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Total Synthesis of Ningalin D

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Abstract: A concise (nine-step) and effective (19% overall yield) total synthesis of ningalin D (**1a**) is disclosed and is based on a key 1,2,4,5-tetrazine → 1,2-diazine → pyrrole Diels−Alder strategy to assemble a fully substituted pyrrole core central to its structure. Additional highlights of the synthesis include a double Dieckmann condensation to introduce the C and D aryl rings enlisting substituents judiciously placed on the dienophile and intrinsic to the widely used tetrazine **2**, a highly effective Suzuki coupling of the resulting C and D phenol triflates for introduction of the sterically demanding F and G aryl rings, and an unusually effective formal oxidative decarboxylation reaction cascade initiated by a Curtius rearrangement to directly provide the biphenylene quinone methide found imbedded in the structure of ningalin D. The cytotoxic and multidrug resistance (MDR) reversal activity of ningalin D, its derivatives, and the key synthetic intermediates are detailed.

First isolated in 1997 by Fenical and Kang,[1] the ningalins constitute a family of structurally interesting and biologically active marine natural products. By far, the most complex of these is ningalin D (**1a**) incorporating a biphenylene quinone methide superimposed on a now oxidized pentasubstituted pyrrole core that characterizes this class of natural products (Figure 1). To our knowledge, the only closely related natural product disclosed to date is purpurone (**1b**),[2] an inhibitor of ATP-citrate lyase, whose name reflects the purple color of the natural products and the sponges from which both **1a** and **1b** were isolated (*Didemnum* sp. and *Iotrochota* sp., respectively). In the course of developing total syntheses of the simpler members of the ningalin family,[3] we disclosed several derivatives that possess potent P-gp inhibitory activity,[3] effective multidrug resistance (MDR) reversal properties in cellular functional assays,[3−5] and efficacious (potentiation/resensitization) in vivo antitumor activity against sensitive and resistant tumors upon coadministration with antitumor therapeutics (vinblastine, taxol) in xenograph animal models.[6] Although such derivatives lack intrinsic cytotoxic activity themselves, they resensitize MDR tumors and hypersensitize sensitive tumors to conventional therapeutics through inhibition of the overexpressed or constitutive drug efflux pump P-gp.[3−6]

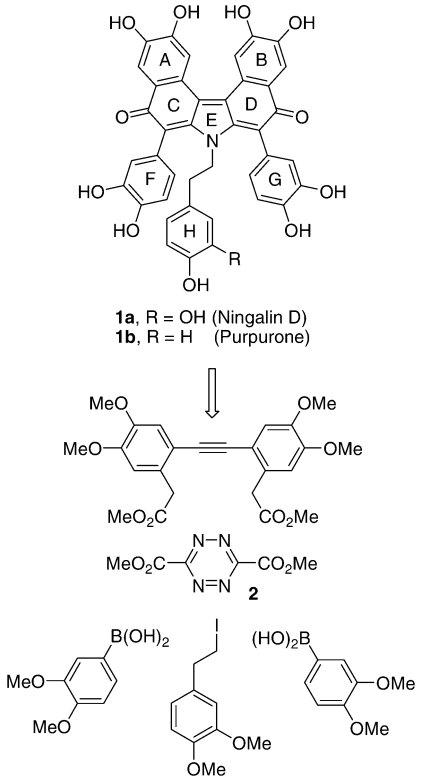

Figure 1. Ningalin D and retrosynthetic analysis.

As a consequence of these biological observations with the simpler ningalin derivatives, and because of the intrinsically interesting structure of ningalin D, we have pursued and herein detail the first total synthesis of ningalin D in studies that complement the only other reported efforts in the area, a

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(2) Chan, G. W.; Francis, T.; Thureen, D. R.; Offen, P. H.; Pierce, N. J.; Westley, J. W.; Johnson, R. K.; Faulkner, D. J. *J. Org. Chem.* **1993**, *58*, 2544.
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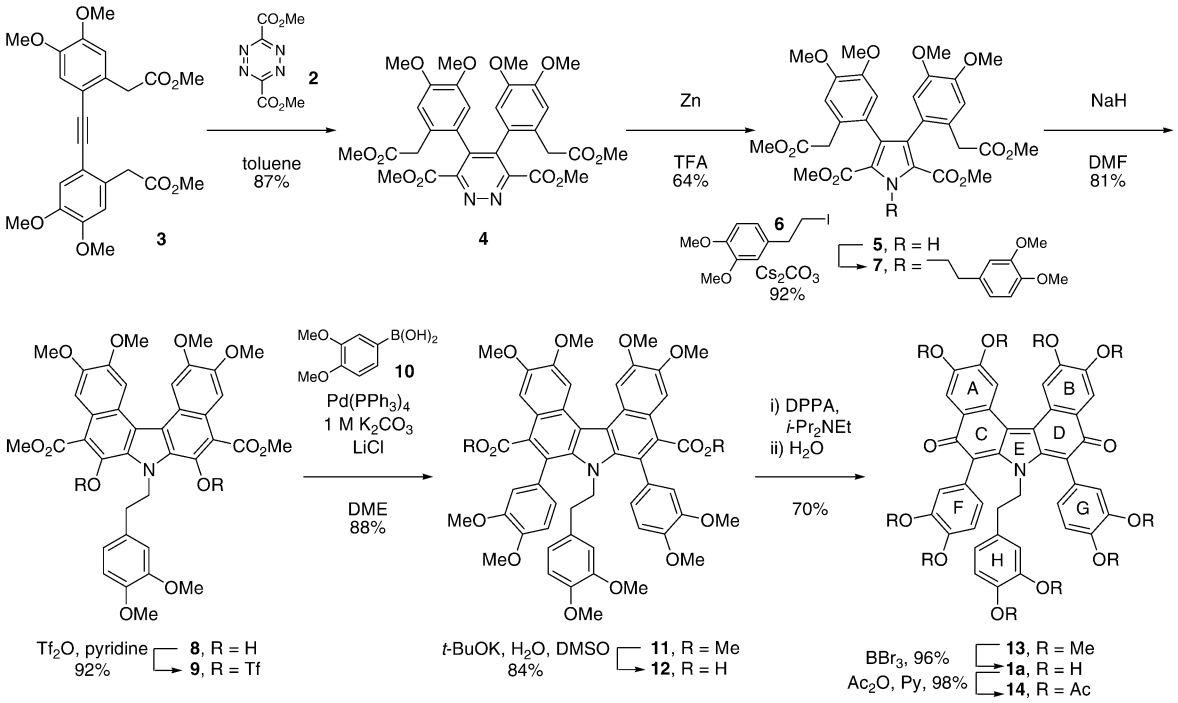
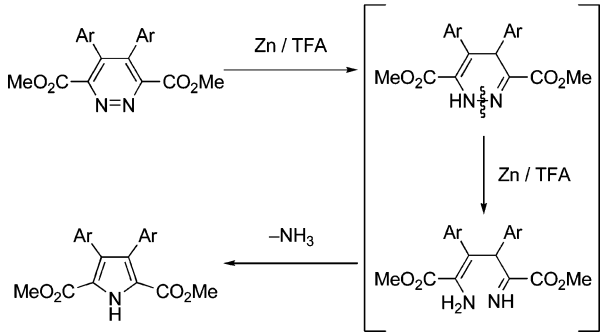
Scheme 1. Total Synthesis of Ningalin D



biomimetic total synthesis of purpurone disclosed by Steglich.[7] Key elements of the concise and nonobvious approach include an inverse electron demand heterocyclic azadiene Diels−Alder reaction (1,2,4,5-tetrazine → 1,2-diazine) followed by a reductive ring contraction of the resultant 1,2-diazine, affording the fully substituted pyrrole core central to the structure of **1** (Figure 1). A double Dieckmann cyclization enlisting substituents judiciously placed on the dienophile (−CH_2CO_2Me) and intrinsic to the widely used tetrazine **2** (−CO_2Me) was used to close the C and D aryl rings; the sterically demanding F and G aryl rings were introduced enlisting a highly effective Suzuki coupling onto the corresponding C and D ring phenol triflates, and an unusually effective formal oxidative decarboxylation reaction cascade initiated by a Curtius rearrangement was discovered to directly provide the biphenylene quinone methide of ningalin D.

Preparation of the symmetrical alkyne **3**, a suitably substituted electron-rich dienophile for Diels−Alder cycloaddition with **2**, was accomplished in two steps from commercially available 2-bromo-4,5-dimethoxyacetonitrile.[8a] Conversion of the nitrile to the known methyl ester[9] (cat. H_2SO_4, MeOH, 90%) followed by a double Stille coupling with 1,2-bis(tributylstannyl)acetylene (0.05 equiv of $(Ph_3P)_4Pd$, toluene, 110 °C, 6 h, 71−80%) provided ready access to **3**. The key [4 + 2] cycloaddition of **3** with dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (**2**)[10] proceeded smoothly in refluxing toluene (110 °C) to provide the symmetrical 1,2-diazine **4** in superb conversions (87%) and was followed by a subsequent reductive ring contraction reaction effected by treatment with Zn/TFA (30 equiv of Zn, TFA, 25 °C, 7 h, 64%) to provide the pyrrole **5** (Scheme 1). Although such reductive ring contraction reactions are typically conducted with Zn/HOAc,[11] we have disclosed that the overall reaction cascade is much faster (4−7 vs 24 h for **4**, 25 °C) with Zn/TFA[12] and often much cleaner (Scheme 2).

Scheme 2. Reductive Ring Contraction



N-Alkylation of **5** with **6**[8b] (3 equiv, 3 equiv of $CsCO_3$,[13] DMF, 60 °C, 2 h, 92%) afforded **7** and set the stage for introduction of the aryl C and D rings via a double Dieckmann condensation, which was effected by treatment of **7** with NaH (6 equiv, DMF, 25 °C, 15 h, 81%), cleanly providing **8**. Notably, the second of the two Dieckmann condensations proceeds more slowly than the first, requiring adoption of a sterically encumbered coplanar arrangement of the A and B aryl rings. Conversion of the bisphenol **8** to the corresponding bistriflate **9** (5 equiv of Tf_2O, py-CH_2Cl_2 (1:1), 0−25 °C, 2 h, 92%) preceded an unusually effective double Suzuki coupling with **10** (0.1 equiv of $(Ph_3P)_4Pd$, 2.2 equiv of LiCl, 1 M aq. K_2CO_3−DME, 80 °C, 15 h, 88%) to provide **11**. Notably, the

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(8) (a) Trans-World Chemicals, Rockville, MD. (b) Sigma-Aldrich library of rare chemicals (commercial) or prepared from the corresponding alcohol (Aldrich): I_2, Ph_3P, imidazole, CH_2Cl_2, 96%.
(9) Ambrose, R.; von Angerer, S.; Weigrebe, W. *Arch. Pharm.* **1988**, *321*, 481.
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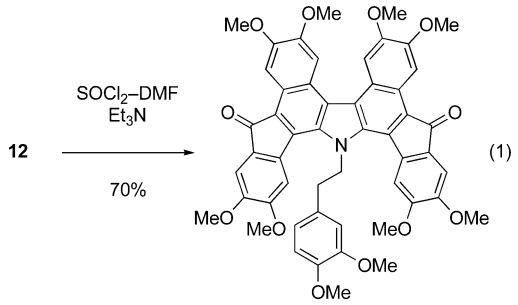
inclusion of LiCl (2.2 equiv) in the reaction mixture[14] was key to the superb conversions, which in its absence proved much lower (0−35%).

The final conversion of **11** to the penultimate ningalin D precursor required conversion of the aryl esters to phenols and was anticipated to arise from Baeyer−Villiger oxidation of the corresponding ketones or a benzylic hydroperoxide rearrangement of the corresponding secondary or tertiary alcohols. Although not investigated in detail, each of these proved problematic to implement due to the sterically hindered nature of the site. However, hydrolysis of diester **11** to the dicarboxylic acid **12** could be effected cleanly with anhydrous hydroxide[15] (26 equiv of *t*-BuOK, 8 equiv of H_2O, DMSO, 80 °C, 24 h, 84%), whereas typical saponification conditions (2 N aq. KOH, reflux, 48 h) failed to touch **11**. Characteristic of the congested nature of the site, attempts to prepare the acid chloride of **12** ($SOCl_2$−DMF, CH_2Cl_2, 25 °C, 4 h) resulted in clean conversion to **15** (70%) derived from a facile double Friedel−Crafts acylation of the proximal and activated F and G aryl rings (eq 1).



(1)

Although several direct conversions of **12** to permethyl ningalin D were examined, including a carboxy inversion reaction and a $Pb(OAc)_4$-catalyzed oxidative decarboxylation reaction, the surprisingly most straightforward approach proved to be initiated by Curtius rearrangement of the dicarboxylic acid **12**, which was anticipated to provide the corresponding diamine. Under the conditions of a modified Curtius rearrangement and in situ hydrolysis of the resulting isocyanate (5 equiv of DPPA,[16] 5 equiv of *i*-Pr_2NEt, CH_2Cl_2, 25 °C, 15−20 h; then H_2O−THF, air atmosphere, reflux, 90 h; 70% **13**), we observed and subsequently adopted conditions that optimized the conversion directly to permethyl ningalin D (**13**). Thus, a remarkably facile in situ oxidation of the expected diamine to the biphenylene quinodiimide and subsequent imine hydrolysis were observed to occur under the reaction conditions, providing **13** directly in superb overall conversions (70%) for the seven transformations from the dicarboxylic acid **12** (Scheme 3). Exhaustive deprotection of **13** (15 equiv of BBr_3, CH_2Cl_2, −78 to 25 °C, 16 h, 96%) requiring the removal of 10 methyl ethers cleanly provided ningalin D (**1a**) identical in all respects with authentic material.[1] Acetylation of **1a** (Ac_2O, py, 25 °C, 3 h, 98%) provided ningalin D decaacetate (**14**), which also displayed properties identical in all respects with those reported for authentic material.[1]

With **1a**, **13**, and the precursor intermediates in hand, they were examined alongside permethyl ningalin B (**16**)[3b] in assays

Scheme 3. Oxidative Decarboxylation Reaction

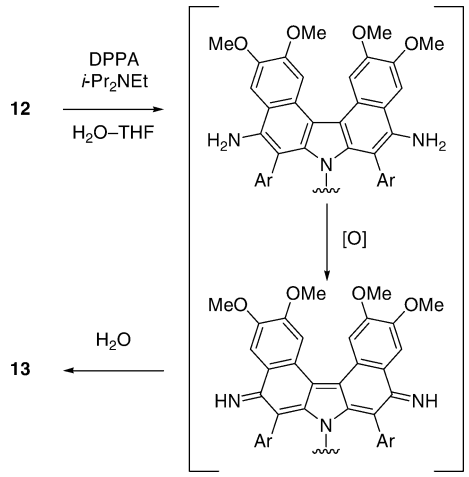


Table 1. Cytotoxic Activity

compound	IC_{50} (μM) L1210	IC_{50} (μM) HCT116
1a	7	70
7	>100	>100
8	>100	>100
11	>100	>100
12	>100	>100
13	1	1
14	7	6
15	>100	>100
16	>100	>100
vinblastine	0.007	0.007
doxorubicin	0.06	0.07

Table 2. MDR Activity

compound (1 μM)	HCT116/VM46 IC_{50} (nM) vinblastine (%)[a]	HCT116/VM46 IC_{50} (nM) doxorubicin (%)[a]
none	100	900
1a	100 (0%)	900 (0%)
7	100 (0%)	750 (8%)
8	10 (70%)	330 (21%)
11	8.5 (82%)	90 (80%)
12	100 (0%)	1000 (0%)
13	8.5 (82%)	100 (70%)
14	100 (0%)	900 (0%)
15	7 (100%)	90 (80%)
16	7 (100%)	90 (80%)

[a] Reversion %, parental HCT116 IC_{50} for vinblastine = 7 nM and doxorubicin = 70 nM.

to establish their intrinsic cytotoxic activity (Table 1, an undesired property) and their ability to reverse multidrug resistance,[17] resensitizing a resistant cell line to front line antitumor drugs (Table 2). Ningalin D (**1a**), permethyl ningalin D (**13**), and ningalin D decaacetate (**14**) exhibited modest cytotoxic activity against a murine leukemia cell line (L1210, IC_{50} = 1−7 μM) and a parental human colon cancer cell line (HCT116, IC_{50} = 1−70 μM). Each of the modestly active compounds contains the embedded biphenylene quinone methide characteristic of ningalin D, suggesting that their use as MDR reversal agents, unlike **16**,[3b] would be complicated by this secondary cytotoxic activity.

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(15) (a) Chang, F. C.; Wood, N. F. *Tetrahedron Lett.* **1964**, 2969. (b) Gassman, P. G.; Schenk, W. N. *J. Org. Chem.* **1977**, *42*, 918.
(16) Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.
(17) Reviews: (a) Robert, J.; Jarry, C. *J. Med. Chem.* **2003**, *46*, 4805. (b) Wiese, M.; Pajeva, I. K. *Curr. Med. Chem.* **2001**, *8*, 685.

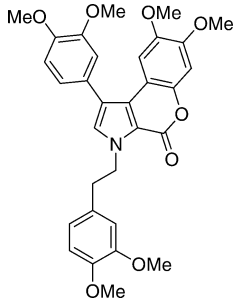

Figure 2. Permethyl ningalin B.

Pertinent to our interests, the compounds were examined against a resistant HCT116 cell line that embodies the MDR phenotype through overexpression of P-glycoprotein (P-gp). This cell line, HCT116/VM46, which is resistant to both vinblastine (IC_{50} = 100 vs 7 nM for parental HCT116) and doxorubicin (IC_{50} = 900 vs 70 nM for parental HCT116), was treated with each of the ningalin derivatives at 1 μM and the IC_{50} established for coadministered vinblastine or doxorubicin (Table 2). Prototypical MDR reversal agents are usually examined at higher concentrations (e.g., 7.5−10 μM verapamil), and few lead series exhibit effective properties when examined at 1 μM as detailed herein.[17] Under these stringent conditions, ningalin D (**1a**) as well as ningalin D decaacetate (**14**) was inactive, whereas permethyl ningalin D (**13**) effectively resensitized the resistant HCT116/VM46 cell line to vinblastine (IC_{50} = 8.5 nM, 80% reversion) and doxorubicin (IC_{50} 100 nM, 70% reversion). This behavior of the hydrophobic permethyl ether derivative of the natural product versus the natural product itself (**13** vs **1a**) is analogous to distinctions we observed earlier with **16** (permethyl ningalin B, Figure 2) versus the inactive natural product ningalin B (free phenols).[3b] More significantly, and of the derivatives that lack the biphenylene quinone methide and its undesired accompanying cytotoxic activity, **11** and especially **15** were found to be active, **8** exhibited an intermediate level of activity, and both **7** and **12** were inactive. The relative inactivity of the polar derivatives **8**, **12**, and **7** was not surprising, and the MDR reversal activity of **11** expectedly approached that of **16**. More unexpectedly, the inadvertently prepared Friedel−Crafts acylation byproduct **15** proved to be a potent MDR reversal agent, matching the efficacy of **16** at 1 μM.

Notably, each of the active compounds incorporates three hydrophobic domains that characterize the P-gp binding pharmacophore models,[18] but unique in ningalin series of MDR reversal agents is the lack of a basic amine central to the structure. As such, they not only constitute efficacious compounds exhibiting unusually potent MDR reversal activity for a lead series, but they also depart from structural features considered central to common pharmacophore models of P-gp binding.

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Supporting Information Available: Full experimental details are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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