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The Photochemistry of *N-p*-Toluenesulfonyl Peptides: The Peptide Bond as an Electron Donor

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ABSTRACT

The scope of photobiological processes that involve absorbers within a protein matrix may be limited by the vulnerability of the peptide group to attack by highly reactive redox centers consequent upon electronic excitation. We have explored the nature of this vulnerability by undertaking comprehensive product analyses of aqueous photolysates of 12 *N-p*-toluenesulfonyl peptides with systematically selected structures. The results indicate that degradation includes a major pathway that is initiated by intramolecular electron transfer in which the peptide bond serves as electron donor, and the data support the likelihood of a relay process in dipeptide derivatives.

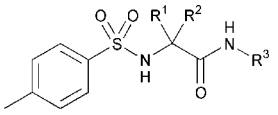
INTRODUCTION

Electron transfer processes within proteins and in particular the mechanistic role of peptide bonds are a topic of considerable biochemical significance and widespread interest (1–5). Hitherto, most attention has been given to the function of peptide chains as bridges in long-range electron transfer between donors and acceptors bound within proteins, and the factors controlling the mechanism of this mediation are topical and highly active areas of study (6). The electron transfer is generally initiated by electronic excitation of either donor or acceptor with the consequent formation of charge-separated intermediates. It might be expected that the proximity of peptide bonds to the resulting highly reactive centers would make them vulnerable to attack with potentially catastrophic consequences for the protein's photobiological properties. That these intramolecular electron transfer (IET) processes seem to leave the protein unaffected would appear to be because of the protective ''design'' features incorporated in their evolution (7–11). The present study uses a simple and convenient model system to investigate the photochemical consequences in peptides that lack such protection.

The arylsulfonyl group confers a significant electron-accepting capacity when electronically excited (12,13). We were interested to examine the extent to which the photochemistry of readily available peptide derivatives containing this group might reveal details of electron transfer processes within these molecules, the results of which could then be applied to the more complex situation present in proteins.

Arylsulfonamides feature prominently among photolabile pharmaceuticals (14–16) and are included among those known for adverse photosensitizing reactions (17,18). Although the relevant photochemistry in this context remains poorly understood, more detailed studies of less-complex arylsulfonyl derivatives, considered as protected and potentially photorecoverable amines and amino acids (19), have provided some useful mechanistic insights. Derivatives of amino acids yield products indicative of IET (20–23), which compromises the anticipated *N*-protection utility, requiring an additional reductant for even modest recovery of the amino compound (24,25). In fact, the charge-separated intermediates seem disposed to alternative modes of degradation through the oxidized component (26).

We have chosen to investigate, by product analysis, the aqueous photochemistry of the *N-p*-toluenesulfonyl derivatives of a representative series of α-amino acid methylamides and four glycyl dipeptides (see general structure below and Table 1), together with an analogous derivative of β-alanine. Changes in product distribution with progressive changes in structure were determined, and the results across the series appear to be most comprehensively and consistently accommodated by pathways initiated by IET that include cleavage of peptide bonds (CO–NH in the structure) remote from the chromophore. The peptide bond may be seen to act in this context as a donor, contrasting with its acceptor role in the photochemistry of underivatized peptides (5,27).



Generalized structure of *N-p*-toluenesulfonyl derivatives.

MATERIALS AND METHODS

General. Melting points (mp) were determined on an electrothermal apparatus and are uncorrected. Infrared (IR) spectra were recorded as potassium bromide discs or as thin films between sodium chloride plates with a Perkin-Elmer 1710 (Beaconsfield, UK) or Nicolet 205 IR spectrometer (Milton Keynes, UK). Nuclear magnetic resonance (NMR) spectra were obtained on a JEOL LAMBDA 300 or JEOL EX400 (Tokyo, Japan). Chemical shifts are reported as parts per million (ppm) downfield of tetramethylsilane, which was used as an internal standard. Microanalytical data were obtained from Medac Ltd., Egham, UK. Accurate mass data were

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Abbreviations: DNP, 2,4-dinitrophenylhydrazone; IET, intramolecular electron transfer; PIET, photoinduced electron transfer; Tol, *p*-tolyl (4-methylphenyl); TsH, *p*-toluenesulfinic acid; TsOH, *p*-toluenesulfonic acid.

Table 1. Structures of the *N-p*-toluenesulfonyl α-amino methylamides and dipeptides

	1	2	3	4	5	6	7	8	9	10	11
R^1	H	H	H	H	H	H	Me	H	H	H	H
R^2	H	$CH(Me)_2$	$(CH_2)_2SMe$	CH_2Ph	$CH_2C_6H_4OMe$	$-(CH_2)_3N$†	Me	H	H	H	H
R^3	Me	Me	Me	Me	Me	Me	Me	CH_2CO_2H	$CH(Me)CO_2H$	$CH(CH(Me)_2)CO_2H$	$(CH_2)_3N–CO_2H$†

† Proline derivative.

obtained by Pfizer Global R & D (Sandwich, UK). UV spectra were recorded in 1 cm quartz cuvettes on a Kontron Model 860 spectrophotometer (Watford, UK), or were extracted from HPLC data. Accurate weighing of samples (<20 mg) was performed on a Perkin-Elmer AD2Z Autobalance (Beaconsfield, UK).

Standard irradiation protocol. A 10^{-2} *M* solution of the compound either in water with pH either unadjusted or at pH 9 (adjusted using 1 *M* NaOH) or in 40% acetonitrile in water, was Suba-sealed (William Freeman, Barnsley, UK) in a quartz irradiation tube and purged with nitrogen (N_2) for 5 min. The tubes were irradiated with a 400 W medium-pressure mercury (Hg) lamp in the inner or outer rings of an Applied Photophysics carousel (Leatherhead, UK) for periods of 1–240 min with an inversion every 30 min. The reactions were quenched on ice for 30 min, which also ensured that all of the product carbon dioxide (CO_2) and ammonia (NH_3) were in solution.

HPLC analyses. HPLC analyses were performed using a Waters System (Elstree, UK) comprising a 616 pump, 600S controller, 717 plus autosampler, 996 photodiode array detector (PDA; 200–700 nm) with a Phenomenex Luna (Macclesfield, UK) C18 250 mm × 4.60 mm column and C18 guard column or security guard cartridge and aqueous acetonitrile gradients plus 10% 10^{-2} M phosphoric acid (H_3PO_4). Products were identified by comparison with standards or, exceptionally, by consistent analytical behavior and liquid chromatography–mass spectroscopy (LC-MS). Quantitation of products was by external calibration with an authentic standard or a related standard with closely similar detector response. Photolysates were treated with Brady's reagent (28) for analysis of aldehydes and ketones as the 2,4-dinitrophenylhydrazone (DNP) derivatives and quantitated relative to formaldehyde DNP if an authentic sample was not available. Amines and NH_3 were analyzed as the AccQTag™ (Waters, Elstree, UK) derivative on a Waters System comprising 474 scanning fluorescent detector, 712 WISP autosampler and a 600 multisolvent delivery system with a Novapak (Waters) 4 μm C18 150 mm × 3.9 mm column and C18 guard column and a 60% aq acetonitrile/buffer (140 mM sodium acetate, 17 mM triethylamine, pH 5.04 [H_3PO_4]) gradient. Data were analyzed using Waters Millennium Software (Elstree, UK). LC-MS data were acquired on a Waters Alliance System with a Waters 996 PDA detector, MS in positive ion-mode detection and a Phenomenex Luna phenyl hexyl 150 mm × 4.60 mm column with an ammonium trifluoroacetate (pH 3.0)/acetonitrile gradient. Prep HPLC was performed with a Waters Deltaprep 3000 pump (Elstree, UK), 600E system controller, 484 tunable absorbance detector and a 745B data module with a Jones Apex ODS 8 μm, 250 mm × 21 mm column.

CO_2 analysis by gas chromatography (GC). Analyses were performed on a Unicam ATI 610GC (Kassel, Germany) with a Unicam methanizer fitted into the injection port. The column was a 10 ft by one-eighth inch SS Porapak Q column, with a mesh size of 80–100. Peak areas were determined using a Spectra-Physics Chromjet integrator (Berkshire, UK). Ten cubic centimeters of a 10^{-2} M solution of the compound (in 40% aq acetonitrile) was treated as for the general photolysis procedure. The photolysate was analyzed by HPLC to calculate sample degradation and by GC as follows for CO_2 quantitation: 1 cm^3 1M hydrogen chloride (HCl) was added to the photolysate in the suba-sealed tube, warmed to 40°C, purged for 2 min with N_2 into an evacuated gas bag, 25 μL of the gas sample was injected into the GC, and CO_2 was detected as methane (CH_4; at room temperature [RT] 2.3 min). Samples and standards (sodium bicarbonate [$NaHCO_3$] in 40% aq acetonitrile) were analyzed in triplicate.

Reagents. *O*-Methyl-L-tyrosine was supplied by Acros Organics (Geel, Belgium). Ammonium acetate, 37% hydrochloric acid, magnesium sulfate (anhydrous), potassium bromide (Spectrosol) and sodium hydroxide were supplied by BDH Chemical Company (London, UK). Acetone, acetonitrile (far UV), dichloromethane, diethyl ether, ethyl acetate, hexane, methanol, tetrahydrofuran (THF) and trifluoroacetic acid (TFA) were supplied by Fisher Scientific (Loughborough, UK). Sodium hydrogen carbonate, tetrabutylammonium bromide, *N*-cetyltrimethylammonium bromide and trisodium citrate were supplied by Fisons Scientific Equipment (Loughborough, UK). Ethanol was supplied by Hayman Ltd. (Witham, UK). 2,4-Dinitrophenylhydrazine was supplied by Hopkin and Williams (Dagenham, UK). 2-Aminoisobutyric acid, methyl 3,3-dimethoxypropionate, phenethylamine, 2-phenyl-5-oxazalone, phenylpyruvic acid Na salt, L-proline and triethylamine were supplied by Lancaster Chemicals (Morecambe, UK). NMR solvents were supplied by Cambridge Isotope Laboratories (Andover, MA) except for deuterium oxide (D_2O) supplied by Goss Scientific Instruments (Great Baddow, UK). All other chemicals were supplied by Sigma-Aldrich (Gillingham, UK). All solvents were reagent grade unless stated otherwise.

General procedures for syntheses of N-*tosyl derivatives*. *N*-tosyl compounds were synthesized by standard methods of tosylation (29). The amino acid was dissolved in H_2O/THF (2:1) with three equivalents of Et_3N. One equivalent of tosyl chloride was added at 0°C and stirred at 20°C for 3 h. THF was removed, and the aqueous solution was extracted with ether, acidified (pH 2), extracted with ethyl acetate and the organic extracts washed with H_2O, dried ($MgSO_4$), filtered, solvent removed and the solid recrystallized. Esterification of tosyl amino acids was by refluxing in MeOH/H_2SO_4 overnight. Amidation was by stirring the ester at RT with an excess of methylamine in THF for 72 h (see Table 2).

N-p-*Tosylmethionine* N′*-methylamide*, **3**. Tosylation of methionine methyl ester hydrochloride (3.00 g, 15 mmol) gave 3.59 g of a white solid (75% yield) that was treated with methylamine to give 1.94 g (41% yield) of white crystals (ethyl acetate): mp 160–162°C; ν_{max} (KBr)/cm^{-1} 3359 (NH), 3325 (NH), 1653, 1647, 1347, 1326, 1162, 673, 573, 553; 1 δ_H (300 MHz; CD_3CN) 1.60–1.73 (1H, m, $CHCH_2$), 1.75–1.85 (1H, m, $CHCH_2$), 1.93 (3H, s, SCH_3), 2.20–2.36 (2H, m, SCH_2), 2.38 (3H, s, $ArCH_3$), 2.46 (3H, d, *J* 4.8, NCH_3), 3.72 (1H, br s, CH), 5.97 (1H, br s, SNH), 6.52 (1H, br s, $NHCH_3$), 7.33 (2H, d, *J* 8.2, Ar), 7.67 (2H, d, *J* 8.2, Ar); 1 δ_C (75 MHz; CD_3CN) 14.66 ($CHCH_2$), 21.00 ($ArCH_3$), 25.67 (NCH_3), 29.89 (SCH_3), 32.93 (SCH_2), 56.28 (NCH), 127.57 (Ar–CH), 130.08 (Ar–CH), 137.63 (Ar–C), 144.44 (Ar–C), 171.21 (CO); Found: C, 49.47%; H, 6.47%; *N*, 8.84% ($C_{13}H_{20}N_2O_3S_2$ requires: C, 49.34%; H, 6.37%; *N*, 8.85%).

N-p-*Tosyl*-O-*methyl*-L-*tyrosine* N′*-methylamide*, **5**. *N-p*-Tosyl-*O*-methyl-L-tyrosine (1.35 g) was converted to the methyl ester and then to the methylamide and recrystallized to give white crystals (53% yield) from ethyl acetate/hexane mp 166°C; ν_{max} (KBr)/cm^{-1} 3321 (NH), 3362 (NH), 1654, 1561, 1513, 1334, 1234, 1157, 1088; 1 δ_H (300 MHz; $CDCl_3$) 2.43

Table 2. Syntheses of known tosyl compounds

Compound	Melting point (°C)	Reference melting point (°C)
N-p-Tosyl-2-amino-isobutyric acid	146–147	(30) 149–150
N-p-Tosyl-β-alanine	118–120	(31) 119.5–121
N-p-Tosylglycylalanine, **9**	168–171	(32) 167
N-p-Tosylglycylglycine, **8**	173–179	(29) 178–179
N-p-Tosylglycylproline, **11**	182–184	(33) 183–184
N-p-Tosylglycyl-DL-valine, **10**	132–137	(34) 131
N-p-Tosyl-DL-methionine	117–118	(35) 104–105
N-p-Tosyl-*O*-methyl-L-tyrosine	139–141	(36) 138–140
N-p-Tosylphenylalanine	145–155	(37) 165.5–167
N-p-Tosyl-L-valine	148–149	(38) 149.5–150.5
N-p-Tosylglycine N′-methylamide, **1**	131–133	(39) 130
N-p-Tosyl-β-alanine N′-methylamide	127–129	(40) 127–130

(3H, s, $ArCH_3$), 2.74 (3H, d, *J* 4.8, NCH_3), 2.84 (1H, d, *J* 6.5, CH_2), 2.86 (1H, d, *J* 6.5, CH_2), 3.77 (3H, s, OCH_3), 3.81 (1H, m, CH), 4.97 (1H, d, *J* 6.8, SNH), 6.38 (1H, br d, *J* 4.8, NHMe), 6.68 (2H, d, *J* 8.7, OAr-CH), 6.82 (2H, d, *J* 8.7, OAr–CH), 7.22 (2H, d, *J* 8.2, SAr-CH), 7.53 (2H, d, *J* 8.2, SAr-CH); 1 δ_C (75 MHz; CCl_3) 21.58 ($ArCH_3$), 26.34 (NCH_3), 37.32 (CH_2), 55.21 (OCH_3), 58.06 (CH), 114.26 (OAr-CH), 127.12 (OAr-C), 127.17 (SAr-CH), 129.77 (SAr-CH), 130.12 (OAr–CH), 135.68 (SAr-C), 143.93 (SAr–C), 158.81 (OAr–C), 170.79 (CO); Found: C, 59.77%; H, 6.15%; *N*, 7.69% ($C_{18}H_{22}N_2O_4S$ requires: C, 59.65%; H, 6.12%; *N*, 7.73%).

N-p-*Tosyl*-N′*-methyl-2-amino-isobutyramide,* **7**. Tosylation of *N*-methyl-2-amino-isobutyramide (1.53 g, 13 mmol) gave 0.90 g (25% yield) of white crystals (diethyl ether/methanol) mp 174–176°C; ν_{max} (KBr)/cm^{-1} 3146 (NH), 1653, 1646, 1645, 1540, 1523, 1330, 1158, 1096, 993, 809, 678, 538; 1 δ_H (300 MHz; CD_3CN) 1.26 (6H, s, $2 \times CH_3$), 2.40 (3H, s, $ArCH_3$), 2.59 (3H, d, *J* 4.8, NCH_3), 6.01 (1H, br s, SNH), 6.66 (1H, br s, CONH), 7.34 (2H, d, *J* 7.9, Ar), 7.71 (2H, d, *J* 7.9, Ar); 1 δ_C (75 MHz; CD_3CN) 20.98 ($ArCH_3$) 25.79 ($2 \times CH_3$), 26.13 (NCH_3), 59.78 (SNC), 127.20 (Ar-CH), 130.03 (Ar–CH), 140.67 (Ar–C), 143.85 (Ar–C), 174.81 (C=O); Found: C, 53.17%; H, 6.72%; *N*, 10.30% ($C_{12}H_{18}N_2O_3S$ requires: C, 53.31%; H, 6.71%; *N*, 10.36%).

N-p-*Tosylphenylalanine* N′*-methylamide,* **4**. *N-p*-Tosylphenylalanine was converted to the methyl ester (3.00 g) and then treated with methylamine (122 cm^3, IMS sol) to give white crystals (ethyl acetate/hexane) mp 160°C; ν_{max} (KBr)/cm^{-1} 3322 (NH), 3263 (NH), 1660, 1334, 1163, 1156, 1091, 1078, 669, 540; 1 δ_H (300 MHz; $CDCl_3$) 2.43 (3H, s, $ArCH_3$), 2.74 (3H, d, *J* 4.6, NCH_3), 2.86 (1H, dd, *J* 6.2 and 13.9, CH_2), 2.96 (1H, dd, *J* 7.0 and 13.9, CH_2), 3.83 (1H, q, *J* 6.8, CH), 4.82 (1H, s, *J* 6.8, SNH), 6.27 (1H, br d, *J* 4.6, CONH), 6.91 (2H, d, *J* 8.4, Ph), 7.14–7.26 (5H, m, Ar), 7.53 (2H, d, *J* 8.4, SAr); 1 δ_C (100 MHz, $CDCl_3$) 21.58 ($ArCH_3$), 26.34 (NCH_3), 38.19 (CH_2), 57.88 (CH), 127.16 (Ar–CH), 127.27 (Ar–CH), 128.93 (Ar–CH), 129.01 (Ar–CH), 129.83 (Ar–CH), 135.24 (SAr–C), 135.55 (Ar–C), 143.96 (SAr–C), 170.63 (CO); Found: C, 61.25%; H, 6.09%; *N*, 8.37% ($C_{18}H_{22}N_2O_4S$ requires: C, 61.43%; H, 6.06%; *N*, 8.42%).

N-p-*Tosylproline* N′*-methylamide,* **6**. *N-p*-Tosylproline was obtained as a pale yellow gum and purified to give a clear gum by column chromatography on silica gel (1:1 vol/vol ethyl acetate:hexane). Then 3.04 g was converted to the ester and then to the methylamide and white crystals (72% yield) were obtained (ethyl acetate/hexane) mp 122–124°C; ν_{max} (KBr)/cm^{-1} 3433 (NH), 3374 (NH), 1669, 1658, 1518, 1347, 1341, 1157, 815, 667, 595, 551; 1 δ_H (300 MHz; CD_3CN) 1.44–1.63 (2H, m, $CH_2CH_2CH_2$), 1.65–1.76 (1H, m, $CHCH_2$), 1.85–1.96 (1H, m, $CHCH_2$), 2.41 (3H, s, $ArCH_3$), 2.69 (3H, d, *J* 4.7, NCH_3), 3.12–3.20 (1H, m, NCH_2), 3.42–3.49 (1H, m, NCH_2), 3.97 (1H, dd, *J* 4.3 and 8.3, CH), 6.99 (1H, br s, NH), 7.40 (2H, d, *J* 8.3, Ar), 7.72 (2H, d, *J* 8.3, Ar); 1 δ_C (75 MHz; CD_3CN) 21.05 ($ArCH_3$), 24.55 ($CH_2CH_2CH_2$), 25.80 (NCH_3), 30.86 ($CHCH_2$), 49.96 (NCH_2), 63.09 (CH), 128.23 (Ar–CH), 130.40 (Ar–CH), 134.02 (Ar–C), 144.90 (Ar–C), 172.62 (CO); Found: C, 55.30%; H, 6.44%; *N*, 9.85% ($C_{13}H_{18}N_2O_3S$ requires: C, 55.30%; H, 6.43%; *N*, 9.92%).

N-p-*Tosylvaline* N′*-methylamide,* **2**. *N-p*-Tosylvaline (3.55 g, 13.1 mmol) was converted to the methyl ester and then to the methylamide to give 1.24 g (33% yield) of white crystals (ethyl acetate) 204–206°C; ν_{max} (KBr)/cm^{-1} 3347 (NH), 3259 (NH),1654, 1320, 1168, 1085, 1068, 664, 576; 1 δ_H (300 MHz; CD_3CN) 0.78 (3H, d, *J* 6.7, $CHCH_3$), 0.81 (3H, d, *J* 6.7, $CHCH_3$), 1.83 (1H, m, *J* 6.7, $CHCH_3$), 2.38 (3H, s, $ArCH_3$), 2.39 (3H, d, *J* 4.8, NCH_3), 3.38 (1H, dd, *J* 6.2 and 8.8, NCH), 5.80 (1H, br d, *J* 8.8, SNH), 6.35 (1H, br s, $NHCH_3$), 7.32 (2H, d, *J* 8.4, Ar), 7.65 (2H, d, *J* 8.4, Ar); 1 δ_C (75 MHz; CD_3CN) 17.60 ($CHCH_3$), 18.85 ($CHCH_3$), 20.98 ($ArCH_3$), 25.44 (NCH_3), 31.88 ($CH(CH_3)_2$), 62.68 (NCH), 127.61 (Ar-CH), 129.89 (Ar-CH), 137.84 (Ar-C), 144.08 (Ar-C), 171.08 (CO); Found: C, 54.86%; H, 7.12%; *N*, 9.73% ($C_{13}H_{20}N_2O_3S$ requires: C, 54.91%; H, 7.09%; *N*, 9.85%).

General procedure for the synthesis of 2,4-dinitrophenylhydrazone derivatives (28). 2,4-Dinitrophenylhydrazine was added to an acidified solution (20 cm^3 solvent plus 2 cm^3 conc. HCl) and heated to boiling. A yellow-orange solid precipitated on cooling and was collected by filtration, dried ($MgSO_4$) and recrystallized. In many cases, two peaks were observed by HPLC analysis corresponding to the *syn* and *anti* stereoisomers. The existence of these was confirmed either by separation of the isomers and acquisition of analytical data or by the interconversion of the two peaks on leaving the DNP derivative in 50% aq acetonitrile at RT for several days. Where present, both stereoisomers were observed in the analysis of photolysates using Brady's reagent (see Table 3).

N-*Methylphenylpyruvamide DNP*. Phenylpyruvic acid (0.50 g) was dissolved in dry THF (10 cm^3) and *N,N*-dimethylformamide (20 μL) under N_2 at 0°C. Oxalyl chloride (0.40 cm^3, 4.6 mmol) was added slowly and stirred for 30 min, warmed to 20°C, solvent and excess $(COCl)_2$ removed to give 0.50 g of an oil, which was dissolved in THF (2 cm^3) and added dropwise to methylamine (5 cm^3, 40% aq wt sol.) at 0°C and stirred for 1 h, warmed to 20°C and the THF removed. The aqueous layer was extracted with diethyl ether (2×20 cm^3) and the organic extracts washed with 2 M HCl (20 cm^3) and H_2O (20 cm^3), dried and the solvent removed to give 0.20 g of an oil, which was dissolved in 1 cm^3 acetonitrile and reacted with DNPH/MeOH solution. Then 0.11 g of yellow crystals were obtained by recrystallization from ethanol, mp 168°C; ν_{max} (KBr)/cm^{-1} 3354 (NH), 1654, 1623, 1522, 1341, 1314, 1259, 1133, 1113; 1 δ_H (300 MHz; CD_3CN) 2.89 (3H, d, $J = 4.95$, CH_3), 4.12 (2H, s, CH_2), 7.20–7.33 (5H, m, CAr), 7.59 (1H, broad s, NH-CH_3), 8.12 (1H, d, *J* 9.51, NAr), 8.37 (1H, dd, *J* 2.55 and 9.51, NAr) 8.92 (1H, d, *J* 2.55, NAr), 11.06 (1H, broad s, NH–Ar); 1 δ_C (75 MHz; CD_3CN) 26.07 (CH_3), 30.75 (CH_2), 117.67 (Ar–CH), 123.24 (Ar–CH), 127.59 (Ar–CH), 129.00 (Ar–CH), 129.50 (Ar–CH), 130.48 (Ar–CH), 131.65 (Ar–C), 134.88 (Ar–C), 140.01 (Ar–C), 144.68 (Ar–C), 148.96 (C=N), 164.35 (C=O); Found: C, 52.8%; H, 4.4%; *N*, 19.3%; ($C_{16}H_{15}N_5O_5$0.0.25 H_2O requires: C, 53.1%; H, 4.3%; *N*, 19.4%).

N-*Oxo-acetylglycine DNP*. *N*-oxo-acetylglycine was synthesized by a known method (46) and converted to the DNP derivative (28); 140 mg of a yellow solid was obtained. HPLC analysis showed two products, which were separated by prep HPLC (45% H_2O:45% acetonitrile:10% 0.1 mol dm^{-3} TFA/5% aq methanol) and recrystallized from ethanol. Product 1: mp 223°C; found MH^+ 312.0585 ($C_{10}H_{10}N_5O_4$ requires 312.0580); 1 δ_H (300 MHz; CD_3OD) 4.07 (2H, s, CH_2), 7.77 (1H, s, N=CH), 8.31 (1H, d, *J* 9.5, Ar), 8.42 (1H, dd, *J* 2.6 and 9.5, Ar), 9.05 (1H, d, *J* 2.6, Ar). Product 2: mp 236°C; found MH^+ 312.0591 ($C_{10}H_{10}N_5O_4$ requires 312.0580) 1 δ_H (300 MHz; CD_3OD) 4.05 (2H, s, CH_2), 7.15 (1H, s, N=CH), 8.16 (1H, d, *J* 9.5, Ar), 8.41 (1H, dd, *J* 2.6 and 9.5, Ar), 9.06 (1H, d, *J* 2.6, Ar). The literature mp (ethanol [46] 214–215°C) was lower than either of the two products formed.

Phenylpyruvic acid DNP. Phenylpyruvic acid sodium salt (0.50 g, 25 mmol) was treated with DNPH/MeOH solution and gave 0.77 g of an orange solid (91% yield). HPLC analysis of a sample (1 mg/cm^3) showed two products, which were separated by prep HPLC (30% H_2O:60% acetonitrile:10% 0.1 M TFA in 5% methanol). The solvents were removed and the residual solids were dissolved in DCM, washed with H_2O, dried and recrystallized from ethyl acetate to give yellow crystals; 0.53 g from peak 1 and 0.17 g from peak 2. Product 1: mp 160–162°C (lit: [47] 192–194°C); 1 δ_H (300 MHz; CD_3CN) 4.11 (2H, s, CH_2), 7.25–7.36 (5H, m, CAr), 8.18 (1H, d, *J* 9.5, NAr), 8.33 (1H, dd, *J* 2.6 and 9.5, NAr), 8.92 (1H, d, *J* 2.6, NAr), 11.14 (1H, br s, NH); Found: C, 52.30%; H, 3.57%; *N*, 16.07%; ($C_{15}H_{12}N_4O_6$ requires: C, 52.33%; H, 3.51%; *N*, 16.27%). Product 2: mp 192–194°C (lit: [47] 192–194°C); 1 δ_H (300 MHz; CD_3CN) 3.94 (2H, s, CH_2), 7.24–7.35 (5H, m, CAr), 8.02 (1H, d, *J* 9.5, NAr), 8.34 (1H, dd, *J* 2.5 and 9.5, NAr), 8.97 (1H, d, *J* 2.5, NAr), 14.12 (1H, br s, NH); Found: C, 52.10%; H, 3.59%; *N*, 15.72%; ($C_{15}H_{12}N_4O_6$ requires: C, 52.33%; H, 3.51%; *N*, 16.27%).

Glycine N-*methylamide*. Glycine methyl ester hydrochloride (1.00 g) was converted to the methylamide and 650 mg of an oil was obtained (93% yield). 1 δ_H (300 MHz; D_2O) 3.73 (3H, s, CH_3), 3.84 (2H, s, CH_2), 4.65 (1H, s, NH). The ^1H NMR spectrum was consistent with the literature (48).

Table 3. Syntheses of known dinitrophenylhydrazine (DNPH) derivatives

DNPH derivative	Solvent	Melting point (°C)	Reference melting point (°C)	Isomers
Acetone	Acetone	121–125	(41) 123–125	no
Benzaldehyde	Brady's (28)	235–241	(42) 238–240	no
N-Methylglyoxamide (43)	MeOH	258–261	(43) 244–245	yes
N-Methylpyruvamide (44)	MeOH	185	(44) 186–187.5	no
3-Oxo-propionic acid	MeOH	146–148	(45) 150	no

Table 4. Mole fractions of products (%) in photolysates of *p*-TsNHC(R^1R^2)CONHR^3 at 10% reaction in 40% aqueous acetonitrile unless indicated otherwise (blank entries and dashes signify ''no data'' and ''not detected,'' respectively)

	1	2	3	4	5	6†	7	8	9‡	10‡	11†‡
10% reaction, *t*/min	18	13	13	13	25	11	14	8§ (11)||	11	7	12
NH_3	74	38	10	58	74	2	67	37 (18)	78	75	59
NH_2R^3	25	10	7	20	3	¶	26	22 (27)	1	1	2
TsH	49	47	37	20	33	65	56	21 (24)	39	32	30
TsOH	10	11	7	10	22	26	11	11 (10)	5	5	3
$TsNH_2$	—	4	3	—	—	—	3	†† (3)	—	—	—
TsNHCHR^1R^2COOH	18	—	—	—	—	—	—	18 (19)	4	—	2
$H_2NC(R^1R^2)CONHR^3$	11	¶	¶	8	¶	¶	34	7 (10)	10	6	5
$R^2COCONHR^3$	78‡‡	23	5	28	12	<56§§	14	58 (71)	86	74	68
$H_2NC(R^1R^2)COOH$	2		—	2	—	—		—	3	2	6
$CHOCONHR^3$	—	—	—	21	>80			—	—	—	—
$R^2COCOOH$	—	4	—	3||||	—	—	—	—	—	—	—
Other DNPs (No.)	—	10§§ (3)	<1§§ (2)	R^2COH 1		<56§§ (2)	$CO(Me)_2$ 6	$CO(Me)_2$ 6	12§§	11§§	—
CO_2							5	1 (10) 12¶¶	10	6	30
Other significant product				$H_2NCH_2R^2$ 1 BzOH	TsX††† 14			Compound **1** <1 (6)			TsX††† 4

† The proline derivative requires small adaptations of some formulas in Column 1.
‡ Data are for 30% reaction.
§ 100% water, unadjusted pH (~3).
|| 100% water, pH 9 with NaOH.
¶ Data consistent with this product but not quantified.
†† No evidence for this product.
‡‡ $R^1 = R^2 = H$ and compound accounted for in row 9.
§§ Uncharacterized 2,4-dinitrophenylhydrazone (DNP) derivatives (except for LCMS) were quantified relative to HCHO standard.
|||| Additionally, 5% PhCHO was found.
¶¶ 40% acetonitrile in water.
††† Unknown tosylamide compound (diagnostic UV spectral data) quantified using substrate as standard.

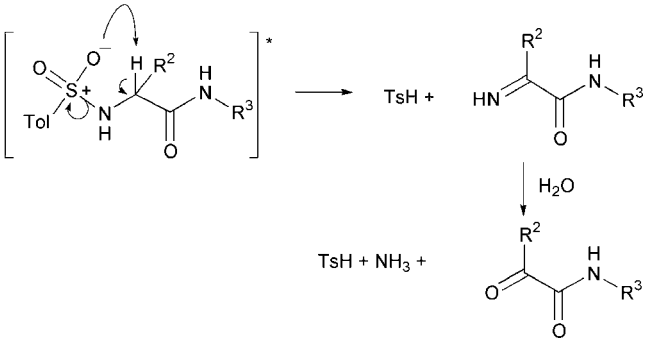
N-*Methyl-2-aminoisobutyramide*. Methyl 2-aminoisobutyrate hydrochloride (49) (4.00 g) was treated with methylamine to give 1.68 g of an oil (55% yield); 1 δ_H (300 MHz; D_2O) 1.26 (6H, s, $2CH_3$), 2.61 (3H, s, NCH_3).

Phenylalanine N-*methylamide*. Phenylalanine methyl ester hydrochloride (0.51 g, 2.36 mmol) was reacted with methylamine to give 0.20 g of an oil (48% yield). 1 δ_H (300 MHz; $CDCl_3$) 1.26 (2H, br s, NH_2), 2.60 (1H, dd, *J* 9.5 and 13.7, CH_2), 2.75 (3H, d, *J* 4.9, CH_3), 3.22 (1H, dd, *J* 4.0 and 13.7, CH_2), 3.53 (1H, dd, *J* 4.0 and 9.5, CH), 7.14-7.31 (5H, m, Ar). The ^1H NMR spectrum was consistent with the literature (50).

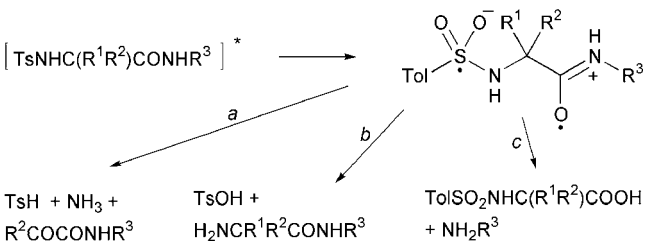
RESULTS AND DISCUSSION

Most solutions of the arylsulfonyl peptides became intensely yellow on irradiation and generated a wide range of products that were identified and quantified by the methods described in the previous section. To minimize complexities from secondary reactions, we determined the relative amounts of products at 10% conversion (Table 4). As might be expected with a common chromophore, we saw little variation in rate (expressed as the time taken to reach 10% conversion of the starting material). We found the major products to be ammonia, *p*-toluenesulfinic acid (TsH) and glyoxylamides or α-ketoamides ($R^2COCONHR^3$) with *p*-toluenesulfonic acid (TsOH) and amines from the carboxamide (NH_2R^3) as significant minor products. Pair-wise correlations of the concentrations of the three major products up to 40% conversion gave coefficients, gradients and intercepts better than 0.99, near unity and close to zero, respectively.

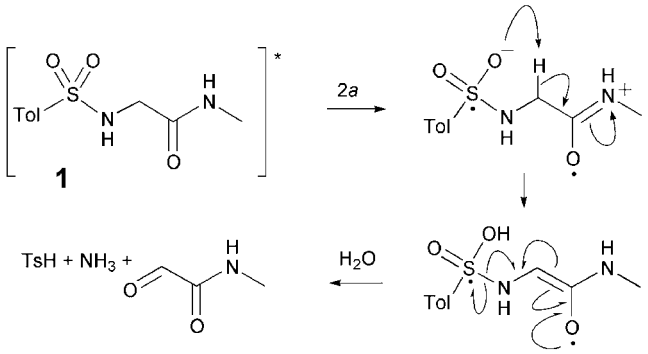
For all but compound **7**, where $R^1 \neq H$, the major products alone could be explained by a concerted β-H abstraction reminiscent of known thermal reactions of arylsulfones (51) but here promoted by electronic excitation (Scheme 1). We suggest, however, that a more comprehensive rationale for this photochemistry (which accommodates both the minor products and the transient coloration of the photolysate) can be expressed as an IET process that makes explicit the photoinduced oxidation by the arylsulfonamide group, a powerful acceptor in its electronically excited state (52) (Scheme 2). The tosylamido chromophore has its least energetic electronic transition near 265 nm with an apparent 0–0 component at 273 nm. This corresponds to 4.54 eV, and with *E* (red) ~ –2.3 V for tosylamido



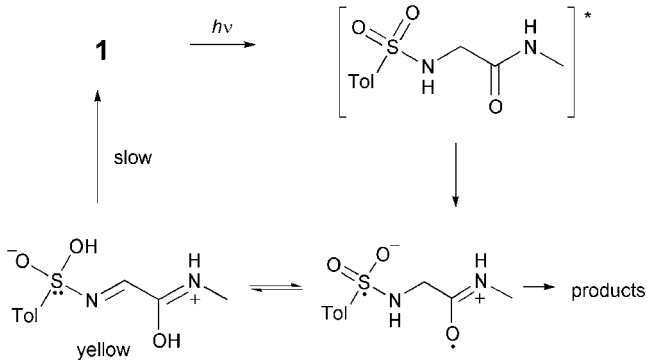
Scheme 1. Converted pathways to principal products.



Scheme 2. IET pathway to all products.



Scheme 3. Possible mechanism for pathway 2*a*.



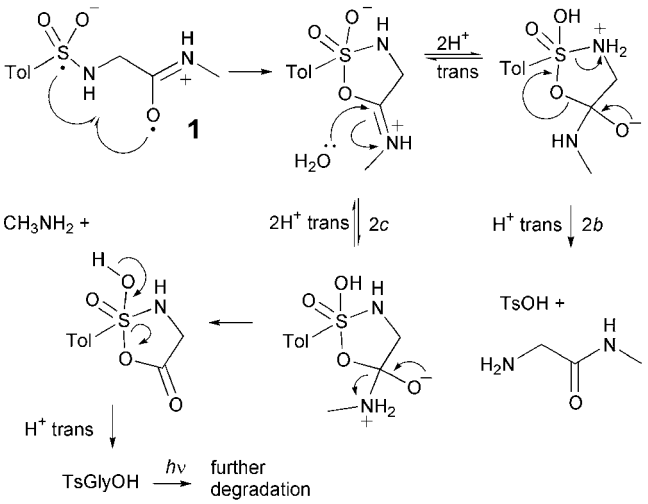
Scheme 5. Possible origin of transient yellow coloration.

(19) and application of the Weller equation (53) is seen to provide the excited state with more than enough driving force (overpotential > (–) 0.7 V) for amide oxidation (E (ox) ~ 1.5 V [54]). Effectively, the excited tosylamido chromophore accepts an electron from the carboxamide moiety and products arise from collapse of the charge-separated intermediate via a choice of competing pathways influenced by substrate structure. (Although short-range IET may proceed via a superexchange [55,56] or an H-bond–mediated [57] rather than an electron-hopping process, the chemical outcomes are qualitatively the same, and the last is more suitable for depicting the changes in bonding that might follow). Scheme 3 depicts pathway 2*a* with the glycyl derivative **1** yielding ammonia, TsH and *N*-methylglyoxamide.

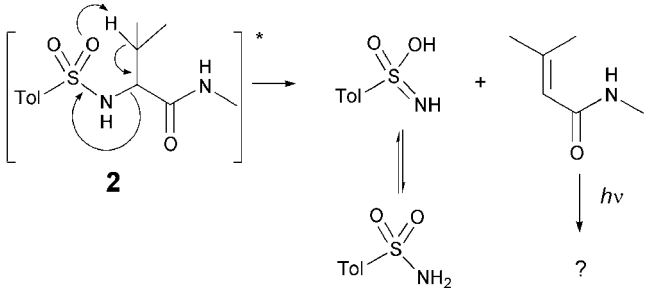
One might expect the choice of pathway to be conformation-dependent in that alignment of appropriate bonds (orbitals) in short-lived intermediates will favor one route or another (6). Competing pathways 2*b* and 2*c* would allow for other products observed, the distribution for the glycine derivative **1** of 2*a*, 65%, 2*b*, 10% and 2*c*, 25% being broadly consistent with the product distribution for **1** in Table 4. (As reported elsewhere, tosylglycine from 2*c* can undergo further photochemistry [26].) Pathways 2*b* and 2*c* might share a common intermediate formed from a conformation allowing biradical ring closure, as suggested with **1** in Scheme 4, from which the two outcomes are seen to depend on competing ring-opening hydrolyses. The products from 2*c*, following C-N bond cleavage remote from the absorbing group, afford the most direct evidence for the participation of the peptide bond as a donor in photoinduced electron transfer, in contrast with its known behavior as an acceptor (5) and as a bridge in long-range processes (1–4,6,58). Photoinduced IET also provides a satisfactory rationale for the coloration of photolysates in this series, which on the basis of its apparent structural requirements, we have attributed elsewhere to photochromism promoted by the charge-separated intermediate (59). This is shown for **1** in Scheme 5.

As can be seen from Table 4, reaction 2*a* is dominant whenever $R^1 = R^2 = H$; *i.e.* when tosylglycyl derivatives are involved (**1**,**8**–**11**). However, when R^2 is a side-chain, the indicator product of 2*a*, $R^2COCONHR^3$, is far less abundant. Although yields of relevant products at 10% conversion of **2** were depressed compared with those from **1** (they became comparable at higher conversion), the introduction of the (bulky) 2-propyl side-chain did not appear to promote significant changes in pathway distribution. Participation of a plausible Norrish Type II competitor hinted by the detection of $TsNH_2$ (Scheme 6) remains tenuous because we failed to find any trace of its anticipated coproduct or of possible secondary photoproducts. A conformational influence might also be expected with the tertiary sulfonamide **6** derived from proline, but the noticeably high value for TsOH and absence of methylamine might also reflect the preference for 2*b* over 2*c* in Scheme 4, the former allowing early strain-relieving ring-opening of what would be a bicyclic intermediate.

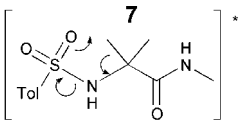
The principal pathway suggested for these degradations, 2*a*, requires a hydrogen atom at C_α. The tosylated methylamide derivative of α-aminoisobutyric acid **7**, lacking this feature, was therefore of some interest and indeed high-mole fractions of the detosylated compound, TsOH and methylamine are consistent with significantly increased use of pathways 2*b* and 2*c*. The values for both ammonia (67%) and TsH (56%) however exceed those that



Scheme 4. Pathways 2*b* and 2*c* may share a common intermediate.



Scheme 6. Unobserved Norrish type II process.

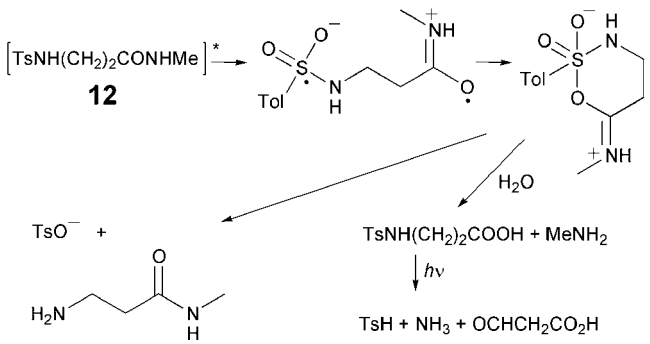


Scheme 7. Concerted pathway applied to C_α-dimethyl concerted derivative.

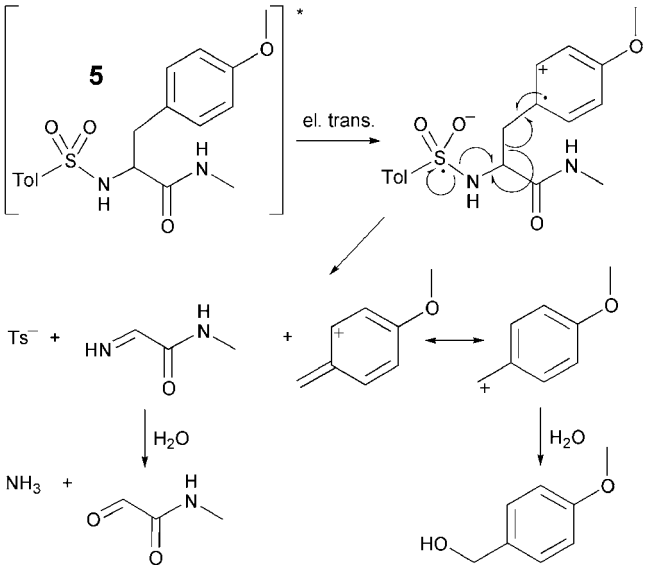
might be expected from further photolysis of tosyl-α-aminoisobutyric acid, the coproduct of methylamine (26%) in 2*c*. Moreover, the unequivocal detection of *N*-methylpyruvamide (14%) requires C–C bond cleavage, and these observations together implicate a process analogous to Scheme 1 (Scheme 7), which might otherwise be considered improbable, even with the enhanced energy available in an electronically excited state.

The mechanism suggested for pathway 2*a* (shown for glycyl **1** in Scheme 3) would be precluded with a β-alanyl derivative **12**, in which the carboxamide and tosyl groups are further separated by an additional carbon atom. The major products at 11% conversion however were found to be analogous to those from the α-aminoacid derivatives: TsH (38%), NH_3 (66%) and $HCOCH_2CONHCH_3$ (41%). A concerted β-H abstraction analogous to that shown in Scheme 1 may therefore operate as a competing pathway in all these degradations, although the detection of a small amount of methylamine in the photolysate of **12** (4%) suggests some IET occurs even at this distance. The complementary product, $HCOCH_2CO_2H$, was also detected (2%), together with more abundant TsOH (23%), all three products being consistent with a process analogous to Scheme 4 (Scheme 8). The theoretical partner of TsOH, β-alanine-*N*-methylamide was seen as a significant amine peak in the AccQTag™ chromatogram.

Compounds **3**–**5** each have a potentially competing electron donor in their side-chains, and we found clear evidence that *p*-methoxyphenyl in **5** reacts in this manner. Thus the side-chain is lost *en route* to the dominant product, *N*-methylglyoxamide, a process that is readily explained by photoinduced electron transfer (PIET; Scheme 9) and is consistent with reports on related systems (60,61). The phenylalanyl derivative (**4**), with a less-polarizable side-chain, shows products from both peptide and side-chain donors (including benzyl alcohol). The methionyl derivative (**3**) also has a readily oxidizable side-chain (62,63), but lack of appropriate standard compounds precluded attributing the reduced amounts of products from the pathways of Scheme 2 to analogous participation of its sulfur atom. Again, we sought but failed to detect any evidence of Norrish Type II chemistry with these side-chains.



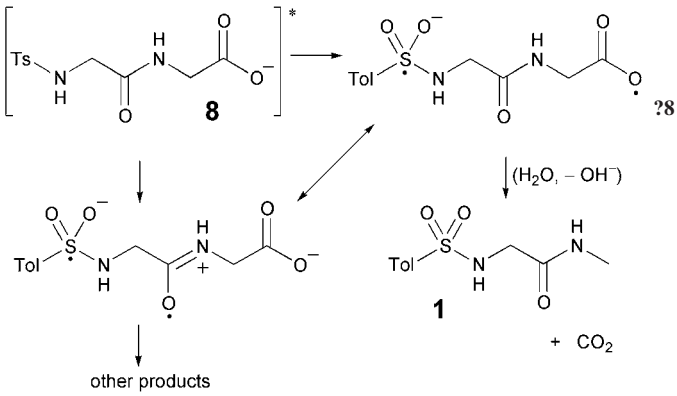
Scheme 8. Possible IET pathway to products from *N*-*p*-tosyl-β-alanine-*N*′-methylamide.



Scheme 9. Possible mechanism for competitive IET pathway involving aryl sidechains.

The competition between a peptide bond and a tyrosyl-related side-chain as donor in electron transfer resembles a situation that could apply in the photooxidation of water by green plants. Constructively, IET from a tyrosyl residue in the protein-based reaction center facilitates the oxidation of water but, destructively, the protein is photodegraded when that process is inhibited (7,8). Mechanistic details of the continual degradation of the D1 protein in Photosystem II during photosynthesis remain unknown (9–11). The oxidation of one or more peptide bonds by the highly reactive intermediate, created in the initial photoinduced charge separation ($P680^{\bullet+}$) when the target required for biological water oxidation (Tyr 116) is unavailable, seems a plausible hypothesis. Moreover it seems possible that this vulnerability will place a general limitation on the ''design'' of photobiological systems incorporating proteins. Optimal redox-active components and spatial arrangements would be critical and, as in Photosystem II, access to a rapid localized regeneration process for any degraded protein might be an additional requirement (7,8).

Observing cleavage of the peptide bond in methylamides prompted us to examine tosylglycyl dipeptides for evidence of longer-range electron transfer (12), the ultimate donor being the terminal carboxyl group. The data for the four compounds **8**–**11** in Table 4 show product distributions broadly consistent with the pathways in Scheme 2 discussed earlier, the significant exception being the presence of the decarboxylation product **1** (6%) seen with the anion of the glycylclycine derivative **8** (values bracketed in Table 4). Although the carbon dioxide observed in this photolysis could have arisen partly from the further degradation of the peptidolysis product tosylglycine, mass balance data in the table indicate most of it arises directly. The reaction thus comprises C–C cleavage remote from the site of initiating absorption and may be depicted as an extended electron transfer process. (See Scheme 10, which includes intermediates closely analogous to those recently cited in the photooxidation of glycylglycine [64].) We looked for possible conformational effects that might be seen with the introduction of side-chains in the second residue, but only *p*-tosylglycylproline **11** showed a significant variation. Here an



Scheme 10. Possible mechanism for relayed IET in the photolysis of *N*-*p*-tosylglycylglycine.

elevated value for carbon dioxide, accompanied by a low value for proline (an indicator of reaction 2*c*, the alternative source of CO_2), suggested that the charge-separated intermediate for this dipeptide derivative could access a conformation particularly suited to direct decarboxylation by extended electron transfer.

CONCLUSIONS

Products from the photodegradation of *p*-toluenesulfonyl α-amino amides in aqueous media are most consistently explained by IET from the carboxamide function (peptide bond) to the sulfonamide group except when a side-chain is aryl, such as in phenylalanyl and tyrosyl derivatives, when these groups provide a competitive electron donor. Collapse of the initial charge-separated intermediates leads to S–N heterolysis (redox and hydrolytic) and to peptide and C_α–C_β cleavage according to substrate structure. The peptide bond is thus seen to act as an electron donor and could act similarly within an irradiated protein having an electronically excited prosthetic group nearby. Further consequences such as longer-range charge separation also seem possible given our observation of an apparently direct decarboxylation in *N*-tosyl dipeptides.

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