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Identification of a Tunable Site in Bryostatin Analogs: C20 Bryologs through Late Stage Diversification

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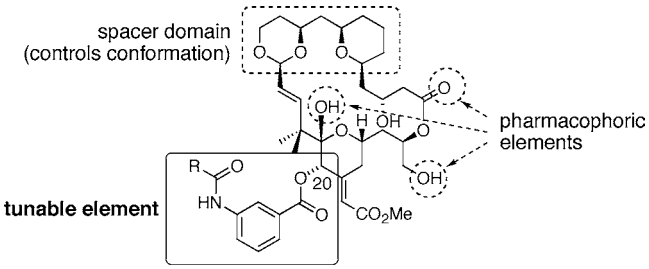
ABSTRACT



The C20 region of our bryostatin analogs was identified as a nonpharmacophoric site that could be varied to tune analogs for function and physical properties without significantly affecting their binding affinity for PKC. The use of this site in a late-stage diversification strategy has enabled the facile synthesis of a variety of new C20 analogs, all of which retain nanomolar affinity for PKC, in agreement with our pharmacophore hypothesis.

Bryostatin 1 (Figure 1) is a highly potent compound with a unique spectrum of biological activities. It is currently in Phase I and II clinical trials for the treatment of cancer. Bryostatin 1 has been shown to inhibit cancer growth in vitro and in vivo, synergize the action of other anticancer agents,[1] reverse multidrug resistance,[2] and stimulate the immune system.[3] While its mode of action is not established, bryostatin 1 is a potent activator of protein kinase C (PKC). Unfortunately, the limited availability[4] and structural complexity of bryostatin have restricted synthetic and biochemical studies that could lead to a better understanding of its molecular mode of action and, equally importantly, the identification of clinically superior agents. Being a marine

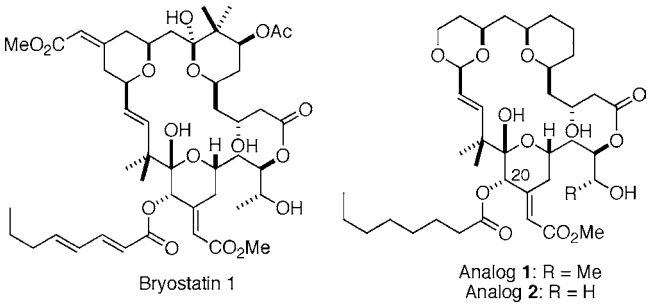


Figure 1. Bryostatin 1 and analogs **1** and **2**.

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natural product, bryostatin is neither produced nor optimized for clinical use in humans.

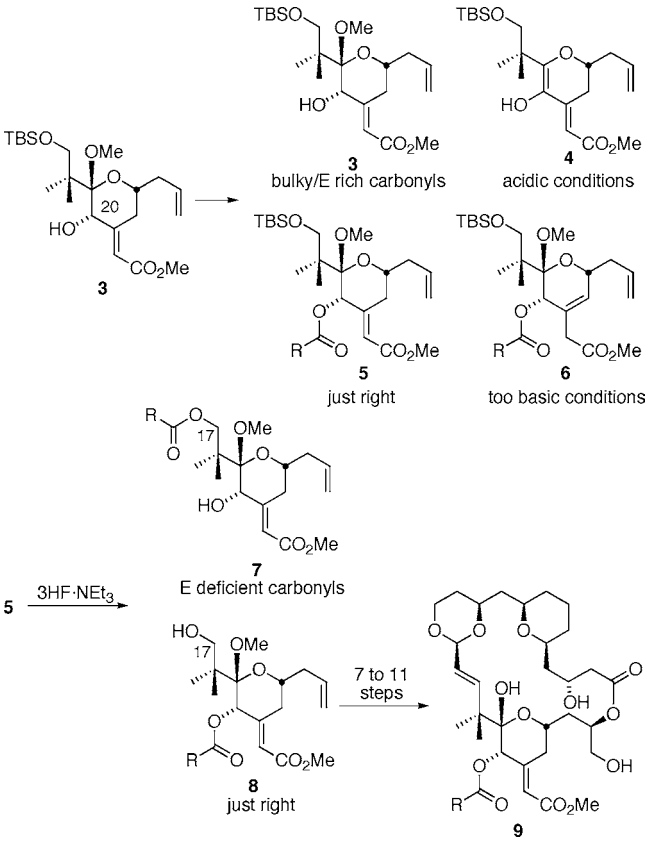
Using pharmacophoric and docking hypotheses, we have been engaged in the design and synthesis of structurally simplified but functionally potent analogs of bryostatin, whose activities, physical properties, side-effect profile, detectability, and other properties could be tuned through synthesis.[5] Analogs **1** and **2** (Figure 1) are representative of this effort. These analogs are readily available synthetically and exhibit bryostatin-like activity but demonstrate greater potency in vitro and in limited in vivo studies when compared to the natural product.[5,6]

With the demonstration that comparable or superior affinity and function can be realized in simplified, synthetically accessible bryostatin analogs, we next sought to identify a region in our analogs that could be readily modified to optimize in vivo performance without sacrificing potency or function. The identification of such a site would allow for the preparation of analogs as needed with improved physical properties and ADME behavior and with tags for use in biochemical and mechanistic studies. Preferably the selected region would be amenable to modification at the end of the current synthesis to reduce the number of steps required to prepare each new analog and to avoid undesired changes in other sensitive functionality.

The above considerations restrict potential sites for modification of our analogs to the C20 ester and C21 enoate as all other functional groups are implicated in binding and/or preorganization. Unfortunately, our previous efforts to modify the C21 enoate at the end of the current synthesis have been frustrated by undesired rearrangements of the densely functionalized C-ring. We were, however, able to prepare ester variants at C20 (Scheme 1: **3** → **5** → **9**) that retained binding potency, but access to such analogs requires early divergence from the current synthesis route, adding as many as eight steps to the synthesis of each derivative and restricting the type of derivative to those compatible with the remaining steps.[7] Attempts to modify our analogs, or their late stage precursors, at C20 have been complicated by steric congestion around C20 and the associated undesired involvement of neighboring functionality in C20 modifications. Notwithstanding these problems, our earlier synthesis of a C20 ester variant and the observation that C20 ester variations are found in active natural bryostatins suggested that the C20 substituent could be used for tuning the performance of our analogs.

Given the steric problems encountered in the attachment of a C20 group at the end of our current synthesis, we sought to install a C20 group at an earlier synthetic point and to

Scheme 1. Installation of C20 Sidechain



incorporate into that group a functionality that would be spatially removed from the steric crowding at C20 and amenable to flexible modification at the end of the synthesis. In our current synthesis (Scheme 1), the C20 ester group is introduced relatively early by acylation of alcohol **3**.[5b,8] Unfortunately, alcohol **3** is sterically hindered and prone to acid-promoted elimination of the C19 OMe group to produce **4** (Scheme 1), as well as base-promoted rearrangement of the C21 enoate. Therefore, the use of less reactive acylation agents is precluded. Electron-rich acids, such as 4-benzyloxybenzoic acid, or even moderately bulky acids, such as bromoacetic acid, failed to react at all or, under forcing conditions, resulted in decomposition. More reactive reagents, such as activated trifluoroacetate or chloroactetate, will acylate alcohol **3**; however, after deprotection of the C17 alcohol, these electron-deficient acyl groups migrate completely to the less hindered C17 alcohol. This "Goldilocks effect",[9] meaning the acyl groups employed must be just right, limits the range of C20 substituents that can be used.

The eventual choice of a 3-aminobenzoate ester as a tunable C20 substituent was made for several reasons. The aniline could be generated by selective reduction of a nitro group without the need to protect other functionalities in

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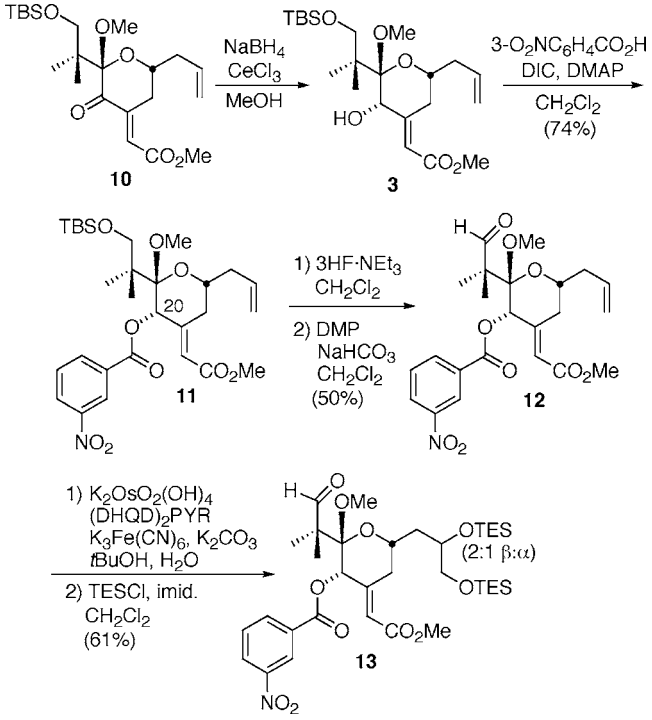
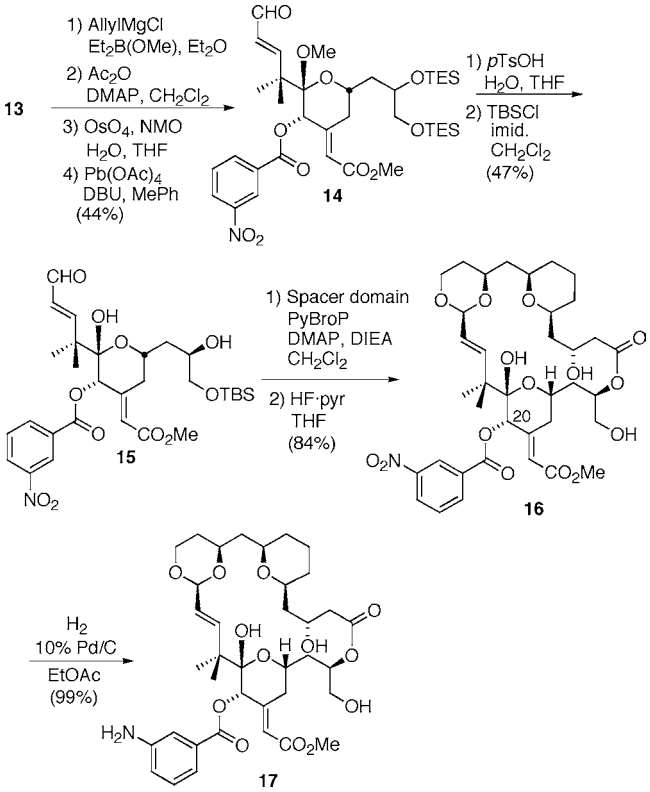
Scheme 2. Synthesis of Intermediate **13**



Scheme 3. Synthesis of Analog **17**



these complex molecules; both the C17−C18 olefin and the C21 enoate are known to be resistant to hydrogenation in bryostatin. The aniline moiety would provide a handle for selective installation of a variety of groups without the need for protection of other groups in the analog, and the phenyl ring would provide spatial separation from the sterically congested C20 position. The choice of a 3-amino species was made to maximize the nucleophilicity of the nitrogen by avoiding conjugation to the carbonyl. For pharmacokinetic and ADME studies, the inclusion of a nitrogen atom is also desirable due to the improved ionization in MS^n analysis, making detection easier, especially at the low doses used for studies with these exceptionally potent compounds.

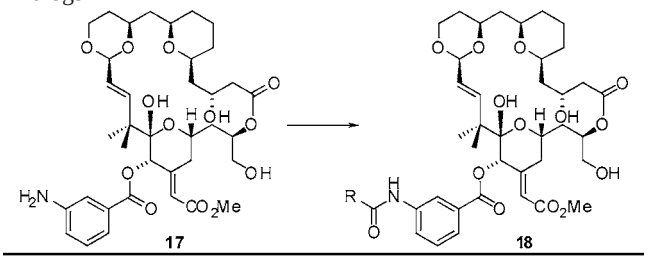
Analog **17** was prepared as shown in Schemes 2 and 3, starting from the γ-ketoenoate **10**.[5] Reduction of the ketone and acylation of the resulting alcohol **3** provided 3-nitrobenzoate ester **11**.

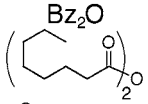
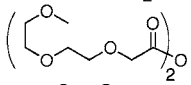
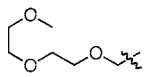
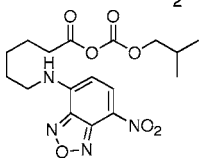
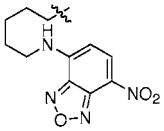
Removal of the C17 TBS protecting group and oxidation of the resulting alcohol generated aldehyde **12**. Dihydroxylation of the terminal alkene provided an inseparable 2:1 mixture of diastereomers. Protection of the diols followed by a four-step homologation of the aldehyde produced enal **14**. Unfortunately, the use of **13** in our previously reported one-step homologation was thwarted by decomposition of the nitroarene moiety induced by the vinyl zincate reagent. Deprotection of the C25−C26 diol and hydrolysis of the C19 ketal was accomplished in one step. The change to the C20 substituent appeared to retard this hydrolysis significantly, lengthening the reaction time from 16 h to 5 days. Selective protection of the primary alcohol and separation of the diastereomers provided recognition domain **15**, which was coupled to the spacer domain used in the synthesis of **2** with PyBroP/DMAP.[5] Macrocyclic transacetalization and concomitant desilylation provided, in a single step and under thermodynamic control, the C20 nitroarene analog **16** in 84% yield. Gratifyingly, the nitrobenzoate could be reduced chemoselectively by hydrogenation to provide the desired aniline **17**.

As shown in Table 1, chemoselective acylation of the aniline moiety of **17** can be accomplished with either symmetrical or mixed anhydrides at room temperature in CH_2Cl_2, providing direct one-step access to a variety of C20 analogs, including aliphatic, aromatic, PEG, and probe amides. The reaction is highly selective for the aniline nitrogen, and acylation of the unprotected alcohols was not observed.

The binding constants for analogs **18a**−**e** to PKC (rat-brain isozyme mixture) were determined as previously described[5] and are shown in Figure 2. It is apparent from these data that there is a good tolerance for substituents with different physical properties. All of the new analogs bind to PKC with potencies sufficient for mechanistic studies. Even the highly hydrophilic glycol chain of **18d** provides a compound that is sufficiently potent. These results suggest that the physical properties of the molecule can be readily tuned by modification of the C20 substituent. The mild reaction conditions also allow moieties with other desirable but potentially sensitive functions to be incorporated into the analogs. An example of such a function is analog **18e**, which incorporates the NBD fluorophore that can be used for receptor identification and imaging studies.

Table 1. Reagents and Yields for Synthesis of Amide Analogs[a]



	reagent	R =	yield
18a	Ac_2O	Me	54%
18b	Bz_2O	Ph	64%
18c		C_7H_{15}	85%
18d			99%
18e			75%

[a] All reactions were run in CH_2Cl_2 at room temperature using 3−4 μmol of **17**. See Supporting Information for details.

Application of a late stage divergence strategy to the synthesis of new C20 analogs of bryostatin 1 has been realized, generating compounds with properties tuned for in vivo use and mechanistic studies. Starting from the aniline analog **17**, analogs with new C20 substituents can be generated in one step and in good yield. Part of the value of this approach arises from its step economy. Generating a similar set of analogs by diversification at the ester formation step that produces **11** (see Scheme 2) would require 13 steps per analog. Any need for additional protecting groups would only lengthen and complicate the preparation of such species. All of the analogs generated exhibit nanomolar binding affinity for PKC. The relatively high affinity of these analogs suggests that changes to this portion of the molecule can be used to improve physical properties, control selectivity, and incorporate additional functionality, a fluorophore, to probe for receptors and mode of action. Further biological testing of the analogs in Figure 2 and related compounds in assays designed to measure PKC activation and functional activity will be reported in due course.

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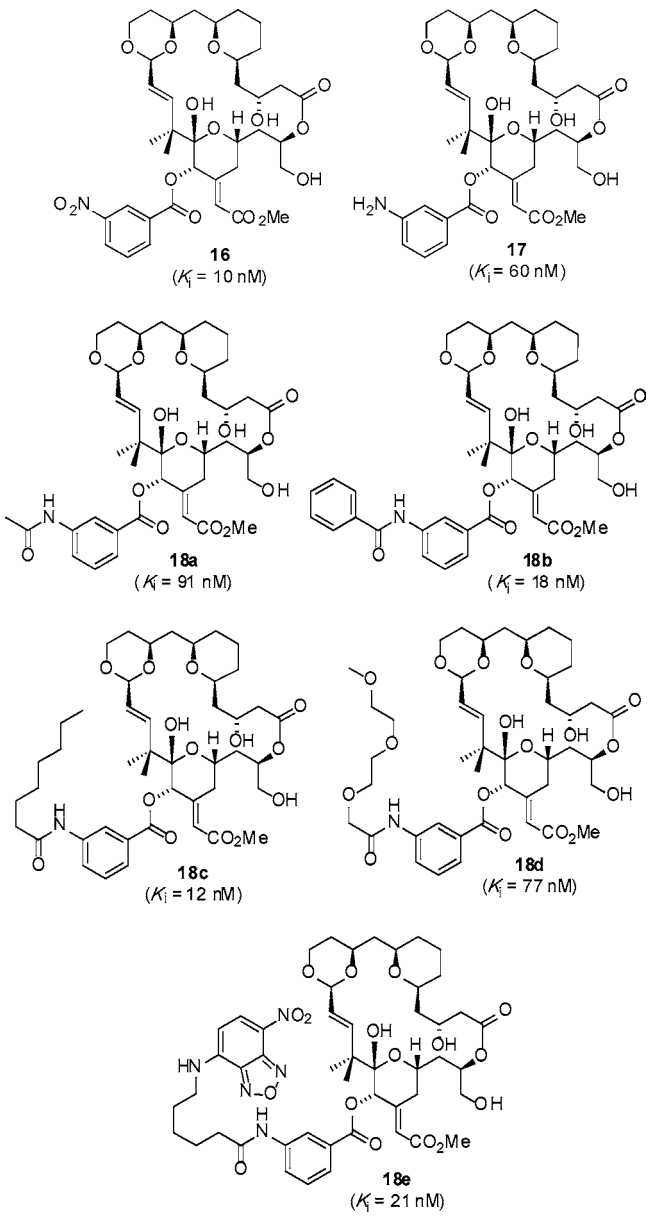

Figure 2. Structures and binding affinities of new analogs to protein kinase C.

Supporting Information Available: Experimental procedures and characterization data for compounds **10**−**18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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