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Convergent Assembly of Highly Potent Analogues of Bryostatin 1 via Pyran Annulation: Bryostatin Look-Alikes That Mimic Phorbol Ester Function

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The bryostatins are a family of complex macrolactone natural products, originally isolated from the marine bryozoan *Bugula neritina* by Pettit and co-workers.[1] Bryostatin 1 (**1**, Figure 1) has shown promising anticancer activity that has led to some 80 clinical trials which are either complete or ongoing.[2] Although bryostatin 1 has shown limited utility as a single agent, it has displayed remarkable synergy with a number of established chemotherapeutic agents.[3] Other recent studies have revealed fascinating effects of bryostatin on memory and have suggested potential for therapeutic use in Alzheimer's disease.[4]

The mode of action of bryostatin 1 is itself a subject of intense research activity, but it is well-known to have exceptionally high affinity for protein kinase C (PKC) isozymes.[5] Unfortunately, therapeutic development of bryostatin has been hampered by the limited and nonrenewable supply of this marine natural product. Although several total syntheses of bryostatins have been reported, these require truly monumental levels of effort.[6]

As reported in an important series of papers, Wender and co-workers have prepared structurally simplified analogues of bryostatin which rival or exceed the activity of bryostatin 1 itself in terms of affinity for PKC isozymes and cytotoxicity toward certain cell lines. One of the most potent of these, compound **2**, is representative and can be seen to have deleted all substitution on the A- and B-rings; in addition, the B-ring pyran has been replaced by an acetal (1,3-dioxane) subunit for ease of synthesis.[7]

We have also established a synthetic program in this area which has as an important subgoal the identification of those structural features which are required both for PKC binding and also for biological function as a bryo 1 mimic. Thus, of the agents known to bind to and activate PKCs, only the bryostatins act as functional antagonists of a subset of biological responses.[8] Toward this end, we have reported the development of powerful new synthetic methodology designed specifically for this problem as well as very flexible strategies for its implementation.[9] Application of our pyran annulation methodology to the synthesis of a bryostatin analogue which contains the complete tricyclic macrocyclic framework of bryostatin 1 has been reported.[9c]We report herein: (1) a more convergent, second generation synthesis of the tricylic macrolactone core via our pyran annulation approach, (2) elaboration of this material to analogues with very high affinity for PKC, and (3) preliminary biological characterization of these materials that suggests a biological profile more akin to that of the tumor-promoting phorbol 12-myristate 13-acetate (PMA) than to that exhibited by bryostatin 1.

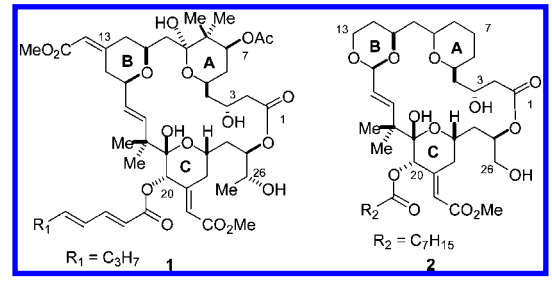

Figure 1. Structures of bryostatin 1 and a bryologue.

The preparation of these analogues is shown in Scheme 1. The A-ring intermediate **5** was prepared by a pyran annulation reaction[9a] between the known[9c] hydroxyallylsilane **4** and aldehyde **3**. The ester moiety was then used to fashion a second hydroxyallylsilane for the next pyran annulation by application of the Bunnelle reaction.[10] Pyran annulation between this new hydroxyallylsilane **5** and C-ring aldehyde **6** then provided tricyclic intermediate **7** in 84% isolated yield. Elaboration at C_1 to give the required carboxylic acid **8** was accomplished via selective deprotection of the BPS group followed by sequential application of the Parikh−Doering and Pinnick oxidations.[11] Removal of the sole TBS group at C_{25} was then followed by a highly efficient Yamaguchi macrolactonization to afford the desired tricylic macrolactone **9** in 87% isolated yield for the two steps.

Introduction of the C_{21} enoate functionality along the lines previously established by Evans[6b] proved difficult. Although the aldol condensation with freshly prepared methyl glyoxalate could be accomplished quite readily using LDA, elimination from the resulting β-hydroxy ketone proved exceedingly difficult with this substrate. Ultimately, a new procedure for this very demanding reaction was devised which involved treatment with carbonyl diimidazole in the presence of $(i\text{Pr})_2\text{NEt}$.[12,13] This very cleanly afforded the desired enoate **10**. Luche reduction of the C_{20} ketone gave the desired alcohol, which was immediately acylated to give protected versions of analogues **11**−**13**. Removal of protecting groups commenced by removal of the PMB group with DDQ. Finally, global deprotection of the remaining groups could be accomplished without incident and in essentially quantitative yield in all three cases using the $LiBF_4$ conditions originally developed by Lipshutz[14] for acetal hydrolysis and previously used successfully on the macrolactone core structure.[9c]

All three of these materials proved to have higher affinity for PKCα than does bryostatin (bryo 1 K_i = 1.35 nM, K_i values given in Scheme 1). Each of these analogues has also been screened for function by examination of proliferation and attachment of U937 leukemia cells.[15] In this assay, phorbol esters inhibit proliferation and induce attachment. Bryostatin has a much reduced effect and

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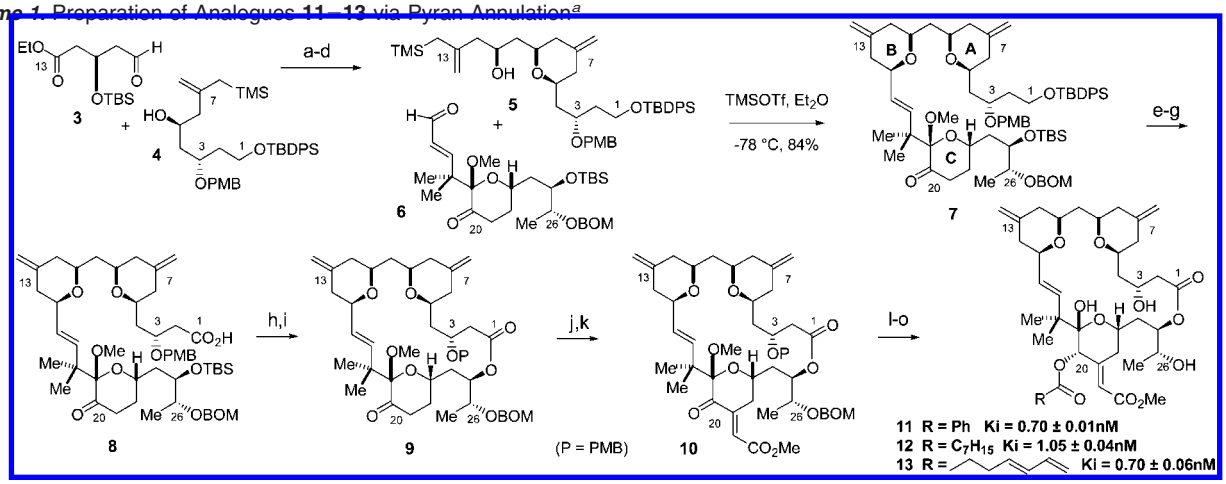
Scheme 1. Preparation of Analogues **11**–**13** via Pyran Annulation[a]



[a] Key: (a) TMSOTf, Et_2O,−78 °C, 96%; (b) TsOH, MeOH, rt, 92%; (c) TMSCl, Et_3N, CH_2Cl_2, 99%; (d) $TMSCH_2MgCl$, $CeCl_3$, THF, 81%; (e) TBAF, AcOH, DMF, 90%; (f) DMSO, SO_3·Py, $(i\text{Pr})_2$NEt, 93%; (g) $NaClO_2$, 2-methyl-2-butene, *t*BuOH, KH_2PO_4, H_2O, 99%; (h) HF·Py, THF, Py; (i) 2,4,6-Cl_3PhCOCl, Et_3N, THF, then DMAP, tol, 40 °C, 87% over 2 steps; (j) LDA, THF, −78 °C, then methyl glyoxate, 76% plus 19% recovered ketone; (k) CDI, DMAP, $(i\text{Pr})_2$NEt, CH_2Cl_2, 75%; (l) $NaBH_4$, $CeCl_3$, MeOH, −40 °C; (m) $(PhCO)_2O$, DMAP, CH_2Cl_2, 91% over 2 steps, dr = 6:1; (n) DDQ, pH 7 buffer, CH_2Cl_2; (o) $LiBF_4$, CH_3CN/H_2O (20:1), 80 °C, quantitative.

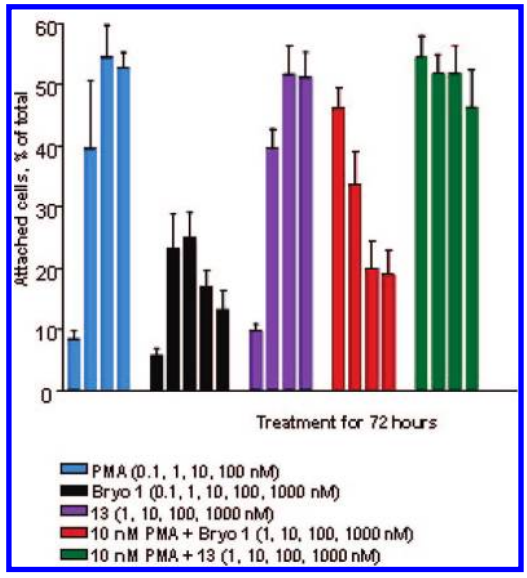


Figure 2. Results for analogue **13** in U937 attachment assay.

correspondingly blocks the effect of the phorbol ester. If the analogues were to act simply as PKC activators, they would inhibit proliferation and induce attachment both alone and in the presence of PMA. If they were to act as functional antagonists, they would show little reduction in proliferation or induction of attachment and would restore proliferation and block attachment in the presence of 10 nM PMA. Results with **13** in the attachment assay are illustrative and are shown in Figure 2.[16]

It is clear that the fingerprint displayed here by **13** is virtually identical to that of the tumor-promoting phorbol ester PMA and distinctly different from that of bryostatin 1. This may have significant implications regarding the projected use of such compounds as therapeutic agents. Additionally, analogue **13** can be seen to differ from bryostatin 1 at just four positions. Efforts to determine, through synthesis, how substitution at each of these sites impacts function are in progress. Further biological characterizations of these highly potent materials will be provided in due course.

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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.

References

(1) For a review of the chemistry and biology of the bryostatins, see: Hale, K. J.; Hummersone, M. G.; Manaviazar, S.; Frigerio, M. *Nat. Prod. Rep.* **2002**, *19*, 413–453.
(2) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216–1238.
(3) Schwartz, G. K.; Shah, M. A. *J. Clin. Oncol.* **2005**, *23*, 9408–9421.
(4) See Alkon, D. L.; Sun, M.-K.; Nelson, T. J. *Trends Pharm. Sci.* **2007**, *28*, 51–60, and references cited therein.
(5) Dell'Aquilla, M. L.; Harold, C. L.; Kamano, Y.; Pettit, G. R.; Blumberg, P. M. *Cancer Res.* **1988**, *48*, 3702–3708.
(6) (a) Kageyama, M.; Tamura, T.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D. C.; Masamune, S. *J. Am. Chem. Soc.* **1990**, *112*, 7407–7408. (b) Evans, D. A.; Carter, P. H.; Carreira, E. M.; Charette, A. B.; Prunet, J. A.; Lautens, M. *J. Am. Chem. Soc.* **1999**, *121*, 7540–7552. (c) Ohmori, K.; Ogawa, Y.; Obitsu, T.; Ishikawa, Y.; Nishiyama, S.; Yamamura, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 2290–2294.
(7) (a) Wender, P. A.; DeBrabander, J.; Harran, P. G.; Jimenez, J.-M.; Koehler, M. F. T.; Lippa, B.; Park, C.-M.; Shiozaki, M. *J. Am. Chem. Soc.* **1998**, *120*, 4534. (b) Wender, P. A.; Horan, J. C.; Verma, V. A. *Org. Lett.* **2006**, *8*, 5299–5302, and references therein.
(8) Blumberg, P. M.; Pettit, G. R. In *New Leads and Targets in Drug Research*; Krosgaard-Larsen, P., Christensen, C. B., Kodof, H., Eds.; Munksgaard: Copenhagen, 1992; pp 273−285.
(9) (a) Keck, G. E.; Covel, J. A.; Schiff, T.; Yu, T. *Org. Lett.* **2002**, *4*, 1189–1192. (b) Keck, G. E.; Truong, A. P. *Org. Lett.* **2005**, *7*, 2149–2152. (c) Keck, G. E.; Truong, A. P. *Org. Lett.* **2005**, *7*, 2153–2156. (d) Keck, G. E.; Welch, D. S.; Vivian, P. K. *Org. Lett.* **2006**, *8*, 3667–3670. (e) Keck, G. E.; Welch, D. S.; Poudel, Y. *Tetrahedron Lett.* **2006**, *47*, 8267–8270.
(10) Bunnelle, W. H.; Narayanan, B. A. *Organic Syntheses*; Wiley & Sons: New York, 1993; Collect. Vol. 8, pp 602−605.
(11) A potential one-step alternative using the procedure of Zhao and co-workers is precluded by the presence of the *exo*-methylene groups. See: Zhao, M.; Li, J.; Mano, E.; Song, Z.; Tschaen, D. M.; Grabowski, E. J. J.; Reider, P. J. *J. Org. Chem.* **1999**, *64*, 2564–2566.
(12) Details of this process will be reported separately.
(13) Although the enoate could have been installed prior to macrolactonization, this would have precluded the systematic introduction of C-ring functionality that we desired.
(14) Lipschutz, B. H.; Harvey, D. F. *Synth. Commun.* **1982**, *14*, 267–277.
(15) Vrana, J. A.; Saunders, A. M.; Srikumar, P. C.; Grant, S. *Differentiation* **1998**, *63*, 33–42.
(16) Results for all analogues in the U937 attachment and proliferation assays are similar and are provided in the Supporting Information.

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