Pergamon

Tetrahedron Letters 41 (2000) 6725–6729

TETRAHEDRON LETTERS

Synthesis and biological evaluation of a new class of bryostatin analogues: the role of the C20 substituent in protein kinase C binding

Paul A. Wender* and Kevin W. Hinkle

Department of Chemistry, Stanford University, Stanford, California 94305-5080, USA

Received 4 June 1999; revised 23 June 2000; accepted 26 June 2000

Abstract

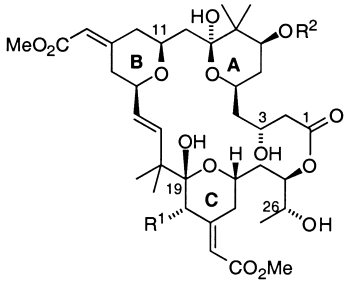
The synthesis and biological assay of a new series of designed bryostatin analogues modified at the C20 position are described. These compounds are found to bind to protein kinase C with affinities of up to 1.5 nM, demonstrating that variations at C20 can indeed be used to tune affinity and potentially function.

The bryostatins are a family of emerging cancer chemotherapeutic candidates isolated from marine bryozoa.[1] These macrocyclic lactones have been shown to exhibit unique biological activities and are currently in expanded phase II human clinical trials for the treatment of various types of cancer.[2] While the molecular mode of action of bryostatin 1 is not known, it is well established that it binds potently to and activates protein kinase C (PKC) in a fashion that is competitive with the phorbol esters but different in physiological consequence.[3,4] Efforts to identify the structural basis for this novel PKC binding and its biochemical ramifications have been hampered thus far by the limited current supply of bryostatin 1 which is obtained with difficulty only from ecologically sensitive marine sources. Two total syntheses of a bryostatin have been completed to date.[5,6] However, both of these impressive efforts required greater than 60 steps. To circumvent this supply problem and more significantly to develop superior clinical candidates, our laboratory has been involved in the design of a series of bryostatin analogues that would exhibit enhanced therapeutic performance and would be readily accessible through synthesis. One such compound, **1**, designed on the basis of computer, X-ray, crystallographic and solution structure analyses, has indeed been shown to exhibit activity similar to bryostatin 1 in several human cancer cell growth inhibitory assays.[7–11]

The similar solution structures and activities of **1** and bryostatin suggest that the more readily available analogue could be used to address questions that cannot be investigated directly with

* Corresponding author.

0040-4039/00/$ - see front matter
PII: S0040-4039(00)01100-X



Bryostatin	R^1	R^2	K_i (nM)
1	*all trans*-OCO(CH)$_4$(CH$_2$)$_2$CH$_3$	Ac	1.35
2	*all trans*-OCO(CH)$_4$(CH$_2$)$_2$CH$_3$	H	5.86
4	OCO *n*-Pr	Piv	1.30
5	OAc	Piv	1.04
6	OAc	CO *n*-Pr	1.18
7	OAc	Ac	0.84
8	OCO *n*-Pr	CO *n*-Pr	1.72
9	OCO *n*-Pr	Ac	1.31
10	H	Piv	3.36
11	H	Ac	
12	*all trans*-OCO(CH)$_4$(CH$_2$)$_2$CH$_3$	CO *n*-Pr	
13	H	CO *n*-Pr	
14	OH	Piv	
15	*all trans*-OCO(CH)$_4$(CH$_2$)$_2$CH$_3$	Ac	

Figure 1. The bryostatins

bryostatin because of its insufficient supply and difficulties associated with its modification.[12] For example, relatively little is known about the role of the C20 ester in binding and activity (Fig. 1), although this site serves as a potential position for tuning pharmacological performance and could be used mechanistically for affinity labeling. Much of the current information on C20 modifications is based on the limited number of naturally derived esters. This information is non-systematic and does not reveal any trends. A few modifications of C20 have been achieved synthetically but this approach is also limited by the complexity of the synthetic intermediates.[5,6] For example, initial efforts in our work to replace directly the C20 ester in the macrocycle **1** proved unsuccessful, presumably due to steric effects and instability problems. As a result, we sought to make this modification on a precursor of **1**, generically represented by **3**, and to carry the latter forward to **1** through an esterification–macrotransacetalization sequence that allows conjunction of the spacer and recognition domains (Fig. 2). We report herein the successful implementation of this strategy and the synthesis and preliminary binding assays of the first members of a new class of C20 analogues.

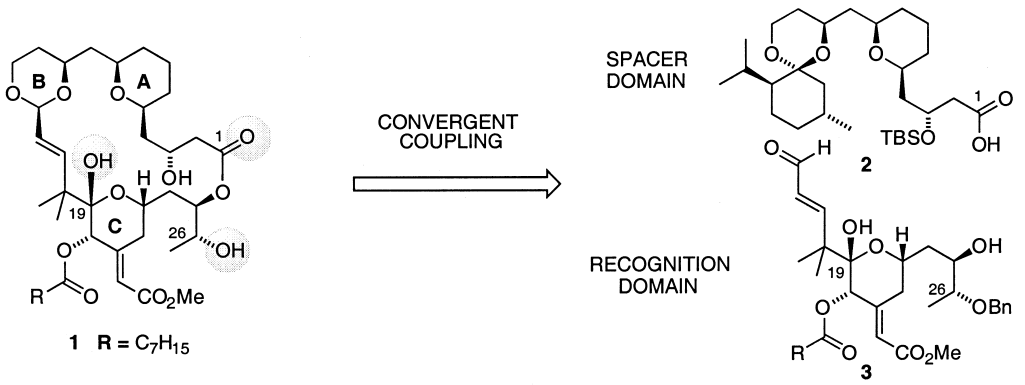
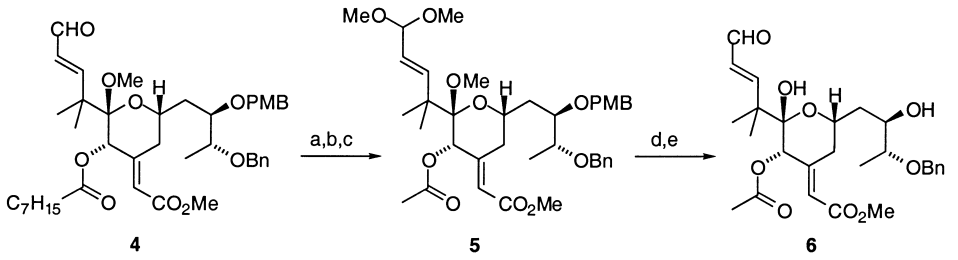


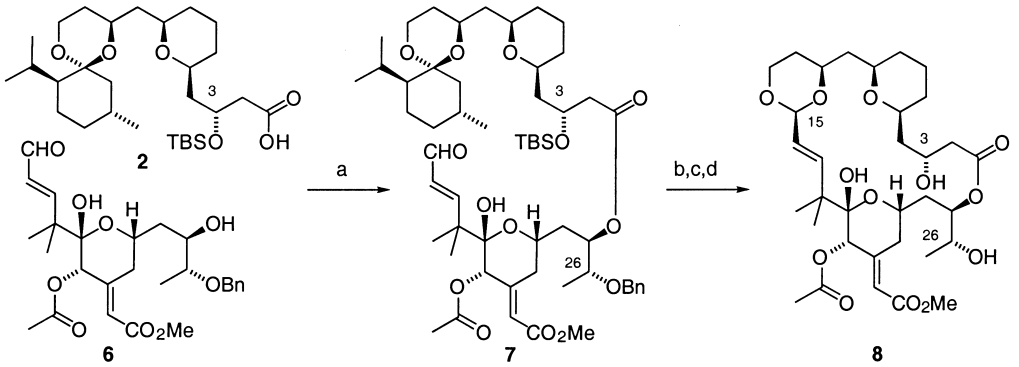
Figure 2. Retrosynthetic analysis

Initial attempts to remove the C20 ester of enal **4** under basic conditions resulted in complete decomposition of the material. In contrast, protection of the base labile aldehyde subunit of **4** provided a more readily modified compound. Thus, **4** was first converted to its dimethyl acetal using trimethyl orthorformate and PPTS. The octanoate ester at C20 was then directly cleaved



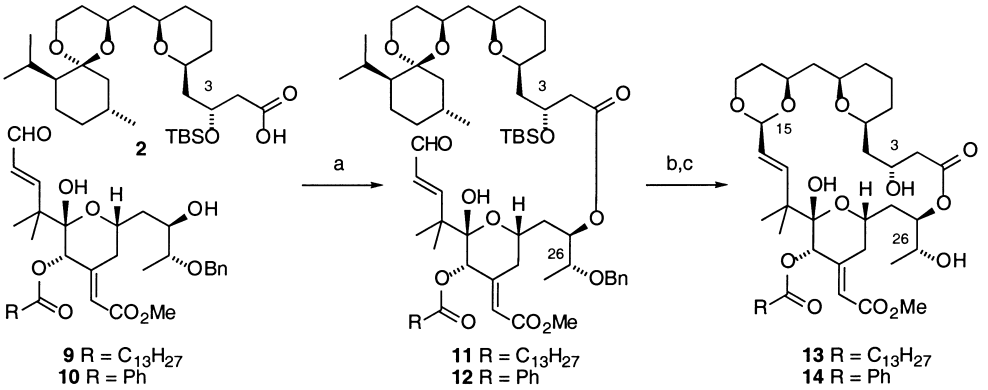
Scheme 1. (a) $CH(OMe)_3$, PPTS, MeOH, rt; (b) K_2CO_3, MeOH, rt; (c) Ac_2O, DMAP, CH_2Cl_2, rt, 82% for three steps; (d) DDQ, CH_2Cl_2/H_2O, rt, 91%; (e) aq. HF, CH_3CN, rt, 89%

using K_2CO_3 in methanol to afford the labile C20 alcohol which was immediately acylated using acetic anhydride and DMAP to give acetate **5**[13] (Scheme 1). Intermediate **5** was prepared for coupling with the spacer domain **2** by cleavage of the *p*-methoxybenzyl ether using DDQ to afford the free hydroxyl at C25. Conveniently, the dimethyl acetal is removed under these conditions. The hemiacetal was revealed with 48% aqueous HF to give enal **6**. Completion of the analogue was then accomplished in a four-step procedure which started with esterification of enal **6** under Yamaguchi's conditions with spacer domain **2** to produce ester **7**[14] (Scheme 2). Cleavage of the C3 silyl ether was accomplished using 1:1 HF·pyridine yielding the *seco*-aldehyde. In a key step, remarkable for it efficiency and tolerance of diverse functionality, macrotransacetalization of the *seco*-aldehyde was accomplished with Amberlyst-15 at room temperature to produce the corresponding benzyl protected analogue.[8] The benzyl ether was removed by hydrogenolysis using $Pd(OH)_2$ as catalyst to afford analogue **8**.



Scheme 2. (a) 2,4,6-Trichlorobenzoylchloride, Et_3N, DMAP then **6**, CH_2Cl_2, rt, 63%; (b) HF·pyridine, CH_3CN, rt, 82%; (c) amberlyst-15 resin, CH_2Cl_2, rt, 83%; (d) $Pd(OH)_2$, H_2, EtOAc, 1 atm, 93%

Syntheses of analogues **13** and **14** were accomplished in good yields using a similar sequence to that used for analogue **8**. Initial esterification of **2** was conducted independently with recognition domains **9** and **10** following Yamaguchi's protocol to afford esters **11** and **12**[14] (Scheme 3). These esters were then individually treated with HF·pyridine in THF at room temperature to afford the macrocyclic products in 83 and 80% yield (for $R = C_{13}H_{27}$ and $R = Ph$, respectively).[15] Hydrogenolysis of the C26 benzyl ethers was accomplished separately using $Pd(OH)_2$ to provide analogues **13** and **14**.

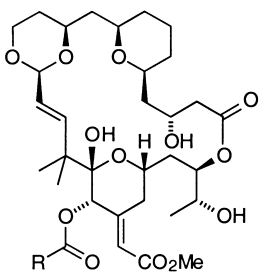


Scheme 3. (a) 2,4,6-Trichlorobenzoylchloride, Et_3N, DMAP then **9** or **10**, CH_2Cl_2, rt, 90% for $R = C_{13}H_{27}$, 89% for R = Ph; (b) HF·pyridine, THF, rt, 83% for $R = C_{13}H_{27}$, 80% for R = Ph; (c) $Pd(OH)_2$, H_2, EtOAc, 1 atm, 97% for **13**, 63% for **14**

The analogues were tested for binding affinity to a rat brain PKC isozyme mixture, a standard reference system. In view of the limited information on bryostatin and the fact that many bryostatins have a C20 acetate, it is noteworthy that the C20 acetate analogue **8** showed a significant reduction in binding affinity relative to the octanoate. Furthermore, increasing the lipophilicity of the analogues (e.g. **13** and **14**) caused a significant increase in the binding affinity. Compound **14**, selected to test preliminarily whether aromatic groups for affinity labeling studies could be introduced at C20, showed a similar binding affinity to the lead analogue. Overall, there was a correlation between the number of carbons on the ester sidechain (*R*) and the binding affinity (number of carbons = 13 > 7 > 6 > 1), which suggested a previously unrecognized role for this subunit in binding, membrane association, and potential function (Table 1).

Table 1
Binding affinity for rat brain protein kinase C

Compound	R	Ki (nM)
1	C_7H_{15}	3.4 (± 0.3)
8	CH_3	232 (± 11)
13	$C_{13}H_{27}$	1.5 (± 0.2)
14	Ph	7 (± 2)



In summary, a novel series of bryostatin analogues has been assembled which reveals, for the first time, a clear role for the C20 group in PKC binding. These analogues exhibit nanomolar affinities to PKC whose relative order correlates with carbon content. This suggests that one might use C20 variations to tune the pharmacological performance of these analogues as well as bryostatin itself. In addition, our ability to introduce aryl groups at C20 without significant loss of affinity bodes well for the use of these compounds in mode of action studies utilizing photoaffinity labels. 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 and will be reported in due course.

Acknowledgements

Support of this work through a grant provided by the National Institutes of Health (CA31845) is gratefully acknowledged. Facilities for PKC assays were supplied by Prof. Daria Mochly-Rosen (Stanford University). HRMS analyses were performed at UC San Francisco and UC Riverside Mass Spectrometry Facilities. Fellowship support from the NIH (K.W.H.) is also gratefully acknowledged.

References

1. Pettit, G. R. *J. Nat. Prod.* **1996**, *59*, 812–821.
2. Current information on the scope and status of bryostatin clinical trials can be found on the NCI web page [http://cancernet.nci.nih.gov/prot/patsrch.shtml].
3. Kraft, A. S.; Smith, J. B.; Berkow, R. L. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 1334–1338.
4. Szallasi, Z.; Du, L.; Levine, R.; Lewin, N. E.; Nguyen, P. N.; Williams, M. D.; Pettit, G. R.; Blumberg, P. M. *Cancer Res.* **1996**, *56*, 2105–2111; and references cited therein.
5. Kageyama, M.; Tamura, T.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D. C.; Masamune, S. *J. Am. Chem. Soc.* **1990**, *112*, 7407–7408.
6. Evans, D. A.; Carter, P. H.; Carreira, E. M.; Prunet, J. A.; Charette, A. B.; Lautens, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2354–2359.
7. Wender, P. A.; Cribbs, C. M.; Koehler, K. F.; Sharkey, N. A.; Herald, C. L.; Kamano, Y.; Pettit, G. R.; Blumberg, P. M. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 7197–7201.
8. Wender, P. A.; De Brabander, J.; Harran, P. G.; Jimenez, J.-M.; Koehler, M. F. T.; Lippa, B.; Park, C. M.; Shiozaki, M.; Pettit, G. R. *J. Am. Chem. Soc.* **1998**, *120*, 4534–4535.
9. Wender, P. A.; De Brabander, J.; Harran, P. G.; Jimenez, J.-M.; Koehler, M. F. T.; Lippa, B.; Park, C. M.; Siedenbiedel, C.; Pettit, G. R. *Proc. Natl. Acad. Sci. USA* **1998**, *55*, 6624–6631.
10. Wender, P. A.; De Brabander, J.; Hinkle, K. W.; Lippa, B.; Pettit, G. R. *Tetrahedron Lett.* **1998**, *39*, 8625–8628.
11. Wender, P. A.; Hinkle, K. W.; Koehler, M. F. T.; Lippa, B. *Med. Res. Review* **1999**, 388–407.
12. Pettit, G. R.; Gao, F.; Sengupta, D.; Coll, J. C.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Van Camp, J. R.; Rudloe, J. J.; Nieman, R. A. *Tetrahedron* **1991**, *47*, 3601–3610.
13. An analogous procedure was originally employed by C. M. Park of our laboratories.
14. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.
15. These conditions for this silyl ether cleavage/macrotransacetalization were developed by B. Lippa of our laboratories: Wender, P. A.; Lippa, B. *Tetrahedron Lett.* **2000**, *41*, 1007–1011.