ORGANIC LETTERS

2006
Vol. 8, No. 20
4581−4584

Synthesis and PKC Binding of a New Class of A-Ring Diversifiable Bryostatin Analogues Utilizing a Double Asymmetric Hydrogenation and Cross-Coupling Strategy

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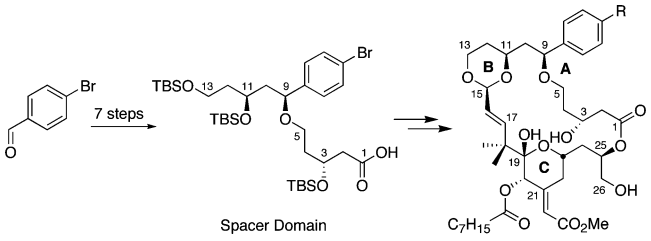
Received July 23, 2006

ABSTRACT



The design, asymmetric synthesis, and biological evaluation of a new class of bryostatin analogues based on a pseudosymmetric spacer domain are described. An aryl bromide diversification site is incorporated allowing access to systematically varied analogues. The new analogues all exhibit potent, nanomolar affinity to PKC.

The marine-derived natural product bryostatin 1[1] (Figure 1) exhibits a remarkable range of biological activities including induction of apoptosis, reversal of multidrug resistance, immune system modulation, and the ability to act synergistically with other oncolytic drugs.[2] As a result of these activities, it is currently being evaluated in phase I and II clinical trials for the treatment of cancer.[3] Recent research has also demonstrated that bryostatin improves memory and learning in animal models, indicating a potential for its use in treating neurological disorders such as Alzheimer's disease and depression.[4]

Despite its broad therapeutic potential, a major hurdle in the advancement of bryostatin 1 in the clinic as well as in the advancement of our understanding of its underlying mode of action is its low natural abundance and the associated difficulty in accessing clinically significant quantities of the natural product through chemical synthesis. Additionally, because it appears that bryostatin was designed by nature as an ichthyic antifeedant,[5] and not as a drug for human therapy, it is unlikely that the natural product is optimized for use as

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10.1021/ol0618149 CCC: $33.50
Published on Web 09/02/2006

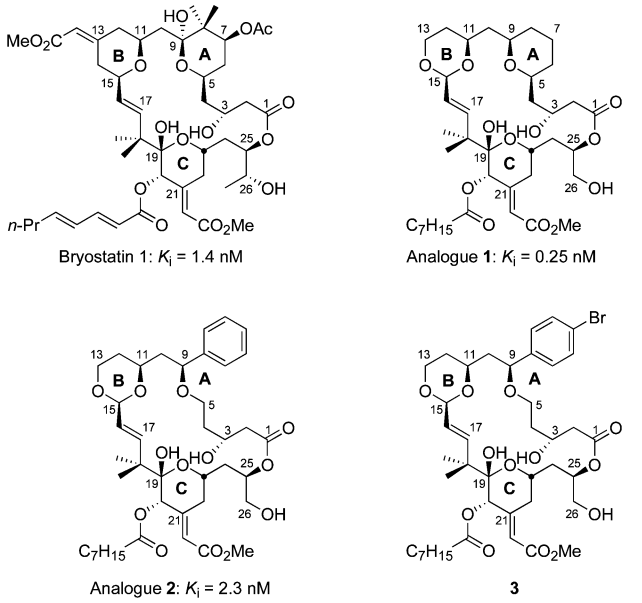


Figure 1. Bryostatin 1 and synthetic analogues.

a treatment for cancer or neurological disease. To address supply, performance, and mode of action issues, our group has been involved in the design and synthesis of simplified bryostatin analogues that can be produced in a practical fashion and tuned for optimal therapeutic performance. Representative of these efforts is analogue **1** (Figure 1), which exhibits in vitro and in vivo biological activities *comparable to or better than* bryostatin 1 in various assays.[6,7] Analogue **2**, with a phenyl group at C9, is representative of a class of simplified analogues that lack an intact A-ring yet retain single-digit nanomolar affinity for PKC.[8]

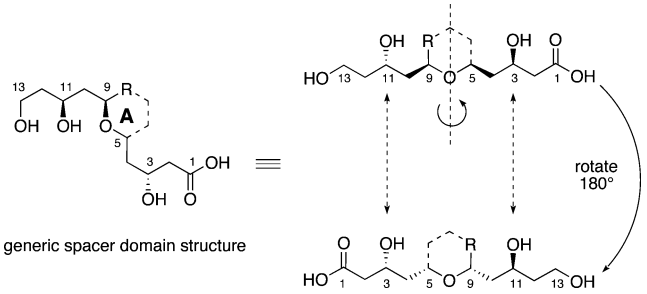
Bryostatin is thought to act by modulating the activity and cellular localization of various C1 domain-containing proteins such as protein kinase C (PKC). In contrast to molecules that target the ATP binding site of PKC and function only as inhibitors, molecules that target the C1 domain can be designed to inhibit or activate enzyme activity. In addition, C1 domains are only present in a small subset of the large family of kinases, offering selectivity in function. The PKC family is divided into three subclasses: the conventional, novel, and atypical isozymes. Of these three, bryostatin binds only to the conventional and novel subclasses (eight isozymes in total). A long-standing goal in the area of C1 domain research is to design agents that can selectively regulate one or a subset of these eight isozymes. Previous work in our group indicates that the A-ring region of the bryostatin scaffold is not directly involved in the key C1 domain binding interactions and is therefore amenable to significant structural modification. Although changes to this portion of the molecule do not have a large impact on PKC affinity in our binding assay, the effect of modifications to the A-ring on isozyme selectivity and function are not known. Analogue **3** (Figure 1), with an aryl bromide substituent in the A-ring region of the molecule, was designed to probe the effect of structural changes in this region on isozyme affinity, selectivity, and overall function.

The aryl bromide functionality was chosen as a diversification site that would allow analogue **3** to be rapidly converted into new, systematically varied analogues with a common core macrocycle and set of binding elements. The general chemical stability of this group in addition to the broad range of C−C, C−N, and C−O bond forming processes described in the literature[9] make this an attractive functionality for late-stage diversification. The para disposition of the bromide substituent was chosen to minimize steric issues that could impede the late-stage coupling reactions and interfere with C1 domain binding.

Because our analogues are synthesized by coupling a top "spacer" domain with a bottom "recognition" domain, the generation of **3** required only the synthesis of a new spacer domain which could be coupled with the preexisting recognition domain. Compared to the existing synthesis of the spacer domain for analogue **2**,[8] a new and more step-economical route was developed for the synthesis of analogue **3**.

This synthesis was designed to exploit the pseudo-C_2 symmetry present in bryostatin analogue spacer domains. The fact that the C3 and C11 carbinols are symmetrically disposed with respect to the axis that bisects the A-ring (Scheme 1)

Scheme 1. Pseudo-C_2 Symmetry of the Spacer Domain



suggests that these two stereocenters could be set in a single transformation through a double asymmetric reduction of a diketone. This type of strategy has precedent in the work of Schreiber et al. on the bidirectional synthesis of mycoticin A.[10]

The synthetic route designed to take advantage of this double asymmetric reduction strategy began with conjugate

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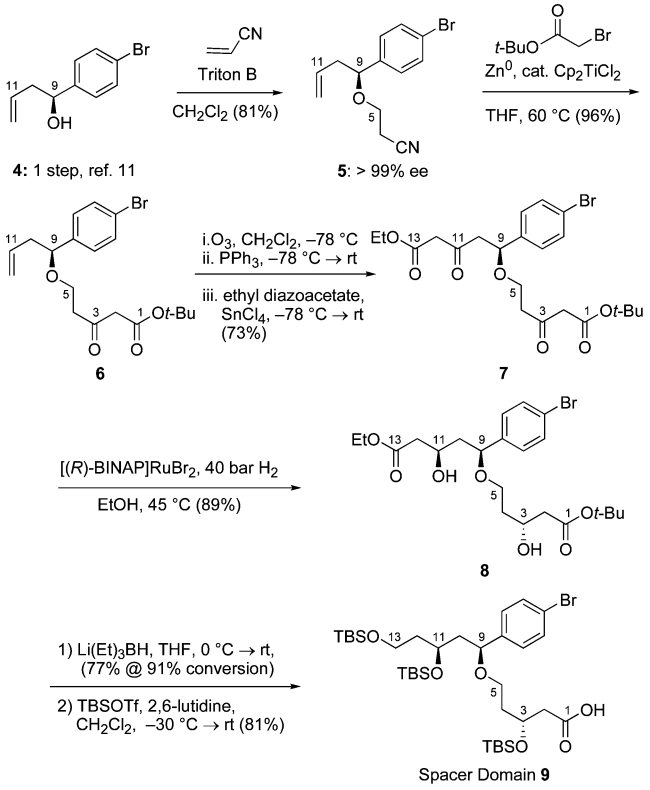
addition of known homoallylic alcohol **4**[11] (available in one step from 4-bromobenzaldehyde) into acrylonitrile to afford **5** (Scheme 2). Recrystallization of **5** from ethyl ether/pentane

Scheme 2. Synthesis of Spacer Domain **9**

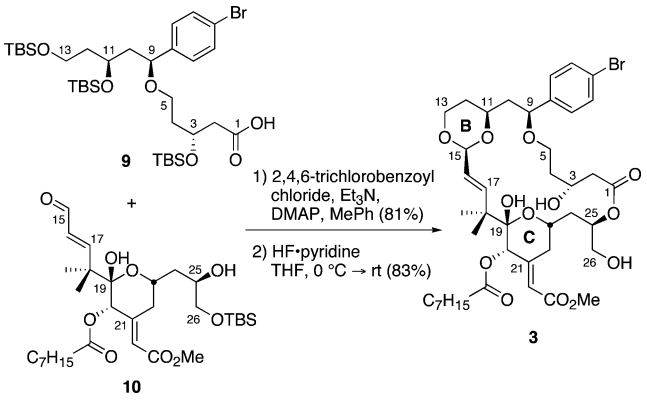


increased the enantiomeric purity from 92% to greater than 99% ee. The structure and absolute configuration of **5** were confirmed by X-ray crystallographic analysis.

Nitrile **5** was subsequently elaborated to β-ketoester **6** using a modified Blaise reaction.[12] Ozonolytic cleavage of the terminal olefin generated an unstable aldehyde, which was immediately used in the next step without isolation. Addition of ethyl diazoacetate and $SnCl_4$ to the flask containing the aldehyde solution effected a Roskamp homologation that produced di-β-ketoester **7** in good yield.[13] Double asymmetric reduction of di-β-ketoester **7** using Noyori's [(*R*)-BINAP]$RuBr_2$ catalyst cleanly produced di-β-hydroxyester **8** in high yield and with superb diastereoselectivity.[14] HPLC analysis of the product mixture revealed that **8** was produced with 98% diastereomeric purity. This mixture could be enriched to greater than 99% isomeric purity by column chromatography. Selective reduction of the ethyl ester in the presence of the *tert*-butyl ester using lithium triethylborohydride afforded the corresponding triol. In an efficient one-step transformation, treatment of the triol with TBSOTf protected the three hydroxyl groups and cleaved the *tert*-butyl ester to reveal the C1 carboxylic acid. *This concise route provided the completed spacer domain* ***9*** *in only seven steps and in 27% overall yield.* This is a dramatic improvement over the synthesis of the spacer domain for analogue **2**, which was produced in 16 steps with an overall yield of 4%.[8]

Spacer domain **9** was coupled to the existing recognition domain **10**[7] using Yamaguchi's esterification protocol (Scheme 3).[15] The macrocycle was then closed, and the silyl protecting

Scheme 3. Completion of Analogue **3**



groups were removed in a remarkably effective, one-step, mild, and diastereoselective macrotransacetalization providing completed analogue **3**. In this process, four silyl protecting groups are removed, and the macrocycle is closed. The newly created C15 stereocenter is formed under thermodynamic control, affording only the bryostatin-like stereochemistry.

Hydrogenolysis of **3** using Pd/C provided the known desbromo analogue **2** (Table 1, entry 1). The product obtained in this reaction is identical (by NMR, MS, and HPLC analyses) with a sample of **2** prepared using our original synthetic route.[8] This result established that the C3 and C11 stereocenters were set with the correct configuration under the Noyori hydrogenation conditions. *This overall sequence establishes a new synthesis of analogue* ***2*** *that is nine steps shorter than the original route and improves the overall yield by a factor of 7.* Importantly, it offers direct access to analogues diversified in the A-ring region, whose influence on activity and function is largely unexplored.

Our attention was next turned to the diversification of analogue **3** using cross-coupling chemistry. Because of the potentially sensitive nature of the analogue to strongly basic conditions, initial diversification efforts focused on using mild, room temperature, and tin-free C−C bond forming reactions. Suzuki coupling of **3** with phenylboronic acid using the Dave-Phos ligand produced new analogue **11** in a single

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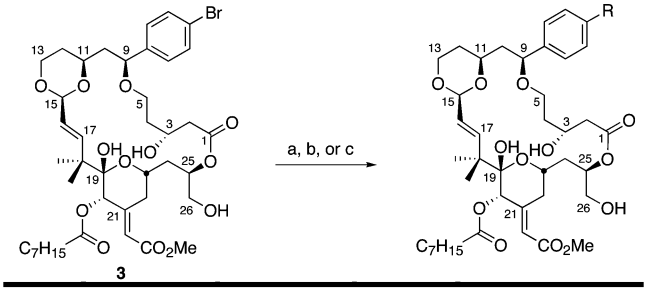
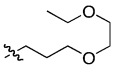
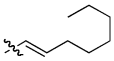
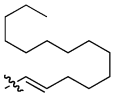
Table 1. Diversification of Analogue **3**[a]



3

entry	conditions	R =	Y =	yield
1	a	H	—	**2**: 73%
2	b			**11**: 53%
3	b			**12**: 20%
4	c			**13**: 62% at 57% conversion
5	c			**14**: 88% at 40% conversion

[a] Conditions: (a) Pd/C, H_2 (1 atm), EtOAc, 50−55 °C; (b) $Pd(OAc)_2$, Dave-Phos, R−Y (see table), CsF, 1,4-dioxane, rt; (c) $Pd(OAc)_2$, S-Phos, R−Y (see table), CsF, 1,4-dioxane, rt.

step (Table 1, entry 2).[16] Using the same conditions with long-chain alkylboranes (chosen to mimic the long acyl chains of the endogenous PKC ligand diacylglycerol) resulted in lower yields and problems with reaction reproducibility (Table 1, entry 3).

As an alternative to alkylboranes, vinylboronic acids were explored. It was discovered that vinylboronic acids could be smoothly coupled using the S-Phos ligand.[17] Coupling of vinylboronic acids with analogue **3** cleanly produced new analogues **13** and **14**; however, these reactions stalled at approximately 50% conversion (Table 1, entries 4 and 5). Despite the moderate yields obtained, these reactions demonstrate that the aryl bromide functionality can successfully be used as a diversification site to rapidly provide new analogues.

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In this initial set of analogues, all compounds exhibit potent nanomolar binding affinity when tested against a rat brain PKC isozyme mixture (Table 2).[18] All new analogues

Table 2. PKC Binding Affinities for Bryostatin Analogues

analogue:	**2**[8]	**3**	**11**	**12**	**13**	**14**
PKC K_i (nM):	2.3	1.6	3.1	3.8	4.6	30

except **14** maintain single-digit nanomolar affinity for PKC, indicating that lipophilic groups substituted at the para position of the phenyl ring are well tolerated. The relatively low binding affinity of analogue **14** might be attributable to the reduced ability of the compound to partition into the phospholipid vesicles over the time course of the assay. A similar trend in PKC binding affinities has been observed for highly lipophilic derivatives of the phorbol esters.[19] More generally, the results obtained with these analogues suggest that the A-ring region can be modified to tune pharmacokinetic and ADME characteristics as needed to improve function and suppress side effects. The ease with which these analogues can be prepared and their potency allow attention to be focused on the previously unexplored influence of this region on biological activity. Studies on the selective binding of these and related analogues to PKC isozymes and other C1 domain containing targets and their functional activities are in progress and will be reported in due course.

Acknowledgment. Support of this work through NIH grant CA31845 is gratefully acknowledged. We thank the Mochly-Rosen group (Stanford Department of Molecular Pharmacology) for assistance with the PKC assay and the Du Bois group (Stanford Department of Chemistry) for assistance with HPLC analyses. HRMS analyses for some compounds were performed at UCSF.

Supporting Information Available: Experimental conditions and spectral data for compounds reported in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) All PKC binding experiments performed against a rat brain isozyme mixture as described in references 6 and 7.

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