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The Bryostatin 1 A-Ring Acetate is Not the Critical Determinant for Antagonism of Phorbol Ester-Induced Biological Responses

Gary E. Keck,* Wei Li, Matthew B. Kraft, Noemi Kedei,† Nancy E. Lewin,† and Peter M. Blumberg†

University of Utah, Department of Chemistry, Salt Lake City, Utah 84112

keck@chemistry.utah.edu

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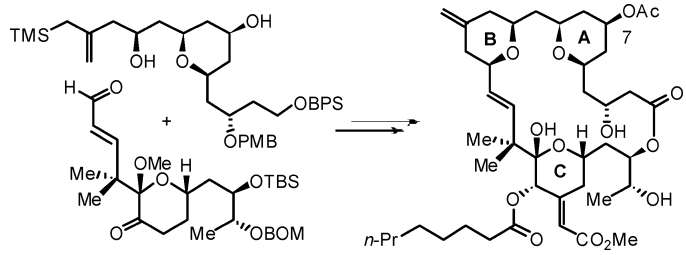
ABSTRACT



The contribution of the A-ring C_7 acetate to the function of bryostatin 1 has been investigated through synthesis and biological evaluation of an analogue incorporating this feature into the bryopyran core structure. No enhanced binding affinity for protein kinase C (PKC) was observed, relative to previously characterized analogues lacking the C_7 acetate. Functional assays showed biological responses characteristic of those induced by the phorbol ester PMA and distinctly different from those observed with bryostatin 1.

The protein kinase C (PKC) family of kinases plays a central role in the regulation of crucial cellular events such as proliferation, differentiation, and apoptosis. Furthermore, alterations in the expression or activity of PKC isoforms have been linked to various human diseases such as cancer,[1] diabetes,[2] and Alzheimer's disease.[3] PKC has thus emerged as an attractive target for drug development. Attempts at targeting the catalytic domain of PKC have been hindered by issues of selectivity since the human genome encodes approximately 500 protein kinases, each containing the highly conserved ATP binding pocket.[4] The novel and classical PKC isozymes possess cysteine-rich C1 domains which recognize endogenous 1,2-diacyglycerols (DAGs) ultimately leading to the activation of the enzyme. Targeting the C1 domain rather than the catalytic domain provides a potential strategic advantage in drug selectivity since only a small number of other enzymes are known to contain a C1 domain, e.g., the chimaerins, RasGRPs, PKDs, Unc-13s, and the diacylglycerol kinases.[5]

The first known exogenous ligands for the PKC C1 domain were the tumor promoting phorbol esters such as phorbol 12-myristate-13-acetate (PMA, **2**) (Figure 1), leading to the hypothesis that their tumor promoting activity was in part

† Laboratory of Cancer Biology and Genetics, Center for Cancer Research, NCI, NIH, Bethesda, MD 20892.

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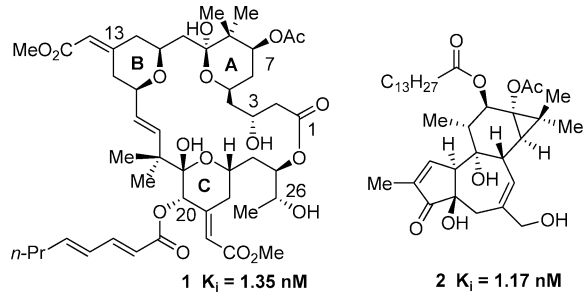

Figure 1. Structures of bryostatin 1 and PMA.

linked to PKC activation.[6] Bryostatin 1 (**1**), isolated by Pettit from the marine bryozoan *Bugula neritina*, is also a potent activator of PKC.[7] However, unlike most phorbol esters, bryostatin 1 is not tumor promoting and antagonizes many of the responses induced by the phorbol esters. Bryostatin 1 has potent activity toward a number of cancer cell lines, displays immunostimulant effects,[8] and shows promise in the treatment of Alzheimer's disease.[9,3] Recently, bryostatin 1 has also been shown to induce repair of neuronal damage resulting from stroke.[10]

In an effort to identify the mechanisms responsible for the unique activity of bryostatin 1, we are attempting to determine, through chemical synthesis, the structural features of bryostatin that are responsible for its unique biological profile. Recently, we have prepared and evaluated analogues (Merle **21**−**23**[11] and Merle **28**,[12] vide infra) which now limit the functionality largely responsible for the unique biological profile exhibited by bryostatin 1 to that present in the C_7−C_9 region of the A-ring.[13] In this paper, we report studies which rule out the A-ring C_7 acetate as being the critical determinant of the unique biological responses characteristic of bryostatin 1, through synthesis and biological evaluation of analogue Merle **27**. The preparation of this new bryopyran is outlined in Scheme 1.

Analogue Merle **27** was prepared using a convergent pyran annulation strategy to unite preformed A- and C-rings with concomitant formation of the B-ring, as shown in Scheme 1. Preparation of the A-ring pyran began with pyran **3**, prepared via pyran annulation as previously described.[11] The exomethylene was used to introduce the requisite C_7 oxygenation by ozonolysis followed by $NaBH_4$ reduction (94%) to give the C_7 alcohol **4** as a single diastereomer. Temporary protection of the alcohol as its TMS derivative[14] was followed by Bunnelle reaction[15] to give the desired hydroxy allylsilane **5** in 71% yield.

Pyran annulation between **5** and C-ring enal **6** using our standard conditions of TMSOTf in ether gave the desired tricyclic adduct, as a single diastereomer, in 92% isolated yield. Protection of the C_7 OH using TBSOTf and 2,6-lutidine gave **7** in 98% yield.

Aldol condensation with methyl glyoxylate gave a diastereomeric mixture of aldol products (92%) which underwent elimination (93%) by treatment with Ac_2O and DMAP in pyridine at 60 °C. The resulting enoate **8** was then subjected to Luche reduction[16] followed by acylation with octanoic anhydride to give **9**. Selective removal of the BPS (*tert*-butyldiphenylsilyl) group at C_1 using TBAF and HOAc in DMF (89%) was followed by oxidation to the acid **10** (quantitative yield over 2 steps) by sequential Parikh−Doering[17] and Pinnick[18] oxidations. Removal of both TBS groups (at C_7 and C_{25}) using HF•Py then gave the corresponding dihydroxy acid.

Attempted macrolactonization of this dihydroxy acid using the Yamaguchi protocol[19] afforded a macrolactone in which the C_7 alcohol had *also* been acylated by 2,4,6-trichlorobenzoyl chloride, in excellent (79%) yield. To preclude the undesired acylation of C_7, this hydroxyl was selectively protected by reaction with TESCl and DMAP to give **11**; the Yamaguchi reaction then afforded the desired macrolactone **12**. Removal of the TES group followed by acetylation gave the desired C_7 acetate derivative **13** (95% over 2 steps). Finally, protecting group removal (DDQ then $LiBF_4$ in aqueous CH_3CN) gave analogue Merle **27** (87% over 2 steps). The C_7 trichlorobenzoate derivative obtained inadvertently was also carried through a parallel sequence to give analogue **14**.

Analogue Merle **27** was found to bind to PKC with slightly lower affinity than that of Merle **21**−**23** (K_i = 3.0 ± 0.6 nM with PKCα). Assays for differential response were conducted using leukemia U937 cells.[20] In this assay, PMA inhibits cell proliferation and induces attachment. Bryostatin 1 has much less effect on either response and blocks the effect of the phorbol ester when both agents are administered together. The results are shown in Figures 2 and 3. Results

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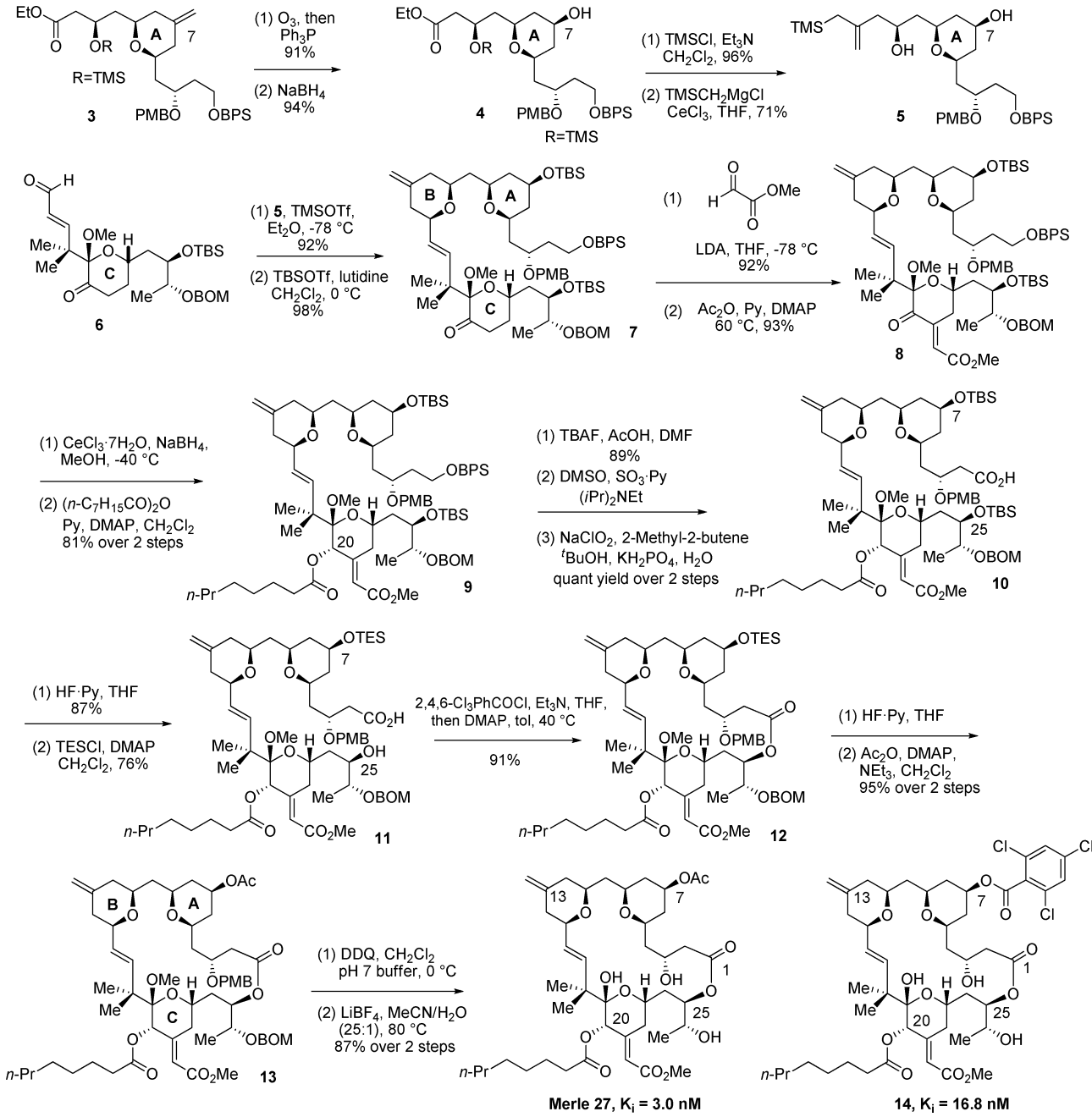
Scheme 1. Synthesis of C_7-Functionalized Bryopyrans



for the previously reported bryostatin-like analogue Merle **28** are also included for comparison.

Merle **27** can be seen to induce biological responses characteristic of those shown by PMA rather than bryostatin 1. Thus, the C_7 acetate cannot be singly responsible for the switch in activity observed between the PMA-like analogues Merle **21**−**23** (and now Merle **27**) and the bryo-like analogue Merle **28**. Instead, it appears that the critical residues must be the C_9 OH and/or the C_8 *gem*-dimethyl. With these results, the puzzling aspects of bryostatin 1 biology have been pinpointed as originating primarily from effects of substitution on, at most, two carbons.[21] Efforts to determine, through synthesis, the role of substituents at each of these carbons are in progress.

It should be noted that Wender and Verma have recently reported the synthesis of the C_7 acetate **16** and a number of other C_7 derivatives, including the C_7−exomethylene compound **15**, using the B-ring acetal platform developed

(21) Since modifications of the bryostatin 1 structure by definition give different compounds, these will of course interact somewhat differently with PKC or other C1 domain-containing proteins. Here we are concerned with substantial changes in the biological profiles exhibited by these analogues and not the minor differences anticipated for different structures, such as relatively minor changes in binding affinity or potency in other assays.

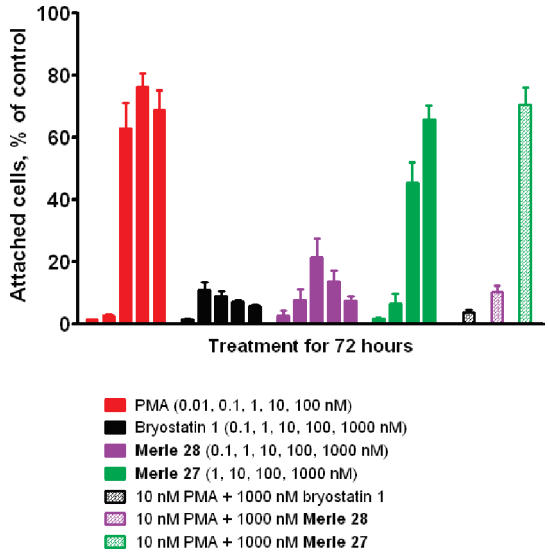


Figure 2. Effect of Merle **27** on U937 cell attachment.

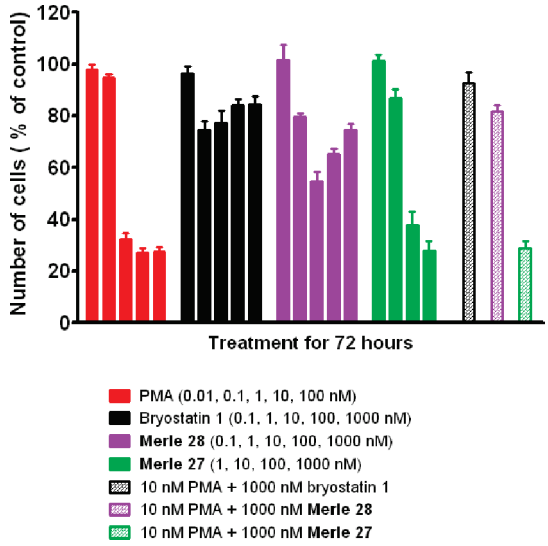


Figure 3. Effect of Merle **27** on U937 cell proliferation.

previously by that group.[22] These authors also utilized our pyran annulation approach for construction of the A-ring bearing the versatile C_7 exomethylene group. Using this platform, the acetate **16** was found to have $K_i = 13 \pm 3.8$ nM against a mixture of PKC isozymes isolated from rat brain, while the exomethylene compound **15** had $K_i = 5.3$ nM.[23] (Note Figure 4.)

Interestingly, of the 12 C_7-functionalized derivatives reported by Wender and Verma, the highest affinity for PKCs was shown by the C_7-exomethylene derivative **15**, i.e., the analogue incorporating the C_7 functionality introduced

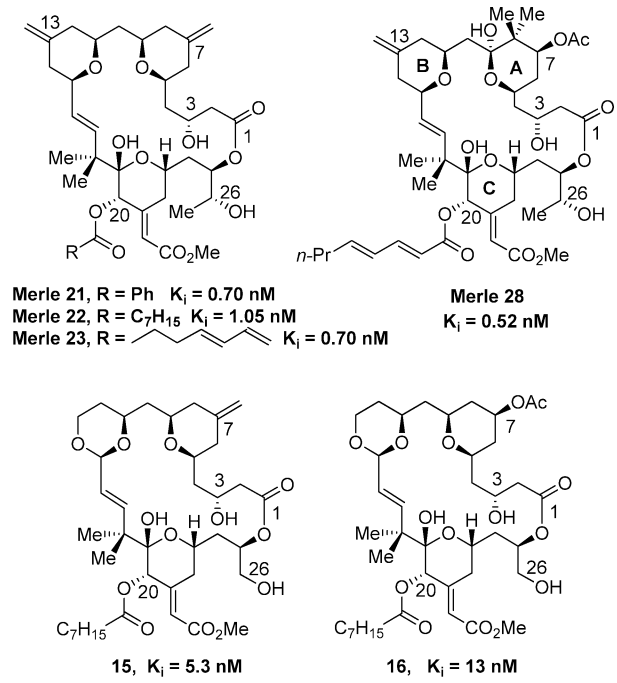


Figure 4. Structures of other recent analogues.

directly by the pyran annulation reaction. All other modifications at C_7 examined gave materials with decreased affinity for PKCs. The same is true of the C_7 acetate Merle **27** and the C_7 trichlorobenzoate **14** ($K_i = 16.8 \pm 4.2$ nM with PKCα) prepared by us using the bryopyran platform, which are less potent than the exomethylene compounds Merle **21**−**23**. Thus, on both platforms, the exomethylene compounds are approximately 2.5−3-fold more potent in terms of binding than the compounds incorporating the natural C_7 acetate functionality.

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

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(23) These authors suggest that the carbonyl oxygen of this acetate substituent may be involved in hydrogen bonding to a residue in the C1 domain.