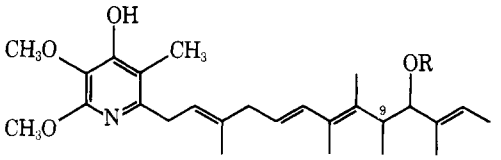
Polyprenylpyridinols. Synthesis of Piericidin Analogues

Franz P. Schmidtchen[1] and Henry Rapoport*

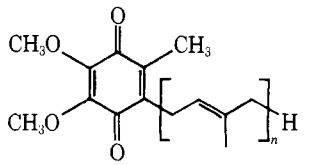
Contribution from the Department of Chemistry, University of California, Berkeley, California 94720. *Received April 27, 1977*

Abstract: Piericidin A, isolated from *S. mobaraensis*, is a specific antagonist of coenzyme Q. Its unusual structure consists of a 2,3-dimethoxy-5-methyl-4-pyridinol nucleus with a polyunsaturated side chain at C-6. We have prepared this nucleus and attached conventional polyprenyl side chains for structure–activity studies on coenzyme Q inhibitors. To prepare the nucleus, 3-methoxyacetylamino-2-methylacrylonitrile was cyclized to a 4-amino-2-pyridone which trimethyloxonium fluoroborate converted to the 4-amino-2,3-dimethoxypyridine. Bromination of the acylated amine formed the 6-bromo derivative in which the 4-amino was then replaced by hydroxy and the latter blocked by conversion to its benzyl ether with a benzylisourea. Transmetalation now gave the 6-lithio compound which was coupled with various prenyl bromides, leading to introduction of all trans polyprenyl side chains. The final 4-pyridinols were formed on selective debenzylation with butyl mercaptide. All the polyprenylpyridinols inhibited coenzyme Q electron transport to some extent, with the farnesyl analogue having the same activity as piericidin A.

Piericidin A is a powerful insecticidal and antimicrobial principle isolated, along with piericidin B, from the soil microorganism *Streptomyces mobaraensis*.[2] Its structure and stereochemistry have been established as *all-trans*-2,3-dimethoxy-5-methyl-6-[10(*S*)-hydroxy-3,7,8,9(*S*), 11-pentamethyl-2,5,7,11-tridecatetraenyl]-4-pyridinol (**1a**), and piericidin B is the corresponding methyl ether **1b**.[3]



1a, R = H
b, R = CH_3

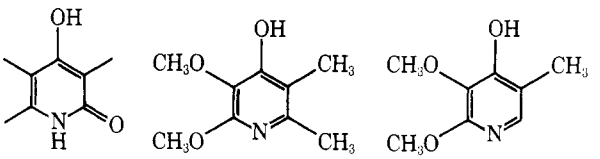


2, n = 6–10

The striking structural resemblance between piericidin A (as its pyridone tautomer) and coenzymes Q (ubiquinones, **2**) suggested that piericidin might be acting as a coenzyme Q antagonist. This has been confirmed by the finding that piericidin is a specific and potent inhibitor to coenzyme Q in the mitochondrial electron transport chain.[4] It is also suggestive of a potential as an antimalarial, along with other antimetabolites of coenzyme Q.[5]

Piericidin is highly toxic[2,3] and this property has restricted its broader application. Thus we have undertaken the synthesis of piericidin relatives in which the coenzyme Q antagonist activity is retained and the toxicity decreased. In considering candidate structures, we determined to retain the 4-pyridinol (or 4-pyridone) portion of the molecule since this moiety seems to mimic the quinone structurally without its parallel oxidation–reduction properties. For the unusual side chain, we proposed to substitute prenyl units of various lengths which would reflect exactly the side chains in the naturally occurring, biologically active quinones.

Our synthetic strategy then focused primarily on the unusual pyridine nucleus with its three contiguous oxygen atoms, two present as methyl ethers and one as an OH group. Nuclei of the 4-hydroxy-2-pyridone (**3**) type were to be avoided at all stages to circumvent any tautomeric ambiguity and possible isomeric mixtures. Thus our specific objectives became 2,3-dimethoxy-5,6-dimethyl-4-pyridinol (**4**) and 2,3-dimethoxy-5-methyl-4-pyridinol (**5**). The former offered the potential of



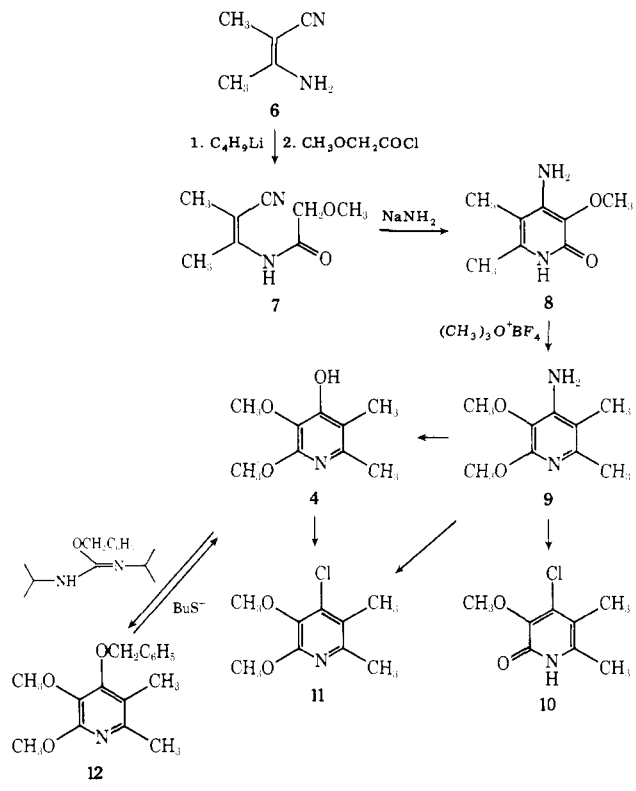
3 **4** **5**

adding the side chain via a carbanionic or otherwise functionalized intermediate derived from the acidic hydrogens of the α-methyl group; the latter, via its 6-halo and 6-metallo derivative, offered the possibility of attaching the side chain by coupling with the necessary allylic bromide, a procedure which has given excellent yields in the synthesis of menaquinones.[6]

Synthesis of 2,3-Dimethoxy-4-pyridinols. With the above plan in mind, we proceeded toward the synthesis of **4**, starting with 3-amino-2-methylcrotononitrile (**6**), itself prepared by sodium catalyzed dimerization of acetonitrile followed by methylation.[7,8] A minor problem in the preparation of **6** is the isolation of a uniform product since *E*/*Z* isomerization is quite facile. One isomer, probably *Z*,[9] is crystalline; however, isomerization takes place rapidly on recrystallization, distillation, or chromatography. When the mixture of isomers is converted to anion with butyllithium and then acylated with methoxyacetyl chloride, the enamide **7** is formed, again as a mixture of isomers. These isomers can be partially separated by distillation, and the *Z* configuration is assigned to the lower boiling isomer, rationalized as the result of hydrogen bonding between the cyano nitrogen and amide hydrogen and assigned on the basis of the upfield shift of its β-methyl group.

Cyclization to the pyridone **8** is effected by heating the dianion, formed with sodamide, in dioxane for several hours.[7,10] Its properties clearly indicate that the cyclic product exists as the 4-amino-2-pyridone as shown and as would be expected,[11] rather than as either other tautomer. Since the usual methylating agents (dimethyl sulfate, methyl iodide, diazomethane) give mixtures of O- and N-methylation,[12] we turned to trimethyloxonium tetrafluoroborate which cleanly converted pyridone **8** to the 2-methoxy derivative **9**.

It was now necessary to replace the 4-amino group directly by hydroxyl or by chlorine which itself could then be easily displaced. We explored replacement by chlorine first, thinking that the usual mild conditions would leave the 2-methoxyl intact.[13] However, the diazonium salt proved to be unexpectedly stable and the prolonged treatment in concentrated hydrochloric acid required for replacement also caused cleavage. The



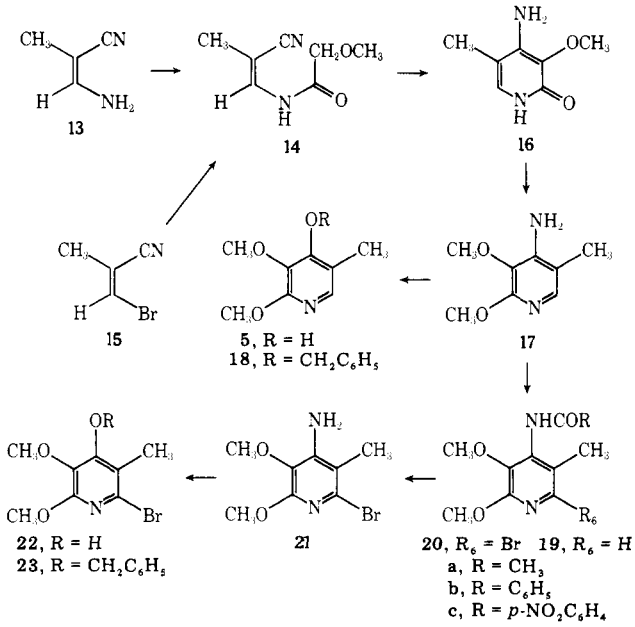
product, in only 15% yield, was 4-chloro-2-pyridone **10.** Addition of cuprous chloride allowed milder reaction conditions and retention of the 2-methoxyl, but the yield of 4-chloropyridine **11** was only 40%. We then turned to direct replacement by hydroxyl and found that diazotization in sulfuric acid followed by brief heating to 100 °C gave the 4-hydroxypyridine **4** in 90% yield. Although **4** may also exist as the 4-pyridone tautomer, the absence of any carbonyl absorption in the IR leads to the conclusion that it is all 4-pyridinol, as would be expected from its substitution pattern.[11]

The projected coupling of the heterocyclic nucleus and side chain through an organometallic derivative at the α-methyl requires the reversible blocking of the 4-hydroxyl function. Furthermore, the protecting group must withstand the reaction conditions of side chain introduction and be removable selectively without cleaving the 2-methoxyl group, under conditions that would not destroy the polyunsaturation in the prenyl side chain. Thus deblocking with acid or by hydrogenolysis, either catalytic or by sodium in liquid ammonia, is not permissible. An ether group, selectively removable by nucleophilic displacement,[14] seemed the ideal solution, so we turned to preparation of the 4-benzyloxypyridine **12.**

Our first effort was to convert 4-hydroxypyridine **4** to 4-chloropyridine **11** with the intent of displacing the 4-chloro with benzyl oxide. This effort failed since treatment of **4** with thionyl chloride, phosphorus oxychloride, phosphorus pentachloride, or even phenylphosphonic dichloride at 185 °C gave the 4-chloro compound in 10% yields at best. A method of direct benzylation of the 4-hydroxyl was then sought, being aware that the usual etherifying agents give mixtures of O- and N-alkylation when applied to 4-pyridinols/4-pyridones.[12] The oxonium salt used in exclusive O-methylation of 2-pyridone **8** was not applicable for benzylation, but we found another general reagent which reacted only at oxygen. This was the *O*-benzyldiisopropylisourea,[15] which afforded an excellent yield of 4-benzyloxypyridine **12** without any detectable N-benzylation. We now could test our hypothesis of specific 4-benzyl cleavage by nucleophilic attack. This was found to be the case when 4-pyridinol **4** was regenerated in practically quantitative yield on heating the benzyl ether with sodium *n*-butylmercaptide for 1 h at 80 °C.

With the appropriately protected, fully substituted 4-benzyloxy-2,3-dimethoxy-5,6-dimethylpyridine (**12**) now at hand, we explored various paths to a functionalized 2-methyl group, suitable for subsequent side chain attachment. These included metalation with butyllithium, oxidation with selenium dioxide, bromination with *N*-bromosuccinimide, and *N*-oxide formation followed by rearrangement. In all cases, reaction occurred but complex mixtures of products were formed. This divergence from the anticipated analogy with α-picoline chemistry was disappointing and foreshadowed a more laborious path than we had hoped. Therefore we interrupted our approach through the fully substituted compounds **4** and **12** and explored the alternative route via the α-free pyridine **5.**

Our plan was to exploit the experience we had gained and follow the same pattern to the synthesis of **5** benzyl ether, **18.** Thus the requisite 3-amino-2-methylacrylonitrile (**13**), pre-



pared by controlled lithium aluminum hydride reduction of methylmalononitrile,[16] was condensed with methoxyacetyl chloride to give an isomeric mixture of enamides, 3-methoxyacetylamino-2-methylacrylonitrile (**14**). On a large scale, this limited reduction of the malonodinitrile proved erratic, so we turned to the more convenient condensation of 3-bromo-2-methylacrylonitrile (**15**)[17] and methoxyacetamide for the preparation of enamide **14.** Cyclization of the enamide with sodium hydride led to 2-pyridone **16** which trimethyloxonium fluoroborate converted to the dimethoxyaminopyridine **17,** as in the previous scheme.

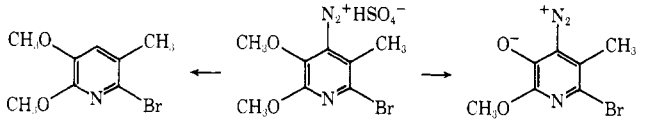
At this point, our plan was to convert the aminopyridine **17** to the pyridinol **5** and its benzyl ether **18,** both of which would be examined for the most efficacious stage for bromination. Both compounds **5** and **18** were prepared, but the yield in replacing the diazonium group by hydroxyl was disappointingly low, using the same conditions that had given excellent yields in the conversion of amine **9** to 4-pyridinol **4.** Deeply colored products were formed which apparently originated from coupling of the diazonium compound with the intermediate 4-pyridinol **5** at its open 6 position. Since such products had not occurred in the previous conversion of amino to hydroxyl, we sought to block their formation by changing the sequence of reactions and brominating first.

Direct bromination of 4-aminopyridine **17** with bromine in a variety of solvents and forms, and also with *N*-bromosuccinimide, was difficult to control. Most of the product was polymeric, overreacted material. To decrease this reactivity,

the amine was acylated and the corresponding *N*-acetyl, benzoyl, and *p*-nitrobenzoyl derivatives, **19a–c,** were prepared. These in turn were brominated to the 6-bromo-2,3-dimethoxy-5-methyl-4-acylaminopyridines (**20a–c**), all in good overall yields. The *p*-nitrobenzoyl derivative was the one of choice because of its ease of isolation and purification, and it was then hydrolyzed to the 4-amino-6-bromopyridine **21.**

Replacement of the amino group by hydroxyl was now undertaken using the conditions of diazotization and decomposition which had been so successful previously. To our dismay there was no parallel in the reactions, and we obtained a product which contained 15 separable components. Since the reaction sequence consists of two steps, these were examined separately to determine the source of trouble. We first found that a yellow, crystalline diazonium hydrogen sulfate could be isolated in practically quantitative yield. It was stable to storage at 10 °C for weeks, thus conveniently allowing exploration of its decomposition under various conditions.

Exposure of this diazonium salt **24** to the previously successful conditions, 6 N H_2SO_4 at 40 °C, led exclusively to a red-brown product. Its spectral (missing OCH_3) and elemental analyses led to the conclusion that it was the diazo oxide **25,**



26 **24** **25**

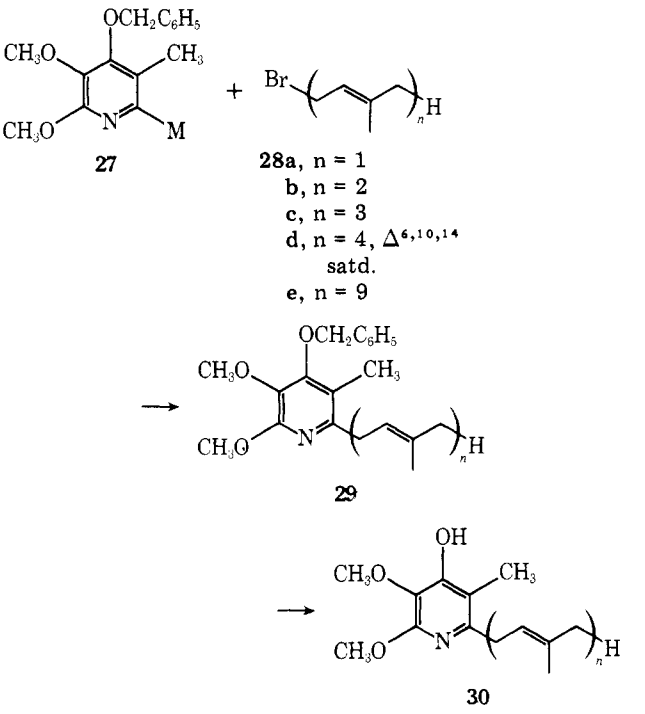
arising by nucleophilic attack of H_2O ortho to the diazonium group with displacement of the 3-methoxyl. Such nucleophilic aromatic substitution in competition with diazonium group displacement occurs frequently.[18] It is interesting to note that changing the substituent at the α position from CH_3 to Br decreases the yield of 4-pyridinol from 90% to zero. Apparently the decreased electron density at C-3, caused by the para bromo substituent, allows exclusive nucleophilic attack at this position.

To direct the attack by water to C-4, we considered a radical reaction in which decomposition of the diazonium salt is catalyzed by copper(I) oxide, followed by oxidation of the intermediate radical by copper(II) salts. This is followed by transfer of OH^- from the ligand sphere of the $Cu(H_2O)$ complex.[19] Decomposition did occur readily under these conditions; however, the product isolated in good yield was not the expected 4-pyridinol **22** but the reduction product, 6-bromo-2,3-dimethoxy-5-methylpyridine (**26**).

As another device for directing attack to C-4, we sought to protonate the very weakly basic ring nitrogen of the diazonium salt. Strong acid obviously would be required and should lead to a much greater increase in electrophilicity at C-4 than at C-3. This concept was put to the test by adding the diazonium salt **23** to 18 N sulfuric acid at 180 °C, and an 84% yield was obtained of the desired 4-pyridinol **22.** With the *O*-benzylisourea this was converted to its benzyl ether **23.** A practical synthesis of the heterocyclic moiety had thus been developed, and we were now ready to attach the side chain.

Attachment of Prenyl Side Chains. Our plan for attaching the side chain followed the procedure used in synthesizing menaquinones[6] and was based on metalation of the α-bromopyridine **23** and coupling of the metallopyridine derivative **27** with the requisite prenyl bromide **28.** The prenyl bromides were prepared by standard procedures[6,20] from prenol, geraniol, farnesol, phytol, and solanesol, all-trans alcohols in all cases. Exploratory experiments with prenyl bromide (**28a**) and the pyridyl Grignard, cuprate, and lithio reagents clearly established the pyridyllithium (**27,** M = Li) as the most promising metallo coupling component.

We then undertook a detailed study of the translithiation of α-bromopyridine **23** and the coupling of the resulting pyridyllithium **27** with prenyl bromide (**28a**) in order to optimize conditions. Using butyllithium and following the consumption of bromopyridine by GC, we found that transmetalation was practically instantaneous at −75 °C and that coupling was complete in 3 h at this temperature. Ether, dimethoxyethane, tetrahydrofuran, and 2,5-dimethyltetrahydrofuran, with and without hexamethylphosphoramide, were tried as solvents, and THF without HMPA gave the best results. Two side products were encountered: a small amount of the 2-butylated pyridine and 10–15% of the α-unsubstituted pyridine **18,** in which bromine has been replaced by hydrogen. The latter arose largely by proton abstraction from the solvent, as was shown by using perdeuteriotetrahydrofuran, although some probably also resulted from adventitious water and from dehydrobromination of the prenyl bromide.



27 **28a**, n = 1; **b**, n = 2; **c**, n = 3; **d**, n = 4, $\Delta^{6,10,14}$ satd.; **e**, n = 9 **29** **30**

That all-trans geometry was maintained in the coupled products was demonstrated for the geranyl and farnesyl components since the geometric isomers of these coupled products are separable by GC. Thus all-trans coupled pyridines **29a–e** were obtained in isolated yields of 60–68%. What remained was to cleave the benzyl ethers, and this was achieved in 65–70% yields without any other changes by warming with butyl mercaptide, as we had previously experienced. In this way, the all-trans piericidin analogues **30a–e** were obtained in 45% overall yields from the 4-benzyloxy-2-bromopyridine **23.** They exist as the 4-pyridinols rather than 4-pyridones, as would be anticipated from their substitution pattern[11] and as was demonstrated by spectral comparison of **30** with the benzyl ethers **23** and **29.**

Compounds **30** were tested for their ability to block the oxidation of NADH-dehydrogenase by CoQ_{10} and compared with piericidin A in this respect.[21] The most inhibitory compound was the farnesyl analogue **30c,** with practically the same activity as piericidin A. Surprisingly, the solanesyl analogue **30e** was 2 orders of magnitude less active.

Experimental Section[22]

3-Methoxyacetylamino-2-methylcrotononitrile (**7**). To a solution of 3-amino-2-methylcrotononitrile[7,8] (**6,** crystalline *Z* isomer, 19.2 g, 0.2 mol; the mixture of *Z* and *E* isomers may also be used)[7,8,9] in 200 mL of THF cooled to −20 °C was added a solution of butyllithium in hexane (2.76 M, 75.5 mL, 0.208 mol). After 10 min at −20 °C, methoxyacetyl chloride (32.6 g, 0.3 mol) in 50 mL of THF was added cautiously and the now clear mixture was allowed to warm to room

temperature. Refluxing for 2 h and standing overnight followed by solvent evaporation gave a residue which was taken up in ether (100 mL), washed with bicarbonate, 1 M HCl, and saturated NaCl solution, dried, and evaporated to leave an oil, which was distilled. After a forerun, containing mostly the *Z* isomer, the main fraction distilled at 124–130 °C (10 Torr) and consisted of the *E* isomer: yield 24.2 g, 0.144 mol, 72%; TLC (I) R_f 0.41, 0.33 (*E*, *Z* isomers); NMR δ *E* isomer 1.84 (q, J = 1 Hz, =C(CH_3)CN), 2.47 (q, J = 1 Hz, =C(CH_3)NH), 3.50 (s, -OCH_3), 3.94 (s, CH_2CH), 8.17 (NH); *Z* isomer 1.90 (br, =C(CH_3)CN), 2.35 (br, =C(CH_3)NH), 3.54 (s, -OCH_3), 3.97 (s, CH_2CO), 8.75 (NH). Anal. ($C_8H_{12}N_2O_2$) C, H, N.

4-Amino-3-methoxy-5,6-dimethyl-2-pyridone (8). To a stirred refluxing suspension of sodamide (from 1.2 g, 52 mmol, of sodium) in 40 mL of liquid ammonia, a solution of the enamide **7** (3.36 g, 20 mmol) in 10 mL of dioxane was added, the ammonia was evaporated and replaced by 40 mL of dioxane, and the resulting brown suspension was refluxed for 3.5 h with vigorous stirring. After cooling, ammonium chloride (5 g in 30 mL of water) was added, and the resulting two-phase mixture was evaporated to dryness and further dried overnight (60 °C, 0.2 Torr) to give a brown powder, which was thoroughly extracted with chloroform. Evaporation of the chloroform and crystallization of the residue from ethanol gave the pyridone **8:** yield 2.35 g, 14 mmol, 70%; mp 279–281 °C; TLC (II) R_f 0.18; NMR (Me_2SO-d_6) δ 1.82 (s, C-5 CH_3), 2.05 (s, C-6 CH_3), 3.62 (s, OCH_3), 5.35 (br, NH_2), 10.84 (br, NHCO). Anal. ($C_8H_{12}N_2O_2$) C, H, N.

4-Amino-2,3-dimethoxy-5,6-dimethylpyridine (9). Pyridone **8** (1.68 g, 10 mmol) and trimethyloxonium tetrafluoroborate (1.55 g, 10.5 mmol) were suspended in 60 mL of CH_2Cl_2. After 75 min of stirring at room temperature, the mixture was chilled, 10 mL of 2 N NaOH was added, and the organic layer was washed with water, dried ($MgSO_4$), and evaporated. Distillation at 90 °C (0.2 Torr) yielded 1.53 g, 8.4 mmol, 84%, of **9** as a colorless oil: TLC (I) R_f 0.51; NMR δ 1.98 (s, C-5 CH_3), 2.33 (s, C-6 CH_3), 3.80 (s, C-3 OCH_3), 3.97 (s, C-2 OCH_3), 4.2 (br, NH_2). Anal. ($C_9H_{14}N_2O_2$) C, H, N.

The dihydrochloride was prepared by dissolving **9** in THF containing excess concentrated HCl, evaporating to dryness, and crystallizing the residue from methanol, mp 143–145 °C dec. Anal. ($C_9H_{14}N_2O_2$·2HCl) C, H, N.

4-Chloro-5,6-dimethyl-3-methoxy-2-pyridone (10). To 20 mL of concentrated HCl, chilled to −7 °C and saturated at this temperature with gaseous HCl, was added 4-amino-2,3-dimethoxy-5,6-dimethylpyridine (**9**, 546 mg, 3 mmol) followed by sodium nitrite (250 mg, 3.6 mmol, in 0.5 mL of H_2O). The solution was allowed to warm to room temperature over 2 h where it was maintained for 20 h. Adding of sodium carbonate, extracting into CH_2Cl_2 (3 × 30 mL), drying, and evaporating gave a residue which was digested with ether and recrystallized from methanol to give 84 mg, 15% yield, of **10:** mp 202–204 °C; TLC (II) R_f 0.40; NMR δ 2.13 (s, 3 H, C-5 CH_3), 2.35 (s, 3 H, C-6 CH_3), 4.00 (s, 3 H, C-3 OCH_3); MS *m/e* (rel intensity) 187, 189 (52, 17.5), 42 (100). Anal. ($C_8H_{10}ClNO_2$) C, H, N.

4-Chloro-2,3-dimethoxy-5,6-dimethylpyridine (11). Aminopyridine **9** (212 mg, 1.16 mmol) in 10 mL of 6 N hydrochloric acid was diazotized with sodium nitrite (83 mg, 1.20 mmol) in 0.8 mL of water at 0 °C; then 0.5 mL of 1 M cuprous chloride in concentrated HCl was added. After 30 min the mixture was neutralized with sodium carbonate, 1 mL of concentrated ammonia was added followed by CH_2Cl_2, and the dried and concentrated organic phase was chromatographed on 20 g of silica gel giving **11** as an oil: yield 93 mg, 40%; TLC (III) R_f 0.53; NMR δ 2.22 (s, C-5 CH_3), 2.38 (s, C-6 CH_3), 3.83 (s, C-3 OCH_3), 3.97 (s, C-2 OCH_3). Anal. ($C_9H_{12}NO_2Cl$) C, H, N.

2,3-Dimethoxy-5,6-dimethyl-4-pyridinol (4). Aminopyridine **9** (2.73 g, 15.1 mmol) in 100 mL of 6 N sulfuric acid was treated with sodium nitrite (1.052 g, 15.3 mmol) dissolved in the minimum amount of water at 0 °C for 20 min. The yellow solution was divided into two portions and each aliquot was heated quickly to 95 °C and kept there for 5 min. After cooling, the combined aliquots were neutralized with sodium carbonate and extracted with CH_2Cl_2 which was filtered through 50 g of silica gel, eluting with $CHCl_3$/CH_3OH (10/1). The eluate was evaporated and the residue sublimed at 35 °C (0.2 Torr) to give **4:** yield 2.43 g, 13.3 mmol, 88%; mp 68–72 °C; TLC (I) R_f 0.60; NMR δ 1.93 (s, C-5 CH_3), 2.33 (s, C-6 CH_3), 3.85 (s, C-3 OCH_3), 3.97 (s, C-2 OCH_3). Anal. ($C_9H_{13}NO_3$) C, H, N.

4-Benzyloxy-2,3-dimethoxy-5,6-dimethylpyridine (12). A mixture of **4** (2.33 g, 12.7 mmol) and *O*-benzyldiisopropylisourea[15] (3.14 g, 13.4 mmol) was heated at 100 °C overnight. After cooling, 20 mL of CH_2Cl_2 was added, the mixture was filtered, and the filtrate was chromatographed on 50 g of silica gel, using CH_2Cl_2/*n*-hexane (4/1) as eluent. Kugelrohr distillation at 120–125 °C (0.3 Torr) of the residue after evaporation gave **12** as an oil: yield 2.66 g, 9.75 mmol, 77%; TLC (I) R_f 0.75; NMR δ 2.02 (s, C-5 CH_3), 2.32 (s, C-6 CH_3), 3.80 (s, C-3 OCH_3), 3.95 (s, C-2 OCH_3), 5.13 (s, OCH_2Ar), 7.33 (br s, C_6H_5). Anal. ($C_{16}H_{19}NO_3$) C, H, N.

Cleavage of 4-benzyloxy-2,3-dimethoxy-5,6-dimethylpyridine (**12**) to the 4-pyridinol **4** was effected by heating **12** (172 mg, 0.63 mmol) for 1 h at 80 °C in 2 mL of DMF with 1.6 mL of sodium *n*-butylmercaptide in DMF (0.79 mmol/mL, 200 mol %, prepared from *n*-butyl mercaptan and excess sodium in ether, filtering, evaporating the filtrate, dissolving the residue in DMF, and standardizing by titration with HCl and phenolphthalein indicator). The solution was then concentrated to half volume, 10 mL of CH_2Cl_2 and 10 mL of phosphate buffer (pH 8) were added, the layers were separated and the aqueous layer was extracted with two more 10-mL portions of CH_2Cl_2, the combined CH_2Cl_2 solution was dried and evaporated, and the residue was sublimed to give a 93% yield of 2,3-dimethoxy-5,6-dimethyl-4-pyridinol (**4**).

3-Methoxyacetylamino-2-methylacrylonitrile (14). A. From 3-Amino-2-methylacrylonitrile (13). To 3-amino-2-methylacrylonitrile[16] (**13,** 4.7 g, 57.3 mmol, mixture of isomers) dissolved in 60 mL of anhydrous THF and cooled to −20 °C under N_2, was added butyllithium (29 mL, 2 M in hexane). After the foaming subsided, methoxyacetyl chloride (7.48 g, 68.8 mmol) in 20 mL of THF was added slowly and the mixture was allowed to warm to room temperature and stirred overnight. The mixture was filtered, the filtrate was concentrated, saturated aqueous ammonium chloride and CH_2Cl_2 were added, and after vigorous shaking, the organic phase was separated. Two further extractions with CH_2Cl_2 followed by drying and evaporating left a residue which was Kugelrohr distilled at 80–105 °C (0.02 Torr) yielding 5.2 g (32.8 mmol, 59%) of 3-methoxyacetylamino-2-methylacrylonitrile (**14**) as a semisolid mixture of isomers which was crystallized from ether to give the *Z* isomer: mp 84–85 °C; GC (120 °C) t_R 4.1 min; TLC (I) R_f 0.41; NMR δ 8.40 (b, NH), 7.58, 7.40 (2 q, J = 1 Hz, =CH), 4.03 (s, 2 H, $COCH_2$), 3.50 (s, 3 H, OCH_3), 1.87 (d, J = 1 Hz, 3 H, CH_3). Anal. ($C_7H_{10}N_2O_2$) C, H, N.

The mother liquors were enriched in the *E* isomer: TLC, R_f 0.22.

B. From 3-Bromo-2-methylacrylonitrile (15). To methoxyacetamide (45.3 g, 0.51 mol, mp 97–98 °C) in 500 mL of dioxane/THF (2/1) kept at 65 °C was added sodium hydride (24.4 g, 0.51 mol) as a suspension in THF. After 30 min of stirring, 3-bromo-2-methylacrylonitrile[17] (70.8 g, 0.49 mol, as a mixture of isomers) was added followed by additional sodium hydride (24.4 g, 0.51 mol) suspension in THF in 15-min intervals over a period of 6 h, maintaining the temperature at 65 °C throughout. After 16 h of stirring, the mixture was cooled, 500 mL of water and 70 mL of acetic acid were added, the mixture was extracted with CH_2Cl_2 (500 mL, then 3 × 300 mL), and the combined organic phase was washed with water (2 × 200 mL), dried, and evaporated. Kugelrohr distillation at 90–100 °C (0.02 Torr) gave 3-methoxyacetylamino-2-methylacrylonitrile (**14,** 30 g, 0.20 mol, 40%), identical in all properties with material prepared from 3-amino-2-methylacrylonitrile (**13**) above.

4-Amino-3-methoxy-5-methyl-2-pyridone (16). To a suspension of sodamide (from 760 mg, 33 mmol, of Na) in 50 mL of liquid NH_3 was added 3-methoxyacetylamino-2-methylacrylonitrile (**14,** 2.52 g, 16.5 mmol, isomeric mixture) in 10 mL of dioxane and the solution was warmed to room temperature to evaporate the NH_3. To the residue was added 50 mL of dioxane, the mixture was refluxed for 14 h and then cooled, ammonium chloride (5 g in 30 mL of water) was added, and the entire mixture was evaporated to dryness. Digestion of the residue for 24 h with $CHCl_3$ gave 1.9 g of solid on evaporation of the $CHCl_3$ and 1.5 g, 9.8 mmol, 60% yield, of the 2-pyridone **16** on crystallization from Me_2SO/ethanol: mp 245 °C; NMR (Me_2SO-d_6) δ 10.81 (br, 1 H, NH), 6.73 (s, 1 H, C-6 H), 5.45 (s, 2 H, NH_2), 3.67 (s, 3 H, OCH_3), 1.88 (s, 3 H, CH_3). Anal. ($C_7H_{10}N_2O_2$), C, H, N.

4-Amino-2,3-dimethoxy-5-methylpyridine (17). Trimethyloxonium tetrafluoroborate (1.31 g, 8.89 mmol) and pyridone **16** (1.29 g, 8.44 mmol) were suspended in 50 mL of CH_2Cl_2 and stirred at room temperature for 75 min, after which the chilled solution was shaken with 5 mL of cold 2 N NaOH. The CH_2Cl_2 phase was washed with water, dried, and evaporated, leaving a residue which was distilled

(Kugelrohr, 85 °C (0.2 Torr)) to give **17**: yield 970 mg, 57.8 mmol, 69%; mp 55–56 °C; NMR δ 2.07 (s, C-5 CH_3), 3.83 (s, C-3 OCH_3), 3.97 (s, C-2 OCH_3), 4.22 (br, NH_2), 7.48 (s, ArH). Anal. ($C_8H_{12}N_2O_2$) C, H, N.

2,3-Dimethoxy-5-methyl-4-pyridinol (5). The 4-aminopyridine **17** (895 mg, 5.33 mmol), dissolved in 30 mL of 6 N H_2SO_4, was diazotized at 0 °C with sodium nitrite (387 mg, 5.6 mmol) in 3.5 mL of water. After 20 min the yellow solution was rapidly heated to 95 °C for 3 min and subsequently to 120 °C for another 3 min. The cooled mixture was neutralized and extracted with CH_2Cl_2 and the residue obtained from evaporation of the CH_2Cl_2 was extracted with hot hexane, from which crystals separated on cooling. Sublimation (70 °C (0.3 Torr)) gave 250 mg (1.48 mmol, 28%) of **5**: mp 89–91 °C; NMR δ 2.14 (s, C-5 CH_3), 3.83 (s, C-3 OCH_3), 4.00 (s, C-2 OCH_3), 7.58 (s, ArH), OH absorption very broad and centered at 6.7. Anal. ($C_8H_{11}NO_3$) C, H, N.

4-Benzyloxy-2,3-dimethoxy-5-methylpyridine (18). The 4-pyridinol **5** (148 mg, 0.877 mmol) and *O*-benzyldiisopropylurea (217 mg, 0.92 mmol) were mixed and kept at 110 °C for 2 h, 2 mL of CCl_4 was then added, the mixture was filtered, and the product was isolated from the filtrate by preparative TLC (III). Distillation (Kugelrohr, 100 °C (0.5 Torr)) gave **18** as an oil: yield 160 mg, 0.63 mmol, 71%; NMR δ 2.03 (s, C-5 CH_3), 3.83 (s, C-3 OCH_3), 3.95 (s, C-2 OCH_3), 4.83 (s, CH_2Ar), 7.39 (s, C_6H_5), 7.65 (s, C-6 H). Anal. ($C_{15}H_{17}NO_3$) C, H, N.

2,3-Dimethoxy-5-methyl-4-*p*-nitrobenzoylaminopyridine (19c). The aminopyridine **17** (252 mg, 1.5 mmol) in 2 mL of pyridine was treated with *p*-nitrobenzoyl chloride (334 mg, 1.8 mmol) at room temperature for 30 min and at 80 °C for 1 h. Evaporation of the solvent and treatment of the residue with 10 mL of saturated bicarbonate solution, followed by filtration and drying, gave the *p*-nitrobenzoyl derivative **19c** as a powder which was crystallized from acetone: yield 380 mg, 1.2 mmol, 80%; mp 200–202 °C; NMR (Me_2SO-d_6) δ 2.13 (s, C-5 CH_3), 3.78 (s, C-3 OCH_3), 8.95 (s, C-2 OCH_3), 7.85 (s, C-6 H), 8.06–8.58 (O_2NArH), 10.5 (br s, NH). Anal. ($C_{15}H_{15}N_3O_5$) C, H, N.

The corresponding 4-acetylaminopyridine **19a** was prepared from aminopyridine **17** and acetic anhydride by refluxing in toluene for 1.5 h. Cooling gave crystals which were recrystallized from toluene, yield 83%, mp 158–160 °C. Anal. ($C_{10}H_{14}N_2O_3$) C, H, N.

The corresponding 4-benzoylaminopyridine **19b** was prepared from aminopyridine **17** by heating with 120 mol % benzoyl chloride in pyridine for 1 h. Evaporation left a residue which was washed with 10 mL of saturated $NaHCO_3$ solution, then crystallized from acetone, yield 80%, mp 129–131 °C. Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

6-Bromo-2,3-dimethoxy-5-methyl-4-*p*-nitrobenzoylaminopyridine (20c). The α-free pyridine **19c** (4.75 g, 15 mmol) was dissolved in a hot mixture of acetic acid and propionic acid (50 mL, 1/1) containing 2.5 g of anhydrous sodium acetate, the solution was cooled to 45 °C, 2.40 g of bromine was added, and it was quickly chilled to 0 °C. Another portion of bromine (9.6 g, 60 mmol) was added and the mixture kept at 0 °C for 30 min and for 30 min at room temperature after which excess bromine was destroyed by addition of 7 g of $NaHSO_3$ and 9 g of Na_2CO_3 in 100 mL of water, and the precipitate was collected dried, and crystallized from methanol: yield 4.04 g, 10.4 mmol, 68%; mp 220–222 °C; NMR (Me_2SO-d_6) δ 2.12 (s, C-5 CH_3), 3.67 (s, C-3 OCH_3), 3.85 (s, C-2 OCH_3), 8.13 (O_2NArH), 10.4 (s, NH). Anal. $C_{15}H_{14}N_3O_5Br$) C, H, N.

The corresponding 6-bromo-4-benzoylaminopyridine **20b** was prepared by bromination of **19b** by the above procedure, mp 176–179 °C. Anal. ($C_{15}H_{15}N_2O_3Br$) C, H, N.

The corresponding 6-bromo-4-acetylaminopyridine **20a** was prepared by heating at 40 °C for 45 min acetylaminopyridine **19a** with 1000 mol % bromine. The solution was evaporated, CH_2Cl_2 was added and evaporated, the residue was dissolved in CH_2Cl_2 and washed with saturated $NaHSO_3$ solution, and the organic phase was dried and evaporated. Preparative TLC (I), removal of the zone at R_f 0.39, and elution with CH_3OH, evaporation, and crystallization from toluene gave **20a**, mp 179–181 °C. Anal. ($C_{10}H_{13}N_2O_3Br$) C, H, N.

4-Amino-6-bromo-2,3-dimethoxy-5-methylpyridine (21). Acylaminopyridine **20c** (3.20 g, 8.1 mmol) was dissolved in 15 mL of 1 N KOH in ethylene glycol, kept for 2 h at 110 °C under nitrogen, diluted with 100 mL of water, and extracted with ether (5 × 30 mL). The oil obtained after washing (H_2O), drying, and evaporating the combined organic phases crystallized and was recrystallized from hexane and sublimed (60 °C (0.2 Torr)): yield 1.73 g, 7.02 mmol, 86%; mp 72–74 °C; NMR δ 2.17 (s, C-5 CH_3), 3.83 (s, C-3 OCH_3), 4.00 (s, C-2 OCH_3), 4.40 (br s, NH_2). Anal. ($C_8H_{11}N_2O_2Br$) C, H, N.

The hydrochloride was prepared by adding HCl in THF to **21** in THF and precipitating the salt by addition of ether, mp 116–118 °C after crystallization from acetone. Anal. ($C_8H_{12}BrClN_2O_2$) C, H, N.

The sulfate of **21** was prepared as above using H_2SO_4 in THF, mp 162–163 °C. Anal. ($C_8H_{13}BrN_2O_6S$) C, H, N.

6-Bromo-2,3-dimethoxy-5-methyl-4-pyridinol (22). 4-Aminopyridine **21** (4.40 g, 17.8 mmol) was dissolved in 12.5 mL of acetic acid/propionic acid (1/1), mixed with 1.98 g of concentrated H_2SO_4, and cooled in ice water. After adding isoamyl nitrite (3.13 g, 26.7 mmol) and keeping the mixture at 0 °C for 15 min, ether (70 mL) was added and the precipitated solid washed thoroughly with ether and air dried. The diazonium hydrogen sulfate **24** obtained in quantitative yield was dissolved in 20 mL of 6 N H_2SO_4 and added to 70 mL of 18 N H_2SO_4 at 180–190 °C over 2–3 min; 30 s after the diazonium salt addition, when nitrogen evolution had ceased, the mixture was rapidly cooled and diluted with ice-water to 250 mL. The precipitate was collected, the filtrate was neutralized and extracted with CH_2Cl_2, and the washed, dried, and evaporated organic phase was combined with the precipitate and crystallized from hexane: yield 3.72 g, 15 mmol, 84%; mp 99–100 °C; TLC (IV) R_f 0.55; NMR δ 2.17 (s, C-5 CH_3), 3.80 (s, C-3 OCH_3), 3.88 (s, C-2 OCH_3); MS *m/e* (rel intensity) 247, 249 (M^+, 48), 231, 233 (46), 96 (100). Anal. ($C_8H_{10}NO_3Br$) C, H, N.

6-Bromo-4-benzyloxy-2,3-dimethoxy-5-methylpyridine (23). A mixture of 4-pyridinol **22** (4.00 g, 16.1 mmol) and *O*-benzyldiisopropylisourea (4.00 g, 16.9 mmol) was kept at 100 °C for 75 min, 5 mL of CCl_4 was added, the mixture was filtered, and the filtrate was chromatographed on 50 g of Al_2O_3 (Woelm, neutral, activity I, CCl_4) to give 4.89 g (14.5 mmol, 90%) of **23** after crystallization from hexane: mp 69–71 °C; TLC (IV) R_f 0.63; NMR δ 2.12 (s, C-5 CH_3), 3.77 (s, C-3 OCH_3), 3.92 (s, C-2 OCH_3), 5.13 (s, CH_2Ar), 7.28 (s, ArH). Anal. ($C_{15}H_{16}NO_3Br$) C, H, N.

6-Bromo-2,3-dimethoxy-5-methylpyridine (26). 4-Aminopyridine **21** was diazotized as described for the preparation of **22.** The diazonium salt (172 mg, 0.5 mmol) was dissolved in a solution of 30 g of $Cu(NO_3)_2 \cdot 3H_2O$ in 50 mL of water at room temperature. Portionwise addition of 7.15 mg (0.5 mmol) of Cu_2O caused decomposition of the diazo compound. After 10 min, 20 mL of ether were added, the ether phase was dried and evaporated, and the residue was sublimed (60 °C (0.3 Torr)) to give 76 mg, 0.328 mmol, 65%, of **26:** mp 47–49 °C; TLC (IV) R_f 0.55; NMR δ 2.30 (s, C-5 CH_3), 3.88 (s, C-3 OCH_3), 4.03 (C-2 OCH_3), 6.95 (s, 4-ArH); MS *m/e* (rel intensity) 231, 233 (M^+, 89), 216, 218 (27), 147, 149 (50), 94 (100). Anal. ($C_8H_{10}NO_2Br$) C, H, N.

General Procedure for the Metalation of 23 and Coupling with Prenyl Bromides 28. Bromopyridine **23** (67.3 mg, 0.20 mmol) was dissolved in 2 mL of anhydrous THF under argon and the solution was cooled to −75 °C. *n*-Butyllithium solution (~2.5 M in hexane, 110 mol %) was then injected through a septum and a sample, withdrawn after 5 min, was partitioned between 20 μL of CH_2Cl_2 and 20 μL of water. The organic phase was analyzed by GC to assure complete conversion to lithiopyridine **27,** M = Li. After addition of 150 mol % of the prenyl bromide,[6,20] the mixture was stirred at −75 °C for 3 h, then 30 μL of water was added and the reaction was allowed to warm to room temperature. The solvent was evaporated in a stream of nitrogen, and the residue was taken up in CH_2Cl_2, filtered through Celite, and separated by preparative TLC (III). The product zone was collected and eluted with ether, and the product was distilled in a small molecular still. Consistent yields of 60–68% of all coupled products were obtained.

4-Benzyloxy-2,3-dimethoxy-5-methyl-6-prenylpyridine (29a): distilled at 80 °C (0.02 Torr); GC (200 °C) t_R 7.3 min; NMR δ 1.72 (br s, $=C(CH_3)_2$), 2.01 (s, $ArCH_3$), 3.32 (br d, J = 8 Hz, $ArCH_2$), 3.80 (s, C-3 OCH_3), 3.93 (s, C-2 OCH_3), 5.17 (s, OCH_2), 5.30 (t, J = 8 Hz, H-C=), 7.33 (m, ArH); MS *m/e* (rel intensity) 327 (M^+, 38), 312 ($M^+ - CH_3$, 28), 236 (M^+ − benzyl, 24), 91 (benzyl, 100); UV (heptane) λ_{max} 212 nm (ε 26 700), 232 (sh), 278 (6700). Anal. ($C_{20}H_{25}NO_3$) C, H, N.

4-Benzyloxy-2,3-dimethoxy-6-geranyl-5-methylpyridine (29b): distilled at 110 °C (0.3 Torr); GC (240 °C) t_R 7.2 min; NMR δ 1.60 (s, terminal cisoid CH_3), 1.66 (s, terminal transoid CH_3), 1.73 (s, β, γ vinyl CH_3), 2.07 (br s, $-CH_2CH_2-$, $ArCH_3$), 3.37 (d, J = 8 Hz, $ArCH_2$), 3.87 (s, C-3 OCH_3), 4.00 (s, C-2 OCH_3), 5.18 (s plus m, OCH_2, ω HC=), 5.33 (br t, J = 8 Hz, β, ω HC=), 7.44 (s, ArH).

Anal. ($C_{25}H_{33}NO_3$) C, H, N.

4-Benzyloxy-2,3-dimethoxy-6-farnesyl-5-methylpyridine (29c): distilled at 140 °C (0.05 Torr); GC (275 °C) t_R 9.1 min; NMR δ 1.60 (s, in chain cisoid CH_3), 1.66 (s, terminal transoid CH_3), 1.74 (s, β, γ $=CCH_3$), 1.97 (s, chain CH_2), 2.07 (br s, chain CH_2 and $ArCH_3$), 3.41 (d, J = 7 Hz, $ArCH_2$), 3.85 (s, C-3 OCH_3), 4.00 (s, C-2 OCH_3), 5.17 (s, OCH_2), 4.95-5.2 (m, in-chain =CH), 5.38 (t, J = 7 Hz, β, γ =CH), 7.37 (m, ArH). Anal. ($C_{30}H_{41}NO_3$) C, H, N.

4-Benzyloxy-2,3-dimethoxy-5-methyl-6-phytylpyridine (29d): distilled at 165 °C (0.02 Torr); GC (270 °C) t_R 16.4 min; NMR δ 0.83, 0.87, 0.93, 1.25, 1.8-2.1 (aliphatic side chain), 1.73 (s, $=CCH_3$), 2.07 (s, $ArCH_3$), 3.38 (d, J = 7 Hz, $ArCH_2$), 3.87 (s C-3 OCH_3), 4.00 (s, C-2 OCH_3), 5.19 (s, OCH_2), 5.37 (br t, J = 7 Hz, HC=), 7.41 (m, ArH). Anal. ($C_{35}H_{55}NO_3$) C, H, N.

4-Benzyloxy-2,3-dimethoxy-5-methyl-6-solanesylpyridine (29e): reaction conducted in dimethoxyethane at −20 °C, purification by column chromatography (CH_2Cl_2/*n*-hexane, 2/3); NMR δ 1.60 (s, in-chain CH_3), 1.67 (s, terminal transoid CH_3), 1.72 (s, β, γ $=CCH_3$), 2.00, 2.02 (two s plus sh at 2.1, CH_2CH_2 and $ArCH_3$), 3.34 (d, J = 7 Hz, $ArCH_2$), 3.80 (s, C-3 OCH_3), 3.95 (s, C-2 OCH_3), 5.13 (s, OCH_2), 4.9-5.3 (m, in-chain =CH), 5.32 (br t, J = 7 Hz, β, γ =CH), 7.37 (m, ArH); UV (heptane) λ_{max} 215 nm (ϵ 26 600), 235 (sh), 277 (6100). Anal. ($C_{60}H_{89}NO_3$) C, H, N.

General Procedure for Cleaving the Benzyl Ethers 29a-e. Formation of Piericidin Analogues 30a-e. To 80 mmol of the benzyl ethers **(29a-e)** dissolved in 0.2 mL of DMF under argon was added 0.60 mL of 0.27 M sodium butylmercaptide in DMF (200 mol %). The mixture was kept at 80 °C for 1 h, then 2 drops of saturated NH_4Cl solution, followed by 50 mL of degassed water, were added. The resulting emulsion was extracted with ether, and the organic layer was washed with water and saturated NaCl solution, dried, and column chromatographed (CH_2Cl_2/ether, 50/1). The eluate was evaporated and Kugelrohr distilled or crystallized from heptane, yields 65-70%.

2,3-Dimethoxy-5-methyl-6-prenyl-4-pyridinol (30a): GC (150 °C) t_R 5.0 min; NMR δ 1.75 (br s, $=C(CH_3)_2$), 2.10 (s, $ArCH_3$), 3.35 (d, J = 6 Hz, $ArCH_2$), 3.87 (s, C-3 OCH_3), 4.00 (s, C-2 OCH_3), 5.35 (br t, J = 6 Hz, HC=). Anal. ($C_{13}H_{19}NO_3$) C, H, N.

2,3-Dimethoxy-6-geranyl-5-methyl-4-pyridinol (30b): GC (200 °C) t_R 10.0 min; NMR δ 1.60 (s, terminal cisoid CH_3), 1.67 (s, terminal transoid CH_3), 1.75 (br s, β, γ $=CCH_3$), 2.03, 2.11 (two s, CH_2CH_2 and $ArCH_3$), 3.35 (d, J = 7 Hz, $ArCH_2$), 3,90 (s, C-3 OCH_3), 4.00 (s, C-2 OCH_3), 5.14 (br, ω =CH), 5.36 (t, J = 7 Hz, β, γ =CH); UV (80% CH_3OH) λ_{max} 208 nm (ϵ 20 700), 225 (sh), 269 (5600). Anal. ($C_{18}H_{27}NO_3$) C, H, N.

2,3-Dimethoxy-6-farnesyl-5-methyl-4-pyridinol (30c): GC (232 °C) t_R 14.0 min; NMR δ 1.62 (s, in-chain cisoid CH_3), 1.68 (s, terminal transoid CH_3), 1.75 (s, β, γ $=CCH_3$), 1.97, 2.03 (two s, CH_2CH_2), 2.10 (s, $ArCH_3$), 3.35 (d, J = 7 Hz, $ArCH_2$), 3.87 (s, C-3 OCH_3), 3.97 (s, C-2 OCH_3), 5.12 (br peak, ω =CH), 5.33 (t, J = 7 Hz, β, γ =CH). Anal. ($C_{23}H_{35}NO_3$), C, H, N.

2,3-Dimethoxy-5-methyl-6-phytyl-4-pyridinol (30d): GC (275 °C) t_R 5.6 min; NMR δ 0.8-2.0 (aliphatic side chain), 1.75 (s, $=CCH_3$), 2.10 (s, $ArCH_3$), 3.35 (d, J = 7 Hz, $ArCH_2$), 3.89 (s, C-3 OCH_3), 4.00 (s, C-2 OCH_3), 5.37 (t, J = 7 Hz, HC=).

2,3-Dimethoxy-5-methyl-6-solanesyl-4-pyridinol (30e): crystals from heptane (−20 °C), mp 57-58 °C; NMR δ 1.60 (s, in-chain cisoid CH_3), 1.68 (s, terminal transoid CH_3), 1.74 (s, β, γ $=CCH_3$), 2.00 (br, s, CH_2CH_2), 2.07 (s, $ArCH_3$), 3.40 (d, J = 7 Hz, $ArCH_2$), 3.85 (s, C-3 OCH_3), 3.95 (s, C-2 OCH_3), 5.16 (HC=), 5.36 (t, J = 7 Hz, β, γ =CH). Anal. ($C_{53}H_{83}NO_3$) C, H, N.

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(22) Melting points are uncorrected. Column chromatography and TLC employed Camag Kieselgel. Four solvent systems were used for TLC: benzene/ethyl acetate, 1/1, system I; $CHCl_3/CH_3OH$, 10/1, system II; CH_2Cl_2/hexane, 4/1, system III; CH_2Cl_2/hexane, 1/1, system IV. All solvent mixtures are v/v and all drying was over $MgSO_4$. GC was carried out on a 6 ft × 0.25 in. column containing 5% SE-30 on 100-120 mesh AW-DMCS treated Chromosorb W with a He flow of 60 mL/min. NMR spectra were determined in $CDCl_3$ solution, unless otherwise stated, with a Varian T-60 instrument and are reported as δ values relative to internal Me_4Si. All solvent evaporations were in vacuo using a Berkeley rotary evaporator. Elemental analyses were performed by the Analytical Laboratory, Department of Chemistry, University of California, Berkeley, and agree with calculated values within ±0.4%.