# Photo-induced electron transfer in small peptides: glycylalanine

PERKIN 2

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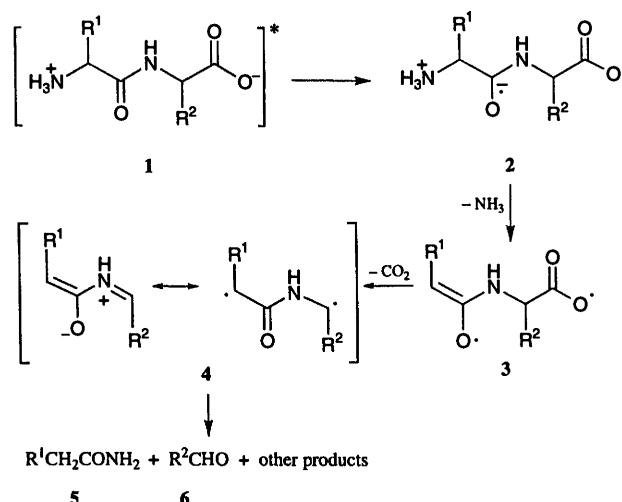
**Participation of the peptide bond in photoinduced electron transfer accounts for product distributions in the photolysis of XG dipeptides and predicts the possibility that the side-chain will be involved in similar reactions of GX dipeptides. Product analysis following exposure of aqueous glycyl-DL-alanine to UV radiation shows this prediction is fulfilled. Thus, unlike XG peptides, glycylalanine degrades to acetamide substantially without co-production of $CO_2$ and to products where bonding in the side-chain is involved in relaxation of the intermediate diradical. At a lower pH, decarboxylation radicals from glycylalanine have the opportunity to disproportionate as well as dimerize, and this accounts satisfactorily for the difference in product distribution when compared with that from XG photolysis under the same conditions.**

## Introduction

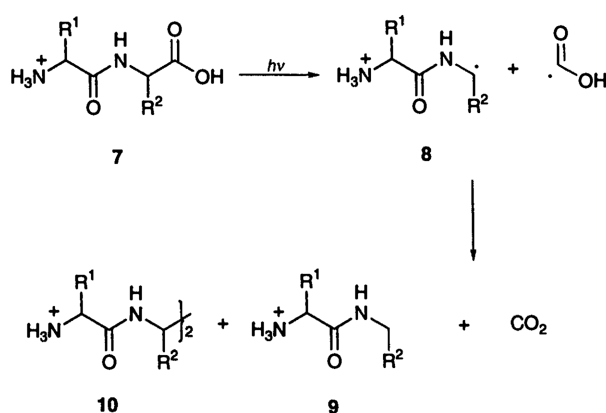
Widespread and an increasing amount of study of long-range electron transfer in proteins[1] requires consideration of the possibility that peptide groups are involved,[2] especially in view of recent debate on whether the polypeptide acts only as a specialised medium[3] or furnishes specific routes for charge separation.[4] Evidence in favour of the latter has accumulated recently,[5] with the participation of peptide groups further implicated in the results from studies with model compounds.[6]

We have found that the peptide group is a photochemically active chromophore that promotes electron transfer processes intramolecularly.[7] Details of an electron-transfer mechanism for the photodegradation of peptides emerged from studies with dipeptides of the general structure, XGly (Scheme 1, $R^2 = H$), and they accommodate satisfactorily variations in product distribution that arise from the influence of the different side-chains in X.[7a] It is clear from Scheme 1 that $R^2$ could also influence product distribution, but with recognizably different outcomes. Studies with dipeptides where $R^2 \neq H$ therefore present opportunities for validation and further development of the electron-transfer hypothesis. For example, $R^2$ could affect the availability of conformations that allow suitable orbital alignment for the ejection of $NH_3$ and/or $CO_2$. It could also present new bond-forming opportunities for radical intermediates. Similar effects, but with different outcomes, might be expected in photolyses at pH 1, contrasting with results for XG dipeptides that prompted the mechanism suggested in Scheme 2 ($R^2 = H$). Here, the most obvious possibility when $R^2$ has a β-hydrogen atom is radical disproportionation after decarboxylation as an alternative or additional pathway to the radical dimerization (**10**) observed when $R^2 = H$.

We have conducted comprehensive product analysis in the photolysis of aqueous glycyl-DL-alanine (**1**, $R^1 = H$, $R^2 = CH_3$) and report that the above predictions are largely correct. The general mechanism advanced earlier continues to be an effective aid in understanding electron-transfer properties of the peptide bond.




Scheme 1




Scheme 2

## Results

The outcome of product analysis in the photolysis of aqueous glycyl-DL-alanine is compared with that of aqueous glycylglycine in Table 1. At pH 6, the most notable effects of introducing methyl as the side-chain are (*i*) the apparent formation of acetamide *via* a route that involves neither concurrent decarboxylation nor a thermally labile intermediate, (*ii*) the inclusion of additional products and (*iii*) the significantly greater yields of **9** and **10**. In photolysis at pH 1, glycylalanine is distinguished by a much higher yield of ammonia and lower yields of **9** and **10**. Moreover, yield profiles for the photolysis of

**Table 1** Product distributions in aqueous photolysis of glycylglycine (GG) and glycylalanine (GA)[a]

| Substrate | $NH_3$ | $CO_2$ | RCHO[b] | $CH_3CONH_2$ (lc) | $CH_3CONH_2$ (gc) | **9** | **10** | Other significant components observed |
|---|---|---|---|---|---|---|---|---|
| (*a*) pH 6 | | | | | | | | |
| GG (**1** R = H)[c] | 65 | 53 | obs | < 4 | 55 | < 5 | < 1 | $CH_3CONHCH_2OH$; $CH_3CONHCH_2GlyOH$[d] |
| GA (**1** R = $CH_3$) | 80 | 30 | obs | 40 | 50 | 20 | 8 | Additional primary products and secondary product by HPLC |
| (*b*) pH 1 | | | | | | | | |
| GG[c] | 12 | 76 | obs | < 5 | — | 31 | 55 | None |
| GA | 40 | 85 | obs | < 10 | — | 16 | 32 | None |

[a] In percent at 20% conversion of peptide and with a relative error of ± 10%. [b] Isolated and characterized as the 2,4-DNP derivative. [c] Data from ref. 7(*a*). [d] Thermally labile acetamide precursor.

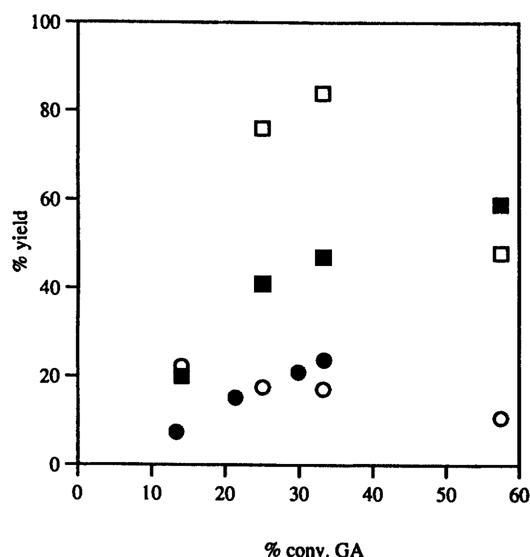



**Fig. 1** Yield profiles for: ammonia at pH 6 (□) and pH 1 (■); glycineethylamide at pH 6 (○) and pH 1 (●)

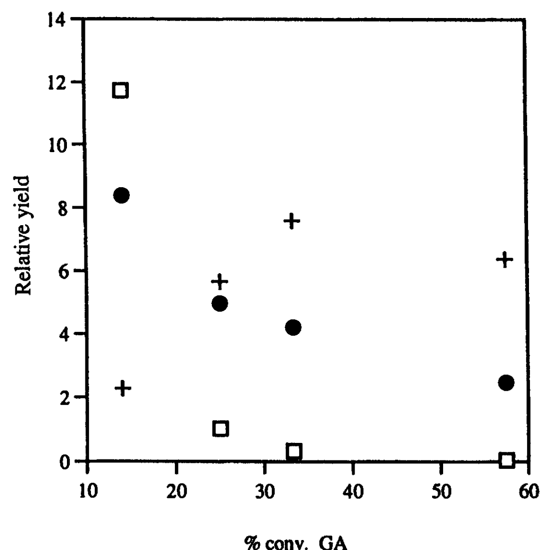



**Fig. 2** Yield profiles for three unidentified products in the photolysis of glycyl-DL-alanine at pH 6 detected by HPLC with retention times 4.8 (●), 8.4 (□) and 9.5 (+) min. Values on an arbitary scale are relative within the data for each product.

glycylalanine suggest ammonia is a primary product (as observed with glycylglycine [7a]), but secondary at pH 1, and the same is true for the decarboxylation product **9** (Fig. 1). On similar criteria, two of the additional products observed in HPLC are primary and the other is secondary (Fig. 2). The new compound **10** ($R^1$ = H, $R^2$ = $CH_3$) is seen in HPLC as a comparatively well retained pair of peaks of equal area, consistent with the racemic and *meso* diastereoisomers expected from a racemic substrate. It was isolated by preparative HPLC and characterized as a mixture of hydrochlorides (see Experimental section).

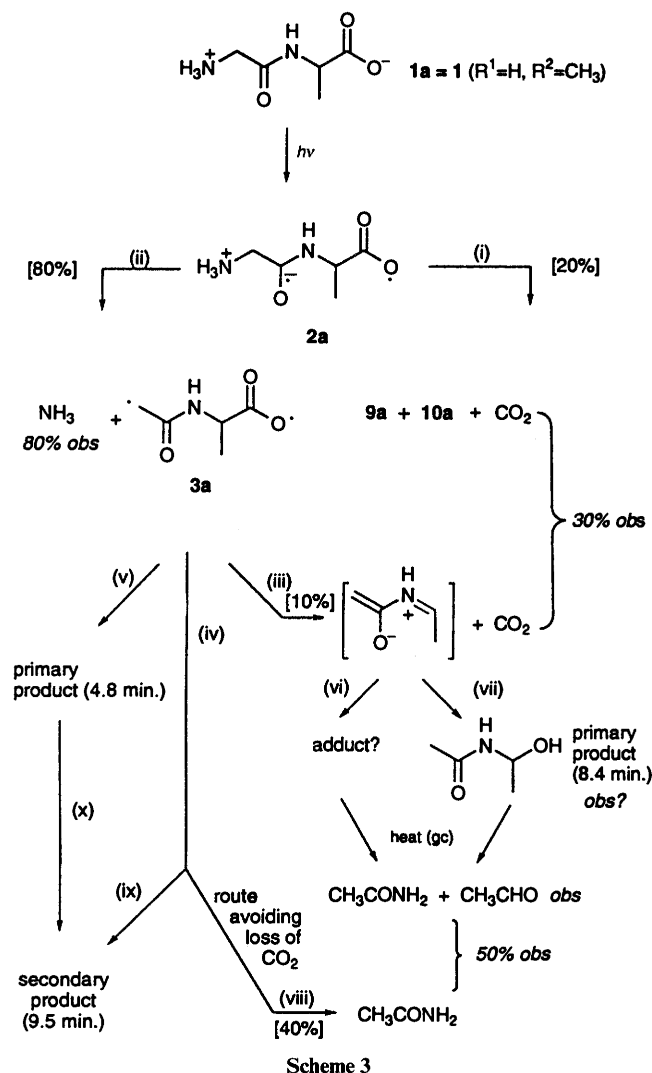
## Discussion

Several reports of the photolysis of glycylalanine are available,[8] but selectivity and lack of quantitation in product analysis severely limit the scope for mechanistic analysis. Some of the present results were anticipated by Leuschner *et al.* who also found acetaldehyde, glycineethylamide and ammonia among the products [8a] and by Riesz and co-workers who observed pH-dependent decarboxylation using spin-trapping techniques.[8d] Of some relevance also are radiolytic studies where electron addition to glycylalanine shares with photoinduced intramolecular electron transfer (PIET) the formation of a peptide radical anion and deamination.[9] Subsequent chemistry differs, however, as PIET leaves a carboxyl radical, whereas the carboxyl function remains initially unaffected by electron addition.

### Photolysis of aqueous free peptide

A major feature of the photolysis of glycylglycine is the trapping of the intermediate **4** ($R^1$ = $R^2$ = H) initially by water, giving *N*-(hydroxymethyl)acetamide but subsequently, as the pH of the photolysate rises with the elimination of ammonia by glycylglycine itself, giving a thermally labile adduct. A significant yield of acetamide is observed only after the photolysate is heated, and it is a co-product of $CO_2$ which is liberated in the formation of **4**.[7a] This pathway, however, appears to be only a minor route in the degradation of glycylalanine. Thus, acetamide yields increase by a factor of less than two after heating and have values significantly exceeding the yield of $CO_2$. No thermally labile component was detected by HPLC, but a peak was observed with appropriate retention for *N*-(1-hydroxyethyl)acetamide at 10% conversion that rapidly loses its significance with increased conversion (labelled 8.4 min in Fig. 2). Route (ii), (iii) and (vii) in Scheme 3 depicts this minor pathway, which is further supported by the detection of acetaldehyde in the photolysate.

The reduced importance of **4** when $R^1$ = H, $R^2$ = $CH_3$ is all the more evident from the appearance of decarboxylation products **9** and **10** which account for at least two thirds of the $CO_2$ observed [Route (i)]. Thus, the first expressed influence of the methyl side-chain seems to be in making less available conformations that allow deamination to be synchronous with electron transfer from carboxylate to peptide (**1**→**3** in Scheme 1). Decarboxylation of **2a** and intramolecular proton transfer would account for **9a** being observed as a significant primary product. Nevertheless, the high yield of ammonia, also as a primary product, suggests immediate deamination, route (ii), remains the principal event at this stage. It appears, however, that unlike the product **3** ($R^2$ = H), **3** ($R^2$ = $CH_3$) has

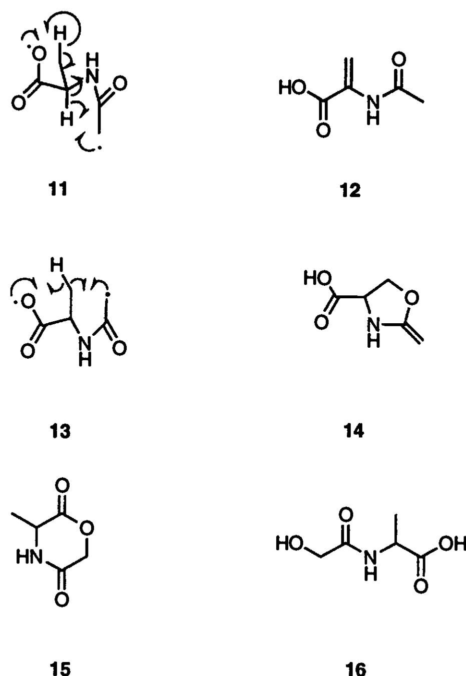



Scheme 3

favourable alternatives to decarboxylation (iv) and (v) of which (iv) also affords acetamide. (Closely analogous behaviour was noted in the photolysis of glycylphenylalanine, the only other GlyX dipeptide examined in detail previously.[7a]) Models show that the CH bonds of the methyl group can participate in the relaxation of the diradical in two ways. A conformation in which the $C_\alpha$–H bond and a methyl C–H bond are antiperiplanar and which also allows each of these hydrogen atoms proximity with an unpaired spin centre (**11**), could promote the formation of *N*-acetyldehydroalanine, **12**. Hydrolysis then affords acetamide and pyruvic acid. Pyruvic acid was not detected, but would be more photolabile than most other components of the photolysate.[10] The secondary and thermally stable, but unknown, product observed by HPLC could be derived from an adduct of **12** with another component of the photolysate [route (iv) and (ix)].

Structure **13** shows a second conformation that could involve a methyl C–H bond in the relaxation of **3** ($R^2$ = $CH_3$). Here, the hydrogen atom can be delivered to an oxygen of the carboxyl radical in a concerted process with attack at the rear of the carbon atom by the other radical centre. The primary product so generated would be pyroglutamic acid if the attacking centre was carbon, or the oxazolidine derivative **14** if it was oxygen. Pyroglutamic acid was detected by HPLC with an exact library match of retention time (4.2 min) and diode array (DA) detector diagnostics, but only as a minor component (< 1% yield). The more significant primary unknown (4.8 min in Fig. 2) could well be **14**, however, which is also the one more readily anticipated in considering requirements in orbital alignment.

Alternatively, the diradical **3a** may find cyclization to **15** a




more favourable process and the latter could then account for the primary product (RT 4.8 min). Moreover, its hydrolysis to **16** would fit the secondary product and, indeed, there does seem to be an inverse linear correlation between the relative yields of these components [routes (v) and (x)] up to 40% conversion.

Scheme 3 summarises the above arguments and illustrates how they would account for the product distribution observed after 20% of the peptide has reacted.

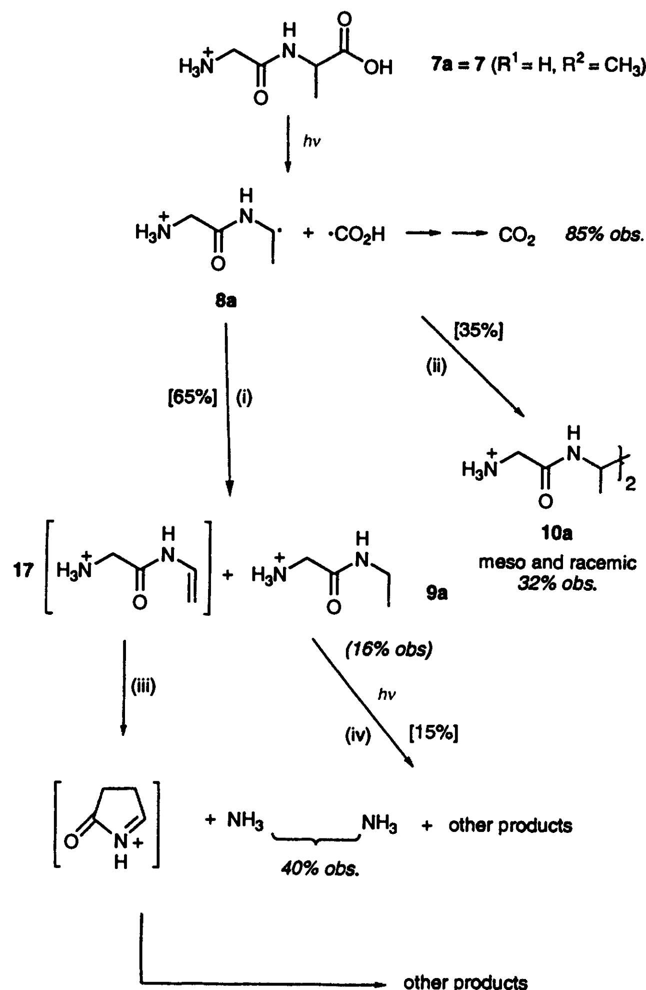
**Photolysis of aqueous protonated peptide**

Protonation of the carboxyl group inhibits electron transfer and photolysis proceeds *via* primary decarboxylation. This follows the behaviour noted by ourselves and others for other dipeptides[7a] and, indeed, has already been observed with glycylalanine.[8d] Products **9** and **10** now arise from disproportionation and combination, respectively, of the decarboxylation radical (Scheme 2) consistent with their assignment as secondary products (Fig. 1). The chemistry also relates consistently to hydroxyl radical promoted processes in radiolysis where both C-terminal α-radicals and dimers are observed.[11]

The main influence on product distribution of the introduction of the C-terminal methyl group appears to be a process that generates ammonia as a secondary product at the expense of **9** and **10**. Disproportionation of **8** ($R^2$ = $CH_3$) in a manner not possible for **8** ($R^2$ = H), would lead to **9** ($R^2$ = $CH_3$) and the enamide **17**. The latter could then undergo a plausible cyclization liberating ammonia and a compound that would be expected to react further.[12] (Glycinamide, expected from the hydrolysis of **17**, is absent from the photolysate.) Scheme 4 summarises all these processes and shows how they could account for the mass balance observed at 20% conversion.

## Conclusions

The validity of an electron-transfer mechanism that accounts for the photochemical behaviour of XGly dipeptides in neutral aqueous solution is sustained by the photolysis of glycyl-DL-alanine. Predictions of the influence of the carboxy-terminal side-chain on the mechanism are seen to be fulfilled in the product distribution, which is best explained by the additional pathways provided by the methyl group for the diradical produced by electron transfer and deamination, to relax.




Scheme 4

As with XGly dipeptides, photolysis at lower pH inhibits electron transfer, but here too, the methyl group of alanyl influences the fate of the intermediate decarboxylation radical, offering disproportionation in competition with dimerization and hydrogen abstraction from other molecules.

## Experimental

### Instrumental

NMR spectra were recorded with a Bruker WH 400 spectrometer with TMS or DSS as standards. Exact mass measurements were carried out with a VG ZAB-E mass spectrometer. Except where indicated otherwise, HPLC analyses were performed with a Varian 5060 LC and a Kratos SF757 UV detector or Polychrom DA detector set at 215 nm, using a 250 × 4.6 mm Partisil 13 column at 30 °C. The mobile phase (flow rate: 1 $cm^3$ $min^{-1}$) was 0.0016 mol $dm^{-3}$ Na octane sulfonate and 0.5 mol $dm^{-3}$ $KH_2PO_4$ in 5:95 acetonitrile–water at pH 2.6. The acetonitrile (ACN) content was increased in a search for more highly retained components. GLC quantitation of acetamide was performed on a Pye Unicom 4500 FID chromatograph fitted with a 10 m × 0.3 mm SAC Superox (BP20-1 micron) column following a 1 m × 0.53 mm deactivated fused silica pre-column and using butanamide as internal standard. Ammonia and carbon dioxide were determined as described previously.[7a]

### Materials

Glycyl-DL-alanine, acetaldehyde, 2,4-dinitrophenylhydrazine (2,4-DNP), acetamide, glycinamide and pyroglutamic acid were obtained from Sigma Chemicals Ltd. Acetaldehyde-2,4-dinitrophenylhydrazone and glycineethylamide[13] were prepared by standard procedures.

### Photolyses

Photolyses were carried out by each of the two procedures described previously as methods A and B.[7a] An amendment to method A was introduced to detect acetaldehyde. A trap containing 2,4-dinitrophenylhydrazine (1.6 × $10^{-2}$ mol $dm^{-3}$) in hydrochloric acid (5 mol $dm^{-3}$) was inserted between the gas outlet of the reactor and the barium hydroxide traps used for $CO_2$ quantitation. Method A was employed in the identification and monitoring of all products by HPLC and, additionally, for acetamide by GLC. Acetaldehyde-2,4-DNP was identified by isolation and comparison with a standard; acetamide, glycineethylamide and pyroglutamic acid were confirmed by HPLC comparisons of capacity factors and DA purity parameters with those of standards; the diastereomeric pair of dehydrodimers **10a** were isolated and characterized (below). Method B was used in the quantitation of **9a** and **10a**.

Photolyses at pH 6 ran without pH adjustment. Those at pH 1 were adjusted close to this value by the addition of the required amount of 70% $HClO_4$.

### *N,N'*-Diglycylbutane-2,3-diamine 10a

Glycyl-DL-alanine (2.44 g, 0.017 mol) in water, to which had been added 70% $HClO_4$ (4 $cm^3$, giving pH 1.31) was irradiated under nitrogen using Method A until 85% of the substrate (HPLC) had reacted. The pH was adjusted to 10 with NaOH and the photolysate passed down an Amberlite IRA 401 ion exchange column converted to the OH form, which removed both unchanged peptide and $ClO_4^-$. The total sample and washings (800 $cm^3$) was reduced to 400 $cm^3$ by rotary evaporation at 35 °C before separation by preparative HPLC (Column: S5 ODS2, 250 × 21 mm; MP: 0.25% heptafluorobutyric acid in 1 : 10 ACN–$H_2O$; 10 $cm^3$ injections; 25 $cm^3$ $min^{-1}$). Samples corresponding to the two diastereoisomer peaks were collected together, the ACN removed by evaporation and the water by lyophylization. The HFBA salts thus recovered were dissolved in ethyl acetate and treated with dry HCl giving, after filtration and drying, the HCl salts as an off-white solid; HRMS (FAB+) $MH^+ = C_8H_{19}N_4O_2$–0.2 mmu; $\delta_H(D_2O)$ 1.1 (d, 6 H) and 3.6–4.1 (m, 6 H); $\delta_C(D_2O)$ 17.1, 18.3, 43.1, 43.3, 51.5, 52.0, 169.0 and 169.1).

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## References

1 S. K. Chapman and A. R. Mount, *Nat. Prod. Rep.*, 1995, **12**, 93.
2 H. B. Gray, *Aldrichim. Acta*, 1990, **23**, 87.
3 C. C. Moser, J. M. Keske, K. Warncke, R. S. Farid and P. L. Dutton, *Nature*, 1992, **355**, 796.
4 D. N. Beratan, J. N. Betts and J. N. Onuchic, *Science*, 1991, **252**, 1285.
5 J. N. Onuchic, D. N. Beratan, J. R. Winkler and H. B. Gray *Ann. Rev. Biophys. Biomol. Struct.*, 1992, **21**, 349.
6 (*a*) J. R. Bolton, J. A. Schmidt, T. F. Ho, J. Liu, K. J. Roach, A. C. Weedon, M. D. Archer, J. H. Wilford and V. P. Y. Gadzepko, in *Electron Transfer in Inorganic, Organic and Biological Systems*, eds. J. R. Bolton, D. Mataga and G. McLardon, ACS, Washington DC, 1991, p 117; (*b*) S. S. Isied, ref. 6(*a*), p. 229; (*c*) S. Song, S. A. Asher, S. Krimm and K. D. Shaw, *J. Am. Chem. Soc.*, 1991, **113**, 1155.
7 (*a*) R. R. Hill, J. D. Coyle, D. Birch, E. Dawe, G. E. Jeffs, D. Randall, I. Stec and T. M. Stevenson, *J. Am. Chem. Soc.*, 1991, **113**, 1805; (*b*) D. Birch, J. D. Coyle, R. R. Hill, G. E. Jeffs, I. Stec and T. M. Stevenson, *J. Chem. Soc., Chem. Commun.*, 1991, 501.
8 (*a*) G. Leuschner, H. Jeschkeit and G. Losse, *Photochem. Photobiol.*, 1966, **5**, 705; (*b*) R. B. Johns and M. G. Seurat, *Photochem.*

*Photobiol.*, 1972, **16**, 413; (*c*) H. Neubacher and G. H. Schnepel, *Radiat. Res.*, 1977, **72**, 48; (*d*) Y. F. Lion, M. Kuwabara and P. Riesz, *J. Phys. Chem.*, 1980, **84**, 3378.
9 M. D. Sevilla and V. L. Brooks, *J. Phys. Chem.*, 1973, **77**, 2954; F. Moriya, K. Makino, N. Suzuki, S. Rokushika and H. Hatano, *J. Phys. Chem.*, 1980, **84**, 3614.
10 D. Budac and P. Wan, *J. Photochem. Photobiol. A*, 1992, **67**, 135.
11 W. M. Garrison, *Chem. Rev.*, 1987, **87**, 381.
12 W. N. Speckamp and H. Hiemestra, *Tetrahedron*, 1985, **41**, 4367.
13 D. S. Kemp, J. M. Dudos, Z. Bernstein and W. M. Welch, *J. Org. Chem.*, 1971, **36**, 157.

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