Total Synthesis of Ningalin B Utilizing a Heterocyclic Azadiene Diels−Alder Reaction and Discovery of a New Class of Potent Multidrug Resistant (MDR) Reversal Agents

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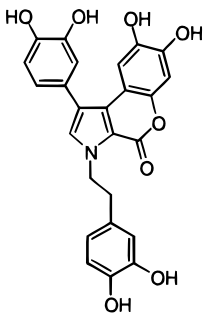
A concise, efficient approach to the total synthesis of ningalin B (**1**) based on a heterocyclic azadiene Diels−Alder strategy (1,2,4,5-tetrazine → 1,2-diazine → pyrrole) ideally suited for construction of the densely functionalized pyrrole core found in the natural product is detailed. Examination of the natural product and a number of synthetic intermediates revealed that while lacking inherent cytotoxic activity, many 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 ningalin class of marine natural products including ningalin B (**1**, Figure 1) possess a common 3,4-diaryl-substituted pyrrole nucleus bearing a 2-carboxylate. Ningalin B (**1**) is the second member of this newly described family of marine natural products which were isolated by Fenical (1997) from an ascidian of the genus *Didemnum* collected in western Australia near Ningaloo Reef.[1] Consequently, **1** and the related ningalins are the newest members of a family of 3,4-dihydroxyphenylalanine (DOPA)-derived *o*-catechol metabolites that include the tunichromes.[2]

The lamellarins are a related rapidly growing class of marine natural products which were first isolated from the prosobranch mollusc *Lamellaria* sp., and important members of this class have been disclosed by Bowden, Faulkner, Fenical, Capon, and Scheuer.[3,4] Recent investigations of several lamellarins demonstrated their cytotoxic activity, revealed equally effective cytotoxic activity against multidrug-resistant (MDR) cell lines, and revealed MDR reversal even at noncytotoxic concentrations by inhibition of P-glycoprotein (P-gp)-mediated drug efflux.[5] Thus, they constitute a new class of antitumor agents which reverse MDR more effectively than verapamil and resensitize resistant malignant cells to front line therapeutics. In recent studies, we were able to define a number of related structures that lack cytotoxic activity but which effectively reverse MDR.[6]



Ningalin B (1)

Figure 1.

Herein we describe an extension of these studies to the total synthesis of ningalin B (**1**) and the subsequent biological evaluation of the natural product and a number of structurally related synthetic intermediates. The synthetic approach, complementary to the efforts described to date,[7] employs a heteroaromatic azadiene Diels−Alder reaction[8] to assemble the substituents onto a six-membered 1,2-diazine core which is followed by a reductive ring contraction reaction[9−11] to provide the corresponding pyrrole (Scheme 1).

Total Synthesis of Ningalin B. The requisite diphenylacetylene **5** was prepared by a palladium(0)-catalyzed

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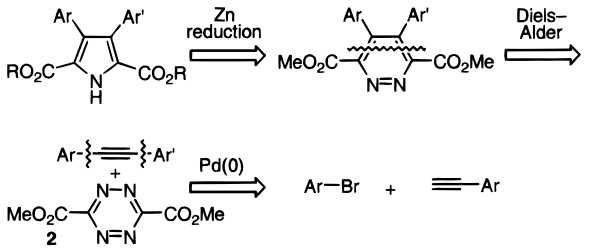
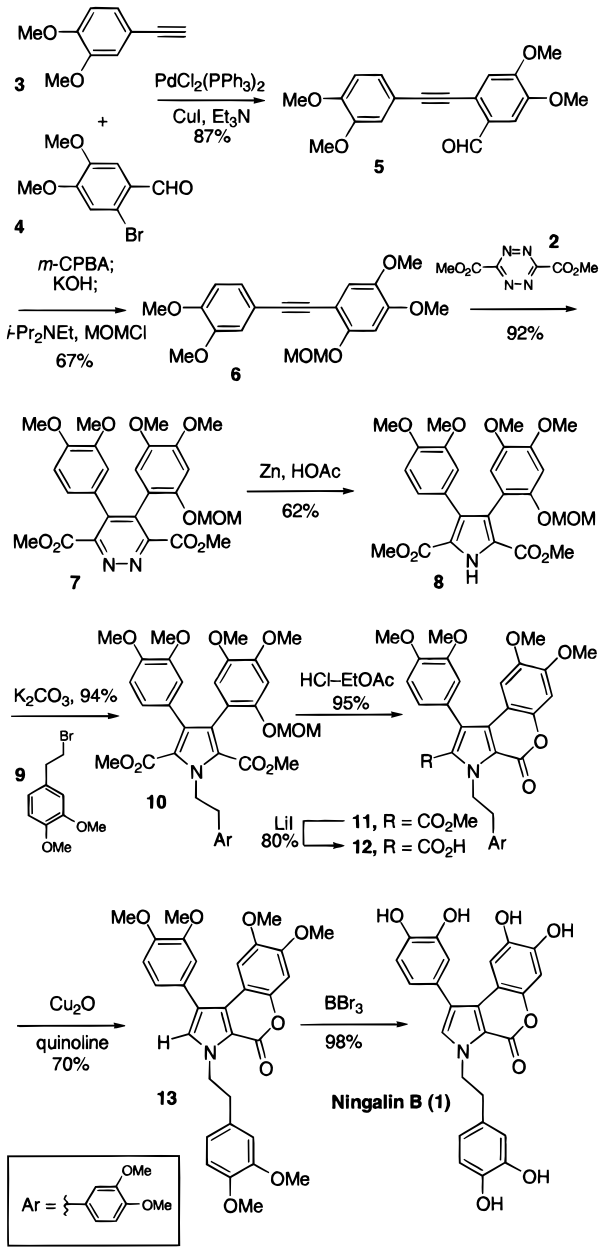
Scheme 1



cross-coupling of the terminal acetylene **3**[12] and **4** (0.05 equiv of Pd(0), 0.3 equiv of CuI, Et_3N, 87%) in which slow addition of the acetylene was necessary to suppress competitive formation of the coupled diacetylene (Scheme 2). Conversion to the methoxymethyl ether **6** was accomplished by Baeyer−Villiger oxidation of aldehyde **5** (1.2 equiv of *m*-CPBA), formate hydrolysis (KOH), and subsequent protection of the phenol (3.0 equiv of MOMCl, 4.0 equiv of *i*-Pr_2NEt, 67% overall). The first of the two key conversions, the Diels−Alder reaction of the electron-rich acetylene **6** with the electron-deficient 1,2,4,5-tetrazine **2**,[11,13] proceeded to give the desired 1,2-diazine **7** in excellent yield (mesitylene, 140 °C, 92%). The relative facility of this inverse electron demand [4 + 2] cycloaddition may be attributed to the electron-donating properties of the dienophile aryl alkoxy groups. Thus, the oxygenation pattern found in the diaryl acetylene **6** increases the nucleophilic character and improves what is typically a poor reactivity of an alkyne toward **2**.[14] Subsequent reductive ring contraction (Zn, HOAc, 62%) of **7** afforded the core pyrrole structure found in the natural product. *N*-Alkylation with the phenethyl bromide **9**[15] (5.0 equiv of K_2CO_3, 94%) and subsequent MOM deprotection with concomitant lactonization (HCl−EtOAc, 95%) provided mono-lactone **11**. Selective hydrolysis of the methyl ester (LiI, 80%) and decarboxylation (Cu_2O, quinoline, 220 °C, 5 min, 70%) afforded hexamethyl ningalin B (**13**). Decarboxylation with alternative copper sources or those conducted at lower temperatures or with longer reaction times resulted in lower yields (0−44%). Exhaustive demethylation with BBr_3 completed the total synthesis of ningalin B and provided material identical in all respects (^1H NMR, ^{13}C NMR, IR, MS) with authentic material.[1]

Initial attempts to promote decarboxylation under acidic conditions resulted in either no reaction (neat TFA, 60 °C, 12 h) or Friedel−Crafts acylation (neat Eaton's acid, 25 °C, 18 h) to provide **14** (Scheme 3). Although not the object of the present efforts, the fused tricyclic ring system consisting of a seven-membered ketone flanked by an aryl group and a pyrrole has been formed by Friedel−Crafts acylation in the synthesis of *cephalotaxus* alkaloids.[16] On the basis of the precedented ease of formation of the seven-membered ring and ^1H and HMBC NMR spectroscopy, formation of the alternative five-

Scheme 2



Scheme 3

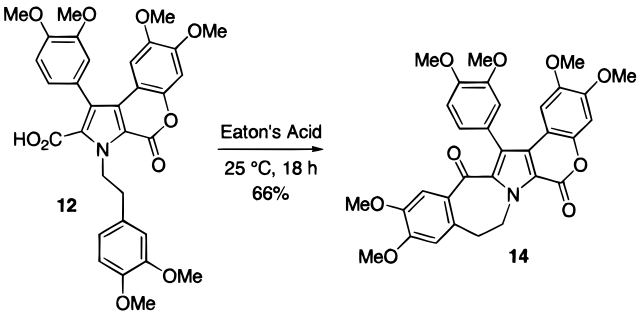


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Table 1. In Vitro Cytotoxic Activity

	IC_{50} (μM)[a]			
compound	L1210	HCT116 wild type	HCT116/VM46 (MDR)	HCT116/VP35 (reduced topo II)
ningalin B (**1**)	10	12	60	30
7	10	40	60	60
8	50	>100	>100	100
10	80	90	>100	70
11	6	6	40	10
12	30	70	>100	>100
13	50	30	40	30
14	90	60	>100	70
vinblastine		0.002	0.07	
doxorubicin		0.01	0.07	0.06
etoposide		0.5	40	40

[a] Duplicate assays, average IC_{50}.

membered ring was excluded. Importantly, **14** proved to be the most potent MDR reversal agent identified in this series, causing hypersensitivity toward vinblastine in the HCT/VM46 MDR cell line.

Cytotoxic Activity and Reversal of Multidrug Resistance. A number of compounds in the structurally related lamellarin class of natural products possess cytotoxic activity.[5] With exception of ningalin A,[6] the biological evaluation of the ningalin family has not been explored. Consequently, ningalin B and a number of structurally related synthetic intermediates were tested in a L1210 cytotoxic assay, and the results are summarized in Table 1. Ningalin B was found to be only moderately active against both the L1210 and HCT116 cell lines, and a number of synthetic intermediates displayed a similar level of activity due to their comparable structures. Notably, the *O*-methyl derivative of ningalin B is 5-fold less active against L1210 and 2.5-fold less active against HCT116 than ningalin B, in agreement with previous studies where an increase in the extent of *O*-methylation results in a decrease in cytotoxic activity.[6]

More importantly, a select set of the naturally occurring lamellarins have been shown to exhibit equally potent cytotoxic activity against multidrug resistant (MDR) cell lines due to overexpression of P-glycoprotein and to reverse MDR at noncytotoxic concentrations, resensitizing the resistant cell lines to conventional therapeutic agents.[5] P-gp is a 170 kDa plasma membrane glycoprotein encoded in humans by the *MDR1* gene which exports drugs out of mammalian cells, lowering their intracellular concentration.[17] Therefore, **7**−**14** were also tested against a wild-type human colon cancer cell line (HCT116) 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 allows an accurate assessment of the MDR sensitivity as well as an assessment of one potential therapeutic target. All of the agents examined showed little or no intrinsic cytotoxic activity against either HCT116 or the resistant cell lines.

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)
ningalin B (**1**)	0.02	4 (10)	0.5	0
7	0.02	4 (10)	0.1	0
8	0.02	4 (10)	0.1	0
10	0.004	18 (50)	0.05	1 (20)
10 (7.5 μM)	0.002	35 (100)	0.02	4 (50)
11	0.002	35 (100)	0.02	4 (50)
11 (7.5 μM)	0.0006	117 (330)	0.009	9 (110)
12	0.02	4 (10)	0.07	1 (14)
13	0.002	35 (100)	0.02	4 (50)
13 (7.5 μM)	0.001	70 (200)	0.01	7 (100)
14	0.0007	100 (290)	0.03	2 (33)
verapamil				
(1.0 μM)	0.02	10 (15)	0.13	
(7.5 μM)	0.003	67 (100)	0.05	1 (24)

[a] IC_{50} (μM) of vinblastine or doxorubicin against the MDR resistant cell line HCT116/VM46 in the presence of 1 μM (unless indicated otherwised) of the indicated compound. IC_{50} values in the absence of added compound are 0.07 μM (vinblastine) and 0.07 μM (doxorubicin). For the wild-type HCT116 cell line not subject to MDR, IC_{50} values are 0.002 μM (vinblastine) and 0.01 μM (doxorubicin). [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.

Fundamentally more important, many of the agents were found capable of reversing MDR at noncytotoxic concentrations, resensitizing HCT116/VM46 to vinblastine and doxorubicin (Table 2). Of the compounds examined, **10**, **11**, **13**, and **14** were able to resensitize HCT116/VM46 to vinblastine and doxorubicin at 1 μM and to do so more effectively than verapamil. While lacking inherent cytotoxicity, **11** and **13** showed complete MDR reversal at this concentration and **14** caused hypersensitivity of HCT116/VM46 to vinblastine, exhibiting an IC_{50} value 3× lower than wild-type treatment with vinblastine alone. At the higher concentrations required for complete reversal by verapamil (7.5 μM), **10** showed complete MDR reversal and the HCT116/VM46 cell line became hypersensitive to vinblastine in the presence of **11** and **13**. The HCT116/VP35 resistant cell line showed no resensitization toward vinblastine or doxorubicin in the presence of the examined agents, indicating that the MDR reversal activity is due to interaction with P-gp. Consistent with its action on Pgp-170, **14** inhibited dye efflux[5] (rhodamine 123) from HT116/VM46, returning the dye retention to levels equivalent to that of wild-type HCT116 (Figure 2).

Conclusions. A concise total synthesis of ningalin B (**1**) was described enlisting a 1,2,4,5-tetrazine → 1,2-diazine → pyrrole Diels−Alder strategy featuring the unusually effective [4 + 2] cycloaddition of the electron-deficient 1,2,4,5-tetrazine **2** with an unsymmetrical, electron-rich alkyne. Ningalin B is a member of a class of marine natural products characterized by a highly functionalized tetra- or pentasubstituted pyrrole which is ideally suited to construction using this strategy. While lacking inherent cytotoxic activity, the ningalin B synthetic precursors **10**, **11**, **13**, and **14**, but not ningalin B itself, were shown to potently reverse MDR, resensitizing a resistant human colon cancer cell line (HCT116/VM46) to vinblastine and doxorubicin. These agents, including **14** bearing a novel ring system, constitute the initial members of a new class of effective MDR reversal agents.

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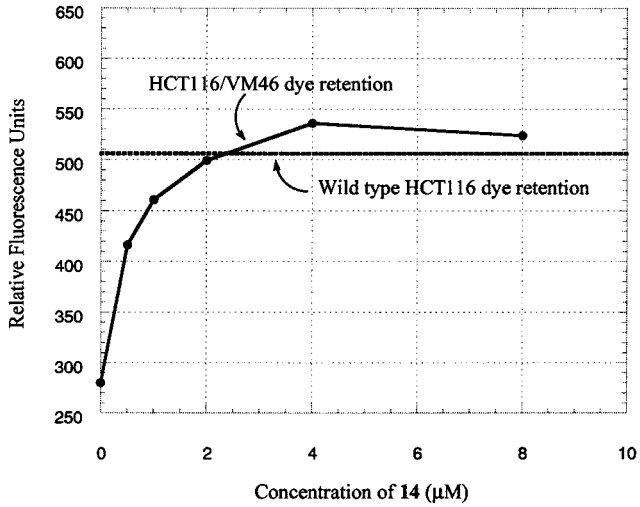

Figure 2. Accumulation of rhodamine 123 in the HCT116/VM46 cell line after 30 min incubation in 40 μM rhodamine in phosphate buffer solution: Quesada, A. R.; Gravalos, M. D. G.; Puentes, J. L. F. *Br. J. Cancer* **1996**, *74*, 677.

Experimental Section

2-[(3,4-Dimethoxyphenyl)ethynyl]-4,5-dimethoxybenzaldehyde (5). A stirred solution of **4** (2.7 g, 11 mmol, 1.0 equiv), $PdCl_2(PPh_3)_2$ (0.39 g, 0.55 mmol, 0.05 equiv), and CuI (0.63 g, 3.31 mmol, 0.3 equiv) in 5:1 DMF−Et_3N (106 mL) under Ar at 75 °C was treated with **3**[12] (2.23 g, 13.8 mmol, 1.25 equiv) in 5:1 DMF−Et_3N (42 mL) over a period of 2.5 h. The reaction mixture was allowed to stir for an additional 1.5 h before it was cooled to 25 °C and concentrated under reduced pressure. Chromatography (SiO_2, 4.5 × 20 cm, CH_2Cl_2) afforded **5** (1.50 g, 87% yield) as a yellow solid: mp 148−149 °C (EtOAc−hexanes); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 10.51 (s, 1H), 7.42 (s, 1H), 7.17 (d, J = 8.0 Hz, 1H), 7.05 (app s, 2H), 6.87 (d, J = 8.4 Hz, 1H), 4.00 (s, 3H), 3.96 (s, 3H), 3.93 (s, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.6, 153.8, 150.1, 149.6, 148.9, 130.0, 125.2, 122.0, 114.7, 114.2, 114.1, 111.2, 108.2, 95.4, 83.6, 56.4, 56.2, 56.06, 56.03; IR (film) ν_{max} 3005, 2933, 2831, 2255, 2203, 1680 cm^{-1}; FABHRMS (NBA/NaI) m/z 327.1228 (M + H$^+$, $C_{19}H_{18}O_5$ requires 327.1232). Anal. Calcd for $C_{19}H_{18}O_5$: C, 69.93; H, 5.56. Found: C, 69.75; H, 5.34.

2-[(3,4-Dimethoxyphenyl)ethynyl]-4,5-dimethoxy-1-(methoxymethoxy)benzene (6). A stirred solution of **5** (3.13 g, 9.60 mmol, 1.0 equiv) in CH_2Cl_2 (380 mL under Ar at 25 °C was treated with Na_2HPO_4 (3.27 g, 23.03 mmol, 2.4 equiv) and *m*-CPBA (3.98 g, 11.52 mmol, 1.2 equiv). After 18 h, the mixture was diluted with saturated aqueous $NaHCO_3$, extracted with EtOAc, washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated under reduced pressure. The formate was redissolved in MeOH (120 mL) and treated with 10% aqueous KOH (7.8 mL, 15.6 mmol, 1.6 equiv), and the mixture was stirred at 25 °C for 1.5 h. The reaction was quenched with the addition of 10% aqueous HCl, extracted with CH_2Cl_2, washed with H_2O, and dried (Na_2SO_4), and the solvent was removed under reduced pressure. An analytically pure sample of the phenol could be prepared by chromatography (SiO_2, 5% EtOAc/CH_2Cl_2): mp 164−165 °C (EtOAc−hexanes); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.13 (dd, J = 2.2, 8.5 Hz, 1H), 7.02 (d, J = 1.9 Hz, 1H), 6.88 (s, 1H), 6.85 (d, J = 8.5 Hz, 1H), 6.56 (s, 1H), 5.59 (s, 1H), 3.915 (s, 3H), 3.911 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 154.1, 152.4, 150.8, 150.1, 143.9, 125.4, 116.9, 116.4, 115.5, 112.6, 101.7, 101.5, 93.8, 84.6, 56.9, 56.2, 56.14, 56.09; IR (film) ν_{max} 3436, 3001, 2934, 2837, 2360, 2340 cm^{-1}; MALDIHRMS (DHB) m/z 337.1058 (M + Na$^+$, $C_{18}H_{18}O_5$ requires 337.1046). A solution of the crude phenol in CH_2Cl_2 (100 mL) under Ar at 0 °C was treated with ^{i}Pr$_2$NEt (6.70 mL, 38.4 mmol, 4.0 equiv) and chloromethyl methyl ether (2.19 mL, 28.8 mmol, 3.0 equiv). The mixture was warmed to 25 °C and allowed to stir for 18 h. Following dilution with H_2O, the mixture was extracted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 4.5 × 15 cm, 5% EtOAc/CH_2Cl_2) afforded **6** (2.32 g, 67% yield) as an orange solid: mp 84−86 °C (EtOAc−hexanes); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.13 (dd, J = 1.5, 8.0 Hz, 1H), 7.04 (d, J = 1.5 Hz, 1H), 6.97 (s, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.75 (s, 1H), 5.24 (s, 2H), 3.90 (m, 12H), 3.58 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.9, 150.2, 149.4, 148.7, 144.4, 124.9, 116.0, 115.2, 114.3, 111.1, 105.7, 102.1, 96.7, 92.2, 84.5, 56.6, 56.4, 56.2, 56.1, 56.0; IR (film) ν_{max} 2995, 2954, 2841, 2267, 2195 cm^{-1}; MALDIHRMS (DHB) m/z 358.1411 (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, 66.81; H, 6.42.

Dimethyl 4-(4,5-Dimethoxy-2-(methoxymethoxy)phenyl)-5-(3,4-dimethoxyphenyl)-1,2-diazine-3,6-dicarboxylate (7). A solution of **6** (1.10 g, 3.07 mmol, 1.0 equiv) and 3,6-dicarbomethoxy-1,2,4,5-tetrazine (**2**,[13] 0.91 g, 4.60 mmol, 1.5 equiv) in mesitylene (15.4 mL) was warmed at 140 °C under Ar for 24 h. Additional **2** (0.91 g, 4.60 mmol, 1.5 equiv) was added, and the mixture was maintained at 140 °C for an additional 24 h before the reaction mixture was cooled to 25 °C, and the solvent was evaporated. Chromatography (SiO_2, 4.5 × 20 cm, 30% EtOAc/CH_2Cl_2) provided **7** (1.49 g, 92% yield) as an orange oil. An analytically pure sample was prepared by recrystallization from EtOAc−hexanes: mp 131−133 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.77 (d, J = 8.4 Hz, 1H), 6.71 (m, 2H), 6.56 (d, J = 1.9 Hz, 1H), 6.35 (s, 1H), 4.93 (d, J = 7.0 Hz, 1H), 4.74 (d, J = 7.0 Hz, 1H), 3.85 (app s, 6H), 3.84 (s, 3H), 3.82 (s, 3H), 3.61 (s, 3H), 3.57 (s, 3H), 3.25 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.1, 165.4, 155.2, 154.8, 150.8, 149.7, 149.3, 148.9, 144.3, 139.0, 136.3, 125.6, 121.7, 114.1, 113.2, 112.3, 110.8, 100.6, 96.4, 56.4, 56.14, 56.11, 56.0, 55.9, 53.3, 53.2; IR (film) ν_{max} 3004, 2955, 2839, 1742, 1608 cm^{-1}; FABHRMS (NBA/NaI) m/z 551.1663 (M + Na$^+$, $C_{26}H_{28}N_2O_{10}$ requires 551.1642). Anal. Calcd for $C_{26}H_{28}N_2O_{10}$: C, 59.09; H, 5.34; N, 5.30. Found: C, 59.05; H, 5.14; N, 5.20.

Dimethyl 3-(4,5-Dimethoxy-2-(methoxymethoxy)phenyl)-4-(3,4-dimethoxyphenyl)pyrrole-2,5-dicarboxylate (8). A solution of **7** (1.01 g, 1.91 mmol, 1.0 equiv) in HOAc (25 mL) under Ar at 25 °C was treated with activated Zn dust (1.25 g, 19.1 mmol, 10 equiv), stirred for 4 h, and then treated with additional Zn dust (1.25 g, 10 equiv). After 14.5 h, the slurry was diluted with 10% MeOH/$CHCl_3$ (25 mL) and stirred 3 h at 25 °C. The mixture was filtered through Celite and rinsed with 10% MeOH/$CHCl_3$, and the filtrate was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated in vacuo. Chromatography (SiO_2, 4.5 × 15 cm, 25% EtOAc/CH_2Cl_2) afforded **8** (0.61 g, 62% yield) as an orange oil. An analytically pure sample could be prepared by recrystallization from EtOAc−hexanes: mp 162−163 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.80 (s, 1H), 6.81 (dd, J = 1.9, 8.1 Hz, 1H), 6.74 (m, 2H), 6.67 (d, J = 1.9 Hz, 1H), 6.49 (s, 1H), 4.82 (d, J = 6.3 Hz, 1H), 4.57 (d, J = 6.3 Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.77 (s, 3H), 3.64 (s, 3H), 3.62 (s, 3H), 3.25 (s, 3H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 161.41, 161.36, 151.1, 150.4, 149.3, 149.1, 144.9, 132.4, 127.9, 127.1, 124.0, 123.7, 122.0, 117.3, 117.0, 115.7, 111.6, 102.7, 97.1, 56.8, 56.2, 56.0, 55.9, 55.8, 51.8, 51.7; IR (film) ν_{max} 3282, 2952, 2834, 1713 cm^{-1}; MALDIHRMS (DHB) m/z 515.1800 (M$^+$, $C_{26}H_{29}NO_{10}$ requires 515.1791). Anal. Calcd for $C_{26}H_{29}NO_{10}$: C, 60.48; H, 5.67; N, 2.72. Found: C, 60.37; H, 5.59; N, 2.68.

Dimethyl 3-(4,5-Dimethoxy-2-(methoxymethoxy)phenyl)-4-(3,4-dimethoxyphenyl)-1-[2-(3,4-dimethoxyphenyl)-ethyl]pyrrole-2,5-dicarboxylate (10). A stirred mixture of **8** (297 mg, 0.58 mmol, 1.0 equiv), 3,4-dimethoxyphenethyl bromide (**9**,[15] 707 mg, 2.88 mmol, 5.0 equiv), and K_2CO_3 (398 mg, 2.88 mmol, 5 equiv) in DMF (5.8 mL) under Ar was warmed to 70 °C. After 2.5 h, the mixture was cooled to 25 °C and solvent was removed in vacuo. Chromatography (SiO_2, 3.5 × 15 cm, 20% EtOAc/CH_2Cl_2) provided **10** (372 mg, 94% yield) as a yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.84 (dd, J = 1.5, 8.1 Hz, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.78 (d, J = 1.5 Hz, 1H), 6.70 (m, 2H), 6.60 (dd, J = 1.9 Hz, 8.1 Hz, 1H), 6.56 (d,

J = 1.8 Hz, 1H), 6.35 (s, 1H), 4.89 (m, 2H), 4.76 (m, 2H), 3.89 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.63 (s, 3H), 3.60 (s, 3H), 3.59 (s, 3H), 3.56 (s, 3H), 3.27 (s, 3H), 3.09 (t, J = 7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.24, 162.19, 149.6, 148.9, 148.6, 147.83, 147.79, 147.65, 143.6, 131.2, 130.9, 127.3, 126.5, 125.1, 123.9, 122.7, 121.2, 117.0, 114.8, 113.8, 112.4, 111.3, 110.2, 101.5, 96.8, 56.2, 56.15, 56.02, 55.91, 55.85, 55.82, 55.7, 51.51, 51.48, 48.7, 38.0; IR (film) ν_{max} 2949, 2834, 1718, 1610 cm^{-1}; FABHRMS (NBA/NaI) m/z 702.2553 (M + Na^+, $C_{36}H_{41}NO_{12}$ requires 702.2526).

Methyl 7,8-Dimethoxy-3-(2-(3,4-dimethoxyphenyl)ethyl)-1-(3,4-dimethoxyphenyl)-[1]-benzopyrano[3,4-*b*]pyrrol-4(3*H*)-one-2-carboxylate (11). A sample of **10** (272 mg, 400 μmol, 1.0 equiv) was treated with 3 M HCl−EtOAc (16 mL) and stirred under Ar at 25 °C for 2 h. Chromatography of the concentrated mixture (SiO_2, 4.5 × 5 cm, 15% EtOAc/CH_2Cl_2) afforded pure **11** (229 mg, 95%) as a light yellow solid: mp 192−193 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.97 (d, J = 8.2 Hz, 1H), 6.90 (dd, J = 2.0, 8.1 Hz, 1H), 6.87 (s, 1H), 6.85 (d, J = 1.9 Hz, 1H), 6.79 (m, 3H), 6.51 (s, 1H), 5.10 (t, J = 7.6 Hz, 2H), 3.94 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.57 (s, 3H), 3.42 (s, 3H), 3.10 (t, J = 7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.4, 155.2, 149.3, 148.95, 148.89, 148.7, 147.9, 146.1, 145.8, 130.6, 129.6, 127.0, 126.9, 124.3, 122.8, 121.3, 117.6, 113.5, 112.4, 111.3, 111.0, 109.6, 104.6, 100.4, 56.2, 56.1 (2C), 56.0, 55.9, 55.6, 51.9, 48.8, 38.0; IR (film) ν_{max} 3001, 2952, 2835, 1732, 1699 cm^{-1}; FABHRMS (NBA/NaI) m/z 626.2017 (M + Na^+, $C_{33}H_{33}NO_{10}$ requires 626.2002).

7,8-Dimethoxy-3-(2-(3,4-dimethoxyphenyl)ethyl)-1-(3,4-dimethoxyphenyl)-[1]-benzopyrano[3,4-*b*]pyrrol-4(3*H*)-one-2-carboxylic Acid (12). A stirred mixture of **11** (120 mg, 0.20 mmol, 1.0 equiv) and LiI (80 mg, 0.60 mmol, 3.0 equiv) in DMF (13 mL) under Ar was warmed at reflux. After 24 and 48 h, the reaction was treated with additional LiI (80 mg, 2 × 3 equiv). The mixture was warmed for a total of 3.5 d before the reaction was diluted with H_2O, acidified with 10% aqueous HCl, extracted with CH_2Cl_2, and dried (Na_2SO_4). Chromatography (SiO_2, 2.0 × 15 cm, 5% MeOH/$CHCl_3$) afforded **12** (94 mg, 80% yield) as a yellow solid: mp 219−220 °C; ^{1}H NMR (10% CD_3OD/$CDCl_3$, 400 MHz) δ 6.94−6.73 (m, 7H), 6.44 (s, 1H), 5.05 (t, J = 7.9 Hz, 2H), 3.86 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 3.36 (s, 3H), 3.06 (t, J = 7.9 Hz, 2H); ^{13}C NMR (10% CD_3OD/$CDCl_3$, 100 MHz) δ 162.5, 155.5, 149.1, 148.82, 148.77, 148.5, 147.6, 145.9, 145.6, 130.8 (2C), 127.4, 127.2, 124.5, 122.8, 121.2, 117.4, 113.6, 112.3, 111.3, 111.0, 109.6, 104.5, 100.3, 56.1, 56.0, 55.93, 55.87, 55.7, 55.4, 37.9, 29.7; IR (film) ν_{max} 3273, 2925, 2851, 1726, 1515 cm^{-1}; MALDIHRMS (DHB) m/z 589.1940 (M^+, $C_{32}H_{31}NO_{10}$ requires 589.1948).

Hexamethyl Ningalin B (13). A solution of **12** (9.3 mg, 16 μmol, 1.0 equiv) and cuprous oxide[18] (2.3 mg, 16 μmol, 1.0 equiv) in degassed quinoline (450 μL) was warmed at 220 °C under Ar for 5 min. The mixture was cooled to 25 °C, and the solvent was removed by a stream of N_2. Chromatography (SiO_2, 0.5 × 10 cm, 10% EtOAc/CH_2Cl_2) provided **13** (6.0 mg, 70% yield) as a white solid: mp 186−187 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.09 (s, 1H), 6.95−6.92 (m, 3H), 6.88 (d, J = 1.2 Hz, 1H), 6.79 (d, J = 8.2 Hz, 1H), 6.74 (s, 1H), 6.71 (dd, J = 1.8, 7.9 Hz, 1H), 6.58 (d, J = 1.5 Hz, 1H), 4.65 (t, J = 7.0 Hz, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.77 (s, 3H), 3.57 (s, 3H), 3.11 (t, J = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 155.7, 149.2, 149.1, 149.0, 148.7, 148.0, 146.4, 145.8, 132.1, 130.7, 127.4, 126.9, 122.3, 121.1, 119.3, 115.1, 113.2, 112.3, 111.4, 111.2, 110.6, 104.9, 100.8, 56.3, 56.2, 56.1 (3C), 56.0, 51.3, 38.0; IR (film) ν_{max} 2931, 2835, 1709, 1591, 1551, 1515 cm^{-1}; MALDIHRMS (DHB) m/z 546.2111 (M + H^+, $C_{31}H_{31}NO_8$ requires 546.2128).

Ningalin B (1). A solution of **13** (5.9 mg, 11 μmol, 1.0 equiv) in CH_2Cl_2 (1.1 mL) under Ar at −78 °C was treated with BBr_3 (1 M in hexanes, 160 μL, 160 μmol, 15 equiv), and the mixture was allowed to warm to 25 °C over 24 h. Following dilution with MeOH (0.50 mL), the solvent was removed by a stream of N_2 to afford synthetic **1** (5.2 mg, 98%) identical in all respects (^{1}H NMR, ^{13}C NMR, IR, MS) when compared to spectra of naturally derived ningalin B: ^{1}H NMR (17% CD_3OD/DMSO-d_6, 400 MHz) δ 7.17 (s, 1H), 7.07 (s, 1H), 6.80 (d, J = 8.2 Hz, 1H), 6.77 (d, J = 2.0 Hz, 1H), 6.75 (s, 1H), 6.63 (m, 2H), 6.59 (d, J = 1.8 Hz, 1H), 6.43 (dd, J = 2.1, 7.9 Hz, 1H), 4.49 (t, J = 7.0 Hz, 2H), 2.87 (t, J = 7.4 Hz, 2H); ^{13}C NMR (17% CD_3OD/DMSO-d_6, 100 MHz) δ 155.0, 146.2, 145.4, 145.3, 145.2, 144.9, 144.0, 142.3, 133.0, 129.4, 126.6, 125.6, 121.0, 119.9, 119.4, 117.2, 116.4, 116.0, 115.8, 114.2, 109.9, 108.8, 103.7, 50.2, 37.4; IR (film) ν_{max} 3333, 2923, 1673 cm^{-1}; MALDIHRMS (DHB) m/z 484.1009 (M + Na^+, $C_{25}H_{19}NO_8$ requires 484.1008).

9,10-Dihydro-12,13-dimethoxy-1-(3′,4′-dimethoxyphenyl)-3,4-dimethoxy- [4,3-*d*]-[1]-benzopyrano-15*H*-benzazepino[3,2-*a*]-[3]-pyrrol-7,15(18*H*)-dione (14). A sample of **12** (3.3 mg, 5.6 μmol) was treated with Eaton's Acid[19] (200 μL, 7.5% P_2O_5−$MeSO_3H$) and stirred under Ar at 25 °C. After 18 h, the reaction was diluted with H_2O, extracted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography (SiO_2, 1.5 × 5 cm, 10% EtOAc/CH_2Cl_2) afforded **14** (2.1 mg, 66% yield) as a yellow solid: mp 225−226 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71 (s, 1H), 7.03 (d, J = 8.1 Hz, 1H), 6.99 (dd, J = 1.8, 8.1 Hz, 1H), 6.94 (d, J = 1.8 Hz, 1H), 6.89 (s, 1H), 6.70 (s, 1H), 6.51 (s, 1H), 5.26 (m, 2H), 3.98 (s, 3H), 3.94 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 3.44 (m, 5H); ^{13}C NMR (20% CD_3OD/$CDCl_3$, 100 MHz) δ 181.9, 156.2, 153.3, 149.2, 149.0, 148.5, 148.1, 145.9, 145.7, 138.6, 136.0, 128.7, 127.5, 127.3, 126.0, 122.4, 115.1, 113.3, 113.1, 112.3, 111.3, 109.6, 104.7, 100.3, 56.1, 56.0 (2C), 55.9 (2C), 55.4, 46.2, 36.0; IR (film) ν_{max} 2995, 2944, 2831, 1711, 1692, 1590 cm^{-1}; MALDIHRMS (DHB) m/z 572.1940 (M + H^+, $C_{32}H_{29}NO_9$ requires 572.1921).

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Supporting Information Available: ^{1}H NMR spectra of **5**−**14** and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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