Thiourea-Enhanced Flavin Photooxidation of Benzyl Alcohol

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Larger Scale Preparation of 4,5-Dimethyl-1,2-dinitrobenzene (1)

The synthesis of 1,2-dinitrobenzene 1 started from 3,4-dimethylaniline 32. The simple nitration method (Scheme ESI-1, method i) was found unreliable, and the amino group was therefore protected by acetylation before the nitration took place (method ii). Nitration of acetonilide 33 described by Monge et al. (method iii) was too energetic and significant amounts of undesired 2,6-dinitro product were obtained. Milder method of Sugaya et al. (method iv) was therefore employed. 2-Nitroacetanilide 34 was then cleaved by hot sulphuric acid to yield the corresponding 2-nitroaniline 35 (method v) with overall yield 46%. Although the two-step method for the oxidation of 2-nitroaniline 35 via 1-nitro-2-nitroso intermediate 36 rendered fair results (methods vi–vii), it was not very elegant due to low solubility of the starting material and product in the aqueous reaction mixture. Far older one-step approach using hydrogen peroxide in acetic acid was therefore used with 46% yield of the dinitro product 1 (method viii).

![Scheme ESI-1](image)

**Scheme ESI-1.** Synthesis of 4,5-dimethyl-1,2-dinitrobenzene 1 from 3,4-dimethylaniline 32; Conditions: (i) 65% nitric acid, 98% sulphuric acid, < 0 °C, (ii) acetic anhydride, acetic acid, reflux, 15 min, (iii) 65% nitric acid, 98% sulphuric acid, < 0 °C, (iv) 65% nitric acid, acetic acid, 10–15 °C, 1 h, (v) 98% sulphuric acid, 100 °C, 25 min, (vi) potassium peroxodisulphate, sulphuric acid, water, room temp., (vii) 30% hydrogen peroxide, sulphuric acid, room temp., (viii) 30% hydrogen peroxide, acetic acid, 50 °C, 16 h. See text for yields.

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Reactivity of the electron-deficient dinitro derivative 1 towards nucleophilic aromatic substitution makes the introduction of the desired substituent possible. Although corresponding 2-fluoronitro derivative was the intermediate of choice in some cases, we found the ipso-substitutions using the dinitro derivative 1 quite straightforward and easy-going.

4,5-Dimethyl-2-nitroaniline (35)

3,4-Dimethylaniline 32 (20.0 g, 165 mmol) was dissolved in acetic acid (25 mL) and acetic anhydride (25 mL, 27 g, 0.26 mol, 1.6 eq.) was added. The reaction mixture was heated to reflux for 15 min, and then added dropwise to water (300 mL) with ice (ad necessitam) and the pink precipitate was filtered off using a Büchner funnel.

A mixture of 65% nitric (95 mL, 1.36 mol, 8.2 eq.) and acetic acid (35 mL) was cooled to 10–15 °C. The filtration cake from the previous step was dissolved in acetic acid (50 mL) and the solution was added dropwise to the mixture of acids. Once the addition was complete, the reaction mixture was stirred for additional 60 min at 10–20 °C. The reaction mixture was poured into water (ca. 1 L) and the resulting yellow precipitate was filtered off using a Büchner funnel, and washed well with water.

The filtration cake was dissolved in concentrated sulphuric acid (125 mL) and heated to ca. 90 °C for 20 min. The reaction mixture was allowed to cool to ambient temperature, added dropwise to ice (ad necessitam), the resulting orange precipitate was filtered off using a Büchner funnel and dried. Yield 12.7 g (46%).

The signals were assigned with the help of a NOESY experiment, and spectra of related N-substituted compounds were solved per analogiam. \(^1\)H NMR (CDCl\(_3\)) \(d = 2.14\ (s, 3\ H, CH_3-4), 2.18\ (s, 3\ H, CH_3-5), 6.54\ (s, 1\ H, H-6), 7.83\ ppm\ (s, 1\ H, H-3).\) EI-MS \(m/z\ (%)\): 166.1 (100) [M]**.

4,5-Dimethyl-1,2-dinitrobenzene (1)

4,5-Dimethyl-2-nitroaniline 35 (12.7 g, 76 mmol) was suspended in acetic acid (300 mL) and 30% aqueous hydrogen peroxide (60 mL, ca. 0.66 mol, 8.6 eq.) was added. The reaction mixture was heated to 45–55 °C for 16 h, and was added dropwise to water (750 mL) with ice

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(ad necessitam). The resulting orange precipitate was filtered off using a Büchner funnel, washed and dried. Yield 6.910 g (46%). $^1$H NMR (CDCl$_3$) δ = 2.42 (s, 6 H, CH$_3$-4,5), 7.68 ppm (s, 2 H, H-3,6). CI-MS m/z (%): 214.2 (100) [M + NH$_4$]$^+$ $\rightarrow$ 184.1 (15) [M + NH$_4$ – NO]$^+$*, 231.2 (43) [M + 2 NH$_3$ + H]$^+$.

**Alternative Observation Methods**

Apart from the direct observation of the catalytic cycle by $^1$H NMR spectroscopy, we have attempted to use other methods for the observation. However, their accuracy (colorimetric methods) or measurement costs (HPLC) did not exceed the observation by $^1$H NMR.

**Colorimetric Determination of Hydrogen Peroxide**

Colorimetric determination concentration by an iodometric method is based on the reactions

$$\text{H}_2\text{O}_2 + 2 \text{I}^- + 2 \text{H}^+ \rightarrow 2 \text{H}_2\text{O} + \text{I}_2$$

and enables indirect detection of hydrogen peroxide by the quantitative conversion to the triiodide anion and its detection by UV at 350 nm.\(^8\) Before analysis of the real samples, a calibration curve was constructed, using calibration solutions prepared from a stock solution whose concentration had been determined by titration with potassium permanganate. In these preliminary experiments, accuracy of this method seemed reasonable; however, when this method was applied to the analysis of the kinetic experiment, measurements exhibited significant experimental errors, presumably due to interaction with the matrix. Although the method indicated that (i) the oxidation of 4-methoxybenzyl alcohol works and that (ii) hydrogen peroxide is formed as a product of oxygen reduction, the results could not be used for the construction of the kinetic curves.

**Determination of Potassium Permanaganate Solution Concentration:** Potassium permanganate solution in water (ca. 0.1 M) was prepared and its concentration was exactly determined by titration by oxalic acid. Thus, oxalic acid dihydrate (ca. 400 mg) was exactly weighed and dissolved in 1 M sulphuric acid (25 mL). Ninety per cent of the expected volume of potassium permanganate solution was then added. The mixture was placed in 60 °C stirred water bath until fizzing and colour disappeared. The titration was then finished, reading the volume when first pink colour appeared. The titration was repeated five times in total,

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excluding one result as far-away, and calculating the potassium permanganate concentration as the average value from the remaining four titrations.

**Determination of Hydrogen Peroxide Concentration:** Commercial 30% hydrogen peroxide solution in water (30 mL) was dissolved in water (up to 1 L in a volumetric flask). Concentration of this dilute solution was determined by titration by potassium permanganate. The dilute hydrogen peroxide solution (10 mL) was pipetted and 1 M aqueous solution of sulphuric acid (25 mL) was added. The mixture was then titrated with the ca. 0.1 M potassium permanganate solution, reading the volume when first pink colour appeared.

**Calibration Curve:** Hydrogen peroxide solution (1.49 mM) was prepared from the dilute hydrogen peroxide solution mentioned above. Seventeen solutions (concentration 0–1.49 mM) were prepared and analysed by the Standard Iodometry Procedure (vide infra). Calibration curve was constructed by plotting corrected absorbance \( A_{corr} = A_{sample} - A_{blank} \) versus concentration of hydrogen peroxide before analysis, and fitting the experimental points by a linear which passes through the intersect. Slope of the curve was 701. Concentration of hydrogen peroxide in an unknown sample analysed by the Standard Iodometry Procedure can be therefore calculated according to the following equation:

\[
 c(H_2O_2) = \frac{A_{sample} - A_{blank}}{701} \text{ mol dm}^{-3}.
\]

**Standard Iodometry Procedure:** Sample of unknown hydrogen peroxide concentration (50 µL) was pipetted into a quartz cuvette (optical path 10 mm). Potassium iodide solution (1 M in water, 600 µL) and acetic acid (15% w/w in water, 1,500 µL) was added. The reaction mixture was placed in a stirred 60 °C water bath for 10 min. UV/VIS spectrum of the mixture was recorded and absorbance at 350 nm \( A_{sample} \) was determined. Blank experiment containing acetonitrile (50 µL) instead of the sample of unknown hydrogen peroxide content was analysed in the same way \( A_{blank} \), and concentration of hydrogen peroxide was calculated as described above.

**Determination of 4-Methoxybenzaldehyde**

This colorimetric method is based on the reaction of 4-methoxybenzaldehyde with 2,4-dinitrophenyl hydrazine (Brady’s Reagent) which leads to an intensively red-coloured hydrazone (Scheme ESI-2) and is detected by UV spectroscopy.\(^9\)

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Maximum of the absorption is located at ca. 450 nm. The method suffers from two drawbacks. Firstly, the difference between the absorption of a blank sample containing no 4-methoxybenzaldehyde and treated in the same way and real samples is quite small at the concentrations required, and secondly the absorption maximum red-shifts with increasing concentration of the aldehyde and overlaps with the absorbance of flavin, making correct evaluation difficult. Before the measurement of real samples, an exponential calibration curve was constructed. Again, accuracy of the method seemed reasonable in these preliminary experiments, but with real samples, interaction with the matrix caused significant experimental errors, leading to unreproducible results.

**Construction of the Calibration Curve:** Fresh 2 mM solution of Brady’s Reagent in a mixture of acetonitrile, methanol and concentrated hydrochloric acid (6:3:1 v/v/v) was prepared. Twenty-one solutions of 4-methoxybenzaldehyde were prepared, covering the range 0 to $2 \times 10^{-3}$ M in $10^{-4}$ M increments. The solutions were then analysed using the Standard Procedure with Brady’s Reagent (*vide infra*). Calibration curve was constructed by plotting the absorbance against the concentration of benzaldehyde, and experimental points were fitted by an exponential curve, which had the equation

$$A = c^{0.055985x-0.69037}$$
where $A$ is the maximum of absorbance at ca. 450 nm and $c$ is the concentration of benzaldehyde in the original solution, expressed in $10^{-4}$ M.

**Standard Procedure with Brady’s Reagent:** Sample (50 µL) of 4-methoxybenzaldehyde solution of unknown concentration was pipetted into a vial. Fresh 2 mM solution of Brady’s Reagent in a mixture of acetonitrile, methanol and concentrated hydrochloric acid (6:3:1 v/v/v, 50 µL), and acetonitrile (1,900 µL) were added. The mixture was allowed to stand at ambient temperature for 5 min. Solution of sodium hydroxide (0.16 M, 500 µL) was then added. Resulting pink to red solution was transferred into a disposable plastic UV/VIS cuvette.\(^\text{10}\) UV/VIS spectrum was recorded, and maximal absorbance at ca. 450 nm was used for the calculation of benzaldehyde concentration according to the formula

$$c = \ln \left( \frac{A + 0.69037}{0.055895} \right)$$

where $c$ and $A$ have the same meaning as above. It was absolutely necessary to prepare the solution of Brady’s Reagent fresh every day or even better twice a day. Quality of the Reagent rapidly deteriorated upon standing, biasing the results even more.

**HPLC Measurements**

Although the observation of the course of the kinetic experiments by high-performance liquid chromatography (HPLC) measurements represents a very accurate method, the delay times between the experiment in question and results and also the price made it somewhat unpractical. It was used for the confirmation of the key results obtained by \(^1\text{H}\) NMR spectroscopy.

The HPLC measurement were carried out on an Agilent 1100 LC system using a Phenomenex Luna C18 column (150 mm length, 4.6 mm diameter, 3 µm particle size) at 25 °C. Injection volume was 10 µL. A mixture of water and acetonitrile (15→95% acetonitrile in 30 min, then 5 min 95% acetonitrile) was used as a mobile phase. 4-Methoxybenzyl alcohol and 4-methoxybenzaldehyde were detected at 220, 225 and 260 nm using a diode array detector. Naphthalene was used as an internal standard to allow for quantitative evaluation of the chromatograms.

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\(^{10}\) The disposable cuvettes have significant absorption under 350 nm, but no absorption in the range important for this analysis.
Kinetic Experiments

Figure ESI-1 shows the course of selected kinetic experiments with flavin--thiourea photocatalyst or with stoichiometric mixtures of simple flavin molecules and thiourea.

Figure ESI-1. Flavin-mediated photo-oxidation of 4-methoxybenzyl alcohol to 4-methoxybenzaldehyde. Conditions: Initial concentration of 4-methoxybenzyl alcohol $2 \times 10^{-3}$ M, concentration of flavin sensitiser $2 \times 10^{-4}$ M. ‘Thiourea’ denotes the addition of thiourea to the reaction mixture ($2 \times 10^{-4}$ M). The conversion was calculated from the ratio of areas under the aromatic signals in $^1$H NMR spectra recorded during the experiment.
**Cyclic Voltammetry I**

Reduction potential of simple flavin 30 and flavin–thiourea 16 were measured by cyclic voltammetry and compared (Figure ESI-2). Reduction potential of the thiourea-containing flavin 16 is shifted by +90 mV. The experiment was measured using the conditions described in the Experimental Section.

Cyclic voltammograms of flavin-thiourea 16 (solid line) and 10-glycol flavin 30 (dashed line). See Experimental Section for the conditions of the measurement.
Cyclic Voltammetry II

Half-wave reduction or oxidation potentials were determined by cyclic voltammetry (Table ESI-1) as described in the Experimental Section. $\Delta G$ energy of various possible redox processes (Table ESI-2) was calculated using the Rehm–Weller equation\textsuperscript{11,12}

$$\Delta G = 96.4 \left( E_{1/2}^{\text{ox}} - E_{1/2}^{\text{red}} \right) - \frac{e^2}{\varepsilon \times a} - E^{O-O}$$

using typical values for the Coulombic ($e^2/(ea) = 5.4 \text{ kJ/mol}$) and flavin excitation term ($E^{O-O} = 241 \text{ kJ/mol}$, neglecting entropy changes from the ground to the excited state (Table ESI-2)).

| Table ESI-1. Redox potentials of species participating in the catalytic cycle |
|-----------------------------|-----------------------------|-----------------------------|
| **Redox process**           | **E$_{1/2}$ vs. SCE [V]**   | **E$_{1/2}$ vs. Fc/Fc$^+$ [V]** |
| 4-Methoxybenzylalcohol ox.  | +1.547                      | +1.086                      |
| Flavin 30 red.              | -0.717                      | -1.178                      |
| Fl$_{\text{red}}$ 30 ox.    | -1.234                      | -1.695                      |
| Oxygen red.                 | -0.923                      | -1.384                      |
| Thiourea ox.                | +0.800                      | +0.339                      |
| Thiourea red.               | -0.720                      | -1.181                      |

| Table ESI-2. $\Delta G$ of redox processes |
|-----------------------------|-----------------------------|-----------------------------|
| **? G [kJ/mol]**            | **Oxidised species**        | **Fl$_{\text{red}}$** |
|                            | Alcohol | Thiourea |                        |
| Reduced species             | Flavin  | 212      | 141                      |
|                            | Flavin* | -29      | -100                     |
|                            | Thiourea| 213      | -55                      |
|                            | Oxygen  | 232      | 160                      |


Determination of Quantum Yields

The quantum yields were measured using ferrioxalate actinometry.\(^{13}\)
Iron(III) sulphate solution (500 µL, 0.3 M in water) and potassium oxalate solution (500 µL, 1.5 M in water) were pipetted into an NMR tube. The reaction mixture was irradiated for 1 min. An aliquot (100 µL) of this solution was pipetted into a 10 mL volumetric flask, 1,10-phenanthroline solution (2 mL, 2% w/w) and buffer solution (1 mL, solution obtained from 0.82 g of sodium acetate trihydrate and 0.1 mL of conc. sulphuric acid, which were diluted up to 10 mL in a volumetric flask). Absorbance at 510 nm was measured. A blank experiment - avoiding irradiation – was carried out.
Intensity of the light source was calculated according to the formula \( I \) (einstein/min) =
\((\text{absorbance sample} - \text{absorbance blank}) \times \text{irradiated volume (L)} \times \text{volume of flask used for the work-up (mL)} / \text{extinction coefficient of the iron(ii)-phenanthroline complex} / \text{path length (cm)} / \text{quantum yield of the actinometry} / \text{time (min)} / \text{taken aliquot (mL)}.\)
Quantum yields were then calculated from the conversion after the time given in the table and the amount of photons emitted by the LED into the reaction mixture. In most cases the reactions exhibited a near-zero-order behaviour and calculation from initial rates was not necessary.

Reported quantum yields of other flavin-mediated photoreactions for comparison:

\[ \text{?} \]
\[ \text{Natural photolyases:}^{14} 0.7 – 0.9 \]
\[ \text{Intramolecular cleavage of an oxetane ring:}^{15} 0.11 \]
\[ \text{Intramolecular cleavage of uracil or thymine dimers:}^{16} 0.005 – 0.1 \]

\(^{13}\) Murov in Handbook of Photochemistry, Marcel Dekker: New York, 1973