A Ratiometric Sensor for the Selective Time-Gated Luminescence Detection of Potassium in Water

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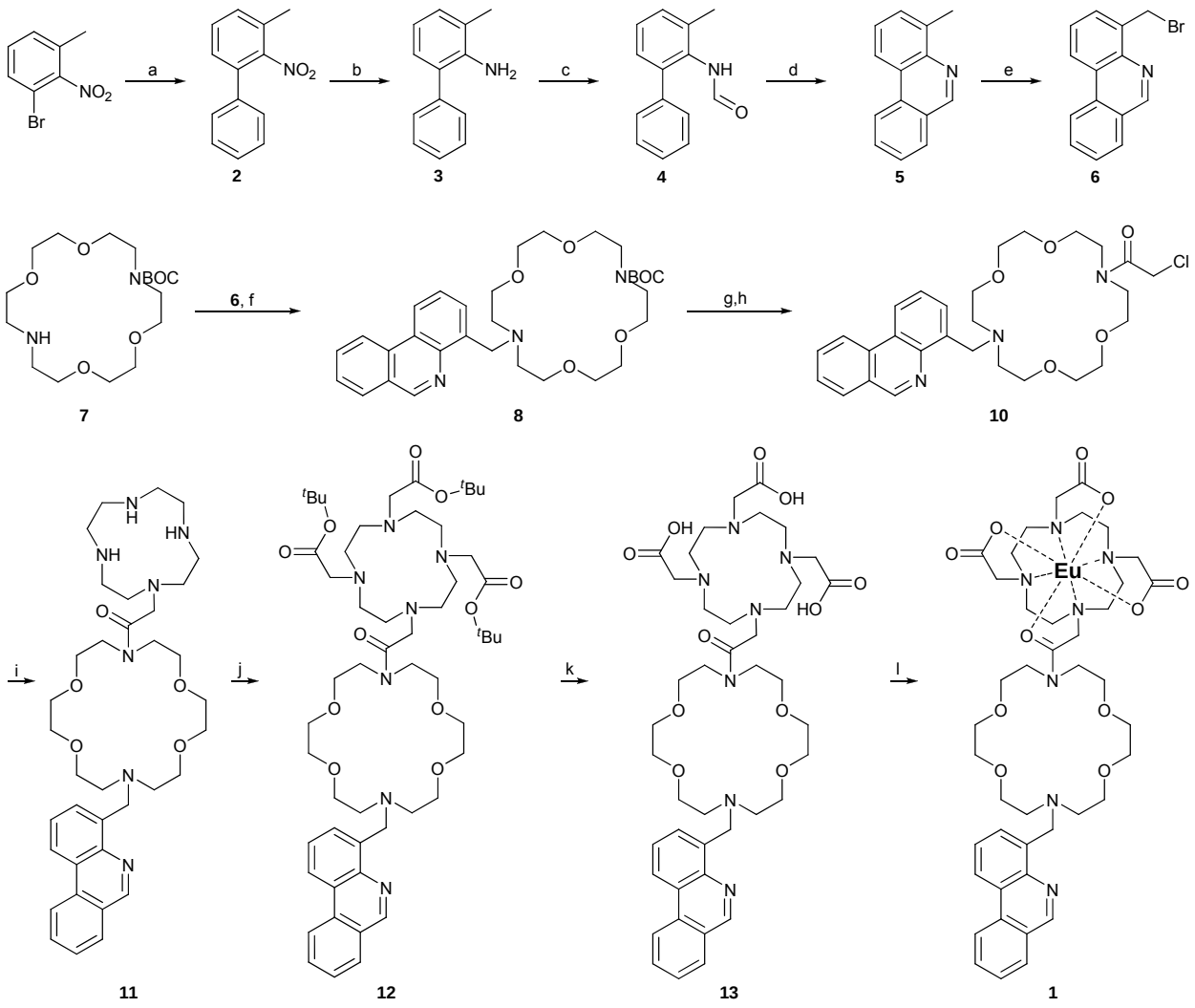
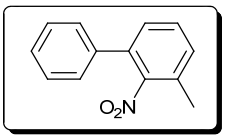
SUPPORTING INFORMATION

Experimental procedures and characterization data for the synthesis of the complex Eu-KPhen (1)

General Considerations. Unless otherwise noted, starting materials were obtained from commercial suppliers and used without further purification. Water was distilled and further purified by a Millipore cartridge system (resistivity 18 x 10^6 Ω). All organic extracts were dried over anhydrous $MgSO_4$ and solvents were removed with a rotary evaporator. Flash chromatography was performed on Merck Silica Gel (40-7 Mesh). ^1H and ^{13}C NMR spectra were recorded on a Varian 500 at 500 MHz and 125 MHz, respectively or on a Varian 400 at 400 MHz and 100 MHz respectively; the residual solvent peak was used as an internal reference. Data for ^1H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s, singlet; b, broad singlet; d, doublet, t, triplet; q, quartet; sept, septet; m, multiplet), integration, coupling constant (Hz). Data for ^{13}C NMR are reported in terms of chemical shift (δ, ppm). Mass spectra (LR = low resolution; HR = high resolution; ES MS = electrospray mass spectrometry) were recorded on a Bruker BioTOF II at the Mass Spectrometry Facility at the Department of Chemistry at the University of Minnesota, Twin-Cities.



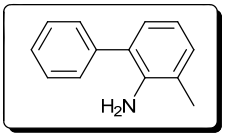
Scheme S1. Synthesis of Eu-KPhen (1).



3-Methyl-2-nitro-1,1'-biphenyl (S2)

1-Bromo-3-methyl-2-nitrobenzene (5.37 g, 24.9 mmol) and phenylboronic acid (3.04 g, 24.9 mmol) were dissolved in toluene (125 mL). Na_2CO_3 (2 M, 75 mL) and tetrakis(triphenylphosphine) palladium(0) (863 mg, 0.747 mmol) were then added to the solution which was purged with N_2 for five minutes. The biphasic mixture was stirred vigorously at 90˚ C under N_2 for 13.5 h. The mixture was diluted with 75 mL mQ H_2O, and the organic phase was separated, dried over Na_2SO_4, and concentrated under reduced pressure to yield a yellow oil. The crude oil was purified by flash chromatography eluting with a gradient of hexanes to 20% $CHCl_3$ / 80% hexanes, yielding the product **S2** as colorless needles (4.93 g, 92.9%).

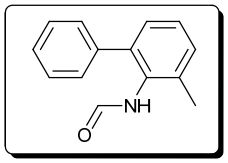
δ_H (500 MHz, $CDCl_3$) 2.39 (3H, s), 7.27-7.31 (2H, m), 7.35-7.37 (2H, m), 7.39-7.45 (4H, m); δ_C(75 MHz, $CDCl_3$) 137.3, 135.0, 131.8, 130.9, 130.7, 130.4, 129.4, 129.3, 129.1, 128.6, 110.3, 109.1, 18.2; HRMS-ESI (m/z): $[M-H]^-$ calcd for $C_{13}H_{11}NO_2$, 212.0706; found, 212.0682.



3-Methyl-[1,1'-biphenyl]-2-amine (S3)

10% Pd on activated carbon (2.95 g) was added to a solution of the biphenyl **S2** (4.90 g, 23.0 mmol) dissolved in methanol (200 mL). The solution was placed in a Parr bomb, the bomb sealed and purged with H_2 gas, then pressurized with H_2 to 36 bar and stirred at room temperature for 48 h. The catalyst was filtered, and the reaction mixture was concentrated to 50 mL under reduced pressure and further dried under high vacuum overnight yielding compound **S3** as a pale yellow oil (4.19 g). The crude oil was used immediately without further purification in the next step.

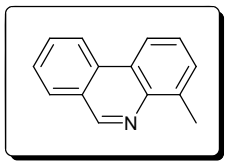
δ_H (500 MHz, CD_2Cl_2) 2.19 (3H, s), 3.72 (2H, br s), 6.70 (1H, t, *J*= 7), 6.95 (1H, dd, *J*=8, *J*=1), 7.03 (1H, dd, *J*=7.5, *J*=1), 7.31-7.35 (1H, m), 7.39-7.45 (4H, m); δ_C (75 MHz, CD_2Cl_2) 142.5, 140.6, 130.2, 129.8, 129.4, 128.7, 127.8, 127.7, 123.1, 118.4, 117.2, 105.0, 18.3; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{13}H_{13}N$, 184.1121; found, 184.1120.



N-(3-Methyl-[1,1'-biphenyl]-2-yl)formamide (S4)

The biphenyl amine **S3** (4.19 g, 22.9 mmol) was dissolved in 95% formic acid (25 mL) and magnetically stirred. The reaction flask was purged with argon for five minutes and the reaction mixture was heated at reflux for 76 h under argon. The formic acid was removed under reduced pressure to yield a light-brown solid (4.58 g, crude), which was used immediately in the next step without further purification.

δ_H (500 MHz, CD_3OD) 2.47 (3H, s), 7.35 (1H, t, *J*=4.5), 7.42-7.58 (8H, m), 8.20 (1H, s); δ_C (75 MHz, CD_3OD) 168.2, 163.6, 142.6, 142.0, 138.4, 133.6, 131.6, 130.8, 130.6, 130.0, 130.0, 129.6, 129.1, 19.6; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{14}H_{13}NO$, 212.1070; found, 212.1076.

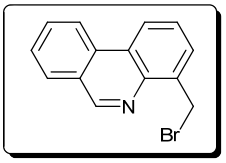


4-Methyl Phenanthridine (S5)

The crude formamide (**S4**) (4.58 g) and polyphosphoric acid (10 g) were heated to 150˚ C under stirring. At 10 min the reaction bubbled and formed a foam layer on the surface. At 25 min the foam layer slowly dissipated to yield a viscous liquid, and at 60 min a dark brown melt was observed. The reaction was allowed to continue for an additional 2 h. The reaction was allowed to cool to ambient temperature then cooled in an ice bath at which point saturated KOH (aq) solution was slowly added to neutralize the acid. The aqueous layer was extracted with $CHCl_3$ (6 × 50 mL) and dried over $MgSO_4$. The

crude product was further purified by flash chromatography eluting with CH_2Cl_2. The phenanthridine **S5** was isolated as a white solid (3.03 g, 68.5% over two steps).

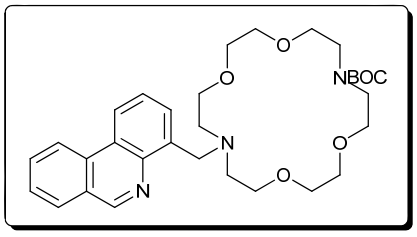
δ_H (500 MHz, $CDCl_3$) 2.91 (3H, s), 7.57-7.63 (2H, m), 7.72 (1H, td, *J*=8, *J*=1), 7.87 (1H, td, *J*=8, *J*=1), 8.08 (1H, d, *J*=8), 8.47 (1H, d, *J*=7), 8.63 (1H, d, *J*=8.5), 9.34 (1H, s); δ_C (75 MHz, $CDCl_3$) 152.8, 138.4, 133.5, 131.5, 130.2, 129.4, 128.0, 127.3, 126.8, 124.6, 122.8, 120.8, 110.7, 19.4; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{14}H_{11}N$, 194.0964; found, 194.0972.



4-Bromomethyl Phenanthridine (S6)

The phenanthridine **S5** (501 mg, 2.59 mmol) and N-bromo succinimide (691 mg, 3.88 mmol) were dissolved in benzene (10 mL) and the reaction mixture was lit with a tungsten lamp for 30 min at room temperature. The solvent was removed under reduced pressure and the crude product was deposited onto silica and purified by flash chromatography eluting with 50% CH_2Cl_2 in 50% hexanes. The product (**S6**) was obtained as a white solid which was further dried under high vacuum overnight (362 mg, 51.4%).

δ_H(500 MHz, $CDCl_3$) 5.33 (2H, s), 7.67 (1H, t, *J*=7.5), 7.75 (1H, t, *J*=7.5), 7.86-7.91 (2H, m), 8.10 (1H, d, *J*=7.5), 8.59 (1H, dd, J_1=8.5, J_2=1.0), 8.62 (1H, d, *J*=8.5), 9.39 (1H, s); δ_C(75 MHz, $CDCl_3$) 153.9, 148.4, 148.0, 137.3, 133.2, 131.9, 130.7, 129.6, 128.4, 127.5, 127.0, 125.1, 123.8, 122.7; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{14}H_{10}BrN$, 272.0069; found, 272.0068. The isotopic distribution matched the calculated one.

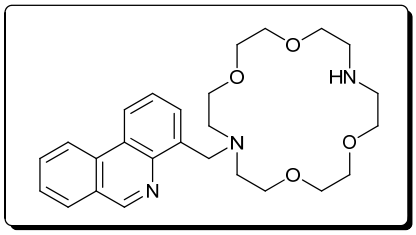


***tert*-Butyl 16-(phenanthridin-4-ylmethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7-carboxylate (S8)**

The mono-BOC protected diaza-18-crown-6 (*tert*-butyl 1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-7-carboxylate, **S7**) was synthesized according to literature procedure.[1] The bromophenanthridine **S6** (362 mg, 1.33 mmol) and Cs_2CO_3 (1.75 g, 5.37 mmol) were dissolved in dry acetonitrile and the reaction mixture was purged with N_2 for five minutes. The crown ether **S7** (468 mg, 1.29 mmol) was added and the reaction mixture was stirred for 48 h at room temperature under N_2, then heated for an additional 48 h at 50° C under N_2. The reaction was then cooled down and the Cs_2CO_3 was filtered off. The solvent was removed under reduced pressure and the resultant crude oil was purified by flash chromatography eluting with 85%/14%/1% CH_2Cl_2/MeOH/NH_4OH. The Lariat ether **S8** was isolated as a brown oil (330 mg, 44.8%).

[1] A. Thibon and V. C. Pierre, *J. Am. Chem. Soc.*, 2009, **131**, 434-435.

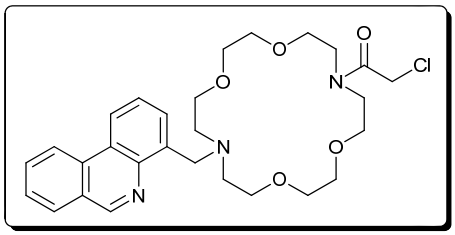
δ_H(500 MHz, $CDCl_3$) 1.46 (9H, m), 3.60-3.72 (24H, m), 4.08 (2H, s), 7.77-7.81 (2H, m), 7.95 (1H, t, *J*=8), 8.11 (1H, t, *J*=8), 8.44 (1H, d, *J*=7), 8.67 (1H, d, *J*=7), 8.73 (1H, d, *J*=8), 9.32 (1H, s); δ_C (125 MHz, $CDCl_3$) 153.1, 135.2, 128.5, 128.0, 126.5, 124.9, 98.8, 73.2, 70.1, 69.1, 66.0, 61.4, 27.0. HRMS-ESI (m/z): $[M + H]^+$ calcd for $C_{31}H_{43}N_3O_6$, 554.3225; found, 554.3225.



7-(Phenanthridin-4-ylmethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane (S9)

The protected amine **S8** (330 mg, 0.596 mmol) was dissolved in CH_2Cl_2 (12 mL) and cooled with an ice bath. Trifluoroacetic acid (2.2 mL, 28 mmol) was added dropwise to the solution over 5 min. The reaction mixture was allowed to warm to ambient temperature and stirred for 1 h. The solvent and the excess TFA were removed under reduced pressure, yielding the deprotected amine **S9** as a yellow oil (225 mg, 83.2%).

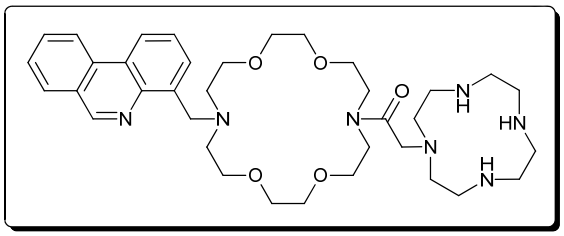
δ_H(500 MHz, $CDCl_3$) 2.88 (4H, br s), 2.95 (4H, t, *J*=5.5), 3.61-3.72 (16H, m), 4.49 (2H, s) 7.70 (2H, t, *J*=7.5), 7.86 (1H, t, *J*=8), 8.06 (2H, t, *J*=9), 8.50 (1H, d, *J*=8), 8.63 (1H, d, *J*=8), 9.28 (1H, s); δ_C(75 MHz, $CDCl_3$) 152.8, 139.3, 133.5, 131.5, 129.6, 129.3, 127.9, 127.6, 126.7, 124.3, 122.8, 121.3, 114.3, 110.3, 71.5, 70.9, 70.6, 55.5, 55.0, 49.9; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{26}H_{35}N_3O_4$, 454.2700; found, 454.2689.



2-Chloro-1-(16-(phenanthridin-4-ylmethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)ethanone (S10)

The amine **S9** (225 mg, 0.496 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled with an ice bath. Triethylamine (208 μL, 1.49 mmol) was added to the solution, and the reaction mixture was purged with N_2 for 3 min. A solution of chloroacetyl chloride (78 μL, 0.992 mmol) dissolved in CH_2Cl_2 (2 mL) was added dropwise to the reaction mixture over ten minutes. The mixture was then allowed to warm to ambient temperature and stirred for 4 h. The solvent and excess chloroacetyl chloride were removed under reduced pressure, and the crude product was purified by flash chromatography eluting with a gradient of CH_2Cl_2 to 13%/2%/85% $MeOH/NH_4OH/CH_2Cl_2$. The chloride **S10** was obtained as a light-brown oil (167 mg, 63.5%).

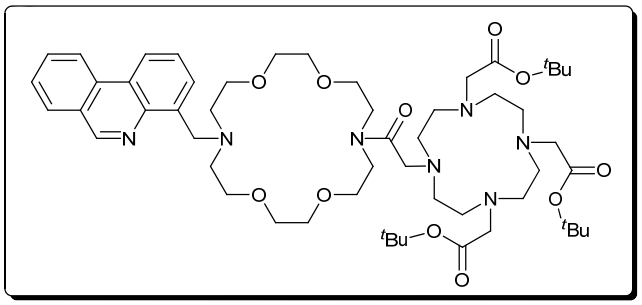
δ_H(500 MHz, $CDCl_3$) 3.07 (4H, m), 3.61-3.80 (22H, m), 4.18 (2H, s), 7.68-7.74 (2H, m), 7.89 (1H, t, *J*=7.5), 8.07 (2H, d, *J*=8), 8.54 (1H, d, *J*=8.5), 8.63 (1H, d, *J*=8.5), 9.29 (1H, s); δ_C(75 MHz, $CDCl_3$) 152.7, 147.1, 131.3, 128.9, 128.0, 127.1, 126.2, 122.2, 109.8, 96.2, 92.7, 71.1, 70.9, 70.6, 69.9, 69.7, 54.7, 49.8, 48.0, 41.6, 37.7; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{28}H_{36}ClN_3O_5$, 530.2416; found, 530.2406.



1-(16-(Phenanthridin-4-ylmethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)-2-(1,4,7,10-tetraazacyclododecan-1-yl)ethanone (S11)

Cyclen (136 mg, 0.789 mmol) and Cs_2CO_3 (308 mg, 0.945 mmol) were dissolved in CH_3CN (60 mL) and the reaction mixture was purged with argon for 2 min. A solution of the chloride **S10** (167 mg, 0.315 mmol) dissolved in CH_3CN (1 mL) was then added dropwise to the reaction mixture over 15 min. The reaction mixture was stirred for 88 h under argon. After which, the Cs_2CO_3 was filtered off, and the mixture was concentrated under reduced pressure to yield a yellow oil. The crude product (273 mg) was used in the next step without purification.

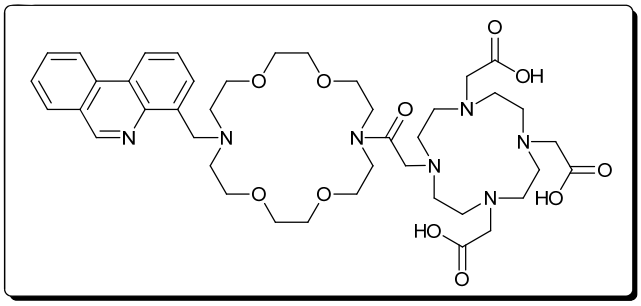
HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{36}H_{55}N_7O_5$, 666.4337; found, 666.4341.



***tert*-Butyl 2,2',2''-(10-(2-oxo-2-(16-(phenanthridin-4-ylmethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (S12)**

The crude amine **S11** (273 mg) was dissolved in CH_3CN (15 mL). Cs_2CO_3 (1.03 g, 3.16 mmol) was added to the reaction mixture which was purged with N_2 for 5 min. *tert*-Butyl-2-bromoacetate (4 × 112 μL, 3.03 mmol) was then added in four batches to the reaction mixture. The mixture was stirred for 20 h under N_2. The solvent was then removed under reduced pressure to yield the crude product which was dissolved in mQ H_2O and purified by reverse phase HPLC (eluent: 0-10% CH_3CN in mQ H_2O), yielding the protected ligand **S12** as a yellow glass that was further dried under high vacuum overnight overnight (189 mg, 59.5% over two steps).

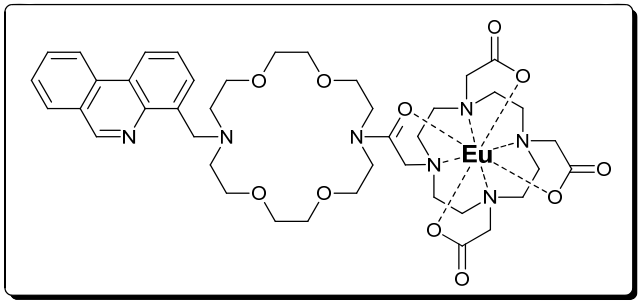
δ_H(300 MHz, $CDCl_3$) 1.44-1.50 (27H, m), 2.21-3.72 (48H, m), 4.49 (2H, s), 7.64-7.75 (2H, m), 7.88 (1H, t, *J*=6.9), 7.99 (1H, d, *J*=6), 8.06 (1H, d, *J*=7.8), 8.51 (1H, d, *J*=6.9), 8.64 (1H, d, *J*=7.8), 9.29 (1H, s); MS-ESI (m/z): $[M + Na]^+$ calcd for $C_{54}H_{85}N_7O_{11}$, 1030.6199; found, 1030.6983.



KPhen, 2,2',2''-(10-(2-oxo-2-(16-(Phenanthridin-4-ylmethyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (S13)

The protected ligand **S12** (189 mg, 0.187 mmol) was dissolved in CH_2Cl_2 (7 mL) and cooled with an ice bath. Trifluoroacetic acid (4 mL) was added dropwise over 5 min, after which the reaction was allowed to warm to ambient temperature and stirred for 2 h. The solvent and TFA were removed under reduced pressure with subsequent additions of methanol (5×3 mL) to drive off the acid. The product was purified by reverse phase HPLC (eluent: 0-100% CH_3CN in mQ H_2O) and lyophilized. The deprotected ligand was obtained as a hygroscopic, white crystalline solid (127 mg, 80.9%).

δ_H(500 MHz, D_2O) 3.01-3.83 (48H, m), 4.87 (2H, s), 7.77 (1H, t, *J*=8), 7.83 (1H, d, *J*=6), 7.89 (1H, t, *J*=7.5), 8.05 (1H, t, *J*=7), 8.23 (1H, d, *J*=7) 8.76 (2H, t, *J*=8.5), 9.27 (1H, s). δ_C (125 MHz, D_2O, δ): 153.5, 131.5, 129.4, 127.4, 124.8, 122.3, 104.3, 55.6, 54.6, 53.6, 52.5, 51.0, 47.9; HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{42}H_{61}N_7O_{11}$, 840.4502; found, 840.4475.



Eu-KPhen (1)

The deprotected ligand **S13** (3.33 mg, 3.96 μmol) and europium chloride hexahydrate (1.02 mg, 3.96 μmol) were dissolved in mQ H_2O (1 mL) and magnetically stirred. The acidic mixture was adjusted to pH 8 with lithium hydroxide. The reaction was purged with N_2 for 1 min and stirred under reflux under N_2 for 19 h. The reaction mixture was then lyophilized, yielding Eu-KPhen (**1**) as an off-white powder (3.91 mg, quant.).

HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{42}H_{58}EuN_7O_{11}$, 990.3479; found, 990.3506. The observed isotopic distribution matched the calculated one.

Selected NMR Spectrum

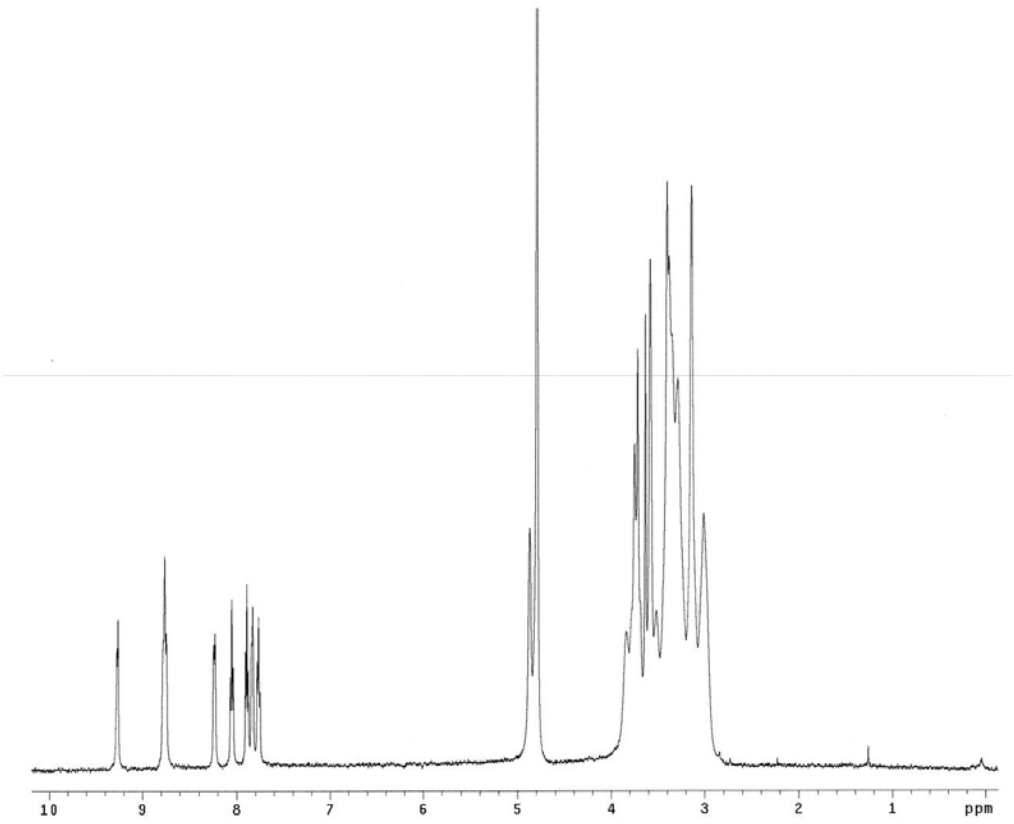


Figure S1. 1H NMR spectra of the ligand KPhen (**S13**) (D_2O, 500 MHz).

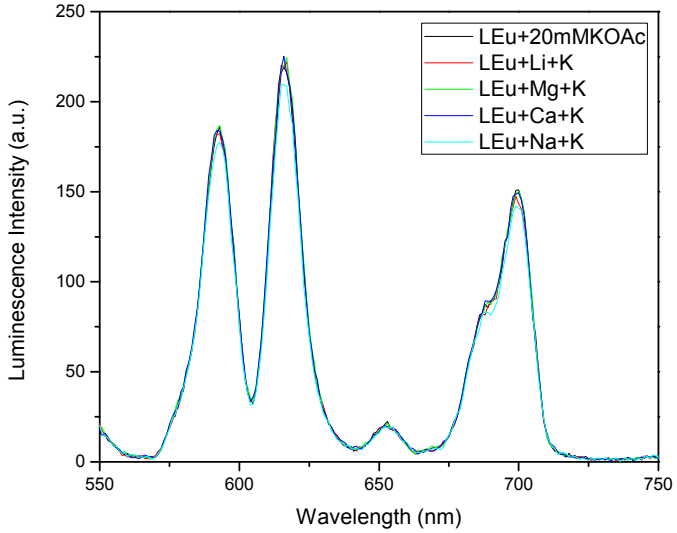


Figure S2. Selectivity of Eu-KPhen (**1**) for K^+ over physiological cations at typical serum concentrations. Time-gated emission spectra of Eu-KPhen in the presence of 50.0 mM Li^+ (red), 0.83 mM Mg^{2+} (green), 2.47 mM Ca^{2+} (dark blue), 107 mM Na^+ (cyan) and water (red) after subsequent addition of 20 mM K^+. Experimental conditions: [Eu-KPhen] = 50.0 μM, 25 mM aqueous HCO_3^- buffer, pH 7, excitation at 267 nm, emission at 593 nm, time delay = 0.1 ms, T = 20° C. The presence of the competing cations does not influence the emission spectra of the probe Eu-KPhen in the presence of K^+. See text for details.

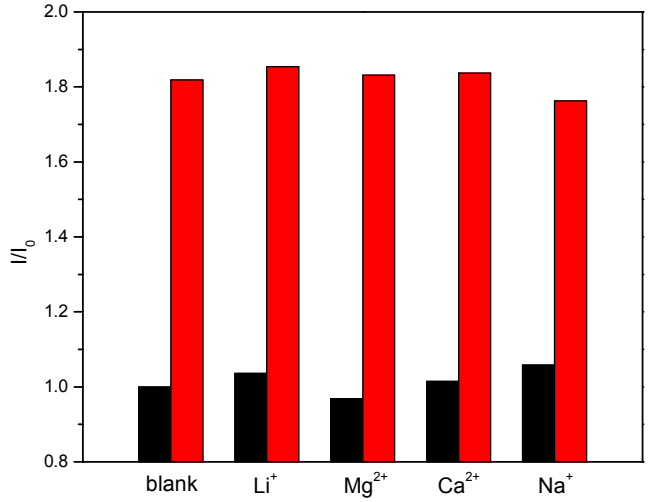


Figure S3. Selectivity of Eu-KPhen to various physiological cations. Black bars represent the time-delayed relative luminescence intensity after addition of an excess of the appropriate cation (107 mM NaOAc, 50.0 mM LiOAc, 0.83 mM $Mg(OAc)_2$ and 2.47 mM $Ca(OAc)_2$). Red bars represent the time-delayed relative luminescence intensity after subsequent addition of 20 mM K^+. Experimental conditions: [Eu-KPhen] = 50.0 μM, 25 mM aqueous HCO_3^- buffer, pH 7, excitation at 267 nm, emission at 593 nm, time delay = 0.1 ms, T = 20° C.

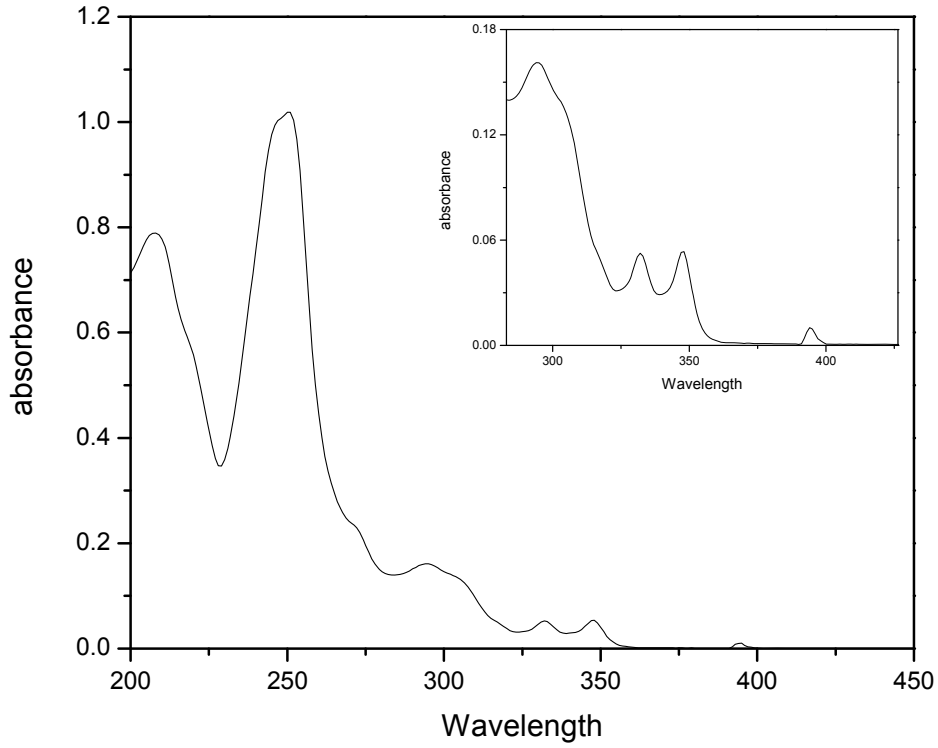


Figure S4. UV-visible absorption spectra of Eu-KPhen in mQ water (25 μM, 20° C). Inset is a zoom of the region from 275 nm – 425 nm.