# Model studies of the (6–4) photoproduct photoreactivation: efficient photosensitized splitting of thymine oxetane units by covalently linked tryptophan in high polarity solvents†

**Qin-Hua Song,*[a] Hong-Bo Wang,[a] Wen-Jian Tang,[a] Qing-Xiang Guo[a] and Shu-Qin Yu[b]**

*Received 20th October 2005, Accepted 15th November 2005*
*First published as an Advance Article on the web 7th December 2005*
**DOI: 10.1039/b514921e**

Three covalently linked tryptophan–thymine oxetane compounds used as a model of the (6–4) photolyase–substrate complex have been prepared. Under 290 nm light, efficient splitting of the thymine oxetane with aromatic carbonyl compounds gives the thymine monomer and the corresponding carbonyl compounds by the covalently linked tryptophan *via* an intramolecular electron transfer, and exhibits a strong solvent dependence: the quantum yield ($\Phi$) is *ca.* 0.1 in dioxane, and near 0.3 in water. Electron transfer from the excited tryptophan residue to the oxetane unit is the origin of fluorescence quenching of the tryptophan residue, and is more efficient in strong polar solvents. The splitting efficiency of the oxetane radical anion within the tryptophan$^{\bullet+}$–oxetane$^{\bullet-}$ species is also solvent-dependent, ranging from *ca.* 0.2 in dioxane to near 0.35 in water. Thus, the back electron transfer reaction in the charge-separated species would be suppressed in water, but is still a main factor causing low splitting efficiencies in the tryptophan–oxetane systems. In contrast to the tryptophan–oxetane system, fast nonradiation processes are the main causes of low efficiency in the flavin–oxetane system. Hence, nonradiative processes of the excited FADH$^-$, rather than electron transfer to oxetane, may be an important factor for the low repair efficiency of (6–4) photolyase.

## Introduction

The two major lesions in DNA induced by UV light (200–300 nm), the cyclobutane pyrimidine dimers (CPDs) and the pyrimidine(6–4) pyrimidone adducts ((6–4) photoproducts) (Fig. 1), are responsible for the harmful effects of UV on organisms, such as growth delay, mutagenesis and death, and constitute 70–80% and 20–30% of the total photoproducts, respectively.[1] The two photolesions can be repaired through DNA photoreactivation catalyzed by CPD photolyase and (6–4) photolyase, respectively.[2]

Although less readily formed than CPDs, (6–4) photoproducts might actually be more effective at causing damaging mutations.[3] The (6–4) photoproduct is thought to form in DNA as follows: [2 + 2] photoaddition (the so-called Paterno–Buchi reaction) of the C4 carbonyl (or amino) of the 3′ thymine (cytosine) across the 5,6 double bond of the 5′ thymine generates an oxetane (or azetidine) ring,[2] which at temperatures above −80 °C undergoes ring opening by C4–O bond cleavage accompanied by a proton shift from N3 to generate the observed (6–4) photoproduct (Fig. 1).[4]

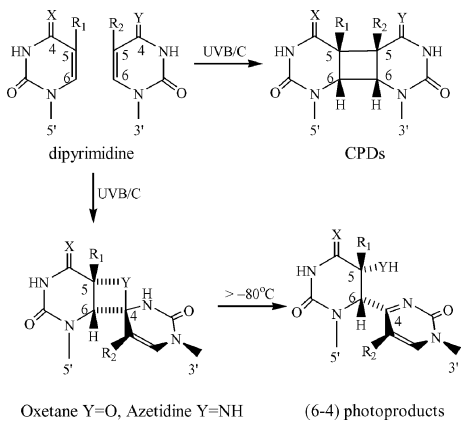



**Fig. 1** Formation of the two major photoproducts in DNA under UV light, CPDs and (6–4) photoproducts.

Whereas CPDs can be restored to their original forms by simply breaking the C5–C5′ and C6–C6′ bonds, the breaking of the C5–OH and C6–C4′ bonds of (6–4) photoproducts would not result in repair. The discovery of (6–4) photolyase and the subsequent identification of structural and cofactor similarities of (6–4) photolyase to CPD photolyase led to a proposal of a reaction scheme very similar to that of CPD photolyase.[5] In the model for (6–4) photolyase, a critical step, in which (6–4) photolyase differs from classical photolyase, is that upon binding to the substrate the enzyme converts the open form of the (6–4) photoproduct to the oxetane intermediate by a dark reaction. The next step is photoinduced electron transfer (ET) to the oxetane intermediate

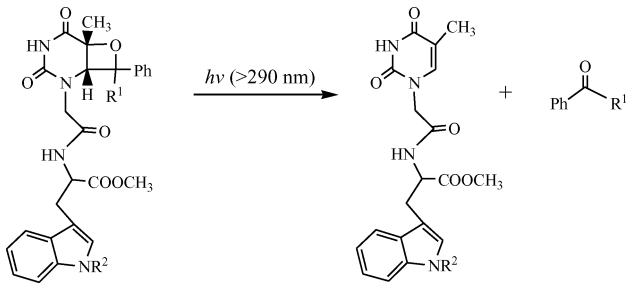
[a]*Department of Chemistry, University of Science and Technology of China, and State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Hefei, 230026, Anhui, P. R. China. E-mail: qhsong@ustc.edu.cn; Fax: +86-551-3601592; Tel: +86-551-3607524*
[b]*Department of Chemical Physics, University of Science and Technology of China, Hefei, 230026, Anhui, P. R. China*

† Electronic supplementary information (ESI) available: UV absorption spectra and/or fluorescence emission spectra of compounds **2a**, **3**, **8b** and **10**; the detail for the measurement of the splitting quantum yield; copies of $^1$H and $^{13}$C NMR spectra of all new compounds. See DOI: 10.1039/b514921e

in a manner analogous to CPD photolyase, and spontaneous splitting of the oxetane anion radical to restore two pyrimidines.[2]

To provide evidence for the ET mechanism, thymine oxetane adducts with carbonyl compounds were prepared. Use of photosensitizers, laser flash photolysis and steady-state studies with thymine oxetane adducts provided extensive support for the facile cleavage of the oxetane anionic radical.[6–8] The strongest support for the ET model came from the investigation of photoinduced cleavage of an oxetane ring covalently linked to flavin.[7] In this model system, it was found that only two-electron-reduced and deprotonated flavin induced more efficient photosplitting of the oxetane ring. This result further demonstrated the close mechanistic similarities between CPD photolyase and (6–4) photolyase.

Although the two classes of enzymes have similar structures, the same chromophores, and the same basic reaction mechanism, an important difference in repair efficiency exists between them, that is, CPD photolyases repair CPDs with a uniformly high quantum yield (0.7–0.98),[2] and the quantum yield of (6–4) photolyases is in the range of 0.05–0.11.[5a,9] X-Ray crystal structures for both CPD photolyase[10] and photolyase–substrate complexes[11,12] have been obtained, and the mechanistic model for CPD photolyase has well been established.[2] However, study of (6–4) photolyase is very insufficient, for example, the mechanism for the low repair efficiency of (6–4) photolyase remains unknown.




**1**: $R^1$ = Ph, $R^2$ = H
**2**: $R^1$ = Ph, $R^2$ = $CH_3$
**3**: $R^1$ = H, $R^2$ = H

**4**: $R^2$ = H
**5**: $R^2$ = $CH_3$

**Scheme 1**

In the CPD model systems, the photosensitized cleavage of the CPD unit by a covalently linked chromophore had low efficiency due to back electron transfer (BET), which competes with the cleavage of the CPD radical anion within a charge-separated species after photo-induced ET between the chromophore and the CPD unit. The photosensitized cleavage of the CPD unit in the model systems by a covalently linked chromophore such as indole,[13a] arylamine,[13b] methoxybenzene,[13c] flavin[14] or tryptophan[15] has been extensively investigated. These studies offer insights into the mechanistic features of CPD repair by DNA photolyase. In contrast to CPD model systems, study of a covalently linked oxetane–chromophore model is very rare.

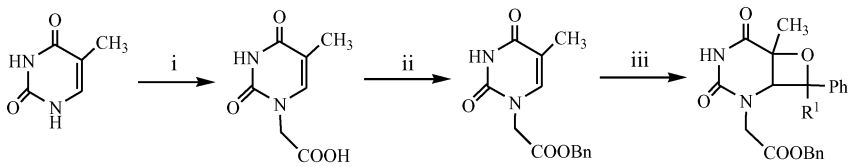
In this work, we prepared three model systems (**1–3**), which are composed of a thymine oxetane adduct with benzophenone or benzaldehyde and a covalently linked tryptophan unit (Scheme 1). The photosensitized cleavage of thymine oxetane units gives the thymine monomer and the corresponding carbonyl compounds under 290 nm light. Fluorescence quenching of tryptophan and the splitting efficiency of the oxetane in these tryptophan–oxetane systems were measured as functions of solvent polarity, and revealed a strong solvent dependence.

## Results and discussion

### Synthesis of the model compounds 1–3

For the synthesis of the model compounds **1–3**, 1-(carboxymethyl)thymine **6**[14b,16] and its benzyl ester **7** were prepared from thymine as shown in Scheme 2. The acetonitrile solution of the benzyl ester and benzophenone was irradiated in a Pyrex photochemical reactor ($\lambda$ > 290 nm) with a 300 W high-pressure Hg lamp, and gave a thymine oxetane adduct **8a** by the Paterno–Buchi reaction. Using benzaldehyde instead of benzophenone, the photochemical reaction would give **8b**.

Tryptophan methyl ester (**9**)[15] and 1-methyltryptophan methyl ester (**10**)[17] were prepared according to the literature methods (Scheme 3). Compound **9** was synthesized with methanol in the presence of $SOCl_2$ in good yield. 1-Methyltryptophan was prepared using tryptophan and iodomethane, then esterified with methanol to give **10**.

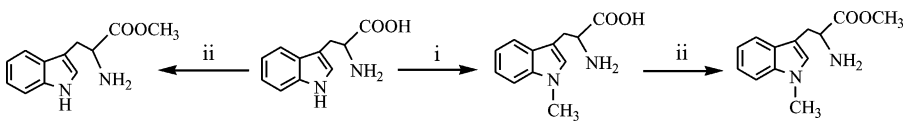



**6**

**7**

**8a**: $R^1$ = Ph
**8b**: $R^1$ = H

**Scheme 2** i) Chloroacetic acid, KOH, $H_2O$, reflux; ii) benzyl alcohol, *p*-toluene sulfonic acid, benzene, reflux; iii) benzophenone or benzaldehyde, h$\nu$ > 290 nm, acetonitrile, room temp.

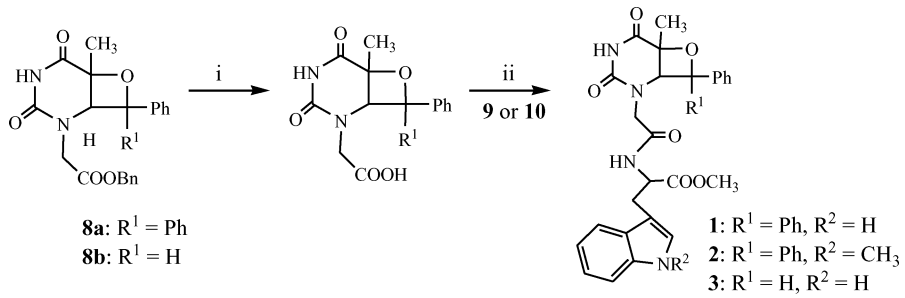



**9**

**10**

**Scheme 3** i) Na, liquid $NH_3$, $Fe(NO_3)_3 \cdot 9H_2O$, MeI; ii) $SOCl_2$, $CH_3OH$.




**Scheme 4** i) Pd/C, $H_2$, acetic acid, room temp., 8 h; ii) HOBt, TBTU, DMF, room temp., 8 h.

The model compounds **1–3** were prepared through condensation of the modified tryptophans **9** and **10** with $N^1$-(carboxylmethyl)thymine oxetanes obtained by hydrogenolytic cleavage of the benzyl esters **8a** and **8b**, as shown in Scheme 4. The condensation was performed in the presence of 1-hydroxy-1*H*-benzotriazole (HOBt) in DMF for 8 h using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate (TBTU) as a coupling reagent, and generated compound **1** from **9** with **8a**, compound **2** from **10** with **8a** and compound **3** from **9** with **8b**. Because racemic **8a** or **8b** was linked to L-tryptophan or *N*-methyl-L-tryptophan, model compounds **1**, **2** and **3** are diastereomeric. The two diastereomers of model compounds **1**, **2** and **3** were separated by flash chromatography on silica gel. The two diastereomers of compound **2**—**2a** and **2b**—were characterized, and only one diastereomer was characterized for compounds **1** and **3** (see Experimental). The characterized diastereomers for compounds **1** and **3** were used in all experiments of analysis and measurements.

### Photo-cleavage properties of compounds 1–3

The model compounds in an acetonitrile solution were irradiated with a 290 nm light beam from a fluorescence spectrometer. The HPLC chromatograms showed the simultaneous cleavage of model compound **1**, **2** or **3** into **4** or **5** and benzophenone or benzaldehyde under 290 nm light. This was confirmed by HPLC co-elution with standards. Fig. 2 shows a representative set of HPLC chromatograms for model compound **2a** irradiated for various times. Obviously, the photochemical reaction of model compound **2a**, with a retention time of 11.3 min, to the photosplitting product **5** and benzophenone with retention times of 4.2 min and 8.9 min, respectively, is a clean conversion as no other product was detectable.

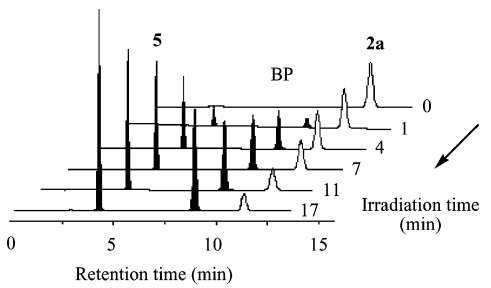



**Fig. 2** Typical HPLC chromatograms obtained from compound **2a** in acetonitrile ($5 \times 10^{-4}$ mol $L^{-1}$, 3 mL) irradiated for 0, 1, 4, 7, 11 and 17 min by a HPLC instrument (HP Agilent 1100) with a C-18 reverse-phase column and detected at 270 nm, methanol–water (70 : 30) as eluent.

The splitting reaction is an intramolecular process because splitting efficiencies of the model compounds are unchanging for measurements at three concentrations—0.01 mM, 0.05 mM and 0.1 mM in methanol—within an experimental error of ±5%. These results ruled out the involvement of intermolecular photosensitization, *i.e.*, the excited tryptophan residue of one model molecule being responsible for the splitting of the oxetane unit of another model molecule.

### Fluorescence emission of model compounds 1–3

Fluorescence emission spectra of model compounds **1**, **2a** and **3** (symbol as Trp–Ox) in various solvents were measured at room temperature on a fluorescence spectrometer. Fig. 3 shows the fluorescence emission of compound **1** compared to the fluorescence emission of the corresponding free tryptophan **9** in methanol at room temperature. The fluorescence of the tryptophan residue in compound **1** is much weaker than that of the free tryptophan **9**.

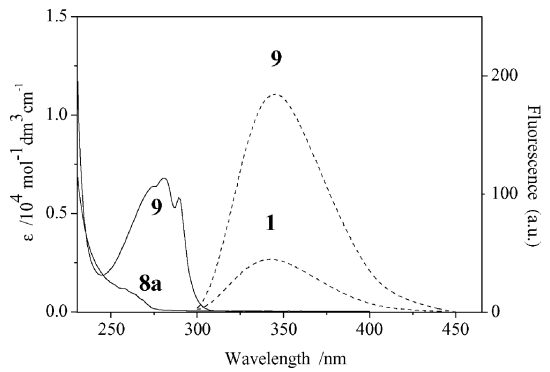



**Fig. 3** UV absorption spectra (solid line) and/or fluorescence emission spectra (dashed line) ($\lambda_{ex}$ = 290 nm) of tryptophan methyl ester (**9**), compounds **1** and **8a**, in methanol.

The concentrations of the tryptophan unit for model compounds **1**, **2a** and **3** and the free tryptophans **9** or **10** were controlled within 0.05 for their absorbance at 290 nm. The fluorescence intensity was further normalized with the absorbance. It was observed that the fluorescence emission of the tryptophan unit in model compounds **1–3** is quenched by the covalently linked thymine oxetane, and the extent of fluorescence quenching [$Q$, eqn (1), where $F$ is fluorescence intensity] was expressed as follows:

$$Q = 1 - F_{\text{Trp–Ox}}/F_{\text{Trp}} \quad (1)$$

**Table 1** Splitting quantum yield and degree of fluorescence quenching of compounds **1** and **3** in various solvents

| | Compound **1** | | | Compound **3** | | |
|---|---|---|---|---|---|---|
| Solvent | $Q$ | $\Phi$ | $\phi_{spl}$ | $Q$ | $\Phi$ | $\phi_s$ |
| $H_2O$–$CH_3CN$ (95 : 5) | 0.80 | 0.24 | 0.30 | 0.82 | 0.28 | 0.34 |
| Acetonitrile | 0.78 | 0.17 | 0.22 | 0.73 | 0.24 | 0.32 |
| Methanol | 0.69 | 0.15 | 0.22 | 0.71 | 0.19 | 0.27 |
| THF | 0.65 | 0.13 | 0.20 | 0.63 | 0.17 | 0.26 |
| Dioxane | 0.49 | 0.10 | 0.20 | 0.61 | 0.11 | 0.18 |

**Table 2** Dependence of the efficiency on the solvent ($\varepsilon_r$, dielectric constants) for the two diastereomers of compound **2**

| | | **2a** | | | **2b** |
|---|---|---|---|---|---|
| Solvent | $\varepsilon_r$ | $Q$ | $\Phi$ | $\phi_{spl}$ | $\Phi$ |
| $H_2O$–$CH_3CN$ (95 : 5) | 78.3[a] | 0.80 | 0.26 | 0.33 | 0.27 |
| Acetonitrile | 37.8 | 0.67 | 0.17 | 0.26 | 0.18 |
| Methanol | 32.7 | 0.68 | 0.16 | 0.24 | 0.17 |
| THF | 7.6 | 0.51 | 0.13 | 0.26 | 0.12 |
| Dioxane | 2.2 | 0.47 | 0.11 | 0.23 | 0.11 |

[a] Dielectric constant of water.

The value of $Q$ for compound **1** in methanol is 0.69 (Fig. 3). The extent of fluorescence quenching is sensitive to the polarity of the solvent. The values of $Q$ increase with increasing polarity of the solvents (Tables 1 and 2).

The fluorescence quenching of the tryptophan residue in the model compounds is not a result of significant absorption of the exciting light (290 nm) by the oxetane unit. Since the oxetanes **8a** (Fig. 3) and **8b** have no significant absorption at 290 nm, an internal filter effect should not be significant.

An intramolecular ET from the excited tryptophan to the oxetane unit may be responsible for the fluorescence quenching. On the basis of laser flash photolysis, fluorescence quenching, and product analysis experiments, Falvey and coworkers[6b] demonstrated that thymine oxetane adducts with aromatic carbonyl compounds undergo a cycloreversion reaction upon photosensitizer reductive ET reactions. The excited state oxidation potentials ($E^*_{ox}$) for the photosensitizers range from −2.45 V to −3.32 V.[6b] Since the value of $E^*_{ox}$ for tryptophan is −2.78 V *vs.* SCE from 1.05 V *vs.* NHE[18] for the oxidation potential of tryptophan, the electron transfer reaction from the excited tryptophan to the thymine oxetane unit is thermodynamically possible. Because there is almost no overlap between the emission spectra of the free tryptophans **9** and **10** and the absorption spectra of the oxetanes **8a** and **8b**, singlet–singlet energy transfer is an improbable pathway of fluorescence quenching in the model compounds. Hence, the ET reaction should be the reason for the fluorescence quenching in the model compounds, and the degree of fluorescence quenching $Q$ should reflect the efficiency of the electron transfer reaction.

The values of $Q$ in Tables 1 and 2 show that ET reactions from the excited tryptophan residue to the linked oxetane become more efficient in high polarity solvents. With increasing polarity of the solvent, decay processes caused by electron transfer would be predominant, and fluorescence and nonradiative processes would be suppressed.

**Quantum yields for cleavage of compounds 1–3**

To measure the splitting quantum yields $\Phi$, which are molecules of oxetane split per photon absorbed, solutions (3 mL) of **1**, **2** and **3** in various solvents were placed in quartz cuvettes (10 × 10 mm) with a Teflon stopper, and then irradiated with 290 nm light from a fluorescence spectrometer. The rates of oxetane unit split were measured by monitoring the increase in absorbance at 270 nm due to the regeneration of the 5,6-double bond of pyrimidine **4** or **5** (symbol as Trp–Thy) and benzophenone or benzaldehyde. The intensity of the light beam was measured by ferrioxalate actinometry.[19] Thus, the rate of photons absorbed was obtained from the absorbance at 290 nm in terms of Beer's law. The observed quantum yields of oxetane splitting of **1**, **2** and **3** were calculated according to $\Phi$ = (rate of oxetane split)/(rate of photon absorbed), and listed in Tables 1, 2 and 3. The splitting reactions of the model compounds reveal notable solvent effects, and more efficient splitting reactions occur in high polarity solvents, ranging from *ca.* 0.1 in dioxane to near 0.3 in water.

There may exist different conformations between two diastereomers for each model compound (**1**, **2** or **3**), which are covalently linked between racemic oxetanes and an L-Trp residue, in solutions, *i.e.*, there are different relative orientations of the tryptophan and oxetane rings. Thus, the different conformations of the diastereomeric model compounds in solution could result in different splitting efficiencies.

To assess the effect of differences in conformation on the splitting quantum yield, we measured the quantum yield of the two diastereomers of compound **2**, **2a** and **2b**, as listed in Table 2. It is obvious that the splitting quantum yield values for **2a** and **2b** are similar within an experimental error of ±5%. Hence, the conformational difference of **2a** and **2b** didn't influence their splitting quantum efficiencies.

Kim and Rose[13b] observed a two-fold higher splitting efficiency of one over another diastereomer in a dimer–arylamine system with a very short spacer. The large difference in splitting efficiency may be a consequence of different BET rates within the diastereomeric charge-separated species. The stereoelectronic explanation was suggested that interaction of the dimer radical anion and the arylamine radical cation electronic system is different owing to differences in the distances and angles between them.[13b] Obviously, these differences in a system with a short spacer are more remarkable than in a system with a long spacer, and the former results in a notable difference in the quantum yield. In our model systems with a longer spacer, a small difference in the distance

**Table 3** The splitting quantum yields of compound **3** in water–dioxane binary solvent

| Water : dioxane(%) | $\Phi$ |
|---|---|
| 100[a] : 0 | 0.28 |
| 80 : 20 | 0.22 |
| 60 : 40 | 0.21 |
| 40 : 60 | 0.20 |
| 20 : 80 | 0.16 |
| 10 : 90 | 0.14 |
| 0 : 100 | 0.11 |

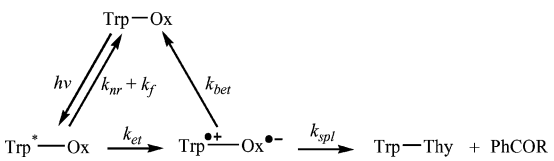
[a] Aqueous solution containing 5% acetonitrile.

resulting from different angles between the donor and the acceptor would give similar quantum yields.

Furthermore, a solvent dependence was observed in solvent mixtures of water–dioxane (Table 3). Upon addition of dioxane, a gradual decrease of the quantum yield was observed until the lowest $\Phi$ was reached in pure dioxane. The systematic reduction of solvent polarity results in a significant decrease of the quantum yield of the splitting reaction, ranging from 0.28 in aqueous solution containing 5% acetonitrile to 0.11 in pure dioxane for compound **3**. This further demonstrates that an efficient splitting reaction occurs in high polarity solvents.

The cleavage of compound **3** is more efficient than that of compound **1**. This is in agreement with the more efficient photosensitized splitting of a free 1,3-dimethyluracil oxetane adduct with benzaldehyde than with benzophenone.[8] The splitting quantum yields of compound **2** are similar to compound **1**. If a deprotonation of the tryptophan radical cation at N1 occurs prior to the cleavage of the oxetane radical anion in the zwitterionic intermediate, the BET would be strongly weakened in a diradical anion, Trp$^{\bullet}$–Ox$^{\bullet -}$, to result in a high splitting efficiency. This also confirmed that the tryptophan radical cation doesn't deprotonate before cleavage of the oxetane radical anion.[15]

The photophysical and photochemical processes of the model compounds were illuminated with the simple mechanism shown in Scheme 5. Under >290 nm light, a tryptophan unit in the model compound absorbs a photon producing an excited state of tryptophan. The excited tryptophan residue has relaxation pathways as follows: fluorescence ($k_f$), internal conversion and intersystem crossing (together represented by $k_{nr}$), and electron transfer to the linked oxetane ($k_{et}$). These efficiencies sum to 1, *i.e.* $\phi_f + \phi_{nr} + \phi_{et} = 1$. The charge-separated species (Trp$^{\bullet +}$–Ox$^{\bullet -}$) formed *via* electron transfer can undergo two processes, splitting ($\phi_{spl}$) or back electron transfer ($\phi_{bet}$) resulting in an unproductive reversal, and their efficiencies sum to 1. Among these processes, $k_{et}$ and $k_{spl}$ contribute to the observed quantum yield of oxetane splitting ($\Phi$), *i.e.* $\Phi = \phi_{et} \times \phi_{spl}$.




**Scheme 5**

The efficiency of forward electron transfer ($k_{et}$) can further be discussed with a reasonable assumption, that is, the rate constants for fluorescence and for the nonradiative relaxation pathways are unaltered by attachment of the oxetane to tryptophan and the electron transfer can occur in the excited model compounds. Utilizing this assumption, it was deduced that the efficiency of the electron transfer is equal to the degree of fluorescence quenching, $\phi_{et} = Q$.[15] Thus, the calculated values of $\phi_{spl}$ were obtained from $\phi_{spl} = \Phi / Q$, and were listed in Tables 1 and 2. It is obvious that the splitting efficiencies of the oxetane radical anions are remarkably dependent on solvent polarity. On the basis of the data of $Q$ and $\phi_{spl}$ in Tables 1 and 2, it might reasonably be expected that the electron transfer ($k_{et}$) and splitting ($k_{spl}$) processes are accelerated in high polarity solvents, or that fluorescence and BET are suppressed.

The quantum yields are 2–3 times as high as the value from the Trp–CPD system.[15] The result is well in agreement with the observation from a covalently linked flavin–oxetane system, *i.e.*, the quantum yield of the flavin–oxetane system was 0.023, and 0.01 for a flavin–CPD system.[7] The two-fold higher splitting quantum yield of the oxetane system than the CPD system can be explained by the splitting rate of the oxetane anion radical (>$10^7$ $s^{-1}$)[6b] being faster than that of the CPD radical anion (~$10^6$ $s^{-1}$).[20] A fast splitting of the oxetane radical anion can efficiently compete with BET within the zwitterionic intermediate.

In contrast to the Trp–Ox model systems, the splitting quantum yield of the flavin–Ox model system is very low, $\Phi = 0.023$.[7] The change in the free energy of BET, $\Delta G^{\circ}$, can be expressed as follows:

$$-\Delta G^{\circ} = E^{\circ}_{ox} - E^{\circ}_{red} - e^2/\varepsilon_r \qquad (2)$$

where $E^{\circ}_{ox}$ and $E^{\circ}_{red}$ are redox potentials for chromophore and oxetane respectively. For the flavin–oxetane system, the BET is a charge shift and no coulomb interaction occurs, and the coulomb term for the Trp–Ox system can be neglected for highly polar solvents such as water. Since $E_{ox} = 0.124$ V[21] for fully-reduced flavin and 1.05 V (NHE)[18] for tryptophan, the value of $-\Delta G^{\circ}$ for the Trp–Ox system is higher than that of the flavin–Ox system, *i.e.* the rate of BET in Trp$^{\bullet +}$–Ox$^{\bullet -}$ would be faster than that in flavin$^{\bullet}$–Ox$^{\bullet -}$. However, the slow BET in the flavin–Ox system[7] and the flavin–CPD system[14] didn't result in higher splitting quantum yields, and they are one order of magnitude lower than that of the Trp–Ox system and the Trp–CPD system,[15] respectively. Because the fluorescence ($k_f$) of fully-reduced flavin can be neglected, the main processes competing with electron transfer $k_{et}$ are nonradiative processes $k_{nr}$. Therefore, the main reason resulting in low efficiency of flavin model systems should be $k_{nr}$ rather than $k_{bet}$. This implies that the apoenzyme of CPD photolyase nonconvalently binding the flavin cofactor can efficiently suppress $k_{nr}$ of the excited $FADH^-$.

Besides, a fast $k_{spl}$ (>$10^9$ $s^{-1}$ in the enzyme,[22] and ~$10^6$ $s^{-1}$ for the model system[20]), which can efficiently compete with the BET process, is also an important factor for high repair efficiency of CPD photolyase. In contrast to CPD photolyase, inefficient suppression of $k_{nr}$ or slow $k_{spl}$ may be responsible for the low repair efficiency of (6–4) photolyase. Cocrystal structures of photolyase–DNA complexes and ultrafast spectroscopic studies on (6–4) photolyase would be helpful for understanding the mechanistic details of the photoreactivation, and might shed some light on the reason for this major difference in the quantum yields of the two types of photolyase.

## Experimental

### General

Melting points are uncorrected. $^1$H and $^{13}$C NMR spectra were measured with a Bruker AV 300 spectrometer operating at 300 and 75 MHz, respectively. The chemical shifts were referenced to $CHCl_3$ ($\delta$ 77.16) in $CDCl_3$ and DMSO ($\delta$ 39.52) in [$D_6$]DMSO for $^{13}$C NMR. IR spectra were recorded in KBr and measured in $cm^{-1}$ on a Bruker Vector22 Infrared Spectrometer. Mass spectra were obtained with a Micromass GCF TOF mass spectrometer. All materials were obtained from commercial suppliers and were used

as received. Solvents of technical quality were distilled prior to use. DMF was dried overnight with $MgSO_4$ and distilled. Acetonitrile and methanol were spectroscopic grade from commercial suppliers and used without further purification.

### Measurement of steady-state fluorescence emission

Fluorescence emission spectra were measured at room temperature on a Perkin-Elmer Instruments LS55 Luminescence Spectrometer. To determine the extent of fluorescence quenching, the fluorescence intensity of **1**, **2** or **3** was compared to that of the corresponding tryptophan without an oxetane attached (**8a** or **8b**), that is $Q = 1 - F_{\mathrm{TrpH\text{–}Ox}}/F_{\mathrm{TrpH}}$. The concentrations of the tryptophan residue of the Trp–Ox and the free tryptophan, **8a** or **8b**, were controlled within 0.05 for absorbance at 290 nm, and the fluorescence intensities were normalized with the absorbances. The wavelength of excitation was 290 nm, which is an absorption peak of the model compounds resulting from the $n \rightarrow \pi^*$ transition of the indole unit.

### Measurements of splitting quantum yields of compounds 1–3

The 3 mL samples were irradiated with a 290 nm light from a Shimadzu RF-5301PC spectrofluorophotomer. After certain time intervals, the absorbance of the irradiated solutions was recorded by a Lambda 45 UV–Vis spectrometer (Perkin-Elmer Instruments). The quantum yields of splitting didn't change with and without $N_2$-bubbling prior to irradiation within an experimental error of ±5%. Hence, the non-deaerated solution was employed in all measurements of quantum yield. Since the photoproducts, the pyrimidines and benzophenone or benzaldehyde, absorb light at 290 nm, in order to avoid competition of absorbing the irradiated light between the model compounds and photoproducts, the splitting extent of model compounds was controlled within 5% in all the measurements.

**1-(Carboxymethyl)thymine 6.** [14b,16] Yield 61%. $\nu_{max}$(KBr)/cm$^{-1}$ = 3026m, 1707s, 1664s, 1632s, 1419m, 1201s, 831m; $^1$H NMR (300 MHz, [D$_6$]DMSO, TMS): $\delta$ = 1.76 (s, 3H, CH$_3$), 4.37 (s, 2H, CH$_2$), 7.50 (s, 1H, CH); $^{13}$C NMR (75 MHz, [D$_6$]DMSO): $\delta$ = 11.9 (CH$_3$), 48.5 (CH$_2$), 108.5 (*C*CH$_3$), 141.9 (CH), 151.0 (NCON), 164.3 (COOH), 169.6 (NCO).

**Benzyl 1,2,3,4-tetrahydro-5-methyl-2,4-dioxopyrimidine-1-acetate 7.** 1-(Carboxymethyl)thymine **6** (1.8 g, 10.0 mmol) and *p*-toluene sulfonic acid (0.8 g) were added to a solution of benzyl alcohol (4 mL, 39 mmol) and benzene (30 mL), and heated to reflux for 15 h; water was continuously removed from the reaction mixture. The reaction mixture was concentrated *in vacuo* and the precipitate was recrystallized twice from methanol to give **7** as colorless needles (2.2 g, 80%). Mp 179–181 °C. $\nu_{max}$(KBr)/cm$^{-1}$ = 1738s, 1460s, 1224s; $^1$H NMR (300 MHz, CDCl$_3$, TMS): $\delta$ = 1.92 (s, 3H, CH$_3$), 4.47 (s, 2H, NCH$_2$), 5.22 (s, 2H, OCH$_2$), 6.92 (s, 1H, CH), 7.34–7.41 (m, 5H, ArH), 8.37 (s, 1H, NH); $^{13}$C NMR (75 MHz, [D$_6$]DMSO): $\delta$ = 11.8 (CH$_3$), 48.5 (NCH$_2$), 66.4 (OCH$_2$), 108.7 (*C*CH$_3$), 127.9, 128.2, 128.4, 135.5, 141.5, 151.0 (NCON), 164.3 (COO), 168.1 (NCO); TOFMS (CI) calcd for (M + 1)$^+$ $C_{14}H_{14}N_2O_4$: 275.1032, found 275.1031.

**Benzyl-*Z*-6-methyl-8,8-diphenyl-7-oxa-3,5-dioxo-2,4-diazabicyclo-[4.2.0]octane-2-acetate 8a.** The benzyl ester **7** (548 mg, 2.0 mmol) and benzophenone (728 mg, 4.0 mmol) were dissolved in acetonitrile (250 mL) and placed in a Pyrex reactor. Under a nitrogen atmosphere, the solution was irradiated for 4 h with a 300 W high-pressure Hg lamp. The solvent in the reaction mixture was removed by rotary evaporation. The residue was dissolved with dichloromethane and subjected to column chromatography (silica gel-H, petroleum ether–ethyl acetate 5 : 1) to yield **8a** as a white powder (196 mg, 21%). Mp 167–169 °C. $\nu_{max}$(KBr)/cm$^{-1}$ = 1748m, 1699s, 1469m, 1199s; $^1$H NMR (300 MHz, CDCl$_3$, TMS): $\delta$ = 1.71 (s, 3H, CH$_3$), 3.55 (d, 1H, NCH$_2$), 4.69 (s, 1H, CH), 4.75 (d, 1H, NCH$_2$), 5.21 (s, 2H, OCH$_2$), 7.29–7.38 (m, 15H, ArH); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 23.5 (CH$_3$), 47.3 (NCH$_2$), 66.4 (OCH$_2$), 67.8 (NCH), 76.6 (*C*CH$_3$), 91.2 (OC), 125.3, 126.0, 128.2, 128.4, 128.6, 128.7, 128.8, 128.9, 135.0, 138.5, 143.9, 151.0 (NCON), 168.2 (COO), 169.6 (NCO); TOFMS (CI) calcd for (M + 1)$^+$ $C_{27}H_{24}N_2O_5$: 457.1763, found 457.1760.

**Benzyl-(6*Z*,8*Z*)-6-methyl-8-phenyl-7-oxa-3,5-dioxo-2,4-diazabicyclo[4.2.0]octane-2-acetate 8b.** Using benzaldehyde instead of benzophenone, the same procedure was performed to give compound **8b** (130 mg, yield 17%) as white needles. Mp 182–184 °C; $\nu_{max}$(KBr)/cm$^{-1}$ = 1735s, 1697s, 1479m, 1206m; $^1$H NMR (300 MHz, CDCl$_3$, TMS): $\delta$ = 1.81 (s, 3H, CH$_3$), 3.96 (d, *J* = 6.6 Hz, 1H, NCH), 4.06 (m, 2H, NCH$_2$), 5.14 (m, 2H, OCH$_2$), 5.75 (d, *J* = 6.6 Hz, 1H, OCH), 7.29–7.36 (m, 10H, ArH), 7.59 (s, 1H, NH); $^{13}$C NMR (75 MHz, [D$_6$]DMSO): $\delta$ = 22.3 (CH$_3$), 48.3 (NCH$_2$), 64.2 (NCH), 66.3 (OCH$_2$), 77.2 (*C*CH$_3$), 85.6 (OCH), 126.3, 128.1, 128.2, 128.4, 128.5, 128.7, 135.5, 139.1, 151.4 (NCON), 168.9 (COO), 170.3 (NCO); TOFMS (CI) calcd for (M + 1)$^+$ $C_{21}H_{20}N_2O_5$: 381.1450, found 381.1456.

**Model compound 1.** A suspension of Pd/C catalyst (15 mg) in AcOH (2 mL) was slowly added to a solution of **8a** (100 mg, 0.22 mmol) in 6 mL of AcOH. The mixture was bubbled with hydrogen and stirred for 8 h at room temperature. The reaction mixture was filtered through a Celite pad. The filtrate was evaporated to dryness *in vacuo*, and a solution of TBTU (141 mg, 0.44 mmol) and HOBt (59 mg, 0.44 mmol) in 8 mL of DMF was added. After the reaction mixture was stirred for 30 min, a solution of tryptophan methyl ester **9** (52 mg, 0.24 mmol) in DMF (2 mL) was added, and stirred for 8 h at room temperature. The reaction mixture was diluted with 100 mL of water and extracted with 200 mL of $CHCl_3$ three times. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The residual crude product was purified by silica gel column chromatography (gel-H, $CHCl_3$–petroleum ether 50 : 1) to give two diastereomers, and the less polar isomer **1** as white powder (15 mg, 12%) was characterized. Mp 204–206 °C. $\nu_{max}$(KBr)/cm$^{-1}$ = 3336m, 1728s, 1686s, 1470w, 1282w, 1211w; $^1$H NMR (300 MHz, CDCl$_3$, TMS): $\delta$ = 1.58 (s, 3H, CH$_3$), 3.37 (m, 3H, CHC$H_2$ + NCH$_2$), 3.74 (s, 3H, OCH$_3$), 4.40 (d, 1H, NCH$_2$), 4.81 (s, 1H, NCH), 4.87 (m, 1H, C*H*CH$_2$), 6.40 (d, 1H, N*H*CH), 7.04–7.46 (m, 15H, ArH + H$_{indole}$), 8.36 (s, 1H, NH), 8.53 (s, 1H, NH$_{indole}$); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 23.3 (CH$_3$), 27.1 (CH*C*H$_2$), 48.7 (NCH$_2$), 52.7 (OCH$_3$), 52.9 (NHCH), 66.2 (NCH), 76.1 (*C*CH$_3$), 91.1 (OC), 108.7, 111.5, 118.3, 119.6, 122.1, 123.9, 125.4, 126.0, 127.4, 128.2, 128.3, 128.5, 128.7, 136.1, 138.6, 143.5, 151.7 (NCONH), 166.8 (COO), 169.7 (NHCO), 172.0 (CH$_2$*C*ONH); TOFMS (CI) calcd for (M + 1)$^+$ $C_{32}H_{30}N_4O_6$: 567.2244, found 567.2247.

**Model compound 2.** Using 1-methyltryptophan methyl ester hydrochloride instead of tryptophan methyl ester **9**, the same procedure as with synthesizing compound **1** was performed, and two diastereomers **2a** (24 mg, 19%) and **2b** (19 mg, 15%) were obtained as white powders. **2a**: mp 162–164 °C. $\nu_{max}$(KBr)/cm$^{-1}$ = 1701s, 1475m, 1282m, 1213m; $^1$H NMR (300 MHz, $CDCl_3$, TMS): $\delta$ = 1.56 (s, 3H, $CH_3$), 3.35 (m, 3H, CHC$H_2$ + $NCH_2$), 3.70 (s, 3H, $NCH_3$), 3.75 (s, 3H, $OCH_3$), 4.43 (d, 1H, $NCH_2$), 4.73 (s, 1H, NCH), 4.88 (m, 1H, C*H*$CH_2$), 6.29 (d, 1H, N*H*CH), 6.84 (s, 1H, $H_{indole}$), 7.04–7.36 (m, 14H, ArH + $H_{indole}$); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 23.3 ($CH_3$), 27.3 (CH*C*$H_2$), 32.8 ($NCH_3$), 49.1 ($NCH_2$), 52.7 ($OCH_3$), 53.4 (NHCH), 66.2 (NCH), 76.5 (*C*$CH_3$), 91.3 (OC), 107.9, 109.6, 118.6, 119.5, 122.1, 125.5, 126.1, 127.9, 128.2, 128.3, 128.4, 128.6, 128.8, 137.1, 138.8, 143.8, 151.0 (NCONH), 167.0 (COO), 169.4 (NHCO), 172.1 ($CH_2$*C*ONH); TOFMS (CI) calcd for (M + 1)$^+$ $C_{33}H_{32}N_4O_6$: 581.2400, found 581.2398. **2b**: mp 120–122 °C. $\nu_{max}$(KBr)/cm$^{-1}$ = 1709s, 1469m; $^1$H NMR (300 MHz, $CDCl_3$, TMS): $\delta$ = 1.38 (s, 3H, $CH_3$), 3.31 (m, 3H, CH*C*$H_2$ + $NCH_2$), 3.71 (s, 6H, $NCH_3$ + $OCH_3$), 4.57 (d, 1H, $NCH_2$), 4.86 (s, 1H, NCH), 4.89 (m, 1H, C*H*$CH_2$), 6.35 (d, 1H, *NH*CH), 6.79 (s, 1H, $H_{indole}$), 7.06–7.46 (m, 14H, ArH + $H_{indole}$); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 22.9 ($CH_3$), 26.9 (CH*C*$H_2$), 32.9 ($NCH_3$), 49.0 ($NCH_2$), 52.7 ($OCH_3$), 53.1 (NHCH), 65.3 (NCH), 76.3 (*C*$CH_3$), 91.3 (OC), 107.8, 109.8, 118.3, 119.8, 122.3, 125.5, 126.1, 127.7, 128.1, 128.3, 128.5, 128.6, 128.8, 137.1, 138.5, 143.6, 150.7 (NCONH), 167.3 (COO), 169.0 (NHCO), 171.9 ($CH_2$*C*ONH); TOFMS (EI) calcd for (M$^+$) $C_{33}H_{32}N_4O_6$: 580.2322, found 580.2319.

**Model compound 3.** Sodium hydroxide (30 mg) and oxetane **8b** (190 mg, 0.5 mmol) were added to a solution of water (10 mL) and methanol (10 mL), and stirred for 2 h at room temperature. The reaction mixture was diluted with 100 mL of water and extracted twice with 100 mL of ethyl acetate. Diluted hydrochloric acid was dropped into the water layer to adjust the pH to 3. The solution was extracted twice with 100 mL of ethyl acetate. The organic phase was washed with water, dried with $MgSO_4$, filtered and concentrated *in vacuo*. Then a solution of TBTU (321 mg, 1.0 mmol) and HOBt (68 mg, 0.5 mmol) in 8 mL of DMF was added. The reaction mixture was stirred for 30 min. After the addition of a solution of tryptophan methyl ester **9** (131 mg, 0.6 mmol) in DMF (2 mL), the reaction mixture was stirred for 8 h at room temperature. Then it was diluted with 100 mL of water and extracted twice with 100 mL of ethyl acetate. The combined organic layers were dried with $MgSO_4$, filtered and concentrated *in vacuo*. The residual crude product was purified by silica gel column chromatography (gel-H, $CHCl_3$–methanol 100 : 1). Two diastereomers were obtained, and the less polar isomer **3** as white powder (26 mg, 11%) was characterized. Mp 249–251 °C. $\nu_{max}$(KBr)/cm$^{-1}$ = 3392s, 1702s, 1478m, 1283m; $^1$H NMR (300 MHz, [$D_6$]DMSO, TMS): $\delta$ = 1.62 (s, 3H, $CH_3$), 3.09 (m, 2H, CHC$H_2$), 3.53 (s, 3H, $OCH_3$), 3.85 (m, 2H, $NCH_2$), 4.12 (d, $J$ = 6.5 Hz, 1H, NCH), 4.49 (m, 1H, NHC*H*), 5.65 (d, $J$ = 6.5 Hz, 1H, OCH), 6.91–7.09 (m, 3H, $H_{indole}$), 7.32–7.46 (m, 7H, $H_{indole + benzene}$), 8.44 (d, 1H, N*H*CH), 10.87 (s, 1H, CNHC); $^{13}$C NMR (75 MHz, [$D_6$]DMSO): $\delta$ = 22.4 ($CH_3$), 27.0 (CH*C*$H_2$), 48.8 ($NCH_2$), 51.7 ($OCH_3$), 53.2 (NHCH), 64.4 (NCH), 77.0 (*C*$CH_3$), 85.2 (OCH), 109.1, 111.3, 117.9, 118.4, 120.9, 123.5, 125.9, 127.0, 128.4, 136.1, 139.5, 151.2 (NCONH), 167.4 (COO), 170.2 (NHCO), 171.9 ($CH_2$*C*ONH); TOFMS (EI) calcd for (M$^+$) $C_{26}H_{26}N_4O_6$: 490.1852, found 490.1833.

**1-(Carboxymethyl)thymine tryptophan methyl ester amide 4.** 1-(Carboxymethyl)thymine **6** (230 mg, 1.25 mmol) and an excess of BOP (608 mg, 1.37 mmol) were dissolved in DMF (6 mL) and stirred at room temperature for 30 min, and a solution of tryptophan methyl ester **9** (272 mg, 1.25 mmol) in DMF (2 mL) was added, and stirred for 5 h at room temperature. The reaction mixture was purified by column chromatography on aluminium oxide (100–200 mesh, ethyl acetate–methanol 20 : 1 → 3 : 1) to give **4** as a white powder (134 mg, 28%). Mp 207–209 °C. $\nu_{max}$(KBr)/cm$^{-1}$ = 3351s, 3311w, 1730s, 1688s, 1646s, 1354w, 1221m; $^1$H NMR (300 MHz, [$D_6$]DMSO, TMS): $\delta$ = 1.73 (s, 3H, $CH_3$), 3.09 (m, 2H, CHC$H_2$), 3.57 (s, 3H, $OCH_3$), 4.34 (m, 2H, $NCH_2$), 4.53 (m, 1H, C*H*$CH_2$), 6.97–7.50 (m, 6H, NCH + $H_{indole}$), 8.68 (d, 1H, N*H*CH), 10.89 (s, 1H, $NH_{indole}$), 11.26 (s, 1H, CNHC); $^{13}$C NMR (75 MHz, [$D_6$]DMSO): $\delta$ = 11.9 ($CH_3$), 27.3 (CH*C*$H_2$), 49.0 ($NCH_2$), 51.9 ($OCH_3$), 53.4 (NHCH), 108.0, 109.1, 111.5, 118.0, 118.5, 121.0, 123.7, 127.1, 136.0, 142.3, 150.9 (NCONH), 164.4 (COO), 167.1 (NHCO), 172.0 ($CH_2$*C*ONH); TOFMS (CI) calcd for (M + 1)$^+$ $C_{19}H_{20}N_4O_5$: 385.1512, found 385.1518.

**1-(Carboxymethyl)thymine (1-methyltryptophan) methyl ester amide 5.** The same procedure as with the synthesis of **4** was performed with 1-methyltryptophan methyl ester hydrochloride and triethylamine instead of **9**, and **5** was obtained as a white powder (300 mg, 60%). Mp 196–198 °C. $\nu_{max}$(KBr)/cm$^{-1}$ = 3435m, 1740m, 1690s, 1666m, 1470w; $^1$H NMR (300 MHz, [$D_6$]DMSO, TMS): $\delta$ = 1.74 (s, 3H, $CH_3$), 3.09 (m, 2H, CHC$H_2$), 3.58 (s, 3H, $NCH_3$), 3.73 (s, 3H, $OCH_3$), 4.33 (s, 2H, $NCH_2$), 4.53 (m, 1H, C*H*$CH_2$), 7.01–7.51 (m, 6H, NCH + $H_{indole}$), 8.67 (d, 1H, N*H*CH), 11.26 (s, 1H, NH); $^{13}$C NMR (75 MHz, [$D_6$]DMSO): $\delta$ = 11.8 ($CH_3$), 27.0 ($NCH_3$), 32.3 (CH*C*$H_2$), 49.0 ($NCH_2$), 51.8 ($OCH_3$), 53.2 (NHCH), 107.9, 108.4, 109.6, 118.2, 118.5, 121.1, 127.4, 128.2, 136.5, 142.2, 150.8 (NCONH), 164.3 (COO), 167.0 (NHCO), 171.8 ($CH_2$*C*ONH); TOFMS (CI) calcd for (M + 1)$^+$ $C_{20}H_{22}N_4O_5$: 399.1668, found 399.1667.

## Acknowledgements

The authors are grateful for the financial support by the National Natural Science Foundation of China (Grant no. 30470444, 20332020).

## References

1 J. Cadet and P. Vigny, in The Photochemistry of Nucleic Acids, *Bioorganic Photochemistry*, ed. H. Morrison, Wiley Interscience, New York, 1990, vol. 1, pp. 1–272; E. C. Friedberg, G. C. Walker and W. Siede, *DNA Repair and Mutagenesis*, ASM Press, Washington DC, 1995; J.-S. Taylor, *Pure Appl. Chem.*, 1995, **67**, 183; J. Cadet, M. Berger, T. Douki, B. Morin, S. Raoul, J.-L. Ravanat and S. Spinelli, *J. Biol. Chem.*, 1997, **378**, 1275.
2 A. Sancar, *Chem. Rev.*, 2003, **103**, 2203.
3 P. E. M. Gibbs, B. J. Kilbey, S. K. Banerjee and C. W. Lawrence, *J. Bacteriol.*, 1993, **175**, 2607; J.-S. Taylor, *Acc. Chem. Res.*, 1994, **27**, 76.

4 R. O. Rahn and J. L. Hosszu, *Photochem. Photobiol.*, 1969, **10**, 131.
5 (*a*) S.-T. Kim, K. Malhotra, C. A. Smith, J.-S. Taylor and A. Sancar, *J. Biol. Chem.*, 1994, **269**, 8535; (*b*) A. Sancar, *Science*, 1996, **272**, 48; (*c*) T. Todo, H. Ryo, K. Yamamoto, H. Toh, T. Inui, H. Ayaki, T. Nomura and M. Ikenaga, *Science*, 1996, **272**, 109; (*d*) X. D. Zhao, J. Q. Liu, D. S. Hsu, S. Y. Zhao, J.-S. Taylor and A. Sancar, *J. Biol. Chem.*, 1997, **272**, 32580.
6 (*a*) A. Joseph and D. E. Falvey, *J. Am. Chem. Soc.*, 1995, **117**, 11375; (*b*) A. Joseph, G. Prakash and D. E. Falvey, *J. Am. Chem. Soc.*, 2000, **122**, 11219; (*c*) A. Joseph and D. E. Falvey, *Photochem. Photobiol. Sci.*, 2002, **1**, 632.
7 M. K. Cichon, S. Arnold and T. Carell, *Angew. Chem., Int. Ed.*, 2002, **41**, 767.
8 Q. H. Song, X. M. Hei, Z. X. Xu, X. Zhang and Q. X. Guo, *Bioorg. Chem.*, 2003, **31**, 357.
9 K. Hitomi, S.-T. Kim, S. Iwai, N. Harima, E. Otoshi, M. Ikenaga and T. Todo, *J. Biol. Chem.*, 1997, **272**, 32591.
10 H.-W. Park, S.-T. Kim, A. Sancar and J. Deisenhofer, *Science*, 1995, **268**, 1866.
11 H. Komori, R. Masui, S. Kuramitsu, S. Yokoyama, T. Shibata, Y. Inoue and K. Miki, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 13560.
12 A. Mees, T. Klar, P. Gnau, U. Hennecke, A. P. M. Eker, T. Carell and L.-O. Essen, *Science*, 2004, **306**, 1789.
13 (*a*) S.-T. Kim, R. F. Hartman and S. D. Rose, *Photochem. Photobiol.*, 1990, **52**, 789; (*b*) S.-T. Kim and S. D. Rose, *J. Photochem. Photobiol., B*, 1992, **12**, 179; (*c*) D. G. Hartzfeld and S. D. Rose, *J. Am. Chem. Soc.*, 1993, **115**, 850.
14 (*a*) R. Epple, E.-U. Wallenborn and T. Carell, *J. Am. Chem. Soc.*, 1997, **119**, 7440; (*b*) J. Bulenandt, R. Epple, E.-U. Wallenborn, A. P. M. Eker, V. Gramlich and T. Carell, *Chem.–Eur. J.*, 2000, **6**, 62.
15 Q. H. Song, W. J. Tang, X. M. Hei, H. B. Wang, Q. X. Guo and S. Q. Yu, *Eur. J. Org. Chem.*, 2005, 1097.
16 A. S. Jones, P. Lewis and S. F. Withers, *Tetrahedron*, 1973, **29**, 2293.
17 S. Yamada, T. Shioiri, T. Itaya, T. Hara and R. Matsueda, *Chem. Pharm. Bull.*, 1965, **13**, 88; M. Cain, O. Campos, F. Guzman and J. M. Cook, *J. Am. Chem. Soc.*, 1983, **105**, 907.
18 M. R. DeFelippis, C. P. Murthy, F. Broitman, D. Weinraub, M. Faraggi and M. H. Klapper, *J. Phys. Chem.*, 1991, **95**, 3416.
19 S. L. Murov, I. Carmichael and G. L. Hug, *Handbook of Photochemistry*, Marcel Dekker Inc., New York, 2nd edn, 1993.
20 L. I. Grossweiner, A. G. Kepka, R. Santus and J. A. Vigil, *Int. J. Radiat. Biol.*, 1974, **25**, 521; S. R. Yeh and D. E. Falvey, *J. Am. Chem. Soc.*, 1991, **113**, 8557.
21 R. F. Anderson, *Biochim. Biophys. Acta*, 1983, **722**, 158.
22 S.-T. Kim, M. Volk, G. Rousseau, P. F. Heelis, A. Sancar and M.-E. Michel-Beyerle, *J. Am. Chem. Soc.*, 1994, **116**, 3115.