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Design, Synthesis, and Investigation of Protein Kinase C Inhibitors: Total Syntheses of (+)-Calphostin D, (+)-Phleichrome, Cercosporin, and New Photoactive Perylenequinones

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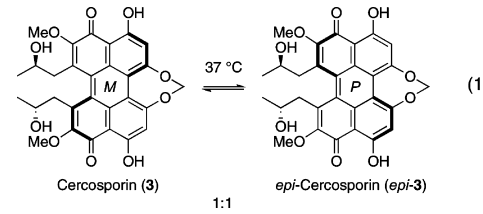
Abstract: The total syntheses of the PKC inhibitors (+)-calphostin D, (+)-phleichrome, cercosporin, and 10 novel perylenequinones are detailed. The highly convergent and flexible strategy developed employed an enantioselective oxidative biaryl coupling and a double cuprate epoxide opening, allowing the selective syntheses of all the possible stereoisomers in pure form. In addition, this strategy permitted rapid access to a broad range of analogues, including those not accessible from the natural products. These compounds provided a powerful means for evaluation of the perylenequinone structural features necessary to PKC activity. Simpler analogues were discovered with superior PKC inhibitory properties and superior photopotentiation in cancer cell lines relative to the more complex natural products.

Introduction

Several years ago we initiated a program to explore the use of the Cu-catalyzed enantioselective oxidative biaryl coupling[1] in the syntheses of enantiopure perylenequinones and demonstrated that systems possessing only helical stereochemistry can be configurationally stable.[2] With the development of a key aldol cycloaddition utilizing a dynamic stereochemistry transfer, we further completed the first total synthesis of hypocrellin A (**4**; Figure 1).[3] Prior to our efforts the total syntheses of the (−)-calphostins A−D (**1a**−**d**) and (+)- and (−)-phleichrome (**2**) were reported involving diastereoselective biaryl couplings.[4] Unfortunately, these couplings afforded mixtures with the wrong diastereomer usually predominating; additional steps were required to establish the correct stereochemistry. Furthermore, cercosporin (**3**) with a bridging seven-membered ring remained a challenging synthetic target. Although the structurally related **1** and **2** are atropisomerically stable, the additional seven-membered ring in cercosporin lowers the atropisomerization barrier, allowing **3** to readily atropisomerize at 37 °C (eq 1).[5]



(1)

Herein, we report the first total syntheses of (+)-**1d** and **3** exploiting a novel double cuprate epoxide opening. The combination of enantioselective oxidative biaryl coupling, double cuprate epoxide opening, and decarboxylative functionalization provides a potentially general means for constructing a diverse array of perylenequinone analogues with complete control of the helical and centrochiral stereochemical elements. As a result, we describe the synthesis of 10 new perylenequinone analogues as well as (+)-**1d**, (+)-**2**, and **3** from a common chiral binaphthyl precursor that can be generated readily in multigram batches. The routes to these new compounds are discussed with respect to chemical efficiency and stereochemistry. Among these new compounds, we identified several with longer wavelengths of absorption, potentially leading to superior photosensitizers. We also report IC_{50} values for all of the analogues against protein kinase C (PKC) establishing which elements are the most

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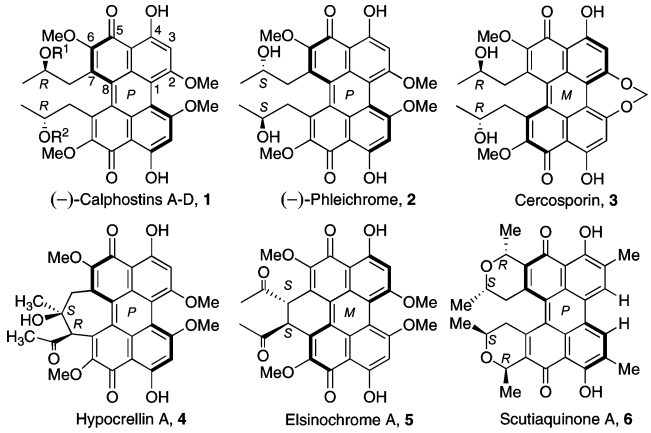

Figure 1. Perylenequinone natural products.

crucial to inhibition of the regulatory domain. Finally, we report CC_{50} values for selected analogues against cancer cell lines.

Background. The perylenequinone family of natural products (Figure 1) is characterized by a helical chiral extended oxidized pentacyclic core combined with C7,C7′-substitution containing centrochiral stereocenters.[6] The perylenequinone portion confers several novel features to these compounds including tautomeric forms that rapidly interconvert, low barriers to atropisomerization, and low barriers to photoexcitation.

Calphostin D, (−)-**1d**, and phleichrome, (−)-**2** are isolates of the *Cladosporium* fungi *Cladosporium cladosporioides* and *Cladosporium phlei*, respectively.[7,8] Cercosporin, **3**, was first isolated by Kuyama and Tamura from *Cercospora kikuchii*,[9] a fungus responsible for the "purple speck disease" of soy beans as well as damage to a host of plant species worldwide.[10] The structures were elucidated through a series of chemical transformations and subsequent spectroscopic analysis.[7,8,11,12] The X-ray structure of **3** was used to assign the absolute and relative configuration.[5] Correlation of the CD and NMR spectra allowed determination of the helical configurations and relative stereochemistries of the remaining natural products. Related natural products, hypocrellin (*ent*-**4**),[13] hypocrellin A (**4**),[14] elsinochromes (**5**),[15] and scutiaquinone (**6**)[16] have also been isolated from several different fungi.

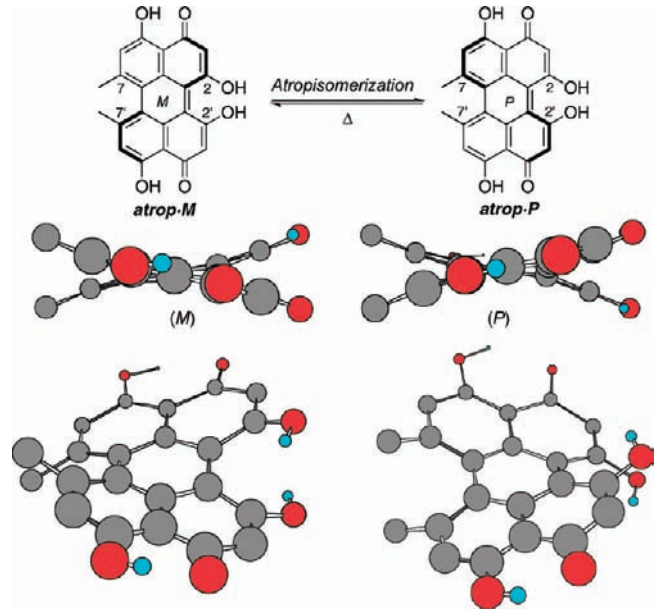

Figure 2. Perylenequinone 3-D helical chirality and atropisomerization.

From a synthetic perspective, **1**−**3** contain the same stereochemical elements: helical chirality and stereogenic C7,C7′-2-hydroxypropyls. Beyond these elements, the perylenequinones exhibit intriguing architectural aspects, namely keto−enol tautomerism[17,18] and potential atropisomerization of the helical perylene core (Figure 2). The barrier to atropisomerization of the helical configuration, which entails rotation of the C2,C2′- and C7,C7′-groups past one another (Figure 2), varies substantially for the compounds in this series. Whereas the calphostins and phleichrome are atropisomerically stable, the additional seven-membered ring in cercosporin lowers the barrier, making it a particularly challenging synthetic target (see Table 1 and discussion below).[5,19] On the other hand, hypocrellin and hypocrellin A atropisomerize rapidly at ambient temperature presenting two sets of sharp peaks in the NMR spectrum.[18]

The perylenequinones exhibit light-induced activity in biological systems making them photodynamic therapeutic candidates.[20] Photodynamic therapy is a treatment that uses a small molecule, called a photosensitizer or a photosensitizing agent, oxygen, and a light source.[21] After the photosensitizers are delivered to target cells/tissues, they are exposed to a specific wavelength of light forming excited states. In turn, these species transfer the energy to molecular oxygen to produce singlet

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oxygen as well as other reactive oxygen species that kill cells. Generation of photosensitizers that can be activated by longer wavelength light, which can penetrate deeper into tissue, is crucial. The nine photosensitizer compounds (none perylenequinones) currently in clinical use as photoactivated anticancer agents encompass three related structural classes: porphyrins, chlorins, and phthalocyanins.[21] While optimization of current photosensitizers is warranted, the multiple criteria of desirable photosensitizers makes discovery of new classes critical to furthering the practice of photodynamic therapy.

The perylenequinone core is a potent chromophore that permits the use of these compounds as photosensitizers. Upon exposure to light, perylenequinones initiate the generation of reactive oxygen species with high quantum efficiency.[20] Furthermore, these compounds selectively bind the C1 regulatory domain of protein kinase C (PKC),[22,23] some forms of which are upregulated in cancer cells and associated with growth.[24] For these reasons, the perylenequinones are candidates for photodynamic therapy of cancer;[20] light-induced activity has been seen with the natural products against selected tumor cell lines.[25] In addition, they have displayed antiviral[26] and immunotherapeutic[27] properties. The development of new classes of PDT agents of defined molecular structure, with lower aggregation tendency, longer absorption wavelengths, high quantum yields, greater stability, and greater selectivity against cancer cells is highly desirable.

The perylenequinones are thought to cause cell apoptosis by two pathways: (1) light-induced production of singlet oxygen and (2) PKC inhibition. Considerable attention has been devoted to the development of potent and specific PKC inhibitors.[24] The catalytic domain-acting PKC inhibitors, such as staurosporin (PKC, IC_{50} = 9 nM) and other indolocarbazoles, are highly potent but not specific for PKC, since they also inhibit other protein kinases.[28] Since the discovery of staurosporin, the structurally related catalytic site inhibitors ruboxistaurin (PKCβI, IC_{50} = 4.7 nM; PKCβII, IC_{50} = 5.9 nM)[29] and enzastaurin (PKCβI, IC_{50} = 30 nM; PKCβII, IC_{50} = 30 nM)[30] have been found to be more selective for the PKCβ isozyme relative to other PKC isozymes and kinases leading to important new diabetes treatments.[31] Significantly, the perylenequinones have demonstrated to be both potent and specific for PKC [Calphostin C (**1c**): PKC, IC_{50} = 0.05−0.46 μM; PKA, IC_{50} = >100 μM; PPK, IC_{50} = >100 μM; Cercosporin (**3**): PKC, IC_{50} = 0.6−1.3 μM; PKA, IC_{50} = >500 μM; PPK, IC_{50} = >180 μM] most likely because they act on a regulatory domain which is unique to PKC.[22,23] To date, evaluation of the perylenequinones as PKC inhibitors has been confined to natural product derivatives or simple analogues due to limitations of available synthetic methods.[20,23,32,33] Novel perylenequinones will allow determination of the structural features necessary for PKC inhibition as well as generation of structures with improved photodynamic properties.

Results and Discussion

Retrosynthetic Considerations. We desired a synthetic strategy to permit a flexible approach to all of the perylenequinone natural products **1**−**6** (Figure 1) as well as several derivatives. Examination of prior approaches to the calphostins[4] revealed a common theme in that the stereochemistry of the C7-subsitution

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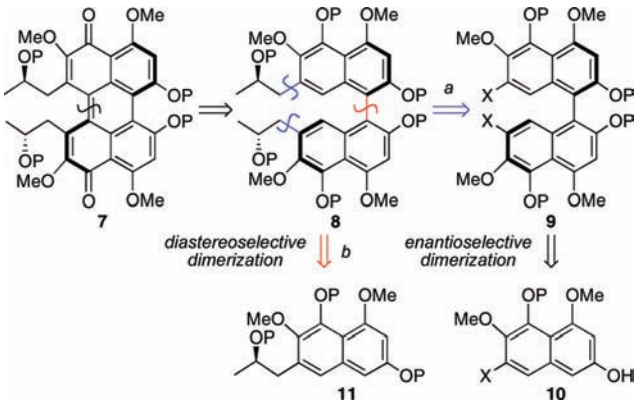
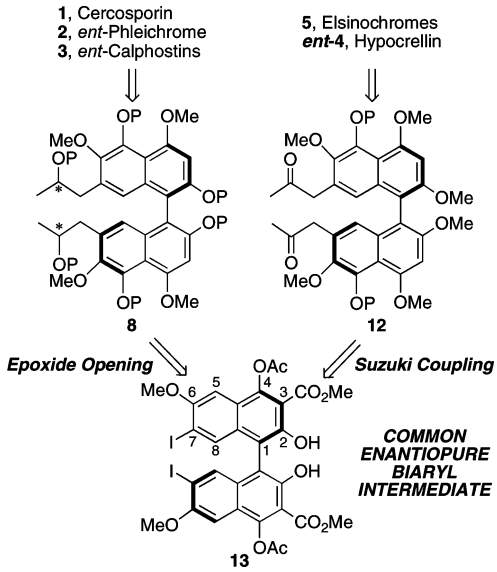
Scheme 1. Retrosynthetic Analysis of the Calphostins



Scheme 2. Common Intermediate to the Perylenequinone Natural Products



was generated first and then diastereoselective dimerizations were undertaken (Scheme 1, path *b*). The moderate diastereoselection (1:1−1:8) afforded by the newly formed C1−C1′ biaryl bond presumably arises from the distance between this center and the C7,C7′-substituents. Furthermore, the predominant diastereomer did not usually correspond to the calphostin array although it does match that needed for phleichrome.

In devising a plan to selectively synthesize all of the possible stereoisomers in pure form, we elected to pursue a different strategy that mimics the likely biosynthesis.[11c,34] By establishing the axial stereochemistry first (path *a* in Scheme 1), the corresponding helical stereochemistry can be generated with complete stereocontrol. The helical stereochemistry can in turn be utilized to control the C7,C7′-stereochemistry with good fidelity as demonstrated in our synthesis of hypocrellin A (Scheme 2).[3] Notably, a synthesis of hypocrellin relying on path *b* in Scheme 1 would require oxidation of the initially formed alcohols resulting in loss of this stereochemical information.

Alternately, the C7,C7′-stereochemistry can be introduced from an external source, a gambit that permits selective synthesis

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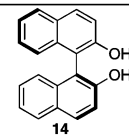
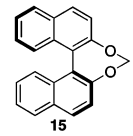
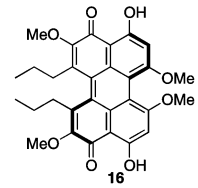
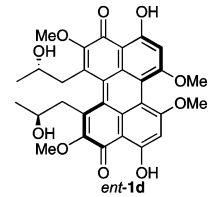
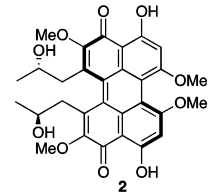
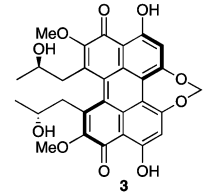
Table 1. Representative Atropisomerization Barriers and Half Lives

Entry		$\Delta G^{\ddagger}$ (kcal/mol)[a]	T (°C)	$t_{1/2}$
1		37.2[35] (naphthalene)	25	5,500,000 y
			60	6,850 y
			90	65.7 y
			180	16 h
			195	270 min
2		33[36] (decalin)[b]	25	10,000 y
			60	13.7 y
			90	80 d
			180	10 min
			195	3 min
3		28.3 (benzene)	25	2 y
			60	4 d
			90	2.5 h
			180	3.2 s
4		30.2 (benzene)	25	51 y
			60	75 d
			90	38 h
			180	27 s
5		30.2[19] (DMSO)	25	51 y
			60	75 d
			90	38 h
			180	27 s
6		28.2 (28.1[19]) (DMSO)	25	2 y
			60	4 d
			90	2.5 h
			180	3 s
		27.4 (benzene)	25	180 d
			60	25 h
			90	50 min
			180	1.2 s
		27.0[5] (acetylacetone)		

[a] Values are given at 65 °C except for entries 1 and 2 for which temperatures could not be located. [b] E_a value.

of all the possible stereoisomers of calphostin D, phleichrome, and cercosporin. We selected an epoxide opening reaction to achieve this goal (Scheme 2). The net result is that all of the target structures (**1**−**5**) devolve onto a common synthetic intermediate, chiral biaryl **13**, or its enantiomer, *ent*-**13** (Scheme 2). Furthermore, bisiodide **13** provides a potential avenue for the synthesis of diverse C7,C7′-analogues by coupling using Heck, Suzuki, and related methods.

In applying such an approach to cercosporin, the sequencing of the transformations is critical due to the relatively low atropisomerization barrier. From measurements that we performed, simple perylenequinones such as **16** enjoy only a moderate degree of atropisomeric stability. Since the C2,C2′,C7,C7′-groups cannot be coplanar due to steric interactions, a helical twist is imposed on the central perylenequinone unit (Figure

2). The strain results in a barrier to atropisomerization for **16** of 28.3 kcal/mol (Table 1, entry 3), substantially lower than that seen with BINOL (**14**) (37.1 kcal/mol, Table 1, entry 1).[35] Larger C7,C7′-substituents would be expected to sterically hinder atropisomerization and to confer a higher degree of atropisomeric stability to the calphostins and phleichromes. Thus, we measured the atropisomerization rate of *ent*-**1d**, which has the same (*M*)-helicity as **16**, in the same solvent (benzene), and as expected, a higher barrier of 30.2 kcal/mol (Table 1, entry 4) was found relative to **16**. As a result, the atropisomerization barriers for *ent*-calphostin D (*ent*-**1d**) and phleichrome (**2**) allow retention of the helical stereochemistry up to 60 °C (Table 1, entries 4−5).[19] The addition of the methylidene bridge to the perylenequinones as seen in cercosporin (**3**) is expected to impose additional strain and lower the atropisomerization barrier further. For example, the addition of a similar bridge to BINOL (**14**) to provide **15** reduces this barrier from 37.2[35] to 33[36] kcal/mol (Table 1, entries 1−2).[37]

Reports in the literature for cercosporin indicated that rapid atropisomerization occurs in boiling ether (37 °C),[5] whereas a slower isomerization was observed in DMSO ($t_{1/2}$ = 2 d at 60 °C).[19] To determine if there is a solvent dependence, we measured the atropisomerization rate in DMSO and benzene at 65 °C. Indeed, the rate was substantially faster in benzene corresponding to a barrier of 27.4 kcal/mol vs 28.2 kcal/mol in DMSO (Table 1, entry 6). These experiments revealed that temperatures at 60 °C or higher would result in unacceptable levels of atropisomerization. From a synthetic standpoint, these results mean that the combination of the perylenequinone and the methylidene bridge must be reserved to as late as possible. Furthermore, all subsequent steps must avoid even moderate temperatures in both the reaction media and purification.

Two distinct approaches fulfill the above requirements. In the first (bottom of Scheme 3), the perylenequinone (i.e., **7**) is constructed prior to formation of the methylidene bridge in **3**. For this strategy to succeed, the conditions for installation of the methylidene bridge need to be relatively mild since the product **3** can undergo significant atropisomerization at 60 °C (Table 1, entry 6). Since model studies indicated temperatures of 65 °C were needed to form the methylidene bridge, we turned to the reverse sequence (top of Scheme 3). Here the methylidene bridge would be installed early (i.e., **19**), also obviating the need for three sets of orthogonal protecting groups in intermediate **8** (bottom of Scheme 3). For this strategy to succeed, the bridged binaphthyl compound needs to be configurationally stable, which is supported by the results from **15** (Table 1, entry 2) which can readily withstand temperatures up to 90 °C. Furthermore, the reaction conditions for formation of the perylenequinone (**17** to **3**) are very mild (room temperature and lower),[3,4c,d] which should prevent any atropisomerization of the sensitive **3** (Table 1, entry 6).

With the general strategy defined, the retrosynthesis evolved to that in the top of Scheme 3. Specifically, helical chiral **3** would arise from axial chiral **17** by means of low temperature reactions to suppress the ready atropisomerization of **3** (Figure 2). Retrosynthetic installation of the C3,C3′-esters in **18** provides a means of protecting the C3,C3′-positions for the next operation and provides functionality needed for highly selective naphthol coupling and rapid naphthalene synthesis. Deoxygenation to **19** and subsequent removal of the methylidene bridge furnishes **20** with a substitution pattern readily generated via biaryl coupling.[2,38] The epoxide opening disconnection was applied at this stage to yield an even simpler biaryl **21**. This stratagem reserved diversification of the synthetic sequence to the latest stage possible providing access to the calphostins, phleichrome, and cercosporin. However, the retron to **21** requires an unprecedented epoxide opening; namely, the combination of a complex biscuprate with the requirement for two epoxide alkylations. Biaryl **21**, in turn, arises from C2,C2′,C4,C4′-protection of **13** (Scheme 2). Thus, common intermediate **13**, available from our previous studies,[3] could be used for the construction of all the perylenequinone natural products reducing the total number of steps that would need to be optimized. Due to the ability to independently generate the two stereochemical elements, the 2-hydroxypropyl group and the helical chirality, this synthetic approach would permit the syntheses of the complete diastereomeric series of **1**, **2**, and **3**, including compounds not isolated from natural sources. In addition, novel perylenequinone structures would be easily accessible from advanced intermediates.

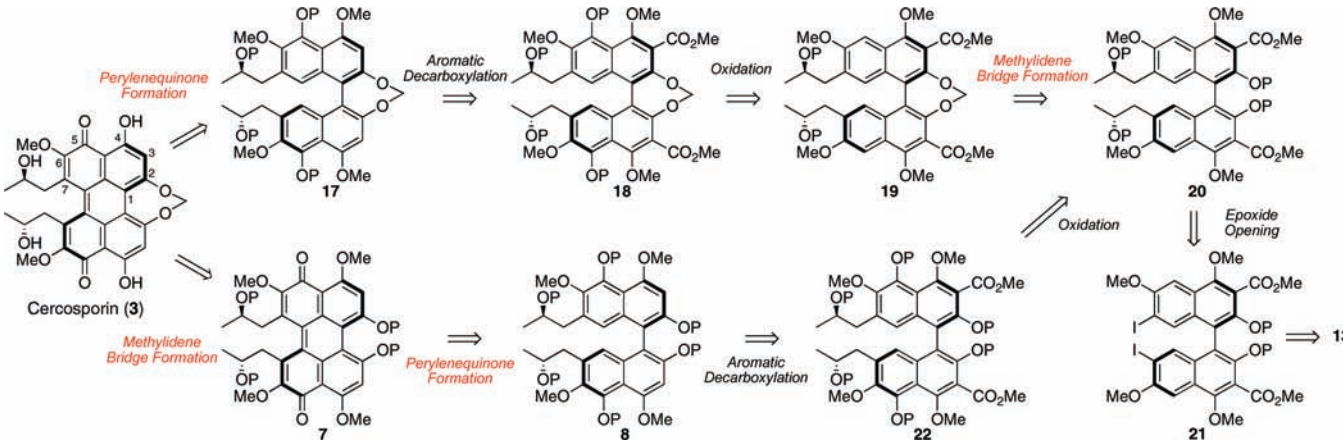
Stereoselective Generation of the C7,C7′ 2-Hydroxypropyls. In prior syntheses of the calphostins and phleichrome, the formation of the C7,C7′-stereochemical array has been a central element. In all cases, incorporation of this element was intimately coupled to formation of the naphthalene skeleton prior to dimerization. Our strategy (Scheme 2) reserves introduction of these substituents until after naphthalene formation and dimerization, a feature which greatly streamlines production of the naphthalene portion and allows for maximum diversity in the synthesis of the natural products and analogues.

To install the stereogenic 2-hydroxypropyl groups we investigated three routes: (1) diastereoselective reduction of diketone **24**; (2) asymmetric methyl-addition to dialdehyde **25**; (3) organocopper epoxide opening with **21** (Scheme 4). Unfortunately, both reduction of the diketone and methyl addition to the dialdehyde proved to be unproductive, proceeding with low yield or diastereoselectivity. Apparently, the biaryl axis is too distant to exercise a high degree of stereocontrol over the additions to the carbonyls of the C7,C7′-substitutents, an outcome reminiscent of the poor stereocontrol observed in the opposite direction (path *b*, Scheme 1). The last pathway, the organocopper epoxide openings (Scheme 4) was particularly attractive in that it allowed access to all of the diastereomers with full stereocontrol without having to rely on the restrictions of relative diastereoselection. Furthermore, such a pathway permits greater flexibility in analogue synthesis. For example, other epoxides or aziridines could lead to a host of analogues not available from the natural products. However, the unprecedented double alkylation of a highly complex biscuprate with two epoxides made this venture risky.

The use of Grignard-derived cuprates in epoxide alkylation has enjoyed a rich history.[39] While complex cuprates have been used successfully[40] and simple biscuprates have been employed

(35) Meka, L.; Reha, D.; Havlas, Z. *J. Org. Chem.* **2003**, *68*, 5677–5680.
(36) (a) Yi, R.; Hoz, S. *J. Phys. Org. Chem.* **2002**, *15*, 782–786. (b) Park, J.-W.; Ediger, M. D.; Green, M. M. *J. Am. Chem. Soc.* **2001**, *123*, 49–56.
(37) Related compounds with the same dioxapine ring are atropisomerically stable up to 85−120 °C but are not stable at 220 °C. Papers on the stability of seven-membered bridged biaryls: (a) Mislow, K.; Hyden, S.; Schaefer, H. *J. Am. Chem. Soc.* **1962**, *84*, 1449–1455. (b) Kurland, R. J.; Rubin, M. B.; Wise, W. B. *J. Phys. Chem.* **1964**, 2426–2427. (c) Zhang, M.; Schuster, G. B. *J. Phys. Chem.* **1992**, *96*, 3063–3067.
(38) Kozlowski, M. C.; Dugan, E. C.; DiVirgilio, E. S.; Maksimenka, K.; Bringmann, G. *Adv. Synth. Catal.* **2007**, *349*, 583–594.

Scheme 3. Retrosynthesis of Cercosporin



in epoxide alkylation,[41,42] we could locate no reports of a highly functionalized dianion effecting two ring openings. Our primary concerns were (1) the metal−halogen exchange on an electron-rich system and in the presence of the C3-methyl esters; (2) the stability of the electron-rich bisarylcuprate, and (3) the requirement that the biscuprate be the limiting reagent rather than in excess as is typical.

To fully evaluate the proposed epoxide-opening reaction, several naphthalenes were investigated revealing that a C7-iodide permitted the best coupling and that robust alkyl protecting groups needed to be employed instead of acetates. Hindered bases (*t*-BuLi, *i*-PrMgBr) were found to attenuate addition to the C3-methyl ester. Encouragingly, the initial epoxide opening with **26** yielded modest amounts of the desired product (40%) with complete regioselectivity (Table 2). Rigorously maintained air- and water-free conditions drastically decreased the amount of protodemetalation, improving the yield of **27** to 77% (entry 1a). An examination of the cuprate addition revealed that additives such as TMSCl or HMPA or lower temperatures (−78 °C) provided no improvement. Upon screening the copper reagent and solvents (Table 2, entries 1a−d), CuI and THF were found to be optimal. Careful purification of the CuI by recrystallization proved to be the most critical element providing product **27** in 87% yield (Table 2, entry 1e).

Due to the requirement for a C4-acetate during biaryl coupling[2] (see below) which is not tolerated in the epoxide opening, the epoxide-opening reaction was reserved until after biaryl coupling to reduce the number of protecting group steps. While the task of forming a biscuprate and undertaking two epoxide alkylations on such a complex dianion was daunting, the opportunity for convergence as late as possible in the synthesis was appealing allowing analogues to be produced from one late intermediate. To examine epoxide opening as late as possible, **28**, in which the oxidation of the C5,C5′-positions had already been accomplished, was utilized (Table 2, entry 2). An arene with acetate groups intact was the major product of this reaction arising from protodemetalation, which indicates that either cuprate formation or epoxide opening was sluggish. Presumably, steric gearing from the C5,C5′-positions hinders the C7,C7′-Grignards slowing subsequent reactions at these positions. Moving the epoxide alkylation prior to C5,C5′-oxidation but after formation of the methylidene bridge was examined next (Table 2, entry 3). While the desired product **31** did form in 40% yield, the methylidene bridge caused the C3,C3′-esters to be less hindered resulting in a competing halodecarboxylation. To alleviate this problem, the double epoxide alkylation was investigated with different C2,C2′-substitution (Table 2, entry 4). The C2,C2′-bismethoxy, bisbenzyloxy, and bisisopropoxy analogues all reacted well providing the corresponding products with two new stereocenters in 65−75% yield, corresponding to the 81−87% yield for each of the two individual coupling reactions. Furthermore, reaction of the same *M*-chiral biaryl **32a** with the enantiomeric epoxide provided **34**, the diastereomer of **33a** in 74% yield (Table 2, entry 5). While different rates might be expected via matched and mismatched reactions of the enantiomeric epoxides (*R*)- and (*S*)-propylene oxide with (*M*)-**32a**, no difference in the reaction rate was seen here. Apparently, the stereochemistry of the biaryl is distant from the reacting centers and exerts little control, in line with previous observations (see Scheme 4). Together, these results validated the use of the epoxide dialkylation as a key bond construction providing a highly flexible synthetic route. For example, biaryl **32** can be used to furnish **33a** corresponding to *ent*-phleichrome (*ent*-**2**) and **34** corresponding to *ent*-calphostin D (*ent*-**1d**) as well as **33b** and **33c** which can either provide analogues with different C2,C2′-subsititution or serve as precursors toward cercosporin (**3**).

Total Synthesis of (+)-Phleichrome and (+)-Calphostin D. Our total synthesis efforts commenced with the simpler perylenequinone compounds lacking the methylidene bridge. As outlined in Scheme 5, the bisiodide **13** was generated in a straightforward manner using the protocol that we reported for its enantiomer.[3] Thus, the *M*-biaryl was produced in good yield and enantioselectivity (81% ee) utilizing the (*S*,*S*)-diaza-*cis*-decalin copper catalyst.[1] The high crystallinity of bisiodide **13**

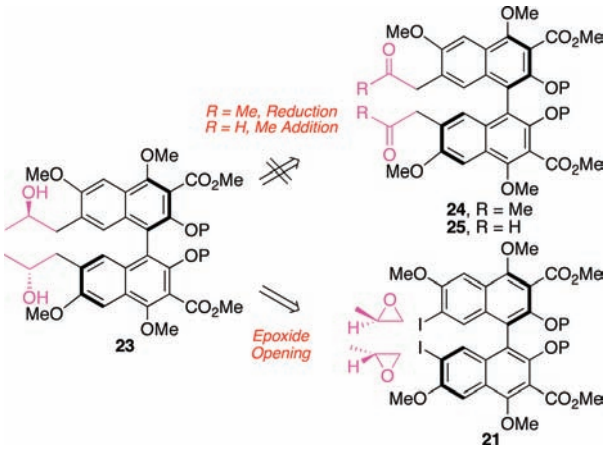
(39) (a) Lipshutz, B. H.; Kozlowski, J.; Wilhelm, R. S. *J. Am. Chem. Soc.* **1982**, *104*, 2305–2307. (b) Smith, J. G. *Synthesis* **1984**, 629–656. (c) Sheperd, T.; Aikins, J.; Bleakman, D.; Cantrell, B.; Rearick, J.; Simon, R.; Smith, E.; Stephenson, G.; Zimmerman, D. *J. Med. Chem.* **2002**, *45*, 2101–2111. (d) Taber, D. F.; He, Y. *J. Org. Chem.* **2005**, *70*, 7711–7714. (e) Nakamura, E.; Mori, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 3751–3771. (f) Review of organocopper reagents: Lipshutz, B. H.; Sengupta, S. *Org. React.* **1992**, *41*, 135.

(40) (a) Ireland, R. E.; Gleason, J. L.; Gegnas, L. D.; Highsmith, T. K. *J. Org. Chem.* **1996**, *61*, 6856–6872. (b) Ireland, R. E.; Liu, L.; Roper, T. D. *Tetrahedron* **1997**, *53*, 13221–13256.

(41) Cahill, S.; Evans, L. A.; O'Brien, M. *Tetrahedron Lett.* **2007**, *48*, 5683–5686.

(42) For simple bis-Grignard and bis-lithium reagents in epoxide opening: (a) Bianchetti, G. *Farmaco, Ed. Sci.* **1957**, *12*, 441–448. (b) Ghorai, P.; Dussault, P. H.; Hu, C. *Org. Lett.* **2008**, *10*, 2401–2404. (c) Gordon, B., III.; Blumenthal, M.; Mera, A. E.; Kumpf, R. J. *J. Org. Chem.* **1985**, *50* (9), 1540–1542.

Scheme 4. Pathways to Installation of the C7,C7′-Stereoarray

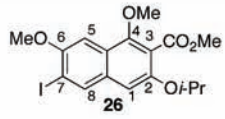
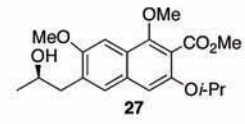
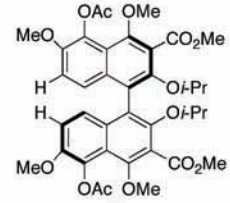
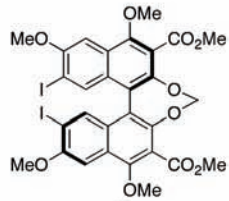
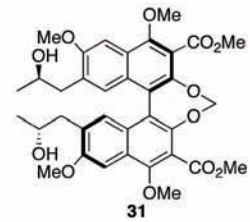
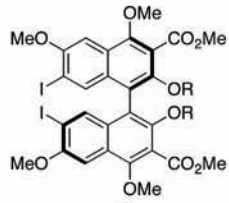
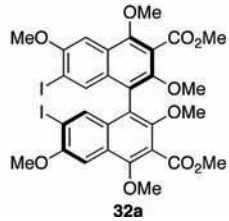
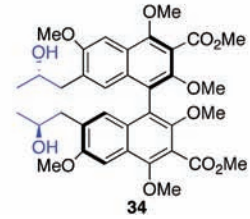


provided a simple means of enantioenrichment with one trituration giving >99% ee material and excellent mass recovery.

The C3-ester is integral to the synthesis of the naphthalene and also provides a necessary coordination site for the biaryl coupling catalyst. Large quantities of common intermediate **13** could be generated, which was used in the synthesis of three natural products and 10 analogues.

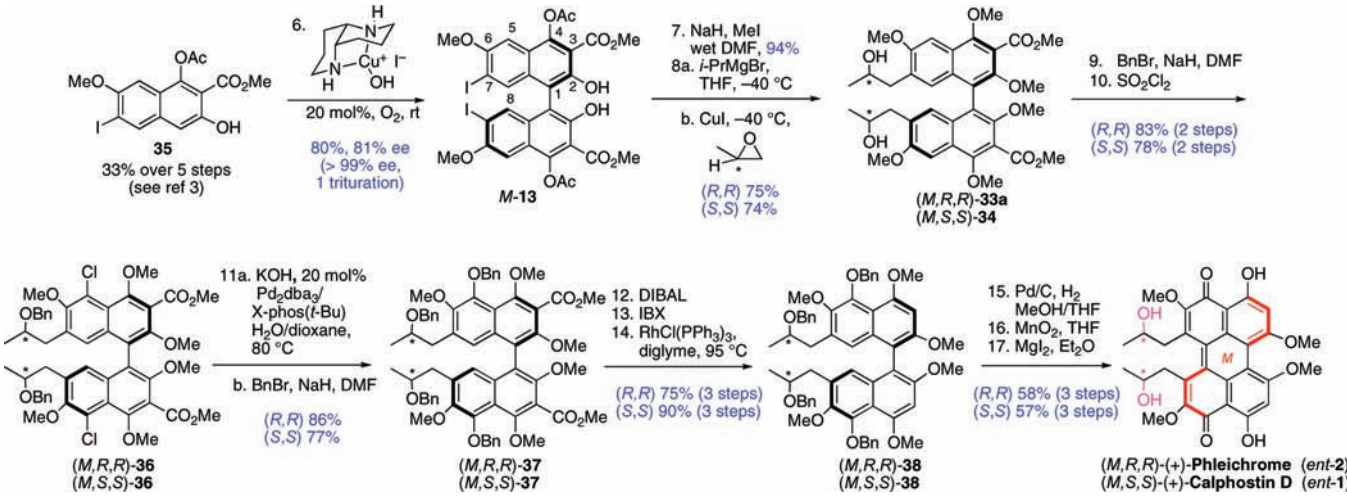
The C2,C2′,C4,C4′-phenols of **13** were methylated by means of excess NaH in undistilled DMF with excess MeI (Scheme 5). The small amount of water in DMF was converted to NaOH, which cleaved the acetates. Subsequently, MeI alkylated all four positions with high efficiency (94% yield). At this stage, formation of the biscuprate and double epoxide alkylation were undertaken following the method described above (see Table 2 and accompanying discussion). Both (*R*)- and (*S*)-propylene oxide were employed to provide the diastereomers (*M*,*R*,*R*)-**33a** and (*M*,*S*,*S*)-**34** in good yield. After benzylation of the newly formed alcohols, the C5,C5′-oxidation was undertaken. We found that our previously reported protocol[3] for this transformation using Kita's method [($PhI(OCOCF_3)_2$, $(CF_3)_2CHOH$,

Table 2. Screening Substrates and Conditions for Epoxide Opening

Entry	Substrate	Conditions[a]	Product	Yield
1	 **26**	a. *i*-PrMgBr, THF, -40 °C; CuI,  b. *i*-PrMgBr, Et_2O, -40 °C; CuI,  c. *i*-PrMgBr, THF, -40 °C; CuCN,  d. *i*-PrMgBr, THF, -40 °C; CuBr,  e. *i*-PrMgBr, THF, -40 °C; CuI (recryst), 	 **27**	a. 77%[b] b. 77%[b] c. 50%[b] d. 71%[b] e. 87%[b] (85%)[c]
2	 **28**	*i*-PrMgBr, THF, -40 °C; CuI (recryst), 	 **29**	ND
3	 **30**	*i*-PrMgBr, THF, -40 °C; CuI (recryst), 	 **31**	40%[d]
4	 **32**	*i*-PrMgBr, THF, CuI (recryst),  a. –78 °C b. –78 °C c. –40 °C	 **33**	a. R = Me, 75% b. R = Bn, 65% c. R = *i*-Pr, 65%
5	 **32a**	*i*-PrMgBr, THF, -78 °C; CuI (recryst), 	 **34**	74%

[a] 2.5 equiv of *i*-PrMgBr; 1 equiv of Cux; epoxide source for entries 1−4 is (*R*)-propylene oxide and for entry 5 is (*S*)-propylene oxide. [b] Conversion. [c] Isolated yield in parentheses. [d] Inseparable byproducts.

Scheme 5. Total Synthesis of (+)-Phleichrome and (+)-Calphostin D



NaOAc][43] was very sensitive to any changes in the oxidation potential of the substrate requiring reoptimization for each new precursor. As a result, we devised a more robust C5,C5′-oxidation utilizing a palladium-catalyzed *O*-arylation. While these transformations have been reported for a host of systems,[44] we aimed to determine their applicability to highly functionalized, hindered, electron-rich systems. Thus, chlorination was effected with high efficiency (>90% yield) using sulfuryl chloride to provide (*M*,*R*,*R*)-**36** and (*M*,*S*,*S*)-**36**. Here, the C3,C3′-esters again proved critical; while not present in the natural products, they serve to protect the C3,C3′-positions from halogenation. Disappointingly, direct *O*-arylation of the bischloride with benzyl alcohol and other alcohols failed, likely due to the hindered nature of the C5,C5′-position. Gratifyingly, hydroxylation using KOH and a palladium catalyst[45] proceeded extremely well and was found to be remarkably general for a range of substrates. While the product bisphenol was not stable, immediate protection with benzyl bromide furnished (*M*,*R*,*R*)-**37** and (*M*,*S*,*S*)-**37**. A control reaction using KOH without palladium catalyst did not produce any of the bisphenol indicating that an addition/elimination pathway of the conjugate ester was not responsible for the results.

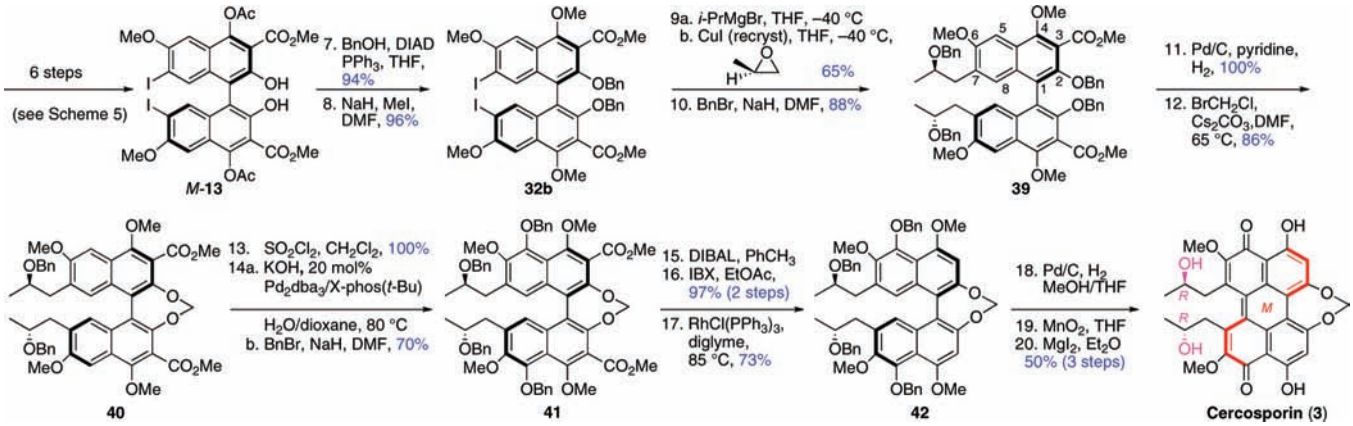
The next task was removal of the C3,C3′-esters which were immune to aqueous basic hydrolysis even at elevated temperatures, due to their steric hindrance. We discovered that an S_N2 displacement of the methyl esters could be accomplished with NaCN in DMSO to provide the bisacid.[3] However, conventional aromatic decarboxylation protocols[46] required temperatures that would atropisomerize the biaryl. As a consequence we developed a palladium-catalyzed decarboxylation protocol,[47] which worked well in these systems.[3] Unfortunately, we discovered a competing C−H insertion reaction occurred with the methylidene bridge series en route to cercosporin (see below). Thus, we undertook development of a new strategy with **37**. Reexamination of other decarboxylation methods[48] yielded no success so our attention turned to decarbonylation.[49] Formation of the requisite dialdehyde proved to be more efficient by overreduction using DIBAL and then oxidation with *ortho*-iodoxybenzoic acid (IBX) (Scheme 5). Decarbonylation with 2 equiv of Wilkinson's catalyst to (*M*,*R*,*R*)-**38** and (*M*,*S*,*S*)-**38** then proceeded smoothly provided that rigorously oxygen-free conditions were employed.

The remaining steps of the synthesis could all be conducted under very mild conditions. Removal of the four benzyl ethers occurred readily and was followed by oxidative cyclization with MnO_2 to reveal the central perylenequinone core.[4c,d] Directed deprotection of the C4,C4′-methyl ethers with MgI_2[4b,e,f] furnished the enantiomers of the natural products phleichrome (*ent*-**2**) and calphostin D (*ent*-**1d**), respectively (Scheme 5). Each synthesis proceeded in a total of 17 steps with *ent*-**2** being formed in an overall yield of 5.3% (average of 87% per step) and *ent*-**1d** being formed in an overall yield of 5.2% (average of 87% per step). With the enantiomers of the natural products available, it was now possible to assess the impact of the helical and centrochiral centers on the biological activity.

First Total Synthesis of Cercosporin. Starting from common intermediate **13** (see Scheme 5), orthogonal C2,C2′-benzyl ethers were installed via a Mitsunobu reaction. A subsequent one-pot deacylation/methylation of the C4,C4′-positions afforded **32b** with high yield and scalability (Scheme 6). The double alkylation of the biscuprate of **32b** with (*R*)-propylene oxide furnished **33b** in high yield as a single diastereomer. The newly formed 2-hydroxypropyl groups were then masked as benzyl ethers furnishing **39**. Subsequent chemoselective debenzylation of the more labile C2,C2′-benzyl ethers of **39** was efficiently accomplished using Pd/C poisoned with pyridine. Installation

(43) Kita, Y.; Tohma, H.; Hatanaka, K.; Takada, T.; Fujita, S.; Mitoh, S.; Sajurai, H.; Oka, S. *J. Am. Chem. Soc.* **1994**, *116*, 3684–3691.
(44) Reviews: (a) Muci, A. R.; Buchwald, S. L. *Top. Curr. Chem.* **2002**, *219*, 131–209. (b) Hartwig, J. F. *Nature* **2008**, *455*, 314–322. (c) Carril, M.; SanMartin, R.; Dominguez, E. *Chem. Soc. Rev.* **2008**, *37*, 639–647.
(45) (a) Torraca, K. E.; Huang, X.; Parrish, C. A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 10770–10771. (b) Vorogushi, A. V.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2005**, *127*, 8146–8149. (c) Anderson, K. W.; Ikawa, T.; Tundel, R. E.; Buchwald, S. L. *J. Am. Chem. Soc.* **2006**, *128*, 10694–10695.
(46) (a) Olah, G. A.; Laali, K.; Mehrotra, A. K. *J. Org. Chem.* **1983**, *48*, 3360–3362. (b) Cohen, T.; Schambach, R. A. *J. Am. Chem. Soc.* **1970**, *92*, 3189–3190. (c) Barton, D. H. R.; Lacher, B.; Zard, S. Z. *Tetrahedron Lett.* **1985**, *26*, 5939–5942.
(47) Dickstein, J. S.; Mulrooney, C. A.; O'Brien, E. M.; Morgan, B. J.; Kozlowski, M. C. *Org. Lett.* **2007**, *9*, 2441–2444.
(48) (a) Garner, P.; Anderson, J. T.; Dey, S. *J. Org. Chem.* **1998**, *63*, 5732–5733. (b) Chimiak, A.; Przychodzen, W.; Rachon, J. *Heteroatom. Chem.* **2002**, *13*, 169–194.
(49) (a) Ohno, K.; Tsuji, J. *J. Am. Chem. Soc.* **1968**, *90*, 99–107. (b) Abu-Hasanayn, F.; Coldman, M. E.; Goldman, A. S. *J. Am. Chem. Soc.* **1992**, *114*, 2520. (c) Kreis, M.; Palmelund, A.; Bunch, L.; Madsena, R. *Adv. Synth. Catal.* **2006**, *348*, 2148–2154.

Scheme 6. First Total Synthesis of Cercosporin



of the methylene bridge was not trivial, presumably due to disfavorable entropy in forming the seven-membered ring combined with ring strain. Of all the potential methylidene equivalents, ring formation was most facile with $BrCH_2Cl$ vs $BrCH_2I$ or $BrCH_2Br$. Apparently, there is a balance between the leaving group ability of the second halide and its size with larger halides greatly decelerating the first alkylation event. Even so, formation of the seven-membered ring of **40** required heating to 65 °C, which was well below the atropisomerization threshold (see Table 1, entry 2).

As described above (see Scheme 5, **33a/34** to **37** and accompanying text), we elected to use a halogenation/*O*-arylation reaction to install the C5,C5′-oxygenation. The C5,C5′-chlorination proceeded uneventfully, but the chloro to alkoxy interchange was difficult. After optimization of the reaction conditions, we found that the catalyst system derived from Pd_2dba_3 and the X-phos(*t*-Bu) ligand proved to be effective in the coupling of **40** with KOH to provide the desired bisphenol. Unfortunately, the resulting product was highly unstable and could not withstand a one-pot alkylation protocol. Isolation of the bisphenol under carefully controlled conditions, however, proceeded well. Immediate exposure to standard benzylation procedures (BnBr, NaH, DMF) furnished key intermediate **41** in 70% yield.

As alluded to above (see Scheme 5, **37** to **38** and accompanying text), decarboxylation of **41** proved difficult. We had developed a palladium-catalyzed protodecarboxylation for this application which succeeded in related systems.[47] With **41**, however, this procedure failed due to a competing C−H insertion reaction of the methylidene in the seven-membered ring. Fortunately, decarbonylation of the corresponding dialdehyde with excess Wilkinson's catalyst proceeded well, provided that rigorously air-free conditions were employed. Furthermore, the relatively high temperatures (85 °C in diglyme) required for this process were well-tolerated in accord with our expectations (Table 1, entry 2) and no atropisomerization was observed.

After deprotection of **42**, the bisphenol was oxidized by MnO_2 to afford perylenequinone (Scheme 6).[4c,d] As discussed in the retrosynthetic analysis, this final cyclization step was held until the end of the synthesis because formation of the perylenequinone reduces the dihedral angle between the upper and lower portions from 50° in axial chiral **42** to 20° in helical chiral **3**, further facilitating atropisomerization. Directed deprotection of the C4,C4′-methyl ethers was accomplished selectively with MgI_2,[4b,e,f] completing the first total synthesis of cercosporin (**3**) in 20 steps and 2.8% overall yield (86% average yield per step). Notably, none of the diastereomer of **3** arising from the facile atropisomerization was observed in this sequence. The synthetic material possessed NMR and CD spectra identical to those from a sample of the natural product.

Design of Novel Perylenequinone Structures. The two perylenequinone apoptosis pathways allow for the therapeutic effects to be localized in cancer therapy two distinct ways: (1) via direction of a specific wavelength of light to the region of the tumor and (2) via sequestration in cells with high levels of PKC. Since most tumor cells appear to have multiple growth and survival mechanisms, the two apoptosis pathways and means of therapeutic localization render the perylenequinones especially promising targets. As a result, we targeted derivatives to both increase the photodynamic activity and also probe PKC binding. To increase the photoresponse in the photodynamic window (600−900 nm), the perylenequinone chromophore was extended through C3,C3′-substitution. Additionally, we designed and synthesized perylenequinones in a systematic manner to examine the effects of the helical chirality, C2,C2′-substitution, C3,C3′-substitution, and C7,C7′-substitution, which have thus far seemed to be the most integral to PKC inhibition.[22] Most perylenequinone analogues examined to date are derived from the natural product themselves;[23] generation of the above variants from the natural products is difficult or not feasible. The lack of information has limited the conclusions drawn between structure and PKC inhibition. On the other hand, our synthetic strategy allows rapid and convergent synthesis of a large number of novel analogues for biological and photophysical evaluation.

The versatility of our perylenequinone strategy (Scheme 2) enabled the syntheses of the stereoisomers of **1**−**4**, including the natural products themselves (Figure 3). Utilizing the enantioselective biaryl coupling we can access both enantiomers of the common intermediate (*P*-**13** and *M*-**13**). In addition, the copper-mediated epoxide openings are integral to this evaluation, allowing entry to the unnatural isomers of **1**, **2**, and **3**, which would not be possible if a stereochemistry transfer reaction was used in the construction of the stereogenic 2-hydroxypropyl groups. Thus, our approach permits a systematic assessment of the helical stereochemistry and the C7,C7′-stereochemistry on PKC inhibition.

Docking studies were performed to determine what structural modifications might lead to higher activity. Starting with the PKCδ C1 regulatory domain obtained from a cocrystal structure with a phorbol ester,[50] *ent*-phleichrome (*ent*-**2**), which contains the helical and centrochiral stereochemistries that led to the best binding (see below), was docked into the phorbol binding domain utilizing Autodock3.[51] The lowest

energy binding mode places the C2,C2′-methoxy groups into a hydrophobic pocket of the binding site. Since there was unfilled space in this pocket, we proposed to modify the C2,C2′-substitution to include the more hydrophobic isopropoxy and *n*-propoxy groups (**48a** and **48b**). Docking of these two analogues of (*ent*-**2**) indicated superior binding and is expected to lead to higher affinity.[52] As seen in Scheme 7, these novel perylenequinones could be synthesized by selective alkylation of an advanced intermediate (**44**) available from the cercosporin synthesis (step 11, Scheme 6). The remaining steps followed the protocol developed above for calphostin/phleichrome (Scheme 5) and cercosporin (Scheme 6) providing unnatural congeners **48a** and **48b** in a straightforward manner (Scheme 7). The yield for the last three steps was lower than those obtained above for cercosporin due to the larger C2,C2′-*n*-propoxy and -*iso*-propoxy groups which slow the perylenequinone forming reaction.

Whereas the docking studies described above indicated a possible interaction of the C2,C2′-substituents with a hydrophobic pocket in the regulatory domain site, the much higher affinity of calphostin A (Figure 1, **1a**, $R^1 = R^2 =$ COPh, $IC_{50} = 0.25\ \mu$M) compared to calphostin D (**1d**, $R^1 = R^2 =$ H, $IC_{50} = 6.4\ \mu$M) points to interaction of the C7,C7′-portion with the binding site.[22b] While it may be possible that the perylenequinone lies in a sufficiently deep pocket to undergo interactions with both the western and eastern portions of the structure, we expect that C2,C2′- and C7,C7′-analogues will be invaluable in elucidating the relative importance of each of these interactions. Our first step was to determine whether the stereogenic hydroxyl groups on the C7,C7′-substituents of the natural products were critical to binding. Thus, the simpler C7,C7′-propyl substitution was incorporated into analogue **16** (Scheme 8). Based upon the *improved* activity of this compound relative to the parent natural products (*ent*-**1d** and *ent*-**2**) with the C7,C7′-2-hydroxypropyl substitution (see below), a series of derivatives

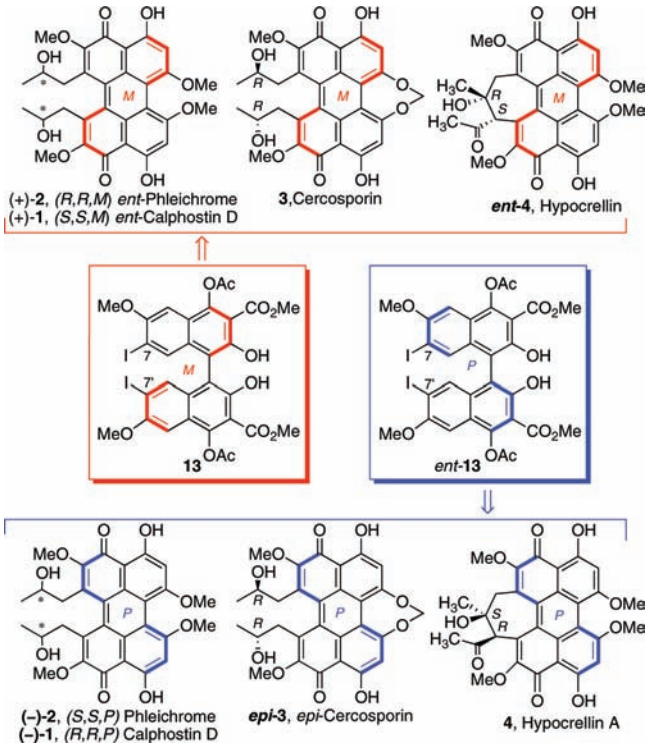
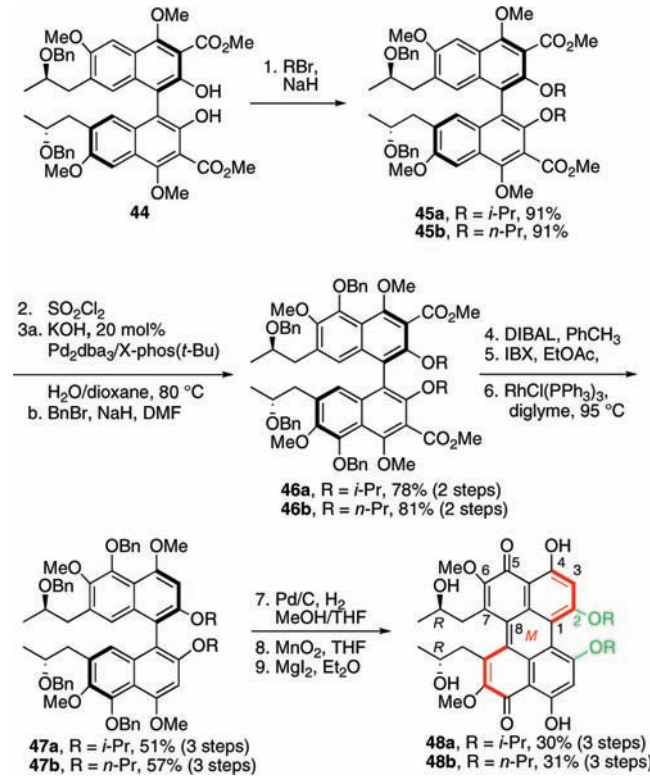


Figure 3. Common enantiopure intermediates to allow evaluation of helical chirality.

Scheme 7. Synthesis of C2,C2′-Analogues



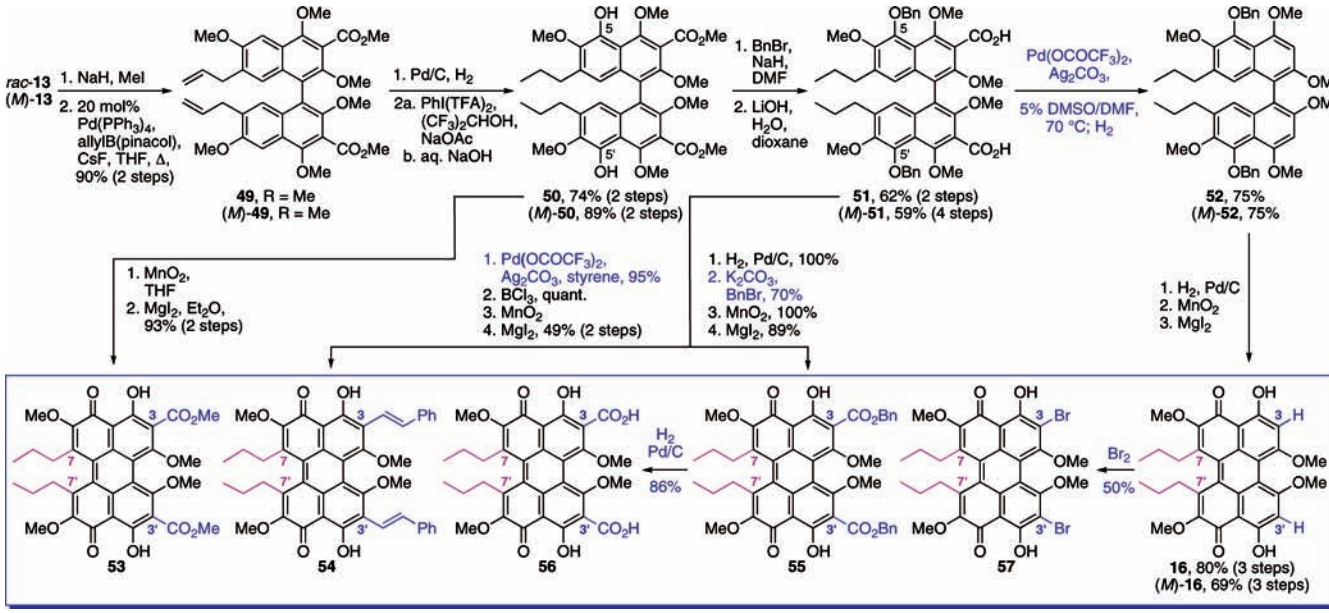
(**53**−**57**) incorporating the C7,C7′-propyl groups were designed to probe the effect of substitution at the C3,C3′-positions. Specifically, bromo (**57**), ester or acid (**53**, **55**, **56**), and vinyl (**54**) C3,C3′-substitutions were proposed to increase the absorption wavelength of the perylenequinone chromophore (Scheme 8). The use of the C3,C3′-methyl ester in our synthetic strategy proved very useful at this juncture allowing all of these structures to be quickly accessed either directly or by decarboxylative functionalization (Scheme 8).

The synthesis of **16** and **53**−**57** (Scheme 8) commenced with racemic bisiodide **13**, a byproduct of the trituration to provide *M*-**13** (see Scheme 5). Cross coupling with the iodides of **13** is a versatile and simple means to introduce any desirable C7,C7′-substitution. In this case, Suzuki coupling with pinacol allylboronate provided bisallyl **49**.[3] Subsequent hydrogenation yielded the bis-*n*-propyl compound. The lack of functionalization of the C7,C7′-groups allowed facile C5,C5′-hydroxylation with Kita's reagent[43] to provide **50** following our first generation protocol.[3]

Compound **50** represents the first branchpoint intermediate in the analogue syntheses; a small amount was subjected to MnO_2 followed by MgI_2 to effect oxidative cyclization[2] and deprotection furnishing analogue **53**. The remainder of branchpoint intermediate **50** was subjected to benzyl bromide and NaH to protect the C5,C5′-naphthols in preparation for ester hydrolysis to provide the next key branchpoint intermediate, biascid **51**. Ester hydrolysis here was surprisingly facile relative to congeners **37** (Scheme 5) and **41** (Scheme 6). Presumably, the smaller C7,C7′-groups alleviate gearing interactions that sterically hinder the C3,C3′-esters.

Bisacid **51** was subject to three different fates. In the first pathway, a palladium-catalyzed decarboxylative Heck reaction of **51** followed by perylenequinone formation provided bisstyryl

Scheme 8. Use of Common Intermediates in the Syntheses of C3,C3′,C7,C7′-Analogues



derivative **54**.[53] In the second pathway, the C5,C5′-benzyl ethers were cleaved and the more acidic carboxylic acids were then selectively benzylated using BnBr and K_2CO_3. This tactic was necessary because the carboxylic acids did not survive the MnO_2-mediated oxidative cyclization, nor was selective hydrogenation of the bisbenzyl ester of **51** successful. Subsequent perylenequinone formation yielded **55**, which could then be further hydrogenated to yield the perylenequinone bisacid **56**. In the third pathway, protodecarboxylation using a palladium source furnished **52**,[47] which was then transformed to perylenequinone **16**[54] following protocols described above. Perylenequinone **16** could, in turn, be treated with Br_2 to provide bisbromide **57**. Subsequent enzyme assays with this analogue series revealed high activity for **16**, stimulating us to also undertake a synthesis of *M*-**16** since we had established that the *M*-isomers are more potent (see below).

Protein Kinase C Inhibition. The activity of the calphostins and hypocrellin A are thought to arise in part by modulation of protein kinase C (PKC), a family of C1 domain containing kinases. The PKCs are membrane bound proteins that play important roles in intracellular signal transduction effecting proliferation, differentiation, and apoptosis and have been implicated in a variety of disease states including diabetes, cancer, heart disease, and cognitive disfunction.[24,55] Because the C1 domain is found in only a small subset of the human kinome,[56] selectivity relative to the many other biologically important kinases may be achievable. In light of this information, the newly synthesized analogues were tested for their ability to inhibit PKC phosporylation, providing a benchmark comparison of a broad range of the natural products and analogues to establish the most critical elements for PKC inhibition.

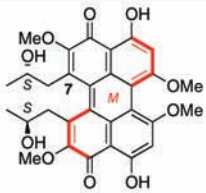
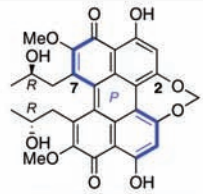
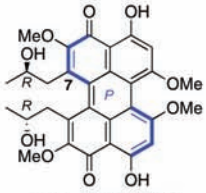
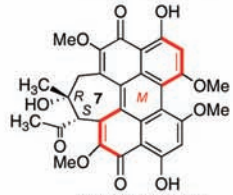
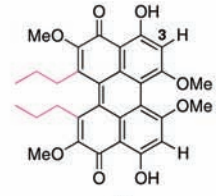
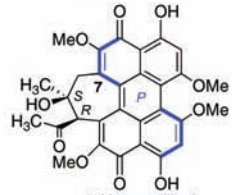
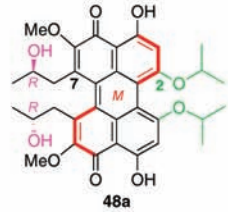
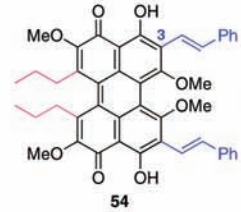
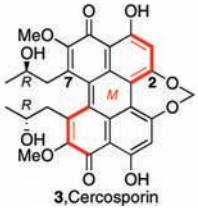
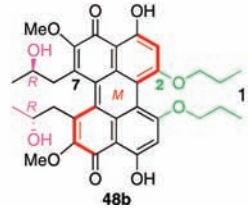
As seen in Table 3, there are clear trends between the perylenequinone architecture and PKC-inhibitory activity. Notably, these results represent the first systematic evaluation of helical chirality on PKC inhibition. We found a direct correlation between helical chirality and inhibition; the *M*-perylenequinones were 1.8−20 times more potent than the corresponding *P*-isomers with the same C7,C7′-stereochemistry [(+)-**1** vs (−)-**2**, (+)-**2** vs (−)-**1**, and **3** vs *epi*-**3**]. For the hypocrellins (**4** and *ent*-**4**), the helical stereochemistry atropisomerizes rapidly at room temperature and the compounds are 4:1 equilibrium mixtures of the two atropisomers.[18] In line with the above results, the greatest PKC inhibition was observed for the compound where the *M*-helicity predominates (*ent*-**4**). Particularly noteworthy is that the unnatural isomers (+)-**1** and (+)-**2** were more potent than the natural products (−)-**1** and (−)-**2**. With respect to the stereochemistry of the C7,C7′-substitution, the *R*,*R*-array was found to offer slightly better (1.7-fold) activity than the *S*,*S*-array [(+)-**1** vs (+)-**2**, (−)-**1** vs (−)-**2**].

Docking models of the perylenequinones indicated that larger hydrophobic groups at the C2,C2′-positions better fill a pocket in the binding site (see above).[52] Analogues of the most active stereochemical array (*M*,*R*,*R*) were thus constructed to probe the role of the C2,C2′-substitution (Scheme 7). Pleasingly, the bisisopropyl **48a** (0.8 μM) and bis-*n*-propyl **48b** (1.5 μM) were more potent than the parent compound (+)-**2** (3.5 μM) providing support for the docking model.

Prior reports indicated that acylation of the C7,C7′-hydroxypropyl group provided superior activity (i.e., calphostin A, **1a**, $R^1 = R^2 =$ COPh, 0.25 μM vs calphostin D, **1d**, $R^1 = R^2 =$ H, 6.4 μM; Figure 1).[22b] To determine if this result arises from a simple increase in hydrophobicity and removal of the two hydroxyl H-bond donors, the C7,C7′-*n*-propyl analogues were synthesized (Scheme 8). Notably, this compound possessed significant potency with racemic **16** exhibiting an IC_{50} of 1.2 μM indicating that hydrophobic C7,C7′-groups are indeed optimal (Table 3). Based upon our discovery that the *M*-helicity

(50) Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917–924.
(51) Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639–1662.
(52) See Supporting Information.
(53) (a) Myers, A. G.; Tanaka, D.; Mannion, M. R. *J. Am. Chem. Soc.* **2002**, *124*, 11250–11251. (b) Tanaka, D.; Romeril, S. P.; Myers, A. G. *J. Am. Chem. Soc.* **2005**, *127*, 10323–10333.
(54) Racemic-**24** has been reported previously. See ref 4f.
(55) Reviews on Protein Kinase C: (a) Nishizuka, Y. *Nature* **1984**, *308*, 693–698. (b) Nishizuka, Y. *Science* **1986**, *233*, 305–312.
(56) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. *Science* **2002**, *298*, 1912–1934.

Table 3. Perylenequinone Inhibition of PKC

	PKC IC_{50}	ε^{670}		PKC IC_{50}	ε^{670}		PKC IC_{50}	ε^{670}
 (+)-**1**, *ent*-Calphostin D	6.0 μM	120 $M^{-1}cm^{-1}$	 *epi*-**3**, *epi*-Cercosporin	12 μM	120 $M^{-1}cm^{-1}$	 *M*-**16**	0.40 μM	480 $M^{-1}cm^{-1}$
 (−)-**1**, Calphostin D	6.4 μM[a]		 *ent*-**4**, Hypocrellin	0.4 μM		 **16**	1.2 μM	480 $M^{-1}cm^{-1}$
 (+)-**2**, *ent*-Phleichrome	3.5 μM	225 $M^{-1}cm^{-1}$	 **4**, Hypocrellin A	4.5 μM		 **53**	3.5 μM	1430 $M^{-1}cm^{-1}$
 (−)-**2**, Phleichrome	11 μM		 **48a**	0.8 μM	150 $M^{-1}cm^{-1}$	 **54**	4.0 μM	5180 $M^{-1}cm^{-1}$
 **3**,Cercosporin	0.60 μM	160 $M^{-1}cm^{-1}$	 **48b**	1.5 μM	360 $M^{-1}cm^{-1}$	 **57**	2.5 μM	1200 $M^{-1}cm^{-1}$

[a] PKC evaluation obtained from ref 23.

provided superior inhibition, enantiopure *M*-**16** was generated (see Scheme 8) and was found to exhibit an IC_{50} of 0.4 μM. Based on these data, *P*-**16** is indeed a poorer inhibitor (2.0 μM) supporting our prior conclusions. Enantiopure *M*-**16** is a 9−25 times better inhibitor than the nearest congeners, (+)-**1** and (+)-**2**, and 16−28 times better than the corresponding natural products, (−)-**1** and (−)-**2**.

The finding that the stereogenic C7,C7′-substitution is not necessary for potency and that architecturally simple structures such as **16** can be employed motivated us to examine C3,C3′-analogues of the C7,C7′-*n*-propyl series (Scheme 8). In doing so, compounds (**53**−**57**) with esters, acids, styryls, or bromo groups at the C3,C3′-positions were designed to alter the chromophore to improve the absorption profile. Analogues **53**−**57** displayed increased absorption in the 600−800 nm range compared to the natural products. Assessing the effects on PKC inhibition, the bisacid **56** was found to decompose rapidly. On the other hand, **53**−**54** and **57** were found to be 2−4 times less potent than the parent analogue **16**, having the same architecture but no C3-substitution.

The assays described above were performed with PKC containing regulatory and catalytic domains. To determine whether these perylenequinones were indeed inhibiting phosporylation by acting on the regulatory domain, assays were performed with PKC containing only the catalytic subunit. Over 85% of the catalytic subunit activity was retained even when 50 μM **16** was employed which is well above the IC_{50} (1.2 μM) seen with the whole enzyme.

Cancer Cell Lines. To determine whether the structural effects on PKC inhibition are related to anticancer activity, eight cancer cell lines were screened against the most potent *M*-isomers: *ent*-phleichrome [(+)-**2**], cercosporin (**3**), and hypocrellin (*ent*-**4**). Finally, the most potent analogue, **16**, was included. All of the compounds exhibited micromolar or submicromolar CC_{50} values for growth inhibition in line with their light activated mechanism of action. Overall, cercosporin (**3**) was the most active compound in every cell line exhibiting CC_{50} values of 0.12−0.27 μM across the series. The remaining compounds exhibited similar activities across all of the cell lines with the exception of simple analogue **16**, which was a poorer inhibitor of HT29

Table 4. CC_{50} (μM) Values of the Perylenequinone against Cancer Cell Lines

	NCI-H460	PC3	SK-MEL-5	SN12C	U251	A2780	MCF7	JHU-012 Head and Neck		
cmpd	lung	prostate	skin	colon	brain	ovary	breast	light	dark[b]	photo-potentiation
(+)-**2**	0.52	0.71	0.86	0.66	0.49	0.28	1.1	0.81	2.8	3.5
3	0.13	0.26	0.15	0.17	0.15	0.12	0.20	0.16	0.78	4.9
ent-**4**	0.49	0.51	0.57	1.0	0.57	0.41	0.84	1.0	1.9	1.9
16	0.51	0.71	0.55	0.58	0.50	0.22	0.84	0.23	1.3	5.8

[a] All assays conducted with a 30 min exposure to a 32 W light at 5 cm. [b] Assays conducted without 30 min light exposure.

and a superior inhibitor of A2780 and JHU-012 relative to all the compounds except cercosporin (**3**). Given the selectivity of cercosporin (**3**) and simple analogue **16** for the head and neck cancer line JHU-012, the compounds were further examined without light exposure in this line. The photopotentiation was strongest with cercosporin (4.9-fold) among the natural products. Remarkably, the photopotentiation was even more pronounced for simple analogue **16** (5.8-fold).

Concluding Remarks

In summary, the first total syntheses of (+)-**1** and **3** and 10 perylenequinone analogues have been accomplished in an efficient manner beginning with a common chiral binaphthalene. The approach employing enantioselective oxidative biaryl coupling, double cuprate epoxide opening, and decarboxylative functionalization permits access with complete control of the helical and centrochiral stereochemical elements of these photoactive compounds. The analogues provided an assessment of the structural features important to PKC inhibition with *M*-helicity and hydrophobic C7,C7′-substitution being identified as advantageous. Substitution at the C2,C2′- and C3,C3′-positions was well tolerated and led to compounds simpler than the natural products, but possessing superior absorption profiles which may provide an avenue to superior photosensitizers. Finally, cercosporin (**3**) and simple analogue **16** provide superior inhibition of growth activity in cancer cell lines, and the latter displayed higher levels of photopotentiation than any of the natural products. Further biological evaluation of these and additional compounds, including selective inhibition among the PKC isoforms, is underway and will be reported in due course.

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Supporting Information Available: Full experimental details including synthetic procedures, atropisomerization data, biological assays, and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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