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Design, Synthesis, and Biological Evaluation of a Potent, PKC Selective, B-Ring Analog of Bryostatin

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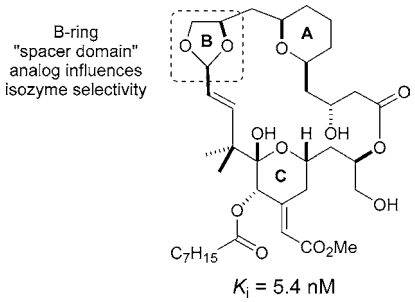
ABSTRACT



The first member of a new class of five-membered B-ring analogs of bryostatin has been synthesized and tested for its ability to bind and translocate protein kinase C (PKC). This synthesis extends the utility of our previously introduced macrotransacetalization strategy to the formation of five-membered dioxolane B-ring analogs. This analog exhibits potent, single-digit nanomolar affinity to PKC and selectively translocates novel PKC isozymes.

In 1968, extracts from *Bugula neritina*, a marine bryozoan, were found to have potent anticancer activity.[1] This activity was ultimately traced to the polyketide natural product bryostatin 1. In addition to its anticancer properties, bryostatin has been shown to synergize the effects of other antineoplastic agents,[2] promote apoptosis,[3] reverse multidrug resistance,[4] and stimulate the immune system.[5] More recently, it has been reported by Alkon and co-workers that bryostatin 1 improves memory and learning,[6] with potential therapeutic value for Alzheimer's disease.[7] Although its mode of action is under investigation, bryostatin is known to be a potent regulator of protein kinase C (PKC), binding to its C1 domain. Targeting the kinase C1 domain offers two advantages. Unlike the ATP binding site common to all kinases, the C1 domain is found in only a small number of kinases in the human kinome and thus offers a selectivity advantage.[8] Second, unlike agents that bind to the ATP binding site and therefore inhibit kinase function, C1 domain binders could

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inhibit or activate kinase activity.[9] The PKC family of isozymes is of additional importance because of its role in numerous therapeutic indications.[10]

A major issue associated with the advancement of bryostatin toward therapeutic goals has been its limited supply for clinical use and for mode of action studies. Low isolation yields as well as environmental concerns make it impractical to obtain clinically relevant amounts of material from its natural ecosystem.[11] Other sources have been identified, but an arduous separation and cost are still serious concerns.[12] Aquaculture has not proven cost effective thus far.[13] Production from the symbiont (*Candidatus endobugula sertula*) or through genetics is promising but would still be limited to bryostatin or its biosynthetic derivatives, agents neither produced nor optimized in nature for human cancer chemotherapy.[14] Total synthesis would provide greater flexibility in achieving an optimized clinical candidate and impressive progress has been made, but at over 70 total steps each, the current syntheses have not impacted supply or advanced investigations toward better candidates.[15]

To address the synthetic, biological, and medicinal challenges in this area, we set out to design simplified analogs of bryostatin that could be synthesized in a practical fashion and that could be tuned for superior clinical function.[16] Initial analog design, using a pharmacophoric model developed in our group, has led to the synthesis of simplified analogs **1** and **2**, which match and surpass, respectively, the potency of the natural product and are synthesized in a highly convergent and efficient fashion (Figure 1).[17] These analogs

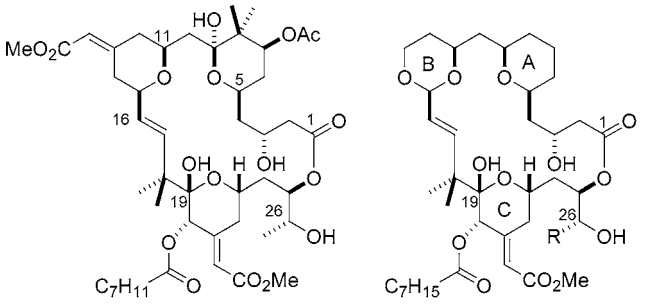

Figure 1. Bryostatin 1 and lead analogs.

can be accessed synthetically in under 30 steps, which makes them viable clinical candidates given the remarkable potency of bryostatin in human therapy (ca. 1.2 mg for a multiweek treatment) and the finding that the analogs are even more potent than bryostatin in cancer cell growth inhibition. A key next objective in this area is the identification of analogs with similarly high potencies but complementary target selectivities. Toward this end, this study describes the advancement of our convergent macrotransacetalization strategy to the synthesis of B-ring modifications of our designed leads and the initial disclosure of the role of this modification in PKC binding and translocation.

Analogs **1** and **2**, when docked to the proposed binding site on the PKCδ-C1B domain in our homology model,[18] have their C-rings deeply embedded in the binding cavity, whereas the A- and B-rings are positioned over and away from the enzyme, potentially interacting with other cellular components, anchoring proteins, or other portions of the enzyme upon binding and activation. As such, modifications to this region of the analog would be expected to retain a high degree of potency and could potentially be used to modulate the dynamics of the interaction with receptors as well as the ADME characteristics of the molecule. To test this possibility, the predictive value of our homology model, and the general utility of our synthetic approach, we set out to make the first B-ring-modified analogs of our lead compounds.

Recent work from our group has targeted variations to the A-ring[19] and the C20 ester.[20] Analogs of the B-ring have not yet been explored. It was envisioned that a five-membered B-ring analog could be generated rapidly and efficiently from 1,2-diols through a macrotransacetalization with the known recognition domain[17] used in the synthesis of **2**. Modeling performed with a conformer of the target analog **10** (1.2 kcal/mol above the global minimum) showed an exceptionally good overlay of the hypothesized pharmacophoric atoms (C1 carbonyl and C19 and C26 hydroxyls,

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RMS = 0.0516 Å) with lead analog **2**, suggesting that this analog would retain potent binding to PKC (Figure 2).

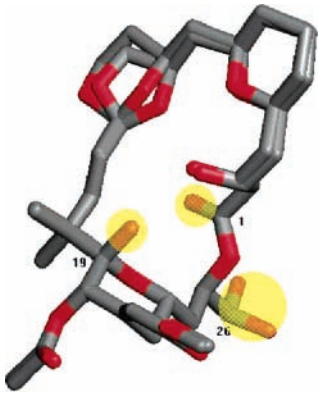


Figure 2. Overlay of proposed analog **10** with lead analog **2** with the pharmacophoric atoms highlighted (RMS = 0.0516 Å).

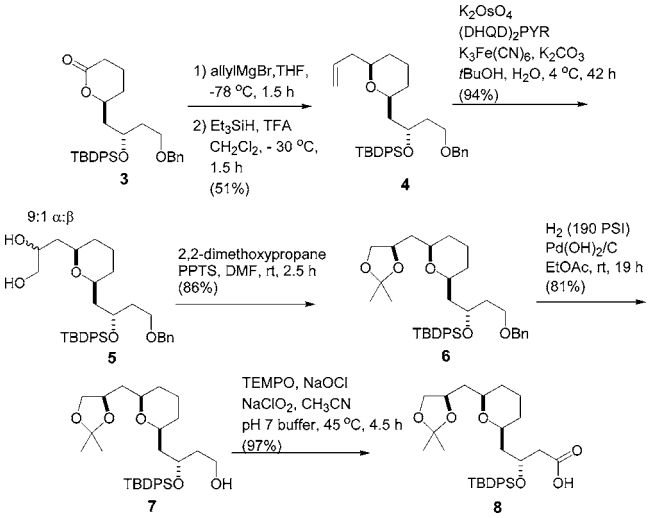
However, the B-ring of this analog and its two heteroatoms would be positioned in a different orientation, potentially influencing interactions with the rim regions of the C1 binding pocket as well as trafficking of the complex upon activation.

The bryologs are synthesized in a highly convergent fashion by coupling, in two steps, a top piece "spacer domain" with a bottom piece "recognition domain", using a macrotransacetalization strategy that we previously introduced for these systems. Such a strategy allows for rapid and efficient analog synthesis wherein the desired spacer domain is synthesized and coupled to give the completed final analog. In this case, the final coupling step would involve an unprecedented macrotransacetalization to a five-membered ring. Modeling of both C15 acetal epimers suggested that the desired product (C15 = β) would be thermodynamically favored.

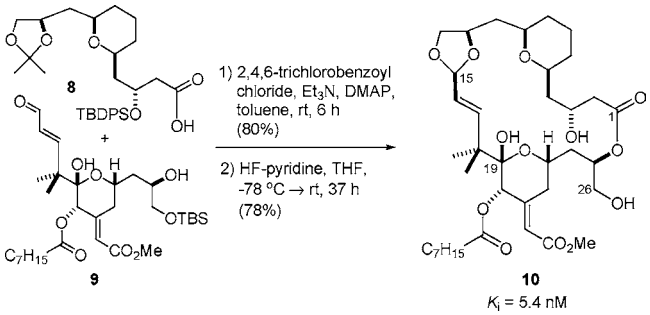
The synthesis of spacer domain **8** began with allyl Grignard addition to the previously generated lactone **3**[21] (four steps from commercial material), followed by selective reduction with triethylsilane to generate the cis stereoisomer **4** (Scheme 1). Sharpless asymmetric dihydroxylation yielded a 9:1 mixture of inseparable diol diastereomers **5**,[22] which were then protected as the acetonide. The selectivity of the dihydroxylation was particularly significant given the influence this stereocenter has on the C15 acetal position. After diol protection, the diastereomers were separated to provide the major isomer **6**. Deprotection of the benzyl ether and subsequent oxidation of the primary alcohol **7** afforded the completed spacer domain **8** in 10 overall steps with an average yield of 75% per step.

Coupling of **8** with recognition domain **9**[17] proceeded via a mild and efficient two-step process (Scheme 2). Yamaguchi

Scheme 1. Synthesis of Spacer Domain **8**



Scheme 2. Convergent, Macrotransacetalization Route to the B-Ring Analog



esterification, followed by a one-step tandem global deprotection and intramolecular transacetalization gave the completed analog **10** as a single diastereomer. In the case of the synthesis of analog **2**, containing the six-membered acetonide, the final deprotection and transacetalization step required 16 h of exposure to HF−pyridine.[21] The five-membered acetonide formed more slowly, requiring 37 h for complete conversion. This macrocyclization is the first in this series involving five-membered ring formation and thus extends the scope of this process to 1,2-diols, which can be readily derived from the dihydroxylation of alkenes. ROESY studies confirmed that the stereocenter at the newly formed acetal position was set under thermodynamic control. The potential C15 epimer of **10** was not observed.

A competitive inhibition binding assay[18] was performed with B-ring analog **10** on a mixture of rat brain PKC isozymes, leading to a binding constant of 5.4 nM. The high affinity of this new analog lends further support to the predictive value of our pharmacophore analysis and homology model. Moreover, it establishes the potential of 1,2-diols as analog precursors. Perhaps most significantly, it suggests that B-ring modifications could be used to tune ADME characteristics without compromising potency.

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In addition to potency, the functional activity of the new analog was addressed in a preliminary PKC translocation assay. PKC, in its inactive form, is located in the cytosol and upon activation translocates to cellular membranes. This translocation can be observed and measured in real time with confocal microscopy using the fusion protein PKC-GFP (green fluorescent protein) as a reporter.[23] Translocation assays[18] were performed on the novel PKC isozymes δ and ϵ and on the conventional isozyme PKCβI. PKCδ is a critical player in various apoptotic pathways and can influence the metastatic potential of cancer cells,[24] and PKCϵ has also been shown to be involved in cancer development.[25] PKCβI is also an essential participant in the apoptotic pathway.[26]

Results for the translocation of the novel PKCs mediated by **10** were similar to those obtained for the parent analog **2** (Figure 3).[27] Translocations of the novel isozymes PKCδ and ϵ were rapid and complete. However, translocation of the conventional isoform PKCβI was marginal, indicating overall a remarkably selective translocation of the novel class over the conventional class. Analog **10** showed a significantly reduced ability to translocate the conventional isozyme PKCβI relative to bryostatin 1.[28] This result is highly significant at both fundamental and applied levels as it indicates that changes to the spacer domain can be made to influence isozyme selectivity, while not affecting potency, as hypothesized from modeling studies. It is not known at present which targets are optimally required for bryostatin's beneficial effects and which contribute to its side effects. The ability to access highly potent analogs with different selectivities thus provides an opportunity to address this clinically significant issue.

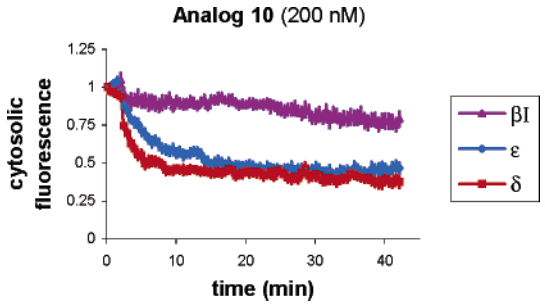

Figure 3. Translocation profile of analog **10** with PKCδ, PKCϵ, and PKCβI.

The efficient synthesis of the first member of a new class of B-ring analogs of bryostatin has been achieved. This study serves to extend our convergent macrotransacetalization strategy to five-membered ring containing targets. Significantly, this new analog retains the potency of the natural product while displaying unique selectivity in the translocation of PKC isozymes. These findings suggest that the B-ring of bryostatin analogs could be modified to accommodate ADME considerations and/or potentially to control isozyme translocation without affecting potency. This represents a new direction for the advancement of bryostatin analogs toward clinical trials.

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Supporting Information Available: Experimental procedures and characterization data for compounds **3**−**10** reported in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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