnovsky's method.[20]

To avoid reduction of the newly installed allyl group, the C1 benzyl group of **16** was deprotected using dissolving metal conditions. Interestingly, these conditions also partially reduced the phenyl substituent of the C3 TBDPS group, which, however, was readily reoxidized with DDQ to provide **17**.[21] The low yield for this two-step procedure was due to extensive migration of the TBDPS group from C3 to the more stable C1 position during the reduction step. Oxidation of the newly revealed primary C1 alcohol to the carboxylic acid completed the synthesis of spacer domain **18**.[22]

Intermediate **16** was separately subjected to oxidative cleavage to reveal a carboxylic acid. Hydrogenolysis of the C1 benzyl ether provided **19**, which was then esterified with allyl alcohol. In the final step, the C1 alcohol was oxidized to the carboxylic acid to provide completed spacer domain **20**.

Each of the four spacer domains was coupled individually to the existing recognition domain **21**[11] using Yamaguchi's esterification procedure (Scheme 3).[23] The macrocycles were closed and the silyl protecting groups removed in a remarkably general one-step, mild, and diastereoselective macrotransacetalization, providing completed analogs **3**−**6**. The

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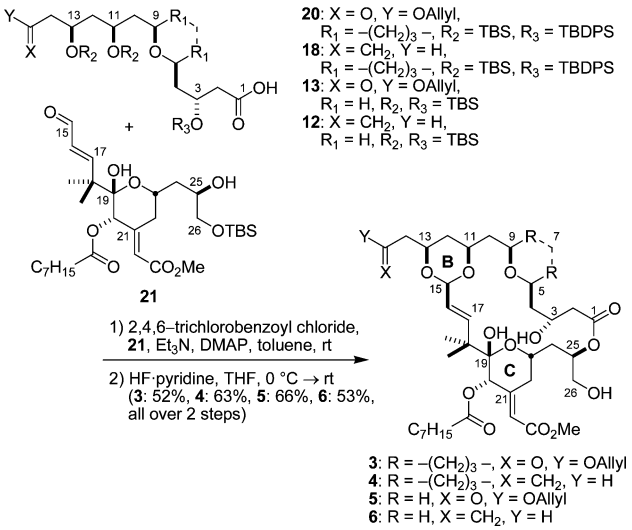
Scheme 3. Convergent Assembly of Analogs **3**−**6**



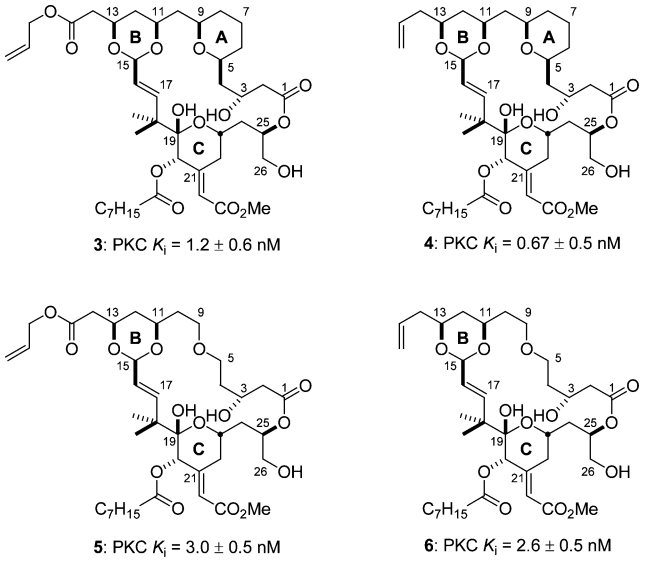


Figure 2. PKC binding affinities for B-ring bryologs.

newly formed C15 stereocenter in each analog was set under thermodynamic control affording only the cis-diequatorial dioxolane B-ring.

These new analogs exhibit potent nanomolar or picomolar binding affinities to PKC when tested in a competition binding assay against the known PKC ligand phorbol 12,13-dibutyrate (Figure 2). Significantly, analogs **5** and **6** exhibit binding potencies superior to analog **2**. As the first C13 modified bryologs, it is especially noteworthy that **3** and **4** exhibit potency on par with if not better than bryostatin.

A series of potent B-ring analogs of bryostatin have been synthesized from four new spacer domains through a convergent esterification/macrotransacetalization strategy. The new bryologs, the first bearing B-ring substitution, exhibit single-digit nanomolar or picomolar affinity to PKC, on par or better than bryostatin, indicating that the B-ring can be modified while maintaining or improving potency. Relative to the parent analog **2**, the side chain in analogs **5** and **6** improves potency. This adds support to the hypothesis that B-ring, and more broadly, spacer domain functionality can be exploited to achieve higher potency and possibly isozyme selectivity. These analogs can be efficiently generated in a scalable, step economical synthesis and provide the basis for probing differences in the isoform surfaces in contact with the B-ring region of bryostatin and the bryologs. Studies on diversification of these new leads through cross-metathesis and further biological evaluation are currently underway.

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Supporting Information Available: Experimental conditions and spectral data for compounds reported in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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