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Deletion of the C-26 Methyl Substituent from the Bryostatin Analogue Merle 23 has Negligible Impact on Its Biological Profile or Potency

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Abstract

Important strides are being made in understanding the effects of structural features of bryostatin 1, a candidate therapeutic agent for cancer and dementia, in conferring its potency for protein kinase C and on the unique spectrum of biological responses which it induces. A critical pharmacophoric element in bryostatin 1 is the secondary hydroxyl at the C26 position, whereas a corresponding primary hydroxyl group plays the analogous role in binding of the phorbol esters to protein kinase C. Herein, we describe the synthesis of a bryostatin homolog in which the C26 hydroxyl group is primary, as in the phorbol esters, and show that its biological activity is almost indistinguishable from that of the corresponding compound with the secondary hydroxyl group.

Graphical Abstract

Presence or absence of the methyl substitution on C26 of bryologues does not affect the induced biological responses.

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Introduction:

The bryostatins are a family of complex macrolactone marine natural products originally isolated through the pioneering efforts of Pettit and coworkers.^[1] The flagship member of the family, bryostatin 1 (**1**) has received enormous attention due to the range of important and medicinally relevant biological activities it induces. Originally isolated due to the potent anticancer activity that extracts of the marine organism *Bugula neritina* showed against a cancer cell line, it has since been found to be a potent activator of protein kinase C (PKC) and other proteins that, like the PKCs, are activated by diacylglycerols (DAGs).^[2] As a potent activator of PKC, the biological activities of bryostatin 1 share similarities with those of other known high affinity ligands for PKC (such as the phorbol esters, exemplified by phorbol 12-myristate 13-acetate, PMA, (**2**)) but importantly, unlike the phorbol esters, bryostatin 1 is not tumor promoting in the 2-stage mouse model for carcinogenesis.^[3] Bryostatin 1 has been utilized in numerous clinical trials for cancer,^[4] and has more recently been of interest with respect to Alzheimer's disease^[5] and HIV infection.^[6] Other members of the bryostatin family mainly differ from bryostatin 1 in having different substituents at C7 and/or C20, although some, such as bryostatin 3, are more heavily modified, but all possess the same basic macrocyclic lactone core structure incorporating 3 pyran rings. Pettit named this common core structural feature a "bryopyran" motif.

As might be expected, the complex structure and potent biological activity of bryostatin 1 has led to great interest in the chemical synthesis of these compounds.^[7] The first synthesis of a bryostatin, bryostatin 7, was reported by Masamune and coworkers in 1990.^[8] Despite the intense interest in these compounds, it was not until 1998 that a second synthesis was reported, this of bryostatin 2, by Evans and coworkers.^[9] This was followed in 2000 by a synthesis of bryostatin 3 by Yamamura and coworkers.^[10] It was roughly a decade later before other syntheses of bryostatins were reported. The first total synthesis of bryostatin 1 was reported by Keck and coworkers in 2011.^[11] Other elegant total syntheses in this area were also published, including bryostatin 16 by Trost,^[12] bryostatin 7 by Krische,^[13] bryostatin 9 by Wender,^[14] and bryostatin 8 by Song.^[15]

However, in 1998 Wender and coworkers reported the synthesis of a simplified analogue of bryostatin 1 (**7**) in which the B-ring pyran was replaced by a 1,3-dioxane, for ease of synthesis of this acetal subunit.^[16] This bryostatin analogue was shown to have similar affinity for PKCs (using a mixture of isozymes isolated from rat brain) to that of bryostatin 1 (3 nM vs 1.4 nM). Subsequently, numerous such acetal analogues of bryostatin 1 were prepared in the Wender group, most notably, perhaps, acetal **8**, which was dubbed "pico" by Wender to reflect the reported very high PKC binding affinity of 0.25 nM (250 pM).^[17] The less potent original acetal analogue **7** was then named "nano"; these identifiers were subsequently used in numerous Wender publications and remain in use today.

Our own interest in these fascinating compounds originally focused on the development of methodology which might be of general utility in accessing the bryopyran core structures. The most powerful of these methodologies, termed “pyran annulation”, described a highly convergent asymmetric methodology by which a hydroxyallyl silane **11** could be annealed with an aldehyde **12** under acidic conditions to provide a disubstituted pyran **13** of the type which occurs repeatedly in the bryopyran core structure. One method by which the hydroxyallyl silanes **11** were readily available was by nucleophilic addition of the conjunctive reagent **10** to an aldehyde **9**; thus, overall, two aldehydes plus the conjunctive reagent were annealed to provide a pyran. (Scheme 3) This initial report^[18] was followed by the demonstration of application to the synthesis of the bryopyran core structure of the bryostatins^[19] and then to the first biologically active bryostatin analogues that possessed the core bryopyran carbon skeleton.^[20] The lead analogue here, Merle 23, also possessed the unusual dienolate C20 substituent found in bryostatin 1 but lacked much of the functionalization in the A- and B-rings. This was found to have binding affinity for the PKC α isozyme very similar to that of bryostatin 1 (0.70 vs 0.4 nM) but surprisingly functioned like the tumor promoting ligand PMA in U937 cells rather than like bryostatin 1. The U937 cells were known^[21] to provide a well characterized biological system that discriminated between PMA and bryostatin 1 activities: PMA induces attachment and blocks proliferation of these cells, while bryostatin 1 has little effect in either assay. Moreover, bryostatin 1 blocks the effect of PMA when the U937 cells are treated with the two agents together, confirming that the bryostatin 1 was not simply being degraded. At the same time, Wender also reported the synthesis of some bryopyrans using our pyran annulation strategy, but in an intramolecular context, and described their binding affinity for a mixture of PKC isozymes from rat brain.^[22] Subsequent studies from our group which examined substituent effects on biological activity led to a model wherein the lower portion of the bryopyran was largely responsible for its binding to PKC, while functional response in living cells depended heavily upon substitution in the upper half of the structure.^[23]

The 2008 paper from the Wender group also made an important correction, namely, that there really was no “pico”! Thus, a footnote to a Table directs the reader to another footnote to a Table in the Supplementary material, which indicates that the original report of binding affinity for “pico” was believed to be an error, and that the new number was 3.1 nM, *i.e.*, essentially the same as that for “nano”. However, a report from the Wender lab published subsequent to this paper still referred to the 0.25 nM value originally claimed for pico.^[24] This confusing state of affairs, and the lack of other biological characterization for pico, prompted us to examine the same structural change on the bryopyran platform, particularly since Merle 23 had been extensively characterized in terms of the biological effects it induced.^[25] Here, we report the synthesis and biological activity of the new bryopyran analogue **Merle 41**, in which the C26 methyl substituent has been deleted from structure Merle 23.

Results

Synthesis of Bryopyran Merle 41.

The synthetic plan for **Merle 41** followed along the lines demonstrated in 2005, utilizing two consecutive pyran annulations to prepare the B- and C-rings. However, the absence of the C26 stereogenic center required that the very beginning steps of the synthesis (which occur in our C-ring fragment) be reworked. Previously, we had used a commercially available starting material containing the C26 stereogenic center, and this stereochemistry was then utilized to construct additional stereogenic centers using a series of diastereoselective reactions. With this center absent, an asymmetric approach to generating the C25 stereogenic center was required.

The synthesis of the C-ring segment commenced from allyl alcohol (**17**). The hydroxyl group was protected, after which ozonolysis afforded aldehyde **18**. The requisite stereocenter at C25 was then established using a catalytic asymmetric allylation (CAA) reaction,^[26] which afforded the desired homoallylic alcohol **19** in excellent yield (93%) and as essentially a single enantiomer (99% ee). The new hydroxyl group was protected as a PMB ether, with an eye to conducting a diastereoselective nucleophilic addition to the aldehyde **20** available from the olefin by oxidative cleavage. Unfortunately, neither chelation-controlled nor nonchelation-controlled reaction conditions provided a satisfying level of stereoselectivity in additions to aldehyde **20**. Finally, a second CAA reaction was applied to afford alcohol **21** as essentially a single diastereomer (minor isomer not detected).

After conversion of the free hydroxyl to a TBS ether, the alkene was regioselectively hydroformylated to give aldehyde **22** in 87% yield.^[27] Next we carried out a prenylation reaction to add a reversed prenyl group to aldehyde **22**. Although we had done such reactions previously using indium metal and an allylic halide, here we found that the use of inexpensive zinc dust in an aqueous medium gave excellent results; subsequent Swern oxidation of the resulting secondary alcohol afforded ketone **23**. Ozonolysis of the vinyl group, followed by a phosphonate Wittig reaction on the resulting aldehyde, afforded the α,β -unsaturated thiol ester **24** in 86% yield over the two steps.^[28] After removal of the TBS group by reaction with aqueous HF buffered with pyridine, acid promoted cyclization of the hydroxy ketone with concomitant dehydration was accomplished using CSA in toluene at reflux to afford glycal **25**. Low temperature reduction with DIBAL in dichloromethane then gave the desired enal **14**.

The hydroxyallylstannane required for the pyran annulation had been previously prepared via CAA reaction in our labs.^[18,19] However, several examples of pyran annulation reactions conducted using glycals such as **14** as substrates had been examined previously and had generally proven to be problematic, in that yields were significantly lower than for most other cases examined. Similar complications were observed in attempted pyran annulations using **14**. Thinking that the problem could potentially be decomposition due to the presence of acid sensitive regions of the substrate, we examined the use of various additives in the pyran annulation using substrate **14**. It was found that the use of substoichiometric amounts of pyridine greatly improved the yields and reproducibility of this reaction, with the yield

improvement being in the 20-30% range. Under optimal conditions, the desired pyran product **26** was obtained much more cleanly, and in 93% isolated yield.

To avoid the impediment of the labile glycal in the remaining synthetic steps, the C-ring was fully functionalized at this point. After chemoselective epoxidation and methanolysis *in situ*, the resulting ketal was subjected to a catalytic amount of PPTS to enrich the mixture of diastereomers (at C19) in favor of the desired and thermodynamically more stable isomer. Dess-Martin periodinane (DMP) oxidation then provided ketone **27**.^[29] This sequence of three steps was accomplished in excellent (89%)yield. To complete the elaboration of the C-ring, an aldol reaction with freshly distilled methyl glyoxalate was used to give enoate **28** in 76% isolated yield. Luche reduction of the ketone^[30] gave an unstable alcohol which was immediately esterified with the requisite octadienoic acid under Yamaguchi conditions.^[31] The desired C20 ester **29** was obtained in an excellent 93% yield over this two-step sequence.

The stage was now set for incorporation of the A-ring. Removal of the BPS group using ammonium fluoride in methanol at reflux gave (92%) the corresponding alcohol, which was oxidized using Dess-Martin reagent to the corresponding aldehyde. At this point, it was necessary to perform a protecting group swap of the PMB group in aldehyde **30**, due to the presence of another PMB group in the hydroxyallyl silane partner for the pyran annulation. This was accomplished by removal of the PMB group by reaction with DDQ, followed by silylation of the alcohol using TBS triflate at -78°C , to give aldehyde **31**.

The A-ring hydroxyallyl silane was easily prepared from homoallylic alcohol **34**, itself available using a CAA reaction as shown (Scheme 8). Protection of the alcohol as a PMB ether was used to allow for a high level of diastereoselectivity in the subsequent allylation of aldehyde **35**.

Thus, after introduction of the PMB group, the vinyl group was cleaved by ozonolysis to afford aldehyde **35**. Chelation controlled addition of the silyl stannane reagent **10**, promoted by magnesium bromide etherate, in dichloromethane at -78°C , afforded, essentially exclusively (>99:1), the desired diastereomer of hydroxyallyl silane **36**, as a consequence of chelation control in the addition to a preformed magnesium chelate.^[32]

With both aldehyde **31** and β -hydroxyallylsilane **36** in hand, the second pyran annulation was conducted to afford the tris pyran **27** in an excellent yield of 90% using the modified (pyridine additive) conditions (Scheme 9). The BPS silyl ether at C1 was selectively deprotected by NH_4F , and the resulting alcohol was subject to DMP oxidation to afford aldehyde **28** in 57% yield over 2 steps. The aldehyde was then oxidized to the corresponding carboxylic acid under Pinnick conditions.^[33] Next, the removal of the C25 TBS group furnished a seco-acid which then underwent macrolactonization using Yamaguchi's protocol under high dilution techniques (slow addition via syringe pump). These three steps transpired in amazingly high overall yield, affording the macrolactone **29** in 95% isolated yield. With the bryopyran skeleton now complete, all that remained was removal of the final three protecting groups. This was accomplished by initial DDQ mediated PMB removal

followed by a global deprotection with LiBF_4 [34] to afford the des-methyl analogue **Merle 41** in 69% yield.

Biological characterization of Merle 41:

Merle 41 bound to PKC α with a K_i of 0.73 ± 0.05 nM ($n = 3$ experiments). This value closely matches the K_i of 0.70 ± 0.06 nM that we reported previously for Merle 23.[20] We have previously described that bryostatin 1 and bryostatin 7 showed little PKC isoform selectivity,[23b] with no more than a 3-fold difference in K_i for human PKC β II, PKC δ , and PKC ϵ compared to that for human PKC α . In preliminary experiments, we found that this was likewise the case for **Merle 41**. K_i values of **Merle 41** for human PKC β II, PKC δ , and PKC ϵ assayed *in vitro* were 2.1 ± 0.6 , 0.8 ± 0.2 , and 0.9 ± 0.2 nM, respectively. While these measurements were not carried out in parallel with the above, and thus do not provide a precise comparison, they give no indication of substantial *in vitro* binding selectivity among PKC isoforms for **Merle 41**.

The Toledo cell line is derived from a Non-Hodgkin's B cell lymphoma. It is among the most sensitive cell lines for growth inhibition by phorbol ester and, unlike leukemia cell lines such as U937, K562, or MV-4-11,[35] is similarly growth inhibited by bryostatin 1. [36, 37] **Merle 41** and Merle 23, like PMA and bryostatin 1, inhibited Toledo cell growth (Figure 1A). **Merle 41** closely resembled Merle 23 both in potency and in the extent of growth inhibition. Unlike the Toledo cells, U937 promyelocytic leukemia cells respond to phorbol ester with growth inhibition and cell attachment, whereas bryostatin 1 causes little growth inhibition and almost no cell attachment. **Merle 41** elicited a response virtually identical to that to Merle 23 (Figure 1 B,C). For both compounds, U937 cell growth was inhibited almost to the level caused by PMA but with a somewhat biphasic dose response, with reduced inhibition at 100 or 1000 nM. Attachment was induced to the same level as for PMA, but again the dose response was biphasic, with reduced attachment at 300 nM or above. The combination of PMA plus **Merle 41** or Merle 23 gave a response similar to that of Merle 23 or **Merle 41** alone and the pattern of partial antagonism of the PMA response at high concentrations of Merle 23 or **Merle 41** was the same for each.

TNF α is released onto the medium of the U937 cells in response to PMA and contributes to the growth inhibition. **Merle 41** and Merle 23 displayed identical dose response curves for release of TNF α with absolute maximal levels of release of $65.2 \pm 2.6\%$ and $66.6 \pm 2.6\%$, respectively, of that for PMA (Figure 1D). Once again, in combination with PMA both compounds reduce the level of release of TNF α in response to PMA to the level induced by the compound alone. By all these measures, **Merle 41** was very similar to Merle 23.

PMA, bryostatin 1, and Merle 23 cause distinct patterns of down regulation of PKC isozymes in the human prostate cancer cell line LNCaP as a function of time.[25] As previously observed, PKC δ was down regulated by PMA, bryostatin 1 caused biphasic down regulation of PKC δ with protection at higher bryostatin 1 doses, and Merle 23 caused more extensive down regulation of PKC δ than did either PMA or bryostatin 1 (Figure 2A). **Merle 41** acted similarly to Merle 23. PKC α was down regulated by PMA, bryostatin 1 was more potent at PKC α down regulation, and Merle 23 was less potent than either and indeed at low

doses caused a modest increase in PKC α (Figure 2B). Merle 41 again acted similarly to Merle 23. PKC ϵ showed modest down regulation in response to PMA, bryostatin 1 caused even less down regulation, and Merle 23 resembled PMA in inducing weak PKC ϵ down regulation (Figure 2C). **Merle 41** showed a pattern of response identical to that of Merle 23. Finally, PMA caused strong down regulation of PKD1. Bryostatin 1 and Merle 23 caused little PKD1 down regulation (Figure 2D). **Merle 41** again closely paralleled Merle 23.

While the data clearly show that Merle 41 resembles Merle 23 in its down regulation of the PKC isoforms examined, in support of the lack of a role for the C26 methyl group in biological activity, it should be noted that the relative dose response curves for down regulation of the PKC isoforms by **Merle 41** do not parallel its *in vitro* binding potencies, as we described above. Likewise, the marked differences between it and bryostatin 1 in the down regulation of the PKC isoforms are not reflected in appreciable differences in their selectivity for *in vitro* binding. While the basis for these differences remains unresolved, down regulation represents the interplay of numerous factors, both reflecting the initial ligand – PKC interaction and feedback from downstream consequences, and multiple pathways. The profound differences in biological response between bryostatin 1 and Merle 23, as previously reported, illustrate how binding alone is only one element, and we and others have speculated that the different surface provided by **Merle 23/Merle 41** versus bryostatin 1 may be an important contributor. [23c]

Comparison of the patterns of down regulation at 100 nM levels of the various ligands emphasizes a very important concept (Figure 3). Whereas “PMA-like” or “bryostatin-like” may afford a convenient summary of the pattern of behavior in a system like the U937 cells, detailed characterization shows that different ligands have different patterns of interaction with various PKC isoforms or other C1 domain containing targets like PKD1. The pattern of down regulation of PKC isoforms by **Merle 41** or Merle 23 was unique and distinct from that of either PMA or bryostatin 1. A powerful prediction therefore is that such derivatives should have a unique pattern of biology, as indeed we observed in our detailed characterization of the action of Merle 23 in the LNCaP cells. [25]

We have previously described that PMA initially induces translocation of PKC δ to the plasma membrane of LNCaP cells, with subsequent localization to internal membranes, whereas bryostatin 1 causes less initial plasma membrane translocation with more staining of nuclear and internal membranes. [38] These differences were likewise found in the current experiments (Figure 4). As previously reported, [25] Merle 23 behaved more like bryostatin 1 than like PMA (Figure 4). **Merle 41** behaved similarly to Merle 23 (Figure 4).

PKC ϵ in LNCaP cells was translocated to the plasma membrane in response to PMA and more weakly in response to bryostatin 1 (Figure 5), as had been reported previously. [23b] Merle 23 and **Merle 41** resembled one another in their behavior, which was intermediate between that of PMA and bryostatin 1 (Figure 5). Because of the extent of variability of response among cells, it should be noted that the comparisons of translocation for PKC δ and PKC ϵ would not be able to define minor differences in behavior.

As a powerful approach to detect more subtle differences in the responses to **Merle 41** and Merle 23, we examined the time and dose dependence of the induction of gene expression by **Merle 41** and Merle 23 and we further compared those responses to those induced by PMA and bryostatin 1. Responses were characterized in both the LNCaP cells (Figure 6) and the U937 cells (Figure 7), two systems that we have studied extensively for their responses to bryostatin derivatives. The genes we examined had been previously observed to illustrate different patterns of response to PMA and bryostatin 1. As we had described before,^[39] bryostatin 1 and Merle 23 caused responses in the LNCaP cells very similar to those to PMA at 2 hr (Figure 6A). By 6 hr, the response to bryostatin 1 had decreased to variable extents for different genes relative to the PMA response; the response to Merle 23 had begun to separate from that to PMA (Figure 6B). By 24 hr the response to Merle 23 had further separated and more approached that to bryostatin 1 (Figure 6C). In each case, the response to **Merle 41** was almost identical to that to Merle 23.

A similar pattern was observed in the U937 cells, except that the response to bryostatin 1 diverged at later times for some genes and the response to Merle 23 remained closer to the PMA response (Figure 7). In each case, the response to **Merle 41** remained very similar to that to Merle 23.

The above summaries reflect behavior at 100 nM of each ligand. The complete dose response curves provide further insight. For example, at 2 hr in the U937 cells the response of TNFAIP3 was greater for bryostatin 1 than it is for PMA or for **Merle 23/41** (Figure 7A). The complete dose response curves revealed that bryostatin 1 was not actually more effective at induction of TNFAIP3 gene expression (Figure 8). Rather, the dose response curves for PMA and for **Merle 23/41** were markedly biphasic, while that for bryostatin 1 was less so. Equal levels of TNFAIP3 were induced at optimal levels of each ligand, but a 1 nM concentration of PMA and bryostatin 1 gave maximal induction, whereas maximal induction by **Merle 23/41** occurred at 10 nM. Because of the biphasic dose response curves, response to bryostatin 1 at 10 nM was now somewhat lower and response to PMA was lower still. Because the dose response to bryostatin 1 was substantially less biphasic than that to PMA or **Merle 23/41**, at 100 nM the response to bryostatin 1 was now greater than that to PMA or **Merle 23/41**. Despite such complexities, **Merle 41** behaved similarly to Merle 23 under virtually all assay conditions. Out of the 15 genes examined in the LNCaP cells and the 12 genes examined in the U937 cells, a modest, statistically significant difference in the responses to Merle 23 and **Merle 41** was observed in only one case, for induction of TNF α expression in the U937 cells (Supplemental Figure 1).

Conclusions

Our extensive analysis failed to reveal any substantial influence of the C26 methyl group on the pattern of biological response of this pair of bryostatin derivatives, Merle 23 and **Merle 41**. Until shown to be otherwise, it therefore seems appropriate to assume that differences in bryostatin structure function relationships reflect the structural differences exclusive of the presence or absence of a C26 methyl group.

As we learn more about bryostatin structure function relationships, the importance of detailed functional characterization has become ever clearer. PKC binding potency represents an initial determinant of potential activity and is predominantly conferred by pharmacophoric elements in the lower half of the molecule.^[16] In contrast, the failure of bryostatin 1 to induce PMA like responses despite PKC activation is largely conferred by the top (A,B ring) portion of the molecule,^[20,40] provided some domain capable of high affinity PKC binding is present. Bryostatin-like activity is not an all-or-none phenomenon, however. A series of bryostatin derivatives with progressive modifications in the patterns of substitution in the A,B-ring portion showed variable extents to which they resembled PMA versus bryostatin 1 in their activity in the U937 cell system.^[39] The extents of bryostatin-like activity correlated with the extent to which they could induce a more bryostatin like pattern of transient gene expression. Comparison of the behavior of Merle 23 in the U937 cells with its activity in the LNCaP cells further emphasized that the cellular pattern of response depended not only on the ligand but also on the cell system.^[25] Thus, in the LNCaP cells Merle 23 was like bryostatin 1, not like PMA, in its inability to inhibit cell proliferation. A plausible rationale is that biological responses in different cells show different dependence on the levels of expression of different PKC isoforms and on the variable sensitivities of these isoforms to the compounds. The biological outcome will depend on the extents of down regulation of the various isoforms as well as on their cellular localization.^[25,39,41]

An important implication for therapeutic development is that different ligands which cause different patterns of PKC isoform down regulation and localization should have different impacts depending on the specific cell type and the specific set of responses of importance for that cell. As illustrated here, **Merle 23/41** have their own pattern of PKC regulation. These compounds may thus be of particular utility for the appropriate targets. By extension, the current efforts in bryostatin structural modification may yield important drug leads beyond the initial goal of structurally simplified, synthetically accessible bryostatin 1 mimetics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Comparison of the biological effects of Merle 23 and Merle 41.

A) Dose response curves for inhibition of growth of Toledo cells after 72 hr of treatment. B) Inhibition of growth of U937 cells after 60 hr of treatment. C) Induction of attachment of U937 cells after 60 hr of treatment. D) Secretion of TNF α from U937 cells after 60 hr of treatment. All points represent the mean $\pm$ SEM of triplicate experiments. Methods were as previously described.^[23b, 39]

Figure 2. Dose response curves for down regulation of PKC isoforms and PKD1 in LNCaP cells. LNCaP cells were treated as indicated for 24 hrs. All points represent the mean $\pm$ SEM of quadruplicate experiments. Methods were as described in the Supplemental Information.

Figure 3. Comparison of the effects of compounds on the levels of PKC isoforms and of PKD1 in LNCaP cells.

Levels following treatment for 24 hr with the indicated compounds (100 nM) are expressed relative to those for the vehicle (DMSO) control (data from Figure 6).

Figure 4. Comparison of the effects of compounds on translocation of mouse GFP-PKCδ. LNCaP cells were imaged at the indicated times after treatment with 1000 nM concentrations of the compounds. Results are representative of 5 experiments for PMA and bryostatin 1, 12 experiments for Merle 23, and 10 experiments for **Merle 41**. Bars indicate 10µm. Methods were as previously described.^[23b]

Figure 5. Comparison of the effects of compounds on translocation of human YFP-PKCe. LNCaP cells were imaged at the indicated times after treatment with 1000 nM concentrations of the compounds. Results are representative of 4 experiments for PMA, bryostatin 1, and Merle 23, and 6 experiments for **Merle 41**. Bars indicate 10 μ m. Methods were as previously described.^[23b]

Figure 6. Induction of gene expression in LNCaP cells as a function of time following treatment with 100 nM concentrations of the various compounds.

Levels of mRNA expression of the indicated genes were measured by qPCR as described previously^[39] after treatment for A) 2 hr, B) 6 hr, and C) 24 hr. Values are expressed relative to those for the vehicle (DMSO) control and represent the mean $\pm$ SEM of triplicate experiments.

Figure 7. Induction of gene expression in U937 cells as a function of time following treatment with 100 nM concentrations of the various compounds.

Levels of mRNA expression of the indicated genes were measured by qPCR as described previously^[39] after treatment for A) 2 hr, B) 6 hr, and C) 24 hr. Values are expressed relative to those for the vehicle (DMSO) control and represent the mean $\pm$ SEM of triplicate experiments.

Figure 8. Dose response for induction of gene expression of TNFAIP3 in U937 cells after treatment for 2 or 6 hrs.

Values are expressed relative to those for the vehicle (DMSO) control and represent the mean $\pm$ SEM of triplicate experiments.

Bryostatin 1

Phorbol-12-myristate-13-acetate (PMA)

Bryostatin 3

1 Bryo 1	$R_1 = \text{OAc}$
4 Bryo 2	$R_1 = \text{OH}$
5 Bryo 4	$R_1 = \text{OCO}i\text{-Bu}$
6 Bryo 9	$R_1 = \text{OAc}$

$R_2 = \text{OCO}(\text{CH}_2)_4n\text{-Pr}$
$R_2 = \text{OCO}(\text{CH}_2)_4n\text{-Pr}$
$R_2 = \text{OCO}n\text{-Pr}$
$R_2 = \text{OCO}n\text{-Pr}$

Scheme 1.
Structures of representative bryostatins and PMA

Nano **Pico****Scheme 2.**

Structures of the Wender acetal analogues nano (7) and pico (8).

Scheme 3. Pyran annulation. a) BITIP, CH_2Cl_2 ; b) R_2CHO (12), TMSOTf, Et_2O , -78°C .

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Scheme 5.
Retrosynthetic Plan for synthesis of **Merle 41**

Scheme 6.
Synthesis of the C-Ring Fragment

Scheme 7.
Pyran Annulation and C-ring Elaboration

Scheme 8.
(Single Column) Preparation of A ring allyl silane

Scheme 9.
Completion of the Synthesis of **Merle 41**