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Synthesis of a Beta-Turn Forming Depsipeptide for Hydrogen Bond Mediated Electron Transfer Studies

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Abstract: Hydrogen bonds are believed to play an important role in the mediation of electron transfer processes in proteins. A porphyrin/depsipeptide/*p*-benzoquinone molecule has been synthesized to help understand this role of hydrogen bond mediated electron transfer in proteins. The synthesis, room temperature folding conformation, and steady-state electron transfer are described. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Outer-sphere electron transfer is a function of nuclear position, electronic coupling, driving force, medium, and electron donor and electron acceptor spatial arrangement. Marcus derived an equation[1] that relates these factors to the rate of electron transfer:

$$k_{et} = \left(\frac{\pi}{\hbar^2 \lambda k_B T}\right)^{\frac{1}{2}} [H_{ab}]^2 \, e^{-\left[\frac{(\Delta G^\circ + \lambda)^2}{4\lambda k_B T}\right]} \qquad [1]$$

where $[H_{ab}]$ is the electronic coupling matrix element of the donor and acceptor, λ is composed of an inner-sphere rearrangement of bonds between the reactants and the products and an outer-sphere component related to rearrangement of solvent orientation, and ΔG° is the electromotive driving force for the reaction. Therefore, the electron transfer is dependent upon electron wavefunction overlap between donor and acceptor and a Frank Condon factor relating solvent effects and driving force.

Electron transfer in proteins is dependent on medium effects. The medium effects are expressed in the electronic coupling matrix element, H_{ab}. Both homogeneous[2] and inhomogeneous[3] tunneling barriers have been used to explain the distance or medium dependence of electron transfer. In the homogeneous model the electronic coupling of the electron donor and electron acceptor decays exponentially with distance by a factor, β, which is independent of the medium. A variation of equation [1] gives the following equation that relates the decay factor, β, to the rate:

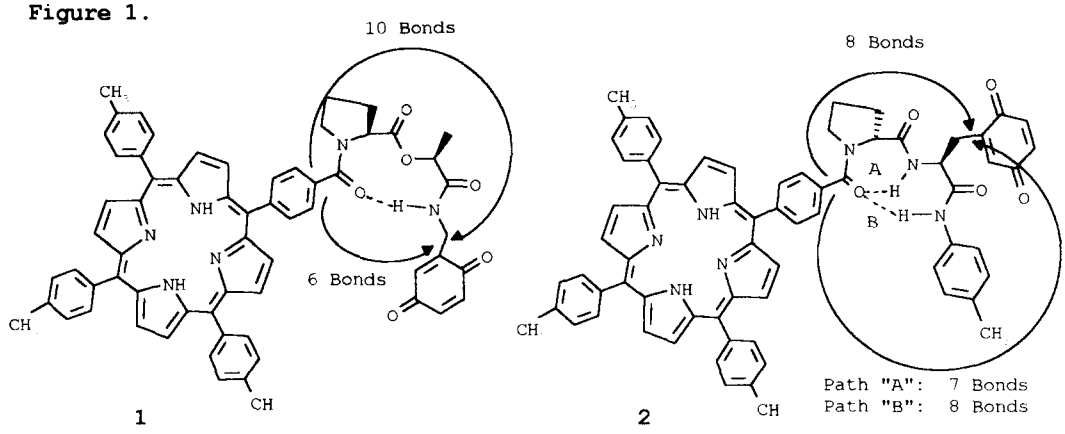
$$k_{et} \propto [H_{ab}]_o^2 e^{-(\beta R)} \qquad [2]$$

The $[H_{ab}]_o^2$ term is the rate at which there is maximum electronic coupling at van der Waals contact ($\sim 1.0 \times 10^{13} s^{-1}$), and R is the donor and acceptor edge-to-edge distance. Dutton et al., found that for certain proteins $\beta = 1.4 Å^{-1}$.[2] Another approach, which assumes an inhomogeneous tunneling barrier, is better known as the Pathway Model. The transfer can be described as valence electrons moving in a pseudo-potential provided by the core electrons and nuclei. The tunneling is more efficient through bonded orbitals than through space due to the potential barrier being lower in covalent bonds. Therefore, the pathway is a combination of covalent bonds, hydrogen bonds, and through space jumps. Gray and coworkers have engineered proteins with modified ruthenium complexes. They have demonstrated that electron transfer reactions in proteins are often better described by the Pathway Model.[4]

Hydrogen bonds have been shown to mediate electron transfer. Electron transfer through hydrogen bonds has been demonstrated for ruthenium complexes coupled to a 3,5-dinitrobenzene acceptor[5a], porphyrins coupled to a 3,5-dinitrobenzene acceptor[5b], porphyrins coupled to quinones[6], and porphyrins coupled together as electron donors and acceptors.[7] Therien and coworkers experimentally found a Beratan-Onuchic decay factor of 0.51 for a hydrogen bond interface in a porphyrin/porphyrin donor/acceptor system. This decay factor of 0.51 is much larger than the value of 0.36 originally proposed[3], indicating that hydrogen bond interfaces may be better mediators of electron transfer than originally believed. This hydrogen bond electron transfer rate is important to understand due to possible hydrogen bond tunneling by electrons in a protein medium. Gray and coworkers have also shown that electron tunneling through β-sheets might be more efficient than through α-helices.[8] The exponential decay constants, β, are $1.26 Å^{-1}$ and $1.00 Å^{-1}$ for the α-helix and β-sheet respectively. Isied and coworkers also have shown the importance of secondary structure since the exponential decay changes from $0.65 Å^{-1}$ to approximately $0.2\text{-}0.3 Å^{-1}$ when oligoproline bridged donor-acceptor compounds undergo a transition from a random coil to a helical trans-polyproline II structure.[9] Thus, one might expect different protein secondary structures to arbitrate electronic coupling in very different manners. Therefore, exploration of the effects of secondary structures on electron tunneling and the relationship of the different types of hydrogen bonding networks of these secondary structures to electronic coupling needs to be explored.

In the work described here, a β-turn **(structure 1 in Figure 1)** has been developed to study the effect of hydrogen bond mediated electron transfer. Maruyama and coworkers have previously reported electron transfer studies with donor/acceptor compounds bridged by β-turn forming peptides **(structure 2 in Figure 1)**.[10a] In our case, we have used a depsipeptide to ensure β-turn folding (C_{10} turn) and eliminate the possibility of γ-turn folding (C_7 turn). The placement of the donor and acceptor has been chosen in our case to favor electron transfer through the hydrogen bond interface. The shortest pathway that results passes through the hydrogen bond and has

Figure 1.



fewer bonds than the pathways in the study by Maruyama and coworkers. The organic solvent of choice for a β-turn forming peptide must be a non-hydrogen bonding solvent so that the hydrogen bond formation is favored. Polar or protic solvents will disrupt the hydrogen bonding of the depsipeptide so that the pathway between the electron donor and acceptor is along the depsipeptide backbone instead of through the hydrogen bond. Chloromethanes should be used, rather than ethers or alcohols, so that the solvent does not hinder the β-turn formation.

EXPERIMENTAL

Chemicals:

All chemicals were used as purchased unless otherwise stated. All chemicals were purchased from Aldrich Chemical Co., Janssen Chemica, or Eastman Chemical Co. tBoc-L-proline and coupling reagents were purchased from Nova Biochem. Monomethylamine was purchased in a lecture bottle from Matheson Gas Co. Solvents were obtained from Mallinckrodt except for tetrahydrofuran and N,N-dimethylformamide which were purchased from Burdick and Jackson. All solvents were reagent grade except for acetonitrile which was spectroscopic grade. Dichloromethane used for the oxidation of the hydroquinone was HPLC (99.9%) grade and was purchased from Aldrich. Upon receipt of the dichloromethane, K_2CO_3 (99.98%) was added directly to the solvent to remove any water or acid present, and it was stored in the refrigerator. Toluene and tetrahydrofuran were dried on molecular sieves, distilled from sodium-benzophenone ketyl, and stored on molecular sieves before use. Triethylamine was distilled from sodium-benzophenone ketyl and placed on KOH pellets to ensure dryness. Reagent grade dichloromethane and benzene were distilled from calcium hydride and stored over sieves before use. N,N-Dimethylformamide was stored on anhydrous $MgSO_4$ before distillation under reduced pressure. N-Bromosuccinimide was recrystallized from water, dried by adding dry toluene, and concentrated to dryness with a rotary evaporator. Pyrrole was distilled under vacuum and stored on molecular sieves prior to use. NMR solvents were purchased from Isotec. TLC plates were silica gel 60, F-254 plates (0.2μm thick), and were

purchased from Selecto Scientific. Silica (260-400 mesh) was purchased from E. M. Science. Florisil (60-100 mesh) was purchased from Mallinckrodt.

Instrumentation:

Melting points were determined on a Thomas-Hoover Capillary Melting Point Apparatus and are uncorrected. Hydrogenation was carried out on a Parr Pressure Reaction Apparatus model #8911. High performance liquid chromatography was performed on a HPLC from Pharmacia LKB Biotechnology (Sweden) with a Vydac C-18 reverse phase preparative column. All ^{1}H-NMR data were obtained using a Chemagnetics 200MHz FT-NMR. ^{1}H-NMR chemical shifts are reported in ppm relative to tetramethylsilane (δ = 0.00ppm) with standard splitting abbreviations. Infrared spectral data were obtained with a Nicolet 5DXC FT-IR and are reported in cm^{-1}. Ultraviolet/visible spectroscopy was performed on a Beckman DU-50 or DU-640 spectrophotometer. Steady-state fluorescence spectroscopy was performed on a Spex Fluorolog II from Spex Industries, Inc. (Metuchen, N. J.). Mass spectral data was obtained using matrix assisted laser desorption ionization time of flight (MALDITOF) mass spectroscopy with a Kratos MALDI III (Manchester, U.K.) instrument at the University of Colorado Health Sciences Center (Denver, CO). Elemental Analyses were performed by Atlantic Microlabs (Norcross, GA). Porphyrin fluorescence lifetimes were determined by time-correlated single photon counting (TCSPC) fluorescence spectroscopy at the National Renewable Energy Laboratories (NREL; Golden, CO).

Handling of Porphyrin-Quinone Moiety:

All vessels containing a porphyrin moiety were placed in the dark after being purged with N_2. The Porphyrin/L-proline/L-lactate/dimethoxybenzylamine compound when dissolved in dichloromethane decomposed when it was exposed to UV radiation. When exposed to UV radiation the porphyrin forms a porphyrin π-cation radical in chlorinated solvents[11] which alters the UV/VIS spectrum giving a yellow color to the solution. Therefore, all porphyrins were assumed to be light sensitive. Before any spectroscopy was performed on a porphyrin moiety, the compound was dried in a drying pistol under vacuum with refluxing methanol overnight. A minimum of ten cycles of freeze-pump-thawing were carried out to remove any oxygen that might be present as a quencher prior to the steady state fluorescence or fluorescence lifetime measurements.

Synthesis of β-Turn:

2,5-Dimethoxybenzylbromide (3). The procedure of A. V. Rama Rao, et. al.[12], was followed for this reaction.

2,5-Dimethoxybenzylamine[13] **(4).** All glassware was dried in an oven overnight. *2,5-Dimethoxybenzylbromide* **(3)** (15.38 g, 66.6 mmol) was dissolved in chloroform (50 ml). Hexamethylenetetramine (10.25g, 73.2 mmol) was dissolved in chloroform (75 ml) and heated to a reflux. The mixture containing *2,5-Dimethoxybenzylbromide* **(3)** was added dropwise to the refluxing hexamethylenetetramine/chloroform mixture over 30 minutes. The mixture was allowed to reflux for three hours and allowed to stand overnight at 4°C. The precipitate was filtered and the filtrate was dissolved into a methanol: concentrated HCl: water mixture (222 ml:54 ml:45 ml). This mixture was refluxed for three days. The mixture was cooled at 4°C overnight. Any precipitate was filtered and the mixture was concentrated under vacuum. The residue was dissolved in water (35ml) and made alkaline with 6N NaOH (~200 ml). The aqueous layer was extracted with diethyl ether. The ether was washed with brine, dried with anhydrous K_2CO_3, and concentrated via vacuum. The residue was purified by vacuum distillation

(122°C, 3.5 torr) to give a clear viscous oil; 6.19g (55.7%). ^{1}H NMR (d_6-DMSO): δ 3.63 (2H, d, -CH_2-Ar), 3.70 (6H, d, -OCH_3), 6.72-6.81 (3H, dd, Ar), 6.95 (2H, d, -NH_2). Anal. calculated for $C_9H_{13}NO_2$: 64.7%C, 7.84%H, 8.38%N. Found: 63.00%C, 7.77%H, 8.06%N.

2-Hydroxy-benzylpropanoate (5). The literature procedure of B. F. Gisin et. al.[14] was followed with the following exception: L-Lactic acid (85%, 47.1ml, 0.631 mol) was added to freshly distilled benzyl alcohol (250ml). The mixture was dried with sodium sulfate (5g) and filtered before reacting with dry HCl.

tBoc-L-proline-L-lactate-Bzl (6). The procedure of B. F. Gisin et. al.[14], was followed. tBoc-L-proline (12.0g, 0.056 mol) was added to dry dichloromethane (36ml). This mixture was added to 1M carbonyldiimidazole in dichloromethane (56ml) over a 30 minute period while being stirred on an ice bath. *2-hydroxy-benzylpropanoate* **(5)** (11.12g, 0.067 mol) was added to the mixture. The reaction was stirred on ice for one hour before it was allowed to stand at room temperature for four days. The reaction was diluted with water and concentrated under vacuum with a rotary evaporator. The residual oil was dissolved into diethyl ether and extracted with water (100ml), 1M citric acid (200ml), 1M sodium bicarbonate (200ml), and water (200ml). The organic layer was dried with anhydrous sodium sulfate and concentrated again to give a clear oil that became a semisolid on standing, 13.41g (62.7%). ^{1}H NMR ($CDCl_3$): δ 1.44 (9H, d, $(CH_3)_3$-C-), 1.51 (3H, d, C<u>H</u>$_3$-CH-), 1.81 (2H, m, γ-CH_2-), 2.09 (2H, m, β-C<u>H</u>$_2$-CH-), 3.45 (2H, m, δ-CH_2-N), 4.31 (1H, dq, α-CH-C=O), 5.15 (1H, q, -C<u>H</u>-CH_3), 5.20 (2H, d, -O-CH_2), 7.34 (5H, s, Ar-H).

tBoc-L-proline-L-lactic acid (7). The procedure of B. F. Gisin et. al.[14], was followed. *tBoc-L-proline-L-lactate-Bzl* **(6)** (13.41g, 0.0745 mol) was dissolved into degassed methanol (65ml). The bottle was purged with N_2, Pd/C (5%, 2.59g) was added to the ester, and the reaction was place on the Parr apparatus with 50 p.s.i. H_2 overnight. The catalyst was filtered, and the mixture was concentrated. The residue was dissolved into 1M sodium bicarbonate (200ml). The aqueous solution was extracted with diethyl ether (200ml), and then the aqueous solution was acidified to litmus paper with 1M citric acid. The aqueous layer was extracted with diethyl ether (200ml). The ether was washed with water (100ml). The organic layer was dried with sodium sulfate and concentrated via rotary evaporation to give a white, blocky solid, 5.19g, (29.0%), m. p. 106°C. ^{1}H NMR ($CDCl_3$): δ 1.44 (9H, d, $(CH_3)_3$-C-), 1.56 (3H, d, C<u>H</u>$_3$-CH-), 1.92 (2H, m, γ-CH_2-), 2.17 (2H, m, β-C<u>H</u>$_2$-CH-), 3.47 (2H, m, δ-CH_2-N-), 3.47 (1H, dq, α-CH-C=O), 5.19 (1H, q, -C<u>H</u>-CH_3-), 9.66 (1H, b, -COOH).

tBoc-L-proline-L-lactate-N-2,5-dimethoxybenzylamide (8). The depsipeptide-amide was prepared in analogy to the procedure of Klausner et. al.[15] *tBoc-L-proline-L-lactic acid* **(7)** (1.00g, 3.5mmol) was dissolved into dry tetrahydrofuran (150ml) and stirred at 0°C for 15 minutes. *7-Azahydroxybenzotriazole* **(9)** (HOAt, 0.56g, 4.18mmol), dicyclohexylcarbodiimide (DCC, 0.721g, 3.5mmol), and *2,5-Dimethoxybenzylamine* **(4)** (0.585g, 3.5mmol) were added to the reaction mixture. The reaction was stirred for an additional two hours at 0°C. The reaction was continued at room temperature under N_2 for five days. The reaction was filtered to remove the dicyclohexylurea that had precipitated. The filtrate was diluted with water (50ml), refiltered, and concentrated with a rotary evaporator. The residue was dissolved into dichloromethane (100ml), and the residual water was removed with a separatory funnel. The organic layer was washed with water (2x100ml) and was dried with anhydrous sodium sulfate. The liquid was concentrated, and the residue was dissolved into hot acetonitrile (50ml). Any precipitation was removed by filtration. The solution was again concentrated and yielded a thick, brown oil. This brown oil was purified by HPLC reverse-phase chromatography (Gradient: 55% acetonitrile: 45% water to 10% acetonitrile: 90% water gradient over 42 minutes, R_t = 18 minutes). 1.41g (93.3%). ^{1}H NMR ($CDCl_3$): δ 1.39

(9H, s, $(CH_3)_3$-C), 1.51 (3H, d, C<u>H</u>$_3$-CH-), 1.90 (2H, m, γ-CH_2-), 2.24, (2H, m, β-C<u>H</u>$_2$-CH-), 3.44 (2H, m, δ-CH_2-N-), 3.77, (6H, d, 2-O-CH_3), 3.83 (2H, d, -CH_2-Ar), 4.34-4.45 (1H, dq, α-CH-C=O), 5.26 (1H, q, -C<u>H</u>-CH_3), 6.77 (3H, d, Ar-H), 7.57, (1H, t, -NH). Mass spectrum (MALDITOF) expected: 436.5. Found m/z (relative intensity, %): 460.2 (M/Na^+, 100), 475.9 (M/K^+, 54). Anal. calculated for $C_{22}H_{32}N_2O_7$: 60.54%C, 7.39%H, 6.42%N. Found: 60.30%C, 7.32%H, 6.39%N.

Synthesis of 7-Azahydroxybenzotriazole (procedures according to L. Carpino, personal communication):

3-Methoxy-2-nitropyridine[16] **(9).** Solid KOH (16.0g, 0.285mol) was crushed and added to dimethyl sulfoxide (150ml). The slurry was stirred in the dark for five minutes. 3-Hydroxy-2-nitropyridine (8.91g, 63.6mmol) was added to the reaction followed by addition of iodomethane (8.0ml, 127.6mmol). The solution was stirred in the dark for an additional 20 minutes. The reaction mixture was diluted with water (200ml) and extracted with dichloromethane (3x200ml). The organic later was reextracted with water (3x200ml). The organic layer was dried by passing over cotton and concentrated with a rotary evaporator to give a light yellow oil that solidifies upon drying under vacuum, 9.80g (90.9%), m.p. 69°C. ^{1}H NMR ($CDCl_3$): δ 3.98 (3H, s, -OCH_3), 7.55 (2H, s, Ar-H), 8.10 (1H, s, Ar-H).

7-Azahydroxybenzotriazole[17,25] **(10).** *3-Methoxy-2-nitropyridine* **(9)** (8.90g, 57.9mmol) was added to ethanol (62ml) and aqueous hydrazine (65%: 40.3ml anhydrous N_2H_4 and 21.7ml water) solution and slowly heated until all the solids were dissolved. The solution was allowed to boil and gently refluxed overnight. The solution was allowed to cool and was concentrated with a rotary evaporator. The residue was dissolved in water (150ml) and acidified with concentrated HCl until the Congo red indicator changed from red to blue. The precipitate was filtered and washed with cold 1N HCl. The solid was dissolved into boiling water (80ml) and filtered while still hot. The filtrate was cooled and stirred to precipitate the product which was isolated by vacuum filtration yielding a light, yellow fluffy solid, 2.01g, (25.9%), m.p. 217°C, darkens at 210°C. ^{1}H NMR (DMSO-$CDCl_3$): δ 7.35 (1H, dd, Ar-H), 8.34 (1H, d, Ar-H), 8.71 (1H, d, Ar-H).

Synthesis of Porphyrin:

5,10,15-Tri(*p*-tolyl)-20-(*p*-methylbenzoate)porphyrin[18] **(11).** All glassware was dried overnight. The porphyrin was prepared by adding methyl-4-formyl benzoate (0.330g, 2.01mmol), *p*-tolualdehyde (0.720g, 5.99mmol), pyrrole (0.540g, 8.05mmol), triethylorthoacetate[19] (1ml, ~8mmol), and 2.5M BF_3:Et_2O in dichloromethane (1ml, ~0.35mol) in a 2l N_2-purged round bottom flask filled with dichloromethane (800ml), ethanol (6ml). The reaction was stirred at room temperature for one hour in the dark. Tetrachloro-1,4-benzoquinone (*p*-chloranil, 1.47g, 5.98mmol) was added to the reaction mixture. The mixture was then heated to a reflux for one hour. The reaction was quenched with triethylamine (1ml) after cooling to room temperature. The mixture was concentrated onto florisil (10g) and purified on a florisil column (60g). The column was eluted with dichloromethane, and any green, yellow, or blue impurities were discarded. The purple fraction was then collected with a 20% diethyl ether/dichloromethane mixture. The purple fraction showed multiple spots on TLC indicating all the different possible condensation products were present. The desired product had an R_f = 0.83 on the silica TLC plate developed with dichloromethane. The purple fraction was concentrated onto silica, and was loaded onto a 1kg silica column. The desired fraction was the second purple fraction eluted with 50% petroleum ether (35-60°C) in dichloromethane. The purple fraction was concentrated again on silica (10g). The porphyrin was further

purified on a smaller silica column (80g). 50% Ethanol/petroleum ether (35-60°C) was used to elute a green impurity, and the product was removed with 20% ethanol/dichloromethane. The purple fraction was concentrated and dried overnight under vacuum to give a dark, purple crystalline material, 0.196g, (32.3%, versus a theoretical yield of 42.2% for the 3:1 isomer), m. p. >350°C. ^{1}H NMR ($CDCl_3$): δ -2.80 (2H, b, N-H), 2.70 (9H, s, CH_3), 4.10 (3H, s, CH_3), 7.55 and 8.09 (12H, d, *p*-tolyl), 8.30 and 8.43 (4H, d, benzoate), 8.76 and 8.87 (8H, d, pyrrole). Mass spectrum (MALDITOF) expected: 714.8. Found m/z (relative intensity, %): 714.6 (MH^+, 100).

5,10,15-Tri(*p*-tolyl)-20-(*p*-carboxyphenyl)porphyrin[20] **(12a).** 5,10,15-Tri(*p*-tolyl)-20-(*p*-methylbenzoate)porphyrin **(11)** (0.050g, 70.0μmol) was dissolved in tetrahydrofuran (1ml). The solution was then diluted with Claisen Alkali (10ml). The solution was refluxed overnight and then allowed to cool to room temperature. The mixture was diluted with dichloromethane (50ml), water (20ml), and adjusted to pH=3 with 2.5M acetic acid. The organic layer was removed, and the aqueous layer was reextracted with dichloromethane (100ml). The combined organic layers were concentrated with toluene (20ml) and dried under vacuum to give a purple crystalline material, 0.046g (94.0%), m. p. >350°C. ^{1}H NMR (d_5-pyridine): δ 2.60 (9H, s, CH_3), 7.57 and 8.27 (12H, d, *p*-tolyl), 8.50 and 8.86 (4H, d, benzoate), 9.09 and 9.14 (8H, d, pyrrole).

Synthesis of Porphyrin-Quinone Moiety:

Porphyrin/L-proline/L-lactate/dimethoxybenzylamide (13). All glassware was dried in an oven overnight. Toluene, dichloromethane, tetrahydrofuran, dimethylformamide, and triethylamine were all freshly distilled before use. *tBoc-L-proline-L-lactate-N-2,5-dimethoxybenzylamide* **(8)** (0.070g, 0.158mmol) was deprotected[21] and prepared for coupling by dissolving into 25% trifluoroacetic acid in dichloromethane (2ml). The solution was stirred at room temperature for 2.5 hours. The solution was then concentrated by rotary evaporation with dry toluene (10ml). The residue was dried under vacuum for four hours before use. The acid chloride of *5,10,15-Tri(p-tolyl)-20-(p-carboxyphenyl)porphyrin* **(12b)** (0.060g, 86μmol) was prepared[10b] by dissolving **(12b)** into dichloromethane (10ml) and adding oxalyl chloride (0.220ml, 22.9mmol). The mixture was stirred at room temperature for four hours. The mixture was concentrated with a rotary evaporator with toluene (25ml). The residue was dried under vacuum for two hours. The coupling[10b] was completed by dissolving the deprotected depsipeptide into N,N-dimethylformamide (2ml) with triethylamine (0.100ml), in a round bottom flask that had been purged with N_2, and cooled to 0°C on an ice bath. The porphyrin acid chloride was dissolved into tetrahydrofuran (20ml) and was added to the depsipeptide solution dropwise over 30 minutes with an addition funnel. The reaction mixture was stirred overnight at room temperature under a $CaCl_2$ drying tube. The mixture was diluted with dichloromethane (50ml), extracted with 5% $KHSO_4$ (3x100ml), extracted with brine (3x100ml), and dried with anhydrous sodium sulfate. The solution was concentrated onto silica (2g), and was added to a silica column (50g) for purification. The column was eluted with 1% methanol in dichloromethane to remove unreacted porphyrin acid; the column was then eluted with 2% methanol in dichloromethane to remove the product. The product was then triturated with acetonitrile (1ml) overnight. The slurry was filtered with celite, and the filtered solid was dissolved into dichloromethane to extract it from the celite. This procedure gave a glassy, purple solid 0.032g (36%). ^{1}H NMR ($CDCl_3$): δ -2.80 (2H, b, pyrrole N-H), 1.65 (3H, d, lactate-CH_3), 2.18 (2H, b, proline-γ-CH_2), 2.50 (2H, b, proline-β-CH_2), 2.70 (9H, s, tolyl-CH_3), 3.74 (6H, d, -OCH_3), 3.98 (2H, t, proline-δ-CH_2-N), 4.57 (2H, d,-CH_2-), 4.85 (1H, b, proline-α-CH-), 5.45 (1H, q, lactate-CH-), 6.73 and 6.94 (3H, m, DMB-aromatic), 7.55 and 8.09 (12H, d, *p*-tolyl), 7.85 and 8.22 (4H, d, benzoate), 8.78 and 8.86 (8H, d, pyrrole).

UV/Vis (DCM): λ_{max} (logϵ): 295 (4.34), 422 (5.49), 517 (4.38), 553 (4.21), 590 (3.94), 649 (3.87). Mass spectrum (MALDITOF) expected: 1019.2. Found m/z (relative intensity, %): 1020.3 (MH^+, 100), 1042.7 (M/Na^+, 18), 1059.2 (M/K^+, 12). Anal. calculated for $C_{65}H_{58}N_6O_6 \cdot H_2O$: 75.27%C, 5.83%H, 8.10%N. Found: 74.87%C, 5.93%H, 7.34%N.

Porphyrin/L-proline/L-lactate/hydroquinone[22] **(14).** *Porphyrin L-proline L-lactate dimethoxybenzylamide* **(13)** (0.0104g, 10.3μmol) was dissolved into dry dichloromethane (4ml) and stirred on an ice bath under N_2. BI_3 (0.065g, 0.166mmol) was transferred to dichloromethane (0.300ml) in a glove bag and was cannulated into the reaction mixture. The reaction was allowed to warm to room temperature for 30 minutes after the ice bath was removed. The reaction was quenched with 10%NH_4OH (50ml). The mixture was diluted with dichloromethane (25ml), and the aqueous layer was removed. The organic layer was reextracted with water (50ml), brine (2x50ml), and dried with anhydrous sodium sulfate. The solution was concentrated onto silica gel. The product was contained in the first purple band removed from a silica gel (50g) column (5% methanol/0.25% triethylamine/dichloromethane). The purple solution was dried by concentrating with dry benzene to remove any residual triethylamine. The solution was again concentrated onto silica gel. The product was the second purple band removed from a silica gel (10g) column (5% tetrahydrofuran/0.25% triethylamine/dichloromethane). The purple solution was again concentrated with dry benzene to remove any residual triethylamine and water. The residue was dried overnight in a drying pistol with refluxing methanol to give a purple solid, 0.0016g (15.3%). Mass spectrum (MALDITOF) expected: 991.2. Found m/z (relative intensity, %): 991.6 (MH^+, 100).

Porphyrin/L-proline/L-lactate/quinone[22] **(1).** *Porphyrin L-proline L-lactate hydroquinone* **(14)** (0.0016g, 1.6μmol) was dissolved in dichloromethane (5ml). This solution was filtered through a disposable pipet with PbO_2 (0.005g, 20.9μmol) and anhydrous K_2CO_3 (0.005g, 36μmol). The solution was added directly to the freeze/pump/thraw fluorescence cell and was checked by UV/Vis. UV/Vis (DCM): λ_{max} (log ϵ): 245 (4.96), 285 (3.83), 419 (5.49), 517 (4.38), 553 (4.21), 590 (3.94), 649 (3.87).

Synthesis of Non-Hydrogen Bonding Standard (procedure according to S. Gellman, personal communication):

D-5-Oxoproline methyl ester[23] **(15).** D-Pyrrolidone-5-carboxylic acid (9.42g, 73mmol) was dissolved into dry methanol (32ml) and stirred on a -20°C ice/salt bath. Thionyl chloride (17.5ml) was added to the slurry over a one hour period with an addition funnel. The reaction was stirred for an additional six hours. After all the solids were dissolved, the reaction was concentrated to dryness with a rotary evaporator. The resulting solid was reconcentrated after toluene (50ml) was added to remove any residual thionyl chloride or methanol. The solid, a hydochloride salt, was dried under vacuum to give a white solid, 12.2g (94.1%), m.p. 97°C. ^1H NMR (d_6-DMSO): δ 2.03 (1H, m, β-CH_2-), 2.14 (2H, t, γ-CH_2-), 2.31 (1H, m, β-CH_2-), 3.67 (3H, s, -O-CH_3), 4.19 (1H, t, α-CH-C=O), 8.01-8.35 (2H, s, N-H).

N-Methyl-D-5-oxoproline methyl ester (16). *D-5-Oxoproline methyl ester* **(15)** (12.2g, 68.7mmol) hydrochloride salt was dissolved into methanol (180ml). The solution was diluted with dichloromethane (75ml). The organic solution was extracted with saturated sodium bicarbonate (100ml) and brine (100ml). The organic layer was dried with anhydrous sodium sulfate and concentrated on a rotary evaporator. The residue (6.72g, 47mmol amine free base) was dissolved in dry toluene (50ml). Sodium metal (1.2g, 51.7mmol) was added to a 3-neck round bottom flask equipped with two addition funnels. Toluene (25ml) was added to the flask to cover

the sodium. The flask was flushed with N_2 and placed on a -20°C ice/salt bath. The free amine was added to the sodium over a period of 30 minutes. Iodomethane (3.22ml, 51.7mmol) was dissolved in dry N,N-dimethylformamide (25ml) and was added to the reaction mixture over a period of 30 minutes. The reaction was allowed to warm to room temperature slowly and to stand overnight. The precipitate (NaI) was removed by vacuum filtration, and the filtrate was concentrated on a rotary evaporator. The residue was purified by silica chromatography (60g, 2% methanol in dichloromethane; R_f=0.93). The product eluted from the column as a light yellow liquid. The elutant was concentrated and provided a light yellow oil, 2.12g, (19.9%). ^{1}H NMR ($CDCl_3$): δ 2.08 (1H, m, β-CH_2-), 2.41 (3H, m, γ-CH_2-, β-CH_2-), 2.85 (3H, s, N-CH_3), 3.78 (3H, s, -O-CH_3), 4.41 (1H, t, α-CH-C=O).

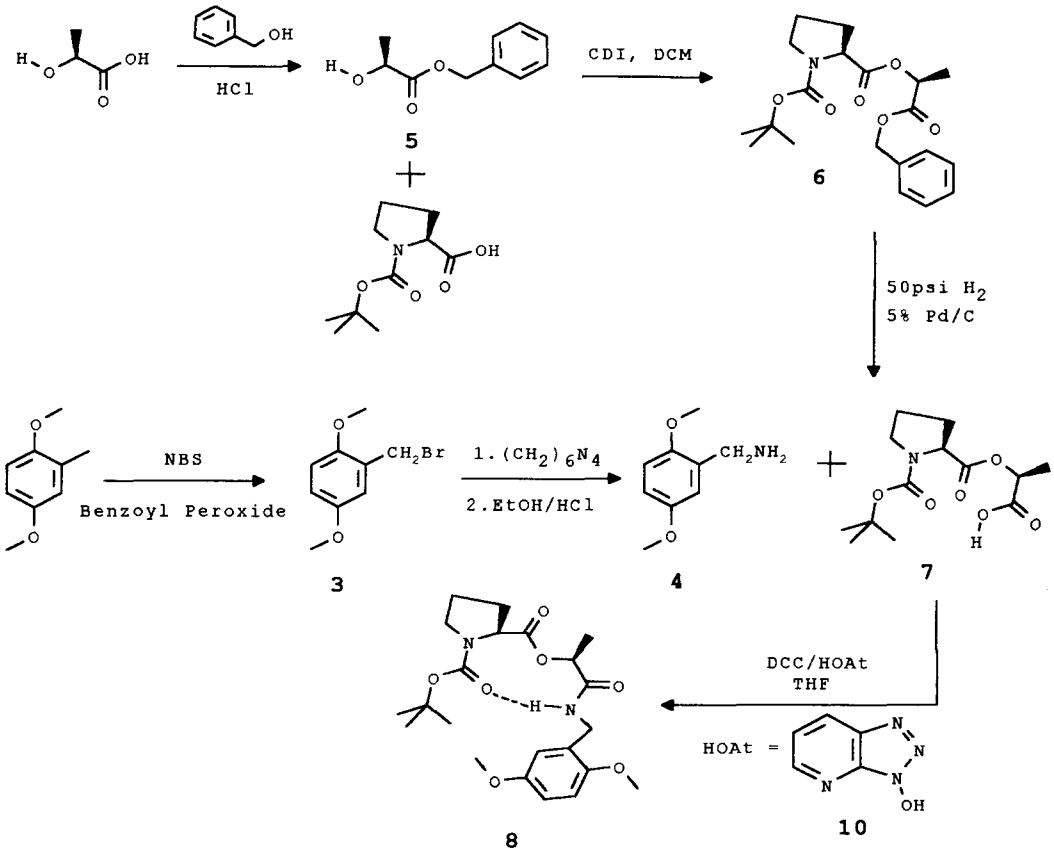
N-Methyl-D-5-oxoproline methyl amide[24] **(17).** *N-Methyl-D-5-oxoproline methyl ester* **(16)** (2.12g, 13.6mmol) was dissolved into dry methanol (200ml). The solution was saturated with monomethylamine gas and stirred for two hours. Potassium cyanide (0.200g, 3.1mmol) was added to the solution. The mixture was stirred at room temperature for three days in a stoppered round bottom flask. The solution was concentrated on a rotary evaporator, and the residue was dissolved into dichloromethane (100ml), was dried with anhydrous sodium sulfate and reconcentrated. The residue was purified by silica chromatography (80g, 5% methanol in dichloromethane; R_f = 0.65). The elutant was concentrated and redissolved into dichloromethane (300ml). The solution was gently boiled with decolorizing carbon. The carbon was removed by vacuum filtration through celite. The clear solution was concentrated to give a light, pink solid, 1.54g (72.9%), m. p. 114°C. ^{1}H NMR ($CDCl_3$): δ 2.05 (1H, m, β-CH_2-), 2.40 (3H, m, γ-CH_2-, β-CH_2-), 2.83 (6H, d, both N-CH_3), 3.98 (1H, t, α-CH-C=O), 6.15 (1H, b, N-H). Mass spectrum (MALDITOF) expected: 156.2. Found m/z (relative intensity, %): 156.8 (MH^+,100).

SYNTHESIS

The β-turn was synthesized by standard solution phase peptide coupling techniques (See Scheme 1). The electron acceptor was synthesized from a 2,5-dimethoxytoluene precursor. The precursor was brominated **(3)** by a standard N-bromosuccinimide reaction and was converted to a primary amine **(4)** through a quaternary bromide salt. L-Lactic acid was protected with a benzyl ester **(5)** so that the alcohol group could be coupled to tBoc-L-proline by ordinary carbonyldiimidazole (CDI) chemistry. The tBoc-L-proline-L-lactate-Bzl **(6)** was hydrogenated under H_2 pressure to produce the free acid **(7)** which was then coupled using dicyclohexylcarbodiimide (DCC) to the amine of the electron acceptor moiety. This coupling to form the depsipeptide β-turn **(8)** was aided with 7-azahydroxybenzotriazole (HOAt, **10**). HOAt is a coupling additive which limits racemization.[25]

The tetra-substituted porphyrin **(11)** was synthesized in a one-pot reaction (see Scheme 2). This reaction[26] yields a product in higher yield than the standard propionic acid method which Loach[27] used to synthesize porphyrins linked by alkane spacers. The porphyrin methyl ester was hydrolyzed by refluxing with strong base and gently acidifying with acetic acid **(12a)**. This porphyrin, 5,10,15-tri(*p*-tolyl)-20-(*p*-carboxyphenyl)porphyrin, was then coupled to the free amine of the depsipeptide. The free amine of the depsipeptide **(8)** was produced by removal of the t-butyloxycarbonyl group by stirring at room temperature with 25% trifluoroacetic acid (TFA) in dichloromethane. A higher concentration of TFA resulted in hydrolysis of the secondary ester linkage. The free amine-depsipeptide was not examined before mixing with the porphyrin acid chloride. This acid chloride **(12b)** was produced by stirring the porphyrin acid with oxalyl chloride. By combining the free amine and the porphyrin

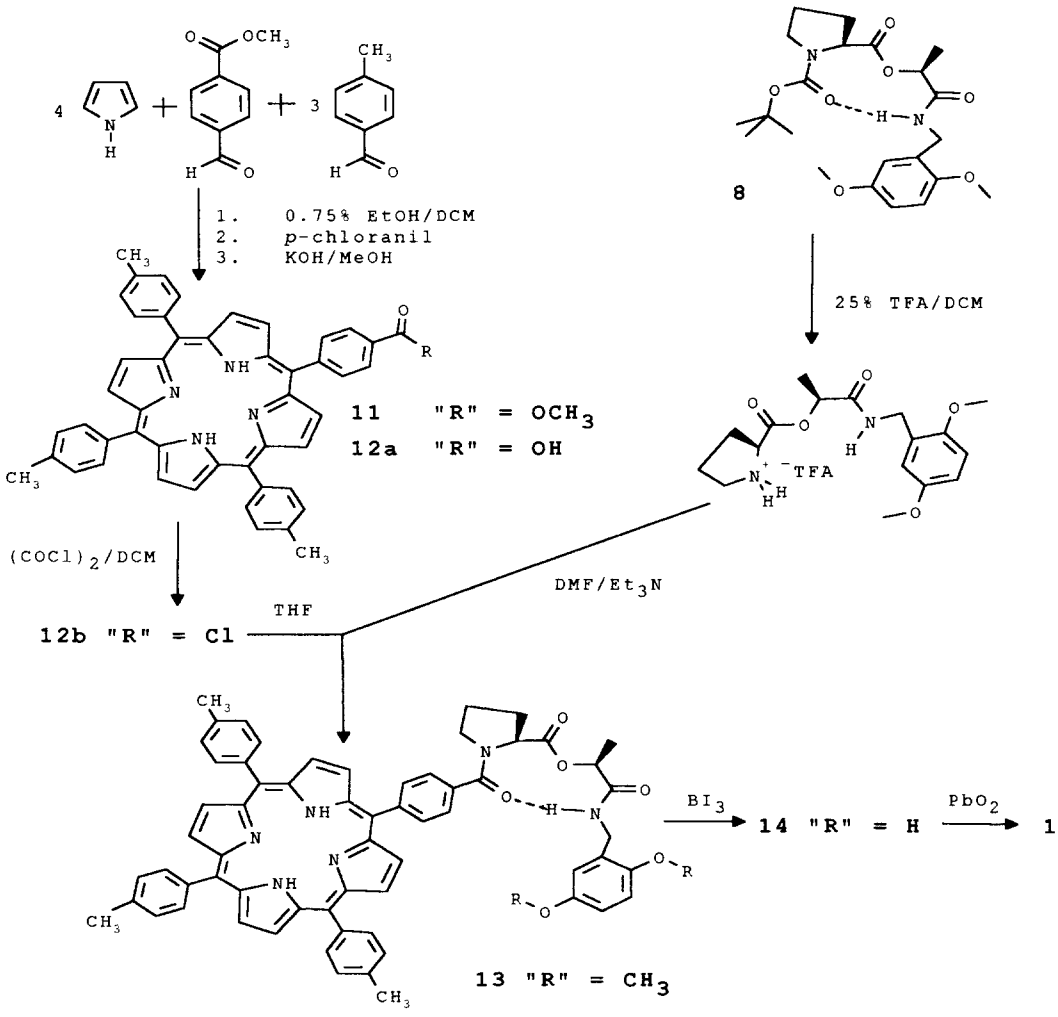
Scheme 1



acid chloride, porphyrin/L-proline/L-lactate/dimethoxybenzylamide (Por/PL/DMB) **(13)** was produced in moderate yields after column chromatography. The coupling via an acid chloride had also been attempted with thionyl chloride, but this reagent was too harsh and cleavage of the secondary ester occurred. Standard peptide coupling techniques such as CDI, DCC, N,N-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic acid[28], and 4-pyrrolidinopyridine[29] were also tried but were found to be ineffective in producing a secondary amide of the benzoic acid moiety of the porphyrin. Maruyama and coworkers comment that only an acid chloride is reactive enough to achieve the coupling of their porphyrin acid to D-proline.[10b]

The deprotection of the electron acceptor moiety was performed by reaction with BI_3 in dichloromethane to give porphyrin/L-proline/L-lactate/hydroquinone (Por/PL/HQ) **(14)**. BBr_3 is the usual method for methoxy-deprotection of the electron acceptor moiety, but BI_3 was chosen to enhance yield, and allow for smaller reagent excess and less porphyrin degradation which is common with BBr_3.[30] However, BI_3 proved to have problems in the deprotection of the electron acceptor as well. The reaction led to breakdown at all the ester and amide bonds within the β-turn. Weedon and coworkers comment that the methoxy-deprotection of a porphyrin-quinone linked

Scheme 2



molecule is much cleaner without ester linkages.[31] We found that all amide and ester linkages are fairly susceptible to cleavage by BI_3. MALDITOF mass spectral data show cleavage products with m/e equal to 699.6 (porphyrin acid), 798.2 (Por/Proline acid), 868.5 (Por/P/Lactic acid), and 991.6 (Por/PL/HQ). All of these cleavage products show peaks of approximately equal intensity in the crude product. The breakdown products were easily removed by column chromotography and do not appear in the final isolated product's mass spectrum. This breakdown causes yields of 10% or less for the Por/PL/HQ moiety. The hydroquinone was oxidized to the quinone (Por/PL/Q) **(1)** by dissolving it into dichloromethane and filtering the solution in the dark through PbO_2 and K_2CO_3. Special care was taken not to expose the quinone to light. Confirmation of quinone production was by UV/VIS spectroscopy.

SPECTROSCOPY

UV/VIS spectroscopy was used to determine if the electronic structure of the tetratolylporphyrin methyl ester had been perturbed by the addition of the depsipeptide, methoxy-deprotection, and quinone oxidation (see Figure 2). All four spectra show a normal porphyrin chromophore at 419nm with no evidence of broadening or shifts in the absorption maximum. Notably, the spectrum of the Por/PL/Q is not perturbed showing that there is little or no electronic interaction between the porphyrin and the quinone, making it unlikely that there is any parallel stacking occurring between the porphyrin and the quinone.[31,32] Thus, any possible through space electron transfer between the porphyrin and quinone is reduced due to the lack of porphyrin-quinone interaction.

Confirmation of production of the quinone moiety (**1**) was performed by comparing the UV/VIS spectra for the Por/PL/DMB, Por/PL/HQ, and Por/PL/Q (see Figure 3). *p*-Benzoquinone has absorbances in the UV

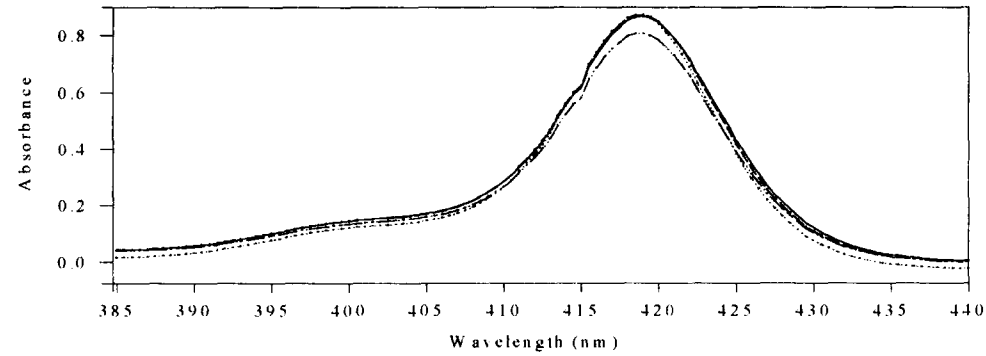


Figure 2. UV/VIS analysis of Soret peak at 419nm. [Porphyrin methyl ester] 2.8uM (solid line), [Por/PL/DMB] = 2.7uM (dashed dotted line), [Por/PL/HQ] 2.8uM (broken line), and [Por/PL/Q] 2.8uM (dotted line).

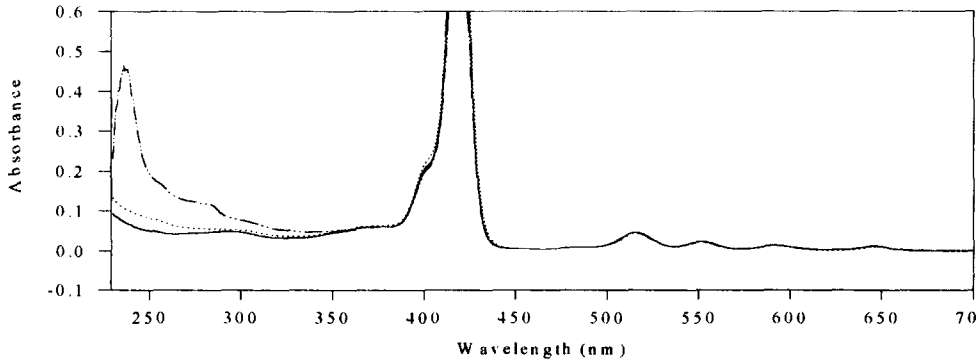


Figure 3. UV/VIS analysis of the p-benzoquinone region (230-350nm). [Por/PL/DMB] (solid line), [Por/PL/HQ] (dotted line), and [Por/PL/Q] (dashed dotted line) 4uM.

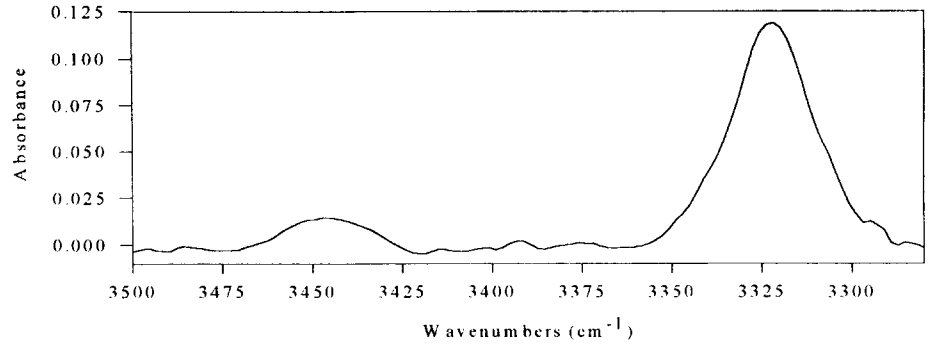


Figure 4. Room temperature FT-IR spectrum of Por/PL/DMB for analysis of folding conformation. Non-hydrogen bonding amide: 3445cm^{-1}. Hydrogen bonding amide: 3321cm^{-1}. [Por/PL/DMB] = 1mM.

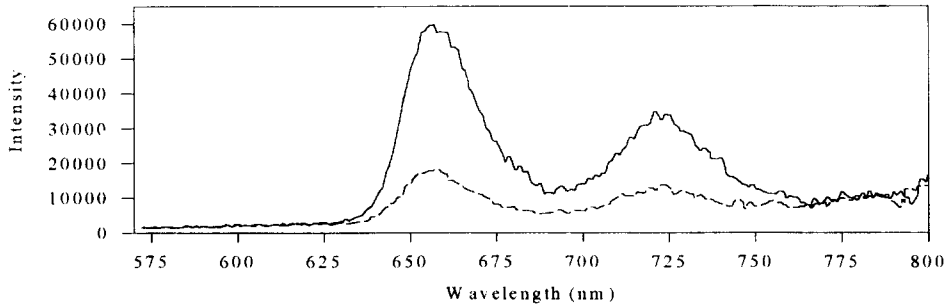
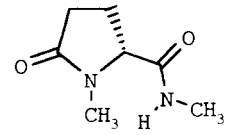


Figure 5. Fluorescence spectrum of Por/PL/DMB (solid line) and Por/PL/Q (broked line) in DCM. Both spectra were recorded at 10uM.

region in chloroform at 245nm (ϵ = 23200M^{-1}cm^{-1}) and 281nm (ϵ = 470M^{-1}cm^{-1}).[33] The quinone compound has absorbances at 245nm and 285nm whereas the Por/PL/DMB compound does not show these same absorbances. The Por/PL/HQ shows an increase in absorbance at 245nm which might indicated some oxidation occurs during the dimethoxy-deprotection with BI_3. Weedon and coworkers report that partial oxidation, up to 25%, of the hydroquinone occurs with methoxy-deprotection.[34] Also, shown are the Q bands of the three compounds. These absorbances do not change between each of the compounds examined.

The β-turn conformation was determined by infrared spectroscopy (see Figure 4). Amide N-H bonds have different infrared stretches dependent upon their environment. If the N-H is involved in a hydrogen bond, then the stretch appears at 3360cm^{-1} in dichloromethane. The non-hydrogen bonding amide will appear around 3450cm^{-1}.[35] The Por/PL/DMB was examined at room temperature to determine the relative amount of β-turn formed versus

the non-hydrogen bonded state. The hydrogen bonded amide stretch was observed at 3321cm^{-1} whereas the non-hydrogen bond N-H came at 3445cm^{-1}. Using the non-hydrogen bonding standard **(17)**, the amount of non-hydrogen bonding depsipeptide was determined by using the extinction coefficient for the standard. The extinction coefficient was found to be 122M^{-1}cm^{-1} at 295.3K, similar to the 130M^{-1}cm^{-1} at room temperature that was reported by Boussard and Marraud.[36] The K_{eq} for the β-turn hydrogen bonded amide/non-hydrogen bonded amide state was calculated using equation [3], and was found to be 5.7 or 86% hydrogen bonded at 295.3K. Qualitative studies with the β-turn **(8)** and Por/PL/DMB in carbon tetrachloride showed that the hydrogen bonded state was the predominant conformation present[37], indicating that the porphyrin moiety has little effect on the hydrogen bonding equilibrium in the β-turn.



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$$K_{eq} = \frac{[Por/PL/Q]_{Total} - \dfrac{Abs_{Non\text{-}hydrogen\ Bonding\ Amide\ Stretch}}{\epsilon_{Non\text{-}Hydrogen\ Bonding\ Amide\ Standard}}}{\dfrac{Abs_{Non\text{-}hydrogen\ Bonding\ Amide\ Stretch}}{\epsilon_{Non\text{-}Hydrogen\ Bonding\ Amide\ Standard}}} \qquad [3]$$

STEADY-STATE FLUORESCENCE

An estimation of the electron transfer rate can be accomplished with a Stern-Volmer analysis.[38] Equation [4] gives an estimated rate constant where I^f_0 is the steady-state fluorescence intensity of Por/PL/DMB, I^f is the steady-state fluorescence intensity of Por/PL/Q, and τ_0 is the Por/PL/DMB lifetime as determined by TCSPC fluorescence spectroscopy. An 8μM solution of Por/PL/DMB yields a single exponential lifetime of 8.95ns.[37] Figure 5 shows the fluorescence emission from 560-800nm for both Por/PL/DMB and Por/PL/Q where the compounds were excited at 550nm. Using the data in Figure 5 and equation [4], we estimate the electron transfer rate constant, k_{et}, to be 2.64x10^{8}s^{-1}. This rate constant is similar to the k_{et} Bolton and coworkers obtained for a Por/Glycine/Q compound (k_{et} = 2.1x10^{8}s^{-1}) in dichloromethane.[39] Direct comparison with the work of Tamiaki and Maruyama[10a] on compound **(2)** (see Figure 1) is less certain since the electron transfer was measured in 2-methyltetrahydrofuran. However, they observed k_{et} = 2.1x10^{8}s^{-1} which is comparable to the k_{et} we have determined. The inherent problems with finding k_{et} using static methods include incomplete oxidation of the hydroquinone to the quinone and difficulty in obtaining concentrations which are exactly the same. The lifetime

$$\frac{I^f_o}{I^f} = 1 + k_{et}(\tau_o) \qquad [4]$$

$$k_{et} = \frac{1}{\tau_1} - \frac{1}{\tau_0} \qquad [5]$$

for the Por/PL/Q, τ_1, calculated from equation [5] is 2.66ns. With considerations for the aforementioned problems with the rate calculation, an additional problem with the rate constant determination for the electron transfer process is that the process might not have a single pathway for electron transfer. Thus, TCSPC fluorescence spectroscopy must be performed to determine the exact rate constant for the transfer process, and to determine if the process has a single pathway or yields a single exponential for the transfer process.

CONCLUSIONS

We have synthesized a β-turn forming depsipeptide to study mediation of electron transfer through a hydrogen bond. The β-turn was determined in dichloromethane to favor folding versus the non-folded form (86% folded). Absorption spectra show that electronic interaction between the porphyrin (electron donor) and the quinone (electron acceptor) is minimal. Finally, steady-state fluorescence shows that electron transfer as observed by fluorescence quenching of the porphyrin excited state by the quinone is taking place.

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