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The Practical Synthesis of a Novel and Highly Potent Analogue of Bryostatin

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The bryostatins are a structurally novel family of marine natural products that exhibit unique and potent biological activities,[1] including the ability to stimulate immune system responses,[2] regulate apoptotic function, reverse multidrug resistance,[3] and act synergistically with other oncolytic agents.[4] Bryostatin 1 is now in phase I and II clinical trials as a single agent and in combination with other therapies.[5] While its mode of action is not established, it has been shown to bind with high affinity to PKC isozymes.[1] Other proteins containing C1 domains, such as RasGRP, Unc/Munc, and the chimaerins, have also been implicated as receptor targets.[6] Studies on the mode of action and clinical use of bryostatin 1 have been hampered by its low natural abundance, difficult isolation, and structural complexity, which collectively have frustrated the search for superior derivatives. While impressive in content, total syntheses of the bryostatins are currently unable to meet preclinical or clinical needs.[7] To address this supply problem and provide potentially superior clinical candidates, we have designed analogues of bryostatin 1 that can be produced in clinically required quantities through synthesis.[8] We previously reported an analogue, **2**, with in vitro activity comparable to that of bryostatin.[9] We now report the practical synthesis and bioassay of a new and superior analogue, **1**, designed on the basis of our pharmacophoric model,[10] that is over 100-fold more potent than bryostatin at inhibiting the growth of numerous human cancer cell lines.

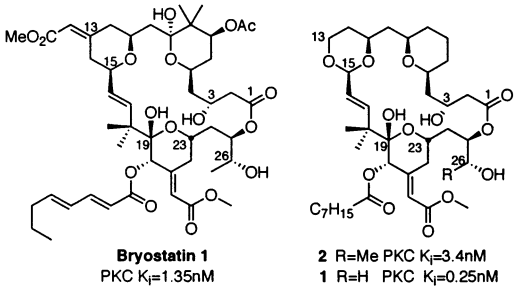
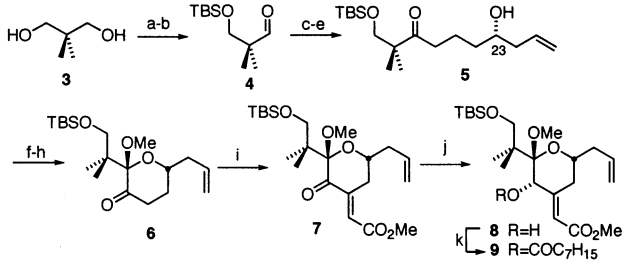


Figure 1.

The synthesis of the C15−C26 recognition domain of **1** began with the monoprotection and oxidation of inexpensive diol **3**[11] on a scale of ≥0.4 mol to generate aldehyde **4**. Reaction of **4** with the Grignard reagent derived from 4-chloro-1-butanol[12] followed by oxidation and asymmetric Keck allylation[13] provided homoallylic alcohol **5**. The use of $B(OMe)_3$ allowed this allylation to be run at room temperature, setting the C23 stereocenter with high enantiomeric control (92% ee).[14] Dehydrative cyclization of **5** followed by epoxidation and in situ methanolysis yielded a mixture of diastereomers, the major of which was oxidized to ketone **6**. Conversion of ketone **6** to enoate **7** was readily accomplished in one step by treatment with K_2CO_3 in MeOH at room temperature with either methyl glyoxylate (72%) or the commercially available methyl 2-hydroxy-2-methoxyacetate (55%). A highly diastereoselective reduction of **7** followed by esterification afforded ester **9** (Scheme 1).

Scheme 1 [a]



[a] (a) NaH, TBSCl, THF, rt; (b) SO_3·pyr, NEt_3, DMSO, CH_2Cl_2, rt; (c) (i) 4-chloro-1-butanol, MeMgCl, THF, −78 °C → rt; (ii) Mg, reflux; (iii) **4**, −78 °C; (d) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, −78 °C, 54% from **3**; (e) 10 mol % R-BINOL, 4 Å MS, 5 mol % $Ti(OiPr)_4$, $B(OMe)_3$, allyl-$SnBu_3$, CH_2Cl_2, rt, 77%; (f) cat. *p*TsOH·H_2O, 4 Å MS, MePh, rt, 85%; (g) MMPP, $NaHCO_3$, 2:1 CH_2Cl_2:MeOH, 0 °C, 78% (4:1 dr); (h) 10 mol % TPAP, NMO, 4 Å MS, 6:1 CH_2Cl_2:CH_3CN, 0 °C → rt, 78%; (i) K_2CO_3, $OHCCO_2Me$, MeOH, rt, 72%; (j) $NaBH_4$, $CeCl_3$·$7H_2O$, MeOH, −30 °C; (k) $C_7H_{15}CO_2H$, DIC, DMAP, CH_2Cl_2, rt, 93% from **7**.

Deprotection of **9** followed by oxidation gave aldehyde **10**. In contrast to our original four-step sequence,[9a] the transformation of aldehyde **10** to enal **11** was achieved in only one step and in 90% yield. Because aldehyde **10** is both sterically encumbered and susceptible to deprotonation at C22, the vinyl zincate derived from (*Z*)-1-bromo-2-ethoxyethene was one of the few nucleophiles effective in this homologation. Sharpless AD conditions[15] were used to convert **11** to diol **12** with 2.5:1 diastereoselectivity. The diastereomers were separated following hydrolysis of the C19 ketal and selective protection of the C26 alcohol. Whereas the fully elaborated recognition domain of analogue **2** was generated in 0.02% and 24 steps,[9a] **14** was produced in >3% yield and only 17 steps (Scheme 2).

The synthesis of the C1−C13 spacer domain began with the ozonolysis and in situ reduction of **15**, which produced pentane-1,3,5-triol in a manner superior to that of previous methods.[16] Desymmetrization of the resulting triol via acetal formation with (−)-menthone[17] and subsequent oxidation yielded a mixture (1.6β:1α) of aldehydes **16**. A hetero Diels−Alder cycloaddition between **16**β and Danishefsky's diene using Jacobsen's tridentate Cr(III) catalyst[18] provided pyranone **17** with exceptional selectivity. The diastereoselectivity (33:1) obtained with this catalyst has far exceeded that of others (1:2 to 4:1) in overcoming the inherent bias (1:3.5) of substrate **16**β. Pyranone **17** was converted to **19** as

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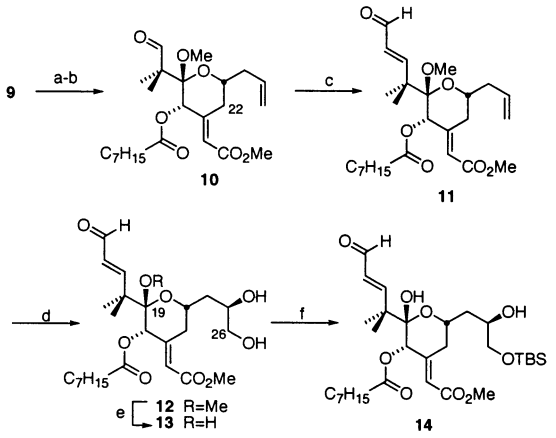
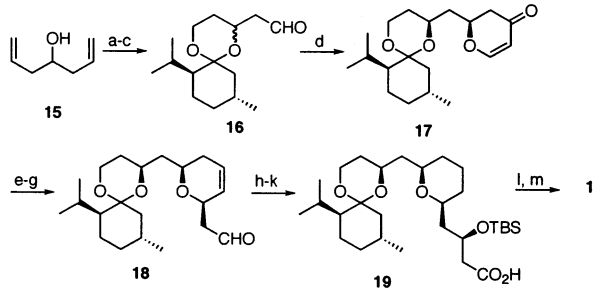
Scheme 2 [a]



[a] (a) 3HF·Et_3N, THF, rt; (b) Dess−Martin periodinane, $NaHCO_3$, CH_2Cl_2, 0 °C → rt, 87% from **9**; (c) (i) (*Z*)-1-bromo-2-ethoxyethene, *t*-BuLi, Me_2Zn, then **10**, Et_2O, −78 °C; (ii) 1M HCl, −78 °C → rt, 90%; (d) $(DHQD)_2PYR$, $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, K_2CO_3, 1:1 *t*-BuOH:H_2O, 0 °C, 71%, (2.5β:1α); (e) *p*TsOH·H_2O, MeCN, H_2O; (f) 1:3 TBSCl: imidazole, CH_2Cl_2, rt, 46% from **12**.

Scheme 3 [a]



[a] (a) (i) O_3, MeOH, −78 °C; (ii) $NaBH_4$, −78 °C → rt, 90%; (b) (−)-menthone, *p*TsOH·H_2O, $CH(OEt)_3$, Et_2O, rt, 71%; (c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 87% (1.6β:1α); (d) Danishefsky's diene, catalyst (see Supporting Information), 4 Å MS, acetone, rt, then TFA, 88%; (e) $NaBH_4$, $CeCl_3$·$7H_2O$, −40 °C, MeOH, 92%; (f) 0.5 equiv $Hg(OAc)_2$, isobutylvinyl ether, rt; (g) decane, 150 °C, 83% over 2 steps; (h) 1 atm H_2, 20% $Pd(OH)_2$/C, EtOAc, rt, 85%; (i) (−)-$(Ipc)_2$BOMe, allyl-MgBr, CH_2Cl_2, −78 °C → rt; (j) TBSCl, imidazole, THF, rt, 69% over 2 steps; (k) $KMnO_4$, $NaIO_4$, rt, 1:1 *t*-BuOH:pH 7 buffer, 84%; (l) Et_3N, 2,4,6-trichlorobenzoyl chloride, then **14**, DMAP, MePh, rt, 87%; (m) 70% HF·pyr, THF, rt, 82%.

shown in Scheme 3, providing the fully elaborated spacer domain in 11 steps and 11% yield overall.[9a]

Fragments **14** and **19** were coupled using the Yamaguchi esterification protocol.[19] Our transacetalization procedure closed the macrocycle, set the C15 stereocenter under thermodynamic control, and allowed for deprotection, providing lactone **1** in 19 steps (LLS) and 2% yield.

In agreement with our pharmacophoric model, **1** displays *picomolar* affinity for PKC (Figure 1). When tested in vitro against various human cancer cell lines, **1** displayed greater potency than bryostatin 1 in 24 of 35 cases. In some cell lines, such as MOLT-4 and NCI-H460, **1** was 3 orders of magnitude more potent than bryostatin 1 at inhibiting cell growth, with GI_{50} values below the limit of detection of 10^{-8} M. Considering the exceptional potency and unique activity of **1**, only gram quantities are required for clinical development. This synthesis meets this requirement, providing a novel strategy for the practical and scalable synthesis of **1** and related analogues. Further synthetic and biological studies are in progress.

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Supporting Information Available: Experimental conditions and spectral data for compounds **1**, **5**−**7**, **9**−**11**, **14**, **16**−**19** and selective assay information for compounds **1** and **2** (PDF). An X-ray crystallographic file in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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