Improved Chemical Syntheses of 1- and 5-Deazariboflavins
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Abstract: The cofactor flavin adenine dinucleotide (FAD) is required for the catalytic activity of a large class of enzymes known as flavoenzymes. Because flavin cofactors participate in catalysis via a number of different mechanisms, isoalloxazine analogues are valuable for mechanistic studies. We report improved chemical syntheses for the preparation of the two key analogues, 5-deazariboflavin and 1-deazariboflavin.

The exceptional chemistry of the flavin cofactor allows flavoenzymes to play a wide variety of roles in vivo. These proteins participate in many biological processes including nitric oxide generation, photosynthesis, soil detoxification, and even apotosis.1 The diversity of functions performed by flavoproteins is due to the ability of the isoalloxazine ring system to participate in an assortment of catalytic mechanisms. It can perform redox and radical reactions, and even apoptosis.1 The diversity of functions of flavoenzymes to play a wide variety of roles in vivo. These enzymes known as flavoenzymes.

FIGURE 1. Flavin analogues are useful in the elucidation of the cofactor’s role in catalysis.

FIGURE 2. Coupling of ribose and 3 provides precursors to both analogues 1 and 2. Previous syntheses required high pressure and temperature hydrogenation to perform this coupling.

steps within the routes to 1 and 2 to be irreproducible. To overcome these limitations, we developed new routes that afford efficient and reproducible syntheses of both analogues.

The syntheses of both 1 and 2 proceed via intermediates 4 and 10, which possess common features (Figure 2). In previous routes, syntheses of these intermediates required high temperature and pressure hydrogenation steps, which are difficult to carry out on larger scales, and require specialized equipment (Figure 2).3 To develop a more convenient route, we surveyed a series of reductive amination conditions. The use of NaCNBH3 in refluxing methanol was found to be optimal, giving the ribitylated aniline 4 in a 90% yield and 10 in a 92% yield.

The published methods for conversion of 4 (or 10) to compound 1 (or 2) involved other problematic steps. Although several syntheses of compound 1 were published around 1970,4 a number of alternative pathways have since appeared in the literature5 because the key coupling step between intermediate 4 and 6-chlorouracil6 was irreproducible (Figure 3). Typically, this reaction is run at high temperature and without any type of base or catalyst. We determined that a catalytic amount of

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malononitrile, which has been reported to catalyze couplings with conjugated vinyl halides, in refluxing methanol is required for efficient and reproducible coupling to form the functionalized uracil, \( \text{5} \) (Scheme 1).

Purification of \( \text{5} \) proved difficult as a result of its high polarity and acid sensitivity. To circumvent this problem, the crude material \( \text{5} \) was acetylated, and the product was isolated by extraction to give protected uracil \( \text{6} \) in a 65% yield over two steps. The last two steps of the synthesis of compound \( \text{1} \) were carried out as previously reported.12

Treatment of compound \( \text{6} \) with the Vilsmeier reagent followed by removal of the acetate protecting groups provided crude 5-deazariboflavin, \( \text{1} \). Purification by high performance liquid chromatography (HPLC) provided the yellow solid \( \text{1} \) as the trifluoroacetic acid (TFA) salt, in 50% yield (19% overall).

The synthesis of compound \( \text{2} \) also presented several challenges. As previously mentioned, the reductive alkylation conditions developed for the synthesis of \( \text{1} \) were effective en route to \( \text{2} \). However, established strategies employed aniline \( \text{4} \), which can be converted to the ribitylated intermediate \( \text{10} \) via further aromatic functionalization (Figure 4). To avoid this late-stage functionalization, the commercially available diamine \( \text{8} \) was utilized. Initial attempts to monorylate the free aniline proved problematic. When compound \( \text{8} \) was desymmetrized with tert-butyloxyformyl anhydride (Boc\(_2\)O), however, the protected aniline \( \text{9} \) was obtained in 66% yield (Scheme 2). Next, \( \text{d}-\text{ribose} \) was coupled with aniline \( \text{9} \) by reductive amination to afford the desired product in a 92% yield. The t-Boc protecting group was then removed using HCl–dioxane to give intermediate \( \text{10} \) in near quantitative yield.

In previous syntheses, the formation of bicyclic intermediate \( \text{12} \) from compounds \( \text{10} \) and \( \text{11} \) was extremely low yielding (19%). These routes utilized potassium carbonate as a base, which is rather insoluble in organic solvents. When cesium carbonate in a mixture of dimethylformamide and dichloromethane was employed, yields up to 55% of \( \text{12} \) were obtained. The isoalloxazine ring system was completed by stirring intermediate \( \text{12} \) in methanolic ammonia to give 1-deazariboflavin, \( \text{2} \). The crude material was purified by HPLC to give the TFA salt of \( \text{2} \) as a purple solid in 33% yield (11% overall).

The routes described here address each of the problematic steps in previous syntheses of flavin analogues \( \text{1} \) and \( \text{2} \). The resulting strategies are more efficient and alleviate the need for specialized equipment. Thus, they can readily be carried out on a multigram scale. Most importantly, the reactions are reproducible. We anticipate that the results presented here will facilitate the syntheses of flavin analogues for chemical and biological studies.

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**Experimental Section**

**Synthesis of 4.** Aniline 3 (3.0 g, 26 mmol), d-ribose (11.2 g, 74.3 mmol), and sodium cyanoborohydride (3.11 g, 49.5 mmol) were dissolved in methanol (150 mL). The solution was heated to 65 °C for 48 h. The solution was stirred at room temperature for 30 min and then heated to 100 °C for 15 min. Ice was added, and the solution was allowed to stir for 30 min. The reaction was cooled to room temperature, and the residue was dissolved in dichloromethane (20 mL) and washed with water. The organic layers were dried with magnesium sulfate, and the solvent was removed under reduced pressure to yield a red oil. Crude material was dissolved in methanol (150 mL), and the mixture was stirred at room temperature for 5 h. The solution was filtered, and the resulting solid was purified by flash chromatography (silica, 3% ethyl acetate in hexanes) to yield 4, 0.35 mmol (a higher mol % of catalyst was necessary for gram-scale reactions) was suspended in dry methanol (5 mL) and heated at reflux for 48 h. Solvent was removed under reduced pressure, and the resulting residue was dissolved in dichloromethane (20 mL) and washed with water (10 mL) and brine (10 mL). The organic layer was dried with magnesium sulfate, and the solvent was removed under reduced pressure. The residue was recrystallized from hexanes and dichloromethane, resulting in the orange solid 5 (80 mg, 65% over two steps): 

**Synthesis of 5.** Aniline 3 (300 mg, 2.63 mmol) was added, and the solution was adjusted to pH 6 with ammonium hydroxide. This solution was stirred at room temperature for 30 min and then heated at 100 °C for 15 min. Ice was added, and the solution was allowed to stir for 30 min. The reaction was cooled to room temperature, and the residue was dissolved in dichloromethane, resulting in the orange solid 6 (408 mg, 65% over two steps): 

**Synthesis of 6.** Ribitylated aniline 4 (300 mg, 1.18 mmol), 6-chlorouracil (207 mg, 1.41 mmol), and malononitrile (23 mg, 0.35 mmol) was added dropwise. This solution was allowed to stir at room temperature for 30 min and then heated at 100 °C for 15 min. Ice was added, and the solution was allowed to stir for 30 min. The reaction was cooled to room temperature, and the residue was dissolved in dichloromethane, resulting in the orange solid 7 (234 mg, 84%): 

**Synthesis of 7.** Bicyclic compound 6 (360 mg, 0.680 mmol) was dissolved in DMF (1.2 mL) to which phosphorus oxychloride (1.28 mmol) was added dropwise. This solution was allowed to stir at room temperature for 30 min and then heated at 100 °C for 15 min. Ice was added, and the solution was allowed to stir for 30 min. The reaction was cooled to room temperature, and the residue was dissolved in dichloromethane, resulting in the orange solid 8 (408 mg, 65% over two steps): 

**Synthesis of 8.** Bicyclic compound 6 (360 mg, 0.680 mmol) was dissolved in DMF (1.2 mL) to which phosphorus oxychloride (119 mL, 1.28 mmol) was added dropwise. This solution was allowed to stir at room temperature for 30 min and then heated at 100 °C for 15 min. Ice was added, and the solution was allowed to stir for 30 min. The reaction was cooled to room temperature, and the residue was dissolved in dichloromethane, resulting in the orange solid 6 (408 mg, 65% over two steps): 

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Synthesis of 1-Deazariboflavin (2). Compound 12 (25 mg, 0.056 mmol) was dissolved in ammonia-saturated methanol (3 mL), and the resulting solution was allowed to stir at room temperature for 48 h. Solvent was removed under reduced pressure. Crude material was purified by HPLC (100% → 60% A:B over 20 min, retention time about 18 min). This resulted in the purple solid 2 (7 mg, 33%) as the TFA salt: mp > 300° (dec); $^1$H NMR (300 MHz, d$_6$-DMSO) δ 11.1 (s, 1 H, exchange D$_2$O), 7.61 (s, 1 H), 7.58 (s, 1 H), 7.22 (b s, 1H, D$_2$O exchange) 5.51 (s, 1 H), 5.20–3.95 (m, 7 H), 2.33 (s, 3 H), 2.23 (s, 3 H) ppm; $^{13}$C NMR δ 164.4 (TFA), 159.9, 144.4, 142.1, 140.9, 133.1, 133.0, 132.5, 131.0, 115.9 (TFA), 86.7, 73.5, 72.9, 63.4, 48.2, 20.6, 18.6 ppm; ESI (m/z) [M + Na] calcd for C$_{18}$H$_{21}$N$_3$O$_6$ 398.1, found 398.0.

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Supporting Information Available: General experimental procedures and $^1$H and $^{13}$C NMR spectra for the complete syntheses of compounds 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.