Concentration-Independent pH Detection with a Luminescent Dimetallic Eu(III)-Based Probe

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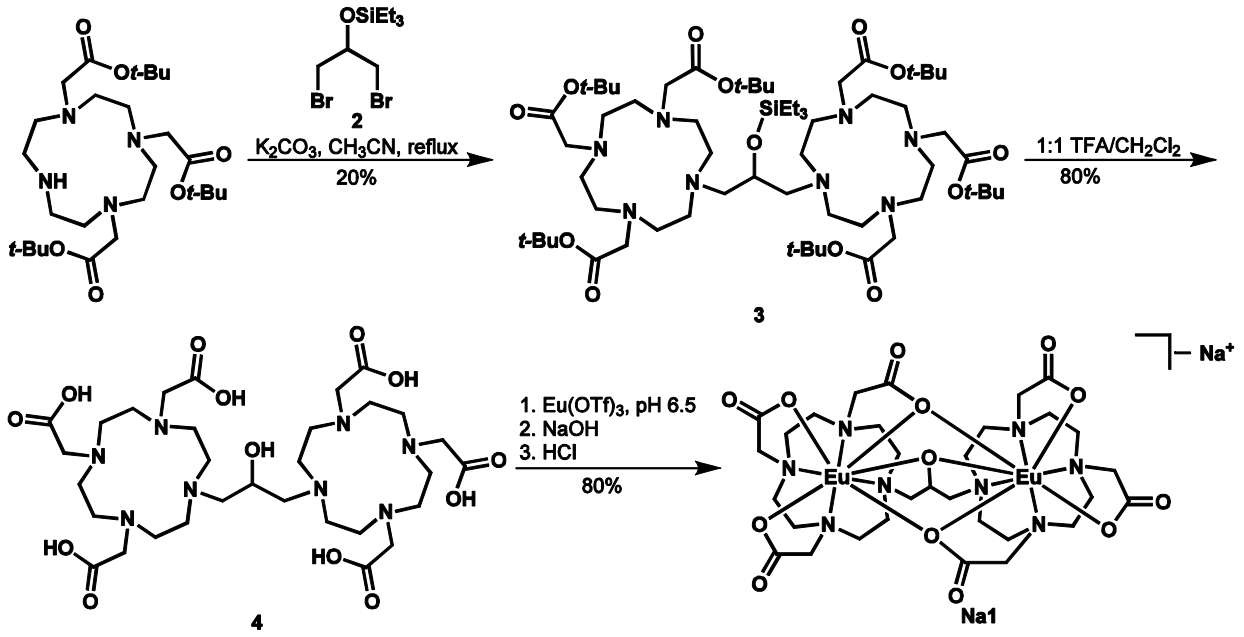
Experimental Procedures

Commercially available chemicals were of reagent-grade purity or better and were used without further purification unless otherwise noted. Water was purified using a PURELAB Ultra Mk2 water purification system (ELGA). Tri-*tert*-butyl-2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (DO3A-*t*-butyl ester) was prepared according to a published procedure.[1]

Flash chromatography was performed using silica gel 60, 230–400 mesh (EMD Chemicals). Analytical thin-layer chromatography (TLC) was carried out on ASTM TLC plates precoated with silica gel 60 F_{254} (250 μm layer thickness). TLC visualization was accomplished by charring with potassium permanganate stain (3 g $KMnO_4$, 20 g K_2CO_3, 5 mL 5% w/v aqueous NaOH, 300 mL H_2O) or by charring with ceric ammonium molybdate stain (4 g cerium(IV) sulfate hydrate complex with sulfuric acid, 100 g ammonium molybdate tetrahydrate, 900 mL H_2O, 100 mL concd H_2SO_4).

^{1}H NMR spectra were obtained using a Varian Unity 400 (400 MHz) spectrometer, and ^{13}C NMR spectra were obtained using a Varian Unity 400 (101 MHz) spectrometer. Chemical shifts are reported relative to residual solvent signals ($CDCl_3$: ^{1}H: δ 7.26, ^{13}C: δ 77.23; H_2O: ^{1}H: δ 4.79). ^{1}H NMR data are assumed to be first order, and the apparent multiplicity is reported as "t" = triplet, "q" = quartet, "quin" = quintet, and "m" = multiplet. Italicized elements are those that are responsible for the shifts. High-resolution electrospray ionization mass spectra (HRESIMS) were obtained using an electrospray time-of-flight high-resolution Waters Micromass LCT Premier XE mass spectrometer.



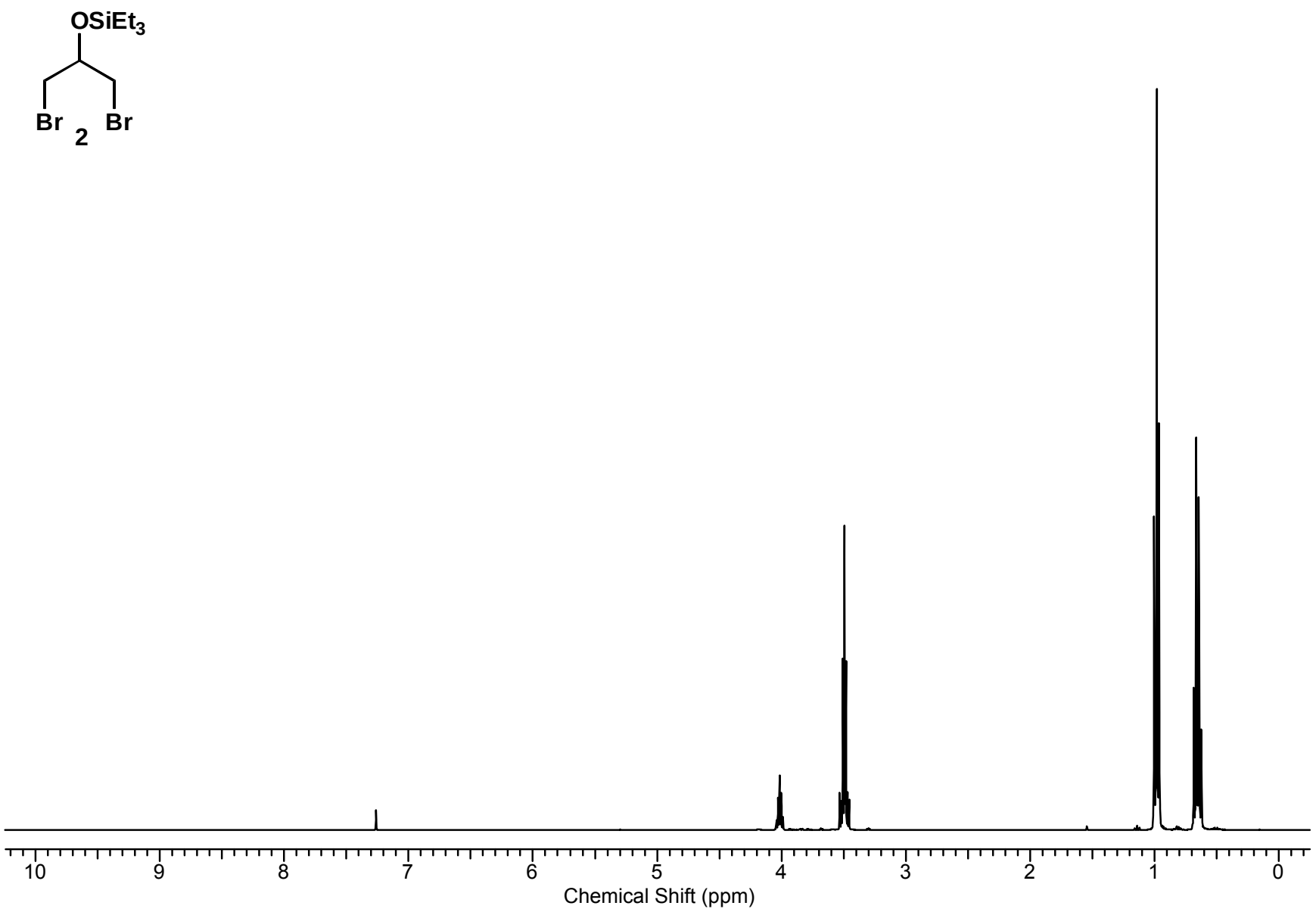
[(1,3-Dibromopropan-2-yl)oxy]triethylsilane (2): To a solution of 1,3-dibromopropan-2-ol (3.0 mL, 6.4 g, 0.030 mol, 1 equiv), pyridine (4.8 mL, 4.6 g, 59 mmol, 2 equiv), and anhydrous dichloromethane (290 mL) under Ar at at 0 °C was added triethylsilyl trifluoromethanesulfonate (12 mL, 15 g, 55 mmol) at a rate of 3 mL/h via syringe pump. The resulting reaction mixture was allowed to warm to ambient temperature after 6 h at 0 °C. Stirring was continued at ambient temperature for 2 h. Volatiles were removed under reduced pressure, and the resulting residue was purified by silica gel chromatography (hexanes) to yield 9.45 g (97%) of **2** as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 0.65 (q, J = 8.0 Hz, SiCH_2, 6H), 0.98 (t, J = 8.0 Hz, CH_3, 9H), 3.44–3.54 (m, BrCH_2, 4H), 4.01 (quin, J = 5.2 Hz, CH, 1H); ^{13}C NMR (101 MHz, $CDCl_3$, δ): 5.1 (*C*H_2), 7.0 (*C*H_3), 35.7 (*C*H_2), 71.3 (*C*H); TLC: R_f = 0.35 (hexanes). Anal. Calcd for $C_9H_{20}Br_2OSi$: C, 32.54; H, 6.07; N, 0.00. Found: C, 32.62; H, 5.96; N, 0.00.

{[1,3-Di(DO3A-*t*-butyl ester)-2-yl]oxy}triethylsilane (3): A mixture of DO3A-tris-*t*-butyl ester (10.01 g, 19.43 mmol, 2.2 equiv), **2** (2.93 g, 8.83 mmol, 1 equiv), and K_2CO_3 (6.12 g, 0.440 mol,

5 equiv) in anhydrous acetonitrile (80 mL) under Ar was heated at reflux. The reaction mixture turned yellow–brown after 2 h and was stirred at reflux for 72 h. The reaction mixture was cooled to ambient temperature and filtered through Whatman 4 paper to remove inorganic solids. The filtrate was concentrated under reduced pressure leaving a viscous yellow residue. The residue was dissolved in $CHCl_3$ (80 mL), and the resulting organic phase was washed with saturated aqueous $NaHCO_3$ (3 × 80 mL). The organic layer was concentrated under reduced pressure to ~10 mL and purified by silica gel chromatography (stepwise gradient $CHCl_3$ to 9:2 $CHCl_3$/MeOH) to yield 2.00 g (20%) of **3** as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 0.46–0.66 (m, SiCH_2, 6H), 0.77–0.97 (m, CH_2CH_3, 9H), 1.16–1.61 (m, CH_3, 54H), 2.02–4.58 (m, 52H); ^{13}C NMR (101 MHz, $CDCl_3$, δ): 5.6 (CH_2), 5.7 (CH_2), 5.9 (CH_2), 7.0–7.3 (m, CH_3), 27.8–28.5 (m, CH_3), 49.5–53.0 (m, CH_2), 55.7–57.4 (m, CH_2), 66.2–66.9 (m, CH), 80.0–83.2 (m), 169.1–171.1 (m), 172.7; TLC: R_f = 0.44 (9:2 $CHCl_3$/MeOH); HRESIMS (*m*/*z*): $[M + H]^+$ calcd for $C_{61}H_{119}N_8O_{13}Si$, 1199.8666; found, 1199.8649.

1,3-Di[1,4,7,10-tetraazacyclododecanyl(1,4,7-triacetate)]propan-2-ol (4): To a solution of **3** (1.58 g, 1.32 mmol, 1 equiv) in CH_2Cl_2 (10 mL) was added a solution of water (0.5 mL) in trifluoroacetic acid (TFA) (10 mL). The resulting solution was stirred for 24 h. The solution was concentrated under reduced pressure, and the resulting residue was dissolved in methanol (2 mL) and added to diethyl ether (11 mL) in a centrifuge tube. The product precipitated as an off-white solid and was centrifuged and washed with diethyl ether (5 × 11 mL). Solvent was removed under reduced pressure to yield 1.15 g (80%) of **4** • 2.7 TFA • 2 H_2O**.** ^{1}H NMR (400 MHz, D_2O, 45 °C, δ): 2.75–4.17 (m, CH_2, 48H), 4.50–4.62 (m, CH, 1H); HRESIMS (*m*/*z*): $[M + Na]^+$ calcd for $C_{31}H_{56}N_8NaO_{13}$, 771.3865; found, 771.3878; Anal. Calcd for $C_{31}H_{56}N_8O_{13}$ • 2.7 $C_2HF_3O_2$ • 2 H_2O: C, 40.01; H, 5.78; N, 10.25. Found: C, 39.76; H, 5.73; N, 10.58.

Sodium{1,3-bis[europium(1,4,7,10-tetraazacyclododecanyl(1,4,7-triacetate))]propan-2-oxide} (Na**1):** A solution of **4** • 2.7 TFA • 2 H_2O (103 mg, 0.0945 mmol, 1 equiv) in water (10 mL) was adjusted to pH 6.5 with 1 M NaOH. An aqueous solution of $Eu(OTf)_3$ (5.0 mL, 52 mM, 2.7 equiv) was added to the solution of **4**. The pH of the solution was maintained at 6.5 with 1 M NaOH while stirring for 18 h. Upon observation of constant pH for 6 h, the pH of the solution was adjusted to 11 with 1 M NaOH to precipitate excess europium. The solution was filtered through a 0.2 μm syringe filter, neutralized to pH 7 with 1 M HCl, and dialyzed in 500 Da molecular weight cut-off tubing against water. The dialysate was transferred to a vial and concentrated under reduced pressure to yield 107 mg (80%) of **1**Na • 0.5 NaOTf • 3 H_2O as a white solid. HRESIMS (*m*/*z*): $[M]^-$ calcd for $Eu_2C_{31}H_{49}N_8O_{13}$, 1043.1816; found, 1043.1830. Anal. Calcd for $Eu_2C_{31}H_{49}N_8O_{13}Na \cdot 0.5\ CF_3SO_3Na \cdot 3\ H_2O$: C, 31.30; H, 4.58; N, 9.27. Found: C, 31.43; H, 4.81; N, 9.04.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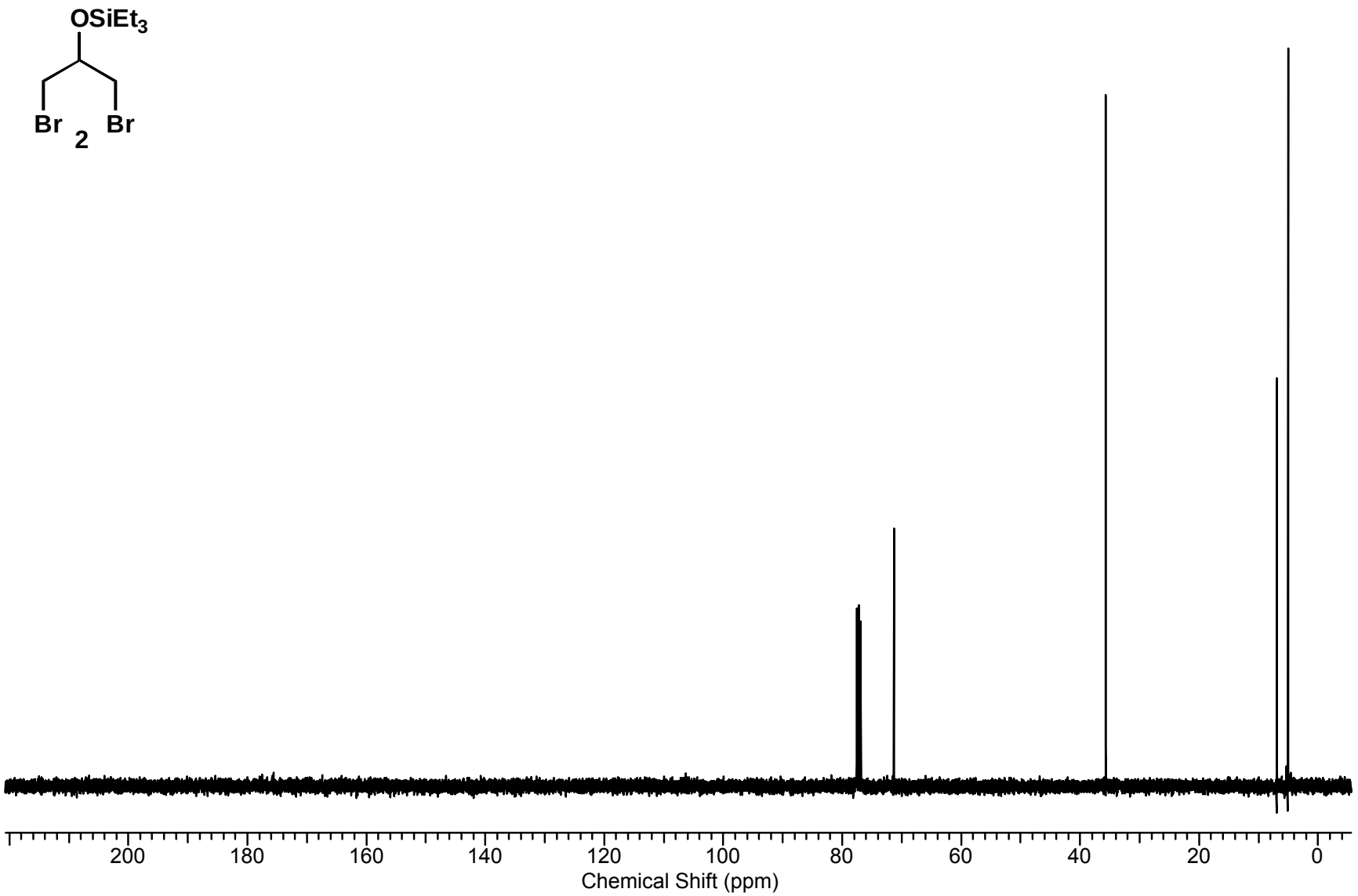
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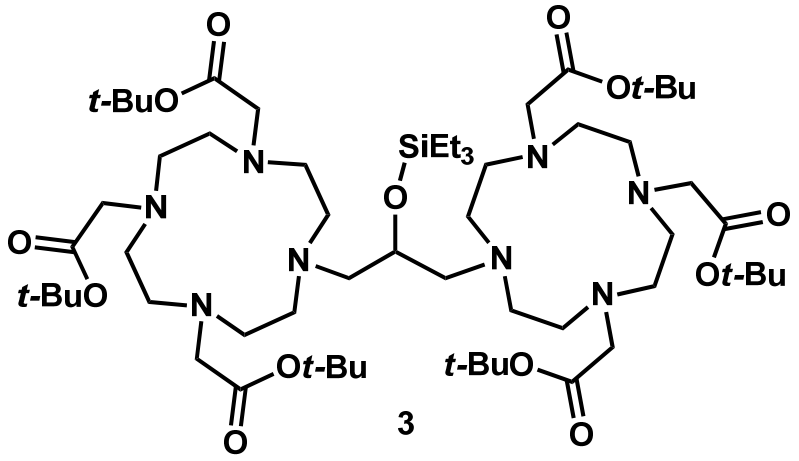
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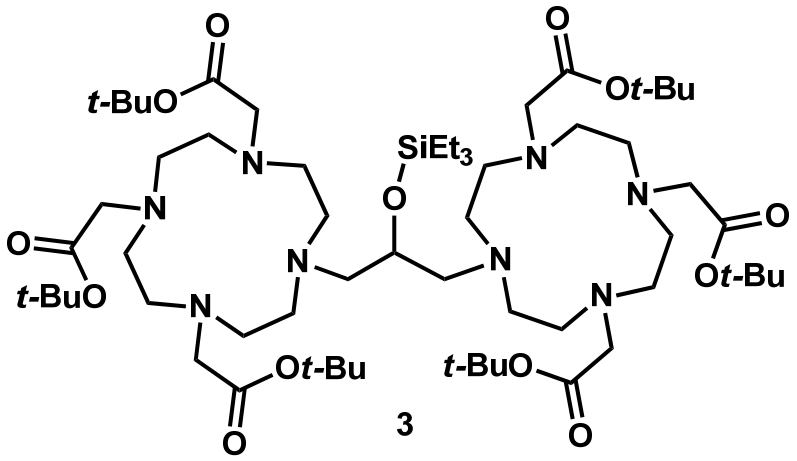
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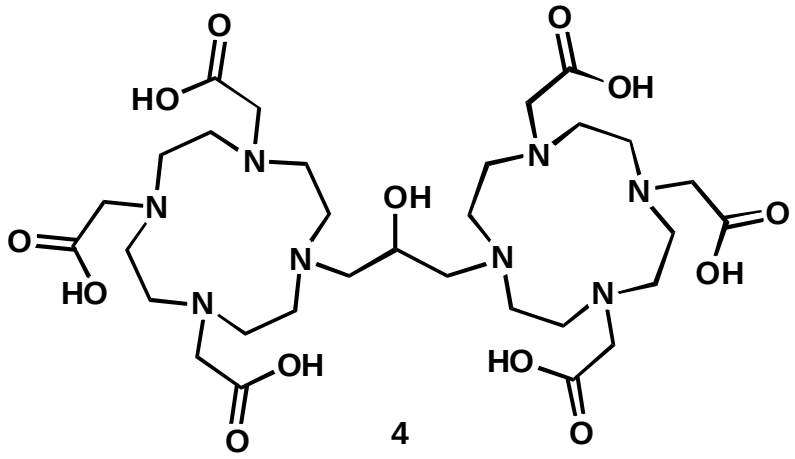
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9
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7
6
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Chemical Shift (ppm)

Determination of the *q* Value of 1

Luminescence-decay measurements were acquired using a HORIBA Jobin Yvon Fluoromax-4 spectrofluorometer in decay by delay scan mode using the phosphorescence lifetime setting. All decay measurements were acquired with a 395 nm excitation wavelength, a 595 nm emission wavelength, excitation and emission slit widths of 5 nm, a flash count of 100, an initial delay of 0.01 ms, a max delay of 3 ms, and a delay increment of 0.1 ms. The natural log plot of the intensity was plotted against time, and the slope was used as the decay rate (Figure S1). The decay rates of **1** in H_2O (pH 5.4) and D_2O were used in eq 1, which was derived by Horrocks and coworkers to calculate q.[2] We accounted for quenching due to an inner-sphere hydroxyl oscillator that we expected to be present at pH 5.4 by subtracting the term $0.45n_{OH}$, where n=1.[2]

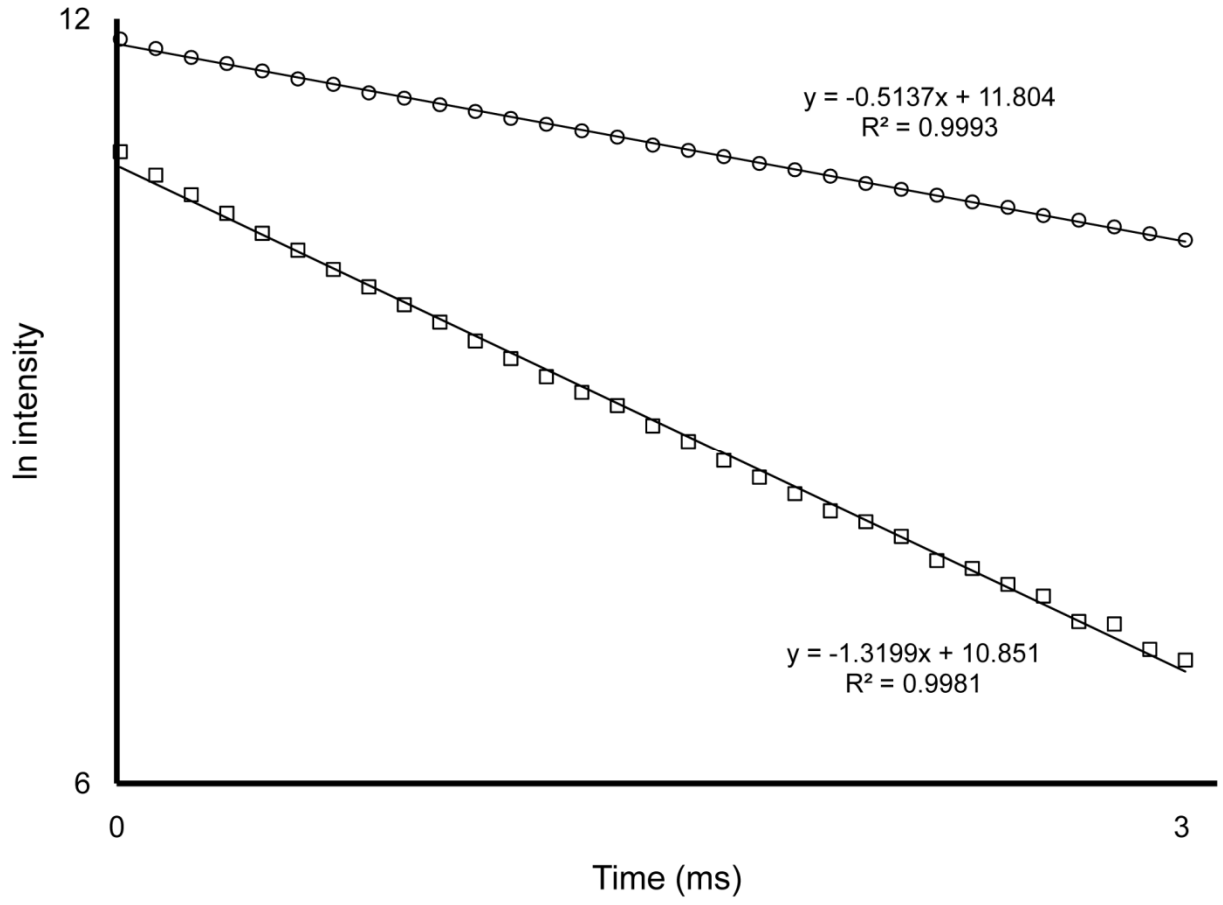


Figure S1. Natural log plot of luminescence intensity of **1** in H_2O (□) and D_2O (○) as a function of time. The luminescence-decay rates were calculated as the slopes of the fitted lines.

eq 1: $q = 1.11(\left|\tau_{H_2O}^{-1} - \tau_{D_2O}^{-1}\right| - 0.31 - 0.45n_{OH})$

Luminescence-Decay Measurements and Spectra of 1 at Different pH Values

Buffer solutions (50 mM citrate and 50 mM phosphate) were prepared from pH 4 to 8 by 0.2 pH units for a total of 21 buffer solutions. Each sample was prepared by mixing **1 (**18 μL, of a 0.50 or1.0 mM in water) with buffer (232 μL). The luminescence decay of each sample was measured within 5 min of sample preparation. All decay measurements were acquired with a 395 nm excitation wavelength, a 595 nm emission wavelength, excitation and emission slit widths of 5 nm, a flash count of 100, an initial delay of 0.01 ms, a max delay of 3 ms, and a delay increment of 0.1 ms. The natural log plot of the intensity was plotted against time, and the slope was used as the decay rate. Spectra were acquired on each of the 21 samples with an excitation wavelength of 395 nm, excitation slit width of 5 nm, and emission slit width of 1 nm.

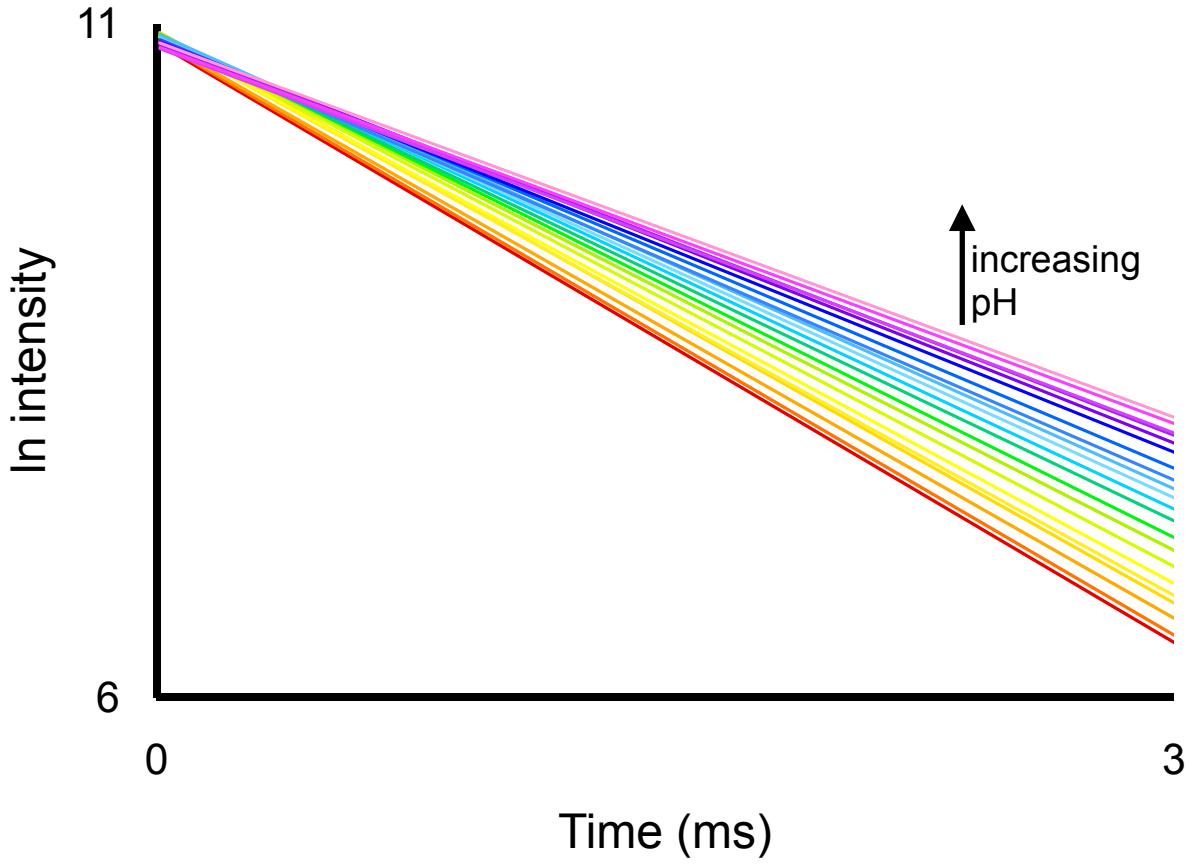


Figure S2. Natural log plots of luminescence intensity of **1** in 50 mM citrate and 50 mM phosphate buffer at pH values of 4 (red) through 8 (pink) by 0.2 pH units.

Table S1. Luminescence-decay rates (τ^{-1}) of **1** at different pH values.

	1 (0.50 mM)	**1** (1.0 mM)
pH	τ^{-1} (ms^{-1})	τ^{-1} (ms^{-1})
4.0	1.48	1.49
4.2	1.47	1.48
4.4	1.43	1.45
4.6	1.42	1.42
4.8	1.39	1.39
5.0	1.36	1.35
5.2	1.32	1.33
5.4	1.28	1.28
5.6	1.25	1.25
5.8	1.20	1.20
6.0	1.18	1.17
6.2	1.15	1.14
6.4	1.13	1.12
6.6	1.09	1.09
6.8	1.07	1.07
7.0	1.01	1.02
7.2	0.998	1.01
7.4	0.972	0.978
7.6	0.955	0.950
7.8	0.934	0.948
8.0	0.929	0.930

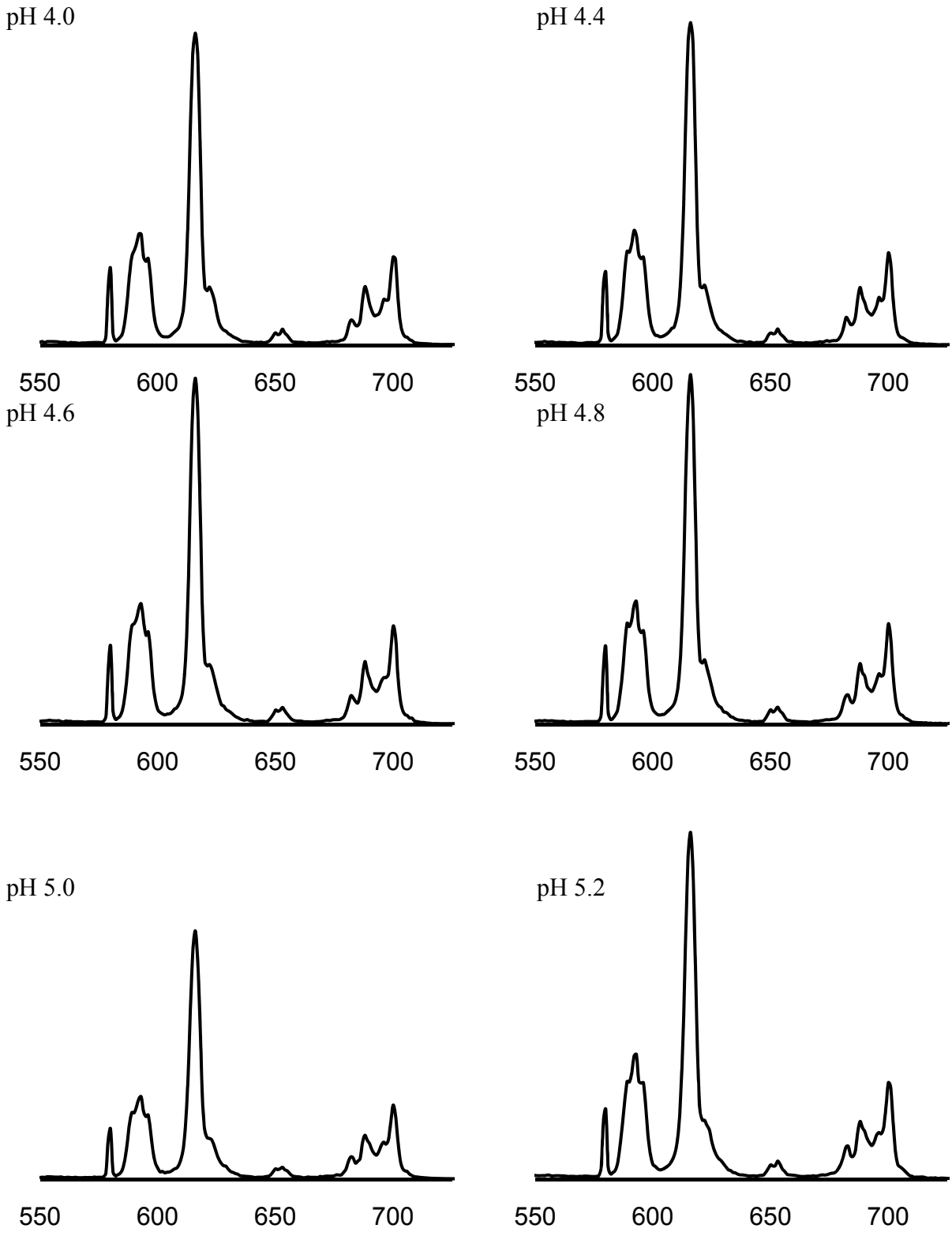


Figure S3. Emission spectra of **1.**

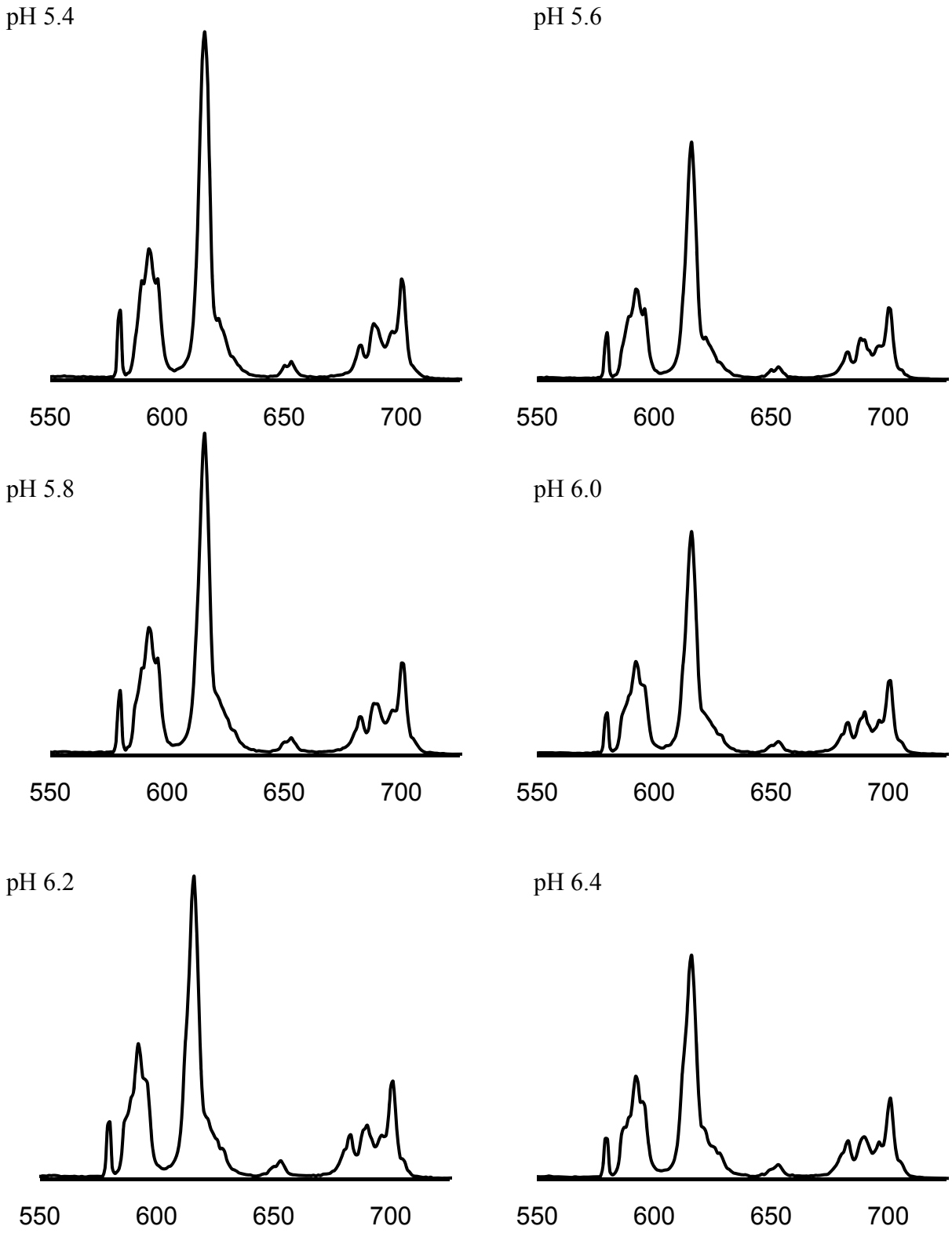


Figure S3 cont'd. Emission spectra of **1**.

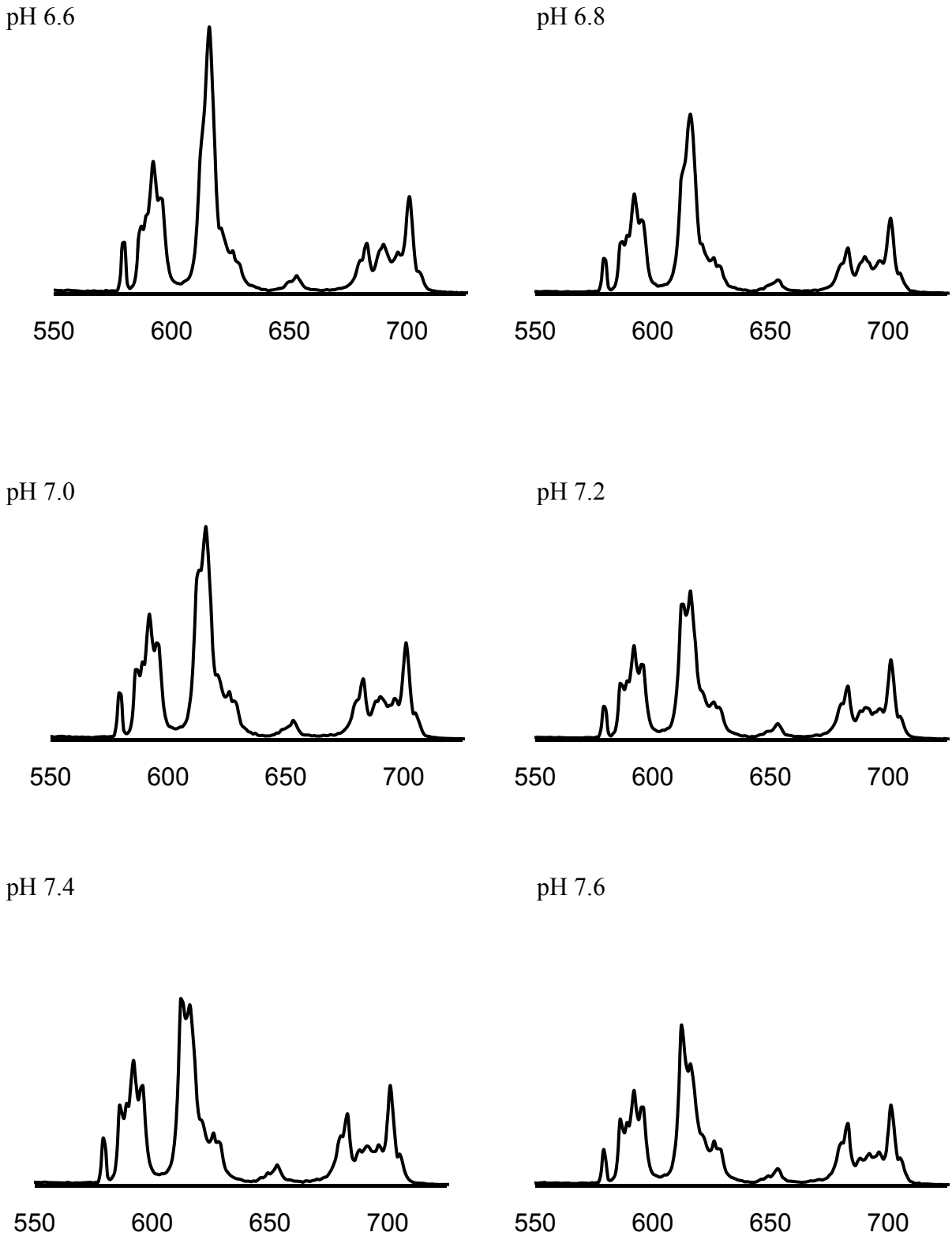
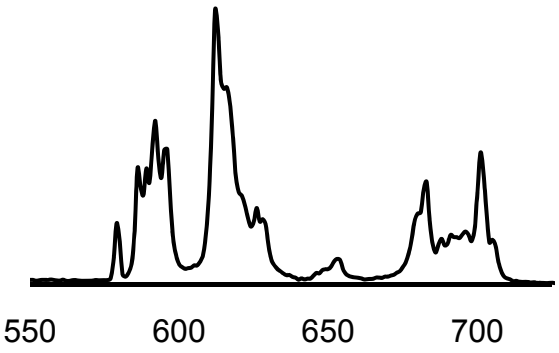


Figure S3 cont'd. Emission spectra of **1**.

pH 7.8



pH 8.0

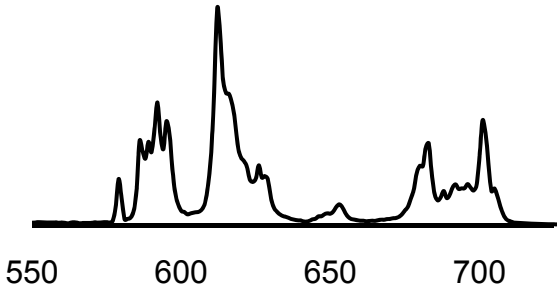


Figure S3 cont'd. Emission spectra of **1**.

Optimized Computational Structure

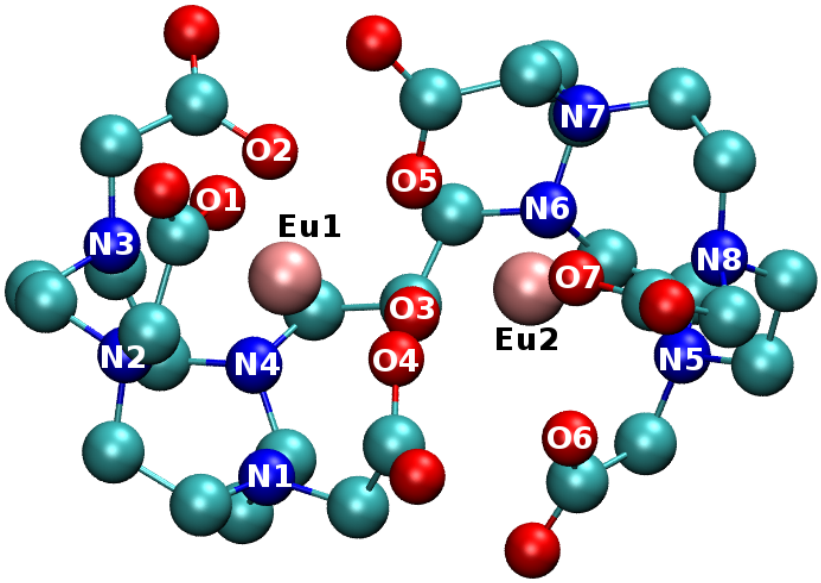


Figure S4. Labels for atoms corresponding to distances in Table S2. The figure corresponds to **1** (see text).

Table S2. Distances between the Eu(III) ions and the first coordination sphere (in Å). Distances between the Eu(III) ions and the bridging hydroxyl O have been bolded.

Distance	**1**	H**1**
Eu1-N1	2.98	2.82
Eu1-N2	2.96	2.84
Eu1-N3	2.91	2.75
Eu1-N4	2.84	2.84
Eu1-O1	2.34	2.31
Eu1-O2	2.38	2.40
Eu1-O3	**2.40**	**2.45**
Eu1-O4	2.42	2.44
Eu1-O5	2.49	2.48
Eu2-O3	**2.36**	**2.91**
Eu2-O4	2.52	2.56
Eu2-O5	2.40	2.34
Eu2-O6	2.33	2.25
Eu2-O7	2.33	2.44
Eu2-N5	2.93	2.95
Eu2-N6	3.26	3.07
Eu2-N7	3.14	2.97
Eu2-N8	2.73	2.71

Table S3. Optimized Cartesian coordinates for **1**.

7	-4.363586	-1.308194	-0.561580
7	-4.293900	1.598668	0.406659
8	-1.928546	-1.756388	-1.761164
8	-2.816247	1.530057	-1.829745
6	-5.548558	-0.538554	-0.106082
1	-5.694288	-0.756751	0.966561
1	-6.471270	-0.893400	-0.623507
6	-5.436393	0.971076	-0.315950
1	-5.307331	1.182090	-1.388084
1	-6.399943	1.442091	-0.009436
6	-4.549963	1.706595	1.862079
1	-5.016362	0.763387	2.194361
1	-5.285507	2.517376	2.080326
6	-4.302512	-2.629946	0.125685
1	-3.707899	-3.311930	-0.499810
1	-5.316473	-3.085768	0.202174
6	-4.300763	-1.474883	-2.047442
1	-4.309071	-0.475883	-2.515250
1	-5.156685	-2.066863	-2.431489
6	-2.981293	-2.176286	-2.438573
6	-3.957250	2.906278	-0.223214
1	-3.148276	3.393477	0.346230
1	-4.827346	3.595288	-0.244652
6	-3.454871	2.672999	-1.659369
7	-2.257977	0.894722	2.570633
7	-2.311327	-2.016520	1.560254
8	-0.256168	1.678251	0.664366
8	0.066839	-1.034738	0.662808
6	-2.618236	-0.294009	3.387700
1	-3.714132	-0.415702	3.348935
1	-2.360156	-0.124524	4.457511
6	-1.919098	-1.577830	2.930802
1	-0.833729	-1.406994	2.933763
1	-2.134613	-2.381734	3.672748
6	-3.701652	-2.542923	1.531413
1	-4.324025	-1.881610	2.156411
1	-3.746836	-3.555716	1.998936
6	-3.284385	1.970422	2.677845
1	-2.809213	2.907236	2.346021
1	-3.573043	2.132698	3.741988
6	-0.925920	1.451939	2.963589
1	-0.215309	0.633378	3.154838
1	-1.007168	2.055854	3.888744
6	-0.362856	2.317989	1.836778
6	-1.357871	-3.012958	0.998240

Table S3 cont'd. Optimized Cartesian coordinates for **1**.

1	-1.714909	-3.273225	-0.008395
1	-1.342146	-3.937174	1.622547
6	0.082941	-2.449465	0.866643
8	-2.991151	-3.093510	-3.313446
8	-0.060672	3.523345	2.031135
8	-3.678296	3.535307	-2.556073
63	-1.831590	0.098782	-0.267848
1	0.634101	-2.659108	1.809990
6	0.790175	-3.123976	-0.317744
7	2.210890	-2.684347	-0.421139
1	0.244578	-2.812404	-1.222344
1	0.727548	-4.235961	-0.243359
6	2.741694	-2.837943	-1.799176
6	3.055203	-3.395538	0.570932
1	3.843268	-2.843067	-1.735711
1	2.453570	-3.827102	-2.224911
6	2.269436	-1.758619	-2.775440
6	4.340212	-2.655841	0.965195
1	2.439896	-3.556533	1.469935
1	3.334084	-4.410521	0.200191
7	2.722466	-0.369008	-2.443824
1	1.169104	-1.744971	-2.809306
1	2.618365	-2.034445	-3.796342
7	4.085855	-1.337879	1.578699
1	4.963086	-2.503090	0.068193
1	4.928524	-3.324958	1.640500
6	4.195621	-0.226991	-2.628552
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6	5.257844	-0.435084	1.567337
6	3.475805	-1.414339	2.925588
6	4.803679	0.995109	-1.936405
1	4.673266	-1.140799	-2.238637
1	4.441686	-0.177303	-3.714105
1	2.330773	1.622630	-3.078992
1	2.136619	0.390146	-4.377796
6	0.461736	0.577227	-3.015149
6	5.658289	0.058018	0.173876
1	4.998697	0.427916	2.200526
1	6.148818	-0.926250	2.032090
1	2.975530	-2.386099	3.054326
1	4.243686	-1.356763	3.728148
6	2.408492	-0.329484	3.186818
7	4.659671	0.959934	-0.451857
1	4.314218	1.910957	-2.299677
1	5.876357	1.068727	-2.229338

Table S3 cont'd. Optimized Cartesian coordinates for **1**.

8	0.160872	0.402093	-1.730102
8	-0.352005	0.733157	-3.957985
1	5.812821	-0.801690	-0.500102
1	6.647054	0.568834	0.254735
8	2.304539	0.615517	2.282864
8	1.692278	-0.448416	4.232002
6	4.737342	2.336525	0.118104
63	1.817000	0.403934	0.010311
1	4.634658	2.270149	1.214348
1	5.705568	2.827184	-0.115753
6	3.585922	3.208968	-0.413241
8	2.471907	2.539654	-0.645033
8	3.769346	4.444524	-0.601403

Table S4. Optimized Cartesian coordinates for H**1**.

7	-4.274561	0.008397	1.404609
7	-4.191426	-1.107736	-1.467416
8	-1.757151	-0.087280	2.394832
8	-2.255979	-2.482853	-0.291067
6	-5.474065	-0.327097	0.584246
1	-5.800593	0.601525	0.083295
1	-6.316861	-0.642690	1.238869
6	-5.240615	-1.426440	-0.450601
1	-4.927504	-2.353479	0.054741
1	-6.208150	-1.649034	-0.954170
6	-4.619575	-0.070656	-2.444629
1	-5.191215	0.699353	-1.900312
1	-5.311890	-0.501730	-3.201665
6	-4.432625	1.356733	2.038121
1	-3.799184	1.385683	2.935739
1	-5.477320	1.497596	2.391072
6	-3.970665	-1.023205	2.454806
1	-3.817949	-2.001852	1.968108
1	-4.794212	-1.111654	3.189470
6	-2.663561	-0.641934	3.178039
6	-3.769675	-2.369095	-2.145369
1	-3.183875	-2.126400	-3.046417
1	-4.642523	-2.971311	-2.464854
6	-2.871309	-3.192938	-1.212440
7	-2.513531	1.341910	-2.282778
7	-2.670927	2.465006	0.596130
8	-0.200944	-0.237257	-1.734755
8	-0.251120	1.566034	0.164569

Table S4 cont'd. Optimized Cartesian coordinates for H**1**.

6	-3.137658	2.644645	-1.883843
1	-4.212053	2.464989	-1.715770
1	-3.071254	3.371340	-2.722697
6	-2.516198	3.288006	-0.640782
1	-1.443945	3.457588	-0.809089
1	-2.978852	4.291321	-0.501137
6	-4.072077	2.514381	1.105668
1	-4.742625	2.507705	0.229661
1	-4.266250	3.471564	1.640896
6	-3.434619	0.564476	-3.172089
1	-2.826138	-0.215174	-3.658220
1	-3.818487	1.217196	-3.987319
6	-1.223892	1.563811	-3.011514
1	-0.717183	2.446159	-2.585705
1	-1.409213	1.777789	-4.081405
6	-0.210284	0.414427	-2.899925
6	-1.686819	2.852234	1.654732
1	-1.917450	2.249800	2.543832
1	-1.796954	3.928310	1.913107
6	-0.218856	2.566124	1.220146
8	-2.545276	-0.838199	4.422012
8	0.601255	0.222657	-3.840753
8	-2.757543	-4.440817	-1.367831
63	-1.925985	-0.213769	-0.001251
1	0.226302	3.482147	0.786265
6	0.629476	2.020863	2.376895
7	2.012964	1.581089	1.979646
1	0.092021	1.142720	2.769217
1	0.695027	2.783208	3.187235
6	2.597670	0.780110	3.103549
6	2.881411	2.756020	1.659372
1	3.694759	0.807275	3.008552
1	2.359835	1.260745	4.077786
6	2.114038	-0.669434	3.151562
6	4.243589	2.423407	1.029145
1	2.318415	3.425545	0.994431
1	3.087689	3.339405	2.586709
7	2.582028	-1.498837	1.989375
1	1.013356	-0.696834	3.164531
1	2.455257	-1.128009	4.104645
7	4.214902	1.796540	-0.325369
1	4.805909	1.751600	1.696732
1	4.821039	3.376279	0.988062
6	4.024487	-1.857892	2.135752
6	1.731359	-2.730088	1.889842

Table S4 cont'd. Optimized Cartesian coordinates for H**1**.

6	5.520095	1.145396	-0.639806
6	3.852907	2.797427	-1.373205
6	4.690323	-2.286068	0.827880
1	4.550046	-0.985159	2.558624
1	4.139920	-2.680449	2.875180
1	2.099527	-3.355902	1.059453
1	1.769170	-3.321672	2.824408
6	0.277906	-2.350223	1.597458
6	5.747016	-0.171737	0.108882
1	5.536439	0.958874	-1.725565
1	6.375310	1.824367	-0.420516
1	4.377474	3.761599	-1.221960
1	4.151180	2.391352	-2.354385
6	2.347300	3.041786	-1.429273
7	4.733188	-1.211097	-0.209088
1	4.144385	-3.137978	0.393007
1	5.717286	-2.650559	1.055155
8	0.158873	-1.311670	0.760908
8	-0.666155	-2.976562	2.126653
1	5.721571	0.005946	1.196382
1	6.774490	-0.532458	-0.123354
8	1.639839	1.921261	-1.354582
8	1.852631	4.199058	-1.525422
6	4.955920	-1.820810	-1.550739
63	1.944126	-0.272259	-0.340350
1	5.179161	-1.022595	-2.278327
1	5.814280	-2.521947	-1.550366
6	3.693863	-2.552993	-2.039913
8	2.562617	-2.069308	-1.553873
8	3.791550	-3.510287	-2.851924
1	0.390473	1.847134	-0.608756

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