Synthesis of the First Members of a New Class of Biologically Active Bryostatin Analogues

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The bryostatins are a novel family of emerging cancer chemotherapeutic candidates isolated from marine bryozoa on the basis of their significant activity against murine P388 lymphocytic leukemia.[1] These macrolactones have been shown to exhibit remarkable and unique activities,[2] leading to the recent entry of bryostatin 1 (Figure 1) into Phase II clinical trials for the treatment of melanoma, non-Hodgkins lymphoma, and renal cancer.[3] While their molecular mode of action is not known, the bryostatins potently inhibit the binding of the tumor-promoting phorbol esters to protein kinase C (PKC) and stimulate enzymatic activity both in vitro and in vivo.[4] However, they induce only a subset of phorbol ester responses and block those actions of phorbol esters which they themselves do not initiate, most notably tumor promotion.[5] Efforts to identify the structural basis for these and related activities and to develop more effective clinical candidates have been hampered by the low natural abundance[1b] of the bryostatins and difficulties associated with their modification. As an alternative approach to these goals, we describe here the first class of simplified, synthetic bryostatin analogues which exhibit a high affinity for PKC and potent growth inhibitory activity against several human cancer cell lines.

Computational studies,[6] limited structure−activity data,[1b,6,7] and analogy to diacylglycerol, the endogenous activator of PKC, suggest that the binding of bryostatin to PKC could be attributed to substituents at C1, C19, and C26 (boxed in Figure 1), whose orientations are remotely controlled by a lipophilic spacer (shaded in Figure 1). Macrocycles of the general structure **1** were designed to test this hypothesis. These systems retain the putative recognition domain of the bryostatins but incorporate a simplified spacer domain to facilitate their synthesis. This design allows access to **1** through a novel, convergent esterification−macrotransacetalization strategy involving coupling of the recognition domain (**2**) with variable spacers (**3**), an approach which has potential for the creation of analogue libraries.

Our first objective in this study was the synthesis of the bryostatin C-ring and its attendant functionality (C15−C27).[8] Scheme 1 depicts a first-generation sequence which has readily

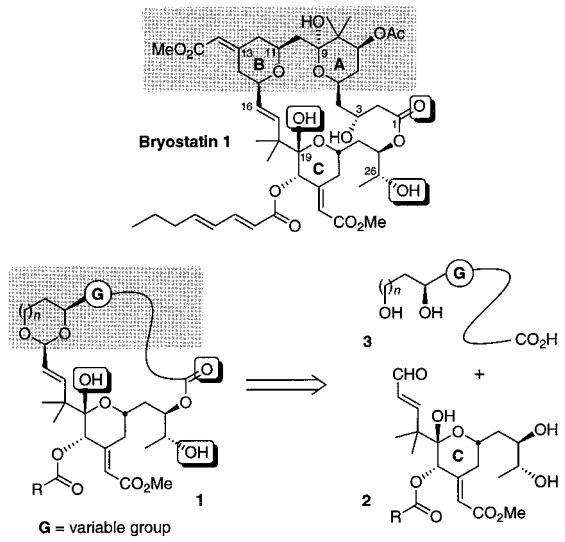
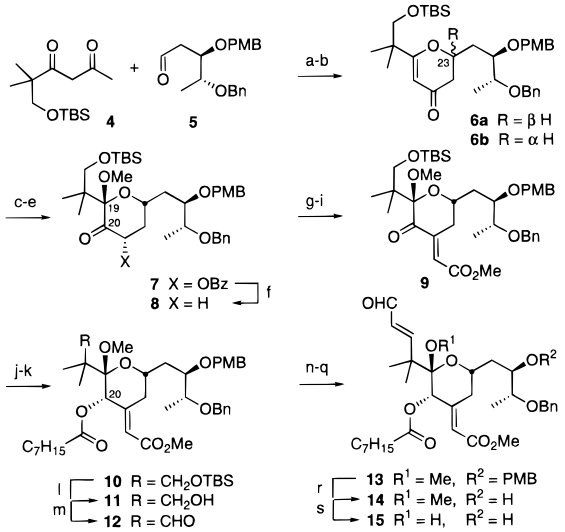


Figure 1.

Scheme 1[a]



[a] (a) **4**, 2 equiv of LDA, THF, −78 °C, 1 h then **5**, −78 °C, 30 min, 98%. (b) cat. *p*TsOH, toluene, room temperature (rt), **6a** (41%), **6b** (49%). (c) **6a**, $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, −20 °C. (d) *m*-CPBA, $NaHCO_3$, 2:1 CH_2Cl_2/MeOH, 71%, two steps. (e) PhCOCl, DMAP, CH_2Cl_2, −10 °C; Dess−Martin periodinane, rt, 90%. (f) SmI_2, THF, MeOH, −78 °C, 95%. (g) LDA, $OHCCO_2Me$, THF, −78 °C, 90% based on recovered **8**. (h) $ClSO_2Me$, Et_3N, CH_2Cl_2, −10 °C. (i) DBU, THF, rt, 78%, two steps. (j) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, −20 °C. (k) $C_7H_{15}CO_2H$, 2,4,6-trichlorobenzoyl chloride, Et_3N, toluene, rt, 93%, two steps. (l) HF/pyridine, THF, rt. (m) Dess−Martin periodinane, CH_2Cl_2, rt, 86%, two steps. (n) allyl-BEt_2, Et_2O, −10 °C. (o) Ac_2O, DMAP, CH_2Cl_2, 95%, two steps. (p) cat. OsO_4, NMO, THF/H_2O. (q) $Pb(OAc)_4$, Et_3N, PhH; DBU, rt, 80%, two steps. (r) DDQ, CH_2Cl_2, H_2O, 79%. (s) HF, CH_3CN, H_2O, rt, ≥95%.

delivered gram quantities of the target fragment **15**. Condensation of the dienolate of **4**[9] with aldehyde **5**[10] followed by acid-catalyzed

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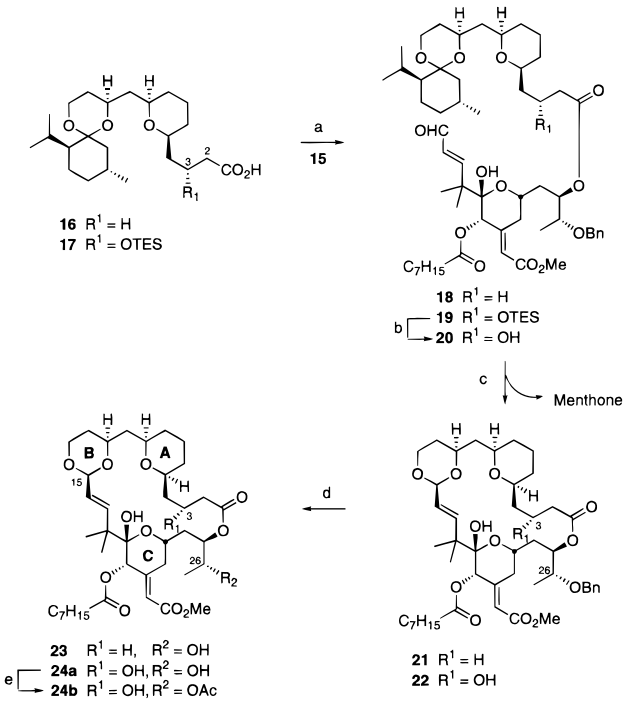
dehydration gave pyranones **6a**,**b** (1:1). The β-isomer (**6a**) was easily separated and reduced under Luche conditions,[11] and the resulting glycal was epoxidized with *m*-CPBA in the presence of MeOH to afford a C19-methoxylated C20,C21-diol (71% from **6a**). Selective benzoylation of the C21 equatorial alcohol followed by oxidation of the remaining C20 hydroxyl group with Dess−Martin periodinane[12] (DMP) provided benzoate **7** (90%, two steps). Treatment of **7** with SmI_2 (2 equiv) selectively deoxygenated C21 to give ketone **8** (95%). From **8**, a straightforward aldol condensation/elimination sequence with $OHCCO_2Me$[13] was sufficient to install the desired *E*-exocyclic unsaturated ester at C21, providing enone **9**. Luche reduction[11] of **9** gave exclusively the C20 axial alcohol which was esterified[14] with octanoic acid to afford compound **10** (93%, two steps). Completion of the target fragment, requiring a demanding two carbon homologation at C17, proceeded with removal of the TBS group with HF/pyridine and oxidation of the resulting alcohol with DMP[12] to give aldehyde **12** (86%, two steps). After much experimentation, this hindered aldehyde was found to react with allyl-BEt_2 followed by Ac_2O to generate an inconsequential mixture of acetates in high yield (95%, two steps). Dihydroxylation of the terminal olefin with catalytic OsO_4 (NMO co-oxidant) and $Pb(OAc)_4$-mediated glycol cleavage in the presence of Et_3N and DBU afforded enal **13** (80%, two steps). Exposure of **13** to DDQ selectively liberated the C25 alcohol to give **14** (79%), which, when treated with aqueous HF, gave the target hemiketal **15** in >95% yield.

With an efficient route to the C15−C27 segment of the bryostatins established, attention turned toward installing our first series of C1−C14 inserts. Molecular modeling indicated that B-ring acetal/A-ring pyrans of type **24a** (Scheme 2) would closely mimic the conformation of bryostatin, allowing for the proper display of putative PKC recognition elements.[6a] Accordingly, menthone-derived spacer segments **16** and **17**[15] were prepared independently and coupled by Yamaguchi esterification[14] with alcohol **15** to provide ester adducts **18** (81%) and **19** (81%), respectively. For **19**, the C3 TES group was removed with HF/pyridine (81%). At this point, a remarkable macrotransacetalization was initiated by stirring **18** and **20** independently in a dilute (0.004 M) solution of Amberlyst-15 acidic resin with 4-Å molecular sieves in CH_2Cl_2. This pivotal reaction served to close the 20-membered macrocycle via acetal formation.[16] In each case, a single isomer of cyclized product was detected in the crude macrocyclization mixture; a result consistent with a thermodynamically controlled acetalization establishing the equatorial configuration at C15.[17] The cyclized products were independently hydrogenated over $Pd(OH)_2$ to afford bryostatin analogues **23** (56% from **18**) and **24a** (88% from **20**).

Scheme 2[a]



[a] (a) 2,4,6-Trichlorobenzoyl chloride, Et_3N, toluene, rt, then **15**, DMAP: **18**, 81% from **16**; **19**, 81% from **17**. (b) 1:1 HF:pyridine, THF, rt, 81%. (c) Amberlyst-15, 4-Å molecular sieves, CH_2Cl_2, rt. (d) cat. $Pd(OH)_2/C$, H_2 (1 atm), EtOAc, rt: **23**, 56% from **18**; **24a**, 88% from **20**. (e) Ac_2O, DMAP, CH_2Cl_2, rt, 85%.

Acetals **23** and **24a** bind rat brain PKC isozymes with K_i = 297 and 3.4 nM, respectively. As previously found for bryostatin,[6] acylation of the C26 hydroxyl group of **24a** produced compound **24b** with substantially reduced affinity for PKC (>10 μM). *Most importantly, analogue **24a** shows significant levels (1.8−170 ng/mL) of in vitro growth inhibitory activity against several human tumor cell lines.*[6a]

In summary, this study establishes a novel and effective route to the first generation of simplified biologically active analogues of bryostatin. An esterification−macrotransacetalization strategy allows for a modified bryostatin ring system to be convergently assembled from readily prepared subunits with high efficiency. Of the first analogues tested, acetal **24a** exhibits potent affinity for PKC and exceptional growth inhibitory activity. Efforts to further simplify these new leads, to elucidate the molecular basis for bryostatin's activity, and to develop improved clinical candidates of bryostatin are in progress.

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Supporting Information Available: Spectroscopic data and experimental procedures for reported compounds (24 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(9) Prepared in four steps from commercially available methyl isopropyl ketone (see the Supporting Information).

(10) Synthesized in five steps from (*R*)-(+)-methyl lactate according to minor modifications of a closely related sequence. See ref 8a.

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(15) Prepared in seven (**16**) and eleven (**17**) steps from the 1,3-menthone acetal of 1,3,5-pentanetriol (see the Supporting Information).

(16) To our knowledge, there is only a single report of a related transformation: Li, G.; Still, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 3804−3805.

(17) The all-equatorial configuration of **24a** was confirmed by 2D-NOESY ^{1}H-NMR analysis. See ref 6a.