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Late-Stage Intermolecular CH Activation for Lead Diversification: A Highly Chemoselective Oxyfunctionalization of the C-9 Position of Potent Bryostatin Analogues

Paul A. Wender,* Michael K. Hilinski, and Alexander V. W. Mayweg

Department of Chemistry, Stanford University, Stanford, California 94305-8050
wenderp@stanford.edu

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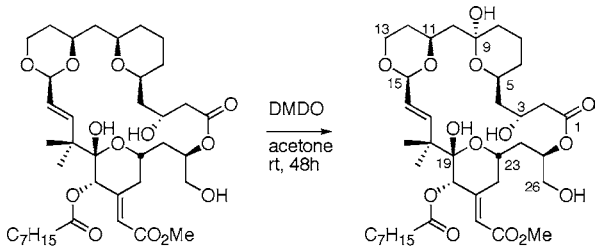
ABSTRACT



1 PKC K_i = 0.3 nM **2** PKC K_i = 2 nM

Treatment of highly potent and densely functionalized bryostatin analogue 1 with dimethyldioxirane afforded the C-9 hydroxylated hemiketal 2 via oxyfunctionalization of the C9-CH bond, one of 12 CH bonds geminal to an oxygen substituent in 1. When bryostatin analogue 3 was subjected to identical conditions, oxidation of a C-26 secondary hydroxyl group was found to compete with C-9 hydroxylation. Complete selectivity for C-9 hydroxylation was restored upon acylation of the C-26 secondary alcohol.

Plants and other organisms have long used late-stage diversification strategies to produce metabolites that provide evolutionary advantages. In the past few decades, such strategies have also been employed in industry and academia to improve the performance of materials and drug candidates. Whereas laboratory approaches have often relied on the *modification* of preexisting functionality (e.g., alcohols to esters to improve the potency or ADME characteristics of drug candidates), Nature frequently relies on the *introduction* of functionality often through CH activation involving various enzymes (e.g., hydroxylases, monooxygenases, and cytochromes P450).[1] The biosynthesis of the anticancer agent taxol (paclitaxel) is a significant example of such late-stage functionality introduction.[2] Nonenzymatic approaches to CH functionalization,[3] particularly those involving intramolecular delivery as represented by the pioneering studies on "remote functionalization",[4] have been used with increasing success in synthesis.[5] Despite its great importance for late-stage diversification, few examples of intermolecular CH oxidation of complex systems have been reported.[6] We describe herein examples of intermolecular DMDO-mediated oxyfunction-

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alization of bryostatin analogues, providing a convenient route to advanced biologically active analogues that would otherwise be difficult to obtain.

Bryostatin 1 is a member of a class of structurally complex marine macrolides that is found in low natural abundance (0.00014%) in the marine bryozoan *Bugula neritina*. It has generated considerable scientific interest as a result of its remarkable potency and unique set of biological activities, including induction of apoptosis, reversal of multidrug resistance, immune system enhancement, and the ability to synergize the activity of other anticancer drugs.[7] It has entered human clinical trials as a single agent and in combination with other agents for the treatment of cancer.[8] Previous efforts in our laboratory have sought to design analogues that would be superior to bryostatin but available through practical total synthesis. Representative of this work, analogues **1** and **3** (Figure 1) have been found to exhibit in vitro and in vivo biological activities comparable or superior to bryostatin 1 in various assays.[9,10]

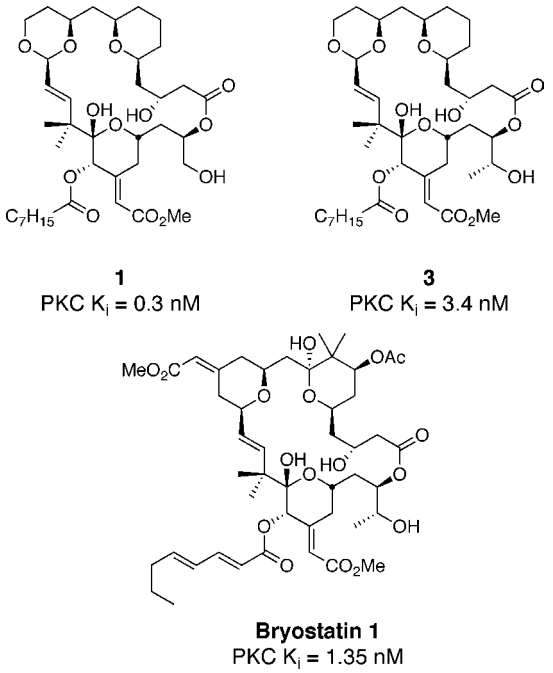

Figure 1. Highly potent simplified bryostatin analogues.

We became interested in pursuing a late-stage oxygenation of our analogues in an effort to learn more about the features of bryostatin that contribute to its activity. For example, we had previously proposed that the hemiketal functionality at C9 is not critical to the binding of bryostatin to its receptor, protein kinase C (PKC).[11] This hypothesis was subsequently supported by the observation that our designed analogues, which lack the C9-OH group, bind PKC as well or better than bryostatin. To further investigate this point we set out to synthesize the C9-hydroxylated version of our analogues. To avoid a lengthy synthesis, we sought to introduce this oxygenation using previously synthesized advanced intermediates.

Dimethyldioxirane is a powerful yet mild oxidant that generates simply acetone as the reaction byproduct.[12] It is known to oxidize a range of functional groups including alkenes to epoxides,[13] alcohols to ketones,[14] saturated hydrocarbons to hydroxylated compounds,[15] and ethers to ketones or hemiketals.[16] Given the broad reactivity range of DMDO, its use for the introduction of functionality in our analogues requires that it react selectively at one out of numerous similar CH sites and not favor epoxidation of one or both olefins.

When analogue **1** was treated with 2 equiv of freshly prepared DMDO as an acetone solution,[17] no reaction was observed at low temperature. However, stirring the reaction at ambient temperature for 48 h with an additional 2 equiv of DMDO resulted in complete conversion of **1** to a single new product in 70% yield (Scheme 1).

The ESI mass spectrum showed a parent ion peak at $m/z = 739$, a mass increase of +16 consistent with the incorporation of only a single oxygen atom. Extensive spectroscopic analysis (1D and 2D NMR) showed that neither the C-16,C-17 alkene nor the enoate had reacted, suggesting that a hydroxylation rather than an epoxidation had occurred. The actual site of oxidation was subsequently revealed by NMR spectroscopy. The ^{13}C spectrum showed a new resonance at 95.58 ppm, consistent with the presence of an additional

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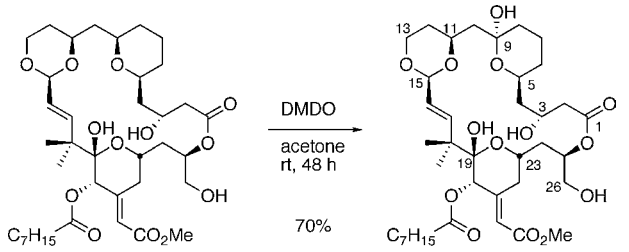
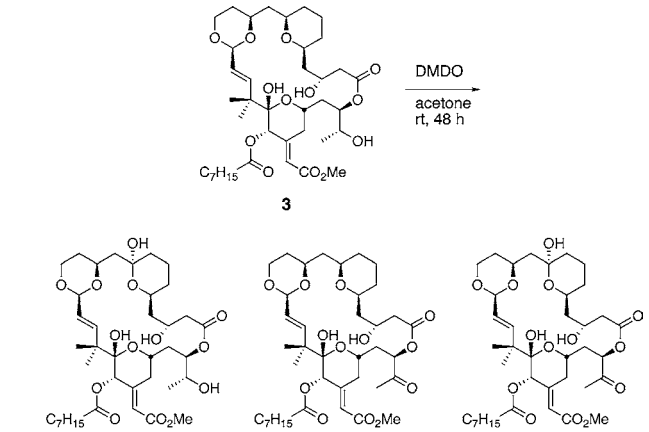
Scheme 1. Reaction of Analogue **1** with DMDO



acetal group in the new analogue. In the ^{1}H NMR spectrum, the C-10 hydrogen was present as a dd, indicating that the adjacent C-9 proton was now absent. The absence of the C-9 proton was further supported by the COSY and HSQC spectra, and the HMBC spectrum showed correlations between the C-10 protons and the C-9 carbon resonance, confirming the site of hydroxylation and the structure of the new analogue as given in **2**. The hydroxylation at C-9 occurred with retention of stereochemistry, presumably via a nonradical C-H insertion mechanism proposed for this type of oxidation.[18]

Because of the remarkable selectivity of the DMDO hydroxylation at C-9 of analogue **1**, we were interested in examining the selective oxidation of other complex analogues (**3**) with DMDO. Interestingly, the reaction of analogue **3** with DMDO in acetone under analogous conditions afforded three new products, identified as **4**, **5**, and **6** (Scheme 2).

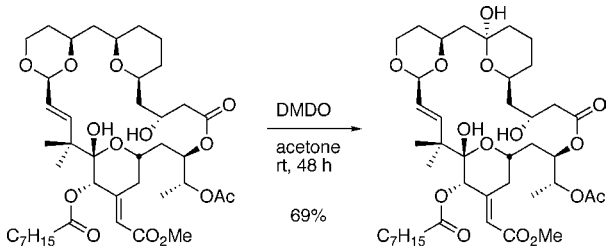
Scheme 2. Reaction of Analogue **3** with DMDO



Both **4** and **6** feature resonances in the NMR spectra consistent with a hemiketal at C-9. In addition, **5** and **6** showed sharp singlets in the proton spectra as well as corresponding resonances in the ^{13}C and 2D spectra, attributed to the presence of a methyl ketone at C26. This was further corroborated by mass spectral data.

The results with analogue **3** suggest that it undergoes competitive oxidation at C9 and C26, with the latter being kinetically favored. The difference in chemoselectivity in the oxyfunctionalization of analogues **1** and **3** is consistent with the observation that secondary alcohols are known to be more reactive than primary alcohols to oxidation by dioxiranes,[19] and that DMDO is known to selectively oxidize secondary alcohols in a molecule containing both functionalities.[14b] Additionally, secondary alcohols are known to react with DMDO at rates an order of magnitude higher than their corresponding ethers.[20] Thus, the selectivity observed in the oxidation of **3** is likely due to the enhanced reactivity of the C26 position rather than an intrinsic diminished reactivity of the C9 position. To test this hypothesis, the C26 position in analogue **3** was deactivated by conversion to the corresponding acetate (**7**).[10a] When acetate **7** was exposed to DMDO, a single product **8** (Scheme 3) was obtained in 69%

Scheme 3. Reaction of Control Substrate **7** with DMDO



yield. Modification of the C-26 alcohol to a deactivating acetate completely suppressed C-26 oxidation and restored the yield of and selectivity for C-9 hydroxylation to the level seen in the case of analogue **1**, confirming our hypothesis.

We had previously proposed that the C9-OH group in bryostatin does not contribute to its high affinity binding to PKC. The availability of analogues **2** and **4** along with their non-hydroxylated variants **1** and **3** allowed us to now test this hypothesis. The binding constants to a PKC isozyme mix, a class of enzymes for which the natural bryostatins and their analogues are known to have high affinity,[9,10a,21] were determined for new analogues **2**, **4**, **5**, and **6** (Table 1).

Table 1. Binding Affinities of Oxidized Analogues for PKC

	analogue					
	1	**3**	**2**	**4**	**5**	**6**
K_i	0.3 nM	3.4 nM	2 nM	4 nM	>500 nM	>500 nM

Analogues **2** and **4** were found to be highly potent, binding to PKC with inhibition constants of 2 and 4 nM, respectively.

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However, the potency is comparable or even less than that found for **1** and **3**, consistent with our previously proposed pharmacophore analysis. Also in accord with this analysis, analogues **5** and **6**, in which the proposed pharmacophore is disrupted, did not exhibit any significant binding affinity to PKC (K_i > 500 nM), further confirming that a hydrogen bond donor at C26 is essential for potent binding of these analogues. Further biological testing of analogues **2** and **4** is currently underway and will be reported elsewhere.

In conclusion, we have found that DMDO can be used to achieve a remarkably selective C−H oxidation of a densely functionalized and delicately appointed complex bryostatin analogue, providing access to a new family of analogues through late-stage diversification. This method afforded the hydroxylated and/or oxidized products **2**, **4**, **5**, and **6** with excellent selectivity and good efficiency, achieving in one step what would otherwise require a lengthy synthesis. Protection of a secondary alcohol as an acetate was found to completely suppress an oxidation that was competitive with C-9 hydroxylation. This example of a mild and selective hydroxylation of a pyran ring embedded in a molecule exhibiting dense and sensitive functionality mimics one of Nature's diversification strategies and may find use when applied to related natural products with promising biological activity that incorporate hydroxy hydropyranyl rings.[22]

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Supporting Information Available: Experimental conditions and spectral data for compounds **2**, **4**, **5**, **6**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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