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Synthesis and Electrochemical Properties of Structurally Modified Flavin Compounds

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Four structurally modified flavin compounds have been synthesized and characterized for their redox potential by chemical reduction with sodium dithionite. Besides the previously reported 1- and 5-deazariboflavin, a 7,8-didemethyl derivative and an 8-isopropylriboflavin have been obtained. The synthesis of these compounds started in all cases from appropriately substituted anilines that were condensed with the ribityl chain, followed by completion of the annealed three-ring structure. The didemethyl- and the isopropyl compounds gave absorption maxima similar to riboflavin (436 and 448 nm, respectively), whereas 1-deazariboflavin showed a bathochromically shifted absorption (λ_{max} = 537 nm), and that of 5-deazariboflavin was hypsochromically shifted (λ_{max} = 400 nm). The midpoint potentials (E_0') of the four modified flavin compounds were determined by potentiometric titration, using riboflavin as a reference compound. Both alkyl-modified flavins showed slightly less negative midpoint potentials, whereas both deaza compounds had more negative midpoint values compared to the reference compound.

Introduction

Flavins are versatile and, in many aspects, essential redox-active compounds which play important roles as enzyme cofactors in various biological processes.[1,2] Recently, the function of flavin derivatives as light-inducible chromophores in photosensory pigment molecules was realised, and their photochemical properties were studied using various spectroscopic methods,[3,4] adding a biological function even to the photochemically generated radical species of flavins.[5] Quite a number of flavin derivatives had been synthesized and studied for their photochemical and electrochemical properties. However, with the exception of riboflavin itself, commonly known as vitamin B_2, only very few structurally or electronically modified flavin derivatives are available. One major problem in the synthesis of novel flavin derivatives is based on their amphipathic character, making handling in either hydrophilic or hydrophobic solutions difficult. Here, we describe the improved chemical synthesis of two deaza compounds (1- and 5-deazariboflavin) that should show electronically changed properties (compared to the parent compound riboflavin) and two sterically modified compounds, 7,8-didemethylriboflavin and 8-isopropylriboflavin. All four compounds were subjected to chemical reduction by sodium dithionite in order to determine their midpoint potential.

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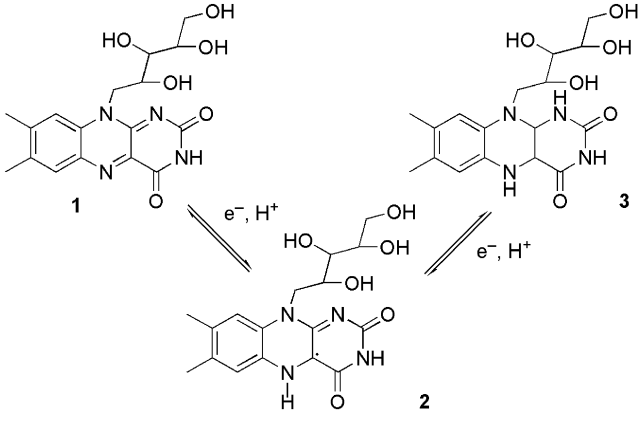
Results and Discussion

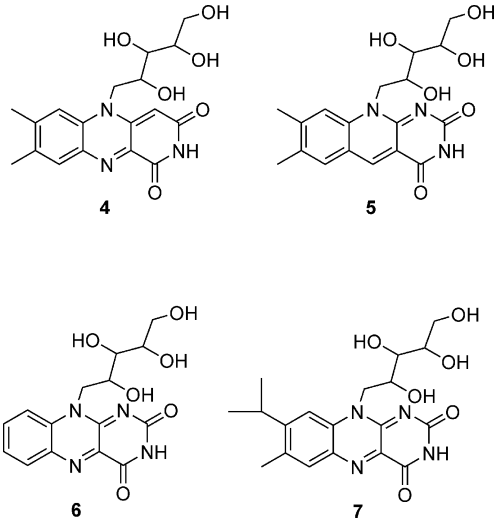
Chemical Synthesis

Flavins can exist in three different redox states: as fully oxidized, (neutral or charged) semiquinoid, and fully reduced forms (Scheme 1).[6] At each of these redox states, the chemical properties of the flavins are significantly different, and are used to support different modes of noncovalent interactions with proteins. To improve our knowledge about the mechanisms of flavin reactivity, four modified flavin analogues were synthesized (Scheme 2).

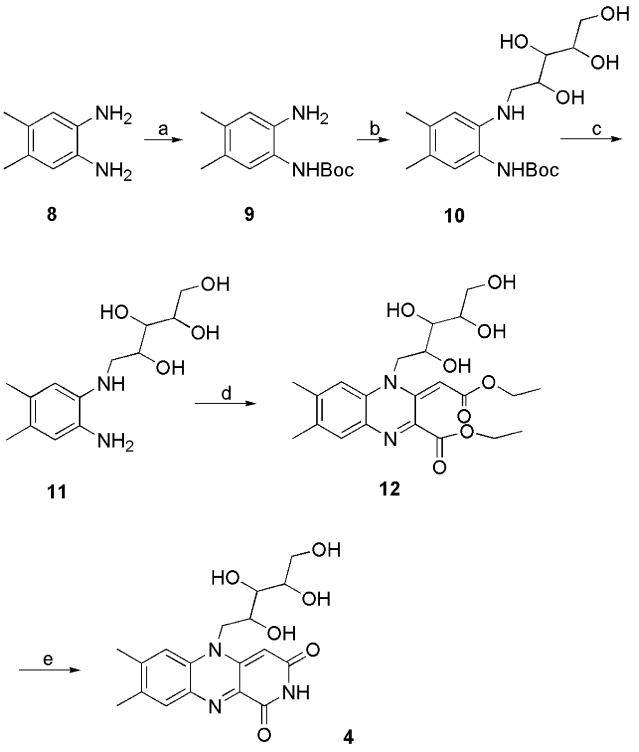


Scheme 1. Oxidized (**1**), "half reduced" (semiquinone **2**) and reduced (**3**) forms of riboflavin.

There are several methods described for the synthesis of flavin derivatives,[7–9] based either on displacement reactions on flavins which contain a good leaving group at the



Scheme 2. Structures of 1-deazariboflavin (**4**), 5-deazariboflavin (**5**), 7,8-didemethylriboflavin (**6**), and 8-isopropylriboflavin (**7**).



Scheme 3. a) Boc_2O; b) D-ribose, $NaCNBH_3$; c) HCl, dioxane; d) $EtOC(O)CH(Br)C(O)CH_2C(O)OEt$, Cs_2CO_3; e) NH_3, CH_3OH.

required positions,[10–12] or undergoing multi-step syntheses from the appropriately substituted aromatic building blocks.[13,14]

1-Deazariboflavin[15,16] **4** and 5-deazariboflavin[17–19] **5** have been previously synthesized. However, their application in biological systems and their physicochemical characterization is still limited due to synthetic difficulties, and improvement of their generation is clearly needed. The synthetic pathways for all four flavins reported in this paper started from available substituted anilines, which were further converted into the corresponding riboanilines in one step and high yield under reductive amination conditions, similar to the proposal by Kiessling et al.[20] The key step in the preparation of compounds **4**–**7** was the elaboration of the corresponding riboaniline derivatives into the complete isoalloxazine ring. According to our aims and commercially available sources, we chose three different synthetic pathways: a) via coupling with 6-chlorouracil; b) via condensation with alloxane, and c) building the deazauracil ring in parts.

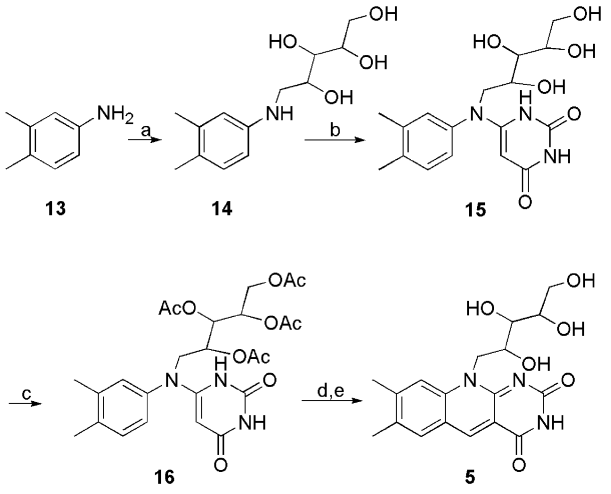
1-Deazariboflavin was synthesized starting from 4,5-dimethyl-2-aminoaniline **8** (Scheme 3). The 2-amino functionality was selectively protected with Boc_2O, and the resulting compound **9** was converted into riboaniline **10** in 86% yield. After the cleavage of the Boc-protection group (HCl in dioxane), compound **11** was coupled with freshly prepared diethyl 2-bromo-3-oxoglutarate in the presence of cesium carbonate. The isoalloxazine ring system was completed by stirring intermediate **12** in methanolic ammonia to afford the desired product **1** in an overall yield of 21%.

For the synthesis of 5-deazariboflavin, the same reductive amination conditions were applied to convert commercially available 3,4-dimethylaniline **13** into riboaniline **14** (Scheme 4). The introduction of the uracil component was performed by coupling **14** with 6-chlorouracil. Although several alternative conditions of this step have been published,[20–25] this coupling still remains the most challenging step due to the relatively low yield and problems with the isolation of the final product **15**. In particular, we found that the use of freshly prepared 6-chlorouracil under carefully maintained dry conditions significantly increases the yield of the reaction. Furthermore, the acetylation of the free hydroxy functions of D-ribose (to reduce the polarity) significantly facilitates the isolation of the intermediate **15**. The isoalloxazine ring closure was performed using a Vilsmeier reaction, followed by cleavage of the acetyl protecting groups to produce 5-deazariboflavin **5** in 36% overall yield.

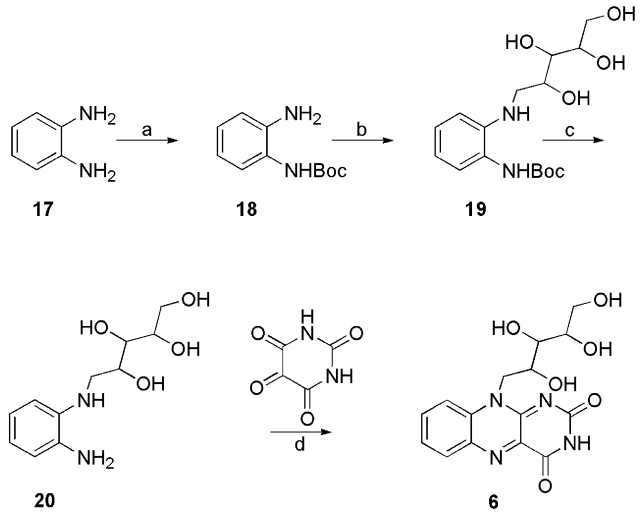
For the generation of 7,8-didemethylriboflavin **6**, 1-*N*-Boc-aminoriboaniline **19** was prepared following a previously established method (Scheme 5).[17,26,27] It was successfully coupled with alloxane under dark conditions in order to avoid photochemical side reactions. HPLC purification yielded the target compound **6** in 56% overall yield.

The synthesis of 8-isopropylriboflavin **7** was similar to that of **5** (Scheme 6). The completion of the isoalloxazine ring was performed by treatment with sodium nitrite[22] in the presence of acetic acid, yielding the *N*-oxide **24**. The desired compound **7** was obtained by reduction with sodium thiosulfite, followed by deprotection of the ribityl OH-groups. The overall yield was 38%.

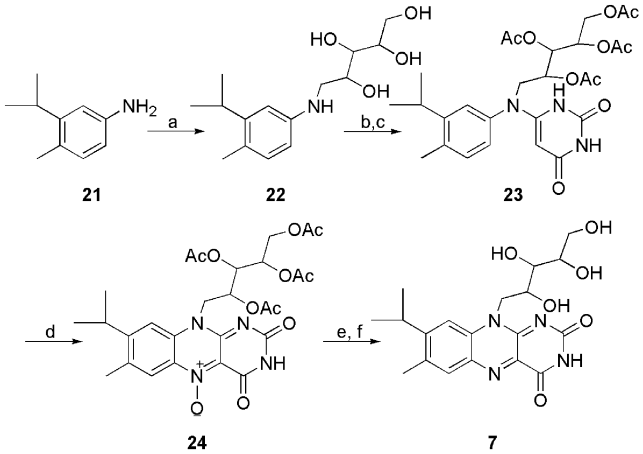
All four target flavins and the intermediate compounds of the synthesis were purified to homogeneity by HPLC (see identification by NMR and high resolution mass spectrometry in the Experimental Section and the Supporting Information).



Scheme 4. a) D-ribose, $NaCNBH_3$; b) 6-chlorouracil; c) Ac_2O, pyridine; d) $POCl_3$, DMF; e) NH_3, CH_3OH.



Scheme 5. a) Boc_2O; b) D-ribose, $NaCNBH_3$; c) HCl, dioxane; d) alloxane, H_3BO_3.



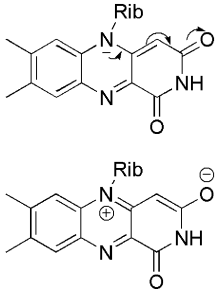
Scheme 6. a) D-ribose, $NaCNBH_3$; b) 6-chlorouracil; c) Ac_2O, pyridine; d) $NaNO_2$, CH_3COOH; e) $Na_2S_2O_4$; f) NH_3, CH_3OH.

UV/Vis Spectroscopic Properties

All four flavins reported here exhibited the flavin-typical double peaked absorption spectra. The spectra of both alkyl-modified flavins, 7,8-didemethylriboflavin (**6**) and 8-isopropylriboflavin (**7**) were practically identical to that of riboflavin, as well as their extinction coefficients (of the $^1S \leftarrow {}^0S$ transition): λ_{max} = 436 nm, ε_{max} = 12500 $M^{-1}cm^{-1}$ for **6**, and λ_{max} = 448 nm, ε_{max} = 12500 $M^{-1}cm^{-1}$ for **7**, respectively. The strongly bathochromically and hypsochromically shifted absorption bands of 1- and 5-deazariboflavin had formerly been noted. The literature data[28] are confirmed by our experiments (λ_{max} = 537 nm, ε_{max} = 6800 $M^{-1}cm^{-1}$ for **4**, and λ_{max} = 400 nm, ε_{max} = 12500 $M^{-1}cm^{-1}$ for **5**, respectively, Table 1). The shifts of the absorption maxima can be explained on the basis of the introduced structural changes. Thus, replacement of the nitrogen atom at position 1 by a methine group generates a conjugated system prone to form a mesomeric, zwitterionic resonance structure where the nitrogen at the 10-position acts as a donor, and the carbonyl at the 2-position acts as an acceptor (Scheme 7). Just the opposite takes place in replacing nitrogen in the 5-position by a methine group in 5-deaza riboflavin. Here, the extended conjugation of the parent compound is reduced by the introduced methine group.

Table 1. Properties of flavin derivatives. The midpoint potential represents the averaged values from three independent measurements.

Sample	E_{av} [mV] (vs. Ag/AgCl)	λ_{max} [nm]	ε_{max} [M^{-1} cm^{-1}]
1-Deazariboflavin	–265 ± 53	537	6800
5-Deazariboflavin	–247 ± 24	400	12500
7,8-Didemethylriboflavin	–203 ± 32	436	12500
8-Isopropylriboflavin	–168 ± 20	448	12500
Riboflavin	–208 ± 12	450	12500



Scheme 7. Formation of a mesomeric, zwitterionic structure upon introducing a methine group at position 1 (1-deazariboflavin **4**).

Electrochemical Characterisation

The midpoint potentials of the four synthesized riboflavin analogues were determined by potentiometric titrations with sodium dithionite. Solutions of riboflavin analogues (≈ 75 μM) were freshly prepared in 100 mM potassium phosphate (KP_i) buffer (pH 7.5), and the samples were maintained in the dark throughout the titration. Freshly pre-

pared sodium dithionite [0.3 M $Na_2S_2O_4$ in 1 M glycine buffer (pH 10.0)] was added in 0.5 μL aliquots to a continuously stirred solution of the riboflavin analogue. The electrochemical potentials were recorded after a steady potential reading was achieved. The absorption spectrum was measured after each equilibration point, from which the concentrations of the oxidised and reduced forms of the riboflavin analogue were determined (Figure 1). The endpoint of the titration was assumed when the redox potential did not change upon further addition of reductant. Application of the Nernst equation allowed the determination of the E_0' of each riboflavin analogue. The titration procedure was calibrated by using riboflavin as a reference compound. Taking the literature value of riboflavin as the reference (–208 mV),[6] we determined the midpoint potentials for 7,8-didemethylriboflavin (–203 mV) and 8-isopropylriboflavin (–168 mV) to be less negative than riboflavin. On the other hand, both deaza compounds exhibited more negative midpoint potentials than riboflavin: the 1-deazariboflavin showed a value of –265 mV, whereas for the 5-deaza compound, a value of –247 mV was determined. A comparison to the literature data showed slight deviations for the latter two compounds, i.e., –280 mV for 1-deazariboflavin,[16,28] and –311 mV for the 5-deaza compound.[28] Whereas the value for the 1-deaza compound is similar to the values reported in the literature, there is a clear deviation for the 5-deaza compound, which cannot be explained.

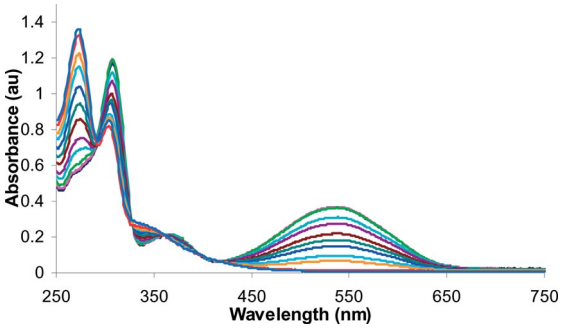


Figure 1. Potentiometric titration of 1-deazariboflavin with sodium dithionite (for details see Experimental Section).

With these novel flavin derivatives at hand, it will be of interest to determine their inherent photochemical properties and their behaviour after incorporation into model peptides or recombinant proteins, in order to extend the understanding of chromophore-protein interactions on the photochemical behavior.

Experimental Section

General: All chemicals were obtained from Sigma–Aldrich unless stated otherwise; 3-isopropyl-4-methylaniline was provided by Daxian Chemical Institute Ltd. All moisture- and oxygen-sensitive reactions were carried out under inert atmosphere (Ar). All reported yields are of isolated pure products. Gel filtration was performed on PD-10 pre-packed desalting columns, purchased from GE Healthcare Life Sciences. Flash chromatography (FC) purification was performed using Merck silica gel 60 (230–400 mesh). High-performance liquid chromatography (HPLC) purification was carried out on a Kromasil C_{18} ODS-5–100 column (RP-C_{18}, 5 μM, 250 mm × 21 mm, flow rate 0.8 mL min^{-1}), eluting with water (A) and acetonitrile (B). The NMR spectra were recorded with a Bruker AVANCE DRX 400 spectrometer. Chemical shifts are given in δ values (ppm) downfield relative to tetramethylsilane (^1H) as an internal standard. Mass spectra (MS) were obtained with a Finnigan MAT 8200 (EI), or with a Bruker Esquire 3000 (ESI). High resolution mass determinations (HRMS) were performed with a Finnigan MAT 95 mass spectrometer. Detailed syntheses of 1-deazariboflavin (**4**) and 5-deazariboflavin (**5**) are given in the Supporting Information.

Potentiometric Methods: All potentiometric redox titrations were performed at 25 °C under anaerobic conditions with Pt wire working and Ag/AgCl reference electrodes. Each titration was performed three times with independently prepared samples. The Ag/AgCl electrode was calibrated against commercially available riboflavin (E_0' = –208 mV vs. standard hydrogen electrode).[6] The reduction potentials were measured using a PHM 63 digital pH meter (Radiometer, Copenhagen, Denmark). All optical absorbance spectra were recorded using an ATI Unicam spectrophotometer model UV2–300.

2-*N*-Boc-amino-ribosylaniline (19): Boc-protected aminoaniline **18** (1 g, 4.8 mmol) was dissolved in MeOH (50 mL). $NaCNBH_3$ (2 equiv.), followed by D-ribose (2 equiv.) was added while stirring. The resulting mixture was stirred under reflux for 23 h, the solvent was removed under reduced pressure, and the remaining material was diluted with 1 M HCl (20 mL) and swirled until gas evolution ceased. The solution was carefully neutralized using a saturated $NaHCO_3$ solution, and extracted with EtOAc (3 × 50 mL). The organic extracts were dried with Na_2SO_4 and concentrated. FC on silica gel, eluting with dichloromethane (DCM)/MeOH (98:2 → 90:10) furnished **19** (1.37 g, 77%) as a white solid and 80 mg (16%) of starting material. ^1H NMR (400 MHz, [D_6]DMSO): δ = 8.24 (br. s, 1 H, NH), 7.05 (d, J = 8 Hz, 1 H, 3-CH), 6.95 (t, J = 8 Hz, 1 H, 4-CH), 6.60 (d, J = 8 Hz, 1 H, 6-CH), 6.53 (t, J = 8 Hz, 1 H, 5-CH), 4.81 (d, J = 6 Hz, 1 H, 2′-CH), 4.76 (d, J = 6 Hz, 1 H, 3′-CH), 4.69 (d, J = 5 Hz, 1 H, 4′-CH), 4.48 (t, J = 6 Hz, 1 H, 5′-C*H*H), 3.76 (m, 1 H, 5′-CH*H*), 3.50 (m, partially hidden under signal of DMSO, 4 OH), 3.25 (m, 1 H, 1′-C*H*H), 2.97 (m, 1 H, 1′-CH*H*), 1.42 (s, 9 H, 3 × CH_3) ppm. ^{13}C NMR (100 MHz, [D_6]-DMSO): δ = 154.4 (CO-Boc), 143.3, 126.3, 126.1, 124.0, 115.9 111.1 (6 × C-Ar), 79.1 (C-Boc), 73.6 (C-3′), 73.0 (C-4′), 70.4 (C-2′), 63.4 (C-5′), 45.8 (C-1′), 28.4 (3 × CH_3-Boc) ppm. EI MS: m/z = 342 [M], 286 [M – $C(CH_3)_3$], 242 [M – Boc], 165 [M – $C(CH_3)_3$-$CH_2OH(CHOH)_3$], 121 [$CH_2OH(CHOH)_3$] ppm. HRMS: calcd. for $C_{16}H_{26}N_2O_6Na$ 365.168307 [M + Na]$^+$; found 365.168017.

2-Amino-ribosylaniline (20): Boc-protected riboaniline **19** (400 mg, 1.17 mmol) was stirred in 4 M HCl/dioxane (20 mL) for 4.5 h. The solvents were removed under reduced pressure, and the resulting mixture was diluted in water (200 mL) and extracted with diethyl ether (3 × 50 mL). The aqueous layer was diluted with water (200 mL) and concentrated by lyophilization, to yield **20** (283 mg, 100%). ^1H NMR (400 MHz, CD_3OD): δ = 7.46 (m, 2 H, 3-CH, 4-CH), 7.40 (t, J = 8 Hz, 1 H, 5-CH), 7.32 (m, 1 H, 6-CH), 4.12 (m, 1 H, 2′-CH), 3.84–3.55 (m, 6 H, ribityl) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 133.2, 129.5, 128.2, 126.9, 125.3, 123.1 (6 × C-Ar), 74.5 (C-3′), 74.0 (C-4′), 69.2 (C-2′), 64.3 (C-5′), 55.9 (C-1′) ppm. EI MS: m/z = 242 [M], 121 [$CH_2OH(CHOH)_3$], 108 [M – rityl]. HRMS: calcd. for $C_{11}H_{18}N_2O_4Na$ 265.115877 [M + Na]$^+$; found 265.115824.

7,8-Didemethylriboflavin (6): A mixture of **20** (130 mg, 0.5 mmol), alloxane (91 mg, 1.2 equiv.) and boric acid (40 mg, 1.2 equiv.) in

acetic acid (3 mL) was stirred at room temp. for 6 h. Water (100 mL) was added, the resulting mixture was adjusted to pH 7 with aqueous NH_3 and then extracted with ethyl acetate (2 × 50 mL). The aqueous phase was evaporated under vacuum, absolute ethanol was added to form the ethyl ester of boric acid, and the mixture was evaporated. The last step was repeated five times until the white crystals stopped appearing. Gel filtration of the residue and HPLC (A:B, 90:10, retention time 9 min) furnished **6** (135 mg, 73%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.41 (m, 1 H, 3-NH), 8.11 (d, J = 8 Hz, 1 H, C-6), 8.07 (d, J = 8 Hz, 1 H, C-9), 7.90 (t, J = 8 Hz, 1 H, C-8), 7.63 (t, J = 8 Hz, 1 H, C-7), 5.09 (d, J = 6 Hz, 1 H, OH), 4.89 (m, 1 H, 2′-CH), 4.83 (d, J = 6 Hz, 1 H, OH), 4.79 (d, J = 6 Hz, 1 H, OH), 4.68 (d, J = 14 Hz, 1 H, 3′-CH), 4.45 (t, J = 6 Hz, 1 H, OH), 4.24 (m, 1 H, 4′-CH), 3.75 (m, 4 H, 5′-CH$_2$, 1′-CH$_2$) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.8 (C-4), 155.6 (C-2), 151.2 (C-10a), 138.2 (C-9a), 135.1 (C-4a), 134.6 (C-8), 133.9 (C-5a), 131.5 (C-6), 126.0 (C-7), 117.8 (C-9), 73.7 (C-3′), 72.7 (C-4′), 68.8 (C-2′), 63.4 (C-5′), 47.6 (C-1′) ppm. ESI MS: m/z = 371 [M + Na]$^+$, 349 [M + H]$^+$, 348 [M]. HRMS: calcd. for $C_{15}H_{16}N_4O_6Na$ 371.096206 [M + Na]$^+$; found 371.096420.

3-Isopropyl-4-methylriboaniline (22): Riboaniline **22** was prepared from 2-isopropyl-3-methylaniline **21** with 79% yield as described for **19**. Reaction time: 72 h. ^{1}H NMR (400 MHz, CD$_3$OD), δ = 6.87 (d, J = 8 Hz, 1 H, 5-CH), 6.63 (d, J = 4 Hz, 1 H, 2-CH), 6.45 (dd, J = 8, 2 Hz, 1 H, 6-CH), 3.91 (m, 1 H, 2′-CH), 3.76 (m, 2 H, 3′-CH, 4′-CH), 3.63 (m, 2 H, 5′-CH$_2$), 3.44 (dd, J = 12, 3 Hz, 1 H, 1′-C*H*H), 3.30 (m, 4 OH), 3.08 (m, 2 H, *i*Pr-CH, 1′-CH*H*), 2.18 (s, 3 H, CH$_3$), 1.19, 1.17 (2s, 2 × *i*Pr-CH$_3$) ppm. ^{13}C NMR (100 MHz, CD$_3$OD), δ = 148.5, 148.4 131.7, 125.2, 112.2, 112.1 (6 × C-Ar), 74.9 (C-3′), 74.3 (C-4′), 72.1 (C-2′), 64.6 (C-5′), 48.1 (C-1′), 30.5 (*i*Pr-CH), 23.6 (2 × *i*Pr-CH$_3$), 18.5 (CH$_3$) ppm. EI MS: m/z = 283 [M], 162 [M – (CHOH)$_3$CH$_2$OH].

Intermediate 23: Ribitylated aniline **22** (200 mg, 0.70 mmol) and 6-chlorouracil (125 mg, 1.2 equiv.), pre-dried together with powdered malonitrile (40 mg), were suspended in dry methanol (5 mL) and stirred under reflux for 3 d. The solvent was removed under reduced pressure, and the resulting solid was dissolved in a mixture of pyridine (4 mL) and acetic anhydride (0.4 mL). The resulting mixture was allowed to stir at room temp. for 6 h. The solvent was removed under reduced pressure with toluene, and the resulting residue was dissolved in DCM (50 mL) and washed with water (3 × 20 mL) and brine (20 mL). The organic extracts were dried with $MgSO_4$, filtered, and the solvent was removed under reduced pressure. FC on silica gel, eluting with DCM/MeOH (95:5), afforded the desired product, **23** (297 mg, 75% after two steps). ^{1}H NMR (400 MHz, CDCl$_3$), δ = 7.18 (m, 1 H, 5α-CH), 7.01 (m, 1 H, 6-CH), 6.89 (m, 1 H, 9-CH), 5.30 (m, 1 H, OH), 5.21 (m, 1 H, OH), 5.08 (m, 1 H, OH), 4.92 (m, 1 H, OH), 4.22 (m, 2 H, 2′-CH, 3′-CH), 4.02 (m, 1 H, 4′-CH), 3.88 (m, 1 H, 5′-C*H*H), 3.66 (m, 1 H, 5′-CH*H*), 3.41 (m, 2 H, 1′-CH$_2$), 3.09 (q, J = 7 Hz, 1 H, *i*Pr-CH), 2.32 (s, 3 H, CH$_3$), 2.06, 2.00, 1.97, 1.83 (4s, 12 H, 4 × Ac-CH$_3$), 1.18 (m, 6 H, 2 × *i*Pr-CH$_3$) ppm. ^{13}C NMR (100 MHz, CDCl$_3$), signals which could be interpreted: δ = 170.5, 170.0, 169.6, 169.5 (4 × CO-Ac), 164.9 (C-4), 153.8 (C-2), 150.4 (C-10a), 150.3 (C-8), 148.7 (C-9a), 142.1 (C-7), 137.2 (C-5a), 137.0 (C-6), 132.5 (C-9), 74.6 (C-4a), 72.8 (C-3′), 71.1 (C-4′), 70.1 (C-2′), 66.2 (C-5′), 51.9 (C-1′), 29.6 (*i*Pr-CH), 23.0, 22.9 (2 × CH$_3$-*i*Pr), 20.9, 20.7, 20.6, 20.5 (4 × CH$_3$-Ac), 18.9 (CH$_3$) ppm. EI MS: m/z = 561 [M], 502 [M – OAc], 442 [M – 2OAc], 259 [M – ribityl].

Tetra-*O*-acetyl-8-isopropyl-5-nitrosoriboflavin (24): $NaNO_2$ (58 mg, 0.5 mmol) was added to a solution of **23** (58 mg, 0.1 mmol) in acetic acid (0.75 mL) in the dark. The mixture was stirred at room temp. for 3 h, and then 0.5 mL of water was added. The suspension was quenched for an additional 3 h before the solvents were removed under reduced pressure. The crude orange solid was washed with water (2 × 0.5 mL), and dried. FC on silica gel, eluting with DCM/MeOH (97:3) furnished **24** (50 mg, 80%). ^{1}H NMR (400 MHz, CD$_3$OD): δ = 8.17 (s, 1 H, 6-CH), 7.72 (s, 1 H, 9-CH), 5.69 (m, 1 H, 2′-CH), 5.53 (t, J = 6 Hz, 1 H, 3′-CH), 5.40 (m, 1 H, 4′-CH), 5.18 (m, 2 H, 5′-CH$_2$), 4.45 (dd, J = 12, 3 Hz, 1 H, 1′-C*H*H), 4.26 (dd, J = 12, 6 Hz, 1 H, 1′-CH*H*), 3.35 (m, 1 H, *i*Pr-CH), 2.50 (s, 3 H, CH$_3$), 2.18, 2.16, 2.00, 1.70 (4s, 12 H, 4 × CH$_3$-Ac), 1.38, 1.36 (2s, 6 H, 2 × CH$_3$-*i*Pr) ppm. ^{13}C NMR (100 MHz, CD$_3$OD): δ = 172.3, 171.8, 171.5, 171.3 (4 × CO-Ac), 158.8 (C-4), 157.1 (C-2), 155.0 (C-10a), 137.3 (C-8), 134.6 (C-7), 134.2 (C-4a), 126.0 (C-5a), 122.5 (C-6), 114.8 (C-9), 71.4 (C-3′), 70.8 (C-4′), 70.6 (C-2′), 63.0 (C-5′), 45.4 (C-1′), 31.7 (*i*Pr-CH), 23.33, 23.30 (2 × *i*Pr-CH$_3$), 21.0, 20.8, 20.60, 20.57 (4 × CH$_3$-Ac), 19.2 (CH$_3$) ppm. EI MS: m/z = 588 [M], 545 [M – Ac], 529 [M – OAc], 286 [M – ribityl], 270 [M – ribityl – O]. HRMS: calcd. for $C_{27}H_{32}N_4O_{11}Na$ 611.195981 [M + Na]; found 611.196507.

Tetra-*O*-acetyl-8-isopropylriboflavin: $Na_2S_2O_4$ (27 mg, 1.5 equiv.) in water (3 mL) was added to a solution of **24** (50 mg, 0.085 mmol) in EtOH (3 mL) under argon. After stirring at room temp. for 4 h, the solvents were evaporated, and the crude yellow solid was washed with water (2 × 2 mL) and dried under vacuum. FC on silica gel eluting with DCM/MeOH (98:2) yielded the acetylated isopropylriboflavin (49 mg, 100%). ^{1}H NMR (400 MHz, CD$_3$OD), δ (ppm): 7.95 (s, 1 H, 6-CH), 7.75 (s, 1 H, 9-CH), 5.71 (q, J = 4 Hz, 1 H, 2′-CH), 5.54 (dd, J = 6, 4 Hz, 1 H, 3′-CH), 5.41 (m, 1 H, 4′-CH), 5.11 (m, 2 H, 5′-CH$_2$), 4.46 (dd, J = 12, 3 Hz, 1 H, 1′-C*H*H), 4.24 (dd, J = 12, 6 Hz, 1 H, 1′-CH*H*), 3.41 (q, J = 7 Hz, 1 H, *i*Pr-CH), 2.52 (CH$_3$), 2.18, 2.17, 2.01, 1.64 (4s, 12 H, 4 × CH$_3$-Ac), 1.39 (m, 6 H, 2 × CH$_3$-*i*Pr) ppm. ^{13}C NMR (100 MHz, CD$_3$OD): δ = 172.3, 171.9, 171.37, 171.27 (4 × CO-Ac), 162.1 (C-4), 159.0 (C-2), 152.4 (C-10a), 137.9 (C-8), 137.2 (C-7), 135.6 (C-4a), 133.6 (2 C, C-5a, C-6), 113.6 (C-9), 71.4 (C-3′), 70.8 (C-4′), 70.3 (C-2′), 63.0 (C-5′), 45.4 (C-1′), 31.9 (*i*Pr-CH), 23.5, 23.4 (2 × *i*Pr-CH$_3$), 21.0, 20.7, 20.6, 20.4 (4 × CH$_3$-Ac), 18.9 (CH$_3$) ppm. EI MS: m/z = 572 [M], 529 [M – Ac], 513 [M – OAc], 284 [M – (CHOAc)$_3$CH$_2$OAc], 270 [M – ribityl]. HRMS: calcd. for $C_{27}H_{32}N_4O_{10}Na$ 595.201063 [M + Na]; found 595.201277.

8-Isopropylriboflavin (7): Tetra-*O*-acetyl-8-isopropylriboflavin (50 mg, 0.09 mmol) was dissolved in methanol saturated with NH_3 (2.5 mL), and the resulting mixture was stirred at room temp. for 5 h. The solvent was removed under reduced pressure, and the crude solid was washed with a mixture of DCM/MeOH (4:1, 0.5 mL) to remove acetylated byproducts. The resulting solid was then dissolved in 5% aqueous NH_4OH (5 mL), filtered, and lyophilized. Pure **7** (28 mg) was obtained in 80% yield. ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.33 (br. s, 1 H, 3-NH), 7.91 (s, 1 H, 6-CH), 7.89 (s, 1 H, 9-CH), 5.15 (m, 1 H, OH), 5.03 (m, 1 H, 2′-CH), 4.78 (m, 2 H, 2 OH), 4.63 (d, J = 12 Hz, 1 H, 3′-CH), 4.46 (t, J = 6 Hz, 1 H, OH), 4.21 (m, 1 H, 4′-CH), 3.63 (m, 4 H, 5′-CH$_2$, 1′-CH$_2$), 3.20 (m, partially obscured by the solvent signal, 1 H, *i*Pr-CH), 2.47 (s, 3 H, CH$_3$), 1.28 (m, 6 H, 2 × CH$_3$-*i*Pr) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 160.0 (C-4), 155.8 (C-2), 155.6 (C-10a), 151.1 (C-9a), 137.1 (C-8), 134.2 (C-7), 133.7 (C-4a), 132.3 (C-5a), 131.5 (C-6), 113.6 (C-9), 73.5 (C-3′), 72.7 (C-4′), 69.0 (C-2′), 63.2 (C-5′), 47.1 (C-1′), 30.0 (*i*Pr-CH), 23.0, 22.7 (2 × *i*Pr-CH$_3$), 18.4 (CH$_3$) ppm. EI MS: m/z = 404 [M], 388 [M – O], 284 [M – (CHOH)$_3$CH$_2$OH], 270 [M – ribityl]. HRMS: calcd. for $C_{19}H_{25}N_4O_6$ 405.176856 [M + H]; found 405.176635.

Supporting Information (see also the footnote on the first page of this article): The detailed syntheses of 1-deazariboflavin and 5-deazariboflavin are described.

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