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Role of the A-Ring of Bryostatin Analogues in PKC Binding: Synthesis and Initial Biological Evaluation of New A-Ring-Modified Bryologs

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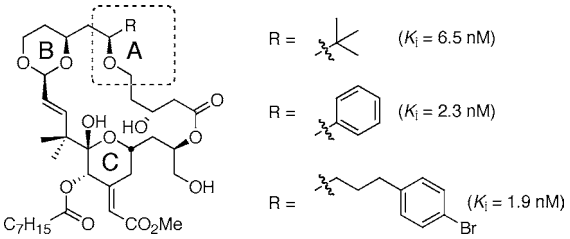
ABSTRACT



The syntheses of three newly designed bryostatin analogues are reported. These simplified analogues, which lack the A-ring present in the natural product but possess differing groups at C9, were obtained using a divergent approach from a common intermediate. All three analogues exhibit potent, single-digit nanomolar affinity to protein kinase C.

The bryostatins are a family of structurally complex, marine-derived macrocyclic polyketides[1] that exhibit a unique range of significant biological activities, including induction of apoptosis, reversal of multidrug resistance, immune system modulation, and efficacy against Alzheimer's disease.[2] 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.[3] Bryostatin 1 is currently in phase I and II clinical trials, as both a single agent and in combination with other therapeutics.[4]

As a natural product, bryostatin is neither produced nor selected by nature for human therapeutic performance. Consequently, its advancement in clinical trials has been slowed by both its scarcity and the associated difficulties in accessing derivatives of this complex system. Harvesting of bryostatin from marine sources is unlikely to provide a sustainable supply due to justifiable concerns about the delicate marine ecosystem. Aquaculture, genetic, and other biosynthetic approaches offer alternative sources but would still provide only bryostatin or its readily accessible derivatives.[5] Total synthesis provides more flexible access to analogues, but its use is limited by the length of existing syntheses (>70 steps).[1] On the positive side, and pertinent to these supply issues, is the exceptional potency of bryostatin: a full multiweek treatment only requires about 1.2 mg per patient.

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Prompted by these considerations and the unique activity profile of bryostatin, we set out to design simplified analogues of bryostatin that could be produced in a practical fashion through total synthesis and tuned for optimal performance in the clinic. Toward this end, we proposed a pharmacophore hypothesis for how bryostatin contacts its putative receptor, protein kinase C (PKC), and designed analogues that incorporate these features into a simplified scaffold.[6] Representative of this effort, analogue **1** (Figure 1), which is a more potent ligand for PKC than bryostatin,

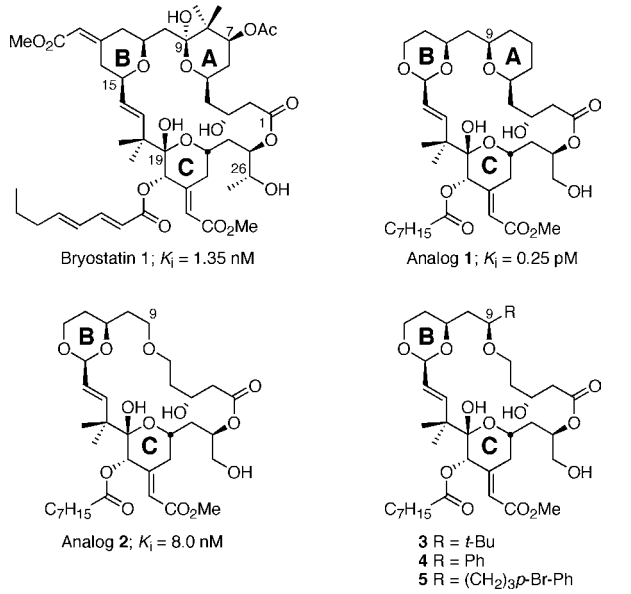


Figure 1. Bryostatin 1 and synthetic analogues.

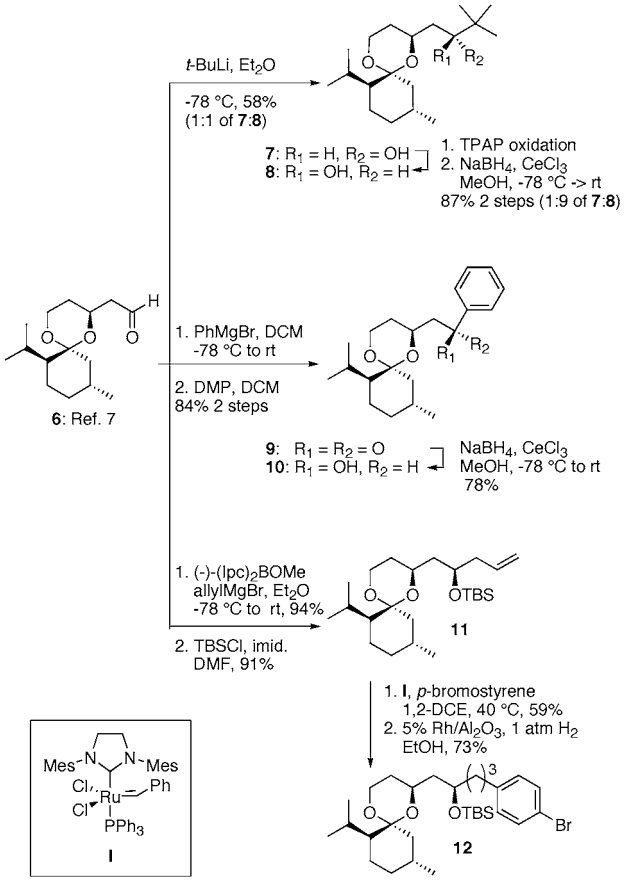
is more effective at inhibiting the growth of a variety of human cancer cell types than the natural product. Compound **1** can be synthesized in less than half the number of steps required to synthesize bryostatin and is available at a fraction of the cost required to obtain the natural product from marine sources.[7]

With the demonstration that simplified analogues retain PKC binding as well as human cancer cell growth inhibitory potency and can be prepared in a practical fashion, the next goal of this program was to refine these new leads[8] to enhance beneficial function, minimize side effects, and explore the mode of action.[9] Toward these goals, a new analogue **2** was recently disclosed wherein the A-ring of the bryostatin analogue skeleton had been removed.[6] Interestingly, this new analogue exhibited single-digit nanomolar affinity for the rat brain PKC isozyme mixture. Comparison of analogues **1** and **2** suggests that the introduction of a substituent at C9 could improve binding affinity by conferring a higher level of conformational rigidity to the macrocycle.[10] At the same time, this new substituent introduces structural elements that could control target selectivity, PK, and ADME characteristics for downstream preclinical evaluation. In this paper, the synthesis and initial biological assay of three A-ring-modified bryostatin analogues, **3**, **4**, and **5**, are reported.

The sterically demanding *tert*-butyl[10a] and phenyl substituents in **3** and **4** were chosen to increase the conformational rigidity in the former A-ring region of the molecule. At the same time, the *p*-bromo-phenylpropyl substituent in **5** was selected to examine the effect of a less sterically demanding substituent and to provide a convenient handle (the aryl bromide) for later diversification.

The new analogues were synthesized in a convergent manner by coupling a top "spacer domain" with a bottom "recognition domain" employing what has proven to be an exceptionally effective two-step macrolactonization sequence.[7] As a consequence of this late-stage convergence of fragments, the generation of new A-ring-modified analogues required only the synthesis of new spacer domains.

Scheme 1. Synthesis of Intermediates **8**, **10**, and **12**[a]



[a] DMP = Dess−Martin periodinane. TPAP = tetrapropylammonium perruthenate.

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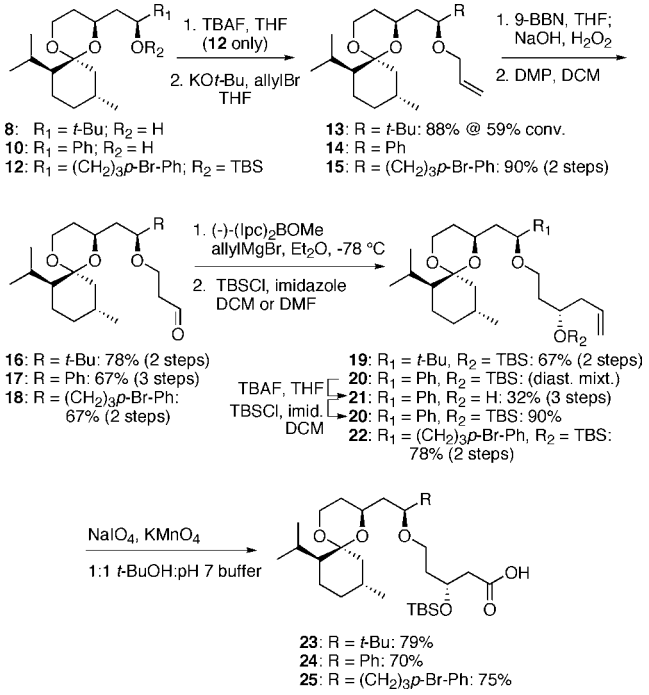
These were then coupled with the preexisting recognition domain for which we have reported a practical synthesis. The synthesis of these new spacer domains began with installation of the appropriate "R" group into known aldehyde **6** (Scheme 1).[7] In the cases where R = *t*-Bu or Ph, the group is installed via addition of the corresponding carbanion to generate a mixture of secondary alcohols. Alcohols **7** and **8** were easily separated, and the undesired epimer was recycled through a two-step oxidation/reduction procedure. For R = Ph, the diastereomers were not easily separable and the mixture was subjected to the same two-step oxidation/reduction sequence to generate diastereomerically pure alcohol **10**. Preparation of the more elaborate intermediate **12** started with an asymmetric Brown's allylation of aldehyde **6** followed by TBS protection to give silyl ether **11**.[11] Cross metathesis with *p*-bromo styrene[12] and subsequent reduction of the olefin using Rh on alumina[13] gave silyl ether **12**.

After desilylation of **12**, each of the secondary alcohols was carried independently through a parallel synthetic sequence to complete the individual spacer domains (Scheme 2). Allylation of the individual alcohols with allyl bromide

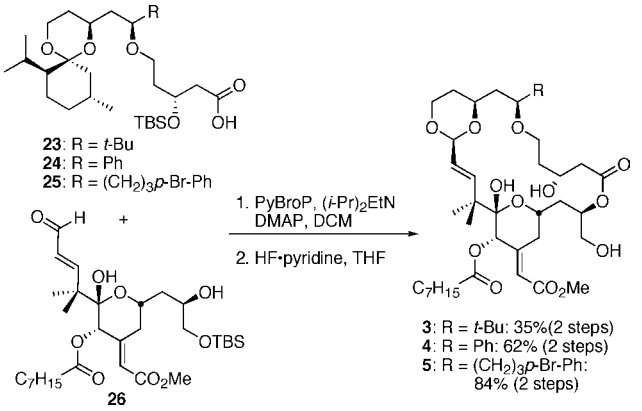
Scheme 2. Completion of Spacer Domains **23**, **24**, and **25**



gave terminal olefins **13**, **14**, and **15**. Hydroboration of these olefins followed by Dess−Martin periodinane oxidation gave aldehydes **16**, **17**, and **18**, respectively.[14] Asymmetric allylation generated inseparable mixtures of the product alcohols and the pinanol byproduct from the allylation reagent. Subsequent TBS protection allowed for isolation of the diastereomerically pure silyl ethers **19** and **22**. Silyl ether **20** (R = Ph) was isolated as an inseparable mixture of diastereomers that could be separated after removal of the silyl group. Reprotection provided silyl ether **20** in diastereomerically pure form. Oxidative cleavage of the terminal olefins using $KMnO_4$ and $NaIO_4$ gave the completed spacer domains **23**, **24**, and **25**.

Each of the spacer domains was coupled individually to the existing recognition domain **26**[7] using the PyBroP coupling reagent (Scheme 3).[15] The macrocycles were closed

Scheme 3. Completion of Analogues **3**, **4**, and **5**



and the silyl protecting groups removed in a remarkably general one-step, mild, and diastereoselective macrotransacetalization, providing the completed analogues **3**, **4**, and **5**. The newly formed stereocenter in each is set under thermodynamic control affording only the cis-diequatorial dioxolane B-ring.

These new analogues exhibited single-digit nanomolar binding affinities for rat brain PKC when tested in a competition binding assay against the known PKC ligand phorbol 12,13-dibutyrate (**3**, K_i = 6.5 nM; **4**, K_i = 2.3 nM; **5**, K_i = 1.9 nM).[16] Significantly, these analogues exhibit binding potencies superior to analogue **2** and on par with bryostatin 1. These data demonstrate that extensive modifications can be made to the A-ring region without affecting binding affinity, indicating that the C9 region could be modified as needed to tune ADME and pharmacokinetic characteristics. To ascertain whether these new analogues will elicit biological responses similar to bryostatin 1, studies exploring the response of individual PKC isozymes to these new ligands, as well as the in vitro functional differences among them, 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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