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The Design, Synthesis, and Evaluation of C7 Diversified Bryostatin Analogs Reveals a Hot Spot for PKC Affinity

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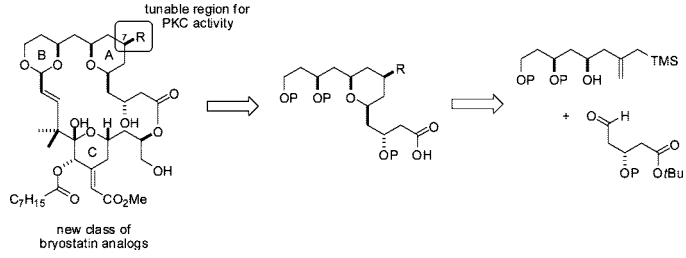
ABSTRACT



The first series of systematically varied C7-functionalized bryostatin analogs (12 members in all) have been synthesized through an efficient and convergent route. A new hotspot for PKC affinity, not present in the natural products, has been discovered, allowing for affinity control and potentially for selective regulation of PKC isozymes. Several analogs exhibit single-digit nanomolar affinity to PKC and display superior activity compared to bryostatin against the leukemia cell line K562.

The bryostatins are structurally complex, marine-derived macrolactones (Figure 1)[1] with a unique and expanding portfolio of biological activities. Bryostatin 1 is currently in clinical trials for the treatment of cancer. It exhibits exceptional potency, being dosed in humans at ca. 50 μg/m^2.[2] It restores apoptotic function in cancer cells,[3] reverses multidrug resistance,[4] and unlike most antineoplastic agents, stimulates the immune system.[5] It also acts synergistically with several anticancer agents when used in combination therapy.[6] Remarkably, bryostatin also facilitates learning and extends memory in animal models,[7] serving as a significant lead for treating cognitive dysfunction including Alzheimer's disease.[8]

There are two major problems that have impeded clinical advancement of the bryostatin leads. Bryostatins are naturally scarce and are isolated in low yields (0.00014%).[9] Second, these complex compounds have proven to be difficult to selectively modify. Thus, relatively few bryostatin derivatives have been prepared and fewer still have been evaluated preclinically.[10] The importance of accessing new functional and tunable analogs

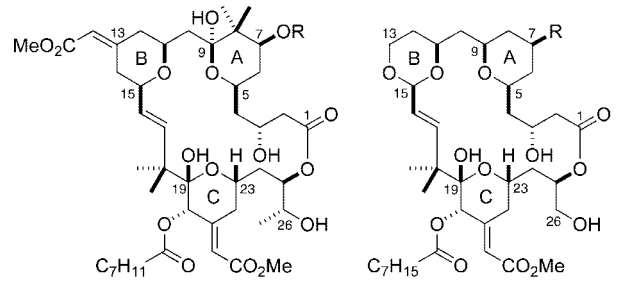


Figure 1. Bryostatins 1 and 2, superior lead analog **1**, and designed C7 analogs.

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is great as the natural product itself elicits off target effects that limit or preclude clinical applications.

Total synthesis offers a means to address this problem, but current syntheses, while impressive in content, are as yet too long (>70 steps) to impact immediate supply needs.[11] Engineered biosynthesis is another notable and promising source under investigation.[12] In 1986, we initiated a third approach to addressing this problem based on function-oriented synthesis.[13] In this approach, the structural features of the complex bryostatin target that putatively influence function (activity) are recapitulated on a simplified scaffold to produce a functional analog that is designed for rapid, step-economical, and practical synthesis.[13a] Illustrative of this approach, designed analog **1** (Figure 1) is found to be more potent than bryostatin and can be readily synthesized in a highly convergent fashion (<30 steps, 19 LL) that can be scaled to meet clinical needs.[14]

The current study is directed at a hitherto unexplored but fundamentally and clinically significant issue, namely the role of A-ring C7 functionality in the activities of bryostatin and analogs related to **1**. Bryostatin 1's activity is mediated through its potent binding to the C1 domain of various proteins, including kinases such as protein kinase C (PKC). This domain is found in only a small subset of the human kinome, but its functional role could be significant because many of these kinases are implicated in major diseases including cancer, cardiovascular, and cognitive indications.[15] Ligands for the selective regulation (activation or inhibition) of C1 domain proteins are not available, adding further importance to the search for new high-affinity, selective agents. Related to the search for ligand structural features that could influence potency, selectivity, and function, it is noteworthy that no systematic studies on the role of C7 functional variations on biological activity have been reported (Figure 1). Significantly, docking studies using PKC subdomain crystal structures and homology models suggest that the C7 functionality of bryostatin is proximate to a conserved tryptophan residue in the novel class of PKC isozymes and a conserved tyrosine residue in the conventional class of PKC isoforms (Figure 2).[16] The biorelevancy of such models has not been tested, but such differences in binding selectivity could have profound therapeutic ramifications. It is noteworthy that these residues of PKC have recently been found to be critical for selective isozyme activation by the endogenous ligand, diacylglycerol.[17] We report herein the efficient and convergent synthesis of 12 members of the first class of C7-functionalized bryostatin analogs and their initial biological evaluation.

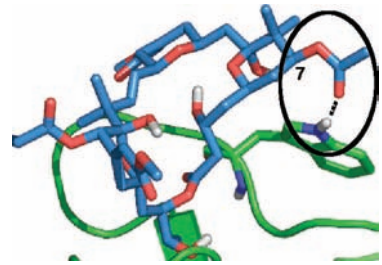

Figure 2. Docking of bryostatin 1 to the C1b domain of novel PKCδ. The distance between the C7 acetate carbonyl and tryptophan hydrogen is 1.9 Å, and the bond angle is $\angle$O−H−N = 152°.

Our overall strategy employs a Yamaguchi esterification followed by a macroacetalization to convergently couple the target recognition (**10**) and spacer domains generated from intermediates **8** or **9**. The synthesis of C7 diversifiable spacer domains draws in turn on a stereospecific Prins cyclization[18] to convergently generate the *syn*-pyran A-ring from bis-nucleophile **4** and 2 equiv of aldehyde **3**, a strategy that exploits the pseudosymmetry of the designed spacer domain. The synthesis of spacer domain intermediate **8** began with

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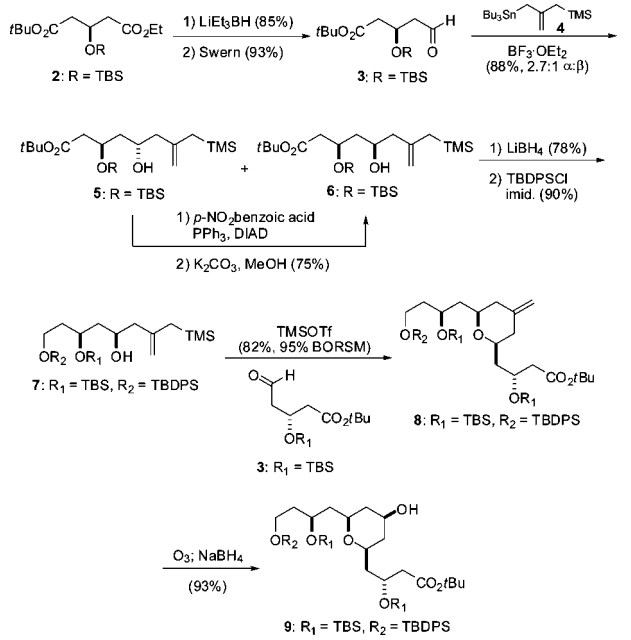
the selective reduction of commercially available diester **2**[19] and oxidation to the key aldehyde **3** (Scheme 1). Allylation

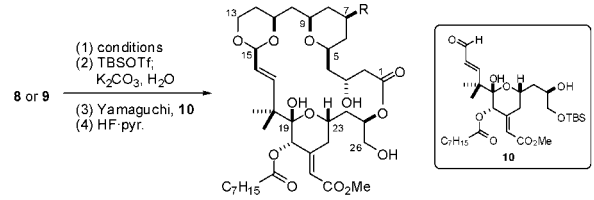
Scheme 1. Highly Convergent Synthesis of C7-Functionalized Intermediates



of this aldehyde with stannane **4**,[20] while efficient, was not highly disatereoselective with various catalytic and stoichiometric Lewis acid systems (Ti/BINOL or Zr/BINOL)[21] as well as additives ($B(OMe)_3$ or *i*-$PrSBEt_2$).[22] The undesired α-alcohol **5** was therefore efficiently converted to the desired *β*-alcohol **6** using Mitsunobu conditions. Reduction of ester **6** and protection of the resultant alcohol led to Prins precursor **7**. The Prins cyclization of **7** using aldehyde **3** a second time proceeded smoothly to give pyran **8** in good yield and with high if not complete diastereoselectivity. The use of the more robust TBDPS protecting group for the primary alcohol was critical, as silyl exchange and deprotection was problematic under TMSOTf-promoted conditions using a primary TBS group. Ozonolysis and reductive workup then selectively installed C7 oxygenation as the desired *β*-alcohol in only seven overall steps from commercial material through **6** or nine steps through **5**. Notably, these syntheses of spacer domains through intermediates **8** and **9** are the shortest of any spacer domain synthesis reported thus far.

An attractive aspect of this strategy is that alkene **8**, alcohol **9**, or their derivatives can be converted into bryostatin analogs in only 3−4 steps (Scheme 2). Toward this end, **8**,

Scheme 2. Completion of C7-Functionalized Analogs[a]



8 or 9	conditions	R	steps (1-2)	steps (3-4)
9	Ac_2O	OAc	94%	71%
9	BzCl	OBz	65%	62%
9	$(tBuC(O))_2O$	$O_2CC(CH_3)_3$	72%	60%
9	2-furoyl chloride	O-furoyl	56%	57%
9	MeOC(O)Cl	OC(O)OMe	59%	48%
9	$Me_2NC(O)Cl$	$OC(O)NMe_2$	35%	52%
9	-	OH	67%	73%
9	Me_3OBF_4	OMe	55%	83%
9	i. *p*NO_2-benzoic acid, PPh_3, DIAD; ii. K_2CO_3, MeOH	α-OH	35%	92%
8	i. *m*CPBA; (2); ii. H_2, Pd/C	CH_2OH	30%	74%
8	O_3; thiourea	carbonyl	58%	62%
8	-	CH_3	82%	68%

[a]See the Supporting Information for experimental details.

9, or their derivatives were selectively converted to the C1 carboxylic acid through an efficient TBSOTf-mediated procedure. The resultant spacer domains were then esterified with recognition domain **10**[14] using Yamaguchi conditions. Our remarkably general[14,23] and mild tandem global deprotection and macroacetalization procedure was then employed to form the B-ring which also serves to complete the macrocycle and set the C15 stereocenter under thermodynamic control, providing the analog in good yield. In total, 12 C7-functionalized analogs were efficiently synthesized in this manner, with the spacer domains prepared in as few as seven overall steps.

The newly synthesized analogs were tested for their ability to bind to PKC, providing a first-ever benchmark comparison to bryostatin and other analogs as well as to whether C7 modifications influence potency (Figure 3). The C7 acetate analog **11** bound with a K_i of 13 nM while the benzoate and pivaloate analogs (**12** and **13**) exhibited comparable but higher potency, indicating that steric bulk is tolerated in this region. The more polar furoyl analog **14** displayed a lower affinity, comparable to the carbonate or carbamate analogs **15** and **16**. Remarkably, the free hydroxyl analog **17** was found to bind with a $K_i = 1000$ nM, a 4 orders of magnitude loss in binding potency compared to lead analog **1** simply due to the incorporation of a hydroxyl moiety at C7. This analog was found to be stable to the assay buffer conditions and ROESY NMR studies indicated that the analog adopts a similar conformation to that of other potent bryologs. Interestingly, inversion of this stereocenter to the C7 α-OH

(19) Item 49.60 from Jülich Chiral Solutions (www.codexis.com) for 33 euros/g.

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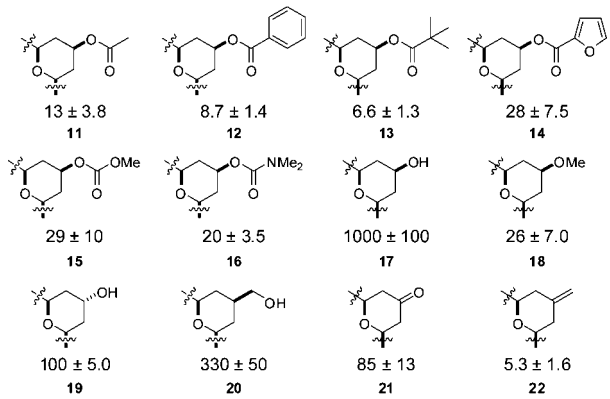

Figure 3. PKC binding affinities of C7 functionalized analogs (nM). Only A-rings are shown.

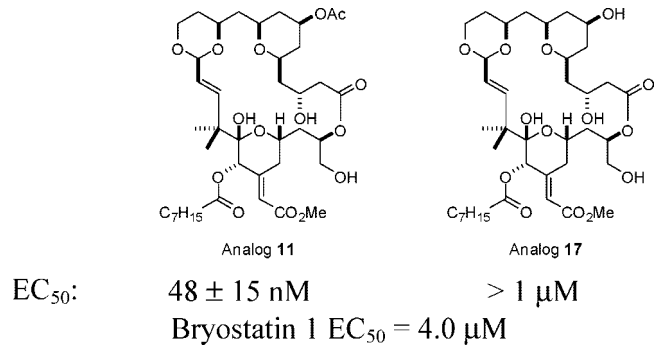

Figure 4. Effective concentrations of analogs **11** and **17** against human leukemia cell line K562 after 48 h incubation.

analog **19** results in a 10-fold increase in binding potency (K_i = 100 nM) compared to **17**, while the homologated hydroxyl analog **20** increases relative affinity by only 3-fold (K_i = 330 nM). It appears that analogs retaining a free hydroxyl in this region of the bryologs lose a significant degree of potency compared to other C7 functionalized analogs, raising the hypothesis that C7 groups are at the protein complex−membrane interface or other hydrophobic region. This hypothesis is further supported by the better binding of methoxy analog **18** relative to **17**. Even more dramatically, exomethylene analog **22**, nearly isosteric with ketone analog **21**, exhibits single digit nanomolar binding.

Interestingly, bryostatin 2 (Figure 1), containing a C7 hydroxyl group as in analog **17**, is only about 4-fold less potent than bryostatin 1 with a C7 acetate. The effect of the neighboring *gem*-dimethyl groups in the natural series could enhance membrane association by offsetting or shielding the C7 differences (Figure 2). The absence of a crystal structure of full length PKC and more significantly, of a solution structure involving membrane association, makes this ligand-based probe of the complex structure a singularly critical means of detailing molecular level interactions. It is clear from the binding data that PKC and the lipid cofactors required for proper protein folding[24] represent a more complex system than the hypothetical binding model of the analogs docked to the C1 domain would indicate. These studies do show that, in contrast to the natural bryostatins, the C7 region of the bryologs can play a significant role in binding affinity and could be potentially exploited for improved pharmacological function such as PKC selectivity.

To further compare the cellular effects of these analogs, **11** and **17** were tested for their effectiveness against the human leukemia cell line K562 (Figure 4). Analog **1** is effective against this line (EC_{50} = 32 nM), while bryostatin 1 is significantly less active (EC_{50} = 4.0 μM).[14] In line with their binding potencies, analog **11** was more effective than analog **17** against this cell line. Importantly, the synthesis of the first C7 acetate bryolog allows for retention of activity when compared with lead analog **1** and superior activity when compared to bryostatin 1.

The first C7 A-ring functionalized bryologs have been synthesized in a scalable and step-economical fashion, with spacer domains available in 7−10 steps (overall 26−29 steps). These 12 analogs represent the first in a series of C7 modified bryologs, some of which retain potent, single-digit nanomolar affinity to PKC. A critical hotspot for PKC affinity has been discovered at the C7 position of the bryostatin analogs. The discovery of this hotspot adds weight to prior data[17] implicating this region of PKC as critical for potency and potentially for controlling selectivity and optimizing pharmacological function. Indeed, the C7 acetate analog **11** exhibits superior in vitro antileukemic activity when compared to bryostatin 1. Further biological evaluation of these analogs, including isozyme selective activity, is currently underway and will be reported in due course.

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Supporting Information Available: 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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