1

Structure and Properties of Flavins

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1.1 Introduction and History of Flavins Discovery

Along with the historical events in 1937 and 1938, such as the explosion of the Hindenburg, the unsolved disappearance of Amelia Earhart, and the inexorable forthcoming of another world war, the world of chemistry was marked by two Nobel Prizes in the discovery and characterization of vitamins. Paul Karrer in Zurich and Richard Kuhn in Heidelberg succeeded almost concurrently in determining the structure of vitamin B2 (riboflavin), which made it possible to produce the vitamin by artificial means [1]. Both scientists received the Nobel Prize for their research on vitamins, Paul Karrer in 1937 for his investigations on carotenoids, flavins, and vitamins A and B2 [2], and Richard Kuhn in 1938 for his work on carotenoids and vitamins [3].

Long before the first synthesis of riboflavin, it had been described as a component of cow milk by an English chemist, A. Wynter Blyth, in 1879. He isolated a bright yellow pigment, which he called lactochrome [4]. It was later described in several reports as a water-soluble substance with green fluorescence found in milk, malt, eggs, liver, and pig heart [5]. Subsequently, in the late 1920s and early 1930s, a significant amount of research in this area was undertaken. The most intensive research outbreak started when it was found that the yellow pigment was a constituent of the vitamin B complex, which plays a vital role in living creatures [6]. The name riboflavin was given to replace the variety of names previously used (lactoflavin, ovoflavin, hepatoflavin), which were related to the source from which the pigment was isolated. Riboflavin represents the D-ribityl derivative of the isoalloxazine heterocycle, whose yellow color gives the second part of the name (from Latin: Flavus = yellow).

Several groups contributed to identification of the first flavin cofactor (flavin mononucleotide [FMN] or riboflavin-5′-phosphate) [5, 7]. Kuhn and co-workers synthesized FMN [7a] and mentioned that it is identical with the “cytoflav” discovered by Banga and Szent–Györgyi [7b]. Almost at the same time, Warburg and Christian isolated a yellow protein from yeast. Theorell found that “yellow

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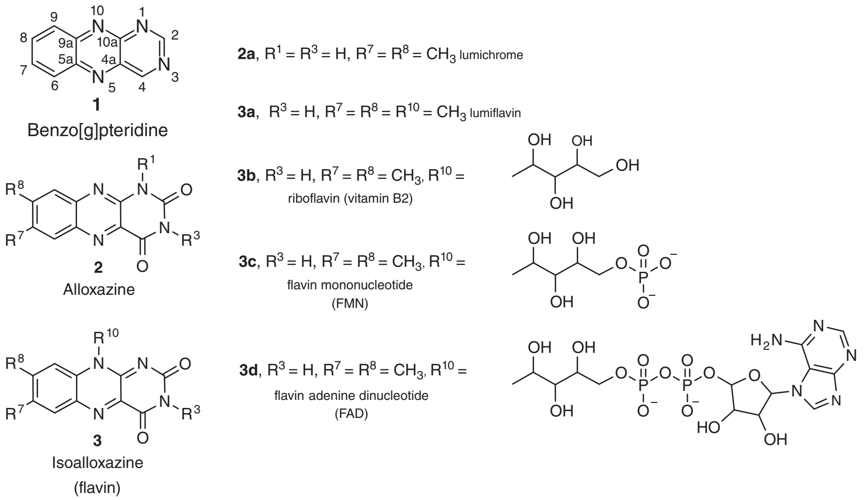


Figure 1.1 The structure of benzo[*g*]pteridine (**1**), alloxazine (**2**), isoalloxazine (flavin, **3**), and their biologically relevant derivatives.

enzyme" consisted of two parts: flavin plus a phosphate group and aprotein called apoenzyme [7c,d]. The second identified flavin cofactor, flavin adenine dinucleotide (FAD), was isolated as a cofactor of D-amino acid oxidase by the same research group in 1938 [8]. In 1954, the structure of FAD was proven by total synthesis [9]. These milestones established riboflavin as a vitamin B2, and FMN and FAD as cofactors in enzymatic catalysis [10]. The phenomenon of covalent attachment of the flavins to proteins was first established in mammalian succinate dehydrogenase in 1955 [11]. Since then, the covalent attachment of the flavins to proteins has been demonstrated through the C6 atom and C8 methyl group (see Figure 1.1 for numbering) of the flavin in many flavoproteins (see Chapter 2). In 2005, X-ray crystallography was used to reveal the first example of a flavin bicovalently linked to a protein in glucooligosaccharide oxidase [12].

Flavins and flavoproteins have been found to play a crucial role in a myriad of metabolic pathways (see Chapter 2 for details). They are involved in aerobic metabolism by catalyzing the two-electron dehydrogenation of various substrates and are responsible for one-electron transfer to different metal centers via their radical states [13]. Flavoproteins play a significant role in soil detoxification processes via the hydroxylation of aromatic compounds, forming parts of multi-redox-center enzymes, such as nicotinamide adenine dinucleotide (NADH) dehydrogenase, xanthine oxidase/dehydrogenase, and cytochrome P450. Flavins take part in the production of light in bioluminescent bacteria and are involved in blue light–initiated reactions, such as plant phototropism and nucleic acid–repair processes, the regulation of biological clocks, energy production, biodegradation, chromatin remodeling, apoptosis, and protein folding [14]. Flavin-dependent light-responsive proteins and enzymes, notably DNA photolyases, cryptochromes, and light-oxygen-voltage (LOV) and blue-light sensors using flavin adenine dinucleotide (BLUF) domains participate in many critical biological processes, including DNA repair, the photoregulation of circadian rhythms, and gene expression [15].

In this chapter, the compounds from the “flavin family” and their properties are reviewed, with a special focus on those, which may be of importance from the viewpoint of flavin catalysis: redox and acid–base properties, reactivity with nucleophiles and electrophiles, and noncovalent interactions. Chapter 2 describes the behavior of flavin derivatives in natural systems. The spectral properties of flavin derivatives are reviewed in detail in Chapter 3. There are also several excellent books focusing on various aspects of the flavins and flavoenzymes [16–18].

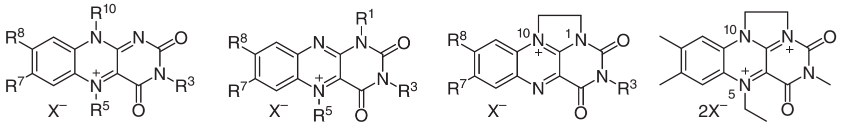
1.2 The Structure of the Flavins and Their Derivatives

According to Massey, the flavins are a class of yellow, water-soluble chemical compounds containing a heterocyclic 7,8-dimethylisoalloxazine ring, which include riboflavin, FMN, and FAD [10]. However, the flavin family has now been extended to all compounds containing an isoalloxazine nucleus as well as the alloxazines, 5-deazaflavins, and other derivatives such as their corresponding N-oxides and flavinium salts.

The underlying heterocyclic structure of flavin molecules is benzo[g]pteridine (**1**; Figure 1.1). Its 2,4-dioxo derivatives are alloxazines with the most cited compound being lumichrome (**2a**). However, the biologically most important molecules, such as lumiflavin (**3a**), riboflavin (**3b**), FMN (**3c**), and FAD (**3d**), are derived from the tautomeric isoalloxazine. Iso- and alloxazines are analogous compounds; however, their spectroscopic and photophysical properties are different (see Chapter 3). Notably, isoalloxazines exhibit intense fluorescence and relatively long fluorescence lifetimes with fluorescence quantum yields one order of magnitude higher than those observed for the alloxazines [19].

Early interest in the photophysical and photochemical properties of the alloxazines **2** was mainly driven by bearing resemblance to the flavins **3**. It is also important to assess the toxicity of lumichromes as products formed during the photochemical reactions of riboflavin. Alloxazines, as products of the biochemical, chemical, or photochemical decomposition of biologically active isoalloxazines, are present in the majority of biological tissues [20]. Lumichrome (**2a**), for example, has been found to inhibit flavin reductase in living *Escherichia coli* cells [21].

The flavins take part in both two-electron processes, such as the oxidation of organic compounds in prokaryotic and eukaryotic respiratory chains, and one-electron transfer in cytochromes and other redox centers [22]. Another key property of the flavin cofactors is their ability to form adducts with different substrates [23]. However, flavin adducts similar to those formed in nature are hard to synthesize, which can be attributed to their low stability outside of an enzyme. The stability of flavin adducts can be increased by introducing substituents at the N5 position [24]. Thus, flavinium salts have been established to mimic the functions of flavin-dependent monooxygenases. Flavinium salts **4•X** possess unique biomimetic

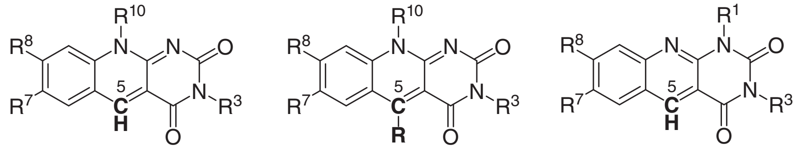


4a•X **4b•X** **4c•X** **4d•X$_2$**

Figure 1.2 The general structures of isoalloxazinium and alloxazinium salts. Source: Modified from Eberlein and Bruice [26].

organocatalytic properties, which promote various chemoselective oxidative reactions under mild conditions and can be derived both from isoalloxazines **4a•X** and alloxazines (**4b•X** and **4c•X**) [25]. A rare example of a 1,5-diblocked quinoid flavinium salt **4d•X$_2$** was expected to possess a high free-energy content and very positive redox potential, which is, however, at the expense of its stability (Figure 1.2) [26].

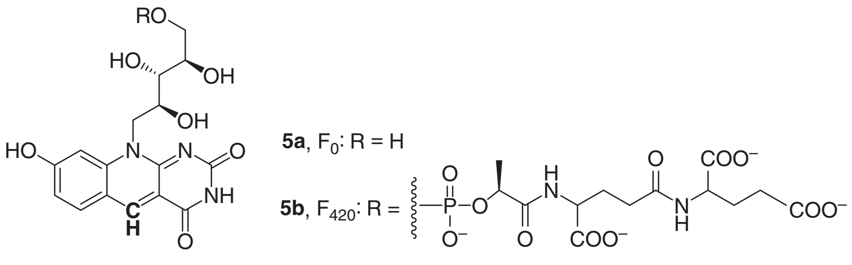
In addition to the flavins (isoalloxazines), structurally similar 5-deazaflavines with the N5 atom replaced by a methine group also occur in biological systems (Figure 1.3) [27]. The 5-deazaflavin family includes 8-hydroxy-7-desmethyl-5-deazariboflavin coenzymes F$_{420}$ (**5b**) and F$_0$ (**5a**). F$_{420}$ was isolated by Wolfe and coworkers in 1978 from various methanogenic bacteria, in which it is involved in the key steps of methanogenesis and carbon assimilation, and represents a derivative of F$_0$ bearing a lactyl-oligoglutamyl group attached via a phosphodiester linkage to the ribityl side chain (Figure 1.3) [28]. Hückel MO calculations have shown that the C5 position of the 5-deazaflavin core is π-electron-deficient in the same way as the C4



5
5-Deazaisoalloxazine
5-Deazaflavin

5-Alkyl(aryl)-5-deazaflavin
R = alkyl, aryl

6
5-Deazaalloxazine



8-Hydroxydeazaflavin

Figure 1.3 The general structures of the 5-deazaflavins **5** and 5-deazaalloxazines **6**, and their biologically relevant cofactors. Source: Greening et al. [27a], Duchstein et al. [27b], Hemmerich et al. [27c], Graml et al. [27d], Eirich et al. [28a], McCormick and Morton [28b], Graham et al. [28c], Selby and Sancar [28d].

position of NAD, which led to the conclusion that the 5-deazaflavins may detach a hydrogen equivalent from hydrogen donors under certain conditions [29]. Thus, the 5-deazaflavins can be regarded as flavin-shaped nicotinamides rather than flavin analogues [27c]. 5-Deazaflavins substituted by alkyl or aryl groups on C5 are also known [27d]. In contrast to the deazaflavins (deazaisoalloxazines), there are very few studies on the 5-deazaalloxazines **6**. The first synthesis of 10-deazariboflavin was achieved in 1970 by Cheng and coworkers [30].

1.3 The Acid–Base and Redox Properties of Flavin Derivatives

Since the first investigations on riboflavin and its derivatives, they have been recognized in terms of their participation in both one-electron and two-electron transfer processes. The electrochemical properties of the flavin derivatives have been mostly studied in aprotic media (usually acetonitrile) using cyclic voltammetry [25a, 31]. When reduced, flavin derivatives (**Fl**) are characterized by two consecutive waves representing two one-electron reductions, first to the radical anion ($\mathbf{Fl} + e^- \rightarrow \mathbf{Fl}^{\bullet -}$) followed by reduction to the anion ($\mathbf{Fl}^{\bullet -} + e^- \rightarrow \mathbf{Fl_{red}}^{2-}$). The first wave is usually reversible, the second one is pseudoreversible or irreversible. The reduction of the flavins (isoalloxazines) has also been studied in protic media [32]. In that case, the formed radical anion ($\mathbf{Fl} + e^- \rightarrow \mathbf{Fl}^{\bullet -}$) is protonated to give a neutral semiquinone ($\mathbf{Fl}^{\bullet -} + H^+ \rightarrow \mathbf{Fl_H}\bullet$), which undergoes a second fast one-electron reduction to give the reduced flavin anion ($\mathbf{Fl_H}\bullet + e^- \rightarrow \mathbf{Fl_H}^-$). Thus, in a protic solvent, the entire electrochemical–chemical–electrochemical (ECE) reaction is faster than the cyclic voltammetry (CV) timescale and observed as a single wave representing an electrochemically reversible process. In Table 1.1, the first redox potentials of the flavins (isoalloxazines) in acetonitrile and water are summarized together with the data obtained for other flavin derivatives in acetonitrile.

Flavins **3** (isoalloxazines) adopt three different redox states: oxidized **3**, semiquinone (one-electron reduced, radical $\mathbf{3_H}\bullet$), and hydroquinone (two-electron reduced, fully reduced $\mathbf{3_{H2}}$), which can be readily distinguished using UV–visible spectroscopy (Scheme 1.1; see Chapter 3 for details on spectroscopy) [10, 33]. Each redox state of the isoalloxazine undergoes acid–base equilibria depending on the pH. At pH 3–12, in which most proteins are stable and functional, the flavins exist in their neutral and anionic forms. At pH <1, there is a considerable amount of their cationic form. The photophysics and photochemistry of the oxidized flavins and hydroquinones have been studied both in solution and in numerous proteins. Oxidized flavin is yellow with absorption maxima at ~370 and 450 nm, which fluoresce in solution. However, electron donors can act as quenchers, a feature responsible for the lowered fluorescence intensity typically observed in the flavoproteins. Hydroquinone usually displays weak fluorescence in solution and exhibits a weak broad absorption in the UV–visible region. However, it shows enhanced fluorescence upon being confined in a rigid protein environment or at low temperature. The flavin semiquinone exhibits two distinctive absorption

Table 1.1 First redox potentials of selected flavin derivatives.

Substitution		**Redox potentials (V vs. SCE) of flavin derivatives**						
		3a)	**3**/H_2Ob)	**2**c)	**5**c)	**4a**d)	**4b**e)	**4c**f)
7	**8**							
MeO	MeO	−0.978	n.d.	−1.320	n.d.	n.d.	−0.280	n.d.
Me	Me	−0.906	−0.509	n.d.	n.d.	+0.253	−0.150	−0.255
H	H	−0.826	−0.460	−1.050	−1.210	+0.335	+0.100	n.d.
Cl	Cl	−0.668	−0.391	n.d.	n.d.	n.d.	+0.235	n.d.
CN	H	n.d.	−0.374	n.d.	n.d.	+0.459	+0.305	n.d.
CF_3	H	−0.679g)	n.d.	−0.719g)	n.d.	n.d.	n.d.	n.d.

a) From Ref. [31a], $E_{1/2}$ for reduction measured by CV with platinum working electrode, in dichloromethane (DCM), 23 °C, 0.1 M tetrabutylammonium perchlorate (TBAP) using ferrocene as standard (−0.38 vs. SCE).

b) From Ref. [32], $E°'$ measured by CV with a glassy carbon working electrode, in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4) vs. Ag/AgCl (−0.053 vs. SCE).

c) From Ref. [31b], $E_{1/2}$ for reduction measured by CV with a glassy carbon working electrode in acetonitrile (ACN), 25 °C, 0.1 M TBAP using ferrocene as internal standard and recalculated to SCE by authors.

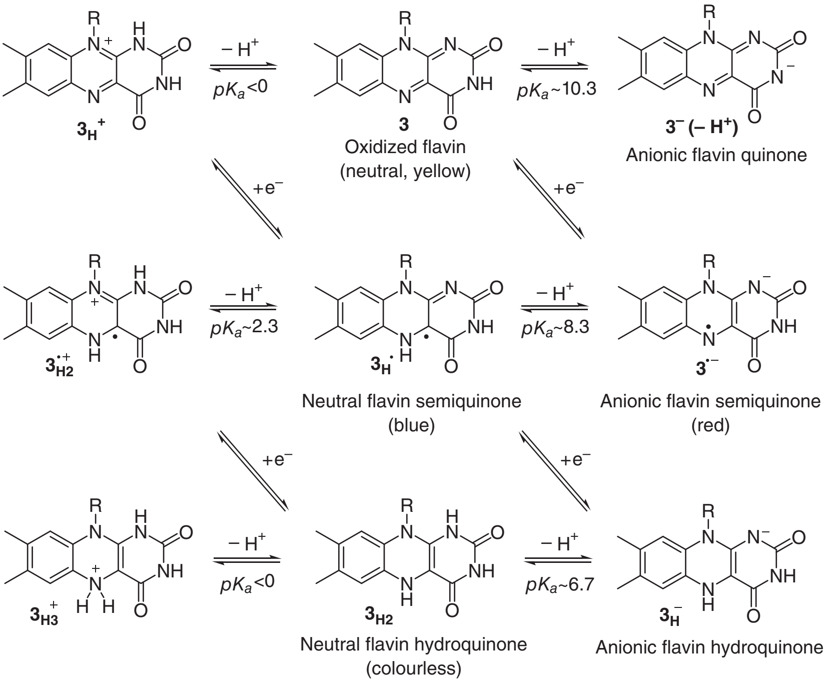
d) From Ref. [31c], $E°'$ measured by CV with a platinum working electrode, in ACN, 25 °C, 0.1 M TBAP vs. Ag/AgCl (−0.053 vs. SCE).

e) From Ref. [31d], $E°'$ measured by CV with a platinum working electrode, in ACN, 25 °C, 0.1 M TBAP using ferrocene as internal standard and recalculated to SCE by authors.

f) From Ref. [25a], $E°'$ measured by CV with a platinum working electrode, in ACN, 25 °C, 0.1 M TBAP vs. Ag/AgCl and recalculated to SCE by authors.

g) From Ref. [31e], $E_{1/2}$ for reduction measured by CV with a glassy carbon working electrode in ACN, 25 °C, 0.1 M TBAP using ferrocene as internal standard and recalculated to SCE by authors.

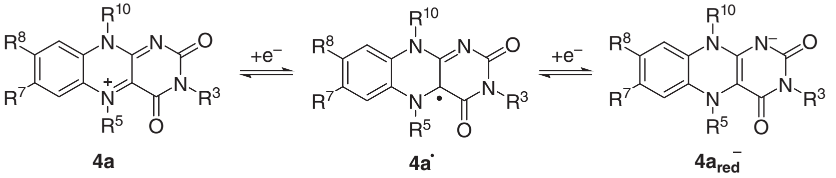
bands depending on the protonation of the N5 atom. Upon the addition of a single electron to the oxidized form **3**, the resulting red radical anion **3**$^{\bullet -}$ is formed with a strong absorption peak observed at 370 nm, which can be protonated with a pK_a value of ~8.5 in a nonenzyme bound system to yield a blue neutral semiquinone species **3**$_{\mathbf{H}}{}^{\bullet}$ with a strong absorbance band at ~570 nm. This form can be further stabilized upon alkylation [34], as shown by its 5-alkyl analogue **4**$^{\bullet}$, or when bound in proteins, such as in blue radical flavoproteins [35]. Whether the semiquinone form is stabilized depends on the enzyme to which the flavin is bound (for more details concerning spectral flavin properties, see Chapter 3).



Scheme 1.1 The redox and protonation states of the flavins (isoalloxazines) (**3**). Source: Heelis [33a], Stanley and Jang [33b], Kowalczyk et al. [33c], Kao et al. [33d], Kabir et al. [33e], and Bailey and Schultz [33f].

The most intensive studies on the acid–base equilibrium of alloxazines **2** have been conducted using lumichrome (**2a**), which can exist in its protonated, neutral, and monoanionic forms in the pH range of 1–14 [20c, 36]. However, studies on lumichrome under different pH conditions are difficult due to the possible transformations between the alloxazine and isoalloxazine-like species. Since these equilibria were mainly the subject of spectroscopic studies, they are summarized in Chapter 3 as well as acid–base equilibria for deazaalloxazines **6** [37, 38].

Isoalloxazinium species **4a** takes part in two one-electron processes. The addition of one electron to the oxidized form gives radical **4a**$^{\bullet}$ and the second one-electron reduction affords anion **4a**$_{\mathbf{red}}{}^-$ or its protonated form (Scheme 1.2) [25a, 39].

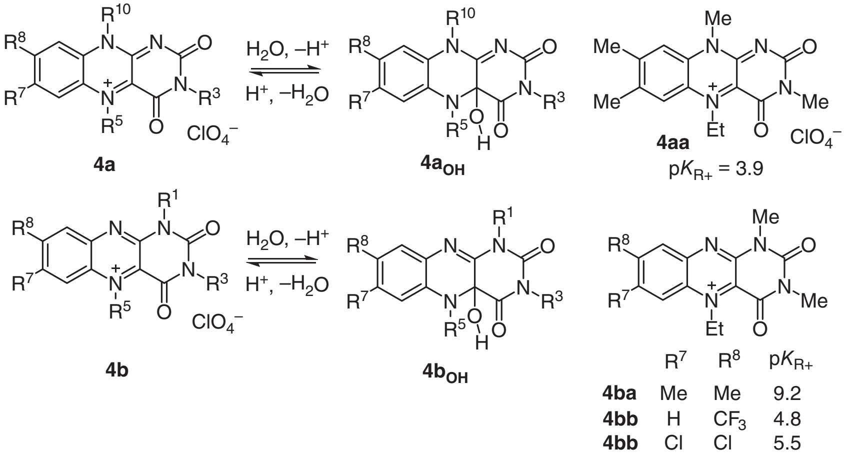


4a **4a•** **$4a_{red}^-$**

Scheme 1.2 The redox states of isoalloxazinium salts (**4a**). Source: Sakai et al. [25a], Ménová and Cibulka [39].

Similar behavior has been observed for alloxazinium **4b**, ethylene-bridged cation **4c**, and dicationic compound **4d** [25a, 26a, 34]. The positive charge makes reduction significantly easier, shifting the potential to positive values by ~1 V when compared with neutral isoalloxazines (**3**) and alloxazines (**2**) (Table 1.1).

5-Alkylflavinium (isoalloxazinium) salts **4a•X** are sensitive toward hydrolysis. However, their solutions are fairly stable toward hydrolysis in slightly acidic solutions and are quite stable in dry organic solvents. 5-Alkyl isoalloxaziniums **4a** and alloxaziniums **4b** exist in aqueous solutions in an equilibrium with their pseudobase forms (**$4a_{OH}$** and **$4b_{OH}$**) with pK_{R+} values of 3.9 and 9.2, respectively (Scheme 1.3) [39]. The pK_{R+} value denotes the pH at which the equilibrium concentrations of the heteroarenium cation and its pseudobase are equal ($pK_{R+} = -\log K_{R+}$; $K_{R+} = [FlOH][H^+]/[Fl^+]$) [40]. The pK_{R+} values of alloxazinium salt **4b** are higher when compared with those obtained for its isoalloxazinium derivatives **4a** by ~5 units, but it can be significantly decreased by an electron-withdrawing group (Scheme 1.3) [31e]. Ethylene-bridged salts **4c** form a pseudobase with a hydroxyl group at the C10a position (see Section 1.4) in an aqueous solution [41].



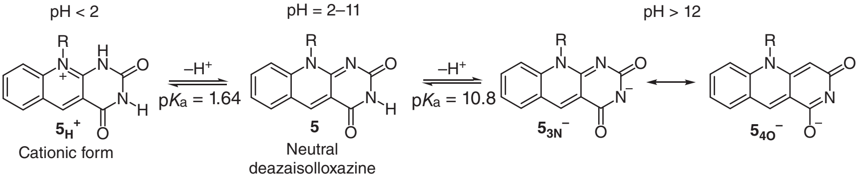
4a **$4a_{OH}$** **4aa** $pK_{R+} = 3.9$

4b **$4b_{OH}$**

	R^7	R^8	pK_{R+}
4ba	Me	Me	9.2
4bb	H	CF_3	4.8
4bb	Cl	Cl	5.5

Scheme 1.3 Flavinium (**4**)-pseudobase (**4_{OH}**) equilibrium for isoalloxazinium **4a** and alloxazinium **4b** cations and their corresponding pK_{R+} values. Source: Ménová and Cibulka [39], Ménová et al. [31e].

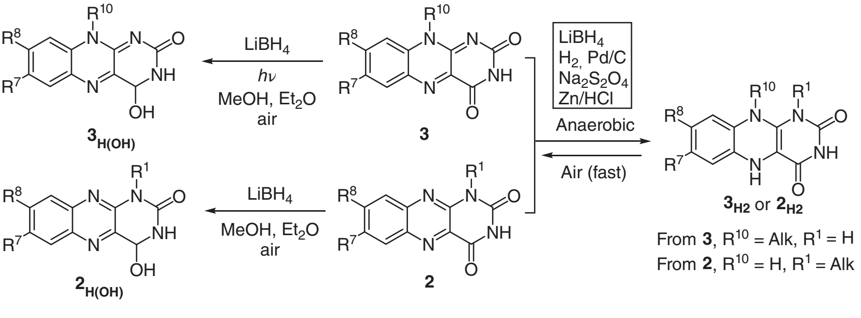
5-Deazaflavins **5** exist in their protonated form at pH <2, neutral form at higher pH values, up to pH 11, and monoanionic form at pH >12 (Scheme 1.4) [37]. The pK_a value of the deazaflavins $\mathbf{5_H}^+$ is higher when compared to that of lumiflavin ($\mathbf{3a_H}^+$), while the pK_a value for the deprotonation of **5** is close to that observed for the flavins **3** [36a]. Electrochemical reduction of yellow deazaflavins **5** occurs analogous to the flavins (isoalloxazines) via two one-electron processes via radical species $\mathbf{5}^{\bullet -}$ although at potentials more negative by ~300–400 mV (see Table 1.1) [31c].



Scheme 1.4 The acid–base equilibrium of the 5-deazaflavins **5**. Source: Modified from Prukała et al. [37].

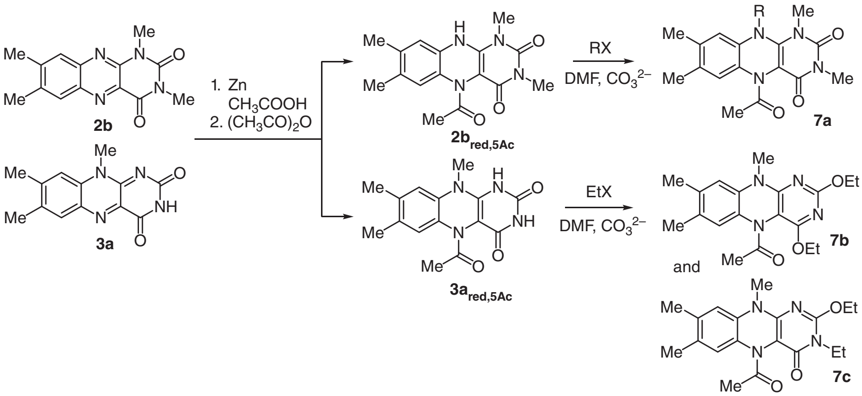
1.3.1 Selected Chemical Reduction and Oxidation Reactions

Natural flavin is a very weak hydride acceptor under dark conditions. Its reaction with cyanoborohydride is >3000 times slower than its slow dark reaction with nicotinamide [42]. This can be attributed to the fact that the neutral flavin is weakly basic similar to a pyridinic nitrogen atom. Alloxazines **2** and isoalloxazines **3** can be reduced under anaerobic conditions to their 5,10- and 1,5-dihydro forms, respectively, using different reducing agents such as sodium or lithium borohydrides, sodium bisulfite, zinc in acetic or hydrochloric acid, and hydrogen in the presence of a palladium or platinum catalyst [43]. Flavins (isoalloxazines), when excited by light, are easily reduceable with tertiary amines or ethylenediaminetetraacetate (EDTA) (see Chapter 4). Unless appropriately substituted at N1, $\mathbf{3_{H2}}$-type dihydroflavins are extremely sensitive to air and are rapidly reoxidized to their oxidized forms (**2** and **3**). Aerobic borohydride reduction of **2** to form $\mathbf{2_{H(OH)}}$ proceeds smoothly in the dark, whereas a similar reduction of the isoalloxazines **3** to their corresponding 3,4-dihydroflavin $\mathbf{3_{H(OH)}}$ requires photoactivation. Unlike the 1,5-dihydroflavins $\mathbf{3_{H2}}$, these reduction products are stable toward molecular oxygen in the dark (Scheme 1.5) [42–44].



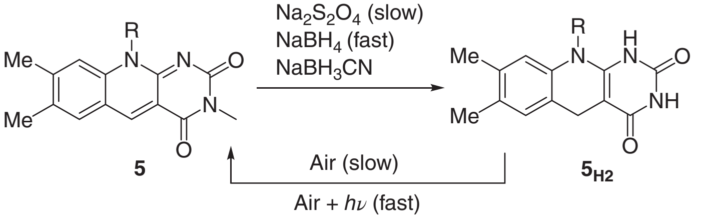
Scheme 1.5 The chemical reduction of the alloxazines **2** and isoalloxazines **3**. Source: Müller et al. [42], Lambeth and Palmer [43a], Hemmerich et al. [43b], Ghisla et al. [43c], Berezovskii [44a], Szczesna et al. [44b].

The stabilization of oxygen-sensitive reduced flavins (isoalloxazines) $\mathbf{3_{H2}}$ can be achieved via selective acylation at the N5 atom, which leads to the formation of 5-acyl-1,5-dihydroisoalloxazines $\mathbf{3_{H,5Ac}}$ and their derivatives. These 5-acyl derivatives are then easily alkylated into their corresponding imino esters **7b** and **7c**. 1,3-Dialkyl isomer **7a**, on the other hand, can only be obtained starting from alloxazines **2**, which are used for the subsequent introduction of the alkyl group at the N10-position [45]. The acylation reaction was carried out simultaneously with reduction by adding zinc dust to a suspension of the alloxazine or isoalloxazine in a 1 : 1 mixture of acetic acid and an organic acid anhydride (Scheme 1.6).



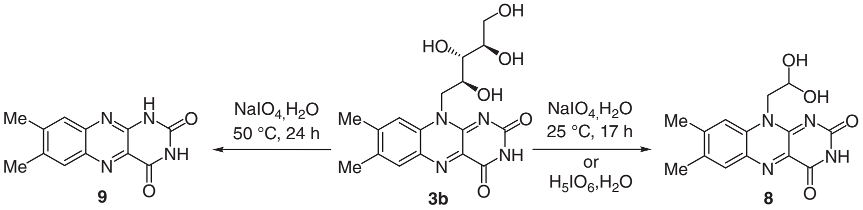
Scheme 1.6 The alkylation and acylation of reduced alloxazines **2** and flavins (isoalloxazines) **3**.

The reduction of 5-deazaflavins **5** using sodium borohydride or cyanoborohydride leads to the quantitative formation of 1,5-dihydrodeazaflavin $\mathbf{5_{H2}}$, and in contrast to the flavins, this process occurs rapidly even without photoactivation (Scheme 1.7). 1,5-Dihydrodeazaflavins $\mathbf{5_{H2}}$ are slow to aerobically oxidize in the dark; at pH 7, the apparent half-time for reoxidation in an air-saturated solution is ~22 h at room temperature. Nevertheless, this reaction is significantly stimulated by light [27d]. 5-Deazaflavin analogs have been found to have redox properties similar to those of ordinary flavins. However, sodium dithionite reduction proceeds considerably more slowly than the flavins with the formation of a fully reduced derivative $\mathbf{5_{H2}}$ [27b].



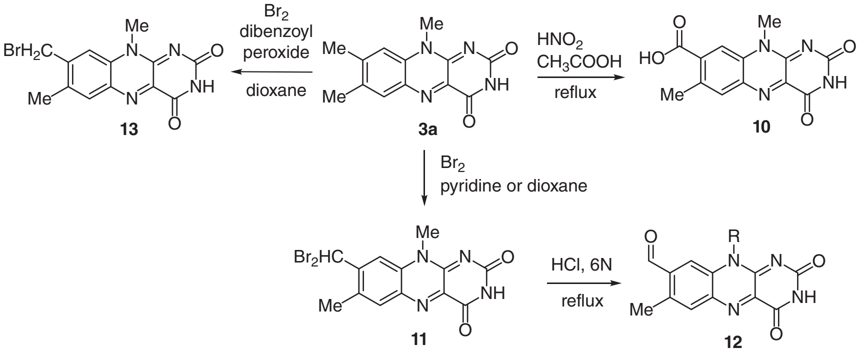
Scheme 1.7 The chemical reduction of deazaflavins **5**.

The isoalloxazine ring is resistant to strong oxidizing agents, such as nitric acid, hydrogen peroxide, and bromine. The oxidation of riboflavin **3b** with periodic acid leads to the cleavage of the side chain between the adjacent hydroxy groups and the formation of 7,8-dimethyl-10-formylmethylisoalloxazine hydrate (**8**) [46]. It has been shown that the oxidation of the side chain of riboflavin (**3b**) with $NaIO_4$ at 25 °C gives flavin derivative **8** in 86% yield, while when the same oxidation was carried out at 50 °C, alloxazine **9** was obtained in 70% yield (Scheme 1.8) [25a].



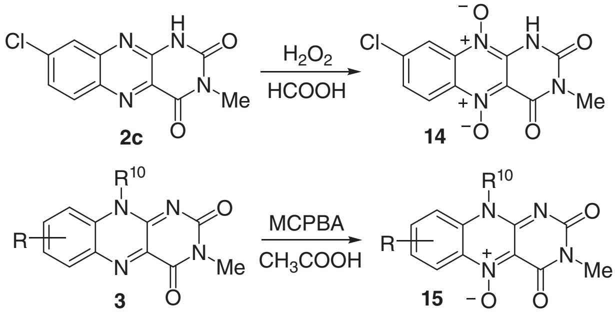
Scheme 1.8 Oxidation of the side chain in riboflavin **3b**. Source: Modified from Sakai et al. [25a].

The enhanced reactivity of the 8-methyl group has been shown in the oxidation reaction of lumiflavin (**3a**) upon heating in glacial acetic acid with nitrous acid with the formation of 8-carboxyderivative **10**. The 8-methyl group is also more susceptible to bromination [43b]. The bromination of riboflavin **3b** in pyridine or dioxane gives 8-α-dibromo derivative **11**, which easily hydrolyzes to 8-formyl derivative **12** after refluxing in 6 N HCl. Treating flavins **3** with bromine in dioxane in the presence of a catalytic amount of dibenzoyl peroxide gives mono-bromo compound **13** in good yield. The following oxidation reactions allow the formation of their corresponding alcohol and acid derivatives (Scheme 1.9) [43b]. Studies on the reactivity of the 8-methyl group of riboflavin conducted in the 1970s by the Ghisla, Walker, and Singer groups have played a significant role in determining the structure of a new flavin coenzyme of succinate dehydrogenase [47].



Scheme 1.9 The reactivity of the 8-methyl group in the flavins. Source: Modified from Hemmerich et al. [43b].

8-Chloroalloxazine (**2c**) can be oxidized using 30% hydrogen peroxide in 88% formic acid, peracetic acid, and monopersulfuric acid into its corresponding alloxazine 5,10-dioxide (**14**) (Scheme 1.10) [48a]. Yoneda reported that the oxidation of isoalloxazines **3** using chloroperbenzoic acid gives their corresponding N5-oxides **15** in moderate yield [48b].

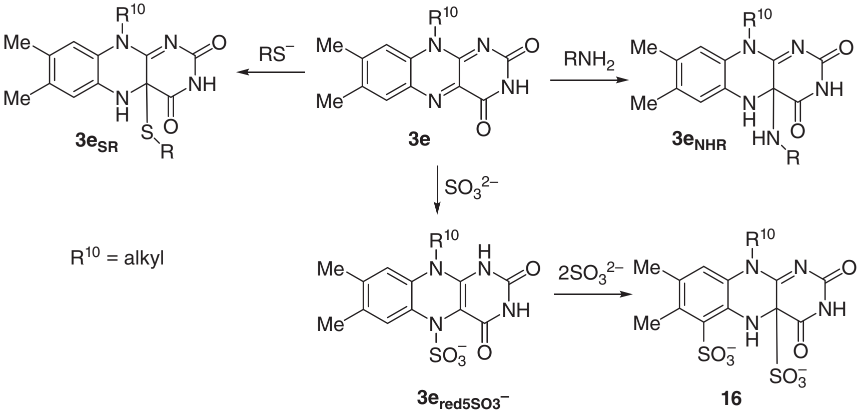


R = H, 7,8-$(CH_3)_2$; R^{10} = CH_3, n-C_3H_7, n-C_4H_9, C_2H_5, D-ribityl

Scheme 1.10 The reactivity of the alloxazine **2c** and isoalloxazines **3** nucleus toward oxidizing agents. Source: Based on Petering and van Giessen [48a].

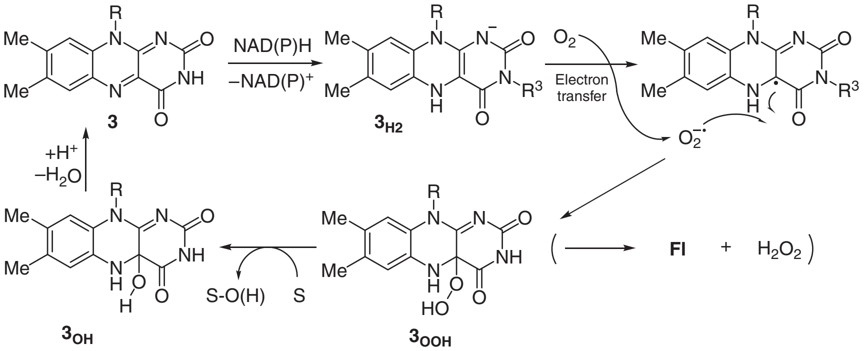
1.4 The Reactions of Flavin Derivatives with Nucleophiles and Electrophiles

Many processes mediated by flavoenzymes utilize the reactivity of flavin cofactors toward nucleophiles (see Chapters 2 and 4 for details). Experimental studies have shown the significance of the N1, N5, C4a, and C10a positions in flavin (isoalloxazine) molecules as potential electrophilic centers [49a]. Among these, C4a and N5 are the most reactive. While soft nucleophiles, including sulfites, phosphines, and nitroalkane carbanions, attack at the N5-position, hard nucleophiles target the C4a-position. Reaction with thiolates and primary or secondary amines leads to 4a-alkylsulfanyl ($\mathbf{3e_{SR}}$) and 4a-alkylamino ($\mathbf{3e_{NHR}}$) derivatives, respectively [49b]. Bruice prepared sulfite adducts via the reaction of isoalloxazines (**3**) with sulfite anion [49c]. The addition reaction is a two-step process. The initially formed N5 adduct ($\mathbf{3e_{H,5SO3}}$) is a kinetic product, while the C4a adduct (**16**) is the thermodynamic product. Attack of the sulfite anion on the aromatic ring proceeds during the second stage of the adduct-forming step (Scheme 1.11). It should be noted that such adducts are very unstable and only exist as metastable intermediates in biological flavin-catalyzed reactions. Such instability may be caused by the low reactivity of the ground state of the flavins (**3**) toward nucleophiles considering the low reduction potential of oxidized flavins (∼–0.4 V vs. SCE in water) [49d]. The stability of the adducts can be significantly increased upon the introduction of a strongly electron-withdrawing group [50] or quaternization of the isoalloxazine ring (see Scheme 1.13).



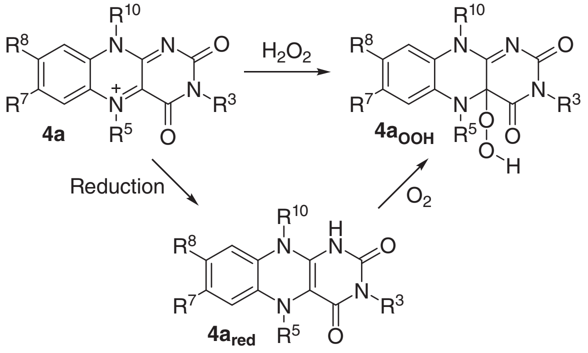
Scheme 1.11 The formation of the C4a adduct upon the reaction of neutral flavins (isoalloxazines) **3** with nucleophiles.

One of the most important adducts is flavin-4a-hydroperoxide (**3_{OOH}**), which is an oxidizing agent in oxygenation reactions mediated by flavin-dependent monooxygenases (see Chapter 7). However, this species is not formed upon simple addition to the oxidized flavin, but by the reaction of the reduced flavin cofactor with oxygen, which occurs via a two-step mechanism (Scheme 1.12). This involves electron transfer to ground state oxygen, followed by radical recombination [51]. In monooxygenases, **3_{OOH}** transfers an oxygen atom to the substrate. Outside of an enzyme, **3_{OOH}** is unstable and dissociates into the oxidized flavin and hydrogen peroxide.



Scheme 1.12 The formation of flavin-4a-hydroperoxide in monooxygenases and its corresponding catalytic cycle. **3** = FMN (**3b**) or FAD (**3c**).

5-Alkylflavinium salts **4a** form similar 5-alkylflavin-4a-peroxides **$4a_{OOH}$** via their reduction into **$4a_{red}$**, followed by its reaction with oxygen. Nevertheless, **$4a_{OOH}$** can also be generated upon the addition of hydrogen peroxide to **4a** (Scheme 1.13) [24b]. This adduct was found to be more stable when compared with N5-unmodified **3_{OOH}** in artificial systems and shows similar reactivity toward various substrates acting as an artificial monooxygenase (see Chapter 5) [25b, c].



Scheme 1.13 The formation of 5-alkylflavin-4a-peroxides (**4a**$_{\mathrm{OOH}}$) from flavinium salt **4a**. Source: Modified from Gelalcha [24b].

When nitrogen-containing heterocycles are converted into their corresponding quaternary salts, the attack of nucleophilic agents is facilitated, which is similar to the nucleophilic substitution of pyridinium, pyrylium, thiapyrylium, and other cations [44a]. Analogously, alkylation at the N5 atom of the isoalloxazine or alloxazine core leads to an increase in the electrophilicity, which is reflected by the less negative reduction potential. Isoalloxazinium species **4a** is significantly more electrophilic than alloxazinium **4b**, which is evident by the 150–300 mV more positive reduction potential (see Table 1.1) and the observation that neutral hydrogen peroxide is readily added to **4a** forming **4a**$_{\mathrm{OOH}}$, while to form **4b**$_{\mathrm{OOH}}$, the hydroperoxide anion is needed [39].

A number of O-adducts have been prepared from both flavinium (**4a•ClO$_4$**) and alloxazinium (**4b•ClO$_4$**) perchlorates, which represent analogs of the adducts found in monooxygenases. Bruice and coworkers first isolated an adduct of hydrogen peroxide to form isoalloxazinium salt **4aa**$_{\mathrm{OOH}}$ [24a]. Later, the structure of adducts **4aa**$_{\mathrm{OH}}$, **4ba**$_{\mathrm{OH}}$, and **4ba**$_{\mathrm{OMe}}$ was determined using X-ray diffraction (Figure 1.4) [52].

In addition, the adducts of amines and anilines with **4a** were isolated and characterized using NMR, UV–Vis, and mass spectroscopy [53]. Alkyl and arylhydrazines are even more nucleophilic substrates than amines and undergo rapid addition to flavinium salts with the formation of their corresponding C4a adducts (**4a**$_{\mathrm{NHNHR}}$) (Scheme 1.14). However, they are unstable and readily fragment into 1,5-dihydroflavin (**4a**$_{\mathrm{red}}$) and their corresponding azo compound (RN=NH or

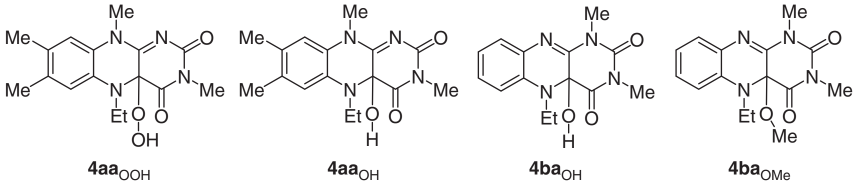
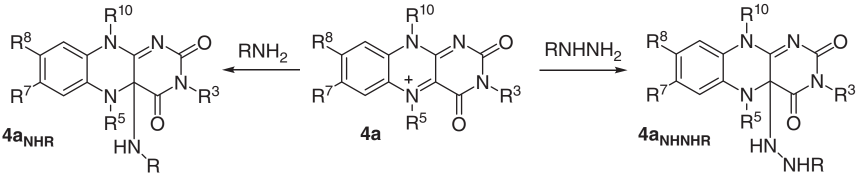


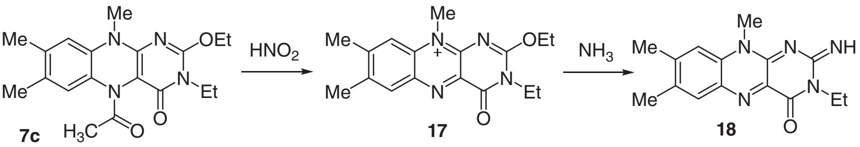
Figure 1.4 The isolated C4a-O-adducts of isoalloxazinium (flavinium) (**4a**) and alloxazinium (**4b**) species. Source: Modified from Menova et al. [52].

ArN=NH), which then decompose into molecular nitrogen and their respective hydrocarbon compound [53b]. Analogously, diimide can be generated from hydrazine and used in hydrogenation reactions (see Chapter 5) [54].



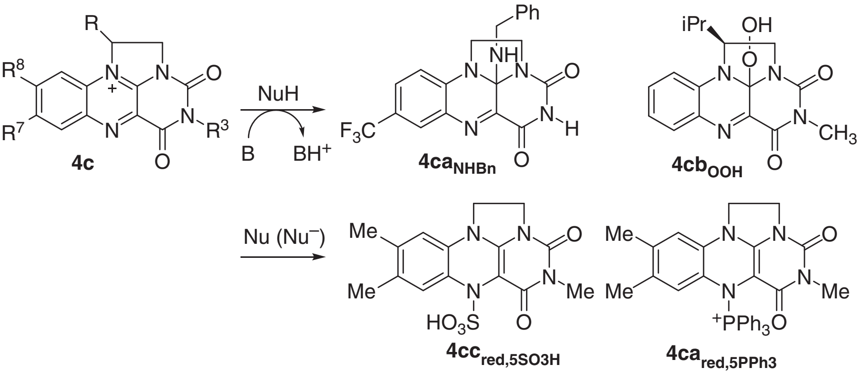
Scheme 1.14 The addition of amines and arylhydrazines to flavinium salts **4a•X**.

It is not usual, but other positions in salts have also been shown to undergo nucleophilic attack. For example, Dudley and Hemmerich showed that 2-ethoxy alloxazinium salt **17**, synthesized from **7c** under oxidizing conditions, reacts with ammonia via a simple addition–elimination mechanism to form imine derivative **18** (Scheme 1.15) [45].



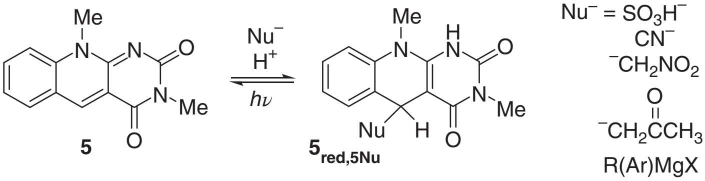
Scheme 1.15 The formation of imine derivative **18** from alloxazinium salt **17**. Source: Modified from Dudley and Hemmerich [45].

Ethylene-bridged salt **4c** also readily reacts with nucleophiles. Several adducts with amines and oxygen nucleophiles at the 10a-position have been generated and confirmed in solution using NMR and mass spectroscopy [41, 55]. Moreover, **4c** was shown to form N5-adducts with soft nucleophiles, such as sulfite or triphenylphosphine (Scheme 1.16) [56].



Scheme 1.16 The adducts of N1,N10-ethylene-bridged alloxazinium salt **4c**. Source: Modified from März et al. [56c].

In contrast to the flavins (isoalloxazines), the addition adducts of the deazaflavins with NH_2OH, CN^-, and SO_3^{2-} are formed rapidly in a reversible reaction [57]. The acetone or nitromethane C5-carbanion adducts of 5-deazaflavins are formed smoothly under basic catalysis in the dark; however, they may undergo oxidative photolysis upon illumination or using dilute acid as a catalyst [27b]. Grignard reagents readily react to form 5-aryl- and 5-alkyl-1,5-deazadeazaflavins, which can be isolated (Scheme 1.17). On the other hand, the attempts to prepare adducts with amines and O-nucleophiles were not successful and were only generated in solution and characterized using UV–Vis spectroscopy [57b].



Scheme 1.17 The formation of the C(5)-carbanion adducts of 5-deazaflavins.

In regard to their reaction with electrophiles, the reactivity of the flavin derivatives is relatively low in their electron-poor oxidized form. Nevertheless, they are able to interact with various metal ions (see Section 1.5). The 8-methyl group in the isoalloxazine ring system is acidic and upon deprotonation, it can react with various electrophiles, such as aldehydes, to provide their corresponding Knoevenagel condensation products [58]. Furthermore, isoalloxazines and alloxazines undergo reactions with strong electrophiles during bromination or nitration reactions. The nitration of nonsubstituted alloxazines using fuming nitric acid in the presence of concentrated sulfuric acid at 20 °C gives a mixture of their 7- and 9-mono-nitro derivatives, while treatment with fuming nitric acid in 25% oleum at 70 °C leads to 7,9-dinitroalloxazines [44a]. Nitration of isoalloxazines with unsubstituted benzene ring leads selectively to C7 position and to C6 for 7,8-dimethylisoalloxazines [59a]. Halogenation of flavin in trifluoroacetic acid in the presence *N*-halogensuccinimides leads to the formation of the C9-substituted product only [59b]. On the other hand, reduced isoalloxazines and alloxazines are nucleophilic enough to undergo alkylations and acylations, which can be utilized for alkylation at the N5 position upon reaction with an aldehyde, followed by reduction of the formed imine [60] or by acylation (see Scheme 1.6).

1.5 Noncovalent Interactions of Flavin Derivatives

Isoalloxazines **3** and alloxazines **2** primarily possess a N5–C4a–C4–O4 chelate site, which can bind to metal centers to form largely planar five-membered chelated ring structures (Figure 1.5a) [61]. Metal ions predominantly bind to oxidized isoalloxazines; however, radical chelates with electrophilic metal ions, such as

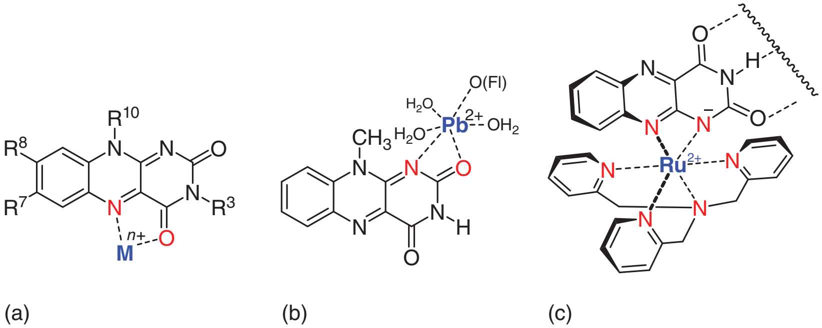


Figure 1.5 The usual coordination modes of the isoalloxazines and alloxazines (a, b). A special example of a Ru(II) complex of an alloxazine (c). Source: (a) Modified from Kaim et al. [61]. (b) Modified from Yu and Fritchie [62].

Zn(II) or Cd(II), coordinated to the one-electron reduced semiquinone state have also been reported [63]. These complexes can also be considered as charge-transfer chelates [64]. The metal ion character influences its relative distance from the N5- and O4-binding atoms in the solid state as evident from X-ray diffraction [61]. While low-valent centers, such as Cu(I), Ag(I), and Mo(IV), bind significantly more tightly to nitrogen when compared to oxygen, high-valent ions such as W(VI), Mo(VI), and Cu(II) are located close to the O4 atom. Ir(III) and Rh(III) ions have similar distances to N5 and O4 in their complexes.

Another metal-binding site has been recognized in the crystal structures of isoalloxazine complexes. Pb(II) is tightly bound to O2 and less strongly to N1 in its complex with 10-methylisoalloxazine (Figure 1.5b) [62]. The same chelating motif was found in the Ag(I) complex, in which it is combined with the N5/O4 binding of another Ag(I) ion [65].

An unusual coordination mode can be seen in a Ru(II) complex of an alloxazine, which contains an unprecedented four-membered chelate ring with the N1 and N10 atoms [66]. Such a novel 1,10-coordination mode in the ruthenium alloxazine complex is allowed by the free N10 position in the alloxazine and simultaneous hydrogen bonding through the available O4, N3-H, and N5 atoms (Figure 1.5c).

As summarized in Ref. [67], in aqueous solutions, flavins **3** (isoalloxazines) do not form stable complexes except with Ag(I) and Cu(I). However, in aprotic solvents, such as acetonitrile or acetone, most metal ions coordinate to the flavin ligand. NMR spectroscopy [67] shows that stable tris-octahedral (Zn(II), Cd(II), Co(II), Ni(II), Fe(II)) and bis-tetrahedral (Ag(I), Cu(I)) or square planar (Cu(II)) complexes can be formed in acetone. Labile complexes with Mg(II), Mn(II), and Fe(III) bound to the carbonyl oxygen atom were also observed. Carbonyl oxygen binding was also observed for other metals when the N5 position was sterically hindered (Table 1.2).

Flavins form relatively stable 1 : 1 complexes with Mg(II) and Zn(II) in dry acetonitrile. The absence of water is essential toward increasing the complex stability to $K_1 = 2.2 \times 10^2\ M^{-1}$ for Mg(II)-**RFTA** [68]. With rare-earth metal ions, such as Sc(III) and Lu(III), the formation of stable 1 : 2 ligand–metal complexes with **RFTA** was observed in dry acetonitrile using UV–Vis spectroscopy with $K_1 = 3.1 \times 10^4\ M^{-1}$ and

Table 1.2 Constants of formation of the complexes $\mathbf{M(RFTA)_N}^{n+}$ in acetone.

Metal ion	N	Log β_N [a)]	Log K_1 [b)]
Cu(I)	2	4.29	3.7
Ni(II)	3		3.3
Ag(I)	2	4.21	3.1
Co(II)	3	5.68	3.1
Cu(II)	2	3.89	2.8
Zn(II)	3	5.72	2.7
Cd(II)	3	5.54	2.6
Fe(II)	3	5.83	2.0
Mn(II)	1		1.6
Mg(II)	1	1.74	1.1

a) Measured by proton magnetic resonance (PMR); [TARF] = 3×10^{-2} M, 38 °C.
b) Measured by optical absorption; [TARF] = 8×10^{-4} M, 25 °C.
Source: Adapted from Lauterwein et al. [67].

$K_2 = 1.4 \times 10^3\ \mathrm{M}^{-1}$ observed for the Sc(III) complex [68, 69]. Based on the significant red shifts observed for the stretching bending vibrations of both C=O bonds in the IR spectrum, the authors suggested both positions, N5-O4 and O2, participate in metal binding. Coordination was shown to significantly affect the electronic properties of flavin, which was manifested by a change in the single excited state reduction potential from 1.67 V for ^{1}**RFTA** to 2.45 V for ^{1}(**RFTA**-2Sc), which makes them extremely strong oxidizing agents. Coordination also changes the lowest excited state from the n,π* triplet state for free RFTA to the π,π* singlet states in the Sc(III) complex [69].

To support the coordination of metal ions, several strong metal-binding sites have been introduced via covalent bonds to the flavin structure, including crown ether [70], cyclen [71], and 1,10-phenanthroline [72]. Such complexes have been mainly used to construct various catalytic systems (see Chapters 6 and 10).

Aromatic stacking interactions and hydrogen bonding play important roles in flavoenzymes. They are crucial in the recognition and binding of flavin cofactors, and also modulate their reactivity. Several artificial model systems have been designed to elucidate the role of these noncovalent interactions, and the results presented by Rotello and coworkers are shown here as illustrative examples (Figure 1.6) [73]. To model the hydrogen bonding of the C2(O)–N3(H)–C4(O) site, for example in lipoamide dehydrogenase, they studied the interaction of 10-isobutyl-7,8-dimethylisoalloxazine with hydrogen donors derived from 2,6-diaminopyridine (Figure 1.6b). Using 2,6-bis(propylamido)pyridine, the complex ($K = 537\ \mathrm{M}^{-1}$) was formed in dichloromethane. Hydrogen bonding was shown to facilitate the electrochemical one-electron reduction, which was attributed to the stabilization of the semiquinone radical. This was manifested by a shift in the reduction potential by 155 mV to less negative values when compared to free flavin [73b, 74]. Interestingly, a similar shift in the potential was caused by flavin

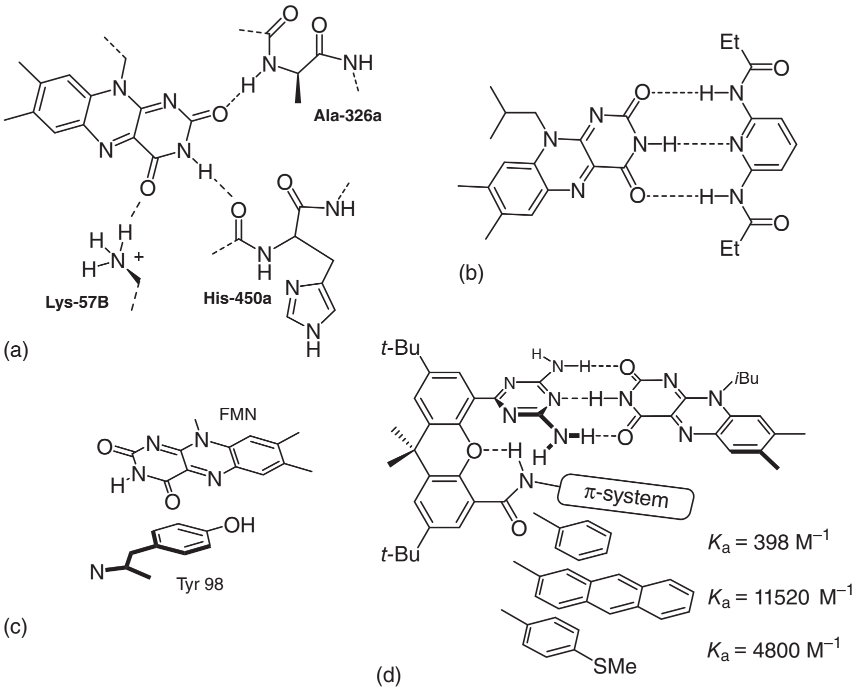


Figure 1.6 Flavin–protein aromatic stacking and hydrogen bonding observed in flavoenzymes (a, c) and model supramolecular systems (b, d). Source: (a–c) Nandwana et al. [73a], Niemz and Rotello [73b], (d) Modified from Nandwana et al. [73a].

substitution using a trifluoromethyl group (cf Table 1.1). Flavin aromatic stacking has been studied using xanthene receptors. It was shown that increasing the π-overlap with the flavin moiety as well as substitution with an electron-donating group supports the interaction as evident from the binding constant. π-Interactions increase the reduction potential showing more favorable stacking when flavin is in its oxidized state [73a] (Figure 1.6d).

1.6 Conclusions

Over the last few decades, there have been a number of studies on the chemistry of flavins and their derivatives reported in the literature. Each decade has provided a resolution to some fundamental problems. The period between the 1930s and 1950s was highlighted by the discovery of the structure of riboflavin and flavoenzymes, which gave a big push toward the development of biological and molecular chemistry, as well as UV–Vis and fluorescent spectroscopy methods. Theoretical calculation methods have been applied to better understand the molecular structure of the flavins and their relationship with proteins, which has also sped up the synthesis of model molecules mimicking enzymatic processes. The past few decades have focused on the investigation and application of electron-transfer processes using flavins with their main applications in photocatalytic reactions, supramolecular systems, and functional materials.

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