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Synthesis and Luminescence Studies of Aryl Substituted Tetraamide Complexes of Europium(III): A New Approach to pH Responsive Luminescent Europium Probes

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DOTA-tetraamide ligands having extended phenol or pyridine substituents have been synthesized. The $^5D_0 \rightarrow {}^7F_J$ $\Delta J = 1$ and 2 emission bands in the corresponding europium(III) complexes differ in their sensitivity to solution pH. This offers the potential for developing pH responsive probes for in vivo imaging that are independent of probe concentration.

Introduction

Luminescent probes that signal changes in pH are of current interest in the analysis of biological and environmental samples.[1] The delayed luminescence of lanthanide ions allows time-gating techniques to be employed in monitoring emission, obviating problems from biological background, autofluorescence, and Raleigh scattering.[2,3] The f−f transitions of the lanthanide ions are LaPorte forbidden and therefore low in intensity. However, the emission sensitivity may be enhanced considerably by exciting the lanthanide ion via energy transfer from an aromatic chromophore,[4] and the use of chromophores capable of sensing pH provides the basis for a number of pH responsive luminescent lanthanide complexes.[5−8] Traditionally, these complexes have operated in one of two ways, by selective excitation of either the protonated or deprotonated form of the chromophore[5] or, alternatively, if the triplet state of one form of the chromophore lies close enough in energy (within 1500 cm^{-1}) to the lanthanide ion excited state, then nonselective excitation may be employed and back energy transfer from the metal to the triplet state of the chromophore will diminish emission intensity in response to changes in pH of the solution.[5] Parker and co-workers have recently described new probes that operate by pH selective dissociation of one ligating group. In this case, the resultant increased hydration and nonradiative quenching alters the emission intensity and, in the case of the terbium complex, the form of the spectrum.[7−9] Typically, the emission intensities of these complexes change over a range of ∼3 pH units, thereby allowing the pH of the sample to be determined provided that the concentration of the probe is known. As part of an ongoing investigation into pH responsive probes,[10−12] ligands **1**−**3** (Chart 1) were prepared and their Eu^{3+} complexes studied by luminescence spectroscopy, NMR, and cyclic voltammetry.

Experimental Section

General Remarks. All solvents and reagents were purchased from commercial sources and used as received unless otherwise stated. ^{1}H NMR spectra were recorded on a JEOL Eclipse 270 or

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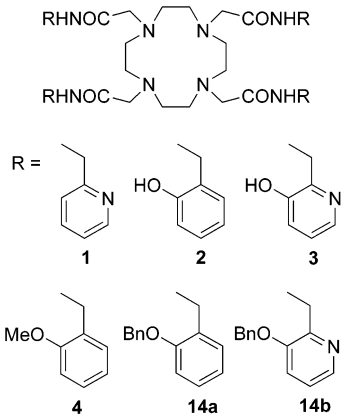
Chart 1



Varian Inova 500 spectrometer operating at 270 or 500 MHz, respectively. ^{13}C NMR spectra were recorded on a JEOL Eclipse 270 spectrometer operating at 67.5 MHz. Infrared spectra were recorded on a Nicolet Avatar 360 FTIR spectrophotometer. Mass spectra were acquired either on a VG70−250SE or Micromass Quattro II electrospray mass spectrometer (Northwest University). Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected.

Luminescence spectra were recorded on a Perkin-Elmer LS55 fluorometer with the slits set to 15 nm (ex) and 5 nm (em) for emission spectra and 5 nm (ex) and 15 nm (em) for excitation spectra. Lifetimes were measured on a Perkin-Elmer LS50B using the pHlemming software provided by Andrew Beeby. pH was measured using a Fisher Accumet 925 pH meter equipped with an Orion 8103 Ross combination pH electrode. The pH was altered by addition of solid lithium hydroxide monohydrate or *p*-toluenesulfonic acid. Cyclic voltammagrams were recorded on a BAS CV-50W voltametric analyzer using a glassy carbon working electrode, platinum wire auxiliary electrode, and Ag/AgCl reference electrode. Samples were prepared at 0.6 mM concentrations using a 50 mM solution of tetrabutylammonium perchlorate in acetonitrile and a buffer solution of either phosphate−citrate or tris buffer. CAUTION: Perchlorate salts may be explosive when dry.

Methyl Salicylate (7a). Salicylic acid (10.30 g, 75 mmol) was dissolved in methanol (150 mL), and after adding concentrated H_2SO_4 (3 mL), the solution was heated under reflux with stirring for 48 h. The solvents were then removed in vacuo, and K_2CO_3 was added until no further effervescence was observed. The residue was taken up into water (30 mL) and extracted with CH_2Cl_2 (2 × 200 mL). The organic extracts were combined and dried (Na_2SO_4) and the solvents removed in vacuo to afford the title compound as a colorless oil (10.62 g, 93%). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.84 (1H dd, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 2 Hz, 3-Ph), 7.46 (1H, ddd overlapping, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 2 Hz, 4-Ph), 6.99 (1H, dd, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 1 Hz, 6-Ph), 6.89 (1H, ddd overlapping, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 1 Hz, 5-Ph), 3.96 (3H, s, CH_3). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 52.3 (CH_3), 112.4 (1-Ph), 117.6 (5-Ph), 119.2 (4-Ph), 130.0 (3-Ph), 135.8 (6-Ph), 161.6 (2-Ph), 170.6 (C═O). ν_{max}/cm^{-1}: 3189 (OH), 2956, 1681 (C═O), 1615, 1586, 1486, 1441, 1305, 1253, 1216, 1158, 1090, 1033, 757, 701. *m*/*z* (EI+): 152 (7% $[M]^+$), 138 (12% $[M - CH_2]^+$), 120 (42% $[M - MeOH]^+$), 92 (100% $[M - HCO_2Me]^+$).

Methyl 2-Hydroxypicolinate (7b). The title compound, synthesized from 2-hydroxypicolinic acid according to the method used for **7a**, was obtained as a colorless solid (11.47 g, 98%). Mp = 75.5−76.5 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 10.66 (1H, s, OH), 8.30 (1H, dd, $^3J_{H-H}$ = 4 Hz, $^3J_{H-H}$ = 1 Hz, 6-Py), 7.44 (1H, dd, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 4 Hz, 5-Py), 7.42 (1H, dd, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 1 Hz, 4-Py), 4.08 (3H, s, OMe). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 53.5 (OMe), 126.8 (5-Py), 130.0 (6-Py), 130.4 (2-Py), 141.8 (4-Py), 159.2 (3-Py), 170.1 (C═O). ν_{max}/cm^{-1}: 3207 (OH), 3049, 3030, 2957, 1674 (C═O), 1445, 1368, 1305, 1216, 1102, 823, 732, 709. *m*/*z* (EI+): 153 (57% $[M]^+$), 123 (46% $[M - CH_2O]^+$), 93 (100% $[M - HCO_2Me]^+$).

2-Hydroxybenzoylamide (8a). Methyl salicylate, **7a** (8.10 g, 53 mmol), was dissolved in aqueous ammonia (150 mL), and the solution was stirred at 50 °C for 2 days. After removal of the solvent in vacuo, the residue was dissolved in water (30 mL) and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent removed in vacuo to afford the title compound as a colorless solid (7.68 g, 96%). Mp = 134.5−135.5 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 12.1 (1H, s, OH), 7.46 (1H, ddd overlapping, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 2 Hz, 6-Ph), 7.40 (1H, dd, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 2 Hz, 5-Ph), 7.02 (1H, dd, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 1 Hz, 4-Ph), 6.89 (1H, ddd overlapping, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 1 Hz, 3-Ph) 6.17 (2H, s br, NH_2). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 113.3 (1-Ph), 118.8 (5-Ph), 118.9 (4-Ph), 126.5 (6-Ph), 135.1 (3-Ph), 162.1 (2-Ph), 172.8 (C═O). ν_{max}/cm^{-1}: 3424 (NH), 3192, 1667 (C═O), 1629, 1589, 1491, 1448, 1425, 1361, 1254. *m*/*z* (EI+): 137 (29% $[M]^+$), 120 (37% $[M - OH]^+$), 92 (100% $[M - CONH_2]^+$).

2-Hydroxypicolinamide (8b). The title compound, synthesized from ester **7b** according to the method used for **8a**, was obtained as a colorless solid (10.13 g, 97%). Mp = 123−124.5 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 11.93 (1H, s, OH), 8.12 (1H, dd, $^3J_{H-H}$ = 4 Hz, $^3J_{H-H}$ = 2 Hz, 6-Py), 7.89 (1H, s br, NH), 7.39 (1H, dd, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 4 Hz, 5-Py), 7.35 (1H, dd, $^3J_{H-H}$ = 8, Hz $^3J_{H-H}$ = 2 Hz, 4-Py), 5.75 (1H, s br, NH). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 125.9 (5-Py), 129.2 (4-Py), 129.3 (2-Py), 139.0 (6-Py), 158.3 (3-Py), 171.7 (C═O). ν_{max}/cm^{-1}: 3391 (OH/NH), 3198, 1693 (C═O), 1594, 1453, 1437, 1300, 1262, 808, 777. *m*/*z* (EI+): 138 (73% $[M]^+$), 93 (48% $[M - CONH_2]^+$).

2-Hydroxybenzylamine Hydrochloride (9a). Amide **8a** (5.35 g, 39 mmol) was dissolved into BH_3·THF (1 M, 200 mL) under argon. This solution was maintained at reflux with stirring for 48 h. Remaining borane was then quenched by dropwise addition of methanol. The solvents were then removed in vacuo and methanol (2 × 50 mL) added and removed under reduced pressure. The residue was dissolved in HCl (2 M, 30 mL) and heated under reflux for 18 h. The solvents were removed under reduced pressure to afford the title compound as sticky gum (6.30 g, quantitative yield). Mp = 147−149.5 °C. ^{1}H NMR (270 MHz, D_2O): δ = 7.28 (2H, m, Ar), 6.92 (2H, m, Ar), 4.11 (2H, s, ArCH_2NH$_2$). ^{13}C NMR (67.5 MHz, D_2O): δ = 39.7 (ArCH_2NH$_2$), 115.7 (4-Ph), 119.3 (1-Ph), 120.7 (6-Ph), 131.1 (5-Ph), 131.2 (3-Ph), 154.9 (2-Ph). ν_{max}/cm^{-1}: 3044 br (NH), 2987, 1599, 1505, 1459, 1380, 1246, 1185, 1123, 755. *m*/*z* (EI+): 123 (84% $[M]^+$), 106 (50% $[M - OH]^+$), 78 (100% $[M - O - CH_2NH_2]^+$).

2-Hydroxymethylaminopyridine Dihydrochloride (9b). The title compound, synthesized from amide **8b** according to the method used for **9a**, was obtained as a colorless solid (14.22 g, quantitative yield). This decomposes at 225 °C. ^{1}H NMR (270 MHz, D_2O): δ = 7.86 (3H, m, Ar), 4.28 (2H, s, ArCH_2NH$_2$). ^{13}C NMR (67.5 MHz, D_2O): δ = 36.8 (ArCH_2NH$_2$), 129.1 (5-Py), 133.2 (4-Py), 133.3 (2-, 3-Py), 155.5 (3-Py). ν_{max}/cm^{-1}: 3400 br (OH/NH), 2887, 2656, 1565, 1476, 1422, 1315, 1173, 1085, 989, 801, 748. *m*/*z* (EI+): 124 (100% $[M]^+$), 96 (48% $[M - CHNH_2]^+$).

***N-tert*-Butoxycarbonyl-2-hydroxybenzylamine (10a).** To the amine hydrochloride **9a** (6.20 g, 39 mmol) in methanol (300 mL) was added $NaHCO_3$ (3.28 g, 39 mmol). After stirring for 5 min,

di-*tert*-butyl dicarbonate (8.73 g, 39 mmol) was added, and the reaction mixture was stirred at room temperature for 18 h before the solvents were removed under reduced pressure. The residue was taken up in CH_2Cl_2 (300 mL) and washed with water (50 mL) and the organic layer dried (Na_2SO_4). The solvents were removed in vacuo and the residue purified by column chromatography over silica gel eluting with 20% Et_2O in hexane followed by 40% Et_2O in hexane. The title compound was obtained as a colorless solid (7.40 g, 85%). $R_f = 0.3$ (SiO_2, 40% Et_2O in hexane). Mp = 80−82 °C. ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 8.91$ (1H, s br, OH), 7.22 (1H, ddd overlapping, $^3J_{H-H} = 8$ Hz, $^3J_{H-H} = 7$ Hz, $^3J_{H-H} = 1$ Hz, 3-Ph), 7.06 (1H dd, $^3J_{H-H} = 7$ Hz, $^3J_{H-H} = 1$ Hz, 4-Ph), 6.96 (1H, dd, $^3J_{H-H} = 8$ Hz, $^3J_{H-H} = 1$ Hz, 5-Ph), 6.82 (1H, ddd overlapping, $^3J_{H-H} = 8$ Hz, $^3J_{H-H} = 7$ Hz, $^3J_{H-H} = 1$ Hz, 6-Ph), 5.25 (1H, s br, NH), 4.23 (2H, d, $^3J_{H-H} = 7$ Hz, ArCH_2NH), 1.54 (9H, s, ^{t}Bu). ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 28.4$ (CH_3), 41.4 ((CH_3)$_3$*C*), 81.3 (Ar*C*H_2NH), 117.7 (5-Ph), 119.9 (6-Ph), 125.0 (1-Ph), 129.9 (4-Ph), 130.7 (3-Ph), 155.8 (2-Ph), 158.5 (C=O). ν_{max}/cm^{-1}: 3345 br (OH/NH), 2978, 1673 (C=O), 1516, 1457, 1367, 1258, 1166, 1109, 1041, 754. *m*/*z* (EI+): 223 (36% [M]$^+$), 167 (86% [M − $(CH_3)_2CCH_2$]$^+$), 123 (100% [M − ^{t}BuCO_2]$^+$), 106 (91% [M − NH_2Boc]$^+$).

***N*-*tert*-Butoxycarbonyl-2-hydroxymethylaminopyridine (10b).** The title compound, synthesized from amine **9b** according to the method used for **10a**, was obtained as a colorless solid (12.98 g, 81%). $R_f = 0.2$ (SiO_2, 40% Et_2O in hexane). Mp = 80−83 °C. ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 9.94$ (1H, s br, OH), 8.07 (1H, dd, $^3J_{H-H} = 6$ Hz, $^3J_{H-H} = 2$ Hz, 6-Py), 7.27 (1H, dd, $^3J_{H-H} = 8$ Hz, $^3J_{H-H} = 2$ Hz, 5-Py), 7.16 (1H, dd, $^3J_{H-H} = 8$ Hz, $^3J_{H-H} = 6$ Hz, 4-Py), 4.73 (1H, s br, NH), 4.42 (2H, d, $^3J_{H-H} = 6$ Hz, Py*C*H_2-NH), 1.47 (9H, s, ^{t}Bu). ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 28.3$ ((CH_3)$_3$C), 42.9 (Py*C*H_2NH), 81.1 ((CH_3)$_3$*C*), 124.5 (4-Py), 125.5 (3-Py), 140.1 (5-Py), 145.3 (6-Py), 152.5 (2-Py), 159.0 (C=O). ν_{max}/cm^{-1}: 3395 br (OH/NH), 2963, 2927, 1683 (C=O), 1507, 1455, 1367, 1258, 1166, 1144, 1102, 1022, 800. *m*/*z* (EI+): 224 (10% [M]$^+$), 168 (100% [M − $(CH_3)_2CCH_2$]$^+$), 124 (79% [M − $(CH_3)_3$-CCO_2]$^+$).

***N*-*tert*-Butoxycarbonyl-2-benzyloxybenzylamine (11a).** The phenol **10a** (8.12 g, 36.4 mmol), benzyl bromide (6.85 g, 40.0 mmol), and K_2CO_3 (5.54 g, 40.0 mmol) in acetonitrile (200 mL) were stirred under an argon atmosphere at 60 °C for 48 h. The solvents were then removed in vacuo and the residue divided between water (50 mL) and CH_2Cl_2 (400 mL). The aqueous phase was further extracted with CH_2Cl_2 (2 × 150 mL), the organic extracts combined and dried (Na_2SO_4), and the solvents removed under reduced pressure. The resulting oil was purified by column chromatography over silica gel eluting first with hexane to remove excess benzyl bromide followed by 20% Et_2O in hexane to elute the title compound which was obtained as a colorless solid (8.40 g, 74%). $R_f = 0.3$ (SiO_2, 20% Et_2O in hexane). Mp = 53−55.5 °C. ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 6.85-7.48$ (9H, m, Ar), 5.15 (1H, s br, NH), 5.07 (2H, s, PhCH_2O), 4.38 (2H, d, $^3J_{H-H} = 6$ Hz, CH_2N), 1.46 (9H, s, ^{t}Bu). ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 28.6$ ((CH_3)$_3$), 40.6 (NCH_2), 70.3 (OCH_2), 79.2 (*C*(CH_3)$_3$), 111.8 (Ar), 120.8 (Ar), 127.3 (3-Ph), 128.0 (Ar), 128.6 (4-Ph), 129.2 (2-Ph), 129.6 (Ar), 130.0 (Ar), 137.2 (Ar), 156.1 (Ar), 156.9 (C=O). ν_{max}/cm^{-1}: 3313 (NH), 3094, 2946, 1731 (C=O), 1666 (C=O), 1548, 1454, 1215, 1187, 1038. *m*/*z* (EI+): 313 (1% [M]$^+$), 257 (19% [M − $(CH_3)_2CCH_2$]$^+$), 167 (26% [M − PhCH − $(CH_3)_2$-CCH_2]$^+$), 123 (31% [M − PhCH − ^{t}BuCO_2]$^+$), 91 (100% [PhCH_2]$^+$), 57 (93% [(CH_3)$_3$C]$^+$).

***N*-*tert*-Butoxycarbonyl-2-benzyloxymethylaminopyridine (11b).** The title compound was synthesized from phenol **10b** according to the method used for **11a**, using 50% Et_2O in hexane as column eluent, and was obtained as a colorless solid (13.0 g, 78%). $R_f = 0.4$ (SiO_2, 40% Et_2O in hexane). Mp = 82−83 °C. ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 8.11$ (1H, dd, $^3J_{H-H} = 4$ Hz, $^3J_{H-H} = 2$ Hz, C*H*N), 7.38 (7H m, Ar), 6.04 (1H, s br, NH), 5.09 (2H, s, Ph*C*H_2), 4.51 (2H, d, $^3J_{H-H} = 6$ Hz, CH_2NH), 1.46 (9H, s, ^{t}Bu). ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 28.7$ ((CH_3)$_3$), 41.3 (NCH_2), 70.1 (OCH_2), 79.4 (*C*(CH_3)$_3$), 118.1 (Ar), 122.8 (Ar), 127.4 (3-Ph), 128.5 (4-Ph), 130.0 (3-Ph), 136.2 (Ar), 140.5 (Ar), 146.6 (Ar), 152.1 (Ar), 156.3 (C=O). ν_{max}/cm^{-1}: 3411 (NH), 3065, 2977, 2931, 1711 (C=O), 1496, 1445, 1365, 1277, 1240, 1169, 1022, 797. *m*/*z* (EI+): 314 (4% [M]$^+$), 258 (16% [M − $(CH_3)_2CCH_2$]$^+$), 168 (21% [M − PhCH − $(CH_3)_2CCH_2$]$^+$), 123 (25% [M − PhCH − ^{t}BuCO_2]$^+$), 91 (80% [PhCH_2]$^+$), 57 (100% [(CH_3)$_3$C]$^+$).

2-Benzyloxybenzylamine Hydrochloride (12a). The Boc-protected benzylamine **11a** (8.60 g, 27.5 mmol) was dissolved in ethanol (40 mL), and a 12 M solution of hydrochloric acid (8 mL) was added. The solution was stirred at room temperature for 1 h, and the solvents were removed in vacuo to afford the title compound as a colorless solid (6.86 g, quantitative yield). Mp = 180−181 °C. ^{1}H NMR (270 MHz, D_2O/[D_3]MeCN 1:2): $\delta = 6.82-7.46$ (9H, m, Ar), 5.18 (2H, s, O*C*H_2Ph), 4.09 (2H, s, *C*$H_2$$NH_2$). ^{13}C NMR (67.5 MHz, D_2O/[D_3]MeCN 1:2): $\delta = 39.3$ (CH_2NH_2), 69.7 (OCH_2), 112.5 (Ar), 121.0 (Ar), 121.3 (Ar), 127.7 (3-Ph), 128.2 (Ar), 128.9 (2-Ph), 130.9 (Ar), 131.0 (Ar), 136.9 (Ar), 156.7 (Ar). ν_{max}/cm^{-1}: 3348 br (NH), 3067, 2881, 1598, 1493, 1454, 1379, 1244, 1006, 748. *m*/*z* (EI+): 213 (14% [M]$^+$), 196 (41% [M − NH_3]$^+$), 122 (41%, [M − PhCH_2]$^+$), 106 (39% [PhCHO]$^+$), 91 (100% [PhCH_2]$^+$).

2-Benzyloxymethylaminopyridine Dihydrochloride (12b). The title compound, synthesized from the Boc-protected benzylamine, **11b**, according to the method used for **12a**, was obtained as a colorless solid (11.21 g, quantitative yield). Mp = 171−174 °C. ^{1}H NMR (270 MHz, D_2O): $\delta = 8.21$ (1H, d, $^3J_{H-H} = 7$ Hz, 6-Py), 8.07 (1H, d, $^3J_{H-H} = 9$ Hz, 4-Py), 7.82 (1H, dd, $^3J_{H-H} = 7$ Hz, $^3J_{H-H} = 9$ Hz, 5-Py), 7.35 (2H d, $^3J_{H-H} = 8$ Hz, 2-Ph), 7.20 (3H, m, 3&4-Ph), 5.24 (2H, s, OCH_2Ph), 4.39 (2H, s, *C*$H_2$$NH_2$). ^{13}C NMR (67.5 MHz, D_2O): $\delta = 36.5$ (*C*$H_2$$NH_2$), 72.0 ($OCH_2$), 128.3 (3-Ph), 129.1 (2-Ph), 129.2 (Ar), 129.4 (Ar), 130.2 (Ar), 134.0 (Ar), 134.3 (Ar), 135.4 (Ar), 155.8 (Ar). ν_{max}/cm^{-1}: 3400 br (NH), 3042, 2866, 1554, 1497, 1472, 1453, 1296, 986, 801, 748. *m*/*z* (EI+): 214 (9% [M]$^+$), 123 (45%, [M − PhCH_2]$^+$), 91 (100% [PhCH_2]$^+$).

***N*-(2-Benzyloxybenzyl)bromoacetamide (13a).** Amine **12a** (6.73 g, 27 mmol) was dissolved in water (20 mL) and $NaHCO_3$ (2.44 g, 29 mmol) added. A solution of bromoacetic acid *N*-hydroxysuccinimide ester (7.31 g, 29 mmol) in THF (80 mL) was added to the amine solution and stirred at room temperature for 18 h. The solvents were removed in vacuo, and the residue was taken up into aqueous sodium bicarbonate solution (50 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The organic extracts were combined, washed with K_2CO_3 solution (pH 10, 30 mL) and HCl (0.1 M 30 mL), and dried (Na_2SO_4) and the solvents removed in vacuo to afford the title compound as a yellow solid. The crude product was eluted through a silica gel column using diethyl ether to remove the yellow coloration. The resulting oil was crystallized from dichloromethane/ether at −20 °C. After drying in vacuo, the title compound was obtained as a colorless crystalline solid (8.50 g, 94%). $R_f = 0.7$ (SiO_2, Et_2O). Mp = 85.5−86 °C. ^{1}H NMR (270 MHz, $CDCl_3$): $\delta = 7.28-7.48$ (7H, m, Ar), 7.13 (1H, t br, $^3J_{H-H} = 6$ Hz, NH), 6.97 (2H, dd, $^3J_{H-H} = 8$ Hz, $^3J_{H-H} = 8$ Hz, 3-Ph), 5.15 (2H, s, OCH_2Ph), 4.53 (2H, d, $^3J_{H-H} = 6$ Hz, CH_2NHCO), 3.85 (2H, s, COCH_2Br). ^{13}C NMR (67.5 MHz, $CDCl_3$): $\delta = 29.4$ (ArCH_2NH), 40.5 (CO*C*H_2Br), 70.2 (OCH_2Ph), 111.7 (Ar), 121.1

(Ar), 125.7 (Ar), 127.6 (2-Ph), 128.3 (Ar), 128.7 (3-Ph), 129.2 (Ar), 130.0 (Ar), 156.9 (Ar), 164.9 (C═O). ν_{max}/cm^{-1}: 3277 (NH), 3064, 2963, 1650 (C═O), 1549, 1497, 1453, 1242, 1024, 756. *m*/*z* (ESI+): 356 (100% $[M + Na]^+$), appropriate isotope patterns were observed.

***N*-(2-Benzyloxymethylenepyridine)chloroacetamide (13b).** The title compound, synthesized from the benzylamine **12b** and chloroacetic acid *N*-hydroxysuccinimide ester according to the method used for **13a**, was obtained as a colorless solid after column chromatography over silica gel eluting with 20% hexane in Et_2O (11.20 g, 98%). $R_f = 0.6$ (SiO_2, Et_2O). Mp = 86−86.5 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.29 (1H, s br, NH), 8.17 (1H, dd, $^3J_{H-H}$ = 4 Hz, $^3J_{H-H}$ = 2 Hz, 5-Py), 7.20−7.45 (7H, m Ar), 5.13 (2H, s, OC*H*$_2$Ph), 4.65 (2H, d, $^3J_{H-H}$ = 5 Hz, C*H*$_2$NHCO), 4.14 (2H, s, COC*H*$_2$Cl). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 40.4 (*C*H$_2$NH), 42.8 (CO*C*H$_2$Cl), 70.1 (O*C*H$_2$Ph), 118.2 (Ar), 123.0 (Ar), 127.4 (3-Ph), 128.4 (Ar), 128.8 (2-Ph), 135.8 (Ar), 140.4 (Ar), 144.9 (Ar), 152.0 (Ar), 166.1 (C═O). ν_{max}/cm^{-1}: 3358 (NH), 3064, 3032, 2957, 1667 (C═O), 1525, 1443, 1278, 1237, 1213, 1178, 1127, 1019, 795, 739. *m*/*z* (EI+): 290 (13% $[M]^+$), 199 (31% $[M - CH_2Ph]^+$), 91 (100% $[PhCH_2]^+$), appropriate isotope patterns were observed.

***N*-(Methylaminopyridine)chloroacetamide (5).** 2-Aminomethyl pyridine (5.0 g, 46 mmol) was added dropwise to a solution of chloroacetyl chloride (5.20 g, 46 mmol) in dichloromethane (100 mL) at 0 °C. The solution was allowed to warm to room temperature whereupon a lilac color ensued. The reaction was stirred at room temperature until the lilac color faded and a colorless precipitate was formed. The reaction mixture was washed with K_2CO_3 solution (pH 10, 50 mL) and dried (Na_2SO_4), and the solvents were removed in vacuo. The crude product was eluted through a silica gel column using 30% THF in dichloromethane. The title compound was obtained as a pale yellow oil (8.2 g, 87%). $R_f = 0.5$ (SiO_2, 30% THF in CH_2Cl_2). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.59 (1H, dd, $^3J_{H-H}$ = 5 Hz, $^3J_{H-H}$ = 2 Hz, 6-Py), 7.89 (1H, s br, NH), 7.70 (1H, ddd overlapping, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 2 Hz, 4-Py), 7.28 (1H, dd, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 2 Hz, 3-Py), 7.24 (1H, ddd overlapping, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 2 Hz, 5-Py), 4.62 (2H, d, $^3J_{H-H}$ = 5 Hz, NHC*H*$_2$Py), 4.15 (2H, s, ClC*H*$_2$CO). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 42.7 (CO*C*H$_2$Cl), 44.6 (NH*C*H$_2$Py), 122.1 (5-Py), 122.6 (4-Py), 136.9 (3-Py), 149.3 (6-Py), 155.5 (2-Py), 166.1 (C═O). ν_{max}/cm^{-1}: 3388 (NH), 3054, 2928, 1693 (C═O), 1632, 1583, 1515, 1461, 1411, 1357, 1301, 1257, 1223, 1099, 1025, 771. *m*/*z* (EI+): 148 (100% $[M - HCl]^+$), 135 (9%, $[M - CH_2Cl]^+$), 119 (81% $[M - HCO - HCl]^+$), 107 (27%, $[M - COCH_2Cl]^+$), 92 (43% $[M - NHCOCH_2Cl]^+$), 79 (91%, $[M - CHNHCOCH_2Cl]^+$).

***N*-(2-Methoxymethylenebenzene)chloroacetamide (6).** To a solution of chloroacetyl chloride (2.5 g, 21 mmol) in dichloromethane (50 mL) at 0 °C was added triethylamine (2.2 g, 21 mmol) and a solution of 2-methoxybenzylamine (3.0 g, 21 mmol) in dichloromethane (50 mL). After ∼1 h, the sample was warmed to room temperature and stirred for an additional hour. The reaction mixture was then washed with water (50 mL) and dried (Na_2SO_4), and the solvents were removed in vacuo. The title compound was obtained as a colorless crystalline solid (4.6 g, 99%). Mp = 76−77 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.29 (2H, m, 3-Ph and 6-Ph), 7.21 (1H, s br, NH), 6.92 (2H, m, 4-Ph and 5-Ph), 4.49 (2H, d, $^3J_{H-H}$ = 6 Hz, NHC*H*$_2$Ar), 4.50 (2H, s, ClC*H*$_2$CO), 3.89 (3H, s, OCH_3). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 40.0 (CO*C*H$_2$Cl), 42.8 (NH*C*H$_2$Ar), 55.4 (OCH_3), 110.5 (5-Ph), 120.8 (4-Ph), 125.4 (1-Ph), 129.3 (6-Ph), 129.9 (3-Ph), 157.7 (2-Ph), 165.6 (C═O). ν_{max}/cm^{-1}: 3289 (NH), 3068, 3016, 2965, 1649 (C═O), 1602, 1559, 1495, 1459, 1422, 1250, 1119, 1049, 1031. *m*/*z* (ESI+): 236 (100% $[M + Na]^+$).

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetamidomethylene-2-benzyloxybenzene (14a). Compound **13a** (3.13 g, 9.4 mmol), cyclen (0.39 g, 2.9 mmol), and K_2CO_3 (1.30 g, 9.4 mmol) were added to acetonitrile (50 mL) and stirred at 60 °C for 4 days. The solvent was removed in vacuo and the residue taken up in water (30 mL) and extracted with CH_2Cl_2 (2 × 200 mL). The combined extracts were dried (Na_2SO_4) and the solvents removed under reduced pressure. The oil obtained was dissolved into a mixture of dichloromethane (10 mL), methanol (20 mL), and water (7 mL) whereupon the title compound crystallized over a period of days as colorless prisms (2.17 g, 63%). Mp = 173−174 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 6.55−7.38 (40H, m, Ar and NH), 4.92 (8H, s, OCH_2Ph) 4.27 (8H, d, $^3J_{H-H}$ = 5 Hz, NHC*H*$_2$Ar), 2.88 (8H, s br, NCH_2CO), 2.45 (8H, s br, ring CH_2), 2.18 (8H, s br, ring CH_2). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 38.3 (N*C*H$_2$CO), 53.6 (ring CH_2), 58.8 (NH*C*H$_2$Ar), 70.0 (Ph*C*H$_2$O), 112.0 (Ar), 121.2 (Ar), 126.8 (Ar), 127.2 (2-Ph), 128.1 (4-Ph), 128.7 (3-Ph), 129.0 (Ar), 129.7 (1-Ph), 136.8 (Ar), 156.5 (Ar), 170.4 (C═O). ν_{max}/cm^{-1}: 3291 (NH), 3062, 2823, 1653 (C═O), 1539, 1491, 1452, 1239, 1117, 1024, 752. *m*/*z* (ESI+): 1207 (16% $[M + Na]^+$), 1185 (77% $[M + H]^+$), 604 (74% $[M + H + Na]^{2+}$), 593 (100% $[M + 2H]^{2+}$). Anal. Found C = 72.6%, H = 6.8%, N = 9.2%. $C_{72}H_{80}N_8O_8$ requires C = 73.0%, H = 6.8%, N = 9.5%.

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetamidomethylene-2-benzyloxypyridine (14b). The title compound, synthesized from chloroacetamide **13b** according to the method used for **14a**, was obtained as a colorless solid (2.0 g, 56%). Mp = 170−171 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.26 (4H, t, $^3J_{H-H}$ = 5 Hz, NH), 8.02 (4H, dd overlapping, $^3J_{H-H}$ = 6 Hz, $^3J_{H-H}$ = 6 Hz, 5-Py), 7.34 (24H, m, Ar), 7.03 (4H, d, $^3J_{H-H}$ = 6 Hz, 6-Py), 4.96 (8H, s, OCH_2Ph), 4.44 (8H, d, $^3J_{H-H}$ = 5 Hz, NHC*H*$_2$Ar), 3.16 (8H, s, NCH_2CO), 2.89 (16H, s br, ring CH_2). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 39.5 (N*C*H$_2$CO), 54.6 (ring CH_2), 60.0 (NH*C*H$_2$Ar), 69.9 (O*C*H$_2$Ph), 117.8 (Ar), 122.7 (Ar), 127.2 (3-Ph), 128.3 (Ar), 128.7 (2-Ph), 136.0 (Ar), 140.3 (Ar), 146.1 (Ar), 151.8 (Ar), 171.2 (C═O). ν_{max}/cm^{-1}: 3369 (NH), 3058, 2957, 1653 (C═O), 1557, 1472, 1443, 1311, 1275, 1174, 1113, 1020, 796. *m*/*z* (ESI+): 1189 (100% $[M + H]^+$). Anal. Found C = 68.6%, H = 6.4%, N = 14.0%. $C_{68}H_{76}N_{12}O_8$ requires C = 68.7%, H = 6.4%, N = 14.1%.

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetamidomethylenepyridine (1). Chloroacetamide **5** (1.73 g, 9.4 mmol), cyclen (0.40 g, 2.3 mmol), and K_2CO_3 (1.30 g, 9.4 mmol) were added to acetonitrile (20 mL) and stirred at 60 °C for 24 h. The solvents were removed in vacuo, and the residue was taken up in water (50 mL) and extracted with CH_2Cl_2 (2 × 200 mL). The combined extracts were dried (Na_2SO_4) and the solvents removed under reduced pressure. The residual oil was purified over silica gel eluting first with 15% methanol in dichloromethane to remove starting materials and then with 5% methanol, 5% aqueous ammonia, and 30% dichloromethane in THF. The oil obtained was taken up into water (5 mL) and freeze-dried to afford the title compound as a pale yellow solid (0.74 g, 41%). $R_f = 0.6$ (SiO_2, 5% methanol, 5% ammonia, 30% dichloromethane, 60% THF). Mp = 85−86 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.38 (4H, d, $^3J_{H-H}$ = 7 Hz, 6-Py), 8.20 (4H, s br, NH), 7.57 (4H, dd overlapping, $^3J_{H-H}$ = 7 Hz $^3J_{H-H}$ = 7 Hz, 5-Py), 7.14 (8H, m, 3- and 4-Py), 4.36 (8H, d, $^3J_{H-H}$ = 6 Hz, ArC*H*$_2$NH), 3.27 (8H, s, NC*H*$_2$CO), 2.68 (16H, s br, ring CH_2). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 44.4 (N*C*H$_2$CO), 54.1 (ring CH_2), 59.1 (Ar*C*H$_2$NH), 122.0 (3-Py), 122.3 (2-Py), 137.1 (4-Py), 148.7 (5-Py), 157.2 (6-Py), 171.8 (C═O). ν_{max}/cm^{-1}: 3335 (NH),

3062, 2965, 2830, 1661 (C═O), 1594, 1539, 1476, 1437, 1367, 1309, 1261, 1241, 1102, 1050, 1026, 1000, 760. *m*/*z* (ESI+): 765 (100% $[M + H]^+$). This compound proved too hygroscopic for a reliable elemental analysis.

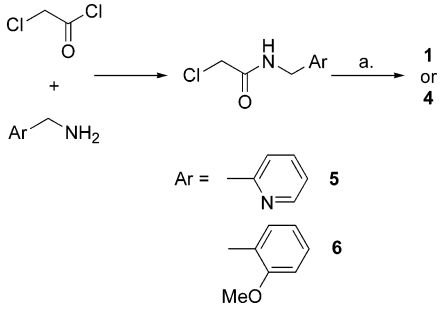
1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetamidomethylene-2-methoxybenzene (4). The title compound, synthesized from chloroacetamide **6** according to the method used for **14a**, was obtained as a colorless solid (1.8 g, 64%). Mp = 192−192.5 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 7.20 (8H, m, 5-Ph and 6-Ph), 7.10 (4H, t, $^3J_{H-H}$ = 6 Hz, NH), 6.89 (4H, d, $^3J_{H-H}$ = 7 Hz, 3-Ph), 6.84 (4H, d, $^3J_{H-H}$ = 8 Hz, 6-Ph), 4.34 (8H, d, $^3J_{H-H}$ = 6 Hz, ArC*H*$_2$NH), 3.79 (12H, s, OCH_3), 2.78 (8H, s, NCH_2CO), 2.41 (16H, s, ring CH_2). ^{13}C NMR (67.5 MHz, $CDCl_3$): δ = 38.3 (N*C*H$_2$CO), 54.0 (OCH_3), 55.5 (ring CH_2), 59.0 (NH*C*H$_2$Ar), 110.6 (6-Ph), 120.8 (3-Ph), 126.3 (1-Ph), 129.1 (5-Ph), 129.8 (4-Ph), 157.5 (2-Ph), 170.3 (C═O). ν_{max}/cm^{-1}: 3301 (NH), 3062, 2943, 2832, 1656 (C═O), 1602, 1547, 1492, 1460, 1308, 1289, 1241, 1108, 1025. *m*/*z* (ESI+): 881 (37% $[M + H]^+$), 903 (100% $[M + Na]^+$), 920 (5% $[M + K]^+$). Anal. Found C = 65.3%, H = 7.4%, N = 12.7%. $C_{48}H_{64}N_8O_8$ requires C = 65.5%, H = 7.3%, N = 12.7%.

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetamidomethylene-2-benzyloxybenzene (2). Tetrabenzyl ether **14a** (0.70 g, 0.6 mmol) was dissolved in methanol (40 mL), and 10% palladium on carbon (0.20 g) was added. The suspension shaken in a Parr Hydrogenator bottle for 72 h under H_2 (50 psi). The catalyst was removed by filtration and the solvent removed in vacuo. The residue was dissolved in water (10 mL) and lyophilized to afford the title compound as a colorless solid (0.48 g, 99%). Mp = 134−135 °C. ^{1}H NMR (270 MHz, D_2O): δ = 7.14 (4H, d, $^3J_{H-H}$ = 7 Hz, 3-Ph), 7.06 (4H, dd overlapping, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 7 Hz, 4-Ph), 6.78 (4H, d, $^3J_{H-H}$ = 8 Hz, 6-Ph), 6.64 (4H, dd overlapping, $^3J_{H-H}$ = 8 Hz, $^3J_{H-H}$ = 7 Hz, 5-Ph), 4.23 (8H, s, ArC*H*$_2$NH), 3.08 (8H, s br, NC*H*$_2$CO), 2.56 (8H, s br, ring CH_2), 2.27 (8H, s br, ring CH_2). ^{13}C NMR (67.5 MHz, [D_6]DMSO): δ = 38.7 (N*C*H$_2$CO), 50.0 (ring CH_2), 55.1 (Ar*C*H$_2$NH), 115.0 (6-Ph), 119.6 (4-Ph), 123.6 (1-Ph), 128.6 (5-Ph), 129.2 (3-Ph), 154.3 (2-Ph), 167.3 (C═O). ν_{max}/cm^{-1}: 3250 br (NH/OH), 3065, 2918, 1658 (C═O), 1548, 1454, 1364, 1237, 1049. *m*/*z* (ESI+): 825 (100% $[M + H]^+$). Anal. Found C = 67.8%, H = 6.6%, N = 13.3%. $C_{44}H_{56}N_8O_8$ requires C = 64.1%, H = 6.8%, N = 13.6%.

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetamidomethylene-2-hydroxypyridine (3). The title compound, synthesized from tetrabenzyl ether **14b** according to the method used for **2**, was obtained as a colorless solid (0.55 g, 99%). Mp = 141−142 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ = 8.06 (4H, d, $^3J_{H-H}$ = 7 Hz, 6-Py), 7.38 (4H, d, $^3J_{H-H}$ = 7 Hz, 4-Py), 7.31 (4H, dd, $^3J_{H-H}$ = 7 Hz, $^3J_{H-H}$ = 7 Hz, 5-Py), 4.66 (8H, s, ArC*H*$_2$NH), 3.38 (8H, s, NC*H*$_2$CO), 2.81 (16H, s br, ring C*H*$_2$). ^{13}C NMR (67.5 MHz, [D_6]-DMSO): δ = 39.9 (N*C*H$_2$CO), 52.5 (ring CH_2), 57.2 (Ar*C*H$_2$NH), 124.2 (4-Py), 124.6 (3-Py), 137.8 (2-Py), 143.3 (5-Py), 152.6 (6-Py), 173.9 (C═O). ν_{max}/cm^{-1}: 3349 (NH), 3070, 2921, 1667 (C═O), 1551, 1456, 1386, 1288, 1163, 1117. *m*/*z* (ESI+): 829 (16% $[M + H]^+$), 415 (100% $[M + 2H]^{2+}$). Anal. Found C = 57.7%, H = 6.4%, N = 20.0%. $C_{40}H_{52}N_{12}O_8$ requires C = 58.0%, H = 6.3%, N = 20.3%.

Synthesis of Europium Complexes. Europium oxide (20 mg, 54 μmol) was added to concentrated HCl (2 mL) and heated until dissolved. The solvent was removed under reduced pressure to afford the chloride salt as a colorless solid. The europium chloride so obtained was dissolved in water (3 mL) and the solution added to a solution of ligand **3** (100 mg, 121 μmol) in water (3 mL). After heating for 18 h at 60 °C, the solvent was removed by freeze-drying to afford the complex as a colorless solid. The complex was used without further purification. HPLC analysis (C18 reversed phase, 5 mL min^{-1}, 280 nm, 0−5 min H_2O (0.1% HCl) → 18 min 80% MeCN/20% H_2O (HCl)) revealed two peaks. One peak was assigned to the excess free ligand and the other to the complex. ^{1}H NMR (500 MHz, D_2O): [Eu(**1**)]$^{3+}$ δ = 24.7 ($H_{ax'}$), 7.3 (H_{Ar}), 6.8 (H_{Ar}), 6.5 (H_{Ar}), 6.1 (H_{Ar}), 3.2 (H_{Bn}), 2.8 (H_{Bn}), −3.1 ($H_{eq'}$), −5.9 ($H_{ax''}$), −8.8 ($H_{eq''}$), −11.8 (H_{ac}), −13.7 (H_{ac}); [Eu(**2**)]$^{3+}$ δ = 27.7 ($H_{ax'}$), 7.2 (H_{Ar}), 7.0 (H_{Ar}), 6.7 (H_{Ar}), 6.6 (H_{Ar}), 3.4 (H_{Bn}), 2.8 (H_{Bn}), −1.7 ($H_{eq'}$), −5.4 ($H_{ax''}$), −7.2 ($H_{eq''}$), −12.0 (H_{ac}), −13.0 (H_{ac}); [Eu(**3**)]$^{3+}$ δ = 24.4 ($H_{ax'}$), 7.4 (H_{Ar}), 6.5 (H_{Ar}), 3.2 (H_{Bn}), 2.8 (H_{Bn}), −3.1 ($H_{eq'}$), −5.7 ($H_{ax''}$), −8.8 ($H_{eq''}$), −11.4 (H_{ac}), −13.2 (H_{ac}); [Eu(**4**)]$^{3+}$ δ = 26.9 ($H_{ax'}$), 7.2 (H_{Ar}), 6.7 (H_{Ar}), 6.4 (H_{Ar}), 6.3 (H_{Ar}), 3.1 (H_{Bn}), 2.7 (H_{Bn}), −2.2 ($H_{eq'}$), −5.8 ($H_{ax''}$), −7.5 ($H_{eq''}$), −12.7 (H_{ac}), −13.3 (H_{ac}).

Scheme 1. The synthesis of ligands **1** and **4**. Conditions and reagents: a) cyclen/MeCN/K_2CO_3/60 °C

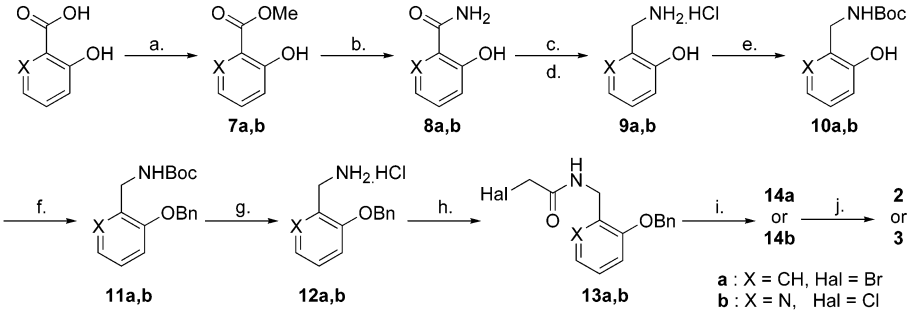


Results and Discussion

Ligand Synthesis. Ligands **1** and **4** were synthesized from the appropriate benzylamines. The benzylamine was condensed with chloroacetyl chloride in dichloromethane (0 °C/2 h) (Scheme 1). In the case of 2-aminomethyl pyridine, no base was employed in this reaction as the pyridine satisfies this role. Triethylamine was added to the reaction of 2-methoxybenzylamine. The resulting chloroacetamides **5** and **6** were then used to alkylate cyclen (K_2CO_3/MeCN/82 °C/48 h). Although ligand **1** was obtained after purification by column chromatography, ligand **4** was obtained by crystallization from aqueous methanol.

Ligands **2** and **3** were synthesized from the corresponding hydroxybenzoic acids (Scheme 2). The hydroxybenzoic acids were esterified in methanol with concentrated sulfuric acid. Protection of the hydroxyl groups of methyl esters **7** proved problematic at this stage in the synthesis so the esters were reacted with aqueous ammonia to form the corresponding primary amides **8**. Borane reduction of **8** afforded hydroxybenzylamines **9** as the hydrochloride salts in quantitative yield. The amino groups of **9** could then be protected with a Boc group by reaction with di-*tert*-butyl dicarbonate in methanol with $NaHCO_3$ as base. Protection of the hydroxyl groups of **10** with benzyl bromide (K_2CO_3/MeCN) was now feasible and proceeded in good yield (74% and 78%). The Boc groups were then removed with ethanolic hydrogen chloride (2 M) to afford protected benzylamines **12** as hydrochloride salts. The corresponding chloroacetamides **13** were synthesized directly from the hydrochloride salt in aqueous THF using the *N*-hydroxysuccinimide ester of chloro- or bromoacetic acid. Alkylation of cyclen with haloacetamides **13** yielded the protected ligands **14** after

Scheme 2. Synthesis of Ligands **2** (X = CH) and **3** (X = N) from the Corresponding Hydroxybenzoic Acids[a]



[a] Reagents and conditions: (a) $MeOH/H_2SO_4$, (b) NH_3(aq), (c) BH_3·THF, (d) 2 M HCl, (e) $(Boc)_2O/MeOH/NaHCO_3$, (f) $BnBr/K_2CO_3/MeCN$, (g) HCl/EtOH, (h) $ClCH_2CO_2Su/THF/NaHCO_3/H_2O$, (i) cyclen/$K_2CO_3$/MeCN, (j) Pd on carbon/$H_2$/EtOH.

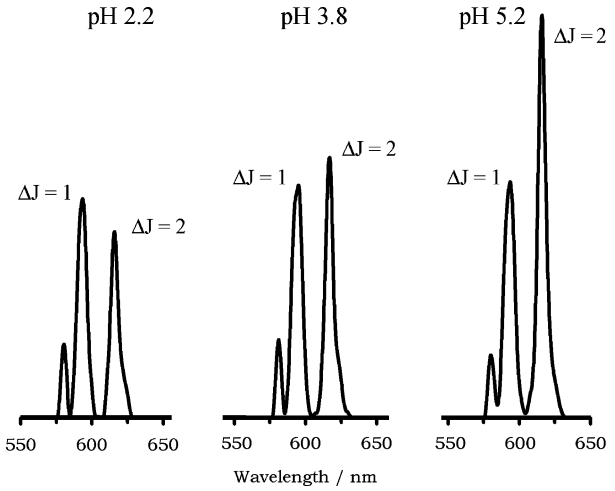


Figure 1. Selected emission spectra of $[Eu(\mathbf{1})]^{3+}$ (λ_{ex} = 363 nm) between pH 2 and 6.

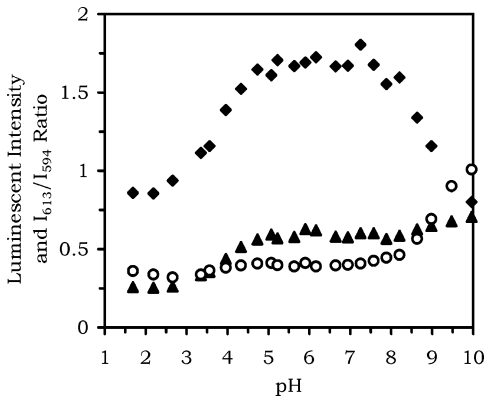


Figure 2. Emission intensity at 594 nm (▲) and 613 nm (○) and the I_{594}/I_{613} ratio (◆) of $[Eu(\mathbf{1})]^{3+}$ plotted as a function of pH.

crystallization from aqueous methanol. Ligands **2** and **3** were then liberated by hydrogenolysis of the benzyl ethers using 10% palladium on carbon as catalyst. The overall yields of 33% and 33% were obtained for **2** and **3**, respectively. The europium complex of each ligand was prepared as a chloride salt from $EuCl_3$ in water (60 °C/18 h).

Luminescence Studies. The emission spectra of $[Eu(\mathbf{1})]^{3+}$, $[Eu(\mathbf{2})]^{3+}$, and $[Eu(\mathbf{3})]^{3+}$ after sensitized excitation (via the aryl chromophore) and direct excitation (397 nm) were recorded as a function of pH. [Due to solubility problems, the spectra of $[Eu(\mathbf{2})]^{3+}$ and $[Eu(\mathbf{4})]^{3+}$ were recorded in 50% aqueous methanol. All other spectra were recorded in water.] The most intense of the $^5D_0 \rightarrow {}^7F_J$ emission bands are usually the $\Delta J = 1$ and $\Delta J = 2$ bands centered at 594 and 613 nm, respectively. The $^5D_0 \rightarrow {}^7F_1$ transition is recognized as being "insensitive" to ligand field effects and therefore the coordination environment of the Eu^{3+} ion while the $^5D_0 \rightarrow {}^7F_2$ transition is considered hypersensitive to coordination environment.[13] However, the exact relationship between the form of the emission spectrum and the coordination environment of Eu^{3+} remains unclear. In the sensitized emission spectrum of $[Eu(\mathbf{1})]^{3+}$ at low pH, the emission at 594 nm is more intense than that at 613 nm (Figure 1). As the solution pH is raised, the emission intensity at 613 nm increases relative to that at 594 nm. Around pH 4, the emission intensities at the two wavelengths are about equal with that at 613 nm continuing to increase until pH 5.5 when it is some 1.5 times the intensity of the emission at 594 nm.

When monitored at 594 nm ($\Delta J = 1$), the emission intensity of $[Eu(\mathbf{1})]^{3+}$ remains constant on passing from pH 2 to 7. However, above pH ~ 7, the intensity of this "insensitive" band also begins to increase with a further increase in pH (Figure 2). A concomitant increase in luminescent lifetime was also observed (Supporting Information). The hydration state, q, measured by using a corrected Horrocks' method ($q = 1.2\{k_{H_2O} - k_{D_2O} - 0.25\}$),[14] was 0.3 at pH 10 and ~1.0 at pH 7 and below. This is consistent with deprotonation of a single water molecule in the inner coordination sphere between pH 7 and 10.[15] The resulting decrease in the number of proximate OH oscillators reduces nonradiative quenching of the Eu^{3+} excited state and increases Eu^{3+} emission. Deprotonation of a Eu^{3+}-bound water molecule would result in a shorter $Eu^{3+}-O$ bond distance, a change in Eu^{3+} ligand field, and a corresponding change in Eu^{3+} emission intensity. Thus, the changes in intensities of the 594 and 613 nm bands above pH 7 can be ascribed to deprotonation of the Eu^{3+}-bound water molecule (plotted as the ratio I_{613}/I_{594} in Figure 2) while the changes

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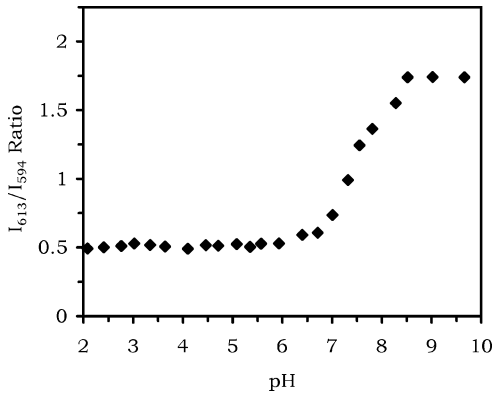

Figure 3. I_{594}/I_{613} ratio for [Eu(**2**)]$^{3+}$ as a function of pH.

observed below pH 7 have a different origin (discussed in following paragraphs).

The pH sensitivity of I_{613}/I_{594} offers the possibility of using this ratio as a direct measure of solution pH. This could prove particularly useful because most other probes used to measure pH required an independent measure of the Eu^{3+} complex concentration.[8] The emission spectra of [Eu(**2**)]$^{3+}$ and [Eu(**3**)]$^{3+}$ also demonstrate a change in I_{613}/I_{594} with pH similar to that observed for [Eu(**1**)]$^{3+}$ (Supporting Information). However, the pH range over which this change is observed is shifted slightly toward higher pH in the latter two complexes (Figure 3).

Clearly, a significant (although possibly subtle) change in the coordination environment of both [Eu(**2**)]$^{3+}$ and [Eu(**3**)]$^{3+}$ occurs over this pH range. Since a similar change in coordination environment is observed for [Eu(**1**)]$^{3+}$, the effect must be common to all three complexes. The excitation spectra of [Eu(**3**)]$^{3+}$ (Supporting Information) show that these changes do not arise from protonation/deprotonation of the pyridyl substituent. However, proton exchange between the amide NH and bulk solvent protons in tetraamide complexes such as these is known to become more rapid with increasing pH.[16] Deprotonation of the amide would result in increased electron donation to the metal center, and this might be detected as a change in Eu^{3+} ligand field and emission intensity. This hypothesis is supported by high-resolution ^{1}H NMR spectra recorded at various pH values in H_2O (Figure 4). NMR spectra of [Eu(**3**)]$^{3+}$ at acidic pH values show a resonance near 0 ppm that integrates to 4 protons and may be assigned to the amide NH protons. As the pH of the solution is raised, the area of this resonance diminishes and totally disappears by pH $\sim$ 7. This is consistent with deprotonation of the four amide groups over the same pH range as the change in I_{613}/I_{594} shown in Figure 3.

One additional feature of the emission spectra of [Eu(**2**)]$^{3+}$ and [Eu(**3**)]$^{3+}$ is that their total emission intensity diminishes with increasing pH. This same feature does not appear in the nonphenol derivative, [Eu(**1**)]$^{3+}$. This diminution in intensity represents a significant drawback to the use of [Eu(**2**)]$^{3+}$ or [Eu(**3**)]$^{3+}$ as a pH indicator since, at one end of the pH curve, accurate measurement of the I_{594}/I_{613} ratio becomes

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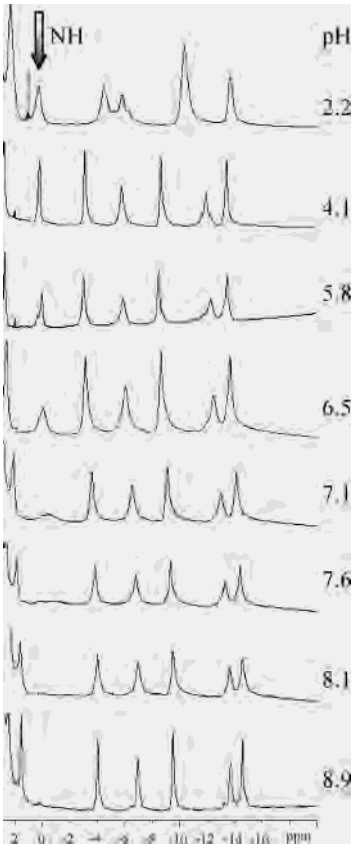

Figure 4. Extended sweep width ^{1}H NMR spectra of [Eu(**3**)]$^{3+}$ recorded at 500 MHz in H_2O (no solvent suppression employed). The amide NH resonance at $\sim$0 ppm can be seen to disappear as the pH is raised.

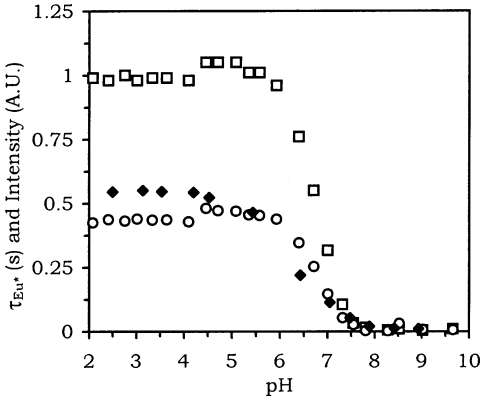

Figure 5. Luminescent lifetime (◆) and emission intensities of [Eu(**2**)]$^{3+}$ at 594 nm of after excitation at 319 nm (○) and 397 nm (□).

problematic due to limited signal. The decrease in emission intensity from both sensitized and direct excitation of [Eu(**2**)]$^{3+}$ and [Eu(**3**)]$^{3+}$ is paralleled by a decrease in the luminescent lifetime in these complexes (Figure 5). The luminescent lifetime of these complexes above pH 8 was estimated to be $\leq$30 μs and is thus substantially shorter than that of the Eu^{3+} aquo ion ($\sim$170 μs). At acidic pH, the hydration state of [Eu(**3**)]$^{3+}$ is 1.0 (k_{H_2O} = 0.54 ms, k_{D_2O} = 1.30 ms). A change in the hydration state (q) of these complexes cannot account for such a dramatic change in the luminescent lifetime. As protonation/deprotonation of the aryl substituents does not occur over this pH range, back energy

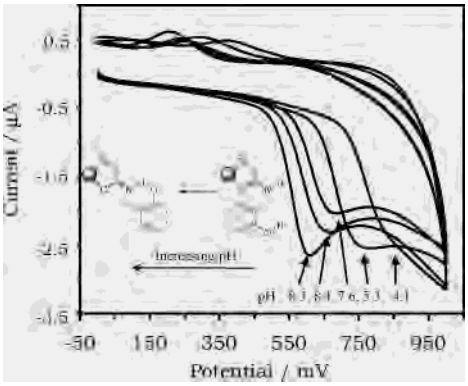

Figure 6. Cyclic voltammagrams of $[Eu(\mathbf{2})]^{3+}$ show that the oxidation potentials of the phenols drop considerably with increasing pH. Recorded in 50% aqueous acetonitrile in the presence of 25 mM Bu_4NClO_4 and 0.6 mM $[Eu(\mathbf{2})]^{3+}$ using a classy carbon electrode and Ag/AgCl reference.

transfer to a low lying triplet state may also be discounted as a cause of this emission quenching.

However, the reduction potential of Eu^{3+} is comparatively low, around −0.8 V, so the presence of an easily oxidized functionality on the ligand could lead to ligand−metal electron transfer and quenching of the Eu^{3+} excited state.[5] The cyclic voltammograms of Figure 6 show that the oxidation potential of $[Eu(\mathbf{2})]^{3+}$, relative to Ag/AgCl, decreases dramatically from 0.85 V at pH 4.1 to 0.61 V at pH 8.3. Although the oxidation of phenol is known to be pH dependent (the deprotonated form is more easily oxidized),[17] the changes observed in Figure 6 occur at pH values too low for a typical phenolic deprotonation. However, coordinating amides can be protonated/deprotonated over this pH range[16] so one could envision intramolecular acid−base pairing between the deprotonated coordinating amides and the phenols in these complexes (Figure 6). Furthermore, acid−base pairing has been shown to reduce the oxidation potential of phenols[18] so we suggest that deprotonation of the amides causes increased electron transfer to the Eu^{3+} excited state thereby quenching luminescence. Such acid−base pairing would also account for the apparent difference in pK_a values.

An analogous complex to $[Eu(\mathbf{2})]^{3+}$ and $[Eu(\mathbf{3})]^{3+}$ was synthesized for comparative purposes. The phenolic groups of $[Eu(\mathbf{4})]^{3+}$ are methylated, and this was expected to reduce the reductive properties of the ligand, preserving emission intensities at higher pH. The emission intensity of $[Eu(\mathbf{4})]^{3+}$ was monitored as a function of pH at 594 nm and, over the pH range 2−10, was found to vary by less than 10%. Unexpectedly no change in the I_{613}/I_{594} ratio was observed over this pH range. This is presumably the result of acid−base pairing between the acidic amide protons and the oxygen of the methoxy substituent, raising the effective pK_a of the amide protons. Measurements at pH > 10 are hampered in these systems by base-catalyzed hydrolysis of the amide bonds, rendering it difficult to further probe this hypothesis. Nonetheless, $[Eu(\mathbf{4})]^{3+}$ clearly demonstrates that ligand to metal charge transfer from the readily reduced phenolic systems is responsible for the quenching of europium based luminescence in $[Eu(\mathbf{2})]^{3+}$ and $[Eu(\mathbf{3})]^{3+}$.

Conclusion

We have shown that Eu^{3+} complexes of DOTA-tetraamide ligands having extended aromatic functional groups have emission spectral characteristics that may allow them to serve as pH sensing agents. Small changes associated with deprotonation of the coordinating amide groups in these complexes cause subtle changes in the coordination environment of Eu^{3+}. These changes, reflected as pH dependent changes in the emission spectra of such complexes, may allow measurement of pH without the need for determining the concentration of the probe. The effective pH range for any given complex may be altered by appropriate selection of the amide substituent. However, the decrease in emission intensity observed with $[Eu(\mathbf{2})]^{3+}$ and $[Eu(\mathbf{3})]^{3+}$ seriously limits the applicability of these two compounds as luminescent probes. When measured by comparison with quinine sulfate (φ = 0.546 in 0.5 M sulfuric acid) using the method of Iki et al.,[19] values of $\varphi = 0.013$, $\varphi = 0.009$, and $\varphi = 0.0125$ were obtained under acidic conditions for $[Eu(\mathbf{1})]^{3+}$, $[Eu(\mathbf{2})]^{3+}$, and $[Eu(\mathbf{3})]^{3+}$, respectively (λ_{ex} = 300 nm). These values are comparable with those obtained for other related europium complexes.[20] Nonetheless, we have successfully been able to demonstrate that, by altering the nature of the amide substituent, one may carefully control the precise pH range over which the coordinating amides deprotonate. Although care must still be taken not to include substituents that have an adverse effect upon quantum yield, this ratiometric method for pH determination, similar to that devised for terbium complexes by Parker and co-workers,[8] should allow the probes to be fine-tuned to fit specific applications.

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Supporting Information Available: Figures showing the variation of luminescent lifetime with pH for $[Eu(\mathbf{1})]^{3+}$, selected emission spectra of $[Eu(\mathbf{3})]^{3+}$, and excitation intensities of $[Eu(\mathbf{3})]^{3+}$ as a function of pH. This material is available free of charge via the Internet at http://pubs.acs.org.

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