Total Syntheses of Ningalin A, Lamellarin O, Lukianol A, and Permethyl Storniamide A Utilizing Heterocyclic Azadiene Diels−Alder Reactions

Dale L. Boger,* Christopher W. Boyce, Marc A. Labroli, Clark A. Sehon, and Qing Jin

Contribution from the Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received June 15, 1998

Abstract: Concise, efficient total syntheses of ningalin A (**1**), lamellarin O (**2**), lukianol A (**3**), and permethyl storniamide A (**5**) are detailed on the basis of a common heterocyclic azadiene Diels−Alder strategy (1,2,4,5-tetrazine → 1,2-diazine → pyrrole) ideally suited for construction of the densely functionalized pyrrole cores found in the three classes of marine natural products. Examination of the natural products and a number of synthetic intermediates revealed that some including lamellarin O (**2**) and lukianol A (**3**) exhibit modest cytotoxic activity against both wild-type and multidrug-resistant tumor cell lines. Fundamentally more important, a new class of agents including permethyl storniamide A (**5**) and its precursor **30**, which lack inherent cytotoxic activity, are disclosed which reverse the multidrug-resistant (MDR) phenotype, resensitizing a human colon cancer cell line (HCT116/VM46) to vinblastine and doxorubicin at lower doses than the prototypical agent verapamil.

The recently identified marine natural products ningalin A (**1**), lamellarin O (**2**), lukianol A (**3**), and storniamide A (**4**) each possess a common 3,4-diaryl-substituted pyrrole nucleus bearing 2- or 2,5-carboxylates. Ningalin A (**1**) is the simplest member of a newly described family of marine natural products isolated by Fenical (1997) from an ascidian of the genus *Didemnum* collected in western Australia near Ningaloo Reef which appear to be derived from condensation of 3,4-dihydroxyphenylalanine (DOPA).[1] Consequently, **1** and the related ningalins B−D are the newest members of a family of DOPA-derived *o*-catechol metabolites that include the tunichromes.

Lamellarin O (**2**)[2] is a prototypical member of a rapidly growing class of marine natural products[3−6] which was first isolated from the southern Australian marine sponge *Dendrilla cactos* by Capon (1994), and important members of this class have been disclosed by Faulkner, Fenical, and Bowden. It has been reported that biological activity could not be obtained for natural lamellarin O due to its instability and limited availability.[2] Lukianol A (**3**), a structurally related compound, was discovered in an unidentified pacific tunicate by Scheuer (1992)[7] and shown to exhibit cytotoxic activity against a cell line derived from human epidermatoid carcinoma (KB). More recent investigations of related lamellarins have confirmed their cytotoxic activity, revealed equally effective cytotoxic activity against multidrug-resistant (MDR) cell lines, and demonstrated that even at noncytotoxic concentrations they reverse MDR by inhibiting P-gp-mediated drug efflux.[8] Thus, they constitute a new class of antitumor agents active against resistant cell lines, and they additionally reverse MDR at noncytotoxic concentrations even more effectively than verapamil, resensitizing the resistant malignant cells to front-line therapeutics.

Storniamide A (**4**) is a member of a new class of secondary metabolites isolated in 1996 from a Patagonian sponge off the coast of Argentina.[9] The crude ethanolic extract of the burrowing yellow sponge *Cliona* sp. showed antibiotic activity against Gram-positive bacteria. Purification of the individual constituents was accomplished by bioassay-guided fractionation to reveal four members of this new family of alkaloids, storniamide A−D. Characterization showed that each differs only in the oxygenation pattern within the peripheral aromatic rings, with **4** being a prototypical member.

Herein we describe total syntheses of ningalin A, lamellarin O, lukianol A, and permethyl storniamide A (**5**), enlisting a common strategy applicable to related natural products and synthetic analogues (Figure 1). The concise, nonobvious approach employs a heteroaromatic azadiene Diels−Alder reaction[10] to assemble the substituents onto a six-membered 1,2-diazine core which is followed by a reductive ring contraction reaction[11−13] to provide the corresponding pyrrole, a five-

(1) Kang, H.; Fenical, W. *J. Org. Chem.* **1997**, *62*, 3254.
(2) Urban, S.; Butler, M. S.; Capon, R. J. *Aust. J. Chem.* **1994**, *47*, 1919.
(3) Anderson, R. J.; Faulkner, D. J.; Cun-heng, H.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492. Reddy, M. V. R.; Faulkner, D. J.; Venkateswarlu, Y.; Rao, M. R. *Tetrahedron* **1997**, *53*, 3457.
(4) Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 4570.
(5) Urban, S.; Capon, R. J. *Aust. J. Chem.* **1996**, *49*, 711.
(6) Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 489.
(7) Yoshida, W. Y.; Lee, K. K.; Carroll, A. R.; Scheuer, P. J. *Helv. Chim. Acta* **1992**, *75*, 1721.
(8) Quesada, A. R.; Gravalos, M. D. G.; Puentes, J. L. F. *Br. J. Cancer* **1996**, *74*, 677.
(9) Palermo, J. A.; Brasco, M. F. R.; Seldes, A. M. *Tetrahedron* **1996**, *52*, 2727.
(10) Boger, D. L. *Chemtracts: Org. Chem.* **1996**, *9*, 149. Boger, D. L. *Bull. Chim. Soc., Belg.* **1990**, *99*, 599. Boger, D. L.; Patel, M. In *Progress in Heterocyclic Chemistry*; Suschitzky, H., Scriven, E. F. V., Eds.; Pergamon: Oxford, 1989; Vol. 1, p 30. Boger, D. L.; Weinreb, S. M. *Hetero Diels−Alder Methodology in Organic Synthesis*; Academic: San Diego, 1987. Boger, D. L. *Chem. Rev.* **1986**, *86*, 781. Boger, D. L. *Tetrahedron* **1983**, *39*, 2869.
(11) Boger, D. L.; Coleman, R. S.; Panek, J. S.; Yohannes, D. *J. Org. Chem.* **1984**, *49*, 4405.
(12) Boger, D. L.; Patel, M. *J. Org. Chem.* **1988**, *53*, 1405. Boger, D. L.; Baldino, C. M. *J. Am. Chem. Soc.* **1993**, *115*, 11418.

10.1021/ja982078+ CCC: $18.00
Published on Web 12/19/1998

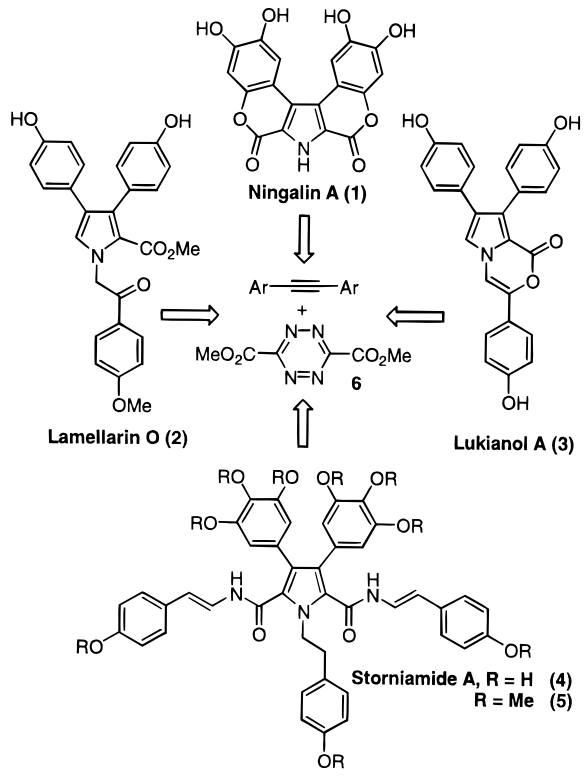
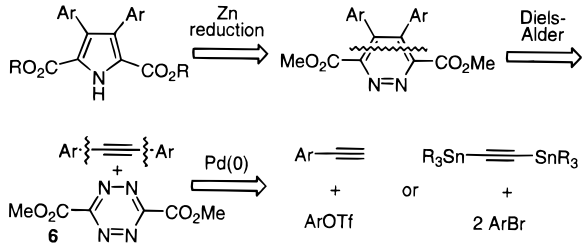


Figure 1.

Scheme 1

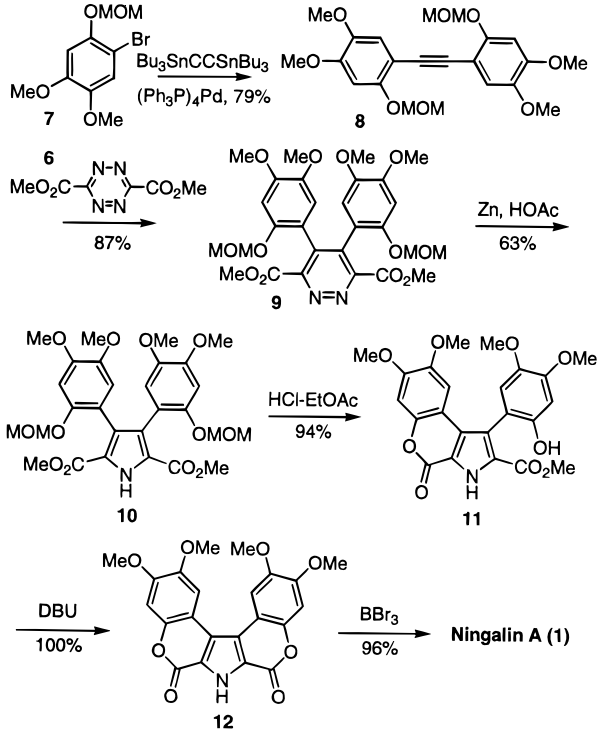


membered heteroaromatic system not commonly assembled by a [4 + 2] cycloaddition reaction (Scheme 1).

Importantly, the oxygenation pattern found in the diaryl groups would be expected to increase the nucleophilic character of the corresponding acetylene and improve what is a typically poor reactivity of the alkynes toward **6**.[14] This approach lends itself to the synthesis of the natural products and a range of analogues by use of alternative acetylenes or by functionalization of the diesters for further elaboration of the common central core with the potential for desymmetrization. As such, its implementation detailed herein complements the limited synthetic efforts disclosed to date on the three classes of marine natural products.[15]

Total Synthesis of Ningalin A. The requisite diphenylacetylene **8** was prepared by a double Stille coupling of 1-bromo-4,5-dimethoxy-2-(methoxymethoxy)benzene (**7**)[16] with bis(tributylstannyl)acetylene[17] ($Pd(PPh_3)_4$, 79%), Scheme 2. The first of the two key conversions in the synthesis, the Diels–Alder reaction of the electron-deficient 1,2,4,5-tetrazine **6**[13] with the electron-rich acetylene **8**, was carried out in toluene at 110 °C to afford the desired 1,2-diazine **9** in excellent yield (87%) as a 2.4:1 mixture of atropisomers. The relative effectiveness of the Diels–Alder reaction of the alkyne **8** in comparison with unactivated alkynes[14] may be attributed to the electron-donating properties of the dienophile aryl alkoxy groups. Subsequent reductive ring contraction of 1,2-diazine **9** effected by treatment with zinc (HOAc, 63%) smoothly afforded the desired pyrrole **10**. Deprotection of the MOM ethers through treatment of **10** with 3 M HCl–EtOAc afforded a mixture of the corresponding diphenol and the monolactone **11**, which completely converted to the monolactone **11** (94%) upon SiO_2 chromatography. Because of the steric congestion and rotational barrier of the two ortho aryl rings, more forcing conditions (DBU, 100%) were required for formation of the second lactone, providing tetramethyl ningalin A (**12**). Exhaustive demethylation with BBr_3 (96%) completed the first total synthesis of ningalin (**1**, 49% overall yield) and provided material that was identical in all respects (^{1}H NMR, ^{13}C NMR, IR, MS)[18] with authentic material.[1]

Scheme 2



Total Synthesis of Lamellarin O and Lukianol A. The acetylenic precursor common to lamellarin O and lukianol A

(13) Boger, D. L.; Panek, J. S.; Coleman, R. S.; Sauer, J.; Huber, F. X. *J. Org. Chem.* **1985**, *50*, 5377. Boger, D. L.; Panek, J. S.; Patel, M. *Org. Synth.* **1991**, *70*, 79.

(14) Sauer, J.; Mielert, A.; Lang, D.; Peter, D. *Chem. Ber.* **1965**, *98*, 1435.

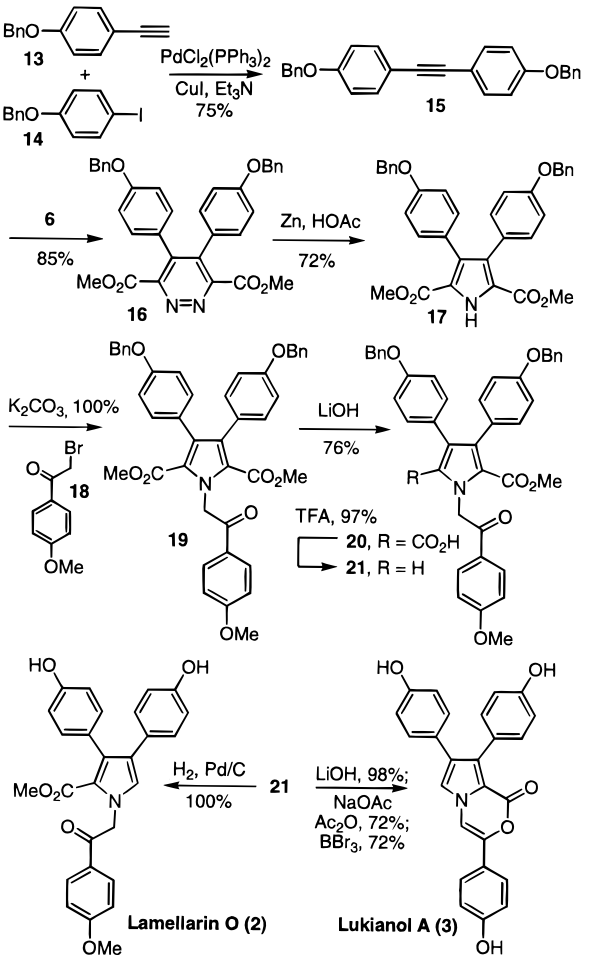
(15) Lukianol A and lamellarin O dimethyl ether: Fürstner, A.; Weintritt, H.; Hupperts, A. *J. Org. Chem.* **1995**, *60*, 6637. Lamellarin O and Q and lukianol A: Banwell, M. G.; Flynn, B. L.; Hamel, E.; Hockless, D. C. R. *Chem. Commun.* **1997**, 207. Lamellarin K: Banwell, M.; Flynn, B.; Hockless, D. *Chem. Commun.* **1997**, 2259. Lamellarin D and H: Ishibashi, F.; Miyazaki, Y.; Iwao, M. *Tetrahedron* **1997**, *53*, 5951. Lamellarin G trimethyl ether: Heim, A.; Terpin, A.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 155.

(16) Donnelly, D. M. X.; Finet, J.-P.; Rattigan, B. A. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1679. Prepared by Baeyer–Villiger oxidation of commercially available 6-bromoveratraldehyde (mCPBA, 97%), subsequent formate methanolysis (catalytic $NaOCH_3$, 72%), and protection as the methoxymethyl ether (MOMCl, NaH, 77%).

(17) Cummins, C. H. *Tetrahedron Lett.* **1994**, *35*, 857.

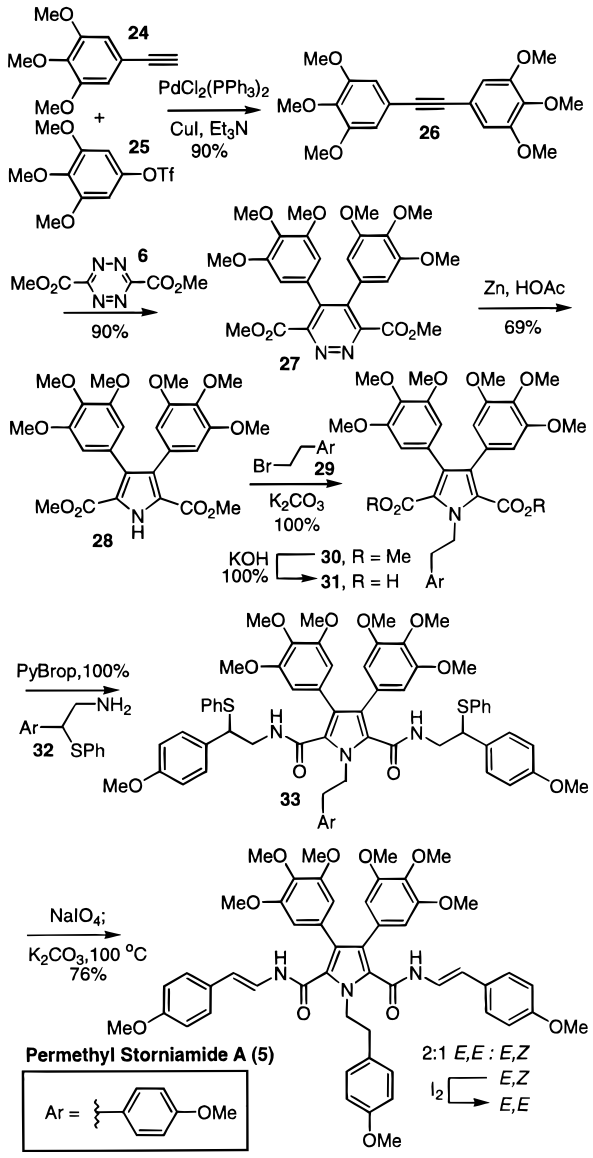
(18) The reported ^{13}C NMR (DMSO-d_6, 50 MHz)[1] for **1** does not list a broadened signal observed at ca. δ 123 (Supporting Information spectrum supplied in ref 1) and assigns two distinct carbons (C2 and C9) to a single resonance (δ 110.3). The ^{13}C NMR (DMSO, 100 MHz) of our synthetic sample of **1** was identical in all respects except this additional broadened singlet was observed as a sharp singlet at δ 122.9, which we tentatively assign as C2.

Scheme 3



was prepared by a palladium(0)-catalyzed cross-coupling of the terminal acetylene **13**[19] and **14**[19] (0.03 equiv of Pd, 0.06 equiv of CuI, Et_3N, 75%) in which slow addition of the acetylene was necessary to suppress formation of the coupled diacetylene (Scheme 3). Initial Stille coupling attempts using bis(tributylstannyl)acetylene[17] and the aryl iodide **14** led predominately to the symmetrical coupled diacetylene.[20] Acetylene **15** was allowed to react with 1,2,4,5-tetrazine **6** to give the desired 1,2-diazine **16** in excellent yield (toluene, 100 °C, 85%). Zinc reductive ring contraction (HOAc, 72%) followed by *N*-alkylation of the resulting pyrrole **17** with the commercially available 2-bromo-4′-methoxyacetophenone (**18**) gave the pentasubstituted pyrrole **19** (100%). The symmetrical diester **19** was subjected to a gratifyingly selective hydrolysis with LiOH (1.3 equiv, 76%) to provide the monoacid **20**. We attribute this selective hydrolysis to phenacyl enolate generation under the reaction conditions followed by enol lactone closure onto either of the dimethyl esters and subsequent preferential hydrolytic cleavage of the enol lactone. The resulting acid **20** was treated with trifluoroacetic acid (5 equiv, CH_2Cl_2, 40 °C, 5 h, 97%) to promote decarboxylation and afford the appropriately substituted and functionalized pyrrole core **21** found in both lamellarin O and lukianol A. This key intermediate could be quantitatively converted to lamellarin O (**2**) by catalytic hydrogenation or, more simply, by conducting a TFA treatment of **20** or **21** at more elevated temperatures (neat TFA, 70 °C, 3 h, 84%). Analogous to the efforts of Fürstner,[15] pyrrole **21** was saponified with LiOH (98%), and the resulting carboxylic acid **22** was efficiently converted to the enol lactone **23** (NaOAc, Ac_2O, 72%). Treatment of **23** with BBr_3 removed both the benzyl ethers and methyl ether in excellent yield (72%) to afford lukianol A (**3**). The ^1H NMR, ^{13}C NMR, IR, MS, and mp of synthetic **2** (45% overall yield) and **3** (23% overall yield) were identical in all respects with those reported for authentic or natural material.

Scheme 4



Total Synthesis of Permethyl Storniamide A. The preparation of the acetylenic dienophile for the storniamide A synthesis, 1,2-bis(3,4,5-trimethoxyphenyl)acetylene (**26**), was accomplished by Pd(0)-catalyzed coupling of the terminal alkyne **24**[21] with the aryl triflate **25**[21] (CuI, Et_3N, 90%), Scheme 4. Initial attempts to couple the terminal acetylene **24** and the aryl triflate **25** under reported conditions[22] (catalytic $PdCl_2(PPh_3)_2$, catalytic CuI, Bu_4NI, 25 and 70 °C) gave an approximately 1:1 mixture

(19) Biftu, T.; Schneiders, G. E.; Stevenson, R. *J. Chem. Res., Synop.* **1982**, 270. Orito, K.; Hatakeyama, T.; Takeo, M.; Suginome, H. *Synthesis* **1995**, 1273.

(20) Data for 1,4-bis(4-benzyloxyphenyl)-1,3-butadiyne: ^1H NMR ($CDCl_3$, 250 MHz) δ 7.44 (d, J = 8.3 Hz, 4H), 7.37−7.33 (m, 10H), 6.94 (d, J = 8.7 Hz, 4H), 5.08 (s, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.4, 136.4, 134.1, 128.7, 128.2, 127.5, 115.0, 114.2, 81.2, 73.0, 70.0; FABHRMS (NBA/NaI) *m/z* 415.1684 ($M + H^+$, $C_{30}H_{22}O_2$ requires 415.1698).

(21) Fürstner, A.; Nikolakis, K. *Liebigs Ann.* **1996**, 2107.

(22) Powell, N. A.; Rychnovsky, S. D. *Tetrahedron Lett.* **1996**, *37*, 7901.

of the desired cross-coupled product **26** and the undesired diacetylene dimer. Modifying the procedure to include slow addition of alkyne **24** to the reaction mixture at 70 °C gave only the desired diphenylacetylene **26** in superb conversion (90%), in which elevating the temperature promotes the slow Pd(0) oxidative addition of the triflate and limiting the relative concentration of **24** avoids the undesired competitive self-coupling reaction. The first of the two key conversions involving the Diels−Alder reaction of the electron-deficient 1,2,4,5-tetrazine **6** with the electron-rich acetylene **26** proceeded in toluene to give the desired 1,2-diazine in excellent yield (110 °C, 90%). The unusual facility with which this [4 + 2] cycloaddition reaction occurs may be attributed to the six methoxy groups donating electron density into the dienophile. Subsequent zinc reductive ring contraction (HOAc, 69%) of **27** afforded the pyrrole **28** and the core structure found in the natural product. *N*-Alkylation with the phenethyl bromide **29**[23] and subsequent saponification with KOH in 4:2:1 dioxane−CH_3OH−H_2O gave the pentasubstituted pyrrole diacid **31** and set the stage for introduction of the sensitive enamides. The diacid **31** was coupled with amine **32**[24] (PyBrOP)[25] to provide the diamide **33**, and the overall conversions of **28** to **33** were sufficiently effective that the three steps could be conducted without intermediate purification and in 99% overall yield. Thioether oxidation ($NaIO_4$) with subsequent thermal sulfoxide elimination gave a separable 2:1 mixture of the *E,E*- and *E,Z*-dienamides **5** in 76% for the two steps and in 47% overall yield.[26] Attempts to isomerize the undesired *E,Z*-isomer with light and mild acid resulted in recovered starting material, decomposition, or undesired side reactions. However, treatment of the *E,Z*-isomer with I_2 (0.5 equiv, $CHCl_3$, 25 °C) under room lighting gave an approximately 2:1 mixture of *E,E*- and *E,Z*-isomers, and a similar treatment of the *E,E*-isomer gave the same thermodynamic 2:1 mixture.

Cytotoxic Activity and Reversal of Multidrug Resistance. A number of compounds in these three classes of natural products have been shown to exhibit cytotoxic activity[8] including lukianol A (**3**), which is reported to be active against a cell line derived from human epidermatoid carcinoma (KB).[7] However, the biological evaluation of most members has not been fully explored, and some, including lamellarin O (**2**), were sufficiently unstable and isolated in quantities that precluded their examination.[2] Consequently, the natural products and a number of structurally related synthetic intermediates were tested in a L1210 cytotoxic assay, and the results are summarized in Table 1. Both lamellarin O (**2**) and lukianol A (**3**) were found to be equally active against L1210 while **3** was 15−20 times less active against HTC116, ningalin A (**1**) was found to be weakly active, and a number of the synthetic intermediates displayed an analogous level of activity, presumably due to their comparable structures. Notably, the *O*-methyl or *O*-benzyl derivatives **12**, **21**, and **23** of ningalin A, lamellarin O, and lukianol A were found to be inactive.

In addition, a select set of the naturally occurring lamellarins have been shown to exhibit equally potent cytotoxic activity against multidrug-resistant (MDR) cell lines arising from overexpression of P-glycoprotein and/or to reverse MDR at noncytotoxic concentrations, resensitizing the resistant cell lines to conventional therapeutic agents.[8] P-gp is a 170 kDa plasma membrane glycoprotein encoded in humans by the gene *MDR1* which functions by exporting drugs out of mammalian cells, lowering their intracellular concentration.[27] Therefore, the active agents and a set of the inactive compounds were also examined against a wild-type human colon cancer cell line (HTC116) and two resistant HCT116 cell lines. The first resistant cell line (HCT116/VM46) embodies the MDR phenotype and overexpresses P-glycoprotein while the second cell line (HCT116/VP35) derives its resistance through underexpression of topoisomerase II. The examination of the latter cell line along with the wild-type HCT116 and their comparison with HCT116/VM46 allow an accurate assessment of the potential MDR sensitivity as well as an assessment of one potential therapeutic target. Of the active compounds examined, each proved equally potent against the three HCT116 cell lines, indicating no MDR or topo II resistance (Table 1). Most notably, lamellarin O (**2**) exhibited a respectable potency against all cell lines examined and exhibited micromolar activity against the HCT116 cell lines including the MDR HCT116/VM46, indicating that it, and related analogues, would not be subject to multidrug resistance phenotypes derived from overexpression of P-glycoprotein.

Table 1. In Vitro Cytotoxic Activity

	IC_{50} (μM)[a]			
compound	L1210	HCT116 wild type	HCT116/VM46 (MDR)	HCT116/VP35 (reduced topo II)
ningalin A (**1**)	80			
lamellarin O (**2**)	2	1	1	1
lukianol A (**3**)	1	20	15	15
permethyl storniamide (**5**)	>100	>100	>100	>100
9	>100	>100	>100	>100
10	>100			
11	10	30	50	60
12	>100			
16	10	90	60	>100
17	>100	>100	>100	>100
19	80	90	>100	>100
21	>100			
23	>100			
27	6	>100	>100	>100
28	20			
30	7	>100	>100	>100
31	>100	>100	>100	>100
vinblastine		0.003	0.2	
doxorubicin		0.2	2.2	0.4

[a] Quadruplicate assays, average IC_{50} (variation from mean, ±8%).

More interesting and fundamentally more important, many of the agents were found to be capable of reversing MDR at noncytotoxic concentrations, resensitizing HCT116/VM46 to vinblastine and doxorubicin (Table 2 and Figure 2). Of the agents examined, **5**, **16**, **17**, and **30** were found to resensitize HCT116/VM46 to vinblastine or doxorubicin at 1 μM and to do so more effectively than verapamil. At this concentration, complete MDR reversal was observed with **5**. Thus, the most effective of the agents capable of reversing MDR was permethyl storniamide A (**5**), which exhibited no inherent cytotoxic activity against the L1210 or HCT116 cell lines and completely reversed the MDR at a concentration of 1 μM, significantly more potent than the protoypical agent verapamil. Both **31** and the corresponding primary carboxamide, which represent potential hy-

(23) Hori, M.; Ozeki, H.; Iwamura, T.; Shimizu, H.; Kataoka, T.; Iwata, N. *Heterocycles* **1990**, *31*, 23.

(24) Somanathan, R.; Rivero, I. A.; Aguirre, G.; Ramirez, M. *Synth. Commun.* **1996**, *26*, 1023.

(25) Frérot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-N.; Jouin, P. *Tetrahedron* **1991**, *47*, 259.

(26) Attempts at methyl ether cleavage under a variety of conditions with *E,E*-**5** to provide **4** resulted in only partial methyl ether deprotection and subsequent decomposition.

(27) Patel, N. H.; Rothenberg, M. L. *Invest. New Drugs* **1994**, *12*, 1. Gottesman, M. M.; Pastan I. *Annu. Rev. Biochem.* **1993**, *62*, 385.

Table 2. MDR Reversal

compound at 1.0 μM	vinblastine IC_{50} (μM)[a]	gain in sensitivity[b] (% reversion)	doxorubicin IC_{50} (μM)[a]	gain in sensitivity[b] (% reversion)
2	0.1	2 (3)	2.0	1.1 (10)
3	0.2	1 (0)	2.2	1 (0)
5	0.003	67 (100)	0.1	22 (200)
5 (7.5 μM)	0.0008	250 (370)	0.08	31 (280)
9	0.09	2.2 (3)	0.8	3 (25)
11	0.15	1.3 (2)	2.2	1 (0)
16	0.05	4 (6)	0.3	7 (67)
17	0.01	20 (30)	0.7	3.1 (28)
19	0.1	2 (3)		
21	0.1	2 (3)		
23	0.1	2 (3)		
27	0.2	1 (0)	2.2	1 (0)
30	0.009	22 (33)	0.6	3.6 (40)
30 (7.5 μM)	0.0008	250 (370)	0.08	31 (280)
31	0.1	2 (3)	2.2	1 (0)
31 (7.5 μM)	0.2	1 (0)	2.2	1 (0)
verapamil (1.0 μM)	0.02	10 (15)		
verapamil (7.5 μM)	0.003	67 (100)	0.2	11 (100)

[a] IC_{50} (μM) of vinblastine or doxorubicin against the MDR resistant cell line HCT116/VM46 in the presence of 1 μM of the indicated compound. IC_{50} values in the absence of added compound are 0.2 μM (vinblastine) and 2.2 μM (doxorubicin). For the wild-type HCT116 cell line, not subject to MDR, IC_{50} values are 0.003 μM (vinblastine) and 0.2 μM (doxorubicin). Average of five experiments, IC_{50} variability (±8%). [b] Gain in sensitivity is measured as $IC_{50}(-)/IC_{50}(+)$ [(−) = without added drug, (+) = with added drug]: Keller, R. P.; Altermatt, H. J.; Nooter, K.; Poschmann, G.; Laissue, J. A.; Bollinger, P.; Hiestand, P. C. *Int. J. Cancer* **1992**, *50*, 593.

drolysis products of **5**, exhibited no MDR reversal at either 1 or 7.5 μM. At the higher concentrations required for complete verapamil reversal (7.5 μM), **5** and **30** (7.5 μM) were still more effective and the HTC116/VM46 cell line became hypersensitive to vincristine and doxorubicin, exhibiting IC_{50} values 2−4× lower than those of the wild type. The concentration dependence of this reversal was examined more carefully with several of the agents including the simpler derivative **30**, enlisting a constant and suboptimal concentration of vinblastine (0.01 μg/mL) in the HCT116/VM46 assay. The results are illustrated in Figure 2 with **30** which exhibited a well-behaved concentration dependence for the MDR reversal. Consistent with their action on P-gp170, both **5** and **30** inhibited dye efflux[8] (rhodamine 123) from HT116/VM46 cells (**5** > **30**), returning the dye retention to levels equivalent to that of wild type HCT116, and both had no significant effect on the intracellular dye concentration in wild type HCT116 cells.

Conclusions. Concise total syntheses of ningalin A (**1**), lamellarin O (**2**), lukianol A (**3**), and permethyl storniamide A (**5**) were completed enlisting a common 1,2,4,5-tetrazine → 1,2-diazine → pyrrole Diels−Alder strategy featuring the unusually effective [4 + 2] cycloadditions of the electron-deficient 1,2,4,5-tetrazine **6** with symmetrical, electron-rich alkynes. The agents constitute prototypical members of three different classes of marine natural products characterized by a highly functionalized tetra- or pentasubstituted pyrrole which is ideally suited to construction using this strategy. Among these agents, lamellarin O (**2**) was found to exhibit micromolar cytotoxic activity against wild-type and multidrug-resistant tumor cell lines, suggesting it may serve as a new lead for the development of antitumor agents insensitive to MDR. Fundamentally more important, permethyl storniamide A (**5**) and its synthetic precursor **30**, which lack inherent cytotoxic properties, were shown to potently reverse MDR, resensitizing a resistant human colon cancer cell line (HCT116/VM46) to vinblastine and doxorubicin, and they constitute the initial members of a new class of MDR reversal agents.

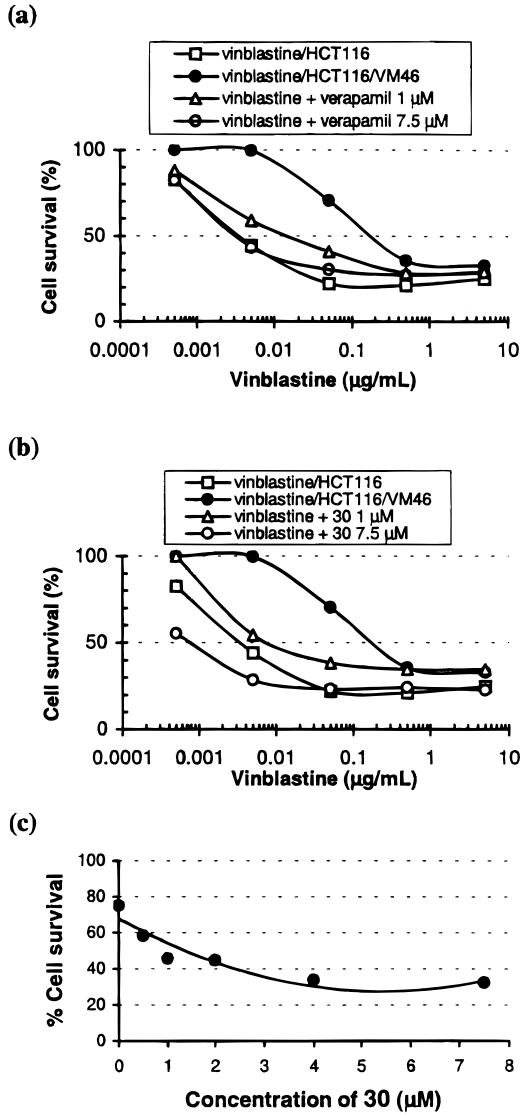


Figure 2. (a) MDR reversal effect of verapamil on the HCT116/VM46 MDR phenotype. The effect of vinblastine on wild-type HCT116 is also included to illustrate the extent of reversal. (b) MDR reversal effect of **30** on the HCT116/VM46 MDR phenotype. The effect of vinblastine on the wild-type HCT116 is also included to illustrate the extent of reversal. (c) Concentration dependence of MDR reversal by **30** at a constant suboptimal vinblastine concentration (0.01 μg/mL), against HCT116/VM46. At these concentrations, **30** alone had no cytotoxic effect on the cell line (see Table 1).

Experimental Section

1,2-Bis(4,5-dimethoxy-2-(methoxymethoxy)phenyl)acetylene (8). Nitrogen gas was bubbled through a slurry of 1-bromo-4,5-dimethoxy-2-(methoxymethoxy)benzene[16] (**7**; 1.67 g, 6.03 mmol, 1.0 equiv) and $Pd(PPh_3)_4$ (0.700 g, 0.60 mmol, 0.1 equiv) in toluene (60 mL) for 15 min. 1,2-Bis(tributylstannyl)acetylene[17] (1.9 mL, 3.62 mmol, 0.6 equiv) was added, and the reaction mixture was warmed to 100 °C for 4 h under N_2. Chromatography (SiO_2, 5.5 × 17 cm, 40% EtOAc−hexane) provided **8** (1.00 g, 79%) as a white crystalline solid: mp 96−97 °C (EtOAc−hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.95 (s, 2H), 6.71 (s, 2H), 5.23 (s, 4H), 3.87 (s, 6H), 3.84 (s, 6H), 3.54 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.7, 150.0, 144.2, 114.9, 105.8, 102.0, 96.5, 88.5, 56.4, 56.3, 56.0; IR (film) ν_{max} 2994, 2932, 2820, 1601, 1508, 1452, 1350, 1211, 1150, 1006 cm^{-1}; FABHRMS (NBA/NaI) *m/z*

418.1620 (M^+, $C_{22}H_{26}O_8$ requires 418.1628). Anal. Calcd for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26. Found: C, 62.99; H, 6.05.

Dimethyl 4,5-Bis(4,5-dimethoxy-2-(methoxymethoxy)phenyl)-1,2-diazine-3,6-dicarboxylate (9). A solution of **8** (0.98 g, 2.3 mmol) and 3,6-dicarbomethoxy-1,2,4,5-tetrazine (**6**;[13] 1.39 g, 7.0 mmol, 3.0 equiv) in toluene (25 mL) was warmed to 105 °C under Ar for 21 h. Additional **6** (0.46 g, 2.3 mmol, 1.0 equiv) was added, and the mixture was further warmed to 105 °C for 40 h before the reaction mixture was cooled to 25 °C and the solvent was evaporated. Chromatography (SiO_2, 3.5 × 20 cm, 6−8% acetone−CH_2Cl_2) provided **9** (1.06 g, 87%) as a yellow crystalline solid: mp 161−162 °C (EtOAc−hexanes); (major atropisomer (2.4:1)) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.68 (s, 2H), 6.30 (s, 2H), 5.05 (d, J = 6.7 Hz, 2H), 4.88 (d, J = 6.8 Hz, 2H), 3.82 (s, 6H), 3.80 (s, 6H), 3.46 (s, 6H), 3.24 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.2, 154.8, 150.3, 148.9, 144.1, 137.3, 114.8, 112.5, 100.0, 96.5, 56.0, 55.8, 55.7, 52.9; IR (film) ν_{max} 3006, 2955, 2832, 1739, 1606, 1503, 1431, 1262, 1216, 1149, 1000 cm^{-1}; FABHRMS (NBA/CsI) *m*/*z* 721.0982 (M + Cs^+, $C_{28}H_{32}N_2O_{12}$ requires 721.1010). Anal. Calcd for $C_{28}H_{32}N_2O_{12}$: C, 57.14; H, 5.48; N, 4.76. Found: C, 57.00; H, 5.72; N, 4.86.

Dimethyl 3,4-Bis(4,5-dimethoxy-2-(methoxymethoxy)phenyl)pyrrole-2,5-dicarboxylate (10). A solution of **9** (30 mg, 0.05 mmol) in HOAc (650 μL) was treated with Zn dust (33 mg, 0.5 mmol, 10 equiv), stirred at 25 °C for 4 h, and then treated with an additional 10 equiv of Zn (33 mg).[28] After 12.5 h, the slurry was diluted with EtOAc (10 mL), filtered through Celite, and rinsed with EtOAc (3 × 10 mL). The filtrate was washed with saturated aqueous $NaHCO_3$ (3 × 10 mL) until effervescence ceased, washed with saturated aqueous NaCl (20 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Radial chromatography (SiO_2, 1 mm plate, 6% acetone−CH_2Cl_2) provided **10** (18.4 mg, 63%) as a white crystalline solid: mp 151−152 °C (EtOAc−hexanes); ^{1}H NMR (acetone-d_6, 400 MHz) δ 11.10 (br s, 1H), 6.78 (s, 2H), 6.55 (br s, 2H), 4.88 (br s, 4H), 3.75 (s, 6H), 3.68 (s, 6H), 3.51 (s, 6H), 3.26 (s, 6H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 161.4, 150.9, 150.1, 144.7, 128.1, 123.5, 117.1, 116.6, 102.8, 97.2, 56.4, 56.1, 55.8, 51.6; IR (film) ν_{max} 3272, 3005, 2944, 2831, 1708, 1615, 1492, 1272, 1221, 1149, 995, 759 cm^{-1}; FABHRMS (NBA/CsI) *m*/*z* 708.1031 (M + Cs^+, $C_{28}H_{33}NO_{12}$ requires 708.1057). Anal. Calcd for $C_{28}H_{33}NO_{12}$: C, 58.43; H, 5.78; N, 2.43. Found: C, 58.19; H, 5.99; N, 2.34.

Methyl 7,8-Dimethoxy-1-(4,5-dimethoxy-2-hydroxyphenyl)-[1]-benzopyrano[3,4-*b*]pyrrol-4(3*H*)-one-2-carboxylate (11). A sample of the **10** (18.8 mg, 32.7 μmol) was treated with 3 M HCl−EtOAc (1.2 mL), and the mixture was stirred at 25 °C for 2 h. Evaporation of the solvent provided a mixture of dimethyl 3,4-bis(2-hydroxy-4,5-dimethoxyphenyl)pyrrole-2,5-dicarboxylate and **11**.[29] Subsequent radial chromatography (SiO_2, 1 mm plate, 8% acetone−CH_2Cl_2), which also promoted cyclization of the diphenol, afforded pure **11** (13 mg, 87%, typically 83−94%) as a white powder: mp > 305 °C dec; ^{1}H NMR (acetone-d_6, 400 MHz) δ 12.09 (br s, 1H), 7.60 (s, 1H), 6.96 (s, 1H), 6.89 (s, 1H), 6.79 (s, 1H), 6.70 (s, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.75 (s, 6H), 3.46 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.3, 154.2, 149.6, 149.5, 148.8, 145.4, 145.3, 141.6, 128.3, 126.9, 120.6, 117.4, 115.9, 110.5, 109.7, 104.5, 100.8, 100.7, 56.4, 55.9, 55.5, 55.0, 51.7; IR (film) ν_{max} 3477, 3262, 2954, 2831, 1728, 1707, 1622, 1548, 1494, 1263, 1214, 1150, 1037 cm^{-1}; FABHRMS (NBA/CsI) *m*/*z* 588.0287 (M + Cs^+, $C_{23}H_{21}NO_9$ requires 588.0271).

Tetramethyl Ningalin A (12). A solution of **11** (8.0 mg, 17.6 μmol) in toluene (1.8 mL) was treated with DBU (8.0 μL, 53.4 μmol, 5.0 equiv) and stirred at 105 °C for 12 h. The solution was diluted with EtOAc (5 mL) and washed with saturated aqueous NH_4Cl (2 × 10 mL) and saturated aqueous NaCl (10 mL) and dried (Na_2SO_4) to provide **12** (7.0 mg, 96%, typically 95−100%) as an ivory powder. An analytically pure sample was prepared by sequential trituration with toluene, Et_2O, and hexane: mp 328−330 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 14.31 (s, 1H), 7.78 (s, 2H), 7.23 (s, 2H), 3.90 (s, 6H), 3.88 (s, 6H); ^{13}C NMR (DMF-d_7, 125 MHz) δ 154.9, 150.7, 146.7, 146.6, 124.5, 122.5, 109.9, 108.8, 102.1, 57.2, 56.2; IR (film) ν_{max} 3231, 2903, 2841, 1711, 1615, 1494, 1228, 830 cm^{-1}; FABHRMS (NBA/NaI) *m*/*z* 423.0976 (M^+, $C_{22}H_{17}H_{17}NO_8$ requires 423.0954).

Ningalin A (1). A solution of **12** (6.5 mg, 15.3 μmol) in CH_2Cl_2 (0.5 mL) at −78 °C was treated with BBr_3 (1 M in hexanes, 230 μL, 230 μmol, 15 equiv), and the reaction mixture was allowed to warm to 25 °C over 24 h. Following dilution with CH_3OH (500 μL), the solvent was removed with a stream of N_2. Subsequent trituration with toluene and 10% CH_3OH−Et_2O afforded pure synthetic **1** (5.4 mg, 96%) identical in all compared respects (^{1}H NMR, ^{13}C NMR, IR, MS)[18] to naturally derived ningalin A: mp > 260 °C dec; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 14.0 (br s, 1H), 9.89 (br s, 2H), 9.44 (br s, 2H), 7.79 (s, 2H), 6.89 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 154.6, 146.4, 144.4, 142.8, 122.9, 122.1, 110.3, 108.4, 104.3; IR (film) ν_{max} 3377, 3179, 1707, 1625, 1497, 1338, 1262, 1164 cm^{-1}; FABHRMS (NBA/CsI) *m*/*z* 499.9364 (M + Cs^+, $C_{18}H_9NO_8$ requires 499.9382).

1,2-Bis(4-benzyloxyphenyl)acetylene (15). A stirred solution of **14**[19] (1.70 g, 5.48 mmol, 1.1 equiv), $PdCl_2(PPh_3)_2$ (0.11 g, 0.15 mmol, 0.03 equiv), and CuI (0.058 g, 0.30 mmol, 0.06 equiv) in Et_3N (58 mL) under N_2 at 70 °C was treated with a solution of **13**[19] (1.04 g, 5.0 mmol) in Et_3N (10 mL) over a period of 1.5 h. The reaction mixture was allowed to stir for an additional 30 min before it was cooled to 25 °C. The mixture was diluted with 10% aqueous HCl (50 mL) and was extracted with $CHCl_3$ (4 × 50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 5.5 × 15 cm, 25% EtOAc−hexane) afforded **15** (1.60 g, 75%) as a white solid. An analytically pure sample was prepared by recrystallization from toluene (2×): mp 168−170 °C (lit.[30] mp 180−182 °C);^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.44 (d, J = 8.3 Hz, 4H), 7.37−7.33 (m, 10H), 6.94 (d, J = 8.7 Hz, 4H), 5.08 (s, 4H); FABHRMS (NBA/NaI) *m*/*z* 390.1631 (M^+, $C_{28}H_{22}O_2$ requires 390.1620). Anal. Calcd for $C_{28}H_{22}O_2$: C, 86.13; H, 5.68. Found: C, 86.31; H, 5.36.

Dimethyl 3,4-Bis(4-benzyloxyphenyl)-1,2-diazine-2,5-dicarboxylate (16). A stirred mixture of **15** (1.00 g, 2.56 mmol) and **6**[13] (0.76 g, 3.84 mmol, 1.5 equiv) in toluene (10 mL) was warmed to 110 °C under N_2 for 48 h. The mixture was cooled to 25 °C, additional **6** (0.76 g, 3.84 mmol, 1.5 equiv) was added, and the mixture was warmed to 110 °C for an additional 24 h. Chromatography (SiO_2, 5.5 × 15 cm, 50% EtOAc−hexane) afforded **16** (1.22 g, 85%) as a yellow solid: mp 169−171 °C (50% EtOAc−hexane); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.41−7.33 (m, 10H), 6.96 (d, J = 8.8 Hz, 4H), 6.86 (d, J = 8.7 Hz, 4H), 5.02 (s, 4H), 3.77 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.6, 159.1, 154.8, 138.0, 136.3, 130.5, 128.6, 127.6, 127.5, 124.8, 114.9, 69.9, 52.9; IR (film) ν_{max} 3025, 2953, 2871, 1743, 1605, 1507, 1435, 1374, 1246 cm^{-1}; FABHRMS (NBA/CsI) *m*/*z* 693.1020 (M + Cs^+, $C_{34}H_{28}N_2O_6$ requires 693.1002). Anal. Calcd for $C_{34}H_{28}N_2O_6$: C, 72.84; H, 5.03; N, 5.00. Found: C, 73.13; H, 5.19; N, 4.87.

Dimethyl 3,4-Bis(4-benzyloxyphenyl)pyrrole-2,5-dicarboxylate (17). A stirred solution of **16** (0.50 g, 0.89 mmol) in HOAc (10.5 mL) under N_2 at 25 °C was treated with powdered Zn (0.52 g, 8.02 mmol, 9 equiv). After 6 h, additional powdered Zn (0.52 g, 8.02 mmol, 9 equiv) was added, and the reaction was allowed to stir for 12 h. The mixture was diluted with EtOAc (25 mL), filtered through a pad of Celite, and rinsed with EtOAc (3 × 20 mL), and the solvent was removed under reduced pressure. Chromatography (SiO_2, 3.8 × 15 cm, 25% EtOAc−hexane) afforded **17** (0.35 g, 72%) as a white solid: mp 164−165 °C (25% EtOAc−hexane); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.78 (br s, 1H), 7.45−7.16 (m, 10H), 7.05 (d, J = 8.7 Hz, 4H), 6.84 (d, J = 8.7 Hz, 4H), 5.02 (s, 4H), 3.78 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.7, 157.8, 136.9, 131.9, 131.2, 128.5, 127.9, 127.6, 125.3, 121.1, 113.8, 69.9, 51.7; IR (film) ν_{max} 3438, 3287, 3032, 2950, 1701, 1465, 1298, 1243 cm^{-1}; FABHRMS (NBA/CsI) *m*/*z* 680.1032 (M +

(28) The initial product, the corresponding 1,4-dihydro-1,2-diazine, could be isolated at intermediate stages of the reaction (10−20%) and characterized: ^{1}H NMR ($CDCl_3$, 400 MHz) major atropisomer, δ 7.05 (br s, 1H), 6.66 (s, 1H), 6.61 (s, 2H), 6.47 (s, 1H), 5.07 (d, J = 7.0 Hz, 1H), 4.95 (d, J = 6.5 Hz, 1H), 4.90 (s, 2H), 4.62 (s, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.66 (s, 3H), 3.50 (s, 3H), 3.47 (s, 3H), 3.42 (s, 3H), 3.39 (s, 3H); FABHRMS (NBA/CsI) *m*/*z* 723.1191 (M + Cs^+, $C_{28}H_{34}N_2O_{12}$ requires 723.1166).

(29) The ratio of diphenol to monolactone **11** ranged from 1.2:1 to 1:7, and the amount of **11** increased with time. The diphenol was never isolated, but in the mixture it exhibited the following ^{1}H NMR (acetone-d_6, 400 MHz): δ 11.24 (br s, 1H), 7.18 (br s, 2H), 6.51 (s, 2H), 6.42 (s, 2H), 3.71 (s, 6H), 3.69 (s, 6H), 3.51 (s, 6H).

(30) Broser, W.; Brockt, M. *Tetrahedron Lett.* **1967**, 3117.

Cs^+, $C_{34}H_{29}NO_6$ requires 680.1049). Anal. Calcd for $C_{34}H_{29}NO_6$: C, 74.57; H, 5.34; N, 2.56. Found: C, 74.70; H, 5.42; N, 2.47.

Dimethyl 3,4-Bis(4-benzyloxyphenyl)-1-[2-(4-methoxyphenyl)-2-oxoethyl]pyrrole-2,5-dicarboxylate (19). A stirred mixture of **17** (0.30 g, 0.55 mmol), **18** (0.138 g, 0.60 mmol, 1.1 equiv), and K_2CO_3 (0.227 g, 1.64 mmol, 3 equiv) in DMF (2.2 mL) under N_2 was warmed to 70 °C for 1 h. The mixture was cooled to 25 °C, diluted with H_2O (10 mL), extracted with EtOAc (3 × 5 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 3.8 × 15 cm, 40% EtOAc−hexane) afforded **19** (0.38 g, 100%) as a white solid: mp: 166.5−167.5 °C (40% EtOAc−hexane); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.05 (d, J = 8.8 Hz, 2H), 7.45−7.16 (m, 10H), 7.00 (d, J = 9.0 Hz, 2H), 6.99 (d, J = 8.7 Hz, 4H), 6.80 (d, J = 8.6 Hz, 4H), 6.38 (s, 2H), 5.01 (s, 4H), 3.90 (s, 3H), 3.49 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 192.1, 163.9, 162.2, 157.4, 137.0, 131.7, 131.6, 131.5, 130.3, 128.5, 128.0, 127.9, 127.6, 127.1, 124.3, 114.0, 113.6, 69.8, 55.5, 53.1, 51.3; IR (film) ν_{max} 3034, 2950, 2910, 2848, 1712, 1601, 1532, 1437, 1299, 1237 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 828.1594 (M + Cs^+, $C_{43}H_{37}NO_8$ requires 828.1574). Anal. Calcd for $C_{43}H_{37}NO_8$: C, 74.23; H, 5.36; N, 2.01. Found: C, 73.87; H, 5.65; N, 1.99.

3,4-Bis(4-benzyloxyphenyl)-5-(methoxycarbonyl)-1-[2-(4-methoxyphenyl)-2-oxoethyl]pyrrole-2-carboxylic Acid (20). A stirred solution of **19** (0.30 g, 0.43 mmol) and LiOH (0.013 g, 0.56 mmol, 1.3 equiv) in 3:2:1 THF−CH_3OH−H_2O (8.6 mL) was warmed to 50 °C for 6 h. The reaction mixture was diluted with 10% aqueous KOH (5 mL) and was extracted with EtOAc (5 mL). The aqueous phase was acidified with 10% aqueous HCl (pH 1) and was extracted with CH_2Cl_2 (3 × 5 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. Chromatography (SiO_2, 3.8 × 15 cm, 5% CH_3OH−CH_2Cl_2) afforded pure **20** (0.22 g, 76%): mp 190−191 °C (*i*-PrOH); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.02 (d, J = 8.8 Hz, 2H), 7.44−7.32 (m, 10H), 7.06 (d, J = 8.6 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 6.81 (t, J = 9.1 Hz, 4H), 6.39 (s, 2H), 5.00 (s, 2H), 4.99 (s, 2H), 3.87 (s, 3H), 3.49 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 192.2, 163.8, 162.1, 157.7, 157.4, 137.0, 136.9, 131.9, 131.6, 130.3, 128.5, 128.0, 127.9, 127.7, 127.6, 114.0, 113.8, 113.6, 69.8, 55.5, 53.2, 51.4; IR (film) ν_{max} 3351−2800, 3031, 2916, 2848, 1708, 1600, 1531, 1435, 1239 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 814.384 (M + Cs^+, $C_{42}H_{35}NO_8$ requires 814.1417). Anal. Calcd for $C_{42}H_{35}NO_8$: C, 74.00; H, 5.17; N, 2.05. Found: C, 73.74; H, 5.53; N, 2.23.

Methyl 3,4-Bis(4-benzyloxyphenyl)-1-[2-(4-methoxyphenyl)-2-oxoethyl]pyrrole-2-carboxylate (21). A stirred solution of **20** (0.10 g, 0.147 mmol) in CH_2Cl_2 (1.5 mL) under N_2 was treated with trifluoroacetic acid (0.056 mL, 0.733 mmol, 5 equiv), and the solution was warmed to 40 °C for 5 h. The mixture was cooled to 25 °C, and the solvent was diluted with saturated aqueous $NaHCO_3$ (5 mL), extracted with CH_2Cl_2 (3 × 2 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 1.9 × 15 cm, 35% EtOAc−hexane) afforded **21** (0.091 g, 97%) as a white solid: mp 120−121 °C (Et_2O); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.04 (d, J = 8.8 Hz, 2H), 7.49−7.34 (m, 10H), 7.18 (d, J = 8.6 Hz, 2H), 7.04−6.99 (m, 4H), 6.93 (s, 1H), 6.92 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.6 Hz, 2H), 5.74 (s, 2H), 5.08 (s, 2H), 5.00 (s, 2H), 3.90 (s, 3H) 3.47 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.8, 164.0, 162.3, 157.5, 157.1, 137.1, 137.0, 131.9, 131.0, 130.3, 129.4, 128.5, 128.2, 127.9, 127.6, 127.5, 127.2, 124.6, 119.7, 114.4, 114.1, 113.8, 88.4, 69.9, 55.5, 50.8; IR (film) ν_{max} 3032, 2948, 1691, 1600, 1532, 1441, 1237, 1171 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 770.1539 (M + Cs^+, $C_{41}H_{35}NO_6$ requires 770.1519).

Lamellarin O (2). Method A. A solution of **21** (10.4 mg, 0.0163 mmol) and Pd/C (1 mg, 0.1 wt equiv) in EtOH (0.16 mL) under H_2 was stirred at 25 °C for 1 h. The solution was filtered through a pad of Celite, and the solvent was removed under reduced pressure to afford **2** (7.5 mg, 100%) as a white solid identical in all compared results (^{1}H NMR, ^{13}C NMR, IR, mp)[31] to authentic material: mp 259−261 °C (lit.[15] mp 259−260 °C; unstable pale yellow oil[2]); ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.08 (d, J = 8.6 Hz, 2H), 7.18 (s, 1H), 7.11 (d, J = 8.6 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 6.76 (d, J = 8.6 Hz, 2H), 6.64 (d, J = 8.6 Hz, 2H), 5.91 (s, 2H), 3.93 (s, 3H), 3.40 (s, 3H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 192.7, 164.9, 162.9, 157.0, 156.6, 132.7, 131.5, 131.1, 130.2, 129.3, 128.3, 128.1, 127.2, 125.2, 120.7, 115.84, 115.80, 115.23, 115.20, 114.9, 56.5, 56.1, 50.7; IR (film) ν_{max} 3430, 2912, 1682, 1600, 1436, 1241, 1169, 1097, 1025 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 590.0601 (M + Cs^+, $C_{27}H_{23}NO_6$ requires 590.0580).

Method B. A sample of crude **21** (7 mg, 0.011 mmol) was warmed in neat trifluoroacetic acid (0.56 mL) at 70 °C for 3 h. The mixture was cooled to 25 °C, and the solvent was diluted with saturated aqueous $NaHCO_3$ (5 mL), extracted with CH_2Cl_2 (3 × 2 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 1.9 × 15 cm, 35% EtOAc−hexane) afforded **2** (4.2 mg, 84%) as a white solid identical in all respects to the material described above.

1*H*-7,8-Bis(4-benzyloxyphenyl)-3-(methoxyphenyl)pyrrolo[2,1-*c*]-[1,4]-oxazin-1-one (23). A stirred solution of **21** (0.025 g, 0.039 mmol) and LiOH (0.0028 g, 0.067 mmol, 1.7 equiv) in 3:2:1 THF−CH_3OH−H_2O (0.4 mL) was warmed to 50 °C for 6 h. The reaction mixture was diluted with 10% aqueous KOH (5 mL) and was extracted with EtOAc (5 mL). The aqueous phase was acidified with 10% aqueous HCl (pH 1), extracted with CH_2Cl_2 (3 × 3 mL), and dried (Na_2SO_4), and the solvent was removed under reduced pressure to afford **22** (0.023 g, 98%). The compound was used without further purification.

A stirred solution of **22** (0.023 g) and NaOAc (0.055 g, 0.67 mmol, 18.1 equiv) in Ac_2O (3.0 mL) was warmed to 100 °C for 1 h. The mixture was cooled to 25 °C, and the solvent was removed by coevaporation with toluene. The residue was diluted with Et_2O (10 mL), washed with saturated aqueous $NaHCO_3$ (3 × 3 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 1.9 × 15 cm, 50% EtOAc−hexane) afforded **23** (0.017 g, 72%) as a white solid: mp 184−185 °C (Et_2O); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.62 (d, J = 8.8 Hz, 2H), 7.48−7.28 (m, 15H), 7.22 (s, 1H), 6.97 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 5.08 (s, 2H), 5.04 (s, 2H), 3.86 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.5, 158.3, 157.8, 154.3, 142.0, 137.0, 136.9, 132.1, 129.8, 129.7, 128.6, 128.5, 128.1, 128.00, 127.96, 127.7, 127.5, 126.1, 125.8, 124.9, 123.1, 118.9, 114.8, 114.3, 114.2, 114.1, 112.9, 102.7, 70.0, 55.3; IR (film) ν_{max} 3108, 3033, 2938, 1732, 1608, 1515, 1428, 1245, 1176 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 738.1279 (M + Cs^+, $C_{40}H_{31}NO_5$ requires 738.1257). Anal. Calcd for $C_{40}H_{31}NO_5$: C, 79.32; H, 5.16; N, 2.31. Found C, 78.97; H, 4.83; N, 2.34.

Lukianol A (3). A stirred solution of **23** (10 mg, 0.0165 mmol) in CH_2Cl_2 (0.33 mL) at −78 °C was treated with BBr_3 (0.148 mL as a 1 M solution in hexanes, 9 equiv) dropwise over a 20 min period. The solution was stirred at −78 °C for 1 h and gradually warmed to 25 °C. The solution was diluted with Et_2O (25 mL) and EtOAc (5 mL) and washed with H_2O (2 × 5 mL) and saturated aqueous sodium chloride (5 mL). Chromatography (SiO_2, 1.9 × 15 cm, 33% EtOAc−hexane) afforded **3** (4.9 mg, 72%) as a white solid identical in all compared respects (^{1}H NMR, ^{13}C NMR, mp)[31] with authentic material: mp 264−266 °C (lit.[15] mp 264−266 °C); ^{1}H NMR (DMSO-d_6, 250 MHz) δ 9.88 (br s, 1H), 9.46 (br s, 1H), 9.42 (br s, 1H), 8.05 (br s, 1H), 7.60 (s, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 6.70 (d, J = 8.6 Hz, 2H), 6.66 (d, J = 8.6 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.4, 156.6, 156.3, 153.6, 140.8, 131.8, 129.4, 128.7, 127.3, 125.5, 123.9, 123.1, 121.3, 120.0, 115.8, 115.2, 114.6, 111.9, 103.1; IR (film) ν_{max} 3406, 1653, 1613, 1420, 1269, 1025, 997 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 412.1201 (M + H^+, $C_{25}H_{17}NO_5$ requires 412.1185).

1,2-Bis(3,4,5-trimethoxyphenyl)acetylene (26). A stirred solution of **25**[21] (0.50 g, 1.58 mmol), $PdCl_2(PPh_3)_2$ (0.11 g, 0.16 mmol, 0.1 equiv), CuI (0.09 g, 0.47 mmol, 0.3 equiv), and Bu_4NI (1.75 g, 4.74 mmol, 3.0 equiv) in 5:1 DMF−Et_3N (8.0 mL) under Ar at 70 °C was treated with **24**[21] (0.43 g, 2.21 mmol, 1.4 equiv) in 5:1 DMF−Et_3N (4.0 mL) over a period of 1.5 h. The reaction mixture was allowed to stir for an additional 30 min before it was cooled to 25 °C, diluted with 10% aqueous HCl (50 mL), and extracted with $CHCl_3$ (4 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 5.5 × 15 cm, $CHCl_3$) afforded **26** (0.51 g, 90%) as a yellow-brown solid. An analytically pure sample could be prepared by recrystallizaton from toluene: mp 192−195 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.77 (s, 4H),

(31) We thank Professors Capon and Scheuer for providing copies of the original spectra of lamellarin O and lukianol A, respectively, for direct comparison.

3.89 (s, 12H), 3.88 (s, 6H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 153.0, 139.0, 118.0, 108.6, 88.4, 60.9, 56.0; IR (film) ν_{max} 2941, 1574, 1508, 1410, 1236, 1128 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 358.1406 (M^+, $C_{20}H_{22}O_6$ requires 358.1416). Anal. Calcd for $C_{20}H_{22}O_6$: C, 67.03; H, 6.19. Found: C, 67.34; H, 6.36.

Dimethyl 4,5-Bis(3,4,5-trimethoxyphenyl)-1,2-diazine-3,6-dicarboxylate (27). A stirred mixture of **26** (25.0 mg, 69.0 μmol) and **6**[13] (20.5 mg, 104 μmol, 1.5 equiv) in toluene (0.14 mL) was warmed to 110 °C under Ar for 24 h and was cooled to 25 °C, and additional **6** (20.5 mg, 104 μmol, 1.5 equiv) was added to the reaction. The mixture was warmed at 110 °C for an additional 24 h and was allowed to cool to 25 °C. Chromatography (SiO_2, 1.0 × 14 cm, 50% EtOAc−hexane) afforded **27** (23.4 mg, 90%) as a light orange solid: mp 143−144 °C (50% EtOAc−hexane); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.28 (s, 4H), 3.83 (s, 12H), 3.64 (s, 12H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 165.6, 154.5, 153.2, 138.6, 137.5, 127.5, 106.6, 61.0, 56.1, 53.2; IR (film) ν_{max} 2918, 1741, 1584, 1411, 1239, 1125 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 661.0824 (M + Cs^+, $C_{26}H_{28}N_2O_{10}$ requires 661.0798). Anal. Calcd for $C_{26}H_{28}N_2O_{10}$: C, 59.09; H, 5.34; N, 5.30. Found: C, 58.94; H, 5.10; N, 5.09.

Dimethyl 3,4-Bis(3,4,5-trimethoxyphenyl)pyrrole-2,5-dicarboxylate (28). A stirred solution of **27** (29.0 mg, 54.9 μmol) in HOAc (0.7 mL) under Ar at 25 °C was treated with powdered Zn (32 mg, 49.5 mmol, 9.0 equiv). After 6 h, additional powdered Zn (32 mg, 49.5 mmol, 9.0 equiv) was added, and the reaction mixture was allowed to stir for 12 h. The mixture was diluted with 5% CH_3OH−$CHCl_3$ (5 mL) and filtered through a pad of Celite which was rinsed with 5% CH_3OH−$CHCl_3$ (50 mL), and the solvent was removed under reduced pressure. Chromatography (SiO_2, 5.5 × 15 cm, 4% acetone−CH_2Cl_2) afforded **28** (19.6 mg, 69%) as a light yellow solid: mp 153−155 °C (4% acetone−CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.86 (br s, 1H), 6.37 (s, 4H), 3.82 (s, 12H), 3.64 (s, 12H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 160.6 (2C), 152.3 (4C), 137.1 (2C), 131.1 (2C), 128.1 (2C), 121.0 (2C), 108.3 (4C), 60.9 (2C), 56.0 (4C), 51.9 (2C); IR (film) ν_{max} 3270, 2938, 1710, 1586, 1465, 1340, 1240, 1125 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 648.0859 (M + Cs^+, $C_{26}H_{29}NO_{10}$ requires 648.0896). Anal. Calcd for $C_{26}H_{29}NO_{10}$: C, 60.58; H, 5.67; N, 2.72. Found: C, 60.94; H, 5.63; N, 2.66.

Dimethyl 3,4-Bis(3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyphenyl)ethyl]pyrrole-2,5-dicarboxylate (30). A stirred mixture of **28** (0.21 g, 0.41 mmol), 4-methoxyphenethyl bromide (**29**;[23] 0.44 g, 2.03 mmol, 5.0 equiv), and K_2CO_3 (0.28 g, 2.03 mmol, 5.0 equiv) in DMF (4.1 mL) under Ar was warmed to 110 °C for 1.5 h. The mixture was cooled to 25 °C, and the solvent was removed under reduced pressure. Chromatography (SiO_2, 2.5 × 15 cm, 2% acetone−CH_2Cl_2) afforded **30** (0.26 g, 100%) as a light yellow solid: mp 118−119 °C (2% acetone−CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.22 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 6.24 (s, 4H), 4.81 (t, *J* = 7.4 Hz, 2H), 3.81 (s, 6H), 3.80 (s, 3H), 3.66 (s, 6H), 3.65 (s, 12H), 3.12 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 162.0, 158.3, 152.2, 136.7, 130.3, 130.0, 129.8, 123.8, 113.9, 107.8, 60.9, 56.0, 55.2, 51.6, 48.9, 37.4; IR (film) ν_{max} 2934, 1719, 1584, 1512, 1410, 1237, 1127 cm^{-1}. Anal. Calcd for $C_{35}H_{39}NO_{11}$: C, 64.70; H, 6.05; N, 2.16. Found: C, 64.39; H, 6.41; N, 2.37.

3,4-Bis(3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyphenyl)ethyl]-pyrrole-2,5-dicarboxylic Acid (31). A stirred solution of **30** (0.13 g, 0.20 mmol) and KOH (0.11 g, 2.00 mmol, 5.0 equiv) in 4:2:1 dioxane−CH_3OH−H_2O (1.0 mL) was warmed to 70 °C for 72 h. The reaction mixture was diluted with 10% aqueous KOH (5 mL) and was extracted with EtOAc (5 mL). The aqueous phase was acidified with concentrated HCl (pH 1), extracted with 5% CH_3OH−$CHCl_3$ (20 mL), and dried (Na_2SO_4), and the solvent was removed under reduced pressure to afford pure **31** (0.13 g, 100%): mp 182−185 °C (Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.13 (d, *J* = 8.5 Hz, 2H), 6.28 (d, *J* = 8.5 Hz, 2H), 6.31 (s, 4H), 4.92 (t, *J* = 7.4 Hz, 2H), 3.81 (s, 6H), 3.77 (s, 3H), 3.65 (s, 12H), 3.12 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 165.4, 158.5, 152.3, 137.1, 132.7, 129.9, 129.8, 129.0, 123.6, 114.0, 108.2, 60.8, 56.0, 55.2, 49.4, 37.2; IR (film) ν_{max} 3189, 2935, 1712, 1585, 1513, 1425, 1240, 1127 cm^{-1}; FABHRMS (NBA/CsI) *m/z* 661.0824 (M + Cs^+, $C_{33}H_{35}NO_{11}$ requires 661.0798). Anal. Calcd for $C_{33}H_{35}NO_{11}$: C, 63.76; H, 5.68; N, 2.25. Found: C, 63.90; H, 5.70; N, 2.35.

3,4-Bis(3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyphenyl)ethyl]-pyrrole-2,5-bis[*N*-(2-(4-methoxyphenyl)-2-phenylthio)ethyl]carboxamide (33). A stirred solution of **31** (5.5 mg, 8.8 μmol), 2-(4-methoxyphenyl)-2-(phenylthio)-1-aminoethane (**32**;[24] 4.6 mg, 18 μmol, 2.05 equiv), and *i*-Pr_2NEt (8.0 μL, 40 μmol, 5.0 equiv) under Ar at 25 °C was treated with PyBrOP[25] (8.8 mg, 19 μmol, 2.1 equiv), and the reaction was stirred for 2 h. Chromatography (SiO_2, 1.5 × 12 cm, 10% EtOAc−hexane) afforded **33** (9.9 mg, 100%) as a light yellow foam: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.21−7.12 (m, 12H), 6.87 (d, *J* = 6.8 Hz, 4H), 6.82 (d, *J* = 6.8 Hz, 2H), 6.69 (d, *J* = 6.8 Hz), 6.21 (s, 4H), 5.69 (t, *J* = 4.0 Hz, 2H), 3.96 (dt, *J* = 6.1, 2.4 Hz, 2H), 3.80 (s, 6H), 3.77 (s, 3H), 3.74−3.67 (m, 2H), 3.72 (s, 6H), 3.61 (s, 12H), 3.56−3.46 (m, 2H), 3.04 (t, *J* = 4.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.5, 158.9, 158.2, 153.1, 137.1, 133.0, 132.8, 130.8, 130.4, 130.3, 128.9, 128.8, 128.6, 127.6, 126.2, 124.8, 113.9, 113.7, 107.3, 60.8, 56.0, 55.2, 55.1, 51.5, 48.4, 43.5, 37.6, 29.7; FABHRMS (NBA) *m/z* 1104.4195 (M + H^+, $C_{63}H_{65}N_3O_{11}S_2$ requires 1104.4138).

***E,E*-3,4-Bis(3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyphenyl)ethyl]pyrrole-2,5-bis[*N*-2-(4-methoxystyryl)]carboxamide (Permethyl Storniamide A, 5).** A stirred solution of **33** (14.5 mg, 13.0 μmol) in CH_3OH (0.2 mL) under Ar at 25 °C was treated with $NaIO_4$ (56 mg, 0.26 mmol, 20.0 equiv) in H_2O (0.1 mL). The mixture was stirred for 6 h at 25 °C, diluted with H_2O (5 mL), and extracted with 5% CH_3OH−$CHCl_3$ (4 × 5 mL) to afford the disulfoxide as a light yellow solid which was used without further purification. The resulting solid and Na_2CO_3 (3.5 mg, 33 μmol, 5.0 equiv) were diluted with toluene (0.26 mL) and were warmed to 80 °C (18 h) and 110 °C (12 h). PTLC (SiO_2, 0.25 mm × 20 cm × 20 cm, 50% EtOAc−hexane, two elutions) afforded a 2:1 mixture of the desired *E,E*-isomer **5** (5.8 mg, 50%) and the *E,Z*-isomer (3.0 mg, 26%). Data for *E,E*-**5**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37 (dd, *J* = 14.6, 10.8 Hz, 2H), 7.16 (d, *J* = 8.1 Hz, 4H), 7.13 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 10.5 Hz, 2H), 6.81 (d, *J* = 8.1 Hz, 4H), 6.74 (d, *J* = 8.4 Hz, 2H), 6.37 (s, 4H), 5.45 (d, *J* = 14.3 Hz, 2H), 5.01 (t, *J* = 7.0 Hz, 2H), 3.85 (s, 6H), 3.79 (s, 6H), 3.68 (s, 15H), 3.15 (t, *J* = 7.0 Hz, 2H);[32] ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 162.0, 158.7, 158.3, 157.9, 153.4, 137.8, 130.4, 128.3, 128.2, 126.6, 126.3, 126.0, 120.2, 114.2, 113.7, 113.0, 107.8, 61.0, 56.3, 55.3, 55.1, 48.6, 37.5; IR (film) ν_{max} 3374, 2936, 2834, 1667, 1650, 1607, 1580, 1511, 1504, 1245, 1177, 1126, 1032, 944, 845 cm^{-1}; FABHRMS (NBA/NaI) *m/z* 906.3607 (M + Na^+, $C_{51}H_{53}N_3O_{11}$ requires 906.3578). Data for *E,Z*-**5**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53 (d, *J* = 11.3 Hz, 1H), 7.37 (dd, *J* = 14.6, 11.1 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 4H), 7.05 (d, *J* = 11.1 Hz, 1H), 6.93 (dd, *J* = 11.3, 9.4 Hz, 1H), 6.80 (d, *J* = 8.6 Hz, 2H), 6.76 (d, *J* = 8.6 Hz, 2H), 6.63 (d, *J* = 8.6 Hz, 2H), 6.41 (d, *J* = 8.6 Hz, 2H), 6.32 (s, 2H), 6.30 (s, 2H), 5.62 (d, *J* = 9.5 Hz, 1H), 5.43 (d, *J* = 14.6 Hz, 1H), 4.93 (t, *J* = 7.0 Hz, 2H), 3.85 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 3.72 (s, 3H), 3.67 (s, 6H), 3.64 (s, 9H), 3.14 (t, *J* = 7.0 Hz, 2H); FABHRMS (NBA/CsI) *m/z* 1016.2777 (M + Cs^+, $C_{51}H_{53}N_3O_{11}$ requires 1016.2734). A trace amount of the *Z,Z*-isomer was also isolated, accumulated, and characterized: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56 (d, *J* = 11.3 Hz, 2H), 7.17 (d, *J* = 8.6 Hz, 2H), 6.92 (dd, *J* = 11.3, 9.4 Hz, 2H), 6.75 (d, *J* = 8.4 Hz, 2H), 6.62 (d, *J* = 8.6 Hz, 4H), 6.41 (d, *J* = 8.6 Hz, 4H), 6.23 (s, 4H), 5.61 (d, *J* = 9.4 Hz, 2H), 4.84 (t, *J* = 7 Hz, 2H), 3.74 (s, 6H), 3.72 (s, 3H), 3.62 (s, 12H), 3.12 (t, *J* = 7 Hz, 2H); FABHRMS (NBA/CsI) *m/z* 1016.2779 (M + Cs^+, $C_{51}H_{53}N_3O_{11}$ requires 1016.2734).

Determination of Cytotoxicity and the Effect on MDR Reversals. The L1210 cytotoxic assays were performed as previously described.[33] Human colon carcinoma cells HCT116, HCT116/VM46 (MDR, overexpression of P-glycoprotein), and HCT116/VP35 (with reduced levels of topoisomerase II)[34] were obtained from Drs. D. M. Floyd and C. R. Fairchild of Bristol-Myers Squibb and were cultured in RPMI 1640 media supplemented with 10% FBS. Upon performing the assay, 3000 cells in 100 μL medium were seeded into each well of 96-well cluster

(32) The ^{1}H NMR of *E,E*-**5** ($CDCl_3$, 400 MHz) was in excellent agreement with that reported for **4**[9] (acetone-d_6−CD_3OD, 9:1), and only the β enamide proton exhibited a significantly altered chemical shift of δ 5.45 (d, *J* = 14.3 Hz) versus δ 5.74 (d, *J* = 14 Hz).[9]

(33) Boger, D. L.; Chai, W.; Jin, Q. *J. Am. Chem. Soc.* **1998**, *120*, 7220.

(34) Long, H. B.; Wang, L.; Lorico, A.; Wang, R. C. C.; Brattain, M. G.; Casazza, A. M. *Cancer Res.* **1991**, *51*, 5275.

dishes, and cell incubation was allowed to proceed for 24 h. Then 98 μL of the medium was added to each well. The antitumor drugs (vinblastine or doxorubicin), verapamil, and our synthetic compounds were dissolved in DMSO and were prepared in serial dilutions. A 1 μL sample of the synthetic compound solution and a 1 μL sample of the antitumor drug solution were added sequentially to the same well for MDR reversal studies, or 2 μL of the synthetic compound solution was added to a well for cytotoxicity studies. The components in the well were mixed by drawing the liquid up and down four times using a pipet. The culture was incubated for another 72 h. At the end of the incubation, the medium was removed and 100 μL of 10 mM acid phosphatase substrate, *p*-nitrophenyl phosphate (Sigma), in 0.1 M sodium acetate (pH 5.5), 0.1% Triton X-100, was added to each well. This incubation was allowed to proceed for 10 h, before 50 μL of 1 N sodium hydroxide was added for colorimetric absorbance determination[35] (*Emax*, Molecular Devices). The wells in the first row of a culture plate contained cultural medium only and were used as blanks. Wells containing cells treated only with DMSO (1%) were used as controls.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (Grant CA42056), the Skaggs Institute for Chemical Biology, the award of an ACS Medicinal Division fellowship sponsored by Bristol-Myers Squibb (1998−1999, C.W.B.), and a National Institutes of Health postdoctoral fellowship (F32 GM19021, C.A.S.). We are especially grateful to D. M. Floyd and C. R. Fairchild of Bristol-Myers Squibb for providing the HCT116, HCT116/VM46, and HCT116/VP35 cell lines enlisted herein.

JA982078+

(35) Connolly, D. T.; Knight, M. B.; Harakas, N. K.; Wittwer, A. J.; Peder, J. *Anal. Biochem.* **1986**, *152*, 136.