Supporting Information for:
Neutral Peptide Biradicals Formed by Dissociative Electron Transfer

Colin S. Burns, Lakecia Rochelle, and Malcolm D. E. Forbes*

Venable and Kenan Laboratories
Department of Chemistry, CB #3290
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599

*to whom correspondence should be addressed. Email: mdef@unc.edu.

Experimental Section

TREPR Methods. The time-resolved experiments were performed at X-band using a JES-RE1X JEOL, USA, Inc. X-Band ESR spectrometer equipped for direct detection using a boxcar integrator as described in previous publications.[1,2] All systems studied were flash photolyzed at 308 nm by an eximer laser (Lambda Physik LPX 110I) operating at a repetition rate of 60 Hz. Sample solutions were bubbled with nitrogen prior to and during photolysis, and were continuously circulated through a 0.5 mm path length quartz flat cell centered in the microwave cavity. Sample concentrations ranged from 0.017 - 0.096 M in peptide (i.e. compounds n = 0-4).

Simulations. Spectral EPR simulations of biradicals were performed using our standard spin-correlated radical pair (SCRP) simulation program. The model used has been described in previous publications.[3] The hyperfine couplings for the tyrosyl radical (Figure 1A) are: H(benzylic) = 7.5 G, H(ortho) = 6.1 G, H(meta) = 1.2 G, H(Cα) = 0.4 G and the g-factor is 2.0041. The solvated electron has a g-factor of 2.0002, but has no hyperfine couplings. The simulation parameters for the phenyl radical (Figure 1B) are: H(ortho) = 17.0 G and H(meta) = 6.0 G, and the g-factor is 2.0032. In this case the couplings for the tyrosyl radical are the same as listed for Figure 1A. The hyperfine couplings and g-factors used for simulation of the biradicals are the same as used in the monoradical simulations and J = -1600 MHz for n = 2 and –1100 MHz for n = 3. The excitation wavelength in all cases is 308 nm.

Solvents and Materials. All reagents and solvents were obtained from the Aldrich chemical company and used without further purification with the following exceptions: The water used for all experiments was obtained from a Millipore Milli-Qplus filtration system (conductivity 18.2 mΩ cm^{-1}), methanol (Burdick and Jackson, 99.9+%), dichloromethane (Mallinckrodt) was distilled from calcium hydride, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)

(Sigma), glycine methyl ester hydrochloride (Gly-Ome•HCl) (Sigma), sodium hydroxide (Mallinckrodt, 98.7%).

(4-bromobenzyl-1-carbonyl)-tyrosine methyl ester (Bba-Tyr-OMe) (6). To a dry 500 mL three-neck roundbottom flask with a stir bar was added 8.3709 g (43 mmol) tyrosine methyl ester, 9.4907 g (47 mmol) 4-bromobenzoic acid, 20.8823 g (47 mmol) BOP, 6.3799 g (47 mmol) 1-hydroxybenzotriazole (HOBt), 50 mL dry dimethylformamide (DMF), and 14.95 mL (86 mmol) diisopropylethylamine (DIEA). The solution was left to stir overnight under nitrogen. The DMF was removed using vacuum and gentle heat to obtain a yellow oil. The oil was dissolved in ethyl acetate (EtOAc) and washed with 10% HCl solution, saturated $NaHCO_3$ solution, and then brine solution. The organic layer was dried with $MgSO_4$ and then filtered. The volume of solvent was reduced with heating and the flask left to sit out open to the air overnight. Obtained four crops of white crystals, **6**, having a combine mass of 14.4884 g (89.3% yield). ^{1}H NMR (acetone-d_6) 8.20 (singlet, 1H), 7.90 (broad doublet, 1H), 7.75 (doublet, 2H), 7.62 (doublet, 2H), 7.10 (doublet, 2H), 6.64 (doublet, 2H), 4.70 (multiplet, 1H), 3.68 (singlet, 3H), 3.08 (multiplet, 2H).

(4-bromobenzyl-1-carbonyl)-tyrosine (Bba-Tyr) (1). Placed 2.4005 g (6.3 mmol) of **6** into a 500 mL roundbottom flask. Added 200 mL 7:3 MeOH/H_2O and 0.8033 g (19 mmol) LiOH•H_2O. The solution was left to stir overnight open to the atmosphere. Removed MeOH under reduced pressure with heating and a white solid precipitated out of solution. Acidified solution by adding HCl and cooled in an ice bath. Filtered white solid and rinsed it with 10% HCl and then with deionized (DI) H_2O. The product was dried in a dessicator. Obtained 2.3115 g (95.0% yield) of **1**. ^{1}H NMR (200 MHz, acetone-d_6) 7.84 (multiplet, 1H), 7.78 (doublet, J = 8 Hz, 2H), 7.62 (doublet, J = 8 Hz, 2H), 7.15 (doublet, J = 8 Hz, 2H), 6.73 (doublet, J = 8 Hz, 2H), 4.84 (multiplet, 1H), 3.14 (multiplet, 2H); ^{13}C NMR (300 MHz, acetone-d_6) 172.3, 166.0, 156.1, 133.8, 131.4, 130.3, 129.2, 128.1, 115.1, 54.4, 36.0; HRMS (FAB, MeOH) *m/z* = 364.0203 (calc. *m/z*

= 364.0184 for $C_{16}H_{14}BrNO_4$). UV/Vis (0.1001M NaOH) λ_{max} = 241.1 nm, $\varepsilon_{241.1}$ = 24,000; λ_{max} = 293.0 nm, $\varepsilon_{293.0}$ = 2200; $\varepsilon_{308.0}$ = 1000.

(4-bromobenzyl-1-carbonyl)-glycine methyl ester (Bba-Gly-OMe) (7). In a 500 mL three-neck flask containing a stir bar was placed 8.37 g (42 mmol) 4-bromobenzoic acid, 5.72 g (46 mmol) Gly-OMe•HCl, 50 mL dry CH_2Cl_2, 22 mL (126 mmol) DIEA, and 1.23 g (10 mmol) 4-dimethylaminopyridine (DMAP) as a catalyst. A yellow oil was obtained. The oil was dissolved in MeOH and H_2O was added until the solution became cloudy. The solution was cooled in an ice bath and sheet-like crystals formed. Collected a total 10.5801 g (93.4% yield) of **7** from three crops of crystals. ^{1}H NMR (acetone-d_6) 8.21 (broad singlet, 1H), 7.85 (doublet, 2H), 7.66 (doublet, 2H), 4.12 (doublet, 2H), 3.68 (singlet, 3H).

(4-bromobenzyl-1-carbonyl)-glycine (Bba-Gly-OH) (8). Saponification of 10.5801 g (39 mmol) of **7** was achieved by stirring it in a solution of 100 mL MeOH and 40 mL H_2O containing 6.54 g (117 mmol) KOH for 3 hours. The solution was acidified with HCl and a white solid slowly precipitated. The solid material was filtered and washed with DI H_2O. Obtained 8.5323 g (85.0% yield) of **8**. ^{1}H NMR (acetone-d_6) 8.12 (broad singlet, 1H), 7.85 (doublet, 2H), 7.66 (doublet, 2H), 4.12 (doublet, 2H).

(4-bromobenzyl-1-carbonyl)-glycyl-tyrosine methyl ester (Bba-Gly-Tyr-OMe) (9). The coupling procedure for the synthesis of **7** was used on 0.9287 g (3.6 mmol) **8**. The product was recrystallized from hexanes/EtOAc to yield 0.9637 g (61.5% yield) of **9**. ^{1}H NMR (acetone-d6) 8.20 (singlet, <1H), 8.08 (broad doublet, 1H), 7.85 (doublet, 2H), 7.67 (doublet, 2H), 7.42 (broad doublet, 1H), 7.00 (doublet, 2H), 6.69 (doublet, 2H), 4.65 (multiplet, 1H), 4.03 (doublet, 2H), 3.63 (singlet, 3H), 2.95 (multiplet, 2H). HRMS (FAB) *m/z* = 435 (M+ for ^{79}Br) and *m/z* = 437 (M+2 for ^{81}Br).

(4-bromobenzyl-1-carbonyl)-glycyl-tyrosine (Bba-Gly-Tyr) (2). The saponification procedure for the synthesis of **1** was used on 0.9637 g (2.2 mmol) of **9**. Obtained 0.5985 g (64.2% yield) of **2**. ^{1}H NMR (200 MHz, acetone-d_6) 8.10 (multiplet,

<1H), 7.85 (doublet, J = 9 Hz, 2H), 7.67 (doublet, J = 9 Hz, 2H), 7.36 (doublet, J = 7 Hz, 1H), 7.03 (doublet, J = 9 Hz, 2H), 6.68 (doublet, J = 9 Hz, 2H), 4.68 (multiplet, 1H), 4.04 (doublet, J = 6 Hz, 2H), 3.01 (multiplet, 2H); ^{13}C NMR (300 MHz, acetone-d_6) 171.5, 168.4, 166. 0, 154.9, 131.6, 130.3, 129.0, 127.9, 126.0, 124.6, 113.8, 52.0, 41.4, 35.0; HRMS (FAB, MeOH) *m/z* = 421.0403 (calc. *m/z* = 421.0399 for $C_{18}H_{17}BrN_2O_5$). UV/Vis (0.1001 M NaOH) λ_{max} = 243.5 nm, $\varepsilon_{243.5}$ = 24,000; λ_{max} = 295.1 nm, $\varepsilon_{295.1}$ = 1,600; $\varepsilon_{308.0}$ = 800.

(4-bromobenzyl-1-carbonyl)-glycyl-glycine methyl ester (Bba-Gly-GlyOMe) (10). Followed the coupling procedure for **7** on 4.0224 g (16 mmol) of **8**. The product was not massed. It used in the next step of the synthesis without purification. ^{1}H NMR (acetone-d6) 8.16 (broad doublet, 1H), 7.86 (doublet, 2H), 7.66 (doublet with broad base, 3H), 4.09 (doublet, 2H), 3.96 (doublet, 2H), 3.64 (singlet, 3H).

(4-bromobenzyl-1-carbonyl)-glycyl-glycine (Bba-Gly-Gly-OH) (11). The saponification procedure for the synthesis of **1** was used on 5.2223 g of **10**. Obtained 4.5348 g (90.7% combine yield for coupling and deprotection steps) of **11**. ^{1}H NMR (acetone-d_6) 7.88 (doublet, 2H), 7.68 (doublet, 2H), 4.09 (doublet, 2H), 3.97 (doublet, 2H).

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-tyrosine methyl ester (Bba-Gly-Gly-Tyr-OMe) (12). The coupling procedure for the synthesis of **7** was followed on 4.5348 g (14 mmol) of **11**. Recrystallized product from acetone/MeOH to obtain 3.6289 g (51.2% yield) of **12**. ^{1}H NMR (CD_3OD) 7.79 (doublet, 2H), 7.62 (doublet, 2H), 7.00 (doublet, 2H), 6.68 (doublet, <2H), 4.59 (multiplet, 1H), 4.03 (singlet, 2), 3.87 (doublet, 2H), 3.64 (singlet, 3H), 2.98 (multiplet, 2H).

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-tyrosine (Bba-Gly-Gly-Tyr) (3). Saponified **12** using procedure for the synthesis of **1** on 3.6289 g (7.4 mmol) of **12**. Obtained 1.5209 g (43.1% yield) of **3**. ^{1}H NMR (250 MHz, CD_3OD) 7.98 (broad doublet, J = 8 Hz, <1H), 7.79 (doublet, J = 8 Hz, 2H), 7.62 (doublet, J = 8 Hz, 2H), 7.03

(doublet, J = 8 Hz, 2H), 6.68 (doublet, J = 8 Hz, 2H), 4.59 (multiplet, 1H), 4.07 (doublet, J = 7 Hz, 2H), 3.91 (doublet, J = 7 Hz, 2H), 3.02 (multiplet, 2H); HRMS (FAB, MeOH) *m/z* = 478.0618 (calc. *m/z* = 478.0614 for $C_{20}H_{20}BrN_3O_6$). UV/Vis (0.1001 M NaOH) λ_{max} = 243.7 nm, $\varepsilon_{243.7}$ = 24,000; λ_{max} = 295.4 nm, $\varepsilon_{295.4}$ = 1,200; $\varepsilon_{308.0}$ = 200.

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-glycine methyl ester (Bba-Gly-Gly-Gly-OMe) (14). The coupling method for the synthesis of **7** was followed using 4.1389 g (13 mmol) of **11**. Obtained 4.20 g (82.8% yield) of **14**. An ^{1}H NMR spectrum was not taken of this compound.

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-glycine (Bba-Gly-Gly-Gly-OH) (15). Followed the de-esterification procedure for the synthesis of **1** using 4.20 g (11 mmol) of **14**. The product was not massed and was used in the next step of the reaction without purification. ^{1}H NMR (DMSO-d^6) 8.90 (triplet, 1H), 8.26 and 8.17 (two overlapping triplets, 2H), 7.83 (doublet, 2H), 7.69 (doublet, 2H), 3.90 (doublet, integral obscured by H_2O), 3.75 (multiplet, integral obscured by H_2O).

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-glycyl-tyrosine methyl ester (Bba-Gly-Gly-Gly-Tyr-OMe) (16). The procedure for the synthesis of **7** was followed using all the material from the synthesis of **15**, assuming a 100% yield from the previous step (i.e. 4.0474 g (11 mmol) of **15**), and using DMAP as the catalyst. Crystallized product from EtOAc to obtain 3.4665 g (58.0% yield for two steps) of **16**. ^{1}H NMR (CD_3OD) 7.79 (doublet, 2H), 7.62 (doublet, 2H), 6.97 (doublet, 2H), 6.68 (doublet, 2H), 4.56 (multiplet, 1H), 4.08 (singlet, 2H), 3.88 (multiplet, 4H), 3.63 (singlet, 3H), 2.93 (multiplet, 2H).

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-glycyl-tyrosine (Bba-Gly-Gly-Gly-Tyr) (4). Used the saponification procedure for the synthesis of **8** on 3.4665 g (6.3 mmol) of **16**. Obtained 2.6415 g (78.2% yield) of **4**. ^{1}H NMR (200 MHz, CD_3OD) 7.68 (doublet, J = 8 Hz, 2H), 7.52 (doublet, J = 8 Hz, 2H), 6.90 (doublet, J = 8 Hz, 2H), 6.56 (doublet, J = 8 Hz, 2H), 4.44 (multiplet, 1H), 3.96 (singlet, 2H), 3.77 (multiplet, 4H),

2.85 (multiplet, 2H); HRMS (FAB, MeOH) *m/z* = 535.0811 (calc. *m/z* = 535.0828 for $C_{22}H_{23}BrN_4O_7$). UV/Vis (0.1001 M NaOH) λ_{max} = 243.8 nm, $\varepsilon_{243.8}$ = 27,000; λ_{max} = 292.6 nm, $\varepsilon_{292.6}$ = 2,000; $\varepsilon_{308.0}$ = 500.

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-glycyl-glycine methyl ester (Bba-Gly-Gly-Gly-Gly-OMe) (17). Followed the coupling procedure described for the synthesis of **7** using 6.3666 g (17 mmol) of **15**, 1.4037 g (11 mmol) DMAP in addition to 2.5602 g (19 mmol) HOBt. Obtained 4.6246 g (61% yield) of **17**. ^{1}H NMR (DMSO-d_6) 8.92 (broad singlet, 1H), 8.25 (broad multiplet, 3H), 7.82 (doublet, 2H), 7.69 (doublet, 2H), 3.91 (broad singlet, 2H), 3.82 (doublet, 2H), 3.74 (broad singlet, 4H), 3.60 (singlet, 3H).

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-glycyl-glycine (Bba-Gly-Gly-Gly-Gly-OH) (18). Saponified **17** using procedure for synthesis of **8** using 4.6246 g (10 mmol) of **17**. Obtained 3.6440 g (81.4% yield) of **18**. No ^{1}H NMR of this compound was taken.

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-glycyl-glycine-tyrosine methyl ester. (Bba-Gly-Gly-Gly-Gly-Tyr-OMe) (19). The coupling procedure for the synthesis of **17** was followed using 3.6440 g (8.4 mmol) of **18**. Obtained 1.8440 g (39.5% yield) of **19**. This compound was not analyzed by ^{1}H NMR and was used in the next step of the reaction.

(4-bromobenzyl-1-carbonyl)-glycyl-glycyl-glycyl-glycine-tyrosine (Bba-Gly-Gly-Gly-Gly-Tyr) (5). Followed the procedure for the de-esterification of **8** using 1.8440 g (3.0 mmol) of **19**. The reaction did not go to completion, but some product was formed (**5**) -- the material obtained was nearly insoluble in all solvents. ^{1}H NMR (hot DMSO-d_6) 9.79 (triplet, .5H), 8.22 and 8.13 (two overlapping triplets, 2.5H), 7.81 (doublet, 2H), 7.69 (doublet, 2H), 6.98 (doublet, .3H), 6.63 (doublet, .3H), 3.90 (doublet, 2H), 3.82 (doublet, 1H), 3.74 (triplet of overlapping resonances, 5H), 3.60 (singlet, 1H).

References

(1) Forbes, M. D. E.; Peterson, J.; Breivogel C. S. Closs, G. L. *Rev. Sci. Instrum.* **1991**, *62*, 2662.

(2) Closs, G. L.; Forbes, M. D. E. *J. Phys. Chem.* **1991**, *95*, 1924.

(3) Closs, G. L.; Forbes, M. D. E.; Norris, J. R. Jr. *J. Phys. Chem.* **1987**, *91*, 3592.