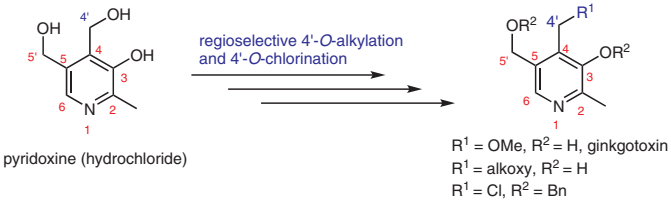
Effective and Versatile Synthesis of Ginkgotoxin and Its 4′-*O*-Derivatives through Regioselective 4′-*O*-Alkylation and 4′-*O*-Chlorination of 3,5′-*O*-Dibenzylpyridoxine

Boshi Huang[a]
Richmond Danso-Danquah[a,b]
Martin K. Safo[a,b]
Yan Zhang*[a,b]

[a] Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, 800 E Leigh Street, Richmond, VA 23298, USA
yzhang2@vcu.edu
[b] The Institute for Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, 800 E Leigh Street, Richmond, VA 23298, USA



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Abstract A regioselective synthesis of ginkgotoxin and its derivatives is described. A blocking–deblocking strategy was employed in this new methodology, which relied on selective ketal protection of the 3- and 4′-hydroxy groups of pyridoxine. The key intermediate, *O*-dibenzylpyridoxine, was prepared in four steps with a reasonable yield. The present synthetic route enables convenient and versatile preparation of diversified 4′-substituted pyridoxine derivatives.

Key words ginkgotoxin, pyridoxine

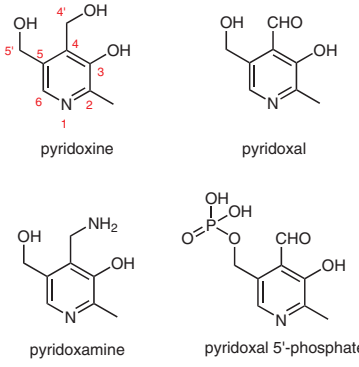


Figure 1 Chemical structures of pyridoxine, pyridoxal, pyridoxamine, and pyridoxal 5′-phosphate

Pyridoxine (PN), an important natural product, is a form of vitamin B_6. Together with the other forms of vitamin B_6, pyridoxal (PL) and pyridoxamine (PM), all three are involved in critical biochemical activities.[1] Meanwhile, pyridoxal 5′-phosphate (PLP) is the active form of these vitamers, acting as a ubiquitous cofactor for more than 160 PLP-dependent enzymes (Figure 1).[2] Furthermore, development of vitamin B_6 analogues based on the 3-pyridinol core has generated many bioactive molecules as anti-diabetic agents,[3] anti-SARS (severe acute respiratory syndromes) agents,[4] and antibacterial agents.[5]

The enzyme pyridoxal kinase (PdxK) plays an important role in catalyzing phosphorylation of PL to PLP in cells, and inhibitors of PdxK have been considered as promising drug candidates in the therapy of protozoan infectious diseases.[6] For example, PdxK is the drug target of the antimalarial agents chloroquine and primaquine.[7] In our previous work, we determined the crystal structure of human pyridoxal kinase in a complex with a potent inhibitor, ginkgotoxin, and MgATP, and provided insight into the molecular basis of human PdxK inhibition.[8] Ginkgotoxin bound at the active site of PdxK and formed multiple interactions with the protein, including hydrogen-bonding interactions and hydrophobic interactions.[8] Ginkgotoxin is a 4′-methoxy-substituted pyridoxine analogue, also named 4′-*O*-methylpyridoxine (**1a**) (Figure 2a).

Considering the critical role of ginkgotoxin in PdxK inhibition, and the scant chemical studies on the diversification at the 4′-position of pyridoxine that have been conducted thus far,[9] we decided to pursue the synthesis of ginkgotoxin and its derivatives with different substituents at the 4′-position, starting from pyridoxine in order to explore the reactivity of the 4′-hydroxy group further.

The synthesis of ginkgotoxin was first accomplished and the synthetic route, then, was applied as a template for the synthesis of its 4′-derivatives. It should be noted that the chemical functionalisation and modification of pyridoxine

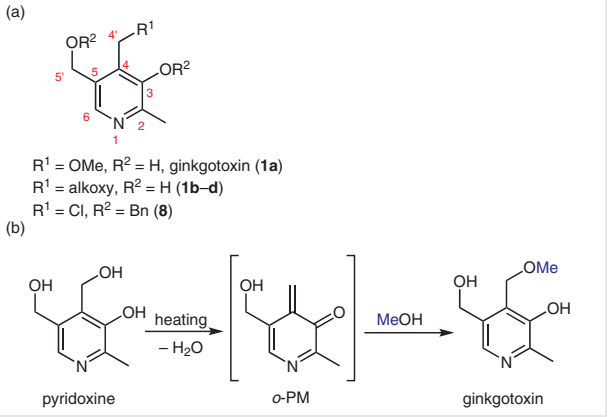


Figure 2 (a) Chemical structures of ginkgotoxin (**1a**) and its derivatives **1b–d** and **8**. (b) Mechanism for generation of ginkgotoxin via formation of *ortho*-pyridinonemethide (*o*-PM) and subsequent trapping with methanol

is fairly challenging due to the high water-solubility of pyridoxine, which may lead to significant mass loss during extraction and isolation.[10]

Moreover, the existence of two similar hydroxyl groups at the 4′- and 5′-positions may result in regioselectivity issues. Achieving regioselectivity between the 4′- and 5′-hydroxy groups commonly requires a ketalization blocking strategy of the 3- and 4′-hydroxy groups, which can be inefficient.[11,12] Utilizing this approach generally would lead to further functionalisation and derivatization at the 5′-hydroxy group.[5,11–13] Meanwhile, the 4′- and 5′-hydroxy groups may also be blocked selectively, which would permit further functionalisation at the 3-hydroxy group.[14] In contrast, few studies have been conducted to modify the 4′-hydroxy group or introduce diverse moieties onto the 4′-position.

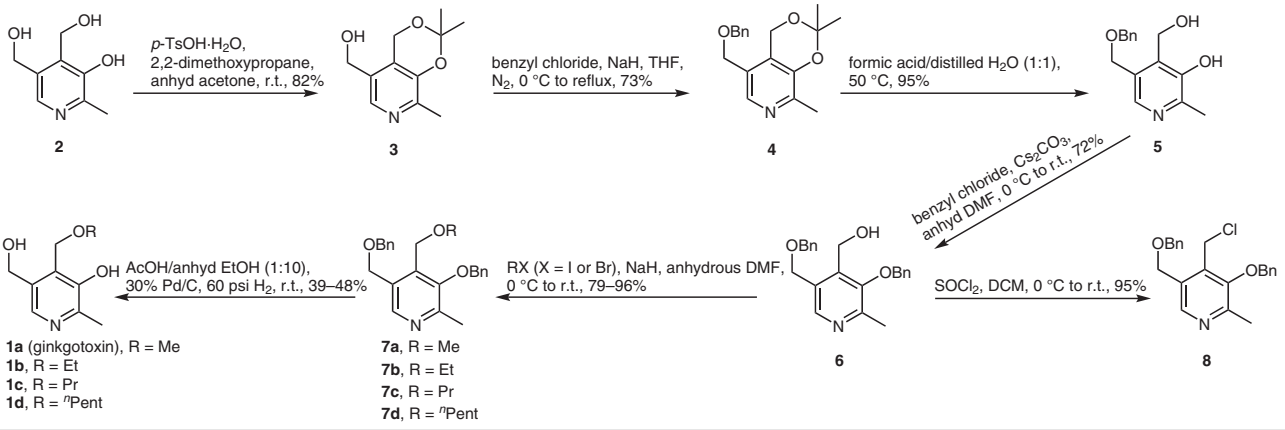
To date, there have been a few studies on the chemical synthesis of ginkgotoxin. Ginkgotoxin as its hydrochloride salt was first obtained by Harrisin in 1940, by heating pyridoxine hydrochloride with one equivalent of sodium methoxide in methanol at 130 °C in a sealed pressure bomb for 8 h in a yield of only 12%.[15] Harrisin noted that condensation or dimerization of pyridoxine took place to a certain extent. In 2014, Liu et al. accomplished the synthesis of ginkgotoxin through a microwave-assisted and *p*-toluenesulfonic acid-catalyzed 4′-O-methylation of pyridoxine by heating pyridoxine hydrochloride at 110 °C with methanol in a sealed tube for 3 h, providing an improved yield of 57%.[16] Subsequently, Lorenzo et al. synthesized ginkgotoxin for toxicology research in 2015 in a similar fashion to that of Harris in a 37.5% yield.[17] In the most recent study, performed by Yazarians et al. in 2017, ginkgotoxin was prepared by mixing pyridoxine with methanol in a pressurized vessel at 105 °C for 144 h in 58% yield.[18] All these methods to prepare ginkgotoxin follow a similar approach; pyridoxine hydrochloride is reacted with methanol at high temperature in a sealed tube. In this process, thermodynamic controlled formation of *ortho*-pyridinonemethide (*o*-PM) followed by subsequent oxa-Michael addition of methanol furnishes ginkgotoxin[19] (Figure 2b), with both steps being reversible, especially for the C–O bond-formation step.[18] Together with potential dimerization of pyridoxine, these may be the reasons for the reported low to moderate yield.

Initially, we tried to obtain ginkgotoxin in a similar way to those reported, but it turned out that the separation process was very challenging. Firstly, after the reaction mixture was cooled to room temperature, a precipitate formed containing the target compound that could not be re-dissolved. Secondly, the reaction mixture was very complex and it proved difficult to purify the target compound. Such observations echoed those by Liu et al. They managed to develop a TLC-based ‘Generally Useful Estimate of Solvent Systems’ (GUESS) method in countercurrent chromatography specifically for separating ginkgotoxin and its two isomers (methylation at 3′-O- or 5′-O-position).[20] Clearly a more effective and versatile synthetic methodology needed to be developed in order to prepare ginkgotoxin and it derivatives.

In the present study, we employed a blocking–deblocking strategy to achieve regioselective synthesis of ginkgotoxin and its 4′-derivatives **1b–d** and **8**.

The synthetic route to ginkgotoxin (**1a**) and its derivatives **1b–d** and **8** is outlined in Scheme 1. Commercially available pyridoxine hydrochloride **2**, was used as the starting material. Blocking the 3- and 4′-hydroxy groups was achieved by the formation of ketal **3** with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in anhydrous acetone. Benzylation of compound **3** with benzyl chloride yielded compound **4**, which was then deblocked with formic acid to afford **5**. The key intermediate **6**, 3,5′-*O*-dibenzylpyridoxine was prepared by a second benzylation of compound **5** at the 3-hydroxy group with benzyl chloride in the presence of cesium carbonate. Methylation of the free 4′-hydroxy of **6** with methyl iodide provided compound **7a**, which was submitted to catalytic hydrogenation in ethanol (with a trace of acetic acid) to give the desired ginkgotoxin (**1a**). Subsequently, synthesis of the other 4′-alkoxy substituted pyridoxine derivatives **1b–d** was efficiently accomplished under the same reaction conditions.

Chlorination of the 4′-hydroxy group of **6** was achieved using thionyl chloride to give the 4-chloromethyl substituted compound **8**, meaning that the 4′-hydroxy group could be further derivatized. For example, compound **8** may be used to react with alkylamines or alkanethiols to furnish 4′-alkylamine or 4′-alkylthio substituted pyridoxine derivatives. These analogues should further enrich the medicinal chemistry of this series of compounds in the future, as -NH- and -S- moieties are classical bioisosteres of the -O- functionality.



Scheme 1 Synthesis of **1a** (ginkgotoxin), **1b–d** and **8**

There were three crucial steps in this new synthetic route. First is the ketal blocking of the 3- and 4′-hydroxy groups, which allows subsequent benzylation of the 5′-hydroxy group without any selectivity issues. Previous studies have demonstrated that treatment of pyridoxine hydrochloride with 14% or 4% (w/v) hydrogen chloride gas in dried acetone gave the six-membered cyclic ketal **3** or seven-membered cyclic ketal **9** (Figure 3), which selectively blocked the 3- and 4′-hydroxy groups or 4′- and 5′-hydroxy groups, respectively.[13,14] However, the same acidic conditions might also lead to hydrolysis of the ketals, as it has been reported that both ketal functional groups could be hydrolyzed to release the hydroxy groups by concentrated hydrogen chloride in water.[5,12] Another reason to avoid using hydrogen chloride gas is that controlling its absolute amount in acetone is quite difficult. For example, Korytnyk et al. reported that the same reaction conditions lead to irreproducible yields of **3**.[21]

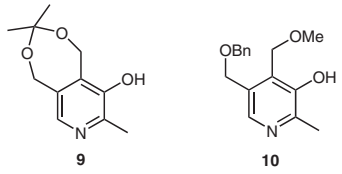


Figure 3 Chemical structures of **9** and **10**

Inspired by a report from Yang et al. in which stereospecifically labeled PLP analogues were synthesized,[9] we adopted the less acidic *p*-toluenesulfonic acid monohydrate. Different ratios between pyridoxine hydrochloride **2** and *p*-toluenesulfonic acid were explored and ultimately the conditions of **2**/*p*-toluenesulfonic acid in a ratio of 1:4 gave a satisfactory yield of 82%.

The second important step was the generation of the key intermediate 3,5′-*O*-dibenzylpyridoxine (**6**). Considering the different acidity between the 3- and 4′-hydroxy groups, chemoselective benzylation of the 3-phenolic group with benzyl chloride and various bases including sodium carbonate, potassium carbonate, and cesium carbonate was explored, with cesium carbonate in DMF giving the optimal outcome.

The final deprotection of the two benzyl groups on the 3- and 5′-positions was somewhat tedious. Initially, compound **7a** was heated to reflux with 4 M aq. HCl for 24 h to afford ginkgotoxin **1a**, but the yield was rather low (<20%). We considered that the low yield might be partially due to extraction losses. We then employed a catalytic hydrogenation approach to complete the deprotection under non-aqueous conditions. To do so, compound **7a** was first dissolved in methanol in the presence of 10% Pd-C(w/w) under 50 psi H_2 and **7a** was consumed completely to furnish a single product, which was confirmed as the mono-deprotected product 4′-*O*-methyl-5′-*O*-benzylpyridoxine **10** by ^{1}H NMR spectroscopic analysis (Figure 3). Different solvent systems and catalyst concentrations were then explored and, ultimately, ginkgotoxin was obtained in a moderate yield of 45% by treatment of **7a** in a mixed solvent (AcOH/anhydrous EtOH = 1:10) in the presence of 30% Pd-C (*w*/*w*) under 60 psi H_2 atmosphere.

In summary, we have utilized a blocking–deblocking strategy to achieve the regioselective synthesis of ginkgotoxin and its 4′-derivatives through 4′-*O*-alkylation and 4′-O-chlorination of the key intermediate 3,5′-*O*-dibenzylpyridoxine (**6**) under mild conditions. This newly developed synthetic route avoids the need for high temperatures and sealed vessel methodologies, potential dimerization, and a challenging separation process, resulting in a versatile and effective preparation of 4′-substituted pyridoxine derivatives.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0040-1707242.

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