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Evaluation of Chromane-Based Bryostatin Analogues Prepared via Hydrogen-Mediated C−C Bond Formation: Potency Does Not Confer Bryostatin-like Biology

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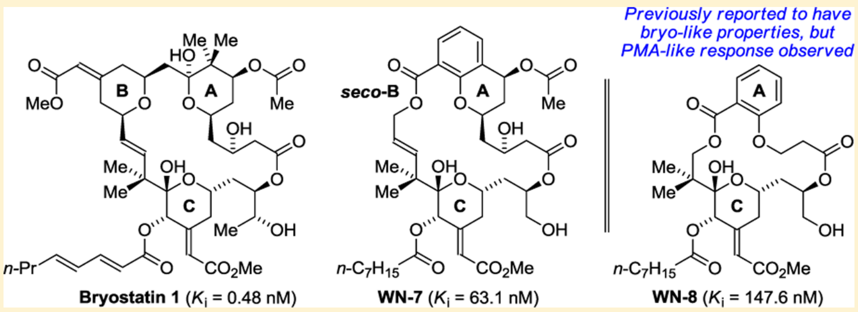
Supporting Information



ABSTRACT: The synthesis and biological evaluation of chromane-containing bryostatin analogues **WN-2**−**WN-7** and the previously reported salicylate-based analogue **WN-8** are described. Analogues **WN-2**−**WN-7** are prepared through convergent assembly of the chromane-containing fragment **B-I** with the "binding domain" fragment **A-I** or its C26-*des*-methyl congener, fragment **A-II**. The synthesis of fragment **B-I** features enantioselective double C−H allylation of 1,3-propanediol to form the C_2-symmetric diol **3** and Heck cyclization of bromo-diene **5** to form the chromane core. The synthesis of salicylate **WN-8** is accomplished through the union of fragments **A-III** and **B-II**. The highest binding affinities for PKCα are observed for the C26-*des*-methyl analogues **WN-3** (K_i = 63.9 nM) and **WN-7** (K_i = 63.1 nM). All analogues, **WN-2**−**WN-8**, inhibited growth of Toledo cells, with the most potent analogue being **WN-7**. This response, however, does not distinguish between phorbol ester-like and bryostatin-like behavior. In contrast, while many of the analogues contain a conserved C-ring in the binding domain and other features common to analogues with bryostatin-like properties, all analogues evaluated in the U937 proliferation and cell attachment assays displayed phorbol ester-like and/or toxic behavior, including **WN-8**, for which "bryostatin-like PKC modulatory activities" previously was suggested solely on the basis of PKC binding. These results underscore the importance of considering downstream biological effects, as tumor suppression cannot be inferred from potent PKC binding.

■ INTRODUCTION

Discovered by Pettit using an assay for inhibitory activity against the P388 leukemia cell system, the bryostatins are a family of structurally complex marine macrolides isolated from the bryozoan *Bugula neritina* (Chart 1).[1] The most abundant and well-studied member of this compound class, bryostatin 1, potently binds the C1 domain of protein kinase C (PKC) isozymes *in vitro*,[2] activating PKC and modulating diverse downstream effects.[3] Most notably, although bryostatin 1 potently binds and activates PKC, it antagonizes most biological responses of the phorbol esters, classic PKC activators that are generally tumor promoting, including phorbol 12-myristate 13-acetate (PMA).[4] This remarkable behavior triggered a good manufacturing practice (GMP) campaign wherein 18 g of bryostatin 1 was isolated from 10 000 gallons of wet bryozoan.[5] This material supported dozens of phase I and phase II clinical trials for cancer treatment[3] and led to the identification of bryostatin 1 as a clinical candidate for the treatment of Alzheimer's disease[6] and human immunodeficiency virus (HIV).[7]

The biological properties of the bryostatins along with their low natural abundance have inspired heroic efforts toward the synthesis of both natural bryostatins[8] and simplified functional analogues.[9−12] In the context of cancer therapy, bryostatin-like activity of analogues was assumed on the basis of potent PKC binding and, in certain cases, membrane translocation assays.[9] However, as demonstrated by the elegant studies of Keck and Blumberg, bryostatin-like biological activity cannot be anticipated from potent PKC binding and membrane translocation

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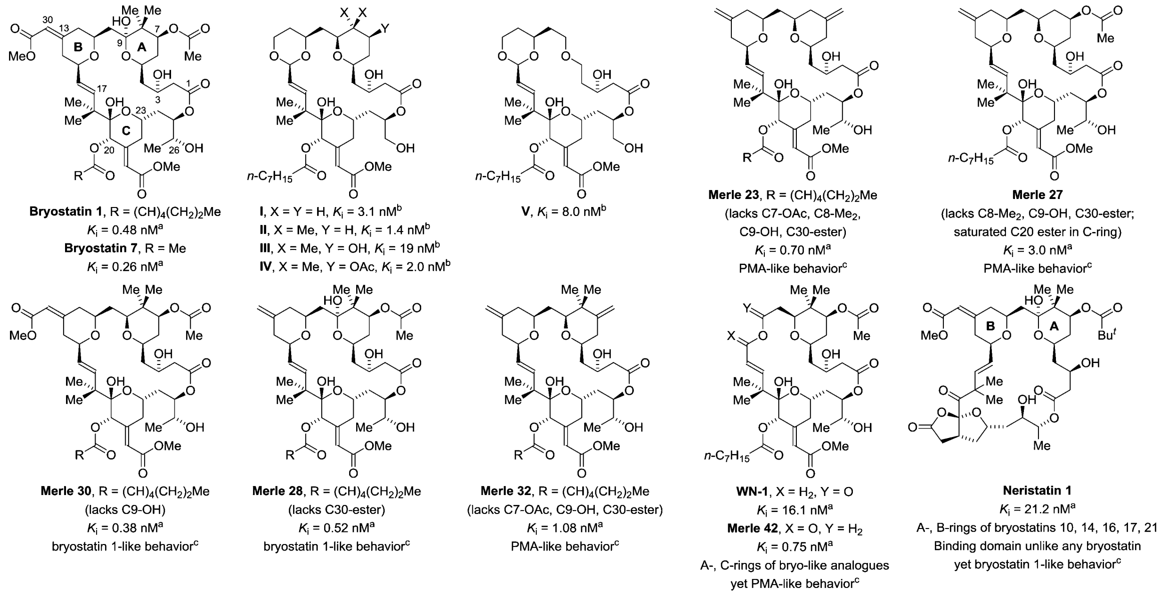
Chart 1. PKC Binding Affinity of Bryostatins 1 and 7, Selected Bryostatin Analogues, and Neristatin 1



[a]Binding affinity to PKCα. See ref 7h for PKCα binding affinity of bryostatin 1 and bryostatin 7. [b]Compounds **I–V**, prepared by Wender,[9] were reported to function similarly to bryostatin 1 with regard to the pattern of PKCδ-GFP translocation induced in rat basophilic leukemia cells.[9h,i,k] Binding affinity refers to a mixture of rat brain PKC isozymes. The initially reported binding affinity of **I** (0.25 nM) has been revised.[9o] [c]For the indicated Merle bryologues prepared by Keck,[10] PMA-like vs bryostatin-like biology was established via U937 attachment and inhibition of proliferation assays.

alone, even for compounds that deviate only slightly from the structure of bryostatin 1 itself (Chart 1).[10] Downstream biological responses must be assessed to determine whether analogues embody the special properties of bryostatin 1. Here, U937 human histiocytic lymphoma cell attachment and inhibition of proliferation assays have proven diagnostic (*vide supra*).[10e] These assays reveal that analogues retaining bryostatin-like activity are relatively intolerant *vis-à-vis* removal or modification of functional groups in the bryostatin A- and B-rings. In contrast, the bottom portion of bryostatin, which incorporates the C-ring and primarily influences PKC binding, appears to be less important in terms of defining PMA-like or bryostatin-like behavior. The biology of neristatin 1 dramatically illustrates these trends.[13] Neristatin 1 incorporates A- and B-rings identical to several bryostatin family members; however, the bottom portion of neristatin is unique. Critically, neristatin 1 displays bryostatin 1-like behavior, not phorbol ester-like behavior, in U937 promyelocytic leukemia cells.[13] These results support the hypothesis that the critical mechanistic feature of bryostatin is formation of a cap by the A- and B-rings over the C1 domain, held in position by interaction of the bryostatin C-region or an equivalent binding group with the binding cleft of the PKC C1 domain. This concept is reflected in recently reported bryostatin analogues that incorporate simple DAG-like substructures in place of the C-region.[14] Extensive simplification of the top portion of bryostatin to furnish analogues mimicking the biological profile of bryostatin remains an elusive, unmet challenge.

Using catalytic C–C bond formations developed in our laboratory,[15] we devised concise routes to the bryostatin A- and C-rings,[16] which, in turn, enabled the total synthesis of bryostatin 7[8g] and the *seco*-B-ring analogue **WN-1**.[12] Although **WN-1** binds PKCα (K_i = 16.1 ± 1.1 nM) and inhibits growth of multiple leukemia cell lines, it displays PMA-like behavior in U937 cell attachment and proliferation assays and in K562 and MV-4-11 proliferation assays. Such PMA-like behavior is surprising, as the A- and C-rings of **WN-1** are shared by analogues that display bryostatin-like behavior in these assays (Chart 1). To assess whether greater conformational rigidity and lipophilicity might restore the desired bryostatin-like behavior in the absence of a B-ring, the synthesis and evaluation of the chromane-based analogues **WN-2–WN-7** and the previously described salicylate-based analogue **WN-8**[9m,n] were undertaken. Beyond probing the biology of the bryostatins, the development of a novel catalytic asymmetric method for the synthesis of chromanes and chromanones, which are privileged substructures in drug discovery, represents a significant outcome of this work.[17]

■ RESEARCH DESIGN AND METHODS

Synthesis of WN-2–WN-8. Our approach to chromane-containing bryostatin analogues **WN-2–WN-8** is illustrated in the retrosynthesis of **WN-7** (Figure 1). Macrodiolide **WN-7** is assembled from fragments **A-II** and **B-I** via successive ester bond formation. As reported in our synthesis of bryostatin 7,[8g] fragment **A-I** is prepared through hydrogen-mediated reductive coupling of glyoxal **1a** and enyne **2a**.[16a] Fragments **A-II** and **A-III** are prepared in a similar fashion from glyoxal **1a** or **1b** and enyne **2b** (Scheme 1). Each reductive coupling forms the C20–C21 bond with control over C20 carbinol stereochemistry and C21 alkene geometry. The C20 hydroxyl groups of the respective reductive coupling products are converted to the octanoates, and then HF·pyridine in methanol is added to the reaction mixtures to furnish fragments **A-I** and **A-II** in eight steps from commercially available compounds.

The synthesis of chromanone containing fragment **B-I** begins with double asymmetric C–H allylation of 1,3-propanediol (Scheme 2).[18] The resulting C_2-symmetric diol **3** is converted to the *mono*-TBS ether **4**. Deprotonation of **4** using sodium hydride followed by addition of the alkoxide to *tert*-butyl 3-

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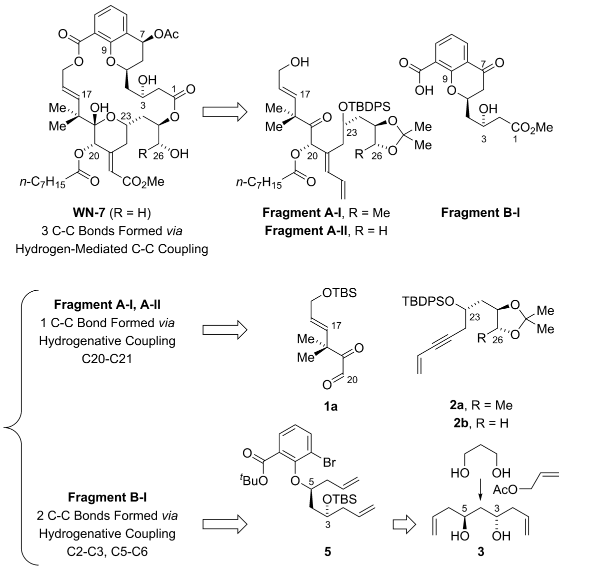
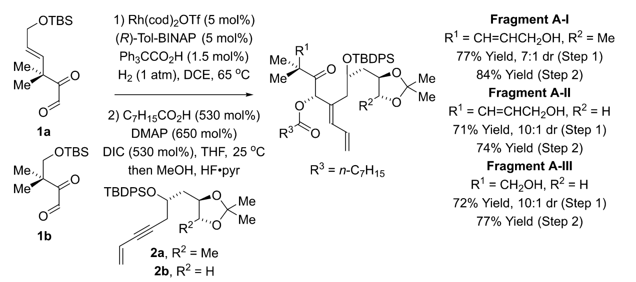


Figure 1. Retrosynthetic analysis of **WN-7** illustrating C−C bonds formed via hydrogenative coupling.

Scheme 1. Synthesis of Fragments A-I−A-III via H_2-Mediated Reductive Coupling of Glyoxals 1a and 1b with 1,3-Enynes 2a and 2b[a]



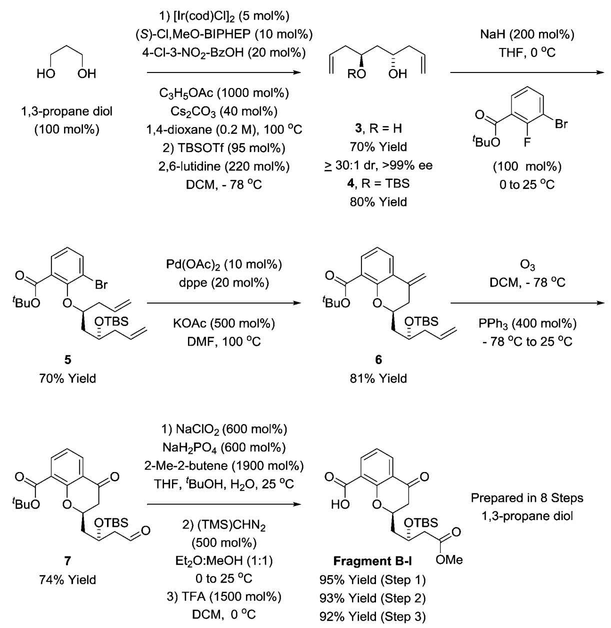
[a]The indicated conditions apply to fragment **A-I**. Similar conditions are used for fragments **A-II** and **A-III**. See ref 8g and Supporting Information for precise experimental details.

bromo-2-fluorobenzoate delivers the S_NAr product **5**.[19] Exposure of **5** to conditions for Heck cyclization provides the desired chromane **6**,[20] which upon concomitant ozonolysis[21] of the terminal olefin moieties provides keto-aldehyde **7**. Finally, Pinnick oxidation[22] followed by treatment with diazomethane and hydrolysis of the *tert*-butyl ester delivers fragment **B-I**.

The synthesis of chromanone-containing macrodiolides **WN-2** and **WN-4** is accomplished as follows (Scheme 3). Fragments **A-I** and **B-I** are treated with PyBroP in the presence of Hunig's base and DMAP to form ester **8** in 85% yield.[23] Exposure of **8** to trifluoracetic acid cleaves the acetonide to provide a triol, which is reacted with TBSOTf to form the *bis*-silyl ether with high levels of chemoselectivity.

Trimethyltin hydroxide[24] enables chemoselective cleavage of the methyl ester in the presence of the C20 octanoate to form the hydroxy acid **9**. Yamaguchi lactonization converts hydroxy acid **9** to macrodiolide **10**.[25] A stepwise protocol[26a] for oxidative cleavage of the diene moiety of **10** is more efficient than direct Lemieux−Johnson oxidation.[26b] Subsequent Pinnick oxidation[22] furnishes the carboxylic acid, which upon removal of the silyl ethers results in spontaneous closure of the macrodiolide C-ring. This strategy for C-ring closure was not possible for the corresponding methyl ester due to lactonization

Scheme 2. Synthesis of Fragment B-I via Transfer Hydrogenative Double Allylation of 1,3-Propanediol[a]



[a]See Supporting Information for experimental details.

onto the C23 alcohol. To our knowledge, **WN-4** is the first carboxylic acid-containing bryostatin analogue. Treatment of **WN-4** with TMS diazomethane delivers the methyl ester **WN-2**.

Syntheses of **WN-3** and **WN-5**, the C26-*des*-methyl congeners of **WN-2** and **WN-4**, respectively, were developed to determine whether potency could be retained or enhanced through this structural simplification.[9o] The construction of **WN-3** and **WN-5** requires the synthesis of 1,3-enyne **2b** (Scheme 4), the precursor of fragment **A-II**. To this end, commercially available (*R*)-butane-1,2,4-triol acetonide **11** is subjected to PCC-mediated oxidation followed by chelation-controlled propargylation of the resulting aldehyde.[27] The homopropargyl alcohol **12** is formed with good levels of diastereoselectivity. Conversion of the secondary alcohol to the TBDPS ether followed by Sonogashira coupling provides the 1,3-enyne **2b**. As described above (Scheme 1), hydrogen-mediated reductive coupling of 1,3-enyne **2b** with glyoxal **1a** proceeds in good yield with excellent control of alkene geometry and C20 carbinol stereochemistry. A one-pot octanoylation−desilylation then affords fragment **A-II**.

With fragment **A-II** in hand, we undertook the synthesis of C26-*des*-methyl chromanone-based macrodiolides **WN-3** and **WN-5** (Scheme 5). Although closely related in structure to analogues **WN-2** and **WN-4**, the C26-*des*-methyl congeners **WN-3** and **WN-5** require a different protecting group strategy. As in the synthesis of **WN-2** and **WN-4**, fragments **A-II** and **B-I** are treated with PyBroP in the presence of Hunig's base and DMAP to form ester **13**.[23] Cleavage of the acetonide using trifluoracetic acid provides a triol. Treatment with TBSOTf leads to selective formation of the *bis*-TBS ether; however, subsequent saponification using trimethyltin hydroxide[24] results in cleavage of the C26-TBS ether. Hence, the more robust C26-TIPS ether was installed, and the C3-alcohol was left unprotected. Saponification in the presence of the C26-TIPS ether mediated by trimethyltin hydroxide[24] provides the dihydroxy acid **14**. Macrolactonization under Shiina con-

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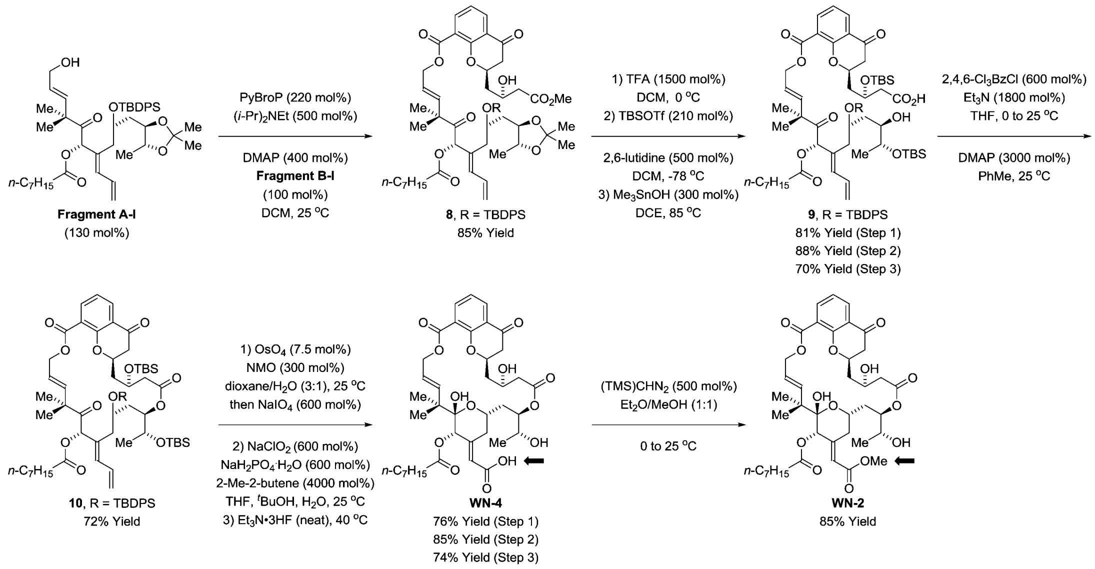
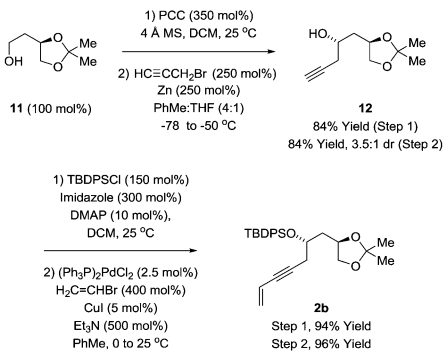
Scheme 3. Synthesis of the Chromanone-Based Macrodiolides WN-2 and WN-4[a]



[a]See Supporting Information for experimental details.

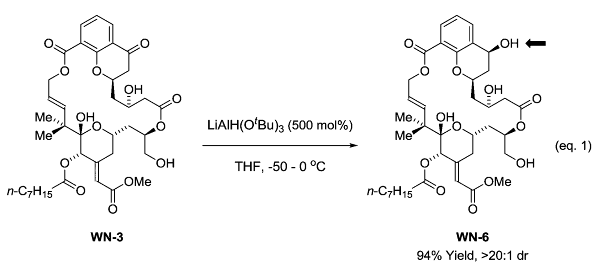
Scheme 4. Synthesis of 1,3-Enyne 2b via Chelation-Controlled Propargylation[a]



[a]See Supporting Information for experimental details.

ditions[28] forms macrodiolide **15**. As in the synthesis of **WN-2** and **WN-4**, one-pot diene oxidative cleavage,[26a] Pinnick oxidation,[22] and exhaustive silyl deprotection provides **WN-5**, which upon methylation of the carboxylic acid delivers **WN-3**.

Reduction of chromanone **WN-3** at the C7 ketone using LiAl(O^{t}Bu)$_3$ occurs with a high level of diastereoselectivity to furnish the C7 alcohol **WN-6** (eq 1).[29] Direct chemoselective



(eq. 1)

acylation of **WN-6** to form the C7 acetoxy compound **WN-7** as found in bryostatin 1 was not possible due to competing functionalization of the C26 hydroxyl. Hence, an alternate sequence was devised (Scheme 6). The C26 hydroxyl of **WN-3** is converted to the TBS ether, and methanolic KBH_4 was added to the reaction mixture.[29] The resulting secondary alcohol **16** is formed as a single diastereomer, as determined by ^{1}H NMR. Acetoxylation of the C7 hydroxyl moiety under conditions developed by Shiina,[28] followed by removal of the TBS protecting group, provides **WN-7**.

The modularity of our synthetic strategy is highlighted by the synthesis of the salicylate-based analogue **WN-8**, previously reported by Wender (Scheme 8).[9m,n] The synthesis of **WN-8** begins with the reaction of fragment **A-III** with the acid chloride derived from fragment **B-II** (Scheme 7, not discussed) to form the neopentyl ester **19**. Concomitant removal of the acetonide and *tert*-butyl ester moieties using trifluoroacetic acid followed by treatment with TBS chloride provides the hydroxy acid **20**. Cyclization under Shiina conditions[28] delivers the macrodiolide **21**. Modified Johnson−Lemieux oxidative cleavage[26b] of the diene terminus followed by Pinnick oxidation[22] and removal of the TBS and TBDPS ethers provides the carboxylic acid **22**. Finally, treatment with methyl iodide delivers **WN-8** in a total of 14 steps (longest linear sequence, LLS), where previously 19 steps (LLS) were required for its preparation.[9m,n]

Biological Evaluation of WN-2−WN-8. *Determination of Binding Affinity to PKCα.* The biological evaluation of **WN-2**−**WN-8** began with the determination of their binding affinities (K_i) toward purified PKCα (Chart 2).[30] The C26-*des*-methyl analogue **WN-3** (K_i = 63.9 ± 16.5 nM) has a 3-fold stronger binding affinity than the parent C26 methyl analogue **WN-2** (K_i = 213.7 ± 33.1 nM).[9o] Compared to the methyl esters **WN-2** and **WN-3**, the carboxylic acids **WN-4** and **WN-5** display a 20−40-fold decrease in potency (K_i = 3988 ± 531 and 2765 ± 738 nM, respectively). The C7-alcohol analogue **WN-6** (K_i = 135.2 ± 22.1 nM) is 2-fold less potent than the C7-OAc analogue **WN-7** (K_i = 63.1 ± 13.6 nM) as well as the C7-ketone analogue **WN-3** (K_i = 63.9 ± 16.5 nM). Recently, Wender reported that **WN-8** bound to PKCβI and PKCδ with K_is = 24 nM and 18 nM, respectively.[9m,n] Our studies have shown that **WN-8** displays weaker binding affinity toward PKCα (K_i = 147.6 ± 17.5 nM). These differences suggested that **WN-8** showed some level of PKC isoform selectivity, as

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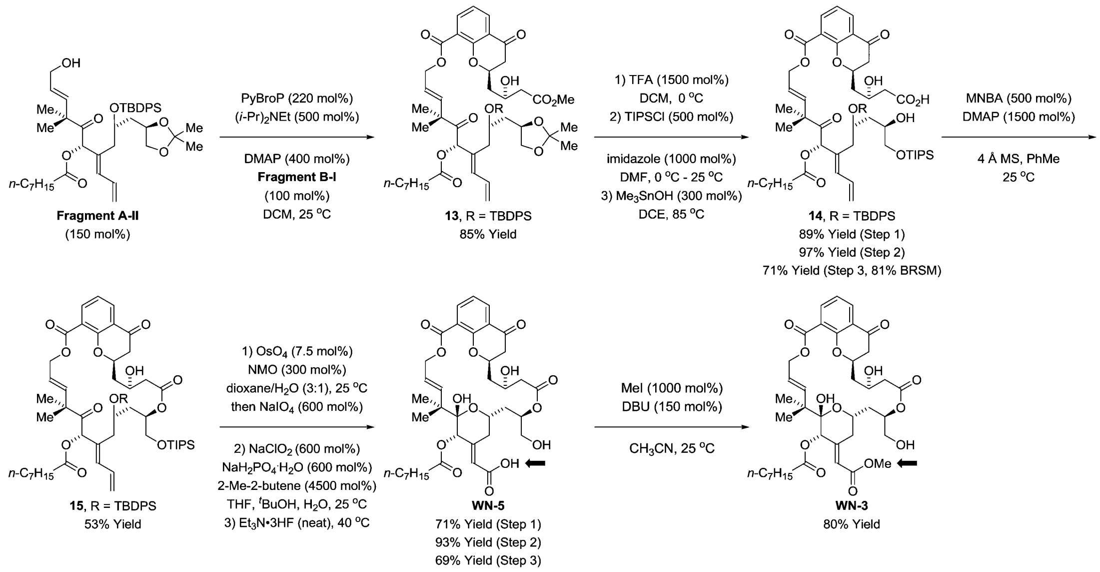
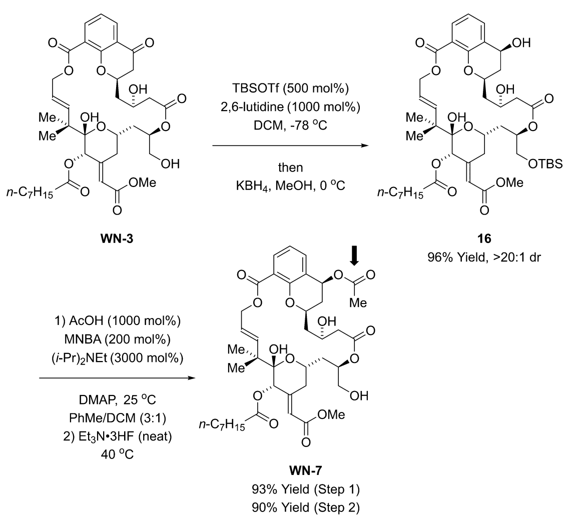
Scheme 5. Synthesis of the C26-*des*-Methyl Chromanone-Based Macrodiolides WN-3 and WN-5[a]



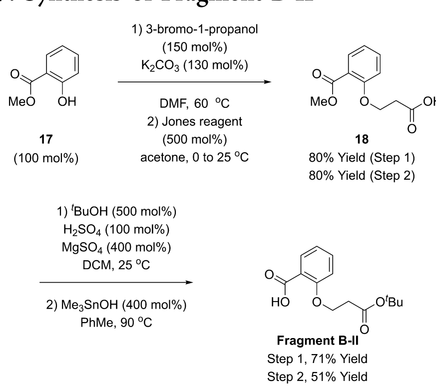
[a]See Supporting Information for experimental details.

Scheme 6. Synthesis of the C26-*des*-Methyl Chromane-Based Macrodiolide WN-7[a]



[a]See Supporting Information for experimental details.

Scheme 7. Synthesis of Fragment B-II[a]



[a]See Supporting Information for experimental details.

has been previously observed for bryostatin 1.[31] The U937 and LNCaP cell lines are the two cell lines in which we have characterized the biological actions of bryostatin analogues in most detail. PKCδ and PKCβII are the major PKC isoforms in the U937 cells; PKCδ and PKCα are the highest expressed PKC isoforms in the LNCaP cells.[32] We therefore measured the affinity of **WN-8** for PKCβII and PKCδ under comparable conditions to those we used for the measurements with PKCα and obtained K_i values of 82.1 ± 14.9 and 56.2 ± 6.0 nM, respectively. We conclude that there is modest selectivity of **WN-8** between various PKC isoforms. The binding affinity of **WN-8** is weaker than that of **WN-3** and **WN-7** and very modestly weaker than that of **WN-6**. Thus, while the chromanone and salicylate analogues retain PKC binding in the nanomolar regime, **WN-1** (K_i = 16.1 nM) remains the most potent compound in the **WN**-series (K_i = 16.1−3988 nM). These data suggest that the northern region of bryostatin analogues not only plays a critical role in determining bryostatin-like vs phorbol ester-like biological activity but strongly influences preorganization (molecular conformation) of the southern binding region and, ultimately, potency.

Activity in U937 Human Histiocytic Lymphoma Cells. The determination of binding affinity to PKC isozymes represents an initial step in understanding the biological properties of the present analogues. Observing downstream biological responses is crucial to determine whether these compounds capture the unique effects associated with bryostatin 1. With U937 human histiocytic lymphoma cells, bryostatin 1 and PMA induce contrasting cellular responses.[10,33] While PMA inhibits the proliferation and promotes attachment of U937 cells, these cells show little response upon treatment with bryostatin 1. Furthermore, co-administration of bryostatin 1 with PMA results in the inhibition of the PMA-like cellular responses, showing that the lack of effect of bryostatin 1 on proliferation and attachment is not due to instability.

In the U937 growth and attachment assays, **WN-2** and **WN-3** display PMA-like behavior. However, at higher concentrations these analogues display toxicity (Figure 2). In the growth inhibition assay, **WN-2** and **WN-3** exhibit strong inhibition at

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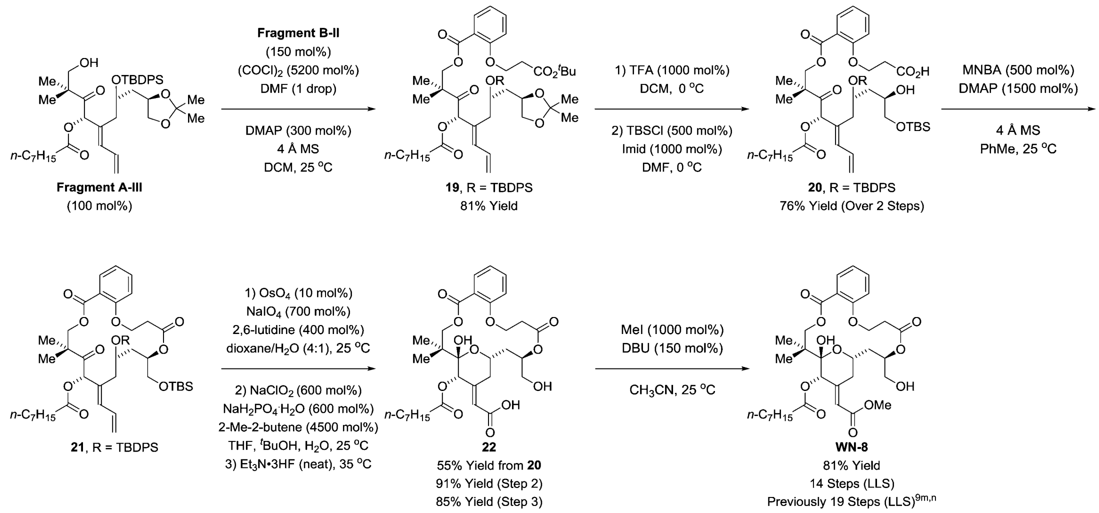
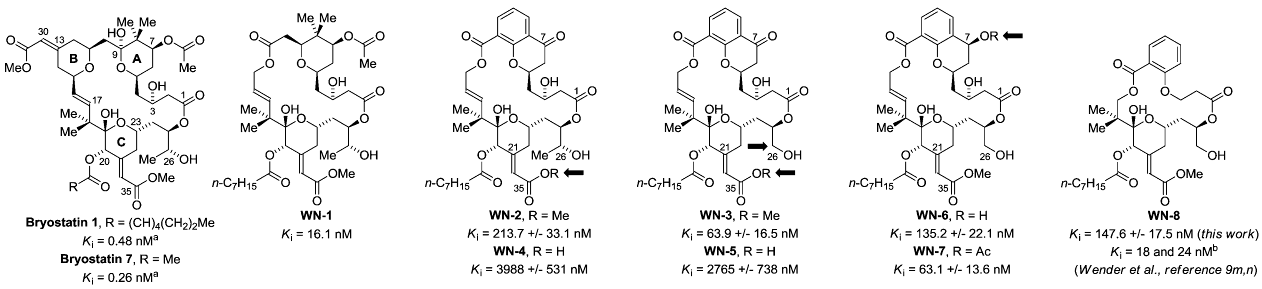
Scheme 8. Synthesis of Previously Reported Salicylate-Based Macrodiolide WN-8[a]



[a]See Supporting Information for experimental details.

Chart 2. PKC Binding Affinity of WN-1−WN-8[a]



[a]Binding affinity to PKCα. See ref 8h for PKCα binding affinity of bryostatin 1 and bryostatin 7. [b]Binding affinity toward PKCδ and PKCβI, respectively.

10 000, 20 000, and 40 000 nM. While bryostatin 1 is able to reverse the antiproliferative effects of PMA in U937 cells, it partially reverses the effects of **WN-2** and **WN-3** at 10 000 nM but not at 40 000 nM. These results are consistent with cell inhibition at 10 000 nM being partially attributable to a PMA-like effect and the further inhibition at higher concentrations being toxicity superimposed on the specific PMA-like inhibition. This trend is also seen in the cell attachment assay for **WN-2** and **WN-3**. The compounds induce the PMA-like response of cell attachment at 10 000 nM and this attachment is antagonized by bryostatin 1. At 20 000 and 40 000 nM, in contrast, the attachment is no longer seen, consistent with toxicity at this higher concentration range.

The biological activities of **WN-6** and **WN-7** in U937 cells are similar to that of **WN-2** and **WN-3**. For **WN-6**, the toxicity predominates. Growth inhibition is not blocked by bryostatin 1, and the minute induction of attachment caused by **WN-6** is also not reversed when coadministered with bryostatin 1. For **WN-7**, a combination of PMA-like and toxic behavior is observed. It inhibits cell growth like PMA but with only modest reversal from bryostatin 1, suggesting that much of the growth inhibition is due to toxicity. In the cell attachment assay, the PMA-like effect is more prominent, with good inhibition by bryostatin 1.

The salicylate analogue **WN-8**, first reported by the Wender group,[9m,n] also was tested in these cell assays. **WN-8** was previously suggested to have "bryostatin-like PKC modulatory activities" solely on the basis of binding.[9m,n] However, **WN-8** behaves like PMA in the U937 growth and attachment assays. Further, in contrast to **WN-2**, **WN-3**, **WN-6**, and **WN-7**, the PMA-like behavior displayed by **WN-8** is not due to a nonspecific toxic effect. Analogues **WN-4** and **WN-5** were not tested in U937 cells given their weak effect relative to **WN-2** and **WN-3** in the Toledo cells (*vide infra*) and the marginal effect of **WN-2** and **WN-3** in the U937 cells.

Effects on TNFα Expression and Activity in Toledo Cells. TNFα secretion from U937 cells was measured after treatment with analogues **WN-2**, **WN-3**, **WN-6**, **WN-7**, or **WN-8** for 60 h (Figure 3). While bryostatin 1 generally has little effect on TNFα secretion, PMA induces secretion in a dose-dependent manner. Results show that high concentrations (10 000 nM) of **WN-2**−**WN-8** are able to induce TNFα secretion even though not to the level induced by PMA. However, this induction is lost at higher concentrations of **WN-2**, **WN-3**, **WN-6**, and **WN-7**, consistent with the higher concentrations being toxic for these analogues.

Unlike their effects in U937 cells, bryostatin 1 and PMA both induce antiproliferative responses in Toledo cells. Compared to bryostatin 1 and PMA, **WN-2**−**WN-8** had IC_{50} values for growth inhibition that are significantly shifted to the right, reflecting weaker potency (Figure 4). The most potent of these analogues in Toledo cells is **WN-7**; **WN-2**, **WN-3**, **WN-6**, and **WN-8** are 3-fold less potent than **WN-7** and all similar to one another. Lastly, within this assay, the C35-acids **WN-4** and

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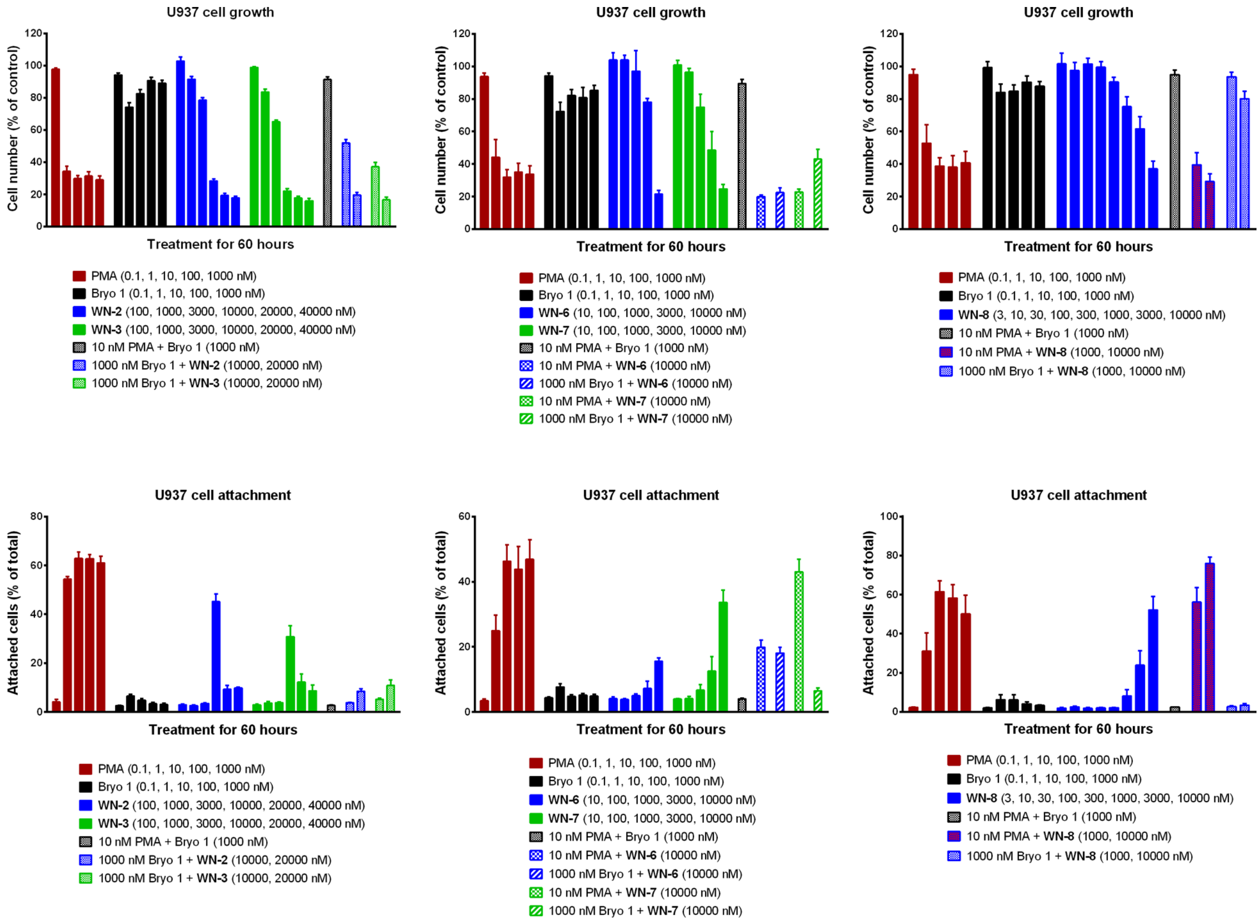

Figure 2. Evaluation of **WN-2**, **WN-3**, **WN-6**, **WN-7**, and **WN-8** in U937 human histiocytic lymphoma cells. See Supporting Information for experimental details.

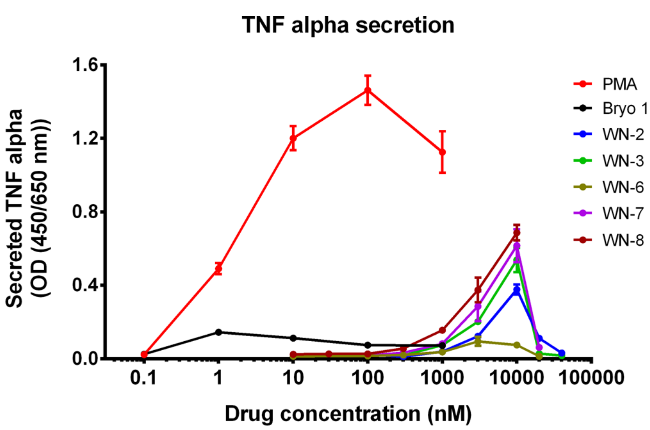

Figure 3. TNFα secretion from U937 cells. See Supporting Information for experimental details.

WN-5 show only minor growth inhibition until concentrations >10 μM are reached.

CONCLUSIONS

In summary, we report the synthesis and biological evaluation of chromane-containing bryostatin analogues **WN-2**–**WN-7** and the previously reported salicylate-based analogue **WN-8**.[9m,n] All **WN**-series analogues conserve the bryostatin C-ring and A-ring features common to analogues with bryostatin-like properties. Despite this structural homology and the observance of nanomolar binding affinities for PKCα, all analogues evaluated in the U937 proliferation and cell attachment assays displayed PMA-like and/or toxic behavior. These data, along with prior studies by Keck and Blumberg,[10] demonstrate the importance of considering downstream biological effects, as potent PKC binding by itself does not predict bryostatin-like biology. Our data further serve as a reminder that the structure of the B-ring region of bryostatin influences PKC binding affinity and profoundly impacts biology, as previously observed.[12]

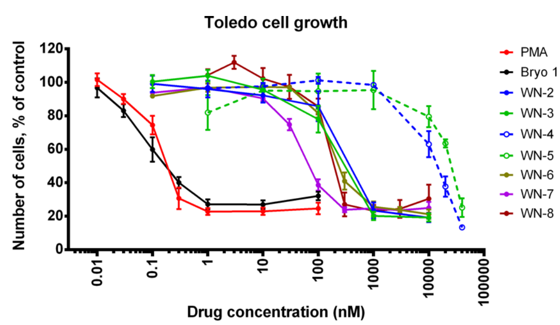

Figure 4. Toledo cell growth assay. See Supporting Information for experimental details.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b08695.

Experimental procedures and spectroscopic data for all new compounds (^{1}H NMR, ^{13}C NMR, IR, HRMS), including images of NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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