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Rational improvement of the synthesis of 1-deazariboflavin

Andrew C. Wood, David W. Knight, Gerald Richter *

School of Chemistry, Main Building, Cardiff University, Cardiff CF10 3AT, United Kingdom

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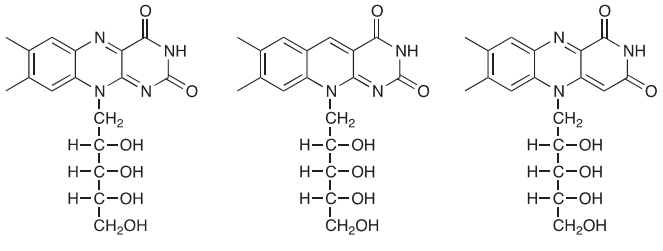
ABSTRACT

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-

* Corresponding author. E-mail address: richterg@cardiff.ac.uk (G. Richter).

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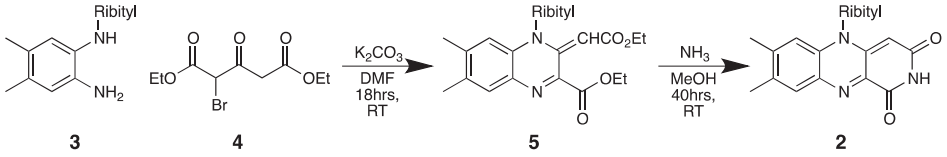
deazariboflavin **2** reported by Ashton et al.[2] This method (with the key steps shown in Scheme 2) features condensation of the ribitylated aniline **3** with diethyl 2-bromo-3-oxoglutarate **4** to form bicyclic intermediate **5**, followed by closure of the isoalloxazine system at the N(3) position 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%.

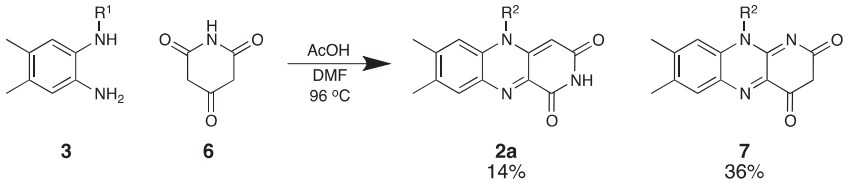
An alternative route also examined by Ashton et al.[2] described the treatment of ribitylated diamine **3** with 2,4,6-piperidine trione **6**, leading to the ribityl-tetraacetate derivatives of 1-deazariboflavin **2a** and 3-deazariboflavin **7** in yields of 14% and 36%, respectively (Scheme 3). However, 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.

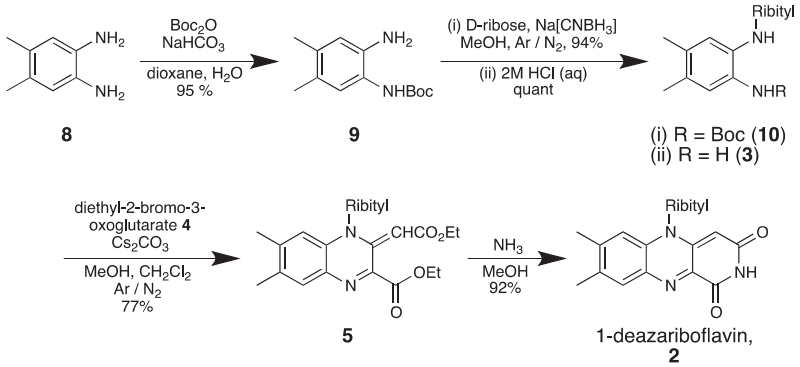
2. Results and discussion

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.