Synthesis and application of flavin based oxidation catalysts
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CHAPTER 2

Synthesis and studies of flavins and flavin catalysts

Laborious workup and difficult purifications are often a standard practice in flavin chemistry. This chapter describes the synthesis of a library of flavins based catalysts. Synthetic procedures and purification methods have been optimized to make flavin compounds more generally accessible.

Part of this chapter has been published:
2.1 Introduction

One of the largest groups of enzymes capable of performing oxidation reactions is the group of flavoproteins. The common motive in all these enzymes is the presence of a flavin cofactor in their active site. Although the basis of the cofactor is an isoalloxazine, some subtle variations exist. The most well known flavin is without doubt riboflavin, also known as vitamin B$_2$, which is the precursor for flavin cofactors and is frequently added to food as a natural colouring agent (Figure 2.1).\textsuperscript{1} Flavin cofactors that are found in proteins are lumiflavin, FMN and FAD.

![Flavin cofactors](image)

Figure 2.1: Natural occurring flavin cofactors.

A common motive in all these cofactors is the alloxazine structure. The main difference between the cofactors is located in the chain present at the N$_{10}$ of the flavin. This chain is often important in binding the flavin to a protein. Interestingly, flavoproteins are active in a diverse range of oxidation and reduction reactions, often steered by interactions between the cofactor and the enzyme and dependent on how substrates interact with the flavin. The active part of the flavins is located at N$_{5}$. Although an impressive amount of modified flavins have been reported, these have mainly been tested in protein binding and activity studies.\textsuperscript{2} Flavin catalysts, used in solution, by necessity are alkylated at the N$_{5}$, since without alkylation of this position the flavins eliminate H$_2$O$_2$ faster than they can react with substrates. A number of flavin catalysts has been syntheses by different groups as described in chapter 1, but there has been no thorough investigation into the effect of different alkyl substituents at the N$_{1}$ and N$_{3}$ of the flavins. In this chapter the synthesis of a library of different N$_{1}$ and N$_{3}$ substituted flavin catalysts is described, while investigations into their activity is described in chapter 3.
The synthesis of the natural cofactors is not trivial but they can be extracted readily from proteins. Synthesis of an unsubstituted isoalloxazine ring system is however not that challenging and can be performed readily in one step starting from a suitable diamine (Scheme 2.1). The alloxazine can undergo alkylation at the N1 and/or N3.

The obtained alloxazines can be turned into a catalyst by reductive alkylation of N5 via a number of pathways. In this fashion, a selection of different flavin catalysts has been prepared. Without N5 alkylation, the respective peroxyflavins readily eliminate H2O2 and cannot be used as a catalyst.
The alloxazine ring system of flavins is the key fragment in the structure of a flavin catalyst. However, there are quite some sites, which can be and have been altered to produce more active or more stable catalysts.

### 2.2 Catalyst design

#### 2.2.1 Alkylating the N<sup>1</sup> and N<sup>3</sup> position of alloxazines

Although the effect of electron donating and withdrawing substituents on the aromatic ring of flavin based catalysts has been investigated, the effect of electron donating or withdrawing groups on the other parts of the molecule has not. To investigate this, first the basic alloxazine structure had to be synthesized, which was performed according to the method described by Bäckvall et al. in which alloxane and a diamine are coupled via imine formation, yielding the desired alloxazine.

![Scheme 2.3: Synthesis of the alloxazine structure.](image)

The introduction of a methyl substituent on the N<sup>1</sup> and N<sup>3</sup> position of flavins has already been reported numerous times. Using a method similar to the synthesis of 2.12a with simplified purification, we introduced a variety of alkyl, benzyl and pyridyl groups. The resulting flavins 2.12a-j and 2.13 a-j were obtained in high yield and the products could be purified by washing with excess water.
Table 2.1: Facile synthesis of a library of alloxazines.

![Chemical Structures]

To a solution of alloxazine in DMF was added 5 equiv K₂CO₃ and 2 equiv of R'-X. Products were isolated by evaporation of solvent and washing of the product with H₂O, with yields in parenthesis.

2.2.2 Alkylation of the N¹ and N³-disubstituted flavins at N⁵

With a library of (iso)alloxazines in hand, the next step was the introduction of an ethyl group at the N⁵ of the flavins to turn them into active catalysts. An often-used method, which also proved successful in this case, was reductive alkylation using palladium on carbon with dihydrogen and acetaldehyde as previously described by Bäckvall et al.⁵

![Scheme 2.4: Reductive alkylation of N¹,N³-disubstituted alloxazines using palladium on carbon.]

2.12a R' = H, R'' = Me 2.13a R' = Me, R'' = Me 2.14a R' = H, R'' = Me (84%) 2.15a R' = Me, R'' = Me (76%)
2.12b R' = H, R'' = Et 2.13b R' = Me, R'' = Et 2.14b R' = H, R'' = Et (73%) 2.15b R' = Me, R'' = Et (80%)
2.12c R' = H, R'' = n-Pr 2.13c R' = Me, R'' = n-Pr 2.14c R' = H, R'' = n-Pr (88%) 2.15c R' = Me, R'' = n-Pr (84%)
2.12d R' = H, R'' = i-Pr 2.13d R' = Me, R'' = i-Pr 2.14d R' = H, R'' = i-Pr (9%) 2.15d R' = Me, R'' = i-Pr (14%)
Most of the catalysts were obtained in high yield, except for 14c and 15c, containing iso-propyl substituents. These were difficult to isolate. We subsequently tried to perform the reductive alkylation on the benzyl substituted flavins despite the risk that benzyl groups can suffer from hydrogenolysis under these conditions. Although benzylic amides are less prone to cleavage under the heterogenous hydrogenation conditions, the desired product could not be obtained and it was noticed that the benzyl and picolyl substituents were cleaved from the alloxazines. Therefore an alternative route was chosen. The group of Imada published a flavin catalyst synthesis in which a isoalloxazine was reduced using sodium dithionite followed by reductive alkylation with sodium cyanoborohydride and acetaldehyde in one pot. ref

Scheme 2.5: Reductive alkylation of N1,N3 disubstituted isoalloxazines using Na2S2O4, NaBH3(CN) and acetaldehyde.

No reduction of the flavins was observed and the starting material was isolated quantitatively after the reaction. As can be seen in Figure 2.3, the flavin catalysts synthesized by Imada et al. are based upon isoalloxazines, while our catalysts are based upon alloxazines and as a result the conjugation in the rings is different. It is very likely that this difference in conjugation in our flavins makes them more resistant to reduction.

Figure 2.3: Catalyst precursors from Imada and our group.

It has been shown that by poisoning the palladium catalyst with diphenylsulfide, olefins can be reduced while no hydrogenolysis takes place. In our case however we found the reduction to be fully inhibited with only 1% diphenylsulfide.
2.2.3 Synthesis of flavin aza-alloxazines

The group of Bäckvall\textsuperscript{8} showed improved activity of flavin catalysts, that had electron withdrawing substituents. The alloxazines were made more electron poor by introducing substituents on the aromatic ring. Another option to tune the alloxazine would be to introduce changes in the aromatic system. Introduction of nitrogen in the left ring should give a less electron rich alloxazine species. Based on these assumptions, starting from commercially available aromatic diamines 14 and 16 we synthesized aza-isoaloxazines 15 and 17 as mixtures of regio-isomers (Scheme 2.7).

![Diagram of flavin aza-alloxazines](image)

Scheme 2.7: Synthesis of pyridopteridines 2.15 and 2.17 from pyridinediamines.

The aza-alloxazines 2.15 and 2.17 were obtained in a ratio of 60:40 (2.15a: 2.15b) and 55:45 (2.17a: 2.17b) as could be determined using \(^1\)H-NMR. Unfortunately, no method for separating the isomers was found, a known problem with flavin isomers. As, however, there is precedent in the literature in which mixtures of regio-isomers were applied in reactions, the investigation was continued.
As expected, aza-alloxazines 2.18 and 2.19, depicted in scheme 2.8, were obtained as isomeric mixtures with ratios of respectively 60:40 (2.18a: 2.18b) and 55:45 (2.19a: 2.19b) and could be extracted from the reaction mixture in moderate yields using a mixture of CHCl₃ and water, instead of isolation via washing with water, due to their high solubility in the aqueous layer. The next step; reduction followed by alkylation of N₅, proved again to be complicated. During this reaction using Pd/C, H₂ with or without HCl and acetaldehyde, full reduction of the pyridine ring was observed and the desired product could not be obtained. Therefore it was decided not to continue the research into this line of substrates.

2.2.4 Synthesis of flavins with larger aromatic systems

Another approach to potentially more active flavin based catalysts is via extending the aromatic system. We looked into two specific possibilities. Starting from the commercial diamines 2.20 and 2.22, alloxazines 2.21 and 2.23 were prepared in good yield. In the case of the dimeric alloxazine system we obtained a mixture of isomers from which the ratio could not be elucidated, although ¹H-NMR and UV/Vis measurements clearly indicated three distinct products.
Synthesis of and measurements on flavins and flavin catalyst

Scheme 2.9: Synthesis of naphthyl and biphenylpteridines 2.21 and 2.23 from aromatic diarnines and alloxane.

[Chemical structures and reactions are shown]

The alloxazines were again 1,3-dimethylated using the same methodology as described before to obtain alloxazine 24 and an isomeric mixture of 25.

Scheme 2.10: Synthesis of naphthyl and biphenylpteridines 2.24 and 2.25 using methyl iodide and K$_2$CO$_3$.  

(55%)

While the methylated products 2.24 and 2.25 were obtained in decent yields, subsequent reductive of the N\textsuperscript{5} position again proved to be unsuccessful, with reduction of either the naphthyl of compound 2.24 taking place or as with compound 2.25 by obtaining a reaction mixture of which either the desired product could be isolated or the resulting product could be identified.

2.2.5 Synthesis of riboflavin based catalysts

Flavin-based catalysts are invariably inspired by naturally occurring riboflavin (vitamin B\textsubscript{2}, Scheme 1) and its derivatives FMN and FAD. As described in chapter 1, the group of Imada\textsuperscript{10} even used riboflavin as starting material for the synthesis of a flavin catalyst in multiple steps. Bruice et al.\textsuperscript{11} already reported reductive alkylation with NaCNBH\textsubscript{3} and aldehydes under aerobic conditions as a general method for the synthesis of N\textsuperscript{5}-ethyl-FAD and FMN. In the catalysts derived from riboflavin by Imada et al., the ribose chain was first protected, followed by methylation of N\textsuperscript{3} and subsequent reductive alkylation. To the best of our knowledge, direct reductive alkylation of riboflavin and lumiflavin had not been reported. We found that in a one-pot synthesis, an active oxidation catalyst could be synthesized from commercially available riboflavin. (Scheme 2.11).

Riboflavin was treated with excess acetaldehyde and H\textsubscript{2}Pd/C in acidic aqueous ethanol. An important observation was that progress of the reaction could be followed using UV/Vis-analysis (see experimental section). After filtration of the reaction mixture over celite and evaporation of the volatiles, catalyst 2.27a was obtained in excellent yield. Although \textsuperscript{1}H-NMR initially gave no indicative spectra,\textsuperscript{12} we found that by adding an excess of sodium dithionite to the NMR tube we could obtain
good spectra. Analysis by LC-MS also showed the expected mass of mono-ethyllated riboflavin. However, the mass spectra also indicated the presence of compounds with higher masses correlating with acetal formation in the ribose chain, together with dimer and trimer formation (Figure 2.4).

We surmised that the formation of acetal bridged species can take place due to the acidic nature of the reaction mixture. To circumvent any acetal formation, we attempted N⁵-alkylation without HCl. In this case the reaction proceeded much slower and we only observed full conversion after one week. Nevertheless, LC-MS measurements did not indicate the formation of acetals (Figure 2.5).
We could exclude competing N\textsuperscript{3} ethylation by comparing the UV-spectra of independently prepared N\textsuperscript{3}-methylated riboflavin with the product. The two compounds showed clearly different UV spectra. Further evidence was that the UV-spectrum of the product correlated well with UV spectra of similar ethylated flavins described by Bruice et al.\textsuperscript{13}

2.3 UV spectra of the synthesised flavins

Flavins have quite distinct UV/Vis spectra. We looked into the UV spectra of our N\textsuperscript{1},N\textsuperscript{3} dialkylated alloxazines and the corresponding catalysts and found a small shift in the N\textsuperscript{1},N\textsuperscript{3} dialkylated alloxazines compared to non alkylated versions. When adding sodium dithionite to the 1,3-disubstituted flavin catalyst 2.14a we obtained the UV spectrum indicative of reduced flavin species (Figure 2.6). In the case of the mixed flavins 2.18, 2.19 and 2.25, the presence of multiple isomers could be confirmed. The riboflavin based catalyst 2.27a turned out to be a special case of which the obtained UV spectra proved to be similar to the spectra as are obtained of flavin cofactors in enzymes. One of these states is a radical species, which needed to be suppressed for \textsuperscript{1}H-NMR measurements, but could be observed in UV-Vis spectra. UV-Vis spectroscopy has been used in this fashion to determine the state of a flavins and is a common ways by which the kinetics of flavoproteins can be investigated.\textsuperscript{14}
Intriguingly, with the riboflavin based catalyst the distinctive “blue colored radical”, ”pink protonated radical” and the “red flavinum cation” described by Bruice et al. could be observed (depicted in Figure 2.7). These states were not observed in the case of the 1,3-dialkylated flavin catalysts. The riboflavin based catalyst can thus be seen as an excellent mimic of the cofactor as present in enzymes. When flavin catalyst 2.27a and 2.27b were compared, no difference between the UV spectra was observed.

Figure 2.6: UV spectrum of a) 2.14a dissolved in H2O. b) 2.14a dissolved in H2O with sodium dithionite.
Synthesis of and measurements on flavins and flavin catalyst

Figure 2.7: UV spectra of \(2.27a\) (0.47 mM, 1.9 mg in 10 ml) under different conditions a) “Blue colored radical”, 0.47 mM \(2.27a\) in pure \(H_2O\) (semiquinone species). b) “Pink protonated radical”, 0.47 mM \(2.27a\) in \(H_2O + 16.6 \mu L\) 6N HCl c) “Red flavinium cation”, 0.47 mM \(2.27a\) in \(H_2O + 16.6 \mu L\) 6N HCl + 50 \(\mu L\) 30% \(H_2O_2\).

The large changes in the UV-spectra of the riboflavin based catalyst \(2.27a\) compared to the UV spectra of riboflavin made it possible to monitor the reaction as depicted in Figure 2.8. This is particularly convenient, because the reaction cannot be followed using techniques like TLC and GC-MS.
2.4 Conclusions

While the synthesis N\(^1\), N\(^3\) substituted is rather straightforward, not all the compounds synthesized proved to be stable under the reductive condition used for the subsequent introduction of an ethyl group on the N\(^5\) position. It however proved to be feasible to prepare a catalyst based upon riboflavin in one step.

2.5 Experimental section

2.5.1 General procedures

General: \(^1\)H-NMR spectra were recorded at 300, 400 or 500 MHz with CDCl\(_3\) or DMSO-D\(_6\) as solvent.

2.5.2 Catalyst precursors

**o-Phenylenediamine (2.10a):** 2-nitroaniline (2.5 g; 166 mmol) was dissolved in 100 mL methanol. 750 mg Pd/C was added and the mixture was hydrogenated for 16 h at 1.1 atm. of dihydrogen. Filtration over celite and evaporation in vacuo gave a brown solid (2.0 g; 96%). \(^1\)H NMR (DMSO, 400 MHz): \(\delta 2.12 \text{ (s, 6H), 6.52 (s, 2H)}\). Analytical data as reported.\(^8\)

**Alloxazine (2.11a):** o-Phenylenediamine (3.38 g; 31 mmol) was dissolved in 50 mL acetic acid. A mixture of alloxane (5.00 g; 31 mmol) and H\(_3\)BO\(_3\) (2.13 g; 34.5 mmol) in 200 mL hot acetic acid was
added to this solution. The reaction mixture was stirred for 75 min at room temperature. The precipitate was removed by filtration and washed with acetic acid and diethylether. Drying gave 2.6 as a yellow solid (6.36 g; 95%); mp: decomposition >390ºC. 1H NMR (500 MHz, dmso) δ 11.94 (s, 1H), 11.76 (s, 1H), 8.17 (d, J = 8.4, 1H), 7.94 (d, J = 2.8, 2H), 7.87 - 7.69 (m, 1H). 13C NMR (126 MHz, DMSO) δ 161.14 (C), 150.81 (CO), 147.55 (CO), 143.31 (C), 139.89 (C), 134.02 (CH), 132.35 (C), 130.82 (CH), 129.10 (CH), 127.65 (CH). IR (KBr): 3430, 3178, 1644, 1618, 1585, 1507, 1451, 1394, 1365, 1336, 1275, 1250, 1154, 1036, 869, 779, 707, 586, 541, 514, 432 cm⁻¹. EI-MS m/z: 214.0487 (100). Analytical data as reported. 8

7,8-dimethylalloxazine (2.11b): 2.10a (0.95 g; 7.0 mmol) was dissolved in 15 mL acetic acid. A mixture of alloxan (1.12 g; 7.0 mmol) and H3BO3 (0.48 g; 7.8 mmol) in 45 mL hot acetic acid was added to this solution. The reaction mixture was stirred for 75 min at room temperature. The precipitate was filtered off and washed with acetic acid and diethylether. Drying gave 2.7 as a yellow solid (1.57 g; 92%); mp: decomposition >380ºC. 1H NMR (DMSO, 400 MHz): δ 1.88 (s, 6H), 7.89 (s, 1H), 7.68 (s, 1H), 11.63 (s, 1H), 11.80 (s, 1H) ppm. Analytical data as reported. 8 IR (KBr): 3962, 3425, 3364, 3326, 3051, 2963, 2652, 251, 1973, 1858, 1723, 1670, 1613, 1561, 1456, 1362, 1298, 1203, 1150, 1109, 1066, 977, 911, 810, 776, 749, 691, 600, 486, 445 cm⁻¹. EI-MS m/z: 242.08167 (100). HRMS ESI (m/z) [M +] calcd for C12H11O2N4 243.08754, found 243.08765. Analytical data as reported. 3

1,3-dimethylalloxazine (2.12a): Alloxazine (2.11a) (2.0 g; 9.2 mmol) and K2CO3 (6.6 g; 47 mmol) were added to 20 mL dry DMF. MeI (1.3 mL; 21 mmol) was added and the reaction mixture was stirred for 2 h at room temperature. 50 mL water was added to the reaction mixture and the precipitate was filtrated and washed with water. No further purification was necessary. Drying gave 2.12a as a yellow solid (2.0 g; 88%); mp: 242.5 - 245.6ºC. 1H NMR (400 MHz, CDCl3) δ 8.54 (d, J = 8.5, 1H), 8.22 (d, J = 7.5, 1H), 8.09 (t, J = 6.0, 1H), 7.95 (t, J = 6.0, 1H), 7.37 (s, 3H), 3.79 (s, 3H). IR (KBr): 3065, 2844, 1737, 1701, 1581, 1487, 1427, 1362, 1281, 1216, 1145, 1030, 1008, 832, 735, 630, 578, 478, 449, 419 cm⁻¹. HRMS ESI (m/z) [M +] calcd for C12H11O2N4 243.0875, found 243.0877. Analytical data as reported. 3

1,3-diethylalloxazine (2.12b): Prepared in a similar fashion as 2.12a with 1 g of 2.11a (4.6 mmol) and ethyl iodide (0.85 mL; 11 mmol) instead of methyl iodide. After drying, 1.1 g (87%) of a yellow solid was obtained. 1H NMR (400 MHz, CDCl3) δ 8.33 (d, J = 8.4, 1H), 7.96 (d, J = 8.5, 1H), 7.37 (d, J = 8.3, 1H), 3.79 (s, 3H), 1.35 (t, J = 7.5, 3H). 13C NMR (126 MHz, CDCl3) δ 159.71 (C), 150.11 (CO), 145.06 (CO), 143.71 (C), 140.17 (C), 133.99 (CH), 131.00 (CH), 130.96 (C), 129.30 (CH), 128.08 (CH), 38.28 (CH2), 38.07 (CH2), 13.37 (CH3), 13.21 (CH3). HRMS ESI (m/z) [M +] calcd for C14H14O2N4 271.119, found 271.119.
1,3-dipropylalloxazine (2.12c): Prepared in a similar fashion as 2.12a with 1 g of 2.11a (4.6 mmol) and n-propyl iodide (1.0 mL, 10.2 mmol). After drying 1.3 g (94%) of a yellow solid was obtained. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.51 (d, $J = 7.5$, 1H), 8.21 (d, $J = 7.6$, 1H), 8.07 (7, 1H), 7.93 (t, 1H), 4.60 (t $J = 7.6$, 2H), 4.34 (t, $J = 7.5$, 2H), 2.00 (m, 4H), 1.23 (t, $J = 7.1$, 3H), 1.21 (t, $J = 6.9$, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 159.90 (C), 150.47 (CO), 145.34 (CO), 143.70 (C), 140.22 (C), 133.95 (CH), 131.04 (CH), 130.03 (C), 129.27 (CH), 128.15 (CH), 44.49 (CH$_2$), 44.33 (CH$_2$), 21.37 (CH$_2$), 21.16 (CH$_2$), 11.63 (CH$_3$), 11.58 (CH$_3$). HRMS ESI (m/z) [M +] calcd for C$_{16}$H$_{19}$O$_2$N$_4$ 299.1503, found 299.1501.

1,3-diisopropylalloxazine (2.12d): Prepared in a similar fashion as 2.12a with 1 g of 2.11a (4.6 mmol) and isopropyl iodide (1.0 mL, 10.2 mmol). After drying, 1.1 g (80%) of a yellow solid was obtained. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.32 (d, 1H), 8.01 (d, 1H), 7.90 (t, 1H), 7.75 (t, 1H), 5.85 (m, 1H), 5.39 (m, 1H), 1.69 (d, $J = 6.8$, 6H), 1.61 (d, $J = 6.8$, 6H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 160.15 (C), 150.75 (CO), 145.53 (CO), 143.18 (C), 139.95 (C), 133.76 (CH), 130.73 (CH), 130.55 (C), 129.15 (CH), 128.00 (CH), 47.85 (s, 7H), 19.90 (CH$_3$), 19.73 (CH$_3$). HRMS ESI (m/z) [M +] calcd for C$_{16}$H$_{19}$O$_2$N$_4$ 299.1503, found 299.1500.

1,3-dibenzyralloxazine (2.12e): Prepared in a similar fashion as 2.12a with 0.5 g of 2.11a (2.3 mmol) with benzylbromide (0.6 mL, 5.0 mmol). After drying, 0.84 g (92%) of a yellow solid was obtained. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.34 (d, $J = 8.4$, 1H), 8.07 (d, $J = 8.5$, 1H), 7.92 (t, $J = 7.7$, 1H), 7.78 (t, $J = 7.7$, 1H), 7.63 (t, $J = 8.1$, 4H), 7.47 - 7.20 (m, 6H), 5.67 (s, 2H), 5.40 (s, 2H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 159.83 (C), 150.70 (CO), 145.15 (CO), 143.46 (C), 140.43 (C), 136.58 (C), 136.48 (C), 134.23 (CH), 131.06 (CH), 130.00 (C), 129.82 (CH), 129.56 (C), 129.33 (CH), 128.82 (CH), 128.80 (CH), 128.29 (C), 128.18 (CH), 128.15 (CH), 45.92 (CH$_3$), 45.87 (CH$_2$). HRMS ESI (m/z) [M +] calcd for C$_{24}$H$_{19}$O$_2$N$_4$ 395.1503, found 395.1502.

1,3-bis(pyridin-2-ylmethyl)alloxazine (2.12f): Prepared in a similar fashion as 2.12a with 0.5 g of 2.11a (2.3 mmol) with 2-picoly chloride hydrochloride (5.1 mmol, 0.83 g). After drying, 0.69 g (76%) of a yellow solid was obtained. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.73 (m, 4H), 8.53 (d, $J = 8.4$, 1H), 8.20 (d, $J = 7.9$, 1H), 8.11 (t, $J = 6.8$, 1H), 7.98 (t, $J = 6.7$, 1H), 7.67 - 7.53 (m, 4H), 5.82 (s, 1H), 5.54 (s, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 159.82 (C), 150.69 (CO), 145.15 (CO), 143.46 (C), 140.43 (C), 136.57 (C), 136.47 (C), 134.23 (CH), 131.06 (CH), 129.99 (CH), 129.81 (CH), 129.56 (CH), 129.32 (CH), 128.82 (CH), 128.79 (CH), 128.28 (CH), 128.17 (CH), 128.14 (CH), 45.92 (CH$_2$), 45.87 (CH$_2$). HRMS ESI (m/z) [M +] calcd for C$_{24}$H$_{17}$O$_2$N$_6$ 397.1408, found 397.1407.
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1,3-bis(pyridin-4-ylmethyl)alloxazine (2.12g): Prepared in a similar fashion as 2.12a with 0.5 g of 2.11a (2.3 mmol) with 4-picoly chloride hydrochloride (5.1 mmol, 0.83 g). After drying, 0.71 g (78%) of a yellow solid was obtained. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.59 (t, $J=6.1$, 4H), 8.37 (d, $J=8.4$, 1H), 8.05 (d, $J=8.4$, 1H), 7.97 (m, $J=7.36$, 1H), 7.84 (m, $J=7.73$, 1H), 7.44 (m, 4H), 5.67 (s, 2H), 5.39 (s, 2H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 159.63 (C), 150.54 (CO), 150.46 (CH), 150.45 (CH), 145.08 (C), 144.82 (CO), 144.72 (C), 143.38 (C), 140.72 (C), 134.84 (CH), 131.15 (CH), 130.15 (CH), 129.55 (C), 128.12 (CH), 124.03 (CH), 123.50 (CH), 45.11 (CH$_2$), 45.00 (CH$_2$). HRMS ESI (m/z) [M +] calcd for C$_{12}$H$_{11}$O$_2$N$_4$ 243.0875, found 243.0877.

1,3-bis(4-methoxybenzyl)alloxazine (2.12h): Prepared in a similar fashion as 2.12a (2.3 mmol) with 0.5 g of 2.11a with 4-methoxybenzyl bromide (1.0 g, 5.1 mmol). After drying, 0.84 g (80%) of a yellow solid was obtained. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.34 (d, $J=8.5$, 1H), 8.09 (d, $J=8.5$, 1H), 7.93 (t, $J=7.7$, 1H), 7.62 (d, $J=8.4$, 1H), 7.39 - 7.22 (m, 2H), 7.12 (d, $J=8.4$, 1H), 7.02 - 6.76 (m, 6H), 5.60 (s, 2H), 5.33 (s, 2H), 4.50 (d, $J=2.5$, 3H), 3.84 (s, 2H), 3.80 (t, $J=2.5$, 3H), 3.79 (s, 2H). HRMS ESI (m/z) [M +] calcd for C$_{26}$H$_{23}$O$_4$N$_4$ 455.1714, found 455.1711.

1,3-bis(4-(trifluoromethyl)benzyl)alloxazine (2.12i): Prepared in a similar fashion as 2.12a with 0.1 g of 2.11a (0.47 mmol) and 1-(chloromethyl)-4-(trifluoromethyl)benzene (244 mg, 1 mmol). After drying, 0.23 g (92%) of a yellow solid was obtained. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.38 (d, $J=8.5$, 1H), 8.09 (d, $J=8.3$, 1H), 7.98 (t, $J=7.2$, 1H), 7.84 (t, 7.2, 1H), 7.77 - 7.71 (m, 4H), 7.65 - 7.60 (m, 4H), 5.73 (s, 2H), 5.46 (s, 2H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 159.71 (C), 150.64 (CO), 144.92 (CO), 143.40 (C), 140.65 (C), 140.30 (C), 140.11 (C), 134.69 (CH), 131.16 (CH), 130.64 (d, $^{2}$J$_{CF}$ = 13.43, C), 130.38 (d, $^{2}$J$_{CF}$ = 13.5, C), 130.08 (CH), 129.99 (CH), 129.72 (C), 129.52 (CH), 128.11 (CH), 125.85 (m, 2x CF$_3$), 45.56 (CH$_2$), 45.52 (CH$_2$). HRMS ESI (m/z) [M +] calcd for C$_{26}$H$_{17}$O$_2$N$_4$F$_6$ 531.1250, found 531.1251.

1,3-bis(3,5-bis(trifluoromethyl)benzyl)alloxazine (2.12j): Prepared in a similar fashion as 2.12a with 0.18 of 2.11a (0.47 mmol) and 1-(chloromethyl)-3,5-bis(trifluoromethyl)benzene (420 mg, 1.6 mmol). After drying, 0.47 g (93%) of a pale yellow solid was obtained. $^1$H NMR (500 MHz, CDCl$_3$) δ 8.17 (s, 2H), 8.13 (s, 2H), 8.09 (s, 1H), 7.84 (s, 2H), 7.81 (s, 1H), 5.74 (s, 2H), 5.48 (s, 2H), 4.50 (d, $J=2.5$, 3H), 3.84 (s, 2H), 3.80 (t, $J=2.5$, 3H), 3.79 (s, 2H). HRMS ESI (m/z) [M +] calcd for C$_{26}$H$_{17}$O$_2$N$_4$F$_6$ 531.1250, found 531.1251.
2.59 (s, 3H), 2.54 (s, 3H). $^{13}$C NMR (126 MHz, CDCl₃) δ 159.78, 150.77, 147.13, 144.33, 142.34, 141.29, 140.01, 138.72, 138.49, 132.16 (qd, J = 33.4, 7.0, CF₃), 130.29, 129.78, 128.47, 126.78, 124.43, 122.53, 45.17 (CH₂), 45.04 (CH₂), 21.26 (CH₃), 20.60 (CH₃). HRMS ESI (m/z) [M +] calcd for C₂₈H₁₅O₂N₄F₁₂ 667.0998, found 667.0999.

1,3,7,8-tetramethylalloxazine (2.13a): 2.11b (0.5 g; 2.1 mmol) and K₂CO₃ (1.51 g, 10.9 mmol) were added to 20 mL dry DMF. MeI (0.3 mL; 4.9 mmol) was added to the mixture and the reaction mixture was stirred for 2 h at room temperature. 50 mL water was added to the reaction mixture, the precipitate was filtered and washed with water. No further purification was necessary. Drying gave 2.13a as a yellow solid (0.52 g, 92%); mp: 253.7-256.1ºC. $^1$H NMR (400 MHz, CDCl₃) δ 8.24 (s, 1H), 7.97 (s, 1H), 3.98 (s, 3H), 3.77 (s, 3H), 2.71 (s, 3H), 2.68 (s, 3H). $^{13}$C NMR (126 MHz, CDCl₃) δ 160.30 (C), 150.99 (CO), 145.90 (CO), 145.17 (C), 142.53 (C), 140.24 (C), 139.31 (C), 129.54 (CH), 128.70 (C), 126.97 (CH), 29.72 (CH₃), 29.37 (CH₃), 21.16 (CH₃), 20.60 (CH₃). IR (KBr): 3430, 3379, 3337, 2976, 2849, 2729, 2521, 2203, 1726, 1674, 1555, 1485, 1360, 1294, 1176, 1106, 1007, 981, 882, 828, 811, 751, 687, 649, 580, 560, 469, 423 cm⁻¹. HRMS ESI (m/z) [M +] calcd for C₁₄H₁₅O₂N₄ 271.1190, found 271.1187.

7,8-dimethyl-1,3-diethylalloxazine (2.13b): Prepared in a similar fashion as 13a with 1 g of 11b (4.1 mmol) and ethyliodide (0.76 mL; 9.8 mmol) instead of methyliodide. After drying 1.17 g (96%) of a yellow solid was obtained. $^1$H NMR (500 MHz, CDCl₃) δ 8.07 (s, 1H), 7.81 (s, 1H), 4.52 (q, J = 7.0, 2H), 4.26 (q, J = 7.1, 2H), 2.55 (s, 3H), 2.52 (s, 3H), 1.41 (t, J = 7.0, 3H), 1.36 (t, J = 7.0, 3H). $^{13}$C NMR (126 MHz, CDCl₃) δ 160.03 (C), 150.12 (CO), 145.70 (CO), 144.77 (C), 142.78 (C), 140.40 (C), 139.39 (C), 129.66 (CH), 129.04 (C), 127.11 (CH), 32.50 (CH₂), 32.21 (CH₂), 16.15 (CH₃), 13.41 (CH₃). HRMS ESI (m/z) [M +] calcd for C₁₆H₁₉O₂N₄ 299.15035, found 299.1501.

7,8-dimethyl-1,3-dipropylalloxazine (2.13c): Prepared in a similar fashion as 13a with 1 g of 11b (4.1 mmol) and $n$-propyliodide (0.91 mL, 9.1 mmol). After drying 1.24 g (93%) of a yellow solid was obtained. $^1$H NMR (500 MHz, CDCl₃) δ 8.06 (s, 1H), 7.80 (s, 1H), 4.41 (s, 2H), 4.16 (t, J = 7.5, 2H), 2.54 (m, J = 7.4, 3H), 2.52 (s, 3H), 1.84 (d, J = 7.4, 2H), 1.80 (s, 2H), 1.06 (m, 3H), 1.03 (m, 3H). $^{13}$C NMR (126 MHz, CDCl₃) δ 160.21 (C), 150.58 (CO), 145.67 (CO), 145.02 (C), 142.75 (C), 140.09 (C), 139.40 (C), 129.65 (CH), 128.92 (C), 127.12 (CH), 44.35 (CH₂), 44.20 (CH₂), 21.38 (CH₃), 21.20 (CH₂), 21.14 (CH₃), 20.61 (CH₃), 11.64 (CH₃), 11.58 (CH₃). HRMS ESI (m/z) [M +] calcd for C₁₈H₂₃O₂N₄ 327.1816, found 327.1814.
7,8-dimethyl-1,3-diisopropylalloxazine (2.13d): Prepared in a similar fashion as 2.13a with 0.5 g of 2.11b (2.1 mmol) and i-propyliodide (0.46 ml, 4.5 mmol). After drying 0.56 g (82%) of a yellow solid was obtained. \( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \) 8.32 (d, 1H), 8.01 (d, 1H), 7.90 (t, 1H), 7.75 (t, 1H), 5.85 (m, 1H), 5.39 (m, 1H), 1.69 (d, \( J = 6.8, 6H \)), 1.61 (d, \( J = 6.8, 6H \)). \( ^{13}C \) NMR (126 MHz, CDCl\(_3\)) \( \delta \) 160.15 (C), 150.75 (CO), 145.53 (CO), 143.18 (C), 139.95 (C), 133.76 (CH), 130.73 (CH), 130.55 (C), 129.15 (CH), 128.00 (CH), 47.85 (s, 7H), 19.90 (CH\(_3\)), 19.73 (CH\(_3\)). HRMS ESI (m/z) [M +] calcd for C\(_{18}\)H\(_{23}\)O\(_2\)N\(_4\) 327.1816, found 327.1815.

7,8-dimethyl-1,3-dibenzylalloxazine (2.13e): Prepared in a similar fashion as 2.13a with 0.5 g of 2.11b (2.1 mmol) and benzylbromide. After drying 0.79 g (89%) of a yellow solid was obtained. \( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \) 8.06 (s, 1H), 7.83 (s, 1H), 7.64 – 7.58 (m, 4H), 7.37 – 7.26 (m, 6H), 5.65 (s, 2H), 5.39 (s, 2H), 2.56 (s, 3H), 2.52 (s, 3H). \( ^{13}C \) NMR (126 MHz, CDCl\(_3\)) \( \delta \) 160.16 (C), 150.80 (CO), 146.06 (CO), 144.85 (C), 142.55 (C), 140.46 (C), 139.63 (C), 136.78 (C), 136.64 (C), 129.77 (CH), 129.64 (CH), 129.28 (CH), 128.85 (C), 128.78 (CH), 128.76 (CH), 128.18 (CH), 128.07 (CH), 127.11 (CH), 45.79 (CH\(_2\)), 45.72 (CH\(_2\)), 21.20 (CH\(_3\)), 20.64 (CH\(_3\)). HRMS ESI (m/z) [M +] calcd for C\(_{28}\)H\(_{23}\)O\(_2\)N\(_6\) 423.1816, found 423.1813.

7,8-dimethyl-1,3-bis(pyridin-2-ylmethyl)alloxazine (2.13f): Prepared in a similar fashion as 2.13a with 0.5 g of 2.11b (2.1 mmol) and 2-picoly chloride hydrochloride (4.6 mmol, 0.75 g). After drying, 0.70 g (79%) of a yellow solid was obtained. \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.73 (m, 4H), 8.53 (d, \( J = 8.4, 1H \)), 8.20 (d, \( J = 7.9, 1H \)), 8.11 (t, \( J = 6.8, 1H \)), 7.98 (t, \( J = 6.7, 1H \)), 7.67 – 7.53 (m, 4H), 5.82 (s, 1H), 5.54 (s, 1H). \( ^{13}C \) NMR (126 MHz, CDCl\(_3\)) \( \delta \) 159.82 (C), 150.76, 150.69 (CO), 145.15 (CO), 143.46 (C), 140.43 (C), 136.57 (C), 136.47 (C), 134.23 (CH), 131.06 (CH), 129.99 (CH), 129.81 (CH), 129.56 (CH), 129.32 (CH), 128.82 (CH), 128.79 (CH), 128.28 (CH), 128.17 (CH), 128.14 (CH), 45.92 (CH\(_2\)), 45.87 (CH\(_2\)). HRMS ESI (m/z) [M +] calcd for C\(_{12}\)H\(_{11}\)O\(_2\)N\(_6\) 243.0875, found 243.0877.

7,8-dimethyl-1,3-bis(4-methoxybenzyl)alloxazine (2.13h): Prepared in a similar fashion as 2.13a with 0.5 g of 2.11b (2.1 mmol) and 4-Methoxybenzyl chloride (0.9 g, 4.6 mmol). After drying, 0.75 g (84%) of a yellow solid was obtained. \( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \) 8.06 (s, 1H), 7.84 (s, 1H), 7.61 (d, \( J = 8.4, 1H \)), 7.37 – 7.26 (m, 2H), 7.11 (d, \( J = 8.6, 0H \)), 6.90 (t, \( J = 11.0, 2H \)), 6.85 (dt, \( J = 9.0, 3.4, 4H \)), 5.57 (s, 2H), 5.32 (s, 2H), 4.63 (d, \( J = 20.7, 1H \)), 4.49 (s, 1H), 3.83 (s, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 2.57 (s, 3H), 2.53 (s, \( J = 7.4, 3H \)). \( ^{13}C \) NMR (126 MHz, CDCl\(_3\)) \( \delta \) 160.15, 159.49, 159.39, 150.76, 145.95, 144.82, 142.51, 140.35, 139.56, 131.43, 131.02, 130.66, 129.98, 129.71, 129.65, 128.97, 128.93, 127.09, 114.15, 114.04, 114.01, 114.00, 71.70, 65.32, 55.55,
55.52, 55.49, 45.19, 45.11, 21.20, 20.63. HRMS ESI (m/z) [M +] calcd for C_{28}H_{27}O_{4}N_{4} 483.2027, found 483.2027.

7,8-dimethyl-1,3-bis(4-(trifluoromethyl)benzyl)alloxazine (2.13i): Prepared in a similar fashion as 2.13a with 110 mg of 2.11b (0.45 mmol) and 1-(chloromethyl)-4-(trifluoromethyl)benzene (244 mg, 1 mmol). After drying, 240 mg (91%) of a yellow solid was obtained. {\textsuperscript{1}H} NMR (500 MHz, CDCl_{3}) δ 8.08 (s, 1H), 7.82 (s, 1H), 7.72 (dd, J = 13.3, 8.1, 4H), 7.60 (dd, J = 8.0, 3.7, 4H), 5.69 (s, 2H), 5.43 (s, 2H), 2.57 (s, 3H), 2.53 (s, 3H). \textsuperscript{13}C NMR (126 MHz, CDCl_{3}) δ 160.02, 150.73, 146.62, 144.58, 142.47, 140.95, 140.53, 139.84, 130.45, 130.31, 130.19, 130.02, 129.70, 129.45, 128.56, 127.04, 125.94 – 125.61 (m), 125.33 (d, J = 4.8), 123.16 (d, J = 4.8), 45.40 (s, CH_{2}), 45.38 (CH_{2}), 21.24 (CH_{3}), 20.66 (CH_{3}). HRMS ESI (m/z) [M +] calcd for C_{28}H_{27}O_{4}N_{4} 483.2027, found 483.2027.

7,8-dimethyl-1,3-bis(3,5-bis(trifluoromethyl)benzyl)alloxazine (2.13j): Prepared in a similar fashion as 2.13a with 173 mg of 2.11b (0.73 mmol) and 1-(chloromethyl)-3,5-bis(trifluoromethyl)benzene (420 mg, 1.6 mmol). After drying, 446 mg (88%) of a pale yellow solid was obtained. {\textsuperscript{1}H} NMR (500 MHz, CDCl_{3}) δ 8.17 (s, 2H), 8.13 (s, 2H), 8.09 (s, 1H), 7.84 (s, 2H), 7.81 (s, 1H), 5.74 (s, 2H), 5.48 (s, 2H), 2.59 (s, 3H), 2.54 (s, 3H). \textsuperscript{13}C NMR (126 MHz, CDCl_{3}) δ 159.78, 150.77, 147.13, 144.33, 142.34, 141.29, 140.01, 138.72, 138.49, 132.16 (qd, J = 33.4, 7.0, CF_{3}), 130.29, 129.78, 128.47, 126.78, 124.43, 122.53, 45.17 (CH_{2}), 45.04 (CH_{2}), 21.26 (CH_{3}), 20.60 (CH_{3}). HRMS ESI (m/z) [M +] calcd for C_{30}H_{19}O_{2}N_{4}F_{12} 695.1311, found 695.1314.

pyrido[2,3-g]pteridine-2,4(1H,3H)-dione (2.15a) and pyrido[3,2-g]pteridine-2,4(1H,3H)-dione (2.15b): 2,3-Diaminopyridine (1.0 g, 9.2 mmol) was dissolved in 20 mL acetic acid. A mixture of alloxane (1.48 g, 9.16 mmol) and H_{2}BO_{3} (0.62 g; 10.0 mmol) in 30 mL of hot acetic acid was added to this solution. The reaction mixture was stirred for 75 min at room temperature. The precipitate was filtered off and washed with water. Drying gave 2.15a as a yellow solid (1.6 g; 81%). The ratio of the products was determined by \textsuperscript{1}H-NMR to be 40:60. \textsuperscript{1}H NMR (500 MHz, DMSO-d_{6}) δ 12.13 (s, 2H), 11.86 (s, 2H), 9.19 (m, 1H), 9.13 (m, 1H), 8.63 (d, J = 8.3, 1H), 8.38 (d, J = 8.4, 1H), 7.95 – 7.91 (m, 1H), 7.83 -7.80 (m, 1H). \textsuperscript{13}C NMR (126 MHz, DMSO-d_{6}) δ 160.13, 159.66, 156.83, 152.79, 150.36, 150.08, 148.97, 147.53, 147.11, 138.89, 138.81, 136.03, 134.03, 133.71, 132.70, 127.92, 124.52, 123.89. HRMS ESI (m/z) [M +] calcd for C_{9}H_{6}O_{2}N_{5} 216.0516, found 216.0515.
pyrido[3,4-g]pteridine-2,4(1H,3H)-dione (2.17a) and pyrido[4,3-g]pteridine-2,4(1H,3H)-dione (2.17b): 3,4-Diaminopyridine, (0.5 g; 4.6 mmol) was dissolved in 20 mL acetic acid. A mixture of alloxane (0.74 g, 4.6 mmol) and H3BO3 (0.31g, 5 mmol) in 30mL hot acetic acid was added to this solution. The reaction mixture was stirred for 75 min at room temperature. The precipitate was filtered and washed with acetic acid and diethylether. Drying gave 2.17 as a yellow solid (0.77 g; 78%); The ratio of the products 2.17a and 2.17b was determined by 1H-NMR to be 45:55.  

1H NMR (500 MHz, DMSO-d6) δ 12.24 (s, 1H), 11.90 (s, 2H), 9.48 (s , 1H), 9.37 (s, 1H), 8.81 (d, J = 5.9, 1H), 7.88 (s, 1H), 7.71 (dd, J = 8.5, 3.9, 1H), 7.65 (dd, J = 8.4, 4.2, 1H), 3.76 (s, 3H), 3.70 (s, 3H), 3.48 (s, 3H), 3.45 (s, 3H). 13C NMR (126 MHz, CDCl3) δ 159.20, 59.12, 158.31, 154.19, 150.97, 150.73, 150.33, 150.06, 148.67, 146.45, 145.89, 142.04, 137.78, 136.75, 135.64, 134.42, 122.28, 120.32. HRMS ESI (m/z) [M+H]+ calcd for C21H16O2N5 244.0834, found 244.0823.

1,3-dimethylpyrido[2,3-g]pteridine-2,4(1H,3H)-dione (2.18a) and 1,3-dimethylpyrido[3,2-g]pteridine-2,4(1H,3H)-dione (2.18b): A mixture of 2.17a and 2.17b (1.0 g, 4.6 mmol) and K2CO3 (3.3 g, 23 mmol) were added to 20 mL dry DMF. MeI (0.7 mL; 11 mmol) was added to the mixture and the reaction was stirred for 16 h at room temperature. 50 mL water was added to the reaction mixture followed by extraction with 3x 25 ml CHCl3. The combined organic layers were dried over MgSO4, filtered and the solvent was removed under reduced pressure. Drying gave a mixture of 2.18a and 2.18b as a greenish solid (0.68 g; 61%). The ratio of and 2.18a and 2.18b was determined by 1H NMR to be 60:40. 1H NMR (400 MHz, CDCl3) δ 9.12 (m, 1H), 9.05 (m, 1H), 8.57 (d, J = 8.4, 1H), 8.26 (d, J = 8.5, 1H), 7.88 (s, 1H), 7.71 (dd, J = 8.5, 3.9, 1H), 7.65 (dd, J = 8.4, 4.2, 1H), 3.76 (s, 3H), 3.70 (s, 3H), 3.48 (s, 3H), 3.45 (s, 3H). 13C NMR (126 MHz, CDCl3) δ 159.20, 159.12, 158.31, 154.19, 150.97, 150.73, 150.67, 148.27, 148.00, 146.13, 139.90, 139.87, 136.92, 135.29, 131.95, 131.09, 128.38, 125.07, 30.36 (CH), 30.25 (CH), 29.63 (CH), 29.61 (CH). HRMS ESI (m/z) [(M+H)+] calcd for C11H16O2N5 244.0834, found 244.0823.

1,3-dimethylpyrido[3,4-g]pteridine-2,4(1H,3H)-dione (2.19a) and 1,3-dimethylpyrido[4,3-g]pteridine-2,4(1H,3H)-dione (2.19b): A mixture of 2.17a and 2.17b (0.5 g; 2.3 mmol) and K2CO3 (1.7 g; 12 mmol) were added to 20 mL dry DMF. MeI (0.35 mL; 5.5 mmol) was added to the mixture and the reaction was stirred for 16 h at room temperature. 50 mL water was added to the reaction mixture followed by extraction with 3x 25 ml CHCl3. The combined organic layers were dried over MgSO4, filtrated and the solvent was removed under reduced pressure. Drying gave a mixture of 2.19a and 2.19b as a dark green solid (307 mg, 55%). The ratio of and 2.19a and 2.19b was determined by 1H NMR to be 46:54. 1H NMR (400 MHz, CDCl3) δ 9.59 (s, 1H), 9.44 (s, 1H), 8.77 (d, J = 6.0, 1H), 8.74 (d, J = 5.8, 2H), 8.04 (d,
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\[ J = 5.8, 1H \], 7.75 (d, \( J = 5.9, 1H \)), 3.76 (s, 3H), 3.74 (s, 3H), 3.51 (s, 6H). HRMS ESI (m/z) [(M+H)+] calcd for C\(_{11}\)H\(_{10}\)O\(_2\)N\(_5\) 244.0834, found 244.0823.

naphtho[2,3-g]pteridine-2,4(1H,3H)-dione (2.21):

\[
\begin{align*}
\text{2,3-Diaminonaphthalene (0.5 g; 3.16 mmol) was dissolved in 20 mL acetic acid. A mixture of alloxane (0.51 g; 3.16 mmol) and H}_3\text{BO}_3 (214 mg; 3.5 mmol) in 30 mL hot acetic acid was added to this solution. The reaction mixture was stirred for 16 h at room temperature. The precipitate was filtered off and washed with water. Drying gave 2.21 (431 mg, 52%) as a red solid.} \\
\text{1H NMR (500 MHz, DMSO-d6) \( \delta \) 12.25 - 11.40 (m, 1H), 7.62 (m, 1H).}
\end{align*}
\]

1,3-dimethylnaphtho[2,3-g]pteridine-2,4(1H,3H)-dione (2.24): 2.21 (200 mg; 0.75 mmol) and K\(_2\)CO\(_3\) (660 mg; 4.7 mmol) were added to 20 mL dry DMF. Mel (0.1 mL; 1.6 mmol) was added to the mixture and the reaction mixture was stirred for 2 h at room temperature. 50 mL water was added to the reaction mixture and the precipitate was filtered and washed with water. No further purification was necessary. Drying gave 2.24 as an orange solid (195 mg; 89%). 1H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 8.94 (s, 1H), 8.54 (s, 1H), 8.08 (m, 2H), 7.60 (m, 2H), 3.83 (s, 3H), 3.59 (s, 3H). 13C NMR (126 MHz, CDCl\(_3\)) \( \delta \) 159.87 (C), 150.92 (CO), 144.66 (CO), 138.87 (C), 137.16 (C), 136.65 (C), 133.45 (C), 131.55 (C), 130.81 (CH), 129.46 (CH), 128.96 (CH), 128.25 (CH), 126.97 (CH), 125.9 (CH), 29.98 (CH\(_2\)), 29.55 (CH\(_2\)). HRMS ESI (m/z) [M +] calcd for C\(_{16}\)H\(_{13}\)O\(_2\)N\(_4\) 293.1033, found 293.1032.

7,8'-bibenzo[g]pteridine-2,2',4,4'(1H,1'H,3H,3'H)-tetraone (2.23a), 7,7'-bibenzo[g]pteridine-2,2',4,4'(1H,1'H,3H,3'H)-tetraone (2.23b) and 8,8'-bibenzo[g]pteridine-2,2',4,4'(1H,1'H,3H,3'H)-tetraone (2.23c):

3,3'-Diaminobenzidine (1.0 g; 4.7 mmol) was dissolved in 30 mL acetic acid. A mixture of alloxan (0.75 g; 4.7 mmol) and H\(_2\)BO\(_3\) (3.16 g; 5.1 mmol) in 200 mL hot acetic acid was added to this solution. The reaction mixture was stirred for 16 h at room temperature. The precipitate was filtered off and washed with water. Drying gave 2.21 (1.7 g, 85% yield) as a dark brown solid. 1H NMR (500 MHz, DMSO-d6 at 100 ºC) 8.71 - 8.61 (m, 1H), 8.53 - 8.43 (m, 1H), 8.27 (d, \( J = 8.4, 1H \)), 8.20 (d, \( J = 8.7, 1H \)), 7.69 (m, 1H), 7.62 (m, 1H).
Synthesis of and measurements on flavins and flavin catalyst

(2.25a), 1,1',3,3'-tetramethyl-7,8'-bibenzo[g]pteridine-2,2',4,4'(1H,1'H,3H,3'H)-tetrone
(2.25b) and 1,1',3,3'-tetramethyl-8,8'-bibenzo[g]pteridine-2,2',4,4'(1H,1'H,3H,3'H)-tetrone (2.25c):

A mixture of 2.23a-c (0.5 g; 1.0 mmol) and K$_2$CO$_3$ (0.8 g; 6 mmol) were added to 20 mL dry DMF. MeI (0.35 mL; 5.5 mmol) was added to the mixture and the reaction was stirred for 16 h at room temperature. 50 mL water was added to the reaction mixture followed by extraction with 3 x 25 mL CHCl$_3$. The combined organic layers were dried over MgSO$_4$, filtrated and the solvent was removed under reduced pressure. Drying gave a mixture of 2.23a-c as a dark brown solid (307 mg, 55%) 1H NMR (500 MHz, CDCl$_3$) δ 9.28 (dd, $J = 4.2, 2.0, 1H$), 9.23 (dd, $J = 4.0, 1.9, 1H$), 8.74 (d, $J = 2.0, 1H$), 8.73 (d, $J = 2.0, 1H$), 8.42 (d, $J = 1.9, 1H$), 8.41 (d, $J = 1.9, 1H$), 8.09 (s, 1H), 8.07 (s, 1H), 7.85 (dd, $J = 8.5, 4.1, 3H$), 7.78 (dd, $J = 8.4, 4.2, 1H$), 6.99 (s, 1H), 6.97 (s, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 3.65 (s, 3H), 3.63 (s, 3H), 3.59 (s, 3H). 13C NMR (126 MHz, CDCl$_3$) δ 162.77, 162.52, 159.65, 159.23, 159.13, 158.33, 154.22, 151.01, 150.86, 150.77, 150.70, 148.34, 148.18, 148.03, 147.23, 146.15, 139.94, 139.91, 138.72, 136.94, 135.35, 131.99, 131.12, 130.88, 128.38, 126.18, 125.07, 122.64, 31.70, 31.26, 30.40, 30.27, 30.01, 29.67, 29.65, 29.38, 28.93. Exact mass could not be determined.

2.5.3 Catalysts preparation

1,3-dimethyl-5-ethyl-5,10-dihydroalloxazine (2.14a):

To a roundbottom flask, charged with 50 ml degassed ethanol/water (5:4) was added 200 mg of flavin 2.12a (0.83 mmol). Hydrogen atmosphere was applied and subsequently 80 mg of 10% Pd/C was added. After 5 minutes, 1.2 ml acetaldehyde (21 mmol) and 1.2 ml concentrated hydrochloric acid were added and the resulting suspension was stirred for 48 h. The reaction mixture was filtered over celite in a double Schlenk flask and the residue was washed with degassed ethanol. All volatiles were removed in vacuo to leave 190 mg (84%) of the product 2.14a. as a
Synthesis of and measurements on flavins and flavin catalysts. The product was kept in a reduced state by storage under a nitrogen atmosphere at −18 ºC.

HRMS ESI (m/z) ([M-H]+) calcd for C14H15O2N4 271.1195, found 271.1181. Analytical data as reported.

1,3-diethyl-5-ethyl-5,10-dihydroalloxazine (2.14a): Prepared in a similar fashion as 2.14b, with 200 mg of flavin 2.12b (0.74 mmol), yielding 160 mg (73%) of an orange/red colored powder.

1,3-diethyl-5-ethyl-5,10-dihydroalloxazine (2.14b): Prepared in a similar fashion as 2.14a, with 200 mg of flavin 2.12b (0.74 mmol), yielding 160 mg (73%) of an orange/red colored powder.

1,3-diethyl-5,10-dihydroalloxazine (2.14c): Prepared in a similar fashion as 2.14a, with 200 mg of flavin 2.12c (0.67 mmol), yielding 150 mg (68%) of an orange/red colored powder.

1,3-diethyl-5,10-dihydroalloxazine (2.14d): Prepared in a similar fashion as 2.14a, with 200 mg of flavin 2.12d (0.67 mmol), yielding 20 mg (9%) of an orange/red powder. Due to insolubility, no indicative 1H and 13C could be obtained. HRMS ESI (m/z) ([M-H]+) calcd for C18H23O2N4 357.1508, found 357.1496.

7,8-dimethyl-1,3-dimethyl-5-ethyl-5,10-dihydroalloxazine (2.15a): Prepared in a similar fashion as 2.14a, with 200 mg of flavin 2.13a (0.74 mmol), yielding 170 mg (76%) of an orange/red powder. Analytical data as reported.

HRMS ESI (m/z) ([M-H]+) calcd for C16H19O2N4 299.1508, found 299.1493.

1,3-diisopropyl-5-ethyl-5,10-dihydroalloxazine (2.14c): Prepared in a similar fashion as 2.14a, with 200 mg of flavin 2.12c (0.67 mmol), yielding 150 mg (68%) of an orange/red colored powder.

1,3-diisopropyl-5-ethyl-5,10-dihydroalloxazine (2.14d): Prepared in a similar fashion as 2.14a, with 200 mg of flavin 2.12d (0.67 mmol), yielding 20 mg (9%) of an orange/red powder. Due to insolubility, no indicative 1H and 13C could be obtained. HRMS ESI (m/z) ([M-H]+) calcd for C20H27O2N4 355.2134, found 355.2119.

7,8-dimethyl-1,3-diethyl-5-ethyl-5,10-dihydroalloxazine (2.15b): Prepared in a similar fashion as 2.14a, with 200 mg of flavin 2.13b (0.67 mmol), yielding 190 mg (86%) of an orange/red powder.
Synthesis of and measurements on flavins and flavin catalysts

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.57 (s, 1H), 6.36 (s, 1H), 3.93 (dt, $J$ = 7.1, 5.1, 4H), 3.32 (q, $J$ = 7.0, 2H), 2.02 (s, 3H), 1.24 (t, $J$ = 7.2, 3H), 1.03 (t, $J$ = 7.0, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 152.91 (C), 144.76 (C), 141.08 (C), 128.32 (C), 127.82 (C), 127.78 (C), 126.73 (C), 118.98 (CH), 118.96 (CH), 110.95 (CH), 110.89 (CH)$_{15}$, 118.82 (C), 45.86 (CH$_2$), 32.14 (CH$_2$), 31.73 (CH$_2$), 14.24 (CH$_3$), 14.10 (CH$_3$), 8.79 (CH$_3$), 8.25 (CH$_3$), 6.38 (CH$_3$). HRMS ESI (m/z) [M + H]$^+$ calcd for $C_{18}H_{23}O_{2}N_4$ 327.1899, found 327.1806.

7,8-dimethyl-1,3-dipropyl-5-ethyl-5,10-dihydroalloxazine (2.15c):
Prepared in a similar fashion as 2.14a, with 200 mg of flavin 2.13c (0.61 mmol), yielding 140 mg (64%) of an orange/red colored powder. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 6.56 (s, 1H), 6.40 (s, 1H), 3.85 (m, 4H), 3.32 (q, $J$ = 7.0, 2H), 2.03 (s, 3H), 2.02 (s, 3H), 1.62 (ddd, $J$ = 22.6, 15.0, 7.4, 4H), 1.03 (t, $J$ = 7.0, 3H), 0.91 (t, $J$ = 7.4, 3H), 0.86 (t, $J$ = 7.4, 3H).

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 158.07 (C), 150.11 (C), 146.36 (C), 133.14 (C), 132.73 (C), 132.68 (C), 131.69 (C), 123.87 (CH), 123.84 (CH), 115.94 (CH), 115.90 (CH)$_{16}$, 99.42 (C), 50.78 (CH$_2$), 43.34 (CH$_2$), 43.03 (CH$_2$), 21.77 (CH$_2$), 21.13 (CH$_2$), 19.14 (CH$_3$), 19.01 (CH$_3$), 11.25 (CH$_3$), 11.24 (CH$_3$), 10.95 (CH$_3$). HRMS ESI (m/z) [(M-H)$^+$] calcd for $C_{20}H_{27}O_{2}N_4$ 355.2134, found 355.2119.

7,8-dimethyl-1,3-diisopropyl-5-ethyl-5,10-dihydroalloxazine (2.15d):
Prepared in a similar fashion as 2.14a, with 200 mg of flavin 2.13d (0.61 mmol), yielding 30 mg (14%) of an orange/red powder. Due to insolvability, no indicative $^1$H and $^{13}$C could be obtained. HRMS ESI (m/z) [(M-H)$^+$] calcd for $C_{20}H_{27}O_{2}N_4$ 355.2134, found 355.2119, [(M-H, -2 i-Pr)$^+$] calcd for $C_{14}H_{15}O_{2}N_4$ 271.1195, found 271.1183.

5-ethyl-riboflavin (2.27a). To a roundbottom flask charged with 100 ml degassed ethanol/water (5:4) was added 1.12 g riboflavin (3.0 mmol). Hydrogen atmosphere was applied and subsequently 200 mg of 10% Pd/C was added. After 5 minutes, 5.6 ml acetaldehyde (100 mmol) and 5 ml concentrated hydrochloric acid were added and the resulting suspension was stirred for 48 h. To follow the progress of the reaction, 50 μL samples were taken from the reaction mixture and directly subjected to UV-analysis in 1 mL H$_2$O followed by UV analysis after addition of 50 μL 6N HCl. Completion of the reaction was indicated by the complete disappearance of the signal (shoulder) in the UV spectrum at 441 nm and full dissolution of the starting material in the reaction mixture. The reaction mixture was filtered over celite in a double Schlenk flask and the residue was washed with degassed ethanol. All volatiles were removed in vacuo to leave 1.29 g of the product 2.27a as a pale yellow/pink air sensitive powder. The product was kept in a reduced state by storage under a nitrogen atmosphere at -18 °C or alternatively by adding a small amount of Na$_2$SO$_3$. Upon air oxidation, 2.27a turned red, but was still active as a catalyst. Using the same conditions, this reaction was performed on 10 g scale with comparable results. (HR)MS data indicate acetal bridged dimers ESI (m/z) [M + H]$^+$ calcd
for C₁₉H₂₅O₆N₄ 405.17796, found 405.17802 ESI (m/z) [M +] calcd for C₂₁H₂₇O₆N₄ 431.19361, found 431.19361 ESI (m/z) [M –H] calcd for C₃₉H₄₉N₈O₁₃⁻ 837.3425, found 837.3804. UV spectra of 2.27a are identical to those of 2.27b.

5-ethyl-riboflavin (2.27b). The same procedure was followed omitting the HCl and after a week the reaction was interrupted, remaining starting material was removed by filtration and the product was isolated in 60% yield. ¹H NMR (400 MHz, DMSO-d₆) δ0.97 (br d, 3H,), δ2.05 (br s, 6H), δ2.48 (DMSO) δ3.08 (br s, 1H), δ3.31 (H₂O), δ3.31-3.69 (br m, 8H), δ4.10 (br s, 1H), δ4.54 (br s, 1H), δ4.93 (br s, 1H), δ5.10 (br s, 1H), δ6.26 (br s, 1H), δ6.53 (br s, 1H), δ6.73 (br s, 1H), δ10.32 (br s, 1H), δ10.59(br s, 1H). Mass spectrum (flow injection analysis on LC-MS, ESI (−)) m/z 405. HRMS ESI (m/z) [M +] calcd for C₁₉H₂₅O₆N₄ 405.1780, found 405.1780. UV: 345 p, 378 sh, 508 p, 584 p.

2.6 References and notes

1 Riboflavin and riboflavin-5’-phosphate are food additives approved by the European Union (EU), with the codes E101 (i) and E101 (ii).
4 Except for the pyridyl substituted flavins which are more water soluble.
9 Whereas the ratio of the isomers could not be determined, the ratio of compounds 25a:25b:25c mixture was not found in a ratio of 2:1:1 as is expected if all products are formed at the same rate.
12 The signal of the N⁵ ethyl group appears in the same region as the signal of the ribose chain. Peak broadening as a result of radical species present, however, blur these NMR data. See: Bruice, T.C.; Yano, Y. J. Am. Chem. Soc. 1975, 97, 5263-5271.
While only two signals for the CH’s were expected, we observed four signals. Using $^{13}$C APT it could be confirmed that all four signals correspond to CH carbons. The peak intensity was different with both peak pairs, indicating that the resulting additional peaks are not the effect of a stereoisomer. Different J-Coupling between both peak pairs did indicate a possible higher order coupling. In the $^1$H spectrum however we observed no coupling effects. After discussions with NMR experts, there is still no clear indication why the CH peaks split up. Since we clearly observed this effect in two different flavins it is probably not trivial, but as of yet cannot be explained.