Effect of C–H Bonds on the Quenching of Luminescent Lanthanide Chelates

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The quenching of europium(III) and terbium(III) chelate luminescence by high-energy C–H vibrational manifolds was studied with two types of stable chelates, i.e., a seven-dentate phenylethynylpyridine derivative and a nine-dentate terpyridine derivative. The replacement of C–H bonds by C–D bonds in the chelating parts of the ligands had a clear positive effect on Eu\(^{3+}\) luminescence but a negligible effect on Tb\(^{3+}\) luminescence. In aqueous solution, however, the positive effect was undetectable, if the chelating ligand did not create complete shielding of the ion against aqueous quenching. In chelates, where the coordination of water molecules to the inner sphere is prevented, the residual quenching through C–H vibrational quanta can be avoided by replacement of all C–H bonds in the vicinity of the emitting ion by C–D bonds.

KEY WORDS: Europium; terbium; luminescence quenching; deuterated ligands.

INTRODUCTION

Delayed detection of luminescence excited with a short pulse is frequently used to avoid background problems in bioaffinity binding assays. Various luminescent lanthanide chelates with long excited-state lifetimes have been developed for the purpose [1]. The stable chelates synthesized so far suffer from relatively low quantum yields as a result of quenching of the emitting ion by the surrounding media, in particular by water [2,3]. Various systems have been developed to avoid this phenomenon, such as using detergents and synergistic compounds [4], using high concentrations of fluoride ions [5], removing water by drying prior to measurement [6], using a polymeric matrix [7], or measuring the luminescence in an organic solvent or in deuterium oxide. An ideal way to avoid direct aqueous quenching is to use stable, nine-dentate chelating agents [8], which do not allow the coordination of water with the chelated ion.

In addition to the quenching caused by O–H stretching, the high-energy vibration of C–H is also known to create a nonradiative energy leakage route [3]. Strong leakage is connected especially with Sm\(^{3+}\) and Dy\(^{3+}\), because compared to Eu\(^{3+}\) and Tb\(^{3+}\), these ions have a narrower energy gap between their lowest excited state and the highest ground-state level and, hence, the number of vibrational quanta required for their nonradiative inactivation [9] is lower. In this work the replacement of C–H bonds in the close vicinity to the emitting ion by C–D bonds is demonstrated to improve the luminescence quantum yield of stable Eu\(^{3+}\) chelates in aqueous media.

MATERIALS AND METHODS

**Instruments**

UV spectra were recorded with a Shimadzu UV 2100 spectrophotometry, IR spectra with a Perkin-Elmer
160 FTIR, $^1$H-NMR at 400 MHz with Jeol GX 400 and luminescence spectra, and decay times and quantum yields with a Perkin-Elmer LS5 spectrotuorometer.


Bromine (33.6 g, 0.21 mol) was added during 10 min into a mixture of $[^2]$H$_3$acetac $[^2]$Hacid (11.6 g, 0.18 mol) and red phosphorus (0.23 g, 7.43 mmol) at 100–105°C. After stirring for 2 h, the product was distilled under reduced pressure: 16.4 g (64%). IR (film): 1730, 1408, 1285 (C=O, C-O).

Methyl Bromo-$[^3]$Hacetate (2)

SOCl$_2$ (27.27 g, 0.230 mol) was dropped slowly to cooled dry MeOH (70 ml). After stirring at r.t. for 0.5 h, 1 (16.28 g, 0.115 mol) was added, and the mixture was refluxed for 6 h and evaporated nearly to dryness. The residue was dissolved in CHCl$_3$ (100 ml), neutralized with sat. NaHCO$_3$, washed with H$_2$O (20 ml), and dried (Na$_2$SO$_4$) and the residue distilled: 3.56 g (20%). IR (film): 1760, 1438, 1261 (C=O, C-O). $^1$H-NMR (CDCl$_3$): 3.81 (s, 3 H).

Methyl Amino-$[^3]$Hacetate Hydrochloride (3)

SOCl$_2$ (2.97 g, 25.0 mmol) was dropped slowly to cooled dry MeOH (10 ml). After stirring at r.t. for 0.5 h, $[^2]$H$_2$amino-$[^2]$H$_2$acetac $[^2]$Hacid (1.00 g, 12.5 mmol) was added, and the mixture was refluxed for 18 h and evaporated to dryness. Yield: 1.60 g (100%). IR (film): 1748, 1433, 1330 (C=O, C-O). $^1$H-NMR (CDCl$_3$): 3.73 (s, 3 H); 8.56 (broad s, 3 H).


A mixture of 3 (1.38 g, 10.8 mmol), dry K$_2$CO$_3$ (7.46 g, 54.0 mmol) and dry MeCN (50 ml) was refluxed for 10 min. After refluxing for 6.5 h, the mixture was filtered and evaporated, and the product purified by flash chromatography (silica gel, petroleum ether (b.p. 40–60°C)/AcOEt 2:5): 1.11 g (62%). IR (film): 3355 (N-H), 1743, 1437, 1280 (C=O, C-O). $^1$H-NMR (CDCl$_3$): 1.98 (broad s, 1 H); 3.74 (s, 6 H).

Dimethyl 2,2':6,2"-Terpyridine-6,6"-dicarboxylate (5)

A mixture of 6,6"-dicyano-2,2',6',2"-terpyridine ([8] 2.40 g, 8.47 mmol), AcOH (25 ml), and H$_2$SO$_4$ (25 ml) was refluxed for 1.5 h. The solution was poured to ice, and the precipitate was filtered, washed, with H$_2$O, and dried. The mixture of dry MeOH (150 ml) and SOCl$_2$ (2.0 ml) was stirred for 15 min, and 2,2':6,2"-terpyridine-6,6"-dicarboxylic acid was added. The mixture was refluxed for 5 h. The solution was evaporated to half a volume and sat. NaHCO$_3$ (250 ml) was added. The mixture was extracted with CHCl$_3$ (3 × 200 ml) and the CHCl$_3$ phase was washed with H$_2$O, dried (Na$_2$SO$_4$), and evaporated: 1.95 g (66%). UV (EtOH): 3.15 (sh), 301 (sh), 288, 248, 216 nm. IR (KBr): 1724 (C=O), 1578 (arom), 1432, 1135 (C-O). $^1$H-NMR (CDCl$_3$): 4.06 (s, 6 H); 8.02 (d, J = 7.6, 2 H); 8.02 (d, J = 7.6, 4 H); 8.18 (dd, J = 1.0 & 7.6, 2 H); 8.63 (d, J = 7.6, 2 H); 8.81 (dd, J = 1.0 & 7.6, 2 H).

Reduction of 5 and Diethyl 4-Bromopyridine-2,6-dicarboxylate [10] with NaBH$_4$ or NaBD$_4$

General Procedure. A mixture of 5 or diethyl 4-bromopyridine-2,6-dicarboxylate (6.23 mmol), abs. EtOH (80 ml), and NaBH$_4$ or NaBD$_4$ (28.0 mmol) was refluxed for 3–20 h. The solvent was evaporated, sat. NaHCO$_3$ (40 ml) was added, and the mixture was heated to boiling. H$_2$O (120 ml) was added, the mixture was cooled to 0°C and filtered.

(2,2':6,2"-Terpyridine-6,6"-diyl)dimethanol (6)

Yield: 58%. UV (EtOH): 315 (sh), 301 (sh), 286, 239 nm. IR (KBr): 3415 (O-H), 1571 (arom). $^1$H-NMR ((D$_6$)-DMSO): 4.70 (s, 4 H); 5.56 (s, 2 H); 7.58 (d, J = 7.7, 2 H); 8.01 (t, J = 7.7, 2 H); 8.08 (t, J = 7.7, 1 H); 8.43 (d, J = 7.7, 2 H); 8.49 (d, J = 7.7, 2 H).

(2,2':6,2"-Terpyridine-6,6"-diyl)di-$[^2]$H$_2$methanol (7)

Yield: 63%. UV (EtOH): 315 (sh), 302 (sh), 286, 239 nm. IR (KBr): 3417 (O-H), 1576 (arom). $^1$H-NMR ((D$_6$)-DMSO): 5.50 (s, 2 H); 7.58 (dd, J = 1.0 & 7.8, 2 H); 8.01 (t, J = 7.8, 2 H); 8.08 (t, J = 7.8, 1 H); 8.43 (d, J = 7.8, 2 H); 8.49 (dd, J = 1.0 & 7.8, 2 H).

(4-Bromopyridine-2,6-diyl)di-$[^2]$H$_2$methanol (8)

After the addition of H$_2$O, the mixture was extracted with CHCl$_3$/EtOH (2:1, 3 × 15 ml) and dried (Na$_2$SO$_4$). Yield: 84%. UV (EtOH): 272, 265 nm. IR (film): 3355 (O-H), 1579 (arom.). $^1$H-NMR ((D$_6$)-DMSO): 5.51 (s, 2 H); 7.52 (s, 2 H).