

## THE SYNTHESIS OF TWO STRONGLY ELECTRON DEFICIENT FLAVIN ANALOGS

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**Abstract** - The synthesis of 7-chloro-8-acetyl-10-methylisoalloxazine (2a) and 7-cyano-8-acetyl-10-methylisoalloxazine (2b) was accomplished by the condensation of appropriately substituted *o*-diamines with alloxan under acidic conditions. The presence of the strongly electron withdrawing chlorine and cyano substituents makes these flavin analogs very effective catalysts of the oxidation of *N*-alkyl-1,4-dihydronicotinamides. Compound 2a was also found to undergo redox reactions with substrates readily forming carbanions (e.g., nitroethane).

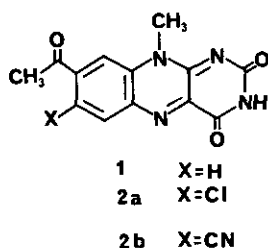
## INTRODUCTION

A major objective of our research has been the design of enzyme-like catalysts capable of carrying out organic transformations with the efficiency and stereospecificity of their naturally occurring counterparts.<sup>1,2,3</sup> Our approach to the creation of "semisynthetic enzymes" is based on the covalent modification of a residue in an existing enzyme active site with a reactive coenzyme analog. This strategy has the goal of producing a new catalytic entity possessing substrate specificity related to that of the parent enzyme and showing the characteristic chemical reactivity of the modifying coenzyme analog.

To carry out this "chemical mutation" of an enzymic active site we have concentrated our efforts on flavin analogs, isoalloxazines, because of their intrinsic catalytic versatility, useful spectral properties and the possibility of manipulating their redox potential by incorporating electronic withdrawing or donating substituents into the phenyl ring portion of the tricyclic flavin nucleus. A number of semisynthetic flavoenzymes have been generated by the alkylation of the Cys-25 residue in the papain active site and Cys-149 in the active site of glyceraldehyde-3-phosphate dehydrogenase with 6 $\alpha$ -, 7 $\alpha$ -, or 8 $\alpha$ -bromoacetyl-10-methylisoalloxazines.

Such "chemical mutation" of the papain active site with 8-acetyl-10-methylisoalloxazine (1) has produced the first example of a semisynthetic oxidase fully functional in all catalytic steps in the oxidation of *N*-alkyl-1,4-dihydronicotinamides. Furthermore, its catalytic efficiency is

exceeded only by the most effective naturally occurring counterparts.<sup>4</sup> More recently, our strategy applied to the glyceraldehyde-3-phosphate dehydrogenase template produced a catalyst with new substrate specificity and exhibiting a high degree of chiral discrimination toward its NADH substrate.<sup>5</sup> In order to expand the repertoire of organic redox transformations mediated by our semisynthetic flavoenzymes we decided to synthesize flavin analogs possessing enhanced oxidation potential by functionalizing the isoalloxazine system with additional electronegative groups. Herein we report the successful preparation and the properties of 7-chloro-8-acetyl-10-methylisoalloxazine (2a) and 7-cyano-8-acetyl-10-methylisoalloxazine (2b).

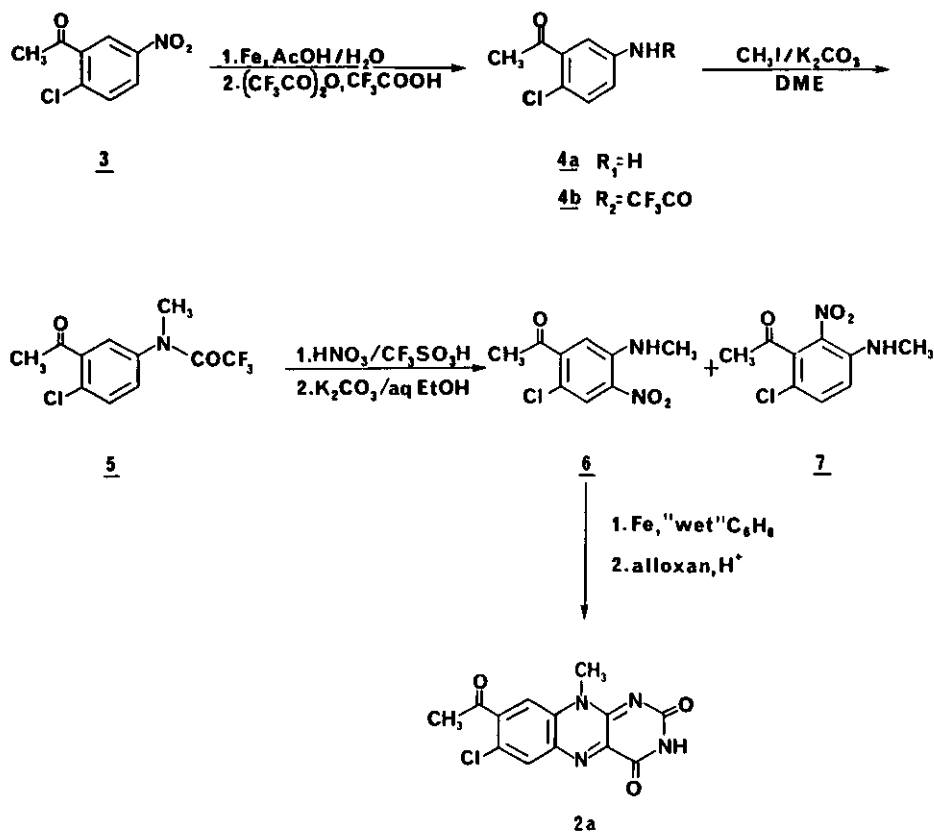


Only a few examples of strongly electron deficient isoalloxazines are known<sup>6,7</sup> notwithstanding the potentially interesting chemistry which these systems offer. In devising our synthetic route to 2a and 2b we could not take advantage of the methodology first developed by Yoneda<sup>8</sup> because of the expected low nucleophilicity of the requisite *N*-methylanilines. Therefore, we faced the rather difficult task of preparing the phenyl ring system derivatized with four different substituents in predetermined orientations.

## RESULTS AND DISCUSSION

Our route for the synthesis of 7-chloro-8-acetyl-10-methylisoalloxazine (2a) begins with 2-chloro-5-nitroacetophenone (3)<sup>9</sup> and follows the sequence of steps outlined in Scheme I. Reduction of the nitro group was achieved by treatment with activated iron in hot aqueous acetic acid to afford the amine 4a as a yellow oil, which could not be purified by vacuum distillation because of acidic contamination leading to decomposition. Reaction of 4a with trifluoroacetic anhydride in trifluoroacetic acid (TFA) provided 4b, purified, as most of our compounds, by vacuum sublimation. Amide 4a was alkylated in excellent yield on treatment with methyl iodide in dimethoxyethane in the presence of dry K<sub>2</sub>CO<sub>3</sub> to furnish 5. Nitration of 5 to obtain 2-chloro-4-nitro-5-(*N*-methylamino)acetophenone (6) required use of a mixture of fuming nitric acid with trifluoromethanesulfonic acid at -20°C. It is interesting to note that other nitrating mixtures led to the formation of 2-chloro-6-nitro-5-(*N*-methylamino)acetophenone (7) as the predominant product. The conditions employed in this work gave 6 and 7 in roughly a 1:1 ratio with a 63% overall yield. The synthesis of 2a was then completed as follows: reduction of the nitro group in 6 by

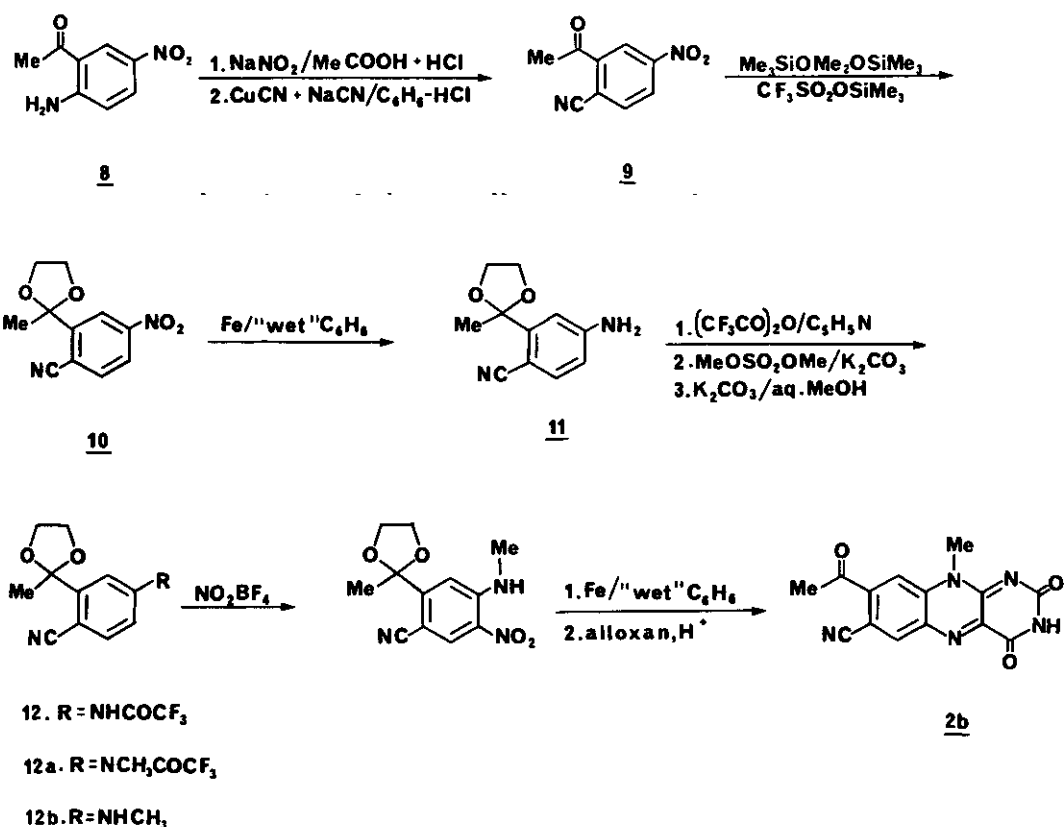
activated iron in refluxing "wet" benzene provided the unstable o-diamine used directly in the acid catalyzed condensation reaction with alloxan to give the target compound in 55% yield.



Scheme I

Scheme II depicts the reactions leading to the formation of 7-cyano-8-acetyl-10-methylisoalloxazine (2b). 2-Amino-5-nitroacetophenone (8) was prepared according to the literature procedure<sup>10</sup> and converted to nitrile 9 by the Sandmeyer reaction. Reduction of the nitro functionality in 9 under a variety of conditions always resulted in several side products. We reasoned that the presence of the other electron withdrawing substituents in the ring could be a contributing factor and decided to mask the carbonyl group. While acid catalyzed ketalization with ethylene glycol gave poor yields of 10, treatment of 9 with bis-trimethylsilyloxyethane in presence of a catalytic amount of trimethylsilyl triflate<sup>11</sup> furnished the desired compound 10 in excellent yield.

Reduction of 10 with activated iron in "wet" benzene proceeded satisfactorily to give 11 which upon methylation and trifluoroacetylation provided 12a. Unfortunately, since this compound did not survive the nitration conditions employed to nitrate 5 and a number of other acidic nitrating mixtures proved too harsh, we decided to focus on the use of the highly reactive nitronium tetrafluoroborate. When 12b was exposed to one equivalent of nitronium tetrafluoroborate for 15 min, the nitro derivative 13 was obtained as a major product along with unreacted starting material and several byproducts. Longer reaction times and higher temperatures led to intractable mixtures. The reduction of 13 followed by condensation with alloxan provided the target compound 7-cyano-8-acetyl-10-methylisoalloxazine (2b).



Scheme II

The introduction of electronegative substituents in addition to the acetyl function substantially increases the reactivity of flavin analogs toward *N*-alkyl-1,4-dihydronicotinamides (Table 1). It is interesting to note that the logarithms of the second order rate constants from Table 1 correlate very well with the corresponding  $\sigma_{\text{meta}}$  Hammett substituents constants. However, we

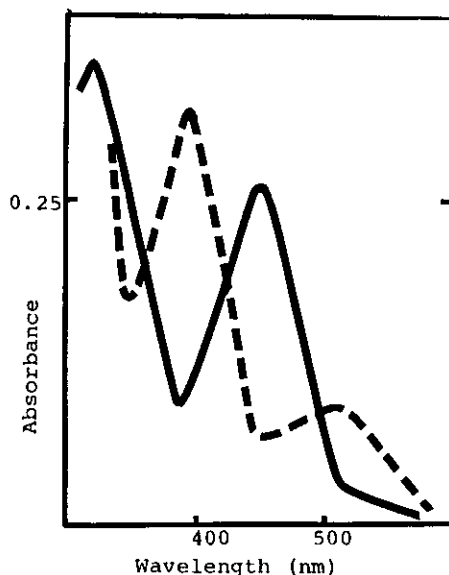
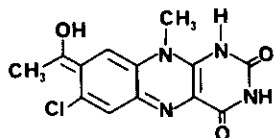
have too few experimental points to test the validity of a linear free energy relationship in this case.

Table 1<sup>a</sup>

| Catalyst                                               | $k_2 (M^{-1} s^{-1})$   |                   |
|--------------------------------------------------------|-------------------------|-------------------|
|                                                        | 1,4-dihydronicotinamide |                   |
|                                                        | <u>N</u> -benzyl-,      | <u>N</u> -propyl- |
| 8-Acetyl-10-methylisoalloxazine                        | 170                     | 880               |
| 7-Chloro-8-acetyl-10-methylisoalloxazine ( <u>2a</u> ) | 485                     | 2700              |
| 7-Cyano-8-acetyl-10-methylisoalloxazine ( <u>2b</u> )  | 920                     | 4900              |

<sup>a</sup> Rates were determined spectrophotometrically for reactions carried out under aerobic conditions in presence of superoxide dismutase at pH 7.5 (pH 6 for 2b)

Under anaerobic conditions at pH 8.5 isoalloxazine (2a) is easily reduced by nitroethane and diethylaminomalonate. An unusual spectrum was observed for the reduced isoalloxazine (Fig. 1) which exhibited a distinct absorption band with a  $\lambda_{max}$  around 500 nm (the solution turned red). We presume that the spectrum results from the presence of the enolic form 14 of the dihydroisoalloxazine<sup>12,13</sup>.



#### Legend

Figure 1. Spectra of the oxidized (solid line) and the reduced (broken line) form of 2a. An aqueous solution of diethylaminomalonate (10 mM, pH 8.5) was made anaerobic and a DMSO solution of 2a was added to a final concentration of 0.025 mM. Spectrum of reduced 2a was recorded after approximately 100 min.

#### EXPERIMENTAL

##### General

Proton magnetic resonance ( $^1\text{Hnmr}$ ) spectra were determined at 60 MHz (Varian HA-60A) 360 MHz (Nicolet 360) and 500 MHz (DS 1000, 500 MHz University of Chicago NMR spectrometer). Chemical shifts are expressed as  $\delta$  values relative to a TMS internal standard. Spectrophotometric determinations were performed on a Perkin-Elmer  $\lambda 5$  spectrophotometer. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectra were obtained on a Finnigan 1015 quadrupole mass spectrometer and VG analytical 70-250 EHF-GC/MS/DS spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee or by the Analytical Laboratory of The Rockefeller University.

##### Preparation of 7-Chloro-8-acetyl-10-methylisoalloxazine (2a)

##### 2-Chloro-5-nitroacetophenone (3)

This compound was prepared according to the procedure of Chapman et al.:<sup>9</sup> mp 61-62°C (lit.<sup>9</sup> 61°C) NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  8.41 (d, 1H), 8.24 (dd, 1H) 7.62 (d, 1H), 2.7 (s, 3H); Anal. Calcd.

for  $C_8H_6NO_3Cl$ : C, 48.14; H, 3.03; N, 7.02; Cl, 17.76. Found: C, 48.18; H, 3.22; N, 6.98; Cl, 18.00.

#### 2-Chloro-5-aminoacetophenone (4a)

Iron powder, 325 mesh (56 g), in a beaker was cooled using an ice bath and then 10 ml of conc. aq. HCl was added slowly with stirring. After the addition of HCl was completed, the activated iron powder was allowed to dry overnight in vacuo over  $P_2O_5$ . To 8.5 g (0.043 mol) of 2-chloro-5-nitroacetophenone (3), in 80 ml of a 1:1 glacial acetic acid/water mixture at 85–90°C was added 20 g of activated iron in three portions over a 15 min period. The reaction was allowed to proceed at the same temperature for 45 min with vigorous stirring, after which the mixture was diluted with 200 ml of water and extracted with 3 x 100 ml of benzene. The benzene solution was dried over anhydrous magnesium sulfate and concentrated in vacuo to yield 7 g of crude amine 4a which was used directly for the next reaction. A sample of analytically pure 4a was obtained by the reduction of 3 with activated iron in "wet" benzene.

#### 2-Chloro-5-(trifluoro)acetylaminacetophenone (4b)

To a solution of 2-chloro-5-aminoacetophenone (4a) (4.8 g, 0.028 mol) in  $CF_3COOH$  (40 ml) was added trifluoroacetic anhydride (4.9 g, 0.034 mol) at 4°C. The reaction mixture was stirred at 4°C for 30 min, then poured on ice, and the resulting solid was collected by suction filtration and washed with cold water. The product was purified by vacuum sublimation to yield 5.8 g (78%) of 4b, mp 108–108.5°C. NMR ( $CDCl_3$ , 500 MHz)  $\delta$  8.56 (s, 1H), 7.76 (d, 1H), 7.86 (dd, 1H), 7.46 (d, 1H), 2.69 (s, 3H); Anal. Calcd. for  $C_{10}H_7ClF_3NO_2$ : C, 45.22; H, 2.64; N, 5.27; Cl, 13.35. Found: C, 45.22; H, 2.69; N, 5.15; Cl, 13.54.

#### 2-Chloro-5-(N-methyl-N-trifluoro)acetylaminacetophenone (5)

A solution of 2-chloro-5-N-trifluoroacetylaminacetophenone (4b) (10.54 g, 0.04 mol) in 100 ml of dry dimethoxyethane (DME) was stirred with methyl iodide (5.3 ml, 0.085 mol) and dry potassium carbonate (30 g) at room temperature for 24 h. Potassium carbonate was removed by filtration and washed with ethyl ether. The DME and ethereal solutions were then combined and concentrated to give a yellow oil containing white crystals of inorganic salt. The crude product was dissolved in ethyl ether and filtered and upon concentration vacuum distillation yielded 10.7 g (96%) of a clear oil: bp 129–131°C (0.25 mm Hg), which solidified upon standing: mp 34–35°C.

#### 2-Chloro-4-nitro-5-(N-methylamino)acetophenone (6)

To a mixture of 90% fuming nitric acid (13 ml) and trifluoromethylsulfonic acid (5 ml) at –20°C was added 2-chloro-5-(N-methyl-N-trifluoroacetyl)aminacetophenone (5) (3.5 g, 0.0125 mol) in small portions over a 15 min period. The reaction mixture was stirred at –20°C for 2 h and then poured on ice to give a viscous oil. The yellow oil was extracted with ethyl ether, the ethereal solution was washed with brine and 0.2 M potassium carbonate, concentrated in vacuo, and the

resulting oil redissolved in ethanol (100 ml). Fifty ml of 0.2 M potassium carbonate solution was added, hydrolysis was carried out for 15 h at room temperature and the reaction mixture was extracted with chloroform. The chloroform extract was washed successively with brine, 0.2 M potassium carbonate, and 0.1 M sodium hydroxide until the aqueous phase was only lightly yellow. The chloroform solution was dried and concentrated to afford 1.7 g of a red solid material. Flash chromatography using benzene afforded 2-chloro-4-nitro-5-(N-methylamino)acetophenone (**6**) (0.82 g, 28%).

2-Chloro-6-nitro-5-(N-methylamino)acetophenone (**7**) was eluted from the column using ethyl acetate and upon crystallization from ethanol yielded 0.95 g (33%), mp 98-99°C. NMR for **6** (CDCl<sub>3</sub>, 500 MHz)  $\delta$ : 8.23 (s, 1H), 7.96 (bs, 1H), 6.89 (s, 1H), 3.03 (d, 3H), 2.64 (s, 3H); Anal. Calcd. for C<sub>9</sub>H<sub>9</sub>ClN<sub>2</sub>O<sub>3</sub>: C, 47.28; H, 3.97; N, 12.25; Cl, 15.51. Found: C, 47.13; H, 3.99; N, 12.13; Cl, 15.66.

NMR for **7** (CDCl<sub>3</sub>, 500 MHz)  $\delta$  8.11 (bs, 1H); 7.42 (d, 1H); 6.84 (d, 1H); 3.03 (d, 3H); 2.61 (s, 3H); Anal. Calcd. for C<sub>9</sub>H<sub>9</sub>ClN<sub>2</sub>O<sub>3</sub>: C, 47.28; H, 3.97; N, 12.25; Cl, 15.51. Found: C, 47.28; H, 4.12; N, 12.08; Cl, 15.35.

#### 7-Chloro-8-acetyl-10-methylisoalloxazine (2a)

2-Chloro-4-nitro-5-(N-methylamino)acetophenone (**6**) (0.75 g, 3.3 mmol) was dissolved in 250 ml of benzene and refluxed with 45 g of activated iron for 30 min. Thereafter, 0.05 ml of water was added, followed by 0.1 ml portions added from time to time, and the reaction was monitored by tlc (silica gel - chloroform). Upon completion of the reduction the hot reaction mixture was filtered and the iron washed with benzene and ethyl acetate. The washings were combined with the primary filtrate. Evaporation afforded the crude diamine, which without further purification was dissolved in 1 N aq. HCl solution (40 ml) saturated with N<sub>2</sub> and added to a solution of alloxan (0.68 g, 4.3 mmol) in 5 ml of 1 N aq. HCl. The reaction mixture was stirred at 75°C for 75 min. During that time a yellow solid precipitated. The reaction mixture was kept at 4°C overnight, and the yellow solid was collected by filtration and dried in vacuo at 60°C. The crude product was dissolved in 98% formic acid (50 ml) and purified by precipitation with ethyl ether (75 ml), giving 0.55 g of a yellow solid (55%).

NMR (DMSO-d<sub>6</sub>, 500 MHz)  $\delta$  11.5 (s, 1H), 8.29 (s, 1H), 8.13 (s, 1H), 3.97 (s, 3H), 2.71 (s, 3H); Anal. Calcd. for C<sub>13</sub>H<sub>9</sub>N<sub>4</sub>ClO<sub>3</sub>: C, 51.24; H, 2.98; N, 18.39; Cl, 11.63. Found: C, 51.02; H, 3.06; N, 18.00; Cl, 11.80. Uv-vis (H<sub>2</sub>O at pH 7.5): max at 445 nm ( $\epsilon = 9.8 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$ ), 335 nm ( $\epsilon = 7.5 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$ ), 269 nm ( $\epsilon = 27.8 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$ ).

Preparation of 7-Cyano-8-acetyl-10-methylisoalloxazine (2b)2-Amino-5-nitroacetophenone (8)

This compound was prepared according to the procedure of Simpson et al;<sup>10</sup> mp 152-153°C (lit.<sup>10</sup> 152-153°C). NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  8.71 (d, 1H), 8.13 (dd, 1H), 6.66 (d, 1H), 2.66 (s, 1H).

2-Cyano-5-nitroacetophenone (9)

A mixture of 2-amino-5-nitroacetophenone (8) (9 g, 50 mmol), 25 ml conc. aq. HCl, 50 ml of water and 35 ml of acetic acid was first brought to a boil and subsequently cooled to 5°C with continuous stirring, followed by the addition of sodium nitrite (54 mmol) in water (15 ml). After ca. 2 h an almost clear solution was obtained, which was carefully neutralized to pH 1 with aqueous sodium carbonate.

The solution of the diazonium salt was filtered and added in portions to a mixture of CuCN (4 g), NaCN (4.4 g), H<sub>2</sub>O (200 ml), and benzene (500 ml) which had been kept at ca. 10°C and stirred vigorously. After the addition of the diazonium salt was completed, the reaction mixture was placed in a water bath and kept at 65°C for 45 min and at room temperature overnight. The benzene layer was separated, washed successively with brine and 0.05 M aq. potassium carbonate. After evaporation of the benzene 7.4 g of crude nitrile was obtained. High vacuum sublimation (115°C, 0.05 mmHg) afforded 6.95 g (73%) of pure 2-cyano-5-nitroacetophenone, mp 130-131°C. NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  8.75 (d, 1H), 8.5 (dd, 1H), 8.05 (d, 1H), 2.8 (s, 3H); Anal. Calcd. for C<sub>9</sub>H<sub>6</sub>N<sub>2</sub>O<sub>3</sub>: C, 56.86; H, 3.16; N, 14.73. Found: C, 56.81; H, 3.30; N, 14.66.

1,3-Dioxolane-2-methyl-2-(2-cyano-5-nitrophenyl) (10)

To 330 mg (1.74 mmol) of 2-cyano-5-nitroacetophenone (9) in 4.2 ml of dry CH<sub>2</sub>Cl<sub>2</sub> at -78°C was added 0.013 ml (0.067 mmol) of trimethylsilyl triflate and 0.425 ml (1.74 mmol) of bis-trimethylsilyloxyethane under argon. Immediately, a precipitate was formed and the reaction mixture was warmed to 23°C and stirred for 48 h. The solution obtained after this period of time was taken up in methylene chloride, washed with 15 ml of saturated aq. sodium bicarbonate, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated to a pale white solid. High vacuum sublimation (100°C, 0.1 mmHg) gave 385 mg (95%) of pure ketal 10.

NMR (CDCl<sub>3</sub>, 360 MHz)  $\delta$  8.54 (d, 1H), 8.26 (dd, 1H), 7.91 (d, 1H), 4.17 (m, 2H), 3.85 (m, 2H), 1.81 (s, 3H). Anal. Calcd. for C<sub>11</sub>H<sub>10</sub>N<sub>2</sub>O<sub>4</sub>: C, 56.41; H, 4.30; N, 11.96. Found: C, 56.37; H, 4.37; N, 11.71.

1,3-Dioxolane-2-methyl-2-(2-cyano-5-aminophenyl) (11)

To 55 mg (0.24 mmol) of 10 in 15 ml of benzene was added 1 g of activated iron, and the mixture was refluxed for 30 min. Thereafter, 0.15 ml of water was added and reflux continued for 45 min. Then the mixture was cooled, filtered, and concentrated to a pale white solid. Vacuum sublimation of the crude product gave 40 mg (83%) of aminoketal 11. NMR (DMSO d<sub>6</sub>, 60 MHz)  $\delta$  7.2 (d,

1H), 6.86 (d, 1H), 6.51 (dd, 1H), 4.03 (m, 2H), 3.71 (m, 2H), 1.67 (s, 3H). Anal. Calcd. for  $C_{11}H_{12}N_2O_2$ : C, 64.71; H, 5.88; N, 13.72. Found: C, 64.50; H, 5.87; N, 13.87.

1,3-Dioxolane-2-methyl-2-[2-cyano-5-(N-trifluoroacetyl)phenyl] (12)

To 142 mg (0.7 mmol) of 11 in 1.7 ml of dry pyridine at 0°C was added 0.125 ml (186 mg, 0.885 mmol) of trifluoroacetic anhydride under argon. The solution was stirred for 30 min and then warmed to 23°C. The solution turned pale red. After 30 min at 23°C the reaction mixture was taken up in 30 ml of chloroform and washed with 20 ml of brine. The aqueous layer was reextracted with chloroform (2 x 20 ml) and ethyl acetate (2 x 20 ml). The organic layers were combined and dried (anhydrous  $MgSO_4$ ). After removal of the solvent 230 mg of crude product was obtained, which was purified by vacuum sublimation to give 200 mg (96%) of 12. NMR ( $CDCl_3$ , 360 MHz)  $\delta$  8.5 (s, 1H), 7.85 (dd, 1H), 7.75 (d, 1H), 4.14 (m, 2H), 3.82 (m, 2H), 7.77 (s, 3H); Anal. Calcd. for  $C_{13}H_{11}F_3N_2O_3$ : C, 52.00; H, 3.67; N, 9.33. Found: C, 52.43; H, 3.72; N, 9.23.

1,3-Dioxolane-2-methyl-2-[2-cyano-5-(N-methyl-(N-trifluoroacetyl)phenyl] (12a)

To 115 mg (0.38 mmol) of 12 in 2 ml of dry acetone were added 327 mg (2.36 mmol) of anhydrous potassium carbonate and 0.05 ml (67 mg, 0.53 mmol) of dimethyl sulfate. The mixture was stirred for 21 h, filtered, and the residue washed with acetone (2 x 10 ml) and concentrated to give 140 mg of a yellow oil. Crude material was used for the next reaction. For analytical purposes crude 12a was purified by vacuum sublimation which afforded a white solid. NMR ( $CDCl_3$ , 60 MHz)  $\delta$  7.8 (d, 1H), 7.6 (d, 1H), 7.31 (d, 1H): 4.1 (m, 2H), 3.83 (m, 2H), 3.43 (s, 3H), 1.81 (s, 3H). Anal. Calcd. for  $C_{14}H_{13}N_2O_3F_3$ : C, 53.50; H, 4.17; N, 8.92. Found: C, 53.88; H, 4.03; N, 8.87.

1,3-Dioxolane-2-methyl-2-[2-cyano-5-(N-methylamino)phenyl] (12b)

To 556 mg of crude 12a in 10.8 ml of a 1:1 methanol-water mixture was added 400 mg of potassium carbonate, and the reaction was allowed to proceed for 14 h under  $N_2$ . The mixture was then taken up in 50 ml of chloroform and washed with 20 ml of brine, the aqueous layer reextracted with chloroform (3 x 20 ml), and the organic layers combined. Vacuum sublimation of the crude product gave 340 mg of pure 12b. NMR ( $CDCl_3$ , 360 MHz)  $\delta$  7.49 (d, 1H), 6.8 (d, 1H), 6.48 (dd, 1H), 4.1 (m, 2H), 3.84 (m, 2H), 2.89 (s, 3H), 1.77 (s, 3H). Anal. Calcd. for  $C_{12}H_{14}N_2O_2$ : C, 66.05; H, 6.42; N, 12.84. Found: C, 65.12; H, 6.43; N, 12.45.

1,3-Dioxolane-2-methyl-2-[2-cyano-4-nitro-5-(N-methylamino)phenyl] (13)

To 64.8 mg (0.3 mmol) of 12b in 0.56 ml of dry sulfolane at 10°C was added 0.59 ml of a 0.5 M nitronium tetrafluoroborate solution in sulfolane, and the mixture was stirred for 15 min under nitrogen. A red color developed within a few minutes of the addition. The mixture was then diluted with 80 ml of ethyl ether, washed with water (4 x 20 ml), dried ( $Na_2SO_4$ ), and concentrated. The resulting brown oil was subjected to preparative thin-layer chromatography using 20% acetone in chloroform as the eluting solvent. Three bands were visible under UV illumination.

The uppermost band ( $R_f$  0.68, 42 mg) was found to contain among other products the desired nitro derivative, while the starting material was isolated as a slightly contaminated band with  $R_f$  0.63. After an initial purification of the uppermost band using the same solvent system, the mixture of compounds was finally separated with three passes of 10% ethyl acetate/benzene solvent to afford 124 mg of 13 in 23% yield (based on recovered starting material). NMR ( $CDCl_3$ , 360 MHz)  $\delta$  8.51 (s, 1H), 7.12 (s, 1H), 4.03 (m, 2H), 3.68 (m, 2H), 3.12 (d, 3H), 1.95 (s, 3H); Mass spectrum: 263 ( $M^+$ , 10%), 248 ( $M^+ - CH_3$ , 40%).

#### 7-Cyano-8-acetyl-10-methylisoalloxazine (2b)

To a solution of 238 mg (0.09 mmol) of 13 in 10 ml of benzene was added 1.27 g of activated iron and this mixture was refluxed for 30 min. After addition of 0.075 ml of water and refluxing for 1 h, an additional 0.44 g of iron and 0.05 ml of  $H_2O$  were introduced. No starting material was seen after 2.5 h. Iron was removed by filtration and washed with benzene (15 ml) and ethyl acetate (15 ml). Concentration of the combined washings afforded the crude diamine as a light blue oil. The diamine as a solution in ethanol (4.5 ml) was added to a solution of alloxan hydrate (15 mg) in degassed 1N aq. HCl (0.4 ml) and the reaction mixture stirred for 75 min at 65°C. During the course of reaction a precipitate was formed. Upon completion of the reaction, the mixture was cooled to 0°C and ethyl ether was added. Centrifugation, followed by washing the precipitate with ethanol (2 x 5 ml) and water (1 x 5 ml) afforded a dirty yellow solid. The crude product was dissolved in a minimum amount of 98% formic acid and reprecipitated with ether to afford 5.5 mg (20%) of 7-cyano-8-acetyl-10-methylisoalloxazine (2b) as a bright yellow powder. NMR ( $CF_3COOD$ , 360 MHz)  $\delta$  8.93 (s, 1H), 8.74 (s, 1H), 4.5 (s, 3H), 3.06 (s, 3H); High resolution mass spectrum: Calcd. for  $C_{14}H_9N_5O_3$ : 295.0705. Found: 295.0708. Uv-vis, maxima at 424 nm ( $\epsilon = 10.9 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$ ) and 274 nm ( $\epsilon = 36.2 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$  (pH 6). Repetitive scanning showed a rapid change in the uv-vis spectrum of 2b at pH 7.5.

#### Kinetics of the oxidation of dihydronicotinamides

The rates of aerobic oxidation of dihydronicotinamides by flavins 2a and 2b were determined by measuring the decrease in dihydronicotinamide absorption (around 360 nm). In a typical experiment 3.0 ml of buffer, pH 7.5, for 2a or pH 6 for 2b was added to a cuvette held in a thermostated cell holder at 25°C. Catalase (100  $\mu\text{g}$ ) and superoxide dismutase (10  $\mu\text{g}$ ) were added and the dihydronicotinamide was introduced as a methanolic solution. Reaction was initiated by the addition of the flavin as a solution in DMSO.

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