Published in final edited form as:

Isr J Chem. 2011 April 1; 51(3-4): 453–472. doi:10.1002/ijch.201100020.

Translating Nature's Library: The Bryostatins and Function-Oriented Synthesis

Paul A. Wender^{*,[a]}, Brian A. Loy^[a], and Adam J. Schrier^[a]

^[a]Department of Chemistry Department of Chemical and Systems Biology Stanford University Stanford, CA 94305, USA

Abstract

We review in part our computational, design, synthesis, and biological studies on a remarkable class of compounds and their designed analogs that have led to preclinical candidates for the treatment of cancer, a first-in-class approach to Alzheimer's disease, and a promising strategy to eradicate HIV/AIDS. Because these leads target, in part, protein kinase C (PKC) isozymes, they have therapeutic potential even beyond this striking set of therapeutic indications. This program has given rise to new synthetic methodology and represents an increasingly important direction of synthesis focused on achieving function through synthesis-informed design (function-oriented synthesis).

Keywords

AIDS; Alzheimer's disease; bryostatin; function-oriented synthesis; protein kinase C (PKC)

1. Introduction

Historically, most chemicals in human use came from natural sources. Over the past two centuries, this changed. Chemical synthesis emerged as an alternative and often superior process for supplying many natural compounds and, significantly, even new compounds beyond what nature can produce. Notwithstanding these advances, many natural and unnatural compounds are too complex to be currently made in a practical, supply-impacting way, causing some to move away from their study and use. Developing step economical^[1] and green approaches to such targets provides one way to address this formidable supply challenge. However, such synthetic advances require time while the need to supply many agents is often immediate.^[2] Function-oriented synthesis (FOS)^[3] offers a strategy to address this problem.^[4] FOS draws on the realization that the value of natural as well as non-natural compounds is driven not only by their structures and the synthetic challenges presented therein but also and significantly by their function (e.g., biological activity or use as a therapeutic, material, nanodevice, probe, imaging agent, diagnostic, ligand, conductor, sensor, catalyst, or molecule of theoretical interest). Consequently, because a specific function can often be achieved with many simple structural types through synthesis-informed design, supplying a specific “function” emerges as an increasingly important and often faster and superior alternative to supplying a specific “structure”. While this requires innovation in designing targets, a successful design can go beyond what nature has produced because it can be used to achieve varied and tunable function in a timely fashion. This review explores this strategy to achieve function with step and time economy in connection

with a fascinating class of molecules, the bryostatins, first reported and impressively advanced by Pettit and coworkers,^[5] and their functional analogs (bryologs),^[6] first reported by our group in 1998.^[7] Collectively, these agents have become promising leads for the treatment of cancer,^[8] Alzheimer's disease,^[9] and for the eradication of HIV^[10] (Figure 1). Not unlike many natural products, the bryostatins are complex and scarce, difficult to modify, and not optimized for human therapeutic use. We started research on the bryostatins and FOS in collaboration with the groups of Blumberg and Pettit in the early 1980s.^[11] We describe below the conceptual foundation for our FOS studies and some recent lessons learned about the bryostatins and their analogs of consequence to synthesis, systems biology and medicine.

2. Results and Discussion

2.1. The Role of Function-Oriented Synthesis

The demand for a compound is driven primarily by its function and less so by its structure. The demand for Taxol, for example, was modest until it showed surprising efficacy in human clinical trials for treating cancer, at which time demand skyrocketed and almost every conceivable technology was explored to address its supply.^[12] The Taxol supply problem, however, conflated structure and function since it would have been eliminated had a more readily available structure been identified with similar function as a microtubule modulator. It was the unique mode of action of Taxol that drove demand. Simply put—and an important aspect of FOS—demand is driven by function, and a specific function could be exhibited and thus supplied by many structures, natural or designed. This point is amply exemplified by functional mimetics of many members of nature's library, including peptoids or morpholino RNAs that exhibit a subset of sought-after functional activities of the more complex natural systems that inspired their design. A second point pertinent to FOS is that the complex structures of many natural products are determined in part by evolutionary selection and not by human therapeutic value. Bryostatin, for example, is proposed to protect bryozoan progeny from predation,^[13] which is a very different function from its role in treating human disease. As such, many complex features of its structure that are important for biosynthesis, distribution, activity, and other functions in its ecosystem are not necessarily needed for its efficacy in human therapy. This point is impressively exemplified with stapled peptides, relatively simple systems designed to emulate a desired subset of functions of more complex proteins.^[14]

It follows from these two considerations that only a subset of functionality in many complex natural products might be needed for a specific function, and this subset could be emulated by many different structures, i.e., functional rather than structural analogs. For example, methyl iodide is an effective, simple functional—but not structural—surrogate of N-adenosyl methionine for many methylation reactions. A third consideration is that if the design process is informed and guided by the goals of step economy and green synthesis, one could match or even exceed natural function with simple designed structures readily assembled in a practical fashion. While not central to function, design can also be used to produce structures that, not unlike natural products, inspire new methodology, challenge the reach of synthesis, and provide a testing ground for the development of new reactions, reactivities, methods, and strategies.^[15] Design also introduces a further creative challenge, since creating a simplified target to emulate a complex natural system requires knowledge of how the latter might work (function)—an exercise rich in biophysics, mechanistic analysis, and physical organic chemistry, especially of weak non-covalent interactions that dominate many functions. The ultimate challenge is thus the integration of hypotheses about function with synthesis goals into the creation of a new synthetically accessible, functional structure. In essence, since the starting point for and ultimate goal of FOS is function (and not initially target structure), it begins with a “retro-function analysis” to set target requirements and

evolves a target iteratively guided by these requirements and synthetic goals (e.g., step economy, cost, waste reduction, scalability, operational ease, environmental impact, and, among others, time—arguably our most important economy). In explicit terminology and intent, FOS consolidates many powerful concepts implicitly directed at function including target-oriented synthesis (TOS), diversity-oriented synthesis (DOS),^[16] biology-oriented synthesis (BIOS),^[17] diverted total synthesis (DTS),^[18] to which might be added new terms like reaction, reagent, and reactivity-oriented synthesis as well as others. FOS does not differentiate between biological and abiological synthesis or combinations thereof. It thus includes function-oriented biosynthesis (FOB), an emerging area of opportunity which places a more specific emphasis on the powerful ways that biosynthetic pathways can be used to make targets with function.^[19]

This proliferation of synthetic strategies to achieve function is expected as the field of synthesis is influenced by the molecularization of the sciences (from molecular astronomy to molecular zoology).^[1] The creation of new molecules with function (whether new or improved) is thus expected and critical to scientific advancement in many disciplines. Thus, all of the above “orientations” in a sense ultimately seek function, differing principally in the means (e.g., generating a target or diversity and screening) by which that end is achieved. By placing explicit emphasis on function in both terminology and intent, FOS provides a unifying framework for directing the power of synthesis toward a goal of great significance: function, whether it be connected to biological activity or an increasing list of exceptional functional opportunities associated with imaging, diagnostics, molecular tools, nanodevices, materials, catalysis, energy, or molecules that test the frontiers of theory, reactivity, and mechanism. Structure-oriented synthesis and structure/function studies have laid a logical and evolutionary foundation for FOS, which retains a structural orientation of the former and functional goals of the latter, but seeks to integrate both by achieving function through synthesis-informed design.

2.2. An Early Example of a Directed FOS Approach

Our early FOS studies on the phorbol esters exemplify and provide a basis for many of the above points.^[11a] This research started with efforts to generate hypotheses about the structural basis for phorbol's activity, which arises at least in part from its interaction with the diacylglycerol (DAG)-dependent isoforms of protein kinase C (PKC) (*vide infra*). Computer-based analysis of all heteroatom arrays in phorbol and other PKC ligands suggested that phorbol's binding to PKC could be determined by only a subset of its functionality; the C3/C4, C9, and C20 oxygens and lipophilic ester side chains were the most likely candidates for controlling PKC affinity. The designed and highly simplified trisubstituted arene **ADMB** fit this blueprint for function, since it positioned hydrogen bonding and lipid groups similarly in space (Figure 2).

Significantly, while dramatically simpler in structure than the phorbol esters, this compound bound to and activated PKC, a function which at the time was only associated with natural products and their derivatives. Furthermore, while we reported a synthesis of phorbol, it required 29 steps;^[20] the simplified analog was prepared in only 7 steps. Although the affinity of this class of analogs was not optimized, this study serves to illustrate an important aspect of this FOS approach, namely, that one can create compounds with functions similar to what nature has produced but with completely new and simplified chemical structures.

2.3. Introduction to Bryostatins

Given the encouraging results of our FOS studies on phorbol, we examined a number of structurally unrelated compounds that bind competitively with the phorbol esters to the regulatory (C1) domain of PKC, with the goal of exploring the generality of this FOS

analysis. The diverse family of known natural PKC ligands (Figure 3) includes the endogenous 1,2-*sn*-diacylglycerols, terpenes (e.g., the phorbol esters, ingenol, and gnidimacrin and related tiglanes), polyketides (e.g., bryostatin, aplysiatoxin), and indole alkaloids (teleocidin). We were particularly interested in the bryostatins, as they were then a new and unique structural type with significant activity, potency, and potential for human therapy.

The bryostatins are a family of structurally complex natural products isolated from the marine bryozoan *Bugula neritina* (Figure 4).^[21] Extracts of this organism harvested from the Gulf of Mexico were found by Pettit and coworkers in 1968^[22] to confer significant life extension in a murine leukemia model, but it was not until 1981 that the structure of bryostatin 1 was elucidated by Pettit, Clardy, and coworkers.^[23] Nineteen additional congeners have since been identified from *B. neritina* isolates that were harvested from the Gulf of Mexico, the Gulf of California, the California Coast, and the Gulf of Japan. Recent studies have demonstrated that the biological source of the bryostatins is *Endobugula sertula*, a bacterial symbiont to *B. neritina*, and it is believed that the bryostatins render host larvae unpalatable to predators.^[13]

Structurally, the bryostatins contain a characteristic 20-member macrolactone ring that supports three densely functionalized pyran rings. Notable features include *gem*-dimethyl groups at C8 and C18, a congested C16/C17-E olefin, exocyclic methyl enoate moieties at C13 and C21, and, in the case of most bryostatins, hemiketal functionality at C9 and C19. Structural diversity within this family occurs primarily in the identity (or lack) of acyloxy groups at positions C7 and C20. Additional variation is observed in C-ring structure. In the case of bryostatins 16 and 17, a dihydropyran C-ring replaces the more common tetrahydropyran motif, and in bryostatins 3, 19, and 20 an additional oxygenation at the C22-position engages the neighboring C21 enoate to comprise a butenolide motif.

As this overview is focused on synthesis and the activities of bryostatin have been reviewed in part,^[5,24] it is sufficient to note at this point that bryostatin and its analogs have since shown significant clinical potential for a number of therapeutic indications including cancer, Alzheimer's disease,^[9] stroke,^[25] HIV eradication,^[10] and problems associated with cognitive dysfunction - functions very different from its putative natural role as a larval antifeedant.^[26] The activity of bryostatin 1 has been evaluated in >35 anticancer clinical trials,^[27] where it has demonstrated an ability to enhance the effects of known oncolytics at remarkably low doses (~50 μgm^{-2} , or ~1–1.5 mg per 8-week treatment cycle). That noted, in addition to supply issues, bryostatin has dose limiting toxicities (e.g., myalgia) that could be minimized or eliminated with designed and more readily available analogs. More fundamentally, bryostatin and its functional analogs^[28] continue to be powerful tools for probing cellular signaling pathways coupled to many major therapeutic indications.

Bryostatin's biological activities are mediated by its interaction with several intracellular targets,^[29] including the DAG-dependent^[30] isoforms of PKC. Since its discovery in 1977, the PKC family has garnered increasing attention from the scientific community because of its central and fundamental role in cellular signal transduction and the potential of its isoforms as targets in the development of new therapies for a remarkable range of indications including cancer, cardiovascular disease, stroke, pain, cognitive dysfunction, and HIV/AIDS eradication. The PKC family consists of 10 related enzymes classified into three major subfamilies, grouped by the cofactors required for activation (Figure 5). Conventional PKCs (α , β I, β II, γ) depend on Ca^{2+} and endogenous DAG for activation, while novel PKCs (δ , ϵ , θ , η) are Ca^{2+} -independent but DAG-responsive. The atypical PKCs (ζ , λ/ι) require neither Ca^{2+} nor DAG. Both conventional and novel PKC isozymes contain a C-terminal catalytic domain, which participates in protein phosphorylation, and an N-terminal

(C1) regulatory domain that binds DAG, bryostatin, the phorbol esters, and other ligands (Figure 5).

Despite the remarkable therapeutic potential of the bryostatins, their clinical development and research on their activities have been impeded by their poor availability. A single large-scale good manufacturing practices isolation of bryostatin 1 provided only 18 grams of pure product from 14 tons of *B. neritina*, a ~0.00014% yield.^[31] Although this effort provided sufficient material for bryostatin 1's preclinical and preliminary clinical investigation, this approach is not appropriate as a long-term supply strategy due to both economic and environmental concerns. Aquaculture of *B. neritina* does not necessarily overcome these issues and was ultimately found to be impractical.^[32] The reported isolation yields for other bryostatin congeners are comparably low,^[21] and variability in their abundance has further complicated reliable isolation. Only recently has the putative biosynthetic machinery responsible for the bryostatins' production been identified from *E. sertula*,^[33] and although modern methods of bioengineering could eventually enable the adaptation of these biosynthetic tools for bryostatin's production in a suitable host organism, such an approach has not yet been realized.

2.4. Bryostatin Total Syntheses

Total chemical synthesis offers an abiological approach to the bryostatins. Indeed, owing to the bryostatins' unique structural complexity and promising biological activities, numerous impressive efforts have been directed toward their synthesis. While a complete discussion of these efforts is beyond the scope of this report, the primary strategies that have been successfully implemented are briefly presented below to provide context. The reader is directed to several recent reviews^[34] for a more comprehensive overview of synthetic strategies and methods employed in these efforts.

2.4.1. Total Synthesis of Bryostatins 2, 3, and 7. (1990–2000)—The first bryostatin to be synthesized was bryostatin 7, which was completed by Masamune and coworkers in 1990.^[35] Evans and colleagues' syntheses of bryostatins 2 (and 6) were reported 8 years later,^[36] with the synthesis of bryostatin 2 also constituting a formal synthesis of bryostatin 1.^[37] Yamamura shortly thereafter reported the preparation of bryostatin 3, which is to date the sole synthesis of a C22-oxygenated congener.^[38] Additionally, a formal total synthesis of bryostatin 7 based on Masamune's approach was reported in 2006 by Hale and coworkers.^[39]

The total syntheses of bryostatins 2, 3, and 7 are grouped as they share several important key fragment disconnections, namely late-stage olefination to link the B- and C-ring substructures via the interceding C16/C17-*E*-olefin and macrolactonization via the C1–C25 ester linkage (Figure 6). The key olefination was accomplished in all three cases by a Julia-type reaction, reacting a lithiated C17-sulfone partner with a C16-aldehyde-containing northern fragment. Although this process capably installed the congested C16/C17-olefin, sensitive functionalities present in the B- and C-rings, notably including the C13–C30 and C21–C34 exocyclic methyl enoate groups, were found to be incompatible with this carbanionic process. As a result, under-functionalized and/or highly protected structural precursors were employed in this olefination, with the required functionality being installed at a late stage. This necessary fragment simplification came at the price of overall convergence; each product required 14 additional linear steps following the union of the respective A-, B-, and C-ring fragments.

2.4.2. Total Synthesis of Bryostatin 16 (2008)—In 2008 Trost and Dong reported the successful synthesis of bryostatin 16, a natural bryostatin with a less complex C-ring

dihydropyran, using a disconnection strategy that was fundamentally distinct from previous syntheses (Figure 7).^[40] Their approach avoided use of the C16/C17-olefin as a strategic fragment disconnection. Instead, the B-ring was constructed via a Ru-catalyzed Alder-ene/oxy-Michael sequence between alkyne **I** and olefin **II**, and the C-ring was subsequently formed via a Pd-catalyzed alkyne/alkyne coupling reaction followed by a gold-catalyzed cycloisomerization. This synthesis is notable for its overall brevity (41 total steps, 28 longest linear sequence (LLS)) and use of highly selective bond-forming reactions. However, because bryostatin 16, which is different in C-ring structure from the other bryostatins, is significantly less active (PKC K_i =118 nM), the issue of supply of bryostatin 1 or similarly potent natural or designed systems remains to be addressed. The low affinity of bryostatin 16 relative to the potent C-ring tetrahydropyranyl family (Figure 4) is likely due to its lack of a C19-hydroxyl group, which is thought to play an important role in mediating bryostatin's affinity to PKC (vide infra). Adaptation of this route to provide the more highly bioactive natural congeners is ongoing.^[41]

2.4.3. Total synthesis of bryostatin 1 (2010)—The most recent total synthesis is the Keck group 2010 synthesis of bryostatin 1^[42] (Figure 8), which follows on recent analog studies.^[43] Their approach also avoided using the C16/C17-olefin as a strategic disconnection and instead employed first an intermolecular Prins cyclization reaction between a northern fragment hydroxyallylsilane and a southern fragment aldehyde to provide the corresponding B-ring pyran product and then closed the macrocycle by lactone formation. Significantly, the Prins reaction tolerated a high level of functionality in the A- and C-ring fragments and was in that regard an impressive extension of Keck's earlier work on more simplified coupling partners. As in the syntheses of bryostatins 2, 3, and 7, a macrolactonization reaction was used to furnish the macrocycle, and further derivatization ultimately provided bryostatin 1 in approximately 31 linear and 57 total steps.

Several additional groups have contributed notably to this field, including those of Thomas,^[44] Vandewalle,^[45] Roy,^[46] Burke,^[47] Krische,^[48] Hoffmann,^[49] Yadav,^[50] and others. Impressive progress continues to be made that could eventually impact clinical supply. At present, published syntheses of highly potent (PKC K_i <10 nM) bryostatins have required >55 total steps and are thus not yet readily adaptable to large-scale preparation. More generally, the clinical studies on bryostatin 1 have now uncovered potential issues such as dose-limiting myalgia that argue for the need for more readily available agents that would not exhibit these off-target effects.

3. Application of FOS to Bryostatin

3.1. Elucidation of Bryostatin's Pharmacophore for PKC

To circumvent issues related to the bryostatin supply problem while providing opportunities for improved therapeutic function, our group has followed a FOS strategy to design simplified bryostatin analogs that exhibit biological activities comparable or superior to the natural product and that can also be accessed in a step-economical manner. The successful application of FOS in this case relied initially on generating hypotheses about which structural elements are required for bryostatin's activity, so that these elements could be incorporated into a simplified molecular scaffold.

The elucidation of bryostatin's putative pharmacophore was informed by several studies.^[11b] A first observation was that most bryostatins, which differ primarily in substitution at C7 and C20 (Figure 4), possess similar affinity for PKC, suggesting against a unique interaction of these sidechains with PKC. Additionally, in our early collaboration with Pettit and Blumberg, we found that hydrogenation of bryostatin 2's B-ring C13–C30 enoate motif and/or C20-octadienoate sidechain had little effect on potency, whereas hydrogenation of the C-

ring C21–C34 enoate significantly diminished affinity.^[51] Similarly, epoxidation of the B-ring enoate in bryostatin 4 had minimal impact on PKC affinity. In contrast, changes to the C26-(*R*)-hydroxyethyl group had a large effect on potency. Inversion of configuration resulted in a 23-fold loss in activity, and acylation completely abrogated specific binding. Thus, while C7/C20-sidechain and B-ring derivatization is well tolerated, changes to the C-ring structure or derivatization of the C26-hydroxyl group causes loss of activity, suggesting the functional importance of these motifs.

Structural comparison of bryostatin 1 with other PKC C1 ligands (Figure 3) also aided in this analysis. Given that these ligands share a common binding site, we reasoned that they might also share a common spatial orientation of a subset of atoms that control affinity, the obvious first candidate being a common hydroxyalkyl group. We had previously suggested a possible pharmacophore for the phorbol esters, in which the C3/C4-, C9-, and C20-hydroxyl groups are preferred candidates for controlling affinity to PKC.^[11a] We therefore compared the spatial disposition of all heteroatomic triads present in bryostatin with those present in the phorbol esters, and found that bryostatin's C1-carbonyl oxygen, C19-hydroxy group, and C26-hydroxyl group gave the best comparative overlay (RMSD=0.16 Å).^[11b] This array also compared favorably with heteroatom triads present in other PKC ligands, including the endogenous diacylglycerols. Taken with the aforementioned structural derivatization studies, it was therefore proposed that bryostatin's C1-carbonyl oxygen, C19-hemiketal hydroxyl group, and C26-hydroxyl group comprise a preferred pharmacophore for its affinity to PKC. More generally, this analysis suggested that bryostatin's southern C-ring region is in direct contact with the PKC C1-domain binding pocket, thus serving as its “recognition domain.” Additionally, this analysis taken in conjunction with crystallographic information^[23] suggested that the A- and B-ring regions act as a scaffolding element, or “spacer domain,” that conformationally controls the spatial orientation of bryostatin's pharmacophore and possibly influences translocation and membrane association (see Figure 1). It was thus proposed that the binding affinity of bryostatin could be achieved with structurally simplified scaffolds having a bryostatin-like recognition domain incorporated into a simplified macrocyclic structure that retains the overall conformational preference of the natural product (Figure 9).^[52] In creating a simpler structure, we initially elected to replace the B-ring pyran with a 1,3-dioxane,^[53] whose retrosynthetic disconnection with the lactone C–O bond would by design produce two fragments of comparable size, allowing for a mild, convergent synthesis thus minimizing post ring closure functionality adjustments. This avoided many problems encountered in prior approaches to the bryostatins and set the stage for mild macroacetalization and Prins macrocyclization strategies.

3.2. Design and Synthesis of Bryostatin Analogs

3.2.1. Synthesis of the First Functional Bryostatin Analogs—In 1998, we reported the synthesis of the first simplified, functionally potent bryostatin analog **1**,^[7] which was designed according to the principles outlined above. By application of the retrosynthetic strategy depicted in Figure 9, analog **1** was envisioned to arise from a convergent coupling of simplified spacer domain **2** and functionalized recognition domain **3** by esterification followed by acid-catalyzed macrotransacetalization.

The synthesis of C1–C13 spacer domain **2** began with bis-olefin **4** and is outlined in Scheme 1. Reductive ozonolysis provided 1,3,5-pentanetriol that was resolved with menthone^[54] and oxidized to afford aldehyde **5**. A hetero-Diels–Alder reaction between **5** and Danishefsky's diene^[55] was effectively catalyzed by Jacobsen's chromium catalyst **6**,^[56] overcoming the inherent substrate bias. Reduction, vinylation, and Claisen rearrangement provided aldehyde **9**, and Brown-type allylation^[57] installed the C1/C2 fragment with excellent C3

stereocontrol. Oxidative cleavage provided the fully elaborated carboxylic acid spacer domain fragment in 11 overall steps and ~11% overall yield.

The C15–C27 recognition domain **3** was synthesized as outlined in Scheme 2. Aldol reaction between the dienolate of ketone **11** and (+)-methyl lactate-derived aldehyde **12** followed by dehydrative cyclization provided a mixture of separable pyranones **13**. Luche reduction followed by epoxidation and in situ methanolysis afforded a dihydroxyketal, which upon selective C21 benzylation followed by oxidation provided α -benzoyloxyketone **14**. SmI_2 -mediated deoxygenation provided ketone **15**, and a 3-step formal condensation with methyl glyoxylate installed the C21-enoate moiety. Luche reduction and octanoylation furnished ester **17**, and C17-desilylation followed by Dess–Martin oxidation^[58] gave aldehyde **19**. Homologation of **19** to enal **20** proceeded in 4 steps via allylation/acylation followed by oxidative cleavage and E1_{CB} elimination. Removal of the C25–OPMB group and C19-hydrolysis thus afforded recognition domain **3** in 29 total steps and in ~2% overall yield over a 24 step linear sequence from (+)-methyl lactate.

Recognition domain **3** was coupled to spacer domain **22** in 81% yield using Yamaguchi's esterification,^[59] and C3-desilylation afforded macrocyclization precursor **23** (Scheme 3). Macrotransacetalization, a key strategic step, was accomplished by treating a dilute solution of **23** with Amberlyst-15 acidic resin over 4 Å molecular sieves, thereby installing the B-ring dioxane motif with concomitant formation of the 20-member macrocycle. A single C15-diastereomer was obtained, consistent with formation of the thermodynamically favored diequatorial acetal. Importantly, only one post-cyclization step, hydrogenation to remove the C26–OBn ether, was needed to afford bryolog **1**.

In accord with our pharmacophore hypothesis, the designed bryostatin analog **1** exhibited excellent affinity for PKC ($K_i=3.4$ nM), which is similar to bryostatin **1** ($K_i=1.4$ nM). Notably, **1** was the first functional analog of bryostatin to be prepared by total chemical synthesis, and was prepared in 51 total steps and ~1% overall yield over a 28-step longest linear sequence. This was at the time >25 steps shorter than the most concise total synthesis of a natural bryostatin. Significantly, **1** exhibited potent in vitro growth inhibitory activity against several human carcinoma cell lines ($1.8\text{--}170$ ngmL⁻¹), and subsequent testing by the National Cancer Institute (NCI) against their 60-cell line panel of human cancers indicated that analog **1** possesses growth inhibitory properties on par with, and in some cases several orders of magnitude superior to, bryostatin **1**. Thus, comparable and in some assays superior function was achieved with a designed simplified structure.

Significantly, the relatively mild conditions required for our late-stage esterification/macrotransacetalization approach tolerated the full complement of C-ring functionality, thereby minimizing late-stage functional group manipulations and maximizing overall convergency. Moreover, the modular nature of this fragment coupling approach rendered it highly amenable to library synthesis, as new analogs could be prepared by coupling recognition domain **3** to a structurally diverse set of C1–C13 spacer domain elements, thereby enabling rapid development of structure-function relationships that would be more difficult to uncover by derivatization of the natural product or adaptation of a total synthesis.

This advantage is exemplified by the facile preparation of simplified bryostatin analogs **26–30** (Figure 10),^[52] which helped elucidate those structural features that are required for selective binding to PKC. For example, crystallographic and NMR solution studies suggest that bryostatin's C3–OH moiety is central to a transannular intramolecular hydrogen bond network, accepting a hydrogen bond from the C19–OH and donating a bifurcated hydrogen bond to the A- and B-ring pyranyl ethers (see Figure 1).^[23,60] To explore the role of this group, we prepared spacer domain **24**, which possesses an inverted C3-stereocenter, and

spacer domain **25**, which lacks the C3-hydroxyl altogether. Using processes analogous to those presented in Scheme 3, these domains were esterified with recognition domain **3** and further elaborated into the corresponding analogs **26** and **27**. Both bryologs were found to be significantly less potent than analog **1**, an observation consistent with the notion that the C3-hydroxyl group plays an important conformational role. Additionally, analog **28**, which retains the C1, C19, and C26 pharmacophoric elements but lacks macrocyclic constraints, also lacks significant PKC affinity. Taken together, the activities of analogs **1** and **26–28** suggest that the spacer domain element is indeed crucial for properly orienting pharmacophoric elements. Lastly, in agreement with our earlier natural product derivatization studies (Section 3.1), oxidation (e.g., **29**) or acylation (e.g., **30**) of the C26-hydroxyl group resulted in abrogation of PKC affinity.

3.2.2. Second Generation Analog Synthesis: C26 Des-Methyl Analog—Our pharmacophoric analysis suggested that the C26-hydroxyl group present in the bryostatins corresponds to the C20-hydroxyl in the phorbol esters, the C24-hydroxyl in teleocidin, and the C3-hydroxyl in the endogenous diacylglycerols (Figure 3). However, it was noted that the hydroxyl groups in these scaffolds are all primary, whereas the C26-hydroxyl group in bryostatin is secondary. Intrigued by the possible impact this structural difference might have on bioactivity, we targeted C26-*des*-methyl analog **31**. By elimination of the C26 stereocenter, it was anticipated that **31** could ultimately be accessed in a fewer number of steps than **1**.

A preliminary synthesis of C26-*des*-methyl recognition domain **32** commenced with intermediate **17**, which had been previously utilized in the synthesis of C26-methyl recognition domain **3** (Scheme 4).^[61] Oxidative cleavage of the C25/C26-glycol motif followed by olefination and dihydroxylation formally removed the C26-methyl group. A 4-step process led to enal **38** from aldehyde **37**, and HF-induced C19-hydrolysis/desilylation followed by re-silylation provided recognition domain **32**.

Recognition domain **32** was coupled to previously described spacer domain **2** using Yamaguchi's esterification,^[59] and subsequent treatment with anhydrous HF-pyridine induced both macrotransacetalization and global desilylation to afford *des*-methyl bryolog **31**. Relative to the sequence originally reported for analog **1** (Scheme 3), this convenient reaction reduced the number of post-fragment-coupling steps from 3 to 1. Significantly, this analog was found to be more potent than bryolog **1**, exhibiting *picomolar* affinity (PKC K_i =0.25 nM). Similar to analog **1**, C26-*des*-methyl analog **31** was found to possess activities orders of magnitude better than bryostatin **1** when tested against the NCI's 60 cell line panel of human cancers.

Justified by its remarkable potency, we sought more efficient synthetic access to **31** and other related and potentially superior analogs. Toward this end, we reported in 2002^[62] a practical and efficient revised synthesis of recognition domain **32** (Scheme 5). Monoprotection of diol **39** and oxidation provided aldehyde **40**, and treatment with the Grignard reagent derived from 4-chloro-1-butanol followed by oxidation and asymmetric allylation^[63] gave homoallylic alcohol **41**. Dehydrative cyclization, in situ epoxidation/methanolysis, and oxidation provided ketone **42**. The C21-enoate motif was then installed in one step by direct aldol condensation with methyl glyoxylate (avoiding the three-step protocol used en route to **3**), and the resulting enone was reduced and octanoylated to provide ester **44**. C17-aldehyde **46** was accessed in two steps by desilylation/oxidation and, in a much more step-economical manner than our original 4-step process (Scheme 2 and 4), was homologated to enal **47** in one step by treatment with the vinyl zincate reagent derived from Me_2Zn and 1-lithio-2-ethoxyethylene followed by acidic hydrolysis. Dihydroxylation of the C25/C26-olefin followed by C19 hydrolysis and C26 silylation furnished recognition

domain fragment **32**. This revised sequence provided **32** in ~3% overall yield over 17 steps, 12 fewer total steps than was required for our original preparation of C26-methyl homolog **3**.

Additionally, in 2003^[64] we reported a concise synthesis of spacer domain **50** (Scheme 6), a fragment amenable to the synthesis of analog **1** or **31**. In contrast to our synthesis of spacer domain **2**, whose chirality originated from a (–)-menthone-based resolution of 1,3,5-pentanetriol, the chirality of **50** was derived from a highly selective Noyori-type catalytic hydrogenation^[65] of ketone **53**, prepared in one step from 4-benzyloxy-2-butanol and methyl glutaryl chloride. Lactonization of resulting diol **54** and subsequent silyl protection provided lactone **55**. Reaction of **55** with the dienolate of ethyl acetoacetate followed by C9-deoxygenation provided *syn* tetrahydropyran **56**. A second asymmetric hydrogenation gave β-hydroxyester **57**, and debenzylation followed by ethyl ester reduction generated a triol that was subsequently protected as its acetonide **58**. Oxidation then provided spacer domain **50**, which was thereby accessed in 10 steps and 25% overall yield. Esterification of **50** with recognition domain **32** followed by HF-pyridine-mediated macrotransacetalization/desilylation afforded analog **31** in 29 total steps and 2% overall yield over a 19-step linear sequence. This was at the time 50 fewer steps than the most concise bryostatin total synthesis, and remains 30 fewer steps than is currently required for a bryostatin of comparable potency.

The clear view that emerged from these studies is that the PKC affinity of bryostatin could be reliably emulated by retaining the features of the southern “recognition” domain, while greatly simplifying the northern “spacer” domain. Leads in this series are currently undergoing preclinical investigation for their use in the treatment of cancer, Alzheimer's disease, and HIV/AIDS eradication.

3.2.3. Des-A-Ring Bryostatin Analogs—Concurrent with the synthetic efforts that led to **1** and **31**, we investigated whether even more simplified spacer domains would serve as scaffolds for achieving high PKC affinity, potentially further reducing step count while maintaining function. Since the B-ring dioxane served as an important synthetic handle for our macrotransacetalization process, we elected to retain that functionality and investigate analogs with simplified A-ring substructures. As described above, we reasoned that retention of the C5–C9 ether linkage would be important to preserve the intramolecular hydrogen bond network that enforces a preferred macrocycle conformation. However, the functional role of the remaining A-ring structure (carbons C6 through C8) was unknown, other than its contribution to conformational rigidity and/or lipophilicity. We therefore targeted *des*-A-ring spacer domains of type **60–63**^[3a,66,67] to explore this hypothesis. By eliminating the C6–C8 linkage, up to two stereocenters could be deleted, thereby facilitating access to these fragments. In spacer domains **61–63** a bulky C9-substituent was incorporated in place of the A-ring for conformational control; modeling studies suggested that this substituent would reinforce the inherent conformational preference imposed by the intramolecular hydrogen bond network by assuming a pseudo-equatorial position on the macrocycle wherein *gauche* interactions with the C11-position would be minimized.

The synthesis of these *des*-A-ring domains began with the menthone-derived 1,3,5-pentanetriol **64** (Scheme 7). Alkylation with allyl bromide provided ether **72** en route to the C9-unsubstituted domain **60**. Alternatively, oxidation followed by treatment with various nucleophiles and subsequent manipulation gave the corresponding C9-substituted derivatives **65–67**. Alkylation with allyl bromide followed by hydroboration and oxidation provided the corresponding aldehydes **76–79**, and Brown-type allylation^[57] followed by protection and oxidation yielded the desired *des*-A-ring spacer domains **60–63**.

As before, the coupling of spacer domains **60–63** with recognition domain **32** proceeded smoothly using either Yamaguchi's esterification or PyBro-P mediated coupling (Scheme 8). Further demonstrating the generality of this B-ring building step, macrotransacetalization and global desilylation induced with anhydrous HF·pyridine provided analogs **84–87**.

Although analogs **84–87** are modestly less potent than the parent A-ring pyran analog **31**, their affinities for PKC are on par with the natural product, demonstrating that the bryostatin spacer domain is an important scaffolding element and that, so long as critical conformational elements are retained (e.g., macrocyclic structure, C5–C9 and C11–C15 H-bond accepting ether linkages, C3-hydroxyl group), high PKC affinity can be obtained. Additionally, given the good tolerance of different C9-substituents, this study suggests that the A-ring region might serve as a tunable site that could be modulated to achieve various activity and pharmacokinetic goals distinct from target affinity. A subsequent route to spacer domains such as **60–63** was identified that requires only 7 steps,^[68] in line with the step-economy goals of FOS.

3.2.4. Functionalized A-Ring Bryostatin Analogs—The work presented above demonstrates that a primary role of bryostatin's northern fragment is to serve as a scaffold for proper orientation of southern fragment recognition domain elements. However, based on our studies examining the biological functions of analogs **1**, **31**, **84–87**, and other related bryologs, we became intrigued by the possibility that northern fragment functionality might influence analog activity in a manner not entirely coupled to PKC affinity. In particular, we sought to determine whether A-ring structure might modulate the translocation selectivity of our agents for different isoforms (or subtypes) of PKC, of great significance given the connection of isoform modulation with various diseases.^[69]

Toward this end, we designed C8-*gem* dimethyl analogs **88–90**, which possess differential substitution at the C7-position (Figure 11).^[70] We were interested in the role of C7 functionality based on the observation that, despite their similar PKC affinities, bryostatins **1** and **2**, which differ only at the C7 position, have markedly different abilities to translocate conventional PKC isoforms. Additional studies from our lab suggested the influence of the A-ring region on PKC isoform selectivity^[71] and that C7-functionality can have significant impact on potency.^[72] For example, C7-OAc analog **91** is a highly potent PKC ligand ($K_i=13$ nM), whereas C7-OH analog **92** is orders of magnitude less potent ($K_i=1000$ nM).

Analog **88–90** were obtained from common A-ring lactone intermediate **93** (Scheme 9). A diastereoselective aldol reaction between the isopinocampheyl-derived boron enolate of **94**^[73] and aldehyde **95**^[74] provided hydroxyketone **96** with 9 : 1 *anti* selectivity, and subsequent *anti*-reduction^[75] followed by lactonization provided hydroxylactone **93**. This intermediate was then silylated for the synthesis of C7-oxy functionalized analogs **88** and **89** whereas a two-step radical-based deoxygenation procedure^[76] gave lactone **99** towards C7-deoxy analog **90**. Lactones **98** and **99** were converted to spacer domains **104** and **105** using protocols similar to those described for spacer domain **50**. Addition of the ethyl acetoacetate dienolate followed by deoxygenation gave pyrans **100** and **101**, and asymmetric hydrogenation followed by acetalization provided acetonides **102** and **103**. Debenzylation and oxidation furnished the desired spacer domains **104** and **105**.

Coupling of these spacer domains to recognition domain **32** was accomplished using Yamaguchi's esterification, and treatment with HF·pyridine effected macrotransacetalization and desilylation to afford bryostatin 2-like C7-OH analog **89** and C7-deoxy analog **90**. Conversion of C7-OH analog **89** into bryostatin 1-like C7-OAc analog **88** was then performed in two steps via *in situ* silylation of the C26 hydroxyl group followed by C7 acylation and subsequent desilylation.

We found that C8 *gem*-dimethyl analogs **88**, **89**, and **90** possess PKC K_i 's of 2.0 nM, 19 nM, and 1.4 nM, respectively, indicating that the C8 *gem*-dimethyl group enhances PKC affinity for analogs possessing polar functionality at the C7 position. This effect was most significant for C7-OH analog **89**, which is 50-fold more potent than C8 *des*-methyl analog **92** (K_i =1000 nM).

Analogues **88–90** were then evaluated for their abilities to translocate PKC δ -GFP and PKC β 1-GFP in CHO-k1 cells (Figure 12).^[77] While all three C8 *gem*-dimethyl analogs showed significant activation of the novel PKC δ isoform (Plot A), they showed a differential ability to translocate the conventional PKC β 1 isoform (Plot B). While C7-OAc analog **88** and C7-deoxy analog **90** were able to translocate PKC β 1 at a 200 nM concentration (Panels A–C and D–E), the rate and extent of translocation were attenuated compared to bryostatin 1. On the other hand, the C7-OH analog **89** induced minimal translocation of PKC β 1 when dosed at 200 nM, despite its activity against PKC δ at the same concentration.

These results demonstrate that the A-ring of bryostatin can be modified to modulate the selectivity for different PKC isoforms. This study further demonstrates that simplified bryostatin analogs can be prepared in a step-economical fashion that possess biological activity comparable or superior to bryostatin 1, while also tuning for activities that are similar or complementary to those exhibited by the natural product.

3.2.5. FOS and New Methodology: A Prins-Driven Macrocyclization—The promising bioactivities of the analogs presented in previous sections clearly demonstrate that a B-ring dioxane motif is an excellent surrogate for bryostatin's B-ring pyran substructure. The value of this designed substitution is apparent in the modularity and overall convergence of our synthetic strategy. This design choice thus simultaneously served to enable rapid access to function and to create methodological opportunity. Given the notable activity of the analogs and the step economy of this strategy, we next sought access to the natural B-ring pyran system so that the influence of this motif on biological activity could be studied (Figure 13).

The primary synthetic challenge associated with accessing the B-ring pyran architecture was doing so in a manner that would conserve the convergency of our strategy for accessing the B-ring dioxane architecture. Thus, we sought a late-stage fragment coupling approach that would be sufficiently mild to tolerate the breadth of functionality present in the C-ring recognition domain fragment. Additionally, given that we had developed a practical, multigram-scale approach to recognition domain **32**, we hoped this fragment or a close derivative could be used as a coupling partner.

Synthetic studies directed toward the natural bryostatins and related model structures provided insight as to the repertoire of methodology that might fulfill these requirements. For example, late-stage application of an intra- or intermolecular olefination to form the C16/C17-E-olefin was expected to be problematic without undesirable simplification of the recognition domain (See Section 2.4). Additionally, though the bryopyran architecture seems to be perfectly suited for an esterification/ring closing metathesis (RCM) strategy,^[78] the application of RCM in synthetic studies aimed at the bryostatins has met with difficulty (Figure 14). For example, although Thomas and coworkers found that bryostatin-like cyclization precursor **106** underwent macrocyclization in modest (25–35%) yield on exposure to Grubbs' generation II catalyst, the corresponding C18-*gem* dimethyl variant **108** failed to react similarly.^[79] This difference in reactivity is likely attributable to the steric congestion associated with the C18-dimethyl group and is consistent with Trost and coworkers' attempted macrocyclization of **110**, a planned precursor to bryostatin 7.^[80] In that case, even a relay ring closing metathesis (RRCM)^[81] strategy failed to generate the

desired 20-member macrocycle; alternatively, ring-expanded product **111** was obtained as a mixture of olefin stereoisomers.

Given the difficulties associated with the late-stage formation of the highly congested C16/C17-olefin, we elected to access the B-ring pyran architecture in a manner that was strategically and mechanistically based on our previously described macrotransacetalization reaction (Figure 15), which putatively proceeds via generation of an oxocarbenium ion at C15 and subsequent capture by a nucleophilic hydroxyl group. We reasoned that substitution of the C13-oxygen nucleophile with a suitable carbon nucleophile would provide direct access to the B-ring tetrahydropyran.^[82] This strategy had the practical benefit of directly employing our previously described aldehyde C-ring fragment **32**, thereby obviating the need for a modified C-ring synthesis.

The intramolecular capture of an oxocarbenium electrophile with a carbon π -nucleophile to form pyran ring systems is a key step in the Prins cyclization reaction.^[83] This process provides facile access to polysubstituted tetrahydropyran motifs, often with excellent stereocontrol. Although the Prins cyclization is a powerful method for pyran synthesis, its application in a macrocyclization mode, which requires the generation and capture of a macrocyclic oxocarbenium ion, was little explored at the time of our initial investigation. A single 1979 report by Shulte-Elte and coworkers documented this reaction's application to macrocycle formation en route to ($\pm$)-muscone (Figure 16).^[84] In that study, a geometric mixture of open-chain precursors **112** underwent macrocyclization to provide 13-member ring product **113** in 75% yield upon treatment with catalytic *p*-TsOH in refluxing toluene.

Given the precedent of our macrotransacetalization reaction, we anticipated that this novel Prins macrocyclization could be achieved with many carbon nucleophiles. Our study of this intramolecular closure strategy initially focused on an allylsilane nucleophile to capture this macro-oxocarbenium ion and was based on methodology originally reported by Marko^[85] and improved upon by Keck and coworkers for the complementary intermolecular Prins process.^[86] At the time, Keck had additionally applied the intermolecular variant of this cyclization to bryostatin-like systems,^[87] though these did not contain requisite functionality for biological activity.

Our synthesis of hydroxyallylsilane-containing spacer domain **114** commenced with β -hydroxyester intermediate **57** (Scheme 10), which we had previously utilized in the synthesis of B-ring dioxane analog **31** (Scheme 6). Silylation of **57** followed by double nucleophilic addition of TMSCH₂MgCl and Peterson-type olefination gave allylsilane **116**.^[88] Debenzylation with the lithium naphthalenide reagent^[89] afforded alcohol **117** in 93% yield, and two-step oxidation with tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO) followed by NaClO₂ provided the fully elaborated carboxylic acid fragment **114** in 89% yield over 2 steps. Esterification of spacer domain **114** with recognition domain **32** provided C11-protected conjugate **118** in 82% yield, and removal of the C11-OTES group with dilute aqueous PPTS furnished desired macrocyclization precursor **119**.

Gratifyingly, we found that the Prins macrocyclization of **119** is readily achieved in 93% yield upon exposure to trimethylsilyl-triflate (TMS-OTf) in Et₂O at a 5 mM substrate concentration. As found with our macrotransacetalization studies, this highly efficient reaction is completely diastereoselective and affords the desired C11/C15-*cis* disubstituted pyran as the sole observable macrocyclization product. Notably, the diversity of functionality found in bryostatin's C-ring is tolerated, thereby minimizing post cyclization functional group manipulations and offering synthetic access to functionalized bryopyran architectures with an unprecedented level of convergence. This macro-cyclization is one of

the more complex examples of a Prins cyclization in complex molecule synthesis. Global desilylation of bryopyran intermediate **120** with HF·pyridine provided our first B-ring tetrahydropyran bryolog **121** in 77% yield.

To probe the role of B-ring enoate functionality on biological activity, the C13-methylidene in bryopyran **120** was chemoselectively cleaved via stoichiometric ozonolysis (Scheme 11). The chemoselectivity of this process further highlights the steric congestion about the C16/C17-olefin, which is left intact even in the presence of this small reactive agent. The resulting ketone **122** was then olefinated using NaHMDS and trimethyl phosphonoacetate, which proceeded in excellent combined yield (87%) but poor stereoselectivity (49 :51 *E*:*Z*). Alternatively, in accord with Evans's and Yamamura's observations, ketone **122** could be olefinated with Fujii's (*R*)-BINOL derived phosphonoacetate **123**^[90] in 65% unoptimized yield and 25 :75 selectivity favoring the natural product-like *Z*-isomer. Treatment of the 49 :51 *E*:*Z* mixture with HF·pyridine provided an 83% combined yield of the corresponding enoate analogs, which were then separated to afford the naturally-configured *Z*-enoate bryolog **125** and the *E*-enoate bryolog **124**.

We found that B-ring tetrahydropyran analogs **121**, **124**, and **125** are among the most potent we have synthesized. In side-by-side comparison, all three had greater affinity for a reference mixture of PKC isoforms than dioxane analog **31** (Table 1). Additionally, these compounds exhibited excellent activity against the K562 and MV411 human leukemia cell lines. In particular, C13-*Z*-enoate analog **125** was found to be our most potent analog in the PKC affinity assay and was nearly 2 orders of magnitude more potent than dioxane analog **31** against the K562 leukemia cell line.

In addition to highlighting the primary goal of an FOS approach, namely step-economical access to molecular function, this study exemplifies that synthetic challenges and opportunities can arise from and indeed be inspired by FOS studies. For example, the excellent efficiency and selectivity associated with the Prins-driven macrocyclization reaction suggest that this strategy might find broad use in the synthesis of natural products, analogs thereof, or even completely novel structures that contain tetrahydropyran function embedded within a macrocycle. Indeed, just prior to our report describing the synthesis of analogs **121**, **124**, and **125**, Scheidt and coworkers reported a related Prins-macrocyclization approach to neopeltolide (Figure 17).^[91] A similar approach toward the same target appeared shortly thereafter from Lee's group,^[92] and Rychnovsky and Bahnck subsequently utilized a Prins-macrocyclization as a key step in their synthesis of kendomycin.^[93] The value of this complexity-building reaction is apparent, and it is anticipated that it will find continued use in synthesis.^[94,95]

4. Future Directions and Outlook

Over the last 200 years, synthesis has emerged as a powerful tool for supplying many of Nature's compounds—a vast, diverse, and functionally rich library representing 3.8 billion years of chemical evolution. Still, many of Nature's compounds are beyond the current reach of synthesis. This reach will no doubt be extended as new reactions and strategies are developed to achieve practical, step-economical, and green routes to targets of interest. At the same time, however, many targets beyond the current reach of synthesis are in demand now. This demand is often driven by function whether it is related to therapy, diagnostics, imaging, smart materials, nanodevices, conductors, energy, catalysis, or other functions of interest and value. Function-oriented synthesis (FOS) offers a strategy to meet this immediate time-sensitive need and at the same time provides many advantages beyond. It is predicated on the view, widely held from evolutionary biology to architecture, that form follows function. In more chemical terms, many structures can exhibit a specific function,

and these structures in turn can be created through synthesis-informed design. Chemists are uniquely skilled to design new structures that would exhibit optimum function and at the same time be supplied in a practical, step-economical, time-economical, and indeed creative fashion. By placing an emphasis on function, one is liberated from the constraints of a single structural solution to a functional need and allowed to more creatively and fully explore all possible solutions to achieve the best. FOS incorporates all of the opportunities typically associated with natural product synthesis, since design can be inspired by Nature's library and design in turn can inspire new methodology. In the near term, it offers a strategy to supply function, both tunable or new, thereby allowing for the more rapid advancement of science and utilization of the many inspirational functions encoded in Nature's library or derivable from de novo design. FOS makes explicit in terminology and intent the towering importance of function and thus consolidates the many powerful strategies such as diversity-oriented synthesis, diverted total synthesis, target-oriented synthesis, and biology-oriented synthesis that serve variably as a means toward that end. Our bryostatin and phorbol studies are representative of these points. They illustrate clearly that a specific function of an otherwise difficult-to-synthesize natural product could be achieved with significantly simpler or totally different structures in a step-economical, time-economical, and practical fashion through synthesis-informed design. Opportunities in the creation of new methodology (e.g., Prins macrocyclization) are realized, as are opportunities to more rapidly and fully explore the therapeutically promising function of a novel natural lead. Practical syntheses—the shortest to function reported thus far—have thus been achieved of designed compounds that exhibit comparable or superior function relative to Nature's lead and can be tuned as needed for improved function or to create new function. These agents are now in preclinical development for the treatment of cancer, Alzheimer's disease, and for the eradication of HIV/AIDS. Given the vast collection of complex natural products with unique function in Nature's library, and others that we can imagine, this FOS strategy can be expected to accelerate access to, and studies on, many complex systems that exhibit function of interest and value.

Biography

Paul A. Wender is a product of Wilkes College (B.S., 1969), Yale University (Ph.D., 1973 with Ziegler) and Columbia University (NIH postdoctoral fellow with Stork). He joined the faculty at Harvard University in 1974 and subsequently moved to Stanford University where he is the Bergstrom Professor of Chemistry and Professor (by courtesy) of Chemical and Systems Biology. His research interests include new reactions, catalysis, total synthesis, novel chemistry-driven approaches to drug delivery, imaging, disease resistance, HIV/AIDS, cognitive dysfunction, and Alzheimer's disease, with an emphasis on the Ideal Synthesis, step economy, and function-oriented synthesis and design.

Adam Schrier graduated from Hope College (Holland, Michigan) with his B.S. in chemistry in 2004. He joined the group of Professor Paul Wender at Stanford University in 2005 and is currently completing his doctoral studies. His research focuses on the design, total synthesis, and biological evaluation of bryostatin analogs, with an emphasis on the development of Prins-driven macrocyclization strategies to the bryostatin core ring structure.

Brian Loy is a native of Ohio and Illinois. He obtained his B.S. in Chemistry in 2006 from Bethel University (St. Paul, MN), graduating with *summa cum laude* honors. After a one-year contract position in the Science & Technology Department at Medtronic, Inc. (Fridley, MN), he joined the graduate program at Stanford University where he is working towards his Ph.D. under the direction of Professor Paul Wender. In 2009, Brian was included as an inaugural fellow of the Center for Molecular Analysis and Design. Brian's research interests include step-economical and function-oriented synthetic approaches to complex molecule synthesis and NMR studies of protein–ligand interactions.

References

- [1]. For pertinent discussions, see: Wender PA, Miller BL. *Nature*. 2009; 460:197–201. Step economy: [PubMed: 19587760] and references cited therein; Trost BM. *Science*. 1991; 254:1471–1477. Atom economy: [PubMed: 1962206] ; Burns NZ, Baran PS, Hoffmann RW. *Angew. Chem.* 2009; 121:2896–2910. Redox economy: ; *Angew. Chem. Int. Ed.* **2009**, 48, 2854–2867; Sheldon RA. *Chem. Ind.* 1992:903–906. E factor: ; Sheldon RA. *Pure Appl. Chem.* 2000; 72:1233–1246.; Anastas PT, Warner JC. *Green Chemistry Theory and Practice*. 1998Oxford University PressNew York.
- [2]. For a superb overview of successes and concerns regarding natural products as drugs, see: Cragg GM, Grothaus PG, Newman DJ. *Chem. Rev.* 2009; 109:3012–3043. [PubMed: 19422222] ; Carlson E. *ACS Chem. Biol.* 2010; 5:639–653. For an overview of natural products as probes, see: [PubMed: 20509672] ; Mann J. *Nat. Rev.* 2003:143–148. For a related discussion pertinent to the intersection of natural products and new technology, see: .
- [3]. a) Wender PA, Baryza JL, Brenner SE, Clarke MO, Craske ML, Horan JC, Meyer T. *Curr. Drug Discovery Technol.* 2004; 1:1–11. b) Wender PA, Verma VA, Paxton TJ, Pillow TH. *Acc. Chem. Res.* 2008; 41:40–49. [PubMed: 18159936] c) Mooberry S, Hilinski M, Clark E, Wender PA. *Mol. Pharm.* 2008; 5:829–838. [PubMed: 18662015] d) Szpilman AM, Carreira EM. *Angew. Chem.* 2010; 122:9786–9823. *Angew. Chem. Int. Ed.* **2010**, 49, 9592–9628.

- [4]. For some recent examples see: Carpenter RD, Verkman AS. *Eur. J. Org. Chem.* 2011; 2011:1242–1248.; Ii K, Ichikawa S, Al-Dabbagh B, Bouhss A, Matsuda A. *J. Med. Chem.* 2010; 53:3793–3813. [PubMed: 20405928]; Gloeckner C, Garnera AL, Merzhad F, Oksove Y, Tricochee N, Eubanks LM, Lustigmane S, Kaufmanna GF, Janda KD. *Proc. Natl. Acad. Sci. USA.* 2010; 107:3424–3429. [PubMed: 20142509]; Nakai Y, Tepp WH, Dickerson TJ, Johnson EA, Janda KD. *Bioorg. Med. Chem.* 2009; 17:1152–1157. [PubMed: 19131252]; Wrona IE, Lowe JT, Turbyville TJ, Johnson TR, Beignet J, Beutler JA, Panek JS. *J. Org. Chem.* 2009; 74:1897–1916. [PubMed: 19191575]; Rizvi SA, Liu S, Chen Z, Skau C, Pytynia M, Kovar DR, Chmura SJ, Kozmin S. *J. Am. Chem. Soc.* 2010; 132:7288–7290. [PubMed: 20455580]; Seletsky BM, Wang Y, Hawkins LD, Palme MH, Habgood GJ, DiPietro LV, Towle MJ, Salvato KA, Wels BF, Aalfs KK, Kishi Y, Littlefield BA, Yu MJ. *Bioorg. Med. Chem. Lett.* 2004; 14:5547–5550. [PubMed: 15482921]; Andresen BA, Du Bois J. *J. Am. Chem. Soc.* 2009; 131:12524–12525. [PubMed: 19678702]; Nicolaou KC, Li Y, Sugita K, Monenschein H, Guntupalli P, Mitchell HJ, Fylaktakidou KC, Vourloumis D, Giannakakou P, Brate AO. *J. Am. Chem. Soc.* 2003; 125:15443–15454. [PubMed: 14664590]; Nakagawa Y, Yanagita RC, Hamada N, Murakami A, Takahashi H, Saito N, Nagai H, Irie K. *J. Am. Chem. Soc.* 2009; 131:7573–7579. [PubMed: 19449873].
- [5]. For an overview and clinical summary, see: Pettit GR, Herald CL, Hogan F, Baguley BC, Kerr DJ. *Anticancer Drug Development.* 2002:203–235. Academic Press San Diego.
- [6]. Wender, PA.; Baryza, JL.; Hilinski, MK.; Horan, JC.; Kan, C.; Verma, VA. *Drug Discovery Research: New Frontiers in the Post-Genomic Era.* Huang, Z., editor. Wiley, Hoboken: 2007. p. 127-162.
- [7]. Wender PA, De Brabander J, Harran PG, Jimenez J-M, Koehler MFT, Lippa B, Park C-M, Shiozaki M. *J. Am. Chem. Soc.* 1998; 120:4534–4535.
- [8]. For a lead reference, see: Barr PM, Lazarus HM, Cooper BW, Schluchter MD, Panneerselvam A, Jacob-berger JW, Hsu JW, Janakiraman N, Simic A, Dowlati A, Remick SC. *Am. J. Hematol.* 2009; 84:484–487. [PubMed: 19536846].
- [9]. a) Nelson TJ, Alkon DL. *Trends Biochem. Sci.* 2009; 34:136–145. [PubMed: 19233655] b) Sun M, Alkon D. *Arch. Pharm.* 2009; 342:689–698. c) Khan TK, Nelson TJ, Verma VA, Wender PA, Alkon DL. *Neurobiol. Dis.* 2009; 34:332–339. [PubMed: 19233276] d) Sun M-K, Alkon DL. *Pharmacol. Ther.* 2010; 127:66–77. [PubMed: 20382181]
- [10]. Mehla R, Bivalkar-Mehla S, Zhang R, Handy I, Albrecht H, Giri S, Nagarkatti P, Nagarkatti M, Chauhan A. *PLoS ONE.* 2010; 5:e11160. [PubMed: 20585398]
- [11]. a) Wender PA, Koehler KF, Sharkey NA, Dell'Aquila ML, Blumberg PM. *Proc. Natl. Acad. Sci. USA.* 1986; 83:4214–4218. [PubMed: 3086877] b) Wender PA, Cribbs CM, Koehler KF, Sharkey NA, Herald CL, Komano Y, Pettit GR, Blumberg PM. *Proc. Natl. Acad. Sci. USA.* 1988; 85:7197–7201. [PubMed: 3174627]
- [12]. A now dated but important account of the early years was edited by Matthew Suffness, a key proponent of Taxol therapy, who regrettably died of cancer at an early age, see: Suffness M. *Taxol: Science and Applications.* 1995 CRC Press Boca Raton.
- [13]. Lopanik N, Lindquist N, Targett N. *Oecologia.* 2004; 139:131–139. [PubMed: 14747940]
- [14]. a) Kutchukian PS, Yang JS, Verdine GL, Shakhnovich EI. *J. Am. Chem. Soc.* 2009; 131:4622–4627. [PubMed: 19334772] b) Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD, Wagner G, Verdine GL, Korsmeyer SJ. *Science.* 2004; 305:1466–1470. [PubMed: 15353804] c) Schafmeister CE, Po J, Verdine GL. *J. Am. Chem. Soc.* 2000; 122:5891–5892.
- [15]. For an impressive recent example, see: Barjau J, Schnakenburg G, Waldvogel SR. *Angew. Chem.* 2011; 123:1451–1455. *Angew. Chem. Int. Ed.* 2011, 50, 1415–1419. and references cited therein.
- [16]. a) Tan DS. *Nat. Chem. Biol.* 2005; 1:74–84. [PubMed: 16408003] b) Nielsen TE, Schreiber SL. *Angew. Chem.* 2008; 120:52–61. *Angew. Chem. Int. Ed.* 2008, 47, 48–56 and references cited therein.
- [17]. Bon RS, Waldmann H. *Acc. Chem. Res.* 2010; 43:1103–1114. [PubMed: 20481515]
- [18]. Njardarson JT, Gaul C, Shan D, Huang X-Y, Danishefsky SJ. *J. Am. Chem. Soc.* 2004; 126:1038. [PubMed: 14746469]

- [19]. Nett M, Gulder TAM, Kale AJ, Hughes CC, Moore BS. *J. Med. Chem.* 2009; 52:6163–6167. [PubMed: 19746976]
- [20]. Wender PA, Rice KD, Schnute ME. *J. Am. Chem. Soc.* 1997; 119:7897–7898. and references therein
- [21]. Pettit GR. *J. Nat. Prod.* 1996; 59:812–821. [PubMed: 8792630]
- [22]. Pettit GR, Day JF, Hartwell JL, Wood HB. *Nature.* 1970; 227:962–963. [PubMed: 4393654]
- [23]. Pettit GR, Herald CL, Doubek DL, Herald DL, Arnold E, Clardy J. *J. Am. Chem. Soc.* 1982; 104:6846–6848.
- [24]. a) Clamp A, Jayson GC. *Anti-Cancer Drugs.* 2002; 13:673–683. [PubMed: 12187323] b) Newman DJ, Cragg GM. *J. Nat. Prod.* 2004; 67:1216–1238. [PubMed: 15332835]
- [25]. a) Sun MK, Hongpaisan J, Nelson TJ, Alkon DL. *Proc. Natl. Acad. Sci. USA.* 2008; 105:13620–13625. [PubMed: 18768786] b) Sun MK, Hongpaisan J, Alkon DL. *Proc. Natl. Acad. Sci. USA.* 2009; 106:14676–14680. [PubMed: 19667190]
- [26]. For an excellent lead reference, see: Trindade-Silva AE, Lim-Fong GE, Sharp KH, Haygood MG. *Curr. Opin. Biotechnol.* 2010; 21:834–842. [PubMed: 20971628] .
- [27]. a) <http://clinicaltrials.gov> b) Kortmansky J, Schartz GK. *Cancer Invest.* 2003; 21:924–936. For a review of bryostatins clinical efficacy prior to 2003, see: [PubMed: 14735696]
- [28]. Stang SL, Lopez-Campistrous A, Song X, Dower NA, Blumberg PM, Wender PA, Stone JC. *Exp. Hematol.* 2009; 37:122–134. [PubMed: 19100522]
- [29]. Several C1-domain-containing proteins are possible targets for bryostatin, see: Kazanietz MG. *Mol. Pharmacol.* 2002; 61:759–767. [PubMed: 11901214] .
- [30]. For reviews of PKC, see: Newton AC. 2001; 101:2353–2364.; Gallegos LL, Newton AC. *IUBMB Life.* 2008; 60:782–789. [PubMed: 18720411] .
- [31]. Schaufelberger DE, Koleck MP, Beutler JA, Vatakis AM, Alvarado AB, Andrews P, Marzo LV, Muschik GM, Roach J, Ross JT, Lebherz WB, Reeves MP, Eberwein RM, Rodgers LL, Testerman RP, Snader KM, Forenza S. *J. Nat. Prod.* 1991; 54:1265–1270. [PubMed: 1800630]
- [32]. Mendola D. *Biomol. Eng.* 2003; 20:441–458. [PubMed: 12919831]
- [33]. a) Hildebrand M, Waggoner LE, Liu H, Sudek S, Allen S, Anderson C, Sherman DH, Haygood M. *Chem. Biol.* 2004; 11:1543–1552. [PubMed: 15556005] b) Sudek S, Lopanik NB, Waggoner LE, Hildebrand M, Anderson C, Liu H, Patel A, Sherman DH, Haygood MG. *J. Nat. Prod.* 2007; 70:67–74. [PubMed: 17253852]
- [34]. For recent reviews of synthetic approaches to the bryostatins, see: Hale KJ, Hummersone MG, Manaviazar S, Frigerio M. *Nat. Prod. Rep.* 2002; 19:413–453. [PubMed: 12195811] ; Hale KJ, Manaviazar S. *Chem. Asian J.* 2010; 5:704–754. [PubMed: 20354984] .
- [35]. Kageyama M, Tamura T, Nantz MH, Roberts JC, Somfai P, Whitenour DC, Masamune S. *J. Am. Chem. Soc.* 1990; 112:7407–7408.
- [36]. a) Evans DA, Carter PH, Carreira EM, Prunet JA, Charette AB, Lautens M. *Angew. Chem.* 1998; 110:2526–2530. *Angew. Chem. Int. Ed.* 1998, 37, 2354–2359. b) Evans DA, Carter PH, Carreira EM, Charette AB, Prunet JA, Lautens M. *J. Am. Chem. Soc.* 1999; 121:7540–7552.
- [37]. Pettit GR, Sengupta D, Herald CL, Sharkey NA, Blumberg PM. *Can. J. Chem.* 1991; 69:856–860.
- [38]. Ohmori K, Ogawa Y, Obitsu T, Ishikawa Y, Nishiyama S, Yamamura S. *Angew. Chem.* 2000; 112:2376–2379. *Angew. Chem. Int. Ed.* 2000, 39, 2290–2294.
- [39]. Manaviazar S, Frigerio M, Bhatia GS, Hummersone MG, Aliev AE, Hale KJ. *Org. Lett.* 2006; 8:4477–4480. [PubMed: 16986929] and references therein
- [40]. Trost BM, Dong G. *Nature.* 2008; 456:485–488. [PubMed: 19037312]
- [41]. Trost BM, Dong G. *J. Am. Chem. Soc.* 2010; 132:16403–16416. [PubMed: 21043491]
- [42]. Keck GE, Poudel YB, Cummins TJ, Rudra A, Covell JA. *J. Am. Chem. Soc.* 2011; 133:744–747. [PubMed: 21175177]
- [43]. a) Keck GE, Kraft MB, Truong AP, Li W, Sanchez CC, Lewin NE, Blumberg PM. *J. Am. Chem. Soc.* 2008; 130:6660–6661. [PubMed: 18452293] b) Keck GE, Poudel YB, Welch DS, Kraft MB, Truong AP, Stephens JC, Keddi N, Lewin NE, Blumberg PM. *Org. Lett.* 2009; 11:593–596. [PubMed: 19113896] c) Keck GE, Li W, Kraft MB, Keddi N, Lewin NE, Blumberg PM. *Org.*

- Lett. 2009; 11:2277–2280. [PubMed: 19419164] d) Keck GE, Poudel YB, Rudra A, Stephens JC, Kedei N, Lewin NE, Peach ML, Blumberg PM. *Angew. Chem. Int. Ed.* 2010; 49:4580–4584.
- [44]. a) Allen JV, Green AP, Hardy S, Heron NM, Lee ATL, Thomas EJ. *Tetrahedron Lett.* 2008; 49:6352–6355. b) Ball M, Bradshaw BJ, Dumunier R, Gregson TJ, MacCormick S, Omori H, Thomas EJ. *Tetrahedron Lett.* 2006; 47:2223–2227.
- [45]. DeBrabander J, Vandewalle M. *Pure Appl. Chem.* 1996; 68:715–718.
- [46]. Roy R, Rey AW, Charron M, Molino R. *Chem. Commun.* 1989:1308–1310.
- [47]. a) Voight EA, Seradj H, Roethle PA, Burke SD. *Org. Lett.* 2004; 6:4045–4048. [PubMed: 15496095] b) Voight EA, Roethle PA, Burke SD. *J. Org. Chem.* 2004; 69:4534–4537. [PubMed: 15202915]
- [48]. a) Lu Y, Krische MJ. *Org. Lett.* 2009; 11:3108–3111. [PubMed: 19586066] b) Cho CW, Krische MJ. *Org. Lett.* 2006; 8:891–894. [PubMed: 16494467]
- [49]. Vakalopoulos A, Lampe TJF, Hoffmann HMR. *Org. Lett.* 2001; 3:929–932. [PubMed: 11263918]
- [50]. Yadav JS, Bandyopadhyay A, Kunwar AC. *Tetrahedron Lett.* 2001; 42:4907–4911.
- [51]. Pettit GR, Sengupta D, Blumberg PM, Lewin NE, Schmidt JM, Kraft AS. *Anti-Cancer Drug Des.* 1992; 7:101–113.
- [52]. Wender PA, De Brabander J, Harran PG, Jiminez JM, Koehler MFT, Lippa B, Park CM, Siedenbiedel C, Pettit GR. *Proc. Natl. Acad. Sci. USA.* 1998; 95:6624–6629. [PubMed: 9618462]
- [53]. For additional and impressive examples of this dioxane design opportunity, see: Smith AB, Razler TM, Meis RM, Pettit GR. *Org. Lett.* 2006; 8:797–799. [PubMed: 16468770] ; Osman S, Albert BJ, Wang Y, Li M, Czaicki NL, Koide K. *Chem. Eur. J.* 2011; 17:895–904. [PubMed: 21226105] ; Lagisetti C, Pourpak A, Goronga T, Jiang Q, Cui X, Hyle J, Lahti JM, Morris SW, Webb TW. *J. Med. Chem.* 2009; 52:6979–6990. [PubMed: 19877647]
- [54]. Harada T, Inoue A, Wada I, Uchimura J-J, Tanaka S, Oku A. *J. Am. Chem. Soc.* 1993; 115:7665–7674.
- [55]. Danishefsky S, Kitahara T. *J. Am. Chem. Soc.* 1974; 96:7807–7808.
- [56]. Joly GD, Jacobsen EN. *Org. Lett.* 2002; 4:1795–1798. [PubMed: 12000301]
- [57]. Jadhav PK, Bhat KS, Perumal T, Brown HC. *J. Org. Chem.* 1986; 51:432–439.
- [58]. Dess DB, Martin JC. *J. Org. Chem.* 1983; 48:4155–4156.
- [59]. Inanaga J, Hirata K, Saeki H, Katsuki T, Yamaguchi M. *Bull. Chem. Soc. Jpn.* 1979; 52:1989–1993.
- [60]. Kamano Y, Zhang HP, Morita H, Itokawa H, Shiota O, Pettit GR, Herald DL, Herald DL. *Tetrahedron.* 1996; 52:2369–2376.
- [61]. Lippa, B. Ph.D. Dissertation. Stanford University; Stanford, CA: 1999.
- [62]. Wender PA, Baryza JL, Bennett CE, Bi FC, Brenner SE, Clarke MO, Horan JC, Kan C, Lacote E, Lippa B, Nell PG, Turner TM. *J. Am. Chem. Soc.* 2002; 124:13648–13649. [PubMed: 12431074]
- [63]. Keck GE, Tarbet KH, Geraci LS. *J. Am. Chem. Soc.* 1993; 115:8467–8468.
- [64]. Wender PA, Mayweg AVW, VanDeusen CL. *Org. Lett.* 2003; 5:277–279. [PubMed: 12556171]
- [65]. Noyori R, Ohkuma T, Kitamura M, Takaya H, Sayo N, Kumobayashi H, Akutagawa S. *J. Am. Chem. Soc.* 1987; 109:5856–5858.
- [66]. Wender PA, De Brabander J, Harran PG, Hinkle KW, Lippa B, Pettit GR. *Tetrahedron Lett.* 1998; 39:8625–8628.
- [67]. Wender PA, Clarke MO, Horan JC. *Org. Lett.* 2005; 7:1995–1998. [PubMed: 15876038]
- [68]. Wender PA, Horan JC. *Org. Lett.* 2006; 8:4581–4584. [PubMed: 16986955]
- [69]. Mackay HJ, Twelves CJ. *Nat. Rev. Cancer.* 2007; 7:554. [PubMed: 17585335]
- [70]. a) Wender PA, Verma VA. *Org. Lett.* 2006; 8:1893–1896. [PubMed: 16623578] b) Wender PA, Baryza JL, Brenner SE, De-Christopher BA, Loy BA, Schrier AJ, Verma VA. *Proc. Natl. Acad. Sci. USA.* 2011 in press.

- [71]. a) Brenner, SE. Ph.D. Dissertation. Stanford University; Stanford, CA: 2004. b) Baryza, JL. Ph.D. Dissertation. Stanford University; Stanford, CA: 2005.
- [72]. Wender PA, Verma VA. *Org. Lett.* 2008; 10:3331–3334. [PubMed: 18588309]
- [73]. Prepared in one step from ethyl acetoacetate
- [74]. Prepared in 4 steps and ~95% ee from acrolein and benzyl alcohol, using an asymmetric allylation to set the C3 configuration
- [75]. Evans DA, Chapman KT, Carreira EM. *J. Am. Chem. Soc.* 1988; 110:3560–3578.
- [76]. Barton DHR, McCombie SW. *J. Chem. Soc. Perkin Trans.* 1975; 1:1574–1585.
- [77]. a) Oancea E, Meyer T. *Cell.* 1998; 95:307–318. [PubMed: 9814702] b) Baryza JL, Brenner SE, Craske ML, Meyer T, Wender PA. *Chem. Biol.* 2004; 11:1261–1267. [PubMed: 15380186]
- [78]. For applications of ring closing metathesis processes in total synthesis, see: Nicolaou KC, Bulger PG, Sarlah D. *Angew. Chem.* 2005; 117:4564–4601. *Angew. Chem. Int. Ed.* 2005, 44, 4490–4527. ; Gradillas A, Perez-Castells J. *Angew. Chem.* 2006; 118:6232–6247. *Angew. Chem. Int. Ed.* 2006, 45, 6086–6101.
- [79]. Ball M, Bradshaw BJ, Dumeunier R, Gregson TJ, MacCormick S, Omori H, Thomas EJ. *Tetrahedron Lett.* 2006; 47:2223–2227.
- [80]. Trost BM, Yang H, Thiel OR, Frontier AJ, Brindle CS. *J. Am. Chem. Soc.* 2007; 129:2206–2207. [PubMed: 17279751]
- [81]. Hoye TR, Jeffrey CS, Tennakoon MA, Wang J, Zhao H. *J. Am. Chem. Soc.* 2004; 126:10210–10211. [PubMed: 15315410]
- [82]. Wender PA, DeChristopher BA, Schrier AJ. *J. Am. Chem. Soc.* 2008; 130:6658–6659. [PubMed: 18452292]
- [83]. For recent reviews of applications of the Prins reaction and cyclization, see: Pastor IM, Yus M. *Curr. Org. Chem.* 2007; 11:925–957.; Olier C, Kaafarani M, Gastaldi S, Bertrand MP. *Tetrahedron.* 2010; 66:413–445.
- [84]. Schulte-Elte KH, Hauser A, Ohloff G. *Helv. Chim. Acta.* 1979; 62:2673–2680.
- [85]. a) Marko IE, Mekhelfia A. *Tetrahedron Lett.* 1992; 33:1799–1802. b) Marko IE, Mekhelfia A, Bayston DJ, Adams H. *J. Org. Chem.* 1992; 57:2211–2213.
- [86]. Keck GE, Covell JA, Schiff T, Yu T. *Org. Lett.* 2002; 4:1189–1192. [PubMed: 11922815]
- [87]. a) Keck GE, Truong AP. *Org. Lett.* 2005; 7:2149–2152. [PubMed: 15901156] b) Keck GE, Truong AP. 2005; 7:2153–2156. c) Keck GE, Welch DS, Poudel UB. *Tetrahedron Lett.* 2006; 47:8267–8270. [PubMed: 17404602]
- [88]. Narayanan BA, Bunnelle WH. *Tetrahedron Lett.* 1987; 28:6261–6264.
- [89]. Liu H-J, Yip J. *Tetrahedron Lett.* 1997; 38:2253–2256.
- [90]. Tanaka K, Ohta Y, Fuji K. *Tetrahedron Lett.* 1993; 34:4071–4074. b) For application of this reagent in bryostatin and analog synthesis, see refs. [36], [38], and [42]; See also: Fuwa H, Sasaki M. *Org. Lett.* 2010; 12:584–587. [PubMed: 20063897]
- [91]. Custar DW, Zabawa TP, Scheidt KA. *J. Am. Chem. Soc.* 2008; 130:804–805. [PubMed: 18161979]
- [92]. Woo SK, Kwon MS, Lee E. *Angew. Chem.* 2008; 120:3286–3288. *Angew. Chem. Int. Ed.* 2008, 47, 3242–3244.
- [93]. Bahnck KB, Rychnovsky SD. *J. Am. Chem. Soc.* 2008; 130:13177–13181. [PubMed: 18767844]
- [94]. For several recent examples, see: Gesinski MR, Tadpetch K, Rychnovsky SD. *Org. Lett.* 2009; 11:5342–5345. [PubMed: 19873984] ; Custar DW, Zabawa TP, Hines J, Crews CM, Scheidt KA. *J. Am. Chem. Soc.* 2009; 131:12406–12414. [PubMed: 19663512] ; Yadav JS, Kumar GGKSN. *Tetrahedron.* 2010; 66:480–487.; Woo SK, Lee E. *J. Am. Chem. Soc.* 2010; 132:4564–4565. [PubMed: 20222708]
- [95]. For a recent review on the use of Prins macrocyclization strategies in complex molecule synthesis, see: Crane EA, Scheidt KA. *Angew. Chem. Int. Ed.* 2010; 49:8316–8326.

Figure 1.
An FOS approach to the bryostatins.

Figure 2.

A function-oriented synthesis approach to the phorbol esters, affording highly simplified functional analogs that bind to PKC.

Figure 3.
A selected set of known competitive PKC C1-domain ligands.

Isr J Chem. Author manuscript; available in PMC 2012 May 30.

Figure 5. Schematic of the diacylglycerol-regulated PKC subfamilies.

Figure 5.
Schematic of the diacylglycerol-regulated PKC subfamilies.

Figure 6.
Bryostatins prepared by total synthesis between 1990 and 2000.

Figure 7.
Trost and Dong's strategy to bryostatin 16.

Figure 8.
Keck's approach to bryostatin 1.

Figure 9.
General bryostatin analog design strategy: maximizing convergency through design.

Figure 10. PKC binding studies of a library of structurally simplified bryostatin analogs to aid in development of structure–function relationships.

Figure 11.

A) Designed C8-*gem* dimethyl analogs and their *des*-methyl counterparts. B) Representative confocal cellular images for translocation of the conventional PKC β 1-GFP isoform induced by treatment with 200 nM bryo 1 (Panels A–C) and bryo 2 (Panels D–F) at t=0 min, 2.5 min, and 38 min post dose. Translocation of fluorescence from the cytosol to the cellular membrane indicates enzyme activation.

Figure 12.

Panels A–C, D–F, and G–I: Representative confocal images for PKCβ1 translocation induced by 200 nM **88**, **90**, or **89**, respectively at $t=0$, 5, and 38 min post dose. Translocation of fluorescence from the cytosol to the cellular membrane indicates analog-induced kinase activation. Panels J–L: Representative confocal images for PKCδ translocation induced by 200 nM **89** at $t=0$, 5, and 38 min post dose. Plot A: Normalized cytosolic fluorescence intensity for PKCδ translocation experiments, $n = 3$. Plot B: Normalized cytosolic fluorescence intensity for PKCβ1 translocation experiments, $n = 3$.

Figure 13.
Envisioned B-ring pyran series of analogs.

An RCM approach to a bryostatin-like macrocycle **Examples of problematic RCM or RRCM strategies** Thomas, E. J. et al. *Tet. Lett.* **2006**, 2223 Trost, B. M. et al. *J. Am. Chem. Soc.* **2007**, 2206

Figure 14.
Ring-closing metathesis studies.

Figure 15.
Envisioned Prins-driven macrocyclization strategy.

Figure 16. Schulte-Elte and coworkers' early studies on a Prins-driven macrocyclization process.

K. Scheidt, 2008

E. Lee, *et al* 2008

Rychnovsky and Bahnck, 2008

Figure 17.

Recent examples of Prins-driven macrocyclizations in complex molecule synthesis.

Scheme 1.

Synthesis of spacer domain **2**. Reagents and conditions: (a) i. O_3 , ii. $NaBH_4$, 90%; (b) (–)-menthone, $pTsOH \cdot H_2O$, $CH(OEt)_3$, 71%; (c) $(COCl)_2$, DMSO, Et_3N , 87% (dr: 1.6 : 1 favoring **5**); (d) i. 1-methoxy-3-(trimethylsilyloxy)-1,3-butadiene, catalyst **6**, then ii. TFA, 88%; (e) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, 92%; (f) $Hg(OAc)_2$, isobutylvinyl ether; (g) decane, $155^\circ C$, 83% over two steps; (h) H_2 , Pd/C, 85%; (i) i. (–)- IpC_2BOMe , allyl-MgBr, then ii. H_2O_2 , NaOH; (j) TBSCl, imidazole, 69% over 2 steps; (k) $KMnO_4$, $NaIO_4$, 84%.

Scheme 2.

Synthesis of recognition domain **3**. Reagents and conditions: (a) **11**, LDA, then **12**, 98%; (b) *p*TsOH, **13a** (41%), **13β** (49%); (c) **13β**, NaBH₄, CeCl₃·7H₂O; (d) *m*-CPBA, NaHCO₃, MeOH, 71% over two steps; (e) PhCOCl, DMAP; Dess–Martin periodinane, 90%; (f) SmI₂, 95%; (g) LDA, OHCCO₂Me, 90% BORSM; (h) ClSO₂Me, Et₃N; (i) DBU, 78% over two steps; (j) NaBH₄, CeCl₃·7H₂O; (k) octanoic acid, 2,4,6-trichlorobenzoyl chloride, Et₃N, 93% over two steps; (l) HF·pyridine; (m) Dess–Martin periodinane, 86% over two steps; (n) allyl-BEt₂; (o) Ac₂O, DMAP, 95% over two steps; (p) cat. OsO₄, NMO; (q) Pb(OAc)₄, Et₃N; DBU, 80% over two steps; (r) DDQ, 79%; (s) HF, 95%.

Scheme 3.

Completion of designed bryostatin analog **1**. Reagents and conditions: (a) HF·pyridine; (b) TESCl, Et₃N, 75% over 2 steps; (c) **22**, 2,4,6-trichlorobenzoyl chloride, Et₃N, then **3**, DMAP, 81%; (d) HF·pyridine, 81%; (e) Amberlyst-15, 4 Å molecular sieves; (f) H₂, cat. Pd(OH)₂, 56% over three steps.

Scheme 4.

Degradative approach to C26-*des* methyl analog **31**. Reagents and conditions: (a) H₂, Pd(OH)₂/C, 67%; (b) Pb(OAc)₄, Et₃N; (c) Cp₂Ti(μ-Cl)(μ-CH₂)AlMe₂, 56% over two steps; (d) HF·pyridine; (e) Dess–Martin periodinane, 60% over two steps; (f) (DHQD)₂AQN, K₂OsO₂(OH)₄, K₂CO₃, K₃Fe(CN)₆, 70%, β:α=2.2 :1; (g) TESCl, pyridine, 100%; (h) allyl-BEt₂; (i) Ac₂O, DMAP, 97% over two steps; (j) cat. OsO₄, NMO; (k) Pb(OAc)₄, Et₃N; DBU, 73% over two steps; (l) HF; (m) TBSCl, imidazole, 57% β 25% α; (n) 2,4,6-trichlorobenzoyl chloride, Et₃N, **2**, then **32**, DMAP, 79%; (o) HF·pyridine, 73%.

Scheme 5.

An efficient synthesis of recognition domain 32. Reagents and conditions: (a) NaH, TBSCl; (b) SO₃·pyridine, Et₃N, DMSO; (c) i. 4-chloro-1-butanol, MeMgCl, ii. Mg, iii. 40; (d) (COCl)₂, DMSO, Et₃N, 54% over 4 steps; (e) cat. (*R*)-BINOL, 4 Å MS, cat. Ti-(*O**i*Pr)₄, B(OMe)₃, allyl-*Sn*Bu₃, 77%; (f) cat. *p*TsOH·H₂O, 4 Å MS, 85%; (g) MMPP, NaHCO₃, MeOH, 78% (dr=4:1); (h) cat. TPAP, NMO, 4 Å MS, 78%; (i) K₂CO₃, OHCCO₂Me, 72%; (j) NaBH₄, CeCl₃·7H₂O; (k) octanoic acid, DIC, DMAP, 93% over two steps; (l) 3HF·Et₃N; (m) Dess–Martin periodinane, NaHCO₃, 87% over two steps; (n) i. (*Z*)-1-bromo-2-ethoxyethene, *t*BuLi, Me₂Zn, 46, ii. 1M HCl, 90%; (o) (DHQD)₂PYR, K₂OsO₂(OH)₄, K₂CO₃, K₃Fe(CN)₆, 71%, β:α=2.5:1; (p) *p*TsOH·H₂O; (q) TBSCl, imidazole, 46% isolated 32 over two steps.

Scheme 6.

Reagents and conditions: (a) i. LDA, **52**, ii. **51**, 68%; (b) Ru-(*S*)-BINAPCl₂, H₂, 92%; (c) silica gel, 95%; (d) TBDPSCl, imidazole, 85%; (e) ethyl acetoacetate, LDA; (f) Et₃SiH, TFA, 70% over two steps; (g) Ru-(*R*)-BINAPCl₂, H₂, 91%, (h) i. H₂, Pd(OH)₂/C, ii. LiBH₄, 96%; (i) i. 2,2-dimethoxypropane, *p*TsOH, ii. silica gel, 93%; (j) TEMPO, NaOCl, NaClO₂, 92%; (k) PyBroP, Hünig's base, **32**, DMAP, 70%; (l) HF·pyridine, 90%.

Scheme 7.

Reagents and Conditions: (a) Dess–Martin periodinane, 97%; (b) *t*BuLi, 58% combined (1:1 d.r.); (c) cat. TPAP, NMO, 4 Å MS; (d) NaBH₄, CeCl₃·7H₂O, 87% over 2 steps (d.r.=9:1); (e) PhMgBr; (f) Dess–Martin periodinane, 84% over 2 steps; (g) NaBH₄, CeCl₃·7H₂O, 78%; (h) (–)-(Ipc)₂BOMe, allylMgBr, 94%; (i) TBSCl, imidazole, 91%; (j) Grubbs' Generation 2 Catalyst, 4-bromostyrene, 59%; (k) cat. Al₂O₃, H₂, 73%; (l) TBAF; (m) *t*BuOK, allyl bromide; (n) i. 9-BBN, ii. H₂O₂, NaOH; (o) Dess–Martin periodinane; (p) (–)-(Ipc)₂BOMe, allylMgBr; (q) For R=H, *t*Bu, and (CH₂)₃-*p*-Br-Ph: TBSCl, imidazole, For R=Ph: i. TBSCl, imidazole, ii. TBAF, iii. TBSCl, imidazole; (r) NaIO₄, KMnO₄.

Scheme 8.

Reagents and Conditions: (a) For **60** (R=H), 2,4,6-trichlorobenzoyl chloride, Et₃N, then recognition domain **32**, DMAP; For **61**–**63**, PyBroP, (*i*Pr)₂Net, DMAP; (c) HF-pyridine.

Scheme 9.

A) Reagents and conditions: (a) Ketone **94**, (+)-Ipc₂BCl, Et₃N, then **95**, -98°C, then H₂O₂, 90:10 d.r., 64% isolated **96**; (b) Me₄NBH(OAc)₃, 85%; (c) (±)-CSA, 90%; (d) *When* X=OTBS: TBS-OTf, 2,6-Lutidine, *When* X=H: i) Im₂CS, ii) Bu₃SnH, AIBN; (e) Ethyl acetoacetate, LDA; (f) TFA, Et₃SiH; (g) cat. Ru[(*R*)-BINAP]Cl₂, H₂; (h) LiBH₄; (i) 2,2-dimethoxypropane, PPTS; (j) Lithium naphthalenide; (k) TEMPO, NaClO, NaClO₂. B) Completion of bryologs **88–90**. Reagents and conditions: (l) 2,4,6-trichlorobenzoyl chloride, Et₃N, then **32**, DMAP; (m) HF·pyridine; (n) TESCl, DMAP, then Ac₂O; (o) HF·pyridine.

Scheme 10.

Reagents and Conditions: (a) TESCl, imidazole, 97%; (b) CeCl_3 , $\text{TMSCH}_2\text{MgCl}$; (c) SiO_2 , 69% from **115**; (d) lithium naphthalenide, 93%; (e) cat. TPAP, NMO, 4 Å MS; (f) NaClO_2 , NaH_2PO_4 , 2-methyl-2-butene, 89% from **117**; (g) **114**, 2,4,6-trichlorobenzoyl chloride, Et_3N , then **32**, DMAP, 82%; (h) PPTS, 84%; (i) TMS-OTf, 93%. (j) HF·pyridine, 77%.

Scheme 11.

Reagents and Conditions: (a) O₃, then thiourea, 88%; (b) **Either**: Trimethyl phosphonoacetate, NaHMDS, 87% combined yield, 49 :51 *E* : *Z*, or: phosphonoacetate **123**, NaHMDS, 65%, 25 :75 *E* : *Z*; (c) Using a 49 :51 *E* : *Z* enoate mixture: HF·pyridine, 83% combined, 32% isolated **124**, 37% isolated **125**.

Table 1

PKC affinity and antiproliferative activity of B-ring pyran analogs, including standard errors of the mean.

ID	PKC K _i (nM)	K562 (nM) ^[c]	MV411 (nM) ^[c]
121	1.63 ± 0.08 ^[a]	2.3 ± 0.6	1.4 ± 0.7
124	2.5 ± 0.1 ^[b]	4 ± 1	0.17 ± 0.05
125	0.9 ± 0.2 ^[a]	0.47 ± 0.09	0.4 ± 0.2
31	3.1 ± 0.3 ^[a]	15 ± 2	1.4 ± 0.3

^[a] Average of two experiments.^[b] Average of four experiments.^[c] EC₅₀ values are an average of three experiments.