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Synthesis and biological evaluation of bryostatin analogues: the role of the A-ring

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Abstract

The first biologically active member and a key intermediate of a new family of simplified bryostatin analogues are synthesized through an optimized esterification–macrotransacetalization strategy. This family incorporates both an ester linkage between C5 and C9 in addition to a C9 *t*-butyl substituent to mimic the bryostatin A-ring. Importantly, a free C7 alcohol is revealed late in the synthesis, allowing access to numerous C7 derivatized analogues.

The bryostatins are a unique and promising class of emerging chemotherapeutic candidates first isolated in trace quantities from the marine invertibrae *Bugula neritina*.[1] Bryostatin **1** has been shown to exhibit therapeutic efficacy in a number of human clinical trials, including the induction of several complete responses to advanced, refractory forms of cancer.[2] While their molecular mode of action is not known, the bryostatins potently bind to protein kinase C (PKC) and stimulate enzymatic activity both in vitro and in vivo.[3] Other noteworthy effects induced by bryostatin include stimulation of immune system responses,[4] increased expression of p53 and decreased expression of bcl-2 coupled to induction of apoptosis in cancer cells,[5] and induction of differentiation of chronic lymphocytic leukemia to a less invasive cell type.[6]

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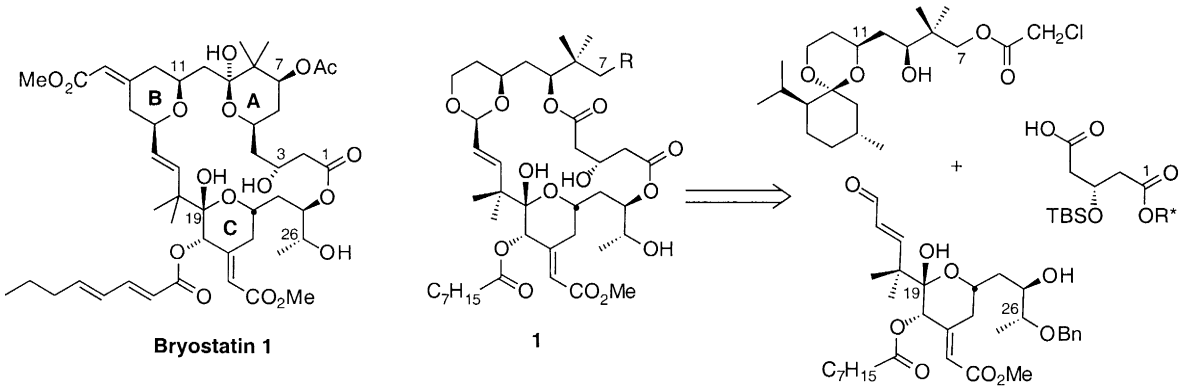
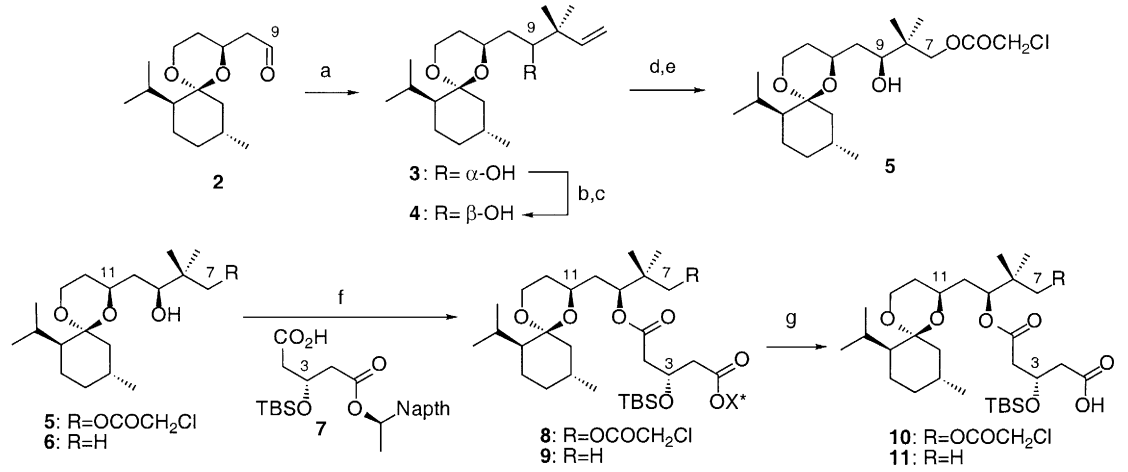


Efforts to identify the structural basis for these and related activities and to develop more effective clinical candidates have been hampered by the low supply of the bryostatins and difficulties associated with their modification.[1b,7] As a potential solution to these problems, we have recently reported the first series of totally synthetic bryostatin analogues based on computer, pharmacophoric, X-ray, and structure activity analyses.[8,1c] These simplified analogues have been shown to have similar or better activity than the bryostatins in terms of PKC binding affinity and in vitro growth inhibition of human cancer cells.

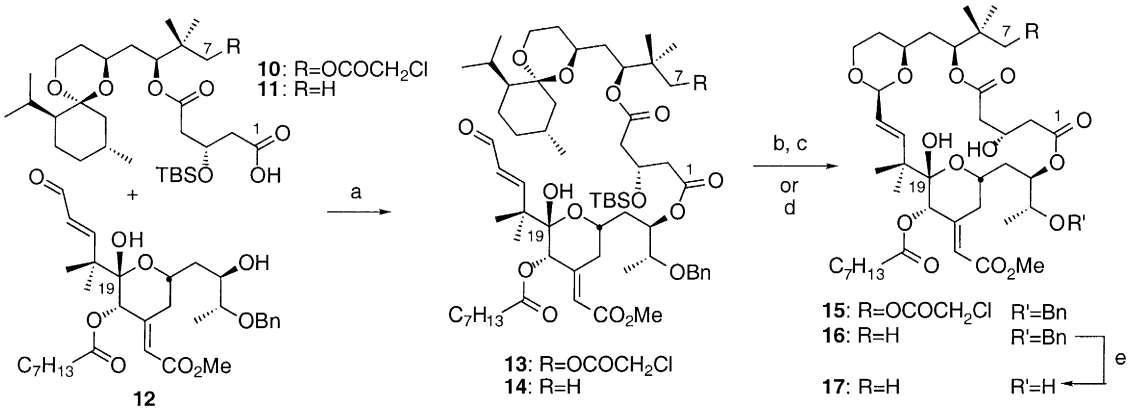
In order to advance this approach to potentially superior clinical candidates and mechanistic probes, it is necessary to simplify the target structure and to allow expedient access to a large number of analogues, possibly in a combinatorial fashion. For these purposes, analogue class **1** has been investigated. Incorporated in the design of **1** is a C7 (bryostatin numbering) alcohol which is designed to be liberated at a late stage of the synthesis to serve as a branching point for the synthesis of a number of analogues. In an effort to systematically simplify the analogue framework, **1** also contains a C9 *t*-butyl substituent to simulate the bryostatin A-ring in addition to an ester linkage between C5 and C9. The ester is expected to prefer the *s-cis* orientation which would, in principle, serve to rigidify this key portion of the target analogue. The incorporation of this ester linkage also allows for an improvement in the convergency of the C1–C13 fragment synthesis. Described herein is the synthesis of the first member and the key branching point intermediate of this new class of bryostatin analogues.

The synthesis of the key C7 hydroxy intermediate began with menthone aldehyde **2** (Scheme 1).[8a] A convenient prenyl indium addition[9] to **2** gave a 3:1 (α:β) diastereomeric mixture of alcohols, which could be recycled through an oxidation reduction protocol to provide **4**. Ozonolysis of **4** followed by a reductive workup yielded a diol which was immediately esterified with chloroacetic anhydride to give the selectively protected chloroacetate derivative **5**. The secondary alcohol of intermediate **5** and the known *t*-butyl alcohol **6**[8c] were then taken through a parallel sequence. Thus, esterification[10] of alcohols **5** and **6** independently with acid **7**,[11] gave the ester derivatives **8** and **9** in good yield. Removal of the chiral auxiliaries then afforded the completed C1–C13 subunits **10** and **11** in only ten and eight linear steps, respectively.

Independent Yamaguchi esterification[10] of both **10** and **11** with the previously synthesized C15–27 enal **12**[8a] gave *seco* aldehydes **13** and **14** (Scheme 2). Utilization of the previously described two step macrotransacetalization conditions[8a] on **13** afforded the target macrocycle **15**, but in only 35% yield. Optimization efforts conducted independently on aldehydes **13** and **14** subsequently revealed that in one step, using excess 70% HF/pyridine, the C3 TBS group and the menthone ketal of **13** and **14** can be removed, and the 20-membered macrocycles **15** and **16** can be formed with the stereocenter at C15 set in both to the desired thermodynamically preferred orientation in high yield. Importantly, this procedure has been found to be superior in yield in all substrates studied to date.[8]



Scheme 1. Syntheses of two C1–C13 bryostatin analogue fragments. (a) PrenylBr, in powder, DMF, 84% (3:1, **3**:**4**); (b) Dess–Martin Periodinane, CH_2Cl_2; (c) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0°C, 71%, (3:1, **4**:**3**), two steps; (d) **4**, O_3, CH_2Cl_2, MeOH, −78°C, then $NaBH_4$, 0°C; (e) $(ClCH_2CO)_2O$, Pyr, CH_2Cl_2, 0°C, 63% (based on recovered diol), two steps; (f) **7**, 2,4,6-trichlorobenzoyl chloride, DMAP, Et_3N, Tol., 83% for **8** from **5**; 79% for **9** from **6**; (g) H_2, $Pd(OH)_2$, EtOAc, 95% for **10** from **8**; 80% for **11** from **9**

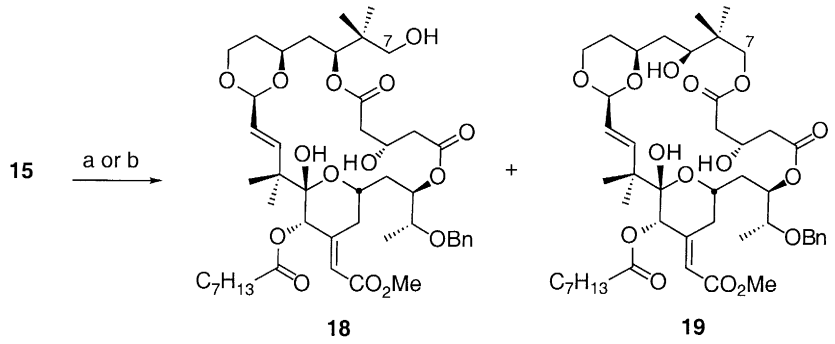


Scheme 2. Convergent esterification–macrotransacetalization approach to bryostatin analogue synthesis. (a) 2,4,6-trichlorobenzoyl chloride Et_3N, DMAP, Tol., then **12**, 75% for **13** from **10**; 70% for **14** from **11**; (b) 1:1 HF:Pyr, THF; (c) Amberlyst-15, 4 Å MS, CH_2Cl_2, 35% for **15** from **13**; (d) excess HF/pyridine, THF, 62% for **15** from **13**; 79% for **16** from **14**; (e) H_2, $Pd(OH)_2$/C, EtOAc, 92%

In order to determine the biological activity of this new analogue framework and to allow a direct comparison to previously synthesized analogues, the C26 benzyl ether of **16** was cleaved to reveal analogue **17**. Binding assays to PKC[12] revealed that **17** binds with high affinity (8.42 ± 0.05 nM), similar to that of the bryostatins. Importantly, **17** is also an effective agent for growth inhibition of the P388 murine lymphocytic leukemia cell line (ED_{50}=113 nM).

Initial efforts to remove the C7 chloroacetate protecting group of **15** with ammonia unfortunately led to low yields and a significant amount of the 1,3-acyl migrated product **19** (Scheme 3).[13] While **19** represents a novel ring expanded bryostatin analogue precursor, our current efforts were focused on the optimization of the yield of **18**. These efforts revealed that the use of excess thiourea in THF gave excellent yields of **18** without any detectable acyl migration despite the complexity of the molecular

framework and the presence of four other ester functional groups. Intermediate **18** is a key precursor for the investigation of the role of the C7 group in binding and functional performance.



Scheme 3. Deprotection of the C7 chloroacetate group. (a) NH_3, MeOH, THF, 1 h, 26%, 1:1, **18**:**19**; (b) excess thiourea, THF, 4 days, 92%, >11.5:1, **18**:**19**

In conclusion, the successful syntheses of macrocycles **15** and **16** utilizing improved macrotransacetalization conditions has been achieved. The synthesis of simplified *t*-butyl analogue **17** has revealed that high affinity binding is maintained even in analogues lacking the A-ring of bryostatin. Selective deprotection of the C7 chloroacetate protecting group of **15** has afforded a pivotal intermediate (**18**) in excellent yield, allowing the study of the role of the C7 substituent in binding and function. Studies on these novel analogues will be reported shortly.

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