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Synthesis and Biological Evaluation of Fully Synthetic Bryostatin Analogues

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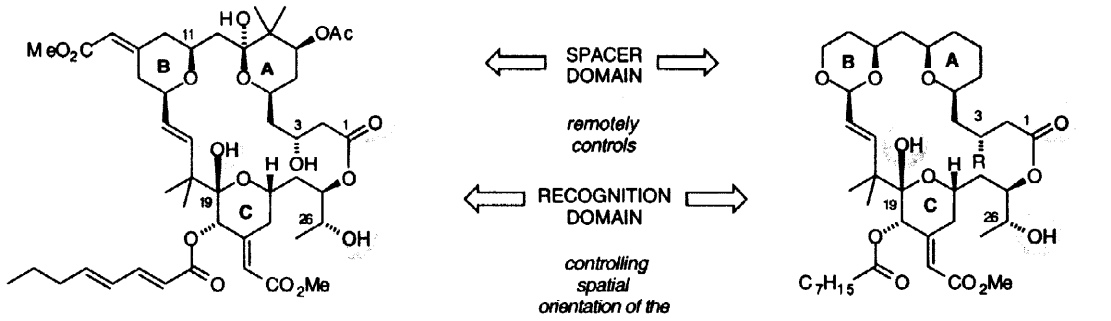
Abstract: The first members of a new class of designed bryostatin analogues are synthesized using a novel, convergent macrotransacetalization strategy. These simplified analogues, lacking the A-ring of bryostatin 1 and possessing a simplified B-ring, exhibit significant protein kinase C binding affinities and growth inhibitory activities against several human cancer cell lines.

The bryostatins are a family of 20 macrocyclic lactones isolated from marine bryozoa and found to exhibit promising cancer chemotherapeutic activity.[1,2] Bryostatin 1 is currently in expanded phase II human clinical trials for the treatment of various types of cancer.[3]



While the molecular mode of action of bryostatin 1 is not known, it is well established that it binds with high affinity to protein kinase C (PKC) competitively with the phorbol esters.[4-7] In addition to blocking a subset of the responses induced by phorbol esters, the most significant of which is tumor promotion, bryostatin 1 potently stimulates PKC activity. Among a range of therapeutic consequences, this activity is thought to provide cellular protection against normally lethal doses of ionizing radiation and to stimulate immune system responses that result in the production of interleukins and interferons.[8,9] The current supply of bryostatin 1 is obtained from ecologically sensitive marine sources. Aquaculture presents a possible alternative source of the natural bryostatins and their biosynthetic analogues. A potentially superior and

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certainly more flexible alternative to these sources could be realized with functional bryostatin mimics designed for optimal therapeutic performance and sufficiently simple in structure to be prepared through practical synthesis. In the first steps toward this end, our laboratory has recently shown that one such mimic (**1**) designed on the basis of computer, x-ray crystallographic and solution structure analyses, does indeed exhibit activity on par with bryostatin 1 in several human cancer cell growth inhibitory assays.[7, 10, 11] This work has clearly provided the first evidence that structurally simpler but biologically potent mimics of bryostatin can be realized through rational design. We report herein a significant evolution of this effort involving the synthesis and biological evaluation of a new class of bryostatin analogues, simplified through deletion of the A-ring pyran and a modification of the B-ring.

Computational studies, limited structure-activity data, and analogy to diacylglycerol, the endogenous activator of PKC, suggest that binding of the bryostatins to PKC could be attributed to the heteroatoms at C1, C19 and C26, whose spatial orientations are in turn remotely controlled by a lipophilic spacer domain associated with the A and B rings.[7, 11] Molecular modeling indicates that replacement of the pyran A-ring with an ether linkage could provide simplified analogues such as **2** or **3** in which the C1-C14 domain of the bryostatins would be accurately simulated, thereby controlling the spatial orientation of the pharmacophoric atoms in the recognition domain. Based on these considerations, two analogues were designed (Figure 1). One analogue utilizes a simple ether linkage (**2**), while the second (**3**) incorporates a *t*-butyl substituent in order to further restrict the conformation of the macrocyclic core. The synthesis of these analogues employed a convergent esterification-macrotransacetalization protocol to couple the recognition (**5**) and spacer (**4**) domains.

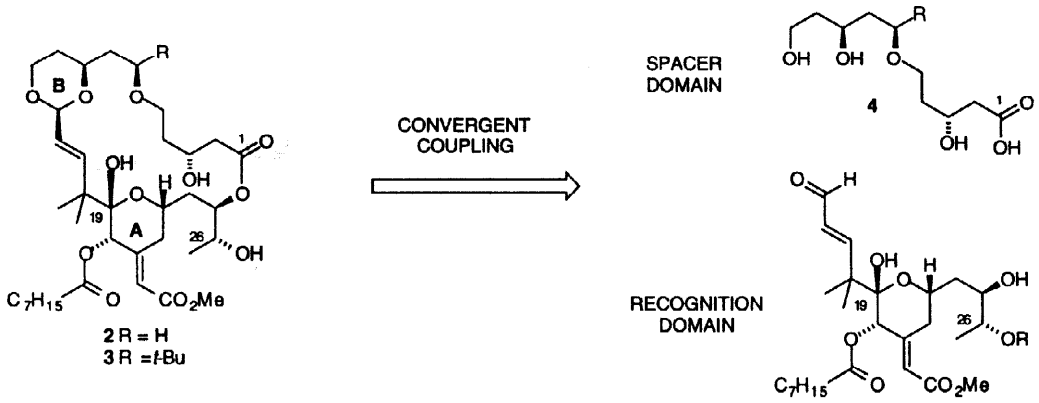
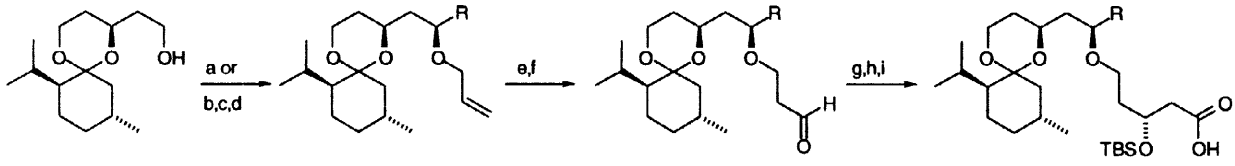


Figure 1

The synthesis of analogue **2** began with direct allylation of menthone derived alcohol **6** to give ether **7** (Scheme 1).[12] For the synthesis of analogue **3**, **6** was first oxidized to the aldehyde and treated with *t*-butyllithium to afford a 1:1 mixture of diastereomers.[10] These diastereomers were separated, and the undesired diastereomer was recycled through an oxidation/reduction protocol, producing a 6:1 mixture favoring the desired product. Allylation of the resulting secondary alcohol gave ether **8**. The two intermediates (**7** and **8**) were then carried independently through a parallel synthetic sequence. Hydroboration of the terminal olefin with an oxidative work-up, followed by oxidation with Dess-Martin periodinane

produced aldehydes **9** and **10**.[13] Asymmetric allylation using Brown's protocol afforded the corresponding homoallylic alcohols, which were subsequently silylated.[14] Oxidative cleavage of the alkenes with $KMnO_4/NaIO_4$ provided the completed spacer subunits **11** and **12**.

Scheme 1

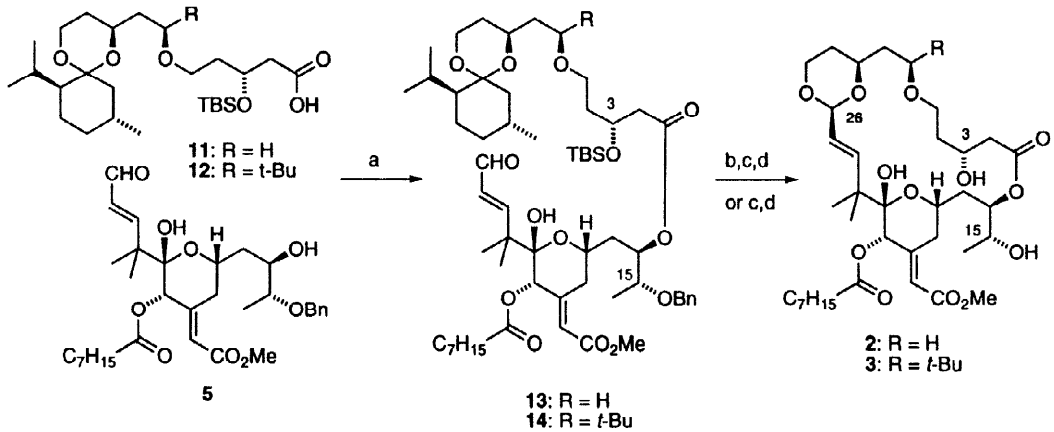


6 — 7: R = H, 8: R = t-Bu — 9: R = H, 10: R = t-Bu — 11: R = H, 12: R = t-Bu

(a) NaH, allylbromide, THF, rt, 76% for 7. (b) Dess-Martin Periodinane, CH_2Cl_2, rt, 90%. (c) *t*-BuLi, Et_2O, -78 °C, 72% (1:1 mixture of diastereomers which can be recycled: Dess-Martin Periodinane, CH_2Cl_2, rt then $NaBH_4$, $CeCl_3 \cdot 7H_2O$, -40 °C, 80% (6:1 α:β)). (d) *t*-BuOK, allylbromide, THF, rt, 90% based on recovered 8. (e) 9-BBN, THF, 65 °C, then NaOH, H_2O_2. (f) Dess-Martin periodinane, CH_2Cl_2, rt, 79% for 9, 80% for 10. (g) $(-)\text{-(Ipc)}_2BOMe$, allylmagnesium bromide, CH_2Cl_2, -78 °C to rt, 83% for R = H, 84% for R = *t*-Bu. (h) TBSCl, imidazole, THF, rt. (i) catalytic $KMnO_4$, $NaIO_4$, rt, 51% for 11, 52% for 12.

Completion of the synthesis of analogues **2** and **3** was achieved in three and four steps, respectively, from **11** and **12**, through a convergent strategy involving esterification, macrotransacetalization, and deprotection.[10] The initial esterification of the richly functionalized **5** was conducted individually with **11** and **12** following Yamaguchi's esterification protocol to afford intermediates **13** and **14**, respectively (Scheme 2).[15] Ester **13** was treated with HF•pyridine to cleave the silyl ether. Then, in the key macrotransacetalization step, remarkable for its mildness and tolerance of the diverse and densely arrayed functionality of the substrates (3 esters, 3 ethers, a hemi-ketal, a ketal, and an unsaturated aldehyde), the seco-aldehydes **13** and **14** were independently stirred at room temperature with Amberlyst-15 resin to provide the 20 membered macrocycles with C15 stereocenters set in the thermodynamically preferred configuration. In the case of **14**, the C3 silyl ether was simultaneously cleaved. Finally, deblocking of the C26 alcohol was accomplished separately using $Pd(OH)_2$ affording target analogues **2** and **3**.

Scheme 2



5 — 13: R = H, 14: R = *t*-Bu — 2: R = H, 3: R = *t*-Bu

(a) 2,4,6-trichlorobenzoylchloride, Et_3N, DMAP then 5, CH_2Cl_2, rt, 94% for R = H, 87% for R = *t*-Bu. (b) HF•pyridine, CH_3CN, rt, 85% for R = H. (c) Amberlyst-15 resin, CH_2Cl_2, rt, 72% for R = H, 49% (83% BORSM) for R = *t*-Bu. (d) $Pd(OH)_2$, H_2, EtOAc, 1 atm, 79% for 2, 60% for 3.

While lacking the bryostatin A-ring, analogues **2** and **3** bind rat brain PKC isozymes with remarkably high affinities (K_i = 47 nM and 8.3 nM, respectively). Of further importance, both analogues show significant levels of growth inhibitory activity against several human cancer cell lines (GI_{50}: 8.0×10^{-3} to 3.3 μg/mL).[11] Interestingly, preliminary results indicate that in some cell lines these analogues have superior activity to bryostatin 1.

In summary, a novel esterification-macrotransacetalization strategy has been used to convergently assemble the first members of a new class of designed bryostatin analogues. These analogues bind to PKC with nanomolar affinities and exhibit functional growth inhibitory activity against human cancer cell lines comparable and in some case superior to bryostatin itself. These studies suggest that further simplification of the bryostatin structure could be realized without adversely affecting the potency, providing an approach to tunable clinical candidates which could be prepared in a practical fashion through synthesis. Efforts to further simplify these new medicinal agents, to elucidate the molecular basis of bryostatin's unique activity, and to develop improved clinical candidates of the bryostatins are in progress.

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