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Model compounds for (6–4) photolyases: a comparative flavin induced cleavage study of oxetanes and thietanes†

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Thietanes were used in the past as mimics for an unstable oxetane intermediate formed during the repair of mutagenic (6–4) lesions. The thietane derivatives were found to be not repaired, raising the question of how well thietanes are cleaved by single electron donation compared to oxetanes. We have prepared two flavin-containing oxetane and thietane model compounds for the (6–4) photolyase catalyzed repair process and we show that both are efficiently cleaved by a reduced and deprotonated flavin. Thietanes are therefore excellent models. The lack of their repair can be attributed to lack of binding.

Introduction

UV irradiation of cells leads to severe damage of the genome.[1,2] The main lesions formed are cyclobutane–pyrimidine dimers (CPD) and (6–4) photoproducts.[3] The (6–4) lesion is presumably more mutagenic than the CPD lesion.[4,5] It is formed in a Paternó–Büchi reaction between adjacent pyrimidine bases giving first an oxetane intermediate, which is thermally not stable and rearranges above −80 °C to the (6–4) photo adduct (Fig. 1a).[6] The lesion is repaired in certain organisms by a particular enzyme called (6–4) photolyase.[7] The enzyme converts the photoproduct back into the parent pyrimidines *via* a light-dependent process. For the homologous CPD photolyases,[8] which are responsible for the repair of the UV-induced CPD lesions, it is known that they contain a reduced and deprotonated flavin ($FADH^-$). The flavin, upon excitation by light, donates an electron to the CPD and cleaves (cycloreverts) the lesion.[9] This mechanism is well understood through crystallographic,[10–12] enzymatic[13,14] and model compound studies.[15–18] In contrast, the repair process catalyzed by the (6–4) photolyase is less well characterized and structural information is lacking.[19,20] Due to the high sequence homology between CPD and (6–4) photolyases and the fact that both enzymes contain a flavin as the essential cofactor, Kim *et al.* proposed the (6–4) repair mechanism depicted in Fig. 1a.[21] They postulate that the binding of the enzyme causes a thermal rearrangement of the (6–4) lesion back to the oxetane intermediate. Although calculations predict that this process may be energetically very costly to proceed spontaneously in the active site,[22] Todo *et al.* identified two histidine residues which might catalyze the rearrangement by a protonation/deprotonation process.[23]

The key step of the proposed repair mechanism is the electron transfer-induced cleavage of the oxetane intermediate by a light-induced electron transfer. Quantum chemical calculations (in the gas phase) predict that oxetanes cleave spontaneously after single electron donation or after single electron abstraction.[24] Laser flash photolysis experiments by Falvey and coworkers[25] and Miranda *et al.*[26] with model compounds showed that the cleavage of oxetanes is indeed possible with different electron donors and acceptors in a light-dependent reaction. With the model compound **1** (Scheme 1) we demonstrated recently that a covalently-linked flavin is also able to split the oxetane upon irradiation when the flavin is in the reduced and deprotonated state providing strong support for the repair mechanism proposed by Kim *et al.*[21]

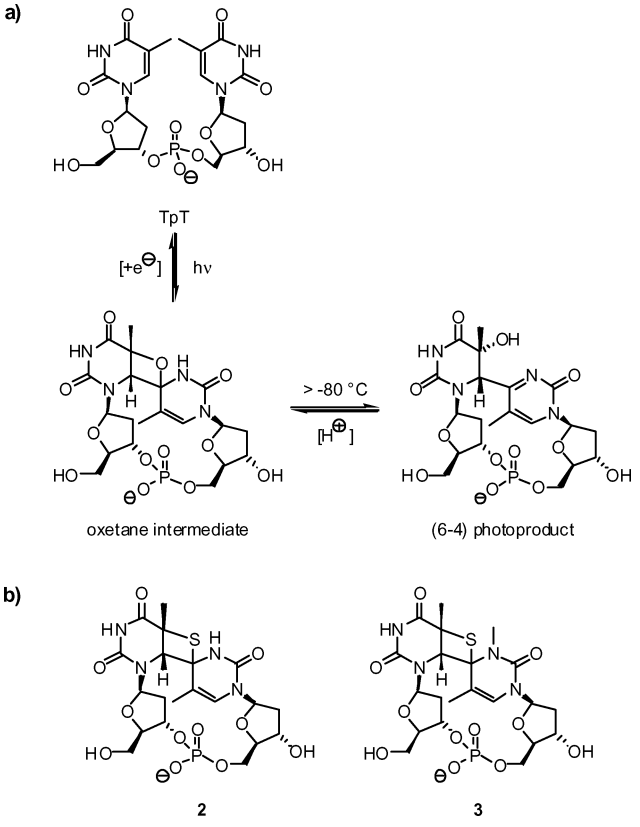

Fig. 1 a) Depiction of the formation and repair of the mutagenic (6–4) photoproduct *via* the oxetane intermediate. b) Stable thietane analogues of the repair intermediate used in enzymatic studies.

In order to investigate the enzymatic repair reaction, Clivio and Fourrey[28–32] and Taylor and Liu[33] prepared the stable thietanes **2** and **3** (Fig. 1b), which were designed to mimic the key oxetane intermediate of the repair reaction. Sancar and co-workers[20] tested these stable thio-analogues and found that both are not repaired by the enzyme due to a lack of binding.

† Electronic supplementary information (ESI) available: MALDI-TOF mass spectra of collected HPLC-peaks. See http://www.rsc.org/suppdata/ob/b5/b503205a/

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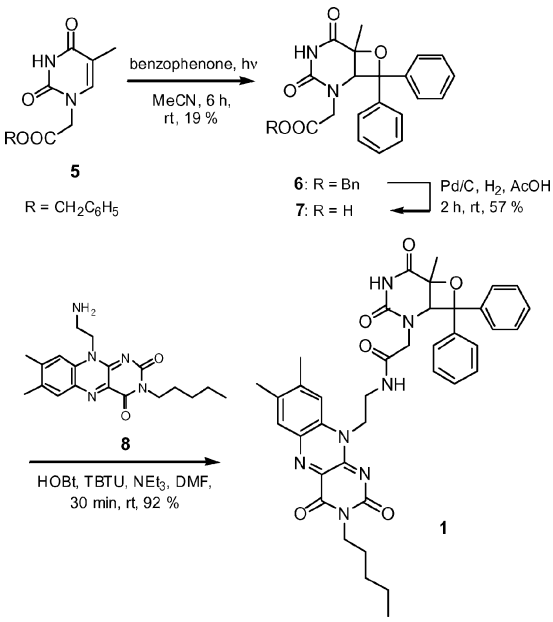
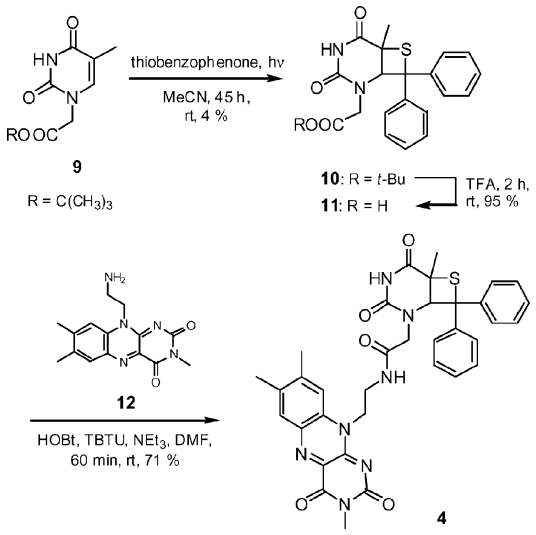


Scheme 1 Synthesis of oxetane model compound **1**.



Scheme 2 Synthesis of thietane model compound **4**.

These results challenge the mechanism and raise three main questions: 1) are thio-analogues of (6–4) lesions suitable models for the (6–4) repair intermediate? 2) Is it possible to cleave a thietane with flavin in an analogous reaction to that of an oxetane? 3) Are thietanes cleaved preferentially by single electron oxidation or reduction? In order to address these three critical questions we synthesized the thio-analogue **4** of our initial model compound **1** and compared both model compounds **1** and **4** in irradiation experiments.

Results and discussion

The covalently linked flavin- and oxetane-containing model compound **1** is able to mimic the critical putative cycloreversion step, catalyzed by (6–4) photolyase with relatively high quantum yield ($\phi = 0.11$).[19] The splitting of the oxetane is only possible *via* the reductive pathway. The cycloreversion is induced by single electron donation to the oxetane from a flavin in the reduced and deprotonated state. The synthesis of this model compound is depicted in Scheme 1. Irradiation of **5** in the presence of benzophenone furnished the stable oxetane **6**. Hydrogenolytic cleavage of the benzyl ester and condensation of the obtained oxetane acid **7** with 10-amino-ethylflavin **8**[18,34] gave model compound **1** in the form of a yellow powder.[27]

The thietane model compound **4** was prepared in a similar way (Scheme 2). Irradiation of **9** in the presence of thiobenzophenone, which was synthesized from benzophenone with the Lawesson-reagent,[35] yielded the stable thietane **10**. The irradiation was repeated five times because of the low reaction yield. Deprotection of the *tert*-butyl ester of **10** with trifluoroacetic acid and coupling of the thietane acid **11** with 10-amino-ethylflavin **12** furnished the model compound **4** in the form of a yellow solid.

To allow a better separation by HPLC of the model compounds **1** and **4** and of the putative cleavage products **13** and **14** (Fig. 2b), the flavins in **1** and **13** were prepared with a pentyl side chain at N3. In contrast, the flavins in **4** and **14** possess a methyl group at N3.

Irradiation experiments were performed with solutions of a mixture of both model compounds (**1** + **4**) in ethylene glycol (10^{-4} M), under anaerobic conditions, in a 500 µL fluorescence cuvette stoppered with a rubber septum to allow direct comparison of both compounds. The flavins were reduced and deprotonated by addition of sodium dithionite solution (100 µL, 0.06 M) and triethylamine (60 µL). Irradiation was carried out in a fluorimeter with monochromatic light (366 ± 5 nm). During the experiments, samples were taken from the assay solution after defined time intervals with a microsyringe. These samples were subsequently reoxidized through shaking with exposure to air for at least 1 h. The samples were analyzed by reversed phase HPLC. The result of a typical irradiation experiment is depicted in Fig. 2a. The chromatogram

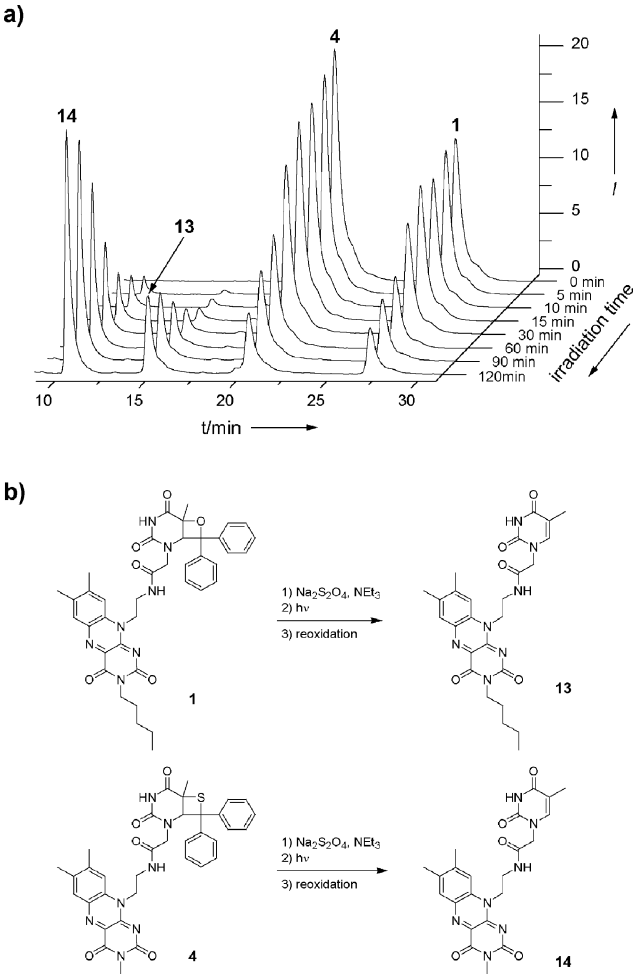


Fig. 2 a) Chromatograms (HPLC) obtained after irradiation of a mixture of the model compounds **1** and **4**. Elution times **14**: 10.7 min, **13**: 15.1 min, **4**: 20.5 min, **1**: 27.1 min. Conditions: Nucleosil RP-C18 (5 µm) column (4 × 250 mm), detection at 450 nm, gradient: A = water, B = acetonitrile, 40–60% B over 25 min, flow rate: 0.7 mL min^{-1}. b) Cleavage reaction of **1** and **4** to give **13** and **14**, respectively.

before irradiation (0 min) shows the mixture of the two model compounds **1** and **4**. Due to the different alkylchains at N3 of the flavins, they elute with very different retention times at 27.1 min (oxetane model compound **1**) and 20.5 min (thietane model compound **4**). After 5 min of irradiation two new peaks elute at 15.1 min and 10.7 min. These peaks increase in intensity during irradiation, while the amount of the model compounds **1** and **4** decrease. The two new peaks are the reaction products (**13** + **14**) of the splitting reaction of compound **1** and **4**. The peak at 15.1 min corresponds to the cleavage product **13**, arising from the oxetane **1**, and the peak at 10.7 min corresponds to the cleavage product **14** from the thietane **4**. This assignment was confirmed by co-injection of both independently synthesized cleavage products (**13** + **14**) and by mass spectrometry. For the MS-analysis all four peaks from the HPLC runs were collected and measured by MALDI-TOF (mass spectra in the supplementary material†). To exclude intermolecular processes the irradiation experiments were repeated with solutions containing the single model compounds. They gave the same results for the cleavage efficiency as in the comparative irradiations.

To investigate if the thietane cleavage required a reduced and deprotonated flavin further irradiation studies were performed. We observed no detectable cleavage with the flavin in the oxidized state or under reductive but acidic conditions, which gives the reduced but neutral flavin FlH_2. The irradiation of thietane **10**, without a flavin, in the presence of sodium dithionite and base gave also no cleavage of the four-membered ring. These control experiments demonstrate that the flavin is strictly needed in its reduced and deprotonated state (FlH^-) to efficiently cleave model compound **4**.

Our experiments show that the reductive splitting reaction of the four-membered ring does not depend on the nature of the heteroatom in the ring. This leads to the conclusion that the thio-analogue compounds, used in the enzyme assay of Sancar *et al.*,[20] should in principle be cleavable with the reduced flavin cofactor present in the (6–4) photolyase. These results confirm the observation from the enzymatic study that the thio-analogue compounds **2** and **3** were not repaired because they were not bound by the (6–4) photolyase. This allows us to finally assume that the enzyme can recognize only the (6–4) lesion and not the oxetane intermediate directly. The lesion then requires rearrangement. It is interesting to note that in our irradiation experiments the thietane is cleaved, by a factor of two, more efficiently.

Conclusions

Two model compounds **1** and **4** were successfully synthesized, containing a stable oxetane and a stable thietane respectively, both covalently linked to flavin moieties. The model compounds mimic the critical intermediate postulated during the repair of UV photo lesions catalyzed by (6–4) photolyase. With these model compounds we show that upon irradiation only the reduced and deprotonated flavin is able to cleave the oxetane and the thietane ring. The result suggests that, in agreement with the proposed mechanism from Kim and co-workers,[21] electron transfer proceeds from the flavin to the oxetane as the key step of the repair reaction. In addition, the cleavage of the thietane model compound under the same conditions shows that the thio-analogues of the (6–4) photoproduct previously used by Sancar *et al.*[20] are suitable model systems. The fact that the repair was not observed is due to a lack of binding and not because thietanes are uncleavable by single electron donation.

Experimental

General

Melting points are uncorrected. ^{1}H-NMR spectra were recorded on Bruker ARX 200 (200 MHz), AMX 300 (300 MHz) and Varian XL400 (400 MHz) spectrometers. The chemical shifts were referenced to DMSO (δ = 2.50 ppm) in DMSO-d_6 and CH_3OH (δ = 3.31 ppm) in CD_3OD. ^{13}C-NMR spectra were recorded on Bruker ARX 200 (50 MHz), AMX 300 (75 MHz), AMX 500 (125 MHz), AMX 600 (150 MHz) and Varian XL400 (100 MHz) spectrometers. The chemical shifts were referenced to DMSO (δ = 39.43 ppm) in DMSO-d_6 and CH_3OH (δ = 49.05 ppm) in CD_3OD. Standard pulse sequences were employed for ^{1}H 2D NOESY, ^{1}H,^{1}H and ^{1}H,^{13}C correlation studies. IR spectra were recorded in KBr and measured with a Bruker IFS 25 Fourier transform infrared spectrophotometer and Perkin-Elmer FT-IR spectrum 100. Mass spectra and high-resolution mass spectra were measured on Finnigan MAT TSQ 700, Finnigan MAT 95, Finnigan MAT 90 and Bruker Autoflex II (MALDI-TOF) mass spectrometers. HPLC was performed with a Merck-Hitachi system equipped with L-7400 UV and L7480 fluorescence detectors. Analytical separations were performed with a Macherey-Nagel Nucleosil 100–5 C18 (250 × 4 mm) column.

All solvents were of the quality puriss. p.a., or purum. Purum solvents were distilled prior to use. The commercially available reagents were used as received without further purification.

Irradiation experiments

A stirred solution of **1** (10^{-4} M) and **4** (10^{-4} M) in ethylene glycol in a fluorescence cuvette, stoppered with a rubber septum, was degassed by bubbling N_2 through the solution for 30 min to ensure anaerobic conditions. For reduction and deprotonation of the flavin, sodium dithionite (100 μL, 0.06 M in water) and triethylamine (60 μL) were added and the solution stirred for further 30 min. Both procedures were carried out under the exclusion of light. The irradiation was carried out within a fluorescence spectrometer (JASCO-FP-750) at 366 nm (±5 nm) with constant flow of nitrogen through the solution. During irradiation samples (20 μL) were taken from the assay solution after defined time intervals *via* a microsyringe and diluted with water (20 μL). Subsequent reoxidation of the flavin was achieved through shaking and exposure to air for at least 1 h under exclusion of light. The samples were analyzed by reversed phase HPLC under the following conditions: Nucleosil RP-C18 (5 μm) column (4 × 250 mm); A = water, B = acetonitrile; 40–60% B over 25 min; detection at 450 nm, flow rate: 0.7 mL min^{-1}.

(6-Methyl-3,5-dioxo-8,8-diphenyl-7-oxa-2,4-diaza-bicyclo-[4.2.0]oct-2-yl)-acetic acid benzyl ester 6. Thymine acetic acid benzyl ester **5** (2.03 g, 7.40 mmol) was dissolved in acetonitrile (200 mL) under sonication. Benzophenone (2.70 g, 14.8 mmol) was added and the solution was degassed by bubbling N_2 through the solution for 0.5 h. The solution was subsequently irradiated for 6 h with a 150 W (TQ-150) medium-pressure mercury lamp in a quartz irradiation apparatus. Acetonitrile was removed *in vacuo* and the product was isolated by flash chromatography (ethyl acetate–*n*-hexane 2 : 8 to 1 : 1). Recrystallisation from ethyl acetate–*n*-hexane (2 : 8) gave **6** (645 mg, 19%) as a white solid. Mp: 158 °C; ν_{max}(KBr)/cm^{-1} = 3445w, 3193w, 3067w, 2931w, 2876w, 1747m, 1726s, 1692s, 1479m, 1448w, 1409w, 1391w, 1370w, 1360w, 1287w, 1253w, 1208m, 1192m, 974w, 833w, 782w, 748w, 727w, 696m, 645w, 596w, 512w; ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): δ = 1.50 (s, 3H; CH_3), 4.09 (d, J = 17.6 Hz, 1H; N–CH_aH), 4.38 (d, J = 17.3 Hz, 1H; N–CHH_b), 4.97 (s, 1H; CH), 5.11 (d, J = 12.6 Hz, 1H; O–CH_aH), 5.17 (d, J = 12.3 Hz, 1H; O–CHH_b), 7.19–7.42 (m, 15H; aryl-H), 10.48 (s, 1H; NH); ^{13}C-NMR (125 MHz, $(CD_3)_2SO$): δ = 24.43, 66.30, 67.69, 69.39, 77.39, 92.22, 126.37 (2C), 126.72 (2C), 128.87 (2C), 129.36 (2C), 129.47 (2C), 129.69 (2C), 129.73 (3C), 136.92, 140.87, 145.65, 152.57, 169.77, 171.36; m/z (ESI$^+$): 479 (100%, M + Na^+); HRMS (ESI$^+$): calc. for $C_{27}H_{24}N_2O_5Na$ (M + Na^+): 479.1583, found: 479.1594.

(6-Methyl-3,5-dioxo-8,8-diphenyl-7-oxa-2,4-diaza-bicyclo-[4.2.0]oct-2-yl)-acetic acid 7. To a stirred solution of **6**

(833 mg, 1.83 mmol) in glacial acetic acid (10 mL) was added a suspension of 10% Pd/C (20 mg, 0.02 mmol) in glacial acetic acid (2 mL). The solution was stirred under a H_2 atmosphere for 2 h at atmospheric pressure. The reaction mixture was filtered through celite and the filter cake was washed with hot acetic acid (80 mL). The solvent was removed *in vacuo*. Recrystallisation from ethyl acetate–*n*-hexane (2 : 3) afforded **7** (384 mg, 57%) as a white solid. Mp: 210 °C; ν_{max}(KBr)/cm^{-1} = 3451w, 3188w, 3061m, 1725s, 1666m, 1492m, 1448w, 1405w, 1391w, 1356w, 1287w, 1256w, 1228w, 1130w, 1082w, 1030w, 996w, 974w, 956w, 939w, 921w, 891w, 807w, 783w, 748w, 704w, 674w, 663w, 640w, 597w, 534w, 513w, 497w, 451w; ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): δ = 1.63 (*s*, 3H; CH_3), 3.91 (d, *J* = 17.6 Hz, 1H; N–CH_aH), 4.30 (d, *J* = 17.6 Hz, 1H; N–CHH_b), 4.94 (s, 1H; C*H*), 7.28–7.47 (m, 10H; aryl-*H*), 10.49 (s, 1H; N*H*), 12.93 (bs, 1H; CO_2H); ^{13}C-NMR (150 MHz, $(CD_3)_2SO$): δ = 23.25, 47.90, 65.16, 76.08, 90.84, 124.96 (2C), 125.43 (2C), 127.50 (2C), 128.07 (2C), 128.39 (2C), 139.61 (2C), 144.48, 151.17, 170.04; *m*/*z* (ESI$^+$): 389.7 (70%, M + Na$^+$), HRMS (ESI$^+$): calc. for $C_{20}H_{18}N_2O_5Na$ (M + Na$^+$): 389.1113, found: 389.1127.

***N*-[2-(7,8-Dimethyl-2,4-dioxo-3-pentyl-3,4-dihydro-2*H*-benzo-[*g*]pteridin-10-yl)-ethyl]-2-(6-methyl-3,5-dioxo-8,8-diphenyl-7-oxa-2,4-diaza-bicyclo[4.2.0]oct-2-yl)-acetamide 1.** Oxetane **7** (100 mg, 0.27 mmol), 1-hydroxybenzotriazole (HOBt) (44 mg, 0.32 mmol) and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium tetrafluoroborate (TBTU) (104 mg, 0.32 mmol) were dissolved in anhydrous DMF (3.6 mL) and stirred for 10 min at room temperature. A solution of flavin **8** (141 mg, 0.03 mmol) in anhydrous DMF (3 mL) and triethylamine (2 mL, 14.3 mmol) were added. The solution was stirred for 30 min at room temperature. The reaction was diluted with chloroform (100 mL), washed with water (3 × 100 mL), dried (magnesium sulfate), filtered and the solvent was removed *in vacuo*. Diethyl ether was added to the orange–red oil, the precipitate was filtered and dried under a reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$–MeOH 20 : 1) and afforded **1** (174 mg, 92%) as an orange solid. Mp: 170 °C; ν_{max}(KBr)/cm^{-1} = 3413w, 3061w, 2954w, 2859w, 1709m, 1657m, 1585m, 1548s, 1463m, 1448m, 1407w, 1383w, 1339w, 1268m, 1230m, 1206m, 1080w, 1017w, 974w, 914w, 807w, 750w, 703w, 668w, 596w, 503w, 435w; ^{1}H-NMR (300 MHz, $(CD_3)_2SO$): δ = 0.65 (t, 3H; CH_3 Pentyl), 1.10 (m, 4H; CH_2 pentyl), 1.36 (m, 5H; CH_2 pentyl, CH_3 oxetane), 2.26 (s, 3H; Ar–CH_3), 2.31 (s, 3H; Ar–CH_3), 3.33 (m, 3H; N–CH_2, N–CH_aH–CO), 3.65 (t, *J* = 7.3 Hz, 2H; CH_2 pentyl), 3.94 (d, *J* = 16.6 Hz 1H; N–CHH_b–CO), 4.41 (s, 1H; C*H* oxetan), 4.44–4.59 (m, 2H; N–CH_2), 7.10–7.20 (m, 10H; aryl-*H*), 7.75 (s, 1H; aryl-*H*), 7.76 (s, 1H; aryl-*H*), 8.13 (s, 1H; N*H*), 10.27 (s, 1H; N*H*); ^{13}C-NMR (100 MHz, $(CD_3)_2SO$): δ = 13.82, 18.65, 20.72, 21.83, 22.81, 26.95, 28.56, 35.70, 38.21, 43.01, 48.89, 65.63, 76.27, 90.73, 115.98, 124.84 (2C), 125.43 (2C), 127.54, 128.11 (2C), 128.47 (2C), 131.07 (2C), 134.13 (2C), 135.83, 136.18, 139.61, 144.48, 146.58, 149.01, 151.18, 154.74, 159.33, 168.35, 169.67; *m*/*z* (ESI$^-$): 378 (18%), 432 (17), 459 (10), 493 (3), 569 (19), 600 (4), 702 (100, M − H$^+$), 738 (64), 765 (52), 792 (12), 837 (8); HRMS (ESI$^+$): calc. for $C_{39}H_{41}N_7O_6Na$ (M + Na$^+$): 726.3016, found: 726.3022.

(6-Methyl-3,5-dioxo-8,8-diphenyl-7-thia-2,4-diaza-bicyclo-[4.2.0]oct-2-yl)-acetic acid *tert*-butyl ester 10. Thymine-acetic acid *tert*-butyl ester **9** (0.30 g, 1.24 mmol) and thiobenzophenone (0.25 g, 1.24 mmol) were dissolved in an ultrasonic bath in acetonitrile (150 mL). The solution was degassed by bubbling Ar through it for 0.5 h. The solution was subsequently irradiated at 20 °C with a 150 W (TQ-150) medium-pressure mercury lamp in a Pyrex irradiation apparatus with a cut-off at 300 nm until the deep blue colour disappeared (45 h). The solvent was removed *in vacuo* and the crude mixture was treated with *i*-hexane–ethyl acetate (4 : 1). The insoluble part, containing unreacted starting material, was separated by filtration and the starting material was reisolated by recrystallisation from chloroform–pentane. The filtrate was evaporated to dryness *in vacuo*. The irradiation was repeated four times. Overall 1.50 g (6.20 mmol) thymine-acetic acid *tert*-butyl ester and 1.25 g (6.20 mmol) of thiobenzophenone were irradiated. The collected soluble fractions were purified by flash chromatography using a gradient of methanol in chloroform (0 to 9%) to give **10** (total amount: 108 mg, 4%) as a reddish–brown foam. Mp: 93–95 °C; ν_{max}(KBr)/cm^{-1} = 3420m, 3254w, 3060w, 2978w, 2931w, 1740s, 1708s, 1467m, 1446m, 1367m, 1274m, 1230m, 1155s, 1086w, 1034w, 984w, 943w, 912w, 842w, 750m, 707m, 666w, 621w, 572w, 547w, 500w, 473w; ^{1}H-NMR (400 MHz, CD_3OD): δ = 1.48 (s, 9 H; C(CH_3)$_3$), 1.80 (s, 3 H; CH_3), 3.96 (d, *J* = 17.6 Hz, 1 H; N–CH_aH), 4.27 (d, *J* = 17.6 Hz, 1 H; N–CHH_b), 4.79 (s, 1 H; C*H*(6)), 7.15–7.45 (m, 8 H; aryl-*H*), 7.78–7.81 (m, 2 H; aryl-*H*); ^{13}C-NMR (100 MHz, CD_3OD): δ = 24.43, 28.34, 46.87, 56.80, 63.27, 66.92, 83.65, 128.15 (2C), 128.36 (2C), 128.85 (2C), 128.99, 129.01, 129.89 (2C), 143.35, 143.96, 152.37, 169.39, 171.93; *m*/*z* (FAB$^+$): 57 (20%), 77 (18), 89 (16), 107 (15), 121 (23), 136 (47), 154 (54), 165 (21), 185 (29), 199 (100, *S*(*Ph*)$_2$ + H$^+$), 221 (11), 241 (14, M − S(Ph)$_2$ + H$^+$), 289 (7), 307 (6), 383 (7), 439 (5, M + H$^+$), 461 (8, M + Na$^+$); HRMS (FAB$^+$): calc. for $C_{24}H_{27}N_2O_4S$ (M + H$^+$): 439.1691, found: 439.1680.

(6-Methyl-3,5-dioxo-8,8-diphenyl-7-thia-2,4-diaza-bicyclo-[4.2.0]oct-2-yl)-acetic acid 11. Thietane **10** (10 mg, 22.0 nmol) was dissolved in TFA (100 µL) and stirred for 2 h. The acid was removed *in vacuo* and the crude product was purified by flash chromatography ($CHCl_3$–MeOH 10 : 1) to give **11** (8 mg, 95%) as a white solid. Mp : >230 °C; ν_{max}(KBr)/cm^{-1} = 3430s, 3060w, 2926w, 2854w, 1697s, 1616m, 1479m, 1445m, 1396w, 1380w, 1360w, 1311w, 1274m, 1209w, 1188w, 1145w, 1087w, 1032w, 911w, 747w, 707w, 578w, 544w, 502w, 468w; ^{1}H-NMR (400 MHz, $(CD_3)_2SO$): δ = 1.65 (s, 3 H; CH_3), 3.58 (d, *J*(NCH$_{2a}$, NCH$_{2b}$) = 16.8 Hz, 1 H; NCH_{2a}), 4.15 (d, *J*(NCH$_{2b}$, NCH$_{2a}$) = 16.8 Hz, 1 H; NCH_{2b}), 4.92 (s, 1 H; C*H*(6)), 7.14–7.44 (m, 8 H; CH_{ar}), 7.66–7.69 (m, 2 H; CH_{ar}), 10.34 (s, 1 H; N*H*); ^{13}C-NMR (100 MHz, $(CD_3)_2SO$): δ = 23.70, 46.15, 54.96, 61.13, 64.56, 126.73 (2C), 126.77 (2C), 127.43 (2C), 127.54, 127.84, 128.24 (2C), 142.10, 142.37, 149.77, 170.01; *m*/*z* (FAB$^-$): 91 (35%), 112 (32), 182 (100), 183 (8, M − S(Ph)$_2$ − H$^+$), 197 (29, *S*(*Ph*)$_2$–H$^+$), 381 (54, M − H$^+$); HR-MS (MALDI$^+$): calc. for $C_{20}H_{18}N_2O_4SNa$ (M + Na$^+$): 405.0885, found: 405.0881.

2-(6-Methyl-3,5-dioxo-8,8-diphenyl-7-thia-2,4-diaza-bicyclo-[4.2.0]oct-2-yl)-*N*-[2-(3,7,8-trimethyl-2,4-dioxo-3,4-dihydro-2*H*-benzo[*g*]pteridin-10-yl)-ethyl]-acetamide 4. Thietane **11** (8.0 mg, 21.0 µmol), HOBt (4.0 mg, 32.0 µmol) and TBTU (10.0 mg, 32.0 µmol) were dissolved in anhydrous DMF (0.5 mL) and stirred for 30 min at room temperature. A solution of flavin **12** (9.0 mg, 21.0 µmol) in anhydrous DMF (0.5 mL) and triethylamine (0.15 mL, 1.11 mmol) were added. The solution was stirred for 60 min at room temperature. The reaction was diluted with chloroform (10 mL), washed with water (3 × 15 mL), dried (magnesium sulfate), filtered and the solvent removed *in vacuo*. Diethyl ether was added to the orange–red oil, the precipitate was filtered and dried under a reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$–MeOH 10 : 1), which afforded **4** (10 mg, 71%) as a yellow solid. Mp: 181–183 °C; ν_{max}(KBr)/cm^{-1} = 3435br, 2924w, 1707m, 1654m, 1584m, 1548s, 1462w, 1354w, 1275w, 1231w, 1202w, 1152w, 1036w, 806w, 748w, 708w, 576w; ^{1}H-NMR (400 MHz, $(CD_3)_2SO$): δ = 1.43 (s, 3 H; C(5)CH_3), 2.41 (s, 3 H; C$_{ar}$CH_3), 2.55 (s, 3 H; C$_{ar}$CH_3), 3.28 (s, 3 H; NCH_3), 3.44–3.49 (m, 2 H; NCH_{2a}CO, NHCH_{2a}CH$_2$N), 3.60–3.66 (m, 1 H; NHCH_{2b}CH$_2$N), 3.73 (s, 1 H; C*H*(6)), 4.14 (d, *J*(NCH$_{2b}$CO, NCH$_{2a}$CO) = 17.2 Hz, 1 H; NCH_{2b}CO), 4.61–4.67 (m, 1 H; NHCH$_2$CH_{2a}N), 4.75–4.83 (m, 1 H; NHCH$_{2b}$CH_{2b}N), 7.13–7.41 (m, 8 H; CH_{ar}), 7.60–7.62 (m, 2 H; CH_{ar}), 7.79 (s, 1 H;

CH_{ar}), 7.94 (s, 1 H; CH_{ar}), 8.07 (t, J(NH, NCH_2) = 6.0 Hz, 1 H; N*H*), 10.40 (s, 1 H; N*H*); ^{13}C-NMR (150 MHz, $(CD_3)_2SO$): δ = 18.73, 20.81, 22.90, 27.92, 35.47, 42.97, 45.71, 54.08, 61.34, 64.82, 115.82, 126.80, 126.95, 127.08 (2C), 127.75 (2C), 127.87 (2C), 128.02 (2C), 131.17, 131.21, 134.02, 135.87, 136.04, 141.48, 142.46, 146.50, 148.79, 149.66, 154.89, 159.51, 167.72, 169.41; *m*/*z* (FAB$^+$): 107 (16%), 137 (66), 154 (100), 289 (14), 307 (32), 460 (3), 664 (4, M + H$^+$); HR-MS (MALDI$^+$): calc. for $C_{35}H_{34}N_7O_5S$ (M + H$^+$): 664.2342, found: 664.2320.

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