

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

I. Hemmilä,^{1,3} V.-M. Mukkala,² and H. Takalo¹

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 Eu³⁺ luminescence but a negligible effect on Tb³⁺ 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 Sm³⁺ and Dy³⁺, because compared to Eu³⁺ and Tb³⁺, 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 Eu³⁺ chelates in aqueous media.

MATERIALS AND METHODS

Instruments

UV spectra were recorded with a Shimadzu UV 2100 spectrophotometry, IR spectra with a Perkin-Elmer

¹ Wallac Oy, P.O. Box 10, FIN-20101 Turku, Finland.

² Department of Chemistry, University of Turku, Turku, Finland.

³ To whom correspondence should be addressed.

1600 FTIR, $^1\text{H-NMR}$ at 400 MHz with Jeol GX 400 and luminescence spectra, and decay times and quantum yields with a Perkin-Elmer LS5 spectrofluorometer.

Bromo- $^{[2}\text{H}_2]$ acetic $^{[2}\text{H}]$ Acid (**1**)

Bromine (33.6 g, 0.21 mol) was added during 10 min into a mixture of $^{[2}\text{H}_3]$ acetic $^{[2}\text{H}]$ acid (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- $^{[2}\text{H}_2]$ acetate (**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_2O (20 ml), and dried (Na_2SO_4) and the residue distilled: 3.56 g (20%). IR (film): 1760, 1438, 1261 (C=O, C–O). $^1\text{H-NMR}$ (CDCl_3): 3.81 (s, 3 H).

Methyl Amino- $^{[2}\text{H}_2]$ acetate 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, $^{[2}\text{H}_2]$ amino- $^{[2}\text{H}_2]$ acetic $^{[2}\text{H}]$ acid (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). $^1\text{H-NMR}$ (CDCl_3): 3.73 (s, 3 H); 8.56 (broad s, 3 H).

Dimethyl Iminobis($^{[2}\text{H}_2]$ acetate) (**4**)

A mixture of **3** (1.38 g, 10.8 mmol), dry K_2CO_3 (7.46 g, 54.0 mmol) and dry MeCN (50 ml) was refluxed for 10 min, and **2** (1.68 g, 10.8 mmol) was added. 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). $^1\text{H-NMR}$ (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_2SO_4 (25 ml) was refluxed for 1.5 h. The solution was poured to

ice, and the precipitate was filtered, washed, with H_2O , 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 \times 200 ml) and the CHCl_3 phase was washed with H_2O , dried (Na_2SO_4), and evaporated: 1.95 g (66%). UV (EtOH): 315 (sh), 301 (sh), 288, 248, 216 nm. IR (KBr): 1724 (C=O), 1578 (arom), 1432, 1135 (C–O). $^1\text{H-NMR}$ (CDCl_3): 4.06 (s, 6 H); 8:02 (t, $J = 7.6$, 2 H); 8.02 (t, $J = 7.6$, 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_2O (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). $^1\text{H-NMR}$ ((D_6) -DMSO): 4.70 (s, 4 H); 5.56 (s, 2 H); 7.58 (d, $J = 7.7$, 2 H); 8.02 (t, $J = 7.7$, 2 H); 8.09 (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- $^{[2}\text{H}_2]$ methanol (**7**)

Yield: 63%. UV (EtOH): 315 (sh), 302 (sh), 286, 239 nm. IR (KBr): 3417 (O–H), 1576 (arom). $^1\text{H-NMR}$ ((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- $^{[2}\text{H}_2]$ methanol (**8**)

After the addition of H_2O , the mixture was extracted with $\text{CHCl}_3/\text{EtOH}$ (2:1, 3 \times 15 ml) and dried (Na_2SO_4). Yield: 84%. UV (EtOH): 272, 265 nm. IR (film): 3355 (O–H), 1579 (arom.). $^1\text{H-NMR}$ ((D_6) DMSO): 5.51 (s, 2 H); 7.52 (s, 2 H).

Bromination of Alcohols 6–8

General Procedure. A solution of PBr_3 (0.42 g, 1.55 mmol) and CHCl_3 (3.5 ml) was added to a suspension of **6–8** (1.03 mmol) and the mixture was refluxed for 4 h, neutralized with 5% NaHCO_3 , and aq. phase was extracted with CHCl_3 (5×10 ml). The CHCl_3 phase was dried (NaSO_4) and the product purified by flash chromatography (silica gel).

6,6''-Bis(bromomethyl)-2,2':6',2''-terpyridine (9)

Yield: 50%. UV (EtOH): 315 (sh), 302 (sh), 289, 245 nm. IR (KBr): 1575, 1566 (arom). $^1\text{H-NMR}$ (CDCl_3): 4.66 (s, 4 H); 7.49 (dd, $J = 0.7$ & 7.7, 2 H); 7.86 (t, $J = 7.7$, 2 H); 7.96 (t, $J = 7.7$, 1 H); 8.52 (d, $J = 7.7$, 2 H); 8.53 (dd, $J = 0.7$ & 7.7, 2 H).

6,6''-Bis($^2\text{H}_2$]bromomethyl)-2,2':6',2''-terpyridine (10)

Yield: 59%. UV (EtOH): 315 (sh), 302 (sh), 288, 246 nm. IR (KBr): 1576, 1567 (arom). $^1\text{H-NMR}$ (CDCl_3): 7.49 (d, $J = 7.8$, 2 H); 7.86 (t, $J = 7.8$, 2 H); 7.96 (t, $J = 7.8$, 1 H); 8.52 (d, $J = 7.8$, 2 H); 8.53 (d, $J = 7.8$, 2 H).

4-Bromo-2,6-bis($^2\text{H}_2$]bromomethyl)pyridine (11)

Flash chromatography: petroleum ether (40–60°C)/AcOEt 10:1. Yield: 58%. UV (EtOH): 274 nm. IR (film): 1556 (arom.). $^1\text{H-NMR}$ (CDCl_3): 7.55 (s, 2 H).

Synthesis of 12–15

General Procedure. A mixture of **9–11** (0.57 mmol), **4**, or di(*t*-butyl) iminobis(acetate) ([11], 1.15 mmol), dry K_2CO_3 , and dry MeCN (10 ml) was stirred for 24 h at r.t. After filtration and evaporation, the product was purified by flash chromatography (silica gel).

*Tetra(*t*-butyl) 2,2',2'',2'''-[(2,2':6',2''-Terpyridine-6,6''-diyl)bis($^2\text{H}_2$]methylenenitrilo)]tetrakis(acetate) (12)*

Flash chromatography: $\text{CHCl}_3/\text{MeOH}$ 19:1. Yield: 73%. UV (EtOH): 316 (sh), 302 (sh), 284, 233 nm. IR (KBr): 1738 (C=O), 1570 (arom), 1433, 1148 (C–O). $^1\text{H-NMR}$ (CDCl_3): 1.47 (s, 36 H); 3.55 (s, 8 H); 7.65 (d, $J = 7.8$ Hz, 2 H); 7.84 (t, $J = 7.8$ Hz, 2 H); 7.90 (t, $J = 7.8$ Hz, 1 H); 8.47 (d, $J = 7.8$ Hz, 2 H); 8.49 (d, $J = 7.8$ Hz, 2 H).

Tetramethyl 2,2',2'',2'''-[(2,2':6',2''-Terpyridine-6,6''-diyl)bis(methylenenitrilo)]tetrakis($^2\text{H}_2$]acetate) (13)

Flash chromatography: $\text{CHCl}_3/\text{MeOH}$ 19:1. Yield: 44%. UV (EtOH): 317 (sh), 304 (sh), 283, 228 nm. IR (KBr): 1746 (C=O), 1569 (arom), 1433, 1170 (C–O). $^1\text{H-NMR}$ (CDCl_3): 3.72 (s, 12 H); 4.16 (s, 4 H); 7.58 (d, $J = 7.8$ Hz, 2 H); 7.84 (t, $J = 7.8$ Hz, 2 H); 7.92 (t, $J = 7.8$ Hz, 1 H); 8.45 (d, $J = 7.8$ Hz, 2 H); 8.50 (d, $J = 7.8$ Hz, 2 H).

Tetramethyl 2,2',2'',2'''-[(2,2':6',2''-Terpyridine-6,6''-diyl)bis($^2\text{H}_2$]methylenenitrilo)]tetrakis($^2\text{H}_2$]acetate) (14)

Flash chromatography: $\text{CHCl}_3/\text{MeOH}$ 19:1. Yield: 54%. UV (EtOH): 315 (sh), 303 (sh), 284, 231 nm. IR (KBr): 1748 (C=O), 1569 (arom), 1434, 1212 (C–O). $^1\text{H-NMR}$ (CDCl_3): 3.72 (s, 12 H); 7.58 (dd, $J = 1.0$ & 7.8 Hz, 2 H); 7.85 (t, $J = 7.8$ Hz, 2 H); 7.93 (t, $J = 7.8$ Hz, 1 H); 8.45 (d, $J = 7.8$ Hz, 2 H); 8.51 (dd, $J = 1.0$ & 7.8 Hz, 2 H).

Tetramethyl 2,2',2'',2'''-[(4-Bromopyridine-2,6-diyl)bis($^2\text{H}_2$]methylenenitrilo)]tetrakis($^2\text{H}_2$]acetate) (15)

Flash chromatography: petroleum ether (40–60°C)/AcOEt, 1:1. Yield: 48%. UV (EtOH): 260 nm. IR (film): 1745 (C=O), 1563 (arom), 1436, 1221 (C–O). $^1\text{H-NMR}$ (CDCl_3): 3.71 (s, 12 H); 7.72 (s, 2 H).

Tetramethyl 2,2',2'',2'''-[[4-(Phenylethynyl)pyridine-2,6-diyl]bis($^2\text{H}_2$]methylenenitrilo)]tetrakis($^2\text{H}_2$]acetate) (16)

A mixture of **15** (62 mg, 0.12 mmol), bis(triphenylphosphine)palladium(II) chloride (2.5 mg, 3.6 μmol), and CuI (1.4 mg, 7.2 μmol) in dry Et_3N (0.9 ml) and dry THF (0.9 ml) was deaerated with N_2 . Phenylacetylene (21 mg, 0.21 mmol) was added and the mixture was heated at 50°C for 24 h. After evaporation, the product was purified by flash chromatography (silica gel, first petroleum ether (40–60°C)/AcOEt 1:1, then AcOEt). Yield: 50 mg (77%). UV (EtOH): 302 (sh), 281, 270 (sh), 245, 238 (sh), 224 (sh) nm. IR (film): 2216 (C \equiv C), 1749 (C=O), 1590 (arom.), 1422, 1216 (C–O). $^1\text{H-NMR}$ (CDCl_3): 3.72 (s, 12 H); 7.31–7.42 (m, 3 H); 7.54 (d, $J = 3.4$, 2 H); 7.56 (s, 2 H).

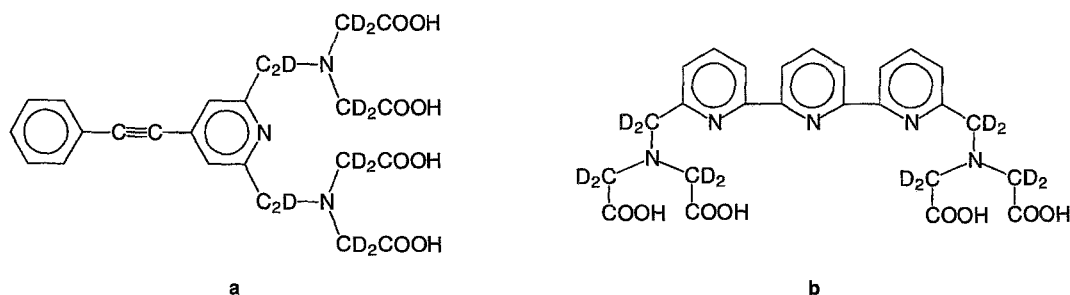


Fig. 1. The structures of seven-dentate (a) and nine-dentate (b) chelating agents of the experiment as their deuterated forms.

2,2',2'',2'''-[2,2':6',2''-Terpyridine-6,6''-diyl]bis([²H₂]methylenetrifluoroacetate))tetrakis(acetic Acid) (17)

A solution of **12** (74 mg, 0.099 mmol) in CF₃COOH (4 ml) was stirred for 2.5 h at r.t. After evaporation, the mixture was triturated with Et₂O and filtered. Yield: 100%. UV (H₂O): 288, 233 nm. UV ([Eu^{III}(**17**), H₂O): 337, 326, 292, 284, 235 nm. IR (KBr): 1734 (C=O), 1570 (arom), 1436, 1195 (C–O). ¹H-NMR ((D₆)-DMSO): 3.75 (s, 8 H); 7.66 (d, *J* = 7.8, 2 H); 8.05 (t, *J* = 7.8, 2 H); 8.12 (t, *J* = 7.8, 1 H); 8.45 (d, *J* = 7.8, 2 H); 8.56 (d, *J* = 7.8, 2 H).

Hydrolysis of Esters 13, 14, and 16

General Procedure. A mixture of **13**, **14**, or **16** (0.087 mmol), 0.5 M KOH/EtOH (3.2 ml), H₂O (0.5 ml) was stirred for 2.5–4 h at r.t., evaporated, dissolved in H₂O (5 ml), and acidified with 2 M HCl (pH ca. 2.0). The precipitate was filtered and washed with cold H₂O.

2,2',2'',2'''-[2,2':6',2''-Terpyridine-6,6''-diyl]bis(methylenetrifluoroacetate))tetrakis([²H₂]acetic Acid) (18)

UV (H₂O): 292, 234 nm. UV ([Eu³⁺(**18**), H₂O): 337, 326, 292, 282, 235 nm. IR (KBr): 1728, 1628 (C=O), 1570 (arom), 1436, 1267 (C–O). ¹H-NMR ((D₆)-DMSO): 4.11 (s, 4 H); 7.63 (d, *J* = 7.6, 2 H); 8.01 (t, *J* = 7.6, 2 H); 8.09 (t, *J* = 7.6, 1 H); 8.42 (d, *J* = 7.6, 2 H); 8.50 (d, *J* = 7.6, 2 H).

2,2',2'',2'''-[2,2':6',2''-Terpyridine-6,6''-diyl]bis([²H₂]methylenetrifluoroacetate))tetrakis([²H₂]acetic Acid) (19)

UV (H₂O): 288, 230 nm. UV ([Eu^{III}(**19**), H₂O): 337, 326, 292, 283, 235 nm. IR (KBr): 1727, 1628 (C=O), 1570 (arom), 1436, 1274 (C–O). ¹H-NMR

((D₆)DMSO): 7.63 (d, *J* = 7.8, 2 H); 8.02 (t, *J* = 7.8, 2 H); 8.10 (t, *J* = 7.8, 1 H); 8.43 (d, *J* = 7.8, 2 H); 8.52 (d, *J* = 7.8, 2 H).

2,2',2'',2'''-[4-(Phenylethynyl)pyridine-2,6-diyl]bis([²H₂]methylenetrifluoroacetate))tetrakis([²H₂]acetic Acid) (20)

UV (H₂O): 313 (sh), 276, 245 (sh) nm. UV ([Eu^{III}(**20**), H₂O): 317 (sh), 273, 260 (sh), 245 (sh) nm. IR (KBr): 2216 (C≡C), 1728 (C=O), 1605 (C–N), 1273 (C–O). ¹H-NMR ((D₆)DMSO): 7.45–7.50 (m, 3 H); 7.57 (s, 2 H); 7.63 (d, *J* = 5.7, 2 H).

Luminometry

The luminescence properties of the chelates prepared were measured in a spectrofluorometer using Eu³⁺ in DELFIA system as a standard. In DELFIA enhancement solution the Eu³⁺-2-naphthoyltrifluoroacetone-TOPO chelate has a molar absorption coefficient of 35,000 at the excitation maximum, 340 nm, and a quantum yield of 0.7 in its main emission line at 613 nm [12]. The luminescences are measured at the most intense emission lines (Tb³⁺ at 544–545 nm and Eu³⁺ at 612–615).

RESULTS

The effect of deuteration was first tested with Eu³⁺ and Tb³⁺ chelates of a seven-dentate 4-(phenylethynyl)pyridine derivative (compound **20**; Fig. 1a) both in aqueous solution and in deuterium oxide (Table I). Deuteration had no effect on Tb³⁺ luminescence but a clear positive effect on Eu³⁺ luminescence was found, however, only in deuterium oxide. The effect of the deuterium substitution in nine-dentate chelating agents (Fig 1b) was further tested by measuring the luminescence

Table I. Effect of deuteration on the luminescence properties of Eu^{3+} and Tb^{3+} chelates of a seven dentate ligand (Fig. 1a)

Parameters	Ln	Deuterated form		Undeuterated	
		H_2O	D_2O	H_2O	D_2O
Excitation max. (nm)	Eu^{3+}	299	306	300	309
	Tb^{3+}	300	299	300	299
Emission max. (nm)	Eu^{3+}	614	614	614	614
	Tb^{3+}	544	544	544	544
Decay time (μs)	Eu^{3+}	400	2620	390	2210
Intensity ($\epsilon \times \Phi$)	Eu^{3+}	263	3790	658	2790
	Tb^{3+}	1180	1530	1160	1570

Table II. Effect of deuterium substitution level on the luminescence properties of Eu^{3+} chelates of a nine dentate ligand (Fig. 1b)

Deuteration level	0	4	8	12
Decay time (μs)	1260	1500	1480	1610
Quantum yield	0.11	0.13	0.13	0.15
Luminescence intensity ($\epsilon \times \Phi$)	940	1120	1230	1470

properties of their Eu^{3+} chelates in aqueous solution and in deuterium oxide. The decay times (from 1260 to 1610 μs), quantum yields (from 0.11 to 0.15), and relative luminescence intensities (from 940 to 1470) increased with increasing deuteration levels from 0 to 12 as shown in Table II. With the nine-dentate chelator the increase in the level of deuteration had an identical effect both in water and in deuterium oxide (data not shown).

DISCUSSION

The energy leakage through the vibrational manifold of O-H stretching is a well-documented phenomenon in luminescent chelates, and various systems have been developed to avoid its negative effect on assay sensitivities when Eu^{3+} or Tb^{3+} chelates are used as labels. Recently, also, quenching of Eu^{3+} and Tb^{3+} through the N-H vibrational overtones has been described [13].

Quenching through C-H stretching quanta is more seldom regarded as a problem. The study of the Eu^{3+} chelates of the seven-dentate ligands showed that the leakage through O-H stretching is so strong that no positive effect of deuteration could be found in water. Tb^{3+} , in contrast, has a wider energy gap (about 14,400 cm^{-1} , compared to 12,200 cm^{-1} with Eu^{3+}), and hence both the O-H and the C-H energy levels have a much less profound effect on Tb^{3+} luminescence. By removing the detrimental effect of water by replacing it with deuterium oxide, the positive effect of deuterium replacement was clearly demonstrated.

The partial substitution of C-H bonds by C-D bonds in the stable, nine-dentate chelate showed that the C-H vibrational manifold, similarly to O-H vibrations [3], has a cumulative quenching effect with respect to the number of C-H bonds present in the vicinity of Eu^{3+} . In defining chelate labels for highly sensitive assays, the replacement of all the hydrogen atoms by deuterium in the chelating moiety of the ligand can be used to maximize the luminescence intensity.

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