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Replacement of the bryostatin A- and B-pyran rings with phenyl rings leads to loss of high affinity binding with PKC

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ABSTRACT

We describe a convergent synthesis of a bryostatin analogue in which the natural A- and B-ring pyrans have been replaced by phenyl rings. The new analogue exhibited PMA like behavior in cell assays, but failed to maintain high affinity binding for PKC, despite retaining an unaltered C-ring 'binding domain'

Introduction

Bryostatin 1 is a marine macrolide that was isolated from *Bugula neritina* in 1982 by Petit and co-workers.[1] In total, 20 naturally occurring bryostatins have been isolated. Bryostatin 1 has been the subject of more than 80 phase 1 and phase 2 clinical trials for the treatment of various cancers,[2] as well as recent phase 1 and 2 trials for the treatment of Alzheimer's disease.[3,4] Additional clinical indications include effects on learning and memory,[5] and activation of latent HIV reservoirs.[6] Bryostatin 1 elicits biological responses primarily through binding the C1 domain of protein kinase C (PKC). However, activation of PKC through binding the C1 domain with an exogenous ligand has been shown to elicit diverse and often contrasting biological responses. Binding of another high affinity ligand, phorbol 12-myristate 13-acetate (PMA) leads to potent tumor promotion in mouse skin[7] whereas prostratin, another phorbol ester, and bryostatin 1 are not tumor-promoting. Bryostatin 1 is unique among C1 domain ligands in that it is capable of antagonizing the biological function of other C1 domain ligands. For instance, PMA induces attachment and inhibits proliferation of U937 leukemia cells; if co-administered with PMA, bryostatin 1 blocks these effects in a dose dependent manner.[8]

Currently, one of the major obstacles to further clinical development of bryostatin 1 is its extremely low natural abundance. Harvesting from natural sources is low yielding and environmentally devastating, culturing *Bugula neritina* or the bryostatin producing symbiotic bacterium *Candidatus endobugula* have yet to be successfully realized, and chemical synthesis requires many steps. Thus, structurally simplified analogues have attracted considerable attention as potential bryostatin substitutes.[9,10]

Structurally, bryostatin 1 is comprised of 3 highly functionalized pyran rings (A, B, and C) contained within a 20-membered macrolactone (Fig. 1). Early studies with synthetic and semi-synthetic analogues suggested that binding with PKC is mediated primarily by substituents on the C-ring. Modification/elimination of the C19 hemiketal or C26 hydroxyl leads to substantial loss in binding affinity.[11] Conversely, modification or omission of A- and/or B-ring substituents has a minimal effect on binding. Additionally, a macrocyclic structure is required, leading to a hypothesis that the A- and B-ring pyrans act as a 'spacer domain,' orienting the C-ring substituents in their optimal binding orientation.[12–14] This initial hypothesis is reasonably accurate with regard to maintaining PKC binding affinity; however, ligand binding is just the first event of a complex biological response mediated by PKC activation. Through a series of detailed studies in which contributions of the A- and B-ring substituents were evaluated, it was shown that this region is not merely a spacer domain but rather imparts to bryostatin its unique biological profile. Analogues **Merle 28**[15] and **Merle 30**,[16] which contain two oxygen 'polar'

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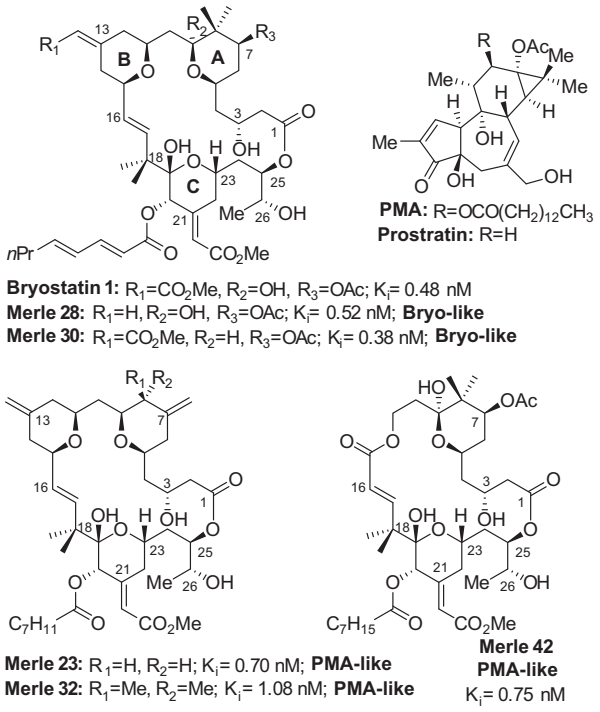


Figure 1. Bryostatin 1, phorbol esters, and selected analogues: K_i values were determined by inhibition of binding of ^{3}H-labeled PDBu to PKCα.[21] 'PMA- or Bryo-like' refers to behavior on U937 cell proliferation and attachment.

functionalities on the A- and B-rings, were found to be bryo-like in U937 cells while **Merle 23**[8] and **Merle 32**,[17] which contain no polar functionalities, were found to be PMA-like. Reintroducing just the C7 acetate on the bis-pyran scaffold (**Merle 27**, not shown) provided an analogue that retained PMA-like behavior, suggesting that a single polar group is not sufficient to confer bryo-like behavior.[18] In addition to investigating the role of the A-, and B-ring substituents both individually and in combination, we have also sought to simplify the synthesis of analogues through omission of non-essential moieties. A recent example is the synthesis of **Merle 42**,[19] which contains 2 polar groups on the A-ring, consistent with **Merle 28**, but has the B-ring deleted in favor of a simple ester linkage. Interestingly, this analogue demonstrated PMA-like behavior suggesting that the B-ring, regardless of polar substituents on the A-ring, plays an important role in maintaining a bryostatin-like response. Similar biological results were also observed with another B-ring truncated analogue, WN-1.[20] Herein, through the synthesis of bis-phenyl analogue **Merle 40**, we report an attempt to develop a new scaffold that encompasses both an A- and a B-ring. This scaffold was designed to be highly modular in that the A-ring, B-ring, or both could be replaced with differentially functionalized phenyl rings, allowing us to further investigate the effect of polar substituents in this region of the molecule.

Results and discussion

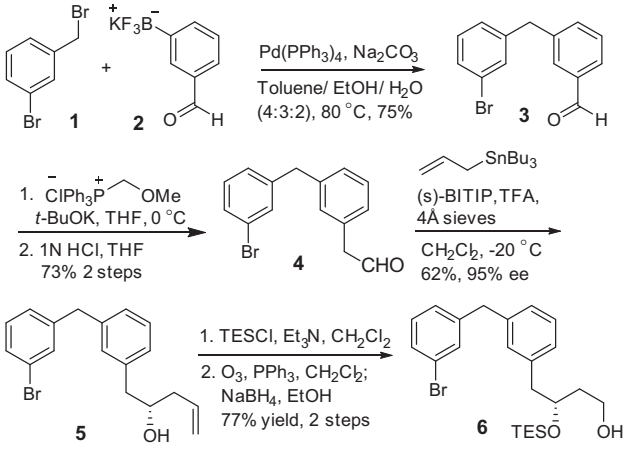
Synthesis of Merle 40

Retrosynthetically, we envisioned accessing **Merle 40** through the union of a bis-phenyl A, B-ring segment with a fully functionalized C-ring subunit. Specifically, we sought to use a Heck insertion to combine a B-ring aryl bromide with a C-ring olefin, followed by oxidation of the C1 alcohol, macrolactonization, and global deprotection. For the C-ring portion of the molecule an acetate ester at C20, as opposed to the natural bryostatin 1 (2E, 4E)-octa-2,4-dienoate ester, was chosen. The C20 ester is synthetically more convenient, and through the synthesis of bryostatin 7 (K_i = 0.26 nM)[22] and a C20 acetate version of Merle 23 (K_i = 0.6 nM)[23] we have shown that this substitution has a minimal effect on PKC binding.

Our first concern for executing this strategy was developing an efficient synthesis of the bis-phenyl A, B-ring subunit. Our route began (Scheme 1) with the coupling of 3-bromobenzyl bromide **1** (B-ring) with the Molander salt **2** derived from 3-bromobenzaldeyde (A-ring).[24] Following homoelongation of aldehyde **3**, using Levine's two-step protocol[25] the sole stereocenter was set using a catalytic asymmetric allylation.[26] The free alcohol at C3 was protected as a TES ether, and the terminal olefin was converted to a primary alcohol in a two-step, single flask, ozonolysis/reduction sequence. The bis-phenyl A, B-ring subunit **6** contains only one stereocenter, compared to six in bryostatin 1, or five in our most studied analogue **Merle 23**. As a result **6** could be synthesized in only 7 chemical manipulations.

Our initial strategy for completing **Merle 40** was to advance glycal **7**[27] with the C26 alcohol protected as a benzyloxymethyl (BOM) ether (Scheme 2). This strategy eventually failed because suitable conditions for removal of the BOM group, as the final step, without decomposition of the analogue, could not be found. The ultimately successful route required that the BOM ether be removed first. This was accomplished using a dissolving metal reduction, which allowed the BOM group to be cleanly removed while leaving the more electron rich PMB ether at C25 intact. The free C26 alcohol was then re-protected as a TBS ether. The first step in oxidizing glycal **8** to the fully functionalized C-ring was epoxidation of the enol-ether with MMPP. In-situ addition of methanol to the axial position opened the epoxide to give a ~4:1 mixture of C19 hemiketal diastereomers. The hemiketal was then equilibrated to a single C19 diastereomer with chloroacetic acid, and the C20 alcohol oxidized to a ketone.[28] At this point the desired C-ring was completed utilizing steps that were previously optimized during our synthesis of bryostatin 1.[29] First, the exocyclic enoate was installed using a one-pot aldol addition/elimination reaction and finally the C20 ketone was reduced and the resulting alcohol esterified with acetic anhydride to give C-ring **11**.

At the outset we were aware that the prenyl olefin of **11** was likely to exhibit very low reactivity. Evans and coworkers encountered challenges using a C17 aldehyde of a fully functionalized C-ring in a Julia olefination during their bryostatin 2 synthesis,[28] and Trost was unable to use a C16-17 prenyl olefin of a similarly functionalized C-ring in metathesis reactions.[30] A Heck reaction on this substrate is particularly challenging, requiring an interme-



Scheme 1. Synthesis of A, B-ring subunit 6.