Ruthenium(II) Phenanthroline-Biotin Complexes: Synthesis and Luminescence Enhancement of upon Binding to Avidin

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Reagents and Buffers

Avidin, and *d*-biotin, DMAP (4-dimethylaminopyridine), HABA (4'-hydroxyazobenzen-2-carboxylic acid) were purchased from Aldrich. $RuCl_3.3H_2O$ was purchased from Pressure Chemical Co. Aluminum oxide, activated, neutral, standard grade, ~ 150 mesh was purchased from Aldrich. All solvent used as obtained from commercial sources. $Ru(bpy)_2Cl_2$ (*1*), $Ru(phen)_2Cl_2$ (*1*), 5-nitrophenanthroline (*2*), 5-aminophenanthroline (*3*), were synthesized according to literature procedures. Buffer A contained 100 mM NaCl, 50 mM NaH_2PO_4, and 1 mM EDTA (ethylenediaminetetraacetic acid), pH 7.5 adjusted using NaOH. Avidin concentration was experimentally calculated using literature procedures (*4*).

Instruments and Methods

^1H NMR, and ^{13}C NMR were recorded on a varian M400 spectrometer operated at 400.140 MHz. UV/VIS spectra were recorded on a Varian Cary 300 spectrophotometer. Fluorescence experiments were recorded on a PTI (Photon Technology International) TimeMaster Model C-720F spectrofluorimeter. The slit widths were 4 nm for both excitation and emission. Quantum yield were measured using litrature procedure (*5*).

Synthesis of Phenanthroline-Biotin Ligand

Biotin (0.425 g, 1.80 mmol) was dissolved in $SOCl_2$ in a dry 25 ml round bottomed flask. The reaction mixture was stirred for 20 minutes, followed by removal of excess thionyl chloride (*6*). The residue of the biotin acyl chloride was redissolved in dry DMF (15 mL). In parallel, 5-amino-phenanthroline (0.400 g, 2.00 mmol), and DMAP (0.030 g, 0.230 mmol) were dissolved in dry DMF (30 mL), to which the biotin-acyl chloride in DMF was transferred. The reaction was allowed to stir for 18 hours at room temperature,

and then concentrated to ~ 3 ml, after which it was poured in ether (30 mL) while stirring. A black precipitate resulted. The solution was decanted and the solid was dissolved in hot methanol. The amide was purified using deactivated alumina chromatography, CH_2Cl_2: MeOH 1:5, R_f = 0.40. Yield 80%. ^{1}H NMR (400 MHz, DMSO-d_6); δ 10.71 (s, 1H, HNC=O), 9.11 (dd, 1H, H2), 9.01 (dd, 1H, H9), 8.60 (d, 1H, H4), 8.43 (d, 1H, H7), 8.15 (s, 1H, H6), 7.81 (dd, 1H, H3), 7.72 (dd, 1H, H8), 6.48 (s, 1H, N*H*-biotin), 6.38 (s, 1H, N*H*-biotin), 4.31 (m, 1H, C*H*), 4.16 (m, 1H, C*H*), 3.16 (m, 1H, SC*H*), 2.86 (m, 1H, SCH_2), 2.58 (m, 1H, SCH_2), 1.70 (m, 2H, CH_2CO), 1.55-1.48 (m, 6H, CH_2CH_2CH_2). ^{13}C NMR (400 MHz, DMSO-d_6); δ 225.48, 22.94, 150.51, 146.25, 136.60, 132.65, 132.51, 124.33, 123.59, 120.65, 61.87, 60.01, 56.28, 31.59. Positive ion ESI-MS: m/z = 422 $[M+H^+]^+$.

Synthesis of Complex 1 $[Ru(bpy)_2(phen\text{-}biotin)](PF_6)_2$

The ligand phen-biotin (0.065 g, 0.154 mmol), and $Ru(bpy)_2Cl_2$ (0.080g, 0.154 mmol) were dissolved in H_2O: EtOH 1:7 (25 ml), the reaction mixture was refluxed for 16 hours under nitrogen atmosphere. After the solvent was reduced to 5 mL, an ammonium hexafluorophosphate solution (5 equiv.) was added, the product precipitated as a yellow-orange solid. The solution was left to stand overnight. The product was collected using vacuum filtration. The solid was further purified by washing with water, ether, then was dried under vacuum. Yield 85%. ^{1}H NMR (400 MHz, acetone-d_6); 9.76 (s, 1H, *H*NC=O), 8.99 (m, 6H, Ar), 8.43 (dd, 1H, Ar), 8.38 (m, 1H, Ar), 8.25 (m, 2H, Ar), 8.15 (m, 4H, Ar), 7.88 (m, 4H, Ar), 7.62 (m, 2H, Ar), 7.39 (m, 2H, Ar), 5.79 (d, 1H, *H*N biotin), 5.72 (s, 1H, *H*N biotin), 4.50 (m, 1H, C*H* biotin), 4.36 (m, 1H, C*H* biotin), 3.28 (m, 1H,

C*HCS*), 2.97 (m, 1H, C*H*$_2$CS), 2.73 (m, 3H, C*H*$_2$CS+ C*H*$_2$C=O), 1.89-1.62 (m, 6H, $CH_2CH_2CH_2$). Positive ion ESI-MS: *m/z* = 980 $[M\text{-}PF_6]^+$. ESI-HRMS 980 $[M\text{-}PF_6]^+$.

Synthesis of complex 2 $[Ru(phen)_2(phen\text{-}biotin)](PF_6)_2$

Complex **2** was synthesised as described above for complex **1**. Yield 75%. ^{1}H NMR (400 MHz, acetone-d_6); 9.80 (s, 1H, *H*NC=O), 8.96 (d, 1H, Ar), 8.77 (m, 4H, Ar), 8.71 (m, 2H, Ar), 8.42 (m, 9H, Ar), 8.28 (dd, 1H, Ar), 7.80 (m, 4H, Ar), 7.73 (dd, 2H, Ar), 5.60 (S, 2H, *H*NCON*H*), 4.49 (m, 1H, C*H*NH), 4.33 (m, 1H, C*H*NH), 3.27 (m, 1H, C*H*CS), 2.95 (m, 1H, C*H*$_2$CS), 2.70 (m, 3H, C*H*$_2$CO + C*H*$_2$CS), 1.91-1.86 (m, 3H, C*H*$_2$), 1.74-1.60 (m, 3H, C*H*$_2$). Positive ion ESI-MS: *m/z* = 1028 $[M\text{-}PF_6]^+$. ESI-HRMS 1028 $[M\text{-}PF_6]^+$.

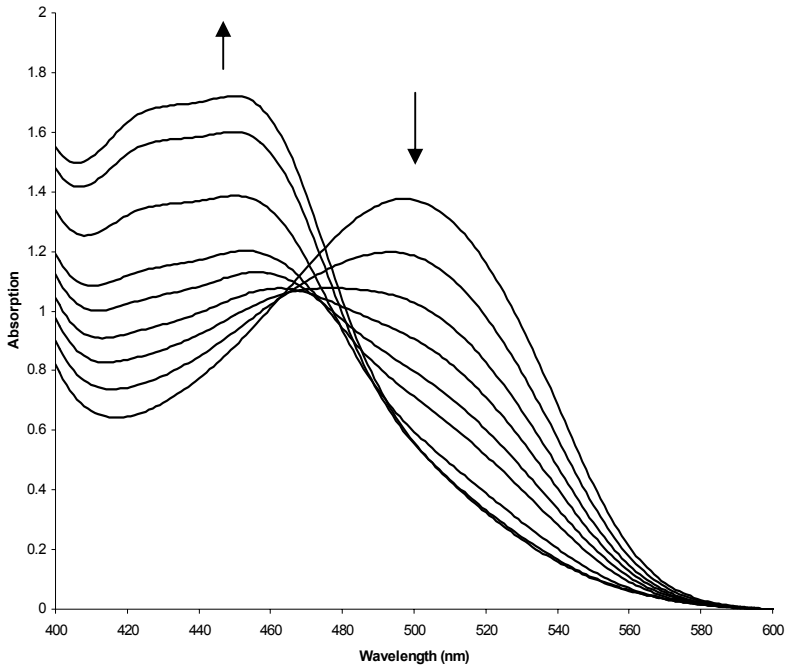
Table 1. Electronic Absorption and Emission Data for Complexes 1, and 2[a]

Complex	ϕ^{b}	Medium	λ_{abs}	λ_{em}
1	0.064	CH_2Cl_2	248, 278, 449	589
		CH_3CN	216, 288, 320, 452	603
		H_2O	285, 452	603
2	0.052	CH_2Cl_2	228, 265, 449	580
		CH_3CN	199, 222, 264, 447	588
		H_2O	263, 447	588

a) All measurements were taken at ambient temperature. b) Quantum yield measured in water purged with N_2

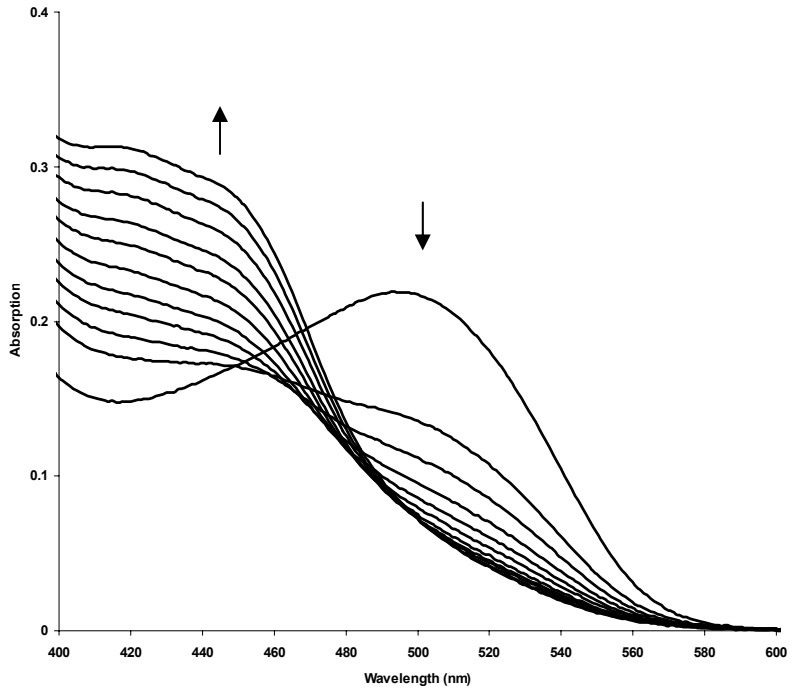
Binding Specificity of Complexes 1 and 2 Towards Avidin: HABA Assays

Avidin (~ 3.5 μM, 990 μL), and HABA (10 mM, 10 μL) were combined and incubated for 30 min. The ruthenium-biotin complex **1**, or **2** (0.25 mM) were added during the titration in aliquots of 5 μL (Figure 1, and 2 respectively). The absorbance was monitored at 500 nm. Approximately 55 μL of ruthenium-biotin complex resulted in total decrease in the absorbance peak at 500 nm, and the appearance of an absorption peak at 450 nm corresponding to the absorption of the ruthenium chromophore.



Titration of HABA:avidin by complex **1**

Figure 1



Titration of HABA:avidin with complex **2**

Figure 2

Competitive Binding Assay: Titration of Ru-biotin: Avidin Complexes 1 and 2 with Biotin

Avidin (11 nmol), and $Ru(bpy)_2$(phen-biotin) **1** (44 nmol) in buffer A (1.00 ml) were combined and incubated in the fluorescence cell. The mixture was titrated by the addition of a solution of biotin in buffer A (16 μM), in aliquots of 5 μL. Luminescence at 615 nm upon irradiation at 450 nm was recorded after 5-8 min of each addition. 40 nmol of biotin were required to displace all the ruthenium-biotin complex **1** from avidin (Figure 3). The experiment was repeated with the same molar quantities of $Ru(bpy)_2$(phen-biotin) **2** and avidin; in this case, 56 nmol of biotin were needed to displace all of complex **2** from avidin (Figure 4) .

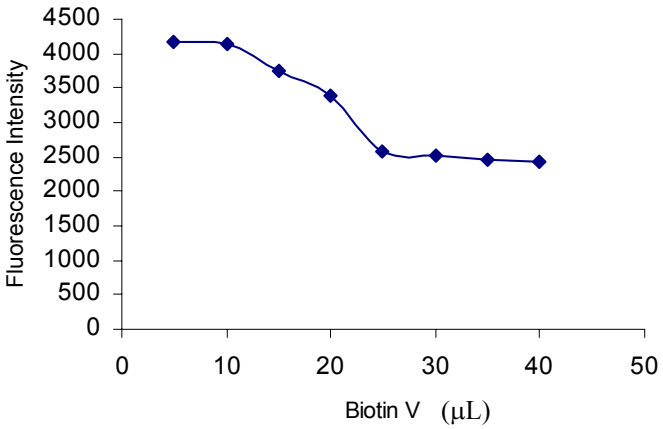


Figure 3

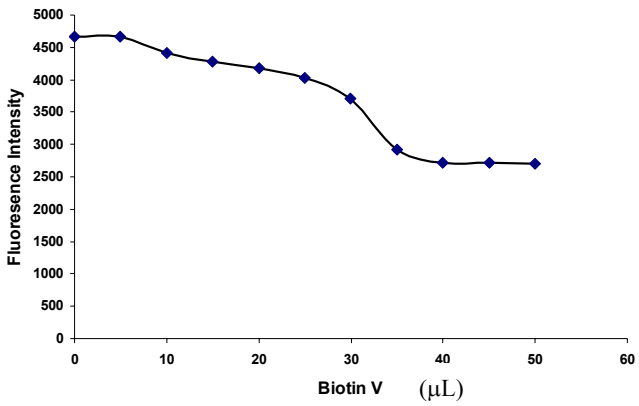


Figure 4

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