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Improved "Oxazole" Method for the Practical and Efficient Preparation of Pyridoxine Hydrochloride (Vitamin B_6)

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ABSTRACT: Vitamin B_6, a well-studied vitamin B, has been synthesized using an oxazole method for the past 20 years. The oxazole method provided 56.2% overall yield but also generated safety, environmental, and health problems, such as using toxic benzene as solvent and unstable, corrosive, and pollutive HCl and $POCl_3$ as reagents. To use the same equipment but the least amount of toxic agents, we developed new reaction conditions for the early steps. For example, we successfully replaced toxic HCl/benzene conditions with $NaHSO_4/PhCH_3$ conditions and also developed a novel and efficient dehydrating agent trichloroisocyanuric acid/Ph_3P/Et_3N to synthesize the key intermediate 5-butoxy-4-methyl oxazole, instead of using phosphorus oxychloride. These improvements resolved safety, waste avoidance, and workup issues that plagued the previous methodologies. Our process comprised six easy synthetic steps and generated vitamin B_6 with 99.4% purity in 56.4% overall yield.

INTRODUCTION

Vitamin B_6 is a natural water-soluble vitamin, which has several isoforms include pyridoxine (PN), pyridoxal (PL), and pyridoxamine (PM) (Figure 1).[1] It plays an important role in various cellular processes, such as metabolism of amino acids, fatty acids, and carbohydrates, biosynthesis of heme, chlorophyll, ethylene, and auxin, and transcriptional regulation.[2] To ensure the vital activity of most organisms, including the human and plant organisms, all living organisms have to obtain vitamin B_6, either from biosynthesis or from foods and nutrients.

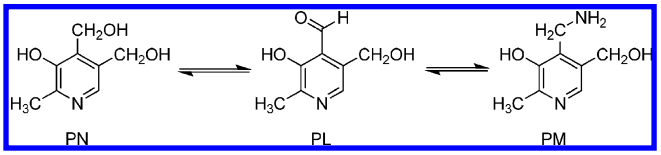


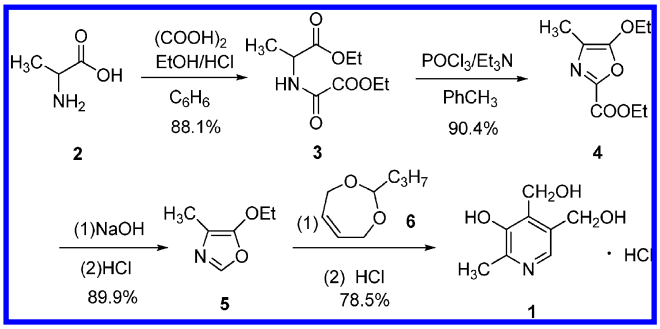
Figure 1. Natural forms of vitamin B_6.

Vitamin B_6 is widely distributed in foods; however, some losses are experienced with prolonged exposure to heat, light, or alkaline conditions during cooking, storage, and processing. The development of convenient synthetic approaches to this vitamin is therefore of great interest to the "nutritional supplements" industry. The main commercial form of vitamin B_6 is pyridoxine hydrochloride.

Harris and Folkers reported the first "pyridone" method[3] for the synthesis of pyridoxine hydrochloride in nine linear steps (<1.3% overall yield) in 1939, which involved the condensation of ethoxyacetylacetone and cyanoacetamide as the main stage. Albeit a pioneering *tour de force*, the lengthy synthesis and low overall yield of **1** could not stimulate a wave of exploration in pyridoxine chemistry. In the early 1960s, Merck and Roche reported a new "oxazole" method for the preparation of pyridoxine,[4] which led to an increase in the output of pyridoxine and to a decrease in its cost on the world market by a wide margin. However, the usage of phosphoric anhydride as the dehydrating agent[5] in the synthesis of key intermediate 4-methyl-5-ethoxy oxazole (**5**) made this operation not very safe. Phosphoric anhydride is corrosive to equipment and is very irritating. It reacts vigorously with water and water-containing substances and forms a hard or powderlike mass which prevented the propeller from working and destroyed the equipment. Considerable efforts were made by many research teams to modify this method.[6] Among which the way involved in the cyclization with $POCl_3$ in the presence of Et_3N, as reported by Zhou,[7] appeared most attractive to people as it drove the reaction to completion without byproduct and avoided the defect which was mentioned above. Therefore, this route was developed and streamlined, resulting in a six-step, higher-yielding (56.2%) route (Scheme 1).

Although many enterprises profitted from the finding of new dehydrating agents, some drawbacks still existed in the process, which would generate safety issues, environmental and health problems, such as using toxic benzene as solvent and unstable, corrosive, and pollutive hydrochloric acid and phosphorus

Scheme 1. "Oxazole" Method for the Synthesis of Pyridoxine Hydrochloride



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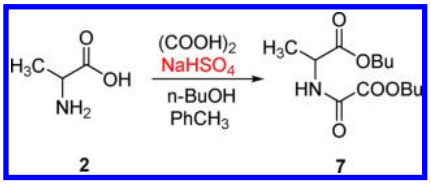
oxychloride as reagents. Considering these disadvantages, we have subjected this synthetic route to further studies and intended to develop an efficient and commercially viable process for vitamin B_6. In this article, we describe an improved process with an overall yield of 56.4% in six steps.

RESULTS AND DISCUSSION

Preparation of Butyl 2-(2-Butoxy-2-oxoacetamido)-propanoate (7). There have been numerous publications on the synthesis of alkyl *N*-alkoxalyl alaninates.[8] In the original methods, the product 7 was acquired by azeotropic removal of the formed water under the condition of using hydrochloric acid as the catalyst, but hydrochloric acid was unstable to store and transport due to its volatility, and it had a detrimental impact on the environment after completion of the reaction. In contrast with hydrochloric acid, sodium bisulfate was safe and easy to handle, and it can reduce plant corrosion. So our improved process used sodium bisulfate to replace hydrochloric acid as the catalyst and reduced the amount of catalyst from 1 to 0.05 equiv.

The present work enables the manufacture of *N*-alkoxalyl alaninates from L-alanine (**2**), which could be purchased commercially. Compound **2** was reacted with oxalic acid in the presence of sodium bisulfate as catalyst with azeotropic removal of the formed water in butyl alcohol by toluene to afford 7 (Scheme 2). After simple washing and extraction removed the catalyst and those unreacted materials, the resulting solution was evaporated to remove the solvent and the dibutyl oxalate. The crude 7 was finally obtained in 89.1% yield with 97.2% purity. Crude 7 was used directly in the next step.

Scheme 2. Simultaneous Esterification and Acylation of 2 To Yield 7



Conversion of 7 to 5-Butoxy-4-methyl Oxazole (10). The key intermediates in the synthesis of **1** by the "oxazole" method were 1,3-oxazoles, which were prepared with dehydration agents such as $POCl_3$.[9] In our improved process, **10** was prepared from 7 with trichloroisocyanuric acid (TCCA)/Ph_3P/Et_3N as dehydrating agents (Scheme 3). $POCl_3$ was a pungent liquid, and it also had the same questions regarding storage and transport as hydrochloric acid. Besides, the byproduct phosphates which formed by $POCl_3$ were difficult to recycle. In contrast with $POCl_3$, TCCA and Ph_3P were inexpensive, stable, and safe. It is worth mentioning that no method had yet been reported to synthesize oxazole derivatives by using TCCA/Ph_3P/Et_3N as dehydrating agents. The experiments indicated that the Ph_3P, solvent, and base were to play a major role in the cyclization reaction.

As expected, the cyclization did not proceed when Ph_3P was absent. Experimental results showed that TCCA was prone to react with Ph_3P acutely to afford the key intermediate **11** (Scheme 4).[10] Because the intermediate **11** was susceptible to hydrolysis, it was not isolated and could be used in the cyclization directly.

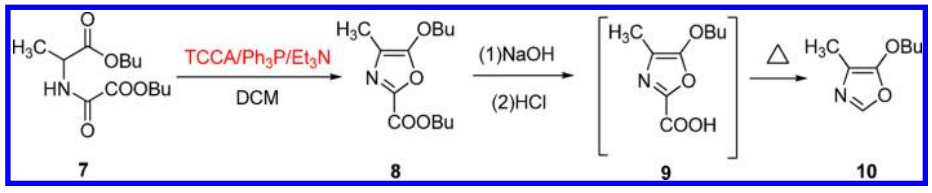
The activity of the dehydrating agents was proven to be strongly solvent-dependent. Various solvents were examined for the cyclization reaction, including dichloromethane, chlorobenzene, toluene, ethyl acetate, acetonitrile, and acetone. As shown in Figure 2, the best results were obtained in dichloromethane and chlorobenzene with higher conversion. Toluene and ethyl acetate furnished lower yields after the same time span. These differences were most likely due to the poor solubility of TCCA in toluene and ethyl acetate. Because intermediate **11** was sensitive to water, this cyclization reaction could not proceed well in undried acetonitrile or acetone, for which absorbing water was easy in an ambient environment. In addition, in order to complete the reaction, it needed a larger amount of chlorobenzene than dichloromethane as a solvent. So the use of dichloromethane would be appropriate for the cyclization process.

After selecting the dichloromethane as a solvent for the cyclization reaction, another key parameter was studied by screening of bases. A series of bases such as Et_3N, pyridine, DBU, DABCO, *N*,*N*-dimethylaniline, *N*,*N*-diisopropylethylamine, and K_2CO_3 were screened in order to find a kind of deacid reagent. The results showed that all of those bases did not work well to afford the desired product except for Et_3N. Therefore, Et_3N was found to be the suitable base for the desired transformation.

After the reaction mixture was washed with water to remove the triethylamine hydrochloride, the layers were separated in order to acquire the product. In the original method for the cyclization with $POCl_3$/Et_3N, it was hard to separate out the water layer from the organic layer for the two layers almost having the same color, which was caused by those black phosphates (Figure 3A). In contrast, the layers were separated easily in our new method (Figure 3B). Hence, these results showed that the dehydrating agent of TCCA/Ph_3P/Et_3N was more suitable in industrialization.

With **8** in hand, the remaining two-step saponification and decarbonylation could be conducted according to the literature[7] to complete the synthesis of **10** after a wet distillation process. Then the distillation residue was cooled to room temperature and filtered to recycle Ph_3PO, which could also be further transformed into Ph_3P again according to the literature.[11] Hence, Ph_3P could be reused in our reaction, and the influence on the environment by the phosphorus compounds had been reduced.

Scheme 3. Improved Process for the Synthesis of 10



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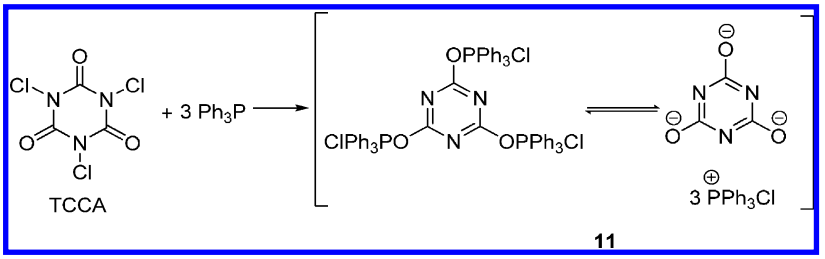
Scheme 4. Formation of the Intermediate 11



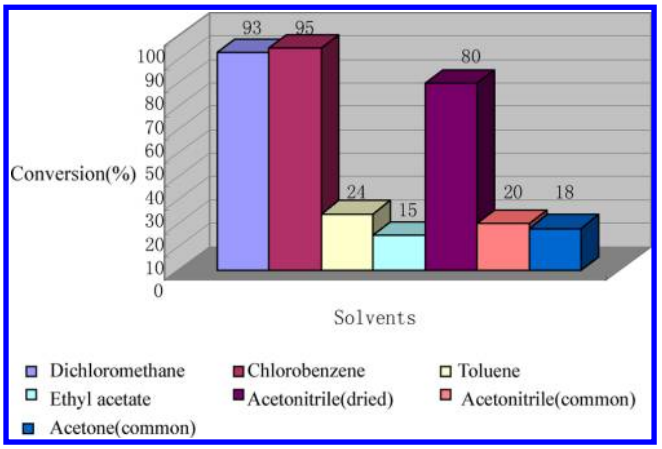


Figure 2. Screening of solvents for the synthesis of **8**. Standard reaction conditions: 1 equiv of **7**, 1.0 equiv of TCCA, 3.0 equiv of Ph_3P, 3.0 equiv of Et_3N. Conversion to **8** determined by GLC analyses.

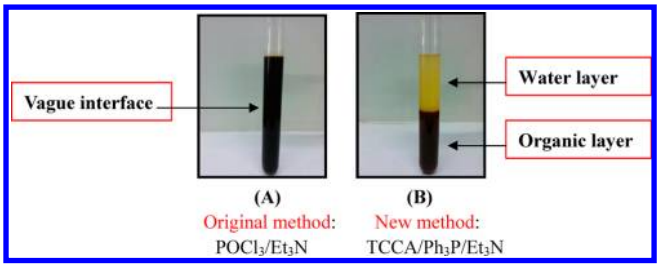


Figure 3. Comparison of two kinds of dehydrating agents after the cyclization reaction.

Based on the above results, the successive cyclization of **7** to oxazole derivative **8** followed by decarboxylation to oxazole **10** would be directly feasible without complex purifications in 79.2% yield. No byproduct appeared in this reaction.

Preparation of Vitamin B_6 (1). Having achieved oxazole **10**, our next task was to study the pyridoxine hydrochloride salt formation. The dienophile **6** was less active and entered into condensation with oxazole **10** at temperatures of 150 °C for 15 h. Using excess **6** to react with **10** was in practice a common solution to promote the reaction. The Diels–Alder reaction was examined under different conditions in order to optimize the reaction conditions. It is well-known that Lewis acid can ameliorate the conditions of the Diels–Alder reaction and enhance its reactivity and selectivity.[12] The Lewis acid mediated Diels–Alder reactions were performed under solvent-free conditions, which produced low yields (Table 1, entries 1–4). These results were most likely due to the instability of **10** under acid environment. Hence, Lewis acid was not suitable for this Diels–Alder reaction.

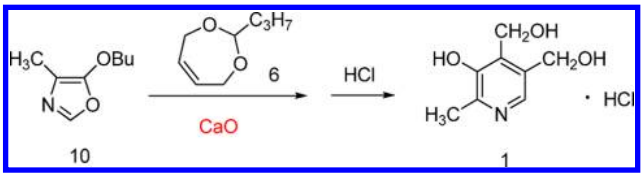
Table 1. Synthesis of 1 by the Diels–Alder Reactions of 10 with 6 under Lewis Acid Catalyzed and Aromatization[a]

entry	Lewis acid	yield (%)[b]
1	$AlCl_3$	70
2	$FeCl_3$	65
3	$ZnCl_2$	68
4	BF_3	66
5	none	78

[a]Standard reaction conditions: 1.0 equiv of **10**, 13 equiv of **6**, 0.05 equiv of Lewis acid, 150 °C, 15 h. [b]The yield was calculated with **1**.

Luckily, calcium oxide was found to increase the yield. When 0.05 equiv of calcium oxide was added, the yield of **1** increased from 78 to 80%. After improving the Diels–Alder reaction, the remaining aromatization could be conducted according to the literature[7] to complete the synthesis of pyridoxine hydrochloride (**1**) (Scheme 5).

Scheme 5. Preparation of 1 by the Diels–Alder Reaction and Aromatization



CONCLUSION

In conclusion, we have developed an efficient and commercially viable process for the synthesis of pyridoxine hydrochloride (**1**). L-Alanine (**2**) was used as the starting material, which was transformed to **1** through six steps including esterification, cyclization, decarbonylation, Diels–Alder reaction, hydrolysis, and hydrochloride salt formation in 56.4% yield with 99.4% purity (Scheme 6). Especially, a new, safe, and efficient dehydrating agent TCCA/Ph_3P/Et_3N was developed for the cyclization reaction to produce this key intermediate **8** with a good yield. Notably, Ph_3PO which formed in the cyclization also could be recycled without complex purification. In the other process of the route, some improvements were also described.

EXPERIMENTAL SECTION

All chemicals were purchased from commercial sources and were used without further purification. All GC analysis was conducted on an Agilent 7890A. GC conditions: SE-30 M column, 30 m × 0.25 mm × 0.25 μm; carrier gas, nitrogen (1.2 mL/min); injection temp = 200 °C; detector temp = 260 °C; oven temp = 190 °C. HPLC analysis for vitamin B_6 was carried out on an Agilent 1200 system equipped with a XDB-C18 250

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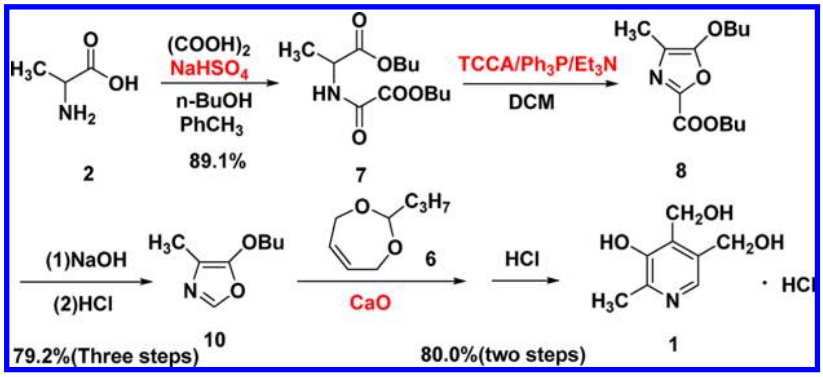
Scheme 6. Optimized Synthesis of Pyridoxine Hydrochloride



mm × 4.6 mm column and detected at 290 nm. Mobile phase: A mixture of methanol, glacial acetic acid, and water (27:1:73) containing 1.40 mg/mL of sodium 1-hexanesulfonate, flow rate = 1 mL/min. ^{1}H (400 MHz) NMR and ^{13}C (101 MHz) NMR spectra were recorded on a Varian spectrometer in $CDCl_3$ or D_2O using tetramethylsilane (TMS) as internal standards.

Butyl 2-(2-Butoxy-2-oxoacetamido)propanoate (7). In a reaction vessel equipped with thermometer, fractionating column, water knockout trap, and reflux condenser, L-alanine 2 (44.5 g, 0.5 mol) and oxalic acid (90.0 g, 1.0 mol) were dissolved in butyl alcohol (350 mL) at 70 °C. Sodium bisulfate (3.0 g, 0.025 mol) and toluene (100 mL) were added into the solution, and then the mixture was distilled off azeotropically to remove the formed water until there no water appeared in the water knockout trap. Toluene and butyl alcohol were recycled from the reaction mixture at a reduced pressure. After being cooled, toluene (100 mL) and water (75 mL) were added to the residue and stirred at 50−55 °C for 5 min. The sodium bisulfate, unreacted L-alanine, and oxalic acid were removed by washing with water. Toluene was recycled by distillation at room pressure followed by distillation to remove dibutyl oxalate at 100 °C/1.4 kPa to afford the crude product 7 (125.2 g) as a faint yellow oil. Yield: 89.1%. GC purity 97.2%. Mass: 274 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, J = 7.0 Hz, 1H), 4.59 (p, J = 7.3 Hz, 1H), 4.29 (t, J = 6.7 Hz, 2H), 4.17 (t, J = 6.6 Hz, 2H), 1.79−1.69 (m, 2H), 1.69−1.59 (m, 2H), 1.52−1.33 (m, 7H), 1.01−0.89 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.52, 159.85, 155.62, 66.95, 65.56, 48.54, 30.48, 30.29, 19.03, 18.99, 18.12, 13.65.

5-Butoxy-4-methyl Oxazole (10). Ph_3P (345.8 g, 1.32 mol) and 7 (125.2 g, 0.44 mol) were dissolved in dichloromethane (1.4 L). Into the solution was added TCCA (102.1 g, 0.44 mol) gradually at 0 °C, and the mixture was heated to reflux for 4 h. After being cooled to room temperature, triethylamine (133.3 g, 1.32 mol) was added into the solution drop by drop, and the reaction mixture was stirred until completion of the reaction (followed by TLC, hexane/ethyl acetate = 3:1). After the reaction mixture had been washed with water to remove the triethylamine hydrochloride, the dichloromethane was distilled off to afford the crude product **8** as a brown liquid. A 105 mL portion of aqueous sodium hydroxide (21.2 g, 0.53 mol) solution was added into the aforementioned crude product **8** and stirred for 30 min. Then the solvent was removed under reduced pressure, and the reaction mixture was cooled to 30 °C. After the pH of the residue was adjusted to 2−2.5 by the addition of 3 mol/L hydrochloric acid, the mixture was heated to 70 °C until no carbon dioxide was produced. The resulting solution was adjusted to pH 8. The wet distillation process was carried out to collect 0.9 L of distillate. Dichloromethane (100 mL) was added to the distillate. The layers were separated, and the organic phase was dried over Na_2SO_4, filtered, and evaporated to give **10** (55.2 g, 79.2%) with 99.2% purity. The distillation residue was cooled to room temperature, and Ph_3PO could precipitate from the water layer. After the mixture had been filtered and distilled off azeotropically to remove water content, the Ph_3PO (361.4 g, rate of recovery = 98.5%) was obtained as a gray solid. Mass: 156 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H), 4.08 (t, J = 6.5 Hz, 2H), 2.04 (s, 3H), 1.70 (dt, J = 14.7, 6.6 Hz, 2H), 1.47 (dq, J = 14.7, 7.4 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.22, 141.83, 111.72, 74.10, 31.39, 18.88, 13.73, 10.04.

Pyridoxine Hydrochloride (1). A mixture of **10** (55.2 g, 0.35 mol), **6** (628.0 g, 4.42 mol), and calcium oxide (1 g, 17.8 mmol) was kept at 150 ± 1 °C for 12 h. After the unreacted **6** was recycled by distillation under reduced pressure, 95% ethanol (100 mL) and 0.1% HCl (300 mL) were added and the mixture was stirred at room temperature for 10 h followed by heating to 60 °C for 2 h. The ethanol and water were removed from the reaction mixture at a reduced pressure. The residue was adjusted to pH 8 by 3 mol/L HCl, and the mixture was then heated under reflux for 1 h. After the solvent had been distilled off, a 100 mL portion of 95% ethanol was added and the mixture was stirred for 10 min. The solvent was then distilled off, and brown yellow crystals of the crude product **1** (65.7 g) were obtained.

Purification. The crude product **1** (65.7 g) was dissolved in water (60 mL). Activated carbon (8.0 g) was added, and the resulting mixture was decolorized at 80 °C for 30 min. The mixture was filtered, and activated carbon (8.0 g) was added into the filter liquor. The resulting slurry was decolorized at 80 °C for 30 min once more. After the activated carbon was removed by filtration, the filtrate was concentrated to give a residue which was further crystallized using absolute alcohol (100 mL) to obtain **1** (58.4 g, 80.0%) as white solid. HPLC purity: 99.4%. Mp = 204.5−205.6 °C (lit. mp 204 °C).[7] Mass: 170 $(M + H)^+$. ^{1}H NMR (400 MHz, D_2O): δ 7.99 (s, 1H), 4.84 (s, 2H), 4.64 (s, 2H), 2.48 (s, 3H). ^{13}C NMR (101 MHz, D_2O): δ 152.95, 143.12, 141.18, 137.16, 130.43, 58.70, 57.62, 15.00.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Huang, S. H.; Zhang, J. Y.; Wang, L. H.; Huang, L. Q. *Plant Physiol. Biochem.* **2013**, *66*, 63−67. (b) György, P. *Am. J. Clin. Nutr.* **1956**, *4*, 313−317.

(2) Huang, S. H.; Zeng, H. B.; Zhang, J. Y.; Wei, S.; Huang, L. Q. *Phytochemistry* **2011**, *72*, 2124−2129.

(3) (a) Harris, S. A.; Stiller, E. T.; Folkers, K. *J. Am. Chem. Soc.* **1939**, *61*, 1242−1244. (b) Harris, S. A.; Folkers, K. *J. Am. Chem. Soc.* **1939**, *61*, 1245−1247.

(4) (a) Harris, E. E.; Firestone, R. A. *J. Org. Chem.* **1962**, *27*, 2705−2706. (b) Hoffmann, F. FR 1343270, 1963. (c) Firestone, R. A.; Harris, E. E.; Reuter, W. *Tetrahedron* **1967**, *23*, 943−955.

(5) (a) Balyakina, M. V.; Zhukova, Z. N.; Zhdanovich, E. S. *Zh. Prikl. Khim.* **1968**, *41*, 2324−2326. (b) Pfister, K., III; Harris, E. E. BE 617499, 1962.

(6) (a) Maeda, I.; Takehara, M.; Togo, K.; Asai, S.; Yoshida, R. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 1435−1437. (b) Rust, H.; Burkart, K.; Faust, T.; Henkelmann, J.; Knoll, C.; Mohry, A.; Kindler, A. US 0120082, 2003. (c) Maeda, I.; Togo, K.; Yoshida, R. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 1407−1410. (d) Zhou, H. Y. CN 86101512, 1988. (e) Chen, T. H.; Li, R. B.; Yang, W. *Zhongguo Yiyao Gongye Zazhi* **2004**, *35*, 1−2.

(7) Zhou, H. Y.; Fang, Z. T.; Ye, D. Y.; Yang, J. M.; Wang, Q. Z. *Zhongguo Yiyao Gongye Zazhi* **1994**, *25*, 385−389.

(8) (a) FR 1533817, 1968. (b) Shirakawa, K.; Tsuda, T. JP 43010614, 1968. (c) Maeda, I.; Asai, S.; Miyashiki, H.; Yoshida, R. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 1917−1918. (d) Gum, A. G.; Fischesser, J.; Haerter, R.; Karge, R.; Jephcote, V. J.; Bonrath, W. WO 2004/087640, 2004.

(9) (a) Murakami, M.; Iwanami, M. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 726−727. (b) Cornforth, J. W.; Cornforth, R. H. *J. Chem. Soc.* **1953**, 93−98. (c) Itov, Z. I.; Gunar, V. I. *Khim.-Farm. Zh.* **1988**, *22*, 207−214.

(10) Sugimoto, O.; Tanji, K. *Heterocycles* **2005**, *65*, 181−185.

(11) (a) Li, Y. H.; Lu, L. Q.; Shoubhik, D.; Sabine, P.; Kathrin, J.; Matthias, B. *J. Am. Chem. Soc.* **2012**, *134*, 18325−18329. (b) Christelle, P.; Alain, F. R.; Belen, A.; Laurent, B.; Gerard, M.; Marc, L. *Organometallics* **2009**, *28*, 6379−6382. (c) Hossein, M.; Javad, A. *Tetrahedron Lett.* **2009**, *50*, 5923−5926. (d) Mikael, B.; Alain, F. R.; Jahjah, M.; Gerard, M.; Gordon, D.; Marc, L. *Synlett* **2007**, *10*, 1545−1548.

(12) (a) Fringuelli, F.; Girotti, R.; Pizzo, F.; Zunino, E.; Vaccaro, L. *Adv. Synth. Catal.* **2006**, *348*, 297−300. (b) Barroso, S.; Blay, G.; Al-Midfa, L.; Muñoz, M. C.; Pedro, J. R. *J. Org. Chem.* **2008**, *73*, 6389−6392.