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Computer-Guided Design, Synthesis, and Protein Kinase C Affinity of a New Salicylate-Based Class of Bryostatin Analogs

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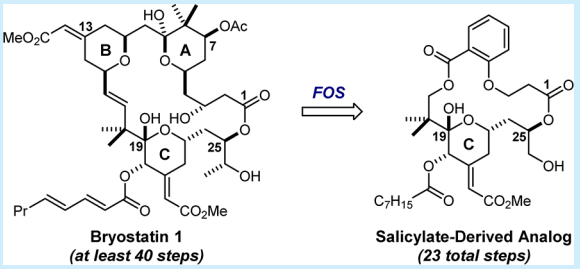
Supporting Information

ABSTRACT: Bryostatin 1 is in clinical trials for the treatment of cancer and Alzheimer's disease and is a candidate for a first-in-class approach to HIV/AIDS eradication. It is neither readily available nor optimally suited for clinical use. Using a function oriented synthesis strategy, a new class of bryostatin-inspired analogs was designed with a simplified salicylate-derived subunit, enabling step-economical synthesis (23 total steps) of agents exhibiting bryostatin-like affinity to protein kinase C (PKC).



The bryostatins are a family of 20 macrolide natural products isolated from the marine bryozoan *Bugula neritina*.[1] Extracts from these animals displayed marked antineoplastic activity when first tested against murine leukemia in the late 1960s by Pettit et al.[2] The structure of bryostatin 1, the biologically active constituent, was subsequently reported in 1982 (Figure 1).[3] Numerous investigations have since sought to

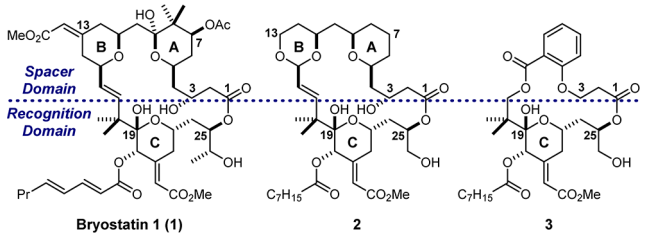


Figure 1. Comparison of bryostatin 1, first generation designed analogs, and new designed analogs.

explore the therapeutic potential of this highly potent agent. Over 37 clinical trials have been conducted to date, principally focused on the utility of bryostatin 1 for the treatment of cancer, both as a single agent and in combination with other oncolytics.[4] New clinical indications have also emerged. The ability of bryostatin to speed recovery after ischemic damage[5] and induce synaptogenesis[6] is particularly noteworthy and could figure in its use to reduce the debilitating effects of stroke relative to current therapies or to treat neurodegenerative diseases. Indeed, bryostatin 1 is the focus of a recently opened clinical trial for Alzheimer's disease.[4a,7] Bryostatin also activates cells latently infected with the HIV provirus,[8] potentially allowing for their destruction by the immune system or cytopathic effects of viral production. Since these proviral reservoirs are thought to resupply active virus, their elimination in conjunction with antiretroviral therapy could lead to reduction of the provirus burden if not eradication of the disease.[9] Of singular significance, the vast majority of research on these indications has been limited to the natural product itself. This raises unresolved issues already encountered in earlier cancer trials regarding bryostatin's supply and off-target effects. In fact, patient accrual in a recent clinical study was stopped due to these concerns.[10] Given that natural products are evolved for purposes other than treating human disease (humans arrived late in this evolutionary process), it is expected that analogs inspired by these information-rich natural prototypes could potentially serve as more accessible and efficacious clinical candidates.[11] The key to realizing this goal is understanding the structural basis for bryostatin's activity and using that information to design simpler and more effective analogs.

The activity of bryostatin is thought to be mediated through binding to the regulatory C1 domains of protein kinase C (PKC) isoforms,[12] a family of serine/threonine kinases integral to proper cellular signal transduction.[13] Two of the three mammalian PKC classes (conventional PKCs: α, βI/βII, γ; novel PKCs: δ, ε, η, θ) are endogenously activated by association of diacylglycerol (DAG) to the C1 domains, with this binding interaction requiring PKC to transiently associate with a phospholipid bilayer.[14] This regulatory mechanism influences a multitude of downstream signaling events, though the basis for its selectivity is still a work in progress.[15] DAG and bryostatin are competitive PKC binders, though the latter is orders of magnitude more potent. Developing small molecule modulators of PKC is key to understanding the system biology and chemistry of this signaling pathway and thus its therapeutic potential.

Early on and now, bryostatin's limited availability has hampered research on its mode of action and clinical use. Isolation yields are prohibitively low (just 18 g of **1** were obtained from 14 tons of the source organism in the only GMP isolation), and large scale harvesting in marine ecosystems raises environ-

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mental issues.[16] Aquaculture has been tested and abandoned.[17] Supply methods based on *de novo* synthesis (the shortest total syntheses of potent bryostatins are around 40 steps)[18] and engineered biosynthesis are still works in progress.[19]

Recognizing these issues, our group started in the 1980s to explore a strategy focused on achieving bryostatin-like function through synthesis- and activity-informed design.[20] The premise behind this function-oriented synthesis (FOS) approach, influenced by our studies on DAG, bryostatin, and phorbol esters—three structurally distinct agents with the ability to bind PKC, albeit with differential selectivities—is that function is not uniquely associated with any one structure. A second premise is that many natural products are encoded with excess structural information peculiar to the needs of their source organism and thus possess structural complexity irrelevant to their intended use in human therapy. It follows that if one could define the minimum set of features needed for a desired target activity, then through design one could incorporate that required functionality into more accessible and effective agents.

Toward this end, our group performed extensive computer-guided comparisons of the spatial orientation of heteroatoms (hydrogen bond donors and acceptors) of then known PKC ligands, resulting in a proposed pharmacophore and the first designed PKC modulators.[21] For bryostatin, this analysis suggested that the key structural elements that govern binding were present in its southern half, termed the "recognition domain," that is proposed to contact and thus be "recognized" by PKC. The role of the northern portion of the molecule, the "spacer domain" (see Figure 1), was proposed to control the conformation of the recognition domain and potentially influence PKC translocation and membrane association. It follows that compounds containing simpler spacer domains that preserve the conformation of the recognition domain should elicit PKC affinities comparable to bryostatin.

In 1998, we reported the first designed bryostatin analog with such a simplified spacer domain.[22] An even more potent analog followed (B-ring dioxane analog **2**), exhibiting PKC affinity comparable to that of bryostatin and requiring ~40 less steps to synthesize than the then contemporaneous syntheses of natural bryostatins.[23] Most designed bryostatin analogs have since been based on this analysis, retaining the recognition domain while employing simplified spacer domains to improve step economy.[24]

Reported herein is the application of the FOS approach to the design of a new and uniquely simplified class of potent functional bryostatin analogs, in which a salicylate subunit was selected as a highly simplified mimic of the A- and B-rings of bryostatin. This drastic reduction in complexity has resulted in the shortest overall step count to a potent bryostatin analog, a goal of significant research and clinical importance. Given the potency of analog **3** (ca. 18 nM) and the ease with which the salicylate subunit could be modified, this scaffold provides the basis for iterative design−synthesis−evaluate approaches to targeting isoform selective modulation of PKC. PKC pathway pharmacology is complex, exhibiting activation or formal inhibition as a function of dose and time.[13b] Step-economical access to tunable leads is thus a key to identifying safe and effective clinical candidates.

The design of analog **3** started with *in silico* studies centered on identifying simple surrogates for the bryostatin spacer domain. In this case, we focused on salicylates to emulate spacer domain function which, being commercially available, would thus reduce step count. It was proposed that an ether at C3 could mimic the function of the natural C3 alcohol by engaging the C19 hemiketal in a hydrogen bond while the aryl group would be positioned for membrane association. This maintains the critical hydrogen bond to the C19 hemiketal, while replacing the bifurcated hydrogen bond network of bryostatin (extending from the C3 alcohol to the A- and B-ring tetrahydropyrans) with covalent linkages.

To evaluate the conformational correspondence between the designed analog and bryostatin 1, the conformations of the analog were determined and compared with the known crystal structure of bryostatin. Analog **3** was subjected to an extensive search of its conformational space within the multiconformer mode of the MMFFs force field provided with the Schrodinger Suite[25] (Macromodel v9.5/Maestro GUI v8.0). The lowest energy structures from this analysis fell within a single conformational class, which was subsequently examined for similarity to the known crystal structure of bryostatin 1. Based on the lowest energy conformers of both compounds, the hydrogen bond from the C19 hemiketal to the C3 oxygenation appeared intact (Figure 2), suggesting that the salicylic acid spacer domain

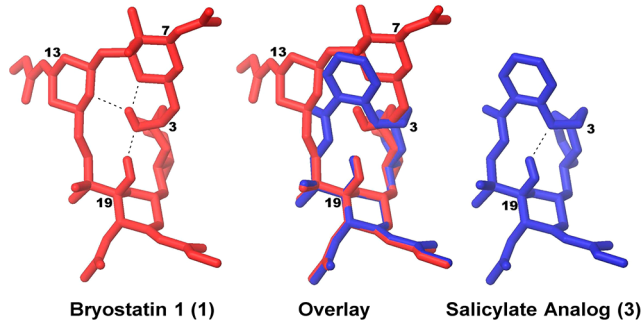
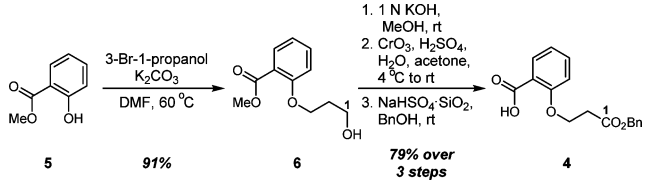


Figure 2. Left: crystal structure of **1** (red). Right: lowest energy conformer of **3** (blue). Center: overlay of both structures. The C20 side chain is depicted as the acetate for clarity.

could be an excellent mimic of that of bryostatin 1. An overlay between this lowest energy conformer of analog **3** and the crystal structure of bryostatin 1 showed remarkable similarity in the spatial location of recognition domain atoms (RMS deviation of 0.05 Å) and prompted our commitment to the synthesis of this new class.

The synthesis of the benzyl ester of spacer domain **4** began with readily available methyl salicylate **5** (Scheme 1). Phenolic

Scheme 1. Synthesis of the Spacer Domain 4



alkylation with 3-bromo-propanol gave the phenyl ether **6**. Saponification of the methyl ester followed by Jones oxidation afforded the desired dicarboxylic acid. Selective benzylation[26] of the aliphatic carboxyl group was then performed utilizing $NaHSO_4{\cdot}SiO_2$ as a weak acid catalyst in benzyl alcohol to provide the desired spacer domain **4** with differentiated functionality (acid and benzylic ester) for coupling to the recognition domain.

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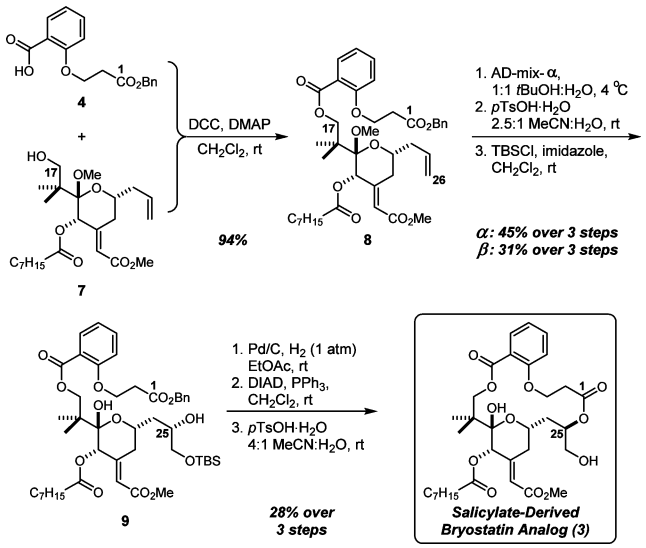
Spacer domain **4** was then attached to known truncated recognition domain fragment **7**[23] via a DCC-mediated esterification (Scheme 2). Sharpless asymmetric dihydroxylation

Scheme 2. Completion of Salicylate-Derived Analog 3



of the C25−C26 olefin in **8** was followed by deprotection of the C19 ketal with *p*-tosic acid. Selective protection of the primary C26 alcohol with TBSCl afforded macrocyclization precursor **9** in 45% yield over three steps along with 31% of the C25 β-epimer. Hydrogenolysis of the C1 benzyl ester provided the requisite seco acid. Reaction of this C25 α-hydroxy seco acid under Mitsunobu conditions followed by acidic hydrolysis of the C26 silyl ether successfully generated designed analog **3** in a three-step yield of 28%, sufficient to reach the critical initial goal of this study, determining whether our analysis would yield new active analogs. That noted, the average yield for these steps (~65%) is not unreasonable given the challenge of closing a 16-membered ring with five contiguous sp^2 centers, two ester linkages with a transoid preference, and an out−out bridgehead across C19/C23. Fortunately, this method did provide the necessary material for initial biological evaluation. For reference, the C25 β-alcohol could also be cyclized using acid activation mechanisms (Yamaguchi macrocyclizations; carbodiimide-mediated esterifications)[27] though that approach provided lower yields, never eclipsing the 20% yield over the final three steps.[28] Attempting the macrolactonization with the C19 ketal in place resulted in little to no product, likely due to the methyl group providing an additional steric barrier to cyclization. In these cases, the primary product was the free phenol resulting from deprotonation at C2 (presumably of the activated form of the C1 acid) and elimination of the phenolate. This elimination byproduct was only isolated in trace amounts with the C19 hemiketal cyclizations, though these substrates likely suffered more from C-ring opening pathways.

Analog **3** was evaluated for its ability to bind PKC via a cell-free competitive binding assay with ^{3}H-phorbol dibutyrate.[29] Using full-length isoform-specific PKC, analog **3** was found to be a potent ligand for novel PKCδ and conventional PKC βI, giving K_i values of 18 and 24 nM respectively, approaching that of bryostatin 1 (K_i = 1.1 and 1.0 nM) and B-ring dioxane analog **2** (K_i = 2.2 nM, PKCδ). This potency and an observed NOE from the C19 hemiketal to C3 (suggesting an intact intramolecular hydrogen bond as anticipated) support both the design strategy aimed at this particular scaffold and the FOS approach in general. These efforts make possible step-economical access to even more potent agents (following study) as is critically needed for various preclinical and intended clinical trials.

By moving from the complex A- and B-ring system of bryostatin 1 to the salicylate-derived spacer domain of analog **3**, six stereocenters, one ring, a di- and a trisubstituted olefin, and two quaternary centers (one of which is an all-carbon quaternary center) were removed from the desired synthetic target, enabling a decreased step count with the only cost being a modest decrease in binding affinity. Further exploration of this scaffold (following manuscript) recovers loss in function through tuning of this accessible structural class. Ultimately, this new, designed scaffold becomes a promising lead for future development of PKC modulators aimed at the treatment of high impact diseases such as cancer, Alzheimer's, and HIV. While almost all research toward these indications has been based on the natural products, function- and synthesis-informed design offers a unique opportunity to supply new leads with improved clinical potential.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures for the above transformations. Characterization data for all novel compounds with ^{1}H and ^{13}C NMR spectra included. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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