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Rational improvement of the synthesis of 1-deazariboflavin

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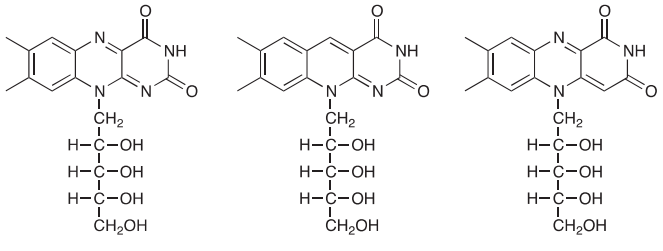
ABSTRACT

The cofactor forms of riboflavin (FMN and FAD) play a crucial role in the mediation of both enzymatic processes and light perception by photo-sensitive proteins, and thus structural analogues of this chromophore are highly useful tools to assist in the elucidation of enzymatic mechanisms. 1-Deazariboflavin has been rarely utilised for this purpose, due in part to its previously difficult and inefficient synthesis. Recent examination has enabled a remarkable improvement in the overall synthetic yield from 11.0 to 61.3%, allowing reliable production of 1-deazariboflavin for use as a tool in enzymatic mechanistic determination.

1. Introduction

Isoelectric analogues of riboflavin have been used to provide evidence of the mechanistic basis for the reactions of flavoenzymes for several decades. The most common of these analogues are the 'deaza-flavins', which have one (or more) of the reactive nitrogen atoms replaced by a carbon atom while maintaining the physical structure of the tricyclic isoalloxazine moiety (Scheme 1). However, substitution of carbon in place of nitrogen at these positions inevitably alters the redox potential of the molecule, leading to a significant change in biological activity. When incorporated into a flavoprotein, deaza-analogues provide useful mechanistic information about the behaviour of the protein, even in cases where structural information has yet to be resolved; this method is therefore complementary to amino acid exchanges within the active site of the protein, representing a 'site-directed mutagenesis' of the cofactor.



Scheme 1. Structures of riboflavin, 5-deazariboflavin **1** and 1-deazariboflavin **2**. Absolute stereochemistry of the ribityl side-chain (as shown above) is assumed in all subsequent schemes.

While 5-deazariboflavin **1** (Scheme 1) has become a widely used probe to examine flavoprotein mechanisms, 1-deazariboflavin **2** has been much less commonly used, for two predominant reasons. Firstly, the synthesis of 1-deazariboflavin **2** is notoriously difficult, with a reported yield of only 11.0% achieved in the 2006 examination described by Carlson and Kiessling[1] (based upon their improvement of an earlier synthetic route[2]). Interestingly, when this method was repeated in 2008 using identical conditions by Mansurova et al.,[3] an overall yield of 21.4% was reported, despite an overwhelming similarity to the previous method. Secondly, the use of 1-deazariboflavin **2** as a mechanistic probe has been somewhat limited by the interesting electronic structure of the molecule, which prevents the formation of a triplet-state,[4,5] and thus precludes interactions, which rely upon these transitions.

This inability of 1-deazariboflavin **2** to form a triplet-state has been of significant benefit in the recent examination of the mechanism of photoadduct formation in the PHOT1-LOV2 domain of *Avena sativa*, providing evidence to support a radical-pair mechanism involving a flavin triplet-state in both the *A. sativa* LOV2 domain,[6] and the YtvA-LOV domain of *Bacillus subtilis*.[7]

The synthetic method used in both cases described above[1,3] is a direct descendant of the earliest reported synthesis of 1-

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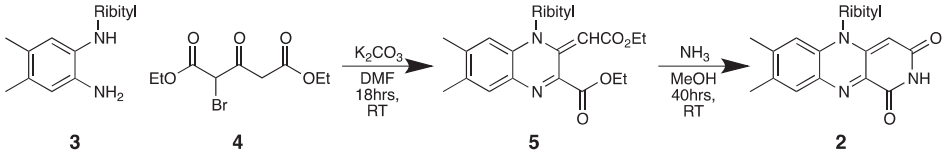
deazariboflavin **2** reported by Ashton et al.[2] This method (with the key steps shown in Scheme 2) features condensation of the ribitylated aniline **3** with diethyl 2-bromo-3-oxoglutarate **4** to form bicyclic intermediate **5**, followed by closure of the isoalloxazine system at the N(3) position using ammonia to give the target compound in a yield of 14% over two steps. Interestingly, during the same study it was also reported that when repeated using a non-ribitylated diamine, the yield of the corresponding 1-deazalumichrome increased to 19% over two steps,[2] suggesting that the ribityl side-group plays some part in the efficiency of this reaction.



Scheme 2. Original method for formation of 1-deazariboflavin **2** reported by Ashton et al.,[2] with the above reaction steps completed in a reported yield of 14%.

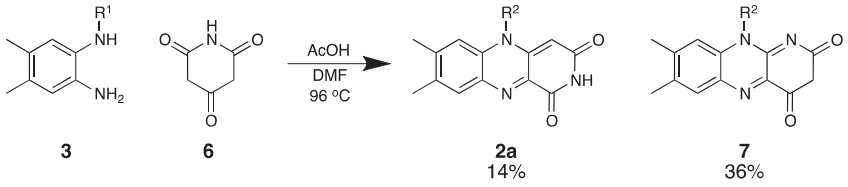
An alternative route also examined by Ashton et al.[2] described the treatment of ribitylated diamine **3** with 2,4,6-piperidine trione **6**, leading to the ribityl-tetraacetate derivatives of 1-deazariboflavin **2a** and 3-deazariboflavin **7** in yields of 14% and 36%, respectively (Scheme 3). However, since this route led predominantly to formation of the alternative deazaflavin isomer 3-deazariboflavin **7**, and the resultant products each required additional deprotection and purification to furnish the target compound, this method has not subsequently been revisited in recent literature.

Hence, in order to discover an improved method for the synthesis of 1-deazariboflavin **2**, each step of the route described by Carlson and Kiessling (overall yield of 11.0%)[1] was scrutinised to determine potential sites for optimisation, and which has led to an approximate five-fold increase in the achievable yield. These improvements are described below.

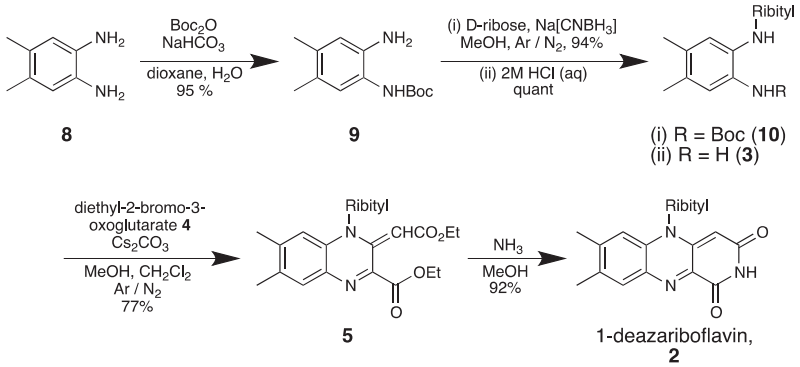
2. Results and discussion

Several key reactions in the synthesis of 1-deazariboflavin **2** were identified, which, although crucial to the success of the synthetic route, had previously been responsible for the greatest reduction in overall efficiency. In each case several alternative strategies were attempted, with the most effective conditions summarised in Scheme 4.

Protection of 4,5-dimethylbenzene-1,2-diamine **8** was necessary in order to allow reliable formation of the mono-ribitylated product **3**. A *tert*-butyloxycarbonyl protecting group was employed as before,[1] with protection achieved at ambient temperature (using a strict stoichiometric amount of the anhydride) in 92% yield, compared to 66% reported previously.[1] Ribitylation of mono-protected intermediate **9** was also performed according to the same procedure;[1] however, it was found that the acid-labile Boc protecting group was readily cleaved during the work-up of the reaction (performed using aqueous hydrochloric acid), even with short exposure times. This was problematic as the resultant deprotected ribitylated intermediate **3** was highly soluble in the aqueous solution, which was also contaminated by other reaction by-products. It was therefore necessary to perform this procedure rapidly in order to maximise recovery of ribitylated protected intermediate **10**.



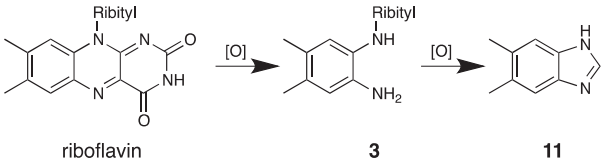
Scheme 3. Alternative route for the synthesis of 1-deazaflavin **2** developed by Ashton et al.[2] R^1 refers to a D-ribityl group, while R^2 describes the tetra-acetylated derivative of this group.



Scheme 4. Overall route for the synthesis of 1-deazariboflavin **2**.

The formation of intermediate **5** devised by Ashton et al.[2] originally described the coupling of ribitylated diamine **3** with diethyl 2-bromo-3-oxoglutarate **4** in the presence of potassium carbonate. Improvement of this inefficient step was key to the method of Carlson and Kiessling,[1] who used caesium carbonate as an alternative base and which was significantly more soluble in their chosen solvent mixture (dichloromethane and dimethylformamide). However, even after this alteration the yield for this crucial reaction was only 55%, and thus remained a target for improvement.

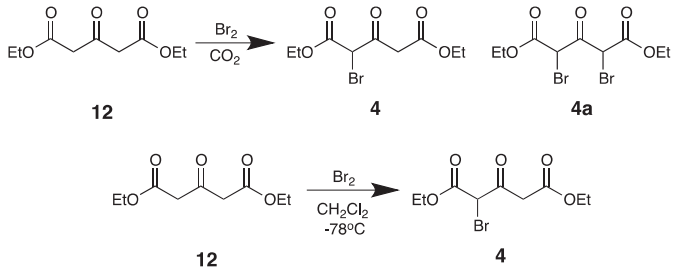
Regarding deprotection of ribitylated intermediate **10**, we presume that the conditions chosen by Carlson and Kiessling (HCl in dioxane) in place of a conventional approach using an aqueous solvent were due to this very water solubility.[3,8,9] However, an additional complication at this stage was identified due to a reported facile oxidation mechanism for the destruction of this intermediate, particularly favoured under aqueous conditions. Formation of 5,6-dimethylbenzimidazole **11** has been reported by an 'oxidative cascade' from riboflavin (or a bio-available flavin derivative) via intermediate **3**, by aerobic oxidation under physiological conditions (pH 7; 37 °C) (Scheme 5).[8,10] However, during the present work it was found that this was an incredibly slow process—a constantly aerated aqueous solution of intermediate **3** (which had been formed under anaerobic conditions) showed only 8% formation of 5,6-dimethylbenzimidazole **11** after 96 h, with 87% recovery of the unreacted starting material.



Scheme 5. Formation of 5,6-dimethylbenzimidazole **11** via an 'oxidative cascade' from riboflavin via intermediate **3**.[8,10]

As the rate of oxidation of intermediate **3** was found to be extremely slow in these conditions, conventional deprotection strategies were examined. The use of aqueous 2 M HCl was found to reliably furnish a quantitative yield of deprotected intermediate **3** within 2 h following simple evaporation and was thus adopted due to its improved convenience.

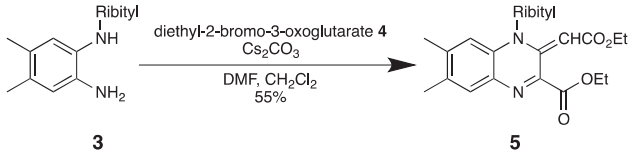
Formation of diethyl 2-bromo-3-oxogluarate **4** following the reported method[1] was found to be ineffective (Scheme 6). The large excess of bromine used in the reaction reported by Carlson and Kiessling[1] was found to produce significant quantities of dibrominated product **4a**, easily identifiable by mass spectrometry by the characteristic isotope pattern of bromine. Although a reduction in temperature (to 0 °C) greatly reduced the formation of this dibrominated species during the timescale of the reaction, the efficiency of subsequent reactions was not improved using intermediate **4** formed in this manner.



Scheme 6. An alternative method for the formation of diethyl 2-bromo-3-oxogluarate **4** [below] prevented additional formation of dibrominated side product **4a**.

An alternative method reported for more reliable formation of diethyl 2-bromo-3-oxoglutarate **4** describes the use of strictly anhydrous conditions in dichloromethane and employing bromine as the determinant reactant.[11] This was found to be significantly more effective, and was further enhanced by cooling to −78 °C during the addition of bromine, with the resultant product being sufficiently stable to allow swift purification through a short plug of silica.

Coupling of the deprotected ribitylated diamine **3** and the so-formed pure diethyl 2-bromo-3-oxogluarate **4** was initially performed as reported by Carlson and Kiessling (using caesium carbonate in a mixture of dichloromethane and dimethylformamide), resulting in the formation of bicyclic intermediate **5** in 55% yield (Scheme 7) as previously reported.[1] However, it was observed that while ribitylated diamine **3** was soluble in high-polarity solvents (and insoluble in non-polar solvents), diethyl 2-bromo-3-oxogluarate **4** was only soluble in non-polar solvents and insoluble in polar solvents. The poor miscibility of the chosen solvents appeared, not unreasonably, to play a deleterious role in the mediocre yield of this reaction stage, and which was highly reproducible; several strategies were considered to improve the mixing of the reactants.



Scheme 7. Original method for the formation of bicyclic intermediate **5** as described by Carlson and Kiessling,[1] using a mixture of DMF and dichloromethane.

Initially, the use of DMAP in place of (or alongside) caesium carbonate was attempted, due to the phase-transfer effect of DMAP;[12–14] however, in both cases a decrease in the yield of product **5** was observed, indicating a sensitivity of this reaction to strong bases. Instead, a range of alternative solvent combinations were examined in place of the previously reported mixture, as summarised in Table 1.

Table 1
Solvent mixtures used to examine the coupling between deprotected ribitylated aniline **3** and diethyl 2-bromo-3-oxoglutarate **4**. All solvents were used in equivolume ratios; the mixture of CH_2Cl_2 and hexane did not allow complete dissolution of starting materials and was performed as a suspension

Solvent 1	Solvent 2	Yield (%)
CH_2Cl_2	DMF	55
CH_2Cl_2	MeOH	77
CH_2Cl_2	EtOH	44
CH_2Cl_2	H_2O	8
CH_2Cl_2	DMSO	0
CH_2Cl_2	Hexane	0
$CHCl_3$	DMF	39
$CHCl_3$	MeOH	65
Et_2O	MeOH	16
Et_2O	H_2O	11

It was found that replacing dimethylformamide with methanol significantly enhanced the yield of bicyclic product **5**, from 55% to 77%. Interestingly, results also indicated a degree of moisture sensitivity for this reaction; subsequent repetitions were therefore performed under anhydrous conditions using thoroughly dried[15] solvents.

Ring-closure of bicyclic intermediate **5** was performed using a solution of ammonia dissolved in methanol. Originally, this was freshly prepared at −5 °C and resulted in moderate to low yields of 1-deazariboflavin **2**; Carlson and Kiessling report a yield of 33% for their analogous reaction. In the present study, we were pleased to find that dissolution of ammonia in methanol cooled to −78 °C produced a solution with reasonable stability and which was suitable for use even when the reaction was subsequently carried out at ambient temperature. When performed in this manner, these conditions were found to be significantly more efficient at furnishing the target product than previous attempts, with a reproducible yield of 92% being achieved.

3. Conclusion

In conclusion, the synthesis of 1-deazariboflavin **2** has been dramatically improved from a method reporting a yield of 11.0%[1] to 61.3%, largely due to improvements made to two key reactions.

Firstly, the reaction between deprotected ribitylated diamine **3** and diethyl 2-bromo-3-oxogluarate **4** was found to be largely influenced by the solvent mixture chosen for the reaction, with poor miscibility between reactant solutions negatively affecting product yields. Strict control in the formation of diethyl 2-bromo-3-oxogluarate **4** was used to ensure the production of a single monobrominated product (with spectroscopic evidence suggesting substantial di-bromination of this compound when prepared according to the original method[1]). While the use of a phase-transfer catalyst (DMAP) was found to be ineffective, a more efficient solvent mixture consisting of 1:1 dichloromethane/methanol was found, improving the yield for this step of the reaction from 55% to 77%.

Secondly, efficient ring-closure of the bicyclic intermediate compound **5** to furnish 1-deazariboflavin **2** was performed using a solution of ammonia in methanol. The concentration of ammonia played a key role, with low concentrations furnishing correspondingly low yields of 1-deazariboflavin **2**. Dissolution of ammonia in methanol cooled to −78 °C was found to produce a highly concentrated solution with sufficient stability for later use at ambient temperature and which allowed yields of 92% to be obtained; a substantial improvement over the 33% reported previously.[1]

4. Experimental detail

4.1. General experimental techniques

All reagents were purchased from Sigma–Aldrich or Fisher Scientific, and were of 98% (or higher) purity. All solvents were purchased from Fisher Scientific. Anhydrous solvents were obtained from an MBraun MB SPS-800 solvent purification system, or (in the case of methanol) distilled over CaH_2 before being further dried over molecular sieves. 'High vacuum' refers to ultra-low vacuum pressure ($<10^{-2}$ Torr), provided by an Oerlikon TRIVAC mechanical oil-pump. Melting points were measured in a Gallenkamp Variable heater apparatus fitted with an internal temperature probe and confirmed using a mercury thermometer and are uncorrected; literature values (where available) are given alongside observed results. NMR spectroscopy was performed using Bruker AVANCE DPX 400 MHz or 500 MHz (Ultrashielded) spectrometers (^{1}H, ^{13}C) or a Bruker AVANCE DPX 250 MHz spectrometer (^{13}C). All spectra were recorded in fully deuterated solvents at 20 °C. Samples for mass spectrometry were analysed via the internal MS Service at Cardiff University, obtained using a Waters LCT Premier XE TOF Spectrometer. Ionisation was performed by EI, ESI or APCI, with detection in positive or negative mode as required for each product.

4.2. *tert*-Butyl (2-amino-4,5-dimethylphenyl)carbamate 9

4,5-Dimethylbenzene-1,2-diamine **8** (1.00 g, 7.30 mmol), di-*tert*-butyl dicarbonate (1.60 g, 7.30 mmol, 1.0 equiv) and sodium hydrogen carbonate (0.62 g, 8.34 mmol, 1.15 equiv) were dissolved in a mixture of dioxane (50 mL) and water (50 mL). The mixture was stirred for 3 h at room temperature, with the original orange colour of the solution changing to brick red. The mixture was diluted with water (150 mL) and extracted into dichloromethane (3×50 mL); the combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (50 mL), brine (50 mL), and further dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting solid was dried under high vacuum for 4 h, yielding *carbamate* **9** as a red-orange solid (1.59 g, 6.73 mmol, 92%), mp 153–155 °C [lit.[16] mp 146 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.02 (s, 1H), 6.57 (s, 1H), 6.19 (br s, 1H), 3.53 (br s, 2H), 2.15 (s, 3H), 2.14 (s, 3H), 1.50 (s, 9H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ=154.1, 137.4, 134.3, 132.3, 127.9, 122.3, 118.6, 80.3, 28.4, 19.3, 18.9; *m*/*z* (EI^+) 236 [M^+, 100%]; no di-protected product was observed ($C_{18}H_{28}N_2O_4$: *m*/*z* 336).

4.3. 1-*N*-(Ribityl),2-*N*(Boc)-1,2-diamino-4,5-dimethylbenzene 10

tert-Butyl (2-amino-4,5-dimethylphenyl)carbamate **9** (3.30 g, 14.0 mmol), D-ribose (5.25 g, 35 mmol, 2.5 equiv) and sodium cyanoborohydride (1.57 g, 25 mmol, 1.8 equiv) were powdered and pre-dried under vacuum in flame-dried glassware, before dissolution in anhydrous MeOH (150 mL) under an Ar/N_2 atmosphere. The mixture was heated at reflux for 48 h before cooling to room temperature, whereupon the solvent was removed by evaporation. The brown residue was dissolved in 1 M $HCl_{(aq)}$ (50 mL) and swirled for 30 s, before swift neutralisation using a saturated aqueous solution of sodium hydrogen carbonate (75 mL). The resulting solution was extracted into ethyl acetate (5×75 mL), with the combined organic layers washed using brine (2×100 mL) and dried over Na_2SO_4. Solvent was removed under reduced pressure and the resultant solid further dried under high vacuum for 8 h, to give the *ribitylated product* **10** as an orange crystalline solid (4.89 g, 13.20 mmol, 94%), mp 110–112 °C; ^{1}H NMR (500 MHz, MeOD-d_3) δ=6.90 (s, 1H), 6.64 (s, 1H), 3.98–3.95 (m, 1H), 3.81 (dd, 1H, J_{AM}=3.5 Hz, J_{AX}=11.0 Hz), 3.79–3.76 (m, 1H), 3.67 (q, 2H, J=6.5 Hz), 3.46 (dd, 1H, J_{AM}=3.0 Hz, J_{AX}=13.0 Hz), 3.17 (dd, 1H, J_{AM}=3.1 Hz, J_{AX}=8.0 Hz), 2.22 (s, 3H), 2.16 (s, 3H), 1.53 (s, 9H); ^{13}C NMR (100 MHz) δ=153.1, 132.9, 132.3, 131.8, 126.4, 122.0, 118.6, 74.4, 73.1, 70.7, 63.4, 46.5, 28.2, 19.3, 18.9; *m*/*z* (EI^+) 394 [($M+H^+$+Na^+), 20%], 370 [($M+H^+$), 100%]; (ES^+) 370 [($M+H^+$), 100%].

4.4. 1-*N*-(Ribityl),2-diamino-4,5-dimethylbenzene 3

1-*N*-(Ribityl),2-*N*(Boc)-diamino-4,5-dimethylbenzene (**10**) (1.20 g, 3.24 mmol) was dissolved in 2 M aqueous HCl (25 mL) and stirred at ambient temperature for 3 h. The solution was lyophilised to furnish *deprotected intermediate* **3** as a green crystalline solid (876 mg, 3.24 mmol, 100%), ^{1}H NMR (400 MHz, D_2O) δ=7.07 (s, 1H), 6.76 (s, 1H), 3.92 (m, 1H), 3.83 (dd, 1H, J_{AM}=3.5 Hz, J_{AX}=11.2 Hz), 3.66 (m, 1H), 3.62 (dd, 2H, J_{AM}=6.5 Hz, J_{AX}=12.8 Hz), 3.47 (dd, 1H, J_{AM}=3.0 Hz, J_{AX}=12.8 Hz), 3.31 (m, 1H), 3.16 (dd, 1H, J_{AM}=3.1 Hz, J_{AX}=8.0 Hz), 2.14 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ=131.1, 130.4, 125.8, 124.0, 115.8, 113.2, 73.8, 72.9, 72.0, 64.7, 47.2, 19.0, 18.5; *m*/*z* (EI^+) 294 [($M+H^+$+Na^+), 20%], 270 [M^+, 100%].

4.5. 5,6-Dimethylbenzimidazole 11

1-(*N*-(Ribityl)),2-diamino-4,5-dimethylbenzene **3** (200 mg, 0.74 mmol) was dissolved in water (100 mL), and the resulting solution stirred vigorously at ambient temperature. Compressed air was continuously bubbled through the solution. After 96 h, the solution was extracted with EtOAc (3×30 mL), and the aqueous fraction lyophilised to furnish *starting material* **3** (174 mg, 0.64 mmol, 87%). Combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate (50 mL) and brine (2×50 mL), before drying over $MgSO_4$. The solvent was evaporated removed and the crude brown solid recrystallised from diethyl ether to furnish 5,6-dimethylbenzimidazole **11** (9 mg, 0.06 mmol, 8%) as a pale yellow crystalline solid, mp 203–205 °C [lit.[17] mp 201–205 °C; lit.[18] mp 205–206 °C]; ^{1}H NMR (400 MHz, $CDCl_3$) δ=8.00 (s, 1H), 7.44 (s, 2H), 2.38 (s, 6H); ^{13}C NMR (100 MHz), δ=139.9, 136.3, 132.0, 115.6, 20.4; *m*/*z* (EI^+) 146 [M^+, 100%].

4.6. Diethyl 2-bromo-3-oxoglutarate 4[11]

In a flame-dried three-neck-round bottom flask, diethyl 3-oxoglutarate **12** (2.90 mL, 3.50 g, 20 mmol) was dissolved under

nitrogen in anhydrous dichloromethane (60 mL) at −78 °C. Bromine (1.03 mL, 3.20 g, 20 mmol, 1 equiv) was added dropwise over 10 min. After 45 min, the reaction mixture was allowed to warm to room temperature and the stoppers removed to release hydrogen bromide as an acidic vapour. The solution was filtered under suction and the solvent evaporated to furnish a red oil, which was purified by swiftly passing it through a short column of silica (eluted with 3:2 hexane/EtOAc), to give *diethyl 2-bromo-3-oxoglutarate* **4** as a rose-coloured oil (ca. 4.0 g). This was used for subsequent reactions immediately, due to fast degradation of the compound; yield calculations were found to be unreliable. Representative spectra were obtained immediately following purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ=5.05 (s, 1H), 4.14 (dq, 4H, J=7.2 Hz), 3.54 (s, 1H), 1.95 (s, 1H), 1.20 (m, 6H); ^{13}C NMR (100 MHz) δ=198.6, 168.6, 165.0, 63.1, 61.4, 60.5, 45.8, 14.4, 14.2, 14.0; m/z (ES^-) 281 [(M(^{81}Br)−H^+), 100%], 279 [(M(^{79}Br)−H^+), 100%].

4.7. Ethyl 3-(2-ethoxy-2-oxoethylidene)-6,7-dimethyl-4-(D-ribityl)-3,4-dihydroquinoxaline-2-carboxylate 5

1-(*N*-(Ribityl)),2-diamino-4,5-dimethylbenzene **3** (3.10 g, 11.5 mmol) was dissolved in anhydrous MeOH (30 mL), and the mixture added dropwise to a stirring solution of diethyl 2-bromo-3-oxoglutarate **4** (4.0 mL) in anhydrous dichloromethane (30 mL) containing caesium carbonate (2.40 g, 7.4 mmol, 0.64 equiv), maintained under an inert atmosphere. The mixture was stirred at ambient temperature for 48 h before filtration under suction; the filtrate was evaporated and the black residue dissolved in water (100 mL). The resulting aqueous solution was extracted into ethyl acetate (5×50 mL), with addition of brine as necessary to facilitate separation of the layers. Combined organic extracts were washed with brine (2×50 mL) and dried over $MgSO_4$, before rotary evaporation furnished a viscous dark red oil, which was purified by silica gel chromatography (5% MeOH/$CHCl_3$). The purified product was dried under high vacuum for 8 h to give *bicyclic intermediate* **5** (4.00 g, 8.89 mmol, 77%) as a dark red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.42 (s, 1H), 7.04 (s, 1H), 5.26 (s, 1H), 4.62–4.49 (m, 4H), 4.38 (q, 2H, J=7.0 Hz), 4.30 (m, 1H), 3.79 (m, 1H), 3.65 (m, 2H), 2.42 (s, 3H), 2.35 (s, 3H), 1.46 (m, 3H), 1.25 (m, 3H); ^{13}C NMR (62.5 MHz) δ=168.3, 162.5, 162.3, 148.3, 132.8, 132.5, 131.6, 130.7, 123.1, 106.5, 77.7, 77.1, 76.6, 73.2, 62.9, 60.8, 40.7, 36.5, 31.4, 29.6, 15.0, 14.4; m/z [ES^+] 473 [(M+Na^+), 45%], 451 [(M+H^+), 100%]; HRMS (ES^+) calcd for $C_{22}H_{31}N_2O_8$: 451.2075; found 451.2080 [M+1].

4.8. 1-Deazariboflavin 2

NH_3 gas was condensed in anhydrous MeOH (5 mL) by bubbling through the solution at −78 °C until the volume had approximately doubled. The solution was allowed to warm slowly to ambient temperature, and bicyclic intermediate **5** (120 mg, 267 μmol) was added. The resulting solution was stoppered, and stirred at ambient temperature for 72 h with a further aliquot of methanolic ammonia solution (10 mL, prepared as above) added after 36 h. Upon completion, the stopper was removed and the mixture stirred at room temperature for 1 h, before the remaining solvent was carefully removed by a gradual reduction of pressure. The resultant purple-brown solid was dried under high vacuum for 8 h to yield *1-deazariboflavin* **2** as a purple-black solid (92 mg, 245 μmol, 92%), ^{1}H NMR (400 MHz, D_2O) δ=7.48 (s, 1H) 7.15 (s, 1H), 5.56 (s, 1H), 3.94 (m, 1H) 3.56 (m, 2H), 3.38 (m, 1H), 2.88 (q, 1H, J=7.6 Hz), 2.79 (m, 2H), 2.11 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (62.5 MHz, MeOD) δ=167.3, 161.1, 148.0, 146.7, 141.1, 136.0, 132.8, 131.3, 130.1, 122.9, 107.3, 76.0, 71.4, 70.2, 65.3, 53.8, 21.5, 18.1; m/z (ES^-) 412 [(M+$^{37}Cl^-$), 15%], 410 [(M+$^{35}Cl^-$), 50%], 374 [(M−H^+), 100%].

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