Pergamon

Tetrahedron 57 (2001) 4507–4522

TETRAHEDRON

N^1,N^{10}-Ethylene-bridged high-potential flavins: synthesis, characterization, and reactivity

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Received 3 August 2000; accepted 14 March 2001

Abstract—N^1,N^{10}-Ethyleneisoalloxazinium chloride and its 8-Cl-, 7-CF_3-, and 3-CH_3-7-CF_3-substituted analogs were synthesized for the purpose of exhibiting thermal reactivity with organic substrates. The new flavins were characterized spectroscopically and electrochemically, and were found to react with amines, thiols, and phenylhydrazine, the latter case exhibiting catalytic aerobic recycling. Reactions of aliphatic benzylic and cyclopropyl amines with the 7-CF_3 analog were also compared to their oxidations by tris(phenanthroline)iron(III). All reactions of the flavinium salts appear to occur through heterolytic rather than homolytic mechanisms. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Flavin-dependent oxidases such as the mitochondrial monoamine oxidase (MAO; E.C.1.4.3.4) and D-amino acid oxidase (E.C.1.4.3.3) are of considerable importance to mammalian biochemistry.[1] These enzymes carry out dehydrogenation of the organic substrate, accompanied by reduction of the tightly- or covalently-bound flavin, followed by O_2-dependent reoxidation of the reduced flavin with by-production of H_2O_2. The mode of transfer of electrons between the flavin moiety and substrate in the dehydrogenation half-reaction remains incompletely defined, the mechanistic proposals being sequential e^-/H^+/e^- transfer,[2] hydrogen atom transfer,[3] hydride transfer, or two-electron transfer by way of a covalent intermediate (addition–elimination).[4] The electron-transfer pathway for flavin-mediated oxidation of amines has been artificially modeled by photochemically driven electron transfer,[5] but normal flavins (e.g. 3-methylumiflavin) exhibit poor ground-state reactivity toward amines (or amino acids) on account of the low reduction potential of the oxidized flavins (~ -0.3 V vs. NHE). Thus it is necessary to consider strategies that activate typical flavins and/or choose analogs with built-in activation, to create a more favorable reaction free energy. It has been determined in the case of monoamine oxidase B that the flavin potential in the enzyme active site is about the same as for simple flavins in solution, but that the potential may be raised significantly upon binding of good substrates.[6] This could result from a conformation change that engages hydrogen bonding interactions that make the isoalloxazine ring a better electron acceptor and/or by enforcing a somewhat bent binding cavity that favors the non-planar conformation of the two-electron reduced flavin as opposed to the planar Fl^{ox} form. Model studies demonstrating both approaches have been published,[7] though the use of such systems in reaction chemistry with reducing substrates has been limited.

Our approach was to use a combination of two factors favoring reduction of the Fl^{ox} form: alkylation at N^1 and attachment of electron-withdrawing groups to the benzene subnucleus. It is well known that electron-withdrawing groups at position C^7 and C^8 as well as alkylation of either N^1 or N^5 increase the flavin redox potential and reactivity toward nucleophiles. In an effort to model a possible C^{4a} addition–elimination reaction for MAO, Mariano and coworkers studied the N^5-ethyl flavinium compound,[4] shown by Bruice years ago to raise the one-electron reduction potential by 600 mV and to permit the generation of stable C^{4a} adducts.[8] In the reaction with amines, the N^5-ethyl flavinium compound generated C^{4a}-amine adducts readily, which however, underwent the desired elimination reaction only upon heating in the presence of a base.[4a] Our choice of examining N^1-alkylation was based on our desire to avoid a bias toward generation of metastable C^{4a} adducts. The decision to focus on the N^1,N^{10}-ethylene-bridged compound rather than another 1,10-alkylated flavinium salt, such as the corresponding N^1,N^3,N^{10}-trimethyl analog, was based on the report that the former is much more stable toward rearrangement and/or degradation,[9] possibly explaining why it acts as a better redox catalyst.[10]

Keywords: flavin analogs; tris(1,10-phenanthroline)iron(III); phenylhydrazine; monoamine oxidase.

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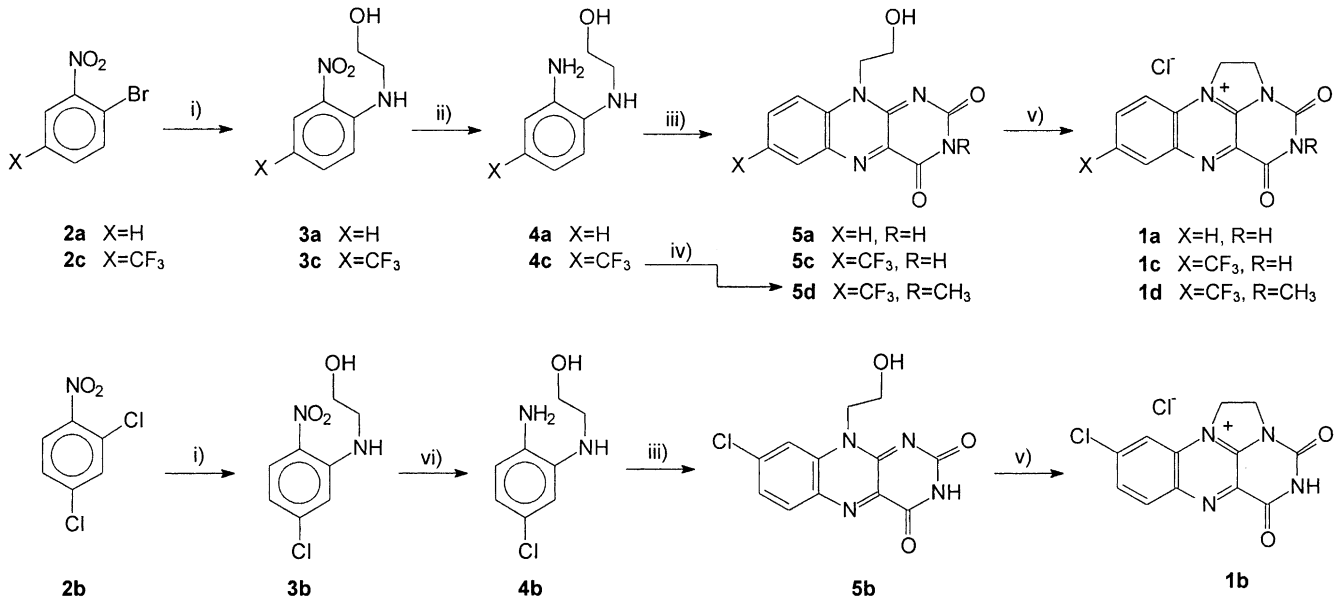
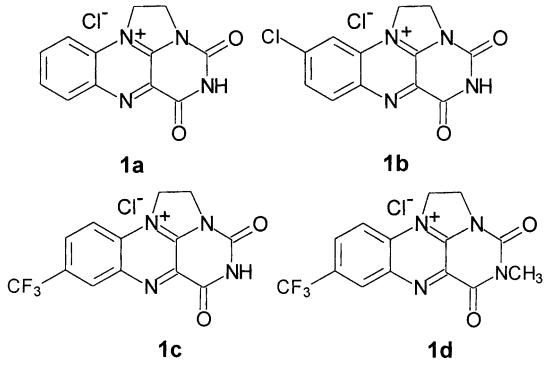
Abbreviations: Fl^{ox}: oxidized flavin; TPIP: tris(1,10-phenanthroline)iron-(III) perchlorate.

PII: S0040-4020(01)00313-1



Scheme 1. Key: (i) ethanolamine, K_2CO_3, DMSO, 90°C; (ii) Pd/C, $NH_4^+HCO_2^-$, MeOH; (iii) alloxan monohydrate, H_3BO_3, HOAc, 60°C; (iv) N-methyl-alloxan monohydrate, H_3BO_3, HOAc, 60°C; (v) $SOCl_2$, room temperature; (vi) Sn/HCl (aq).

Thus, the N^1,N^{10}-ethylene-bridged flavinium analogs **1a–d** were synthesized, along with the N^3-methyl compound **1d** needed in order to evaluate the possibility of N^3-deprotonation in **1c** in the reaction with basic reductants, a situation made likely by the combination of partial positive charge at N^1 and the C^7–CF_3 group. Details on the synthesis and characterization of the analogs are provided along with initial reactivity studies with thiols, hydrazines, and amines. In the latter case, comparison of relative reactivity data to that obtained for reaction of the same amines with the well-known one-electron transfer oxidant $Fe^{III}(phen)_3$, suggests that the flavin reactions utilize a mechanism other than single-electron transfer. The following paper[11] focuses on the mechanism of the reaction of amines with the flavinium compounds.



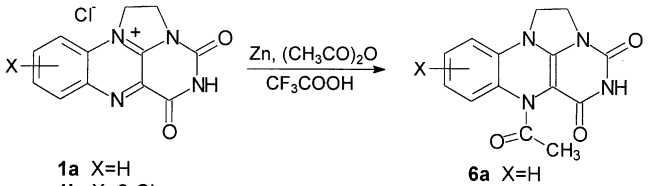
2. Results and discussion

2.1. Synthesis

Synthesis of the flavinium compounds **1a–d** was achieved by an intramolecular cyclization reaction effected by the action of $SOCl_2$ on the corresponding N^{10}-(2-hydroxyethyl)-flavin,[12] in turn prepared by the classical condensation reaction[13] of alloxan with the appropriately substituted N-(2-hydroxyethyl)-*ortho*-phenylenediamine compound (Scheme 1). The syntheses started with the respective 2-bromonitrobenzene **2a–c**, which was reacted with ethanolamine in the presence of K_2CO_3 as base to give the 2-nitroaniline derivatives **3a–c**. Note that the substitution of **2b** was regioselective, as reported.[14] The 2-nitroanilines were purified by chromatography and by recrystallization from methanol to give orange crystalline solids. Catalytic transfer hydrogenation of **3a** and **3c** in dry MeOH at 0°C, using ammonium formate and 10% palladium on activated carbon as a catalyst, gave the diamine compounds **4a** and **4c**. In the case of the chloro analog **3b**, catalytic hydrogenation would result in dehalogenation, and thus its reduction was accomplished with mossy tin in aqueous HCl.

The oxygen-sensitive diamine compounds **4a–c** were condensed anaerobically with alloxan monohydrate (or N-methylalloxan monohydrate for **1d**) in acetic acid in the presence of boric acid to give 10-(2-hydroxyethyl)iso-alloxazines **5a–d**, which were subjected to reaction with $SOCl_2$ to afford the desired flavin compounds **1a–d**. Obtaining a good yield and purity in this final reaction required keeping the volume of $SOCl_2$ as small as possible and keeping the temperature at or below room temperature.

The final flavinium salts **1a–d** were characterized by ^1H and ^{13}C NMR spectroscopy, and formation of the cationic N^1,N^{10}-ethylene bridge was confirmed by the $\geq$1 ppm downfield shift of the four methylene protons relative to the precursor ethanolamines **5a–d**. Further verification of **1a–c** was achieved by isolation and full characterization (^1H and ^{13}C NMR, HRMS) of the N^5-acetyl derivatives **6a–c** (using zinc in acetic anhydride,[12] Scheme 2) of the reduced dihydroflavins, which themselves were unstable to air oxidation. Interestingly, whereas reductive acylation of **1a** and **1b** required 1 h at room temperature or 30 min at reflux,



Scheme 2.

reduction of **1c** was complete in 5 min at room temperature, consistent with an expected higher reduction potential for this analog.

2.2. Electrochemical properties

Both cyclic voltammetry and the OSWV method were used for electrochemical characterization of **1a–c**. From the measured one-electron redox potentials (Table 1), it is clear that all of these N^1,N^{10} bridged flaviniums have much higher potentials than the normal lumiflavin analog N^3,N^{10}-dimethylisoalloxazine (DMI). Compound **1a** exhibits a potential about 200 mV positive of DMI, showing the effect of the positive charge associated with the N^1,N^{10}-ethylene bridge alone. In addition, the substitution of Cl at C^8 and CF_3 at C^7 increases the potential further, such that the most highly oxidizing 7-CF_3 analog **1c** has a potential more than 300 mV positive of DMI. Although obtaining reversible CV waves for **1a** and **1b** required rapid scan rates, suggesting instability of the resulting flavin radicals, the cyclic voltammogram for the 7-CF_3 analog **1c** exhibited a reversible one-electron redox wave even at scan rate below 50 mV/s.

Table 1. Redox potentials for flavinium salts **1a–c**

Isoalloxazine	Cyclic voltammetry method		OSWV Method
	$E_{p,c}$ (mV)[a]	$E^{o'}$ (mV)[b]	E (mV)[c]
1a	−178	−167	−152
1b	−124	−103	−84
1c	−94	−72	−64
DMI[d]	−413	−376	−396

[a] Cathodic peak potential vs. SCE in water using 1 mM flavin, 0.167 M phosphate (pH=6), glassy carbon electrode, scan rate=50 mV/s.
[b] Formal reduction potential ($E_{p,a}+E_{p,c}$)/2 vs. SCE in water using 1 mM flavin, 0.167 M phosphate (pH=6), glassy carbon electrode. Scan rate=50 mV/s for **1c** and DMI, 6000 mV/s for **1b**, and 3000 mV/s for **1a**.
[c] Osteryoung square-wave voltammetry (vs. SCE) in water using 1 mM flavin, 0.167 M phosphate (pH=6), glassy carbon electrode.
[d] N^3,N^{10}-dimethylisoalloxazine.

Despite the potential-raising effect of the N^1,N^{10}-ethylene bridge, the increase in 200–250 mV observed is significantly less than the 600 mV increase seen for N^5-alkylation. This distinction is probably a reflection of the fact that the N^1,N^{10}-ethylene bridge resists the conformational change from planar to bent that normally accompanies flavin reduction,[15] whereas this is allowed in the case of N^5-alkylation. In this way, even the most electrophilic of the analogs studied here (7-CF_3 analog **1c**) possesses only about half of the increase in redox potential induced by N^5-alkylation,[8] and thus might be considered more biologically relevant.

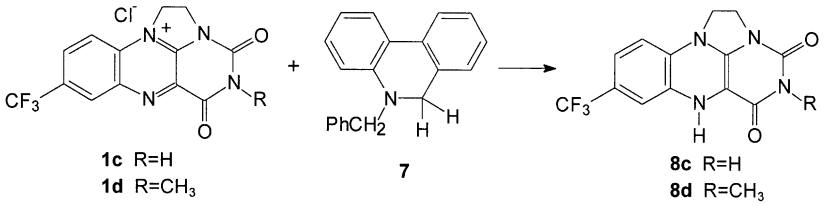
2.3. UV–visible spectroscopic properties

The long wavelength absorption bands of flavinium salts **1a–1c** and their reduced forms are listed in Table 2. A conventional isoalloxazine derivative such as DMI is known to have two characteristic absorption bands at ca. 329 and 437 nm for the oxidized form.[16] The shorter-wavelength absorption band is known to be sensitive to solvent polarity, shifting to shorter wavelength in a hydrophobic environment, whereas the longer wavelength absorption band is fairly insensitive to solvent polarity.[17] For the flavinium salts **1a–c**, the shorter wavelength peak is red shifted and the longer wavelength peak is blue shifted compared with those bands exhibited by DMI. The parent analog **1a** exhibited λ_{max} values at 356 and 403 nm at pH 6, each about 20 nm blue-shifted from that reported for the

Table 2. Long wavelength absorption of flavinium salts **1a–c** and their reduced forms[a]

Compound	Oxidized form[a]		Reduced form[b]		Solvent
	λ_{max} (nm)	ε ($mM^{-1}\,cm^{-1}$)	λ_{max} (nm)	ε ($mM^{-1}\,cm^{-1}$)	
1a	356	13.4	301	7.2	H_2O
	403	10.8			
	352	13.7	306	8.7	CH_3CN
	396	10.4			
1b	368	16.6	300	7.5	H_2O
	402	14.3			
	392	15.7	308	7.6	CH_3CN
1c	340	12.5	312	7.9	H_2O
	396	100	362	2.6	
	340	10.1	304	8.1	CH_3CN
	388	11.5			

[a] Stock solutions (5 mM) of **1a–c** in DMSO were diluted 1:100 into either H_2O–CF_3COOH 50:1 (pH 6) or CH_3CN.
[b] Stock solutions (5 mM) of **1a–c** in DMSO were diluted 1:100 into either H_2O or CH_3CN, followed by the addition of $Na_2S_2O_4$ (final concentration 0.06 mM) or $NaBH_4$ (final concentration 0.03 mM), respectively.



Scheme 3.

corresponding 7,8-dimethyl derivative.[9] Compared to **1a**, whereas the 8-Cl substituent resulted in a slight red shift of the most intense absorption, the 7-CF_3 substituent was associated with a blue shift of both absorption bands. The 7-CF_3 N^3-methyl analog **1d** exhibited spectral properties nearly identical to those of **1c**, with λ_{max} at 336 and 388 nm in CH_3CN. The red shift seen for **1b** probably reflects resonance donation by chlorine, even though the redox potential indicates that the 8-Cl substituent is overall electron-withdrawing. In contrast to conventional isoalloxazines (e.g., DMI), a change in solvent from H_2O to CH_3CN resulted in little or no hypsochromic shift of either band of **1a–c**, a finding that must reflect the permanent (delocalized) positive charge.

Reduced flavins absorb strongly around 300 nm, with a shoulder around 390–400 nm characteristic of the bent geometry.[18] The dihydro derivatives of **1a–c** generated by reduction in degassed (anaerobic) solution, using $Na_2S_2O_4$ in water or $NaBH_4$ in CH_3CN, displayed the expected absorption around 300 nm, but lacked a discernible shoulder around 400 nm, consistent with the conformational restriction imposed by the 1,10-ethylene bridge. The shoulder at ~360 nm seen for **1c** probably reflects the electronic effect of the 7-CF_3 substituent, since an absorption in this region is consistently observed for dihydroflavins containing other strongly electron-withdrawing substituents.[19] Despite the clear generation of dihydroflavins in solution, all attempts to isolate them by treatment with traditional chemical reductants failed to yield material of sufficient analytical purity for full characterization. However, preparative reduction of flavins **1c** in aqueous CH_3CN with $Na_2S_2O_4$, hydrazine, or 1,3-propanedithiol (see below) followed by evaporation of solvent anerobically and quenching with acetic anhydride in degassed CF_3COOH, led to the isolation of the same N^5-acetyl derivative **6c** prepared above by the Zn/Ac_2O method. The N^5-acetyl derivatives **6a–c** exhibited absorptions in DMSO near 260 nm (stronger) and near 300 nm (weaker), consistent with previous reports.[20]

2.4. Reduction of 1c by N-benzyl 5,6-dihydrophenanthridine

Since the reaction of phenylhydrazine with the 7-CF_3 indicated that reoxidation of the reduced flavin by O_2 is slow (see below), it was hoped that use of 1 equiv. of a mild reducing agent might permit isolation of the reduced flavin, at least in this case. Trial and error led to the finding that reaction of **1c** and **1d** with N-benzyl-5,6-dihydrophenanthridine (**7**) in acetonitrile generated the reduced forms **8c** and **8d** as light yellow solids that precipitated from solution (Scheme 3).[21] Although insufficiently soluble for ^{13}C NMR study, **8c** and **8d** were characterized by ^1H NMR and by mass spectrometry, which to our knowledge, constitutes the first such achievement for a reduced N^1,N^{10}-dialkyl dihydroflavin.[22]

2.5. Reactions with phenylhydrazine

Alkyl- and arylhydrazines have been widely studied as MAO inactivators[23] owing to their antidepressant properties.[24] Catalytic turnover of these compounds involves

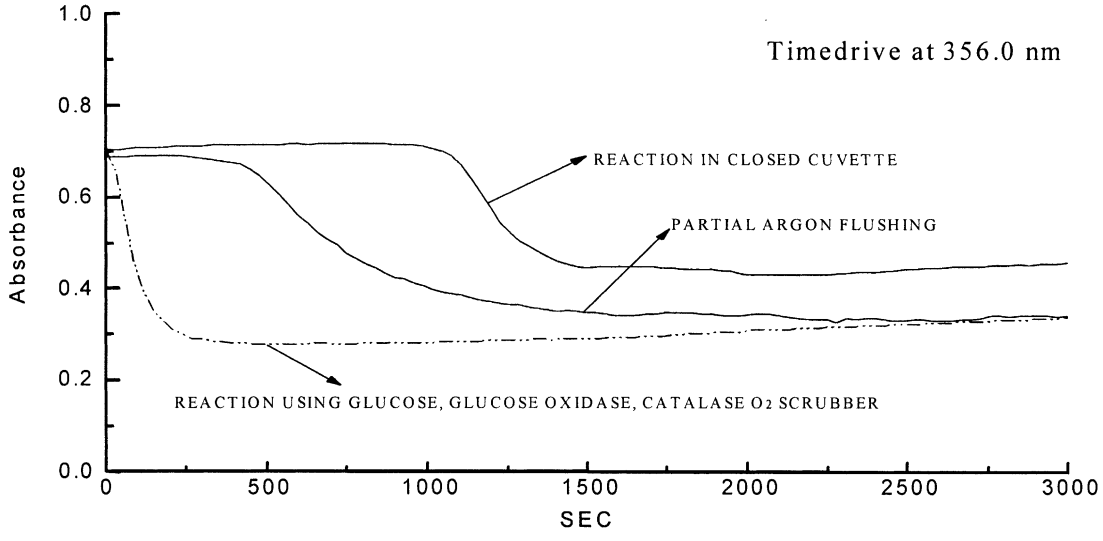


Figure 1. Reaction of **1a** with phenylhydrazine.

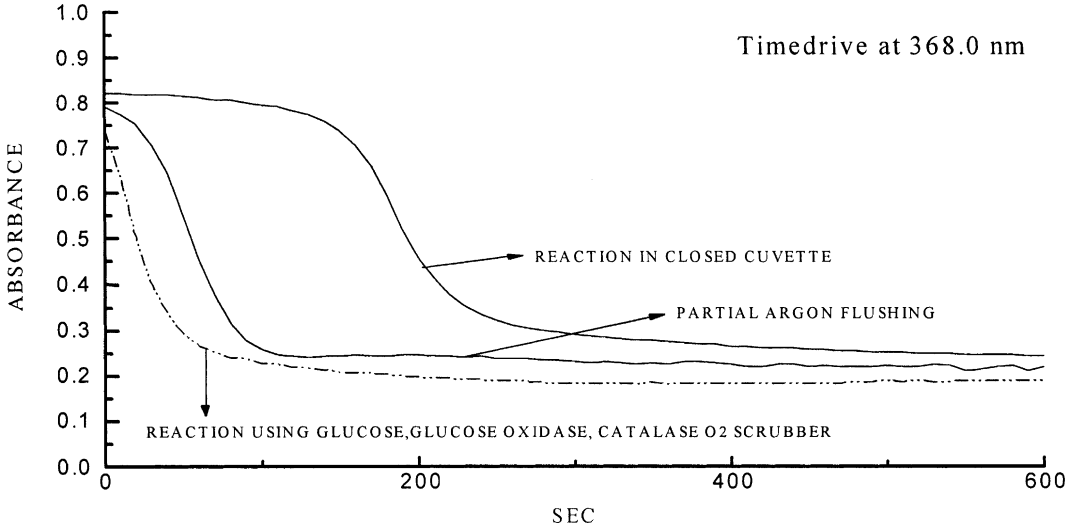


Figure 2. Reaction of **1b** with phenylhydrazine.

dehydrogenation to the corresponding azo compounds RN=NH and ArN=NH, which decompose to N_2 and the respective hydrocarbon. Inactivation results from a competing reaction resulting ultimately in the attachment of the alkyl or aryl group to the flavin.[25]

The reactions of flavinium salts **1a–c** with phenylhydrazine in aqueous buffer were followed spectrophotometrically in a *closed* cuvette (monitoring $-\Delta A$ of the long-wavelength flavin band, Figs. 1–3), such that if catalytic turnover were to occur, involving reoxidation of the reduced flavin by O_2, the extent of turnover would be limited by the initial amount of dissolved O_2. Upon mixing of **1a** with phenylhydrazine, there was observed a lag period for the first 1000 s, followed by reduction of [**1a**] over the next 300 s, at which time the absorbance again plateaued (Fig. 1). However, the lag period was reduced by more than two-fold if argon was bubbled through the solutions of the two reactants. When the reaction was run in O_2-free solution afforded by the presence of a glucose/glucose oxidase/catalase 'scrubber' system, the lag period was eliminated. These results suggest that **1a** is being reduced by phenylhydrazine in a manner that the resulting reduced flavin is immediately reoxidized when O_2 is present. The end of the lag period coincides with the point where the dissolved O_2 is consumed.

The reaction of 8-Cl analog **1b** with $PhNHNH_2$ exhibited basically the same behavior (Fig. 2) as seen for **1a**. The lag seen without argon bubbling indicates that reoxidation of the reduced flavin resulting from oxidation of $PhNHNH_2$ by **1b** is faster than its generation. However, for the reaction of 7-CF_3 analog **1c**, the decrease in absorbance did not exhibit a lag period, and the increase in A_{340} observed following the initial decrease was very slow (Fig. 3), suggesting that the reduced flavin in this case is reoxidized very slowly by O_2. Upon argon bubbling or use of the 'O_2 scrubber', the rate of decrease in A_{340} was accelerated, consistent with an interpretation that the initially observed A_{340} decrease without argon bubbling represents a composite of simultaneous

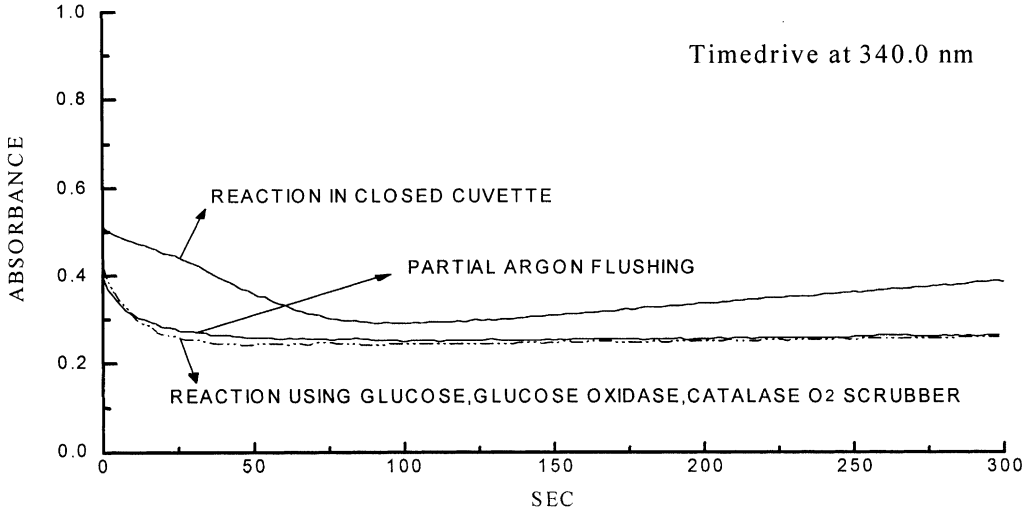


Figure 3. Reaction of **1c** with phenylhydrazine.

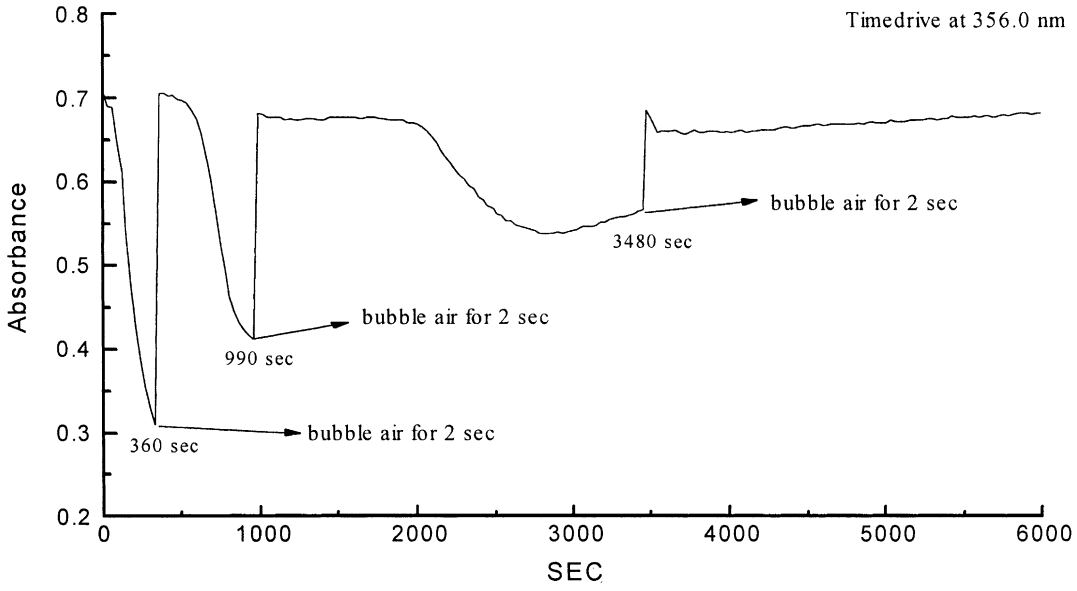


Figure 4. Reaction of **1a** with phenylhydrazine under controlled atmosphere conditions.

flavin reduction (faster) and reduced flavin reoxidation (slower).

The nature of the reaction products was investigated in the case of flavin **1c** in CH_3CN. Although the solubility of **1c** in this solvent was low, the reaction of 1.1 equiv. of $PhNHNH_2$ resulted in a homogeneous solution that when quenched with acetic anhydride, permitted isolation of the N^5-acetyl derivative **6c** of the reduced flavin **8c**. In addition, when the reaction was followed in an NMR tube in CD_3CN, the formation of phenyldiazene (PhN=NH) was confirmed through detection of its well-known decomposition product, benzene.[26]

To further confirm that the lag period observed for parent flavinium analog **1a** reflects immediate O_2-mediated reoxidation of the reduced flavin, reaction of **1a** with $PhNHNH_2$ was studied under a condition where either argon or air could be bubbled along the side of the cuvette at a flow rate of 5 mL/min. The reaction was initiated under argon bubbling, and the A_{356} decreased steadily over the first 6 min (Fig. 4). At that point, air was bubbled for 2 s, resulting in immediate return to the initial absorbance and then a decrease over the next 10 min *but to a lesser extent than initially*. Again air was bubbled for 2 s, but the decrease in A_{356} following the initial rapid rise now exhibited a lag period and then a slower decrease, again by a reduced margin. The third cycle of O_2 introduction exhibited a further deterioration of the initial cycling seen. Our interpretation of this data is that repetitive redox cycling of **1a** with $PhNHNH_2$ results in eventual irreversible conversion of **1a** to a form that is inactive as a redox catalyst, but which absorbs at 356 nm with an extinction similar to that of **1a**. Although no product analysis was performed on this system, it is possible that the irreversible reaction reflects covalent modification of the flavin by the phenyl group of $PhNHNH_2$ as occurs when MAO is irreversibly inactivated by $PhNHNH_2$.[25]

Overall, these results represent the first report of catalytic activity of a model flavin for the O_2-dependent oxidation of $PhNHNH_2$. With use of an O_2 scrubber, the initial rate of decrease of the oxidized flavinium absorbance can be taken to reflect the pseudo-first-order rates for reduction of each flavinium salt **1a–c** by $PhNHNH_2$, and these values are listed in Table 3. As expected, the rate rank order reflects the increase in oxidizing strength **1a**<**1b**<**1c**, evident from the measured redox potentials (Table 1). At the same time however, this rank order does not reflect the efficiency of the flavinium compound as a redox *catalyst*, since catalysis depends on both the rate of reduction by $PhNHNH_2$ and the rate of reoxidation by O_2. The reoxidation rate follows **1a**>**1b**≫**1c**, with the latter reduced 7-CF_3 analog being nearly resistant to O_2-mediated reoxidation.

Table 3. Pseudo-first-order rates for reaction of flavinium salts **1a–c** with $PhNHNH_2$·HCl[a]

Flavin	Rate (s^{-1})[b]
1a	0.0102
1b	0.032
1c	0.104

[a] *Reaction conditions*: 0.05 mM flavin, 0.67 mM $PhNHNH_2$·HCl, 16 mM glucose, 165 mM potassium phosphate buffer (pH 6.01), 15°C, containing 6.7 μg glucose oxidase and 1.0 μg catalase per mL.
[b] Monitoring the decrease in the long wavelength Fl^{ox} absorption.

2.6. Reaction with thiols

It has been reported that flavins can effect the oxidation of thiols to disulfides by an addition–elimination mechanism proceeding via a C^{4a}-mercapto adduct.[27] It was thus of interest to determine whether flavinium salts **1a–c** could also oxidize thiols such as thiophenol. In the case of the 8-chloro analog **1b**, the reaction with thiophenol resulted in a nearly isosbestic spectral change (Fig. 5) indicative of conversion of the flavin to a highly delocalized species (for a short time an intermediate can be detected at 410 nm). The

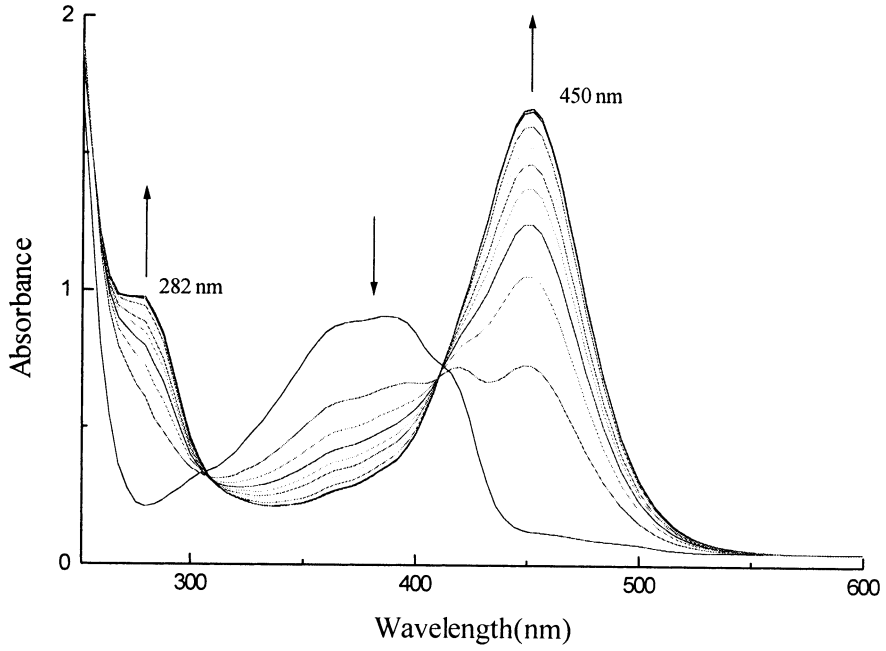
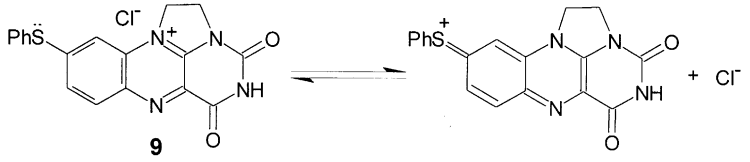


Figure 5. Time course of the spectral changes for the reaction of 8-chloro flavin **1b** with thiophenol: [**1b**] 5×10^{-5} M, [thiophenol] 5×10^{-4} M, CH_3CN, 25°C.



Scheme 4.

intense product absorptions, λ_{max} (CH_3CN) 450 nm (ε=34 $mM^{-1}\,cm^{-1}$) and 282 nm (ε=17 $mM^{-1}\,cm^{-1}$), are consistent with the spectrum reported for 8-(arylmercapto)-riboflavin [λ_{max} 475 nm (ε=26 $mM^{-1}\,cm^{-1}$) at pH 7],[28] suggesting that the reaction involves nucleophilic displacement of the 8-chloro substituent to give 8-(phenylmercapto)-N^1,N^{10}-ethyleneisoalloxazinium chloride (**9**). The marked bathochromic shifts must reflect conjugative electron release by the 8-thio substituent, as exemplified by the paraquinoid resonance form (Scheme 4).

Table 4. Oxidation of benzylamines by flavins **1a**, **1c**, and **1d**[a]

Flavin	Amine	Yield of PhCHO (%)[b]
1a	$PhCH_2NH_2$	25.0
	$PhCH_2NHCH_3$	5.0
	$PhCH_2N(CH_3)_2$	~0.6
1c	$PhCH_2NH_2$	147.0
	$PhCH_2NHCH_3$	44.0
	$PhCH_2N(CH_3)_2$	4.8
1d	$PhCH_2NH_2$	118.0
	$PhCH_2NHCH_3$	20.0
	$PhCH_2N(CH_3)_2$	1.6

[a] *Conditions*: [Flavin]:[Amine]:[Amine hydrochloride]=10 mM:55 mM:5 mM, 50°C, 48 h under argon with exposure to air (5 min) after 6 and 12 h. PhCHO was removed by azeotropic distillation and quantified as the 2,4-DNP derivative.

[b] Yield based on flavins after correction for minor spontaneous autoxidation.

Although the nuclear substitution undergone by 8-Cl analog **1b** with thiols prevented an investigation of the ability of **1b** to catalyze thiol→disulfide oxidation, reactions of **1a** and **1c** with thiols (thiophenol or 1,3-propanedithiol) showed spontaneous bleaching of absorptions of the oxidized flavins, concomitant with appearance of new absorptions at 303 nm characteristic of the reduced flavins. Furthermore, NMR tube reactions of **1c** with 1,3-propanedithiol in either CD_3CN or D_2O exhibited rapid appearance of signals corresponding to 1,3-dithietane. Additional evidence for thiol-mediated reduction was obtained for **1c** by isolation of the N^5-acetyl derivative **6c** following quenching of the thiol reaction with acetic anhydride. These findings support the ability of **1a** and **1c** to mediate oxidation of organic reductants, though no information on mechanism could be obtained due to the rapidity of the reactions.

2.7. Oxidation of benzylamines

Reactions of benzylamine and its N-methyl and N,N-dimethyl analogs were explored with flavinium salts **1a** and **1c** (it was feared that the 8-Cl analog **1b** would be susceptible to nuclear substitution). In order to evaluate relative reactivity as a function of both amine and flavin, preparative-scale reactions were conducted with quantitation of the oxidative debenzylation product PhCHO as its 2,4-dinitrophenylhydrazone. The reactions were run in stoppered flasks under argon to prevent evaporative loss of

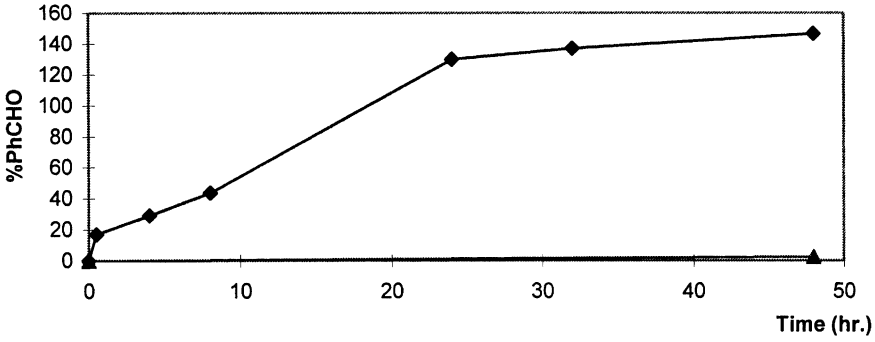
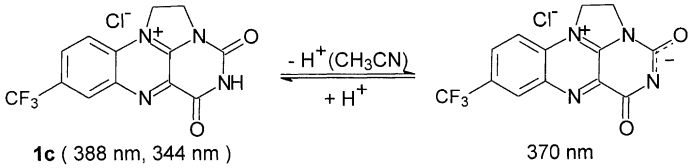


Figure 6. Time course of benzylamine oxidation by **1c**. *Reaction conditions*: 55 mM $PhCH_2NH_2$/5 mM $PhCH_2NH_2$·Cl/50°C/CH_3CN/2 days in the dark under argon with exposure to air for 5 min after 6 h and 12 h, in the presence (◆) and absence (▲) of 10 mM **1c**. Yield of PhCHO based on flavin catalyst.



Scheme 5.

solvent, but O_2 was admitted periodically to allow for catalytic recycling. The background-corrected yields after 48 h of reaction for flavins **1a**, **1c**, and **1d** are listed in Table 4. It is apparent that the 7-CF_3-substituted flavins **1c** and **1d** are stronger debenzylating agents than the unsubstituted parent **1a**, and that the amine reactivity order is 1°>2°>3°. In addition, it should be noted that for **1c** and **1d**, the yield of PhCHO based on flavin exceeds 100%, indicative of some aerobic recycling. As can be seen from the time course of PhCHO generation in the reaction of **1c** with benzylamine (Fig. 6), PhCHO was produced rapidly in the first 0.5 h and then more slowly over 24 h. The substantial slowing of PhCHO production in excess of 1 equiv. based on flavin suggests that O_2-dependent regeneration of oxidized flavin **1c** occurs slowly, consistent with what was seen in its reaction with phenylhydrazine.

The rates of reaction of **1c** with benzylamine, its N-methyl and N,N-dimethyl analogs, and tributylamine were followed spectrophotometrically in amine/amine·HCl (10:1) buffered CH_3CN solution. In every case there was an immediate shift of the oxidized flavin spectrum (λ_{max}=388 nm) to one exhibiting a λ_{max} of 370 nm. Since this change was observed even for tertiary amines, it appeared that the spectral shift represented equilibrium deprotonation at N^3 (Scheme 5) rather than formation of an amine–flavin adduct, which would be sterically difficult for tertiary amines. Using substoichiometric amounts of amines, the shift was shown to be isosbestic and titratable (data not shown). Evidence that the spectral change represented a reversible equilibrium was that the original spectrum of **1c** could be reestablished when the incubations of **1c** with amines were quenched with excess HCl or CH_3COOH. Confirmation of the occurrence of N^3-deprotonation[29] was that no immediate spectral shifts were observed when the same reactions were conducted with N^3-methyl analog **1d**.

Table 5. Pseudo-first-order rates for reaction of 7-CF_3 flavin **1c** with benzylamines

Amines	Rate (min^{-1})[a]
$PhCH_2NH_2$	0.59
$PhCH_2NHCH_3$	0.085
$PhCH_2NMe_2$	0.075
n-Bu_3N	0.014

[a] From $t_{1/2}$, monitoring reduction at 370 nm (observed immediately upon mixing amine with flavin). *Reaction conditions*: 0.05 mM **1c**, 1 mM amines/amine·HCl (10:1), CH_3CN, 50°C.

Subsequent to the rapid shift from 388 nm to 370 nm, irreversible reactions of the amines with **1c** could be assessed by measuring the rate of decay of the 370 nm band. The rate constants obtained under pseudo-first order reaction conditions are listed in Table 5. The apparent amine reactivity towards **1c** again follows the order 1°>2°>3°, though the nature of the reaction mechanism is not revealed by this data, and may not be the same for both primary/secondary and tertiary amines. In fact, whereas for primary and secondary amines, the decrease in A_{370} was accompanied by appearance of a band at 390 nm, the slower reaction of the tertiary amines was accompanied by appearance of a band at 376 nm. For the reactions with N^3-methyl analog **1d**, there was no initial spectral shift in the starting 388 nm chromophore, but the same bands at 390 nm (primary and secondary amines) or 376 nm (tertiary amines) were still generated in the same relative rate order (data not shown). Structural interpretations of these spectral changes, accomplished using ^{13}C-labeled flavins and ^{15}N-labeled amines, permitted ascribing the 390 nm band to the C^{10a}-amine adducts.[11] Although unstable and previously identified tentatively only on the basis of spectral changes,[30,31] C^{10a}-nucleophile adducts in N^1,N^{10}-dimethyl flavinium salts have been invoked as intermediates to rationalize the isolation of spirohydantoin rearrangement products

Table 6. Oxidation of benzylamines by $(phen)_3Fe(III)$[a]

Amines	Products	Yield (%) for [amine]=28mM[b]	Yield (%) for [amine]=560mM[b]
$PhCH_2NH_2$	PhCHO	8.3	29.6
$PhCH_2NHCH_3$	PhCHO	11.1	42.0
	CH_2O		0.5
$PhCH_2N(CH_3)_2$	PhCHO	20.6 (23.0)[c]	31.2
	CH_2O	0.5 (6.5)[c]	21.9

[a] *Reaction conditions*: 25°C for 1 h in dry CH_3CN under argon, $[Fe(phen)_3(ClO_4)_3]$=56 mM.
[b] Yield of the 2,4-dinitrophenylhydrazone based on 2 equiv. of $Fe(phen)_3(ClO_4)_3$.
[c] Solid anhydrous K_2CO_3 (5 equiv. based on Fe(III)) was added to the reaction flask.

resulting from reaction of the flavinium salts with water,[30,32,33] alcohols,[31,33] or amines[33] under basic conditions.

2.8. Oxidation of benzylamines by tris(1,10-phenanthroline)iron(III) perchlorate

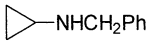
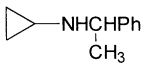
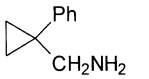
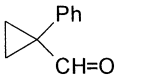
Preparative-scale reactions of benzylamine and its N-methyl and N,N-dimethyl analogs with the well-known outer-sphere electron-transfer oxidant tris(1,10-phenanthroline)-iron(III) perchlorate (TPIP) were conducted using an amine:TPIP stoichiometry of 1:2 as well as 20:1. Higher total levels of N-dealkylation were observed for the secondary and especially tertiary as opposed to primary amine in both reaction conditions (Table 6). Although an overall two-electron oxidation of amine should theoretically consume 2 equivalents of Fe(III), the total yield of N-dealkylation products based on Fe(III) increased 2–3-fold by increasing the amine concentration 20-fold. In addition, variation in the reaction conditions for one case (third entry in Table 6) revealed that addition of solid K_2CO_3 to the reaction flask resulted in a substantial increase in reaction yield, suggesting that the difference in yield between the 1:2 and 20:1 conditions reflects increased basicity of the medium using excess amine. It is interesting that the competition between N-debenzylation and N-demethylation of the tertiary amine varied markedly with the amine:TPIP stoichiometry, though the reason for this is not clear. Nonetheless, the finding that the order of amine reactivity, $3°>2°>1°$, is exactly opposite to that found using flavins **1** as oxidants (Tables 4 and 5), indicates that *the flavin reactions do not follow an electron-transfer mechanism*.

2.9. Oxidation of cyclopropyl-containing amines

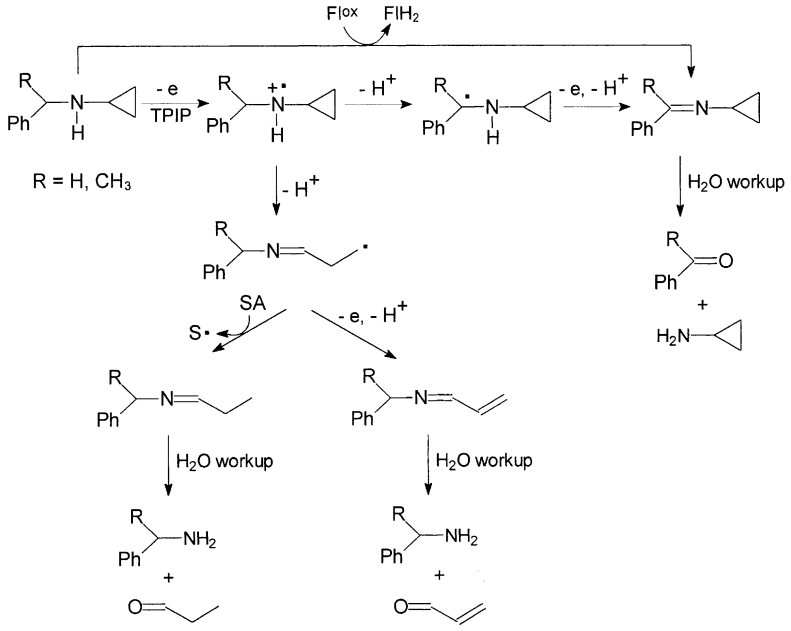
In the reaction with amines, the suggestion of an electron-transfer oxidation mechanism in the case of TPIP but not in the case of the flavinium salts, led us to investigate the interaction of these two oxidants with cyclopropylamines, which are known to undergo one-electron oxidative ring-openings. TPIP-induced oxidation of N-cyclopropylbenzyl-amine, N-cyclopropyl-α-methylbenzylamine, (1-phenyl-cyclopropyl)methylamine, and the primary amine 'control' of the latter reactant, phenethylamine, was studied under either the K_2CO_3-enhanced or excess-amine-enhanced condition. Reactivity was evaluated by quantification of the 2,4-dinitrophenylhydrazine derivatives of the aldehyde products, and the results are listed in Table 7. Oxidation of the two cyclopropylamines resulted in products of benzylic oxidation, but also products of cyclopropyl ring-opening, as expected for generation of an N-cyclopropyl aminium cation-radical intermediate. In contrast, the homologous cyclopropylcarbinylamine exhibited only the ring-closed product, as expected. The primary amine control of the latter amine (same carbon atom spacing) underwent reaction only sluggishly.

A mechanism consistent with formation of the aldehyde products arising from oxidation of the two cyclopropyl-amines by TPIP is shown in Scheme 6. This mechanism

Table 7. Oxidation of cyclopropylamines by $Fe(phen)_3(ClO_4)_3$ (TPIP)

Conditions[a] Amine:TPIP:(added base) ratio	Substrates	Products[b]	Yield (%)[c]
1:2:10 (K_2CO_3)		PhCHO	10.0
		CH_3CH_2CHO	3.6
		$CH_2=CHCH=O$	<0.5
1:2:10 (K_2CO_3)		$PhC(CH_3)=O$	3.5
		CH_3CH_2CHO	3.5
		$CH_2=CHCH=O$	1.1
20:2			4.1
20:2(Δ80°C)	$PhCH_2CH_2NH_2$	$PhCH_2CHO$	9.9

[a] *Standard conditions*: 0.56 mmol TPIP+amines, 25°C, 1 h, 10 mL CH_3CN, under Ar.
[b] Products were isolated as 2,4-dinitrophenylhydrazine derivatives.
[c] Yields are based on 2 equiv. of $Fe(phen)_3(ClO_4)_3$.



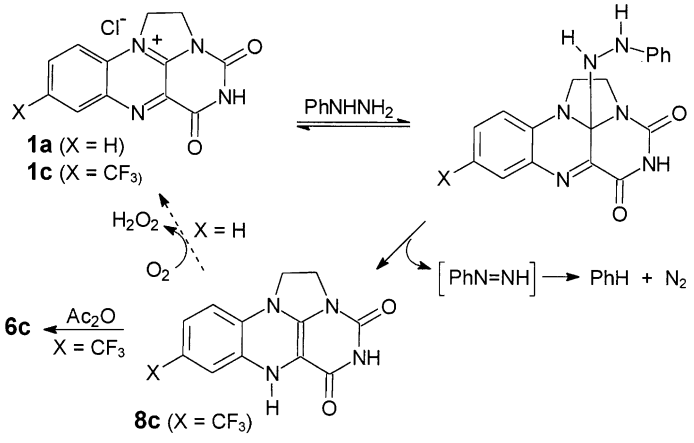
Scheme 6.

suggests a partitioning of the initial aminium cation-radical between benzylic deprotonation, leading to PhCHO or $PhC(=O)CH_3$, and ring opening. On the other hand, reaction of the same two cyclopropylamines with flavin **1c** gave no evidence for ring-cleavage products, as determined by monitoring the reactions in an NMR tube. Instead, the spectrum revealed 27% conversion of N-cyclopropylbenzylamine to benzylidenecyclopropylamine, and a low level (~1%) of the analogous conversion in the case of N-cyclopropyl-α-methylbenzylamine. In the latter case, α-branching may sterically retard whatever mechanism is responsible for oxidation by **1c**. Nonetheless, the absence of ring-cleavage products supports the previous conclusion that oxidation of amines by the flavinium salts **1** involves a mechanism distinct from electron transfer.

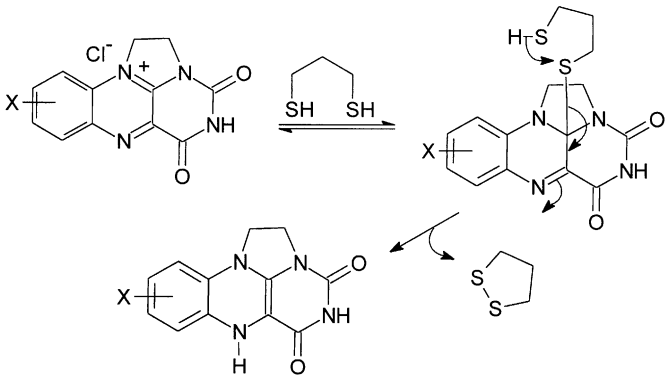
Interestingly, even the unsubstituted parent flavin **1a** effected oxidative cleavage of the highly activated compound 2-phenylcyclopropylamine, leading to isolation of the 2,4-dinitrophenylhydrazone of cinnamaldehyde in 45% yield. However, such conversion was also observed in the reaction using N^5-ethyl flavinium cation,[4b] where an addition–elimination mechanism was proposed. It is well known that cyclopropylamines can become the subject of heterolytic[4b,34,35] as well as homolytic ring-cleavage processes. Thus, the result for 2-phenylcyclopropylamine does not alter the conclusion reached above.

3. Conclusions and mechanistic proposals

This paper describes new high-potential N^1,N^{10}-ethylene-bridged flavinium salts, which have been characterized spectroscopically and electrochemically, and found to induce thermal reactions with thiols and phenylhydrazine. In the latter case, catalytic recycling of the parent analog **1a** was demonstrated, though catalysis by the higher-potential analog **1c** appears to be limited by slow O_2-dependent oxidation of the reduced flavin species. In addition, initial reactivity studies on the reaction of benzylic and cyclopropyl-containing amines with the flavinium salts have been compared to the reactions with the well-known



Scheme 7.



Scheme 8.

one-electron oxidant tris(phenanthroline)iron(III) perchlorate (TPIP). Whereas both the effect of alkyl substitution on amine reactivity (3°>2°>1°) and the observation of ring-cleavage products from benzylic cyclopropylamines are consistent with an electron-transfer oxidation mechanism for TPIP, the opposite reactivity rank order and lack of ring-cleavage products for benzylic cyclopropylamines indicate that the flavin reactions do not involve electron transfer. For the related N^5-ethyl flavinium salts, an addition–elimination mechanism via a C^{4a}-adduct intermediate was demonstrated for the observed oxidation of amines.[4] In the following paper, we provide evidence that dehydrogenation of primary and secondary amines by N^1,N^{10}-ethylene-bridged flavins **1** also involves mainly an addition–elimination mechanism, but in this case proceeding via C^{10a} adducts.[11] For tertiary amines with strong hydride donor capacity such as **7**, a hydride transfer mechanism appears to occur.[21]

The evidence for C^{10a} nucleophile adducts formed from N^1,N^{10}-dimethyl flavinium salts[30–33] and for the N^1,N^{10}-ethylene-bridged compounds **1**[10] suggests that the oxidations of phenylhydrazine and thiols by flavins **1** also proceed via C^{10a} adducts. Our proposed mechanisms are shown in Schemes 7 and 8. For the reaction of $PhNHNH_2$ with flavin **1a**, addition of $PhNHNH_2$ and subsequent β-elimination affords reduced flavin and PhN=NH, which decomposes to N_2 and benzene (Scheme 7). The reduced flavin is then aerobically recycled, allowing for catalytic turnover. In the case of flavin **1c**, the reduced flavin **8c** is only slowly reoxidized and can be trapped with acetic anhydride to give **6c** (Scheme 7). For the reaction with 1,3-propanedithiol, Scheme 8 depicts the C^{10a}-adduct version of the C^{4a}-adduct mechanism previously worked out in detail in the case of normal flavins.[27]

Overall, the information revealed by studies such as those described here and elsewhere,[4,11,21] should provide important information relevant to evaluating proposed non-radical oxidation mechanisms mediated by flavoenzymes.

4. Experimental

4.1. General experimental information

^1H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Gemini 300 spectrometer. Chemical shifts are reported relative to the standard resonance of the residual protons in the deuterated solvents. High-resolution mass spectra (HRMS) were obtained on a Kratos MS25RFA spectrometer using electron impact ionization (20–40 eV). UV–visible spectra were recorded with a Perkin–Elmer model Lambda 3B spectrophotometer fitted with a water-jacketed multiple cell holder for maintenance of constant temperature. Spectral scan and kinetics data (at constant temperature) were obtained using PECSS software (Perkin Elmer Corporation, 1988). Thin-layer chromatography was performed with Merck silica gel G plates. The solvents used for kinetics determinations (e.g., CH_3CN was distilled from P_2O_5) and reactive reagents such as thionyl chloride were purified by standard methods.[36] The water used in this study was deionized and doubly distilled. 3,10-Dimethylisoalloxazine (DMI) was synthesized as described.[16]

4.2. Electrochemistry

Cyclic voltammetry and Osteryoung square-wave voltammetry measurements in water were performed using a BAS100 Electrochemical Analyzer with a three-electrode system consisting of a glassy carbon working electrode with s=6 mm, a platinum auxiliary electrode, and a Ag/AgCl (saturated KCl) reference electrode. The glassy carbon electrode was polished with 0.3 and 0.05 mm alumina powder, sonicated (to remove polishing powder), and washed with water. All of the solutions were prepared at 1 mM and contained 0.167 M potassium phosphate (pH 6.01) as a supporting electrolyte. All measurements were carried out under an atmosphere of nitrogen using N_2-purged solutions, and the potentials listed are given vs SCE.

4.3. Preparation of substituted 2-nitro-N-(2-hydroxyethyl)anilines 3a–c

In a typical procedure, a solution of 4-bromo-3-nitrobenzotrifluoride (5.03 g, 18.5 mmol) and ethanolamine (3.39 g, 55.5 mmol) in 50 mL n-butyl alcohol containing solid K_2CO_3 (2.8 g, 20 mmol) was refluxed for 5–6 h with constant stirring and then cooled. The reaction mixture was filtered through a layer of Celite, and the filtrate was concentrated under reduced pressure. The resulting suspension of orange crystalline solid was poured onto saturated aqueous NaCl (50 mL) and extracted with EtOAc (2×100 mL). The organic extract was washed with saturated

aqueous NaCl (3×20 mL), dried ($MgSO_4$), evaporated, and purified by silica gel flash chromatography (2:1 CH_2Cl_2–EtOAc eluant) to give 4-(trifluoromethyl)-2-nitro-N-(2-hydroxyethyl)aniline (**3c**) as an orange solid in 94% overall yield: ^{1}H NMR ($CDCl_3$) δ 2.86 (br m, 1H, OH), 3.51 (q, 2H, J=5.2 Hz), 3.95 (t, 2H, J=5.2 Hz), 6.96 (d, 1H, J=9.0 Hz), 7.57 (dd, 1H, J=9.0 and 2.2 Hz), 8.36 (s, 1H), 8.47 (br t, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 45.0, 60.5, 114.7, 117.5 (q, J=34.3 Hz, C–CF_3), 123.6 (q, J=270 Hz, CF_3), 124.8, 130.8, 132.2, 147.0.

2-Nitro-N-(2-hydroxyethyl)aniline (**3a**)[37] was obtained in the same manner in 54% yield based on 1-bromo-2-nitrobenzene: ^{1}H NMR ($CDCl_3$) δ 2.08 (br t, 1H, OH), 3.53 (q, 2H, J=5.4 Hz), 3.97 (t, 2H, J=5.4 Hz), 6.67 (t, 1H, J=7.7 Hz), 6.90 (d, 1H, J=8.7 Hz), 7.45 (t, 1H, J=7.7 Hz), 8.17 (d, 1H, J=8.7 Hz), 8.24 (br m, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 45, 60.7, 113.9, 115.6, 126.9, 131.9, 136.4, 145.6.

5-Chloro-N-(2-hydroxyethyl)-2-nitroaniline (**3b**)[38] was obtained in the same manner in 84% yield based on 2,4-dichloronitrobenzene: ^{1}H NMR (DMSO-d_6) δ 3.41 (2H), 3.63 (2H), 5.00 (t, 1H, J=5.2 Hz, OH), 6.68 (dd, 1H, J=8.7, 2.1 Hz), 7.14 (d, 1H, J=2.1 Hz), 8.07 (d, 1H, J=8.7 Hz), 8.31 (t, 1H, J=5.2 Hz, NH). ^{13}C NMR (DMSO-d_6) δ 44.8, 58.9, 113.0, 115.2, 128.1, 129.8, 141.5, 145.9.

4.4. Preparation of substituted 2-((2-hydroxyethyl)-amino)anilines 4a–c

To a solution of **3c** (5.71 g, 22.8 mmol) in 80 mL of dry MeOH at 5°C was added ammonium formate (7.2 g, 114.2 mmol) and 10% Pd on carbon (3.82 g). The reaction was allowed to warm to 25°C, where it was stirred for 1 h. After this time, the reaction mixture was filtered through a bed of Celite, and then the solids were collected and washed with methanol. The filtrate was concentrated under reduced pressure to afford a reddish residue, which was diluted with saturated NaCl (100 mL) and extracted twice with EtOAc (50 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford 2-((2-hydroxyethyl)amino)-5-(trifluoromethyl)-aniline (**4c**) as a white powder in 80% yield: ^{1}H NMR (DMSO-d_6) δ 3.19 (t, 2H, J=5.4 Hz), 3.65 (t, 2H, J=5.4 Hz), 4.78 (br m, 1H, OH), 4.92 (br, 2H, NH_2), 4.99 (br t, 1H, NH), 6.50 (d, 1H, J=8.3 Hz), 6.84 (d, 2H, J=8.3 Hz); ^{13}C NMR (DMSO-d_6) δ 45.6, 59.3, 108.0, 109.5, 114.6, 116.4 (q, J=31.4 Hz, C–CF_3), 125.5 (q, J=270 Hz, CF_3), 135.1, 139.1.

2-((2-Hydroxyethyl)amino)aniline (**4a**)[39] was obtained from **3a** in the same manner in 91% yield: ^{1}H NMR (DMSO-d$_6$) δ 3.08 (q, 2H, J=5.5 Hz), 3.60 (q, 2H, J=5.5 Hz), 4.34 (br t, 1H), 4.43 (s, 2H, NH_2), 4.69 (t, 1H, J=5.5 Hz, NH), 6.41–6.56 (4H); ^{13}C NMR (DMSO-d$_6$) δ 46.0, 59.6, 109.8, 114.2, 116.9, 117.7, 135.3, 136.2.

4-Chloro-2-((2-hydroxyethyl)amino)aniline (**4b**) was obtained according to a modification of the published method.[40] To a solution of **3b** (4.13 g, 19.1 mmol) and mossy tin (6.80 g, 57.3 mmol) in water (60 mL) at 100°C was added 35 mL conc. HCl dropwise. After being cooled for 30 min at 5°C, the reaction mixture was made basic with 50% aqueous NaOH and extracted with EtOAc (3×50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to provide **4b** as a white solid (1.46 g, 41%): ^{1}H NMR (DMSO-d_6) δ 3.16 (br, 2H), 3.69 (br, 2H), 4.58 (br, 2H, NH_2), 4.68 (br, 1H), 4.82 (br, 1H), 6.49 (d, 2H, J=8.4 Hz), 6.59 (d, 1H, J=8.4 Hz); ^{13}C NMR (DMSO-d_6) δ 45.8, 59.5, 109.2, 114.9, 115.9, 121.7, 133.9, 137.7.

4.5. Preparation of substituted 1,10-ethyleneisoalloxazinium chlorides 1a–c

To a solution of the corresponding N-(hydroxyethyl)-*o*-phenylenediamine **4a–c** (4.02 g, 18.3 mmol) in glacial acetic acid (40 mL) at 50°C that was thoroughly flushed with argon, was added alloxan (2.93 g, 18.3 mmol) and boric acid (1.17 g, 19.0 mmol). After 1 h the solution was cooled, and the yellow-orange solid formed was filtered, washed with CH_2Cl_2 (30 mL), and dried under vacuum. Without delay, the yellow-orange solid was placed in a round-bottom flask that had been flushed with N_2, and thionyl chloride (30 mL) was added gradually with constant stirring. The reaction solution was stirred for 16 h at 50°C under N_2 then cooled and filtered, and the solid was washed with CH_2Cl_2 (50 mL). The crude product was dissolved in a minimum amount of 98% formic acid and re-precipitated with ether.

1,10-Ethyleneisoalloxazinium chloride (**1a**) was obtained in 61% yield. mp 216–219°C; ^{1}H NMR (CD_3COOD–CF_3COOH, 1:6) δ 4.94 (t, 2H, J=8.9 Hz), 5.53 (t, 2H, J=8.9 Hz), 8.04–8.57 (4H); ^{13}C NMR (CD_3COOD–CF_3COOH, 1:6) δ 45.9, 51.9, 117.2, 130.3, 131.9, 133.2, 134.2, 142.1, 142.2, 144.1, 146.7, 159.4. A sample of the chloride salt **1a** was converted to the perchlorate salt for purposes of elemental analysis. Anal. Calcd for $C_{12}H_9ClN_4O_6$: C, 42.31; H, 2.66; N, 16.45. Found: C, 42.13; H, 2.60; N, 16.35.

8-Chloro-1,10-ethyleneisoalloxazinium chloride (**1b**) was obtained in 56% yield. mp >350°C; ^{1}H NMR (CD_3COOD–CF_3COOH, 1:6) δ 4.92 (t, 2H, J=8.9 Hz), 5.49 (t, 2H, J=8.9 Hz), 8.06 (d, 2H, J=8.4 Hz), 8.50 (d, 1H, J=8.4 Hz); ^{13}C NMR (CD_3COOD–CF_3COOH, 1:6) δ 45.9, 51.9, 117.2, 130.9, 131.8, 134.2, 135.1, 140.6, 144.6, 146.6, 150.1, 159.1. Anal. Calcd for $C_{12}H_8N_4O_2{\cdot}H_2O$: C, 43.79; H, 3.06; N, 17.02. Found: C, 43.78; H, 2.56; N, 17.02.

7-(Trifluoromethyl)-1,10-ethyleneisoalloxazinium chloride (**1c**) was obtained as a bright yellow powder in 67% yield. mp 193–196°C; ^{1}H NMR δ (CD_3COOD–CF_3COOH, 1:6) 4.96 (t, 2H, J=8.9 Hz), 5.58 (t, 2H, J=8.9 Hz), 8.20 (d, 1H, J=8.8 Hz), 8.47 (d, 1H, J=8.8 Hz), 8.81 (s, 1H); ^{13}C NMR (CD_3COOD–CF_3COOH, 1:6) δ 46.2, 52.1, 122.6 (q, J=272.6 Hz, CF_3), 118.9, 131.4, 131.7, 133.9, 135.4 (q, J=35.5 Hz, C CF_3), 136.8, 141.1, 145.2, 146.4, 158.8.

4.6. Preparation of N-methylalloxan

According to a modification of established procedures,[41] potassium chlorate (5 g, 40.8 mmol) was added slowly to a 4.5N HCl solution (40 mL) of theobromine (Acros

Organics) (10 g, 55.5 mmol) over a period of 40 min at 50°C. After the mixture was stirred for 10 min, a conc. HCl (5 mL) solution of tin(II) chloride (6.5 g, 34.3 mmol) was added dropwise over a period of 10 min at 0°C. Then the solution was cooled to 5°C and the white solid was separated rapidly by filtration, washed with cold water followed by cold ether, and dried *in vacuo* to yield 6.1g (70%) of N-methylalloxantin. ^{1}H NMR (DMSO-d_6) δ 3.04 (s, 3H), 3.08 (s, 3H), 3.06 (s, 1H), 5.05 (s, 1H), 11.0 (s, 1H), 11.3 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 26.8, 27.5, 69.5, 111.3, 148.8, 150.9, 168.5, 169.3. A suspension of the N-methylalloxantin (6.0 g, 19.1 mmol) in water (9 mL) was treated at 45°C with concentrated nitric acid dropwise until the solid dissolved. The solution was kept in a desiccator over sulfuric acid until the product crystallized. The N-methylalloxan separated as fine white crystals (4.8 g, 72%). mp 150–151°C; ^{1}H NMR (DMSO-d_6) δ 3.06 (s, 3H, $-CH_3$[hydrate form]), 3.12 (s, 3H, $-CH_3$[keto form]), 11.4 (s, 1H, –NH[hydrate form]), 11.9 (s, 1H,– NH[keto form]); ^{13}C NMR (DMSO-d_6) (signals from both hydrate and keto forms) δ 27.5, 85.2, 150.1, 155.8, 156.9, 165.7, 168.0, 168.9. Direct isolation of N-methylalloxan from oxidation of theobromine (bypassing N-methylalloxantin) was unsuccessful.

4.7. Preparation of 3-methyl-7-(trifluoromethyl)-1,10-ethyleneisoalloxazinium chloride (1d)

The same procedure described above for **1a–c** was followed except for the used of N-methylalloxan instead of alloxan. In this case the intermediate 3-methyl-7-(trifluoromethyl)-10-(2-hydroxyethyl)isoalloxazine generated prior to $SOCl_2$-mediated cyclization was characterized: mp 234–235.5°C; ^{1}H NMR (CD_3COOD–CF_3COOH, 1:6) δ 3.51 (s, 3H), 4.42 (t, 2H, J=4.7 Hz), 5.21 (t, 2H, J=4.7 Hz), 8.38 (2H), 8.66 (s, 1H); ^{13}C NMR (CD_3COOD–CF_3COOH, 1:6) δ 29.1, 51.5, 60.1, 119.5, 123.0 (q, J[^{19}F,^{13}C]=272.6 Hz, CF_3), 131.3, 135.1, 133.7 (q, J[^{19}F,^{13}C]=35.5 Hz, C-7), 134.0, 135.7, 139.0, 145.2, 151.6, 159.3. Following cyclization and recrystallization, **1d** was obtained in 60% yield: mp 186–189°C; λ_{max} (CH_3CN) 336, 388 nm; ^{1}H NMR (CD_3COOD–CF_3COOH, 1:6) δ 3.48 (s, 3H), 4.91 (t, 2H, J=9.1 Hz), 5.46 (t, 2H, J=9.1 Hz), 8.11 (d, 1H, J=8.8 Hz), 8.39 (d, 1H, J=8.8 Hz), 8.73 (s, 1H); ^{13}C NMR (CD_3COOD–CF_3COOH, 1:6) δ 29.5, 46.9, 51.7, 118.8, 131.4, 136.4, 122.8 (q, J[^{19}F,^{13}C]=272.5 Hz, CF_3), 131.8, 133.6, 134.9 (q, J[^{19}F,^{13}C]=35.3 Hz, C-7), 141.1, 144.0, 146.8, 159.0; HRMS (FAB) m/z calcd for $C_{14}H_{10}N_4O_2F_3$ 323.0756, found 323.0751. A sample of the chloride salt **1d** was converted to the perchlorate salt for purposes of elemental analysis. Anal. Calcd for $C_{14}H_{10}ClF_3N_4O_6\cdot H_2O$: C, 38.15; H, 2.74; N, 12.71. Found: C, 37.67; H, 2.63; N, 12.65.

4.8. Preparation of substituted 1,10-ethano-5-acetyl-1,5-dihydrolumiflavins (6a–c)

According to a published procedure,[12] the isoalloxazinium salts **1a–c** were allowed to react with Zn (0.27 g per mmol) and $(CH_3CO)_2O$ (5.9 g per mmol) in CF_3COOH solvent with heating at reflux for 1 h (**1a** and **1b**) or stirring at room temperature for 5 min (**1c** and **1d**). The inorganic material was filtered off, and the filtrates were evaporated to give light yellow oils, which were precipitated on addition of 5 mL of H_2O. The solids were collected, washed and dried to give light yellow solids.

1,10-Ethano-5-acetyl-1,5-dihydrolumiflavin (**6a**). 54% yield based on **1a**: mp 274–276°C, dec.; UV (DMSO) 256 nm (ε 9600 M^{-1} cm^{-1}), 294 nm (ε 7937 M^{-1} cm^{-1}); ^{1}H NMR (DMSO-d_6) δ 2.16 (s, 3H), 4.11 (4H), 6.96–7.49 (4H), 10.97 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 21.3, 43.3, 47.2, 91.6, 113.3, 122.7, 126.1, 126.4, 126.5, 136.6, 147.7, 152.3, 158.5, 171.7; EIMS m/z (relative intensity) 284 (M^+, 3.5), 242 (100), 171 (59), 143 (21), 129 (7); HRMS (EI) m/z calcd for $C_{14}H_{12}N_4O_3$ 284.0910, found 284.0909.

7-(Trifluoromethyl)-1,10-ethano-5-acetyl-1,5-dihydrolumiflavin (**6c**). 67% yield based on **1c**: mp 316–320°C, dec.; UV (DMSO) λ_{max} 258 nm (ε M^{-1} cm^{-1}), 306 nm, (ε 7571 M^{-1} cm^{-1}); ^{1}H NMR (DMSO-d_6) δ 2.20 (s, 3H, CH_3), 3.95–4.14 (4H, CH_2CH_2), 7.19–7.77 (3H), 11.09 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 21.1, 43.4, 47.3, 91.7, 113.9, 122.5 (q, J=36.3 Hz), 122.7, 123.6, 124.0 (q, J=272.6 Hz, CF_3), 126.7, 140.1, 147.7, 151.9, 158.4, 171.9; EIMS m/z (relative intensity) 353 ([M+1]$^+$, 0.3), 352 (M^+, 1.5), 324 (0.7), 310 (100); HRMS (EI) m/z calcd for $C_{15}H_{11}N_4O_3F_3$ 352.0784, found 352.0781.

8-Chloro-1,10-ethano-5-acetyl-1,5-dihydrolumiflavin (**6b**). 41% yield based on **1b**; the above typical procedure led to the production of the N^5-acetyl derivative (**6b**) as a mixture of two inseparable diastereomers in the ratio of 5:3. mp 304–310°C, dec.; UV (DMSO) λ_{max} 261 (ε 17,800 M^{-1} cm^{-1}), 310 nm (ε 9013); ^{1}H NMR (DMSO-d_6) δ 1.90 and 2.15 (s, 3H), 3.93–3.95 and 4.05–4.09 (4H), 7.00–7.67 (3H), 11.03 and 11.09 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 20.9 and 21.2, 43.3, 46.8 and 47.3, 90.2 and 91.8, 113.3 and 113.8, 122.2 and 122.5, 123.6 and 125.3, 124.7 and 127.3, 130.6 and 130.9, 136.6 and 138.1, 147.2 and 147.6, 149.3 and 151.9, 157.9 and 158.4, 171.8 and 172.2; EIMS m/z (relative intensity) 318 (M^+, 0.9), 276 (83), 242 (9), 204 (30), 177 (26), 143 (5); HRMS (EI) m/z calcd for $C_{14}H_{11}N_4O_3Cl$ 318.0520, found 318.0522.

4.9. Reaction of 8-chloro-1,10-ethyleneisoalloxazinium chloride (1b) with thiophenol

A solution of freshly distilled thiophenol (500 μM) and **1b** (50 μM) in CH_3CN was monitored by repetitive scan UV–Vis spectrophotometry in the range of 250–600 nm at 25°C.

4.10. Reduction of flavinium salt 1c under anaerobic conditions by dithionite, 1,3-propanedithiol, or hydrazine

To a suspension of **1c** (8.1 mg, 0.023 mmol) in 0.5 mL of deaerated CH_3CN was added under argon either $Na_2S_2O_4$ (48 mg, 0.023 mmol) in 80 μL of H_2O, or 1,3-propanedithiol (2.4 μL, 0.023 mmol) or anhydrous hydrazine (0.73 μL, 0.023 mmol) in 80 μL of CH_3CN. In either case, an instantaneous color change occurred from green to yellow. Without delay, the solutions were concentrated in vacuo to give a yellow residue, to which was added 0.5 mL of deaerated CF_3COOH and 0.6 mL of deaerated

acetic anhydride under argon (an instantaneous color change occurred from yellow to red and then again to yellow). After being stirred for 5 min at room temperature, the reaction mixture was directly evaporated under reduced pressure. The product was identical to N^5-acetyl derivative **6c** prepared above.

4.11. Preparation of substituted 1,10-ethano-1, 5-dihydrolumiflavins 8c and 8d

The reaction of flavinium salts **1c** or **1d** (35 mg) and N-benzyl-5,6-dihydrophenanthridine[21,42] (35 mg) were dissolved together in 1 mL of CH_3CN–CH_3OH (4:1) under argon. Following complete dissolution, a bright yellow solid emerged immediately. The solid was collected by centrifugation and washed with methanol to afford the reduced flavin.

7-Trifluoro-1,10-ethylene-1,5-dihydroisoalloxazine (**8c**). ^{1}H NMR (DMSO-d_6): 3.64 (t, 2H, J=7.4 Hz), 3.99 (t, 2H, J=7.4 Hz), 6.33 (d, 1H, J=8.0 Hz), 6.59 (s, 1H), 6.74 (d, 1H, J=8.0 Hz), 7.01 (s, 1H), 10.60 (s, 1H). HRMS (EI) m/z calcd for $C_{13}H_9F_3N_4O_2$ 310.0678, found 310.0674.

7-Trifluoro-3-methyl-1,10-ethylene-1,5-dihydroisoalloxazine (**8d**). ^{1}H NMR (DMSO-d_6): 3.06 (s, 3H), 3.64 (t, 2H, J=7.3 Hz), 4.04 (t, 2H, J=7.3 Hz), 6.33 (d, 1H, J=7.8 Hz), 6.62 (s, 1H), 6.73 (d, 1H, J=7.8 Hz), 7.02 (s, 1H). HRMS (EI) m/z calcd for $C_{14}H_{11}F_3N_4O_2$ 324.0834, found 324.0844.

4.12. Kinetics of phenylhydrazine reduction of flavinium salts 1a–c

The rates of reduction of flavinium salts by $PhNHNH_2$ were determined by measuring the decrease in the long wavelength flavin absorption (**1a**, 356 nm; **1b**, 368 nm; **1c**, 356 nm). The cuvettes contained a solution of 0.67 mM $PhNHNH_2$·HCl dissolved in 165 mM potassium phosphate buffer, and the reaction was initiated by adding 0.03 mL of a 0.05 mM solution of the flavinium salt in DMSO. Three different conditions were employed: (a) use of a tight-fitting Teflon stopper to retard continuous exposure to atmospheric O_2; (b) purging of solutions with argon prior to initiation of reaction, and continuous bubbling of argon (and intermittently air) from a needle inlet along the side of the cuvette; (c) use of an in situ 'O_2 scrubber' system, consisting of glucose (16 mM), 20 μg of glucose oxidase, and 3 μg of catalase per 3 mL reaction solution.

In order to verify that the lag time seen for $PhNHNH_2$ reduction of flavin **1a** was due to oxidative recycling of reduced flavin by the O_2 content of the sample, we repeated the experiment using periodic intentional bubbling of O_2 following initial complete degassing. The cuvette reaction content was established as above except that all stock solutions were degassed by bubbling argon. The reaction was monitored as usual at 356 nm under a controlled atmosphere condition using bubbling of air into the cuvette for 2 s at a flow rate of 5 mL/min at different time points (360, 990 and 3480 s).

4.13. Kinetics of oxidation of benzylamines by 7-CF_3 flavin 1c

The rates of oxidation of benzylamine and its N-methyl and N,N-dimethyl derivatives by **1c** were determined by following the decrease of the A_{370} (anion form of **1c**) in a closed cuvette. In a typical experiment, the cuvette contained a solution of amine/amine·HCl (10:1) dissolved in CH_3CN. The reactions were initiated by adding 30 μL of a 0.05 mM solution of **1c** in DMSO to 2.97 mL of 1 mM amine/amine·HCl (10:1), pre-equilibrated in a thermostatted holder at 50°C.

4.14. Product analysis of the oxidation of benzylamines

A mixture of flavinium salt **1a**, **1c** or **1d** (10 mM), benzylamine (55 mM) and benzylamine hydrochloride (5 mM) in dry CH_3CN (20 mL) was stirred at 50°C for 2 days in the dark under argon with exposure to air for 5 min after 6 h and again after 12 h. The reactions were quenched with 1N HCl. Benzaldehyde was removed by azeotropic distillation and quantified as its 2,4-dinitrophenylhydrazine derivative.

4.15. Preparation of tris(1,10-phenanthroline)iron(III) perchlorate monohydrate (TPIP)

$Fe(phen)_3(ClO_4)_3$ was prepared by the method of Schilt and Taylor.[43] Chlorine gas was introduced into a solution of $Fe(NH_4)_2(SO_4)_2 6{\cdot}H_2O$ (0.3 g, 0.76 mmol), phenanthroline monohydrate (0.6 g, 3.0 mmol), H_2SO_4 (0.2 mL) and H_2O (100 mL) until the color changed from red to light blue. NaOH (0.12 g) and 70% $HClO_4$ (0.43 g) were mixed together, the resulting concentrated $NaClO_4$ solution was added into the reaction solution, and the mixture was cooled in an ice bath. The blue crystalline product was washed three times with cold water and dried in vacuo at room temperature to give TPIP 0.40 g (30%). The IR spectrum was identical to that reported.[43]

4.16. Cyclopropylamines

N-Cyclopropylbenzylamine was prepared[44] by $NaBH_4$ reduction of the Schiff base formed from benzaldehyde and cyclopropylamine. N-Benzylidenecyclopropylamine: ^{1}H NMR ($CDCl_3$) δ 0.99 (m, 2H), 1.01 (m, 2H), 3.06 (m, 1H), 7.44 (3H), 7.76 (m, 2H), 8.46 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 9.06, 42.1, 127.7, 128.7, 130.2, 136.7, 158.4. N-Cyclopropylbenzylamine: ^{1}H NMR ($CDCl_3$) δ 0.49 (m, 4H), 1.88 (s, 1H, NH), 2.21 (m, 1H), 3.90 (s, 2H), 7.38 (5H); ^{13}C NMR ($CDCl_3$) δ 6.64, 30.2, 53.9, 126.9, 128.3, 128.5, 140.8. N-Cyclopropyl-1-phenethylamine[45] was also prepared in the same way, except with acetophenone. N-1-Phenethylidenecyclopropylamine: ^{1}H NMR (CD_3CN) δ 0.84 (m, 2H), 0.94 (m, 2H), 2.32 (s, 3H), 3.17 (m, 1H), 7.37 (3H), 7.78 (m, 2H); ^{13}C NMR (CD_3CN) δ 9.61, 15.8, 34.7, 127.2, 129.0, 129.9, 142.1, 163.5. N-Cyclopropyl-1-phenethylamine: ^{1}H NMR ($CDCl_3$) δ 0.39 (m, 4H), 1.42 (d, 3H, J=6.7 Hz), 1.83 (s, 1H, NH), 2.01 (m, 1H), 3.90 (q, 1H, J=6.7 Hz), 7.35 (5H); ^{13}C NMR ($CDCl_3$) δ 6.58, 23.8, 29.0, 58.5, 126.7, 126.9, 128.4, 146.2. 1-(Phenylcyclopropyl)-methylamine was prepared by $LiAlH_4$ reduction of 1-phenylcyclopropanecarbonitrile by the reported method.[46]

4.17. Product analysis of the oxidation of benzylamines and cyclopropylamines by TPIP

To a solution of $Fe(phen)_3(ClO_4)_3$ (56 mM) in dry CH_3CN (10 mL) at 25°C was added the amine (28 mM). After being stirred for 1 h, the reaction mixture was acidified with 1 mL of 2,4-dinitrophenylhdyrazine solution (144 mM in a 3:4:14 mixture of H_2SO_4, H_2O, and EtOH) and concentrated under reduced pressure. Flash chromatography (3×3 cm SiO_2, CH_2Cl_2 or EtOAc/$CHCl_3$, 1:2) afforded the desired 2,4-dinitrophenylhydrazones. Quantitative aldehyde/ketone analyses were carried out by dissolving the hydrazones and a known weight of hexamethylbenzene in DMSO-d_6 and calculating the molar ratio using ^{1}H NMR integrals. In some cases, the 2,4-dinitrophenylhydrazones were collected, concentrated, dried and weighed. The nature of the hydrazone derivatives were confirmed by their independent synthesis. The 2,4-dinitrophenylhydrazone of 1-phenylcyclopropanecarboxaldehyde[47] had ^{1}H NMR (DMSO-d_6) δ 1.33 (m, 2H), 1.44 (m, 2H), 7.31–7.39 (5H), 7.62 (d, 1H, J=9.4 Hz), 8.18 (s, 1H), 8.29 (d, 1H, J=9.4 Hz), 8.83 (s, 1H), 11.4 (s, 1H).

4.18. Anaerobic reactions of 1c with hydrazines, amines, and thiols monitored by ^{1}H NMR spectroscopy

Stock solutions of **1c** (40–80 mM final concentration) in degassed DMSO-d_6 and of the hydrazine, amine, or thiol in either degassed DMSO-d_6 or CD_3CN (40–80 mM final concentration) were added to an NMR tube containing degassed DMSO-d_6 or CD_3CN under argon at 25°C, and the spectra were recorded at various time intervals. The reaction of **1c** with 1,3-propanedithiol was also followed by ^{1}H NMR spectroscopy in D_2O.

Acknowledgements

We are grateful for support of this research by the National Institutes of Health through grant GM 48812. We also thank Professor Fred Urbach and his group for use of their electrochemistry equipment and for helpful advice.

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