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Synthesis of a *des*-B-Ring Bryostatin Analogue Leads to an Unexpected Ring Expansion of the Bryolactone Core

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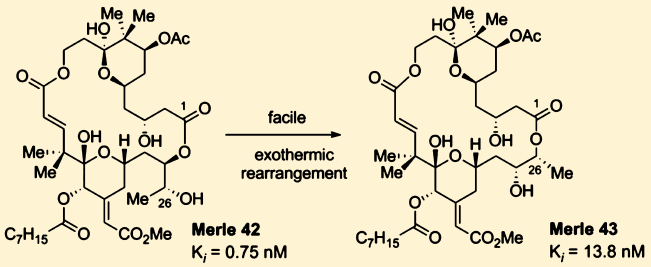
Supporting Information

ABSTRACT: A convergent synthesis of a *des*-B-ring bryostatin analogue is described. This analogue was found to undergo an unexpected ring expansion of the bryolactone core to generate the corresponding 21-membered macrocycle. The parent analogue and the ring-expanded product both displayed nanomolar binding affinity for PKC. Despite containing A-ring substitution identical to that of bryostatin 1 and displaying bryostatin-like biological function, the *des*-B-ring analogues displayed a phorbol-like biological function in cells. These studies shed new light on the role of the bryostatin B-ring in conferring bryo-like biological function to bryostatin analogues.



INTRODUCTION

The bryostatins are a family of 20 closely related marcrolide natural products of marine origin. Bryostatin 1 (Figure 1), the most abundant and well-studied member of this family, was initially isolated by Pettit and co-workers from the bryozoan *Bugula neritina*.[1] It has since been found that the bryostatins are not synthesized by the bryozoan itself but rather by a bacterial symbiont called "*Candidatus* Endobugula sertula".[2] Bryostatin 1 initially garnered interest in the treatment of cancer after showing potent cytoxicity toward the murine P388 lymphocytic leukemia cell line.[1a] In addition to activity against cancer, bryostatin shows potential in the treatment of a wide range of other indications, including ischemic stroke,[3] Alzheimer's disease,[4] and HIV.[5] The broad therapeutic potential of bryostatin 1 has been attributed to its ability to activate protein kinase C (PKC) by binding to the cysteine-rich C1 domain in place of the endogenous lipid messenger diacylglycerol,[6] a property which is also shared by the structurally dissimilar phorbol esters.

Bryostatin, however, is unique in that it is able to antagonize a subset of biological responses induced by the phorbol esters. A particularly important difference is that the phorbol esters are generally tumor-promoting, whereas bryostatin 1 is not. Furthermore, bryostatin is able to block tumor promotion by the phorbol esters.

Numerous synthetic research groups, including our own, have taken interest in bryostatin's unique biological activity and structural complexity, resulting in numerous total syntheses[7] as well as the syntheses of structurally simplified analogues.[8] We have initiated a program aimed at identifying, through the synthesis of analogues, the structural features of bryostatin that confer bryostatin-like biological responses as opposed to the responses induced by the phorbol esters. Previously, we reported the synthesis of **Merle 23** (Figure 1), which differs from the flagship compound bryostatin 1 in that it has two simplified tetrahydropyrans in place of bryostatin's functionalized A- and B-rings.[8c] Despite bearing a close structural resemblance to bryostatin, **Merle 23** displayed a pattern of biological responses that more closely resembled those exhibited by phorbol-12-myristate-13-acetate (PMA).[9] This led to the conclusion that the structural features conferring bryostatin-like biological responses must be located within the four A,B-ring substituents that were omitted in **Merle 23**. Analogues have since been prepared that re-introduce one or a combination of these substituents, and **Merle 28**[8e] and **Merle 30**[8f] have been identified to be bryo-like in their behavior. While work to understand how A,B-ring functionality governs the biological function exhibited by these analogues is still ongoing, we also sought to use our previous findings to guide the design of simplified constructs that might be capable of mediating bryostatin-like biological effects. Here, we report one such attempt, through the synthesis of the *des*-B-ring analogue **Merle 42** (Figure 2).

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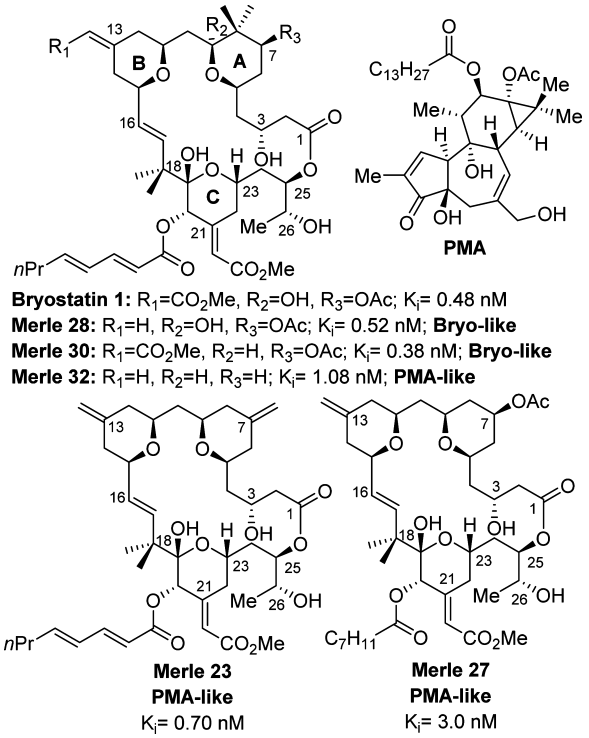


Figure 1. Bryostatin 1, PMA, and selected analogues.

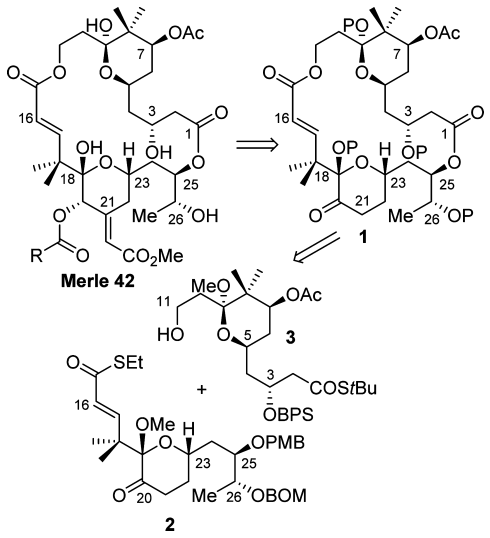


Figure 2. Retrosynthetic analysis of **Merle 42**.

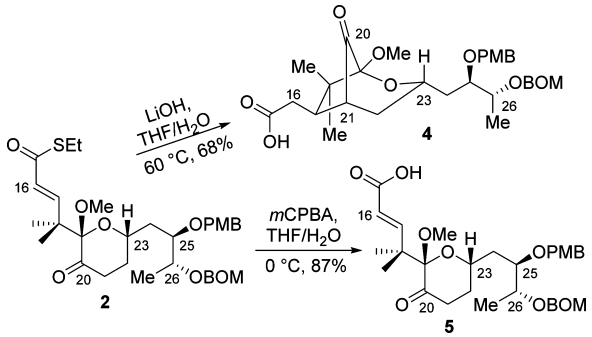
We had previously found, through the synthesis of **Merle 28**, that removal of the B-ring methyl ester did not affect binding affinity or the analogue's ability to mimic bryostatin. This led us to hypothesize that the A-ring substituents were the major structural features that confer bryo-like function and led to the consideration that the B-ring might present as a site of further simplification. Thus, we considered synthesizing analogues that would omit the B-ring pyran altogether and simply join a C-ring subunit with a functionalized A-ring subunit with a simple acyclic tether. **Merle 42**, which contains an ester linkage in place of the B-ring pyran, represents such a simplified structure. Replacing the B-ring pyran with an ester greatly simplifies the synthesis, as it removes the need for two stereoselective carbon−carbon bond-forming events and replaces these steps with a simple esterification reaction. Moreover, it appeared that such a compound could be prepared in a single trivial synthetic operation from subunits previously prepared in our laboratories.

RESEARCH DESIGN AND METHODS

Synthesis of Merle 42. Retrosynthetically, we anticipated that **Merle 42** could be obtained from a late-stage functionalization of the C-ring in macrodilactone **1** (Figure 2). Further simplification of this unsymmetrical macrodilactone reveals C-ring subunit **2** and A-ring subunit **3**, which in a forward sense could be joined via esterification and subsequent macrolactonization.

Focus was initially turned toward preparing the C-ring carboxylic acid coupling partner **5**, which was expected to be available in a single step from the previously described C_{15} thioester **2** via hydrolysis.[8c] This hydrolysis was first attempted under basic conditions using lithium hydroxide, but the oxobicyclic derivative **4** was obtained instead (Scheme 1).

Scheme 1. Hydrolysis of Thioester 2



This byproduct results from Michael addition of the C_{21} enolate into the α,β-unsaturated thioester, followed by hydrolysis of the thioester. Since basic hydrolysis failed to provide any of the desired carboxylic acid, the transformation was then attempted using silver nitrate in aqueous THF.[10] These conditions did provide acid **5** in 80% yield; however, the reaction time was slow, and purification was difficult due to the presence of multiple low-level impurities. We were also drawn to an earlier report by Masamune and co-workers describing the activation of thioesters by other oxidants such as mCPBA and $Hg(TFA)_2$ to drive hydrolysis and transesterification.[11] To our delight, we found that treatment of **2** with mCPBA in aqueous THF cleanly afforded the desired carboxylic acid in 87% yield.

With the successful preparation of the C-ring acid subunit **5**, focus was shifted toward accomplishing the requisite bimolecular esterification with the previously described A-ring alcohol **3** (Scheme 2).[12] Initial attempts at esterification using DCC or EDCI were low yielding. However, esterification was successful using the Yamaguchi conditions, providing ester **6** in 87% yield. In preparation for macrolactonization, the C_{25} PMB group was removed using DDQ to give the corresponding C_{25} alcohol. Due to the difficulties that were encountered during the hydrolysis of thioester **2** and concerns over competitive hydrolysis of the two oxo-esters, we decided to employ mCPBA again for the hydrolysis of the C_1 *tert*-butyl thioester **6** in lieu of basic hydrolysis. Under conditions identical to those described earlier, seco-acid **7** was obtained in 85% yield. Seco-acid **7** was then used in a Yamaguchi macrolactonization to provide the corresponding 20-membered macrodiolide **8** in 90% yield.

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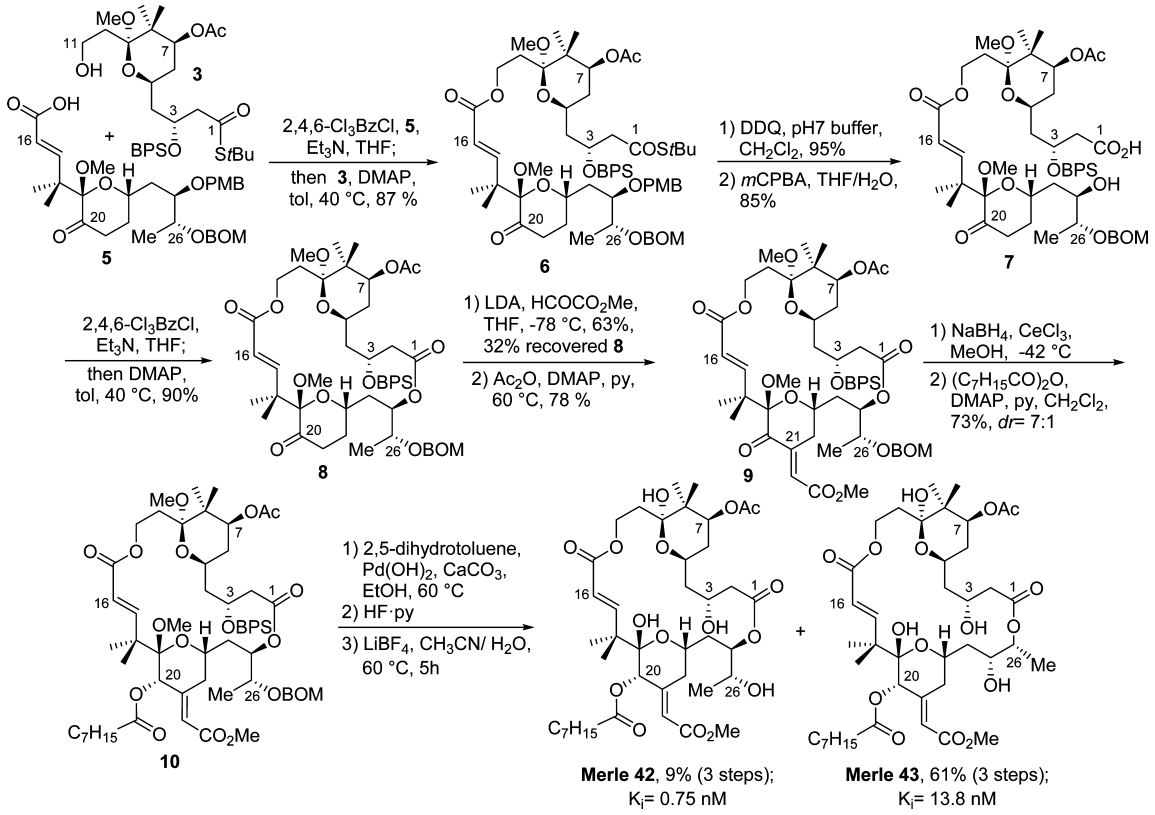
Scheme 2. Synthesis of Merle 42 and Merle 43



The C_{21} unsaturated ester was installed by an aldol reaction between the C_{20} ketone in **8** and methyl glyoxylate. This reaction was complicated by the presence of the acetate at C_7, which concomitantly underwent an aldol reaction when even a slight excess of base was used. When 1 equiv of LDA was used, the desired aldol adduct was obtained in 63% yield as an inconsequential mixture of diastereomers; however, 32% of the starting ketone **8** remained, which could be recovered and reused as needed. Elimination of the β-hydroxyketone intermediate was accomplished using acetic anhydride in pyridine to give the C_{21} enoate **9** in 78% yield as a single olefin isomer. The elimination product **9** was used immediately in a Luche reduction to provide the intermediate C_{20} alcohol. The crude alcohol was taken on to esterification to install the C_{20} side chain by treatment with octanoic anhydride, providing analogue precursor **10** in 73% yield as a 7:1 mixture of diastereomers. The undesired diastereomer was successfully removed by preparative thin-layer chromatography (TLC).

With analogue precursor **10** in hand, the only task that remained was protecting group removal. Our initial approach was to remove all protecting groups in a single step using the conditions of Lipshutz ($LiBF_4$, CH_3CN/H_2O),[13] as these had proven to be mild and successful in our previous preparations of bryostatin analogues. This attempt, however, was unsuccessful and led to substrate decomposition. It was thought that the failure of this deprotection might be related to the presence of the C_3 *tert*-butyldiphenylsilyl (BPS) protecting group, as there was no precedent for the removal of this group under these conditions. Thus, we were led to investigate performing the deprotection in a two-step sequence, where the BPS group would be removed with HF·pyridine, followed by the removal of the two methyl ketals and the C_{26} BOM ether under Lipshutz conditions.

Deprotection of the C_3 BPS group occurred without incident using HF·pyridine. Careful monitoring of the subsequent step revealed that the C_9 and C_{19} methyl ketals were the first to be hydrolyzed, which occurred within 5 h. On the other hand, the hydrolysis of the C_{26} BOM group took much longer, and the substrate ultimately proved to be unstable toward the extended reaction time required to remove this group. It is worthy of note, however, that hydrolysis of the two methyl ketals was observed to give a product with significantly *higher* TLC R_f than that of the starting material, which is of course highly unusual. Normally, conversion of a protected alcohol to the free alcohol is accompanied by a significant decrease in TLC R_f. This higher TLC mobility for the deprotected derivative is a consequence of formation of the intramolecular H-bonding network that is found in bryostatin 1 and other similar bryopyrans. Here, the C3-OH makes a bifurcated H-bond to the two pyran oxygens of the A- and B-rings, and the C19-OH proton is hydrogen-bonded to the oxygen of the C3 OH.[1b]

In the end, we settled on a three-step deprotection strategy. First, the BOM ether was removed by transfer hydrogenolysis using $Pd(OH)_2$ and 2,5-dihydrotoluene, without observable reduction of the C_{16},C_{17} olefin.[14] Next, the resulting C_{26} alcohol was then subjected to reaction with HF·pyridine to remove the C_3 BPS ether. Finally, the C_9 and C_{19} methyl ketals were hydrolyzed under Lipshutz conditions at 60 °C for 5 h. This process yielded two isomeric products. The first was identified as the desired analogue, **Merle 42**, which was obtained in only 9% isolated yield over three steps. The other was the ring-expanded product, **Merle 43**, wherein the C1 carbonyl had undergone a 1,2-acyl migration to the C_{26} hydroxyl to give the 21-membered macrodiolide in 61% yield over three steps. It is thought that this ring expansion is driven by significant ring strain brought about by the presence of the internal C_{15}–C_{17}

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unsaturated ester, which is relieved upon ring expansion. This is supported by Krische and co-worker's recently reported synthesis of the *des*-B-ring analogue **WN-1**, which differs from **Merle 42** only in that the C_{15} carbonyl is transposed across the macrolactone to the C_{11} position and the hydroxyl moiety at C_9 is absent (Figure 3).[15] When **WN-1** was subjected to the

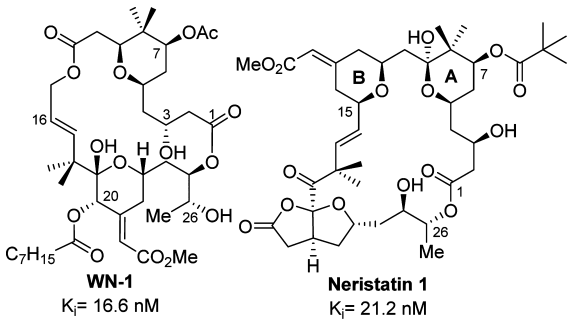

Figure 3. Structures of **WN-1** and neristatin 1.

identical deprotection conditions used to generate **Merle 42** and **Merle 43**, no ring expansion was observed. **WN-1** is conceivably less strained than **Merle 42** and likely does not have the thermodynamic incentive to rearrange. All attempts to prevent the ring expansion of **Merle 42** by altering the deprotection strategy were unsuccessful.

Biological Evaluation of Merle 42 and Merle 43. The newly prepared *des*-B-ring bryostatin analogues, **Merle 42** and **Merle 43**, were examined for binding toward purified PKCα and were both found to be potent ligands with binding affinities (K_i) in the low nanomolar range. **Merle 42**, however, was found to have approximately 20-fold higher binding affinity (K_i = 0.75 nM) than its ring-expanded counterpart **Merle 43** (K_i = 13.8 nM). The high activity of **Merle 43** toward PKC was surprising, as the C_{26} hydroxyl moiety has previously been proposed to be a critical binding motif; however, these results demonstrate that C_{26} bryolactones are capable of binding PKC.[16] While **Merle 43** is the first synthetic C_{26} bryolactone, the PKC-binding natural product neristatin 1 (Figure 3) bears a striking resemblance to bryostatin and also has a macrocyclic linkage at the C_{26} hydroxyl moiety.[17]

After establishing that the newly prepared analogues were potent ligands for PKC, we sought to characterize them as either bryostatin-like or phorbol-like with regard to their biological function in cells. Thus, they were compared to both bryostatin 1 and PMA in the U937 cell line (Figure 4). In this cell line, bryostatin and PMA elicit distinctly different responses.[18] PMA inhibits proliferation and induces attachment of U937 cells, whereas bryostatin shows little activity for inducing either phenotypic response. Moreover, bryostatin is able to block the action of PMA in a dose-dependent manner. Interestingly, both **Merle 42** and **Merle 43** were found to behave in a manner consistent with PMA rather than bryostatin, despite bearing the fully functionalized A-ring that was previously found to bestow bryostatin-like function in the analogue **Merle 28**. These results further demonstrate that bryostatin-like behavior is not driven by a single set of substituents in the A- and B-ring region and that perturbations within this entire region can dramatically affect how bryolactones function in a biological setting.

It is of interest to note that, while **Merle 42** is more potent than **Merle 43** in terms of binding, this difference is not

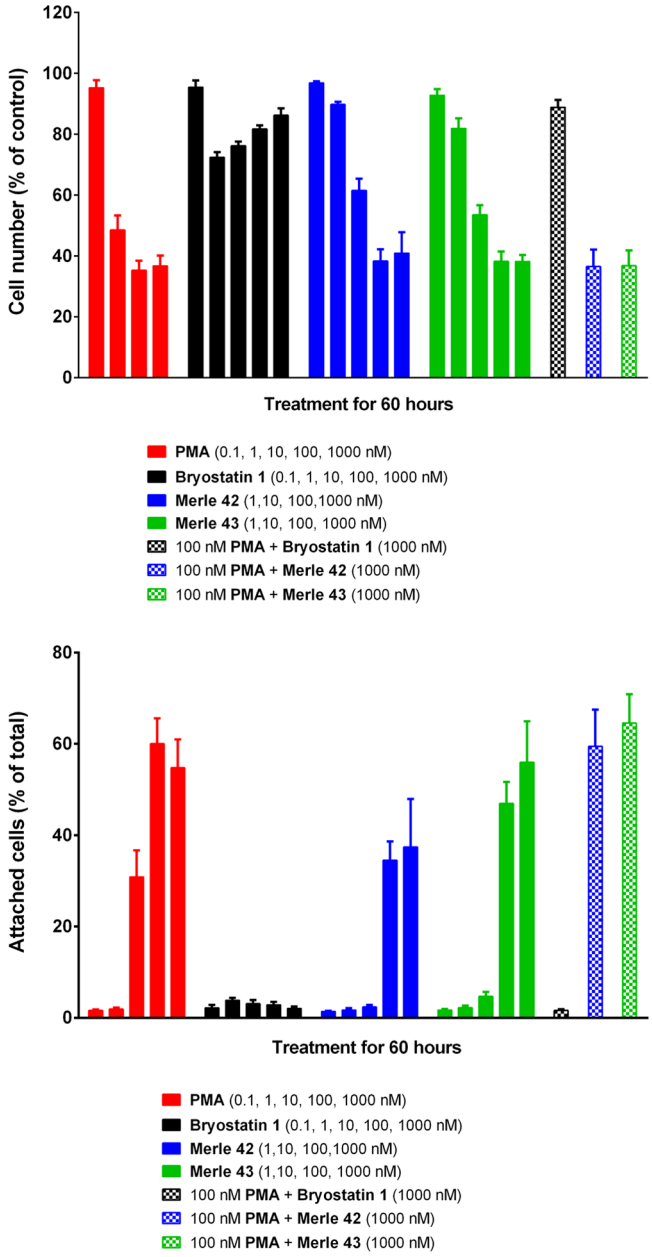

Figure 4. U937 proliferation and attachment assays.

reflected in the U937 proliferation and attachment assay, where both compounds acted with similar potency. On the other hand, when the analogues were tested for their ability to induce phosphorylation of the downstream kinase ERK in the LnCAP cell line, it was found that **Merle 42** was more potent than **Merle 43**, with an EC_{50} of 21.9 ± 4.2 nM compared to 385.6 ± 97.9 nM for **Merle 43** (see SI Figure 1). A similar difference in potency was also observed for the induction of PKD phosphorylation. These differences in potency (factor of 17.6) are essentially identical to the differences in PKC binding affinity observed with PKCα (factor of 18.4). Since the attachment and proliferation assays require 60 h to complete, compared to just 30 min to assay for phosphorylation, it is possible that **Merle 42** is rearranging to **Merle 43** over the longer period of time required for the U937 cell-based assays. It should be noted that rearrangement is not expected to be the sole cause of PMA-like response observed in the U937 cell line,

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as **WN-1** is also reported to induce a PMA-like response (see the accompanying paper[15]).

Computational Investigations on Merle 42 and WN-1. We were intrigued by the propensity of **Merle 42** to undergo ring expansion under very mild conditions, while the structurally similar compound **WN-1** (in which the carbonyl group is 1,3-transposed and hence not conjugated) did not. We had noted during simple model building that **Merle 42** appeared to be significantly strained, which might provide a driving force for the observed ring expansion. To further investigate these observations, we turned to a computational approach.

To analyze the effect of replacing the B-ring with an ester linkage on the overall conformation of the macrolide ring, we performed a thorough conformational search of **Merle 42** in octanol solvent. The lowest-energy conformation found retained a strong similarity to the crystal conformation of bryostatin 1[1] (Figure 5). The A- and C-rings can be overlaid

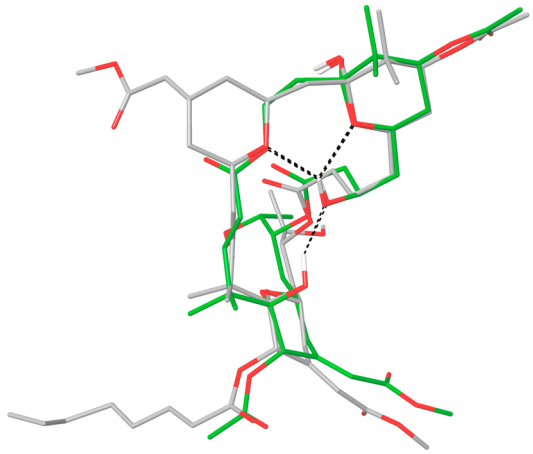

Figure 5. Overlay of the crystal structure of bryostatin 1 (gray) with the low-energy conformer of **Merle 42** (green). Intramolecular hydrogen bonds are shown as black dashed lines.

nearly exactly, and the ether oxygen in the ester linkage aligns with the pyran oxygen in the bryostatin B-ring. This allows the internal hydrogen-bonding structure of bryostatin to be preserved.

We then docked **Merle 42** into the crystal structure of the C1b domain of PKCδ (Figure 6) and found, as expected on the basis of the conformational analysis, that this analogue reproduces the binding mode of bryostatin,[8f] with the C26 hydroxyl hydrogen-bonding to the backbone at Thr 242 and Leu 251, and the C-ring methoxycarbonyl group hydrogen-bonding to Gly 253. The C9 hydroxyl in **Merle 42** forms an additional hydrogen bond to the backbone carbonyl of Met 239. The new carbonyl oxygen in the ester linkage remains solvent exposed and does not form any interactions with the C1 domain. The conformational analysis and docking results suggest that the ~20-fold difference in binding affinity between **Merle 42** and **WN-1** is not due to any significant change in conformation or loss of favorable interactions with the PKC C1 domain, although it is possible that, in the absence of the B-ring, the C9 hydroxyl has a much stronger effect on binding than it does in the context of the full A+B-ring structure.[8f]

Finally, we calculated the energetics of the two ring-expansion reactions, i.e., the observed conversion of **Merle 42** into **Merle 43** and the theoretically equivalent conversion of

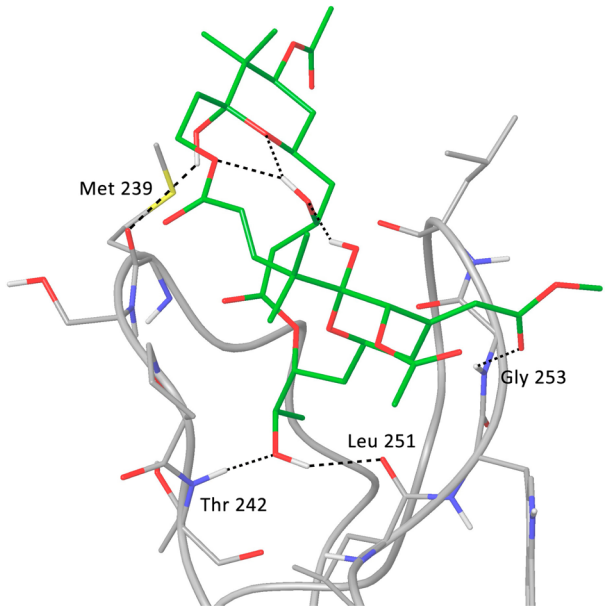

Figure 6. Computed binding model for **Merle 42** with the C1 domain of PKCδ. See the Supporting Information for details of the computational methodology.

WN-1 into **isoWN-1**, using the lowest-energy conformer for each compound. Geometry optimizations for each structure were run at the B97-D3/6-31G(d) level, and subsequent single-point energies were calculated at the ωB97X-D/6-311G(2d,2p) level. The reaction energy for **Merle 42** → **Merle 43** was −6.54 kcal/mol, whereas the energy for **WN-1** → **isoWN-1** was 2.27 kcal/mol, confirming that the rearrangement of **Merle 42** into **Merle 43** is energetically favorable while the equivalent rearrangement of **WN-1** into **isoWN-1** is not. We also calculated the energies for the hypothetical strictly isomeric compounds wherein the differences at C9 were removed, i.e., C9-deoxy **Merle 42** and C9-deoxy **Merle 43**, and the analogous C9-hydroxy **WN-1** and C-9 hydroxy **isoWN-1**. The results are interesting in that **Merle 42** has the highest energy of all the structures, and that the presence of the C9 hydroxyl group was found to make the rearrangement even more facile (−6.538 kcal/mol for the **Merle 42** case) than when it was absent (−4.96 kcal/mol for the C9-deoxy **Merle 42** case). In addition, **Merle 42** was found to be 6.54 kcal/mol less stable than the isomeric C9-hydroxy **WN-1**. The complete energetic relationships are detailed in the Supporting Information.

■ CONCLUSIONS

In conclusion, we report the synthesis of **Merle 42**, which was found to be unstable and to undergo an unexpected ring expansion to generate the 21-membered bryolactone analogue **Merle 43**. Both compounds proved to be potent ligands for PKC, which demonstrates that deletion of the bryostatin B-ring pyran does not preclude effective PKC binding. Additionally, these studies revealed that bryolactones whose C_{26} oxygen serves as the macrolactone linkage partner are also capable of binding PKC. Computational studies revealed a lowest energy conformer for **Merle 42** very similar to that of bryostatin 1, and with an internal H-bonding array like that of bryostatin. Likewise, the bound conformations were very similar. The unexpected ring expansion by a 1,2-acyl migration was calculated to be energetically favorable. The addition of other

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ring-expanded bryolactones to the repertoire of bryostatin analogues could be valuable, as their biological potential remains unexplored. The impetus to target **Merle 42** resulted from our previous studies that identified the A-ring substitution of bryostatin 1 as a key player in conferring bryo-like function in bryopyran analogues of bryostatin 1. Both **Merle 42** and **Merle 43**, however, were found to be phorbol-like in U937 cells, which suggests that the bryostatin B-ring also plays an important role in conferring bryo-like biological function to analogues of bryostatin 1.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, assay results, computational methods, and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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