Reduced Molecular Flavins as Single-Electron Reductants after Photo-Excitation

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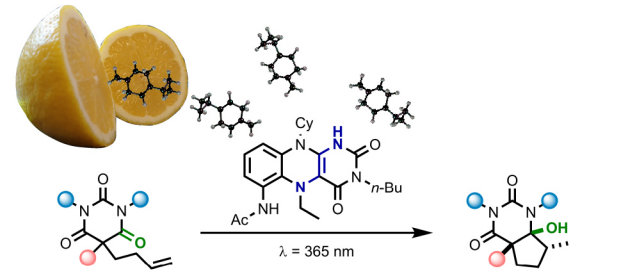


• Air-Stable Reduced Flavin Catalyst • γ-Terpinene Reductant
• Mimicking DNA Photolyase Activity

Abstract

Flavoenzymes mediate a multitude of chemical reactions and are catalytically active both in different oxidation states and in covalent adducts with reagents. The transfer of such reactivity to the organic laboratory using simplified molecular flavins is highly desirable and such applications in (photo-)oxidation reactions are already established. However, molecular flavins have not been used for the reduction of organic substrates yet, although this activity is known and well-studied for DNA photolyase enzymes. We report a catalytic method using reduced, molecular flavins as photo-reductants and γ-terpinene as sacrificial reductant. Additionally, we present our design for air-stable, reduced flavin catalysts, which is based on a conformational bias strategy and circumvents the otherwise rapid reduction of O_2 from air. Using our catalytic strategy, we were able to replace super-stoichiometric amounts of the rare-earth reductant SmI_2 in a 5-*exo*-trig cyclization of substituted barbituric acid derivatives. Such flavin-catalyzed reductions are anticipated to be of broad applicability and their straightforward synthesis indicates future use in stereo- as well as site-selective transformations.

Main Text

Flavins are versatile cofactors in enzymes and are involved in a variety of chemical transformations either as flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN).[1] This diversity stems from the occurrence of different catalytically active cofactor states and depends on oxidation or reduction (FAD *vs*. $FADH_2$) as well as photochemical excitation. Among the known reactions of flavoenzymes, the cleavage of thymine dimers **1** by DNA photolyase

is a particularly interesting and relevant reaction, which is mediated by excited, hydroquinoid FADH^{-*} (**2**$^{-*}$). Thymine dimers (T<>T) **1** are the result of UV-light mediated [2+2]-cycloaddition reaction between adjacent thymines in DNA and their formation hampers correct transcription or replication. The enzymatic strategy to revert such DNA damage relies on the excitation of the reduced cofactor **2**$^{-}$ and subsequent one-electron reduction of (T<>T) **1** (Figure 1).[2] The ketyl radical intermediate **1**$^{\bullet-}$ undergoes rapid cyclobutane ring-opening and back electron transfer to the semiquinone **2**$^{\bullet}$, which leads to the release of both thymines and closes the catalytic cycle. The very negative redox potential E (**1**/**1**$^{\bullet-}$) = –2.2 V *vs.* SCE (in CH_3CN) of thymine dimer **1** highlights the strong reducing capacity of excited cofactor **2**$^{-*}$.[3] Despite the great potential for synthetic transformations, such one-electron reductions have not yet been applied using molecular flavin catalysts in the organic laboratory, although the photosensitized[4] and oxidative[5] cleavage of thymine dimers with molecular flavins has been studied. The general usefulness of reduced flavin catalysts is highlighted by recent reductive catalytic reactions with flavoenzymes[6] and a deazaflavin.[7] We found it remarkable, that in organic synthesis molecular flavins are applied for oxidations extensively,[8] for example using commercially available (–)-riboflavin,[9] but not for reductions. Therefore, we aimed for finding a strategy to use molecular flavins in the reductive 5-*exo*-trig cyclization of barbituric acid derivatives, which had previously only been reported with super-stoichiometric metal reductants (Figure 1B).[10,11]

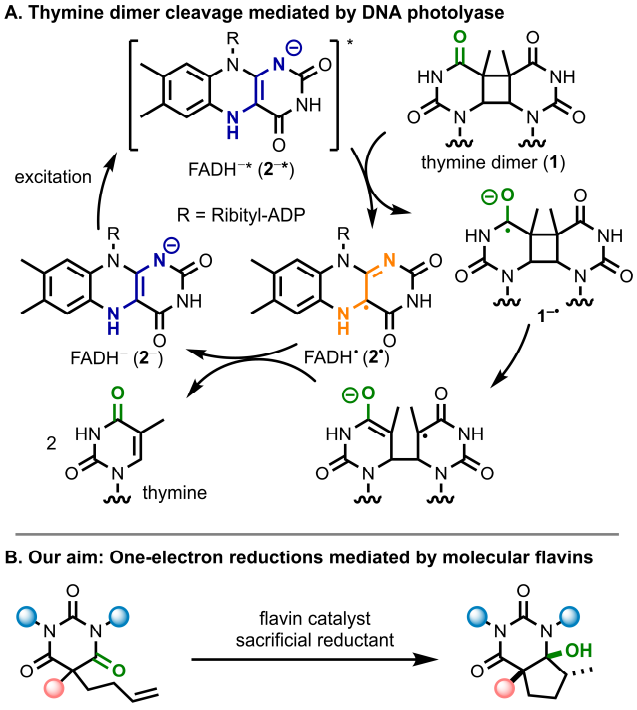


Figure 1. Mechanism of enzymatic thymine dimer **1** cleavage mediated by DNA photolyase (A). In the key step, an electron is transferred from the excited cofactor **2**$^{-*}$ to the dimerized thymine **1**. Our envisioned transformation using molecular, reduced flavin catalysts relies on similar one-electron transfer (B). Carbonyl groups involved in reduction are highlighted in green.

We hypothesized that the instability of reduced flavins such as **2** towards oxygen from air is one reason for the above-described discrepancy since it renders studies of the reduced cofactor very impractical. The instability itself is the result of a rapid reduction of O_2 to $O_2^{\bullet-}$ and subsequent formation of covalently bound flavin hydroperoxides.[12] The initial reduction of molecular oxygen [E ($O_2/O_2^{-\bullet}$) = –0.55 V *vs.* SCE] by a reduced flavin [E ($Fl^{\bullet+}/Fl$) = –0.05 V *vs.* SCE] (both values in aqueous buffer at pH = 4.6) is disfavored, but the net reaction becomes exergonic upon formation of the thermodynamically favored oxidized flavin and hydrogen peroxide.[13] We decided to base our strategy for air-stable, reduced flavin catalysts on a conformational bias for the reduced form,[14] which is typically bent along the N5-N10 axis with a ring puckering angle of 27.3°.[15] Both oxidized, as well as semiquinone states, are (almost) planar.[16] The conformational bias was achieved by double substitution of the N5- and C6-positions *via* reductive alkylation of flavin **3** and reduced catalyst **4** was obtained as an air-stable solid (Figure 2A). However, unsubstituted analog **5** did not yield the reduced flavin and was instead oxidized by air. Both oxidized flavins have similar redox properties: $E_{1/2}$ = –0.78 V *vs.* SCE (**3**) and $E_{1/2}$ = –0.85 V *vs.* SCE (**5**). The bent structure of flavin **4** (Figure 2B) was further characterized by single-crystal diffraction and a ring puckering angle of 32.1° was found along the N5-N10 axis (Figure 2C). Cyclic voltammetry confirmed that oxidation [$E_{1/2}$ = +0.46 V *vs.* SCE (**4**) in CH_3CN] of reduced flavin **4** is at least partially reversible (Figure 2C). When the measurement was continued to more positive potentials, irreversible processes were detected which is in line with our strategy of destabilizing the planar oxidized states.

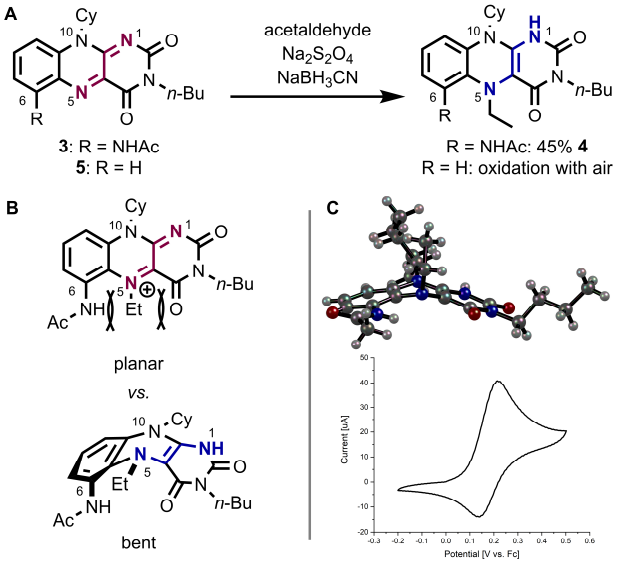


Figure 2. Synthesis and characterization of an air-stable reduced flavin. Conformational bias is realized by reductive alkylation (A) which results in steric destabilization of the planar states (B). The bent structure of flavin **4** is visible in the X-ray structure (C) and cyclic voltammetry in CH_3CN shows partially reversible electron transfer.

We then turned our attention to the photophysical properties of air-stable flavin **4** and its oxidized counterpart **3** (Figure 3). The first major difference is the blue-shift of the absorption maximum, which is at $\lambda_{A,max}$ = 324 nm for the reduced catalyst. The same trend is observed for the emission with fluorescence occurring at $\lambda_{E,max}$ = 567 nm.

In analogy to the natural cofactor ($FADH_2$ *vs.* $FADH^-$), the emission of **4** is shifted to $\lambda_{E,max}$ = 533 nm upon addition of triethylamine base.[2c] We observed two long lifetime components of flavin **4** in the excited state (see SI): τ = 1.1 ns (78%) and τ = 11.7 ns (22%).

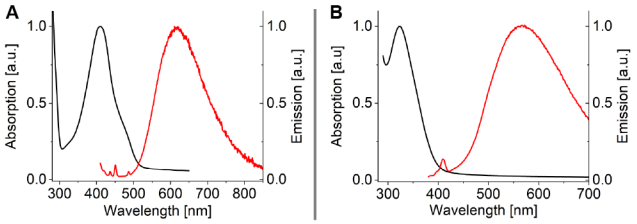
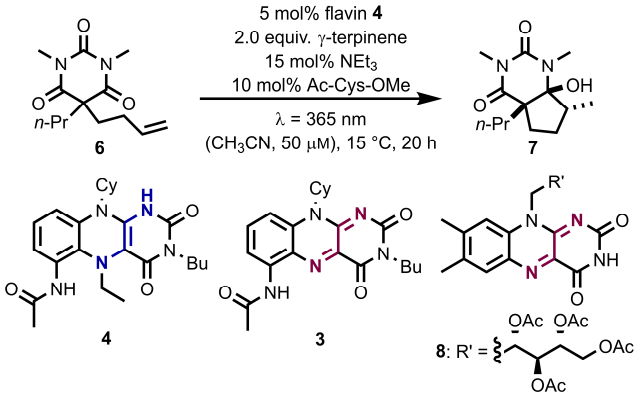


Figure 3. Normalized absorption (in black) and emission (in red) spectra of flavin **3** (A) and reduced flavin **4** (B) in CH_3CN solution. Quinoid flavin **3**: $\lambda_{A,max}$ = 411 nm; $\lambda_{E,max}$ = 620 nm. Hydroquinoid flavin **4**: $\lambda_{A,max}$ = 324 nm; $\lambda_{E,max}$ = 567 nm.

With this information in hand, we started our catalysis studies and chose barbituric acid derivative **6** as a promising substrate for one-electron reduction due to its structural and electronic similarity to thymine dimer **1**. The 5-*exo*-trig cyclization of substrate **6** had previously been reported using six equivalents of rare-earth reductant SmI_2,[10] thus making this reaction an ideal target for a mild catalytic protocol. Optimization of the catalytic conditions (see SI for details) quickly revealed that **4** is indeed a suitable electron donor in the excited state. Several sacrificial reductants turned out to be applicable, but we decided to move forward with γ-terpinene which is an inexpensive and commercially available essential oil.[17] With a catalytic amount of triethylamine and a catalytic amount of cysteine as a hydrogen atom donor,[18] we obtained bicycle **7** in 90% yield (Table 1, Entry 1).

Table 1. Flavin-catalyzed net-reductive cyclization of substituted barbituric acid derivative 6.



Entry	Deviation from standard conditions	Yield[a]
1	None	90%
2	No NEt_3	nd
3	No γ-terpinene	<5%
4	No Ac-Cys-OMe	39%
5	No flavin catalyst	nd
6	No irradiation	nd
7	With 4.0 equiv. NEt_3	quant.[b]
8[c]	10 mol% $(Ac\text{-}Cys\text{-}OMe)_2$	quant.
9[c]	5 mol% flavin catalyst **3**	46
10[c]	5 mol% flavin catalyst **8**	88

a: Measured by NMR spectroscopy with internal standard. b: Isolated yield on 0.1 mmol substrate scale. c: With 4.0 equiv. NEt_3. nd: Not detected.

Leaving out the amine base resulted in no conversion. This is in analogy to the enzymatic process, which relies on the deprotonated cofactor $FADH^-$ (Entry 2).[2f] The γ-terpinene is required as a sacrificial reductant and only trace product is formed in its absence due to irreversible catalyst oxidation (Entry 3). Cysteine improves the yield significantly but is not essential for catalysis (Entry 4). We also verified that catalyst and irradiation are necessary (Entries 5,6). Increasing amounts of triethylamine led to quantitative conversion (Entry 7) and other bases such as quinuclidine are also suitable (see SI). Cystine and cysteine perform equally well, which implies that the former is efficiently reduced under the reaction conditions as well (Entry 8). Under our optimized conditions, *in-situ* reduced quinoid flavin **3** and even (–)-riboflavin tetraacetate **8** also led to product formation (Entries 9,10), which highlights the applicability of our method.

According to our mechanistic proposal, the catalytic reaction is initiated by a one-electron reduction of barbiturate **6**, very similar to the first step in DNA photolyase (Figure 4). Our flavin catalysis proceeds relatively slowly with a quantum yield of $\Phi = 1.2 \cdot 10^{-3}$ (see SI) and in line with this observation, no significant fluorescence quenching

of **4**$^{\bullet-}$* occurs upon addition of substrate **6** presumably due to inefficient electron transfer. The intermediate ketyl radical next undergoes rapid 5-*exo*-trig cyclization to anion **7**$^{\bullet-}$ which is further reduced and protonated to yield bicyclic product **7**. Hydrogen atom transfer from γ-terpinene (BDE of 1,4-cyclohexadiene is 77 kcal mol^{-1})[19] resulting in *para*-cymene *via* **9** seems plausible for reduction of both intermediate **7**$^{\bullet-}$ and semiquinone **4**$^{\bullet}$. In agreement with this line of events, hydroquinone **4** is regenerated quickly and is the only catalyst species we observe when conducting the reaction in an NMR tube under inert conditions (see SI). During the catalytic reaction, photoexcitation of intermediate **4**$^{\bullet}$ will also occur given the typical absorption properties of flavin semiquinones.[20] These species are also strong reductants in the excited state,[7] however, we have not observed any quinoid flavin under our reaction conditions by NMR. Catalytic amounts of cysteine as a hydrogen atom donor ensure rapid conversion of the carbon-centered radicals.

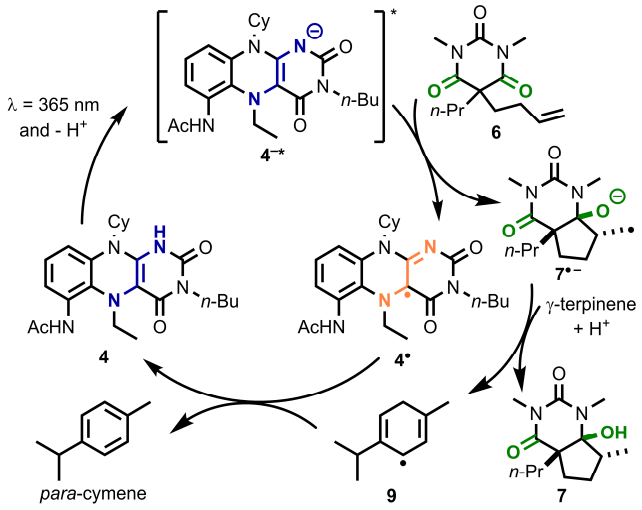


Figure 4. Simplified mechanistic proposal for the flavin-catalyzed reduction of barbituric acid derivative **6**. The carbonyl groups, one of which is initially reduced, are highlighted in green.

Gratifyingly, flavin catalysis also allows efficient reductive conversion of a series of barbituric acid derivatives (Figure 5). The 5-*exo*-trig cyclization is initiated with different substituted alkene side chains (**10**-**12**) and di- as well as tri-substitution is tolerated. With allene substrates, terminal alkene product **13** is formed. An analogous cyclization forming six-membered rings is also possible, but bicycle **14** was obtained as a mixture of diastereomers. With terminal alkynes, 5-*exo*-dig cyclization results in exocyclic alkene **15**.

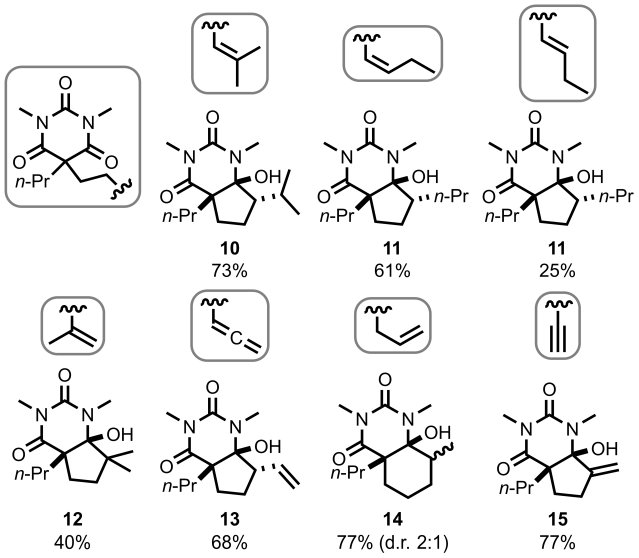


Figure 5. Application of the flavin catalysis to a variety of barbituric acid derivatives. Reaction conditions: 5 mol% **4** (10 mol% for product **11**), 2.0 equiv. γ-terpinene, λ = 365 nm, (CH_3CN), 15 h. Yields were determined by NMR spectroscopy with internal standard.

We next probed for potential differences between flavin-mediated reductions and conventional reactions using SmI_2. In this context, barbituric acid derivative **16** was chosen, which was reported to result in pinacol coupling product **17** upon reduction with SmI_2 (Figure 6).[21] Here, three carbonyl sites compete for the one-electron reduction, and the formation of the pinacol product was rationalized based on a favored (E = –2.2 V *vs.* SCE) initial reduction of a barbituric acid carbonyl group compared to the ketone (E < –2.5 V *vs.* SCE).[21] In contrast, when applying flavin catalyst **4**, no pinacol product was obtained and secondary alcohol **18** was formed instead. This reactivity difference could be explained by substrate chelation when using SmI_2, which allows flavin **4** to leave the carbonyl groups in the heterocycle fully intact.

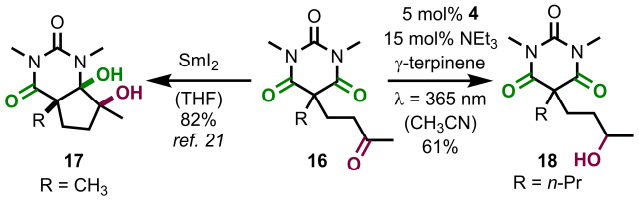


Figure 6. Reduction of ketone **16** shows distinct differences between SmI_2-mediated and flavin-catalyzed reactions.

In summary, we report reduced, molecular flavins as catalysts in organic transformations. While so far such reduced flavins have been exclusively used as O_2-reductants, our combination of the essential oil reductant γ-terpinene, photochemical excitation, and mild basic conditions allow the conversion of other, useful organic substrates. Inspired by DNA photolyase, we focused on barbituric acid derivatives in this study but envision broad applicability

to other substrate classes based on the strong reducing power of excited flavins. Additionally, we observed differences between traditional metal reductants and flavin catalysts, which stimulate our continuing search for other challenging transformations as well as chiral catalyst versions for stereoselective reductions.

Supporting Information

The Supporting Information (PDF) contains experimental procedures, analytical data for all new compounds, additional experiments, and NMR data. The X-ray crystallographic coordinates for **4** are deposited at the Cambridge Crystallographic Data Center (CCDC) under deposition number CCDC 2126062.

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Notes

The authors declare no competing financial interests.

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