Organic LETTERS

Letter

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Improved Protein Kinase C Affinity through Final Step Diversification of a Simplified Salicylate-Derived Bryostatin Analog Scaffold

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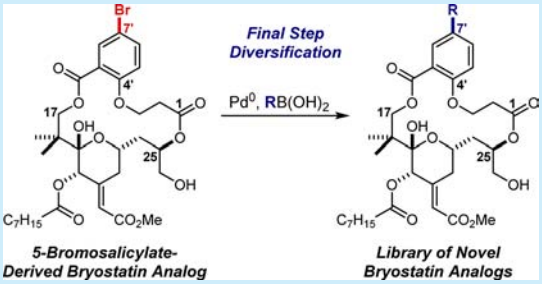
Supporting Information

ABSTRACT: Bryostatin 1, in clinical trials or preclinical development for cancer, Alzheimer's disease, and a first-of-its-kind strategy for HIV/AIDS eradication, is neither readily available nor optimally suited for clinical use. In preceding work, we disclosed a new class of simplified bryostatin analogs designed for ease of access and tunable activity. Here we describe a final step diversification strategy that provides, in only 25 synthetic steps, simplified and tunable analogs with bryostatin-like PKC modulatory activities.



Discovered over 46 years ago by Pettit and collaborators in a marine extract, bryostatin 1 (**1**) is a densely functionalized macrolide that has since attracted much interest due to its novel structure[1] and its clinical potential (evaluated in 37 clinical trials to date[2]). In addition to its proposed clinical use for the treatment of cancer and its largely under-explored potential as a small molecule immunomodulator,[3] bryostatin has also shown promise for the treatment of stroke based on animal models of ischemic damage.[4] It has also been shown to facilitate learning and extend memory in animal conditioning experiments, apparently through induction of synaptogenesis,[5] leading to its recent entry into clinical trials for the treatment of Alzheimer's disease.[2a,6] Bryostatin and other PKC modulators[7] (e.g., prostratin) also activate transcription of the HIV provirus,[8] which, when combined with antiretroviral therapy, could eliminate latent viral reservoirs, thus potentially providing a first-of-its-kind strategy to reduce disease burden or eradicate HIV.[9,10]

These clinical indications have justifiably driven interest in bryostatin, but the lack of a reliable natural supply and off target effects (dose-limiting toxicity of myalgia) have provided barriers to its development as a therapeutic.[2c] Aquaculture, engineered biosynthesis, and total synthesis have been explored as sources of bryostatin with varying degrees of success,[11] but a practical supply of bryostatin has not yet been realized.

This situation has no doubt contributed to the relative lack of advanced studies and absence of clinical studies on analogs. Unlike most other natural product leads, the clinical case for bryostatin has thus far been exclusively based on one compound, bryostatin 1 itself. Significantly, the supply of the natural product and issues related to off-target effects of bryostatin 1 could be ameliorated or eliminated with simplified designed analogs that retain efficacy using a function-oriented synthesis (FOS) strategy.[12] While natural products represent a vast and exceptional library of leads, encoding 3.8 billion years of chemical experimentation, their availability is often variable and limited, as found for bryostatin, and they are rarely optimal candidates for human use (taxol notwithstanding), more often serving as the inspiration for more effective derivatives or designed clinical agents.[13] More generally, analogs of a natural product containing only those substructures critical for activity could be designed to enable facile synthesis, thereby solving the issue of supply while generating clinical candidates with improved function. Toward this end, our group has extensively investigated the bryostatin scaffold both *in silico* and synthetically[14] in an effort to better understand its interaction with protein kinase C, the intracellular target putatively responsible for its desirable effects.[15] There are eight isoforms of PKC (conventional PKCs: α, βI/βII, γ; novel PKCs: δ, ε, η, θ) that contain the regulatory C1 domains allowing for allosteric regulation by bryostatin, phorbol esters, or the endogenous ligand, diacylglycerol.[16] Using this FOS approach, our group has prepared analogs that either mimic or differ from the *pan*-PKC selectivity profile of bryostatin, some of which perform as well or even better than the natural product in various *in vitro* and *ex vivo* evaluations.[17]

In the preceding manuscript, we reported the development of a 16-membered macrolactone analog (**2**, Figure 1; contracted from the natural 20-membered ring) that substituted the densely functionalized A- and B-ring system of bryostatin with a salicylate-derived linker.[18] Since the southern half of the molecule contains the functionality implicated in binding PKC, this drastic simplification of the northern fragment did not have a major deleterious effect on PKC affinity (K_i = 18 nM vs 1.1 nM for bryostatin 1), a promising first data point for this new scaffold. Described herein is a *final step* diversification strategy of this salicylate-derived bryostatin analog scaffold based on a C7′-bromide handle and requiring only one step from the point of diversification to reach library members. The diversifiable C7′-Br

Received: August 22, 2014
Published: September 19, 2014

   dx.doi.org/10.1021/ol502492b | *Org. Lett.* 2014, 16, 5140–5143

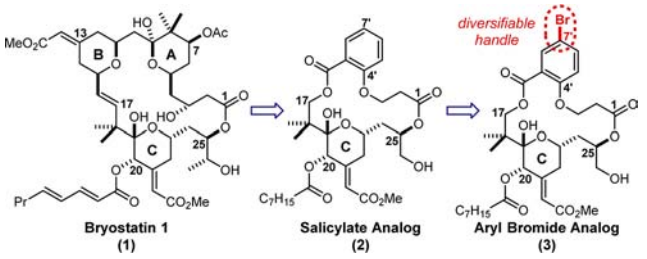


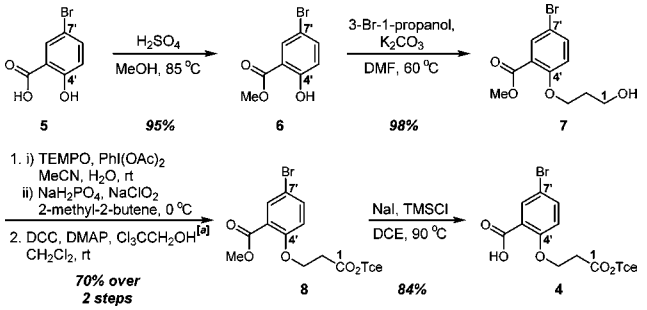
Figure 1. Simplification through function-oriented, synthesis-informed design: new diversifiable analog **3**.[19]

analog **3** is reached in just 24 total steps. The reduced step count and ability to tune PK properties provide a practical foundation for the synthesis and study of agents with improved accessibility and clinical potential.

The synthesis of analog **3**, incorporating a diversifiable bromide group, was achieved in a similar manner to that for the original salicylate-derived analog (**2**),[18] although a different northern fragment was used and improvements were made in the late-stage macrocyclization step.

Northern fragment **4** was prepared in 5 steps (Scheme 1) from commercially available 5-bromosalicylic acid (**5**). Fischer

Scheme 1. Synthesis of the Northern Fragment 4

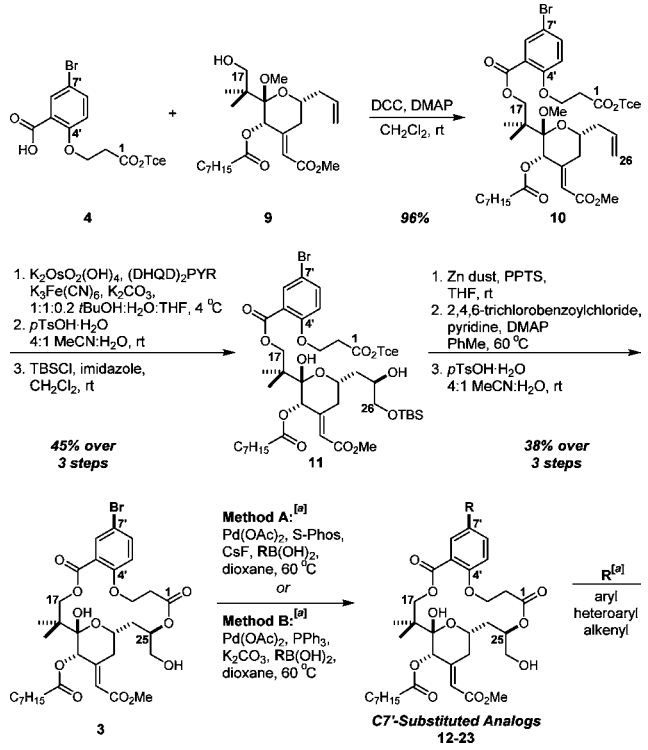


[a]Tce = 2,2,2-trichloroethyl.

esterification afforded the desired methyl salicylate (**6**), allowing for clean alkylation of the C4′ phenol with 3-bromo-1-propanol to generate phenyl ether **7**. Oxidation of the resultant C1 alcohol and esterification with 2,2,2-trichloroethanol provided *bis*-ester **8**. This was then demethylated without loss of the C1 protecting group with *in situ* generated TMSI in refluxing DCE in 84% yield, thus affording northern fragment **4** in 55% yield over five steps.[20]

The southern fragment of analog **3** was derived from C17 alcohol **9**,[21] a versatile intermediate available in 12 steps and used en route to numerous other bryostatin analogs including parent salicylate analog **2** (Scheme 2). The northern and southern fragments were coupled through simple DCC-mediated esterification, cleanly bringing together the two pieces in 96% yield when run on gram scale. Sharpless asymmetric dihydroxylation of the C25−C26 olefin proceeded with a modest ∼2:1 dr and was followed by deprotection of the C19 hemiketal and selective TBS protection of the primary C26 alcohol to generate C25 *β*-alcohol **11**. Notably, 10% THF was required for sufficient solvation of olefin **10** during dihydroxylation. Deprotection of the 2,2,2-trichloroethyl ester with nucleophilic (NaSeH or NaTeH) or reductive methods (e.g., SmI_2) was either inefficient or led to decomposition. However, a slight modification of standard Troc removal conditions (Zn, PPTS in THF rather than

Scheme 2. Fragment Coupling and Preparation of C7′-Br Analog 3



[a]See Figure 2 for structures of R and yields.

Zn, AcOH in DMF) cleanly afforded the desired seco acid and set the stage for the necessary macrolactonization.

While macrocyclizations driven by acid activation did not compete with a Mitsunobu-based lactonization in the synthesis of the parent salicylate scaffold, it was speculated that simply reducing the basicity of these methods could improve the yield. Gratifyingly, substituting pyridine for Hünig's base during the anhydride formation in a traditional Yamaguchi cyclization led to a reproducible 38% yield over the final three steps (C1 deprotection, macrocyclization, C26 deprotection). Notably, the best yields were achieved on ∼350 mg scale. Acidic hydrolysis of the silyl ether furnished the free C26 alcohol, affording C7′-Br analog **3** in 24 steps overall (19 longest linear sequence) from commercial materials. Two sets of Suzuki conditions were employed to modify C7′, using $Pd(OAc)_2$ in both cases: S-Phos and CsF or PPh_3 with K_2CO_3. The monodentate ligand strategy (drawing from previous diversification efforts in our group[22]) generally outperformed the more basic PPh_3/K_2CO_3 conditions, though the latter were necessary for certain boronic acids (e.g., 3,5-dimethylisoxazole-4-boronic acid). Of note, only boronic acids proved to be useful for this scaffold. Thermal instability at 60 °C under the reaction conditions limited the time course of the reaction to 2−3 h. Boronic esters or trifluoroborate salts resulted in slow reactions and were thus not further used.

The 13 C7′-substituted analogs were tested for their affinity to two full-length PKC isoforms, *β*I and *δ*,[24] in a heterogeneous competitive binding assay with [^{3}H]-phorbol dibutyrate.[25] These isoforms are representative members of the conventional and novel PKC subfamilies. The 4-substituted electron-rich arenes of **14** and **15** showed enhanced potency for PKC*δ*, while PKC*β*I affinity was more or less unchanged from the parent scaffold (Figure 2). Moving the methoxy or isopropoxy groups to the 2

dx.doi.org/10.1021/ol502492b | Org. Lett. 2014, 16, 5140−5143

Compound	Bryo 1	2	3	12	13	14	15	16	17
R[a]	-	H	Br	C_6H_{13}		OMe	OiPr	OMe	OiPr
CC yield[b]	-	-	-	32%[d]	55%	45%	83%	60%	80%
PKCβI[c] K_i (nM)	1.0	24	38	12	49	30	16	9.6	8.2
PKCδ K_i (nM)	1.1	18	28	4.4	9.1	4.0	2.7	5.1	3.4

Compound	18	19[e]	20	21	22	23
R[a]	MeO, OMe	iPrO, OiPr	CO_2iPr	HN	O_2S-NEt_2	O–N
CC yield[b]	66%	64%	79%	69%	33%	45%
PKCβI[c] K_i (nM)	4.9	3.1	14	>250	49	22
PKCδ K_i (nM)	1.3	1.9	7.6	>250	5.9	2.8

Figure 2. Diversified library of salicylate-derived analogs. [a] R represents functional group cross-coupled onto diversifiable scaffold **3** (for analogs **12**–**23**); see Scheme 2 for full structure. [b] CC yield = yield of Pd-based cross-coupling; analogs **12** and **14**–**21** were prepared with Method A whereas analogs **13**, **22**, and **23** were prepared with Method B (see Scheme 2 for methods). [c] K_i values (nM) from a heterogeneous competitive binding as against [^{3}H]-phorbol dibutyrate. [d] Two-step yield resulting from use of Method A with *trans*-octenyl boronic acid followed by hydrogenation: Pd/C, THF, H_2 (1 atm), rt. [e] 2,6-Bis(isopropoxy)phenyl boronic acid was prepared in two steps from 2-bromoresorcinol.[23]

position generated the first compounds (**16** and **17**) with single-digit nanomolar affinity for both isoforms, each favoring PKCδ by only about 2-fold (as opposed to 5+-fold for **14** and **15**) and reaching affinities comparable to bryostatin itself. While further research is needed, this modest difference in selectivity might be explained by a hydrogen bonding interaction between the −OMe or −O*i*Pr of analogs **16** and **17** with serine 110 of PKCβI (9th C1b residue; methionine in PKCδ[26]), an interaction that would likely not be accessible to the 4-substituted variants, as their substituents point away from the binding pocket. This interaction would also explain the additional gain in potency when moving to the 2,6-bis-substituted analogs **18** and **19**, as the ligand would no longer need to preorganize itself to one atropisomer over the other for favorable ligand−protein interactions. Given the lack of information on the interaction between bryostatin and PKC, a more detailed understanding of the basis for these effects is not possible.[26] However, we are attempting to rectify this problem through molecular dynamics and REDOR NMR studies currently in progress, as the structure of PKC in a membrane environment is currently not known.

At this time, the electron-rich nature of the arene substituents and overall lipophilicity of certain analogs (e.g., C7′-octyl analog **12**) raised concerns about potential metabolic toxicities and pharmacokinetic properties, respectively. The isopropyl benzoate analog **20** was prepared as a comparator to the 4-O*i*Pr-Ph analog **15** in terms of lipophilicity but with inverted electronic properties. Since the two analogs displayed comparable function, the electron-rich nature of the arene is, at least in this first generation study, not significant. While the introduction of an indole moiety greatly reduced PKC binding affinity, both a sulfonamide (**22**) and isoxazole (**23**) were successfully incorporated and afforded potent ligands, suggesting that hydrogen-bond acceptors but not donors are tolerated. This is intriguing given that these ligands are generally expected to provide a hydrophobic cap for the PKC C1 domain,[27] but again, more structural detail is needed on the spatial orientation of the ligand−substrate complex within a membrane before these new results can be rationalized. Interestingly, these analogs displayed the largest selectivity difference between PKCδ and PKCβI at nearly a full order of magnitude, perhaps facilitated by an S−π interaction with M239 of PKCδ (M9 of C1b domain). While this difference is still modest, it differs from the *pan*-selectivity of bryostatin 1.

The consequences of these various selectivities with respect to functional activity remains to be established, especially given that it is not known whether *pan*-PKC selectivity is required for activity or contributes to side effects, but even slight perturbations in selectivity could lead to unique phenotypic results given the time- and dose-dependent nature of PKC-driven signal transduction.[28] The incorporation of such polar functionality is particularly intriguing, as it implies that late-stage control over PK properties is possible without carrying major concerns over the abrogation of activity.

This 5-bromosalicylate-derived analog **3** has thus proven to be an effective scaffold for versatile, final step diversification of a potent yet highly simplified variation on bryostatin 1. Transitioning to less basic Yamaguchi conditions improved the macrocyclization relative to the closure for parent salicylate analog **2**, and importantly, it was most efficient when run on scale (~350 mg). The library resulting from standard Suzuki coupling conditions with C7′-Br analog **3** demonstrated that a variety of functionalities are tolerated by this approach while still producing highly potent (<10 nM) analogs. Electron-rich or -poor arenes as well as lipophilic or hydrophobic moieties (at least when avoiding hydrogen bond donors) were all tolerated, a valuable trait when tuning ADME properties for clinical needs. Interestingly, several of these agents displayed modest selectivity profiles between PKCδ and PKCβI which could reveal distinct trends when moving to more advanced biological evaluations of these compounds. Efforts to assess the therapeutic potential of this library toward Alzheimer's disease, HIV eradication, and other high priority indications are underway and will be reported in due course. It is clear from these studies that readily tunable analogs comparable in potency to the natural product can be accessed in significantly fewer steps than that required to access the natural product. This FOS strategy thus provides an alternative to the "all or nothing" efforts to achieve clinical relevancy based on the use of the natural product alone.

■ ASSOCIATED CONTENT

Supporting Information

Expanded description of PKC C1 domain structure and implications for targeting isoform selectivity. Synthetic procedures for all transformations shown above. 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.

dx.doi.org/10.1021/ol502492b | *Org. Lett.* 2014, 16, 5140−5143

■ ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health (CA031845 and CA031841). Additional funding was provided by the Amgen Graduate Fellowship.

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(19) For ease of comparison with prior work, the bryostatin 1 carbon numbering is used. The C7′ designation arises because the arene portion is not part of a contiguous carbon chain with the C-ring; thus, C3 is connected to C4′ through the phenyl ether linkage.
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