

ESI for 'Lighting-up Protein-Ligand Interactions with Fluorescent PET (Photoinduced Electron Transfer) Sensor Designs' by Bernard McLaughlin, Esther M. Surender, Glenn D. Wright, Brian Daly and A. Prasanna de Silva

S1. Synthesis procedures and characterization details for sensors 1 and 3.

N-(2-(diethylamino)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide, 4

Biotin (60 mg, 0.246 mmol) and hydroxybenzotriazole (6.6 mg, 0.049 mmol) were suspended in anhydrous dimethylformamide (2 ml) and heated alongside 4Å molecular sieves. The solution, when clear, was cooled to room temperature and a solution of dicyclohexylcarbodiimide (0.27 ml, 1 M solution in DCM) was added dropwise and the mixture was stirred at room temperature for 3 hours. N,N-diethylethylenediamine (0.042 ml, 0.295 mmol) was added to the now-cloudy reaction mixture and stirred at 60°C for 4 hours, followed by a further 24 hours at room temperature. The initial precipitate of dicyclohexylurea was filtered off, and the DMF was removed via high vacuum. The residue that remained was thoroughly washed with water, and filtered. The filtrate was collected and water removed by high vacuum, leaving an off white solid. (Yield: 62%, melting point: 123-4°C)

Found:	C ₁₆ H ₃₁ N ₄ O ₂ S	[M+H ⁺] 343.2165
Calculated:	C ₁₆ H ₃₁ N ₄ O ₂ S	[M+H ⁺] 343.2168
¹ H NMR (CDCl ₃)	δ7.00(s, CONH, 1H), 6.39(s, NHCONH, 1H), 5.69(s, NHCONH, 1H), 4.51(t, NHCHC, 1H, J = 4 Hz), 4.32(t, NHCHC, 1H, J = 5 Hz), 3.36(m, CH ₂ S, 2H), 3.14(q, CHS, 1H, J = 5 Hz), 2.93(m, CH ₂ O, 2H), 2.68(m, CH ₂ N, 6H), 2.25(q, CH ₂ CH ₂ N, 2H, J = 5 Hz), 1.74(m, CH ₂ CH ₂ CH ₂ , 4H), 1.45(qt, CH ₂ CH ₂ CH ₂ , 2H, J = 5 Hz), 1.08(t, CH ₃ CH ₂ N, 6H, J = 5 Hz).	
¹³ C NMR (CDCl ₃)	δ173.5, 163.8, 61.8, 60.2, 55.5, 51.9, 46.8, 40.6, 36.5, 35.9, 28.2, 28.1, 25.7, 10.8.	
i.r. (u _{max}) KBr	3292, 3081, 2969, 2937, 2864, 2810, 1698, 1646, 1550, 1465, 1419, 1385, 1324, 1266, 1241, 1203, 1181, 1153, 1077, 871, 693, 656, 552 cm ⁻¹ .	

N-((10-(3,4-dimethoxybenzyl)anthracen-9-yl)methyl)-N,N-diethyl-2-(5-((3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)ethanaminium, 1

4 (0.0684 g, 0.2 mmol) was dissolved in dichloromethane (3 ml) along with **2** (0.084 g, 0.2 mmol). Sodium carbonate was added (0.032 g, 0.3 mmol) and the reaction mixture was refluxed overnight. The sodium carbonate was removed via filtration and the solvent removed via rotary evaporation. A DCM: H₂O extraction was performed on the yellow residue. The organic phase was removed and dried with anhydrous magnesium sulphate. The solvent was removed to give a yellow oil, which was triturated in diethyl ether to give a yellow solid which was filtered and dried. (Yield: 41%, melting point: 135-6°C)

λ _{Abs} /nm (ε/M ⁻¹ cm ⁻¹):	400(8000), 380(9100), 360(6700)	
Found:	C ₄₀ H ₅₁ N ₄ O ₄ S ⁺	[M ⁺] 683.3617
Calculated:	C ₄₀ H ₅₁ N ₄ O ₄ S ⁺	[M ⁺] 683.3631
¹ H NMR (CDCl ₃)	δ8.50(d, AnthH, 2H, J = 6 Hz), 8.29(d, AnthH, 2H, J = 6 Hz), 7.50(m, AnthH, 4H), 6.79(s, ArH, 1H), 6.66(d, ArH, 1H, J = 5	

Hz), 6.48(d, ArH, 1H, J = 5 Hz), 5.50(m, AnthCH₂N, 2H) 4.98(s, AnthCH₂Ar, 2H), 4.02(m, NHCH₂CH, 2H), 3.75(d, OCH₃, 6H, J = 15 Hz), 3.48(d, CH₂NHCO, 2H, J = 5 Hz), 3.01(d, N⁺CH₂, 2H, J = 5 Hz), 2.82(m, N⁺CH₂/CH₂S, 5H), 2.27(m, CH₂S, 2H), 1.68(m, CH₃CH₂N, 6H), 1.37(m, NHCOCH₂, 2H), 1.21(m, CH₂CH₂CH₂, 4H), 0.948(m, CH₂CH₂CH₂, 2H).

¹³C NMR (CDCl₃) δ173.5, 163.8, 149.1, 147.6, 138.7, 132, 128.0, 125.4, 122.6, 118, 112.3, 65.4, 63.8, 58.1, 55.6, 52.9, 40.6, 36.5, 35.9, 28.2, 28.1, 25.7, 10.8.

i.r. (u_{max}) (KBr) 3422, 2930, 2361, 1695, 1589, 1513, 1447, 1348, 1234, 1183, 1139, 1026, 848, 760, 730, 694, 602, 542 cm⁻¹.

N-((10-(3,4-dimethoxybenzyl)anthracen-9-yl)methyl)-3-hydroxy-N,N-dimethylpropan-1-aminium, 3

2 (0.084 g, 0.2 mmol) was added to a round-bottom flask and dissolved in dichloromethane (DCM) (3 ml). 3-Dimethylamino-1-propanol (0.024 ml, 0.2 mmol) was added and the reaction mixture was refluxed overnight. DCM was removed and an extraction was performed with DCM and water. The organic phase was separated, dried and the solvent removed via rotary evaporation and dried on a high vacuum line. The remaining brown oil was triturated in acetone to give a yellow solid, which was filtered and dried. (Yield: 70%, melting point: 126-7°C)

λ_{Abs}/nm (ε/M⁻¹cm⁻¹): 402(7900), 381(9300), 363(6200)

Found: C₂₉H₃₄NO₃⁺ [M⁺] 444.2521

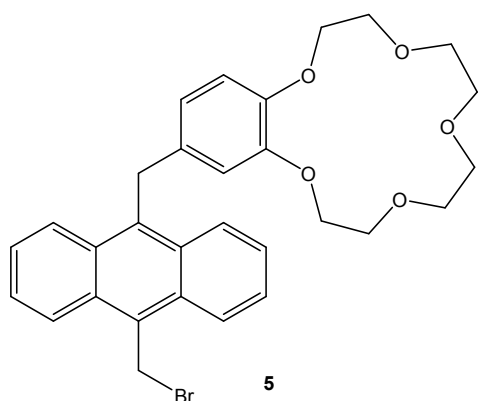
Calculated: C₂₉H₃₄NO₃⁺ [M⁺] 444.2533

¹H NMR (CDCl₃) δ8.76(d, AnthH, 2H, J = 9 Hz), 8.27(d, AnthH, 2H, J = 9 Hz), 7.70(t, AnthH, 2H, J = 7 Hz), 7.48(t, AnthH, 2H, J = 7 Hz), 6.79(s, ArH, 1H), 6.66(d, ArH, 1H, J = 8 Hz), 6.42(d, ArH, 1H, J = 8 Hz), 6.07(s, AnthCH₂N, 2H), 4.95(s, AnthCH₂Ar, 2H), 4.55(t, CH₂OH, 2H, J = 7 Hz), 3.96(t, NCH₂, 2H, J = 5 Hz), 3.56(d, OCH₃, 6H, J = 7 Hz), 3.06(s, NCH₃, 6H), 2.33(m, CH₂CH₂OH, 2H).

¹³C NMR (CDCl₃) δ149.1, 147.6, 137.4, 133.0, 132.4, 130.3, 128.0, 126.0, 125.2, 120.0, 117.8, 111.6, 111.4, 65.0, 60.5 59.9, 58.7, 55.9, 50.4, 34.0, 26.6.

i.r. (u_{max}) KBr 3395, 2931, 1622, 1508, 1448, 1410, 1268, 1250, 1184, 1141, 1114, 1059, 1020, 933, 838, 822, 780, 726, 694, 651, 604 cm⁻¹.

S2. Synthesis procedures and characterization details for sensors 6 and 7.



N,N-diethyl-N-((10-((2,3,5,6,8,9,11,12-octahydrobenzo[b][1,4,7,10,13]pentaoxacyclopentadecin-15-yl)methyl)anthracen-9-yl)methyl)-2-(5-((3aR,4R,6aS)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)ethanaminium, 6

4 (0.0684 g, 0.2 mmol) was dissolved in dichloromethane (3 ml) along with **5** (0.11 g, 0.2 mmol). Sodium carbonate was added (0.032 g, 0.3 mmol) and the reaction mixture was refluxed overnight. The sodium carbonate was removed via filtration and the solvent removed via rotary evaporation. A DCM: H₂O extraction was performed on the yellow residue. The organic phase was removed and dried with anhydrous magnesium sulphate. The solvent was removed to give a yellow oil, which was triturated in diethyl ether to give a yellow solid which was filtered and dried. (Yield: 54%, melting point: 105°C)

$\lambda_{\text{Abs}}/\text{nm}$ ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$): 400(8700), 380(9100), 360(6000)

Found:	C ₄₆ H ₆₁ N ₄ O ₇ S ⁺	[M ⁺] 813.4265
Required:	C ₄₆ H ₆₁ N ₄ O ₇ S ⁺	[M ⁺] 813.4261

¹H NMR (CDCl₃) 8.44(d, AnthH, 2H, J = 9 Hz), 8.18(d, AnthH, 2H, J = 8 Hz), 7.44(m, AnthH, 4H), 6.65(s, ArH, 1H), 6.55(d, ArH, 1H, J = 9 Hz), 6.46(d, ArH, 1H, J = 9 Hz), 5.66(s, AnthCH₂N, 2H), 4.87(s, AnthCH₂Ar, 2H), 4.01(m, NHCH₂CH, 2H), 3.98(m, CH₂O/ NCH₂, 20H), 3.42(m, NCH₂CH₃/SCH, 5H), 3.05(m, NHCH₂CH, 2H), 1.59(m, NCH₂CH₃/CHCH₂CH₂, 8H), 1.20(m, NHCOCH₂, 2H), 0.948(m, CH₂CH₂CH₂, 4H)

¹³C NMR (CDCl₃) δ 173.5, 163.8, 149.1, 147.6, 138.7, 132, 128.0, 125.4, 122.6, 118, 112.3, 70.6, 68.1, 65.4, 63.8, 58.1, 52.9, 40.6, 36.5, 35.9, 28.2, 28.1, 25.7, 10.6

i.r. (u_{max}) KBr 3854, 3413, 3056, 2920, 2864, 1696, 1509, 1448, 1357, 1269, 1135, 1054, 933, 851, 764, 722, 695, 602, 541 cm⁻¹.

3-Hydroxy-N,N-dimethyl-N-((10-((2,3,5,6,8,9,11,12-octahydrobenzo[b][1,4,7,10,13]pentaoxacyclopentadecin-15-yl)methyl)anthracen-9-yl)methyl)propan-1-aminium, 7

5 (0.11 g, 0.2 mmol) was added to a round-bottom flask and dissolved in dichloromethane (DCM) (3 ml). 3-Dimethylamino-1-propanol (0.024 ml, 0.2 mmol) was added and the reaction mixture was

refluxed overnight. DCM was removed and an extraction was performed with DCM and water. The organic phase was separated, dried and the solvent removed via rotary evaporation and dried on a high vacuum line. The remaining brown oil was triturated in acetone to give a yellow solid, which was filtered and dried. (Yield: 84%, melting point: 124-5°C)

$\lambda_{\text{Abs}}/\text{nm}$ ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$):	402(8100), 381(9200), 362(6300)	
Found:	$\text{C}_{35}\text{H}_{44}\text{NO}_6^+$	$[\text{M}^+]$ 574.3196
Required:	$\text{C}_{35}\text{H}_{44}\text{NO}_6^+$	$[\text{M}^+]$ 574.3168
^1H NMR (CDCl_3)	δ 8.64(d, AnthH, 2H, $J = 9$ Hz), 8.21(d, AnthH, 2H, $J = 8$ Hz), 7.64(t, AnthH, 2H, $J = 7$ Hz), 7.43(t, AnthH, 2H, $J = 7$ Hz), 6.63(s, ArH, 1H), 6.60(d, ArH, 1H, $J = 9$ Hz), 6.41(d, ArH, 1H, $J = 9$ Hz), 5.98(s, CH_2N , 2H), 4.86(s, Anth CH_2 Ar, 2H), 4.45(m, CH_2OH , 2H), 3.98-3.65(m, CH_2O , 16H), 2.97(s, NCH_3 , 6H), 2.25(m, NCH_2 , 2H), 1.64(m, $\text{CH}_2\text{CH}_2\text{CH}_2$, 2H)	
^{13}C NMR (CDCl_3)	δ 149.6, 148, 138, 133.3, 130.6, 128.3, 126.3, 126.2, 121.1, 118.2, 114.7, 71.3, 70.8, 69.9, 69.4, 69.3, 59, 50.8	
i.r. (ν_{max}) KBr	3384, 2870, 1624, 1510, 1481, 1451, 1419, 1350, 1259, 1221, 1133, 1059, 978, 937, 848, 808, 792, 769, 729, 667, 602 cm^{-1} .	

S3. Solvent-dependent fluorescence quantum yields of 3.

Solvent	π^*	ϕ_{F}
Xylene	0.43	0.24
Toluene	0.54	0.089
Ethyl acetate	0.55	0.11
Dioxan	0.55	0.11
Chloroform	0.58	0.057
Tetrahydrofuran	0.58	0.062
2-Butanone	0.67	0.007
Acetone	0.68	0.009
Chlorobenzene	0.71	0.042
Anisole	0.73	0.038
Acetonitrile	0.75	0.009
Dichloroethane	0.81	0.01
Dichloromethane	0.81	0.019
Dimethylformamide	0.88	0.014
Dimethylsulfoxide	1.00	0.014

S4. Fluorescence emission spectra of 3 as a function of added bovine serum albumin.

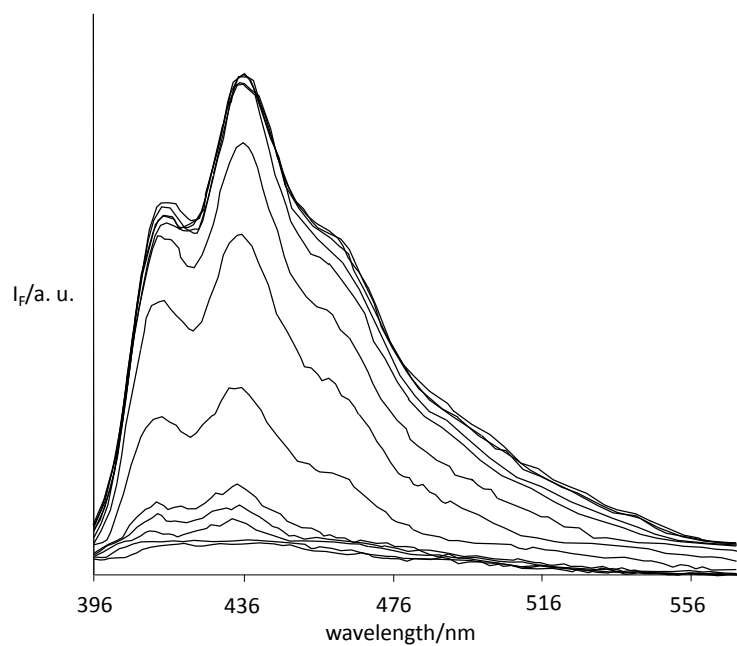


Figure S1. Fluorescence emission spectra for 10^{-5} M **3** (excited at 380 nm) in aqueous phosphate buffer (pH 7.2, 0.1 M) solution in the presence of bovine serum albumin at 0.0, 1.0, 5.0, 10.0, 50.0, 100, 200, 350, 550, 650, 750, 850 and 950 μ M concentrations (in order of increasing intensity at 436 nm).