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Role of the C_8 *gem*-dimethyl group of bryostatin 1 on its unique pattern of biological activity

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ABSTRACT

The role of the C_8 *gem*-dimethyl group in the A-ring of bryostatin 1 has been examined through chemical synthesis and biological evaluation of a new analogue. Assays for biological function using U937, K562, and MV4-11 cells as well as the profiles for downregulation of PKC isozymes revealed that the presence of this group is not a critical determinant for the unique pattern of biological activity of bryostatin.

Bryostatin 1 is the most thoroughly studied member of a family of over 20 complex macrolide natural products originally isolated by Pettit and coworkers from the marine bryozoan *Bugula neritina*.[1] It was later discovered that the bacterial endosymbiont *Candidatus endobugula sertula* was the true source of the bryostatins.[2] The bryostatins are composed of three highly functionalized pyran rings embedded in a macrocycle and differ primarily in substitution at the C_7 and C_{20} positions.[3] Bryostatin 1 has been found to display a remarkably wide range of biological activity. Due to its initially promising anticancer activity, bryostatin 1 has been used in numerous phase I and II clinical trials for cancer chemotherapy. Moreover, bryostatin 1 has been shown to reverse multidrug resistance[4] as well as to synergize[5] with other well known oncolytic drugs such as paclitaxel, vincristine and cisplatin. However, unlike most anticancer drugs, bryostatin 1 stimulates the immune system, a property that has recently been utilized to overcome HIV latency in lymphocytes.[6] Additionally, bryostatin 1 has displayed promising neurologic effects. Bryostatin 1 has also been shown to enhance memory and learning in animal models[7,8] and has shown activity against Alzheimer's disease in transgenic mice. Moreover, it has recently been reported that bryostatin 1 is able to stimulate repair of the neural damage and reestablishment of synapses for up to 24 h after stroke in rats.[9]

The mechanism by which bryostatin 1 evokes these diverse biological effects is believed to arise largely from the modulation of protein kinase C isozymes (PKCs) and other C1 domain containing proteins upon their binding of bryostatin 1.[10] Due to their important role in signaling, PKCs have emerged as an attractive target in drug discovery.[11] Several natural products such as phorbol esters, bryostatins, indolactams, and aplysiatoxin are known high affinity exogenous ligands for PKCs. An important distinction among various PKC ligands is that, although they bind to the same C1 domain of PKCs, the biological responses subsequent to bindings can be very different. For example phorbol esters such as phorbol 12-myristate-13-acetate (PMA) and bryostatin 1 bind to the same C1 domain of PKC with high affinity; however, PMA is tumor promoting whereas bryostatin 1 is not.[12] In addition, bryostatin 1 antagonizes many of the PMA induced response which it does not induce itself.

Our group is endeavoring to understand the structural features of bryostatin 1 responsible for its unique biological activity. To this end, we have synthesized and analyzed various bryopyran structures[13] using the pyran annulations methodology[14] developed in our laboratories that has proven critical in recently reported total syntheses of bryostatins.[15] Representative examples of our bryopyran analogues are shown in Figure 1. Examination of analogue Merle 23 revealed that substitution on A- and B-rings is critical for obtaining antagonism of phorbol ester induced biological responses. The roles of individual substituents in this region including the C_{30} carbomethoxy group (Merle 28), C_9 hydroxy group

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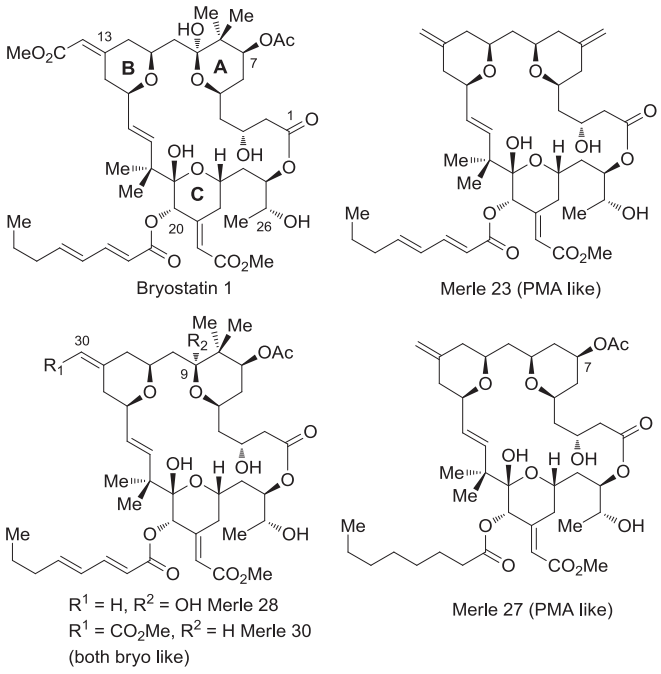


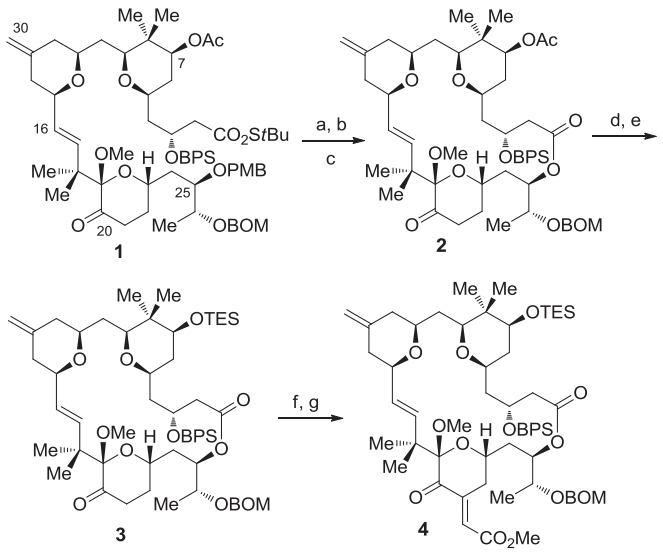
Figure 1. Structure of bryostatin 1 and bryopyrans.

(Merle 30) and C_7 acetate group (Merle 27) have been addressed, revealing that none of these substituents alone acts as a functional switch for PMA versus bryostatin like activity. Herein we examine the role of the final such substituent, the C_8 *gem*-dimethyl group, through chemical synthesis and biological evaluation of the new bryopyran analogue Merle 32.[16]

The synthesis of Merle 32 commenced from intermediate **1** which had been prepared previously enroute to analogues Merle 28 and Merle 30 (Scheme 1). The C_{25} alcohol was first freed by removal of the PMB group using DDQ. Subjection of the resulting hydroxy-thioester to oxidative hydrolysis using *m*-CPBA in aqueous THF selectively cleaved the thioester in the presence of the C_7 ester and provided the corresponding seco acid. Although both the C_{16}–C_{17} olefin and C_{20} ketone proved unreactive towards *m*-CPBA, it was necessary to stop the reaction after three and half hours in order to prevent the epoxidation of the C_{13}–C_{30} olefin. Yamaguchi macrolactonization of the seco acid then furnished the macrolactone **2** in excellent yield.[17] To preclude competing enolization of the C_7 acetate in the ensuing aldol reaction,[13c,d] the C_7 acetate was removed using K_2CO_3/MeOH (without interference from the macrolactone ester functionality) and the resulting C_7 alcohol was protected as the TES ether. The aldol reaction of the C-ring ketone **3** with freshly distilled methyl glyoxylate using LDA then provided the corresponding aldol adduct as a mixture of diastereomers which were subjected to elimination by stirring with acetic anhydride. It proved necessary to keep the reaction at room temperature in order to avoid deprotection of the C_7 TES group. This reaction provided the desired α,β-unsaturated ester **4** as a single isomer.

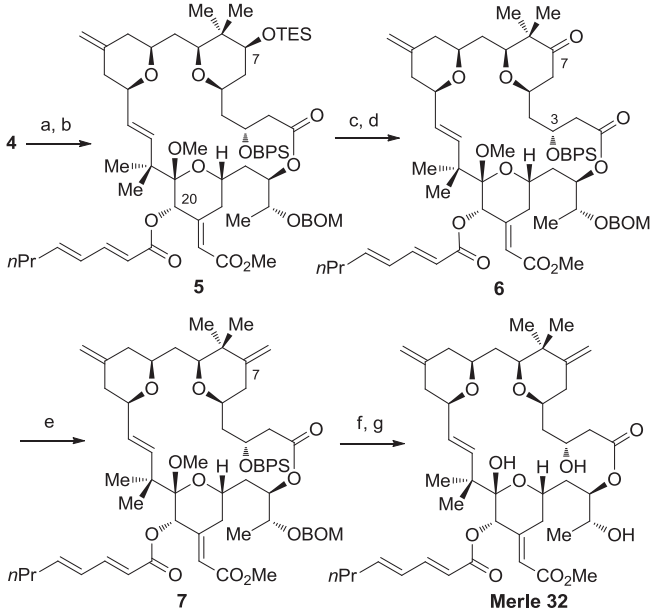
Luche reduction of the C_{20} ketone provided the alcohol which was found to be unstable for isolation and purification purposes (Scheme 2). Thus, this intermediate was immediately subjected to esterification by reaction of the crude alcohol with 2,4-octadienoic anhydride. The C_7 alcohol was then revealed by removal of the TES group by reaction with PPTS in MeOH.

Oxidation of the alcohol to the corresponding ketone proved uneventful using the Dess-Martin oxidation.[18] However, subsequent conversion of the ketone **6** to the desired alkene proved to be a daunting task. Attempted olefination of the ketone using Petasis reaction conditions provided a complex mixture of products.[19] On the other hand, we were initially reluctant to use the Wittig reaction[20] on such a complex substrate due to the following concerns: (i) low reactivity of the sterically hindered C_7 ketone towards the Wittig reagent, leading to enolization, (ii) potential β-elimination of the C_3-OBPS group, and (iii) migration of the C-ring olefin to an internal position. The latter two processes had been observed in our laboratory during the synthesis of related structures. However, when the ketone **7** was subjected to Wittig reaction



(a) DDQ, CH_2Cl_2/pH 7 buffer, 91%; (b) *m*-CPBA, THF/H_2O, 83%; (c) 2,4,6-$Cl_3PhCOCl$, Et_3N, THF, then DMAP, 40 °C, 79%; (d) K_2CO_3, MeOH, 94%; (e)TESCl, Et_3N, DMAP, CH_2Cl_2, 93%; (f) LDA, $OHCCO_2Me$, THF, -78 °C; (g) Ac_2O, py, DMAP, CH_2Cl_2, 60% (2 steps)

Scheme 1. Preparation of macrolactone and further functionalization.



(a) $NaBH_4$, $CeCl_3$•$7H_2O$, MeOH, -40 °C; (b) $(C_8H_{11}O)_2O$, DMAP, py, CH_2Cl_2, dr = 5:1, 75% (2 steps); (c) PPTS, MeOH; (d) Dess-Martin reagent, py, CH_2Cl_2 84% (2 steps); (e) $Ph_3P^+MeBr^-$, *n*-BuLi; then **6**, 70%; (f) HF•Py, THF/MeOH/py; (g) $LiBF_4$, $MeCN/H_2O$, 80 °C, 68% (2 steps)

Scheme 2. Completion of the C_8 *gem*-dimethyl analogue Merle 32.

conditions, we were pleasantly surprised to find that the reaction provided the desired product in excellent yield in just 15 min. Removal of the BPS group followed by global deprotection using $LiBF_4$ then completed the synthesis of Merle 32.[21]

The biological evaluation of Merle 32 began by determining its binding affinity (K_i) towards PKC in vitro.[22] Its K_i of 1.08 ± 0.16 nM proved similar to that of bryostatin 1 (K_i = 1.35 nM) and to other bryopyran analogues prepared previously. The biological profile of Merle 32 was initially addressed in the U937 human lymphoma cell line using proliferation and attachment assays (both measures of differentiation in this cell line). The U937 cells display differential response towards tumor-promoting PMA and bryostatin 1.[23] Specifically, PMA induces attachment and inhibits the proliferation of U937 cells whereas bryostatin 1 has little effect. On the other hand, bryostatin 1 blocks both responses to PMA in a dose dependent manner when the two agents are administered together. It can be seen (Fig. 2) that Merle 32 induced the attachment of U937 cells in a manner largely similar to PMA, although the maximal attachment was not quite as great. Similar observations were made in the proliferation assay, but with even closer resemblance to the PMA response.

Merle 32 was also evaluated using the human leukemia cell lines K562[24] and MV4-11,[25] which both show distinctive dose dependent patterns for inhibition of proliferation in the presence of PMA or bryostatin 1. As can be seen in Figure 3, Merle 32 was strongly antiproliferative, as was PMA but not bryostatin 1. Moreover Merle 32 did not block the response to PMA, while bryostatin 1 did. These results indicate that the behavior shown by Merle 32 is not limited to just the U937 cell line and indeed was even more PMA-like in these other two cell lines. Here Merle 32 showed no ability to block the effect of 10 nM PMA, while bryostatin 1 was effective in that regard.

Downregulation of PKC isoforms and other C1 domain containing proteins subsequent to ligand binding is another characteristic phenomenon displayed by PKCs and is also important in determining their functional response to different ligands.[26] Dose dependent patterns of downregulation were determined for PMA, bryostatin 1, and Merle 32 in the K562 cells (Fig. 4). Here bryostatin 1 and PMA showed distinctly different patterns. Bryostatin 1 was more effective in downregulating PKC-α and β and showed biphasic downregulation of PKC-δ (Fig. 4). Moreover, it did not cause the prominent induction of PKC-ϵ and RasGRP3 observed

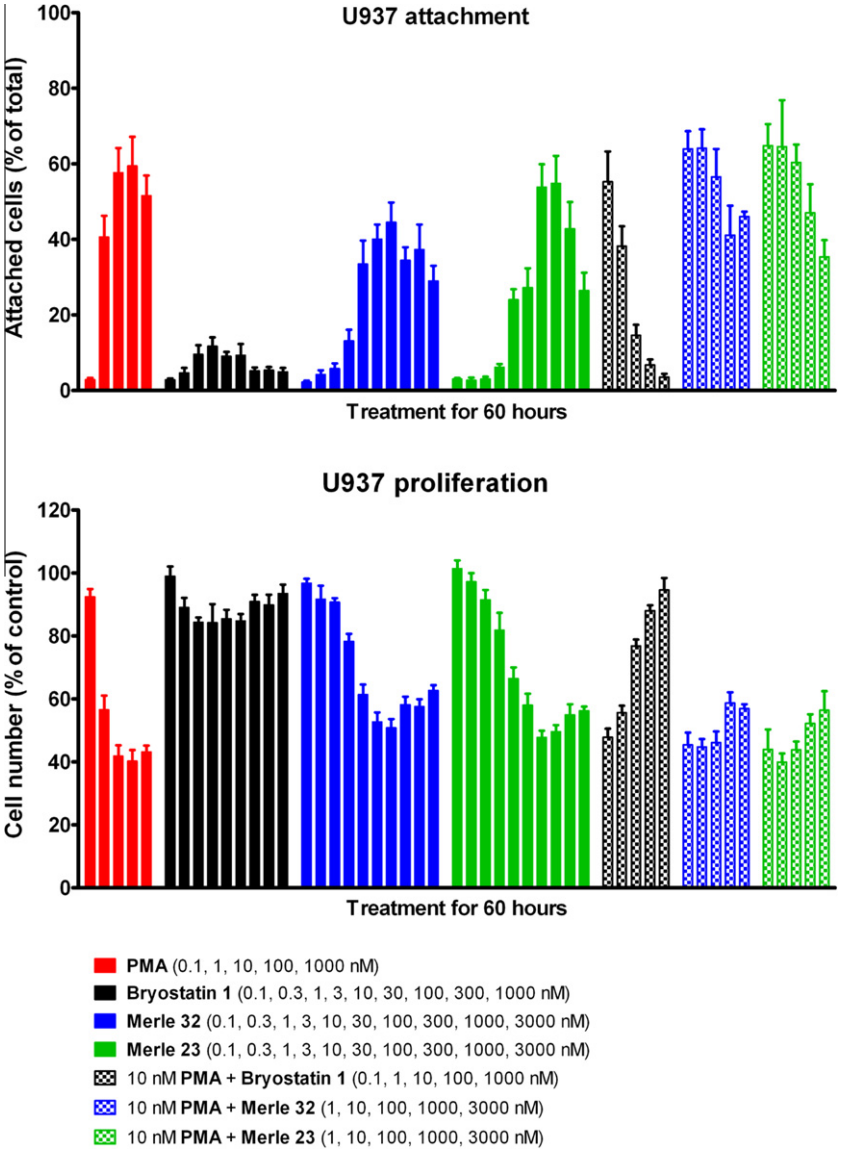


Figure 2. Effect of Merle 32 on proliferation and attachment of U937 cells. Cells were treated 24 h after seeding with the indicated concentrations of the different compounds. The floating and attached cells were counted 60 h after treatment as described earlier (**13a**). Values represent the mean ± SEM of five independent experiments.

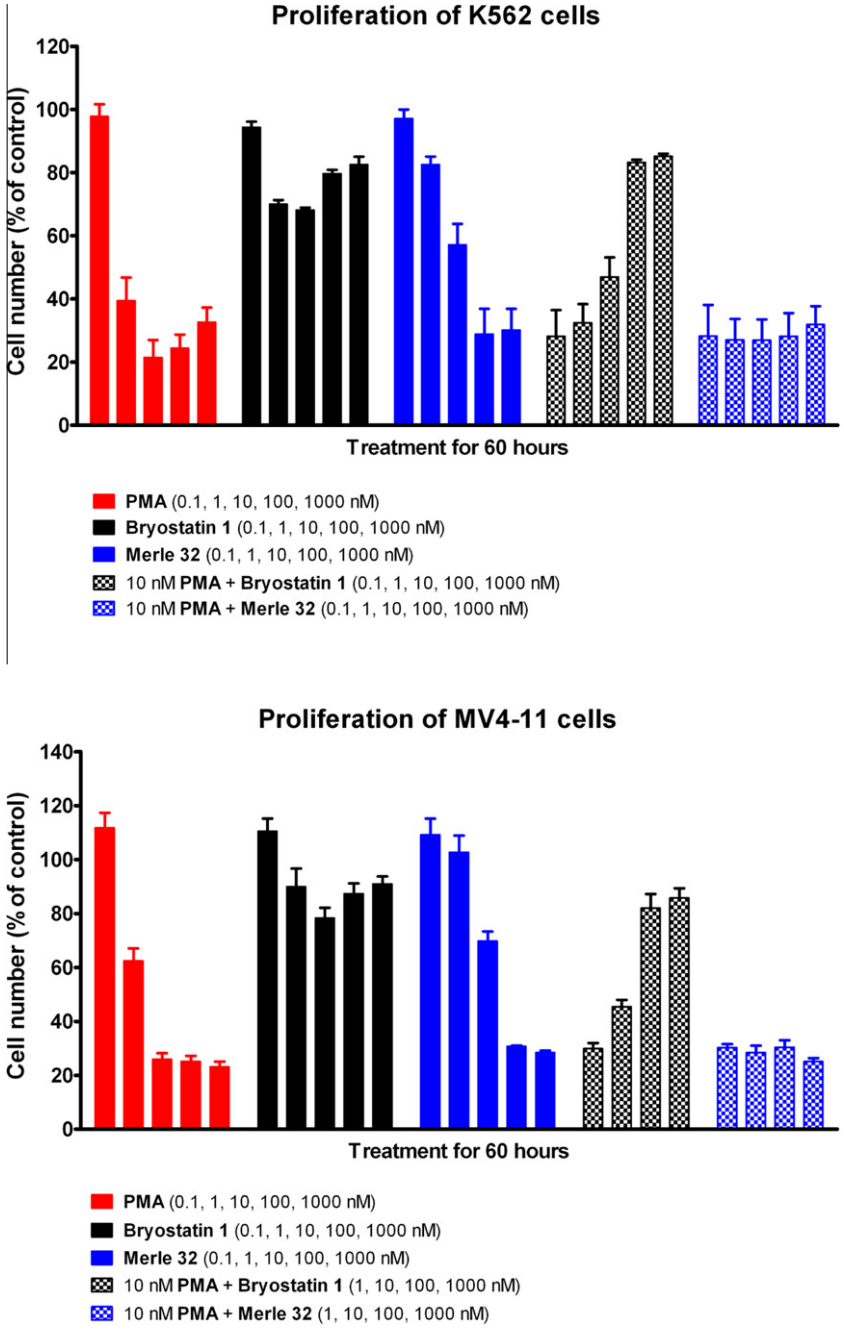


Figure 3. Effect of Merle 32 on proliferation of K562 and MV4-11 cells. Cells were seeded and treated as described for the U937 cells (**13a**). Total cell numbers were determined 60 h after treatment. Values represent the mean ± SEM of three independent experiments.

in these cells with PMA. Merle 32 showed a pattern largely similar to that of PMA but with approximately 10-fold weaker potency.

The results from the proliferation assays in the various cell lines together with these downregulation assays indicate that Merle 32 induces biological responses largely similar to those of PMA rather than bryostatin 1. If the C_8 *gem*-dimethyl group was to serve as a functional switch, the biological results displayed by Merle 32 in these systems would be closer to those of bryostatin 1 than to those of Merle 23, which differs from Merle 32 only at C_8, whereas they very closely resemble those of Merle 23.

To date, we have studied the role of four individual groups in the A–B region of bryostatin 1 that have been implicated as responsible for its unique biological activity. None of these groups was found to function singly as a biological switch, thus it appears that the unique activity of bryostatin 1 relies on a more subtle interplay between some combinations of these groups. It is interesting to note that those analogues with two polar substituents in the A–B ring region (Merle 28 and 30) give biological results similar to those for bryostatin 1, while those with fewer or no polar groups (Merle 32, 27 and 23) are PMA-like or largely so. Thus, it appears that a proper combination of polar substituents in the A–B ring region may be needed for mimicking the activity of bryostatin 1. Efforts to identify suitable combinations of groups in this region are in progress and will be reported in due course.

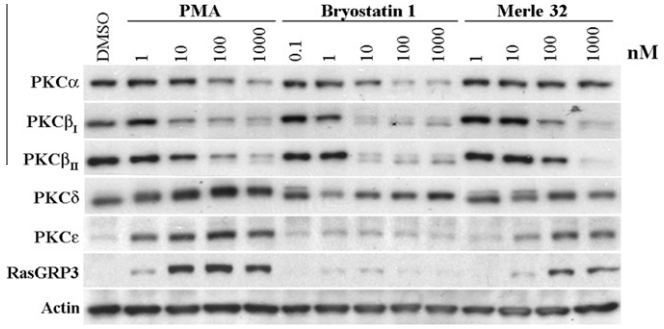


Figure 4. Modulation of C1 domain containing proteins upon 24 h treatment of K562 cells. K562 cells (4 ml of 150,000 cells/ml) were treated 24 h after plating with the indicated concentrations of the compounds for 24 h. Western blot analysis was performed on the total cell lysates as described earlier.[27] Actin provides a loading control. A representative image from three independently performed experiments is shown.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.073.

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